The repertoire of epithelial morphogenesis on display: Progressive elaboration of *Drosophila* egg structure.

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Abstract

Epithelial structures are foundational for tissue organization in all metazoans. Sheets of epithelial cells form lateral adhesive junctions and acquire apico-basal polarity perpendicular to the surface of the sheet. Genetic analyses in the insect model, *Drosophila melanogaster*, have greatly advanced our understanding of how epithelial organization is established, and how it is modulated during tissue morphogenesis. Major insights into collective cell migrations have come from analyses of morphogenetic movements within the adult follicular epithelium that cooperates with female germ cells to build a mature egg. Epithelial follicle cells progress through tightly choreographed phases of proliferation, patterning, reorganization and migrations, before they differentiate to form the elaborate structures of the eggshell. Distinct structural domains are organized by differential adhesion, within which lateral junctions are remodeled to further shape the organized epithelia. During collective cell migrations, adhesive interactions mediate supracellular organization of planar polarized macromolecules, and facilitate crawling over the basement membrane or traction against adjacent cell surfaces. This review surveys the repertoire of follicle cell morphogenesis, to highlight the coordination of epithelial plasticity with progressive differentiation of a secretory epithelium. Technological advances will keep this tissue at the leading edge for interrogating the precise spatiotemporal regulation of normal epithelial reorganization events, and provide a framework for understanding pathological tissue dysplasia. Comparative studies with other insects are revealing the diversification of morphogenetic movements for elaboration of epithelial structures.

Introduction

Substantial advances have been made toward understanding the mechanisms that pattern the body plan and generate diverse cell types, using developmental genetics of model organisms. In parallel, cell culture studies have yielded interaction maps for the protein networks associated with either differentiation of specific cell types or with cell migration. However, the full integration of patterning, cell type diversification, and morphogenesis of functional architecture remains one of the major challenges in developmental biology. With recent advances in live imaging of whole animals or explanted tissues, we are uncovering the diversity of mechanisms by which embryonic cells can change shape, migrate, and reorganize. The impact of this exploration extends beyond the goal of understanding embryogenesis.

Aberrant cell migration can be associated with pathological states, either through an overt developmental defect or a from a subtler syndrome that arises because the affected cell population does not generate the proper tissues or end up in the correct places, e.g. (Hirotsune et al., 1998), and reviewed in (Gleeson and Walsh, 2000). Moreover, morphogenetic movements also are used throughout adulthood to maintain the functional integrity of tissues with high cell turnover rates, or during repair and regeneration of damaged tissues (reviewed in Goichberg, 2016). The majority of adult tissues with these characteristics are epithelial, because epithelia line tissue surfaces with direct environmental exposure (for example, airway epithelium, reviewed in Iosifidis et al., 2016) or repeated disruptions (for example, ovarian surface epithelium, reviewed in Ng and Barker, 2015). Adult epithelia are stable structures, in which cells are interconnected by adhesion junctions, desmosomes, and occluding junctions. Because of distinctions in behaviors of cultured epithelial cells when they are diffusely seeded versus in a confluent monolayer, cell biologists sometimes conflate the formation of epithelial junctions with an inhibition of motility (a concept reviewed and critiqued by Martz and Steinberg, 1973; Stramer and Mayor, 2016).

However, as pointed out by (Gumbiner, 1996), many epithelia are stable, but dynamic, and normal adult epithelia retain or modify some of the morphogenetic behaviors displayed during embryonic development. Importantly, adult epithelia must be sufficiently flexible to permit the orderly movement of differentiating daughters away from the stem cell niche, such as in normal cell turnover in the intestinal epithelium (as shown by Genander et al., 2009), and (reviewed by Clevers, 2013), or recruitment of newborn keratinocytes from hair follicles in severe skin wounding models (reviewed by Plikus et al., 2012). Understanding the mechanisms that allow organizational flexibility of epithelia will provide new insights into pathological states marked by the loss of tissue-appropriate epithelial organization, such as in progression to

invasive cancer (reviewed in Bobrow et al., 1994; Cheung and Ewald, 2016; Hanahan and Weinberg, 2000; Jen et al., 1994).

The *Drosophila* ovary provides an enduring model for studies of morphogenesis in an intact epithelium. During oogenesis, the developing oocyte is interconnected with 15 sister cells, called nurse cells; forming a syncytium, or cyst. The germ cell cyst is surrounded by a somatic epithelium, which undergoes multiple rounds of reorganization to create the shape of the egg. The end result is an egg encased in an elaborate eggshell with features that permit fertilization of the egg, enhance respiration and environmental stress resistance of the developing embryo, and, ultimately, rupture to release the larva from its protective casing {Hinton, 1981 #4064;King, 1970 #550;Mahowald, 1980 #3971;Margaritis, 1980 #606}. Amongst the genus *Drosophila*, the characteristic eggshell morphology of several species have been examined in detail and correlated with the substrate where eggs are deposited (summarized in Hinton, 1981; King, 1970; Mahowald and Kambysellis, 1980). We will return to the underlying variation in patterning and morphogenesis later in this review. The majority of our discussion will focus on the genetic model species, *Drosophila melanogaster*.

The shape of the eggshell has provided a valuable point of entry into genetic and morphometric analyses of the mechanisms for epithelial morphogenesis. The somatic epithelium becomes progressively subdivided into several regional domains. Within each domain the cells undergo stereotypical reorganizations and migrations, remodeling their lateral adhesive interfaces to different degrees, and sometimes expressing distinct sets of cell adhesion molecules. One such domain of migratory follicle cells, the border cells, is frequently featured in collective cell migration reviews (e.g. in Haeger et al., 2015; Scarpa and Mayor, 2016). This migration is only one of several striking morphogenetic events that are essential for formation of a viable *Drosophila* egg. Understanding the transitions between each phase of collective migration, and the precise orchestration of disparate cell movements within close proximity to each other, will provide important information about the mechanisms that ensure tight control of cell behaviors in adult tissues.

Here we highlight recent advances in understanding specific morphogenetic movements within this compact tissue. We highlight open questions about the initiation and mechanism for each type of movement, with emphasis on transitions between successive types of movements or to subsequent differentiation. In section 1, we start with an overview of the developmental progression of *Drosophila* oogenesis, and the distinct morphogenetic movements that occur as the egg is formed. In sections 2 through 8, we discuss current thoughts on the five major epithelium reorganizations that together define the shape of the egg and its surrounding eggshell. Each event involves distinct patterning and morphogenetic mechanisms, yet these

cells are all in close proximity to one another and were all derived from the same simple epithelium earlier in oogenesis. The transitions between each event reveal the fundamental roles for differential expression of cell adhesion proteins and remodeling of adhesive interactions. Understanding the gene regulatory networks and molecular machinery that organize the choreography of oogenesis will provide a template for understanding morphogenesis of more complex cellular systems.

1. Overview of egg chamber origin, developmental progression, and critical morphogenetic movements

The morphogenetic events of *Drosophila* egg formation are easily accessible to the investigator, for each oocyte develops in concert with its surrounding somatic epithelium within a follicle, or egg chamber, and independently from other oocytes. A female has two ovaries, each of which contains 16-20 ovarioles, or assembly lines of maturing egg chambers (King, 1970). Multiple developmental stages are displayed at once, due to the organization into parallel ovarioles, which are tubes of developing follicles (Fig. 1A). Thus, the successive morphogenetic events are laid out in the array of egg chambers available in a single ovary (previously reviewed by Bastock and St Johnston, 2008; Horne-Badovinac and Bilder, 2005; McLaughlin and Bratu, 2015). Investigation of these morphogenetic events has intensified with the advent of methods for ex vivo culture combined with improvements in time-lapse fluorescence imaging (reviewed by Peters and Berg, 2016b).

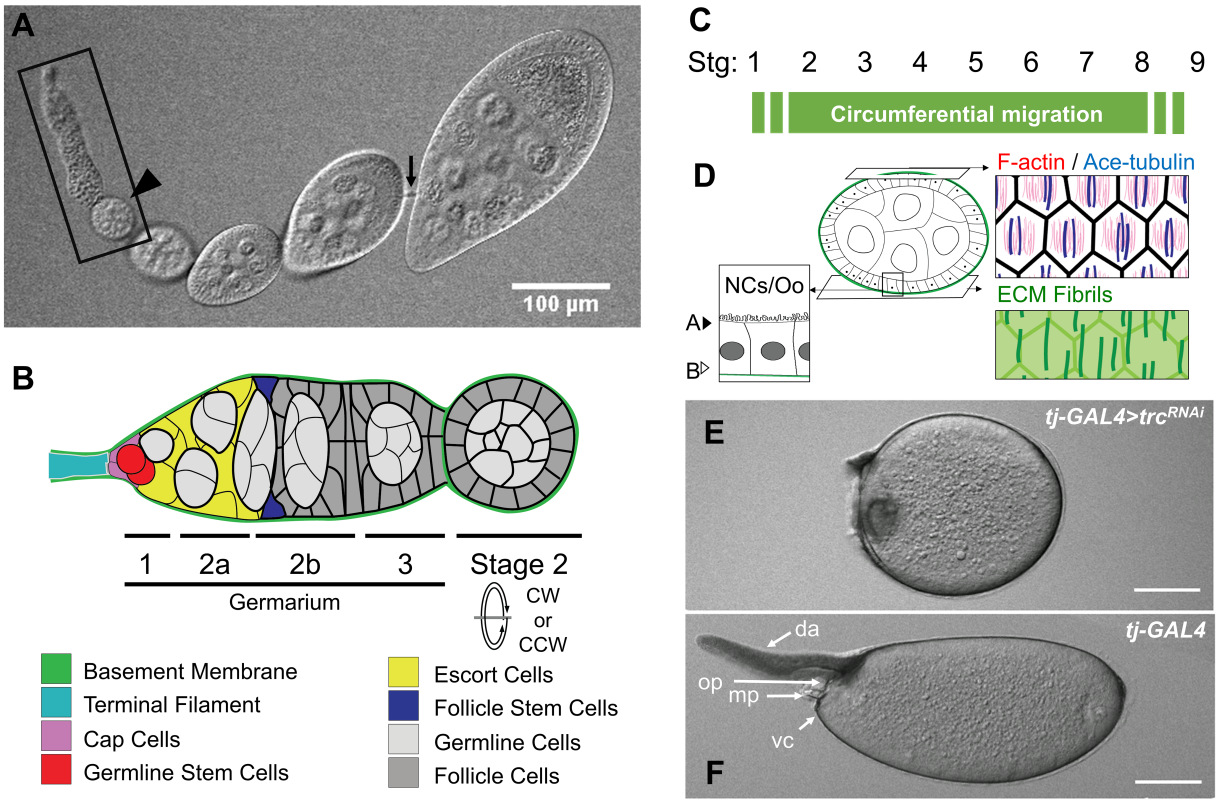

Figure 1. *Drosophila* eggs are produced in the ovary within ovarioles.

***A*: Anterior portion of a single ovariole.** Eggs develop from egg chambers, which are assembled de novo in the germarium. Successively formed egg chambers develop in an assembly-line manner as they make their way towards the posterior of the ovariole, such that each egg chamber is older than its more anterior neighbor. Egg chambers are linked in a "chain" by stalk cells (arrow). The anterior-most region of each ovariole contains a stem cell niche for egg chamber formation. The boxed region includes this anterior-most region at the upper left, with the terminal filament at the tip, the long, germarium structure, and a newly formed egg chamber that has budded from the germarium (arrowhead, lower right of box). ***B*: A schematic of a Drosophila germarium, which is composed of several cell types that coordinate their behaviors to continuously produce egg chambers.** Egg chamber formation begins with the asymmetric division of germline stem cells, which are associated with the terminal filament and cap cells of the niche in region 1. Asymmetric division leads to the formation of a daughter germ-line stem cell that stays associated with the cap cells. The more distant daughter, now a cystoblast, undergoes four rounds of mitotic division as it moves through region 2a, surrounded by escort cells. Incomplete cytokinesis during cystoblast divisions gives rise to a 16-cell syncytium interconnected via cytoplasmic bridges. The cystoblast syncytium, or germ cell cyst, encounters prefollicle cell progeny of the follicle stem cells situated at the boundary of region 2b. Prefollicle cells coat the posterior side of the cyst, separating it from escort cells, and from the now completed stage 1 egg chamber that resides in region 3. Organization of stalk cells and polar cells accompanies the budding of a stage 2 egg chamber from the germarium. By mid-stage 2, egg chambers are fully encapsulated with a specialized basal extracellular matrix (ECM), or basement membrane. Egg chambers begin to rotate early in their developmental program (stage 1/2) in either a clockwise or counter clockwise direction (black arrows under stage 2) relative to their anterior-posterior axis (gray horizontal line under stage 2). ***C:* Circumferential migration is maintained through stage 8 and arrests by stage 9.** The timeline is depicted here as a thick green bar. ***D:* Circumferential migration requires the planar polarization of the follicular epithelium.** Cytoskeletal structures composed of F-actin (red) and Acetylated-tubulin (Ace-tubulin, blue) are arranged in planar polarized basal bundles, oriented perpendicular to the anterior-posterior axis. Lower left inset shows follicle cell apicobasal polarity, with the apical surface (filled arrowhead) in contact with the nurse cells (NCs) or oocyte (Oo) and the basal surface (unfilled arrowhead) in contact with the basement membrane (ECM; green). During this migration, FCs secrete ECM components including Collagen, Laminin, and Perlecan.

Though not essential, migration facilitates the polarized deposition of long, ECM fibrils, oriented perpendicular to the AP axis (dark green; lower right). Disruption of planar cell polarity and/or circumferential migration leads to failure in egg elongation. ***E:*** **Defects in planar cell polarity are associated with the production of round eggs.** A stage 14 egg, resulting from RNA interference-mediated depletion of *trc* in the follicle cells using the traffic-jam-Gal4 driver, which gives a similar round egg phenotype to the published mosaic analysis with traditional mutations (Horne-Badovinac et al., 2012). ***F:*** **Drosophila melanogaster eggs are normally ovoid in shape, with elongated dorsal appendages.** A stage 14 egg from a parallel control experiment is shown. Anterior eggshell structures are indicated: dorsal appendage (da), operculum (op), micropyle (mp), and ventral collar (vc). Scale bars are 100 micrometers (μm). For detailed electron micrographs of these structures, see Margaritis et al, 1980. In panels A, B, and D-F, anterior is left and posterior is right. In E and F, dorsal is up and ventral is down.

Insect ovarioles have a common general organization, in which oogonia, or germline stem cells, reside in the anterior terminal region, the germarium (Fig. 1B), whereas subsequent oogenesis occurs in progressively more posterior regions, sometimes called the vitellarium (reviewed in Simmons, 2013). Each follicle progresses through fourteen developmental stages, as detailed in (King and Koch, 1963), some diagrammed in Figs. 1 and 2. The features used to define the stages include the relative positioning of the egg chamber within the ovariole, the size of the oocyte relative to the egg chamber or other germ cells, accumulation of certain organelles, and follicle cell numbers, organization and morphology. A succinct "field guide" to egg chambers using differential interference contrast images combined with DAPI-staining was provided in a widely-used review (Spradling, 1993). Recently, the Deng lab developed an automated method to stage DAPI-stained egg chambers, which uses egg chamber size, aspect ratio, and follicle cell numbers (Jia et al., 2016). With either method, dysregulation of morphogenesis, oocyte growth, or follicle cell proliferation can uncouple the associations between morphogenesis in different cell populations, making staging of mutant egg chambers problematic.

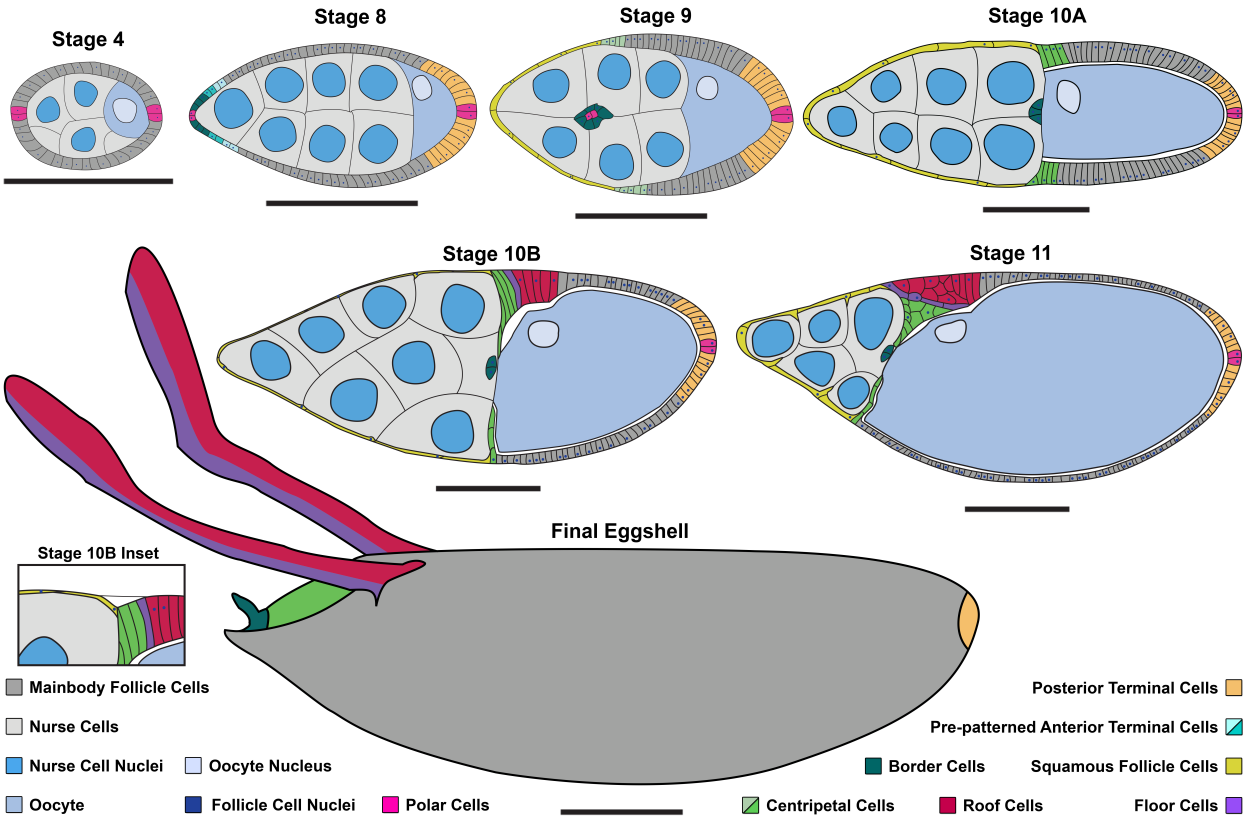

Figure 2. Egg chamber diagrams from selected stages, showing morphogenesis of relevant follicle cell populations and their contributions to the eggshell.

Six stages of *Drosophila* oogenesis are depicted, as well as the final mature eggshell. Key cell populations are color coded according to their patterning and final fate. *Stage 4*: Prior to this stage, the polar cells are specified and reside at both ends of the developing egg chamber. Nurse cells and their nuclei are visible, as well as the oocyte, oocyte nucleus, and epithelial follicle cells (FCs). *Stage 8:* As the oocyte grows larger, additional patterning specifies at least one posterior terminal FC domain, as well as three anterior FC domains. *Stage 9:* During this stage, the border cell cluster, composed of the anterior polar cells and neighboring terminal FCs, delaminates from the epithelium and migrates posteriorly between the nurse cells. Concurrently, epithelial FCs reorganize into squamous and columnar domains, starting from the anterior and posterior poles of the egg chamber, respectively. Some evidence suggests that centripetal FCs are specified by this time (pre-patterned anterior follicle cells in diagram). *Stage 10A:* The centripetal FCs are organized in rows at the anterior edge of the columnar FCs, and the border cells reach the nurse cell/oocyte boundary and begin to migrate dorsally. *Stage 10B:* Centripetal migration is underway, and the roof and floor cells that will comprise the dorsal appendages are specified. *Stage 10B inset*: After initial elongation, individual centripetal cells appear to detach from the basement membrane and move inward over their more posterior neighbors (TTP and LAR, unpublished observations). *Stage 11:* Centripetal migration nears completion as the nurse cells dump their contents into the oocyte. Squamous FCs begin to wrap around individual nurse cells, ultimately to promote their phagocytosis. *Mature Egg:* The columnar FCs secrete the final eggshell resulting in two dorsal appendages, the operculum, the outer portion of the mycropylar structure, and the aeropyle located at the posterior. Border cells secrete the interior material of the micropyle, and shape a path for sperm entry. The approximate cell populations are color coded as indicated at the bottom of the figure. The egg chambers are not depicted at the same scale; indicated by its individual scale bar (100 μm) just below. Each egg chamber was hand traced from micrographs; positions of FC lateral interfaces were estimated.

The following sections of this review focus on each morphogenetic event in order of their developmental progression. In this section, we provide a general summary of A) the origin of the follicular epithelium and formation of egg chambers in the germarium, and B) the subsequent stages of follicular epithelium development in the vitellarium, ending with a summary of the five major epithelial reorganizations that create the shape of the egg and the eggshell. A summary of proteins or *Drosophila* genes mentioned in this review can be found in Table 1. A unique Flybase gene identifier is listed; interested readers can use this number as a portal to find links to more detailed protein family information through the Flybase database (Flybase.org) (Gramates et al., 2017).

1A. Formation of an egg chamber within the germarium

The germarium houses a stem cell niche for both somatic and germline stem cells, as reviewed elsewhere (Chen et al., 2011; Losick et al., 2011). Within this structure, newborn germ cells and somatic follicle cells organize to form an egg chamber. The development of the oocyte within each egg chamber is summarized briefly, before we turn our attention to the formation and morphogenesis of the follicular epithelium.

Germline stem cells are maintained at the anterior tip of the germarium (Fig. 1B). In *Drosophila*, only some progeny of the germline stem cells become oocytes, while other progeny become nurse cells that remain associated in a syncytial cyst with their oocyte sister (early work reviewed in King, 1970; Mahowald and Kambysellis, 1980; Telfer, 1975). Several germ cells within one cyst initiate the early events of meiotic prophase I within region 2B of the germarium (reviewed in Lake and Hawley, 2012). Subsequent meiotic progression becomes restricted to the oocyte by the time the egg chamber leaves the germarium. The regulation of meiotic progression, and evidence for linkage to somatic developmental progression, are reviewed by (Von Stetina and Orr-Weaver, 2011). The fifteen nurse cells contribute their cytoplasmic contents to their sister oocyte during stages 11-12, augmenting the accumulation of cytoplasmic mass in the oocyte (reviewed in Mahajan-Miklos and Cooley, 1994), a process we return to in section 5A. Completion of meiosis and egg activation occurs when the mature stage 14 egg moves into the oviduct, prior to fertilization (reviewed by Krauchunas and Wolfner, 2013). The rate of egg production is regulated by nutritional and other inputs, some examples of these inputs can be found in recent studies (Laws et al., 2015; Shimada et al., 2011; Sun and Spradling, 2013) and reviews by (Belles and Piulachs, 2015; Bloch Qazi et al., 2003; Bownes, 1982).

The mid-region of the germarium houses the somatic stem cells that give rise to daughter cells that form the follicular epithelium (Fig. 1B) (Margolis and Spradling, 1995). The

numbers and positions of these stem cells, called follicle cell stem cells, appears to be determined during pupal stages (Vlachos et al., 2015). Follicle cell stem cell maintenance requires the nuclear protein Castor, which continues to be expressed in their differentiating daughters, the precursor follicle cells (Chang et al., 2013). In adult germaria, follicle cell stem cells are maintained via contact with neighboring somatic cells and paracrine germarium signals (Song and Xie, 2002; Song and Xie, 2003; Zhang and Kalderon, 2001). Laminin A expression by these stem cells is important for maintenance, as are α-integrins PS1 and PS2, along with β-integrin$_{PS2}$ (Hartman et al., 2015; O'Reilly et al., 2008). More recent studies indicate that follicle cell stem cells require basal polarity proteins Discs large (Dlg) and Lethal giant larvae (Lgl) to compete for niche occupancy (Kronen et al., 2014). These stem cells appear to have extended baso-lateral domains and adhesion junctions, but no detectable apical domains, an organization maintained by high Epidermal Growth Factor Receptor activity (EGFR). Apical domains, identified by localization of apical polarity proteins Bazooka (Baz) and atypical Protein Kinase C (aPKC), are not detected until after division to produce a differentiating daughter (Castanieto et al., 2014; Tanentzapf et al., 2000). This newborn precursor follicle cell matures its apical membrane domain as it move inward to contact a germ cell cyst, thus acquiring initial apico-basal polarity as it forms a simple, cuboidal epithelium (Castanieto et al., 2014), and the apical domain genes *crumbs* (*crb*) and *discs lost (dlt)* are required at this time to generate a contiguous epithelium over the early egg chambers (Tanentzapf et al., 2000). These data seem to contradict the original view that precursor follicle cells undergo an epithelial-mesenchymal transition (articulated in a review by Tepass et al., 2001), a flexible process thought to underlie much of the epithelial plasticity exhibited during epithelial morphogenesis in embryos (nicely articulated in a recent mini-review of developmental morphogenesis for cancer researchers, Nakaya and Sheng, 2013).

Neither the follicle cell stem cells nor the precursor follicle cells appear to fit the strict definition of epithelial cells articulated by (Nakaya and Sheng, 2013). The evidence supports an essential role of apical cues in the emergence of apico-basal polarity (Franz and Riechmann, 2010; Goode et al., 1996; Tanentzapf et al., 2000), followed by later elaboration of a basement membrane (Chen et al., 2016) as the stage 1/2 egg chamber emerges from the germarium (Fig. 1A,B, this stage reviewed in (Horne-Badovinac and Bilder, 2005)). This appears distinct from recent discussions of de novo apical membrane polarization and lumen formation, initiated via basal cues such as attachment to a basement membrane generated by another cell type (e.g. in Bedzhov and Zernicka-Goetz, 2014), recently reviewed by (Roman-Fernandez and Bryant, 2016).

Inward migration of precursor follicle cells separates a 16-germ cell cyst from its older, posterior neighbor, and reinforces the posterior position of the oocyte within the egg chamber

(reviewed by Huynh and St Johnston, 2004; Roth and Lynch, 2009). Additional precursor follicle cells are recruited to form the somatic epithelium that encases one germ cell syncytium to form an egg chamber, or follicle (Fig. 1B) (Margolis and Spradling, 1995; Tworoger et al., 1999). As the epithelium forms over the germ cell cyst, some of the epithelial follicle cells (FCs) begin to express Eyes absent (Eya) (Bai and Montell, 2002), which blocks the polar cell fate through repression of Castor expression (Chang et al., 2013). The follicle cells can organize into an epithelial-like structure in the absence of germ cells; however, their apico-basal polarity depends on contact with the germ cells (Goode et al., 1996; Margolis and Spradling, 1995; Tanentzapf et al., 2000). The importance of apico-basal polarity for follicular epithelial structure, and the gradual acquisition of epithelial characteristics in early egg chambers are discussed elsewhere (Bastock and St Johnston, 2008; Franz and Riechmann, 2010).

A few precursor follicle cells become either epithelial FCs or pre-polar cells, which subsequently become either the polar cells or the stalk cells (Tworoger et al., 1999), although a recent report challenges this initial epithelial versus prepolar lineage restriction (Nystul and Spradling, 2010). Stalk cells have a distinct organization, through which they separate individual egg chambers throughout the ovariole (arrow in Fig. 1A). Polar cells are embedded in the epithelium at the anterior and posterior poles of an egg chamber (Fig. 2, stage 4 and subsequent); initially, these cells are contiguous with the stalk cells that connect to an egg chamber (Margolis and Spradling, 1995; Tworoger et al., 1999). Even though the polar cells remain contiguous with the follicular epithelium, they undergo a distinct developmental program during stages 2-8 (Besse and Pret, 2003; Borensztejn et al., 2013; Grammont and Irvine, 2001; Khammari et al., 2011; Niewiadomska et al., 1999; Ruohola-Baker et al., 1991).

1B. Overview of egg chamber development and epithelial morphogenesis

Once the egg chamber forms, the follicular epithelium initiates a circumferential migration, which helps constrain the egg to an ovoid shape. The entire follicular epithelium takes part in this migration, which we discuss in section 2. Circumferential migration overlaps with other significant events of oogenesis, including follicle cell proliferation during stages 1-6 (represented by stages 1-2 in Fig. 1A, and stage 4 in Fig. 2)(Calvi et al., 1998), and into the beginning of vitellogenesis during stages 8-9, when the oocyte takes up vitellogenins, the major storage lipoprotein (all diagrammed in Fig. 2) (Cummings and King, 1970; Schonbaum et al., 2000). Circumferential migration does not appear to disrupt juxtacrine interactions between FCs and the underlying germ cells; for example, Delta ligand on germ cell membranes can activate

its receptor Notch on FC apical surfaces, for FC mitotic proliferation is maintained by high Delta ligand expression in the germ cells (reviewed by Klusza and Deng, 2011).

As oogenesis proceeds, maturation of the oocyte is tightly coordinated with the subdivision of FCs into distinct groups (indicated as different colors in Figs. 2, 3). Each group will form a distinct structure of the eggshell, or else interact with the 15 nurse cells to ensure their final elimination. When the epithelium forms, the epithelial FCs show fluctuations in gene expression, but seem equivalent in fate (Skora and Spradling, 2010). Subdivision of the terminal and middle, or mainbody, regions of the follicular epithelium occurs by stage 5 (Gonzalez-Reyes and St Johnston, 1998; Grammont and Irvine, 2002; McGregor et al., 2002) Polar cells begin expressing the gene for the Unpaired (Upd) ligand when they are specified in the posterior of stage 1 egg chambers (McGregor et al., 2002); Upd activates the Janus kinase/STAT signaling pathway in the FCs to induce terminal cell fate (reviewed by Denef and Schupbach, 2003). Anterior-posterior symmetry is broken in the FCs slightly later, when Gurken (Grk) ligand from the oocyte activates EGFR in overlying FCs. This signal blocks the later formation of anterior eggshell structures at the posterior pole of the egg chamber (Peri and Roth, 2000; Queenan et al., 1997). We revisit anterior-posterior patterning in section 3.

A striking diversity of morphogenetic events occurs in a near-simultaneous fashion beginning in the vitellogenic stages (stages 8-10, Fig. 2), and continuing into the post-vitellogenic stages, sometimes called the choriogenic stage (stages 11-14, Fig. 2). FC circumferential migration has stopped by stage 9 (Chen et al., 2016). Prior to stage 7/8, all epithelial FCs are cuboidal in shape, as discussed in section 3. During stage 9, the follicular epithelium begins to reorganize into distinct squamous or columnar domains, as discussed in section 5. At the same time, the border cells cluster together at the anterior pole of the egg chamber, and detach to migrate between the nurse cells to the oocyte. Border cell migration has been extensively reviewed in recent years, so we only summarize key events in section 4.

Patterning of dorsal mainbody FCs occurs as they form the columnar epithelium during stage 9, as we discuss in section 6. Radial symmetry of the oocyte is broken during stage 8, when the oocyte nucleus migrates along the cortex to an anterior corner, defining the dorsal midline. Localized secretion of Grk moves with the position of the nucleus, creating a Grk morphogen gradient that spreads from anterior to posterior over the dorsal surface of the growing oocyte. High levels of Grk accumulate over the oocyte nucleus during stage 10, further elevating EGFR signaling to specify dorsal anterior FC fates. Diversification of dorsal anterior FCs occurs in two hours or less, during stages 10-11 (Figs. 2,3).

Completion of epithelial reorganization results in abutting squamous and columnar domains of the follicular epithelium. The junction aligns neatly over the nurse cell-oocyte

interface, and occurs at a characteristic wild-type ratio of oocyte to nurse cell volume of 1:1 (King, 1970). The border cells reach the anterior oocyte at about the same time. This distinctive morphology defines stage 10A (Fig. 2), which coincides with the initial diversification of dorsal anterior FC types. Although cell morphology appears stable, refinement of gene expression domains presages the next rounds of morphogenesis. Onset of stage 10B is defined by the visible inward elongation of the centripetally migrating FCs, when a ring of FCs reach inward between the nurse cells and oocyte (Fig. 2) (King, 1970). Centripetal migration ensures that the anterior oocyte will be covered by eggshell; we discuss this poorly understood migration in section 7.

The onset of bulk transfer of nurse cell cytoplasmic contents to the oocyte marks the beginning of stage 11 (Figs. 2 and 3A,B) (Gutzeit and Koppa, 1982; King, 1970). It should be noted that RNA binding proteins, endoplasmic reticulum and Golgi proteins are synthesized in the nurse cells and transported to the oocyte by selective transport during stages 6-7 (not diagrammed here), mediated by polarized microtubules in the oocyte (Shimada et al., 2011). In contrast, the bulk cytoplasmic transfer that begins after the elongation of the centripetal FCs is non-selective; the nurse cells transfer maternal organelles, macromolecular machinery, RNAs and proteins that will be needed for early embryogenesis, while retaining their nuclei (King, 1970; Mahajan-Miklos and Cooley, 1994; Spradling, 1993; Telfer, 1975). Colloquially called "nurse cell dumping" (e.g. in Spradling, 1993), this bulk cytoplasm movement results in rapid expansion of the oocyte, and concomitant shrinkage of the nurse cells, which retain their nuclei due to anchoring by an actin cytoskeletal matrix assembled during stage 10B (shown in Fig 2), demonstrated by (Cooley et al., 1992), and reviewed in (Hudson and Cooley, 2002; Huelsmann and Brown, 2014). Subsequently, squamous FCs enwrap individual shrunken nurse cells and induce programmed cell death in a phagocytic process (Timmons et al., 2016); these events are briefly covered in section 5A.

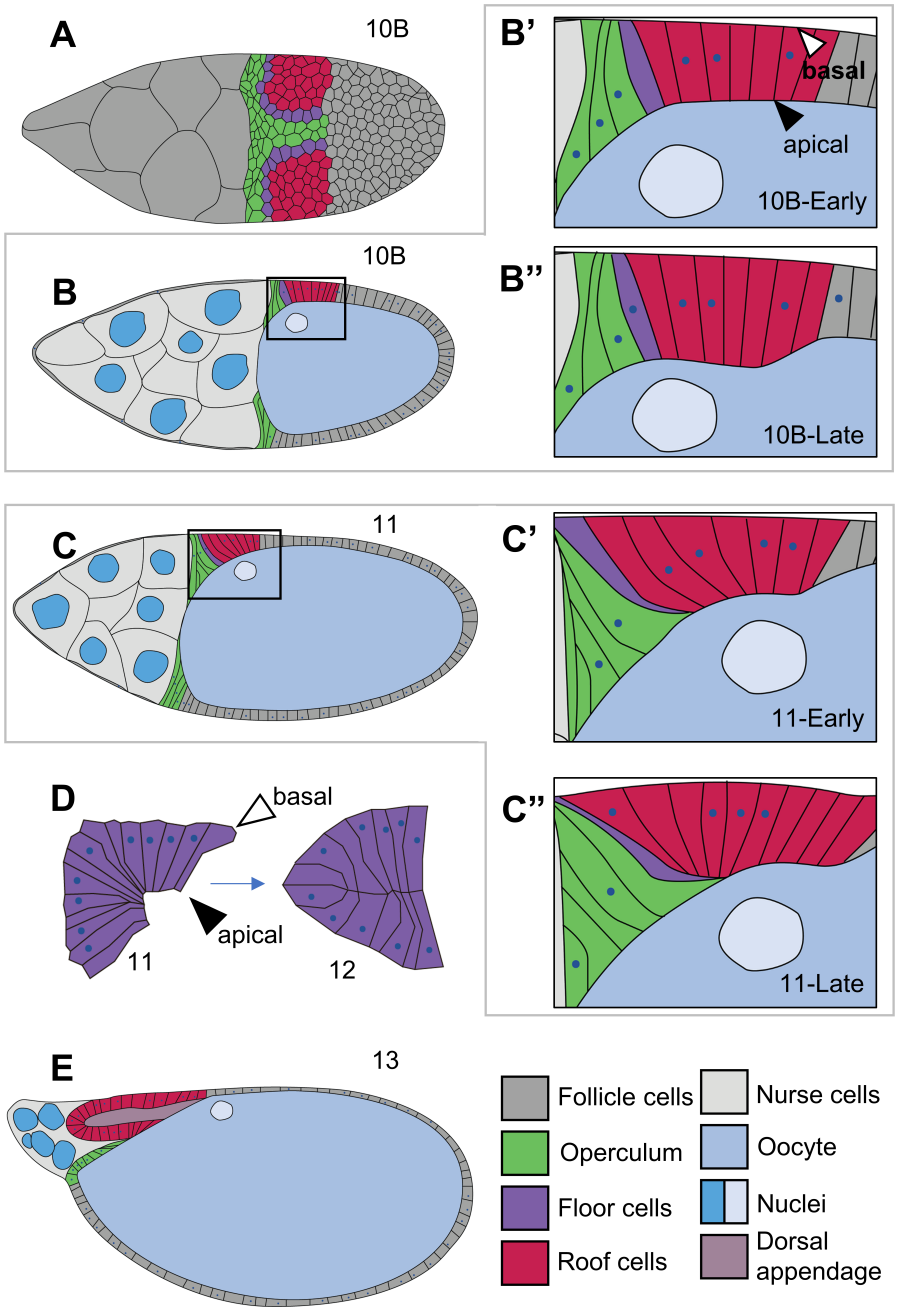

Figure 3. Dorsal appendage morphogenesis requires de novo tube formation.

A-B: **Dorsal appendage placodes become evident by stage 10B.** By stage 10B, the dorsal appendage placode is composed of two cell types, the floor and roof cells, which can be identified by gene expression patterns, as discussed in the text. The dorsal appendage placodes abut the dorsal operculum-forming cells that populate the "T-region" along their anterior and medial borders. ***B:*** **Dorsal appendage morphogenesis begins with follicle cell elongation along the apicobasal axis.** The boxed region of interest in B, is depicted as a "magnified" view in B' (early stage 10B) and B" (late stage 10B). By late stage 10B, the follicle cells of the dorsal appendage placode have elongated substantially along their apicobasal axis and are morphologically distinct from neighboring cells that do not participate in dorsal appendage morphogenesis. ***C-D:*** **Floor cells "dive" underneath the roof cells to form a tube.** The boxed region of interest in C, is depicted as a "magnified" view in C' (early stage 11) when the floor cells begin to dive underneath the roof cells and in C" (late stage 11) when the floor cell apices of the anterior and medial

floor cell populations are approaching each other underneath the roof cells, better appreciated from the dorsal view in D. ***D:*** **Apical extension by the floor cells gives rise to the dorsal appendage tube.** By stage 12, the anterior and medial floor cells meet under the roof cells and form new lateral contacts with each other, thereby sealing off the tube (along a ventral seam) and generating a lumen between the roof and floor cells. ***E:*** **The dorsal appendage tube elongates during stages 12-13 and eggshell components are sequentially secreted into the dorsal appendage lumen.** Dorsal appendage elongation and morphological maturation requires the coordination of several behaviors that include, convergent extension, concerted cell migration, and remodeling of cell shape. As the dorsal appendage tube elongates, it rotates such that the roof cells face outwards (or laterally), while the floor cells face inwards (towards the nurse cell compartment). Eggshell components begin to be secreted into the lumen during stage 11, however the bulk of secretion taking place between stages 12-14. The mature dorsal appendage has a narrow stalk (proximal) and a wide paddle (distal; Fig. 1F). This figure is adapted from figures in Dorman et al. (2004), which presents more detailed descriptions of dorsal appendage morphogenesis; additional information for specific details are available in references cited within the text. In all panels, anterior is left and posterior right. Panel A depicts a dorsal surface view. Panel D, depicts a "flattened" dorsal view of floor cells only. Panels B-C" and E depict cross-sectional views (dorsal side up). Developmental stage is indicated in each panel.

The last morphogenetic movement of FCs is the combined tubulogenesis and collective cell migrations that form the two dorsal appendages of the eggshell (Berg, 2005; Ward and Berg, 2005). In section 8, we cover recent advances in understanding morphogenesis of dorsal appendages from the two FC domains that each reorganize to initiate tubulogenesis, and then collectively migrate anteriorly during an elongation phase (Fig. 3). Eggshell proteins are secreted into the lumen of each tube, generating the characteristic respiratory horns, or dorsal appendages, of the *D. melanogaster* egg (Hinton, 1981; Hinton, 1969).

Each egg chamber synthesizes prostaglandins that coordinate the events of oogenesis from stage 9 onward (Tootle and Spradling, 2008). Complex dynamics of this signaling system coordinates the temporal program for expression of eggshell and vitelline membrane genes, proper transfer of nurse cell cytoplasm to the oocyte, and oocyte maturation (Groen et al., 2012; Spracklen et al., 2014; Tootle et al., 2011). Columnar FCs and border cells sequentially secrete components of the eggshell, beginning with the vesicles of vitelline membrane components during stage 9 (Margaritis, 1985; Waring, 2000). The elaborate structure of the eggshell is defined by the final architecture of the FCs covering the oocyte. Eggshell structures vary, even across the *Drosophilid* species, and are thought to enhance embryonic survival in the species-specific substrate where eggs are deposited (Hinton, 1981; Kambysellis, 1974; Kambysellis, 1993).

Once an oocyte is mature, it may be held within the ovariole for a variable period of time before it is released for egg activation and fertilization (reviewed by Bloch Qazi et al., 2003; Spradling, 1993). Ovulation signals trigger the degradation of posterior FCs, leaving behind a mass containing the rest of the FCs (Deady et al., 2015; Deady and Sun, 2015). The resultant follicle rupture separates the egg and the anterior FC mass as they move into the lateral oviduct. Now activated, the egg proceeds to the uterus for fertilization, while the FCs remain *en masse* in

the oviduct. Surprisingly, immunofluorescence analysis suggest that this FC mass retains nuclear localization of Hindsight, cortical β-Catenin, and mitochondrial localization of the Ecdysone biosynthetic enzyme Shade. It has been proposed that this corpus luteum-like structure regulates maturation of successive follicles within the same ovariole (Deady et al., 2015).

2. Circumferential migration of the follicle cell epithelium

Circumferential migration is a notable example of the expanded repertoire of morphogenetic events that became accessible when a reliable protocol for prolonged, ex vivo culture of vitellogenic stage egg chambers was developed, early examples in (Bianco et al., 2007; Prasad et al., 2007; Zimyanin et al., 2008). Identification of this migration stemmed from the surprising observation that previtellogenic egg chambers rotate about their anterior-posterior axis with either left- or right-handed chirality (Haigo and Bilder, 2011). Further studies indicated that the entire follicular epithelium migrates over an immobile basement membrane that encapsulates the egg chamber (Cetera et al., 2014; Chen et al., 2016; Isabella and Horne-Badovinac, 2015b; Isabella and Horne-Badovinac, 2016; Lerner et al., 2013; Lewellyn et al., 2013); thus FCs migrate along a path that traces the circumference of the egg chamber. As we mentioned in section 1B, this does not disrupt Notch-Delta signaling between FCs and germ cells, and the egg chamber rotation seen in time-lapse imaging may be facilitated by continuing *D*E-Cadherin-mediated adhesive interactions between the FC apical surface and the underlying germ cell syncytium (FC-germ cell adhesion reviewed in Muller, 2000).

Whether other epithelial tissues engage in circumferential migration or whether the observed *Drosophila* FC mechanisms and functional outputs are conserved for all such migrations, are open questions. Several recent review articles address these questions from different perspectives. Horne-Badovinac (Horne-Badovinac, 2014) makes the case that circumferential rotation may be conserved in egg development across distantly related insects, citing evidence for circumferential cell organization or related features in egg chambers of other insects (also addressed by Gates, 2012). Intriguingly, egg rotation occurs in the avian shell gland and is essential to break radial symmetry of the blastocyst embryo (Kochav and Eyal-Giladi, 1971), but it is unknown whether the mechanism for this rotation is related to that of insects. Taking a distinct perspective, Bilder and Haigo (Bilder and Haigo, 2012) describe a set of criteria that may identify tissue-level rotation in other systems. These authors argue that rotation might occur in tissues with partially closed epithelia of similar topologies to the FCs, such as tubular and acinar structures (e.g., mammalian breast, lung, and kidney). Indeed, a recent study

reported that culture of a non-tumorigenic breast epithelial cell line in a three-dimensional matrix can yield acinus-like structures that rotate (Squarr et al., 2016).

Returning to the *Drosophila* follicular epithelium, it is now appreciated that the onset of circumferential migration begins very early. One group detected it as soon as stage 1 follicles are formed (Cetera et al., 2014), another traced the onset to stage 2 follicles (Chen et al., 2016); these stages are difficult to distinguish, so this discrepancy may reflect differences in staging criteria. The latter study correlates the initiation of circumferential migration with enclosure of the budding egg chamber by a Collagen IV α 2-GFP-positive basal extracellular matrix (Fig. 1B). Formation of a basement membrane effectively separates the stalk cells from the egg chamber FCs (Pearson et al., 2016); however, it remains unclear whether this transition is required for the onset of migration, or is simply coincident with it.

Planar polarized organization of the cytoskeleton is the earliest evidence for circumferential organization of FCs, and is functionally required for this migration. The orientation of actin filaments and the biased polarity of microtubules provide early markers for planar cell polarity (Chen et al., 2016; Frydman and Spradling, 2001; Viktorinova and Dahmann, 2013). This organization is evident in the prefollicle cells that populate germarial region 2b (Fig 1B), prior to egg chamber formation; here, basal bundles of cytoskeletal elements are oriented perpendicular to the long axis of the germarium (Fig. 1D). This planar polarization is maintained throughout circumferential migration, even though computational analysis indicates that ordered cytoskeletal organization is dynamic (Aurich and Dahmann, 2016; Cetera et al., 2014; Chen et al., 2016). Several recent reviews discuss the effect of disrupting cell- and tissue-level cytoskeletal organization in FCs (Bilder and Haigo, 2012; Cetera and Horne-Badovinac, 2015; Gates, 2012; Horne-Badovinac, 2014). Generally, the actin cytoskeleton is essential throughout circumferential migration, whether disruption is accomplished by genetic or pharmacological means (Cetera et al., 2014; Chen et al., 2016; Squarr et al., 2016). This organization includes parallel F-actin bundles that are reminiscent of stress fibers. In contrast, microtubule cytoskeletal dynamics appear essential to initiate migration (Chen et al., 2016), but are largely dispensable after migration commences. Once initiated, this migration is unidirectional.

Several molecular markers distinguish the leading and trailing edges of actively migrating FCs, an asymmetry that is coordinated across the entire follicular epithelium and is required for persistent, on-axis rotation (planar cell polarity of this tissue is reviewed by Cetera and Horne-Badovinac, 2015). Notably, the atypical Cadherin Fat2 becomes localized to the trailing edge (Viktorinova et al., 2009). The role of Fat2 in establishing and reinforcing FC planar cell polarity is intriguing. Fat2 is a poorly understood member of the Fat family of Cadherins,

which engage in either heterotypic cell-cell adhesion interactions, and possibly also homotypic adhesive interactions (Saburi et al., 2012). However, comparative studies indicate that Fat2 may have distinct functions from the founding family member, Fat, which has a well-characterized function in establishing planar cell polarity of other tissues (Sharma and McNeill, 2013; Sopko and McNeill, 2009).

During FC circumferential migration, Fat2 is necessary for organized microtubule polarity, so that the growing, plus-end of each microtubule orients toward the leading edge (Chen et al., 2016; Viktorinova and Dahmann, 2013). Biased organization of microtubule polarity precedes egg chamber rotation, and is predictive of the chirality for rotation. Fat2 function is necessary to establish biased microtubule polarity. Conversely, Fat2 trafficking to the trailing edge depends on this microtubule orientation. These observations suggest a positive feedback loop that strengthens the organization of microtubule polarity on the one hand, and localization of Fat2 to the trailing edge membrane, on the other. The mechanisms that trigger this positive feedback loop to establish planar cell polarity are currently unclear.

Stepping back from the cytoskeletal organization, the physiological function for this migration is an outstanding question. Early studies proposed the "molecular corset" hypothesis, in which FC planar organization is needed to generate a physical constraint to outward growth, so that the egg chamber elongates along its anterior-posterior axis to form an ovoid structure (Gutzeit et al., 1991). This work first identified the disrupted planar cell polarity in rounded egg chambers from *fat2* mutant females. Supracellular organization of FCs was implicated as a determinant of egg shape, because both F-actin within FCs and Laminin fibrils of the adjacent basement membrane were disorganized in the round egg chambers of mutant females (Gutzeit et al., 1991). These authors posited that structural rigidity from cytoskeletal and basement membrane organization would oppose growth along the radial axis.

In support of the molecular corset hypothesis, the normal ovoid shape of an egg chamber is disrupted in vitro by enzymatic depletion of Collagen, or in vivo by genetic depletion of Collagen-IV (Fig. 1E, F, (Haigo and Bilder, 2011)). Furthermore, each egg chamber dynamically remodels its basement membrane as the ovoid shape becomes apparent, during stages 5-8. Migrating FCs deposit fibrillar structures composed of Collagen IV, Laminin, and Perlecan (Gutzeit et al., 1991; Haigo and Bilder, 2011; Isabella and Horne-Badovinac, 2015a; Isabella and Horne-Badovinac, 2016; Lerner et al., 2013; Schneider et al., 2006). These extracellular fibrils are planar polarized, mirroring the organization of underlying F-actin bundles (Fig. 1D). Consistent with a critical role for the basement membrane in egg elongation, altered egg aspect ratio results from experimental manipulation of fibrillar constituents of the extracellular matrix (ECM), achieved by modulating the secretory apparatus (Isabella and

Horne-Badovinac, 2016). Thus, in wild-type egg chambers, migrating FCs lay down polarized arrays of long ECM fibrils. Circumferential migration was disrupted in FCs that expressed only a truncated form of Fat2, and these FCs deposited polarized ECM fibrils that were abnormally short (Aurich and Dahmann, 2016). However, even without circumferential migration, egg chamber elongation occurred and ovoid eggs were produced, strengthening the link between polarized ECM fibrils and elongated egg shape.

In sum, circumferential migration appears strongly coupled with cytoskeletal organization, basement membrane structure, and egg chamber elongation, for mutations that abrogate migration are predominantly associated with defects in the others. However, differing results from different mutant alleles or experimental manipulations reveal distinct temporal and structural sensitivities in this process (e.g. compare Aurich and Dahmann, 2016; Cetera et al., 2014; Chen et al., 2016; Viktorinova et al., 2009). Understanding the choreography and essential functions of this full-scale migration will be important for understanding the interplay between morphogenetic force generation and epithelial plasticity.

3. Roles of lateral adhesion in the simple, cuboidal FC epithelium

3a. Maturation and maintenance of the cuboidal epithelium during proliferative stages.

As circumferential migration is beginning, epithelial FCs become distinct from polar cells, a process regulated by antagonism between Eyes absent (Eya), a bHLH transcriptional repressor (Bai and Montell, 2002) and the nuclear protein Castor (Chang et al., 2013). Distinct features of epithelial FCs include functional criteria, such as continued survival and proliferation (Besse and Pret, 2003; Khammari et al., 2011), and morphological criteria, such as their apico-basal polarity (Franz and Riechmann, 2010), cuboidal shape, and lateral FC-FC junctions. Polar cells retain high levels of Fasciclin 3 (Fas3, (Ruohola-Baker et al., 1991)) and *D*E-Cadherin (Niewiadomska et al., 1999) throughout their plasma membranes, whereas epithelial FCs restrict Fas3 homophilic adhesion molecules to their lateral FC-FC interfaces, and gradually down-regulate its levels through stage 5 (Bai and Montell, 2002). (A comparative summary of cell adhesion genes in the *Drosophila melanogaster* genome can be found in (Hynes and Zhao, 2000)). Epithelial FCs maintain lower levels of *D*E-Cadherin at their lateral junctions, and high levels are seen at the FC-germ cell interface, e.g. in (Niewiadomska et al., 1999).

Apico-basal polarity is a major feature of epithelial organization, and the general relationships between epithelial polarity and morphogenesis is reviewed in (Laprise and Tepass,

2011; St Johnston and Sanson, 2011; Tepass, 2012). We mentioned some aspects of apico-basal polarization of FCs in sections 1 and 2, including the maturation of apical membrane domains upon contact with the germ cells, and secretion of basement membrane components during circumferential migration, respectively. Lateral adhesive junctions are organized with an apico-basal polarity (reviewed in Knust and Bossinger, 2002; Muller, 2000), and the formation of adherens junctions can be a driving force in polarization of epithelial cells (articulated for cuboidal FCs in Franz and Riechmann, 2010; Tanentzapf et al., 2000; Tanentzapf and Tepass, 2003). However, cuboidal FCs have some distinctions from the well-organized basolateral and occluding junctions of commonly described columnar epithelia. Cuboidal FCs have an apico-lateral junction, an adherens junction, and an extended basolateral junction from stages 2-7 (Fig. 2) (Zhao et al., 2008). The adherens junctions contain both *D*E-Cadherin and N-Cadherin, and both must be knocked out to disrupt the associated localization of β-Catenin (Peifer et al., 1993; Tanentzapf et al., 2000). A number of studies suggest that the basolateral junction is critical for FC epithelial organization and monolayer homeostasis independent of the well-recognized circuitry that regulates apico-basal organization.

The cuboidal FC basolateral adhesive junction is mediated by Fasciclin 2 (Fas2) and Neuroglian (Nrg). These transmembrane cell adhesion proteins co-localize with Dlg, which is responsible for recruiting Fas2 and Nrg to the lateral junction complex (Szafranski and Goode, 2007; Wei et al., 2004). Nrg, in turn, recruits the αβ–Spectrin complex to the basolateral membrane cytoskeleton (Szafranski and Goode, 2007; Wei et al., 2004). Fas2, Nrg, and Dlg also have been identified as septate junction components (Genova and Fehon, 2003; Lamb et al., 1998; Woods et al., 1996; Woods et al., 1997), and "incipient septate junctions" have been detected as early as stage 6 in electron micrographs (Muller, 2000). These incipient junctions are morphologically distinct from the well-described insect occluding junctions, pleated septate junctions (reviewed by Harden et al., 2016), which are detected in the columnar FCs overlying the oocyte during stage 10 (Muller, 2000). The maturation of pleated septate junctions is circumstantially related to subsequent FC morphogenesis, but whether the "incipient septate junctions" are related to continued morphogenetic plasticity of the cuboidal FC epithelium is less clear (reviewed by Harden et al., 2016). Remodeling of adhesive junctions is central to morphogenesis (Lecuit, 2005), and the remodeling of the elongated basolateral junctions of cuboidal FCs is critical for subsequent morphogenesis of three populations: the border cells, the anterior squamous FCs, and the posterior columnar FCs, as we discuss in sections 4 and 5. Notably, FCs retain their cuboidal shape until stage 7/8 (Fig. 2), when Fas2 levels and localization are modulated (Bergstralh et al., 2015; Szafranski and Goode, 2004).

In contrast, several lines of evidence support the role of the extended basolateral junctions and associated membrane cytoskeleton in maintenance of the epithelial monolayer during proliferative stages. Nrg, Fas2, Dlg, and Lgl seem to have tumor suppressor function within the FC cuboidal epithelium; FCs that lack any one of these proteins abnormally invade between the germ cells of an egg chamber (Goode and Perrimon, 1997; Szafranski and Goode, 2004; Szafranski and Goode, 2007; Zhao et al., 2008). In the case of Dlg, this capability seems to be limited to terminal FCs, at the anterior and posterior of the egg chamber (Goode et al., 2005), and can occur as early as stage 4 (Goode and Perrimon, 1997). During proliferative stages, FC basal adhesion and apico-basal polarity regulate spindle orientation, which determines whether a new cell is born into the epithelium, or separate from it (Bergstralh et al., 2013a; Bergstralh et al., 2013b; Fernandez-Minan et al., 2007). When a new FC is born outside of the epithelium due to altered spindle orientation, the monolayer is restored by Fas2 and Nrg-mediated adhesive interactions that reintegrate the misplaced cell (Bergstralh et al., 2015). After proliferation ends, during stages 7-9 (Fig. 2), Nrg is down-regulated at lateral interfaces (Bergstralh et al., 2015).

Changes to the extended basolateral junction/incipient septate junction interface of cuboidal FCs appears to be strongly linked to the morphological plasticity of these cells. As we highlight in section 4, the distinct behaviors of the border cells, squamous FCs, and columnar FCs are linked to remodeling of their adhesive interfaces. These changes are viewed as increasing or ongoing morphological plasticity for the border cells and squamous FCs respectively, as we discuss in sections 4 and 5a. For the columnar cells, the transition is sometimes termed a maturation to pleated septate junctions and adherens junctions in the columnar FCs (e.g. in Muller, 2000). However, as we describe in sections 5-8, substantial remodeling occurs in regions of columnar FCs, even as they begin differentiation to a secretory epithelium.

3b. Anterior and posterior patterning influence organization of the cuboidal epithelium.

Terminal and mainbody FCs initially share an equivalent cuboidal shape (Fig. 2). FC anterior-posterior asymmetry is established before stage 7/8 by Grk-EGFR signaling, which induces expression of posterior FC transcription factors (Fregoso Lomas et al., 2013). Recent studies have confirmed the roles of opposing gradients for establishing the prepattern of columnar FC fates, involving anterior BMP activity and posterior Grk + Upd activity (Fregoso Lomas et al., 2016). Posterior FC fate is maintained by the combination of oocyte-stimulated EGFR/Ras/MAP kinase signaling and polar cell-stimulated Domeless/Janus kinase/STAT

signaling. The posterior terminal FCs seem particularly vulnerable to loss of apicobasal polarity and monolayer organization, as we briefly discuss here.

The specification of posterior terminal FCs may be linked to the maintenance of a monolayer epithelium in this population, but the specific relationship remains unclear. Early studies demonstrated a requirement for the cortical cytoskeleton protein, α-Spectrin, to maintain monolayer organization in FCs over the curved posterior end of the oocyte, but not in the mainbody FCs (Lee et al., 1997). These authors speculated that the differential sensitivity of the posterior FCs to loss of cortical cytoskeleton components might be due differential tension caused by the curvature at the posterior egg chamber, or alternatively, due to the signaling milieu from interactions with the oocyte. They also noted that the hyperplasia caused by clones could displace the posterior polar cells, and disrupt the localization of oocyte posterior determinants (the role of FCs in oocyte polarization is reviewed by Bastock and St Johnston, 2008; Roth and Lynch, 2009).

The interplay between apico-basal polarity, paracrine signals from the oocyte or polar cells, and maintenance of monolayer organization is a timely topic, but progress has been hampered by technical issues associated with mosaic analyses. Notably, mosaic FC analyses of the maintenance of monolayer organization were challenged by a report that damaged cells can develop a multi-layered appearance and also lose GFP, so that they can be mistaken for negatively-marked mutant cells in in a commonly used mosaic analysis strategy (Haack et al., 2013). In at least one instance, this report stimulated a careful statistical reanalysis to confirm their original interpretation of mosaic analysis data (Conder et al., 2007; Conder et al., 2016). Additional technical issues have been uncovered with regard to specific mutations or chromosomes used in the analyses. Linkage to cryptic mutations can confound analyses of either mosaic tissues (Horne-Badovinac et al., 2012) or whole animal mutant phenotypes (e.g. Ng et al., 2016), and the specific nature of the mutant alleles used may provide a confounding factor (e.g. Shahab et al., 2015). These examples emphasize the importance of using more than one allele or method to manipulate FCs within the intact egg chamber.

Notwithstanding, multiple types of experiments indicate the importance of the Hippo signaling pathway (Meignin et al., 2007; Polesello and Tapon, 2007) and cortical cytoskeleton components β-Spectrin and myosin II in maintenance of apico-basal polarity of posterior terminal FCs overlying the curved end of the oocyte (Wong et al., 2015; Yan et al., 2011).

Unlike the posterior FCs, the anterior terminal and mainbody FC organization seems impervious to these manipulations until late stage 8/early stage 9. Returning to the patterning of anterior fates, current evidence for graded BMP activity prior to stage 8 is indirect (Fregoso

Lomas et al., 2016); additional evidence supports this earlier BMP activity throughout the cuboidal FCs (Chen and Schupbach, 2006). However, detectable expression of the BMP ligand Decapentaplegic (Dpp) is seen in anterior terminal FCs by the end of stage 8 (Twombly et al., 1996). Multiple BMP responses are detected readily in anterior terminal FCs and neighboring mainbody FCs during stage 9 (Deng and Bownes, 1997; Dobens and Raftery, 1998; Dobens et al., 2000; Peri and Roth, 2000; Twombly et al., 1996). Importantly, anterior-posterior polarity of the FC epithelium becomes visible during stage 8, as a graded transition to a more columnar shape at the posterior (Fig. 2).

4. Formation and migration of the border cell cluster

Border cell migration provided an early and prominent example of collective cell migration in living tissues. This group of cells becomes morphologically distinct early in stage 9 (King and Koch, 1963), when they migrate posteriorly between the nurse cells to reach the oocyte. With the finding that *slow border cells* (*slbo*, the fly homolog of C/EBP) is essential for determination and migration of the border cells (Montell et al., 1992; Rorth and Montell, 1992), a critical set of transgenic tools was developed for studies of border cell migration (Dai and Montell, 2016; Prasad et al., 2015).

Pre-patterning of border cells and neighboring anterior terminal cell types is detected by stage 6, using enhancer trap reporters that are responsive to Upd (shown at stage 8 in Fig. 2, Xi et al., 2003). The timing for onset of border cell morphogenesis is regulated by rising steroid hormone levels (evidence summarized in Bai et al., 2000; Jang et al., 2009), in part from temporal coordination by rising levels of Ecdysone (Buszczak et al., 1999). At the beginning of stage 9, border cells cluster around anterior polar cells, a process induced by Upd stimulation of the Domeless receptor (Ghiglione et al., 2002), and Jak-STAT signaling (Beccari et al., 2002; Silver et al., 2005). Highest levels of Stat activity induce *slbo* expression, which is required for border cell migration ((Monahan and Starz-Gaiano, 2013; Montell et al., 1992; Rorth and Montell, 1992; Starz-Gaiano et al., 2008) and reviewed in (Saadin and Starz-Gaiano, 2016)), and down-regulates Ecdysone nuclear hormone receptor signaling (Jang et al., 2009). Continued activation of the Jak/STAT pathway maintains *slbo* expression within the border cells, and is necessary throughout their migration (Silver et al., 2005). The border cell gene regulatory network that progressively deploys migratory gene products is reviewed by (Saadin and Starz-Gaiano, 2016).

Remodeling of lateral junctions plays a critical role in assembling the migrating border cell cluster. During stages 3-5, polar cells retain high levels of the basolateral adhesion molecule

Fas3 (Bai and Montell, 2002). During stages 6-8, the polar cells appear to form a straight adhesive interface with each other, which accumulates *D*E-Cadherin along its length (Niewiadomska et al., 1999). At the beginning of stage 9, nearby anterior terminal cells remodel *D*E-Cadherin localization to preferentially adhere with the polar cells, organizing the cluster that delaminates from the FC epithelium. Within the migrating border cell cluster, cell surface markers of apicobasal polarity localize to distinct cell-cell interfaces; in a recent study, apical markers Sdt, Patj, and Crb asymmetrically localized to border cell interfaces with each other and with polar cells, whereas lateral membrane markers Coracle and Dlg are found at the border cell interface with polar cells (Felix et al., 2015). The distinctive border cell cluster's asymmetric distribution of these and other apicobasal polarity and lateral adhesion proteins, as well as their requirements are further described by (McDonald et al., 2008; Medioni and Noselli, 2005; Niewiadomska et al., 1999; Pinheiro and Montell, 2004). E-cadherin aids the cluster in direction sensing, while holding the cells together and polarizing each cell individually (Pinheiro and Montell, 2004).

Border cell migration is guided by redundant signaling through two receptor tyrosine kinases: the PDGF/VEGF receptor homolog PVR-1 (Duchek and Rorth, 2001; Duchek et al., 2001) and EGFR (Duchek and Rorth, 2001; Duchek et al., 2001), with a minor contribution by the FGF receptor, Breathless (Murphy et al., 1995). At this stage, two additional EGFR ligands act to stimulate the receptor in border cells, Spitz and Keren (McDonald et al., 2006). Surprisingly, migration is accomplished in two distinct phases that utilize different mechanisms and exhibit unique behavior. Migration through the first half of the nurse cells requires ELMO-Mbc and involves 1 - 2 highly polarized, fast moving leading cells that are located at the leading edge of the cluster. The second half of migration is slower, and requires Raf/MAPK or PLC-γ. During this latter phase, the cell that leads the migration "shuffles" with its neighbors, resulting in a frequent change of which cell leads the cluster. The leading cell appears to be selected by virtue of its responsiveness to guidance cues; in mosaic clusters, wild type cell are found at the leading edge, while cells that are mutant for relevant signaling pathway genes end up in the back of the migrating cluster (Bianco et al., 2007; Wang et al., 2010). In-depth reviews of this migration include (Montell, 2003; Montell et al., 2012; Rorth, 2009).

When the border cell cluster reaches the anterior side of the oocyte; it migrates dorsally along this surface, guided by Grk ligand signaling through the EGFR (Bianco et al., 2007; Duchek and Rorth, 2001). After this movement, another FC population, the centripetal FCs, begins to invade inward between nurse cells nurse cell and oocyte; we discuss this migration in section 7. Later in oogenesis, centripetal FCs build anterior eggshell structures, while the border

cells build a canal inside the micropyle, which allows for sperm entry into the egg (Montell et al., 1992; Montell et al., 1991; Zarani and Margaritis, 1985; Zarani and Margaritis, 1991).

5. Morphogenesis of the cuboidal follicular epithelium into columnar and squamous domains

Global reorganization of the cuboidal FC epithelium occurs concurrently with border cell migration, during a period when the germ cell volume increases several fold (King and Koch, 1963; Kolahi et al., 2009). Specifically, anterior terminal FCs form a distinct domain of squamous FCs, whereas the mainbody and posterior terminal FCs form an abutting domain of columnar FCs. In each case, reorganization is initiated by at the pole, and progresses along the plane of the epithelium. In the case of anterior terminal FCs, the polar cells and border cells have left the epithelium. In the case of posterior FCs, the polar cells remain in place, and do not change shape. The forces and mechanisms that establish planar organization of cell shape have attracted substantial interest from cell biologists and computational biologists (Lancaster and Baum, 2011; Mao and Baum, 2015; Martin, 2010; McNeill, 2000). This reorganization sometimes is viewed as the period when columnar FCs lose plasticity, even though further migrations will occur, as we discuss in sections 6-8, .

5A. Morphogenesis of anterior terminal cells to form a squamous epithelium

Recent studies have illuminated some of the mechanisms by which the anterior terminal FCs transition from a simple cuboidal epithelium to a squamous epithelium (a process we refer to as 'flattening'). Cell flattening begins at the anterior and proceeds posteriorly at the same pace as border cell migration, progressing in a radially symmetric fashion. In other words, the flattening of one circumferential row leads to the flattening of the next (Brigaud et al., 2015). At completion, a squamous epithelium overlies all of the nurse cells. We refer to the flattened FCs as squamous FCs; sometimes they are called "stretch follicle cells", or "nurse cell follicle cells". In this section, we discuss the mechanisms for this reorganization, and the later squamous FC functions that may be enhanced by their flattened morphology.

Squamous FCs do not contribute to the final production of the eggshell (King and Koch, 1963; Parks et al., 1986). Whereas the oocyte-associated FCs lengthen their lateral interfaces, form occluding junctions, and mature into a columnar, secretory epithelium (see next section), the flattening anterior FCs shorten their lateral interfaces and form an extensive interface with one underlying nurse cell (Grammont, 2007). Notably, each squamous FC subsequently engulfs one nurse cell, and the squamous FCs are non-autonomously required for nurse cell programmed cell death, which is completed during stage 13 (Fig. 2) (Timmons et al., 2016). This

work also supplies evidence that nurse cell dumping fails to occur when squamous FCs are genetically ablated. The mechanism by which squamous FCs enable nurse cell dumping is currently unknown, and might be mediated by a signaling event or by a mechanical contribution such as "squeezing" the nurse cells to promote the emptying of their contents.

The remodeling of squamous FCs' lateral junctions facilitates their flattening, and the sterile 20-like kinase Tao has a critical role in promoting this process. When *tao* is mutant in the anterior terminal cells, they retain their cuboidal shape and fail to flatten (Gomez et al., 2012). During stages 7/8 (Fig. 2), Fas2 is strongly down-regulated in anterior FCs (Szafranski and Goode, 2004). Tao promotes Fas2 endocytosis, and this process is a prerequisite for cell flattening to occur (Gomez et al., 2012). Down regulation of N-Cadherin also is required for flattening, supporting a parallel remodeling of adherens junctions (Grammont, 2007). Altogether, the morphogenetic transition of a cuboidal cell to a squamous cell requires a significant depletion of lateral adhesive junctions; cells defective in this process cannot flatten due to a physical inability to reduce their lateral surface area. Further work in this area will be needed to test whether the down regulation or remodeling of other adhesion molecules is similarly important for flattening.

Formation of the squamous epithelium is regulated in many aspects by BMP and Notch signaling (Brigaud et al., 2015; Grammont, 2007). BMP signaling pathway components are necessary for flattening of each cell, and ectopic BMP ligand expression is sufficient to induce formation of a few squamous FCs among posterior terminal cells (Peri and Roth, 2000); although not in mainbody FCs (Dobens and Raftery, 2000). BMP signaling controls the dynamics, degree, and timing of anterior cell flattening. This signal coordinates multiple systems required for flattening, through down-regulation of N-Cadherin, and modulation of actomyosin contractility and Notch and Delta expression levels (Grammont, 2007). One report found flattening of posterior terminal FCs with removal of basolateral regulators (Li et al., 2009). BMP and Notch signaling might make the anterior terminal FCs more susceptible to increased planar tension during this stage, as suggested by (Kolahi et al., 2009). This model suggests that flattening occurs in response to mechanical forces, from growth of the underlying germ cells combined with the inelasticity of main body FCs, due the lengthening lateral interfaces of FCs in the columnar FC epithelium (section 5b). Tools are accumulating within the fly community to critically test this model for differential responses to mechanical forces.

The sequential mode of flattening suggests a planar polarity or other juxtacrine signaling mechanism for recruiting each successive row of flattening cells. Another unresolved question is why the flattening stops at the nurse cell-oocyte junction. Does differential adhesion to the nurse cells play an essential role in this process? Are mainbody FCs cells non-responsive to the

juxtacrine signal that triggers flattening? Some data suggest that anterior mainbody FCs have distinct gene expression responses to BMP signaling, compared to squamous FCs (Deng and Bownes, 1997; Dobens et al., 2005; Dobens and Raftery, 1998; Dobens et al., 2000; Peri and Roth, 2000); however, when squamous FCs do not fully flatten, more rows of flattening FCs are recruited from the presumptive mainbody FCs (Brigaud et al., 2015).

Programmed cell death of nurse cells occurs after flattening of the anterior FCs (Buszczak and Cooley, 2000; Cummings and King, 1970; McCall, 2004); a recent study provides strong evidence that squamous FCs actively promote nurse cell death (Timmons et al., 2016). During stage 11, the squamous FCs wrap around the nurse cells, completely enveloping them by stage 12 (Tran and Berg, 2003). Programmed cell death of nurse cells is detected by several means (Timmons et al., 2016). Nurse cells' nuclei persist for prolonged periods, and nurse cell dumping is aberrant when squamous FCs are genetically ablated. Squamous FCs utilize phagocytic mechanisms to induce programmed cell death; nurse cell nuclei remain intact when the phagocytosis genes Draper/CED-1 and CED-12/ELMO are knocked down in the squamous FCs alone.

Together, these new data indicate that the squamous FCs have a more active role in the progression of egg development than was appreciated previously. Emerging evidence suggests that these FCs promote the delivery of nurse cell cytoplasm to the maturing oocyte (Timmons et al., 2016), and thus to ensure successful development of progeny. FCs' function in this process remains to be determined. The accessibility of developing egg chambers and the wealth of genetic tools make the squamous FCs a useful model system for understanding cuboidal-to-squamous epithelial transitions, how they are regulated, and the mechanisms that drive them. In particular, these types of rearrangements require extensive remodeling of lateral adherens junctions, in which they could serve as a key model for mechanistic understanding of how these processes occur. Furthermore, understanding how squamous FC's transition to phagocytic function will be important for understanding the roles of non-professional phagocytes in tissue responses to necrosis and apoptosis.

5B. Formation of a columnar epithelium overlying the oocyte

In parallel with flattening of the anterior terminal FCs, the mainbody FC's progressively become columnar as the growing oocyte expands to underlie this portion of the FC epithelium (Kolahi et al., 2009). This process is often referred to as a posterior migration (e.g. in reviews by Dobens and Raftery, 2000; Horne-Badovinac and Bilder, 2005; Spradling, 1993), because formation of the FC columnar epithelium was originally thought to include migration of more

anterior mainbody FCs to cover the oocyte (King and Koch, 1963). Our understanding of the mechanisms controlling the formation of the columnar epithelium comes in part from incidental observations made during studies of border cell migration or flattening of the squamous FCs. Well before the onset of dramatic changes in FC aspect ratios, a gradation in FC lateral membrane length is observed, with the tallest cells in the posterior (noted in King and Koch, 1963; Kolahi et al., 2009; Ng et al., 2016). Although the squamous and columnar reorganizations occur in parallel, they each begin with FCs at opposite ends of the egg chamber.

Cuboidal FCs show a graded transition in apico-basal(Mahowald and Kambysellis, 1980; Trougakos et al., 2001) "height" above the oocyte during stages 7/8 (Fig. 2) (Kolahi et al., 2009). Posterior FCs acquire a distinctly columnar aspect ratio at this time, prior to the anterior detachment of border cells, whereas the anterior-most FCs are mostly cuboidal (for clear example see Medioni and Noselli, 2005). FC lengthening to a columnar aspect ratio begins in posterior-most FC, which encompasses ~29% of FCs at this stage (Kolahi et al., 2009). During stage 9, columnar FCs further elongate their lateral faces, and the region with cells that are lengthening lateral faces progresses to more anterior FCs until it meets the squamous FCs (King and Koch, 1963). By stage 10, about 95% of FCs are in the columnar epithelium (Kolahi et al., 2009); this massive increase in numbers led to the early notion of a posterior-directed migration.

Morphometric analysis of oocyte growth during columnar epithelium formation challenged the posterior migration model (Kolahi et al., 2009). This study tracked the position of individual FCs during columnar epithelium formation, and also calculated the surface area of the oocyte and multiple metrics for FCs, including apical circumference, lateral membrane length, and others. The data clearly indicate that anterior elongation of the underlying oocyte accounts for the large increase in numbers of overlying columnar FCs. Furthermore, the transition to columnar aspect ratio is not due to an apical constriction; indeed the FCs also grow in volume during this time, with an accompanying increase in apical surface area. Instead, mainbody FCs lengthen their lateral membrane domains, thus changing their aspect ratio (Gomez et al., 2012; Kolahi et al., 2009; Ng et al., 2016). Mainbody FCs undergo oscillating contractions of basal stress fibers during this period, presumably maintaining a planar counter force to outward pressure from the growing oocyte (He et al., 2010).

Elongation of the lateral membrane interface in columnar FCs involves the transition from an elongated basolateral junction (discussed in section 3A) to a shorter occluding junction just below the adherens junction. Invertebrate occluding junctions are the pleated septate junctions, which share components with vertebrate tight junctions (reviewed recently in Harden et al., 2016). Consistent with this, pleated septate junctions are apparent in electron micrographs of the columnar epithelium at stage 10 (Mahowald, 1972; Muller, 2000). These barriers are

thought to be important during the secretion and cross-linking of the insect eggshell layers (reviewed in Harden et al., 2016; Muller, 2000; Tepass et al., 2001; Waring, 2000).

Fas3, Fas2, and Dlg become localized to a more apico-lateral region as posterior FCs elongate, and α-Spectrin is necessary to lengthen the lateral surface of columnar FCs (Ng et al., 2016). As discussed in section 5A, Tao kinase regulates endocytosis of Fas2 in anterior FCs; strikingly, *tao* mutant columnar cells exhibit slightly longer lateral interfaces than wild type FCs (Gomez et al., 2012). This observation supports an active trafficking of septate junction components from the basolateral region to a more apico-lateral region during lengthening of columnar FCs. In contrast, adherens junction markers appear to have constant localization. These data specifically implicate remodeling of septate junctions as a critical component for the lengthening of columnar FCs, and emphasize the importance of the Spectrin membrane cytoskeleton.

A recent computational model examined the relative roles of adhesive and contractile forces as parameters driving three-dimensional morphogenesis of planar epithelia in theoretical spherical or cylindrical structures (Hannezo et al., 2014). This theory suggests that cell shape variation within an epithelium can undergo a stochastic phase transition from a continuous shape variation to a bistable, discontinuous transition, similar to that observed for the FCs. However, this minimal model must be directly tested for its relevance to FCs; for example, the models' constrained parameters of apical constriction and constant tissue size are invalid in the stage 9 remodeling of the FC epithelium, even though the spherical structure approximates an ovoid egg chamber.

Columnar FCs differentiate as secretory cells. They synthesize and secrete vesicles of vitelline membrane components first, during stages 8-10 (Fig. 2) (Cavaliere et al., 2008; Cavaliere et al., 1997; Margaritis, 1986; Margaritis et al., 1980; Waring, 2000). Long apical microvilli appear during stage 10 (Mahowald, 1972), initially interdigitating with the oocyte's microvilli in an organization that is dependent on the proto-cadherin, Cad99C (Schlichting et al., 2006; Trougakos et al., 2001). This microvillar proto-cadherin is trafficked to the apical domain via MyosinV on polarized microtubules, which are anchored to the apical cortical domain via β_H-Spectrin/Patronin and by Shot, which appear genetically redundant (Khanal et al., 2016). Subsequent shortening of FC microvilli is essential for proper organization of the impenetrable vitelline membrane that forms adjacent to the oocyte plasma membrane. FCs continue to follow a precise temporal and spatial gene expression program as they sequentially produce and secrete proteins and other components for the eggshell layers, during a period when the oocyte enlarges rapidly from nurse cell dumping (Cavaliere et al., 2008; Margaritis, 1985; Margaritis et al., 1980; Tootle et al., 2011; Waring, 2000). Structural integrity of the columnar epithelium is

maintained, even as membrane domains are remodeled so that FC shape accommodates oocyte growth (e.g. Sherrard and Fehon, 2015).

The continued remodeling of FC membrane domains challenges our concepts of epithelial plasticity and differentiation, which may be instructive for consideration of human pathologies, such as the epithelial dysfunction that accompanies loss of airway clearance in chronic airway disorders such as asthma or chronic obstructive pulmonary disorder (Vladar et al., 2016). The dynamic nature of the FCs during terminal differentiation phases provides a valuable platform for further exploration.

6. Diversification of dorsal anterior columnar FC fates during stages 10A-11

When the squamous and columnar epithelial domains are completed, the developing egg chamber pauses its morphogenesis, in what appears to be a critical 'respite', when new patterns of gene expression arise, and cells prepare to perform the morphogenetic movements that will create the shape of the eggshell. This pause encompasses stage 10A, which begins when the columnar epithelium completely overlies the oocyte and ends with the visible elongation of centripetal migrating FCs. The border cells have just reached the nurse cell/ oocyte interface and are moving dorsally, a relatively subtle change that is easy to overlook.

Columnar FCs are nearly indistinguishable from one another, except that dorsal FCs are taller than ventral FCs. This distinction arises during stage 9 (Fig. 2), when dorsal FC fates are specified as they pass over the dorsal-anterior Grk-secreting region of the rapidly growing oocyte (Boisclair Lachance et al., 2009; Goentoro et al., 2006; Roth and Schüpbach, 1994; Simakov et al., 2012; Zartman et al., 2011). Tightly regulated trafficking and translation of Grk ligand ensures its production at the oocyte cortex nearest the oocyte nucleus (Cáceres and Nilson, 2005; Cáceres and Nilson, 2009; Clark et al., 2007; Davidson et al., 2016; Delanoue et al., 2007; Jaramillo et al., 2008; Tian et al., 2013). Localized ligand secretion from only a small portion of the oocyte is central to axial patterning of the egg, and also of the resultant embryo (González-Reyes et al., 1995; Nilson and Schupbach, 1999; Roth and Lynch, 2009).

Now, at stage 10A, both the Grk source and the overlying columnar FCs are stationary, and dorsal-anterior cell fates are further refined. During this brief period, the Grk ligand gradient peaks at the dorsal-anterior midline of the oocyte surface, and diminishes posteriorly and ventrally (Goentoro et al., 2006). This gradient is central to combinatorial regulation of gene expression that defines at least four distinct dorsal-anterior FC populations (Figs. 2,3), a process that has attracted the attention of computational biologists (Fauré et al., 2014; Yakoby et al.,

2008a; Zartman et al., 2011). Each of these FC groups will undergo the morphogenetic behaviors necessary to build the distinctive anterior structures of the *Drosophila melanogaster* eggshell.

As discussed in section 3b, Dpp expression in the anterior terminal FCs provides a BMP source as early as stage 8 (Brigaud et al., 2015; Deng and Bownes, 1997; Dobens and Raftery, 1998; Dobens and Raftery, 2000; Twombly et al., 1996). The BMP gradient refines the dorsal-anterior FC domain through negative regulation at the anterior edge (Deng and Bownes, 1997; Dobens et al., 2000), through cooperative positive regulation within the dorsal-anterior peak of Grk signaling (Charbonnier et al., 2015; Chen and Schupbach, 2006; Fauré et al., 2014; Fregoso Lomas et al., 2016; Shravage et al., 2007; Yakoby et al., 2008b), and by negative regulation of the posterior FC transcription factors, Mid and H15 (Fregoso Lomas et al., 2016; Fregoso Lomas et al., 2013). Positioning of BMP and Grk morphogen sources during egg development is conserved in eggs lacking dorsal appendages from another Dipteran, *Ceratitis capitata*, suggesting a general role in patterning higher insect eggs (Vreede et al., 2013). Consistent with this, a distinct dorsal eggshell feature of other *Drosophilids* is patterned by Grk signaling (Niepielko and Yakoby, 2014).

During stage 9 (Fig. 2), BMP responses extend several cell rows beyond the Dpp-expressing anterior terminal FCs (Niepielko and Yakoby, 2014). The anterior BMP signal could be augmented at the nurse cell-oocyte interface by additional Dpp expression detected by microarray analysis in the border cells (Wang et al., 2006). The mainbody BMP response domain refines, to mark an ~2-cell wide ring of anterior-most columnar FCs that will be the centripetally migrating FCs, as we discuss next in section 7. During this period, responses to morphogen gradients and the subdivision of cell types are revealed by detection of activated signal transducers or by gene expression patterns (Boyle and Berg, 2009; Duchek and Rorth, 2001; Hsu et al., 2001; Jekely and Rorth, 2003; Muzzopappa and Wappner, 2005; Peri et al., 1999; Ward and Berg, 2005; Ward et al., 2006; Yakoby et al., 2008a; Yakoby et al., 2008b). These processes extend through stages 10A/B (Figs. 2, 3A), which last an estimated 5-12 hours (King, 1970; Lin and Spradling, 1993; Mahowald and Kambysellis, 1980). Thus, these stages are accessible to time-lapse studies to interrogate the mechanisms that interpret patterning cues to organize differentially directed migrations.

A large dorsal-anterior FC domain is established by the end of stage 9 (Atkey et al., 2006; Goff et al., 2001; Nilson and Schupbach, 1998; Pai et al., 2006). Refinement of the dorsal appendage-competent domain occurs through integrated BMP and Grk responses that increase expression of the zinc finger transcription factor Broad (Charbonnier et al., 2015; Chen and Schupbach, 2006; Deng and Bownes, 1997; Fauré et al., 2014; Yakoby et al., 2008a; Yakoby et al., 2008b). This domain into further subdivided into two patches of dorsal appendage forming FCs

separated by a strip of midline FCs (Fig. 3A) (Boisclair Lachance et al., 2009; Zartman et al., 2009a). At least some of these midline cells will form the dorsal extension of the operculum. As centripetal migration commences, the roof and floor cell of the dorsal appendages become distinct, with a 1-cell-wide, L-shaped domain of floor cells situated at the dorsal anterior/midline faces of the two roof cell domains (Fig. 3A, Berg, 2005; Ward and Berg, 2005). Floor cells continue to show the highest levels of EGFR responses (Mayer and Nüsslein-Volhard, 1988; Peri et al., 1999; Ruohola-Baker et al., 1993), up-regulate Fas3, and do not intermingle with the roof cells (Mayer and Nüsslein-Volhard, 1988; Peri et al., 1999; Ruohola-Baker et al., 1993). In section 8, we will continue with the rearrangements of these two cell types during morphogenesis of the two dorsal appendage tubes.

7. Inward migration of an anterior ring of columnar FCs.

When the columnar epithelium abuts the squamous FCs during stage 10A, a thin ring of columnar FCs, about 2 cells wide, continues to show BMP-responsive gene expression (Deng and Bownes, 1997; Dobens et al., 2000). Current evidence suggests that this domain becomes the centripetal FCs, which migrate inward to cover the anterior end of the oocyte (Bernardi et al., 2006; Charbonnier et al., 2015; Chen and Schupbach, 2006; Jekely and Rorth, 2003; Levine et al., 2007). Centripetal migration is initiated when the most-anterior columnar FCs extend apically into the germ cell cyst, and then are followed by their posterior neighbors (Dobens et al., 2000). Time-lapse imaging suggests that cells progressively begin to elongate from the anterior columnar FC edge to the next more posterior cell; detachment of the more anterior cell occurs after elongation of their neighbor begins (Fig. 2 inset for stage 10B' TTP and LAR, unpublished observations). Subsequent events are poorly understood; emerging technology to obtain time-lapse images deep into tissues with multiphoton fluorescence microscopy will assist in delineating the behaviors of centripetal FCs after they detach from the basement membrane. We can infer that centripetal FCs continue to move inward to generate a contiguous sheet, with the inner most cells abutting the border cell cluster, consistent with studies of fixed egg chambers ((King and Koch, 1963), see also images in (Dobens et al., 2000)). Surface images reveal that the centripetal FC shrink in basal circumference as they elongate, and exchange lateral neighbor junctions before they delaminate from the basement membrane (Levine et al., 2010). During stages 11-12, the border cell cluster is displaced more ventrally (TTP and LAR, unpublished observations), consistent with the asymmetrical position of the micropyle within the operculum of the eggshell (well-described in Margaritis et al., 1980). Once the centripetal FCs cover the anterior oocyte, they form a secretory epithelium that will build the operculum and outer edges

of the micropyle (Cavaliere et al., 1997; King and Koch, 1963; Levine et al., 2007; Mahowald and Kambysellis, 1980; Margaritis et al., 1980).

The mechanisms by which centripetal FCs move inward are poorly understood. *D*E-cadherin-mediated homotypic adhesion between the centripetal FCs and the germ cells is essential; when the germ cells are mutant for *shotgun* (shg, the DE-cadherin gene, Niewiadomska et al., 1999), the centripetal FCs fail to migrate inward, even though they accumulate *D*E-cadherin. Similarly, centripetal FCs that lack *D*E-cadherin fail to migrate inward, even when germ cells are otherwise wild type. The centripetal FCs accumulate *D*E-cadherin extensively along their elongated lateral faces, suggesting that they substantially remodel their lateral adhesive junctions. Consistent with this, centripetal FCs accumulate high levels of lateral Fas3 prior to their inward migration (Shravage et al., 2007). The significance of this accumulation is unknown, modulation of basolateral adhesion may be involved in this migration, or formation of pleated septate junctions may be delayed.

Some evidence suggests that anterior columnar FCs are not alone in their inward migration. The posterior-most squamous FCs extend inward adjacently to the elongating centripetal FCs ((Tran and Berg, 2003), and TTP, Anna Kabanova, and LAR, unpublished observations; at this point the squamous FC is too thin to draw proportionately in the Fig. 2 stage 11B inset). Thus, the posterior-most squamous FCs may be the location for *dpp* expression observed in centripetally migrating FCs (Dobens and Raftery, 1998; Dobens et al., 2000; Twombly et al., 1996). Whether this process represents an infolding at the junction between squamous FCs and columnar FCs is an open question. However, infolding, or sheet flexing, is a common feature in epithelial morphogenesis, that is described as initiating with apical constrictions (discussed for FCs in Osterfield et al., 2013). This general model does not fit centripetal migration at first glance; both apical and basal constriction occurs in the columnar FCs, whereas the adjacent squamous FCs do not constrict their apices, but rather appear to remodel their apical membranes to form extensive contact with an underlying nurse cell (Brigaud et al., 2015; Timmons et al., 2016).Whether adjacent squamous and centripetal FCs maintain some form of lateral adhesion as they extend inward, and how these contacts are eliminated later, are open questions.

How centripetal migration is initiated is currently unknown; at least three, non-exclusive models are consistent with current evidence from *D. melanogaster*. One model is that these cells are fated to migrate inward due to early prepatterning by Upd signaling through the Jak/STAT pathway (Fig. 2, and Xi et al., 2003), followed by a subsequent round of anterior patterning by BMP signaling through Tkv/Mad (Chen and Schupbach, 2006; Dobens et al., 2005; Fauré et al., 2014; Fregoso Lomas et al., 2016; Shravage et al., 2007). BMP signaling is a prominent feature of

squamous FC-centripetal FC interactions. Beginning in stage 8, the BMP activated form of Mad is detected in anterior terminal cells (D.J. Sutherland and LAR, unpublished observations), and this pattern spreads with squamous FC flattening throughout stage 9 (Brigaud et al., 2015). During stage 10B however, activated Mad is highly enriched in the ring of centripetal FCs (Jekely and Rorth, 2003; Yakoby et al., 2008b, and TTP and LAR, unpublished data). *dpp* transcripts are detected continuously throughout centripetal migration and eggshell formation, including cells surrounding the micropyle at stage 14 (Twombly, 1995).

A gene regulatory network links BMP signaling to increased accumulation of *D*E-Cadherin and Myosin II, through BMP down-regulation of the negative regulator BunB (Dobens et al., 2005; Levine et al., 2010; Levine et al., 2007). Within stage 10 centripetal and operculum-forming FCs, BMP signaling down-regulates genes encoding other transcriptional regulators: Broad, and Brinker (Charbonnier et al., 2015; Chen and Schupbach, 2006; Deng and Bownes, 1997; Dobens et al., 2005; Yakoby et al., 2008b). Local BMP accumulation from adjacent, inward-spreading squamous FCs may be responsible for changing expression of these genes during stage 10, perhaps with additional BMP from the border cells (Wang et al., 2006). However, it remains unclear whether there are late functions for BMP signaling in formation of the operculum or micropylar structures, or whether the squamous FCs are necessary for centripetal migration in *D. melanogaster*.

In a second model for initiation, inward movement may be triggered by a temporally regulated signal. Perhaps consistent with this model, centripetal migration is sensitive to Ecdysone signaling, which provides a temporal, and possibly systemic status input for progression of egg chamber development (Domanitskaya et al., 2014; Hackney et al., 2007; Romani et al., 2009; Romani et al., 2016, and others cited within; Sieber and Spradling, 2015). Alternatively, the centripetal FCs may sense their position at the interface between the nurse cells and oocyte, perhaps due to a distinct signal emitted by the border cells. Border cells are not essential for centripetal migration, however; because fully eggshell-encased eggs are produced after laser-ablation of the border cells (Montell et al., 1991).

A third model was proposed by Edwards and Kiehart (Edwards and Kiehart, 1996), in which the actomyosin network may provide a contractile force that draws the centripetal FCs inward. Additional genes known to disrupt normal centripetal migration when mutated in either the FCs or in whole animals are *notch, spaghetti squash, zipper, 18-wheeler, tramtrack, shotgun*, and *capping protein beta* (Dobens et al., 2005; Edwards and Kiehart, 1996; Kleve et al., 2006; Ogienko et al., 2013; Wang et al., 2006; Wheatley et al., 1995). Mutations that disrupt centripetal migration may not be uncovered readily, if the affected gene is required earlier in oogenesis, resulting in adsorption of the aberrant egg chambers (reviewed in Buszczak and Cooley, 2000;

McCall, 2004). Alternatively, it may imply that centripetal migration is a more robust morphogenetic process than other migrations such as those of the border cells or the dorsal appendages.

Notably, more basal insect species with meroistic polytrophic ovaries utilize a variety of mechanisms to enclose the anterior face of the oocyte, including epithelial folding, and in general are less "aggressively" invasive than the centripetal FCs of *Drosophila* (Garbiec and Kubrakiewicz, 2012; Garbiec et al., 2016; Tworzydlo and Kisiel, 2011). In Neuroptera, both the anterior and posterior poles of the oocyte initially contact nurse cells, and must be covered by an epithelial layer later in oogenesis. The two centripetal migrations that fulfill this requirement are thought to occur via leading cells that passively drag the mainbody FCs behind them (Garbiec and Kubrakiewicz, 2012). These observations suggest that the specific movements that take place during *Drosophila* centripetal migration may be a more recently acquired evolutionary trait.

A central role for the oocyte is indicated by impaired centripetal migration when gene disruption results in failed oocyte differentiation or import of nutrients; including mutations in *Cup, chalice, bicaudal, kelch,* and *quit* (Schupbach and Wieschaus, 1991). In these cases, centripetal FCs may be competent to migrate, but they may not move inward because of the absence of external cues from the oocyte (Berg, 2005; Keyes and Spradling, 1997; Mahone et al., 1995; Swan and Suter, 1996). Failure in centripetal migration results in formation of eggshells that have a "cup"-like shape due to the open anterior (Dobens et al., 2005; Edwards and Kiehart, 1996; Schupbach and Wieschaus, 1991; Twombly et al., 1996).

Genome-wide approaches have identified sets of genes expressed in FCs during stages 9-12. One lab employed micro-array analysis combined with a high-throughput assay for genes showing patterned FC expression during stages 10A – 12 (Figs. 2, 3A) (Yakoby et al., 2008a). These results were used to define a combinatorial code for patterns of FC gene expression, in which one pattern unit corresponds to the ring of centripetal FCs during stage 10. Another lab focused specifically on migratory FCs that express high levels of the C/EBP bZIP transcription factor gene *slbo*, a procedure that enriched for centripetal and border FC RNAs (Wang et al., 2006). This approach netted 413 genes that were more highly expressed in migratory FCs than other FCs. Importantly, this screen identified *18-wheeler*, a Toll-like IgG domain gene that is necessary for normal centripetal migration (Kleve et al., 2006). Altogether, these datasets provide a starting point for RNA interference surveys to identify genes that that are required in centripetal FCs for inward migration.

Although many questions remain, these data create a working model for how centripetal migration is initiated and carried out. Anterior patterning by BMP signaling establishes a

domain of FCs competent for centripetal migration, by indirectly up-regulating genes important for migration. The resultant gene regulatory network involves intermediary regulatory genes, such as *bun* and *slbo*. Once made competent, these cells might elongate inward in response to some combination of an activating signal and direct interactions with the underlying oocyte. Elongation occurs through modulation of the actomyosin contractile network and *D*E-cadherin-mediated adhesive interactions with the oocyte. This elongation occurs prior to initiation of nurse cell dumping, and therefor does not disrupt the cytoplasmic connections between nurse cells and oocyte. Centripetal migration continues into stage 11, when nurse cell dumping begins. Events necessary for eggshell protein production are delayed in the centripetal FCs (Bernardi et al., 2006; Bernardi et al., 2007; Bernardi et al., 2009; Cavaliere et al., 2008; Cavaliere et al., 1997; Hackney et al., 2007; Parks and Spradling, 1987; Waring, 2000), so that the entire anterior end of the egg is encased prior to secretion of eggshell components. The regulation that determines the specific time and place for centripetal migration remains an open question.

8. Morphogenesis of two dorsal appendage tubes

The progressive formation of two, elongated tubes from dorsal-anterior FCs provides an accessible genetic system for study of tubulogenesis. In general, tubulogenesis refers to a broad class of morphogenetic processes that produce tubes or tubular structures, which are essential functional structures in organs and tissues throughout the animal kingdom (Andrew and Ewald, 2010). In the specific case of dorsal appendage morphogenesis, a developmentally programmed process transforms a two-dimensional columnar epithelial sheet into a three-dimensional tube (reviewed by Berg, 2005; Berg, 2008). Of the five general types of tubulogenesis (reviewed by Andrew and Ewald, 2010; Berg, 2008; Iruela-Arispe and Beitel, 2013; Lubarsky and Krasnow, 2003), dorsal appendage formation appears to be an amalgam of wrapping and budding morphogenesis (see also Osterfield et al., 2013). We will briefly introduce general features and classic examples of wrapping and budding morphogenesis then summarize the specific cellular behaviors that take place during DA formation.

The wrapping type of tube formation is exemplified by vertebrate neurulation, in which cells of the neural plate bend via regulated cell shape changes, thus forming a fold with an emerging lumenal space. Subsequently the lateral borders of the fold meet at the midline, where cells differentially adhere to form the neural tube (Suzuki et al., 2012). The budding mechanism is typically deployed during branching morphogenesis (as seen in the development of lung, vascular, and glandular tissues, Ochoa-Espinosa and Affolter, 2012). During budding, discrete

groups of cells extend perpendicularly to the tissue of origin, via changes in cell shape and/or concerted cell migration.

Dorsal appendage morphogenesis may be broken up into three processes: 1) tube formation 2) tube elongation and 3) tube maturation (Fig. 3) (Dorman et al., 2004; French et al., 2003). The two dorsal appendage primordia form a pair of appendages with mirror-image symmetry on either side of the dorsal midline. Though they develop in concert, each dorsal appendage forms independently of the other from a separate placode, a dorsal anterior patch of ~55-65 cells that is displaced by two cell diameters on either side of the dorsal midline (Deng and Bownes, 1997). Each primordium consists of two FC types: the roof and the floor cells, which behave in strikingly different ways during tube formation.

At the earliest phase of dorsal appendage morphogenesis during stage 10B (Fig. 3B-B″), the placodes become morphologically distinct from neighboring columnar FC and adopt an elongated form (Dorman et al., 2004; French et al., 2003). By stage 11 (Fig. 3C-C″), tube formation begins: the roof cells intercalate, constrict apically and expand basally. These dramatic changes in cellular architecture lead to a local tissue bending that is characteristic of wrapping tubulogenesis. However, bending alone is insufficient for tube completion. To close this nascent tube and create a lumen, the floor cells must undergo a concomitant reorganization. Like the roof cells, floor cells elongate along their apico-basal axis, but floor cells of each DA primordium "dive" underneath the roof cells (Fig. 3C″). A single row of floor cells is situated at the anterior and medial borders of the roof cells; these two groups of floor cells come together, forming new lateral cell-cell adhesive junctions that close the nascent tube along a ventral seam at stage 12 (Fig. 3D) (Dorman et al., 2004; Osterfield et al., 2013). The newly formed tubes now elongate through combined processes of convergent extension, anterior-directed migration and concomitant remodeling of cellular architecture during stages 12-13 (Fig. 3D-E). Once the tubes have elongated over the anterior egg chamber, appendage eggshell components are sequentially secreted into the lumens. Secretion finishes by stage 14 (Fig. 2), at which point the entire egg reaches maturity and awaits muscular contractions that will move it down the oviduct for fertilization.

FC behaviors during dorsal appendage morphogenesis were revealed by time-lapse imaging, combined with studies of aberrant morphogenesis in mutant genotypes (summarized in Berg, 2005; Berg, 2008; Dorman et al., 2004). Morphometric studies indicate that *D. melanogaster* FCs undergo a more extensive series of movements to form these respiratory appendages than *Drosophilid* species that have simpler, but more numerous appendages (Osterfield et al., 2013; Osterfield et al., 2015). Future studies will reveal the types of forces and molecular mechanisms that underlie this plastic feature of *Dipteran* eggshells. We will focus on

recent work that is beginning to unravel the mechanisms that translate patterning information into the dorsal appendage morphogenetic program.

An early output of dorsal-anterior patterning is the differential expression of cell adhesion molecules. Patterned expression of distinct cell adhesion molecules has multiple roles in tissue morphogenesis (as reviewed by Fagotto, 2015; Lecuit, 2005). Several of these are on display during dorsal appendage tube formation; for example, floor cells must "dive" into the space between roof FCs and the oocyte, necessitating a weakening of any adhesive forces between the roof cells and the oocyte. Furthermore, tube closure requires formation of new lateral cell interfaces between the anterior and medial floor cells that seal the DA tube. Recent work has highlighted the dynamic expression of Cadherin superfamily cell adhesion molecules during DA tubulogenesis.

Spatial and temporal precision in remodeling of *D*E-Cadherin-based adherens junctions is required for tube closure and elongation. Short and wide dorsal appendages form following experimental manipulation of *D*E-Cadherin levels, either by localized over-expression or by RNA interference-mediated knockdown (Peters and Berg, 2016a). Dorsal midline cells, which separate the dorsal appendage primordia, have elevated levels of *D*E-Cadherin RNA and protein during stages 10B-12 (Fig. 3B-E) (Zartman et al., 2009b). Conversely, *D*E-Cadherin levels are decreased in the roof cells as DA morphogenesis takes place. In addition to dynamic transcriptional regulation, *D*E-Cadherin levels at lateral membranes are modulated by Dynamin-mediated endocytosis (Peters and Berg, 2016a; Peters et al., 2013).

The atypical Cadherin Fat2 was introduced in section 2. *fat2* mutant FCs shape round eggs that have severely misshapen dorsal appendages (Viktorinova et al., 2009, example of a similar phenotype in Fig. 1E). Dorsal appendage primordia FCs have the only detectable *fat2* RNA accumulation during stage 10B (Fig. 3a, B-B"), with higher levels in the floor cells and lower levels in the roof cells (Zartman et al., 2009b). This expression pattern suggests that dorsal appendage defects result from a direct requirement for Fat2 in dorsal appendage morphogenesis. However, further experiments will be needed to eliminate the alternative of a secondary effect that arises as the primordia elongate over a rounded egg chamber.

The Cadherin superfamily includes 17 *Drosophila* genes in total (Hill et al., 2001); several more are expressed in the FCs and exhibit dynamic expression patterns throughout egg chamber development (Zartman et al., 2009b). One, *Cad74A*, is expressed in all columnar FCs until stage 10B (Figs. 2, 3B-B"). Cad74A accumulates at FC apical membranes, and may facilitate adhesion to the underlying oocyte. Transcriptional down-regulation of *Cad74A* in roof cells is important for proper morphogenesis. Flat and short dorsal appendages are frequently formed

with forced expression of *Cad74A*, suggesting that its differential expression is important for both tube closure and elongation (Berg, 2008).

Other lateral cell adhesion molecules are differentially expressed in dorsal appendage forming FCs, including the IgCAMs Fas3 and Echinoid. Floor cells, which do not intermingle with the roof cells, up-regulate Fas3, though the function of this adhesive molecule has not been tested (Ward and Berg, 2005; Ward et al., 2006). In contrast, a requirement has been demonstrated for Echinoid, which is recruited to adherens junctions (Wei et al., 2005). Echinoid is present in all FCs until stage 10B, when it is down-regulated in most, and fully repressed in the roof cells (Laplante and Nilson, 2006). Now roof cells that lack Echinoid are juxtaposed with Echinoid-expressing FCs. Echinoid-mediated cell sorting excludes roof cells, and maintains a smooth boundary for this domain. Roof cells react to the adhesive discontinuity by assembling a supracellular, contractile actomyosin cable, which is thought to promote their apical constrictions. Consistent with this, large patches of *echinoid* mutant cells disrupt normal assembly of the supracellular actomyosin cable, fail to close the dorsal appendage tube, and produce misshapen dorsal appendages. Many intriguing questions remain for this adhesion molecule, such as how cells sense their location at an Echinoid expression boundary and interpret this information to assemble a supracellular actomyosin cable. Dorsal appendage morphogenesis is an attractive model to tackle these questions, which are relevant to other examples of tissue morphogenesis that require actomyosin cable assembly and function such as salivary gland tubulogenesis, embryonic dorsal closure, and wound-healing (Röper, 2013).

Different paracrine signals may coordinate roof and floor cells for morphogenesis of distinct sub-regions of dorsal appendage primordia. For example, both roof and floor cells are included in a region showing active BMP/Tkv responses during stages 11/12 (Fig. 3D,E), visualized by immunostaining for activated Mad (Niepielko et al., 2011). Similarly, the growth factor regulated-bZIP transcription factor Fos is expressed in a domain of cells that includes both floor and roof cell types (Boyle et al., 2010; Dequier et al., 2001; Souid and Yanicostas, 2003). Genetic depletion of Fos, its BMP or EGFR regulators, or its transcription factor partners leads to several defects in egg chamber development that include defects in DA morphogenesis (Boyle et al., 2010; Dequier et al., 2001; Souid and Yanicostas, 2003). Taken together, these data suggest that distinct morphogenetic activities may occur within different regions along the lengths of the dorsal appendage tubes. In a critical test of this model, laser ablation studies revealed that elongation requires the FCs in the anterior third of the dorsal appendage tube, whereas more posterior tube FCs appear to be dispensable (Boyle et al., 2010). Additional support for diversity of cell behaviors or morphogenetic forces across the length of one tube comes from observations of positional heterogeneity in phenotypic outcomes of mosaic

experiments, in which the roof cells that contact floor cells have distinctive roles from the rest of the roof cells. These studies point to the coordinated action of anterior roof cells and floor cells as a critical factor in elongation.

How is tube elongation facilitated by coordinated behaviors of the anterior-most dorsal appendage FCs? The closed end of the tube shares features of a leading edge, but this aspect of elongation remains poorly understood. Notably, floor cells extend filopodia-like protrusions as they conform to the squamous FCs (Ward and Berg, 2005). Another report observed protrusions from the underlying squamous FCs, suggesting an active interaction between the basal surface of floor cells and with the squamous FCs as a substrate (Tran and Berg, 2003).

The dorsal appendage tubes move between the squamous FCs and the basement membrane that surrounds the egg chamber, most clearly visualized by GFP-tagged Collagen IV in (Haigo and Bilder, 2011). In that work, collagenase treatment of stage 12 egg chambers results in disorganized dorsal appendage FCs overlying the rounded egg chamber. Consistent with a role for basement membrane interactions in dorsal appendage morphogenesis, Integrin mis-expression results in aberrant morphology, suggesting that proper modulation of Integrin levels is important (Duffy et al., 1998; Peters and Berg, 2016a; Peters et al., 2013). This is further supported by the accumulation of higher levels of basally localized βPS-Integrin when Dynamin function is impaired, perhaps due to reduced recycling. A tempting model is that Integrin levels must be tightly regulated to support this anterior-directed migration.

Taken all together, these observations suggest a working model in which both ventral floor cells and leading edge-roof cells take active part in an anterior-directed migration. Ventral floor cells might provide an adhesive traction mechanism over squamous FCs, whereas leading edge-roof cells might provide an Integrin-based crawling mechanism through interactions with the basement membrane. More work is needed to define the mechanisms that drive sustained, directional migration during the elongation phase of dorsal appendage morphogenesis.

9. Conclusions and Perspectives:

Morphogenesis of the *Drosophila* egg continues to provide a system for addressing significant questions at the forefront of biology. Different questions have become accessible with each new technological advance, beginning with the identification of follicle cell diversity and migrations revealed with improvements in optical microscopy (e.g.King, 1970; King and Koch, 1963), quickly followed by the elaboration of ultrastructural morphology and eggshell diversity revealed by electron microscopy (e.g. Kambysellis, 1974; Mahowald and Kambysellis,

1980; Mahowald, 1972; Margaritis, 1985; Margaritis et al., 1980). We have said little about the elegant gene expression program that leads to spatial and temporal regulation of eggshell component production during a period of less than 24 hours (e.g. Cavaliere et al., 2008; Kafatos, 1975; Margaritis, 1986; Petri et al., 1978; Tootle et al., 2011; Waring, 2000). The rate of eggshell deposition is greatly enhanced by developmentally-controlled amplification of FC genomic regions, which has provided important leads to understanding the regulation of origins of DNA replication (e.g. Calvi et al., 1998; Orr-Weaver, 1994; Smith et al., 1993; Smith and Orr-Weaver, 1991).

Most recently, our understanding of the cell biological processes that orchestrate epithelial morphogenesis has blossomed through the combination of sophisticated genetics and high-resolution time lapse imaging of FC behaviors and egg chamber-wide morphogenesis. Examination of the FC-specific functions of individual gene products has been driven by advances in genetic tools that enabled detailed mosaic analyses and spatio-temporally-controlled RNA interference to perform systematic, genome-wide screens (recent ovary specific reviews include Cheung and Shvartsman, 2015; Hartman et al., 2015; Hudson and Cooley, 2014; Rubin and Huynh, 2015). Understanding the precise deployment of specific cellular behaviors across time and space requires high resolution, time-lapse imaging under appropriate ex vivo culture conditions (Peters and Berg, 2016b, provide an excellent history of this rapidly advancing technology). Publication of detailed protocols for new technologies and established workhorse approaches ensures that they can be adopted readily by a growing number of research laboratories (e.g. Hurd et al., 2015; Jambor et al., 2016; Prasad et al., 2007; Thompson et al., 2015; Zimmerman et al., 2013).

Improvements in imaging technology are likely to drive new discoveries in FC morphogenetic movements. The ability to follow events through time has enabled the discovery of new organ-shaping modalities for migration, and, in some cases, the delineation of morphogenetic events too quick to capture from fixed tissues. We anticipate that new imaging modalities, such as multiphoton fluorescence microscopy or micro-computed tomography of high-resolution X-ray scans, will open up areas that were previously inaccessible due to light scattering within thick tissues or the presence of opaque structures (recently demonstrated by Mattei et al., 2015), respectively. Availability of these instruments through regional imaging core facilities, equipped with advanced computing and large data storage systems, will extend the scope for transformative discoveries using this otherwise economical model organism.

As we deepen our understanding of epithelial plasticity during morphogenesis of the highly structured *D. melanogaster* follicular epithelium, an increasing number of studies are uncovering the divergent morphogenetic behaviors associated with distinctive eggshell features

across a broadening range of insect species (e.g. Garbiec and Kubrakiewicz, 2012; Garbiec et al., 2016; Jaglarz et al., 2008; Jaglarz et al., 2010; Niepielko et al., 2014; Niepielko and Yakoby, 2014; Osterfield et al., 2015; Tworzydlo et al., 2005; Tworzydlo and Kisiel, 2011; Vreede et al., 2013). In a few cases, divergent eggshell features have been related to the stresses imposed on developing embryos, due to egg deposition on different substrates by different insect species (Hinton, 1981; Hinton, 1969; Kambysellis, 1993). With a new appreciation for the time scale for evolutionary radiations of insects in general, and *Dipterans* in particular (Misof et al., 2014; Wiegmann et al., 2011; Yeates et al., 2016), we anticipate that future studies of follicular epithelium morphogenesis will establish a deep understanding for the progressive elaboration of diversity in epithelial morphologies.

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Figure Legends

Figure 1. *Drosophila* eggs are produced in the ovary within ovarioles.

***A*: Anterior portion of a single ovariole.** Eggs develop from egg chambers, which are assembled de novo in the germarium. Successively formed egg chambers develop in an assembly-line manner as they make their way towards the posterior of the ovariole, such that each egg chamber is older than its more anterior neighbor. Egg chambers are linked in a "chain" by stalk cells (arrow). The anterior-most region of each ovariole contains a stem cell niche for egg chamber formation. The boxed region includes this anterior-most region at the upper left, with the terminal filament at the tip, the long, germarium structure, and a newly formed egg chamber that has budded from the germarium (arrowhead, lower right of box). ***B*: A schematic of a Drosophila germarium, which is composed of several cell types that coordinate their behaviors to continuously produce egg chambers.** Egg chamber formation begins with the asymmetric division of germline stem cells, which are associated with the terminal filament and cap cells of the niche in region 1. Asymmetric division leads to the formation of a daughter germ-line stem cell that stays associated with the cap cells. The more distant daughter, now a cystoblast, undergoes four rounds of mitotic division as it moves through region 2a, surrounded by escort cells. Incomplete cytokinesis during cystoblast divisions gives rise to a 16-cell syncytium interconnected via cytoplasmic bridges. The cystoblast syncytium, or germ cell cyst, encounters prefollicle cell progeny of the follicle stem cells situated at the boundary of region 2b. Prefollicle cells coat the posterior side of the cyst, separating it from escort cells, and from the now completed stage 1 egg chamber that resides in region 3. Organization of stalk cells and polar cells accompanies the budding of a stage 2 egg chamber from the germarium. By mid-stage 2, egg chambers are fully encapsulated with a specialized basal extracellular matrix (ECM), or basement membrane. Egg chambers begin to rotate early in their developmental program (stage 1/2) in either a clockwise or counter clockwise direction (black arrows under stage 2) relative to their anterior-posterior axis (gray horizontal line under stage 2). ***C:* Circumferential migration is maintained through stage 8 and arrests by stage 9.** The timeline is depicted here as a thick green bar. ***D:* Circumferential migration requires the planar polarization of the follicular epithelium.** Cytoskeletal structures composed of F-actin (red) and Acetylated-tubulin (Ace-tubulin, blue) are arranged in planar polarized basal bundles, oriented perpendicular to the anterior-posterior axis. Lower left inset shows follicle cell apicobasal polarity, with the apical surface (filled arrowhead) in contact with the nurse cells (NCs) or oocyte (Oo) and the basal surface (unfilled arrowhead) in contact with the basement membrane (ECM; green). During this migration, FCs secrete ECM components including Collagen, Laminin, and Perlecan. Though

not essential, migration facilitates the polarized deposition of long, ECM fibrils, oriented perpendicular to the AP axis (dark green; lower right). Disruption of planar cell polarity and/or circumferential migration leads to failure in egg elongation. ***E:*** **Defects in planar cell polarity are associated with the production of round eggs.** A stage 14 egg, resulting from RNA interference-mediated depletion of *trc* in the follicle cells using the traffic-jam-Gal4 driver, which gives a similar round egg phenotype to the published mosaic analysis with traditional mutations (Horne-Badovinac et al., 2012). ***F:*** **Drosophila melanogaster eggs are normally ovoid in shape, with elongated dorsal appendages.** A stage 14 egg from a parallel control experiment is shown. Anterior eggshell structures are indicated: dorsal appendage (da), operculum (op), micropyle (mp), and ventral collar (vc). Scale bars are 100 micrometers (μm). For detailed electron micrographs of these structures, see Margaritis et al, 1980. In panels A, B, and D-F, anterior is left and posterior is right. In E and F, dorsal is up and ventral is down.

Figure 2. Egg chamber diagrams from selected stages, showing morphogenesis of relevant follicle cell populations and their contributions to the eggshell.

Six stages of *Drosophila* oogenesis are depicted, as well as the final mature eggshell. Key cell populations are color coded according to their patterning and final fate. *Stage 4*: Prior to this stage, the polar cells are specified and reside at both ends of the developing egg chamber. Nurse cells and their nuclei are visible, as well as the oocyte, oocyte nucleus, and epithelial follicle cells (FCs). *Stage 8:* As the oocyte grows larger, additional patterning specifies at least one posterior terminal FC domain, as well as three anterior FC domains. *Stage 9:* During this stage, the border cell cluster, composed of the anterior polar cells and neighboring terminal FCs, delaminates from the epithelium and migrates posteriorly between the nurse cells. Concurrently, epithelial FCs reorganize into squamous and columnar domains, starting from the anterior and posterior poles of the egg chamber, respectively. Some evidence suggests that centripetal FCs are specified by this time (pre-patterned anterior follicle cells in diagram). *Stage 10A:* The centripetal FCs are organized in rows at the anterior edge of the columnar FCs, and the border cells reach the nurse cell/oocyte boundary and begin to migrate dorsally. *Stage 10B:* Centripetal migration is underway, and the roof and floor cells that will comprise the dorsal appendages are specified. *Stage 10B inset*: After initial elongation, individual centripetal cells appear to detach from the basement membrane and move inward over their more posterior neighbors (TTP and LAR, unpublished observations). *Stage 11:* Centripetal migration nears completion as the nurse cells dump their contents into the oocyte. Squamous FCs begin to wrap around individual nurse cells, ultimately to promote their phagocytosis. *Mature Egg:* The columnar FCs secrete the final

eggshell resulting in two dorsal appendages, the operculum, the outer portion of the mycropylar structure, and the aeropyle located at the posterior. Border cells secrete the interior material of the micropyle, and shape a path for sperm entry. The approximate cell populations are color coded as indicated at the bottom of the figure. The egg chambers are not depicted at the same scale; indicated by its individual scale bar (100 μm) just below. Each egg chamber was hand traced from micrographs; positions of FC lateral interfaces were estimated.

Figure 3. Dorsal appendage morphogenesis requires de novo tube formation.

A-B: **Dorsal appendage placodes become evident by stage 10B.** By stage 10B, the dorsal appendage placode is composed of two cell types, the floor and roof cells, which can be identified by gene expression patterns, as discussed in the text. The dorsal appendage placodes abut the dorsal operculum-forming cells that populate the "T-region" along their anterior and medial borders. ***B:*** **Dorsal appendage morphogenesis begins with follicle cell elongation along the apicobasal axis.** The boxed region of interest in B, is depicted as a "magnified" view in B′ (early stage 10B) and B″ (late stage 10B). By late stage 10B, the follicle cells of the dorsal appendage placode have elongated substantially along their apicobasal axis and are morphologically distinct from neighboring cells that do not participate in dorsal appendage morphogenesis. ***C-D:*** **Floor cells "dive" underneath the roof cells to form a tube.** The boxed region of interest in C, is depicted as a "magnified" view in C′ (early stage 11) when the floor cells begin to dive underneath the roof cells and in C″ (late stage 11) when the floor cell apices of the anterior and medial floor cell populations are approaching each other underneath the roof cells, better appreciated from the dorsal view in D. ***D:*** **Apical extension by the floor cells gives rise to the dorsal appendage tube.** By stage 12, the anterior and medial floor cells meet under the roof cells and form new lateral contacts with each other, thereby sealing off the tube (along a ventral seam) and generating a lumen between the roof and floor cells. ***E:*** **The dorsal appendage tube elongates during stages 12-13 and eggshell components are sequentially secreted into the dorsal appendage lumen.** Dorsal appendage elongation and morphological maturation requires the coordination of several behaviors that include, convergent extension, concerted cell migration, and remodeling of cell shape. As the dorsal appendage tube elongates, it rotates such that the roof cells face outwards (or laterally), while the floor cells face inwards (towards the nurse cell compartment). Eggshell components begin to be secreted into the lumen during stage 11, however the bulk of secretion taking place between stages 12-14. The mature dorsal appendage has a narrow stalk (proximal) and a wide paddle (distal; Fig.

1F). This figure is adapted from figures in Dorman et al. (2004), which presents more detailed descriptions of dorsal appendage morphogenesis; additional information for specific details are available in references cited within the text. In all panels, anterior is left and posterior right. Panel A depicts a dorsal surface view. Panel D, depicts a "flattened" dorsal view of floor cells only. Panels B-C" and E depict cross-sectional views (dorsal side up). Developmental stage is indicated in each panel.