hyp Loci Control Cell Pattern Formation in the Vegetative Mycelium of *Aspergillus nidulans*

Susan G. W. Kaminskyj[1] and John E. Hamer

Department of Biological Sciences, Hansen Life Science Research Building, Purdue University, West Lafayette, Indiana 47907-1392

Manuscript received September 18, 1997
Accepted for publication October 20, 1997

ABSTRACT

Aspergillus nidulans grows by apical extension of multinucleate cells called hyphae that are subdivided by the insertion of crosswalls called septa. Apical cells vary in length and number of nuclei, whereas subapical cells are typically 40 μm long with three to four nuclei. Apical cells have active mitotic cycles, whereas subapical cells are arrested for growth and mitosis until branch formation reinitiates tip growth and nuclear divisions. This multicellular growth pattern requires coordination between localized growth, nuclear division, and septation. We searched a temperature-sensitive mutant collection for strains with conditional defects in growth patterning and identified six mutants (designated *hyp* for *hyp*ercellular). The identified *hyp* mutations are nonlethal, recessive defects in five unlinked genes (*hypA-hypE*). Phenotypic analyses showed that these *hyp* mutants have aberrant patterns of septation and show defects in polarity establishment and tip growth, but they have normal nuclear division cycles and can complete the asexual growth cycle at restrictive temperature. Temperature shift analysis revealed that *hypD* and *hypE* play general roles in hyphal morphogenesis, since inactivation of these genes resulted in a general widening of apical and subapical cells. Interestingly, loss of *hypA* or *hypB* function lead to a cessation of apical cell growth but activated isotropic growth and mitosis in subapical cells. The inferred functions of *hypA* and *hypB* suggest a mechanism for coordinating apical growth, subapical cell arrest, and mitosis in *A. nidulans.*

MULTICELLULAR organisms use a variety of molecular signals to establish patterns of cell growth, cell cycle arrest, cell differentiation, and cell death. For example, pattern formation in insect and animal embryos initially depends on morphological gradients of positional determinants that are laid down during oogenesis (Duffy and Perrimon 1996; Hammerschmidt *et al.* 1997). In plants, only a provisional body plan is established in the embryo, and asymmetric cell divisions and highly localized patterns of gene expression continually establish new organs as the plant grows (Meyerowitz 1997). One of the simplest patterns of multicellular growth occurs in the hyphae of filamentous fungi.

In filamentous fungi, growth is localized to the tips of hyphae, which are tubular, uni-, or multinucleate cells. Cells within hyphae are delimited by the insertion of crosswalls called septa (Fiddy and Trinci 1976; Gull 1978; Harris *et al.* 1994). In some fungi, nuclear division cycles are synchronous within cell compartments delimited by septa (Rosenberger and Kessel 1967; Robinow and Caten 1969). Septa may play a variety of roles, such as preventing loss of cytoplasm during hyphal damage (Richle and Alexander 1965) and partitioning cell growth and differentiation (Gull 1978). Although much is known about fungal cell wall synthesis (Gooday 1994) and cytoskeletal structure (Heath 1994; Ayscough and Drubin 1996), little is known about the molecular mechanisms that establish the apical growth patterns in fungal mycelia.

Cell morphogenesis in fungi is best understood in the polarized growth processes of budding and mating projection in the yeast *Saccharomyces cerevisiae* (Kron and Gow 1995; Pringle *et al.* 1995; Drubin and Nelson 1996). These processes resemble hyphal growth in filamentous fungi in that they involve polarization of the actin cytoskeleton and localized cell wall synthesis. In filamentous fungi, calcium gradients (Jackson and Heath 1993; Pierson *et al.* 1994), pH gradients (Kropf *et al.* 1995), cyclic AMP signaling (Kore-eda *et al.* 1991; Bruno *et al.* 1996), calcium signaling (Rasmussen *et al.* 1994; Dayton and Means 1996), and *ras* function (Som and Kolaparthi 1994) also play roles in tip growth, and some of these processes are essential for growth in *S. cerevisiae* (Cunningham and Fink 1994, 1996; Mosch *et al.* 1996; Ward *et al.* 1995).

Aspergillus nidulans forms a multicellular mycelium with a highly regulated pattern of growth and nuclear division (Figure 1). In the vegetative mycelium, only tip growing cells contain actively dividing nuclei, which have synchronous mitotic cycles. Septa are formed with

Corresponding author: Dr. John Hamer, Department of Biological Sciences, 333 Hansen Life Science Research Building, Purdue University, West Lafayette, IN 47907-1392.
E-mail: jhamer@bilbo.bio.purdue.edu
[1]*Present address:* Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N 5E2 Canada.
E-mail: Susan.Kaminskyj@usask.ca

a uniform spacing along vegetative hyphae (Fiddy and Trinci 1976), and septum formation is dependent on mitosis, nuclear positioning, and attainment of a critical cell size (Wolkow *et al.* 1996). After septation, subapical cells cease growth and nuclei arrest in interphase. This state can continue indefinitely, unless branching reestablishes polarized growth and active nuclear division. Nuclear division, however, is not essential for maintaining polarized growth, since many conditional nuclear division mutants of *A. nidulans* are able to produce a polarized growing germ tube at restrictive temperature (Morris 1976). Conversely, overproduction of the activated form of the GTPase, *A-ras*, results in swollen spores or cells containing abundant nuclei (Som and Kolaparthi 1994). Thus, the mechanisms that restrict cell growth and mitosis to tip cells in *A. nidulans* are unknown.

A large collection of conditional mutants of *A. nidulans* has been generated (Morris 1976; Harris *et al.* 1994) and used to identify loci involved in various growth processes. We screened this mutant collection for strains with defective patterns of growth and septation. Here, we describe the characterization of six conditional mutants defining five genes that appear to play roles in mycelial pattern formation. We call these mutations *hypA1-hypE2* for *hyp*ercellular because they have abnormally short subapical cells with abundant nuclei at restrictive temperature. Phenotypic analyses suggest that *hypC* controls the spacing of septa and may be involved in cell size control. Mutations in *hypA*, *hypB*, *hypD*, and *hypE* cause the proliferation of nuclei in subapical cells and cause defects in cell polarization. Intriguingly, *hypA* and *hypB* appear to play dual roles as positive effectors of tip cell growth and negative regulators of subapical cell growth. All *hyp* mutants are able to complete the asexual life cycle at restrictive temperature, suggesting that these mutants mislocalize growth cues that are required to establish wild-type mycelial growth patterns.

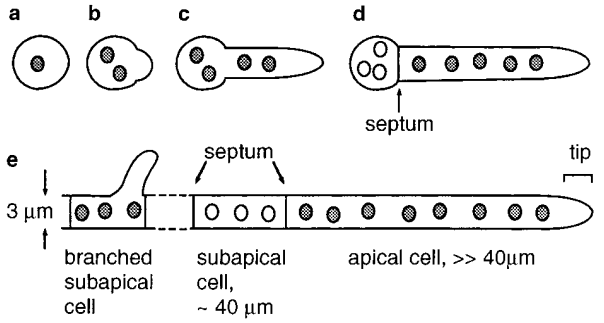

Figure 1.—Cartoon summarizing the morphology of cells in wild-type *A. nidulans* hyphae grown at 28°. This cartoon is not to scale. A conidium (a) germinates (b), and nuclei migrate into the germ tube as it grows (c). The first septum is deposited at the base of the germ tube (d) when the germling has eight or more nuclei. Hyphae (e) are tubular, ~3 μm in diameter. Apical cells are variable in length and usually much longer than 40 μm. Apical cells contain many (sometimes exceeding 50) nuclei (circles) that are evenly spaced along the length of the cell. Apical cell growth is indeterminate and occurs only at the hyphal tip. Subapical cells are defined by septa averaging 40 μm apart. Subapical cells contain three to four evenly spaced nuclei. Subapical cells can branch, and then the branched cell grows like an apical cell. Apical and branched subapical cells have ongoing nuclear cycles (filled circles), while nuclei in unbranched subapical cells are arrested in interphase (empty circles).

MATERIALS AND METHODS

The strains used in this study are shown in Table 1. Methods are described in Harris *et al.* (1994) and Kafer (1977). For phenotypic analyses, we used strains grown on complete medium (CM) with supplements as described by Kafer (1977). Where required, media contained 1 m sucrose or 0.01–0.1% Calcofluor (a gift of American Cyanamid, Princeton, NJ).

Mutagenesis and screening: Six hundred fifty temperature-sensitive mutants were generated by 4-nitroquinoline oxide mutagenesis of strain A28, extending the collection screened by Harris *et al.* (1994) to 1800 mutants. Temperature-sensitive strains were screened microscopically after 16 hr growth at 28° and 42° on thin agar plates (Harris *et al.* 1994). Strains were selected for having abnormally short subapical cells when grown at 42°. These strains were mated with GR5. Cleistothecia were cleaned of Hülle cells by rolling on 4% agar containing 1% diatomaceous earth (Sigma, St. Louis; Kaminskyj and Hamer 1996). Segregation of the temperature-sensitive phenotype was determined for ascospore progeny grown on replica plates at 28° and 42°. Six or more temperature-sensitive progeny from each cross were examined after growth on coverslips at 42° to confirm the mutant phenotype. Mutant progeny were crossed to strain AH12.

Pairwise crosses between all combinations of mutants were used to assess allelism. Diploids were generated from heterokaryons in these pairwise crosses (Kafer 1977) and used to test for genetic complementation. Diploids made from crosses between *hyp* and wild-type strains were used to test for dominance/recessivity. Haploid sectors showing segregation of parental spore colors were generated from these diploids using benlate-induced instability (Hastie 1970). Gene designations were assigned after Clutterbuck and Arst (1995).

Microscopy: Conidia were inoculated in liquid and allowed to attach to glass coverslips for microscopic examination. Conidia were inoculated into liquid media (10^5 conidia/ml) with nutritional supplements and 100 μg/ml ampicillin, and they were incubated at 28° and 42°. Germlings were fixed and stained for nuclei using Hoechst (Warrington, PA) and for walls using Calcofluor (Harris *et al.* 1994). Some germlings were stained with 1 mg/ml FITC-conjugated wheat germ agglutinin (FITC-WGA; Sigma), which binds to the chitinous cell wall. Slides were mounted in Citifluor (Ted Pella, Redding, CA) and examined with a BH-2 microscope (Olympus, Lake Success, NY) using phase contrast and UV or FITC epifluorescence. Germling features were measured with an ocular micrometer. Statistical analyses used Statview SE+Graphics (Abacus Concepts, Berkeley, CA).

Subapical cell characteristics: Germlings were grown on coverslips for 18–24 hr at 28° and 42° and prepared for microscopy (Harris *et al.* 1994). Unbranched subapical cells were chosen for having a complete septum at each end, with these septa being perpendicular to the long axis of the hypha. Cell length was defined as the distance between adjacent septa, measured at the junction between the septa and the lateral hyphal wall. Unlike other *hyp* strains, *hypC4* germlings

TABLE 1

Genotypes of strains used in this study

Strain	Genotype
ASK 30[a]	*hypA1; wA3; pyroA4*
ASK 39[b]	*hypA1 biA1 pabaA6*
ASK 32[b]	*biA1 pabaA6; hypB5*
ASK 80[a]	*pabaA6; hypB5; chaA1*
ASK 70[a]	*biA1 pabaA6 pyrG89; hypC4*
ASK 158[b]	*hypC4; pyroA1*
ASK 65[a,b]	*biA1 pabaA6 hypD3*
ASK 53[a]	*pabaA6; wA3; hypE2*
ASK 54[b]	*biA1 pabaA6; pyroA1; hypE2*
A28[a,b,c]	*biA1 pabaA6*
A104[c]	*yA2 adE20; AcrA1; phenA2; pyroA4; lysB5; sB3; nicB8; coA1*
A297[c]	*lysF51 biA1; sB3*
AH12[d]	*argB2; chaA1*
ASH20[e]	*sepA1 yA2; argB2*
GR5[c]	*pyrG89; wA3; pyroA4*

All strains used in this study were *ve*$^-$.

[a] Strains generated in this study used for microscopic analysis.

[b] Strains generated in this study used for colony phenotype analysis.

[c] Available from Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City, KS 66160-7420.

[d] Available from John Hamer.

[e] Harris *et al.* 1994.

grown at 42° often had thick septa, so their cell lengths were measured from center to center of adjacent septa. At least 50 subapical cells were analyzed for each strain and growth temperature. Hyphal width was measured across septa that were complete and perpendicular to the long axis of the hypha. At least 30 width measurements were made for each strain and growth temperature.

Germling morphogenesis: Germination and growth characteristics were determined microscopically, as described below, for wild-type and *hyp* germlings grown for up to 16 hr at 28° and 42°. Only germlings growing from isolated spores (at least one spore diameter from all other spores) were chosen for this analysis. *A. nidulans* conidia adhere tightly to glass coverslips after 3–4 hr in complete media (CM) at either temperature, regardless of germination. As a result, ungerminated conidia were not lost from the original population.

Germination was defined as the production of a germ tube that was at least half the spore diameter. At least 100 spores were counted for each data point. Results are shown as percentage germination.

Total hyphal lengths were measured with an ocular micrometer and 20×, 40×, or 100× objectives, as appropriate. Total hyphal length was defined as the distance along the long axis of all hyphae in each germling plus all branches that were longer than half the hyphal diameter. At least 50 germlings were measured for each strain and growth temperature. Total hyphal length per germling is shown as the mean value ± SE of the mean.

The total number of nuclei in wild-type and *hyp* strains was determined by counting them in spores and germlings. Mitosis is synchronous in wild-type germlings (Bergen and Morris 1983), but not in some *hyp* strains grown at 42°. To compare the number of nuclei between *hyp* and wild-type germlings, these values are shown as the population mean ± SEM. This method is not intended to convey that germlings could contain partial nuclei. Data point markers obscured small error bars when these data were plotted.

Septa were counted in isolated wild-type and *hyp* germlings grown at 28°and 42°, using fixed germlings stained with Hoechst and Calcofluor. Unlike the cell length measurements described above, in this analysis, septa were counted whether they were perpendicular or oblique to the long axis of the hypha, or if they were incomplete. If incomplete, a continuous ring of Calcofluor-stained material had to extend more than half of the hyphal circumference. *hypA1* and *hypE2* strains frequently had double septa (morphologically normal septa <1 μm apart). Double septa usually defined anucleate cells, but some had single nuclei (*e.g.*, Figure 3a), so they were counted as two septa. The broad septa characteristic of *hypC4* strains were counted as single septa because an intervening space could not be resolved. Septation is asynchronous in *hyp* germlings growing at 42°, so the number of septa per germling was expressed as population mean ± SEM, as described above for nuclei.

RESULTS

Genetic analysis: Six mutant strains with abnormally short subapical cells in their vegetative hyphae were found by visually screening 1800 temperature-sensitive mutants (Harris *et al.* 1994). *hyp* mutants did not have terminal arrest phenotypes, and they grew indefinitely at restrictive temperature, albeit with abnormal morphologies. Table 2 summarizes the genetic analysis of the *hyp* mutants. The mutants defined six single-gene mutations in five complementation groups. Two alleles were recovered for *hypA*, and single alleles were recovered for the remaining mutants. All mutations were recessive to wild type.

hypA and *hypE* were mapped to chromosomes by fortuitous linkages to markers segregating in backcrosses. *hypA* is linked to *pabaA* (4%) on chromosome I. Because of the proximity of another hyphal morphology gene in this region (*sepA*; Harris *et al.* 1997), we investigated the linkage between *hypA* and *sepA*. *hypA* is tightly linked to *sepA* (2%) but is not allelic with it. The near-centromere chromosome 1R marker *lysF* is linked to both *hypA* (0.3%) and *sepA* (2.7%). *hypE2* is linked to *chaA* (8%) on chromosome VIII. *hypC4* and *hypD3* were mapped to chromosomes by parasexual genetic crosses, as described by Kafer (1977), using strain A104.

Phenotype analysis: Figure 2 shows typical morphologies of wild-type and *hypA1* hyphae grown for 18 hr at 28° and 42°. Wild-type cells grown at 28° are shown in Figure 2a. *hypA1* hyphae grown at 28° (Figure 2b) closely resemble those of the wild-type strain. This is typical of all *hyp* strains grown at 28°. Figure 2c shows the dramatic difference between *hypA1* and wild-type cells grown in mixed culture at 42°. The *hypA1* germlings were shorter and wider, and their walls and septa stained more brightly with Calcofluor. The *hypA1* germlings had made one or two closely spaced septa despite

TABLE 2

Summary of the genetic characteristics of *hyp* strains

Mutant	Segregation of temperature-sensitive(ts) phenotype		Dominant (D) or recessive (R)	Linkage group	Allele
	ts−:ts+[a]	ts−:ts+[b]			
E8	263:306	19:23	R	I	*hypA1*
B47	87:90	61:87	R	VII	*hypB5*
B81	18:10	33:46	R	III	*hypC4*
D66	21:17	35:21	R	II or VII	*hypD3*
B42	48:66	26:24	R	VIII	*hypE2*
8-160[c]	NA	NA	R	I	*hypA6*[d]

[a] Segregation of temperature-sensitive phenotype in single ascospore progeny from crosses between mutant strains and GR5.

[b] Segregation of temperature-sensitive phenotype in single ascospore progeny from crosses between temperature-sensitive progeny from (a) and AH12.

[c] Strain recovered independently by Steve Harris and Michelle Momany from temperature-sensitive collection of Harris *et al.* (1994).

[d] *hyp* designation based on morphological assessment after growth at 42°. Allelic assignment based on crosses with *hypA1-hypE2* strains. *hypA1*/*hypA6* diploids were temperature sensitive and phenocopied *hypA1*.

NA, not assessed

their limited length, compared to ~10 septa per germling for the wild-type strain. Despite these abnormalities, septum placement was always asymmetric, producing a relatively long apical cell. Figure 2d shows older *hypA1* germlings that were labeled with FITC-WGA. The arrows indicate the base of hemispherical hyphal tips, behind which the apical cells continued to increase substantially in width, distinctly unlike cylindrical wild-type hyphae (Figure 2, a and c) or *hyp* strains grown at 28° (Figure 2b). *hypA1* germlings were pulse labeled with FITC-WGA and allowed to grow at 42° for another 3 hr. Walls formed during this interval were relatively faint (Figure 2e).

Figure 3 shows *hyp* germlings grown for 24 hr at 42°, then fixed and stained for walls and nuclei. Quantitative analysis of the *hyp* phenotypes is summarized in Table 3, and subapical cell characteristics are shown graphically in Figure 4. With the exception of *hypC4*, *hyp* germlings grown at 42° were significantly wider than wild type (see also Figure 2c), and all *hyp* mutants had significantly shorter subapical cells. The increased hyphal width in *hyp* mutants might compensate for the decrease in subapical cell length and thus conserve subapical cell volume. To test this hypothesis, average subapical cell volumes were calculated using the data in Table 3, A and C. Average cell volumes for wild-type and *hyp* strains varied considerably at 28°, suggesting that these calculations represent modest approximations. At 42°, the majority of the *hyp* mutants showed only modest changes (±50%) in average cell volume, with *hypD3* and *hypE2* increasing and *hypB5* and *hypC4* decreasing. In contrast, *hypA1* underwent a dramatic increase in subapical cell volume. This result suggests that changes in cell width in *hypA1* mutants result in increased cell volumes.

Wild-type hyphae typically had three to four nuclei per cell, regardless of growth temperature, as did *hyp* strains grown at 28° (Figure 4; Table 3B). In contrast, the short subapical cells produced by *hypA1*, *hypB5*, *hypD3*, and *hypE2* strains at 42° had significantly more nuclei. *hypA1* produced the most dramatic changes, with some cell compartments containing up to 20 nuclei. *hypA1* nuclei appeared smaller and more compacted than wild-type nuclei. The dramatic increase in cell volume in *hypA1* mutants may activate additional rounds of nuclear division.

Other defects observed in *hyp* mutants included a high frequency of dichotomous apical branches in *hypA1* and *hypE2* germlings (Figure 3a). *hypB5* and *hypD3* germlings had near-apical, lateral branches (Figure 3, b and d). About 25% of *hypA1* and *hypE2* germlings had double, oblique, or incomplete septa, unlike wild-type or other *hyp* strains. About 5% of *hypB5* germlings had anucleate subapical compartments. *hypC4* germlings had cell compartments that were half the size of wild-type at 42°, and ~20% of the septa were thickened or appeared as double septa (Figure 3c, arrow). All *hyp* mutants could be grown indefinitely at 42°, and all mutants were able to complete the asexual life cycle and produce viable conidia at restrictive temperature (data not shown).

Figure 5 shows plate phenotypes for wild-type and *hyp* mutants growing at 28° and 42°. Wild-type and *hypA1-hypD3* colonies grew well at 28°, but growth was restricted for the *hypE2* strain (Figure 6a). All *hyp* strains were clearly restricted for growth at 42°, com-

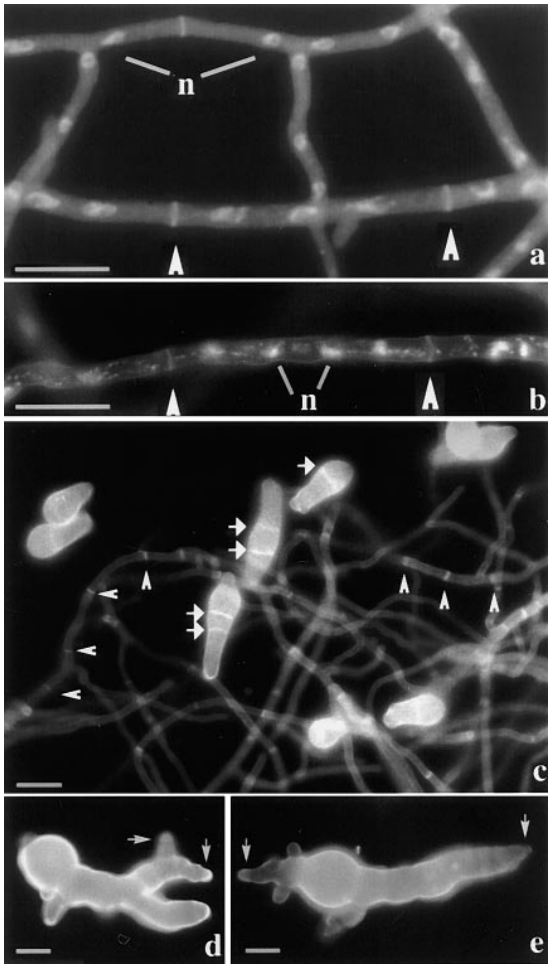

Figure 2.—Wild-type (a) and *hypA1* (b) strains growing at 28°. Wild-type and *hypA1* strains growing in mixed culture at 42° (c). *hypA1* germlings grown at 42° (d and e). Germlings were grown for 20 hr on coverslips, and were then fixed and stained with Calcofluor / Hoechst (a and b) or Calcofluor (c). Septa are indicated by arrowheads for wild type and by arrows for *hypA1* strains. Germlings were grown for 24 hr at 42° and stained with FITC-WGA, and were then fixed (d) or rinsed to remove the unbound FITC-WGA and allowed to grow for another 3 hr at 42° (e). The arrows in d and e indicate the base of hemispherical hyphal tips, which in wild-type and *hyp* strains grown at 28° marks the limit of most hyphal expansion. In *hypA1* germlings grown at 42°, however, the hypha continues to expand in subapical regions (d and e). The hyphal tips in e are stained less brightly than the lateral wall, likely because of the incorporation of new wall material. n, nuclei. Bars, 10 μm.

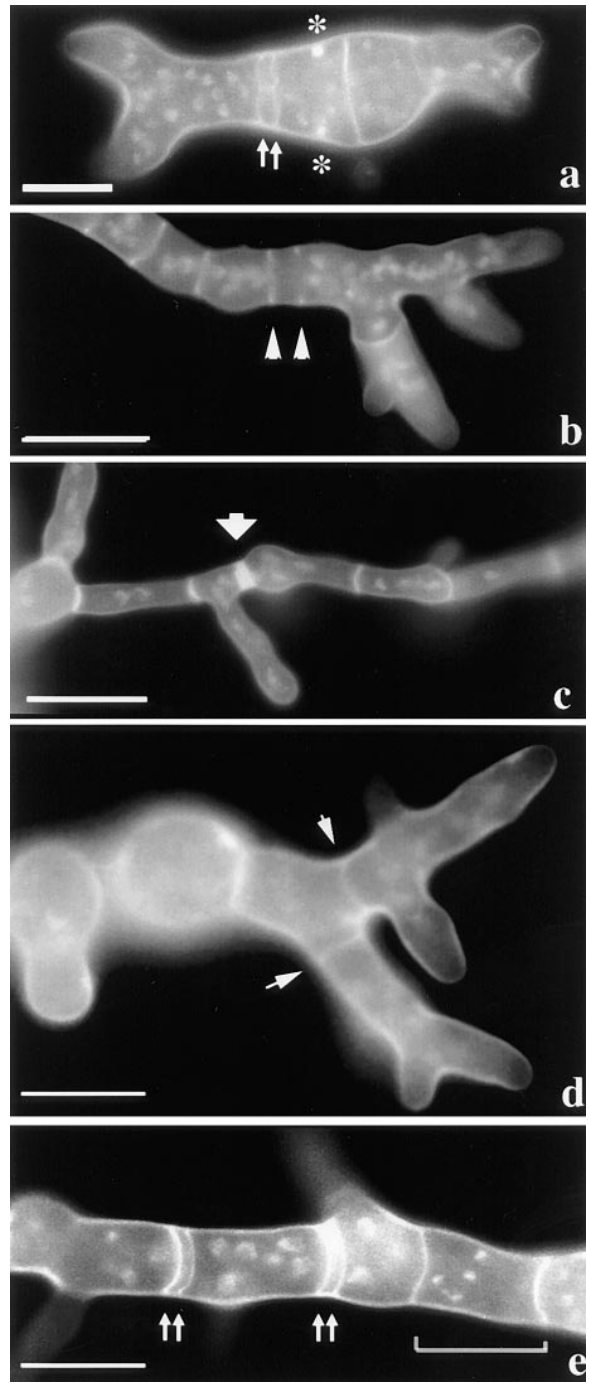

Figure 3.—*hypA1* (a), *hypB5* (b), *hypC4* (c), *hypD3* (d), and *hypE2* (e) germlings grown at 42°. Double septa are indicated for *hypA1* and *hypE2* germlings by paired arrows (a and e). The double septa in panel a enclose a cell with a single nucleus. An incomplete septum is indicated by asterisks in panel a. Septa enclosing an anucleate compartment are indicated by arrowheads in a *hypB5* germling (b). A broad septum typical of a *hypC4* germling is indicated by a broad arrow (c). Septa in a *hypD3* germling are indicated by single arrows (d). The walls of *hypD3* germlings stain brightly with Calcofluor, obscuring nuclear staining. An unbranched subapical cell that appears to have mitotic nuclei is identified by a bracket (e). The nuclei in the bracketed cell are distinctly more condensed than those in the compartment to the left that is bordered by pairs of double septa. Bars, 10 μm.

pared to wild-type (Figure 5b) or to their growth at 28° (Figure 5a). The *hypD3* defect was osmotically remedial at 42°, shown by increased colony growth on medium containing 1 m sucrose (Figure 5c). *hypD3* germlings were grown for 18 hr at 42° in CM or CM plus sucrose and were prepared for microscopy. Figure 5e shows that the restrictive phenotype of *hypD3* germlings grown in CM at 42° was fully remediated by growth on CM plus sucrose (Figure 5f). Under these conditions,

TABLE 3

Characteristics of subapical cells of *A. nidulans* strains

	A28	*hypA1*	*hypB5*	*hypC4*	*hypD3*	*hypE2*
A. Subapical cell length (μm) ± SE[a]						
28°	39.1 ± 2.2	25.3 ± 1.2	27.8 ± 1.3	30.6 ± 1.8	24.8 ± 1.2	22.9 ± 1.0
42°	27.4 ± 1.2	4.6 ± 0.3	6.3 ± 0.4	12.4 ± 0.6	8.1 ± 0.2	8.4 ± 0.6
B. Number of nuclei per subapical cell ± SE[b]						
28°	3.6 ± 0.2	3.6 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	3.0 ± 0.2	3.5 ± 0.2
42°	3.6 ± 0.2	9.1 ± 0.5	5.2 ± 0.5	3.0 ± 0.2	6.2 ± 0.2	4.6 ± 0.4
C. Hyphal width (μm)± SE[c]						
28°	3.0 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	3.5 ± 0.1
42°	3.6 ± 0.1	10.6 ± 0.2	5.4 ± 0.1	4.0 ± 0.1	6.4 ± 0.2	7.1 ± 0.2
D. Average subapical cell volume (μm^3)[d]						
28°	276	179	267	202	175	220
42°	279	406	144	155	260	332

[a] Subapical cell lengths were measured in unbranched cells having complete septa that were perpendicular to the long axis of the hypha. Fifty cells were measured for each strain and growth temperature. Analysis was by ANOVA ($P \leq 0.05$). For each strain, subapical cell lengths were significantly greater at 28° than at 42°.

[b] Number of nuclei in subapical cells (nuclei per cell) was counted in the same cells as used to measure subapical cell lengths. Analysis was by ANOVA ($P \leq 0.05$). For all but A28 and *hypC4*, nuclei per cell was significantly greater at 42° than at 28°. At 28°, there were no significant differences in nuclei per cell between any strains.

[c] Hyphal widths were measured at complete septa that were perpendicular to the long axis of the hypha. Thirty measurements were made for each strain and growth temperature. Analysis was by ANOVA ($P \leq 0.05$). For each strain, hyphal widths were significantly greater at 42° than at 28°.

[d] Calculated using average hyphal width and length values using the following formula: *Average cell volume* = π (*average hyphal width*/2)^2 × (*average cell length*).

the *hypD3* hyphae resembled wild-type strains in width, branching pattern, and nuclei per cell.

hyp strains might be hypersensitive to growing in the presence of Calcofluor, which binds to cell walls and interferes with crosslinking between chitin and nonfibrillar components. Growth of the *hyp* strains was reduced in the presence of Calcofluor, but none of the strains were notably sensitive compared to wild type (Figure 5, a *vs.* d).

Germling development and morphogenesis: The *hyp* mutant defects were examined in detail by growing all strains on coverslips at 28° and 42° for microscopy and following the kinetics of germination, nuclear division, growth, and cell division (septation). At hourly intervals, coverslips were fixed and stained for walls and nuclei (Figure 6).

A. nidulans conidia are apolar (Bergen and Morris 1983) and must polarize to form a germ tube. The *hyp* phenotypes could result from defects in polarity establishment. Wild-type strains germinate synchronously after a short lag (Bergen and Morris 1983), and germination was faster at 42° than at 28°. In contrast, all *hyp* strains had delayed germination compared to wild type, even at 28° (Figure 6a). Despite delays, spore germination in *hyp* strains eventually exceeded 95% at both temperatures. The delay in germination was exacerbated at 42° for all but *hypC4* strains. We conclude that *hypA1*, *hypB5*, *hypD3*, and *hypE2* have delayed germ tube formation, perhaps because of defects in cell polarization.

hyp morphologies could result from abnormally rapid mitotic cycles, because nuclei were abundant in *hypA1*, *hypB5*, *hypD3*, and *hypE2* germlings grown at 42° (Figure 3). Accumulation of nuclei in the wild-type strain was more rapid at 42° than at 28°, as shown in Figure 6b. The data for wild-type growth (Figure 6, open symbols) are plotted with the data for each of the *hyp* strains to facilitate comparisons. The nuclear cycle of *hyp* germlings was delayed compared to wild type when grown at 28° and 42° (Figure 6b). The length of the nuclear cycle was unaffected by growth at 42° for *hypA1*, *hypB5*, and *hypE2* germlings, and like the parental strain, it was shorter at higher temperature for *hypC4* and *hypD3* strains. Most notably, the nuclear cycle of the *hyp* strains did not accelerate more than the wild type at 42°.

In the wild-type parental strain, the first nuclear division occurred before germination, and most germinating conidia had two nuclei, as did *hyp* conidia germinating at 28°. At 42°, *hypC4* strains typically had two nuclei

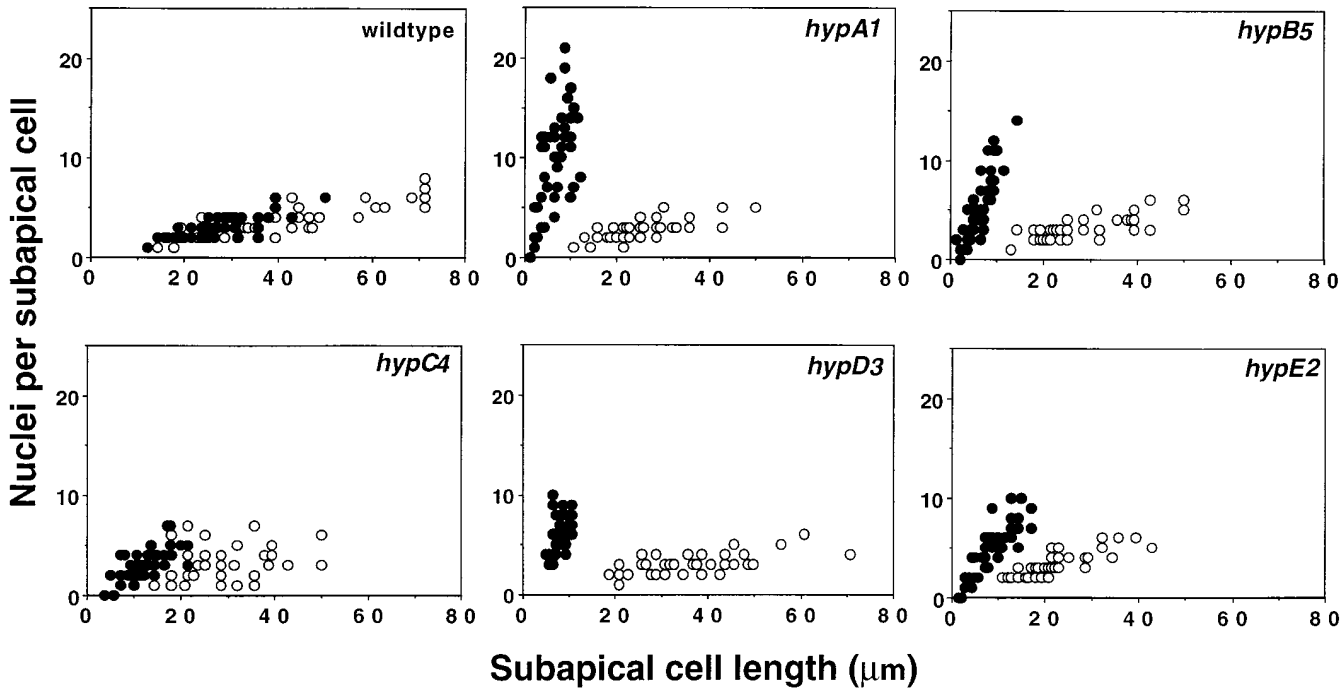

Figure 4.—Distributions of subapical cell lengths and corresponding numbers of nuclei in subapical cells of the wild-type parental and hypA1-hypE2 mutant strains. Strains were grown on coverslips of 18–24 hr at 28° (○) or 42° (●) and prepared for microscopy. Data are from unbranched subapical cells defined by complete septa that were perpendicular to the long axis of the hypha.

per spore when they germinated, but the *hypA1*, *hypB5*, *hypD3*, and *hypE2* strains had four to eight nuclei. Therefore, germination was delayed with respect to accumulation of nuclei in *hypA1*, *hypB5*, *hypD3*, and *hypE2* strains growing at 42°, consistent with a temperature-sensitive delay in germ tube emergence. These findings further demonstrate that *hypA1*, *hypB5*, *hypD3*, and *hypE2* mutations affect cell polarization.

The *hyp* morphologies may also be caused by defects in the maintenance of cell polarity, that is, by reduced hyphal extension rates. Maximum hyphal length determines colony diameter (Figure 5, a–d), but this does not account for additional hyphal growth through branching. Hyphal growth was measured for individual germlings as the total hyphal length, including all branches longer than half the hyphal diameter. Figure 6c shows the average germling lengths for wild-type and *hyp* germlings growing at 28° and 42°. For wild-type strains, the total hyphal length per germling increased rapidly after germination, with the rate being faster at the higher temperature. Growth of all *hyp* strains was delayed because of slow germination. Growth of *hypA1* and *hypB2* strains was inhibited at 42° compared to 28° (Figure 6c). The colony growth of *hypC4* and *hypD3* strains was reduced at 42° (Figure 5, a and b). These strains, however, branched extensively at 42°, so the cumulative length of all branches increased more rapidly than at 28° (Figure 6c). The growth rate of *hypE2* germlings was very slow (Figure 5a) and was relatively unaffected by growth temperature (Figure 6c), although *hypE2* germlings were more branched at 42°. Therefore, *hyp* strains have a variety of defects in maintaining tip growth, all of which produce compact colonies at 42°.

Septation in *hyp* strains growing at 28° was qualitatively similar to wild type. All *hyp* germlings produced septa at 42° (Figures 2 and 3). For *hypA1*, *hypB5*, *hypC4*, and *hypD3* strains, the onset of septation was delayed at 42° and occurred in short cells that contained ≥16 nuclei. Like wild type, septation in *hyp* strains appeared to correlate with mitosis (data not shown). Since tip growth was reduced in *hyp* strains at 42°, however, additional rounds of septation produced significantly shorter subapical cells (Figures 2–4; Table 3).

Temperature shift analysis: The *hyp* mutations appear to perturb aspects of hyphal morphology without blocking completion of the life cycle. To gain further insight into how the loss of *hyp* gene function affects cell morphogenesis, we grew *hyp* mutants on coverslips or agar at permissive temperature for 12–15 hr and then shifted the cells to restrictive temperature for varying lengths of time. Little or no change was observed for *hypC* mutants. Figure 7 illustrates changes associated with loss of *hypA* function. Similar results were obtained with *hypB5* (data not shown). Within 3 hr of shifting to the restrictive temperature, apical cell growth ceased and only subapical cells began to enlarge isotropically (Figure 7, a–c). Subapical cells stained brightly with Calcofluor, indicative of cell wall synthesis

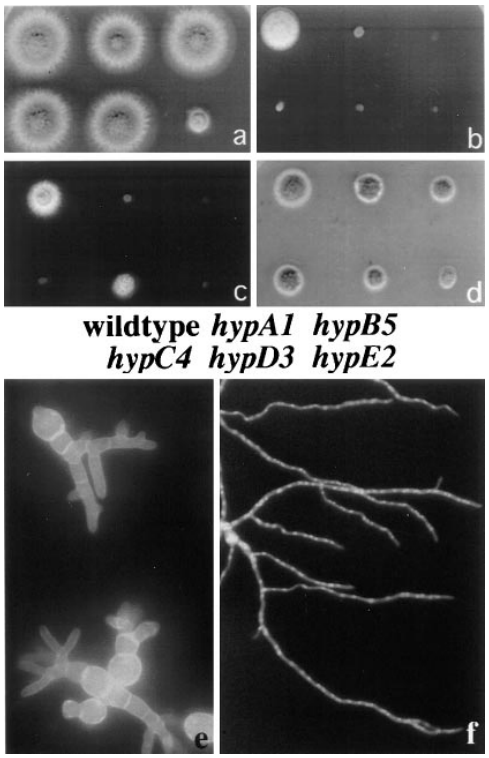

Figure 5.—Colony phenotypes of *hyp* mutations. [Upper panel (a–d)] Plate phenotypes of wild-type and *hyp* strains. Relevant genotypes are listed below the panel. [Top row (right to left)] Wild-type, *hypA1*, and *hypB5*; (bottom row) *hypC4*, *hypD3*, and *hypE2*. [Lower panel (e and f)] *hypD3* strain grown for 18 hr at 42° on CM or on CM containing 1 m sucrose, then fixed and stained for microscopy. Plate phenotypes of colonies that were grown for ~2 d on CM at 28° (a) and 42° (b), on CM amended with 1 m sucrose at 42° (c), and on CM containing 0.1% Calcofluor at 28° (d). The *hypD3* strain is osmotically remedial at 42° (c). Wild-type and *hyp* colonies have slightly reduced growth on media containing 0.1% Calcofluor (d), but none of the *hyp* strains were notably hypersensitive compared to wild type. (e) Microscopic examination of the restrictive phenotype of *hypD3* germlings grown at 42°; (f) demonstration that this phenotype was fully remediated at 42° by growth on 1 m sucrose. Bars, 10 μm.

(Figure 7b). Concomitant with their increased cell size, subapical cells accumulated abundant nuclei (Figure 7c). During this time, apical cells (arrows) underwent a progressive loss of nuclear staining and never resumed growth. This *hypA1* defect was readily reversible (Figure 7d). If germlings were grown at restrictive temperature and then returned to permissive temperature, they quickly reestablished multiple new apical tip cells.

Loss of *hypA1* overcomes the growth and nuclear cycle arrest of subapical cells. To test if the loss of *hypA* was capable of overriding nuclear cycle blocks, we constructed double mutants containing *hypA1* and temperature-sensitive mutations in a variety of cell cycle genes (*nimA5*, *nimE6*, *nimT23*, and *nimX1*) that arrest cells at the G2-M boundary (Doonan 1992; Osmani *et al.* 1994). In all cases, the double-mutant phenotypes were additive. Subapical cell compartments swelled, but nuclear division remained blocked (data not shown). Similarly, when germinated at restrictive temperature, double mutants produced pear-shaped germlings that contained single nuclei (data not shown). We conclude that *hypA* (and *hypB*) are required for apical cell growth but are also necessary to suppress growth in subapical cells until the onset of branch formation.

Temperature shift analysis with *hypE2* is shown in Figure 8. Similar results were obtained with *hypD3* (data not shown). When shifted to restrictive temperature, the apical and subapical cells widened and accumulated nuclei, suggesting that loss of *hypD* and *hypE* function causes a generalized perturbation in growth polarity.

DISCUSSION

Wild-type *A. nidulans* hyphae grow indefinitely by the extension of mitotically active apical cells. Septation results in the formation of subapical cells that are quiescent for growth and mitosis unless they branch (Figure 1). We have identified five genes, mutations in which cause markedly abnormal cell growth patterns. The *hyp* mutations define a class of temperature-sensitive mutations distinct from cell cycle mutants (Morris 1976), septation mutants (Harris *et al.* 1994), or conidiation mutants (Clutterbuck 1969; Champe and Simon 1992; Wieser *et al.* 1994). At restrictive temperature, all *hyp* mutants are able to complete the asexual life cycle. Cumulative data from phenotypic analyses suggest that at least some of the *hyp* genes play novel roles in controlling the pattern of cell growth in fungal mycelia.

The *hypA1*, *hypB5*, and *hypE2* phenotypes are unlikely to be caused by defects in cell wall synthesis. Five chitin synthase genes have been cloned in *A. nidulans*, and none of the strains disrupted for these genes phenocopy the *hyp* mutants (Motoyama *et al.* 1994; Borgia *et al.* 1996a; Specht *et al.* 1996). The genes *gcnA*, *manA*, *orlA-D*, and *tsE* encode additional components of the cell wall biosynthetic pathway (Borgia and Dodge 1992; Smith and Payton 1994; Borgia *et al.* 1996b). Unlike the *hyp*s, these mutants are chitin deficient and/or unable to grow without osmotic stabilization, which suggests that their walls do not mature properly. Although *hyp* mutants, particularly *hypD3*, have phenotypes suggestive of cell wall structural defects, all *hyp* strains were able to grow and complete their asexual life cycles at 42° without osmotic stabilization. Therefore, *hyp* mutants appear to define a class of genes that is phenotypically distinct from those directly involved in cell wall biosynthesis.

hypC4 is distinct from the other *hyp* mutations because its effect is largely restricted to septation. Like wild type, but unlike the other mutants, *hypC4* germinates faster at 42° than at 28°, and it has normal hyphal

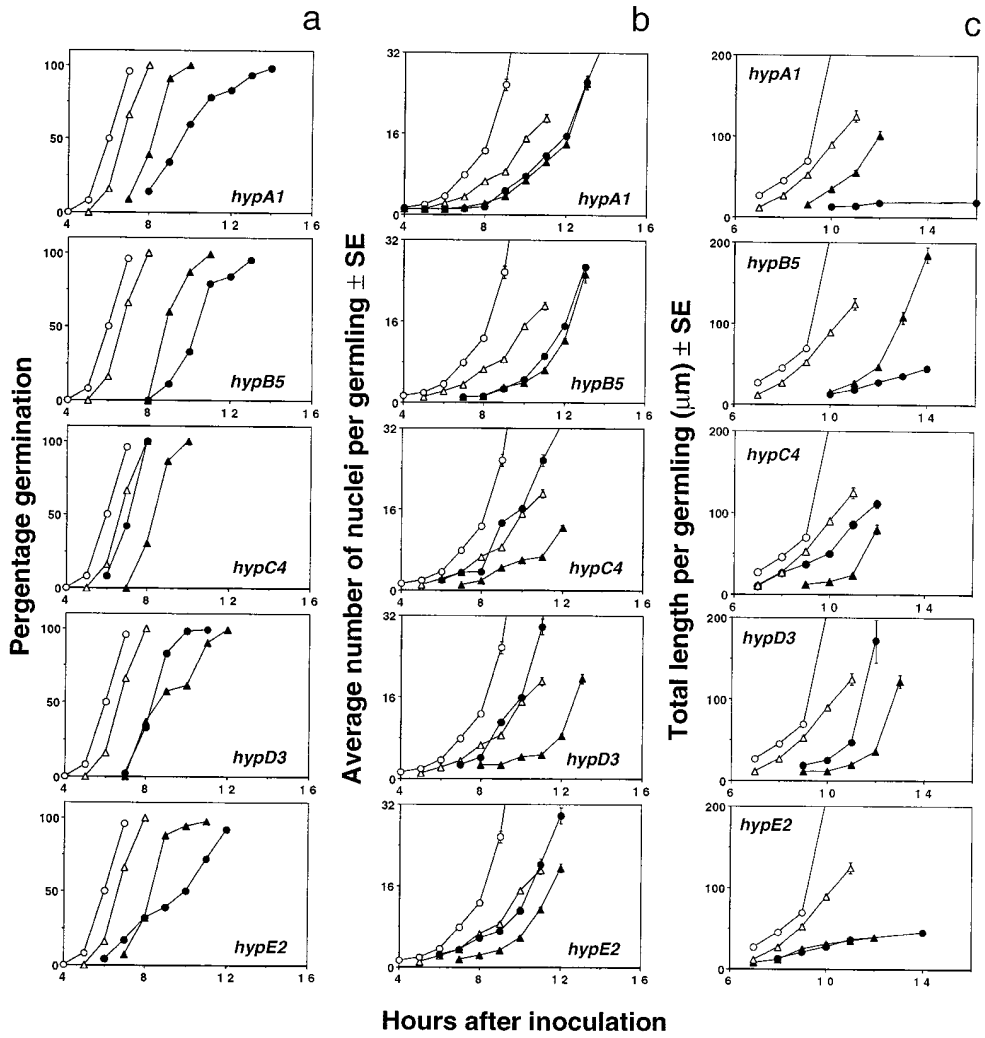

Figure 6.—Spore germination (a), nucleus accumulation (b), and hyphal growth (c) in wild-type (○, △) and *hyp* (●, ▲) germlings grown at 28° (△, ▲) and 42° (○, ●). Data from wild-type germlings are shown on each graph to facilitate comparison with individual *hyp* strains. Data points obscure small error bars. Germination (a) was defined as production of a germ tube longer than half the spore width. Nuclei per germling (b) data are expressed as population averages (see materials and methods). Hyphal length (c) was defined as the total length including all branches that were longer than half the hyphal width. *hypC4* and *hypD3* strains branched extensively at 42°, and so their total hyphal length increased compared to growth at 28°, but the colonies remained compact (compare with Figure 5b).

width and nuclei per cell when grown at restrictive temperature. *hypC4* strains branch profusely at 42°, so that total germling length increases even though colony diameter is restricted compared to growth at 28°. *hypC4* has more septa per hyphal length and thus shorter subapical cells than wild-type strains. *hypC4* hyphal walls are not stained abnormally with Calcofluor, and the defect is not osmotically remedial; however, about one-third of the septa in *hypC4* strains grown at 42° are unusually thick. In *A. nidulans*, a threshold cell size controls the ability of nuclear divisions to activate septation (Wolkow *et al.* 1996). Thus, *hypC4* may lower the threshold cell size required to activate septation. Alternatively, loss of *hypC* may alter nuclear distribution in a way that septa are formed in closer proximity. Experiments to distinguish between these possibilities are underway.

In contrast to the *hypC4* mutation, *hypA1*, *hypB5*, *hypD3*, and *hypE2* mutations seem to primarily affect hyphal morphogenesis and, consequently, affect septation. Kinetic studies of germ tube polarization and nuclear division suggest that these *hyp* mutations delay cell polarization. Even more unusual, *hyp* germlings continue to get wider behind the growing tip and in subapical cells. The lateral walls of *hypA1*, *hypB5*, *hypD3*, and *hypE2* germlings stain more brightly with Calcofluor than do those of wild type, suggesting that *hyp* wall thickness may be excessive. The tips of *hyp* germlings stained less brightly with Calcofluor than their lateral walls, suggesting that *hyp* cell walls may not be evenly thick (Figure 3, a, b, and d). This growth pattern implies that wall deposition in *hyp* mutants may not be restricted to hyphal tips. This is consistent with colony growth (hyphal extension) rates being reduced compared to wild type at 42°. Thus, at restrictive temperature, *hypA1*, *hypB5*, *hypD3*, and *hypE2* germlings may grow by both expansion and extension.

A variety of genes affect growth polarity in filamentous fungi. These include components of the actin cytoskeleton (McGoldrick *et al.* 1995), components of the cAMP signaling pathway (Kore-eda *et al.* 1991), and small GTPases of the *ras* family (Som and Kolaparthi 1994). In addition, some of these genes also affect patterns of septation. Mutations in the *Neurospora*

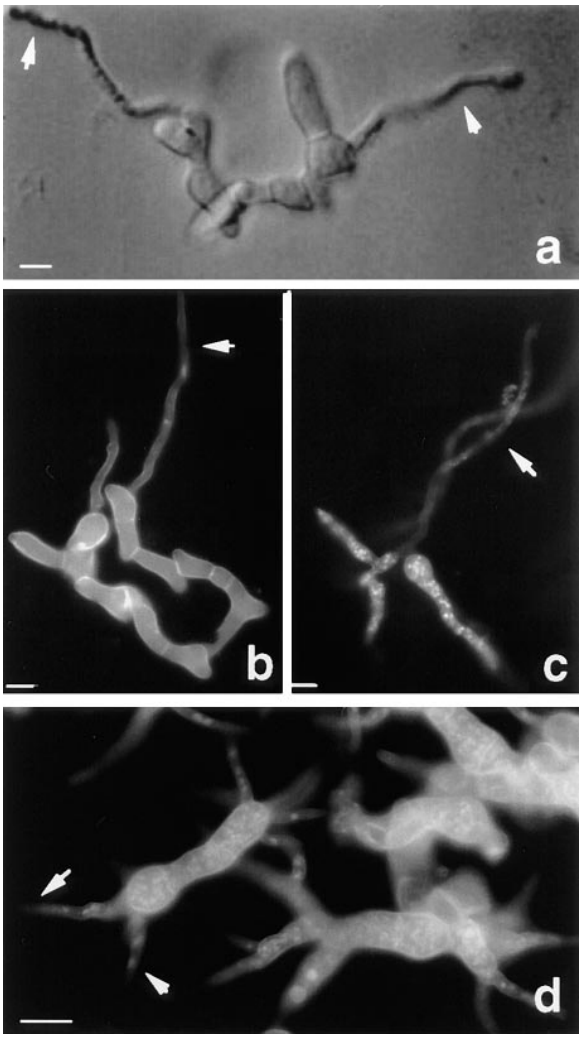

Figure 7.—Temperature shift analysis of *hypA1*. *hypA1* spores were germinated on agar media (a) or on coverslips (b and c) for 12–15 hr at 28° and then shifted to 42° for 3 hr. Cells were either visualized directly (a) or fixed and stained for cell walls (b) or nuclei (c and d). After a shift to the restrictive temperature, the apical cells in *hypA1* germlings (arrows) cease growing, while subapical cells swell (a). Subapical cells show intense Calcofluor staining (b), indicative for new cell wall synthesis, and they accumulate numerous nuclei (c). During reciprocal shifts (42° to 28°; d), *hypA1* germlings reestablish numerous apical tips. Similar results were obtained with *hypB5* mutants (data not shown). Bars, 10 μm.

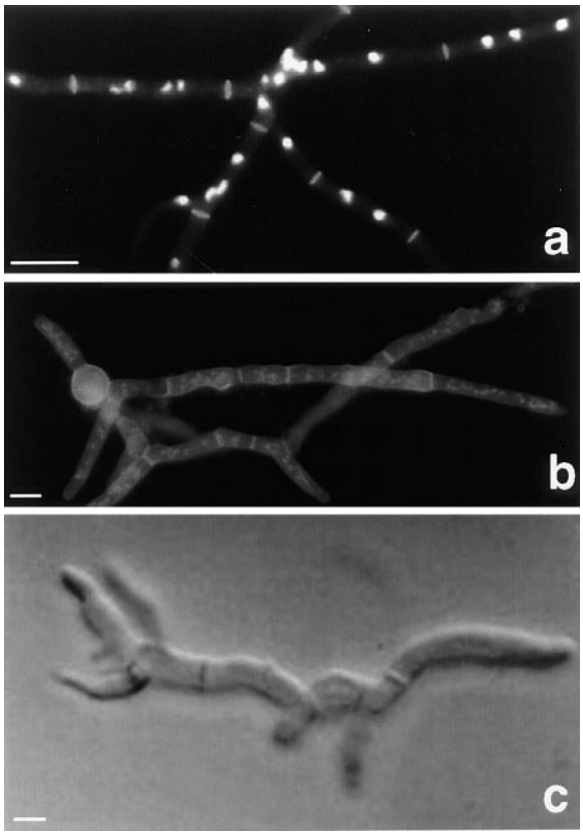

Figure 8.—Temperature shift analysis of *hypE2*. In contrast to *hypA1* (see Figure 7), *hypE1* germlings undergo expansion and accumulate nuclei within both apical and subapical cells. Temperature shift analysis was performed as described in Figure 7. (a) Wild-type germling stained for nuclei and cell walls. (b) hypE2 mutant stained for nuclei and cell walls or (c) visualized directly on plates after a shift from 28° to 42°. Similar results were obtained for *hypD3* mutants (data not shown). Bars, 10 μm.

crassa regulatory subunit of protein kinase A (*mcb-1*) cause major defects in growth polarity, actin localization, and septation (Bruno *et al.* 1997). In particular, temperature shift experiments with the *mcb-1* mutants resulted in phenotypes similar to *hypD3* and *hypE2* (Figure 8). *hyp* phenotypes also resemble the effects of moderate overproduction of the constitutively active Aspergillus ras homolog *A-ras*. In this case, extension but not growth was limited, so the germlings were wide and had thickened walls and septa and abundant nuclei compared to wild type. Germination was not affected by moderate *A-ras* overproduction, but it was inhibited by high *A-ras*. Nuclear division and wall deposition were largely unaffected by *A-ras* activity (Som and Kolaparthi 1994). Molecular cloning of *hyp* genes may provide clues to their function in growth polarity.

Temperature shift analysis revealed that *hypA* and *hypB* have cell-specific functions in hyphal morphogenesis. Unlike other mutations affecting growth polarity in fungi, loss of *hypA* function resulted in the rapid onset of subapical cell growth and nuclear division and a complete cessation of apical growth. Subapical cell growth occurred isotropically, with cells becoming swollen and rounded. In contrast, the apical cell underwent vacuolization, and cytoplasmic and nuclear staining were rapidly lost (data not shown). Thus, the *hypA* gene product must play two roles in distinct cell types. In apical cells, *hypA* is required for continued growth polarity. After septation, *hypA* must activate a growth arrest in subapical cells. It is not currently known how a single gene product could play contrasting roles in different cell types; however, the apparent dual function of *hypA* (and *hypB*) provides the mycelium of *A. nidulans* with a

simple regulatory mechanism for patterning growth. We have recently cloned *hypA* by virtue of its close linkage to *sepA* (Harris *et al.* 1997). *hypA* encodes a 5.3-kb gene that has no significant similarity to any gene or expressed sequence tag in current databases (S. Kaminskyj and J. E. Hamer, unpublished data). Thus, *hypA1* and perhaps other *hyp* mutations will define novel genes involved in cell polarity and cell pattern formation.

We thank Drs. Lis Hamer, Kelly Kinch, Michelle Momany, John Marhoul, and Tom Wolkow for critical reading of the manuscript. This research was funded by the National Institutes of Health (GM-49867; J.E.H.), the Indiana Elks Cancer Research Fund (J.E.H. and S.G.W.K.). S.G.W.K. is a recipient of a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

Ayscough, K. R., and D. G. Drubin, 1996 Actin: general principles from studies in yeast. Annual Review in Cell Biology **12:** 129–160.

Bergen, L. G., and N. R. Morris, 1983 Kinetics of the nuclear division cycle of *Aspergillus nidulans.* J. Bacteriol. **156:** 155–160.

Borgia, P. T., and C. L. Dodge, 1992 Characterization of *Aspergillus nidulans* mutants deficient in cell wall chitin or glucan. J. Bacteriol. **174:** 377–383.

Borgia, P. T., N. Iartchouk, P. J. Riggle, K. R. Winter, Y. Koltin *et al.*, 1996a The *chsB* gene of *Aspergillus nidulans* is necessary for normal hyphal growth and development. Fung. Genet. Newsl. **20:** 193–203.

Borgia, P. T., Y. Miao and C. L. Dodge, 1996b The *orlA* gene from *Aspergillus nidulans* encodes a trehalose-6-phosphate phosphatase necessary for normal growth and chitin synthesis at elevated temperatures. Mol. Microbiol. **20:** 1287–1296.

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.

Champe, S. W., and L. D. Simon, 1992 Cellular differentiation and tissue formation in the fungus *Aspergillus nidulans*, pp. 63–91 in *Morphogenesis: An Analysis of the Development of Biological Form*, edited by E. F. Rossomando and S. Alexander. Dekker, New York.

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

Clutterbuck, A. J., and H. Arst, 1995 Genetic nomenclature guide. *Aspergillus nidulans.* Trends Genet. **11:** 13–24.

Cunningham, K. W., and G. R. Fink, 1994 Ca^{2+} transport in *Saccharomyces cerevisiae.* J. Exp. Biol. **196:** 157–166.

Cunningham, K. W., and G. R. Fink, 1996 Calcineurin inhibits VCX1-dependent H^+/Ca^{2+} exchange and induces Ca^{2+} ATPases in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **16:** 2226–2237.

Dayton, J. S., and A. R. Means, 1996 $Ca^{(2+)}$/calmodulin-dependent kinase is essential for both growth and nuclear division in *Aspergillus nidulans.* Mol. Biol. Cell **7:** 1511–1519.

Doonan, J. R., 1992 Cell division in *Aspergillus nidulans.* J. Cell Sci. **103:** 599–611.

Drubin, D. G., and W. J. Nelson, 1996 Origins of cell polarity. Cell **84:** 335–344.

Duffy, J. B., and N. Perrimon, 1996 Recent advances in understanding signal transduction pathways in worms and flies. Curr. Opin. Cell Biol. **8:** 231–238.

Fiddy, C., and A. P. J. Trinci, 1976 Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans.* J. Gen. Microbiol. **97:** 169–194.

Gooday, G. W., 1994 Cell walls, pp. 43–62 in *The Growing Fungus*, edited by N. A. R. Gow and G. M. Gadd. Chapman & Hall, London.

Gull, K., 1978 Form and function of septa in filamentous fungi, pp. 78–93 in *The Filamentous Fungi, Developmental Mycology* edited by J. E. Smith and D. R. Berry. John Wiley & Sons, New York.

Hammerschmidt, M., A. Brook and A. P. McMahon, 1997 The world according to hedgehog. Trends Genet. **13:** 14–21.

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. Sharpless and J. E. Hamer, 1997 *sepA* is a member of a conserved family of cytokinesis genes with formin homology. EMBO J. (in press).

Hastie, A. C., 1970 Benlate induced instability of *Aspergillus* diploids. Nature **226:** 771.

Heath, I. B., 1994 The cytoskeleton, pp. 99–134 in *The Growing Fungus*, edited by N. A. R. Gow and G. M. Gadd, Chapman & Hall, London.

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

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

Kaminskyj, S., and J. Hamer, 1996 A medium for rapid cleaning of *Aspergillus* cleistothecia. Fung. Genet. Newsl. **43:** 71.

Kore-eda, S., T. Murayama and I. Uno, 1991 Isolation and characterization of the adenylate cyclase structural gene of *Neurospora crassa.* Jpn. J. Genet. **66:** 317–334.

Kron, S. J., and N. A. R. Gow, 1995 Budding yeast morphogenesis: signalling, cytoskeleton and cell cycle. Curr. Opin. Cell Biol. **7:** 845–855.

Kropf, D. L., C. A. Henry and B. C. Gibbon, 1995 Measurement and manipulation of cytosolic pH in polarizing zygotes. Eur. J. Cell Biol. **68:** 297–305.

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

Meyerowitz, E. M., 1997 Genetic control of cell division patterns in developing plants. Cell **88:** 299–308.

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

Mosch, H. U., R. L. Roberts and G. R. Fink, 1996 *Ras2* signals via the *Cdc42/Ste20*/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **93:** 5352–5356.

Motoyama, T., N. Kojima, H. Horiuchi, A. Ohta and M. Takagi, 1994 Isolation of a chitin synthase gene (*chsC*) of *Aspergillus nidulans.* Biosci. Biotechnol. Biochem. **58:** 2254–2257.

Motoyama, T., M. Fujiwara, N. Kojima, H. Horiuchi, A. Ohta *et al.*, 1996 The *Aspergillus nidulans* genes *chsA* and *chsD* encode chitin synthases which have redundant functions in conidia formation. Mol. Gen. Genet. **253:** 520–528.

Osmani, A. H., N. V. Peij, M. Mischke, M. J. O'Connell and S. A. Osmani, 1994 A single p34cdc2 protein kinase (encoded by nimXcdc2) is required at G1 and G2 in *Aspergillus nidulans.* J. Cell Sci. **107:** 1519–1528.

Pierson, E. S., D. D. Miller, D. A. Callaham, A. M. Shipley, B. A. Rivers *et al.*, 1994 Pollen tube growth is coupled to the extracellular calcium ion flux and the intracellular calcium gradient: effect of BAPTA-type buffers and hypertonic media. Plant Cell **6:** 1815–1828.

Pringle, J. R., E. Bi, H. A. Harkins, J. E. Zahner, C. De Virgilio *et al.*, 1995 Establishment of cell polarity in yeast. Cold Spring Harbor Symposium Quantitative Biology **60:** 729–744.

Rasmussen, C., C. Garen, S. Brining, R. L. Kincaid, R. L. Means *et al.*, 1994 The calmodulin-dependent protein phosphatase catalytic subunit (calcineurin A) is an essential gene in *Aspergillus nidulans.* EMBO J. **13:** 3917–3924.

Richle, R. E., and J. V. Alexander, 1965 Multiperforated septations, Woronin bodies and septal plugs in *Fusarium.* J. Cell Sci. **24:** 489–496.

Robinow, C. F., and C. E. Caten, 1969 Mitosis in *Aspergillus nidulans.* J. Cell Sci. **5:** 403–413.

Rosenberger, R. F., and M. Kessel, 1967 Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans.* J. Bacteriol. **94:** 1464–1469.

Smith, D. J., and M. A. Payton, 1994 Hyphal tip extension in *Aspergillus nidulans* requies the manA gene which encodes phosphomannose isomerase. Mol. Cell. Biol. **14:** 6030–6038.

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

Specht, C. A., Y. Liu, P. W. Robbins, C. E. Bulawa, N. Iartchouk *et*

al., 1996 The *chsD* and *chsE* genes of *Aspergillus nidulans* and their roles in chitin synthesis. Fung. Genet. and Biol. Newsl. **20:** 153–167.

Ward, M. P., C. J. Gimeno, G. R. Fink and S. Garrett, 1995 *SOK2* may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. Mol. Cell. Biol. **15:** 6854–6863.

Wieser, J., B. N. Lee, J. W. Fondon III and T. H. Adams, 1994 Genetic requirements for initiating asexual development in *Aspergillus nidulans*. Curr. Genet. **27:** 62–69.

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.

Communicating editor: R. H. Davis