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# Amino acid acquisition, cross-pathway control, and virulence in *Aspergillus*

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Supply of all amino acids required for translation is crucial for the synthesis of new proteins. Fungal amino acid biosynthesis has to be coordinated with amino acid uptake as well as protein degradation. A global regulator that connects amino acid biosynthesis and developmental programs is the transcription factor CpcA/Gcn4p. This transcriptional activator is conserved within the fungal kingdom and the cellular levels of this protein are carefully regulated. Deletion of the encoding *cpcA* gene in the opportunistic pathogen *Aspergillus fumigatus* results in impaired virulence in immuno-compromised mice, suggesting a role of the cross-pathway control system in fungal pathogenicity.

**Keywords** amino acid supply, cross-pathway control, CpcA/Gcn4p

## Introduction

Regulation of metabolism and the coordination of developmental programs are interwoven processes in fungi. The metabolic and developmental response to a changing environment requires the transcription of appropriate genes and, subsequently, translation of the newly synthesized mRNAs. Amino acids are substrates for translation and therefore necessary for fungal growth and development in reaction to an external stimulus. Amino acids are either: (i) taken up from the environment by transport processes, or (ii) synthesized from precursors, which are derivatives of the carbon and nitrogen primary metabolism, or (iii) result from degradation of proteins which are no longer required under specific conditions. All three processes have to be coordinated and are carefully regulated in fungi (reviewed in [1]). Uptake of amino acids in fungi includes a sophisticated system of amino acid permeases connected to sensing systems localized in the plasma membrane. Most insight was gained from studies in the budding yeast *Saccharomyces cerevisiae*, where the Ssy1p-Ptr3p-Ssy5p (SPS) system has been identified as amino acid sensing site [2]. The SPS sensor system seems not to be conserved in filamentous fungi, and therefore it is yet an open question how filamentous fungi monitor the amino acids that are available from the environment.

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## The cross-pathway control of amino acid biosynthesis

Regulation of amino acid biosynthesis includes the transcriptional and post-transcriptional control of genes encoding biosynthetic enzymes or the regulation of enzymatic activities. In numerous yeasts as well as filamentous fungi, an imbalanced amino acid diet results in amino acid starvation and activates a complex genetic network including a signal transduction cascade that determines the cellular levels of the transcriptional activator CpcA/Gcn4p. This genetic network, which has been named 'cross-pathway control' (*cpc*) in filamentous fungi and 'general amino acid control' (*gc*) in yeast, coordinately regulates expression of hundreds of genes involved in numerous biosynthetic pathways [3]. Additional pathway-specific transcriptional activation mechanisms also exist in fungi but are restricted to a limited number of amino acids.

Comparative evaluation of the recently published genome sequences from three *Aspergillus* species – *A. nidulans*, *A. fumigatus*, and *A. oryzae* – has revealed a high degree of conservation between the fungal cross-pathway control systems. Scanning the three genomes



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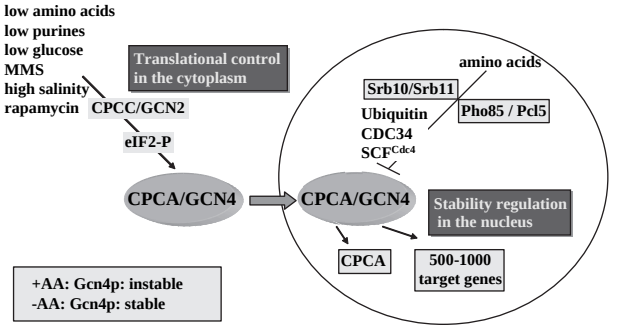


**Fig. 1** Regulation of CpcA/Gcn4p in the fungal cell. Simplified overview is given of: (i) the cytoplasmic translational regulation of CpcA/Gcn4p synthesis via controlling initiation of translation, (ii) the transcriptional auto-regulation in the nucleus, which seems to be restricted to filamentous fungi, and (iii) the nuclear regulation of CpcA/Gcn4p protein stability that has yet only been demonstrated in yeasts. Gene designation follows the yeast nomenclature. All genes for components of a corresponding CpcA/Gcn4p degradation pathway are present in the *A. fumigatus* genome. The only genes which have been analysed in *A.f.* are CpcA [9] and CpcC [unpublished results].

for high-scoring sequences that represent functional motifs found the palindromic 5′-TGASTCA-3′ site that is bound by the CpcA protein to be enriched upstream of genes involved in amino acid transport and metabolism, and translation [4]. Essential components of the cross-pathway control system required for signal transduction, translational regulation, or creation of the transcriptional read-out were found among the list of automatically annotated genes (our unpublished data), and future studies will have to address the regulatory interplay of transcriptional auto-regulation and translational de-repression that results in appropriate levels of the transcriptional activator protein CpcA.

In addition to its role as metabolic transcription factor, CpcA/Gcn4p interrelates metabolism and development. In the yeast *S. cerevisiae*, activation of Gcn4p supports cell-cell and cell-surface adhesion [5,6], whereas in the pathogenic dimorphic yeast *C. albicans* Gcn4p affects filament formation [7].

In the model *Aspergillus* species *A. nidulans*, activation of the cross-pathway control impairs the developmental program of fruiting bodies formation [8]. *A. nidulans* is homothallic and therefore able to form fruitbodies, named cleistothecia, either by mating of two strains or by selfing in the absence of a partner. The three-dimensional *A. nidulans* cleistothecium is the most complicated structure this fungus is able to form. This includes an energy and material consuming process during which parts of hyphae have to be dissolved and locally rearranged. Increased amounts of CpcA impair fruitbody formation in this fungus suggesting that the synthesis of novel proteins is a prerequisite for the formation of the cleistothecial structure and/or ascosporogenesis.

The *A. fumigatus* transcription factor CpcA is required during periods of amino acid starvation but also for full virulence as determined in a murine model of pulmonary aspergillosis [9]. The molecular mechanism for this observation is unknown, because only the basal activity of CpcA but not the induction of its expression by the cross-pathway control signal transduction cascade appears to be required for full virulence. Current approaches aim at the evaluation of CpcA target genes, accompanied by comprehensive studies on the CpcA-directed transcriptome; preliminary data suggest a highly conserved role of the transcriptional activator protein in regulating the expression of genes involved in the biosynthesis of amino acids. However, uncharacterised proteins of *A. fumigatus* appear to be regulated by the cross-pathway control system, making them attractive candidates in upcoming research projects.

## CpcA/Gcn4p translational control

The amount of CpcA/Gcn4p in the fungal cell is regulated by translational control of the corresponding mRNA in the cytoplasm. Amino acid starvation is sensed in fungi by the eIF2α kinase CpcC/Gcn2p. This sensor has a protein kinase activity and is located in the cytoplasm attached to the ribosome, where it monitors whether tRNAs are charged with amino acids. The protein controls yeast CpcA/Gcn4p synthesis but also affects the overall cellular translation rate. Gcn2p-like kinases seem to be typical for eukaryotic cells, because several mammalian counterparts exist that are regulated by various environmental stimuli of stress.

CpcC/Gcn2p consists of a more C-terminal histidyl-tRNA synthetase (HisRS)-related domain and an N-terminal protein kinase domain. The HisRS domain responds to the presence of uncharged tRNAs by activating the adjacent kinase moiety. Several deacetylated tRNAs are recognized with similar affinities, whereas the charged form of a given tRNA shows a significantly reduced binding affinity. This mechanism resembles the *relA*-dependent stringent reponse as it is described for the bacterium *Escherichia coli* [10]. The stringent response is also induced by amino acid starvation and is part of the control of ribosome and amino acid biosynthesis. In *E. coli*, limitation for an amino acid also results in accumulation of uncharged tRNAs in the cytoplasm. When uncharged tRNAs

accumulate at the ribosomal A site, the relA protein synthesizes ppGpp and initiates the stringent response.

CpcC/Gcn2p activation is assumed to be a two-step mechanism in which several protein domains are necessary to overcome an auto-inhibitory function of the protein kinase domain. The activated kinase inhibits overall translation by phosphorylation of the initiation factor of translation eIF2. However, a limited number of transcripts including the mRNA of *CpcA/GCN4* are not repressed in their translation when the CpcC/Gcn2p kinase is active. Therefore, this kinase activity couples the availability of amino acids to expression of the mRNA encoding the transcriptional activator of the cross-pathway control system.

## Protein degradation and CpcA/Gcn4p protein levels within the fungal cell

An additional control mechanism for protein stability that depends on the availability of amino acids has been demonstrated for the yeast transcription factor Gcn4p [11]. The Gcn4p degradation pathway is restricted to the yeast nucleus and is initiated via phosphorylation of the transcription factor by the cyclin dependent kinase Pho85p/Pcl5p. Pcl5p is one of ten cyclins which are able to interact with Pho85p in yeast, providing a wide range of different substrate specificities. The analysis of the corresponding cyclin CaPcl5p in the opportunistic fungal pathogen *Candida albicans* revealed a role in modulating the filamentous response in amino acid rich media and therefore presumably in virulence [12]. Yeast Pho85p as well as *Aspergillus* PhoAp and PhoBp are fungal counterparts of human Cdk5, which is assumed to promote neurodegenerative processes such as Alzheimer's disease [13]. The initial step for Gcn4p stabilization during amino acid limitation includes the dissociation of Pho85p and the highly instable cyclin Pcl5p. Additional factors like the stable cyclin Pcl7p and the inhibitor Pho81p are also required for Gcn4p stabilization [14]. In a second step of degradation, phosphorylated Gcn4p is a substrate for the nuclear $SCF^{Cdc4p}$ E3 ubiquitin ligase, which mediates the kovalent attachment of ubiquitin chains as prerequisite for the degradation of Gcn4p within the proteasome distruction machinery. All yeast genes that are necessary for Gcn4p stability control are also present in the genome of *A. fumigatus*, suggesting a similar conserved fungal mechanism to control the stability of CpcA.

Moreover, SCF ubiquitin ligases seem to play a prominent role in *Aspergillus* differentiation: Besides a role of the $SCF^{GrrA}$ complex in ascosporogenesis and likely meiosis [15], the COP9 signalosome (CSN), which is involved in the control of SCF E3 ubiquitin ligase activities, could be identified as an additional key regulator of fungal development in *A. nidulans* [16]. These finding support an important role of protein degradation in the specialization of fungal cells. The ubiquitinylation activity of SCF complexes can be modulated by neddylation, a reversible conjugation of the ubiquitin-related protein NEDD8/Rub1 on the cullin subunit. SCF E3 ubiquitin ligases have to be neddylated and deneddylated at their cullin subunit to perform their function *in vivo*. Fungal *csn* mutant strains accumulate neddylated cullins during vegetative growth and therefore the intrinsic COP9 signalosome deneddylase activity seems to be critical for fungal development. Detailed inspection of the proteome of a *csn* deletion strain prior to any visible mutant phenotypes suggests a failure to induce an appropriate oxidative stress response. Since higher eukaryotes are not able to survive the embryonic state without a functional COP9 signalosome, we use *A. nidulans* as tractable model system to investigate principal mechanisms in the coordination of protein degradation during growth and development.

## Outlook

Major goals of the study of the cross-pathway control system in an opportunistic pathogen as *A. fumigatus* will include the identification of CpcA target genes that require this transcription factor to mediate virulence. Another question will be whether the CpcA degradation pathway is required for virulence in *A. fumigatus* as it has been suggested in the yeast pathogen *C. albicans*. It will be interesting to perform similar experiments as in yeast in the future with a focus on the *A. fumigatus* counterparts of Pcl5p and the $SCF^{Cdc4p}$ ubiquitin ligase as crucial players of the destruction machinery for yeast Gcn4p.

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## References

1 Braus GH, Pries R, Düvel K, Valerius O. Molecular biology of fungal amino acid biosynthesis regulation. In Kück U, (ed.) *The Mycota II – Genetics and Biotechnology*. 2nd edn. Berlin-Heidelberg-New York-Tokyo: Springer Press; 2004: 239–269.

2 Forsberg H, Gilstring CF, Zargari A, Martinez P, Ljungdahl PO. The role of the yeast plasma membrane SPS nutrient sensor in the metabolic response to extracellular amino acids. *Mol Microbiol* 2001; **42**: 215–228.

3 Natarajan K, Meyer MR, Jackson BM, *et al*. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol* 2001; **21**: 4347–4368.
4 Galagan JE, Calvo SE, Cuomo C, *et al*. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* 2005; **438**: 1105–1115.
5 Braus GH, Grundmann O, Brückner S, Mösch HU. Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2003; **14**: 4272–4284.
6 Kleinschmidt M, Grundmann O, Bluthgen N, Mösch HU, Braus GH. Transcriptional profiling of *Saccharomyces cerevisiae* cells under adhesion-inducing conditions. *Mol Genet Genomics* 2005; **273**: 382–393.
7 Tripathi G, Wiltshire C, Macaskill S, *et al*. Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in *Candida albicans*. *Embo J* 2002; **21**: 5448–5456.
8 Hoffmann B, Valerius O, Andermann M, Braus GH. Transcriptional autoregulation and inhibition of mRNA translation of amino acid regulator gene *cpcA* of filamentous fungus *Aspergillus nidulans*. *Mol Biol Cell* 2001; **12**: 2846–2857.
9 Krappmann S, Bignell EM, Reichard U, *et al*. The *Aspergillus fumigatus* transcriptional activator CpcA contributes significantly to the virulence of this fungal pathogen. *Mol Microbiol* 2004; **52**: 785–799.
10 Chatterji D, Ojha AK. Revisiting the stringent response, ppGpp and starvation signaling. *Curr Opin Microbiol* 2001; **4**: 160–165.
11 Irniger S, Braus GH. Controlling transcription by destruction: the regulation of yeast Gcn4p stability. *Curr Genet* 2003; **44**: 8–18.
12 Gildor T, Shemer R, Atir-Lande A, Kornitzer D. Coevolution of cyclin Pcl5 and its substrate Gcn4. *Eukaryot Cell* 2005; **4**: 310–318.
13 Patrick GN, Zukerberg L, Nikolic M, *et al*. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* 1999; **402**: 615–622.
14 Bömeke K, Pries R, Korte V, *et al*. Yeast Gcn4p stabilization is initiated by the dissociation of the nuclear Pho85p/Pcl5p complex. *Mol Biol Cell* 2006; **17**.
15 Krappmann S, Jung N, Medic B, *et al*. The *Aspergillus nidulans* F-box protein links SCF activity to meiosis. *Mol Microbiol* 2006; **61**: 76–88.
16 Busch S, Eckert SE, Krappmann S, Braus GH. The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. *Mol Microbiol* 2003; **49**: 717–730.