Septins AspA and AspC Are Important for Normal Development and Limit the Emergence of New Growth Foci in the Multicellular Fungus \textit{Aspergillus nidulans}

Rebecca Lindsey,† Susan Cowden, Yainitza Hernández-Rodríguez, and Michelle Momany*

Department of Plant Biology, University of Georgia, Athens, Georgia 30602

Received 14 September 2009/Accepted 23 November 2009

Septins are cytoskeletal proteins found in fungi, animals, and microsporidia, where they form multiseptin complexes that act as scaffolds recruiting and organizing other proteins to ensure normal cell division and development. Here we characterize the septins AspA and AspC in the multicellular, filamentous fungus \textit{Aspergillus nidulans}. Mutants with deletions of \textit{aspA}, \textit{aspC}, or both \textit{aspA} and \textit{aspC} show early and increased germ tube and branch emergence, abnormal septation, and disorganized conidiophores. Strains in which the native \textit{aspA} has been replaced with a single copy of \textit{aspA-GFP} driven by the native septin promoter or in which \textit{aspC} has been replaced with a single copy of \textit{aspC-GFP} driven by the native promoter show wild-type phenotypes. \textit{AspA-GFP} and \textit{AspC-GFP} show identical localization patterns as discrete spots or bars in dormant and expanding conidia, as rings at forming septa and at the bases of emerging germ tubes and branches, and as punctate spots and filaments in the cytoplasm and at the cell cortex. In conidiophores, \textit{AspA-GFP} and \textit{AspC-GFP} localize as diffuse bands or rings at the bases of emerging layers and conidial chains and as discrete spots or bars in newly formed conidia. \textit{AspA-GFP} forms abnormal structures in \textit{ΔaspC} strains while \textit{AspC-GFP} does not localize in \textit{ΔaspA} strains. Our results suggest that \textit{AspA} and \textit{AspC} interact with each other and are important for normal development, especially for preventing the inappropriate emergence of germ tubes and branches. This is the first report of a septin limiting the emergence of new growth foci in any organism.

Septins are novel cytoskeletal proteins first discovered in a screen for \textit{Saccharomyces cerevisiae} cell cycle mutants (14). The core septin proteins, Cdc3, Cdc10, Cdc11, and Cdc12, localize to the mother/bud neck, where they assemble into heteropolymers that organize proteins necessary to complete cytokinesis and ensure proper coordination between bud formation and nuclear division (4, 16, 37, 38).

\textit{S. cerevisiae} septins first appear as a cortical patch at the future bud site. They later form a ring through which the bud emerges and then develop into an hourglass-shaped complex at the base of the bud that splits into two rings to complete cytokinesis (8, 10, 23). In the dimorphic fungus \textit{Candida albicans}, septins assemble during the formation of buds and pseudohyphae, localize to prebud sites, and form rings at the mother/bud neck. During hyphal growth septins localize to hyphal tips and transiently as a basal band within germ tubes (33, 39). The genome of the filamentous fungus \textit{Ashbya gossypii} is 90\% homologous and syntenic with the genome of \textit{S. cerevisiae}, though it grows in the filamentous morphology rather than the yeast morphology (6). In \textit{A. gossypii} septins localize as discrete filamentous bars at septation sites, at tips of hyphae, and at the bases of emerging branches. \textit{A. gossypii} septins are not essential, though they have been shown to be involved in mitosis, sporulation, hyphal morphogenesis, and septum formation (5, 9).

In mammals, septins ensure proper growth, cell migration, vesicle trafficking, and cell division (19, 32, 35, 42). Mammalian septins form distinct filaments that colocalize with and appear to organize the actin and microtubule cytoskeletons (19, 31, 35), punctate patterns at neuron terminals and vesicles, and rings in sperm cells (2, 15, 18, 43).

\textit{Aspergillus nidulans} is a multicellular filamentous fungus which has five septins, AspA, AspB, AspC, AspD, and AspE (29). All five septins are expressed during vegetative and asexual growth, with AspB having the highest expression levels (29). Immunofluorescence studies showed that AspB localizes to septa and conidiophore layers and anticipates the sites of branch emergence (40). To better understand the roles of septins in shaping the growth of multicellular organisms, we characterized \textit{A. nidulans} septins AspA and AspC, orthologs of \textit{S. cerevisiae} Cdc11 and Cdc12, respectively (30). Septin deletion mutants were characterized throughout vegetative and asexual development. AspA and AspC are necessary for normal development and morphogenesis as well as sporulation. AspA and AspC were found to localize as rings, caps, puncta, or filaments throughout development. Localization of AspA and that of AspC appear to be mutually dependent, as AspC was unable to localize in the \textit{ΔaspA} strain and AspA localization was abnormal in the \textit{ΔaspC} strain.

\section*{Materials and Methods}

\textbf{Strains and Media.} Strains used in this study are listed in Table 1. Media used were previously described (13). Strain construction and growth were done by standard \textit{A. nidulans} techniques (13, 17). All incubations were at 30°C and in
TABLE 1. *A. nidulans* strains used in this paper

| Strain          | Genotype                        | Source or reference |
|-----------------|---------------------------------|--------------------|
| A28             | pabaA6 bvA1                    | FGSC               |
| A773            | pyrG89 wA3 pyrG44              | FGSC               |
| A850            | bvA1 argB::trpC_B methG1 veA1 trpC801 | FGSC               |
| A1147           | pyrG89 argB2 pabaB22 nkaA::argB riboB2 | FGSC               |
| A1145           | pyrG98 pyroA4 nkaA::argB riboB2 | FGSC               |
| A505            | anp::argB2 bvA1 argB::trpC_B methG1 veA1 trpC801 | FGSC               |
| A526            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | FGSC               |
| A528            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | A28, A773          |
| A529            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | A505, A526         |
| A530            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | A526, A528         |
| A531            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | A529, A530         |
| A532            | anp::argB2 pyrG98 wA3 argB::trpC_B methG1 veA1 trpC801 | A531, A532         |

a The symbol \( \sim \) indicates haploids fused to make diploid strain.

b FGSC, Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center (Kansas City, KS).

table 2. Primers

| Construct and primer type or target | Sequence |
|------------------------------------|----------|
| Flank 1                            | Forward: GCCTCGAGACCCGACGCGACGAGGAAAG |
|                                    | Reverse: CCGGATCCGATGGAAGTACGGGAAAG |
| Flank 2                            | Forward: CCTAAGCTTCTTGTTCGAGGTGAAG |
|                                    | Reverse: CCGGATCCGAGGAAAGTACGGGAAAG |
| ΔaspA check                        | Forward: CCTCACTCCTTCTCTTCTCACCAC |
|                                    | Reverse: TCTTATCAGTCAAGGACGAG |
| aspA deletion                      | Forward: ATGTGCTCGCTCTAATAACGG |
| Plasmid pFNO3 (GA5-aspA amplification) | Reverse: GTTGAGCCGAGCGCAGCTTTCTT |
| Downstream native aspA             | Reverse: CTGTCGCGCTCTAATAACGG |
| aspA-gfp check                     | Reverse: CTGTCGCGCTCTAATAACGG |
| aspC deletion                      | Forward: ATGGCCCCGCTACAAGGCAAGG |
| Plasmid pFNO3 (AfpR amplification)  | Reverse: GGGGAGCCGAGGAAAGTACGGG |
| Downstream native aspC             | Reverse: GGGGAGCCGAGGAAAGTACGGG |
| ΔaspC check                        | Reverse: GGGGAGCCGAGGAAAGTACGGG |
| aspC-gfp                           | Forward: ATGGCCCCGCTACAAGGCAAGG |
| Plasmid pFNO3 (GA5-aspA amplification) | Reverse: GGGGAGCCGAGGAAAGTACGGG |
| Downstream native aspC             | Reverse: GGGGAGCCGAGGAAAGTACGGG |
| ΔaspC check                        | Reverse: GGGGAGCCGAGGAAAGTACGGG |

Preparation and growth of conidiophores was as previously reported (20). Briefly, conidia were inoculated on the edges of a square of complete agar medium which was sandwiched between two coverslips and placed on top of water agar to prevent the complete agar from drying out. Plates were incubated inverted at 30°C for 2 days. To observe conidiophore structures, coverslips with complete medium (1% glucose, 0.2% peptone, 0.1% Casamino Acids, 0.1% yeast extract, trace elements, nitrate salts, and 0.01% vitamins, pH 6.5) with amino acid supplements (17) except where noted. Green fluorescent protein (GFP) diploid strains were generated as previously reported (12) by mixing conidiospores from a wild-type strain (A773 or A850) with those from the AspA-GFP (ARL141) or AspC-GFP (ARL159) strain, respectively. Heterokaryotic germ-spores were plated into minimal agar from which only diploids could emerge. The resulting diploid conidia were streaked for isolation two times to yield strains ARL205 and ARL206, respectively. Localization was examined in four GFP bright strains for each diploid.

**Growth conditions and microscopic observations.** Preparation and growth of cultures were as previously reported (26). Briefly, conidia were inoculated on sterile coverslips in liquid complete or minimal medium and incubated at 30°C in a petri dish. Cells were fixed, septa were stained with calcifluor (American Cyanamid, Wayne, NJ), and nuclei were stained with Hoechst 33258 (Sigma, St. Louis, MO). Microscopic observations were made using a Zeiss (Thornwood, NY) Axiosplan microscope with appropriate filters, and digital images were acquired using an AxioCam (Axion Technologies) digital imaging system. For all GFP fusion observations, recipient strains that had not been transformed with GFP cassettes were viewed under identical settings to verify that there was no autofluorescence. Images were prepared using Photoshop cs version 8.0 (Adobe, Mountain View, CA). For quantitation of phenotypes, counts of 200 cells were done. All experiments were repeated at least three times with similar results. A representative data set is shown.

**Asexual structures.** Preparation of conidiophores was as previously reported (20). Briefly, conidia were inoculated on the edges of a square of complete agar medium which was sandwiched between two coverslips and placed on top of water agar to prevent the complete agar from drying out. Plates were incubated inverted at 30°C for 2 days. To observe conidiophore structures, coverslips with...
endogenous promoter was obtained. Southern blotting to ensure that a single homologous integration under the formants were checked by PCR (primers listed in Table 2) and confirmed by transform gene. After pOAS15 was confirmed by restriction digestion, it was used to by a complete replacement of the aspC gene (pFNO3 plasmid template), which included 30 bp before the aspC gene (flank 1) was located at the 3' end of the aspA gene, and flank 2 is located at the 5' end of the aspA gene (flank 2) were PCR amplified using the Molecular Bio Products EasyStart Micro 20 kit (San Diego, CA) with primers AspAko_HindIII_2 and AspAko_KpnI_2 (Table 2), which introduced HindIII and KpnI sites, respectively. The PCR products were cut with the appropriate restriction enzymes and then ligated on either side of the argB gene in the pArgB2 plasmid, resulting in plasmid pOAS15. Flank 1 is located at the 3' end of the argB gene, and flank 2 is located at the 5' end of the argB gene. After pOAS15 was confirmed by restriction digestion, it was used to transform A. nidulans A850 by the protoplasting method. The resulting transformants were checked by PCR (primers listed in Table 2) and confirmed by Southern blotting to ensure that a single homologous integration under the endogenous promotor was obtained.

All PCRs and fusion PCRs were conducted using Invitrogen AccuPrime Pfx DNA polymerase as previously described (44). The ΔaspC strain was generated by a complete replacement of the aspC gene with the AfpyrG gene. This was done by amplifying three separate fragments: the first fragment was 1 kb upstream of the aspC start codon (A850 template), the second fragment was 1 kb downstream of the aspC stop codon (A850 template), and the third fragment was the AfpyrG gene (pFNO3 plasmid template), which included 30 bp before the aspC start codon and 30 bp after the stop of the aspC stop codon added to facilitate fusion PCR (primers listed in Table 2). Each DNA fragment was run on an 0.8% agarose gel, and the bands were excised and cleaned with the Qiagen QIAquick gel extraction kit (Maryland). Equal amounts of each fragment were used for the fusion PCR. After fusion PCR the DNA was run on a gel, excised to ensure that a single band was obtained, and gel purified with the Qiagen QIAquick gel extraction kit (Maryland). The cleaned DNA was used to transform A. nidulans ΔnkuA strains by protoplasting. The resulting transformants were checked by PCR, which utilized primers that flanked the entire fusion PCR product (Table 2). Each PCR product was run on an 0.8% agarose gel, and the band was excised and cleaned with the Qiagen QIAquick gel extraction kit (Maryland). Conidia of wild-type and mutant strains were inoculated into liquid medium and incubated for 5, 8, or 14 h; fixed; and photographed with differential interference contrast optics. Panels show composites of multiple cells from each time point. Scale bar, 5 μm.

RESULTS

In ΔaspA, ΔaspC, and ΔaspA ΔaspC strains, germ tubes emerge early, germ tube and branch emergence increases, and septation and conidiation are reduced. In the filamentous fungus A. nidulans development begins when uninucleate asexual
conidiospores break dormancy in the presence of a carbon source. Soon after conidia break dormancy, nuclear division begins and a single primary germ tube emerges and grows by tip extension (11, 13, 27). After germlings pass a critical size threshold, nuclear division triggers septum formation (41). This normally occurs when germlings contain 8 to 16 nuclei. At about the same time a secondary germ tube emerges from the conidium in a bipolar pattern, opposite from the site where the first germ tube emerged. After subapical and apical compartments are formed through septation, generally only one branch emerges per active subapical compartment of the hypha (21). To determine if the septins AspA and AspC play roles in A. nidulans development, we compared wild-type strains to strains in which individual septin genes were deleted (ΔaspA, ASH5, and ΔaspC, ARL161) or in which both septins were deleted (ΔaspA ΔaspC, ARL162). After 5 h at 30°C, no germ tubes were yet visible in the wild type, while 52 to 58% of deletion strain cells had germ tubes (n = 200; Fig. 1 and 2). When incubated for 8 h, 92% of wild-type cells had one germ tube, while 75 to 82% of single deletion mutants and 95% of double deletion mutants had two or more germ tubes (Fig. 1 and 2). If two germ tubes formed in the wild-type strain, they were usually separated by 180°. In the septin deletion mutants multiple germ tubes were adjacent to each other or separated by 45°, 90°, or 180° (data not shown). Branch emergence was assayed by counting the number of hyphae that had formed at least one branch. When cells were incubated for 14 h, branches emerged in 7% of wild-type cells, 58% of ΔaspA cells, 56% of ΔaspC cells, and 82% of the double deletion mutants (n = 200; Fig. 1 and 2). In addition to branching early, the mutants also showed more branches or branch initials relative to wild type.

Because septin deletion mutants appeared to develop more rapidly than wild type and because nuclear division is known to trigger septation (41), we scored septation based on nuclear number rather than time of incubation. After 11 h of incubation, nuclei and septa were labeled and only hyphae with 16 nuclei were scored. All wild-type cells, 12% of ΔaspA cells, and 3% of ΔaspC cells with 16 nuclei had septa (n = 200). No ΔaspA ΔaspC cells with 16 nuclei contained septa (data not shown). To determine whether septation in the septin deletion mutants was reduced or simply delayed, we examined cells after 15 h of incubation, a time when all hyphae had >32 nuclei. All wild-type cells and 95% of ΔaspA cells had at least one septum. In contrast, only 28% of ΔaspC and 38% of ΔaspA ΔaspC cells had at least one septum (n = 200; Fig. 2). The intensity of septum staining with calcofluor was reduced and difficult to see and photograph in ΔaspC and ΔaspA ΔaspC cells compared to wild-type and ΔaspA cells (data not shown). In plate assays of growth, we saw no obvious defect of septin deletion mutants with addition of the cell wall-perturbing agent calcofluor and no obvious difference in radial growth rate at restrictive temperature (data not shown).

During asexual reproduction (conidiation), an aerial hypha emerges from the main hypha and swells at its tip to form a vesicle. From the vesicle two ordered layers of cells emerge sequentially (metulae and phialides), ultimately giving rise to chains of conidiospores. We compared conidiophores (asexual structures) of wild type to septin-deleted strains (Fig. 3). Wild type had regular layers and a chain of conidiospores (Fig. 3A). ΔaspA, ΔaspC, and ΔaspA ΔaspC mutants had irregular and fused layers in the conidiophore and produced fewer spores (Fig. 3B to H).

AspA and AspC localize to dormant conidia, emerging germ tubes, emerging branches, septa, and emerging conidiophore layers. To localize the septins, we made in-frame fusions of GFP to the 3′ end of the open reading frames encoding AspA and AspC. To make expression as close to wild-type levels as
possible, fusions were integrated at the \textit{aspA} or \textit{aspC} locus behind the native \textit{asp} promoter, replacing the wild-type gene. To determine whether the GFP tag might interfere with septin function, we compared the phenotypes of Asp-GFP fusion strains (AspA-GFP, ARL141, and AspC-GFP, ARL161) with those of wild-type and septin-deleted strains. Germ tube and branch emergence, septation, and conidiation were all wild type in the septin-GFP strains, indicating that the GFP tag does not interfere with function (Fig. 2 and data not shown).

AspA-GFP was visible as spots or short rods in dormant conidia. There was generally a single very bright spot either alone or with dimmer spots (Fig. 4A). As the conidium expanded, AspA-GFP localization became more punctate and cortical (Fig. 4A and B), and as polarization occurred, AspA-GFP was found at the base and growing tip of the germ tube (Fig. 4B). In hyphae, AspA-GFP showed cytoplasmic localization that was often punctate at the cortex and brighter at hyphal tips and emerging branches (Fig. 4C, D, E, and G). AspA-GFP was also visible as a ring or cap at forming septa and emerging branches (Fig. 4E and F). In conidiophores, AspA-GFP localized transiently to each individual layer as it emerged, persistently to the phialide-conidiospore interface at the base of the forming chain of spores and generally as a single bright spot in each conidium, either alone or with dimmer spots (Fig. 5). Localization of AspA-GFP was also examined in a heterozygous diploid strain containing one copy of \textit{aspA-GFP} and one of native \textit{aspA} (ARL205). Localization of AspA-GFP in the heterozygous diploid strains was identical to localization in the haploid strain (data not shown). Localization of AspC-GFP was virtually indistinguishable from AspA-GFP at all stages examined in both haploid and heterozygous diploid strains (data not shown).

AspA localizes abnormally in \textit{ΔaspC} cells, and AspC fails to localize in \textit{ΔaspA} cells. In most cases where septins have been studied, different septins interact to form heteropolymers. To investigate whether AspA and AspC require each other for localization, we made strains in which one septin was fused to GFP and the other septin was deleted. From crosses of AspC-GFP and \textit{ΔaspA} strains, we examined four progeny that contained both the deletion and the GFP fusion based on PCR analysis (ARL182 to ARL185). In all cases we no longer observed specific localization of AspC in the \textit{ΔaspA} background in vegetative or asexual growth (Fig. 6A). From crosses of AspA-GFP and \textit{ΔaspC} strains, we examined two progeny that contained both the deletion and the GFP fusion based on PCR analysis (ARL198 and ARL201). AspA-GFP in \textit{ΔaspC} cells localized as a tiny bright dot in conidia (Fig. 6B). AspA-GFP

\textbf{FIG. 3.} \textit{ΔaspA}, \textit{ΔaspC}, and \textit{ΔaspA ΔaspC} strains show abnormal conidiophores. (A) Wild-type conidiophores develop regular layers and conidiospore chains. (B to H) \textit{ΔaspA}, \textit{ΔaspC}, and \textit{ΔaspA ΔaspC} mutants had irregular and fused conidiophore layers and produced fewer spores, with the double mutant phenotype being more severe than either single mutant. Scale bar, 5 μm.
continued to localize as a single very bright dot or a short bar at different locations during the remaining developmental stages (Fig. 6B), except in the conidiophore where multiple dots were seen (Fig. 6B).

**DISCUSSION**

**AspA and AspC interact genetically and have similar functions in germ tube and branch emergence, septation, and conidiation.** Our finding that ΔaspA, ΔaspC, and ΔaspA ΔaspC strains are viable is in sharp contrast to findings in *S. cerevisiae*, where the orthologous genes, *CDC11* (aspA ortholog) and *CDC12* (aspC ortholog), are essential (7). Phenotypes of ΔaspA and ΔaspC strains were almost identical in *A. nidulans*, with both strains showing early and increased germ tube emergence, increased branch emergence, delayed or reduced septation, and disorganized conidiophores. The only phenotype that differed between the deletion strains was seen after 15 h of incubation, at which time ΔaspA cells made normal levels of septa after an initial delay, while ΔaspC cells showed a reduction in the total number of septa.

The very similar phenotypes in ΔaspA and ΔaspC strains suggest that AspA and AspC play very similar roles in germ tube and branch emergence, septation, and conidiation. The identical localization patterns of AspA-GFP and AspC-GFP are consistent with the view that these septins have similar functions. However, the increased severity of all phenotypes in the ΔaspA ΔaspC double mutant suggests that there might be subtle differences between these septins. One possible difference would be the individual positions of AspA and AspC within multiseptin complexes. In *S. cerevisiae*, AspA ortholog Cdc11 and AspC ortholog Cdc12 associate directly with each other in the bud neck septin complex, where Cdc12 is thought to be the central septin associating with both Cdc11 and Cdc3 and linking them into the complex (3, 37). Our finding that AspA localizes to abnormal structures in strains lacking AspC and that AspC does not localize at all in strains lacking AspA suggests that the Cdc11 ortholog AspA might be the central septin in the *A. nidulans* septin complex and/or that AspA might be needed for the retention of AspC in complexes.

**AspA and AspC localize as spots, rings, collars, and filaments.** Consistent with the idea that AspA and AspC play similar roles, AspA and AspC show virtually identical localization patterns. Typically, fungal septins localize as rings or collars through which new growth emerges, cross walls delineating compartments, or caps at hyphal tips (22). AspA and AspC show these typical fungal localization patterns, forming rings or collars through which germ tubes, branches, and conidiophore layers emerge; septa delineating hyphal compartments; and diffuse caps at tips of actively growing hyphae (Fig. 4 and 5). In addition to these typical fungal localization patterns, AspA and AspC localize as dots or short bars, puncta, or elongated filaments, patterns previously described mainly for animal septins (Fig. 4) (22).

**AspA and AspC are involved in, but not required for, septation.** While *S. cerevisiae* strains with mutations in septin core complex members make no septa and cannot complete cytokinesis (16), *Schizosaccharomyces pombe* septin mutants form septa and divide, though the process is delayed in some mutants (24, 34). Thus, it was not too surprising that *A. nidulans* ΔaspA, ΔaspC, and ΔaspA ΔaspC strains made at least some septa. After an initial delay, ΔaspA strains made near-wild-type

**FIG. 4.** AspA localizes to dormant conidia, emerging germ tubes, emerging branches, and septa. The AspA-GFP strain was incubated, and live cells were photographed using fluorescence microscopy. Panels show composites of multiple cells for each developmental stage. (A) Dormant conidia; (B) newly emerging germ tubes; (C) germlings; (D) hyphae; (E) septating hyphae; (F) branching hyphae. Scale bar, 5 μm.
levels of apparently normal septa. This is very close to the situation in *S. pombe*, where a mutant lacking Spn3, the AspC ortholog, shows normal cytokinesis (1). In contrast, ∆aspC strains made only one-third of the number of septa that the wild type did and these septa appeared to be abnormal based on staining with the chitin-binding dye calcofluor, suggesting that AspC has a unique role in septation and that this role cannot be filled by other septins. This is somewhat similar to the case in *S. pombe*, where a mutant in Spn4, the AspC ortholog, shows delayed cytokinesis, a more severe effect than loss of Spn3 (1). These differences in septin defects are consistent with recent literature showing that though different septins interact for proper function, septin dynamics within a complex can vary (5).

**AspA and AspC influence the number of new growth foci and their patterns.** During vegetative growth ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants showed increased emergence of new growth foci, making extra germ tubes and branches (Fig. 1). In addition to having too many germ tubes, their spatial pattern is disrupted in ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants. In wild-type *A. nidulans*, a single germ tube emerges from the conidium and is generally followed later by emergence of a second germ tube 180° relative to the first (27). In contrast, second germ tubes in the ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants emerge axial or at 45° or 90° relative to the first germ tube, indicating a disruption of spatial pattern. In wild-type hyphae generally a single branch emerges from each compartment delineated by septa (21). Thus, we cannot predict the normal position for a second branch within a compartment and so cannot determine whether the multiple, closely spaced branches that emerge from compartments of ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants indicate a disruption of spatial pattern or simply an increase in the number of new growth foci.

During asexual reproduction in *A. nidulans*, a regular, organized, multilayered conidiophore that bears chains of spores (conidia) is made by a process that closely resembles budding in yeast (36). During conidiation in ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants, the multiple layers of the conidiophore are disorganized and the number of conidia is reduced (Fig. 3). This disorganization appears to affect different layers of the multilayered structure in individual conidiophores within a population. In some conidiophores the deletion phenotype appears to be a patterning defect, and in others it appears to be a cell division or separation defect.

In *S. cerevisiae*, septin mutants show disruption of normal axial and bipolar budding patterns (24, 26), and so the disruption of germ tube emergence patterns in ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants was not too surprising. However, we were very surprised to see the increased germ tube and branch emergence in the *A. nidulans* septin mutants. Though *S. cerevisiae* septin mutants form cells with multiple buds, this multibud phenotype is thought to result from failure to complete cytokinesis, which normally separates buds from the mother cell, not from simultaneous emergence of new buds (25). During vegetative growth of filamentous fungi like *A. nidulans*, there is no separation of germ tubes and branches from the hypha comparable to the separation of buds from the mother cell at cytokinesis in yeast. Cytokinesis in filamentous fungi results in partitioning of hyphal compartments by septa, a process which still takes place in the ∆aspA, ∆aspC, and ∆aspA ∆aspC mutants, although it is delayed or reduced. This is, to our knowledge, the first report of an increase in the number of new growth foci associated with loss of septin function in any or-

**FIG. 5.** AspA localizes to emerging conidiophore layers. The AspA-GFP strain was incubated on agar between glass coverslips, and live cells were photographed using fluorescence microscopy. AspA-GFP at vesicle-metula interface (A) and phialide-conidiospore interface (B and C). Scale bar, 5 μm.
ganism and raises the intriguing possibility that septins might limit new growth foci in other multicellular organisms.

ACKNOWLEDGMENT
This work was supported by National Science Foundation grant MCB-0211787 to M.M.

REFERENCES
1. An, H., J. Morrell, J. Jennings, A. Link, and K. Gould. 2004. Requirements of fission yeast septins for complex formation, localization, and function. Mol. Biol. Cell 15:5551–5564.
2. Beites, C. L., H. Xie, R. Bowser, and W. S. Trimble. 1999. The septin CDCrel-1 binds syntaxin and inhibits exocytosis. Nat. Neurosci. 2:434–439.
3. Bertin, A., M. McMurray, P. Grob, S. Park, G. Garcia, I. Patanwala, H. Ng, T. Alber, J. Thorner, and E. Nogales. 2008. Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. Proc. Natl. Acad. Sci. U. S. A. 105:8274–8279.
4. Caviston, J. P., M. Longtine, J. R. Pringle, and E. Bi. 2003. The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast. Mol. Biol. Cell 14:4051–4066.
5. DeMay, B. S., R. A. Meseroll, P. Occhipinti, and A. S. Gladfelter. 2009. Regulation of distinct septin rings in a single cell by Elm1p and Gin4p kinases. Mol. Biol. Cell 20:2311–2326.
6. Dietrich, F. S., S. Voegeli, S. Brachat, A. Lerch, K. Gates, S. Steiner, C. Mohr, R. Pohlmann, P. Luedi, S. Choi, R. A. Wing, A. Flavier, T. D. Gaffney, and P. Philippsen. 2004. The Ashbya gossypii genome as a tool for mapping the ancient Saccharomyces cerevisiae genome. Science 304:304–307.
7. Frazier, J. A., M. L. Wong, M. S. Longtine, J. R. Pringle, M. Mann, T. J. Mitchison, and C. Field. 1998. Polymerization of purified yeast septins: evidence that organized filament arrays may not be required for septin function. J. Cell Biol. 143:737–749.
8. Gladfelter, A., J. Pringle, and D. Lew. 2001. The septin cortex at the yeast mother-bud neck. Curr. Opin. Microbiol. 4:681–689.
9. Gladfelter, A. S. 2006. Control of filamentous fungal cell shape by septins and formins. Nat. Rev. Microbiol. 4:225–229.
10. Gladfelter, A. S., L. Kozubowski, T. R. Zyla, and D. J. Lew. 2005. Interplay between septin organization, cell cycle and cell shape in yeast. J. Cell Sci. 118:1617–1628.
11. Harris, S. D. 1999. Morphogenesis is coordinated with nuclear division in germinating Aspergillus nidulans conidiophores. Microbiology 145:2747–2756.
12. Harris, S. D. 2001. Genetic analysis of ascomycete fungi, p. 56–57. In N. J. Talbot (ed.), Molecular and cellular biology of filamentous fungi. Oxford University Press, New York, NY.

FIG. 6. AspC fails to localize in ΔaspA cells, and AspA localizes abnormally in ΔaspC cells. (A) An AspC-GFP, ΔaspA strain was incubated, and live cells were photographed using fluorescence microscopy. (B) An AspA-GFP, ΔaspC strain was incubated, and live cells were photographed using fluorescence microscopy. Scale bar, 5 μm.
13. 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.

14. Hartwell, L. H. 1971. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp. Cell Res. 69:265–276.

15. Ihara, M., A. Kinoshita, S. Yamada, H. Tanaka, A. Taniyagi, A. Kitano, M. Goto, K. Okubo, H. Nishiyama, and O. Ogawa. 2005. Cortical organization by the septin cytoskeleton is essential for structural and mechanical integrity of mammalian spermatozoa. Dev. Cell 8:343–352.

16. Iwase, M., J. Luo, E. Bi, and A. Tobe. 2007. Shs1 plays separable roles in septin organization and cytokinesis in Saccharomyces cerevisiae. Genetics 177:215–229.

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

18. Kartmann, B., and D. Roth. 2001. Novel roles for mammalian septins: from vesicle trafficking to oncogenesis. J. Cell Sci. 114:839–844.

19. Kinoshita, M., C. Field, M. Coughlin, A. Straight, and T. Mitchison. 2002. Self- and actin-templated assembly of mammalian septins. Dev. Cell 3:791–802.

20. Lin, X., and M. Momany. 2003. The Aspergillus nidulans swoC1 mutant shows defects in growth and development. Genetics 165:543–554.

21. Lin, X., and M. Momany. 2004. Identification and complementation of abnormal hyphal branch mutants ahbA1 and ahbB1 in Aspergillus nidulans. Fungal Genet. Biol. 41:998–1006.

22. Lindsey, R., and M. Momany. 2006. Septin localization across kingdoms: three themes with variations. Curr. Opin. Microbiol. 9:559–565.

23. Longtine, M., and E. Bi. 2003. Regulation of septin organization and function in yeast. Trends Cell Biol. 13:403–409.

24. Longtine, M., D. DeMarini, M. Valencik, O. Al-Awar, H. Fares, C. De Virgilio, and J. Pringle. 1996. The septins: roles in cytokinesis and other processes. Curr. Opin. Cell Biol. 8:106–119.

25. Longtine, M., C. Thesfeld, J. McMillian, E. Weaver, J. Pringle, and D. Lew. 2000. Septin-dependent assembly of a cell cycle-regulatory module in Saccharomyces cerevisiae. Mol. Cell. Biol. 20:4049–4061.

26. Madden, K., and M. Snyder. 1998. Cell polarity and morphogenesis in budding yeast. Annu. Rev. Microbiol. 52:687–744.

27. Manyany, M., and I. Taylor. 2000. Landmarks in the early duplication cycles of Aspergillus fumigatus and Aspergillus nidulans: polarity, germ tube emergence and septation. Microbiology 146:3279–3284.

28. Manyany, M., P. J. Westfall, and G. Abramowsky. 1999. Aspergillus nidulans two mutants show defects in polarity establishment, polarity maintenance and hyphal morphogenesis. Genetics 151:557–567.

29. Manyany, M., J. Zhao, R. Lindsey, and P. J. Westfall. 2001. Characterization of the Aspergillus nidulans septin (asp) gene family. Genetics 157:969–977.

30. Pan, F., R. L. Malmberg, and M. Momany. 2007. Analysis of septins across kingdoms reveals orthology and new motifs. BMC Evol. Biol. 7:103.

31. Spiliotis, E., and W. Nelson. 2006. Here come the septins: novel polymers that coordinate intracellular functions and organization. J. Cell Sci. 119:4–10.

32. Spiliotis, E. T., M. Kinoshita, and W. J. Nelson. 2005. A mitotic septin scaffold required for mammalian chromosome congression and segregation. Science 307:1781–1785.

33. Sudbery, P. E. 2001. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. Mol. Microbiol. 41:19–31.

34. Sudbery, P. E., and A. S. Gladfelter. 2008. Pathocycles. Fungal Genet. Biol. 45:1–5.

35. Surka, M., C. Tsang, and W. Trimble. 2002. The mammalian septin MSF localizes with microtubules and is required for completion of cytokinesis. Mol. Biol. Cell 13:3532–3545.

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

37. Versele, M., R. Guilbrand, M. Shulewitz, V. Cid, S. Bahmanyar, R. Chen, P. Barth, T. Alber, and J. Thorner. 2004. Protein-protein interactions governing septin heteropentamer assembly and septin filament organization in Saccharomyces cerevisiae. Mol. Biol. Cell 15:4568–4583.

38. Versele, M., and J. Thorner. 2004. Septin collar formation in budding yeast requires GTP binding and direct phosphorylation by the PAK, ClpA4. J. Cell Biol. 164:701–715.

39. Warren, A. J., and J. B. Konopka. 2002. Septin function in Candida albicans morphogenesis. Mol. Biol. Cell 13:2732–2746.

40. Westfall, P. J., and M. Momany. 2002. Aspergillus nidulans septin AspB plays pre- and postmitotic roles in septum, branch, and conidiophore development. Mol. Biol. Cell 13:110–118.

41. 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.

42. Xie, H., M. Surka, J. Howard, and W. Trimble. 1999. Characterization of the mammalian septin H5: distinct patterns of cytokinetic and membrane association from other septin proteins. Cell Motil. Cytoskeleton 43:52–62.

43. Xue, J., C. Tsang, W. Gai, C. Malladi, W. Trimble, J. Rostas, and P. Sudbery. 2004. Septin localization across kingdoms reveals orthology and new motifs. BMC Evol. Biol. 7:103.

44. Yang, L., L. Ukil, A. Osmani, F. Nahm, J. Davies, C. P. De Souza, X. Dou, A. Perez-Balaguer, and S. A. Osmani. 2004. Rapid production of gene replacement constructs and generation of a green fluorescent protein-tagged centromeric marker in Aspergillus nidulans. Eukaryot. Cell 3:1359–1362.