Isolation of Two *apsA* Suppressor Strains in *Aspergillus nidulans*

Miriam Krüger and Reinhard Fischer

*Philips Universität Marburg, Laboratorium für Mikrobiologie and Max-Planck-Institut für terrestrische Mikrobiologie, D-35043 Marburg, Germany*

Manuscript received April 4, 1996
Accepted for publication July 15, 1996

ABSTRACT

*Aspergillus nidulans* reproduces asexually with single nucleated conidia. In *apsA* (anucleate primary sterigmata) strains, nuclear positioning is affected and conidiation is greatly reduced. To get further insights into the cellular functions of *apsA*, aconidial *apsA* strains were mutagenized and conidiating suppressor strains were isolated. The suppressors fell into two complementation groups, *samA* and *samB* (suppressor of anucleate metulae). *samA* mapped on linkage group I close to *pyrG*. The mutant allele was dominant in diploids homozygous for *apsA*. Viability of conidia from *samA* suppressor strains (*samA*; *apsA* ) was reduced to 50% in comparison to wild-type conidia. Eighty percent of viable spores produced small size colonies that were temperature- and benomyl-sensitive. *samB* mapped to chromosome VIII and was recessive. Viability of conidia from *samB* suppressor strains (*apsA*; *samB* ) was also affected but no small size colonies were observed. Both suppressors produced partial defects in sexual reproduction and both suppressed an *apsA* deletion mutation. In wild-type background the mutant loci affected hyphal growth rate (*samA*) or changed the colony morphology (*samB*) and inhibited sexual spore formation (*samA* and *samB*). Only subtle effects on conidiation were found. We conclude that both suppressor genes bypass the *apsA* function and are involved in microtubule-dependent processes.

CELL biology, cellular differentiation and development in eukaryotic organisms are subjects of intensive studies. The filamentous fungus *Aspergillus nidulans* is a model organism for the analysis of these processes at a molecular level (TIMBERLAKE 1990; MABBITO and OSMANI 1994). Regulation of the cell cycle and cytoskeletal dependent organelle movements have been studied for more than 20 years (MORRIS 1976; OAKLEY and RINEHART 1985; MORRIS et al. 1995). Initial findings in the mold could often be generalized to higher eukaryotes. The most prominent example is γ-tubulin that is involved in microtubule assembly in all eukaryotes (OAKLEY and OAKLEY 1989; MORRIS and ENOS 1992).

Another aspect of general interest is the asexual development in *A. nidulans*. The asexual reproductive structures, called conidiophores, consist of only five different cell types, and morphological changes throughout differentiation are easy to observe. Each conidiophore arises from a specialized hyphal cell, the foot cell. Then, a stalk with a vesicle is formed that produces two layers of cells, metulae and phialides, respectively. Phialides continuously generate spores, named conidia (CLUTTERBUCK 1977; TIMBERLAKE 1991).

The morphological changes during conidiophore development are characterized by three striking transitions: (1) In contrast to unlimited hyphal growth apical extension of the stalk ceases after ~100 μm. Furthermore, vegetative hyphae grow at the surface of the substrate whereas the stalks are specialized aerial hyphae. (2) Hyphal tip growth changes to a budding-like process in conidiophores. (3) Hyphal compartments are multinucleated with mitosis and cytokinesis uncoupled. In contrast, a transition to uninucleated cells occurs in the conidiophore. Metulae, phialides and conidia contain only a single nucleus.

The molecular processes and their genetic regulation underlying the asexual differentiation have been elucidated by analyzing morphological mutants defective in conidiogenesis (CLUTTERBUCK 1969). Initial genetic studies and subsequent molecular approaches revealed at least two classes of developmental genes, transcriptional regulators and structural genes. After acquisition of developmental competence and exposure of hyphae to air and light a central cascade of at least four transcription factors leads to the initiation of the observed morphological changes (CLUTTERBUCK 1969, 1990; MARSHALL and TIMBERLAKE 1991; MILLER et al. 1992; PRADE and TIMBERLAKE 1993; ANDRIANOPoulos and TIMBERLAKE 1994).

In addition to transcriptional activators and structural genes another interesting class of genes has been identified to be necessary for completion of asexual development. These genes couple basic cellular functions like nuclear migration, cytokinesis and mitosis with developmental processes. Temperature-sensitive mutants of *A. nidulans* with a defect in nuclear migration only form very small colonies at restrictive temperature (MORRIS et al. 1995). Some nuclear distribution
mutants (nudF), however, form microcolonies at restrictive temperature that do not conidiate. Under semipermissive conditions abnormal conidiophores are produced (Xiang et al. 1995). Similar phenotypes were observed in some suppressor strains isolated as extragenic suppressors of nudA at permissive temperature. These mutants specifically stopped development at the metula stage (Goldman and Morris 1995).

A similar phenotype is found in apsA (anucleate primary sterigmata) mutants, in which nuclear positioning is affected. The mutation is not temperature-sensitive and the defect is most pronounced during asexual differentiation. In hyphae the nuclei are clustered instead of the evenly distributed nuclei in wild type, and in conidiophores nuclei accumulate in the vesicles without further distribution. This implies a function of the apsA gene in hyphal nuclear positioning and a crucial role for metular nucleation and completion of development (Morris et al. 1992). It encodes a 183-kD coiled-coil protein with three direct repeats and a PH-domain at the C-terminus. This suggests an interaction with cytoskeletal proteins like microtubules or a function in a signal transduction pathway (Musacchio et al. 1993; Gibson et al. 1994; Fischer and Timberlake 1995).

To further elucidate the cellular and developmental functions of the ApSA protein we isolated extragenic suppressors of the apsA mutation. Suppressor analysis has been shown to be a powerful tool for identifying proteins in multicomponent pathways in fungi. For example, in Saccharomyces cerevisiae suppressors of actin mutants were found that are involved in actin related processes (Novick et al. 1988). In A. nidulans, nudF was identified as an extracopy suppressor of nudC. It was shown that the nudC3 mutation reduced the protein level of NudF, suggesting a functional interaction between these two proteins (Chiu and Morris 1995; Xiang et al. 1995). Furthermore, y-tubulin, isolated as a suppressor of b-tubulin, was shown to physically interact with the latter protein (Oakley and Oakley 1989; Morris and Enos 1992).

In this paper we describe the isolation and characterization of two extragenic suppressors of apsA.

MUTAGENESIS AND ISOLATION OF REVERTANTS: apsA mutant strains were grown on complete media plates to a lawn and then sealed to induce the sexual life cycle of the fungus. After 2 weeks, mature cleistothecia were collected by scraping them off the plates. Cleistothecia were crushed in sterile water with a homogenizer. The resulting suspension was filtered through sterile miracloth and the derived ascospore suspension was counted and used for mutagenesis. Ascospores at concentrations of 5.0 × 10^7/ml were mutagenized in 1 ml of phosphate buffer (pH 7) with 0.05 M diethylsulfate. The suspension was shaken 30 min at 37°C and washed two times afterwards. The survival rate under these conditions was ~0.25%. Aliquots of 100 µl were plated on desoxycholate plates (see above), incubated for 3 days at 37°C and screened for the revertant phenotype. Revertants were colony purified on pure complete media.

STAINING AND MICROSCOPY: For examination of conidiophores by fluorescence microscopy we point inoculated microslide slides, which were covered with a thin film of media (solidified with 0.7% agarose), and grew the cultures at 37°C for 1–2 days in a petri dish with 25 ml of media not covering the microscope slide but making contact to the agarose film. During the fixation procedure with 8% formaldehyde in PME buffer (50 mM PIPES pH 6.7, 25 mM EGTA pH 8, 5 mM MgSO_4) the colonies came off the slide and floated on the surface. They were transferred to several washes in PME and stained with 0.1 µg/ml DAPI and 1 mg/ml phenylendiamine in PME with 0.1% triton added. Fluorescence microscopy was performed with a Zeiss Axioshot microscope with the appropriate filter combination (Clutterbuck 1994; Fischer and Timberlake 1995).

For scanning electron microscopy (SEM) colonies grown on plates were transferred with a piece of agar into 5% glutaraldehyde for fixation. After several steps of washes with water the pieces were transferred to ethylene glycol monothyl ether and incubated over night at room temperature. Then they were transferred to water free acetone, critical point dried, sputter coated with gold and observed in a Hitachi S-530 (Hitachi S-530, Japan) scanning electron microscope.

RESULTS

Phenotypic studies of apsA mutant strains suggested a role for ApSA in nuclear positioning in A. nidulans. To learn more about the molecular functions of apsA we took the approach of a suppressor analysis. Although the apsA mutation is neither temperature-sensitive nor sensitive to antimitotic drugs like benomyl, the aconidial phenotype of apsA mutants allowed a simple suppressor screening method to be applied: apsA mutants conidiate only very poorly and appear brown in contrast to the green-colored wild-type colonies. Thus, after mutagenesis of apsA spores we screened for green colonies.

Isolation of extragenic apsA suppressor mutations: Since apsA strains are nearly aconidial, but sexual spore formation is quite normal, we used ascospore suspensions for mutagenesis. Ascospores from seven different apsA mutant alleles were treated with diethylsulfate, spread on complete medium agar plates containing desoxycholate and incubated at 37°C. Among 50,000 brown colonies, 12 strains produced significantly more conidia than apsA strains (Figure 1). The pigmented conidia hide the brown color of the conidiophore stalks and vesicles and thus the suppressor colo-
Suppressors of \textit{apsA} in \textit{A. nidulans} 

\section*{TABLE 1}

\textbf{Aspergillus nidulans strains}

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCl.2</td>
<td>\textit{biA}; \textit{apsA}2</td>
<td>Clutterbuck (1969)</td>
</tr>
<tr>
<td>AJCl.17</td>
<td>\textit{biA}; \textit{apsA}36</td>
<td>Clutterbuck (1969)</td>
</tr>
</tbody>
</table>
| DES2X    | \textit{samA}; \textit{biA}; \textit{apsA}2 | This study\textsuperscript{a}
| SM12     | \textit{biA}; \textit{apsA}36; \textit{samB} | This study\textsuperscript{a}
| WTC2     | \textit{samA}; \textit{biA} | This study\textsuperscript{a}
| SM120    | \textit{biA}; \textit{samB} | This study\textsuperscript{a}
| SRF33    | \textit{biA}; \textit{waA}; \textit{\Delta apsA}; \textit{pyr4} | This study\textsuperscript{a}
| GR5      | \textit{pyrG}89; \textit{waA}; \textit{pyroA4} | G. May, Houston
| G1102    | \textit{pyroB12}; \textit{suAI}; \textit{diAI} | J. Clutterbuck, Glasgow
| G95      | \textit{sucA}10E20; \textit{yaA}; \textit{ade20}; \textit{acrA}; \textit{galA}; \textit{pyroA4}; \textit{facA}303; \textit{sB3}; \textit{nicB8}; \textit{riboB2} | J. Clutterbuck, Glasgow
| FGSC26   | \textit{biA} | Fungal Genetics Stock Center, Kansas |

\textsuperscript{a} Obtained by mutagenesis of AJCl.2.
\textsuperscript{b} Obtained by mutagenesis of AJCl.17.
\textsuperscript{c} Obtained by crossing DES2X to GR5.
\textsuperscript{d} Obtained by crossing SM12 to GR5.
\textsuperscript{e} Obtained by crossing SRF33 (Fischer and Timberlake 1995) to GR5.

Nies appeared light green. The strains were colony purified on complete medium without desoxycholate. Only four strains displayed a stable phenotype. In subsequent genetic studies two of them were further analyzed.

To test whether the suppression was due to extragenic or intragenic mutations, the revertants were crossed with the \textit{\textit{apsA}+} strain GR5. About 25% of the segregants in each cross showed again an \textit{\textit{apsA}+} phenotype, proving that the increasing number of conidia in the two revertants was caused by second site, extragenic mutations (Table 2, cross 1 and 2). These mutations were designated \textit{sam} (suppressor of anucleate 	extit{Eutulae}).

The ratio between wild-type-like conidiating strains (\textit{\textit{apsA}+}) and the sum of brown (\textit{\textit{apsA}−}) and light-colored suppressor strains was 1:1. This suggested that either suppressor mutation had nearly no effect in a \textit{\textit{apsA}−} background. However, a closer look at the wild-type-like progeny showed subtle differences in their phenotypes, including morphological changes in colony growth and reduced cleistothecia formation (see below). These differences appeared in \(\sim50\%\) of the wild-type colonies. Backcrosses of the latter segregants with \textit{\textit{apsA}+} strains showed Mendelian inheritance of the suppressor mutations, exemplified in cross 3 (Table 2). On the other hand, crosses with colonies without any detectable changes in phenotypes with \textit{\textit{apsA}−} strains led to wild-type and \textit{\textit{apsA}−} progeny. No suppressor phenotypes were found. Thus, these strains had been truly wild type. The two isolated suppressor strains were subjected to further genetic analyses.

\textbf{Genetic analyses:} Heterokaryon formation, nuclear fusion and meiotic segregation are prerequisites for classical genetic analyses, like the determination of complementation groups, mapping and dominance assays. Although all four suppressor strains originally isolated from the desoxycholate plates produced viable ascospores in crosses with wild-type strains, several attempts to cross the suppressors with each other failed. Three of the four were found to be self-sterile. Hence, it was impossible to determine complementation groups with this approach. However, in two cases, crosses between \textit{\textit{sam}−} \textit{\textit{apsA}+} strains produced hybrid cleistothecia and we were able to analyze the progeny. The subtle differences in phenotype allowed us to distinguish strains with the suppressor mutations (\textit{\textit{sam}−}; \textit{\textit{apsA}+}) and wild-type strains (\textit{\textit{sam}+}; \textit{\textit{apsA}+}). The genotypes of the corresponding strains were confirmed by backcrosses to \textit{\textit{apsA}−}. From this we concluded that the two suppressors belonged to two different complementation groups, named \textit{samA} and \textit{samB}.

To test whether \textit{samA} or \textit{samB} were dominant or recessive, diploids homozygous for \textit{\textit{apsA}+} and heterozygous for \textit{samA} and \textit{samB}, respectively, were constructed. The diploid \textit{samA−}; \textit{\textit{apsA}+}/\textit{samA+}; \textit{\textit{apsA}−} displayed a \textit{samA−}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Scanning electron microscopy (SEM) study of conidiation of an \textit{\textit{apsA}+} and suppressor strains of \textit{A. nidulans}. Colonies were grown at 37°C on plates, fixed and examined. (A) AJCl.2 (\textit{\textit{apsA}2}). (B) DES2X \textit{\textit{samA}; \textit{apsA}2}. (C) SM12 \textit{\textit{apsA}36; \textit{samB}}. Only relevant genotypes are given. For complete genotypes see Table 1. (A) Bar, 5 \textmu m. (B and C) Bar, 10 \textmu m.}
\end{figure}
TABLE 2
Segregation of *apsA*<sup>-</sup> and revertant phenotypes in crosses 1–3

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parent strains</th>
<th>Progeny</th>
<th>wt</th>
<th>Nonconidiating (<em>apsA</em>&lt;sup&gt;-&lt;/sup&gt;)</th>
<th>Conidiating (revertant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GR5 × DES2X</td>
<td>P1</td>
<td>64</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>GR5 × SMI12</td>
<td>P2</td>
<td>102</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>AJCl.2 × WTG2</td>
<td></td>
<td>114</td>
<td>55</td>
<td>58</td>
</tr>
</tbody>
</table>

In cross 1 and 2 the revertants were crossed with an *apsA*<sup>+</sup> strain, GR5. In cross 3, a wild-type like segregant from cross 1 was crossed with an *apsA*<sup>-</sup> strain, AJCl.2. wt, wild type.

Phenotypic characterization of *sam* mutants: In *apsA*<sup>-</sup> mutant strains nuclear positioning is affected in hyphae and conidiophores, giving rise to an irregular nuclear distribution pattern in hyphae and to a specific block of development. Conidiophore development stops at the metula stage (Figure 1A), because nuclei fail to migrate from vesicles into metulae. Anucleate primary sterigmata do not produce phialides and no spores. However, the mutation is leaky and some nuclei escape from the vesicles into metulae (Figure 1B). These metulae differentiate further and then single chains of conidia were formed. Mutation of either suppressor gene, *samA* or *samB*, increased the number of conidia five- to 10-fold (Figure 1, B and C) and thus caused a color change of colonies from brown to light green. The spore number is still much lower than in wild-type strains and the phenotypic rescue therefore only partial. Microscopic analysis confirmed that nuclear distribution in suppressor conidiophores was still *apsA*<sup>-</sup>-like. Metulae and phialides were often multinucleated and misshapen (Figure 3, C and D). Additionally, also multinucleated conidia were found (data not shown). The number of nuclei in conid-
Suppressors of \( \text{apsA} \) in \( A. \) nidulans

Nuclear distribution in conidiophores of \( \text{apsA} \) suppressor and wild-type \( A. \) nidulans strains. (A) FGSC26 (wild type). Metulae (m) and phialides (p) contain one nucleus each. (B) AJC1.2 (\( \text{apsA} \)). Development is inhibited at the metula stage. Nuclei are gathered in the vesicle (v) and one escaped into a metula. This metula proceeded development (arrowhead). The corresponding metula and phialide are multinucleated. (C) DES2X (\( \text{samA; apsA} \)) and (D) SM112 (\( \text{apsA} \); \( \text{samB} \)). Many metulae and phialides contain several nuclei. Bar, 10 \( \mu \)m.

Conidiophore vesicles appeared to be increased in comparison to wild-type strains (Figure 3, A, C and D). In addition, nuclear distribution in hyphae was also irregular with clumps of nuclei (data not shown). \( \text{samA} \) and \( \text{samB} \) had only minor effects in \( \text{apsA} \) wild-type strains. Conidiophore morphology sometimes appeared to be affected, with metulae, phialides and conidia with irregular size and shape (Figure 4, A–C). The frequency of bi- or multinucleated conidia was increased.

Besides these common properties both suppressors displayed allele-specific phenotypes. \( \text{samA} \) suppressor strains (\( \text{samA}^{-} \); \( \text{apsA}^{-} \)) grew slower than wild-type strains and they were self-sterile in all genetic backgrounds tested. Conidiation in the suppressor strain was delayed. Older colonies appeared green after 5 days of growth at 37°. Nutrient limitation or osmotic supplementation of the media had no effect on the phenotype. Viability of conidia of the suppressor strain was reduced to 50% in comparison to wild-type spores. In addition, \( \sim \)80% of viable spores produced only small size colonies, 3 mm in diameter after 2 days of growth (microcolonies). The remaining 20% produced normal size colonies (1 cm after 2 days). After prolonged incubation of the microcolonies at 37° fast growing sectors grew out (Figure 5). Spores of the small and the normal size colonies produced colonies of either type. This phenotype resembled the growth properties of aneuploid \( A. \) nidulans strains, which grow poorly until they lose their extra chromosome(s) (S. Assinder, personal communication). In the presence of the microtubule drug benomyl or incubation at 16°, growth of microcolonies was severely reduced whereas growth of "normal" colonies and of wild-type strains was only slightly inhibited (Figure 6). This difference in growth was also found in germlings of suppressor strains. Surprisingly, conidia of different \( \text{apsA}^{-} \) alleles (AJC1.2, AJC1.17) and the deletion strain SRF53 also produced small colonies, although with a very low frequency (Table 3). This sug-
Figure 6.—Growth of the two types of colonies from DES2X and of wild type at different conditions. Strains were grown on complete media for 3 days at 37°C (first column) or for 11 days at 16°C (second column). Complete media was supplemented with 0.5 µg/ml benomyl (third column).

suggested that the effect of samA− was only an enhancement of an apsA defect. The samA mutation in a wild-type background (samA−; apsA+) had only minor effects (see above). Growth rate was reduced and the strains failed to produce cleistothecia.

Colonies of samB suppressor strains (apsA−; samB−) grew like wild type but they appeared rather “flat.” Some cleistothecia were produced but they failed to generate ascospores. Conidia appeared after 2 days of growth at 37°C but lysed after prolonged incubation. The lysis was not observed when osmotically stabilized media was used. Viability of conidia was reduced like in samA− suppressor strains but microcolonies were observed only with the frequency of apsA strains (Table 3).

The samB− mutation in a wild-type background (apsA−; samB−) had also minor effects. Colonies displayed a flat phenotype and only nonproductive cleistothecia were found.

DISCUSSION

A suppressor analysis was initiated to learn more about functions of the ApsA protein that appears to be involved in nuclear positioning. When apsA is mutated, metulae remain anucleated and fail to differentiate further. However, the mutation is leaky and a residual number of conidia is produced in some conidiophores. The molecular function of the 183-kD protein remained unclear, although a coiled-coil motif at the amino-terminus and a PH-domain at the carboxy-terminus suggested a function either in interaction with cytoskeletal proteins or in a signal transduction pathway (Musacchio et al. 1993; Clutterbuck 1994; Gibson et al. 1994; Fischer and Timberlake 1995).

In this study two apsA suppressor strains (samA and samB) were isolated and characterized. The suppressor mutations partially rescued the oligosporogenic phenotype of apsA mutant strains. The number of conidia increased significantly, but conidiophore morphology in samA and samB suppressor strains still markedly differed from wild-type conidiophores. Moreover, metulae, phialides and conidia were often multinucleated in contrast to the uninucleated cells in wild-type strains. This phenotype was also observed in apsA null mutants when suppressed by samA or samB. This suggested that the suppressors probably bypass the apsA function than directly interact with the ApsA protein. Nevertheless, molecular analysis of the suppressor genes will help to elucidate the molecular function of apsA. Similarly, Willins et al. (1995) isolated a suppressor of the nuclear migration gene nudF and found also a suppression of nudA, nudC, nudG and the deletions of nudA and nudF. Sequence analysis showed that the suppression was due to a mutation in the α-tubulin gene tufA.

In a conidiospore analysis samA suppressor strains (samA−; apsA−) produced, with a high frequency, microcolonies with fast growing sectors. This phenotype resembled aneuploid A. nidulans strains, which grow poorly until the nuclei become haploid (Ushall and Mortimore 1984; S. Assinder, personal communication). Given that a misdistribution of chromosomes leads to the observed microcolonies in samA strains, this also could explain the reduced number of viable spores. Interestingly, microcolonies were also observed with conidia of apsA strains, although at a very low rate. Hence, samA drastically enhanced this apsA defect. One possible explanation is an involvement of samA and apsA in microtubule-dependent processes, with apsA being involved in nuclear positioning and apsA and samA in mitosis. This idea is supported by results found in CIN (chromosome instability) mutants in yeast, which are also involved in microtubule-dependent processes (Hoyt et al. 1990). The samA suppressor mutation did not rescue the nuclear positioning defect in hyphae and conidiophores but increased the number of nuclei in metulae, giving rise to the production of more conidia than in apsA strains. Since in apsA mutants metulae also are multinucleated, samA also enhanced this

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of small colonies (%)</th>
<th>No. of regular sized colonies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES2X</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>AFC1.2</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>SRF53</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>AFC1.17</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>SMI12</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

Conidia suspensions were harvested from strains incubated on complete medium plates at 37°C for 5 days. Conidia were plated on complete media and colonies were counted after 2 days of growth at 37°C.
apsA phenotype. Additionally, samA mutant strains were sexually self-sterile, which could be due to a defect in meiosis, another microtubule-dependent process. These possible functions for samA and apsA are supported by results recently obtained in the S. cerevisiae NUM1 mutant. These strains have a defect in nuclear distribution and also generate aneuploid daughter cells with high frequency. Production of meiotically derived ascospores is also impaired. For NUM1, a homologue of apsA, a stabilizing function of astral microtubules has been proposed (Kormanec et al. 1991; Revarel and Agle 1993; Farkasovsky and Kuntzel 1995).

In samB strains the suppression of the apsA mutation was also due to an increased number of nuclei in metulae as compared to apsA strains. Although samB resembled samA in this respect, in samB no defect in mitosis was observed. No increase in production of microcolonies could be detected. One possible molecular function of samB could be the sensing of the number of nuclei in single cellular compartments like the metulae. Besides the multinucleated cells in the conidiophores, the following phenotypic observations were indicative of another possibility for a molecular function of samB. samB suppressor strains produced conidia that lysed after prolonged incubation. The destruction could be prevented by osmotically stabilized media. Interestingly, osmotically sensitive yeast mutants were isolated. Molecular analysis of these mutants revealed that mutagenesis of actin was responsible for the phenotype (Novick and Botstein 1985; Chowdhury et al. 1992; Wertman et al. 1992; Mulholland et al. 1994). If the actin cytoskeleton would be involved in nuclear sensing in A. nidulans, both effects, multinucleated cells and the osmotic dependency, could be explained. Surprisingly, Plamann et al. (1994) and Robb et al. (1995) characterized rtpy mutants in Neurospora crassa and observed that an actin related protein was involved in nuclear positioning in this fungus.

Only subtle changes of phenotypes were found in wild-type strains containing the mutated suppressor genes samA or samB. Similar phenomena have been described in strains with mutations in α-tubulin (tubα), γ-tubulin (mipA) and with the development affecting mutation sthenyo, where only the null mutants led to the functions of the genes (Weil et al. 1986; Oakley and Oakley 1989; Kirk and Morris 1991; Kirk and Morris 1993; Gems and Clutterbuck 1994). Therefore, a molecular analysis with cloning, sequencing and deletion of the two genes will be crucial for a detailed understanding of their cellular functions and their interplay with apsA.

We thank Dr. J. Clutterbuck for providing the apsA mutant and other A. nidulans strains. We thank Dr. S. Assinder for the help with the analysis of the suppressor strains. We are grateful to Dr. G. Kost and his laboratory for their assistance with SEM. We thank also our colleagues for helpful discussions and critical reviews of the manuscript. We thank DePont for the gift of Benomyl. This work was supported by the Deutsche Forschungsgemeinschaft, the Philipps-Universität Marburg and the Max-Planck-Gesellschaft.

LITERATURE CITED


Communicating editor: D. BOTSTEIN