III
Transcriptional Regulation

Chair:

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Regulation by blue light and heat shock of gene transcription in *Phycomyces* proteins: proteins required for photoinduction and identification of a novel mechanism for adaptation to light

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The gene hspA for the heat-shock protein HSP100 is induced by blue light and heat shock in the zygomycete fungus *Phycomyces blakesleeanus*. HSP100 proteins are ATPases involved in the tolerance to high temperatures, proteolysis, and protein disaggregation. The activation of hspA by light in *Phycomyces* could be required to deal with damaged proteins after an exposure to light. HSP100 could also play a role in the phototransduction pathway mediating the disaggregation of regulatory elements. We have cloned and characterized 1.9 kb of hspA upstream DNA sequence. The hspA promoter contains six heat-shock elements that are presumably involved in the activation of hspA after a heat-shock. In addition, the hspA promoter contains four sequences that are also present in the promoter of other light-regulated genes, and are probably binding sites for light-regulated transcription factors. We have identified the gene products required for hspA photoactivation and found that many of them are also required for mycelial photoresponses, a suggestion for a common signal transduction pathway. The photoactivation of hspA requires the protein MADA, with a Zn finger and a flavin-binding site, the madB gene product, which is also required for all the *Phycomyces* photoresponses, and a regular supply of beta-carotene. None of the madA alleles that result in mutations in the flavin-binding site or that lacks the Zn finger allow hspA photoactivation suggesting that MADA is a photoreceptor and a transcription factor responsible for gene photoactivation probably through the formation of a complex with a protein similar to WC-2 from *Neurospora*. The activation of hspA after blue light-exposure or a heat shock is transient. The adaptation of hspA photoactivation is not the result of photoreceptor desensitization, but the result of a novel mechanism causing a light-dependent blockage of gene transcription. The light-dependent blockage of gene transcription does not prevent the activation of hspA by a heat shock and may be caused by the depletion of the MADA transcription factor from the hspA promoter. Our results have allowed us to propose a model for the regulation of hspA transcription by blue light and heat shock.

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The GATA factor AreA regulates localization and *in vivo* binding site occupancy of the nitrate activator NirA

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The GATA factor AreA is a wide-domain regulator in *Aspergillus nidulans* with transcriptional activation and chromatin remodelling functions. AreA interacts with the nitrate-specific Zn(2)-C(6) cluster protein NirA and both proteins cooperate to synergistically activate nitrate-responsive genes. We have previously established that NirA *in vivo* DNA binding site occupancy is AreA dependent and in this report we provide a mechanistic explanation for our previous findings. We now show that AreA regulates NirA at two levels: (i) through the regulation of nitrate transporters, AreA affects indirectly the subcellular distribution of NirA which rapidly accumulates in the nucleus following induction; (ii) AreA directly stimulates NirA *in vivo* target DNA occupancy and does not act indirectly by chromatin remodelling. Simultaneous overexpression of NirA and the nitrate transporter CrnA bypasses the AreA requirement for NirA binding, permits utilization of nitrate and nitrite as sole N-sources in an *areA* null strain and leads to an AreA-independent nucleosome loss of positioning.

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mRNA turnover and gene regulation in *Aspergillus nidulans*

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The stability of any given transcript plays a key role in determining the level of the mRNA and subsequent gene expression. Transcript instability permits a cell to adapt its pattern of gene expression continuously to changing physiological needs, and therefore providing a cell with flexibility in effecting rapid change. Very stable transcripts are optimal for achieving high expression levels. As a consequence RNA stability varies significantly between genes, and in some cases this is directly regulated as a means of controlling gene expression. We have previously demonstrated the differential rate of decay for the *areA* transcript *(eg* Morozov*et al., 2001 Mol Microbiol* 42:269-277), which encodes the major transcription factor responsible for mediating nitrogen metabolite repression. The *areA* transcript is destabilised by the presence of intracellular Gln, a signal of nitrogen sufficiency. The same mechanism is important to the expression of various structural genes involved in nitrogen metabolism, including *niiA* and *niaD*. Intriguingly, some of these genes are also subject to an additional regulatory mechanism which acts at the level of RNA stability, stabilising the transcripts in the presence of the respective protein's substrates. Based on *in silico* analysis of the utranslated regions of *Aspergillus* genes, the presence of a number of conserved motifs suggests a large number of genes are likely to be regulated at the level of RNA stability. Most notably, many are genes that include conserved elements likely to be involved in interactions with the Pumilio-homology domain (Puf) RNA-binding proteins (Galagan, *et al.*, (2005) *Nature* 438: 1105-1115). In other organisms, including *Saccharomyces cerevisiae* and *Drosophila melanogaster,* these proteins have been shown to coordinate expression of specific groups of genes. Utilising a combination of approaches we have identified a number of genes of interest, and we are currently investigating their function. Genomic analysis has led to the identification of putative orthologues of yeast proteins involved in mRNA deadenylation and turnover. Affinity purification, using RNA sequences known to be critical for regulated degradation, has revealed a number of RNA-binding proteins. Finally, proteomic analysis, focused on nitrogen metabolism and the proteins subject to regulation by AreA, has led to the identification of additional candidates which may play a role in the regulated stability of genes involved in nitrogen metabolism. We have selected a number of these proteins for functional characterisation and, utilising homologous gene replacement, are assessing their various roles in *A. nidulans*.

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Role of sumoylation in the function of the transcription activator AreA

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The *Aspergillus nidulans areA* gene encodes a GATA transcription activator of genes involved in nitrogen metabolism. The activity of AreA is altered according to the availability of environmental nitrogen sources such that *areA*-dependent genes are expressed at a low level in the presence of a favourable nitrogen source such as ammonium, and at a high level in the presence of a poor nitrogen source such as alanine. For at least some *areA*-regulated genes, AreA-dependent expression is further elevated during nitrogen starvation. AreA expression and activity are regulated by autogenous transcriptional control, differential stability of the *areA* mRNA, interaction of AreA with the co-repressor NmrA and by regulated nuclear accumulation of AreA. We have investigated the role of post-translational modification on the function of AreA. We have shown that AreA is multiply phosphorylated and its phosphorylation status differs under nitrogen-sufficient, nitrogen-limiting and nitrogen-starvation conditions. AreA contains a putative attachment site for a Small Ubiquitin-like Modifier (SUMO). We have altered the putative SUMO-conjugating lysine (K713) to arginine, which is predicted to prevent covalent binding to the SUMO peptide. The AreA-K713R protein does not accumulate in the nucleus during nitrogen starvation. We have examined the effects on AreA function of the SUMO peptide encoded by the *smoA* gene. Deletion of *smoA* confers reduced growth and prevents nuclear accumulation of AreA. However, overexpression of SmoA from the *xylP* promoter does not affect growth. We are assessing whether SmoA overexpression affects nuclear accumulation and whether AreA is a direct target of sumoylation. Neither *areA*-K713R nor *smoA* deletion mutations affect the elevated *areA*-dependent expression of genes in response to nitrogen starvation. Therefore nuclear accumulation of AreA and elevated *areA*-dependent gene expression in response to nitrogen starvation are separable.

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The nitrogen regulation network in *Fusarium fujikuroi* - from AreA to TOR

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In *F. fujikuroi*, the biosynthesis of gibberellins (GAs) and bikaverin, both nitrogen-free metabolites, is under control of AreA-mediated nitrogen metabolite repression. However, the signalling components acting upstream of AreA,and regulatory proteins, affecting AreA activity by protein-protein interactions, are still unknown. Searching for other components of the nitrogen regulation network, we investigated the role of the glutamine synthetase (GS) as a potential candidate. In contrast to our expectation, loss of function mutants ΔglnA) were unable to express GA- and bikaverin- biosynthetic genes despite the strong starvation due to the depletion of the intracellular glutamine pool. Differential hybridization of macroarrays with cDNA from the wild-type and ΔglnA mutant as probes revealed a set of genes, dramatically up- or down-regulated in the mutant. Among them are genes encoding nitrogen starvation-response proteins, polyubiquitins, the homologue of the yeast multiprotein bridging factor MBF1, and proteins involved in translation control and ribosome biogenesis[2]. Glutamine can overcome the up-and down-regulation of most but not all genes. This finding and the fact that an intact GS protein is essential for the expression of GA-and bikaverin biosynthetic genes led us to the assumption that the GS is probably an important regulatory protein which might be involved in protein-protein interactions with other components of the nitrogen regulation system. Beside GS, the TOR kinase was shown to be a player of this regulation network. Rapamycin treatment resulted in partial de- repression of GA-and bikaverin biosynthesis genes with glutamine and ammonium in the medium. Interestingly, a set of genes was found to be under control of both, TOR and GS. However, genes which were down-regulated in the *glnA* mutant were up-regulated by rapamycin treatment. In addition, MepB, one of three ammonium permeases, seems to be involved in sensing and transduction of the nitrogen signal. ΔmepB mutants show severe growth defect on low ammonium oncentrations suggesting that ammonium uptake occurs mainly by MepB. The GA- and bikaverin biosynthetic genes, normally repressed by ammonium, are de-repressed in mepB mutants under the same conditions. We suggest a model of interaction of the so far identified components of the nitrogen regulation network in *F. fujikuroi*.

[1] Mihlan, M., Homann, V., Liu, T., Tudzynski, B.(2003) Mol. Microbiol., 47: 975.
[2] Teichert, S., Schönig, B., Richter, S. Tudzynski, B. (2004) Mol. Microbiol: 53: 1661.

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Two transcriptional regulators, AraR and XlnR, control growth of *Aspergillus niger* on L-arabinose and D-xylose

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The pentose catabolic pathway enables fungi to use L-arabinose and D-xylose as carbon sources. L-arabinose is converted to xylitol by L-arabinose reductase (ArdA), L-arabitol dehydrogenase (LadA) and L-xylulose reductase (LxrA), while D-xylose is converted in a single step to xylitol by D-xylose reductase (XyrA). Xylitol is then converted by xylitol dehydrogenase (XdhA) and D-xylulose kinase (XkiA) to D-xylulose 5-phosphate that enters the pentose phosphate pathway. D-Xylose and L-arabinose catabolism thus share common steps. The xylanolytic regulator XlnR is involved in the utilisation of D-xylose and indications for a L-arabinose responsive activator have been described.

We identified the L-arabinose responsive transcriptional activator that controls L-arabinose catabolism. This gene is a member of the GAL4-family of transcriptional regulators, which also includes XlnR. The *araR* disruptant had significantly reduced growth on L-arabinose, but not on D-xylose. The activity of extracellular L-arabinose releasing enzymes and expression of the corresponding genes was reduced in this strain. Moreover, activity of all enzymes involved in L-arabinose catabolism was strongly decreased, which was accompanied by a reduced expression of the encoding genes. The *xlnR* disruptant demonstrated reduced growth on D-xylose but not on L-arabinose and only had a strong effect on the expression of *xyrA*, encoding D-xylose reductase. The expression of *xdhA* and *xkiA* was only slightly decreased. This demonstrates that both AraR and XlnR regulate the shared steps in L-arabinose and D-xylose catabolism.

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The key player of the hydrolytic enzyme system in *Trichoderma reesei* - regulation of and by Xyr1

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In nature, cycling of carbon is of great importance to living systems. Xylans are heteropolysaccarides with a backbone of β-1,4-linked xylopyranosyl units which compose 20 - 35 % of the approximate 830 Gt of annually formed renewable plant biomass. Enzymes capable of degrading the xylan backbone of *Hypocrea jecorina* (anamorph *Trichoderma reesei*) have received strong attention because of their application in feed and paper industry. Thus, the filamentous ascomycete *H. jecorina* is an industrial important organism not only for production of xylanases but also of cellulolytic enzymes.

We have recently demonstrated that Xyr1 (Xylanase regulator 1) plays an essential role in transcriptional regulation of *xyn1* gene expression. In the presented study we will show that expression of the major cellulolytic and xylanolytic enzymes of *H. jecorina* such as XYNI, XYNII, CBHI, CBHII and EGI are governed by Xyr1. The mode of regulation of the respective genes via Xyr1 is neither influenced by the substances mediating induction nor the level of gene expression (basal, derepressed, induced). Furthermore, we will demonstrate that Xyr1 takes part in control of the fungal xylose pathway in particular in the regulation of the xylose reductase.

Transcription of *xyr1* is strictly under control of the carbon catabolite repressor Cre1. Consequently, the repression of *xyn1* gene expression is caused by a Cre1 double lock mechanism (functional Cre1-elements in the *xyn1*- and *xyr1*-promoters). In addition to this wide domain regulation a direct competition of Xyr1 with Ace1 for the essential binding element in the *xyn1*-promotor completes the *xyn1* shut-off.

Nevertheless *xyn1* is a repression/derepression and induction dependent system, transcription of its main *in trans* acting factor Xyr1 is not controlled by induction specific signals, but only dependent on carbon catabolite repression.

Moreover, we can demonstrate that no *de novo* synthesis of Xyr1 is required for mediating initial induction of *xyn1* expression. Evidence for a post-translational modification changing Xyr1 into its transactivating form will be provided.