Mass Transit: Epithelial Morphogenesis in the *Drosophila* Egg Chamber

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Epithelial cells use a striking array of morphogenetic behaviors to sculpt organs and body plans during development. Although it is clear that epithelial morphogenesis is largely driven by cytoskeletal rearrangements and changes in cell adhesion, little is known about how these processes are coordinated to construct complex biological structures from simple sheets of cells. The follicle cell epithelium of the *Drosophila* egg chamber exhibits a diverse range of epithelial movements in a genetically accessible tissue, making it an outstanding system for the study of epithelial morphogenesis. In this review, we move chronologically through the process of oogenesis, highlighting the dynamic movements of the follicle cells. We discuss the cellular architecture and patterning events that set the stage for morphogenesis, detail individual cellular movements, and focus on current knowledge of the cellular processes that drive follicle cell behavior. *Developmental Dynamics 232:559–574, 2005.* © 2005 Wiley-Liss, Inc.

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INTRODUCTION

The dynamic movements of epithelial cells are critical to the shaping of individual organs and the overall structure of embryos. Within adults, sheets of tightly adherent cells divide the body into physiologically distinct compartments; however, the somewhat static nature of mature epithelia belies the virtually constant and highly complex movements of these tissues during development. Cells can change their positions within a two-dimensional epithelium through cell shape changes, intercalation, and directed migration. Furthermore, these two-dimensional sheets often reorganize to form three-dimensional structures such as sacs or tubes, which can undergo subsequent remodeling to construct elaborate epithelial networks. The cellular mechanisms that drive epithelial morphogenesis include cytoskeletal rearrangements and changes in cell–cell and cell–matrix adhesion, but we are only just beginning to understand how these cellular processes integrate with one another and with patterning information in the embryo to create complex biological structures from simple sheets of cells.

The follicle cell epithelium (FCE) of the *Drosophila* ovary provides an excellent system for the study of epithelial morphogenesis. The FCE is a somatic monolayer, which surrounds the cluster of germ cells that give rise to the *Drosophila* egg. These cells produce yolk and eggshell components and participate in signaling events with the germline that determine the future embryonic axes. Although the FCE is an adult tissue, its dynamic and varied morphogenetic movements are reminiscent of epithelial rearrangements during embryonic and larval development. During oogenesis, the follicle cells (FCs) recognize and encapsulate another tissue type, undergo dramatic cell shape changes and directed migrations, and two subsets of cells reconfigure to form extended tubes. Until recently, genetic studies of these processes were limited to examining rare female sterile alleles. However, with the advent of genetic mosaic techniques, comprehensive screens to uncover the mechanisms underlying FCE morphogenesis are

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now feasible. Given this variety of morphogenetic movements and genetic accessibility, the FCE is an ideal tissue in which to investigate the diverse cell biological processes that drive epithelial morphogenesis.

In this review, we provide an overview of the many roles that somatic cells play during oogenesis and focus on the morphogenetic movements of the FCE. We begin with the stem cell origin of the FCs and their early migration to surround the germ cell cluster. We next discuss basic properties of the FCE, such as proliferation dynamics and apicobasal and planar polarity, which set the stage for subsequent morphogenetic events. Finally, we highlight two periods of high morphogenetic activity and briefly describe the cellular patterning events that precede them. The first period occurs during mid-to-late oogenesis when the majority of the FCs move toward the posterior of the egg chamber to surround the oocyte. The second period occurs slightly later in oogenesis when subsets of FCs reorganize to create specialized eggshell structures, including two extended tubes that will form the respiratory appendages.

OVERVIEW OF OOGENESIS

The mature *Drosophila* oocyte is an amazingly complex cell. It is over 500 μm long, contains the patterning information to establish the anterior–posterior (A-P) and dorsal–ventral (D-V) axes of the embryo and comes complete with a highly complex eggshell that facilitates embryonic development in harsh external environments. However, the oocyte does not attain this level of sophistication on its own. Each oocyte develops within a group of cells known as an egg chamber (or follicle), which consists of a cluster (or cyst) of 16 germ cells surrounded by an epithelial monolayer of somatic follicle cells (FCs). The germline cyst originates from a single cell, the cystoblast, which undergoes four rounds of division to form the 16-cell cluster. Cytokinesis is incomplete during these mitoses, and the germ cells remain connected through large cytoplasmic bridges during much of oogenesis. Among the 16 germ cells, 1 differentiates as the oocyte and the other 15 become nurse cells, which contribute maternal mRNAs and proteins and eventually dump their collective cytoplasm into the oocyte helping it to swell to its very large size. The FCs, which envelop the cyst, are derived from ovarian mesoderm and are indispensable for patterning the oocyte, the production of yolk proteins, and the secretion and construction of a highly complex eggshell. After the completion of the eggshell and the dumping of the nurse cell cytoplasm, the FCs and nurse cells degenerate, leaving the mature egg behind.

The entire process of oogenesis occurs within the *Drosophila* ovary, which consists of 16 to 20 long tube-like structures called ovarioles. Each ovariole acts as an individual egg assembly line, with younger egg chambers near the anterior and a series of progressively older egg chambers marching toward the posterior until the mature egg reaches the oviduct, is fertilized, and exits the body. The most anterior region of the ovariole is called the germarium, and it is from within this structure that somatic and germline components of the follicle originate and come together to create the basic structure of the egg chamber. When a newly formed egg chamber buds from the germarium, it enters the larger, more posterior region of the ovariole called the vitellarium where it joins a line of six to seven progressively older follicles. The approximate age of an egg chamber can be determined by morphological criteria and assigned to 1 of 14 stages of oogenesis. These stages are outlined in Figure 1; for a more complete description, see Spradling (1993).

CONSTRUCTION OF AN EGG CHAMBER

FC precursors become morphogenetically active shortly after their birth, when they encapsulate the 16-cell germline cyst. These cells are derived from somatic stem cells within the germarium and give rise to three somatic cell types within the egg chamber that exist at the time of encapsulation: the epithelial FCs, the polar cells, and the stalk cells. During encapsulation the precursors extend processes around the cyst and migrate to cover its surface. Polar and stalk cells begin to differentiate concurrent with this morphogenesis and further contribute to the shape of the egg chamber by helping to orient the germline cyst within the follicle and by creating the narrow stalk that separates adjoining egg chambers later in oogenesis. In this section, we discuss the stem cell origin of the FC precursors and focus on the dynamic and varied roles that somatic cells play in determining the basic structure of the egg chamber at the time it buds from the germarium.

Somatic Stem Cells

To sustain a lifetime of egg production, the germarium of each ovariole contains two populations of stem cells: the germline stem cells (GSCs) and the somatic stem cells (SSCs). Intriguingly, these two stem cell populations are strategically placed in different regions of the germarium to facilitate the timely interaction of the germline cyst with the FC precursors that will encapsulate it (Fig. 2). The two to three GSCs reside at the anterior tip of the germarium and produce a single cystoblast with each division. The cystoblast is then pushed posteriorly through the germarium by subsequent GSC divisions as it begins its transformation into a 16-cell cyst. The two to three SSCs are located approximately one third of the way down the germarium at the boundary of region 2a and 2b (Margolis and Spradling, 1995; Zhang and Kalderon, 2001). Although there are currently no molecular markers for the SSC population, their position has been inferred through lineage tracing (Margolis and Spradling, 1995). SSC divisions create a proliferating population of FC precursors that line the walls of the germarium just posterior to the SSCs. By the time a germline cyst passes the SSCs, it has completed its four rounds of division and is ready to be encapsulated by the FC precursors to produce an individual egg chamber.

The precise location of stem cell populations within the germarium is not only important for timely encapsulation but is also required to provide the microenvironment necessary to ensure GSC and SSC self-renewal. GSCs directly adhere to terminal filament and cap cells at the anterior tip of the germarium. These two somatic cell types provide signaling molecules

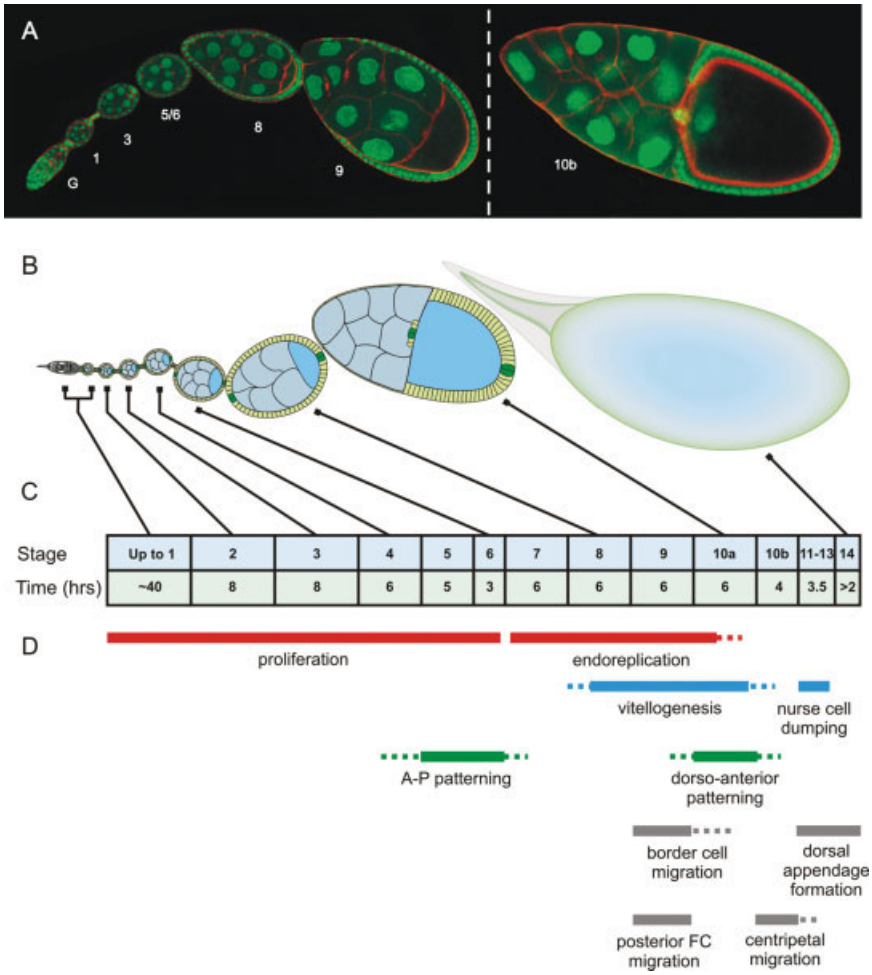


Fig. 1. Overview of oogenesis. Wild-type ovariole with stage 10b egg chamber. Cell outlines are labeled with rhodamine-phalloidin (red) and cytoplasm and nuclei are labeled with green fluorescent protein (green). **A:** The number below each egg chamber denotes the stage of oogenesis, and the germarium is marked with a G. **B:** Drawing of a wild-type ovariole, with somatic cells in green and germ cells in blue. **C:** Timeline showing the relative length of oogenic stages adapted from Spradling (1993) and Margolis and Spradling (1995). **D:** Representation of the temporal overlap of critical events relating to follicle cell morphogenesis.

that regulate GSC proliferation and maintenance and appear to comprise the physical niche that confers stem cell identity for the germ line (reviewed in Lin, 2002). The SSCs also receive critical signals from the terminal filament and cap cells even though these two cell populations are separated by two to five cell diameters. The cells that lie between this signaling center and the SSCs are the inner germarial sheath (IGS) cells, which are quiescent somatic cells that form the walls of the germarium anterior to the SSCs. There is strong accumulation of E-cadherin and β-catenin between the SSCs and their neighboring IGS cells,

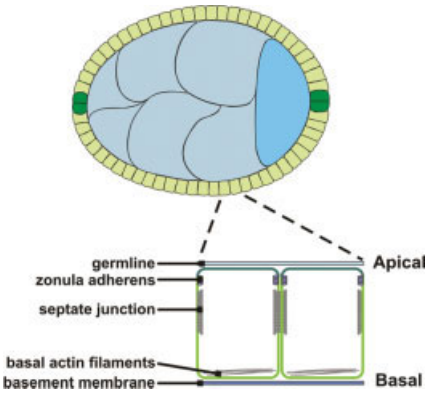


Fig. 3. Apicobasal polarity in the follicle cell epithelium. The drawing shows a stage 8 egg chamber with a magnified view of two follicle cells. This magnification reveals the proximity of the apical surface to the germline and basal surface to a basement membrane as well as the relative positions of certain junctional complexes.

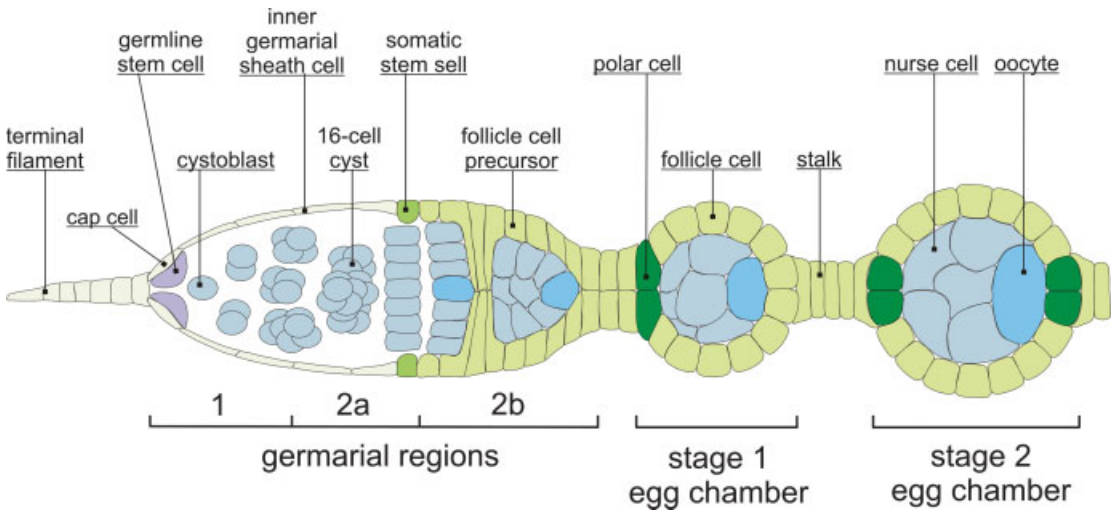


Fig. 2. Construction of an egg chamber. The drawing shows events early in oogenesis that contribute to the basic structure of the egg chamber, including the encapsulation of the germline cyst by follicle cell precursors, the differentiation of polar and stalk cells, and the posterior positioning of the oocyte. The large variety of cell types found within the germarium and early egg chambers are indicated.

and SSCs mutant for the E-cadherin gene *shotgun* are readily lost from the germarium (Song and Xie, 2002). These data indicate that the IGS cells likely anchor the SSCs near the signaling center that maintains their identity as stem cells, while allowing their daughters to move away from the niche to differentiate into FC precursors.

Several factors have been identified that are required within the terminal filament and cap cells for SSC division and maintenance. Hedgehog (Hh) is a secreted signaling molecule that appears to be one of the primary regulators of SSC fate (Forbes et al., 1996a,b; Zhang and Kalderon, 2000, 2001). When SSCs are unable to transduce Hh signals due to a mutation in *smoothened*, they are incapable of proliferating as stem cells. Conversely, activation of Hh signaling within the SSCs through mutation of *patched* has been reported to lead to an expansion of the SSC population (Zhang and Kalderon, 2001). Wingless (Wg) is a second secreted signaling molecule expressed in the terminal filament and cap cells that is required for SSC maintenance (Song and Xie, 2003). Unlike Hh, however, either increasing or decreasing Wg signaling leads to stem cell loss. Because β-catenin participates in both Wg signaling and cadherin-mediated cell adhesion, it is not clear whether precise levels of Wg signaling play a direct role in regulating stem cell self-renewal, are required for retention of the SSCs in their niche, or both.

To ensure the proper ratio of FCs to germline cysts at the time of encapsulation, there are also regulatory mechanisms that may help to synchronize the division rate of the SSCs and GSCs. The *Yb* gene encodes a novel cytoplasmic protein that is required in terminal filament and cap cells to control the expression of both *Hh* and *piwi*, which are key regulators of SSCs and GSCs, respectively (King and Lin, 1999; King et al., 2001). In addition, the division rate of both stem cell populations depends on the nutritional state of the female, such that when a female is undernourished SSC and GSC division rates can coordinately drop fourfold (Drummond-Barbosa and Spradling, 2001). Synchronicity between the two stem cell populations is not perfect, however. When SSCs are lost due to reductions in Wg signaling, germline cysts accumulate in the anterior of the germarium—presumably because there are insufficient FC precursors to encapsulate them (Song and Xie, 2003).

A second level of regulation may help to ensure the proper ratio of FCs to germline cysts at the time of encapsulation. In cases where GSC output exceeds that of the SSCs, apoptosis of germline cysts increases near the border of germarial regions 2a and 2b. This observation indicates that an apoptotic checkpoint may exist at the point where the FCs first contact germline cysts to further coordinate the germ cell and somatic cell populations (Drummond-Barbosa and Spradling, 2001).

Encapsulation of Germline Cysts

Encapsulation is the process in which FC precursors come to surround the 16-cell germline cyst to produce the basic structure of the egg chamber (described in King, 1970; Spradling, 1993). The FC precursors first contact the germline cyst in region 2b of the germarium, just posterior to the SSCs (Margolis and Spradling, 1995). After this contact, the cyst flattens until it extends across the diameter of the germarium. To separate the cyst from its neighbors, the FC precursors extend thin centripetal processes between the posterior edge of one cyst and the anterior edge of the next older cyst. After this initial separation, several somatic cells migrate between the two cysts and form a population of intercyst cells, many of which will go on to form the polar cells and the stalk that will separate the follicles once they leave the germarium (see below). The somatic cells then repeat this action at the anterior of the cyst, separating it from its younger neighbor (Fig. 2).

The mechanisms by which FC precursors precisely recognize and engulf an individual cyst are largely unknown and investigations have been hampered by the difficulty in unambiguously identifying mutations that disrupt this process. The presence of compound follicles, in which more than one germline cyst is packaged into a single egg chamber, is often cited as evidence for a defect in encapsulation; however, this phenotype can arise in more than one way. Compound follicles can be formed within the germarium when FC precursors fail to migrate between neighboring cysts, indicating a true defect in the encapsulation process. Compound follicles can also arise later in oogenesis, however, through the fusion of individual egg chambers within the vitellarium (see King, 1970). The tendency of egg chambers to fuse often correlates with the absence of interfollicular stalks (Bai and Montell, 2002; Torres et al., 2003), although lack of stalks has not been conclusively shown to cause fusions. An example of this phenotype can be seen when Notch signaling in the FCE is disrupted by depleting *Delta* from the germline. In this case, individual cysts are completely surrounded by FCs when they leave the germarium, but they lack interfollicular stalks, which causes adjacent egg chambers to be tightly apposed. The two FC layers that separate the germline cysts eventually degenerate, resulting in compound follicles predominantly after stage 7 of oogenesis (Torres et al., 2003).

Several mutations have been identified that appear to disrupt encapsulation within the germarium. *cheerio* encodes the actin binding protein Filamin and mutation of this gene within the FC precursors impairs their ability to extend processes and/or migrate between individual 16-cell cysts (Sokol and Cooley, 2003). Two other mutations that appear to specifically disrupt the encapsulation process are *egghead* (*egh*) and *brainiac* (*brn*). Both mutations produce compound follicles, but this more-common phenotype is accompanied by the tendency to split 16-cell cysts between adjoining egg chambers (Goode et al., 1996). Because *egh* and *brn* are both required in the germline, there must be a signal on germ cells that allows FC precursors to correctly recognize the boundaries of individual cysts and migrate between them. Egh and Brn function within the biosynthetic pathway for glycosphingolipids (Muller et al., 2002; Schwientek et al., 2002; Wandall et al., 2003). What role these enzymes play in signaling from the

germline to the FC precursors remains to be determined. Finally, a deficiency in the number of FC precursors as compared with germline cysts can also lead to the packaging of more than one cyst into a single follicle within the germarium (Song and Xie, 2003).

Polar Cells, Stalk Cells, and Positioning of the Oocyte

The FC precursors that encapsulate the germline cyst give rise to the epithelial FCs, which comprise the majority of the somatic cells in the egg chamber. However, these precursors also produce two other somatic cell types: the polar cells and stalk cells. Each egg chamber contains two pairs of polar cells: one pair at the anterior pole and one pair at the posterior pole. Although nestled within the monolayer of FCs, the polar cells can be easily distinguished from their neighbors by their round morphology, distinct pattern of gene expression, and early postmitotic state. The stalk cells initially form a thick cluster of six to eight wedge-shaped cells that separates one egg chamber from the next younger egg chamber at the time of budding. These cells subsequently intercalate with one another to produce a narrow bridge of linearly arranged disc-shaped cells that links adjacent egg chambers within the vitellarium.

The polar cells, stalk cells, and epithelial FCs appear to differentiate from the FC precursors in a stepwise manner, roughly concurrent with the encapsulation process. The first split occurs when a common precursor population for the polar and stalk cells becomes distinct from the epithelial FC lineage and exits the mitotic cycle while still in the germarium (Margolis and Spradling, 1995; Tworoger et al., 1999). The polar/stalk precursors can first be distinguished at the border of regions 2b and 3 of the germarium, where they lie between two 16-cell cysts and down-regulate the expression of *eyes absent* (*eya*) while selectively expressing *fringe*. *eya* is a transcription factor that acts in epithelial FCs to suppress polar cell fate, and exclusion of *eya* from the polar/stalk precursors requires Hh signaling (Bai and Montell, 2002). Expression of the glycosyltransferase Fringe within the polar/stalk precursors appears to enable these cells to receive a Delta signal from the germline, which activates Notch in a subset of the polar/stalk precursors to induce the polar cell fate (Grammont and Irvine, 2001; Lopez-Schier and St. Johnston, 2001). Initially four to five polar cells are induced at each pole, but several undergo apoptosis by stage 5 to produce the two characteristic pairs (Besse and Pret, 2003). The newly formed polar cells express the JAK/STAT pathway ligand Unpaired, which subsequently induces the remainder of the precursors to differentiate as stalk cells (McGregor et al., 2002). The induction of stalk cells by polar cells also requires Notch/Delta signaling, this time with Delta acting within the soma (Lopez-Schier and St. Johnston, 2001).

Also concurrent with the encapsulation process, the somatic cells help arrange the germline cyst within the forming egg chamber to place the oocyte at the posterior. The posterior localization of the oocyte is a critical event during oogenesis, as it is required for the subsequent A-P patterning of the FCE, the oocyte itself, and ultimately the embryo (reviewed in Lopez-Schier, 2003). Proper oocyte localization relies on a cell-sorting event in which the oocyte is chosen from among the other germ cells to specifically adhere to the posterior FCs. Preferential adhesion between the oocyte and posterior FCs is mediated by E-cadherin, which is up-regulated in both of these cell types (Godt and Tepass, 1998; Gonzalez-Reyes and St. Johnston, 1998a). It has been unclear, however, how the posterior FCs become different from the anterior FCs so early in egg chamber development. Torres et al. have recently proposed an elegant relay model in which A-P information from one follicle is transferred to the next younger follicle (Torres et al., 2003). A key observation that led to this model is that the anterior polar cells for a given egg chamber differentiate approximately 12 hr before the posterior polar cells (Torres et al., 2003). By examining pairs of follicles in which one follicle contained cells that were deficient for Notch signaling and the other was entirely wild-type, Torres et al. demonstrated that it is the anterior FCs that induce the formation of the stalk between their own egg chamber and the next younger follicle, while the posterior polar cells appear to be dispensable for stalk formation (Torres et al., 2003). Furthermore, when a stalk is missing between two egg chambers, the younger egg chamber fails to upregulate E-cadherin in the posterior FCs and oocyte positioning is defective. This result indicates that it is the stalk induced by the anterior polar cells in the older egg chamber that positions the oocyte in the next younger egg chamber (Torres et al., 2003). The mechanism for determining A-P asymmetry for the first follicle in the chain remains to be determined.

MATURATION OF THE CUBOIDAL EPITHELIUM

Egg chambers emerge from the germarium with a uniform monolayer of cuboidal FCs. During vitellogenesis, when the oocyte swells due to yolk uptake, these cells will undergo a dramatic series of cell shape changes and migrations that result in the majority of the FCs moving to surround the oocyte. However, there is a period of several days between encapsulation and the FC migrations (stages 1–8) in which the FCE is morphogenetically quiescent. During this time, the FCs proliferate, the epithelial structure of the tissue continues to mature, and the FCE is patterned along its A-P axis. In this section, we discuss three topics that relate to general properties of the FCE and events that largely take place between stages 1 and 8. A-P patterning will be covered in the next section in conjunction with the discussion on FC migrations.

Proliferation, Endoreplication, and Growth

Approximately 80 FCs surround the germline cyst at the time that an egg chamber buds from the germarium (King and Vanoucek, 1960). The germ cells cease dividing before encapsulation but then enter a phase of endoreplication and growth, in which DNA synthesis occurs in the absence of cytokinesis and the germ cells dramatically increase in size. To accommodate the rapidly growing germ cells, the FCs continue to proliferate

through the early stages of oogenesis. In well-fed females, the FCs have a doubling time of approximately 10 hr, but similar to SSC divisions, the proliferation rate can drop fourfold in response to poor nutrition (Margolis and Spradling, 1995; Drummond-Barbosa and Spradling, 2001). FC divisions cease at the end of stage 6, at which time the FCs undergo three rounds of endoreplication and growth and selectively amplify four chromosomal regions rich in genes required for eggshell production and patterning (reviewed in Calvi and Spradling, 1999; Botchan and Levine, 2004). King and Margaritis originally estimated that there are approximately 1,000 FCs in the egg chamber when divisions cease at the end of stage 6 (King and Vanoucek, 1960; Margaritis et al., 1980); however, direct counts of FC nuclei place the number closer to 650 (Margolis and Spradling, 1995).

The transition from proliferation to endoreplication occurs when a Delta signal from the germline activates Notch in the FCs. In egg chambers that contain either *Delta* germline clones or *Notch* FC clones, the FCs continue to proliferate beyond stage 6 (Deng et al., 2001; Grammont and Irvine, 2001; Lopez-Schier and St. Johnston, 2001). How is it that Notch signaling regulates this transition? In addition to continuing to divide, FCs that fail to receive the Delta signal also express FasIII, a protein whose expression is normally restricted to early stages of oogenesis (Grammont and Irvine, 2001; Lopez-Schier and St. Johnston, 2001). This ectopic FasIII expression has led to the suggestion that *Notch* mutant FCs continue to divide because they have failed to differentiate. However, FasIII is normally down-regulated long before the pulse of Delta signaling at stage 6, and *Notch* mutant FCs do express some late differentiation markers (Deng et al., 2001). A second, but not mutually exclusive, possibility is that the Notch pathway acts more directly on cell cycle components independent of FC differentiation. In fact, during the switch to endocycles, the transcription of key cell cycle components required for the G1/S, G2/M, and M/G1 transitions appear to be downstream of Notch activation (Deng et al., 2001; Schaeffer et al., 2004; Shcherbata et al., 2004). One area for further investigation is to determine what factors trigger the up-regulation of Delta in the germline to stop FC divisions.

FC proliferation occurs during the early stages of oogenesis, and there is, therefore, little overlap between cell division and major cell rearrangements within the FCE. Although the process of encapsulation occurs while the somatic cells are actively dividing, all subsequent movements of the FCE occur in the absence of cell division. These morphogenetic processes, thus, can be attributed entirely to changes in cell position and/or cell shape within a stable population of FCs.

Apicobasal Polarity and Junctions

The FCE, like other *Drosophila* epithelia, is architecturally similar to vertebrate epithelia and, thus, provides an outstanding system in which to study features such as apicobasal polarity. There are many excellent reviews on epithelial polarity (Tepass et al., 2001; Knust and Bossinger, 2002; Johnson and Wodarz, 2003), and we particularly refer the reader to a discussion of FCE polarity by Muller (2000). In this section, we will limit our discussion to some unique aspects of FC architecture, such as the interaction between the FCs and germ cells during polarization and the specialized array of cell junctions that FCs contain.

One of the most intriguing properties of the FCE is that its apical surface contacts the germ cells throughout much of oogenesis (Fig. 3). For most epithelia, the apical domain constitutes a free surface facing the exterior of the body or the lumen of a tube. Although unusual, this close proximity between the FCE and germline is essential for many aspects of FC biology. During later stages of oogenesis, FCs function as protein factories, synthesizing yolk proteins and eggshell components, which are secreted from their apical surfaces toward the oocyte. The interaction between the apical surface and germ cells also facilitates numerous signaling events between the germline and soma. We have discussed previously two signaling events during oogenesis where the Delta ligand in the germline activates the Notch receptor on the apical surface of the FCs. One interesting question that arises is whether the close apposition between the FCE and germline also provides a positional cue for the establishment and maintenance of apicobasal polarity in this tissue.

Typically, the early cues that establish apicobasal polarity come in the form of basal cues, through cell–substrate adhesion, and lateral cues in the form of cadherin-based cell–cell adhesion. These adhesive events then trigger the formation of protein complexes at the cell surface, which further refine the apical and basolateral membrane domains and lead to the localization of a cadherin-based junctional complex, the zonula adherens (ZA), at their interface. These protein complexes have been best studied in the embryonic ectoderm where three groups, known as the Baz (Bazooka/Par-6/aPKC), Scrib (Scribble/Discs Large/Letha Giant Larvae), and Crumbs (Crumbs/Stardust/PATJ) complexes, have been shown to act in a functional hierarchy to delineate the apical and basolateral membrane domains (Bilder et al., 2003; Tanentzapf and Tepass, 2003). The Baz complex localizes to the marginal zone just apical to and overlapping with the ZA and acts first in the hierarchy to specify the apical domain. The Scrib complex is found just basolateral to the ZA and functions as a basolateral determinant by repressing the apicalizing activity of the Baz complex. Finally, the Baz complex recruits the Crumbs group to the apical domain, to antagonize the activity of the Scrib complex.

The FC monolayer is unusual among epithelia in that the cells have the potential to receive adhesion-based polarizing signals at their basal, lateral and apical surfaces. Before encapsulation, the FC precursors adhere to a basement membrane that surrounds the germarium and contact each other laterally through adherens junctions. These contacts appear to be sufficient to establish a basal membrane domain; however, apical and lateral markers are intermixed, suggesting that contact with the germ cells is necessary to resolve the apical and lateral domains (Tanentzapf et al., 2000). In support of this assertion,

the apical determinant Crumbs is not expressed in the FCs of agametic ovaries (Tanentzapf et al., 2000). One interesting question is whether the interactions between the Baz, Scrib, and Crumbs complexes mirror those seen in the ectoderm or have been modified to exploit the unique cellular environment of this tissue. It is known that all three complexes are required for cell polarity in the FCE, as loss of function of any one component leads to rounded cells and multilayering at the follicle poles (Manfruelli et al., 1996; Goode and Perrimon, 1997; De Lorenzo et al., 1999; Bilder et al., 2000; Genova et al., 2000; Tanentzapf et al., 2000; Abdelilah-Seyfried et al., 2003; Benton and St. Johnston, 2003; Hutterer et al., 2004). More work will be required, however, to explore the interactions between these various players. Finally, contact between the apical surface and the germline also appears to be required to maintain polarity in the FCE, as loss of function of *egghead* or *brainiac*, which are both required in the germline, can lead to multilayering and loss of polarity during mid-oogenesis (Goode et al., 1996).

A second interesting property of the FCE is the array of cellular junctions that connect the FCs to one another and to the germline. The FCs assemble a functional ZA at the apical side of their lateral surfaces from the time that the egg chamber first buds from the germarium (Muller, 2000). Of interest, the adherens junctions of the ZA contain both E and N cadherin up to stage 10 of oogenesis, at which time N-cadherin disappears (Tanentzapf et al., 2000). Overlap of two classic cadherins in the same tissue is unusual in *Drosophila* and could exist because of the dual role E-cadherin plays in adhesion between the FCs and adhesion between the FCs germline. In fact, the large Maf transcription factor Traffic Jam, which is required for proper interactions between the germline and soma, regulates the expression of E-cadherin but not N-cadherin in the FCs (Li et al., 2003). The septate junction (SJ), which acts as a transepithelial diffusion barrier, forms along the lateral membrane, just basal to the ZA. SJ formation begins when proliferation is complete at stage 6, but these junctions do not fully mature until stage 10 after the posterior migration of the FCs is finished (see below; Mahowald, 1972; Muller, 2000). Functional gap junctions have been observed between cells within the FCE and between FCs and the germline (Mahowald, 1972; Giorgi and Postlethwait, 1985; Bohrmann and Haas-Assenbaum, 1993; Tazuke et al., 2002). What information passes through these gap junctions is currently unknown, but there is evidence that gap junctions between the FCs and oocyte are required for the oocyte to take up yolk (Waksmonski and Woodruff, 2002). Cytoplasmic bridges, presumably resulting from incomplete cytokinesis, have also been observed to connect groups of up to eight FCs within the FCE (Giorgi, 1978; Woodruff and Tilney, 1998). The cytoplasmic bridges in the FCs are much smaller than those found within the germline cyst, and their function remains to be determined.

Planar Polarity and Egg Elongation

In addition to apicobasal polarity, many epithelia also display polarity within the plane of the epithelium. Planar polarity can govern tissue morphogenesis in several ways (for review, see Adler, 2002). In the case of the FCE, it is involved in creating the elongated shape of the egg. Each FC contains a dense array of polarized actin filaments at its basal cortex that is arranged such that the fibers run perpendicular to the A-P axis of the egg chamber (Gutzeit, 1990). This pattern is first established during stages 5–6 in FCs that lie near the egg chamber poles. The effect of this bilateral initiation is that actin filaments appear to swirl around each pair of polar cells, which has led to the suggestion that these cells may function as organizers in this process (Frydman and Spradling, 2001). The circumferential orientation of the actin bundles then spreads medially until all FCs display this pattern by stage 7; this organization in maintained in cuboidal and columnar FCs through stage 14 (Gutzeit, 1990; Frydman and Spradling, 2001). Intriguingly, laminin A is organized into complementary circumferential fibers within the basal extracellular matrix (ECM) that surrounds each follicle (Gutzeit et al., 1991).

This striking circumferential organization of actin and laminin within the FCE has led to a model proposing that the planar array of filaments acts as a molecular corset that forces the follicle to grow preferentially along its A-P axis. Egg chambers are roughly spherical until stage 6, but lengthen along their A-P axis roughly concurrent with the polarization of the actin bundles. This A-P elongation is particularly apparent after nurse cell dumping at stage 11 and leads to the production of a lozenge-shaped egg. Although it is not yet known how these actin fibers exert morphogenetic forces to shape the egg, their proper orientation within the plane of the epithelium is absolutely required for them to perform this function. Several genes have been identified that, when mutated in the FCs, produce spherical eggs (Gutzeit et al., 1991; Bateman et al., 2001; Frydman and Spradling, 2001; Deng et al., 2003). Examining clones of these mutations within the FCE reveals that actin bundles form normally at the basal side of each cell, but their orientation is random with respect to the A-P axis. Surprisingly, this phenotype is not restricted to the mutant clone, but spreads to adjacent wild-type tissue, suggesting a need for cell communication within the epithelium to establish the planar pattern of actin. Evidence for communication between the cells is also seen within mutant clones where small groups of cells often coordinate their actin bundles with respect to each other, whether or not they match the global orientation of actin bundles in the tissue.

Several mutations that cause a spherical egg phenotype have been shown to disrupt proteins that mediate interactions between the actin cytoskeleton and the ECM. These disruptions include the Dystrophin-associated glycoprotein complex component Dystroglycan (Deng and Ruohola-Baker, 2000), the receptor-like tyrosine phosphatase Dlar (Bateman et al., 2001; Frydman and Spradling, 2001), and the β-integrin subunit encoded by the *myospheroid* (*mys*) gene (Duffy et al., 1998; Bateman et al., 2001). It is not yet known how these proteins work to align the actin filaments along the A-P axis, but studies

of DLar indicate that it may play an early role in the establishment of the planar pattern. *DLar* and *myospheroid* genetically interact and their proteins colocalize at the actin filament termini. Whereas β-integrin is maintained at the termini throughout oogenesis, the localization of DLar is transient, occurring only during the stages when the planar pattern is established. Furthermore, genetic rescue experiments have shown that expression of DLar before stages 7–8 is sufficient to rescue the mutant phenotype, whereas expression at stage 10 is not. These data have led to a model postulating that DLar is required to modulate the early interaction between integrins, the basal actin filaments and the ECM (Bateman et al., 2001). An opposing model stipulates that DLar is actually required for polar cell specification and that the misorientation of actin in DLar mutants is secondary to this defect (Frydman and Spradling, 2001). This model is based on the observation that DLar mutant follicles often have additional polar cells and that the circumferential actin pattern is first seen in the polar region. Mosaic analysis has shown, however, that DLar is not required in polar cells to correctly orient the actin bundles (Frydman and Spradling, 2001). This observation, in conjunction with the protein localization pattern makes it more likely that DLar functions independently in these two processes.

An interesting and open question is to what extent the planar organization of actin filaments within the FCE is governed by conserved mechanisms that control other planar polarity systems in the fly such as the proximal–distal positioning of wing hairs (for reviews, see Adler, 2002; Tree et al., 2002). One striking feature shared by both systems is that mutant clones that disrupt the planar organization of the cytoskeleton have nonautonomous effects on neighboring wild-type cells. To date, however, no overlap has been found between the genes that appear to control planar polarity in the FCE and the genes that have been indicated in other planar polarity systems. This finding includes the serpentine receptor Frizzled and its downstream effector Disheveled, which are required for most examples of planar polarity in *Drosophila* as well as in vertebrates. Future experiments will be required to determine whether an entirely unique system is used to create planar polarity in the FCE or if conserved mechanisms are operating that have not yet been recognized.

FOLLICLE CELL MIGRATIONS TO SURROUND THE OOCYTE

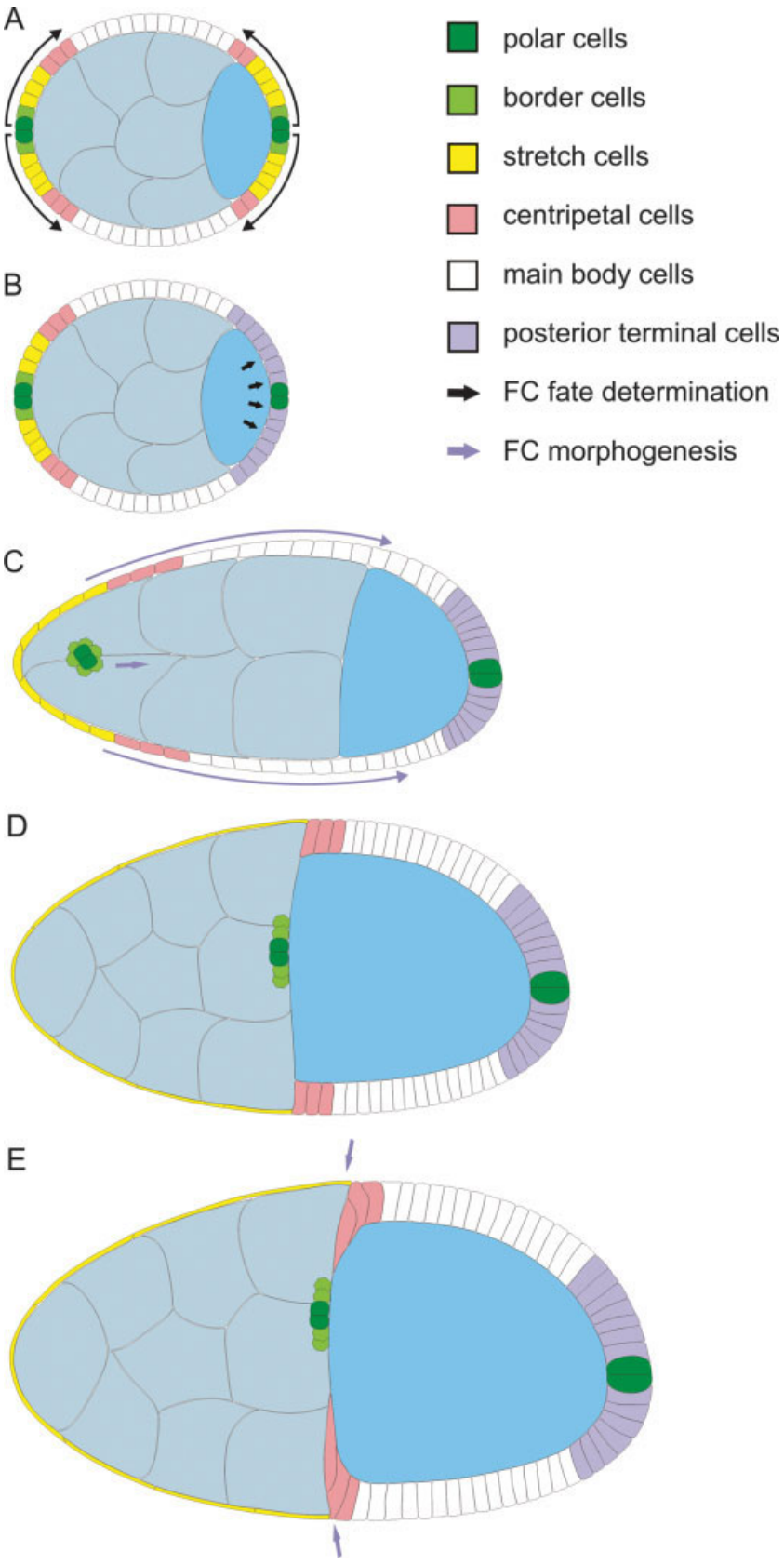
During vitellogenesis, the oocyte grows much larger than the nurse cells and the FCE undergoes a complex series of morphological changes designed to bring the majority of the FCs into contact with the oocyte to allow them to secrete and pattern the eggshell. These morphological changes are preceded by the differentiation of the uniform cuboidal epithelium into at least five cell types along its A-P axis, known as border, stretch, centripetal, main body, and posterior terminal cells. In this section, we will review the signaling events that create these various subpopulations within the FCE and discuss the unique morphogenetic and migratory properties used by each cell type during the mass movement of cells to surround the oocyte.

A-P Patterning of the FCE

The dynamic cell shape changes and migrations that occur within the FCE during vitellogenesis are entirely dependent on the specification of different FC fates along the A-P axis. Posterior placement of the oocyte creates the earliest A-P asymmetry in the egg chamber (see above), but it is not until stages 5–6 that this germline asymmetry is exploited to create A-P differences within the FCE. These early differences have been primarily assayed using enhancer–trap reporters, because few differentially expressed proteins have been documented. The expression of these reporters corresponds well to the morphologically and morphogenetically distinct cell types that will appear later in development, however, making them a useful tool to study FCE patterning.

The first division in FC fates seems to occur when two terminal domains, extending 10–11 cell diameters from each pole, differentiate from the more medial group of main body cells (Gonzalez-Reyes and St. Johnston, 1998b; Fig. 4A). Because the polar cells lie at the center of each terminal domain and differentiate early in oogenesis, a signal from these cells could induce the formation of the terminal domains. The best candidate for this signal is the Jak/Stat ligand Unpaired (Upd), which is expressed specifically in polar cells (Silver and Montell, 2001; Beccari et al., 2002; McGregor et al., 2002). Consistent with this model, loss of Jak/Stat components from the termini of the egg chamber disrupts the formation of the terminal domains (Xi et al., 2003). Curiously, loss of Upd from the polar cells does not appear to affect the differentiation of terminal cells (Grammont and Irvine, 2002); however, this discrepancy could be due to redundancy with other Upd-like ligands encoded in the *Drosophila* genome (Hombria and Brown, 2002; Hou et al., 2002; Denef and Schupbach, 2003).

The two terminal domains do not remain uniform but rather are patterned to form several anterior and posterior FC fates. Experimental manipulations indicate that the three anterior FC fates (border, stretch, and centripetal cells) are the default state for the cells near both poles of the egg chamber and that a signal from the oocyte, the TGFα-like molecule Gurken, is required to make the posterior terminal cells unique from their anterior counterparts (Gonzalez-Reyes et al., 1995; Roth et al., 1995; Fig. 4B). Once the posterior terminal cells are specified by the Gurken signal, these cells send an unknown signal back to the oocyte that is required to reorganize the oocyte cytoskeleton and establish the A-P axis of the future embryo (reviewed in Lopez-Schier, 2003). Some data suggest that a gradient of Jak/Stat activity arising from polar Upd expression may induce the three anterior cell fates within the terminal domains (Xi et al., 2003; Fig. 4B). Although it is clear that the Jak/Stat pathway is required for the formation of the most anterior cell type, the border cells (Silver and Montell, 2001; Beccari et al., 2002; Ghiglione et al., 2002; Grammont and Irvine, 2002; Xi et al., 2003), its role in the specification of the stretch and centripetal cells is more controversial (Beccari et al., 2002; Denef and



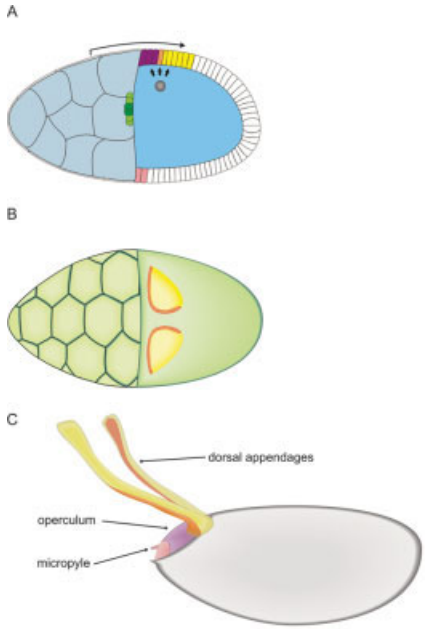


Fig. 5. Specialized eggshell structures. **A:** During stage 10, signals from the oocyte nucleus and stretch cells induce a patch of dorsoanterior columnar cells to take on the fate of either operculum (purple) or dorsal appendage forming cells (orange and yellow). Dorsal surface view of the follicle cells in a stage 10 egg chamber highlighting the dorsal appendage-forming regions. **B:** Roof cells are yellow, and floor cells are orange. **C:** Mature egg. The colors imposed on the anterior eggshell structures match those shown in A and indicate which cells contributed to each eggshell structure.

Fig. 4. Early patterning and morphogenesis. A,B: A model for the determination of anterior and posterior cell fates in the follicle cell epithelium. **A:** A signal from the polar cells induces the terminal domains to differentiate from the main body cells and subdivides them terminal into the three anterior cell types. **B:** Next, a signal from the oocyte instructs the posterior terminal cells to adopt a different fate from their anterior counterparts. C–E: Follicle cell (FC) migrations to surround the oocyte. **C:** During stage 9, the majority of the FCs migrate toward the posterior of the egg chamber. **D:** At stage 10a, the migrations are complete, the stretch cells, which are associated with the nurse cells, are squamous and the cells over the oocyte are columnar. **E:** During stage 10b, the centripetal cells migrate between the nurse cells and oocyte to enclose the anterior of the oocyte in FCs.

Schupbach, 2003; Xi et al., 2003). Whether Upd acts alone to establish cell fates within the terminal domains, or operates within a more complicated framework of A-P patterning signals remains to be determined.

Posterior Migration of the FCE

From the time that the egg chamber first buds from the germarium, through the stage when A-P patterning occurs, the FCs form a uniform cuboidal epithelium over the germline cyst. However, during stage 9, the FCs undergo a dynamic rearrangement toward the posterior of the egg chamber. The vast majority of the FCs, including those specified to form posterior terminal, main body and centripetal cells, undergo a cuboidal to columnar transition and migrate posteriorly to cover the oocyte (Fig. 4C). The switch to columnar morphology begins at the pole and spreads in a posterior to anterior wave that helps to draw the cells toward the oocyte. To complement the reduction in apical surface area that occurs when the cuboidal cells become columnar, the stretch cells become increasingly squamous until this population of only 50 cells is thin enough to cover the 15 nurse cells. Although posterior migration is typically only attributed to the columnar cells, to fully cover the nurse cell cluster the stretch cells must also move toward the posterior concurrent with their change in morphology. By stage 10A of oogenesis, these dynamic cell shape changes and migrations result in a sharp border between the columnar and squamous cells that lies directly over the oocyte–nurse cell boundary.

The cellular mechanisms that drive the dramatic and opposing cell shape changes within the FCE appear to be specified along with cell fate during the A-P patterning of the epithelial cells. Although it is tempting to speculate that contact with the oocyte could trigger the cuboidal to columnar transition, in mutations like *Bicaudal-D* where the oocyte is small, columnar FCs form over the nurse cells (Swan and Suter, 1996). Likewise, when posterior FCs fail to receive the Gurken signal, squamous cells can form over the oocyte (Gonzalez-Reyes et al., 1995; Roth et al., 1995). As these cell shape changes occur over a relatively short period of time (~ 6 hr), it is reasonable to assume that the cell volume does not significantly change during the time that cell shape is being altered. So, for the cuboidal to squamous transition, the apical and basal surfaces likely expand at the expense of the lateral domain, whereas a columnar transition would require an increase in the lateral domain with a corresponding constriction of the apical and basal surfaces. To date, no mutations have been identified that specifically disrupt the cuboidal to squamous transition and only one mutation has been identified that mildly affects the cuboidal to columnar transition. In *karst* mutants, cells become columnar, but the apical surface does not constrict sufficiently for all cells to fit onto the oocyte (Zarnescu and Thomas, 1999). *karst* encodes β_H-spectrin, which is a component of the membrane skeleton that is associated with the apical surface and ZA. *karst* mutant FCs show a general disruption in the structure of the ZA, which likely contributes to the defect in apical constriction (Zarnescu and Thomas, 1999).

Although cell shape changes likely provide much of the force driving posterior migration of the FCE, the oocyte itself also plays a significant role in drawing the FCs toward its surface. One fact that must be noted when discussing migration of the FCs onto the oocyte is that yolk uptake also occurs during this period, which causes the oocyte to grow until it occupies nearly half of the egg chamber (Spradling, 1993). Therefore, some FCs come to reside over the oocyte simply because the oocyte surface itself has expanded. However, several lines of evidence suggest that there is also a specific cue from the oocyte that attracts the FCs. For instance, when the oocyte is ectopically located in the center of the egg chamber, the cells that are fated to become columnar do not migrate toward the posterior but rather converge medially (Gonzalez-Reyes and St. Johnston, 1994). Furthermore, three genes known to act specifically in the germline affect the posterior migration of the FCs when mutated. *toucan* (*toc*) encodes a novel protein of unknown function, that appears to interact with the Notch pathway during stalk formation. Loss of *toc* function can also completely block the posterior rearrangement of the FCE in egg chambers where stalks formed normally (Grammont et al., 1997). Moreover, *egh* and *brn*, which act during encapsulation to ensure that a single cyst is packaged into each egg chamber (see above), may also contribute to the timely posterior rearrangement of the FCs, as loss of function of either gene causes the FCs to migrate onto the oocyte faster than in wild-type (Goode et al., 1996).

The nature of the attractive signal from the oocyte is unclear but may involve adhesion between the apical surface of the FCs and the oocyte membrane. In *karst* mutants, where some FCs that are fated to become columnar remain over the nurse cells, the oocyte–nurse cell boundary can become distorted as the ectopically positioned FCs appear to pull the oocyte membrane toward themselves (Zarnescu and Thomas, 1999). Posterior migration of the FCE is quite different from more-commonly studied examples of epithelial migration such as dorsal closure where specialized cells at the leading edge of the epithelium largely control the direction of movement (reviewed in Jacinto et al., 2002). There is no "leading edge" in the FCE; therefore, selective adhesion between the columnar cells and oocyte may provide this directional function. The identification of new mutants that disrupt the posterior migration of the FCE should shed light on the molecular nature of the interaction between the FCs and oocyte and may reveal a novel mechanism for cell migration in which the apical surface of an epithelium migrates along a cellular substrate.

Border Cell Migration

Coincident with the posterior migration of the FCE, the border cells exit the epithelium and strike out on their own path toward the oocyte. Among the three anterior cell fates, the border cells lie closest to the pole and comprise four to eight cells surrounding the two anterior polar cells. At stage 9, the border cells detach from the FCE, carrying the anterior polar cells with them, and actively migrate as an epithelial cluster between the

nurse cells. The border cells change direction upon reaching the oocyte and migrate dorsally to their final destination across from the oocyte nucleus. Border cell migration has been studied intensively for its potential to contribute to our understanding of cell invasion and migration both in the context of normal development and metastatic cancer. As such, the extensive literature on this topic is beyond the scope of this review. For more information on border cells, please see the recent review by Montell (2003).

Centripetal Migration

Posterior migration of the FCE brings most FCs in contact with the oocyte so that they can secrete and construct the eggshell. However, formation of a complete eggshell requires that FCs also enclose the anterior of the oocyte. To accomplish this, the columnar cells that lie at the anterior edge of the oocyte begin to elongate at stage 10B and dive between the oocyte and adjoining NCs in a process known as centripetal migration (Fig. 4E). Centripetal migration occurs roughly concurrent with NC dumping, and these two processes must be carefully coordinated to allow the nurse cell contents to be transferred to the oocyte before the time that the oocyte becomes completely ensheathed in FCs. Centripetal migration ends when the ingressing cells contact the border cells to form a confluent epithelium over the anterior of the oocyte. Failure to undergo centripetal migration results in an "open chorion" phenotype where the mature egg contains a hole in the anterior eggshell.

Competence for centripetal migration appears to be conferred on a subset of FCs during AP patterning (see above) as presumed centripetal cells do not need to lie at the NC–oocyte border to ingress. In *Bicaudal-D* mutants, where the oocyte is small and the oocyte–NC border is shifted toward the posterior, centripetal cells can migrate aberrantly between the nurse cells (Swan and Suter, 1996). Furthermore, in cases where the posterior terminal domain is not specified, the "mirror image" centripetal cells occasionally ingress into the middle of the oocyte (Gonzalez-Reyes et al., 1995; Roth et al., 1995). Among the centripetal cells, at least two subpopulations can be identified based on cell behavior and gene expression. The leading edge cells are the first tier to penetrate the germ cells. These cells express Dpp and accumulate high levels of actin and nonmuscle myosin II at their medial surfaces (Edwards and Kiehart, 1996; Twombly et al., 1996). In contrast, the remainder of the population fails to express Dpp and instead expresses the A359 enhancer trap both before and during migration (Dobens et al., 2000).

The cellular processes that drive centripetal migration share features in common with two other more intensively studied morphogenetic events in *Drosophila*: dorsal closure and border cell migration. During dorsal closure, the epithelia that make up the lateral regions of the embryonic epidermis elongate their cells and migrate dorsally to enclose a gaping hole in the embryo that is exposed after germ band retraction (reviewed in Jacinto et al., 2002). In both centripetal migration and dorsal closure, a thick actomyosin cable is assembled at the leading edge of the migrating epithelia and loss of function of nonmuscle myosin II disrupts both processes (Young et al., 1993; Edwards and Kiehart, 1996). Although the exact function of these cables is controversial, they may act through a purse-string contraction mechanism to help enclose the anterior of the oocyte and the dorsal hole in the embryo, respectively.

A second similarity between centripetal migration and dorsal closure is that Dpp is selectively expressed in the leading edge cells of the migrating epithelium. In the case of dorsal closure, Dpp is believed to act in a paracrine fashion to induce the rows of cells behind the leading edge to elongate and drive migration (reviewed in Jacinto et al., 2002). To date, mutational analysis has not revealed a clear role for Dpp in centripetal migration; however, these studies used conditional partial-loss-of-function alleles of *dpp*, and mosaic analysis with null alleles may be required to assess any centripetal phenotype (Twombly et al., 1996; Dobens et al., 2000). Both the FCE and germline express the Dpp type I receptor *saxophone* (*sax*), and either the expression of a dominant negative form of *sax* throughout the follicle or mutation of *sax* specifically in the germline has been shown to disrupt centripetal migration (Twombly et al., 1996). What role Dpp has in signaling to the germline is unclear, but the non–leading edge centripetal cells must also receive the Dpp signal, as phospho-Mad accumulates in their nuclei during centripetal migration (Jekely and Rorth, 2003). Once again mosaic analysis of *sax* in the FCE could reveal whether or not Dpp secretion from the leading edge affects the following cells in a manner similar to dorsal closure. Although the role of Dpp in inducing cell elongation may be conserved between these two morphogenetic processes, the upstream signaling events that lead to its restricted expression in the leading edge are not. During dorsal closure the Jun kinase signaling pathway is responsible for the transcription of *dpp* in leading edge cells (reviewed in Jacinto et al., 2002). Components of the Jun kinase pathway are expressed in the FCE during centripetal migration, but this pathway does not seem to control *dpp* expression in this tissue (Suzanne et al., 2001).

Centripetal migration and dorsal closure may share some mechanistic properties for closing an epithelial hole, but they differ in the way that the epithelia interact with adjacent cell types. During dorsal closure, the epithelia crawl over the underlying cells in the embryo, while in centripetal migration the epithelia actually penetrate the cluster of germ cells. In this way, centripetal migration resembles border cell migration, during which a small epithelial cluster invades the germline cyst and migrates toward the oocyte (reviewed in Montell, 2003). Both the border cells and centripetal cells express high levels of DE-cadherin during their migration and removal of DE-cadherin from either the FCs or the germline disrupts both processes (Oda et al., 1997; Niewiadomska et al., 1999). Cadherin-based adhesion between the FCs and germ cells, therefore, may provide the traction necessary for both the border and centripetal cells to penetrate the germ cell cluster. These data indicate that purse-string contraction of the actomyosin cable and cell elon-

gation are unlikely to be the only forces driving the centripetal cells toward the center of the egg chamber; the centripetal cells may also actively migrate between the germ cells using a mechanism involving homophilic adhesion.

FORMATION OF SPECIALIZED EGGSHELL STRUCTURES

Around the time that the FCs complete their migrations to cover the oocyte, they begin to secrete the eggshell between the epithelium and oocyte membrane. The complexity of the mature eggshell can be seen both in its multilayered radial construction and through the elaboration of distinct architectural features that perform critical functions for the egg and/or developing embryo (reviewed in Dobens and Raftery, 2000; Waring, 2000). Although these specialized eggshell structures are largely proteinaceous and completely acellular once the egg is laid, their morphology reflects the morphogenetic movements of the FCs that secreted them (Fig. 5). In this section, we describe the location and function of three anterior eggshell structures, discuss the signaling events that instruct the dorsoanterior FCs to contribute to two of these elements, and, finally, take a closer look at the specific morphogenetic movements of FCs that construct the best studied of these structures, the dorsal appendages.

Specialized Eggshell Structures

The bilateral pair of dorsal appendages is the most prominent of three chorionic elaborations situated at the anterior of the eggshell. Each dorsal appendage consists of a narrow cylindrical stalk with a flattened "paddle" at the tip that extends from the anterior of the eggshell just lateral to the dorsal midline. The specialized chorionic structure of the paddle is believed to facilitate gas exchange when the egg is submerged in liquids such as water or rotting fruit. The dorsal appendages arise from two patches of dorsoanterior columnar FCs, which reorganize to form tubes that extend out over the nurse cells during stages 11 to 14 of oogenesis. The FCs that make up these tubes then secrete eggshell components into the tubes' lumen to construct the mature chorionic appendages.

The operculum is a nearly flat plate at the anterior of the mature egg that is characterized by a distinct pattern of FC imprints and a raised "collar" at its periphery. The operculum represents a weakened region of the eggshell through which the larva escapes at the end of embryogenesis. This structure is formed by approximately 50–70 centripetal FCs (Margaritis et al., 1980), and the dorsal centripetal cells are specifically induced to adopt the operculum fate before their migration over the anterior face of the oocyte (see below).

The centripetal FCs also participate in the construction of the most anterior eggshell component, the micropyle. The micropyle is a narrow channel that extends at an oblique angle from the ventral aspect of the operculum and functions as the sperm entry point during fertilization. After their respective migrations, the border cells and leading edge centripetal cells come together at the anterior of the oocyte to construct this channel (Zarani and Margaritis, 1986). Manipulations that prevent border cells from reaching the oocyte have shown that the centripetal cells play the major role in the construction of the cone projecting from the front of the egg, whereas the border cells create a pore within the cone for sperm entry (Montell et al., 1992).

Patterning of the Dorsoanterior FCs

Two major signaling pathways converge to induce a population of the dorsoanterior columnar FCs to adopt cell fates that will give rise to the dorsal appendages and operculum (Fig. 5A). The first of these signals comes through the epidermal growth factor receptor (EGFR) pathway. In addition to its earlier role in establishing the follicular A-P axis, Gurken/EGFR is used a second time during oogenesis to distinguish FC fates along the D-V axis. Once the signal from the posterior terminal cells induces a rearrangement of the oocyte cytoskeleton (reviewed in Huynh and St. Johnston, 2004), the oocyte nucleus migrates toward the anterior oocyte membrane to an asymmetric position adjacent to the FCs. *gurken* mRNA is tightly associated with the oocyte nucleus and the FCs that pass over this nucleus during their posterior migration become exposed to the Gurken signal and consequently take on a dorsal fate. The dorsal Gurken signal has two seemingly independent effects on the FCs. The first is to restrict the action of *pipe* to a ventral stripe in the FCE—an event that is necessary for the subsequent D-V patterning of the embryo (reviewed in Nilson and Schupbach, 1999; van Eeden and St. Johnston, 1999). The second is to instruct dorsoanterior FCs to produce the operculum and dorsal appendages and to direct the placement of the two dorsal appendages on either side of the midline.

To produce two laterally placed dorsal appendages, the Gurken signal from the oocyte triggers an amplification of EGFR signaling within the FCE itself, through the activation of a second EGFR ligand, Spitz (Wasserman and Freeman, 1998). Although Spitz is uniformly expressed in the FCE throughout oogenesis, this ligand cannot activate EGFR until it is cleaved by the intramembrane protease Rhomboid. Gurken induces the transcription of *rhomboid* in a dorsoanterior patch of FCs at stages 9–10 of oogenesis. Presumably, cleavage of Spitz in this region leads to an increase in EGFR signaling, which is evidenced by a dorsoanterior patch of increased Map kinase activation (Wasserman and Freeman, 1998). This higher level of EGFR signaling then triggers a negative feedback loop by inducing the expression of an inhibitory ligand, Argos, in a T-shape at the dorsoanterior midline. Argos subsequently inhibits the EGFR receptor in the midline, splitting the region of EGFR activation into two bilateral patches, which go on to express dorsal appendage markers such as members of the *broad-complex* (*BR-C*) group of transcription factors. EGFR signaling also induces the transcription of a third EGFR ligand, *Vein*, in the FCE, which may further refine the lateral positioning of the pair of dorsal appendages (Wasserman and Freeman, 1998).

The restriction of *pipe* to the ventral

side of the FCE occurs along the entire length of the egg chamber, and yet dorsal chorionic structures only form at the anterior. How is this second response to the dorsal Gurken signal localized? The answer is that the formation of the operculum and dorsal appendages requires a combination of the dorsal Gurken signal and an anterior signal, the TGF-β family member Dpp (Deng and Bownes, 1997; Peri and Roth, 2000). *dpp* is expressed in an anterior region of the FCE, including the stretch cells and leading edge centripetal cells, from stage 8 of oogenesis (Twombly et al., 1996; Dobens et al., 2000). Furthermore, Dpp appears to set the A-P boundary between the operculum and dorsal appendages. Decreased Dpp signaling greatly reduces the operculum and shifts the position of the dorsal appendages toward the anterior; conversely, increased Dpp signaling expands the operculum at the expense of the dorsal appendages (Twombly et al., 1996; Dobens et al., 2000). The positioning of these two structures by Dpp appears to be mediated by the transcription factor *bunched*, an inhibitor of the operculum fate whose transcription is negatively regulated by the anterior Dpp signal (Dobens et al., 2000).

Dorsal Appendage Morphogenesis

Once the dorsal appendage cells are specified by the combined actions of Gurken and Dpp, each two-dimensional array of cells undergoes a complex reorganization to form a three-dimensional tube, a key morphogenetic process that underlies much of animal organogenesis. Dorman et al. recently have used sophisticated live imaging techniques on postvitellogenic egg chambers to reveal in startling detail the cellular movements that convert the dorsoanterior columnar cells into the two extended tubes that will secrete the chorion of the dorsal appendages (Dorman et al., 2004). These studies have identified two distinct cell populations within each of the dorsal appendage-forming regions, based on patterns of gene expression, morphology, and cell behavior (Fig. 5B). The roof cells comprise an ovoid patch of ~50 cells that express high levels of *BR-C* and will go on to form the dorsal roof of the tube. The floor cells consist of a single row of 10–15 cells that bend through a 90-degree angle to flank the anterior and medial borders of the roof cell population. The floor cells fail to express elevated levels of *BR-C*, but rather can be identified through use of the *rhomboid-lacZR1.1* enhancer trap. As their name suggests, these cells will form the ventral floor of the tube.

Morphogenesis begins during stage 10b of oogenesis when the dorsoanterior columnar cells extend along their apicobasal axes to form a thickened placode from which the dorsal appendages will form. After placode formation, Dorman et al. describe the specific movements of the roof and floor cells in three phases: tube formation, anterior extension, and paddle maturation (Dorman et al., 2004). Tube formation occurs during stages 10b–12 of oogenesis. During this phase, the roof cells constrict their apices, which presumably leads to a dorsal curvature in this region of the epithelium, and the roof population as a whole narrows in the mediolateral direction and extends along the A-P axis. As the roof cells undergo these changes, the apical and medial rows of floor cells reorient such that their apical surfaces begin to extend under the roof population. The floor cells continue to elongate beneath the roof cells until the apices from the two rows meet to close the tube. The newly formed tubes are quite short and sit atop the cells that have migrated centripetally. Anterior elongation occurs during stages 12–13, when the tubes extend out over the remnants of the nurse cell population after cytoplasmic dumping. The tubes migrate between the basal lamina of the egg chamber and the stretch cells that continue to encompass the nurse cell remnants. Finally, paddle maturation occurs during stages 13–14 when the anterior portion of the tube becomes wider and flatter through a combination of a deconstriction of the roof cell apices and an increase in the number of roof and floor cells in the paddle region as compared with the stalk.

Mutant studies have revealed several genes that are specifically required for dorsal appendage morphogenesis. In *bullwinkle* (*bwk*) mutants, the patterning of the dorsal appendage primordia and early tube formation appears normal, yet the tubes fail to elongate over the nurse cells (Rittenhouse and Berg, 1995; Dorman et al., 2004). *bwk* mutants affect a family of Sox/TCF transcription factors that are required in the germline to promote dorsal appendage morphogenesis (Rittenhouse and Berg, 1995) as well as other events during oogenesis (Jimenez et al., 2000; Goff et al., 2001). Through a screen to isolate genetic modifiers of the *bwk* phenotype, Tran and Berg identified the SH2-ankyrin-repeat tyrosine kinase, *shark*. Intriguingly, *shark* and *src42a* are required in the stretch cells to mediate the action of *bwk* (Tran and Berg, 2003). Although it is not known what action these genes perform within the stretch cells, the stretch cells act as a substrate for the migrating dorsal appendages and have been observed to extend processes toward the elongating tubes (Tran and Berg, 2003). Several members of the Jun kinase signaling pathway are also expressed in stretch cells and are required for dorsal appendage morphogenesis; however, careful clonal analysis will be required to confirm whether these genes actually function within the stretch cells to affect tube elongation (Dequier et al., 2001; Dobens et al., 2001; Suzanne et al., 2001). A downstream member of the Jun kinase pathway, Fos, displays an additional domain of expression within the floor cells (Dequier et al., 2001; Dorman et al., 2004). As Fos is required for cell elongation during dorsal closure, it will be interesting to determine whether this gene plays a similar role during floor cell elongation. Finally, the nonmuscle myosin gene *spaghetti squash* (Edwards and Kiehart, 1996) and the transcription factor *tramtrack 69* (French et al., 2003) have both been implicated in anterior elongation, but it is not known in which tissue they function. Further genetic screens will be required to identify genes that act autonomously within the dorsal appendage primordia during anterior elongation as well as during tube formation and paddle maturation.

PERSPECTIVE

The combination of diverse morphogenetic behaviors and genetic tractability make the *Drosophila* FCE an optimal tissue in which to investigate the cellular process that drive epithelial

movement. Previous studies have made great strides in elucidating the signaling events that pattern the FCE into a variety of cell types. Now the challenge is to understand the various morphogenetic properties that are programmed into these cells along with cell fate. Because the majority of epithelial movement in the egg chamber occurs in the absence of cell division, most morphogenetic changes in the FCE can be attributed to changes in cell shape, greatly simplifying studies of how form is generated. Detailed visual analyses are required to better characterize the cellular movements that underlie wild-type morphogenesis as well as to document how these processes go awry in mutant egg chambers. In pursuit of this goal, beautiful live imaging studies have now revealed the complex cell shape changes that lead to dorsal appendage formation (Dorman et al., 2004). The future development of culture conditions for younger egg chambers should allow these indispensable analyses to be extended to earlier stages as well.

From a genetic perspective, the ease of producing mutant clones within the FCE will also greatly facilitate the identification of the molecules responsible for the dynamic movements of epithelial cells. Several mutants that affect epithelial morphogenesis in the FCE currently exist, and more are being generated. One of the great advantages of screening for mutants within the FCE is the ability to make mosaic clones within the somatic stem cells (Duffy et al., 1998). This technique allows for the production of large mutant clones that have the potential to disrupt any one of the morphogenetic processes detailed in this review. In the future, the development of culture conditions supporting egg chamber morphogenesis could also facilitate the identification of molecular players through RNAi or small molecule screens. The information gained from genetic and molecular screening in the FCE will likely make significant contributions to our understanding of frontier issues in epithelial biology, such as the regulation of epithelial stem cells, control of cell shape changes, the formation of a tube, and the study of convergent extension movements. In addition, these studies have the potential to inform our understanding of more novel epithelial behaviors such as the migration of an epithelium along its apical surface or the use of a stable epithelial structure to drive the morphogenesis of an adjacent tissue.

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