The *Aspergillus nidulans sepA* gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity

Steven D. Harris  
*University of Nebraska - Lincoln*, sharris2@unl.edu

Lisbeth Hamer  
*Purdue University, West Lafayette, IN*

Kathryn E. Sharpless  
*University of Connecticut Health Center, Farmington, CT*

John E. Hamer  
*Purdue University, West Lafayette, IN*

Follow this and additional works at: http://digitalcommons.unl.edu/plantpathpapers

Part of the Plant Pathology Commons

http://digitalcommons.unl.edu/plantpathpapers/50

This Article is brought to you for free and open access by the Plant Pathology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Plant Pathology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
The Aspergillus nidulans sepA gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity

Steven D. Harris1, Lisbeth Hamer2, Kathryn E. Sharpless and John E. Hamer2

Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06030-3205 and 2Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392, USA

1Corresponding author

Cytokinesis (septation) in the fungus Aspergillus nidulans occurs through the formation of a transient actin ring at the incipient division site. Temperature-sensitive mutations in the sepA gene prevent septation and cause defects in the maintenance of cellular polarity, without affecting growth and nuclear division. The sepA gene encodes a member of the growing family of FH1/2 proteins, which appear to have roles in morphogenesis and cytokinesis in organisms such as yeast and Drosophila. Results from temperature shift and immunofluorescence microscopy experiments strongly suggest that sepA function requires a preceding mitosis and that sepA acts prior to actin ring formation. Deletion mutants of sepA exhibit temperature-sensitive growth and severe delays in septation at the permissive temperature, indicating that expression of another gene may compensate for the loss of sepA. Conidiophores formed by sepA mutants exhibit abnormal branching of the stalk and vesicle. These results suggest that sepA interacts with the actin cytoskeleton to promote formation of the actin ring during cytokinesis and that sepA is also required for maintenance of cellular polarity during hyphal growth and asexual morphogenesis.

Keywords: Aspergillus/cellular polarity/cytokinesis/FH1/2 protein/septation

Introduction

Cytokinesis requires assembly of the division apparatus at a specified cortical site. In animal cells, a contractile ring forms at the division site and then acts to cleave the cell such that two daughter cells are produced (reviewed by Satterwhite and Pollard, 1992). Amongst the major components of the contractile ring are actin and myosin, whose interaction provides the mechanical forces necessary for contraction of the ring and cell cleavage (reviewed by Fishkind and Wang, 1995). Although signals emanating from the mitotic spindle are known to specify the location of the division site (Rappaport, 1986), the mechanisms which lead to organization of the actin cytoskeleton at the selected site remain poorly understood. Initial insights into the nature of these mechanisms may be drawn from the observation that small GTP binding proteins of the rho class are required for cytokinesis in a number of cell types (Kishi et al., 1993; Mabuchi et al., 1993; Larochelle et al., 1996). This class of GTPases (including rho, rac and cdc-42) controls the re-organization of the actin cytoskeleton required for formation of cellular extensions in fibroblasts (Chant and Stowers, 1995; Ridley, 1996). Conceivably, activated rho-related GTPases may trigger recruitment of proteins required for nucleation and polymerization of actin filaments to the division site. Proteins such as the septins (Longtine et al., 1996) and the actin-associated proteins anillin and radixin (Sato et al., 1991; Field and Alberts, 1995) may then act in a downstream manner to control formation of the contractile ring.

The actin cytoskeleton also plays a critical role in cytokinesis in fungal cells. In particular, the division apparatus is thought to organize an actin ring at the incipient division site (Raudaskoski, 1970; Hoch and Howard, 1980; Kilmartin and Adams, 1984; Marks and Hyams, 1985). Presumably, the actin ring directs vesicle-mediated delivery of cell wall biosynthetic precursors to this site. Ultimately, this leads to cell division via the centripetal formation of a cross-wall, termed a septum. Genetic screens in both the fission yeast Schizosaccharomyces pombe and the budding yeast Saccharomyces cerevisiae have resulted in identification of a number of genes required for septum formation. In particular, six different genes have been shown to be required for assembly of the actin ring in S. pombe (Chang et al., 1996). Two of these genes, cdc3 and cdc8, encode the actin-associated proteins profilin and tropomyosin respectively (Balasubramanian et al., 1992, 1994). A third gene, cdc4, may function in a calcium-dependent fashion to regulate actin ring formation (Collum et al., 1995). In S. cerevisiae, the septins (encoded by the CDC3, CDC10, CDC11 and CDC12 genes; reviewed by Longtine et al., 1996) may play a role in the re-organization of the actin cytoskeleton which precedes septum formation (Adams and Pringle, 1984). Despite the successful application of genetic approaches to identify numerous gene products required for septum formation in yeast cells, it is not yet clear how the actin ring is assembled.

We have used a genetic approach to identify gene products required for septum formation in the filamentous fungus Aspergillus nidulans. Previous studies have demonstrated that a transient actin ring forms at the incipient division site in A. nidulans and that loss of functional microfilaments prevents septum formation (Harris et al., 1994). Amongst a group of eight genes required for cell division in A. nidulans, four (sepA, sepD, sepG and sepH) appear to encode products which function late in septum formation (Harris et al., 1994). These gene products could be components of the division apparatus or factors which regulate its assembly. Evidence supporting this notion comes from the observation that unlike the ‘early acting’ sep mutants (Harris and Hamer, 1995),
temperature-sensitive (Ts) mutations in any of these genes do not affect cellular growth or nuclear division (Harris et al., 1994). Moreover, the septation defects caused by these mutations are readily reversible. Shifting sepA, sepD, sepG or sepH mutants from the restrictive to the permissive temperature leads to the formation of septa with rapid kinetics and proper spatial distribution throughout the previously aseptate hyphae (Harris et al., 1994). These results suggest that these mutations do not block early events required for septum formation (i.e. specification of the division site).

Mutations in sepA confer additional defects which may reflect a failure to properly organize the actin cytoskeleton at sites of localized cell wall deposition. In particular, sepA mutants display mild defects in establishment and maintenance of hyphal polarity, such that mutant hyphae are wider than wild-type and exhibit an aberrant dichotomous branching pattern (Harris et al., 1994). Cell wall deposition at growing hyphal tips is similar to septum formation in that it also requires recruitment of actin to specific cortical sites (Mulholland et al., 1994; Gow, 1995). The observation that both septum formation and polarized growth have gone awry in sepA mutants suggests that the sepA gene product has a general role in promoting formation of actin-rich structures. We have investigated this possibility by pursuing a detailed phenotypic analysis of sepA mutants and by cloning and characterizing the sepA gene. Here we report that sepA is a member of the expanding FH1/2 class of proteins and that the previously described figA gene is actually an allele of sepA. Furthermore, we show that sepA is required for actin-related functions in A. nidulans and that it acts post-mitotically to promote septum formation. Finally, we show that sepA function is necessary for maintenance of cellular polarity during asexual development.

Results

Cloning of the sepA gene

The sepA locus was originally defined by three Ts mutations (sepA1, sepA2 and sepA3) which cause defects in septum formation at restrictive temperatures and also lead to aberrant hyphal branching patterns as well as increased hyphal width at both restrictive and permissive temperatures (Morris, 1976; Harris et al., 1994). Microscopic observations of mutant hyphae using both cell wall (Calcofluor) and nucleic acid (Hoechst 33258) stains revealed that all associated phenotypes cause similar defects. Genetic analyses indicated that each mutation is recessive and that all associated phenotypes cosegregate with the mutation. Parasexual genetic analysis was used to show that the sepA locus resides on chromosome I. Furthermore, meiotic mapping experiments revealed that sepA is closely linked to the lysF locus near the chromosome I centromere (Figure 1).

Linkage between the sepA and lysF loci was exploited to clone the sepA gene. Amongst the cosmids known to contain the cloned lysF gene (S.Harris, unpublished results), coslys12 was found to complement all of the phenotypes caused by the sepA1 mutation. Additional co-transformation experiments using fragments derived from this cosmid showed that the complementing activity was located on a HindIII fragment (S.Harris, unpublished results). Since restriction mapping revealed that coslys12 may contain rearrangements, pooled HindIII fragments from this cosmid were used to screen a chromosome I-specific cosmid library (Brody et al., 1991). Two of the cosmids identified, L29G03 and W12E03, were shown to possess sepA-complementing activity when transformed into either sepA1 or sepA3 mutants (Figure 1). Additional experiments revealed that the complementing activity resided on a 6.1 kb HindIII–Spbl fragment (pON48 and pKES1, Figure 1). RNA blot analyses indicated that the sequences contained on this fragment hybridized to a single transcript of ~6.0 kb in vegetative mRNA (L.Hamer, unpublished results).

sepA is a member of the FH1/2 family of proteins

Both strands of the 6.1 kb HindIII–Spbl fragment contained on pKES1 were sequenced. Analysis of the DNA sequence revealed the presence of two open reading frames (ORFs) of 4617 and 753 bp interrupted by a 52 bp intron. The presence of the intron was confirmed by the isolation and sequencing of a 2.2 kb cDNA clone which encompasses the spliced region. Since repeated attempts to recover cDNA clones spanning additional regions of the ORF were unsuccessful, we cannot rule out the possibility that other small in-frame introns exist. The combined ORFs are capable of encoding a 1790 amino acid residue protein (Figure 2A). A pronounced feature of the predicted sepA protein is the presence of an ~100 amino acid region located on pKES1 was sequenced. Analysis of the DNA sequence indicated that coslys12 was found to complement all of the events required for septum formation (i.e. specification of the division site).

Mapping data were generated from the crosses obtained: sepA–yA, 26 mu; sepA–pabaA, 8 mu; sepA–lysF, 3 mu. Details of genetic mapping in A. nidulans are summarized in Clutterbuck (1987). (B) Cloning of the sepA gene. Isolation of cosmids coslys12, L29G03 and W12E03, as well as descriptions of plasmids pON48, pKES1 and pKES2, are presented in the text. A positive result for complementation of sepA indicates restoration of 4°C of ability both to grow and to form septa. A positive result for complementation of lysF indicates ability to grow on supplemented MN plates without lysine. H. HindIII; X, XbaI; S, Spbl, nd, not determined.

Fig. 1. Genetic and molecular analysis of the sepA gene. (A) Genetic mapping of sepA. Mapping data were generated from the crosses ASH20×A28 and ASH51×A376. The following map distances were obtained: sepA–yA, 26 mu; sepA–pabaA, 8 mu; sepA–lysF, 3 mu. Details of genetic mapping in A. nidulans are summarized in Clutterbuck (1987). (B) Cloning of the sepA gene. Isolation of cosmids coslys12, L29G03 and W12E03, as well as descriptions of plasmids pON48, pKES1 and pKES2, are presented in the text.
clusters of 12 consecutive proline residues and additional strings of nine and seven consecutive prolines. The predicted sepA protein contains two regions which are predicted to adopt a coiled-coil conformation (Figure 2B).

Searches of the available databases revealed that sepA may oligomerize or be part of a multi-protein complex (Figure 2A and C). The FH1 and FH2 domains of sepA possess significant levels of identity to the BNI1 gene from S.cerevisiae (Jansen et al., 1996; Zahner et al., 1996; H.Fares and J.Pringle, personal communication), the fus1+ and cdc12+ genes from S.pombe (Petersen et al., 1995; Chang et al., 1997), the diaphanous and cappuccino genes from Drosophila melanogaster (Castillon and Wasserman, 1994; Emmons et al., 1995) and the formin 4 gene from Mus musculus (Woychik et al., 1990). The similarities between sepA and the other members of this family are mainly confined to the FH1 and FH2 domains, which reside within the C-terminal region of each protein. An additional feature shared by sepA and most of the FH1/2 proteins is the presence of regions capable of adopting a coiled-coil conformation (Lupas et al., 1991; Berger et al., 1995).

**figA is a dominant negative allele of sepA**

Database searches showed that the C-terminus of the predicted sepA protein (residues 563–1539) is identical to an A.nidulans sequence previously designated figA (Figure 2C). The figA sequence was identified in a screen for A.nidulans genes which cause dominant negative inhibition of growth when over-expressed (Marhoul and Adams, 1995). A number of observations indicate that figA represents a dominant negative allele of the sepA gene. First, all cosmids and plasmids which possess sepA-complementing activity (L29G03, pON48 and pKES1) also contain the entire figA sequence. Second, DNA blot analysis using a probe spanning the entire sepA gene demonstrates that the figA transformant (TTAP22) contains an over-expression plasmid integrated at the sepA locus (L.Hamer, unpublished results). As a result, TTAP22 possesses an intact sepA gene and a truncated version missing sequences from the 5'-end. Over-expression of the truncated allele is controlled by the inducible alcA promoter and over-expression leads to inhibition of growth on inducing media (Marhoul and Adams, 1995; Figure 3B). Notably, the region of sepA which prevents growth when over-expressed includes both the FH1 and FH2 domains. Hereafter we refer to figA as sepA5figA.

Additional evidence demonstrating that sepA5figA is an allele of sepA comes from transformation experiments.
which show that sepA<sup>586B</sup> sequences can repair the sepA<sup>1</sup> mutation by homologous recombination. The three classes of transformants recovered in these experiments and their growth phenotypes are shown in Figure 3. Two classes of transformants remain Ts due to the presence of the sepA<sup>1</sup> mutation. One of these classes (class 2) is unable to grow on media which lead to induction of the alcA promoter and is due to ectopic integration of sepA<sup>586B</sup>. The second class (class 3) is not inhibited for growth on inducing media and represents conversion of the argB2 mutation present in the recipient strain by the plasmid-borne argB gene. The third class of transformants (class 1) are distinctive in that they are no longer Ts and are also able to grow under alcA-inducing conditions. The explanation for this class of transformants is that recombination between the plasmid-borne sepA<sup>586B</sup> sequences and the chromosomal sepA gene has led to: (i) repair of the sepA<sup>1</sup> mutation; (ii) transfer of the Ts mutation into the sepA<sup>586B</sup> sequence such that it can no longer inhibit growth when over-expressed. These observations also indicate that the sepA<sup>1</sup> mutation resides within the 3′-end of the sepA gene in the region spanned by the sepA<sup>586B</sup> sequence.

The finding that sepA<sup>586B</sup> is a dominant negative allele of sepA prompted us to examine the phenotype of sepA<sup>586B</sup> transformants grown under inducing conditions. However, microscopic observations revealed that the transformants possess no defects in septum formation.

subP is essential for growth at high temperature

The sepA gene was disrupted to determine if it is essential for growth. Plasmid pLH9, in which a 2.2 kb BamHI fragment internal to the sepA gene was replaced with the argB gene, was introduced into a haploid strain (ATW17) and Arg<sup>+</sup> transformants were selected. DNA blot analysis indicated that two transformants (ALH1 and ALH2) had undergone a homologous integration event which resulted in disruption of the sepA gene (Figure 4B). No wild-type segregants were obtained when ALH1 (sepA<sup>4ΔBm</sup>) was crossed to ASH34 (sepA<sup>1</sup>), indicating that we had indeed cloned and disrupted the bona fide sepA gene.

Surprisingly, sepA<sup>4ΔBm</sup> mutants were able to grow at 28°C, although they exhibited a highly restricted colonial phenotype (Figure 4C). This result indicates that the sepA gene is not essential for growth. However, sepA<sup>4ΔBm</sup> mutants failed to grow at 42°C (Figure 4C), conditions under which Ts sepA mutants form small, tightly restricted colonies. At 42°C, sepA<sup>4ΔBm</sup> spores germinate to form aberrantly branched hyphae devoid of septa. These results demonstrate that sepA function is essential for growth at higher temperature and also indicate that the Ts mutants retain partial activity at 42°C.

sepA mutants fail to make an actin ring

We have previously shown that Ts sepA mutants fail to make septa, display an increase in hyphal width and exhibit an aberrant pattern of apical branching when incubated at the restrictive temperature (Harris et al., 1994). Examination of sepA<sup>4ΔBm</sup> hyphae by fluorescent microscopy using Calcofluor and Hoechst 33258 revealed that they display similar phenotypes. Specifically, the disruptants form broad hyphae which display the aberrant branching pattern that is characteristic of sepA mutants (Figure 5A and C). Although the hyphae are initially aseptate, scattered septa do begin to appear following prolonged incubation (i.e. >20 h) at 28°C (Table II). Following 40 h incubation at 28°C, all hyphae formed by the disruptant contained septa (Table II), but the number of septa/hyphae was much lower than wild-type levels. These observations indicate that a second gene product, perhaps another FH1/2 protein, can functionally substitute for the loss of sepA (see below).

Previous attempts to assess the status of both the actin and tubulin cytoskeletons in the Ts sepA mutants were confounded by the inability to effectively remove the cell wall from hyphae incubated at higher temperatures. We were able to circumvent these difficulties by performing immunofluorescent microscopy on sepA<sup>4ΔBm</sup> hyphae grown at 28°C for 12 h and stained with either anti-actin or anti-α-tubulin antibodies. In wild-type cells, an intense patch of actin staining is observed at the hyphal tip (Harris et al., 1994). Even though sepA<sup>4ΔBm</sup> mutants exhibit defects consistent with an inability to maintain hyphal polarity, each hyphal tip does contain a dense patch of actin staining (Figure 5D). Wild-type cells also form an actin ring in association with the developing septum (Figure 5B, arrows). In contrast, actin rings are not observed in sepA<sup>4ΔBm</sup> hyphae (Figure 5D). Staining with anti-α-tubulin antibodies revealed that inactivation of sepA has no effect upon the structure of cytoplasmic or spindle microtubules (Figure 5E and F).

sepA functions after mitosis to promote septum formation

The septation defects observed in Ts sepA mutants were previously shown to be readily reversible (Trinci and Morris, 1979; Harris et al., 1994). Specifically, following a shift from restrictive to permissive temperature, properly spaced septa were formed throughout the previously aseptate hyphae within an interval of 90–120 min. These results suggested that septation sites were marked by a mechanism which may involve the sepA gene product. Conceivably, SepA could function as a positional marker which is activated by adjacent mitotic nuclei. Alternatively, SepA could be required for septum formation at marked time of mitosis.

Fig. 3. fggA sequences can repair the sepA<sup>1</sup> mutation. (A) The different classes of transformants obtained when pTAP22 is transformed into ASH34. The open boxes represent either the argB gene, fggA sequences or the sepA gene and are labeled accordingly. The arrow represents the sepA<sup>1</sup> mutation. The stippled box depicts the alcA promoter. (B) The colony growth phenotypes of the three different classes of transformants. The extent of growth on glucose or threonine plates at either 30 or 42°C is shown. +, ++ and +++ represent increasing extents of colony growth. – indicates failure to form a colony.
situates in a manner which is independent of mitosis. To distinguish between these possibilities, we determined if blocking progression through mitosis with either hydroxyurea (HU) or benomyl (BEN) could prevent formation of septa when a sepA1 mutant is returned to the permissive temperature.

Growth of a sepA1 strain (ASH34) at semi-permissive temperature (37°C) for 12 h led to the formation of aseptate hyphae (Table I). Within 2 h following a return to the permissive temperature >70% of the hyphae had formed at least one septum (Table I). However, <20% of sepA1 hyphae could form septa when returned to the permissive temperature in the presence of either HU or BEN (Table I). The residual septation observed in the presence of these drugs is likely due to hyphae completing mitosis at 28°C prior to imposition of the block. These results show that sepA function is tightly coupled to the completion of mitosis.

sepa is required for maintenance of cellular polarity during conidiation

Although hyphae possessing a disruption of the sepA gene display the same spectrum of morphogenetic defects (i.e. failure to form septa, aberrant branching, increased hyphal width) when incubated at 28°C as do Ts sepA mutants incubated at restrictive temperature, they are still able to grow and produce a conidiating colony. This observation suggested that the sepA gene product is not required for conidiophore development. To test this idea further, we examined wild-type and sepA4ΔBm hyphae by DIC microscopy (Figure 6). Conidiophores produced by sepA4ΔBm mutants do not exhibit gross developmental defects and form abundant conidia. However, they do exhibit striking defects in the maintenance of cellular polarity, leading to formation of branched stalks (Figure 6B) and split vesicles (Figure 6C, arrowhead). These observations show that the sepA gene product is required for maintenance of cellular polarity in additional A.nidulans cell types other than growing hyphae.

Examination of conidiophores formed by sepA4ΔBm mutants revealed that they are capable of undergoing cell division and possess abundant septa (Figure 6; K.Sharpless and S.Harris, unpublished results). This observation lends additional support to the notion that a second gene product may be able to functionally substitute for sepA during conidiation. Since the initiation of conidiophore development is known to be suppressed when hyphae are grown in submerged culture (Timberlake, 1991), the delayed appearance of septa in sepA4ΔBm cultures (Table II) indicates that expression of the other gene(s) cannot be subject to developmental control. However, the unfavorable growth conditions found in older cultures and associated with initiation of conidiation may contribute to expression of this gene(s). If a functional homolog of sepA does exist, it is not detectable in hybridization experiments using DNA probes derived from the sepA gene.

Discussion

A characteristic feature of cell division is that it requires dramatic re-organization of the actin cytoskeleton. In fungal cells, septum formation proceeds via formation of an actin ring at a precise cortical location with proper timing relative to the cell cycle. Amongst the growing list of proteins shown to be required for septum formation are those whose known function is to control the dynamics of actin polymerization (Balasubramanian et al., 1992, 1994). We have found that the A.nidulans sepA gene, which is required for septum formation, encodes a protein whose predicted properties suggest that it may interact with the actin cytoskeleton.

sepa is required for septum formation and maintenance of hyphal polarity

Temperature-sensitive mutations of the sepA gene cause a failure to form septa, an increase in hyphal width and aberrant branching from hyphal tips (Trinci and Morris, 1979; Harris et al., 1994). We have found that disruption of the sepA gene results in the same pleiotropic effects. A number of observations support the notion that the
**Fig. 5.** sepA mutants fail to form an actin ring. Wild-type (A and B) and sepAΔBm hyphae (C–F) were examined by immunofluorescence microscopy following incubation at 28°C for 12 h. Hyphae were stained with either Calcofluor and Hoechst 33258 (A and C), a mouse anti-actin monoclonal antibody (B and D) or a mouse anti-α-tubulin monoclonal antibody (E and F). The arrows in (A) and (B) show septal staining with either Calcofluor (A) or the anti-actin antibody (B). sepAΔBm mutants fail to form septa (C) and do not contain actin rings (D). They do display normal arrays of cytoplasmic (E) and spindle (F) microtubules. Scale bars in (A) and (C) represent 2 µm.

**Table I.** sepA functions after mitosis to promote septum formation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time following return to permissive temperature (h)</th>
<th>No. of septa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>200 0 0 0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>107 41 25 27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55 42 34 69</td>
</tr>
<tr>
<td>HU</td>
<td>0</td>
<td>200 0 0 0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>152 30 13 5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>171 26 2 1</td>
</tr>
<tr>
<td>BEN</td>
<td>0</td>
<td>200 0 0 0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>163 20 10 7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>159 19 11 11</td>
</tr>
</tbody>
</table>

*ASH34 hyphae were incubated at 37°C (semi-permissive temperature) for 12 h, after which they were transferred to media containing either 20 mM HU, 2.5 mg/ml BEN or no addition. Samples were taken at 1 and 2 hours following the temperature shift. Calcofluor was used to visualize septa.*

defects in septation caused by sepA mutations are due to an inability to promote formation of actin rings at incipient division sites. First, septum formation in *A. nidulans* is known to require formation of contractile actin rings (Harris et al., 1994; M.Momany and J.Hamer, unpublished results). Second, we have not been able to detect actin rings by indirect immunofluorescence microscopy in sepA mutants. In contrast, sepA mutations have no effect upon the appearance of the actin patch located at hyphal tips. Finally, molecular analysis of the sepA gene shows that it encodes a protein which may be capable of promoting formation of actin filaments.

Loss of sepA function, while not preventing accumulation of actin at hyphal tips, does lead to defects in polarized growth. Preliminary observations indicate that the increased hyphal width observed in sepA mutants is due to a severe delay in establishment of spore polarity (S.Harris, unpublished observations). Difficulties in properly organizing the actin cytoskeleton at the site of
two genes encoding FH1/2 proteins (see below). However, since all sepA mutants are defective in septum formation at 42°C, the redundant activity must be inherently temperature sensitive.

**sepA is a member of the FH1/2 class of proteins**

Molecular analysis of the sepA gene product demonstrates that it is a member of the FH1/2 class of proteins. This expanding family includes members in species ranging from yeast to mice. The level of similarity between sepA and the other FH1/2 proteins ranges from 28 to 65% and is mainly confined to the FH1 and FH2 domains. An additional feature shared by sepA and most of the other FH1/2 proteins is the presence of regions capable of adopting a coiled coil conformation. This suggests that these proteins may function as dimers or oligomers.

The participation of FH1/2 proteins in cellular processes which require formation of microfilaments suggests that they may be functionally related. The most striking conservation of function occurs between sepA and both cdc12+ and diaphanous. We have shown that mutations in the *A. nidulans* sepA gene lead to defects in septum formation, presumably due to a failure to form actin rings. The cdc12+ gene is required for assembly of the contractile actin ring during septum formation in *S. pombe* (Chang et al., 1996). Mutations in cdc12+ cause delocalized clusters of actin patches to accumulate in the middle of the cell, suggesting that it functions early during actin ring assembly. The *Drosophila* diaphanous gene is required for cytokinesis in all tissues which have been examined (Castrillon and Wasserman, 1994). Furthermore, the absence of cleavage furrows in mutant larval neuroblasts suggests that *diaphanous* may also act early during contractile ring formation. Functional similarities also exist between sepA and the *S. cerevisiae* BN11 gene product. *bni1* mutants exhibit partial defects in cytokinesis (forming a broader mother–bud junction) and fail to display a bipolar pattern of bud site selection (Jansen et al., 1996; Zahner et al., 1996). The increase in hyphal width and the aberrant branching of both hyphae and conidiophores caused by sepA mutations may represent defects in polarized growth which are analogous to those caused by *bni1* mutations. Collectively, these observations suggest that the re-organization of the actin cytoskeleton which precedes cell division in animal and fungal cells occurs via an evolutionarily conserved mechanism involving FH1/2 proteins. These proteins may stimulate assembly of actin rings or contractile rings by nucleating formation of actin filaments.

The presence of multiple prolines within the FH1 domain suggests at least two possible mechanisms by which FH1/2 proteins could stimulate formation of actin filaments. One mechanism is through interactions with proteins containing either SH3 or WW domains. These proteins bind to ligands containing a proline-rich motif (Sudol, 1996) and, in the case of SH3 proteins, have been implicated in mediating signal transduction to the actin cytoskeleton (Pawson, 1995). Consistent with this possibility, the mouse formins have recently been shown to interact with SH3 and WW proteins (Chan et al., 1996). The polyproline tracts may also mediate interactions with the actin binding protein profilin. Profilin is known to modulate the kinetics of actin filament assembly (Pantaloni...
et al., 1991). Furthermore, genetic and/or two-hybrid interactions have been observed between profilin and FH1/2 proteins in both flies and yeast (Manseau et al., 1996; Chang et al., 1997; Evangelista et al., 1997). It is tempting to speculate that the inhibition of growth caused by overexpression of the FH1/2 region in the sepAΔ5Bm allele is due to titration of an interacting protein(s) which is required for organization of the actin cytoskeleton.

**Roles for FH1/2 proteins in cytokinesis**

Our results, together with other studies on proteins possessing FH1 and FH2 domains, suggest that these gene products may stimulate assembly of actin filaments at specified cortical sites through interactions with actin-associated proteins. In this manner, sepA may play a critical role in directing formation of the actin ring at the division site in *A. nidulans*. The sepA gene product may be recruited to the incipient division site by interacting with a class of proteins termed septins. Septins are a novel class of cytoskeletal proteins required for cell division in numerous organisms (Longtine et al., 1996). One of their primary functions may be to serve as a scaffold for assembly of the division apparatus. The observation of genetic interactions between a septin and an FH1/2 protein in *S. cerevisiae* (Longtine et al., 1996) indicates that similar interactions between sepA and septins may also occur in *A. nidulans*. At least three septin-encoding genes have been identified in *A. nidulans* and their function is currently being assessed (Momany et al., 1995). The observation that mitotic progression is required for sepA to promote septum formation indicates that the sepA gene product may localize to the incipient division site in response to mitotic signals. Alternatively, mitotic signals may ‘activate’ sepA and trigger actin filament formation. In either case, these signals could be transduced to the division apparatus by small GTP binding proteins of the rho class, which are known to be required for cytokinesis in a number of cell types (Kishi et al., 1993; Mabuchi et al., 1993; Larochelle et al., 1996).

**Table II. Septation defects caused by the sepAΔBm mutation**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Septation</th>
<th>Strain</th>
<th>ATW17 28°C</th>
<th>42°C</th>
<th>ASH34 28°C</th>
<th>42°C</th>
<th>ALH1 28°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>100</td>
<td>18</td>
<td>97</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>0</td>
<td>82</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>75</td>
<td>0</td>
<td>74</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>25</td>
<td>100</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>97</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Materials and methods

**Strains, media and growth conditions**

The following strains were used in this study: A28 (pabaA6 bIAJ), A376 (ulaa1de20 lysF88 pabaA1 yA2 udE20), ASH20 (sepA1 argB2 yA2), ASH34 (sepA1 pyrG89 wA2), ASH51 (sepA1 pyrG89 wA2), ATW17 (ΔargB::tpCBa), ALH1 (sepAΔBm) and TTAP22 (ΔAI ΔargB::tpCBa methG1 trpC801 alcA(p)::figA). Strains were either obtained from the Fungal Genetics Stock Center (Department of Microbiology, University of Kansas Medical Center, Kansas City, KS) or constructed specifically for this study.

Media used [complete medium (CM), minimal medium (MN) and yeast extract glucose (YEG)] and general growth conditions were as described previously (Harris et al., 1994). YGV is yeast extract glucose + 0.1% vitamins (Kafet, 1977). For induction of the alcA promoter, MN containing 100 mM threonine and no glucose was used.

*Aspergillus nidulans* was grown on coverslips in liquid media as described previously (Harris et al., 1994). Temperature down-shift experiments were initiated by growing strain ASH34 on coverslips at 37°C in YGV for 12 h. Coverslips with adherent cells were then transferred to YGV containing either no additions, 20 mM HU or 2.5 mg/ml BEN and shifted to 28°C. Coverslips were removed at 1 and 2 h following the temperature shift and processed for microscopy. To examine the phenotype of strains overexpressing sepAΔBm, strain TTAP22 was grown on coverslips at 28°C in MN for 12 h. Coverslips with adherent hyphae were then transferred to either MN or MN+Thr medium and incubated for an additional 6 h at 30°C before being processed for microscopy. Septation time course experiments were initiated by growing strains ATW17, ASH34 and ALH1 on coverslips at either 28 or 42°C in CM supplemented with arginine. Coverslips were removed at 0, 10, 12, 15, 20 and 40 h post-incubation and processed for microscopy. Samples removed at 0 and 10 h were inoculated at a density of 10⁶ conidia/ml, samples removed at 12, 15 and 20 h were inoculated at a density of 10⁷ conidia/ml and samples removed at 40 h were inoculated at a density of 2.5×10⁷ conidia/ml.

**Genetic and recombinant DNA manipulations**

Methods for the genetic analysis of *A. nidulans* were as described previously (Kafet, 1977; Harris et al., 1994).

All recombinant DNA manipulations followed standard protocols (Sambrook et al., 1989). Previously described procedures were employed for isolation of nucleic acids from *A. nidulans* mycelia (Timmerlake, 1980; Dobinson et al., 1993) and for transformation of *A. nidulans* (Oakley and Osmani, 1993).

**Plasmid constructs**

The plasmids pSalArgB, pDHG25 and pTAP22 have been previously described (Gems et al., 1991; Marhoul and Adams, 1995). Plasmid pON48 contains the ~13 kb HindIII fragment from cosmid L13G11.
Plasmid pKES2 contains the partially digested 4 kb Sph fragment from pON48 subcloned into pUC119. Plasmids pKES1 and pKES2 were obtained by subcloning the following fragments from pON48 into pBK KS−: ~2.2 kb BamHI (pHL14)−1.6 kb XhoI (pHL15); ~0.9 kb XbaI–SphI (pHL19); ~1.7 kb XbaI–BamHI (pHL20); ~1.0 kb EcoRI (pHL21). An ~2.0 kb DNA fragment from a λgt10 library was cloned into pBK KS− to form plH22. pHL22 and pHL23 (containing an ~1.4 kb Cld fragment from plH22) were used for sequencing of the 3′ sepA region. The sepA disruption plasmid pLH9 was constructed in three steps. First, the 6.1 kb HindIII–SphI fragment from pON48 was subcloned into pBR322 (pLH5). Second, pLHS5 was digested with BamHI and re-ligated (pLH7). Third, the 1.8 kb BamHI fragment containing the argB gene was ligated to BamHI-digested, dephosphorylated pLH7.

**Sequencing and sequence analysis**

Sequencing of the plasmids pHL14, pHL15, pHL19, pHL20, pHL22 and pHL23 was performed on a Pharmacia A12 sequencer using the T7, T3, Universal and Reverse primers. The sequence was analysed using XFERDB database search tools (Bassett et al., 1995) and the Coils (Lupas et al., 1991) and Paircoil (Berger et al., 1995) software.

**Microscopy**

Coverslips with adherent cells were processed for microscopy and stained with the Hoechst 33258 and Calcein orange as described previously (Harris et al., 1994). Immunofluorescence microscopy for detection of actin and tubulin cytoskeletons was performed using standard protocols (Oakley and Osmani, 1993; Harris et al., 1994). Primary antibodies used were the mouse anti-actin N350 monoclonal (Amersham) at 1:500 and the mouse anti-α-tubulin DM1A monoclonal (Sigma Immunochemicals) at 1:200. Secondary antibodies used were FITC-conjugated anti-mouse (Sigma Immunochemical) at 1:200.

**Acknowledgements**

We thank John Marhoul and Tom Adams (Texas A&M University) for kindly sharing strains and plasmids. We also thank Johnny Fares, John Pringle and Fred Chang for the communication of unpublished results and Susan Kaminsky for assistance in the genetic mapping of sepA. This work was supported by grants from the American Cancer Society (IRG-1521) and the National Science Foundation (MCB 5-22270) to S.D.H. and the National Institutes of Health (GM49867) to J.E.H.

**References**


FH1/2 protein required for cytokinesis


Received on November 18, 1996; revised on February 17, 1997