Dystroglycan down-regulation links EGF receptor signaling and anterior–posterior polarity formation in the *Drosophila* oocyte

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Anterior–posterior axis formation in the *Drosophila* oocyte requires activation of the EGF receptor (EGFR) pathway in the posterior follicle cells (PFC), where it also redirects them from the default anterior to the posterior cell fate. The relationship between EGFR activity in the PFC and oocyte polarity is unclear, because no EGFR-induced changes in the PFC have been observed that subsequently affect oocyte polarity. Here, we show that an extracellular matrix receptor, Dystroglycan, is down-regulated in the PFC by EGFR signaling, and this down-regulation is necessary for proper localization of posterior polarity determinants in the oocyte. Failure to down-regulate Dystroglycan disrupts apicobasal polarity in the PFC, which includes mislocalization of the extracellular matrix component Laminin. Our data indicate that Dystroglycan links EGFR-induced repression of the anterior follicle cell fate and anterior–posterior polarity formation in the oocyte.

axis specification | gurken | intercellular communication | microtubules | oogenesis

Formation of the main body axes is a critical stage in the development of most multicellular organisms. In *Drosophila melanogaster*, the main body axes are determined by the polarization of the developing oocyte. Each oocyte develops in the posterior region of an individual egg chamber, which is comprised of the oocyte, 15 germ-line nurse cells, and a surrounding monolayer of somatic follicle cells. The anterior–posterior (AP) body axis is established during stages 9–10 of oogenesis by the microtubule-dependent localization of *bicoid* (*bcd*) and *oskar* (*osk*) RNAs to the anterior and posterior ends of the developing oocyte, respectively (1–4). Formation of the correct microtubule arrangement and AP polarity requires activation of the EGF receptor (EGFR) signaling pathway in the follicle cells directly contacting the oocyte at the posterior of the egg chamber, causing these cells to differentiate as posterior follicle cells (PFCs). EGFR [Torpedo (Top) in *Drosophila*] is activated in the PFC in early oogenesis by secretion of Gurken (Grk; a TGF-α homologue) from the adjacent oocyte. Previous studies have demonstrated that EGFR activation in the PFC cues a complete reorganization of the oocyte microtubule cytoskeleton, such that by stage 9, there is a distinct accumulation of microtubule plus ends in a well defined compartment at the posterior cortex of the oocyte, and the minus ends appear to be concentrated predominately at the anterior region of the oocyte, with some extending along the lateral cortex (3–5). It is this microtubule polarity within the oocyte that serves as the basis for the localization of the RNAs and associated proteins that define the AP axis. Mutations inhibiting EGFR activation in the PFCs preclude this microtubule reorganization and, thus, axis formation. In these cases, *osk* RNA is mislocalized to the center of the oocyte, and *bcd* RNA accumulates at both poles of the oocyte (1, 2).

Mutations disrupting EGFR activation also inhibit differentiation of the follicle cells contacting the oocyte to the PFC fate, causing these cells to take the default anterior follicle cell (AFC) fate (1, 2). The basis for this misexpression of AFC markers stems from an initial equivalency of the terminal follicle cell groups, as established by JAK-STAT signaling at both poles before stage 6 (6). This symmetry is broken by EGFR activation in the PFCs but not the AFCs, which leads to the expression of different sets of genes/markers in the two cell groups. Despite the importance of EGFR signaling in AP axis formation, information on the molecular function of EGFR activation in the PFC is extremely limited, because no connection has yet been made between specific EGFR-induced changes in the PFCs and formation of the AP axis in the oocyte. Thus, the essential questions remain: What are the downstream targets of EGFR activation in the PFCs, and which are involved in AP axis formation?

Results

EGFR Signaling Regulates Dystroglycan (DG) Expression. To determine the relationship between EGFR-induced cell differentiation and establishment of the AP axis, we looked for genes differentially expressed in the follicle cells along the AP axis of the egg chamber and found the transmembrane protein DG. DG is an adhesion molecule known to function as an essential link between the extracellular matrix and the actin cytoskeleton through its role in the dystrophin–glycoprotein complex; in mammals, disruption of DG function in this complex is believed to contribute to several forms of muscular and neurodegenerative disorders (7). In oogenesis, antibody staining for DG shows relatively even expression on apical, basal, and lateral follicle cell surfaces (Fig. 1*A*), until about stage 6/7 when the protein is down-regulated in the PFCs (Fig. 1*A*, arrow), and main body cells, leading to an AP gradient of DG by stage 8/9 (Fig. 1*B*, antibody staining; Fig. 5 *A* and *C*, which is published as supporting information on the PNAS web site, RNA *in situ*). After stage 8, the minimal level of DG in the main body and PFCs is restricted to the basal surface.

The down-regulation of DG in the PFC at stage 6/7 coincides with EGFR-induced differentiation of the PFC. To determine whether EGFR signaling is involved in the down-regulation, we used the flipase (FLP)–flipase recombination target (FRT) mitotic recombination technique to generate PFC clones mutant for top^{CO} and stained them for DG protein. Loss of EGFR function in the PFC caused a cell-autonomous up-regulation of DG (Fig. 1 *E* and 1*E*′). As a result of difficulties in reliably generating follicle-cell clones with the top^{CO} stock, we tested a null allele of *Ras*, known to transduce the EGFR signal. Employing the "mosaic analysis with a repressible cell marker" (MARCM) technique (8), we found that $Ras^{\Delta C40b}$ PFC clones also up-regulate DG (Fig. 1 *F* and *F*′), confirming the involvement of Ras in EGFR signaling in these cells, which is consistent with a previous report that ectopic expression of activated *Ras* in the AFC causes misexpression of PFC markers (9). The misexpression of DG in top^{CO} and $Ras^{\Delta C40b}$ clones was specific

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Abbreviations: AFC, anterior follicle cell; AP, anterior–posterior; CAM, clone adjacent mislocalization; DG, Dystroglycan; EGFR, EGF receptor; MARCM, mosaic analysis with a repressible cell marker; PFC, posterior follicle cell.

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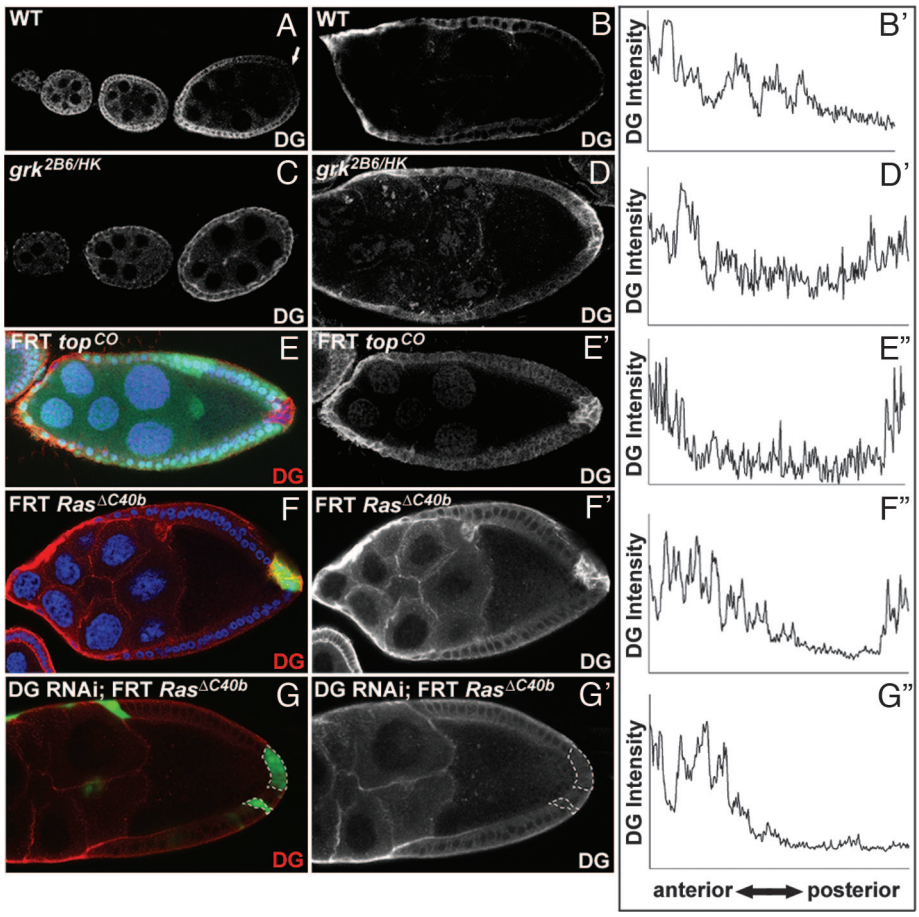

Fig. 1. Expression patterns of Dystroglycan (DG). (*A*) Antibody staining for DG expression in the early stages of wild-type oogenesis reveals even expression in all follicle cells before stage 6/7, when DG is down-regulated in the PFC (arrow). (*B*) Stage 9 wild-type egg chambers have reduced DG staining in all but the AFCs, although some DG persists on the basal surfaces of non-AFC (arrow). (*B′*) A plot of the intensity of the DG signal at the apical surface of the follicle cells based on a transect running from the anterior to the posterior of the egg chamber shown in *B* (all other plots are based on the image shown to their left; plots were generated by using ImageJ version 1.32j software). (*D*) Transheterozygous *gurken* mutant egg chambers fail to down-regulate DG in mid-oogenesis. (*E* and *F*) FRT top^{CO} clones (*E* and *E′*; clones lack GFP) and $Ras^{\Delta C40b}$ MARCM clones (*F* and *F′*; clones are GFP-positive) in PFC of poststage-6 egg chambers display significantly higher levels of DG in all cell-membrane domains. (*G* and *G′*) $Ras^{\Delta C40b}$ MARCM clones (GFP-positive), which also express a UAS DG RNAi construct, have DG levels comparable with the wild type. DG staining in red (*E*–*G*) or in white (*A*–*D* and *E′*–*G′*). DAPI staining of nuclei in blue. Posterior is on the right for all images.

to the PFC, because lateral or anterior clones did not change DG expression (Fig. 5 *E* and *E′* and data not shown). In addition, we observed up-regulation of DG in the PFC of egg chambers mutant for the activating ligand *grk* (Fig. 1*D*, antibody staining; Fig. 5 *B* and *D*, RNA *in situ*). In *top*, *Ras*, and *grk* mutants, there were also defects in the basal localization of DG within PFCs after stage 7, because up-regulated DG was present in basal, lateral, and apical surfaces. To investigate the sufficiency of EGFR activation in down-regulating DG in follicle cells, we misexpressed a constitutively active form of EGFR (λTop) and found that DG expression was down-regulated cell-autonomously in the AFC during mid-oogenesis (Fig. 5 *F* and *F′*); no obvious effect was detected in lateral follicle cells (data not shown). These experiments indicate the importance of EGFR activity for the down-regulation of DG in the PFC. The fact that DG levels remain high in the AFC throughout oogenesis, and because mutations affecting EGFR activity in the PFC cause those cells to misexpress AFC markers (Fig. 3 *B*, *B′*, *D*, and *D′*; ref. 10), suggests that the presence of ectopic DG in these EGFR pathway mutants is a consequence of the misexpression of the AFC fate. Taken together, these experiments demonstrate that EGFR signaling is necessary to repress AFC fate, which is required for the down-regulation and basal restriction of DG in the PFC.

DG Down-Regulation Is Required for Oocyte Polarity Formation. To investigate a possible relationship between EGFR, DG down-regulation, and AP axis formation, we used Staufen (Stau) as a measure of oocyte polarity. Stau is an RNA-binding protein known to colocalize with *osk* RNA to the oocyte posterior by stage 9 (Fig. 2*A*; ref. 11), and previous studies have shown that Stau and *osk* RNA are mislocalized to the center of the oocyte in *grk* and *top* mutants (1, 2). We also observed this Stau phenotype in *Ras* PFC clones (Fig. 6 *B* and *B′*, which is published as supporting information on the PNAS web site), but the large PFC clones of *top* or *Ras* required to completely mislocalize Stau were recovered rarely in our experiments. More frequently, we observed a milder polarity defect in *Ras* clones that we refer to as the "clone adjacent mislocalization" (CAM) phenotype. In 78% of egg chambers with *Ras* clones on only one side of the posterior of the egg chamber, Stau was mislocalized away from the area of the oocyte cortex adjacent to the clones and toward the wild-type cells (Fig. 2*E*; $n = 81$); this CAM phenotype also occurred in egg chambers containing top^{CO} PFC clones (data not shown). The CAM phenotype was observed for other posterior polarity markers such as Vasa (12) and Kin:βGal (a fusion of Kinesin (Kin) and β-Gal) (ref. 4; Fig. 6 *D*, *D′*, *F*, and *F′*). Kin is a plus-end microtubule motor protein that also localizes to the oocyte posterior in mid-oogenesis and is required for

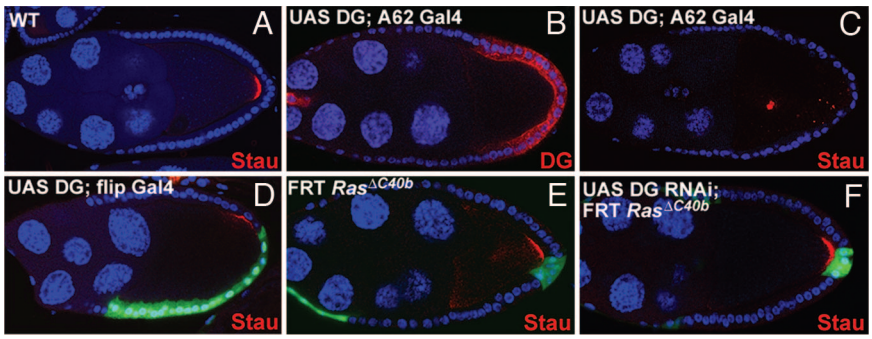

Fig. 2. DG is involved in establishing oocyte polarity. (*A*) Stau is localized to the posterior cortex of wild-type stage 9 oocytes. (*B* and *C*) Overexpression of DG by the A62 Gal4 driver results in ectopic expression of DG in all membrane domains of the PFC (*B*) and causes a complete mislocalization of Stau toward the center of the stage 9 oocyte (*C*). (*D*) Use of the flip-out Gal4 driver creates GFP-positive DG overexpression clones that, when present on one side of the posterior end of the egg chamber, cause mislocalization of Stau away from the clones and toward wild-type cells, the CAM phenotype. The CAM phenotype also results from similarly positioned $Ras^{\Delta C40b}$ MARCM clones (*E*; clones are GFP-positive). Reduction of DG levels by RNAi in $Ras^{\Delta C40b}$ MARCM clones (GFP-positive) leads to the wild-type pattern of Stau localization at the posterior (*F*). DAPI staining of nuclei is in blue. Stau staining is in red (*A* and *C–F*). DG staining is in red (*B*). Posterior is on the right.

the localization of *osk* RNA, Stau, and various other posterior determinants. Therefore, the mislocalization of Kin:βGal suggests that the microtubule reorganization initiated by EGFR signaling has not occurred properly in egg chambers bearing the CAM phenotype. The CAM phenotype has been reported in similarly positioned PFC clones of the phosphatase *Dlar* (13) and the JAK-STAT component *hopskotch* (6), but the mechanism underlying these two cases has not yet been identified.

Because disruption of EGFR activation in the PFC caused both oocyte polarity defects and DG up-regulation, DG down-regulation may be required for AP axis formation. To examine this possibility, we induced overexpression of DG by using a UAS construct of the full-length DG protein (14) under control of either the A62-GAL4 (a PFC driver) (15) or flip-out GAL4 driver (16). Overexpression of DG by A62-GAL4 resulted in high levels of DG in all membrane domains of the PFC (Fig. 2*B*) and mislocalizations of Stau [complete mislocalization in 8% of stage 9 egg chambers (Fig. 2*C*) and partial mislocalization in 30% (Fig. 6*J*); $n = 362$]. We also observed the CAM phenotype in 71% of oocytes in which *UAS DG;flip-out GAL4* clones were positioned to one side of the posterior end of the egg chamber (Fig. 2*D*; $n = 49$), similar to that described above in *Ras* clones. The polarity defects caused by overexpression of DG affected not only Stau but also Kin:βGal (Fig. 6 *G*, *G′*, *H*, and *I*). These results indicate that the presence of ectopic DG in the PFC after stage 6 can inhibit proper localization of posterior determinants in the oocyte, which is likely a result of mislocalization of the microtubule plus ends.

Given the similarity of the CAM phenotypes caused by DG overexpression and *Ras* clones, and the up-regulation of DG present in *Ras* PFC clones, we hypothesized that up-regulation of DG is at least partially responsible for oocyte polarity defects in mutants affecting EGFR signaling. To test the hypothesis, we performed a rescue cross by using the MARCM technique to generate *Ras* follicle-cell clones but also incorporated a GAL4-UAS-driven DG RNAi hairpin construct (14) to knock down DG expression in the *Ras* clones by RNAi. DG expression was reduced to approximately wild-type levels in *UAS DG-RNAi;FRT* $Ras^{\Delta C40b}$ PFC clones (Fig. 1 *G* and *G′*; compare to 1 *B* and 1 *F* and *F′*). Furthermore, in egg chambers possessing *Ras* PFC clones positioned so as to create the CAM phenotype, we noted a 3-fold increase in the percentage of oocytes with wild-type localization of posterior polarity markers in the *Ras* rescue egg chambers (Fig. 2*F*) over that of the *Ras* clones alone [66% in rescue clones (*UAS DG-RNAi;FRT Ras*); 22% in *Ras* clones; $\chi^2 = 36.6$; d.f. = 1; $P < 0.001$; $n = 81$ *Ras*, $n = 122$ *Ras* rescue]. These findings indicate that up-regulation of DG in *Ras* clones is responsible, at least in part, for the associated oocyte polarity defects.

Modification of DG Expression Does Not Affect Cell Fate. As noted previously, EGFR activation is essential for the differentiation of the PFC to their unique fate, which is believed to allow them to provide a polarizing signal back to the oocyte (1, 2). We therefore tested the possibility that manipulations of DG levels in our experiments affected cell fate. We initially confirmed the role of Ras in PFC differentiation by generating *Ras* clones and incorporating the AFC markers slbo-lacZ (17) and dpp-lacZ (18), which were both misexpressed in *Ras* PFC clones (Fig. 3 *A* and *A′* shows wild-type slbo-lacZ, Fig. 3 *B* and *B′* shows *Ras* clones, and dpp-lacZ not shown). We also used a new AFC marker, Eya (19) (Fig. 3 *C* and *C′*; see *Materials and Methods*). In *grk* and *Ras* mutant egg chambers, Eya was misexpressed in the affected PFCs after stage 8 (Fig. 3 *D* and *D′* and data not shown), but in the experiment involving *Ras* rescue by DG RNAi, Eya continued to be misexpressed (Fig. 3 *E* and *E′*). Therefore, the down-regulation of DG by RNAi, although able to rescue the CAM polarity phenotype, does not appear able to compensate for loss of Ras function in the differentiation of the PFC fate. We also found no capacity for DG overexpression to disrupt EGFR-induced PFC differentiation (Fig. 7, which is published as supporting information on the PNAS web site). Taken together, these experiments indicate that modification of DG levels alone does not affect fate determination in the PFC.

Ras Mutation Disrupts Follicle Cell Polarity and Laminin Localization. We have shown that overexpression of DG results in reduced accumulation of the apically localized β Heavy-Spectrin (βh-Spec) and Bazooka/Par3 proteins, indicating that DG overexpression can impair follicle-cell polarity (14). Because disruption of EGFR signaling caused up-regulation of DG in the PFC, we asked whether apicobasal polarity is intact in these cells. Staining for the apical marker βh-Spec (20) and a lateral membrane marker, Discs Large (Dlg) (21), indicates a weakened accumulation of βh-Spec on the apical surface, and a loss of lateral Dlg localization, in both *Ras* loss-of-function (Fig. 4 *A*, *A′*, *B*, and *B′*) and *UAS DG;flip-out GAL4* clones (Fig. 4 *C*, *C′*, *D*, and *D′*). These findings demonstrate the ability of high levels of DG to disrupt apicobasal polarity, which probably contributes to the phenotypic similarities observed in *Ras* PFC clones and DG overexpression clones. The finding that mutations in EGFR signaling lead to defects in apicobasal polarity is perhaps not surprising, because misexpression of AFC fate in the PFC caused by *grk* and *top* mutations causes the affected cells to express behavioral and morphological characteristics associated

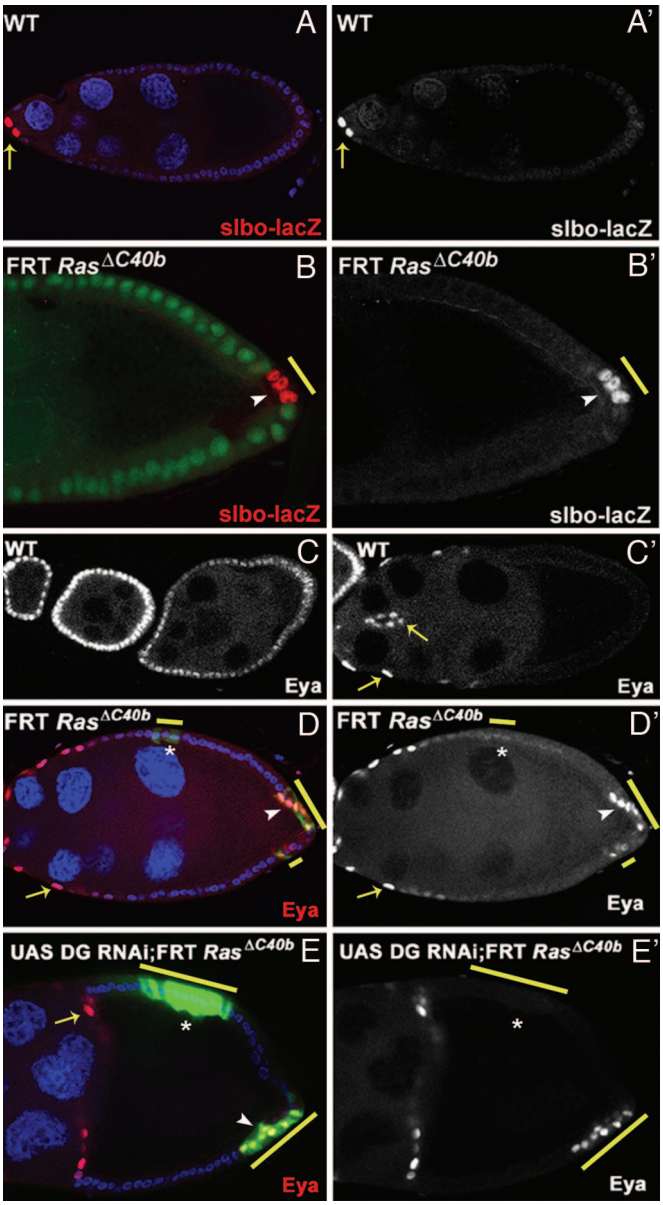

Fig. 3. DG does not affect cell fate. (*A* and *A′*) The AFC marker slbo-lacZ (red) is expressed exclusively in the border cells at the anterior pole of stage 9 egg chambers (yellow arrow). (*B* and *B′*) FRT $Ras^{\Delta C40b}$ clones (GFP-negative) misexpress slbo-lacZ in the PFC (white arrowhead). (*C*) The wild-type pattern of the follicle-cell fate marker Eya shows constitutive expression in all follicle cells before stage 7, except for the polar cells. (*C′*) After stage 8, Eya is down-regulated in all but the AFCs (yellow arrows). (*D* and *D′*) GFP-positive $Ras^{\Delta C40b}$ MARCM clones misexpress Eya (red in *D*; white in *D′*; example of wild-type AFC expression marked by yellow arrow, misexpression in PFC clones indicated by white arrowhead). (*E* and *E′*) PFC $Ras^{\Delta C40b}$ MARCM clones (GFP-positive) with the UAS DG RNAi construct also misexpress Eya (red in *E*; white in *E′*). Note that $Ras^{\Delta C40b}$ clones, with or without DG RNAi, in main-body cells do not misexpress Eya (marked by asterisks in *C*, *C′*, *D*, and *D′*). Clones are indicated by yellow lines.

with the AFC fate, and, subsequently, they tend to lose the columnar morphology typical of the PFC (1, 10), which is almost certainly linked to their apicobasal polarity.

To extend our understanding of the relationship between EGFR-induced DG down-regulation, apicobasal polarity, and oocyte polarity, we examined the effect of DG up-regulation on its extracellular binding partner, Laminin (Lan) (7). If Lan transduces the effects of ectopic DG on oocyte polarity, then disruptions in Lan localization/expression seem likely. In wild-type egg chambers, Lan has an expression pattern (Fig. 4 *E–G*) very similar to that of DG (Fig. 1 *A* and *B*), although Lan does not have an obvious AP gradient characteristic of DG expression after stage 6 (compare Fig. 4*G* to Fig. 1*B*). Lan localization in DG overexpression (Fig. 4 *H*, *H′*, and *I*) and *Ras* knockout (Fig. 4 *J* and *J′*) experiments revealed distinct mislocalizations of Lan to the lateral and apical surfaces of the affected cells, directly adjacent to the oocyte. This phenotype was alleviated significantly in *UAS DG-RNAi;FRT* $Ras^{\Delta C40b}$ egg chambers (Fig. 4 *K* and *K′*) [wild-type Lan pattern in 17% of $Ras^{\Delta C40b}$ PFC clones ($n = 36$) compared with 65% in *UAS DG-RNAi;FRT* $Ras^{\Delta C40b}$ PFC clones ($n = 26$)]. Together these experiments demonstrate a clear relationship between DG and Lan expression/localization patterns and suggest a role for ectopic Lan in mediating the deleterious effects of misexpressed DG on oocyte polarity.

Discussion

Here we have identified DG as a gene whose expression pattern is both regulated by EGFR signaling in the PFC and necessary for oocyte polarity. These findings provide a mechanistic link between EGFR activity in the PFC and polarization of the oocyte. Furthermore, we have discovered that defects in apicobasal polarity caused by ectopic DG also are present in the PFC where EGFR signaling is disrupted, possibly due to the misexpression of DG in these cells. In addition, our findings that ectopic DG leads to mislocalizations of Lan at the apical surface of the PFC indicates a process of cell–cell communication in which EGFR-regulated DG expression in the PFC controls Lan organization in the ECM that in turn may affect localization of posterior determinants in the oocyte.

It was reported that loss of LanA in the PFC disrupts oocyte polarity (22), which seems to be in conflict with the suggestion that high levels of apical Lan in the PFC perturbs oocyte polarity. However, a model in which Lan is required in early oogenesis, but must be localized basally after EGFR activation and DG down-regulation, reconciles these findings. In the previous research on loss-of-function *lanA* mosaic egg chambers, oocyte polarity defects observed at stage 9/10 could be generated only by larger *lanA* PFC clones (22). Because follicle cells are only mitotically active until stage 6/7 of oogenesis, these large PFC clones present at stage 9/10 would have represented sizeable *lanA* clones in prestage-6 follicle cells. Because Lan is present on the apical surface of these pre-PFCs (Fig. 4 *E* and *F*, arrow) (23), the polarity defects observed at stage 9/10 may have resulted from perturbation of some earlier Lan-dependent processes, such as organizing receptors on the facing surfaces of the oocyte or follicle cells. Consistent with this model, the addition of Lan to myotubes in culture is sufficient to organize the receptors integrin and DG, as well as their respective cytoplasmic counterparts, vinculin and dystrophin (24). Alternatively, it could be that the role of Lan in mediating the relationship between the PFC and oocyte is sensitive to any disruption of the ECM stemming from either the loss or misexpression of Lan, which then is sufficient to negatively affect oocyte polarity. Either of these models demonstrates the importance of the ECM in this process and ultimately may lead to a mechanistic understanding of the oocyte polarity defects caused by mutation in the putative Lan receptor Dlar (13).

Precisely how ectopic DG on the surface of the PFC translates to mislocalizations of posterior polarity markers in the adjacent oocyte remains to be determined, however, several different explanations for this process can be considered. First, DG down-regulation in the PFC may be necessary to allow the actin-based cortical anchoring of the posterior determinants in the oocyte (25), but ectopic DG did not appear to disrupt this mechanism (Fig. 8, which is published as supporting information on the PNAS web site). Second, the down-regulation of DG after EGFR activation might serve as a cue to the oocyte, which leads directly to MT reorganization and AP axis

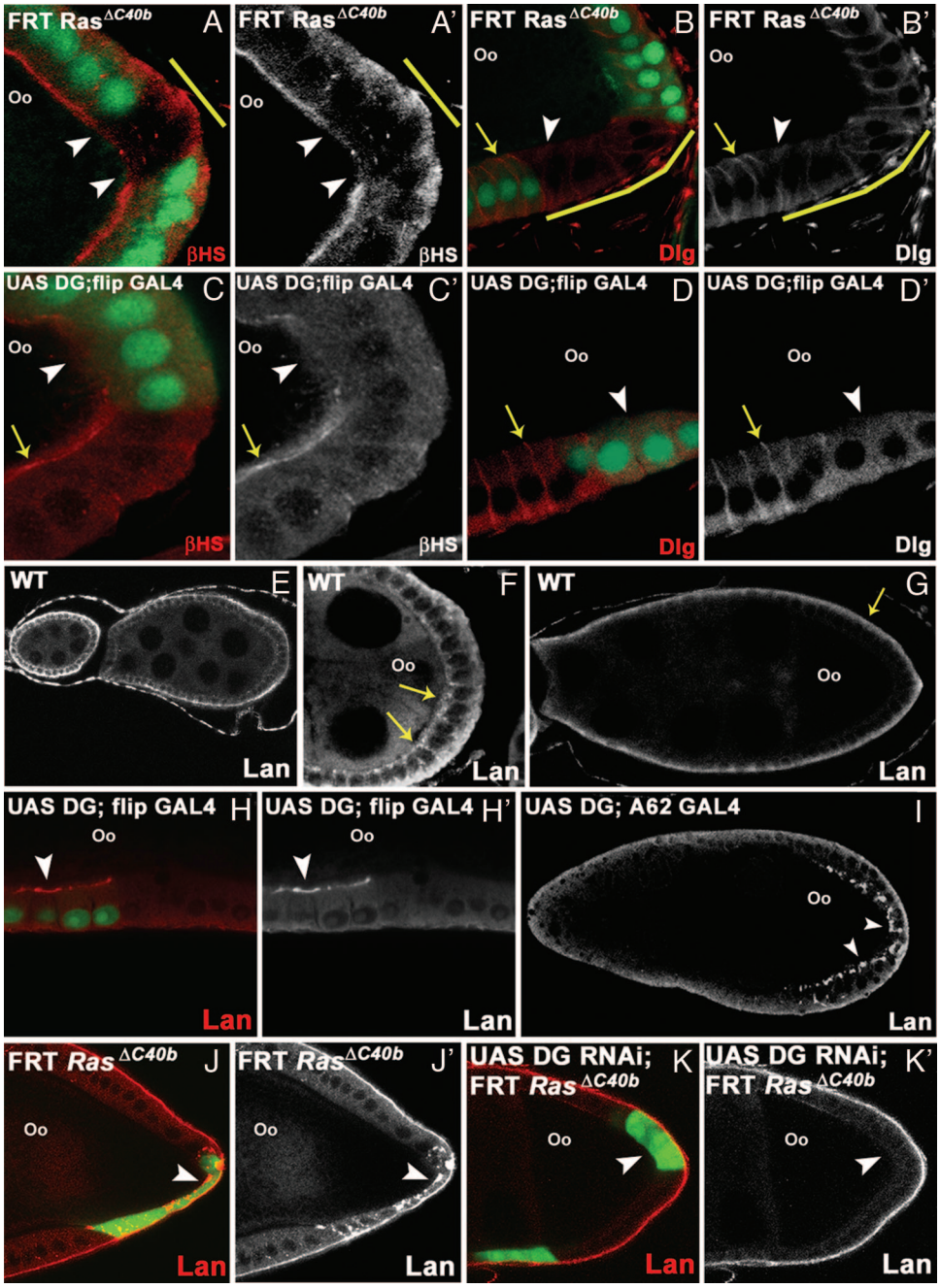

Fig. 4. Ectopic DG disrupts apicobasal polarity. (*A*, *A′*, *B*, and *B′*) FRT $Ras^{\Delta C40b}$ clones (GFP-negative) have reduced apical staining of βH-Spectrin (βHS) (red in *A*; white in *A′*; arrowheads) and reduced lateral accumulations of Dlg (red in *B*; white in *B′*; yellow arrow, wild-type pattern; white arrowhead, loss of Dlg staining). (*C*, *C′*, *D*, and *D′*) Overexpression of DG by the flip-out GAL4 also causes loss of apical βHS (red in *C*; white in *C′*) and lateral Dlg staining (red in *D*; white in *D′*). Lan protein is present in all follicle cell membrane domains in early oogenesis (*E* and *F*; yellow arrows in *F* highlight apical Lan in pre-stage 6 egg chambers), but, by midoogenesis, Lan is evenly restricted to the basal surfaces of all follicle cells (*G*; yellow arrow). (*H* and *H′*) Overexpression of DG by the flip-out (clones are GFP-positive) and posterior-follicle-cell (PFC) driver A62 GAL4 (I) result in distinct apical mislocalizations of Lan (arrowheads). Similar phenotypes are present in GFP-negative FRT $Ras^{\Delta C40b}$ PFC clones (*J* and *J′*; white arrowhead). These Lan mislocalizations are not present in GFP-positive $Ras^{\Delta C40b}$ MARCM clones, which also express the UAS DG RNAi construct (K, *K′*; white arrowhead). Lan staining in red (*H*, *J*, and *K*) or white (*H′*, *J′*, and *K′*). In all images, ''Oo'' labels the oocyte, yellow arrows mark wild-type expression patterns, white arrowheads indicate changes in marker of interest, and yellow lines indicate clone cells.

formation. In our analyses, however, DG overexpression did not result in defects in global microtubule organization or mislocalization of anterior oocyte polarity markers (Fig. 9, which is published as supporting information on the PNAS web site), phenotypes that have been reported in *grk* and *top* mutant egg chambers (1, 2). Furthermore, simply reducing DG levels in non-PFCs by RNAi was not sufficient to mislocalize Stau to nonposterior regions of the oocyte (data not shown). Therefore, DG down-regulation alone probably cannot serve as the signal to repolarize the microtubule network and, thus, establish oocyte polarity, but it is possible that changes in cell adhesion mediated through the DG/Lan complex could be part of a complex signal involving additional ECM receptors or even other signaling mechanisms that have yet to be identified. A similar model has been proposed for this signal in which changes in cell adhesion between the oocyte and PFCs serve as a nontraditional signal initiating AP axis formation (26). Alternatively, EGFR-mediated changes in DG/Lan patterns could regulate a novel mechanism that is required specifically for localization of posterior determinants at the oocyte cortex but is independent of the signal provided by the PFC to repolarize the oocyte microtubule cytoskeleton.

Third, the apicobasal defects caused by up-regulation of DG may have led to the loss of apical targeting of the polarizing signal from the PFC, as has been proposed for oocyte polarity defects caused by *Merlin* mutation (27). This explanation does not seem likely here, however, given the ability of DG RNAi to rescue the CAM phenotype even though the *Ras* clones still should be unable to produce the signal, because they do not take the PFC fate. Instead, we favor a model in which the apicobasal defects caused by ectopic DG results in apical accumulations of Lan, thereby modifying the ECM between the clones and oocyte so as to preclude diffusion of a secreted signal from the adjacent wild-type cells. Therefore, in the

Ras rescue experiment, down-regulation of DG allows the basal restriction of Lan, facilitating diffusion of the polarizing signal from the remaining wild-type cells. The fact that the rescue of the CAM phenotype by DG RNAi in *Ras* clones was not complete (34% of these egg chambers continued to show some defect in Stau localization) may support this model, because the diffusion of a signal from the neighboring cells probably would not be expected to replace fully the endogenous signal absent from the clone cells in every case. Whether mutations in other genes required for both apicobasal polarity and oocyte polarity also disrupt the ECM will be interesting to discover.

The study of axis formation in the *Drosophila* oocyte has demonstrated the importance of cell–cell communication in the tightly regulated patterning of the follicle cells, which ultimately leads to the establishment of those axes. The key findings presented here suggest a multifaceted role for EGFR signaling in PFC differentiation and oocyte polarization, highlighting the need for further study of EGFR activity, differentiation of the PFC, and formation of the AP axis.

Materials and Methods

Fly Stocks and Crosses. Mosaic clones of top^{CO} (28) had the following genotype: *hsFLP*; *FRT 42D GFP/FRT 42D* top^{CO}. Flies used for the *Ras* MARCM clones had the following genotype: *hsFLP*; *actin-GAL4 UAS-GFP*; *FRT82B tubGAL80/FRT82B* $Ras^{\Delta C40b}$. The $Ras^{\Delta C40b}$ allele is a null, deletion allele of Ras (29). The *Ras*-rescue flies had the same genotype as the previous flies but included a UAS DG RNAi hairpin loop construct (14): *hsFLP*; *actin-GAL4 UAS-GFP/UAS DG RNAi*; *FRT82B tubGAL80/FRT82B* $Ras^{\Delta C40b}$. Standard mitotic *Ras* clones were generated in flies with genotype *hsFLP*; *FRT82B GFP/FRT82B* $Ras^{\Delta C40b}$. The AFC markers slbo-lacZ and dpp-lacZ, as well as the microtubule polarity marker Kin:βGal, were incorporated into this *Ras* clone background.

Flip-out GAL4 clones were generated in flies of genotype *hsFLP*; *UAS-DG*; *act>cd2>GAL4 UAS-GFP* (16). The UAS-DG construct expresses the full-length transcript of the long isoform of DG (14). Flip-out GAL4 clones including the pointed-lacZ reporter line 998/12 (10) had genotype *hsFLP*; *UAS-DG*; *act>cd2>GAL4 UAS-GFP/pointed-lac-z*. Flip-out GAL4 clones including the Kin:βGal reporter had genotype *hsFLP*; *UAS-DG*; *act>cd2>GAL4 UAS-GFP/Kin:βGal*. Flip-out DG RNAi clones had the genotype *hsFLP*; *UAS-DG RNAi*; *act>cd2>GAL4 UAS-GFP*. Flip-out GAL4 clones of the activated form of EGFR had the genotype *hsFLP/UAS-λtop*; *act>cd2>GAL4 UAS-GFP* (30). The A62-GAL4 overexpression flies had the genotype *UAS-DG/pin*; *A62-GAL4/+*. The A62-GAL4 stock (15) crossed to a UAS-GFP line indicates expression in all PFC beginning around stage 6 and also in the border cells beginning shortly thereafter (data not shown). From the current understanding of AP axis formation, we believe that any of the phenotypes described herein for the A62-GAL4 overexpression crosses resulted from its expression in the PFC and not from its expression in the border cells, particularly because the phenotypes described here can be observed well before the border cells have migrated to the oocyte. Wild-type fly stocks used were *OregonR or* w^{1118}.

Clone Generation and Immunocytochemistry. MARCM mutant clones (8) were generated by 37°C heat shock of adult flies twice daily for 1 h. Flip-out GAL4 clones were created by a single 45-min heat shock of adult flies. Ovaries were dissected 5–6 days after heat shock. Ovaries were fixed in 5% formaldehyde solution, and the following primary antibodies were applied according to a standard antibody staining protocol (22): rabbit anti-β-gal (1:5,000; Sigma, St. Louis, MO), rabbit anti-βH-Spec (1:1,000) (20), rabbit anti-DG (1:3,000) (14), guinea pig anti-Laminin (1:1,000; a gift from T. Volk, Weizmann Institute, Rehovot, Israel), rabbit anti-Staufen (1:2,000; a gift from D. St Johnston, University of Cambridge, Cambridge, U.K.) (11), rat anti-Vasa (1:1,000; a gift from P. Lasko, McGill University, Montreal, QC, Canada) (12), mouse anti-Cut (1:20), anti-Dlg (1:20), and anti-Eya (1:10) (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). (See *Supporting Materials and Methods* for further details.)

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