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Differential Expression of the Chitin Synthase Genes of *Aspergillus nidulans*

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Chitin is an important structural component of the vast majority of fungal cell walls, but is not present in plant or mammal cells. Chitin synthases (UDP-*N*-acetyl-D-glucosamine:chitin 4-β-*N*-acetylglucosaminyl transferase, EC 2.4.1.16) are thus thought to be indispensible for fungal growth, and therefore, deserve attention as they could be utilized in the development of antifungal drugs with high selectivity and low toxicity, as well as in the elucidation of the molecular mechanism of hyphal growth.

In *Aspergillus nidulans*, five chitin synthase genes, *chsA* (Class II) (Culp et al., 2000; Yanai et al., 1994), *chsB* (Class III) (Borgia et al., 1996; Yanai et al., 1994), *chsC* (Class I) (Motoyama et al., 1994), *chsE* (Class IV, *CHS3/CAL1* homolog; identical to *chsD* reported by Motoyama *et al.* (Motoyama et al., 1996)) (Specht et al., 1996), and *csmA/chsD* (Class V) (Fujiwara et al., 1997; Specht et al., 1996), have been cloned and identified. Disruption of *chsA* (Yanai et al., 1994), *chsC* (Motoyama et al., 1994), or *chsE* (Motoyama et al., 1996; Specht et al., 1996) does not cause any defect in cell growth or morphology during the asexual cycle, which suggests that none of the three genes is essential for hyphal growth. Double disruption of *chsA* and *chsE*, however, causes a remarkable decrease in the efficiency of conidia formation, which supports that *chsA* and *chsE* serve redundant functions in conidia formation (Culp et al., 2000; Motoyama et al., 1996). Double disruption of *chsA* and *chsC* causes a loss of integrity of the hyphal wall and remarkable abnormalities during asexual development (Fujiwara et al., 2000). *chsA* is mainly expressed in the metulae, phialides, and conidia, whereas *chsC* is expressed in hyphae and in conidiophores, which implies that ChsA and ChsC share critical functions in hyphal wall integrity and differentiation (Fujiwara et al., 2000). The haploid disruptant of *chsB* grows as minute colonies without conidia, and produces hyphae with enlarged tips, high degree of branching, and disorganized lateral walls. However, its mycelium is not deficient in chitin and shows no evidence of lysis, suggesting that chitin synthesized by the ChsB enzyme does not substantially contribute to the rigidity of the cell wall but is necessary for normal hyphal growth and organization (Borgia et al., 1996). The deduced amino acid sequence of the C-terminal half of CsmA protein, a chitin synthase with a myosin motor-like domain, is almost identical to that of ChsD (Specht et al., 1996), in fact the ORF of *chsD* seems to be a partial sequence of *csmA* (Fujiwara et al., 1997). Disruption of *csmA* causes chitin deficiency and lysis in conventional medium, indicating that this gene contributes to the rigidity and osmotic integrity of the cell wall, but that it is not necessary for normal cell morphology (Specht et al., 1996). The presence of a myosin motor-like domain in CsmA protein suggests that the localization of chitin synthesis may be guided by association with cytoskeletal structures (Fujiwara et al., 1997).

In the present study, to understand the role of the chitin synthase genes in *A. nidulans*, we analyzed the expression modes of *chsA*, *chsB*, *chsC*, and *chsD* both by Northern blot analysis and by the reporter system with a codon-modified version of green fluorescent protein, sGFP, as a vital reporter.

chsA was mainly expressed during the process of conidiophore development, *i.e.*, the formation of stalks, vesicles, metulae, and phialides, and budding of conidia, but was not expressed during either vegetative growth or sexual differentiation. Consequently, it is suggested that *chsA* is specifically involved in asexual differentiation. The expression of *chsB* was not only ubiquitous throughout the whole fungal body but was almost constant and independent of the change in developmental status of fungal cells, *i.e.*, vegetative growth, asexual development, and sexual development. The expression of *chsC* was much more specific to the period of sexual development than to the period of asexual development, and furthermore, that this expression is spatially differentiated, *i.e.*, very strong in ascospores, moderate in cleistothecial shells, and negligible in Hülle cells. Although *chsD* was expressed during the process of conidiophore development as well as during vegetative growth, it was most strongly expressed during the early sexual differentiation, *i.e.*, in young cleistothecia and Hülle cells. Consequently, it is suggested that *chsD* is more specifically involved in the initial stage of sexual differentiation. Osmostress stimulates the expression of *chsA* mainly in conidiophores and neighboring substrate hyphae, and the expression *chsC* in the whole fungal body. On the other hand, *chsB* was not intimately responsive to salt stress or osmostress. Heat shock did not stimulate the expression of either *chsA*, *chsB*, or *chsC*. Sodium acetate, especially at high concentaions (3%), strongly enhances the expressions of the three chitin synthase genes. Quite differently from the case of sodium acetate, the expressions of the chitin synthase genes is not responsive to the sugars supplied as carbon sources.

Consequently, all the the results of the present study suggest that the chitin synthase genes of *A. nidulans* are subject to differential expression in response to developmental status, carbon sources, and environmental stresses.

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