The Second *Aspergillus* Meeting

March 13-15, 2005
Asilomar Conference Center

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

2004 AGRPC:

Gerhard Braus (*A. nidulans*) 2004
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Jiujiang Yu (*A. flavus*), 2003-04
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David Denning, Past Chair (*A. fumigatus*), 2003
North Manchester Gen Hospital, UK; Email: ddenning@man.ac.uk
Ex Officio: Kevin McCluskey (Fungal Genetics Stock Center, USA)

THANKS TO OUR MEETING SPONSORS!!!!!

Second *Aspergillus* Meeting Program
All sessions in Merrill Hall

March 13, Sunday
3:00-6:00 Registration
6:00 Dinner
7:00-9:00 Welcome Reception (sponsored by Gilead Sciences, Inc)

March 14, Monday

7:30-9:00 Breakfast
Session 1: New labs/ young investigators Chair: Michelle Momany
9:00-9:15 Welcome, introductions and announcements

9:15-9:45 Evolutionary Processes in the Aflatoxin Gene Cluster in *Aspergillus*
Ignazio Carbone, North Carolina State University, USA

9:45-10:15 Analysis of the Regulatory MultiProtein Complex COP9 signalsome in *Aspergillus nidulans*
Silke Busch, Georg-August University-Goettingen, Germany

10:15-10:45 Coffee Break (Sponsored by Monsanto)

10:45-11:15 A Mitochondrial Short-Chain Acyl-CoA Dehydrogenase from *Aspergillus nidulans*
Lori Maggio-Hall, University of Wisconsin, USA

11:15-11:45 Two *Aspergillus nidulans* Developmental Mutants (SWOM1 and SWON1) are Defective in Genes Involved in Synthesis of Cell Wall Components
Brian Shaw, Texas A&M University, USA

12:00-1:00 Lunch

Session 2: Genome updates Chairs: Jiujiang Yu and Masayuki Machida
1:00 – 1:20 The six *Aspergillus* genomes and microarrays
William C. Nierman, TIGR, USA
1:20 – 1:30 The *A. flavus* genome
Gary Payne, North Carolina State University, USA
1:30 – 1:40 The DSM *A. niger* genome
Noel van Peij, DSM, The Netherlands
1:40 – 1:45 The DOE *A. niger* genome
Scott Baker, Pacific North West National Laboratory, USA
1:45 – 1:50 The *A. flavus* microarray
Jiujiang Yu, USDA/ARS, Southern Regional Research Center, USA
1:50 – 1:55 The *A. oryzae* microarray
Masayuki Machida, National Institute of Advanced Industrial Science and Technology (AIST), Japan

2:00-2:30 Community directions discussion Moderator: Michelle Momany
2:30-2:45 Coffee Break (Sponsored by Monasanto)

Session 3: Talks selected from abstracts Chair: Gerhard Braus

2:45-3:00 The role of RACA and the identification of RACA interacting proteins during polarized growth in *Aspergillus niger*
Arthur F. J. Ram, Leiden University, The Netherlands

3:00-3:15 The *Aspergillus nidulans* uvsB-ATR and scaA-NBS1 genes show genetical interactions during recovery from the HU-replicational stress
Marcia Regina von Zeska Kress Fagundes, Sao Paolo University, Brazil

3:15-3:30 *Aspergillus oryzae* ATFB encodes a transcription factor which is required for stress tolerance of conidia
Kazutoshi Sakamoto, National Res. Institute of Brewing Hiroshima, Japan

3:30-3:45 Absence of free iron induces the expression of non-ribosomal peptide synthetases *in Aspergillus fumigatus*
Katrhin Reiber, National University of Ireland Maynooth, Ireland

3:45-4:00 Break

4:00-4:15 Nitrate metabolite and nitrate signalling
Mark Caddick, The University of Liverpool, UK

4:15-4:30 Identification of a natural antisense transcript of AFLR, the transcriptional regulator of the aflatoxin biosynthesis pathway in *Aspergillus flavus*
Carrie Jacobus, North Carolina State University, USA

4:30-4:45 Some genes expressed during the stationary phase in *Aspergillus fumigatus*
Amin Mouslavi, University of Kerman, Iran

4:45-5:30 Pontecorvo Lecture: What would he think of this?
John Clutterbuck, University of Glasgow, UK

6:00-7:00 Dinner

7:00-10:00 Posters and drinks (sponsored by DSM)

March 15, Tuesday

7:30-9:00 ***Breakfast***
9:00-9:15 Elections--2 new AGRPC members

9:15-11:30 Session 4: Tools Chair: Michael Hynes

9:15 -9:25 CADRE
M.J.Anderson, University of Manchester, UK
9:25 -9:35 TAP tagging
Özgür Bayram, Georg-August-University Goettingen, Germany
9:35 -9:45 S-tag
Steve Osmani, University of Ohio, USA
9:45-9:55 RNAi
Tom Hammond , University of Wisconsin, USA
9:55-10:05 NHEJ
Berl Oakley, University of Ohio, USA

10:05-10:15 Discussion of vectors/manipulations

10:15-10:35 Coffee Break (Sponsored by Monsanto)

10:35-10:50 Microarrays
H. Stanley Kim, TIGR, USA
10:50-11:00 Microarrays
Luisa Trindade, Wageningen University, The Netherlands

11:00-11:10 Discussion of microarrays

11:10-11:20 Proteomics
Ken Oda, National Research Institute of Brewing, Japan

11:20-11:30 Discussion of proteomics

11:30 Announce election results and take any further discussion

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

1:00-3:00 Subgroup meetings, TBA
4:00 New AGPRC Meets - elect officers and get organized

Abstract Titles, Alphabetical by presenting Author
(Complete author list at back of program booklet)

1. Aguirre, Jesus	The TMPA Gene Regulates Asexual Development in Aspergillus nidulans
2. Anderson, Michael	Viewing Annotated Aspergillus Genomes on the Central Aspergillus Data Repository (Cadre) Website
3. Arentshorst, Mark	The role of RacA and the identification of RacA interacting proteins during polarized cell growth in Aspergillus niger
4. Bayram, O.	Adaptation of the TAP Tag Method to Decipher Protein Complexes in Filamentous Fungi
5. Burns, Claire	Isolation, cloning and characterisation of three glutathione-s-transferases in Aspergillus fumigatus
6. Busch, Silke	Analysis of the Regulatory MultiProtein Complex COP9signalsome in Aspergillus nidulans
7. Caddick, Mark	Nitrogen Metabolite and Nitrate Signalling
8. Campbell, Bruce	Targeting Stress-Response Genes for Control of Aspergillus Using Antifungal Natural Compounds and Target-Gene Based Bioassays
9. Canovas, David	Arsenic Detoxification in a Hypertolerant Aspergillus Strain
10. Carbone, Ignazio	Evolutionary Processes in the Aflatoxin Gene Cluster in Aspergillus
11. Clutterbuck, John	RIP-affected and unaffected transposable elements in Aspergillus
12. Dzikowska, Agnieszka	Nitrogen Metabolite Repression of Arginine Catabolism Genes in Aspergillus nidulans is Meidated by Negatievly Acting Factor AREB
13. Fagundes, M. R. Z.	Aspergillus nidulans uvsBATR AND scaANBS1 Genes Show Genetic Interaction s During Recovery From the HU-Replicational Stress
14. Fedorova, Natalie	Phylogenomic Analyses of Heterocaryon Incompatibility Proteins in Aspergilli
15. Flitter, Simon	RAMOSA-1: an Evolutionary Convserved Protein Involved in Fungal Morphogenesis
16. Gautam, Poonam	Comparative Proteomic Analysis of Culture Proteins of Aspergillus fumigatis From Clinical Isolates of ABPA and Invasive Patients of Aspergillosis
17. Gomi, Katsuya	Carbon Source-Dependent Alternative Transcription of the Enolase-Encoding Gene (ENOA) in Aspergillus oryzae
18. Goosen, Theo	Unfolded Protein Response in Aspergillus niger Chemostat Fermentations
19. Helmstaedt,Kerstin	Analysis of Dynein Regulatory Complexes Using a Tandem Affinity Purification Tag for Filamentous Fungi
20. Khew, Gillian	Aspergillus nidulans mutants unable to localise glyoxylate cycle enzymes to the peroxisomes are able to utilise acetate
21. Hammond, Tom	Investigating abnormal phenotypes associated with RNA silencing enzymes and RNA-dependent RNA polymerases in Aspergillus nidulans
22. Hoffman, Gerald	Transcription analysis of carbon repression of Aspergillus nidulans using High Density Microarrays

23. Jacobus, Carrie	Identification of a Natural Antisense Transcript of AFLR, the Transcriptional Regulator in the Aflatoxin Biosynthese Pathway in Aspergillus flavus
24. James, Steven	Identification and Molecular Analysis of SNOA (Suppressor-of-NIMO), a Novel Regulator of DNA Synthesis in Aspergillus nidulans
25. Jones, Meriel G.	Protemics Ananlysis of Regulation and Signalling
26. Kaminskyj, Susan	A Model for Branch Establishment in Aspergillus nidulans
27. Kato, Masahi	In Vitro Analysis oon the Assembly Mechanisms of the Aspergillus CCAAT-Binding Factor
28. Keszenman-Pereyra, D.	New Conditional Promoter Cassesttes for Aspergillus Carrying the Pyrithiamine Resistance Gene
29. Kim, H.Stanley	Temperature-dependent gene expression in Aspergillus fumigatus examined by DNA microarray
30. Kusumoto, Ken Ichi	Characterization of the telomere-attached vectors and their transformants of Aspergillus oryzae
31. Lindsey, Rebecca	A single septin gene from Aspergillus nidulans induces filamentous growth in Saccharomyces cerevisiae
32. Machida, Masayuki	Analyses of metabolic pathways and their expression of Aspergillus oryzae
33. Maggio-Hall, Lori	A Mitochondrial Short-Chain Acyl-CoA Dehydrogenase from Aspergillus nidulans
34. Martens, Elena	Cross species gene discovery using microarray analysis allows for the identification of D-galacturonic acid utilization pathway in Aspergillus niger and Aspergillus nidulans
35. Mousavi, Amin	Some Genes Expressed During the Stationary Phase in Aspergillus fumigatis
36. Nahlik, Krystyna	The JAMM motif of the COP9 signalosome is essential for Aspergillus nidulans sexual development
37. Nielsen, Michael Lynge	Genetic Stability of Direct and Inverted Repeats in Aspergillus nidulansLANS
38. Nierman, William C.	Secondary Metabolite Biosynthetic Gene Clusters in Aspergilli
39. Oakley, Berl R	Deletion of the KU70 Homolog of Aspergillus nidulans Facilitates Gene Replacement and Gene Tagging
40. Oda, Ken	Proteome Analysis of secreted proteins from Aspergillus oryzae in liquid and solid-state culture conditions.
41. Osami, Stephen	A Single Step Affinity Purification of Protein Complezes from Aspergillus nidulans
42.Payne, Gary	Aspergillus flavus Genome Sequence Initial Analysis
43 .Peij-Van, Noel	Comparison of the Aspergillus niger genomic DNA sequence with its genetic map
44. Punt, Peter	Proteolysis and Protein Processing in Filamentous Fungi
45. Ram, Arthur	Characterization of CWPA, a Putative Glycosyl-Phosphatidylinositol Anchored Cell Wall Mannoprotein in the Fungus Aspergillus Niger
46.Ram, Arthur	A New Study of Cell Wall Integrity and Disturbing Antifungal Compounds with a Novel GFP-Based Reporter Method
47. Reiber, Kathrin	Absence of free iron induces the expression of non-ribosomalpeptide synthetases *in Aspergillus fumigatus*

48. Sakamoto, Kazutoshi	Aspergillus Orzyae ATFB Encodes a Transcription Factor Which is Required for Stress Tolerance of Conidia
49. Schwier, E.	Characterization pf Subunit One and Two of the A. nidulans COP9 Signalsome
50 Shaw, Brian D.	Two Aspergillus nidulans Developmental Mutants (SWOM1 and SWON1) are Defective in Genes Involved in Synthesis of Cell Wall Components
51. Suzuki, Yumi	Regulation of Gluconeogenesis in Aspergillus Nidulans
52. Trindade, Luisa	Micorarray Analysis of the Aspergillus niger Transcriptome Revealed that XLNR Plays an Important Rolse in the Regulation of Different Pathways
53. Wong, Chris	Post-Translational Regulation of AreA, the Global Transcriptional Activator of Nitrogen Metabolism in Aspergillus Nidulans
54. Wortman, Jennifer	Examination of the phylogeny and possible function of genes specific to A. fumigatus and A. fischerianus
55.Yu, Jiujiang	Functional Genomics Studies for Identifying Genes Involved in Aflatoxin Formation in Aspergillus Flavus
56. Yuan, Xiaolian	Identification and transcriptional Regulation of New Starch Modifying Enzymes in the Aspergillus Niger Genome

ABSTRACTS

1. THE TMPA GENE REGULATES ASEXUAL DEVELOPMENT IN ASPERGILLUS NIDULANS.

Gabriela Soid-Raggi, Olivia Sánchez and Jesús Aguirre. Instituto de Fisiología Celular-UNAM, Apartado postal 70-242, 04510 México, D.F., Tel: (5255) 5622 5651, Fax: (5255) 5622 5630, E-mail: jaguirre@ifc.unam.mx.

In A. nidulans, the study of "fluffy" mutants showing delayed asexual development (conidiation) has led to the identification of a genetic pathway, composed by fluG, fadA, flbA-D and other genes, involved in the regulation of this process. Genetic characterization of a fluffy mutant isolated by REMI mutagenesis has led us to identify tmpA as a new gene that regulates conidiation in a fluG-independent pathway. TmpA defines a new family of putative transmembrane proteins of unknown function, present in fungi and plants. As TmpA appears to bind FAD and seems distantly related to the ferric reductase family, we propose that TmpA participates in the production of a small metabolite involved in regulation of asexual development. Supported by CONACYT 400346-5-25115N and SAGARPA-CONACYT 2002-C01-1713.

2.VIEWING ANNOTATED ASPERGILLUS GENOMES ON THE CENTRAL ASPERGILLUS DATA REPOSITORY (CADRE) WEBSITE.

JE Mabey Gilsenan MJ Anderson TK Attwood SG Oliver NW Paton GD Robson DW Denning. 1.800 Stopford Building, The University of Manchester, Oxford Road, Manchester M13 9PT, UK. t: +441612753918; f: +441612755656; e: m.j.anderson@manchester.ac.uk

The Central Aspergillus Data Repository (CADRE) has been funded to house publicly available genomic data for all Aspergillus species. The Ensembl database schema and associated software for housing and displaying eukaryotic genomic data have been adapted and used to set up a Web site (http://www.cadre.man.ac.uk/). This system has enabled us to establish a database for housing the sequence and annotation of each genome and will provide users with several means of searching for and viewing the data. Using a Web browser, the position of mapped features, such as protein-coding genes, can be viewed on sequence contigs. The highly flexible display means that users can turn views and features on and off as desired. For each feature, links are provided to allow the user to retrieve further data. For protein-coding genes, such data include: chromosomal co-ordinates; a description of the encoded protein's function; similarity matches; and displays of transcript structure and protein features. Further links from this Gene Report provide sequences and additional information for transcripts, proteins and exons/introns. The readily adaptable Ensembl system will enable us in the future to display syntenic regions between genomes and map additional features, such as ESTs, onto the sequence contigs. Two complete Aspergillus genomes are currently available on the CADRE Web site. The Aspergillus nidulans genomic sequence and automatically generated annotation has been provided by the Broad Institute and consists of 28.6 Mbases of DNA with 9520 predicted genes. The sequence consists of 248 contigs assembled into 18 supercontigs. One hundred and sixty four of the contigs were assigned to the 8 linkage groups and these contigs have been assembled into virtual chromosomes. Our next release of the A. nidulans sequence will incorporate manually corrected gene reports (including splits/merges) brought to our attention and representations of chromosomal arms. The Aspergillus fumigatus genomic sequence was determined by The Institute for Genomic Research (TIGR) and the Sanger Institute. Annotation was generated automatically using the TIGR pipeline with some manual

correction. The genome consists of 28.8 Mbases of DNA with 10,034 predicted genes. The sequence has been assembled into 16 chromosomal arms stretching from the telomere to the centromere. Two arms contain gaps: one of these gaps is only 5 kb and the other one represents the ribosomal DNA repeat.

3. THE ROLE OF RACA AND THE IDENTIFICATION OF RACA INTERACTING PROTEINS DURING POLARIZED CELL GROWTH IN ASPERGILLUS NIGER.

M. Arentshorst[1], R.A. Damveld[1], S.M.J. Langeveld[1], M.S. Roelofs[1], C.A.M.J.J. van den Hondel[1,2] and A.F.J. Ram[1,2].
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The establishment of cell polarity in filamentous fungi is critical for the control of many cellular and developmental processes, including polarized hyphal growth, intracellular movement of organelles, protein secretion, cell wall biosynthesis and the development of conidiophores. In yeasts, but also in other eukaryotic cells, polarized cell growth is driven by Rho-related GTPases (Rho, Rac and Cdc42). Analysis of the function of the small GTPase RacA in A.niger has implicated a role for RacA during polarized cell growth. Deletion of racA showed abnormal branching at the hyphal tip and delayed sporulation. Overexpression of dominant active RacA resulted in the loss of polarized hyphal growth. To identify RacA interacting proteins Gateway compatible yeast two hybrid cDNA libraries have been constructed. The effects of alteration of expression of these RacA interacting proteins on fungal morphology will be determined.

4. ADAPTATION OF THE TAP TAG METHOD TO DECIPHER PROTEIN COMPLEXES IN FILAMENTOUS FUNGI

Özgür Bayram, Kerstin Helmstaedt, Sven Krappmann and Gerhard H. Braus
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Protein purification methods employing tandem affinity purification (TAP) tags have become an increasingly useful tool to gain information about the composition of cellular protein complexes and interactions among proteins. Generally, TAP tag constructs consist of two tandemly repeated Staphylococcus aureus protein A domains, one TEV protease cleavage site and a small peptide comprising a calmoduline binding domain. Originally designed for yeast expression, when expressed in the filamentous fungus Aspergillus nidulans detectable levels of TAP tag fusion proteins are rather low owing to the species' codon usage.
By application of site directed mutagenesis, we have altered all the rarely used codons in the commonly employed TAP tag construct to achieve higher rates of translation in the endogenous host. Both versions of the tag suited for N-terminal and C-terminal fusions were modified. Expression levels of these modified TAP tags were tested by construction of fusions to the green flourescent protein (GFP), the expression of which was driven by the inducible alcA promoter. After functionality could be validated, chimeric constructs with one regulator of A. nidulans fruit body formation were expressed and a purification protocol for complex enrichment could be established.

5. ISOLATION, CLONING AND CHARACTERISATION OF THREE GLUTATHIONE-S-TRANSFERASES IN ASPERGILLUS FUMIGATUS

Claire Burns, Rachel Geraghty, Kevin Kavanagh and Sean Doyle
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Aspergillus fumigatus is a severe fungal pathogen of immunocompromised patients commonly treated with antifungal agents such as amphotericin B. However, treatment is often ineffective and infection frequently leads to death. We postulate that detoxification enzymes such as glutathione-s-transferases (GST) may play a role in survival of the fungus during infection, and would thus provide a novel therapeutic target.
Several putative GSTs were identified from the A. fumigatus genome. Three of these genes, namely gst1, gst2 and gst3, have been cloned and heterologously expressed, yielding proteins of 29 kDa, 29 kDa and 30kDa. The recombinant proteins exhibited GST activities against CDNB of 0.025 U/mg, 0.006 U/mg and 0.004 U/mg, and glutathione peroxidase activities against cumene hydroperoxide of 0.145 U/mg, 0.025 U/mg and 0.019 U/mg respectively.
Gst2 and gst3 were basally expressed, and were induced 4-fold and 10-fold in the presence of CDNB, and 3-fold and 5-fold in the presence of hydrogen peroxide. Gst1 was not basally expressed, and was induced by CDNB only. Experiments are ongoing to examine expression in the presence of other xenobiotics. Preliminary results when induced with amphotericin B indicate possible downregulation of gst2 and gst3 and upregulation of gst1.

6. ANALYSIS OF THE REGULATORY MULTIPROTEIN COMPLEX COP9 SIGNALOSOME IN ASPERGILLUS NIDULANS.

Silke Busch[1], K. Helmstaedt, Ö. Bayram, O. Valerius, Gerhard H. Braus[2]Institut für Mikrobiologie und Genetik, Georg-August-Universität,
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The COP9 signalosome (CSN) is a conserved eukaryotic multiprotein complex that connects various signals with multiple downstream molecular regulatory pathways controlling cellular differentiation and development. In higher eukaryotes, disfunction of the CSN is embryonic lethal, whereas in Aspergillus nidulans deletion of CSN subunits leads to viable strains with severe developmental defects. We want to understand the molecular networks that connect integration of external signals and subsequent regulation of multiple molecular pathways by the action of the CSN in A. nidulans as a genetically amaneable eukaryotic model system
In higher eukaryotes, the CSN is composed of eight conserved subunits. We identified proposed open reading frames for all of them in the A. nidulans genome sequence. By application of the tandem affinity purification method (TAP tag) we purified a multiprotein complex containing several CSN subunits as proven by mass spectrometry. We are currently improving the method to additionally identify CSN associated proteins.Function of the CSN involves 26S proteasome-dependent regulation of cellular protein levels, mainly by the signalosome's intrinsic deneddylation and associated kinase activities. In agreement with this, we observed a different protein expression pattern in an A. nidulans wild-type strain compared to a csn deletion strain in two-dimensional gel analyses. Differentially expressed proteins were identified by mass spectrometry. These potential downstrem targets of CSN action in A. nidulans hint at a contribution of the CSN to regulation of metabolism, cytoskeleton composition and stress response in A. nidulans. Our experiments on CSN associated proteins and downstream targets are in progress and current results will be presented.

7. NITROGEN METABOLITE AND NITRATE SIGNALLING

Mark X Caddick[1], Igor Morozov[1], Greg Fitzgibbon[1], Amar Razak[1], Joseph Straus[2] and Meriel Jones[1]. [1] Biological Sciences, The University of Liverpool, Biosciences Building, Crown St. Liverpool, L69 7ZB UK Tel (44)151 795 44566, email caddick@liv.ac.uk; [2] Institut für Angewandte Genetik und Zellbiologie, BOKU-University, Vienna, Austria

The GATA transcription factor AreA, which mediates nitrogen metabolite signalling in the filamentous fungus Aspergillus nidulans, is modulated by at least four distinct mechanisms. Our recent work has investigated two of these; the TOR kinase pathway and regulated transcript stability. In S. cerevisiae the TOR pathway acts via Ure2p to modulate the AreA orthologue Gln3p, and represents the predominant signalling mechanism. A. nidulans does not have a Ure2p orthologue but we have shown that the TOR pathway still contributes to nitrogen metabolite signalling through AreA. Previously we have shown that the areA transcript degrades rapidly in the presence of primary nitrogen sources (glutamine, ammonia) whilst remaining stable under conditions of nitrogen limitation. This 3' UTR-dependent degradation is preceded by rapid deadenylation. We have now demonstrated that nitrogen metabolites modulate the stability of further transcripts in these pathways, either accelerating or retarding decay. These include the niaD and niiA transcripts which are required for the reduction of nitrate to glutamine. Both transcripts degrade rapidly in the presence of glutamine and conversely, are stabilised by intracellular nitrate. Furthermore, when both glutamine and nitrate are present, these transcripts are stable, ensuring that nitrate and the toxic intermediate nitrite are always removed. Degradation of the niaD transcript is triggered by deadenylation, which is dependent on its 5' UTR. Nitrate inhibits poly(A) shortening even in the presence of glutamine. Therefore mRNA degradation, mediated through the poly(A) tail, is a fundamental part of the adaptive response to specific environmental signals.

8. TARGETING STRESS-RESPONSE GENES FOR CONTROL OF ASPERGILLUS USING ANTIFUNGAL NATURAL COMPOUNDS AND TARGET-GENE BASED BIOASSAYS

Jong H. Kim[1], Bruce C. Campbell[1], Jiujiang Yu[2], Deepak Bhatnagar[2], and Thomas E. Cleveland[2]. [1]Plant Mycotoxin Research Unit, Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, California 94710 USA, phone: 510.559.5846, fax: 510.559.5737, email: bcc@pw.usda.gov. [2] Food and Feed Safety Unit, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, Louisiana, 70124 USA, phone: 504.286.4387, fax: 504.286.4533, email: eclevela@srrc.ars.usda.gov

Signal transduction and stress-response genes of fungal pathogens play important roles for exerting virulence and pathogenesis. For example, mutants of fungal pathogens lacking a two-component histidine kinase or a Mitogen Activated Protein Kinase (MAPK) gene have been shown to have significantly reduced virulence compared to respective wild-type strains. To discover more stress-response genes critical for virulence, and as potential targets for control, we constructed an in silico database of transduction/stress-response pathway genes of Aspergillus flavus. The database was based on orthologs of the yeast, Saccharomyces cerevisiae, because its entire genome had been sequenced and well annotated; whereas that of A. flavus had not. Moreover, many genes in S. cerevisiae are interrelated to genes of many fungal pathogens. For example we demonstrated functional complementation of an antioxidative stress gene from A. flavus, mitochondrial superoxide dismutase (sodA), in a sod2□yeast mutant. This complementation verified that S.

cerevisiae deletion mutants could serve as a model system for indirectly studying A. flavus functional genomics and discovery of target genes for fungal control.
We next developed a high throughput bioassay, using yeast, to screen phenolic natural compounds for antiaflatoxigenic or antifungal activity. Many fungitoxic phenolics are produced during fungal infection in plants, and detoxification of these compounds/fungicides by fungi is necessary for their successful pathogenesis. Deletion mutants of yeasts were used to ascertain genes (or their products) being affected by any active compounds. After identifying active compounds using the yeast screening system, we tested the active compounds on A. flavus. The results were parallel between the fungi, demonstrating the usefulness of yeast for rapid screening. We were able to identify signal transduction and antioxidative stress response genes important to fungal tolerance. Targeting the antioxidative stress response system with certain compounds (e.g., vanillyl acetone) in combination with strobilurin-fungicides had a synergistic antifungal effect against both fungi. This provides evidence that antifungal activity of known fungicides can be enhanced with natural compounds. Application of yeast bioassays and functional genomics for controlling other fungal pathogens will be discussed.

9. ARSENIC DETOXIFICATION IN A HYPERTOLERANT ASPERGILLUS STRAIN

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The Tinto river in Spain is an example of an environment hostile to life, holding high acidity and heavy metal concentrations (As, Cu, Cr, Zn). Regardless of these extreme conditions, this ecosystem possesses a great diversity of eukaryotic life forms. Aspergillus sp. P37 is an arsenate-hypertolerant fungus isolated from this river. This strain was able to grow in the presence of 200 mM arsenate, i. e., 20-fold higher than the reference strains, Saccharomyces cerevisiae, and Aspergillus nidulans. The physiological properties of both Aspergillus strains were compared. Uptake of arsenate was slightly increased in Aspergillus sp. P37. Both strains reduced As(V) to As(III), which was slowly pumped out of the cell. Increasing levels of arsenic in the medium did not diminish the intracellular pool of reduced glutathione in Aspergillus sp. P37, in sharp contrast with the decline of glutathione in A. nidulans under the same conditions. Furthermore, addition of 50 mM arsenate to Aspergillus sp. P37 resulted in accumulation of thiols in the vacuoles. Exposure of Aspergillus sp. P37 (but not A. nidulans) to high As concentrations ($\geq$150 mM) induced the production of small quantities of a distinct thiol species indistinguishable from plant phytochelatin-2. Yet, the very low levels of such phytochelatin fail to account for arsenic resistance in Aspergillus sp. P37. On the contrary, $As(GS)_3$ complexes are the species playing a role in arsenic detoxification in this strain. Data will be shown suggesting that increased arsenate reduction together with the maintenance of the intracellular pool of glutathione are the elements responsible for the hyper-tolerant phenotype of this fungus.

10. Evolutionary Processes in the Aflatoxin Gene Cluster in *Aspergillus*

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Aflatoxins are carcinogenic bioreactive compounds produced by several species in the genus *Aspergillus*. In addition to being a serious threat to human health, the widespread contamination of peanuts, cottonseed and corn with *A. flavus* and *A. parasiticus* has resulted in significant yield losses and pose significant challenges in maintaining a safe and abundant food supply. In 2003, a nonaflatoxigenic *A. flavus* strain was approved by the U.S. Environmental Protection Agency for commercial use on cotton in Arizona and Texas and another strain was approved in 2004 for use on peanuts in the U.S. Nonaflatoxigenic strains out-compete aflatoxigenic strains during plant invasion thereby reducing aflatoxin contamination. Although this is reported as an effective method for aflatoxin control, we have no knowledge of the long-term effects that introduced strains have on the evolution of aflatoxigenicity in the environment.

We hypothesize that a low level of recombination and gene flow among *Aspergillus* species occupying the same ecological niche are significantly contributing to the persistence and further evolution of aflatoxigenic strains and to the evolution of new species. To address this we are applying macro- and micro-evolutionary approaches to reconstruct the evolutionary processes influencing the organization and further evolution of genes in the aflatoxin cluster in *Aspergillus*. On a micro-scale we examined nucleotide sequence variation in 21 intergenic regions across the entire aflatoxin gene cluster of *A. parasiticus* and found evidence of recombination blocks – groups of two or more genes in the aflatoxin cluster with different evolutionary histories. Each recombination block infers a perfect phylogeny; recombination is between but not within blocks. On a macro-scale the same blocks appear to be conserved for putative orthologs of these genes in *A. nidulans, A. flavus,* and *A. fumigatus*. Collectively, our macro- and micro-evolutionary inferences indicate the potential for an introduced biocontrol strain to acquire toxigenicity genes from indigenous strains via recombination or from sympatric species via horizontal transfer. We are currently investigating the timing and frequency of these events in nature, specifically whether they coincide with speciation or are occurring among individuals in populations.

11. RIP-AFFECTED AND UNAFFECTED TRANSPOSABLE ELEMENTS IN *ASPERGILLUS*

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In *Neurospora crassa* almost all transposable elements are inactivated by Repeat Induced Point mutation (RIP), characterized by multiple C-T and G-A transition mutations relative to the consensus for that element. In contrast, many transposon element families in *Aspergillus* species include both RIP-affected an unaffected copies. Can we find clues as to which elements are affected by RIP, and which are not? To this end, some of the younger families, identified by minimal divergence from their consensus, are examined for correlations of RIP with chromosomal position, other mutations, fragmentation, and invasion by other elements.

12. NITROGEN METABOLITE REPRESSION OF ARGININE CATABOLISM GENES IN ASPERGILLUS NIDULANS IS MEDIATED BY NEGATIVELY ACTING FACTOR AREB.

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The arginine catabolism genes agaA and otaA, coding for arginase and ornithine transaminase (OTAse) respectively, are specifically induced by arginine and repressed by ammonia (Dzikowska et al. 1999 ; Borsuk et al. 1999 ; Dzikowska et al. 2003). In A. nidulans nitrogen metabolite repression is mediated by transcriptional activator AREA from GATA family. areA 600 loss of function mutant does not grow on arginine as a nitrogen source but we have shown that agaA and otaA expression does not depend on AREA. Arginase and OTAse enzymatic activities are fully inducible in areA600 mutant. The same was shown for agaA and otaA mRNA. It is possible that an arginine permease gene(s) can be a target for AREA activator. Preliminary data suggest that there are two arginine transport systems and only one depends on AREA activator. We have also shown that another negatively acting factor from GATA family (AREB) participates in the ammonia repression of agaA and otaA. In areB loss of function mutant the ammonia repression of both agaA and otaA is changed at the level of enzyme activity and at the level of mRNA.

13. THE ASPERGILLUS NIDULANS uvsB^{ATR} AND scaA^{NBS1} GENES SHOW GENETIC INTERACTIONS DURING RECOVERY FROM THE HU-REPLICATIONAL STRESS

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The Mre11-Rad50-Nbs1 protein complex has emerged as a central player in the cellular DNA damage response. Here, we investigate possible interactions between A. nidulans uvsB^{ATR} and the Mre11 complex. We showed that there is an epistatic relationship between uvsB^{ATR} and scaA^{NBS1} during growth on the anti-topoisomerase I poison camptothecin and in the DNA replication checkpoint, and that correct ScaA expression is dependent on uvsB^{ATR} integrity during recovery from the replication stress. The scaA^{NBS1} and ΔuvsB^{ATR} mutations are epistatic to monitor and/or repair UV light-sensitivity when conidiospores are germinating. In addition, we also show that the formation of UvsC foci during recovery from the replication stress is dependent on the uvsB^{ATR} and scaA^{NBS1} integrity. We also examined interactions between the uvsB^{ATR} and musN^{RecQ} genes. When quiescent conidia were exposed to UV light, the uvsB110 ΔmusN double mutant strain was more sensitive to UV irradiation than the single mutant strains suggesting that these genes are interacting during recovery from the UV light stress in quiescent conidia. The double mutant ΔmusN uvsB110 also showed a synergistic interaction during the DNA replication checkpoint. Our results suggest that UvsB^{ATR}, MusN^{RecQ} and the Mre11 complex are interacting during progression and/or recovery from S-phase upon DNA damage.

14. PHYLOGENOMIC ANALYSES OF HETEROCARYON INCOMPATIBILITY PROTEINS IN ASPERGILLI

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Heterokaryon incompatibility is a form of programmed cell death in filamentous fungi, triggered by the fusion of filaments from two strains with different sets of het loci. The analyses of the completely sequenced genomes of Aspergillus fumigatus, Aspergillus fischerianus, Aspergillus nidulans and Aspergillus oryzae reveal remarkable diversity of heterokaryon incompatibility systems in filamentous fungi. The Aspergillus genomes encode numerous homologs of heterokaryon incompatibility proteins from Neurospora crassa and Podospora anserina. Yet the number of homologous proteins, their domain architecture and, perhaps, the underlying mechanisms are quite different. Notable examples include an aspergilli-specific expansion of the putative NACHT NTPases that function as signaling hubs during heterokaryon incompatibility in filamentous fungi, pathogen resistance in plants, or apoptosis in mammals. The Aspergillus NACHT proteins are most similar to GTP-binding protein Het-E and Het-D from P. anserina, but they do not have the HET domain, found at the N-terminus of the Podospora proteins. Instead many of Aspergillus NACHT proteins have a highly divergent nucleoside phosphorylase domain. Still unknown is whether all NATCH NTPases are involved in self/non-self recognition during heterokaryon incompatibility or some are part of another signaling pathway in aspergilli.

15. RAMOSA-1: AN EVOLUTIONARY CONSERVED PROTEIN INVOLVED IN FUNGAL MORPHOGENESIS

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Polarised growth of leading hyphae of many filamentous fungi is characterised by the presence of a Spitzenkörper (Spk) or a Vesicle Supplying Centre (VSC). This structure is thought to allow the efficient delivery of vesicles to the growing apex. To identify proteins involved in this process we have characterised the previously isolated apical branching mutant, ramosa-1 (1), and by complementation of the temperature sensitive phenotype have cloned the ramosa-1 gene. Homology searches indicate that the protein belongs to an evolutionary conserved family of proteins, in all eukaryotic cells. The ramosa-1 homolog gene in S. cerevisiae, YOL078w/AVO1, is an essential gene. Over-expression of the ramosa-1 cDNA using the GAL4 promoter could rescue the S. cerevisiae gene deletion mutant (Yol078w), indicating a functional conservation between the two homologs. Ramosa-1 depletion studies in S. cerevisiae indicate that the gene is required for proper morphogeneisis, as is seen in Aspergillus niger. Ramosa-1 homologs have been shown to be involved in several signalling mechanisms in the cell including MAPK signals (S. pombe), cyclic AMP signalling (Dictyostelium) and PI signaling (S. cerevisiae). Current research is aimed at the identification of Ramosa-1 interacting proteins to elucidate its function in fungal cells.

16. COMPARATIVE PROTEOMIC ANALYSIS OF CULTURE FILTRATE PROTEINS OF *ASPERGILLUS FUMIGATUS* FROM CLINICAL ISOLATES OF ABPA AND INVASIVE PATIENTS OF ASPERGILLOSIS

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Aspergillus fumigatus is the second most prevalent airborne fungal pathogen, causing allergic bronchopulmonary aspergillosis (ABPA), a severe respiratory disease in immunocompetent patients and invasive aspergillosis, usually fatal disease in immunocompromised patients. A. fumigatus genome sequencing data proposes approximately 10,000 genes and 30,000 proteins but information is available for very less number of proteins. Currently, out of over 2334 entries of Aspergillus protein/protein subunits sequences in Swiss-Prot and TrEMBLdatabase, 605 are from Aspergillus fumigatus. Of these 156 are hypothetical proteins and 124 are putative proteins. A number of secretory proteins are reported to be allergenic/antigenic/virulent factors e. g. ribonucleases (18kD), proteases (45kD, 33kD), superoxide dismutase (27kD, 67kD). We have identified 19 novel allergens/antigens of A. fumigatus from three week culture filtrate proteins by immunoproteomic studies. In the current study, comparative proteomic analysis of three week culture filtrate proteins of two clinical isolates of A. fumigatus (from ABPA and invasive aspergillosis patients) has been carried out to identify the proteins specifically expressed in clinical isolate of invasive aspergillosis which may have relevance to virulence and pathogenesis. The proteomic analysis of three week culture filtrate proteins of clinical isolates of ABPA and invasive aspergillosis patient showed expression of 112 and 101 proteins respectively. Strain isolated from patient of invasive aspergillosis specifically expressed 63 proteins, 39 proteins expressed in both the strains and 73 proteins expressed specifically in clinical isolate of ABPA patient. MALDI-TOF analysis of four distinct proteins highly expressed in invasive strain identified glyceraldehyde-3-phosphate (involved in glycolysis), exocyst complex subunit Sec5 (component of exocyst complex involved in secretion of proteins), cullin like protein (involved in regulation of the cell cycle and as components of ubiquitin-protein ligases) and a hypothetical protein. Identification of these proteins will be further confirmed by ESI-MS/MS and relevance with respect to virulence and pathogenesis will be examined.

17. CARBON SOURCE-DEPENDENT ALTERNATIVE TRANSCRIPTION INITIATION OF THE ENOLASE-ENCODING GENE (ENOA) IN ASPERGILLUS ORYZAE

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Enolase generally plays a pivotal role in the glycolysis and is therefore one of the most highly expressed proteins in most organisms. We have cloned an enolase-encoding gene (enoA) of Aspergillus oryzae and identified a cis-element of 15-bp located between -195 and -181 relative to the translation start codon responsible for the transcriptional regulation of the enoA by promoter deletion analysis and gel mobility shift assay[1)]. Surprisingly, recent comparative analysis using ESTs and whole genomic sequence data of A. oryzae suggested the existence of alternative transcription initiation sites in the enoA dependent on culture conditions. In the absence of carbon

source or in the presence of ethanol or acetic acid as a carbon source, transcription of the enoA is initiated mainly at -530 upstream of the start codon and results in the accumulation of transcript with an intron of approximately 440-bp in the 5'-untranslated region (5'UTR) removed. In contrast, the enoA is transcribed from -50 relative to the start codon, which is located within the intronic sequence in the 5'UTR, in the presence of carbon sources such as glucose or glycerol. To our knowledge, this is the first finding that alternative transcription initiation sites are utilized depending on carbon sources in the genes involved in the primary metabolic pathway in filamentous fungi, except for the developmental regulatory gene, brlA, of A. nidulans[2)].

18. UNFOLDED PROTEIN RESPONSE IN ASPERGILLUS NIGER CHEMOSTAT FERMENTATIONS.

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Unfolded Protein Response (UPR) is a universal reaction of eukaryotic cells to protein folding stress in the endoplasmic reticulum (ER). The expression of heterologous secreted proteins often results in such stress and thus UPR, also in filamentous fungi like Aspergillus niger which are renowned for their high secretion capacity. As a consequence, the production yield of secreted heterologous proteins usually is very low (milligrams/L at best).
UPR results in induction of expression of genes that allow the cell to cope with the surplus of protein folding intermediates. Identification of these genes and understanding the response pathway(s) should give leads to improve the folding and secretion capacity of A. niger. Towards this, two approaches are taken: transcriptomics with Affymetrix genome arrays to identify up- or down-regulated genes and genetic screening to select regulatory mutants.
For transcriptomics analysis, A. niger strains are constructed in which poorly folded (scFv) or unfoldable (yeast CPY*) proteins can be expressed under control of the regulated glaA promoter. RNA analysis is first performed with shake flask cultures, both under induced and non-induced conditions for the expression of the poorly folded proteins
For a sound transcriptomics analysis, tight control of all cultivation conditions is an absolute prerequisite. We therefore are developing protocols for steady-state fermentation of A. niger under repressing conditions for the glaA promoter and for transition to a glaA induced steady-state. The samples collected throughout these fermentations are used for transcriptomic and protein analysis.Tools are developed to improve the genetic screen for the isolation of regulatory mutants with altered UPR characteristics.We will report on the recent progress made in these project goals.This research is carried out within the Kluyver Centre for Genomics of Industrial Fermentation.

19. ANALYSIS OF DYNEIN REGULATORY COMPLEXES USING A TANDEM AFFINITY PURIFICATION TAG FOR FILAMENTOUS FUNGI

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In filamentous fungi, migration of nuclei is established through the interplay of the molecular motor dynein in combination with dynactin and microtubuli. Several proteins like NUDF, NUDE, and NUDC had been identified as regulators of dynein-mediated movement, which are not part of the dynein/dynactin motor complex itself (Xiang et al, 1995; Efimov & Morris, 2000). We intend to identifiy interaction partners of these regulatory proteins in Aspergillus nidulans in order to clarify the regulatory pathway leading to dynein activation. For this biochemical approach we used the tandem affinity purification (TAP) tag, which was originally developed for yeast and was optimized for expression in filamentous fungi. The nuclear migration gene nudF was fused to the new N-terminal tag and expressed in A. nidulans from its native promoter for gentle purification of active regulatory complexes at their natural level. Proteins which co-purified with NUDF were identified by mass spectrometry. In first affinity purifications, several hypothetical proteins with homologs in other filamentous fungi and higher eucaryotes, respectively, were found. The method is currently improved and interactions are verified by yeast two-hybrid analysis.

20. ASPERGILLUS NIDULANS MUTANTS UNABLE TO LOCALISE GLYOXYLATE CYCLE ENZYMES TO THE PEROXISOMES ARE ABLE TO UTILISE ACETATE

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Growth on acetate and fatty acids results in the production of acetyl-CoA which is channeled into the TCA and glyoxylate cycles. The TCA cycle occurs in the mitochondria and the glyoxylate cycle in the peroxisomes. Peroxisomal proteins are directed to the peroxisomes via one of two classes of peroxisomal targeting signals – PTS1 and PTS2. PTS1 targeting occurs via the Pex5 receptor and PTS2 via the Pex7 receptor. These receptors are recycled to the cytosol by Pex6, an ATPase. The A. nidulans genes, acuD (isocitrate lyase) and acuE (malate synthase), encode enzymes specific to the glyoxylate cycle. AcuE possesses a peroxisomal targeting signal 1 (PTS1) whereas AcuD lacks any identifiable PTS1 or PTS2. We are interested in the effects of peroxisomal protein localisation on fatty acid utilisation in A. nidulans. A knockout mutant of the A. nidulans PEX5 orthologue (pexE) mislocalises AcuE to the cytoplasm but is able to target AcuD to the peroxisomes. A mutant in the PEX7 orthologue (pexG), however, mislocalises AcuD but is unaffected in peroxisomal targeting of AcuE. A mutant defective in the PEX6 orthologue (pexF) mislocalises both AcuD and AcuE to the cytoplasm but is nonetheless able to utilise acetate. Most of the peroxisomal mutants studied in our laboratory are sensitive to the presence of fatty acids. The growth of the pexE mutant is particularly inhibited by the presence of all fatty acids tested, including acetate. A ΔpexEΔpexG double mutant shows relief of acetate inhibition and is able to grow on acetate.

21. INVESTIGATING ABNORMAL PHENOTYPES ASSOCIATED WITH RNA SILENCING ENZYMES AND RNA DEPENDENT RNA POLYMERASES IN ASPERGILLUS NIDULANS.

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Of the three Aspergilli with fully sequenced and publicly available genomes, A. fumigatus, A. nidulans, and A. oryzae, A. nidulans encodes the fewest enzymes commonly attributed to RNA silencing related processes. For example, A. nidulans is predicted to have only one 'Dicer', one Paz and Piwi domain (PPD) protein and two RNA dependent RNA polymerases (RDRPs), while the other two sequenced Aspergilli, and most other studied filamentous ascomycetes, are predicted to have at least two Dicers, two PPD proteins, and three RDRPs. The reduced number of these types of genes in A. nidulans suggests that it is an ideal model organism to study the biological function of these genes in filamentous fungi. In previous studies we have shown that the predicted A. nidulans PPD protein (RsdA) is required for RNA silencing during the vegetative cycle but that its two RDRPs (RrpA and RrpB) are not required for the same process. Interestingly, the simple experiments required to determine that the one predicted Dicer (DcrA) is required for RNA silencing in A. nidulans have not been performed because of an abnormal level of difficulty encountered during our attempts to obtain a dcrA deletion strain. In addition to our continual pursuit of a dcrA deletion strain, we are currently investigating two peculiar phenotypes associated with RNA silencing related genes but not thought to be directly related to RNA silencing during the vegetative cycle. These include a 'slow-growth' phenotype associated with dcrA over-expression and a 'selfing proficient' but 'crossing deficient' phenotype associated with rrpB deletion.

22. TRANSCRIPTION ANALYSIS OF CARBON REPRESSION OF *ASPERGILLUS NIDULANS* USING HIGH DENSITY MICROARRAYS

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Glucose repression has been the subject of fungal research for many years now, and still there is a lot to be resolved, for example the many pleiotropic effects of mutations of the *creA* gene. Therefore this study aims at a better understanding of glucose and/or carbon repression in *A. nidulans*, utilising high density DNA-microarrays for high throughput transcriptional analysis.
The effect of CreA was investigated by comparison of the gene-expression of the *creA* mutant against a reference strain under growth conditions considered to be repressing (glucose) and de-repressing (ethanol) . Statistical analysis based on biological triplicates showed 200 significantly regulated genes (p-value less than 1.0×10^{-2}). Cluster analysis, based on the expression profiles of all four conditions, reveals a number of complex patterns, whose interpretation is hampered by the relative poor annotation of the majority of open reading frames (ORFs) the *A. nidulans* genome sequence. On the other hand, this enhances the value of this study, since it increases the knowledge about putative roles/functions of ORFs. For further analysis and interpretation of the data, promotor

analysis has been performed and the response of metabolic genes has been compared with data from C^{13} metabolic flux analysis of the same *creA* mutant strain.

23. IDENTIFICATION OF A NATURAL ANTISENSE TRANSCRIPT OF AFLR, THE TRANSCRIPTIONAL REGULATOR IN THE AFLATOXIN BIOSYNTHESIS PATHWAY IN ASPERGILLUS FLAVUS.

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Natural antisense transcripts (NATs) are involved in gene expression in several eukaryotic systems. Cases of transcriptional interference, RNA masking, and double-stranded RNA dependent mechanisms have all been shown to involve NATs. Recently, antisense transcripts have been identified in the filamentous fungus Neurospora crassa that are complementary to frq, a key component of the circadian clock. An antisense transcript was identified in a cDNA library of another filamentous fungus, Aspergillus flavus. This species among others in the genus Aspergillus produces aflatoxin, the most potent naturally occurring carcinogen. The antisense transcript identified in the cDNA library corresponds to aflR, which is the transcriptional regulator of aflatoxin biosynthesis. This NAT, aflRas, overlaps with the 5' end of aflR and extends into its promoter. RT-qPCR analysis was used to compare aflR and aflRas levels
with respect to aflatoxin production at different time points. In addition, an over-expression construct utilizing the alcA promoter to drive expression of
alfRas was transformed into A. flavus. These over-expression transformants will provide insight as to the function of aflRas.

24. IDENTIFICATION AND MOLECULAR ANALYSIS OF SNOA (SUPPRESSOR-OF-NIMO), A NOVEL REGULATOR OF DNA SYNTHESIS IN *ASPERGILLUS NIDULANS*

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In Aspergillus nidulans, $nimO^{Dbf4}$ and cdc7 encode regulatory and catalytic subunits of the conserved Dbf4-dependent kinase (DDK). In budding yeast, Dbf4p escorts Cdc7p to origins of replication, and origin unwinding is triggered through phosphorylation of pre-RC components by Cdc7p. Dbf4p is subsequently targeted for destruction by the Anaphase Promoting Complex, thus preventing reinitiation of DNA synthesis. In Aspergillus, the temperature sensitive nimO18 mutation can be partially suppressed by mutations in two genes, snoA and snoB. Semi-dominant snoB mutations lie within the cdc7 gene, and suppress by stabilizing the association of cdc7p with nimO18p. Recessive snoA suppressors not only rescue nimO18 ts-lethality, but they also rescue growth of strains hypomorphic for expression of $nimO^{+}$. However, snoA suppressors cannot bypass a complete loss of nimO. Thus, snoA suppression is not allele-specific, and snoA appears to act by influencing the level or stability of nimOp. Bioinformatic and positional cloning approaches were combined to localize snoA within a 340,000 bp interval on Linkage Group VII. snoA was discovered by systematic ORF deletion. Deletion of one ORF phenocopied the snoA suppressor phenotype by partially rescuing nimO18 ts-lethality. Current efforts include snoA overexpression

studies, mapping snoA functional domains, and biochemical analysis of tagged nimOp and cdc7p in snoA mutants. (Supported by NSF-RUI #01-14446)

25. PROTEMICS ANALYSIS OF REGULATION AND SIGNALLING

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Our work investigates regulatory responses to extracellular pH and the availability of nitrogen and phosphate in the filamentous fungus A. nidulans. All are essential biological processes and have been implicated in fungal pathogenicity. The related species A. fumigatus is an important opportunistic pathogen in immunocompromised patients but the biology of its multifactoral pathogenicity determinants is poorly understood. The combination of proteomics with the recently released genome sequences of both A. nidulans and A. fumigatus, combined with the genetic resources offered by A. nidulans, offers an opportunity to advance knowledge of these processes. Although the pacC/pal system is known to regulate response to pH in A. nidulans, the combination of proteomics and mutant strains can provide leads to the extent, and identity, of additional factors. We have examined the response of the intracellular soluble and external secreted proteomes *of* A. nidulans to external pH during growth. We are also interested in applying proteomics to investigate signalling mechanisms, by looking at the primary response to an environmental shift. We are examining the response of the proteome to altered nitrogen status within two minutes to identify early consequences of nitrogen metabolite signalling. We will present a brief description of the biology of the systems under study and our recent work.

26. A MODEL FOR BRANCH ESTABLISHMENT IN ASPERGILLUS NIDULANS.

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Aspergillus nidulans establishes cell polarity during germination; polarity is maintained during tip growth, which produces cylindrical hyphae. Secondary polarized sites are established for the formation of branches, which are essential for vegetative colony formation and for asexual sporulation. Undisturbed wildtype hyphae branch from basal regions, typically close to a septum. Following mild mechanical stress one or more branches can develop near hyphal tips. Apical branching can be induced by treatment with cytoskeletal poisons, and also is typical of many morphogenetic defects. The precise timing and positioning of branch initiation is difficult to predict, complicating attempts to understand the events prior to formation of a visible branch initial. A. nidulans hypA1 temperature sensitive mutants form poorly polarized cells at 42°C due to a defect in endomembrane trafficking. When released from restrictive temperature, hypA1 cells form wildtype branches. Formation of branch initials in stress-released hypA1 cells appears comparable to polarization events during wildtype vegetative branching, but is highly temporally and spatially predictable. This system can be used to study early events in recruitment of exocytic machinery prior to external evidence of branch formation. As expected, branch initiation requires the actin cytoskeleton and endomembrane arrays. Extension of a branch initial is accompanied by investment of one or more cytoplasmic microtubules.

27. IN VITRO ANALYSIS ON THE ASSEMBLY MECHANISMS OF THE ASPERGILLUS CCAAT-BOX BINDING FACTOR

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The CCAAT-box is one of the most common cis-elements present in the promoter regions in eukaryotes. It has been shown to be important in the high-level expression of many genes in Aspergillus species. The factor which binds to the CCAAT-box, so-called the Hap complex, belongs to the NF-Y family and consists of three subunits, HapB, HapC and HapE. Previously we have shown that the number of HapE is strictly dependent on the number of HapE, suggesting the number of HapC could adjust that of HapE by forming stable heterodimers prior to assembly of the Hap complex (Kato et al. 2002 FEBS Lett. 512, 227-229). We performed a reconstitution study with the recombinant subunits and S35-labeled in vitro translated subunits. Significant amounts of the translated HapE were recovered in the insoluble fraction while the other two subunits were in the soluble fraction. However, HapE was recovered in the soluble fraction when the recombinant HapC was added in the translation reaction. These results indicate that the HapC subunit plays a role in increasing the solubility of the HapE subunit as a chaperon-like factor specific to the HapE subunit. Although many CCAAT complexes have been characterized from various eukaryotes, there is little information regarding the stability of their subunits. These findings suggest that similar mechanisms that control the quantity of the subunits may exist in the other eukaryotes. Furthermore, we also carried out pull-down assays of the labeled subunits with recombinant GST or MBP fusion subunits to examine the interactions among the subunits. The mechanisms of subunit assembly of the Aspergillus Hap complex will be discussed.

28. NEW CONDITIONAL PROMOTER CASSETTES FOR ASPERGILLUS CARRYING THE PYRITHIAMINE RESISTANCE GENE

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New tools are needed for systematic analysis of genes revealed by the sequence of Aspergillus sp. Recently, the plasmid pMZ1A carrying a cassette containing P_{alcA} and Neuropora crassa pyr-4 has been reported (Zarrin et al., Fungal Gen. Biol. 42:1). We have developed similar types of cassette using ptrA, a dominant selectable marker from A. oryzae (Kubodera et al. 2000, Biosci. Biotechnol. Biochem. 64:1416-1421). In pDKP4699 the ptrA gene is transcribed in the same direction as P_{alcA}; in pDKP4685 ptrA is in the opposite orientation. During the construction of the cassettes, 2 intermediates plasmids have been also developed. They are pCR2.1 backbone with a PCR fragment carrying ptrA. Restriction sites available, flanking the ptrA marker, expand the possibilities for cloning and excision of the ptrA marker. Since pMZ1A and several vectors carrying P_{alcA} and pyr-4 have the transcription toward the same direction, we also constructed pDKP4652 where pyr-4 is transcribed in the opposite direction to P_{alcA}. The application of these vectors to the analysis of genes will be described.

29. TEMPERATURE-DEPENDENT GENE EXPRESSION IN *ASPERGILLUS FUMIGATUS* EXAMINED BY DNA MICROARRAY

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Recent completion of the genome sequencing of the opportunistic human pathogen *Aspergillus fumigatus* provided a great opportunity to study its biology in greater detail. One of the characteristic phenotypes of *A. fumigatus* is its high adaptability to elevated environmental temperatures not tolerated by other members of the species. These environments, including soil, animal hosts, and compost, requires the fungus to adjust its biochemical constitution and physiology to survive and possibly resume growth after exposure to a temperature as high as 70^{o} C. We examined genome-wide expression upon temperature shift from low to high and from high to low, within the range of 30˚C to 52˚C. We found that different genes have specific windows of expression over the temperature range and the duration at a given temperature. For example, many heat shock and stress-responsive genes were immediately up-regulated when the culture was shifted to high temperatures (above 48˚C), but some of these were also up at 37˚C after about an hour. On the other hand, many putative virulence genes were readily expressed at 37˚C, as if the fungus were in the host. This suggests that temperature change is an environmental signal by which the fungus adjusts its physiology in an environment appropriate manner. Another finding we made from this study is that many transposases, especially those of the Mariner-4 type, are highly expressed at high temperatures. This suggests that transposition might be activated at high temperatures perhaps as a means to allow improved adaptability to the high temperature stress through genome alterations. We are extending this study relating the temperature-responsive genes to the Comparative Genomic Hybridization (CGH) data with closely related stains.

30. Characterization of the telomere-attached vectors and their transformants of Aspergillus oryzae

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The authors isolated the telomere sequences at the chromosomal terminal regions of Aspergillus oryzae, and showed that their structure was a repeated sequence constituted of 12bp repeat unit (TTAGGGTCAACA) (AMB61: 247, 2003). As telomere is participated in the replication of the chromosomal DNA and its stabilization of their ends, development of new type of the replicating vectors is expected. The replicating function of the telomeres of A. oryzae and the conservation of the chromosomal ends by them are not investigated yet. In this study, we constructed the two types of the telomere-attached vectors to examine their function. The vector I was constituted of single copy of TEL134, a clone of A. oryzae telomere (TTAGGGTCAACA)X10 with its associated sequence, inserted into a vector of a integrated type, pPTRI (ptrA as a marker gene, TaKaRa Bio, JAPAN). The vector II was constituted of two copies of TEL134 inserted into pPTRI, where the two TEL134 were inserted as inverted repeat. A 0.8 kb DNA fragment derived from a vector for <I>Escherichia coli</I> was put as a spacer sequence between those TEL134 DNA. Namely, the spacer sequence was positioned as vector-(TTAGGGTCAACA)X10-spacer-(TGTTGACCCTAA)X10-vector. A. oryzae NFRI1599 were transformed with those

telomere-attached vectors and pPTRI as a control plasmid. We are going to characterize the structure of the introduced vectors and the transformants.

31. A single septin gene from Aspergillus nidulans induces filamentous growth in Saccharomyces cerevisiae

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Septins were first discovered in S. cerevisiae where they are thought to form a scaffold that organizes the bud site and they are a component of the morphogenesis checkpoint that monitors bud shape and coordinates budding with cytokinesis. Here we show that one of the A. nidulans septins, AspC, induces filaments and elongated asci containing up to twelve spores in S. cerevisiae. AspC-induced filaments are noninvasive, form in haploid and diploid strains and require the presence of a functional yeast septin Cdc10. Based on comparisons of amino acid sequence, AspC is most similar to the S. cerevisiae septin Cdc12. The A. nidulans aspC gene complements cdc12 and cdc3 yeast mutants. When GFP-Cdc12 and AspC are co-expressed Cdc12 localizes to the necks of buds, it does not localize to the necks of AspC-induced filaments. In budding yeast, the Bni1 formin and Cdc12 are known to interact. When aspC is introduced into an S. cerevisiae Δbni1 strain, a novel bent filament phenotype is seen. This phenotype is suppressed by extra copies of CDC12. Our results suggest that the A. nidulans septin AspC competes with Cdc12 for incorporation into the septin scaffold at the S. cerevisiae neck and that when AspC is present it perturbs normal yeast bud morphology causing it to take on the more elongated shape common to filamentous fungi.

32. Analyses of metabolic pathways and their expression of *Aspergillus oryzae*

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Aspergillus oryzae is extensively used in Japanese traditional fermentation industries and in enzyme productions. The whole genome sequencing of *A. oryzae* has been almost completed at the end of 2004 (in submission). Approximately 12,000 genes with longer than 100 amino acids in length were predicted from the *A. oryzae* genome of 37.6 Mb in size. Comparison of the number of genes in each COG functional category[1] revealed that *A. oryzae* had genes more redundant specifically for those concerning to metabolism than *Aspergillus fumigatus*, *Aspergillus nidulans* and *Neurospora crassa*. Homology search (E-value <= 10^{-10}) showed that approximately 1,200 entries among 1,830 entries in the BRENDA database[2] were covered by the *A. oryzae* genes. The analysis of redundancy of the genes showed that the genes supposed to be important for fermentation were significantly higher than the others. Further, some of the highly redundant genes are known as highly expressed genes. Based on the genome sequence, the DNA microarray consisting of 11,000 oligonucleotides were prepared, which showed reasonable consistency of the result with that obtained by the EST microarray. Preliminary oligo microarray analysis indicated low expression of the genes on the loci specifically existing on the *A. oryzae* genome but not in *A. fumigatus* and *A. nidulans* as was already observed from the expression by ESTs. The results of detailed analysis of pathways, gene redundancy, localization on chromosome and expression will be discussed.

33. A MITOCHONDRIAL SHORT-CHAIN ACYL-COA DEHYDROGENASE FROM ASPERGILLUS NIDULANS

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We have recently described a mitochondrial fatty acid □eta-oxidation pathway in Aspergillus nidulans (Maggio-Hall & Keller. 2004. Mol. Microbiol. 54: 1173-85). Here we describe the disruption and characterization of another gene in the pathway, that encoding the acyl-CoA dehydrogenase (scdA). The phenotypes of the scdA deletion strain further confirmed this pathway's role in degrading short-chain fatty acids (C_4-C_6) as well as the amino acids isoleucine and valine. Unlike the previously described disruption of the enoyl-CoA hydratase enzyme in the pathway, the scdA mutant showed no defect during growth on long- and very long-chain fatty acids. Furthermore, the scdA; echA double mutant also grew just as well as wild type on these fatty acids, indicating that the echA disruption phenotype—extremely restricted growth on long- and very long-chain fatty acids—was due entirely to toxicity of accumulated intermediates and not a failure to metabolize the fatty acids. Biochemical analysis of the scdA (dehydrogenase) mutant 12 h after transfer to hexanoate-containing medium showed that whole-cell enoyl-CoA hydratase activity was significantly diminished, suggesting a build-up of short-chain fatty acids inhibits other □eta-oxidation pathways in the cell. This inhibition is not at the level of transcription, based on Northern analysis of identified enoyl-CoA hydratase-encoding genes (echA and foxA) and other uncharacterized homologs.

34. CROSS SPECIES GENE DISCOVERY USING MICROARRAY ANALYSIS ALLOWS FOR THE IDENTIFICATION OF D-GALACTURONIC ACID UTILIZATION PATHWAY IN ASPERGILLUS NIGER AND ASPERGILLUS NIDULANS

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Contrary to the phosphorolytic degradation of D-galacturonic acid in bacteria and plants, non-phosphorolytic metabolism of D-galacturonic acid has been shown to occur in Aspergillus species (Uitzetter et al., 1985). This short pathway begins with the conversion of D-galacturonic acid to galactonate by an aldoketo reductase. Subsequently a dehydratase or racemase modifies galactonate to 2-keto-3-deoxygalactonate and an aldolase splits 2-keto-3-deoxygalactonate into pyruvate and glyceraldehyde. Although, A. nidulans mutants defective in the racemase and aldolase activities, known as gaaB and gaaA, respectively, were previously reported (Uitzetter et al., 1985), the genes encoding these activities have not been described yet. Transcriptome analysis of Aspergillus niger cultures grown on D-galacturonic acid, enabled the identification of a cluster of co-expressed genes that, among others, encode the necessary putative aldoketo reductase, racemase and aldolase. Sequence analysis of the genes orthologous to the A. niger gaaA and gaaB from the two A. nidulans D-galacturonate non utilizing mutants demonstrated the presence of point mutations in the coding regions of both A. nidulans gaaA and gaaB leading to the production of non-functional proteins and thus to the mutant phenotype. Comparative analysis of the A. niger gaaA and gaaB genes in publicly available fungal genomes revealed the presence of conserved orthologs to both genes in various distantly related fungi which suggests the presence of a common utilization pathway of D-galacturonic acid in fungi.

35. SOME GENES EXPRESSED DURING THE STATIONARY PHASE IN ASPERGILLUS FUMIGATUS

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A hallmark of Programme Cell Death or Apoptosis is the active participation of the cell in its own death requiring specific mRNA and protein synthesis. Subtractive hybridisation (SH) can be used to aid the isolation of genes showing altered expression in target cells following exposure to a stimulus. Most importantly, using such an approach, no prior knowledge of the specific genes is required. This procedure increases the effective concentration of induced sequences expressed in an experimental mRNA population (target) but not in a control mRNA population (driver) by hybridisation of target with an excess of driver to remove sequences common to both. Any residual driver present after the second PCR can not be amplified and thus can not contaminate the next round of hybridisation. Historically, mRNA was isolated from two sources, cDNA made from one and hybridised to an excess of cDNA from the other. The unhybridised material was either cloned or, more commonly, used as a probe to screen a library. As we found evidence of caspase, TUNEL activity and the externalisation of PS to the outer leaflet of the plasma lemma in the early stages of the stationary phase in life-threating fungus (*Aspergillus fumigatus*), we used SH from samples taken from mid-log phase growth and from the stationary phase to enrich for genes expressed in the stationary phase. As different genes may be expressed at different times during the stationary phase, two samples from the stationary phase were selected, one sample 2 h post-stationary phase and a second 4 h post-stationary phase.

36. THE JAMM MOTIF OF THE COP9 SIGNALOSOME IS ESSENTIAL FOR *ASPERGILLUS NIDULANS* SEXUAL DEVELOPMENT

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The COP9 signalosome (CSN) is a conserved multiprotein complex, playing an essential role in development of many eukaryotes, including the filamentous fungus *Aspergillus nidulans*. The deduced amino acid sequences of four recently identified *A. nidulans* genes: *csnA*, *csnB*, *csnD* and *csnE*, show high identities to the respective subunits of higher eukaryotes. Considering the conservation of the proteasome-related machinery in *A. nidulans*, it provides an easily amenable model for studying the broad array of CSN developmental functions.
Deletion of either *csnD* or *csnE* results in pleiotropic phenotypes affecting formation of fruit bodies during sexual development, light-dependent signalling and secondary metabolism. We are trying to determine how exactly the observed phenotypes arise: are they associated with disturbed deneddylation activity of COP9 (the removal of NEDD8 tag from cullin, a subunit of ubiquitin ligase), are kinases associated with CSN responsible for these effects, or is it a combination of both? We have addressed the first part of this question by point mutagenesis of the *csnE*, containing the JAMM metalloprotease motif, which has been shown to convey the deneddylation activity. Mutant strains with *csnE* bearing point mutations in the codons for H134, H136 and D145 of the JAMM motif were constructed. The JAMM mutant phenotype is identical with that of the csnE deletion strain, indicating that the COP9 deneddylation activity alone is essential for sexual development in *A. nidulans*. Analysis of cullin neddylation state in these mutants is underway.

37. GENETIC STABILITY OF DIRECT AND INVERTED REPEATS IN ASPERGILLUS NIDULANS

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Homologous recombination facilitates error free repair of DNA double strand breaks because it uses a homologous sequence as a template for the repair. However, most genomes contain repetitive sequences such as transposable elements, rDNA, centromeric repeats, etc, which in some cases cause inadvertent homologous recombination leading to chromosomal translocations or genomic instabilities. In many filamentous fungi, including Aspergillus nidulans, transformation of recombinant DNA often leads to the integration of multiple copies of this DNA either as inverted or tandem repeats. For many industrial applications transformants with multiple gene copies are obtained from screening because such strains often have high gene expression levels, but consequently, the repeated sequences result in genetic instability. For filamentous fungi there are no accurate methods for assessing this type of genetic instability. Therefore, we have developed a sensitive conidiospore color assay in A. nidulans that allows the detection of as little as a single spontaneous or induced recombination event within a fungal colony and also determines whether the event proceeded as a loop-out or a gene conversion event. The assay provides a unique way to measure the recombinogenic effects of specific direct or inverted repeats, and can effectively be used to measure the genotoxicity of compounds or the effect of various gene mutations on recombination.

38. SECONDARY METABOLITE BIOSYNTHETIC GENE CLUSTERS IN ASPERGILLI

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Aspergillus fumigatus pathogenicity and competition for resources may be augmented by its numerous secondary metabolites. Secondary metabolite biosynthetic genes are typically organized in clusters containing most if not all of the structural enzyme genes required for product biosynthesis. The A. fumigatus genome contains 24 clusters with polyketide synthase, non-ribosomal peptide synthase or dimethylallyl tryptophan synthase genes which range in size from 20 kb to 60 kb and contain from 6 to 22 genes. These clusters are dispersed throughout the genome with only 8 of the 24 located in subtelomeric regions. Many A. fumigatus clusters contain regulatory genes, genes associated with resistance to the metabolite, and apparently unrelated genes with no obvious role in production of the metabolite in question. Twelve clusters contain genes

encoding transcription factors involved in the regulation of polyketide production and other secondary metabolite biosynthetic pathways. With the availability of several other sequenced Aspergilli and related filamentous fungi, we have undertaken a comparative analysis of the secondary metabolite biosynthetic genes and clusters. Some A. fumigatus clusters have orthologs in Aspergillus nidulans or Aspergillus oryzae while most are A. fumigatus specific. We will report on these and other comparative aspects of these clusters, including chromosomal localizations, cross-species cluster integrity, inter- and intra- species relatedness of paralogous genes and the likelihood of interspecific and perhaps interkingdom horizontal gene transfer.

39. DELETION OF THE KU70 HOMOLOG OF *ASPERGILLUS NIDULANS* FACILITATES GENE REPLACEMENT AND GENE TAGGING.

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Deletion of the *Neurospora crassa* homologs of the human KU70 and KU80 genes, which encode proteins involved in the non-homologous end joining of double-stranded DNA breaks, greatly increases the frequency of correct gene targeting during transformation (Ninoiya et al., 2004, Proc. Natl. Acad. Sci. USA, 101: 12248-12253). While homologous recombination, which is required for gene targeting, is significantly more efficient in *A. nidulans* than in *N. crassa*, during transformation other integration events occur at a relatively high frequency. As a result, identifying a transformant with correct gene targeting often requires screening several to many transformants. In an effort to obtain more efficient gene targeting, we have identified the *Aspergillus nidulans* homolog of KU70 and have deleted it, replacing it with the *A. nidulans arg*B gene. We have verified the replacement by Southern hybridization and PCR. The KU70 deletion causes no apparent growth phenotype and, unlike in *N. crassa,* does not cause sensitivity to methyl methanesulfonate. To test the effects of the KU70 deletion on gene targeting, we have carried out two sets of experiments. Both involved transformation with linear DNA fragments carrying a non-*A. nidulans* selectable marker. One involved gene replacement using a glufosinate resistance marker and the second involved GFP-tagging using the *A. fumigatus pyr*G gene as a selectable marker. Preliminary results indicate that the KU70 deletion dramatically improved gene targeting in both cases.

40. Proteome Analysis of secreted proteins from Aspergillus oryzae in liquid and solid-state culture conditions.

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Filamentous fungi are widely used for the production of homogeneous and heterogeneous proteins. Aspergillus oryzae is focused on because of its ability of production of heterogeneous proteins in solid-state culture. To clarify whole overview of protein secretion by A.oryzae in solid-state culture, we carried out comparative proteome analysis of secreted proteins in solid-state culture and submerged culture. Secreted proteins, which were prepared from both culture for 0, 12, 24, 32, and

40hr, were subjected to 2-D electrophoresis, and protein spots of 40hr were identified by peptide mass finger printing using MALDI-TOF MS. Cell-wall bound proteins of 40hr submerged culture were also identified. One hundred twelve from solid-state and 110 spots from submerged culture were analyzed. From them, 82 spots were positively identified derived from 29 gene products in total. The protein secretion profile was revealed and the identified proteins were classified into 4 groups by secretion pattern. Northern analyses of 7 genes (TAA, bgl1, glaA, glaB, xynG1, xynG2, dppV) from these 4 groups were carried out to examine the mechanism that control solid-state specific protein production. These data suggested that A. oryzae altered the manner of protein secretion at posttranscriptional and transcriptional level with respond to culture condition.

41. SINGLE STEP AFFINITY PURIFICATION OF PROTEIN COMPLEXES FROM ASPERGILLUS NIDULANS

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To uncover regulatory networks in Aspergillus nidulans we have previously used two-hybrid and genetic suppression analysis. Both approaches are time consuming and require biochemical confirmation of potential interactions. Direct biochemical identification of binding partners of a target protein is, in contrast, generally very quick. Because interactions take place under physiological conditions, identification of spurious interactions are also minimized. Expression of the target protein under control of its own promoter in single copy also helps limit non-physiological interactions. We have utilized the S-tag as a rapid and efficient affinity tag by which to purify proteins from Aspergillus nidulans. An S-tag gene replacement cassette has been made incorporating a Gly/Ala repeat between the target protein and the 15 amino acid S-tag sequence to improve functionality of tagged proteins. As a test case, we have endogenously S-tagged SonA, a nuclear pore complex component, using fusion PCR to generate the replacement cassette. This protein was chosen as we have previously shown that SonA interacts with SonB and this physical interaction serves as a positive control for the approach. We are additionally interested in identifying other proteins with which SonA interacts. From 1000 ml of exponentially growing cells we typically extracted ~200 mg crude protein extract through lyophilization of mycelia followed by grinding in a mortar and pestle. After S-tag affinity purification from 100 mg total protein, the affinity purified proteins were separated using SDS-PAGE and visualized by Commassie blue staining. A strain not containing an S-tag was similarly purified as a negative control. The bands specifically purified from the SonA-S-tag strain, but lacking from a control strain, were identified using in gel trypsin digestion and mass spectroscopy. As expected, both SonA and SonB were identified in affinity purified SonA-S-tag samples. Two additional co-purifying proteins were identified as nuclear pore complex proteins. One, AN1157.2 is related to yeast Gle1. The other, AN4594.2, is related to yeast Nup42. Both Gle1 and Nup42 have been found to physically and genetically interact with Gle2, the S. cerevisiae ortholog of SonA, indicating that these are true interacting proteins in A. nidulans. The data demonstrate that single step affinity purification of endogenously S-tagged proteins, and identification of co-purifying proteins, is a viable approach to discover meaningful protein-protein interactions in A. nidulans. The methodology developed should be readily transferable to other Aspergilli and help facilitate the rapid identification of protein-protein interactions in this important group of fungi.

42. ASPERGILLUS FLAVUS GENOME SEQUENCE: INITIAL ANALYSIS

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Aspergillus flavus is a widely distributed filamentous fungus that normally occurs as a saprophyte in the soil or on decaying organic matter. It is pathogenic to plants, insects and animals and produces aflatoxin, one of the most toxic and carcinogenic naturally occurring compounds. A. flavus is also the second leading cause of aspergillosis in humans. A whole genome sequencing project funded by the USDA/NRI and USDA/ARS and conducted at TIGR is nearing completion. Preliminary studies of the 33.1 Mb draft sequence, which consists of 17 scaffolds representing 2995 contigs, indicate that the genome contains approximately 13,800 genes. Thus the A. flavus genome appears to be larger than either A. nidulans or A. fumigatus. Aspergillus species are of interest in part because of the large range of clinically and industrially important secondary metabolites they synthesize. An initial scan of the A. flavus genome for proteins involved in the production of these secondary metabolites revealed the presence of 24 putative polyketide synthases and 25 putative non-ribosomal peptide synthases. Manual annotation and analysis of the genome is being coordinated through North Carolina State University and will be made available at www.Aspergillusflavus.org.

43. Comparison of the *Aspergillus niger* genomic DNA sequence with its genetic map

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Aspergillus niger is a key organism for the production of proteins, primarily enzymes. DSM sequenced the complete 35.9 Mb genome of *A. niger* CBS 513.88 (a natural derivative of strain NRRL 3122). The 7.5-fold coverage random sequencing of carefully selected large insert BACs allowed the assembly of the DNA sequence data into 19 large so-called supercontigs, providing a detailed physical map. Previously, the electrophoretic karyotype of *A. niger* strain CBS 120.49 was estimated to contain 37 Mbp of DNA located on 8 chromosomes. We compared the genetic location of a number of genes with known DNA sequence to their location on the supercontigs. For *A. niger*, only few of such markers are known, therefore we used pulsed field gel electrophoresis in combination with Southern blot analysis to assign more cloned genes and complete this comparison. All supercontig localizations confirm with the assigned genes, indicating that there are no major translocations between *A. niger* CBS 513.88 and CBS 120.49. Two supercontigs were found to represent the major part of chromosome III. Six genetic markers on chromosome III were identified based on their annotations and subsequent complementation with PCR clones. Mitotic recombination revealed the orientation of the supercontigs and an improved genetic map of

chromosome III was constructed. Recombination of these markers in a chromosome specific tester strain will enable us to assess possibilities for mitotic mapping in *A. niger*. In addition, we think that the sequence information of *A. niger* CBS 513.88 facilitates chromosome assignment and positional cloning using *A. niger* sequences.

44. Proteolysis and protein processing in filamentous fungi

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Filamentous fungi are being as production organisms for fungal and, more recently, non-fungal proteins. Our research is focused on three different areas of research related to efficient protein production: ***protease mutants***, ***protein processing in the secretion pathway***, ***vacuolar proteases***. Already early on, protease production was addressed as a bottleneck in achieving high levels of secreted protein. Several approaches to isolate strains with reduced protease production, including a new direct selection approach and options for functional genomics and HTS-type of approaches will be discussed. A "secretion carrier" approach results in increased levels of secreted heterologous protein. To analyze the role of the KEX2/furin-like protein processing protease in this approach, we have isolated the corresponding fungal *kexB/pclA* genes and analyzed the effect of gene-disruption on protein secretion. The resulting mutant strains show significantly increased levels of several fusion proteins. Detailed analysis of some of the secreted fusion proteins revealed the presence of alternative, PclA- independent, protein processing pathways. Also protein targeting to the vacuole and release of vacuolar proteases is identified as possible reasons for low levels of secreted proteins. To study this we have isolated vacuolar protease mutant strains to study proteolysis and protein processing in vacuolar
protease mutants.

45. CHARACTERISATION OF CWPA, A PUTATIVE GLYCOSYL-PHOSPHATIDYLINOSITOL ANCHORED CELL WALL MANNOPROTEIN IN THE FILAMENTOUS FUNGUS ASPERGILLUS NIGER.

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In fungi, glycosylphosphatidylinositol (GPI)–anchored proteins are found at the cell surface, either attached to the plasma membrane (GPI-PMPs) or attached via a remnant of the GPI-anchor to the cell wall. In the yeasts S. cerevisiae and C. albicans, it has been shown that GPI-anchored cell wall mannoproteins (GPI-CWPs) become attached to the beta-1,3-glucan or chitin part of the cell wall through a flexible beta-1,6-glucan moiety. GPI-CWPs can be extracted from the fungal cell wall by treatment with hydrofluoric acid (HF), which cleaves the phosphodiester bonds which is present in the remnant of the GPI-anchor. We show that A. niger contains at least five HF-extractable cell wall mannoproteins. A gene encoding an HF-extractable cell wall mannoproteins was cloned and named cwpA. The protein sequence of CwpA indicated the presence of two hydrophobic signal sequences at the N-terminus and C-terminus of the protein, for entering the ER and the addition a

GPI-anchor, respectively. A CwpA specific antiserum was raised and in combination with simple fractionation experiments, we confirmed that this protein was hardly present in the membrane fraction and abundantly present as a HF-extractable protein in the cell walls. Deletion of cwpA did not show an apparent difference in growth under normal growth conditions. The cwpA deletion strain displayed however a more sensitive phenotype towards the cell wall disturbing compound Calcofluor White. The latter result indicate the CwpA might have a structural role in cell wall integrity and that the absence of CwpA leads to weakening of the cell wall which makes the fungal more sensitive to Calcofluor White.

46. STUDY OF CELL WALL INTEGRITY AND DISTURBING ANTIFUNGAL COMPOUNDS WITH A NOVEL GFP-BASED REPORTER METHOD

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Activation of the fungal cell wall integrity pathway is a mechanism used by fungi to escape from cell wall threatening conditions. In Aspergillus niger we have previously shown that the *agsA* gene, encoding an alpha-1,3-glucan synthase, is transcriptionally activated in response to cell wall stress. We have used the agsA promoter region to set up a GFP-based reporter system to identify compounds that activate the agsA expression by fusing the PagsA to the cytosolic GFP or to a nuclear targeted version of GFP. The reporter strains confirmed earlier observation that the agsA gene is activated in response to various cell wall disturbing compounds such as CFW, caspofungin and tunicamycin. Other forms of stress (osmotic or oxidative stress) did not activate agsA expression, indicating that the induction of agsA is cell wall stress specific and not a general stress response. We have used the cell wall stress reporter system to study the effect of various known antifungal compounds in relation to cell wall remodeling. The results with be presented on the poster.

47. Absence of free iron induces the expression of non-ribosomal peptide synthetases in Aspergillus fumigatus.

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The filamentous fungus *Aspergillus fumigatus* is a human pathogen, causing devastating, invasive diseases mainly in the respiratory system of the immunocompromised hosts. Apart from an array of mycotoxins, contributing to its aggressive appearance, the fungus produces siderophores which are responsible for iron accumulation. Siderophore production appears to contribute to organismal virulence and it is becoming clear that non-ribosomal peptide synthetases (NRPS) are involved in hydroxamate siderophore biosynthesis. We report here the characterisation and analysis of two NRPS encoding genes, termed *pesB* and *pesF*. RT-PCR analysis confirms that expression of both *pesB* and *F* is up-regulated under conditions of iron limitation concomitant with increased siderophore production. In addition, a protein corresponding to the predicted molecular mass of pesB (~200 kDa) has been identified by MALDI TOF peptide mass fingerprinting and MALDI LIFT TOF/TOF peptide sequencing following SDS-PAGE and 2D-PAGE separation of protein extracts obtained from mycelia grown under iron-limiting conditions. Proteomic characterisation of

other high molecular mass proteins is ongoing to enable identification of other siderophore synthetases in *Aspergillus fumigatus*. Because siderophore synthetases are unique in bacteria and fungi they display an attractive target for anti-fungal chemotherapy to reduce the virulence of *Aspergillus fumigatus*.

48. ASPERGILLUS ORYZAE ATFB ENCODES A TRANSCRIPTION FACTOR, WHICH IS REQUIRED FOR STRESS TOLERANCE OF CONIDIA

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In the solid-state culture, Aspergillus oryzae exhibits phenotypes such as the high production of enzymes, conidiophore development, and the production of various kinds of secondary metabolites. Though these characteristics should involve various gene expressions, the only a few regulatory systems have been understood. From the EST database of A.oryzae, we found two genes encoding transcription factors that show high homology to atf1 of Shizosaccharomyces pombe and named atfA and atfB respectively. The atfB gene was upregulated at the late phase of solid-state culture, while atfA gene was constitutively expressed. We tried to identify atfB target genes by microarray analyses. In the atfB-overexpression strain, about 30 genes including catalase gene (catA) were upregulated. Almost all of these genes were upregulated at the late phase of solid-state culture in wild type strain. Furthermore the expressions of these genes were downregulated in the atfB-disrupted strain. The disruptant strain grew normaly but its conidia lost several stress tolerances.

49. CHARACTERIZATION OF SUBUNIT ONE AND TWO OF THE A. NIDULANS COP9 SIGNALOSOME

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The COP9 Signalosome (CSN) is a highly conserved protein complex critical to the accurate development of numerous multicellular organisms. It shares structural similarities with the lid subcomplex of the proteasome and the translation initiation factor 3 (eIF3). The subunits of these complexes contain either a PCI or a MPN domain. Recently two subunits of the COP9 Signalosome were isolated in the filamentous ascomycete A. nidulans. Deletion of either csnD or csnE resulted in pleiotrophic phenotypes affecting the sexual cycle, secondary metabolism and light signaling.
We report the isolation and characterization of csnA and csnB from A. nidulans. The csnA coding sequence of 1497 bp is interrupted by three introns resulting in a protein of approximately 55,7 kDa. The approximately 54,2 kDa Protein CSNB is encoded by a DNA sequence of 1670 bp. Both deduced amino acid sequences contain a PCI domain and show high identity to the analogous CSN subunits of higher eukaryotes. Deletion of either csnA or csnB resulted in viable strains with hyphae appearing partly red. The sexual cycle is initiated but the fruit body formation is blocked at the stage of primordia. These defects are identical to those found for the csnD and csnE deletion strains. We conclude that csnA and csnB code for the first two subunits as defined by database searches of the COP9-Signalosome in A. nidulans. At present, Aspergillus nidulans strains carrying a csnA::gfp fusion are

constructed to verify where the protein is localised at different developmental stages. Our results reinforce this fungus as an appropriate model organism for studying the extensive functions of the complex in a manageable eukaryote.

50. TWO ASPERGILLUS NIDULANS DEVELOPMENTAL MUTANTS (SWOM1 AND SWON1) ARE DEFECTIVE IN GENES INVOLVED IN SYNTHESIS OF CELL WALL COMPONENTS

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Two temperature sensitive developmental mutants of Aspergillus nidulans have been isolated and characterized. Both swoM1 and swoN1 show polarity maintenance defects when incubated at restrictive temperature. The swoM1 mutant extends a primary germ tube that quickly losses polarity and swells to a uniform cell of approximately 20 µm diameter, while the swoN1 mutant is roughly wild type, with the exception of numerous periodic swellings along the hyphae that give rise to multiple germ tubes leading to a hyper-branched appearance. Both mutants are restored to near wild type growth when supplemented with 1.2 M sorbitol as osmoticum, indicating they posses a cell wall defect. Upon downshifting swoM1 germlings from restrictive to permissive temperature multiple germ tubes emerge from both the conidium and the depolarized primary germ tube. The swoM1 mutant is fully complemented by gene An6037.2 which encodes a homolog of a phosphoglucose isomerase. This enzyme interconverts glucose-6-phosphate and fructose-6-phosphate. This is the first step in glycolysis, as well as chitin synthesis. The swoN1 mutant is fully restored to wild type growth when transformed with a construct containing the gene An5586.2. The An5586.2 gene encodes a GDP-mannose pyrophosphorylase. This enzyme synthesizes GDP-mannose from GTP and mannose-1-phosphate. These two enzymes are both involved in mannose utilization and protein glycosylation. The mutant swoM1 locus is hypothesized to encode a protein that contains a single base substitution, A305P, at a highly conserved residue. This mutant grows poorly on the standard growth media even at the permissive temperature but when supplemented with fructose, mannose, or ethanol wild type growth is restored even at restrictive temperature, but conidiation is absent. Previous work with the swoA mutant defective in a protein mannosyl transferase also implicated mannoprotein synthesis in cell morphogenesis (Shaw and Momany, 2002). All three of these swo genes encode enzymes that participate in protein mannosylation, therefore we hypothesize that a mannosylated protein(s) is critical for proper cell wall assembly that leads to maintenance of polar growth.

51. REGULATION OF GLUCONEOGENESIS IN ASPERGILLUS NIDULANS

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Gluconeogenesis is required for the utilization of carbon sources metabolized via the TCA cycle. Gluconeogenesis requires the enzymes fructose-1,6-bisphosphatase and PEP-carboxykinase that mediate irreversible steps in glycolysis. There is at present little known about the mechanisms underlying the regulation of gluconeogenic genes in A. nidulans. The expression level of genes

encoding these enzymes is significantly lower in strains with mutations of the acuK and acuM genes. These genes encode Zn(II)2Cys6 binuclear cluster proteins (Sealy-Lewis, personal communication) and are likely to be involved in transcriptional regulation of gluconeogenic genes. A double deletion strain of acuK and acuM shows a phenotype equivalent to that of the single deletion strains. It can therefore be concluded that these two proteins are involved in a single regulatory mechanism. Expression studies have been conducted on maeA, which encodes an NADP-dependent malic enzyme. Malic enzyme is required for growth on carbon sources metabolized via 2-oxoglutarate, such as proline. It converts malate to pyruvate, which is used to produce acetyl-CoA, and also provides NADPH. Mutations in acuK and acuM genes significantly reduce maeA expression supporting their role as regulatory proteins. However, the study showed that maeA had a different regulatory pattern from the gluconeogenic genes studied previously. One significant difference is that the high induction level brought about by proline is abolished in the presence of acetate. Acetate does not need to be utilized for this effect.

52. MICROARRAY ANALYSIS OF THE *ASPERGILLUS NIGER* TRANSCRIPTOME REVEALED THAT *XLNR* PLAYS AN IMPORTANT ROLE IN THE REGULATION OF DIFFERENT PATHWAYS

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The XlnR protein was initially identified as a transcription activator of different genes encoding cellulolytic and xylanolytic enzymes in *Aspergillus niger* (van Peij *et al*., 1998). A number of experiments have been performed to elucidate the regulation mechanism of *xlnR* and the results suggest that the transcription of the *xlnR* gene is induced by the presence of D-xylose in the culturing media and repressed by D-glucose. As the mRNA levels of XlnR gene are very low, and they can hardly be detected by Northern blot analysis, further research is necessary to confirm these results. To study the XlnR regulon, XlnR mutants were grown under inducing (D-xylose and xylan), repressing (D-glucose) and neutral (sorbitol) culturing conditions. The whole transcriptome was examined by microarray analysis. The XlnR mutants used in these experiments are an *xlnR* knock out mutant, a mutant where XlnR is constitutively expressed, and the wild type phenotype. Comparison of the transcriptome of different XlnR strains under inducing and repressing conditions showed that XlnR regulates several genes that are involved in different pathways. Among these are genes encoding proteins involved in signal transduction, in the regulation of transcription, in sugar transport but also genes encoding enzymes.

Previous work of Graaff *et al*. (1994) suggested that the CreA transcription factor might play a role in the transcriptional regulation of the XlnR regulon. This has been shown for the enzyme encoding genes of the regulon, but whether CreA regulates the transcription of the *xlnR* gene still was uncertain. In order to investigate this the transcriptome of different single and double CreA derepressed and XlnR mutants were compared using both microarray and qPCR analysis. The results of these experiments lead to a new model for the transcription regulation of XlnR.

53. POST-TRANSLATIONAL REGULATION OF AreA, THE GLOBAL TRANSCRIPTIONAL ACTIVATOR OF NITROGEN METABOLISM IN ASPERGILLUS NIDULANS.

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In Aspergillus nidulans, the global nitrogen regulatory gene areA, encoding a positively acting GATA transcription factor AreA, is required for activating genes involved in nitrogen metabolism. When a good nitrogen source like ammonium or glutamine is available, areA-dependent genes are expressed at a low level, while the expression of these genes is up regulated when only a poor nitrogen source, like alanine, is present (nitrogen limiting condition). This differential expression is controlled by areA transcript stability and the interaction of AreA with a negative regulator NmrA. A further increase in gene expression, which is not mediated by these two regulatory mechanisms, is observed under nitrogen starvation and correlates with AreA accumulation in the nucleus. Nuclear accumulated AreA is rapidly exported upon addition of a nitrogen source via the CrmA exportin. We therefore sought to investigate the importance of post-translational modifications on nuclear localization and function of AreA.
We have shown that AreA is multiply phosphorylated and its phosphorylation status differs under nitrogen sufficient, limiting or starvation conditions. A number of conserved potential phosphorylation sites on AreA have been mutated and assessed for their roles in AreA function. In addition, AreA contains a highly conserved small ubiquitin-like modifier (SUMO) modification site adjacent to a putative CrmA exportin binding motif. We have deleted the gene encoding the SUMO peptide and mutated the putative sumoylation site on AreA to address the involvement of sumoylation in the regulation of AreA.

54. Examination of the phylogeny and possible function of genes specific to *A. fumigatus* and *A. fischerianus*

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Comparative analysis of the genomes of *Aspergillus fumigatus*, a human pathogen, with the model organism *Aspergillus nidulans* and the industrial agent *Aspergillus oryzae* has led to the identification of more than 500 genes that appear specific to *A. fumigatus*. Provocatively, many of these *A. fumigatus* genes have few or no homologs in the genomes of other eukaryotes available in public databases. Instead, a subset shows significant sequence similarity to bacterial or archaeal proteins. Interesting examples include heat shock protein GroES, death-on-curing family protein Doc, and arsenate reductase ArsC. The preliminary genomic sequence and automated annotation of a fourth Aspergillus genome, *Aspergillus fischerianus* (*Neosartorya fischeri*), has allowed us to investigate further *the A. fumigatus*-specific gene set. As *A. fischerianus* is the most closely related species to *A. fumigatus* that has been identified (apart from the sister species *A fumigatus var. ellipticus*), we can now determine that approximately half of the genes previously identified as *A. fumigatus*-specific genes are actually shared between *A. fumigatus* and *A. fischerianus*. We are also investigating genes that appear unique to *A. fischerianus*. Some of these *A. fumigatus* and *A. fischerianus*-specific genes are likely to confer significant selective advantage to these fungi in competing for resources and in adapting to diverse environments. We are currently exploring the

origin of these genes using phylogenetic methods in order to posit whether they were selectively retained by these species or acquired through lateral gene transfer.

55. FUNCTIONAL GENOMICS STUDIES FOR IDENTIFYING GENES INVOLVED IN AFLATOXIN FORMATION IN *Aspergillus flavus*

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Aflatoxins are secondary metabolites produced mainly by the molds *Aspergillus flavus* and *A. parasiticus*. Because these compounds are toxic and extremely carcinogenic to animals, they pose a serious risk to human health. The aflatoxin biosynthetic pathway and its genetic regulation have been studied for decades, revealing a well organized aflatoxin pathway gene cluster consisting of 25 genes within a 70 kb DNA region. Many nutritional and environmental factors were also found to affect expression of aflatoxin pathway genes. In order to better understand the molecular mechanisms that control or regulate aflatoxin production, identification of genes (gene profiling) using *A. flavus* expressed sequence tags (ESTs) and microarrays is currently being performed. Sequencing and annotation of *A. flavus* ESTs from a normalized *A. flavus* cDNA library identified 7,218 unique EST sequences. Genes that are putatively involved in aflatoxin biosynthesis, regulation and signal transduction, fungal virulence or pathogenicity, stress response or antioxidation, and fungal development were identified from these ESTs. Microarrays containing over 5,000 unique *A. flavus* gene amplicons were constructed at The Institute for Genomic Research (TIGR). Gene profiling has thus far identified hundreds of genes that are highly expressed under aflatoxin-producing conditions. Further investigations on the functions of these genes are underway. This research is expected to provide information for developing new strategies for control of aflatoxin contamination of agricultural commodities.

56. IDENTIFICATION AND TRANSCRIPTIONAL REGULATION OF NEW STARCH MODIFYING ENZYMES IN THE ASPERGILLUS NIGER GENOME

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Aspergillus niger is well known for its ability to secrete a wide variety of plant Carbohydrate Modifying Enzymes (CMEs). The secreted CMEs are able to degrade the plant cell wall materials as well as its storage carbohydrates, like starch. Based on its recently finished genomic sequence by Dutch life sciences company DSM, it is estimated that only a fraction of the potential of enzymes produced by A. niger is currently exploited.

Database mining of the A.niger genome resulted in the identification of twelve new enzymes, which contain motifs indicating that they might act on starch. In addition to the five known starch degrading enzymes of A. niger, glucoamylase (glaA), two alpha-amylase (amyA and amyB), an acid amylase (aamA) and an alpha-glucosidase (aglU), six putative alpha amylases, five putative alpha-glucosidases and one alpha1,4-glucan branching enzyme were identified. It is well established that the expression of starch modifying enzymes is coordinately regulated at the transcriptional level by a pathway specific transcription factor AmyR. AmyR binds to sequences ($CGGN_8CGG$ or $CGGN_8AGG$) in the promoter region of amylolytic genes. Inspection of the promoter regions of the newly identified amylolytic genes revealed that only five of them contain AmyR consensus binding sites. The expression of the newly identified genes in relation to the presence of starch and the role of AmyR during regulation has been examined using microarrays and will be presented.

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