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MINIREVIEW

Septin Function in Yeast Model Systems and Pathogenic Fungi

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The septins were first discovered in the budding yeast *Saccharomyces cerevisiae* and were named for their role in cytokinesis and septum formation (69). Septins are now known to be highly conserved in fungi and animals, although absent in plants and many protozoans (e.g., *Plasmodium fasciculatum* and *Dictyostelium discoideum*). The septin proteins are characterized by presence of a distinct type of GTPase domain and by their ability to form filaments. The *S. cerevisiae* septin proteins form a series of 10-nm filaments that assemble into a ring on the inner surface of the plasma membrane at the bud neck. Septin rings are thought to function as a scaffold to recruit proteins to the bud neck and to act as a boundary domain to restrict diffusion during budding and cytokinesis (30, 39, 66, 68). However, septins are now implicated in a broad range of dynamic membrane events. In *S. cerevisiae*, septins have been found to also play a role in conjugation and sporulation. Moreover, analyses of septin function in other organisms, including fungi that undergo different patterns of growth and differentiation, are revealing new aspects of septin function. Therefore, we will provide an overview of the current understanding of the relatively well-studied roles of septins during *S. cerevisiae* budding and then use this as a context to review studies of septin function during other developmental pathways in *S. cerevisiae* and other fungi. The other fungi will include the model fission yeast *Schizosaccharomyces pombe* and two opportunistic fungal pathogens: the multimorphic *Candida albicans* and the filamentous fungus *Aspergillus nidulans*.

SEPTIN PROTEINS FORM FILAMENTS

The *S. cerevisiae* genome encodes seven septin proteins, five of which associate during vegetative growth to form a ring at the bud neck (Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7). Two other septins are only expressed during sporulation (Spr3 and Spr28). A common structural feature of all septins from yeast to man is the presence of a GTPase domain (Fig. 1) (21, 82). This domain is not highly homologous to the well-known Ras family of GTPases but does contain the characteristic motifs conserved in other GTPase domains (65). (However, as described below, not all septins appear to function as GTPases.) The GTPase domain is flanked on the N-terminal side by a short basic rich region and on the C-terminal side by

a conserved domain that is unique to the septin protein family. The region of basic amino acids is thought to mediate binding to phosphoinositide containing membranes, similar to what has been shown for a basic domain in the mammalian septin H5 (12, 115). Most *S. cerevisiae* septins also contain a C-terminal coiled-coil domain, although it is absent in Cdc10. The coiled-coil region appears to act together with the adjacent conserved domain to bind to other septins (106).

Septin proteins assemble to form a ring at the bud neck that correlates with the position of 10-nm filaments seen by electron microscopy (9, 33, 89). The 10-nm filaments are thought to be composed of septins, since purified septin proteins bind each other and form similar types of filaments in vitro (33, 106). Recent studies indicate that the five septin proteins produced in vegetative cells form a pentameric complex with the orientation shown in Fig. 2 (1, 106). Based on the ability of purified septin proteins to interact in vitro and their relative roles in septin ring formation in vivo, Cdc3, Cdc11, and Cdc12 are thought to form the main filament polymer, and Cdc10 is proposed to promote cross-linking. Shs1/Sep 7 is not essential for filament formation (10, 79) but plays a role in stabilizing the structure and is a target for regulation by phosphorylation (26). The septin proteins are indicated as dimers in Fig. 2 to signify that the individual septin proteins also interact in a homotypic fashion (106). Previous analysis of septin complexes purified from yeast showed a stoichiometry of 2:2:1:2 for Cdc3, -10, -11, and -12 (33), but other studies suggested that this might be due to loss of some subunits during purification (106).

Studies on other fungi have identified general similarities for septin filament assembly. For example, the *S. pombe* orthologs of Cdc3 (Spn1), Cdc10 (Spn2), Cdc11 (Spn3), and Cdc12 (Spn4) interact with each other in a manner similar to that described above for *S. cerevisiae* septins (1). Although there are some differences, such as the absence of an ortholog of Shs1/Sep7 in *S. pombe*, the relative importance of the septins for ring formation in vivo seems conserved. The orthologs of Cdc3 (Spn1) and Cdc12 (Spn4) play a central role in septin ring formation, whereas the Cdc10 ortholog (Spn2) was less important (1). Similarly, in *C. albicans* Cdc3 and Cdc12 are essential but Cdc10 is not (110). However, the *C. albicans* ortholog of Cdc11 was also not required for septin ring formation (although the rings were partially defective). The ability of *C. albicans cdc11∆* mutants to form septin rings is not compatible with the model of *S. cerevisiae* septin filament formation shown in Fig. 2. One possibility is that Cdc3 and Cdc12 can form filaments in vivo and that the other septins help to stabilize the filaments or recruit other proteins to do so. In this regard it is

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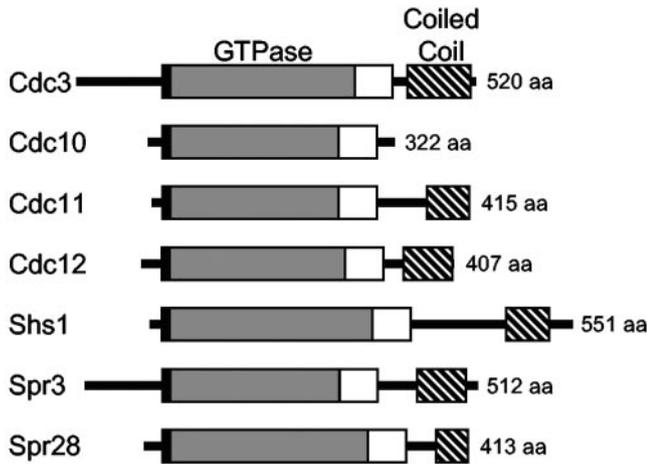
FIG. 1. Comparison of *S. cerevisiae* septin proteins. Diagrams of the *S. cerevisiae* septin protein structures are shown. ■, Region rich in basic amino acids that is implicated in binding to phosphinositide-containing membranes; ▨, GTPase domain; □, conserved domain characteristic of septins; ▧, coiled-coil domain. The length of each septin is listed after the protein diagram. aa, Amino acids.

interesting that the microsporidium *Encephalitozoon cuniculi*, an obligate intracellular parasite representing a very divergent branch of fungi with a compact genome, appears to contain only three septin homologs (57). Multicellular organisms can also get by on a small complement of septins; the *Caenorhabditis elegans* genome appears to contain only three septin genes (20).

REGULATION OF SEPTIN RING ASSEMBLY AND DISASSEMBLY DURING THE *S. CEREVISIAE* CELL CYCLE

The dynamic localization of septins during the cell cycle has made the septins both an interesting target for analysis of cell-cycle regulation and a useful landmark for monitoring cell
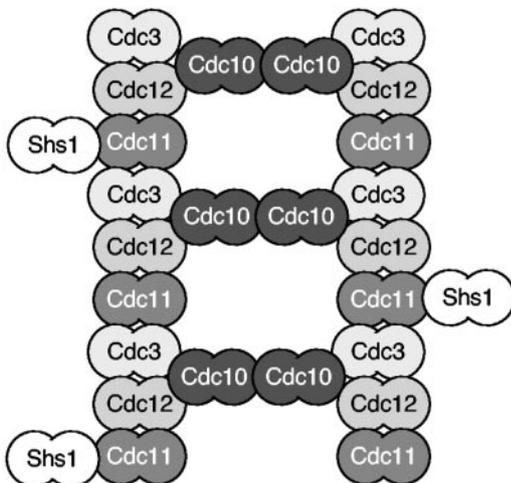

FIG. 2. Model for septin filament assembly. Recent students predict that septin proteins assemble into filaments based on a repeating structure of the five mitotic septins that assemble into a pentameric complex (1, 106). Septin proteins are indicated as dimers to signify that the septin proteins associate with themselves.
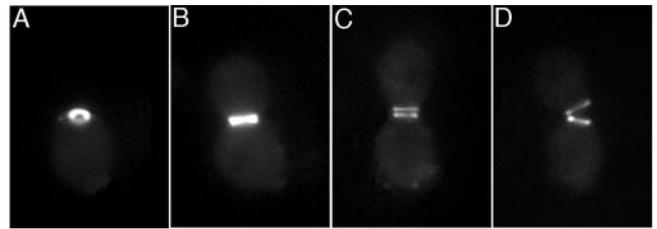

FIG. 3. Examples of septin ring structures during budding. Fluorescence microscope photographs of *C. albicans* producing Cdc10-GFP. Representative cells are shown as examples of ring formation (A), septin collar at junction between mother and daughter cell (B), ring splitting (C), and cytokinesis and ring disassembly (D).

cycle progression. Examples of septin localization during budding are shown in Fig. 3 for *C. albicans* cells to demonstrate that the patterns are identical to those observed in *S. cerevisiae* and are thus likely to be conserved in other budding yeasts.

In *S. cerevisiae*, a septin ring forms at the future site of bud formation about 15 min before the bud is observed to emerge (32, 42, 59, 69). As budding progresses, the septin ring extends into the daughter cell and forms an hourglass-shaped structure that is often described as a collar. The collar then changes from a fluid state, wherein the septin subunits are interchangeable, to a frozen state in which the septin subunits are stable during S, G_2, and M phase, as judged by FRAP (fluorescence recovery after photobleaching) (14, 26). Ultrastructural studies suggest the possibility that septin filaments may be more compact in the frozen state (89). Interestingly, the septins transiently become fluid again when the septin ring splits at the start of actomyosin ring contraction during cytokinesis (26, 67). The split septin rings then become frozen again and then later disassemble in order to reassemble at the next bud site in G_1. Thus, the alternating periods of fluid and frozen states are thought to permit the septin ring to alter its shape to match the changes in neck morphology during bud emergence and cytokinesis. The frozen state is thought to allow septins to form a stable structure that can act as a scaffold or as a barrier domain, as will be described in the following sections.

A key aspect of septin function is that ring formation is regulated during the cell cycle by assembly and disassembly rather than by synthesis and degradation. A large number of genes have been implicated in influencing septin regulation (30, 39, 68). Therefore, we will summarize below three aspects of septin regulation that have been relatively well studied: regulation by the Rho-family GTPase Cdc42, phosphorylation, and GTP binding. Some of the other proteins that bind septins and may regulate their function include Bni5, Iqg1, and Hof1 (18, 30, 40, 64).

Septin ring assembly. Septins are thought to initially form a patch at the future bud site that subsequently coalesces into a ring (14, 68, 106, 107). The role of the patch is not well defined, however, since it was discovered in mutants that are defective in forming septin rings. This has suggested to others that the patch may be an abnormal structure and not a true intermediate in septin ring assembly (40). Either way, Cdc42 plays an important role in septin ring formation, in addition to its other roles in promoting actin localization and polarized cell growth. Analysis of specific Cdc42 point mutations showed that Cdc42

carries out a special role in septin ring formation that is independent of its role in actin polarization (38). In particular, these studies indicated that cycles of Cdc42 GTPase activity are needed for proper septin ring formation. Cdc42 GTPase activity was also implicated by the phenotypes of mutants lacking the GTPase-activating proteins (GAPs) for Cdc42 (*rga1Δ*, *rga2Δ*, and *bem3Δ*) (14). Several possibilities have been suggested for the role of Cdc42 in septin assembly, including a direct role for Cdc42 in shuttling septin subunits to the ring (36). Alternatively, the Cdc42 GAPs themselves may act as effectors to promote septin organization, as was suggested by colocalization of the Cdc42 GAPs and septins and by the ability of overproduced GAPs to suppress septin mutant phenotypes (14). It is also possible that there is a downstream effector of Cdc42 that has not yet been discovered, since in mammalian cells the Borg proteins, which are downstream effectors of Cdc42, regulate septin assembly (50). In addition, it has also been proposed that the primary role of Cdc42 is to recruit the PAK kinase Cla4 to the future bud site and activate it to phosphorylate septins, as will be described next.

At least six protein kinases are thought to contribute to septin regulation. Cla4, a PAK kinase family member that binds to Cdc42, interacts with the septins and phosphorylates Cdc3 and 10 in vitro and possibly other septins in vivo (107). Septin phosphorylation by Cla4 appears to play a direct role in stabilizing septin filaments, since it coincides with the change to a frozen septin structure in vivo and because the septins remain fluid in *cla4Δ* cells (107). *cla4Δ* mutants form aberrant septin structures, including rings that move away from the bud neck. The Gin4 protein kinase also localizes to the bud neck and is thought to regulate septin function by phosphorylating at least one septin, Shs1/Sep7 (10, 26, 70). Interestingly, *gin4Δ* cells do not form a septin ring; instead, the septins are present as a series of bars across the bud neck that run parallel to the mother-daughter axis (39, 70). Similar bars are observed at the neck of mating pheromone-induced shmoos that are arrested in G_1 by the pheromone signal pathway. These results have suggested that Gin4 may stabilize the ring by promoting lateral association of septin filaments. Septins are also phosphorylated by the cyclin-dependent kinase Cdc28 to influence septin ring disassembly, as will be discussed below. Elm1, Cdc5, and Yck2 protein kinases have also been implicated in septin regulation, although the specific mechanisms are not clear, since they have other roles in morphogenesis.

GTP has long been implicated in septin function, given that all septins contain a similar GTPase domain. Analysis of septin proteins purified from yeast and other organisms confirmed that septins bind GTP and hydrolyze it in vitro (31, 33). Analysis of individual recombinant septin proteins showed that Cdc10 and Cdc12 display GTPase activity, but not Cdc3 or Cdc11 (106). In fact, Cdc3 and Cdc11 may not bind GTP. Cdc10 and Cdc12 mutants that block GTP binding caused a temperature-sensitive phenotype in vivo and block septin filament formation in vitro, indicating an important role for GTP binding as a prerequisite for filament formation (106). Mutations that prevent GTP binding did not impair the ability of septins to associate with each other into a pentameric complex, suggesting the role of GTP binding is to promote association of these complexes into filaments. GTP binding was also found to promote filament formation for septins from other organisms

(80). In contrast, *S. cerevisiae* septin mutants that block GTP hydrolysis did not affect septin filament assembly or disassembly, in vivo or in vitro (106). In addition, most GTP bound to septins does not appear to get hydrolyzed during the cell cycle (108). Thus, GTP binding to septins may play a structural role similar to the binding of GTP to α-tubulin rather than the role of GTP binding and hydrolysis in signaling by Ras (106, 108).

Septin disassembly. The mechanisms that trigger septin ring disassembly at the end of the cell cycle are not as well understood as those that promote assembly. One likely mechanism is the dephosphorylation of septins by the PP2A phosphatase bound to Rts1, a B′ regulatory subunit. PPA2^{RTS1} is thought to directly dephosphorylate the septins in part because it localizes to the bud neck after the Tem1 GTPase promotes spindle breakdown and septin ring splitting (25). Also, septin rings are stabilized in *rts1Δ* mutants (25). Interestingly, phosphorylation of Cdc3 by Cln-activated Cdc28 cyclin-dependent kinase in G_1 phase appears to play a role in septin disassembly (102). *cdc3-S1S2* mutants that lack the target sites for Cln-Cdc28 show a delay in septin ring disassembly. Other mechanisms may contribute in addition to phosphorylation/dephosphorylation. For example, Cdc42 localizes to the septin rings late in the cell cycle and may potentially affect septin filament disassembly. Septin modification by SUMO (Smt3), the small ubiquitin-like protein, was also implicated in disassembly (51). However, this now seems unlikely, since mutation of the E3 attachment factor (*siz1Δ*) blocked sumoylation of the septins but did not cause a delay in septin ring disassembly (52). Nonetheless, the observation that bud neck proteins other than the septins are SUMO modified in *C. albicans* suggests a conserved role for SUMO at the bud neck (75).

SEPTIN RINGS ACT AS A SCAFFOLD TO RECRUIT PROTEINS TO THE BUD NECK

The distinctive pattern of septin localization has facilitated the identification of about 40 proteins that associate, directly or indirectly, with the septins at the bud neck (18, 39). Thus, the septin filaments at the bud neck are thought to act as a scaffold for anchoring proteins. However, the septin scaffold is not a simple hitching post since proteins can be recruited to specific subdomains of the ring, and subsets of proteins are found to bind or dissociate from the ring at different stages of the cell cycle (18, 39). For example, Bni4 initially binds in G_1 to the mother cell side of the ring and then is later found throughout the ring (22). In contrast, Hsl1 binds to the daughter cell side (4, 71). Given the large number of septin-binding proteins, we will review below only three well-studied examples of protein networks that bind to the septins, including complexes involved in bud site selection, chitin ring deposition, and a cell cycle checkpoint.

Bud site selection. The process of bud site selection is an interesting example of how distinct types of protein networks can be recruited to the septins to specify the future site of cell polarization and bud formation. Each new bud in *S. cerevisiae* is positioned relative to the previous bud site in a specific manner. Haploid **a** or α cells bud in an axial manner adjacent to the previous division site, and diploid **a**/α cells bud in a bipolar pattern near the poles (16, 55). A central role of septin proteins in this process is indicated by the random budding

pattern of septin mutants (15, 93). Axial budding requires recruitment to the bud neck of the Bud3, Bud4, and Axl2/Bud10 proteins (11, 15, 93). It has been suggested by several groups that Bud3 and Bud4 may remain at the cortex after septin disassembly to define the position of the septin ring in the next cell cycle (11, 85). Bipolar budding is specified in part by persistent markers involving Bud8 at the pole distal to the birth scar and Bud9p at the proximal pole, along with the interacting integral membrane proteins Rax1 and Rax2 (55). Correct localization of Bud9 depends upon the septin proteins and the actin cytoskeleton, while proper targeting of Bud8p relies on other factors including actin and the formin Bni1 (16, 45, 55, 94). Proteins needed both for proper axial and bipolar budding are also recruited to septin rings, including the Bud5 guanine-nucleotide exchange factor (GEF) and the Bud2 GAP, that act on the Bud1/Rsr1 Ras-like GTPase (56, 72, 87).

Chitin ring formation. The formation of a chitin ring in the cell wall at the bud site provides an important example of how some proteins are recruited to the septin ring by intermediary proteins that bind to the septins. Just prior to bud emergence and coincident with formation of a septin ring at the incipient bud site in G_1, chitin deposition occurs in the cell wall to form a ring. The chitin ring remains throughout the cell cycle, ultimately marking the cortical site of division as the bud scar. Synthesis depends upon activity of Chs3 (chitin synthase III) and its localization to the proper site. Chs3 is tethered to the septin ring by a hierarchy of proteins involving the binding of Chs3 to Chs4, which then binds to Bni4 (22). Bni4 binds Cdc10 and is therefore thought to directly anchor this protein complex to the septin ring in vivo. Bni4 also binds to the Glc7 serine-threonine phosphatase and recruits it to the septin ring, which also facilitates interaction of Bni4 with septin proteins (63). The recruitment of Bni4 to the septin ring in G_1 is also interesting, because it indicates that the septins can act as a scaffold while they are in a fluid state.

Cell cycle checkpoints/sensors. Septins organize protein networks that sense the normal progression of bud morphogenesis and spindle position (18). The role of the septins in the spindle checkpoint has not been well defined (13, 18), so this section will focus on the bud morphogenesis checkpoint because it provides an interesting example of how proteins can be sequentially recruited to the septin ring (58, 66). Perturbation of bud morphogenesis was discovered to activate a checkpoint by stabilizing the Swe1 protein kinase, which then phosphorylates the cyclin-dependent kinase Cdc28, leading to arrest of cell division in G_2 (4, 66, 71, 78). Swe1 also regulates cell size in *S. cerevisiae*, similar to the function of its ortholog Wee1 in *S. pombe* (48, 58). Thus, Swe1 plays a key role in sensing the progression of bud morphogenesis (48, 58, 66, 90, 91). If bud morphogenesis proceeds normally during the cell cycle, components of the morphogenesis/size checkpoint are sequentially localized to the septin ring to promote phosphorylation of Swe1 and consequently its degradation. The first protein to be recruited is the Hsl1 protein kinase, which binds to the daughter side of the septin collar, and then recruits Hsl7 and Swe1 (4, 19, 71, 96). Binding of Hsl1 to the Cdc11 and Cdc12 septins activates its protein kinase activity, but Hsl1 does not appear to phosphorylate Swe1 directly (44). Instead, two other protein kinases, Cla4 and Cdc5, are targeted sequentially to the bud neck and directly phosphorylate Swe1 and appear to contribute

to its degradation via a ubiquitin-dependent pathway (92). Abnormal morphogenesis prevents the proper function of this regulatory module, leading to Swe1 stabilization and cell cycle arrest in G_2. The Swe1-mediated delay in G_2 contributes to the elongated bud morphology characteristic of septin mutants in *S. cerevisiae*. Recent studies suggest that the checkpoint may sense either the shape of the neck region or the transition of the septin ring from a fluid to a frozen state at the bud neck (which may only occur in cells undergoing proper morphogenesis) (66, 104).

SEPTIN RINGS FORM A BARRIER DURING BUDDING AND CYTOKINESIS

Bud neck barrier. Septins also contribute to cell polarization by mediating a barrier function that blocks diffusion between the daughter and mother cells. One line of evidence for this came from analysis of the integral membrane protein Ist2p, whose mRNA was discovered to localize to the bud tip (101). Interestingly, green fluorescent protein (GFP)-tagged Ist2 in the plasma membrane of the daughter cell was not able to diffuse across the bud neck to the mother cell. The asymmetric localization of Ist2p depends on the septins: GFP-Ist2p diffused into the mother cell when *cdc12-6* cells were shifted to the restrictive temperature to disrupt septin rings (101). Septins are peripheral membrane proteins, making it possible that the septin ring acts directly as a physical barrier to diffusion of integral membrane proteins, such as Ist2. However, septin barrier function is probably more complex, since septin rings are also required to maintain the polarization to the bud of actin patches, as well as exocyst and polarisome components such as Sec3p, Sec5p, and Spa2p (3). Septins were also reported to act as a boundary to restrict to the bud Lte1, the GEF for the TEM1 GTPase, that promotes exit from mitosis (13). In septin mutants Lte1 can diffuse into the mother cell, where it activates Tem1 and thereby promotes premature exit from mitosis prior to proper nuclear division.

Cytokinesis barrier. Later in the cell cycle the septin ring splits and acts as a boundary to trap between the rings some of the proteins involved in cytokinesis and septum formation (25). During cytokinesis, the polarisome component Spa2p, exocyst component Sec3p, and chitin synthase II (Chs2p) localize between the split septin rings, as does the actomyosin ring. Cytokinesis then proceeds by actomyosin ring contraction and the fusion of secretory vesicles to form the septum. However, shifting a *cdc12-6* septin mutant to the restrictive temperature to disrupt septin rings revealed that Spa2, Sec3, and Chs2 were rapidly lost from the bud neck, indicating that their diffusion was normally constrained by the split septin rings. In contrast, the actomyosin ring was maintained at the bud neck after the temperature shift. Although the septins were not required for actomyosin ring maintenance during cytokinesis or its subsequent contraction, septins were required for proper abscission (25).

SEPTIN FUNCTION DURING ALTERNATE DEVELOPMENTAL PATHWAYS IN *S. CEREVISIAE* (MATING AND SPORULATION)

Septin function during mating. *S. cerevisiae* cells conjugate when cells of the opposite mating type (a and α) signal each

other with secreted mating pheromones (27, 28). Mating pheromone-stimulated cells arrest in G_1 phase and then undergo polarized morphogenesis to form a conjugation bridge that connects the mating cells. Pheromone-stimulated cells are distinct from budding cells in that they form an acute projection of growth, which is commonly referred to as a shmoo. Interestingly, septins localize to the neck of shmoos even though these pheromone-arrested cells are not progressing through the cell cycle and do not undergo cytokinesis at this site (32, 59). However, several observations suggest that septins carry out distinct roles during mating. For one, septins at the shmoo neck do not form a tight ring as seen during budding and instead form a more diffuse ring that appears to be composed of a series of bars that run parallel to the long axis of the cell (70). These septin bars are similar in appearance to the septins in budding cells that lack Gin4 (gin4Δ). Also, the septin bars function differently than rings, since Gin4 does not localize with septins in mating cells, even though it is present (70).

Septins are thought to act as a scaffold during mating because they are required to localize Afr1 (35, 62) and Glc7 (8) to the shmoo neck, and they are required for the proper chitin localization at this position. Afr1 may bind directly to the septins, since it interacts with Cdc12 in the Two-Hybrid Assay. Afr1 is required for the proper formation of an acute projection of shmoo morphogenesis (23, 35). Localized chitin deposition at the neck of the mating projection also requires the function of septins and Afr1 (62). Interestingly, the C terminus of Afr1 is highly similar to the C-terminal region of Bni4 that binds to Glc7 and recruits it to bud necks, suggesting that Afr1 plays an analogous role in recruiting the Glc7 phosphatase to the neck of shmoos.

The septins may also act as a barrier at the neck of shmoos to prevent diffusion of factors away from the tip. Polarization of mating pheromone receptor signaling to the shmoo tip is thought to help stimulate highly polarized growth of mating projections to form the conjugation bridge (49). Receptor polarization results from a combination of the removal of old receptors by ligand-induced endocytosis, along with the targeting of new receptors to the shmoo tip by the secretory pathway. The septins may help to polarize pheromone signaling by acting as a barrier to prevent diffusion away from the tip of receptors and possibly other pheromone pathway components. The localization of Afr1 to the septin ring at the shmoo neck is thought to further narrow the active zone of pheromone signaling to the shmoo tip, since Afr1 can negatively regulate receptor signaling (23, 35). Consistent with this model, mutation of either *AFR1* or the septins results in cells that form a broad shmoo tip with a more diffuse localization of receptors. It is also possible that septins could play a role as a barrier during cell fusion and zygote formation by creating a special zone in the conjugation bridge. However, septin mutants do not display a significant mating defect.

Septin proteins display unique localization during sporulation. In *S. cerevisiae*, nutrient limitation stimulates diploid a/α cells to undergo DNA replication and meiosis that results in development of four haploid spores within a sac-like ascus derived from the mother cell. This process is quite different from budding in that there is no equivalent of the bud neck or septation site. Instead, the spore membranes result from de novo synthesis of membrane around the postmeiotic nuclei.

Septins were first implicated in sporulation because transcription of *CDC3* and *CDC10* is increased more than tenfold, and expression of the sporulation-specific septins, *SPR3* and *SPR28*, is highly induced late in meiosis at the time of spore development (17, 24, 29, 86).

Septins are not observed at the plasma membrane of sporulating cells and are instead first observed in meiosis II when chromosomes divide into four nuclear lobes. Cdc3, Cdc11, Spr3, and Spr28 were detected in ring-like structures surrounding each of the four spindle pole bodies (24, 29). However, these rings are distinct from those seen during budding. They initially form at the leading edge of prospore membrane and then achieve a broader localization. As the prospore membrane elongates and expands along the outer surface of each nucleus, septins are detected in sheets that appear as pairs of bars in optical sections (24, 29, 99). Upon closure of the prospore membranes around each nucleus, the septins then display a generally uniform distribution around each nascent spore. The septins appear to colocalize with each other in the prospore membranes, and the localization of Cdc3 and Cdc11 was less intense in *spr3Δ* cells, suggesting the septins form a complex (29). However, Spr3 did not detectably localize at bud necks when expression of *SPR3* was engineered in vegetative cells. Further work will be required to determine the regulation of septin protein interactions during sporulation.

Septins appear to act as a scaffold during sporulation. The Glc7 phosphatase is recruited to the septins by Gip1, a targeting subunit, which is only expressed in sporulating cells (99). Interestingly, Gip1 and Glc7 are essential for the organization of septin complexes, although it is unclear whether this is due to a direct dephosphorylation of septin proteins (99). Thus, an interesting similarity between budding and sporulating cells is that they each have a unique protein that targets Glc7 to the septins; Bni4 in budding cells and Gip1 in sporulating cells. This similarity may also extend to mating cells since Afr1 shows similarity to the domain of Bni4 that binds Glc7 and thus may recruit Glc7 to the septins in mating cells. Septin localization to the spore periphery after prospore membrane closure has been proposed to act as a scaffold for recruitment of factors that contribute to spore wall formation within the lumen of the prospore double membrane (29).

Although these studies implicate septins in spore formation, septin mutant strains display no significant sporulation phenotype. *spr3Δ*, *cdc10Δ*, and double mutant *spr3Δ spr28Δ* strains exhibited little or no reduction in sporulation efficiency (24, 29, 99). It has been proposed that the lack of severe sporulation defects upon septin gene deletion may be due to "functional redundancy" among the septin proteins (24).

SEPTIN FUNCTION IN *C. ALBICANS*, *S. POMBE*, AND *A. NIDULANS*

The essential role of the septins has lead to their study in other fungi, including *S. pombe*, *C. albicans*, and *A. nidulans*. A common feature of these organisms is that the septins assemble into a ring that functions in cytokinesis. However, the study of these other fungi is also providing new insights into how septins are regulated during different cell cycle programs as *S. pombe* divides by fission, *C. albicans* grows in different morphologies ranging from buds to filamentous hyphae, and *A.*

nidulans is a filamentous fungus. In addition, these studies are revealing new aspects of septin function in developmental pathways not seen in *S. cerevisiae*, such as the formation of chlamydospores in *C. albicans* and asexual conidiospores in *A. nidulans*. Studies on *C. albicans* and *A. nidulans* are also significant in that they are expected to help to identify mechanisms underlying the ability of fungal pathogens to grow invasively in human hosts.

Septins in *S. pombe*. *S. pombe* undergoes a very distinct type of cell division from *S. cerevisiae* in that it divides by fission rather than by budding. Consistent with this, septins are not localized at early stages in the cell cycle and do not appear to contribute to morphogenesis (1, 6, 103). Later in the cell cycle at anaphase, the septins form a ring around the inner surface of the plasma membrane at the medial region of the cell, marking the future site of cytokinesis. The general behavior of the septins at this stage appears to be similar to what happens in budding yeast cells in that assembly and disassembly of the septin ring is regulated during the cell cycle. However, septins are not needed for cytokinesis; deletion of septins appears to cause primarily a delay in cell separation (1). The localization of septins to the septation site late in anaphase is similar to what was observed in animal cells (60), suggesting *S. pombe* may be a good model system for understanding septin regulation in animals.

The *S. pombe* genome contains seven genes that are homologous to the septins in *S. cerevisiae*. Four of the septins (Spn1, Spn2, Spn3, and Spn4) function during vegetative growth and appear to be orthologous to the key septins in *S. cerevisiae* (Cdc3, -10, -11, and -12, respectively) (1). For example, Spn1 and Spn4 are required to form a septin ring in vivo just as Cdc3 and Cdc12 are essential for *S. cerevisiae*. Additional analysis of the localization of septins in vivo, together with biochemical studies of the ability of different septin proteins to interact, suggests that the *S. pombe* septins associate in a manner similar to the *S. cerevisiae* septins (1, 6, 103) (See section above on septin proteins forming filaments.) *S. pombe* does not appear to contain an ortholog of Shs1/Sep7, which is a target for regulation by phosphorylation in *S. cerevisiae*, indicating that future studies will likely identify significant differences in the regulation of *S. pombe* septins by phosphorylation. The remaining three *S. pombe* septin genes (*spn5*, *-6*, and *-7*) are induced during sporulation (77), but their role in this process has not been reported.

Although initial studies identified many similarities in septin function between *S. pombe* and *S. cerevisiae*, there are also several significant differences. For example, the anillin homolog Mid2 colocalizes with septins in *S. pombe* and is also required for septin ring formation (6, 103). In contrast, the similar septin-binding proteins Bud4 in *S. cerevisiae* and Int1 in *C. albicans* colocalize with septins in their respective organisms, but they are not required for ring formation. However, recent studies have implicated Bud4 in contributing to proper septin organization in *S. cerevisiae* (37). (It should also be noted that the low level of sequence identity of Mid2 with these other proteins makes it unclear that their functions are orthologous.) Mid2 also influences septin ring disassembly, since overproduction of Mid2 leads to a delay in septin ring disassembly (6, 103). This indicates that Mid2 degradation, which is regulated by the Skp1/Cdc53/F-box (SCF)-dependent proteol-

ysis, facilitates septin disassembly in *S. pombe* (6, 103). Another difference between these organisms is that *S. pombe* septins are not involved in recruiting the ortholog of Gin4 (Cdr2), which in *S. pombe* functions independently of the septins (83). Thus, further differences in septin regulation are likely to be found in future studies of *S. pombe*.

Septins in *C. albicans*. In response to different environmental stimuli *C. albicans* will form either rounded buds, elongated chains of cells known as pseudohyphae, or long filamentous cells with parallel cell walls known as hyphae (97). In addition, certain low-nutrient conditions induce *C. albicans* to form chlamydospores, which are thought to be asexual resting spores. *C. albicans* has not been observed to undergo meiotic spore formation. Studies on the regulation of *C. albicans* morphogenesis are important for understanding the mechanisms of fungal pathogenesis, since the ability to switch between budding and hyphal cells has been linked to virulence. The budding and hyphal growth phases differ in the production of virulence factors, the ability to grow invasively, and the ability to escape the immune system in human hosts (7, 112).

The budding phase in *C. albicans* shows general similarity to *S. cerevisiae* with some key differences. Five septins form the septin ring at the bud neck that are orthologous to Cdc3, -10, -11, and -12 and Shs1/Sep7 (34, 54, 98, 110). Deletion analysis indicates that the overall contribution of most septins is similar; *CDC3* and *CDC12* are essential, and *SHS1*/*SEP7* plays a relatively minor role (110). Deletion of *CDC10* and *CDC11* resulted in defects in cytokinesis and spindle orientation but, in contrast to *S. cerevisiae*, these septins were not essential for growth, even at 42°C. The observation that *cdc11Δ* cells still form a septin ring, even at elevated temperatures, contradicts current models that propose an essential role for Cdc11 in filament formation (1, 106). This suggests either that the current models are wrong or that *C. albicans* septins are organized in a different manner or are stabilized in vivo by different factors. The higher thermostability of *C. albicans* septins may relate to this organism being adapted for growth at 37°, the temperature of its host. Septins act as a scaffold to recruit proteins to the bud neck, including the *C. albicans* homologs of Hsl1, Gin4, Int1, and Bni4 (34, 73, 105, 113). However, the role of the bud neck proteins may be altered. For example, *C. albicans* lacks a homolog of Hsl7 that in *S. cerevisiae* binds to Hsl1 and is important for its function. Septins are not detectably modified by SUMO (Smt3) as they are in *S. cerevisiae*, but they do act as a scaffold to recruit SUMO-modified proteins, suggesting a role for SUMO in regulation of bud neck proteins (75).

The role of septins in pseudohyphae is thought to be similar to budding, since in both cases a septin ring forms at the junction with the mother cell. In contrast, hyphal cells form the septin ring about 10 μm away from the junction with the mother cell (see below). Thus, GFP-tagged septins can be used to help distinguish between pseudohyphal and hyphal cells (34, 97, 98) and can also be used to analyze septin structures in *C. albicans* cells taken from infected mice (41).

C. albicans hyphae are commonly seen at sites of infection, and their formation can be induced in vitro at 37°C by various stimuli, such as serum. Three types of septin localization were observed in hyphae:

(i) A classic septin ring forms in the initial protrusion of

hyphal growth (known as a germ tube) about 10 to 15 μm from the mother cell and also at subsequent sites of cell division as the hypha elongates (34, 98, 110). This ring functions similarly to the bud neck ring to promote septum formation. Hyphae are distinct from budding cells in that the previous septin rings are not fully disassembled, particularly on the mother cell side, after septation. An interesting possibility is that the subapical cells arrest at a stage prior to the signal for septin ring disassembly, since these subapical cells lag before beginning a new cell cycle.

(ii) A diffuse ring of septins was detected at the junction between the mother cell and the germ tube (98, 110). This basal septin band is regulated differently from the septin rings at sites of cytokinesis because it still forms in a *gin4*Δ strain, whereas septin rings do not (113). It is not clear that the basal septin band acts as a scaffold, since Gin4 and Int1 do not localize to this region, but the basal band may have other functions as described below. The basal septin band appears to be similar to the septin localization in pheromone-induced shmoos in *S. cerevisiae* (70). Thus, shmoo formation may be a good model for aspects of germ tube formation in that both processes can occur in G_1, whereas budding initiates as cells enter the S phase of a new cell cycle.

(iii) A faint cap of septins was also detected at the leading edge of growth in germ tubes and hyphae (98, 110). Interestingly, this localization coincides with an ergosterol-rich region of the plasma membrane at hyphal tips that was identified by filipin staining (74). A similar ergosterol rich domain was also seen at shmoo tips in *S. cerevisiae* and at hyphal tips in *A. nidulans* (2, 88). There may be a general connection between septins and the organization of ergosterol in the plasma membrane, since filipin staining coincides with septin rings during cytokinesis in *C. albicans* hyphae and in *S. pombe* (74, 100). Thus, septins may play a role in cell polarization by facilitating the organization of specific plasma membrane domains, such as sterol-rich lipid rafts.

C. albicans *cdc10*Δ and *cdc11*Δ mutants displayed abnormalities in hyphal growth in addition to defects in septum formation that suggest that septins in the basal band and at the hyphal tip contribute to proper morphogenesis (110). Both *cdc10*Δ and *cdc11*Δ mutants form hyphae that are more curved than the relatively straight hyphae formed by wild-type cells. The sites of extreme bending or curvature are associated with altered Calcofluor staining of cell walls, suggesting that the septins may play a direct role in promoting even hyphal growth. The *cdc10*Δ and *cdc11*Δ mutants were also defective in selecting sites of secondary germ tube formation. Wild-type cells typically initiate a second germ tube at a distal site so that the two germ tubes form at an angle of >90° apart on the mother cell. In contrast, *cdc10*Δ and *cdc11*Δ mutants often formed a secondary germ tube adjacent to the initial hypha and in some cases from within the hypha. This may be related to the defects in bud site selection seen for septin mutants in *C. albicans* and *S. cerevisiae*. Although the septin mutants form hyphae, they were defective in invasive growth, both in vitro in agar and in vivo in a mouse model of *Candida* infection (109). The *cdc10*Δ and *cdc11*Δ mutants grew to high levels in kidneys of infected mice but did not cause a disseminated infection, as did the wild type. Instead, the septin mutants formed large clumps of fungal cells that were surrounded by lymphocytes. Thus, it may not be

necessary to completely block hyphal formation to have significant effects on preventing the spread of *C. albicans* infections.

Analysis of chlamydospore formation identified a novel pattern of septin localization. Chlamydospores are large thick-walled cells whose role in infection is unclear, but they act as a resting form in other species. During chlamydospore morphogenesis, cells switch to filamentous growth and then develop elongated suspensor cells that in turn give rise to chlamydospores. The *cdc10*Δ and *cdc11*Δ mutants were defective in forming chlamydospores, primarily because of a failure to undergo septation (73). Interestingly, analysis of GFP-tagged septin proteins in chlamydospores revealed that, after septation, the septins are present throughout the plasma membrane in a series of filamentous structures that run parallel to the axis of the chlamydospore-suspensor cell junction. This peripheral localization of septins is reminiscent of septin localization in meiotic spores in *S. cerevisiae* and suggests that septins may play a role in the formation of the specialized cell walls observed in these cell types.

Septins in *A. nidulans*. *A. nidulans* provides an interesting system for comparative analysis of septins, since it undergoes distinct patterns of cell division characteristic of many filamentous fungi and can also form aerial hyphae that produce asexual conidiospores (46). These studies also have application to fungal pathogenesis, since *A. nidulans* is an opportunistic pathogen, and it is related to the most common filamentous fungal pathogen of humans, *Aspergillus fumigatis*.

Five septin genes, *aspA* to *-E*, were identified in *A. nidulans* (82). Comparison of the predicted protein sequences indicates that four septins—AspA, -B, -C, and -D—correspond to the key septins in *S. cerevisiae* and appear to be orthologs of Cdc11, -3, -12, and -10. As predicted for an ortholog of *CDC3*, *aspB* is an essential gene (111). The remaining septin-like protein, AspE, does not show strong similarity to any of the *S. cerevisiae* septins, but all five septins are expressed during vegetative growth (82). There do not appear to be any sporulation-specific septins.

A unique aspect of septin localization in *A. nidulans* is that the timing of septin ring formation varies, depending on the type of cell division. Germination of a spore results in the growth of a germ tube that elongates without septation until a critical size is reached. After this size threshold is passed the next mitosis triggers formation of the first septum (46, 47, 114). Comparison of the timing of septin localization and nuclear division indicates that septins localize postmitotically (111). Thus, *A. nidulans* is similar to *S. pombe* in forming a septin ring late in the cell cycle. After the septin ring splits, the ring on the apical side persists and may act as a marker of cell polarity. In contrast, the timing of septin localization was different during formation of a secondary germ tube from the conidiospore or when branching occurs and a filament emerges from a subapical cell within a hypha. In these latter cases, a septin ring was observed before mitosis, similar to the timing of septin ring formation in *S. cerevisiae*. An *aspB* temperature-sensitive mutant demonstrated hyperbranching when shifted to the restricted temperature, suggesting septins might also recruit components that regulate induction of mitotic events (111). It will be interesting to determine how cell cycle regulation of septin localization is controlled in these different cell types. Another interesting question is how the sites of branching and

secondary germ tubes are selected, since the bud genes are not conserved.

Septins also localize to cell junctions during the formation of the conidiophore, the specialized structure that bears asexual conidiospores (81, 111). During this developmental process, the tip of an aerial hypha becomes swollen and then gives rise to two layers of small bud-like cells, the metulae and phialides. The conidiospores themselves emerge from the second layer (the phialides) in a budding process. Immunofluorescence localized AspB to a diffuse band at the base of each forming metula, which disappeared as the structure elongated (111). Additional AspB rings were observed at the interfaces of metulae and developing phialides, which similarly were no longer seen as the phialides matured, and septins were also present at the interface of each phialide and emerging conidiospore. An *aspB* temperature-sensitive mutant exhibited a lack of metulae formation when shifted to the restrictive temperature, suggesting that AspB may function as a scaffold or barrier to direct specific components to sites within the developing conidiophore (111). *A. nidulans* septins are therefore suggested to act as an "organizational scaffold" that targets specific components to designated sites at different points in development.

EXPANDING ROLES OF SEPTINS: FROM YEAST TO HUMANS

The yeast bud neck septins have provided a valuable model for the ability of septins to form filaments, act as scaffolds, and create diffusion barriers. Analysis of septins in other developmental pathways, and in fungi with distinct modes of cell division, has identified many common aspects of septin function. The study of septins in these other contexts is also identifying many critical differences that are broadening our knowledge of different types of septin organization and functions. A further example of this is the new insight into septin function coming from the analysis of septins in animal cells. Many septin functions are conserved in animal cells, such as the ability of GTP binding to promote septin filament formation, the role of septins in cytokinesis, and a likely role for septins in a diffusion barrier at the cleavage furrow of dividing mammalian cells (60, 95). Studies on fungal septins may therefore prove to be a good model for understanding the role of septins in human disease; septin mutations have been implicated in cancer, neurodegenerative conditions, and other pathologies (43). Similarly, the studies on animal cells are also helping to identify novel septin functions that are not seen in budding yeast, such as a role in regulated secretion (5, 76). In animal cells it is also interesting that actin, together with the adaptor protein anillin, acts as a template for septin filament assembly (61). The relationship between actin and septins in fungal cells is not well defined, but an anillin homolog (Mid2) promotes septin organization in *S. pombe* (6, 103) and several recent studies in *S. cerevisiae* also indicate that actin influences septin assembly (53, 84). Thus, the synergism of research from yeast to humans will aid future studies on the septin family of proteins.

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