Septins and Generation of Asymmetries in Fungal Cells

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Annu. Rev. Microbiol. 2015. 69:487–503

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev-micro-091014-104250

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Keywords

asymmetry, septins, diffusion barrier, scaffold, polar growth

Abstract

Polarized growth is critical for the development and maintenance of diverse organisms and tissues but particularly so in 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. Septins, a family of GTP-binding proteins, are key regulators of spatial compartmentalization in fungi and other eukaryotes. Septins form higher-order structures on fungal plasma membranes and 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.

Contents

INTRODUCTION

The establishment and maintenance of cell asymmetries is necessary for polarized cell growth, cell-to-cell communication, intracellular transport, and many other biological functions critical for cells across the kingdoms. Asymmetries are a particularly important phenomenon for fungal cells, which are often immobile and must achieve polarized growth to obtain nutrients, communicate with other cells, and reproduce; thus, polarity is essential for spore germination, germ tube extension, branch formation, hyphal extension, and mating projections (13, 50). Polarity establishment in filamentous fungi requires that new compartments arise from within a large, streaming cytoplasm and that multiple sites of cell polarity coexist (101). Septins, a family of GTP-binding proteins, are critical to the development of cell asymmetry and define cell shape in organisms from yeast to mushrooms.

Discovered by Lee Hartwell (60) in 1971 during a screen for *Saccharomyces cerevisiae* mutants with temperature-sensitive cell division, septins are critical contributors to cytokinesis, membrane remodeling, scaffolding, and potentially other cell functions (36, 49, 82). 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 (42, 59). The assembly and organization of these septin higher-order structures require the GTPase Cdc42 (52, 64). Conserved across eukaryotic species, except higher-order plants, septins assemble nonpolar, heteromeric rods from two to five proteins (varying in number among species) (**Figure 1*a***, **Table 1**) (2, 7, 16, 39, 43, 65, 114). *S. cerevisiae* has four core septins—Cdc3, Cdc10, Cdc11, and Cdc12—and a nonessential septin, Shs1 (**Table 1**). These nonpolar rods further assemble end-on to form filaments, and ultimately a variety of higher-order structures (8, 14, 94, 98).

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. As bud protrusion occurs, septins adopt an hourglass shape at the mother-bud neck. Just prior to the onset of cytokinesis, the septin hourglass splits into a double ring that persists throughout cytokinesis and cell separation (**Figure 1*b***) (42, 72). Assemblies similar in both shape and dynamics occur in the yeast form of dimorphic and filamentous pathogenic fungi, including *Candida albicans*, *Cryptococcus neoformans*, and *Ustilago maydis* (1, 57, 76, 115, 126). During filamentous growth of many hyphae-forming species, septins localize diffusely as bars at the base of and along hyphae and are enriched at hyphal tips; these assemblies demarcate future septa and form puncta and cables in cytosol (**Figure 1*c*,*d***) (5, 62, 63, 90). How the size and shape of different kinds of septin assemblies are determined is still unclear in most contexts, with the exception of some aspects of ring formation in *S. cerevisiae*

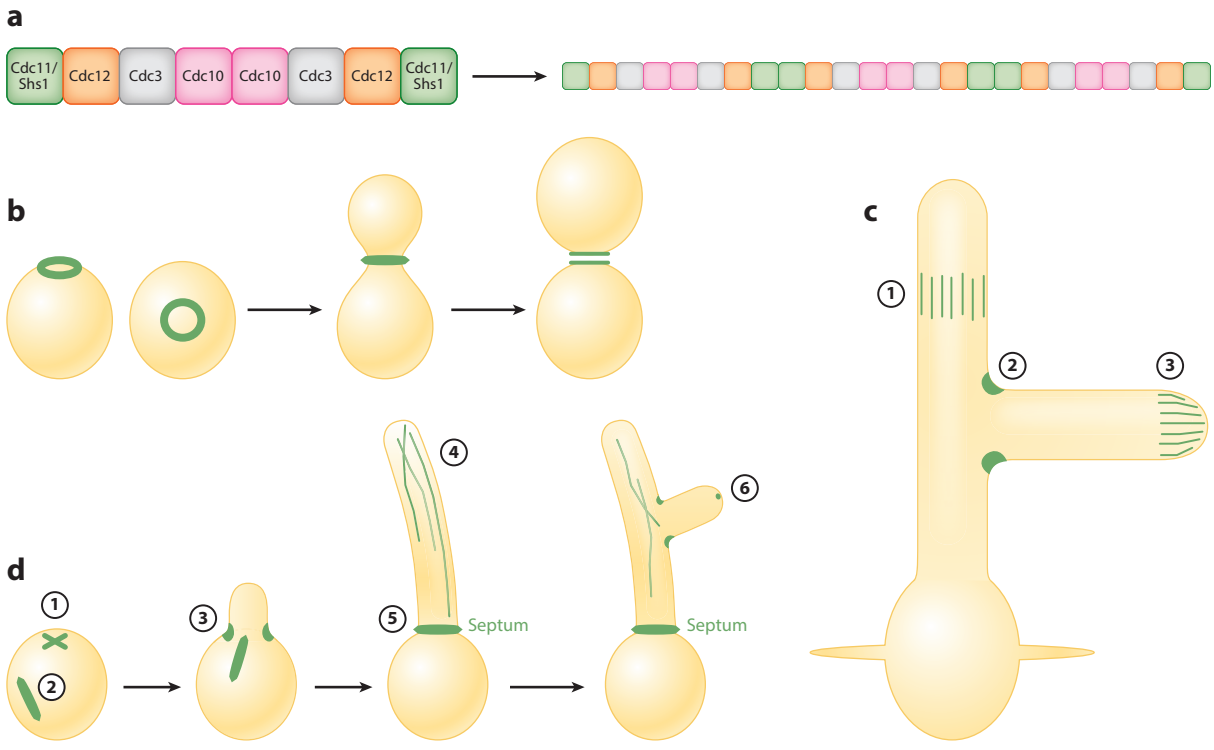

Figure 1

Septin localization in various fungi. (*a*) Schematic of *Saccharomyces cerevisiae* septin hetero-octamer and septin filament. (*b*) *S. cerevisiae* septin organization: Before budding, septins (*green*) form rings (shown in both side and top-down views; *left*). The ring then transitions into an hourglass-shaped structure at the mother-bud neck (*middle*), which splits to form a double ring (*right*) just prior to cytokinesis. (*c*) *Ashbya gossypii* septin organization: Septins form a number of higher-order structures throughout the hyphae and branches. These include interregion rings ① along the length of the hyphae and branch rings that form ② at the base of lateral branches. In addition, septins form a diffuse cloud at the growing tip in *A. gossypii* ③. (*d*) *Aspergillus nidulans* septin organization: During isotropic growth of the conidia, septins assemble into Xs ① and bars ②. The assembled bars are observed in the conidia, even after the emergence of the germ tube. Germ tube emergence accompanies septin assembly into higher-order structures ③ at the germ tube base and into long, dynamic filaments ④ throughout the germ tube's length during growth. Septins also form rings ⑤ at the sites of septa. As branching occurs, septins also form dots ⑥ at the tips of branches.

(discussed below). Variation in form and function could arise from posttranslational 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 to be associated with polarized structures, the degree to which septins generate, stabilize, and/or recognize cell polarity is still under investigation. Several proposed models 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 (54, 85). Both scaffold and membrane organization functions may depend on the local plasma membrane lipid composition and posttranslational modifications of septins. In this review we discuss how septins contribute to cellular asymmetries in fungal cells in light of how their roles as scaffolds and membrane organizers impart function.

Table 1 Septins and their roles in fungi

Organism	No. of septins	Septins	Functions	Reference(s)
Ashbya gossypii	8	Cdc10, Cdc3, Cdc12, Cdc11a, Cdc11b, Shs1, Spr3, Spr28	Cell morphology, ascospore formation	87, 88
Aspergillus fumigatus	5	AspA, AspB, AspC, AspD, AspE	Septation, conidiation, cell wall stress	67, 68, 123
Aspergillus nidulans	5	AspA, AspB, AspC, AspD, AspE	Septation, growth emergence (germ tube and branching), conidiation	62, 127
Candida albicans	7	Cdc10, Cdc3, Cdc12, Cdc11, Shs1, Spr3, Spr28	Morphogenesis, germ tube site selection, invasive growth and virulence	80, 125, 126
Coprinopsis cinerea	6	Cdc3, Cdc10, Cdc12, Cdc11a, Cdc11b, AspE	Cell polarity	111
Cryptococcus neoformans	4	Cdc10, Cdc3, Cdc12, Cdc11	Morphogenesis during sexual reproduction, nuclear distribution during monokaryotic fruiting, virulence	76
Magnaporthe oryzae	5	Sep1, Sep3, Sep4, Sep5, Sep6	Plant cell invasion	26, 102, 106
Neurospora crassa	6	Cdc10, Cdc3, Cdc12, Cdc11, Asp-1, Asp-2	Cell polarity, septation, asexual spore formation	5
Saccharomyces cerevisiae	7	Cdc10, Cdc3, Cdc12, Cdc11, Shs1, Spr3, Spr28	Cytokinesis, bud site selection, mating	18, 20, 60, 71, 83
Schizosaccharomyces pombe	7	Spn1, Spn2, Spn3, Spn4, Spn5, Spn6, Spn7	Localize at the site of cell division and affect cell division timing, mating, sporulation	95, 132
Ustilago maydis	4	Sep1, Sep2, Sep3, Sep4	Morphogenesis, invasive growth and virulence	12

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 prespecified locations of growth), or new sites can be determined stochastically through symmetry-breaking mechanisms. Studies have supported the idea that septins have a cardinal role in positioning landmarks (20, 41, 70, 105, 107). This is best established in *S. cerevisiae*, where, depending on ploidy, cells will place new buds adjacent (axial) to 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 (69, 70, 105, 131). The association between Bud4 and septins is not as straightforward as septins simply tethering Bud4; instead, there is 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 (35, 69, 70, 105, 129, 131). The synergistic links between Bud4 and septins are conserved across a variety of fungi, including *Schizosaccharomyces pombe*, where the Bud4 homolog Mid2 stabilizes the septin rings during cytokinesis, similar to what occurs in budding yeast (6). Likewise, Int1, the *C. albicans* homolog, affects axial bud site selection and interacts with septins (44), and in both *Aspergillus nidulans* and *Neurospora crassa*, Bud4 homologs control septation (66, 112). Similarly, Axl2, another bud-site-selection protein,

has been shown to both promote polarity site selection via septins and have a conserved ability to regulate septin organization (3, 46, 112). This indicates septins have an ancient interdependence with these proteins, establishing the landmarks of polarity and at the same time relying on these factors for their own organization. It is unclear why site selection machinery simultaneously uses and regulates septin organization, but this may indicate that polarity site selection has evolutionary origins 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 (99). Similarly, in another filamentous fungus, *N. crassa*, septin mutants show an increase in germ tube emergence and hyperbranching 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—something never observed in wild-type cells (80). 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 GTPase-activating protein (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 (121). Our own work examining septins by TIRF microscopy in *Ashbya gossypii* indicates that in addition to its organized septin rings, the cortex has many individual septin filaments and bundles that had previously been unappreciated when conventional imaging was employed (14). 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 (74, 83). 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 that insertion of new membrane at these sites is septin dependent (52). In the absence of well-organized septins, *S. cerevisiae* cells have difficulty tracking pheromone gradients; thus, this could be considered a function in the persistence of polarity in mating projections (71). 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*, deletion of nonessential septins Cdc10 and Cdc11 results in curved hyphae and defective cell wall deposition. Likewise, although septin mutants in the pathogenic fungi *U. maydis* display a loss of cell polarity, this results from defective cell wall deposition, and the shape can be restored in the presence of sorbitol (1). Septins are thus not essential for targeted exocytosis needed to build a bud or hypha, but they clearly can influence the precise shape of the protrusion. Alternatively, in the plant pathogen *Magnaporthe oryzae*, septins are essential for maintaining a robust appressorium projection, which is essential for penetrating the plant host tissue (26). Additionally, the functions may not be so clear in more complex morphologies such as mushroom formation in *Coprinopsis cinerea*, where the septin Cdc3 is required for the extreme polarity needed for stipe cell elongation. It is possible that in highly robust and rapidly polarized growth, septins become critical to driving polarity and are not simply regulators of site choice and fine-tuning of shape (111).

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 affect septins? Septins require Cdc42 signaling and nucleotide hydrolysis cycles to form stable and functional assemblies (19, 53, 64, 97). There are many Cdc42 effectors implicated in some way in either recruiting septins to the correct site, leading to the formation of a stable ring, or both; however, it has not been easy to tease apart precisely what molecular contributions specific effectors are making to septin organization (55). Work from the Lew (51) and Bi (19) labs showed that the GAPs of Cdc42 are required for normal septin ring assembly and that Cdc42 needs 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. 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. Given that septin assembly can be delayed in the absence of actin (but does not require actin), it was not clear whether the Gics play a direct role in septin assembly (22, 64). Importantly, the Gic effectors are conserved in humans, where the orthologs are called Borgs, indicating this is likely an evolutionarily conserved player in assembly. The Farkasovský and Raunser groups' (103) structural studies with electron microscopy (EM) and purified proteins suggested a mechanism linking Cdc42 nucleotide cycling, the Gics, and septin assembly. They showed that Gic1 can bundle septin filaments and that when Cdc42-GTP binds Gic1, it can prevent Gic1 from binding to septins. These experiments suggested that Cdc42-GTP-Gic1 complexes might regulate the lateral association of septin filaments. Additionally, Cdc42-GDP could influence septins, leading to septin filament disassembly in this case. Thus, this work points to distinct functional consequences of Cdc42 being in GTP- versus 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, Okada et al. (93) showed 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 (93). This study suggests 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 promoting complexes with Shs1 (which in vitro promotes rings) into joining the assembly in such a way as to promote the formation of a ring shape (47). Dynamic in vitro studies of the role of Cdc42, Gics, and Shs1 in shaping septins should prove useful in unifying the in vitro and in vivo data.

It has been suggested that once a septin ring is formed, the assembly traps Cdc42 and restricts the zone of bud emergence to the center of the ring (93, 96). Recent work linking the septin-associated kinase Gin4 to regulation of local membrane composition, specifically levels of phosphatidylethanolamine (PE) through inhibition of the flippase Fpk1, suggests a possible means of limiting the zone of activity of the Cdc42 to the center of the ring (27, 100, 104). 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 scaffold and 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 (18, 77, 91). Elm1 is somehow regulated by the ubiquitin ligases Dma1 and Dma2 for this checkpoint function. The bud-neck-localized activities of Elm1 control the checkpoint kinase Kin4 to ensure both mother and daughter receive a nucleus after anaphase (86). 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 (79). Septins, along with the Hsl1 kinase and a CDK-based feedback loop, help promote degradation of the Swe1 (the Wee1 ortholog) kinase as long as there is a bud (74, 75, 83, 120). Similarly, morphogenesis sensing requires Swe1 in the yeast form of *C. albicans* (45). *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 (61). The degree to which septin-based control of nuclei is conserved merits further 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 uniparental mitochondrial inheritance (118). 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 barriers to free diffusion of integral membrane proteins in the plasma membrane; and as barriers, they could promote the maintenance of cell polarity (25). A principal way of fulfilling this role may be by acting like a gasket, or physical barrier, to prevent diffusion of molecules along membranes. EM studies have shown septin-dependent filamentous and highly-ordered arrays at the bud neck (8, 10, 15, 98). Recent studies using EM visualization of platinum-replica spheroplasts in *S. cerevisiae* revealed a diverse set of filaments of varying widths, lengths, and orientations (94). This is consistent with live cell fluorescence polarization microscopy studies in both yeast and hyphal fungi, and EM of *A. gossypii* hyphae (28, 30). Polarized fluorescence imaging has also shown that septin assemblies are highly anisotropic, suggesting they are highly ordered within assemblies (28, 30, 124). 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 (40, 116, 119). Additionally, septins can trap exocytotic components at the cleavage furrow, although this is not required for either the timing or fidelity of cytokinesis (32, 129). At the time these articles were published, it was proposed that septins act as plasma membrane diffusion barriers. In addition, studies of the sperm annulus, ciliary base, and midbody of mammalian cells have found septin-dependent asymmetries in the localization of proteins and thus implicated septins as plasma membrane diffusional barriers in these contexts and systems (24, 56, 78).

In the case of *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 (130). The ER function was also supported by experiments using fluorescence recovery after photobleaching (FRAP): Although lumen-targeted green fluorescent protein diffused freely between mother and bud, FRAP data showed that the translocon component Sec61 was not able to move between mother and bud ER (84). In another study using EM tomography, septin filaments were observed juxtaposed with the ER; apparently, they are associated with ER membranes as well 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 (21). A plasma membrane barrier function is still possible. 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 (122). One possibility is that membrane-membrane junctions between organellar membranes and the plasma membrane can create stable contacts to reduce membrane protein diffusion (**Figure 2*a***). Evidence of this mechanism has been found in mammalian cells in which septins are required for ER–plasma membrane interactions used for calcium channel function and phosphatidylinositol 4,5-bisphosphate (PIP_2) organization (17, 110). Such contacts could create immobile sites along the plasma membrane where membrane proteins cannot freely diffuse.

A second possible explanation for septin-dependent maintenance of membrane compartments is a role in organizing lipids (**Figure 2*b***). 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 on and coincident with restricted sphingolipid localization (25). Septins are also attracted to negatively charged lipids; thus, they might restrict the mobility of membranes by generating lipid nanodomains enriched with phosphatidylinositols (PIs) (9, 14, 48, 133). 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 (100). This asymmetry in 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* (29, 42, 59, 72, 126). Sites of curved membranes have distinct lipid properties because as a membrane curves sharply the distance between lipid head groups changes (**Figure 2*c***). The degree to which septins establish membrane compartments due to building lipid domains or reacting to topologically induced lipid domains merits further work.

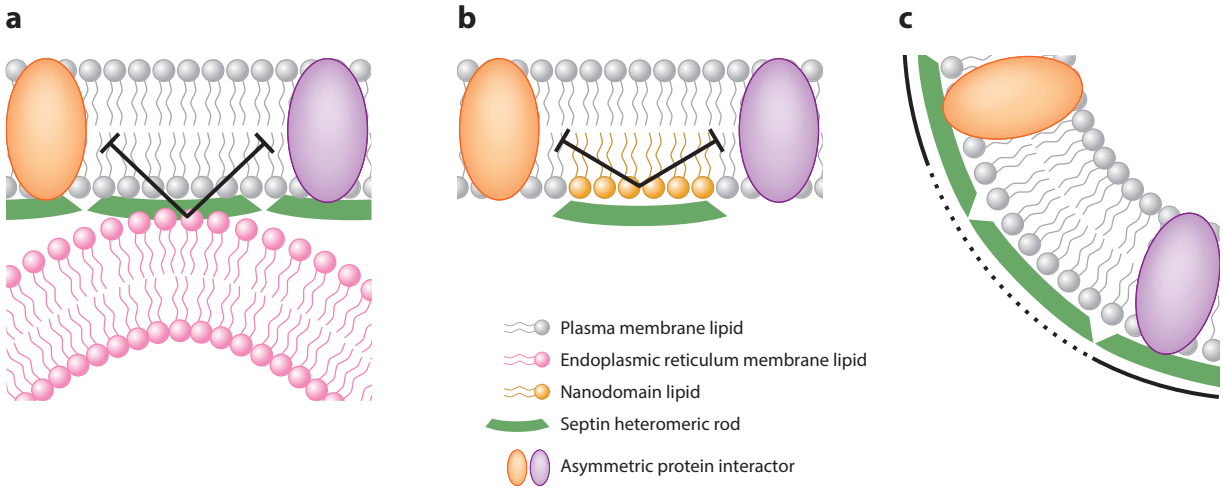

Figure 2

Models for septins as physical diffusion barriers. (*a*) Membrane-membrane junctions between the endoplasmic reticulum and the plasma membrane. Black inhibition lines indicate a restriction of protein movement in the designated area. (*b*) Lipid nanodomains restrict membrane protein diffusion. (*c*) Highly curved membranes create differences in membrane protein diffusion rates. Solid lines indicate normal diffusion rates, whereas the dotted line indicates slowed or nonexistent diffusion.

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 have been studied in *S. cerevisiae*, where the higher-order septin structures 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 for investigating how differences in cell morphology relate to septin-dependent membrane organization, because the same proteins can be studied in cells of different shapes. 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 because 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 such that 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? 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 (54, 85). 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 (54, 85). One possibility is that posttranslational modifications of septins are not

uniformly delivered across assemblies, and spatial variation in modification can create different scaffolding zones on the mother, bud, and middle of the hourglass.

Shs1 is highly phosphorylated in *S. cerevisiae* and in *A. gossypii* (34, 87). Shs1 has been implicated as a substrate of the Cla4 and Gin4 kinases, and it may also be a substrate of the protein phosphatase 2A subunit, Rts1 (33, 92). Shs1, however, is not the only septin to be posttranslationally modified in vivo: Cdc3 and Cdc11 are also acetylated (38). Septins are acetylated in *S. cerevisiae* and *C. albicans*, implicating this modification in asymmetry generation (80, 89, 113). In addition, Cdc3 is phosphorylated by Cdc28. This seems to affect septin disassembly, although there has been minimal investigation of this (117). It is possible that even these modifications of the septins themselves are asymmetric, meaning there is a spatial heterogeneity of posttranslational modifications across higher-order structures. Potentially, other septin binding partners only bind the hourglass under certain posttranslational 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* (113). 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 of how there are spatially heterogeneous distributions of septin posttranslational modifications.

Another possible mechanism allowing 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 to control protein interactions and regulation (81). 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., PIP_2) (31, 37, 58). Altering regulators of bilayer asymmetries influences the activity of GAPs and Rho guanine dissociation inhibitors to control Cdc42 activity (23, 27). Therefore, by controlling Cdc42 activation, bilayer asymmetry contributes to cell polarity and morphogenesis (11, 22). Could different lipid compositions within the yeast mother-bud neck influence septin interactions and the asymmetric localization of septin interactors?

An alternative cause of heterogeneity even within a single assembled septin assembly would be 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 at the terminal position in the rod can interact with other terminal Cdc11 molecules to mediate filament assembly required for the formation of higher-order structures (14). The terminus is also a site of diversification in the heteromeric complex. In vitro, this terminal position can be occupied by Cdc11 or Shs1 (8, 47). Does this plasticity in composition give rise to different rod complexes in vivo? Genetic work on Shs1 and Cdc11 in *S. cerevisiae* indicates this is possible (40a). Does the cell use different hetero-octamers 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. Although the filamentous-fungi-specific septin AspE was 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 (63). Different complexes exist in the same cell type in mammals as well (73, 108, 109). EM studies of the mother-bud neck have revealed that septins transition from a radial arrangement along the mother-bud axis before cytokinesis to a circumferential orientation during cytokinesis (94). During this process, a number of different proteins that localize to the septin cortex change locations along the septin collar; these have been reviewed thoroughly (54, 85). 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 of the variation in septin complex composition, abundance, and localization will help evaluate this possible mechanism of building asymmetries into septin scaffolds.

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 toward one end of a septin assembly rather than directly in the middle, and in *A. nidulans* it was noted that septin rings disassemble in an asymmetric manner (29, 127). The molecular basis of asymmetry is not known in these systems, but 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, in *A. gossypii* the kinases AgElm1 and AgGin4 are required for the formation of interregion rings (formed along hyphae) but not branch rings (formed at the base of lateral branches) (29). Similarly, Gin4 plays roles in a subset of rings in *C. albicans* (128). This suggests the regulatory role of Gin4 may be conserved across filamentous fungi, although for *A. gossypii* it is not clear whether this is a direct effect on septins or an indirect effect through regulating flippases. Notably, a mutant form of Shs1 lacking Ser/Thr phosphorylation sites in *A. gossypii* does not phenocopy a *gin4* mutant, indicating a possible role for Gin4 outside of Shs1 regulation (87, 88). In contrast, in *C. albicans*, a single Gin4-dependent phosphorylation site is required to prime phosphorylation by the hyphal-specific CDK-cyclin complex (113). The septin rings present in filamentous fungi likely also act as scaffolds for growth and division; however, the picture of the interacting proteins in these systems is much less complete.

CONCLUSION

Polar growth and asymmetries are an important aspect of fungal biology; long cells need to compartmentalize their cortical domains to grow toward nutrients and mates. 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 to how septins act as scaffolds and membrane organizers; remarkably, the molecular mechanisms that enable these functions remain open to investigation. With a diversity of assemblies but relative simplicity in terms of number of septin proteins, fungi are powerful systems to demonstrate how septins define compartments on the cell cortex.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by GM100160 from the National Institutes of Health and MCB-1212400 from the National Science Foundation.

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Erratum

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