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Protein Degradation and Fungal Growth and Development

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The regulation of metabolism and the control 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 the translation of the newly synthesised mRNAs. Amino acids are essential precursors of translation and therefore necessary for a fungal growth and development reaction to an external stimulus.

Amino acids are either (i) taken up from the environment by transport processes, or (ii) synthesised from precursors which are derivatives of the carbon and nitrogen primary metabolism, or (iii) result of the degradation of proteins which are no more required under specific conditions. All three processes have to be coordinated and are carefully regulated in fungi.

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 pathway and the transcriptional activator CpcAp/Gcn4p. This genetic network, which has been named 'cross-pathway control' in filamentous fungi and 'general amino acid control' in yeast, coordinately regulates hundreds of genes in numerous biosynthetic pathways (Braus et al., 2004).

In addition to its role as metabolic transcription factor, CpcAp/Gcn4p coordinates metabolism and development. In the yeast *S. cerevisiae*, activation of Gcn4p results in cell-cell and cell-surface adhesion (Braus et al., 2003), in the pathogenic dimorphic yeast *C. albicans* Gcn4p affects filament formation (Tripathi et al., 2002).

The genome of the opportunictic pathogen *A. fumigatus* is one of three Aspergilli genomes which have recently become available (Galagan et al., 2005). The *A. fumigatus* transcription factor CpcAp is required during amino acid starvation but also for full virulence in the mouse model (Krappmann et al., 2004). In another Aspergillus, *A. nidulans,* activation of the cross-pathway control impairs developmental programs as the formation of fruitbodies (Hoffmann et al., 2001). *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 where parts of hyphae have to be dissolved and locally rearranged. High amounts of CpcAp impair fruitbody formation in this fungus suggesting that the synthesis of novel proteins is a prerequisite for the formation of the structure and/or ascosporogenesis.

The amount of CpcAp/Gcn4p in the fungal cell is regulated by the translational control of the corresponding mRNA in the cytoplasm. For yeast Gcn4p, an additional control of protein stability in the nucleus (Pries et al., 2002), which depends on the availability of amino acids, has been demonstrated. The Gcn4p degradation pathway includes the phosphorylation by the cyclin dependent kinase Pho85p/Pcl5p, the subsequent attachement of ubiquitins by Cdc4-SCF E3 ubiquitin ligase and the degradation within the proteasome. The initial step for Gcn4p stabilization during amino acid limitation includes the dissociation of Pho85p and Pcl5p (Boemeke et al., 2006).

The COP9 signalosome which is involved in the control of SCF E3 ubiquitin ligase activities has been identified as a key regulator of fungal sexual development in *Aspergillus nidulans* (Busch et al., 2003). This finding further supports an important role of protein degradation in fungal development. The ubiquitinylation activity of SCF can be modulated by neddylation, a reversible conjugation of the ubiquitin-related protein NEDD8/Rub1 on the cullin subunit. Among the ubiquitin-like protein family, the gene for NEDD8 is most homologous to the gene for ubiquitin. 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. SCF E3 ubiquitin ligases have to be neddylated and deneddylated at their cullin subunit to perform their function *in vivo*. Prior to the visible mutant phenotypes, the proteome of a *csn* deletion strain 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 model system to understand principal mechanisms of the coordination of protein degradation in growth and development as supply for amino acids. Recent results of our laboratory on this topic will be discussed.

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