A Sponge-like Structure Involved in the Association and Transport of Maternal Products during *Drosophila* Oogenesis

Michaela Wilsch-Bräuninger, Heinz Schwarz, and Christiane Nüsslein-Volhard

Max-Planck-Institut für Entwicklungsbiologie, D-72076 Tübingen, Germany

Abstract. Localization of maternally provided RNAs during oogenesis is required for formation of the antero–posterior axis of the *Drosophila* embryo. Here we describe a subcellular structure in nurse cells and oocytes which may function as an intracellular compartment for assembly and transport of maternal products involved in RNA localization. This structure, which we have termed "sponge body," consists of ER-like cisternae, embedded in an amorphous electron-dense mass. It lacks a surrounding membrane and is frequently associated with mitochondria. The sponge bodies are not identical to the Golgi complexes. We suggest that the sponge bodies are homologous to the mitochondrial cloud in *Xenopus* oocytes, a granulo-fibrillar structure that contains RNAs involved in patterning of the embryo.

Exuperantia protein, the earliest factor known to be required for the localization of *bicoid* mRNA to the anterior pole of the *Drosophila* oocyte, is highly enriched in the sponge bodies but not an essential structural component of these. RNA staining indicates that sponge bodies contain RNA. However, neither the intensity of this staining nor the accumulation of Exuperantia in the sponge bodies is dependent on the amount of *bicoid* mRNA present in the ovaries. Sponge bodies surround nuage, a possible polar granule precursor. Microtubules and microfilaments are not present in sponge bodies, although transport of the sponge bodies through the cells is implied by their presence in cytoplasmic bridges. We propose that the sponge bodies are structures that, by assembly and transport of included molecules or associated structures, are involved in localization of mRNAs in *Drosophila* oocytes.

THE antero–posterior axis of the *Drosophila* embryo is determined by the localization of maternal mRNAs in the egg. Two groups of genes are involved in the localization of the RNAs and thereby initiate the formation of the segmented regions along the antero–posterior axis: the anterior system is necessary for the formation of head and thoracic structures, whereas the posterior system is responsible for abdomen formation and for assembly of the germ plasm. Embryos from females homozygous mutant for any member of these two groups of genes show pattern defects in the respective body regions (for review see St Johnston and Nüsslein-Volhard, 1992).

The key event in the anterior system is the localization of the *bicoid* (*bcd*)[1] mRNA to the anterior pole, which results in a graded distribution of the transcription factor Bicoid throughout the embryo (Frigerio et al., 1986; Frohnhöfer and Nüsslein-Volhard, 1986; Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988*a*,*b*; Driever and Nüsslein-Volhard, 1989; St Johnston et al., 1989). Three genes, *exuperantia* (*exu*), *swallow*, and *staufen*, are involved in different steps in the localization of the *bcd* mRNA (Berleth et al., 1988; Stephenson et al., 1988; St Johnston et al., 1989).

In addition to its role in the formation of the anterior pattern, *staufen* is required for the localization of the *oskar* (*osk*) mRNA to the posterior pole of the embryo, which is necessary for abdominal segmentation (Ephrussi et al., 1991; Kim-Ha et al., 1991). A second requirement for *osk* mRNA localization exists in the formation of the germ plasm at the posterior pole (Lehmann and Nüsslein-Volhard, 1986). The germ plasm is characterized by the presence of electron-dense polar granules that contain Osk protein (Mahowald, 1962, 1992; Breitwieser et al., 1996). An additional factor shown to be present in the polar granules is Vasa, one of the members of the posterior system, which directly interacts with Oskar protein (Breitwieser et al., 1996; Hay et al., 1988*a*,*b*). In addition to its location in the polar granules, Vasa is concentrated in the nuage (Hay et al., 1988*a*,*b*; Liang et al., 1994). This granular structure of the

Address all correspondence to Michaela Wilsch-Bräuninger, Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35/III, D-72076 Tübingen, Germany. Tel.: (07 071) 601 490. Fax: (07 071) 601 384. E-mail: mibr@gen.mpib-tuebingen.mpg.de

1. *Abbreviations used in this paper*: *bcd*, *bicoid*; *capu*, *cappuccino*; *exu*, *exuperantia*; GFP, green fluorescent protein; MT, microtubule; *osk*, *oskar*; *spir*, *spire*.

nurse cells has been proposed to be a stage in the life cycle of the polar plasm, and it, as well as the polar granules, contains RNA (Mahowald, 1971*b*; Eddy, 1975). Since *vasa* encodes for an ATP-dependent RNA helicase of the D-E-A-D family of proteins, it might act on the RNAs included in the nuage and polar granules, and therefore it might mediate their localization to these cytoplasmic organelles (Mahowald, 1971*a*; Hay et al., 1988*a*,*b*; Lasko and Ashburner, 1988, 1990).

The localization of *osk* and *bcd* mRNA is initiated during oogenesis (Berleth et al., 1988; St Johnston et al., 1989; Ephrussi et al., 1991; Kim-Ha et al., 1991). A *Drosophila* egg chamber consists of 16 germ line cells surrounded by follicle cells of somatic origin. The germ line cells arise from a common stem cell by four rounds of incomplete divisions and remain interconnected by cytoplasmic bridges (for a review of oogenesis see Spradling, 1993). One cell of this arising cluster is specified during development to form the oocyte. The other 15 cells develop into nurse cells with highly polyploidized nuclei. Most of the maternal mRNAs, as well as the factors required for RNA localization, are produced in the nurse cells and then transported into the oocyte. An extensive cytoskeletal network (for review see Cooley and Theurkauf, 1994) extending throughout the germ line cells and through the ring canals may allow this transport. Transport along microtubules is necessary for both *bcd* and *osk* mRNA localization (Pokrywka and Stephenson, 1995). The polarity of the microtubule network within the oocyte, in combination with plus and minus end–directed motor molecules, might explain the different target sites of the RNA transport (Theurkauf et al., 1992, 1994*a*). However, the motor molecules involved have not been identified so far, nor has a direct association of the RNAs with microtubules been shown.

Translocation and localization of RNAs can occur via a three-step pathway as proposed by Wilhelm and Vale (1993). In this model, the formation of RNP particles precedes the transport along cytoskeletal elements and subsequent anchoring of the RNA. In the case of the *bcd* mRNA, the RNP particles could be formed by the binding of *trans*-acting factors to the 3′ untranslated region, which is essential for the localization process (Macdonald and Struhl, 1988; Macdonald et al., 1993; Ferrandon et al., 1994). A possible candidate for such a *trans*-acting factor is the Exuperantia protein (Exu). After an initial phase in oogenesis of wild-type flies when *bcd* mRNA is present throughout the oocyte, Exu is required to mediate *bcd* mRNA localization in an anterior ring in stage 6–9 oocytes (King, 1970; St Johnston et al., 1989). Subsequently, Swallow, as a possible component of the cytoskeleton, seems to anchor the *bcd* mRNA at the cortex of the oocyte during stage 10B–11 (Stephenson et al., 1988; St Johnston et al., 1989; Chao et al., 1991; Hedge and Stephenson, 1993). The third protein known to be involved, Staufen, does not act on *bcd* mRNA localization until stage 12 of oogenesis; it then releases the transcript from the cortex into the anterior cytoplasm of the embryo, where it is localized until degradation at cellularization (St Johnston et al., 1989, 1991). *bcd* mRNA fails to be localized during oogenesis of females homozygous mutant for *exuperantia* (*exu* females). As a result, the *bcd* mRNA is initially equally distributed in the egg (Berleth et al., 1988; St Johnston et al., 1989), although a weak gradient forms later by destabilization of the *bcd* mRNA at the posterior pole by *nanos* activity (Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988*a*; Wharton and Struhl, 1989). The intermediate Bcd protein levels extending throughout the anterior two thirds of the egg result in a lack of head structures and extended thoracic body regions in *exu* embryos (Frohnhöfer and Nüsslein-Volhard, 1987; Driever and Nüsslein-Volhard, 1988*a*).

Exu was proposed to be necessary for an association of *bcd* mRNA with the microtubule-dependent localization machinery (Pokrywka and Stephenson, 1991). It is not known so far whether Exu binds directly to *bcd* mRNA. No significant RNA-binding domains or other functional domains within the Exu sequence have been identified that would help to understand the mode of its function. The protein does not accumulate at the anterior end of the oocyte (Macdonald et al., 1991; Marcey et al., 1991). In contrast to Swallow and Staufen, which are still present in early embryos, Exu is present only during oogenesis (Macdonald et al., 1991; Marcey et al., 1991). It is first detectable in stage 4 oocytes. Starting at stage 7, it is accumulating in the nurse cells, where a high concentration can be observed during midoogenesis. The content of Exu in the oocyte increases again by stage 10, when the protein is transported from the nurse cells into the oocyte. Subcellularly, the protein shows a patchy distribution. Wang and Hazelrigg (1994) have shown a fusion protein of Exu and green fluorescent protein (GFP) to be present in particles that are transported through the germ line cells, but the nature of these particles has not been resolved.

In this paper we describe a subcellular structure in the female germ line of *Drosophila* that contains RNA and is strongly enriched for Exu protein. Our data suggest that the so-called sponge bodies might form an intracellular compartment for the assembly and transport of *cis*- and *trans*-acting elements involved in RNA localization.

Materials and Methods

Fly Strains

The wild-type strain was OregonR. Unless noted otherwise we used flies homozygous for the exu^{VL} allele to obtain ovaries that express neither *exu* RNA nor protein at detectable amounts (Hazelrigg et al., 1990). Transgenic flies carrying six additional copies of the *bcd* locus were constructed by T. Berleth (Berleth et al., 1988). The *serendipity* flies, which in transheterozygous combination give rise to ovaries not expressing *bcd* mRNA (Payre et al., 1994), were a kind gift of A. Vincent (University of Toulouse, France). The transgenic flies that express an Exu–GFP fusion gene under the pCaSper promoter were constructed by Wang and Hazelrigg (1994).

Antibody Generation and Preadsorption

The 3rd exon of the *exu* gene was amplified out of genomic DNA. The fragment was cloned into the pQE12 vector (Qiagen, Chatsworth, CA). A fusion protein containing six COOH-terminal His-residues and amino acids 100–532 of the *exu* gene product was expressed in *Escherichia coli* and purified via a Ni-affinity column. The purified protein was used for subcutaneous injection of two rabbits. The sera of both rabbits were tested against the preimmune sera on Western blots with bacterially expressed Exu–6×His fusion protein and extracts of wild-type or *exu Drosophila* ovaries. Both sera showed very high specificity for the expected band of ~60 kD in extracts of wild-type but not *exu* ovaries. We therefore used the unpurified rabbit serum for all further experiments. Before use, an aliquot of the serum was preadsorbed overnight at an equal volume of paraformaldehyde-fixed *exu* ovaries or embryos.

Ovary Dissection and Fixation

Dissections were carried out in PBS at room temperature (or on ice, which did not lead to different results). The ovaries were transferred into a fixative consisting of 85 volume parts 4% paraformaldehyde in 200 mM Pipes, pH 7, and 15 parts saturated picric acid (improved protocol of Stefanini et al., 1967) for immunohistochemistry. For structural analysis, the fixative also contained 2% glutaraldehyde. The ovaries were fixed for 15–30 min and afterwards washed several times with PBS.

Embedding and Sectioning

For structural analysis, the ovaries were embedded in Epon 812 (Glycidether 100; Roth, Karlsruhe, Germany). Fixed (or freshly dissected for membrane analysis) ovaries were incubated in 1% OsO_4 and 1% tannic acid in PBS for 1 h on ice and stained with 1% aqueous uranyl acetate for 1 h at 4°C. These heavy metal treatments were omitted for samples used for RNA detection (see below). The samples were subsequently dehydrated through an ethanol series at room temperature. They were infiltrated twice with a 1:1 mixture of ethanol and Epon and finally with pure Epon resin overnight. After a second step in the pure resin for several hours, they were embedded in flat wells and heat polymerized at 60°C for 48 h.

For immunogold and immunofluorescent labeling, the ovaries were embedded in the methacrylate resins Lowicryl K4M or HM20 (Polyscience Ltd, Eppenheim, Germany). (All pictures shown here are taken from sections embedded in HM20 since they yielded better contrast but similar labeling.) The ethanol dehydration for the Lowicryl resins was performed with progressive lowering of the temperature (1 h in each ethanol step). Infiltration with 2:1 and 1:1 mixtures of ethanol and resin was performed at −35°C, as well as the final infiltration steps (overnight/several hours) with the pure resin. Polymerization was performed by UV irradiation at 366 nm and −35°C in closed 750-μl reaction tubes (Sarstedt, Nümbrecht-Rommelsdorf, Germany) for oxygen exclusion.

The sample blocks were trimmed on an LKB pyramitome. Sections of 50–100 nm were cut by an LKB ultramicrotome, and Lowicryl sections were mounted either on pioloform-coated, carbon-coated copper grids, or on polylysine-treated coverslips. Epon sections used for studying the ultrastructure were mounted on uncoated copper grids.

Immunofluorescence and Immunoelectron Microscopy

For postembedding labeling, the Lowicryl sections (on grids or coverslips) were blocked with PBG (0.2% gelatine, 0.5% BSA in PBS) twice for 10 min. The first antibody was incubated in the same solution [1 h for rabbit anti-Exu antiserum 1:100, for mouse anti–α-tubulin IgG 1:125 (Sigma Chemical Co., St. Louis, MO], and for mouse anti-actin IgG 1:300 [Cedarlane Laboratories, Hornby, Ontario, Canada]; and 10 min for mouse anti-Vasa mAb46F11 1:100, as described in Hay et al. [1988*a*]). Sections were washed with PBG six times for 5 min and incubated with the secondary antibody in PBG for 1 hr (Cy3-conjugated anti–rabbit IgG 1:500 [Jackson ImmunoResearch, West Grove, PA]; protein A–15-nm gold [noncommercial]; Cy3-conjugated anti–mouse IgG 1:500 [Jackson ImmunoResearch]; anti–mouse IgG 18-nm gold-conjugated 1:20 [Jackson Immunoresearch]; Cy3-conjugated anti–mouse IgM 1:500 [Jackson ImmunoResearch]; 10-nm gold–conjugated anti–mouse IgM 1:10 [Janssen Biotech N.V., Olen, Belgium]). Six washes with PBG followed, and two with PBS.

The immunofluorescently labeled sections were treated with 0.4 μg/ml DAPI for 5 min and washed again with PBS before mounting in Moviol 4-88 (Hoechst) containing 25 mg/ml DABCO as antifading agent.

For immunoelectron microscopy, the grids were washed for several times with distilled water after the antibody labeling. They were incubated on 1% aqueous uranyl acetate for 10 min and subsequently washed again thoroughly with water. A contrasting step with 0.4% lead citrate trihydrate followed for 3 min. The immunolabeling was studied with a transmission electron microscope (model CM10; Philips Electronics Instruments, Inc., Mahwah, NJ).

Cytoskeletal Drug Treatment

OrR flies were starved overnight and subsequently fed on medium containing either 100 μM taxol or 20 μg/ml colchicine (or no drugs for control) for 2 d. The paired ovaries of one female were split; one half was further processed for Epon embedding (see above), whereas the other was embedded in Lowicryl HM20 (see above). In parallel, ovaries from the same batch of flies were processed for in situ hybridization with a DIG-labeled *bcd* antisense RNA probe according to the protocol of Tautz and Pfeifle (1989).

RNA Staining

The RNA staining was performed by a modification of a protocol of Bernhard (1969). Epon sections were mounted on pioloform-coated, carbon-coated copper grids. They were treated with 5% aqueous uranyl acetate for 10 min. One half of the sections was directly processed with lead citrate (2.5 min) as controls, whereas the other half was partially destained again by incubation in 0.2 M EGTA for 15 min. These grids were then contrasted with lead citrate for 2.5 min as well.

Results

Exu Is Localized to Sponge-like Structures

To study the function of the Exuperantia protein (Exu) in the female germ line, we investigated the intracellular protein distribution. On ultrathin methacrylate sections of ovaries, we observed a patchy distribution of Exu, as described earlier for whole mounts and paraffin sections, and for an Exu–GFP fusion construct (Fig. 1; Macdonald et al., 1991; Marcey et al., 1991; Wang and Hazelrigg, 1994).

To understand the nature of this patchy intracellular distribution pattern, we performed immunogold labeling of Exu on the ultrathin Lowicryl sections for the transmission electron microscope. We could observe a distinct, electron-dense structure in the cytoplasm in which Exu is highly en-

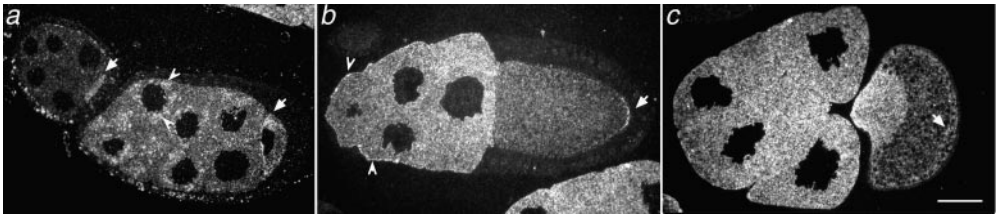

Figure 1. Exu distribution in wild-type ovaries. Ovaries embedded in Lowicryl HM20 were sectioned (100 nm) and afterwards immunofluorescently labeled with anti-Exu antiserum. The staining was studied by light microscopy. (*a*) Stage 6 and 8 follicles showing a punctate distribution pattern of Exu (frequently perinuclear) in the nurse cells (*arrowhead*) and an accumulation of Exu in the oocyte (*arrows*). The follicle cells show no staining. (*b*) Stage 10A follicle with apical accumulation of Exu in the nurse cells (*arrowheads*) and transient enrichment of Exu at the posterior end of the oocyte (*arrow*). (*c*) Stage 10B follicle after the onset of the bulk flow of cytoplasm. The follicle is sectioned at a slightly oblique angle; therefore, the oocyte appears smaller than the area covered by nurse cells. The nurse cell–derived cytoplasm containing high amounts of Exu enters the oocyte, and the yolk-rich (darker) cytoplasm is restricted to more central regions of the oocyte. A cortical enrichment of Exu in the oocyte can be observed (*arrow*). The columnar follicle cells covering the oocyte do not stain for Exu and therefore are not visible. Bar, 100 μm.

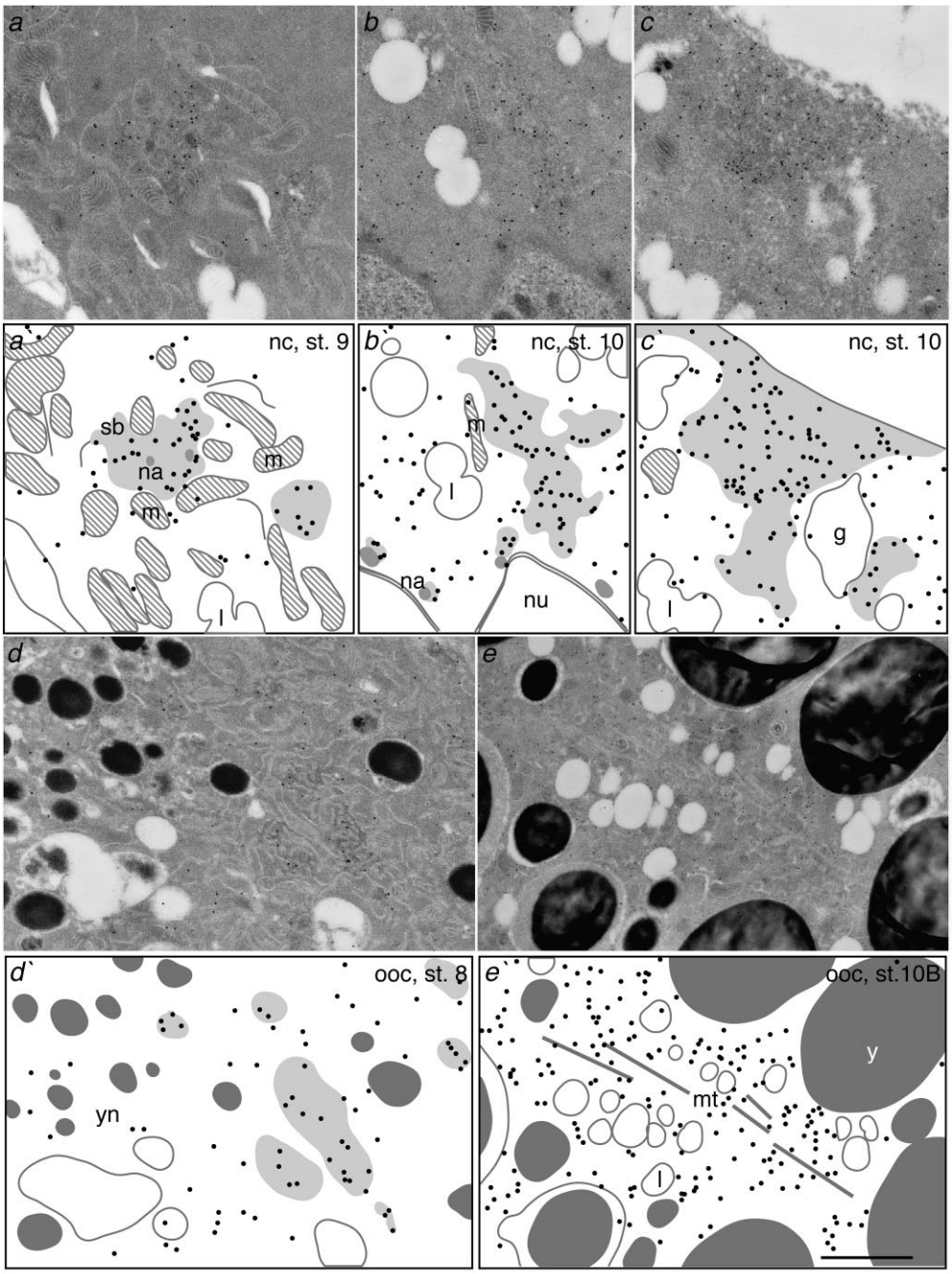

Figure 2. Ultrastructural distribution of Exu. Electron micrographs (*a–e*) and schematic drawings (*a′–e′*) of ultrathin Lowicryl sections on which Exu was indirectly immunolabeled by 15-nm colloidal gold particles. (*a* and *a′*) Nurse cell, stage 9. Exu is highly enriched in the sponge body in comparison to the surrounding mitochondria and cytoplasm. The nuage particles surrounded by the sponge body contain no Exu. (*b* and *b′*) Nurse cell, stage 10. Exu is accumulated in the elongated sponge body, which extends from the nucleus into the cytoplasm. Nuage particles next to the nucleus are partly surrounded by higher amounts of Exu present in small sponge bodies. (*c* and *c′*) Nurse cell, stage 10. A large sponge body is present at the apical border of the nurse cell. A small fragment of the overlying follicle cell can be seen at the upper right edge of the micrograph. The gap between these cells is due to the embedding. (*d* and *d′*) Oocyte, stage 8. Sponge bodies with high concentrations of Exu are present in the ooplasm. However, the central region of the oocyte where the yolk granules first accumulate (yolk nucleus) contains only very little Exu or sponge bodies. (*e* and *e′*) Oocyte, stage 10B. Thick parallel bundles of microtubules run at this stage in the cortical layer of the oocyte. No sponge bodies are present, but Exu is equally distributed between the large yolk granules. No accumulation of Exu on the microtubules can be observed either. *g*, Golgi vesicles; *l*, lipid droplet; *m*, mitochondria (*hatched*); *mt*, microtubule; *na*, nuage (*middle gray*); *nc*, nurse cell; *nu,* nucleus; *ooc*, oocyte; *sb*, sponge body (*light gray*); *y*, yolk granule (*dark gray*); *yn*, yolk nucleus. Each black dot in the drawing corresponds to a 15-nm gold particle in the micrograph. Bar, 1 μm.

riched (Fig. 2). Strong immunogold labeling and high immunofluorescence were concentrated at the same position in the cells of the adjacent ultrathin sections. The distribution of these structures on the electron microscopic sections therefore directly corresponds to the patchy staining pattern in nurse cells and oocyte seen by light microscope.

The densely Exu-labeled structure consists of elongated elements embedded in an electron-dense matrix (as shown in Fig. 2 [Lowicryl embedding] and in Fig. 3 [Epon embedding]). We will therefore refer to this structure as "sponge body." The elongated elements of the sponge bodies are formed by ER-like cisternae or small vesicles interspersed between an electron-dense, amorphous material. These ER-like tubules become most obvious after osmiumtetroxide treatment without previous fixation of the follicles, thereby removing most of the soluble components of the cytoplasm, including the electron-dense, amorphous matter of the sponge bodies (Fig. 4). However, the spacing between the tubules in these preparations appears larger than between the elongated elements in unextracted follicles (compare Fig. 3 *a* and Fig. 4), so that we can not exclude the possibility that in addition to the membrane-

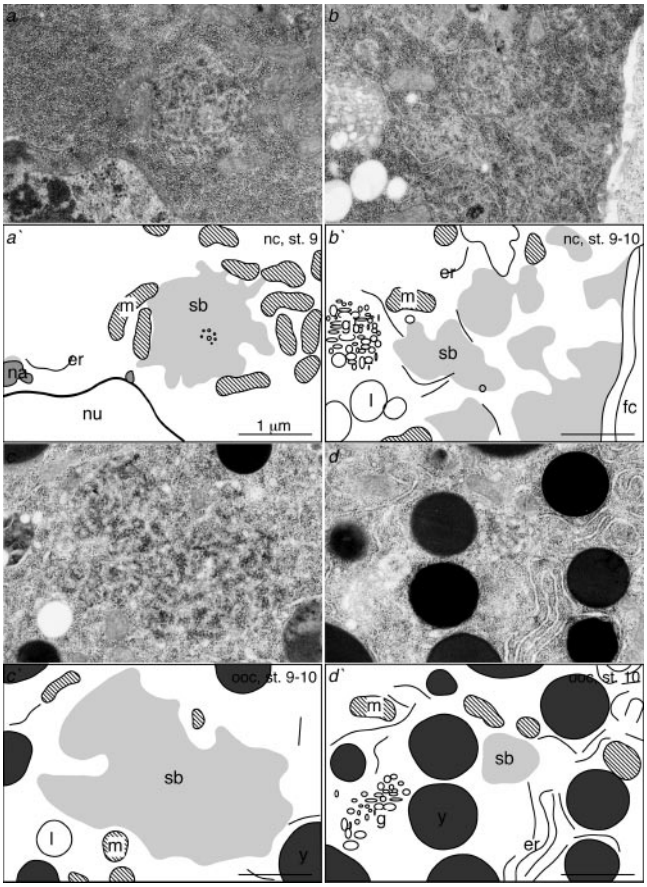

Figure 3. Ultrastructural distribution of the sponge bodies. Electron micrographs *(a–d)* and schematic drawings *(a′–d′)* of ultrathin Epon sections of glutaraldehyde- and osmiumtetroxide-fixed wild-type follicles. (*a* and *a′*) Nurse cell, stage 9. The sponge body consists of electron-translucent, elongated elements that are interspersed between an electron-dense amorphous mass. It hardly contains ribosomes in contrast to the surrounding cytoplasm. Small vesicles are present within the sponge body. The sponge body is situated close to the nucleus and is surrounded by mitochondria. (*b* and *b′*) Nurse cell, stage 9–10. Adjacent to the flattened follicle cells, sponge bodies are accumulated in the apical regions of the nurse cell. ER tubules and Golgi vesicles are present between the sponge body clusters. (*c* and *c′*) Oocyte, stage 9–10. The large sponge body can be distinguished from the surrounding ooplasm by the accumulation of elongated elements in an electron-dense matter, excluding ribosomes and other organelles. (*d* and *d′*) Oocyte, stage 10A. Most sponge bodies have disappeared by this stage; however, some small clusters are left in the cytoplasm, which is densely packed with yolk granules, ER tubules, mitochondria, and Golgi vesicles. *er*, endoplasmic reticulum; *fc*, follicle cells; *g*, Golgi complex; *l*, lipid droplet; *m*, mitochondria (*hatched*); *na*, nuage (*middle gray*); *nc*, nurse cell; *nu*, nucleus; *ooc*, oocyte; *sb*, sponge body (*light gray*); *y*, yolk granule (*dark gray*). Bar, 1 μm.

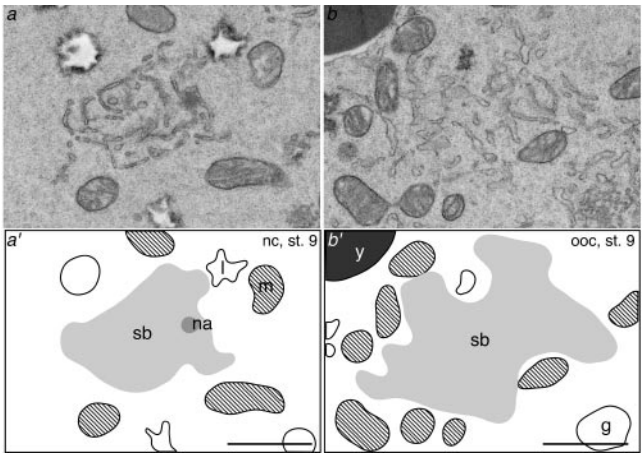

Figure 4. Ultrastructure of sponge bodies after extraction. Micrographs *(a* and *b)* and schematic drawings *(a′* and *b′)* of ultrathin Epon sections of wild-type follicles that were only fixed with osmiumtetroxide, not with aldehyde, for better visualizing the membranes in the sponge bodies. (*a* and *a′*) Nurse cell, stage 9. The sponge body contains ER-like cisternae. No amorphous matter is visible between these due to the strong extraction of the cytoplasm. However the electron-dense nuage particle contained in the sponge body is not affected by this procedure. (*b* and *b′*) oocyte, stage 9. The lumen of the cisternae of the sponge body in the oocyte appears larger than that in the nurse cells. As in the nurse cells no amorphous matter is left after the extraction. *g*, Golgi complex; *l*, lipid droplet; *m*, mitochondria; *na*, nuage; *nc*, nurse cell; *nu*, nucleus; *sb*, sponge body; *y*, yolk granule. Bar, 1 μm.

bounded cisternae, other electron-translucent elements exist in the central regions of the sponge bodies that lack bounding membranes and are removed by strong osmiumtetroxide fixation together with the electron-dense component of the sponge bodies. In addition to the elongated elements, occasionally small membrane-bounded vesicles are present in the sponge body structures (Fig. 3 *a*). The outlines of the sponge body structures often appear indistinct, and they are not limited by a surrounding membrane. Mitochondria are frequently associated with the sponge bodies in the nurse cells, which is most obvious between stages 4 and 9 (Figs. 2 *a* and 3 *a*). In summary, we define the sponge bodies as cytoplasmic aggregations of cisternae and vesicles embedded in amorphous material that exclude most ribosomes. They are frequently situated in close proximity to mitochondria.

Sponge Bodies Are Not a Specialized Golgi Apparatus

Since the sponge bodies include membrane-bounded cisternae and small vesicles, we were interested to know whether these structures correspond to the Golgi apparatus of the cells. Only few typical Golgi consisting of ordered membranous stacks and small vesicles are present within the ovarian germ line cells. However, the majority of Golgi complexes in nurse cells and oocytes consist of aggregates of different sized vesicles and few membranous stacks (Fig. 3, *b* and *d*). Golgi vesicles are frequently found in proximity to the sponge bodies or even contained therein. However, these Golgi vesicles are tightly packed and never separated by electron-dense amorphous material like the cisternae of the sponge bodies. We can therefore conclude that the sponge bodies are not identical with the Golgi apparatus of the *Drosophila* germ line cells.

Sponge Body Morphology Changes with Progressing Development

Sponge bodies are first observed in the cytoplasm of stage

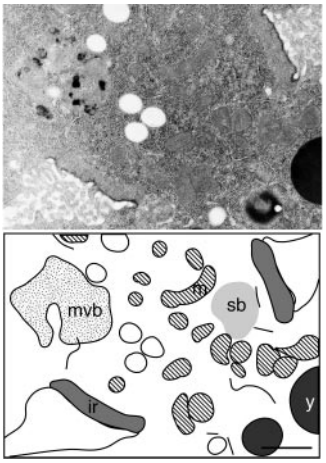

Figure 5. Sponge bodies are present in ring canals. Micrograph and schematic drawing of a stage 10 follicle that was treated as described for Fig. 3. The cytoplasmic bridge, connecting a nurse cell (to the left) with a yolk-containing oocyte, includes a sponge body as well as other cytoplasmic organelles (ER and lipid droplets). A multivesicular body containing electron-dense, yolk-like material is present in close proximity to the ring canal. *ir*, inner ring; *m*, mitochondria; *mvb*, multivesicular body; *sb*, sponge body; *y*, yolk. Bar, 1 μm.

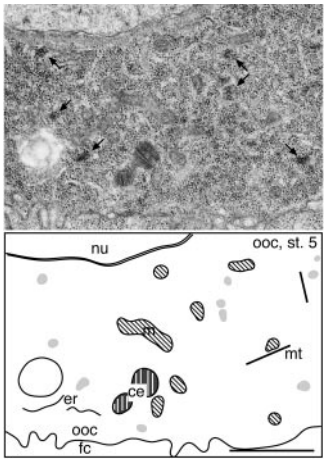

Figure 6. Cytoplasm of previtellogenic oocyte. Micrograph and schematic drawing of a stage 5 oocyte that was treated as described for Fig. 3. The microtubule network of the oocyte (*ooc*) is organized by the centrioles (*ce*) at the posterior pole adjacent to the follicle cells (*fc*). The cytoplasm is packed with short microtubules (*mt*), ER-like tubules (*er*), ribosomes, and electron-dense granule (*am*, amorphous matter; some of the granules were marked by *arrows* in the micrograph). The appearance of these granules resembles the amorphous matter in the sponge bodies. *nu*, nucleus. Bar, 1 μm.

3–4 germ line cells and persist until stage 10–11. They are found in the nurse cells as well as in the oocyte. The transport of the sponge bodies between the germ line cells is suggested by their presence in the cytoplasmic bridges (Fig. 5). Throughout development, with the exception of the very first sponge bodies forming that hardly label for Exu (in stage 3–5 nurse cells, not shown), all sponge bodies contain higher amounts of Exu than the surrounding cytoplasm (Fig. 2).

The morphology of the sponge bodies differs slightly at the different developmental stages and cell types. In nurse cells (stage 4–9), the tight association of mitochondria with the sponge bodies exists until mid-vitellogenesis (Figs. 2 *a* and 3 *a*). At early stages (3–7), large aggregates of mitochondria exist, between which small sponge bodies are interspersed (not shown). Although there is a superficial resemblance to fusomes, which are present in younger stages, the fusomes never contain the electron-dense amorphous matter, and they show a wider spacing of the cisternae than the sponge bodies. Taken together with the presence of α-spectrin in fusomes but not in sponge bodies (Lin et al., 1994; Lee et al., 1997), this observation argues against the sponge bodies being fusome-derived structures. In the nurse cells of stage 7–9, the mitochondrial aggregates, which earlier have been restricted to one or two large areas close to the nucleus, fractionate into smaller clusters still including sponge bodies. In stage 10 nurse cells, the association of sponge bodies with mitochondria has mostly disappeared (Figs. 2, *b* and *c*, and 3 *b*). The sponge bodies in these cells are mostly elongated (Fig. 2 *b*) (in comparison to their rounded shape earlier on) and form apical clusters next to the thin layer of follicle cells (Figs. 2 *c* and 3 *b*). At this stage, the amorphous material in the sponge bodies is hardly visible between the ER-like cisternae (Fig. 3 *b*); however, Exu protein is still enriched in these structures (Fig. 2 *c*). As development proceeds, the sponge bodies dissociate further and the distribution of Exu becomes uniform in the nurse cells (Fig. 1 *c*, not shown).

In the oocytes, mitochondrial aggregates like these in the nurse cells are never observed. Instead, at stage 3–7 the cytoplasm is densely packed with small organelles (ER, vesicles, lysosomes, Golgi, and microtubules), between which small particles of electron-dense, amorphous material, resembling that of the later sponge bodies, are interspersed (Fig. 6). A clear distinction between sponge body and non–sponge body material is still difficult, and Exu staining is rather uniform in these early oocytes. This early situation in the oocyte is terminated by the formation of yolk granules that first aggregate in a central cluster (sometimes termed yolk nucleus). The forming sponge bodies are restricted to lateral regions up to the time when the yolk granules disperse throughout the oocyte (Fig. 2 *d*). From then on, the sponge bodies are situated throughout the cytoplasm of the oocyte, which accumulates organelles and yolk granules of increasing number and size during the vitellogenic stages (Fig. 3, *c* and *d*). By stage 10B, sponge bodies are no longer visible within this densely packed ooplasm. However, Exu protein remains detectable up to stage 12. It is then no longer restricted to a specific structure within the cytoplasm (Fig. 2 *e*).

Sponge Body Morphology Is Unchanged in Ovaries Mutant for exu

Since Exu is highly enriched in the sponge bodies, we were interested to see whether this protein is a structural component of the sponge bodies. We investigated ovaries from females homozygous for the exu^{VL} allele, which has a 700-bp deletion due to imprecise P-element excision. Neither RNA nor protein is detectable in these ovaries (exu^{VL} ovaries) (Hazelrigg et al., 1990). However, the morphology of the sponge bodies shows no obvious difference to that of the wild type (Fig. 7). The same result was obtained with the EMS-induced alleles exu^{SC} and exu^{XL1}, which do not produce detectable Exu (Hazelrigg et al., 1990; Marcey et al., 1991; data not shown). This implies that Exu is not an essential structural component of the sponge bodies, although it is closely associated with these structures in wild-type ovaries.

RNA Is Present in the Sponge Bodies

To investigate whether the sponge bodies contain RNA,

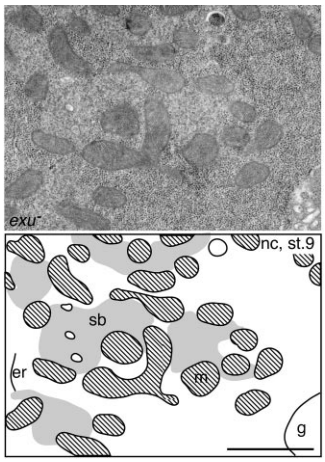

Figure 7. Sponge bodies in exu^{VL}/exu^{VL} ovaries. Micrograph and schematic drawing of a stage 9 exu^{VL}/exu^{VL} nurse cell that was treated as described for Fig. 3. No obvious difference in the morphology of sponge bodies that do not contain Exu can be observed compared with sponge bodies in wild-type follicles by electron microscopy. As in wild-type follicles, these sponge bodies exclude ribosomes and consist of electron-translucent elements in an amorphous matter with some small vesicles in between. *er*, endoplasmic reticulum; *g*, Golgi; *m*, mitochondria; *nc*, nurse cell; *sb*, sponge body. Bar, 1 μm.

we performed RNA staining on the ultrathin sections. This staining procedure enhances the contrast of RNA-containing structures, as for example the euchromatin in the nuclei. Indeed, sponge bodies in sections treated with this procedure are darkly contrasted in comparison to the surrounding cytoplasm (Fig. 8). This result suggests that the sponge bodies do contain some RNA. Next, we studied the contrast of the sponge bodies in follicles that lack *bcd* mRNA. No *bcd* mutation exists that eliminates the expression of the RNA. However, ovaries lacking *bcd* mRNA can be obtained taking advantage of mutations in the *serendipity* gene family. One of its members, *serendipity* δ, is required for viability, as well as for the activation of transcription of *bicoid*. Replacement of two domains within the Serendipity δ protein by the corresponding region of the closely related Serendipity β protein rescues the zygotic phenotype of *serendipity* δ but not the activation of *bcd* transcription (Payre et al., 1994). In ovaries of such flies, *bcd* mRNA levels are strongly reduced or absent (at 18°C). However, no difference in the contrast of the sponge bodies can be observed in these follicles containing no *bcd* mRNA. The same result was obtained for follicles containing additional copies of *bcd* mRNA, transcribed from multiple copies of the gene (not shown). This implies that *bcd* mRNA, if present in the sponge bodies, is not the only or predominant RNA component of the sponge bodies. However, this method is not suited to directly detect *bcd* mRNA in the sponge bodies. So far, we have failed to visualize *bcd* mRNA in the nurse cells under conditions sufficiently preserving the structure of the sponge bodies. Therefore, we cannot directly show whether *bcd* mRNA is present in the sponge bodies. However, the distribution of the *bcd* mRNA in stage 10 nurse cells is patchy (Fig. 9, and as described in St Johnston, 1989), which might indicate an accumulation of *bcd* mRNA on some subcellular structure.

Exu Localization Is Not Dependent on bcd Copy Number

We were interested to know whether the localization of Exu in the sponge bodies depends on the presence of *bcd* mRNA. We investigated ovaries that lack *bcd* mRNA (see above). We find that the level and distribution of Exu in the sponge bodies is similar to normal in these ovaries. Likewise, ovaries that have threefold-increased *bcd* mRNA levels because of four additional copies of *bcd* show the same distribution of Exu as wild-type ovaries (data not shown). These experiments show that the localization of Exu in the sponge bodies occurs independently of the presence and amount of *bcd* mRNA.

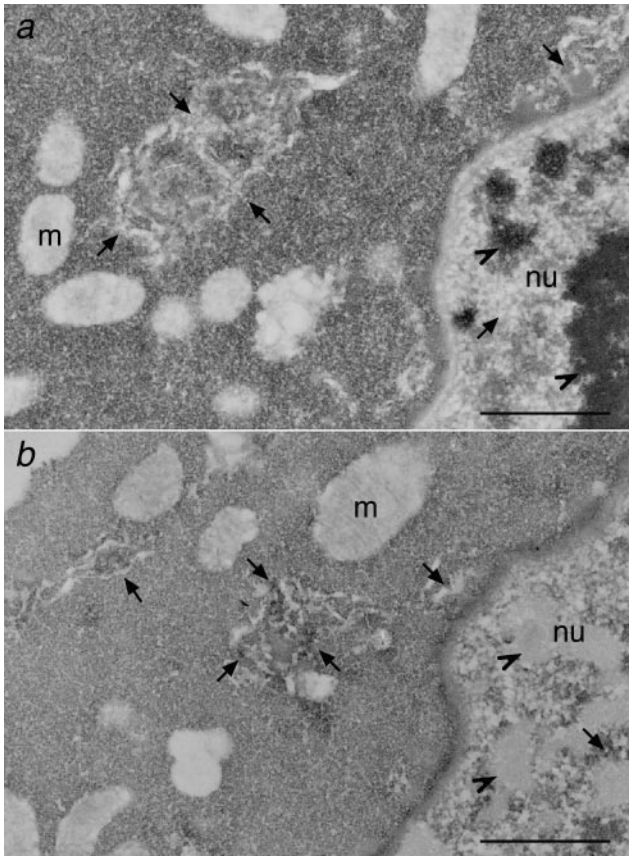

Figure 8. RNA staining of wild-type stage 9 nurse cells. (*a*) Electron micrograph of an ultrathin Epon section with normal contrast. DNA-rich regions of heterochromatin in the nucleus (*nu*) are darkly stained (*arrowheads*), whereas the extended euchromatin and sponge bodies show little contrast (*arrows*) in comparison to the ribosomes in the cytoplasm. (*b*) Modified Bernhard staining of an ultrathin Epon section. Note the dark contrast of the RNA rich regions (*arrows*) of the nucleus (*nu*) and the sponge bodies in contrast to the bright regions of the heterochromatin (*arrowheads*). *m*, mitochondria. Bars, 1 μm.

Microtubules Are Not a Component of the Sponge Bodies

Pokrywka and Stephenson (1991) reported that in stage 10 oocytes, taxol-induced, additional microtubules result in ectopic *bcd* mRNA localization in wild-type but not in *exu* ovaries. Wang and Hazelrigg (1994) observed that Exu is also ectopically localized close to the taxol-induced microtubules. These experiments suggest a function of Exu in connecting *bcd* mRNA to the microtubular molecular motor. We stained microtubules (MTs) within the ovaries with an anti–α-tubulin antibody in order to investigate the possibility of a colocalization of Exu and MTs. However, no accumulation of α-tubulin in Exu-labeled sponge bod-

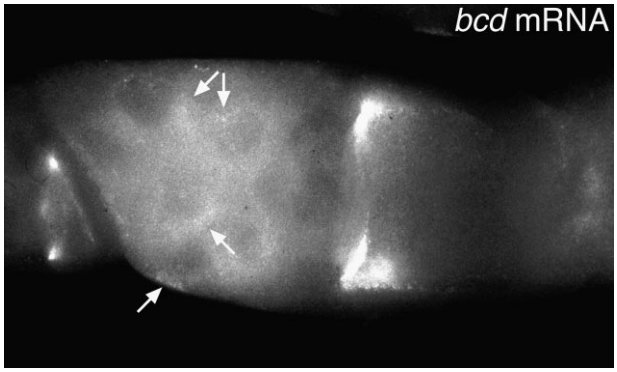

Figure 9. Patchy *bcd* mRNA localization in the nurse cells. *bcd* mRNA in a whole wild-type stage 10 follicle was fluorescently labeled by in situ hybridization. In the nurse cells, a patchy distribution of *bcd* mRNA is observed at the apical side of the cells and around the nuclei (*arrows*). The nurse cells adjacent to the oocyte are depleted of most of the *bcd* mRNA (as it was described by Wang and Hazelrigg [1994] for the Exu–GFP fusion protein).

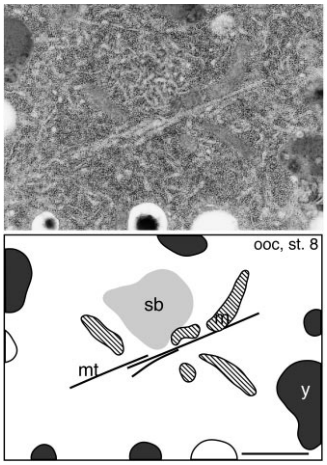

Figure 11. Subcellular localization of sponge bodies versus microtubules. Micrograph and schematic drawing of a wild-type stage 8 oocyte that was treated as described for Fig. 3. Microtubule bundles (*mt*) run across the cytoplasm. Although sponge bodies (*sb*) are present close by, no microtubules are present in these structures. *m*, mitochondria; *y*, yolk. Bar, 1 μm.

ies could be observed either by double immunofluorescence of ultrathin sections (Fig. 10) or by confocal microscopy of double-labeled ovaries (not shown). In rare cases, MTs can be observed running close to the sponge bodies, but even here MTs are never present near the center of the sponge bodies (Fig. 11). Clearly, the majority of sponge bodies in the cells is not associated with MTs. At stage 10B, MT bundles form at the oocyte cortex (Fig. 2 *e*). Again, no colocalization can be observed, but rather Exu accumulates cortically (Figs. 1 *c* and 2 *e*). Consistent with these data, double immunogold labelings with differently sized gold particles did not reveal a colocalization of α-tubulin and Exu (data not shown).

In *cappuccino (capu)* or *spire (spir)* ovaries, unusually thick, cortical bundles of MTs are found within stage 8–10 oocytes as described by Theurkauf (1994*b*) and Emmons et al. (1995). These two genes are required for the transport of all posterior factors to the posterior pole of the oocyte and for transport of Nanos protein from the posterior pole to its site of action in the abdomen (Manseau and Schüpbach, 1989). Theurkauf (1994*b*) interpreted the thick MT bundles in *capu* or *spir* ovaries as premature formation of the thick MT bundles leading to the cytoplasmic streaming of wild-type stage 10B oocytes. We observed not only thick bundles of MT forming at stage 8 (instead of stage 10B) parallel to the oocyte cortex but also much higher amounts of MT bundles in later stages, compared with wild-type follicles (Fig. 12 *b*), although Emmons et al. (1995) could not detect higher concentrations of α- or β-tubulin in *capu* or *spir* ovaries.

Double immunolabeling of these mutant oocytes for α-tubulin and Exu protein did not reveal any additional accumulation of Exu at these ectopic microtubules, on neither light- nor electron microscopic levels (Fig. 12, not shown). An association of sponge bodies with these microtubule bundles is not observed either (not shown). Consistent with these data, a change in the *bcd* mRNA localization in mutant ovaries compared with wild type has not been found (Manseau et al., 1996).

Further evidence against a direct association of sponge

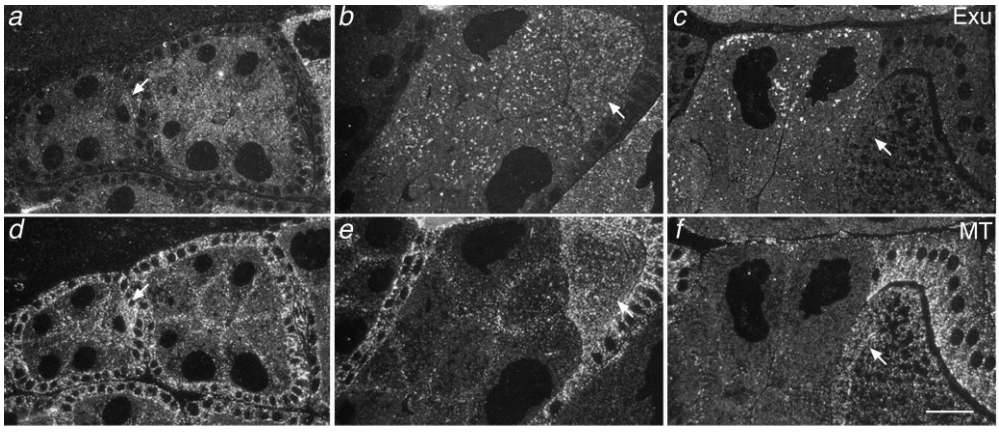

Figure 10. Comparison between Exu (*a–c*) and α-tubulin (*d–f*) distribution in wild-type follicles. Ultrathin Lowicryl sections were immunofluorescently labeled with either anti-Exu serum *(a–c)* or anti–α-tubulin antibodies *(d–f)* and observed by fluorescent light microscopy. Follicle cells harbor much higher amounts of microtubules than the germ line cells, but no Exu is present in the follicle cells. (*a* and *d*) Stage 4 and 6. High amounts of α-tubulin are found at the borders of the nurse cells and in the oocyte (*arrows*), but the punctate Exu staining is not enriched at these sites. (*b* and *e*) Stage 9. Many sponge bodies (*b*, *bright dots*) are present but not accumulated at the borders of the nurse cells or in the oocyte (*arrows*) where tubulin is enriched. (*c* and *f*) Stage 10. Hardly any microtubules are labeled, but sponge bodies labeled by Exu (*c*, *bright dots*) are accumulated in apical patches in the nurse cells. In the oocyte, Exu is not accumulated along the microtubules running parallel to the anterior border of the oocyte (*arrows*). Bar, 50 μm.

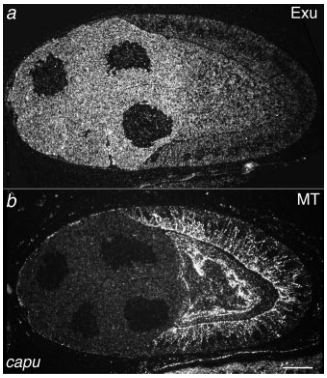

Figure 12. Exu is not accumulated on the thick microtubules forming in *cappuccino* oocytes. Adjacent ultrathin Lowicryl sections of $capu^{RK}/capu^{RK}$ follicles were immunofluorescently stained for Exu (*a*) or α-tubulin (*b*). Unusual thick bundles of microtubules run in the cortical cytoplasm of the stage 10A oocyte. However, in the same oocyte Exu is equally distributed. The same pattern can be observed in *spire* follicles. Bar, 50 μm.

bodies with MTs came by performing a drug treatment in a similar manner as described by Pokrywka and Stephenson (1991). After feeding flies for several days with either colchicine- or taxol-containing medium, large sponge bodies were observed (Fig. 13). In both cases, these structures still contained high amounts of Exu (Fig. 14, *e* and *f*), although in comparison to untreated follicles a clear difference in the tubulin distribution was observed (Fig. 14, *g*, *b,* and *c*), and the colchicine feeding had completely abolished *bcd* mRNA localization in sibling ovaries (Fig. 14, *c* versus *a*). However, we were not able to reproduce the ectopic localization of *bcd* RNA nor of Exu in follicles of taxol-treated flies (Fig. 14, *b* and *e*), which has been described by Pokrywka and Stephenson (1991) and by Wang and Hazelrigg (1994).

Since no microtubules were detectable in the sponge bodies, we wondered whether the second major component of the cytoskeleton, actin filaments, might be associated with the sponge bodies. Neither double fluorescence of ovaries expressing the Exu–GFP fusion protein with rhodamine-coupled phalloidin, nor immunogold labeling of ultrathin sections with antiactin antibodies could reveal actin cables present in the sponge bodies (data not shown). The amount of detectable actin in the nurse cells or oocyte is very low during midoogenesis. However, in stage 10 the apical borders of the nurse cells are densely lined with actin. Sponge bodies are accumulated there at the same time (Figs. 2, *c* and *b*), and at this location an association with the microfilament network might occur.

Sponge Bodies Surround Nuage

During stage 7–10A, Exu is accumulated around the nurse cell nuclei (Fig. 1 *a*), and sponge bodies are enriched on the cytoplasmic face of the nuclear membrane as determined by electron microscopy (Figs. 2 *b*, 3 *a*, and 15 *a*). An electron-dense structure called the nuage or fibrous body has been described previously at this location (Eddy, 1975; Mahowald, 1962; Mahowald, 1971*a*; Mahowald and Kambysellis, 1980). The nuage of *Drosophila* follicles shows an electron-dense granular or fibrous structure similar to the polar granules at the posterior pole of the oocyte, and in contrast to the sponge bodies it does not contain elongated elements. In addition to their conspicuous perinuclear location, smaller nuage particles are present in the cytoplasm further away from the nuclear membrane (as previously described by Mahowald, 1971*a*) for germarial stages and frequently observed by us in vitellogenic stages (Figs. 2 *a* and 4 *a*). Interestingly, the nuage is surrounded by sponge bodies in the nuclear lobes and in particular in the cytoplasm of the nurse cells (Figs. 2 *a*, 4 *a*, and 15 *a*). The nuage does not contain Exu (Fig. 2 *a*). Vasa protein is present in the nuage at the nuclear membrane of the nurse cells and in the polar granules at the posterior pole of the oocyte (Hay et al., 1988*a*,*b*; Liang et al., 1994). Vasa is required for the assembly of the polar granules and is a member of the posterior system. We used Vasa as a marker for nuage particles and found Vasa-staining nuage material surrounded by sponge bodies in the cytoplasm away from the nuclei (Fig. 15), where localization of Vasa in nuage particles had not been reported previously. Mahowald and Kambysellis (1980) reported some nuage particles to enter the oocyte through the ring canals. We confirmed this observation and, in addition, found a few, small Vasa-labeling nuage particles surrounded by sponge bodies anterior to the position where the polar granules form in stage 9 oocytes (Fig. 15 *b*).

In stage 9–10 follicles, a transient accumulation of Exu at the posterior pole is observed in comparison to the uniform distribution of Exu in the remaining ooplasm (Wang and Hazelrigg, 1994; Fig. 1 *b*). However, Exu is excluded from the polar granules that are forming in the posterior cytoplasm of the oocyte at this same time (Fig. 16). Since the nuage and polar granules are surrounded by regions enriched for Exu in wild-type follicles, Exu might be re-

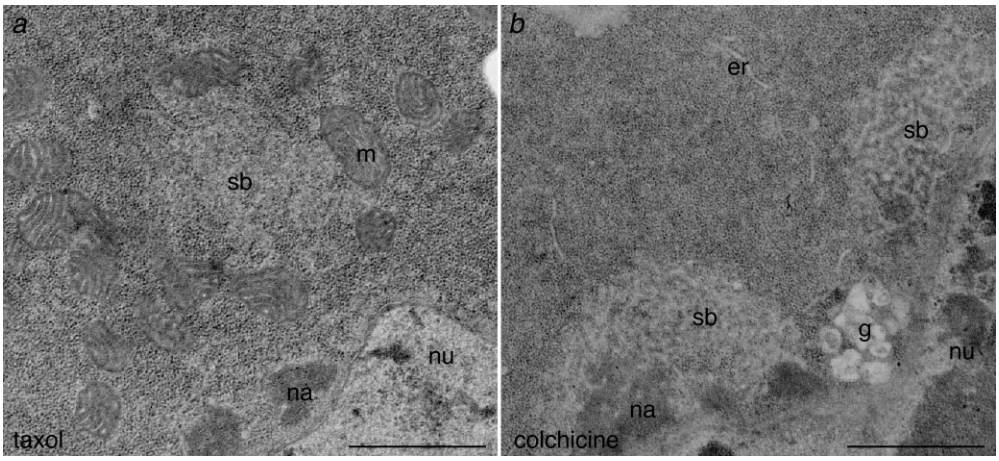

Figure 13. Ultrastructure of sponge bodies in ovaries after taxol or colchicine treatment. Micrographs of stage 9–10 nurse cells of flies that have been fed with cytoskeletal drugs for several days. The follicles were treated as described for Fig. 3. (*a*) Taxol; (*b*) colchicine. *er*, endoplasmic reticulum; *g*, Golgi; *m*, mitochondria; *na,* nuage; *nu*, nucleus; *sb*, sponge body. Bars, 1 μm.

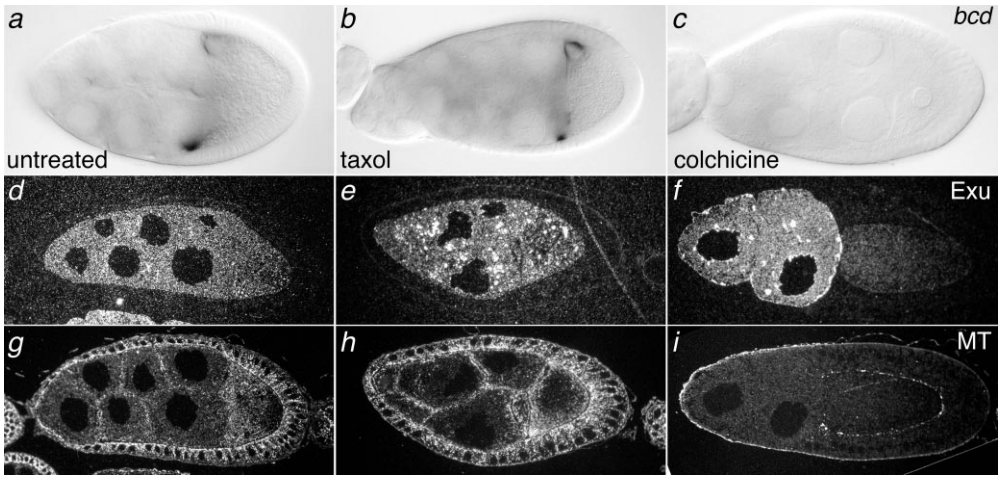

Figure 14. Effects of taxol or colchicine on localization of maternal factors. Ovaries of flies fed without any drug *(a, d,* and *g)*, with taxol (*b*, *e*, and *h*) or colchicine (*c*, *f*, and *i*) were stained for *bcd* mRNA (*a–c*), Exu (*d–f*) or α-tubulin (*g–i*). (*a–c*) *bcd* mRNA in situ hybridization on whole ovaries. After taxol treatment (*b*) no striking difference is to be seen compared with wild type (*a*), however after colchicine treatment (*c*) the localization of *bcd* mRNA is completely abolished. Note the central position of the oocyte nucleus in *c*, which can serve as a control for the MT destabilization. (*d–f*) Ultrathin Lowicryl sections of ovaries treated with taxol (*e*) or colchicine (*f*) were immunofluorescently labeled for Exu and compared with the wild-type staining (*d*). Neither treatment eliminates the punctate distribution pattern of Exu; however, in colchicine-treated follicles the staining seems to be preferentially close to the nucleus. The size difference of the sponge bodies observed here does not exceed the variance in the size of sponge bodies observed in different wild-type follicles. (*g–i*) Adjacent ultrathin Lowicryl sections of *d–f* stained for α-tubulin. After taxol treatment (*h*), clear microtubules are visible while the staining is diffuse and strongly reduced after colchicine treatment (*i*) compared with the wild-type staining (*g*). (The vitelline membrane in *i* is stained by cross-reaction of the secondary antibody.)

quired for the organization and distribution of these structures. However, we could not detect a change in the Vasa distribution in *exu* ovaries or an altered number of pole cells in *exu* embryos (data not shown). Therefore, Exu is not necessary for the accumulation of Vasa in polar granules and related organelles.

Discussion

The Nature of the Sponge Bodies

In this paper we describe a new subcellular structure in *Drosophila* germ line cells. According to its sponge-like appearance, we have called this structure "sponge body." In wild-type ovaries, Exu is enriched in the sponge bodies, as we observed by electron and light microscopy. We therefore assume that the sponge bodies correspond to the migrating fluorescent particles found in fly ovaries that express an Exu–GFP fusion protein (Wang and Hazelrigg, 1994). By light microscopic staining methods, structures have been detected previously in *Drosophila* ovaries that show a distribution within the germ line cells that is similar to that in our sponge bodies (Hsu, 1952, 1953). Hsu termed these structures yolk flakes, judging them to be proteid

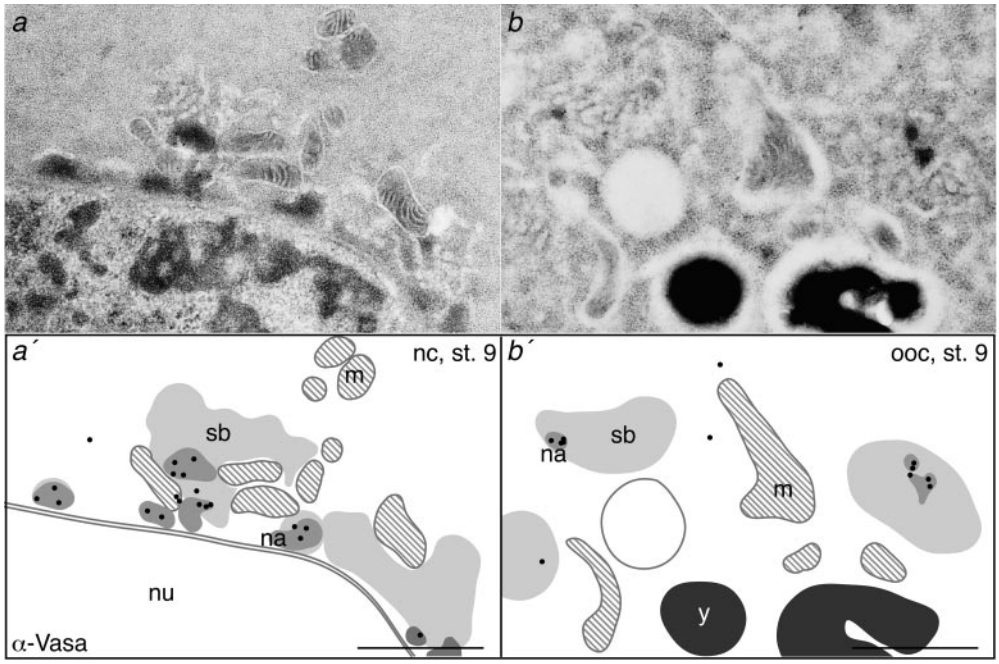

Figure 15. Vasa distribution in nuage particles. Micrographs (*a* and *b*) and schematic drawings (*a′* and *b′*) of follicles embedded in Lowicryl. Vasa protein was indirectly immunolabeled by 10-nm colloidal gold particles. (*a*) Nurse cell, stage 9. Vasa is accumulated in the nuage (*na*), which is present next to the nuclear membrane (*nu*, nucleus) and delaminating into the cytoplasm. The electron-dense, compact nuage particles are surrounded by sponge bodies (*sb*). (*b*) Oocyte, stage 9. Small Vasa-labeled nuage particles (*na*) are found at low frequency in the sponge bodies (*sb*) in the anterior cytoplasm of the oocyte. *m*, mitochondria; *y*, yolk. Bars, 1 μm.

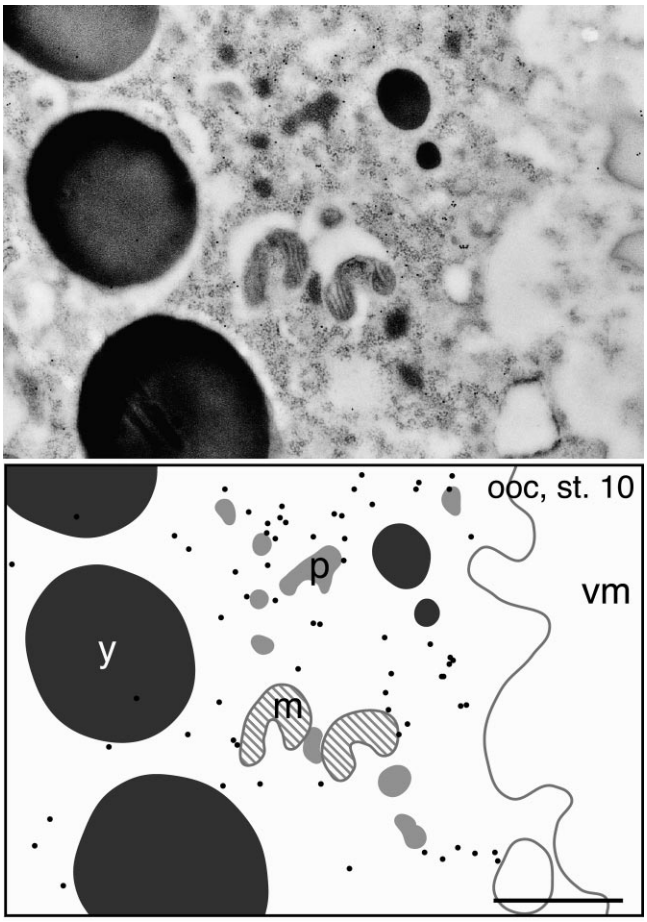

Figure 16. Transient posterior localization of Exu. Micrograph and schematic drawing of an ultrathin Lowicryl section of the posterior pole of a stage 10 oocyte that was immunogold-labeled for Exu (15-nm gold). The gold particles are accumulated in the posterior cytoplasm next to the vitelline membrane (*vm*), whereas the cytoplasm between the large yolk granules (*y*) is hardly labeled for Exu. The polar granules (*p*) at the posterior pole are free of Exu. *m*, mitochondria. Bar, 1 μm.

yolk precursors. Although the distribution of the sponge bodies parallels that of the yolk flakes, the sponge bodies are not yolk precursors. Yolk granules are formed in distinct, so-called multivesicular bodies in young oocytes and some nurse cells (Fig. 5). Furthermore, the central region of young oocytes, where the first accumulation of yolk granules can be observed (yolk nucleus), hardly contains sponge bodies (Fig. 2 *d*). However, in ovaries of many invertebrates and vertebrates, a structure with a morphology that is strikingly similar to the sponge bodies has been described, which is termed Balbiani's vitelline body (Henneguy, 1887; Guraya, 1979). It shares the presence of a structured, electron-dense mass and ER within an area enriched in mitochondria and Golgi with the sponge bodies of *Drosophila*. Although Guraya was not able to identify a corresponding structure in insect ovaries, we assume that our sponge bodies are the Balbiani's vitelline bodies of *Drosophila*. The high variability in the morphology might be one possible reason why the sponge body structure had not been described earlier. So far we have not been able to determine precisely which factors are responsible for the variance in size and consistency of the sponge bodies, but genetic background is definitely involved, as well as to a minor degree feeding conditions of females and temperature.

Comparison of the Sponge Bodies with the Mitochondrial Cloud of Xenopus Oocytes

The mitochondrial cloud in *Xenopus* oocytes is also thought to be homologous to Balbiani's vitelline bodies (Heasman et al., 1984). The morphology of the mitochondrial cloud, which consists of granulo-fibrillar material and surrounding mitochondria, closely resembles that of the sponge bodies. The mitochondrial cloud migrates from an early position next to the germinal vesicle to the vegetal pole of *Xenopus* oocytes while partitioning into large islands. RNA molecules like *Vg1*, *Xcat2*, *Xwnt11* mRNAs, and *Xlsirts*, thought to be involved in axis determination in *Xenopus*, comigrate with this structure (Forristall et al., 1995; Kloc and Etkin, 1995). The granular material of the mitochondrial cloud is thought to form the germinal granules in later stages of *Xenopus* embryogenesis, which contain a Vasa-related protein of the D-E-A-D family of proteins (Watanabe et al., 1992). Interestingly the sponge bodies surround the granular, Vasa-containing nuage particles, which are putative polar granule precursors. It is tempting to speculate that homologous molecules are transported and localized via conserved structures in insect and vertebrate ovaries.

Transport of the Sponge Bodies

In addition to their similarity in structure, the different Balbiani's vitelline body homologues may involve similar transport mechanisms. In *Xenopus*, both microtubule- and microfilament-based transport mechanisms have been shown to be required for localization of different RNAs to the mitochondrial cloud (Kloc and Etkin, 1995). In *Drosophila*, involvement of both microtubule- and microfilament-based transport in RNA localization is suggested by the finding that a cytoplasmic tropomyosin (cTmII) is required for *osk* mRNA localization (Erdelyi et al., 1995), in addition to a previously shown microtubule dependency (Pokrywka and Stephenson, 1995). So far, no effect of microfilament-destabilizing drugs (cytochalasin B and D) has been observed on *osk* or *bcd* mRNA localization (Pokrywka and Stephenson, 1995), but the microfilament network in general seems to be less sensitive to inhibitor treatment than the microtubule network (Emmons et al., 1995). In contrast to *osk* mRNA localization, no hint at an involvement of an actin-based transport or anchoring has been described for *bcd* mRNA localization. Consistent with this, no actin is detectable in the Exu-containing sponge bodies. However, we were not able to confirm a microtubule-dependent transport of the sponge bodies or Exu either, since a clear colocalization of Exu or sponge bodies with microtubules was not observed. Nevertheless, it is still possible that some proportion of Exu that is not localized to the sponge bodies is connected with the microtubule network, although we consider this to be rather unlikely.

Bohrmann and Biber (1994) have shown that intracellular transport of cytoplasmic particles within the germ line cells is dependent on microtubules, but microfilaments are involved in the transport through the ring canals. In addi-

tion, Pelham et al. (1996) have recently shown that some organelles are able to move along both microtubules and actin filaments depending on the concentration of a tropomyosin isoform in cultured rat epithelial cells. Furthermore, there is now growing evidence that microtubules can be required to organize actin bundles and vice versa in cultured cells (Challacombe et al., 1996; Fishkind et al., 1996). A similar link between the two systems is indicated by the organization of the microtubule and microfilament network by *cappuccino* or *chickadee* gene products in *Drosophila* germ line cells (Manseau et al., 1996). Therefore, the sponge bodies might migrate both along microtubules and microfilaments depending on their cytoplasmic environment in nurse cells and oocyte and they might require the two systems for different steps of their transport.

Association of Sponge Bodies with bcd mRNA

Many early transcripts and proteins are accumulated in early *Drosophila* oocytes. In this respect it is an interesting observation that these oocytes show small electron-dense granules resembling the amorphous matter of the sponge bodies, which form slightly later. RNA-rich regions of cells usually show up as electron-dense areas in electron micrographs, therefore it can be assumed that these granules in the early oocytes are accumulations of transcripts. These might then assemble with the elongated elements of the sponge bodies for subsequent transport or interaction with other factors.

To fulfill its function in *bcd* mRNA localization, we expect Exu to interact directly or indirectly with *bcd* mRNA at some point during oogenesis. Sponge bodies are good candidates for an intracellular compartment where this interaction could occur. Nevertheless, we still do not know whether the sponge bodies contain *bcd* mRNA and whether they are essential for the function of Exu in *bcd* mRNA localization. The manner in which Exu functions in *bcd* mRNA localization appears to be very different from that of Staufen. Staufen directly binds to double-stranded RNA, and its localization to the anterior pole of the embryo depends on the amount of *bcd* mRNA present in the embryos (Ferrandon et al., 1994). In contrast, the formation of the sponge bodies and the Exu distribution in the germ line cells is not dependent on the presence of *bcd* mRNA, and no direct interaction of RNA and Exu has been shown. In *exu* mutants, the *bcd* mRNA localization is disrupted within the oocyte, but the transport into the oocyte is not, and therefore Exu (unlike Staufen) might not be involved in the actual transport of the *bcd* mRNA. Since Exu is mostly localized on the sponge bodies in the nurse cells at the time of its action, we assume that its function is performed at this location. The function of Exu could somehow be to modulate a *bcd* mRNA–binding protein or *bcd* mRNA itself (e.g. by connecting it to a *trans*-acting factor), thereby allowing an Exu-independent but microtubule-dependent transport of the *bcd* mRNA to the anterior pole after entry into the oocyte. This assumption would be able to account for the different effect of additional microtubules on the *bcd* mRNA localization in wild-type and *exu* ovaries that was observed by Pokrywka and Stephenson (1991) without involving a colocalization of Exu and microtubules. While this model does not specify how the *bcd* mRNA is transported into the oocyte, it does not rule out a function for the observed migration of the sponge bodies in the transport of other factors.

Cotransport of Sponge Bodies and the Nuage

The movements of the Exu–GFP particles (Wang and Hazelrigg, 1994) and the presence of the sponge bodies in the ring canals (Fig. 6) imply that these structures migrate through the germ line cells. The remarkable accumulation of mitochondria next to the sponge bodies might provide the energy for this migration. Golgi complexes are found close to or in the sponge bodies. These can serve to transport molecules from the ER to their site of secretion. In a similar manner, the ER-like tubules within the sponge bodies might function as storage containers for transported proteins. In addition, it is an interesting observation that the sponge bodies surround the nuage, as this implies that the nuage could migrate in concert with the sponge bodies, at least on its way from the nuclei into the cytoplasm of the nurse cells. Because of their size and abundance, there is less evidence for a simultaneous migration of these two structures into and within the oocyte, although the transient, posterior localization of some proportion of Exu argues in favor of this possibility. This localization is not observed after colchicine treatment (Fig. 14 *f*), and polar granule-like particles do not localize to the posterior pole of colchicine-treated follicles (Clark et al., 1994; Pokrywka and Stephenson, 1995). Therefore the migration of the nuage and the sponge bodies might be mediated by a shared motor molecule. Exu has no effect on the formation of the polar granules nor is Vasa involved in the anterior system. Therefore, we cannot deduce so far whether the association of the nuage and sponge bodies results from a common transport mechanism, which might be required for the function of these structures in localization of the maternal mRNAs.

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