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Characterization of the *Aspergillus nidulans* Septin (*asp*) Gene Family

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ABSTRACT

Members of the septin gene family are involved in cytokinesis and the organization of new growth in organisms as diverse as yeast, fruit fly, worm, mouse, and human. Five septin genes have been cloned and sequenced from the model filamentous fungus *A. nidulans*. As expected, the *A. nidulans* septins contain the highly conserved GTP binding and coiled-coil domains seen in other septins. On the basis of hybridization of clones to a chromosome-specific library and correlation with an *A. nidulans* physical map, the septins are not clustered but are scattered throughout the genome. In phylogenetic analysis most fungal septins could be grouped with one of the prototypical *S. cerevisiae* septins, Cdc3, Cdc10, Cdc11, and Cdc12. Intron-exon structure was conserved within septin classes. The results of this study suggest that most fungal septins belong to one of four orthologous classes.

SEPTINS are key players in cellular organization processes ranging from cytokinesis to surface growth. Members of the septin gene family have been found in budding and fission yeast, fruit fly, worm, mouse, and human (reviewed by Longtine *et al.* 1996; Field and Kellog 1999; Trimble 1999). In *Saccharomyces cerevisiae* there are seven septins. Multiple septins are also found in other fungi and animals, although the total number in most of these organisms is not yet known because of incomplete genome information. At least some of the septins are specific to a particular life cycle stage or tissue type.

Septin proteins are highly homologous, and all contain a P-loop nucleotide binding motif (reviewed by Longtine *et al.* 1996). Septin complexes isolated from Drosophila possess nucleotide binding and GTPase activity and can polymerize *in vitro* (Field *et al.* 1996). It has been suggested that the septins may represent a novel cytoskeletal element and that, like actin and tubulin, they may be regulated by nucleotide hydrolysis (Sanders and Field 1994). Most septins also contain coiled-coil domains thought to be involved in protein-protein interactions.

Septins were first described as temperature-sensitive mutations in the cell division cycle genes *CDC3*, *CDC10*, *CDC11*, and *CDC12* of *S. cerevisiae* (reviewed by Longtine *et al.* 1996). At restrictive temperature these mutants make elongated buds, do not complete cytokinesis, and lack the 10-nm filament ring normally seen just inside the plasma membrane at the neck (Byers and Goetsch 1976). It now seems clear that the major components of the 10-nm filament ring are Cdc3p, Cdc10p, Cdc11p, and Cdc12p. In addition to their location at the neck, Cdc3p, Cdc10p, Cdc11p, and Cdc12p are found at the site of bud emergence and at the base of the mating projection, or schmoo (Haarer and Pringle 1987; Kim *et al.* 1991). A mutation in any one of these septins results in loss of neck localization of the other three septins. A fifth septin, Sep7p, is found at the bud neck and site of bud emergence. Deletion of *SEP7* gives only a mild cytokinesis and budding defect (Carroll *et al.* 1998). The remaining septins, *SPR3* and *SPR28*, are only expressed during sporulation. The proteins Spr3p and Spr28p are found at the leading edge of developing spore walls (DeVirgilio *et al.* 1996; Fares *et al.* 1996). Strains deleted in *SPR3* are less efficient in sporulation, while those deleted in *SPR28* show no phenotype. Evidence suggests that Ccd3p, Cdc10p, and Cdc11p play some role in spore formation along with their roles in vegetative growth (Haarer and Pringle 1987; Kim *et al.* 1991).

In addition to their interactions with each other, *S. cerevisiae* septins interact with a wide variety of other proteins. Many important cytokinetic proteins localize to the neck region in a septin-dependent manner. The myosin responsible for actin ring contraction, Myo1p, is found only in the middle of the septin ring at the neck (Bi *et al.* 1998; Lippincott and Li 1998). Proteins Bni4p and Chs4p, which are involved in the deposition of the bud scar on the mother cell, are found specifically on the mother side of the neck (DeMarini *et al.* 1997). The cell cycle regulators Hsl1p and Hsl7p are found on the daughter side of the neck where they may form part of the morphogenesis checkpoint delaying nuclear division when budding is delayed (Barral *et al.* 1999; Shulewitz *et al.* 1999; Lew 2000).

There are major differences in the septin-associated processes of cytokinesis and deposition of new growth

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in budding yeast *vs.* filamentous fungi. In yeast the mother cell grows isotropically, adding new cell wall in every direction. Late in G1, growth shifts to a spot on the surface previously defined by a ring of septins, actin, and chitin. The polar growth of the bud continues through S phase until the middle of G2, when the bud switches to isotropic growth. As M phase ends, septation occurs, separating the mother and daughter cells, each containing a single nucleus (reviewed by Pringle and Hartwell 1981; Lew *et al.* 1997).

In *Aspergillus nidulans*, a brief period of isotropic growth is followed by apical extension of the germ tube (reviewed by Harris 1999; Momany and Taylor 2000). Once these tip-growing germ tubes reach a predetermined size, they are partitioned by septa. Only the apical cell remains mitotically active. Basal compartments, which contain three to four nuclei, arrest in interphase until a new branch is formed (Kaminskyj and Hamer 1998). In addition to this partitioning during vegetative growth, *A. nidulans* uses budding to form its asexual reproductive spores known as conidia (reviewed by Adams *et al.* 1998). During conidiation, specialized basal cells give rise to aerial hyphae. The tips of these aerial hyphae swell to form a vesicle, and layers of cells bud off of the vesicle. Finally, uninucleate conidia bud from this multi-layered structure.

In previous work *aspB*, a septin gene cloned from *A. nidulans*, was shown to be essential (Momany and Hamer 1997). The *aspB* gene product localizes to forming septa, emerging branches, and layers of the conidiophore (P. Westfall and M. Momany, unpublished results). Described here are the identification and characterization of four new septins in *A. nidulans, aspA*, *aspC*, *aspD*, and *aspE*. Comparison of septin sequences and intron-exon structure from a variety of fungi suggest that there are at least four classes of orthologous fungal septins.

MATERIALS AND METHODS

***A. nidulans* strains and growth methods:** *A. nidulans* strain A28 (*pabaA6*; *biA1*) was purchased from the Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City. All incubations were in complete medium except where noted (1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, nitrate salts, trace elements, and 0.01% vitamins, pH 6.5). Trace elements, vitamins, nitrate salts, and amino acid supplements are described in the appendix to Kafer (1977).

DNA and RNA isolation: Standard molecular biology procedures were used (Sambrook *et al.* 1989). DNA was isolated from *A. nidulans* using previously described methods (Hamer and Givan 1990). Total RNA from *A. nidulans* was extracted by using Trizo Reagent (GIBCO BRL, Grand Island, NY). Isolation of RNA from synchronous cultures undergoing conidiation was as described by Miller *et al.* (1992). Briefly, synchronous development was induced by exposing liquid cultures to an air interface. Cultures were examined microscopically to verify appropriate developmental stage at harvest times. At 0 hr vegetative hyphae were observed, at 2.5 hr aerial hyphae, at 5.0 hr vesicles and metulae initials, at 7.5 hr metulae and phialides, at 10.0 hr immature conidia, and at 13.0 hr mature pigmented conidia. Poly(A) RNA was isolated from total RNA using GIBCO BRL message maker reagent assembly kit with Ambion (Austin, TX) oligo(dT) following the manufacturer's recommendations.

First-strand cDNA synthesis: First-strand cDNA for RT-PCR was synthesized from 1.0 μg RNA using an RT-PCR cDNA synthesis kit as described by the manufacturer (Boehringer Mannheim, Indianapolis). Reverse transcriptions were carried out in 20-μl reaction mixtures containing 20 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim), 15 pmol of oligo$(dT)_{15}$, and 50 units of Rnase inhibitor (Boehringer Mannheim). Thirty cycles were performed under the following conditions: at 25° for 10 min, at 45° for 60 min, and at 99° for 5 min.

Cloning of septin genes: The PCR amplification of a highly conserved 300-bp region of *aspA*, *aspB*, and *aspC* and the cloning of *aspB* have been previously described (Momany and Hamer 1997). cDNA clones for *aspA* and *aspC* were isolated from an *A. nidulans* library as previously described (Momany and Hamer 1997). Genomic clones containing *aspA* and *aspC* were identified by hybridizing the corresponding cDNA clones to an *A. nidulans* chromosome-specific library (Brody *et al.* 1991). The *aspA* probe hybridized with cosmid SW18A08 on contig IIIA. The *aspC* probe hybridized with cosmids SL32D02, SW08F07, and SW24G01, which are all on contig IID. Partial sequences of *aspD* and *aspE* septin homologues were identified through a BLAST search of the Cereon Genomics *A. nidulans* sequence database using *S. cerevisiae CDC3*, *CDC10*, *CDC11*, and *CDC12* sequences as queries. Partial sequence information was used to design PCR primers to amplify fragments of *aspD* and *aspE* from *A. nidulans* genomic DNA. The resulting PCR products were used to probe an *A. nidulans* chromosome-specific library (Brody *et al.* 1991). The *aspD* PCR product hybridized with cosmids SW08H01 and SW11B09, which are mapped to contig VIIIG. The *aspE* PCR product hybridized to cosmids SW10A02, SW04B03, and SW08E11 on contig IIIB and SL04C11 and SL06G03 on contig IIIF.

DNA sequencing and analysis: Both strands of *aspA*, *aspC*, *aspD*, and *aspE* genomic clones were completely sequenced using Taq polymerase cycle sequencing and an automated DNA sequencer (Model ABI 310, Perkin-Elmer, Norwalk, CT; Momany and Hamer 1997). Primer walking was performed beginning with the PCR primers used in gene identification as described above. Both strands of *aspA* and *aspC* cDNA clones were sequenced using primer walking from highly conserved regions and from flanking vector regions. To identify introns in *aspA* and *aspC*, cDNA and genomic sequences were compared. To identify introns in *aspD* and *aspE*, RT-PCR from *A. nidulans* RNA was used. Primers for RT-PCR were based on regions in which the genomic sequence disagreed with the conserved septin consensus sequence or in which the open reading frame ended prematurely. Consensus fungal sequences were identified for all introns (Ballance 1986). Analysis of sequences was performed using SeqLab 10.0 of the Genetics Computer Group package of the University of Wisconsin.

Northern hybridization: Six identical denaturing gels containing 3 μg of poly(A) RNA for each time point were transferred to nitrocellulose membranes by standard methods (Sambrook *et al.* 1989). Probes were made by incorporation of ^{32}P by random priming of PCR products from *aspA*, *aspB*, *aspC*, *aspD*, *aspE*, or the housekeeping gene *argB*. After hybridization and washes, the blot was exposed to a phosphor screen (Kodak) overnight at room temperature. The hybridization signals were read three times on a Storm Imager (Molecular Dynamics, Sunnyvale, CA). Data reported are relative to the average *argB* signal.

Genomic data acquisition and processing: Septin homologues were acquired through searches of GenBank using septins from *A. nidulans* as the query. All expressed sequence tagged (EST) data were acquired from the *A. nidulans* EST database consisting of ∼13,400 sequences from a 24-hr mixed vegetative and asexual culture (http://www.genome.ou.edu/fungal.html). Yeast microarray data are described by Spellman *et al.* (1998). Nucleotide and amino acid sequences were aligned by using ClustalX 1.8 (Thompson *et al.* 1994). Phylogenetic trees were constructed using the neighbor-joining method from ClustalX 1.8 with default settings (Saitou and Nei 1987). Bootstrap values are based on 1000 replicates.

Nucleotide sequence accession numbers: Nucleotide sequences for *aspA*, *aspC*, *aspD*, and *aspE* were assigned GenBank accession nos. AF299320, AF299321, AF299322, and AF299323, respectively.

RESULTS

Septin family alignment: The *asp* (for *A*spergillus *sep*tin) family consists of at least five genes: *aspA*, *aspB*, *aspC*, *aspD*, and *aspE*. Predicted products of the *asp* genes range from 343 (*aspD*) to 469 (*aspE*) amino acids in length with an estimated mass range of 38–52 kD. The *A. nidulans* septin genes display >35% amino acid identity over their entire lengths. Alignment of the deduced amino acid sequences of *A. nidulans* and *S. cerevisiae* septins is shown in Figure 1. As is true for the entire septin family, the similarity among Asp proteins is greatest in their central regions, while the amino-terminal and carboxyl-terminal regions are divergent in both length and sequence. All septin genes, including those from *A. nidulans*, have three predicted P-loop GTPase domains, G1 (GXXXXGKT), G3 (DTPG), and G4 (XKXD) (Saraste *et al.* 1990). Most septins, including all *asp* products except that of *aspD*, contain predicted coiled-coil domains at or near the carboxyl termini (Lupas *et al.* 1991). These domains are thought to be involved in homotypic or heterotypic interactions among the septins themselves or in interactions between the septins and other proteins (Longtine *et al.* 1996).

Phylogenetic analysis: Currently available fungal septin sequences were analyzed for similarities. This analysis established four main septin classes with a nearly one-to-one correspondence among proteins from *A. nidulans*, *Schizosaccharomyces pombe*, and *S. cerevisiae* (Figure 2). Each class contained one of the prototypical *S. cerevisiae* septins, Cdc3, Cdc10, Cdc11, or Cdc12. The Cdc3 class also contains AspB from *A. nidulans*, Cacdc3 from *Candida albicans*, Abs2 from the basidiomycetous *Agaricus bisporus*, and Spn1 from *S. pombe*. The Cdc10 class contains AspD, Nccdc10 from *Neurospora crassa*, *C. albicans* Cacdc10, Spn2, and Ums1 from the basidiomycete *Ustilago maydis*. The Cdc11 class contains AspA, Spn3, Abs1, and Pbs1 from the ascomycete *Pyrenopeziza brassicae* (Singh *et al.* 2000). The Cdc12 class contains AspC, Spn4, and the *Mucor circinelloides* septin Mcs1. The predicted products of the *aspE*, *SPN5*, *SPPC584.09*, and *SPR3* genes did not cluster with septins from other fungi. Except for the *S. cerevisiae* protein Spr28, the septins from within a single fungal species were dispersed among the classes.

Intron-exon structure: The locations of introns in the *A. nidulans* septins were determined by comparing sequences of genomic and cDNA (Figure 3). The number of introns varied for the *asp* genes, with one in *aspB* and *aspE*, two in *aspA*, and five in *aspC* and *aspD*. Intron-exon junctions of *asp* genes contained the consensus-splice sequences seen in other filamentous fungi, specifically, PuPy (usually GT) at the 5′ end and AG at the 3′ end (Ballance 1986). Intron sizes varied from 51 bp (*aspD* intron III) to 180 bp (*aspA* intron I). All five *asp* genes contained an intron upstream of the G1 GTP binding domain. In *aspB*, *aspC*, *aspD*, and *aspE* the first intron is within 15 nucleotides of the codon of the G1 domain. In *aspA* the first intron is 51 nucleotides upstream of the codon for the first G1 glycine. There was no sequence conservation detected in the intron upstream of the G1 domain. Neither the positions nor the sequences of the other *asp* introns appeared to be conserved.

The conservation of the intron position upstream of G1 within the *A. nidulans* septin family prompted us to examine intron positions in the other fungal septins for which genomic sequence was available (Figure 3). None of the *S. cerevisiae* septins contain introns, but this is not surprising as introns are unusual in budding yeast (Fink 1987). Within the Cdc11 class of fungal septins, the sister sequences encoding *aspA* and *pbs1* both have an intron in the same position relative to the G1 domain. The more diverged *spn3* lacks this intron. Genomic sequence is not available for the *A. bisporus* septins. Within the Cdc3 class of septins, *S. pombe spn1* and *C. albicans cacdc3* have no introns, and *aspB* contains a single intron. The divergent *spn5*, which does not fall within one of the four septin classes, lacks the intron upstream of G1. Within the Cdc12 class, the sister sequences *aspC* and *mcs1* both have an intron immediately upstream of the G1 domain, while the more distant *spn4* lacks introns. The Cdc10 class contains the most conserved intron positions. *AspD* and *nccdc10* have two introns in identical positions relative to the conserved G1 and G3 domains, while *spn2* shares three identical intron positions with *aspD*. The single intron in *aspE*, which does not fall into one of the main septin classes, does not share its position with any of the other septins.

Genomic organization: To determine the genomic organization of the *A. nidulans* septin family, a chromosome-specific cosmid library (Brody *et al.* 1991) was probed with either cDNA clones (*aspA*, *aspB*, and *aspC*) or PCR products (*aspD* and *aspE*). The hybridizing clones were then located on the *A. nidulans* physical map (Prade 2000) as shown in Figure 4. Localization of *aspB* to chromosome I has been previously described (Momany and Hamer 1997). The *aspA* and *aspC* cDNA clones hybridized with cosmids mapped to contig IIIA and

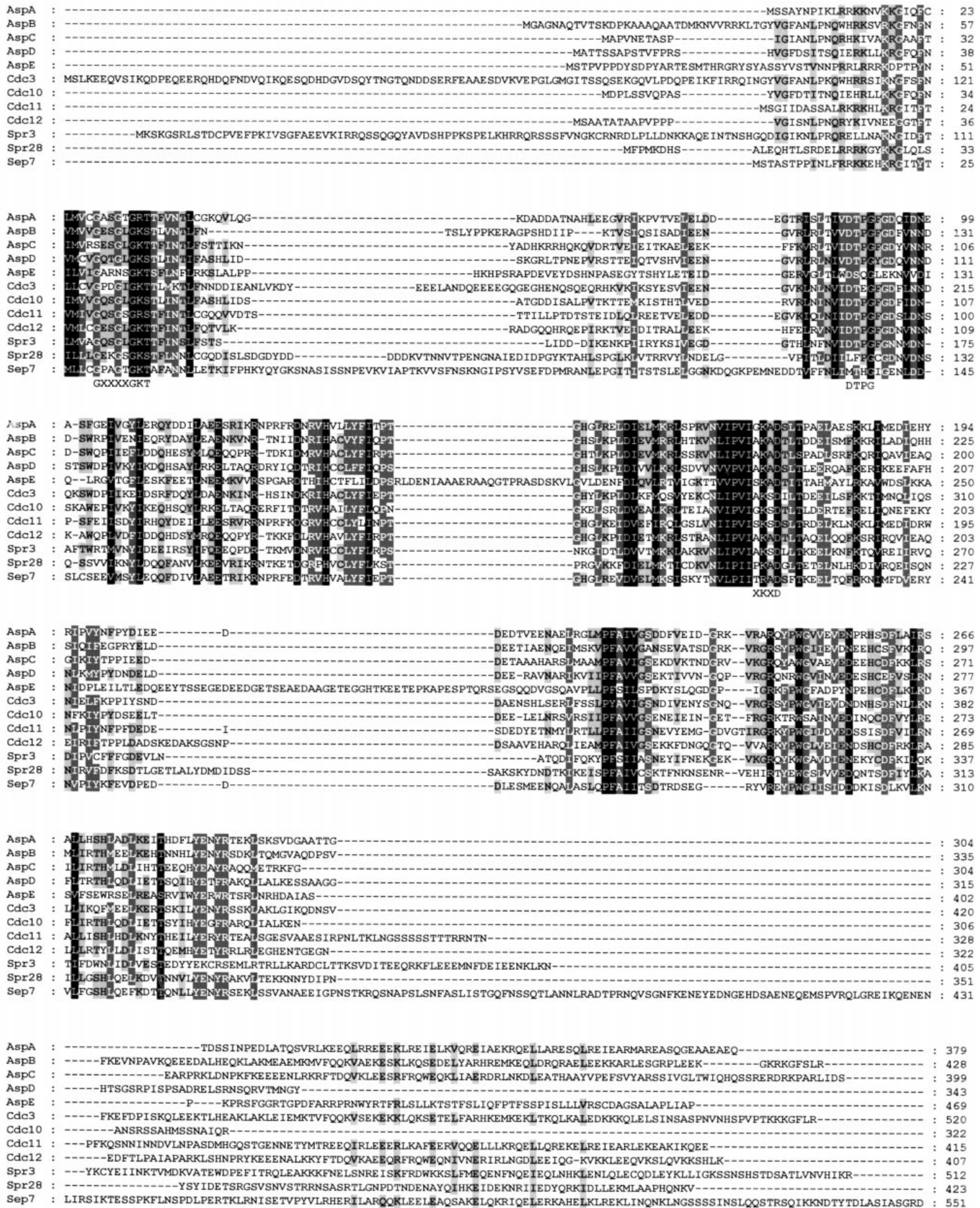

Figure 1.—Alignment of *A. nidulans* and *S. cerevisiae* septins. Alignment of deduced amino acid sequences of septins from *A. nidulans* (AspA–E) with septins from *S. cerevisiae* (Cdc3, 10–12, Spr3, Spr28, and Sep7) is shown. Alignment was generated using ClustalX 1.8 with default settings. Gaps have been introduced for optimal alignment and are indicated by dashes. The positions of G1 (GXXXXGKT), G3 (DTPG), and G4 (XKXD) nucleotide binding domains are indicated under the alignment. Darker shading indicates higher homology. GenBank accession nos. for *S. cerevisiae* genes are: Cdc3, AAB64515.1; Cdc10, CAA42339.1; Cdc11, AAB39301.1; Cdc12, AAB68863.1; Spr3, CAA97061.1; Spr28, CAA88498.1; Sep7, CAA98804.1.

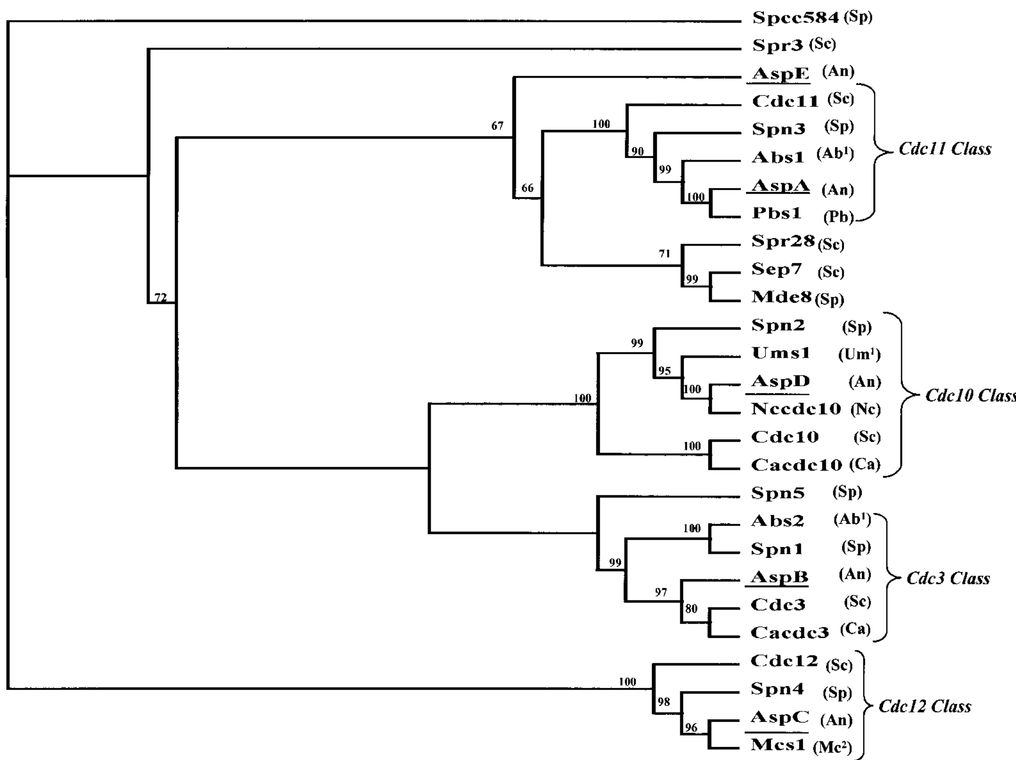

Figure 2.—Phylogenetic analysis of septins from *A. nidulans* and other fungi. Phylogenetic tree was constructed using neighbor-joining. Bootstrap values >50% based on 1000 replicates are shown above the line. *A. nidulans* septins are underlined. GenBank accession numbers for *S. cerevisiae* and *A. nidulans* sequences are as for Figure 1. GenBank accession numbers for other fungal genes are: Spn1, AAB53692.1; Spn2, CAB57440.1; Spn3, AAB53691.1; Spn4, CAB-11495.1; Spn5, CAB11273.1; Mde8, CAA19121.1; Spcc-584.09, CAB41232.1 (all from Field *et al.* 1999); Cacdc3, S43298; Cacdc10, P39826; Abs1, ABZ82019.1; Mcs1, CAB61437.1; Nccdc-10, CAC09398; Pbs1, AJ132791.1. No GenBank numbers are available for Ums1 sequence Michael Bolker, (personal communication) and Abs2 (Ospina-Giraldo *et al.* 2000). Ab, *A. bisporus*; An, *A. nidulans*; Ca, *C. albicans*; Mc, *M. circinelloides*; Nc, *N. crassa*; Pb, *P. brassicae*; Sc, *S. cerevisiae*; Sp, *S. pombe*. All septins are from ascomycetes except where indicated by footnote. [1]Septin from basidiomycete. [2]Septin from zygomycete.

IID, respectively. The *aspD* PCR product hybridized with cosmids mapped to contig VIIIG. The location of *aspE* is somewhat ambiguous as its PCR product hybridized with four cosmids mapped to contig IIIB and two cosmids mapped to contig IIIF.

Expression of *asp* genes: To determine expression during asexual development, mRNA isolated from synchronized cultures undergoing conidiation was probed with the *asp* genes (Figure 5). The *asp*B gene was the most highly expressed of the *A. nidulans* septins, ranging from 10.2- to 27.1-fold above the levels of a housekeeping gene (*argB*). The *asp*D gene was the most poorly

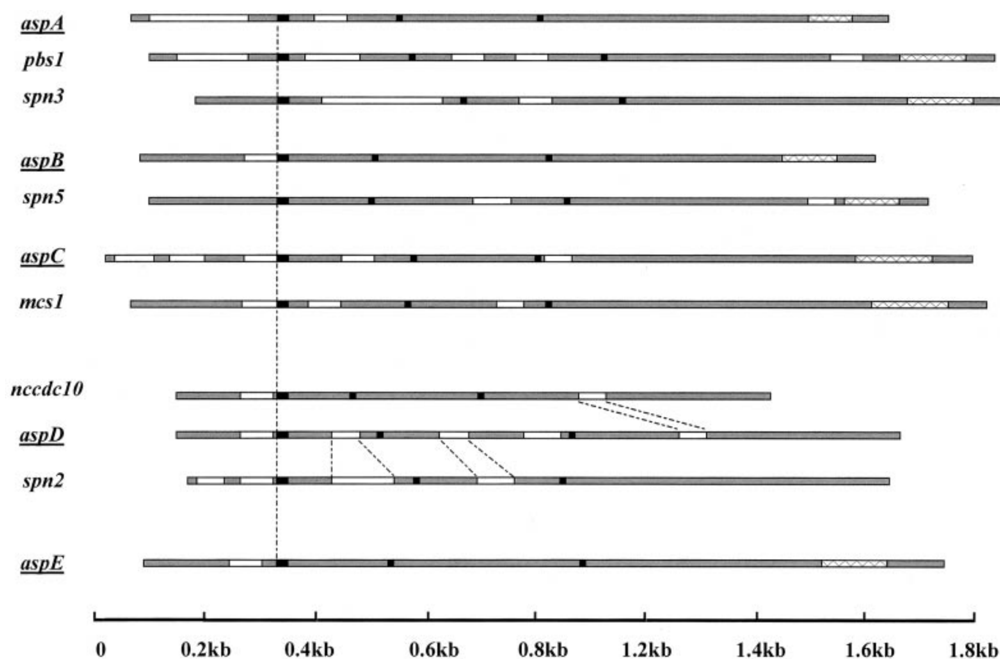

Figure 3.—Comparison of intron-exon structure of septin genes from *A. nidulans* and other fungi. Open boxes represent introns and shaded boxes represent exons. The predicted positions of GTPase domains are indicated by solid boxes and of coiled-coil domains by stippled boxes. Beginning of the first G1 GTPase domain is indicated by a vertical dashed line. Intron positions are shown relative to predicted amino acid sequence of exons. Dashed lines connecting introns between aspD and spn2 or nccdc10 indicate identical positions relative to predicted amino acid sequence of exons.

Figure 4.—Genomic organization of *A. nidulans* septin genes. Probable locations of *A. nidulans* septin genes are shown superimposed on the current *A. nidulans* map of chromosomes I, II, III, and VIII (www.Aspergillus-genomics.org). Aligned boxes represent contigs and shadowing represents cosmids that contain repeats.

expressed with mRNA levels ranging from 0.7- to 2.3-fold that of *argB*.

As the culture progressed from vegetative growth through conidiation, the levels of *aspB* fell, recovering slightly as conidia matured. Both *aspA* and *aspD* showed a similar declining expression pattern, although at a much lower level. The expression of *aspC* peaked as metulae budded from the vesicle layer. The expression of *aspE* was lowest during phialide development (1.1-fold above *argB*) and then increased in mature conidia (3.1-fold above *argB*). The *aspE* gene also showed the highest ratio of minimum/maximum expression (5.8), which may indicate that it is highly regulated.

To verify our RNA hybridization results we searched

Figure 5.—Expression of *A. nidulans* septin genes during conidiation. (A) Hybridization of *A. nidulans* septin genes to poly(A)-RNA isolated from synchronized cultures undergoing conidiation. (B) Plot of signal intensities read from blots in A. Error bars show standard deviation of three replicate reads. (C) Diagram representing conidial stages at each time point.

the *A. nidulans* EST database (http://www.genome.ou.edu/fungal.html) for the *asp* genes and the *argB* control. *aspB* was found twice and *aspC* was found four times. None of the other *asp* genes were detected. The *argB* control was found once.

DISCUSSION

Members of the septin gene family are highly homologous and have been found in several species of fungi and animals where they play critical roles in cytokinesis and cell surface organization (reviewed by Field and Kellogg 1999). Interestingly, septins have not been reported in algae or land plants. It seems most likely that septins arose in a common ancestor of fungi and animals after divergence of the green plants (Wainright *et al.* 1993). It is also possible that septins arose in an earlier common ancestor shared by green plants, fungi, and animals and were later lost in the green plant lineage by nonorthologous gene displacement or lineage-specific gene loss. Regardless of the evolutionary path, it is not surprising that septins are present in animals and fungi and absent in plants in view of the major differences in division mechanisms. Animal and fungal cells divide centripetally; that is, the division furrow progresses from the cortex to the interior. Plant cells, however, divide centrifugally, and the cell plate proceeds from the interior outward to the cortex (reviewed by Field *et al.* 1999).

The septins occur as multigene families in both fungi and animals (reviewed by Field and Kellogg 1999; Trimble 1999). In fungi, septins have been found in three of the four phyla (for fungal phylogeny see Bruns *et al.* 1991, 1993). Multigene septin families are found in each major branch of the phylum Ascomycota. In the archaeascomycete *S. pombe* there are at least seven septins (http://www.sanger.ac.uk/Projects/s_pombe). In the hemiascomycetes *S. cerevisiae* and *C. albicans* there are seven and at least two septins, respectively (http://sequence-www.stanford.edu/group/candida/search.html). In the euascomycete *A. nidulans* there are at least five septins. Within the phylum Basidiomycota, a *U. maydis* septin has been identified through a cytokinesis mutant (Michael Bolker, University of Marburg, unpublished results), and two *A. bisporus* septins have been identified in an EST project (Ospina-Giraldo *et al.* 2000). Within the less-derived phylum Zygomycota, a single septin was found in GenBank from *M. circinelloides*. The absence of septins from the fourth fungal phylum, the Chytridiomycota, and the relative paucity of septins outside the Ascomycota are undoubtedly because there are fewer genomic sequencing efforts underway in these organisms.

Even with incomplete genome data, it seems clear that most fungal septins fall into one of four classes, each containing one of the prototypical *S. cerevisiae* septins, Cdc3, Cdc10, Cdc11, or Cdc12 (Figure 2). It seems likely that septins in each group arose by vertical descent from a common ancestor. In other words, members of each of these four septin classes appear to be orthologs. Our finding of conserved intron positions between the most similar members within septin classes also supports the concept of orthology (Figure 3). The apparent lack of Cdc3 class septins with shared intron positions is probably because so few sequences from this class were analyzed. Spn1 and *C. albicans* Cdc3 lack introns. While none of the *S. cerevisiae* septins contain introns, this is not surprising as there are very few introns in budding yeast. A mechanism for intron loss involving reverse transcription of processed mRNA followed by homologous integration has been proposed to explain the general rarity of yeast introns (Fink 1987; Derr *et al.* 1991). Such a mechanism might also explain the apparent loss of the conserved introns from the *S. pombe* septins Spn3 and Spn4.

There is evidence for both the gain and loss of introns during evolution (Frugoli *et al.* 1998; Venkatesh *et al.* 1999). It is not possible to predict whether individual septin introns are the products of gain or loss during evolution without more sequence data, especially from the basal Chytridiomycota and Zygomycota. Nor can we yet make an informed prediction about how many septins were present in the putative common ancestor of fungi and animals. As more sequence data become available, clustering analysis and intron position in septins from lower fungi and animals should tell us more about the evolution of this important group of proteins. Indeed, intron-exon structure has been used to ascertain evolutionary relationships among angiosperm catalase genes (Frugoli *et al.* 1998) and to define clades in vertebrate evolution (Venkatesh *et al.* 1999).

Most of the Aspergillus septin genes are found on separate chromosomes rather than being clustered. Only *aspC* and *aspE* are on the same chromosome (III), although they are not closely linked. The *S. cerevisiae* septins are also on different chromosomes, except for *SPR28* and *SEP7*, which are 300 kb apart on chromosome IV. Because duplicated genes are often clustered (Stewart and Cullen 1999), such genomic organization is consistent with the idea that fungal septins are related by vertical descent rather than duplication.

Cdc3, -10, -11, and -12 interact to form the yeast 10-nm filament neck ring. Mutation in any one of these genes abolishes neck localization of the other three. We speculate that the ancestors of the Cdc3, -10, -11, and -12 classes also formed complexes; thus interacting domains have been highly conserved in different fungal species throughout evolution. Perhaps septins that participated in more specialized tasks such as spore formation did not have to interact with each other and so were free to diverge. Consistent with this notion, the *S. cerevisiae* nonessential septin (Sep7) and the sporulation-specific septins (Spr3 and Spr28) do not fall within one of the four main septin classes. Also of interest, the Mde8 septin of *S. pombe*, which groups close to Sep7 and Spr28,

appears to be a most unusual septin, lacking most of the G1 consensus domain.

On the basis of the idea that individual members of the four septin classes participate in complexes together, it is predicted that AspA, -B, -C, and -D proteins will interact in a complex that is critical in development, while the AspE protein will probably participate in a more specialized, nonessential process. To begin to address function, septin gene expression levels during vegetative growth and asexual development were investigated in *A. nidulans* (Figure 5). In *S. cerevisiae*, the septins *SPR3* and *SPR28* are expressed only during sporulation. However, all five *A. nidulans* septins showed expression during vegetative growth as well as asexual sporulation, although mRNA levels did fall during sporulation. It seems likely that sporulation-specific septin orthologs are present in *A. nidulans* but have not yet been uncovered by genome projects. Comparison of the expression profiles of septins from *A. nidulans* with orthologous septins from *S. cerevisiae* revealed an interesting similarity. On the basis of Northern analysis and frequency in the EST database, *aspB* and *aspC* are the most highly expressed *A. nidulans* septins in vegetative and conidiating cultures. And, on the basis of publicly available microarray data (Spellman *et al.* 1998), *CDC3* and *CDC12*, the *S. cerevisiae* orthologs of *aspB* and *aspC*, respectively, are also the most highly expressed septins. Further experiments are needed to clarify how much functional conservation exists among fungal septin orthologs. Newly available *A. nidulans* microarrays (Prade *et al.* 2001, this issue) should allow a more thorough investigation of septin expression. As originally suggested by Longtine *et al.* (1996), it does seem likely that conserved sequence domains among the septins are important for their ability to assemble into complexes, whereas the nonconserved sequence domains are involved in interactions with other proteins.

To identify putative *A. nidulans* septin-interacting proteins, we have exploited the recently developed genomic concept of "interlogs" (reviewed by Galperin and Koonin 2000). Interlogs are interacting proteins from one species whose orthologous proteins from another species also interact. In *S. cerevisiae*, several septin-interacting proteins have been identified through genetic and biochemical methods (reviewed by Longtine *et al.* 1996; Field and Kellogg 1999). Two mitosis-specific kinases from *S. cerevisiae*, Gin4p and Kcc4p, regulate cell cycle progression and localize to the bud neck in a septin-dependent manner. The *S. cerevisiae* chitin synthases Chs3p and Chs4p have also been shown to interact with septins in budding yeast. Using septin-interacting proteins from *S. cerevisiae* to query the *A. nidulans* EST database has allowed identification of several putative septin interlogs (data not shown). Interestingly, potential orthologs of Gin4p, Hsl1p, Chs3p, and Chs4p were all highly expressed in the *A. nidulans* EST library. Two-hybrid analysis and immunoprecipitation are being used to identify septin-interacting proteins in *A. nidulans*. It is expected that a directed identification of interlogs by a combination of computational and experimental methods will significantly accelerate studies of septin function in *A. nidulans*.

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