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The state of the septin cytoskeleton from assembly to function

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Abstract

Septins are conserved guanine nucleotide-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 the cytokinetic machinery, organization of the plasma membrane, and orientation of cell polarity. Mutations in septins or their misregulation are 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 with 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.

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Keyword

Amphipathic helix (AH) domain.

Introduction

Septins are found in many eukaryotes including fungi and metazoans but missing in land plants. The basic unit of septins are nonpolar, hetero-oligomers 17–32 nm 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 of cellular processes including cell division, cell migration, ciliogenesis, and neuronal branching [7–11]. Given their varied cellular functions, a growing body of literature implicates 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.

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 appressorium — 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 endoplasmic reticulum 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.

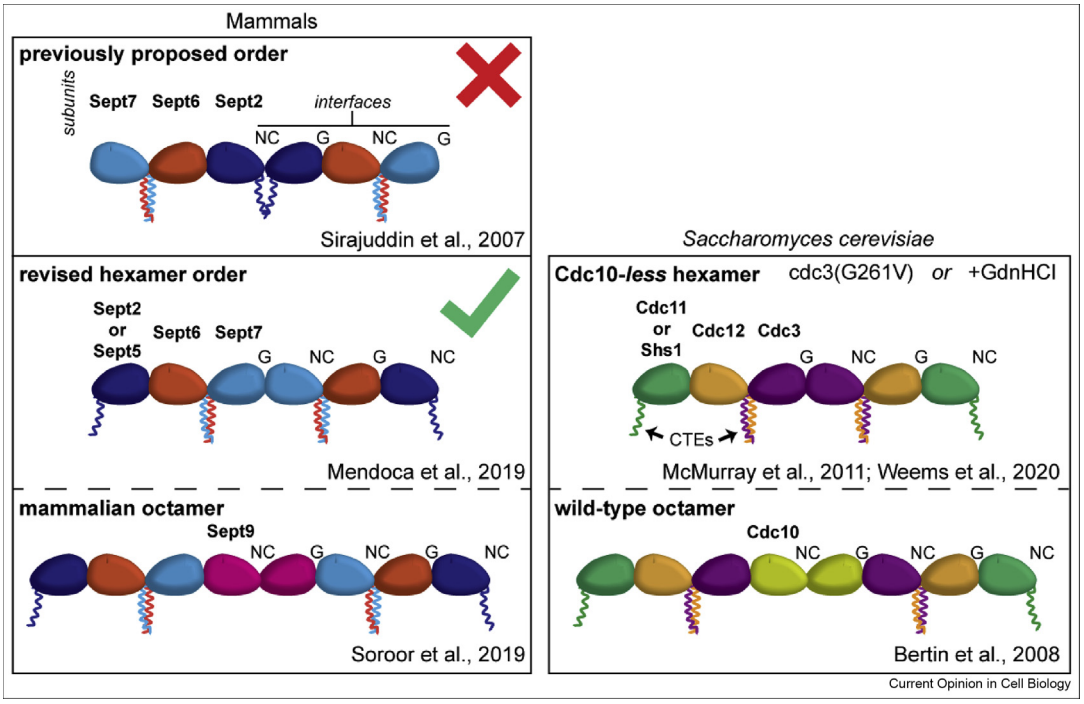
Although there is growing appreciation for the functional roles of septins in many different cellular processes, how septins perform these functions is less well understood. Nonetheless, recent research is shedding light on mechanistic principles of septin complex formation, membrane binding, and the 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 a 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 the alternating N- and C-terminal interface (NC) and G-domain interface (G). 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 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 strength, similar to yeast septin complexes which polymerize into filaments via NC-interface interactions between terminal Cdc11 subunits [1,31,32].

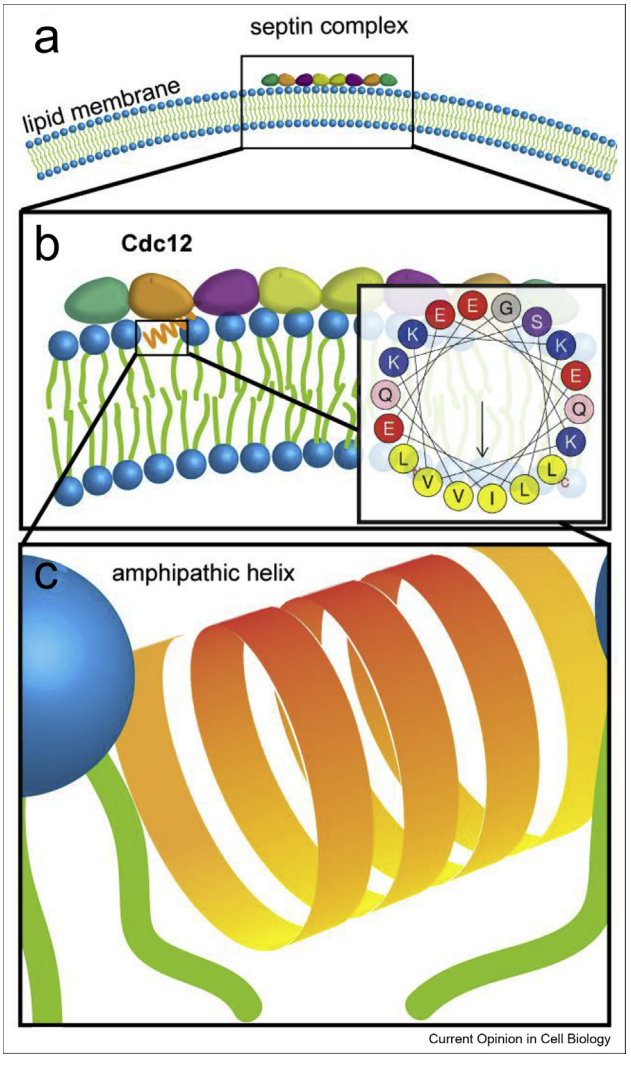
Figure 1



The 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-domain 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 innermost 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.

A second study, posted synchronously on bioRxiv as [31], also supports the 'reordering' of the septin complex [33]. Sooror et al. [33] investigated whether oligomers with different subunits (e.g. octamers with Sept9 and hexamers without) could co-polymerize into filaments. To their surprise, hexamers and octamers did associate and co-polymerized 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 because 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) are 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].

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 is necessary and sufficient for septins to recognize membrane curvature. The 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.

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 (ability to hydrolyze bound guanosine triphosphate) [36]. Cdc3 subunits were found to dimerize between their G–interfacesin 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 *cdc10Δ* 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 et al. [37] point out that the residues most conserved across species are those that mediate septin complex formation via monomer interactions. 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

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 (Bin, Amphiphysin and Rvs) 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].

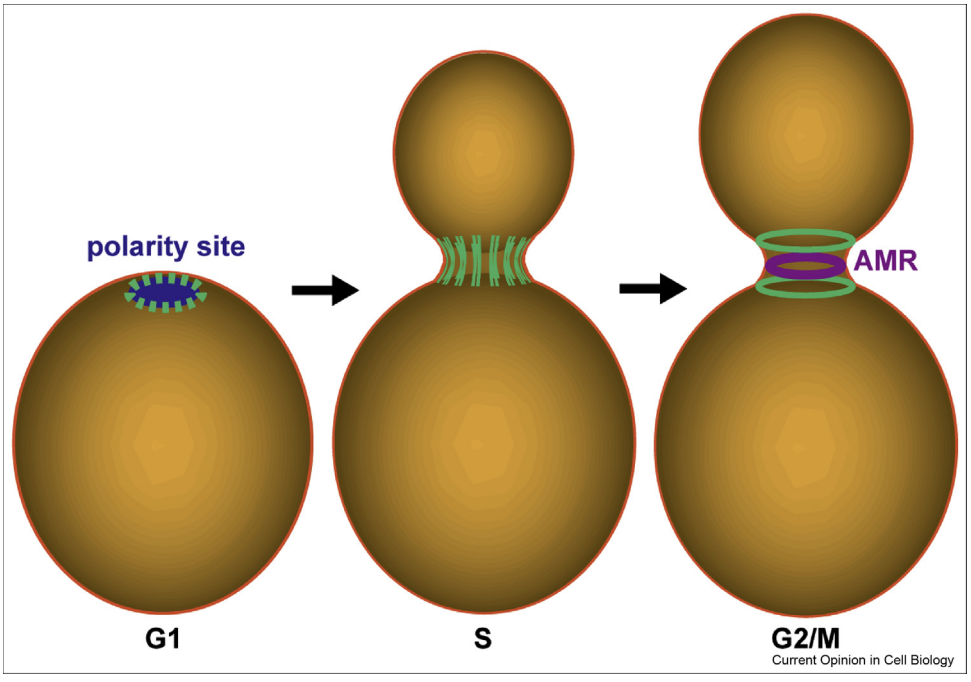
Membrane curvature is thought to induce lipid-packing defects thereby presenting binding sites for AH domains. Although the nature of these defects is unclear at relatively shallow, micron-scale membrane curvatures [42], AH domains are conserved in other micron-curvature sensitive proteins such as SpoVM 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 et al. [45] determined that the septin AH domain is both necessary and sufficient for septins to distinguish membrane curvature (Figure 2). In addition, 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. [46] 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. The discrepancy in filament alignment could stem from several experimental differences including the use of lipid bilayers versus monolayers, the topology of the substrate of the membrane support, and 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

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. Before cytokinesis septins rearrange into separate rings on other side of the bud neck and the actomyosin ring (AMR).

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 before bud emergence [51]. New evidence suggests, however, that vesicle trafficking is not necessary for septin recruitment or initial collar formation (Figure 3) [52]. Furthermore, Lai et al. [52] demonstrated that the initial assembly of septins is critically dependent on the cell cycle–dependent kinase, Cdc28 (budding yeast homolog of Cdc2). 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 cell cycle–dependent kinase, 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]. Before cytokinesis, septins undergo a dramatic rearrangement in which filaments change orientation by 90°, 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 the contractile actomyosin ring (AMR) is precisely positioned between the split septin rings before 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 rings at the cleavage furrow [63–67]. Septins are also important for abscission in the final stages of cytokinesis through their regulation of the (endocytic sorting complex required for transport (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 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 a 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] and 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.

Conflict of interest statement

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Bertin A, McMurray MA, Grob P, Park SS, Garcia 3rd G, Patanwala I, Ng HL, Alber T, Thorner J, Nogales E: **Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly**. *Proc Natl Acad Sci U S A* 2008, **105**:8274–8279.
2. Sirajuddin M, Farkasovsky M, Hauer F, Kuhlmann D, Macara IG, Weyand M, Stark H, Wittinghofer A: **Structural insight into filament formation by mammalian septins**. *Nature* 2007, **449**: 311–315.
3. McMurray MA, Bertin A, Garcia 3rd G, Lam L, Nogales E, Thorner J: **Septin filament formation is essential in budding yeast**. *Dev Cell* 2011, **20**:540–549.
4. DeMay BS, Meseroll RA, Occhipinti P, Gladfelter AS: **Regulation of distinct septin rings in a single cell by Elm1p and Gin4p kinases**. *Mol Biol Cell* 2009, **20**:2311–2326.
5. Kinoshita M, Field CM, Coughlin ML, Straight AF, Mitchison TJ: **Self- and actin-templated assembly of Mammalian septins**. *Dev Cell* 2002, **3**:791–802.
6. Bridges AA, Gladfelter AS: **Septin form and function at the cell cortex**. *J Biol Chem* 2015, **290**:17173–17180.
7. Oh Y, Bi E: **Septin structure and function in yeast and beyond**. *Trends Cell Biol* 2011, **21**:141–148.
8. Hall PA, Russell SH, Pringle JR: *The septins*. John Wiley & Sons; 2008.
9. Longtine MS, DeMarini DJ, Valencik ML, Al-Awar OS, Fares H, De Virgilio C, Pringle JR: **The septins: roles in cytokinesis and other processes**. *Curr Opin Cell Biol* 1996, **8**:106–119.
10. Mostowy S, Cossart P: **Septins: the fourth component of the cytoskeleton**. *Nat Rev Mol Cell Biol* 2012, **13**:183–194.
11. Dolat L, Hu Q, Spiliotis ET: **Septin functions in organ system physiology and pathology**. *Biol Chem* 2014, **395**:123–141.
12. Montagna C, Sagie M, Zechmeister J: **Mammalian septins in health and disease**. *Res Rep Biochem* 2015, **5**. 59-14.
13. Peterson EA, Petty EM: **Conquering the complex world of human septins: implications for health and disease**. *Clin Genet* 2010, **77**:511–524.
14. Angelis D, Spiliotis ET: **Septin mutations in human cancers**. *Front Cell Dev Biol* 2016, **4**:122.
15. Katz ZB, Zhang C, Quintana A, Lillemeier BF, Hogan PG: **Septins organize endoplasmic reticulum-plasma membrane junctions for STIM1-ORAI1 calcium signalling**. *Sci Rep* 2019, **9**:10839.
16. Deb BK, Chakraborty P, Gopurappilly R, Hasan G: **SEPT7 regulates Ca(2+) entry through Orai channels in human neural progenitor cells and neurons**. *Cell Calcium* 2020, **90**:102252.
17. Nurullin LF, Khuzakhmetova VF, Khaziev EF, Samigullin DV, Tsentsevitsky AN, Skorinkin AI, Bukharaeva EA, Vagin O: **Reorganization of septins modulates synaptic transmission at neuromuscular junctions**. *Neuroscience* 2019, **404**:91–101.
18. Shen YR, Wang HY, Tsai YC, Kuo YC, Wu SR, Wang CY, Kuo PL: **The SEPT12 complex is required for the establishment of a functional sperm head-tail junction**. *Mol Hum Reprod* 2020, **26**:402–412.
19. Robertin S, Mostowy S: **The history of septin biology and bacterial infection**. *Cell Microbiol* 2020, **22**:e13173.
20. Krokowski S, Lobato-Marquez D, Chastanet A, Pereira PM, Angelis D, Galea D, Larrouy-Maumus G, Henriques R, Spiliotis ET, Carballido-Lopez R, *et al.*: **Septins recognize and entrap dividing bacterial cells for delivery to lysosomes**. *Cell Host Microbe* 2018, **24**:866–874 e864.
21. Pfanzelter J, Mostowy S, Way M: **Septins suppress the release of vaccinia virus from infected cells**. *J Cell Biol* 2018, **217**: 2911–2929.
22. Phatarpekar PV, Overlee BL, Leehan A, Wilton KM, Ham H, Billadeau DD: **The septin cytoskeleton regulates natural killer cell lytic granule release**. *J Cell Biol* 2020:219.
23. Dulal N, Rogers A, Wang Y, Egan M: **Dynamic assembly of a higher-order septin structure during appressorium morphogenesis by the rice blast fungus**. *Fungal Genet Biol* 2020, **140**: 103385.
24. Zhang X, Gonzalez JB, Turgeon BG: **Septins are required for reproductive propagule development and virulence of the maize pathogen Cochliobolus heterostrophus**. *Fungal Genet Biol* 2020, **135**:103291.
25. Chao JT, Pina F, Onishi M, Cohen Y, Lai YS, Schuldiner M, Niwa M: **Transfer of the septin ring to cytokinetic remnants in ER stress directs age-sensitive cell-cycle Re-entry**. *Dev Cell* 2019, **51**:173–191 e175.
26. Mitchison TJ, Field CM: **Cytoskeleton: what does GTP do for septins?** *Curr Biol* 2002, **12**:R788–R790.
27. Frazier JA, Wong ML, Longtine MS, Pringle JR, Mann M, Mitchison TJ, Field C: **Polymerization of purified yeast septins: evidence that organized filament arrays may not be required for septin function**. *J Cell Biol* 1998, **143**:737–749.
28. Oegema K, Desai A, Wong ML, Mitchison TJ, Field CM: **Purification and assay of a septin complex from Drosophila embryos**. *Methods Enzymol* 1998, **298**:279–295.
29. Field CM, Al-Awar O, Rosenblatt J, Wong ML, Alberts B, Mitchison TJ: **A purified *Drosophila* septin complex forms filaments and exhibits GTPase activity**. *J Cell Biol* 1996, **133**: 605–616.
30. Nakahira M, Macedo JN, Seraphim TV, Cavalcante N, Souza TA, Damalio JC, Reyes LF, Assmann EM, Alborghetti MR, Garratt RC, *et al.*: **A draft of the human septin interactome**. *PloS One* 2010, **5**, e13799.

31. ** Mendonca DC, Macedo JN, Guimaraes SL, Barroso da Silva FL, Cassago A, Garratt RC, Portugal RV, Araujo APU: **A revised order of subunits in mammalian septin complexes**. *Cytoskeleton (Hoboken)* 2019, **76**:457–466.
Using negative stain EM and molecular dynamics simulations, the authors demonstrate that Sept2 subgroup subunits (Sept2 or Sept5) are positioned at the termini of mammalian septin complex. This revises the original model where Sept2 subunits were positioned on the inside.

32. Bridges AA, Zhang H, Mehta SB, Occhipinti P, Tani T, Gladfelter AS: **Septin assemblies form by diffusion-driven annealing on membranes**. *Proc Natl Acad Sci U S A* 2014, **111**: 2146–2151.

33. * Forooz Soroor MSK, Oliva Palander, Balachandran Yadu, Collins Richard, Benlekbir Samir, Rubinstein John, William S: **Trimble: revised subunit order of mammalian septin complexes explains their in vitro polymerization properties**. *bioRxiv* 2019, https://doi.org/10.1101/569871.
The authors reconstitute septin filament assembly co-incubating hexameric and octameric mammalian septin complexes with or without Sept9 revealing hexamers and octamers can co-polymerize into the same filament. These experiments suggest Sept9, when present, occupies the inner most subunit position, contrary to the original model positioning them at the termini.

34. Pan F, Malmberg RL, Momany M: **Analysis of septins across kingdoms reveals orthology and new motifs**. *BMC Evol Biol* 2007, **7**:103.

35. McMurray MA, Thorner J: **Turning it inside out: the organization of human septin heterooligomers**. *Cytoskeleton (Hoboken)* 2019, **76**:449–456.

36. * Johnson CR, Steingesser MG, Weems AD, Khan A, Gladfelter A, Bertin A, McMurray MA: **Guanidine hydrochloride reactivates an ancient septin hetero-oligomer assembly pathway in budding yeast**. *Elife* 2020, **9**.
The authors provide genetic and biochemical analyses that provides an evolutionary context as to why fungal septin complexes are biased towards forming octamers as opposed to hexamers.

37. * Auxier B, Dee J, Berbee ML, Momany M: **Diversity of opisthokont septin proteins reveals structural constraints and conserved motifs**. *BMC Evol Biol* 2019, **19**:4.
A thorough bionformatic and evolutionary analysis of the septin gene family across species.

38. Bridges AA, Jentzsch MS, Oakes PW, Occhipinti P, Gladfelter AS: **Micron-scale plasma membrane curvature is recognized by the septin cytoskeleton**. *J Cell Biol* 2016, **213**: 23–32.

39. Cannon KS, Woods BL, Gladfelter AS: **The unsolved problem of how cells sense micron-scale curvature**. *Trends Biochem Sci* 2017, **42**:961–976.

40. Drin G, Casella JF, Gautier R, Boehmer T, Schwartz TU, Antonny B: **A general amphipathic alpha-helical motif for sensing membrane curvature**. *Nat Struct Mol Biol* 2007, **14**: 138–146.

41. Gimenez-Andres M, Copic A, Antonny B: **The many faces of amphipathic helices**. *Biomolecules* 2018, **8**.

42. Hatzakis NS, Bhatia VK, Larsen J, Madsen KL, Bolinger PY, Kunding AH, Castillo J, Gether U, Hedegard P, Stamou D: **How curved membranes recruit amphipathic helices and protein anchoring motifs**. *Nat Chem Biol* 2009, **5**:835–841.

43. Ramamurthi KS, Lecuyer S, Stone HA, Losick R: **Geometric cue for protein localization in a bacterium**. *Science* 2009, **323**: 1354–1357.

44. Ursell TS, Nguyen J, Monds RD, Colavin A, Billings G, Ouzounov N, Gitai Z, Shaevitz JW, Huang KC: **Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization**. *Proc Natl Acad Sci U S A* 2014, **111**: E1025–E1034.

45. ** Cannon KS, Woods BL, Crutchley JM, Gladfelter AS: **An amphipathic helix enables septins to sense micrometer-scale membrane curvature**. *J Cell Biol* 2019, **218**:1128–1137.
The authors find that septin assembly onto positively curved membranes is cooperative and that septins have a conserved amphipathic helix (AH) domain. Using biochemical and genetic analyses the authors demonstrate that the AH is both necessary and sufficient for membrane curvature sensitivity.

46. * Beber A, Taveneau C, Nania M, Tsai FC, Di Cicco A, Bassereau P, Levy D, Cabral JT, Isambert H, Mangenot S, *et al.*: **Membrane reshaping by micrometric curvature sensitive septin filaments**. *Nat Commun* 2019, **10**:420.
Reconstituting septin assembly on giant unilamellar vesicles and supported lipid monolayers on undulating surfaces, the authors investigate the spatial patterning of septin filaments in membrane-curvature specific contexts.

47. Gladfelter AS, Bose I, Zyla TR, Bardes ES, Lew DJ: **Septin ring assembly involves cycles of GTP loading and hydrolysis by Cdc42p**. *J Cell Biol* 2002, **156**:315–326.

48. Gladfelter AS, Kozubowski L, Zyla TR, Lew DJ: **Interplay between septin organization, cell cycle and cell shape in yeast**. *J Cell Sci* 2005, **118**:1617–1628.

49. Gladfelter AS, Zyla TR, Lew DJ: **Genetic interactions among regulators of septin organization**. *Eukaryot Cell* 2004, **3**: 847–854.

50. Chollet J, Dunkler A, Bauerle A, Vivero-Pol L, Mulaw MA, Gronemeyer T, Johnsson N: **Cdc24 interacts with septins to create a positive feedback loop during bud site assembly in yeast**. *J Cell Sci* 2020:133.

51. Okada S, Leda M, Hanna J, Savage NS, Bi E, Goryachev AB: **Daughter cell identity emerges from the interplay of Cdc42, septins, and exocytosis**. *Dev Cell* 2013, **26**:148–161.

52. * Lai H, Chiou JG, Zhurikhina A, Zyla TR, Tsygankov D, Lew DJ: **Temporal regulation of morphogenetic events in Saccharomyces cerevisiae**. *Mol Biol Cell* 2018, **29**:2069–2083.
The authors investigate the dynamics of septin assembly relative to the cell-cycle and the timing of exocytosis revealing that septin recruitment to the polarity site is finely tuned by the CDK, but not by actin.

53. Kukhtevich IV, Lohrberg N, Padovani F, Schneider R, Schmoller KM: **Cell size sets the diameter of the budding yeast contractile ring**. *Nat Commun* 2020, **11**:2952.

54. DeMay BS, Bai X, Howard L, Occhipinti P, Meseroll RA, Spiliotis ET, Oldenbourg R, Gladfelter AS: **Septin filaments exhibit a dynamic, paired organization that is conserved from yeast to mammals**. *J Cell Biol* 2011, **193**:1065–1081.

55. Ong K, Wloka C, Okada S, Svitkina T, Bi E: **Architecture and dynamic remodelling of the septin cytoskeleton during the cell cycle**. *Nat Commun* 2014, **5**:5698.

56. Vrabioiu AM, Mitchison TJ: **Structural insights into yeast septin organization from polarized fluorescence microscopy**. *Nature* 2006, **443**:466–469.

57. Vrabioiu AM, Mitchison TJ: **Symmetry of septin hourglass and ring structures**. *J Mol Biol* 2007, **372**:37–49.

58. Marquardt J, Chen X, Bi E: **Architecture, remodeling, and functions of the septin cytoskeleton**. *Cytoskeleton (Hoboken)* 2019, **76**:7–14.

59. ** Chen X, Wang K, Svitkina T, Bi E: **Critical roles of a RhoGEF-anillin module in septin architectural remodeling during cytokinesis**. *Curr Biol* 2020, **30**:1477–1490 e1473.
Using live cell fluorescence microscopy and platinum replica electron microscopy on unroofed yeast spheroplasts, the authors provide exquisite images of septin hourglass and double ring structures in wild-type cells and in different regulatory mutants.

60. Kang H, Tsygankov D, Lew DJ: **Sensing a bud in the yeast morphogenesis checkpoint: a role for Elm1**. *Mol Biol Cell* 2016, **27**:1764–1775.

61. Tamborrini D, Juanes MA, Ibanes S, Rancati G, Piatti S: **Recruitment of the mitotic exit network to yeast centrosomes couples septin displacement to actomyosin constriction**. *Nat Commun* 2018, **9**:4308.

62. Spiliotis ET: **Spatial effects - site-specific regulation of actin and microtubule organization by septin GTPases**. *J Cell Sci* 2018:131.

63. Calvo F, Ranftl R, Hooper S, Farrugia AJ, Moeendarbary E, Bruckbauer A, Batista F, Charras G, Sahai E: **Cdc42EP3/BORG2 and septin network enables mechano-transduction and the emergence of cancer-associated fibroblasts**. *Cell Rep* 2015, **13**:2699–2714.

64. Verdier-Pinard P, Salaun D, Bouguenina H, Shimada S, Pophillat M, Audebert S, Agavnian E, Coslet S, Charafe-Jauffret E, Tachibana T, *et al.*: **Septin 9_i2 is downregulated in tumors, impairs cancer cell migration and alters subnuclear actin filaments**. *Sci Rep* 2017, **7**:44976.

65. Kinoshita M, Kumar S, Mizoguchi A, Ide C, Kinoshita A, Haraguchi T, Hiraoka Y, Noda M: **Nedd5, a mammalian septin, is a novel cytoskeletal component interacting with actin-based structures**. *Genes Dev* 1997, **11**:1535–1547.

66. Spiliotis ET, Kinoshita M, Nelson WJ: **A mitotic septin scaffold required for Mammalian chromosome congression and segregation**. *Science* 2005, **307**:1781–1785.

67. Mavrakis M, Azou-Gros Y, Tsai FC, Alvarado J, Bertin A, Iv F, Kress A, Brasselet S, Koenderink GH, Lecuit T: **Septins promote F-actin ring formation by crosslinking actin filaments into curved bundles**. *Nat Cell Biol* 2014, **16**:322–334.

68. Karasmanis EP, Hwang D, Nakos K, Bowen JR, Angelis D, Spiliotis ET: **A septin double ring controls the spatiotemporal organization of the ESCRT machinery in cytokinetic abscission**. *Curr Biol* 2019, **29**:2174–2182 e2177.

69. Smith C, Dolat L, Angelis D, Forgacs E, Spiliotis ET, Galkin VE: **Septin 9 exhibits polymorphic binding to F-actin and inhibits myosin and cofilin activity**. *J Mol Biol* 2015, **427**:3273–3284.

70. Dolat L, Hunyara JL, Bowen JR, Karasmanis EP, Elgawly M, Galkin VE, Spiliotis ET: **Septins promote stress fiber-mediated maturation of focal adhesions and renal epithelial motility**. *J Cell Biol* 2014, **207**:225–235.

71. Pruyne D, Gao L, Bi E, Bretscher A: **Stable and dynamic axes of polarity use distinct formin isoforms in budding yeast**. *Mol Biol Cell* 2004, **15**:4971–4989.

72. ** Garabedian MV, Wirshing A, Vakhrusheva A, Turegun B, Sokolova OS, Goode BL: **A septin-Hof1 scaffold at the yeast bud neck binds and organizes actin cables**. *Mol Biol Cell* 2020, **31**:1988–2001.

Using genetics and biochemical reconstitution experiments, the authors demonstrate how septins regulate actin cable patterning through direct association and bundling via the F-Bar protein, Hof1.

73. * Farrugia AJ, Rodriguez J, Orgaz JL, Lucas M, Sanz-Moreno V, Calvo F: **CDC42EP5/BORG3 modulates SEPT9 to promote actomyosin function, migration, and invasion**. *J Cell Biol* 2020:219.

The authors use a melanoma model system to explore how septins regulate cortical actin assembly, and by extension, amoeboid-like movement of metastatic cancer cells.

74. Lo JA, Fisher DE: **The melanoma revolution: from UV carcinogenesis to a new era in therapeutics**. *Science* 2014, **346**:945–949.

75. Georgouli M, Herraiz C, Crosas-Molist E, Fanshawe B, Maiques O, Perdrix A, Pandya P, Rodriguez-Hernandez I, Ilieva KM, Cantelli G, *et al.*: **Regional activation of myosin II in cancer cells drives tumor progression via a secretory cross-talk with the immune microenvironment**. *Cell* 2019, **176**:757–774 e723.

76. * Nakos K, Radler MR, Spiliotis ET: **Septin 2/6/7 complexes tune microtubule plus-end growth and EB1 binding in a concentration- and filament-dependent manner**. *Mol Biol Cell* 2019, **30**:2913–2928.

Reconstitution experiments demonstrate how septins can tune microtubule polymerization dynamics in a concentration dependent manner.

77. Nakos K, Rosenberg M, Spiliotis ET: **Regulation of microtubule plus end dynamics by septin 9**. *Cytoskeleton (Hoboken)* 2019, **76**:83–91.

78. * Karasmanis EP, Phan CT, Angelis D, Kesisova IA, Hoogenraad CC, McKenney RJ, Spiliotis ET: **Polarity of neuronal membrane traffic requires sorting of kinesin motor cargo during entry into dendrites by a microtubule-associated septin**. *Dev Cell* 2018, **46**:204–218 e207.

The authors discover that Sept9 is a dendritic microtubule-associated protein that modulates specific kinesin motor activity that biases directional membrane trafficking.