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The *Drosophila* shell game: patterning genes and morphological change

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What are the mechanisms that convert cell-fate information into shape changes and movements, thus creating the biological forms that comprise tissues and organs? Tubulogenesis of the *Drosophila* dorsal eggshell structures provides an excellent system for studying the link between patterning and morphogenesis. Elegant genetic and molecular analyses from over a decade provide a strong foundation for understanding the combinatorial signaling events that specify dorsal anterior cell fates within the follicular epithelium overlying the oocyte. Recent studies reveal the morphogenetic events that alter that flat epithelial sheet into two tubes; these tubes form the mold for synthesizing the dorsal appendages – eggshell structures that facilitate respiration in the developing embryo. This review summarizes the mutant analyses that give insight into these patterning and morphogenetic processes.

Introduction

In the ovaries of the female fruit fly *Drosophila melanogaster*, oogenesis occurs in the context of individual egg chambers, which mature in assembly-line fashion within long strings called ovarioles [1]. Each egg chamber progresses through 14 developmental stages over the course of several days. The egg chamber consists of 16 interconnected germ-line cells, 15 nurse cells and a single oocyte, surrounded by a layer of ~650 somatically derived follicle cells [2,3]. The highly polyploid nurse cells synthesize various components required by the developing oocyte and future embryo and transport these molecules and organelles into the oocyte through cytoplasmic bridges called ring canals [4]. The follicle cells secrete ligands or activators that establish polarity within the oocyte and embryo [5]; the follicle cells also synthesize the layers and specializations of the eggshell [6].

The dorsal appendages are proteinaceous eggshell structures synthesized by two groups of dorsal, anterior follicle cells. These structures arise at the end of oogenesis following a series of signaling events that specify two dorsal appendage primordia within the follicle cell layer. Subsequent shape changes and rearrangements transform these regions of the epithelium into two tubes [7]. The follicle cells then secrete eggshell proteins into the tube lumens to produce the two dorsolateral eggshell appendages. The appendages serve as breathing tubes for the developing embryo and provide a mechanism for air exchange if the egg becomes submerged [8]. The signaling processes that initially specify the appendage primordia also define a ventral prepattern, laid down within the eggshell, that is used to establish dorsoventral polarity in the embryo [9–13].

Patterning the appendage primordia

Figure 1 depicts the later events in oogenesis that specify the dorsal appendage primordia. At stage ten (Figure 1a), the oocyte occupies the posterior half of the egg chamber, the nurse cells occupy the anterior half, and the oocyte nucleus is positioned at the dorsal anterior corner of the oocyte. Most follicle cells form a columnar layer over the oocyte, although a few follicle cells form a thin layer stretched out over the nurse cells (not visible in Figure 1a; schematized in Figure 2b). Previously, all follicle cells entered an endoreplication cycle and reached a ploidy of 45C; thus, both patterning and morphogenesis of the dorsal appendage-forming cells occur in the absence of cell division [2].

By stage ten, transcripts encoding the transforming growth factor α (TGF-α)-like signaling molecule Gurken (GRK) are localized in a cap above the oocyte nucleus (Figure 1b) [14]. Gurken signals through the epidermal growth factor (EGF) receptor homolog (EGFR), activating a transduction cascade involving the RAS–RAF–MAPK pathway [5]. This signaling event defines a set of dorsal-anterior cells (Figure 1c) and induces a second signaling cascade involving three additional EGFR ligands, including the inhibitory molecule Argos. This second cascade amplifies and refines the initial GRK signal, leading to the definition of two separate populations of dorsal follicle cells (Figure 1d) [15–17].

Information along the anterior–posterior (A–P) axis also contributes to cell-fate determination within the dorsal-appendage primordia. The BMP2,4 homolog encoded by *decapentaplegic* (*dpp*) is expressed in the stretched cells and a single row of centripetally migrating cells (Figure 1e). This morphogen radiates posteriorly and alters columnar-cell fates [18]. High levels of DPP repress dorsal identities and specify the operculum (anterior face of eggshell). Moderate levels synergize with GRK to define dorsal anterior. At low levels of DPP, cells cannot respond to EGFR signaling and become 'main body' follicle cells [19–22].

Through a mechanism that is not yet clear, the gradients of DPP and EGFR activity are sharpened into precise domains along the A–P axis. By analogy to the

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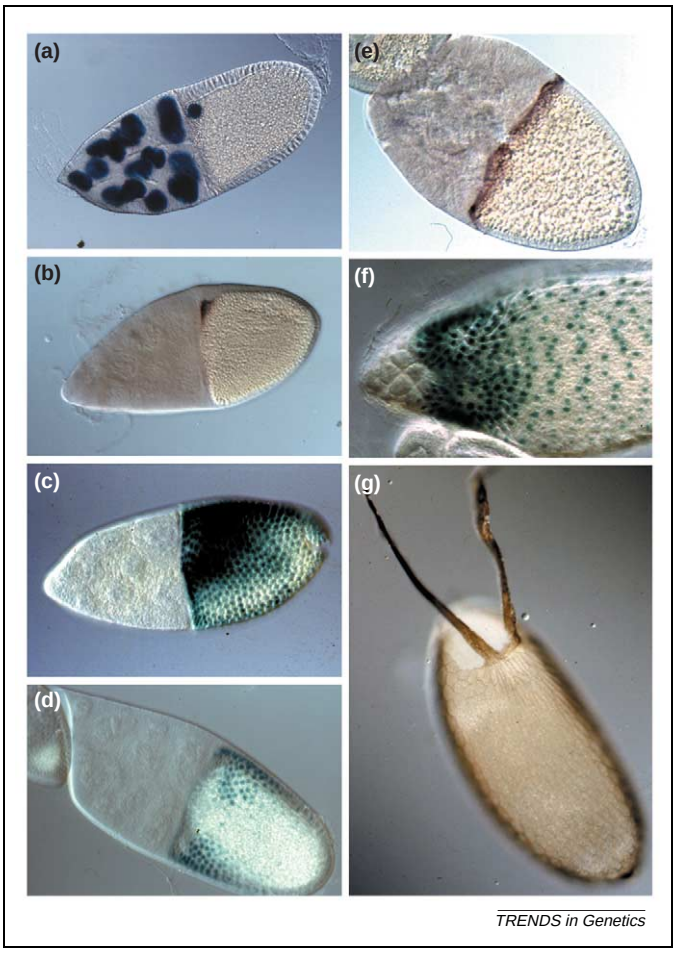

Figure 1. Late oogenesis: differential interference contrast microscopy of enhancer trap lines that express *lacZ* in ovarian cells (a, c, d, f), *in situ* hybridization to mRNA (b, e), and a mature egg (g). (a–e) stage ten, (f) stage 12, (g) stage 14. Anterior to the left, (a–c) dorsal up, (d–f) dorsal is facing out of the page, and (g) dorsal is facing to the upper right and out of the page. **(a)** Stage ten egg chamber from $Ras85D^{05703}$, which labels the germ-cell nuclei. The 15 nurse cells reside at the anterior of the egg chamber and the yolk-filled oocyte is at the posterior. The oocyte nucleus marks the dorsal anterior corner of the oocyte. Somatic follicle cells form a columnar epithelium over the oocyte. **(b)** *gurken* mRNA lies in a cap over the oocyte nucleus. **(c)** Dorsal anterior follicle cells that receive the GRK signal activate expression of the *lacZ* reporter *PZ(2)03225*. **(d)** Additional signaling through the EGFR pathway induces the inhibitory ligand Argos, which represses dorsal fates on the midline, as revealed by enhancer trap line *PZ(2)05660*. **(e)** *decapentaplegic* is expressed in the squamous stretched follicle cells over the nurse cells and in a single row of columnar cells over the oocyte. Secretion of the BMP2,4 ligand patterns anterior cell fates. **(f)** At stage 12, the nurse cells have dumped their contents into the oocyte and are undergoing apoptosis. The dorsal follicle cells, highlighted by *PZ(2)03225*, have formed two tubes that move out over the apoptosing nurse cells. **(g)** A mature egg has a curved ventral surface that is longer than the flat dorsal side. The respiratory appendages lie just lateral to the midline, extending out of the collar and partially obscuring the view of the operculum, which appears white in this image. Secretion of the chorion by the main body follicle cells leaves hexagonal imprints, visible in the eggshell after the follicle cells slough off during oviposition.

process that occurs in the *Drosophila* embryo, this refinement might involve competition between downstream transcription factors. For example, GRK and DPP induce expression of Bunched and Mirror in a broad saddle overlying the oocyte nucleus. By the end of stage ten, their expression patterns resolve into discrete anterior and posterior domains [23–25]. These proteins in turn regulate expression of Notch-pathway components, which could facilitate the resolution of distinct domains [25–29]. Components of the WNT pathway also function downstream of EGFR and could contribute to the process of defining dorsal anterior cell types [30]. The exact mechanisms that achieve this process, the identity of all the molecules and the exact role that each has in patterning remain unclear. Nevertheless, the combination of GRK and DPP signaling produces distinct populations of cells (Figure 2) [31]. Knowledge of these signaling processes helps us to distinguish different classes of eggshell patterning and morphogenesis mutants.

Dorsal appendage formation

At the end of stage ten, ecdysone signaling from the germ cells [32,33] and DPP signaling from the stretched cells [18] trigger a series of carefully orchestrated events. The follicle cells closest to the nurse cell–oocyte boundary migrate centripetally, between nurse cells and oocyte [34]. At later stages, the centripetal cells secrete the operculum (a thin layer of chorion that acts as an escape hatch for the larva), the collar (a hinge on which the operculum swings), and the micropyle (a cone-shaped structure that allows sperm entry) [31].

At stage 11, the nurse cells rapidly transfer their contents into the oocyte, then begin a process of programmed cell death (stages 12–14) [4,35]. At the same time, the dorsal-anterior follicle cells begin their morphogenesis (Figure 1f) [7,36]. About 65–70 cells synthesize each dorsal appendage. Two subpopulations contribute to each tube: cells expressing the transcription factor Broad [19] constrict apically (apical is down, facing the oocyte), flexing the epithelium to form the roof and sides of each tube (Figure 3a,b,e). At the same time, *rhomboid* expression marks a hinge-shaped pattern of cells [37] just anterior and medial to the Broad-expressing cells; this subpopulation elongates and dives beneath the roof cells, fusing apically to make the floor (Figure 3c,d,f). At first, the nascent tube lies between the oocyte and nurse cells, resting on centripetal cells. During stages 12–13, the dorsal follicle cells relinquish contact with the centripetal cells and migrate out over the stretched cells that cover the nurse cells (Figures 1f and 3g–l) [7,36,38]. Beginning in stage 11, the dorsal follicle cells secrete chorion proteins into the tube lumens to create the two dorsolateral eggshell appendages (Figure 1g) [39].

By stage 13, the nurse cells are almost gone. The dorsal follicle cells stop moving but continue to secrete chorion proteins, thickening the appendages. Distal roof and floor cells shorten along their apical–basal axes, reversing their earlier elongation. These shape changes produce the characteristic flattened paddle of the *melanogaster* species (Figure 4a) [40]. At stage 14, the follicle cells begin to degenerate, then slough off during passage of the mature egg through the oviduct [41]. As the egg is laid, meiosis is completed and a single sperm fertilizes the egg by entering through the micropyle [1].

Dorsal appendage mutants

What are the molecular mechanisms that regulate shape generation during dorsal-appendage formation? Many genetic screens identified mutants with defects in this process [2]. Most investigators focused their efforts on female sterile mutants, biasing the recovery of mutations towards those that affect patterning. Indeed, >60 genes

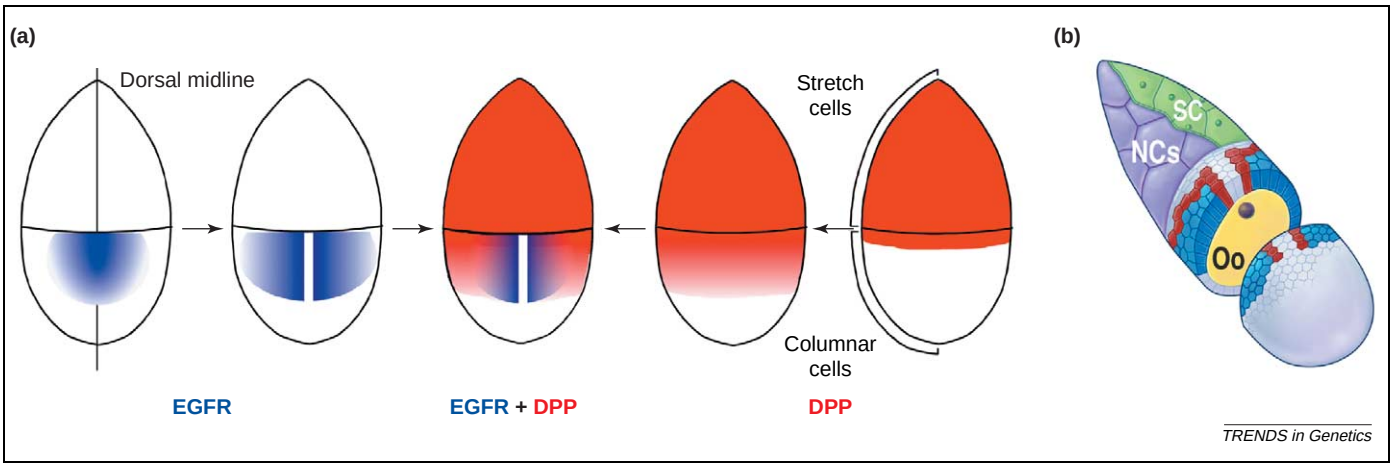

Figure 2. Patterning the dorsal appendage primordia. **(a)** In the left panels, blue indicates dorsal fates induced by EGFR signaling, which modify over time into two lateral domains. In the right panels, red indicates a gradient of BMP2,4 signaling from anterior to posterior. The central panel depicts the combination of signaling information that defines two dorsal-appendage primordia. **(b)** Through a mechanism that is not yet clear, the gradients of EGFR and DPP signaling are refined into a sharp boundary that creates two distinct subpopulations of cells within each appendage primordium. This three-dimensional schematic shows nurse cells (NCs) in light purple, the thin layer of stretched follicle cells (SCs) that surround the nurse cells (green, cut away to reveal nurse cells), oocyte (Oo, yellow), *rhomboid*-expressing cells (red), which form the floor of each tube, high-Broad-expressing cells (blue), which form the roof and sides of each tube, and centripetal and dorsal midline cells (white), which secrete the operculum. Figure 2b was reproduced with permission from Ref. [7].

are required for specifying the dorsal-appendage-forming cells (Table 1). Mutagenesis screens that analyzed eggshell structures *per se* identified mutants with defects either in patterning or in morphogenesis, in addition to a third class that disrupts amplification or transcription of the chorion genes [2,42]. The overall translucence of the main-body eggshell distinguishes these chorion mutants from those with defects in regulatory processes affecting patterning or morphogenesis of the dorsal appendages. Analysis of the eggshell phenotypes gives clues to the mechanisms that govern distinct aspects of these regulatory processes.

Table 1. Dorsal appendage patterning genes[a]

Ventralizing		Dorsalizing	Midline minus	Anterior	Oocyte differentiation
aubergine	*Merlin*	*capicua (fettuccini)*	*argos*	*bunched*	*Bicaudal-C*
Beadex	*mirror*	*cappuccino*	*brainiac*	*CTP:phosphocholine cytidylyltransferase 1 (Cct1)*	*Bicaudal-D*
blistered	*Misexpression suppressor of KSR 2 (MESK2)*	*Cbl*	*egghead*	*decapentaplegic*	*Cup*
cactus	*modifier of mdg4 (E-(var)3–93)*	*Chorion factor 2 (Cf2)*	*fringe*	*Mothers against dpp*	*CyclinE*
cAMP-dependent protein kinase 1 (Pka-C1)	*multiple ankyrin repeats single KH domain (mask)*	*female sterile (1) K10*	*Notch*	*Myocyte enhancing factor 2* (*Mef2*)	*Kelch*
cap-n-collar	*mutagen sensitive 301 (spindle-C)*	*GTPase-activating protein 1 (Gap1)*	*pointed*	*saxophone*	*Nup154*
COP9 complex homolog subunit 5 (CSN5)	*okra*	*Heterogeneous nuclear ribonucleoprotein at 27C (Hrb27C)*	*spitz*	*Signal-transducer and activator of transcription protein at 92E* (*Stat92E*)	*Shutdown*
corkscrew	*oo18 RNA-binding protein (orb)*	*kekkon-1*	*toucan*	*thickveins*	*stand still*
cornichon	*pole hole* (RAF)	*Lamin*	*vein*	*ultraspiracle*	
dodo	*Ras oncogene at 85D*	*ovarian tumor*			
Downstream of raf1 (MEK1)	*rhomboid*	*pipsqueak*			
DP transcription factor	*Sec61β*	*poly U binding factor 68D (half pint)*			
encore	*SHC-adaptor protein (Shc)*	*rhino*			
Epidermal growth factor receptor	*spindle-A*	*short gastrulation* (through *dpp*)			
gurken	*spindle-B*	*spire*			
gustavus	*spindle-D*	*sprouty*			
Kinesin heavy chain	*spindle-E (homeless)*	*squid*			
licorne	*Star*				
Lissencephaly-1	*tsunagi*				
maelstrom	*vasa*				
mago nashi					

[a]For information about these genes, the proteins they encode, their site of action, alleles, phenotypes and references, please see FlyBase at http://flybase.bio.indiana.edu. Only cloned genes with loss-of-function phenotypes are shown. For some genes, the embryonic phenotypes are not known.

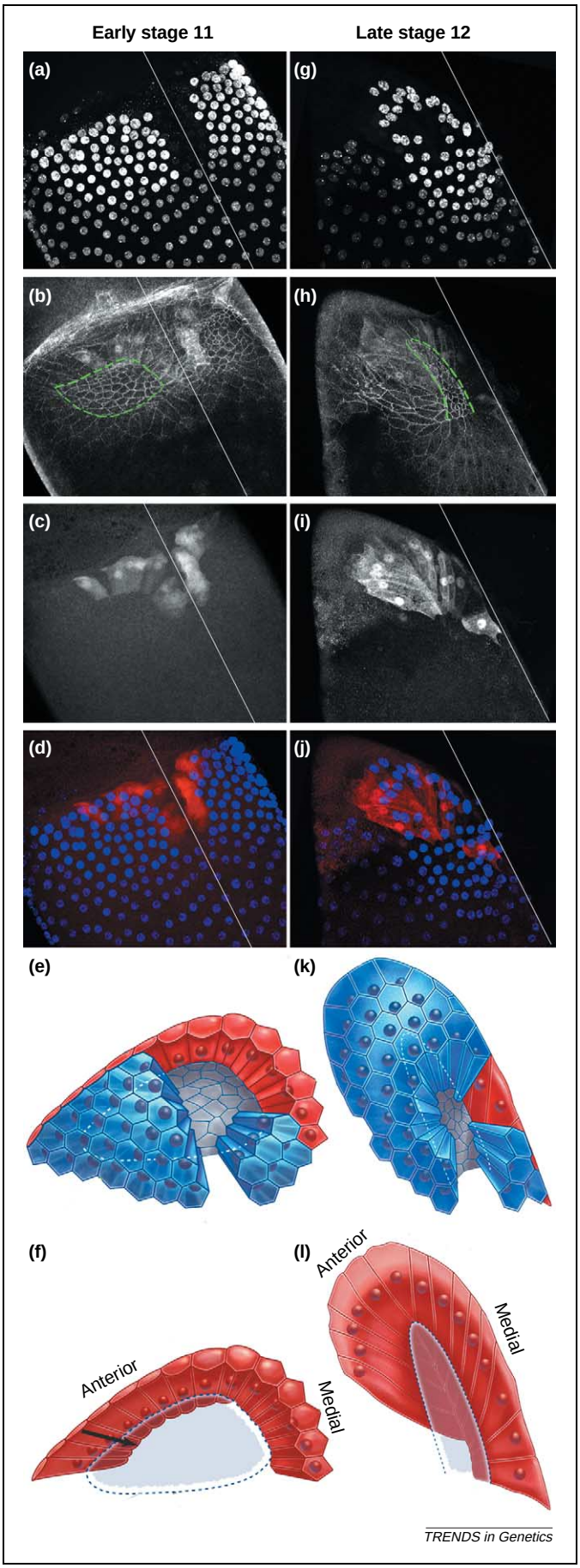

Figure 3. Dorsal appendage formation. In all panels, anterior is towards the top. In **(a–d)** and **(g–j)**, a white line marks the dorsal midline; in **e**, **f**, **k** and **l** the schematic drawings are presented in the same orientation as the confocal images shown above. In e–l, only the left primordium is shown. Roof-forming cells, represented in blue in the schematic drawings, express high levels of Broad (a,g). They constrict their apices (b,h, outlined in green), as visualized by antibodies against E-cadherin. Convergent extension brings lateral roof cells towards the midline (a→g, e→k). Floor-forming cells, represented in red in the schematic drawings, express *rho-lacZ* (c,i) and extend underneath the high-Broad-expressing cells (d,j,k,l). During anterior extension, the roof-cell population narrows and lengthens into a skinny triangle (g, green outline in h); the floor-forming *rho-lacZ*-cell population narrows and elongates (i,j). This figure was modified with permission from Ref. [7].

Patterning mutants

Dorsoventral (D–V) patterning mutations are of three general types, ventralizing (e.g. *gurken*), dorsalizing (e.g. *squid*) and midline-minus (e.g. *argos*) (Table 1; Figure 4b–d). All genes in these classes are required to establish the correct cell fate of the dorsal-appendage-forming follicle cells. Ventralizing and dorsalizing mutations also disrupt the establishment of the embryonic D–V axis, principally because they affect the production, localization, activity or reception of the signaling molecule Gurken, which is required for the establishment of D–V polarity in both eggshell and embryo [43]. Gurken synthesis is linked to other important processes in oogenesis such as egg chamber growth and successful meiotic recombination, revealing the key role of GRK in fly development [44–52].

Loss of the initial GRK–EGFR signal ventralizes the egg, producing elongated eggs lacking dorsal structures (Figure 4b). Null alleles produce spindle-shaped eggs with a micropyle (normally found only at the anterior) at both ends; this phenotype occurs due to disruption of GRK–EGFR signaling earlier in oogenesis, during the establishment of posterior follicle cell fates [53,54]. Hypomorphic alleles of *grk–Egfr* pathway members produce a single, thin, dorsal appendage residing on the dorsal midline; here, low levels of signaling are insufficient to specify the normal number of appendage-forming cells and to induce the inhibitory ligand whose activity divides the dorsal-appendage primordium in half. By contrast, a ring of dorsal appendage material on a shortened egg results when all the anterior follicle cells act like dorsal follicle cells and synthesize 'dorsal' appendages (Figure 4c). This phenotype results when *grk* mRNA is mislocalized in a ring at the anterior of the oocyte [14], or when a repressor of dorsal fate is lost [55–57]. The characteristic features that distinguish these patterning mutants include embryonic D–V patterning defects; elongated or shortened egg length; thin, absent, or ringlike appendage material; aberrant numbers of high-Broad-expressing or *rhomboid*-expressing cells; and, in many cases, mislocalized *grk* mRNA or protein.

Midline-minus mutations produce eggs of normal egg shape but with a single, very broad, dorsal appendage. The width of this structure is that of two separate dorsal appendages plus the midline space that normally lies between them (Figure 4d). This phenotype results from defects in genes required for modulating the initial GRK signal, genes such as *pointed* or *spitz* [15,17,58,59], or from certain allelic combinations of EGFR pathway genes

[60–64]. Germ-line loss of the neurogenic genes *brainiac* (*brn*) or *egghead* (*egh*) also produces a similar phenotype through interactions with *Egfr* and *Notch* in the follicle cells [65,66]. *brn* and *egh* encode biosynthetic enzymes that produce glycosphingolipids, which form lipid rafts in the *trans* Golgi and help sort membrane proteins to apical regions of the cell [67,68]. The authors speculate that Brainiac and Egghead modulate receptor function, affecting Notch-mediated germ cell–follicle cell adhesion and EGFR signaling dependent on this interaction [65,68]. More work is needed to elucidate the precise role of these genes in dorsal appendage formation, including a determination of the time at which these genes act. The distinguishing features of all these midline-minus mutants are the single, thick, dorsal appendage of normal length and the aberrant pattern of *rhomboid*- and high-Broad-expressing cells [36].

Mutations in *dpp*-pathway genes disrupt the patterning of all anterior columnar follicle cells, affecting dorsal appendage formation by altering the number and shifting the relative position of cells that can respond to GRK [18–22]. Loss of function results in eggs with small anterior faces and reduced appendage material, whereas overexpression of *dpp* using a heat-shock promoter produces eggs with enlarged opercula and antler-like dorsal appendages [18,23]. Overexpression can also generate four appendages, similar to those found on *Drosophila virilis* eggs [19,69]. The phenotypes vary for both loss and gain of function, in part because of the methods used to alter function, but also because DPP regulates several distinct processes that affect dorsal appendage morphology. First, DPP signaling determines a competency state in anterior follicle cells. For example, expression of an activated EGFR in all follicle cells alters cell fates only in the anterior, where DPP signaling occurs [20]. That DPP is the relevant molecule is shown by coactivation of both EGFR and DPP pathways in the posterior, which induces ectopic dorsal appendage formation [21]. DPP signaling also modulates boundary formation between populations of anterior follicle cells [24,31]. During these patterning processes, BMP2,4 signaling induces expression of Jun-kinase pathway genes that probably regulate cell-shape changes [22]. Furthermore, loss of function causes defects in nurse-cell cytoplasmic transfer, which can indirectly alter follicle cell functions by disrupting the coordination between germ layers [18] (see discussion below on *bwk*). Finally, parallel pathways involving the steroid hormone ecdysone [32,33] and the lipid phosphatidylcholine [70] feed into anterior patterning and contribute to the overall process, complicating analysis. These studies reveal a complexity to anterior patterning, and BMP2,4 signaling in particular, that will require further experimentation to unravel. Methods that disrupt tissue- and stage-specific functions, after cell division has ceased, are needed to distinguish the contributions of these various signaling molecules to patterning and morphogenesis. Nevertheless, these mutants are readily identifiable by their altered opercula and concomitant changes in anterior cell-fate markers [36].

Cup eggs result from defects in both anterior and dorsoventral patterning. These eggs exhibit open-ended anteriors, similar to the porcelain cups used to hold soft-boiled eggs (e.g. *cup*; Figure 4h). A single, teardrop-shaped dorsal appendage resides on the dorsal midline. Thus far, the best-characterized genes in this class, such as *Bicaudal-D, Bicaudal-C* and *cup*, actually regulate oocyte differentiation or the transport of molecules into the oocyte at early stages [71–74]. One hypothesis to explain the eggshell defects follows. The small size of the oocyte prevents the bulk of follicle cells from moving over the oocyte during the middle stages of oogenesis. Those cells competent to carry out centripetal migrations are too far

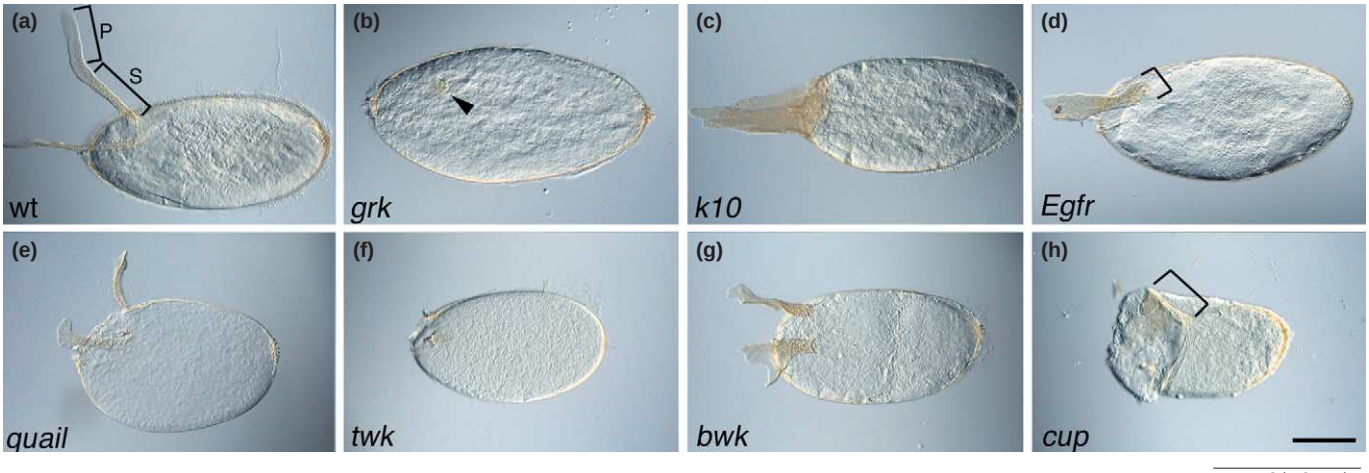

Figure 4. Patterning and morphogenetic mutants. Anterior is to the left, dorsal is up. **(a)** Wild type. Labels indicate the stalk (S) and paddle (P). **(b)** Ventralizing mutant *gurken*. Note elongated egg shape and nub of appendage (arrowhead) on dorsal midline. **(c)** Dorsalizing mutant *fs(1)K10*. The ring of appendage material is broken by a gap on the dorsal midline. **(d)** Midline-minus phenotype represented by an allelic combination of *Egfr* mutations. Bracket shows that the width of this single appendage encompasses the region normally downregulated by increased EGFR signaling. **(e)** The small egg and paddleless appendages of this *quail* (Villin) mutant are typical phenotypes due to loss of actin regulatory components. **(f)** An unusual allele of *tramtrack-69* creates the two nubs of this *twin peaks* mutant. Tubes form but fail to elongate. **(g)** Moose antler appendages give *bullwinkle* its name. Information from the germ line is needed to coordinate nurse cell dumping, stretched cell differentiation, and dorsal follicle cell behaviors. **(h)** In *cup* mutants, effects on anterior and dorsal patterning cause centripetal cell migration problems and generate a disorganized mass of dorsal appendage material (marked by a bracket).

anterior to migrate between nurse cells and oocyte, resulting in an open-ended eggshell. Follicle cells directly dorsal to the oocyte nucleus receive a GRK signal, but these cells are too far posterior relative to DPP signaling. A reduced population of cells attempts to synthesize an eggshell appendage, but the structure is abnormal, being blocked by the stationary centripetal cells. Thus, the open-ended eggshells exhibited by *cup* mutants probably result from patterning defects compounded by morphogenetic problems.

Morphogenesis mutants

Dorsal appendage formation involves the coordinated movement of roof and floor cells progressing through three distinct phases of morphological change. These cell-shape changes and rearrangements take place in the context of the follicular epithelium and its associated germ cells and basement membrane. Not only do the cells reorganize to form a tube, but the tube extends over the apoptosing nurse cells and then alters shape to create paddles. Thus, morphogenesis mutations affect the cell biological processes that regulate cell shape, cell–cell and cell–matrix adhesion, the reorganization of cells within an epithelial sheet, the movement of the sheet itself, or the signaling pathways that coordinate populations of cells (Table 2). Unlike patterning mutants, in which the number or spatial position of the dorsal appendages is altered, morphogenesis mutants produce two, properly positioned, dorsal appendages but with defective shape. With the exception of *bullwinkle*, discussed below, no one has examined the exact cellular defects that occur in mutants affecting the morphogenesis of the dorsal appendages. Nevertheless, the eggshell phenotypes, the known biological functions of the affected molecules and, in some cases, knowledge of the tissue requirement of the genes, suggest hypotheses for the roles of these genes.

The first phase of dorsal-appendage formation resembles other tubulogenesis processes that employ a wrapping mechanism [75], such as primary neural tube formation in vertebrates [76,77] and ventral furrow formation in *Drosophila* gastrulation [78]. The appendage tubes form parallel to the epithelial sheet by apical constriction of the roof-forming cells, which flexes the epithelium and causes it to curve out of the flat plane. During this time, lateral roof cells converge towards the midline, extending and narrowing each tube. Directed elongation and apical fusion of floor cells closes off the tubes [7,36]. Interestingly, null mutations in *Fasciclin 3*, which encodes a homophilic adhesion molecule [79], result in dorsal appendages with thick stalks, as if tube formation (e.g. floor-cell fusion) or lumen size were not faithfully regulated [36]. Although only this one mutant exists that specifically disrupts this phase of dorsal-appendage formation, analysis of patterning genes has shed light on this process. Mosaic analysis of Ras^{null} follicle cells demonstrates that cells lacking all D–V patterning information fail to perform the earliest steps of morphogenesis. These cells do not reorganize their actin cytoskeleton and do not constrict apically. They also misexpress the cell adhesion molecule E-cadherin. Eventually, wild-type neighbors reorganize and leave these mutant cells behind [63]. Gain-of-function experiments further illuminate this process. Ectopic expression of *rhomboid*, which encodes a protease that activates EGFR signaling [80,81], enlarges the dorsal appendage primordium when expressed in anterior follicle cells, presumably benefiting by endogenous DPP signaling. In the posterior, however, *rhomboid* expression produces only roof cells, which are unable to form a tube in the absence of floor cells [36]. Thus, both cell types are essential for making the tube.

The second phase of dorsal-appendage formation, anterior extension, involves remodeling cell shapes, relinquishing cell and matrix contacts to the posterior of the tube, and establishing new contacts towards the

Table 2. Dorsal appendage morphogenesis genes[a]

Actin dynamics or regulation	GPCR signaling	Adhesion	Tubulogenesis
Btk family kinase at 29A (*Tec29A, Src29A*)	*Dunce*	*Fasciclin 3*	*basket*
cheerio (Filamin)	*G protein-coupled receptor kinase 2* (*Gprk2*)	*inflated* (αPS2 integrin)	*broad*
chickadee (Profilin)	*locomotion defects* (*loco*)	*multiple edematous wings* (αPS1 integrin)	*bullwinkle*
dreadlocks		*myospheroid* (β integrin)	*Cdc42 (dominant negative)*
Ets at 97D		*shotgun* (E-cadherin)	*Ecdysone-induced protein 78C* (*Eip78C*)
female sterile (1) Yb (phenotype like *DLar*)			*hemipterous*
Leukocyte-antigen-related-like (*DLar*)			*Jun-related antigen* (JUN)
peanut			*kayak* (FOS)
Pendulin			*puckered*
Plenty of SH3s (*POSH*)			*Rac1* (*dominant negative*)
quail (Villin)			*shark*
quaking related 58E-3 (*kep1*)			*skittles*
singed (Fascin)			*smt3* (*SUMO*)
spaghetti-squash (myosin light chain)			*squeeze*
			Src homology 2, ankyrin repeat tyrosine kinase (*shark*)
			Src oncogene at 42A (*Src42A*)
			tramtrack-69 (*twin peaks*)

[a]For information about these genes, the proteins they encode, their site of action, alleles, phenotypes and references, please see FlyBase at http://flybase.bio.indiana.edu. Only cloned genes with loss-of-function phenotypes are shown. Some genes are listed as actin regulators based on work in other tissues.

anterior. Two classes of mutants exhibit defects in this process. First, mutations that affect the Jun-kinase (JNK) pathway, for example *basket* (JNK) and *kayak* (FOS), or the zinc-finger transcription factor Tramtrack-69 (*twin peaks*, Figure 4f), inhibit tube elongation. These mutations result in eggs with two distinct but short, sticklike, dorsal appendages that lack paddles [22,82–84]. Although Tramtrak-69 is expressed uniformly throughout the germ line and follicular layer, JUN and FOS are expressed at high levels in the floor-forming *rho-lacZ* cells and could regulate the elaborate shape changes that accompany anterior extension and paddle maturation. Indeed, given the function of JNK pathway genes in controlling other cell elongation processes such as those that mediate dorsal closure [85], clonal analysis with null alleles might reveal a role for these genes in the directed elongation of tube formation itself. Unfortunately, the precise cellular defects that occur in these mutants are not known; thus, these genes bear further investigation for their potential role in regulating all three phases of tube morphogenesis.

A second class of mutations that inhibit anterior extension are loss-of-function alleles affecting cell adhesion molecules, which disrupt overall egg shape and dorsal eggshell structures. Egg chambers with follicle cell clones lacking integrin subunits or signaling components are short and round with drumstick-like dorsal appendages [86,87]. Loss of these molecules disrupts the actin network that maintains elongated egg shape; presumably it also prevents attachment of roof and floor cells to the basal lamina or other matrix while crawling anteriorly over the stretched cells. Similarly, mutations in *shotgun*, which encodes *Drosophila* E-cadherin, disrupt two other cell migration processes in the ovary and might also affect dorsal appendage formation. Individual border cells lacking E-cadherin lag behind their wild-type neighbors, always forming the rearguard of the population. Loss of E-cadherin within the centripetal cell population produces cell-autonomous and nonautonomous effects, disrupting both localized and distant regions of the follicle cell ring that encloses the anterior face of the oocyte [88,89]. Loss-of-function clones specifically in dorsal anterior follicle cells are needed to clarify the exact role of all these molecules in dorsal appendage formation.

The last phase of dorsal appendage formation is paddle maturation, in which elongated cells flatten and become more cuboidal. The best characterized 'mutant' in this process is *Drosophila virilis*, which produces four elongated stalks that lack paddles. The appendage cells in this species form tubes normally and extend anteriorly but never regress and flatten out [69]. It is likely that paddle maturation in *D. melanogaster* involves regulated actin dynamics because hypomorphic mutations in five actin-regulatory genes produce small eggs with two appendages composed of elongated stalks and thin, ragged or absent paddles. Elegant genetic and biochemical studies reveal that these mutations disrupt actin-filament formation in the nurse cells, inhibiting the transfer of cytoplasm to the oocyte and thereby creating 'dumpless' eggs (e.g. *quail* Figure 4e) [90]. These same genes also probably control the structural elements that drive cell-shape changes throughout dorsal-appendage formation [91]. Functional analysis of *spaghetti-squash* (*sqh*), which encodes the regulatory light chain (RLC) of non-muscle myosin [92], supports this hypothesis by demonstrating temporally distinct requirements for this motor protein in nurse-cell dumping and dorsal-appendage formation [34].

Mutations in two other genes, *G protein-coupled receptor kinase 2* (*Gprk2*) and *locomotion defects* (*loco*), produce phenotypes similar to those of known actin regulatory mutants and implicate G-protein signaling in this process [93,94]. GPRK2 is thought to act by desensitizing serpentine receptors, enabling their continued response to stable or high levels of signaling molecules [93]. *loco* encodes a Regulator of G-protein Signaling (RGS) that enhances the intrinsic GTPase activity of Gα subunits, facilitating the transition to the GDP-bound state and thereby terminating trimeric G-protein signaling [95]. These proteins could act directly in follicle cells to alter the activity or distribution of molecules that govern actin dynamics. Alternatively, they might act globally to control long-range signals, such as cAMP [96], thereby coordinating the follicle cells and/or germ-cell layers. Interestingly, germ-line loss of *chickadee* (Profilin) or somatic disruption of *loco* (RGS) produces weakly ventralized eggs [64,94,97]. These phenotypes reveal additional roles in patterning, potentially by affecting the localization of the GRK ligand, its receptor or other signaling components.

Finally, *bullwinkle* (*bwk*) encodes a high mobility group (HMG) box transcription factor that functions in the germ line to regulate dorsal appendage formation [7,98] (C.A. Berg *et al.,* unpublished). Loss-of-function mutations result in eggs with enlarged opercula and short, broad, dorsal appendages, resembling moose antlers, hence the name (Figure 4g). *bwk* interacts genetically with two genes encoding non-receptor tyrosine kinases; loss-of-function mutations in *shark* (*Src homology 2, ankyrin repeat, tyrosine kinase*) and *Src42A* (*Src oncogene at 42A*) enhance the bullwinkle eggshell phenotype whereas overexpression of these genes specifically in the stretched follicle cells that surround the nurse cells suppresses the appendage defects [38]. These results suggest that BWK controls cell migrations during dorsal-appendage synthesis by regulating the expression of signaling molecules that affect the identity or function of the stretched cells. The stretched cells then produce guidance cues or cell-adhesion molecules that modulate the activities of the dorsal follicle cells during tube elongation and paddle maturation.

Concluding remarks

From these studies, several facts are clear. Dorsal appendage formation exhibits features similar to those of many other complex morphogenetic events. Multiple signaling molecules determine the fate of the dorsal follicle cells and regulate cell-shape changes and anterior migration. Much of the work carried out thus far has focused on the initial steps of the pathway, the patterning of the appendage primordia. These studies reveal elaborate combinatorial signaling and feedback mechanisms. Less is known about the morphogenetic program, although initial analyses demonstrate similarities to neural tube formation in vertebrates and ventral furrow formation in flies.

Many questions remain: What are the precise molecular mechanisms that regulate GRK synthesis and tie it to other key events in oogenesis? How are the *Egfr* and *dpp* signaling pathways integrated to create a sharp boundary between distinct cell types? What are the exact roles of Argos, Notch and WNTs in this process? Within each cell, what molecules regulate diverse cell-shape changes: apical constriction, directed elongation, apical fusion and cell shortening? What signals drive convergent extension, directing tube formation towards the dorsal midline? What are the guidance cues that elicit anterior extension? What are the species-specific regulators that induce the cell-shape changes of paddle maturation? And finally, what are the mechanisms that coordinate these events – between roof and floor cells, and with nurse cell apoptosis?

Dorsal appendage formation has provided a tremendous model for understanding epithelial patterning [31]. This system is beginning to yield information about the mechanisms that generate shape, including the discovery that two distinct cell types mediate tube formation [7,36]. Overall, dorsal appendage formation is an outstanding forum for investigating the link between patterning and morphogenesis.

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