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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*

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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 cdc42) 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 (McCollum 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),
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 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 containing numerous clusters of consecutive proline residues (underlined in Figure 2B). This region contains two

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. A positive result for complementation of sepA indicates restoration at 32°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, SphI. nd, not determined.
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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), indicating that sepA may oligomerize or be part of a multi-protein complex.

Searches of the available databases revealed that sepA is a member of the FH1/2 family of proteins (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 (Castrillon 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).

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.

**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.

**Table 1. Levels of sequence similarity between **sepA** and the other FH1/2 proteins.**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Identity %</th>
<th>Similarity %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sepA</strong></td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td><strong>BN1</strong></td>
<td>45</td>
<td>69</td>
</tr>
<tr>
<td><strong>CDC12</strong></td>
<td>30</td>
<td>69</td>
</tr>
<tr>
<td><strong>FUS1</strong></td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td><strong>DIAPHANOUS</strong></td>
<td>25</td>
<td>59</td>
</tr>
<tr>
<td><strong>CAPPUCCINO</strong></td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td><strong>FORMIN4</strong></td>
<td>28</td>
<td>57</td>
</tr>
</tbody>
</table>

**Fig. 2.** sepA is a FH1/2 protein. (A) Structure of the sepA gene. A restriction map of the sepA gene is shown on the top line. Shown underneath is a schematic drawing of the sepA gene product. The thin grey line represents the sepA coding region, with the FH1 and FH2 domains depicted as square black boxes. X, XbaI; Sp, Spel; B, BamHI; E, EcoRI; S, SplI. (B) The predicted amino acid sequence of the sepA gene product. The DDBJ/EMBL/GenBank accession No. for the DNA sequence is U83658. The FH1 (polyproline) and FH2 domains are indicated by underlining. Regions of sepA capable of adopting a coiled-coil conformation (Lupas et al., 1991; Berger et al., 1995) are depicted by lines drawn in the left column. (C) Levels of sequence similarity between sepA and the other FH1/2 proteins. FH1+FH2 refers to the sequence spanning the FH1 and FH2 domains and including the intervening region. FH1 refers to the sequence encompassed solely by the FH2 domain. Comparisons were made using the software provided in the XREFdb package (Bassett et al., 1995).
The star represents the sepA1 mutation and is due to ectopic integration of sepA5 alcA 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.

which show that sepA5#58#A sequences can repair the sepA1 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 sepA1 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 sepA5#58#A. 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 sepA5#58#A sequences and the chromosomal sepA gene has led to: (i) repair of the sepA1 mutation; (ii) transfer of the Ts mutation into the sepA5#58#A sequence such that it can no longer inhibit growth when over-expressed. These observations also indicate that the sepA1 mutation resides within the 3′-end of the sepA gene in the region spanned by the sepA5#58#A sequence.

The finding that sepA5#58#A is a dominant negative allele of sepA prompted us to examine the phenotype of sepA5#58#A transformants grown under inducing conditions. However, microscopic observations revealed that the transformants possess no defects in septum formation.

**sepA is essential for growth at high temperature**

The sepA gene was disrupted to determine if it is essential for growth. Plasmid pH9, 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+ 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 (sepA4ΔBm) was crossed to ASH34 (sepA1), indicating that we had indeed cloned and disrupted the bona fide sepA gene. Surprisingly, sepA4ΔBm 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, sepA4ΔBm mutants failed to grow at 42°C (Figure 4C), conditions under which Ts sepA mutants form small, tightly restricted colonies. At 42°C, sepA4ΔBm 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 sepA4ΔBm 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 sepA4ΔBm 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). 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).

**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 the plasmid-borne argB gene and are labeled accordingly. 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 (sepA4ΔBm) was crossed to ASH34 (sepA1), indicating that we had indeed cloned and disrupted the bona fide sepA gene. Surprisingly, sepA4ΔBm 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, sepA4ΔBm mutants failed to grow at 42°C (Figure 4C), conditions under which Ts sepA mutants form small, tightly restricted colonies. At 42°C, sepA4ΔBm 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.

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polarity during conidiation dramatic re-organization of the actin cytoskeleton. In presence of these drugs is likely due to hyphae completing exist, it is not detectable in hybridization experiments permissive temperature in the presence of either HU or with initiation of conidiation may contribute to expression hyphae could form septa when returned to the 

sepA1 formed at least one septum (Table I). However, 

[53x393].

In 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 sepAΔBm hyphae by DIC microscopy (Figure 6). Conidiophores produced by sepAΔBm 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 sepAΔBm 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 sepAΔBm 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 known 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></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>2</td>
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</tr>
<tr>
<td>HU</td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>171</td>
</tr>
<tr>
<td>BEN</td>
<td>0</td>
<td>200</td>
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<tr>
<td></td>
<td>1</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>159</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 division cause the mutants to fail to form a contractile ring. This results in improper septum formation and a failure to generate a normal array of microtubules.

**Table I.** sepA functions after mitosis to promote septum formation.
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; Zahnert 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...
and Cartier, 1993) and avidly binds to polyproline (Janmey, 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 SepA5 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).

**Materials and methods**

**Strains, media and growth conditions**

The following strains were used in this study: A28 (pabaA6 biaA), A376 (uacA1adE20 lysF88 pabaA1 yA2 adE20), ASH20 (sepA1 argB2 yA2), ASH34 (sepA1 pyrG89 wA2), ATW17 (ΔargR::trpC58), ALH1 (sepAΔBm) and TTA22 (bia1 ΔargR::trpC58 mepG1 trpC801 alcA::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 SepA55Bm, strain TTA22 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, 15, 20 and 40 h post inoculation 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 (Timberlake, 1980; Dobinson et al., 1993) and for transformation of *A nidulans* (Oakley and Osmami, 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 cosmide L13G11.
(Brody et al., 1991) subcloned into pBS SK+ (J.Marhoul and T.Adams, personal communication). Plasmid pKE31 contains the 6.1 kb HindIII–SphI fragment from pON48 subcloned into pUC19. Plasmid pKE22 contains the partially digested 4.1 kb SphI fragment from pON48 subcloned into pUC19. Plasmids for sequencing the 5' sepb region were obtained by subcloning the following fragments from pON48 into pBK KS−: ~2.2 kb BamHI (pLH14); ~1.6 kb XhoI (pLH15); ~0.9 kb XbaI–SphI (pLH19); ~1.7 kb XbaI–BamHI (pLH20); ~1.0 kb EcoRI (pLH21). An ~2.0 kb CDNA fragment from a λgt10 library was cloned into pBS SK+ to form pLH22. pLH22 and pLH23 (containing an ~1.4 kb Clal fragment from pLH22) were used for sequencing of the 3' sepb region. The sepb disruption plasmid pLH9 was constructed in three steps. First, the 6.1 kb HindIII–SphI fragment from pON48 was subcloned into pBR322 (pLH5). Second, pLH5 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
Sequence of the plasmids pLH14, pLH15, pLH19, pLH20 and pLH23 was performed on a Pharmacia ALF sequencer using the T7, T3, Universal and Reverse primers. The sequence was analysed using XREFdb 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 Hoechst 33258 and Calcein 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-α-tubulin DM1A monoclonal (Sigma Immunochemicals) at 1:500 and the mouse anti-α-tubulin DMI1A monoclonal (Sigma Immunochemicals) at 1:200. Secondary antibodies used were FITC-conjugated anti-mouse (Sigma Immunochemical) at 1:200.

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References
FH1/2 protein required for cytokinesis


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