Septins at a glance

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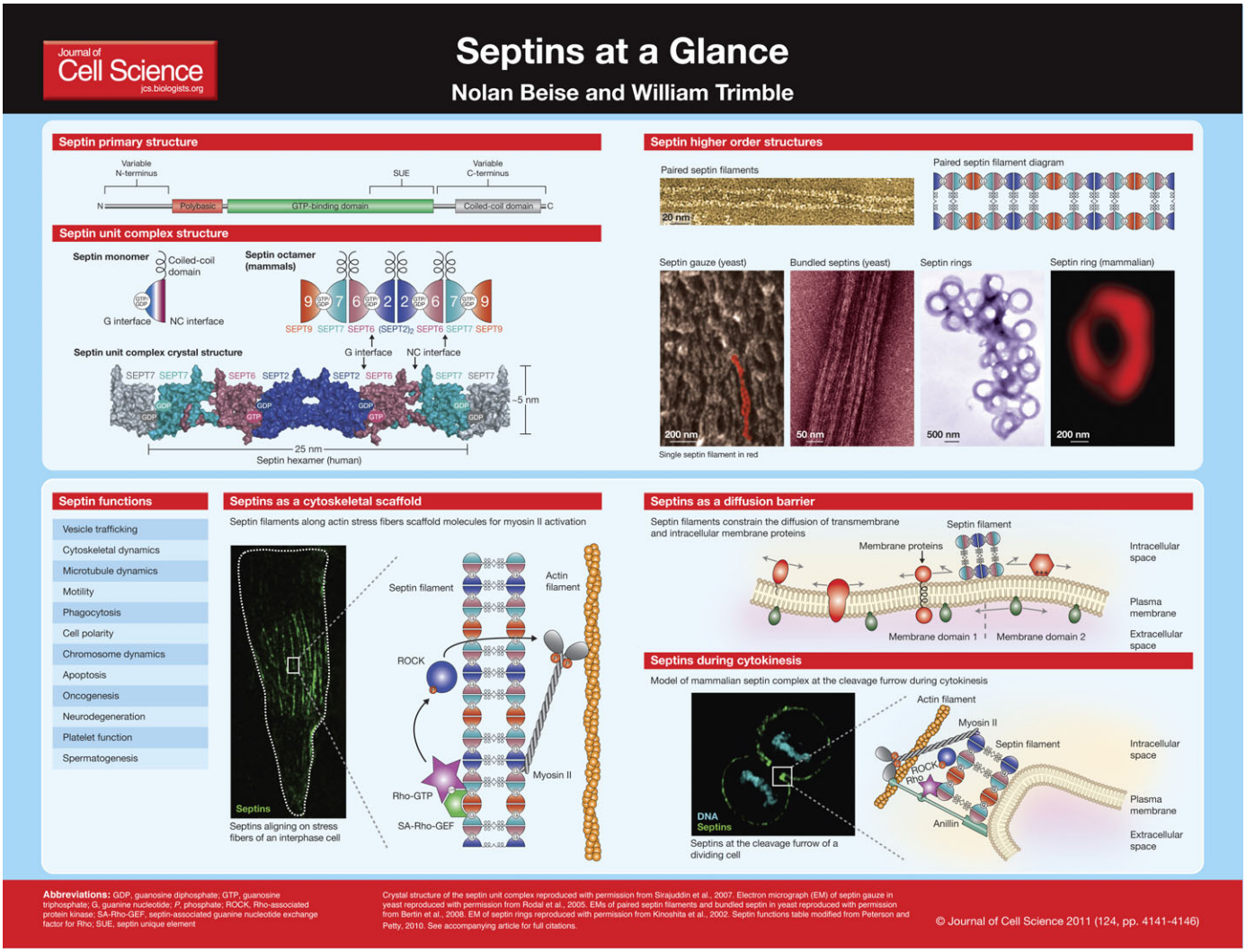
The pioneering cell division cycle screens carried out by Hartwell in 1971 first identified septins as genes that are essential for yeast cytokinesis (Hartwell, 1971). However, significant characterization of the evolutionarily conserved proteins they encode did not begin until the late 1980s. Since then, septins have been shown to be fundamental for cytokinesis in many organisms and are recognized as important components of the cytoskeleton. In addition to cytokinesis, septins have been implicated in a wide range of biological processes, including cell polarity, spermatogenesis, exocytosis, ciliogenesis, phagocytosis, motility and apoptosis. As septins can form filaments and interact directly with membranes, microfilaments and microtubules, they have also been suggested to act as diffusion barriers and multimolecular scaffolds. Furthermore, alterations in septin amino acid sequence or their expression levels have been linked to neurological disorders and a multitude of cancers. In this Cell Science at a Glance article and the accompanying poster, we highlight recent studies that have provided important information about the structure of septin proteins and that have defined the underlying principles of septin polymer assembly. In addition, we discuss the unique biological properties of septins and explain how each of these contributes to their diverse functions. We then give examples of where the perturbation of septin function is linked to human disease and, finally, discuss some of the many questions that remain pertinent to the septin field.

Septin structure

A multitude of septins exists across many species. Which specific septins are expressed, as well as their proportion to each other, can vary in different cell types. In this section, we review the diversity of septin subunits, their properties and how they assemble into filaments.

Septin subunits

Septins are conserved from yeast to humans and are present in widely divergent eukaryotic species – including some algae – but they are notably absent in plants (Nishihama et al., 2011; Pan et al., 2007). The number of genes that encode septins ranges from one in *Chlamydomonas reinhardtii* to 13 in humans. Why some eukaryotic organisms have conserved – and amplified – septin genes, whereas others have presumably lost them is unclear but might reflect distinct mechanisms of cytokinesis in different organisms.



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Comparison of septins from many different species has revealed that these proteins share several conserved domains and, in species with multiple septin genes, can be organized into subgroups. In mammals, four such distinct groups – or families – are represented by SEPT1, SEPT3, SEPT6 and SEPT7, and these share some structural similarities with the four main septins in yeast (Cao et al., 2007). Nearly all septins contain a GTPase domain that belongs to the GTPase superclass of P-loop nucleotide triphosphatases, and whose other members include kinesin, myosin and Ras proteins (Leipe et al., 2002). However, the GTPase domain of septins is unique among this family in that it contains a conserved sequence near its end – the septin unique element (SUE) (Pan et al., 2007; Steels et al., 2007; Versele et al., 2004). Most septins in yeast and metazoans can bind and hydrolyze GTP (Field et al., 1996; Mendoza et al., 2002; Sheffield et al., 2003; Versele and Thorner, 2004). In vitro, this hydrolysis is slow. For example, the apparent k_{cat} value for SEPT2 is 2.7×10^{-4} seconds^{-1} (Huang et al., 2006), which is similar to that of the structurally related Ras GTPase (k_{cat} 3.4×10^{-4} seconds^{-1}) (Neal et al., 1988) and the Cdc3–Cdc12 complex in yeast (apparent k_{cat} of 3×10^{-4} seconds^{-1}) (Farkasovsky et al., 2005). In vivo, septins also display slow rates of GTP exchange (Frazier et al., 1998; Huang et al., 2006; Vrabioiu et al., 2003).

Septins have additional conserved elements that flank the GTPase domain. N-terminal to the GTPase domain, most septins possess a polybasic region that has been shown to bind phosphoinositides (Casamayor and Snyder, 2003; Zhang et al., 1999). Moreover, the C-terminus of most septins contains an extension, which is predicted to form coiled coils that might be important for certain septin–septin and septin–substrate interactions (Casamayor and Snyder, 2003; Versele and Thorner, 2005).

Septins have two conserved interfaces that are involved in the formation of septin–septin interactions and subsequent complex assembly: the guanine nucleotide-binding domain (G interface), and the N- and C-terminal extensions (NC interface) (Sirajuddin et al., 2007). Interestingly, the SUE partly overlaps with the G interface, where it might also contribute to septin assembly into complexes (Sirajuddin et al., 2009; Versele et al., 2004). Within the cytosol, most septins are found assembled into hetero-oligomeric filaments that are composed of octameric non-polar unit complexes (Bertin et al., 2008; John et al., 2007; Kinoshita et al., 2002; Sellin et al., 2011; Sheffield et al., 2003). In fact, mammalian septins exist solely as 6- to 8-unit heteromeric complexes within the cell (Sellin et al., 2011).

Unit complex

Septin assembly into filaments begins with their incorporation into unit complexes. Typically these complexes are hetero-oligomers that are composed of four, six or eight septin monomers polymerized into a linear, non-polar polymer. Complexes of septin units were first identified when the initial purification of septin filaments from *Saccharomyces cerevisiae* (Cdc3, Cdc10, Cdc11, Cdc12) and from *Drosophila melanogaster* (Pnut, Sep1, Sep2) showed that these proteins are present in near equal amounts, suggesting 1:1:1:1 and 1:1:1 stoichiometries, respectively (Field et al., 1996; Frazier et al., 1998; Oegema et al., 1998). Electron microscopy studies of septin complexes in *S. cerevisiae* and *Caenorhabditis elegans* showed that septin complexes (Cdc11–Cdc12–Cdc3–Cdc10–Cdc10–Cdc3–Cdc12–Cdc11 and Unc59–Unc-61–Unc61–Unc59, respectively) are non-polar (Bertin et al., 2008; John et al., 2007). In mammals, a septin complex consisting of SEPT2, SEPT4, SEPT6 and SEPT7 was first identified in brain tissue (Hsu et al., 1998). Two other groups were able to isolate a complex from mammalian cell lines and mouse brain that contained SEPT2, SEPT6 and SEPT7 with a stoichiometry of approximately 1:1:1 (Joberty et al., 2001; Kinoshita et al., 2002). Further structural characterization of recombinant complexes of these septins revealed them to be non-polar, rod-shaped oligomers in which the order of septins is SEPT7–SEPT6–SEPT2–SEPT2–SEPT6–SEPT7 (Sirajuddin et al., 2007). Most mammalian cells appear to express members of each of the four septin families. It is, therefore, unclear where members of the SEPT3 family fit within the unit complex. However, recent biochemical approaches have been used to show that, in HeLa cells, the SEPT3 family member SEPT9 localizes to the ends of these hexamers in octameric complexes (Kim et al., 2011a; Sellin et al., 2011). Interestingly, the composition of the septin unit complex can be flexible because septins from the same subgroup, and even isoforms of the same septin, can substitute for each other within this structure (Sellin et al., 2011).

Septin monomers within the unit complex interact through G–G and NC–NC interfaces, thereby pairing up with each other (Sirajuddin et al., 2007). These G–G and NC–NC interactions alternate along the unit complex, and are necessary for its formation (Sirajuddin et al., 2007). The coiled-coil C-termini of the septin monomers have also been implicated in the formation of septin unit complexes, by directly interacting with each other (Moshe S. Kim, Carol D. Froese and W. T., unpublished results) (Low and Macara, 2006; Shinoda et al., 2010). Unfortunately, the detailed structure of septin coiled-coil interactions in the mammalian septin complex has not yet been visualized, as these regions of the proteins were not resolved in the septin crystal structure (Sirajuddin et al., 2007). However, electron microscopy (EM) images of the yeast septin complex show finger-like projections from the unit complex, which are consistent with the coiled-coil domains that project perpendicular to the septin complex (Bertin et al., 2008; Hsu et al., 1998).

Guanine nucleotides also have a role in the formation and stability of septin unit complexes. They fully saturate recombinant septin octamers in yeast and septin hexamers in human, although the function of these nucleotides in the assembly of septin complexes is still not fully understood (Farkasovsky et al., 2005; Sirajuddin et al., 2007; Vrabioiu et al., 2003). Mutation of residues that contribute to the binding of septin to GTP alters the formation, appearance, localization and/or function of septin polymers (Casamayor and Snyder, 2003; Ding et al., 2008; Hanai et al., 2004; Kinoshita et al., 1997; Nagaraj et al., 2008; Robertson et al., 2004; Steels et al., 2007; Vega and Hsu, 2003). Structural studies of SEPT2 have shown that binding to guanine nucleotide (GTP or GDP) induces stable conformational changes in the G and NC surfaces that – in turn – might regulate septin–septin interactions (Sirajuddin et al., 2007; Sirajuddin et al., 2009). GTP hydrolysis might, therefore, act as a switch that regulates complex assembly (Sirajuddin et al., 2007; Sirajuddin et al., 2009).

Interestingly, when expressed alone, SEPT2 forms a dimer at its G interface, yet SEPT2-GDP dimers can interact through both G and NC interfaces and, in unit complexes, SEPT2 only interacts with other SEPT2 molecules through the NC interface (Moshe S. Kim, Carol D. Froese and W. T., unpublished results) (Sirajuddin et al., 2007). Indeed, we found that when any two septins are overexpressed together, they preferentially interact at their G interface, even though they might normally interact at their NC interface in the unit complex (Moshe S. Kim, Carol D. Froese and W. T., unpublished results). Despite this apparent promiscuity, septin filaments assemble in an ordered manner, suggesting that preferential pairwise G interface assembly is likely to precede the formation of septin unit complexes. Because SEPT2–SEPT6 and SEPT7–SEPT9 pairs interact in the octamer at their G interfaces, these might assemble first as GTP-bound forms. Subsequent GTP hydrolysis might then trigger conformational changes at the NC interfaces to allow subsequent assembly of the octamer

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(Moshe S. Kim, Carol D. Froese and W. T., unpublished results) (Sirajuddin et al., 2009). In addition, interactions of SEPT6–SEPT7 and NC might also be promoted by the binding of Cdc42 effector protein 5 (Borg3) to the septin coiled-coil domains (Sheffield et al., 2003). In the same way that septin monomers are the building blocks of the unit complex, those unit complexes that become polymerized end-to-end subsequently form the building blocks of septin filaments.

Filaments

Septin filaments are the predominant septin structure within the cell. As septin unit complexes vary in composition, septin filament composition is likely to change correspondingly. These filaments primarily localize to the cleavage furrow in dividing cells and to stress fibers in interphase cells, but are also present at other structures, which relate to their functions. The basic septin filament is 4–5 nm wide with varying lengths, and has the capacity to form rings, bundles, linear filaments and gauzes (Bertin et al., 2008; Kinoshita et al., 2002; Rodal et al., 2005). The mechanism of septin assembly into this diverse array of structures is not completely known, but there is some evidence that links Cdc42, the Borg family, anillin, guanine nucleotides and the membrane association of septins to the regulation of septin filament assembly (Caviston et al., 2003; Gladfelter et al., 2002; Joberty et al., 2001; Kinoshita et al., 2002; Nagaraj et al., 2008; Smith et al., 2002; Tanaka-Takiguchi et al., 2009). In yeast, mutations in the Rho-type GTPase Cdc42p abrogate ring formation by causing defects in the polarized localization of septins to the presumptive bud site (Gladfelter et al., 2001; Jeong et al., 2001). Joberty et al. have shown that Cdc42-GTP is involved in negatively regulating Borg-mediated septin polymerization in mammals (Joberty et al., 2001). Additionally, defects in anillin cause loss of septin recruitment to the cleavage furrow, and overexpression of the septin-interacting fragment of anillin perturbs septin organization (Field et al., 2005; Kinoshita et al., 2002). As guanine nucleotides might regulate unit complex formation, it would not be surprising if they also were to affect higher order septin filament assembly. In yeast, GTP binding by all four septins is necessary for the formation of the septin ring under stressful growth conditions (37°C), and a combination of septin mutants that are defective in GTP-binding is often lethal (Longtine et al., 1996; Nagaraj et al., 2008; Versele and Thorner, 2004). Both yeast and mammalian septins show increased rates of polymerization into filaments following their association with lipid bilayers (Bertin et al., 2010; Tanaka-Takiguchi et al., 2009). The formation of septin filaments is required for stable association of septins with the plasma membrane and successful completion of yeast cytokinesis (McMurray et al., 2011). This suggests that septin membrane associations and septin filament assembly are inextricably linked (McMurray et al., 2011). Interestingly, despite septin unit complexes being very stable, septin filaments can be highly dynamic during certain stages of the cell cycle (Dobbelaere and Barral, 2004; Sellin et al., 2011; Vrabioiu and Mitchison, 2006). Septin filament characteristics also depend on the properties of their monomer constituents, which indicates that the function of septin filaments varies depending on their composition.

Septin functions

Septins as scaffolds

Yeast

As diverse and relatively static polymers, septins are able to interact with a large variety of molecules. As such, they have largely been described as scaffolds that recruit molecules to their sites of function and promote protein–protein interactions. In *S. cerevisiae*, more than 40 proteins localize to the mother-bud neck in a septin-dependent manner (Gladfelter et al., 2001); formation of a functional contractile ring at the mother-bud neck requires septin scaffolding of several proteins that are essential for cytokinesis. For example, degradation of the Wee1-family kinase Swe1p by its negative regulators leads to progression of mitosis, and occurs when septins provide a scaffold for the recruitment of a multi-protein kinase cascade to the mother-bud neck (Barral et al., 1999; Hanrahan and Snyder, 2003; Longtine et al., 2000; Sakchaisri et al., 2004; Szkotnicki et al., 2008). Interestingly, septin scaffolding in the form of microtubule capture at the cleavage plane ensures correct positioning of the spindle pole body and subsequent segregation of replicated chromosomes into mother and daughter cells (Kusch et al., 2002). Despite septins being essential for cytokinesis in budding yeast, they are not necessary for cell division in fission yeast *S. pombe*. In fact, deletion of all four vegetatively expressed septins results in only mild cell separation defects in fission yeast (Berlin et al., 2003; Tasto et al., 2003). Thus, although they are dispensable for cytokinesis, septins are important for subsequent cell separation in *S. pombe* (Wu et al., 2010).

Metazoans

Septin scaffolding of kinases and their substrates also seems to occur in mammals, as demonstrated by phosphorylation of myosin II at sites at which septin filaments are located near actin stress fibers (Joo et al., 2007). Because septins bind the septin-associated Rho guanine nucleotide exchange factor (SA-Rho-GEF) and myosin (Joo et al., 2007; Nagata and Inagaki, 2004), the entire GEF–Rho–ROCK–myosin signaling kinase cascade, which is essential for full myosin activation, might be scaffolded by septins on stress fibers. In addition, septin scaffolds at stress fibers are also important in regulating the DNA damage response by interacting with suppressor of cytokine signaling 7 (SOCS7) (Kremer et al., 2007). As septins are conserved between fungal and animal species, it is not surprising that many septin functions are also conserved among these species. An essential role for septins in cytokinesis has also been shown in *D. melanogaster* and mammals (Nagata et al., 2003; Neufeld and Rubin, 1994; Surka et al., 2002). Interestingly, SEPT5-containing scaffolds additionally regulate neuronal exocytosis through interactions with syntaxin and SNARE proteins (Beites et al., 1999; Yang et al., 2010).

Septins as membrane modulators

Yeast

In addition to acting as scaffolds, septins modulate membrane dynamics by binding lipids through their polybasic domains. For example, septin filaments at the cortex of the mother-bud neck in yeast limit the diffusion of membrane-associated components between daughter and mother cells (Barral et al., 2000; Takizawa et al., 2000). This diffusion-barrier-dependent polarized bud localization is essential for successful mitosis (Barral et al., 2000). In addition, the septin structure at the mother-bud neck transitions from an hourglass-shaped structure to two parallel rings on either side of the mother-bud neck. Between them, the rings define a specialized cortical compartment that retains diffusable factors such as the exocyst and polarizome complexes at the mother-bud neck, which are important for cytokinesis (Dobbelaere and Barral, 2004). The yeast cleavage furrow septin ring also participates in limiting the diffusion of ER and nuclear envelope proteins through the bud neck by recruiting other membrane-associated factors such as Bud6 (Luedeke et al., 2005; Shcheprova et al., 2008). Interestingly, a septin diffusion barrier is not necessary for the successful completion of cytokinesis, as shown in *S. cerevisiae* mutants that cannot form a diffusion-limiting hourglass structure (Wloka et al., 2011).

Metazoans

In mammals, a diffusion barrier does exist at the cleavage furrow (and septins are found at this location), but it remains to be determined

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whether this barrier is septin-dependent (Schmidt and Nichols, 2004). Mouse SEPT2-containing filaments at the base of primary cilia, however, have been shown to function as a diffusion barrier to ciliary membrane proteins that are essential for correct ciliary structure and function (Hu et al., 2010). A septin diffusion barrier also forms a ring at the sperm annulus, which is required for the cortical organization, morphology and normal motility of sperm flagella (Ihara et al., 2005; Kissel et al., 2005; Lin et al., 2009). Phagocytosis requires septins at the base of the phagocytic cup and it is tempting to speculate that, in addition to scaffolding actomyosin components, this functions to segregate lipid distribution at this structure (Huang et al., 2008; Yeung et al., 2009; Yeung et al., 2006). Interestingly, septins also form another ring at the base of dendritic spines in neurons, which is essential for their morphology (Cho et al., 2011; Xie et al., 2007). Neuronal SEPT6-containing rings at dendritic branch points might act as diffusion barriers or membrane braces, thereby maintaining polarized molecular distributions and dendritic membrane structure (Cho et al., 2011).

In addition to acting as diffusion barriers, filamentous, cortical septins are also able to modulate cellular membrane rigidity as shown by studies in T-cells (Tooley et al., 2009). Interestingly, decreases in septin-mediated membrane bracing does not block motility, but cells become poorly persistent and uncoordinated (Tooley et al., 2009). This link is further supported by septin-dependent neuronal migration (Shinoda et al., 2010). Additionally, Tanaka-Takiguchi and co-workers showed that septin-containing brain extracts are able to tubulate giant liposomes following membrane-associated septin filament formation, which further supports a role for septins as membrane modulators (Tanaka-Takiguchi et al., 2009).

Additional roles for septins in metazoans

In addition to their roles as molecular scaffolds and membrane modulators, septins have been shown to carry out other functions in metazoans. For example, mammalian septins are required for cell polarity, although they act through a different mechanism than in yeast. SEPT2 participates in epithelial cell polarity by facilitating apical and basal factor vesicle transport along polyGlu microtubule tracks by preventing the binding of microtubule-associated protein 4 (MAP4) (Spiliotis et al., 2008). In host cell defence, ring-like septin structures form cages around intracellular bacteria, thereby restricting their proliferation and facilitating their degradation by autophagy (Mostowy et al., 2010). Finally, one specialized type of septin function involves an alternative transcript of SEPT4 called ARTS, which translocates from mitochondria to the nucleus upon exposure to an apoptotic agent and induces apoptosis by activating caspase 3 (Gottfried et al., 2004; Larisch, 2004; Larisch et al., 2000).

Septin-associated diseases

Because mammalian septins interact with a variety of molecules and are essential in many cellular processes, it is not surprising that they are implicated in multiple human diseases. Several septins have been found to associate with protein aggregates that are common in neurodegenerative disorders such as Parkinson and Alzheimer disease (Garcia et al., 2006; Ihara et al., 2003; Kinoshita et al., 1998). Septins are also able to interact with parkin, an E3 ubiquitin ligase that is involved in Parkinson disease (Choi et al., 2003; Zhang et al., 2000). Further evidence that links septins to neurological disorders stems from mutations within *SEPT9*, causing hereditary neuralgic amyotrophy (HNA) (Hannibal et al., 2009; Kuhlenbaumer et al., 2005; Landsverk et al., 2009). As cytokinetic defects are linked to cancer (Sagona and Stenmark, 2010), it is not surprising that septins, which are essential to this process, have also been linked to cancer (Russell and Hall, 2005). Several septins have been identified as mixed lineage leukemia (MLL) fusion partners that contribute to the pathogenesis of leukemia (Borkhardt et al., 2001; Cerveira et al., 2006; Kojima et al., 2004; Osaka et al., 1999). Notably, septins are also being used in a diagnostic context, with the methylation of the *SEPT9* promoter serving as an effective biomarker for colorectal cancer (deVos et al., 2009; Grutzmann et al., 2008; Lofton-Day et al., 2008). The role of septins in neurological disorders and cancer is complex and poorly understood. For more detail on this subject, we refer readers to a recent review by Peterson and Petty (Peterson and Petty, 2010).

Perspectives

Since the initial characterization of septins in the late 1980s, research in this field has grown impressively, especially when considering the number and diversity of septins that have been characterized across species. This research has led to some very interesting questions related to septin structure and function. Determining the mechanism of both the septin unit complex and septin filament assembly will be paramount in elucidating the myriad of properties and functions that septins exhibit within the cell. Specifically, it will be interesting to illuminate how the variable N- and C-termini of septins regulate filament polymerization, stability and scaffolding functions. Additionally, it will be exciting to elucidate how the coiled-coil domains of septin proteins interact and function within the septin polymer and how septin-associated factors influence septin filament assembly and disassembly. In conjunction with this it will be interesting to characterize heterogeneous septin filaments and the way in which varying filament composition relates to different properties and functions. Septin-lipid association is another relevant topic of future research. Not only are lipids implicated in promoting septin polymerization, but septin filaments might also inhibit the lateral diffusion of lipids forming distinct domains. These and many more questions will hopefully be resolved in the coming years, thus making it a very exciting time in septin research.

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Individual poster panels are available as JPEG files at http://jcs.biologists.org/cgi/content/full/124/24/4141/DC1

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