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The Partner of Inscuteable/Discs-Large Complex Is Required to Establish Planar Polarity during Asymmetric Cell Division in *Drosophila*

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Summary

Frizzled (Fz) signaling regulates cell polarity in both vertebrates and invertebrates. In *Drosophila*, Fz orients the asymmetric division of the sensory organ precursor cell (pI) along the antero-posterior axis of the notum. Planar polarization involves a remodeling of the apical-basal polarity of the pI cell. The Discs-large (Dlg) and Partner of Inscuteable (Pins) proteins accumulate at the anterior cortex, while Bazooka (Baz) relocalizes to the posterior cortex. Dlg interacts directly with Pins and regulates the localization of Pins and Baz. Pins acts with Fz to localize Baz posteriorly, but Baz is not required to localize Pins anteriorly. Finally, Baz and the Dlg/Pins complex are required for the asymmetric localization of Numb. Thus, the Dlg/Pins complex responds to Fz signaling to establish planar asymmetry in the pI cell.

Introduction

In unicellular and multicellular organisms, acquisition of cell polarity is essential for the formation and physiology of epithelia, oriented migration, neurite outgrowth, and asymmetric cell division (Drubin, 2000). Receptors of the Frizzled (Fz) family and their Wnt ligands have been implicated in the regulation of cell polarity in several organisms. For instance, Fz signaling polarizes migratory cells during gastrulation in zebrafish and *Xenopus* embryos (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000). In *Drosophila,* Fz regulates the planar polarity of epithelial cells (Mlodzik, 1999; Shulman et al., 1998). Fz receptors are also known to orient asymmetric divisions in *Caenorhabditis* and *Drosophila* (Gho and Schweisguth, 1998; Rocheleau et al., 1997; Sternberg and Horvitz, 1988; Thorpe et al., 1997).

In the dorsal thorax (notum) of the *Drosophila* pupa, the pI cell divides unequally with its spindle axis aligned with the anterior-posterior (a-p) axis of the fly body (Gho et al., 1999; Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989). It produces two different daughter cells, pIIa and pIIb. During this division, Numb and its adaptor protein Partner of Numb (Pon) form an anterior crescent and segregate unequally into the anterior pIIb cell (Bellaiche et al., 2001; Gho and Schweisguth, 1998; Lu et al., 1998; Rhyu et al., 1994; Roegiers et al., 2001). In *fz* mutant pupae, the division of the pI cell is oriented randomly relative to the a-p axis (Bellaiche et al., 2001; Gho and Schweisguth, 1998; Roegiers et al., 2001) and the crescent of Numb forms, but at a random position. Thus, Fz is not required to establish planar asymmetry per se, but is necessary to orient the axis of the asymmetric cell division. This indicates that additional genes may be required for establishing, rather than orienting, planar asymmetry in the pI cell.

In *Drosophila* epithelial cells, the establishment and maintenance of polarity depends on the activity of cortical proteins (Muller, 2000). Among these, the proteins Bazooka (Baz; known as PAR-3 in other species), DmPAR-6, and *Drosophila* atypical protein kinase C (DaPKC) form an evolutionarily conserved complex that localizes just above the adherens junction (AJ) (Etemad-Moghadam et al., 1995; Hung et al., 1999; Joberty et al., 2000; Kuchinke et al., 1998; Lin et al., 2000; Petronczki and Knoblich, 2001; Qui et al., 2000; Watts et al., 1996; Wodarz et al., 2000). In neuroblasts, this complex acts as an apical cue inherited from the epithelium and establishes asymmetry by recruiting the Inscuteable (Insc) and Partner of Inscuteable (Pins) proteins (Kraut et al., 1996; Parmentier et al., 2000; Schaefer et al., 2000; Schober et al., 1999; Wodarz et al., 1999; Yu et al., 2000). Pins binds Gαi/o proteins and may act as an activator of G protein signaling (De Vries et al., 2000; Parmentier et al., 2000; Schaefer et al., 2000). The Baz/DmPAR-6/DaPKC/Insc/Pins complex regulates the basal localization of the cell-fate determinants Numb and Prospero and orients the mitotic spindle (Kaltschmidt et al., 2000; Schaefer et al., 2000; Schober et al., 1999; Wodarz et al., 1999; Yu et al., 2000).

The maintenance of cell polarity also requires the Discs large (Dlg) and Scribble (Scrib) proteins that localize at the septate junctions, basal to the AJ (Bilder and Perrimon, 2000; Budnik et al., 1996; Goode and Perrimon, 1997; Woods and Bryant, 1991; Woods et al., 1996). Dlg is a membrane-associated GUanylate kinase homolog (MAGUK) protein that contains three PDZ domains, one SH3 domain and a C-terminal GUK domain, and Scrib is a LAP protein containing 16 leucine-rich repeats and four PDZ domains. Dlg and Scrib cooperate with the Lethal(2)giant larvae (Lgl) protein in the regulation of cell polarity (Bilder et al., 2000). Dlg accumulates at the apical cortex of the neuroblast and is required together with Lgl to localize Numb and Miranda (Ohshiro et al., 2000; Peng et al., 2000). However, *dlg* and *lgl* are

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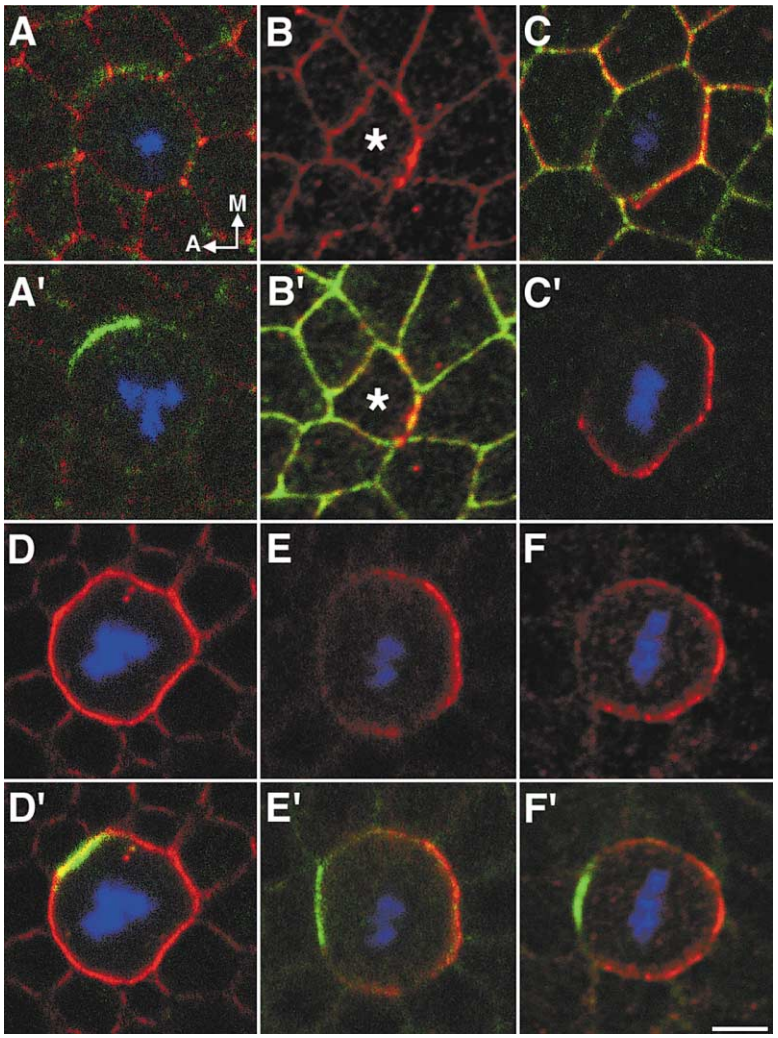

Figure 1. Baz and DaPKC Localize at the Posterior Cortex in the pI Cell

(A and A′) Numb (green) localizes below the AJ (Cad in red). The basal z-section (A′) is 1.7 μm below the apical section (A).

(B and B′) Baz (red) colocalizes with Cad (green) in all epithelial cells. It becomes enriched at the posterior pole at late interphase in the pI cell (asterisk in B and B′).

(C and C′) Baz (red) accumulates below the AJ (Cad in green) at metaphase. The basal z-section (C′) is 1.96 μm below the apical section (C).

(D and D′) Fas3 (red) overlaps with Numb (green, D′).

(E and E′) Baz (red) forms a posterior crescent opposite to Numb (green, E′) at metaphase.

(F and F′) DaPKC (red) is also enriched at the posterior cortex, opposite to Numb (green, F′) at metaphase.

The pI cells were identified using the mitotic stage marker histone2B-yellow fluorescent protein (H2B-YFP) (Bellaiche et al., 2001), shown here in blue. H2B-YFP was specifically expressed in the pI cells using the neu^{P72} GAL4 driver. This allowed us to simultaneously identify the pI cells and determine their mitotic stage. In this and all following figures, anterior is on the left and medial is up in surface views (see orientation arrows in [A]). Scale bar is 4 μm in this and all other figures.

not required for the asymmetric localization of the Baz/Insc/Pins complex.

Here, we show that Baz, Pins, and Dlg establish and maintain planar polarity in the pI cell. Pins and Dlg form a protein complex localizing at the anterior cortex of the dividing pI cell and this complex cooperates with Fz to localize Baz at the posterior-lateral cortex. Baz directs the localization of Numb to the opposite pole but is not essential to establish planar asymmetry in the pI cell. We conclude that establishment of planar polarity in the pI cell involves a partial remodeling of the preexisting apical-basal polarity.

Results

Baz and DaPKC Localize to the Posterior Cortex, Opposite to Numb

In the dividing pI cell, Numb and Pon colocalize at the anterior pole of the lateral cortex, marked with Fasciclin3 (Fas3), below the AJ, marked with DE-Cadherin (Cad) (Figures 1A, 1A′, 1D, and 1D′, and data not shown).

Baz is known to regulate the asymmetric distribution of Miranda and to localize asymmetrically in pI cells of the embryo (Schober et al., 1999). This prompted us to study the distribution of Baz in the developing pupal notum. In epithelial cells in interphase, Baz colocalizes with Cad at the AJ around the apical cortex (Figures 1B and 1B′). In the pI cell, Baz accumulates at the posterior cortex during mitosis. Prior to chromosome condensation, this accumulation is seen at the level of the AJ (Figures 1B and 1B′). Then, during prophase and metaphase, Baz forms a posterior crescent below AJ (Figures 1C and 1C′) and opposite to Numb (Figures 1E and 1E′). At telophase, the pIIa cell inherits a higher level of Baz than its sister cell (not shown). DaPKC shows a similar distribution to Baz in the pI cell (Figures 1F and 1F′).

Pins and Dlg Accumulate at the Anterior Cortex, Opposite to Baz

In neuroblasts, a key function of the Baz/DaPKC/DmPAR-6 complex is to recruit the Insc and the Pins proteins (Schaefer et al., 2000; Yu et al., 2000). However, in the pI cell, Insc is not expressed (Roegiers et al., 2001; see also below) and Pins does not colocalize with Baz at the posterior cortex. Rather, it localizes to the anterior pole in early prophase (Figures 2A and 2A′) and colocalizes with Numb at the anterior lateral cortex at metaphase (Figures 2B and 2B′).

Because DaPKC and Baz have a dual function in epithelial polarity and asymmetric neuroblast division, we

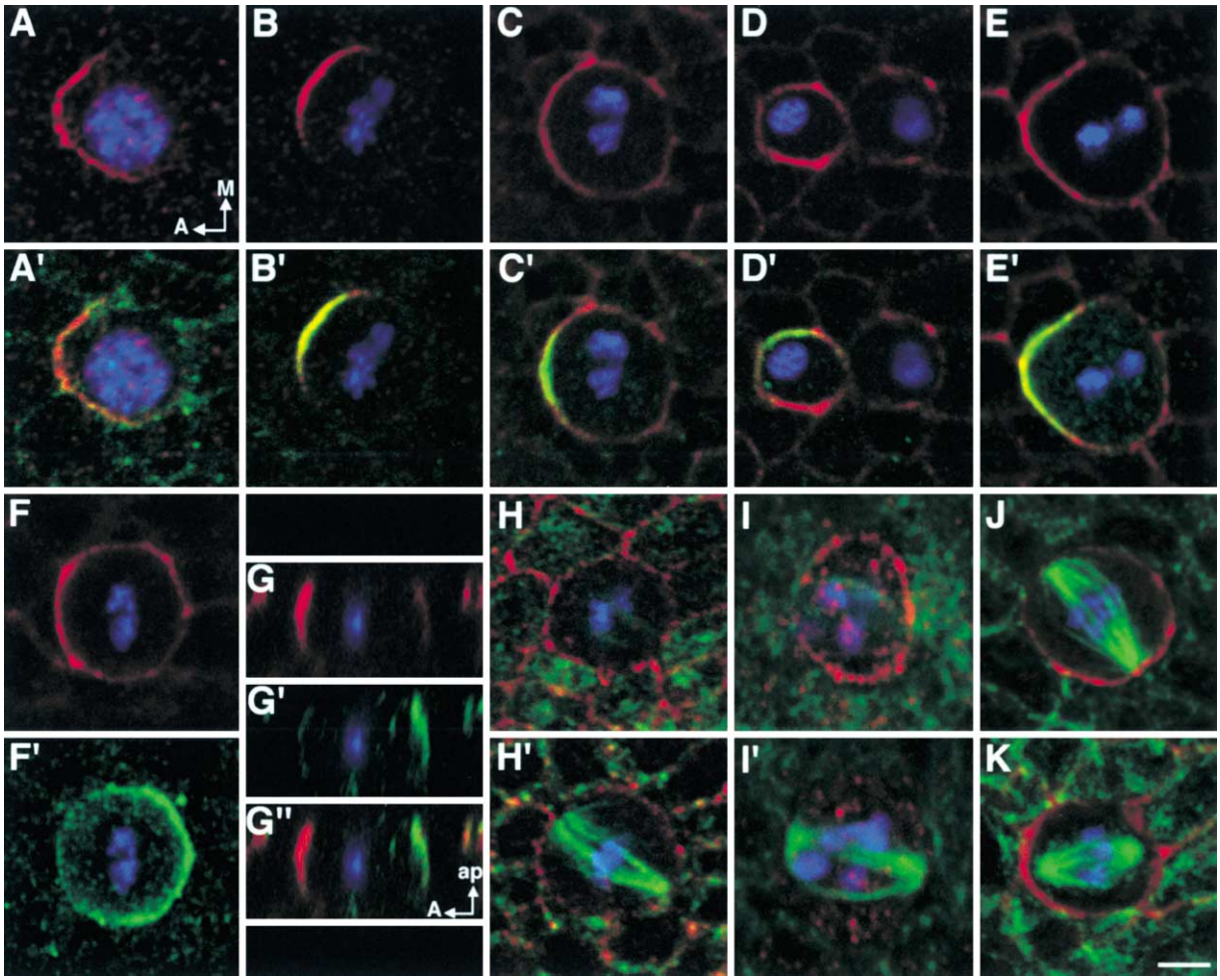

Figure 2. Pins and Dlg Accumulate at the Anterior Cortex in the pI Cell

(A–B′) Pins (red) localizes to the anterior-lateral cortex prior to the formation of the Numb crescent (green) at early prophase (A and A′) and colocalizes with Numb at metaphase (B and B′).

(C–D′) Dlg (red) localizes preferentially to the lateral anterior cortex, together with Numb (green), at metaphase (C and C′). Dlg is preferentially, but not exclusively, inherited by the pIIb cell at telophase (D and D′).

(E and E′) The anterior accumulation of Dlg (red) colocalizes with Pins (green).

(F–G′′) Dlg (red) is enriched at the anterior-lateral cortex opposite to Baz (green), as seen on planar (F and F′) and vertical (G–G′′) sections.

(H–K) The mitotic spindle (α-tubulin in green) lies below the AJ. Arm (H and H′) and a Myc-tagged version of Arm expressed under the control of neu^{P72} GAL4 (I and I′) are in red. The z sections shown in H′ and I′ are 2.0 and 1.8 μm below the z sections shown in H and I, respectively. The posterior spindle pole is centered with the posterior crescent of Baz (red in J), while its anterior pole is more basal and centered with the anterior accumulation of Dlg (red in K).

hypothesized that genes required for epithelial polarity might also regulate planar polarity in the pI cell. To test this hypothesis, we studied the planar distribution of various proteins known to be distributed asymmetrically along the apical-basal axis of epithelial cells (see Experimental Procedures). Of these, only Dlg was identified as a protein localizing asymmetrically along the planar axis in the pI cell. Dlg overlaps with Fas3 below the AJ in interphase cells (not shown). In dividing pI cells, Dlg redistributes in part along the planar a-p axis. From late prophase onward, Dlg becomes enriched at the anterior cortex, where it colocalizes with Numb (Figures 2C and 2C′) and Pins (Figures 2E and 2E′). During this time, Dlg does remain detectable at the posterior lateral cortex. At telophase, a higher level of Dlg segregates into the pIIb cell (Figures 2D and 2D′). Thus, the accumulation of Dlg/Pins and Baz at opposite poles of the cell defines two complementary cortical domains oriented along the a-p planar axis of the pI cell (Figures 2F–G′′ and 3E). The position of the mitotic spindle at metaphase correlates with the localization of these two cortical domains. The posterior spindle pole is positioned near the accumulation of Baz (Figure 2J), and the anterior spindle pole lies near the accumulation of Dlg (Figure 2K). In both pI and epidermal cell, the mitotic spindle poles are found below the AJ, which appear to remain functional as they retain their ability to recruit Arm (Figures 2H–2I′).

Baz Is Required to Localize Numb but Not Pins at the Anterior Cortex

To determine the possible function of Baz in the planar polarization of the pI cell, we studied clones of *baz* mutant cells in the notum. Loss of *baz* activity did not affect the localization of Cad and Dlg (Figures 3A–A′′),

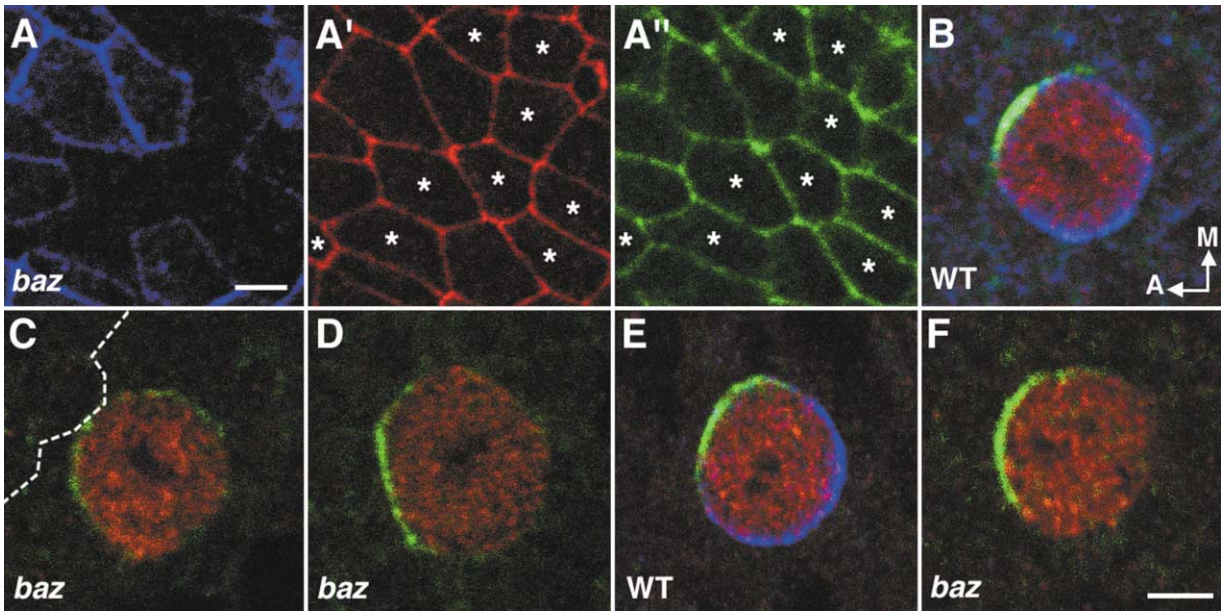

Figure 3. Baz Regulates the Asymmetric Localization of Numb

(A–A′′) Apical section of a *baz*^{Xi106} clone in the notum of a 16.5 hr APF pupa. Mutant cells (asterisks in A′ and A′′) are detected by the lack of Baz protein shown in blue (A). Loss of *baz* function does not affect the localization of Cad (A′) and Dlg (A′′).

(B–F) Localization of Numb (green in B–D) and Pins (green in E and F) in wild-type (B and E) and *baz*^{Xi106} mutant (C, D, and F) cells. The pI cells are identified using Cut (red). Numb does not localize asymmetrically (C) or forms a weak anterior crescent (D) in *baz* mutant pI cells. In contrast, Pins localizes asymmetrically to the anterior cortex of the pI cell in the absence of Baz. We found that the Pins crescent is more extended than in wild-type controls in only one of the 31 *baz* mutant pI cells (F). The *baz* mutant cells are detected by lack of Baz staining. These pI cells are in the center of large *baz* clones (D and F) or near the clone border (indicated by a dashed line in C). The wild-type pI cells (B and E) are from the nota that carry the *baz* clones, and can therefore be directly compared to the *baz* mutant pI cells (C, D, and F).

indicating that apical-basal polarity in the notal epithelium is maintained in the absence of Baz. In the dividing pI cell, Numb either does not localize asymmetrically (Figure 3C; n = 7/21) or forms a weak crescent at the anterior cortex at prometaphase (Figure 3D; n = 12/21). In contrast, Pins localizes asymmetrically at the cortex of the pI cell during division (Figure 3F; n = 31). Moreover, *baz* mutant pI cells divide within the plane of the epithelium with a normal a-p orientation with Pins localizing at the anterior cortex (wild-type: 31° ± 29, n = 35; *baz*: 37° ± 20, n = 31; these angles correspond to the mean orientation of pI divisions relative to the a-p axis using Pins as a polarity marker; see plots in Supplementary Material available online at http://www.cell.com/cgi/content/full/106/3/355/DC1). This shows that *baz* is required for the asymmetric localization of Numb but is not essential to establish asymmetry nor to orient polarity along the a-p axis.

Dlg and Pins Regulate Numb and Pon Localization

The function of Pins during the asymmetric division of the pI cell was analyzed using a viable null allele of *pins*$^{\Delta 1\text{-}50}$ that does not affect epithelial cell polarity (Schaefer et al., 2000). To study the function of Dlg, we used two hypomorphic alleles, *dlg*^{SW}and *dlg*^{1P20}, which are predicted to encode truncated proteins lacking the C-terminal 14 and 43 amino acids, respectively (Woods and Bryant, 1991; Woods et al., 1996) and which do not perturb apical-basal polarity. The GUK domain of Dlg is partly deleted in the mutant Dlg^{1P20} protein, but should be unaffected in the mutant Dlg^{SW} protein. In the pI cell, the Dlg^{SW} protein accumulates normally at the anterior cortex, whereas the mutant Dlg^{1P20} protein is cortical, but fails to accumulate anteriorly (not shown).

We first investigated the possible role of Dlg and Pins in regulating the position of the mitotic spindle. Spindle movements were analyzed in living pupae using Tau-GFP (Bellaiche et al., 2001; Kaltschmidt et al., 2000; Roegiers et al., 2001). We found that the a-p orientation of the pI division does not depend on the activity of *pins* and is not affected in the *dlg*^{1P20}mutant (wild-type: 30° ± 29, n = 64; *dlg*^{1P20}: 28° ± 38, n = 44; *pins*$^{\Delta 1\text{-}50}$: 28° ± 36, n = 40). In wild-type and *pins* mutant pI cells, the spindle lines up with the planar polarity axis 3–4 min prior to the metaphase-anaphase transition (Figures 4A and 4C). In contrast, the spindle often rotates throughout metaphase in *dlg* mutant pI cells (Figures 4B and 4C). We conclude that Dlg regulates the localization or the activity of factors responsible for spindle rotation.

We then analyzed the role of Dlg and Pins in the asymmetric localization of Numb and Pon. The interphase localization of Numb at the cortex and of Pon around the nucleus (Bellaiche et al., 2001) does not depend on the function of the *dlg* or *pins* genes (not shown). At metaphase, however, the anterior localization of both proteins requires the activity of both *pins* and *dlg* (Figures 4D–4F and 4H). Thus, in *pins* mutant cells at prometaphase, the crescent of Numb and Pon is either not detected (Figure 4E; n = 17/32) or weak (Figure 4F; n = 15/32). Nevertheless, both proteins segregate into the

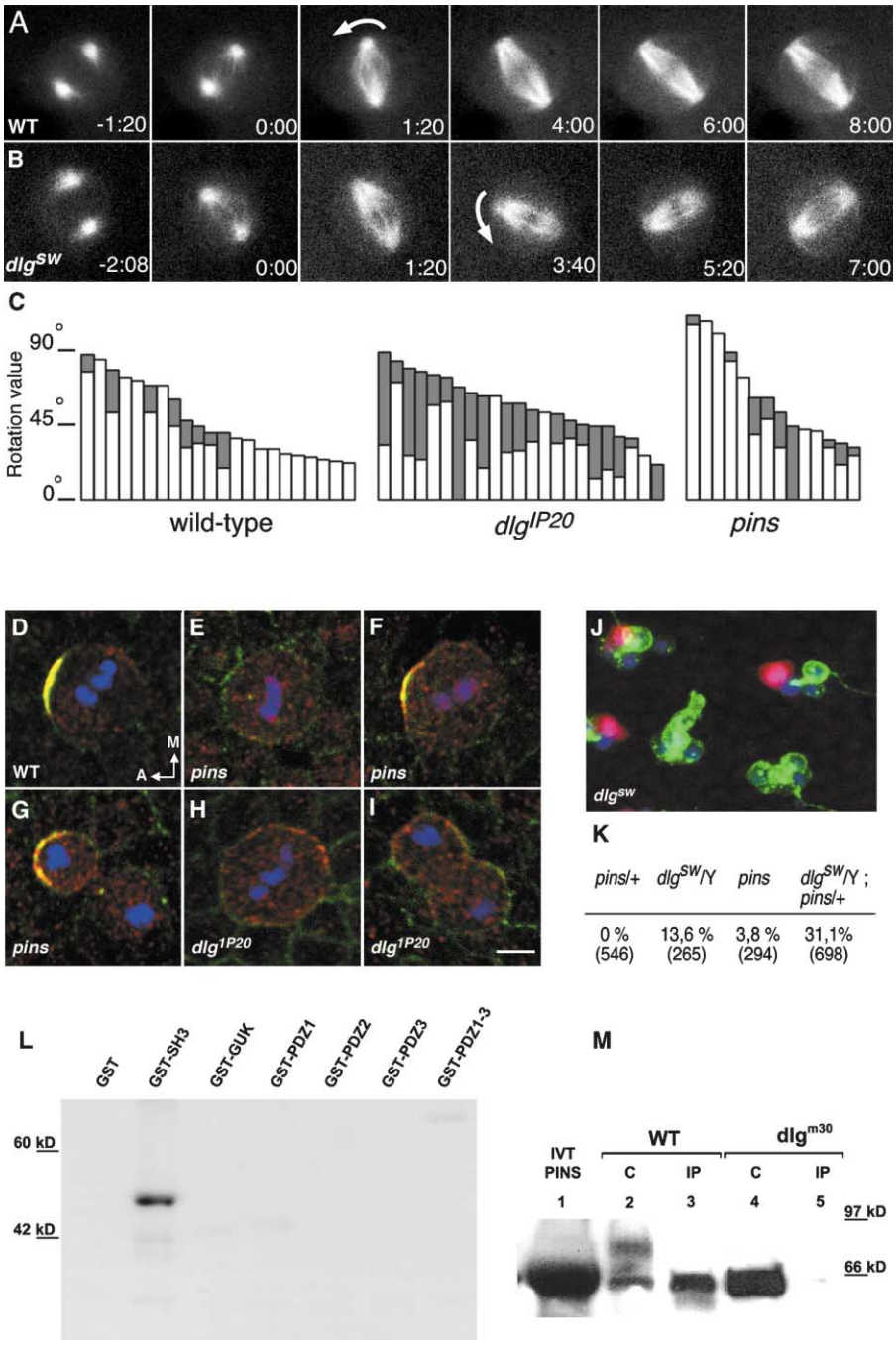

Figure 4. Dlg and Pins Form a Protein Complex and Regulate the Localization of Numb

(A and B) Time-lapse imaging of pI cells expressing tau-GFP under the control of neu^{P72} in wild-type (A) and *dlg^{sw}* mutant (B) pupae, from late prophase (t = 0:00 corresponds to the formation of the mitotic spindle) to the metaphase-anaphase transition (at t = 8:00 and t = 7:00, respectively). The mitotic spindle starts to rotate immediately after entry into prometaphase in the wild-type pI cell (arrow at t = 1:20), while the onset of rotation is delayed in the *dlg^{sw}* mutant pI cell (arrow at t = 3:40). No rotation is observed between t = 4:00 and t = 8:00 in the wild-type pI cell. In contrast, spindle rotation is observed in late metaphase in the *dlg^{sw}* mutant pI cell (after t = 5:20).

(C) Diagram showing angle values corresponding to spindle rotation measured between prometaphase and anaphase. Each bar corresponds to the division of one pI cell. The white part of the bar shows the angle value of the early spindle rotation that takes place between prometaphase and mid-metaphase, and the black part gives the value of the late spindle rotation that occurs between mid-metaphase and anaphase, respectively. The time value of the mid-metaphase is determined as the mid-time-point between spindle formation and metaphase-anaphase transition. All recorded pI cells with a spindle rotation value greater than 20° are shown. Similar rotation values are observed in wild-type, *dlg^{sw}*(not shown), *dlg^{1P20}* and *pins$^{\Delta 1-50}$* pupae. However, late spindle rotation is minor in wild-type and *pins$^{\Delta 1-50}$* mutant pupae, but is more important in *dlg^{SW}*(not shown) and *dlg^{1P20}* mutant pupae.

(D–I) Distribution of Numb (green) and Pon (red) in the pI cells (H2B-YFP in blue) of wild-type (D), *pins$^{\Delta 1-50}$* (E–G) and *dlg^{1P20}* (H and I) mutant

(*Figure 4 continued on next page*)

anterior cell at anaphase and telophase, (Figure 4G; n = 22/23). In dlg^{1P20} mutant pI cells, Numb does not accumulate at the anterior cortex and Pon remains cytoplasmic at metaphase (Figure 4H). At telophase, Numb and Pon segregate equally into both daughter cells (Figure 4I). These results show that Dlg and Pins are required to localize Numb and Pon at the anterior cortex in the pI cell. Consistently, nonsensory cells are transformed into neurons leading to a bristle loss phenotype in adult flies (Figures 4J and 4K; data not shown). Furthermore, the genetic interaction seen between dlg^{sw} and *pins* (Figure 4K) suggests that *pins* and *dlg* act in the same process to specify the fate of the pI daughter cells.

Dlg and Pins Interact Directly

Pins colocalizes with the anterior accumulation of Dlg (Figures 2E and 2E′) and *pins* and *dlg* mutations genetically interact (Figure 4K). This raises the possibility that the two proteins interact directly. Indeed, in a yeast two-hybrid screen using full-length Dlg as bait, one Pins clone (encoding amino-acid residues 235 to 658) was isolated (D.F.W., unpublished data). To further test for a direct interaction between Dlg and Pins and to identify the Pins interaction domain of Dlg, blot overlay experiments were performed using GST-fusion proteins. A biotinylated Pins protein is found to interact with the SH3 domain but not with the PDZ1, PDZ2, PDZ3, HOOK, or GUK domains of Dlg (Figure 4L). The Dlg-Pins complex is also detected in brains and imaginal discs by coimmunoprecipitation experiments (Figure 4M, lane 3). This interaction is abolished by a single amino-acid substitution (L556P) in the SH3 domain, which does not noticeably affect Dlg stability in dlg^{m30} mutant larvae (Figure 4M, lane 5; Woods et al., 1996; data not shown) but does result in disc overgrowth and late larval lethality.

Consistent with this direct interaction, Pins and Dlg are mutually dependent for their accumulation at the anterior cortex. A very weak crescent of Pins is seen at the anterior cortex in dlg^{1P20} mutant pI cells at metaphase (Figures 5A and 5B), suggesting that the GUK domain might facilitate the interaction between the SH3 domain of Dlg and Pins. Conversely, Dlg does not become enriched at the anterior cortex of *pins* mutant pI cells at metaphase (Figure 5C′). We conclude that Dlg directly interacts with Pins via its SH3 domain, and that this interaction is important for the anterior accumulation of both Dlg and Pins.

Dlg Is Required to Maintain Baz at the Lateral Cortex

We next studied the role of Pins and Dlg in localizing Baz asymmetrically. In *pins* mutant pI cells, Baz accumulates at the posterior cortex at metaphase, but the asymmetry is less pronounced than in wild-type cells (Figure 5C, compare with Figure 5F′). This raises the possibility that Pins participates in the asymmetric localization of Baz (see below). In dlg^{1P20} mutant pupae, Baz is correctly localized to the apical posterior cortex prior to chromosome condensation (Figures 5D and 5E), but does not form a cortical crescent below the AJ during late prophase and prometaphase (Figures 5G–G′′). Instead, Baz accumulates in the cytoplasm and remains cortical only at the level of the AJ. Thus, the initial posterior localization of Baz at the level of the AJ does not depend on the activity of the GUK domain of Dlg, but its cortical localization below the AJ does require *dlg* activity. We conclude that planar polarization of the pI cell cannot be maintained without Dlg activity.

Fz Cooperates with Pins to Localize Baz Posteriorly

To test whether the initial Dlg-independent localization of Baz at the posterior cortex depends on Fz signaling, we studied the distribution of Baz in *fz* mutant pupae. In wild-type pupae, a clear accumulation of Baz is seen at the level of the AJ in 61% (n = 51) of the interphase pI cells (Figure 6A). By contrast, an asymmetric distribution of Baz at the apical cortex is detected in only 19% (n = 83) of the interphase pI cells in *fz* mutant pupae. In the remaining 81% of the cells, the asymmetric accumulation of Baz is either weak (Figure 6B) or similar to that seen in the surrounding epithelial cells (Figure 6C). This indicates that Fz signaling regulates the initiation of the asymmetric localization of Baz at the posterior cortex. At metaphase, however, Baz and Pins form misoriented crescents relative to the a-p axis that localizes at opposite poles in *fz* mutant pI cells (Figures 6D–6F and data not shown; see plots in Supplementary Material online at http://www.cell.com/cgi/content/full/106/3/355/DC1). We conclude that the formation of the two opposite Baz and Pins domains does not depend on *fz* activity, and that planar asymmetry can be established in the absence of Fz signaling. However, as previously seen for *pins*, the asymmetric distribution of Baz is less pronounced in *fz* mutant pI cells than in wild-type cells

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pupae. At prometaphase, Numb and Pon redistribute to the lateral anterior cortex in wild-type pI cells (D). In contrast, the anterior crescent of Numb and Pon is either not detectable (E; n = 17/32) or weak (F; n = 15/32) in $pins^{\Delta 1\text{-}50}$ mutant pI cells. Nevertheless, Numb and Pon segregate to the anterior daughter cell during anaphase and telophase (G; n = 22/23). The dlg^{sw} phenotype is very similar to the one described for $pins^{\Delta 1\text{-}50}$ (not shown). The dlg^{1P20} phenotype is, however, more severe (H and I). No crescent of Numb and Pon is detected at metaphase (H), and both Numb and Pon are equally segregated to the two pI daughter cells (I).

(J) Confocal image showing five sense organs in a dlg^{sw} 24 hr APF pupa stained for Cut (blue), Su(H) (a socket cell marker, in red), and HRP (a neuronal marker, in green). Two of these five sense organs are composed of only HRP-positive cells.

(K) The quantification of the cell-fate transformations observed in 22–24 hr APF pupal nota stained for Cut, Su(H), and HRP is given as the percentage of sense organs composed of four to six HRP-positive cells and devoid of Su(H)-positive cells. The total number of counted sense organs is indicated in brackets.

(L) Pins binds to the SH3 domain of Dlg. The binding of GST-tagged individual domains of Dlg to the biotinylated C-terminal half of Pins was tested by blot overlay. Of the tested constructs, only the SH3 domain of Dlg showed significant binding to Pins.

(M) Pins is coimmunoprecipitated with wild-type Dlg but not with mutant Dlg^{m30}. This shows that Pins and Dlg form a complex in vivo that depends on the SH3 domain of Dlg. Pins produced by in vitro transcription/translation (lane 1) was used as a control for Pins size. Lysates were prepared from third-instar larval heads of wild-type (lanes 2 and 3) and dlg^{m30} mutant larvae (lanes 4 and 5). Lanes 2 and 4 are whole-lysate controls, and lanes 3 and 5 are the immunoprecipitates. Pins was detected by Western blotting.

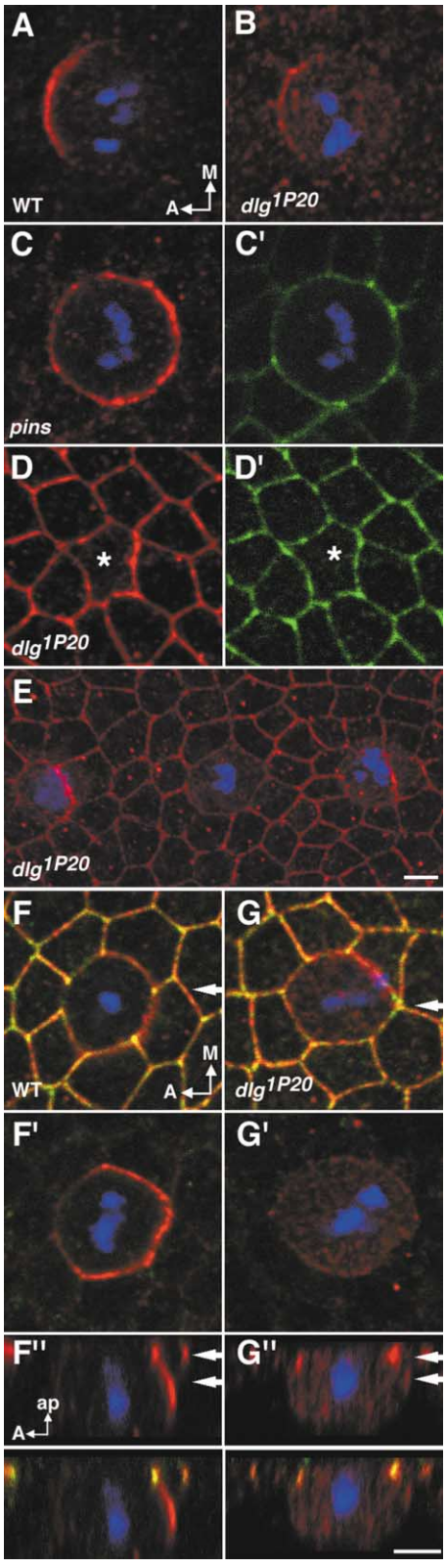

Figure 5. Dlg Is Required for the Asymmetric Localization of Baz

(A and B) Distribution of Pins (red) in a wild-type (A) and *dlg*^{1P20} mutant (B) pI cell. A very weak accumulation of Pins is detected at the anterior cortex in the *dlg*^{1P20} mutant pI cell at metaphase. A higher gain was set to detect the anterior accumulation of Pins in panel B.

(C and C′) Localization of Baz (red in C) and Dlg (green in C′) in a *pins*$^{\Delta 1\text{-}50}$ mutant pI cell at metaphase. Dlg appears to be distributed uniformly at the basal-lateral cortex, while Baz is still enriched at the posterior pole.

(D and D′) The posterior accumulation of Baz (red) localizes apically with Cad (green) in early prophase *dlg*^{1P20} pI cell (asterisk in D and D′).

(E) Baz (red) accumulates to the posterior cortex in the early prophase *dlg*^{1P20} pI cell (pI cell on the left). This posterior cortex persists during prophase (pI cell on the right), but is not detectable at metaphase (pI cell in the middle). To show the accumulation of Baz at the apical cortex and in the cytoplasm, all z-sections were projected in this panel.

(F–G′′) Apical-basal localization of Baz (red) relative to Cad (green) at prometaphase in wild-type (F–F′′) and *dlg*^{1P20} (G–G′′) pI cells. (F) and (G) show apical sections, whereas (F′) and (G′) show more basal confocal sections, as indicated by the arrows in (F′′) and (G′′). The z section axes are indicated by arrows (F and G). Baz colocalizes with Cad at the posterior cortex of *dlg*^{1P20} pI cells but does not form a posterior crescent basal to Cad. Instead, Baz accumulates in the cytoplasm.

In all panels, the pI cells express H2B-YFP (in blue) under the control of neu^{P72}.

(Figure 6G). Moreover, Dlg is distributed around the entire cell cortex, indicating that Fz signaling is required for the anterior accumulation of Dlg (Figure 6G′).

Since Pins localizes asymmetrically in a Fz-independent manner, we asked whether Pins is necessary to localize Baz at one pole of the pI cell in the absence of Fz. We find that Baz localizes uniformly around the basal-lateral cortex in 82% of the *fz pins* double mutant pI cells at metaphase (Figure 6H; n = 22). Moreover, although Numb forms a crescent at anaphase in pI cells mutant for *pins* or *fz*, no Numb crescent is seen at either metaphase or anaphase in *fz pins* double mutant pI cells (Figures 6I–6K). Consistently, loss of *fz* activity enhances the *pins* bristle loss phenotype (not shown). These data show that Pins and Fz act in a redundant manner to exclude Baz from the anterior cortex and to establish planar asymmetry in the pI cell.

Ectopic Expression of Inscuteable Reverses Planar Polarity

Our results show that Pins localizes in a Baz-independent manner to the anterior cortex, with Numb and opposite to Baz, and that Pins cooperates with Fz to exclude Baz from the anterior cortex of the pI cell. In contrast, in neuroblasts, Pins localizes in a Baz-dependent manner to the apical pole, opposite to Numb, and stabilizes the Insc/Baz/DmPAR-6/DaPKC complex (Peng et al., 2000; Schaefer et al., 2000; Yu et al., 2000). Nevertheless, Pins promotes the localization of Numb in both cell types.

One important difference between pI cells and neuroblasts is the lack of *insc* expression in pI cells (Figure 7A; see also Roegiers et al., 2001) To test the functional significance of this lack of Insc, we expressed Insc in the pI cell. Under these circumstances, Insc and Pins localize at the anterior cortex (Figures 7C and 7E). Insc triggers the anterior relocalization of Baz (Figure 7G), while Numb forms a posterior crescent at anaphase (n = 18/19, Figures 7C–7F). The pI cell division remains

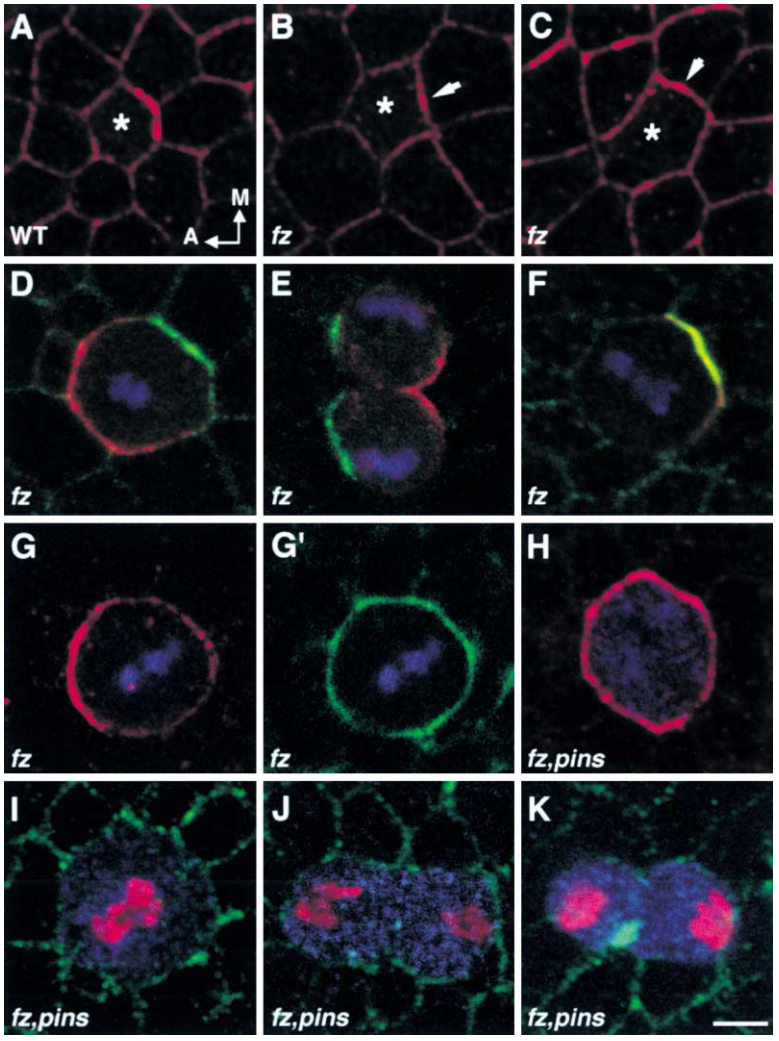

Figure 6. Fz Cooperates with Pins to Localize Baz Asymmetrically

(A–C) Apical views showing the posterior accumulation of Baz (red) at interphase in wild-type (A) and *fz* mutant pI cell (B and C). The position of the pI cells is indicated with asterisks. Arrows indicate where Baz accumulates in the *fz* mutant pI cells. The interphasic pI cells were identified as Cut-positive and pH3-negative cells (not shown). All the pI cells analyzed were from nota in which most of the pI cells have already divided in the dorso-central region.

(D–F) Baz (red in D and E) localizes opposite to Numb (green), while Pins (red in F) colocalizes with Numb in *fz* mutant pI cells. The asymmetric localization of Baz and Pins define two opposite cortical poles that are randomly oriented relative to the body axis. In approximately 5% of the *fz* mutant pI cells, the mitotic spindle fails to line up with the center of these two domains at telophase (E). In two *fz* mutant cells, the Pins crescent is more extended than in wild-type controls (n = 35). In two others, Baz localizes around the cortex at prometaphase (n = 32), and in one *fz* mutant cell, Pins and Baz form bipolar crescents (n = 32).

(G and G′) Dlg (green) does not accumulate to the cortical domain opposite to Baz (red) in *fz* mutant pI cells at metaphase. In (D)–(G′), the pI cells express H2B-YFP (in blue) under the control of neu^{P72} . Mutant backgrounds are: fz^{R54} / fz^{K21} (D and E), and fz^{K21} / fz^{KD4a} (F–G′).

(H–K) Distribution of Baz (red in H) and Numb (green in I–K) in fz^{K21}, $pins^{\Delta 1\text{-}50}$/ fz^{KD4a}, $pins^{\Delta 1\text{-}50}$pI cells. Baz is uniformly cortical in 82% of the *fz pins* double mutant pI cells (H). No crescent of Numb forms at metaphase (I) and anaphase (J). However, Numb accumulates at the site of cytokinesis in one of the two pI daughter cells at late anaphase and telophase (K). A similar accumulation of Numb at the site of cytokinesis is not seen in wild-type pI cells at telophase. Nevertheless, this indicates that Numb retains the ability to form a crescent in a Fz- and Pins-independent manner. The pI cells were identified with Cut (in blue). The DNA was visualized using either DAPI (in H; not shown) or anti-pH3 (red in I–K).

planar (Figure 7F). This contrasts with the effect of Insc in epithelial cells. In these cells, Insc localizes apically and orients the spindle along the apical-basal axis (Kraut et al., 1996). This further indicates that the apical-basal polarity is remodeled in the pI cell. We conclude that the ectopic expression of Insc is sufficient to reverse the planar polarity axis of the pI cell and to modify the activity of Pins relative to Baz. In the absence of Insc, the Dlg/Pins complex excludes Baz, while expression of Insc leads to the formation of a Pins/Insc/Baz complex. In both cases, Numb localizes opposite to Baz.

Discussion

Apical-Basal Polarity Remodeling

In *Drosophila*, Fz signaling regulates the establishment of planar polarity in the epidermis, the eye, and the pI cell. In the epidermis, Fz organizes the actin cytoskeleton at the site of hair formation (Shulman et al., 1998). In the eye, it signals to the nucleus to bias a Notch-dependent cell-fate decision in the eye (Cooper and Bray, 1999; Fanto et al., 1999). Here, we show that planar polarity, in the pI cell, is established by a novel mechanism that involves a remodeling of the previously established apical-basal polarity. During the pI cell division, Baz and DaPKC relocalize from the apical cortex to the posterior lateral cortex, while Dlg and Pins accumulate asymmetrically at the anterior lateral cortex. This redistribution along the a-p axis leads to the formation of two complementary planar domains at the cell cortex (Figure 8A). This mechanism of polarity establishment is distinct from the one described in *Drosophila* neuroblasts. In these cells, Pins is recruited via Insc by Baz to the apical cortex, and acts in a Dlg-independent manner to maintain the Baz/DmPAR-6/DaPKC/Insc complex at the apical cortex (Figures 8B and 8D). We discuss below the roles of Pins, Dlg, Baz, and Fz in establishing and orienting polarity in the pI cell (see also model, Figure 8C).

Role of the Dlg/Pins Complex in Establishing Planar Polarity

Pins is recruited to the anterior cortex of the pI cell opposite to Baz and in a Baz-independent manner. Dlg

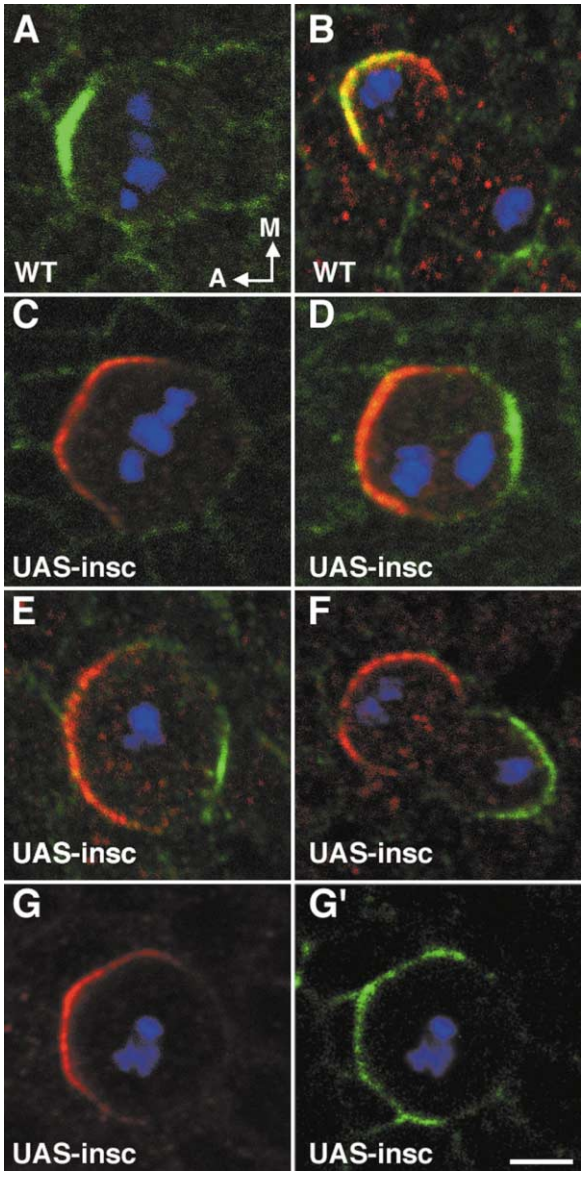

Figure 7. Insc Reverses the Planar Polarity of the pI Cell

(A) At prometaphase, Numb (green) localizes at the anterior cortex in wild-type pI cells, while Insc (red) is not detectable. H2B-YFP is in blue in (A)–(G′).

(B) Numb (red) and Pins (green) specifically segregate to the anterior daughter cell in wild-type pI cells.

(C–F) Insc (red in C and D) and Pins (red in E and F) localize to the anterior cortex of neu^{P72}-GAL4/UAS-Insc pI cells at metaphase (C and E) and anaphase-telophase (D and F). Numb (green in C–F) forms a posterior crescent. This crescent can be hardly seen at metaphase (C and E), suggesting that it forms later than in wild-type pI cells.

(G and G′) Baz (red) is relocalized to the anterior cortex and Dlg (green) accumulates asymmetrically in neu^{P72}-GAL4/UAS-Insc pI cells.

binds directly to Pins and is required for the efficient localization of Pins at the anterior cortex. Conversely, the anterior accumulation of Dlg depends on Pins. Therefore, one function of the Pins/Dlg complex is to further recruit Dlg and Pins at the anterior cortex.

How Pins is initially recruited to the anterior cortex is not known. This involves Fz signaling, since Fz specifies where Pins accumulates along the a-p axis. In the wing epithelium, it has been proposed that Fz establishes planar polarity by signaling at the distal edge of the cell, i.e., where the distal-pointing epithelial hair will grow (Strutt, 2001). Since epithelial hairs point posteriorly in the notum, we suggest that Fz signals at the posterior pole of the pI cell. Fz signaling might therefore promote the posterior localization of Baz and conversely destabilize the Dlg/Pins complex at the posterior pole.

The analysis of the *fz-pins* double-mutant phenotype reveals that Pins is sufficient to exclude Baz from one pole of the cell cortex and to establish asymmetry. Furthermore, the asymmetry in the localization of Baz is less pronounced in *pins* mutant pI cells than in wild-type cells. We propose that Pins cooperates with Fz by excluding Baz from the anterior cortex, thereby reinforcing the initial asymmetry established by Fz. Both Pins and Baz promote the asymmetric localization of Numb. Pins may either act locally to localize Numb at the anterior cortex, or indirectly by excluding Baz from the anterior cortex.

Pins binds Gαi/o, and AGS3, a mammalian homolog of Pins, activates G$\beta\gamma$ signaling by binding to the GDP-bound form of Gαi (De Vries et al., 2000; Parmentier et al., 2000; Schaefer et al., 2000). In the pI cell, Gαi localizes at the anterior cell cortex (Y.B., unpublished data). This raises the possibility that Pins localizes Baz and Numb by activating G protein signaling.

Dlg Acts as a Lateral Scaffold

Dlg is required to maintain Pins and Pon at the anterior cortex and Baz at the posterior cortex, suggesting that Dlg acts as a scaffold around the lateral cortex of the pI cell. Dlg also promotes spindle rotation. Therefore, it may scaffold the molecules required for spindle rotation. A scaffolding function has been proposed for Dlg in neuroblasts (Ohshiro et al., 2000; Peng et al., 2000). In these cells, the localization of Numb at the basal cortex appears to require the cortical anchoring of the Lgl protein by Dlg. Since Lgl is also required to localize Numb in the pI cell (Ohshiro et al., 2000), Dlg may also anchor Lgl in the pI cell.

Distinct Modes of Baz Localization

Baz is not required to maintain epithelial polarity in the notum nor to orient the division of the pI cell. Baz is, however, required for the efficient localization of Numb at the anterior cortex of the pI cell. Baz has a conserved function in localizing cell-fate determinants asymmetrically. In the *C. elegans* zygote, PAR-3 localizes asymmetrically to the anterior cortex in response to a signal provided by the sperm aster and directs the posterior localization of P granules and the anterior accumulation of the MEX-5 determinant (Etemad-Moghadam et al., 1995; Schubert et al., 2000; Wallenfang and Seydoux, 2000). In *Drosophila* neuroblasts, Baz localizes apically and directs the basal localization of Numb and Prospero (Schober et al., 1999; Wodarz et al., 1999). In the pI cell, Baz is redistributed to the posterior-lateral cortex and is required to localize Numb to the anterior pole. In each case, Baz/PAR-3 probably functions in a multiprotein complex with aPKC and PAR-6 (Petronczki and Knoblich, 2001; Wodarz et al., 2000).

While the function of Baz appears to be conserved,

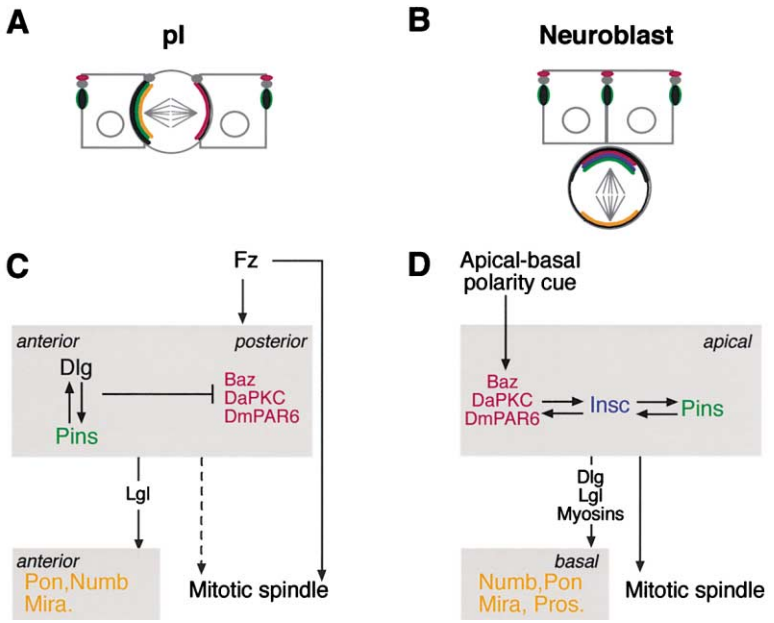

Figure 8. Mechanisms of Polarization in the pI Cell and in the Neuroblast

(A and B) Diagram showing the distribution of Baz (red), Dlg (black), Pins (green), Numb (orange), Miranda (Mira, orange), Prospero (Pros, orange), and Insc (blue in B) in the pI cell (A) and in the neuroblast (B). AJs are indicated by gray dots (A and B). The pI cell divides within the plane of the epithelium, while the neuroblast delaminates from the neuroepithelium prior to division.

(C and D) Regulatory network responsible for the planar and apical-basal polarization of the pI cell (C) and of the neuroblast (D), respectively. See text for details.

the molecular mechanisms involved in the asymmetric localization of this complex may vary. In *C. elegans*, the anterior localization of PAR-3 depends on PAR-2, which is not conserved in *Drosophila*. PAR-2 localizes at the posterior cortex in response to sperm entry and thereby excludes PAR-3 from this pole (Boyd et al., 1996; Etemad-Moghadam et al., 1995). In *Drosophila*, Pins and Dlg have distinct functions in regulating Baz localization in the neuroblast and in the pI cell. In neuroblasts, Pins and Baz colocalize and are mutually dependent for their asymmetric localization (Schaefer et al., 2000; Yu et al., 2000), and Dlg is not required to maintain Baz at the cortex (Ohshiro et al., 2000; Peng et al., 2000). Insc is specifically expressed in neuroblasts and molecularly links Baz and Pins. In contrast, in the dividing pI cell, Pins cooperates with Fz to exclude Baz, and Dlg is required to anchor Baz at the posterior-lateral cortex. Finally, while Pins excludes Baz-containing complexes in the absence of Insc, Insc appears to be sufficient to promote the stabilization of Baz-containing complexes by Pins. These variations illustrate that distinct regulatory strategies have been selected during evolution to establish polarity by localizing Baz/PAR-3 asymmetrically.

Experimental Procedures

Flies

The hypomorphic $dlg^{sw}$and dlg^{1P20} mutant alleles are described in Woods and Bryant, 1991 and Woods et al., 1996. The $pins^{\Delta 1\text{-}50}$mutant allele is a null allele that corresponds to a small deletion (Schaefer et al., 2000). Recombinant chromosomes carrying the $pins^{\Delta 1\text{-}50}$mutant allele were selected by PCR analysis (details and primer sequences available upon request). KD4a and K21 are null alleles of *fz*, while R54 is a strong loss-of-function allele (Jones et al., 1996). The neu^{P72}-GAL4 (Bellaiche et al., 2001) line was used to express the H2B-YFP (Bellaiche et al., 2001), tau-GFP (Kaltschmidt et al., 2000), Arm-Myc (Pai et al., 1997), and Insc (Knoblich et al., 1999) proteins using the UAS/GAL4 expression system. The function of *baz* was analyzed in somatic clones using the null allele baz^{Xi106} (Wodarz et al., 1999). Clones were recovered *y*, baz^{Xi106}, FRT18E / ovo^{D}, FRT18E; hs-flp^{38}/ + pupae that had been heat shocked for 1 hr at 37°C at 80 ± 12 hr after egg laying.

Immunocytochemistry

Pupal nota were dissected from staged non-*Tb* and/or *y* pupae selected from the following crosses: (1) *w*; UAS-H2B-YFP; neu^{P72}/SM5 :TM6b, *Tb* x *w*/Y; (2) *y*, dlg^{sw}/Basc x *w*/Y; UAS-H2B-YFP; neu^{P72}/SM5 :TM6b, *Tb*; (3)

y, dlg^{1P20}, *f*/Basc x *w*/Y; UAS-H2B-YFP; neu^{P72}/ SM5 :TM6b, *Tb*; (4) UAS- H2B-YFP/+; fz^{K21}/TM6b, *Tb* x fz^{KD4a}, neu^{P72}/TM6b, *Tb*; (5) UAS-H2B-YFP/Cyo; fz^{R54}/TM6b, *Tb* x fz^{KD4a}, neu^{P72}/TM6b, *Tb*; (6) *w*, UAS-H2B-YFP; $pins^{\Delta 1\text{-}50}$/SM5 :TM6b, *Tb* x $pins^{\Delta 1\text{-}50}$, neu^{P72}/SM5 :TM6b, *Tb*; (7)

fz^{K21}, $pins^{\Delta 1\text{-}50}$/TM6b, *Tb* x fz^{KD4a}, $pins^{\Delta 1\text{-}50}$/TM6b, *Tb*; and (8) fz^{K21}/TM6b, *Tb* x fz^{KD4a}/TM6b, *Tb*.

Primary antibodies were rabbit anti-Pon (gift from Y.N. Jan; 1:1000), guinea-pig anti-Numb (gift from Y.N. Jan; 1:1000), rat anti-α-tubulin (Serotec; 1:3000), rabbit anti-HRP (gift from J.-R. Martin; 1:1000), rat anti-Su(H) (1:1000), mouse anti-Cut (2B10 obtained from the DSHB; 1:500), rat anti-DE-cadherin (gift from T. Uemura; 1:50), guinea-pig anti-Dlg (1:3000), rabbit anti-Baz (gift from A. Wodarz; 1:3000), rabbit anti-pH3 (Upstate Biotechnology; 1:2000), mouse anti-Arm (gift from M. Peifer; 1:50), mouse anti-Fas3 (7G10, obtained from the DSHB 1:10), rabbit anti-Pins (gift from J. Knoblich; 1:1000), rat anti-Pins (1:1000), rabbit anti-Myc (Upstate Biotechnology; 1:1000), and rabbit anti-PKCzeta (Santa Cruz Biotechnology; 1:1000). We studied the distribution of Discs-lost, Lethal(2)giant larvae, Canoe, Crumbs, Coracle, and Spectrin. The Cy3- and Cy5-coupled secondary antibodies were from Jackson's Laboratories, and Alexa-488-coupled secondary antibodies were from Molecular Probes. Images were acquired on Leica TCS 4D and SP2 confocal microscopes, and assembled using Adobe Photoshop.

GFP Imaging

GFP imaging was carried out as described in Bellaiche et al. (2001) on non-*Tb* and/or *y* pupae from the following crosses: (1) *y*, *w* x *w*/Y; neu^{P72}, UAS-tau-GFP/TM6b, *Tb*; (2) *y*, dlg^{1P20}, *f*/Basc x *w*/Y; neu^{P72}, UAS-tau-GFP/TM6b, *Tb*; (3) *y*, dlg^{SW}/Basc x *w*/Y; neu^{P72}, UAS-tau-GFP/TM6b, *Tb*; and (4) *w*/Y; UAS-tau-GFP/+; $pins^{\Delta 1\text{-}50}$/TM6b, *Tb* x *w*; neu^{P72}, $pins^{\Delta 1\text{-}50}$/TM6, *Tb*

Blot Overlay and Coimmunoprecipitation

DNA fragments encoding the Dlg domains (PDZ1[109–399]; PDZ2[439–756]; PDZ 3[1393–1695]; SH3[1810–2001]; HOOK[2002–2340]; and GUK[2329–2805]) were made by PCR amplification and

inserted into the pGEX expression vector. The purified GST-fusion proteins were resolved by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% dry milk TPBS, and incubated with biotinylated Pins overnight. Biotinylation was performed using aminohexanoylbiotin N-hydroxysuccinimide according to the manufacturer's instructions (PIERCE). After washing, the bound biotinylated Pins was detected using avidin-HRP and the ECL detection kit (Amersham Life Sciences).

The brain and imaginal discs from third-instar larval heads (~5 days old for wild-type and ~10 days old for *dlg*^{m30} mutant larvae) were dissected in Ringer's solution. Approximately 20 heads were placed in lysis solution (100 mM Tris [pH 7.5], 1% NP40) on ice and homogenized with a small dounce homogenizer, centrifuged at 14,000 rpm for 15 min, and the soluble fraction was collected. The extracts were then incubated in an Eppendorf tube with the UltraLink™ immobilized protein A/G (PIERCE) and Guinea pig anti-Dlg for 2 hr at 4°C. The immunoprecipitates were analyzed by Western blotting using Guinea pig anti-Pins antibodies (1:500), peroxidase-conjugated goat anti-Guinea pig secondary antibodies (Jackson immunoresearch; 1:2000), and ECL (Amerham Life Sciences). Guinea pig anti-Pins antibody was made using the methods already described for rat anti-Pins (Parmentier et al., 2000).

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