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***Drosophila* follicle cells: morphogenesis in an eggshell**

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Abstract

Epithelial morphogenesis is important for organogenesis and pivotal for carcinogenesis, but mechanisms that control it are poorly understood. The *Drosophila* follicular epithelium is a genetically tractable model to understand these mechanisms in vivo. This epithelium of follicle cells encases germline cells to create an egg. In this review, we summarize progress toward understanding mechanisms that maintain the epithelium or permit migrations essential for oogenesis. Cell-cell communication is important, but the same signals are used repeatedly to control distinct events. Understanding intrinsic mechanisms that alter responses to developmental signals will be important to understand regulation of cell shape and organization.

Keywords

review; oogenesis; migration; epithelial-mesenchymal transition; apical-basal polarity

Introduction

Studies in cultured cells have driven our understanding of mechanisms that regulate cell shape and migration. The application of this mechanistic information to living tissues remains a challenge. Genetic studies in the model invertebrate *Drosophila melanogaster* have been instrumental for illuminating how cell biological mechanisms are orchestrated during tissue morphogenesis. An important tissue for such studies is the follicular epithelium that surrounds the maturing fly oocyte. Elegant developmental and genetic studies have revealed that this epithelium develops coordinately with the oocyte and establishes the body axes of the resultant embryo. Cell-cell communication coordinates the morphogenesis of the follicular epithelium with the associated germ cells, in much the same way as epithelial-mesenchymal interactions drive tissue formation in vertebrates.

Once formed, the follicular epithelium is subdivided to yield distinct regions of epithelial organization, small groups of migratory follicle cells, and ultimately a secretory epithelium that progressively deposits the layers of the eggshell. The follicular epithelium has been an important mode for studies of receptor tyrosine kinase signal transduction (recently reviewed by [1-3]), epithelial cell polarity (recently reviewed by [4,5]), and stem cell/niche biology (recently reviewed by [6,7]). Here we highlight recent progress towards understanding the mechanisms of morphogenesis.

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Overview of oogenesis

Each adult ovary has 14-16 ovarioles, which contain strings of developing oocytes of progressive ages (Fig. 1). At the end of each ovariole are 2-3 germline stem cells (GSCs) in a structure called the germarium. Differentiating daughters of GSCs, the cystoblasts, undergo four mitotic divisions to form a 16-cell syncytium, or cyst. One germ cell becomes an oocyte; the remaining 15 become nurse cells. Nearby, follicle cell stem cells (FCSCs) give rise to precursor follicle cells [8]. About 16 precursor follicle cells invade between cysts, cease division and become pre-polar cells, which ultimately become polar cells and stalk cells [9-11]. Other precursor cells form a simple epithelium around the cyst, creating an egg chamber. Differentiation of the 5-8 stalk cells separates the newly formed egg chamber from the germarium. The oocyte grows substantially, due to uptake of yolk protein synthesized in the follicle cells and fat, and occupies almost half the egg chamber by stage 10A [12-14].

The follicle cells (FCs) remain in a cuboidal epithelium through stage 8. Beginning in stage 9, they reorganize in a series of migrations. The 6-10 anterior-most FCs become the border cells, which migrate through the nurse cells to the oocyte. Another fifty anterior FCs form a squamous epithelium overlying the nurse cells. Most FCs become a columnar epithelium over the oocyte. During stage 10B, FCs move inward to cover the anterior end of the oocyte.

During stages 10B to stage 14, nurse cells transfer their cytoplasmic contents to the oocyte [15]. The FCs deposit the vitelline membrane and eggshell over the oocyte. The oocyte completes maturation; nurse cells and FCs undergo apoptosis. The anterior end of a mature egg has a pair of dorsal appendages for embryonic respiration, an operculum for larval exit, and a micropyle for sperm entry (Fig. 2) [12,13,16].

The germarium: a complex niche with multiple stem cell types

The adult ovary develops from a larval primordium, which contains mitotically active populations of mesodermally-derived somatic cells and primordial germ cells (PGCs). A central mass of mesenchymal and primordial germ cells forms the ovarioles. During larval development, signaling between PGCs and intermingled somatic cells balances their numbers [17]. At the larval to pupal transition, posterior PGCs differentiate into cystoblasts, which become the first eggs laid by the adult female.

Formation of the niche and stem cells

Ovariole formation starts in third instar, when somatic cell migrations form ovarian structures, the niche for GSCs, and the FCSCs [13]. The central mesenchymal cells form a niche that maintains anterior PGCs in an undifferentiated state [20]; these PGCs become the GSCs [13, 14]. The terminal filaments, which sit at the anterior end of each germarium (Fig. 3), differentiate first, forming an array of 8-9 cell stacks. The Bric-a-brac (Bab) transcription factor is expressed in niche-forming somatic cells, and required for terminal filament formation [18,19], which can occur in the absence of PGCs [13]. Terminal filament cells form clusters by cell intercalation and cell recruitment [18,19]. The organization of terminal filament cells requires the transcription factor Engrailed, but subsequent cell flattening does not [21].

The mature germarium

Niche cells, GSCs and mitotically dividing cystoblasts lie in region 1 of the germarium (Fig. 3). The newly formed 16-cell cyst moves into region 2a as oocyte differentiation progresses [reviewed in 23]. FCSCs are located at the 2a/2b boundary [8]. In region 2b, the cyst reorganizes with the oocyte located in its center. Here, precursor FCs envelope the cyst, replacing the escort cells. A nascent egg chamber comprises region 3 [12-14,24].

In adults, a terminal filament and cap cells form a niche that maintains GSCs in every germarium [6,7,17,22,25-27]. At the base of the terminal filament, a cluster of cap cells contact the GSCs. GSCs are identified by the location of their fusomes, a large membrane cytoskeleton complex [38,39]. As differentiating daughters of GSCs move away from the cap cells, somatic escort cells wrap around them and support cystoblast differentiation [22]. The terminal filament and cap cells promote GSC self-renewal and prevent their differentiation. Somatic cells regulate germ cell proliferation and differentiation in other well-studied animal models. Mammalian Sertoli and Leydig cells regulate spermatogonia [28-31]; *C. elegans* distal tip cells maintain the mitotic population of germ cells [32-35]. Even in the mammalian ovary, which lacks GSCs after birth, cumulus cells regulate the oocyte cell cycle [29]. The *Drosophila* germarium has easily identifiable, actively dividing stem cells and an architecturally defined niche, providing a powerful system to study niche function.

Contacts between cap cells and GSCs [36] are necessary to maintain GSCs in an undifferentiated state. Adherens junctions, composed of DE-cadherin (Shotgun) and β -catenin (Armadillo), between cap and germ cells are required for GSC maintenance; indeed cap cells are identified by their high Armadillo levels [37]. Endosomal trafficking is important for this association; Rab11 null GSCs detach from the cap cells, undergo abnormal divisions, and arrest cyst development [40]. Gap junctions are present between germ cells and somatic cells, and may be important for maintenance of GSCs and their progeny [41].

Cap cells also regulate GSCs via secreted signals, notably Decapentaplegic (Dpp), the *Drosophila* homolog of Bone Morphogenetic Proteins (BMP) 2/4 [42]. Dpp is necessary to maintain GSCs in an undifferentiated state [43-48]. The niche regulates numbers of both GSCs and escort cell progenitors through the Jak/Stat (Janus kinase/signal transducer and activators of transcription) pathway. This pathway is activated by Unpaired ligands (Upd, and related genes, Upd2 and Upd3), acting through the transmembrane receptor Domeless (reviewed by [57,58]). Conversely, GSCs regulate cap cell number and function via Delta signaling to its receptor Notch [49,50]. Ectopic expression of Notch or Delta leads to ectopic cap cells that are sufficient to maintain GSCs through local Dpp signaling [50]. Thus, a GSC signal stimulates the somatic niche to produce factors for stem cell maintenance [49].

Follicle cell stem cells

Production of both germ cells and FCs is coordinately regulated by the same niche cells. FCs are born from 2-3 somatic stem cells, which we call follicle cell stem cells (FCSCs) as in [21]. FCSCs are 2-5 cell diameters away from the cap cells and terminal filament, which maintain FCSCs by producing Hh, Wingless (Wg), and Dpp [52,59-61]. Like GSCs, FCSCs are short-lived if they cannot respond to BMP signals [59]. Hh regulates the number of FCSCs [52,61]; Wg regulates their proliferation and differentiation [60].

A few inner germarial sheath cells lie between the cap cells and the FCSCs. DE-cadherin and Armadillo are strongly localized at the interface between FCSCs and inner germarial sheath cells, and are required to maintain the FCSCs long term [62]. The role of adherens junctions for either GSCs or FCSCs remains unclear: they may mediate a structural or tensile signal, or they may hold the stem cells near the source of other niche signals.

Disruption of the coordinated proliferation of GSCs and FCSCs leads to defective egg chamber development [52]. When niche cells are mutant for *Yb*, FCSCs are fewer and GSCs are lost, whereas overexpression increases the numbers of both populations [53,54]. In addition to developmental regulation, the proliferation rate of both GSCs and FCSCs responds to nutrition of the female over at least a four-fold range [63]. The mechanisms that mediate this response are unknown, but may occur through *Drosophila* insulin-like peptides produced by the nervous system [64].

Precursor follicle cells and egg chamber formation

Inward migration of precursor FCs separates individual germline cysts into discrete egg chambers. This process involves the specification of three FC types: polar cells, stalk cells, and epithelial FCs.

Prepolar cells and separation of cysts

A polyclonal group of precursor FCs migrates between cysts, stops dividing and becomes prepolar cells, founding the polar/stalk cell lineage [65]. This migration requires Hedgehog (Hh) signaling through a Fused-dependent response pathway [66]. β -integrin is required in precursor FCs to prevent them from migrating inside cysts [21]; additional mechanisms are reviewed elsewhere [5]. Prepolar cell differentiation into polar and stalk cells maintains egg chamber separation.

Selection of the polar cell fate requires a Delta signal from the germline to stimulate Notch activity in the prepolar cells. The Notch receptor has two membrane-bound ligands, Delta and Serrate; the Fringe glycosyltransferase biases Notch to bind Delta preferentially [67][68-70]. Fringe is expressed in prepolar cells as they intercalate between cysts and determines the balance between polar and stalk cells [71]. No polar cells form without Fringe; whereas elevated Fringe increases their numbers. Importantly, egg chambers mosaic for *Notch* mutant FCs develop normally whenever the polar cells are wild type. Absence of Delta in the germline is associated with absence of polar cells and large, multicyst egg chambers [11,72-74]. Thus, polar cells are essential to separate cysts into individual egg chambers.

An early function for polar cells is to specify the stalk cells. Prepolar cells between stage 1 and stage 2 egg chambers are induced to form stalk cells by Delta ligand from anterior polar cells of the stage 2 egg chamber [75]. Different levels of Notch activation distinguish the polar and stalk cell fates; high levels induce polar cells. In polar cells, the metalloprotease Kuzbanian-like attenuates the Delta signal to induce stalk cells [76]. Overexpression of either Notch or Delta in the FCs leads to abnormally long stalks between egg chambers [77].

Additional signals modulate polar and stalk cell differentiation. Upd/Jak-Stat signaling is necessary for stalk cells; when FCs cannot respond to Upd, there are excess polar cells [78, 79]. The stacked organization of stalk cells requires Hh signaling through Fused; stalk cells without Fused differentiate in a cluster [66]. Conversely, Fused-independent Hh signaling biases prepolar cells become polar cells [80]. Even within the epithelial FCs, ectopic Hh activity can induce ectopic polar cells [52], by repressing an epithelial FC-determining gene [81]. We will discuss the maturation of polar cells and epithelial FCs below.

Formation of the follicular epithelium

The initial encapsulation of germline cysts involves a mesenchymal to epithelial transition. Mesenchymal precursor FCs divide a few more times, then encapsulate the egg chamber in the follicular epithelium [8,65]. Differential adhesion, in which tissues reorganize by altering adhesion to their neighbors (reviewed by [82]), drives many of the FC reorganizations during oogenesis. Homotypic association of DE-cadherin mediates adhesion of precursor FCs to germline cells during epithelium formation. Prepolar cells maintain the oocyte at the posterior of the egg chamber via higher levels of DE-cadherin [83-85].

Glycosphingolipids are required for FCs to encapsulate the egg chamber. *egghead* and *brainiac* encode proteins that add sugar residues to glycosphingolipids [86]; both are required in germ cells to separate cysts into individual egg chambers [87,88]. Similarities in mutant phenotypes between *egghead*, *brainiac*, and *Notch* suggested that they might be involved in a common signaling pathway; however, *egghead* and *brainiac* are not required for polar and

stalk cell specification. *brainiac* may also modulate Gurken-EGFR signaling, as simultaneous reduction in *brainiac* and *gurken* produces multicyst follicles with discontinuities in the follicular epithelium [88,89]. It is unlikely that all signals needed for this transition have been identified; indeed, the Upd-Domeless/Jak-Stat pathway is also involved [90].

Some Polycomb group (PcG) genes promote differentiation of both the prepolar and epithelial lineages during egg chamber formation [99]. Because PcG proteins mediate gene silencing and chromatin remodeling (recently reviewed by [98]), it is tempting to speculate that these two lineages are irreversibly determined in the germarium. However, forced expression of certain signals, such as Hh [52,81] or Delta [76], can convert early epithelial FCs to polar cell or intermediate fates, respectively. Thus, a gradual differentiation of polar cells and epithelial FCs is a more likely scenario.

Maturation of polar cells

Polar cells reside at the anterior and posterior termini of egg chambers (Fig. 3) [14,94] and organize FC fates throughout oogenesis. They provide cues for axial patterning of the embryo, create a pore for sperm entry, and organize the specialized eggshell domains thought to enhance embryonic survival [61,65,80,91-93]. Initially, they are distinguished from epithelial FCs by their expression of a reporter in *neuralized* [11]. During stages 1 and 2, both polar cells and epithelial FCs have high levels of Fasciclin III (Fas3), a homophilic adhesion molecule [95], on their lateral membranes. Polar cells maintain high Fas3 levels as oogenesis proceeds (Fig. 3).

Initially, clusters of about 3-5 polar cells join each end of an egg chamber [80]. During stages 2-5, excess polar cells undergo apoptosis, leaving only two in every cluster. During stages 6-8, polar cells become rounded, reorganize adherens junction proteins, undergo transient apical capping of basement membrane proteins, and detach from the basement membrane [11,96,97]. Thus, polar cell maturation involves a progressive loss of epithelial cell features.

Maturation of epithelial follicle cells

One of the earliest markers to distinguish epithelial FCs is Eyes absent (Eya) [81], a DNA binding protein phosphatase [100,101]. Eya represses polar/stalk cell fates; epithelial FCs that lack Eya form ectopic polar cells; whereas overexpression of Eya in this lineage prevents or delays their differentiation. The Hh signal transducer Cubitus interruptus (Ci) may reinforce the decision between the polar cell and epithelial FC fates.

Extra machrochaetae (Emc), is required in the epithelial FCs for Eya expression, and for other aspects of their maturation [102]. Emc inhibits the transcriptional activity of basic helix-loop-helix proteins, like its mammalian homologs, Id proteins [103,104]. Emc is detected in precursor FCs, but is not detected in polar cells or stalk cells beginning at stage 2. In the absence of Emc, epithelial FCs continue to express high levels of Fas3, and do not stop proliferating.

Epithelial FCs stop dividing at the end of stage 6, and enter a modified cell cycle where DNA is duplicated without cell division [105,106]. Hh signaling through activated Ci promotes FC proliferation [52,61]. Delta in the germline and Notch in the FCs are required for the switch from mitotic cycles to endocycles. Notch activates this switch by inducing expression of the transcription factor Hindsight, which down-regulates expression of Cut, Eya, and Ci [109]. The Salvador-Warts-Hippo pathway also limits FC proliferation by regulating expression of Hindsight and Cut [107,108].

This change in cell cycle is sometimes called the onset of differentiation, or the switch from immature to differentiated FCs (e.g. [105,109,110]). The notion that Notch triggers

differentiation is a tempting parallel to Notch function in some mammalian tissues, such as epidermis [113]. Many observations seem to support it, for *Notch* is required in all epithelial FCs to stop proliferation and to express differentiation markers [11,71,72,105,110]. However, epithelial FCs begin differentiating at stage 2, with progressive down-regulation of Fas3. Furthermore, studies that manipulate Notch activity late in oogenesis have demonstrated requirements for Notch signaling during FC migrations [114-116], when epithelial FCs reactivate expression of early, 'immature' markers such as Fas3 [111], and Emc [112]. Thus, a nuanced model for dynamic modulations in Notch activity throughout FC morphogenesis is more appropriate.

During stages 7-9, FCs complete three endocycles [117]; the resultant polyploidy is necessary for their final size [105,106]. *Drosophila* Myc is required for endocycles and resultant growth [118]. As in other tissues, Akt kinase is required for normal FC size and PTEN limits the size increase [119-121]. Some isoforms of the transcription factor Bunched are required for FCs to achieve normal size during these stages (Wu et al, submitted); however, these isoforms are dispensable for the endocycles. The mechanisms that link cellular growth and DNA replication in the FCs are unknown.

Subdivision of epithelial follicle cell types

At about stage 5, FCs at the ends of the egg chamber, terminal FCs, become distinct from those in the center, mainbody FCs. Polar cells induce the terminal FCs [74]. Genetic ablation of polar cells at the anterior results in loss of the border, stretched, and centripetal fates; at the posterior, it results in failure of FCs to polarize the oocyte axis. Moreover, ectopic polar cells recruit neighboring FCs to become border cells or posterior terminal cells, depending on their location [52,93,122,123].

The terminal domains are symmetrically patterned in concentric rings, leading to the model that polar cells produce a morphogen that specifies different cell types at different concentrations [74]. Consistent with this model, Upd is produced from polar cells [78,79], and one study supports a morphogen function [92]. However, conflicting data exist; the model is reviewed critically elsewhere [124]. Overall, it is clear that the Upd/Jak-Stat pathway subdivides terminal and mainbody FCs, with mainbody FCs marked by expression of the homeodomain gene *mirror* [92].

The terminal domains have the default, anterior fate, until stage 6, when the TGF α -like ligand Gurken (Grk) activates sufficient levels of epidermal growth factor receptor (EGFR) activity in posterior FCs [9,10,74]. Strikingly, both the Jak-Stat and EGFR/Ras pathways must be activated to induce expression of posterior markers in mainbody cells [92]. Posterior FCs signal back to polarize the oocyte cytoskeleton, which localizes determinants to the anterior and posterior poles of the oocyte. This polarization also directs the oocyte nucleus to migrate anteriorly. Beginning in stage 9, the newly positioned oocyte nucleus specifies the dorsal FCs, which define the dorsal-ventral axis of the embryo (reviewed in [125]).

Reorganization of the epithelium

Starting in stage 9, FCs undergo extensive cell shape changes and morphogenetic movements. Different groups of cells undergo different kinds of reorganization and migration (Fig. 4), discussed separately here.

Border cell migration

At the beginning of stage 9, anterior polar cells recruit 4-6 adjacent FCs to become border cells, which delaminate from the epithelium, surround the polar cells, and migrate posteriorly

between nurse cells. The centrally localized polar cells are not migratory; they are carried by the border cells. Elegant studies have identified signaling systems important for border cell migration, including Jak-Stat [90,91,126,127], Notch [128,129], PDGF/VEGF receptor (PVR) [130], and EGFR pathways [131].

Notch must be activated in border cells for delamination [128,129]. Border cells then form an extension several cell diameters in length; similar extensions may generally initiate invasive migration [132]. DE-cadherin-mediated adhesion is necessary for movement of the border cell cluster between nurse cells [85,133,134]. Since loss of apical/basal cell polarity is associated with invasion and metastasis in cancer [135], one might expect that border cells would lose polarity. Surprisingly, this is not the case; polarity is required for both reorganization and migration [136].

Border cell migration is guided by chemotaxis. Two growth factor receptors, PVR and EGFR, have partially redundant roles in guiding migration towards the oocyte, which expresses their ligands, PDGF/VEGF factor 1 (PVF1) and Grk, respectively [130,137]. Only EGFR is required for subsequent migration along the oocyte to appose the dorsal nucleus [131]. Initially, border cells migrate toward a localized signal detected by a few cells [138]. Later, differences in signal strength detected by different cells within the cluster guides migration [139].

Transcription factors are the link between extracellular signals and expression of genes required for adhesion and motility. In border cells, Upd from anterior polar cells induces expression of the basic leucine zipper transcription factor, Slow border cells (Slbo), the fly CCAAT/enhancer binding protein (C/EBP) [91,126]. Slbo is necessary and sufficient for border cell migration [140], and regulates expression of genes such as FAK [141,142], DE-cadherin [133,134], the FGF receptor Breathless [143], and genes encoding other nuclear factors, such as *jing* and *yan* [144,145]. Microarray studies have identified a large set of genes differentially expressed in border cells, many of which are induced by Slbo [146,147].

Formation of squamous and columnar domains

Concurrent with border cell migration, mainbody FCs migrate posteriorly to cover the oocyte. This reorganization involves the formation of a squamous epithelium from the anterior terminal FCs, and a columnar epithelium of the mainbody and posterior terminal FCs. At the beginning of stage 10, the boundary for squamous and columnar FCs is aligned with the nurse cell-oocyte interface. Columnar FCs are a secretory epithelium, which sequentially deposits eggshell components.

About 50 anterior terminal FCs stretch flat to cover the nurse cells. These stretched cells are also called nurse cell FCs or squamous FCs. The name “stretched” implies that flattening is a passive event. However, stretched cells require Notch activation to disassemble their adherens junctions in an ordered fashion [116]. Furthermore, inactivation of Notch signaling in anterior FCs, either by lack of Fringe glycosylase alone, or both Delta and Serrate ligands, impedes posterior migration, so that clusters of mainbody cells linger over the nurse cells. It is likely that anterior cells must interact with each other and the underlying nurse cells to flatten.

Terminal FCs will flatten unless the posterior identity is induced by EGFR activity [9,10]. However, absence of EGFR activity is not sufficient for flattening; posterior FCs lacking EGFR activation become columnar while expressing anterior markers [74]. Constriction of the apical surface is important for cuboidal FCs to become columnar; this requires *karst*, the gene for apical β_{Heavy} -spectrin (β_{H}) [148]. Mainbody FCs that lack β_{H} -spectrin do not all migrate to overlie the oocyte; some lag over the nurse cells. This suggests that posterior migration is driven in part by compaction as cells become columnar.

The oocyte is important for the change to columnar shape. When the oocyte is mislocalized at the center of the egg chamber, the columnar cells form at this position [149]. When the germline is mutant for either *egghead* or *brainiac*, posterior migration is accelerated relative to border cell migration [87]. Conversely, posterior migration is blocked when the germline lacks the microtubule-associated protein Toucan [151]. However, contact with the oocyte is not necessary for the cuboidal to columnar transition. When the oocyte is abnormally small, columnar FCs form over nurse cells [150]. Altogether, it appears that the oocyte initiates the transition to columnar FCs.

Centripetal migration

At stage 10B, columnar FCs migrate inward at the interface between the oocyte and nurse cells. These cells are called centripetal migrating FCs, and form the operculum and ventral collar of the eggshell. Centripetal migration is regulated by a now-familiar set of signaling pathways, including Dpp, Notch, Jak-Stat, and EGFR.

At the end of stage 9, Dpp is permissive for centripetal migration by downregulating expression of *bunched* (*bun*), which encodes multiple isoforms of a TSC-22/DIP/BUN type leucine zipper transcription factor [153]. At this time, Dpp also stimulates Fas3 expression in a broad domain [111]; the Fas3-positive cells are likely competent for migration. Dpp expression continues in leading edge cells during centripetal migration [152,153]; perhaps it stimulates migration of the following cells, as in embryonic dorsal closure [156].

Notch is required for centripetal migration [114]. In nearby cells, *bun* down-regulates Notch receptor, ligand, and target genes, as well as other migration markers such as non-muscle myosin [162]. Thus, Bun maintains the columnar epithelium by preventing recruitment of excess centripetal cells. Slbo is expressed in centripetally migrating cells [154]. Bun and Slbo mutually antagonize each other's expression, maintaining a boundary between stationary and migrating cell fates.

Centripetal migrating FCs elongate apically to move over the oocyte surface. Consistent with remodeling of membrane cytoskeletal domains, lateral α -spectrin levels increase [114], and β_H -spectrin mutant egg chambers show a mild defect in centripetal migration [148]. DE-cadherin-mediated adhesion promotes centripetal migration, which is delayed in its absence [85]. The ecdysone receptor (EcR) is required for normal centripetal migration, and up-regulates DE-cadherin levels [155]. EcR activity is negatively regulated by EGFR/Ras activity, whereas *bun* expression is upregulated by this pathway [153]. Intriguingly, both DE-cadherin and β_H -spectrin influence the site of centripetal migration. FCs that lack either protein occasionally migrate between nurse cells, instead of along the anterior oocyte. However, for β_H -spectrin mutants, defects in columnar epithelium formation may misposition FCs competent for centripetal migration.

Centripetal migration is fundamentally different from border cell migration, and has superficial similarities to convergent extension [157]. Understanding the commonalities and the differences between these processes will provide important insights into the highly regulated process of normal epithelial to mesenchyme transitions and resultant migrations.

Formation of dorsal appendages

The most complex set of migrations forms the dorsal appendages. Two patches of dorsal appendage-forming cells are specified by combinatorial signaling by BMP, EGF, and Notch pathways during stages 9-10 [2,3,115,158]. Within each patch, two cell types, the floor cells and roof cells [159], cooperate to form a tube. Specialized chorion proteins are secreted into each lumen. Each tube extends anteriorly between the basal lamina and the squamous FCs. By

stage 13, a flattened paddle is formed at the anterior tip of each tube, by shortening of cells along their apicobasal axes. These morphogenetic movements are reviewed in detail elsewhere [160].

Maintenance of the epithelium

Border cell and centripetal migration have features reminiscent of epithelial-to-mesenchymal transition (EMT). In both cases, FCs detach from the epithelium, invade the germline tissue, and share mechanisms with cancer metastasis. Jak-Stat signaling is required for border cell migration in flies [91,126] and promotes cell invasion in certain human cancers [161]. Notch signaling regulates both centripetal and border cell migration [74,146][162]. For human cancers, Notch can be either a tumor suppressor or an oncogene (reviewed in [163]); it can promote metastasis in primary melanomas [164]. VEGF recruits new blood vessels, which support tumorigenesis (reviewed in [165]). Interestingly, *Drosophila* VEGF is an attractant for border cell migration [130]. Elevated BMP expression appears to be associated with metastatic state of some human cancers (reviewed in [163]); fly BMP signaling makes epithelial cells competent for migration [166]. Despite mechanistic similarities with carcinogenesis, migrating FCs are well-behaved, and most FCs remain in an epithelial monolayer. Many factors influence maintenance of the monolayer epithelium, including cell junctions, apical/basal cell polarity, and association with the extracellular matrix (ECM).

Loss of adherens junctions, as from *armadillo* mutations, disrupts the membrane cytoskeleton, resulting in irregular FC morphology and degeneration of follicles [170]. Spectraplakins, a cytoskeleton protein encoded by *short stop*, is localized at adherens junctions; when absent, the epithelium is double layered [171]. Some mechanisms that are required for initial formation of the epithelium in the germarium are also required to maintain a monolayer epithelium, particularly Notch signaling and sphingolipid production by germline cells [172].

FCs are polarized cells, with differential distribution of membrane proteins along the apical/basal axis (reviewed in [4,167-169]). Disruption of different apical/basal polarity complexes has different consequences. Early loss of the apical determinant Crumbs leads to multilayering or discontinuity of the epithelium [170]; however, once the epithelium has formed, Crumbs is dispensable. In contrast, the loss of basolateral proteins has profound consequences. *discs large (dlg)* mutant FCs invade between germline cells and overproliferate in the pole regions [173-175]. FCs lacking either Scribble or Lethal giant larvae (Lgl) show the same phenotypes [174], consistent with their function as an interdependent protein complex. Two basolateral proteins, Fasciclin-2 and Neuroglian, suppress epithelial invasion [176]. The apical protein Bazooka is required for invasion by *dlg* mutant FCs; implicating the apical domain in aberrant adhesion between FCs and the germ cells [177]. These studies led to identification of *Drosophila* neoplastic tumor suppressors, such as Lgl, Dlg and Scribble (reviewed in [178]). Interestingly, human basolateral proteins, such as Dlg1, are implicated in tumorigenesis [179].

Protein trafficking is essential for apical/basal polarity by targeting proteins to specific intracellular locations. Two core components of the vesicle trafficking machinery, a syntaxin (Avl) and a Rab protein (Rab5) are required to maintain the epithelium. Loss of either results in accumulation of membrane proteins such as Notch and Crumbs, loss of cell polarity, and overproliferation [180].

Epithelial organization of posterior terminal FCs is particularly vulnerable. The membrane cytoskeleton is necessary here; loss of α -spectrin leads to multilayering and hyperplasia at this location [181]. The proteoglycan Perlecan is required here for monolayer organization and proper localization of polarity proteins [184]. The ECM receptor Dystroglycan is required to maintain apical/basal polarity of posterior FCs [182]. Integrin, another transmembrane receptor

that links ECM to the cytoskeleton, maintains monolayer organization by orienting planar FC division independent of apical/basal polarity [183]. The vulnerability of this domain has been attributed to the curvature of the epithelium [181], or to adoption of the migratory border cell fate [74]. A third possibility is the activity of two potentially oncogenic signaling pathways in these cells: the Jak-Stat and EGFR/Ras pathways [92].

Summary

Several themes emerge from studies of FC morphogenesis. Cell-cell communication and adhesion are important for every change in cell shape and organization. However, the same signals are used repeatedly; what mechanisms generate different responses in different locations and at different times? Multiple signals are active in every FC rearrangement; it is unlikely that combinatorial signaling alone confers the different responses. More likely, each round of cell-cell interactions induces cell-intrinsic changes that confer distinct responses to the same signals. One example is the expression of different modulators for Notch signaling at different locations and stages. Transcription factors such as *Slbo* (*C/EBP*), *Mirror*, and *Bunched* also may change cells' competence to respond to certain signals.

In summary, the follicular epithelium is an excellent model to study EMT and migration as well as mechanisms for normal organogenesis. Each egg chamber, comprised of about 1000 cells, undergoes many of the morphogenetic events of larger organs, and uses most of the same regulatory networks. Importantly, sophisticated genetic methods can manipulate individual cells to separate events in space and time. Thus, FCs are a powerful system both to integrate newly identified effector molecules into known networks, and to identify novel mechanisms for cell shape, organization and migration in vivo.

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Figure 1.

Cell outlines visualized by phalloidin staining of the actin cytoskeleton (white). Stages of egg chambers are indicated by the numbers; and G is for germarium. Anterior is to the left. Follicle cells form an epithelium to cover the underlying germline cells. As oogenesis proceeds, follicle cells undergo shape changes and morphogenetic movements and eventually give rise to the complex structure of the eggshell. CFC: columnar follicle cells; NC: nurse cells; Oo: oocyte; SFC: squamous follicle cells.

Figure 2.
Scanning electron micrograph of an eggshell (dorso-lateral view). Image adapted from Ref. [5] copyright 2000 Wiley-Liss.

Figure 3.

A. Diagram of a *Drosophila* germarium. This illustration shows the location of germline stem cells (GSCs; red) and follicle cell stem cells (FCSCs; yellow) and their niche cells. Two GSCs cells are located close to their niche composed of cap cell (bright green) and terminal filament (turquoise). The GSCs and cap cells also contact the Escort stem cells (blue). The GSCs differentiating daughters cystoblasts (light orange) move away from the niche in close contact with escort cells (moon shape pale blue) until escort cells die (star shaped pale blue) or replaced by prefollicular cells (light yellow). The prefollicular cells are derived from follicle cell stem cells (yellow), located at the junction of regions 2a and 2b. FCSCs are in close contact with inner germarial sheath cells (pink) (This illustration is based on figures and data in [4,5, 22,25,27,185,186]). B. Egg chambers stained with Fas3 (red) and counterstained with the nuclear dye Topro3 (white). Polar cells (arrow) stain strongly with Fas3. Epithelial follicle cells (arrowhead) down-regulate Fas3 as they mature. Anterior is to the left.

Figure 4.

A-D. Follicle cell morphology visualized by α -spectrin staining. Anterior is to the left. A. At stage 8, epithelial follicle cells are morphologically uniform and have a cuboidal shape. B. At stage 9, follicle cells change their shapes as they migrate posteriorly (arrows). C. At stage 10A, follicle cells that are in contact with the oocyte (Oo) become columnar, while those covering the nurse cells (nc) become squamous. D. At stage 10B, a group of anterior columnar follicle cells elongate and migrate at the interface between the oocyte and the nurse cells (indicated by the arrows). These are centripetally migrating follicle cells. E. A stage 9 egg chamber stained with Fas3 (red) to label polar cells and counterstained with Topro3 (white) to visualize nuclei. Border cells (arrowhead) migrate posteriorly between the nurse cells. A pair of polar cells is located in the center of the border cell cluster. Image adapted from Ref. [5] copyright 2000 Wiley-Liss.