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Genes Controlling Sexual Development of *Aspergillus nidulans*

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Many fungi produce spores through sexual or asexual development to resist unfavorable conditions or to propagate to other habitats. The mechanisms of spore formation are quite different among fungal genera and various specialized morphological structures are generated. A homothallic ascomycete, *Aspergillus nidulans*, which develops both sexual and asexual spores through complicated morphogenic processes, is a model fungus to study spore formation in molecular level. After a certain period of vegetative growth, asexual sporulation begins by producing spore-bearing structures, conidiophores, from foot cells of aerial hyphae. Sequential development of metula and phialides takes place and is followed by formation of conidia by budding at the tip of phialides. A number of genes have been identified that are associated with asexual development and the regulatory network of these genes is well established.

A. nidulans also produces sexual spores developed in closed fruiting bodies, called cleistothecia, which are matured from primordia. Sexual development of *Aspergillus nidulans* is greatly affected by carbon sources and environmental stresses. When higher concentration of glucose was supplied, the more cleistothecia were formed. On fermentable carbon sources including lactose, galactose and glycerol cleistothecia developed more favorably than conidiophores, whereas on acetate which could be utilized only via aerobic respiration, no cleistothecia developed. Low level of aerobic respiration caused by either plate-sealing(Fig.1) or treatment with various inhibitors favored for sexual development. Light exposure, high osmolarity or high concentration of salts inhibits cleistotheicial development but promotes conidiation, suggesting that the environmental stresses affect developmental fate of *A. nidulans.* Since sexual sporulation takes longer period and more complicated than asexual sporulation, it is predicted that much more genes are involved in sexual development. However, still only a small number of genes have been identified.

We have been interested in the regulatory mechanism how the developmental fate is decided in response to environmental factors and what kinds of genes are involved in that process. Several genes have been identified by forward genetics using the developmental characteristics in response to environmental stresses described above. In order to isolate genes that positively control sexual development, we first have screened the mutants that failed to produce any sexual reproductive organs even in hypoxic condition and characterized several NSD (never in sexual development) mutants and identified four complementation groups, *nsdA, nsdB, nsdC* and *nsdD*. Two genes, *nsdC* and *nsdD*, were isolated and identified as positive regulators of sexual development.

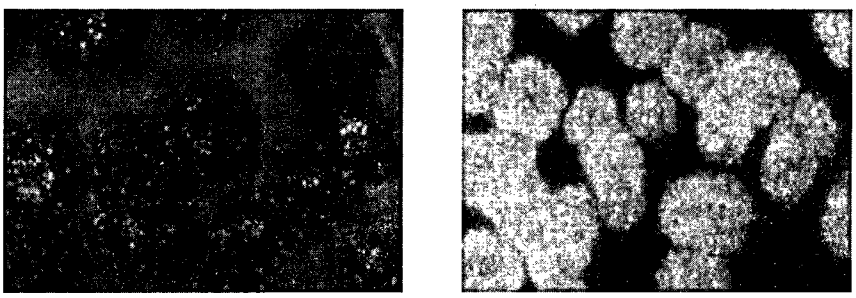

Fig. 1 Effect of hypoxic condition on development of *A. nidulans*

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. 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.

The *nsdC* gene, which is predicted to encode a 643 aa polypeptide with a novel C2H2-type zinc finger DNA binding domain. While deletion of *nsdC* resulted in the typical NSD phenotype, i.e., lack of sexual development, overexpression of *nsdC* not only enhanced formation of sexual fruiting bodies (cleistothecia) but also overcame inhibitory effects of certain stresses on cleistothecial development, indicating that NsdC is a key positive regulator of sexual development. The *nsdC* gene generates two distinct transcripts, 3.2 and 2.8 kb, and its 5' UTR is ~1.5 kb with two relatively long introns (168 bp and 212 bp). The ratio of the two transcripts of *nsdC* is different in different stages of growth or development. When the submerge-cultured mycelia were transferred on plate and cultured further with air-tight seal for more than 6h, the amount of *nsdC* mRNA, especially the 2.8kb mRNA, was dramatically increased. However, after the mycelia were exposed to fresh air for more than an hour, the 2.8kb mRNA was almost disappeared(Fig.2). This result

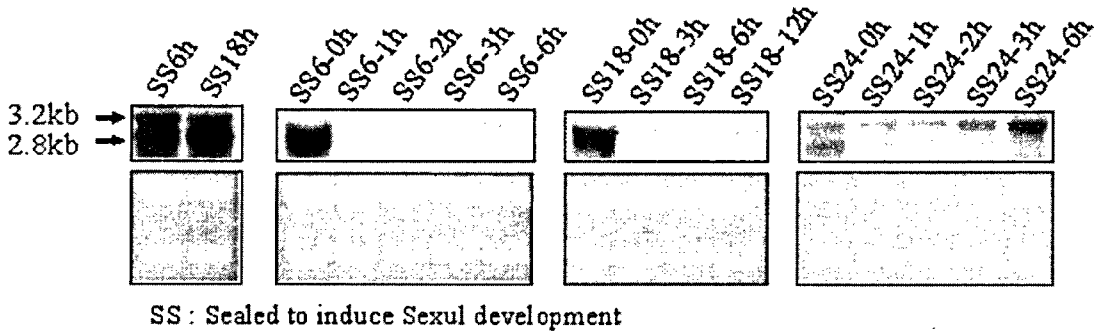

Fig. 2 Two different *nsdC* mRNAs in size are transcribed and smaller mRNA accumulates in hypoxic condition. SS; Sealing period. -h; Time after removal of seal.

strongly suggests that *nsdC* mRNA was accumulated in hypoxic condition but is repressed or degraded quickly after exposure to oxygen. However, in 20h sealed culture, where mycelia were determined to develop only sexually, the 2.8kb mRNA reappeared again in the mycelia cultured further for 6h and 12h after exposure to fresh air while not in that for 9h, implying that *nsdC* was periodically expressed during early sexual development. The expression of *nsdC* is not apparently dependent upon other regulatory genes, such as, *veA* and *nsdD* and also not significantly affected by any other environmental stress than oxygen. However, 2.8kb mRNA was greatly reduced in catalases deficient strains, implying that *nsdC* gene expression is sensitive to oxidative stress. Beside to the defect in sexual development of *nsdC* mutants, mycelial growth was impaired, especially much more on the medium where glycerol or lactose was supplied as a sole carbon source. Interestingly, conidia were formed in submerged culture grown in the media supplied with those carbon sources but not in glucose and acetate media. This phenotype suggested that NsdC played a certain role on growth and development on those moderate favorite carbon sources.

Over-expression of *nsdD*, which drives cells to sexual development in wild type, rescued the growth defect in *nsdC* null mutants, but not the deficiency of sexual development. NsdC over-expression also failed to recover the sexual defect in *nsdD* null mutants as well. The interplay between NsdC and NsdD during growth and sexual development is hard to recognize right now but the results indicate that the sexual defect in *nsdC* mutants is not a consequence of the impaired mycelial growth.

The *veA* gene has been known to control sexual development positively. The *veA1* mutation delays and reduces the development of sexual organs, which eventually results in the preferential development of asexual spores. The asexual development of *veA1* mutant is much less affected by various environmental factors, including nutrients, light and temperature.

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. 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(Fig.3). 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*

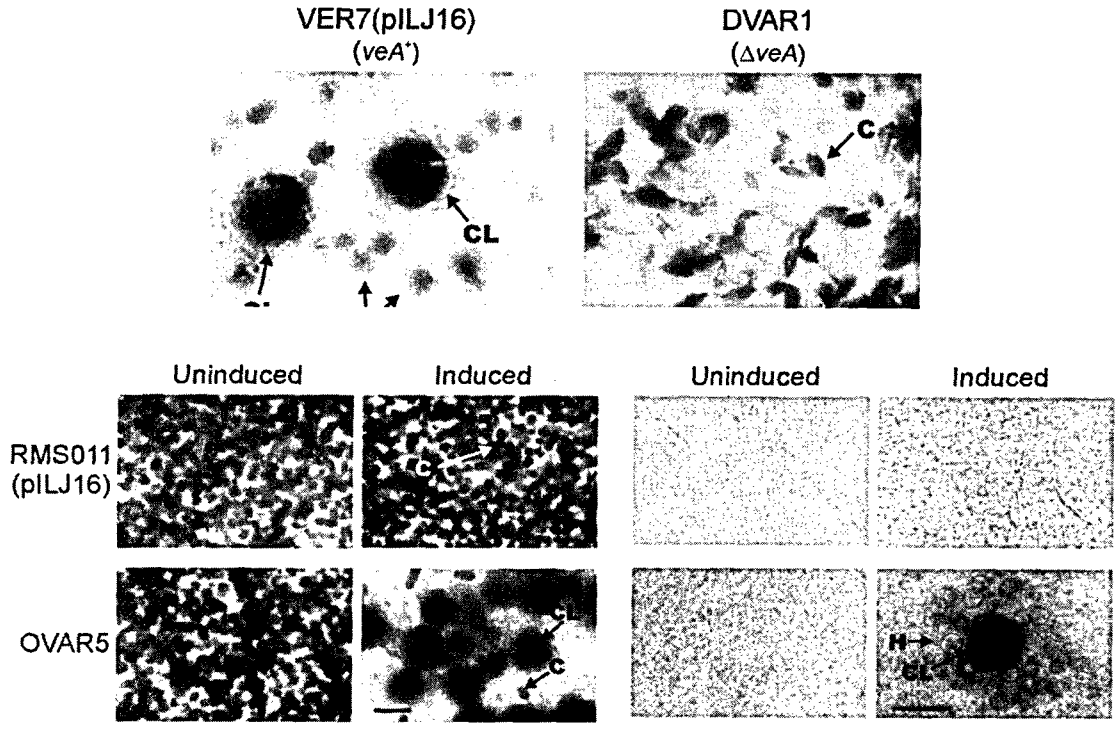

Fig. 3 Phenotypes of veA deletion and over-expression mutants.

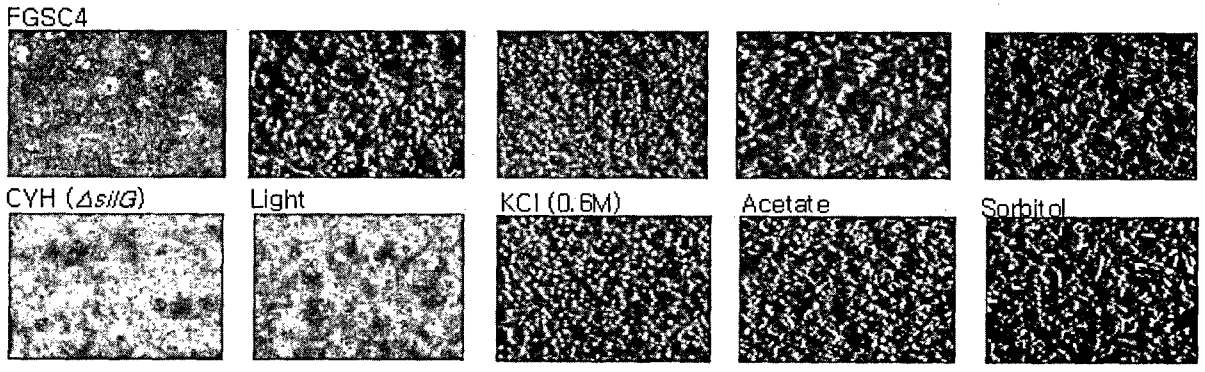

Fig. 4 Phenotype of *silG* deletion mutant.

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.

We have also screened and characterized several genes that negatively regulate sexual development in response to environmental stresses. These include *silG* and *silH* encoding zinc finger proteins, which repress sexual development in response to light exposure(Fig.4). Also *sndA* and *nrsA* encoding a Zn2Cys6 protein and a homeoprotein, respectively, control sexual development negatively in response to various stresses. Characteristics and inter-relationship among those regulators and with positive regulator will be discussed.

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