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Primer

Septins

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Septins are a family of proteins highly conserved in eukaryotes that have been under intense scrutiny lately because they appear to comprise a novel component of the cytoskeleton. As such, septins play important roles in many cellular processes by providing rigidity to the cell membrane, serving as scaffolds to recruit proteins to specific subcellular locales, and creating membrane diffusion barriers to establish discrete cellular domains.

Septins were first discovered in yeast during screens for genes that are critical for cell division. Orthologs have since been identified in animals, fungi, and algae; but, interestingly, septins are absent from higher plants. The number of septin genes ranges from a minimum of two in *Caenorhabditis elegans* (UNC-59 and UNC-61) to 14 in humans (SEPT1 through SEPT14). To complicate matters, the transcripts of many mammalian septin genes undergo alternative splicing, making the number of unique septin isoforms even greater. Despite this complexity, all septins bind guanosine-5'-triphosphate (GTP), and interact with each other to form complexes. Septin complexes further associate with each other to form large filamentous structures, as is observed with other cytoskeletal proteins. In this primer, we provide an overview of the structural and functional features of septins, and highlight their involvement in various human diseases.

Septin structure

Septin architecture

All septins contain a central Ras-like domain which binds GTP (Figure 1A). This domain is flanked by more variable regions and septins can be divided into groups based on their degree of sequence similarity and other shared features within the variable regions. All septins bind to GTP and most hydrolyze it to GDP. Structural studies of human SEPT2 suggests that septins interact with each other using two distinct interfaces: one composed of the nucleotide-binding site, called the G interface, and the other involving the amino and carboxyl termini, called the NC interface (Figure 1B). GTP hydrolysis appears to induce stable conformational changes in the septin–septin interaction interfaces. This situation is in contrast to classical small GTPases, like RhoA and Rac1, which are known to act as molecular switches by cycling between an active GTP-bound state and inactive GDP-bound state. Structural differences between GTP-bound and GDP-bound septins are small at the G interface, but more pronounced at the larger NC interface. Structural studies have suggested that GTP hydrolysis may alter the NC interface to facilitate septin–septin interactions. Nucleotide-dependent changes in structure that facilitate or block septin–septin interactions may be a common theme in septins that cycle between GDP and GTP. Interestingly, the subgroup of septins that is most similar to SEPT6 lack a key threonine residue, which prevents them from hydrolyzing GTP to GDP. These septins are conformationally locked in the GTP-bound state, and their ability to interact at the NC- and G- interfaces thus cannot be regulated by GTP hydrolysis.

Septins interact to form complexes

A variety of different heteromeric septin complexes have been identified, although the exact composition remains unclear in most cases. The best characterized septin complex is isolated from budding yeast, which comprises four septins found in equal amounts. This complex contains two copies of each septin, making up a core octamer unit. Electron microscopy studies revealed that, in the octamer, each tetrameric arm is identical and an axis of two-fold rotational symmetry runs orthogonal to the middle of the central pair of protomers (Cdc11–Cdc12–Cdc3–Cdc10–Cdc10–Cdc3–Cdc12–Cdc11). Therefore, the octameric rod lacks polarity. In humans, the 14 septin genes appear to fall into four families, raising the possibility that human septin complexes may also be octamers composed of two copies of a member of each family. By this logic, a large number of septin complexes would be possible if different family members could substitute for each other at a conserved position within the octamer. Although no mammalian octamer has been characterized to this level, the structure of the GTP-binding domains of a complex of three human septins (SEPT2, SEPT6 and SEPT7) co-expressed in bacteria was recently solved. Analogous to the yeast octamer, the structure was an apolar hexamer with two copies of each septin arranged symmetrically with each septin occupying either an inner, middle or outer position. Similar to the septin–septin interfaces in the homomeric SEPT2 structures, the heteromeric structure shows alternating NC and G interfaces within the complex (Figure 1B). Recent work suggests that, *in vivo*, SEPT9 may occupy the ends of this structure, thereby generating an octamer, as seen in yeast.

Septin complexes associate to form filaments

Septin complexes from different organisms vary in number and composition, but all complexes studied so far possess the ability to form filaments. When viewed by electron microscopy, *in vitro* purified septin complexes are rod-shaped. These rod-shaped septin complexes are the building blocks for septin polymerization. Each rod-shaped structure can be induced to join end-on-end, to form filaments under conditions of low salt concentration (Figure 1B). These filaments typically pair, and associate laterally to form bundled filaments. Ultimately, bundled filaments tend to self-assemble into even higher ordered structures, including rings.

Although the ordered arrangement of individual septins within complexes and filaments would appear to be fixed, recent evidence from budding yeast suggests that the removal of specific septins from the complex results in alternative filaments as they adopt a next-best configuration and, in some cases, these are able to maintain septin function, albeit less efficiently than the full-length wild-type complex. For example, if the septins normally at the ends of the yeast octamer are not expressed, the remaining hexamer appears capable of assembling into filaments and supporting yeast viability. This apparent plasticity in septin interactions may also partially explain the fact that disruption of many septin genes in mice have failed to produce dramatic phenotypes.

Intriguingly, there is also the possibility that changes in the end subunits of the complexes may alter their properties. In the case of the yeast octamer, the septin Shs1 can substitute for Cdc11 at the end position in the complex and during sporulation Spr3 and Spr28 replace Cdc12 and Shs1 at the ends. In the case of mammalian septins, preliminary data places SEPT9 at the ends and this protein undergoes extensive alternative splicing at its amino terminus. These distinct end units may provide distinct polymerization properties to the septin complex.

Septin filaments act as a macromolecular scaffold

Septin filaments appear to act as a macromolecular scaffold that directly interacts with several proteins (Figure 1B). As a result, septins play a key role in recruiting these proteins to particular cellular locales in order to mediate specific functions. The best characterized example occurs during cytokinesis, the process by which a mother cell divides to generate two distinct daughter cells. In budding yeast, septins form a filamentous hourglass structure at the site of division. The septins in this structure recruit several proteins that play important roles in cytokinesis. In the absence of the septin hourglass structure, these proteins fail to accumulate at the division site, and cytokinesis fails. Mammalian septins also form a filamentous hourglass structure at the site of division, where they too recruit specific proteins that are critical for cytokinesis.

Mammalian septins may also act as a scaffold during exocytosis. Septin filaments localize to sites of exocytosis, where they associate with tethering proteins (such as the exocyst complex) involved in holding vesicles at the plasma membrane and with SNARE proteins that drive the fusion of vesicles with the plasma membrane. Since septins also interact with the plasma membrane itself (see below), they may serve as a scaffold for the different components involved in exocytosis by coordinating them at the plasma membrane.

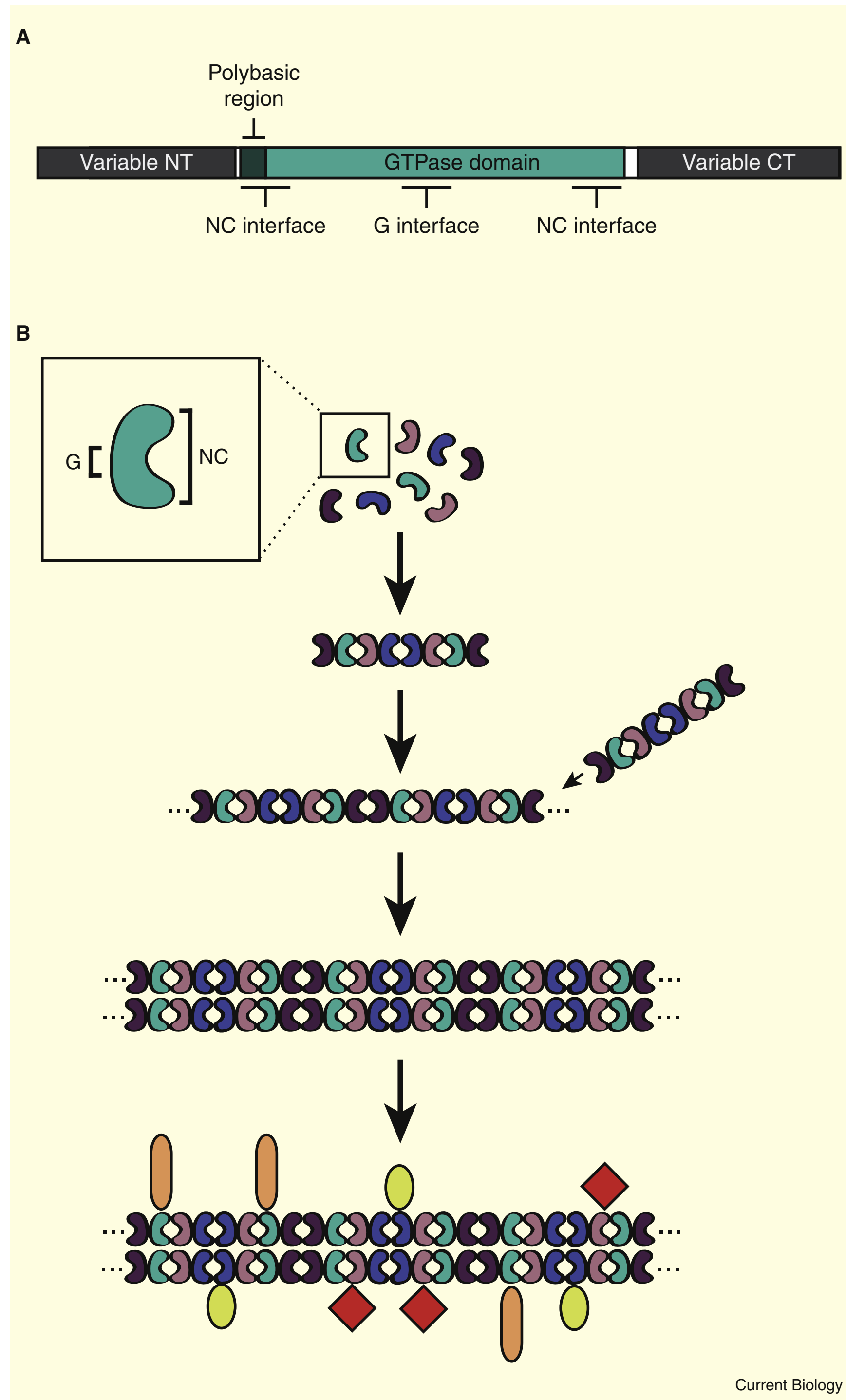

Figure 1. Septin complex and filament assembly.
(A) Schematic of the primary structure of a prototypical septin. NT, amino terminus; CT, carboxyl terminus. (B) Septin monomers (depicted as jellybeans) interact via their G and NC interfaces to form ordered oligomeric complexes, which further assemble into large filamentous structures. The different color jellybeans represent different members of the septin family. Septin filaments are thought to function as scaffolds by interacting with other proteins (depicted as red diamonds, yellow ovals, and orange rods).

Septins associate with membranes

Polymerization of septin filaments and membrane rigidity

Individual septins bind weakly to specific glycerophospholipids, namely certain phosphorylated phosphatidylinositols (polyphosphoinositides), in part by using a stretch of basic residues near the septin amino terminus. Phospholipids associate into a bilayer that self-assembles into sheets or spherical vesicles.

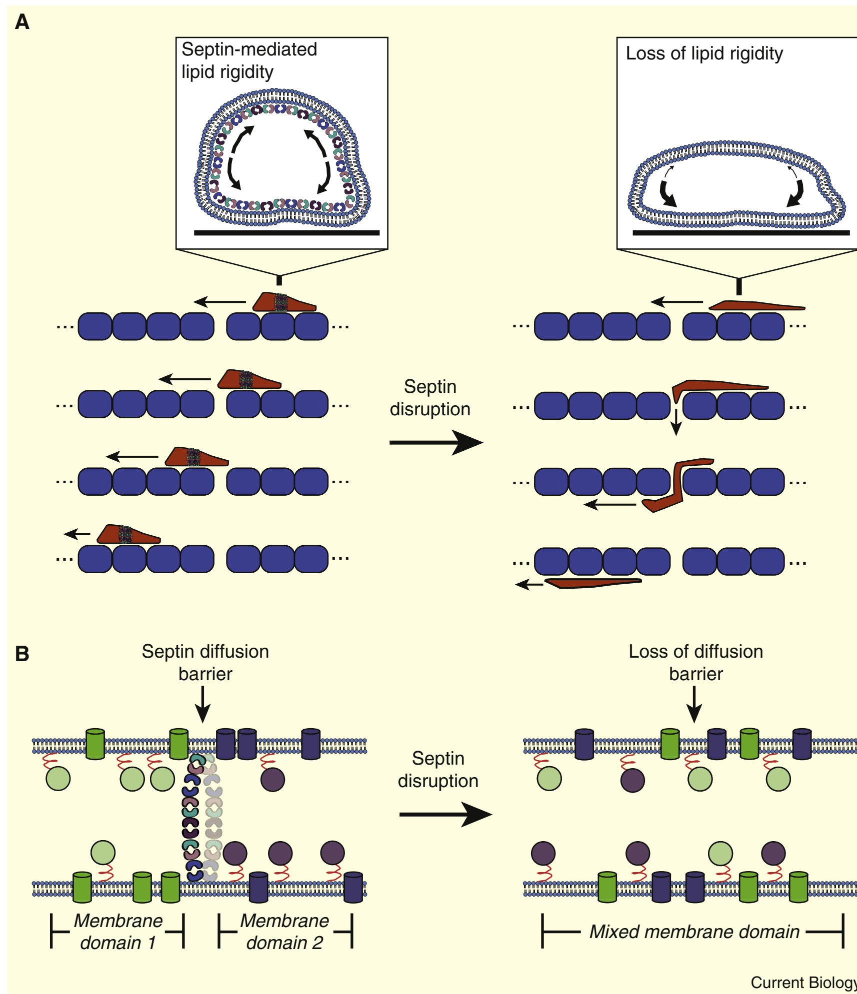

Figure 2. The association of septin filaments with the plasma membrane provides rigidity to the cell and establishes membrane diffusion barriers.
(A) Septin filaments provide rigidity to the cell by interacting with the plasma membrane. This is important for maintaining cell shape (left). Upon septin disruption, cell rigidity is lost, allowing cells to change their shape and squeeze through tight spaces (right). (B) The association of septin filaments with the plasma membrane forms a barricade called a membrane diffusion barrier that restricts the mobility of membrane proteins in the plane of the membrane. This leads to the formation of distinct membrane domains (shown in green and purple; left). Upon septin disruption, this membrane diffusion barrier is lost, resulting in a mixed membrane domain (right).

The addition of septins to phosphatidylinositol-containing phospholipid sheets promotes the formation of septin filaments. These bilayer sheets are even able to efficiently polymerize septin complexes at concentrations below those necessary for polymerization in solution. In addition, when septins are added to large spherical vesicles containing phosphatidylinositols, these vesicles undergo dramatic changes in shape and begin to extrude thin tubules, which appear to be wrapped in septin filaments. Thus, phosphatidylinositols appear to promote septin polymerization into filaments, and septin filaments seem to affect membrane shape. The presence of multiple membrane-binding regions on the same surface of a filament may provide sufficient avidity to maintain stable membrane association. This tight interaction may also be capable of organizing specific lipids within the membrane. By directly interacting with the plasma membrane, septin filaments are thought to confer rigidity to the cell (Figure 2A). Plasma membrane rigidity is not only important for regulating cell shape, but also plays an important role in controlling the directional movement of cells. In the absence of septin filaments, cells are able to change their shape to squeeze through tight spaces. In addition, they exhibit disorganized movement.

Septin filaments act as a membrane diffusion barrier

The association of septin filaments with the plasma membrane is also thought to form a barricade called a membrane diffusion barrier (Figure 2B). This barricade acts to confine membrane proteins to specific membrane domains. In budding yeast, the filamentous septin hourglass structure splits into two rings prior to cytokinesis. These rings confine key membrane proteins to the site of division in order to ensure complete cytokinesis. A diffusion barrier has also been described at the division site in mammalian cells, and mammalian septins are important for various stages of cytokinesis. This raises the possibility that mammalian septins also form a membrane diffusion barrier during cytokinesis, although this hypothesis remains to be tested.

Mammalian septins do, however, form membrane diffusion barriers in other cellular contexts. Septins are the major component of a membrane-associated ring-like structure called the annulus, which is present in mammalian sperm. This structure forms a membrane diffusion barrier between two different regions of the sperm tail. Sperm from *Sept4* knockout mice, which have no annulus, lack membrane protein compartmentalization in these regions. This results in defects in sperm mobility, causing male-specific sterility.

A similar diffusion barrier exists at the base of the primary cilium. This organelle functions like a cellular antenna to sense extracellular cues through membrane protein receptors and transmit them into the cell. These receptors can diffuse freely within the membrane of the primary cilium, but are restricted from diffusing across the base of this organelle to other parts of the plasma membrane. SEPT2, likely in conjunction with other septin family members, forms a ring-like structure at the base of the primary cilium. Decreasing the expression of SEPT2 not only

impairs the formation of the primary cilium, but also increases the rate of diffusion of membrane proteins across its base.

Septins may also act as a diffusion barrier in neurons of the brain. These specialized cells contain projections called dendrites that are involved in relaying signals from neighbouring neurons. Signals are received at protrusions in the dendrite referred to as dendritic spines. Several septins are found at the base of these spines, where they may act to compartmentalize the membrane of the dendrite as is observed in the primary cilium.

It should be noted that the scaffolding, rigidity, and membrane diffusion barrier functions of septins are not necessarily mutually exclusive. It is possible that they act in concert to mediate some septin-dependent processes.

Septins and human disease

Given their roles in many different cellular processes, it is not surprising that the septin family of proteins is also implicated in several human diseases.

Alterations in septin structure and expression are associated with many different types of cancer. For example, several septin genes have been identified as fusion partners of the *MLL* gene in leukemias. Specific septins are overexpressed in some types of cancer and are inactivated in other cancers. However, the relationship between the levels of septin protein and cancer appears to be complex and remains poorly understood. Of note, the DNA of the *SEPT9* gene is highly modified by methyl groups in colon cancer tissue compared with healthy tissue. As a result, methylation of the *SEPT9* gene is being used as a biomarker for colon cancer.

Mutations in *SEPT9* also cause hereditary neuralgic amyotrophy (HNA). Patients with this rare disorder experience sudden, severe pain in the shoulder and/or arm, and weakness and wasting of arm muscles. Interestingly, mutations that cause this disease are found in the amino-terminal region of the longest isoforms of SEPT9, suggesting that this unique region is somehow involved in the pathogenesis of HNA. However, the physiological role of the SEPT9 amino-terminal region remains poorly understood.

A common feature of neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, is the formation of protein aggregates in the brain. Some members of the septin family are found in these aggregates, suggesting that septins may also be involved in neurodegenerative diseases. However, the specific role they may be playing is unknown thus far.

Unanswered questions and future directions

While great progress has been made in understanding the structure and function of septins, many important questions remain unanswered. Aside from in yeast, the composition of septin complexes that form in different cell types is poorly understood. As a result, it is unclear whether general rules exist that dictate septin complex composition and the arrangement of individual septins within these complexes. Likewise, little is known about the composition of septin filaments and the order of individual septins within these large structures, aside from in yeast. It is not known whether septin complexes are simply building blocks for making septin filaments, or whether they are sufficient to mediate certain septin-dependent functions. The factors that regulate the assembly and disassembly of septin filaments also remain to be identified. However, it is clear that septins undergo a number of post-translational modifications (including phosphorylation, SUMOylation, and acetylation) in a cell-cycle- and stress-dependent manner.

Since individual members of the septin family have variable regions within their protein sequences (Figure 1A), it is likely that different septins have unique properties (this may also be the case with different isoforms of a single septin family member). For example, septin-interacting proteins have been identified that interact with one septin family member, but not others. As a result, the individual components of a given septin complex or filament may confer unique properties, and specific combinations of septins may be required to carry out specific cellular functions. Indeed, it is not known if septins can only function as part of a complex, or if some septin functions may involve individual subunits. It is also not known if they always function in association with the membrane or if some septin functions may occur in other locations.

A complete understanding of the many roles of the septin cytoskeleton will not only require a detailed analysis of the composition of septin complexes and filaments, but will also require investigation into the properties of individual septin family members. Such an understanding could shed light on the pathogenesis of many human disorders, including cancer, ciliopathies, HNA, Parkinson's and Alzheimer's disease.

Further reading

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