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Septin Form and Function at the Cell Cortex*

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Septins are GTP-binding proteins that form filaments and higher-order structures on the cell cortex of eukaryotic cells and associate with actin and microtubule cytoskeletal networks. When assembled, septins coordinate cell division and contribute to cell polarity maintenance and membrane remodeling. These functions manifest themselves via scaffolding of cytosolic proteins and cytoskeletal networks to specific locations on membranes and by forming diffusional barriers that restrict lateral diffusion of proteins embedded in membranes. Notably, many neurodegenerative diseases and cancers have been characterized as having misregulated septins, suggesting that their functions are relevant to diverse diseases. Despite the importance of septins, little is known about what features of the plasma membrane influence septin recruitment and alternatively, how septins influence plasma membrane properties. Septins have been localized to the cell cortex at the base of cilia, the mother-bud neck of yeast, and branch points of filamentous fungi and dendritic spines, in cleavage furrows, and in retracting membrane protrusions in mammalian cells. These sites all possess some degree of curvature and are likely composed of distinct lipid pools. Depending on the context, septins may act alone or in concert with other cytoskeletal elements to influence and sense membrane properties. The degree to which septins react to and/or induce changes in shape and lipid composition are discussed here. As septins are an essential player in basic biology and disease, understanding the interplay between septins and the plasma membrane is critical and may yield new and unexpected functions.

Septins are a conserved family of cytoskeletal GTP-binding proteins that function in cytokinesis, cell polarity, and membrane remodeling in many eukaryotic cell types (1, 2). To contribute to these diverse process, septins polymerize into filaments and higher-order structures that organize the cell cortex into domains that are tightly controlled in time and/or space (3). Higher-order septin structures act to recruit and/or integrate protein networks at specific locations, an example being contractile ring components in cell division (4–7). In addition, septins are thought to alter lateral diffusion of proteins embedded in the plasma and endoplasmic reticulum (ER)[2] membranes and may influence local lipid composition (8–10). In addition to membrane association, septins interact with, respond to, and organize the actin and microtubule cytoskeletons (11). Although septins were discovered by Hartwell, Pringle, and colleagues (12) in cell division cycle screens decades ago, understanding of septins has lagged behind other cytoskeletal systems. Recent biochemical, structural, and biophysical approaches, however, have made septins highly tractable and brought about exciting advances. Despite this recent progress, many fundamental questions remain in the septin field, in particular, the mechanisms by which septins associate with and influence the cell cortex.

A better understanding of septin biology is of both biomedical and ecological importance. Human septin misregulation is associated with numerous disease states, which range from cancers to neurodegenerative disorders (13). For example, septin expression levels are altered in a variety of solid tumors, and recently, it has been shown that cell lines overexpressing individual septins are better at crawling in two-dimensional and three-dimensional matrices (14, 15). In addition, the methylation status of septin 9 DNA is gaining use as a blood screening tool for colorectal cancers (16, 17). In neurons, septins are thought to play an important role in the migration of neural precursors and later in establishing proper morphology (18–20). Moreover, in fungal pathogens, septins are generally required for virulence and are thought to be directly involved in host tissue entry (21). This includes a variety of human pathogens, such as *Candida albicans*, as well as devastating plant crop pathogens, including *Magnaporthe oryzae*, the cause of rice blast disease (22–24). Given this context, our current understanding of septin form and function must be broadened.

Fortunately, numerous studies in the last 10 years have begun to reveal basic properties of the septin proteins. The number of septin genes varies widely between eukaryotic organisms, from 2 in nematodes to 13 in humans (25–27). Despite this variability, single particle electron microscopy and crystallography studies have shown that septins assemble into a conserved rod-shaped heteromeric complex (also referred to as a protofilament) (Fig. 1*A*) (28–30). Septins interact with one another via two interfaces: a surface comprising the GTP-binding domain and a surface created by the N and C termini, which are brought into close proximity upon folding (28). Septins are thought to interact with anionic phospholipids via a highly conserved polybasic region near the N terminus composed of 2–6 positively charged residues (31, 32). The polybasic region of an individual septin monomer is brought into the proximity of a polybasic region of an adjacent septin protein upon interaction at the N- and C-terminal interface (Fig. 1*A*). In addition, most septins are predicted to have a C-terminal coiled coil domain. This region remains functionally mysterious as it has failed to resolve by x-ray crystallography, possesses high flexibility, and

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[2] The abbreviations used are: ER, endoplasmic reticulum; PI, phosphatidylinositol; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate.

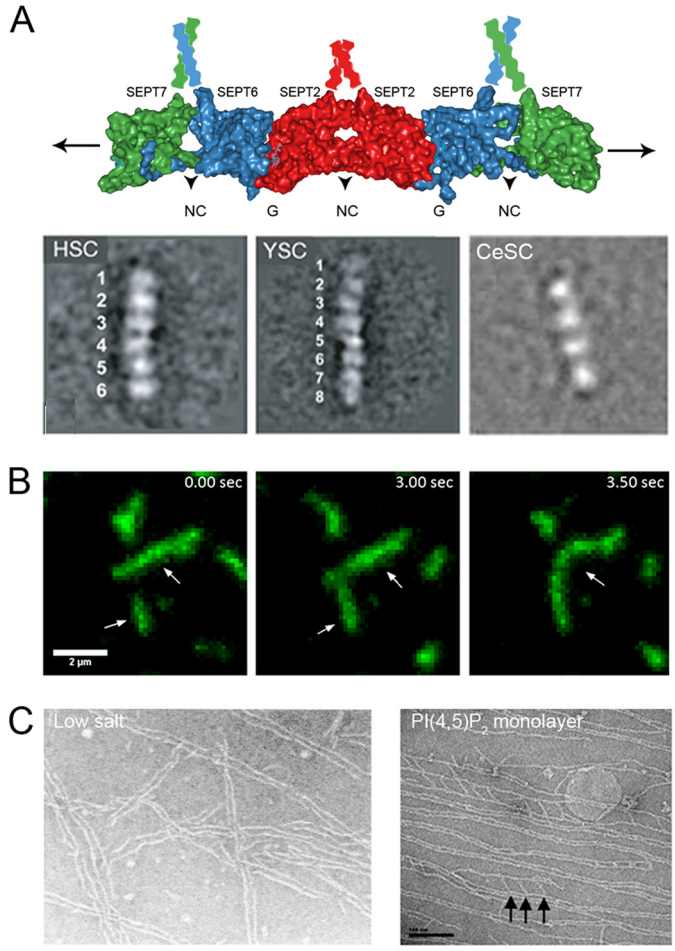

FIGURE 1. **Septin complex and filament formation.** *A*, structure of the human septin complex. The septin complex is arranged in a palindromic order by alternating N and C termini (*NC*) and G-interface (*G*) associations. Each septin contains predicted C-terminal coiled coil extensions, which have remained mysterious in function. A polybasic stretch of amino acids is thought to be important for membrane association (*arrowheads*), and polymerization occurs through terminal subunit interactions (*arrows*). The palindromic and rod nature of the complex is conserved in yeast and *Caenorhabditis elegans*. *HSC*, human septin complex; *YSC*, yeast septin complex; *CeSC*, *C. elegans* septin complex. *B*, recent studies have begun to analyze the mechanism by which septins polymerize. Septin polymerization is characterized as involving frequent annealing, or joining, of two short filaments into a longer one on supported lipid bilayers. *C*, structural studies of septin filaments in low salt solution and on model lipid membranes. Septins polymerize into paired filaments in low salt solutions and single, branched, and tightly paired filaments on lipid monolayers. *Panel A* is modified, with permission, from Ref. 28 (human and yeast complexes) © 2007 Macmillan Publishers Ltd., and Ref. 29 (*C. elegans* complex), © 2007 European Molecular Biology Organization. All rights reserved. *Panel B* is reproduced from Ref. 37, © 2014 National Academy of Sciences. All rights reserved. *Panel C* is reproduced from Ref. 36, © 1998 J. A. Frazier *et al.*, originally published in *J. Cell Biol.*, and Ref. 33, © 2010 Elsevier Ltd. All rights reserved.

produces variable phenotypes when removed from individual septins (28, 29, 33, 34). These rod-shaped complexes serve as the basic subunit of filament assembly.

Insights into how septins form filaments have come from both structural studies and reconstitution of septin assembly on synthetic lipid bilayers. Unlike actin and microtubules, the septin complex is non-polar along the long axis in recombinant systems, with individual septins arranged in palindromic order (Fig. 1*A*) (28, 29, 35). Pure yeast septin complexes form filaments upon transfer to low salt buffer (~50 mM) or upon contact with a synthetic phospholipid surface (33, 36, 37). Consistent with a non-polar organization, yeast septins on supported lipid bilayers polymerize at both filament ends via a process that involves annealing of short filaments into long filaments (Fig. 1*B*) (37). These assembled filaments may exist as individual polymers, pairs, bundles, "gauzes," and sheet-like arrangements (Fig. 1*C*) (30, 33, 38–41). Collectively, these structures are referred to as septin assemblies or higher-order structures. It is generally thought that septins function in cells as higher-order assemblies (42).

Septin function is intimately linked with cortical and cytoskeletal networks; however, detailed mechanistic studies of the septin-membrane interactions are just beginning to be undertaken. The goal of this review is to highlight both what is known and what is unknown about the interplay between the septins and the cell cortex. We will begin by discussing established and emerging septin functions at the cell cortex followed by a review of recent progress in understanding the dynamics and organization of septin higher-order structures.

Function of Higher-order Structures at the Cell Cortex

When assembled on the cell cortex, septins integrate both spatial and timing information to coordinate highly regulated processes such as cytokinesis, ciliogenesis, or phagocytosis (43–45). Frequently, septins localize to the cortex at boundaries between cell compartments: the base of cell outgrowths (cilia, dendritic spines, filopodia, and hyphal branches), as well as sites of cell division (45–48). At these transition zones, septins act as platforms to convene cytoplasmic and cytoskeletal proteins. This scaffold property is best described at the bud neck of *Saccharomyces cerevisiae* to which >60 proteins have been shown to localize, most in a septin-dependent manner (Fig. 2*A*) (5, 49–51). Proteins that interact with septins can be categorized into several functional groups: those involved in the cell cycle, cell shape, polarity, and cytokinesis. Thus, assembled septins at the bud neck of yeast integrate cell cycle regulation and morphogenesis (52). In mammalian systems, higher-order septin assemblies, in concert with anillin, are involved in organizing cytokinetic machinery but do not appear to play any mechanical role in division. Septins collaborate with anillin at the cell cortex to recruit and stabilize myosin, actin, and regulatory kinases at the site of cell division for coordinated contraction (6, 53–58). Subsequently, septins are important for the morphology and maturation of the intercellular bridge and midbody and thus for the resolution of division in animal cells (53, 58, 59). Despite an abundance of interacting proteins, no general septin interaction motifs have been described in the population of proteins that depend on septins for localization. Likewise, it is not known what septin domains are involved in scaffolding as opposed to polymerization and membrane association. Although it is clear that septins act as a scaffold, the mechanisms controlling how septins interact with specific proteins is ripe for investigation in cells and by reconstitution.

Increasing evidence suggests that septins influence animal cell shape and cortical rigidity in interphase and during cell division. In both cases, the effect of septins on plasma membrane properties could simply be through organizing the actomyosin cortex, or may be due to a direct effect of septin fila-

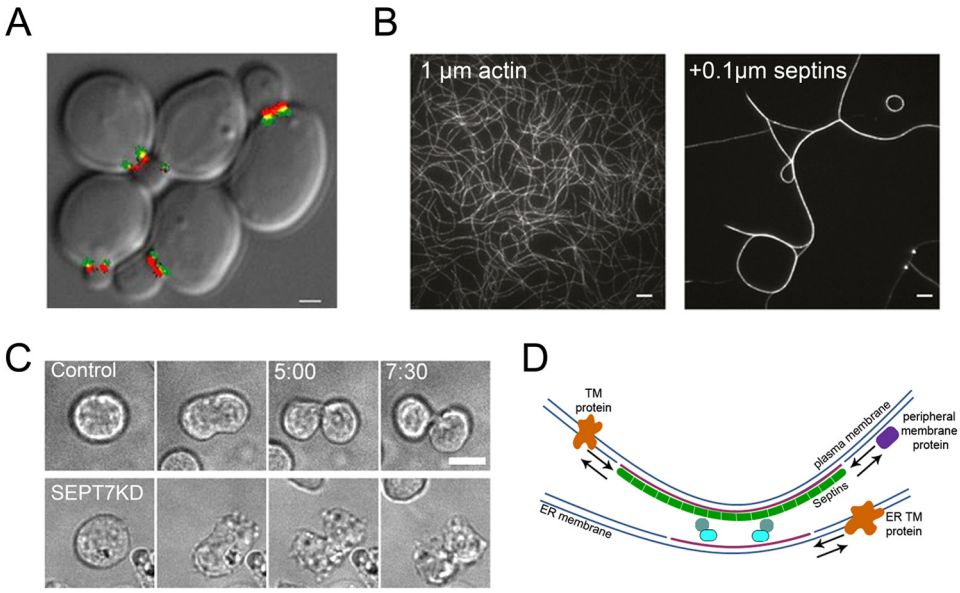

FIGURE 2. **Septin function in eukaryotic cells.** *A*, septins at the cell cortex scaffold proteins involved in cell polarity, cell shape, cell cycle, and cytokinesis. Septin scaffolding is best studied at the mother-bud neck of *S. cerevisiae*, which serves as a site that integrates complex signals. *B*, septins have increasingly been implicated in cytoskeletal remodeling. Here, septins have been shown to bundle and bend actin *in vitro*. *C*, septins are important players in animal cell cortical rigidity. Cells that lack SEPT7 during cell division displayed an altered cell shape characterized as containing abundant blebs indicative of an unstable cell cortex. *D*, septins are thought to act as lateral diffusion barriers in the plasma membrane and adjacent organelles. This may occur through enrichment of lipid species, rendering the plasma membrane semipermeable, or it may occur through a structural mechanism. Recently, adjacent ER membranes in *S. cerevisiae* have been shown to contain a diffusion barrier that is also septin-dependent. *TM protein*, transmembrane protein. *Panel A* is reproduced from Ref. 51 © 2013 by The American Society for Cell Biology. *Panel B* is reproduced from Ref. 61, © 2007 Macmillan Publishers Ltd. All rights reserved. *Panel C* is reproduced from Ref. 63, © 2012 J. K. Gilden *et al.*, 10.1083/jcb.201105127, originally published in *J. Cell Biol.*

ments on the rigidity or composition of the plasma membrane (1, 60). Septin function via actin coordination is supported by recent analysis of septins in cellularizing *Drosophila* embryos, in which it is clear that septins are required for normal cell shape and septins contribute to the formation of tight, curved, actin bundles in cells and with pure components (Fig. 2*B*) (61). In addition, knockdown of septins in HeLa cells has been shown to reduce cell stiffness to similar levels as treatment with latrunculin B, as measured by atomic force microscopy (62). In T-cells, septin knockdown results in abnormal cell morphology characterized by an increased frequency of blebs and extended cell protrusions (Fig. 2*C*) (63). Notably, septin influence on T-cell shape seems to occur as a response to malformed regions of the plasma membrane, rather than being proactive in preventing such deformations (63). Moreover, cells exposed to hypotonic media, followed by regulatory volume decrease, form a variety of septin structures including puncta and rings on membrane invaginations (63). Cells lacking septins return to a normal volume substantially slower than control cells, similar to latrunculin treatment, yet no additive effect was observed when septin knockdown was paired with actin depolymerization. Might this work through septin coordination of actin to drive retraction, or could septins themselves direct changes in cell shape? Taken together, increasing evidence suggests that septins are a major player in cortical integrity and cell shape, likely in part through coordination with the actin cortex.

In addition to influencing actin at the cell cortex, cortical septins also associate with microtubules directly and indirectly in various contexts. In budding yeast, septins are important for spindle positioning by scaffolding proteins responsible for aligning the spindle between mother and bud (64). Similarly, in developing axon collateral branches, SEPT7 is important for directing microtubules into filopodia, a process required for the successful formation of branches (18). In non-adherent myeloid K562 cells, which possess an abundance of cortical microtubules, septins form striking disc or ring-like structures at the cell cortex (65). In the absence of microtubules, these disc structures disperse and septins localize diffusely to the cell cortex. Notably, in these cells, actin depolymerization had no effect on septin localization. What are the properties of the membrane at these microtubule-dependent sites of septin assembly and what function do these septin discs serve? In addition, septins have been implicated in microtubule remodeling away from the plasma membrane by numerous studies (66–69). The emerging appreciation of septin interaction with other cytoskeletal networks is exciting, and coordination of these networks is likely an important conserved function.

Although septins respond to and recruit proteins to the cell cortex, there are likely roles for septins in regulating membrane properties. In combination with other mechanisms that generate cellular asymmetry, septins may compartmentalize cell membranes by functioning as diffusion barriers (Fig. 2*D*) (9, 70). Septins are closely apposed to the plasma membrane, and this may restrict the passage of integral membrane proteins of a sufficient size. Alternatively, or in addition, septins or septin-binding proteins may recruit, modify, or maintain a pool of specific lipids leading to a selectively permeable barrier that operates based on lipid preferences of the impacted proteins (8, 71). The diffusion barrier property of septin assemblies was noticed, and has been most studied at the yeast mother-bud neck where septins are thought to restrict diffusion of membrane-associated proteins and lipids between mother and bud,

in effect maintaining asymmetrically distributed membrane proteins (72, 73). Interestingly, the septin hourglass at the bud neck has also been shown to impose a diffusion barrier on other organelles from the cortex, namely the ER. Septins interact with membrane-associated ER proteins that may locally enrich sphingolipids, allowing for selective inheritance of ER proteins (Fig. 2*D*) (8, 10, 74). Septins have also been localized to plasma membrane-ER junctions in HeLa cells. At these sites, septins organize $PI(4,5)P_2$ microdomains and are involved in store-operated Ca^{2+} entry (75). In addition, septins have been proposed to act as a diffusion barrier for cytokinetic machinery during cytokinesis, although an intact diffusion barrier is not required for successful cell division (76, 77). A diffusion barrier function has been evoked in mammalian systems as well at the base of cilia and dendritic spines and in the diffusion of the Glutamate Aspartate Transporter (GLAST) (20, 45, 78). Despite numerous studies that have suggested septins are important for a diffusion barrier function in cells, how septins restrict lateral diffusion in and on membranes is unclear, and analysis *in vitro* could be informative. Reconstitution of such behavior will help dissect whether the diffusion barrier phenotype is a septin intrinsic property if other proteins are involved. How lipid distribution and membrane diffusibility are influenced by septins is an important frontier for the field, and further study will help in understanding how this property contributes to the many mechanisms used by cells to generate asymmetry. Next, we will discuss the dynamics of assembly and states of organization of septin higher-order structures.

Path to the Plasma Membrane

The process by which septin complexes, the units of polymerization, are formed has only recently begun to be addressed. A breakthrough in septin complex biochemistry came when Sheffield *et al.* (79) were able to co-express three mammalian septin genes on two plasmids in the same *E. coli* cell. When a single septin protein is epitope-labeled, all three septins co-purify in rod-shaped complexes that resemble those immunoprecipitated from native cells (79). This expression system has been adopted for yeast and *Drosophila* septins and demonstrates the true "self-assembling" propensity of the septin complex (80, 81). Despite this, recent work has shown that yeast cells monitor the quality of septin complexes being incorporated into the higher-order structure at the bud neck. The McMurray group (82) showed that septin proteins carrying mutations in the G-interface are not assembled into higher-order structures in the presence of wild type proteins. Are other proteins, such as chaperones, involved in monitoring the formation of stable septin complexes? The fact that these mutations are localized to the G-interface may suggest a role for GTPase function in the assembly of septin complexes, rather than in the assembly or dynamics of septin filaments. Indeed, septins have been found to hydrolyze and exchange nucleotide very slowly in cells and *in vitro*, and the half-life of septin complexes is correspondingly slow (83–86). Thus, although progress has been made in understanding the assembly process of septin complexes, much work remains.

The existence of quality control systems in assembly of septin complexes raises the possibility that septin protein synthesis could be spatially or temporally coordinated. A particularly interesting recent study in the highly polarized cells of *Ustilago maydis*, a fungal plant pathogen, showed that septin *CDC3* mRNA is tethered to and likely translated on endosomes being transported to the growing tip (87). Could organelle membranes such as the endosome be a platform for coordinating the local synthesis of different septins and for assembly of septin complexes? Because of the variety of septin genes and isoforms in higher eukaryotes (expressed in the same tissues), the number of possible septin complexes, as well as their potential unique functions within a single cell, is extremely complex. Understanding the process of septin complex assembly, such as a localized production in territories of the cytosol or on endosomal membranes, may shed light on septin complex diversity and function.

Once stably assembled, septin complexes are competent for polymerization into filaments and higher-order structures. In the cytoplasm, septins are thought to exist primarily as single complexes as demonstrated by density centrifugation of soluble proteins in lysates and by fluorescence correlation spectroscopy in living cells (37, 88). Notably, the cytoplasmic septin complex concentration in three fungal organisms has been measured at ~200 nM by fluorescence correlation spectroscopy, a concentration well above a "critical concentration" for filament formation with pure protein at low salt concentrations; however, only non-filamentous complexes were detected in cytosol (37). Could a "capping protein" or posttranslational modification prevent septin polymerization in the cytoplasm, or is the cytoplasmic ionic strength too high to promote filament formation? In contrast, analysis of the fungal cell cortex by total internal reflective fluorescence microscopy has revealed that septins exist as a distribution of short filaments and dense higher-order structures (37). The assembly and function of higher-order structures on the cortex are the topics of the following section.

Formation of Higher-order Structures

How are septins recruited and stabilized on the plasma membrane? As briefly described above, the septin-plasma membrane association is thought to occur via an N-terminal polybasic stretch interacting with anionic phospholipids. Mutational analysis of the polybasic domain has produced variable results and is complicated by the fact that it is within a region important for polymerization and that most septin proteins have the conserved domain (33, 89). Indeed, avidity through multiple binding sites and polymerization could complicate the analysis of a single protein's polybasic mutations, and multiplicity of weak binding sites could be a driving factor in stable septin association with membranes (89). Notably, removal of the polybasic region of a single septin in yeast results in inviability, although the interpretation of such a mutation is complicated (42). In animal cells, it will be important to discern whether septin cortical localization is due to direct association with membrane or via association with other components of the cell cortex. There is clear room for further structural and kinetic analysis of how septins associate with membranes and how this association may be regulated.

The role of specific lipids in promoting the recruitment and shape of higher-order assemblies has also been challenging to

dissect. Experiments assessing phospholipid specificity *in vitro* have produced variable results, depending on the septin construct used and the organism of origin. Early experiments performed with individual recombinantly expressed septins from humans and yeast showed a preference for phosphorylated phosphatidylinositides (31, 32). More recently, several groups have shown that heteromeric septin complexes bind to and form filaments on monolayers and bilayers containing phosphatidylinositol, phosphatidylinositol phosphate, and/or $PI(4,5)P_2$, but only a small number of different membrane compositions have been assessed (33, 37). In budding yeast, septins appear mislocalized when PI 4-kinases are deleted or when PI 3-kinase is overexpressed, although these conditions risk being highly pleiotropic and phenotypes could be the result of mislocalized septin regulators (32, 90). Experiments assessing lipid binding specificity of pure heteromeric complexes across species are needed. It is important to consider and test the possibility that septins bind negatively charged phospholipids in general beyond phosphatidylinositides and that septin-binding proteins, many of which also contain lipid-binding motifs, assist in septin recruitment to the cell cortex. It is also possible that lipid composition influences the geometry of higher-order septin organization, as demonstrated by Bertin *et al.* (33) on monolayers containing $PI(4,5)P_2$, where less paired, and more sheet-like arrangements were observed as compared with assemblies formed in low salt solution. Indeed, further work should investigate what role local lipid composition has on the location and morphology of higher-order assemblies.

It has recently been shown in fungi that upon binding to the plasma membrane, septin complexes diffuse and collide to form short septin filaments (37). Short filaments can anneal to make longer filaments, which merge into higher-order structures. In addition, reconstitution of septin assembly on supported lipid bilayers showed that relatively long septin filaments form in a matter of seconds from nanomolar septin complex concentrations by a process that is largely driven by annealing of short filaments on the bilayer, rather than solely by the addition of single complexes to filament ends. Both the *in vitro* and *in vivo* data in fungi suggest that the membrane can promote polymerization by restricting diffusion to two dimensions and promoting favorable end-on interactions for polymerization. However, it is also worth noting that in higher eukaryotes, septins are seen to associate with other cytoskeletal systems in the cytoplasm, and if septins are polymerized in this context, it is conceivable that other cytoskeletal assemblies could play a role in septin polymerization.

What determines the cortical locations where higher-order septin structures form? Local signaling by Cdc42 and other Rho-GTPases frequently demarcates sites of assembly at the cell cortex (91). Cdc42 GTP cycling has been shown to be critical for septin ring formation in yeast and Cdc42-GDP, and its effector Gic1 can directly associate with and regulate the stability of septin filaments (40, 92–94). Although Cdc42 has been shown to promote assembly of the septin higher-order structures, it is possible that features of the plasma membrane at sites of assembly such as composition and shape also play a role (93, 95). Recent work has shown that Cdc42 recruitment of septins to an incipient bud site in yeast is followed by an exocytosis-

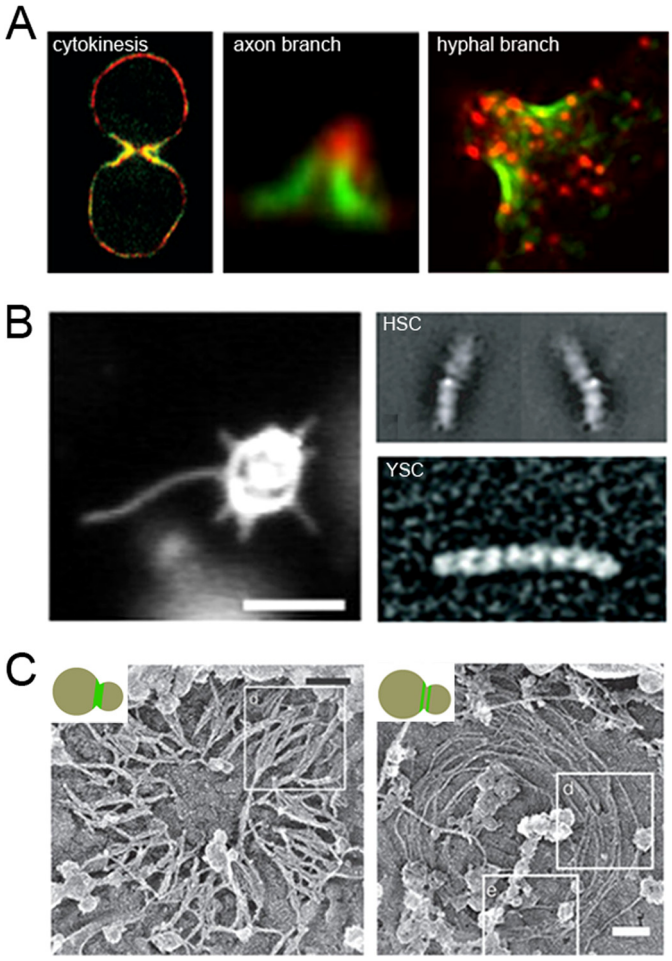

FIGURE 3. **Septin localization at sites of membrane curvature and higher-order structure organization.** *A*, septins localize to curved membranes in diverse contexts. Septins localize to the cytokinetic furrow (*green*, SEPT2; *red*, myosin IIA), the base of axon branches (*green*, SEPT7; *red*, F-actin), and at the base of branches in filamentous fungi (*green*, SEPT7; *red*, F-actin). *B*, septins display properties that indicate their localization may be driven by membrane shape. Septins tubulate giant unilamellar vesicles *in vitro*, and the human and yeast septin complexes have been shown to hinge and curve, respectively, along their length. *HSC*, human septin complex; *YSC*, yeast septin complex. *C*, structural organization of septin filaments in the yeast bud neck. Prior to ring-splitting, septins localize along the mother-bud axis. After cell division, septin filaments reorganize to run circumferentially. *Panel A* is reproduced (from left to right) from Ref. 6, © 2007 Elsevier, Ref. 18, © 2012 Elsevier, and Ref. 48, ©2009 by The American Society for Cell Biology. All rights reserved. *Panel B* is reproduced (from left to right) from Ref. 89, © 2009 Elsevier, Ref. 28, © 2007 Macmillan Publishers Ltd., and Ref. 35, © 2008 National Academy of Sciences. All rights reserved. *Panel C* is reproduced from Ref. 41, 2014 Macmillan Publishers Ltd., All rights reserved.

driven sculpting of a septin ring or hourglass. Could it be that a change in membrane properties, the result of exocytosis, helps establish a stable septin higher-order structure (95)? Many sites of higher-order septin assemblies, including the cytokinetic furrow, the yeast bud neck, the base of filamentous fungal branches and cilia, and the dendritic spines, have some degree of membrane curvature (Fig. 3*A*). Do septins recognize and/or influence plasma membrane shape? Evidence for inducing shape change comes from experiments using septins from brain tissue and recombinant co-expression, which demonstrated their propensity to tubulate giant unilamellar vesicles, although more experiments are necessary to determine whether this property is relevant *in vivo* (Fig. 3*B*) (89). If, in fact, septins do

prefer and/or promote curvature, how might this work? Single particle EM studies of the human septin complex have shown that it is able to hinge in the middle up to 30° (28). Interestingly, the yeast septin complex was not observed to hinge, but rather, a high frequency of complexes was determined to possess curvature along their length (Fig. 3*B*) (30). What role might a curved membrane play on the septin assembly process? Are plasma membrane curvature and lipid composition separable factors? Are septins simply responding to curvature, or can they also induce curvature in cells? Beyond looking at localized small GTPase activity, it is critical to understand how membrane shape and composition function to organize and stabilize higher-order assemblies.

Once recruited to the membrane, filaments come together into a variety of higher-order structures. The organization of septin filaments within functional higher-order structures has been extensively studied, particularly in *S. cerevisiae*, starting in 1976 with Byers and Goetsch's detection of prominent filaments at the mother-bud neck (96). As demonstrated by fluorescence polarization microscopy, electron tomography, and platinum replica EM of unroofed spheroplasts, septin filaments possess a high degree of organization in higher-order structures (38, 39, 41, 97, 98). Indeed, in the septin hourglass, pairs of septin filaments were found to run parallel to the mother-bud axis (Fig. 3*C*) (41). Immediately prior to cell division, the septin hourglass splits into two rings, and this process has been characterized as involving a major reorientation of septin filaments to a circumferential organization (41, 97). The reorganization at the hourglass to split ring transition is coincident with a substantial loss of septin filaments as well as the rearrangement and assembly of new filaments (Fig. 3*C*). How are transitions between different septin organizations, such as single strands, pairs, bundles, and gauzes, controlled in living cells? (33, 36). Are septin filaments capable of self-assembling into complex organizations, or do other proteins control their organization (40)? In meshes, which population of filaments contacts the membrane, and how does this relationship influence large rearrangements seen at cytokinesis? As the *in situ* higher-order assemblies become clearer, future work will need to address how regulatory proteins execute changes in dynamics and organization in sync with the cell cycle. Additionally, ultrastructure studies of assemblies in the context of animal cells will be critical.

Although substantial progress has been made in understanding the assembly of septin higher-order structures, the process by which septins disassemble remains elusive. What role does changing membrane shape and lipid composition during cytokinesis play in their disassembly? Although other proteins involved in the process of *S. cerevisiae* septin ring splitting have been identified, their mechanism of action remains unclear. Numerous septins and septin regulators are phosphorylated at some time in the cell cycle, and it is possible that opposing dephosphorylation could trigger rearrangement or septin disassembly (99). In addition, septins on supported lipid bilayers have been shown to fragment (37). Might this property be central to dynamic rearrangements?

Concluding Comments

In conclusion, the septin cytoskeleton is an important and remarkably exciting player in organizing the complex eukaryotic cell cortex. Recent studies have revealed that the septins are more dynamic than previously appreciated, they are intricately associated with other cytoskeletal systems, and they have an emerging role in responding to changes in plasma membrane shape. Despite these advances, many fundamental questions pertaining to their interplay with the plasma membrane and cell cortex remain to be addressed. Recent advances in imaging, the development of *in vitro* reconstitution techniques, and ever increasing interest in the field have put the septins in a place where these fundamental questions are capable of being addressed in the near future.

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