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SPOTLIGHT

The septin cytoskeleton: Heteromer composition defines filament function

Gizem Özbaykal Güler[1] and Serge Mostowy[1]

Septins are an evolutionarily conserved protein family whose members form hetero-oligomeric complexes that assemble into filaments and higher-order structures. In this issue, Martins et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202203016) and Cannon et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202204063) report that heteromer composition impacts the physiological role of septin filaments in yeast and human cells.

Septins are GTP-binding proteins discovered in yeast for their role in cytokinesis. Since then, septins have been shown to participate in many important cellular processes, ranging from morphogenesis to host–pathogen interactions (1, 2). Different members of the septin protein family are grouped together based on sequence homology, and septins from different groups can form non-polar hetero-oligomeric complexes that assemble into filaments and higher-order structures such as rings or gauzes (Fig. 1 A). Septins preferentially localize to membranes presenting micron-scale curvature and are well known to associate with actin filaments and microtubules (1, 3), but whether they do so as heteromers, polymers, or higher-order structures is poorly understood.

Curiously, the number of septin genes varies across eukaryotes (4). For example, *Saccharomyces cerevisiae* has 7 septin genes, while *Caenorhabditis elegans* has 2, *Drosophila melanogaster* has 5, and *Homo sapiens* has 13. In humans and other vertebrates (including zebrafish and mice), the increase in the number of septin genes increases the pool of possible heteromer combinations, suggesting a greater potential for functional specificity. It is widely viewed that septins from the same homology group can replace each other when forming a heteromer, referred to as "Kinoshita's rule" (5), but it is unclear if this type of replacement translates into functional differentiation. While functional redundancy within members of the same septin group may provide robustness, the vast number of possible septin heteromer combinations has hindered our ability to decipher the function of each septin or the assemblies they collectively form. In this issue of *JCB*, two independent studies set out to address the division of labor among septin filaments comprised of distinct heteromer combinations and report that the molecular composition of septin heteromers have important consequences on their physiological role as filaments in yeast and human cells (6, 7).

A gene duplication in *Ashbya gossypii* impacts septin function

The filamentous fungus *A. gossypii* is a model organism that has provided fundamental insights into septin assembly and function. The septins in *A. gossypii* assemble into octamers with palindromic arrangement of two hetero-tetramers (Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11; Fig. 1 A). In this issue, Cannon et al. (6) exploit a gene duplication event unique to species of *Ashbya* resulting in two Cdc11 proteins of high sequence similarity (i.e., Cdc11a and Cdc11b) to investigate if their presence in octamers differentially impacts filament properties and function (Fig. 1 B). The authors first observed that the localization and relative expression levels of Cdc11a and Cdc11b were dependent upon *A. gossypii* developmental stage and local cell morphology. Motivated by these observations, the authors tested functional redundancy between Cdc11a and Cdc11b by characterizing the impact of gene deletions on *A. gossypii* growth. In this case, each single mutant had a different consequence on fungal morphology (e.g., tip-splitting defects or larger hyphal diameters), indicating distinct roles for Cdc11a and Cdc11b in cellular development.

Septins are curvature sensing proteins (3), and septin filaments are known to be enriched at regions of *A. gossypii* that exhibit micron-scale curvature such as tip splits and branching points. However, the precise cellular localization of septin filaments may depend on their heteromer composition and biophysical properties. To test this, Cannon et al. (6) used an in vitro reconstitution system with supported lipid bilayers and studied properties of septin filaments composed of octamers capped by either Cdc11a or Cdc11b. The two filament types similarly localized to regions of micron-scale curvature but displayed significantly different length distributions and membrane surface coverage abilities. Here, Cdc11b-capped octamers were found to form shorter filaments than Cdc11a-capped octamers with increased density on membranes. The

[1]Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, UK.

Correspondence to Serge Mostowy: serge.mostowy@lshtm.ac.uk.

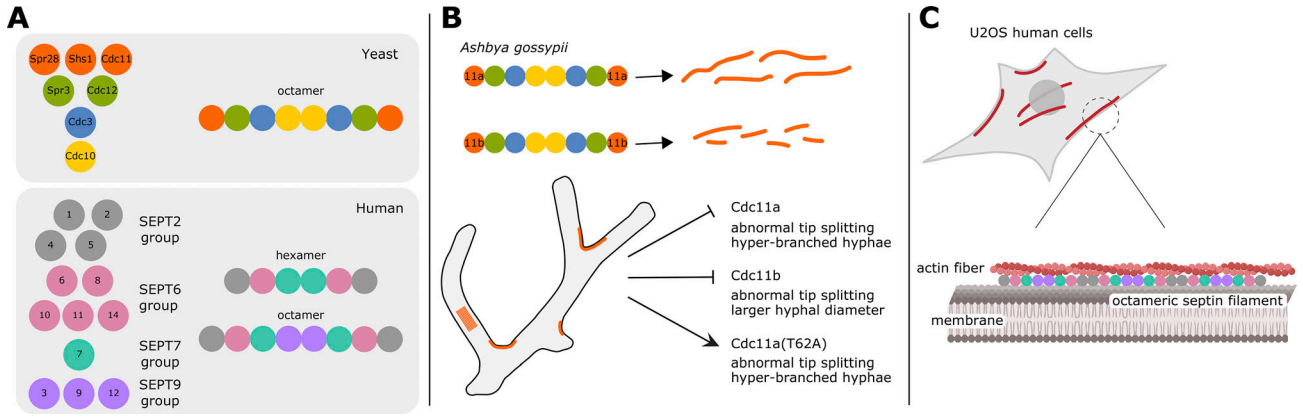

Figure 1. **Septin filament and function in yeast and human cells. (A)** Illustration showing members of the septin protein family in yeast (top) and humans (bottom), as well as homology groups in humans and proposed hetero-oligomeric complexes. **(B)** Illustration showing Cdc11a- or Cdc11b-capped octamers that form long and short filaments, respectively (top). Cartoon of *A. gossypii* morphology, sites of septin enrichment, and shape defects observed upon listed mutations (bottom; as shown by Cannon et al.). **(C)** Cartoon showing actin stress fibers in U2OS human cells (top), and illustration of how septins connect actin to membranes (bottom; as shown by Martins et al.).

authors suggest that different end-on interaction strengths between Cdc11 proteins can dictate filament length, which in turn influences membrane off-rates, leading to differences in packing efficiency on membranes.

Sequence and structural variation between the two septin proteins led authors to discover that a single amino acid mutation in Cdc11a(T62A) controls in vitro biophysical properties of Cdc11a-capped filaments, causing them to mimic features of Cdc11b-capped filaments. Next, the authors introduced the Cdc11a(T62A) mutation in *A. gossypii* and followed its development in vivo. Strikingly, the mutant displayed morphological defects that are similar to the Cdc11a deletion mutant (i.e., hyperbranching and abnormal tip splits), showing that a single amino acid mutation which alters filament properties can significantly impact septin function.

Collectively, these data from *A. gossypii* highlight that heteromer composition endows septin filaments with distinct biophysical properties and cellular functions, and may suggest physiological relevance for the increased number of septin genes observed in higher eukaryotes.

Human septins function as octamers when associated with actin stress fibers

In human cells, septins are predominantly found as hexamers and octamers with palindromic arrangement of two hetero-trimers (SEPT2-SEPT6-SEPT7-SEPT7-SEPT6-SEPT2) or two hetero-tetramers (SEPT2-SEPT6-SEPT7-SEPT9-SEPT9-SEPT7-SEPT6-SEPT2), respectively (8, 9; Fig. 1 A). Although the role of septins in human cells has been the subject of intense investigation for many years, whether hexamers or octamers contribute distinct cellular roles is poorly understood. In this issue of *JCB*, Martins et al. (7) use the association of septins to actin stress fibers in epithelial cells as a model system to study the relationship between septin heteromer composition and filament function (Fig. 1 C).

By using a clever combination of split-GFP constructs and septin–septin interaction mutants, the authors show that septins associate with actin stress fibers as filaments and not as individual heteromers. By targeting heteromer formation, they show stress fiber associated septin filaments are purely composed of octamers, and not hexamers, consistent with previous observations testing for SEPT9 (10). Experiments using atomic force microscopy showed cell stiffness was significantly decreased in the absence of octamers, and not in the absence of hexamers, indicating that stress fiber associated octamers play a major role in cellular mechanics.

In vitro reconstitution studies using purified septin proteins and supported lipid bilayers have shown that septins can bind synthetic membranes (3, 11); however, the molecular details of membrane binding, and whether in vitro binding reflects in vivo conditions, is not fully known. To validate septin–membrane associations in vivo, the authors used an approach based on metal-induced energy transfer and showed that septin filaments, unlike actin stress fibers, directly associate with membranes in cells. Furthermore, using an in vitro reconstitution system, the authors observed that actin was membrane associated only in the presence of septin filaments. Taken together, these experiments showed that septins can simultaneously associate with both membrane and actin, and suggest that septin filaments comprised of octamers anchor actin stress fibers to the plasma membrane.

In summary, Martins et al. (7) highlight the importance of human septin octamers in filament function and cellular physiology. This work also generates molecular biology tools of great interest to address key questions in the septin field regarding heteromer composition and the interaction of septin assemblies with membrane and/or other cytoskeleton components.

Perspectives

The first x-ray structure of a human septin complex transformed the field, revealing interaction interfaces essential for septin heteromer composition and higher-order assembly (8). The septin protein family, although highly conserved across eukaryotes, presents a vast combinatorial diversity (e.g., Kinoshita's rule) and structural plasticity (e.g., filaments, bundles, gauzes, rings, cage-like structures). The use of purified septin proteins and in vitro reconstitution systems have revolutionized our fundamental understanding of septin biology, shedding light on septin polymerization characteristics and membrane interactions (3, 11, 12).

In yeast or human cells, the cell cycle or tissue-specific expression of different septins may modulate the composition of septin heteromers and may therefore determine the building blocks of filaments and their biophysical properties. However, the co-existence of different septin heteromers may point towards a "septin code" underlying a functional specificity essential for different cellular roles. In agreement with this, Cannon et al. (6) show that a gene duplication in *A. gossypii* results in functionally distinct septin filaments crucial for development, and Martins et al. (7) show that octamer organization in human cells underlies the role of septin filaments interacting with membrane and actin stress fibers. Considering both studies, it is next of great interest to understand the breadth of septin assemblies available in cells and what this implies for their higher-order assembly and function.

Septins are involved in a wide variety of physiological processes by acting as curvature sensors, molecular scaffolds, and diffusion barriers (1, 2). Hexamer- and octamer-based polymers remain the most studied septin filament types; however, work is required to build a more complete view of septin assemblies and their many cellular functions. Considering the important roles for septins in human health and disease (1), a better understanding of septin heteromers and filament function may one day have clinical implications.

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