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Cytoskeletal Functions During *Drosophila* Oogenesis

Lynn Cooley and William E. Theurkauf

Organismal morphogenesis is driven by a complex series of developmentally coordinated changes in cell shape, size, and number. These changes in cell morphology are in turn dependent on alterations in basic cytoarchitecture. Elucidating the mechanisms of development thus requires an understanding of the cytoskeletal elements that organize the cytoplasm of differentiating cells. *Drosophila* oogenesis has emerged as a versatile system for the study of cytoskeletal function during development. A series of highly coordinated changes in cytoskeletal organization are required to produce a mature *Drosophila* oocyte, and these cytoskeletal transformations are amenable to a variety of experimental approaches. Genetic, molecular, and cytological studies have shed light on the specific functions of the cytoskeleton during oogenesis. The results of these studies are reviewed here, and their mechanistic implications are considered.

Drosophila ovaries are composed of parallel bundles of developmentally ordered egg chambers, each of which supports the development of a single oocyte. These bundles, called ovarioles, are divided into anterior and posterior compartments [Fig. 1A; for a comprehensive review of *Drosophila* oogenesis, see (*1*)]. Oogenesis is initiated in the anterior compartment of the ovariole, or germarium (Fig. 1B), by a stem cell division that produces a cystoblast and regenerates a stem cell (Fig. 1C). The cystoblast proceeds through four mitotic divisions to produce a cyst of 16 germline cells that will differentiate to form the single oocyte and 15 nurse cells found in each egg chamber. During oogenesis, the nurse cells synthesize maternal components for transport to the oocyte (Fig. 1D). Cytokinesis is incomplete at each of the cystoblast divisions, which leaves the 16 germline cells interconnected by large cytoplasmic bridges called ring canals, which are maintained through the completion of oogenesis.

Germariums are divided into four cytologically distinct regions that contain developmentally arrayed germline cysts (Fig. 1B). The stem cells and the mitotically dividing cystoblasts lie within germarial region 1, whereas newly formed 16-cell cysts are located in region 2a. When cysts progress into region 2b, they become lens-shaped and span the width of the germarium. The future oocyte is positioned at the center of the lens-shaped cysts from region 2b. By the time the 16-cell cyst occupies region 3 of the germarium, the oocyte is located at the posterior pole. The oocyte remains at the posterior of the germline cell cluster through the completion of oogenesis. In region 2a, somatic follicle cells begin to migrate between the 16-cell germline cysts. When they reside in region 3, the cysts are surrounded by a monolayer of follicle cells and are referred to as stage 1 egg chambers.

Stage 2 egg chambers bud from the germarium and enter the posterior compartment of the ovariole, or vitellarium (Fig. 1A). During stages 2 through 6, the egg chambers increase in size while remaining roughly spherical. The oocyte grows at approximately the same rate as a single nurse cell. Oocyte growth during stages 2 through 6 is the result of the transport of nutrients into the oocyte from the nurse cells.

During stages 7 through 10a, the oocyte endocytoses yolk proteins synthesized by fat bodies and follicle cells. Consequently, oocyte growth is more rapid than nurse cell growth, and by stage 10 the oocyte occupies the entire posterior half of the egg chamber (Fig. 1D). The morphogenetic molecules that specify the embryonic axes are asymmetrically positioned within the oocyte during these stages. Messenger RNA (mRNA) of *bicoid*, the primary anterior morphogen, is localized to the anterior cortex (*2*); the Vasa (*3*, *4*) and Staufen proteins and *oskar* mRNA (*5*), which are required for pole cell formation and posterior patterning, are positioned at the posterior pole; and *gurken* mRNA, which plays a key role in dorsoventral axis specification, accumulates between the dorsally located oocyte nucleus and the cortex (*6*).

During stages 10b through 12, the remaining nurse cell cytoplasm is transferred to the oocyte. As the nurse cells shrink, the oocyte expands (*7*). Nurse cell cytoplasm enters the oocyte and is mixed with the existing ooplasm by rapid ooplasmic movements. During stages 13 and 14, these ooplasmic movements stop and the meiosis I spindle assembles. The oocyte remains in the metaphase of the first meiotic division until it enters the oviduct and egg activation and fertilization initiate embryonic development.

Oocyte Specification

As outlined above, oogenesis in *Drosophila* begins with the formation of a cyst of 16 cells. Although the 16 sibling cells are interconnected by cytoplasmic bridges, only a single oocyte is produced. Oocyte differentiation thus reflects the establishment of a specialized region of cytoplasm within a syncytium.

The pattern of the four incomplete cystoblast divisions is precisely controlled and leads to the production of a cyst containing two cells with four ring canals, two cells with three ring canals, four cells with two ring canals, and eight cells with a single ring canal (Fig. 1C). One of the two cells with four ring canals invariably forms the oocyte, indicating that specification of the cytoplasmic compartment that will ultimately form the oocyte is linked to this cystoblast division pattern. The geometry of the cystoblast divisions, in turn, appears to depend on a structure called the fusome (*8*). The fusome is a region of cytoplasm that is rich in vesicles and membrane-associated cytoskeletal proteins that forms along mitotic spindle remnants during the cystoblast divisions (*9*). At the completion of each division, newly formed segments of fusome merge with material from previous mitoses. As a result, the fusome becomes a continuous branched structure that extends through the intercellular bridges that connect all of the germline cells (Fig. 2D). One spindle pole in each mitotic cystoblast is always anchored in the fusome (Fig. 2C). Because the orientation of the spindle determines the mitotic cleavage plane, the fusome has a direct effect on the geometry of the cystoblast divisions. Once all four mitotic cell cycles are complete, the fusome disappears.

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The cystoblast division pattern may place one of the cells with four ring canals in a position to receive oocyte-specifying signals from the surrounding somatic tissue. This seems unlikely, however, because newly formed region 2a cysts appear to be randomly oriented with respect to the axis of the germarium (*10*). An alternative model is that oocyte specification occurs during the cystoblast divisions. For example, oocyte specification could be achieved through the partitioning of a key oocyte-specification factor to a single cell during the cystoblast mitoses (*7*, *11*). This model is formally analogous to the process of P-granule segregation to the germline precursors that occurs during the early *Caenorhabditis elegans* embryonic mitoses, a process that also requires a stereotyped division pattern (*12–14*). In this model, the fusome is critical for oocyte specification because it controls the geometry of the cystoblast divisions, which allows the asymmetric segregation of an oocyte-specification factor to a single cell (9). At present, no clear candidates exist for the hypothesized oocyte-specification factor.

Oocyte Differentiation

Ultrastructural analyses indicate that oocyte differentiation involves a dynamic redistribution of components within early germline cysts. When in region 2a, synaptonemal complexes (SCs), which are normally associated with paired meiotic chromosomes, begin to assemble in both of the cells with four ring canals and in the two cells with three ring canals (*15*). As the cysts mature, the SCs break down in all but one of the cells with four ring canals. This cell becomes the oocyte, whereas the remaining 15 cells become polyploid nurse cells. Serial section electron microscope studies have further demonstrated that the centrioles that are initially associated with the progenitor (pro) nurse cells migrate through the ring canals toward the pro-oocyte and are largely restricted to the oocyte by the time the cysts enter germarial region 3 (*10*). These observations suggest that the centrioles and at least one limiting factor for SC formation are actively recruited to the future oocyte during germarial development. Differences in the composition of the oocyte and of the nurse cell cytoplasm are first detectable when the cysts are located in germarial region 2a, when a number of mRNAs and proteins accumulate specifically in the pro-oocyte. The oocyte is transcriptionally inactive during most of oogenesis, which suggests that oocyte-specific mRNAs and proteins are synthesized in the nurse cells and then transported to the oocyte (*16*).

Cytoskeletal organization during early

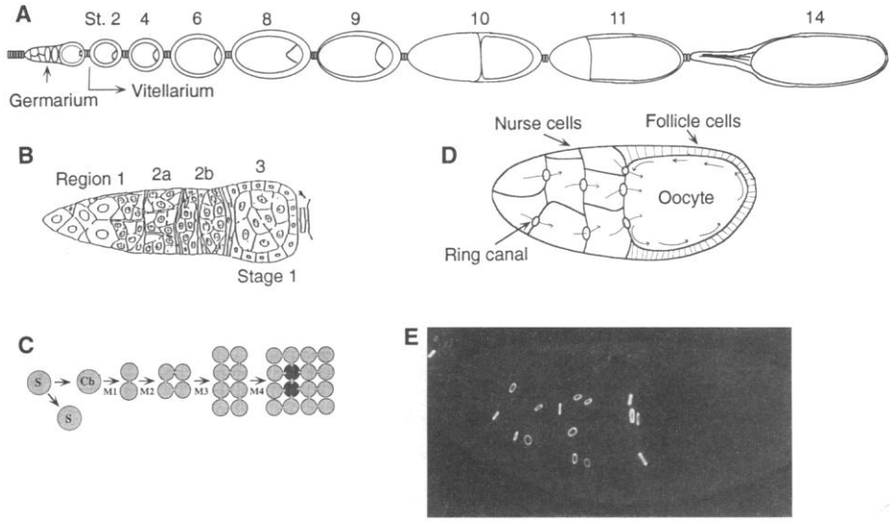

Fig. 1. Oogenesis in *Drosophila*. Egg chambers develop assembly-line fashion in tubular ovarioles (**A**), several of which make up each ovary. The oocyte of each egg chamber is at the posterior (right). Oogenesis begins in region 1 of the germarium (**B**), with the division of a germline stem cell to produce a cystoblast and regenerate a stem (S) cell (**C**). The cystoblast (Cb) proceeds through four incomplete mitotic divisions to yield a cyst of 16 interconnected cells. One of the cells with four intercellular bridges [(C), dark shading] becomes the oocyte. The remaining 15 cells develop as polyploid nurse cells that synthesize most of the ooplasmic components. Late in oogenesis, nurse cell cytoplasm flowing into the oocyte is vigorously mixed in the oocyte [arrows in oocyte in (**D**)]. The cytoplasmic bridges connecting the cells develop into ring canals. A protein from the *hts* gene required for ring canal development is located specifically at ring canals as seen by immunofluorescence with an antibody to the Hts protein (*23*) (**E**). (B) is adapted from (*10*).

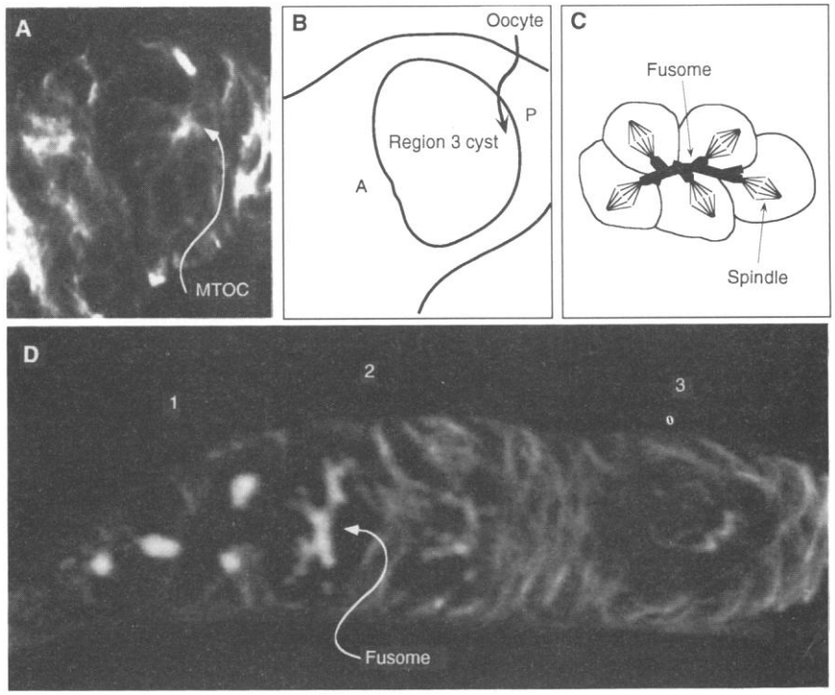

Fig. 2. Microtubules and the fusome in germariums. In regions 2 and 3 of the germarium, a MTOC forms in the oocyte, with microtubules extending into the nurse cells. (**A**) Region 3 cyst stained with tubulin antibodies and a fluorescent secondary antibody. (**B**) Diagram of the cyst in (A), showing the position of the oocyte. A, anterior; P, posterior. The fusome forms during the germline mitotic divisions. The anchoring of one pole of each mitotic spindle in the fusome [(**C**), adapted from (*67*)] is involved with orienting the division plant. The fusome can be seen by staining germariums with antibodies to spectrin or a protein from the *hts* gene [different from the Hts ring canal protein (*9*)]. In (**D**), staining with antibodies to Hts reveals the fusomes (bright staining in regions 1 and 2) and the follicle cell membranes. The fusome begins to disappear when the mitoses are complete and is very faint by region 3.

oogenesis is consistent with a role for microtubules in the establishment of these nuclear and cytoplasmic asymmetries (*17*). In the newly formed cysts in region 2a, microtubules are uniformly distributed among the 16 germline cells. As cysts pass through region 2a, however, a single dominant microtubule focus or microtubule organizing center (MTOC) develops within each 16-cell cyst. In region 2b and 3 cysts, the MTOC clearly lies within the future oocyte (Fig. 2, A and B). Double label immunofluorescence analyses demonstrate that microtubules extend from this MTOC, through the ring canals, and into the nurse cells (*17*). The microtubule cytoskeleton therefore structurally differentiates the pro-oocyte from the remaining cells in the cyst.

Inhibitor studies provide evidence that the asymmetric microtubule cytoskeleton within the 16-cell cysts is required for oocyte differentiation. Adult female flies fed small amounts of microtubule assembly inhibitors produce young egg chambers that contain 16 nurse cells and no oocyte (*17*, *18*). These treatments disrupt microtubules within the 16 germline cells in each cluster but have relatively little effect on microtubules in the overlying follicle cells (*17*). The germline microtubule cytoskeleton thus appears to play a critical role in oocyte differentiation. Time course studies with these inhibitors indicate that the functional requirement for microtubules in oocyte differentiation is specific to germarial region 2, when the MTOC assembles and synaptonemal complexes become restricted to a single cell (*15*, *17*). The functional requirement for microtubules in oocyte differentiation is therefore temporally correlated with the morphological differentiation of the pro-oocyte from the pro-nurse cells.

Cytological analyses of mutations that prevent oocyte differentiation provide further evidence that the polarized germline microtubule cytoskeleton is essential for oocyte differentiation and reveal steps in the oocyte differentiation pathway (*17*). Loss-of-function mutations at both the *egalitarian* (*egl*) and *Bicaudal-D* (*Bic-D*) loci lead to the production of cysts that contain 16 nurse cells and no oocyte (*19*, *20*). In *egl* mutant germariums, microtubules initially reorganize normally to form an MTOC in a single cell. However, the MTOC is unstable, which indicates that its establishment and maintenance are independent processes. Two hypomorphic alleles of *Bic-D* (*R26* and *PA66*) block establishment of the polarized microtubule array (*20*, *21*). However, in *Bic-D*^{R26} mutant germariums, initial oocyte specification appears to take place. Both *oskar* mRNA and mutant Bic-D protein accumulate in a single cell of each cyst (*21*). Therefore, the polarized microtubule array appears to be downstream of initial oocyte specification.

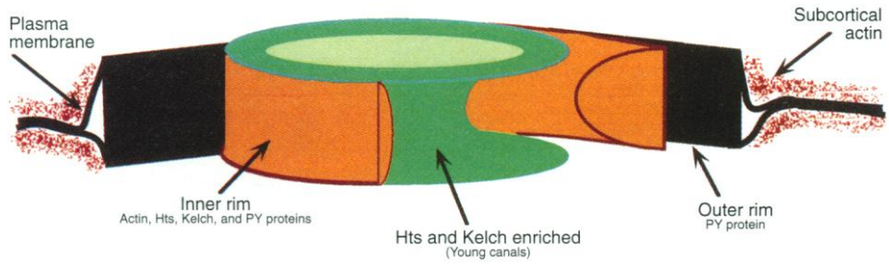

Fig. 3. Ring canal structure (*23*). The outer and inner rim of a ring canal are diagrammed partially peeled away from the channel. The outer rim is associated with the plasma membrane and contains at least one protein phosphorylated on tyrosine (PY protein). The inner rim contains actin filaments that colocalize with proteins from the *hts* and *kelch* genes, as well as one or more PY proteins. In ring canals from young egg chambers (up to stage 6), the Kelch and Hts proteins are also enriched on the innermost surface of the inner rim. The diameter of the channel through a ring canal is about 1 μm when it is established in the germarium and increases to about 8 μm in stage 10 egg chambers.

Microtubules are intrinsically polar filaments with distinct plus and minus ends. Microtubule depolymerization studies on germariums and early egg chambers suggest that the minus ends are located in the oocyte and that the plus ends extend into the surrounding nurse cells (*22*). Microtubule assembly inhibitors disrupt the oocyte-specific accumulation of at least three mRNAs within germarial cysts and early egg chambers, implying that polarized microtubules provide a scaffold for transport to the oocyte (*17*). These observations, combined with the cytological studies reviewed above, suggest that these mRNAs associate with specific microtubule motors and move to the oocyte along the microtubule scaffold.

These data support a three-step model for oocyte differentiation. The first step generates the asymmetry that specifies the cytoplasmic compartment that will form the oocyte. This step appears to occur during the cystoblast divisions or in the earliest region 2a cysts. The second step is a reorganization of the germline microtubules in order to form a polarized scaffold focused on the oocyte. Finally, the transport of mRNAs and proteins to the pro-oocyte along the polarized microtubule scaffold extends the asymmetry that ultimately leads to the biochemical and cytological differentiation of the oocyte.

Ring Canal Assembly

The transport of mRNA and protein from the nurse cells to the developing oocyte is made possible by the presence of stable intercellular bridges that link the cells in each cyst (Fig. 3). These intercellular bridges, called ring canals, are the end products of a complex elaboration of the arrested mitotic cleavage furrows that form during the cystoblast divisions. Initially, an electron-dense thickening of the plasma membrane forms at the rims of the intercellular canals. As cysts pass through the germarium, their ring canals acquire a less electron-dense inner rim, which leaves a channel about 1 μm in diameter.

Ring canal maturation involves the sequential addition of proteins. An early ring canal component is identified by immunoreactivity to a phosphotyrosine-specific antibody (*23*). This antibody recognizes intercellular bridges within germarial region 1 mitotic clusters and may react with the arrested cleavage furrows. Because this antibody recognizes ring canals of mutant cysts that lack the inner rim, at least one phosphoprotein it recognizes is likely to be a part of the membrane-associated outer rim. The number of ring canal components recognized by this antibody remains to be determined.

The three identified ring canal components include filamentous actin and the products of the *hu-li tai shao* (*hts*) and *kelch* genes. In region 2a, just after the cystoblast divisions are completed, both filamentous actin (*24*) and a product of the *hts* gene (*23*) associate with the ring canals. Although actin is also present at the plasmic membranes, Hts protein is completely specific to the ring canals (Fig. 1E). Because actin is not detected in the ring canals of *hts* mutant ovaries (*25*), Hts protein is probably required to assemble or stabilize actin at the rim of the ring canal.

The Hts ring canal protein is produced from an ovary-specific transcript with an open reading frame that could encode a 128-kD protein. The NH_2-terminal half of the predicted protein is homologous to the vertebrate actin binding protein adducin, and the COOH-terminal half shows no significant homology to previously characterized proteins (*25*, *26*). The ring canal–specific form of Hts is only 60 kD and is derived from the COOH-terminal half of the *hts* open reading frame (*23*). Efforts are under way to determine how this protein is produced.

A product of the *kelch* gene is the last well-characterized protein to be recruited specifically to the ring canal (*27*). Kelch protein is not detected on ring canals until stage 1 and does not associate with all ring canals until stage 2 or 3 (*23*, *27*). Because ring canal assembly begins in germarial region 2, Kelch does not appear to be required to initiate ring canal formation. In support of this hypothesis, the phosphotyrosine protein actin and Hts are normally localized to the germarial ring canals in *kelch* mutant ovaries. In later stage *kelch* egg chambers, however, actin and Hts, which normally form a compact rim, extend into the channel (*23*). Consequently, mutant ring canals are partially occluded, which suggests that the undersized and infertile eggs produced by *kelch* females are the result of insufficient flow of nurse cell cytoplasm through these obstructed intercellular bridges.

Ring canal deterioration in *kelch* egg chambers coincides with the initiation of ring canal growth, which suggests that Kelch protein is required to maintain the structure of the actin-based ring canals as they increase in diameter. The predicted amino acid sequence of one of the Kelch proteins is consistent with this hypothesis. At least two proteins are produced by the *kelch* gene: One terminates at a stop codon near the middle of the long open reading frame and another, a full-length protein, appears to be produced by suppression of the stop codon (*27*). Ths shorter 80-kD protein contains a 120–amino acid motif near its NH_2-terminus, termed a BTB (BR-C, ttk, and bab) box or POZ (poxvirus and zinc finger) domain, that functions as a specific protein-protein interaction domain in a number of transcription factors (*28*, *29*). The Kelch BTB box made in *Escherichia coli* can dimerize in solution, as shown by dynamic light scattering (*30*). The COOH-terminal half of the 80-kD Kelch protein contains six copies of a 50–amino acid motif, termed the Kelch repeat, also found in the actin binding protein scruin (*31*). Scruin has six Kelch repeats at the NH_2-terminus and six Kelch repeats at the COOH-terminus, and each of these two Kelch repeat domains appears to bind actin monomer (*32*, *33*). As a result, a single scruin molecule can make contacts with two actin monomers within an actin filament or can cross-link adjacent actin filaments. These observations suggest a model in which Kelch dimerizes through its BTB box to produce a bifunctional actin binding molecule that cross-links and stabilizes actin filaments in the ring canal.

In a wide range of species, male and female germline cells are connected by intercellular bridges during some or most of their development, and ring canal–like structures are found at these bridges. For example, mammalian female germline cells are present as syncytial clusters during early ovary development in the fetus (*34*), and spermatogonia remain interconnected by cytoplasmic bridges until a cluster of postmeiotic spermatids separate from the syncytial residual body. In these cases, syncytial development may be required for the synchronization of the mitotic and meiotic cell cycles, rather than for the transport of nutrients to a particular cell. In *Drosophila*, ring canals are also present between somatic cells in imaginal disc epithelia (*35*) and the follicle cell epithelium of egg chambers. The function of these somatic ring canals is not known.

Selective Transport Through Ring Canals

A subset of the mRNAs and proteins that are synthesized in the nurse cells, which includes several products of early pattern formation genes, are selectively transported to the oocyte during oogenesis stages 2 through 6. In contrast, organelles and many proteins and mRNAs appear to travel into the oocyte in a constant unselective nutrient stream (*36*). Recent work suggests that ring canals and associated cytoskeletal elements may participate in these transport processes. With the use of video-enhanced contrast microscopy, single particles have been observed moving through the ring canals and into the oocyte of egg chambers from stages 7 through 10 (*37*). Only a subset of the particles in the vicinity of a ring canal move through the channel, which indicates that this transport process is selective. Movement of the particles through ring canals is inhibited by cytochalasin, which suggests that it is actin-based. Actin filaments that extend through ring canals have not been identified, but the filaments that mediate this process may not be preserved by standard cytochemical procedures.

The particles seen moving into the oocyte may include complexes of mRNA and proteins that are targeted specifically to the oocyte. One such complex appears to contain *bicoid* mRNA and Exuperantia (Exu) protein. Exu protein is required for the localization of *bicoid* mRNA to the anterior cortex. This protein is found in particles in the nurse cells and accumulates transiently at the anterior cortex of the oocyte (*38*, *39*). An Exu–green fluorescent protein fusion protein has been found to localize to particles that are concentrated near ring canals in a microtubule-dependent manner (*40*). These observations suggest that selective transport into the oocyte might involve both microtubule-based recruitment to the vicinity of a ring canal and actin-based passage through the canal.

Axis Specification

The axes of the *Drosophila* embryo are specified through the asymmetric localization of morphogenetic determinants within the oocyte during stages 7 through 10. The movement of these determinants to the proper position at the oocyte cortex, like oocyte differentiation, is dependent on microtubule function. The oocyte-focused microtubule cytoskeleton that forms in the germarium persists until stage 6. During stages 7 and 8, however, the oocyte MTOC is lost, and a posterior cluster of centrioles that colocalizes with the MTOC degenerates (*10*, *22*). Concomitantly, microtubules begin to associate with the anterior cortex of the oocyte. Initially, microtubules are concentrated at the anterior margin of the oocyte. By stage 9, however, an anterior to posterior gradient of oocyte microtubules is present (Fig. 4A) (*22*).

Inhibitor studies support a direct role for the polarized cortical microtubule network in anteroposterior patterning. The primary anterior morphogen, *bicoid* mRNA, is localized to the anterior cortex during stages 8 through 10 (*2*). Upon microtubule depolymerization, *bicoid* mRNA is released into the ooplasm, and it returns to the cortex when the depolymerizing drug is removed (*41*). The anterior localization of the Exu protein is also disrupted by microtubule depolymerization (*40*). Proteins and mRNAs that are required for posterior patterning also become localized in the oocyte during stages 8 through 10. Posterior localization of at least two of these molecules, the Staufen protein and *osk* mRNA, is disrupted by microtubule depolymerizing drugs (*42*). These observations suggest that microtubules are required to localize morphogenetic molecules to both the anterior and posterior poles of the oocyte.

There is indirect evidence that the plus and minus ends of oocyte microtubules are oriented with respect to the anterior-posterior axis. In oocytes from stages 8 through 10, limited inhibitor-induced depolymerization leaves short microtubules associated with the anterior cortex, which suggests that the microtubule nucleating sites that anchor the minus end are at the anterior pole (*22*). In addition, a fusion protein that contains the mechanochemical head domain of kinesin, a plus end–directed microtubule motor, accumulates at the posterior pole of the oocyte (*42*). This evidence suggests that most of the microtubules in oocytes from stages 7 through 10 are nucleated at the anterior pole, with their plus ends extending into the oocyte toward the pos-

terior. However, cytoplasmic dynein, a microtubule motor that is minus end–directed, accumulates at the posterior of stage 9 oocytes (*43*). This observation suggests either (i) that the oocyte contains two populations of polarized microtubules or (ii) that microtubule motor localization is not a reliable indicator of microtubule polarity.

One model for microtubule-dependent anteroposterior axis specification is that microtubule nucleating activity is redistributed from the posterior MTOC to the anterior cortex during stages 7 and 8, which leads to the nucleation of oocyte microtubules at the anterior cortex. Morphogenetic molecules that are destined for the anterior pole then complex with minus end–directed microtubule motors and move toward the anterior pole along microtubules. Conversely, proteins and mRNAs destined for the posterior pole associate with plus end–directed motors and move toward the posterior along the same microtubule scaffold. Once localized to the appropriate pole, the morphogens appear to be anchored by an as yet unidentified cortical structure. This anchoring is needed to maintain axial asymmetry during the vigorous ooplasmic streaming that accompanies the final stages of oogenesis (*44*).

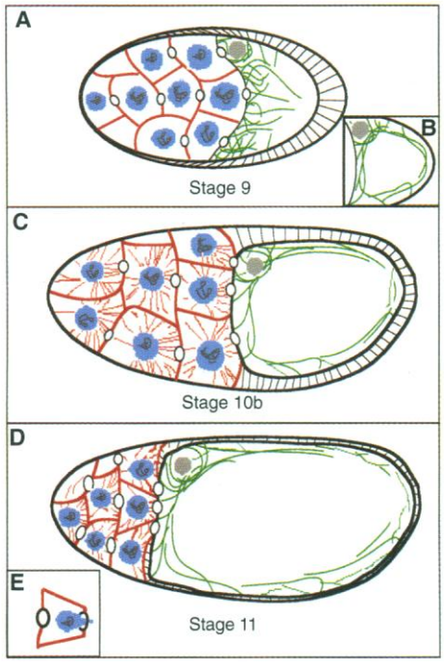

Fig. 4. Cytoskeleton rearrangements in late stage egg chambers. (**A**) In stage 9 egg chambers, actin filaments are present subcortically in the nurse cells (highlighted in red), oocyte, and follicle cells. Microtubules (green) are present in an anterior to posterior gradient in the oocyte and form a cage around the oocyte nucleus (gray). (**B**) In *cappuccino* and *spire* mutants, the gradient of microtubules is replaced by subcortical microtubule bundles that are associated with a premature initiation of ooplasmic streaming. (**C**) Stage 10b egg chambers contain actin filament bundles in the nurse cell cytoplasm that extend from the plasma membrane to the nuclear membranes (nuclei in blue). Microtubules in the oocyte cortex mediate coplasm mixing. (**D**) As the nurse cells regress in stage 11, rapid oocyte growth is accompanied by follicle cell flattening and follicle cell migration around the anterior of the oocyte. The nurse cell nuclei remain in the center of the nurse cells. (**E**) In *chickadee*, *singed*, and *quail* mutants, the cytoplasmic actin bundles are absent in nurse cells and the nuclei become lodged in ring canals.

The mechanism of dorsoventral patterning is not clear, but available data suggest that microtubules are involved. The movement of the oocyte nucleus to the dorsal surface, which is the earliest morphological indication of dorsal-ventral asymmetry, is inhibited by microtubule depolymerization (*18*). Microtubules could play a role in moving the oocyte to the dorsal cortex or they could simply maintain the asymmetric position of the nucleus after it is in position. The effects of microtubule depolymerization on the dorsal localization of *gurken* mRNA (6) have not been reported.

The microtubule dependence of axial patterning raises the possibility that a subset of the genes that are required for axis specification affect microtubule organization. The *cappuccino* (*capu*) and *spire* (*spir*) mutations affect both dorsoventral and anteroposterior patterning and thus occupy a unique position in the genetic hierarchy that controls axis specification. Mutations at both of these loci induce a dramatic reorganizing of the oocyte microtubule cytoskeleton (*45*). In *capu* and *spir* mutants, the anterior to posterior cortical gradient of microtubules that is normally present during axial patterning is not established, and prominent microtubule bundles can be observed just beneath the oocyte cortex (Fig. 4B). In addition, microtubule-dependent ooplasmic streaming, which normally occurs after axis asymmetry is established, during stages 10b through 12, is prematurely initiated. The patterning defects associated with the *capu* and *spir* mutations could be caused either by the changes in microtubule organization or by the mechanical disruption of morphogen localization by the streaming itself. A combination of these factors may also be responsible for the pleiotropic patterning defects that are induced by these mutations.

Final Transport of Nurse Cell Cytoplasm

During stage 11, the remaining nurse cell cytoplasm is rapidly transferred to the oocyte (Fig. 4D). This results in a doubling of the oocyte volume and complete regression of the nurse cells in about 30 min. The nurse cell nuclei become permeable just before the onset of nurse cell regression, which allows karyoplasm to enter the oocyte (*46*). The remnants of nurse cell nuclei, including the membranes and condensed chromatin, are excluded from the flow of cytoplasm through ring canals.

Egg chambers from stage 10 undergo major cytoskeletal rearrangements in preparation for final cytoplasm transport. An extensive array of cytoplasmic actin filament bundles forms in the nurse cells (*47*). One end of these bundles is anchored in the plasma membrane, and the other is embedded in the nuclear membrane. As a result, each nurse cell nucleus becomes surrounded by a cytoskeletal halo (Fig. 4C). Mutations at the *chickadee*, *singed*, and *quail* loci prevent the assembly of these cytoplasmic actin bundles. In each of these mutant backgrounds, the nurse cell nuclei become lodged in the ring canals as the final rapid flow of cytoplasm begins (Fig. 4E). As a result, the flow of cytoplasm from the nurse cells is blocked, and small, infertile eggs are produced. These observations indicate that the cytoplasmic actin networks keep the nurse cell nuclei from moving into the ring canals during nurse cell regression.

The genes *chickadee*, *singed*, and *quail* encode proteins that are homologous to an actin binding protein. The *chickadee* gene encodes *Drosophila* profilin, a small actin monomer binding protein (*46*); *singed* and *quail* encode homologs of actin filament crosslinking proteins (*48*, *49*). The in vitro properties of profilin suggest that it is involved in controlling actin filament polymerization and may also play a role in the regulation of signal transduction (*50*, *51*). The *chickadee* gene produces a generally expressed transcript and a transcript that is restricted to the female germ-line, each of which encodes the same polypeptide. Mutations that disrupt these different transcripts produce distinct development defects (*52*). Null alleles of *chickadee* are lethal, which indicates that profilin may be essential for cell function during many stages of development. Alleles of the gene that disrupt the germline-specific *chickadee* transcript induce the defects described above: Adult mutant females are viable but sterile and produce egg chambers in which cytoplasmic actin bundles fail to assemble during stage 10. Significantly, the more general *chickadee* transcript is expressed in these mutant egg chambers. These observations suggest that basal levels of profilin may be sufficient for some actin-dependent processes and signal transduction, whereas higher concentrations are needed during the assembly of the specialized actin filament bundles during oogenesis.

The *singed* and *quail* genes encode proteins that cross-link actin filaments into bundles. The Singed protein is homologous

to sea urchin fascin (*48*, *53*, *54*), and the *quail* gene encodes a villin-like protein (*49*). Sea urchin fascin cross-links actin filaments into hexagonal arrays and produces periodic crossbanding in electron micrographs of the bundled filaments (*55*, *56*). Villin, in contrast, does not give actin bundles a consistent, organized packing pattern (*57*). The genetic analysis of these genes demonstrates that the functions of the two bundling proteins are not redundant. Similar to other systems with two bundling proteins (*58*), the Singed and Quail proteins may be required for distinct aspects of actin bundling, such as organization and stability.

Oogenesis requires the action of cytoskeletal proteins that are required throughout development, as well as the products of germline-specific genes. For example, the *armadillo* gene is first required during segmentation of the embryo (*59*). This gene encodes a protein that is related to vertebrate plakoglobin and β-catenin, which appear to function in anchoring the cytoskeleton to sites of cell-cell adhesion (*60*). The germline function of Armadillo protein has been analyzed by the induction of clones of mutant cells within the ovary. These studies indicate that Armadillo is required for the assembly of the stage 10 cytoplasmic actin bundles and for the assembly of more generalized actin-based structures in the egg chamber (*61*).

The oocyte microtubule cytoskeleton also undergoes a major rearrangement in preparation for nurse cell regression. Late in stage 10, microtubules in the oocyte reorganize into parallel arrays that lie 5 to 10 μm below the surface (*22*) (Fig. 4, B and C). At the time that these subcortical arrays assemble, the ooplasm begins swirling vigorously (*44*). When cytoplasm transfer to the oocyte is complete, ooplasmic streaming ceases, the subcortical microtubules are disassembled, and the meiotic spindle forms (*62*). This streaming, which mixes the incoming nurse cell cytoplasm with the existing ooplasm, is inhibited by microtubule-depolymerizing agents (*63*). These observations suggest that ooplasmic streaming is driven by organelle transport along the subcortical microtubule bundles.

Force generation during rapid cytoplasm transport is not well understood. Inhibitor studies indicate that this process is dependent on actin filaments (*47*), but these experiments do not identify the cells of the egg chamber in which actin filaments are required. The nurse cells, oocyte, and follicle cells all undergo dramatic shape changes during nurse cell regression: The nurse cells contract, the oocyte expands, and the follicle cells flatten. Transfer of nurse cell cytoplasm could be driven by an concerted contraction of the nurse cell cortex (*46*), by an actin-dependent oocyte expansion, or by follicle cell flattening (*1*). It is likely that cell shape changes in all three cell types contribute to the dramatic shift of cytoplasm to the oocyte.

Conclusions

Cytoskeletal elements clearly have a critical role in the assembly of a functional oocyte. However, the studies reviewed here only hint at the underlying molecular mechanisms. Before these mechanisms can be fully understood, several key features of cytoskeletal organization must be rigorously examined. For example, the structural polarity of microtubules within early egg chambers and later oocytes has been inferred by indirect means but has not been unambiguously determined. The ultrastructure of the microtubule and actin filament systems also remain to be studied in detail. In addition, the signaling pathways responsible for triggering cytoskeletal rearrangements are unknown, and the functions of cytoskeletal elements within the somatic follicle cells have not been thoroughly explored.

The genetic analyses of oogenesis in *Drosophila* reviewed here have largely focused on the relatively small set of genes that can be mutated to produce female sterility. Assembly of a mature oocyte, however, depends on interactions between the specialized proteins identified in these studies and a much larger group of zygotically active genes that are required throughout development. Mutations in these zygotically active genes will usually be lethal during pre-adult stages, precluding a direct analysis of their functions during oogenesis. Techniques are now available for the clonal analysis of these lethal mutations within the developing oocyte (*64*–*66*). Systematic analysis of the germline functions of genes required throughout development with this technology should help define additional cytoskeletal functions and regulators important for oocyte morphogenesis.

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Vertebrate Embryonic Induction: Mesodermal and Neural Patterning

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Within the fertilized egg lies the information necessary to generate a diversity of cell types in the precise pattern of tissues and organs that comprises the vertebrate body. Seminal embryological experiments established the importance of induction, or cell interactions, in the formation of embryonic tissues and provided a foundation for molecular studies. In recent years, secreted gene products capable of inducing or patterning embryonic tissues have been identified. Despite these advances, embryologists remain challenged by fundamental questions: What are the endogenous inducing molecules? How is the action of an inducer spatially and temporally restricted? How does a limited group of inducers give rise to a diversity of tissues? In this review, the focus is on the induction and patterning of mesodermal and neural tissues in the frog *Xenopus laevis*, with an emphasis on families of secreted molecules that appear to underlie inductive events throughout vertebrate embryogenesis.

A fundamental experiment in the history of embryology was the organizer transplant of Spemann and Mangold (*1*). In this impressive demonstration of induction in the newt, transplantation of a gastrula dorsal blastopore lip to a region fated to form ventral mesoderm resulted in formation of a second body axis. In the chick, fish, and mouse, transplants of the node, the anatomical equivalent of the amphibian blastopore lip, resulted in similar axial organization (*2–5*). This type of experiment became a much discussed example of induction (*6*) and has challenged biologists for decades to explain how one group of cells controls the fate of its neighbors.

Over the past century, numerous inductive events have been described in vertebrates including multiple interactions between the three germ layers (endoderm, mesoderm, and ectoderm) and within each germ layer. Reciprocal inductions occur throughout early development, and later multiple mesenchymal-epithelial inductions underlie organogenesis (*7*). In essence, virtually every vertebrate tissue and organ is formed by some type of induction. Mesoderm and neural induction have received considerable attention in recent years, and the molecules and principles used in these early events may be relevant to subsequent tissue and organ formation. With this in mind, we examine current advances in mesoderm and neural induction in vertebrates.

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Mesoderm Induction

The importance of endoderm in the induction of mesoderm in the frog, *Xenopus laevis*, was established by Nieuwkoop and colleagues. In isolation, explanted blastula animal and vegetal pole cells form only ectoderm and endoderm, respectively, but ectoderm can be induced to form mesodermal structures in recombinants containing both presumptive ectoderm and endoderm (*8–10*). In addition, although explants of the marginal zone (presumptive mesoderm; Fig. 1) from a 32-cell stage embryo fail to form mesoderm, blastula stage explants will form mesoderm, implicating a progressive interaction between endoderm and ectoderm to form mesoderm (*11*, *12*). These observations suggest that vegetal endoderm produces a mesoderm-inducing signal during cleavage stages.

In addition to inducing mesoderm, vegetal endoderm can confer a dorsal-ventral pattern on mesoderm. Dorsal vegetal cells induce dorsal mesoderm (notochord and muscle), whereas lateral and ventral vegetal cells induce ventrolateral mesoderm (mesenchyme, blood, and small amounts of muscle) (*13–15*). In addition, transplanted dorsal vegetal blastomeres can induce ectopic dorsal axial structures, an activity that has led to these cells being designated the endodermal organizer or Nieuwkoop center (*16–19*).

In *Xenopus*, the dorsal-ventral axis is established at fertilization with sperm entry stimulating a reorganization of egg contents by cortical rotation, leading to demarcation of future dorsal tissues opposite the site of sperm entry. Cortical rotation, a displacement of the surface (or cortex) of the egg relative to the inner cytoplasm, is thought to result in the formation of a "dorsal determinant" in the presumptive endoderm (Fig. 1) (*20*). Disruption of cortical rotation by ultraviolet (UV) irradiation results in the loss of dorsal axial structures (*19–22*). Both cortical rotation and subsequent axis formation can be rescued by manual tipping of the egg, which causes gravity-induced rearrangements (*23*, *24*). Gravity-driven rotation during the blastoderm stage is also responsible for axis formation in the chick (*25*).

Mesodermal patterning continues during gastrulation, as evidenced by the fact that as late as the gastrula stage explanted lateral marginal zone tissue forms ventral mesoderm rather than the intermediate mesodermal tissues (muscle and kidney) predicted from the fate map. Organizer tissue can induce ventral and lateral marginal zones to form intermediate mesoderm, suggesting that the gastrula stage organizer "dorsalizes" neighboring ventral mesoderm (Fig. 1) (*15*, *26*, *27*). Furthermore, examination of muscle formation indicates that local communication within a single tissue type is also required for differentiation. These studies showed that blastula or gastrula explants of presumptive muscle fail to form differentiated muscle if fewer than 100 cells are present. This community effect (*28*, *29*), distinct from other inductive interactions, appears to regulate the coordinate differentiation of specified mesodermal tissues and may be essential to the orderly patterning of the marginal zone (*30*, *31*).

These studies illuminate the cellular basis of mesoderm induction. Cortical rotation generates a dorsal determinant that resides in dorsal vegetal blastomeres, the endodermal organizer (Nieuwkoop center), and that subsequently induces formation of the mesodermal organizer (Spemann organizer) (Fig. 1). Although promising candidates for endogenous mesoderm inducers have been identified, it has not yet been possible to assign them to specific inducing functions in vivo.