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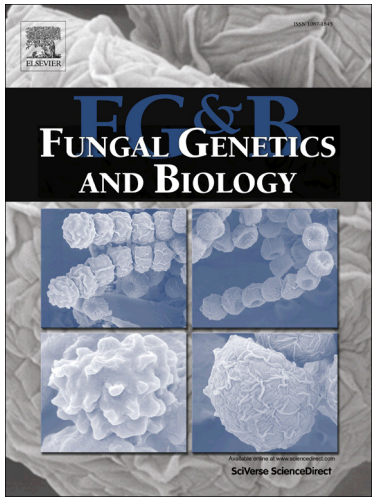

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Dissecting the function of the different chitin synthases in vegetative growth and sexual development in *Neurospora crassa*

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Abstract

Chitin, one of the most important carbohydrates of the fungal cell wall, is synthesized by chitin synthases (CHS). Seven sequences encoding CHSs have been identified in the genome of *Neurospora crassa*. Previously, CHS-1, -3 and -6 were found at the Spitzenkörper (Spk) core and developing septa. We investigated the functional importance of each CHS in growth and development of *N. crassa*. The cellular distribution of each CHS tagged with fluorescent proteins and the impact of corresponding gene deletions on vegetative growth and sexual development were compared. CHS-2, -4, -5 and -7 were also found at the core of the Spk and in forming septa in vegetative hyphae. As the septum ring developed, CHS-2-GFP remained at the growing edge of the septum until it localized around the septal pore. In addition, all CHSs were located in cross-walls of conidiophores. A partial co-localization of CHS-1-mCherry and CHS-5-GFP or CHS-2-GFP occurred in the Spk and septa. Analyses of deletion mutants suggested that CHS-6 has a role primarily in hyphal extension and ascospore formation, CHS-5 in aerial hyphae, conidia and ascospore formation, CHS-3 in perithecia development and CHS-7 in all of the aforementioned. We show that *chs-7*/*csmB* fulfills a sexual function and *chs-6/chsG* fulfills a vegetative growth function in *N. crassa* but not in *A. nidulans*, whereas vice versa *chs-2*/*chsA* fulfills a sexual function in *A. nidulans* but not in *N. crassa*. This suggests that different classes of CHSs can fulfill distinct developmental functions in various fungi. Immunoprecipitation followed by mass spectrometry of CHS-1-GFP, CHS-4-GFP and CHS-5-GFP identified distinct putative interacting proteins for each