

Apical, Lateral, and Basal Polarization Cues Contribute to the Development of the Follicular Epithelium during *Drosophila* Oogenesis

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Abstract. Analysis of the mechanisms that control epithelial polarization has revealed that cues for polarization are mediated by transmembrane proteins that operate at the apical, lateral, or basal surface of epithelial cells. Whereas for any given epithelial cell type only one or two polarization systems have been identified to date, we report here that the follicular epithelium in *Drosophila* ovaries uses three different polarization mechanisms, each operating at one of the three main epithelial surface domains. The follicular epithelium arises through a mesenchymal–epithelial transition. Contact with the basement membrane provides an initial polarization cue that leads to the formation of a basal membrane domain. Moreover, we use mosaic analysis to show that Crumbs (Crb) is required for the formation and maintenance of the follicular epithelium. Crb localizes to the apical membrane of follicle cells that is in contact with germline cells. Contact to the germline is required for the accumulation of Crb in follicle cells. Discs Lost (Dlt), a cytoplasmic PDZ domain pro-

tein that was shown to interact with the cytoplasmic tail of Crb, overlaps precisely in its distribution with Crb, as shown by immunoelectron microscopy. Crb localization depends on Dlt, whereas Dlt uses Crb-dependent and -independent mechanisms for apical targeting. Finally, we show that the cadherin–catenin complex is not required for the formation of the follicular epithelium, but only for its maintenance. Loss of cadherin-based adherens junctions caused by *armadillo* (β -catenin) mutations results in a disruption of the lateral spectrin and actin cytoskeleton. Also Crb and the apical spectrin cytoskeleton are lost from *armadillo* mutant follicle cells. Together with previous data showing that Crb is required for the formation of a zonula adherens, these findings indicate a mutual dependency of apical and lateral polarization mechanisms.

Key words: epithelial polarity • follicular epithelium • Crumbs • Discs Lost • Armadillo

Introduction

The polarization of epithelial cells is a fundamental process in animal development. The study of mammalian culture cells and the genetic analysis of epithelial differentiation in *Drosophila* have made significant contributions to the understanding of the mechanisms involved in epithelial polarization (Tepass, 1997; Yeaman et al., 1999; Müller, 2000). Transmembrane proteins that specifically localize to one of three surface domains found in epithelial cells mediate asymmetric cues that control cell polarization. Work on mammalian culture cells, which allow the reversible induction of epithelial morphology, has revealed important roles for cadherin- and integrin-mediated adhesion in epithelial polarization. Cadherin and integrin activity causes the assembly of a domain-specific cytocortex at the lateral and basal membrane, respectively. Cell surface polarization is

followed by a reorganization of the cytoskeleton that in turn facilitates asymmetric distribution of organelles and the polarized targeting of transport vesicles to the apical or basolateral membranes. Polarized delivery of proteins and lipids is critical for solidifying and maintaining the polarized membrane domains of epithelial cells (Drubin and Nelson, 1996; Yeaman et al., 1999). Genetic studies in *Drosophila* have also revealed evidence for lateral and basal polarization cues (for review, see Tepass, 1997). However, the best understood factor that controls epithelial polarization in *Drosophila* is Crumbs (Crb),¹ a single pass transmembrane protein that is part of the apical membrane (Tepass et al., 1990; Tepass, 1996). Crb is a powerful regulator of epithelial polarization as lack of Crb causes the apical membrane to disappear, and overexpression of Crb

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¹Abbreviations used in this paper: Arm, Armadillo; Crb, Crumbs; Dlt, Discs Lost; FE, follicular epithelium; IEM, immunoelectron microscopy; ZA, zonula adherens.

leads to an “apicalization” of much of the cell surface. Both conditions cause the breakdown of epithelial cell and tissue structure (Tepass et al., 1990; Tepass and Knust, 1990; Wodarz et al., 1993, 1995).

Analysis of epithelial development in *Drosophila* has led to a distinction between primary and secondary epithelia (Tepass and Hartenstein, 1994a; Tepass, 1997). Primary epithelia derive directly from the blastoderm epithelium without mesenchymal intermediates, and differentiate a zonula adherens (ZA) as part of their junctional complex. In contrast, secondary epithelia form by a mesenchymal–epithelial transition and do not contain a ZA. Both types of epithelia require lateral adhesion mediated by DE-cadherin to maintain integrity (Tepass and Hartenstein, 1994b; Tepass et al., 1996; Uemura et al., 1996; Haag et al., 1999). Differences in the mechanisms that orient the apical–basal axis in primary and secondary epithelia exist as primary epithelia depend on apical Crb for maintaining polarity, whereas secondary epithelia require basal cues that involve Laminin (reviewed in Tepass, 1997). Evidence for a role of basal cues in the polarization of primary epithelia is lacking. On the other hand, Crb is not expressed in secondary epithelia, and an alternative apical polarization factor in secondary epithelia is not known. Mammalian culture cells used in studies on epithelial polarization are derived from epithelia that form late in development in the presence of extracellular matrix. For example, MDCK and CACO-2 cells, which are related to kidney epithelia and intestinal epithelial cells, respectively (Pinto et al., 1983; Yeaman et al., 1999), depend on integrins for normal polarization and could therefore be regarded as models for secondary epithelia. A well-conserved homologue of Crb has recently been described in humans, but its role in epithelial development remains unclear (den Hollander et al., 1999). Other apical polarization factors are currently not known in vertebrates or other invertebrates, although circumstantial evidence suggests that apical polarity might be specified independent of lateral and basal cues in early sea urchin, *Xenopus*, and mouse embryos (Reeve and Ziomek, 1981; Nelson and McClay, 1988; Müller and Hausen, 1995). Taken together, these findings suggest that epithelial polarity is governed by two polarizing cues in a given cell type. Lateral cadherin-mediated adhesion is critical for the assembly of the lateral membrane domain, and organizes epithelial cells into two-dimensional sheets. One additional polarization cue, either apical or basal, is required to establish the apical–basal axis.

This study characterizes the mechanisms that are involved in the formation of the follicular epithelium (FE) during *Drosophila* oogenesis. *Drosophila* follicle cells are widely used as a model to study pattern formation and morphogenesis in an epithelial context. The FE combines features of primary and secondary epithelia as it has a ZA and expresses Crb (Mahowald, 1972; Tepass and Knust, 1990). On the other hand, the FE forms through a mesenchymal–epithelial transition, during which follicle cells are in contact with a basement membrane (King, 1970). Our observations suggest that the FE uses mechanisms operating on the apical, lateral, and basal membranes for epithelial differentiation. Contact of follicle cells to the basement membrane leads to an initial polarization of follicle cells, generating a basal membrane that is distinct from the rest of the plasma membrane.

Full epithelial polarization is initiated by the contact of follicle cells to germline cells. This contact leads to a demar-

cation of apical and lateral membranes and the accumulation of Crb and the Crb-associated PDZ domain protein Discs Lost (Dlt; Bhat et al., 1999; Klebes and Knust, 2000) at the apical membrane. Crb and Dlt are involved in the formation of the FE and at least Crb is also required for its maintenance. Although Crb and Dlt are likely to form a complex at the apical membrane, our data also suggest that Dlt interacts with a second apical factor different from Crb, which may explain the variable defects observed in *crb* mutant clones. Finally, we explored the requirement of the cadherin–catenin complex in the differentiation of the FE by analyzing mutations in *armadillo* (*arm*), which encodes the *Drosophila* homologue of β -catenin (Peifer and Wieschaus, 1990). An essential role for *arm* in the formation of embryonic epithelia has been documented (Cox et al., 1996; Müller and Wieschaus, 1996). We were therefore surprised to find that disruption of the cadherin–catenin complex does not prevent follicle cells from forming a FE. However, *arm* mutant follicle cells show defects that indicate a disruption of the lateral and apical membrane domains.

Materials and Methods

Fly Stocks

We used Oregon R as wild-type stock. Agametic ovaries were recovered from female flies homozygous mutant for *oskar*³⁰¹ that were raised at 18°C (Lehmann and Nusslein-Volhard, 1986). Other mutations and transgenic lines are described below.

Clonal Analysis

Homozygous mutant clones for *crb*, *dlt*, and *arm* were generated with the FRT/FLP system (Golic, 1991; Xu and Harrison, 1994). Two different strategies were used to induce clones either before the formation of the FE (“early clones”), or after the FE has been established (“late clones”). For induction of early clones, FLP expression was driven in follicle stem cells using the system developed by Duffy et al. (1998). Here, the *FLP* gene, which is under the control of upstream activator sequences (*UAS>FLP*), is driven by the Gal4 line *e22c-Gal4* that is expressed in follicle stem cells. Late clones were induced by driving FLP under the control of a heat shock promoter (*hsFLP*; Xu and Harrison, 1994).

Homozygous *crb* mutant clones were induced for the null allele *crb*^{11A22} and *crb*^{B82} (Tepass and Knust, 1990; Brook et al., 1993). The genotypes of flies, in which clones were induced are as follows. Early clones: *w; e22c-Gal4, UAS>FLP/+; FRT^{82B} crb⁻/FRT^{82B}+*. Late clones: *w hsFLP1/+; FRT^{82B} crb⁻/FRT^{82B}+*.

Homozygous *dlt* mutant clones were induced for the null allele *dlt*^{MY10} and the hypomorphic, protein-positive allele *dlt*^{drel} (Bhat et al., 1999). The *dlt*^{MY10} allele is a deletion that uncovers the genes *dlt*, *cdc37*, and α -spectrin. To compensate for the loss of *cdc37*, clones were induced in the background of a *cdc37* rescue construct, *P[w⁺, cdc37^{res}]*. However, *dlt*^{MY10} clones are mutant for both *dlt* and α -spectrin. *FRT, dlt* recombinant lines were provided by Manzoor Bhat (Mount Sinai School of Medicine, New York, NY). The genotypes of flies, in which clones were induced are as follows. Early clones: *w; e22c-Gal4 UAS>FLP/P[w⁺, cdc37^{res}]; FRT^{80B} dlt^{MY10}/FRT^{80B}+*. *w; e22c-Gal4 UAS>FLP/+; FRT^{80B} dlt^{drel}/FRT^{80B}+*. Late clones: *w hsFLP1/+; P[w⁺, cdc37^{res}]/+; FRT^{80B} dlt^{MY10}/FRT^{80B}+*. *w hsFLP1/+; FRT^{80B} dlt^{drel}/FRT^{80B}+*.

Homozygous *arm* mutant clones were induced for the null allele *arm*^{YD35}, the strong hypomorph *arm*^{XK22}, and the intermediate hypomorph *arm*^{XP33} (Peifer and Wieschaus, 1990). The genotypes of flies in which clones were induced are as follows. Early clones: *w arm⁻ FRT¹⁰¹/w, FRT¹⁰¹; e22c-Gal4 UAS>FLP/+*. Late clones: *w, arm⁻ FRT¹⁰¹/w, FRT¹⁰¹; hsFLP2/+*.

For early clones, flies were grown at 25° or 29°C; adults were maintained in yeast vials and dissected 2–4 d after eclosion. For late clones, freshly eclosed females were collected, heat shocked for 1–2 h at 37°C, and transferred to yeast vials for 2–5 d before dissection.

Clonal overexpression of full-length Crb (*UAS>crb^{w12c}*; Wodarz et al., 1995) and of a truncated form of Crb that lacks most of the extracellular part, including all EGF-like domains and the LG domains (*UAS>crb^{trunc2b}*; Wodarz et al., 1995) was induced following the technique of Ito et al. (1997). Clones were induced in flies of the genotypes *w hsFLP1; AyGal4*

UAS>lacZ/UAS>crb^{w^{2c}} or *w hsFLPI; AyGal4 UAS>lacZ/+; UAS>crb^{intra2b/+}*. Newly eclosed females were heat shocked for 15 min at 37°C and kept in yeasted vials for 2–4 d before dissection.

Production of Dlt Antibodies

A full-length cDNA encoding Dlt (accession No. AF 274350) as isolated by screening a *Drosophila* embryo (4–6 h) cDNA expression library (Novagen) with a peptide corresponding to the carboxy terminus of the Inscuteable protein. Polyclonal antibodies were generated by immunizing rabbits with a peptide (NH₂-SMGAEPDLIPDWRN-COOH) corresponding to amino acids 858–872 of Dlt. An amino-terminal cysteine was added to facilitate keyhole limpet hemocyanin conjugation and to couple the peptide to a Sulfolink column (Pierce Chemical Co.) used to affinity purify the anti-Dlt serum.

Antibody Stainings

For immunostainings, the following primary antibodies were used: rat monoclonal antibody anti-DE-cadherin (DCAD2, 1:50; Oda et al., 1994); rat mAb anti-DN-cadherin (Ex8, 1:50; Iwai et al., 1997); mouse mAb anti-Crb (Cq4, 1:25; Tepass and Knust, 1990); mouse mAb anti-Fasciclin III (7G10, 1:50; Patel et al., 1987); mouse mAb anti-Armadillo (N2-7A1, 1:100; Peifer et al., 1993); rabbit polyclonal antibody anti-Armadillo (N2, 1:200; Riggelman et al., 1990); mouse mAb anti- α -spectrin (3A9, 1:100; Byers et al., 1987); rabbit pAb anti- β _{Hcavy}-spectrin (243, 1:500; Thomas and Kiehart, 1994); rabbit pAb anti-Dlt (1:250); rabbit pAb anti- β -galactosidase (1:1,000; Cappel); mouse mAb anti- β _{PS}-integrin (G11, 1:50; a gift from L. Fessler, University of California at Los Angeles, Los Angeles, CA).

Anti-DE-cadherin and anti-DN-cadherin stainings were carried out as previously described (Niewiadomska et al., 1999). For other antibody stainings, ovaries from 2–5-d-old well-fed female flies were dissected in PBS and fixed in 5% formaldehyde PBS, pH 7.4, for 10 min, and then treated with methanol for 5 min. Methanol treatment was not used for all phalloidin stainings, and the β _{PS}-integrin, DE-cadherin double-labeling experiment. Tissues were washed in PBT (PBS, 0.2% Triton X-100) for 4 × 15 min, followed by a 1-h incubation in blocking solution PBTB (PBT, 0.2% BSA, 5% goat serum). Incubation with primary antibodies, diluted in blocking solution, was done at 4°C overnight. Ovaries were washed in PBT for 4 × 15 min and incubated in blocking solution for 1 h. Secondary antibodies conjugated with Cy3, Cy5, FITC (Jackson ImmunoResearch Laboratories), or Oregon green 488 (Molecular Probes) were used at a dilution of 1:400 in PBTB at 4°C overnight. Ovaries were washed in PBT for 4 × 15 min and mounted in Antifade [70% glycerol + 2.5% DABCO (Sigma-Aldrich) in PBS].

F-actin was detected with phalloidin. After antibody staining, ovaries were washed with PBS, incubated in Oregon green 488-phalloidin (Molecular Probes) at a dilution of 1:20 in PBS at 4°C overnight, washed in PBS, and mounted in Antifade. Cell nuclei were visualized with Picogreen (Molecular Probes). After antibody staining, ovaries were treated with 0.4 mg RNaseA/ml in PBT for 1 h, rinsed with PBT, incubated with Picogreen at a dilution of 1:1,000 in PBT at 4°C overnight, washed in PBT, and mounted in Antifade.

Confocal images were obtained with a scanning laser confocal microscope (LSM510; Carl Zeiss, Inc.) using Plan-Neofluar 40×/1.30 oil and Plan-Apochromat 100×/1.40 oil lenses. Images were processed in Adobe Photoshop or Adobe Illustrator.

Immunoelectron Microscopy

Immunoelectron microscopy (IEM) on embryos was carried out as described previously (Tepass, 1996). For IEM on follicles, ovaries were dissected in PSS buffer (100 mM Na-glutamate, 25 mM KCl, 15 mM MgCl₂, 5 mM CaSO₄, 2 mM sodium phosphate buffer, pH 6.9) (Woodruff and Tilney, 1998), and fixed for 30 min in 8% formaldehyde, 0.02% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2. Further treatment was as described previously (Tepass, 1996). The dilution of the anti-Dlt antibody in these experiments was 1:150.

Results

Expression and Subcellular Distribution of Crb and Dlt during Oogenesis

Drosophila ovaries are composed of ovarioles, each representing an anterior–posterior series of follicles of increas-

ing age (King, 1970; Spradling, 1993). The assembly of follicles takes place in the germarium that is located at the anterior tip of each ovariole (Fig. 1). During follicle formation, ~30 follicle cells form an epithelial monolayer that surrounds a cluster of 16 germline cells (Margolis and Spradling, 1995). Whereas the germline cells increase in size but not in number, the cells in the FE proliferate so that the FE in a mature follicle contains ~650 cells. A second population of follicle cells that does not establish direct contact with germline cells forms short stacks by cell intercalation, giving rise to the interfollicular stalk (Godt and Laski, 1995).

Crb is initially expressed in all follicle cells at stage 1 of oogenesis, but its expression becomes rapidly restricted to the cells of the FE, and is not maintained in the interfollicular stalk (Fig. 2 A; Tepass and Knust, 1990). Crb is found in the apical membrane of the cells of the FE (Fig. 2 A), similar to many other *Drosophila* epithelia (Tepass et al., 1990; Tepass and Knust, 1990). Dlt shows a similar temporal and spatial distribution as Crb in follicle cells (Fig. 2, B–D), suggesting that both proteins might interact physically in follicle cells as they do in embryonic epithelia (Bhat et al., 1999; Klebes and Knust, 2000). Dlt also associates in vitro with Neurexin IV, a transmembrane component of the septate junction (Baumgartner et al., 1996; Bhat et al., 1999). The septate junction, which blocks paracellular diffusion similar to the chordate tight junction, is located basally to the ZA (Mahowald, 1972; Tepass and Hartenstein, 1994a,b). To reconcile these data, it was proposed that Dlt interacts with Crb apically to the ZA, with the ZA, and with Neurexin IV basally to the ZA (Bhat et al., 1999).

To clarify the subcellular localization of Dlt, we determined the distribution of Dlt in the FE and in the embryonic ectoderm and epidermis by IEM (Fig. 3). IEM reveals that Dlt, similar to Crb (Tepass, 1996), is confined to the apical membrane. Within the apical membrane, Dlt accumulates at the marginal zone, an area of cell–cell contact apical to the ZA, and shows a lower concentration throughout the apical surface (Fig. 3). The signal for Dlt at the ZA or basally to it does not exceed background levels, and no signal is observed at septate junctions (Fig. 3 F). These findings suggest that Dlt does not physically interact with Neurexin IV at the septate junction, and further corroborates the notion that Dlt and Crb form a complex at the apical membrane of epithelial cells.

Crb and Dlt are also expressed in the germline, although the distribution of both proteins overlaps only partially. Dlt and Crb colocalize at the membrane of the nurse cells during early and mid oogenesis. High levels of Crb are seen in the plasma membrane of the oocyte, whereas Dlt is not detectable (Fig. 2, C and D; Niewiadomska et al., 1999). Dlt accumulates transiently at the ring canals that connect nurse cells (data not shown). During late stages of oogenesis, when the content of the nurse cells is rapidly transferred to the oocyte (Cooley and Theurkauf, 1994), Dlt is found at the nuclear membrane in close association with actin filaments that connect the nuclei of the nurse cells with the plasma membrane (data not shown). Previous germline clone experiments did not reveal a function for *crb* in the germline (Tepass and Knust, 1990). In contrast, maternal expression of *dlt* is essential for egg production, suggesting that *dlt* plays an important role in germline development (Bhat et al., 1999). The function of *dlt* in germline development remains to be studied in more detail.

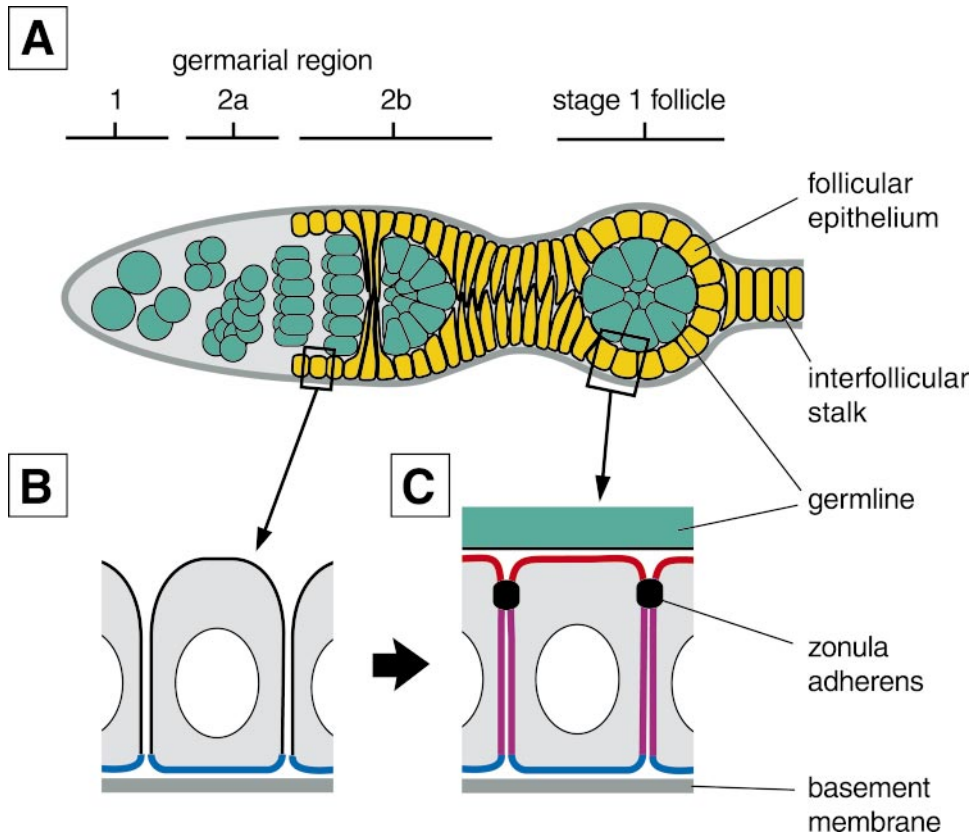


Figure 1. Schematic of the formation of the FE. (A) A germarium and a stage 1 follicle. Follicle cells originate from two stem cells that are located at the transition of regions 2a and 2b (Margolis and Spradling, 1995). Follicle cells surround a cluster of 16 germline cells in region 2b. Follicle cells that contact the germline cells fully polarize and form the FE, whereas a second group of follicle cells forms the interfollicular stalk by cell intercalation. (B) A follicle cell before contact to the germline. The cell is in contact to the basement membrane and has established a basal membrane (blue), whereas apical and lateral membranes have not been clearly defined. (C) A follicle cell after contact to the germline has been established. Apical (red) and lateral (purple) membranes have been formed and a zonula adherens has differentiated.

Polarization of the FE Requires Germline-dependent and -independent Mechanisms

The follicle cells are generated by two stem cells that are located in the middle of the germarium at the boundary of region 2a and 2b (Margolis and Spradling, 1995). Offspring of these stem cells establish contact with the basement membrane that surrounds the germarium and the follicles (King, 1970; Fig. 1). Analysis of agametic ovaries has

shown that the contact between follicle cells and germline cells is required for the formation of the FE. Follicle cells continue to proliferate in agametic ovaries and form a column that is two to three cells wide (Margolis and Spradling, 1995; Goode et al., 1996b). To determine whether, and to what extent, contact between follicle cells and the basement membrane contributes to the polarization of the FE, we examined follicle cells in agametic ovaries (Fig. 4).

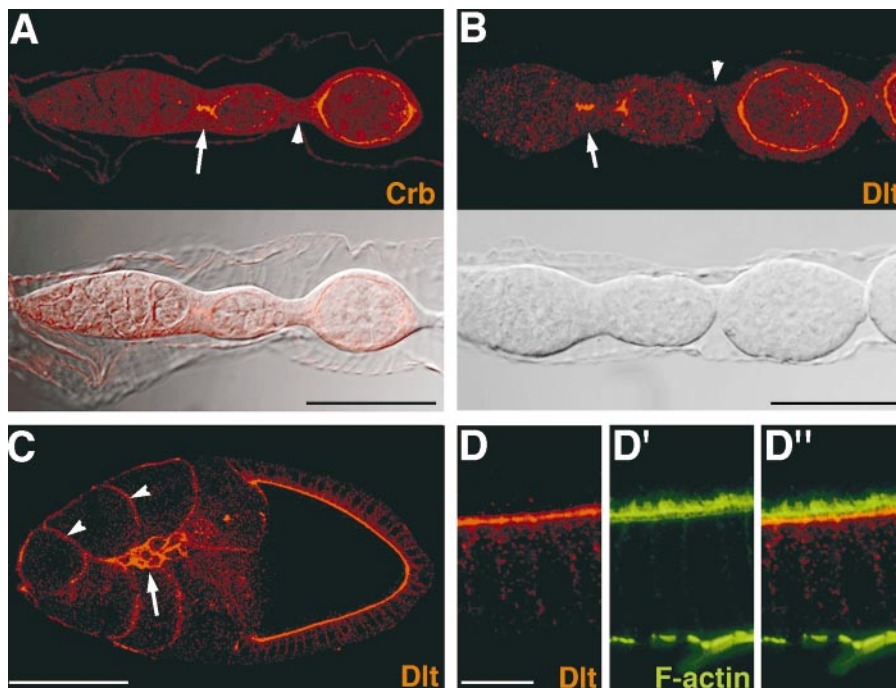


Figure 2. Expression of Crb and Dlt in wild-type ovaries. (A) Crb is first detected in follicle cells located between region 2b and the stage 1 follicle that will form the interfollicular stalk (arrow) and in the FE of the stage 1 follicle. Note that interfollicular stalk cells lose Crb rapidly (arrowhead), but that Crb expression is maintained in the FE, where it associates with the apical membrane. (B) Dlt shows the same distribution as Crb in early stages of oogenesis. (C) Expression of Dlt at stage 9 of oogenesis. Dlt is expressed in the FE and the border cells (arrow). In germline cells, Dlt is found at a contact site between nurse cells (arrowheads). (D) Dlt is found at the apical surface of follicle cells, but is absent from the oocyte membrane that is associated with large amounts of F-actin (D'). (D'') A merged image of D and D'. By contrast, Crb strongly accumulates in the oocyte membrane, as was shown previously (Niewiadomska et al., 1999).

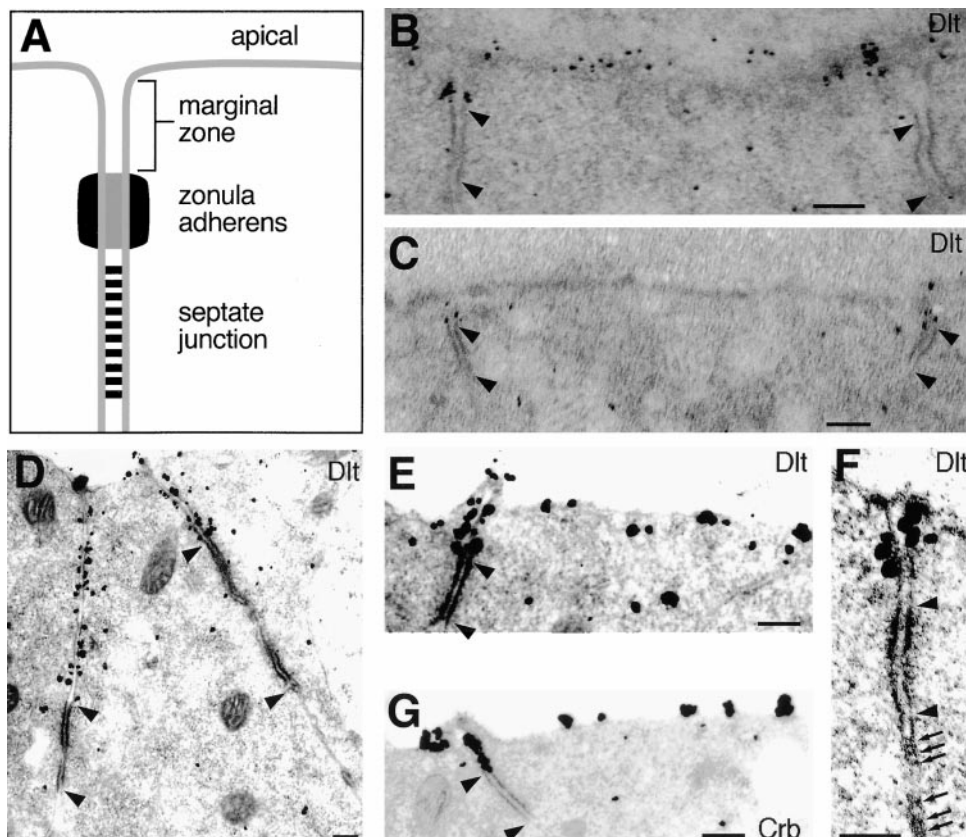


Figure 3. IEM of Dlt in the FE and in embryonic ectoderm and epidermis. (A) Schematic of the apicolateral region of an epithelial cell after the junctional complex composed of a ZA and a septate junction has formed. The marginal zone of the apical membrane domain is an area of cell–cell contact that is found apical to the ZA (Tepass, 1996). (B and C) Dlt is found throughout the apical membrane of follicle cells, but is concentrated just apical to the ZA (between arrowheads) in the marginal zone. The higher concentration of Dlt in the marginal zone is particularly apparent when the signal intensity is low as in C, where the signal is confined to the marginal zone. (D, E, and G) Examples of Dlt (D and E) and Crb (G) immunolabeling in ectodermal cells of stage 11 embryos. Labeling for Dlt and Crb are confined to the apical surface and concentrated at the marginal zone immediately apical to the ZA (between arrowheads). (F) Dlt is found apical to the ZA (arrowheads) in stage 16 embryos after the septate junction has formed (arrows). Bars, 100 μm .

In agametic ovaries, β_{PS} -integrin localizes to the basal membrane of follicle cells as in wild type (Fig. 4, A and B). Markers that normally localize to the lateral membrane [Fascilin III (Fig. 4 C), and DN-cadherin (data not shown)], to the lateral and apical membranes [DE-cadherin (Fig. 4, A and B), Armadillo (data not shown)], or to the apical membrane (β_{Heavy} -spectrin, Fig. 4 D) are excluded from the basal cell pole. Apical and lateral markers show an overlapping distribution at the non-basal cell surface, but are concentrated at the cell pole that opposes the basal membrane. These findings suggest that contact to the basement membrane causes a partial polarization of follicle cells. The basal membrane is established and an asymmetric distribution of apical and lateral markers is observed, but the apical and lateral membrane domains are not clearly demarcated. As the follicle cells express the cadherin–catenin complex constitutively (Peifer et al., 1993; Oda et al., 1997; Godt and Tepass, 1998; Niewiadomska et al., 1999; this work), it appears that basal polarization cues together with the activity of the cadherin–catenin complex are insufficient to fully polarize follicle cells.

Interestingly, the presence of germline cells is required for the accumulation of Crb in follicle cells, as Crb is not detected in follicle cells in agametic ovaries (Fig. 4 E).

Function of Crb and Discs Lost in the Formation of the FE

To study the role of *crb* and *dlt* in the development of the FE, we generated homozygous mutant follicle cell clones. Clones were induced for the two protein-negative *crb* null

alleles *crb^{11A22}* and *crb^{B82}*, for the *dlt* protein negative null allele *dlt^{MY10}*, and the hypomorphic protein-positive allele *dlt^{drel}*. We examined two types of mutant follicle cell clones. Clones were generated either before the formation of the FE by inducing mitotic recombination in the follicle stem cells or their immediate offspring. Alternatively, clones were induced after the formation of the FE by inducing mitotic recombination in region 2b or stage 1 follicles (Fig. 1; see Materials and Methods). Early induced clones can be distinguished from late induced clones by their larger size as all follicle cells derive from only two stem cells, and follicle cells continue to proliferate during early stages of follicle development (Margolis and Spradling, 1995). We considered clones as induced early (early clones) if they comprise 15% or more of the cells of the FE of an individual follicle. Early clones can make up the entire FE of a single follicle. On the other hand, late-induced clones (late clones) typically comprise less than 10 cells at mid to late stages of oogenesis.

A variable phenotype is observed when *crb* mutant follicle cells are generated before the FE forms. Many experimental follicles show an incomplete FE with areas in which the germline cells are not covered by follicle cells (Fig. 5, A and D), a defect that is never seen in wild-type follicles. The area of a follicle not covered by a FE varied greatly in size and in some cases comprised most of the follicle. These gaps in the FE are likely caused by the failure of *crb* mutant follicle cells to integrate into the FE. We were unable to track the missing follicle cells and presume

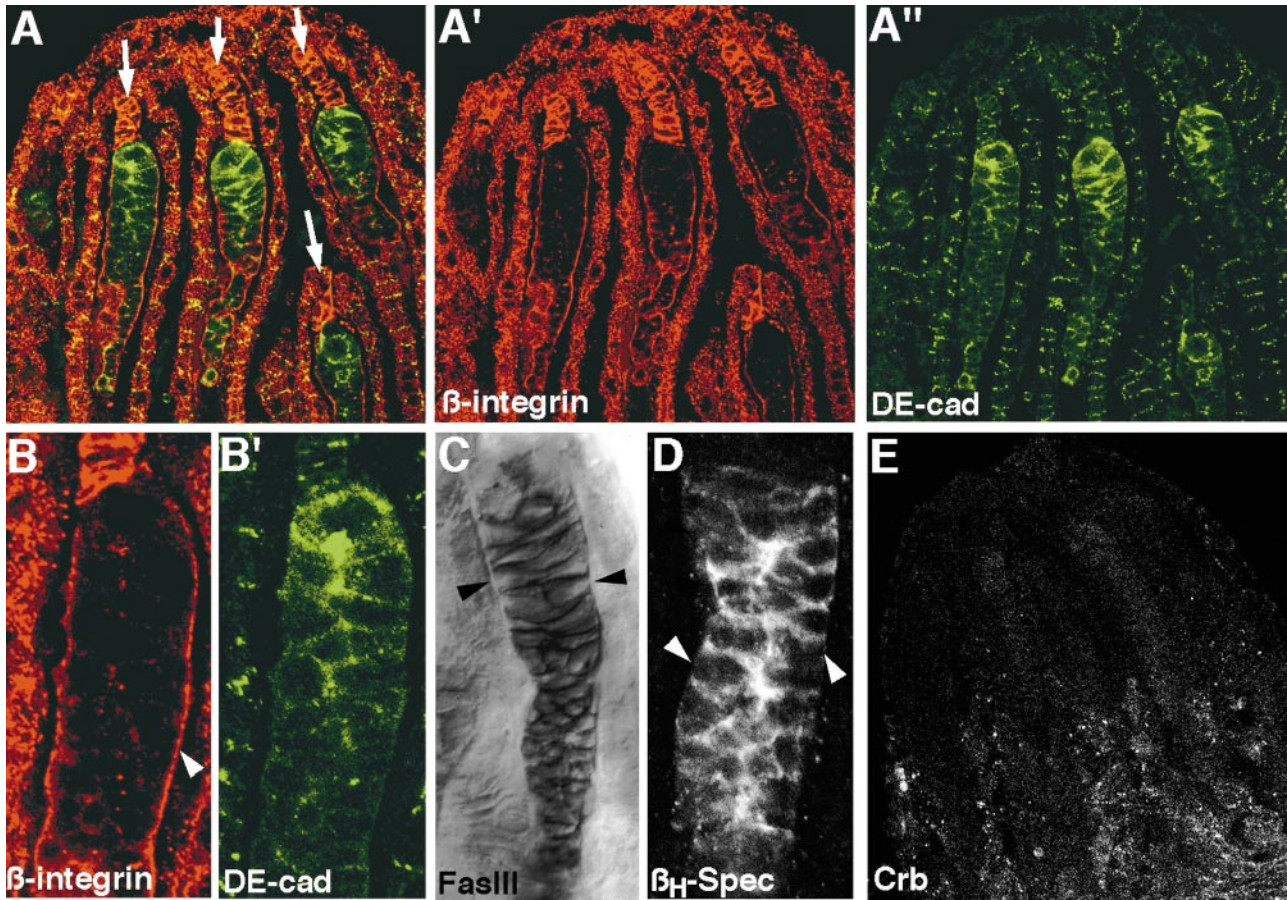


Figure 4. Follicle cells in agametic ovaries show partial polarization. Distribution of epithelial polarity markers in agametic ovaries of flies derived from *oskar³⁰¹* homozygous mutant females (Lehmann and Nusslein-Volhard, 1986). (A) The anterior half of a *oskar³⁰¹* mutant ovary double labeled for β_{PS} -integrin (red) and DE-cadherin (green). The arrows point to individual ovarioles in which the intense DE-cadherin labeling highlights the follicle cells. (B) As in wild type (data not shown), β_{PS} -integrin localizes to the basal membrane of follicle cells in agametic ovaries (arrowhead), whereas DE-cadherin is largely excluded from the basal membrane and accumulates in the remaining cell surface. (C) Fascilin III is excluded from the basal membrane (arrowheads). (D) The apical marker β_{H} -spectrin is excluded from the basal membrane (arrowheads) as well. Apical and lateral markers show an overlapping distribution and concentrate at the center of the follicle cell column at the cell pole that opposes the basal cell surface. (E) An anterior half of an *oskar³⁰¹* mutant ovary (compare with A) labeled for Crb. Crb is not expressed in the follicle cells of *oskar³⁰¹* mutant ovaries.

that they degenerate within the germarium. On the other hand, some *crb* mutant follicle cells can form a FE with apparently normal morphology (Fig. 5, D and E). The distribution of various markers was analyzed in *crb* mutant follicle cells that were part of the FE. Such *crb* mutant follicle cells retained apical Dlt, although the level of Dlt associated with the apical membrane was reduced in these cells (Fig. 5, D and E). The level of β_{Heavy} -spectrin is also slightly reduced compared with wild-type follicle cells (Fig. 5 F). These findings indicating that the apical localization of Dlt and β_{Heavy} -spectrin depends on Crb, but also on other mechanisms. No significant alteration was noticed in the level or distribution of Arm (Fig. 5 G).

dlt^{MY10} and *dlt^{drel}* mutant follicle cells clones, if generated before the formation of the FE, displayed gaps of variable size in the FE similar to those caused by *crb* mutations (Fig. 6, B–D). As *dlt^{drel}* is a protein-positive allele, we were not able to determine whether some *dlt^{drel}* mutant follicle cells become part of the FE. In contrast to *crb* mutant follicle cell clones, we did not find any *dlt^{MY10}* mutant follicle cells that participate in the formation of the

FE in early clones. *dlt^{MY10}* is a deletion that removes the genes encoding *cdc37* and α -spectrin in addition to *dlt* (Bhat et al., 1999; our unpublished observations). The loss of *cdc37* is compensated for by a transgene (see Materials and Methods). Thus, *dlt^{MY10}* mutant clones lack *dlt* and α -spectrin, raising the possibility that the observed mutant phenotype is the consequence of a synergistic effect between *dlt* and α -spectrin mutations, although it was shown that α -spectrin is not required for the formation of the FE (Lee et al., 1997). These findings suggests that Dlt is required, and may be essential for the formation of the FE.

Function of Crb and Discs Lost in Maintaining the FE

Large *crb* mutant clones within the FE that survive until mid-oogenesis often form a multilayered epithelium indicating that Crb is important for the maintenance of the FE. The multilayering of follicle cells in *crb* mutant clones is limited to posterior follicle cells that cover the oocyte (Fig. 5 B). To determine whether removal of Crb and Dlt from cells of the FE disrupts the integrity of the FE, we also examined small *crb*

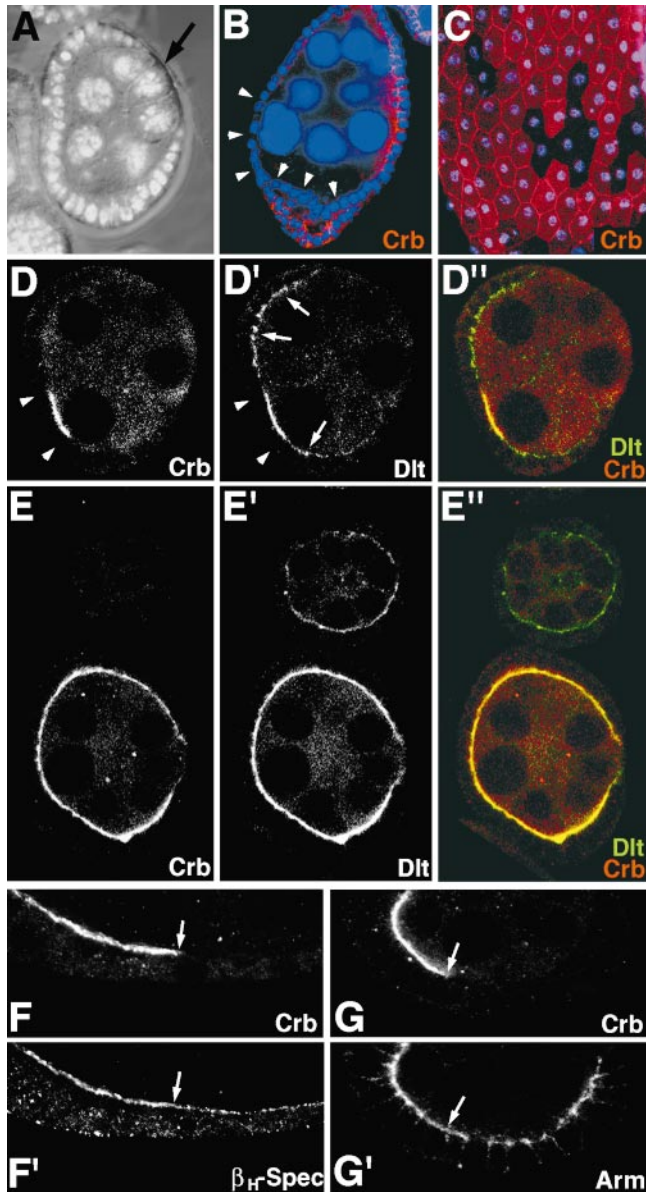


Figure 5. Crb is required for the formation and maintenance of the FE. (A and B) *crb*^{11A22} mutant follicle cell clones induced before the formation of the FE may lead to epithelial discontinuities (A, arrow), or multilayering defects in posterior follicle cells (B, arrowheads indicate *crb* mutant cells). (C) Small *crb*^{11A22} mutant cell clones, induced after the FE has formed, show no morphological defects. A nuclear counterstain has been used in A–C. (D) Follicle with mostly *crb*^{11A22} mutant follicle cells and some *crb* positive cells (between arrowheads). Note the large gap in the FE between 12 and 4 o'clock. The remaining *crb* mutant cells, which have formed an epithelial layer retain apical Dlt (arrows), which, however, is reduced in concentration as compared with wild-type cells. (E) Wild-type follicle (bottom) and a follicle in which all cells of the FE are *crb* mutant (top). Small amounts of Dlt have been retained at the apical membrane of the *crb* mutant follicle cells. (F) *crb*^{11A22} mutant clone shows reduced concentration of β_{Heavy} -spectrin associated with the apical membrane. (G) The distribution and levels of Arm appear normal in *crb* mutant follicle cells. Arrows in F and G point to the boundary between mutant and wild-type cells.

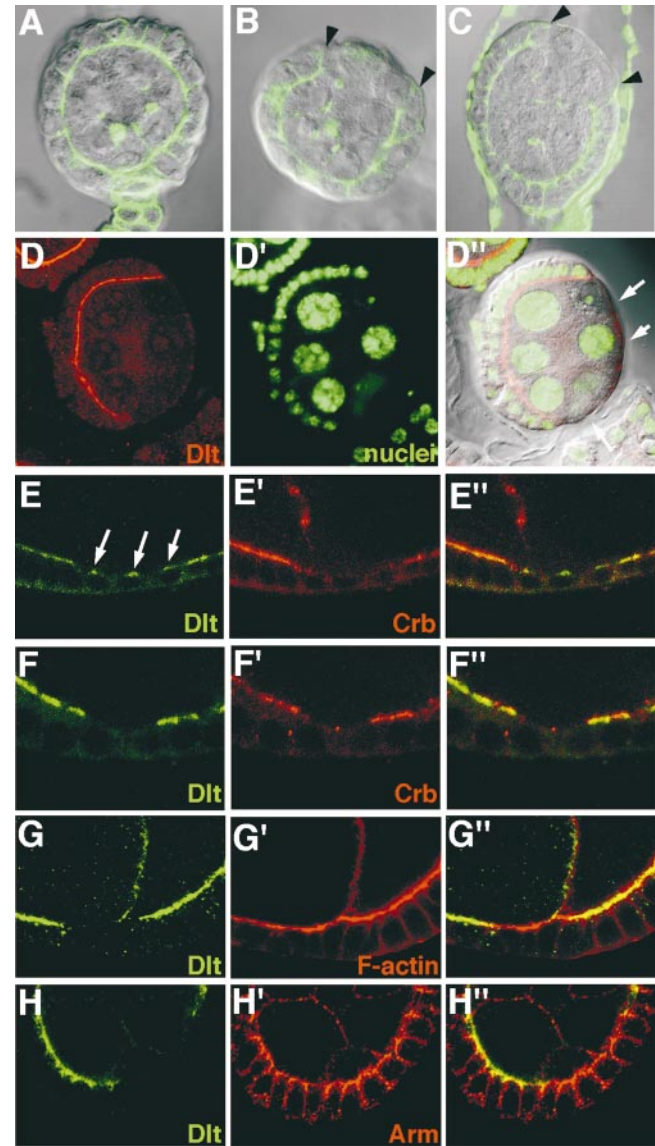


Figure 6. Dlt is required for the formation of the FE and apical localization of Crb. (A) Wild-type stage 2 follicle stained with phalloidin. Note the prominent accumulation of F-actin at the apical surface of the cells of the FE. (B) Stage 2 and (C) stage 4 follicles containing *dlt*^{dre1} mutant early clones. The FE shows gaps (between arrowheads) into which germline cells have penetrated. (D) *dlt*^{MY10} mutant follicle cells, induced before the FE forms, do not form a FE, resulting in follicles with gaps in the FE (arrows). (E–H) Late *dlt* mutant clones. (E) *dlt*^{dre1} and (F) *dlt*^{MY10} mutant follicle cells have lost Crb. Dlt forms a central cap in the apical membrane in *dlt*^{dre1} mutant cells (E, arrows). (G and H) *dlt*^{MY10} mutant follicle cell clones show normal distribution of F-actin (G) and Arm (H).

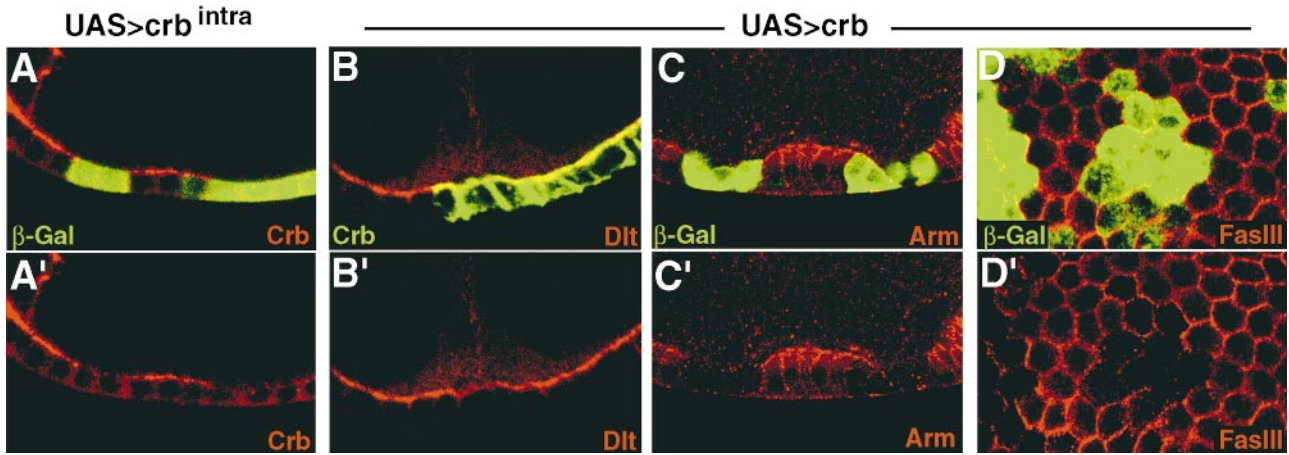


Figure 7. Overexpression of Crb disrupts lateral markers but has little effect on Dlt distribution. (A) Follicle cells overexpressing *UAS>crb^{intra}* (β -Gal) do not contain detectable amounts of endogenous Crb as detected by mAbCq4, which recognizes the extracellular part of Crb that is missing in *Crb^{intra}*. (B) In follicle cells that overexpress full-length Crb (*UAS>crb*), Crb localizes to the apical and lateral membranes, but does not cause a substantial mislocalization of Dlt. (C and D) Levels of Arm (C) and Fasciclin III (D) are strongly reduced in some follicle cells that overexpress Crb (*UAS>crb*). Clones are detected by anti- β -Gal staining.

or *dlt* mutant follicle cell clones that were induced after the FE had been established. Small *crb* mutant cell clones developed normally until late in oogenesis (Fig. 5 C), although they showed a substantial reduction in the level of Dlt (data not shown). Cells in small *dlt* mutant clones develop with apparently normal morphology, and show a normal distribution of F-actin and Arm (Fig. 6, G and H). Crb was lost from most *dlt* mutant clones, indicating that Dlt is important for maintaining apical Crb (Fig. 6, E and F). Some *dlt* mutant clones

retained Crb, suggesting that a mechanism other than binding to Dlt can contribute to maintaining Crb at the apical membrane. Crb was also undetectable in many cell clones mutant for *dlt^{drel}*. Dlt protein in *dlt^{drel}* mutant cells forms a “cap” in the center of the apical membrane rather than being distributed throughout (Fig. 6 C). Together, these findings suggest that the *dlt^{drel}* allele may carry a mutation that specifically disrupts the interaction of Dlt and Crb, but not the interaction of Dlt with other apical factors.

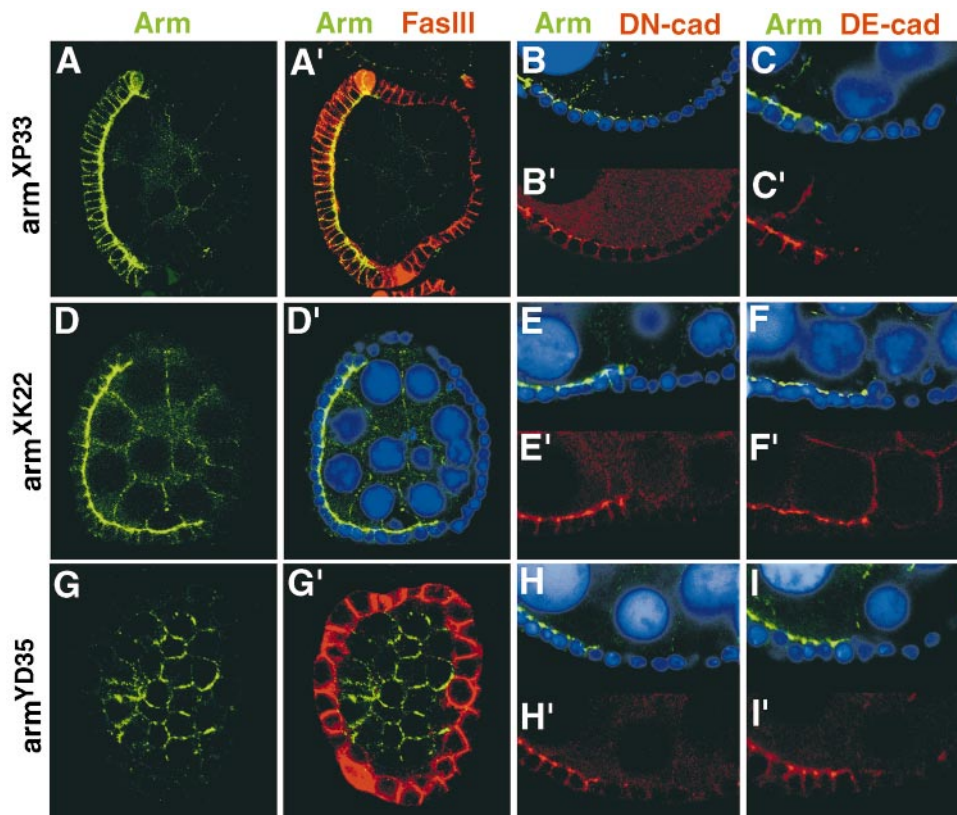


Figure 8. Disruption of the cadherin–catenin complex by *arm* mutations does not interfere with the formation of the FE. Large follicle cell clones mutant for intermediate (*arm^{XP33}*; A–C), strong (*arm^{XK22}*; D–F), and null (*arm^{YD35}*; G–I) alleles of *arm* form a FE. *arm* mutant cells are indicated by the absence of Arm immunoreactivity, and highlighted by Fasciclin III staining (FasIII; A', G') or by a nuclear stain (blue). *arm* mutant cells do not express detectable amounts of DN-cadherin (DN-cad; B, E, and H) or DE-cadherin (DE-cad; C, F, and I).

Crb Overexpression Disrupts Polarity of the FE

We examined the consequences of Crb overexpression in the FE to analyze the response of the FE to mislocalization of Crb, and to further study the interactions between Crb and Dlt. Overexpression of Crb in embryonic epithelia causes a severe disruption of epithelial integrity that includes an extension of the apical cell surface and multilayering of epithelial tissues (Wodarz et al., 1995; Grawe et al., 1996; Klebes and Knust, 2000). We first expressed a truncated form of Crb that lacks all EGF and laminin G domains ($UAS>crb^{intra}$; Wodarz et al., 1995). In embryos, this construct causes a similar phenotype as overexpression of full-length Crb, but, as it is not recognized by our anti-Crb antibody, the effect of the expression of crb^{intra} on the distribution of endogenous Crb can be examined. Expression of $UAS>crb^{intra}$ causes a strong reduction in the level of endogenous Crb, suggesting that crb^{intra} acts competitively with Crb for interaction to binding partners that are important for maintaining Crb in the plasma membrane (Fig. 7 A). Overexpression of full-length Crb leads to misdistribution of Crb into the lateral membrane, at levels that are similar to the apical membrane. In most of these cell clones, we did not see a significant mislocalization of Dlt (Fig. 7 B), suggesting again that the apical localization of Dlt is at least in part Crb independent. A fraction of the Crb overexpressing follicle cells show mislocalization of Dlt, a thinning of the epithelium, and a strong reduction of lateral markers such as Arm and Fasciclin III (Fig. 7, C and D). These findings indicate that overexpression of Crb disrupts the lateral membrane in follicle cells.

Role of the Cadherin–Catenin Complex in the Differentiation of the FE

The cadherin–catenin complex plays a major role in epithelial polarization as cadherin-mediated adhesive contacts cause the assembly of the lateral surface domain. Consequently, lack of cadherin activity compromises epithelial integrity in many tissues or epithelial cell culture lines (Drubin and Nelson, 1996; Tepass, 1997). Removal of DE-cadherin from follicle cells causes only mild defects in the development of the FE (Godt and Tepass, 1998). In follicle cells that lack DE-cadherin, some Arm is retained at adherens junctions, raising the possibility that the FE coexpresses a second cadherin that interacts with Arm. Indeed, we find that DN-cadherin (Iwai et al., 1997) is expressed in follicle cells in a pattern that overlaps with DE-cadherin in early to mid oogenesis (Fig. 8; data not shown). DN-cadherin disappears from the FE at stage 10 of oogenesis, whereas DE-cadherin is expressed throughout oogenesis. In contrast to DE-cadherin, DN-cadherin is not expressed in the cells of the germline (data not shown). On the other hand, Arm appears to be the only β -catenin homologue in *Drosophila*, in contrast to vertebrates, where β -catenin can be functionally replaced by plakoglobin in the cadherin–catenin complex (e.g., Huelsken et al., 2000). Thus, to effectively remove the cadherin–catenin complex from follicle cells, we generated clones that lack Arm, which was previously shown to interact with both DE- and DN-cadherin (Peifer, 1993; Oda et al., 1993, 1994; Iwai et al., 1997).

Clones were induced for three different mutant *arm* alleles that carry premature stop codons in the 6th (arm^{YD35}), 7th (arm^{XK22}), and 10th (arm^{XP33}) *arm* repeat. arm^{XP33} is an intermediate hypomorph, arm^{XK22} is a strong hypomorph, and arm^{YD35} is believed to be a null allele (Peifer and Wieschaus, 1990). Embryos derived from germline clones mutant for arm^{XP33} show a dramatic disruption of epithelial morphology that occurs at the onset of gastrulation and is substantially more severe than the defects in epithelial structure seen in *crb* null mutant embryos (Cox et al., 1996; Müller and Wieschaus, 1996; Tepass, 1996, 1997). Moreover, it has been shown previously that Crb is needed for the formation of the ZA in embryonic epithelia (Tepass, 1996; Grawe et al., 1996). If the failure to form the ZA is the major consequence of compromising Crb or Dlt activity in the FE, we would expect the lack of the cadherin–catenin complex to cause similar defects as seen in *crb* and *dlt* mutant follicle cells; that is, a failure to form a FE. Surprisingly, we find that follicle cells mutant for any of the three *arm* alleles form a FE (Fig. 8, A, D, and G). No follicles were observed in these experiments that show epithelial discontinuities, as seen in *crb* and *dlt* mutant follicles. *arm* mutant follicle cells often show an irregular morphology at early stages of follicle development. The irregularities in epithelial structure increase in severity until the FE is compromised and the follicle degenerates at mid to late oogenesis. To determine whether adherens junctions were effectively disrupted in *arm* mutant follicle cells, we examined the expression of DE- and DN-cadherin in those cells. Neither DE- nor DN-cadherin are detected in follicle cells mutant for any of the three *arm* alleles studied (Fig. 8, B, C, E, F, H, and I). Taken together, these findings suggest that cadherin-based adherens junctions are not essential for the formation of the FE, but are important for maintaining its epithelial structure.

We took advantage of the fact that *arm* mutant cells in the FE are maintained for several days and analyzed their molecular architecture. arm^{XP33} mutant follicle cells (see also Müller, 2000), which in most cases have a normal cuboidal to columnar shape, show a decrease of F-actin and α -spectrin at the lateral membrane, and an accumulation of these molecules at the apical cell pole (Fig. 9, A and B). In contrast, the apical marker β_{Heavy} -spectrin shows a normal distribution in arm^{XP33} mutant cells (Fig. 9 C), suggesting that the apical spectrin cytoskeleton is intact. Follicle cells mutant for arm^{XK22} or arm^{YD35} often develop a squamous cell morphology (Fig. 9, D–F) or show a multilayered structure. α -Spectrin remains associated with the narrow lateral membranes in squamous *arm* mutant cells (Fig. 9 D). β_{Heavy} -Spectrin, on the other hand, is lost from these follicle cells, suggesting that the apical spectrin cytoskeleton is disrupted (Fig. 9 E). To further examine the apical surface domain of *arm* mutant follicle cells, we studied the distribution of Crb and Dlt in these cells. arm^{XP33} mutant follicle cells show typically a normal apical localization of Crb and Dlt (Fig. 10, A and B). In contrast, Crb is lost from the apical membrane of follicle cells mutant for strong *arm* alleles, whereas apical Dlt is retained in these cells (Fig. 10, C–E). Similar to *dlt^{dre1}* mutant cell clones, Dlt forms a cap in the center of the apical membrane of *arm* mutant follicle cells. These observations suggest that the disruption of adherens junctions leads to a

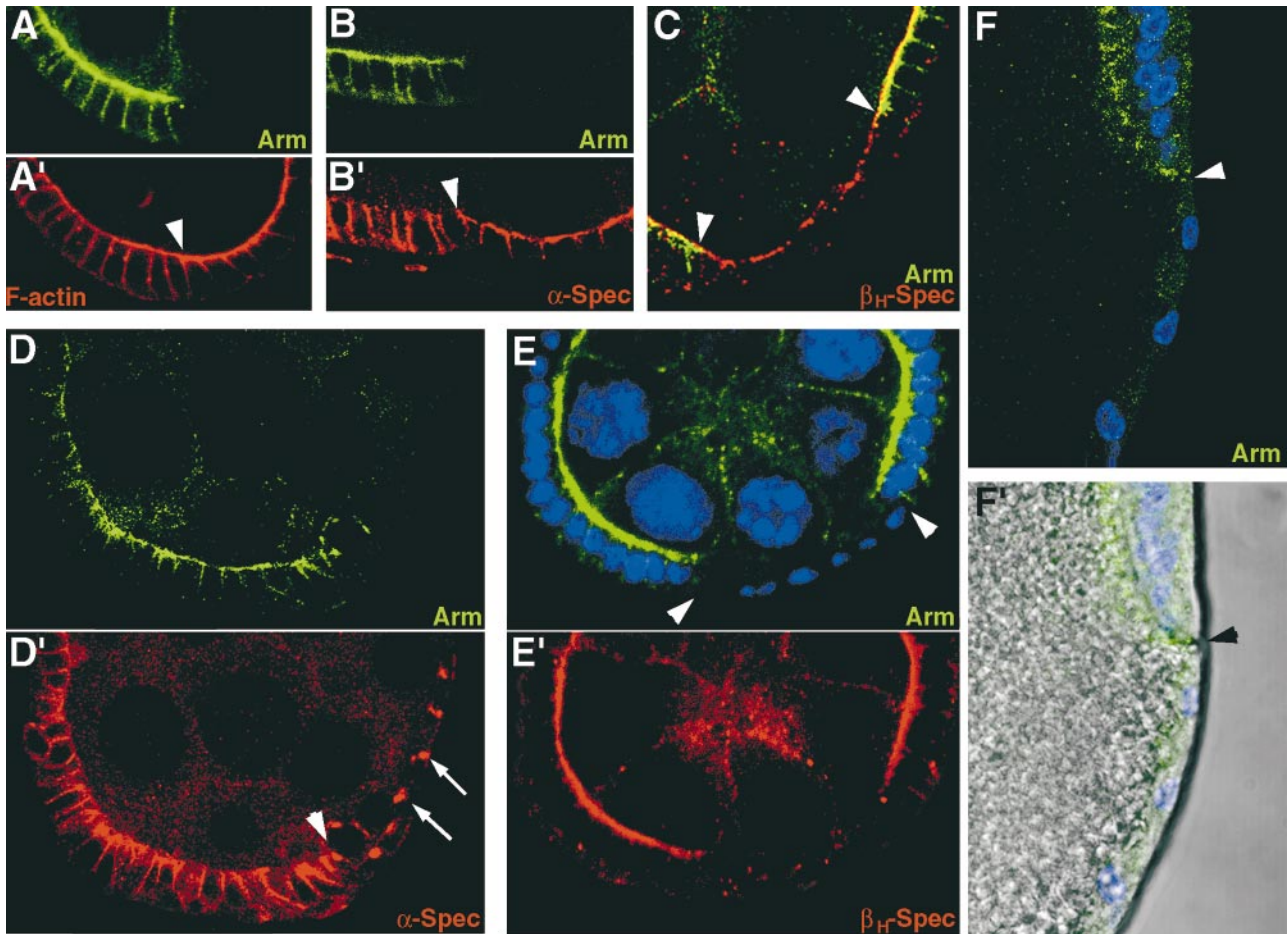


Figure 9. Distribution of F-actin, α -Spectrin (α -Spec) and β_{Heavy} -Spectrin (β_{H} -Spec) in *arm* mutant follicle cells. (A–C) *arm*^{XP33} mutant follicle cells (arrowheads point to clone boundary) show a strong reduction at the lateral membrane and an apical accumulation of F-actin (A) and α -Spectrin (B). Normal amounts of β_{Heavy} -Spectrin are associated with the apical membrane in *arm*^{XP33} mutant cells (C). (D and E) Follicle cells mutant for *arm*^{XK22} (arrowheads) have a flat cell shape and show a reduction in the size of the lateral membrane domain as indicated by the α -Spectrin staining (arrows). Apical β_{Heavy} -Spectrin is lost from *arm*^{XK22} mutant follicle cells (E). (F) *arm*^{YD35} mutant follicle cells (below arrowhead) show squamous cell morphology in this stage 10 follicle. Nuclei are stained blue in E and F.

breakdown of the lateral membrane domain, as expected, but also compromises the apical surface domain. The differential behavior of Crb and Dlt in strong *arm* mutant cell clones again emphasizes that Dlt can rely on a Crb-independent apical targeting mechanism, and shows that apical Dlt can be retained in the absence of an apical spectrin cytoskeleton.

Discussion

The polarization cues that contribute to the differentiation of the FE described here participate in the development of a number of other epithelia (Eaton and Simons, 1995; Drubin and Nelson, 1996; Tepass, 1997; Yeaman et al., 1999). They include contact with the basement membrane, lateral adhesion mediated by the cadherin–catenin complex, and apical polarization that, in *Drosophila*, involves Crb. The FE is unique among epithelia characterized to date as it is the first epithelium for which polarization cues operating in parallel at all three cell surfaces have been documented. Further, our observations concerning the interactions between Crb and Dlt suggest that both proteins

interact functionally in epithelial polarization, but also that the activity of Dlt is in part independent of Crb.

Activity of Apical, Lateral, and Basal Cues in the Differentiation of the FE

Contact of follicle cells with basement membrane appears sufficient to establish a basal membrane from which apical and lateral markers are excluded. Contact to extracellular matrix (ECM) material is important for the polarization of a number of epithelia including the *Drosophila* midgut epithelium and the dorsal vessel (Yarnitzky and Volk, 1995; Haag et al., 1999), as well as vertebrate kidney epithelia and cell culture lines such as MDCK (Eaton and Simons, 1995; Yeaman et al., 1999). Contact of MDCK cells to ECM not only establishes a basal membrane, but also leads to the formation of microvilli throughout the non-basal plasma membrane (Vega-Salas et al., 1987; Ojakian and Schwimmer, 1988). Such long-range effects of basal contacts in the differentiation of epithelial membrane domains may also contribute to the differentiation of the FE as apical and lateral markers, such as β_{Heavy} -Spectrin and DE-cadherin, are not uniformly distributed over the non-

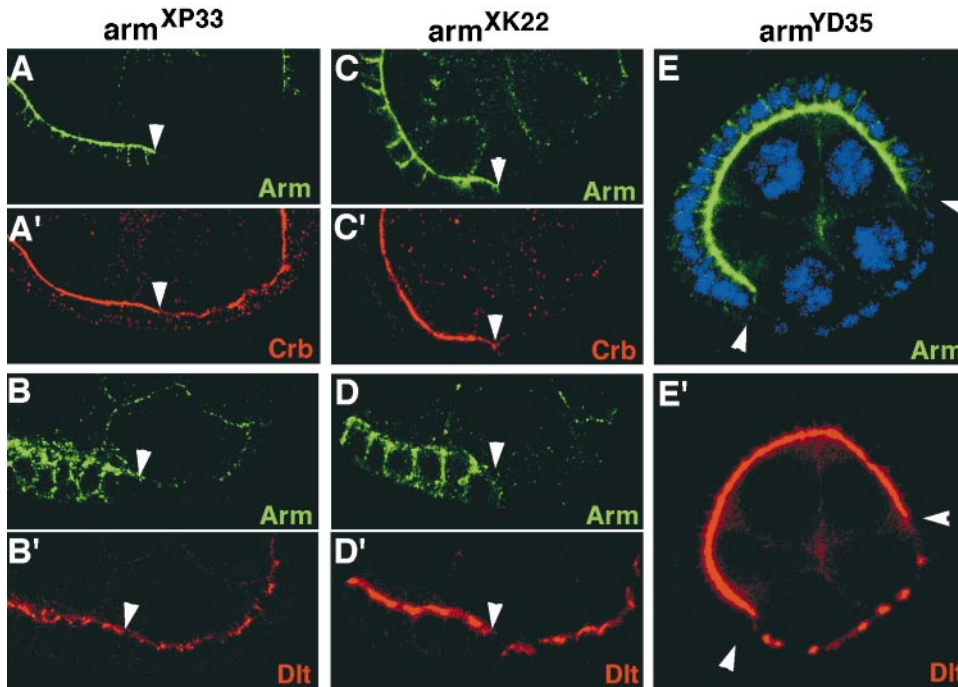


Figure 10. Crb and Dlt distribution in *arm* mutant follicle cells. (A and B) *arm^{XP33}* mutant follicle cells show a normal apical distribution of Crb and Dlt. (C–E) Crb is lost in *arm^{XK22}* and *arm^{YD35}* mutant follicle cells (C), but Dlt is retained at the apical membrane in these cells (D and E). Nuclei are stained blue in E. Clone boundaries are marked by arrowheads.

basal plasma membrane of follicle cells in agametic follicles, but are concentrated at the cell pole that opposes the basement membrane. These findings suggest that follicle cells express extracellular matrix receptors that contribute to epithelial polarization. However, these extracellular matrix receptors together with the activity of the cadherin–catenin complex are not sufficient to cause full epithelial polarization of follicle cells.

One unusual feature that sets the FE apart from most other epithelia is that its apical surface is not a free surface but contacts the cells of the germline. Contact of follicle cells to the germline is essential for the formation of the FE (Margolis and Spradling, 1995; Goode et al., 1996b). Interaction between germline and follicle cells is also required for the accumulation of Crb in follicle cells, as indicated by the absence of Crb from follicle cells in agametic ovaries. In wild-type ovaries, Crb and Dlt are initially detected in all follicle cells, but both proteins rapidly disappear from the cells that form the interfollicular stalk. Both proteins are retained only in those cells that give rise to the FE in which they are found at the apical membrane. The subcellular distribution of Crb and Dlt in the FE and the fact that both proteins are required for the differentiation of the FE suggest that the mechanism of apical polarization of the FE is similar to epithelia that have a free apical surface (Tepass et al., 1990; Tepass and Knust, 1990; Bhat et al., 1999).

Crb and Dlt are the first identified structural components of the FE that are essential for the formation of this epithelium. The activity of two other genes, *brainiac* (*brn*), which encodes a secreted protein, and *egghead* (*egg*), which encodes a transmembrane protein, are also important for the formation of the FE as mutations in both genes cause epithelial discontinuities in the FE similar to mutations in Crb and Dlt (Goode et al., 1992, 1996a,b). Both *brn* and *egg* are expressed exclusively by the germline, suggesting that these genes contribute to germline-

follicle cell interactions critical for FE formation. Interestingly, the EGF receptor (*Egfr*) pathway mediates interactions between germline and follicle cells that contribute to the formation of the FE. Compromising the activity of Gurken, a transforming growth factor- α -like ligand of *Egfr* expressed by the germline, or of *Egfr* causes gaps in the FE similar to those seen in *crb* or *dlt* mutant follicle cell clones (Goode et al., 1996a). These findings raise the possibility that the germline-dependent expression of Crb in follicle cells may be controlled by *Egfr* signaling.

Crb is also required for the maintenance of the FE as Crb mutant clones develop a multilayered structure by mid oogenesis. This defect, which is seen in posterior follicle cells that cover the oocyte, was only observed in large *crb* mutant clones induced before the formation of the FE, but not in small *crb* mutant clones. Absence of structural defects in small *crb* mutant cell clones might be due to perdurance; that is, the cytoplasmic inheritance of *crb* gene product, after a *crb* mutant clone was induced. We believe that this possibility is unlikely as small *crb* mutant clones do not contain detectable levels of Crb 2 d after they were induced, and are then maintained in the FE for several days until late oogenesis. An alternative possibility that would explain the differences in the behavior of small and large *crb* mutant clones is to assume that the structural defect caused by the lack of Crb is rather weak, so that effects on epithelial tissue structure are manifested only as a “community effect;” that is, when small structural defects in a large number of cells enhance each other, leading eventually to a collapse of normal tissue architecture.

Mutations in a number of other genes, including α -*spectrin*, *brn*, *egg*, and *Notch*, also cause multilayering of the FE that is limited to the follicle cells that cover the oocyte and develop by mid oogenesis (stage 7 and beyond; Goode et al., 1996b; Lee et al., 1997). Defects in epithelial architecture that are more severe than those seen in large *crb* mutant cell clones are detected in follicles mutant for *discs*

large, lethal giant larva, and scribble (Manfrulli et al., 1996; Goode and Perrimon, 1997; De Lorenzo et al., 1999; Bilder et al., 2000), as well as *arm* (this work). These four genes encode cytoplasmic factors that are predominantly associated with the lateral cytocortex of epithelial cells. Taken together, the multitude of factors now known to contribute to maintaining the FE, and the fact that most factors cause only moderate defects in epithelial structure if removed, suggest that the stability of the FE is controlled by several molecular pathways that act in parallel and may overlap in function.

The cadherin–catenin complex is required for maintaining the integrity of the FE, but, surprisingly, not for its initial formation. Embryos mutant for the intermediate allele *arm*^{XP33} show a dramatic and rapid collapse of the ectodermal epithelium at gastrulation after the blastoderm epithelium has been established (Cox et al., 1996; Müller and Wieschaus, 1996). In contrast, follicle cell clones mutant for this allele of *arm* are maintained with normal gross morphology for several days. The lateral cytocortex is disrupted in these cells as F-actin and α -spectrin are strongly decreased in concentration at the lateral plasma membrane, and have apparently relocated to the apical cell pole, where they may associate with the apical membrane or the ZA. Also, follicle cells mutant for an *arm* null allele form a FE, but display an irregular morphology already at early stages of oogenesis. These mutant cells often develop a squamous cell shape, which suggests a decrease in the size of the lateral membrane. A correlation between the expression level of the cadherin–catenin complex and epithelial cell shape has also been found in *Xenopus* ectodermal cells that develop a cuboidal or columnar shape in response to the overexpression of N-cadherin (Detrick et al., 1990; Fujimori et al., 1990).

The finding that the cadherin–catenin complex is dispensable for the formation of the FE, and that in its absence integrity is lost not rapidly, but slowly, over a period of several days, implies that the FE has mechanisms that can compensate for the loss of this complex, although this complex plays a key role in epithelial development in other *Drosophila* and vertebrate epithelia (Tepass, 1997; Yeaman et al., 1999). One possibility is that the basal and apical polarization cues are sufficient to establish epithelial polarity in follicle cells, and align these cells in an epithelial layer. Moreover, apical adhesion to germline cells and basal adhesion to a basement membrane might constrain the follicle cells in a monolayered sheet. Finally, the FE might express an alternative lateral adhesion system that can assemble a lateral cytocortex and even a ZA, as exemplified by *Caenorhabditis elegans* epithelia that form and are maintained in the absence of a classic cadherin and associated catenins (Costa et al., 1998; Raich et al., 1999).

One interesting observation in *arm* mutant follicle cells is the disruption of the apical cell pole. Whereas several apical markers are maintained in *arm*^{XP33} mutant follicle cells, Crb and β _{Heavy}-Spectrin are lost from the apical surface in cells mutant for strong or null alleles of *arm*. In contrast, Dlt still associates with the apical membrane of *arm* mutant follicle cells, suggesting that the apical membrane has not disappeared entirely, but shows specific molecular defects. Previously, it was shown that Crb is needed for the formation of the ZA in early *Drosophila* embryos (Grawe et al., 1996; Tepass, 1996) and that β _{Heavy}-

spectrin is required for maintaining a continuous ZA in imaginal disc epithelia and in the FE (Thomas et al., 1998; Zarnescu and Thomas, 1999). Taken together, these data reveal mutual dependencies in the stability of molecular complexes that contribute to epithelial polarity. These complexes may interact directly at the transition zone between the apical and lateral membranes, where both complexes are enriched, Crb and the apical spectrin cytoskeleton at the marginal zone, and the cadherin–catenin complex at the ZA.

Interactions between Crb and Dlt

Dlt was shown to interact with the cytoplasmic tail of Crb in vitro and in vivo, an interaction that involves the COOH-terminal five amino acids in Crb and presumably the first of four PDZ domains in Dlt (Bhat et al., 1999; Klebes and Knust, 2000). In epithelia that express Crb and Dlt, both proteins colocalize at the apical membrane, where they concentrate just apical to the ZA, in the marginal zone of the apical membrane, and are found at a lower concentration in the remaining apical membrane (Fig. 4; Tepass, 1996). IEM shows that Dlt, like Crb, straddles the apical edge of the ZA, but is not a component of the ZA or the septate junction. This finding suggests that Dlt does not interact with Neurexin IV at the septate junction, as was proposed previously (Bhat et al., 1999).

Comparative analysis of the *crb* and *dlt* mutant phenotypes thus far did not directly support a functional interaction between these genes. Removal of Dlt from embryos causes severe defects at pregastrula stages that mask potential defects at later embryonic stages when Crb and Dlt are co-expressed (Bhat et al., 1999). Dlt is also required for the development of imaginal disc epithelia, whereas Crb is not, although Crb and Dlt are coexpressed in those epithelia (Tepass and Knust, 1990; Bhat et al., 1999). Thus, our finding that *crb* and *dlt* mutant follicle cell clones show similar defects is the first direct phenotypic evidence that these genes interact functionally to support epithelial development. Dlt is required to maintain Crb at the apical surface. On the other hand, four observations support the conclusion that apical Dlt localization is at least in part independent of Crb. (a) Dlt is found at the apical membrane, although at reduced levels, in Crb mutant follicle cell clones. Not only is the retention of Dlt at the apical membrane independent of Crb, but also its initial localization as follicle cells that have been rendered *crb* mutant before they would normally express Crb still show apically localized Dlt. (b) Dlt is also retained apically in *dlt*^{dre1} mutant follicle cells that have lost Crb, and (c) in *arm*^{XK22} and *arm*^{YD35} mutant follicle cells that have lost Crb as well. (d) Dlt retains its apical localization in many follicle cells that overexpress Crb and in which Crb accumulates at the lateral membrane. Moreover, reduced levels of Dlt are retained at the apical membrane in embryonic and imaginal epithelia that have not lost integrity in *crb* mutants (our unpublished observations). These findings suggest that Dlt interacts with another apical factor or factors different from Crb, which remain to be identified.

Assuming that Crb activity is mediated through apical Dlt, the Crb-independent retention of Dlt might explain the variability in the *crb* mutant phenotype observed in follicle cells and other epithelia (Tepass and Knust, 1990). A finding that corroborates this explanation of the vari-

able nature of the *crb* mutant phenotype is that *dlt*^{MY10} mutant follicle cells induced before the formation of the FE show no variability and, consistently, do not form a FE. A caveat of this conclusion is that *dlt*^{MY10}, the only available null allele of *dlt*, is a deletion that uncovers α -spectrin and *cdc37*, in addition to *dlt* (Bhat et al., 1999). The function of *cdc37* has been restored by a *cdc37* transgene in our experiments so that *dlt*^{MY10} mutant clones lack only Dlt and α -spectrin. Although it was shown that the lack of α -Spectrin does not interfere with the formation of the FE (Lee et al., 1997), we cannot rule out the possibility that the consistent defects cause by *dlt*^{MY10} are a result of synergistic interactions between α -spectrin and *dlt*. Analysis of a second, hypomorphic *dlt* allele, *dlt*^{dre1}, which also causes epithelial discontinuities in the FE, demonstrates that *dlt* similar to *crb* is required for the formation of the FE. Together, these results suggest that Crb and Dlt form a complex at the apical membrane of follicle cells that plays an important role in the formation and maintenance of the FE.

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