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State of the septin cytoskeleton from assembly to function

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Abstract

Septins are conserved GTP-binding proteins that polymerize into filaments at the cell cortex or in association with other cytoskeletal proteins, such as actin or microtubules. As integral players in many morphogenic and signaling events, septins form scaffolds important for the recruitment of cytokinetic machinery, organization of the plasma membrane, and orientation of cell polarity. Mutations in septins or their misregulation is associated with numerous diseases. Despite growing appreciation for the importance of septins in different aspects of cell biology and disease, septins remain relatively poorly understood compared to other cytoskeletal proteins. Here in this review, we highlight some of the recent developments of the last two years in the field of septin cell biology.

Introduction

Septins are GTPases found in many eukaryotes including fungi and metazoans but missing in land plants. The basic unit of septins are non-polar, hetero-oligomers 17 to 32 nanometers long (depending on the species, see Figure 1), which polymerize into larger filaments by annealing end-to-end at the plasma membrane [1-3]. Filaments can be organized into a variety of distinct, higher-order assemblies such as bundles, lattices, rings, and bars each of which have different functions in different cellular contexts [4,5]. Higher-order septin assemblies act as scaffolds for microtubules and F-actin, regulate cell morphogenesis, recognize micron-scaled membrane curvature, and organize the plasma membrane [6]. Septins have been implicated in a range cellular processes including cell division, cell migration, ciliogenesis, and neuronal branching[7-11]. Given their varied cellular functions, a growing body of literature implicate septins in various diseases including cancer[12-14].

Septins are finding new prominence and importance in diverse cell functions including neuronal signaling, fertility and immunity. Several new studies have expanded the role of septins in neuronal signal transduction. Calcium signaling and homeostasis are dependent on septin regulation of calcium entry channels at the plasma membrane in neurons [15,16]. Moreover, disruption of septins in neurons delays neurotransmitter release in mice through a separate, calcium signaling-independent mechanism [17]. Another recent study in mice demonstrated that septins are important in male fertility [18]. Knock-in septin mutants yielded immotile sperm cells, and septins were found to be required for the formation of a functional sperm head-tail junction.

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Septins also have important functions in innate cellular immunity and virulence [19]. New studies expand our understanding of how septins recognize the membrane of actively dividing bacteria providing evidence for a cardiolipin-dependent interaction [20]. Similarly, septins coat the outside of vaccinia virus particles in inhibitory cage-like structures that limit cell-to-cell spread [21]. In another link to innate immunity, septin stability is associated with natural killer cell lytic granule release [22]. These roles in infection are not limited to animal hosts, as recent studies are revealing important roles for septins in fungal plant pathogens. Plant infection by the rice blast fungus *Magnaporthe oryzae* requires assembly of a septin ring at the base of the fungal appresporium – a cellular structure that breaks into plant cells via turgor pressure [23]. In the maize pathogenic fungus *Cochilobolus heterostrophus* septins are required for sexual reproduction and virulence [24]. Finally, a recently described role of septins in coping with ER-stress in budding yeast highlights a potential new function to consider in pathogenic fungi [25]. Thus, immunity and pathogenesis are growing areas of functional relevance for septin biology.

Although there is growing appreciation for the functional roles of septins in many different cellular processes, *how* septins perform these functions is less well understood. Nonetheless, recent research is shedding light on mechanistic principles of septin complex formation, membrane binding, and the assembly of higher order cytoskeletal structures. Here, we review these most recent advances.

Revisiting the subunit order of the septin complex

The structure of the septin complex and its subunit order has been a challenging and controversial subject of investigation in the septin field [26]. Studies of budding yeast and *Drosophila* septin complexes revealed that multiple septin proteins were arrayed in rod-shaped hetero-oligomeric complexes [27-29]. The supramolecular order of septin subunits within the oligomeric complex remained mysterious for over decade. A seminal paper in the septin field analyzed the structure of a mammalian complex [2]. Crystal structures revealed that within the oligomer, subunits interacted with one another between alternating N- and C-terminal and G-domain interfaces (NC and G interfaces, respectively). The mammalian oligomer, as well as was later shown for yeast [1], is an apolar hexamer and the data suggested Sept2 was positioned in the middle, with Sept6 in the penultimate position and Sept7 at the termini with its G interface facing out (Figure 1). Determination of the mammalian septin complex order was further complicated by inclusion of Sept3 subfamily subunits (e.g. Sept9), which interact strongly with Sept7 between their G-interfaces [30]. Based on the previously established order it was reasoned Sept9 would be at the termini of octameric complexes with its NC interface exposed [2].

A recent manuscript now provides new evidence that the mammalian septin complex as first postulated is actually inside out (Figure 1) [31]. The authors revisited the ordering using individual particle negative stain EM analysis of hexameric mammalian complexes. At physiological ionic conditions it was found that Sept2 was actually positioned at the hexamer termini [31]. This revised order has the critical implication that filament polymerization is actually dependent on Sept2's NC interface [2,31]. Molecular dynamics simulations suggest interactions between Sept2 NC interfaces is more sensitive to ionic

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strength, similar to yeast septin complexes which polymerize into filaments via NC interactions between terminal Cdc11 subunits [1,31,32].

A second study, posted synchronously on bioRxiv as [31], also supports the "reordering" of the septin complex [33]. Sooror et al. investigated whether oligomers with different subunits (e.g. octamers with Sept9 and hexamers without) could copolymerize into filaments [33]. To their surprise, hexamers and octamers did associate and copolymerized into long (micrometer) filaments. Based on the old proposed subunit order (e.g. 7-6-2-2-6-7 and 9-7-6-2-2-6-7-9), this would not be possible, since the model was that G-domain interface interactions and NC interface interactions were mutually exclusive. This data suggested Sept2 is at the termini of both hexamers *and* octamers and that Sept9, when present, is incorporated into the middle between Sept7 subunits Consistent with this hypothesis, budding yeast Cdc10, which is most closely related to Sept9 evolutionarily, is at the center of the yeast septin complex [34]. The ability of hexamers and octamers of different subunit compositions to polymerize into filaments raises the interesting possibility that basic biophysical septin filament properties (e.g. polymerization kinetics, filament flexibility, septin mediated association with downstream signaling proteins) is regulated by differentially swapping subunits within the core particle, and should prove a fruitful avenue for future research. A recent review covers in more detail the history of the field concerning the septin complex subunit ordering [35].

The interchangeability of supramolecular ordering between hexamers appears to be an ancient evolutionary trait that was once also shared by fungi and is dependent on septin GTPase activity [36]. Cdc3 subunits were found to dimerize between their G-interfaces in the absence of Cdc10 (much like the newly proposed model for Sept7 in the absence of Sept9), and this interaction is stabilized by a single point mutation (G261V) which is responsible for rescuing viability of *cdc10* mutants (Figure 1) [3]. The hexameric Cdc10-less fungal septin complex can alternatively be stabilized by additional guanidine hydrochloride [36]. Mutation and chimeric analysis suggest that Cdc3 preferentially dimerizes when bound to GDP - thus bypassing Cdc10 incorporation - to form Cdc10-less hexamers [36]. In certain fungal lineages including budding yeast, GTPase activity of Cdc3 was lost, thereby "enforcing" Cdc10 incorporation into octamers. Biochemical studies investigating the ordering of the septin complex are well supported by a recent bioinformatic analysis investigating septin evolution, which highlights the extraordinary conservation of septins across animals and fungi [37]. Auxier and colleagues point out that the residues most conserved across species are those that mediate septin complex formation via monomer interactions [37]. These recent studies [31,33,36,37] underscore how the supramolecular structure of septins is remarkably more conserved between species than had been previously surmised [1,2]. A major goal for the field is to now understand the functional and biophysical implications of oligomers that are made of distinct septin subunits.

Sensors of micron-scale membrane curvature

Septins bind membranes where they diffuse, collide with one another, and anneal into higher order assemblies [32]. Some higher order septin assemblies are at sites of membrane curvature, such as the mother-bud neck in yeast, or at the base of hyphal branches in

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filamentous fungi. Remarkably, septins can intrinsically "sense" positive-membrane curvature at the micron scale [38]. How do septins - rod-shaped protein complexes tens of nanometers in length - perceive membrane curvature orders of magnitude larger [39]? Other membrane curvature sensors, such as BAR domain proteins, possess an amphipathic helix (AH) domain that is critical for recognizing nanometer scale membrane curvature [40]. The AH has polybasic residues on one face and hydrophobic residues on the other, which is thought to enable the AH interact with the membrane by binding exposed hydrophobic lipid acyl chains (Figure 2) [39,41].

Membrane curvature is thought to induce lipid packing defects thereby presenting binding sites for amphipathic helixes. Although the nature of these defects is unclear at relatively shallow, micron-scale membrane curvatures [42], AH domains are conserved in other micron-curvature sensitive proteins such as SpoVM and MreB [43,44]. A recent study investigating the mechanisms of septin membrane-curvature sensitivity discovered that some septin subunits possess an AH at their C-terminus [45]. Using genetic and biophysical approaches, Cannon and colleagues determined that the septin AH domain is both necessary and sufficient for septins to distinguish membrane curvature (Figure 2) [45]. Additionally, the authors demonstrated that septin adsorption to curved membranes is a cooperative process, and that polymerized septin filaments co-align along optimal positive membrane curvature.

In a parallel study, another group found that septins at high concentrations could reshape membranes in giant unilamellar vesicles, and that septin filaments differentially assemble on positively and negatively curved membranes [46]. However, Beber et al. found that filaments avoid positive curvature preferentially aligning in dense filaments aligning with flat curvatures at the crest of supported lipid monolayer ridges or in the troughs with negative curvature [46]. The discrepancy in filament alignment could stem from several experimental differences including the use of lipid bilayers vs monolayers, the topology of the substrate of the membrane support, the lipid composition [45,46]. It will be important for future investigations to disentangle how variable conditions influence filament alignment on curved membranes.

Organizing higher order septin assemblies

Other recent work has investigated the dynamics and organizational progression of septin assemblies from cell polarity establishment through cytokinesis. Early genetic studies in the budding yeast *Saccharomyces cerevisiae* showed that recruitment and assembly of septins during cell polarity establishment at the plasma membrane is critically dependent on the master polarity regulator Cdc42, a small Rho GTPase, and numerous other polarity proteins [47-49]. Some polarity proteins directly interact with septins, and it has been recently documented that the Cdc42-activator also directly binds septins [50]. Polarity proteins can also recruit septins during ER stress to the previous cytokinesis site [25]. It has been unclear, however, how septins are initially assembled at the polarity site. It was previously proposed that septins localize to the polarity site as a diffuse patch, and that subsequent directed vesicle trafficking sculpted septins into a collar encircling the polarity site prior to bud emergence [51]. New evidence suggests, however, that vesicle trafficking is not necessary

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for septin recruitment or initial collar formation (Figure 3) [52]. Furthermore, Lai, et al. demonstrated that the initial assembly of septins is critically dependent on the cell-cycle dependent kinase (CDK), Cdc28 (budding yeast homolog of Cdc2) [52]. Interestingly, perturbations to the cell-cycle or cell polarity revealed that cell size and septin ring size are directly correlated [52,53]. These studies suggest that the CDK, in addition to Cdc42 and cell polarity proteins, is an important regulator in septin higher-order assembly.

After bud emergence in *S. cerevisiae*, septins are arrayed in an hourglass structure at the bud neck. Paired, axial filaments run along the neck parallel to the mother-bud axis (Figure 3) [54-57]. Prior to cytokinesis, septins undergo a dramatic rearrangement in which filaments change orientation by 90 degrees, ultimately forming two separate rings on opposing sides of the bud neck (Figure 3) [54,55]. Two new studies from the same group use fluorescence microscopy and platinum replica EM begin to investigate how specific regulators orchestrate the hourglass to split ring transition (Figure 3) [58,59]. EM analysis shows that that the contractile actomyosin ring (AMR) is precisely positioned between the split septin rings prior to cell division. Localization of the LKB1-like kinase Elm1 - a regulator of the budding yeast morphogenesis checkpoint pathway - to the septin hourglass structure is critical for the stability of the paired axial filaments, particularly filaments on the daughter side of the bud neck [58,60]. On the other hand, the anillin, Bud4 is important for hourglass organization on the mother side of the bud neck [59]. Mutants that perturb septin hourglass organization lead to poorly formed or nonexistent double split rings at cell division. Remarkably, even in the absence of the double split septin rings, activity of the AMR is unaffected [59], in agreement with another recent study which showed that the septins actually inhibit AMR during cytokinesis [61]. These investigations demonstrate how budding yeast remains an important model system in unraveling the regulatory processes orchestrating the assembly of higher order septin structures.

Regulators of actin and microtubules

There is growing appreciation for septins as important regulators in positioning and organizing *other* cytoskeletal networks [62]. Septins colocalize with a variety of actin assemblies and are thought to be important in the formation or maintenance of actin stress fibers, perinuclear actin networks, and actomyosin contractile ring at the cleavage furrow [63-67]. Septins are also important for abscission in the final stages of cytokinesis through their regulation of the ESCRT machinery [68]. In some instances, actin regulation is thought to be mediated by direct association of septins with actin [63,67,69,70]. In budding yeast, septins recruit the formin, Bnr1, which nucleates actin cables from the bud neck into the mother [71]. Newly published work demonstrates that septins pattern actin cables at the bud neck into “axial pillars” running parallel to the mother-bud axis [72]. The axial pillars are septin-actin cable filaments bundled by the F-BAR protein Hof1 and are important for the distribution and organization of actin cables into the mother cell. Another recent study found important roles for septins in the organization and activity of actin networks necessary for amoeboid migration and tissue invasion in melanoma cells [73]. The authors found that the septin regulator Cdc42EP5/Borg3 induces actomyosin rearrangements in a Sept9-dependent manner necessary for amoeboid-like cell migration and matrix invasion, both hallmarks of

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malignant melanoma [74,75]. In the absence of Sept9 or Cdc42EP5, melanoma cells round up and cortical actin networks are reduced.

There is also an emerging role for septins regulating microtubule bundling via post-translational modifications [62]. More recently, reconstitution experiments demonstrate that septins can modulate microtubule polymerization and stability dynamics [76,77]. In a concentration dependent manner, septins directly bind microtubules to differentially regulate microtubule polymerization kinetics. At nanomolar concentrations septins enhance plus end microtubule growth and suppress catastrophe events, whereas at micromolar concentrations septins stabilized microtubule lengths by suppressing dynamic instability [76,77]. Moreover, septins were found to displace the plus-end binding protein EB1 from microtubules, adding a further layer of septin regulation on microtubule dynamics [76]. Sept9 can also differentially tune kinesin motor activity [78]. In neurons, kinesin-3 motor activity is enhanced on microtubules decorated with Sept9, whereas kinesin-1 motor activity is inhibited. This biases cargo transport in dendrite specific manner in neurons, as dendrite-bound cargo is transported via kinesin-3 whereas axonal-bound cargo is transported via kinesin-1. These recent studies highlight how septins are finding newly appreciated roles as important scaffolds and regulators of both actin and microtubule assemblies.

Conclusion

Recent research in septin biology provide new perspectives on the evolution of the septin complex subunit order [31,33,36,37] give mechanistic insights into how septins recognize micron-scale membrane curvature [45,46], how higher order assemblies are dynamically arranged via septin regulators [52,58,59], and provide new evidence into how septins regulate other cytoskeletal networks [72,73,76,78]. Although septins are a burgeoning research topic within cell biology, many aspects remain uncharted. Future research will hopefully address the broader implications of the newly appreciated subunit ordering, uncover more mechanistic insights into septin-membrane interactions, and investigate septin filament polymerization dynamics to name a few open questions in the field of septins.

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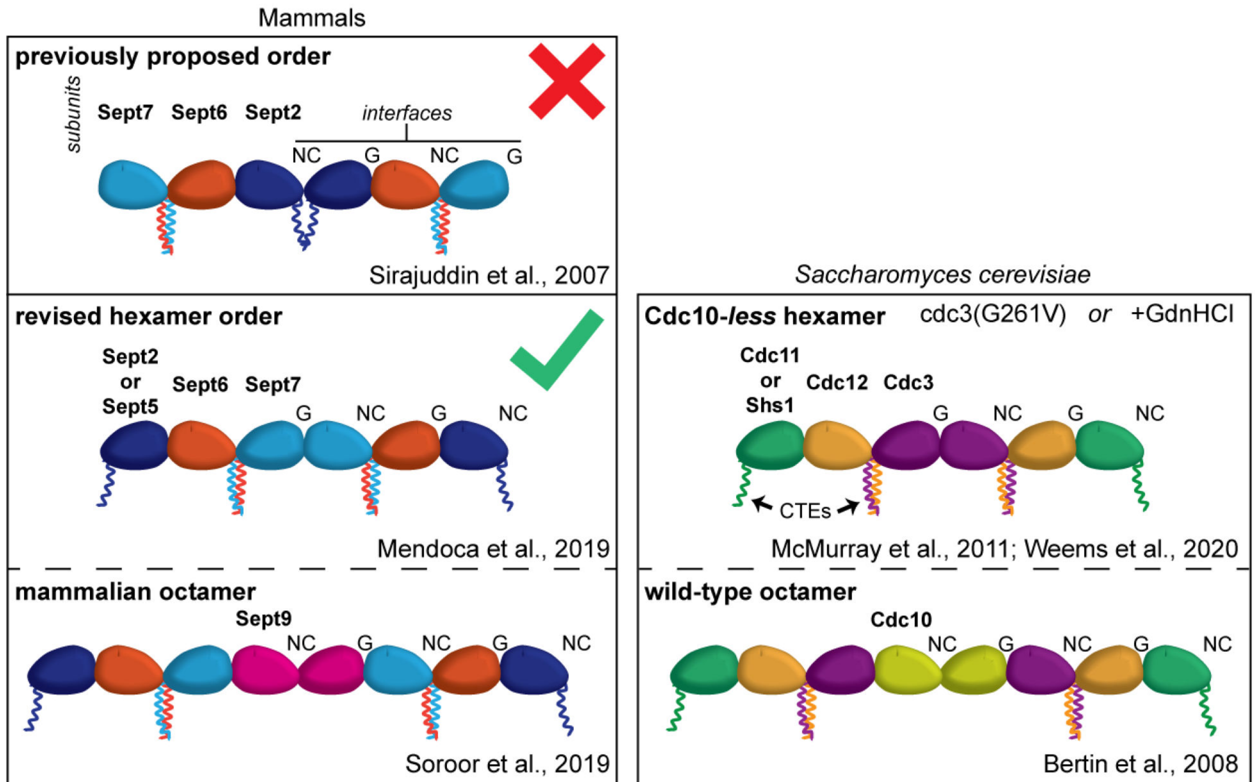

Figure 1. Subunit order of the mammalian and budding yeast septin complexes.

The original proposed subunit ordering of the mammalian septin complex positioned Sept2 in the middle and Sept7 at the termini, with Sept7's G-interface facing outward (top left). The subunit ordering has now been revised, with Sept7 positioned in the middle of the hexamer and Sept2 on the outside with its NC-interface facing outward. When present, Sept9 becomes the inner most subunit. This revised mammalian subunit ordering resembles the established ordering of the budding yeast septin complex (right panels).

NC: N- and C- terminal interface, G: G-domain interface, CTE: C-terminal extension.

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Figure 2. An amphipathic helix (AH) domain enables septins to recognize micron-scale membrane curvature.

(A) Septins preferentially bind lipid membranes with micron-scale curvature. (B) The penultimate subunit within the complex (e.g. Cdc12 in budding yeast, Sept6 in mammals) harbors an AH domain, which necessary and sufficient for septins to recognize membrane curvature. Inset is a helical wheel diagram of the Cdc12 AH domain (Heliquest). (C) Cartoon representation of an amphipathic helix within a lipid packing defect generated by membrane curvature.

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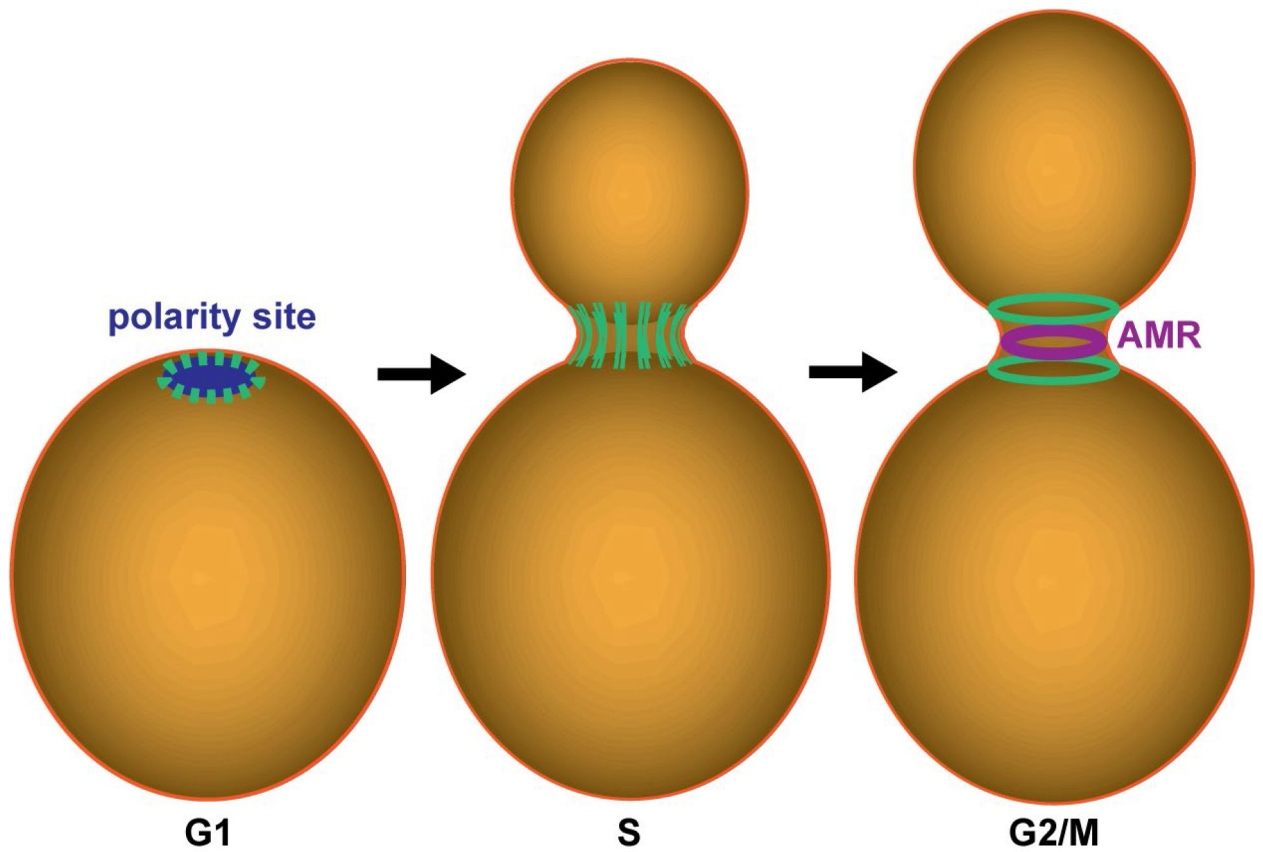

Figure 3. Subcellular localization of septins through the cell cycle in budding yeast.
Septins (green) localize around the polarity site early in the cell cycle. After bud emergence, septins are arrayed in parallel pairs at the bud neck - the site of positive plasma membrane curvature. Prior to cytokinesis septins rearrange into separate rings on other side of the bud neck and the actomyosin ring (AMR).