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Septins and Generation of Asymmetries in Fungal Cells

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Abstract

Polarized growth is critical for the development and maintenance of diverse organisms and tissues, but particularly central to fungi where nutrient uptake, communication, and reproduction all rely on cell asymmetries. To achieve polarized growth, fungi spatially organize both their cytosol and cortical membranes. The septins, a family of GTP-binding proteins, have emerged as key regulators of spatial compartmentalization in fungi and other eukaryotes. By forming higher-order structures on fungal plasma membranes, septins are thought to contribute to the generation of cell asymmetries by acting as molecular scaffolds and forming diffusional barriers. Here we discuss the links between septins and polarized growth and consider molecular models for how septins contribute to cellular asymmetry in fungi.

Keywords

Asymmetry; Septins; Diffusion Barrier; Scaffold; Polar Growth

Introduction

The establishment and maintenance of cell asymmetries is necessary for polarized cell growth, cell-to-cell communication, transport, and many other biological functions critical for cells across the kingdoms. Asymmetries are particularly important phenomenon for fungal cells, which are often immobile and must achieve polarized growth to obtain nutrients, communicate with other cells, reproduce and thus polarity is essential for spore germination, germ tube extension, branch formation, hyphal extension and mating projections (12, 46). Polarity establishment in filamentous fungi requires that new compartments arise from within a large, streaming cytoplasm and multiple sites of cell polarity coexist (96). Septins, a family of GTP-binding proteins, are central to the development of cell asymmetry and define cell shape from yeast to mushrooms.

Discovered by Lee Hartwell in 1971 during a screen for temperature sensitive cell division mutants in *Saccharomyces cerevisiae*, septins are critical contributors to cytokinesis, membrane remodeling, scaffolding, and potentially other cell functions (33, 56, 62, 78). Over the ensuing decades septins were named and localized to the mother-bud neck by John Pringle and colleagues and it became clear that septins form higher order structures to function (39, 55). The assembly and organization of these septin higher order structures

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requires the GTPase, Cdc42 (48, 60). Conserved across eukaryotic species, except higher-order plants, septins assemble non-polar, heteromeric rods from 2–5 different proteins (varying in number among species) (Figure 1A) (2, 7, 15, 36, 40, 61, 108). In *S. cerevisiae* these are comprised of the four core septins (Cdc3, Cdc10, Cdc11, and Cdc12) and the nonessential septin, Shs1. These non-polar rods further assemble end-on in order to form filaments, and ultimately a variety of higher-order structures (8, 13, 89, 92).

Septin filaments are joined into diverse higher-order assemblies in different fungi, including rings, bars, and patches on membranes at sites of growth and division (Figure 1). In *S. cerevisiae* septins localize to the site of polarized growth as a ring, which transitions into the hourglass (mother-bud neck), and then just prior to cytokinesis the hourglass splits into a double ring (Figure 1B) (39, 55, 68). Similar assemblies both in terms of shape and dynamics are also seen in the yeast form states of dimorphic and filamentous pathogenic fungi, including *Candida albicans*, *Cryptococcus neoformans* and *Ustilago maydis* (1, 53, 72, 109, 120). During filamentous growth of many hyphal-forming species, septins localize diffusely as bars at the base and along hyphae, and are enriched at hyphal tips; these assemblies demarcate future septa as well as forming puncta and cables in cytosol (Figure 1C and D) (5, 58, 59, 64, 86, 119). How the size and shape of different kinds of septin assemblies is determined is still unclear in most contexts with the exception of some aspects of ring formation in *S. cerevisiae* (discussed below). Variation in form and function could arise from post-translational modifications to septins, the specific septin subunits present or local membrane composition. Filamentous fungi are important model systems for determining how morphologically distinct septin assemblies form.

Although septins are frequently found associated with polarized structures, the degree to which septins generate, stabilize and/or recognize cell polarity is still under investigation. Several models have been proposed which could explain how the septin cortex promotes the asymmetric distribution of proteins and lipids in the cell. Septin assemblies, in some way, prevent the free diffusion of lipids, lipid organizers and proteins along membranes and also act as scaffolds to recruit proteins to specific locations at specific times (50, 81). Both scaffold and membrane organization functions may depend on the local plasma membrane lipid composition and post-translational modifications of septins. Our goal in this review is to discuss how septins contribute to cellular asymmetries in fungal cells in light of how their roles as scaffolds and membrane organizers impart function.

How septins pattern and shape cells

The obvious first step in generating polarized cell growth is choosing a site or direction. There can be landmark-based sites which are pre-specified locations of growth or new sites can be determined stochastically through symmetry-breaking mechanisms. Studies so far support that septins are central to positioning landmarks (18, 38, 66, 99, 100). This is most well established in *S. cerevisiae*, where depending on ploidy, cells will place new buds adjacent to (axial) or at the opposite pole from the previous bud location. At cytokinesis, septins split into a double ring, so the mother and the daughter cells each inherit one half of the original septin ring. These septin rings then act as a scaffold for landmark proteins such as Bud4, a fungal anillin-like protein (65, 66, 99, 125). The association between Bud4 and

septins is not as straightforward as septins simply tethering Bud4, but instead there is clearly a conserved reciprocal relationship. In *S. cerevisiae*, Bud4 is required for normal septin organization and the splitting of the septin ring; the very process Bud4 itself relies on for segregation to each cell after division (32, 65, 66, 99, 123, 125). The synergistic links between Bud4 and septins are conserved across a variety of fungi including *S. pombe*, where the Bud4 homolog, Mid2 acts to stabilize the septin rings during cytokinesis similar to that in budding yeast (6). Likewise Int1, the *C. albicans* homolog, affects axial bud site selection and interacts with septins (41) and in both *Aspergillus* and *Neurospora* Bud4 homologues control septation (63, 105). Similarly, another bud-site selection protein called Ax12, has been shown to both promote polarity site selection via septins and have a conserved ability to regulate septin organization (3, 43, 105). This indicates an ancient interdependence between septins controlling the landmarks of polarity and at the same time also relying on these factors for their own organization. Why site selection machinery simultaneously uses and regulates septin organization is not clear but may indicate that the evolutionary origins of polarity site selection were in factors that were localized to and involved in septin organization.

In addition to specifying the site of polarity by scaffolding landmarks, septins in some fungi actually limit the number of polarity sites through an unknown mechanism. For reasons that are not yet clear, loss of septins in some filamentous fungal contexts can result in the emergence of excess polarity sites. This can be readily seen in *A. nidulans*, where deletion of AspB (Cdc3 homolog) resulted in a dramatic increase in germ tubes and branching (93). Similarly in another filamentous fungus, *Neurospora crassa* septin mutants show an increase in germ tube emergence and hyper-branching in mature colonies (5). Additionally, a *cdc12-6* allele in *C. albicans* led to the generation of a second germ-tube adjacent to the primary germ-tube which is never observed in wild-type cells (76). These results suggest a conserved septin function in limiting the number of polarity sites in branching hyphal cells with multiple sites of polarity in one cytoplasm, possibly through a negative feedback mechanism that inhibits the activation of the polarity machinery. Perhaps this functions in a way analogous to how septins fine-tune the location of the bud by scaffolding the Cdc42 GAP Rga1 at the end of the cell cycle to ensure cells do not “reuse” the previous bud site and instead bud adjacent to the site (116). Our own work examining septins by TIRF microscopy in *A. gossypii* indicates that in addition to organized septin rings, the cortex also has many individual septin filaments and bundles that had previously been unappreciated with conventional imaging (13). Perhaps these arrays provide an inhibitory role for branching? Hyperpolarized hyphal cells may be especially useful systems for uncovering ways by which septins influence negative feedback programs needed to limit polarity establishment.

Septins clearly can inform the position of polarity sites but do they influence the emergence and maintenance of polarized growth? In *S. cerevisiae*, septin mutants can still form a well-polarized bud with a failure in cytokinesis (70, 79). Septin mutants however, do show subtle morphological defects. While the base of the bud neck forms a curved hourglass in normal cells, septin mutants display a cylindrical neck as a result of misdirected growth suggesting the insertion of new membrane at these sites is in a septin-dependent manner (48). In the absence of well-organized septins, *S. cerevisiae* cells have difficulty tracking pheromone gradients and thus this could be considered a function in the persistence of polarity in mating

projections (67). Similar to budding yeast, many other fungi display abnormal morphologies in the absence of normal septins but still display polarized growth. In the dimorphic fungi *C. albicans*, the deletion of non-essential septins Cdc10 and Cdc11 results in curved hyphae and defective cell wall deposition. Likewise, while superficially septin mutants in the pathogenic fungi *Ustilago maydis* display a loss in cell polarity, this is a result of defective cell wall deposition and the shape can be restored in presence of sorbitol (1). Septins thus are not essential for targeted exocytosis needed to build a bud or hypha but they clearly can influence the precise shape of the protrusion. However, the functions may not be so clear in more complex morphologies such as mushroom formation where the septin Cdc3 is needed for the extreme polarity needed for stipe cell elongation in *Coprinopsis*. It is possible in highly robust and rapidly polarized growth, septins become critical to driving polarity and not simply regulators of site-choice and fine-tuning of shape (104).

Polarized growth and control of septin assembly

There are conserved roles for septins in choosing polarity sites, but once the site is picked how does the ensuing establishment of polarity impact septins? Septins require Cdc42 signaling and nucleotide hydrolysis cycles in order to form stable and functional assemblies (17, 49, 60, 91). There are many Cdc42 effectors implicated in some way to either recruit septins to the correct site and/or to lead to the formation of a stable ring, however precisely what molecular contributions specific effectors are making to septin organization has not been easy to tease apart (51). Work from the Lew and Bi labs showed that the GAPs of Cdc42 were required for normal septin ring assembly and that Cdc42 needed to cycle between GTP- and GDP-bound states. However, for over a decade it has not been clear why Cdc42 nucleotide hydrolysis cycles contribute to septin assembly (17, 47). Similarly, the Cdc42 effectors Gic1/2 have a role in septin organization but are also critical for the association between Cdc42 and the formin Bni1. As septin assembly can be delayed in the absence of actin (but does not require actin) it was not clear if the Gics played a direct role in septins assembly (20, 60). Importantly, the Gic effectors are conserved through humans, where the orthologues are called Borgs, indicating this is likely an evolutionarily conserved player in assembly. A mechanism linking Cdc42 nucleotide cycling, the Gics and septin assembly was suggested by structural studies with electron microscopy and purified proteins from the Farkasovský and Raunser groups (97). They showed that Gic1 can bundle septin filaments and that when Cdc42-GTP binds Gic1, it can prevent Gic1 binding to septins. These experiments suggested that Cdc42-GTP-Gic1 complexes might regulate the lateral association of septin filaments. Additionally, Cdc42-GDP could also influence septins but in this case led to septin filament disassembly. Thus, this work points to distinct functional consequences of Cdc42 being in GTP-vs. GDP-bound states and it is possible that cycling between the two states allows for an iterative process of ring sculpting by both effectors and Cdc42 for ring assembly.

How can we put this *in vitro* data in the context of septin ring assembly *in vivo*? Using Gic2 CRIB-domain as a reporter of active Cdc42, it was shown that shortly after Cdc42 recruits septins, the active pool of Cdc42 moves to an adjacent territory likely in part due to the recruitment of the Cdc42 GAP Rga1 to septins. Thus a Cdc42 GAP-based negative feedback zone, similar to what ensures a bud does not form inside an old site, is implicated in shaping

the septin ring (88). This study suggested that areas in the incipient bud site with assembled septins are areas of limited exocytosis due to Cdc42 inhibition by septins, but as further Cdc42 activity is achieved, increased exocytosis may be able to “punch” a hole in the zone of septins building a ring. It is alternatively/additionally possible that spatially discrete Cdc42-GDP and Cdc42-GTP/Gic zones are pruning nascent septin assemblies or complexes with Shs1 (which *in vitro* promotes rings) are joining the assembly in such a way as to promote the formation of a ring shape (44). Dynamic studies *in vitro* of the role of Cdc42, Gics and Shs1 in shaping septins should prove useful in the future to unify the *in vitro* and *in vivo* data.

Once a septin ring is formed, it has been suggested that the assembly then traps Cdc42 and restricts the zone of bud emergence to the center of the ring (88, 90). Recent work linking the septin-associated kinase Gin4 to regulating local membrane composition, specifically levels of PE through inhibition of the flippase Fpk1, present a possible means of limiting the zone of activity of the Cdc42 to the center of the ring (24, 94, 98). The connections between septins, Cdc42, lipid composition and cell morphology are observed throughout eukaryotes. Future work will surely disentangle the regulatory links in the Cdc42-septin complex and the relative roles of septins as a scaffold and as a membrane organizer in the process of establishing cell polarity.

Septin function in transmitting polarity cues to organelles

In addition to contributing to polarity site choice, septins themselves persist through the cell cycle as markers for different organelles to sense and orient along the polarity axis. At the level of the nucleus, septins are required for controlling both the nuclear position and the timing of the cell division cycle. The spindle position checkpoint uses septins and Elm1 kinase at the neck to control microtubules and spindle location before the nucleus transits to the neck (16, 73, 114). Elm1 is somehow regulated by the ubiquitin ligases Dma1 and Dma2 for this checkpoint function (82). The bud-neck localized activities of Elm1 control the checkpoint kinase Kin4 to ensure both mother and daughter receive a nucleus after anaphase. The septins are also integral for the morphogenesis checkpoint that ensures the cell cycle is paused in G2 in cases where bud morphology is perturbed (75). Septins, along with the Hsl7 kinase and a CDK-based feedback loop help promote degradation of the Swe1 (the Wee1 orthologue) kinase as long as there is a bud (70, 71, 79, 115). Similarly, morphogenesis is sensed via Swe1 in the yeast form of *C. albicans* (42). In *A. gossypii*, septin mutants show a reduced frequency of mitotic nuclei at branches and may spatially regulate branching in response to a local nutrient gradient (57). The degree to which septin-based control of nuclei is conserved merits future study.

Other cytoplasmic organelles take advantage of septin assemblies to distribute into buds in yeast cells. This is increasingly clear for the endoplasmic reticulum (ER) during vegetative growth and will be discussed further below. An intriguing study recently showed that septins perform a “gatekeeper” function that regulates the distribution of mitochondria from each parent in *S. cerevisiae* zygotes. Septins serve to delay nuclear fusion to ensure uni-parental mitochondrial inheritance (112). It can be challenging to disentangle direct and indirect effects of septins as landmarks for informing the cell of its polarity axis but it is worth

keeping an open mind in investigating how septin assemblies are linked to the polarized distribution of a variety of organelles.

A role for septins in organizing membranes

After polarity sites are established and Cdc42-dependent feedback loops promote F-actin polymerization and targeted secretion, what roles do septins play in maintaining cell asymmetry? Septins have been proposed to serve as a barrier to free diffusion of integral membrane proteins in the plasma membrane and as barriers, they could promote the maintenance of cell polarity (23). One way in principal they may serve this role is by acting like a gasket or physical barrier to prevent the diffusion of molecules along membranes. Electron microscopy studies have shown septin-dependent filamentous and highly ordered arrays at the bud neck (8, 10, 14, 92). Recent studies using EM visualization of platinum-replica spheroplasts in *S. cerevisiae* revealed a diverse set of filaments of varying widths, lengths, and orientations (89). This is consistent with live cell fluorescence polarization microscopy studies in both yeast and hyphal fungi, and EM of *A. gossypii* hypha (25, 27, 89). Polarized fluorescence imaging has also shown that septin assemblies are highly anisotropic suggesting they are highly ordered within assemblies (25, 27, 118). Based on all of these studies, it is clear septins are closely associated with the plasma membrane and form potentially quite rigid structures in the narrow opening between mother and bud. Because of the organization and the topology of the neck, septins could serve as blocks to diffusion of proteins in adjacent membranes that encounter the septin assembly.

Multiple studies in *S. cerevisiae* indicate that septins are required for the establishment or maintenance of distinct membrane domains that have variable composition (4, 95). When septins are disassembled at the restrictive temperature of the septin mutant *cdc12-6*, the membrane protein Ist2 and the polarisome/exocyst complex, which are normally restricted to bud cells, are no longer restricted in their localization (37, 110, 113). Additionally, septins can trap exocytotic components at the cleavage furrow, although this is not required for either the timing or fidelity of cytokinesis (29, 123). At the time these papers were published, it was proposed septins act as plasma membrane diffusion barriers. In addition, studies in the sperm annulus, the ciliary base, and midbody of mammalian cells have shown septin-dependent asymmetries in the localization of proteins and thus implicated septins as plasma membrane diffusional barriers in these contexts and systems (22, 52, 74).

In the case of the *S. cerevisiae*, additional studies indicated that it was likely an ER-based barrier that was being detected. In reality Ist2 resides primarily in the ER membrane, and not the plasma membrane, allowing reinterpretation of the previous studies and implicating septins instead as ER diffusional barriers (124). The ER function was also supported by work showing that while lumen-targeted GFP diffused freely between mother and bud that the translocon component Sec61 was not able to move between mother and bud ER based on FRAP experiments (80). Another study used EM tomography and observed septins filaments juxtaposed to the ER, making them apparently associated with ER membranes as the plasma membrane (8). Finally, recent work demonstrated that the ER proteins, Epo1 and Scs2, associate with Shs1 and this is important for asymmetric function and compartmentalization of the ER (19). It is still possible there is a plasma membrane barrier function that needs

further elucidation. Critically, even with the reassignment of septin-dependent membrane compartmentalization to the ER, it is still not clear on a molecular level how these compartments form in a septin-dependent manner.

What then is the molecular mechanism by which septins are required for building membrane compartments? In addition to the presence of physical barriers adjacent to membranes, there are multiple means by which inhomogeneous membrane organization may emerge on the micron scale (117). One possibility is that membrane-membrane junctions between organellar membranes and the plasma membrane can create stable contacts to reduce membrane protein diffusion (Figure 2A). Support for this mechanism has been shown in mammalian cells in which septins have been shown to be required for ER-PM interactions used for calcium channel function and phosphatidylinositol 4,5-bisphosphate (PIP₂) organization (103, 106). Such contacts could create immobile sites along the plasma membrane where membrane proteins cannot freely diffuse.

A second possible means by which septins are required for maintaining membrane compartments is through a role in organizing lipids (Figure 2B). Clustering of lipids in the form of nanodomains could influence protein localization and diffusion, and thus, lead to locally decreased membrane mobility. Consistent with this mechanism, in *S. cerevisiae* the ER-compartment is dependent upon and coincident with restricted sphingolipid localization (23). Septins also are attracted to negatively charged lipids, and thus, septins could also restrict the mobility of membranes by generating lipid nanodomains enriched with phosphatidylinositols (PIs) (9, 13, 45, 126). Recently, as noted above, the septin-associated kinase Gin4 was found to negatively regulate the flippase, Fpk1, which is important for asymmetry in the bilayer in which PE is on the extracellular leaflet at sites of polarized growth (94). This asymmetry in phosphatidylethanolamine (PE) is likely important for localized regulation of Cdc42 activity but may also be critical for setting up the uneven distribution of other lipids and proteins. Finally, septins frequently, but not exclusively, form higher-order structures at sites of micron-scale membrane curvature such as dendritic spines, the base of cilia, and in the buds and branches of fungi such as *S. cerevisiae*, *C. albicans*, and *A. gossypii* (26, 39, 55, 68, 120). Sites of curved membranes have distinct lipid properties because as a membrane curves sharply the distance between lipid head group changes. (Figure 2C). The degree to which septins establish membrane compartments due to building lipid domains or reacting to topologically-induced lipid domains merits further work. Advancements in single molecule tracking in live cell membranes and the development of less-perturbing probes for lipid distributions should be illuminating in deciphering the role of septins in membrane organization.

Although the possible roles of septins in organizing membranes has been studied in *S. cerevisiae* where the higher-order septins structure are enriched at the bud neck cortex, little has been studied in other fungi as to how septins influence membrane diffusion. Dimorphic fungi such as *C. albicans* will be great systems to compare how cell morphology changes relate to septin-related membrane organization, as the same proteins can be studied in different shaped cells. The varied shapes of filamentous fungi and developmental contexts of septin localization will likely be important for dissecting the relationship between septin assembly and membrane compartmentalization.

Scaffold mediated asymmetry by septins

Septins are frequently referred to as molecular “scaffolds” whereby they bring specific proteins to a specific site at a certain time. What makes septin assemblies such effective molecular scaffolds is not well understood. Are interacting proteins recognizing specific septin proteins in the heteromeric complex and so the assemblies serve as a multivalent platform for interactions? Are interacting proteins recognizing monomers or filament organization? Could interactors be sensitive to the nucleotide state of septins themselves? It has been well documented that many proteins that localize to septins in *S. cerevisiae* change their localization within the septin hourglass throughout the cell cycle and frequently are asymmetrically distributed relative to the cell axis (50, 81). What determines the asymmetric localization of interacting proteins? These proteins include kinases, regulatory subunits of protein phosphatase 2A, and many other modifying enzymes needed for specific regulatory tasks during the cell cycle (50, 81). One possibility is that post-translational modifications of septins are not uniformly delivered across assemblies and spatial variation in modification can feedback and create different scaffolding zones on the mother, bud and middle of the hourglass.

It is known that Shs1 is highly phosphorylated in *S. cerevisiae* and in *A. gossypii* (31, 83). Shs1 has been implicated as a substrate of the Cla4 and Gin4 kinases, and may also be a substrate of the protein phosphatase 2A subunit, Rts1 (30, 87). Shs1, however, is not the only septin to be post-translationally modified *in vivo*, Cdc3 and Cdc11 have also been shown to be acetylated (35). Septins are acetylated in *S. cerevisiae*, *C. albicans*, and *A. nidulans*, implicating this modification in some way (76, 85, 107). In addition to acetylation, Cdc3 is phosphorylated by Cdc28 and this seems to impact septin disassembly although there has been minimal investigation of this to date (111). It is possible even these modifications of the septins themselves are asymmetric, meaning there is a spatial heterogeneity of post-translational modifications across higher-order structures. Potentially, other septin binding partners may only bind the hourglass under certain post-translational modification conditions of the septins themselves. In the case of kinase regulation, one can imagine this as a priming step as has been seen for Cdc11 phosphorylation in *C. albicans* where priming for binding could be quite generalizable to different protein associations that vary in time and space across the septin assembly. If so, this just elevates the question as to how then there are spatially heterogeneous distributions of septin post-translational modifications.

Another possible mechanism that could allow septins to differentially scaffold protein interactors is plasma membrane asymmetry at sites of septin localization. Lipid composition in the eukaryotic plasma membrane is highly complex and organized in order to control protein interactions and regulation (77). Flippases are necessary to control the individual lipid content of each leaflet. The inner leaflet, where septins and many of their regulators interact, is enriched in PE, phosphatidylserine (PS), PI, and phosphoinositides (*e.g.*, PIP₂) (28, 34, 54). It has been seen that altering regulators of bilayer asymmetries influences the activity of GAPs and Rho GDI to control Cdc42 activity (21, 24). Therefore, by controlling Cdc42 activation, bilayer asymmetry contributes to cell polarity and morphogenesis (11, 20).

Could different lipid compositions within the yeast mother-bud neck influence septin interactions and the asymmetric localization of septin interactors?

An alternative means to create heterogeneity even within a single assembled septin assembly would be for the existence of different combinations of septin complexes in different areas. Multiple septins can interact with each other to form nonpolar, rod-shaped hetero-oligomers. In budding yeast, this oligomer adopts the following palindromic linear arrangement: Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11. Cdc11, found at the terminal position in the rod, can interact with itself to mediate filament assembly required for the formation of higher-order structures (13). This terminal position has also been shown to be a site of diversification in the heteromeric complex. *In vitro* this terminal position can be occupied by Cdc11 or Shs1 (8, 44). Does this plasticity in composition give rise to different rod complexes *in vivo*? Does the cell use different heterooctomers to generate distinct higher-order structures? A recent study in *A. nidulans* demonstrated the presence of at least two distinct septin heteropolymers during vegetative growth. The investigators showed that while the filamentous fungi specific septin, AspE is not required for the formation of either type of complexes, it was needed by one class of heteropolymers to form higher-order structures during multicellular development (59). The coexistence of different complexes in the same cell type is also established in mammalian cells (69, 101, 102). EM studies of the mother-bud neck over the cell cycle have revealed that septins transition from being arranged radially along the mother-bud axis before cytokinesis, to being oriented circumferentially when the cell undergoes cytokinesis (89). During this process, a number of different proteins that localize to the septin cortex change locations along the septin collar and these have been reviewed thoroughly (50, 81). Are different rod complexes required for the radial versus circumferential arrangement of septin filaments at the bud neck? Is there intrinsic asymmetry during the assembly of different septin rod complexes? Future studies on the variation in septin complex composition, abundance, and localization will help evaluate this possible mechanism of building asymmetries into septin scaffolds.

Are there asymmetries in the septin scaffold in other fungi? Unfortunately, few studies have looked at the asymmetry of interactors along the septin structures in different organisms. In *A. gossypii*, rings of F-actin are asymmetrically localized towards one end of a septin assembly rather than directly in the middle and in *Aspergillus* it was noted that septin rings disassemble in an asymmetric manner (26, 121). The molecular basis of asymmetry is not known in these systems but it has been shown that there is also heterogeneity between different classes of septin assemblies in filamentous fungi. Different types of septin rings are controlled by different septin regulators. For example, it is known in *A. gossypii* the kinases AgElm1 and AgGin4 are required for the formation of inter-region rings (that form along hyphae) but not branch rings (that form at the base of lateral branches) (26). Similarly, Gin4 plays roles in a subset of rings in *C. albicans* (122). This suggests the regulatory role of Gin4 may be conserved across filamentous fungi although it is not clear in *Ashbya* if this is a direct effect on septins or through regulating flippases. Notably, a mutant form of Shs1 lacking Ser/Thr phosphorylation sites in *Ashbya* does not phenocopy a *gin4* mutant indicating there is a possible role for Gin4 outside of Shs1 regulation (83, 84). In contrast, in *C. albicans*, a single Gin4-dependent phosphorylation site is required to prime phosphorylation by the hyphal-specific CDK-cyclin complex (107). The septin rings present

in filamentous fungi likely also act as scaffolds for growth and division however there is a much less complete picture of the interacting proteins in these systems.

Conclusion

Polar growth and asymmetries are an important aspect of fungal biology where long cells need to compartmentalize their cortical domains to grow toward nutrients and mates in a particular direction. Similar to fungi, many animal cells are polarized and therefore need to distribute materials asymmetrically. Septins play an important role in this compartmentalization by amplifying molecular-scale asymmetries that translate to functional asymmetries in the cell. Recent studies about septin assembly and structure have begun to provide clues on how septins act as scaffolds and membrane organizers but the molecular mechanisms that enable these functions remain remarkably open to investigation. With a diversity of assemblies but relative simplicity in terms of number of septin proteins, fungi serve as powerful systems to understand how septins act to define compartments on the cell cortex.

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Figure 1.

Septin localization in various fungi. **A.** Schematic of *S. cerevisiae* septin hetero-octamer. **B.** *S. cerevisiae* septin organization: Before budding, septins form rings (left) shown in both side top-down views. This ring then transitions into an “hourglass” structure at the mother-bud neck (middle), which splits to form a double ring (right) just prior to cytokinesis. **C.** *A. gossypii* septin organization: Septins form a number of higher order structures throughout the hypha and branches in *A. gossypii*. These include inter-region rings (1) along the length of the hypha and branch rings (2) that form at the base of lateral branches. In addition septins form a diffuse cloud at the growing tip in *Ashbya* (3). **D.** *A. nidulans* septin organization: During isotropic growth of the conidia, septins assemble into “X”s (1) and “bars” (2). The assembled bars are observed in the conidia, even after the emergence of the germ tube. With the emergence of the germ tube, septins form higher order structures at the germ tube base (3), and into long, dynamic filaments (4) throughout the germ tube’s length during growth. Septins also form “rings” at the sites of septa (5). As branching occurs, septins are seen to form “dots” (6) at the tips of branches.

Figure 2.

Models for septin physical diffusion barriers. **A.** Membrane-membrane junctions between ER membranes and the plasma membrane. “T” junctions indicate an inhibition of protein movement in the designated area. **B.** Lipid nano-domain restricting membrane protein diffusion. **C.** Highly curved membranes creating differences in membrane protein diffusion. Solid lines indicate normal diffusion rates, while dotted lines indicate slowed or non-existent diffusion.