

Septins May Form a Ubiquitous Family of Cytoskeletal Filaments

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THIS minireview discusses recent information about the septin family of proteins, which suggests that the septins may be elements of a new filament system that functions in all or most eukaryotic cells. Septins are found in a wide variety of eukaryotes, are important for cytokinesis, and may compose or regulate a ubiquitous filament system that has not been previously recognized. A more extensive review of septins was recently published (26). This minireview also presents a new comparison of septin sequences.

Actin filaments, microtubules, intermediate filaments, and myosin thick filaments, have been extensively studied over the past several decades, using methodologic advances in electron and light microscopy, detergent extraction of cells, and purification of proteins. This collection of filaments has been assumed to represent a fairly complete picture of the cytoskeletal filaments commonly found in eukaryotes. However, recent work suggests that cells augment these common cytoskeletal elements with additional elements, at least one of which, the septins, appears to be widely expressed and used for essential cell processes.

Neck Filaments and Septins

A set of ~10-nm filaments associated with the mother/bud neck of *Saccharomyces cerevisiae*, was discovered by thin section electron microscopy (4, 5). These "neck filaments" are in a plane perpendicular to the mother/bud axis and are very near the plasma membrane. These properties are similar to those of the actin filaments in the contractile ring associated with cytokinesis in animal cells. However, the yeast neck filaments are observed during bud growth but not cytokinesis, unlike the actin filaments of the contractile ring of animal cells. Subsequent localization of actin in budding yeast confirmed the disparity between the neck filaments and actin, in that actin is present at the neck during bud emergence and cytokinesis, but not during bud growth, a temporal pattern clearly distinct from that of the neck filaments (1, 22).

A family of proteins, termed septins, are essential for neck filament assembly and may indeed be the primary structural components of the neck filaments. Septins were discovered in *S. cerevisiae* through the analysis of cell divi-

sion cycle (*cdc*)¹ mutants by Pringle, Hartwell, and colleagues. Mutations in any one of four septin genes—*CDC3*, *CDC10*, *CDC11*, and *CDC12*—prevent cytokinesis, leading to an accumulation of large multinucleated multiply budded cells in conditional mutants at the restrictive temperature (1, 17). The protein products of these four genes are located at the mother/bud neck by immunofluorescence microscopy (15, 16, 24).

Sequence Comparison

The amino acid sequences predicted for the Cdc3, 10, 11, and 12 proteins are similar to each other and originally defined the septins as a family (26). Septins have now been identified in a broad spectrum of eukaryotes by molecular and genetic approaches (12, 26, 31, 32). Their amino acid sequences are not similar to those of other proteins, except for the presence of a P-loop consensus for nucleotide binding (26).

One important feature of the sequence analysis is the absence of a high degree of sequence similarity between individual septins in evolutionarily distant species. That is, a given septin from a metazoan species, such as *Drosophila* Pnut, Sep1, or Sep2, is not the obvious orthologue of any particular yeast septin (Fig. 1). The metazoan sequences cluster together away from those of the lower organisms. The sequences of the lower organism septins do define groups that cross a wide evolutionary distance (*S. cerevisiae* to *S. pombe*), but the evolutionary distances between these groups are not large, as manifest by branch points that are relatively close to the center of the tree (Fig. 1). In such a phylogenetic analysis, septins appear to differ from tubulins, myosins, and actin-related proteins, where orthologues in widely divergent organisms are readily identified, and groups are separated by larger distances (29, 33, 41). Current information from biological analyses indicates that orthologues have similar functions in different organisms (e.g., 29, 33, 41). Therefore, the absence of orthologues in the septin sequence analysis suggests that individual septins may not have unique conserved functions. All four yeast genes are necessary at wild-type expression levels, but whether overexpression of one can suppress the loss of another is not yet known. In this scenario, septins may function in cell division as a group, and individual septins could substitute for each

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1. Abbreviation used in this paper: *cdc*, cell division cycle.

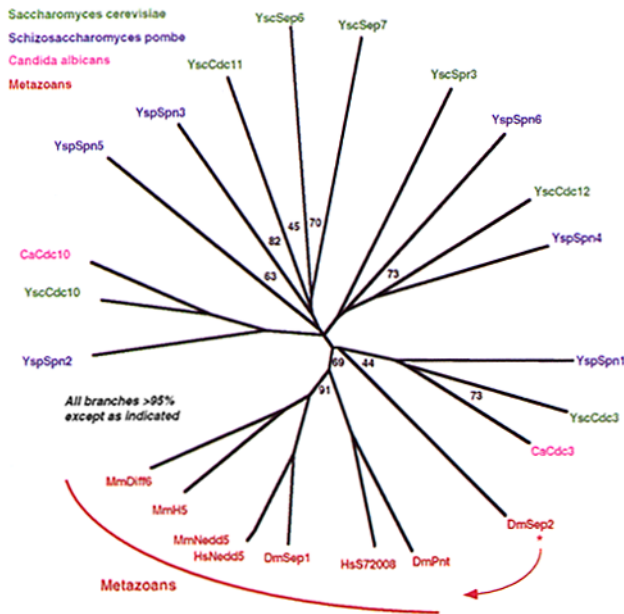


Figure 1. Phylogenetic tree of septins. GenBank was searched with TBLastN (2) using *Saccharomyces cerevisiae* Cdc10p, which was chosen because it lacks the predicted coiled-coil sequence of most septins. ClustalW was used for the analysis (42). The numbers on the tree indicate branch points occurring with a frequency <95% in the bootstrap analysis. All unlabeled branch points have frequencies $\geq 95\%$. All the metazoan sequences fall into one group, with the exception of *Drosophila* Sep2 (DmSep2). However, DmSep2 is connected to the group with YscCdc3 by a low frequency branch point (41%), and in analyses where NH₂-terminal or COOH-terminal nonhomologous regions are removed from the alignment, DmSep2 does fall into the adjacent metazoan group. Therefore, placement of DmSep2 in the phylogenetic tree is uncertain. Abbreviations on the diagram: Ysc, *Saccharomyces cerevisiae*; Ysp, *Schizosaccharomyces pombe*; Ca, *Candida albicans*; Dm, *Drosophila melanogaster*; Mm, mouse; Hs, human. The accession numbers for these sequences are: YscSpr3-L31767, YspSpn6-Z66569, YscCdc12-L16551, YspSpn4-U29890, YspSpn1-U31742, YscCdc3-L16548, CaCdc3-Z25869, DmSep2-U28966, DmPnt-U08103, HsS72008-S72008, DmSep1-L33246, HsNedd5-D28540, MmNedd5-D49382, MmH5-X61452, MmDiff6-M37030, YspSpn2-U29888, YscCdc10-L16549, CaCdc10-Z25870, YspSpn5-U29891, YspSpn3-U29889, YscCdc11-L16550, YscSep6-Z48612, YscSep7-D45 or D50, Chromosome IV 50,000 to 55,000.

other to a certain extent. Additional evidence supporting the hypothesis that septins have different roles in different cell types and organisms are the observations that septins are found outside of the cell division site in *Drosophila* (12) and that septins are apparently involved in spore-wall formation in yeast (11).

Purification and Characterization of Septin Proteins

A recent issue of this Journal includes an important new piece of the story, purified septins can form filaments in vitro (13). Field and colleagues purified a complex of three septin polypeptides from *Drosophila* embryos using affinity chromatography with antibodies prepared against a

COOH-terminal peptide from one septin. The three septins in the complex, Pnut, Sep1, and Sep2, had been identified previously by genetic or molecular biological approaches. The complex appears to contain two copies of each of the three polypeptides, possibly as a heterotrimer of homodimers. Homodimer formation might be mediated by short regions of predicted coiled-coil structure near the COOH terminus of each septin.

The purified septin complex formed 7-nm-wide filaments. The lengths of individual septin filaments were multiples of 26 nm, suggesting that the septin filament is a linear array of subunits 26-nm long and 7-nm wide. However, individual filaments did not have a detectable 26-nm periodic substructure along their length or any other properties indicative of a helical structure. Many short filaments and a few long filaments were seen. This initial analysis of filament length distribution suggests that the septin assembly process is simple linear polymerization without nucleation, in contrast to actin and microtubules, which are nucleation/condensation polymerization systems (19, 34). Individual septin filaments aggregated laterally into coarse bundles at high concentration.

The 7-nm width of the purified septin filaments suggests that they may correspond to the 10-nm-wide neck filaments in vivo. However, the neck filaments are spaced 28-nm apart in vivo, so the 26-nm length of the septin subunits and the observation that filaments aggregated laterally prompted Field and colleagues to suggest an alternative model in which the septin filaments are oriented perpendicular to the neck filaments and parallel to the mother/bud axis. In this model, what is observed as the neck filament in thin section electron microscopy corresponds to a linear array of one heavily stained region of the septin subunits.

The purified *Drosophila* septin complex contains ~ 1 mol of guanine nucleotide per mol of septin polypeptide, and the complex can bind and catalyze the hydrolysis of GTP, consistent with the presence of a P-loop consensus in the primary sequence (13). The presence of bound nucleotide and the hydrolysis of that nucleotide are important determinants of the assembly properties of actin and tubulin, but as yet septin assembly is not known to depend on nucleotide binding or hydrolysis.

The finding by Field and colleagues that three *Drosophila* septins, Pnut, Sep1, and Sep2, are purified as a complex supports and extends other recent observations. Two *Drosophila* septins, Pnut and Sep1, immunoprecipitate with each other and cosediment from whole-cell extracts (12). The three *Drosophila* septins colocalize in vivo (12, 26, 32), and loss of Pnut function causes a loss of Sep1 staining (12). Four yeast septins (Cdc3, 10, 11, and 12) colocalize at the mother-bud neck, and mutation of any one of these four genes causes a loss of all four septins from the neck (15, 16, 24, 26).

Current Questions and Future Directions

The study of the septins is still in its infancy, and many basic questions remain to be addressed.

(1) *Are the Neck Filaments Composed Primarily of Septins?* In budding yeast, mutations in septin genes abolish the neck filaments, and the location of septins by light mi-

crosscopy overlaps with that of the neck filaments by electron microscopy. However, septins and the neck filaments have never been visualized and identified in the same ultrastructural image, to confirm that the distributions overlap at high resolution.

In unpublished work with conditional septin mutants, a loss of the neck filaments visualized by EM is reported to correlate over time with the loss of septin immunofluorescence staining after a shift to the restrictive temperature (26). On the other hand, the septins are seen at the neck, by immunofluorescence staining, at times in the cell cycle before and after neck filaments are observed by EM (26). The new data from Field et al. argue that the septins can assemble into filaments *in vitro* (13), and the evidence cited above establishes that the septin genes are necessary for neck filament formation. Nevertheless, whether the septins are the primary structural elements of the neck filaments, are regulators of neck filament formation, or are simply essential for neck filament formation, has yet to be determined.

Additional support for the hypotheses that the neck filaments are composed primarily of septins, and that septins are sufficient for neck filament formation, might come from a detailed ultrastructural comparison of neck filaments *in situ* with septin filaments formed *in vitro*. A model suggested by Field et al. (13) for how septins might compose the neck filaments predicts that the spacing between the neck filaments should depend on the length of the septin molecules because the septin molecules are oriented perpendicular to the filaments. This model may be testable using recombinant septins of differing length, following the approach used by Kilmartin and colleagues to show that a spacer protein, Spc110p/Nuflp, bridges a gap in the spindle pole body of yeast (23).

(2) Do Septins Always Form Filaments *In Vivo*? Many organisms contain septins—do those organisms also contain filaments like the yeast neck filaments? To date, such filaments have only been observed in the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Comparable filaments have not been observed in other dividing cells even though the septins are concentrated in the division furrows of animal cells and the ultrastructure of animal division furrows has been studied intensively. Perhaps septin filaments exist but are difficult to observe amid the dense background of actin filaments and intermediate filaments in animal cells, especially because both actin and intermediate filaments are ~10-nm wide. Perhaps septin filaments are short-lived, as are the yeast neck filaments. Based on the Field model for the structure of the yeast neck filaments, perhaps subtle differences in lateral interactions between septins prevents the registration necessary for their observation.

(3) What Are the Functions of Septins and Neck Filaments? Another critical question is the relationship, if any, between the formation of filaments and function(s) of septins.

Cytokinesis. In budding yeast, defective cytokinesis defined the original mutations that led to the discovery of the septin genes, and septin proteins are located at the site of cytokinesis. The *Drosophila pnut* mutant also shows multinucleate cells, consistent with a defect in cytokinesis, and septins are located at cytokinesis sites (12, 32).

What is the role of septins in cytokinesis? Cytokinesis appears to be quite different in budding yeast and animal cells. In budding yeast, the location of the site of cell division is specified at the start of the cell cycle, and a constriction between mother and daughter exists from the beginning as well. Septins and the neck filaments are present at this mother/bud neck, but there is no evidence that they undergo constriction during cytokinesis. Indeed, the ring splits in the plane of the ring, so that both mother and bud receive an intact ring reduced in thickness but not diameter. In addition, the cell wall may grow inward, in response to the local secretion of appropriate enzymes and/or vectorial assembly of cell wall polymer.

On the other hand, cytokinesis in animal cells involves a contractile ring that forms late in the cell cycle, at a position specified by the mitotic apparatus. The contractile ring includes actin and myosin, and myosin is absolutely required for cytokinesis to drive constriction of the ring that divides mother and daughter (8, 20, 25, 28). In *S. cerevisiae*, by contrast, myosin contributes to cell wall synthesis and bud site selection, but is not required for cytokinesis *per se* (39, 43). The fact that the septins have essential roles in cell division mechanisms that seem so different highlights the question of whether septins play a common or different role in yeast and animal cytokinesis.

The mechanism of cell division in plants is different from that of yeast and animals, and septins have not yet been described in plants. If septins do exist in plants, then understanding their role in cytokinesis will be important.

Other functions in yeast. Septin mutations also affect the budding pattern in yeast. The observed effects on the haploid budding pattern are readily rationalized because the septins may be a physical landmark for the positioning of spatial cues for the next bud site, which is adjacent to the previous bud site (6, 7). Genetic interactions between septin genes and genes necessary for the diploid budding pattern have also been observed (14, 44); possible mechanisms for these interactions are more difficult to perceive since the tight correlation between septin location and selection of the next bud site does not hold for the diploid pattern.

In addition to the four septins involved in vegetative cell division, yeast also have at least two additional septins, Spr3p and Spr28p, that are expressed specifically in sporulating cells and seem to be involved in spore formation (9, 11).

Other functions in higher organisms. In higher organisms, septins may have roles in addition to cytokinesis. In *Drosophila*, septins are found in locations where cytokinesis is not occurring but where actin and myosin appear to collaborate to drive contractility (12).

Other Novel Filament Systems

Recent work has also highlighted the existence of additional filament systems that may contribute to the cytoskeleton. For example, filaments formed from the 14-kD major sperm protein (MSP) from nematodes do not only augment but completely replace the actin cytoskeleton in motility (reviewed in 18, 38). Currently, there is no evidence that MSP is found in any other cell types based on sequence analysis.

In prokaryotes, the product of the *ftsZ* gene is essential for bacterial division, is distantly related to tubulins, and can form protofilament sheets with a lattice structure comparable to microtubule walls (3, 10, 27, 30). However, filaments of *ftsZ* have not been observed in situ by electron microscopy of fixed specimens. Eukaryotic homologues with high sequence similarity to *ftsZ*, which function in the cytoplasm, may not exist because they have not appeared in the *Saccharomyces cerevisiae* genome. On the other hand, a nuclear gene that encodes a chloroplast homologue of *ftsZ* has been identified and may play a role in chloroplast structure (35). Additional proteins, including titins (21) and centrins (40), may contribute to filament systems in eukaryotes.

Conclusion

The septins are a family of proteins found in all eukaryotes thus far examined. They are critical for cytokinesis in fly and budding yeast and perhaps other functions. They either form or regulate the assembly of a set of 10-nm filaments, the neck filaments, seen at the site of cell division in yeast.

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