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A Balbiani body and the fusome mediate mitochondrial inheritance during *Drosophila* oogenesis

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SUMMARY

Maternally inherited mitochondria and other cytoplasmic organelles play essential roles supporting the development of early embryos and their germ cells. Using methods that resolve individual organelles, we studied the origin of oocyte and germ plasm-associated mitochondria during *Drosophila* oogenesis. Mitochondria partition equally on the spindle during germline stem cell and cystocyte divisions. Subsequently, a fraction of cyst mitochondria and Golgi vesicles associates with the fusome, moves through the ring canals, and enters the oocyte in a large mass that resembles the Balbiani bodies of *Xenopus*, humans and diverse other species. Some mRNAs, including *oskar* RNA, specifically associate with the oocyte fusome and a region of the Balbiani body prior to becoming localized. Balbiani body development requires an intact fusome and microtubule cytoskeleton as it is blocked by mutations in *hu-li tai shao*, while *egalitarian* mutant follicles accumulate a large mitochondrial aggregate in all 16 cyst cells. Initially, the Balbiani body supplies virtually all the mitochondria of the oocyte, including those used to form germ plasm, because the oocyte ring canals specifically block inward mitochondrial transport until the time of nurse cell dumping. Our findings reveal new similarities between oogenesis in *Drosophila* and vertebrates, and support our hypothesis that developing oocytes contain specific mechanisms to ensure that germ plasm is endowed with highly functional organelles.

Key words: Mitochondria, Oogenesis, Fusome, Balbiani body, Patterning, Germ plasm

INTRODUCTION

Oocytes provide virtually all the mitochondria of the zygote at the time of fertilization. Embryonic viability and vitality depend on the egg acquiring functional cellular components in appropriate numbers and locations. Studies of mitochondria during oogenesis hint that processes other than simple growth and duplication may exist to address these needs. In particular, mitochondria, Golgi bodies, endoplasmic reticulum and other organelles form aggregates or ‘clouds’ in pre-follicular germ cells and then congregate within a large structure known as the Balbiani body in the early oocyte cytoplasm of many species (reviewed by Raven, 1961; Guraya, 1979; de Smedt et al., 2000). Mitochondrial clouds sometimes arise in interconnected germ clusters shortly before cyst breakdown (Al-Mukhtar and Webb, 1971) and the apoptotic death of many germ cells (Pepling and Spradling, 2001). Balbiani bodies may also contain RNAs and organelles destined for future germ cells, leading to the idea that they play a role in the development of germ plasm (see Matova and Cooley, 2001). However, it has been difficult to test the function of mitochondrial clouds and Balbiani bodies genetically, in part because they have not been described in model organisms such as *Drosophila* or *C. elegans*.

Drosophila oogenesis (reviewed by Spradling, 1993; Johnstone and Lasko, 2001) (Fig. 1A) provides an attractive system for studying the origin of oocyte organelles. At the anterior tip of each ovariole, germline stem cells divide asymmetrically to produce daughters known as cystoblasts. Cystoblasts undergo four rapid, asymmetric divisions with incomplete cytokinesis to generate interconnected 16-cell groups known as germline cysts. During these cell divisions, the cysts elaborate a cytoskeletal polarity that ultimately causes one cell to develop as an oocyte, while the others become nurse cells (reviewed by Reichmann and Ephrussi, 2001). Cyst polarity originates within a special cytoplasmic organelle rich in membrane skeleton proteins known as the fusome whose branches grow thinner with each cystocyte division (Lin et al., 1994; Lin and Spradling, 1997; de Cuevas and Spradling, 1998). As cysts move through germarium region 2, their microtubule minus ends accumulate on the large fusome segment within one of the two pro-oocytes (Grieder et al., 2000; Huynh et al., 2001), resulting in the detection of a new microtubule organizing center (Theurkauf et al., 1993). The movement of cystocyte centrioles along the fusome toward the future oocyte during this time may cause this microtubule reorganization (Mahowald and Strassheim, 1970; Grieder et al., 2000; Bolivar et al., 2001). The meiotic gradient within

developing cysts may also respond directly to fusome polarity (Huynh et al., 2000). The cytoskeletal structures that have developed in region 2b cysts causes specific proteins such as Bicaudal-D (Bic-D), Orb and Cup, and mRNAs from *oskar*, *orb* and *Bic-D* to accumulate within an initial cell and stimulate it to differentiate as the oocyte, while the other 15 cells become nurse cells.

Maternal organelles may play developmental roles, rather than just accumulating passively in the oocyte. Mitochondria are enriched in the germ plasm, a special region found in many eggs that specifies germ cells. Mitochondria in some vertebrate eggs associate with germ plasm precursors known as granulofibrillar material or nuage beginning very early in oogenesis (Al-Mukhtar and Webb, 1971; Heasman et al., 1984) (reviewed by Saffman and Lasko, 1999). Specific RNAs destined for germ plasm also associate with the Balbiani body and/or forming germ granules (Forristall et al., 1995; Kloc and Etkin, 1995; Bradley et al., 2001). In *Drosophila* eggs, mitochondria associate closely with germ plasm constituents known as polar granules (Mahowald, 1968). Moreover, exported mitochondrial ribosomes and/or their component rRNAs have been proposed to actively contribute to *Drosophila* germ plasm function shortly after fertilization (Iida and Kobayashi, 1998; Amikura et al., 2001).

Recent studies of mitochondria in yeast and cultured animal cells document three important properties that are relevant to understanding the behavior of mitochondria during oogenesis. First, mitochondria are highly plastic and can readily alter their shape from spheres to ellipsoids to complex reticuli (Bereiter-Hahn and Voth, 1994). Specific forms depend on the relative number of fusion and fission events controlled by specific genes (Jensen et al., 2000). A candidate *Drosophila* mitofusin encoded by the *Marf* (also known as *dmfn*) gene is expressed during oogenesis (Hwa et al., 2002). Second, mitochondria are frequently mobile within the cytoplasm, and can move actively along microtubules in most animal cell types, or actin in plant and yeast cells (Bereiter-Hahn and Voth, 1994). Finally, studies of mitochondrial genomes document a high rate of somatic mutation (Denver et al., 2000; Nekhaeva et al., 2002), and it has been suggested that maternal mitochondrial genomes pass through a 'mitochondrial bottleneck' in order to maintain their average fitness (Bergstrom and Pritchett, 1998).

To begin to uncover the molecular mechanisms that support the inheritance of mitochondria and other organelles, we have visualized their number, shape and movement during *Drosophila* oogenesis. Our observations show that a fraction of the mitochondria, Golgi and other organelles within each 16-cell cyst are delivered into the cytoplasm of the single forming oocyte as a typical Balbiani body under the control of the fusome-dependent system of cyst polarity. The Balbiani-body-derived mitochondrial population of the oocyte remains largely separate from nurse cell mitochondria until nurse cells break down late in oogenesis. Before then, members of this population associate with the polar plasm, leading us to propose that the genomes in Balbiani body-associated mitochondria will be preferentially inherited in grandchildren. Similar mechanisms may act during oogenesis in other species, and these events may underlie the frequent presence of a Balbiani body and the origin of the mitochondrial bottleneck.

MATERIALS AND METHODS

Drosophila strains and culture

Fly stocks were cultured at 22-25°C on standard food. The *y; ry^{506}* strain (Karpen and Spradling, 1992), the *hts^{1}* mutation (Yue and Spradling, 1992) and *egl^{wu50}* (Schupbach and Wieschaus, 1991) have been described. Other genes and balancer chromosomes are described in FlyBase.

Construction and integration of transgenes

We constructed a mitochondrial marker transgene ('mito-GFP') by fusing the human COX VIII mitochrondrial targeting signal (Rizzuto et al., 1995) to the N terminus of EGFP (Clontech). We synthesized the targeting signal using overlapping oligos (CGG CTA CGG CTG ACC GTT TTT TGT GGT GTA CTC CGT GCC ATC ATG TCC GTC CTG ACG CCG CTG CTG and CTT GGC GCG CGG CAC TGG GAG CCG CCG GGC CGA GCC TGT CAA GCC CCG CAG CAG CAG CGG CGT CAG GAC), and then added a 5′ *Kpn*I site (TAT GGT ACC GGC TAC GGC TGA CCG TTT) and a 3′ *Bam*HI site (TAT GGA TCC CTT GGC GCG CGG CAC TGG). A 5′ *Bam*HI site (TAT GGA TCC AGT AAA GGA GAA GAA CTT) and 3′ *Xba*I site (TAT TCT AGA TTT GTA TAG TTC ATC CAT) were added to EGFP. The EGFP fragment was subcloned downstream of the targeting sequence in the pUASp vector (Rørth, 1998) and transformed into *y; w* flies (Grieder et al., 2000). Males containing the transgene were crossed to *w; NGT40; nosGAL4::VP16* females to drive expression of the transgene. Flies bearing single integrated copies displayed green ovaries, embryos and larvae, but the signal level in their germaria was variable. However, strong expression was observed in the germaria of flies carrying multiple copies of the transgene; hence, they were used in these experiments.

Immunostaining and fluorescence microscopy

One-day-old females were fed wet yeast overnight prior to analysis. Ovaries were dissected in room temperature Grace's media (BioWhittaker) and fixed for 20 minutes in 3.7% formaldehyde (Sigma) in Grace's, then rinsed in PBT (1×PBS, 0.1% Triton-X100, 1 mg/ml BSA). To fix GFP-expressing ovaries, 3.2% EM grade formaldehyde (Ted Pella) was used. Primary antibodies were incubated overnight at 4°C. Primary antibodies were diluted at follows: rabbit α-*Drosophila* ATP synthase, β subunit (1:350, gift from Dr Rafael Garesse), mouse α-human cytochrome c_1 oxidase, subunit I (COX1, 1:500, Molecular Probes), rabbit α-rat mannosidase 2 (1:300, Dr Kelley Moremen), rabbit α-*Drosophila* α-Spectrin (1:300) (deCuevas et al., 1996), mouse α-*Drosophila* 1B1 (specific for Hts protein, 1:100) (Zacci and Lipshitz, 1996), rabbit and rat α-*Drosophila* Vasa (1:2000 and 1:200, respectively, gift of Dr Paul Lasko), mouse α-*Drosophila* Orb (1:100, Dr Paul Schedl, Developmental Studies Hybridoma Bank), rat α-*Drosophila* Cup (1:1000) (Keyes and Spradling, 1997), mouse and rat anti-α-tubulin (1:350, Sigma; 1:20, Oxford Biotechnology, respectively), mouse α-phosphotyrosine PY20 (1:1000 ICN Biomedicals), and mouse α-*Drosophila* lamin ADL67 (1:4 Nico Stuurman). Ovaries were then washed in PBT three times for 20 minutes, then secondary antibody was added in PBT either overnight at 4°C, or for 4 hours at room temperature. The following secondary antibodies were used: goat α-rabbit and α-mouse AlexaFluor488, AlexaFluor568, AlexaFluor546 and AlexaFluor633 (1:200, Molecular Probes); and goat α-rabbit, α-mouse and α-rat Cy3 and Cy5 (1:1000, Jackson ImmunoResearch) After secondary antibody incubation, ovaries were washed three times for 10 minutes in PBT, then equilibrated overnight at 4°C in VectaShield (Vector Laboratories) before mounting. Tubulin antibody labeling was carried out as described (Grieder et al., 2000). Fluorescent in situ hybridization was performed according to Wilkie et al. (Wilkie et al., 1999). Phalloidin staining was carried out according to Frydman and Spradling (Frydman and Spradling, 2001)

using both phalloidin AlexaFluor546 (1:200, Molecular Probes) and rhodamine phalloidin (1:200, Jackson ImmunoResearch). For DNA labeling, ovaries were incubated in 20 μg/ml RNAseA for 2 hours during secondary antibody incubation and TOTO3 (1:1500, Molecular Probes) was added for 20 minutes. Confocal analysis was carried out using Leica TCS NT and Leica TCS SP2 confocal microscopes.

Live ovary imaging

For live ovary imaging, ovaries were dissected from flies expressing mito-GFP, or wild-type ovaries were incubated with MitoTracker GreenFM (1:1000, Molecular Probes) for 15 minutes, then briefly rinsed in Grace's. The ovaries were mounted on a petriPerm 50 hydrophobic plate (Vivascience) in Grace's and covered with an 18 mm^2 cover slip. Using the petriPerm plates allows the ovarioles to stay alive for 2-3 hours. Ovariole quality was assessed based on mitochondrial swelling; experiments terminated prior to its onset. Ooplasmic streaming was defined as vigorous directional movement of bulk cytoplasm; it contrasted sharply with the relatively slow, random movement of mitochondria and yolk droplets normally seen. All live imaging and analysis was carried out using a spinning disk confocal [Leica DM IRE2 inverted microscope, Ultraview confocal system (Perkin Elmer) and MetaMorph work station (Universal Imaging)].

Electron microscopy

Whole ovaries were dissected in Grace's media, then fixed for 1 hour at 4°C in 1% gluteraldehyde, 1% OsO_4, 0.1 M cacodylate buffer, 2 mM Ca (pH 7.5). The ovaries were then washed three times for 5 minutes in cacodylate buffer and embedded in agarose at 55°C. They were then rinsed for 5 minutes in 0.05 M maleate (pH 6.5), and incubated for 1.5 hours in 0.5% uranyl acetate, 0.05 M maleate. After rinsing in H_2O, they were put through an ethanol dehydration series (35% twice for 5 minutes, 50% for 10 minutes, 75% for 10 minutes, 95% for 10 minutes and 100% three times for 10 minutes) then incubated twice for 10 minutes in propylene oxide and for 1 hour in 1:1 propylene oxide:resin [Epon 812:Quetol 651 (2:1)], 1% silicone 200, 2% BDMA. After three changes of resin (1 hour each), the ovaries were polymerized at 50°C overnight then at 70°C overnight. Images were captured with a Phillips Tecnai 12 microscope and recorded with a GATAN multiscan CCD camera using Digital Micrograph software.

RESULTS

Visualizing individual *Drosophila* mitochondria using confocal microscopy

Mitochondria in developing female germ cells have been studied previously by electron microscopy (Mahowald and Strassheim, 1970; Carpenter, 1975) (Fig. 1B). To facilitate more detailed studies, we developed methods to visualize these organelles in fixed or living tissues using confocal microscopy. An antibody to *Drosophila* ATP synthase β-subunit was found to label mitochondria in both germline and somatic cells (Fig. 1C), as the labeled organelles appear similar in size and number to those revealed by electron microscopy. In addition, a mitochondrial-GFP marker ('mito-GFP') constructed by fusing the human COX VIII mitochrondrial targeting signal to the N terminus of EGFP and expressing the construct from the UASp promoter (see Materials and Methods) produced an indistinguishable pattern of labeling. By visualizing mitochondria within living ovarian tissue from strains expressing mito-GFP (Fig. 1D) or after incubation with fluorescent molecules such as 'Mitotracker' (Fig. 1E), we could study their dynamic behavior. Time-lapse confocal movies of mitochondrial behavior could be obtained using a special mounting technique and a spinning disc confocal microscope with a relatively low rate of sample bleaching (see Materials and Methods).

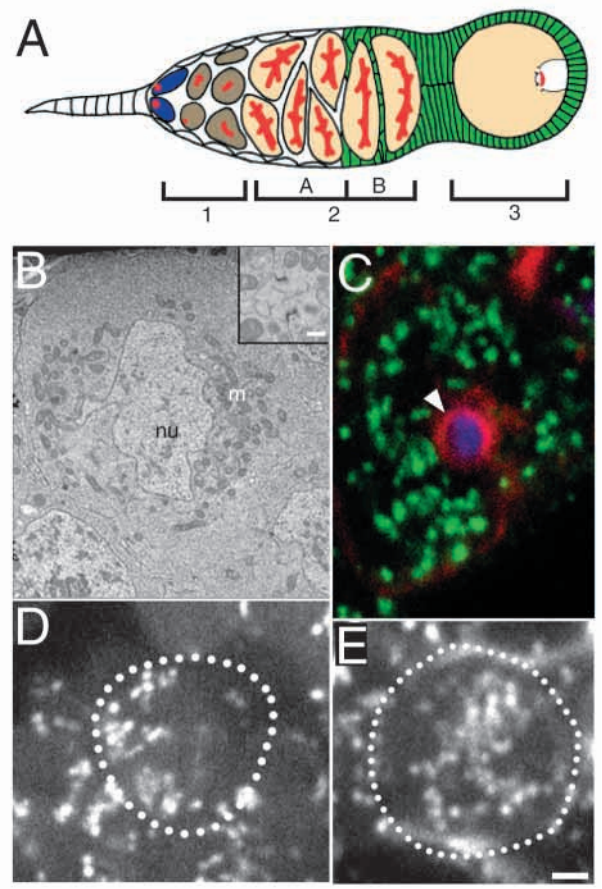

Fig. 1. High resolution detection of mitochondria. (A) The germarium in cross-section; at the anterior tip (region 1, left) reside two germline stem cells (blue) as well as cystoblasts and growing cysts (gray). Region 2a contains 16-cell germline cysts that enter meiosis and begin centrosome migration, while in region 2b, more mature cysts associate with follicle cells (green), and stretch to span the entire width of the germarium. Subsequently, in region 3, a new follicle with a well-defined oocyte (white) prepares to bud off. The fusome (red) grows, branches and eventually breaks down, leaving a remnant in the oocyte; it provides a distinctive marker for each stage. (B) A transmission electron micrograph showing the nucleus (nu) and mitochondria (m) of a region 1 cystocyte. The inset shows a cross-section of a ring canal containing a fusome plug surrounded by mitochondria. (C) Another region 1 cystocyte sectioned facing a ring canal (arrowhead): mitochondria (anti-ATP synthase) are green, the fusome (1B1 antibody) is blue and ring canal actin (rhodamine phalloidin) is red. (D,E) Mitochondria within living 16-cell cystocytes (broken circles) are labeled with the mito-GFP fusion gene (D) or using Mitotracker (E). Scale bars: in E, 1 μm for B-E; 100 nm in inset in B.

Mitochondria change shape and distribute equally during asymmetric stem cell and cystocyte divisions.

Germline stem cells (GSCs) and their progeny divide

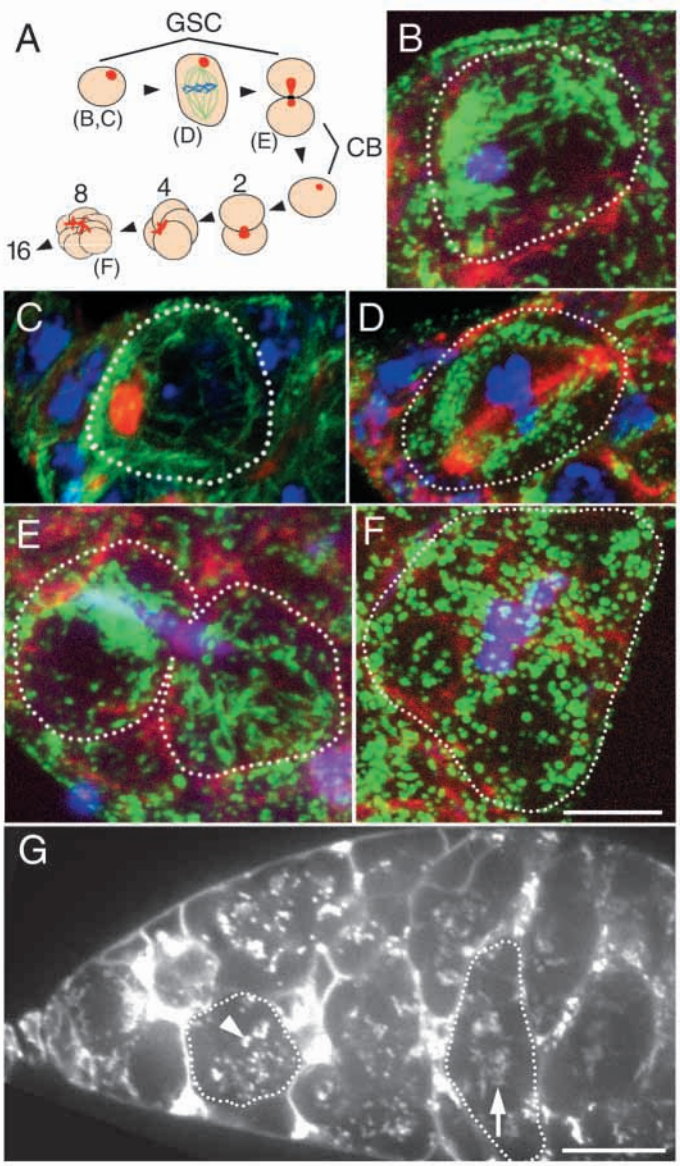

Fig. 2. Equal distribution of mitochondria during cyst formation. (A) Asymmetric fusome behavior during stem cell and cystocyte divisions. The fusome (red) remains in the stem cell (GSC) following mitosis (green, mitotic spindle; blue, chromosomes), while a smaller fusome grows in the cystoblast (CB) before cell separation is complete. The fusome branches during subsequent divisions that produce two-, four-, eight- and 16-cell cysts. The letters under each diagram correspond to figure panels illustrating the indicated stage. (B) A germline stem cell (broken circle) in G2 phase. Mitochondria (green) vary in length but most associate with the fusome (blue). Green, ATPsynthase; red, phalloidin; blue, 1B1. (C) Another GSC (broken circle), revealing the clustering of microtubules (green) around the fusome (red). Green, tubulin; red, 1B1; blue, DNA. (D) A mitotic GSC (broken circle). Round mitochondria associate with the outer region of the mitotic spindle (red) and segregate equally (see Table 1). Green, ATP synthase; red, tubulin; blue, DNA. (E) A stem cell (broken circle, left) and daughter cystoblast (broken circle, right) in S phase after mitosis remain connected via a ring canal (red) containing the fusome (blue). Mitochondria aggregate around the stem cell fusome (upper left), but show little or no association with the cystoblast fusome (lower right). Green, ATPsynthase; red, phalloidin; blue, 1B1. (F) Spherical mitochondria (green) within an eight-cell cyst (broken circle) do not associate with the fusome (blue). Green, ATPsynthase; red, phalloidin; blue, 1B1. (G) The anterior region of a living germarium labeled with Mitotracker to reveal the position and movement of mitochondria. Mitochondria in region 1 cysts do not associate with the fusome (arrowhead) while mitochondria in region 2 do (arrow). B-F are projections of multiple confocal *z*-sections. Scale bars: in F, 5 μm for B-F; 10 μm for G.

unequally during cyst production (Fig. 2A) (de Cuevas and Spradling, 1998). To learn if mitochondria were distributed unequally in forming cysts, we followed the behavior of mitochondria, fusomes and actin using specific antibodies. GSCs in G2 (Fig. 2B) contain between 100-200 unbranched, non-reticulate mitochondria that are about 0.35 μm in diameter and range in length up to 3 μm. Most stem cell mitochondria are preferentially located near the stem cell fusome (Fig. 2B, blue sphere) in the same position as most cellular microtubules (Fig. 2C). Were they to remain near the fusome during mitosis, mitochondria would be distributed very unevenly between the daughter cells, and there would be a strong bias for cystoblasts to acquire the mitochondria not bound to the fusome. However, we found that in stem cells, mitochondria fragment and associate with the outer edge of the mitotic spindle just prior to mitosis (Fig. 2D). Equal-sized daughter cells are produced that contain approximately the same number of mitochondria (Table 1). Mitochondria in the daughter stem cell elongate and re-associate with the fusome (Fig. 2E, left cell). By contrast, those in the cystoblast remain more spherical and distribute evenly throughout the cell cytoplasm (Fig. 2E, right cell). During the remaining cystocyte divisions, the mitochondria remain round and fusome-independent (Fig. 2F), but continue to associate with the spindle during mitosis. Thus, despite cytoplasmic asymmetries, mitochondria segregate equally into each cell of the 16-cell cyst.

Cystocytes fail to double in size prior to mitosis during their rapid, synchronous cell cycles. Consequently, the overall size of a newly completed cyst is only about fourfold larger than a stem cell, despite containing 16 times as many cells (King, 1970). To determine if mitochondria follow a similar pattern, we measured their number and shape within growing cysts. Mitochondrial number increases about eightfold and mitochondrial volume rises 14- to 20-fold during cyst formation, compared with only a 3.6-fold gain in total cytoplasm (Table 1). Hence mitochondrial capacity approximately doubles prior to each mitosis, despite the cleavage-like cystocyte cell cycle. Mitochondrial growth must be regulated differently than total cytoplasmic volume.

Mitochondria move along the fusome toward the oocyte in developing 16-cell cysts

Completed cysts continue to undergo programmed changes in their fusome and microtubule cytoskeletons that are central to oocyte development during the 2-4 days they spend traversing germarium region 2 (see Fig. 1A). To determine whether mitochondria are affected by these cytoskeletal changes, we next studied the subcellular location and movement of mitochondria within region 2 cysts (Fig. 3). These experiments revealed that many mitochondria in developing 16-cell cysts associate with the fusome, like centrioles. These mitochondria begin to localize along the branched arms of the

Table 1. Mitochondrial numbers and concentrations in germline cysts

Stage (number of cells)	Mitochondrial diameter (nm)*		Number of mitochondria/ cyst†	Number of mitochondria/ cell	Cyst cytoplasmic volume (μm^3)	Cell cytoplasmic volume‡ (μm^3)	Volume/ mitochondrion (μm^3)	Mitochondrial volume§/cell (μm^3)
	Major axis	Minor axis						
Germline stem cell (10)	n.d.	n.d.	140±32	140	1600	1600±520	0.20	28±12
Cystoblast (10)	n.d.	n.d.	150±23	150	1600	1600±630	0.27	40±17
Two-cell cyst (16)	n.d.	n.d.	280±46	140	2600	1300±110	0.096	27±17
Four-cell cyst (24)	430±43	340±36	410±72	100	3800	950±130	0.26	26
Eight-cell cyst (25)	460±30	380±29	810±31	100	4500	560±110	0.30	29
16-cell cyst (25)	550±36	460±34	1100±43	69	5800	360±99	0.53	36

*Mitochondrial diameters were measured by applying NIH image software to confocal images of individual mitochondria from germaria stained with ATP synthase.

†Mitochondrial number was determined using ATP synthase staining and confocal microscopy.

‡Cell cytoplasmic volume was determined by measuring major and minor axes, as outlined by phalloidin staining, and then subtracting the nuclear volume, as determined by either the region without Vasa staining or enclosed by Lamin staining. Calculations assumed the structures were ellipsoids. The cell radii were very similar to those we measured in our transmission electron micrographs. Cyst cytoplasmic volume was determined by extrapolation for any unmeasured cells.

§Mitochondrial volume was measured for germline stem cells, cystoblasts and two-cell cysts using confocal microscopy of germaria stained with ATP synthase and using NIH Image software. Total pixel volume was determined at the highest setting where labeling remained confined to mitochondria. For four-, eight- and 16-cell cysts, which contain ellipsoidal mitochondria, volume was calculated from the mitochondrial diameters.

Values without standard errors are calculated from the other data.

fusome in region 2a cysts (Fig. 3A). As cyst development proceeds, they move towards the center of the fusome while remaining tightly associated (Fig. 3B,C). In so doing, they follow the developmental behavior of microtubule minus ends and migrating centrioles (Grieder et al., 2000; Bolivar et al., 2001). However, the mitochondria are less centrally concentrated on the fusome in region 2b than microtubule minus ends, possibly because there is insufficient space to further concentrate such a large number of organelles. A small percentage of other mitochondria, indistinguishable in size and shape from those undergoing translocation, remain free in all the cystocytes.

Directed mitochondrial migration toward the center of the fusome was associated with significant reductions in random intracellular movement. *Drosophila* germaria were labeled using the fluorescent dye 'Mitotracker' (Fig. 2G) and movies that spanned up to 3 hours of developmental time were recorded with a confocal microscope (see Materials and Methods). At all stages of germ cell development, mitochondria unassociated with the fusome are mobile, and sometimes undergo bursts of directed motion within a cell (Fig. 2G, arrowhead). By contrast, mitochondria associated with the fusome in stem cells or developing cysts show little net movement within the cytoplasm (Fig. 2G, arrow). They remain on the fusome but their translocation toward its center occurs too slowly to be clearly visualized in these experiments.

Mitochondria are delivered along the fusome into the oocyte to form a Balbiani body

The translocating centrioles and microtubule minus ends enter the anterior of the oocyte at about the time the 16-cell cyst reaches region 3 of the germarium and prepares to bud. We observed that the translocating mitochondria in cysts just prior to region 3 form aggregates or 'clouds' at the ring canals that connect the last four nurse cells to the oocyte (Fig. 3C, arrowhead). Shortly thereafter, these mitochondrial clusters enter the oocyte, where they coalesce into a single mass anchored at the large segment of fusome in the anterior of the cell (Fig. 3D, arrow). We term this mitochondrial mass a Balbiani body because it appears very similar in the light and electron microscope (Fig. 3E, arrow) to the Balbiani bodies described in the early oocytes of other species (Raven, 1961; Guraya, 1979). Moreover, as in many other species, we found that the *Drosophila* Balbiani body persists in the oocyte near the germinal vesicle during early follicle development (see below).

An asymmetric fusome is essential for oocyte determination and for all known aspects of cyst and follicle polarity. To ask whether the fusome is functionally required for Balbiani body formation, we studied the behavior of mitochondria in *huli-tai-shao* (*hts*) mutant flies. *hts* encodes homologs of the mammalian spectrin-binding protein Adducin, and is required to maintain the structural integrity of the fusome (Lin et al., 1994) and to organize microtubules in region 2 cysts (Grieder et al., 2000). We found that in contrast to wild type, *hts* mutant cysts contain mitochondria that are broadly distributed (Fig. 3F,G). Mitochondrial clumps remain around presumptive centrosomes in stem cells (Fig. 3F, arrowheads), but this is expected because *hts* flies retain functional centrosomes and microtubules. Thus, the organized movement of mitochondria into the oocyte and their association into a Balbiani body requires an intact fusome.

To investigate the relationship between Balbiani body formation and oocyte determination, we analyzed mitochondria in *egalitarian* (*egl*) mutant females. *egl* mutants initially specify all 16 cyst cells as oocytes, but fail to maintain the oocyte fate, so that all the cells eventually differentiate as nurse cells (Schupbach and Wieschaus, 1991). Mitochondria behave normally in dividing *egl* cysts (data not shown). However, in 16-cell cysts, they do not localize into a single Balbiani body, even though centrosomes migrate along the fusome and end up in one cell (Bolivar et al., 2001). Instead, most of the mitochondria in each cystocyte aggregate on the fusome remnants at the ring canals (Fig. 3H). These mitochondrial aggregates are retained and grow in size during subsequent follicle development (not shown).

Balbiani bodies contain Golgi vesicles

Balbiani bodies in other organisms contain additional organelles and vesicles besides mitochondria (see Raven, 1961). Electron micrographs of *Drosophila* Balbiani bodies in early region 3 oocytes revealed the presence of Golgi (Fig. 4A),

ER-like vesicles in the residual fusome (Fig. 4A,B) and centriole clusters (Fig. 4B,C). Using anti-α-mannosidase 2 antibody as a marker, we confirmed that Golgi are present by confocal microscopy (Fig. 4D). Furthermore, we found that Golgi elements arrive by much the same pathway as mitochondria. Golgi vesicles begin to associate with the fusome in region 2 (arrowhead), become enriched near its center, and move into the oocyte in stage 1 (arrow). The α-mannosidase 2-positive vesicles remain near the fusome and Balbiani body within the oocyte anterior early in region 3, but soon disperse throughout the oocyte cytoplasm. Some of the Golgi vesicles located in region 2 cysts never associate with the fusome or move to the oocyte, but remain scattered throughout the 15 nurse cells.

A subset of mitochondria may be enriched in the Balbiani body

To investigate whether more than one population of mitochondria might be present in pre-follicular cysts, we examined the staining of a variety of other antibodies to mitochondrial proteins. We observed that crossreacting antibodies against human cytochrome c_1 oxidase subunit I (COXI) label round cytoplasmic structures that associate with the fusome in region 2 and move into the oocyte in stage 1, much like mitochondria and Golgi vesicles (Fig. 4E). Double-labeling experiments showed that the small COXI reactive particles represent a subset of germ cell mitochondria because they co-localize with ATP-synthase staining (Fig. 4F) but are distinct from Golgi (Fig. 4G). However, it was not clear what property might set these mitochondria apart from others. Very few mitochondria in region 1 or in somatic cells were labeled by the anti-COXI antisera, and it was difficult to explain the pattern of labeling based on signal strength or accessibility to staining (somatic cells should be more accessible). Moreover, two other antisera, anti-human COXII and anti-yeast porin, produced a similar pattern. These results suggest that some of the mitochondria entering the oocyte in the Balbiani body have distinctive properties.

Balbiani bodies are associated with localized RNAs and proteins

In *Xenopus* the 'METRO' region of the Balbiani body associates with RNAs that will later become localized in germ plasm at the vegetal pole (Forristall et al., 1995; Kloc and Etkin, 1995), and similar links between Balbiani bodies and germ plasm constituents have been noted in diverse species (Kobayashi and Iwamatsu, 2000; Tsunekawa et al., 2000; Bradley et al., 2001). Consequently, we investigated whether *Drosophila* mRNAs that are known to localize to the early oocyte and participate in germ plasm formation (reviewed by Saffmann and Lasko, 1999) associate with the *Drosophila*

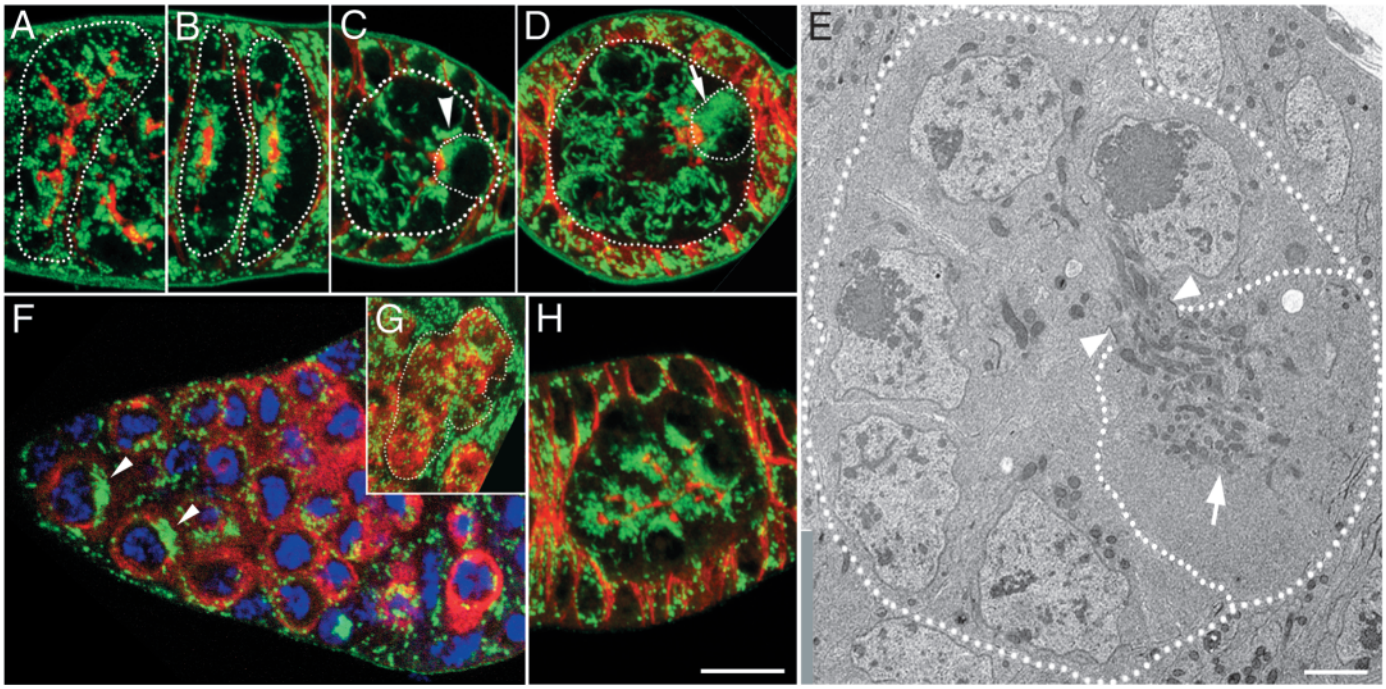

Fig. 3. Fusome-dependent formation of a Balbiani body. (A-D) Green, mitochondria (ATPsynthase); red, fusome (1B1). (A) Mitochondria in a 16-cell cyst (broken outline) midway in region 2a begin to associate with the fusome (red); in an older adjacent cyst (not outlined), they move toward the center of the fusome (yellow). (B) Mitochondrial movement along the fusome has progressed further in these two region 2b cysts (broken outline). (C) In cysts just entering region 3, clouds of mitochondria (arrowhead) accumulate near the ring canals that connect to the oocyte (small broken circle). (D) In region 3 follicles and young budded egg chambers, a Balbiani body containing many aggregated mitochondria (arrow) is visible in the anterior of the oocyte (small broken circle). (E) Electron micrograph of a region 3 follicle (large outline) reveals mitochondria entering the oocyte (small outline) via a ring canal (arrowheads) to form the Balbiani body (arrow). (F,G) Balbiani bodies fail to form in cysts from *hts* mutant females that lack fusomes. Arrowheads show mitochondrial clusters around presumptive centrosomes in the stem cells. Green, mitochondria (ATPsynthase); red, germ cells (Vasa); blue, DNA. (G) A stage 1 *hts* cyst (broken outline) showing the absence of a Balbiani body or mitochondrial clusters near presumptive ring canals. (H) Cysts mutant for egalitarian (*egl*) contain a normal fusome (red) but mitochondrial aggregates (green) of reduced size arise in all 16 cells at stage 1. Green, mitochondria (ATPsynthase); red, fusome (1B1). Scale bars: in H, 10 μm for A-D,F-H; in E, 2 μm for E.

Balbiani body using antibody/in situ hybridization double labeling experiments. We found that *osk* mRNA is associated with the center of the fusome as soon as it can be detected in region 2a cysts (Fig. 5A). This is before centrosomes and microtubules begin to move to the center. *osk* mRNA remains on the fusome of the pro-oocyte where it associates with the newly formed Balbiani body (Fig. 5B). In oocytes at this stage (Fig. 5B and 5B′), the fusome (blue, arrowhead) is the most anterior structure, followed by the mitochondria (red, arrow), and finally the RNA (green). The *osk* RNA (Fig. 5B, green) lies partially within the Balbiani body (Fig. 5B, yellow line) and partially outside its posterior side. However, this state is transient, as the RNA moves to the oocyte posterior by the end of stage 1 (Fig. 5A, rightmost cyst). *orb* RNA also localizes on the central fusome and associates with the Balbiani body in early stage 1 cysts in a similar manner (Fig. 5C-D′), although it may associate with a slightly larger region of the fusome. While it was known previously that *hts* is required to localize *orb* and *osk* RNAs to a single cystocyte in region 2 (de Cuevas et al., 1996; Deng and Lin, 1997), these are the first indications that localized RNAs directly associate with the fusome.

Several proteins, including Orb and Cup, also localize within the future oocyte in developing region 2-3 cysts. We examined the relationship between these proteins and the Balbiani body using specific antibodies (Fig. 5E-I). Cup protein (Fig. 5E, red) is located on and adjacent to the central fusome (Fig. 5E, blue) within the pro-oocyte in late region 2b cysts, near the clustered mitochondria (Fig. 5E, green). As the mitochondria enter the ooctye early in region 3 to form a Balbiani body, Cup associates briefly along the posterior edge (Fig. 5H) but soon moves towards the oocyte posterior (Fig. 5F). The behavior of Orb protein is very similar at these stages (Fig. 5G,I), but Orb is always slightly more diffuse. These proteins may be associated with some of the localized mRNAs that follow a similar path. Our experiments show that at least for a brief period the *Drosophila* Balbiani body is regionally organized, with fusome material located at the anterior and specific RNAs and proteins located toward the posterior.

Balbiani body-derived mitochondria preferentially associate with the germ plasm

The previous experiments indicate that *Drosophila* eggs

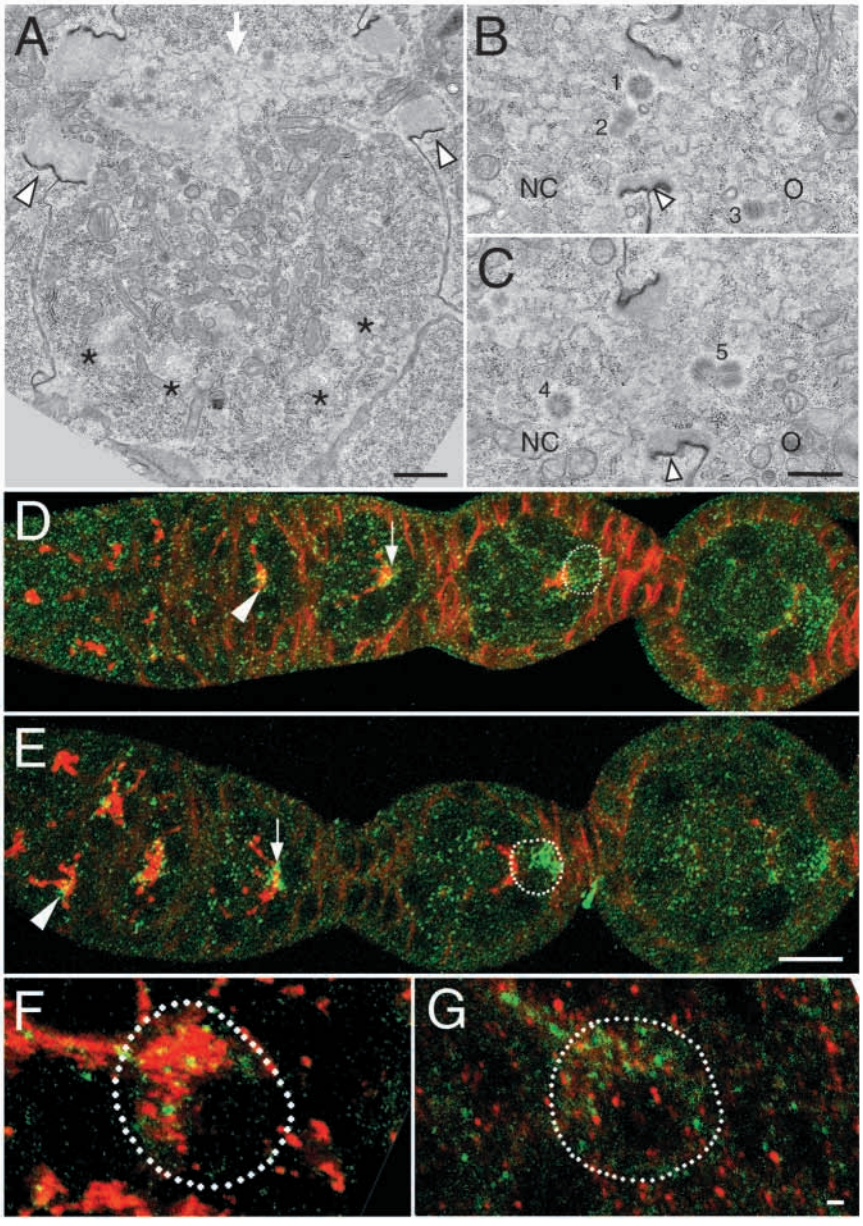

Fig. 4. Multiple organelles associate with the Balbiani body. (A) An electron micrograph of the Balbiani body in the anterior region of a young oocyte. The residual fusome (white arrow), still rich in ER-like vesicles, lies at the anterior near the ring canals (white arrowheads) it formerly occupied. Numerous mitochondria are located adjacent to the fusome, while scattered Golgi stacks lie further below (asterisks). (B,C) Electron micrographs showing nearby sections of a single ring canal (white arrowhead) connecting a nurse cell (NC) and a young ooctye (O). Five centrioles or centriole pairs (numbered) lie within the material moving in to form the Balbiani body. (D) The anterior portion of an ovariole stained with anti-α-mannosidase 2 to reveal Golgi vesicles (green) and with 1B1 to show the fusome (red). The Golgi elements associate with the fusome beginning in region 2b (yellow, arrowhead), move into the oocyte with the Balbiani body (arrow), but in a slightly older oocyte (broken line) spread throughout the ooplasm. (E) COXI-reactive vesicles (green) associate with the fusome (red) in region 2b (yellow, arrowhead) and move into the oocyte as part of the Balbiani body (arrow). (F) COXI-positive particles (green) appear to be mitochondria because they are co-labeled with the mitochondrial ATP synthase marker (red). Broken circle, oocyte. (G) COXI-positive particles (green) are not co-labeled with a Golgi marker (red, α-mannosidase 2). Broken circle, oocyte. Scale bars: in E, 10 μm for D,E; in A, 1 μm for A; in C, 1 μm for B,C; in G, 1 μm for F,G.

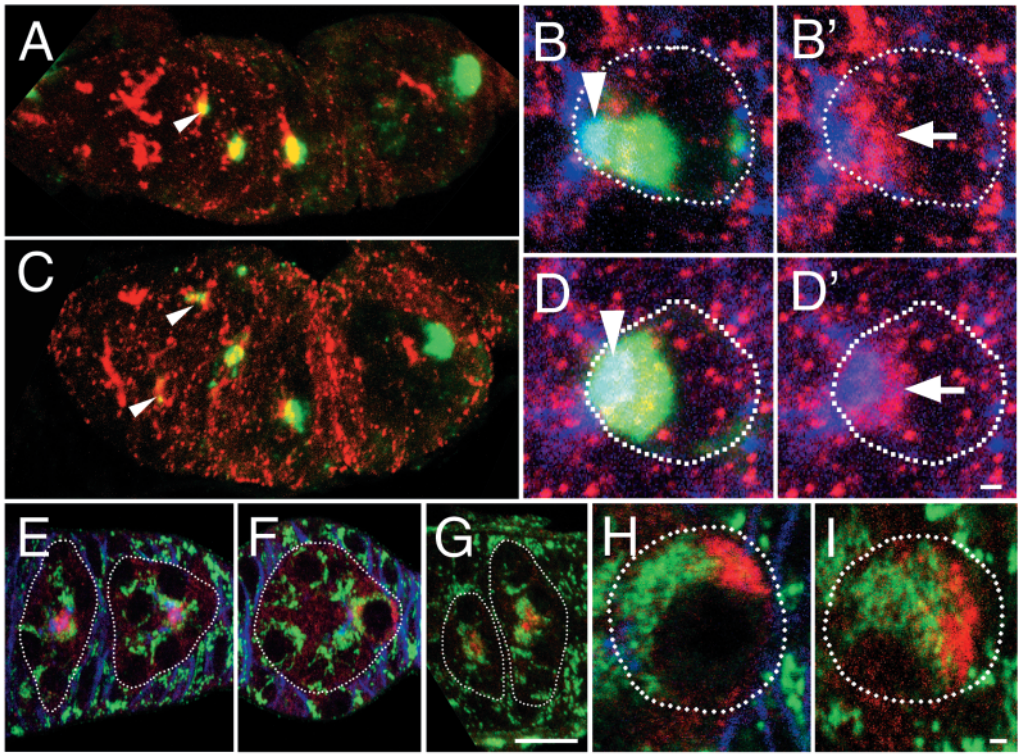

Fig. 5. Localized RNAs and proteins associate with the fusome and Balbiani body. (A) In situ hybridization reveals that *osk* RNA (green) associates with the central fusome (red) within the future oocyte beginning in region 2 (arrowhead). The RNA moves to the posterior of the oocyte during stage 1 (rightmost follicle). (B) An early region 3 oocyte (broken line) shown at higher magnification, reveals that *osk* RNA (green) partially overlaps (yellow) the Balbiani body on the posterior side of the mitochondrial mass (red, anti-ATP synthase). The arrowhead indicates the fusome (blue, 1B1). B′ is the same image as B without the green channel, in order to better show the Balbiani body (arrow). (C) *orb* RNA (green), like *osk* RNA, associates with the center of the fusome (red) beginning early in region 2 (arrowheads), and then moves to the oocyte posterior in stage 1 (rightmost follicle). (D) Like *osk* RNA, *orb* RNA (green) also overlaps with the Balbiani body (red). D′ is the same as D without the green channel, in order to better show the Balbiani body (arrow). (E,F,H) Cup protein (red) associates with the center of the fusome (blue) and with mitochondria (green) in region 2 cysts. (G,I) Orb protein (red) localizes to a similar position as Cup (green, mitochondria). (H,I) Both Cup (red, H) and Orb (red, I) briefly associate with the edge of the Balbiani body mitochondria (green) in early region 3 oocytes and then move to the oocyte posterior (not shown). Scale bars: in G, 10 μm for A,C,E-G; in D′ 1 μm for B,B′,D,D′; in I, 1 μm for H,I.

acquire mitochondria from two sources. Oocytes receive an initial consignment of these organelles from the Balbiani body at the time of follicle formation. However, a large number of mitochondria are also transported into the oocyte from the nurse cells much later in oogenesis, during nurse cell dumping in stage 11. These dual sources raise the question of which mitochondrial subpopulation furnishes the organelles that associate with the germ plasm and whose genomes found the mitochondrial DNA of subsequent generations. To investigate whether Balbiani body mitochondria are used preferentially in constructing germ plasm, we studied the behavior of both mitochondrial populations during follicle development using mito-GFP.

These studies revealed that Balbiani body-derived mitochondria preferentially associate with forming germ plasm. We could be sure of this because we found that the nurse cell mitochondria are blocked from moving through ring canals into the oocyte before nurse cell dumping, well after germ plasm has formed. This conclusion is based on studies of the movement of nurse cell mitochondria into the oocyte using movies of mito-GFP expressing follicles of various ages (Table 2). Before stage 11 nurse cell mitochondria build up in large masses just outside the oocyte ring canals instead of moving into the oocyte (Fig. 6B, arrowheads). Only at the time of nurse cell dumping do nurse cell-derived mitochondria enter the oocyte.

To examine when Balbiani body-derived mitochondria begin to associate with the germ plasm, we carried out double labeling experiments using germ plasm markers. In early follicles, the Balbiani body mitochondria remain clustered around the germinal vesicle, often at its posterior (Fig. 6A). After the microtubules re-organize at stage 7, Balbiani body mitochondria disperse throughout the cytoplasm (Fig. 6B). By stage 9, they begin to contact the forming germ plasm at the posterior pole (Fig. 6C). Later, in stage 10A, many more

Table 2. Nurse cell mitochondria do not enter the oocyte until stage 11

	Rate of ring canal transit*	Ooplasmic streaming†
Stage 8	0.1/minute	0/10
Stage 9	1.5/minute	0/7
Stage 10A/B	3.6/minute	1/8
Stage 11‡	⩾60/minute	4/4

*Mitochondrial movement through the ring canals was measured by counting the number of organelles that crossed the nurse cell/oocyte boundary in movies of the indicated stages during a 5 minute period. No movement was seen prior to stage 8, and the measured rates prior to stage 11 probably overestimate true nurse cell/oocyte transfer because some organelles only appeared to cross the boundary because of the twisting of the egg chamber in the observation chamber, rather than because of true translocation.

†The number of egg chambers with ooplasmic streaming (see Materials and Methods)/the total number of egg chambers analyzed at the indicated stage.

‡The measured rate during stage 11 (dumping) strongly underestimates the true value of mitochondrial translocation because individual frames required 2-3 seconds of exposure, during which time most mitochondria became blurred and could not be followed.

mitochondria associate with the expanded region containing germ plasm (Fig. 6D,D′, arrowheads). Although these studies show a gradual association of Balbiani body mitochondria with the forming germ plasm beginning in stage 9 and 10A, we cannot rule out that a small subpopulation of these mitochondria, such as those labeled by COXI antisera, associate with germ plasm precursors at an earlier time. At stage 11, most of the oocyte cytoplasm begins to undergo vigorous cytoplasmic streaming, mixing the two mitochondrial populations (Table 2). However, streaming does not dislodge the germ plasm, so the Balbiani body-derived mitochondria in this location are likely to remain associated at the oocyte posterior and eventually be inherited by forming germ cells.

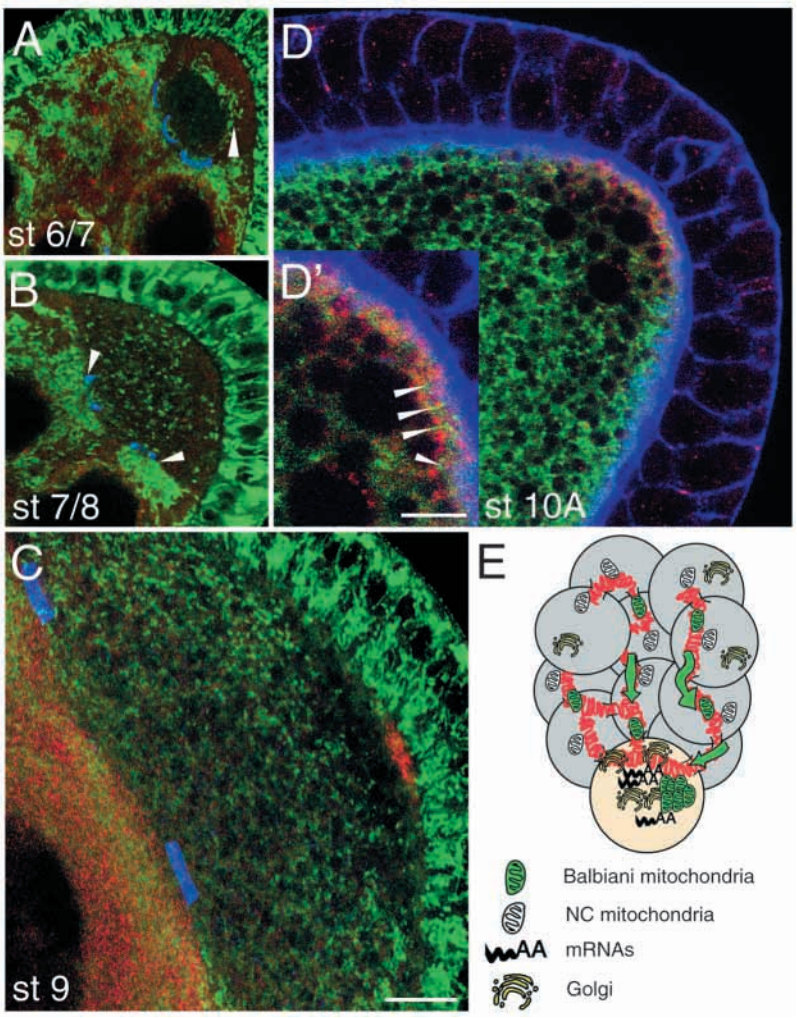

Fig. 6. Balbiani body-derived mitochondria associate with the germ plasm prior to nurse cell dumping. (A) Mitochondria labeled with anti-ATP synthase (green) congregate on the posterior side of the GV (arrowhead) in stage 6-7 egg chambers. Green, ATP synthase; red, germ cells (Vasa); blue, ring canals (anti-Phosphotyrosine). (B) By contrast, mitochondria (green) in the nurse cells adjacent to the oocyte accumulate (arrowheads) at the ring canals (blue), but fail to enter the oocyte until nurse cell dumping (see Table 2). Green, ATPsynthase; red, Vasa; blue, phosphotyrosine. (C) At stage 9, a few mitochondria (green) associate with the germ plasm, which has begun to coalesce at the oocyte posterior as revealed by Vasa staining (red). Green, ATPsynthase; blue, Phosphotyrosine. (D,D′). By stage 10A, mito-GFP-labeled mitochondria (green) are abundant in the germ plasm (arrowheads in D′). D′ shows a higher magnification. Green, mito-GFP; red, Vasa; blue, actin-rich membranes (phalloidin). (E) A summary of the structure and assembly of the *Drosophila* Balbiani body. Green arrows indicate direction of movement along the fusome (red) towards the oocyte. Scale bars: in C, 10 μm for A-D; in D′, 5 μm for D′.

DISCUSSION

Tools for a genetic analysis of mitochondrial inheritance

Our experiments revealed that mitochondria behave in a distinctive but previously unknown manner during *Drosophila* oogenesis. They associate with the spindle to facilitate equal inheritance, fragment into small spheres and associate with the fusome, damping their normal intracellular movement. One major subpopulation moves along the fusome to form an oocyte Balbiani body, while another group is blocked from entering the oocyte by ring canals. Each of these biological events can now be visualized and analyzed dynamically using the spinning disk confocal microscope, which will make it possible to screen for genes that developmentally control these behaviors.

Drosophila oocytes contain a Balbiani body

Drosophila oocytes were found to contain a typical Balbiani body at the time follicles form in region 3 of the germarium. In a wide range of animal species, including *Xenopus* (Heasman et al., 1984), chick (Ukeshima and Fujimoto, 1991), mouse (Pepling and Spradling, 2001) and human (Hertig and Adams, 1967), young oocytes at a similar developmental stage display these distinctive aggregates of mitochondria and other organelles near their germinal vesicles (see Raven, 1961). In a typical Balbiani body, centrioles and associated cytoplasm are surrounded by a ring of Golgi bodies and encased in a large mass of mitochondria. As the oocyte grows, the mitochondria first spread around the nuclear periphery and later disperse throughout the oocyte cytoplasm. *Drosophila* Balbiani bodies, like those described in other species, were shown to contain clustered mitochondria, Golgi vesicles and centrioles. Moreover, as young follicles develop from stage 1-6, the mitochondria move around the germinal vesicle and disperse after microtubules re-organize in stage 7. The fact that Balbiani bodies occur in a genetically tractable system will now make it easier to decipher the function of these enigmatic structures, which have been postulated to play an early role in RNA transport and in patterning the mammalian egg (de Smedt et al., 2000).

The Balbiani body is assembled by regulated movement of organelles along the fusome

The studies reported here provide new insight into the origin of Balbiani bodies. *Drosophila* Balbiani bodies do not arise de novo within oocytes, but are built by the transport of organelles

from neighboring cells within interconnected germline cysts. Our experiments make clear that many components of oocyte cytoplasm arise in this manner. Centrioles were the first cytoplasmic organelle whose movement through ring canals into the oocyte in 16-cell germarial cysts was described (Mahowald and Strassheim, 1970). Studies of serial sectioned germaria in the electron microscope also suggested that mitochondria move between cystocytes and toward the oocyte in region 2b cysts (Mahowald and Strassheim, 1970; Carpenter, 1975; Carpenter, 1994). However, despite a description of the mitochondrial cluster in the region 3 oocyte (Mahowald and Strassheim, 1970) and the proposal that it arose from transport, these movements were not commonly viewed as a special process. Rather, they were seen as just the start of an extended process of generalized 'cytoplasmic flow' from nurse cell to oocyte that formed an ongoing 'nutrient stream' responsible for the growth of the oocyte relative to the nurse cells throughout oogenesis. (Little such growth takes place in the germarium.) Nonetheless, these studies described the onset of mitochondrial movement, their transient association with downstream ring canals, and the fact that mitochondria appear to be constricted in diameter as they pass through ring canals.

Our experiments document that virtually all of the newly formed mitochondria in oocytes are derived from the Balbiani body. The great majority are transported from other cystocytes along the fusome but 1/16th or more might simply originate in the oocyte. Like oocyte determination itself, Balbiani body formation was shown to depend on the fundamental cyst polarity manifested in the fusome. Arising in embryonic germ cells (Lin and Spradling, 1997), the fusome builds up a framework of cyst polarity during the cystocyte divisions (Lin and Spradling, 1995; de Cuevas and Spradling, 1998). Fusome polarity probably acts directly to control centriole migration (Grieder et al., 2000; Bolivar et al., 2001) and the meiotic gradient (Huynh and St. Johnston, 2000), and acts indirectly to differentiate and maintain the oocyte by regulating the microtubule cytoskeleton (Grieder et al., 2000; Huynh et al., 2001). Deciphering the molecular mechanisms that define fusome polarity and allow the fusome to control microtubule organization remains a central issue for understanding Balbiani body formation and oocyte development.

Oocytes develop from germline cysts or syncytia in diverse species (reviewed by Pepling et al., 1999) so Balbiani bodies may arise through intercellular transport in a wide range of organisms besides *Drosophila*. In both *Xenopus* (Al-Mukhtar and Webb, 1971; Kloc and Etkin, 1995) and the mouse (Pepling and Spradling, 2001), mitochondrial clouds present within interconnected germ cells are thought to be precursors to the Balbiani bodies that arise shortly after the cysts break down and form primordial follicles. In *Drosophila*, the large chunk of fusome at the anterior of the early stage 1 oocyte contains clustered centrioles and is likely to act as a microtubule-organizing center (Grieder et al., 2000). It may attract and retain mitochondria, Golgi and localized macromolecules as they enter the oocyte, thereby creating the Balbiani body. *Xenopus* Balbiani bodies may arise in a similar fashion as they have a similar organization consisting of a spectrin-rich zone, mitochondria, Golgi and the Metro region containing RNAs in transit. However, there has been insufficient study of the *Xenopus* larval ovary to identify a fusome or some other material with microtubule organizing properties that might play an analogous role. In most other systems whose Balbiani bodies share the same basic structure in young oocytes, very little is known about their origin during earlier stages of germ cell development.

The Balbiani body may facilitate RNA localization

The Balbiani bodies in many species contain structures resembling germinal granules. In *Xenopus*, these granules are found in a region containing specific RNAs that are also destined to be localized in the egg and incorporated in germ cells. Consequently, the Balbiani body has been proposed to function as a messenger transport organizer (METRO) that organizes and mediates the delivery of RNAs and germinal granules to the vegetal pole of the egg (Forristall et al., 1995; Kloc and Etkin, 1995; Kloc et al., 1998). Specific elements have been mapped in the 3′ UTR of the *Xcat2* mRNA that are sufficient for localization to the Balbiani body (Zhao and King, 1996) or to the germinal granules themselves (Kloc et al., 2000).

Our studies revealed that the *Drosophila* Balbiani body may play a related role. *oskar* RNA, a key component that is capable of inducing germ plasm formation, was associated with the posterior segment of the Balbiani body in early stage 1 oocytes, much as *Xcat2* is localized in the *Xenopus* Balbiani body. A few hours later, towards the end of stage 1, *osk* RNA moves to the oocyte posterior along with the other Balbiani-associated RNAs and proteins we studied, presumably in response to the shift in microtubule polarity that occurs at this time. Thus, at least some molecules that participate in germ plasm assembly associate with the Balbiani body in early *Xenopus* and *Drosophila* oocytes.

We found that *Drosophila* RNAs that become associated with the Balbiani body, like organelles, first interact with the fusome during early stages of cyst development. However, there were significant differences in these fusome interactions that probably reflect different molecular mechanisms of delivery to the Balbiani body. Organelles associate next to the fusome along much of its length and subsequently move toward the center, in concert with microtubule minus ends. By contrast, the RNAs associate with one or a few cells at the center of the fusome from the earliest stages they could be detected, and are located within it, as well as nearby. These observations suggest that localized RNAs may read the fusome polarity directly, and need not rely on changes in microtubule organizing activity to get to the oocyte or be stabilized within it.

Potentially significant differences exist in the role of RNA transport played by the *Drosophila* and *Xenopus* Balbiani bodies. The *Drosophila* Balbiani body associates with germ plasm RNAs for only 5-10 hours during early stage 1. By contrast, *Xenopus* Balbiani bodies associate throughout stage 1 of oogenesis, a process requiring many days, with at least 11 RNAs. When the RNAs leave the *Drosophila* Balbiani body, mitochondria mostly remain behind, only to follow much later in oogenesis. By contrast, in *Xenopus*, both mRNAs and mitochondria are reported to proceed together to the vegetal pole (Kloc and Etkin, 1995). These differences may simply reflect differences in the timing of cytoskeletal remodeling that control these events. Moreover, our observation that a small subset of mitochondria recognized by COXI antisera do translocate with the RNAs in stage 1 indicates that certain

Drosophila mitochondria may follow a *Xenopus*-like pattern. However, it remains possible that RNAs in transit to the oocyte posterior may simply pass through the Balbiani body without begin affected in any way.

Previously, Wilsch-Bräuninger et al. (Wilsch-Bräuninger et al., 1997) described sponge-like structures in the cytoplasm of stage 4-10 nurse cells that were associated with Exu protein, RNA, and (frequently) mitochondria and nuage. They proposed that these structures were analogous to classical Balbiani bodies and that they mediate transport of localized transcripts such as *bicoid* RNA. Our results suggest that the ooctye contains a true Balbiani body much earlier – in stage 1 follicles. The sponge bodies described by Wilsch-Bräuninger et al. (Wilsch-Bräuninger et al., 1997) more likely represent transport complexes organized at the surface of nurse cell nuclei that subsequently move through the follicle and into the ooctye. However, there may be structural and molecular similarities between nurse cell transport complexes and those mediating transport out of the Balbiani body.

Ring canal behavior and fusome breakdown controls mitochondrial movement

Our studies provide further evidence that the ring canals that join the cystocytes play an important role in regulating Balbiani body formation. Mitochondria appear to first enter the oocyte when fusome segments within the adjoining ring canals break apart, unplugging the channels. Subsequently, a novel mechanism blocks further mitochondrial passage through these canals, because we observed large backups of mitochondria outside each oocyte ring canal in young oocytes and documented a lack of mitochondrial movement into the oocyte in movies. Mitochondria did not accumulate in the same manner around the ring canals that join nurse cells, but were spread throughout the cell and in the nuclear periphery. This behavior has the effect of limiting the mitochondrial genotypes within the oocyte to those found in Balbiani body mitochondria until well after mitochondria have begun to associate with the germ plasm at the oocyte posterior pole. Despite the importance of these regulatory steps, we still know very little about how movement through ring canals is controlled.

Fusome-mediated transport may be selective

Our studies suggest that centrioles, mitochondria, Golgi, RNAs and other key components of oocyte cytoplasm are added to the *Drosophila* oocyte by a special mechanism that may have been widely conserved in evolution. It is remarkable that in the oocyte, the lone cell that will contribute cytoplasm for the next generation of organisms, many fundamental components of cytoplasm do not arise by random partitioning among daughter cells. Rather, an elaborate mechanism is used to transport materials from multiple cells and maintain them in a large aggregate for an extended period of time. It is possible that Balbiani bodies do not play a specific role in ooctye development, but represent a byproduct of the unusual centrosome behavior in these cells. However, we favor an alternative hypothesis.

One of the potentially most interesting reasons that oocyte organelles might be delivered en mass via the fusome would be to increase organelle fitness (Pepling et al., 1999; Pepling and Spradling, 2001). Mitochondrial DNAs are known to accumulate mutations that have frequently been postulated to affect the aging of cells and tissues (Chinnery and Turnbull, 2001) (reviewed by Partridge and Gems, 2002). If only mitochondria with functional genomes were able to associate with the fusome and move into the oocyte, damaged genomes might be weeded out when they still represent a small fraction of the total. Such a system would be far more efficient than eliminating defective genomes by inducing the apoptosis of entire germ cells. A purifying mechanism based on organelle selection might be particularly important in organisms that need to produce eggs with a high average viability, or that must support long intergenerational life spans.

Several other observations may also be explained by the need to eliminate defective mitochondrial genomes. The exclusion of nurse cell mitochondria from passing through the oocyte ring canals prior to dumping would ensure that only the 'selected' mitochondria in the Balbiani body populated the germ plasm. Mitochondria may break up into small, nearly round, organelles during this period so that each will contain a single genome whose fitness can be tested. The cytoplasmic streaming of the ooctye may serve to mix the two populations of organelles so each somatic cell type inherits at least some of the selected mitochondrial population. Finally, a requirement for translation on mitochondrial ribosomes in the early embryonic germ plasm might serve as a concluding selective step to ensure that viable germ cells are well supplied with intact mitochondrial genomes. If female germ cells do possess mechanisms to remove defective mitochondria, they would probably have contributed to the evolutionary conservation of germ line cysts and Balbiani bodies.

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