Protein family review

The septins

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Summary

The septins make up a family of guanine-nucleotide binding proteins, most of which polymerize to form filaments. Septin genes have been found in fungi and animals but not in protozoa or plants; yeasts have seven septin genes and humans have twelve, but *Caenorhabditis elegans* has only two. Some septin genes generate multiple polypeptides by alternative splicing or alternative translation start sites. Of the five conserved motifs found in other members of the GTPase superfamily, three are highly conserved in septins. Septin filaments are thought to form a cytoskeletal system that organizes higher-order structures by self-assembly and templated assembly. These multifunctional proteins are best known for their role in cytokinesis, but other functions in dividing and non-dividing cells have evolved in different lineages: budding yeast has septins specific for sporulation; nematode septins are implicated in postembryonic morphogenesis of multiple cell lineages; fly septins are associated with the development of germ cells, photoreceptor cells and nervous system; and mammalian septins are implicated in exocytosis, tumorigenesis, apoptosis, synaptogenesis and neurodegeneration.

The septin genes were originally discovered through genetic screening for budding yeast mutants defective in the cell-cycle progression [1]. Mutants of any one of the genetic loci *CDC3*, *CDC10*, *CDC11* or *CDC12* commonly form multinucleated cellular clusters [2-4]. These mutants cannot organize the 'bud neck filaments' that normally encircle and demarcate the cell cortex between a mother cell and the bud (daughter) [5]. From these and other data, the septins have been regarded as the major constituents of the bud-neck filaments, which have essential roles in cytokinesis [2-4]. Molecular genetic studies revealed that the four *CDC* genes encode similar polypeptides, each with some of the set of conserved motifs found in GTPases. The four encoded proteins, termed septins, thus founded a protein family within the GTPase superfamily [2-4]. The septins that were later found in other fungi, nematodes, flies, and mammals have also been shown to have roles in cytokinesis and other cellular processes.

Gene organization and evolutionary history

Septins have been found in diverse eukaryotes, including animals and fungi but not protozoa and plants. Most septin genes generate one or more polypeptides by alternative splicing and/or multiple translation start sites; the number of variants is not yet established for many of the genes. The septin genes in five organisms, and the largest product of each gene known from the current databases, are shown in Table 1, and a phylogenetic tree illustrating their structural relationships and molecular evolution is shown in Figure 1. It is noteworthy that considerable diversity has been generated within each species; for example, the human septins are 39-63% identical to human Sept2 at the amino-acid level. It may be possible to classify the septins in each species into two to four groups by sequence homology. Orthologs can be found within the fungi (such as *Saccharomyces cerevisiae CDC3*, *Schizosaccharomyces pombe Spn1* and their *Candida albicans* orthologs, not shown) and within metazoa (such as *Drosophila Sep1* and mammalian *Sept2*), but not between distant lineages (fungi and metazoa). This pattern suggests that there have been independent expansions of the family in different lineages. Thus, any rules and functions found in the fungal septin systems may not necessarily apply to the metazoan ones, and *vice versa*.

Table 1

The septin genes and proteins in five representative organisms

Species	Approved gene name	Chromosomal location*	Size (amino acids)	Mass (kDa)	Isoelectric point (pI)	Charge	Number of predicted coiled coils	Accession number
Saccharomyces cerevisiae	*Cdc3*	XII:764137	520	60.0	5.3	A	2	L16548
	Cdc10	III:118342	322	37.0	5.6	A	0	L16549
	Cdc11	X:576294	415	47.6	4.8	A	1	L16550
	Cdc12	VIII:328038	407	46.7	8.2	B	1	L16551
	Spr3	VII:607564	512	59.8	7.3	N	3	U24129
	Spr28	IV:905043	423	48.2	5.8	A	1	NP_010504
	Shs1/Sep7	IV:52446	551	62.6	5.3	A	2	Z74273
Schizosaccharomyces pombe	*spn1*	I:1067997	469	53.7	5.3	A	1	U31742
	spn2	I:960487	331	38.1	8.0	B	0	U29888
	spn3	II:2682372	412	46.6	4.7	A	1	U29889
	spn4	I:1962024	380	44.7	7.0	N	1	U29890
	spn5	I:3044705	464	53.1	8.2	B	1	U29891
	spn6	III:421493	380	44.0	6.9	N	1	AL032824
	spn7	II:161221	428	49.2	5.2	A	0	AF417166
Caenorhabditis elegans	*unc-59*	I:21.15	459	52.9	8.9	B	1	NM_060987
	unc-61	V:6.66	530	60.7	8.9	B	1	NM_182356
Drosophila melanogaster	*Pnut*	44C2	539	60.2	9.0	B	1	NM_165597
	Sep1	19F5	361	41.1	6.1	A	2	NM_167747
	Sep2	92F2	419	48.5	7.4	N	1	NM_079693
	Sep4	15A1	427	49.0	6.9	N	2	NM_167530
	Sep5	43F8	422	48.5	7.3	N	1	NM_165578
Homo sapiens	*Sept1*	16p11.1	366	41.8	5.5	A	2	NM_052838
	Sept2	2q37.3	361	41.5	6.2	A	2	NM_004404
	Sept3	22q13.2	345	39.3	6.8	N	0	NM_145733
	Sept4	17q23	478	55.1	5.7	A	2	NM_004574
	Sept5	22q11.2	369	42.3	6.4	A	2	NM_002688
	Sept6	Xq24	427	48.9	6.4	A	1	NM_145799
	Sept7	7q36.1	418	48.8	9.0	B	1	NM_001788
	Sept8	5q31	483	55.8	5.9	A	1	XM_034872
	Sept9	17q25.3	586	65.4	9.3	B	0	AF189713
	Sept10	8q11.23	517	60.0	6.6	N	1	BC020502
	Sept11	4q21.22	429	49.4	6.4	A	1	NM_018243
	Sept12	16p13.3	358	40.8	6.7	N	0	NM_144605

The data refer to the largest gene products for each gene, deduced from cDNAs on the sequence databases. *The numbers given for the yeast genes refer to the position of the gene along the sequence of the chromosome. A, acidic (pI < 6.5); B, basic (pI > 7.5); N, neutral. The algorithm COILS [56,57] was used to predict coiled coils, and the peaks above an arbitrary threshold ($p > 0.8$ at a window size of 14) were counted. The mouse genome has counterparts to each of the 12 human septin genes (not shown). For comparative nomenclature of the mouse and human *Sept1-Sept10* genes and the products, see [58].

Characteristic structural features

The full-length septin cDNAs in the current sequence databases encode polypeptides of 30-65 kDa. Most of these gene products have a set of GTPase motifs, G-1, G-3 and G-4, found in members of the GTPase superfamily, (Figure 2 and not shown). The GTPase motifs of the septins are closer to those of the Ras family than of other members of the GTPase superfamily [6] such as the other cytoskeletal GTPases, tubulins in eukaryotes or FtsZ in bacteria. The G-1 motif (which has a consensus in the superfamily of GxxxxGK[S/T] in the single-letter amino-acid code) is well conserved, and the consensus around the G-1 motif of septins is **G**E**S**G**L**G**K**-**S**TLINT**L**F (where the bold residues are strictly conserved). The G-3 motif (DxxG) is moderately conserved, with the

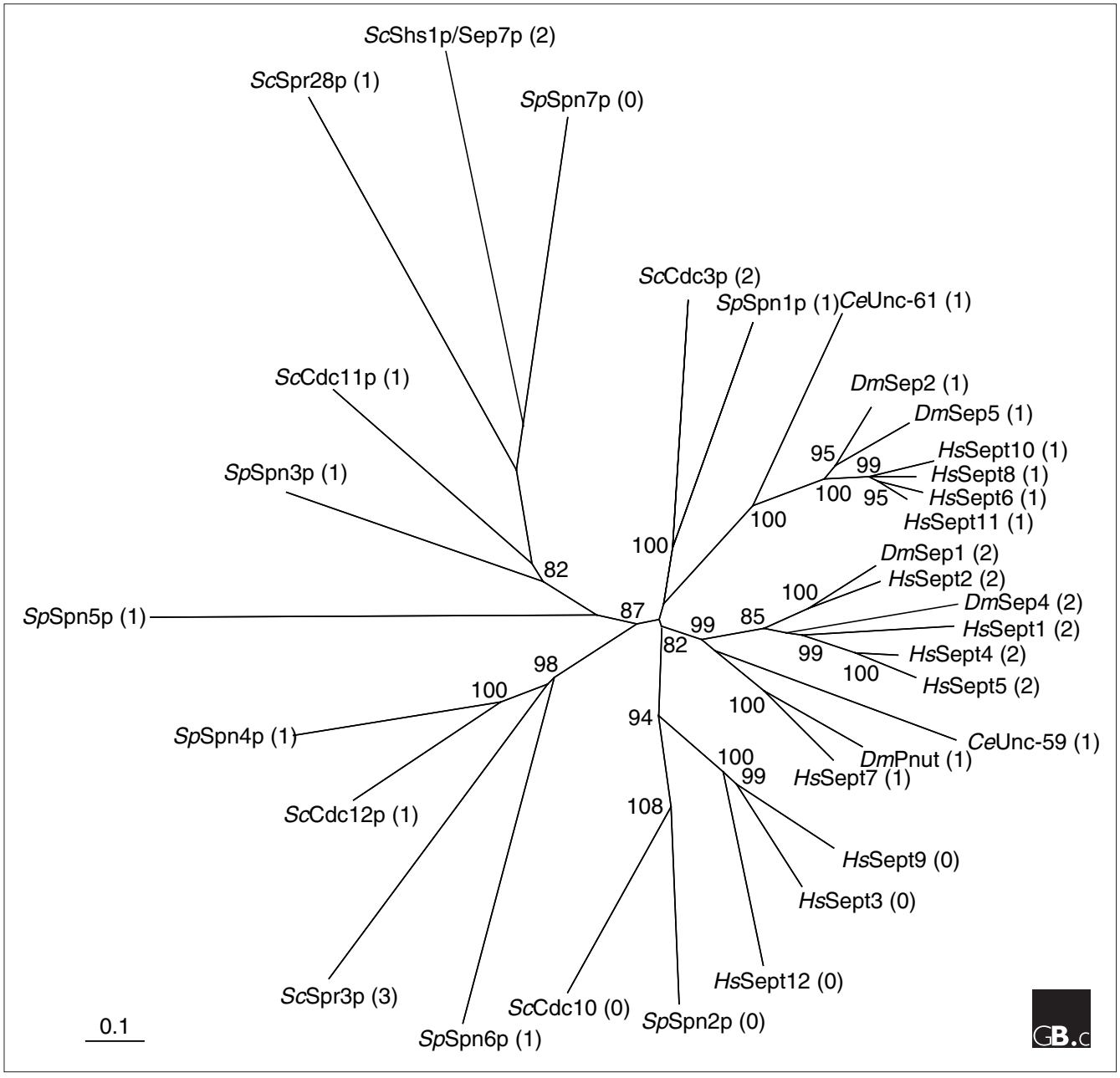

Figure 1
A phylogenetic tree of the septins in *Saccharomyces cerevisiae* (*Sc*), *Schizosaccharomyces pombe* (*Sp*), *Caenorhabditis elegans* (*Ce*), *Drosophila melanogaster* (*Dm*) and humans (*Hs*). The longest amino-acid sequence among the putative polypeptides generated by each gene was analyzed with the software Phylip [59] using the default mode with the UPGMA method, 1,000 bootstrap replicates and systematic tie-breaking, and Poisson-corrected distances with proportionally distributed gaps. The numbers of predicted coiled coils are shown in parentheses. The scale bar represents 0.1 substitutions.

consensus sequence DTP**G**; the G-4 motif (xKxD) is strictly conserved with a unique septin consensus of A**K**A**D**. The G-2 and G-5 regions cannot be defined in septins; some other classes of GTPases also lack these motifs. GTP-binding and GTP-hydrolyzing activities of the purified and recombinant septin complexes or polypeptides have been demonstrated *in vitro* ([7-11] and M.K., C.M. Field, M.L. Coughlin and T.J. Mitchison, unpublished observations). The biochemical and biological significance of septin GTPase activity remains a conundrum in the field, however.

Septins polymerize to form rod-shaped hetero-oligomeric complexes, which in turn are arranged in tandem arrays to form filaments that appear by electron microscopy to be

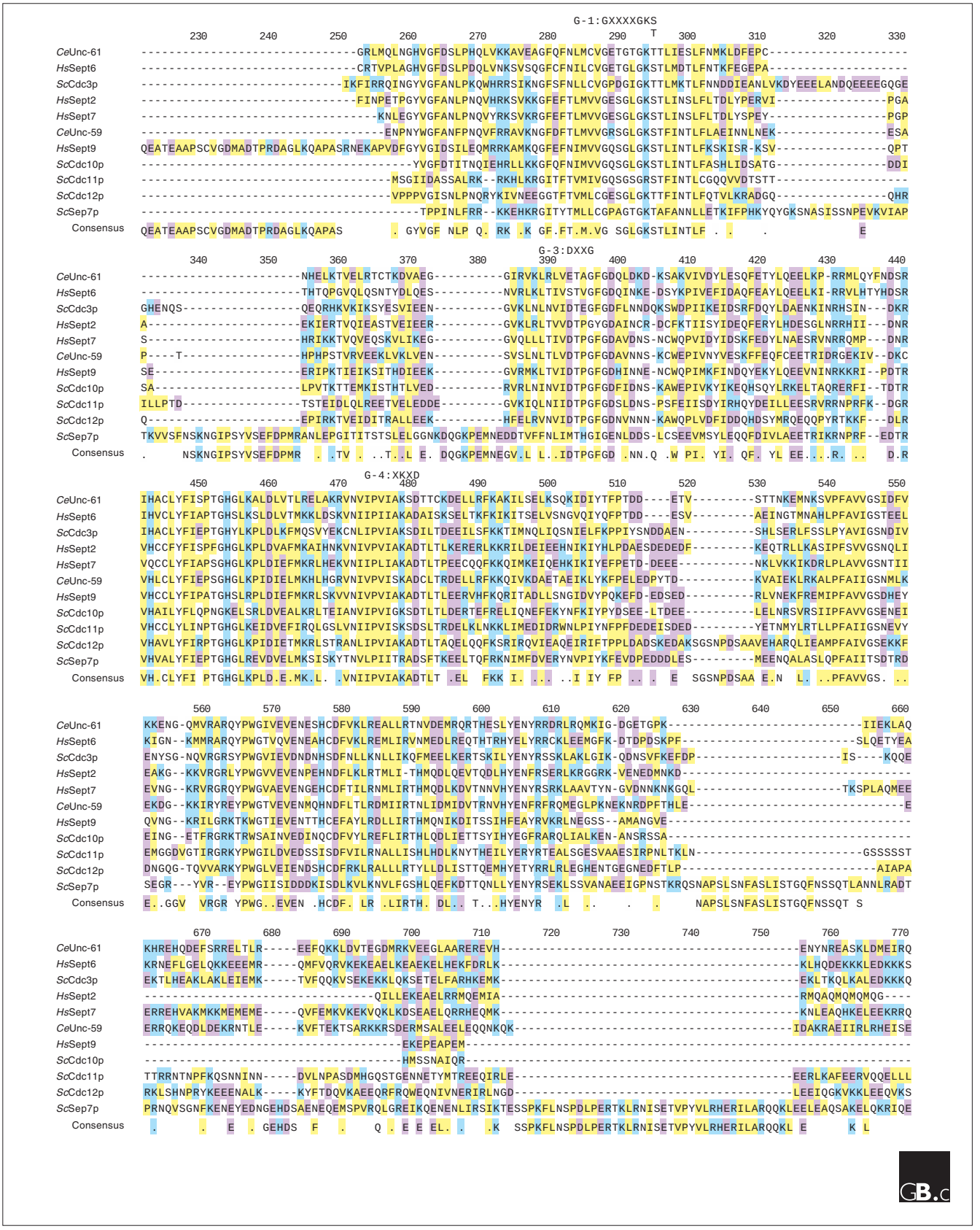

Figure 2 *(see the legend on the next page)*

7-9 nm thick. These filaments can assemble *in vitro* into even higher-order structures by self-assembly and templated assembly. Repeating unit complexes made up of Cdc3p, Cdc10p, Cdc11p, and Cdc12p in budding yeast, Sep1, Sep2 and Pnut in flies, and Sept2, Sept6, and Sept7 in mouse and human have been purified and characterized [4,7,10,12-14]. The majority of septins are predicted to have one or more coiled-coil regions, each spanning about 50-100 amino-acid residues, mostly near the carboxyl termini. In the metazoan septins, proteins that are close on the phylogenetic tree have the same number of coiled coils (Figure 1). Some of the coiled-coil regions are necessary for intermolecular interaction upon septin complex formation [10], whereas others are dispensable ([11,15] and M.K., C.M. Field, M.L. Coughlin and T.J. Mitchison, unpublished observations). Some septins have no predictable coiled-coil region (for example, *S. cerevisiae* Cdc10p, *S. pombe* Spn2p and Spn7p, and human Sept3, Sept 9 and Sept12). The mechanism of inter-septin interaction other than through coiled coils is unknown.

The isoelectric points of most septin polypeptides are within the acidic to neutral range, but each organism has one or two septins of basic charge (for example, *S. cerevisiae* Cdc12p, *S. pombe* Spn2p and Spn5p, both *C. elegans* septins, *Drosophila* Pnut and human Sept7 and Sept9; these are indicated in Table 1). The nematode is exceptional in that it has only two septin genes, both of which encode highly basic proteins. The significance of the isoelectric points of septins is currently unknown. Regardless of the total charge, a short stretch of basic residues preceding the G-1 region is shared by most, but not all, of the septins. Some of these basic residues are critical for interactions with phospholipids *in vitro* [9,11].

The budding yeast septins Cdc3p, Cdc11p and Shs1p have one or more motifs for sumoylation, [I/V/L]KX[E/D]; the lysine is the attachment site for the ubiquitin-like protein SUMO. Mutating these sites results in loss of bud-neck-associated SUMO and persistent septin rings [16]. Thus, SUMO conjugation is a prerequisite for septin-ring disassembly. This discovery provided a breakthrough towards an understanding of the regulatory mechanism of yeast septin dynamics, and it also suggests that the significance of the sumoylation motifs found in septins from other organisms should be tested.

Localization and function

Expression of the septin genes seems to be regulated according to the cell cycle, cell lineage, and developmental stage. In accordance with a generally accepted notion that the hetero-oligomeric complex is the main functional unit of the septin system [4], the cell-type distributions of different septin proteins largely overlap one another. Paradoxically, however, their subcellular localization is not necessarily identical; this is demonstrated, for example in postmitotic cells in the mouse brain [17]. The differential localization of septin proteins or complexes may reflect their distinct roles *in vivo*. Besides the best-known functions in cytokinesis, the septin system seems to have evolved to fulfill multiple roles in dividing and non-dividing cells. The normal localization, mutant phenotypes, and possible functions inferred from genetic and cell biological data are summarized for key organisms below.

S. cerevisiae and *S. pombe*

The 'classical' septins of budding yeast (Cdc3p, Cdc10p, Cdc11p, Cdc12p, and Shs1p/Sep7p) predominantly occur as ring(s) encircling the mother-bud neck, but they also localize at the cell cortices near the presumptive bud site, at the bud scar after cytokinesis, and at the tapering part and the tip of the shmoo, a pheromone-induced protrusion [2,18,19]. As described above, the main phenotype of the original temperature-sensitive mutants (*cdc3, cdc10, cdc11* and *cdc12*) is a lack of bud-neck filaments and cytokinesis defects. The *CDC3Δ* and *CDC12Δ* mutants are lethal; the *CDC10Δ* and *CDC11Δ* mutants are viable but are unable to organize the bud-neck filaments (the septin ring), and the other septins localize to the bud neck to partially fulfill the functions of the missing septins [12,19].

The septin ring is a multifunctional structure that serves several functions: firstly, as a spatial landmark to establish cell polarity for bud-site selection, in cooperation with other proteins (such as the bud-site selection proteins Bud3p and Bud4p) [20,21]; secondly, as a barrier that prevents bud-specific cortical molecules (Spa2p, Sec3p, Sec5p, Ist2p and others) from diffusing laterally into the mother-cell cortex [22,23]; thirdly, as a scaffold to recruit molecules for cell-wall synthesis (for example, the chitin synthases Chs4p and Chs3p and the scaffold protein Bni4p) [24] and for positioning of the mitotic spindle [25]; and finally, as an apparatus to monitor and control progression of mitosis in conjunction with the cell-cycle regulatory kinases Gin4p, Hsl1p and Kcc4p [26-28], and a component of the mitosis exit network, Tem1p [29,30].

The 'non-classical' *S. cerevisiae* septins (Spr3p and Spr28p) are expressed in a temporally limited manner during spore formation and are targeted beneath the developing prospore

Figure 2 *(see the figure on the previous page)*
Multiple alignment of the central regions of representative septins. Amino-acid sequences of the representative septins were aligned using MacVector. Acidic, basic and hydrophobic residues are in purple, blue, and yellow respectively. The GTPase motifs that are conserved in this family - G-1, G-3 and G-4 - are indicated above the sequence. A few other conserved stretches of hydrophobic and charged residues are also recognizable. Species abbreviations are as in Figure 1.

wall [15,31,32]. Deletion of the *SPR3* or *SPR28* genes causes no obvious phenotype, and a double mutant has minimal defects in sporulation, suggesting that there is compensation by the other septins [15,32].

S. pombe Spn1p, Spn3p, and Spn4p localize to medial ring(s) around the circumference of the dividing cell, where they functionally interact with Mid2p (which is related to the actin-binding protein anillin in animals). The *spn1Δ* and *spn4Δ* mutants show mild cytokinetic defects such as delayed cell-cell separation and accumulation of cells with one or more septa [2,33,34].

Animals

The *C. elegans* UNC-59 and UNC-61 septin proteins localize to the leading edge of the cleavage furrow and the spindle midbody. Mutants of either or both of them exhibit minimal defects in embryonic cytokinesis, but abnormalities in post-embryonic morphogenesis occur in multiple organs; these include vulva protrusion, germ-cell defects including gonad extrusion, egg-laying defects, and deformities in the male tail and male sensory neurons. The uncoordinated movement defect through which the mutants were originally isolated also indicates some functional defects in the mutants' nervous systems. Some of these phenotypes are recapitulated by silencing *unc-59* and/or *unc-61* through siRNA microinjection of small interfering RNAs (siRNAs) [35,36].

In the *Drosophila* embryo, the Pnut, Sep1, and Sep2 septin proteins have been found in the front of cellularization moving along the early embryo, in the cleavage furrows of dividing cells, and at the leading edges of the epithelium during embryonic dorsal closure. Later in development, they are found in the apical and basal cell cortices of larval imaginal discs, in the cell cortices of the embryonic and larval central nervous system and of photoreceptor cells in the eye imaginal discs [37-39], and in ring canals (stable intercellular bridges formed by incomplete cytokinesis of male and female germ cells) [7,40,41]. The *pnut* gene was identified as an enhancer of the *seven in absentia* defect, which results in loss of the R7 photoreceptor cells; *pnut*-null mutant larvae have severely reduced cell number, with multinucleated cells in the imaginal discs and brain, and they die shortly after pupation [37]. Mutant embryos lacking the Pnut contribution from both the mother and the zygote have abnormal organization of actin rings in the late cellularization stage of embryogenesis and extensive morphological defects during gastrulation and in the formation of cuticle, head, tail, and denticles [39].

Mammalian septins have been found in the cell cortex, contractile ring and midbody of mitotic cells (Sept2, Sept4, Sept6, Sept7, and Sept9) and in the cell cortex, actin stress fibers (Sept2, Sept4, Sept6, Sept7, and Sept9) and microtubules (Sept9) of interphase cells ([8,9,13,14,42-46] and M.K., C.M. Field, M.L. Coughlin and T.J. Mitchison, unpublished observations). In the nervous system, they are seen on the cytoplasmic side of presynaptic membranes (Sept7) and synaptic vesicles (Sept5 and Sept6) and in the endfeet of astroglia (Sept4 and Sept7) [17]. Cytokinesis is perturbed by microinjection of anti-septin antibodies (against Sept2 and Sept9) or transfection of siRNAs (against *Sept2, Sept7, Sept9*) [8,45,46]. Depletion of Sept2 or Sept7 protein by RNA interference also causes disorganization of actin stress fibers, leading to a flat cell morphology in interphase cells [14]. Although Sept5 is highly expressed in mature nervous systems, no brain abnormality is seen in the *Sept5*-null mice, probably because of compensation by redundant septin species [47]. *Sept5*-null mice do, however, aggregate and release granules from blood platelets more readily than do wild-type mice [48].

Frontiers

A number of open questions remain with regard to the septins. Firstly, the fine structures of septins beyond the ultrastructural level are totally unknown. Resolving the atomic structures of septin monomers, oligomers and polymers should help us to address the major questions in septin biochemistry, such as the mechanisms of septin polymer assembly and disassembly and how GTP hydrolysis might be coupled to changes in the structure and activity of the proteins. It will be important to elucidate the mechanisms by which sumoylation and phosphorylation might control septin assembly and disassembly at the structural, biochemical, and cellular levels [16,49].

The interactions of septins with non-septin molecules - such as actin and anillin [8,14,33,34], microtubules [25,45,46], mitosis-associated proteins (see above), and lipids [9,11] - should help to reveal their unknown cellular functions and to clarify the mechanisms underlying the events in which they are involved. Likewise, the discovery of new subcellular localizations of septins may also lead to discoveries of novel roles for the proteins, as is illustrated by a mitochondrial septin variant that has been implicated in apoptosis [50].

Many research groups have found independently that two human septin genes from different groups (*Sept6* and *Sept9*; see Figure 1) have translocated to, and fused in-frame with, the mixed lineage leukemia (*MLL*) gene, and that a few human and mouse septin genes (*Sept2*, *Sept4*, and *Sept9*) are amplified and/or aberrantly expressed in a variety of malignancies, including leukemia, lymphoma and solid tumors (see, for example, [51,52]). Although the hypothetical oncogenic activities of these septins and the fusion proteins remain to be tested, exploring the involvement of septins in carcinogenesis should bring novel perspectives to cancer research as well as to septin biology.

As mentioned above, a subset of septins are abundantly expressed in metazoan nervous systems, but the biological

significance of septins in postmitotic neurons and glial cells has not been understood. Considering the functional redundancy and complexity of the septins, determining their precise roles in neural development and in synaptic or glial functions is challenging, even using systematic genetic analysis including multiple and conditional gene disruption. The septins of nematodes have the potential to lead the field of septin neurobiology, given their relative simplicity.

Finally, in addition to the elusive functions of septins in normal brains, aberrant deposits of septins have been found in neurofibrillary tangles in Alzheimer's disease, in Lewy bodies in Parkinson's disease, and in related pathological aggregates in human brains [53,54]. Exploring the possible linkages between septins and the major players in each disease (such as amyloid-precursor protein, presenilins, and tau proteins in Alzheimer's disease and parkin, the Pael receptor, and synucleins in Parkinson's disease [54,55]) is expected to reveal functions for septins in the brain and help to clarify the unknown pathophysiology underlying these disorders.

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