

The septins: roles in cytokinesis and other processes

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The septins are a novel family of proteins that were first recognized in yeast as proteins associated with the neck filaments. Recent work has shown that septins are also present in other fungi, insects, and vertebrates. Despite the apparent differences in modes of cytokinesis amongst species, septins appear to be essential for this process in both fungal and animal cells. The septins also appear to be involved in various other aspects of the organization of the cell surface.

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Current Opinion in Cell Biology 1996, 8:106–119

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Abbreviations

EM electron microscopy
PCR polymerase chain reaction
ts temperature-sensitive

Introduction

The septins were first recognized in *Saccharomyces cerevisiae* as a set of homologous proteins (the products of the *CDC3*, *CDC10*, *CDC11*, and *CDC12* genes) associated with the ~10 nm filaments found at the cytoplasmic face of the plasma membrane in the mother–bud neck [1–7]. Two additional *S. cerevisiae* septin-encoding genes, *SPR3* [8,9] and *SPR28* (chromosome IV, cosmid 9934) have been identified subsequently, and related proteins are now known to be present in other fungi ([10,11]; T Pugh, HB Kim, O Al-Awar, JR Pringle, unpublished data), insects (*Drosophila*) [12,13], amphibians (*Xenopus*) (M Glotzer, T Hyman, personal communication), and mammals (both mouse and human) ([14–17]; L Schaefer, H Zoghbi, personal communication; M Kinoshita, S Kumar, M Noda, GenBank entry D63878). The name ‘septins’ alludes to the widespread involvement of this family of proteins in cytokinesis and septum formation. However, data from both fungal and animal systems suggest that the septins also have a variety of other roles in morphogenesis and the organization of the cell surface. As the septins and interacting proteins have been the subject of only one previous brief review [18], we attempt to provide here a

concise but comprehensive review of the topic up to the present time.

Septin properties, interactions, and assemblies

Properties of septin proteins

The known septins range from 275 to 539 amino acids in length (31 990–60 200 kDa) and display ≥26% identity in amino acid sequence over their entire lengths (Fig. 1). The sequence similarity amongst the proteins is greatest in their central regions; the amino-terminal and carboxy-terminal regions are more divergent in both length and sequence (Fig. 2). Except for the presence of a P-loop consensus (see below), the septins are not closely homologous to other known proteins. Although multiple septins are present in each organism in which these proteins have been found, there is not in general a close one-to-one correspondence between the individual septins of distantly related species (Fig. 1). This suggests either that the specific functions of individual septins have not been closely conserved during evolution, or that the expansion of the septin families occurred after the divergence of the major phyletic lines. In this regard, it may be significant that septins have not yet been identified in plants, algae, or slime molds, and that only three septins, all apparently coexpressed (see below), have been identified in *Drosophila*, as opposed to the six which have been identified in each of two yeasts. However, the searches done to date have probably not been exhaustive.

All of the known septins contain P-loop consensus sequences (predicted nucleotide-binding sites) (Fig. 2), and recent evidence indicates that at least some of the *Drosophila* and *S. cerevisiae* septins bind GTP and GDP (C Field, T Mitchison, personal communication; MS Longtine, JR Pringle, unpublished data). By analogy to actin and tubulin, nucleotide binding might be involved in the control of septin assembly or function, and its importance is suggested by the strong conservation of the P-loop sequence motif. However, nucleotide hydrolysis has not been demonstrated, and the role of nucleotide binding remains unclear.

In addition, all of the known septins except for *S. cerevisiae* Cdc10p, *C. albicans* CaCdc10p, and *S. pombe* Spn2p contain predicted coiled-coil domains of 35 to 98 amino acids at or near their carboxyl termini (see Fig. 2). These domains may be involved in homotypic or heterotypic interactions

Figure 1

	S.c. Cdc10p	S.c. Cdc11p	S.c. Cdc12p	S.c. Spr3p	S.c. Spr28p	S.p. Spn1p	S.p. Spn2p	S.p. Spn3p	S.p. Spn4p	S.p. Spn5p	C.a. Cdc3p	C.a. Cdc10p	D.m. Pnut	D.m. Sep1	D.m. Sep2	M.m. Diff6	M.m. Nedd5	M.m. H5	H.s. H5	H.s. 'hCdc10'	H.s. Nedd5
S.c. Cdc3p	38	35	46	29	27	45	36	36	32	31	57	34	38	43	37	39	41	35	35	45	39
S.c. Cdc10p		38	39	33	31	37	52	35	35	32	41	66	41	43	36	39	41	40	39	40	41
S.c. Cdc11p			32	30	35	31	35	42	33	34	34	35	35	41	33	37	37	39	39	35	32
S.c. Cdc12p				37	28	34	40	33	51	29	39	38	38	41	36	35	38	42	42	38	37
S.c. Spr3p					31	30	34	31	40	26	33	31	31	36	28	33	37	32	31	34	33
S.c. Spr28p						31	27	32	28	27	31	28	32	34	31	32	29	31	31	33	27
S.p. Spn1p							37	35	36	33	50	35	41	44	38	42	44	39	38	45	42
S.p. Spn2p								33	36	32	37	51	38	38	35	37	38	40	39	38	38
S.p. Spn3p									33	35	39	31	40	39	33	33	39	38	38	38	37
S.p. Spn4p										32	36	35	38	41	35	36	39	39	40	37	39
S.p. Spn5p											32	30	33	32	29	32	33	30	31	35	33
D.m. Pnut														53	38	44	51	43	43	67	48
D.m. Sep1															43	54	77	62	61	56	75
D.m. Sep2																38	42	40	39	41	42
M.m. Diff6																	55	63	63	47	55
M.m. Nedd5																		63	62	56	99
M.m. H5																			93	53	62
H.s. H5																				53	62
H.s. 'hCdc10'																					56

The septin family of proteins. Each entry is the percentage of identical amino acids in the pairwise comparison over the full length of the shorter protein, as determined by the GCG 'GAP' program using the default parameters. Percentage identities $\geq 50\%$ are shown in bold face. GenEmbl Accession Numbers and references are as follows: *S. cerevisiae* (S.c.) Cdc3p (L16548), Cdc10p (L16549, P25342), Cdc11p (L16550), Cdc12p (L16551), Spr3p (L31767, U24129), and Spr28 (Z48612) ([8*,9,128]; BK Haarer, SH Lillie, JR Pringle, unpublished data; Chromosome IV, cosmid 9934); *Schizosaccharomyces pombe* (S.p.) Spn1p (U31742), Spn2p (U29888), Spn3p (U29889), Spn4p (U29890), and Spn5p (U29891) (T Pugh, HB Kim, OS Al-Awar, JR Pringle, unpublished data); *Candida albicans* (C.a.) Cdc3p (P39826) and Cdc10p (P39827) [10]; *Drosophila melanogaster* (D.m.) Pnut (P40797), Sep1 (L33246), and Sep2 (U28966) ([12*,13*]; OS Al-Awar, M Peifer, JR Pringle, unpublished data); *Mus musculus* (M.m.) Diff6 (P42209), Nedd5 (D49382), and H5 (P28661) [14-16]; and human (H.s.) H5 (L Schaefer, HY Zoghbi, personal communication; no accession number; H5 name assigned here because of its close similarity to mouse H5), 'hCdc10' (JC2352) ([17]; the name 'hCdc10' should probably be changed because the protein is not particularly close to yeast Cdc10p and, in particular, possesses a predicted coiled-coil domain, in contrast to Cdc10p), Nedd5 (D63878) (unpublished 'ORF4' of M Kinoshita, S Kumar, M Noda; Nedd5 name assigned here because of the close similarity to mouse Nedd5). Full sequences are not yet available for a sixth *S. pombe* septin (Spn6) (HB Kim, T Pugh, OS Al-Awar, JR Pringle, unpublished data), three *Aspergillus nidulans* septins [11], and two *Xenopus laevis* septins (M Glotzer, T Hyman, personal communication).

among the septins themselves and/or in interactions between the septins and other proteins (see below).

Inter-septin Interactions

Extensive data suggest that the septins interact intimately with each other. First, multiple septins typically colocalize. For example, in *S. cerevisiae* Cdc3p, Cdc10p, Cdc11p, and Cdc12p colocalize to the mother-bud neck in vegetative cells ([5-7]; HB Kim, BK Haarer, JR Pringle, unpublished data), whereas Cdc3p, Cdc11p, and Spr3p (and perhaps other septins) colocalize to the region of the developing

prospore wall in sporulating cells ([8*]; see below). Similarly, in *Drosophila*, Pnut, Sep1, and Sep2 colocalize in every situation examined to date ([12*,13*]; O Al-Awar, M Peifer, JR Pringle, unpublished data). Moreover, localization of the septins appears to be interdependent. In *S. cerevisiae*, a temperature-sensitive (ts) mutation in *CDC3*, *CDC10*, *CDC11*, or *CDC12* causes a disappearance of all four proteins from the mother-bud neck ([5-7]; HB Kim, BK Haarer, JR Pringle, unpublished data), and deletion of *SPR3* appears to cause a partial loss of Cdc3p and Cdc11p localization to the prospore wall [8*]. Similarly, mutant

Figure 2

<i>S. c. Cdc3p</i>	MSLKEEQVSI KQDPEQEERQ HDQFNDVQIK QESQDHDGVD SQYNTGTQND DSERFRAAES DVKVELPGLG MGITSSQSEK GOVLPDQPEI	90
<i>S. c. Cdc10p</i>		MD
<i>S. p. Spn1p</i>	MASM VLDAGMPTVK DDSTRSRGSD VDSFTSTDMV TQINVEAAIS ENKNE.....EKPIQD NSEQEFNPHV	65
<i>D. m. Sep1</i>		MADTK
<i>M. m. Diff6</i>		5
<i>S. c. Cdc3p</i>	KFIRRRQINGV VGFAMLPKQW HRRSIRNGFS FMLLCVQPDG IGTITLMKTL FMNDIDIANL VDYEWELAN DOEEEGQGE GHENOSQEQR	180
<i>S. c. Cdc10p</i>	PLSSVQPAZY VGFDTITNQI EHRLLKKGQF FMNVVVGQSG LQKSTLINTL FASHLIDSAT GDDISALPVT KT	74
<i>S. p. Spn1p</i>	SIQRLQNGY VGFASLFWQW HRRCVRGQFN FMVLVLGSGG SGKSTLVNLT LMRDVYPTPT .KSLTGDFG	133
<i>D. m. Sep1</i>	GFSIETPGY VGFAMLPKQV HRRSVKKGFE FTLMVVVSGG LQKSTLVNLT FLTDLYPERI IPDAIEKQK QT	76
<i>M. m. Diff6</i>	MDKEY VGFAPALPQL HRRSVKKGFD FTLMVVGSGG LQKSTLVNLT FLTNLYEDRQ VFDASARTA QT	66
	=====	
<i>S. c. Cdc3p</i>	HKYKIKSYES VIKESGVKLS LNVIDTEPGF DFLNMDQKSW DPIIKEIDSR FDQYLDANK IMRHS .INDK RIRACLYTIE PTGHYLPKLD	269
<i>S. c. Cdc10p</i>	..TEMKISTH TLVEDRVRLS INVIDTEPGF DFIDMSK .AM EPIVKYIKKQ HSQYLAKKLT AQRERFITDT RVRHAILYFLQ PNGKELSRID	161
<i>S. p. Spn1p</i>	..VMINSNAV HIVENGISLQ LNVIDTEPGF DFIDMTD .CW QFVLTDIEGR YDQYLELEKH NPRST .IQDP RVRACIFFFIQ PTGHAIISAME	219
<i>D. m. Sep1</i>	..VKLEASTV HIERGVKLR LFVVDTPGFG DAIDMSN .SF GALLEYIDEG YERFLRDESG LMRRM .IVDN RIRCCFFYIS PTGHGLEKPLD	162
<i>M. m. Diff6</i>	..LTIERRGV HIERGGIKVK LTLVDTGPGF DSVDFSD .CW LFVVRFINEG FEQYLKDESG LMRRM .IQDS RVRCCLYTIS PTGRAPAP .R	151
	=====	
<i>S. c. Cdc3p</i>	LKPMQSVYRK CHLIPVIAKS DILTDERILS FKKTIDMQLI QSMIELFKFP IYSNDDAE .N .SHLSERLFS SLPYAVIGSN DIVENYSQNG	357
<i>S. c. Cdc10p</i>	VEALKRLTEI ANVIPVIGKS DTLTLDERTE FRELIQMEFE KYMFKIIPYD SEKLTDEHLE LNRSVRSII ..PFAVVGSE NEIMI .NGET	247
<i>S. p. Spn1p</i>	LVRMLALSKK VMIIPIIAKA DTLTLDERLN TKRMELRPQI YHMIRIFPPP TYTIDDEPE ..SVAENADIMS RIPFAIIAM TFVVMNEGRK	307
<i>D. m. Sep1</i>	VEPMCKLSEK VMIVPVIAKA DCLTKKRIILR LKCRIMQIE SEGIKIYPLF DCDSDEHLE .D YKEQVKQLKE AVFFAVCGAN TLLEV .KGRK	250
<i>M. m. Diff6</i>	CGFLRAVHEK VMIIPIVIGKA DALMPRETQA LKQKIRDQLK EEEINITYQFF ECDSDEHLE .E FKQSMENKE NIFFAVVGSC EVVRD .GTRP	239
	=====	
<i>S. c. Cdc3p</i>	VRGRSYPMGV IVNDIMHSD FNLLKNLLIK QPMELAKERT SKILYENYRS SKLAKLGI .. KQDNVFP KFPDPISKQ HEKTLHEAKL	442
<i>S. c. Cdc10p</i>	FRGRKTMSA INVEDIMQCD FVYLKREPLIR TELQDLIFT SYIHYESFRA RQLIAL .. KEMA .NS RSSAHMSSNA	319
<i>S. p. Spn1p</i>	VRGRKYPGV VENDEEHSD FPKLREMLIR TELKELAKQT NK LYEAYRT ERLLSSGI .. SQDHVFP REVNPSAKLE HERALHEKEL	391
<i>D. m. Sep1</i>	VRGRLYPMGV VEVENPDHCD FIKLRTMLI TEMQDLQVFT QEVHYENYRS DRILAK .. G IKGKMGVKA ERDSS	320
<i>M. m. Diff6</i>	VRGRKYSWT VEVENPHCD FLNLRLMLVQ TELQDLKEVT HDLLYCYRA RCLQSLARPG ARDRASREKL SRQSA	314
<i>S. c. Cdc3p</i>	AKLEIEKRTV FOQKVSEKK KLOKSETELF ARRKKEKEL TEQLKALEDK KKOLELSINS ASPNNVHSPV PTKKGFLR *	521
<i>S. c. Cdc10p</i>	IQR *	322
<i>S. p. Spn1p</i>	MMKRAEKRTV FSQKVSEKK RLKQSENELR TREREMKAL EKKQADLIDH KNRLMOAKAA AEN EKSERKFFK *	463
<i>D. m. Sep1</i>	..SQV ...V SNSVLGKDR TLOKELAKLR .FMQEM LAQMOARMOA QQ *	361
<i>M. m. Diff6</i>	..TEIPLPML PLA ...DTEK LIRKDEDEL .RMQEM LEKQOQMOQ SQAGEQSDV L *	365

Sequences of representative septins. The proteins shown are *S. cerevisiae* Cdc3p and Cdc10p, *S. pombe* Spn1p, *D. melanogaster* Sep1, and *M. musculus* Diff6 (for references, see Fig. 1 legend). Sequences were aligned using the GCG 'Pretty' program; amino acid symbols are in bold face wherever two or more of the septins shown have identical amino acids. Dots indicate gaps introduced to maximize sequence alignment. Dashed underlining indicates the elements of the P-loop motifs [129]. Thick underlining indicates regions predicted (probability > 89%) to form coiled coils by the program of Lupas *et al.* [130]. Thin underlining indicates regions of Cdc3p and Sep1 that show a smaller predicted probability (32–55%) of forming coiled coils.

Drosophila embryos deficient in Pnut also fail to localize Sep1 normally to neurons of the central nervous system (the only tissue examined to date) [13*].

Other genetic and biochemical data from *S. cerevisiae* and *Drosophila* also support the hypothesis that there are close interactions among the septins. For example, most pairs of *cdc3^{ts}*, *cdc10^{ts}*, *cdc11^{ts}*, and *cdc12^{ts}* mutations display synthetic lethality (i.e. the double mutants are inviable at temperatures at which each of the single mutants is viable); mutations in *CDC3* can suppress mutations in *CDC10* and vice versa; and overexpression of *CDC12* and *CDC10* can partially suppress mutations in *CDC11* and *CDC3*, respectively ([19]; J Robinson, R Preston, JR Pringle, unpublished data). These data also raise the possibility of functional redundancy among the *S. cerevisiae* septins; this possibility might also explain why *cdc10* and *cdc11* deletion strains are viable (although abnormal) [8*,20]; MS Longtine, JR Pringle, unpublished data). In addition, Cdc3p, Cdc10p, Cdc11p, and Cdc12p

co-immunoprecipitate in experiments using antibodies to Cdc3p or Cdc11p (A Healy, M Longtine, JR Pringle, unpublished data), and Pnut, Sep1, and Sep2 behave as a robust complex as judged by immunoprecipitation, sucrose-density-gradient centrifugation, and gel filtration in several buffers ([13*]; C Field, T Mitchison, personal communication; O Al-Awar, M Peifer, JR Pringle, unpublished data). Finally, two-hybrid assays [21] have also revealed strong interactions among the *S. cerevisiae* septins (C De Virgilio, JR Pringle, unpublished data). Interestingly, these interactions do not seem to be mediated solely by the coiled-coils found in the carboxy-terminal domains (see above).

Association with higher-order structures and the plasma membrane

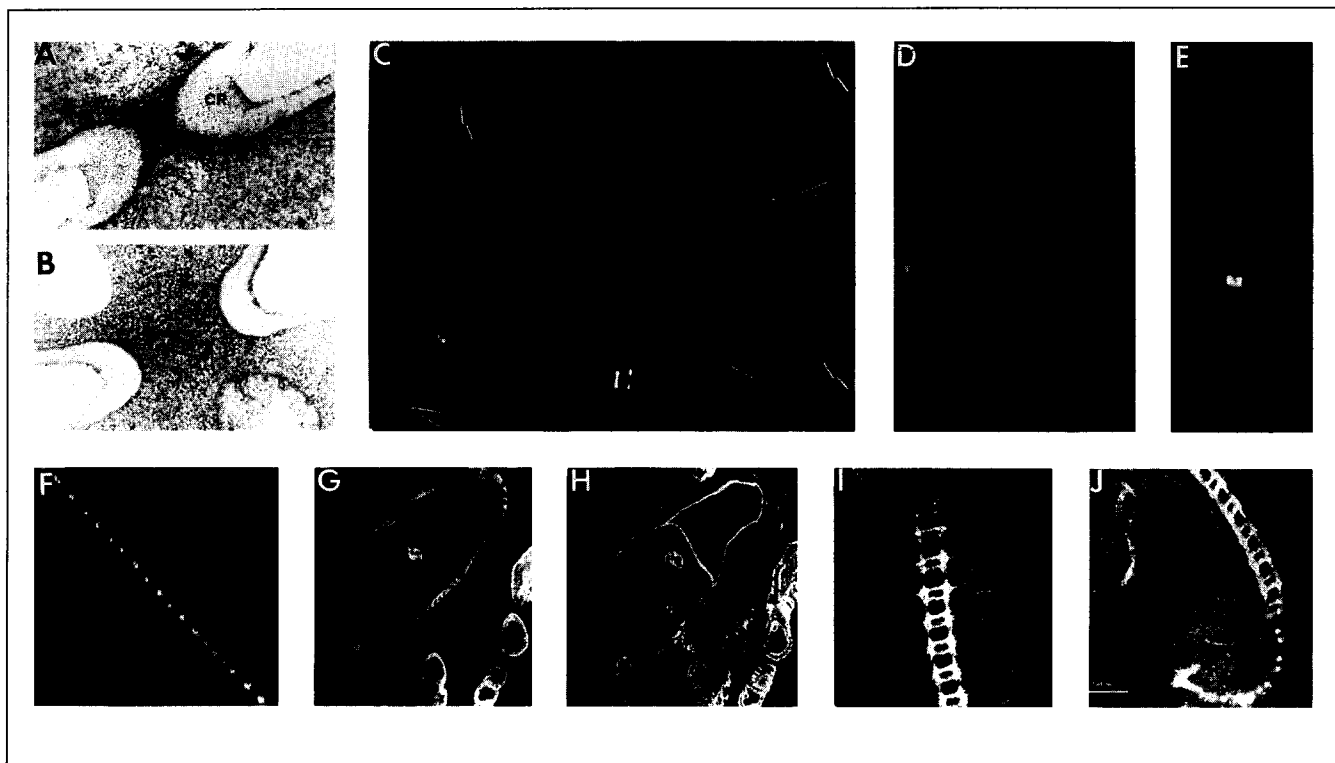
Electron microscopy (EM) studies of *S. cerevisiae* revealed a novel filament system in the neck that connects the mother cell and bud [1,3]. The filaments are ~10 nm

in diameter and form a ring that encircles the neck in close apposition to the cytoplasmic face of the plasma membrane (Fig. 3A,B). Cytochemical and genetic data show that Cdc3p, Cdc10p, Cdc11p, and Cdc12p are closely associated with these filaments. First, in immunofluorescence and immunoEM studies on wild-type cells, antibodies specific for each of the four proteins decorate the neck region as would be expected if the proteins were constituents of the neck filaments (Fig. 3C) ([5–7]; HB Kim, BK Haarer, JR Pringle, unpublished data; D Preuss, J Mulholland, D Botstein, personal communication). Moreover, when a *ts cdc3*, *cdc10*, *cdc11*, or *cdc12* mutant is shifted to restrictive temperature, the immunofluorescence signals for all four proteins disappear with the same kinetics (differing from mutant to mutant) as do the filaments (as judged by EM) [2,4,5–7]. The simplest interpretation of these data is that Cdc3p, Cdc10p, Cdc11p, and Cdc12p are major structural components of the neck filaments. However, it also seems likely that the

filament system contains proteins in addition to these four, and it has not been demonstrated that these four proteins are sufficient to form the filaments.

One discrepancy between the EM and immunofluorescence data may be important. As judged by EM, the neck filaments appear to form coincident with bud emergence, and to disappear prior to cytokinesis [1,3]. In contrast, immunofluorescence observations clearly show that the septins appear in a well-defined ring at the presumptive bud site ~15 min before bud emergence (Fig. 3C) and persist as a ring at the division site on mother and daughter cells for much of the unbudded phase following cytokinesis (Fig. 3C) [5–7]. It is possible that this discrepancy merely reflects the difficulty of visualizing the neck filaments by EM in unbudded cells. However, it also seems possible that the assembly of the proteins into a higher-order structure that is visible by EM is regulated

Figure 3



(A,B) EM visualization of the *S. cerevisiae* neck filaments in tangential (A) and cross-section (B) (reproduced, with permission, from [3]). CR, chitin ring in the cell wall. (C) Immunofluorescence localization of *S. cerevisiae* Cdc3p in vegetative cells [6]. Arrows indicate assembly of septins at presumptive bud sites; arrowheads indicate residual septin assemblies at recent division sites. (D) Double staining of sporulating cells for tubulin and for Cdc11p [8], illustrating the meiosis II spindles (straight lines) and septin assemblies at the leading edges of the 'prospore walls' (rings around the ends of the spindles). (E) *Drosophila* S2 tissue culture cell stained for Sep1, illustrating septin concentration in the cleavage furrow (reproduced from [13] by copyright permission of the American Society for Cell Biology). (F) Cellularizing *Drosophila* embryo stained for Sep1, illustrating the concentration of septins at the leading edge of the furrow [12,13]. (G,H) *Drosophila* ovarioles double stained for Sep1 (G) and actin (H), illustrating the differences in protein distributions (reproduced from [13] by copyright permission of the American Society for Cell Biology). (I,J) *Drosophila* embryo stained for Sep1, illustrating the high concentration of the protein in neurons of the central and peripheral nervous systems ([I] reproduced from [13] by copyright permission of the American Society for Cell Biology; [J] H Fares, M Peifer, JR Pringle, unpublished data).

independently of their colocalization to the correct part of the cell.

Filaments similar to the *S. cerevisiae* neck filaments have also been seen at the mother-bud neck and near the bases of hyphae in the dimorphic yeast *C. albicans* [22], and it is likely, although not yet demonstrated, that these filaments contain the *C. albicans* septins [10]. However, *C. albicans* is morphologically similar and relatively close phylogenetically [23,24] to *S. cerevisiae*, and it is not yet clear whether septin function in other types of cells involves assembly into a higher-order structure such as the neck filaments. To date, no similar structures have been observed at the developing prospore wall of *S. cerevisiae* [3,25], at the septation site in *S. pombe* ([26]; see below), or in the likely locations in animal systems (for example, see [27–33]). (One possible exception is the periodic densities seen in the intercellular bridges that persist after division of some embryonic *Drosophila* cells [34]; these densities might represent cross-sections of structures comparable to those seen in yeast.) However, it has recently been observed that a purified *Drosophila* septin complex containing Pnut, Sep1, and Sep2 can assemble into filaments of varying length *in vitro* (C Field, T Mitchison, personal communication), which suggests that similar higher-order structures will also be found to exist *in vivo*.

EM studies in *S. cerevisiae* suggest that the septin-containing structures are closely associated with the plasma membrane [1,3], and such an association is also suggested by immunofluorescence studies of *S. pombe* (see below), *Drosophila* [12*,13*], and mammalian cells (see below). Consistent with this notion, both *S. cerevisiae* septins and *Drosophila* septins fractionate largely with the crude membrane fraction in cell fractionation experiments (A Healy, M Longtine, JR Pringle, unpublished data; M Peifer, personal communication). Because none of the septins displays a predicted transmembrane domain, or other obvious motif that could explain the apparent membrane association, it is likely that this association is mediated by another protein(s) that has yet to be identified.

Regulation of septin localization and assembly

In both *S. cerevisiae* (see above) and *S. pombe* (see below), septin-containing structures detectable by immunofluorescence appear and disappear at particular times in the cell cycle. In addition, in *Drosophila*, Sep1 (at least) undergoes orderly, cell-cycle-coordinated rearrangements both in syncytial blastoderm embryos and in the cells of post-gastrulation embryos [13*]. Thus, it appears that the localization and/or assembly of the septins must be regulated, directly or indirectly, by cell-cycle controls. There is, as yet, little information as to how this regulation is achieved. In both *S. cerevisiae* and *Drosophila*,

the immunofluorescence observations suggest that total protein levels do not change much, if at all, during the cell cycle [5–7,13*], and Western blots on synchronized *S. cerevisiae* cultures also failed to detect significant changes in the levels of the individual septins (SK Ford, JR Pringle, unpublished data). These data suggest that the control of septin localization and assembly may be mediated by a post-translational modification such as phosphorylation, but the putative modification(s) have not yet been identified.

Moreover, in *S. cerevisiae*, synthetic lethality has been observed between *cdc12* and a mutant allele of *SWI4*, which encodes a cell-cycle-dependent transcriptional activator (reviewed in [35]), and deletion of *SWI4* has been found to decrease by several-fold the steady state levels of *CDC10* and *CDC11* mRNAs in an asynchronous cell population (H Fares, JR Pringle, unpublished data). Although it is not yet clear whether the synthetic lethality observed in *cdc12 swi4* mutants is due to the effects on expression of *CDC10* and *CDC11* or on the expression of an as-yet-unidentified gene, these results, together with the observation that there is no detectable septin immunofluorescence signal throughout most of the cell cycle in *S. pombe* (see below), keep open the possibility that changes in protein level may play some role in the cell-cycle-coordinated changes in septin localization and assembly.

In *S. cerevisiae*, both the septins and the proteins of the actin cytoskeleton assemble at the presumptive bud site ~15 min before bud emergence (reviewed in [36–38]). Septin assembly and actin assembly appear to be independent of each other ([7,39]; BK Haarer, HB Kim, JR Pringle, unpublished data; K Ayscough, D Drubin, personal communication), but both are dependent on the activity of the 'polarity-establishment proteins', including the Rho-type GTPase Cdc42p and its activating factor Cdc24p ([36–41]; BK Haarer, HB Kim, JR Pringle, unpublished data). Recent observations suggest that activated (i.e. GTP-bound) Cdc42p may promote septin assembly, at least in part, by interacting with, and thereby activating, a pair of functionally redundant protein kinases, Cla4p and Ste20p [42*,43,44]. When a strain deleted for *STE20* and carrying a *ts* allele of *CLA4* is shifted to the restrictive temperature, Cdc11p (the only septin examined to date) appears to assemble normally at the presumptive bud site, but then fails to remain in the normal tight ring at the mother-bud neck; instead, it disperses over the surface of the growing bud. This mislocalization of Cdc11p (and presumably of the other septins) is associated with an abnormally elongated cell shape and lethality, apparently due to a failure to complete cytokinesis [42*]. *cla4* and *cdc12* mutations also show a synthetic-lethal genetic interaction [42*]. How Cdc42p promotes the initially normal localization of the septins in the absence of Cla4p and Ste20p activity is not understood.

Differential expression of septin genes

In *S. cerevisiae*, expression of *SPR3* is undetectable in vegetative cells but is high in sporulating cells [8*,9,45,46]. The expression of *CDC10* is also ~fivefold greater in sporulating cells than it is in vegetative cells [47]. The differential expression of these septins appears to be related to their role(s) in spore formation (see below). In mouse, the Diff6 septin is abundant in the central nervous system and in lymphoid tissue, but is absent or present at very low levels in many other tissues, and the relative levels of *DIFF6* and *H5* (see Fig. 1) mRNAs are also strikingly different in different tissues (M Valencik, JR Pringle, unpublished data). These results suggest that different cell types in mammals may express different subsets of an extended septin family. In this context, it is remarkable that the three known septins in *Drosophila* (one found by serendipity, two by non-exhaustive polymerase chain reaction [PCR] screens) are co-expressed and colocalized (and, indeed, are apparently present as a complex that contains predominantly these three proteins) in all situations examined to date ([12*,13*]; OS Al-Awar, M Peifer, JR Pringle, unpublished data; C Field, T Mitchison, personal communication).

Septin function in *S. cerevisiae*

Analyses of mutant phenotypes, protein localization, and protein interactions have given many clues to septin function in *S. cerevisiae*; however, we are still far from understanding the functions of these proteins at the molecular level.

Cytokinesis

The paradigmatic septin genes *CDC3*, *CDC10*, *CDC11*, and *CDC12* were originally defined by a set of very similar ts-lethal mutants that continue the nuclear cycle but are defective in cytokinesis, and thus form multibudded, multinucleate cells at the restrictive temperature [48]. This phenotype, together with the localization of the septins to the mother–bud neck [5–7], suggests that the septins and associated proteins play a direct, positive role in cytokinesis, but the precise nature of this role is still unknown. The septins might be involved directly in the localization of septal cell-wall synthesis (see below); they might mediate the presumed role(s) of actin and myosin in cytokinesis; or they might play a role in both processes. For example, the septins may be involved in the reorganization of actin to the mother–bud neck that occurs shortly before cytokinesis [39,49–51] and that appears (from the phenotypes of actin mutants [52–54]) to be necessary for cytokinesis. This reorganization does not appear to occur in the septin mutants [39] despite the continuation of the nuclear cycle and hence, presumably, of the cyclic changes in the levels of cell-cycle-control elements that normally trigger actin reorganizations [37,50]. However, attempts to understand the possible septin–actin interaction in cytokinesis are currently hindered by the fact that we do not know whether actin itself is involved exclusively in the localized deposition of new cell-surface material

or whether it also participates in a contractile-ring-like structure. Whatever the role of the septins in cytokinesis, it does not appear to involve a contractile function, because both the neck-filament ring (as judged by EM) and the septin ring (as judged by immunofluorescence) retain their original diameters (or even expand slightly) during cytokinesis and septum formation [1,3,5–7]. EM evidence suggests that the septin mutants may form abortive septumlike structures [55], raising the possibility that some early step(s) in the organization of the septation site can be completed in the absence of septin function.

Morphogenesis and cell wall deposition

In ts *cdc3*, *cdc10*, *cdc11*, and *cdc12* mutants, the buds that form at the restrictive temperature are much longer and narrower than normal from the time that they first appear [39,48]. As the actin system is responsible for the polarized delivery of vesicles carrying new cell-surface material to the growing bud [54,56], the hyperpolarization of bud growth in the mutants presumably reflects an alteration in the structure and/or function of the actin cytoskeleton. Indeed, the actin cytoskeleton does appear to be hyperpolarized in the mutant cells [39]. The details of the apparent interaction between the septins and the actin cytoskeleton remain to be elucidated.

The septins may also be directly involved in localizing the deposition of chitin, a cell-wall component that is confined largely to a ring at the base of the bud and to the primary septum, and hence to the ‘bud scar’ found at each previous division site on mother cells [57]. At restrictive temperatures, ts *cdc3*, *cdc10*, *cdc11*, and *cdc12* mutants synthesize chitin early in the cell cycle, as do wild-type cells, but the chitin is deposited diffusely in the cell wall of the bud rather than in the normal tight ring at its base [4]. This suggests a role for the septins and/or associated proteins in the localization of chitin synthase and/or of relevant activating factors. Recently, a synthetic-lethal interaction has been observed between *cdc12* and mutations in *CHS4* (*CSD4*) (D DeMarini, H Fares, JR Pringle, unpublished data); *CHS4* encodes a protein that was identified previously as a putative regulatory subunit for chitin synthase III, the enzyme that produces the bulk of the cellular chitin [57]. Perhaps a Cdc12p–Chs4p interaction serves to localize the activity of Chs3p, the chitin synthase III catalytic subunit.

Bud site selection

Wild-type yeast cells select bud sites in one or the other of two non-random patterns. In the axial pattern (as normally displayed by *MATa* or *MATα* haploid cells), new buds form adjacent to the immediately preceding division site on both mother and daughter cells, suggesting that the division site is marked by a transient signal that can direct assembly of the next bud site at an adjacent location [58]. In the bipolar pattern (as normally displayed by *MATa/MATα* diploid cells), a daughter cell can bud either adjacent to its division site or at the tip of the distal pole,

and a mother cell can also bud adjacent to any of its previous bud sites; this pattern suggests that persistent or permanent signals mark the potential bud sites [58]. Recent evidence suggests that the septins may be involved in localizing both types of signal molecules.

The *BUD3* gene product appears to be a component of the transient signal used in axial budding. *bud3* mutations specifically disrupt the axial pattern without affecting the bipolar pattern [59,60*]. In addition, immunofluorescence studies have shown that Bud3p assembles at the mother–bud neck, in the precise region occupied by the septins, beginning at about the time of mitosis [60*]. After cell division, the assembled Bud3p remains as a ring marking the cell cortex at the division site on both the mother and daughter cell; these rings then disappear as the new bud sites begin to assemble. In a *cdc12^{ts}* mutant that loses both its neck filaments and localization of all four septins within 10 minutes after temperature shift, the Bud3p immunofluorescence signal is lost with the same kinetics, suggesting that the localization of Bud3p to the neck region is dependent on the septins and/or associated proteins. Consistent with this conclusion, mutations in *CDC10* and *CDC11* have been found to disrupt the axial pattern [20,60*]. The mechanism by which the septins determine the localization of Bud3p remains to be elucidated.

Genetic evidence suggests that the septins also interact with proteins involved specifically in bipolar budding. One such protein is the *SPA2* gene product. Spa2p localizes to a patch at the presumptive bud site prior to bud emergence, to the tips of small buds, and to the neck region prior to cytokinesis [61,62]; deletion of *SPA2* randomizes the budding pattern of a/α diploid cells but has little or no effect on the axial budding pattern of a or α haploid cells [38,63]. Deletion of *SPA2* alone has little effect on growth rate [61,64], but such a deletion is lethal in combination with a non-lethal *cdc10* mutation [20], suggesting an interaction between Spa2p and the septins.

A second protein involved in bipolar budding is encoded by *BNI1*, which was identified in screens both for bipolar-specific budding-pattern mutants [63] and for mutations synthetically lethal in combination with a *cdc12* mutation (H Fares, MS Longtine, JR Pringle, unpublished data). The localization of Bni1p is similar to that of Spa2p, and deletion of *BNI1* also disrupts bipolar budding without affecting axial budding. In addition, deletion of *BNI1* causes morphological abnormalities (including a partial defect in cell division) that suggest defects in the structure or function of the mother–bud neck; the severity of these abnormalities varies with genetic background and mating type, but the effect on bipolar budding is seen even in strains whose morphology is otherwise essentially normal. Bni1p is predicted to contain coiled-coil domains flanking a proline-rich region. It shares at least the latter of these features (and in most cases also the former), in

addition to a block of strong sequence similarity, with other members of a newly recognized family of proteins that includes: *Aspergillus nidulans* FigA [65], which appears to be involved in septation (S Harris, J Hamer, personal communication); *S. pombe* Cdc12p, which is involved in cell division ([66,67]; F Chang, P Nurse, personal communication); *S. pombe* Fus1p, which is involved in cell fusion during mating [68]; *Drosophila* Diaphanous, which is essential for cytokinesis [69*]; *Drosophila* Cappuccino, which is involved in embryonic patterning [70]; and the mouse formins, which are also involved in embryonic patterning [71].

Mating

Haploid yeast cells of mating types a and α can fuse to form diploid zygotes in a process that involves considerable remodeling of the cell surface, including the deposition of chitin [72,73]. Although an early analysis [74] suggested that Cdc3p, Cdc10p, and Cdc11p do not have a direct role in mating (Cdc12p was not tested), more recent data are prompting a re-examination of this issue. First, at least two of the vegetatively expressed septins localize to a band at the base of the projections formed by mating cells (or by cells responding to mating pheromones in the absence of partner cells) [6,7]. This band is more diffuse than that seen at the neck of budding cells and corresponds approximately to the region of chitin deposition in the mating cells. Second, Spa2p, which may interact with the septins ([20]; see above), is necessary for projection formation and for mating under some conditions [64,75]. Third, two-hybrid interactions [21] have been observed between Cdc12p and Afr1p [76*]. Afr1p is normally not expressed in vegetative cells and is induced by mating pheromone [77]. Afr1p localizes to the bases of mating projections very much as do the septins, and appears to interact with the carboxyl terminus of the mating-pheromone receptor to promote a normal morphogenetic response [76*,77]. Upon ectopic expression in vegetative cells, Afr1p localizes to the mother–bud neck and causes abnormal morphogenesis similar to that seen in septin mutants, suggesting that it interferes with septin function [76*]. Finally, although strains deleted for *CDC10* or *CDC11* can mate reasonably well with wild-type cells, they display a strong mating defect in mutant-by-mutant crosses (H Fares, MS Longtine, unpublished data). However, it is possible that this defect is secondary to the morphological abnormalities of the mutant cells and does not reflect a direct role of the septins in mating. Further studies will be required to clarify these issues.

Sporulation

When PCR was used to investigate whether *S. cerevisiae* contains septins other than Cdc3p, Cdc10p, Cdc11p, and Cdc12p, a fifth gene was identified that proved to be *SPR3*, a gene identified previously on the basis of its specific expression late in sporulation [8*,9,45,46]. As expected from this expression pattern, knockout mutations in *SPR3*

produce no obvious phenotype in vegetative cells but, at least in some genetic backgrounds, cause a sporulation defect [8*,46]. In *spr3* mutant strains, meiosis appears to be completed successfully, but spore formation is inefficient, with often only two or fewer spores per ascus being encapsulated. These data suggest that Spr3p (and perhaps other septins) might be involved in the fascinating and poorly understood process by which flattened membrane sacs (the 'prospore walls') appear adjacent to the outer plaques of the spindle pole bodies during meiosis II, extend to envelop (and eventually enclose) the nuclear lobes containing the haploid chromosome sets, and then lay down the multiple layers of the spore wall while also giving rise to the spore plasma membrane [3,8*,25,78–80].

Consistent with this hypothesis, immunofluorescence observations suggest that Spr3p, Cdc3p, and Cdc11p (other septins have not yet been examined) are indeed localized to the prospore wall (Fig. 3D) [8*]. Moreover, although an earlier study using ts mutants suggested that Cdc3p, Cdc10p and Cdc11p were not involved in sporulation [81], deletion of *CDC10* has now been shown to produce a partial block of spore formation in at least some genetic backgrounds [8*]. This rationalizes the previously puzzling observation that *CDC10* expression increases ~fivefold in sporulating cells [47]. The incomplete blockage of sporulation in the *spr3* and *cdc10* knockout strains may well reflect redundancy of function among the septins. Testing this possibility has been hampered by the difficulty of constructing a viable *spr3lspr3 cdc10cdc10* double-mutant diploid and by the inviability (in the cases of *cdc3* and *cdc12*) or unhealthiness (in the case of *cdc11*) of the other deletion strains. However, the recent realization that a sixth *S. cerevisiae* septin (Spr28p, identified by the genome-sequencing project) also shows sporulation-specific expression (C De Virgilio, JR Pringle, unpublished data) may allow more definitive investigation both of the redundancy issue and of the actual functions of the septins during spore formation.

As the septins appear to be concentrated at the leading edge of the prospore wall during at least part of its period of extension [8*], they might be involved in this membrane extension; such a role would be analogous to one possible role for the septins during cellularization in *Drosophila* (see below). By the time that the haploid nuclei are enclosed and cell wall formation is beginning, the septins appear to be uniformly distributed over the surface of the developing spore [8*]. This suggests that they might also play a role in formation of one or more of the layers of the spore wall. In this regard, it is interesting that formation of the spore wall chitosan layer [78] depends on chitin synthase III [57,82], and that Shc1p, a homologue of Chs4p (implicated in the apparent septin–chitin synthase interaction in vegetative cells; see above), is expressed specifically in sporulating cells [57].

Pseudohyphal growth

Some strains of *S. cerevisiae* display an alternative vegetative growth form when grown under nitrogen limitation; during this pseudohyphal growth, budding is unipolar, cells are elongated, cell separation is delayed, and the cells can grow invasively into agar [83,84]. The morphology of certain septin mutants resembles that of wild-type cells undergoing pseudohyphal growth, suggesting that development of the pseudohyphal form may be mediated, in part, by effects on septin function [85].

Septin function in other fungi

There is, as yet, little information about septin function in either *C. albicans* or *A. nidulans*. However, in *C. albicans*, the spatial correlation between the position of the filament ring (at the mother–bud neck in budding cells, and ~2 µm from the base of the hypha in cells beginning mycelial growth) with the position of chitin deposition [22] reinforces the suggestion from observations in *S. cerevisiae* (see above) that the septins may be involved in the localization of chitin synthase activity.

Like *S. cerevisiae*, the fission yeast *S. pombe* is genetically tractable, but the two yeasts are phylogenetically distant from each other [24,86]. They are also morphogenetically distinct: instead of budding, *S. pombe* cells grow by extending the ends of the cylindrical cell and then divide by forming a medial septum, the production of which depends, at least in part, on actin and associated proteins that seem to form a contractile ring [67,87–90]. Thus, comparative studies of septin function in *S. pombe* and *S. cerevisiae* should be a powerful approach to elucidating the mechanism(s) of septin action in cytokinesis and other processes. Six septins (Spn1p–Spn6p) have now been identified in *S. pombe* by screening a λ gt11 library with antibodies generated against the *S. cerevisiae* septins, by PCR-based cloning, and by complementation of the *S. cerevisiae* septin mutations with an *S. pombe* cDNA library (T Pugh, HB Kim, O Al-Awar, JR Pringle, unpublished data).

Like septins in other organisms, Spn1p appears to function in cell division; in particular, it seems to participate in a late stage of septum formation or in subsequent events (perhaps localized cell-wall dissolution) leading to separation of the daughter cells. Spn1p is undetectable by immunofluorescence analysis during most of the cell cycle, and it becomes localized to the presumptive septation site late in the cycle, after the chromosomes are clearly separated during mitotic anaphase; this localization of Spn1p occurs some minutes after actin and associated proteins have become localized to the same site. Spn1p then remains concentrated at the newly formed cell ends for a short time after septum formation appears to have been completed, but it disappears by the time that cell separation occurs. In addition, although an *spn1* knockout

mutant grows well, it has a delay in the completion of septum formation or in cell separation and thus forms chains of cells. It is not yet clear why the absence of Spn1p leads only to a delay, and not to a full blockage, of cell division; this may reflect functional redundancy among the septins that will be clarified by genetic studies of the other septins. The *spn1* mutant phenotype resembles that of the *sep1* mutant [91], but the mutations are not allelic (M Sipiczki, personal communication).

Septin function in *Drosophila*

Evidence concerning septin function in *Drosophila* comes from protein localization and from the phenotypes of *pnut* mutants; *sep1* and *sep2* mutants are not yet available, although the map locations of both genes are known (*sep1*: region 19F4–5; *sep2*: region 92E8–9). Analysis of the mutant phenotypes is complicated because *pnut* is an essential gene; thus, *pnut/pnut* homozygotes are inviable and must be generated by a cross between heterozygous flies. In such a cross, the mother apparently contributes sufficient wild-type Pnut to the zygote to sustain early development, and the mutant individuals survive until pupation [12*,13*]. Although methods are available to reduce the maternal contribution, and thus to allow analysis of the role(s) of Pnut in embryonic and larval development, these methods are not trivial to apply. Thus, to date, only limited (but important!) information is available from the mutant analyses.

Cytokinesis and cellularization

Both Pnut and Sep1 localize to the cleavage furrows and residual intercellular bridges of cultured cells and of cells in postgastrulation embryos (Fig. 3E) [12*,13*]. In addition, *pnut* mutant larvae display severely reduced imaginal discs (suggesting a defect in cell division [92,93]) and multinucleate and/or polyploid cells in several tissues [12*]. Moreover, when homozygous *pnut* mutant flies were rescued to adulthood by use of a construct placing *pnut*⁺ under control of the heat-shock promoter, and then placed at non-inducing temperature, the ovaries degenerated and displayed atypically large follicle cells with multiple, large nuclei [12*]. Thus, it seems clear that the septins are involved in ordinary cytokinesis. However, the studies to date have given no clue as to what step in cytokinesis is dependent on septin function. Cytokinesis in *Drosophila*, as in other animal cells, depends on the function of an actin/myosin contractile ring [27–29,93,94]. Thus, the septins might be involved in selection of the site for furrowing, organization of the actin/myosin ring at that site, regulation of contraction of the actin/myosin ring, or attachment of the actin/myosin ring to the plasma membrane. Alternatively, the septins might be involved in the addition of new plasma membrane and/or extracellular matrix that presumably must accompany furrow formation [95,96]. Also unclear is how septin function relates to the functions of other proteins involved in cytokinesis, such as Pebble [97,98], anillin [99], and potential *Drosophila* homologues of proteins implicated in cytokinesis in other

systems, such as radixin [100], the INCENPs [101], TD60 [102], CD43 [103], coronin [104], and Rho [105]. Of particular interest is the probable interaction between the septins and the product of *diaphanous*, a gene detected in a screen for male-sterile mutants [69*]. When homozygous, the *diaphanous* mutation produces a cytokinesis defect very similar to that of *pnut* [69*], and Diaphanous shares structural similarity and sequence homology with the product of the *S. cerevisiae BNI1* gene, which was identified on the basis of its genetic interaction with *cdc12* (see above).

Protein localization data also suggest that the septins are involved in the specialized cytokinesis known as cellularization, in which an actin/myosin network divides the ~6000 nuclei of the syncytial blastoderm into separate cells after nuclear cycle 13 [31]. In particular, Pnut, Sep1 and Sep2 are all highly concentrated both in the furrows that invaginate from the egg surface during cellularization (Sep1 localization is shown in Fig. 3F) and in the necks that connect the epithelial cells to the underlying yolk at the end of this process [12*,13*]. Detailed comparison of septin localization to that of other proteins involved in cellularization [13*] shows that the septins are, like myosin [106], localized at the leading edge of the furrow canal; this localization contrasts with that of spectrin, which is most concentrated a short distance behind the leading edge [107]. These data suggest that the septins may be involved in the mechanism of furrow invagination itself rather than in the subsequent stabilization of the membrane and cortical cytoskeleton. As in the case of ordinary cytokinesis, the septins might mediate the function of the actin/myosin network or be involved in the process of cell-surface extension that occurs during cellularization [32,108]; generation of *pnut* mutant embryos lacking maternally contributed Pnut (see above) should allow testing of these possibilities.

Also of interest is the relationship between septin function and the functions of proteins involved specifically in cellularization, such as Nullo [109,110], Serendipity α [111], and Bottleneck [112]. The only available information is that Sep1 (unlike Serendipity α [110]) is able to colocalize with the actin/myosin network in a *nullo* mutant [13*]; the organization of Sep 1 then becomes perturbed (in parallel with that of actin [109]) as cellularization proceeds [13*]. On the other hand, the behavior of Nullo and Sep1 prior to cellularization (see below) is consistent with the possibility that the septins might be involved in recruiting Nullo to the proper location.

Other processes

The syncytial cell cycles

During the syncytial cell cycles that immediately precede cellularization, the actin cytoskeleton undergoes a series of regular rearrangements in the cortex of the embryo. These rearrangements are believed to be responsible for keeping the nuclei evenly spaced and maintaining

the integrity of individual mitoses [31,94,99,113]. During this stage, Sep1 (and presumably the other septins) also undergoes a cycle of localization that is related to, but distinct from, that of actin [13[•]]. During interphase, while actin is concentrated in a cap over each nucleus, Sep1 is localized in a more circumferential array. At metaphase, both proteins are concentrated in the pseudocleavage furrows, which begin to invaginate between the nuclei but then retract. This behavior of Sep1 is very similar to that of anillin [99], and suggests that these proteins might be involved in recruiting proteins of the actin cytoskeleton to, or stabilizing their arrangements at, the sites of pseudocleavage furrow formation. The behavior of Sep1 and anillin is also very similar to that of Nullo ([110]; see above), except that Nullo first becomes visible at nuclear cycle 13, whereas Sep1 and anillin are visible throughout the syncytial blastoderm stage (i.e. nuclear cycles 10–13). It should be interesting to observe Pnut-deficient mutants at the syncytial blastoderm stage, and to determine the behavior of the septins in other mutants in which the syncytial cell cycles are abnormal [113–116].

Organization of the cell cortex in non-dividing cells

Several lines of evidence suggest that the septins are involved in the organization of the cell surface in both interphase and non-dividing cells. First, in cells of the postgastrulation embryo, Sep1 appears to be distributed rather uniformly over the cell cortex, and then relocalizes, via the cytoplasm, to the cleavage furrows at the time of cytokinesis [13[•]]. Second, during the morphogenetic movements that result in 'dorsal closure' of the embryo, Sep1 is concentrated in the leading edges of the cells at the front of the extending epithelial sheets [13[•]]; the septin distribution resembles, but is distinct from, that of actin and myosin [117]. Third, in the polarized epithelial cells of the imaginal discs, salivary glands, and ovarian follicle cells (Fig. 3G,H), Sep1 is concentrated in particular surface domains that are typically distinct from the domains in which actin is most concentrated [13[•]]. Thus, the septins may be involved in establishing or maintaining the asymmetric cortical domains characteristic [118,119] of such polarized cells. Fourth, both Sep1 and Pnut are concentrated in neurons of the embryonic central and peripheral nervous systems (Fig. 3I,J) [12[•],13[•]]. Further genetic studies (to look at, for example, the effects of septin mutations on development of the cell types in question and the effects of other relevant mutations upon septin distribution) should clarify the roles of the septins in these contexts.

Photoreceptor differentiation

pnut was discovered serendipitously during studies of photoreceptor differentiation [12[•]]; heterozygosity for a *pnut* loss-of-function mutation exacerbates the phenotype that results from a weak allele of *sina* [120]. *sina* encodes a nuclear protein that functions downstream of Ras1 in the signal-transduction pathway by which the R8 photoreceptor cell induces (by means of the Boss

protein in its plasma membrane) the presumptive R7 cell (by means of the Sev receptor tyrosine kinase in its plasma membrane) to differentiate into a photoreceptor [121]. Although the genetic interaction between *sina* and *pnut* might possibly reflect a division delay (due to Pnut insufficiency) that would make the presumptive R7 cell insensitive to the R8 cell's inductive stimulus during a critical period [12[•]], it might also reflect an effect of Pnut insufficiency on the localization of (and therefore the efficiency of signalling by) cell-surface signalling molecules such as Boss and Sev. Consistent with this possibility, Pnut [12[•]], Boss [119,122,123], and Sev [119,124,125] are all concentrated at the apical surfaces of developing photoreceptor cells in the eye imaginal disc.

Septin function in vertebrates

There is as yet little information on the functions of vertebrate septins. One potentially significant clue is that the expression levels of mouse Diff6 in different lymphocyte lines correlate with those of gp90^{MEL-14}, a cell surface glycoprotein (an 'L-selectin' or 'homing receptor') that is implicated in lymphocyte adhesion and transmigration through endothelial layers [14]. Although the correspondence of expression levels could be coincidental, it is possible that there is a functional connection between the septin and the transmembrane L-selectin; for example, the former could serve as a subplasmalemmal anchor for the latter. With this in mind, it is intriguing that a human homologue of the mouse L-selectin localizes to ruffles and pseudopods in migrating lymphocytes, and to the cleavage furrow in dividing cells [126]. Antibodies to Diff6 have now also been shown to stain the cleavage furrows and midbodies of dividing mouse T cells and neuroblastoma cells (M Valencik, JR Pringle, unpublished data), suggesting that the septins also have a role in cytokinesis in mammalian cells. Consistent with the high levels of septin expression in brain cells ([15,16]; M Valencik, JR Pringle, unpublished data), the Diff6 antibodies also stain the growth cones of differentiating PC-12 cells, suggesting a role in the organization of the cell surface in these highly asymmetric cells (M Valencik, JR Pringle, unpublished data).

Conclusions

For many years, it seemed possible that the neck filaments of *S. cerevisiae* and their associated septin proteins might be only of parochial interest to yeast workers. However, it has recently become clear that septin proteins are widely, if not ubiquitously, distributed in eukaryotic organisms; that the septins are widely, if not ubiquitously, involved in the centrally important process of cytokinesis; and that the septins also have a variety of other roles that appear to be unrelated to cytokinesis. Thus, study of the septins should now move into the mainstream of cell biology, and the usual combination of genetic, cytological, and biochemical approaches in various appropriate systems should lead to rapid progress in the next few years. The issues that need to be addressed include the following.

Size and distribution of the septin family

We do not know the full size of the septin family in any organism. Although this question will soon be answered for *S. cerevisiae* by the imminent completion of the genome sequence, it is of even more interest in multicellular organisms, in which it remains unclear how large a role is played by differential expression of particular septins in different cell types. We also need to know whether septins are present in other organisms, such as plants, algae, and slime molds.

Biological roles of the septins

Although mutant studies and protein localization analyses have provided many insights into the roles of the septins, our understanding is clearly far from complete, particularly in multicellular organisms. For example, we have no clear idea why the septins are expressed at high levels in the central nervous systems of both *Drosophila* and mammals, or why the septins are concentrated in particular domains of polarized epithelial cells. More detailed studies of the *pnut* mutant (and of other septin mutants when available) should be illuminating.

Structure and function of the septin proteins

To date, we have no clear picture of septin function at the molecular level in any of the processes in which these proteins are involved. In the case of cytokinesis, we do not know whether the septins are mediating the function of the actin/myosin contractile ring or controlling a parallel pathway; we do not even know whether the septin roles in cytokinesis are the same in fungi and in animal cells. (If septins are present in plants, it will be of great interest to learn whether they are also involved in the seemingly very different cytokinetic mechanisms [127] of plant cells.) We do not yet know the function of the nucleotide-binding site, or whether the coiled-coil domains are involved in homotypic or heterotypic interactions among the septins or in interactions between the septins and other proteins. It is unclear why the septin family has been so widely conserved without, in general, a close conservation of the structure of individual family members, and the amount of overlap in function among the individual septins is not known. In a general way, we suppose that the domains of similar sequence among the septins reflect their common ancestry and their common ability to assemble into appropriate complexes, whereas the domains of different sequence reflect their differential roles in interacting with other proteins at the cell cortex. However, the details of this picture remain obscure.

Relationship between septin function and the formation of higher-order structures

In *S. cerevisiae*, the septins are associated with, and apparently constituents of, a set of filaments found in close association with the plasma membrane. Despite a rough similarity in filament diameter, there is no evidence that these filaments are related (structurally or functionally)

to the intermediate filaments of animal cells. It is not yet clear whether similar higher-order structures exist in organisms other than *S. cerevisiae* and the related *C. albicans* (although the formation of filaments *in vitro* by *Drosophila* septins is suggestive), and it is not clear what (if any) is the connection between the formation of such a higher-order structure and the function of the proteins.

Acknowledgements

We particularly thank Brian Haarer, Hyong Kim, Sue Lillie, Annette Healy, Stuart Ketcham, Tom Pugh, Jane Robinson, Rob Preston, Alison Adams, and Susan Ford, all of whom played critical roles in the development of the septin story in our laboratory, and Mark Peifer, for his indispensable collaboration in our *Drosophila* work. We thank Breck Byers for his interest and generous sharing of unpublished information over many years. We also thank the numerous colleagues (many, but not all, cited by name in the text above) who have provided unpublished information, research materials, valuable discussions, and/or comments on this manuscript. Work in our laboratory has been supported by National Institutes of Health (NIH) research grants GM31006 and GM52606. Mark S Longtine was supported by NIH postdoctoral fellowship GM15766; Maria L Valencik was supported by American Cancer Society Postdoctoral Fellowship PF-3882; Claudio De Virgilio was supported by fellowships from the Ciba-Geigy-Jubiläums-Stiftung and the L and Th LaRoche-Stiftung. Funds from the RJEG Trust also supported our work.

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