S4-1

Control of Sexual Development *via* NsdD in *Aspergillus nidulans*

Dong-Min Han*, Keon-Sang Chae[1], Suhn-Kee Chae[2]
Division of Life Science, Wonkwang University, Iksan City, Chonbuk, 570-749
[1]Division of Biological Science, Chonbuk University, Chonju City, Chonbuk, 561-756
[2]Division of Life Sciences, Paichai University, Taejeon 302-735

The ability to reproduce both sexually and asexually is one of the characteristics of the homothalic ascomycete *Aspergillus nidulans*. Unlike the other *Aspergillus* species, *A. nidulans* undergoes complete sexual development that seems to be regulated by internal and external stimuli. We have isolated and characterized lots of mutants that showed defects in sexual development or abnormal responses in sexual development to various environmental factors. During the first screening of mutants, several NSD (*n*ever in *s*exual *d*evelopment) mutants that failed to produce any sexual reproductive organs were isolated and four complementation groups, *nsdA*, *nsdB*, *nsdC*, and *nsdD* were identified (Han *et al.*, 1994a). The *nsdD* gene was isolated and predicted to encode a GATA-type transcription factor carrying the type IVb zinc finger DNA-binding domain at C terminus (Han *et al.*, 2001). The *nsdD* gene was expressed during vegetative growth, and the expression level increased as sexual development proceeded. Deletion of *nsdD* resulted in no cleistothecia (fruiting bodies) formation, even under the conditions that preferentially promoted sexual development, indicating that *nsdD* is necessary for sexual development. In contrast, when the *nsdD* gene was over-expressed, the number of cleistothecia was dramatically increased on solid medium and also a sexual-specific organ (Hulle cells) was formed even in submerged culture, which normally completely blocked sexual development. These results lead us to propose that the *nsdD* gene functions in activating sexual development of *A. nidulans*.

In several allelic mutants of *nsdD* that resulted in early chain termination and lacked zinc finger motif, the accumulation of mRNA was greatly increased. And when *nsdD* gene was over-expressed by *niiA* promoter, the transcription under its own promoter was reduced. The mRNA levels in the strains with multiple copies of *nsdD* gene were not increased although the cleistothecial formation was dramatically increased. These results suggest that the expression of *nsdD* is negatively autoregulated and the NsdD protein in the cell is maintained within a certain level.When the *nsdD* gene was over-expressed, cleistothecia were formed in excess amount even in the presence of 0.6 M KCl that inhibited sexual development of wild type. Northern blot analysis revealed that the expression of *nsdD* was repressed by 0.6M KCl. These results strongly suggest that the inhibition of sexual development by salts was carried out *via nsdD* involved regulatory network.

We have isolated another gene, *veA*, which has been known to play an important role in the regulation of development. The *veA* gene has been known to control the development in response to

some environmental. The *veA1* mutation delays and reduces the development of sexual organs, which eventually results in the preferential development of asexual spores (Champe *et al.* 1981). The asexual development of *veA1* mutant is much less affected by various environmental factors, including nutrients (Han *et al.* 1994b), light (Mooney and Yager, 1990) and temperature (Champe *et al.* 1981).

The *veA* gene has an open reading frame (ORF) of a 573 amino acid polypeptide, which matched some clones of which functions were not assigned yet (Kim *et al.*, 2003). The *veA* transcript was present in the conidia and in mycelia cultured for up to 14 h and expressed almost constitutively at an increased level throughout the asexual and sexual developmental processes, suggesting that it may act from a relatively early developmental stage. Null mutants of the gene never formed sexual structures, even under conditions where sexual development preferentially occurs in wild types. Over-expressors of the gene formed larger numbers of sexual structures with a much reduced number of conidial heads than a control strain (a *veA1* mutant), even under conditions where wild type strains form little sexual structure but form conidial heads very well, such as in the presence of a salt at high concentration. Furthermore, over-expressors could form Hulle cells and cleistothecia, even in a liquid culture. These results indicated that the *veA* gene is a positive regulator of sexual development and simultaneously a negative one of asexual development.

There is no direct regulatory relationship between *veA* and *nsdD*. In *veA* deletion mutant, the expression of *nsdD* was not reduced, indicating that control of sexual development by VeA is not mediated *via* the regulation of *nsdD* transcription. Also the expression of *veA* is not significantly affected by *nsdD*.

To identify another regulatory components of sexual development in relation to NsdD function, proteins interacting with NsdD were screened using the yeast two-hybrid system. Two IND (interactor of NsdD) proteins, INDB and INDD were isolated and characterized further. Determination of *indB* and *indD* cDNA sequences revealed open reading frames of 648 bp and 642 bp, encoding polypeptides of 215 and 213 amino acids, respectively. INDB and INDD showed 42% amino acid sequence identity and also shared similarities to an ORF in *Neurospora crassa*. NsdD-IndB and NsdD-IndD interactions were confirmed *in vitro* using a GST-pull down assay. Both proteins interacted with the Zn-finger domain of NsdD. The N-terminus of IndB and the C-terminus of IndD were responsible for the NsdD interaction. IndB and IndD were able to interact each other. Self-association of IndB but not for IndD was observed, too. In Northern analysis, 1.2 kb transcripts were detected for both genes. The *indB* transcripts expressed to similar levels during asexual development, but increased at 20-30 hour after induction of sexual development, then decreased. In contrast, the *indD* transcript was the most abundant at the initial asexual developmental stage. Furthermore, both kinds of transcripts were highly produced in *veA* mutant strains, but repressed in *veA*+ wild type background, indicating *veA*-dependent expressions. The *veA*$^{-}$ mutations conferred defectiveness in sexual differentiation. These results suggest that IndB and IndD might affect NsdD function negatively by binding to the Zn-finger region of NsdD to block DNA binding. And they also suggest that VeA controls sexual development by repressing the transcription of *indB* and *indD*, which leads the NsdD to function in regulating the sexual development positively.

References

1. Han, D.M., Y.J. Han, J.H. Kim, K.Y. Jahng, Y.S. Chung, J.H. Chung, and K.S. Chae. 1994a. *Kor. J. Mycol.* 22, 1-7.
2. Han, D.M., Y.J. Han, K.S. Chae, K.Y. Jahng, and Y.H. Lee. 1994b. *Kor. J. Genet.* 22, 332-337.
3. Han, K.H., K.Y. Han, J.H. Yu, K.S. Chae, K.Y. Jahng, and D.M. Han. 2001. *Mol. Microbiol.* 41, 299-309.
4. Champe, S.P., M.B. Kurtz, L.N. Yager, N.J Butnick, and D.E. Axelrod. 1981. pp 255-276. *In* G. Turian, and H.R. Hohl(ed.), The fungal spore-morphogenetic controls-1981. Academic Press, New York.
5. Kim, H.S., K.J. Kim, K.Y. Jahng, S.K. Chae, D.M. Han, and K.S. Chae. 2002. *Fungal Genet. Biol.* 37, 72-80
6. Mooney, J.L., and L.N. Yager. 1990. *Genes Dev.* 4, 1473-1482.