1999

Identification and Characterization of Genes Required for Hyphal Morphogenesis in the Filamentous Fungus *Aspergillus nidulans*

Steven D. Harris
University of Nebraska-Lincoln, Steven.Harris@umanitoba.ca

Amy F. Hofmann
University of Connecticut Health Center

Hugo W. Tedford
University of Connecticut Health Center

Maurice P. Lee
University of Connecticut Health Center

Identification and Characterization of Genes Required for Hyphal Morphogenesis in the Filamentous Fungus *Aspergillus nidulans*

Steven D. Harris, Amy F. Hofmann, Hugo W. Tedford and Maurice P. Lee

Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06030-3205

Manuscript received July 21, 1998
Accepted for publication December 7, 1998

ABSTRACT

In the filamentous fungus *Aspergillus nidulans*, germination of an asexual conidiospore results in the formation of a hyphal cell. A key feature of spore germination is the switch from isotropic spore expansion to polarized apical growth. Here, temperature-sensitive mutations are used to characterize the roles of five genes (*sepA*, *hypA*, *podB–podD*) in the establishment and maintenance of hyphal polarity. Evidence that suggests that the *hypA*, *podB*, and *sepA* genes are required for multiple aspects of hyphal morphogenesis is presented. Notably, *podB* and *sepA* are needed for organization of the cytoskeleton at sites of polarized growth. In contrast, *podC* and *podD* encode proteins that appear to be specifically required for the establishment of hyphal polarity during spore germination. The role of *sepA* and the *pod* genes in controlling the spatial pattern of polarized morphogenesis in germinating spores is also described. Results obtained from these experiments indicate that the normal pattern of germ-tube emergence is dependent upon the integrity of the actin cytoskeleton.

A characteristic feature of fungal morphogenesis is its highly polarized nature. Fungal cells grow by inserting new membrane and cell wall at localized sites on the cell surface. Vesicles laden with components required for cell-surface expansion are thought to be transported to these sites along a polarized cytoskeletal network (reviewed by Gow 1995). Different types of fungal cells acquire their unique morphologies by employing distinctive patterns of polarized morphogenesis (reviewed by Mata and Nurse 1998). For example, budding yeast cells attain their ellipsoidal shape by undergoing alternating periods of apical and isotropic growth (Lew and Reed 1993), whereas fission yeast cells become rod shaped by restricting growth to the cell poles (Mitchison and Nurse 1985). Notably, both yeasts cease polarized growth during mitosis and relocalize the morphogenetic machinery to the incipient division site to allow septum formation and cytokinesis. In contrast to yeast cells, the distinct tubular shape of hyphal cells is achieved by confining growth to the apical pole. Furthermore, hyphal cells do not depolarize during mitosis and instead assemble an apparently distinct morphogenetic complex at cell division sites (reviewed by Harris 1997).

Budding yeast cells are capable of initiating polarized growth in response to either internal or external spatial cues (reviewed by Roemer *et al.* 1996). Internal cues (*i.e.*, Bud3p, Bud4p) direct bud site selection during the cell cycle (Chant *et al.* 1995; Sanders and Herskowitz 1996; Zahner *et al.* 1996), whereas external cues (*i.e.*, bound pheromone receptor) dictate the site at which mating projections form prior to conjugation (Jackson *et al.* 1991; Valtz *et al.* 1995). In both cases, spatial information is relayed to the morphogenetic complex by a series of GTPase signaling modules (reviewed by Pringle *et al.* 1995). Ultimately, polarized growth requires numerous gene products involved in cytoskeletal and secretory functions (reviewed by Govindan and Novick 1995). Notably, many of the gene products that control polarized growth in yeast were initially identified by mutations that alter normal cellular morphology (Sloat and Pringle 1978; Adams *et al.* 1990; Mondesert *et al.* 1997).

Many approaches have been employed to experimentally investigate polarized morphogenesis in hyphal cells. Detailed physiological studies have established that Ca^{2+} flux plays an important role in regulating the establishment and maintenance of hyphal polarity in filamentous fungi (Jackson and Heath 1993; Grinberg and Heath 1997). Molecular and genetic studies have demonstrated that the maintenance of hyphal polarity in *Neurospora crassa* requires both the cAMP-dependent protein kinase (PKA) and the COT1 kinase (Yarden *et al.* 1992; Bruno *et al.* 1996). The relevant targets of these kinases remain unknown. Furthermore, the establishment and maintenance of hyphal polarity depends upon both actin-based (McGoldrick *et al.* 1995) and microtubule-based (Lehmler *et al.* 1997; Seiler *et al.* 1997; Wu *et al.* 1998) motor proteins. Although the role of GTPase signaling modules in controlling polarized morphogenesis in hyphal cells has not been fully addressed, the analysis of a Ras homologue in *A. nidu-*

Corresponding Author: Steven Harris, Department of Microbiology, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030-3205. E-mail: sharris@nso2.uchc.edu

lans suggests that it regulates the establishment of hyphal polarity (Som and Kolaparthi 1994). Despite these insights, a coherent picture of the molecular mechanisms underlying polarized morphogenesis in hyphal cells has yet to emerge.

A. nidulans forms dormant asexual conidiospores that germinate to form hyphal cells. Spore germination occurs in three sequential stages: (i) activation of the dormant spore, (ii) isotropic expansion driven by hydration and wall growth, and (iii) initiation of polarized growth at the site of germ-tube emergence (d'Enfert 1997). Concomitant with these events, the uninucleate spore reenters the cell cycle and undergoes nuclear division. As a result, *A. nidulans* hyphal cells are multinucleate (Fiddy and Trinci 1976; Harris 1997). In mature hyphae that have formed septa, the tip cell continues to grow at the apical pole, whereas subapical cells establish new axes of polarized growth and form lateral branches (Fiddy and Trinci 1976; Harris 1997). In this report, we present the identification and phenotypic characterization of temperature-sensitive (ts) mutations that define four genes required for normal hyphal morphogenesis (*hypA*, *podB–podD*) in *A. nidulans*. We demonstrate that two of these genes, *hypA* and *podB*, are needed for both the establishment and the maintenance of hyphal polarity. Results obtained from microscopic analyses suggest that the *podB* gene is required for cytoskeletal organization in the hyphal tip. In contrast, we show that the *podC* and *podD* genes are required only for the establishment of hyphal polarity. We also characterize the polarity defects caused by mutations in the previously described *sepA* gene (Harris *et al.* 1997). Analysis of double mutants suggests that the function of the PODD protein depends upon the SEPA formin. Finally, we show that mutations in *sepA*, and to a lesser extent *podB* and *podD*, alter the spatial pattern of polarized morphogenesis in germinating *A. nidulans* spores.

MATERIALS AND METHODS

Strains, media, and growth conditions: The genotypes of all strains used in this study are presented in Table 1. Media used [complete medium (CM) and minimal medium (MN)] were as described previously (Harris *et al.* 1994). YGV (0.5% yeast extract, 2% glucose, and 0.1% vitamins; Kafer 1977) was also used.

A. nidulans was grown on glass coverslips in liquid media as described previously (Harris *et al.* 1994). For all experiments, permissive temperature was 28°, semipermissive temperatures ranged from 35.5° to 37°, and restrictive temperature was 42°. Temperature shift experiments were initiated by inoculating conidiospores into YGV and allowing them to germinate into hyphal cells at 28° for 12 hr. Coverslips with adherent germlings were removed for staining and microscopy just prior to the temperature shift. Cultures were then shifted to 42°, and additional coverslips were removed at 3 and 6 hr postshift. The polarity index was calculated as the length of a hypha (measured from the spore/hypha junction to the hyphal tip) divided by its width (measured at the approximate midpoint of the hypha).

TABLE 1

***A. nidulans* strains**

Strain	Relevant genotype	Source
A28	*pabaA6 biA1*	FGSC[a]
GR5	*pyrG89*; *pyroF4*; *wA3*	G. May
AH13	*argB2*; *wA2*; *chaA1*	Lab stock
ASH5[b]	*sepA1*; *wA2*	Lab stock
ASH35	*sepA1 yA2*; *argB2*	Lab stock
ALH1[c]	*sepA4ΔBm*	L. Hamer
ASH80	*hypA6 pabaA6*	This study
ASH83	*podB1*; *pabaA6*; *pyroA4*	This study
ASH130	*podC1*; *pabaA6*	This study
ASH134	*podD1*; *pabaA6*; *wA3*	This study
ASH308	*podC1 wA2*; *argB2*	This study
ASH311	*podD1*; *pabaA6*; *argB2*; *wA3*	This study
ASH334	*podB1*; *argB2*; *chaA1*	This study
AAS100	*sepA1 yA2*; *podD1*; *argB2*	This study
AAS202	*sepA3 pyrG89*; *podD1*; *wA3*	This study

[a] Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center (Kansas City, KS 66160-7420).

[b] The *sepA4ΔBm* allele was created by replacing an internal 2.2-kb *Bam*HI fragment with the *argB* gene. Phenotypic analyses suggest that this is a null allele (Harris *et al.* 1997), and preliminary Western analyses with anti-SEPA sera detected only degradation products (K. Sharpless and S. Harris, unpublished results).

[c] Based on phenotypic analyses (Harris *et al.* 1997), the *sepA1* and *sepA3* alleles are presumed to be hypomorphic. Preliminary Western analyses with anti-SEPA sera detected full-length protein in these mutants (K. Sharpless, S. Avatapalli and S. Harris, unpublished results).

To monitor polarization kinetics in *sepA* and *pod* mutants, conidiospores were inoculated into YGV and incubated at 42°. Samples were collected at regular intervals, and the percentage of spores with a visible germ tube was determined (n = 200). The first sample in which 10–20% of the spores had germinated was chosen for further analysis, as we reasoned that most of the spores at this timepoint were on the verge of forming a germ tube. Samples were stained, and both nuclear number and cell volume were determined for 25 randomly selected swollen, ungerminated spores. The nonparametric Mann-Whitney test (Zar 1984) was used to determine if values obtained for a given mutant were significantly different from those of wild-type strains.

To determine the effects of cytochalasin A (CA) or nocodazole on the spatial pattern of germ-tube emergence, wild-type conidiospores were inoculated into YGV and incubated at 37° for 6 hr. Germinated spores were subsequently treated for 3 hr with either 5μg/ml CA or 2μg/ml nocodazole or were left untreated. They were then released into fresh YGV at 37° for an additional hour. Coverslips were then removed and processed for staining and microscopy.

Genetic manipulations: Methods used for the genetic analysis of *A. nidulans* were as described previously (Kafer 1977; Harris *et al.* 1994). Putative double mutants were backcrossed to a wild-type strain to confirm the presence of the parental mutations. Candidate *pod* mutants were identified by screening a previously described collection of 1156 ts mutants that were generated using 4-nitroquinoline 1-oxide (4NQO) as mutagen (Harris *et al.* 1994). For each mutant, the original isolate was patched onto a CM plate and examined under a

dissecting microscope following 48 hr incubation at 42°. Mutants displaying morphologies consistent with defects in polarized morphogenesis were subjected to a secondary screen by growing them on coverslips at 42° in liquid YGV, followed by staining and microscopy. Mutants unable to establish hyphal polarity were selected and backcrossed to a wild-type strain at least two times prior to subsequent genetic and phenotypic analyses.

Staining and microscopy: Coverslips with adherent cells were processed for microscopy and stained with Calcofluor (to visualize cell walls and septa) and Hoechst 33258 (to visualize nuclei) as described previously (Harris *et al.* 1994). For labeling cell walls, coverslips with adherent cells were stained with 1 mg/ml FITC-conjugated wheat germ agglutinin (FITC-WGA; Sigma, St. Louis). Immunofluorescence microscopy for the detection of the actin and tubulin cytoskeletons was performed using standard protocols (Oakley and Osmani 1993; Harris *et al.* 1994). The protocol for detecting the actin cytoskeleton was modified by substituting PEM (50 mm Pipes, pH 6.7; 25 mm EGTA, pH 7.0; 5 mm $MgSO_4$) + 3% BSA for the PBS (phosphate-buffered saline) + BSA washes (Oakley and Osmani 1993; Harris *et al.* 1994). Primary antibodies used were the mouse anti-actin C4 monoclonal (ICN Biomedicals, Inc.) at 1:400 and the mouse anti-α-tubulin DM1A monoclonal (Sigma Immunochemicals) at 1:200. The secondary antibody was FITC-conjugated sheep anti-mouse (Sigma Immunochemicals) at 1:200.

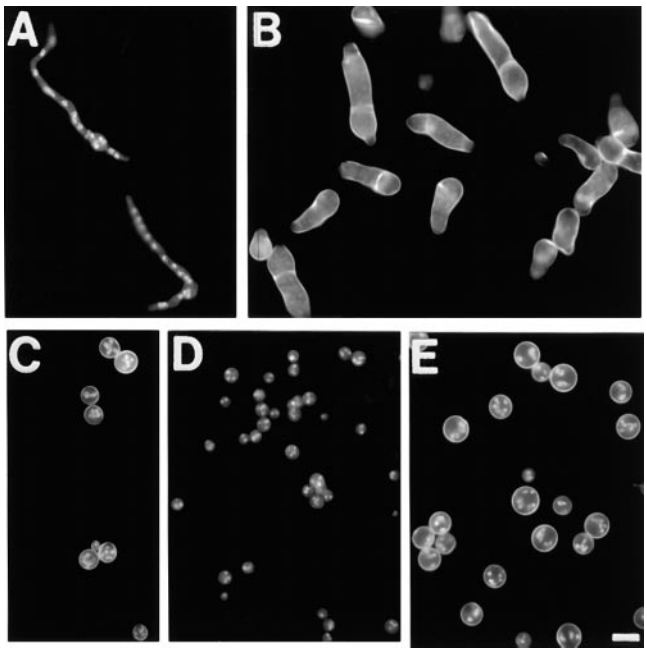

Figure 1.—Cellular morphology of the *pod* mutants. Conidiospores were incubated on coverslips for 12 hr at 42°. Preparations were stained with Calcofluor and Hoechst 33258 to visualize cell walls and nuclei, respectively. (A) A28 (wild type), (B) ASH80 (*hypA6/podA1*), (C) ASH83 (*podB1*), (D) ASH130 (*podC1*), and (E) ASH134 (*podD1*). Bar, 5 μm.

RESULTS

Identification and phenotypic characterization of the *pod* mutants: To systematically identify gene products required for polarized morphogenesis in *A. nidulans*, we screened for mutants defective in the establishment of hyphal polarity. Because we sought mutations that cause specific defects in morphogenesis without adversely affecting cellular growth and/or nuclear division, we screened for mutants displaying the following phenotypes: (i) failure of germinating spores to produce a germ tube, (ii) continued growth and enlargement of spores in a depolarized manner, and (iii) accumulation of multiple nuclei. A collection of 1156 ts mutants was screened as described in materials and methods. Five mutants displaying defects in the establishment of hyphal polarity were recovered following the secondary screen. Several backcrosses to a wild-type strain demonstrated that the morphogenetic defect cosegregated with temperature sensitivity in four of these mutants, and, in each case, both phenotypes were caused by a recessive mutation in a single gene. Complementation and linkage analyses revealed that these four mutations define four distinct genes, which were named *podA–D* for *po*larity *d*efective (Figure 1).

Analysis of multiple segregants from each backcross showed that the *podB1*, *podC1*, and *podD1* mutations prevent the establishment of hyphal polarity at restrictive temperature. These mutations lead to the accumulation of swollen round spores possessing 4–8 (*podC1*), ≥8 (*podB1*), or ≥16 (*podD1*) nuclei (Figure 1, C–E). Under the same conditions, the *podA1* mutation causes a severe delay in the establishment of hyphal polarity. Germ-tube emergence occurs when *podA1* spores typically possess 4 nuclei and have swollen to an average volume of 141.4 ± 52.1 μm^3 (*vs.* 81.2 ± 28.7 μm^3 for wild-type spores). Upon germination, *podA1* mutants form multinucleate hyphae that are abnormally wide (Figure 1, A and B). The *pod* mutations are not ts lethal, because, to varying extents, they were able to grow at permissive temperature following an extended incubation (*i.e.*, ~12 hr) at 42° (S. Harris, unpublished data). Furthermore, in no case were the phenotypes caused by these mutations osmotically remedial.

Linkage analyses revealed that the *podA1* mutation maps to chromosome I and displays tight linkage to the *sepA* locus (3/100 recombinant segregants). Another mutation that causes morphogenetic defects, *hypA1*, also maps to this region of chromosome I (Kaminskyj and Hamer 1998). Complementation tests demonstrated that the *podA1* and *hypA1* mutations are allelic (S. Kaminskyj, personal communication; see discussion); hence the *podA1* mutation has been renamed *hypA6/podA1*. The *podB1* mutation maps to chromosome II and displays linkage to the *wA* locus (75/800 recombinant segregants). The *podC1* mutation also maps to this region of chromosome II (43/395 recombinants with respect to *wA*), but the observation that ts^+ recombinants can be readily obtained (33/100) from a *podC1* × *podB1* cross shows that *podC1* defines a locus distinct from *podB1*. The *podD1* mutation has not yet been unambiguously assigned to a chromosome.

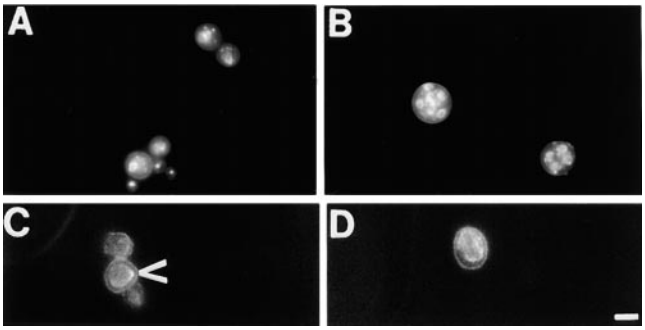

Figure 2.—Mutations in the *sepA* gene delay the establishment of hyphal polarity. Conidiospores from strains A28 (wild type; A and C) and ALH1 (*sepA4*; B and D) were incubated on coverslips for 5 hr (A28) or 6 hr (ALH1) at 42°. Coverslips with adherent cells were processed for immunofluorescence microscopy as described in materials and methods. (A and B) Spores stained with Calcofluor and Hoechst 33258. (C and D) The same spores stained with the C4 monoclonal anti-actin antibody. Note that actin patches accumulate at the presumptive germination site in the wild-type spore (arrowhead), whereas they exhibit an apparently random cortical distribution in the *sepA4* spore. Bar, 5 μm.

SEPA is required for hyphal morphogenesis: Temperature sensitive mutations in the *sepA* gene were first identified as mutants unable to form septa at the restrictive temperature (Morris 1976). Recently, it was demonstrated that SEPA is a member of the formins (Harris *et al.* 1997), a class of proteins thought to facilitate localized organization of the actin cytoskeleton by serving as a molecular scaffold (Frazier and Field 1997; Wasserman 1998). In addition to the previously characterized defects in septum formation, *A. nidulans sepA* mutants form wide hyphae that are unable to properly maintain a polarized axis of growth (Morris 1976; Harris *et al.* 1994). Because the increase in hyphal width could also reflect a delay in the establishment of hyphal polarity, the kinetics of germ-tube emergence were monitored in a *sepA* deletion mutant (*sepA4*) and compared to those of a wild-type strain. At 42°, germ-tube emergence typically occurs when wild-type spores possess two nuclei and have swollen to an average volume of 81.2 ± 28.7 μm^3. However, in a *sepA4* mutant, germ-tube emergence does not occur until spores possess at least four nuclei and have attained an average volume of 198.1 ± 87.3 μm^3. The difference in cell volume at the time of germination in the *sepA4* mutant is statistically significant when compared to the wild-type strain ($\alpha = 0.05$, $Z = 5.50$). Furthermore, the large swollen spores formed by the *sepA4* mutant possess an actin cytoskeleton that has not become polarized (Figure 2). These observations demonstrate that the establishment of hyphal polarity is severely delayed in the absence of *sepA* function. In addition, they suggest that SEPA may be needed to properly organize the actin cytoskeleton at the germination site.

Characterization of *sepA pod* double mutants: The characterization of SEPA as a member of the formins suggests that it may be required for organization of the actin cytoskeleton at sites of localized cell-wall deposition (Harris *et al.* 1997). According to this model, the localization and/or activity of gene products involved in polarized morphogenesis may be dependent upon SEPA function. In such a scenario, mutations in the *sepA* gene may exacerbate the phenotypes caused by inactivation of these gene products. This notion was investigated by constructing *sepA pod* double mutants and testing for synthetic phenotypes (*i.e.*, defects that exceed those caused by either parent).

The analysis of *sepA1*, *hypA6/podA1*, *sepA1*; *podB1*, and *sepA1*; *podC1* double mutants failed to reveal detectable synthetic growth defects at permissive or semipermissive temperatures. In contrast, unlike the parent single mutants, *sepA1*;*podD1* double mutants failed to form colonies at 37° (Figure 3A). Whereas *sepA1* or *podD1* mutants can establish hyphal polarity at this temperature, double mutant spores underwent considerable swelling, accumulated ≥16 nuclei, and failed to produce germ tubes (Figure 3, B–D). Moreover, the ability of *podD1* mutants to form colonies on agar-solidified media at 42° was abolished by the *sepA1* mutation (Figure 3A). These synthetic defects were not allele-specific, as *sepA3*;*podD1* double mutants displayed an identical spectrum of phenotypes (*sepA4*;*podD1* double mutants could not be constructed). These observations suggest that the activity and/or localization of PODD may depend upon SEPA function.

The *podC* and *podD* genes are required only for the establishment of hyphal polarity: Phenotypic characterization of the *pod* mutants has demonstrated that the *podB*, *podC*, and *podD* genes are required for the establishment of hyphal polarity. To determine if these genes are also needed for the maintenance of polarity, mutant spores were allowed to form germ tubes at permissive temperature and were then shifted to 42°. The polarity index (hyphal length/hyphal width; see materials and methods) was determined for samples taken prior to the shift and during the subsequent period of incubation at 42°. Under these conditions, mutants able to maintain hyphal polarity will continue to elongate and will possess a polarity index that increases with time. In contrast, the polarity index will remain unchanged or will decrease in mutants that cease hyphal elongation due to a failure to maintain polarity.

Following growth at 28°, hyphae formed by *hypA6/podA1* and *podC1* mutants displayed a polarity index that was noticeably lower than that of wild type or any of the other *pod* mutants (Figure 4A). This observation indicates that the *hypA6/podA1* and *podC1* mutations caused mild defects in polarized gowth even at permissive temperature. When shifted to restrictive temperature, *hypA6/podA1* and *podB1* mutants ceased hyphal elongation within 3 hr (Figure 4A). The polarity index for *podB1* mutants subsequently declined due to exten-

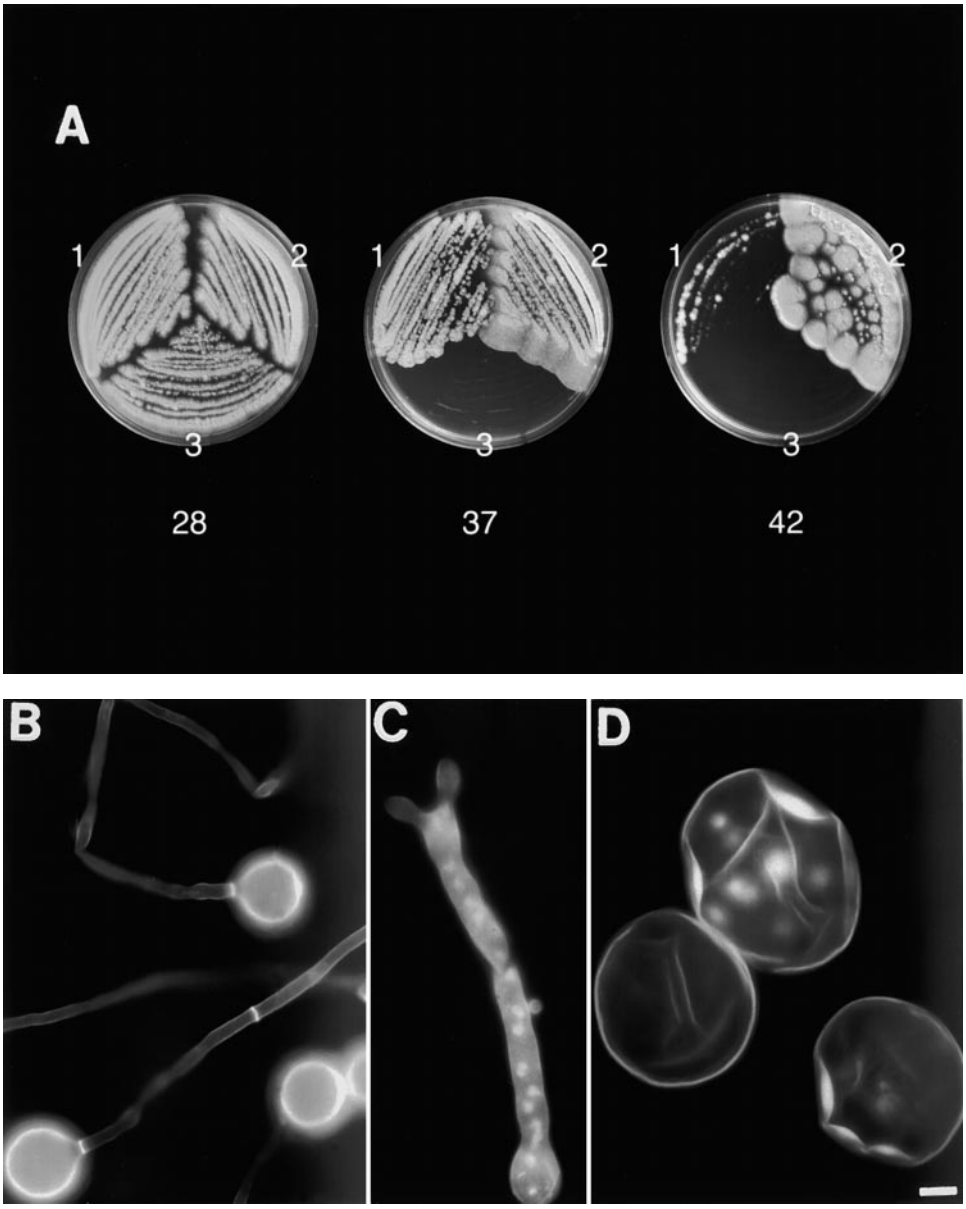

Figure 3.—Growth phenotype and cellular morphology of *sepA1 podD1* double mutants. (A) Growth phenotype. Conidiospores were streaked onto triplicate CM plates. Plates were incubated at 28° for 3 days, 37° for 2 days, or 42° for 2 days. The following strains were used: (1) ASH35 (*sepA1*); (2) ASH134 (*podD1*); (3) AAS100 (*sepA1*;*podD1*). Note that *podD1* mutants are able to grow on agar-solidified media at restrictive temperature. Under these conditions, mutant hyphae are swollen and display considerable lysis. They do not undergo asexual development. (B–D) Cellular morphology. Conidiospores were germinated on coverslips for 12 hr at 37°. Cells were stained with Calcofluor and Hoechst 33258 to visualize cell walls and nuclei, respectively. (B) ASH134 (*podD1*), (C) ASH35 (*sepA1*), (D) AAS100 (*sepA1*; *podD1*). Bar, 5 μm.

sive hyphal swelling in both apical and subapical regions (Figure 4, B and C). Further incubation of *hypA6*/*podA1* mutants at 42° resulted in considerable cellular lysis, which precluded determination of the polarity index. In contrast to the *hypA6*/*podA1* and *podB1* mutants, *podC1* and *podD1* mutants continued hyphal elongation when shifted to restrictive temperature (Figure 4, A, D and E). Whereas the apparent rate of hyphal elongation was similar for both wild-type and *podC1* mutants, it was notably slower for *podD1* (Figure 4A). Collectively, these results suggest that the *podC* and *podD* genes are specifically needed for the establishment of hyphal polarity and, unlike *hypA* and *podB*, are not required for general polarized growth.

The *podB1* mutation affects organization of the hyphal tip: The temperature shift experiments described above demonstrated that *podB1* mutants are defective in the maintenance of hyphal polarity. Typically, ~65% of *podB1* hyphae exhibit swollen apical and/or subapical regions within 2 hr of a shift from permissive to restrictive temperature. Hyphal swelling could conceivably be caused by disruption of the normal pattern of cell-wall deposition in upshifted *podB1* mutants. To test this notion, *podB1* hyphae incubated at permissive temperature were pulse labeled with FITC-WGA. As expected, intense staining was observed at hyphal tips, whereas subapical regions displayed relatively weak staining (Figure 4F). Labeled hyphae that were allowed to continue growth at permissive temperature displayed no staining at the immediate hyphal tip and were weakly stained throughout the rest of the hyphae (Figure 4G). This labeling pattern presumably reflects the restriction of new cell-wall deposition to the hyphal tip. In contrast, labeled *podB1* hyphae that were shifted to restrictive tempera-

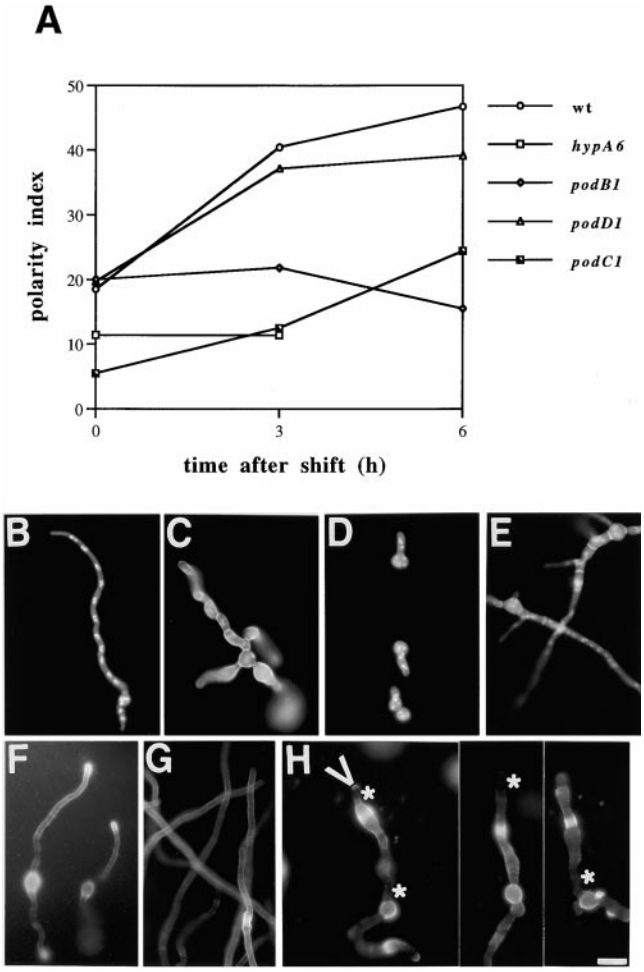

Figure 4.—Temperature shift analysis of the *pod* mutants. Conidiospores germinated on coverslips for 12 hr at 28° were shifted to restrictive temperature. Samples were taken at the time of shift, as well as 3 and 6 hr postshift. Coverslips were stained with Calcofluor and Hoechst 33258 to visualize cell walls and nuclei, respectively. (A) The polarity index was determined as described in the materials and methods. Strains used were as follows: A28 (wild type), ASH80 (*hypA6*/*podA1*), ASH83 (*podB1*), ASH130 (*podC1*), and ASH134 (*podD1*). (B and C) *podB* is needed for the maintenance of hyphal polarity. Different *podB1* hyphae are shown (B) at the time of shift and (C) at 6 hr postshift. Note that the shifted hypha has become Calcofluor bright and has undergone considerable swelling. (D and E) *podC* is not needed for the maintenance of hyphal polarity. Different *podC1* hyphae are shown at (D) the time of shift and (E) at 6 hr postshift. Note that apical extension has continued in the shifted hyphae. *podB1* hyphae incubated for 12 hr at 28° were pulse-labeled with FITC-WGA. Hyphae were then (F) fixed or rinsed to remove unbound FITC-WGA and allowed to grow at (G) 28° or (H) 42° for an additional 2 hr. Arrowheads depict stained hyphal apices, and asterisks indicate unstained subapical regions. Bar, 2 μm.

ture exhibited an aberrant pattern of staining (Figure 4H). In particular, hyphae possessing stained apices subtended by an unstained swollen region were frequently observed (43% of hyphae, $n = 100$). In addition, unstained subapical and basal regions were noted in almost all upshifted hyphae (97%, $n = 100$). These results suggest that the normal pattern of cell-wall deposition in *A. nidulans* requires a functional *podB* gene.

The altered pattern of cell-wall deposition in *podB1* mutants could be attributed to an underlying defect in cytoskeletal organization. This possibility was investigated by using indirect immunofluorescence with monoclonal anti-actin and anti-tubulin antibodies to examine cytoskeletal organization in upshifted *podB1* mutants. The organization of the actin and microtubule cytoskeletons in wild-type hyphae subjected to a temperature shift are shown in Figure 5. The pattern of actin localization displays two features (Figure 5A, left): (i) a dense patch at the immediate hyphal tip and (ii) cortical spots extending in a basal direction from the tip. Wild-type hyphae also form actin rings at septation sites (not shown; Harris *et al.* 1994). Cytoplasmic microtubules are found as longitudinal arrays that parallel the axis of hyphal growth and extend into the region subtending the tip (Figure 5A, right).

Following the shift to 42°, *podB1* mutants displayed apparent defects in actin cytoskeletal organization in hyphal tips (Figure 5). In particular, swollen hyphal tips did not contain a dense patch of actin staining (Figure 5, B and C). Instead, actin spots were distributed in a somewhat irregular fashion throughout the cortical region of the hyphal tip. Furthermore, atypical clusters of actin spots were observed in basal regions of swollen hyphae (Figure 5D). These clusters may correspond to the subapical sites of cell-wall deposition noted in the WGA-labeling experiment. Note that septation-associated actin rings were observed in upshifted *podB1* hyphae (Figure 5C), which suggests that the mutation does not affect the organization of all actin-based structures. In contrast to the actin cytoskeleton, microtubule organization did not appear to be dramatically affected by the *podB1* mutation (Figure 5E). However, it was noted that cytoplasmic microtubules did not form organized arrays in the vicinity of swollen tips. Collectively, these observations suggest that the *podB* gene is needed for proper organization of the cytoskeleton at the hyphal tip.

Patterns of polarized morphogenesis in *sepA* and *pod* mutants: Germinating *A. nidulans* conidiospores typically produce two or three germ tubes in a sequential manner; following the formation of the first germ tube, the spore establishes a second axis of polarity upon which the next germ tube emerges. To determine if the first axis of polarity exerts any influence over the selection of the second axis, the pattern of germ-tube emergence was examined in wild-type spores possessing two germ tubes. Three patterns were observed (Table 2): (i) bipolar, whereby the second germ tube emerges from the opposite hemisphere (~180°) relative to the first, (ii) quarterpolar, whereby the second germ tube emerges at a ~90° angle relative to the first, and (iii) random, whereby the second germ tube emerges at a

random site within the same hemisphere as the first. At all temperatures tested, the majority of wild-type conidia (>80%) displayed the bipolar pattern of germination (Table 2). Notably, <3% of wild-type spores exhibited the random pattern of germination. These results define the normal spatial pattern of germ-tube emergence in germinating *A. nidulans* conidia.

During routine observations, it was noted that the normal pattern of germ-tube emergence appeared to be altered in *sepA* mutants. Indeed, when examined at restrictive temperature, two different alleles of *sepA* dramatically perturb this pattern (Table 2; Figure 6). The bias toward the bipolar pattern is abolished by the *sepA1* and *sepA4* mutations, and both mutations also lead to an increase in the percentage of spores displaying the random pattern of germ-tube emergence (Table 2; Figure 6). Similar, but less severe, disruption of the normal pattern of polarized morphogenesis was observed when *podB1* and *podD1* mutants were incubated at the maximum permissive temperature that allowed formation of two germ tubes (Table 2). In contrast, *hypA6*/*podA1* and *podC1* mutations had no effect. These results suggest that SEPA and, to a lesser extent, PODB and PODD are needed for the spatial control of polarized morphogenesis in germinating *A. nidulans* conidia.

Two possible explanations could account for the ability of *sepA* mutations to disrupt the normal pattern of germ-tube emergence. First, the absence of a septum could permit the use of inappropriate germination sites. However, the failure of the *sepD5* mutation, which also prevents septum formation (Harris *et al.* 1994), to perturb the normal pattern of germ-tube emergence suggests that this possibility is unlikely (Table 2). Alternatively, the failure of *sepA* mutants to properly organize the actin cytoskeleton at presumptive germination sites could affect the pattern of germ-tube emergence. If so, other perturbations of the actin cytoskeleton should have a similar effect. To test this notion, wild-type spores were treated with CA following the formation of the first germ tube. In *A. nidulans*, CA treatment causes loss of normal actin cytoskeletal organization and blocks actin-dependent processes such as polarized growth, secretion, and septation (Harris *et al.* 1994; Torralba *et al.* 1998). Following CA treatment, spores were released into fresh medium and the site at which the second germ tube emerged was determined. As was observed in *sepA* mutants, the bias toward the 180° bipolar pattern was reduced by CA treatment (Table 2). Furthermore, the fraction of CA-treated spores displaying the random pattern was much larger than that of untreated controls. Because disruption of the microtubule cytoskeleton with nocodazole does not dramatically alter the pattern of

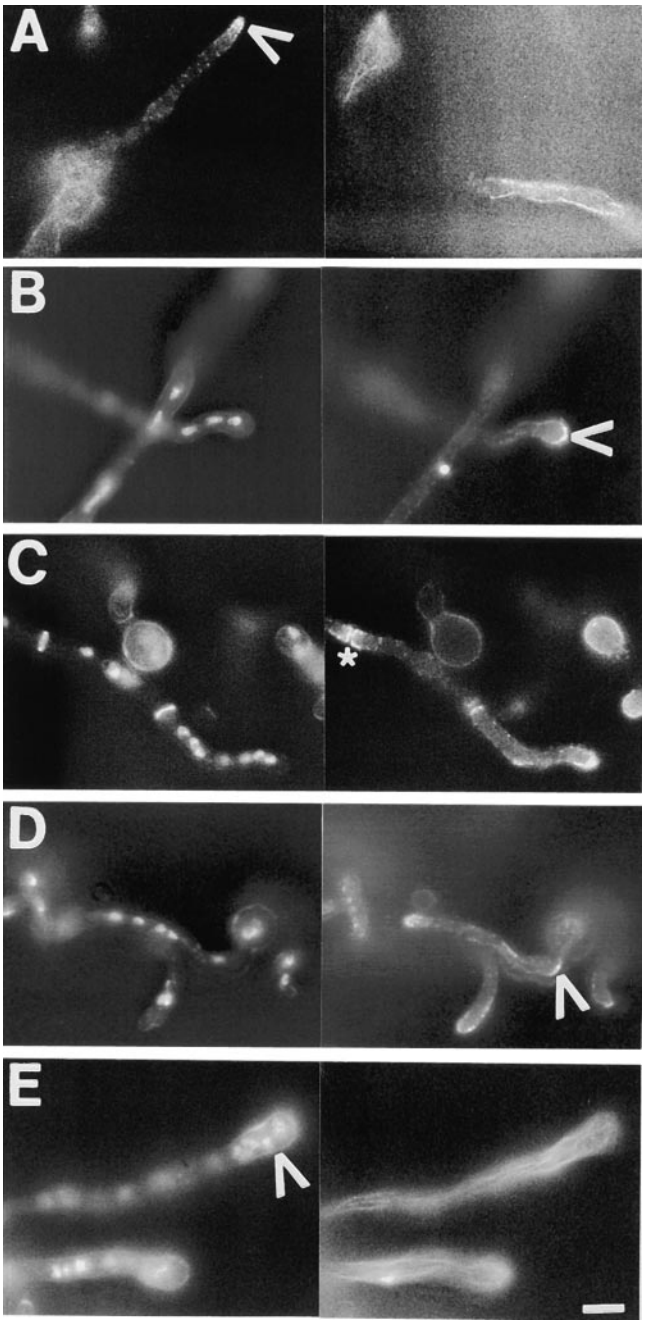

Figure 5.—*podB* mutants display defects in cytoskeleton organization. (A) Wild type and (B–E) *podB1* conidiospores germinated on coverslips for 12 hr at 28° were shifted to restrictive temperature. Samples were removed at 3 hr postshift, stained with mouse anti-actin or anti-α-tubulin monoclonal antibodies, and examined by indirect immunofluorescence microscopy. (A) Left, actin localization in wild-type hyphae. The accumulation of actin at the hyphal tip is depicted by the arrowhead. Right, tubulin localization in wild-type hyphae. (B–D) Left, nuclei (stained with Hoechst 33258) and septa (stained with Calcofluor) in *podB1* mutants. Right, actin localization in *podB1* mutants. The arrowhead in B indicates random arrays of cortical patches in the tips of swollen *podB1* hyphae. The arrowhead in D depicts a subapical region where aggregates of actin patches have formed. (E) Left, nuclei (stained with Hoechst 33258) and septa (stained with Calcofluor) in *podB1* mutants. The arrowhead points to a cluster of nuclei in a swollen hyphal tip. Right, tubulin localization in *podB1* mutants. Bar, 5 μm.

TABLE 2

Spatial patterns of polarized morphogenesis

Genotype	Temperature	180° biopolar[a]	90° quarterpolar[b]	random[c]
1 wt	42°	84	16	0
2 wt	37°	83.5	15	1.5
3 *sepA1*	42°	44	40	16
4 *sepA4*	42°	34	40	26
5 *hypA6/podA1*	35.5°	68	31	1
6 *podB1*	37°	52	36	12
7 *podD1*	35.5°	67	15	18
8 *sepD5*	42°	87	13	0
9 wt[d]	37°	76	21	3
10 wt + CA[d]	37°	60	21	19
11 wt + Noc[d]	37°	67	31	2

Conidiospores were germinated on coverslips for 9–12 hr at the indicated temperature. Coverslips were stained with Calcofluor and Hoechst 33258. For each strain, the pattern of germ-tube emergence was scored only in those conidia that possessed two germ tubes ($n = 100$). wt, wild type.

[a] In this pattern, the second germ tube emerges from the opposite hemisphere (~180°) of the spore relative to the first.

[b] In this pattern, the second germ tube emerges at ~90° relative to the first.

[c] In this pattern, the second germ tube emerges from a random site within the same hemisphere as the first.

[d] For these experiments, conidia were germinated on coverslips for 6 hr at 37°. Germinated conidia were subsequently treated for 3 hr with either 5 μg/ml CA or 2 μg/ml nocodazole (Noc) or were left untreated. They were then released into fresh media at 37° for an additional hour.

germ-tube emergence (other than causing increased use of the quarterpole; Table 2), these observations suggest that the spatial control of polarized morphogenesis in germinating *A. nidulans* spores primarily depends upon the integrity of the actin cytoskeleton.

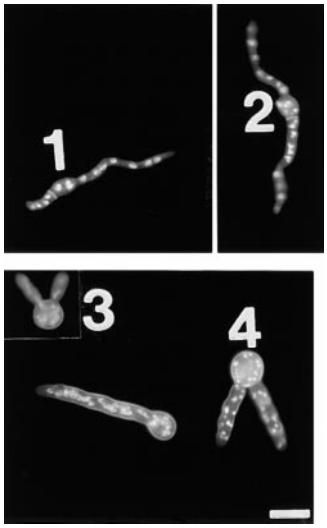

Figure 6.—*sepA* mutants exhibit defects in the spatial control of polarized morphogenesis. Conidiospores were germinated on coverslips for 9 hr at 42°. Coverslips were stained with Calcofluor and Hoechst 33258 to visualize cell walls and nuclei, respectively. Cells 1 and 2 are A28 (wild type) spores displaying bipolar germ-tube emergence. Cells 3 and 4 are ALH1 (*sepA4*) spores in which the second germ tube has emerged from a site adjacent to the first (*i.e.*, a random site as defined in the text). Bar, 2 μm.

DISCUSSION

During the process of germination, *A. nidulans* spores undergo a morphogenetic switch from isotropic expansion to polarized apical growth. Thereafter, cell-surface expansion is confined to the hyphal tip. Here, we report a first systematic attempt to identify and characterize genes that are required for the establishment and/or maintenance of hyphal polarity in *A. nidulans*. In particular, we show that: (i) the *sepA*, *hypA*, and *podB* genes are required for multiple aspects of polarized morphogenesis, (ii) the *podC* and *podD* genes are specifically needed for the establishment of hyphal polarity, and (iii) polarized morphogenesis relies upon spatial controls that are dependent upon the integrity of the actin cytoskeleton.

Genes required for the establishment and maintenance of hyphal polarity: Mutations in the *sepA*, *hypA*, and *podB* genes cause pronounced defects in multiple aspects of polarized morphogenesis in *A. nidulans* hyphae. In *podB1* mutants, spores and hyphal cells undergo isotropic expansion without defining polarized axes of growth. In contrast, spores possessing *sepA* or *hypA6/podA1* mutations are able to switch from isotropic expansion to polarized apical growth, but only after a severe delay. In addition, they form hyphal cells that

are abnormally wide and have a tendency to undergo apical branching (Harris *et al.* 1994; Kaminskyj and Hamer 1998). These observations imply that the *sepA*, *hypA*, and *podB* genes encode products that are involved in both the establishment and the maintenance of hyphal polarity in *A. nidulans*.

Previous molecular analyses demonstrated that SEPA is a member of an expanding family of proteins known as the formins (Frazier and Field 1997; Harris *et al.* 1997; Wasserman 1998). It has already been shown that SEPA is required for the formation of actin rings during septation in *A. nidulans* (Harris *et al.* 1997). Here, we provide evidence that SEPA is also needed to efficiently organize the actin cytoskeleton at the site of germ-tube emergence. As with other formins, the morphogenetic functions performed by SEPA are likely to be mediated by actin-associated proteins such as profilin and Bud6p (Manseau *et al.* 1996; Chang *et al.* 1997; Evangelista *et al.* 1997). In addition, spatial regulation of SEPA function presumably requires interactions with Rho-related GTPases (Evangelista *et al.* 1997; Imamura *et al.* 1997; Watanabe *et al.* 1997). However, it remains unclear how these interactions control the apparently distinct functions of SEPA during septation (*i.e.*, formation of an actin ring) *vs.* germination (*i.e.*, formation of a dense patch of cortical actin). Perhaps interactions between SEPA and different Rho-related GTPases permit spatial discrimination of its function. Alternatively, particular SEPA functions may be mediated by specific interactions with other proteins (such as PODD).

Complementation and linkage analyses have revealed that the *podA1* mutation resides in the recently characterized *hypA* gene (S. Kaminskyj, personal communication). Mutations in *hypA* cause two distinct phenotypes: (i) delocalized cell-wall deposition in tip hyphal cells and (ii) failure to arest growth and nuclear division in normally quiescent subapical hyphal cells (Kaminskyj and Hamer 1998). Molecular characterization of the *hypA* gene has failed to provide any clues to its function. In particular, *hypA* encodes a 5.3-kb open reading frame that does not show significant similarity to any sequence in current databases, nor does it possess informative motifs (Kaminskyj and Hamer 1998). Thus, *hypA* encodes a novel gene that is required for localized cell-surface expansion and cell-pattern formation in fungal hyphae. Because it is not known if *hypA1* and *hypA6*/*podA1* are null alleles, further functional characterization of *hypA* will require the construction and phenotypic analysis of a null mutant.

Although the molecular nature of PODB has yet to be determined, two observations suggest that it is required for the proper localization and/or function of the morphogenetic machinery at the hyphal tip. First, temperature shift and cell-wall labeling experiments show that the *podB1* mutations leads to isotropic cell-surface expansion. Second, results from indirect immunofluorescence microscopy demonstrate that the organization of the actin and microtubule cytoskeletons in the hyphal tip is disrupted by the *podB1* mutation. These phenotypes suggest that PODB may function in a manner analagous to the *Saccharomyces cerevisiae* polarity establishment proteins (*i.e.*, Cdc24p, Cdc42p, and Cdc43p; Pringle *et al.* 1995). For example, it could be a component of a signal transduction pathway (*i.e.*, a GTPase cascade; Chant and Stowers 1995) that orchestrates multiple events at sites of polarized morphogenesis. In this context, it should be noted that expression of the *A. nidulans* homologue of the Rho-related GTPase Cdc42p does not alter the morphogenetic defects caused by the *podB1* mutation (M. Lee, A. Hofmann and S. Harris, unpublished results).

Genes specifically required for the establishment of hyphal polarity: Temperature shift experiments show that the *podC* and *podD* genes are needed only for the establishment of hyphal polarity. If spores possessing a mutation in either of these genes are allowed to form a germ tube prior to imposition of restrictive conditions, hyphal elongation continues despite the apparent absence of PODC or PODD. Preliminary results obtained from indirect immunofluorescence experiments indicate that actin localizes normally in hyphal tips of upshifted *podD1* mutants, but is arranged in an apparently random array of cortical spots in the extremely swollen subtending spore (S. Harris, unpublished results). These observations suggest that the *podC* and *podD* genes encode functions specifically required for the switch from isotropic expansion to polarized apical growth that precedes spore germination. It should be noted that other aspects of the germination program (*i.e.*, breaking of spore dormancy, isotropic expansion, reentry into the cell cycle; d'Enfert 1997) occur normally in *podC1* and *podD1* mutants. This is the first evidence that spore germination in *A. nidulans* depends upon specific morphogenesis-related functions beyond those involved in general polarized growth.

Although the specific function of PODD remains to be determined, the observation that *sepA* mutations exacerbate the phenotypes caused by the *podD1* mutation implies that its localization and/or activity is dependent upon the presence of functional SEPA. In this context, the delayed switch to polarized apical growth caused by *sepA* mutations may in part be due to their effects on PODD.

Spatial control of polarized morphogenesis: *A. nidulans* conidiospores show a consistent spatial pattern of polarized morphogenesis, which was quantified in spores by scoring sites of germ-tube emergence. Because of the absence of morphological markers in ungerminated spores, the possibility that specific rules govern the emergence of the first germ tube could not be addressed. However, by using the first germ tube as a marker, it was possible to define the pattern in which the second germ tube emerged. Our experiments revealed the existence of a pronounced bias toward the

use of the opposite pole when the second germ tube is emerging from a spore.

To explore potential mechanisms that may be involved in specifying the site of germ-tube emergence, the spatial orientation of the second germ tube was scored in mutants that are defective in polarized morphogenesis. Notably, mutations in the *sepA* gene, and to a lesser extent in the *podB* and *podD* genes, reduce the bias toward bipolar germination. More strikingly, the use of random sites increased significantly in *sepA* mutants. Thus, SEPA, PODB, and PODD may control the pattern in which germ tubes emerge from a conidiospore. The observation that other mutations affecting polarized morphogenesis (*hypA6*/*podA1*, *podC1*) or septation (*sepD5*) do not perturb the site selection process is important for three reasons. First, it demonstrates that the effects of *sepA*, *podB*, and *podD* mutations are specific. Second, because *hypA6*/*podA1* and *sepA* mutants form large swollen spores prior to establishment of hyphal polarity, yet the *hypA6*/*podA1* mutation does not affect site selection, the effect of *sepA* mutations is most likely not due to increased spore size (*i.e.*, which may dilute a repressive signal). Third, although *sepD5* mutants fail to form a septum at the base of the extending germ tube (Harris *et al.* 1994; Wolkow *et al.* 1996), they still display the wild-type pattern of site selection. This suggests that the septum does not play a role in specifying subsequent axes of polarized morphogenesis.

SEPA and PODB are likely to control organization of the actin cytoskeleton in *A. nidulans*, so the observation that mutations in these genes alter the normal pattern of polarized morphogenesis suggests an underlying role for the actin cytoskeleton. Consistent with this notion, treatment of wild-type spores with CA, which prevents actin polymerization (Brown and Spudich 1981), mimics the effect of *sepA* mutations on selection of the site from which the second germ tube emerges. Thus, in addition to its known roles in the establishment and maintenance of hyphal polarity in *A. nidulans* (Torralba *et al.* 1998), the actin cytoskeleton also plays a role in defining the pattern of polarized morphogenesis. Although the function of cytoplasmic microtubules in the spatial control of hyphal morphogenesis in *A. nidulans* is less obvious, two observations do suggest a possible role. First, depolymerization of microtubules causes increased used of the quarterpole when the second germ tube is emerging from a spore. Second, loss of microtubules affects the maintenance of polarized axes of morphogenesis (Jochova *et al.* 1993).

The unicellular yeasts each display a bipolar pattern of polarized morphogenesis during vegetative growth (reviewed by Mata and Nurse 1998). The bipolar pattern of germ-tube emergence exhibited by *A. nidulans* shares a number of similarities with bipolar budding in *S. cerevisiae*. In both conidiospores and yeast cells, opposite poles are used for successive polarization events. Furthermore, in both organisms, the site selection process depends upon the integrity of the actin cytoskeleton (Yang *et al.* 1997) and is compromised by mutations in related proteins (*i.e.*, SEPA in *A. nidulans*, Bni1p in *S. cerevisiae*; Zahner *et al.* 1996). In contrast, cytoplasmic microtubules play a predominant role in establishing the bipolar mode of growth in *S. pombe* (Mata and Nurse 1997). Despite these differences, both yeasts use the appropriate cytoskeletal network to position morphological landmarks at specific cortical sites (Chant *et al.* 1995; Sanders and Herskowitz 1996; Mata and Nurse 1997; Yang *et al.* 1997). Thus, it is tempting to speculate that a functionally analogous cortical marking system may exist in *A. nidulans* conidiospores.

We thank Kim Nichols and Thaphne Hadjimichael for assistance during the genetic analysis of the *pod* mutants. We also thank John Hamer, Tom Wolkow, Susan Kaminskyj, and the anonymous reviewers for comments that greatly improved the manuscript. This work was supported by a grant from the National Science Foundation (MCB-9723711).

LITERATURE CITED

Adams, A. E. M., D. I. Johnson, R. M. Longnecker, B. F. Sloat and J. R. Pringle, 1990 *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell. Biol. **111:** 131–142.

Brown, S. S., and J. A. Spudich, 1981 Mechanism of action of cytochalasin: evidence that it binds to actin filament ends. J. Cell Biol. **88:** 487–491.

Bruno, K. S., R. Aramayo, P. F. Minke, R. L. Metzenberg and M. Plamann, 1996 Loss of growth polarity and mislocalization of septa in a *Neurospora* mutant altered in the regulatory subunit of cAMP-dependent protein kinase. EMBO J. **15:** 5772–5782.

Chang, F., D. Drubin and P. Nurse, 1997 cdc12p, a protein required for cytokinesis in yeast, is a component of the cell division ring and interacts with profilin. J. Cell Biol. **137:** 169–182.

Chant, J., and L. Stowers, 1995 GTPase cascades choreographing cellular behaviour: movement, morphogenesis, and more. Cell **81:** 1–4.

Chant, J., M. Mischke, E. Mitchell, I. Herskowitz and J. R. Pringle, 1995 Role of Bud3p in producing the axial budding pattern of yeast. J. Cell Biol. **129:** 767–778.

d'Enfert, C., 1997 Fungal spore germination: insights from the molecular genetics of *Aspergillus nidulans* and *Neurospora crassa*. Fungal Genet. Biol. **21:** 163–172.

Evangelista, M., K. Blundel, M. S. Longtine, C. J. Chow, N. Adames *et al.*, 1997 Bni1p, a yeast formin linking Cdc42p and the actin cytoskeleton during polarized morphogenesis. Science **276:** 118–122.

Fiddy, C., and A. P. J. Trinci, 1976 Mitosis, septation, branching, and the duplication cycle in *Aspergillus nidulans*. J. Gen. Microbiol. **97:** 169–184.

Frazier, J. A., and C. M. Field, 1997 Are FH proteins local organizers? Curr. Biol. **7:** 414–417.

Govindan, B., and P. Novick, 1995 Development of cell polarity in budding yeast. J. Exp. Zool. **273:** 401–424.

Gow, N. A. R., 1995 Tip growth and polarity, pp. 277–299 in *The Growing Fungus*, edited by N. A. R. Gow and G. M. Gadd. Chapman & Hall, London.

Grinberg, A., and I. B. Heath, 1997 Direct evidence for Ca^{2+} regulation of hyphal branch induction. Fungal Genet. Biol. **22:** 127–139.

Harris, S. D., 1997 The duplication cycle in *Aspergillus nidulans*. Fungal Genet. Biol. **22:** 1–12.

Harris, S. D., J. L. Morrell and J. E. Hamer, 1994 Identification and characterization of *Aspergillus nidulans* mutants defective in cytokinesis. Genetics **136:** 517–532.

Harris, S. D., L. Hamer, K. E. Sharpless and J. E. Hamer, 1997

The *Aspergillus nidulans sepA* gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity. EMBO J. **16:** 3474–3483.

Imamura, H., K. Tanaka, T. Hihara, M. Umikawa, T. Kamei *et al.*, 1997 Bni1p and Bnr1p: downstream targets of the Rho family small G-proteins which interact with profilin and regulate actin cytoskeleton in *Saccharomyces cerevisiae.* EMBO J. **16:** 2745–2755.

Jackson, C. L., J. B. Konopka and L. H. Hartwell, 1991 *S. cerevisiae* α pheromone receptors activate a novel signal transduction pathway for mating partner discrimination. Cell **67:** 389–402.

Jackson, S. L., and I. B. Heath, 1993 Roles of calcium ions in hyphal tip growth. Microbiol. Rev. **57:** 367–382.

Jochova, J., I. Rupes and J. F. Peberdy, 1993 Effect of the microtubule inhibitor benomyl on protein secretion in *Aspergillus nidulans.* Mycol. Res. **97:** 23–27.

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

Kaminskyj, S. G. W., and J. E. Hamer, 1998 *hyp* loci control cell pattern formation in the vegetative mycelium of *Aspergillus nidulans.* Genetics **148:** 669–680.

Lehmler, C., G. Steinberg, K. M. Snetselaar, M. Schliwa, R. Kahmann *et al.*, 1997 Identification of a motor protein required for filamentous growth in *Ustilago maydis.* EMBO J. **16:** 3464–3473.

Lew, D. J., and S. I. Reed, 1993 Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. J. Cell Biol. **120:** 1305–1320.

Manseau, L., J. Calley and H. Phan, 1996 Profilin is required for posterior patterning of the *Drosophila* oocyte. Development **122:** 2109–2116.

Mata, J., and P. Nurse, 1997 *tea1* and the microtubule cytoskeleton are important for generating global spatial order within the fission yeast cell. Cell **89:** 939–949.

Mata, J., and P. Nurse, 1998 Discovering the poles in yeast. Trends Cell Biol. **8:** 163–167.

McGoldrick, C. A., C. Gruver and G. S. May, 1995 *myoA* of *Aspergillus nidulans* encodes an essential myosin required for secretion and polarized growth. J. Cell Biol. **128:** 577–587.

Mitchison, J. M., and P. Nurse, 1985 Growth in cell length in the fission yeast *Schizosaccharomyces pombe.* J. Cell Sci. **75:** 357–376.

Mondesert, G., D. J. Clarke and S. I. Reed, 1997 Identification of genes controlling growth polarity in the budding yeast *Saccharomyces cerevisiae*: a possible role of *N*-glycosylation and involvement of the exocyst complex. Genetics **147:** 421–434.

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

Oakley, B. R., and S. A. Osmani, 1993 Cell cycle analysis using the filamentous fungus *Aspergillus nidulans*, pp. 127–142 in *The Cell Cycle: A Practical Approach*, edited by P. Fantes and R. Brooks. Oxford University Press, Oxford.

Pringle, J. R., E. Bi, H. Harkins, J. E. Zahner, C. De Virgilio *et al.*, 1995 Establishment of cell polarity in yeast. Cold Spring Harbor Symp. Quant. Biol. **60:** 729–743.

Roemer, T., L. G. Vallier and M. Snyder, 1996 Selection of polarized growth sites in yeast. Trends Cell Biol. **6:** 434–441.

Sanders, S., and I. Herskowitz, 1996 The Bud4 protein of yeast, required for axial budding, is localized to the mother/bud neck in a cell cycle-dependent manner. J. Cell Biol. **134:** 413–427.

Seiler, S., F. E. Nargang, G. Steinberg and M. Schliwa, 1997 Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa.* EMBO J. **16:** 3025–3034.

Sloat, B., and J. R. Pringle, 1978 A mutant of yeast defective in cellular morphogenesis. Science **200:** 1171–1173.

Som, T., and V. S. R. Kolaparthi, 1994 Developmental decisions in *Aspergillus nidulans* are modulated by Ras activity. Mol. Cell. Biol. **14:** 5333–5348.

Torralba, S., M. Raudaskoski, A. M. Pedregosa and F. LaBorda, 1998 Effect of cytochalasin A on apical growth, actin cytoskeleton organization and enzyme secretion in *Aspergillus nidulans.* Microbiology **144:** 45–53.

Valtz, N., M. Peter and I. Herskowitz, 1995 *FAR1* is required for oriented polarization of yeast cells in response to mating pheromones. J. Cell Biol. **131:** 863–873.

Wasserman, S., 1998 FH proteins as cytoskeletal organizers. Trends Cell Biol. **8:** 111–115.

Watanabe, N., P. Madaule, T. Reid, T. Ishizaki, G. Watanabe *et al.*, 1997 p140mDia, a mammalian homolog of *Drosophila diaphonus*, is a target protein for Rho small GTPase and is a ligand for profilin. EMBO J. **16:** 3044–3056.

Wolkow, T. D., S. D. Harris and J. E. Hamer, 1996 Cytokinesis in *Aspergillus nidulans* is controlled by cell size, nuclear positioning and mitosis. J. Cell Sci. **109:** 2179–2188.

Wu, Q., T. M. Sandrock, B. G. Turgeon, O. C. Yoder, S. G. Wirsel *et al.*, 1998 A fungal kinesin required for organelle motility, hyphal growth, and morphogenesis. Mol. Biol. Cell **9:** 89–101.

Yang, S., K. R. Ayscough and D. G. Drubin, 1997 A role for the actin cytoskeleton of *Saccharomyces cerevisiae* in bipolar bud-site selection. J. Cell Biol. **136:** 111–123.

Yarden, O., M. Plamann, D. Ebbole and C. Yanofsky, 1992 *cot-1*, a gene required for hyphal elongation in *Neurospora crassa*, encodes a protein kinase. EMBO J. **11:** 2159–2166.

Zahner, J. E., H. A. Harkins and J. R. Pringle, 1996 Genetic analysis of the bipolar pattern of bud site selection in the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol. **16:** 1857–1870.

Zar, J. H., 1984 *Biostatistical Analysis.* Prentice-Hall, Englewood Cliffs, NJ.

Communicating editor: J. J. Loros