The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation

Haifan Lin, Lin Yue and Allan C. Spradling

Howard Hughes Medical Institute Research Laboratories, Department of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, MD 21210, USA

1Present address: Howard Hughes Medical Institute Research Laboratories, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA

### SUMMARY

Oogenesis in *Drosophila* takes place within germline cysts that support polarized transport through ring canals interconnecting their 15 nurse cells and single oocyte. Developing cystocytes are spanned by a large cytoplasmic structure known as the fusome that has been postulated to help form ring canals and determine the pattern of nurse cell-oocyte interconnections. We identified the adducin-like *hts* product and α-spectrin as molecular components of fusomes, discovered a related structure in germline stem cells and documented regular associations between fusomes and cystocyte centrosomes. *hts* mutations completely eliminated fusomes, causing abnormal cysts containing a reduced number of cells to form. Our results imply that *Drosophila* fusomes are required for ovarian cyst formation and suggest that membrane skeletal proteins regulate cystocyte divisions.

Key words: *Drosophila*, fusome, germline, oocyte, membrane skeletal protein, cyst formation

### INTRODUCTION

Gametogenesis in diverse organisms takes place in groups of cells known as germline cysts (reviewed in Spradling, 1993a). Cyst cells are interconnected in specific patterns by small intercellular bridges with a characteristic structure (Burgos and Fawcett, 1955; Meyer, 1961; Gondos, 1973). The formation of ovarian germline cysts by a series of synchronous, incomplete cell divisions has been extensively studied in insects (reviewed in Telfer, 1975). In *Drosophila*, intercellular bridges differentiate into ring canals that mediate transport of materials between the 15 nurse cells and the oocyte throughout most of oogenesis (reviewed in King, 1970; Mahowald and Kamby-sellis, 1980; Spradling, 1993b). Recently, polarized intercellular transport through ring canals in newly formed cysts has been suggested to determine which of the 16 cells differentiates into the oocyte (Suter and Steward, 1991; Yue and Spradling, 1992; Spradling, 1993a; Theurkauf et al, 1993).

A large, novel cytoplasmic structure, the fusome (Fig. 1), is associated with cyst formation in several insect orders (reviewed in Telfer, 1975). The fusome was originally described during oogenesis in the diving beetle *Dytiscus marginalis* (Giardina, 1901). Fusomes arise from mitotic spindle ‘residues’ during the four cystocyte divisions; following completion of each cystocyte mitosis, new material arising at the site of the disaggregated cystocyte spindles contacts and fuses with residues from earlier divisions. The resulting ‘fusome’ consequently takes on a branched appearance with arms that extend through each intercellular bridge in the cyst. Telfer proposed that the fusome plays a key role in cyst formation. The aggregation of newly formed fusomes, and their association with one pole of each cystocyte spindle (Maziarski, 1913; Fig. 1), provides an explanation for the specific pattern of branching interconnections characteristic of these cysts (Knaben, 1934; Koch and King, 1966). The generation of cyst cells with 4 ring canals by this mechanism was proposed to turn off further cystocyte divisions and be a prerequisite for oocyte determination (Koch and King, 1966; King et al., 1982; King and Storto, 1988; Storto and King, 1989).

Germline cysts develop in an assembly-line fashion within the germarium, a structure at the anterior end of each ovariole that is conventionally divided into three regions (Fig. 1C). Just behind a distinctive stack of somatic cells known as the terminal filament lies region 1, home of 2-3 germline stem cells, and 2-4 forming cysts. Region 2a begins with the first 16-cell cyst and continues for 4-6 additional cysts until the flattening of a cyst to span the entire width of the germarium signals region 2b (which ends with the first budding egg chamber). In the electron microscope, fusomes can be readily identified as a distinctive region of cystocyte cytoplasm filled with small vesicles and deficient in ribosomes and mitochondria (Telfer, 1975). Fusome material forms a branching network that passes through and entirely fills each ring canal within region 1 cysts. As cysts move through region 2a, cytoplasmic organelles including mitochondria and centrioles (Mahowald and Strassheim, 1970) and specific RNAs (Wharton and Struhl, 1989; Suter et al., 1989; Ephrussi et al., 1991; Kim-Ha et al., 1991; Lantz et al., 1992; Yue and
Spradling, 1992) begin to be transported from nurse cells into the future oocyte despite the continued presence of fusomal material. Fusomes disappear by the end of region 2, about the same time that ring canals acquire their mature ultrastructure (King et al., 1982) and actin content (Warn et al., 1985). Despite these detailed descriptions, fusome structure has remained unknown at the molecular level.

Genes required for cyst formation are likely to provide further insight into the structure and function of fusomes. Tumorous mutants such as ovarian tumor (otu) (King et al., 1978), benign gonial cell neoplasm (bgcn) (Gateff, 1982) and bag-of-marbles (bam) (McKearin and Spradling, 1990) disrupt the orderly steps of cyst development. Electron microscopic studies of the proliferating cells in otu1 germaria revealed that most lacked intercellular junctions, or were members of aberrant cysts of 2-4 cells interconnected by small fusomes (King, 1979). Since these cysts lack any cells with 4 ring canals, their proliferation and failure to form an oocyte was consistent with the regulatory role proposed for the fusome (King et al., 1982). However, it remained unclear whether ovarian tumor genes encode fusome components or act indirectly by disrupting upstream or parallel processes such as germline sex determination (see Pauli and Mahowald, 1990; Bopp et al., 1993).

Females mutant for egaliitarian (egl) or Bicaudal-D (Bic-D), still produce 16-cell cysts. However, an oocyte never forms and all 16 cells differentiate as nurse cells. Although it is not known if fusomes behave normally in these mutants, early defects in intercellular transport through ring canals have been documented (Wharton and Struhl, 1989; Suter et al., 1989). Bic-D encodes a cytoplasmic protein that has been proposed to play a role in intercellular transport within developing cysts. The molecular nature of egl remains unknown.

The properties of hu-li tai shao (hts) render it a strong candidate as a fusome component. Females homozygous for hts produce cysts containing an average of only 4 cells (Yue and Spradling, 1992). These reductions are accompanied by little or no abnormal proliferation of germinal cells, unlike the case of tumorous mutants. At later developmental stages, the ring canals in these small cysts are defective and oocytes form only rarely. The first 600 amino acids of the 1156 residue hts protein are homologous to mammalian adducin (Joshi et al., 1991), an actin and spectrin-binding protein constituting part of the membrane skeleton (Gardner and Bennet, 1987; Mische et al., 1987). The studies reported here now identify hts protein as a fusome component and provide new insight into the mechanism of germline cyst formation.

MATERIALS AND METHODS

Drosophila stocks

Stocks were raised at 22-25°C except as indicated. Genetic markers are described in Lindsley and Zimm, 1992. The bam1 (McKearin and Spradling, 1990) and hts1 (Yue and Spradling, 1992) mutations were induced by single P element insertion, bgcn112, Bic-D126PA56 and eglp127 (Schüpbach and Wieschaus, 1991) and otu1 (King and Storto, 1988) are EMS-induced alleles that have been described previously. All homozygous mutant females were aged for 3-5 days on yeast-containing corn meal/agar medium prior to examination.

Antisera

Anti-hts polyclonal antisera were generated in mice from the amino terminal portion of the predicted hts protein expressed as a fusion to E. coli. glutathione S-transferase protein (K. Cant and L. Cooley, personal communication). Briefly, the 1.2 kb hts12 cDNA that corresponded to amino acids 49-500 (Yue and Spradling, 1992) was digested with EcoRI and ligated to the pGEX vector (Smith and Johnson, 1988). Sera recovered from three separate mice produced identical immunofluorescence patterns. Western blots revealed several proteins, but only a single species at 140×103 Mr was affected by hts mutations; the labeling of this protein could not be detected in hts1 ovaries (D. Robinson, K. Cant and L. Cooley, personal communication). The relative molecular mass of this protein is close to the

![Fig. 1. Fusome and germarial structure. Drawings of developing germline cysts in synchronous metaphase from a wasp ovary (A; after Maziarski, 1913) or a beetle testis (B; after Hegner, 1914) as they appear after fixation in Carnoy’s fluid and staining with iron haematoxylin. The dark, branched structure figured in the center of each cyst is the fusome (F). Note that one pole of each spindle is associated with the fusome. (C) A drawing illustrating the organization of cells in the Drosophila germarium, including subregions 1, 2a, 2b and 3 (adopted from Lin and Spradling, 1993). See text for details. CB, cystoblast; CC, cystocyte; ES, sheath; FC, follicle cell; GSC, germline stem cell; NC, nurse cell; O, oocyte; TF, terminal filament.](image-url)
128×10^3 predicted by the hts cDNA sequence (Yue and Spradling, 1992; Ding et al., 1993). The polyclonal rabbit sera specific for α-spectrin was described previously (Pescacreta et al., 1989) and was a gift of D. Kiehart. The affinity-purified polyclonal rabbit anti-Drosophila-γ-tubulin antibody was prepared by Raff et al. (1993) and was a gift of M. Moritz.

**Immunostaining and fluorescence microscopy**

For immunofluorescence experiments, the anti-hts antibody was used at 1:100 dilutions, anti-spectrin antibody at 1:200 dilutions, and anti-γ-tubulin antibody at 1:150 dilutions. Ovaries were dissected and fixed as described previously (Lin and Spradling, 1993) and immunologically stained based on an embryo-staining protocol by Patel et al. (1989), except that fluorophore-conjugated secondary antibodies were used for immunofluorescence microscopy. The TRITC- or FITC-conjugated AffilPure™ donkey anti-rabbit or anti-mouse antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. and were used at 1:200 dilutions. The stained ovaries were then mounted in 1× phosphate-buffered saline buffer with 50% glycerol and 1 mg/ml of anti-quenching agent phenyl diamine for microscopy.

A Zeiss Axioscope microscope with epifluorescence was used, and ovaries were examined under oil using a 100× Neofluor lens. Preparations were also analyzed by confocal microscopy using a Zeiss Laser Scan Microscope. Images were collected using the Zeiss software. The Image-I program (version 4.0, Universal Imaging Corporation, West Chester, PA) were used for overlaying the double-labeled images. Files containing single images, or electronically ‘stacked’ z-series were processed on a Macintosh Quadra using Adobe Photoshop™ and Aldus Pagemaker™ programs and printed on a Kodak dye transfer printer.

**Electron microscopy of ovaries**

Whole ovaries were dissected in Ringer’s solution (EBR: 130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, and 10 mM Hepes at pH 6.9), and processed for transmission electron microscopic analysis as described in Yue and Spradling (1992).

**RESULTS**

**A hts product and α-spectrin are present in distinctive structures within developing germline cysts**

In order to learn more about the possible roles of hts protein in cyst formation, we used antisera raised by K. Cant and L. Cooley against a fusion protein containing residues 49-500 of the hts protein to determine its pattern of expression (see Materials and Methods). Confocal immunofluorescence microscopy of wild-type ovarioles revealed that hts protein was present during many stages of oogenesis (Fig. 2). Heavy labeling was observed just underneath the follicle cell membranes, as might be expected for a putative membrane skeleton protein (Fig. 2A, ‘FC’). The signal was concentrated in basolateral regions where adjacent follicle cells lie in contact, and was particularly prominent in the interfollicular stalk cells that join budded egg chambers (Fig. 2A, ‘IF’). The follicle cell precursors in the germarium were also labeled (Fig. 2C, ‘FC’).

The antigen was distributed very differently in ovarian germline cells. At the tip of each germarium, the cell membranes were conspicuously free of antigen but several large internal structures were labeled (Fig. 2C). Their shape changed in concert with cyst development. At the very tip, where 2-3 stem cells reside, several cells contained a single large sphere (Fig. 2C, ‘SS’) in a perinuclear location (see below). Posterior to the stem cell region where growing 2-, 4- and 8-cell cysts are located, the labeled structures became elongated and branched (Fig. 2C, ‘F’). The number of labeled structures with various levels of branching in this region varied between germlia, reflecting the known fluctuations in the number of developing cysts. Older cysts in region 2 stained more weakly and labeling could no longer be detected in the germline cells of budded chambers. The location, size and developmental modulations of the branched structures were identical to those previously described for Drosophila fusomes (see Storto and King, 1989).

Since mammalian adducin is frequently associated with spectrin in vivo, and binds to it in vitro, we examined the distribution of this protein as well. Antibodies specific for α-spectrin have been raised (Byers et al., 1987) and used to study its location during Drosophila embryogenesis (Pesacreta et al., 1989). When ovarioles were labeled with α-spectrin-specific antibodies, a pattern similar but distinct from that seen with anti-hts serum was observed (Fig. 2E). Submembranous follicle cell regions labeled by the α-spectrin probe were strongly labeled with the spectrin-specific probe. In addition, the terminal filament and sheath cells were stained. The putative fusomes within stem cells and early cysts were strongly labeled, and could be seen to lie within cysts (Fig. 2E, ‘1-8’) whose outer walls were highlighted, probably due to their association with inwardly migrating prefollicle cells. Double-label experiments revealed no differences between the fusome-like structures labeled by anti-spectrin and anti-hts antisera (data not shown). Neither antisera labeled the ring canals in budded egg chambers.

Although it remains possible that polyclonal antisera raised against the hts fusion protein recognized fusomes but not a hts-encoded protein, this was unlikely for several reasons. When similar studies were carried out on ovaries from hts mutant females, which contain less than 5% of normal hts mRNAs (Yue and Spradling, 1992), no staining could be detected in either germ line or somatic cells (Fig. 2B, D). A 140×10^3 M₁ protein recognized by the antisera was affected by hts mutations (see Materials and Methods). This protein is similar in mobility to the 128×10^3 M₁ predicted product of the major hts ovarian transcript. In ovaries from hts mutants, spectrin labeling of the putative fusome structures was abolished specifically (Fig. 2F). Spectrin labeling did not change within any of the other cells, and the outlines of the developing cysts were still visible. Therefore, the association of spectrin with the fusome-like structures, but not other germline structures, required the presence of hts protein.

**The branched structures labeled in early cysts are fusomes**

We carried out several additional experiments to verify that the structures labeled in early germline cysts were fusomes. First, the behavior of fusomes was examined in the electron microscope. Previous studies have identified fusomes as zones of light, ribosome-deficient cytoplasm containing conspicuous small vesicles that span the ring canals of region 1 and 2 cysts (reviewed in Mahowald and Kambsellis, 1980; see Fig. 3). Three-dimensional reconstructions from serial electron micrographs have verified that these membrane-rich zones of amorphous cytoplasm corresponded to fusomes (King et al.,
When we examined electron micrographs of germaria from hts1 females, fusomal material was absent or greatly reduced (Fig. 3 inset). This paralleled the general disruption of the hts and spectrin-rich structures in hts1 mutants, supporting their identity as fusomes. Immunoelectron microscopy will be required to learn what structures within the zones of distinctive cytoplasm actually contain hts and α-spectrin protein; several attempts at such experiments were unsuccessful.

A second characteristic property of fusomes is their association with centrosomes during cystocyte mitoses (Maziarski, 1913; King et al., 1982; Storto and King, 1989; see Fig. 1). We documented a similar association between the spectrin and hts-containing structures and cystocyte centrosomes by studying cysts undergoing synchronous mitoses in germaria that had been stained with anti-hts sera and DAPI. For example, a 4-cell mitotic cyst containing a bar-like fusome is shown in Fig. 4A. The metaphase chromosomes are oriented such that four of the eight spindle poles would be projected to lie adjacent to the fusome. A similar cyst is shown in Fig. 4B that was labeled

Fig. 2. Fusome-like structures are labeled by adducin and spectrin antisera in ovarioles. Sera specific for an amino-terminal region of the hts protein (A-D) or for α-spectrin (E-F) were used to label whole mounts of ovarioles from wild-type (A,C,E) and hts1 (B,D,F) females. (A) Immunofluorescence microscopy reveals labeling of interfollicular cell membranes (FC, for follicle cell) within a stage 3 egg chamber. The interfollicular stalk cells (IF) joining the chambers are strongly labeled. A similar field (B) from the hts1 ovariole was not labeled above background levels. A stacked series of confocal z-sections spanning 6 microns from a wild-type germarium (panel C) revealed specific labeling of fusome-like structures (F) and spherical structures (SS) in germline cysts and stem cells near the anterior end. More posteriorly, labeling of pre-follicle cell membranes (FC) was also prominent. No specific labeling was detected in hts1 germaria (D). (E) A 6 µm stack of confocal z-sections showed α-spectrin labeling in wild-type ovarioles near follicle cell membranes, and in the same fusome-like structures as seen with anti-hts sera. Among them, fusome-like structures in 16-cell cysts were labeled in numerical order according to their approximate developmental age. In addition, strong labeling was observed in terminal filament cell and sheath cell membranes. In hts1 ovarioles, only the putative fusome labeling was eliminated (F). Bars in A and E denote magnifications for panels A-D and E-F, respectively.
The fusome contains membrane skeletal proteins with anti-spectrin antibody and anti-α-tubulin (a centrosome marker). Eight centrosomes defining the orientation of the 4 mitotic spindles were labeled by the anti-α-tubulin antibody; four lie close to the spectrin-rich material. Centrosome staining was observed near the putative fusomes in earlier cysts as well (Fig. 4C).

Third, we looked to see if anti-hts and anti-α-spectrin antisera recognized fusomes during early stages of male germline development. 16-cell cysts form in the testis by a mechanism similar to that observed in the ovary (Rasmussen, 1973) and contain elongated fusomes (Hegner, 1914: see Fig. 1B). When whole mounts of testes from third instar larvae were stained with these antisera, highly branched structures were labeled within cysts located near the apical end (Fig. 4D). The approximate size, shape and developmental profile of these branched structures were exactly as expected for male fusomes; similar structures were not seen in any other tissue examined (data not shown). In light of these diverse supporting data, we concluded that the structures labeled by the anti-hts and anti-α-spectrin antisera were fusomes.

**Fusomes begin to regress at the time that intracyst transport initiates**

Fusomes achieved maximum thickness and labeling intensity early in region 2a. However, in all later (more posterior) cysts, they underwent further changes (see Fig. 2E: 16-cell cysts numbered in order of increased age). The diameter of the individual branches decreased greatly (compare cyst 1 and cyst 2). By the time that cysts widen to form a ‘lens-shaped’ chamber (cyst 5), their fusomes had assumed the form of elongated fibers running along the length of the chamber. By late in region 2b (cyst 7), the staining had almost entirely disappeared, and only weak submembranous labeling was seen in subsequent egg chambers. The first sharp reduction in fusome thickness occurred in mid-region 2a cysts at just the time that intracyst transport of organelles and RNA has been observed.
to initiate (Mahowald and Strassheim, 1970; Wharton and Struhl, 1989; Suter et al., 1989; Lantz et al., 1992; Yue and Spradling, 1992).

**Spectrin and hts protein are located in a novel structure in germline stem cells**

Although the branched structures observed in region 1 cysts were similar in shape to previously described fusomes, the presence of large spherical structures near the tip of each germarium had not been previously described in *Drosophila*. However, Giardina (1901) visualized similar structures in *Dysticus* oogonia that appeared to be fusome precursors. To identify the spherical structures in electron micrographs, we first noted that in the distalmost cells, presumably stem cells, the labeled spheres within were located between the nucleus and that portion of the plasma membrane in contact with the terminal filament (Fig. 4E). We then examined the corresponding region in serial section electron micrographs, and observed an unusual spherical region of cytoplasm (Fig. 3). This region was about the expected diameter for the adducin-rich sphere (3 µ). Like the fusome it excluded most ribosomes and mitochondria, and was rich in membrane vesicles, although their density appeared lower than in later fusomes. Similar structures could occasionally be seen in electron micrographs of other anterior germarial cells.

The membrane-rich spherical regions seen in electron micrographs were frequently seen to lie near centrioles. For example, serial sectioning demonstrated that the stem cell in Fig. 3 contained two recently separated centrioles (C), one of which lies about 0.8 mm from the membrane-rich spherical region. The association of fusomes with cystocyte centrosomes was described above (Fig. 4). Furthermore, in *hts* mutant germaria, the spherical organelles in electron micrographs (data not shown) and the spherical structures staining with anti-hts and anti-α-spectrin antisera (Fig. 2B) were absent. We suggest that these spectrin-rich spheres represent an early stage of fusome development similar to that described by Giardina (1901).

**Tumorous mutations disrupt distinct aspects of cyst formation**

A variety of genes are known that produce phenotypically similar ovarian tumors; however, the reasons that germline cells proliferate in such strains have not been elucidated (reviewed in Spradling, 1993b). Females deficient for *Sex-lethal* or for *sans fille* ([=fs(1)1621] partially transform ovarian germline cells to a male identity (Bopp et al., 1993), suggesting failures in germline sex determination as an underlying cause. In contrast, *otu* has been proposed to regulate fusome formation; the effects of *otu* mutations form the basis for models of fusome function (Storto and King, 1989). Finally, *bam* has been suggested to act directly in the process of 16-cell cyst formation (McKearin and Spradling, 1990). We

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**Fig. 4.** Structure and location of fusomes and spectrin-rich spheres as visualized by anti-α-spectrin or anti-hts antisera. The association of a bar-like fusome with one pole of each dividing cell in a 4-cell cyst is illustrated in A. The fusome was labeled with anti-hts antiserum (and visualized with a secondary antibody containing horseradish peroxidase) while the four metaphase nuclei were stained with DAPI. Each metaphase chromosomal set is equidistant and perpendicular to the fusome material, suggesting that one centrosome is associated with the fusome. This association is clearly shown in a 6 µ z-stack of confocal sections (B), in which the four pairs of centrosomes (labeled green, detected by anti-γ-tubulin antibody) are radially oriented towards the fusome (labeled red, detected by anti-α-spectrin sera), so that one centriole from each pair is in close juxtaposition with the fusome. (One of the centrioles lies under the edge of the fusome and consequently appears yellow.) The association of centrioles with fusomes in a younger interphase cyst is also evident (C). In D (same magnification as C) an example of the structure labeled with anti-α-spectrin in each early cyst from a third instar larval testis is shown. It consisted of blobs of material interconnected by thin filaments; the shape and location of the stained material within the developing cysts appeared identical to the fusome previously described in male germline cysts (compare Fig. 1B). Anti-α-spectrin staining (E) shows a spectrin-rich sphere adjacent to the base of the terminal filament. A second spectrin-rich sphere in a separate anterior cell is also located near the terminal filament, but lies below the plane of focus in this photograph.
The fusome contains membrane skeletal proteins

expected that the use of a fusome antiserum would more clearly reveal the effects of these mutations on cyst development. Consequently, we used anti-α-spectrin sera to stain ovaries from females homozygous for a variety of tumorous ovary mutations.

Analysis of germaria and egg chambers from *otu* females using the anti-*hts* and anti-spectrin sera revealed that this gene blocks cyst development at multiple steps. Nearly 80% of germaria contained highly branched fusomes (not shown), while the rest (Fig. 5A) contained a greatly increased number of small cells that appeared to contain a *hts* and spectrin-rich sphere or early fusome. Some groups of tumor cells still managed to acquire a follicle cell layer. Approximately 30% of these chambers were filled with small cells as revealed by staining with DAPI (Fig. 5B), which always contained spectrin-rich spheres and fusomes (Fig. 5C). In contrast, when the cells in such chambers had formed polyploid pseudonurse cells (Fig. 5D), then fusome staining was always absent (Fig. 5E).

Developing *bgcn* cysts appeared quite different when visualized with anti-α-spectrin antibodies. The anterior end of the germarium contained 25-50 cells with prominent spectrin-rich spheres; branched fusomes were rarely seen but some cells contained two or more small blobs of labeled material instead of a single sphere (Fig. 5F). Electron micrographs showed that some early *bgcn* germine cells contained cytoplasmic zones rich in membranes that differed from wild type and resembled normal endoplasmic reticulum (Fig. 5G). Because of their size, location and number per cell, we suspected (but could not prove) that these aggregates contained α-spectrin and were responsible for the observed staining.

Analysis of *bam* mutant germaria suggested that cyst development was arrested at an early stage. Cells contained only spectrin-rich spheres or very small fusomes associated with a single ring canal. Unlike *bgcn* mutant cells, the spheres and fusomes in *bam* mutant germaria appeared to be normal in the electron microscope (data not shown). We concluded that *bam*

Fig. 5. Alterations in cyst development in the ovarian tumor mutants *otu* (A-E) and *bgcn* (F-G). A germarium is shown following staining for α-spectrin, revealing many cells with labeled spheres or small fusomes (A). Tumorous egg chambers were stained with DAPI to visualize DNA (B,D) or anti-α-spectrin antibodies to visualize fusomes (C,E). Chambers that retained only small cells (B) contained spectrin-rich spheres and fusomes as shown by the 6 µm z-stack of confocal sections (C); chambers that developed pseudonurse cells (D) lacked α-spectrin staining within the germ cells as seen in the confocal z-stack of same thickness (E). A germarium and two tumorous egg chambers from a female homozygous for *bgcn* were labeled with anti-α-spectrin antibodies, revealing the presence of a greatly increased number of cells which contained a single structure resembling a spectrin-rich sphere (F). An electron micrograph of the anterior region of a *bgcn* germarium is shown (G). An aggregate of membranes is present that differed from the spectrin-rich structures seen in wild-type cells. The bar in A denotes the magnification in A-F.
arrests cyst development prior to or just after the 2-cell cyst stage. Thus, the fusome structures seen in all three mutants provided valuable insight into their distinctive developmental defects.

**Bic-D and egl have normal fusomes**

*Bic-D* and *egl* mutations block the differential transport of mRNAs that normally accumulate in the presumptive oocyte (Wharton and Struhl, 1989; Suter et al., 1989), and causes all 16 cells to differentiate as nurse cells. Two possible effects of these mutations on the fusome might have explained these results. *Bic-D* and/or *egl* may be needed to generate cytoplasmic asymmetry at each division that might subsequently be the basis for the transport polarity. Alternatively, fusome regression might have been blocked, preventing the free flow of materials through the ring canals. In either case, alterations in fusome structure and kinetics would have been expected. When we examined germaria from *Bic-D*<sup>26ipabb</sup> and *egl*<sup>pV27</sup> females, cysts containing fusomes in various stages of development were observed (Fig. 6). The morphology of the fusomes appeared similar to wild type, suggesting that the 16-cell cysts produced by these females form normally. Therefore, *Bic-D* and *egl* do not block oocyte formation by altering fusome development.

![Image](image_url)

**Fig. 6.** The fusomes of *Bic-D* and *egalitarian* cysts are similar to wild type. The pattern of labeling seen with spectrin and adducin antisera is shown for *Bic-D*<sup>26ipabb</sup> transheterozygotes (A) and *egl*<sup>pV27</sup> homozygotes (B). Although the number of developing cysts in each germarium was reduced, the structure of individual spectrin-rich spheres and fusomes appear normal. The bar in A denotes the magnification for both panels.

**DISCUSSION**

Fusomes have been proposed to control at least four critical aspects of *Drosophila* oogenesis. First, fusomes may be needed to block cytokinesis physically, leading to the production of ring canals and interconnected cysts of cells. Second, they may be required to control the pattern of cystocyte interconnections by orienting mitotic spindles (Telfer, 1975). Third, it has been suggested that fusomes synchronize the cystocyte divisions, and signal individual cystoctyes to stop dividing in response to the production of cells with 4 normal ring canals (Storto and King, 1989). Finally, by causing cells to divide unequally during the four cystocyte mitoses, fusomes may create the polarity that is subsequently used to distinguish one cell as an oocyte (Spradling, 1993a). The research reported here, by identifying specific fusome components and a mutation abolishing fusome structure, provides new insights into all four of these proposed roles.

**Fusomes contain membrane skeletal proteins**

Our studies provide the first information on the molecular composition of fusomes. The acquisition of spectrin and hts-protein may be part of the process that prevents spindle remnants from dispersing following mitosis and creates a stable cytoplasmic structure. How these proteins accomplish this task and the nature of additional fusome components remain to be determined. Adducin is a component of junctional complexes linking spectrin via short actin filaments in characterized vertebrate membrane skeletons (reviewed in Bennet and Gilligan, 1993). Actin is not a prominent component of fusomes, however, since they are not specifically labeled with phalloidin (Warn et al., 1985; Spradling, 1993a). Thus the arrangement of these proteins in fusomes is likely to differ from that present in previously studied membrane skeletons.

Fusomes also appear to lack microtubules. Spindle microtubules can be seen in the electron microscope within forming ring canals until late in telophase (Koch and King, 1966; Mahowald and Strassheim, 1970; Mahowald, 1971; Telfer, 1975; King et al., 1982). They subsequently disappear and the cytoplasmic region they occupied immediately acquires the characteristics of a fusome. α-tubulin-specific antibodies do not label fusomes in region 1 cysts (Theurkauf et al., 1993). However, as fusomes regress in region 2a, a multiply branched collection of microtubules forms and at least some of them pass through ring canals. Perhaps fusome regression templates the polymerization of an ordered array of microtubules in its place.

**Fusomes are not needed to form ring canals**

Previously, fusomes have been thought to block physically the contractile ring from separating daughter cystocytes. However, *hts<sup>1</sup>* mutants still form ring canals containing outer rims (Yue and Spradling, 1992; Fig. 3). It was not completely surprising to learn that a fusome is not needed to effect incomplete cytokinesis and to initiate ring canal formation. The material that forms the dense outer rim of each ring canal begins to accumulate while spindle microtubules are still present (Mahowald, 1971; Telfer, 1975) and prior to fusome formation. Cytokinesis may be blocked during cyst formation by the assembly of these outer rims, rather than by fusomes. However, the efficiency of ring canal formation in *hts* females could not be
compared to wild type, so the fusome might facilitate ring canal formation even though it is dispensable.

**Fusomes associate with cystocyte centrosomes.**

Our experiments showed that centrosomes associate with fusomes during cyst formation. This juxtaposition was observed in 2- and 4-cell cysts, supporting previous observations on nearly complete cysts (Maziaszki, 1913; King et al., 1982; see Fig. 1). During the interphase of each successive cystocyte cell cycle, a new fusome branch must associate with the centrosome that was not located near a fusome during the previous metaphase. Perhaps transport of fusome vesicles along microtubules plays some role, much as is thought to occur with the Golgi complex.

Telfer (1975) realized that the fusome-centrosome association would guarantee the non-random branching pattern characteristic of *Drosophila* ovarian cysts. Without a fusome, this pattern of interconnections might be changed. Although the small size of *hts* cysts renders tests of this idea difficult, it may be possible to learn if the fusome controls the orientation of cytosome mitotic spindles through additional study of weak *hts* alleles (Yue and Spradling, 1992).

**Involvement of fusomes in intercellular signaling**

Previous studies of cyst development in *ottu* mutant females suggested that the growth of germline cells might be negatively regulated by the fusome (Storto and King, 1989). Germline cells in *hts* ovarioles developed in the complete absence of fusomes, yet they did not undergo the extensive proliferation characteristic of tumorous ovary mutations. Consequently, fusomes are unlikely to be needed to shut off germ cell divisions. However, they may act as positive regulators of proliferation during cystocyte division. Adducin and spectrin are known to be modified by phosphorylation in vertebrates (Bennet and Gilligan, 1993). Cyclic modifications of the fusome may choreograph the cystocyte cell cycles. This would provide a simple mechanism for cystocyte synchronization. Tumorous ovary genes might encode regulatory proteins that modify fusome constituents as part of such a cycle, inactivating a transient, division-promoting state. Genes such as *bgy* and *bum* would be specific for this function, since they only affect cyst formation. However, genes such as *ottu* might also modify other structures containing cytoskeletal proteins. This would explain why *ottu* alleles affect diverse processes in oogenesis (King and Storto, 1988).

**Asymmetric cystocyte divisions may determine cyst polarity and oocyte formation**

A variety of evidence suggests that polarized intercellular transport through ring canals is necessary for oocyte differentiation (Suter and Steward, 1991; Yue and Spradling, 1992; Spradling, 1993a) and that microtubules are likely to be required (Koch and Spitzer, 1983; Theurkauf et al., 1993). The presence of the fusome at only one pole of each cystocyte spindle emphasizes that these divisions are inherently unequal. Fusome development, by virtue of its associations with centrosomes and the microtubule cytoskeleton, may be required to establish a properly polarized system of differential transport and therefore to differentiate an oocyte. The fusomes within *Bic-D* and *egi* cysts appeared to be normal, even though an oocyte never forms. These gene products may be required for transport or may themselves be oocyte-determining factors. Several RNAs, including *oskar* and *Bic-D*, do not accumulate differentially in these mutants (Wharton and Struhl, 1989; Suter et al., 1989) or in *hts* cysts (Yue, 1992). hs protein may disrupt oocyte formation by two possible mechanisms. *hts* is required during the cystocyte divisions for fusome formation, and later for the acquisition by ring canals of actin and an ‘inner ring’ of electron-dense material (Yue and Spradling, 1992). Either or both functions might be necessary for the polarized transport of oocyte-determining substances.

**Are membrane skeletal proteins used more generally to regulate asymmetric cell divisions?**

*Drosophila* cyst formation may provide a genetically tractable model for the function of spectrin and adducin-like proteins outside of the membrane skeleton. A fraction of mature human lymphocytes contain a large aggregate of spectrin that is rich in small membranous vesicles and located in a perinuclear position reminiscent of the spheres that we observed in germline stem cells (Black et al., 1988). The distribution of spectrin is altered upon lymphocyte activation (Gregorio et al., 1992). The organization of spectrin and hs protein into spectrin-rich spheres may be a more general characteristic of cells that respond to certain signals or that undergo asymmetric divisions.

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