Localization and Possible Functions of *Drosophila* Septins

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The septins are a family of homologous proteins that were originally identified in *Saccharomyces cerevisiae*, where they are associated with the “neck filaments” and are involved in cytokinesis and other aspects of the organization of the cell surface. We report here the identification of Sep1, a *Drosophila melanogaster* septin, based on its homology to the yeast septins. The predicted Sep1 amino acid sequence is 35–42% identical to the known *S. cerevisiae* septins; 52% identical to Pnut, a second *D. melanogaster* septin; and 53–73% identical to the known mammalian septins. Sep1-specific antibodies have been used to characterize its expression and localization. The protein is concentrated at the leading edge of the cleavage furrows of dividing cells and cellularizing embryos, suggesting a role in furrow formation. Other aspects of Sep1 localization suggest roles not directly related to cytokinesis. For example, Sep1 exhibits orderly, cell-cycle-coordinated rearrangements within the cortex of syncytial blastoderm embryos and in the cells of post-gastrulation embryos; Sep1 is also concentrated at the leading edge of the epithelium during dorsal closure in the embryo, in the neurons of the embryonic nervous system, and at the baso-lateral surfaces of ovarian follicle cells. The distribution of Sep1 typically overlaps, but is distinct from, that of actin. Both immunolocalization and biochemical experiments show that Sep1 is intimately associated with Pnut, suggesting that the *Drosophila* septins, like those in yeast, function as part of a complex.

INTRODUCTION

The septins are a family of proteins first identified in the budding yeast *Saccharomyces cerevisiae* (see below) and subsequently found also in other fungi (DiDomenico *et al.*, 1994; Momany *et al.*, 1995; Pugh, Kim, Healy, and Pringle, unpublished results), *Drosophila* (Neufeld and Rubin, 1994; this report), *Xenopus* (Glotzer and Hyman, personal communication), and mammals (Kato, 1990; Nottenburg *et al.*, 1990; Kumar *et al.*, 1992; Nakatsuru *et al.*, 1994). The name septin refers to the widespread involvement of these proteins in cytokinesis and septum formation (Sanders and Field, 1994; Longtine *et al.*, 1996). The known septins all contain sequences conserved among nucleotide-binding proteins, and most (but not all) contain predicted coiled-coil domains near their C-termini. The

septins are not otherwise closely related to other proteins of known function.

In *S. cerevisiae*, the septins encoded by the *CDC3*, *CDC10*, *CDC11*, and *CDC12* genes are closely associated with, and apparently constituents of, a ring of filaments, ~10 nm in diameter, that are closely apposed to the cytoplasmic face of the plasma membrane in the mother-bud neck (Byers and Goetsch, 1976a; Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim *et al.*, 1991; Mulholland, Preuss, and Botstein, personal communication; Kim, Haarer, and Pringle, unpublished results). A temperature-sensitive mutation in any of the four genes causes loss of the neck filaments as observed by electron microscopy, loss of localization of all four of the proteins (suggesting that their assembly at the neck is interdependent), a failure to form a chitin ring in the cell wall at the base of the bud (suggesting a defect in the localization of chitin synthase or of an associated regulatory factor), the pro-

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duction of abnormally elongated buds (apparently associated with a hyperpolarization of the actin cytoskeleton), and a failure of cytokinesis (presumably reflecting a defect in actin/myosin organization and/or in the localization of cell-wall deposition) (Hartwell, 1971; Byers and Goetsch, 1976b; Adams, 1984; Adams and Pringle, 1984; Slater *et al.*, 1985). Septin mutants also show defects in the axial budding pattern (Flescher *et al.*, 1993; Chant *et al.*, 1995), apparently reflecting an inability to localize Bud3p, a putative component of the axial positional signal (Chant *et al.*, 1995). In addition, expression studies, mutant analysis, and protein localization suggest that the septins (including a fifth family member, Spr3p, that appears to play no role in vegetative cells) are also involved in spore-wall formation (Kaback and Feldberg, 1985; Holaway *et al.*, 1987; Fares *et al.*, 1996). Thus, the *S. cerevisiae* septins appear to play a variety of roles in the organization and development of the cell surface.

There is as yet little information about the functions of the septins in nonfungal systems. An important recent advance was the serendipitous isolation of a septin mutant, *pnut*, during a screen for genes that interact with *sina* (Carthew and Rubin, 1990) in the specification of the R7 photoreceptor cell fate in *Drosophila* (Neufeld and Rubin, 1994). The *pnut* product has 31–43% identity in amino-acid sequence to the fungal septins. Flies deficient for Pnut die as pupae with small imaginal discs, apparently due to defects in cytokinesis, and Pnut localizes to the cleavage furrows of dividing cells and cellularizing embryos (Neufeld and Rubin, 1994).

We report here the identification of another *Drosophila* septin gene, *sep1*, on the basis of its similarity to the fungal septin genes. We have determined the localization of the *sep1* product relative to that of other relevant proteins at different stages of the cell cycle and at different stages of development. Sep1 localizes to the leading edge of the cleavage furrows in dividing cells and in cellularizing embryos. Sep1 also is concentrated in other locations that suggest additional roles unrelated to cytokinesis. Immunolocalization and biochemical data suggest that Sep1 and Pnut probably function as parts of a complex.

MATERIALS AND METHODS

Drosophila Strains

The wild-type Canton S strain of *Drosophila melanogaster* was used except as noted. The embryonic cDNA library (Poole *et al.*, 1985) was derived from strain Oregon R. A heterozygous *pnut* mutant strain of genotype *Plry+ lacZ/rN498-e17/CyOBE3(Plftz-lacZ)* (Neufeld and Rubin, 1994) and a heterozygous *nullo* mutant strain of genotype *Df(1)LMDF,y z w cm ct fFM7* (Simpson and Wieschaus, 1990) were kindly provided by T. Neufeld and G. Rubin, and by E. Wieschaus, respectively. In both stocks, one-fourth of the embryos produced are homozygous for the mutation of interest; such mutant embryos were identified by staining with the Pnut antibody (for the

pnut mutant) or with fluorescein isothiocyanate (FITC)-phalloidin (for the *nullo* mutant). The Schneider S2 cells were kindly provided by M. Murray and L. Searles.

DNA Manipulations

Standard DNA cloning techniques were used except as noted (Sambrook *et al.*, 1989). *Escherichia coli* strain DH5 α F' (Life Technologies, Gaithersburg, MD) was used for all plasmid manipulations. The Sequenase kit (version 2.0) was obtained from United States Biochemicals (Cleveland, OH). Overlapping clones for sequencing were generated in the pBluescript vectors (Stratagene, La Jolla, CA) using a modification (Beltzer *et al.*, 1986) of the exonuclease III method of Henikoff (1984). Single-stranded sequencing was done for both strands as described in the United States Biochemicals' Sequenase manual.

The polymerase chain reaction (PCR) was done in two ways. For the initial isolation of *sep1* (see RESULTS), *Taq* DNA polymerase was used as described by Gould *et al.* (1989). To generate full-length *sep1* DNAs for cloning into fusion-protein vectors (see below), Vent DNA polymerase was used according to the New England Biolabs (Beverly, MA) specifications.

Protein Manipulations

For extraction of total protein, *Drosophila* embryos (0- to 10-h old) were collected on apple-juice-agar plates and homogenized in 2 $\times$ Laemmli buffer (Laemmli, 1970). Proteins from *E. coli* insoluble fractions were also resuspended in 2 $\times$ Laemmli buffer. In both cases, the preparations were boiled for 5 min to solubilize proteins, which were then electrophoresed on SDS-PAGE and transferred electrophoretically to nitrocellulose membranes as described previously (Ford and Pringle, 1991). For immunodetection, the nitrocellulose membranes were cut into longitudinal strips. In the case of *Drosophila* embryonic proteins, each strip contained $\sim$ 40 embryos' worth of total protein; in the case of *E. coli* protein preparations, each strip contained $\sim$ 1 μ g of fusion protein (see below). Proteins were detected using the primary antibodies and alkaline phosphatase-conjugated secondary antibodies. For the affinity purification of antibodies, a transverse strip of membrane carrying the fusion protein was excised and used as described below; each such strip had $\sim$ 400 μ g of immobilized fusion protein, and eight such strips were used for every 0.6 ml of serum.

To prepare extracts for fractionation and immunoprecipitation, 0- to 15-h-old embryos were dechorionated with 50% bleach (Orsulic and Peifer, 1994), rinsed, and homogenized in either NET (50 mM Tris, pH 7.5, 400 mM NaCl, 5 mM EDTA, 1% NP-40) or RIPA (50 mM Tris, pH 8.5, 300 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40) buffer using a Dounce homogenizer. Both NET and RIPA buffers also contained protease and phosphatase inhibitors (50 μ g/ml phenylmethylsulfonyl fluoride, 1 μ g/ml leupeptin, 1.4 μ g/ml pepstatin, 1 mM NaF, 0.4 mM NaVO₃, 0.4 mM NaVO₅). Extracts were then spun at 3500 rpm in a microcentrifuge to remove nuclei, eggshells, and unlysed embryos. Immunoprecipitations were carried out as in Peifer (1993), using affinity-purified polyclonal anti-Sep1 (see below) or monoclonal 4C9 anti-Pnut hybridoma culture supernatant (Neufeld and Rubin, 1994). Immunoprecipitates were analyzed by SDS-PAGE and immunoblotting as in Peifer (1993), using the ECL-chemiluminescence system (Amersham, Arlington Heights, IL). The same blot was probed sequentially with anti-Sep1 and anti-Pnut antibodies, with stripping between probings as recommended by Amersham. Alternatively, extracts were fractionated on 5–20% sucrose gradients as in Peifer (1993), and the fractions were analyzed by SDS-PAGE and immunoblotting as described above.

Preparation of Sep1 Antigen and Antibodies

The entire open reading frame of *sep1* was amplified by PCR using the cDNA clone (see RESULTS) as template. The primers for PCR

had *Bam*HI sites incorporated at their ends, and the amplified product was inserted at the *Bam*HI site of pATH3 (Koerner *et al.*, 1991), resulting in a *trpE-sep1* fusion, and at the *Bam*HI site of pMal-2c (Maina *et al.*, 1988), resulting in a *malE-sep1* fusion. *E. coli* cells transformed with the resulting plasmids were induced for expression of the fusion proteins, and the insoluble fractions were isolated from both strains (Maina *et al.*, 1988; Koerner *et al.*, 1991). In both cases, these fractions were highly enriched (>95% of the total protein) for proteins of the sizes predicted for the desired fusion proteins (79.3 kDa for TrpE-Sep1 and 82 kDa for MalE-Sep1; our unpublished results) as judged by SDS-PAGE. Each insoluble fraction was injected into rabbits, using standard protocols (Cocalico Biologicals, Reamstown, PA). Fusion protein (800 μ g) was used for each primary injection, and 400 μ g of fusion protein was used for the subsequent boosts. The resulting sera were tested by staining blots of proteins from the appropriate *E. coli* strains (see above). When strong signals were seen (after two boosts of the rabbits injected with each fusion protein), antibodies were affinity purified as described previously (Pringle *et al.*, 1989), using nitrocellulose blots of the fusion proteins (see above). Two steps of purification were used. Antibodies raised against TrpE-Sep1 were purified first on MalE-Sep1 and then on TrpE-Sep1; antibodies raised against MalE-Sep1 were purified first on TrpE-Sep1 and then on MalE-Sep1. All experiments were done using the twice-affinity-purified antibodies except where noted.

Antibody-depleted preparations were prepared as follows. Antibodies raised against TrpE-Sep1 were purified once on MalE-Sep1 and then placed on nitrocellulose strips containing TrpE-Sep1 under the same conditions as used for affinity purification. The solution containing unbound antibodies was saved as the once-depleted fraction. One-half of that fraction was then placed on nitrocellulose strips containing MalE-Sep1, and the solution containing unbound antibodies was saved as the twice-depleted fraction. Antibodies that had bound to TrpE-Sep1 during the first depletion step were eluted as described (Pringle *et al.*, 1989) and used in parallel to the depleted fractions at comparable dilutions to serve as a control.

Other Reagents

Immunofluorescence localization of Pnut used the mouse monoclonal antibody 4C9 hybridoma culture supernatant (Neufeld and Rubin, 1994). Localization of myosin and α -spectrin used rabbit antibodies (Kiehart and Feghali, 1986; Pesacreta *et al.*, 1989). Actin was localized using FITC-labeled phalloidin (Sigma, St. Louis, MO). Armadillo was localized using the mouse monoclonal antibody 7A1 (Peifer *et al.*, 1994). D4.1, the product of *coracle* (Fehon *et al.*, 1994), was localized using the mouse monoclonal antibody c615-16b. The mouse monoclonal antibody BP102 (Seeger *et al.*, 1993) was used to stain the axons of the developing central nervous system. Propidium iodide was obtained from Molecular Probes (Eugene, OR). Goat anti-rabbit-IgG and anti-mouse-IgG secondary antibodies conjugated to rhodamine, FITC, or alkaline phosphatase were purchased either from Jackson Immunoresearch Labs (West Grove, PA) or from Boehringer Mannheim (Indianapolis, IN). Before use in immunofluorescence experiments, secondary antibodies were preadsorbed on embryos prepared as if for immunofluorescence (see below) but not treated with primary antibody.

Immunofluorescence

S2 cultured cells were immobilized on slides coated with 0.1% polylysine, fixed for 20 min at room temperature in PEM (0.1 M piperazine-*N,N'*-bis(2-ethanesulfonic acid), pH 6.5, 2 mM MgSO₄, 1 mM EGTA) containing 1% NP-40 and 1% formaldehyde, and blocked for 1 h in blocking solution (Orsulic and Peifer, 1994). Incubations with primary and secondary antibodies and the washes following each incubation were all done in antibody-wash solution (Orsulic and Peifer, 1994). Following the washes after incubation with secondary antibody, a drop of mounting medium (Pringle *et*

al., 1989) containing bisBenzimide (Sigma) as a DNA stain was added before covering the cells with a coverslip.

Ovaries, salivary glands, and imaginal discs were dissected in Ringer's solution and fixed for 20 min at room temperature in PEM containing 1% NP-40 and 1% formaldehyde. The fixative was removed and the tissues were washed once in blocking solution, incubated for 1 h in the same solution, and then treated exactly as described for embryos stained for Sep1 (see below).

Embryos were collected after various times on apple-juice agar at room temperature. For staining with the Sep1 antibody alone or in combination with the Pnut, D4.1, Armadillo, or BP102 antibodies, the embryos were treated as described previously (Orsulic and Peifer, 1994). Minor variations of these procedures were used for double staining of Pnut and α -spectrin or myosin. To visualize Pnut and α -spectrin, fixation was performed for 35 min at room temperature in PEM containing 3.7% formaldehyde. To visualize Pnut and cytoplasmic myosin, fixation was performed for 5 min at room temperature in a mixture of nine parts 37% formaldehyde to one part 500 mM EGTA, pH 7.5. In both cases, all incubations subsequent to devitellinization were done in phosphate-buffered saline containing 0.3% Triton X-100 and 50 mM EGTA, and with normal goat serum added to 25% for blocking and to 5% for antibody incubations and washes. Washes were done four times for 5 min each.

To double stain with phalloidin and Sep1 antibody, dechorionated embryos (Orsulic and Peifer, 1994) were fixed with shaking for 35 min at room temperature in a 1:1 mixture of heptane and 3.7% formaldehyde in PEM. The aqueous phase was removed, and the embryos were washed in one change of heptane, laid on double-stick tape, and covered with water. The vitelline membrane was then removed using a tungsten needle (Wieschaus and Sweeton, 1988), and the embryos were washed three times in antibody-wash solution and then post-fixed and treated as described previously (Orsulic and Peifer, 1994). Following the washes after incubation with secondary antibody, staining with FITC-labeled phalloidin was done as described previously (Peifer *et al.*, 1993). Nuclei were stained as described previously (Orsulic and Peifer, 1994), except that the microwave step following the RNaseA treatment was omitted.

Microscopy

For conventional fluorescence microscopy, a Nikon Optiphot-2 microscope was used. Confocal microscopy was performed using a Zeiss (Thornwood, NY) LSM410 Inverted Laser Scan confocal microscope with a krypton/argon laser. Black-and-white prints of scanned images were obtained using a Focus Graphics (Foster City, CA) ImageCorder Plus analogue film recorder or a Presentation Technologies (Sunnyvale, CA) Montage FR2 film recorder. Color prints were made using Fujix Pictography 3000 (Fuji Photo Film, Elmsford, NY).

RESULTS

Isolation of *sep1*

The septin family of proteins was first identified in fungi (see INTRODUCTION). To isolate a *Drosophila* homologue, two highly degenerate primers were designed based on regions conserved among the fungal genes and proteins known at the time (Figure 1, A and B). PCR was then performed as described by Gould *et al.* (1989) on genomic DNA from the Oregon R strain of *D. melanogaster*. The major amplified product was ~400 bp, larger than the ~275 bp expected from the fungal sequences (our unpublished results). This product was isolated, cut with *Eco*RI and *Pst*II at sites

Figure 1. (A and B) Design of the PCR primers used to isolate *sep1*. (A) Alignment of the relevant parts of the septin proteins used to design the primers (B). Cdc3p, Cdc10p, Cdc11p, and Cdc12p are from *S. cerevisiae* (Haarer, Lillie, and Pringle, unpublished results; GenEmbl Nos. L16548, L16549, L16550, and L16551); Spn1p is from *S. pombe* (Pugh, Kim, Healy, and Pringle, unpublished results; GenEmbl No. U31742); and CaCdc3p and CaCdc10p are the *C. albicans* homologues of Cdc3p and Cdc10p, respectively (DiDomenico *et al.*, 1994; GenEmbl Nos. Z25869 and Z25870). Primer 1 is 8,192-fold degenerate and primer 2 is 110,592-fold degenerate. (C and D) Analysis of the *Sep1* protein sequence. (C) Comparison of the *Sep1*, *Pnut*, and *Cdc11p* sequences. Both *Cdc11p* and *Pnut* were aligned with *Sep1*. Vertical bars indicate identical amino acids and asterisks indicate similar ones (I/L/V; S/T; R/K; N/Q; and D/E). Dots indicate gaps introduced to maximize sequence alignment. Double underlining indicates amino-acid motifs conserved in nucleotide-binding proteins (Dever *et al.*, 1987; Saraste *et al.*, 1990); light underlining indicates the predicted coiled-coil domains; arrows indicate the regions corresponding to the PCR primers (panel A). The single-nucleotide discrepancy between the PCR product and the cDNA would correspond to a replacement of Arg by Pro at position 129 if it represents a bona fide polymorphism. The putative intron (GenEmbl No. L33246) occurs within codon 133. (D) Probabilities of coiled-coil formation for *Sep1* (solid line) and *Pnut* (dashed line) calculated using the program of Lupas *et al.* (1991) with a window size of 28.

included in the primers (see Figure 1B), and cloned into pBluescript vectors for sequencing and to provide the probes used for hybridization analysis. DNA sequencing (our unpublished results) revealed an open reading frame that was interrupted by a putative intron that had all the conserved splicing motifs (Mount *et al.*, 1992); the predicted protein showed homology to the fungal proteins.

A radioactive probe prepared from the cloned PCR product was used to screen a *D. melanogaster* embryonic (3–12 h) cDNA library (Poole *et al.*, 1985) by plaque hybridization. Several clones were isolated that had overlapping inserts as determined by restriction mapping and partial sequence analysis (our unpublished results). The longest insert, 2052 bp in length, was sequenced in its entirety, revealing an open reading frame of 361 codons together with 116 bp of apparent 5' noncoding sequence and 850 bp of apparent 3' noncoding sequence (our unpublished results, GenEmbl #L33246; an identical sequence was deposited as "Diff6 protein homologue" by Hayward *et al.*, GenEmbl #S25063). Except for the absence of the putative intron and a single nucleotide discrepancy (presumably a PCR error or a polymorphism), the cDNA sequence was identical to that of the PCR product within the region of overlap. In situ hybridization to the salivary gland polytene chromosomes (kindly performed and analyzed by T. Lavery, T. Neufeld, and G. Rubin) revealed hybridization only to one region, 19F4–5, at the base of the X-chromosome. We have designated this gene *sep1*.

Characterization of the *sep1* Product

The known fungal septins are 25–66% identical in amino-acid sequence. The predicted Sep1 protein (41.1 kDa) is not strikingly more similar to any one of the fungal proteins than it is to the others; it has sequence identity values that range from 35–42% with the *S. cerevisiae* proteins (Figure 1C), from 37–41% with the *Candida albicans* proteins, and from 32–41% with the *Schizosaccharomyces pombe* proteins. Sep1 is more closely related (52% identity in amino acid sequence) to another known *Drosophila* septin, Pnut (Neufeld and Rubin, 1994; Figure 1C), as well as to three mouse septins, DIFF6 (Nottenburg *et al.*, 1990; 53% identity), H5 (Kato *et al.*, 1990; 61% identity), and NEDD-5 (Kumar *et al.*, 1992; 73% identity), and a human septin, hCDC10 (Nakatsuru *et al.*, 1994; 57% identity). Like all other known members of the septin family, Sep1 has amino acid motifs that are conserved among nucleotide-binding proteins (Dever *et al.*, 1987; Saraste *et al.*, 1990; Figure 1C). In addition, Sep1, like most (but not all) of the other known septins, has a domain at its carboxyl terminus that is strongly predicted by the empirical equation of Lupas *et al.* (1991) to form a coiled-coil (Figure 1, C and D). Another domain com-

prising amino acids 68–95 has a smaller calculated probability of forming a coiled-coil (Figure 1D).

TrpE-Sep1 and MalE-Sep1 fusion proteins were used to raise and affinity purify Sep1-specific antibodies as described in MATERIALS AND METHODS. To test the specificity of the purified antibodies, total proteins were isolated from *Drosophila* embryos and analyzed by immunoblotting. The affinity-purified antibodies from all four rabbits immunized recognized a protein of the appropriate size for Sep1 (41 kDa) as well as other proteins that were specific to the antibodies from particular rabbits (Figure 2A). At the dilution used, the antibodies from one rabbit appeared to recognize only the ~41-kDa protein (Figure 2A, lane 2); this band was absent when the depleted fractions of antibodies (see MATERIALS AND METHODS) from the same rabbit were used (Figure 2, B and C). The purified antibodies from this rabbit were used in all immunolocalization experiments shown below, although key aspects of Sep1 localization were confirmed using the antibodies from the other three rabbits and the depleted fractions shown in Figure 2, B and C.

Cell-Cycle-coordinated Rearrangements of Sep1 and Localization to the Cleavage Furrow in Dividing Cells

To begin exploring the function(s) of Sep1, we first examined its localization in *Drosophila* S2 tissue-culture cells. During interphase, no strong localization of Sep1 was detected (Figure 3A). As cells began cytoki-

Figure 2. Specificity of anti-Sep1 antibodies. Proteins were extracted and analyzed by immunoblotting as described in MATERIALS AND METHODS. (A) Total *Drosophila* embryo proteins stained with the affinity-purified antibodies from the four rabbits immunized (lanes 1–4, respectively). All antibodies were twice affinity-purified except for those shown in lane 3, which were only once affinity-purified (see MATERIALS AND METHODS). (B) Insoluble proteins from *E. coli* producing the MalE-Sep1 fusion protein stained with antibody preparations from the rabbit of panel A, lane 2. Lane 1 was stained with the depleted fraction (see MATERIALS AND METHODS), lane 2 with the depleted fraction, and lane 3 with the twice-affinity-purified serum. (C) Total *Drosophila* embryo proteins stained with the same antibody preparations as shown in panel B. Arrowheads indicate the putative Sep1 band at ~41 kDa (A and C) and the MalE-Sep1 fusion protein at ~82 kDa (B).

Figure 3. Localization of Sep1 in S2 tissue-culture cells. Images were obtained by conventional fluorescence microscopy except where noted. (A) Absence of Sep1 staining in interphase cells. Cells were double-stained for Sep1 (upper panel) and DNA (lower panel). The interphase cell (left) showed no detectable Sep1 localization; the telophase cell in the same field (right) showed strong Sep1 localization to the midbody. (B and C) Localization of Sep1 to the leading edge of the cleavage furrow during cytokinesis. (B) Cells double stained for actin (left panels) and Sep1 (right panels) at early (top), intermediate (middle), and late (bottom) stages of cytokinesis. (C) Optical section obtained by confocal microscopy through a cell stained for Sep1 at an intermediate-late stage of cytokinesis. The inset shows an enlargement (and shorter exposure) of the same section. (D) Co-localization of Sep1 (right) and Pnut (left) in the cleavage furrow.

nesis, the Sep1 antibodies weakly stained the cleavage furrow region where actin was concentrated (Figure 3B, upper). As furrow formation progressed, Sep1 staining became more intense but was more restricted than actin staining (Figure 3B, middle); Sep1 appears to be confined to the vicinity of the plasma membrane at the leading edge of the advancing furrow and/or to the cytoplasm just under the leading edge, a conclusion supported by confocal microscopy images of cells at a similar stage (Figure 3C). Sep1 remained concentrated in the furrows of cells late in cytokinesis (Figure 3A; Figure 3B, bottom). Pnut shows a similar localization in dividing cells (Neufeld and Rubin, 1994), and double staining revealed that the two proteins are in fact precisely co-localized (Figure 3D).

Examination of the dividing cells of post-gastrulation (extended-germ-band) embryos also revealed a localization of Sep1 to the cleavage furrows, but in addition revealed a more complex pattern of cell-cycle-coordinated changes in Sep1 localization. During interphase, Sep1 antibodies stain the cell cortices in a punctate pattern (Figure 4C, arrowhead). As the cells enter mitosis, Sep1 staining is not confined to the cell cortices and is seen in a punctate pattern throughout the cytoplasm (Figure 4C, arrow), and at the onset of cytokinesis it becomes concentrated at the cleavage furrows between the pairs of daughter nuclei (Figure 4, A and B, arrows). In addition, some sections of post-gastrulation embryos revealed sharp foci of staining at the junctions between pairs of cells (Figure 4D). Such foci have also been observed using Pnut antibodies (Neufeld and Rubin, 1994), and in double-stained embryos it is clear that the foci of Sep1 and Pnut staining are identical (Figure 4D). At least some of these foci probably represent the intercellular bridges that transiently connect recently divided embryonic cells (Rickoll and Counce, 1980).

Figure 4. Cell-cycle-coordinated rearrangements of Sep1, its localization to the cleavage furrow in dividing cells, and its co-localization with Pnut in intercellular bridges in cells of postgastrulation (extended-germ-band) embryos. Optical sections were obtained by confocal microscopy. (A and B) An embryo double-stained for Sep1 (A) and for DNA (panel B; for orientation, the Sep1 staining is superimposed at reduced intensity compared with that in panel A). Arrows indicate Sep1 localization to cleavage furrows in dividing cells. (C) An embryo stained for Sep1. The arrowhead and arrow indicate domains of interphase cells and cells early in mitosis, respectively. (D) An embryo double stained for Sep1 (top) and Pnut (bottom).

Distinct Localizations of Sep1 and Actin during the Cell Cycle in Pre-cellularization Embryos

Following fertilization, the nuclei of the *Drosophila* embryo undergo 13 rapid and synchronous divisions that are unaccompanied by cell divisions except in the pole cells. During these first 13 nuclear cycles, the cytoskeletal systems of the embryo undergo a series of ordered rearrangements (reviewed in Schejter and Wieschaus, 1993a). We used fluorescence staining and confocal microscopy to determine the localization of Sep1 in pre-cellularization embryos and to compare this localization to that of actin.

The first nine nuclear divisions occur in the interior of the embryo; during this period, actin is found mainly at the embryo cortex. During the early nuclear cycles, Sep1 is distributed throughout the cytoplasm. However, as development proceeds, Sep1 gradually relocates to the embryo cortex; by the interphase between nuclear divisions 9 and 10, it is found predominantly in a nonuniform, grainy layer at the cortex (Figure 5a). At this time, most of the nuclei have moved to the embryo cortex, forming the syncytial blastoderm, and cortical actin is concentrated in a cap above each nucleus (Schejter and Wieschaus, 1993a; Figure 5A). As the nuclei undergo the mitosis of cycle 10, these actin caps expand and then divide to form two caps, one above each daughter nucleus (Figure 5B). During this nuclear division, Sep1 retains its dispersed and grainy localization in the embryo cortex but is concentrated into rings that surround the dividing nuclei (Figure 5b).

During nuclear cycles 11–13, actin is found in a cap over each nucleus during interphase (Figure 5, C, E, and G) and reorganizes during each mitosis to associate with the membrane invaginations (pseudocleavage furrows) that partially separate the dividing nuclei (Figure 5, D, F, and H). During these cycles, Sep1 is found during interphase in a distribution strikingly different from that of actin. Sep1 appears as a nonuniform, rather grainy network in the embryo cortex that generally surrounds the actin caps (Figure 5, c and g), although superposition of the images (our unpublished results) shows that the distributions of the two proteins overlap at the edges of the actin caps. In addition, although Sep1 appears to be associated in part with the plasma membrane, it is also found in the underlying cortical cytoplasm surrounding each nucleus (Figure 5, e and g). During the mitoses of nuclear cycles 11–13, Sep1 remains localized in a network surrounding each nucleus (Figure 5, d, f, and h). This localization resembles that of actin (Figure 5, D, F, and H), but the Sep1 staining appears less uniform and more grainy than that of actin. Sep1 appears to localize mainly to the tip of the invaginating membrane, although a fainter staining is also seen along the sides of the pseudocleavage furrow and at the embryo cortex.

During these mitoses, although Sep1 is intimately associated with the advancing membrane, it also appears to be concentrated in the cytoplasm just beyond the tip of the furrow: in some but not all embryos and optical sections examined, when the actin and Sep1 images were superimposed, the Sep1 staining appeared to extend beyond that of actin at the leading edge of the pseudocleavage furrow (Figure 5, H and h; our unpublished results).

Localization of Sep1 to the Leading Edge of the Furrow in Cellularizing Embryos

During the interphase between nuclear divisions 13 and 14, actin first reorganizes from the set of caps over the nuclei to form a hexagonal actin-myosin network at the embryo cortex. Then, in the process of cellularization, the cleavage furrows move into the embryo around each nucleus, with actin and myosin concentrated at their leading edges (Schejter and Wieschaus, 1993a) and spectrin concentrated slightly behind the leading edges (Pesacreta *et al.*, 1989; Thomas and Kiehart, 1994). At the beginning of cellularization, Sep1 also assumes a hexagonal pattern similar to, but apparently less uniform than, that of actin and myosin (Figure 6A, left). The nonuniformity of the Sep1 staining is maintained until the end of cellularization (Figure 6A, middle and right panels). As cellularization proceeds, Sep1 is concentrated at the leading edges of the advancing cleavage furrows, although some diffuse staining is still observed at the embryo cortex and in the underlying cytoplasm (Figure 6B). A similar localization has been observed for Pnut (Neufeld and Rubin, 1994), and double staining revealed that the two septins co-localize at least at the resolution of the light microscope (Figure 6B). Moreover, examination of double-stained embryos revealed that the septins co-localize with actin (Figure 6C) and myosin (Figure 6D) at the very leading edge of the cleavage furrows, in a position distinctly ahead of that of spectrin (Figure 6E).

As the membranes start to constrict at the bases of the forming cells, Sep1, like actin, continues to associate with the invaginating membrane (Figure 6A, middle). At the completion of cellularization, Sep1 and Pnut remain concentrated in the necks that connect the newly formed epithelial cells to the underlying yolk (Neufeld and Rubin, 1994; Figure 6A, right). This localization to the yolk channels persists through gastrulation until later in development (our unpublished results).

Several genes are known whose products are involved in the restructuring of the cortical actin-based cytoskeleton during cellularization (Schejter *et al.*, 1992; Schejter and Wieschaus, 1993a). In *nullo* and *serendipity- α* mutant embryos, the hexagonal network of actin forms normally during the cycle 13–14 inter-

Figure 5. Distinct localizations of Sep1 and actin during the cell cycle in pre-cellularization embryos. Surface views and optical cross-sections were obtained by confocal microscopy of embryos that had been double stained for actin (capital letters) and for Sep1 (lower case letters). Each pair of panels shows the same optical section. (A–b) Surface views of embryos in interphase or early prophase (A and a) or in anaphase (B and b) of nuclear cycle 10. (C–h) Surface and cross-section views of syncytial blastoderm embryos (nuclear cycles 11–13). (C, c, E, and e) An interphase cycle 13 embryo viewed at the surface (C and c) and in an optical section 2 μm below the surface (E and e). (G and g) An interphase cycle 12 embryo viewed in cross-section. (D, d, F, and f) A mitotic cycle 12 embryo viewed in optical sections 2 μm (D and d) and 4 μm (F and f) below the surface. (H and h) A mitotic cycle 12 embryo viewed in cross-section.

phase, but as cellularization proceeds, the actin network becomes nonuniform and exhibits areas that have either more or less actin than the wild type (Schweisguth *et al.*, 1990; Simpson and Wieschaus, 1990; Figure 7B). The functions of Nullo and Serendipity- α appear to be linked, as both proteins normally co-localize with the actin/myosin network and Serendipity- α fails to do so in a *nullo* mutant (Schweisguth *et al.*, 1990; Postner and Wieschaus, 1994). In contrast, Sep1 does localize to the invaginating furrows in *nullo* embryos; however, as cellularization proceeds in such

embryos, the Sep1 distribution, like that of actin, becomes nonuniform (Figure 7A).

Sep1 Localization Suggesting Septin Roles Not Related to Cytokinesis

To gain further insights into the roles of Sep1, we used confocal microscopy to examine the localization of this protein during later stages of embryonic development, in the larval imaginal discs and salivary glands, and in the ovaries of adult flies. The results suggest strongly

that the septins have a variety of roles unrelated to cytokinesis.

Following gastrulation and germ-band retraction, the epithelial cells on the lateral surfaces of the embryo extend and migrate dorsally to cover the amnioserosa and eventually meet at the dorsal midline ("dorsal closure"). Sep1 is concentrated in the cells at the front of the extending epithelia (Figure 8A, large arrow), although some nonuniform staining is also seen on the lateral membranes of these cells. Actin is concentrated in the same regions (Figure 8B, large arrow), but double labeling revealed that the Sep1 staining in the migrating epithelial cells, as well as in the cortices of the amnioserosa cells (Figure 8, A and B, small arrows), is less continuous than that of actin. Moreover, the staining of Sep1 at the tips of the migrating cells seems to extend further back into their cytoplasm than does the staining of actin.

Later in embryogenesis and during the early development of the larva, Sep1 becomes concentrated in the neurons of the developing central (Figure 8C, arrow) and peripheral (Figure 8C, arrowhead) nervous systems. Localization of Pnut to the central nervous system has also been observed (Neufeld and Rubin, 1994), and, in double-stained embryos, the localizations of Sep1 and Pnut are indistinguishable (Figure 9, A and B).

Examination of larval imaginal discs (epithelial tissue that will give rise to adult structures) revealed that Sep1 is concentrated mainly at the apical and basal surfaces of the cells (Figure 8, D and E, green) and is apparently less abundant at the lateral surfaces. In imaginal discs, Armadillo is found in the adherens junctions near the apical surfaces of the cells (Peifer and Wieschaus, 1990), and D4.1, the product of the *coracle* gene, is concentrated in the septate junctions basal to the adherens junctions on the apico-lateral membrane (Fehon *et al.*, 1994). Double-staining for Sep1 and D4.1 (Figure 8D) and for Sep1 and Armadillo (Figure 8E) revealed that the localization of Sep1 to the apical membranes extends laterally at least as far basally as the septate junctions (Figure 8D, yellow), although there may be a partial exclusion from the region of the adherens junctions (Figure 8E, red).

Examination of salivary glands revealed that Sep1 was found predominantly at the lateral and basal cell surfaces (Figure 8F, arrow) as well as in a reticular network in the cytoplasm. The patterns of actin and Sep1 localization at the lateral cell surfaces are overlapping but not identical (color variegation in Figure 8G); moreover, actin, but not Sep1, is heavily concentrated at the apical membranes of the cells facing the lumen (Figure 8F, arrowhead).

The localizations of Sep1 and actin also displayed interesting differences in the ovaries of adult females. Actin is concentrated at the plasma membranes of the nurse cells (Figure 8I, small arrowhead), in the ring

canals that connect them (Figure 8I, black arrows), and at the plasma membranes of the follicle cells, particularly on their apical surfaces (Figure 8I, large arrowhead) (Warn *et al.*, 1985; Theurkauf *et al.*, 1992). In contrast, Sep1 staining was weak or undetectable at the nurse-cell plasma membranes and ring canals but was evident in the cytoplasm of these cells (Figure 8H). In addition, although Sep1 is clearly associated with the plasma membranes of the follicle cells, it seems to be more concentrated at their baso-lateral surfaces (Figure 8, H and J, arrowheads). Some Sep1 may also be present in the cytoplasm of the follicle cells. This pattern of Sep1 staining of the follicle cells was seen in all egg chambers of an ovariole (Figure 8, H and J), including in the stalk cells that connect neighboring egg chambers (Figure 8J, black arrow). Furthermore, in the border cells (a subset of follicle cells that migrates between the nurse cells to the anterior end of the oocyte), Sep1, like actin, appears to be concentrated at the plasma membranes and may also be present in the cytoplasm (Figure 8, H and I, asterisk).

Evidence that Sep1 and Pnut Function as Parts of a Complex

The co-localization of Sep1 and Pnut suggests that these proteins may function as parts of a complex like that in yeast. To explore the possible Sep1-Pnut interaction further, we examined Sep1 localization in *pnut* mutant flies. In a cross between flies heterozygous for the *pnut* mutation, one-fourth of the resulting embryos should be homozygous for the mutation. Previous analysis had shown that such *Pnut*⁻ individuals die as pupae with severely reduced imaginal discs, apparently due to a defect in cytokinesis (Neufeld and Rubin, 1994). Analysis of Pnut localization in the embryos arising from such a cross revealed that the maternally contributed Pnut protein could be detected readily at least until germ-band retraction and appeared to be localized normally in all embryos (our unpublished results). Pnut staining became noticeably reduced in about one-fourth of the older embryos, most conspicuously in the developing nervous system, presumably due to the dilution and/or decay of the maternally contributed product in the absence of new synthesis in individuals homozygous for the *pnut* mutation (Figure 9B, right). The same individuals also showed little or no staining with the Sep1 antibodies (Figure 9B, left). In contrast, the remaining three-fourths of the embryos (presumably those homozygous or heterozygous for *pnut*⁺) showed strong, indistinguishable staining of both proteins (Figure 9A). No embryos showed staining for one protein without the other. The absence of Pnut and Sep1 did not grossly disrupt development of the nervous system: when the progeny from the same cross were double-stained with Sep1 antibodies

Figure 6. Localization of septins to the leading edge of the furrow in cellularizing embryos. Images were obtained with the confocal microscope. (A) Optical sections parallel to the surface and through the leading edge of the furrow of embryos stained for Sep1 early in cellularization (left), at an intermediate stage (middle), and at the end of cellularization (right). (B–E) Cross-sections of embryos double stained for Sep1 and Pnut (B), for Sep1 and actin (C), for Pnut and myosin (D), or for Pnut and α -spectrin (E). Each row of images shows the same optical section; the image on the left was stored in the green channel of the confocal microscope (regardless of the fluorophore actually used), the image in the middle was stored in the red channel, and the image on the right represents the superimposed images from both channels. In the right hand images, a green or red color indicates that the two stainings do not coincide, whereas an intermediate color (orange or yellow) indicates co-localization (at least at the level of resolution of the light microscope).

and with the CNS-specific antibody BP102, individuals that did not stain for Sep1 showed no obvious differences from normal embryos in the appearance of the developing CNS (Figure 9, C and D).

To explore the apparent Sep1-Pnut interactions biochemically, we used anti-Pnut and anti-Sep1 antibodies to immunoprecipitate proteins from *Drosophila* embryonic extracts. Analysis of the immunoprecipitates by SDS-PAGE and immunoblotting revealed that both proteins were present, in roughly equal amounts, in each immunoprecipitate (Figure 10A, middle lanes). In contrast, neither protein was detected in control immunoprecipitates prepared with anti-Armadillo antibodies (Figure 10A, right lane). To investigate further the nature of the apparent Sep1-Pnut complex, we fractionated the same types of cell extracts by sucrose density-gradient centrifugation. Sep1 and Pnut co-sedimented at about 8 S (Figure 10B), suggesting a molecular mass of 160–200 kDa (assuming globular proteins). This is much larger than expected for a Sep1 or Pnut monomer and would be consistent with the presence of a complex containing approximately four polypeptides of the sizes of the septins. Both co-immunoprecipitation and co-sedimentation were observed in two different detergent-containing buffers (see MATERIALS AND METHODS; Figure 10; our unpublished results), one of which is quite stringent (1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS), suggesting that the complex between Sep1 and Pnut is quite stable.

DISCUSSION

Sep1 and the Septin Family of Proteins

Sep1 was identified on the basis of its similarity to fungal septins. As this family of proteins has now been found also in amphibia and mammals (see INTRODUCTION), it may be ubiquitous in eukaryotes, although septins have not yet been reported from plants. In each organism examined to date, there are multiple septins; for example, *S. cerevisiae* has at least five septins (see INTRODUCTION) and *S. pombe* has at least six (Al-Awar, Pugh, Kim, and Pringle, unpublished results). Thus, it is unlikely that Sep1, Pnut, and Sep2 (Al-Awar, Peifer, and Pringle, unpublished results) represent the only *D. melanogaster* septins. As at least one septin is expressed only in differentiating (sporulating) yeast cells (Fares *et al.*, 1996), it seems likely that individual septins will show differential expression in different cell types in multicellular organisms. However, there are as yet no data to support this possibility; in particular, Sep1 and Pnut have shown identical patterns of expression in all cell types examined to date.

Although the septin family is broadly conserved, there is not in general a close one-to-one correspondence between individual family members in dis-

Figure 7. Optical section obtained by confocal microscopy through a *null* mutant embryo double stained for Sep1 (A) and actin (B). The embryo was at the cellularization stage.

tantly related organisms. For example, the most closely related pair among the known *S. cerevisiae* and *S. pombe* septins are only 51% identical in amino acid sequence, and Sep1 is only 42% identical to the most closely related of the known fungal septins. Against this background, the 73% identity between Sep1 and the mouse *NEDD-5* gene product (Kumar *et al.*, 1992) is striking, and suggests that these proteins have some common function(s) that have been conserved during animal evolution.

Like all of the other known septins, Sep1 has sequence motifs suggesting the presence of a nucleotide-binding site. Although the conservation of these motifs argues that they are important for protein function, nucleotide binding has not been demonstrated, and the function of these sites remains unclear. In addition, all of the known septins except Cdc10p (Haarer, Lillie, and Pringle, unpublished results), CaCdc10p (DiDomenico *et al.*, 1994), and *S. pombe* Spn2p (Al-Awar, Pugh, and Pringle, unpublished results) contain a predicted coiled-coil domain at or near their C-termini, a feature that is also conserved in Sep1. These domains are likely to be involved in interactions between septins or between septins and other proteins. Like Cdc3p (Haarer, Lillie, and Pringle, unpublished results), Sep1 also contains a short domain in its N-terminal half that has a smaller predicted probability for coiled-coil formation; this domain might represent an element of secondary or tertiary structure that is conserved between these two proteins.

In both *S. cerevisiae* (see INTRODUCTION) and *S. pombe* (Valencik, Al-Awar, and Pringle, unpublished results), the septins appear to be interdependent for assembly and function. The same appears to be true for Sep1 and Pnut in *Drosophila*: the two proteins co-localize in all situations examined to date, they are associated in a complex as judged by co-immunoprecipitation and co-sedimentation, and Sep1 fails to accumulate normally in the developing nervous system of *pnut* mutant embryos. It is not yet clear whether the last observation represents a failure of

Figure 8. Sep1 localization suggesting septin roles not related to cytokinesis. Optical sections were obtained by confocal microscopy through embryos and isolated imaginal discs, salivary glands, and ovaries. Symbols mark features discussed below or in the text. In panels D–G, intermediate colors (orange or yellow) indicate co-localization. (A and B) An embryo late in dorsal closure double stained for Sep1 (A) and actin (B). (C) The developing nervous system of a late-stage embryo stained for Sep1. (D and E) Superimposed images of imaginal discs double stained (D) for Sep1 (green) and D4.1 (red) or (E) for Sep1 (green) and Armadillo (red). Arrows indicate apposed apical surfaces and arrowheads indicate apposed basal surfaces of the cells in this tightly folded epithelial layer. (F and G) Superimposed images of a salivary gland double stained for Sep1 (green) and actin (red). Two optical sections of the same gland are shown, one through its center (F) and one (at higher magnification) displaced some distance from the center (G). (H–J) Isolated ovarioles double stained for Sep1 (H) and actin (I; H and I show the same optical section) or stained for Sep1 (J).

Sep1 to localize properly in the absence of Pnut, or whether Sep1 is actually expressed at a lower level, or turned over more rapidly, if Pnut is not available for complex formation.

In *S. cerevisiae*, the septins expressed in vegetative cells are associated with a higher-order structure, the ~10-nm filaments found at the cytoplasmic face of the plasma membrane in the mother-bud neck (see INTRODUCTION). Although the localization of the *Drosophila* septins also generally suggests an association with the cytoplasmic face of the plasma membrane, it

is not yet clear whether Sep1 and Pnut are associated with filaments like those seen in *S. cerevisiae*. However, the information now available from immunofluorescence studies on the sites and timing of septin localization should facilitate investigation of this issue by electron microscopy.

Role(s) of the Septins in Drosophila Cytokinesis

The *S. cerevisiae* septins are involved in cytokinesis (see INTRODUCTION). The *S. pombe* septins are also

Figure 9. Loss of Sep1 localization in embryos lacking Pnut. Optical sections were obtained by confocal microscopy through embryos from a stock heterozygous for the *pnut* mutation. Embryos were double stained for Sep1 and Pnut (A and B) or for Sep1 and the CNS-specific antigen recognized by antibody BP102 (C and D). Paired images represent the same optical section. The embryos in panels A and C were apparently homozygous or heterozygous for *pnut*⁺; the embryos in panels B and D were presumably homozygous for the *pnut* mutation.

involved either in the completion of septum formation or in some subsequent event leading to the separation

of daughter cells (Pugh, Kim, Healy, and Pringle, unpublished results). Remarkably, despite the apparent differences in mechanism, the septins are also clearly involved in cytokinesis in *Drosophila*. Both Sep1 and Pnut localize to the cleavage furrows of dividing cells and to the intercellular bridges that sometimes persist after division (see RESULTS; Neufeld and Rubin, 1994). Moreover, the *pnut* mutant exhibits defects in cytokinesis in several tissues (Neufeld and Rubin, 1994). The localization of Sep1 and Pnut to the pseudocleavage furrows of the syncytial blastoderm and to the furrows of cellularizing embryos (see RESULTS; Neufeld and Rubin, 1994) strongly suggests that the septins are involved in these processes as well, although the presence of maternal gene product has thus far precluded determining whether the *pnut* mutant shows the expected defects.

The precise role(s) of the septins in cytokinesis, however, remain unclear. Ordinary cytokinesis in *Drosophila*, as in other animal cells, depends on the action of an actin/myosin contractile ring (Salmon, 1989; Conrad and Schroeder, 1990; Karess *et al.*, 1991; Satterwhite and Pollard, 1992). The formation of pseudocleavage furrows and cellularization also depend on the function of an actin/myosin network (Warn and Magrath, 1983; Warn *et al.*, 1984; Karr and Alberts, 1986; Katoh *et al.*, 1991; Young *et al.*, 1991; Schejter and Wieschaus, 1993a). Thus, the septins might be involved in the organization of the actin/myosin ring or network, in its contraction, or in its attachment to the plasma membrane. Alternatively, the septins might be involved in the extensive remodeling of the cell surface that must accompany furrow formation (Byers and Armstrong, 1986; Planques *et al.*, 1991); for example, the septins might be involved in the alignment or fusion of the vesicles that appear to give rise to new plasma membrane during cellularization (Loncar and Singer, 1995). The apparent localization of the septins to the very tip of the invaginating membrane, and indeed sometimes apparently to a grainy (perhaps vesicular) layer just ahead of that tip, is consistent with this last possibility. Also suggestive is the observation that a fraction of the Sep1 and Pnut remains at the embryo cortex during cellularization. It seems possible that the cortical septins sequester components that are gradually recruited into the invaginating membrane to allow its progression into the embryo.

Further studies, including more precise localization of the septins by immunoelectron microscopy, characterization of the nature of the cytokinesis (and possible cellularization) defect in *pnut* and other septin mutants, and the identification of other *Drosophila* proteins that interact with the septins, will be necessary to clarify the role(s) of the septins. It will also be important to determine how septin function relates to the function of other proteins involved specifically in ordinary cytokinesis (such as Pebble: Lehner, 1992;

Figure 10. Identification of a complex containing Sep1 and Pnut. An embryonic cell extract was prepared using the detergent-containing NET buffer (see MATERIALS AND METHODS; identical results were obtained with RIPA buffer). (A) Samples of the extract and of immunoprecipitates prepared using anti-Pnut antibodies (Pnut IP), anti-Sep1 antibodies (Sep1 IP), or anti-Armadillo antibodies (Arm IP) were fractionated by SDS-PAGE and analyzed by immunoblotting using anti-Sep1 antibodies (upper panel) or anti-Pnut antibodies (lower panel). The positions of relevant molecular size markers are indicated. (B) A portion of the extract was fractionated on a 5–20% sucrose gradient (see MATERIALS AND METHODS). Fractions were analyzed by SDS-PAGE and immunoblotting

Hime and Saint, 1992) or in cellularization (such as Nullo, Serendipity- α , and Bottleneck: Simpson and Wieschaus, 1990; Schweisguth *et al.*, 1990; Schejter and Wieschaus, 1993b; Postner and Wieschaus, 1994). Other proteins of great interest include anillin, which also localizes both to cleavage furrows and to the tip of the cellularization furrow (Field and Alberts, 1995), and Diaphanous, which was discovered on the basis of a mutant that has a cytokinesis defect like that of *pnut* (Castrillon and Wasserman, 1994). Diaphanous is homologous to the product of the *S. cerevisiae* BNI1 gene, which was discovered on the basis of genetic interaction with the CDC12 septin gene and appears to be involved in the selection of bud (and hence division) sites and in the process of division itself (Fares and Pringle, unpublished results).

Roles of Septins Not Related to Cytokinesis in Drosophila

The localization of Sep1 and Pnut also suggests that the septins have roles unrelated to cytokinesis in *Drosophila*. In both syncytial-blastoderm and post-gastrulation embryos, Sep1 undergoes regular rearrangements during the nuclear/cell cycle. The significance of these rearrangements is unknown. However, the cortical localization of Sep1 in interphase cells suggests that the septins may play a role in the organization of the cell surface in such cells. In the syncytial blastoderm, the distribution of Sep1 is largely complementary to (but overlapping) the distribution of actin, suggesting that actin and the septins may play complementary (but overlapping) roles in the organization of the embryo cortex. The Sep1 staining is also distinctly grainier than that of actin, perhaps indicating an association with vesicles rather than with continuous structures along the plasma membrane. A variety of proteins identified as actin-binding proteins have also been localized in syncytial-blastoderm embryos, and some (notably anillin) have distributions rather similar to that of Sep1 (Miller *et al.*, 1989; Field and Alberts, 1995). The situation may be similar to that in *S. cerevisiae*, where the septins and actin appear to behave largely independently (Ford and Pringle, 1991; Kim, Haarer, and Pringle, unpublished results), but some interaction apparently occurs as evidenced by the effect of septin mutations on the degree of polarization of the actin cytoskeleton and of cell-surface growth (Adams and Pringle, 1984). It will be interesting to examine the behavior of the septins in mutants in which cytoskeletal organization in the syncytial

Figure 10 (cont). using anti-Sep1 antibodies (upper panel) or anti-Pnut antibodies (lower panel). Bovine serum albumin (4.6 S), aldolase (7.4 S), and catalase (11.4 S) were fractionated on a parallel gradient as molecular size standards (Peifer, 1993).

blastoderm is disrupted (Sullivan *et al.*, 1990, 1993; Postner *et al.*, 1992), as well as the distribution of the other proteins in septin mutants that reach the syncytial blastoderm stage in the absence of maternal gene product.

During dorsal closure, the cells of the lateral epidermis undergo a dramatic elongation and move over the underlying amnioserosa (Young *et al.*, 1993). As in cellularization, an actin/myosin network is believed to generate the directional force for movement. As in the syncytial blastoderm and in cellularizing embryos, the distribution of Sep1 is overlapping, but distinct from and more grainy than, the distribution of actin. The Sep1 distribution also resembles that of myosin (Young *et al.*, 1993), but, interestingly, anillin does not co-localize with the other proteins at this stage. These data suggest hypotheses for septin function similar to those noted above in relation to cytokinesis. It should be informative to determine septin localization in mutants defective in dorsal closure (Young *et al.*, 1993), as well as the effects of septin mutations on dorsal closure.

Sep1 is also strikingly concentrated in other cell types not undergoing active division. These include neurons of the embryonic central and peripheral nervous systems and polarized epithelial cells of the imaginal discs, salivary glands, and ovarian follicle. In the epithelial cells, Sep1 staining seems generally most prominent in the basolateral membrane domains, although the details of localization appear to vary with cell type (note the strong staining of the apical domain in imaginal-disc cells). At least in the salivary gland and follicle cells, the distribution of Sep1 is strikingly different from that of actin, which is most concentrated in the apical domains. It seems likely that the septins play roles in the specific organization of differentiated cell-surface domains in these polarized cells. Staining of ovaries revealed other intriguing differences in the localization of Sep1 and actin. Although both proteins are prominent at the surfaces of the migrating border cells, Sep1 is found in the cytoplasm of the nurse cells (presumably as part of the eventual maternal contribution to the oocyte) but is not conspicuous at their surfaces, in contrast to actin, and actin, but not Sep1, is conspicuous in the ring canals.

There is as yet no strong evidence as to the significance of the septin distributions in nondividing cells. It should be informative to examine more closely the development of the nervous system and the several epithelial tissues in septin mutants. Meanwhile, it seems reasonable to speculate that, as appears to be the case in yeast, the *Drosophila* septins have common structural features that allow their interdependent assembly into a functional complex, and structural differences that allow them to interact with various other proteins and thus organize these other proteins at the cell surface.

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