

F041

### The *smtA* Gene Encoding a Putative SAM Methyl Transferase are Necessary for Progression of Both Sexual and Asexual Development of *Aspergillus nidulans*

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The *smtA* (S-adenosylmethionin Methyl Transferase) gene was identified as a multi-copy suppressor of various mutations such as *sndE80*, *silC188*, *silD6*, *silE181* and *silF174*, which overcame the inhibitory effect of stresses on sexual development. The gene is predicted to encode a putative methyl transferase carrying an S-adenosylmethionin binding domain. The *smtA* ORF consists of 353 amino acids and is disrupted by 9 introns. The null mutant showed defects both in sexual and asexual developments. Conidiation and cleistothecia maturation were delayed and the amounts were reduced. The *smtA* gene was expressed in high level during vegetative growth and maintained only upto the early stages of asexual and sexual development. The *smtA* mRNA level was largely reduced in *veA* or *fluG* null mutant indicating that the *smtA* gene expression may be controlled by those genes. The *veA* null mutation was epistatic to *smtA* null mutation. These results together suggest that SmtA play some role in progression of both sexual and asexual development downstream of VeA and FluG.

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### Identification of Amino Acid Residues Critical for Binding of a 7mer Peptide to TiO<sub>2</sub> Nanoparticles

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We have isolated peptides specifically binding to TiO<sub>2</sub> nanoparticles using phage display peptide libraries and biopanning procedure. A 7mer, C-7-Cmer, and 12mer peptide libraries were screened. The target particles were either 7 nm, 15 nm, or 25 nm TiO<sub>2</sub>. More than 20 independent clones were selected. The binding was confirmed by electron microscopy analysis. The binding was shown to be specific by dose-dependency tests. After cross-binding experiments, the bindings were not shown to be particle size-specific. To identify the nature of the binding between a selected peptide and nanoparticles, an alanine scanning mutagenesis was done for the peptide. The putative role of amino acids responsible for binding are discussed.

F043

### The *sndA*, a Suppressor Gene of *nsdD*, Encodes a Zn(II)2Cys6 Protein That Negatively Regulates Sexual Development of *Aspergillus nidulans*

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The *Aspergillus nidulans nsdD* gene encodes a GATA-type transcription factor that is required for sexual development. To further understand the NsdD-mediated regulatory cascade, we isolated various suppressors of *nsdD* (SND) that restored cleistothecia development in the absence of *nsdD* function. Two mutant alleles (*sndA16* and *sndA88*) were identified in *sndA*, one of five complementation groups (*sndA-E*). They were all recessive to wild type allele and located on linkage group II. The *sndA* gene, which has an ORF with 693 bp and disrupted by an intron, is predicted to encode a putative transcription factor carrying a GAL4-like Zn(II)2Cys6 binuclear cluster DNA-binding domain. ORF deleted mutant developed a largely increased amount of cleistothecia, even in the presence of environmental stresses such as high osmolarity, visible light or acetate as sole C source. When the *sndA* gene was over expressed, cleistothecia were not formed. These results suggested that SndA is a negative regulator of sexual development. The *sndA* gene was expressed throughout the life cycle and not significantly affected by stresses. The gene expression was negatively regulated by *nsdD* or *veA*, the positive regulators of sexual development.

F044

### The 5'-end Nucleotide Sequences of the Porcine Reproductive and Respiratory Syndrome Virus Are Essential for RNA Replication: Discovery of Novel 5'-end Nucleotide Sequence Acquisition

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Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to the family *Arteriviridae* in the order *Nidovirales* together with equine arteritis virus, simian hemorrhagic fever virus, and the lactate dehydrogenase-elevating virus of mice. Like the other arteriviruses, PRRSV is a small-enveloped virus with a positive-sense, single-stranded RNA genome of ≈15 kb in length. The genome has a cap structure at its 5' end and a poly (A) tail at its 3' end. We have recently developed an efficient reverse genetics system for PRRSV by construction of an infectious bacterial artificial chromosome. Using our system, the viral cap structure and poly (A) tail are shown to be essential for replication. Serial mutations deleted up to seven nucleotides at the PRRSV 5' end in genomic RNAs show a gradually decrease in their replicability, while mutations deleted more than nine nucleotides completely abolish their replicability. Various pseudo-revertants are isolated and acquisition of novel 5' sequences composed of mostly A and T bases with various sizes appears to be responsible for restoring their replicability. Thus, this study provides new insight into functional elements in the PRRSV 5' end during RNA replication.