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Review

Patterning and morphogenesis of the follicle cell epithelium during *Drosophila* oogenesis

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CONTENTS

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Introduction

The *Drosophila* egg is not only polarized in its shape along the two major axes, the anterior-posterior (AP) axis and the dorsal-ventral (DV) axis, but also carries localized maternal signals to instruct embryonic pattern formation during subsequent embryogenesis. The development of the polarized egg requires cell signaling interactions between germline cells and somatic follicle cells, and between sub-populations of follicle cells. In recent years, a number of developmentally important questions relating to follicle cell activity and function have been studied. For example, how are the follicle cells interacting with the germline cell in the determination of polarity of both the embryo and the eggshell? The egg chamber, which is the developmental unit of oogenesis, contains sixteen germline cells encapsulated by a sheet of epithelial follicle cells: how do the germline cells and somatic cells co-operate to form an egg chamber? What are the germline signals that trigger follicle cell differentiation? Other areas of follicle cell related studies include the regulation of vitellogenesis and hormonal regulation in development. In this review, we will discuss recent progress in the understanding of the interactive roles the follicle cells play during oogenesis.

Morphology of *Drosophila* oogenesis

The adult *Drosophila* female has a pair of ovaries, each of which contain 16-20 ovarioles that consist of developmentally ordered egg chambers. Every egg chamber supports the development of a single oocyte/egg. The egg chambers, according to their size and morphology, can be divided into 14 developmental stages (Fig. 1A) (for reviews see King, 1970; Mahowald and Kambysellis, 1980; Spradling, 1993; Bownes, 1994a; Lasko, 1994).

Oogenesis starts within the anterior compartment of the ovariole, the germarium (Fig. 1B). The germline stem cells divide to produce a daughter stem cell and a cystoblast. The latter undergoes four mitotic divisions to form a 16-cell-cyst, one of which is determined to become the oocyte; the others become nurse cells. Cytokineses are incomplete at each of the cystoblast divisions, so the cells are connected by cytoplasmic bridges called ring canals. The germarium can be subdivided into four regions. The stem cell and the mitotically divided cystoblasts lie within germarium region 1, whereas newly formed 16-cell cysts are located in region 2a. In region 2b, the 16-cell cysts become lens-shaped with the pro-oocyte positioned at the centre of the cysts. By the time the cyst occupies germarium region 3, the oocyte is located at the posterior pole, where it will

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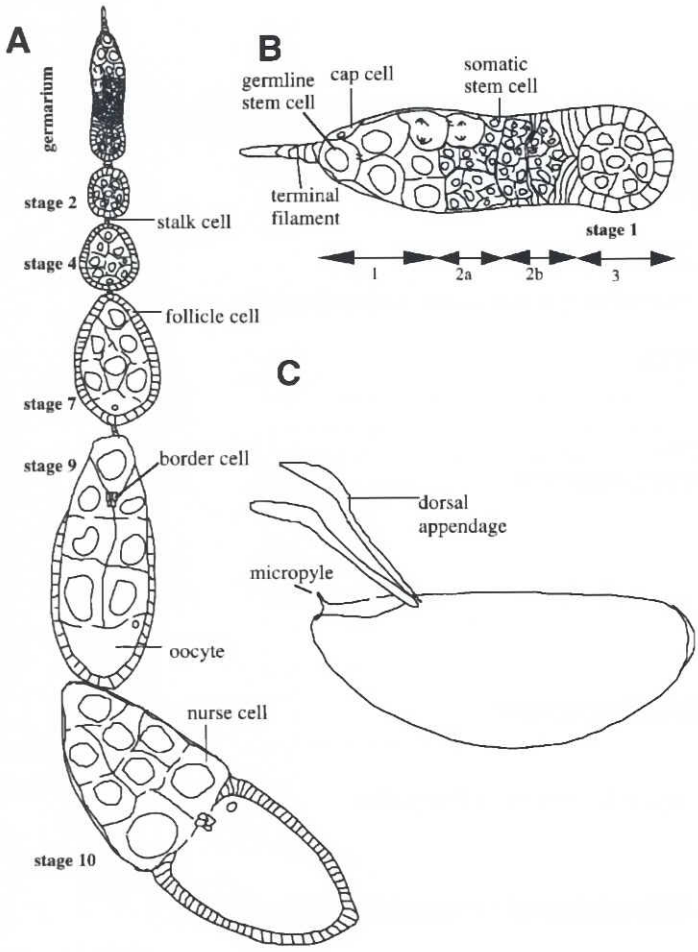

Fig. 1. *D. melanogaster* ovaries and the developmental sequence of oogenesis. (A) *Drawing of an adult wild-type ovariole.* **(B)** *Drawing of a germarium.* **(C)** *A mature egg.*

remain throughout the completion of oogenesis. Somatic stem cells are located at the border between germarium regions 1 and 2 (Margolis and Spradling, 1995). In region 2, follicle cells migrate from the wall of the germarium to encapsulate the 16-cell cysts. When they reach region 3, the cysts are surrounded by a single layer of follicle cells and referred to as stage-1 egg chambers (Fig. 1B). At the anterior-most tip of the germarium is the terminal filament, containing a stack of 6-9 non-dividing somatic cells. The terminal filament is closely associated with another group of somatic cells called cap cells. Both the basal cells of the terminal filament and the cap cells lie in close proximity to the germline stem cells.

Starting from stage 2, egg chambers leave the germarium (Fig. 1A). The developing egg chambers are connected by a stack of specialized interfollicular stalk cells (Fig. 2A). The nurse cells and the oocyte are approximately the same size from stages 1 to 6. At stage 2, the oocyte nucleus, or germinal vesicle, is similar in size to the nurse cell nuclei. The germinal vesicle is positioned at the posterior of the oocyte up to stage 7. When egg chambers reach stage 4, they are no longer oval as in previous stages, but more elongated in shape. From stage 8 to stage 10B, the egg chambers grow quickly; the oocytes grow at a greater rate than the nurse cells as a consequence of the uptake of yolk proteins, which are synthesized in follicle cells and fat bodies. In a stage-10A egg chamber, the oocyte occupies about half of the egg chamber.

A sheet of somatic follicle cells is uniformly distributed over the germline cells from stage 1 to 8 (Fig. 1A). However, starting at stage 9, a series of follicle cell migrations take place. The majority of follicle cells, which originally overlay the nurse cells, elongate and migrate posteriorly so that by stage 10A, the oocyte is covered by a sheet of thick columnar follicle cells, while only a thin layer of stretched cells are left covering the nurse cells (Fig. 2D). At the same time, a group of about 6 to 10 anterior follicle cells move through the nurse cell cluster to reach the nurse cell-oocyte border (Fig. 2C). During stage 10B, the anterior columnar follicle cells migrate centripetally along the DV axis to cover the anterior end of the oocyte (Fig. 2F). Up to this stage, the oocyte is surrounded by a layer of follicle cells, which secrete eggshell protein to protect the mature egg.

During stages 10B to 12, nurse cell cytoplasm is rapidly transferred through the ring canals into the oocyte. The transfer of nurse cell cytoplasm is quite rapid; most will be transported into the oocyte within 30 min. At stages 13 and 14, the remaining nurse cells and follicle cells shrink and undergo apoptosis, leaving behind the mature egg, wrapped with a complete eggshell (chorion) and its specialized structures: a pair of dorsal appendages (filaments) at the anterior end of the egg that facilitate embryonic respiration; operculum (used for the larvae to hatch) and micropylar apparatus (for sperm entry) (Fig. 1C).

Egg chamber formation and early follicle cell differentiation

Within germarium region 2, the 16-germ cell cysts are enveloped by a monolayer of epithelial follicle cells. These follicle cells are descendants of somatic stem cells. Using an FLP-catalyzed mitotic recombination technique, Margolis and Spradling (1995) suggested that there are two somatic stem cells located near the border of germarium regions 2a and 2b (for germarium regions refer to Fig. 1B). It is also suggested that each cyst in region 2b is covered by about 16 follicle cells, which are produced by one division of both somatic stem cells and four rounds of division of their progeny (Margolis and Spradling, 1995). The division of the somatic stem cells and the germline stem cells are not co-ordinated. In agametic flies, somatic stem cells continue to divide in the absence of the germline cells. Therefore, a general co-ordination mechanism may exist to maintain the balance between germline and somatic cell populations (Margolis and Spradling, 1995).

Within germarium region 2a, the envelopment of the germline cysts starts when somatic follicle cells migrate from the wall of the germarium to surround the 16-cell cysts. This is the earliest migration these somatic cells perform during oogenesis. The individual cysts are not separated by the inwardly migrating follicle cells until the cysts reach germarium region 2b. Polar follicle cells, which are located at the anterior and posterior pole of each egg chamber (Fig. 2B), are specified shortly after cyst encapsulation (Margolis and Spradling, 1995). They specifically express Fascilin III and *neuralized* (Ruohola *et al.*, 1991). FLP-catalyzed mitotic recombination analysis reveals that the anterior and posterior polar cells are derived from the same cell lineage (Margolis and Spradling, 1995). They stop dividing long before those follicle cells around them. These cells are required for polarity organization of the egg chambers (Ruohola *et al.*, 1991).

Since the follicle cells do not attach to the 8-cell cysts, it is suggested that the association of the follicle cells with the 16-cell cysts is due to an inducing signal(s) produced in the 16-cell cysts (Spradling, 1993; Goode *et al.*, 1996a). The EGF-R signaling pathway may mediate this inductive event. The *Drosophila* homolog of the EGF receptor, encoded by *torpedo (top/DER),* is required in the follicle cells, while its ligand, a TGF-α homolog encoded by *gurken (grk)* is required in the germline cells (Goode *et al.*, 1996a,b). *grk* is expressed in the 16-cell cyst, but not in the 8-cell cyst. Strong mutations of either *grk* or *Top/DER* produce ovarian egg chambers with multiple sets of nurse cell-oocyte complexes, indicating that Grk-DER signaling is essential for cyst encapsulation (Goode *et al.*, 1996b).

Several other signaling pathways from different sources are also involved in the regulation of cyst encapsulation. *Hedgehog* (Hh), a secreted intercellular molecule, is expressed in the terminal filament and cap cells, which are located at the anterior most region of the germarium (Fig. 1B). Forbes et *al.* (1996a) suggested that *Hh* acts as a morphogen to regulate the proliferation and specification of somatic cells during germline cyst encapsulation in region 2b, which is about 2 to 5 cells away from the *Hh* expressing cells. Reducing Hh activity in germaria blocks cyst encapsulation, resulting in misincorporated egg chambers. Weakly affected germaria continue to produce large, abnormal egg chambers that contain more than 15 germline cells. Severely affected ovarioles are also produced that lack any budded egg chambers. Ectopic *Hh* expression in the ovary results in a dramatic increase in the number of somatic cells accumulating between the egg chambers. These interfollicular somatic cells fail to express a stalk cell marker, suggesting Hh activity is restricted in the proliferation of pre-follicle cells rather than stalk cells (Forbes *et al.*, 1996a,b).

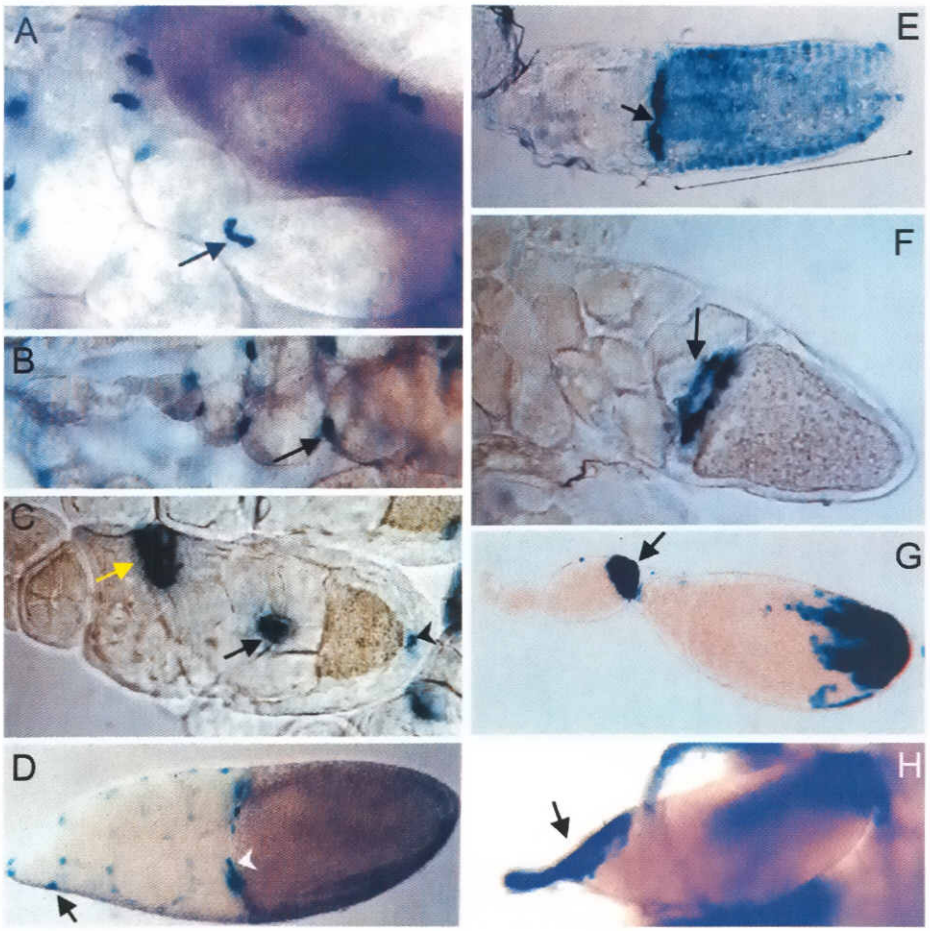

Fig. 2. ß-Galactosidase staining shows subsets of follicle cells during oogenesis. *A,B,C,G and H are P[Gal4] enhancer-trap lines (Deng et al., 1997). D,E and F are P[lacZ] enhancer-trap lines.* **(A)** *The stalk cells interconnecting the egg chambers are stained (arrow).* **(B)** *Staining is observed in the anterior polar follicle cells (arrow).* **(C)** *Staining in the anterior follicle cells (yellow arrow), the migrating border cells (black arrow) and the posterior polar follicle cells (arrowhead) in a stage-9 egg chamber.* **(D)** *The decapentaplegic(dpp)-lacZ line shows staining in the nurse cell associated follicle cells (arrow) and the centripetal cells (arrowhead) during stage 10.* **(E)** *The columnar follicle cells (bracket) and the centripetal cells (arrow) are stained.* **(F)** *Staining in a ring of leading follicle cells centripetally migrating along the nurse cell-oocyte border.* **(G)** *Staining is observed in a cap of posterior follicle cells (arrow).* **(H)** *Staining in chorionic appendage associated follicle cells.*

Another signaling pathway, including the neurogenic genes, *Notch* and *Delta*, is also essential for follicle cell differentiation and cyst encapsulation (Ruohola *et al.*, 1991; Xu *et al.*, 1992; Bender *et al.*, 1993). *Notch* and *Delta* signaling has been shown to be involved in a number of developmental processes. They exhibit a lateral inhibition mechanism in specifying cell fate (Muskavitch, 1994). During early oogenesis, constitutively active N*otch* arrests follicle cells at a precursor stage. Long stalk-like structures are generated when constitutively active *Notch* is expressed in the germarium. On the contrary, "loss-of-function" alleles result in hyperplasia of stalk cells early in oogenesis and later, a loss of polar cells (Ruohola *et al.*, 1991; Larkin *et al.*, 1996). These observations indicate that *Notch* functions in holding the follicle cells in a precursor stage of development. *Notch* is also required in the follicle cells for germline cyst envelopment. This has been shown by the analysis of some *Notch* mutant egg chambers that contain 32 nurse cells, resulting from encapsulation of two germline cysts (Ruohola *et al.*, 1991; Goode *et al.*, 1996a; Zhao and Bownes, unpublished data). *fringe (fng),* a gene encoding a secreted protein which mediates the *Notch-Delta* signaling in imaginal disc patterning (Kim *et al.*, 1995; Panin *et al.*, 1997), is also required in the germarium. It is expressed as a cap in the follicle cells as they surround the germline cyst. Antisense-*fng* expression leads to fusions of egg chambers, cysts containing two oocytes and cysts with too many nurse cells (D. Zhao and M. Bownes, unpublished data).

A novel signaling pathway, involving the neurogenic genes, *egh* and *brn*, is also essential for cyst encapsulation. Both *egh* and *brn* are required in germ cells. *egh* encodes a novel secreted or

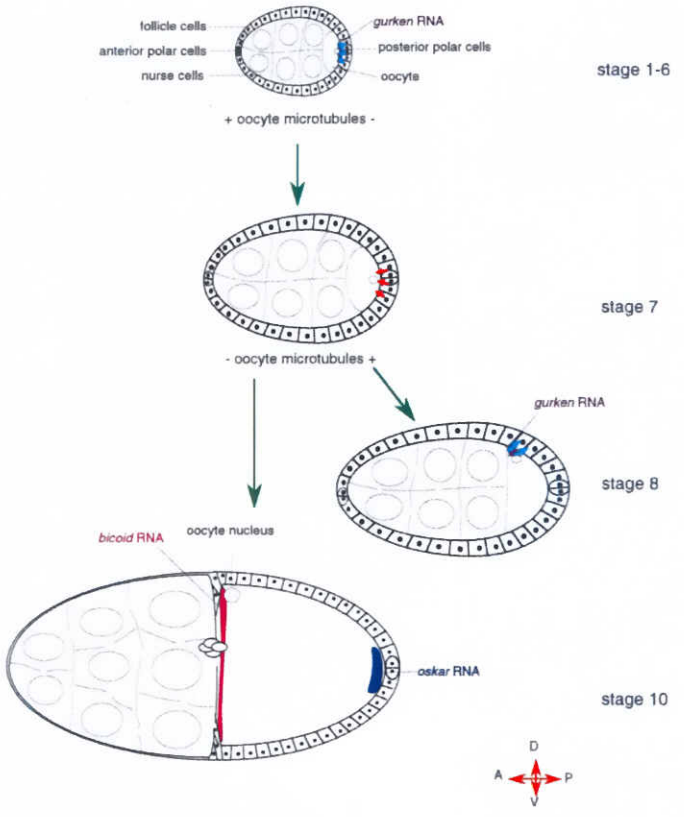

Fig. 3. Gurken signaling is required for both anterior-posterior (AP) and dorsal-ventral (DV) axis determination during oogenesis. *Arrows between the oocyte and the follicle cells show the direction of signaling. During stages 1-6, both the oocyte nucleus and the Gurken signal are located at the posterior pole of the oocyte. The Gurken signal causes the adjacent follicle cells to adopt a posterior fate. These follicle cells later send an unidentified signal back to the oocyte to re-orientate the microtubules in the germ cell cluster. This in turn leads to the localization of the anterior determinant, bicoid, at the anterior and the posterior determinant, oskar, at the posterior of the oocyte. The re-orientation of the oocyte microtubules also leads to movement of the oocyte nucleus towards the anterior and localization of the Gurken signal into the future dorsal-anterior corner during stage 8. The Gurken signal is again received by the adjacent follicle cells, and in turn it determines the dorsal-ventral axis of both the eggshell and the embryo.*

transmembrane protein, while *brn* encodes a novel, putative secreted protein (Goode *et al.*, 1995,1996a,b). *egh* and *brn* expression in germline cells correlates with follicular epithelium morphogenesis throughout oogenesis. In the absence of *egh* or *brn* function, formation of the follicular epithelium is less efficient. As a consequence, several cysts are wrapped in one chamber. Additionally, both genes are required for maintaining the follicular epithelium once the egg chamber is established. Mutations in both genes in the germline result in loss of apical-basal polarity and accumulation of follicle cells in multiple layers, particularly surrounding the oocyte (Goode *et al*, 1992,1995,1996a,b). The maintenance of the follicular epithelium is also part of *Notch*'s function in oogenesis. In *Notch* mutants, a similar phenotype to mutants of both *egh* and *brn* is observed. This may suggest that *brn* and *egh* mediate *Notch* function, which is required in the somatic cells. However, neither *brn* nor *egh* is essential for polar/stalk cell fate specification, while *Notch* plays an active role in this function (Goode *et al.*, 1996a).

It is suggested that *brn* acts in a parallel, but partially overlapping pathway to the Grk-DER signaling pathway (Goode *et al.*, 1996b). Doubly mutant for *brn* and weak *grk* or *Top/DER* mutations produce ovarian follicles with multiple sets of nurse cell-oocyte complexes. The *brn* pathway may help to provide specificity to Grk-DER function during oogenesis (Goode *et al.*, 1996b).

Polarity determination and germline-soma interactions during oogenesis

Systematic genetic analysis has identified four separate genetic hierarchies that control axis formation in embryos, three of which are designed for anterior-posterior (AP) axis formation, while only one is used to define the dorsal-ventral (DV) axis (for review see St Johnston and Nüsslein-Volhard, 1992). The stepwise establishment of polarities is initiated during oogenesis. In stage-10 oocyte, *bicoid (bcd)* and *oskar (osk)* mRNAs are localized at the anterior and posterior end respectively to define the AP axis of the future embryo, while *grk* transcripts are at the dorsal-anterior region to induce the DV polarity of the egg.

Origin of polarity

In the wild-type egg chamber, the oocyte is always located posterior to the nurse cells, which marks the AP polarity of the egg chamber. However, in germarium region 2, the pro-oocyte is located near the centre of the lens-shaped 16-cell cyst. It moves towards the posterior to make contact with the somatic follicle cells that have migrated from the germarium wall to envelop the cyst. In germarium region 3, the oocyte is positioned at the posterior of the newly formed stage-1 egg chamber. This movement appears to be among the earliest morphological signs of polarity formation, and is a key step for axis establishment. In mutant egg chambers where the oocyte is positioned in the middle, localization of *bcd* and *osk* mRNAs is disrupted, which in turn disrupts the two axes (Peifer *et al.*, 1993; González-Reyes and St. Johnston, 1994).

The movement of the oocyte to the posterior requires the functions of several genes, including *armadillo,* five *spindle* genes *(spindle A* to *E)*, and *dicephalic* (González-Reyes and St. Johnston, 1994). When one of these genes is mutated, the oocyte is usually mis-positioned in the egg chamber. *armadillo (arm)* is a segment polarity gene and encodes a *Drosophila* homolog of adhesion junction components plakoglobin and β-catenin (Peifer, 1995). Germline *arm* mutations disrupt the cell arrangement and cytoskeletal system, resulting in mis-positioning of the oocyte. Armadillo protein is distributed in the vicinity of cell-cell adhesive junctions in *Drosophila* ovaries. In the germarium, Armadillo is distributed at the posterior pole of the earliest egg chamber, in the follicle cells (Peifer *et al.*, 1993). This may suggest it functions to hold the oocyte at the posterior of the egg chamber. In *arm* mutant egg chambers when the oocyte is positioned at the anterior end, maternal determinants appear to be localized correctly, although the AP polarity is inverted relative to that of the ovariole (Peifer *et al.*, 1993). However, when the oocyte is positioned at the middle in an *arm* mutant egg chamber, the localization of *osk* and *orb* mRNAs is disrupted (Peifer *et al.*, 1993). These observations indicate that

the establishment of polarity requires the oocyte to occupy one end of the egg chamber in order to make contact with the somatic follicle cells. The follicular epithelium seems to form a symmetrical pattern along the AP axis prior to oocyte movement, with the polar cells located at both ends. Although the polar cells are likely to be important in holding the oocyte at the posterior pole once it reaches there, it is unclear whether the polar cells send signals to attract the oocyte to move or not. Since the polar cells at both poles have the potential to develop towards either anterior or posterior fate, they should not be distinguishable from each other before the oocyte movement, therefore, other sources of "signals" may initialize the movement of the oocyte towards the posterior end in the wild-type egg, and these remain unidentified.

Genetic evidence suggests that the origin of oocyte polarity is directly linked to the initial cell fate determination that singles out the oocyte from its 15 sister cells. *spindle (spindle A-E)* genes are also required for oocyte specification. In some *spindle* mutants, *grk* and other RNAs are localized to the two cystocytes with four ring canals, indicating that two oocytes are specified in one 16-cell cyst (González-Reyes and St Johnston, personal communication). In these egg chambers, oocytes cannot be properly positioned at the posterior, which in turn disrupts polarity formation. Since the cloning of the *spindle* genes have not been reported, it is unclear what roles they play in oocyte specification and positioning.

In summary, the origin of polarity within the egg chamber and the oocyte is strongly linked to oocyte specification. Once the oocyte is singled out from its sister cells, a polarized cytoskeletal network is established in the germline cyst, which will lead to the posterior movement of the oocyte. The maintenance of the oocyte at the posterior pole probably requires the function of polar follicle cells. Disturbance of any of these steps will lead to the disruption of polarity formation.

AP axis formation

When the oocyte is correctly positioned at the posterior end of the egg chamber, it is in contact with the posterior follicle cells. A signal, which has been identified as the TGF-α homolog, Grk, is sent out and received by the adjacent posterior follicle cells (Neuman-Silberberg and Schüpbach, 1993; González-Reyes *et al.*, 1995; Roth *et al.*, 1995). The Grk RNA and protein are localized at the posterior pole of the oocyte, in association with the posteriorly positioned germinal vesicle. It is proposed that the Grk protein is the ligand of Top/DER, the *Drosophila* homolog of the EGF receptor tyrosine kinase, which is expressed in follicle cells (Price *et al.*, 1989; Clifford and Schüpbach, 1992,1994; Neuman-Silberberg and Schüpbach, 1993; González-Reyes *et al.*, 1995; Roth *et al.*, 1995). The binding of Grk to Top/DER activates a signal transduction pathway, involving Ras, DRaf, and Dsor1, in adjacent follicle cells and induces these cells to adopt a posterior fate (González-Reyes *et al.*, 1995; Lee and Montell, 1997). In *grk* and *top* mutant egg chambers, follicle cells at the posterior pole express an anterior marker, indicating that they adopt the default anterior fate when Grk-DER signaling is disrupted (González-Reyes *et al.*, 1995).

Additionally, the differentiation of posterior follicle cells also requires signaling among the somatic cells. The pre-differentiation of the polar follicle cell fate is essential for this process. Therefore, signaling pathways required for polar cell differentiation, such as Notch-Delta and Hh signaling pathways, are also essential for axis formation. fng is also expressed in the polar follicle cells. In flies expressing antisense-*fng* during oogenesis, the posterior polar follicle cells over-proliferate. This leads to an abnormal localization of *oskar* transcripts in the oocyte (Zhao and Bownes, unpublished data).

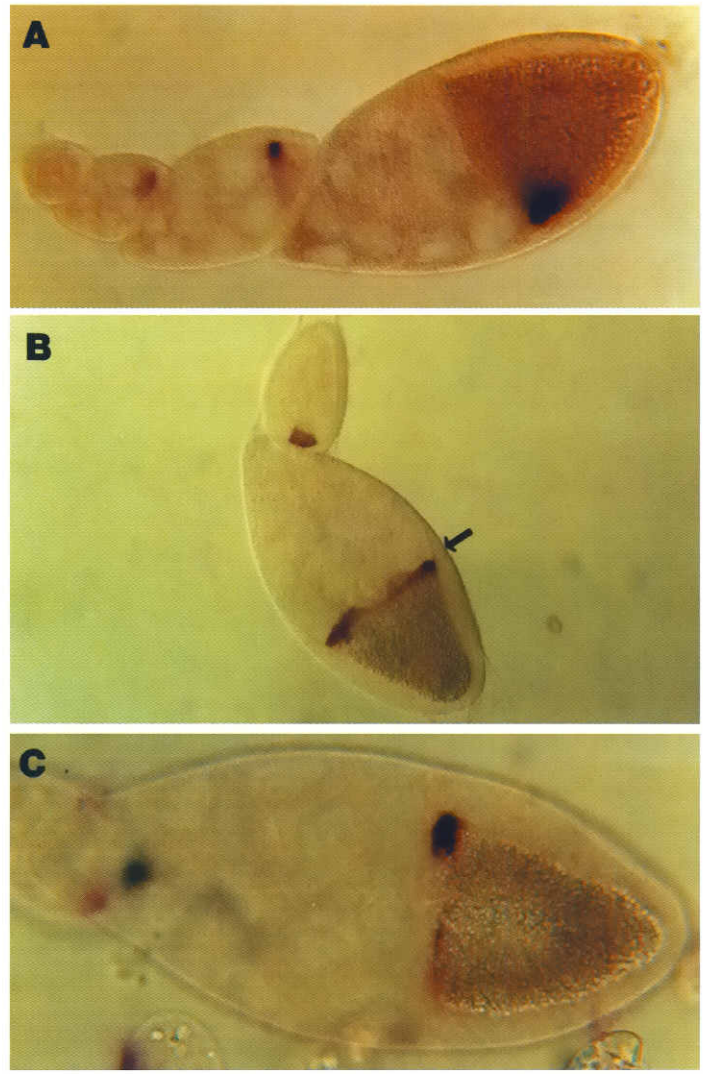

Fig. 4. Whole-mount *in situ* hybridization to show *grk* mRNA localization in the wild-type (A) and *fs(1)K10*^{1} (B,C) mutants during stages 8-10 of oogenesis. (A) *In wild-type egg chambers, grk mRNA is localized at the dorsal-anterior corner of the oocyte during stages 8-10. This localization is disrupted in fs(1)K10*^{1} *egg chambers. It is shown in* **(B)** *that grk mRNA is ectopically localized at the anterior end of the oocyte during stages 9-10A. However, the signal is stronger at the dorsal-anterior corner when compared to the rest of the anterior region (arrow). During stage 10B, ectopically localized grk mRNA nearly disappears, whilst strong signals remain at the dorsal-anterior corner of the oocyte* **(C)**.

The posterior follicle cells that have received the Grk signal from the oocyte are presumed to send an unidentified signal back to the oocyte. Consequently, the microtubule network in the germline cells is re-arranged. The microtubules extend from the MTOC at the posterior end of the oocyte into the adjacent nurse cells during stages 1 through 6. This posterior MTOC degenerates during

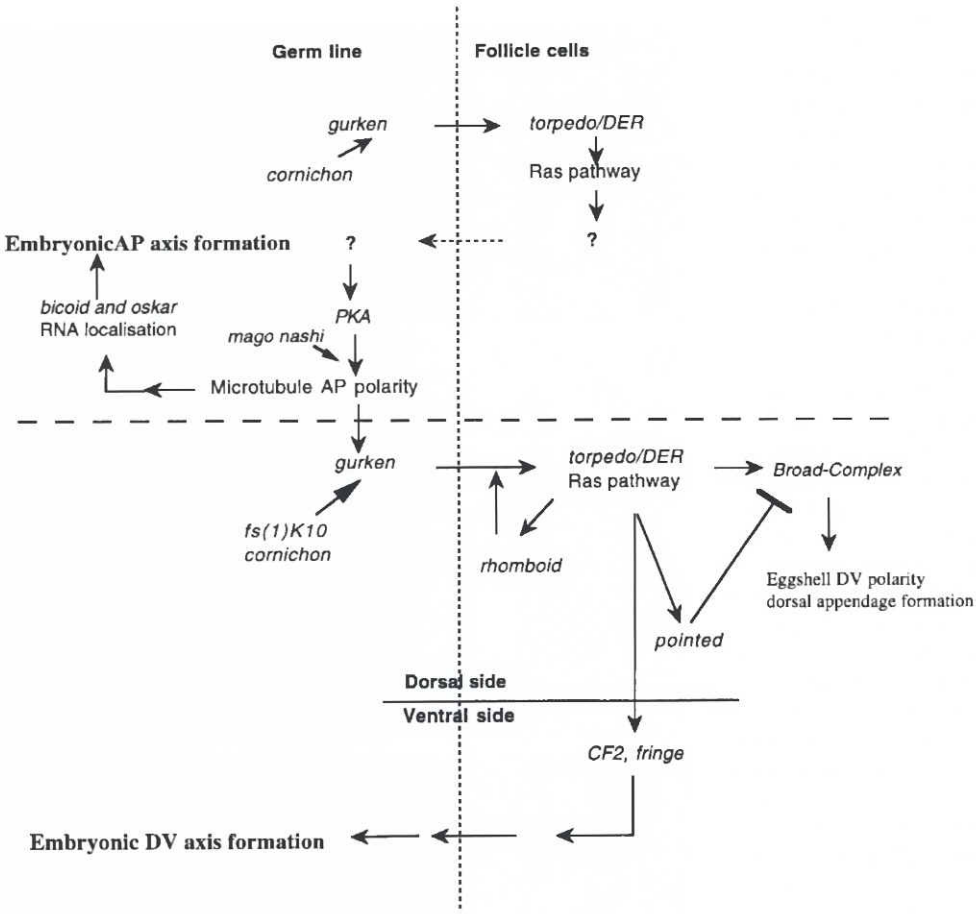

Fig. 5. A pathway of genes required for establishment of the AP and DV axes in *D. melanogaster* during oogenesis. *A key step in this pathway is the re-orientation of the microtubules in the germ cell cluster during mid-oogenesis, which requires germline-soma cell-cell interactions. The microtubule re-orientation leads to localization of morphogenetic determinants bicoid and oskar at both poles of the oocyte, which is crucial for the establishment of the anterior-posterior axis. The re-orientation of microtubules also leads to the dorsal-anterior localization of Gurken mRNA and protein, which activates a number genes that are required for DV patterning of the eggshell and the embryo. For details see text.*

stages 7 and 8 and microtubules begin to associate with the anterior margins of the oocyte (Theurkauf *et al.*, 1993; Theurkauf, 1994). This in turn leads to the establishment of the AP polarity in the oocyte. Protein kinase A (PKA) is required in the germline and may be involved in the transmission of the signal from the posterior follicle cells to the oocyte. This has been shown by the analysis of a *PKA* mutant in which the microtubule fails to be reorganized (Lane and Kalderon, 1994,1995). Additionally, *mago nashi* also appears to be required in the germline for the re-organization of the microtubules (Micklem *et al.*, 1997).

During early stages, microtubules are required for preferential accumulation of mRNAs and proteins in the previtellogenic oocyte, consistent with the idea that these molecules are transported by a microtubule-dependent mechanism to the oocyte (Pokrywka and Stephenson, 1995). When the microtubule polarity is re-oriented at stage 7, maternal determinants that localize to the oocyte during early stages are re-distributed, as shown by the posterior localization of *osk* RNA, anterior localization of *bcd* RNA, and dorsal-anterior localization of *grk* RNA. The central role played by microtubules in axis formation has been shown by using inhibitors of microtubule function to study the effect on maternal determinant localization. Maternal determinants, such as *bcd* and *osk*, fail to be localized when the microtubule reorganization is disrupted (Pokrywka and Stephenson, 1991,1995; for reviews see Cooley and Theurkauf, 1994; Theurkauf, 1994; Pokrywka, 1995).

DV axis formation

The formation of the DV axis also requires an interaction between the germline and the follicle cells. First, a signal from the germline determines the dorsal follicle cell fate, and then, follicle cells signal back to pattern the DV axes of the egg/embryo (Schüpbach, 1987) (Fig. 2).

During stage 8 of oogenesis, the germinal vesicle migrates from the posterior to the anterior margin of the oocyte. It appears that this movement is crucial for the establishment of DV polarity: in egg chambers with the germinal vesicle laser ablated, DV polarity fails to be established (Montell *et al.*, 1992). The germinal vesicle movement is microtubule-dependent (Roth *et al.*, 1995). The reorganization of the germline microtubule network at stage 7, a key step in AP polarity formation, is the major force for germinal vesicle migration. This indicates that DV axis formation is dependent on the existing AP axis. As the germinal vesicle moves to the anterior margin, *grk* mRNA, which is associated with the germinal vesicle at the posterior pole during earlier stages, remains associated with the oocyte nucleus. As a result, *grk* mRNA and protein are localized at the dorsal-anterior region of the oocyte (Fig. 3), and this induces the follicle cells facing the germinal vesicle to adopt a dorsal fate (Haenlin *et al.*, 1995). The receptor of the Grk signal is still Top/DER, which is used earlier in inducing posterior follicle cell fate. Ras, Draf and Dsor1 are involved in transmitting the signal in the dorsal anterior follicle

cells (Brand and Perrimon, 1994; Lu *et al.*, 1994; Schnorr and Berg, 1996).

The localization of *grk* RNA to the anterior-dorsal region surrounding the germinal vesicle requires the function of *fs(1)K10 (K10)*, a gene encoding a helix-loop-helix DNA binding protein (Wieschaus *et al.*, 1978; Haenlin *et al.*, 1987; Fig. 3). K10 RNA is transported to the anterior oocyte and its protein product is localized in the oocyte nucleus. In *fs(1)K10*^{1} mutant egg chambers, *grk* RNA is localized in the anterior apical region of the oocyte, which induces a ring of anterior follicle cells to become dorsalized. In addition to *K10*, a number of other genes also appear to be required in *grk* RNA localization, such as *orb*, *Bic-D*, and *squid* (Lantz *et al.*, 1992,1994; Kelly, 1993; Christerson and McKearin, 1994; Ran *et al.* 1994; Swan and Suter, 1996)

A small number of genes have been identified that are expressed in the follicle cells, downstream of the Grk-DER signaling pathway (Fig. 4). rhomboid, the first to be identified, encodes a transmembrane protein (Bier *et al.*, 1990). It shows an asymmetric expression pattern in the follicle cells along the DV axis. It has been suggested that it intensifies the action of Grk-DER signaling, as has been shown for some other developmental pathways (Ruohola-Baker *et al.*, 1993). *pointed*, a gene encoding a transcription factor with ETS domains, is expressed in the dorsal midline follicle cells at stage 10A and is required for DV polarity formation in the eggshell (Klämbt, 1993; Klaes *et al.*, 1994; Morimoto *et al.*, 1996). It is expressed in response to Grk-DER signaling and is thought to down-regulate this signaling pathway in cells where it is expressed (Morimoto *et al.*, 1996). The *Broad-Complex (BR-C)*, which encodes a family of zinc-finger transcription factors, is found to be downstream of the Grk-DER signaling pathway expressed in two groups of lateral-dorsal-anterior follicle cells. *pnt* is thought to repress the expression of the *BR-C* in the dorsal midline follicle cells. Genetic analysis suggested that the *BR-C* is required for the morphogenesis of the dorsal appendages (Deng and Bownes, 1997).

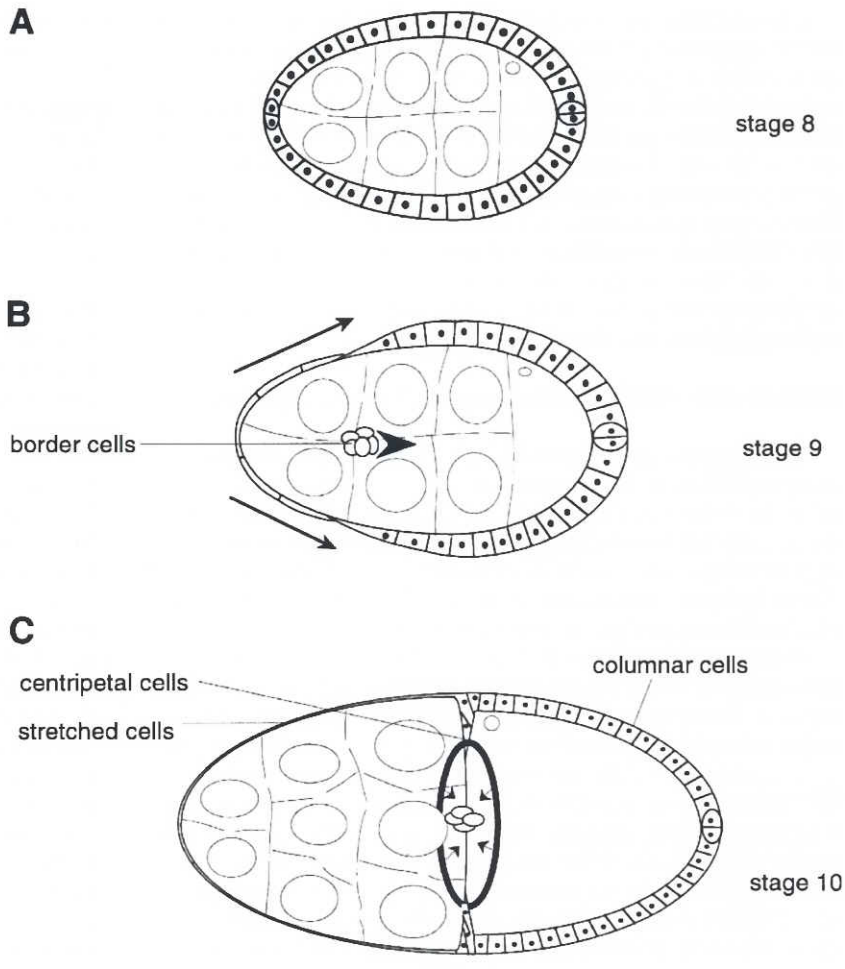

Fig. 6. Follicle cell migrations during mid-oogenesis. (A) *Shows a stage-8 egg chamber which has a monolayer of follicle cells over the germ cell cluster. During stage 9, a group of anterior follicle cells delaminate from the follicular epithelium and migrate through the nurse cell cluster* (**B**, *arrowhead). These cells stop migration at stage 10 and locate at the nurse cell-oocyte border* (**C**). *During stage 9, most follicle cells retract from the nurse cell cluster to cover only the oocyte (arrows in B), leaving only a few stretched cells to be associated with the nurse cells (B,C). During stage 10b, the anterior most columnar cells migrate centripetally along the nurse cell-oocyte border to cover the anterior end of the oocyte (arrows in C).*

CF2 is a zinc-finger transcription factor which is expressed in follicle cells except those in the dorsal-anterior region (Hsu *et al.*, 1992). It is required for DV polarity formation of both the embryo and the eggshell. CF2 is probably a mediator between the dorsal and ventral signals. Since the expression of CF2 along the DV axis is also specified by Grk-DER signaling, a gene must exist with an expression pattern which is dependent on Grk-DER signaling and which acts to suppress CF2 expression in the dorsal anterior follicle cells (Hsu *et al.*, 1996). *fng* is also expressed in the follicle cells except those receiving the Grk signal. In an *fs(1)K10* mutant, the repression of *fng* expands ventrally. Using antisense-*fng* expression to inhibit fng function in the posterior ventral follicle cells leads to a proliferation of the adjacent anterior-dorsal follicle cells. These cells often detach from the follicle cell layer (Zhao and Bownes, unpublished data).

In the absence of dorsal signals, follicle cells will follow a ventral fate, and contribute to a signal that is stored in the fluid between the oocyte and vitelline membrane. Twelve genes have been characterized in the signaling pathway from follicle cells to the oocyte. Mutations in these genes affect the DV axis of the embryo. But unlike the upstream genes, they have no effect on the eggshell. The ventral signal, which is thought to be produced by *pipe, nudel or windbeutel*, has not been characterized (Anderson *et al.*, 1985b; Hong and Hashimoto, 1995). It is thought to be a ligand of Toll, a transmembrane protein that is evenly distributed on the oocyte membrane. The binding of the ventral signal and Toll induces a gradient of nuclear translocation of Dorsal protein, the embryonic DV determinant, which is initially evenly distributed in cytoplasm of the zygote (Fig. 4; Anderson *et al.*, 1985a,b; Roth *et al.*, 1989).

It is interesting that the position of the germinal vesicle and *grk* RNA is crucial to both AP and DV axes formation. González-Reyes *et al.* (1995) suggested that AP is the primary axis, with DV axis formation dependent on the prior establishment of the AP axis. The movement of the germinal vesicle to the anterior margin –the first step in DV axis formation– requires the already established AP polarity of the microtubule system within the oocyte, which results from AP axis specification. In some mutant egg chambers, where the microtubule cytoskeleton develops a symmetrical organization, with 'minus' ends at both poles, the germinal vesicle can be localized at either end of the oocyte. As a result, *grk* RNA is mislocalized and the DV axis is disrupted.

Follicle cell migration and eggshell morphogenesis

Cell migration, which involves dynamic cell shape changes and rearrangements of the cytoskeleton, is one of the major cellular activities in the morphogenesis of multicellular organisms. During oogenesis, follicle cells undergo a series of migrations in order to form egg chambers and specific structures of the eggshell (Fig. 2C-F). These epithelial cells provide an ideal model to study the molecular mechanisms regulating cell motility and morphogenesis.

The first major follicle cell migration is that from the wall of the germarium to surround the 16-cell germinal cysts in germarium region 2. This is a significant step in the formation of egg chambers, which has been discussed earlier. During middle and late oogenesis, a series of follicle cell migrations occur. These include: (1) During stage 9, a group of 6-10 follicle cells at the anterior tip of egg chambers migrate through the nurse cell cluster to the oocyte border (Fig. 2C); (2) At the same stage, the majority of the follicle cells move towards the posterior to form a columnar epithelium covering the oocyte, while the remaining follicle cells stretch to cover the nurse cell cluster (Fig. 2D,E); (3) During stage 10b, the anterior columnar cells migrate centripetally between the oocyte/nurse cell border to cover the anterior end of the oocyte (Fig. 2F); (4) Two groups of columnar cells at the dorsal-anterior region migrate anteriorly to produce a pair of dorsal appendages (filaments) (Spradling, 1993; Deng *et al.*, 1997).

Border cell migration

Starting at stage 9, a group of 6-10 anterior cells delaminate from the follicle cell layer and migrate between the nurse cells (Fig. 6). These cells are called the border cells because they complete their migration at the border between the nurse cells and the oocyte at stage 10. The border cells participate in the formation of an anterior eggshell structure, the micropyle, thus maintaining an opening through which the sperm enters at fertilization (Montell *et al.*, 1992).

The border cells are good models to study the temporal regulation of cell migration. The initiation of border cell migration is controlled by the *slow border cell (slbo)* locus (Montell *et al.*, 1992). Weak *slbo* mutations result in retarded border cell migration, whereas stronger alleles cause complete failure of migration and female sterility. Null *slbo* alleles result in embryonic lethality. *slbo* encodes the *Drosophila* homolog of C-EBP, a basic region/leucine zipper transcription factor (Friedman *et al.*, 1989; Rørth and Montell *et al.*, 1992).

breathless (btl) is a key target gene of *slbo* in regulating border cell migration. During oogenesis, *btl* is specifically expressed in the border cells, and is dependent on *slbo*$^+$ function. *slbo*-independent *btl* expression is able to rescue the migration defects in weak *slbo* mutants. *btl* mutations are dominant enhancers of weak *slbo* alleles. In the *btl* 5' regulatory region, there are eight *Drosophila* C/EBP binding sites, this therefore suggests that *btl* may be a direct target of *slbo* (Murphy *et al.*, 1995).

btl encodes a homolog of vertebrate fibroblast growth factor receptor (FGFR), a membrane receptor tyrosine kinase (RTK). It has been found that heat-shock-induced expression of other RTKs, such as Sevenless, could partially rescue the migration defects in *slbo* weak alleles (Lee *et al.*, 1996). These observations therefore indicate that RTK signaling pathways may play a key role in border cell migration. Lee *et al.* (1996) found that Ras, a common downstream effector for RTKs, is required for border cell migration. A dominant-negative Ras protein inhibits cell migration when it is expressed specifically in the border cells during the period when these cells normally migrate. When it is expressed prior to migration, the dominant-negative Ras promotes premature initiation of migration. Furthermore, reducing Ras activity in border cells prior to migration is able to rescue the migration delay in weak *slbo* alleles. These observations suggest that different levels of Ras activity are required for border cell migration at different stages. Moreover, it appears that the role Ras plays in regulating border cell migration acts via a Raf-independent pathway (Lee *et al.*, 1996).

Non-muscle myosin-II could be a downstream target of Ras signaling in border cell migration, as in egg chambers where the light chain gene, *sqh*, is mutated, border cell migration is blocked (Karess *et al.*, 1991; Edwards and Kiehart, 1996). A class VI myosin, unconventional myosin 95F (Kellerman and Miller, 1992), is expressed in the anterior follicle cells and the migrating border cells (Deng and Bownes, unpublished data) and it may therefore also play a role in border cell migration.

Border cells migrate along a track between nurse cells. This process requires properly built nurse cell intercellular bridges. The protein kinase A (PKA) catalytic subunit, DC0, seems to be involved in interfollicular bridge formation in *Drosophila* oogenesis. Intercellular bridges in egg chambers from *PKA* deficient females are unstable, leading to the formation of multinucleate nurse cells by the fusion of adjacent cells. As a result, border cell migration is disrupted, implying that nurse cell junctions provide an essential path for border cell migrations. Furthermore, the highest levels of PKA catalytic subunit protein are associated with germ cell membranes, suggesting that the targets of PKA are associated with the membrane or membrane skeleton and contribute to the stabilization of intercellular bridges (Lane and Kalderon, 1995).

Migration over the oocyte

During stage 9, the majority of the follicle cells begin to be displaced in a posterior direction. Eventually more than 95% of the cells will stack up to form a columnar epithelium in contact with the oocyte, leaving only about 50 cells remaining over the nurse cells (Fig. 5). In order to cover the whole nurse cell cluster, the remaining nurse cell associated follicle cells are stretched and very thin. The nuclei of these stretched cells are normally located in the small valleys created by the joining of two adjacent nurse cells (Fig. 2D). The function of these cells has not been fully characterized. They may be involved in sending signals to the nurse cells since a *Drosophila* homolog of TGF-β, *decapentaplegic (dpp)*, is expressed

in these cells (Fig. 2D; also see Twombly *et al.*, 1996). We have also observed a number of *P[Gal4]* lines directing reporter gene expression specifically in these cells (Deng *et al.*, 1997).

The follicle cells that have migrated posteriorly to cover the oocyte are more columnar in shape. The migrating cells move only relative to their underlying substrates but not in relation to their immediate neighbors-they remain interconnected at all times. This may be due to co-ordinated changes in the cytoskeleton within each follicle cell that act in conjunction with an anchoring point in the posterior pole of the oocyte. Spradling (1993) suggested that the oocyte probably has a signal on its surface at stage 8 to instruct this movement. However, little is known about the genes involved in this movement. Goode *et al.* (1996a) suggested that *brainiac*, *egghead*, and *Notch* act to inhibit the late migration of the follicular epithelium over the oocyte. Non-muscle myosin-II is expressed in these follicle cells, however, their migration appears not be disrupted in egg chambers of the *sqh* mutant. which encodes the regulatory light chain of non-muscle myosin-II (Edwards and Kiehart, 1996). Unconventional myosin 95F is also expressed in these follicle cells, but targeted expression of antisense RNA of this myosin gene seems to have no effect on this type of follicle cell migration (Deng and Bownes, unpublished data).

Centripetal migration

By stage 10B, when the majority of follicle cells originally covering the nurse cells have completed their migration towards the posterior, the columnar cells over the anterior of the oocyte start to migrate centripetally along the DV axis between the oocyte and the nurse cells (Fig. 5). These cells will secrete the anterior end of the eggshell, which along with the eggshell secreted by the columnar cells, will eventually cover the entire egg.

Mutations of a few genes have been identified that block centripetal migration and result in mature eggs with an opened anterior end (Schüpbach and Wieschaus, 1991). *fs(2)cup* is one of these genes. However, it seems that it is not directly involved in regulating centripetal migration. More likely, the failure of migration in *fs(2)cup* mutants is due to the growth of the oocyte being slower than in the wild-type. By stage 10, when centripetal cells start to migrate, the *fs(2)cup* mutant oocytes reach only 1/4 to 1/2 the size of corresponding wild-type oocytes, and occupy no more than 33% instead of 50% of the egg chamber. The under-sized oocyte seems to be too small to accommodate all the columnar cells, many of which are still in contact with the nurse cells. As a result, a centripetal migration never occurs in these egg chambers (Keyes and Spradling, 1997). These observations indicate that the initiation of the centripetal migration requires the anterior-most columnar cells reach the border between the nurse cells and the oocyte. Thus, there may be a signal sent by the oocyte and received by the anterior-most columnar cells to initiate the migration.

Non-muscle myosin-II, a molecular motor encoded by *zipper (zip)*, appears to be directly involved in centripetal follicle cell migration. Antibody staining shows that the centripetal cells specifically accumulate this myosin at the edge of the apical (inner) surface that leads the penetration between the nurse cells and the oocyte. F-actin is also concentrated at this edge. The 'leading edge' of each centripetal cell joins to form a continuous band of actomyosin staining around the egg chamber, which decreases in diameter as the cells move centripetally. Contraction of this actomyosin band could provide the force to pull the centripetal cells inwards. In egg chambers where the regulatory light chain (RLC) gene, *sqh*, is mutated, centripetal cells fail to elongate. Consequently, all mature eggs display the 'open chorion' phenotype similar to that of *fs(2)cup* mutants (Edwards and Kiehart, 1996; Keyes and Spradling, 1997). However, since *sqh* mutants also produce undersized oocytes, it is not clear if the failure of centripetal migration is due to the relative position of the anterior-most columnar cells and the oocyte-nurse cell border. Furthermore, RLC may regulate other myosins. Thus the mutant phenotype caused by *sqh* could be due to the disruption of the organization of a few myosins that may have a joint function in follicle cell migration. We have found that another myosin, unconventional myosin VI, is also expressed in the centripetal cells and the border cells. Targeted silencing experiments reveals that this myosin appears to be essential for centripetal migration. It is possible that several myosins co-operate to drive the follicle cell migration during oogenesis (Deng and Bownes, unpublished data).

Centripetal cells might provide a signal source for the patterning of the columnar cells, because the signaling molecule, Dpp, is also expressed in these follicle cells. A decrease in the expression level of Dpp in these cells results in a diminished operculum (Twombly *et al.*, 1996). It is hypothesized that the Dpp signal is involved in the AP patterning of the eggshell (Deng and Bownes, 1997). Dpp has been found to be expressed in cells at the leading edge during dorsal closure in embryogenesis, and is essential for this epithelial morphogenetic movement which is very similar to centripetal migration. Dpp may be directly involved in the regulation of centripetal migration.

Dorsal-anterior follicle cell migration and dorsal appendage formation

At the dorsal-anterior region of the mature egg of *D. melanogaster*, lies a pair of chorionic appendages, also called the dorsal appendages or dorsal filaments. Each dorsal filament is formed by a group of follicle cells that start migrating over the anterior part of the oocyte at stage 11. Spradling (1993) suggests that about 150 follicle cells form each dorsal appendage. This number may be exaggerated. As shown by staining of a dorsal appendage precursor cell marker, the *Broad-Complex (BR-C)*, there are about 55-65 follicle cells in each group (Deng and Bownes, 1997). Filament-producing cells begin to secrete the filament bases to attach to the main body of the eggshell before they begin migrating towards the anterior. As cells migrate past the growing end, they join a cylinder of cells secreting chorion proteins and commence secretion themselves. The dorsal appendages complete their elongation at stage 14.

The production of the dorsal appendages is dependent upon the Grk-DER signaling pathway which induces dorsal-ventral polarity of the eggshell and the embryo. Thus, the regulation of appendage formation provides a good model for the study of signal instructed morphogenesis. In strong *grk* mutants, the dorsal appendages are absent. In *fs(1)K10*^{1} mutant egg chambers, *grk* RNA is ectopically localized to form an anterior ring (Fig. 3), which induces the dorsal appendages to fuse at the ventral side. The downstream effector gene that directly regulates dorsal appendage formation is likely to be *Broad-Complex*, a gene

complex encoding a family of zinc-finger transcription factors (Bayer *et al.*, 1996; Deng and Bownes, 1997). In addition, non-muscle myosin-II and unconventional myosin 95F seems to be downstream of Grk-DER signaling and are likely to be required for dorsal follicle cell migration (Edwards and Kiehart, 1996; Deng and Bownes, unpublished data).

We have not discussed in this review the synthetic activities of the follicle cells, specifically the secretion of yolk proteins, endocytosed into the oocyte during stages 8-10, the vitelline membrane proteins and the chorion proteins needed to build the protective eggshell around the oocyte. These have been reviewed elsewhere (Spradling, 1993; Bownes, 1994a,b).

Summary

The follicle cells have proved to be much more than a simple layer of cells over the egg chamber which will produce some of the biochemical building blocks of the egg such as the yolk and egg membranes. They are constantly interacting with each other and the germ-line to co-ordinate and build the egg chamber, oocyte and eggshell. Although we now understand much more about these dynamic cells there remains a lot more to discover.

It seems to us that some of the key issues that remain to be resolved are 1) how the follicle cells and germ cells communicate and interact to assemble the egg chambers in the germarium; 2) precisely how the polar follicle cells are specified; 3) the nature of the signaling from the posterior follicle cells to the oocyte to specify its anterior-posterior axis; 4) how follicle cell migration is regulated and then executed at the cellular level; and 5) how a dorsal signal initiated by Gurken is translated by the follicle cells such that ventral follicle signal to the embryo by producing Nudel, Pipe, Windbeutel and determine its DV polarity.

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