Integrated activity of PDZ protein complexes regulates epithelial polarity

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Polarized cells contain numerous membrane domains, but it is unclear how the formation of these domains is coordinated to create a single integrated cell architecture. Genetic screens of *Drosophila melanogaster* embryos have identified three complexes, each containing one of the PDZ domain proteins — Stardust (Sdt), Bazooka (Baz) and Scribble (Scrib) — that control epithelial polarity and formation of zonula adherens. We find that these complexes can be ordered into a single regulatory hierarchy that is initiated by cell adhesion-dependent recruitment of the Baz complex to the zonula adherens. The Scrib complex represses apical identity along basolateral surfaces by antagonizing Baz-initiated apical polarity. The Sdt-containing Crb complex is recruited apically by the Baz complex to counter antagonistic Scrib activity. Thus, a finely tuned balance between Scrib and Crb complex activity sets the limits of the apical and basolateral membrane domains and positions cell junctions. Our data suggest a model in which the maturation of epithelial cell polarity is driven by integration of the sequential activities of PDZ-based protein complexes.

Metazoan cells often polarize along a single axis, such as the somatodendritic axis of neurons or the apicobasal axis of epithelia. However, the organization of cellular components is not limited to a simple bipartite decision. For instance, the basolateral membrane of epithelia contains distinct regions, such as the tight junction (TJ), zonula adherens and hemidesmosomes, as well as apical and basolateral compartments. Differentiation of the varied epithelial membrane domains requires multiple systems of organization, but the generation of a unified architecture requires coordination between these systems. Although molecules that regulate polarity are being rapidly identified, the functional links between different polarizing systems have been difficult to analyse. Epithelial morphogenesis in the *Drosophila* embryo provides a simple system in which to investigate these questions.

Genetic analysis has identified eight proteins, divided into three groups (Table 1), that are required for epithelial polarity. Formation of the apical domain is dependent on the Crb complex, which is composed of the transmembrane protein Crumbs (Crb) and its PDZ domain partner Sdt[1–4]. As ectopic expression of *Crb* on basolateral surfaces is sufficient to recruit other apical proteins, Crb has been proposed to be necessary and sufficient for the determination of apical membrane identity[5]. A second protein complex, the Baz complex, contains the PDZ proteins Baz, dmPar-6 and the atypical protein kinase aPKC[6–8]. Embryos maternally and zygotically mutant for *baz* (*baz* M/Z) display disrupted cell junctions during gastrulation[9]. A third complex, the Scrib complex, contains the PDZ proteins Scrib and Discs-large (Dlg) and is required for apical polarity. The Scrib complex recruits the apparently non-complexed Lethal giant larvae (Lgl) (J. Zeitler and D. B., unpublished observations) to the cell membrane[10–14]. In *scrib* mutant embryos (*scrib* M/Z), apical proteins and zonula adherens components become distributed throughout the basolateral domain.

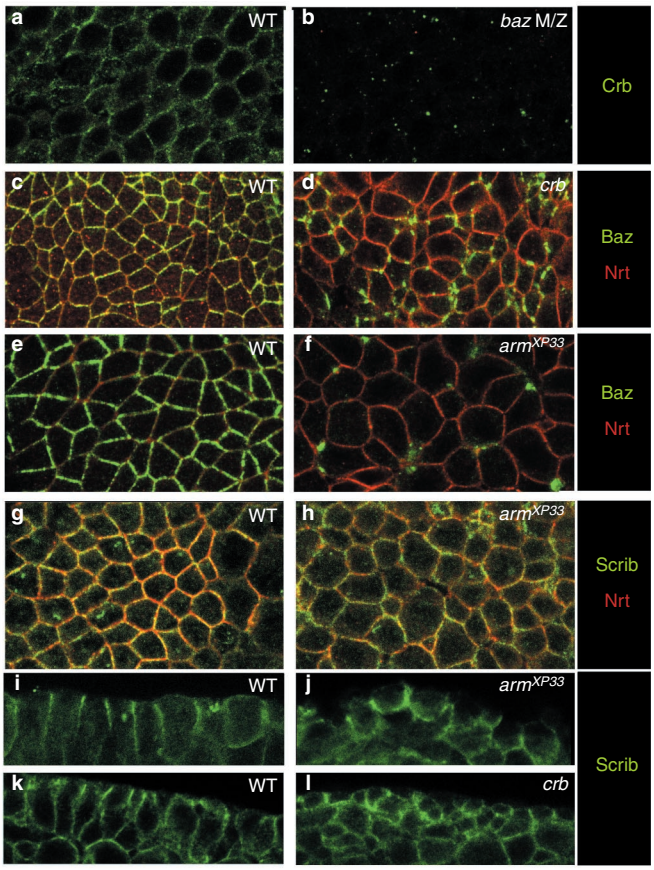

Figure 1 **Localization of PDZ protein complexes.** Confocal microscopy images of embryos stained for PDZ complex proteins (green). Staining for neurotactin (Nrt) (red in **c**–**h**) outlines cell membranes. **a**, **b**, Crb is localized to apical surfaces in stage-7 wild-type embryos (**a**), but not in stage-7 *baz* M/Z mutant embryos (**b**). **c**, **d**, Baz is localized to apical surfaces in stage-9 wild-type embryos (**c**), but not in stage-9 *crb* embryos (**d**). **e**–**j**, Compared with wild-type embryos (**e**), Baz is also lost apically in stage-6 arm^{XP33} M/Z mutants (**f**), whereas Scrib remains associated with basolateral membranes (**h**, **j**; compare with wild-type embryos, **g** and **i**). Compared with wild-type embryos (**k**), loss of *Crb* does not cause apical mis-localization of Scrib at stage 11 (**l**).

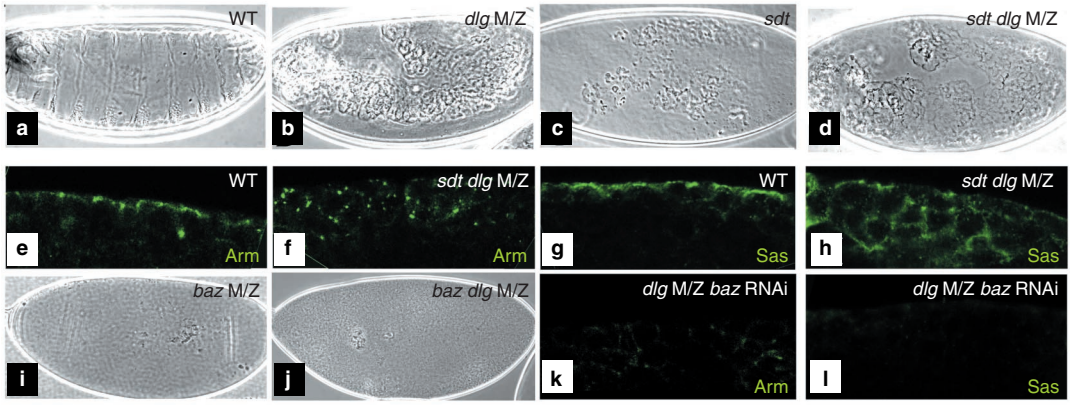

Figure 2 **Epistasic analysis of *dlg* with *sdt* and *baz*. a–d**, Cuticle preparations of wild-type (**a**), *dlg* M/Z (**b**), *sdt* (**c**) and *sdt dlg* M/Z (**d**) embryos are shown. Note the distinct phenotypes of *dlg* M/Z and *sdt*, and that the double mutant has a *dlg* M/Z-like phenotype. **e–h**, Confocal cross-sections reveal that adherens junctions (anti-Arm) are basolaterally mis-localized in stage-11 *sdt dlg* M/Z embryos (**f**, compare with wild-type embryos in **e**), as is the apical marker Sas (**h**, compared with wild-type embryos in **g**); these are *dlg*-like phenotypes. **i**, **j**, Cuticle preps of *baz* M/Z (**i**) and *baz dlg* M/Z embryos (**j**). Both animals produce little to no cuticle. **k**, **l**, *dlg* M/Z *baz* RNAi embryos at stage 11 show mis-localization of adherens junctions (**k**; anti-Arm) and depletion of Sas (**l**), which are *baz* M/Z-like phenotypes.

Although the roles of individual PDZ protein complexes in polarizing different cell types are the subject of intense scrutiny, it is unclear how such complexes are coordinated to construct a unified cell architecture. Here, we investigated the relationship between the complexes that regulate epithelial polarity in *Drosophila* and showed that the three PDZ-based complexes are integrated into a single, sensitively balanced, regulatory network.

To evaluate possible interactions between the Baz, Crb and Scrib complexes, we examined their subcellular localization during embryogenesis (summarized in Fig. 5). Crb is found apical to the zonula adherens, whereas Baz complex proteins are localized to both the zonula adherens and to regions apical to the zonula adherens (data not shown)[6,7,15]. In contrast, Scrib complex proteins are found basal to the zonula adherens[10,12]. The high colocalization of Baz and Crb suggests that these complexes are engaged in positive regulatory relationships, whereas the distinct localization of Scrib suggests a negative regulatory relationship with the other two complexes.

First, we investigated the relationship between Baz and Crb. Zonula adherens defects in *baz* M/Z embryos occur at stage 6, preceding defects in *crb* M/Z embryos, which occur at stage 8 (refs 9,15,16 and data not shown). In *baz* M/Z gastrulae, Crb protein levels were reduced and distributed in a non-polarized fashion (Fig. 1b). In *crb* cellular blastoderms, however, Baz localized normally to the primitive zonula adherens, but became markedly mis-localized after gastrulation (Fig. 1d). Thus, Baz functions before Crb and is required to establish the apical localization of Crb, whereas Crb maintains Baz at apical membranes.

We wondered what factors regulate the initial localization of Baz to apical membranes. In epithelial cell culture systems, polarization is initiated by primitive zonula adherens formation at regions of cell–cell contact[17]. We tested whether formation of the primitive zonula adherens is required for the localization of Baz by examining arm^{XP33} M/Z mutants, which are defective in the early stages of zonula adherens formation[18]. In these embryos, localization of Baz was lost from stage 6 onwards (Fig. 1f). Localization of Crb, Discs lost (Dlt) and the apical protein Stranded at second (Sas) was also defective (data not shown), further demonstrating that apical polarity in *Drosophila* requires zonula adherens formation.

We performed similar experiments to identify the stage that Scrib interacts with the regulatory apical pathway. In common with *crb* mutants, *scrib* M/Z mutants fails to assemble a continuous zonula adherens. However, in *scrib* M/Z mutants, adherens junction material reaches the cell surface, but is mis-localized to basolateral membranes[10]. The onset of zonula adherens disruption occurs almost simultaneously in *scrib* M/Z and *crb* mutants (data not shown), suggesting that these complexes function at a similar stage of zonula adherens assembly. Because Scrib restricts Crb to apical membranes, we asked whether Crb might function reciprocally to restrict Scrib to basolateral membranes. In stage-12 *crb* epidermis, Scrib is excluded from apical membranes, although it is not enhanced at baolateral membranes, as in wild-type epidermis (Fig. 1l). These data exclude the possibility that apical mis-localization of Scrib causes the loss of apical characteristics in *crb* mutants. Furthermore, Scrib remained localized to basolateral membranes in arm^{XP33} M/Z embryos (Fig. 1h, j). Thus, the basolateral localization of Scrib does not require apical polarity or formation of the zonula adherens.

The independence of Scrib localization from the activities of Crb and Baz raises the possibility that Scrib functions in a distinct polarity pathway. However, we identified genetic interactions between Scrib and Crb, suggesting that these components do, in fact, function in a common pathway. Overexpression of *Crb* in the wing resulted in the production of excess cuticle, consistent with an expansion of apical membrane (see Supplementary Information, Fig. S1). These defects resemble those observed in *dlg* mutant clones, indicating that in the wing, as well as in the embryo[10], overexpression of *Crb* can mimic *scrib* group loss-of-function. Notably, heterozygosity for either *dlg* or *scrib* enhances the ectopic Crb wing phenotype, causing extensive cuticular elaboration. Halving *scrib* activity does not affect localization of the wild-type protein, but apparently renders the tissue more sensitive to apical polarizing activity. These genetic interactions demonstrate that Scrib and Crb functionally interact, and that Scrib antagonizes apical polarity in a dose-dependent manner.

The data presented above are consistent with a pathway in which Baz and Crb direct apical polarity and in which Scrib antagonizes apical regulatory activity along basolateral membranes, preserving basolateral characteristics. We wondered what step in the apical regulatory pathway Scrib antagonizes, that is, whether Scrib inhibits the activity of either Baz, Crb, or some unidentified apical regulator.

Because of the similar onset of *scrib* M/Z and *crb* phenotypes, we first examined the relationship between these two complexes. Ectopic basolateral localization of Crb is sufficient to phenocopy *scrib* M/Z embryos[5,10], suggesting that Scrib might antagonize apical

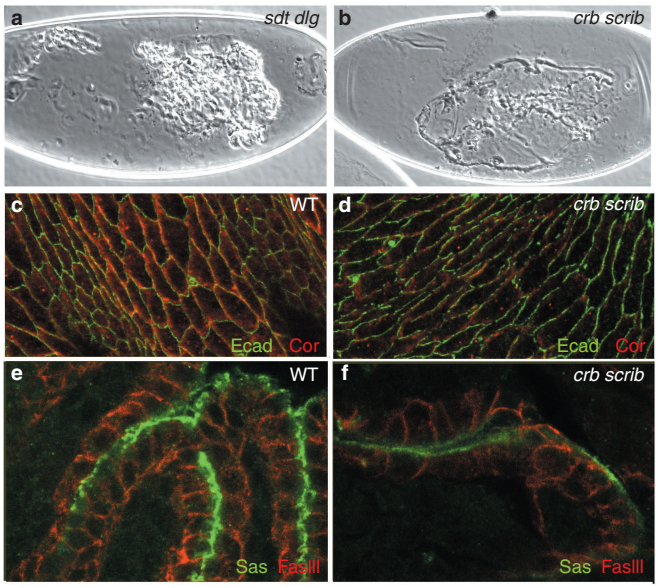

Figure 3 **Reduction of *scrib* group activity suppresses *crb* group phenotypes. a**, **b**, Zygotic double-mutant cuticles of *sdt dlg* (**a**) and *crb scrib* (**b**) embryos show rescued production of a continuous cuticular sheet. **c**, **d**, Surface sections of wild-type (**c**) or *crb scrib* (**d**) stage-15 epithelia stained for the zonula adherens protein E-cad (green) and the septate junction protein Coracle (Cor; red). The zonula adherens of *crb scrib* embryos (**d**) resembles that of wild-type embryos. Cor localization is also restored. **e**, **f**, Cross sections of wild-type segmental grooves at stage 14 (**e**) shows apical localization of Sas (green) and basolateral localization of FasIII (red) in an epithelial monolayer. Monolayering and polarized organization is also observed in stage-15 *crb scrib* embryos (**f**).

polarity by directly repressing the localization of Crb. However, similar to the *scrib* M/Z mutation, overexpression of *Crb* results in the mis-localization of many apical proteins, perhaps including additional apical regulators. To test whether the Scrib complex specifically antagonizes the Crb complex, we generated *sdt dlg* M/Z embryos, which are mutant for both complexes. Surprisingly, these embryos had cuticle phenotypes that were indistinguishable from *dlg* M/Z embryos (Fig. 2d). At mid-embyrogenesis, adherens junctions and apical markers were mis-localized to basolateral surfaces (Fig. 2f, h). These results demonstrate that the *scrib* group is epistatic to the *crb* group, and that the ectopic apical polarity observed in *scrib* M/Z mutants does not require Crb.

Interestingly, we found that *crb* epithelial defects were markedly suppressed merely by reducing *scrib* levels through zygotic mutation alone. *sdt dlg* and *crb scrib* zygotic mutants showed pronounced phenotypic rescue, displaying continuous cuticle patches that resemble those of *crb* hypomorphs (Fig. 3a, b). Loss of adherens junctions was less severe in stage-9 *crb scrib* zygotic mutants than in *crb* mutants (data not shown), and most stage-13 epidermal cells showed significant restoration of epithelial polarity, junctions and tissue organization (Fig. 3d, f). The fact that reducing the dose of *scrib* can rescue the epithelial organization of *crb* mutants provides additional evidence that the Scrib complex antagonizes a Crb-independent apical polarity pathway.

We then asked whether Scrib might antagonize Baz, instead of Crb. We generated embryos that lack *baz* and *dlg* and assayed their phenotype. *baz dlg* M/Z embryos had a severe loss of post-gastrulation adherens junctions and apical markers, and failed to produce cuticle (Fig. 2j–l), a phenotype that is identical to *baz* M/Z embryos[9,19]. Therefore, we conclude that *baz* is epistatic to *dlg*. This result shows that the Crb-independent protein localization to apical membranes seen in *scrib* M/Z embryos, similar to wild-type apical polarity itself, is regulated by Baz. Together, these data demonstrate that Scrib antagonizes the apical polarization activity of Baz.

While analysing zygotic *crb scrib* embryos, we noticed that improved junctional organization is most evident in late embryos. This improvement could result from the decline of maternal *scrib* gene product, or could indicate additional, uncharacterized polarity signals in the late embryo. To investigate the latter possibility, we assayed polarity in late epithelia that were mutant for *crb* or *scrib* M/Z alone. Immunohistochemical analysis of late *crb* epidermis revealed many multicellular highly polarized cyst-like structures (Fig. 4b). Apical markers were expressed on the cyst interior, whereas lateral markers were excluded from both the apical domain and the opposing basolateral domain. Furthermore, the localization of markers for adherens junctions and septate junctions, which are disrupted during mid-embryogenesis, was restored (Fig. 4e, h). These results confirm and extend previous reports[4] suggesting that *crb* mutant cells that avoid apoptosis can repolarize in an epithelial manner.

Next, we examined the phenotype of late *scrib* and *dlg* epidermis. In stage-15 *dlg* M/Z embryos, the loosely adherent and rounded mutant epidermal cells re-associated to form coherent strips of cells (Fig. 4c) that were also highly polarized. Apical markers were restricted to the outer, free surface, whereas basolateral markers were found throughout the contacting surfaces. Although mislocalized in mid-embryogenesis (Fig. 4f), adherens junction proteins became restricted to the junction of apical and basolateral surfaces, similar to wild-type epithelia (Fig. 4i). Although septate junction proteins were excluded from apical membranes, they were found throughout the basolateral membrane, rather than being enhanced laterally and excluded basally, as in wild-type cells. Ultrastructural analysis of late *dlg* M/Z mutant epithelia confirmed that the zonula adherens formed normally; however, basal to the zonula adherens, septa were not found (Fig. 4k). The absence of the septate junction and basal domain in repolarized *scrib* epithelia demonstrates that, in addition to restricting apical protein localization, Scrib actively organizes the basolateral membrane. The similarity of junctions in late *sdt dlg* M/Z animals (Fig. 4l), which lack both Crb and Scrib complexes, emphasizes that unidentified secondary polarizing mechanisms partially substitute for the absence of both these critical epithelial regulators.

Forward and reverse genetic approaches have demonstrated that three protein complexes — Baz, Crb, and Scrib — control epithelial polarity in *Drosophila* [20,21]. A current challenge is to understand the relationship between these different polarizing systems during the ontogenesis of cell architecture. In this work, we outline a regulatory pathway for apical polarity (Fig. 5) that initiates with adherens junction-dependent recruitment of the Baz complex to cell junctions. The Scrib complex, which localizes to basolateral domains independently of apical polarity, represses apical membrane identity in these areas. Our data suggest that the Scrib complex antagonizes the apical polarizing activity initiated by the Baz complex, whereas the Crb complex, recruited apically by the Baz complex, antagonizes Scrib activity to maintain the apical membrane domain. This sensitive equilibrium between PDZ complex activities determines the proper placement of apical and basolateral membrane domains, as well as cell junctions. We also identified additional cues that can polarize the cell membrane, even in the absence of the Crb and Scrib complexes. In conclusion, our studies highlight the complex integration of distinct polarizing activities that construct a coordinated cell architecture.

The first genetic requirements for epithelial polarity can be identified by comparing the onset of mutant phenotypes. The earliest known mutations that affect embryonic polarity are mutations that compromise cell–cell adhesion. In arm^{XP33} M/Z embryos, neither Baz, nor other polarized proteins, are efficiently recruited to the apicolateral membrane. Notably, *baz* M/Z embryos have similar defects and are also incapable of assembling a primitive zonula adherens[9]. Baz and Arm have a similar localization and the absence

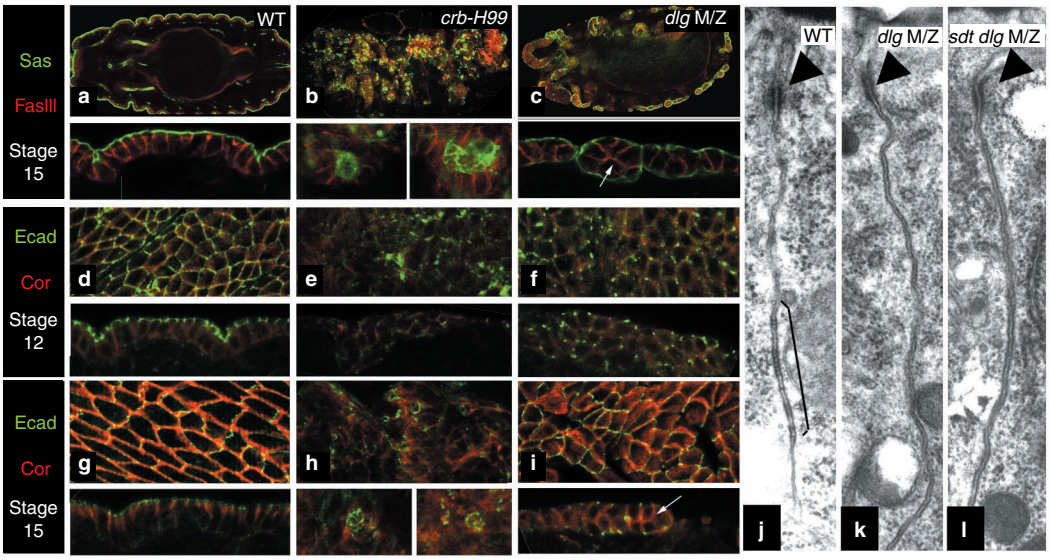

Figure 4 **Recovery of apicobasal polarity in late *crb* and *dlg* M/Z embryos.** **a**–**c**, Confocal microscopy images of wild-type (**a**), crb^{H99} (**b**) and *dlg M/Z* (**c**) stage-15 embryos stained for Sas (green) and FasIII (red). High-magnification cross-section images are shown below. **d**–**i**, Confocal microscopy images of wild-type (**d**, **g**), crb^{H99} (**e**, **h**) or *dlg M/Z* (**f**, **i**) embryos stained for E-cadherin (green) and Cor (red). High-magnification cross-section images are shown below. Cells in crb^{H99} and *dlg* M/Z embryos that are disorganized and have severe polarity defects at stage 12 (**d**–**f**) show polarized localization of Sas and FasIII (**a**–**c**) and apical adherens junctions (**g**–**i**) at stage 15. Furthermore, *dlg* cells do not contain a basolateral domain that excludes Fas III and Cor (**c**, **i**, arrows). **j**–**l**, Transmission electron microscopy images of wild-type (**j**), *dlg* M/Z (**k**) and *sdt dlg* M/Z (**l**) epidermis from stage-15 embryos. Normal zonula adherens (arrowheads) are observed at the apical surface of epidermal cells. However, there is little sign of the pleated septate junction (**j**, bracket) in *dlg* M/Z and *sdt dlg* M/Z embryos.

of either protein results in a similar phenotype. Currently, we cannot determine whether the loss of Baz in arm^{XP33} M/Z mutants or the loss of adherens junctions in *baz* M/Z mutants is primarily responsible for the loss of apical polarity.

One major consequence of *baz* M/Z mutations is the absence of the essential apical regulator Crb. A number of results suggest that Crb regulates apical polarity primarily through its effects on Baz. First, *crb* M/Z embryos have normal polarity at stage 7, by which time *baz* M/Z embryos show severe defects[9,15,16]. Second, *baz* is required for the initial polarization of Crb, whereas *crb* is only required for Baz maintenance. Third, apical polarity can be achieved in the absence of Crb, in late embryos and/or in contexts where Scrib function is reduced; interestingly, in all of these cases, localization of Baz is normal (data not shown). The similar phenotype of *baz* M/Z and *crb* mutations in embryos makes it difficult to distinguish epistatic relationships. However, removal of zygotic *baz* enhances the *sdt* embryonic phenotype[9], and in follicle cells where the phenotypes can be distinguished, *baz sdt* cells resembled *baz* cells (data not shown). All these data suggest that the major function of the Crb complex in polarity is the maintenance of Baz complex localization.

Scrib group proteins restrict the localization of apical proteins and adherens junctions, although the mechanism is unknown. One possibility, suggested by the independence of Scrib localization from adherens junction and Baz/Crb function, is that Scrib participates in a distinct pathway regulating epithelial organization, and only affects Baz and Crb localization incidentally. However, genetic interactions, in which *scrib* levels sensitively modify *crb* loss- and gain-of-function phenotypes, place *scrib* within the *baz* and *crb* pathway. These interactions also raise the possibility that Scrib function is limited to localizing the apical determinant Crb. As a powerful regulator of apical polarity, misexpression of Crb is highly toxic, so Crb must be carefully restricted. However, the mis-localization of apical proteins evident in *sdt dlg* M/Z embryos reveals that Scrib does not restrict only Crb. In contrast to *sdt dlg* M/Z embryos, the lost apical polarity of *baz dlg* M/Z embryos indicates

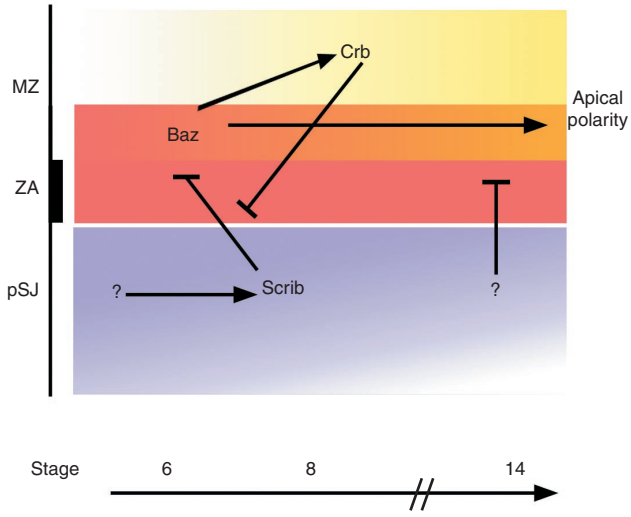

Figure 5 **A model of the embryonic epithelial polarization pathway.** A schematic representation of the localization and regulatory relationships between Crb, Baz and Scrib. The complexes function in a single pathway to control apical polarity. Baz is the initial and critical apical regulator. Scrib allows basolateral development by repressing the ability of Baz to promote apical characteristics. We propose that Crb maintains Baz at apical membranes by antagonizing repression of Scrib. Note that Crb represses the activity, not the localization, of the Scrib complex. The sensitive balance between Crb and Scrib complex activities allows proper polarity to be achieved.

that the *baz* group is epistatic to the *scrib* group. Thus, Baz is required for both the ectopic apical polarity of *dlg* M/Z embryos, as

Table 1 Protein complexes required for apicobasel polarity in *Drosophila*

Group	Protein	Structure	Homologs
Crb	Crb	Transmembrane	ceCRB-1/2, hsCrb1/2
	Sdt	MAGUK (1 PDZ)	hsPALS-1
Baz	Baz	3 PDZ	cePar-3, hsPar-3
	dmPar-6	1 PDZ	cePar-6, hsPar-6
	daPKC	Cytoplasmic kinase	cePar-3, hsPKC z
Scrib	Scrib	LAP (4 PCZs)	ceLet-413, hsScrib1
	Dlg	MAGUK (3 PDZs)	ceDlg-1, hsPSD-95 et al.
	(Lgl)	(Myosin/SNARE-binding)	(ceM01A01.2a, HUGL/LLGL)

well as apical polarity in wild-type embryos. Overall, the epistasis studies demonstrate that Crb exerts its effects on polarity by repressing Scrib activity, whereas Scrib exerts its effects on polarity by repressing Baz. This is consistent with a model in which Scrib establishes basolateral identity by antagonizing the ability of Baz to induce apical characteristics.

If Scrib obstructs apical polarization, it is interesting to consider what factors prevent it from disrupting the apical domain itself. Our results suggest an intimate and finely calibrated antagonism through which Crb regulates the activity of Scrib. The onset of *crb* and *scrib* M/Z loss-of-function phenotypes is almost identical, and the lost apical polarity observed in *crb* group mutants is at least partly a result of increased Scrib activity, as this phenotype is suppressed in embryos that also lack zygotic *scrib* gene product. Furthermore, ectopic expression of *Crb* is sufficient to phenocopy *scrib* mutants, suggesting that Crb can block Scrib function. Therefore Crb may promote apical identity by locally opposing the antagonistic effects of Scrib on an apical polarizing activity that originates with Baz itself. We propose that during embryogenesis, Baz recruits Crb apically to specifically counterbalance the antagonism of Scrib. As Scrib does not expand apically in *crb* mutants, our model proposes that Crb interferes with Scrib polarizing activity, rather than Scrib localization. As Scrib also fails to expand apically in *baz* or arm^{XP33} mutants, the mechanisms that recruit and restrict Scrib to the basolateral membrane remain unknown.

Although the Baz, Crb and Scrib groups are molecularly and genetically distinct, our data suggest that they interact in a single pathway to regulate apical protein localization. The mechanism of this interaction is currently unclear. The Baz, Crb and Scrib complexes are widely expressed in metazoans and current work has highlighted the evolutionarily conserved role of these complexes in organizing epithelial membrane domains[22–28]. Furthermore, human Baz, Crb and Scrib complex proteins have recently been implicated in malignant and degenerative pathologies[24,29–31]. Thus, future studies should continue to explore in mechanistic detail the activity and integration of these complexes during cell polarization. □

Methods

Genetics

The following amorphic alleles were used: baz^{X1106}, $scrib^{2}$, $dlgm^{52}$, lgl^{4}, crb^{11A22}, sdt^{XP96} and sdt^{EH1}. To specifically examine the epidermis of *crb* mutants, we used an apoptosis-deficient background (*Df(3L)H99*)[32]. Ectopic expression of *Crb* in adults was performed at 18 °C using the wing pouch driver *71B*. Germline clones to remove both maternal and zygotic gene product (M/Z mutants) were produced using FLP-ovoD chromosomes for *FRT 101* (X) and *FRT 82B* (3R), except for *baz* and *baz sdt*, which were produced using the UAS-OvoA system (E. Selva, D. Pauli and N. Perrimon, unpublished observations) and *dlg baz* which were produced using X-ray irradiation *in trans* to *ovoD*. Embryos lacking *dlg* and *baz* were also generated using RNA interference stratagies. For these experiments, *baz* dsRNA was produced and injected, as previously described[33].

Immunohistochemistry

Embryos were fixed as previously described[10]. Baz and Arm was detected by using a heat–methanol fixation procedure[34]. To narrowly stage *scrib* M/Z and *crb* mutants, 10-min collections were taken and aged at 25 °C. Mutant embryos were identified with *ftz-lacZ* balancer chromosomes. Mouse anti-Arm, -Neurotactin and -FasIII were obtained from the Developmental Studies Hybridoma Bank. Other antibodies were: mouse anti-Cor (R. Fehon), rat anti-Ecad (H. Oda), rabbit anti-Crb and anti-Dlt (M. Bhat), rabbit anti-Sas (D. Cavener), rabbit anti-Baz (A. Wodarz), rabbit anti-Dlg (V. Budnik) and rabbit anti-Scrib. Images were obtained using a Leica TCS confocal microscope.

Transmission electron microscopy

Embryos were fixed and sectioned in accordance with standard protocols[35]. Approximately 90-nm sections were examined on a JEOL JEM1200 EX electron microscope (JEOL, Peabody, MA).

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COMPETING FINANCIAL INTERESTS
The authors declare that they have no competing financial interests.

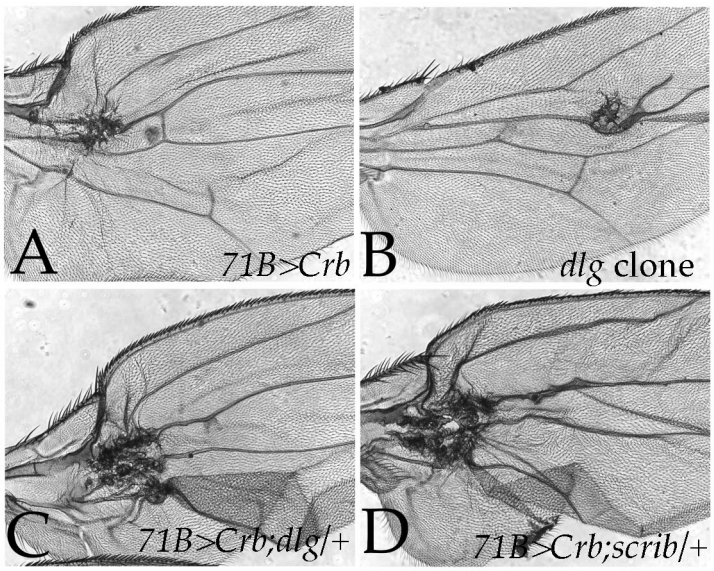

Figure SI *scrib* and *dlg* enhance *crb* gain-of-function phenotypes. Overexpression of Crb in the wing pouch (*71B>Crb*, A) causes phenotypes similar to *dlg* mutant clones (B). Heterozygosity for *dlg* (C) or *scrib* (D) enhances the ectopic Crb phenotype.