The planar cell polarity protein Strabismus promotes Pins anterior localization during asymmetric division of sensory organ precursor cells in *Drosophila*

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Summary

Cell fate diversity is generated in part by the unequal segregation of cell-fate determinants during asymmetric cell division. In the *Drosophila* bristle lineage, the sensory organ precursor (pI) cell is polarized along the anteroposterior (AP) axis by Frizzled (Fz) receptor signaling. We show here that Fz localizes at the posterior apical cortex of the pI cell prior to mitosis, whereas Strabismus (Stbm) and Prickle (Pk), which are also required for AP polarization of the pI cell, co-localize at the anterior apical cortex. Thus, asymmetric localization of Fz, Stbm and Pk define two opposite cortical domains prior to mitosis of the pI cell. At mitosis, Stbm forms an anterior crescent that overlaps with the distribution of Partner of Inscuteable (Pins) and Discs-large (Dlg), two components of the anterior Dlg-Pins-Gαi complex that regulates the localization of cell-fate determinants. At prophase, Stbm promotes the anterior localization of Pins. By contrast, Dishevelled (Dsh) acts antagonistically to Stbm by excluding Pins from the posterior cortex. We propose that the Stbm-dependent recruitment of Pins at the anterior cortex of the pI cell is a novel read-out of planar cell polarity.

Supplemental data available online

Key words: Planar cell polarity, Strabismus, Asymmetric cell division, Sensory organ

Introduction

During development, cell fate diversity is generated in part by asymmetric cell division, in which a mother cell divides to produce two daughter cells with distinct developmental potentials. Adoption of different fates often relies on the unequal segregation of cell-fate determinants at mitosis. To ensure unequal partitioning of cell-fate determinants at anaphase, the mitotic spindle must be lined up with the polarity axis revealed by the position of cell-fate determinants at the cortex. Polarization of the mother cell regulates both mitotic spindle positioning and asymmetric localization of cell-fate determinants.

Recent studies have shed light on the mechanisms that regulate cell polarization during asymmetric division in the *Drosophila* peripheral nervous system. Each bristle sensory organ is composed of four distinct cells that are generated from a single sensory organ precursor cell (pI for primary precursor) via a series of stereotyped asymmetric divisions (Fichelson and Gho, 2003; Gho et al., 1999; Reddy and Rodrigues, 1999). In the pupal notum, the pI cell divides asymmetrically within the plane of the single-layered epithelium, along the anteroposterior (AP) axis of the pupa, to generate an anterior pIIb cell and a posterior pIIa cell (Gho and Schweisguth, 1998). The cell-fate determinants Numb and Neuralized (Neur) localize at the anterior cortex of the pI cell during prophase (Le Borgne and Schweisguth, 2003; Rhyu et al., 1994). At prometaphase, the mitotic spindle rotates and lines up with these cell-fate determinants (Bellaiche et al., 2001a; Roegiers et al., 2001). Numb and Neur co-segregate into the anterior cell, where they both act to bias the Notch-mediated pIIa/pIIb fate decision (Berdnik et al., 2002; Guo et al., 1996; Le Borgne and Schweisguth, 2003; Rhyu et al., 1994). The anterior localization of these two cell-fate determinants and the AP orientation of the mitotic spindle depend on the activity of the *frizzled* (*fz*) gene (Bellaiche et al., 2001a; Gho and Schweisguth, 1998; Roegiers et al., 2001). In *fz* mutant pupae, the division of the pI cell is oriented randomly relative to the AP axis, with Numb localizing asymmetrically at one pole of the mitotic spindle in most pI cells. Thus, *fz* activity is necessary to orient the planar cell polarity (PCP) of the pI cell along the AP axis but is not required for asymmetric localization of cell-fate determinants.

Fz encodes a seven-pass transmembrane receptor that signals to polarize cells within the plane of the epithelium (Adler, 2002). Recent studies have indicated that Fz signaling must be spatially restricted within the cell to ensure establishment of PCP (Strutt, 2003; Strutt, 2002). In pupal wing epidermal cells, which are polarized along the proximodistal axis of the wing, Fz becomes asymmetrically localized at the distal edge of the apical cortex of each epidermal cell (Strutt, 2001b). The asymmetric distribution of Fz is regulated by PCP proteins, three of which also localize asymmetrically. Dishevelled (Dsh)

co-localizes with Fz at the distal-apical cortex (Axelrod, 2001; Shimada et al., 2001), whereas Prickle (Pk), a LIM- and PET-domain protein, and Strabismus (Stbm; also know as Van Gogh), a four-pass transmembrane protein, form a complex and localize opposite to Fz (Bastock et al., 2003; Gubb et al., 1999; Jenny et al., 2003; Tree et al., 2002; Wolff and Rubin, 1998). The activity of each of these PCP proteins is required for each of the others to become localized asymmetrically. Thus, formation of two opposite domains at the apical cortex appears to be essential to spatially restrict Fz signaling to a specific domain at one pole of the apical cortex, which, in turn, is translated into the planar polarization of wing epidermal cells.

How Fz regulates the anterior localization of Numb and Neur as well as the AP orientation of the spindle is not well understood. Two signaling complexes localizing opposite to each other have, however, been implicated in this process. The first complex includes two PDZ-containing proteins, Bazooka (Baz; the fly homolog of Par3) and Par-6, together with the atypical Protein Kinase C (aPKC) (for a review, see Henrique and Schweisguth, 2003). This complex re-localizes from the apical cortex to the posterior lateral cortex at mitosis (Bellaiche et al., 2001b). The second complex includes the proteins Discs-large (Dlg), Partner of Inscuteable (Pins) and the Gαi subunit of heterotrimeric G proteins (Bellaiche et al., 2001b; Schaefer et al., 2001). This complex localizes at the anterior lateral cortex of the dividing pI cell. The Dlg protein contains three PDZ domains, one SH3 domain and a C-terminal GUK domain. In epithelial cells, Dlg localizes at septate junctions and regulates epithelial cell polarity (Bilder et al., 2000; Woods and Bryant, 1991; Woods et al., 1996). In mitotic pI cells, Dlg interacts via its SH3 domain to Pins and regulates the accumulation of Pins at the anterior cortex (Bellaiche et al., 2001b). Pins is a modular protein with seven tetratricopeptide repeats (TPR) and three G protein regulatory (GPR) motifs (Schaefer et al., 2000; Yu et al., 2000). The GPR motif has the ability to bind the GDP-bound form of Gαi and has been proposed to promote Gβγ signaling by dissociating the Gβγ dimer from Gαi•GDP (De Vries et al., 2000; Schaefer et al., 2001). The downstream targets of the Pins-Gαi, Gβγ and aPKC signaling activities in the pI cell are not known.

We have investigated the mechanisms by which PCP signaling regulates the anterior localization of the Dlg-Pins-Gαi complex during the asymmetric pI cell division. Our data indicate that Fz localizes at the posterior cortex prior to mitosis, whereas Stbm and Pk co-localize at the anterior cortex. At mitosis, Stbm forms an anterior crescent that overlaps with the distribution of Pins and Dlg, and promotes Pins cortical localization at prophase. We propose that a read-out of PCP in the pI cell is the Stbm-dependent localization of the Dlg-Pins-Gαi complex at the anterior cortex.

Materials and methods

Flies

The null fz^{K21}, fz^{Kd4a}, $stbm^{6c}$, baz^{XJ106} alleles and the dsh^{1} allele are described in FlyBase (http://flybase.bio.indiana.edu/). $pk^{pk\text{-}sple14}$ is a null allele (D. Gubb, personal communication).

Somatic clones were generated in: (1) *Ubx*-flp baz^{XJ106} FRT9-2/*ubi-nlsGFP* FRT9-2; (2) *Ubx*-flp dsh^{1} baz^{XJ106} FRT9-2/*ubi-nlsGFP* FRT9-2; and (3) *Ubx*-flp baz^{XJ106} FRT9-2/nlsGFP FRT9-2; $stbm^{6c}$ pupae. The *Ubx*-flp stock was a kind gift of J. Knoblich.

The *arm* –Fz::GFP line is described elsewhere (Strutt, 2001b). The $neur^{P72}$GAL4 (Bellaiche et al., 2001a) line was used to express Histone2B::YFP (Bellaiche et al., 2001a), Pon::GFP (Lu et al., 1999a), tau::GFP (Kaltschmidt et al., 2000), Pk (Gubb et al., 1999), GFP::Stbm, GFP::StbmΔPBM and Stbm using the UAS/GAL4 expression system (Brand and Perrimon, 1993). The UAS-Stbm, UAS-GFP::Stbm, UAS-GFP::StbmΔPBM, *arm*-Stbm, *arm*-GFP::Stbm, *arm*-StbmΔPBM and *arm*-GFP::Stbm transgenic flies were generated by P-element transformation. In all GFP::Stbm constructs, the sequence of m6GFP (gift of A. Brand) was fused in frame to the first codon of Stbm (the *stbm* cDNA was a gift of T. Wolff). Cloning details for the UAS-GFP::Stbm, UAS-GFP::StbmΔPBM, *arm*-Stbm, *arm*-StbmΔPBM and *arm*-GFP::Stbm plasmids are available upon request.

GFP imaging

Live GFP imaging was carried out as described (Bellaiche et al., 2001a). Orientation of the pI cell division was measured at telophase using tau::GFP or Pon::GFP in living pupae of the following genotypes: (1) $neur^{P72}$GAL4/UAS-tau::GFP; (2) $neur^{P72}$GAL4/UAS-Pon::GFP; (3) fz^{K21} UAS-tau::GFP/fz^{Kd4a} $neur^{P72}$GAL4; (4) $stbm^{6c}$; $neur^{P72}$GAL4/UAS-Pon::GFP; (5) $pk^{pk\text{-}sple14}$; $neur^{P72}$GAL4/UAS-Pon::GFP. For all other genotypes, the orientation of the pI cell division was measured at telophase in fixed pupae described previously (Bellaiche et al., 2001a). Localization of Pon::GFP was analyzed in: (1) dsh^{1}/Y; $neur^{P72}$GAL4 UAS-Pon::GFP/+; (2) $stbm^{6c}$; $neur^{P72}$GAL4 UAS-Pon::GFP/+; (3) $pk^{pk\text{-}sple14}$; $neur^{P72}$GAL4 UAS-Pon::GFP/+; and (4) UAS-Pk/+; $neur^{P72}$GAL4 UAS-Pon::GFP/UAS-Stbm pupae.

Fluorescence Recovery After Photo-bleaching (FRAP) analysis was performed on *arm*-Fz::GFP pupae. Photobleaching was achieved by scanning a region of interest using the 488 nm laser source at maximal intensity.

All images were acquired on a SP2 confocal microscope and assembled using NIH image and Photoshop software.

Antibodies

Rabbit polyclonal anti-Stbm antibodies were raised against a mixture of the two peptides CNVLAEEVVDPKSNKFV and MENESVKSEHSGRSRC.

Pupal nota were dissected and processed as previously described (Gho et al., 1999). Primary antibodies were: guinea pig anti-Senseless (Sens; gift from H. Bellen; 1:3000); mouse anti-Cut (2B10 obtained from the DSHB; 1:500); rat anti-DE-Cadherin (gift from T. Uemura; 1:50); guinea pig anti-Dlg (gift from P. Bryant; 1:3000); rabbit anti-Baz (gift from A. Wodarz; 1:3000); rabbit anti-Pins (gift from J. Knoblich; 1:1000); rat anti-Pins (gift from P. Bryant; 1:1000); rabbit anti-Pk (gift from J. Axelrod; 1:500); rabbit anti-Stbm (affinity-purified;1:400); and rabbit anti-GFP (Molecular Probes; 1:1000). Cy3- and Cy5-coupled secondary antibodies were from Jackson's Laboratories and Alexa-488-coupled secondary antibodies were from Molecular Probes.

Protein interaction assays

A cDNA fragment encompassing the intracellular domain of Stbm with (303-584) or without (303-581) the PBM was subcloned into pGEX2KG. A PCR product encompassing Stbm553-584 was subcloned into pGEX2KG to generate GST-PBM. Similarly, cDNA fragments encoding the full-length DlgA isoform (GST-Dlg) or the PDZ domains of Dlg (GST-PDZ1-3: amino acid 39-640) were subcloned into pGEX2KG. Met ^{35}S radiolabelled Stbm and Dlg proteins were in vitro synthesized using the TNT kit (Promega). Stbm was synthesized in presence of Canine Pancreatic Microsomal Membranes (Promega). Pull-down assays were performed as previously described (beads were incubated in PBS, NP40 0.5% and 0.5 M NaCl in the final wash) (Brou et al., 1994).

Results

Fz localizes at the posterior apical cortex of the pI cell

In the notum of wild-type pupae, pI cells divide within the plane of the epithelium and along the AP axis (Fig. 1A) (Gho et al., 1999; Gho and Schweisguth, 1998). In *fz* mutant pupae, the mitotic spindle is randomly oriented relative to the AP axis (Fig. 1B). This PCP phenotype was fully rescued by a transgene directing the ubiquitous expression of a Fz::GFP fusion protein (Fig. 1C) (Strutt, 2001b). Using this functional Fz::GFP protein, we have examined the localization of Fz in the developing notum at 16-17 hours APF, when pI cells divide. Fz::GFP co-localized with Baz and E-Cadherin (Cad) at the apical cortex of epidermal cells (not shown), and showed no obvious sign of planar asymmetry. In pI cells, however, Fz::GFP predominantly co-localized with Baz at the posterior cortex at prophase (Fig. 1D-D″). Posterior localization of Fz::GFP was confirmed using Fluorescence Recovery After Photo-bleaching (Fig. 1E-E″). The posterior cortex of the pI cell and the epidermal cells posterior to the pI cell were photo-bleached prior to division of the pI cell. If Fz::GFP accumulates at the anterior cortex of epidermal cells, then no significant fluorescence recovery should be observed at the contact region with the pI cell because the entire pool of Fz::GFP molecules of these posterior epidermal cells has been photo-bleached. By contrast, if Fz::GFP accumulates at the posterior cortex of the pI cell, then fluorescence recovery should be observed because of the redistribution of fluorescent Fz::GFP molecules within the pI cell. After photo-bleaching, a recovery of Fz::GFP was seen at the posterior cortex of the pI cell (Fig. 1E-E″). We therefore conclude that Fz::GFP accumulates at the posterior pole of the pI cell.

Time lapse-analysis revealed that Fz::GFP localized uniformly at the apical cortex of pI cells in *dsh*^{1}, *stbm*^{6c} and *pk*$^{pk\text{-}sple14}$ mutant pupae (Fig. 1F-H; data not shown), indicating that posterior accumulation of Fz::GFP in pI cells required the activity of the *dsh*, *stbm* and *pk* genes. Thus, our results extend to the pI cell the observation first made in wing epidermal cells showing that unipolar accumulation of Fz::GFP depends on PCP signaling (Strutt, 2001b; Strutt, 2002).

Stbm and Pk co-localize at the apical anterior cortex of the pI cell

The *stbm* and *pk* genes not only regulate the posterior localization of Fz::GFP in pI cells but also polarize the pI cell along the AP axis. In *stbm*^{6c} and *pk*$^{pk\text{-}sple14}$ mutant pupae, pI cells divided with a random orientation (Fig. 2A,D) and Numb localized asymmetrically but at a random position relative to the AP axis (not shown). Thus, *stbm* and *pk* mutant pI cells exhibit planar polarity defects that are similar to the ones previously described for *fz*, *dsh*^{1} and *fmi* mutant pupae (Gho and Schweisguth, 1998; Lu et al., 1999b).

To study the distribution of Stbm, we generated antibodies that specifically detect Stbm on western blots and on fixed tissues (data not shown; see Fig. S1 at http://dev.biologists.org/supplemental). In epidermal cells, Stbm co-localized with Cad, apical to Dlg, with no clear sign of planar asymmetry (Fig. 2F-G). In pI cells, however, Stbm localized asymmetrically at the anterior cortex prior to division, and formed a crescent opposite to Baz upon mitosis (Fig. 2H-J″).

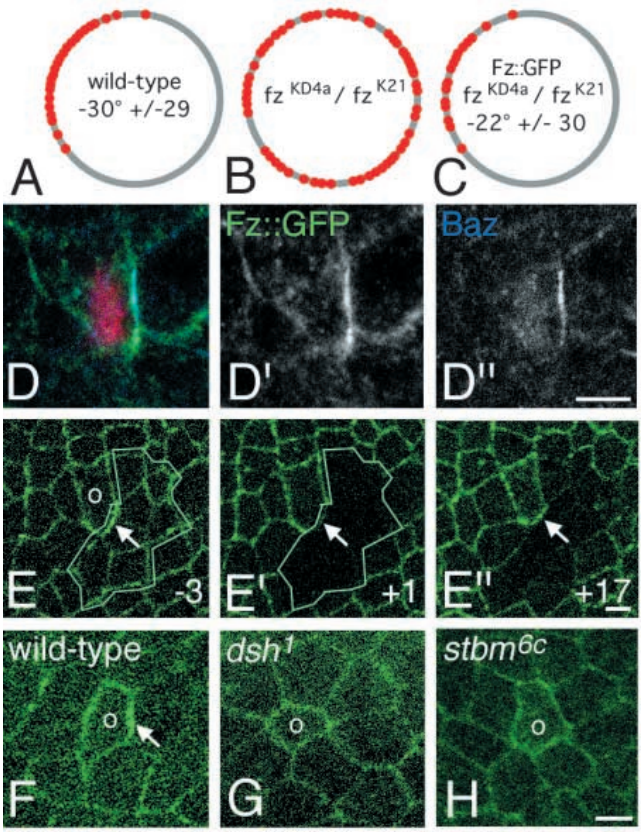

Fig. 1. Fz-GFP localizes to the posterior cortex. (A-C) Orientation of the pI cell division in neur^{P72}GAL4 UAS-tau::GFP (wild type; A), *fz*^{K21} UAS-tau::GFP/*fz*^{Kd4a} neur^{P72}GAL4 (B) and *arm*-Fz::GFP/+; *fz*^{K21} UAS-tau::GFP/*fz*^{Kd4a} neur^{P72}GAL4 pupae (C). Expression of Fz::GFP rescued the *fz* PCP phenotype. Each red dot indicates the orientation of one division measured by live imaging as described elsewhere (Bellaiche et al., 2001a). Anterior is leftwards, midline is towards the top. Mean and standard deviation values are given for genotypes exhibiting a stereotyped orientation. (D-D″) Fz::GFP (anti-GFP; green in D,D′) co-localized with Baz (blue in D,D″) at the apical posterior cortex of pI cells at early prophase. Sens (red in D) was used as a pI cell marker. (E-E″) FRAP analysis indicates that Fz::GFP localizes at the posterior cortex in the pI cell. Live GFP imaging of a 16.5 hours APF *arm*-Fz::GFP pupa showing a pI cell (circle) prior to photobleaching (E; *t*=–3 minutes), soon after photobleaching (E′; *t*=1 minute) and 17 minutes after photobleaching (E″). The photobleached region includes the posterior cortex of the pI cell (arrow) and the epidermal cells posterior to this pI cell. (F-H) Live GFP imaging of wild-type (F), *dsh*^{1} (G) and *stbm*^{6c} (H) mutant pupae expressing *arm*-Fz::GFP. The arrow in F indicates the posterior accumulation of Fz::GFP in the pI cell prior to mitosis. Asymmetric Fz::GFP localization depends on *dsh* and *stbm* activities. pI cells (circle) were identified based on their higher level of Fz::GFP accumulation at 16.5 hours APF and on the stereotyped morphology of their daughter cells. In this and all other figures, anterior is towards the left. Scale bars: 5 μm.

We further studied the distribution of Stbm using a GFP::Stbm fusion protein. Uniform low-level expression of GFP::Stbm fully rescued the *stbm*^{6c} mutant PCP phenotype (Fig. 2B), indicating that GFP::Stbm is functional (GFP::Stbm was expressed at a level similar to endogenous Stbm: see Fig. S1 at http://dev.biologists.org/supplemental). Expression of

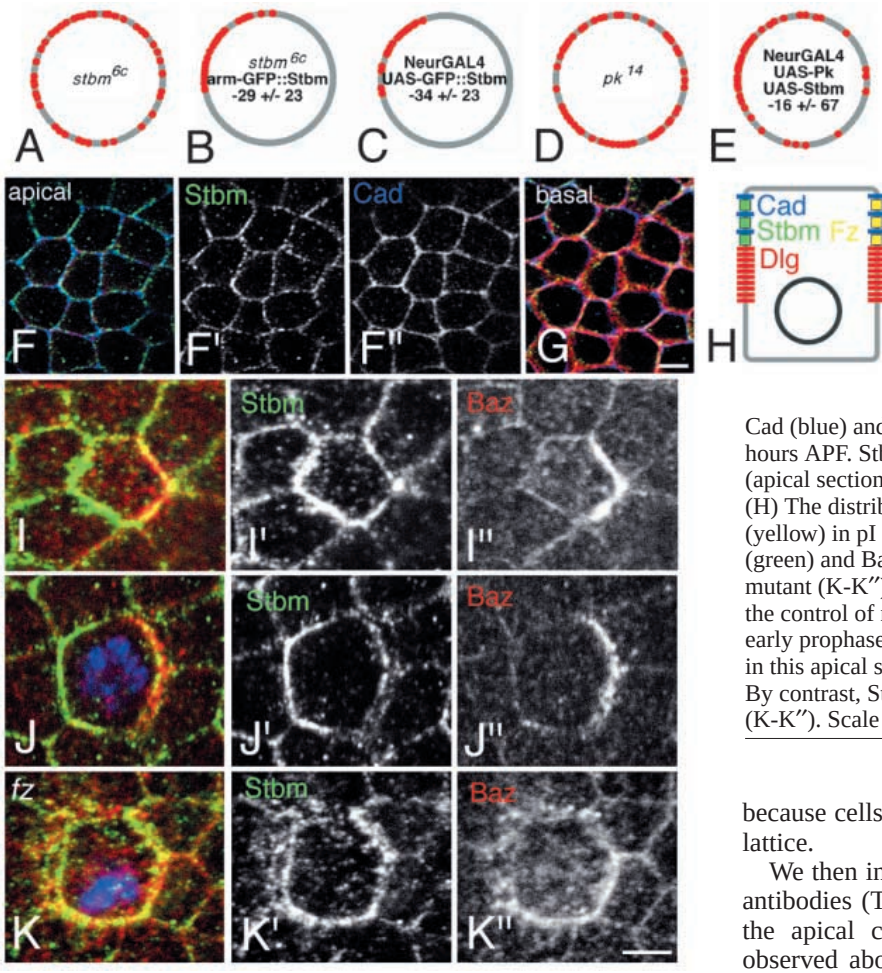

Fig. 2. Stbm localizes to the anterior apical cortex. (A-E) Orientation of the pI cell division in *stbm*^{6c} (A), *stbm*^{6c}; *arm*-GFP::Stbm (B), neur^{P72}GAL4/UAS-GFP::Stbm (C), *pk*$^{pk\text{-}sple14}$ pupae (D) and UAS-Pk/+; neur^{P72}GAL4/UAS-Stbm (E). Low-level ubiquitous expression of GFP::Stbm rescued the *stbm*^{6c} PCP phenotype, whereas overexpression of Pk and Stbm in the pI cell partially disrupted planar polarity. Orientation of cell division was measured at anaphase and telophase in fixed pupae stained for Sens and either Numb or Pins as described elsewhere (Bellaiche et al., 2001a). (F,G) Localization of Stbm (green), Cad (blue) and Dlg (red) in dorsal thorax epithelial cells at 16.5 hours APF. Stbm co-localized with Cad at the apical cortex (apical section in F-F″), apical to Dlg (basal section in G). (H) The distribution of Cad (blue), Dlg (red), Stbm (green) and Fz (yellow) in pI cells prior to division. (I-K″) Localization of Stbm (green) and Baz (red) in wild-type (I-I″,J-J″) and *fz*^{K21}/*fz*^{Kd4a} mutant (K-K″) pI cells expressing Histone2B::YFP (blue) under the control of neur^{P72}GAL4. Stbm localized opposite to Baz at early prophase (I-I″; note that Histone2B::YFP was not detected in this apical section) and prometaphase (J-J″) in wild-type cells. By contrast, Stbm localized rather uniformly in *fz* mutant pI cells (K-K″). Scale bar: 5 μm.

GFP::Stbm (or Stbm) in pI cells did not modify the stereotyped orientation of the pI cell division (Fig. 2C; data not shown). GFP::Stbm localized at the apical anterior cortex of the pI cell prior to division (Fig. 3A) and formed an anterior crescent at mitosis (Fig. 3B,B′). Anterior accumulation of GFP::Stbm was also seen in epidermal cells (Fig. 3C). This suggests that endogenous Stbm may also localize asymmetrically in epidermal cells but that this asymmetry is difficult to visualize because cells in the notum do not form a regular hexagonal lattice.

We then investigated the localization of Pk using anti-Pk antibodies (Tree et al., 2002). Pk co-localized with Cad at the apical cortex of epidermal cells (Fig. 4A-A″). As observed above for Fz::GFP and Stbm, no sign of planar asymmetry was detected in the distribution of Pk in epidermal cells. However, the higher level of Pk accumulation in pI cells allowed us to detect an accumulation of Pk at the anterior cortex of pI cells, opposite to Baz (Fig. 4A-B). Consistent with the notion that Pk and Stbm directly interact (Bastock et al., 2003; Jenny et al., 2003), Pk co-localized with GFP::Stbm (Fig. 4C,C′). We conclude that Pk and Stbm co-localize at the anterior apical cortex of the pI cell, opposite to Fz, prior to mitosis.

The asymmetric accumulation of Pk and Stbm was dependent on the activity of *fz* and *dsh*. In *fz*^{KD4a}/*fz*^{K21} and *dsh*^{1} mutant cells, Pk, Stbm and GFP::Stbm were uniformly distributed at the apical cortex (Fig. 2K-K″,

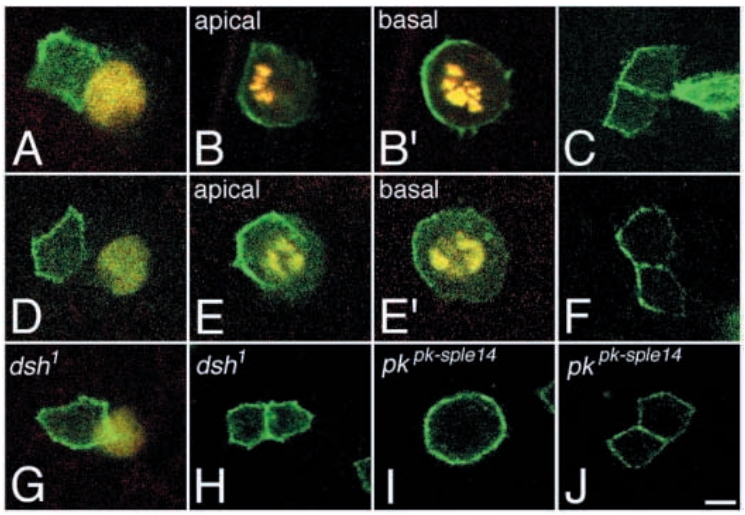

Fig. 3. Distribution of GFP::Stbm and GFP::StbmΔPBM. Localization of GFP::Stbm (green in A-C and G-J) and GFP::StbmΔPBM (green in D-F) in living pI cells expressing Histone2B::YFP (yellow in A-H) under the control of neur^{P72}GAL4. GFP::Stbm and GFP::StbmΔPBM similarly localized at the apical anterior cortex of both pI (A,D) and epidermal cells (C,F; a few isolated epidermal cells expressed low levels of GAL4 in the notum of neur^{P72}GAL4 pupae). In mitotic pI cells, low levels of GFP::Stbm are seen at the apical cortex (B). By contrast, GFP-StbmΔPBM remained apical (E) and did not accumulate laterally as GFP::Stbm (B,B′ and E,E′ show two different confocal sections of the same cell). Anterior localization of GFP-Stbm in pI and epidermal cells was disrupted in *dsh*^{1} (G,H) and *pk*$^{pk\text{-}sple14}$ (I,J) mutant pupae. Scale bars: 5 μm.

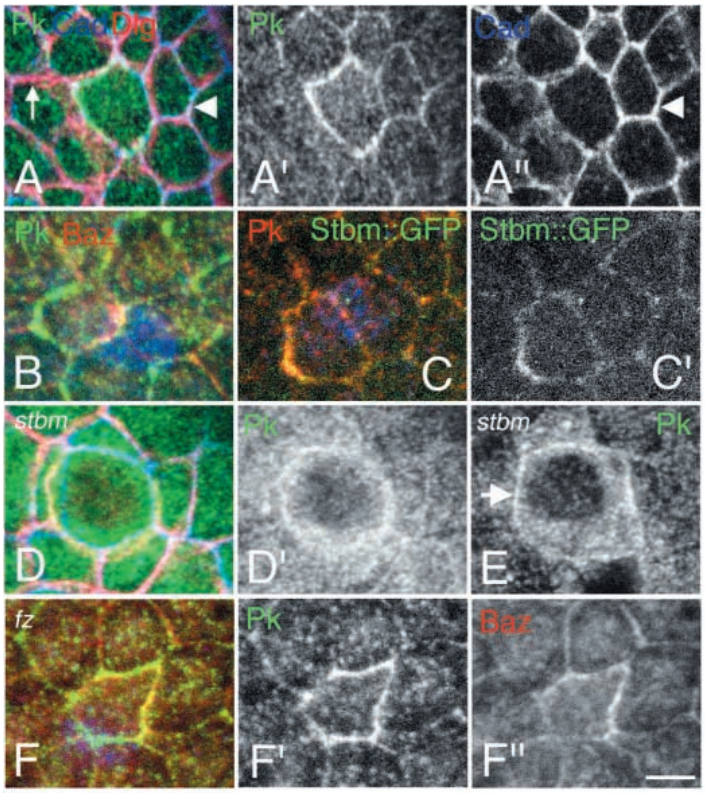

Fig. 4. Pk colocalizes with Stbm at the anterior cortex. (A-A″) Localization of Pk (green), Cad (blue) and Dlg (red). Pk accumulated at a higher level in pI cells where it localized at the anterior pole. This slightly oblique section shows that Pk co-localized apically with Cad (arrowheads) apical to Dlg (arrow) in epidermal cells. (B) Pk (green) localized at the anterior apical cortex of the pI cell (Sens in blue), opposite to Baz (red) at prophase. (C,C′) Pk (red) co-localized with GFP::Stbm (green) at the anterior cortex of *stbm*^{6c} *arm*-GFP::Stbm pI cells. (D-E) Pk (green) accumulated in the cytoplasm of *stbm*^{6c} mutant cells whereas Cad (blue) and Dlg (red) remained cortical. A faint, uniformly distributed cortical Pk staining was detected in pI cells (arrow in E). (F-F″) Pk (green) localized uniformly at the cortex and did not accumulate opposite to Baz (red) in *fz*^{K21}/ *fz*^{Kd4a} mutant pI cells (Sens in blue). Scale bar: 5 μm.

Fig. 3D,E and Fig. 4F-F″; and data not shown). Similarly, Stbm and Stbm::GFP localized uniformly at the apical cortex of *pk*$^{pk\text{-}sple14}$ mutant pI cells (Fig. 3I,J and data not shown). By contrast, Pk was mostly cytoplasmic in *stbm*^{6c} mutant cells, with only a small amount of Pk remaining at the cell cortex (Fig. 4D-E) (Bastock et al., 2003), indicating that Stbm is required for the cortical localization of Pk. Furthermore, we found that the intracellular tail of Stbm promotes dimerization in vitro and that Stbm forms oligomers in vivo (see Fig. S1 at http://dev.biologists.org/supplemental). Thus, oligomerization of both Pk and Stbm may contribute to the clustering, hence asymmetric localization, of Pk- and Stbm-containing complexes at one pole of the pI cell (Bastock et al., 2003; Jenny et al., 2003).

Overlapping distribution of Stbm, Pins and Dlg in dividing pI cells

The anterior accumulation of Stbm suggests that Stbm may co-localize with Pins and Dlg at the anterior cortex in dividing pI cells (Bellaiche et al., 2001b; Schaefer et al., 2001). Cortical staining for Pins was first detected at prophase. Pins co-localized with Stbm at the anterior apical cortex at this stage, whereas Dlg predominantly accumulated at a more basal position, with only the apical-most fraction of Dlg overlapping with Pins and Stbm (Fig. 5A-B‴; arrowhead in B). This suggests that Pins is first recruited at prophase at the anterior apical cortex where Stbm and Dlg appear to overlap. At prometaphase, Stbm localization appeared to extend to a slightly more lateral position where it overlapped with the apical-most region of the Dlg-Pins crescent (Fig. 5C-C‴ and E-E‴; arrowhead

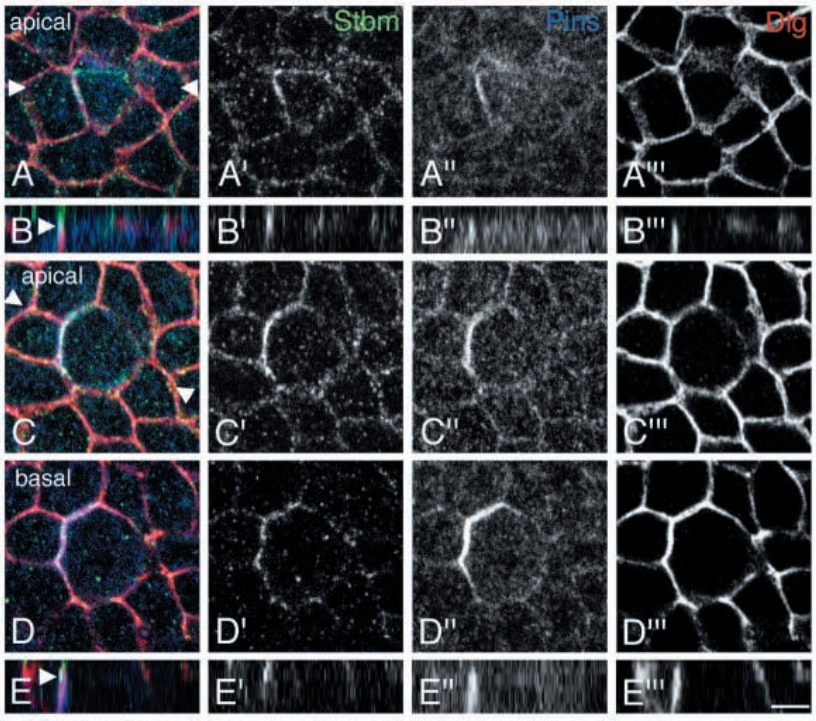

Fig. 5. Stbm overlaps with Pins and Dlg at the anterior lateral cortex. Localization of Stbm (green), Pins (blue) and Dlg (red) in dividing pI cells. At prophase (A-B‴), Stbm (A′,B′) co-localized with Pins (A″,B″) at the anterior apical cortex whereas Dlg (A‴,B‴) was predominantly basal to Stbm and Pins. Although, in this particular cell, the rounding up of the posterior cortex resulted in a weak Dlg posterior staining, no asymmetry in Dlg accumulation is usually detected at this stage. At prometaphase (C-E‴), Stbm (C′,D′,E′) localized at the anterior lateral cortex where it overlapped with Pins (C″,D″,E″) and Dlg (C‴,D‴,E‴). Only low levels of Stbm (C′) were seen at a more basal position, where Pins (C″) and Dlg (C‴) predominantly accumulated. A *z*-section view is shown in (E-E‴). *z*-section axes are indicated by arrowheads in A,C. pI cells were identified based on the anterior accumulation of Pins. Mitotic staging was deduced from cell morphology and accumulation of Pins. Scale bar: 5 μm.

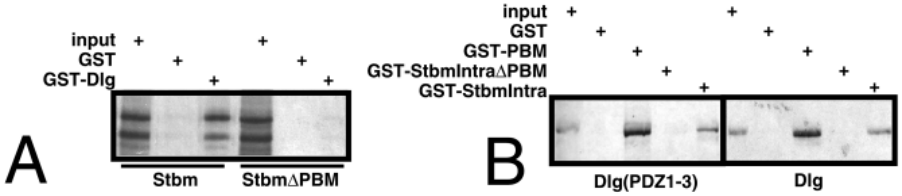

Fig. 6. Stbm interacts with Dlg. (A,B) GST pull-down assays showing that Stbm interacts in vitro with Dlg via its PBM. GST served as a negative control and input lanes correspond to 10% of the amount of radiolabelled proteins used for interaction. GST-Dlg interacts with Stbm but not with StbmΔPBM (A). GST-PBM and GST-Stbm, but not GST-StbmΔPBM, interact with Dlg or with the PDZ1-3 domain of Dlg (B).

in E; see Fig. S2 at http://dev.biologists.org/supplemental in order to view the complete stack). At a more basal position, high levels of Pins and Dlg were detected anteriorly, whereas Stbm levels were very weak (Fig. 5D-E‴). Thus, Stbm overlapped with the apical-most part of the cortical domain containing the Dlg-Pins complex at the anterior pole of the dividing pI cell from prophase onwards.

The PBM of Stbm binds Dlg and regulates the re-localization of Stbm at mitosis

As the distribution of Stbm overlapped with the localization of Dlg during mitosis, we tested whether Stbm interacts with Dlg. The presence of a class I PDZ Binding Motif (PBM) at the C terminus of Stbm indeed suggests that Stbm may directly interact with the PDZ domains of Dlg that are known to bind class I PBM. GST-Dlg was found to directly interact with Stbm in vitro. This interaction required the PBM of Stbm (Fig. 6A). Conversely, the C-terminal intracellular tail of Stbm [GST-StbmIntra(303-584)] bound both Dlg and a N-terminal fragment of Dlg containing the PDZ domains in a PBM-dependent manner (Fig. 6B). Similarly, the PBM motif of Stbm was sufficient to bind Dlg or the PDZ domains of Dlg (Fig. 6B). These data indicate that Dlg may directly interact via its PDZ domains with the PBM of Stbm.

The function of the PBM of Stbm was tested using a phenotypic rescue assay. Uniform low-level expression of Stbm rescued the *stbm*^{6c} mutant PCP phenotype in pI cells (division angle relative to the AP axis: –10±43°; to be compared with –30±29° in wild-type). A similar result was obtained with StbmΔPBM, a Stbm protein deleted of its last three amino acids (division angle relative to the AP axis: –25±28°). Thus, the PBM is dispensable for the PCP function of stbm in pI cells [see Bastock et al. (Bastock et al., 2003) for a similar result in wing epidermal cells]. Furthermore, anterior localization of Stbm at interphase does not require its PBM (Fig. 3D). At prometaphase, GFP::Stbm relocalized from the anterior apical cortex to a more lateral position in 72% of the pI cells at prometaphase (*n*=18; Fig. 3B,B′), while GFP::StbmΔPBM remained predominantly apical in 86% of the pI cells (*n*=14; Fig. 3E,E′). This suggests that a PDZ-containing protein, possibly Dlg, regulates localization of Stbm at mitosis in pI cells.

Stbm promotes Pins localization at the anterior cortex at prophase

We next investigated the role of Stbm in the anterior localization of Pins. In wild-type pupae, Pins localized at the anterior cortex of 92% of the pI cells at prophase (*n*=24, Fig. 7A). By contrast, Pins localized asymmetrically in only 42% of the pI cells in *stbm*^{6c} mutant pupae at prophase (*n*=17). In the remaining 58% of the pI cells, Pins was not restricted to a single pole at the cortex and was also found in the cytoplasm (Fig. 7B). This initial defect in Pins localization appeared to be corrected during prometaphase as Pins localized asymmetrically (but at a random position) in *stbm*^{6c} mutant pI cells at prometaphase (Fig. 7E,F). These results indicate that Stbm is required for the initial localization of Pins at the anterior cortex.

Conversely, co-expression of Stbm and Pk using the neur^{P72}GAL4 driver disrupted PCP in pI cells (Fig. 2E) and resulted in a broadened accumulation of cortical Pins in 78% of the pI cells at prophase (*n*=9; Fig. 7D). Again, this initial defect in Pins localization was partly corrected because the Pins crescent appeared to be extended in only 30% of the pI

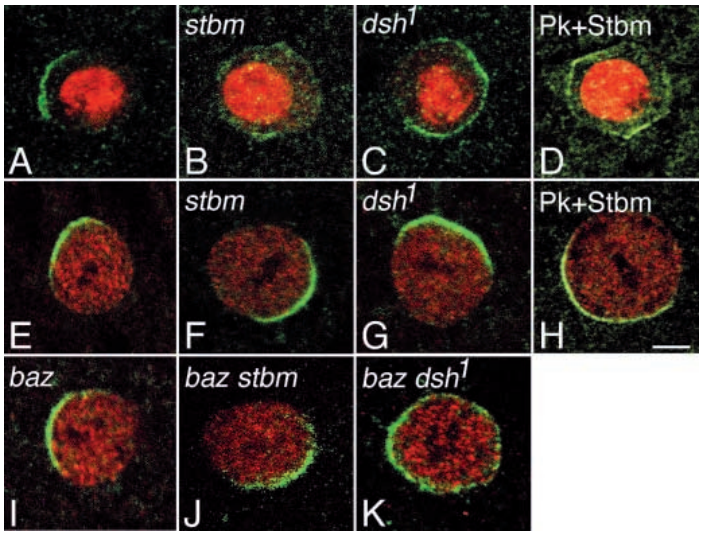

Fig. 7. Stbm promotes and Dsh inhibits Pins cortical localization at prophase. Localization of Pins (green) in wild-type (A,E), *stbm*^{6c} (B,F), *dsh*^{1} (C,G), *baz*^{XJ106} (I), *baz*^{XJ106}; *stbm*^{6c} (J), *baz*^{XJ106} *dsh*^{1} (K) and UAS-Pk/+; neur^{P72}GAL4/UAS-Stbm (D,H) pI cells (Sens in red) at late prophase (A-D; the onset of nuclear breakdown was detected by the weak cytoplasmic Sens staining) and prometaphase/metaphase (E-K). At prophase, reduced Pins cortical staining was observed in *stbm*^{6c} pI cells (B), whereas increased cortical staining was seen in *dsh*^{1} (C) and UAS-Pk/+; neur^{P72}GAL4/UAS-Stbm (D) pI cells. Despite this early defect, Pins localized asymmetrically at prometaphase/metaphase in all genotypes analyzed here (E-J) except in *baz*^{XJ106} *dsh*^{1} double mutant pI cells (K). Note that Pins localized randomly relative to AP axis in UAS-Pk/+; neur^{P72}GAL4/UAS-Stbm, *stbm*^{6c} and *dsh*^{1} mutant pupae. Scale bar: 5 μm.

cells at prometaphase (Fig. 7H; *n*=20). We conclude that Stbm acts together with Pk to promote the anterior localization of Pins in mitotic pI cells.

Dsh excludes Pins from the posterior cortex at prophase

In contrast to the Stbm-Pk complex shown above to promote Pins cortical localization, Dsh appears to antagonize it. In 63% of the *dsh*^{1} mutant pI cells, Pins localized in a broad cortical region at prophase (Fig. 7C, *n*=33). This defect was rescued during prometaphase because in 75% of the *dsh*^{1} mutant pI cells, Pins accumulated asymmetrically (but at a random position) at prometaphase (*n*=28; Fig. 7G) opposite to Baz (data not shown). This suggests that Dsh acts antagonistically to the Pk-Stbm complex at prophase. As Dsh acts downstream of Fz, we propose that activated Dsh at the posterior cortex prevents Pins from accumulating there.

Dsh and Pk-Stbm act antagonistically to organize the anterior cortex

The anterior cortex has been proposed to regulate mitotic spindle positioning. First, the anterior pole of the mitotic spindle is more tightly associated with the anterior cortex than the posterior pole is with the posterior cortex (Roegiers et al., 2001). Second, in the *fz* mutant pI cells in which Pon::GFP, a marker for Numb localization, is mis-partitioned into the two daughter cells, both poles of the spindle appeared to be tightly associated with the 'anterior-like' Pon::GFP-positive cortex (Bellaiche et al., 2001a; Doe, 2001; Roegiers et al., 2001). It is thought that a broadening of the 'anterior-like' cortex results in the capture of both spindle poles, hence leading to a mis-partitioning of Pon::GFP (Fig. 8A,B). To further test the role of Dsh, Stbm and Pk in organizing the anterior cortex, we have used the defective partitioning of Pon::GFP as a read-out for the broadening of the anterior domain. In wild-type pI cells, Pon::GFP was asymmetrically localized at the anterior cortex from prophase onwards and was unequally segregated into the anterior cell at anaphase (100%; *n*=54). By contrast, Pon::GFP was mis-partitioned in 15% of *fz* mutant pI cells (*n*=33) (Bellaiche et al., 2001a) and in 11% of the *dsh*^{1} mutant pI cells (*n*=114). Such a defect was not seen in *pk*$^{pk\text{-}sple14}$ mutant pupae (0%; *n*=17) and was very rare in *stbm*^{6c} mutant pupae (1%; *n*=84). We conclude that the loss of *fz* or *dsh* but not of *pk* or *stbm* activities leads to a broadening of the anterior domain of the pI cell. Conversely, overexpressing Pk and Stbm resulted in defective partitioning of Pon::GFP (16%; *n*=70; Fig. 8D-E′). Moreover, a transient extension of the Pon::GFP-positive cortical domain could also be seen in cells that divide unequally with Pon::GFP segregating in only one of the two cells (Fig. 8C-C″). Thus, our analysis of both Pins localization (Fig. 7A-D) and Pon::GFP mis-partitioning (Fig. 8) indicate that the Pk-Stbm complex promotes the 'anteriorization' of the cortex and that Fz/Dsh signaling acts antagonistically to restrict this 'anteriorization'.

Dsh and Stbm have distinct functions at prometaphase

Finally, we have studied the mechanism that acts during prometaphase in *stbm*^{6c} and *dsh*^{1} mutant pI cells to correct the initial defects in Pins localization (Fig. 7A-D). This mechanism may possibly involve the Baz-Par6-aPKC complex that localizes opposite to Pins. We have therefore analyzed the localization of Pins in pI cells that are double mutant for *dsh*^{1} and *baz*^{XJ106} or for *stbm*^{6c} and *baz*^{XJ106}. Although Pins correctly localized at the anterior cortex of *baz*^{XJ106} mutant pI cells (Fig. 7I) (Bellaiche et al., 2001b), asymmetric localization of Pins was strongly reduced and/or lost in 71% of the *dsh*^{1} *baz*^{XJ106} double mutant pI cells at prometaphase (*n*=24, Fig. 7K). This shows that Baz functions in the rescue mechanism operating in *dsh*^{1} mutant pupae, possibly by excluding Pins. It further indicates that Dsh acts redundantly with Baz to maintain Pins at the anterior cortex of the pI cell during prometaphase. By contrast, Pins was found to localize asymmetrically in 67% of the *baz*^{XJ106} *stbm*^{6c} double mutant pI cells at prometaphase (*n*=15, Fig. 7J). This indicates that Baz does not play an essential role in the rescue mechanism operating in *stbm*^{6c} mutant pupae. These data also show that Stbm and Dsh have distinct functions at

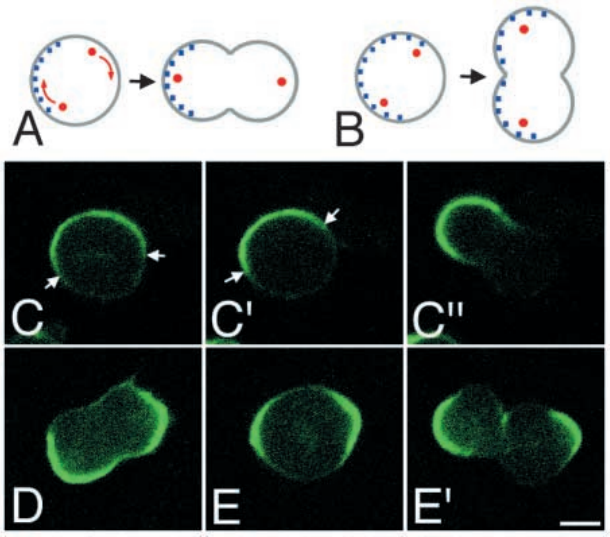

Fig. 8. Stbm and Dsh have opposite activities in organizing the anterior cortex. (A,B) Schematic interpretation of the Pon::GFP mis-partitioning phenotype. In wild-type pI cells (A), centrosomes (red) are randomly oriented at late prophase and rotate to line up with the AP polarity axis during prometaphase (Bellaiche et al., 2001a; Roegiers et al., 2001). An anterior 'centrosome-attracting activity' (blue) has been suggested to attract and anchor the anterior centrosome, thereby regulating spindle rotation (Doe, 2001). Alignment of the spindle with Pon::GFP (Pon::GFP co-localizes with the blue dots of the 'centrosome-attracting activity') results in the unequal segregation of Pon-GFP at anaphase (A). We suggest that this 'centrosome-attracting activity' is distributed over a larger cortical region in *fz*^{K21}/*fz*^{Kd4a}, *dsh*^{1} and UAS-Pk/+; neur^{P72}GAL4/UAS-Stbm pI cells such that both centrosomes can be simultaneously anchored to this cortical domain. This would therefore lead to a mis-partitioning of Pon::GFP at anaphase (B). (C-E) Co-expression of Pk and Stbm in pI cells led to a broadening of the Pon::GFP domain. In 84% of the pI cells, the crescent of Pon::GFP was progressively restricted to a single pole during prometaphase and was unequally inherited by only one daughter cell (C-C″). Arrows indicate the extent of the Pon::GFP crescent at late prophase (C) and metaphase (C″). In the remaining 16% of the cells, Pon::GFP accumulated along a broad cortical domain (D) or in a bipolar manner (E) and was mis-partitioned in the two daughter cells at anaphase (D,E′). Scale bar: 5 μm.

prometaphase in dividing pI cells, at least in the absence of *baz* activity. We therefore conclude that the asymmetric localization of Pins is maintained in a *baz*-dependent manner in *dsh*^{1} mutant pupae and in a *baz*-independent manner in *stbm*^{6c} mutant pupae.

Discussion

PCP proteins define two opposite cortical domains prior to mitosis

Asymmetric localization of Fz, Stbm and Pk at the apical cortex define two opposite domains in the pI cell prior to its division. Unipolar accumulation of Fz, Stbm and Pk depends on PCP signaling: Fz appears to exclude Stbm and Pk from the posterior cortex and, conversely, Stbm and Pk appears to exclude Fz from the anterior cortex. In addition, the cortical localization of Pk depends in part on Stbm. As Stbm is an integral membrane protein and as Pk directly binds Stbm (Bastock et al., 2003; Jenny et al., 2003), one function of Stbm is to tether Pk at the membrane.

Recent studies have shown that Fz, Dsh, Stbm and Pk become asymmetrically distributed during planar polarization of the wing epidermis (Axelrod, 2001; Bastock et al., 2003; Shimada et al., 2001; Strutt, 2001b; Tree et al., 2002). In this tissue, planar polarity is established along the proximodistal axis. Fz and Dsh preferentially accumulate at the distal vertex of wing epidermal cells, whereas Pk and Stbm localize at the opposite proximal pole. Planar polarization of the eye epithelium also involves the asymmetric distribution of Fz, Dsh, Stbm and Pk (Das et al., 2002; Rawls and Wolff, 2003; Strutt et al., 2002; Strutt, 2002). Thus, our analysis of the distribution of Fz, Stbm and Pk in the pI cell reinforces the idea that unipolar distribution of PCP proteins is a landmark of planar polarization.

Pins cortical localization as a novel read-out for PCP

Planar polarization of the pI cell occurs prior to division and is required, upon entry into mitosis, to direct the Dlg-Pins-Gαi and Baz-Par6-aPKC complexes at the anterior and posterior cortex, respectively (Bellaiche et al., 2001b). We have shown that the localization of Pins at the anterior cortex is regulated positively by the Stbm-Pk complex and negatively by Dsh. First, loss of *stbm* activity results in a delay in the cortical localization of Pins during prophase. Second, concomitant expression of Stbm and Pk leads to a broadening of the cortical crescent of Pins at prophase. Third, loss of *dsh* PCP activity similarly results in an extended Pins crescent at prophase. Moreover, our analysis of the defective partitioning of Pon::GFP suggests that the Stbm-Pk complex acts antagonistically to Dsh to localize at the anterior cortex a centrosome-attracting activity. We propose that the Stbm-Pk complex organizes the anterior cortex and recruits the Dlg-Pins-Gαi complex as well as molecules regulating spindle positioning.

Cortical localization of Pins is a novel read-out of PCP signaling in the pI cell that is distinct from the ones previously identified in wing and eye cells (Fig. 9). In wing epidermal cells, Fz promotes the formation of a polarized actin cytoskeleton via a pathway that possibly involves a direct interaction between Dsh and a Daam1-Rho complex (Habas et al., 2001) and a Rho Kinase-dependent phosphorylation of cytoplasmic myosin (Strutt, 2001a). Whether Dsh also regulates microfilament assembly in pI cells remains to be studied. In photoreceptor cells, the read-out for PCP signaling is the transcriptional regulation of the *Delta* gene in R3 (Cooper and Bray, 1999; Das et al., 2002; Fanto and Mlodzik, 1999; Mlodzik, 1999; Strutt et al., 2002). Thus, the conserved core of PCP signaling molecules have different, cell-type specific read-outs.

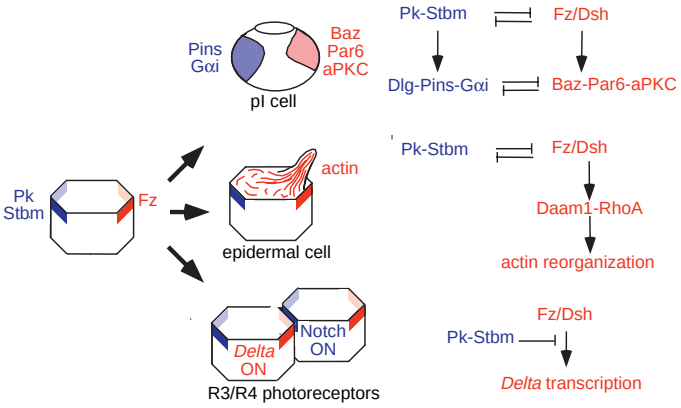

Fig. 9. Different read-outs for PCP in *Drosophila*. In pI cells of the notum (top), Fz localizes opposite to the Stbm-Pk complex prior to mitosis. Upon division, PCP signaling localizes the Dlg-Pins-Gαi and Baz-Par6-aPKC complexes along the AP axis. The Sbm-Pk complex recruits the Dlg-Pins-Gαi complex at the anterior apical cortex. Conversely, Dsh restricts the localization of Pins at the cortex, at least partly via the regulation of the localization of Stbm as well as via Baz. In wing epidermal cells (center), Fz signals at the distal vertex to promote the formation of an actin-based structure, the epidermal hair, via a pathway that may involve direct interactions between Dsh, the fly homolog of Daam1 and Rho. Localized activation of Rho would control the spatial regulation of myosin activity via the Rho Kinase and the Myosin Light Chain Kinase (Strutt, 2001a). In the eye (bottom), higher levels of *fz* activity in the equatorial cell leads to an up-regulation of the expression of the *Delta* gene (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Mlodzik, 1999; Strutt et al., 2002). This in turn leads to the activation of Notch in the polar cell that, therefore, becomes R4.

How does Stbm direct the localization of Pins to the anterior cortex? One hypothesis is that Stbm directs the anterior localization of Pins via the regulated assembly of a Stbm-Dlg-Pins complex. We have previously shown that the anterior accumulation of Pins depends on its interaction with Dlg (Bellaiche et al., 2001b). We provide evidence that Stbm may bind Dlg. First, in vitro binding studies indicate that Stbm interacts with Dlg. We note, however, that PDZ-containing proteins other than Dlg may also bind Stbm in this assay. Second, the localization of Stbm overlaps with the distribution of Pins and Dlg in dividing pI cells. Third, the PBM motif of Stbm appears to regulate the re-localization of Stbm in pI cells. Our data are therefore consistent with a model in which, upon mitosis, the binding of Stbm to Dlg in turn promotes the binding of Pins to Dlg and, hence, localization of Pins at the anterior cortex where Stbm and Dlg accumulations overlap. This model predicts that the PBM of Stbm should be required for the anterior localization of Pins. We have found, however, that StbmΔPBM is fully functional and that Pins is properly recruited at the anterior cortex in

stbm^{6c} mutant pI cells expressing StbmΔPBM (F.S., unpublished). One interpretation of this result is that Stbm regulates the localization of Pins not only via the PBM-dependent assembly of the Dlg-Pins complex but also via a second PBM-independent mechanism. As Dsh acts redundantly with Baz to localize Pins asymmetrically, we suggest that this second mechanism may involve Dsh. Accordingly, in *stbm*^{6c} mutant pI cells, uniformly distributed Dsh activity would prevent Pins cortical localization. By contrast, as the PCP function of *stbm* does not depend on its PBM, the activity of Dsh should be restricted to the posterior cortex in *stbm*^{6c} mutant pI cells expressing StbmΔPBM. Dsh should therefore restrict Pins localization to the anterior cortex in this mutant background. Another interpretation of the correct localization of Pins in *stbm*^{6c} mutant pI cells expressing StbmΔPBM is that Stbm recruits Pins via a mechanism that does not involve an interaction with Dlg (or any other PDZ-containing proteins). Future studies will address how the Stbm-Pk complex regulates the localization of Pins in the pI cell.

Maintenance of Pins anterior localization in the mitotic pI cell

Different mechanisms appear to cooperate to maintain Pins asymmetric localization. We have shown here that *baz* is required for the asymmetric localization of Pins in the absence of *dsh* PCP activity. This indicates that Baz can regulate the maintenance of Pins asymmetric localization at prometaphase. The loss of asymmetric localization of Pins in *dsh baz* mutant pI cells suggests that Dsh may also contribute to maintain Pins asymmetric localization at prometaphase. Dsh does not merely act by excluding Stbm, a positive regulator of Pins localization in prophase, because Pins localizes asymmetrically in *baz stbm* double mutant pI cells. The mechanisms by which Baz and Dsh regulates Pins localization are not known. However, as Pins regulates its own localization via a Gβ13F-dependent positive feedback loop (Fuse et al., 2003; Schaefer et al., 2001), one hypothesis is that Baz and/or Dsh negatively regulates Gβ13F signaling activity.

Implications for the read-out of PCP in vertebrates

One of the best examples of PCP in mammals is the stereotyped planar orientation of the stereociliary bundles that are located at the apical cortex of each mechanosensory hair cells within the cochlea. In these cells, the first sign of polarization is the stereotyped movement, at the luminal surface of the cell and along the neural-abneural axis, of the kinocilium, the single tubulin-based cilium, from the center towards the abneural pole of the cell (Montcouquiol et al., 2003). Recently, a mutation in a *stbm* homolog, *Vangl2*, has been shown to result in the defective orientation of the stereociliary bundles. This planar cell polarity defect appears to result from the randomly oriented center-to-periphery movement of the kinocilium (Montcouquiol et al., 2003). Because LGN, a mammalian homolog of Pins (Yu et al., 2003), is known to regulate microtubule stability (Du et al., 2002), it is tempting to speculate that Vangl2 may regulate via LGN a microtubule-dependent process regulating kinocilium movement along the neural-abneural axis. Future studies will reveal whether the regulation of Pins/LGN cortical localization is a conserved read-out of PCP.

Note added in proof

A recent study by Lee et al. (Lee et al., 2003) indicates that Stbm directly interacts with Dlg via its C-terminal PBM. Dlg and Stbm can be co-immunoprecipated from a *Drosophila* embryonic extract. This direct interaction was also observed with mammalian homologs of Stbm and Dlg. The authors propose that the Dlg-Stbm interaction is important for the formation of new plasma membrane during cellularisation of the *Drosophila* embryo.

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References

Adler, P. N. (2002). Planar signaling and morphogenesis in Drosophila. *Dev. Cell* **2**, 525-535.

Axelrod, J. D. (2001). Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. *Genes Dev.* **15**, 1182-1187.

Bastock, R., Strutt, H. and Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during Drosophila planar polarity patterning. *Development* **130**, 3007-3014.

Bellaiche, Y., Gho, M., Kaltschmidt, J. A., Brand, A. H. and Schweisguth, F. (2001a). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. *Nat. Cell Biol.* **3**, 50-57.

Bellaiche, Y., Radovic, A., Woods, D. F., Hough, C. D., Parmentier, M. L., O'Kane, C. J., Bryant, P. J. and Schweisguth, F. (2001b). The Partner of Inscuteable/Discs-large complex is required to establish planar polarity during asymmetric cell division in Drosophila. *Cell* **106**, 355-366.

Berdnik, D., Torok, T., Gonzalez-Gaitan, M. and Knoblich, J. (2002). The endocytic protein alpha-adaptin is required for Numb-mediated asymmetric cell division in Drosophila. *Dev. Cell* **3**, 221.

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* **289**, 113-116.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brou, C., Logeat, F., Lecourtois, M., Vandekerckhove, J., Kourilsky, P., Schweisguth, F. and Israel, A. (1994). Inhibition of the DNA-binding activity of Drosophila suppressor of hairless and of its human homolog, KBF2/RBP-J kappa, by direct protein-protein interaction with Drosophila hairless. *Genes Dev.* **8**, 2491-2503.

Cooper, M. T. and Bray, S. J. (1999). Frizzled regulation of Notch signalling polarizes cell fate in the Drosophila eye. *Nature* **397**, 526-530.

Das, G., Reynolds-Kenneally, J. and Mlodzik, M. (2002). The atypical cadherin Flamingo links Frizzled and Notch signaling in planar polarity establishment in the Drosophila eye. *Dev. Cell* **2**, 655-666.

De Vries, L., Fischer, T., Tronchere, H., Brothers, G. M., Strockbine, B., Siderovski, D. P. and Farquhar, M. G. (2000). Activator of G protein signaling 3 is a guanine dissociation inhibitor for galpha i subunits. *Proc. Natl. Acad. Sci. USA* **97**, 14364-14369.

Doe, C. Q. (2001). Cell polarity: the PARty expands. *Nat. Cell Biol.* **3**, E7-E9.

Du, Q., Taylor, L., Compton, D. A. and Macara, I. G. (2002). LGN blocks the ability of NuMA to bind and stabilize microtubules. A mechanism for mitotic spindle assembly regulation. *Curr. Biol.* **12**, 1928-1933.

Fanto, M. and Mlodzik, M. (1999). Asymmetric Notch activation specifies photoreceptors R3 and R4 and planar polarity in the Drosophila eye. *Nature* **397**, 523-526.

Fichelson, P. and Gho, M. (2003). The glial cell undergoes apoptosis in the microchaete lineage of Drosophila. *Development* **130**, 123-133.

Fuse, N., Hisata, K., Katzen, A. L. and Matsuzaki, F. (2003). Heterotrimeric

g proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions. *Curr. Biol.* **13**, 947-954.

Gho, M. and Schweisguth, F. (1998). Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in Drosophila. *Nature* **393**, 178-181.

Gho, M., Bellaiche, Y. and Schweisguth, F. (1999). Revisiting the Drosophila microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. *Development* **126**, 3573-3584.

Gubb, D., Green, C., Huen, D., Coulson, D., Johnson, G., Tree, D., Collier, S. and Roote, J. (1999). The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. *Genes Dev.* **13**, 2315-2327.

Guo, M., Jan, L. Y. and Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* **17**, 27-41.

Habas, R., Kato, Y. and He, X. (2001). Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell* **107**, 843-854.

Henrique, D. and Schweisguth, F. (2003). Cell polarity: the ups and downs of the Par6/aPKC complex. *Curr. Opin. Genet. Dev.* **13**, 341-350.

Jenny, A., Darken, R. S., Wilson, P. A. and Mlodzik, M. (2003). Prickle and Strabismus form a functional complex to generate a correct axis during planar cell polarity signaling. *EMBO J.* **22**, 4409-4420.

Kaltschmidt, J. A., Davidson, C. M., Brown, N. H. and Brand, A. H. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. *Nat. Cell Biol.* **2**, 7-12.

Le Borgne, R. and Schweisguth, F. (2003). Unequal segregation of Neuralized biases Notch activation during asymmetric cell division. *Dev. Cell* **5**, 139-148.

Lee, O. K., Frese, K. K., James, J. S., Chadda, D., Chen, Z. H., Javier, R. T. and Cho, K. O. (2003). Discs-Large and Strabismus are functionally linked to plasma membrane formation. *Nat. Cell Biol.* **5**, 987-993.

Lu, B., Ackerman, L., Jan, L. Y. and Jan, Y.-N. (1999a). Modes of protein movement that lead to the asymmetric localization of Partner of Numb during Drosophila neuroblast division. *Mol. Cell* **4**, 883-891.

Lu, B., Usui, T., Uemura, T., Jan, L. and Jan, Y. N. (1999b). Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in Drosophila. *Curr. Biol.* **9**, 1247-1250.

Mlodzik, M. (1999). Planar polarity in the Drosophila eye: a multifaceted view of signaling specificity and cross-talk. *EMBO J.* **18**, 6873-6879.

Montcouquiol, M., Rachel, R. A., Lanford, P. J., Copeland, N. G., Jenkins, N. A. and Kelley, M. W. (2003). Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. *Nature* **423**, 173-177.

Rawls, A. S. and Wolff, T. (2003). Strabismus requires Flamingo and Prickle function to regulate tissue polarity in the Drosophila eye. *Development* **130**, 1877-1887.

Reddy, G. V. and Rodrigues, V. (1999). A glial cell arises from an additional division within the mechanosensory lineage during development of the microchaete on the Drosophila notum. *Development* **126**, 4617-4622.

Rhyu, M. S., Jan, L. Y. and Jan, Y. N. (1994). Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell* **76**, 477-491.

Roegiers, F., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N. (2001). Two types of asymmetric divisions in the Drosophila sensory organ precursor cell lineage. *Nat. Cell Biol.* **3**, 58-67.

Schaefer, M., Shevchenko, A. and Knoblich, J. A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in Drosophila. *Curr. Biol.* **10**, 353-362.

Schaefer, M., Petronczki, M., Dorner, D., Forte, M. and Knoblich, J. A. (2001). Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. *Cell* **107**, 183-194.

Shimada, Y., Usui, T., Yanagawa, S., Takeichi, M. and Uemura, T. (2001). Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. *Curr. Biol.* **11**, 859-863.

Strutt, D. (2001a). Planar polarity: getting ready to ROCK. *Curr. Biol.* **11**, R506-R509.

Strutt, D. I. (2001b). Asymmetric localization of frizzled and the establishment of cell polarity in the Drosophila wing. *Mol. Cell* **7**, 367-375.

Strutt, D. I. (2002). The asymmetric subcellular localisation of components of the planar polarity pathway. *Semin. Cell Dev. Biol.* **13**, 225-231.

Strutt, D. (2003). Frizzled signalling and cell polarisation in Drosophila and vertebrates. *Development* **130**, 4501-4513.

Strutt, D., Johnson, R., Cooper, K. and Bray, S. (2002). Asymmetric localization of frizzled and the determination of notch-dependent cell fate in the Drosophila eye. *Curr. Biol.* **12**, 813-824.

Tree, D. R., Shulman, J. M., Rousset, R., Scott, M. P., Gubb, D. and Axelrod, J. D. (2002). Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. *Cell* **109**, 371-381.

Wolff, T. and Rubin, G. M. (1998). Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in Drosophila. *Development* **125**, 1149-1159.

Woods, D. F. and Bryant, P. J. (1991). The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. *Cell* **66**, 451-464.

Woods, D. F., Hough, C., Peel, D., Callaini, G. and Bryant, P. J. (1996). Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. *J. Cell Biol.* **134**, 1469-1482.

Yu, F., Morin, X., Cai, Y., Yang, X. and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of Drosophila asymmetric divisions, reveals two distinct steps in inscuteable apical localization. *Cell* **100**, 399-409.

Yu, F., Morin, X., Kaushik, R., Bahri, S., Yang, X. and Chia, W. (2003). A mouse homologue of Drosophila pins can asymmetrically localize and substitute for pins function in Drosophila neuroblasts. *J. Cell Sci.* **116**, 887-896.