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The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the *Drosophila* follicular epithelium

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Background: Regulation of actin structures is instrumental in maintaining proper cytoarchitecture in many tissues. In the follicular epithelium of *Drosophila* ovaries, a system of actin filaments is coordinated across the basal surface of cells encircling the oocyte. These filaments have been postulated to regulate oocyte elongation; however, the molecular components that control this cytoskeletal array are not yet understood.

Results: We find that the receptor tyrosine phosphatase (RPTP) Dlar and integrins are involved in organizing basal actin filaments in follicle cells. Mutations in *Dlar* and the common β-integrin subunit *mys* cause a failure in oocyte elongation, which is correlated with a loss of proper actin filament organization. Immunolocalization shows that early in oogenesis Dlar is polarized to membranes where filaments terminate but becomes generally distributed late in development, at which time β-integrin and Enabled specifically associate with actin filament terminals. Rescue experiments point to the early period of polar Dlar localization as critical for its function. Furthermore, clonal analysis shows that loss of *Dlar* or *mys* influences actin filament polarity in wild-type cells that surround mutant tissues, suggesting that communication between neighboring cells regulates cytoskeletal organization. Finally, we find that two integrin α subunits encoded by *mew* and *if* are required for proper oocyte elongation, implying that multiple components of the ECM are instructive in coordinating actin fiber polarity.

Conclusions: Dlar cooperates with integrins to coordinate actin filaments at the basal surface of the follicular epithelium. To our knowledge, this is the first direct demonstration of an RPTP's influence on the actin cytoskeleton.

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Background

Throughout development, cells are organized into a monolayer of identical units called an epithelium. All epithelia possess an intrinsic polarity; an apical surface faces "out" from the monolayer, while the basolateral face contacts neighboring cells and adheres to the substratum (reviewed in [1]). In adults, these sheets of interacting cells function as selective barriers between the body and the external world. However, during development, epithelia are often involved in determining the morphology of nascent tissues; for example, the vertebrate neural tube is created by an invagination of its neuroepithelial precursor cells [2]. Similarly, the wings of *Drosophila* adults are created by an epithelium that undergoes extensive morphological changes during pupation [3]. The influence of epithelia in coordinating the growth of nascent tissues is a common theme in organogenesis.

The integrity of an epithelium is maintained by several classes of cell-surface adhesion molecules, which are coupled to the cytoskeleton to regulate the cell's cytoarchitecture. For example, cadherin-rich adherens junctions facilitate cell-cell interactions through homophilic interactions (reviewed in [4]). Similarly, the attachment of the epithelium to its substrate is largely mediated by integrins, heterodimers of α and β subunits that bind components of the extracellular matrix (ECM) at focal adhesions (FAs) along basal surfaces [5]. In each of these cases, various intracellular components are associated with the adhesion molecules as part of large complexes and act as physical links to tether actin filaments to the cell surface and establish structural integrity (reviewed in [6]). Thus, extracellular engagement of adhesion molecules coordinates the underlying actin cytoskeleton through multiple protein-protein interactions.

The ability of an epithelium to alter its morphology during development requires that the function of adhesion molecules be modulated, frequently by phosphorylation of junctional components [7, 8]. It is therefore common to

find kinases and phosphatases as part of adhesion complexes, both at adherens junctions and at FAs. For example, it has been demonstrated that PTPα regulates integrin adhesivity by dephosphorylating and activating Src family tyrosine kinases, which subsequently phosphorylate other junctional components required for adhesion [9, 10]. Our understanding of cytoskeletal remodeling by adhesion molecules requires the identification of regulatory molecules that control interactions within the adhesion complex.

The follicular epithelium surrounding developing germ tissue in *Drosophila* females is a well-studied example of an epithelium required for tissue morphogenesis [1]. Follicle cells are necessary for creating specialized structural components of the egg, such as the dorsal respiratory appendages and the micropyle required for sperm entry, and also participate in signaling events that help to pattern the oocyte [11]. The follicular epithelium has been useful in identifying genes required for the maintenance of epithelial cytostructure. For example, mutations in the β-catenin homolog Armadillo (Arm) disrupt follicle cell structure, consistent with a loss of cytoskeletal organization and adhesion [1]. Furthermore, mosaic screening has identified many loci whose function is required for organization of the follicular epithelium and its underlying cytoskeleton (e.g., [12–14]).

Characterization of the actin cytoskeleton within follicle cells has previously revealed a dense system of actin filaments at the basal surface, highly reminiscent of actin stress fibers observed in cultured cells [15]. Interestingly, the filaments in middle-stage follicle cells are invariably perpendicular to the anterior-posterior (A-P) axis of the oocyte and envelop the germ layer in an interconnected ring of filamentous (F-) actin [15–17]. This actin array is postulated to aid in oocyte elongation by acting as a "molecular corset" to restrict growth in the short axis. Supporting this model, analysis of the mutant *kugel*, which frequently fails to elongate its oocytes, shows a significant disruption in the organization of basal F-actin [18]. However, the molecular mechanisms by which these actin fibers are organized are poorly understood.

In this study, we show that the RPTP Dlar is involved in coordinating the polarity of basal F-actin in the *Drosophila* follicular epithelium. Dlar is known to have a putative role regulating the actin cytoskeleton in the developing embryonic nervous system [19–22]. In *Dlar* mutant oocytes, follicle cell basal actin filaments lose their normal orientation; this loss is associated with a failure to elongate the developing oocyte. Immunolocalization of Dlar shows that it is specifically associated with membranes in which actin filaments terminate early in development. We further show that actin filament terminals localize with β-integrin throughout the middle to late stages of oogenesis and that integrin function is required for proper actin filament polarity. Finally, genetic interactions between Dlar and the β-integrin subunit encoded by *myospheroid* (*mys*) suggest a cooperation between the two molecules in realizing actin filament polarity.

Results

Mutations in Dlar alter oocyte shape

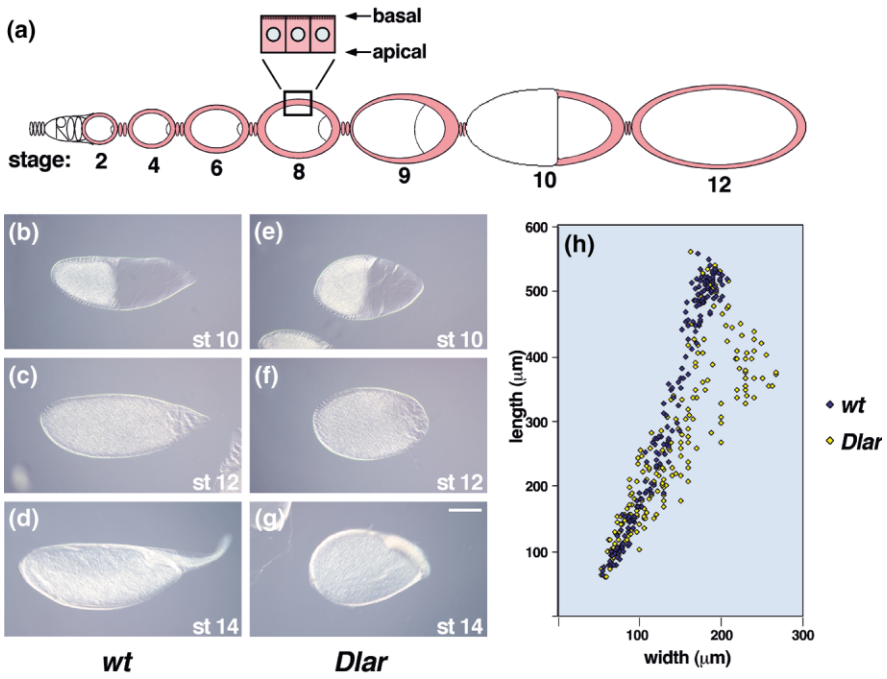
To gain further insight into RPTP function, we sought to identify phenotypes caused by loss of *Dlar* in nonneuronal *Drosophila* tissues. During our analysis, we discovered an egg-shaped defect among late-stage oocytes in dissected mutant ovaries (Figure 1). In wild-type ovaries, oocytes begin as small and relatively spherical bodies and grow more elongated through their later stages (Figure 1a–d,h). In *Dlar* mutants, some oocytes fail to elongate properly, such that late-stage oocytes appear rounded (Figure 1e–h). This phenotype is moderate in penetrance (14.1% defective stage 14 oocytes, n = 297), similar to the mild effect caused by loss of *Dlar* in the embryonic nervous system [19–22]. We find no defects in major aspects of oocyte patterning in *Dlar* mutants; both the micropyle and the dorsal appendages are formed in their correct positions, although the latter are often shortened relative to those of the wild-type (Figure 1d,g). Similarly, the oocyte nucleus is correctly positioned in the dorsal-anterior compartment in rounded oocytes (data not shown). Thus, mutations in *Dlar* disrupt normal oocyte shape determination without altering the gross polarity of the oocyte.

Dlar mediates the organization of follicle cell actin filaments

Previous studies suggested that insect oocyte elongation is mediated by the follicular epithelium [15, 16, 23, 24]. In particular, mutations in the "round egg" gene *kugel* disrupt the polarity of follicular basal F-actin [18], which is postulated to restrict oocyte growth in the short axis. We therefore examined follicle cell actin structures in *Dlar* mutant egg chambers.

Wild-type and *Dlar* mutant oocytes stained with Texas Red-phalloidin display actin bundles at the basal surface of the follicular epithelium throughout the vitellogenic stages of development (stages 8–13). In wild-type stage 8 oocytes, bundles are strictly perpendicular to the A-P axis (Figure 2b). This wild-type pattern is also observed in many *Dlar* mutant oocytes at this stage, consistent with the partial penetrance of the elongation defect. However, in some *Dlar* mutant egg chambers, actin bundles are poorly organized; the strict polarization perpendicular to the A-P axis is lost, although the actin filaments that do form appear polarized within a given cell (Figure 2c). Additionally, cell shapes are generally less regular than the wild-type array, with F-actin accumulating abnormally at the boundaries of some cells (Figure 2c). Thus, loss of

Figure 1



Mutations in *Dlar* alter oocyte morphology. **(a)** Schematic of oocyte development shows the follicular epithelium (pink) surrounding the germ tissue. Staging is according to [11]. **(b–d,h)** In wild-type ovaries, oocytes elongate as they develop, while **(e–h)** in $Dlar^{5.5}/Dlar^{13.2}$ mutants, some oocytes fail to elongate significantly. The scale bar represents 100 μm for (b–g). (h) Distribution plot of individual oocyte dimensions through oogenesis (roughly stage 6 through stage 14) in wild-type (blue) and $Dlar^{5.5}/Dlar^{13.2}$ (yellow) ovaries. This distribution does not reflect the actual penetrance of mutant oocytes but rather presents the overall developmental progress of normal and mutant egg chambers.

Dlar causes a distinct disruption of the actin cytoskeleton in follicle cells.

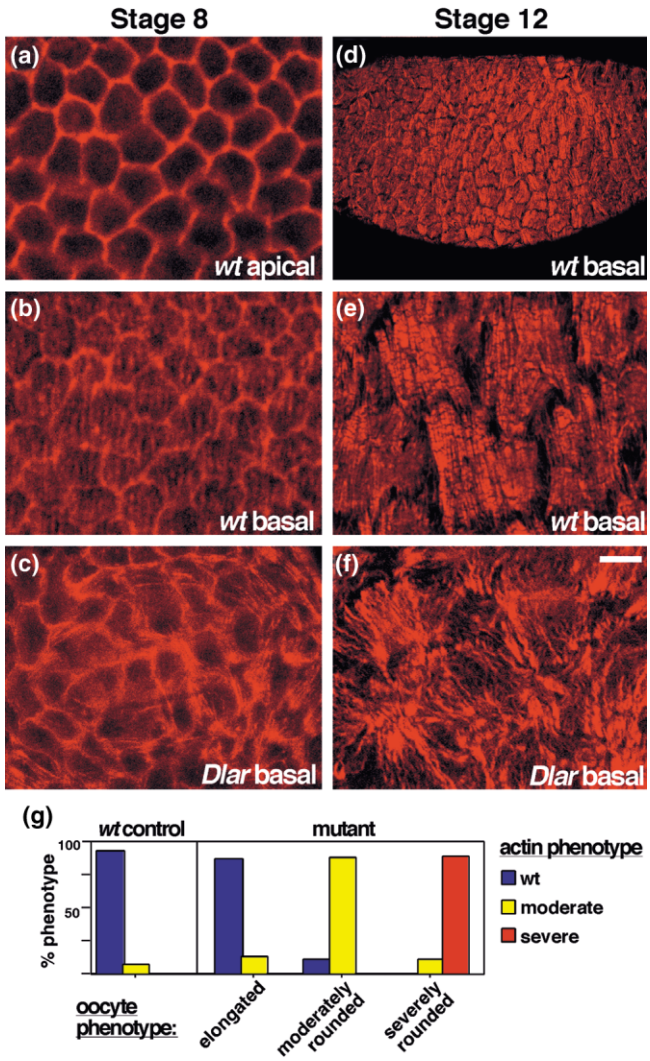
At early stages, it is difficult to correlate cytoskeletal defects with oocyte elongation phenotypes due to the relatively spherical shape of wild-type oocytes at this time in development. However, polarized basal actin arrays are also seen at late stages (stage 12), when disruptions are observed in *Dlar* mutants (Figure 2d–f), and oocyte elongation can be assessed. At this stage, we find a strong correlation between defects in actin polarity and a failure to elongate the oocyte in various genetic backgrounds (Figure 2g; see below). Mutant oocytes that elongate properly display a wild-type pattern of basal actin filaments, while oocytes that fail to elongate show disruptions in the organization of these fibers. We conclude that Dlar functions in either establishing or maintaining the polarity of actin filaments in the follicular epithelium and thereby influences oocyte morphology.

We next asked whether Dlar functions within follicle cells to affect actin fiber polarity. First, we determined the wild-type pattern of Dlar expression by using an anti-Dlar antibody. Consistent with Dlar's role in actin filament organization, staining of stage 7–8 oocytes shows that Dlar is specifically concentrated at basal follicle cell contacts, with most of the protein localized in a polarized manner at membranes where actin filaments terminate (Figure 3a–c). At medial planes of focus, Dlar is concentrated where the borders of three follicle cells meet (Figure 3e). This site is also rich in F-actin (Figure 3d) and may represent a specialized region for cytoskeletal assembly.

At later stages, follicle cells begin to migrate over the germ tissue [11], which is associated with a loss of polarized Dlar localization. Instead, oocytes at stage 10 and beyond show an even distribution of Dlar staining around the borders of cells at all planes of focus (Figure 3g–i). We observe no detectable staining at any stage in *Dlar* null oocytes (either $Dlar^{5.5}$ or $Dlar^{13.2}$), indicating that the antibody is specific. Additionally, we do not observe Dlar staining in germ tissues, implying a function specific to follicle cells.

To confirm that Dlar acts in the follicular epithelium, we examined mutant oocytes in which a wild-type *Dlar* transgene was expressed specifically in follicle cells. We made use of the GAL4 driver T155-GAL4, whose expression is detected in all follicle cells beginning at stage 7–8. While mutant ovaries lacking a driver showed a 16.0% (n = 192) round-egg phenotype (stage 14), we observed no defects (n = 236) among *Dlar* mutant oocytes expressing the transgene ($Dlar^{5.5}/Dlar^{13.2}$;*UAS-Dlar/T155-GAL4*) (Figure 3j). In contrast, we do not see significant rescue with the GAL4 driver 198Y-GAL4, which expresses in all follicle cells at stage 10 and beyond (13.0% round stage 14 oocytes, n = 276). These data were confirmed by Texas Red-phalloidin staining of oocytes from both driver backgrounds, which showed rescue of cytoskeletal defects by T155-GAL4 but not by 198Y-GAL4 (see Materials and methods). Although we cannot entirely rule out an

Figure 2



Mutations in *Dlar* disrupt actin filament organization in follicle cells. In this and all subsequent figures, the anterior-posterior axis is oriented left to right. Panels **(a)** and **(b)** show two optical sections of the same stage 8 follicle cells stained with Texas Red-phalloidin. Panel (a) shows a plane near the apical face, where the majority of F-actin is localized to the cortical region of the cell. Panel (b) is focused on the basal surface of the cells, where bundles of actin are perpendicular to the A-P axis. These bundles are more pronounced at later stages of development. **(d,e)** Low- and high-magnification lateral vews of a wild-type stage 12 oocyte. **(c,f)** In $Dlar^{5.5}/Dlar^{13.2}$ mutants, basal actin bundles are still apparent, but their orientation is disrupted, accompanied by the overall rounding of the oocyte at late stages. The scale bar represents 5 μm for (a–c,e,f), and 40 μm for (d). **(g)** Disruptions in basal actin filaments correlate with defects in oocyte elongation at stage 12. Oocytes were scored as either elongated (wild-type morphology), moderately rounded, or severely rounded, and corresponding defects in cytoskeletal organization were scored as wild-type, moderate, or severe (see Materials and methods). Data represent a pool of 67 mutant oocytes lacking either Dlar (n = 32) or integrin (n = 35). Twenty-eight wild-type oocytes were scored as controls.

earlier role for Dlar in oogenesis, both the period of polarized Dlar localization and the temporal specificity of rescue are consistent with a requirement for Dlar in stage 7–8 follicle cells.

Dlar acts nonautonomously to influence actin structure in follicle cells

To further explore Dlar function in organizing actin, we generated *Dlar* mutant clones in follicle cells by using the FLP recombinase and analyzed their effects on basal actin filament polarity [12]. As expected from the incomplete penetrance of *Dlar* nulls, many individual clones show no obvious defects in cytoskeletal organization. However, in cases where disruptions in F-actin polarity are evident, defects are always observed in both mutant cells and in wild-type cells surrounding the clone (Figure 4), indicating that the influence on the actin cytoskeleton is nonautonomous in *Dlar* mutants. Although the global organization of actin polarity is lost around these clones, actin filaments tend to be similarly polarized in adjacent cells, implying that neighboring cells influence one another's cytoskeletal organization (Figure 4c). Similar disruptions were observed in and around mutant clones in late-stage oocytes, where they correlated with a failure to elongate the oocyte, as observed in homozygous females (Figure 6g–i).

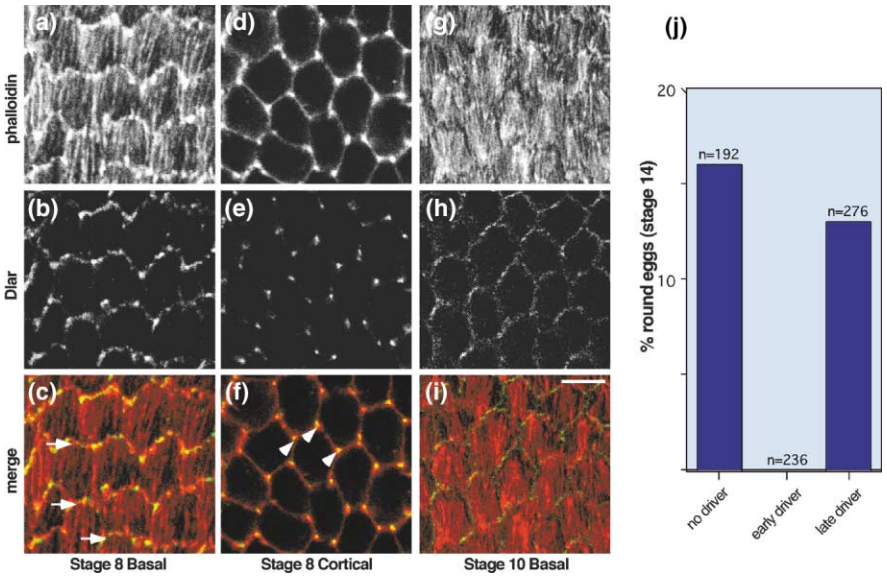
In addition to nonautonomous influences on the cytoskeleton, we also observed defects in the normal pattern of Dlar localization in wild-type cells surrounding mutant clones; rather than showing the polarized localization observed at the basal surface of wild-type stage 8 oocytes, Dlar became evenly distributed around the borders of many wild-type cells surrounding mutant tissue (Figure 4b). A failure to properly localize Dlar was occasionally observed around clones with no obvious effect on actin filaments (data not shown), implying that Dlar localization is not a strict determinant of actin filament polarity. Rather, it is likely that Dlar plays a regulatory role in ensuring the fidelity of basal F-actin polarity in early follicle cells.

Integrins associate with basal actin filaments

Our finding that Dlar influences but does not dictate basal F-actin organization led us to seek other components that may interact with actin structures. Actin filaments in *Drosophila* follicle cells are highly reminiscent of stress fibers, which are bundles of F-actin observed at the basal surface of many cultured cells. Stress fiber formation is dependent upon the activation of integrins, transmembrane proteins that link the actin cytoskeleton to the outlying ECM through the focal adhesion complex [5, 25]. To assess whether the actin bundles of follicle cells are related to stress fibers, we asked whether *Drosophila* integrins play a role in their formation and maintenance.

Wild-type oocytes stained with anti-β-integrin show intense labeling at the basal surface throughout develop-

Figure 3



Dlar is localized to cell-cell contacts. Developing oocytes were stained with **(a,d,g)** Texas Red-phalloidin and **(b,e,h)** anti-Dlar; merged channels are shown in **(c,f,i)** with actin in red and Dlar in green. (a–c) At the basal surface of stage 8 oocytes, Dlar protein is primarily localized to follicle cell membranes where actin bundles terminate (arrows). (d–f) At deeper planes of focus where actin is restricted to cortical regions, the majority of Dlar protein is observed at cell-cell contacts where three follicle cells meet (i.e., the "corners" of the cells) (arrowheads). (g–i) As follicle cells migrate toward the posterior through stage 9, the polarity of Dlar protein is lost; at stages 10–12, Dlar protein is evenly distributed around the cell cortex, as shown in cells above the oocyte-nurse cell boundary. The scale bar represents approximately 5 μm. **(j)** Defective egg morphology is rescued by wild-type Dlar expression in early follicle cells. Corresponding genotypes are *Dlar*$^{5.5}$/*Dlar*$^{13.2}$*;UAS-Dlar* (no driver), *Dlar*$^{5.5}$/*Dlar*$^{13.2}$*;UAS-Dlar/T155-GAL4* (early driver), *Dlar*$^{5.5}$/*Dlar*$^{13.2}$*;UAS-Dlar/198Y-GAL4* (late driver).

ment (Figure 5a), consistent with a strong association with basal actin structures and the underlying laminin-rich ECM (Figure 5b). Higher magnification views of stage 7–8 follicles show that β-integrin staining is somewhat diffuse at the basal surface, with significant staining over cell-cell contacts and where actin filaments terminate (Figure 5c–e). Often, integrin staining is observed along an individual actin filament, reflecting a close association with the actin cytoskeleton (Figure 5e). A similar pattern of staining is observed for the ECM component laminin, a ligand for PS1 integrin heterodimers, as previously described [15] (Figure 5f–h). As with Dlar, integrin staining is intense at cell-cell contacts where 3 cells meet at this early stage (Figure 5e). However, unlike the pattern observed for Dlar, β-integrin remains localized to the terminals of actin bundles until late stages of oogenesis, primarily highlighting cell membranes parallel to the A-P axis (Figure 6a–c). This continued association of β-integrin with actin bundles throughout development is consistent with a role in actin filament formation and maintenance, as observed between stress fibers and FAs in cultured cells.

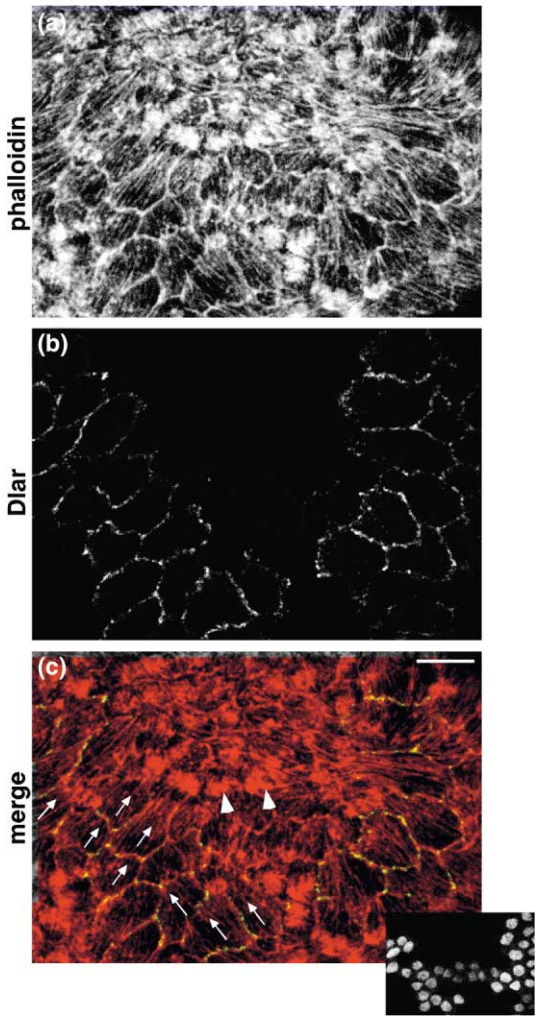
The coupling of integrins to the cytoskeleton is mediated by a group of intracellular proteins that bind the cytoplasmic tails of receptors and interact with actin filaments. For example, members of the Vasodilator-stimulated phosphoprotein (VASP) family localize to FAs, where they are thought to regulate actin assembly [26]. To explore the relationship between stress fibers and basal actin filaments of follicle cells, we stained oocytes with antibodies to the *Drosophila* VASP homolog Enabled (Ena). At early stages (stage 7–8), Ena was relatively diffuse throughout the cytoplasm, with little obvious concentration at cell membranes (data not shown). However, at stages 10–12, we observed significant Ena staining at actin filament terminals, coinciding with the period of specific β-integrin association with filaments (Figure 6d–f). Staining was also observed along actin filaments that traverse the cell, consistent with the VASP family's proposed role in actin regulation ([27]; Figure 6f). The association of actin bundles with multiple FA markers supports our hypothesis that the basal cytoskeleton of *Drosophila* follicle cells is analogous to stress fibers observed in vitro.

Due to the association between actin filaments and β-integrin in late-stage oocytes, we asked if integrin staining was affected in cells within and surrounding *Dlar* mutant clones. Indeed, cells lacking *Dlar* show improper localization of integrin associated with a loss of F-actin polarity (Figure 6g–i). β-integrin staining remains highest at the filament terminals of mutant cells regardless of their orientation, resulting in a loss of staining at membranes parallel to the A-P axis. In some cases, β-integrin staining shows no restriction to opposite sides of a cell as it does in the wild-type but instead appears generally distributed around the cell border (Figure 6h–i).

Integrins are required for proper oocyte elongation

Because strong defects in β-integrin localization are only observed in cells with actin defects, it is unclear whether these errors reflect a direct effect of Dlar on integrins

Figure 4



Dlar acts nonautonomously to influence actin filament organization. Shown is a *Dlar*$^{5.5}$ clone in a stage 8 oocyte stained with **(a)** Texas Red-phalloidin and **(b)** anti-Dlar. Panel **(c)** merges actin in red and Dlar in green; inset (one-third size) shows GFP marking wild-type cells. In this example, actin in the mutant region forms unpolarized bundles and amorphous aggregates (arrowheads), while filaments in wild-type cells surrounding the clone also lose their normal polarity (orientation within subsets of wild-type cells indicated with arrows in [c]). In (b), only wild-type cells stain with anti-Dlar, where the normally polarized distribution observed in wild-type oocytes is lost. Scale bar represents approximately 5 μm.

or whether integrin localization is dependent upon prior orientation of F-actin by Dlar. To address this issue, we asked whether integrins determine the polarity of basal actin filaments, which would suggest an upstream instructive role rather than a downstream dependence. Strong integrin mutants do not survive to adulthood, so we created clones of mutant tissue. We were aided by Duffy and colleagues [12], whose screen for genes required in follicular development identified a mutation (*968*) with a round-egg phenotype indistinguishable from *Dlar* mutants; mapping data placed the insertion near the *myospheroid* (*mys*) locus encoding β-integrin. We confirmed that *968* is allelic to *mys* by its failure to complement the allele *mys*^{nj42} and by loss of β-integrin staining within *968* clones (data not shown). As observed with *Dlar*, Texas Red-phalloidin staining of oocytes carrying *mys*^{968} clones shows disruptions in basal actin fiber polarity within mutant cells and in wild-type cells beyond the clonal boundary (Figure 7e–f). Follicle cell clones of the independent allele *mys*^{10} also result in round eggs (Figure 7b), confirming that integrins are required in follicle cells for elongation of the oocyte.

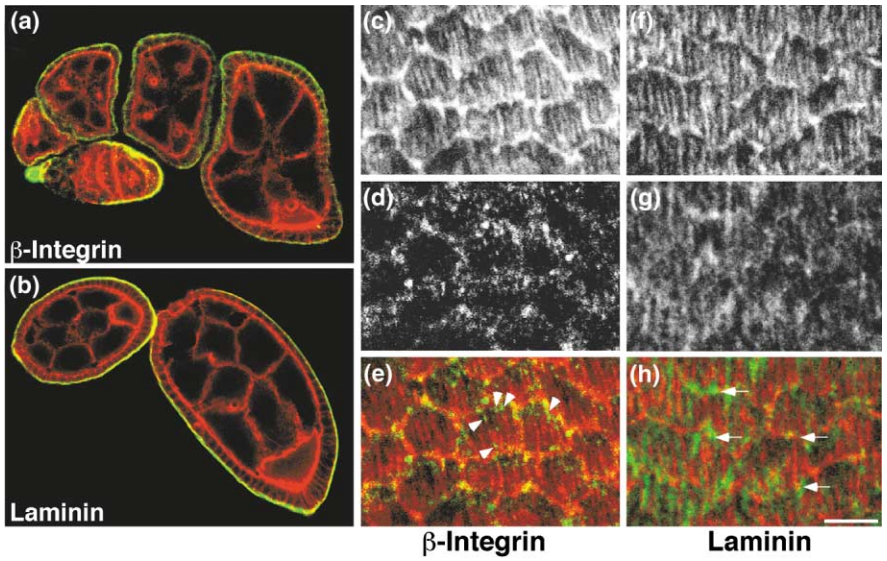
Drosophila integrins function as heterodimers of a common β subunit (*mys*) with a series of α subunits, creating functional receptors with different binding properties. For example, the α integrin encoded by *multiple edematous wings* (*mew*) is a receptor for laminin when combined with β-integrin, while the α-integrin encoded by *inflated* (*if*) confers specificity to ECM components carrying the amino acid motif RGD [28–31]. To determine if these ligands are relevant to integrin function in follicle cells, we created clones of *if* and *mew*. We observed rounded eggs in oocytes with clones of either subunit (Figure 7c–d), implying that both laminin and RGD components of the ECM are involved in mediating follicle cell control of oocyte shape.

The similarity in phenotypes caused by loss of either Dlar or integrins suggests a cooperative relationship in organizing F-actin. To explore this, we examined whether a genetic relationship exists between *Dlar* and *mys*. We reasoned that the mild penetrance of round eggs in *Dlar* mutants may be sensitive to the dosage of genes in the same pathway. Indeed, removal of half of the gene dosage of *mys* in a *Dlar* null background caused a substantial increase in the penetrance of the round egg phenotype (Figure 7g). For example, *Dlar*$^{5.5}$/*Dlar*$^{13.2}$ females show defective rounding in 14.1% (n = 297) of their stage 14 oocytes, while in *mys*^{1}/+;*Dlar*$^{5.5}$/*Dlar*$^{13.2}$ females, the penetrance is increased nearly 4-fold to 48.7% (n = 265). This enhancement is not due to changes in the overall fitness of mutant flies since *Dlar* mutants carrying the balancer TM6B, which decreases the viability of *Dlar* escapers, show little change in mutant oocyte penetrance (22.0% round stage 14 oocytes, n = 122). Thus, genetic interactions support a model wherein Dlar and integrins cooperate in organizing basal actin filaments.

Discussion

Drosophila oogenesis provides a useful system for the study of relationships between cytoskeletal organization and cell-cell interactions. Previous analyses described a system of coordinated actin fibers at the basal surface of follicle cells, but the molecular mechanisms that organize these filaments are largely unknown. Here, we provide

Figure 5



β-integrin is tightly associated with actin filaments and the extracellular matrix in developing oocytes. **(a,b)** low-magnification crosssections of early stage oocytes stained with (a) Texas Red-phalloidin (red) and anti-β integrin or (b) anti-laminin α chain. Both proteins are concentrated immediately above the basal surface of the follicular epithelium. High-magnification views of stage 8 cells show **(c–e)** β integrin and **(f–h)** Laminin in the same optical section as basal actin bundles. Although more diffuse than Dlar, β-integrin is most abundant around cell-cell contacts, and is often associated with individual filaments (arrowheads in [e]). Laminin is more generally spread over the basal surface, but sometimes accumulates over follicle cell membranes (arrows in [h]). Scale bar represents approximately 25 μm for (a–b) and 5 μm for (c–h).

evidence that the RPTP Dlar functions with integrins to control F-actin polarity in the follicular epithelium.

Dlar organizes actin fibers

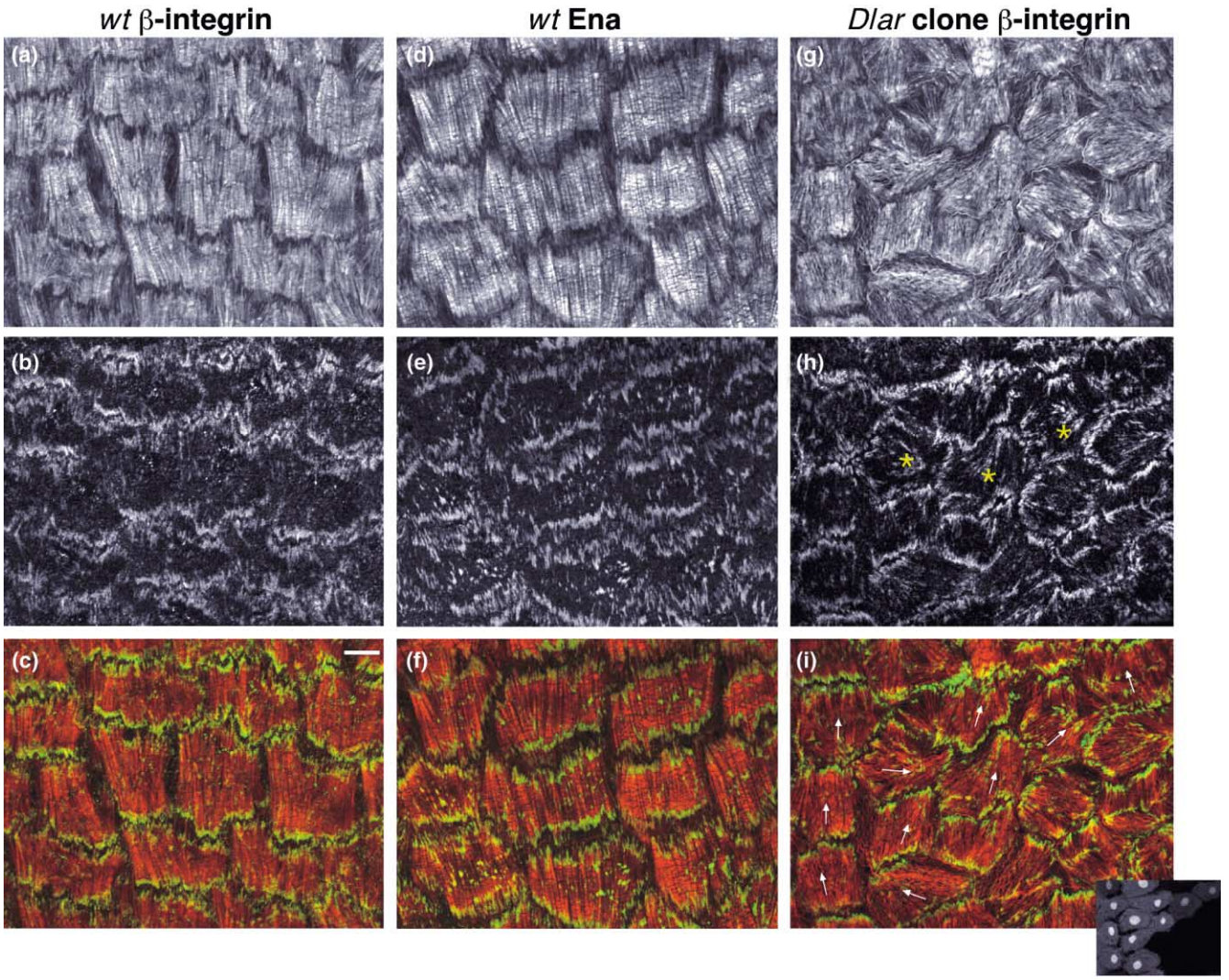
Mutations in *Dlar* and integrin subunits disrupt the normal pattern of basal actin filament organization observed at stage 8 and stage 12. In wild-type oocytes, these filaments are perpendicular to the A-P axis, while in mutants this global organization is lost. In early vitellogenic follicle cells, *Dlar* preferentially localizes to actin filament terminals, and this localization overlaps the distribution of β-integrin at the basal surface. As development continues, this preferential localization of *Dlar* is lost, while β-integrin becomes further restricted to actin terminals. This suggests a model wherein *Dlar* serves a modulatory role in organizing actin filament interactions with FAs during early stages of oocyte growth. Our rescue data support this early function for *Dlar* because expression of a wild-type *Dlar* transgene in stage 7–8 follicle cells, but not in cells at stage 10 and beyond, rescues the round oocyte phenotype associated with loss of strict actin filament polarity.

Analysis of human LAR in cultured cells has shown that it is preferentially localized to portions of FAs undergoing disassembly, suggesting a role in modulating the adhesive function of integrins [32]. Consistent with this, studies of Rho-dependent stress fiber formation suggest that PTPs function upstream of Rho in integrin signaling [33]; indeed, follicle cell clones of hypomorphic RhoA mutations cause a failure in oocyte elongation as observed in *Dlar* and integrin mutants (J.B. and D.V.V., unpublished data). Furthermore, LAR associates with an activator for Rho called Trio, which has been functionally implicated in RPTP signaling in both the *Drosophila* embryo and in the chick retina [22, 34, 35].

Previous analyses have shown that Dlar cooperates with related RPTPs to regulate axon outgrowth and guidance during development of the embryonic nervous system [19, 36]. Since other PTPs are also expressed during oogenesis [37], it is possible that the low penetrance of defects observed in *Dlar* mutant oocytes again reflects an overlapping function with other RPTPs. Several observations have implied that Dlar and other RPTPs signal to the actin cytoskeleton to regulate neural development. For example, neural phenotypes caused by the loss of *Dlar* are copied by direct perturbation of actin with low doses of cytochalasin D and by the loss of molecules that have been directly tied to actin regulation, including the Rho family GTPase Drac1 and the actin regulator Ena [20, 21]. The loss of basal actin filament polarity in *Dlar* mutants clearly demonstrates that the RPTP influences actin organization. However, the molecular mechanisms by which this occurs are not yet clear. It is unlikely that Dlar is directly responsible for nucleating actin since filaments are still formed in cells lacking *Dlar*. Rather, Dlar might bias the localization or activity of other factors that directly influence actin such as Ena, which binds the cytoplasmic domain of Dlar [21]. Ena is expressed in follicle cells throughout oogenesis, and the protein becomes restricted to actin filament terminals in late-stage oocytes, as observed with β-integrin.

The laminin-rich ECM underlying the follicular epithelium is intimately associated with basal F-actin and may aid in signaling to the actin cytoskeleton [16, 18]. Binding

Figure 6



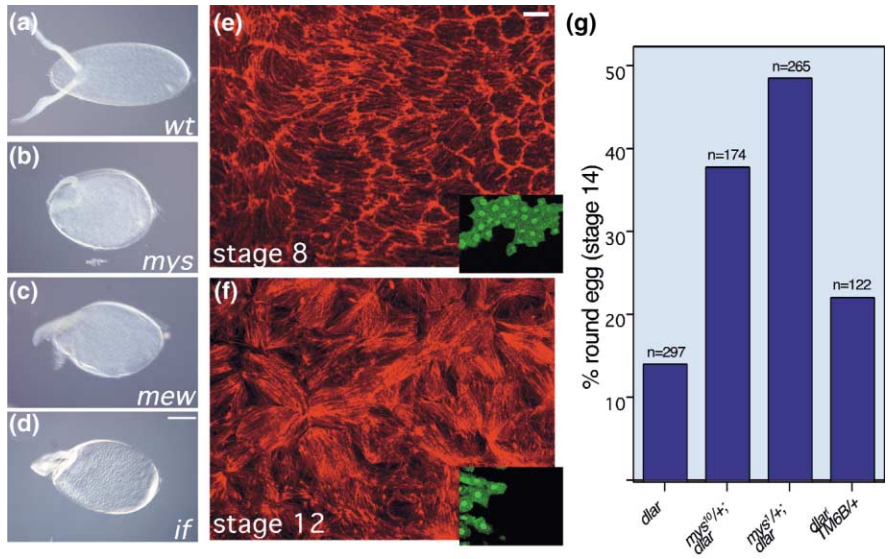
β-integrin and Ena associate with actin filaments through late stages of oogenesis. Stage 12 egg chambers were stained with **(a,d,g)** Texas Red-phalloidin and **(b,h)** anti-β-integrin or **(e)** anti-Ena; merged channels in **(c,f,i)** show actin in red and β-integrin or Ena in green; the inset in (f) shows GFP marking wild-type cells at one-third scale. (a–f) In wild-type oocytes, β-integrin and Ena are concentrated at the terminals of actin filaments and highlight cell boundaries parallel to the A-P axis. (e,f) Additional staining is observed on filaments within the cell, particularly for Ena. (g–i) In *Dlar* mutant clones, β-integrin staining is highest at filament terminals in most cells, including those in which filaments are misoriented. However, some mutant cells show little specificity in β-integrin localization (asterisked cells in [h]), with staining spread around the perimeter and occasionally within the cell. As in early oocytes, defects in filament orientation are observed in wild-type cells surrounding a mutant clone (mean orientation of filaments in wild-type cells is indicated with arrows). The scale bar represents approximately 5 μm for (a–f) and 8 μm for (g–i).

studies using human LAR have shown that laminin can serve as part of a ligand complex for the RPTP [38] and could thus influence actin filaments in follicle cells through Dlar signaling. Laminin also serves as a ligand for the α/β integrin heterodimer encoded by *mys* and *mew* [30] and may associate with the underlying actin cytoskeleton through coupling of integrins. Supporting this notion, mutant clones of either *mys* or *mew* cause a failure of oocyte elongation, indicative of defects in basal actin filament organization. However, loss of the PS2 α-integrin encoded by *if* also causes an oocyte elongation defect, suggesting that other components of the ECM containing RGD motifs are also instructive in organizing basal actin fibers. RGD-encoding ligands in *Drosophila* include tiggrin [39], Tenm [40], and the newly characterized laminin α2 [41]. It is not yet clear whether these ligands play a substantial role during oogenesis.

Basal actin filament polarity

The organization of basal actin filaments observed in stage 8 follicle cells shows similarities with previously characterized instances of planar polarity, such as the organization

Figure 7



Integrin function is required for proper elongation of oocytes. **(a–d)** Shown are stage 14 oocytes dissected from (a) wild-type adult females and (b–d) females carrying follicle cell clones of *mys*^{10}, *mew*^{498}, and *if*^{k27e}. (Identical defects are observed with the independent alleles *mys*^{968} and *if*^{B2}). **(e,f)** As with *Dlar*, clones of *mys*^{968} affect actin filament organization nonautonomously both at early stages (stage 8 in [e], compare with Figure 2b) and late stages (stage 12 in [f], compare with Figure 2e). Insets show wild-type cells expressing GFP at one-third size. We carefully examined 51 oocytes carrying *mys*^{968} clones of at least 10%–20% of the follicle cell field (the smallest clone size associated with an actin phenotype) and found that approximately half (25) induced disruptions in the wild-type pattern of basal actin filaments. The scale bar in (d) represents approximately 100 μm for (a–d); the scale bar in (e) represents 5 μm for (e–f). **(g)** Removal of one copy of *mys* enhances the penetrance of rounded stage 14 oocytes in *Dlar*$^{5.5}$/*Dlar*$^{13.2}$ ovaries (note that *mys* heterozygotes alone do not produce round eggs). This effect is observed by using two independent *mys* alleles, *mys*^{1} and *mys*^{10}, but not by decreasing the overall fitness of *Dlar* mutant flies by addition of the balancer TM6B.

of hairs in the *Drosophila* wing [42, 43]. In both cases, all cells of the epithelium align aspects of their cytoskeleton in a common orientation; in the case of the follicular epithelium, basal actin filaments are aligned perpendicular to the A-P axis, while in the wing epithelium actin protrusions that will form the wing hairs are restricted to the distal end of each cell. In addition to this morphological relationship, there also appear to be mechanistic similarities between the wing and the follicular epithelium. For example, clonal analysis reveals the importance of cell-cell communication in establishing planar polarity in the wing because misoriented cells within a mutant clone of *fz* or other tissue polarity mutants are able to disrupt the orientation of wing hairs in wild-type cells surrounding the clone [44]. Mutant cells tend to orient hairs in the same direction as their nearest neighbors, but not necessarily with the global tissue. Both of these phenomena have been observed with respect to actin filament orientation in and around follicle cell mutant clones of *Dlar* and *mys*. Furthermore, the polarized localization of Dlar to opposing membranes of the cell is similar to the localization of the seven-pass transmembrane cadherin Flamingo (Fmi) in the wing, which is controlled by tissue polarity genes such as Fz [45]. However, despite these similarities, considerable differences also exist between planar polarity in the wing and the organization of follicle cells. Perhaps most significantly, the events that organize actin filaments in follicle cells must operate at the basal surface where filaments are localized, whereas tissue polarity gene products such as Fz and Fmi localize to and function at the apical surface of other epithelia [45, 46]. Moreover, the follicular epithelium is a highly dynamic tissue relative to other epithelia, with cells undergoing major migrations over the developing germ layer during later stages of development [11]. During these migrations, cells must alter their cytoskeletal organization in order to establish proper orientation of filaments once migrations cease. In light of these similarities and differences, it will ultimately require a more thorough genetic analysis of follicle cell actin polarity in order to determine whether there is a conserved molecular logic.

An alternative model for the communication of polarity information from cell to cell in the follicular epithelium is presented by analyses of stress fibers in cultured cells. Stress fibers form in response to the application of force to a cell, and they themselves create tension across the cell parallel to their filaments [47–51]. One could thus imagine a model wherein outward stresses from the growing oocyte create tension across the epithelium, forming stress fibers that are biased to an orientation perpendicular to the A-P axis by the action of proteins such as Dlar and integrins. These stress fibers would create further tension on neighboring cells in this axis, creating positive feedback to maintain proper filament orientation across the tissue. This model would explain the observation that neighboring mutant cells organize filaments in similar orientations. It would also explain the nonautonomous

effects around *Dlar* and *mys* clones. Although this model accounts for the maintenance of actin filaments and for phenomena observed in mutants, some polarizing factor must function upstream of Dlar and integrins in order to initialize the bias of filament orientation. The nature of this signal is currently unclear.

Follicular epithelia as determinants of oocyte morphology

Evidence from several analyses supports a role for follicular epithelia in mediating oocyte elongation during insect oogenesis. Analysis of the round-egg mutant *kugel* demonstrated a correlation between actin polarity defects and a failure to elongate developing oocytes [18]. We observe the same correlation in both *Dlar* and *mys* mutants, supporting the "corset" model wherein actin filaments restrict growth in the short axis of the oocyte. Although confirmation of this model will require direct perturbation of the actin cytoskeleton during oogenesis, the proposed mechanism is similar to actin-dependent morphogenesis in *C. elegans*, in which parallel actin filaments in the hypodermis constrict to elongate the embryo to its characteristic adult shape [52]. In this case, elongation is blocked in embryos incubated in cytochalasin D, resulting in an abnormally rounded embryo. In other insect species, a restrictive network of parallel microtubules is oriented circumferentially around the germ layer, and stripping the follicular epithelium from nascent oocytes results in round eggs similar to those we observe in *Dlar* mutants [23, 53]. It is not clear whether the parallel actin filaments in *Drosophila* are either restrictive or constrictive in vivo, but in vitro analysis of cultured oocytes demonstrated an actin-dependent contractile force over the nurse cell-oocyte boundary where basal F-actin encircles the oocyte [16]. Thus, the basal actin network of the follicular epithelium combined with the underlying ECM appears to represent a conserved function for cytoskeletal control of morphology during development.

Conclusions

We have shown that the loss of Dlar results in a disorganization of cytoskeletal structures in the follicle cells of *Drosophila* oocytes. Although many previous analyses have implicated roles for RPTPs in communicating to the actin cytoskeleton, to our knowledge this study represents the first direct demonstration of an RPTP's influence on actin organization. Furthermore, our analysis shows that integrins are required for organizing the same subset of actin filaments, suggesting a function for Dlar in cooperation with integrins and other members of the focal adhesion complex.

Materials and methods

Fly husbandry

Several lines used in this work were obtained from the Bloomington Stock Center, including mys^{10}, mew^{498}, if^{B2}, and if^{k27e} lines and those carrying FRT40A and FRT18A. In addition, stocks for generating follicle cell clones by using the GAL4 system were a kind gift from Joe Duffy, and the *968* stock and FRT stocks carrying a ubiquitin-GFP marker were a kind gift from David Bilder. A w^{1118} stock was used as a wild-type control throughout this study. All stocks and crosses were maintained at 25°C unless otherwise noted.

Scoring defects in oocyte elongation and actin organization

For scoring the penetrance of egg shape defects, 2- to 5-day-old mated females were dissected in PBS, and their ovaries were dissected with forceps and examined under a microscope. Stage 14 oocytes were scored as either wild-type (elongated, with long dorsal appendages) or rounded (significantly shorter and thicker, with reduced dorsal appendages; for this analysis, only severely rounded oocytes as pictured in Figure 1g were scored as mutant). Genotypes were scored blindly.

To score the correlation between oocyte elongation and actin filament organization, we analyzed 67 stage 12 oocytes stained with Texas Red-phalloidin from females mutant for either Dlar (n = 32) or integrin (n = 35). Oocytes were binned according to overall morphology (elongated, n = 39; moderately rounded, n = 9; severely rounded, n =19) and overall stage 12 actin morphology (wild-type, virtually all lateral cells show filaments perpendicular to the A-P axis; moderate, regional disturbances in the wild-type pattern of less than approximately one-third of visible cells; severe, large disturbances of the wild-type pattern affecting the majority of visible cells).

To assess the rescue of actin organization with GAL4 drivers expressing a wild-type Dlar transgene, we scored filament organization in oocytes at multiple stages, with the following results: $Dlar^{5.5}/Dlar^{13.2}$*;UAS-Dlar/198Y-GAL4*, 12.5% severly affected actin, 10% moderate, 77.5% wild-type (n = 40); $Dlar^{5.5}/Dlar^{13.2}$*;UAS-Dlar/T155-GAL4*, 100% wild-type phenotype (n = 52); wild-type control, 0% severe actin phenotype, 3.7% moderate, 96.3% wild-type.

Supplementary material

An additional Materials and methods section is available at http://images.cellpress.com/supmat/sumatin/htm.

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