Identification of differentially expressed genes in the *nuc*-2 mutant strain of *Neurospora crassa*

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The filamentous mould *Neurospora crassa* synthesizes a number of phosphate (Pi)-repressible phosphatases and permeases that have the function of making more Pi available to the cell, i.e., these enzymes are synthesized and secreted in response to the signaling of phosphorus starvation. The genetic and molecular mechanisms controlling this response in *N. crassa* include four regulatory genes, *nuc-2*, *preg*, *pgov*, and *nuc-1*, involved in a hierarchical relationship. Nuc-1 is a positive wide domain transcription factor. Nuc-2 senses the availability of Pi and transmits the metabolic signal downstream the regulatory pathway. Also, Nuc-2 is an ankyrin repeat protein, a class of proteins that regulate, for example, important biological functions of the cell cycle and of the cytoskeleton organization. Here, we describe genes which are differentially expressed in response to *nuc-2* mutation, by employing the suppression subtractive hybridization (SSH) approach. The driver and tester cDNAs were obtained from mycelium of the *St.L.74A* and *nuc-2* (FGSC# 1996) strains of *N. crassa*, respectively. Out of a total of 550 cDNA clones analyzed by dot-blot macro-arrays, we confirmed 50 clones differentially expressed in the *nuc-2* mutant strain, representing 40 different genes. Based on BLAST homology, the clones selected from these libraries are largely of fungal origin. The hypothetical proteins represent 52% of the total, and the putative proteins identified are involved in metabolism, cell signaling, and cell fate. These studies provide data that are useful for identifying the role of gene *nuc-2* in the expression of proteins other than those involved in the acquisition of Pi from the ambient.

IIIp-2

Identification of regulators of the *Ustilago maydis mig* genes

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The *Ustilago maydis mig* genes are drastically upregulated upon entry of *U. maydis* into its host plant maize. The *U. maydis* genome comprises the *mig1* and the six highly homologous *mig2* genes, *mig2-1* to *mig2-6*, with five arranged as direct repeats in a 7.1-kb cluster. *mig* genes encode small, secreted, cysteine-containing proteins that lack homologies in data bases and whose function is unknown. Detailed analysis of the *mig2-5* promoter, which confers the strongest inducible activity within the cluster, uncovered a consensus motif (5'-CCA$^{C}/_{A}{}^{C}/_{A}$-3') present in multiple copies in all *mig2* promoters and whose activity specifically relies on the sequence triplet CCA. On this basis zinc finger transcription factors of the Cys_2His_2-type were considered candidates for *mig2* regulation. We have identified one *U. maydis* gene (*mzr1*) encoding a putative Cys_2His_2-type zinc finger protein that is essential for inducible *mig2-5* expression *in planta*. We could further demonstrate that conditional overexpression of *mzr1* is sufficient to induce transcription of a number of *mig2* genes as well as of *mig1*. Together with the observation that the action of *mzr1* relies on the same *mig2-5* promoter motifs that are required for inducible activity *in vivo*, we infer that *mzr1* encodes a putative activator of *mig* genes.

Identification and characterization of a cutinase transcription factor in *Trichoderma harzianum*

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Strains of *Trichoderma* genus are free-living fungi that are common in soil, roots, decaying wood, and other forms of plant organic matter. They are able not only to antagonize phytopathogenic fungi, but also to establish symbiotic interactions with plants, inducing localized or systemic resistance responses and exerting beneficial effects on plant growth and development. A cDNA library was constructed by mixing RNAs obtained from different growth conditions of *T. harzianum* CECT 2413. A total of 1508 cDNA clones were sequenced from the 5' end. Finally, 914 unique ESTs were obtained from this library. The EST L03T34P022R02092 was selected for futher characterization in this study, based on its high homology with a cutinase transcription factor (*ctf*) described in *Fusarium solani* f. sp. *pisi*. We are interested in knowing the role of this gene in *T. harzianum* CECT 2413. The cDNA partial sequence was used as a probe for screening a *T. harzianum* CECT 2413 genomic library previously built. A positive clone was used to obtain the complete sequence of the gene by means of a "primer walking" strategy. Slot blots were carried out with total RNA obtained from mycelia of *T. harzianum* CECT 2413 grown for 4, 8 or 24 h in different culture conditions. The signal was increased along the time except when olive oil was used as carbon source. To determine the biological relevance of this gene, transformants containing a disrupted *ctf* gene were obtained. Morfological, physiological and biochemical assays, and proteomic analyses by 2DE maps are being used to characterize the knock-out mutants.

IIIp-4

Insights into RIP and DNA methylation in the *Aspergillus* section *Flavi* complex

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DNA methylation of cytosine is an epigenetic mechanism found in many eukaryotic genomes, with the dual roles of gene regulation and protection of the genome. Control and function of DNA methylation are still largely unknown in fungi and, apart from detailed work in *Ascobolus immersus* and *Neurospora crassa*, few studies have been conducted in other species. In *N. crassa* DNA methylation is involved in a process called Repeat-Induced Point Mutation (RIP), which acts at a precise stage in the sexual cycle, detecting sequence duplications and introducing C:G and T:A transitions leading to dense cytosine methylation. Recently RIP-like transitions have been reported in transposons in *Aspergillus nidulans* and *A. fumigatus*,but *Aspergillus* species were thought to be devoid of DNA methylation, although this has now been contradicted in work detecting methylation by HPLC for *A. flavus*. We isolated a transposase sequence from *A. parasiticus* using a DNA methyl-binding column. The sequence has 67% identity with *Tan 1* from *A. niger* and is present in at least 20 copies in the *A. oryzae* genome sequence database. Comparison of these copies indicates the presence of RIP-like transitions similar to RIP changes in *N. crassa*. Although the original sequence was isolated from a methyl-binding column, no evidence of methylation was found by Southern Blotting. The preference for CpA to TpA changes could increase the introduction of termination codons (TAA and TAG) leading to gene inactivation by mutation alone. It is also possible that the light patterns of RIP are a source of genetic diversity.

A long terminal repeat (LTR) of basidiomycetous mushroom *Lentinula edodes* retrotransposon as a regulator of transcription initiation and termination

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We identified a long terminal repeat (LTR)-type retrotransposon, designated *Le*.RTn1 in the basidiomycetous mushroom *Lentinula edodes*. *Le*.RTn1(6,213 bp) carried 474-bp LTRs and contained two internal overlapping open reading frames (ORFs) putatively encoding Gag (group-specific antigens), and Pro (protease) plus Pol (reverse transcriptase, RNase H, and integrase). Translation of the second ORF was suggested to occur by ribosomal frameshifting of the *gag* domain. *L. edodes* chromosomes seemed to carry more than ten copires of *Le*.RTn1 (and its derivatives). About two hundreds copies of solo LTRs were considered to be present on the chromosomes. The LTR contains four consecutive, eukaryotic promoter consensus TATA-(like) boxes, two relatively long direct repeats, and terminator consensus sequences TAG---TAGT---TTT first identified in yeast. Analysis of the 5' end and 3' end of the cDNAs synthesized from several sorts of mRNAs revealed that the four TATA-(like) boxes and the TAG---TAGT---TTT do work as a regulator of transcription initiation or terimination.

IIIp-6

The homocysteine regulon in *Aspergillus nidulans*

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Homocysteine is the intermediary amino acid in methionine, cysteine, and AdoMet metabolism. Levels of homocysteine are normally kept low by remethylation to methionine catalyzed by the methionine synthase (MS) in reaction that requires folate (MTHF). Homocysteine can also be catabolized to cysteine in the transsulfuration pathway involving cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CGL). Impaired activity of either of these pathways results in the accumulation of homocysteine. An excess of homocysteine is toxic to the cell, which is manifested by growth inhibition of mutants impaired in CBS by an exogenous methionine or homocysteine. We found that several *Aspergillus nidulans* genes are similarly regulated. Some of the homocysteine-induced genes encode enzymes of the folate cycle – as MS and MTHFR (methylenetetrahydrofolate reductase which synthesizes MTHF) and the enzymes of the transsulfuration pathway (CBS and CGL). We have shown that this regulation occurs both on transcriptional and enzymatic level. It appears therefore, that a new regulatory system – we call “homocysteine regulon” - exists in *A. nidulans*. This regulatory system controls genes that participate in the conversion of homocysteine to less harmful sulfur amino acids.

Transcriptional activators of *Aspergillus nidulans* sulphur metabolism

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Aspergillus nidulans metR gene encodes a bZIP transcription factor specific for activation of several sulphur metabolism genes including those encoding the sulphate assimilation pathway enzymes. METR protein level and/or activity is negatively regulated by a SCF ubiquitin ligase complex which consists of the proteins encoded by the *scon* (sulphur controller) genes. Both *metR* and *scon* genes constitute a regulatory system known as sulphur metabolite repression (SMR). Loss of function mutations in the *metR* gene cause methionine auxotrophy while mutations in the *scon* genes lead to a derepression of the sulphate assimilation pathway enzymes.

All *metR* and *scon* mutants isolated so far were recessive. Recently, we have isolated three dominant suppressor mutants in a diploid strain homozygotic for *metB3* allele. Genetic and molecular analysis showed that Phe48 of *metR* gene is affected in every of the dominant mutants obtained (*MetR20, 21* and *22*). The orthologs of *metR* gene were also found in other fungal species and Phe48 appears as one of the most conserved amino acids of the N-terminal domain which suggests that this residue may be important for METR protein function and/or stability.

MetR20 mutant strain has elevated activity of sulphur metabolism enzymes in comparison to the wild type strain. Suppressor *metR* mutant strains have also an increased level of sulphur compounds in mycelia which suggests that they are resistant to SMR.

By searching of the *A.nidulans* genome sequence, we have found a paralogue of METR protein that has similar basic region and leucine zipper sequences. The gene we named *metZ* is well conserved among other *Aspergilli* and it apparently has a large intron as long as intron in the *metR* gene. Northern blot analysis indicates that *metZ* gene is regulated by SMR. Preliminary results suggest also that *metZ* splicing efficiency may be dependent on METR protein, because *metR1* mutant accumulates *metZ* unspliced transcript. *MetZ* gene interaction with other SMR regulated genes is under investigation.

IIIp-8

Regulation of a toxin, sirodesmin PL, in *Leptosphaeria maculans*

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Sirodesmin PL is a phytotoxin produced by *Leptosphaeria maculans*, which causes blackleg (phoma stem canker), the major disease of *Brassica napus* worldwide. Sirodesmin PL belongs to the epipolythiodioxypiperazine (ETP) class of fungal toxins, which includes the immunosuppressive molecule, gliotoxin. A cluster of 18 genes with predicted roles in the biosynthesis of sirodesmin PL has been cloned. The expression of these genes coincides with the expression of sirodesmin PL and the deletion of the peptide synthetase, *sirP*, within the cluster, results in the dramatic reduction of sirodesmin PL. The *sirP* deletion mutant also displays reduced virulence on *B. napus*, suggesting sirodesmin PL may be a virulence factor.

The sirodesmin PL biosynthetic gene cluster includes a zinc binuclear (Zn2Cys6) transcription factor, SirZ. RNA interference was used to silence *sirZ* expression, which resulted in the dramatic reduction of sirodesmin PL production, and of the expression of the seven genes in the sirodesmin PL biosynthetic gene cluster that were analysed. This suggests that *sirZ* regulates sirodesmin PL biosynthesis.

To isolate regulators of sirodesmin PL production outside the sirodesmin PL biosynthetic gene cluster, a high throughput screen of random T-DNA insertional mutants for sirodesmin-deficient isolates was carried out. Two sirodesmin-deficient mutants have thus far been isolated, both of which show very low levels of *sirZ* expression compared to wild type. In one mutant the T-DNA has inserted into the coding region of a gene with best match to a fungal-specific transcriptional regulator of unknown function. In the other mutant, the T-DNA inserted into the 3' untranslated region of a gene with a best match to a gene involved in the regulation of amino acid biosynthesis. Indeed, this mutant grows poorly under conditions of amino acid starvation. The involvement of these two regulatory genes in the regulation of sirodesmin PL biosynthesis is currently being investigated.

The putative transcription factor CrzA forms specific DNA-protein complexes with the *Aspergillus giganteus afp* promoter

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Aspergillus giganteus secretes a biotechnologically promising protein with antifungal activity, denoted antifungal protein (AFP). Transcription of the corresponding *afp* gene is up-regulated by several environmental stimuli such as alkaline pH, osmotic stress, carbon starvation and heat shock. In a recent study, we could show that alkaline pH-induced up-regulation of the *afp* gene is not mediated by the wide-domain transcription factor PacC. Instead, the increase in *afp* mRNA and AFP levels can be completely prevented by the calcineurin inhibitor FK506, suggesting that the calcineurin signalling pathway might control the *in vivo* activation of the *afp* promoter by alkaline pH.

In yeast it has been shown that one role of the phosphatase calcineurin is to activate gene expression through its regulation of the zinc-finger transcription factor Crz1p. As the 5' upstream region of the *afp* gene comprises five putative Crz1p binding sites (CDREs, calcineurin dependent responsive element), we have isolated the Crz1p homolog from *A. nidulans* and designated it CrzA. The zinc-finger domain of CrzA consists of three zinc-fingers and shows 66 % identity to the corresponding domain of Crz1p, suggesting that CrzA might bind to the same consensus sequence. The entire zinc-finger domain of CrzA expressed as GST fusion was used *in vitro* binding studies with *afp* promoter fragments harbouring the putative CDREs. For four out of five CDRE`s, distinct binding complexes could be detected, giving a further indication for a possible involvement of the calcineurin signalling pathway in *afp* regulation. Interestingly, a truncated version of the zinc finger domain (lacking finger 3) was not able to bind to those sites, suggesting that the "non-conventional" finger 3 is required for efficient DNA binding.

(1) Meyer V, Spielvogel A, Funk L, Tilburn J, Arst HN Jr, Stahl U.(2005) Alkaline pH-induced up-regulation of the *afp* gene encoding the antifungal protein (AFP) of *Aspergillus giganteus* is not mediated by the transcription factor PacC: possible involvement of calcineurin. Mol Genet Genomics. 274:295-306

IIIp-10

DsRed as a reporter to study regulation of *cre1* gene expression in the beta-lactam-antibiotic producer *Acremonium chrysogenum*

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Homologs of the fungal *cre1* gene encode a carbon catabolite repressor protein (CRE1). Previous analyses revealed that the *cre1* gene is differently regulated in wild-type strains of *Acremonium chrysogenum* in comparison to a semi-producer strain. The wild-type shows a positive (auto-) regulation of *cre1* gene expression in the presence of glucose, whereas no regulation has been found in the semi-producer strain. These effects are contrary to *cre1* gene expression in *Trichoderma reesei* and *Aspergillus nidulans* where a negative regulation has been observed [1,2]. In the presence of glucose, the biosynthesis genes *pcbC* and *cefEF* are down regulated in the *Acremonium chrysogenum* wild-type strain while only the *cefEF* transcript level is reduced in the semi-producer strain [3]. Overexpression of the *cre1* gene in the semi-producer strain leads to changes in its expression profile showing wild-type-like glucose repression of *pcbC* and enhanced repression of *cefEF* [4].

In order to measure promoter strengths and to analyse the localization of the CRE1 polypeptide, the *DsRed* reporter gene [5] has been used in two different approaches.

In the first approach, we have constructed different promoter deletions in order to detect regulatory sequences in the promoter region of the *cre1* gene. For comparison the full-length promoter sequence was used in all experiments. A total of nine promoter derivatives were fused to the *DsRed* reporter gene. Using fluorescence spectroscopy the effect of glucose on gene expression was measured by determining the amount of the DsRed protein in crude cell extracts.

In a second approach, we have constructed a set of fungal expression vectors for localization-studies. Using fluorescence microscopy we demonstrate that a CRE1-DsRed fusion protein is localized in the nucleus when glucose is present in the culture medium. In the absence of glucose, the chimeric protein has been observed in the cytoplasm. Based on this experiments, the glucose regulation in *Acremonium chrysogenum* should be elucidated in detail.

[1] Dowzer CE and Kelly JM (1991) Mol Cell Biol 11: 5701-5709
[2] Strauss J, Mach RL, Zeilinger S, Hartler G, Stoffler G, Wolschek M, Kubicek CP (1995) FEBS Lett 376 : 103-107
[3] Jekosch K and Kück U (2000 a) Curr Genet 37 : 388-395
[4] Jekosch K and Kück U (2000 b) Appl Microbiol Biotechnol 54 : 556-563
[5] Matz MV *et al.* (1999) Nature Biotechnol 17 : 969-957

Molecular investigations of *Botrytis cinerea*: reporter genes and gene silencing

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Unlike many plant pathogens, *Botrytis cinerea* is prominent year round and causes infection under a broad range of environmental conditions. Its ability to destroy large quantities of agricultural produce has resulted in the manufacture of numerous fungicides with an annual market size of US $15-25 million dollars and discovery of various factors involved in host-pathogen reactions.

The use of gene silencing is a well-established technique in animal, plant and fungal systems. The benefits of down regulating genes are vast, including the potential to identify the function of targeted genes. Although gene silencing has been demonstrated in various fungal species, we know of no reports to date in *B. cinerea*. Therefore, an effective gene silencing model must be demonstrated and developed before useful targets may be identified. Here, to test if gene silencing is applicable in the fungus, the superoxide dismutase (*BcSOD1*) gene was targeted by the transformation of a sense and antisense silencing constructs. In addtion, the laccase (*BcLCC2*) gene has been targeted for silencing with sense, antisense and hairpin silencing constructs; successful silencing of the superoxide dismutase and laccase gene may indicate which construct is most efficient during silencing. Phenotypic tests indicate that gene silencing is most frequently observed with the hairpin (80%) or antisense (47-60%) silencing constructs in *B. cinerea*. Furthermore, because levels of gene silencing vary between transformants, we hope to determine the level of superoxide dismutase required for full or partial virulence and further investigate the function of laccase during host-pathogen interactions.

To further explore the intricacies of *B. cinerea*, in addition to these advances in gene silencing we are developing reporter systems, such as enhanced green fluorescent protein (eGFP), luciferase (LUC) and ß-glucuronidase (GUS), which may allow for greater visualization and spatial localization of interactions within and between the fungus and its host. Here we introduce an efficient *B. cinerea* expression vector for the induction of various reporter genes, including eGFP, LUC and GUS. We observed successful transformation of each reporter gene through molecular and protein expression studies. Both GFP and GUS have been successfully visualized in *B. cinerea* and LUC expression has been demonstrated by fluorimetry. These were all expressed under the control of the *oli*C promoter from *A. nidulans,* a strong promoter giving good levels of expression. With this vector system we hope that the ease of construct production and simple detection for GFP, GUS and LUC activity will be exploited in uncovering gene function, especially in conjunction with gene silencing experimentation.

IIIp-12

Regulation of the gene *cut-1* of *Neurospora* and relation with the *ovc* phenotype

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The mutant *ovc* of *Neurospora crassa* has a pleiotropic phenotype that includes carotenoid overproduction in the light, hyperosmotic sensitivity and alterations in aerial development. This phenotype is complemented by the gene *cut-1* (Youssar *et al.* 2005, Mol. Microbiol. 55:828-838), coding for a protein of the haloacid dehalogenase family, a group of enzymes usually associated to phosphatase or phosphotransferase activities. Molecular analysis of the *ovc* mutant indicates the absence of a large DNA segment affecting the gene *cut-1* and an undetermined extension of neighbor DNA. A PCR walking approach allowed the identification of the precise deletion limits, covering 77,078 bp on linkage group IV with 21 predicted ORFs, of which *cut-1* is the only known genetic marker. Four of the deleted genes belong to a large transmethylase gene family. The wild-type phenotype exhibited by the mutant upon restoration of *cut-1* indicates no relevant biological role for any of the 20 neighbor ORFs.

To gain more information on the biological role of *cut-1*, a detailed analysis of its transcriptional regulation was carried out. As expected, targeted *cut-1* mutation lead to a hyperosmotic phenotype. Accordingly, *cut-1* mRNA levels increase rapidly following osmotic shock. In addition, they are moderately induced by heat-shock, but they are not affected by other stressing conditions tested. The relation of this gene with carotenoid photoinduction led us to investigate the effect of light on *cut-1* mRNA levels. The modest *cut-1* transcript amounts found in the dark disappeared following illumination, indicating a negative light control. A similar light-triggered down-regulation of *cut-1* mRNA was found in a bd mutant, and a less pronounced reduction was also found in *wc-1*, *wc-2* and *vvd* and *cut* mutants, the latter holding a premature *cut-1* stop mutation. This response is at least partly mediated by the WC system, as shown by the lack of effect of blue light in a *wc-1* mutant. Unexpectedly, *cut-1* mRNA levels are much higher in the dark in the *wc*, *vvd*, *bd* and *cut* mutants, showing the involvement of these genes in a *cut-1* dark repressing mechanism. This is the first report of a dark-effect for the *vvd* gene. In the cases investigated, the increase in the mutants was additive with the one produced by high osmotic conditions, indicating different action mechanisms. The environmental or genetic factors influencing *cut-1* expression have no significant effect on the mRNA levels of two additional *Neurospora* genes encoding HAD proteins.

Mutations in a gene repressor or a glucose transporter result in sustained gene photoactivation in *Neurospora*

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The gene con-10 of *Neurospora* is expressed during conidiation and after illumination of vegetative mycelia. Photoactivation of con-10 is transient and disappears after two hours of light. The adaptation to light of gene photoactivation has been described for other light-regulated genes in fungi and plants and, in *Neurospora*, requires the product of the gene vivid and its modified by inhibitors or mutations in the protein kinase C. The rco mutants were isolated for the abundant expression of gene con-10 in vegetative mycelia. The gene rco-1, encoding a putative gene repressor, and the gene rco-3, encoding a putative glucose sensor, are required for the repression of con-10 in vegetative mycelia. We have observed that, in addition, rco-1 and rco-3 mutants have an enhanced and sustained photoactivation of con-10 and con-6, a phenotype they share with vivid mutants. The abundant photactivation of con-10 and con-6 in rco and vivid strains is detected with short light exposures, but it is best observed after five hours of light. The rco and vivid mutations do not alter the stability of the con-10 and con-6 mRNAs, suggesting that the sustained photoactivation is due to a high transcriptional rate that is not subjected to adaptation to light. The threshold of con-10 and con-6 photoactivation is significantly lowered in the rco-1 mutant, but remains unchanged in the rco-3 and vivid mutants. The circadian clock in these mutants does not seem to be altered, but the induction of carotene biosynthesis by light is slightly enhanced in the rco-3 strain. Our observations indicate that the mutations in these genes specifically affect a subset of light-regulated genes. We propose that VVD, RCO-1, and RCO-3 participate in the mechanism responsible for transient gene photoactivation in *Neurospora*.

IIIp-14

MCMA, a transcriptional regulator from a MADS – box family participates in regulation of arginine catabolism in *Aspergillus nidulans*

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The arginine catabolism genes *agaA* and *otaA* are specifically induced by arginine. This induction depends on Zn2Cys6 transcriptional activator ARCA. We have shown that MCMA, a transcriptional regulator from a MADS-box family also participates in this process. In $mcmA_{I70A}$ mutant, the induced levels of arginase and ornithine transaminase activities are lower than in a wild type. We have isolated a cDNA clone of mcmA (Gene Bank Acc. No. AY957455). MCMA is a homologue of *Saccharomyces cerevisiae* Mcm1p transcriptional regulator. Similarly as the yeast protein, MCMA posses a MADS domain and glutamine rich region.

Identification of the XlnR regulon in *Aspergillus niger* by microarray analysis

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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*. 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 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.

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IIIp-16

Identification of differentially expressed genes in the filamentous fungus *Penicillium glabrum* submitted to heat stress conditions

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Penicillium glabrum is a filamentous fungi frequently involved in food spoilage of different products such as cereals, ham, fruit juices or mineral water. The different steps of an industrial food process represent numerous stress conditions (temperature, pH, a_w) for this microorganism whose growth and conidiation could be modified, affecting its contaminating ability. Beyond basic knowledge, study of stress effects on *P. glabrum* has an interest to improve the reliability of consumption limiting dates which are usually defined without considering stress.

Our research work aims to identify some specific and general stress molecular markers that could characterize the physiologic state (stress or not) of *P. glabrum.* The first experimental phase was devoted to study thermal stress effects on mycelium.

Thermal stress impacts on fungal growth was explored using Central Composite Design with 9 different experimental stress couples ''temperature-time''. Statistical analysis of our results produced a ''growth response surface'' from which **[40°C; 120 min]** could be considered as convenient thermal stress condition as growth was reduced without lethal effect.

Once stress temperature and duration were defined, a transcriptional study was performed using the Suppression Subtractive Hybridization method (SSH) in order to compare gene expression of *P. glabrum* when submitted or not to thermal stress **[40°C; 120 min]**. Two reciprocal experiments (forward and reverse) were performed and produced two subtracted cDNA libraries, forward and reverse, containing approximately 270 down-regulated and 200 up-regulated genes after this thermal stress. The analysis of both gene lists suggests that ribosomal proteins, transcription / translation proteins and respiratory chain proteins were partially or entirely repressed after this thermal stress. Some different transcription / translation proteins and few known stress markers (hsp 30, hsp 98/104, sod) were up-regulated in thermal stress conditions. In order to confirm those results, to observe the differential genome expression and identify some efficient stress molecular markers, a custom cDNA microarray, containing the whole libraries from SSH is about to be spotted.

Trans-sensing Mechanism in a Diploid Strain of *Aspergillus flavus*

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Aspergillus flavus is an asexual filamentous fungus that produces the toxic and carcinogenic compound aflatoxin. The parasexual cycle can be induced in this fungus for genetic analyses, including assessment of gene dominance in stable diploids. All known mutations in genes for aflatoxin biosynthesis are recessive in diploids except for *afl-1* in strain 649. Diploids between 649 and 86 (wild type) lack transcripts for the aflatoxin biosynthetic genes and fail to produce aflatoxin. We are characterizing this mutant to better understand the mechanism of this inhibition of aflatoxin biosynthesis. Loss of aflatoxin production in 649 is due to a 317 kb deletion that includes the aflatoxin gene cluster. In addition, this strain contains a 939 kb duplication. Failure to produce aflatoxin in 649 x 86 diploids does not appear to be due to a repressor of the transcriptional regulator AflR as diploids between 86 and a strain of 649 carrying ectopic copies of *aflR* produce aflatoxin. These data suggest that the location of *aflR* in the genome dictates whether it is functional in the 649 x WT diploid. One explanation for this is that some form of transvection or trans-sensing mechanism is preventing aflatoxin production in the diploid strains. Investigations are currently underway to characterize the possible trans-sensing phenomenon.

IIIp-18

Tryptophan's effects on aflatoxin biosynthesis and its regulation in *Aspergillus flavus*

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Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These compounds are toxic and carcinogenic. Many nutritional and environmental factors are known to affect aflatoxin formation. In order to better understand the molecular mechanisms that control or regulate aflatoxin production changes in aflatoxin biosynthesis in *A. flavus* and *A. parasiticus* grown in yeast extract sucrose media supplemented with 50 mM tryptophan was examined. *A. flavus* grown in the presence of 50 mM tryptophan was found to have significantly reduced aflatoxin B1 and B2 biosynthesis, but A. parasitiucs cultures had significantly increased B1 and G1 biosynthesis. To determine if the effects of tryptophan were due to decreased levels of biosynthesis, microarray analysis of the effects of tryptophan on regulation of aflatoxin biosynthesis was performed. Preliminary analysis by MeV (TIGR) revealed 1174 genes occurred in both *A. flavus* and *A. parasiticus*. Nine genes were found to be significant across the experiments by T-test. Further investigation of these candidate genes may identify potential regulators involved in aflatoxin biosynthesis.

Investigation of the role of the transcriptions factors Cre and Ace in gene regulation in *Talaromyces emersonii*

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Cellulose is the most abundant organic macromolecule on earth. Hydrolysis of cellulose to glucose is achieved by microorganisms, which produce a "complete" cellulase system. One such organism is *Talaromyces emersonii*, a moderately thermophilic fungus with generally regarded as safe (GRAS) status. Filamentous fungi play a key role in the degradation of cellulose and other plant cell wall polysaccharides. These microorganisms are critical for the recycling of carbon back into the ecosystem and thus, for the maintenance of the global carbon cycle. The use of fungal cellulases for the hydrolysis of plant biomass to sugars that can be fermented to ethanol has received much attention as the production of bio-ethanol represents an environmentally friendly alternative to the use of fossil fuels.

The environmental and biotechnological importance of these enzymes has provoked intensive study into the mechanisms by which they are regulated at the genetic level. Studies conducted on the regulation of cellulase gene expression in certain other fungal species, e.g. *Trichoderma reesei*, have established that certain cellulase promoters are among the strongest *cis*-acting elements reported to-date. Consequently there has been significant effort in identifying key regulators (*trans*-acting factors), such as the zinc-finger transcription factors, CRE (Catabolite repressor element) and ACE (Activator of cellulase expression), that bind to specific regions of cellulase promoters and regulate gene expression. In this work, we report on the studies aimed at deciphering the regulation of the expression of cellulase (and key hemicellulase) genes in *T. emersonii* and the role of CRE and ACE in this process. Through this research, key differences between the mechanisms of regulation in *T. emersonii* and *Trichoderma reesei* have been identified and we suggest that such differences may reflect strategies that have evolved to enable fungi to compete in nature.

IIIp-20

Regulation of aurofusarin gene cluster in *Fusarium graminearum*

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Fusarium graminearum and related species produces the red pigment aurofusarin. Aurofusarin is a homodimeric polyketid. Its synthesis is dependent on *pks12* [1,2], *aurJ*, *fmo* and *gip1* [1], all located in the 24 kb gene cluster [1].

The biosynthesis of the polyketide aurofusarin is regulated by multiple factors. A bioinformatic analysis of the aurofusarin gene cluster, has revealed two transcription factors (*aurR1* and *aurR2*) of the binuclear zinc cluster type (Cys_6Zn_2) [1]. *Agrobacterium* mediated transformation was used to replace these genes with *hygR*. The mutants have been analysed for morphological changes, by HPLC-UV and for expression of genes in the aurofusarin gene cluster by RT-PCR.

Replacement of *aurR1* results in a milky white mutant and aurofusarin is not detectable by HPLC. RT-PCR analysis shows that the lack of aurofusarin production is due to a lack of expression of *pks12*, *aurJ*, *fmo* and *gip1*. Replacement of *aurR2* results in a red mutant, with a growth rate similar to the wild type. HPLC analysis shows that the mutant produces less aurofusarin than the wild type, but RT-PCR analysis does not show any changes in expression of genes in the aurofusarin gene cluster.

Random mutagenesis has resulted in the identification of two additional genes (pr5.1 and pr35.1), located outside the aurofusarin gene cluster, being required for production of aurofusarin. The pr5.1 mutant is mutated in a single gene which is found in a number of pathogenic fungi. The strain has a white phenotype and does not produce aurofusarin, due to a lack of expression of PKS12 and additional PKS-genes. The pr35.1 mutant produces a novel yellow pigment. The lack of aurofusarin production is however not due to a lack of expression from the gene cluster. This indicates that pr35.1 is involved in regulation on a post-transcriptional or directly in the biosynthesis.

[1] Malz *et al.* 2005. Identification of a gene cluster responsible for the biosynthesis of aurofusarin in the *Fusarium graminearum* species complex. Fungal Genet. Biol. 42(5):420-433
[2] Kim *et al.* 2005. Putative polyketide synthase and laccase genes for biosynthesis of aurofusarin in *Gibberella zeae*. Appl. Environ. Microbiol. 71(4):1701-1708

Spliced variants of the transcription factor CLSTE12 display opposite roles in the pathogenicity of *Colletotrichum lindemuthianum*

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STE12-like proteins are transcription factors that regulate invasive growth in yeast and pathogenicity in parasitic fungi. Such proteins harbour a homeodomain involved in DNA binding. In filamentous fungi, an additional domain is found, containing two Cys_2 His_2 zinc fingers, whose role is still unknown. We isolated a *STE12*-like gene (*CLSTE12*) from the bean pathogen *Colletotrichum lindemuthianum*. Gene expression studies revealed that two mRNAs are produced during saprophytic growth, resulting from the alternative splicing of the third exon. The loss of this exon leads to the deletion of one zinc finger in the corresponding protein. During pathogenesis, only the mRNA encoding the full-length protein is detected. The function of both proteins was analyzed by heterologous expression in yeast. The full-length CLSTE12 protein is able to complement a yeast *ste12*Δ mutant by restoring invasive growth, whereas the expression of the truncated protein blocks invasive growth in the wild-type yeast. Accordingly, constitutive expression of the truncated protein in *C. lindemuthianum* strains has a negative effect on pathogenicity. Overall, these results suggest that the modification of the zinc finger domain through alternative splicing allows the production of a transcriptional repressor whereas the full-length protein acts as a transcriptional activator.

IIIp-22

Pathogenic development of *Ustilago maydis* is controlled by a network of transcriptional regulators

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In the phytopathogenic basidiomycete *Ustilago maydis*, the *b*- mating type locus, encoding two distinct homeodomain proteins bE and bW, represents the sole control authority for the decision between saprophytic growth and infection of the host plant. The heterodimeric complex formed by the bE and bW proteins is thought to achieve its function as a transcriptional regulator of pathogenicity genes either directly by binding to cis regulatory sequences or indirectly via *b*-dependent regulatory cascades.

To get a comprehensive view of the *b*-dependent processes, we have employed genome-wide DNA arrays to analyze changes in the transcription profile of haploid sporidia resulting from induced expression of an active b heterodimer. Among the more than 300 *b*-dependently regulated genes that were identified, we have focused so far on genes with potential regulatory functions. This has led to the identification of four novel pathogenicity genes: *rbf1*, *biz1* and *hdp2*, encoding two zinc finger and one homeodomain transcription factor, respectively, and *clp1*, encoding a protein with unknown function. Rbf1 is required for both filamentous growth as well as pathogenic development. DNA-array experiments revealed that this transcription factor is responsible for the regulation of a majority of the *b*-dependent genes, and thus has a central role within the *b*-regulatory network. *biz1* and *hdp2* are Rbf1 dependently induced. Both are required for plant penetration and have been shown to control distinct subsets of genes found to be activated during the infection process. Clp1 is thought to act as a modulator of the b-heterodimer required to release the b and Rbf1 dependent cell cycle arrest in order to allow proliferation in planta. *clp1* mutant strains are able to infect, but are blocked in development before the first cell division. Clp1 interacts with the bW and the Rbf1 protein. Furthermore b-dependent gene regulation is almost completely blocked by simultanous induction of *clp1*, although the expression of *b* itself is not affected. The emerging picture shows that the network of these regulatory proteins is used to control the expression of pathogenicity related proteins during infection and to synchronize cell cycle and cell division during in planta propagation.

Nuclear export of the transcription factor NirA is a regulatory checkpoint for nitrate induction in *Aspergillus nidulans*.

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NirA, the specific transcription factor of the nitrate assimilation pathway of *A. nidulans*, accumulates in the nucleus upon induction by nitrate. A nirA^{c}1 mutation leads to constitutive nuclear localisation, remodelling of chromatin and *in vivo* binding to a NirA UAS. The expression of the *niiA* and *niaD* genes (nitrate and nitrite reductase) is partially constitutive. Except for nuclear localisation, these phenotypes are dependent on an active AreA GATA factor. The nirA^{c}1 mutation maps in a putative nuclear export signal (NES). NirA interacts with the nuclear export factor KapK, which bridges an interaction with a protein of the nucleoporin-like family (NplA). These interactions are nearly abolished in NirA^{c}1 and in NirA proteins mutated in canonical leucine residues in the NirA NES. Leptomycin-sensitive *kapK*1 mutants are constitutive for NirA nuclear accumulation in the presence of the drug. This phenotype is not associated with a constitutively active NirA protein. The results are consistent with a model in which activation of NirA by nitrate would disrupt the interaction of NirA with the NplA/KapK nuclear export complex, thus resulting in nuclear retention, leading to AreA-facilited DNA binding of the NirA protein and subsequent chromatin remodelling and transcriptional activation.

IIIp-24

Normalization of specific mRNA quantification data in *Aspergillus niger*

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For optimization of cultivation processes of the filamentous fungus *Aspergillus niger* used as expression system for heterologous proteins, a thorough characterization of product formation is required. Therefore, the productivity of *A. niger* AB 1.13 for the model protein glucoamylase is examined with respect to the dependency on cultivation time and morphology. This includes the quantification of separate steps of the product pathway from transcription to the release of the protein into the culture broth. The mRNA specific for glucoamylase is quantified by real-time PCR. To correct the real-time data for variations, e.g. in sample preparation and enzymatic efficiencies, accurate normalization is necessary. Normalization against reference genes can compensate these varieties. However, validation of potential control genes is very important. Vandesompele *et al.* [2] showed that the conventional use of a single reference gene leads to relatively large errors. They validated the geometric mean of the expression level of multiple reference genes as an accurate normalization factor. This approach is based on the assumption that an increasing variation of the expression ratio of two reference genes means a decreasing expression stability.

Following the proposed validation method of Vandesompele *et al.* [2], 10 housekeeping genes for *A. niger* were selected and their expression patterns determined in the study presented. First of all, a small number of samples were examined. The 6 genes that gave the best results were chosen for further experiments. For these genes, mRNA transcription levels of samples from various stages of 6 different cultivations were evaluated by comparing the X_{SDM} value (SDM: second derivative maximum) determined by real-time PCR. This value is inversely correlated with the amount of template present at the beginning of the PCR reaction [1]. By determining the average pair-wise variation between a particular reference gene and all other control genes, the gene stability M was calculated. A ranking of the examined genes according to their expression stability was established by stepwise exclusion of the least stable gene (highest M value) and recalculation of the M values. A normalization factor was calculated that is based on the geometric mean of the expression level of the most stable reference genes. Furthermore, it was calculated how many reference genes should be used according to Vandesompele *et al.* [2].

As a result, the 3 most stable genes (*ß-act*, *cox5* and *sarA*) were selected as proper reference genes. They are supposed not to be co-regulated and can therefore be used for normalization of specific mRNA quantification data in *Aspergillus niger*.

References:

[1] Tichopad A, Dilger M, Schwarz G, Pfaffl W. 2003. Standardized determination of real time PCR efficiency from a single reaction set-up. Nucleic Acids Res 13(20):1-6.
[2] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. 2002. Accurate normalization of Real-Time quantitative RT-PCR by geometric averaging of multiple internal control genes. Genome Biol 3:34.

Analysing gene expression in submerged cultivations of *Aspergillus niger*

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Filamentous fungi such as *Aspergillus niger* are widely used in biological production processes. Besides traditional homologous product formation the production of heterologous proteins is becoming more and more important. Therefore, *A. niger* provides several advantages as a production strain. The fungus offers an efficient system for protein secretion and unlike bacterial hosts it is able to carry out post-translational modification of proteins. In addition, *A. niger* shows two different types of morphology in submerged cultures -pelleted or freely dispersed mycelia. Depending on the desired product, the optimal morphology has to be determined from case to case.

Especially in production processes of heterologous proteins, yields are 100 to 1000 fold below those of homologous ones. Reasons for that are manifold and can be found in the complete production path from gene to product. In the work presented, an approach that includes extensive bioprocess monitoring based on the quantification of the specific transcription activity for the desired protein is shown. Therefore, detection of bottlenecks limiting the bioprocess already on the transcriptional level is possible. Currently, homologous protein production is investigated with *A. niger* strain AB 1.13 considering glucoamylase as model product. The glucoamylase gene (*glaA*) is regulated by a strong inducible promoter.

The above mentioned monitoring strategy of bioprocesses on the transcriptional level is based on three molecular biological methods:

The first approach is the quantification of mRNA specific for the desired product by means of real time polymerase chain reaction (real time PCR). Thereby, effects of different process parameters and influences of varying carbon sources and concentrations are tested. Thus insights into the induction behaviour of the *glaA* promoter are expected. These conclusions are of great importance for subsequent production of heterologous proteins under the control of the *glaA* promoter.

Furthermore, detection of product specific cDNAs and monitoring process related gene transcription is performed with a DNA array system.

Finally, an *in situ* hybridization based method is intended to show the activity of gene transcription across pellet sections.

The above mentioned methods should allow a detailed insight into the protein producing fungal cell and a thorough characterisation of bioprocesses with respect to transcript formation.

IIIp-26

RpdA, a classical histone deacetylase, is essential for viability of *Aspergillus nidulans*

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In eukaryotic cells DNA and histone proteins constitute the nucleosome core particle, which is the fundamental structural and organizational unit for packing DNA into the nucleus. Moreover, nucleosomal chromatin is a highly dynamic structure that represents an important regulatory factor affecting nuclear processes such as DNA replication, recombination, DNA repair and transcription by tuning the accessibility of DNA for various factors. Crystallographic analysis revealed that the free amino terminal tails of the core histones protrude from the core octamer and contain conserved amino acid residues that are subject for posttranslational modifications like acetylation, methylation and phosphorylation. The most prominent modification is the acetylation, where an acetyl group from acetyl-CoA is linked to the epsilon amino group of highly conserved lysine residues by histone acetyltransferases (HATs). This reaction is reversed by the action of histone deacetylases (HDACs). The identification of HATs as transcriptional co-activators and HDACs as transcriptional (co)-repressors has confirmed the importance of these enzymes for gene regulatory processes. Today the classical HDACs are classified into several classes and filamentous fungi contain members of all of these groups. Recently, we have started to delete representatives of each HDAC class in *A. nidulans*. Since deletion of RpdA, a class 1 HDAC of the fungus, failed, we generated rpdA-knock-down strains. Here we report the down regulation of RpdA in *A. nidulans* using different promoter systems and demonstrate that repression of RpdA leads to a remarkable deficiency in viability of the fungus. Further examinations revealed that an exceptional C-terminal tail of RpdA comprises distinct motifs that cannot be deleted without affecting the functionality of RpdA *in vivo*. Since these C-terminal motifs are absent in RpdA-type HDACs of other eukaryotes, they may display ideal targets for HDAC-inhibitors specific for filamentous fungi.

Identification of genes whose expression is dependent on the predicted transcription factor CON7p in the rice blast fungus *Magnaporthe grisea*

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We generated a library of ~6000 insertional mutants in *Magnaporthe grisea* using *Agrobacterium* mediated transformation. These transformants were screened for pathogenicity toward rice and barley and ten non-pathogenic mutants were recovered. A 'step-down' PCR based approach was used to successfully recover DNA from the site of the insertion in eight mutants. Sequencing of the sequences recovered revealed that in one of these mutants the insertion had occurred within the promoter of the *CON7* gene. This gene is predicted to encode a transcription factor and has previously been shown to be essential in *M. grisea* for appressorium formation and growth *in planta*. We have identified several potentially CON7p regulated genes using microarray based global gene expression analysis. This analysis has revealed that the expression of genes coding for the pathogenicity factor PTH11p. and several other PTH11p related predicted G-protein coupled receptor like proteins are CON7 dependant. A second group of genes, whose expression is partially or fully dependant on CON7p, was also identified through microarray analysis. These genes are predicted to encode factors which might play a role in the remodelling of the cell wall, either through the degradation and re-synthesis of glucan and chitin during appressorium formation or by their predicted association with the cell wall. Among this group of genes is *CBP1*, which is predicted to encode a chitin binding protein and has previously been reported to be required for full levels of appressorium formation on hydrophobic surfaces. Microscopy and biochemical analyses have been used to test the view that the product of the CON7 gene influences the remodelling of the cell wall. Recent progress will be presented.

IIIp-28

Functional analysis of protein arginine methyltransferases in *Aspergillus nidulans*

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Our research interests focus on protein arginine methyltransferases (PRMTs) which are part of the epigenetic gene regulation machinery of eukaryotic organisms. PRMTs catalyze the transfer of methyl groups of S-adenosylmethionine to arginine residues of a variety of proteins including core histones. Histone methylation is known to regulate gene expression and contribute to the histone code. The diverse functions of PRMTs, mainly demonstrated for higher eukaryotes, implicate a role in transcriptional regulation, RNA processing (transport, splicing, translation), signal transduction, and DNA repair. We have recently identified and cloned 3 *Aspergillus nidulans* PRMTs, termed RmtA, RmtB, and RmtC, all of which exhibit enzymatic activity *in vitro*, when expressed as GST-fusion proteins in *E. coli*. Based on sequence alignments and the unique substrate specificity, RmtB most likely represents a novel member of the PRMT family, which is only present in filamentous fungi. Furthermore, we show that *Aspergillus* histone H4 is methylated at arginine 3 *in vivo*. To analyze specific functions of *A. nidulans* PRMTs we have disrupted *rmtA*, *rmtB*, and *rmtC* via gene displacement using *argB* as selection marker. Initial results indicate that the *ΔrmtA* mutant exhibits growth retardation under oxidative-stress conditions compared to *wt* strains. In order to elucidate the mechanism underlying this phenotype we perform Northern analysis of genes involved in the detoxification of reactive oxygen species (eg *catA, catB, sodA, sodB*). We further conduct the biochemical characterization of mutant strains which includes the purification of endogenous enzyme form(s) and the analysis of substrate and site-specificity by fluorography and site-specific antibodies, respectively. The long-term goal of these studies is to understand the role of protein arginine methylation in filamentous fungi, since these organisms are important in biotechnology and also cause human diseases. This work was supported by a grant of the Tiroler Wissenschaftsfonds to I. B.

Identification of *Aspergillus nidulans* genes regulated by the transcription factor, NsdD

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Sexual differentiation of *Aspergillus nidulans* is regulated by NsdD (Never in Sexual Development), which encodes a GATA-type transcription factor with the type IVb zinc finger DNA-binding domain. Although expression of the *nsdD* is detected in the early phase of vegetative growth, the level increased as sexual development proceeded and expressed in high level after 30h of sexual induction (SI-30). To identify genes regulated by the NsdD, the DEG (Differentially Expressed Gene) analysis were performed using the RNA preparations from cells of various stages with wild type and *nsdD* deletion background. DEG analysis revealed at least 4 signatures with vegetative cells, 17 with SI-0 cells, and 11 with SI-30 cells. PCR-cloning and sequence analysis of the 11 DEG signatures of the SI-30 revealed one known gene and 12 unknown genes, of which expression might be regulated by the NsdD.

To confirm the results of the DEGs of SI-30, RNAs were isolated various stages from wild type strain and *nsdD* deletion strain and subjected to real time PCR using specific primers for DEGs. As a result, up-regulation genes by *nsdD* are AN0501.2 and AN8466.2, down-regulation gene by *nsdD* was AN1630.2. By BLAST searching, AN0501.2's function conjectured as a maltose permease according to in *A. fumigatus* and AN8466.2's function guessed for chitin binding protein by means of in *Pichia acaciae*. Also AN1630.2's function guessed for mitochondrial cytochrome permease in *Cryptococcus neoformans* by BLAST searching.

IIIp-30

Exploitation of "omics" tools to study protease regulation in *Aspergillus niger*

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The *A.niger* genome-sequencing project was the basis for developing 'omics' techniques such as transcriptomics, proteomics and metabolomics and the trigger for organizing a firm BioIT infrastructure within DSM. Having these technologies up and running, challenging questions can be formulated such as: Can bottlenecks in (heterologous) protein production be identified or can fermentation processes be improved using the data obtained by 'omics' techniques?

A. niger is well known for its capability to secret a vast amount of homologous proteins, however, the production of heterologous proteins was shown to be hampered by proteolytic degradation. Using the *A. niger* genome information around 200 protease-encoding genes have been identified. It is well known that environmental factors such as C-source, N-source and pH can effect the expression of proteases. The regulation can be often very complex and on various levels (transcriptional and post-translational). Furthermore, only one protease specific transcription factor was described thusfar.

In the enzyme production processes, fermentation conditions are set before hand to allow producing as many as possible different products using a standardized protocol. Therefore production properties of a production strain should be improved in such a way that they optimally fit a uniform production concept.

Data on protease expression in wt and in enzyme producing *A. niger* strains will be discussed. All the experiments were performed in fermentors under strictly controlled fed-batch culture conditions. Furthermore insight to the PrtT function in regulation of proteases in *A. niger* will be disclosed.

Heterochromatin protein HepA regulates secondary metabolism gene expression in the filamentous fungus *Aspergillus nidulans*

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Heterochromatin is a repressive chromatin involved in gene silencing phenomena. The genes placed in the neighbourhood of the heterochromatin are susceptible to silencing due to the extension in cis of the heterochromatin structure.

One of the principal components of the heterochromatin is the heterochromatin protein 1, HP1. *Su[var]*2–5, the coding gene, was isolated as a dominant suppressor of Position effect variegation in Drosophila melanogaster. The deletion of the gene coding HP1 homologous causes different phenotypes in all organisms studied so far, i.e. growth defects and lost of DNA methylation in Neurospora crassa, defects in mitotic segregation of chromosomes in Sacharomyces pombe or developmental abnormalities in Caenorhabditis elegans.

We identified, cloned and deleted the unique HP1 homologous gene, *hepA*, in the filamentous fungus *A. nidulans*. Surprisingly, the deletion of *hepA* does not lead to any evident phenotype. DNA microarray analysis was performed under standard growth conditions (18 hours at 37°C, ammonia and glucose containing media) comparing *ΔhepA* to an isogenic wild type strain in order to identify genes to affected by this deletion.

In this screen several ORFs were identified being up and downregulated. Among the upregulated genes we found ORFs related to the secondary metabolism, including genes belonging to the Sterigmatocystin (ST) biosynthetic cluster.

Further studies revealed that deletion of *ΔhepA* strain results in precocious ST gene expression and ST production. These results are consistent with the hypothesis that *HepA* is involved in the regulation of secondary metabolism.

IIIp-32

Functional analysis of the subterminal repeats of the transposon *Restless*

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Restless is the first identified fungal member of the widely distributed *hAT* transposon family (1,2). It has been proven to be active in its homologous host *Tolypocladium inflatum* (1) and has also shown exision activity in a heterologous host (3), the filamentous fungus *Neurospora crassa*. The *Restless* transcript shows alternative splicing (1) - a process regarded as rare in fungi - which seems to have influence on *Restless* activity (4). As *Restless* has already been used for gene tagging in *T. inflatum* (2), and as it is at least partially active in a heterologous host *Restless* is of interest as a genetic tool. So far the *Restless* transposase, the molecule that catalyzes *Restless* transposition, has not been analysed. So effords were made to establish a heterologous expression system in *N. crassa* by strongly expressing the transposase using the *ccg*1 promotor (5). Protein preparations will be used for biochemical experiments. Band-shift experiments were done with transposase overexpressing and non-expressing strains as well as with the homologous host *T. inflatum*.

(1) Kempken F, Kück U (1996) Mol Cell Biol 16:6563-6572,
(2) Kempken F, Kück U (2000) Mol Gen Genet 263:302-308,
(3) Windhofer F, Hauck K, Catcheside DEA, Kück U, Kempken F (2002) Fungal Genet Biol 35:171-182,
(4) Kempken F, Windhofer F (2004) Curr Genet 46:59-65, (5) McNally MT, Free JF (1988) Curr Genet 14:545-551

Reducing argininosuccinate lyase transcript levels by RNAi in *Agaricus bisporus*

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Argininosuccinate lyase (ASL) is a key enzyme of the urea cycle converting argininosuccinate to fumarate and arginine. In *Agaricus bisporus* fruitbodies the levels of both ASL transcript and urea increase in the mushroom after harvest. This stimulated urea cycle activity is hypothesized to have the role for processing amino acids (released from protein degradation) i.e. directing the carbon for oxidation via the citric acid cycle and sequestering nitrogen to urea which acts as an osmoticum driving cell growth and fruitbody expansion postharvest. This hypothesis was tested down-regulating transcript levels of ASL. The experiments conducted describe the first example of the use of RNAi technology in *A. bisporus*. A RNAi vector was constructed for the use in *A. bisporus*. The fungus was transformed with the ASL-specific RNAi vector using *Agrobacterium tumefaciens*-mediated transformation of mushroom gill tissue. Transformants were screened for reduced ASL gene expression and for postharvest quality attributes. Quantitative RT-PCR data showed 87% reduction in ASL transcripts levels in one transformed strain 2 days after fruitbody harvest. For this strain the RNAi vector was observed to have integrated three times into the *Agaricus bisporus* genome. Phenotypic examination of the transformants revealed a reduced rate of mushroom growth during postharvest storage. Urea measurements were also made to assess the effect of down-regulation on the urea cycle.

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Gene silencing technologies in the basidiomycetes *Coprinus cinereus* and *Agaricus bisporus*

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Post Transcriptional Gene Silencing (PTGS) methods such as RNAi have emerged as exciting new technologies in the repression of gene expression. In this report, we describe the development of gene silencing technologies within *Agaricus bisporus* and *Coprinus cinereus*.

Using *C. cinereus* we describe the utilization of green fluorescent protein (GFP) in the evaluation of three different silencing cassettes, both in terms of ease of construction and in silencing capabilities. All three constructs, untranslatable sense orientated, antisense and hairpin, were transformed into a GFP expressing *C. cinereus* strain. Lines exhibiting complete silencing, and strains exhibiting partial repression of GFP were recovered from transformations with all three cassettes.

In *A. bisporus* we chose the endogenous serine protease gene as a targeted for silencing. Serine protease has been implicated in post-harvest and age-related senescence of sporophores. On harvesting, mushrooms degenerate rapidly to give browned caps and loss of texture in the fruit body, and such problems can dramatically reduce sale ability of the mushrooms. Silenced lines have been generated and these show a range of biological effects depending on the degree of silencing. Data on these studies will be presented. Suppression of genes involved in these pathways could increase mushroom shelf-life and profitability for mushroom growers, or help to further elucidate the complex biochemical pathways involved in post-harvest degradation.

Gene silencing would appear to be an effective tool for the study of gene function in these fungi, and is of particular use where the dikaryotic or diploid nature of species precludes effective gene disruption.

A histone deacetylase acts as a negative regulator of secondary metabolism in *Aspergillus nidulans*

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Secondary metabolites produced by fungi include medically and economically important molecules such as antibiotics and toxins. Understanding the pathways that regulate production of these metabolites may allow this production to be manipulated in ways that benefit a variety of efforts such as the development of new medicines and the control of crop diseases. In the model filamentous fungus *Aspergillus nidulans*, the gene *laeA* is required for production of secondary metabolites including the mycotoxin sterigmatocystin (ST), and the antibiotic penicillin (PN). Deletion of this gene (delta *laeA*) prevents the expression of gene clusters responsible for producing these compounds. We hypothesize that regulation of such gene clusters occurs through fluctuations in chromatin structure between repressive heterochromatin and active euchromatin. Because the acetylation state of histone proteins has been shown to play a key role in determining chromatin structure, we examined the effects that loss of a histone deacetylase (HDAC) has on the secondary metabolism of *A. nidulans*. We show that replacement of the HDAC gene *hdaA* with a marker gene results in overexpression of the ST and PN gene clusters and a corresponding increase in the production of these compounds. This increase in secondary metabolite production is enhanced in a strain mutated in three HDAC genes (*hdaA*, *hosB* and *sirA*). Furthermore, combination of *hdaA* and *laeA* mutations results in partial remediation of the delta *laeA* phenotype. These results suggest a role for chromatin structure in the regulation of *A. nidulans* secondary metabolism.