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

Miriam Krüger and Reinhard Fischer

Philipps 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 of *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; MIRABITO 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

Corresponding author: Reinhard Fischer, Philipps Universität Marburg, Laboratorium für Mikrobiologie, Karl-von-Frisch-Str., D-35043 Marburg, Germany. E-mail: fischerr@mailer.uni-marburg.de

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 (CLUTTERBUCK 1994).

The *apsA* gene has been analyzed at a molecular level. 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, γ-tubulin, isolated as a suppressor of β-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*.

MATERIALS AND METHODS

Aspergillus strains and growth methods: *A. nidulans* strains (Table 1) were grown at 37° on complete media plates (2% glucose, 1.5% agar, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, nitrate salts, trace elements and vitamins) or on appropriately supplemented minimal media plates (1% glucose, 1.5% agar, nitrate salts and trace elements). For osmotic stabilization 1.2 M sorbitol or 0.6 M potassium chloride was added. After mutagenesis spores were plated on complete media supplemented with 0.1% sodium-desoxycholate to keep the colonies smaller (MACKINTOSH and PRITCHARD 1963). Standard genetic techniques for *A. nidulans* were used for strain constructions essentially as described in KÄFER (1977) and GOLDMAN and MORRIS (1995).

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 homogenisator. 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° 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° 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 microscope slides, which were covered with a thin film of media (solidified with 0.7% agarose), and grew the cultures at 37° 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 Axiophot 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 monoethyl 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 alelles were treated with diethylsulfate, spread on complete medium agar plates containing desoxycholate and incubated at 37°. 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-

TABLE 1

***Aspergillus nidulans* strains**

Strain	Genotype	Source
AJC1.2	*biA1; apsA2*	CLUTTERBUCK (1969)
AJC1.17	*biA1; apsA36*	CLUTTERBUCK (1969)
DES2X	*samA, biA1; apsA2*	This study[a]
SMI12	*biA1; apsA36; samB*	This study[b]
WTG2	*samA, biA1*	This study[c]
SMI20	*biA1; samB*	This study[d]
SRF53	*biA1; wA3; ΔapsA::pyr4*	This study[e]
GR5	*pyrG89; wA3; pyroA4*	G. MAY, Houston
G1102	*pyroB12, sulA1; dilA1*	J. CLUTTERBUCK, Glasgow
G95	*suA1adE20, yA2, adE20; acrA1; galA1; pyroA4; facA303; sB3; nicB8; riboB2*	J. CLUTTERBUCK, Glasgow
FGSC26	*biA1*	Fungal Genetics Stock Center, Kansas

[a] Obtained by mutagenesis of AJC1.2.
[b] Obtained by mutagenesis of AJC1.17.
[c] Obtained by crossing DES2X to GR5.
[d] Obtained by crossing SMI12 to GR5.
[e] Obtained by crossing SRF30 (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 *apsA*$^+$ strain GR5. About 25% of the segregants in each cross showed again an *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 *sam* (suppressor of anucleate metulae).

The ratio between wild-type-like conidiating strains (*apsA*$^+$) and the sum of brown (*apsA*$^-$) and light-colored suppressor strains was 1:1. This suggested that either suppressor mutation had nearly no effect in an *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 cleisthothecia formation (see below). These differences appeared in ~50% of the wild-type colonies. Backcrosses of the latter segregants with *apsA*$^-$ strains showed Mendelian inheritance of the suppressor mutations, examplified in cross 3 (Table 2). On the other hand, crosses with colonies without any detectable changes in phenotypes with *apsA*$^-$ strains led to wild-type and *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.

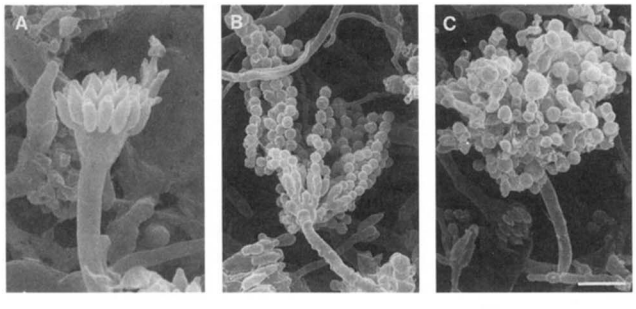

FIGURE 1.—Scanning electron microscopy (SEM) study of conidiation of an *apsA* and suppressor strains of *A. nidulans*. Colonies were grown at 37° on plates, fixed and examined. (A) AJC1.2 (*apsA2*), (B) DES2X (*samA;* apsA2), (C) SMI12 (*apsA36; samB*). Only relevant genotypes are given. For complete genotypes see Table 1. (A) Bar, 5 μm. (B and C) Bar, 10 μm.

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 *sam*$^-$ *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 (*sam*$^-$; *apsA*$^+$) and wild-type strains (*sam*$^+$; *apsA*$^+$). The genotypes of the corresponding strains were confirmed by backcrosses to *apsA*. From this we concluded that the two suppressors belonged to two different complementation groups, named *samA* and *samB*.

To test whether *samA* or *samB* were dominant or recessive, diploids homozygous for *apsA* and heterozygous for *samA* and *samB*, respectively, were constructed. The diploid *samA*$^-$; *apsA*$^-$/*samA*$^+$; *apsA*$^-$ displayed a *samA*$^-$

TABLE 2

Segregation of *apsA*$^-$ and revertant phenotypes in crosses 1–3

Cross	Parent strains P1		P2	Progeny wt	Nonconidiating (*apsA*$^-$)	Conidiating (revertant)
1	GR5	×	DES2X	64	29	29
2	GR5	×	SMI12	102	45	41
3	AJC1.2	×	WTG2	114	55	58

In cross 1 and 2 the revertants were crossed with an *apsA*$^+$ strain, GR5. In cross 3, a wild-type like segregant from cross 1 was crossed with an *apsA*$^-$ strain, AJC1.2. wt, wild type.

phenotype, indicating that it was a dominant mutation. In contrast, the diploid constructed to test the *samB* mutation (*apsA*$^-$; *samB*$^-$/*apsA*$^-$; *samB*$^+$) showed an aconidial *apsA* phenotype, which is expected for a recessive mutation. However, both diploids were not stable on complete medium and haploidized even in the absence of benomyl.

The *samA* and *samB* suppressor mutations were isolated from the two *apsA* mutant strains AJC1.2 (*apsA2*) and AJC1.17 (*apsA36*), respectively. Interestingly, all *apsA* mutant alleles including a null mutant of *apsA* (SRF53) were suppressed by either suppressor. This suggested that in the suppressor strains the *apsA* function was probably bypassed.

To map the two suppressor genes on the linkage groups of *A. nidulans* we studied the cosegregation of the genes with known auxotrophy marker genes after haploidization of diploids or after meiotic segregation. Diploid analysis of *samB* placed the gene on linkage group VIII. *samA* mapped on chromosome *I*. It cosegregated with *pyrG*. In subsequent crosses it mapped close to *pyroB* and *sulA* (Figure 2).

Phenotypic characterization of *sam* mutants: In *apsA* 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 3B). 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*$^-$-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-

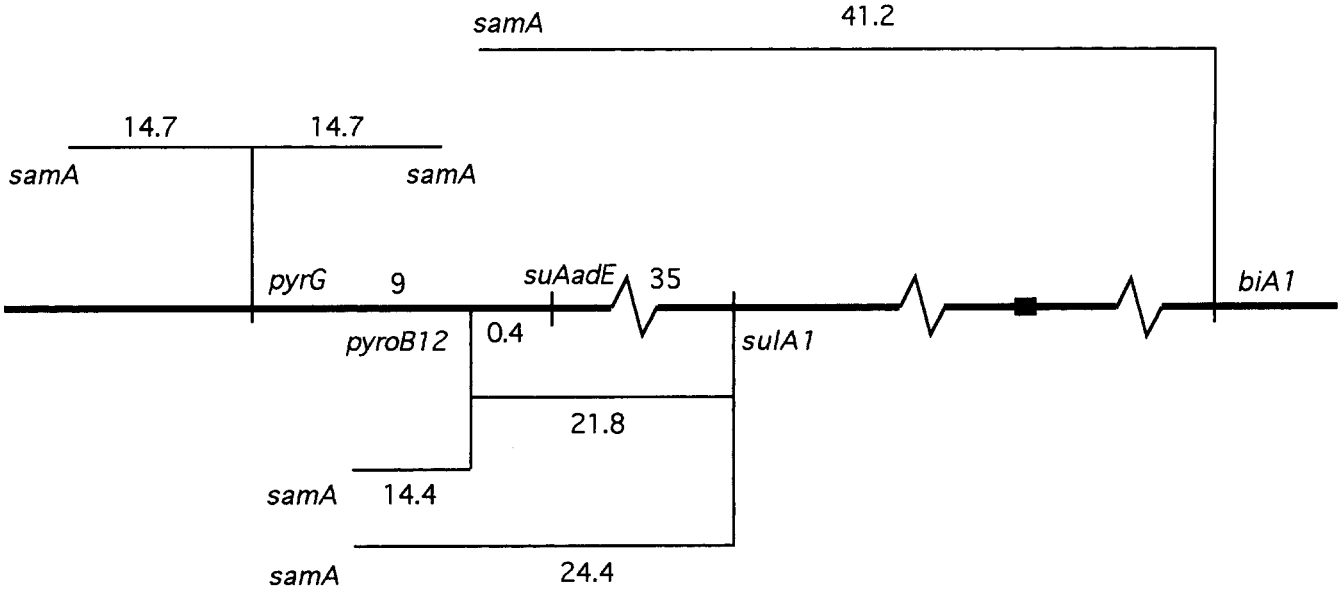

FIGURE 2.—Mapping of *samA* on linkage group I. The distance relative to *pyrG89* was derived from cross GR5 × DES2X; the relation to *sulA1* and *pyroB12* was derived from cross G1102 × DES2X (distances indicated in cM).

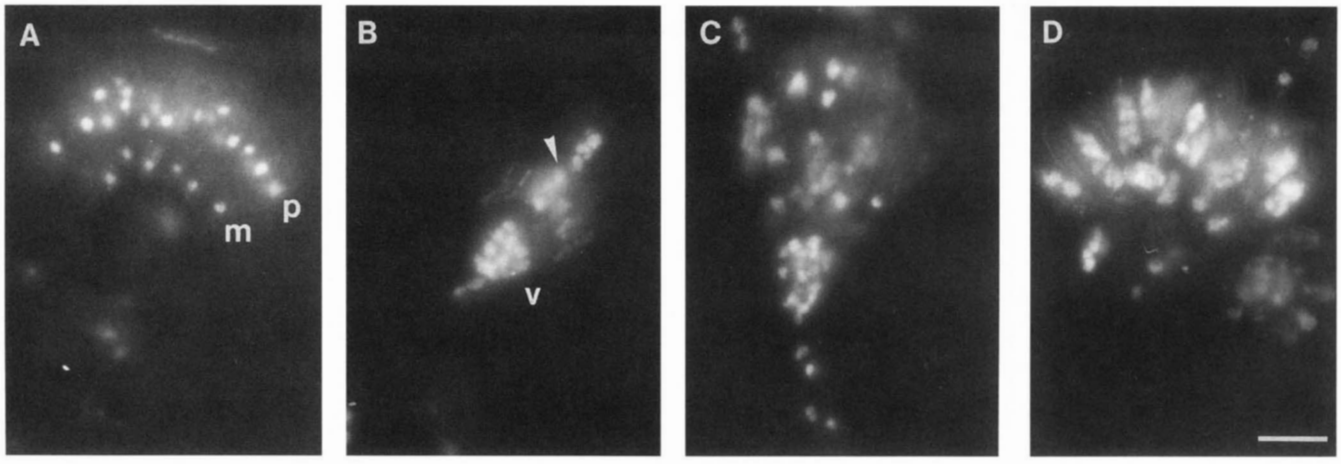

FIGURE 3.—Nuclear distribution in conidiophores of *apsA*, suppressor and wild-type *A. nidulans* strains. (A) FGSC26 (wild type). Metulae (m) and phialides (p) contain one nucleus each. (B) AJC1.2 (*apsA2*). Development is inhibited at the metula stage. Nuclei are gathered in the vesicle (v) and one escaped into a metula. This metula proceeded development (arrowhead). The corresponding metula and phialide are multinucleated. (C) DES2X (*samA; apsA2*) and (D) SMI12 (*apsA36; samB*). Many metulae and phialides contain several nuclei. Bar, 10 μm.

iophore 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). *samA* and *samB* had only minor effects in *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. *samA* suppressor strains (*samA*$^-$; *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, ~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 loose 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 *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-

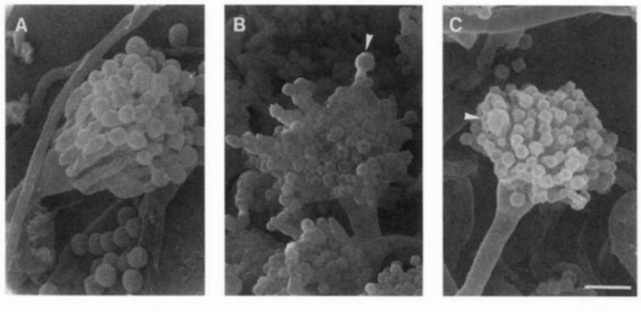

FIGURE 4.—SEM study of conidiophores of a wild-type *A. nidulans* strain and of suppressor mutations in wild-type background. Colonies were grown at 37° on plates, fixed and examined. (A) FGSC26 (wild-type) and suppressor mutations in wild-type background. (B) WTG2 (*samA*). (C) SMI20 (*samB*). Misshapen conidia were found frequently in suppressor strains (arrowhead). (A) Bar, 5 μm. (B and C) Bar, 10 μm.

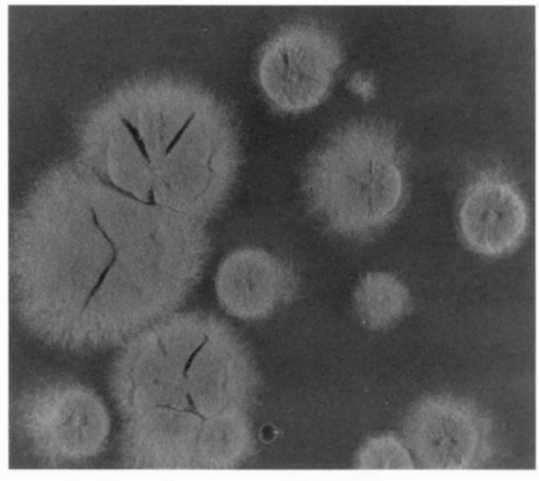

FIGURE 5.—DES2X (*samA; apsA2*) colonies grown from conidia (4 days at 37°). Normal size colonies and microcolonies with outgrowing sectors were observed.

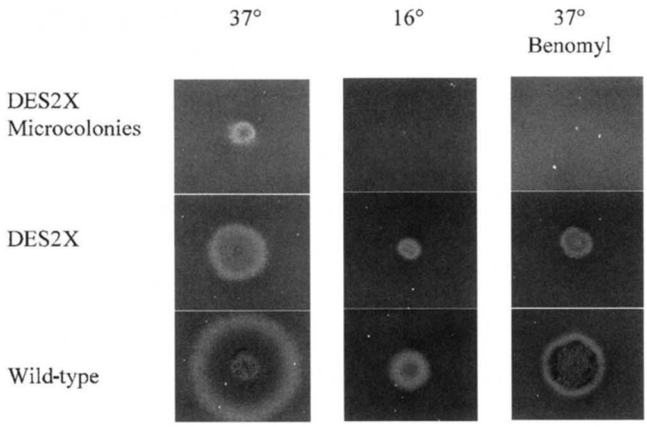

FIGURE 6.—Growth of the two types of colonies from DES2X and of wild type at different conditions. Strains were grown on complete media for 3 days at 37° (first column) or for 11 days at 16° (second column). Complete media was supplemented with 0.5 μg/ml benomyl (third column).

gested 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° but lyzed 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 conidiospores 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 *tubA*.

TABLE 3

Analysis of microcolonies

Strain	No. of small colonies (%)	No. of regular sized colonies (%)
DES2X	82	18
AJC1.2	17	83
SRF53	7	93
AJC1.17	3	97
SMI12	5	95

Conidia suspensions were harvested from strains incubated on complete medium plates at 37° for 5 days. Conidia were plated on complete media and colonies were counted after 2 days of growth at 37°.

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 (UPSHALL 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

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; REVARDEL and AIGLE 1993; FARKASOVSKY and KÜNTZEL 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 *ropy* 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 (*tubB*), 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 DuPont for the gift of Benomyl. This work was supported by the Deutsche Forschungsgemeinschaft, the Philipps-Universität Marburg and the Max-Planck-Gesellschaft.

LITERATURE CITED

ANDRIANOPOULOS, A., and W. E. TIMBERLAKE, 1994 The *Aspergillus nidulans abaA* gene encodes a transcriptional activator that acts as a genetic switch to control development. Mol. Cell. Biol. **14:** 2503–2515.

CHIU, Y., and N. R. MORRIS, 1995 Extragenic suppressors of nudC3, a mutation that blocks nuclear migration in *Aspergillus nidulans.* Genetics **141:** 453–464.

CHOWDHURY, S., K. W. SMITH and M. C. GUSTIN, 1992 Osmotic stress and the yeast cytoskeleton: phenotype-specific suppression of an actin mutation. J. Cell Biol. **118:** 561–571.

CLUTTERBUCK, A. J., 1969 A mutational analysis of conidial development in *Aspergillus nidulans.* Genetics **63:** 317–327.

CLUTTERBUCK, A. J., 1977 The genetics of conidiation in *Aspergillus nidulans,* pp. 305–318 in *Genetics and Physiology of Aspergillus,* edited by J. E. SMITH and J. A. PATEMAN. Academic Press, London.

CLUTTERBUCK, A. J., 1990 The genetics of conidiophore pigmentation in *Aspergillus nidulans.* J. Gen. Microbiol. **136:** 1731–1738.

CLUTTERBUCK, A. J., 1994 Mutants of *Aspergillus nidulans* deficient in nuclear migration during hyphal growth and conidiation. Microbiol. **140:** 1169–1174.

FARKASOVSKY, M., and H. KÜNTZEL, 1995 Yeast Num1p associates with the mother cell cortex during S/G2 phase and affects microtubular functions. J. Cell Biol. **131:** 1003–1014.

FISCHER, R., and W. E. TIMBERLAKE, 1995 *Aspergillus nidulans apsA* (anucleate primary sterigmata) encodes a coiled-coil protein necessary for nuclear positioning and completion of asexual development. J. Cell Biol. **128:** 485–498.

GEMS, D. H., and J. CLUTTERBUCK, 1994 Enhancers of conidiation mutants in *Aspergillus nidulans.* Genetics **137:** 79–85.

GIBSON, T. J., M. HYVÖNEN, A. MUSACCHIO and M. SARASTE, 1994 PH domain: the first anniversary. Trends Biol. Sci. **19:** 349–353.

GOLDMAN, G. H., and N. R. MORRIS, 1995 Extragenic suppressors of a dynein mutation that blocks nuclear migration in *Aspergillus nidulans.* Genetics **139:** 1223–1232.

HOYT, M. A., T. STEARNS and D. BOTSTEIN, 1990 Chromosome instability mutants of *Saccharomyces cerevisiae* that are defective in microtubule-mediated processes. Mol. Cell. Biol. **10:** 223–234.

KÄFER, E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:** 33–131.

KIRK, K. E., and N. R. MORRIS, 1991 The *tubB* α-tubulin gene is essential for sexual development in *Aspergillus nidulans.* Genes Dev. **5:** 2014–2023.

KIRK, K. E., and N. R. MORRIS, 1993 Either alpha-tubulin isogene product is sufficient for microtubule function during all stages of growth and differentiation in Aspergillus nidulans. Mol. Cell. Biol. **13:** 4465–4476.

KORMANEC, J., I. SCHAAFF-GERSTENSCHLÄGER, R. K. ZIMMERMANN, D. PERECKO and H. KÜNTZEL, 1991 Nuclear migration in *Saccharomyces cerevisiae* is controlled by the highly repetitive 313 kDa NUM1 protein. Mol. Gen. Genet. **230:** 277–287.

MACKINTOSH, M. E., and R. H. PRITCHARD, 1963 The production and replica plating of micro-colonies of *Apergillus nidulans.* Genet. Res. **4:** 320–322.

MARSHALL, M. A., and W. E. TIMBERLAKE, 1991 *Aspergillus nidulans wetA* activates spore-specific gene expression. Mol. Cell. Biol. **11:** 55–62.

MILLER, K. Y., J. WU and B. L. MILLER, 1992 *StuA* is required for cell pattern formation in *Aspergillus.* Genes Dev. **6:** 1770–1782.

MIRABITO, P. M., and S. A. OSMANI, 1994 Interactions between the developmental program and cell cycle regulation of *Aspergillus nidulans.* Dev. Biol. **5:** 139–145.

MORRIS, N. R., 1976 Mitotic mutants of *Aspergillus nidulans.* Genet. Res. **26:** 237–254.

MORRIS, N. R., and A. P. ENOS, 1992 Mitotic gold in a mold: *Aspergillus* genetics and the biology of mitosis. Trends Genet. **8:** 32–37.

MORRIS, N. R., X. XIANG and S. M. BECKWITH, 1995 Nuclear migration advances in fungi. Trends Cell. Biol. **5:** 278–282.

MULHOLLAND, J., D. PREUSS, A. MOON, A. WONG, D. DRUBIN *et al.,* 1994 Ultrastructure of the yeast actin cytoskeleton and its association with the plasma membrane. J. Cell Biol. **125:** 381–391.

MUSACCHIO, A., T. GIBSON, P. RICE, J. THOMPSON and M. SARASTE, 1993 The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biol. Sci. **18:** 343–348.

NOVICK, P., and D. BOTSTEIN, 1985 Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40:** 405–416.

NOVICK, P., B. C. OSMOND and D. BOTSTEIN, 1988 Suppressors of yeast actin mutations. Genetics **121:** 659–674.

OAKLEY, B. R., and J. E. RINEHART, 1985 Mitochondria and nuclei move by different mechanisms in *Aspergillus nidulans*. J. Cell Biol. **101:** 2392–2397.

OAKLEY, C. E., and B. R. OAKLEY, 1989 Identification of gamma-tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. Nature **338:** 662–664.

PLAMANN, M., P. F. MINKE, J. H. TINSLEY and K. S. BRUNO, 1994 Cytoplasmic dynein and actin-related protein Arp1 are required for normal nuclear distribution in filamentous fungi. J. Cell Biol. **127:** 139–149.

PRADE, R., and W. E. TIMBERLAKE, 1993 The *Aspergillus nidulans brlA* regulatory locus consists of two overlapping transcription units that are individually required for conidiophore development. EMBO J. **12:** 2439–2447.

REVARDEL, E., and M. AIGLE, 1993 The *NUM1* yeast gene: length polymorphism and physiological aspects of mutant phenotype. Yeast **9:** 495–506.

ROBB, M. J., M. A. WILSON and P. J. VIERULA, 1995 A fungal actin-related protein involved in nuclear migration. Mol. Gen. Genet. **247:** 583–590.

TIMBERLAKE, W. E., 1990 Molecular genetics of *Aspergillus* development. Ann. Rev. Genet. **24:** 5–36.

TIMBERLAKE, W. E., 1991 Temporal and spatial controls of *Aspergillus* development. Curr. Opin. Genet. Dev. **1:** 351–357.

UPSHALL, A., and I. D. MORTIMORE, 1984 Isolation of aneuploid-generating mutants of *Aspergillus nidulans*, one of which is defective in interphase of the cell cycle. Genetics **108:** 107–121.

WEIL, C. F., C. E. OAKLEY and B. R. OAKLEY, 1986 Isolation of *mip* (microtubule-interacting protein) mutations of *Aspergillus nidulans*. Mol. Cell. Biol. **6:** 2963–2968.

WERTMAN, K. F., D. G. DRUBIN and D. BOTSTEIN, 1992 Systematic mutational analysis of the yeast *ACT1* gene. Genetics **132:** 337–350.

WILLINS, D. A., X. XIANG and N. R. MORRIS, 1995 An alpha-tubulin mutation suppresses nuclear migration mutations in *Aspergillus nidulans*. Genetics **141:** 1287–1298.

XIANG, X., A. H. OSMANI, S. A. OSMANI, M. XIN and N. R. MORRIS, 1995 *NudF*, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human *LIA-1* gene required for neuronal migration. Mol. Biol. Cell **6:** 297–310.

Communicating editor: D. BOTSTEIN