Approaching the secrets of N-glycosylation in *Aspergillus fumigatus*: characterization of the AfOch1 protein.

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Abstract

The mannosyltransferase Och1 is the key enzyme for synthesis of elaborated protein N-glycans in yeast. In filamentous fungi genes implicated in outer chain formation are present, but their function is unclear. In this study we have analyzed the Och1 protein of Aspergillus fumigatus. We provide first evidence that poly-mannosylated N-glycans exist in A. fumigatus and that their synthesis requires AfOch1 activity. This implies that AfOch1 plays a similar role as S. cerevisiae ScOch1 in the initiation of an N-glycan outer chain. A Δafoch1 mutant showed normal growth under standard and various stress conditions including elevated temperature, cell wall and oxidative stress. However, sporulation of this mutant was dramatically reduced in the presence of high calcium concentrations, suggesting that certain proteins engaged in sporulation require N-glycan outer chains to be fully functional. A characteristic feature of AfOch1 and Och1 homologues from other filamentous fungi is a signal peptide that clearly distinguishes them from their yeast counterparts. However, this difference does not appear to have consequences for its localization in the Golgi. Replacing the signal peptide of AfOch1 by a membrane anchor had no impact on its ability to

complement the sporulation defect of the Δafoch1 strain. The mutant triggered a normal cytokine response in infected murine macrophages, arguing against a role of outer chains as relevant Aspergillus pathogen associated molecular patterns. Infection experiments provided no evidence for attenuation in virulence; in fact, according to our data the Δafoch1 mutant may even be slightly more virulent than the control strains.

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