

NOTE

Regulation of *nsdD* Expression in *Aspergillus nidulans*

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The *nsdD* gene has been predicted to encode a GATA type transcription factor with the type IVb zinc finger DNA binding domain functions in activating sexual development of *A. nidulans*. In several allelic mutants of *nsdD* producing truncated NsdD polypeptides lacking the C-terminal zinc finger, the transcription level of *nsdD* gene was greatly increased. Also in an over-expressed mutant, the transcription under its own promoter was reduced. These results suggest that the expression of *nsdD* is negatively autoregulated. When the *nsdD* gene was over-expressed, cleistothecia were formed in excess amounts even in the presence of 0.6 M KCl that inhibited sexual development of the wild type. Northern blot analysis revealed that the expression of *nsdD* was repressed by 0.6 M KCl. These results strongly suggest that the inhibition of sexual development by salts was carried out via the *nsdD* involved regulatory network.

Key words: *Aspergillus nidulans*, sexual development, *nsdD*, autoregulation

Aspergillus nidulans reproduces both sexually and asexually. Distinctive reproductive organs are produced after morphogenic development takes place. A number of genes have been identified that are involved in asexual development and the regulatory network of these genes is well established (for review see Adams *et al.*, 1998). Only a few genes have been identified that are associated with sexual development. They include the *veA*, *phoA*, *stuA*, and *medA*, the functions of which are not well known yet (Clutterbuck, 1969; Busby *et al.*, 1996; Bussink and Osmani, 1998).

We isolated mutants that were unable to form any sexual structures (NSD: never in sexual development) and were analyzed for their genetic and morphological characteristics (Han *et al.*, 1994b; Han *et al.*, 1998). At least four complementation groups were identified (*nsdA*, *nsdB*, *nsdC* and *nsdD*). Among them, the *nsdD* gene was isolated and characterized (Han *et al.*, 2001). The *nsdD* gene was predicted to encode a putative GATA type transcription factor that contains a type IVb zinc finger domain at the C-terminus. Deletion of *nsdD* resulted in

the absence of sexual development, and forced expression of *nsdD* induced sexual sporulation even under conditions that do not allow sexual development. Based on these results, we proposed that *nsdD* positively controls the early step(s) of sexual reproduction and is necessary for sexual development of *A. nidulans*. For the expression of *nsdD*, normal FlbA function is necessary. FlbA is known to be a RGS protein that blocks FadA mediated growth signaling, leading to asexual sporulation (Yu *et al.*, 1996). The requirement for FlbA in *nsdD* expression indicates that the FlbA- FadA regulation is necessary for sexual as well as asexual development.

The relationship between the copy number of the *nsdD* gene and the rate of cleistothecial development was described (Chae *et al.*, 1995). The increased copy number of the *nsdD* gene resulted in a proportional increment in the rate of development of the sexual organs. Four phenotypically discriminative transformants were selected and the copy number and the expression level of the *nsdD* gene were examined by Southern and Northern blot analysis, respectively. As seen in Fig. 1, the difference of copy number was easily detectable. However, the increase of mRNA was not observed in multicopy transformants.

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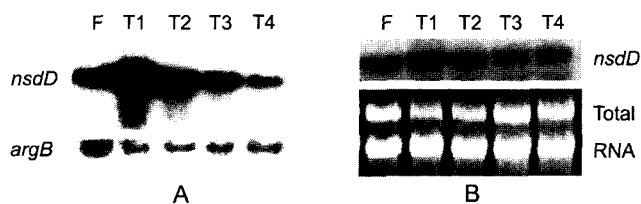


Fig. 1. Transcription of *nsdD* in multicopy transformants. The genomic DNA and total RNA were extracted from 16 h cultured mycelia and hybridized with *nsdD* specific probe. (A) Southern hybridization (B) Northern hybridization

This result implies that the amount of *nsdD* mRNA is maintained at a constant level regardless of the copy number of the gene. The increment in the rate of development of sexual organs by increased *nsdD* copy number is not due to the increased expression of the gene. The phenotypical change by multicopy transformation is caused by some other unknown mechanism. Both pre- and post-transcriptional control may be responsible for the maintenance of the *nsdD* mRNA level.

Several different alleles were identified in *nsdD*, including *nsdD16*, *nsdD19*, and *nsdD20*, and these allelic mutants showed the typical NSD phenotype. Fig. 2A represents the mutation sites of three different alleles. The *nsdD19* mutant allele was predicted to cause a reading frame shift which resulted in early termination of translation in *nsdD19* and the truncated polypeptide was predicted to have only 81 amino acids. In the *nsdD16* allele an early termination codon (TAG) is generated after 30 out-of-frame codons, resulting in truncation of the NsdD polypeptide ($\Delta 130-461$ aa). The *nsdD20* alleles have mutations in the consensus lariat forming sequence of the 2nd intron. Such mutations are predicted to interfere with the splicing of introns. In all cases, the mutant polypeptides lack the zinc finger domain. The amount of mRNA in those mutants were highly increased as compared with the wild type (Fig. 2B). ORF-deletion mutant also contained a largely increased amount of mRNA. The increased amount of *nsdD* mRNA in *nsdD*⁻ mutants implies that the *nsdD* transcript is controlled at a constant level in the wild type. The C-terminus of NsdD is important in controlling the amount of *nsdD* mRNA. It is probable that *nsdD* expression is self-regulated by NsdD. NsdD may bind to the *nsdD* promoter by the zinc finger domain and negatively control the expression of *nsdD*. The possibility of autoregulation of *nsdD* expression is supported by the fact that the *nsdD* mRNA transcribed by its own promoter is reduced when it is overexpressed. Fig. 2C represents the Northern blot analysis examined with total RNA from *nsdD* over-expressed strain which carried *nsdD* ORF downstream from the *niiA* promoter. Upper lanes are Northern blot data obtained using the *nsdD* coding region as a probe. The *nsdD* transcript was greatly increased in an over-expressed transformant, KHH62, but not in an isogenic control strain, KHH60, when cultured

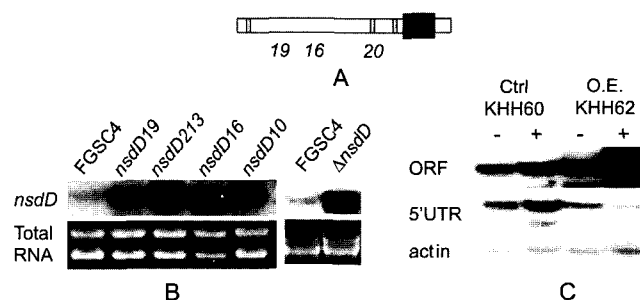


Fig. 2. Autoregulation of *nsdD*. (A) Schematic structure of *nsdD* ORF. Black box represents the zinc finger domain. Introns are indicated by small gray boxes. Figures represent mutation sites. (B) The *nsdD* transcript levels in allelic and ORF deleted mutants. Total RNA was extracted from 16 h cultured mycelia of *nsdD* allelic and deletion mutants and hybridized with a probe specific for *nsdD* ORF (left 5 lanes) or 5'UTR (right 2 lanes). *nsdD213* and *nsdD16* are the same alleles. (C) Effect of *nsdD* over-expression under the *niiA* promoter on the expression of *nsdD* by its own promoter. Total RNA was extracted from *nsdD* over-expressed strain (KHH62) and its isogenic control strain (KHH60) cultured for 16 h in MM broth containing 0.2% ammonium tartrate (-) or 0.6% sodium nitrate (+) as the sole nitrogen source, and Northern hybridized. The upper lane was probed with *nsdD* ORF and the middle lane with 5'UTR. When induced by sodium nitrate, the amount of *nsdD* transcript by the *niiA* promoter in KHH62 dramatically increased (upper, lane 4), while that by the *nsdD* promoter declined (middle, lane 4).

on sodium nitrate as the sole nitrogen source. In the presence of ammonium tartrate, however, expression was reduced to the control level, indicating that the controllable expression was achieved. When the 5'UTR region was used as a probe, the reduction of *nsdD* transcript in KHH62 was observed. This result implies that increased NsdD by over-expression represses the expression of *nsdD* by its own promoter. All of these results strongly suggest that *nsdD* transcript is maintained at a certain level and is achieved at least by autoregulation of NsdD.

There are several conditions that promote or inhibit cleistothecia formation. For instance, when sufficient glucose (3%) is supplied, cleistothecia formation is greatly increased (Han et al., 1994a). However, salt such as KCl at a higher concentration than 0.6 M repressed sexual development completely (Song et al., 2001). The *nsdD* deletion mutant did not respond to the increase of glucose concentration (data not shown). Over-expression of *nsdD* results in the cleistothecial formation even in the presence of high concentration of salt (Han et al., 2001). It is possible that the function of NsdD is related to those environmental changes. Therefore, we examined whether *nsdD* transcription was affected by those environmental changes (Fig. 3). Expression of *nsdD* was not affected by the increase of glucose concentration (Fig. 3A), indicating that preference of sexual development under conditions where a sufficient C source was supplied was not concerned with the change of *nsdD* expression. However, the transcription of *nsdD* was significantly reduced in the

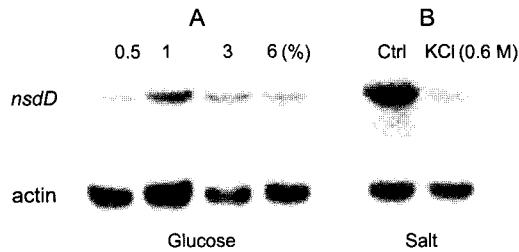


Fig. 3. Effect of high concentration of glucose or KCl. Total RNA was isolated from the mycelia cultured for 16 h in MM broth supplied with various concentration of glucose (A) or in the presence of 0.6 M KCl (B).

presence of 0.6 M KCl (Fig. 3B), indicating that *nsdD* is repressed by KCl. This result suggests that the inhibition of sexual development by a high concentration of salts is achieved via repression of *nsdD*. Expression under the *niiA* promoter is not repressed by KCl and thus the over-expressed strain (KHH62) can develop cleistothecia in the presence of a high concentration of salts.

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