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The planar polarity pathway promotes coordinated cell migration during *Drosophila* oogenesis

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Cell migration is fundamental in both animal morphogenesis and disease. The migration of individual cells is relatively well-studied; however, in vivo, cells often remain joined by cell-cell junctions and migrate in cohesive groups. How such groups of cells coordinate their migration is poorly understood. The planar polarity pathway coordinates the polarity of non-migrating cells in epithelial sheets and is required for cell rearrangements during vertebrate morphogenesis. It is therefore a good candidate to play a role in the collective migration of groups of cells. *Drosophila* border cell migration is a well-characterised and genetically tractable model of collective cell migration, during which a group of about six to ten epithelial cells detaches from the anterior end of the developing egg chamber and migrates invasively towards the oocyte. We find that the planar polarity pathway promotes this invasive migration, acting both in the migrating cells themselves and in the non-migratory polar follicle cells that they carry along. Disruption of planar polarity signalling causes abnormalities in actin-rich processes on the cell surface and leads to less-efficient migration. This is apparently due, in part, to a loss of regulation of Rho GTPase activity by the planar polarity receptor Frizzled, which itself becomes localised to the migratory edge of the border cells. We conclude that, during collective cell migration, the planar polarity pathway can mediate communication between motile and non-motile cells, which enhances the efficiency of migration via the modulation of actin dynamics.

KEY WORDS: Cell migration, Frizzled, Planar polarity, Strabismus, *Drosophila*

INTRODUCTION

The ability of cells to undergo directed migration is a prerequisite for the morphogenesis of complex animal body plans. Such migration can generally be divided into two forms: single-cell migration or collective cell migration (Friedl, 2004). In single-cell migration, junctional contacts are lost, although cells might still remain loosely associated in small groups or chains. Because this can be easily studied in cultured cells, it is relatively well-characterised. During collective cell migration, junctional contacts are maintained between groups of moving cells. Such coordinated movement of cell groups is a key event in organogenesis and has been implicated in disease states such as cancer metastasis (Friedl et al., 2004; Lecaudey and Gilmour, 2006). Well-studied examples of collective cell migration include the movement of relatively small groups of cells, such as border cell migration during *Drosophila* oogenesis (Montell, 2003) and movement of the lateral line primordium in fish and amphibians (Ghysen and Dambly-Chaudière, 2004), as well as the rearrangement of sheets of cells such as that which occurs during *Drosophila* dorsal closure (Jacinto et al., 2002) and vertebrate gastrulation (Keller, 2002). A key feature of collective cell migration is that it permits the coordinated movement of both motile and non-motile cells together in a single group. However, because collective cell migration can only be studied in the context of developing organisms, it remains poorly understood.

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In recent years, a great deal of progress has been made in understanding how cell polarity is coordinated in developing tissues. In many contexts in both *Drosophila* and vertebrates, a conserved planar polarity pathway mediates local cell-cell interactions to ensure that neighbouring cells adopt the appropriate polarity (Strutt, 2003; Veeman et al., 2003; Klein and Mlodzik, 2005). Central to this is a core group of polarity proteins, which includes the seven-pass transmembrane receptor Frizzled (Fz), the four-pass transmembrane protein Strabismus (Stbm, also known as Van Gogh) and the cytoplasmic proteins Dishevelled (Dsh) and Prickle (Pk). This core is involved in diverse patterning events, from bristle and hair polarity on the body surface of flies to convergent extension and neural crest migration in vertebrate embryos (Strutt, 2003; Wallingford et al., 2002; De Calisto et al., 2005).

Given the requirement for planar polarity pathway function for efficient convergent extension movements, it is a good candidate to coordinate the collective migration of cells in other contexts. Therefore, we examined its requirement in border cell migration in the *Drosophila* egg chamber, which represents a particularly well-characterised and genetically tractable model of collective cell migration. The early egg chamber consists of an oocyte and nurse cells, of germline origin, surrounded by a single layer of follicular epithelium of somatic origin (Fig. 1A, stage 8). At each end of this epithelium is a pair of differentiated cells known as the polar follicle cells (Ruohola et al., 1991). During egg chamber maturation, the outer follicle cells undergo a series of stereotypic cell movements (Fig. 1A, stage 9). The anterior polar follicle cells signal to their neighbours, inducing a partial epithelial-to-mesenchymal transition (Silver and Montell, 2001). These neighbouring cells, known as the border cells, delaminate from the epithelium, invade between the nurse cells and migrate to the anterior border of the oocyte, carrying the polar follicle cells along with them. Concurrently, the outer follicle cells also rearrange such that they all come into contact with the oocyte (Fig. 1A, stage 10) (Montell, 2003).

The direction of border cell migration is determined by gradients of ligands for receptor tyrosine kinases, produced in the oocyte (Duchek and Rørth, 2001; Duchek et al., 2001). Within the migrating cluster, the border cells remain attached to one another and to the polar follicle cells by stable epithelial junctions (Niewiadomska et al., 1999). The region of the border cells that is linked neither to the polar follicle cells nor to each other appears to be mesenchymal and is motile.

Here, we show that planar polarity gene function is required for efficient border cell migration. Our results support a model in which the planar polarity pathway is required both in the polar follicle cells and the border cells, promoting the production of actin-rich protrusions during migration.

MATERIALS AND METHODS

Genetics and scoring

Fly culture and crosses were done at 25°C unless indicated. Strains are described in FlyBase, except *Upd-Gal4* (Tsai and Sun, 2004). Mutant follicle cell clones were induced using the FLP/FRT system (Xu and Rubin, 1993): 1–3-day-old females of genotypes *hs-FLP; FRT42 stbm^{6} / FRT42 arm-lacZ* or *hs-FLP; fz^{15} FRT80 / arm-lacZ FRT80* were heat-shocked for 1 hour twice daily at 37°C for 3 days, then dissected 3-6 days after the last heat shock.

Border cell migration experiments were scored blind. For wholly mutant egg chambers, all alleles tested (except for the *slbo* and *Wnt4* controls) were crossed out for 10-20 generations to *w^{1118}*, to provide a common genetic background, and *w^{1118}* was used as the control. For GAL4/UAS overexpression and RNAi-knockdown experiments, the controls were siblings lacking the UAS insert or the GAL4 driver, both these controls being significantly different from the experimental samples but not from each other. To test for defects caused by insertion of the RNAi transgenes, lines containing only the *slbo-lacZ* marker and the insertion were also scored (data not shown). Significance was scored between an experimental line and its control using the significance test for a difference in two proportions (Statistics at Square One, www.bmj.com). The mosaic data was analysed using Chi-squared tests, incorporating the Yates' correction for small sample sizes. Expected values were calculated by counting the frequency of wild-type to mutant cells in each cluster and calculating the probability of a leading cell being wild-type due to random assortment.

To quantitate the actin protrusions in border cell clusters, egg chambers from control and mutant flies were dissected in a single experiment and processed in parallel. Confocal *z*-stacks were captured throughout the entire depth of representative clusters for each genotype and the total number of actin protrusions was then counted for eight clusters selected at random. There was no statistically significant difference between the numbers of protrusions observed between different mutant genotypes (*fz^{21}*, *stbm^{6}* and *dsh^{1}*), whereas each mutant genotype showed a highly significant difference from the control *w^{1118}* chambers ($P<10^{-7}$, *t*-test). As with the border cell migration experiments, the mutant alleles had previously been crossed out to the *w^{1118}* control stock for 10-20 generations to provide a common genetic background. Mutant chambers were additionally compared to control chambers processed in parallel in at least two independent experiments and, in each case, more protrusions were observed in the control chambers than in the mutant chambers.

Levels of GFP-RhoA in the cytoplasm versus the membranes of border cells were quantitated from confocal XY sections through border cell clusters, using NIH Image. The average level of GFP-RhoA fluorescence in the border cell cytoplasm was compared to the peak levels of GFP-RhoA fluorescence in the border cell membrane.

Molecular biology

RNAi constructs were made in the pWIZ vector (Lee and Carthew, 2003) against the first exon of *fz* (bp 682-1332, Accession: AY051808), the second exon of *stbm* (bp 413-1312, Accession: AF044208) and a 1000 bp segment within *dsh* (bp 705-1728, Accession: AF044208). Sequence analysis showed no off-target matches of more than 17 bp for the *fz* construct, 18 bp for the *stbm* construct and a single off-target match of 20 bp for the *dsh* construct. The RNAi lines gave the expected loss-of-function phenotypes in the wing, eye and notum, accompanied by loss of Fz/Dsh/Stbm immunolabelling as appropriate. As an additional control for specificity, the *fz* and *stbm* RNAi phenotypes were also found to be enhanced in backgrounds heterozygous for *fz* and *stbm* gene function, respectively. To make *Actin-EGFP-RhoA*, the *RhoA* ORF was tagged at the N-terminus with *EGFP* and inserted downstream of the *Actin5C* promoter in pCasper4. This construct recapitulates known RhoA localisation patterns (Magie et al., 2002).

Histology

Ovaries were generally dissected, fixed and antibody/X-gal stained as described (Verheyen and Cooley, 1994). To preserve the actin cytoskeleton, egg chambers were dissected and fixed as previously described (Frydman and Spradling, 2001), except that Schneider's medium was used in place of Grace's.

Primary antibodies used were 1:4000 rabbit anti-β-galactosidase (Cappel), 1:100 mouse anti-Armadillo-N2 7A1 (DSHB), 1:400 rabbit anti-Strabismus (Rawls and Wolff, 2003), 1:50 mouse anti-Rho-p1D9 (DSHB) (Magie et al., 2002), 1:1000 rabbit anti-Stat92E (Chen et al., 2003) and 1:10 rat anti-DE-Cad2 (Oda et al., 1994). Rabbit antibodies against Fz were raised using a His-tagged fusion protein containing residues 40-252, and affinity purified using a GST-tagged fusion protein containing residues 40-240. Secondary antibodies used were anti-rabbit-Alexa-Fluor-568, anti-rabbit-Alexa-Fluor-488 and anti-mouse-Alexa-Fluor-488 at 1:1000 (Molecular Probes), and anti-rat-Cy2 and anti-mouse-Cy5 at 1:400 (Jackson). Actin was visualised with phalloidin-Texas Red and phalloidin-FITC at 1:200 (Molecular Probes). Fluorescent images were captured on a Leica SP confocal and processed using NIH Image and Adobe Photoshop.

RESULTS

Loss or gain of core planar polarity gene function in the border cells delays migration

To test the hypothesis that core planar polarity gene function might be involved in border cell migration, we examined egg chambers from flies carrying mutations in a number of these loci. During stage 9, when border cell migration is in progress (Fig. 1A), we found that mutations in the planar polarity genes *fz*, *stbm*, *dsh* and *pk* all caused significant delays (see Fig. S1A in the supplementary material). In wild-type egg chambers, border cell migration was usually complete by stage 10 and, at this stage, most chambers lacking *fz* or *stbm* function had also completed migration (see Fig. S1B in the supplementary material), indicating that loss of planar polarity pathway function delays but does not block migration. These results suggest that border cell migration is less efficient without planar polarity pathway function. However, because gene function is removed from the entire egg chamber throughout its development in these experiments, this does not prove a specific function in the border cell cluster itself.

To address whether core planar polarity gene function is required specifically in the border cell cluster, we used RNAi-mediated knockdown of gene function, coupled with tissue-specific expression under the control of the GAL4/UAS system (Brand and Perrimon, 1993). The *slbo-GAL4* driver expresses specifically in the border cells upon initiation of migration (dark green cells in Fig. 1A) (Rørth et al., 1998). Knockdown of *fz*, *stbm* or *dsh* transcripts using this driver results in a significant delay in border cell-cluster migration, relative to the concomitant movement of the outer follicle cells over the egg chamber surface (Fig. 1B). This suggests that planar polarity gene function is required in the border cells for cluster migration to occur efficiently. Two aspects of the phenotype are particularly noteworthy: first, most border cells eventually reach the oocyte (not shown but see Fig. S1B in the supplementary material); second, we never observed any guidance defects, such as border cell clusters failing to migrate in the correct direction. Hence,

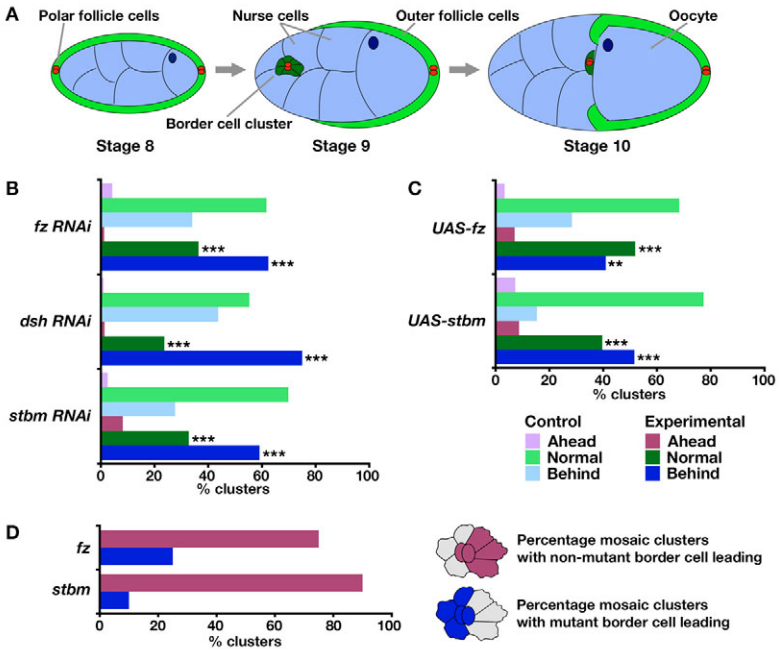

Fig. 1. Core planar polarity gene function is required in the border cells. Anterior is to the left and border cells are migrating towards the right, in this and subsequent figures. Statistical significances are indicated on charts as ***P<0.001 and **P<0.01; all P values and numbers of clusters examined are shown in Table S1 in the supplementary material for UAS/GAL4 experiments and Table S2 in the supplementary material for mosaic cluster analysis. (**A**) Schematic of border cell migration and outer follicle cell rearrangement. Anterior polar follicle cells (red) recruit adjacent outer follicle cells (light green) to form the border cells (dark green). The border cell cluster delaminates from the follicular epithelium and begins to migrate posteriorly at the beginning of stage 9, normally completing its journey by the end of this stage. Concomitantly, the outer follicle cells rearrange so that they no longer cover the nurse cells. In wild-type chambers, the border cell cluster migrates at such a rate that it approximately keeps up with the posterior movement of the outer follicle cells. (**B**,**C**) Charts showing the extent of border cell migration relative to outer follicle cell rearrangement for clusters in which either *fz*, *dsh* or *stbm* transcripts have been either knocked-down by *UAS*-RNAi constructs at 29°C (B) or overexpressed using *UAS* constructs at 25°C (C) under the control of the border cell-specific *slbo-GAL4* driver (Rørth et al., 1998). Coloured bars indicate the proportion of clusters found ahead of the outer follicle cells, in approximately the same position ('normal') or lagging behind, for either sibling controls or experimental conditions. 'Ahead' or 'behind' are defined as being more than the diameter of a nurse cell nucleus away from the trailing edge of the rearranging outer follicle cells. Either knockdown of *fz*, *dsh* or *stbm* or overexpression of *fz* or *stbm* causes a significant increase in the number of clusters 'behind' and an accompanying decrease in the number of clusters showing a 'normal' rate of migration. (**D**) Chart showing the proportions of genetically mosaic clusters recovered for the strong alleles fz^{15} and $stbm^{6}$ with both polar follicle cells retaining gene function, but with either wild-type border cells leading (pink bars) or mutant border cells leading (blue bars). In both genotypes, there is a statistically significant (P=0.003) preponderance for wild-type border cells to be found at the leading edge of the migrating cluster. Mutant cells in the cartoons are represented by grey shading, with leading cells to the right and lagging cells to the left.

we conclude that the previously identified RTK-mediated guidance cues (Duchek and Rørth, 2001; Duchek et al., 2001) are intact, but that the ability of the border cell cluster to efficiently migrate in response to these cues is impaired.

An intriguing feature of planar polarity pathway function in other contexts is that overexpression and loss-of-function of pathway components give similar defects (Krasnow and Adler, 1994; Strutt et al., 1997). Consistent with this, we found that overexpressing either *fz* or *stbm* in the border cells results in delayed migration (Fig. 1C). This suggests that the planar polarity pathway functions in a similar manner in border cells as in other tissues.

Taken together, the observed delays in border cell migration following three independent methods of altering core planar polarity gene function (i.e. classical loss-of-function mutations, transcript knockdown by RNAi and overexpression of the gene products), for two independent core polarity genes (*fz* and *stbm*), provides strong evidence that the planar polarity pathway is required in border cells for efficient migration.

To further characterise planar polarity gene function in border cells, we used mitotic recombination to generate genetically mosaic clusters. Border cells, polar follicle cells and the nurse cells through which the cluster migrates are derived from different cell lineages (Margolis and Spradling, 1995); therefore, it is possible to generate clusters in which a subset of the migratory border cells lack gene function, but the non-migratory polar follicle cells and the substrate nurse cells retain function. It has recently been demonstrated that the relative position of an individual border cell within the migrating cluster is very fluid, with an individual cell potentially able to occupy leading, lateral and lagging roles during migration (Prasad and Montell, 2007). Furthermore, earlier studies have shown that, if cells within a cluster lack activity of a gene that is required for proper motility, then these cells will partition to the lagging (anterior) edge of the cluster, whereas cells that retain gene function are found at the leading (posterior) edge (Niewiadomska et al., 1999; Rørth et al., 2000). We examined clusters in which either *fz* or *stbm* activity was removed from a subset of border cells. In both cases, mutant border cells were predominantly found at the lagging edge of the clusters, whereas cells that retained gene function showed a strong preference to migrate at the leading edge (Fig. 1D). This confirms that the planar polarity pathway promotes border cell motility, and, furthermore, demonstrates that pathway function is required autonomously in the border cells themselves.

Planar polarity pathway function is not required for normal *slow border cells*, *DE-Cadherin* or *Stat92E* expression

The planar polarity pathway has been implicated in the regulation of both gene expression and cell fate, as well as in the modulation of the cytoskeleton (Strutt, 2003). To investigate how planar polarity signalling promotes border cell migration, we examined the expression of factors previously shown to be important for border cell fate and motility.

The transcription factor encoded by the *slow border cells* (*slbo*) gene (Montell et al., 1992; Rørth et al., 2000) and the cell adhesion molecule DE-Cadherin (DE-Cad, also known as Shotgun – FlyBase) (Niewiadomska et al., 1999) are both required for efficient migration of individual border cells. Therefore, it is possible that the planar

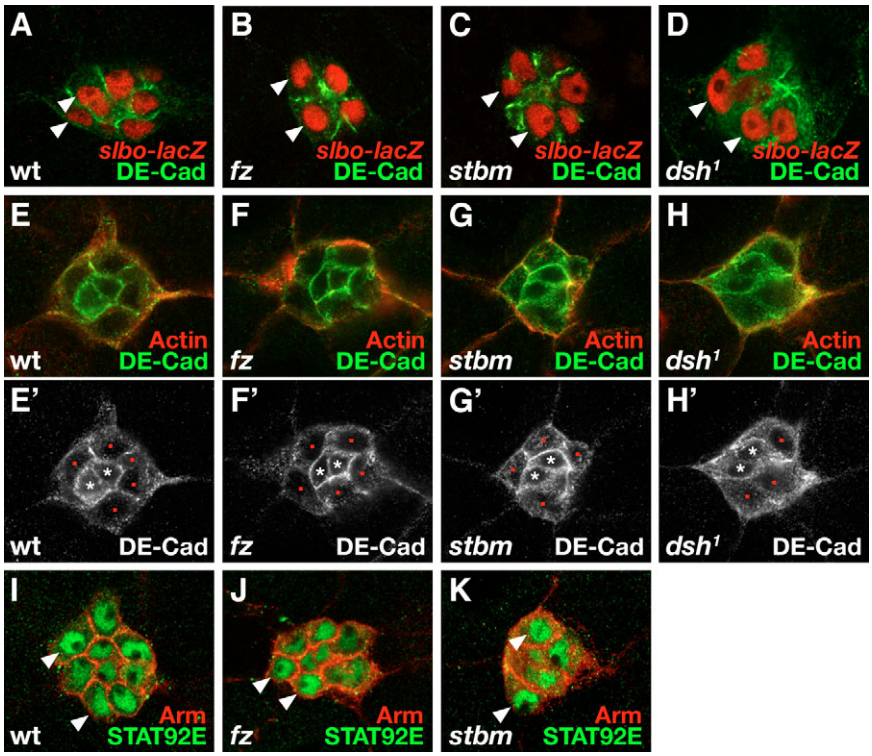

Fig. 2. Border cell clusters lacking core planar polarity gene function show normal expression of *slow border cells*, DE-Cadherin and Stat92E. (**A-D**) *slow border cells* expression as revealed by the *slbo-lacZ* reporter (Montell et al., 1992); β-gal immunolabelling (red) and DE-Cadherin (DE-Cad) expression (green) in migrating border cell clusters from wild-type (A), fz^{21}/fz^{15} (B), $stbm^{6}$ (C) and dsh^{1} (D) individuals. High levels of nuclear-localised *lacZ* gene product in border cells is indicated by arrowheads. We observed that, in wild-type clusters, β-gal levels were lower at early stage 9 than at the end of stage 9, whereas, in mutant clusters, β-gal levels were generally higher throughout migration. We assume that β-gal accumulates progressively within the border cells after the onset of gene expression, and that the delayed migration seen in the mutant backgrounds results in higher accumulation at equivalent stages of migration. (**E-H'**) DE-Cad (green/white) and actin (red) distribution in migrating border cell clusters from wild-type (E,E'), fz^{21} (F,F'), $stbm^{6}$ (G,G') and dsh^{1} (H,H') individuals. Border cells are marked by red dots and polar follicle cells by white asterisks. (**I-K**) Stat92E (green) and Armadillo (Arm, red) distribution in migrating border cell clusters from wild-type (I), fz^{21} (J) and $stbm^{6}$ (K) individuals. High levels of nuclear-localised Stat92E in border cells is indicated by arrowheads.

polarity pathway could regulate the levels of these proteins. However, in egg chambers wholly lacking planar polarity gene function, *slbo* expression (Fig. 2A-D), and DE-Cad expression and subcellular distribution (Fig. 2E-H) appear normal.

Activity of the transcription factor Stat92E is also required for border cell migration (Silver and Montell, 2001; Silver et al., 2005), and the JAK/STAT pathway has been previously implicated in planar polarity signalling (Zeidler et al., 1999). However, we found that Stat92E expression and nuclear localisation was also normal in border cell clusters lacking *fz* and *stbm* function (Fig. 2I-K). Thus, judging from these examples, border cell fate and gene expression is normal in these mutants.

Border cells with altered planar polarity pathway function have abnormal actin protrusions

By contrast, examination of the actin cytoskeleton of border cells from mutant egg chambers did reveal significant defects. Wild-type border cells showed prominent actin-rich protrusions (Fig. 3A). Removal of *fz*, *stbm* or *dsh* activity resulted in the loss of prominent protrusions and a more even actin distribution over the border cell surface (Fig. 3B-D). Whereas border cell clusters from the control w^{1118} stock showed an average of 94.8 protrusions per cluster (n=8), clusters in an fz^{21} background showed an average of 38.4 protrusions (n=8), $stbm^{6}$ showed 37.0 (n=8) and dsh^{1} showed 42.4 (n=8). We observed no clear directional bias, suggesting that the planar polarity pathway affects the frequency but not the orientation of such actin-rich protrusions. However, given the complex morphology of the border cell cluster, an effect on protrusion orientation cannot be ruled out.

A similar phenotype was observed upon knockdown of *fz* transcripts specifically in border cells (Fig. 3E). Furthermore, overexpression of *fz* and *stbm* in border cells also disrupted the production of large actin protrusions (Fig. 3F,G), suggesting that precise levels or spatial distribution of pathway activity is important for the correct production of stable actin structures. These results support the view that the motility defects observed in border cells with altered planar polarity pathway function are due to abnormal cytoskeletal dynamics.

RhoA GTPase function is required for border cell migration and is regulated by the planar polarity pathway

In the *Drosophila* wing and eye, the planar polarity pathway positively regulates the activity of the cytoskeleton modulator RhoA GTPase. Loss of RhoA function leads to defects in both the rotation of ommatidial clusters in the eye and the production of actin-rich trichomes in the wing (Strutt et al., 1997). Thus, RhoA is a good candidate for mediating the effects of the planar polarity pathway in border cells.

We examined the effects of the inactivation and activation of RhoA in migrating border cells. We found that expression of a dominant-negative form of RhoA led to the normally compact border cell cluster becoming spread out along the anteroposterior axis, with the trailing edges of cells failing to retract towards the cell bodies (Fig. 3H). This is consistent with studies in other migrating cells in which RhoA is required for retraction of the trailing edge (Raftopoulou and Hall, 2004). Overall, border cell migration is strongly delayed and, in any particular cluster, many cells never reach the oocyte. By contrast, expression of a constitutively active RhoA produced clusters in which the border cells were tightly rounded with no large actin protrusions, indicative of excessive retractive activity (Fig. 3K). This also delayed migration, although less severely than expression of dominant-negative RhoA, with approximately 50% of clusters

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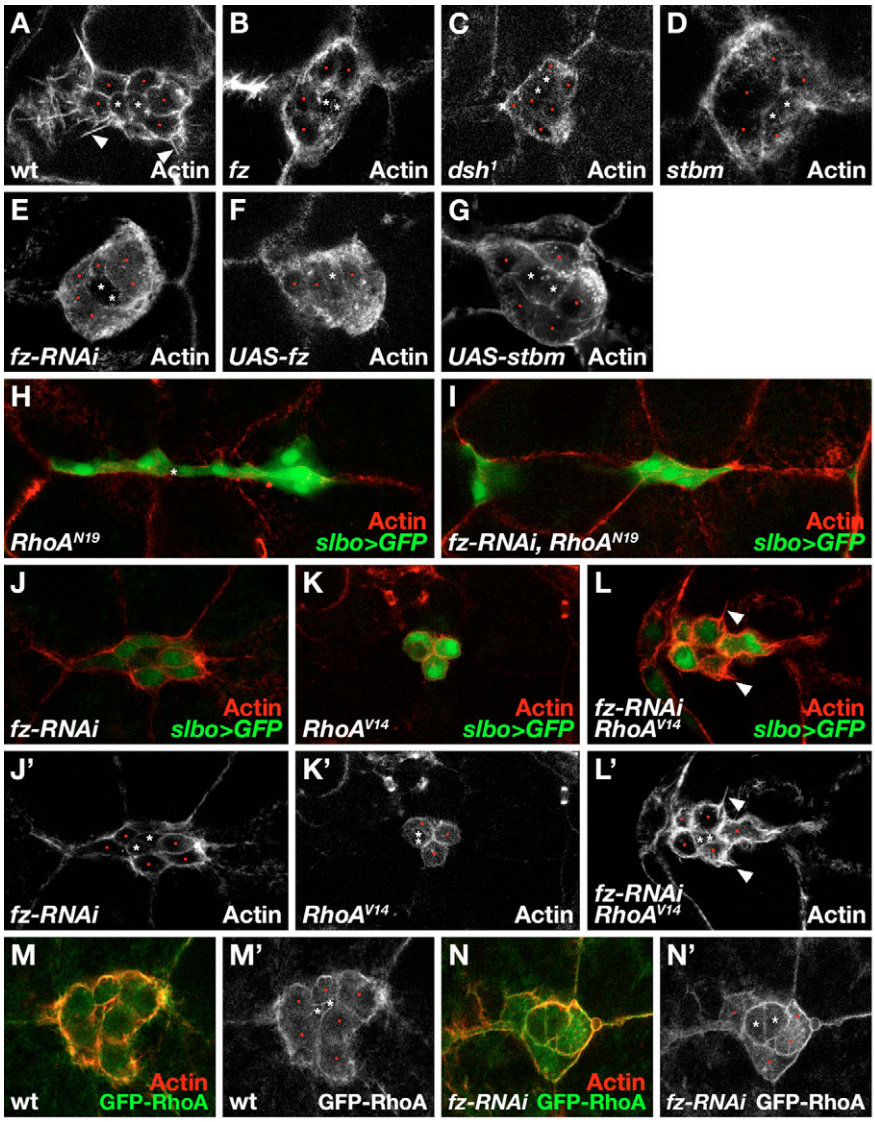

Fig. 3. Core planar polarity genes regulate the border cell actin cytoskeleton. Polar follicle cells are marked with white asterisks and border cells are marked with red dots. (**A-G**) Migrating border cell clusters, fixed to enhance preservation of the actin structures (see Materials and methods). In wild-type clusters (A) large actin-rich protrusions can be seen (arrowheads). In fz^{15}/fz^{23} (B), dsh^{1} (C), $stbm^{6}$ (D) and *slbo-GAL4/UAS-fz*-RNAi (E) mutants, the cytoskeleton appears fuzzy and large protrusions are rarely seen. Overexpression of *fz* (F) and *stbm* (G) under the control of *slbo-GAL4* also disrupts the formation of large actin-rich protrusions. GAL4 experiments were carried out at 29°C. (**H-L'**) Migrating border cell clusters stained for actin (red/white), expressing *slbo-Gal4, UAS-GFP* (green) at 25°C. Expression of dominant-negative Rho^{N19} (H), Rho^{N19} and *fz*-RNAi (I), *fz*-RNAi (J), dominant-active Rho^{V14} (K), and Rho^{V14} and *fz-RNAi* (L). The *UAS-fz*-RNAi insertion used was chosen because it gives weaker phenotypes than the insertion used for other experiments (e.g. panels E and N), with some actin-rich protrusions still being visible (J). Expressing dominant-negative Rho^{N19} results in border cells becoming long, thin and not migrating effectively (H), and co-expressing *fz*-RNAi has no effect on this phenotype (I). Cells expressing dominant-active Rho^{V14} become very round with an even cytoskeleton (K), and co-expressing *fz*-RNAi (L) ameliorates this phenotype, with the cells appearing less round and producing actin-rich protrusions (arrowheads). (**M-N'**) Migrating border cell clusters, stained for actin (red), expressing *GFP-RhoA* (green/white). In wild-type clusters, GFP-RhoA colocalises with actin-rich protrusions at the cell surface (M), which are lost in cells expressing *fz*-RNAi under the control of *slbo-GAL4* at 25°C (N), resulting in a partial redistribution of GFP-RhoA to the cytoplasm. Border cell clusters expressing *fz*-RNAi under the control of *slbo-GAL4* showed an average cytoplasmic level of GFP-RhoA of 24.0% of peak membrane levels (n=10), compared with 15.4% for control clusters lacking the *slbo-GAL4* driver (n=9), these results being statistically significant at the $P<10^{-5}$ level (t-test).

showing an overall delay relative to controls. Consistent with its positive role on RhoA activity in other tissues (Strutt et al., 1997), reduction of planar polarity pathway function was able to ameliorate the effects of RhoA activation, leading to less-rounded cells showing obvious actin protrusions (Fig. 3L), but did not alter the effects of RhoA inactivation (Fig. 3I).

Using either a GFP-RhoA fusion in transgenic flies (Fig. 3M), or an antibody against RhoA (data not shown), we observed that, in wild-type border cell clusters, RhoA localises with actin at the cell periphery, again consistent with a role regulating the cytoskeleton in these cells. Knockdown of *fz* activity specifically in the border cells resulted in a partial redistribution of RhoA to the cytoplasm

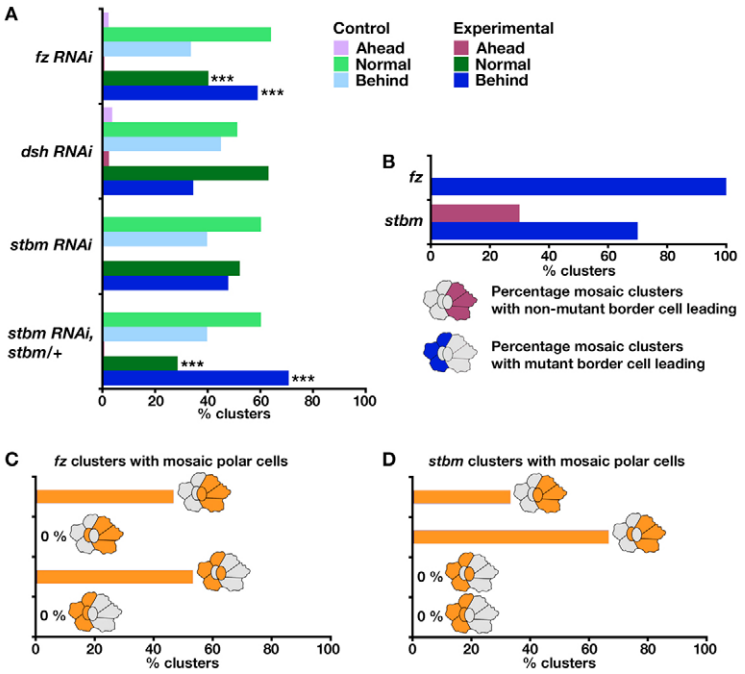

Fig. 4. Core polarity gene function in the polar follicle cells affects border cell migration. (**A**) Chart showing the extent of border cell migration for clusters in which either *fz*, *dsh* or *stbm* transcripts have been knocked-down by *UAS*-RNAi constructs under the control of the polar follicle cell-specific *upd-GAL4* driver at 29°C (Tsai and Sun, 2004). Knockdown of *fz* transcripts causes a significant increase in the number of clusters 'behind' (see Fig. 1), whereas knockdown of *dsh* causes no delay in migration. Knockdown of *stbm* in flies carrying two copies of the endogenous *stbm* locus causes a mild delay in border cell migration, which is greatly enhanced by the removal of one copy of the endogenous locus. (**B**) Chart showing the proportions of genetically mosaic clusters recovered for the strong alleles fz^{15} and $stbm^{6}$ with both polar follicle cells lacking gene function, and either wild-type border cells leading (pink bars) or mutant border cells leading (blue bars). Mutant cells in the cartoons are represented by grey shading, with leading cells to the right and lagging cells to the left. In the small number of *fz* mosaic clusters recovered (n=6), we saw no clusters with a wild-type border cell leading, which only deviates from the null hypothesis that border cell position is random at a significance level of P=0.034. In the *stbm* mosaic clusters recovered (n=10), both wild-type and mutant border cells are seen leading, and the result fits the null hypothesis that border cell position is random (P=0.5). (**C**) Chart showing the proportions of genetically mosaic clusters recovered for the strong fz^{15} allele with only one polar follicle cell lacking gene function. Two classes of clusters were recovered (n=15); both had the non-mutant polar follicle cell touching the leading border cell, with the genotype of this leading border cell approximately equally distributed between wild type and mutant. The leading position of the polar follicle cells strongly deviates from the null hypothesis that polar cell position is random (P=0.0003), whereas the position of the border cells fits the hypothesis that this is random with respect to the genotype of the border cell (P=0.71). The data suggest that border cell position is determined by the genotype of the polar follicle cell with which they make junctional contact, regardless of the genotype of the border cell. (**D**) Chart showing the proportions of genetically mosaic clusters recovered for the strong $stbm^{6}$ allele with only one polar follicle cell lacking gene function. Two classes of clusters were recovered (n=9); both had non-mutant border cells leading the cluster, with the genotype of the polar follicle cell touching the leading border cell being either mutant or non-mutant. The leading position of wild-type border cells does not fit the null hypothesis that position is random (P=0.018). The position of the wild-type polar cells fits the hypothesis that this is randomly determined (P=0.51).

(compare Fig. 3M′ with Fig. 3N′). Quantitation showed the relative cytoplasmic levels of Rho-GFP upon *fz* knockdown to be over 50% higher than in control clusters.

Therefore, we conclude that the planar polarity pathway controls the actin cytoskeleton in border cells and positively regulates RhoA activity, and that RhoA itself is required for normal border cell migration.

Core planar polarity gene function is additionally required in the non-migratory polar follicle cells

Border cell migration depends not only on the motile border cells, but also on the presence of the non-migratory polar follicle cells in the cluster; these cells form adherens junctions with the border cells and signal to them (Niewiadomska et al., 1999; Han et al., 2000; Silver and Montell, 2001). We investigated whether border cell migration also requires planar polarity gene function in the polar follicle cells by using RNAi-mediated knockdown of transcripts specifically in these cells. Knockdown of either *fz* or *stbm* resulted in delayed border cell migration, but no delay was observed upon knockdown of *dsh* (Fig. 4A). Recent work in the *Drosophila* wing has shown that *dsh* is not required for intercellular communication mediated by the planar polarity pathway, but is necessary to couple such signals to downstream effectors (Strutt and Strutt, 2007). These results suggest that Fz/Stbm-dependent cell-cell communication in the polar follicle cells is required for efficient border cell migration, but that downstream pathway effectors are not required in these non-migratory cells.

To verify the specificity of the requirement for *fz* and *stbm* in the polar follicle cells, we again examined genetically mosaic border cell clusters. We obtained border cell clusters in which both polar follicle cells lacked either *fz* or *stbm* function, but in which some of the border cells retained activity. The number of such clusters was small, because loss-of-function clones are rarer in the polar follicle cell lineage than in the border cell lineage (Margolis and Spradling, 1995). Contrary to what was observed in mosaic clusters in which both polar cells retained *fz* or *stbm* activity, in clusters in which both polar follicle cells lacked activity, we no longer observed a preference for non-mutant border cells to partition to the leading edge of the cluster (Fig. 4B). From this we deduce that planar polarity pathway function only confers a migratory advantage on border cells if the polar follicle cells also have *fz* and *stbm* function. Therefore, a Fz/Stbm-dependent signal must pass from the polar cells, either directly or indirectly, to the border cells, and this signal is required in the border cells for planar polarity pathway function to enhance their migration.

These results indicate a requirement for *fz* and *stbm* in the polar follicle cells, but do not address whether the requirement is in one or both cells, or whether it requires direct contact between polar follicle cells and responding border cells. Therefore, we examined the positions of wild-type and mutant cells within mosaic clusters in which only one polar cell retained *fz* or *stbm* activity.

In such mosaic clusters lacking *fz* activity in one polar follicle cell, we made an important observation (Fig. 4C): the polar cell that retained *fz* function was always positioned towards the

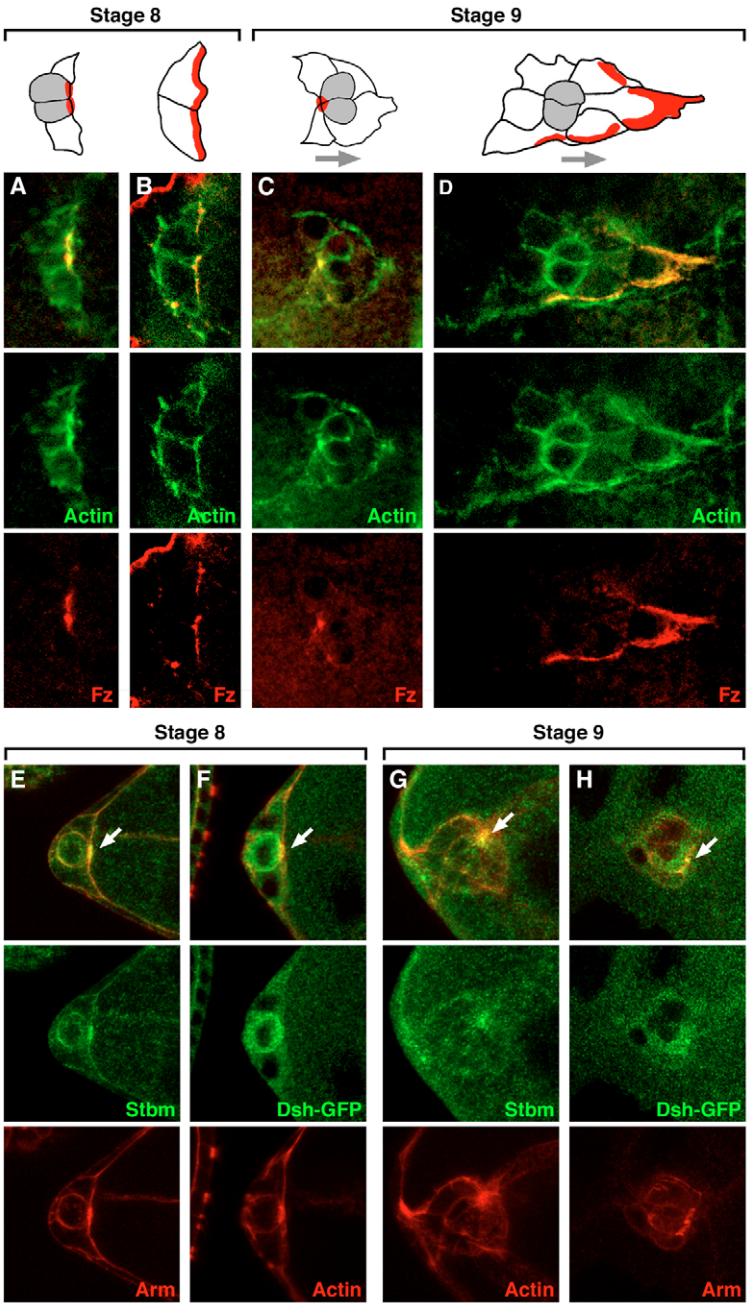

Fig. 5. Fz and Stbm proteins are localised within the border cell cluster. Illustrations (top) show border cells (white), polar follicle cells (grey) and Fz (red); the direction of migration is towards the right (grey arrows). (**A-D**) Border cell clusters stained for Fz (red) and actin (green). Fz is localised in the adherens junctions of the polar follicle cells (A) and apical regions of the border cells (B) prior to migration. During migration, Fz localisation is retained in the junctional region that the polar follicle cells share with the border cells (C) and is within the migratory regions of the border cells (D). This pattern of localisation is lost in egg chambers mutant for *fz*, consistent with the immunolabelling being specific (data not shown). (**E**,**G**) Egg chambers stained for Armadillo or actin (red) and Stbm (green). Stbm is localised to the polar follicle cell adherens junctions (arrow). This pattern of localisation is lost in egg chambers mutant for *stbm* (data not shown). (**F**,**H**) Egg chambers stained for Armadillo or actin (red) and Dsh-GFP (green). Dsh-GFP is seen in a punctate pattern in polar follicle cell and border cell cytoplasm, and also partially overlaps the adherens junction region (arrow).

leading edge of the cluster, contacting the leading border cells, whereas the *fz* mutant polar cell was always positioned towards the lagging end of the cluster, contacting the lagging border cells. This result was independent of the genotype of the border cells. Consequently, border cells are positioned within the cluster according to the genotype of the polar cell with which they make junctional contact. Thus, we conclude that the motility of either wild-type or *fz* mutant border cells is enhanced by contact with a *fz*-expressing polar cell.

Examination of mosaic clusters containing one polar follicle cell lacking *stbm* function revealed a different requirement for *stbm* activity. In the small number of such clusters that we obtained, we found that the polar cell that retained *stbm* activity could contact either the leading or the lagging border cells, and that *stbm* mutant border cells always lagged at the back of the cluster (Fig. 4D). Thus, unlike the situation observed for *fz*, there is no migratory advantage conferred on a border cell that is in contact with a *stbm*-expressing polar cell. However, because the wild-type border cells are always at the leading edge of the clusters, unlike in clusters that lack *stbm* activity in both polar cells (Fig. 4B), we can conclude that *stbm* activity in at least one polar cell does confer increased motility on *stbm*-expressing border cells but that this effect is not contact-dependent.

We can summarise our findings from the mosaic analysis data as follows: in clusters that retain *fz* and *stbm* activity in the non-migratory polar follicle cells, border cell motility is cell-autonomously enhanced by *fz* and *stbm* function; second, this enhancement of border cell motility by planar polarity pathway function requires direct contact with a *fz*-expressing polar follicle cell; and third, this enhancement also requires *stbm* activity in at least one polar follicle cell, but this cell does not need to directly contact a border cell to enhance its migration.

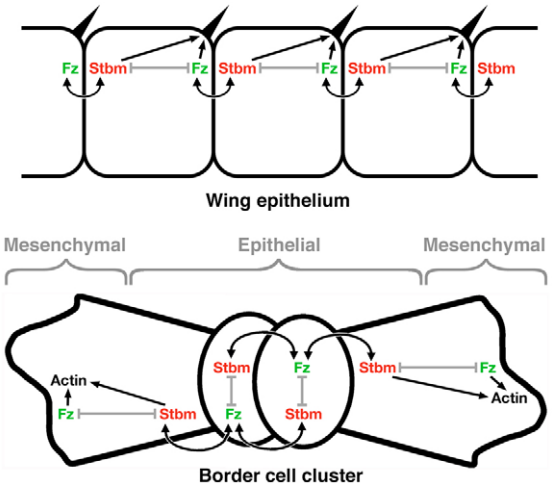

Fig. 6. Model of Fz and Stbm interactions in the pupal wing epithelium and border cell cluster. In the wing, Fz and Stbm mutually reinforce the localisation of each other in opposing junctions of neighbouring cells (rounded black arrows) and inhibit the localisation of each other in adjacent regions of the same cell (grey bars). Distally localised Fz within the same cell promotes the production of a single distal actin-rich trichome, via Dsh and RhoA function (Axelrod, 2001; Strutt, 2001; Strutt et al., 1997). In addition, proximally localised Stbm is thought to promote trichome formation at the opposite end by an uncharacterised mechanism (Adler et al., 2004). In the border cell cluster, Fz and Stbm are localised to the junctional regions in which the epithelial polar follicle cells and the partly epithelial border cells make contact. Because Fz-expressing polar follicle cells promote the migration of Stbm-expressing border cells in a contact-dependent manner, we infer that Fz in the junctions of polar cells promotes the localisation of Stbm to the junctions of border cells. In turn, this would lead to Fz localisation to the non-junctional (mesenchymal) migratory regions of the border cells. Fz in border cells locally modulates the formation of appropriate actin structures, probably via Dsh and RhoA, as in the wing. In addition, Stbm in the junctions of border cells promotes the formation of actin structures at a distance in the migratory region. In this way, Stbm localised to junctions and Fz in the migratory region both independently promote migration. Consistent with our mosaic analysis, this scheme predicts that (i) contact with an Fz-expressing polar cell promotes border cell migration (Fig. 4B,C), (ii) Fz-expressing polar cells are only able to promote the migration of border cells that express Stbm (Fig. 1D, Fig. 4D), and (iii) Stbm is required in the polar cells for efficient border cell migration (Fig. 4B), but the border cells do not need to touch the Stbm-expressing polar cell (Fig. 4D).

Fz and Stbm proteins are localised within migrating border cell clusters

In other contexts, such as in the eye and wing, Fz and Stbm are believed to mediate intercellular communication via the formation of asymmetric protein complexes at the adherens junctions, in which Fz in one cell is juxtaposed with Stbm in the neighbouring cell (Strutt, 2001; Bastock et al., 2003). Because the polar cells contact each other and the border cells via an adherens junction-like region (Niewiadomska et al., 1999), we studied whether Fz and Stbm localise to this region, which would be consistent with the Fz/Stbm-dependent signalling that our mosaic analysis revealed between these cells.

Immunolabelling of border cell clusters prior to migration (late stage 8) or during migration (stage 9) revealed the presence of Fz in the adherens junction region joining the polar and border cells (Fig. 5A,C). Similarly, Stbm was also seen localised to the junctional region at these stages (Fig. 5E,G), consistent with a role in mediating intercellular communication.

We also observed Fz (but not Stbm) localised to the migratory edges of the border cells, both prior to and during migration (Fig. 5B,D). Fz localisation has not previously been observed in migrating cells, but tagged forms of the *Xenopus* Dsh homologue (a Fz-binding partner) are enriched at the actin-rich bilateral tips of elongating cells undergoing convergent extension (Kinoshita et al., 2003). Using available reagents (Shimada et al., 2001; Strutt et al., 2006), we were unable to detect a specific distribution of endogenous Dsh within migrating border cell clusters (data not shown). Therefore, we investigated the distribution of a Dsh-GFP fusion protein, which accurately reflects the junctional distribution of Dsh in the *Drosophila* pupal wing (Axelrod, 2001). We found that this protein accumulated at high levels in a punctate pattern in the cytoplasm of both polar follicle cells and border cells (Fig. 5F,H). Although there was evidence of enrichment in the junctional region of the clusters, this was partly obscured by the high cytoplasmic levels. Similarly, we were unable to determine whether Dsh-GFP was specifically localised to the migratory edges of the border cells.

DISCUSSION

The planar polarity pathway involving Fz/Stbm is implicated in an increasing number of processes in development, organogenesis and disease. These include cell rearrangements during gastrulation, neural crest migration, neuronal pathfinding, sensory hair orientation, heart formation, and cell invasion in cancer (Curtin et al., 2003; De Calisto et al., 2005; Heisenberg et al., 2000; Jessen et al., 2002; Montcouquiol et al., 2003; Wallingford et al., 2000; Phillips et al., 2005; Weeraratna et al., 2002). Many of these processes involve coordinated cell movement, and yet this aspect of planar polarity pathway function is poorly understood.

Here, we use the *Drosophila* ovary to study the control of coordinated cell movements by the planar polarity pathway, taking advantage of its relative simplicity and the ability to precisely manipulate gene function in individual cell populations. Activity of the core polarity genes facilitates invasive migration of the border cell cluster through the nurse cells. Of particular interest is our observation that migration of the border cells is enhanced by planar polarity activity in the non-migratory epithelial polar follicle cells, suggesting a key role for interactions between migratory and non-migratory cell types.

In the *Drosophila* wing, the planar polarity pathway regionalises cells via the formation of proximal and distal domains at the level of the adherens junctions. The distal domain contains Fz (Strutt, 2001), which acts via the downstream factors Dsh (Axelrod, 2001) and RhoA (Strutt et al., 1997) to ensure local production of a single actin-rich trichome, while, in the proximal domain, Stbm (Bastock et al., 2003) recruits factors that locally inhibit trichome formation (Adler et al., 2004) (Fig. 6). During border cell migration, the coordinated movement of the non-migratory polar follicle cells and the migratory border cells is achieved in part by the border cells retaining epithelial character in the region contacting the polar follicle cells, but also having an actin-rich partly mesenchymal migratory region (Niewiadomska et al., 1999). Taking these observations together, we propose that, in border cells, localised Fz in the migratory region and localised Stbm in the junctional region might promote the production of actin-rich structures (Fig. 6), which, in turn, would increase the motility both of individual cells and the cluster as a whole.

Our mosaic analyses suggest a mechanism for how this localised Fz and Stbm activity is established within the border cells. Fz and Stbm mediate intercellular communication between the polar cells and the border cells via the production of junctional complexes. Because contact with an Fz-expressing polar cell enhances the migration of border cells, we surmise that Fz in each polar cell interacts with Stbm in the contacting border cell. Junctionally localised Stbm in the border cell can then act as a cue to indirectly promote actin-rich protrusion formation in the migratory region, at least in part via the localisation of Fz (Fig. 6).

Although the planar polarity pathway has been known for some years to promote cell rearrangements during vertebrate gastrulation (Wallingford et al., 2002), surprisingly little is understood about its mechanisms of action in cell movement and the particular roles of this pathway in cell-cell communication. We have demonstrated that Fz/Stbm-mediated intercellular communication can enhance the invasive migration of a group of cells. Migration of groups of cells, sometimes including both motile and non-motile types, is important for many processes in animal morphogenesis and in disease processes, such as cancer metastasis (Friedl, 2004; Lecaudey and Gilmour, 2006). Our work provides evidence that planar polarity pathway function could be generally important in coordinated cell migration, providing a mechanism by which cells within a group can communicate and establish the proper regional production of actin structures required for efficient movement.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/134/17/3055/DC1

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