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Planar polarity genes in the *Drosophila* wing regulate the localisation of the FH3-domain protein Multiple Wing Hairs to control the site of hair production

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The core planar polarity proteins play important roles in coordinating cell polarity, in part by adopting asymmetric subcellular localisations that are likely to serve as cues for cell polarisation by as yet uncharacterised pathways. Here we describe the role of Multiple Wing Hairs (Mwh), a novel formin homology 3 (FH3)-domain protein, which acts downstream of the core polarity proteins to restrict the production of actin-rich prehairs to distal cell edges in the *Drosophila* pupal wing. Mwh appears to function as a repressor of actin filament formation and, in its absence, ectopic actin bundles are seen across the entire apical surface of cells. We show that the proximally localised core polarity protein Strabismus acts via the downstream effector proteins Inturned, Fuzzy and Fritz to stabilise Mwh in apico-proximal cellular regions. In addition, the distally localised core polarity protein Frizzled positively promotes prehair initiation, suggesting that both proximal and distal cellular cues act together to ensure accurate prehair placement.

KEY WORDS: Planar polarity, Multiple Wing Hairs, *Drosophila*, Cell polarity, Frizzled, Actin

INTRODUCTION

The development of multicellular organisms requires the coordinated specification of both cell fates and cell polarities. One example of coordinated cell polarisation is planar polarity, whereby cells within epithelial sheets adopt uniform polarities relative to the plane of the tissue. Although widespread in nature, the mechanisms underlying the establishment of planar polarity have been most often studied in insect cuticles; nevertheless, essential roles in vertebrate development are now recognised (reviewed by Strutt, 2003; Klein and Mlodzik, 2005; Wang and Nathans, 2007).

In many contexts, a key event in the coordinated planar polarisation of epithelia is the asymmetric subcellular localisation of a group of 'core' planar polarity proteins to opposite cell edges. This process is best characterised in the *Drosophila* pupal wing, in which the seven-pass transmembrane protein Frizzled (Fz) and the cytoplasmic proteins Dishevelled (Dsh) and Diego (Dgo) localise to distal apicolateral junctional regions, the four-pass transmembrane protein Strabismus (Stbm, also known as Van Gogh) and the cytoplasmic protein Prickle (Pk) localise proximally, and the seven-pass transmembrane cadherin Flamingo (Fmi, also known as Starry Night) localises both distally and proximally (reviewed by Klein and Mlodzik, 2005). The localisations of the core proteins define distinct distal and proximal apicolateral membrane domains, which are thought to act as cues for subsequent cell polarisation events mediated by downstream effector genes.

The best-characterised morphogenetic event regulated by the asymmetric localisation of the core polarity proteins is the production of a single, distally pointing trichome from each cell of the wing blade (Gubb and García-Bellido, 1982; Wong and Adler, 1993). Trichome formation begins with increased actin bundling close to the distal cell vertex at ~32 hours of pupal life, leading to formation of a prehair that contains both F-actin and microtubules (Mitchell et al., 1983; Wong and Adler, 1993; Eaton et al., 1996; Turner and Adler, 1998). Electron microscopy has revealed that shortly prior to prehair formation, the apical cell surface is covered in electron-dense 'pimples' (Guild et al., 2005), which are believed to be precursors for microvillus formation. Prehair initiation is manifested by actin bundles sprouting from a region covering several pimples close to the distal vertex. This suggests that it is the local activation of pimples to polymerise actin that specifies the site of prehair formation.

Four genes have been identified that act downstream of the core polarity proteins to specify the site of prehair initiation, namely *inturned* (*in*), *fuzzy* (*fy*), *fritz* (*frtz*) and *multiple wing hairs* (*mwh*) (Gubb and García-Bellido, 1982; Wong and Adler, 1993; Lee and Adler, 2002; Collier et al., 2005). Interestingly, whereas loss of core polarity protein function (and thus of asymmetric localisation) causes formation of a single prehair in the cell centre, loss of function of these downstream effectors leads to the formation of multiple prehairs at cell edges. This led to the suggestion that the downstream effectors repress prehair formation throughout the cell periphery, whereas the core planar polarity proteins promote prehair formation at the distal cell edge by locally counteracting the effectors (Wong and Adler, 1993).

The best-studied effectors are *in*, *fy* and *frtz*, which all act cell-autonomously upstream of *mwh* (Gubb and García-Bellido, 1982; Wong and Adler, 1993; Park et al., 1996; Collier and Gubb, 1997; Collier et al., 2005) and encode, respectively, a putative two-pass transmembrane protein, a putative four-pass transmembrane protein and a WD40-repeat cytoplasmic protein. Interestingly, the ectopic trichome phenotype of null alleles of all three loci is enhanced at lower temperatures, leading to the suggestion that these proteins act in a microtubule-dependent process (Adler et al., 1994).

An important recent observation is that the In protein localises at the proximal apicolateral edges of wing cells under control of the core polarity proteins, shortly prior to prehair formation (Adler et al., 2004). This localisation also requires the activity of *fy* and *frtz*, but not *mwh*. Two alternative models have been put forward to

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explain the requirement of proximally localised In for distal prehair initiation: In might promote local formation of a repressor of prehair initiation, thus restricting initiation to distal regions; alternatively, In could act positively to promote polarised transport of a factor required for prehair initiation to the distal cell edge (Adler et al., 2004). Both models challenged the existing assumption that distally localised Fz/Dsh act as the primary determinants for prehair initiation, and instead suggest that proximally localised Stbm/Pk might be the crucial cue.

The phenotype of *mwh* mutants is stronger than that of *in*, *fy* or *frtz*, displaying a greater number of inappropriate prehair initiations per cell (Wong and Adler, 1993). By genetic criteria, *mwh* acts downstream of the other effectors and is therefore the factor most likely to interact directly with the actin cytoskeleton, perhaps by acting as a repressor of pimple activation. However, thus far, the molecular identity of the gene product of *mwh* is unknown.

The widespread requirements of core planar polarity protein activity in invertebrate and vertebrate morphogenesis, and the associated observation of asymmetric core protein subcellular localisation, suggest that it will be important to understand how the 'distal' Fz/Dsh cue and/or the 'proximal' Stbm/Pk cue control cell shape and behaviour by modulating the cytoskeleton. Significantly, homologues of the effectors In and Fy have already been found to play crucial roles in vertebrate embryogenesis (Park et al., 2006). In this study, we sought to understand the mechanisms by which the core planar polarity proteins and their effectors restrict prehair initiation to the distal cell vertex during *Drosophila* wing development.

MATERIALS AND METHODS

Fly strains and genetics

Alleles and transgenes are described in FlyBase, except for *P{w+, ActP-FRT-PolyA-FRT-EGFP-Fy}* and *P{w+, ActP-FRT-PolyA-FRT-EGFP-CG13913}* (this study). Mitotic clones were generated using the FLP/FRT system (Xu and Rubin, 1993) and *Ubx-FLP* (Emery et al., 2005); twin clones were generated as previously described (Strutt and Strutt, 2007). Overexpression was using GAL4/UAS (Brand and Perrimon, 1993).

Immunolabelling and imaging

Pupae were aged at 25°C unless indicated otherwise and wings and cells were processed for immunofluorescence as described previously (Strutt, 2001), except that in some cases, to improve labelling of actin structures, the fixative was supplemented with 1% Triton X-100 and 1:200 Alexa568-phalloidin (Molecular Probes). Primary antibodies were 1:400 mouse monoclonal anti-β-galactosidase (β-gal) (Promega), 1:4000 rabbit anti-β-gal (Cappel), 1:4000 rabbit anti-GFP (Abcam), 1:10 mouse monoclonal anti-Fmi (#74, DSHB) (Usui et al., 1999), 1:200 mouse monoclonal anti-Arm (DSHB), 1:1000 rat anti-Dsh (Strutt et al., 2006), 1:1000 rat or 1:100 rabbit anti-Mwh, 1:1000 rat or 1:100 rabbit anti-Frtz, 1:200 mouse monoclonal anti-α-tubulin (DM1A, Sigma). Actin was visualised using 1:200 Texas Red or Alexa568-phalloidin. Confocal *z*-stacks were captured on a Leica SP confocal microscope, and average projections of several *z*-planes were made to provide a final image depth of ~1 μm. Fluorescent intensities were quantitated using ImageJ.

Molecular biology and tissue culture

ESTs containing the coding sequences of *fy* (AT05453), *frtz* (RH72421) and *CG13913* (RE53394) were obtained from the DGRC. Flies expressing EGFP-Fy and EGFP-CG13913 were generated by fusing EGFP to the N-terminus of the coding sequence and cloning into the transformation vector p*P{w+, ActP-FRT-PolyA-FRT-PolyA}* (Strutt, 2001). Germline transformations were performed by BestGene. EGFP-CG13913 was expressed in cultured *Drosophila* S2 cells using p*P{w+, ActP-FRT-PolyA-FRT-EGFP-CG13913}* transfected using Effectene (Qiagen), with cotransfection of pActP-FLP to excise the FRT-PolyA-FRT cassette. Cells were plated on Concanavalin A-treated coverslips.

Generation of antibodies

Frtz and Mwh antibodies were generated in rats and rabbits against His-tagged fusion proteins corresponding to amino acids 670-951 and 440-836, respectively; rabbit sera were affinity purified against the same fusion protein.

Characterisation of the *mwh* lesion

The breakpoint in mwh^1 was isolated by inverse PCR, identifying an inversion following amino acid 367 that breaks within the conserved FH3 domain, leading us to believe that this might result in a null allele. In support of this, we scored mwh^1 as amorphic in the wing, with the adult trichome phenotype of mwh^1 homozygotes being indistinguishable from that of hemizygotes (D.S., unpublished).

Immunoblotting

Protein extracts for immunoblotting were prepared by dissecting pupal wings directly into sample buffer (141 mM Tris base, 2% lithium dodecyl sulphate, 10% glycerol, 0.51 mM EDTA, 100 mM dithiothreitol, pH 8.5) and running the equivalent of one wing per lane. For phosphatase experiments, wings were dissected into ice-cold lysis buffer [50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% Triton X-100, protease inhibitors (Roche)] supplemented with phosphatase inhibitor (1 μM microcystin) for control samples. Experimental samples were treated with 400 U lambda phosphatase (NEB) for 30 minutes at room temperature. Proteins were detected using 1:500 affinity-purified rabbit anti-Mwh, 1:5000 anti-actin (AC40, Sigma) or 1:10,000 anti-α-tubulin (DM1A, Sigma).

RESULTS

Roles of the 'proximal' and 'distal' cues in specifying the site of prehair initiation

To assess the requirements for potential prehair initiation cues provided by localisation of the core polarity proteins Fz/Dsh/Dgo distally or Stbm/Pk proximally, we generated cells that contained only one of these cues at a known cell edge. In cells lacking *fz*, Dsh/Dgo are no longer recruited to the junctions (Axelrod, 2001; Shimada et al., 2001; Das et al., 2004), but, at cell edges touching non-mutant cells, Stbm/Pk are strongly recruited (Tree et al., 2002; Bastock et al., 2003), giving rise to a localised 'proximal' cue in a cell lacking a 'distal' cue. Strikingly, in such cells containing just a proximal cue, prehair initiation is still seen to occur at the opposite cell edge (Fig. 1B). However, in *fz* cells away from the clone edge, prehair initiation occurs in the cell centre as previously reported (Wong and Adler, 1993), but is noticeably delayed.

Patches of cells lacking *stbm* activity present the opposite situation, in which no proximal cue is present, but, in cells touching non-mutant cells, a distal cue containing Fz/Dsh assembles (Strutt, 2001; Bastock et al., 2003). Cells containing only a localised distal cue were also seen to initiate a prehair at the site of this cue, despite the lack of a proximal cue at the opposite cell edge (Fig. 1C). Notably, *stbm* mutant cells within the clone showed a shorter delay in prehair initiation than observed with *fz* tissue. In addition, prehair initiation was only consistently seen in the cell centre in the fourth row of cells away from the clone edge. In the third row of cells, about half produced a prehair that was positioned towards the cell edge nearest to the clone boundary, whereas in the second row of mutant cells, almost all prehairs initiated closer to the cell edge nearest the clone boundary.

To compare further the effects on prehair initiation of cells only having a distal or proximal cue, we generated wings containing clones of *fz* mutant cells abutting clones of *stbm* mutant cells (*fz*; *stbm* twin clones) (Strutt and Strutt, 2007). At the boundary between the *fz* and *stbm* clones, the core polarity proteins showed normal asymmetric localisation, but the neighbouring cells were all mutant and failed to asymmetrically localise core polarity proteins. Hence, we can rule out any potential influence of asymmetric core protein

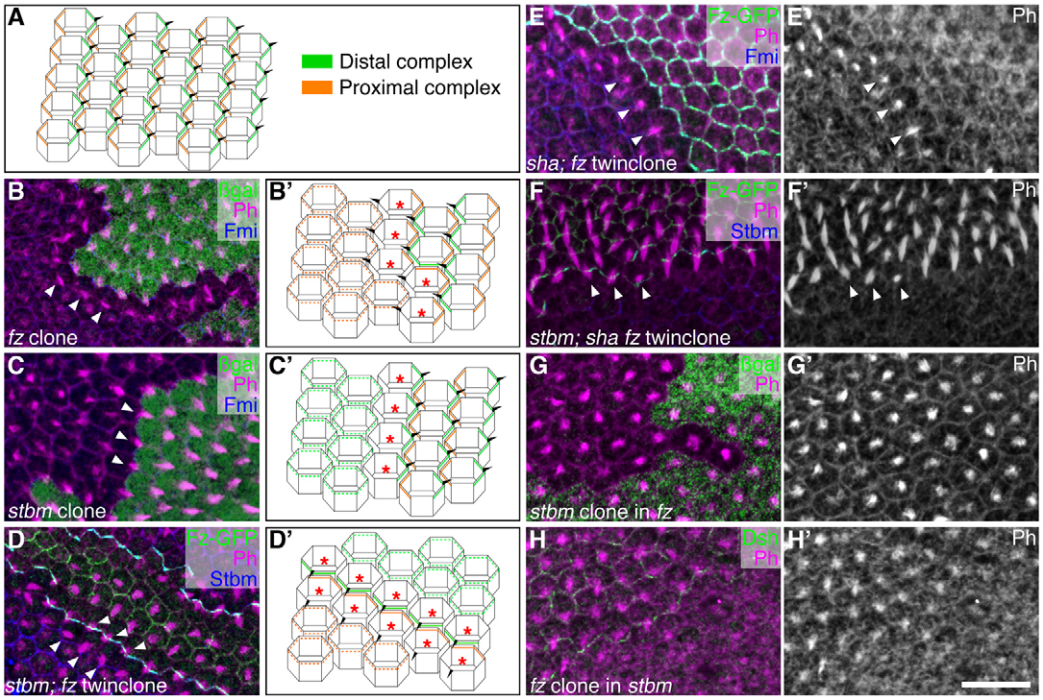

Fig. 1. Core polarity gene function and the site of prehair initiation. *Drosophila* 32-hour pupal wings immunolabelled for actin [Ph; magenta in B-H, white in E′-H′], clonal marker (*lacZ*, *fz-EGFP* or *dsh* expression; green) and Fmi or Stbm (blue). Arrowheads indicate prehairs initiating at edges of mutant cells touching a mitotic clone boundary. Distal is to the right in this and subsequent figures. Diagrams illustrate sites of core polarity protein localisation (Fz/Dsh distal complex in green, Stbm/Pk proximal complex in orange) in wild type or on edges of clones, and observed sites of prehair initiation; first row of mutant cells in each clone marked with asterisks. Sites of prehair initiation in mutant cells away from clone edges are not illustrated but are located at the cell centre (except as noted in the text for the first rows of cells within *stbm* tissue). (**A**) Diagram of normal localisation of distal and proximal core polarity proteins and site of prehair initiation in wild-type wings. (**B**,**B′**) fz^{21} clone. Note that prehair initiation is delayed in mutant cells away from clone edge. Normal polarity is reversed in non-mutant cells owing to the influence of the clone (Vinson and Adler, 1987). (**C**,**C′**) $stbm^{6}$ clone. Note that prehair initiation is less delayed than in *fz* clones and is more likely to be towards a cell edge. (**D**,**D′**) $stbm^{6}/fz^{21}$ twin clone. (**E**,**E′**) sha^{1}/fz^{21} twin clone. (**F**,**F′**) $stbm^{6}/sha\ fz^{21}$ twin clone. (**G**,**G′**) $stbm^{6}$ clone in a fz^{21} background. (**H**,**H′**) fz^{21} clone in a $stbm^{6}$ background. Note that *fz* mutant cells are marked by loss of Dsh junctional recruitment (Shimada et al., 2001). Scale bar: 10 μm.

localisation in neighbouring cells on the site of prehair initiation, and focus on the effects of the asymmetric localisation straddling a single cell-cell boundary. Consistent with the effects observed around isolated *fz* and *stbm* clones, cells containing a distal cue initiated a prehair at this cell edge, whereas the neighbours containing a proximal cue assembled a prehair towards the opposite cell edge (Fig. 1D). We again saw a greater delay in prehair initiation in *fz* mutant cells that were not on the clone edge, versus *stbm* mutant cells, and also found that the second row of *stbm* mutant cells had a site of prehair initiation that was influenced by their polarised neighbours on the clone edge.

Taken together, these results suggest that asymmetric localisation of the core polarity proteins across cell junctions provides both a distal cue for prehair initiation, which promotes initiation at the same cell edge, and a proximal cue that promotes initiation towards the opposite cell edge. It should be noted, that a distal cue is always associated with a proximal cue in the next cell, and vice versa, by virtue of the mutually dependent localisations of the core polarity proteins. Thus, it is formally possible that only one of these cues has a direct effect on the site of prehair initiation, and that the apparent effect of the other cue is in fact the result of a signal from the adjacent cell.

One way in which an adjacent cell might influence prehair formation in a neighbour would be if assembly of a prehair produced a physical cue that influences the cytoskeleton of the neighbouring cell and induces prehair formation at the opposite edge. To test this, we generated mutant cells containing only a proximal or distal polarity cue, juxtaposed to cells that are unable to form a prehair by virtue of being mutant for *shavenoid* (*sha*) (Ren et al., 2006). Interestingly, cells on the edges of a *fz* clone, adjacent to *sha* mutant cells, formed prehairs at the expected cell edge despite the lack of prehairs in neighbouring cells (Fig. 1E). Similarly, cells containing only a distal cue also formed prehairs close to the cell edge (Fig. 1F). Hence, prehair formation in neighbouring cells is not necessary to enable cells with just a proximal or distal cue to position prehairs correctly, although cell-cell communication of another form cannot be excluded.

Additionally, we investigated the increased delay in prehair initiation in *fz* mutant cells versus *stbm* mutant cells. In theory, Fz and Stbm could be required simply to localise a prehair-promoting cue and a prehair-repressing cue, respectively, and/or could also be required for the activity of the cue. Hence, cells mutant for both *fz* and *stbm* might contain neither cue, or could contain one or both cues uniformly distributed.

Interestingly, *stbm* clones in a uniformly *fz* mutant background show no difference in the time of prehair initiation between single- and double-mutant tissue (Fig. 1G). This is consistent with Stbm acting to modulate the distribution of a cue but not altering its overall activity, such that the cue is equally active in cells with and without Stbm. Conversely, *fz* clones in a *stbm* mutant background show

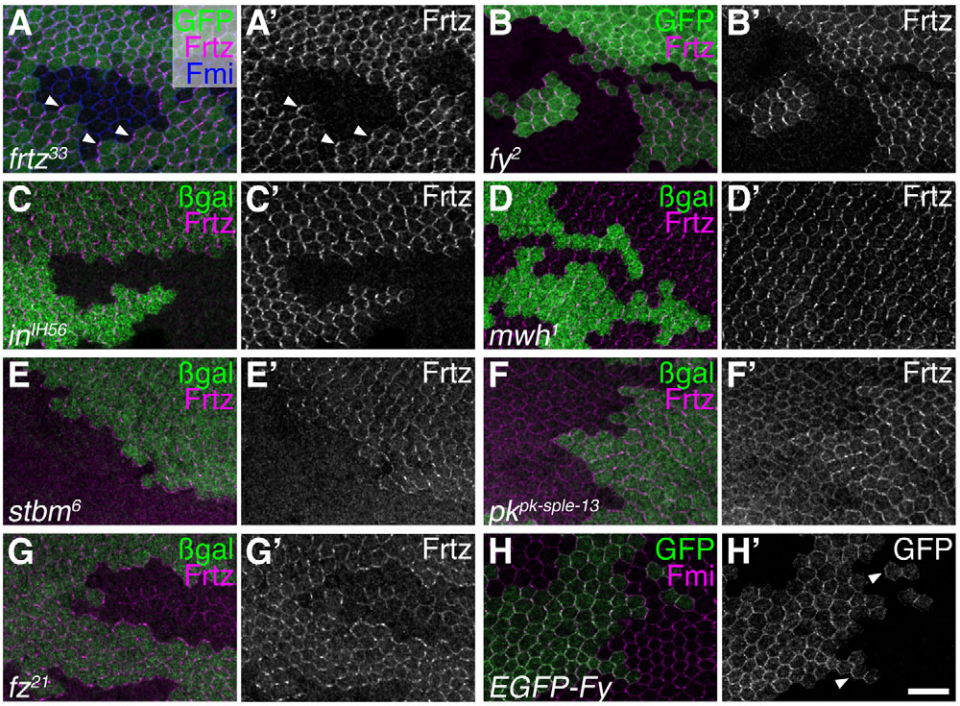

Fig. 2. Frtz is proximally localised under control of polarity gene activity. *Drosophila* 28-hour pupal wings immunolabelled for Frtz (magenta in A-G, white in A′-G′) and/or Fmi (A, blue; H, magenta), and clonal marker (*lacZ* or *GFP*, green) or EGFP-Fy (green in H, white in H′). Arrowheads indicate proximally enriched localisation of protein. (**A**,**A′**) $frtz^{33}$ clone. (**B**,**B′**) fy^{2} clone. (**C**,**C′**) in^{IH56} clone. (**D**,**D′**) mwh^{1} clone. (**E**,**E′**) $stbm^{6}$ clone. (**F**,**F′**) $pk^{pk\text{-}sple\text{-}13}$ clone. (**G**,**G′**) fz^{21} clone. (**H**,**H′**) Mosaic expression of EGFP-Fy induced from a *P{w+, ActP-FRT-PolyA-FRT-EGFP-Fy}* transgene using *hsFLP* expression. Scale bar: 10 μm.

prehairs initiating sooner in *stbm* mutant tissue than in *stbm; fz* double-mutant tissue (Fig. 1H). This suggests that Fz is able to promote prehair initiation positively and that in the absence of Fz this promoting activity is lost.

Together, our results suggest that prehair initiation is controlled by an inhibitory cue that is normally localised proximally in a Stbm-dependent manner, but is not strictly dependent upon Stbm for its activity, and by a Fz-dependent cue that positively promotes prehair formation.

Fy, Frtz and In are putative effectors of the proximal prehair initiation cue

Loss of activity of *fy*, *frtz* or *in* results in more than one prehair initiating in ectopic positions in the cell, consistent with inappropriate activation of a prehair-promoting cue or the loss of a prehair-repressing cue (Gubb and García-Bellido, 1982; Wong and Adler, 1993; Collier et al., 2005). Notably, In localises proximally with Stbm, in a Stbm-dependent manner (Adler et al., 2004), suggesting that In mediates the proximal cue. Proximal localisation of In also depends upon *fy* and *frtz* activity (Adler et al., 2004), consistent with the three gene products acting together to regulate prehair initiation.

We raised an antibody against Frtz and found that Frtz also localises to proximal junctions (Fig. 2A), and that this localisation depends upon *in* and *fy* activity (Fig. 2B,C), but not on the downstream-acting gene *mwh* (Fig. 2D). Frtz junctional localisation also required *stbm* activity (Fig. 2E), but was only reduced in *pk* mutants (Fig. 2F), consistent with the effects of loss of *pk* on the distribution of Stbm (Bastock et al., 2003). Similarly, in *fz* mutant cells away from clone borders, significant junctional localisation was retained (Fig. 2G). Fluorescent intensities of Frtz immunolabelling in the junctional regions of mutant tissue were quantitated. Interestingly, within *in* and *fy* tissue, levels were the same as within *frtz* tissue, indicating that junctional Frtz protein is undetectable. However, in *fz* tissue, fluorescent intensities were ~50% of wild-type levels, and, in *stbm* tissue, fluorescent intensities still achieved 25% of wild-type levels. This suggests that although Frtz is not noticeably localised to junctions in *stbm* tissue, the protein is nevertheless still present within the cell, supporting the contention that Stbm acts to localise Frtz activity but not necessarily to control its levels. (As our Frtz antibodies did not work in immunoblotting, we were unable to more directly assess these protein levels.)

These results indicate that Frtz, like In, localises proximally in a Stbm-dependent manner. We also attempted to determine the localisation of Fy using an EGFP-tagged form. We again saw preferential localisation to the apicolateral junctions, with enrichment at proximal cell edges (Fig. 2H). These results are consistent with In, Fy and Frtz all colocalising proximally and (at least in the case of In and Frtz) each requiring the activity of the other two to become localised.

mwh encodes a novel FH3-domain protein that is more strongly localised proximally in cells

The *mwh* locus has been mapped genetically to the cytological position 61E-F. As the most downstream known effector of core polarity gene function, we hypothesised that *mwh* might encode a protein that interacts directly with the cytoskeleton. Searching by gene ontology in FlyBase for 'cytoskeletal protein binding' revealed a single uncharacterised candidate gene, *CG13913*, in this region. A strain carrying a transgene that expresses an inducible RNAi hairpin against the *CG13913* transcript (Dietzl et al., 2007) closely phenocopied the *mwh* phenotype (Fig. 3B). Importantly, transgenic flies expressing the *CG13913* gene product fused to EGFP also showed complete rescue of the *mwh* phenotype (Fig. 3C). In addition, we isolated the predicted coding region of *CG13913* from mwh^{1} flies by PCR and determined that the locus has been subject to a rearrangement in which an inversion breaks the coding sequence after amino acid 367 (Fig. 3D). Taken together, this is good evidence that *mwh* corresponds to *CG13913* and also suggests that the mwh^{1} allele is likely to correspond to a null allele (see also Materials and methods).

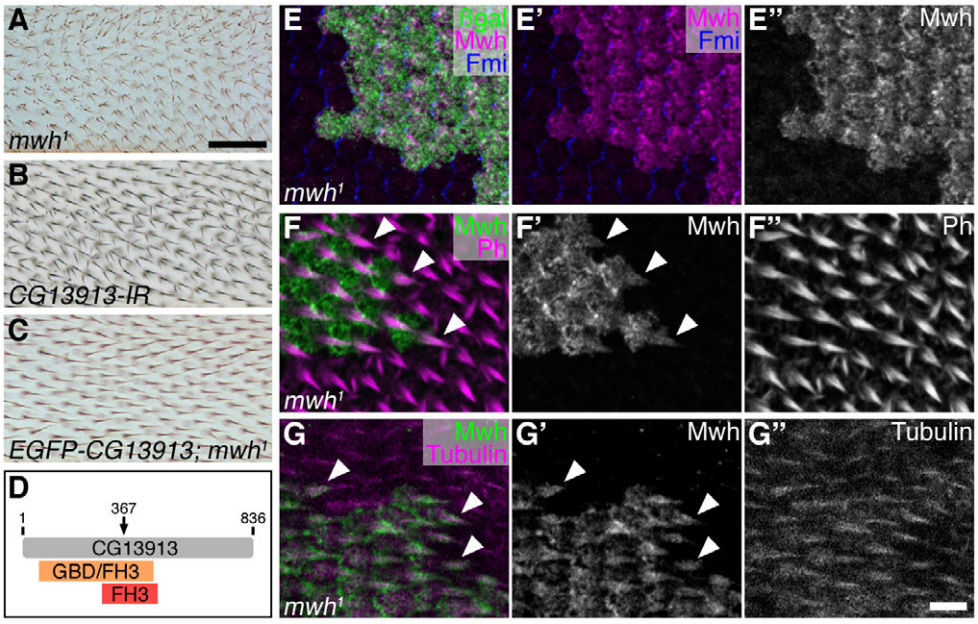

Fig. 3. Mwh is an FH3-domain protein that localises more strongly proximally in cells. Adult (A-C) or pupal (E-G″) *Drosophila* wings. (**A**) *mwh*^{1} adult wing between veins 3 and 4, showing trichomes with characteristic multiple wing hair phenotype. (**B**) Wing expressing *CG13913-IR* under control of *ptc-GAL4* between veins 3 and 4 showing a multiple wing hair phenotype. (**C**) *mwh*^{1} wing expressing EGFP-CG13913 from a *P{w+, ActP-EGFP-CG13913}* transgene showing rescue of the multiple wing hair phenotype. (**D**) Diagram of the CG13913 coding sequence, showing the extent of the Interpro GBD/FH3 (IPR014768) and FH3 (IPR010472) domains, and the point at which the inversion in *mwh*^{1} breaks the coding sequence (367, arrow). (**E-E″**) *mwh*^{1} clone in a 32-hour wing, labelled for Mwh (magenta), clonal marker (*lacZ*, green) and Fmi (blue). (**F-F″**) *mwh*^{1} clone in a 32.5-hour wing, labelled for Mwh (green) and actin (Ph, magenta). Arrowheads indicate trichomes in which Mwh does not colocalise strongly with actin bundles. (**G-G″**) *mwh*^{1} clone in 32.5-hour wing, labelled for Mwh (green) and α-tubulin (magenta). Arrowheads indicate trichomes in which Mwh does not colocalise with α-tubulin. Scale bars: 50 μm in A; 10 μm in G″.

Analysis of the *CG13913* coding sequence for known protein domains (Labarga et al., 2007) revealed the presence of the Interpro domains Diaphanous formin homology 3 (FH3) and GTPase-binding/formin homology 3 (GBD/FH3) (Fig. 3D). Formins are a class of proteins involved in actin nucleation that generally consist of three conserved domains known as the FH1, FH2 and GTPase-binding (GBD) domains (Wallar and Alberts, 2003). In addition, some formins also contain a further conserved domain known as the FH3 domain, which partly overlaps the GBD and is thought to be involved in subcellular localisation of the protein (Petersen et al., 1998; Kato et al., 2001). Homology searches identified homologues of Mwh only in insects, with the closest mammalian matches to the GBD/FH3 domain being found in conventional formins also containing an FH2 domain. However, a GBD/FH3 domain is present in the absence of FH2 domains in some *Dictyostelium* RasGEFs (Rivero et al., 2005). Thus, Mwh is a novel protein implicated in actin cytoskeleton regulation, but which lacks the functional domains normally found in formins that mediate actin nucleation.

We raised an antibody against Mwh and used it to determine the subcellular distribution of the protein. At around the time of prehair initiation, Mwh exhibited a punctate distribution in apical regions of the cell, which was stronger proximally and weaker distally (Fig. 3E; see Fig. S1A,B in the supplementary material). Interestingly, unlike In, Fy and Frtz, Mwh showed no direct colocalisation with core polarity proteins in the junctional region, although it was present in a similar apical plane. Mwh was also seen at uniformly low levels within growing prehairs, but did not strongly colocalise with either actin filaments or microtubules (Fig. 3F,G).

Mwh localisation and phosphorylation are regulated by *frtz*

Next we asked whether Mwh levels and distribution were regulated by *in*, *fy* or *frtz*. Interestingly, in all three genotypes, the apical punctate labelling of Mwh was dramatically reduced (Fig. 4A-C). By contrast, in *fz* and *stbm* clones, the proximal enrichment of Mwh was lost (consistent with loss of proximal Frtz localisation), but the levels of apical punctate labelling were not greatly altered (Fig. 4D,E; see Fig. S1C-F in the supplementary material).

To further investigate the effects of *frtz* on Mwh, we used our Mwh antibody for immunoblotting. In extracts from wild-type flies, the antibody detected a number of bands in the molecular weight range expected for Mwh (~91 kDa). Only a broad band migrating at ~110 kDa was lost in *mwh* mutants or *CG13913-RNAi* extracts, consistent with this representing the Mwh protein (Fig. 5A). Expression of EGFP-Mwh gave rise to bands ~20-30 kDa larger, reflecting the expected shift in molecular weight due to the tag. Extracts from *fz* and *stbm* flies showed negligible changes in Mwh levels, consistent with the immunolabelling results (Fig. 5A). However, loss of *frtz* activity resulted in a slight increase in Mwh gel-mobility, with the loss of a higher-molecular-weight form (Fig. 5A,C). We surmise that the higher-molecular-weight form constitutes the apical and proximally enriched punctate populations of Mwh seen by immunolabelling, as both were reduced in *frtz* cells.

A plausible explanation for the presence of the higher-molecular-weight form of Mwh is that Frtz promotes post-translational modification of Mwh. When wild-type protein extracts were treated with phosphatase (Fig. 5B), the slower-migrating form of Mwh was

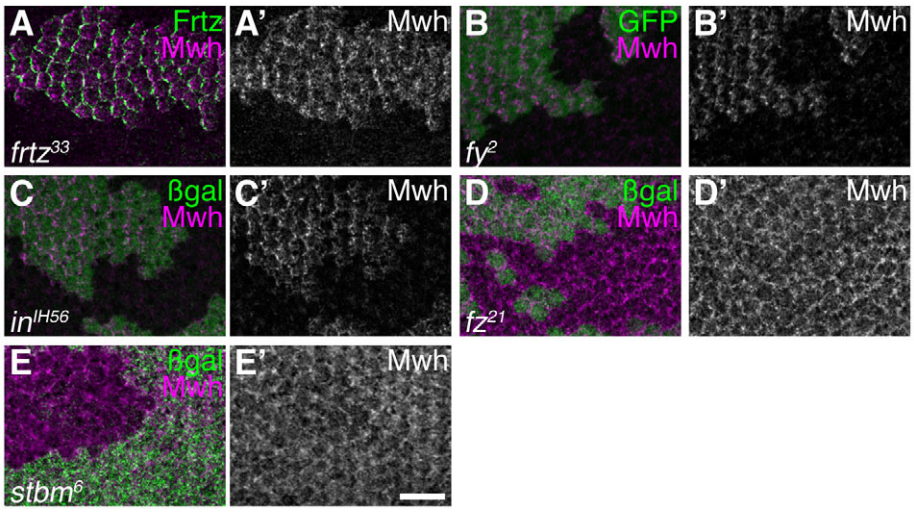

Fig. 4. Mwh localisation is regulated by *in*, *fy* and *frtz*. *Drosophila* 32-hour pupal wings immunolabelled for Mwh (magenta in A-E, white in A′-E′) and clonal marker (*frtz* expression, *GFP* or *lacZ*, green). (**A**,**A′**) $frtz^{33}$ clone. (**B**,**B′**) fy^{2} clone. (**C**,**C′**) in^{lH56} clone. (**D**,**D′**) fz^{21} clone. (**E**,**E′**) $stbm^{6}$ clone. Scale bar: 10 μm.

lost and an increase in gel-mobility similar to that seen upon loss of *frtz* was observed, consistent with Frtz promoting Mwh phosphorylation.

Interestingly, the *frtz*, *in* and *fy* null phenotypes increase in strength at lower temperatures (Adler et al., 1994; Collier et al., 2005). This could be explained if Mwh stability was temperature dependent. On immunoblots, we observed a dramatic reduction in Mwh levels in wings from animals raised at 18°C, as compared with 29°C (Fig. 5C). This was additive to the effects of *frtz* on Mwh gel-mobility, consistent with the cold-sensitivity of *in*, *fy* and *frtz* nulls being a result of reduced stability of Mwh.

mwh mutant cells show ectopic actin bundles across their apical surface

The earliest reported manifestation of the *mwh* phenotype is ectopic prehair initiation at the cell edge (Wong and Adler, 1993). Using fixation conditions optimised for preservation of F-actin structures, we re-examined the earliest stages of the *mwh* phenotype, looking in wings from animals raised at 18°C, 25°C and 29°C. A similar phenotype was seen at each temperature (Fig. 6A-D, and data not shown). We observed that prior to and during the appearance of prehair structures at the cell periphery, cells showed excess actin bundling across the apical surface of the cells, sometimes in a 'starburst' pattern, with actin bundles radiating from the cell centre (Fig. 6A,B). Subsequently, prehair structures were seen at cell edges, as previously reported. Interesting, the excess actin bundles often extended at least 1 μm basally into the cytoplasm from the apical surface, particularly once prehair initiation was underway (Fig. 6C,D). These results suggest that excess actin polymerisation across the apical surface of the cell is the primary defect in *mwh*, and that ectopic prehairs forming at cell edges might be a secondary consequence.

Mwh affects actin structures in cultured cells

From the molecular homology of Mwh with other proteins of known function, one can suggest that it could directly interact with the cytoskeleton or cytoskeletal modulators via its GBD/FH3 domain. However, the lack of other functional domains seen in formins might indicate that Mwh negatively influences actin filament formation, which would explain the unrestricted actin bundling seen across the apical surface of cells in its absence.

To gather more evidence for Mwh repressing actin filament formation, we transfected Mwh into cultured *Drosophila* S2 cells and assayed the effect on their actin cytoskeleton and behaviour. Intriguingly, cells expressing high levels of EGFP-Mwh showed an altered morphology compared with their contacting neighbours, characterised by a less rounded shape, the appearance of slender projections at the cell periphery and a reduction in F-actin bundles visible at the cell periphery (Fig. 6E). When we quantitated the reduction in fluorescent labelling intensity at the edges of transfected cells we found, on average, a 63% reduction. Furthermore, we observed that isolated transfected cells progressively developed a more dramatically altered morphology, with radially projecting slender extensions (Fig. 6F, present in 84% of transfected cells, compared with only 6% of control cells). This phenotype is reminiscent of the effects of reducing the activity of a number of proteins involved in actin dynamics, including the Arp2/3 complex, which is required for nucleation of actin filaments (Kiger et al., 2003; Kunda et al., 2003). Together, these results suggest that EGFP-Mwh overexpression can inhibit actin filament formation.

DISCUSSION

The site of prehair initiation is influenced by both proximal and distal cellular cues

Activity of the core planar polarity proteins is required in cells of the *Drosophila* pupal wing to specify prehair initiation at the distal vertex (Wong and Adler, 1993). Here we present evidence that core polarity protein localisation at both proximal and distal cell edges provides redundant cues for specifying distal prehair initiation.

Regarding the mechanistic basis of the proximal cue, this and previous work provide evidence for a plausible model (Fig. 7). The downstream effectors In, Fy and Frtz all colocalise at the proximal cell edge with Stbm and in a Stbm-dependent manner. Activity of In, Fy and Frtz is required for Mwh phosphorylation and its sub-apical subcellular localisation, which is thus concentrated towards the proximal side of the cell. Genetic studies have shown that loss of *fy*, *in*, *frtz* or *mwh* activity leads to excess prehair initiation (Wong and Adler, 1993; Collier et al., 2005), and we find that the initial defect in *mwh* mutant cells is excess actin bundling across the entire apical face of cells. Thus, proximal restriction of Mwh activity in the cell results in actin bundling and prehair initiation specifically in distal regions.

Additional evidence for the sufficiency of a Stbm-dependent cue for prehair initiation at opposite cell edges comes from experiments in the *Drosophila* abdomen (Lawrence et al., 2004). Here, it was reported that cells lacking *fz* activity, but juxtaposed to cells with *fz* activity, were able to produce polarised trichomes, as we also observed in the first row of cells within a *fz* clone in the wing.

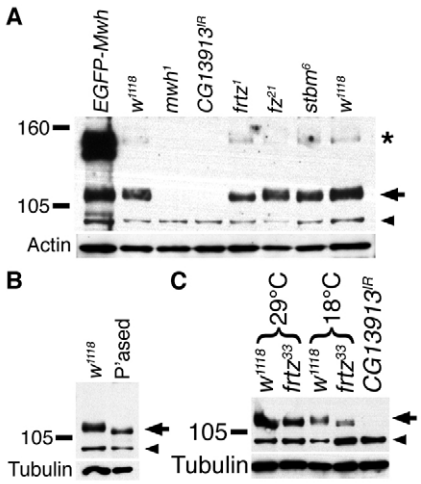

Fig. 5. Mwh gel-mobility is modified by *frtz* activity and phosphorylation. Immunoblots using anti-Mwh on extracts from *Drosophila* pupal wings of the indicated genotypes, with w^{1118} as wild-type control. Positions of molecular weight markers shown on left; arrow on right indicates Mwh signal; arrowhead points to non-specific band that is not altered in mutant extracts. Some extracts also show a weak higher-molecular-weight band which appears to be specific (asterisk), but which varies in intensity. Blots were reprobed for actin or tubulin as loading controls. (**A**) Extracts from 32-hour pupal wings at 25°C. (**B**) Extracts from 32-hour pupal wings at 25°C, either treated with the phosphatase inhibitor microcystin (control, w^{1118}) or with lambda phosphatase (P'ased). (**C**) Extracts from 28-hour pupal wings raised at 29°C or 64-hour pupal wings raised at 18°C (both equivalent to 32 hours at 25°C).

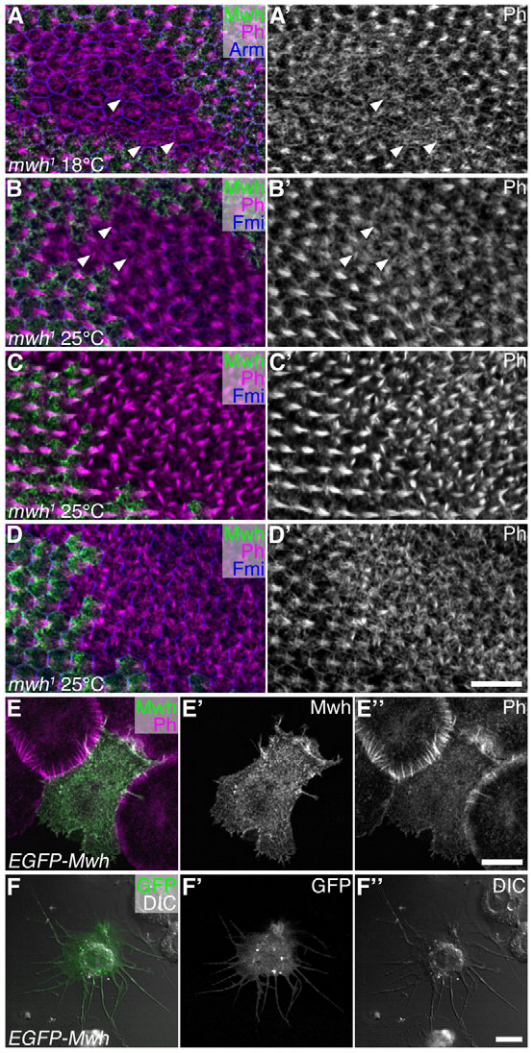

Fig. 6. Loss of *mwh* activity results in excess apical actin. *Drosophila* (A-D′) pupal wings immunolabelled for Mwh (green), actin (Ph, magenta or white) and Arm or Fmi (blue), and (E-F″) S2 cells transfected with EGFP-Mwh. (**A**,**A′**) mwh^1 clone in wing aged 65 hours at 18°C. Arrowheads indicate examples of cells with actin bundles radiating across the entire apical surface, prior to formation of distinct prehair structures. (**B**,**B′**) mwh^1 clone in wing aged 32.25 hours at 25°C. Prehair formation is more advanced than in the wing shown in A, but some cells still show actin bundles radiating across the entire apical surface (arrowheads). (**C**,**C′**) mwh^1 clone in wing aged 32.5 hours at 25°C. All mutant cells now showing multiple prehairs forming at the cell periphery. (**D**,**D′**) Same clone as in C, but sectioned ~1 μm deeper, showing excess actin bundles below the apical surface. (**E-E″**) S2 cell transfected with EGFP-Mwh (green), labelled for actin (Ph, magenta) and plated on Concanavalin A for 1 hour, shows altered morphology with projections extending from cell edges and a loss of actin bundles at cell periphery. (**F-F″**) DIC image of an S2 cell transfected with EGFP-Mwh (green) and plated on Concanavalin A for 24 hours, showing more extreme phenotype with long filopodia-like extensions around the cell periphery. Scale bars: 10 μm.

We have less information regarding the distal cue. Its existence is based upon two pieces of evidence. First, if prehair initiation were entirely dependent on Stbm-mediated localisation of Mwh activity, then prehairs should show no bias in their site of initiation in cells lacking *stbm* activity. In fact, *stbm* mutant cells with Fz localised at one cell edge show a strong bias towards initiating prehairs at that edge. Second, if prehair initiation were controlled only by a Stbm-dependent repressive cue, then in the absence of *stbm* activity, Fz would have no influence over prehair initiation. Instead, in a *stbm* background, *fz* activity still weakly promotes prehair formation. Taken together, these data support the view that distally localised Fz acts as a prehair-promoting cue.

A possible mechanism of action of the distal cue is that localised Fz might be able to repress Mwh activity in distal cell regions, possibly via its known effectors RhoA and Drok (Rho1 and Rok – FlyBase) (Strutt et al., 1997; Winter et al., 2001). Alternatively, Fz might promote prehair initiation in a Mwh-independent fashion, via RhoA/Drok or other effectors.

It is notable that the absence of *fz* activity results in a delay in prehair formation and in a greater tendency, compared with loss of *stbm*, for prehairs to form in the cell centre rather than towards a cell edge. We surmise that in *fz* mutant cells, there is no Fz-dependent prehair-promoting cue, and the Stbm-dependent repressive cue is evenly distributed around the cell edge, resulting in delayed prehair initiation in the cell centre. Conversely, in *stbm* mutant cells, there is no change in the activity of the repressive cue, but the Fz-dependent prehair-promoting cue is localised to cell edges, albeit more thinly spread than in the wild-type situation. This results in approximately normally timed prehair initiation near the cell edges.

An unexplained observation is that within *stbm* mutant tissue, the site of prehair initiation appears to be biased towards that seen in neighbouring cells. Thus, in the first rows of cells within a clone, prehairs tend to point towards the adjacent wild-type tissue. This phenomenon is presumably independent of core protein asymmetric

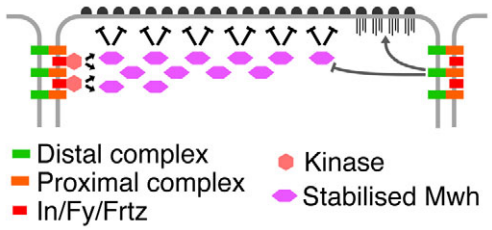

Fig. 7. Model for placement of prehair initiation in *Drosophila*. Diagram representing a *z*-section of apical regions of a cell, showing distal complexes (green) and proximal complexes (orange). The proximal complex recruits In, Fy and Frtz (red), which in turn recruit or activate an unknown kinase (pink) that modifies Mwh (magenta), which is consequently stabilised in apical/proximal regions. Mwh acts as an inhibitor of actin filament formation in electron-dense pimples on the surface of cells (dark grey), possibly by interfering with the activity of conventional formins. Distally in the cell, pimples become activated as a consequence of a lack of Mwh activity. The distal complex promotes pimple activation, either by repression of Mwh activity or via an alternative mechanism.

localisation and might depend upon some mechanical linkage between cells. In this context, there is already evidence to suggest that the microtubule cytoskeletons of adjacent cells are linked and that this could coordinate cell polarity (Turner and Adler, 1998). An alternative, core-protein-independent mechanism to align wing hairs that relies on the activities of Gliotactin and Coracle, has also been reported (Venema et al., 2004).

How is Mwh activity regulated?

Loss of *in*, *fy* and *frtz* results in a similar phenotype to loss of *mwh*, with multiple ectopic prehairs at the cell edge preceded by excess apical actin bundling (D.S., unpublished) (Wong and Adler, 1993). As In, Fy and Frtz are all required for the apical punctate distribution of Mwh within cells, and also appear to stabilise each other (this work) (Adler et al., 2004), this suggests that In, Fy and Frtz act together to activate Mwh and promote its apical localisation. Conversely, although Stbm plays a role in localising Mwh within the cell, it is not required for its activity, as loss of *stbm* does not phenocopy *mwh* mutants in which increased apical actin bundling is observed. This role of Stbm in localising but not regulating Mwh activity is most simply explained by Stbm acting to localise, but not regulate, the activity of In, Fy and Frtz. This is supported by the observation that whereas loss of *fz* or *stbm* has a strong effect on the distribution of Frtz to the apicolateral junctions, it has a negligible effect on the apparent phosphorylation state of Mwh.

The regulation of Mwh activity appears to be largely post-translational, as although the subcellular distribution of Mwh changes dramatically in *frtz* mutant cells, total levels of Mwh are not similarly altered. Further evidence that In, Fy and Frtz regulate Mwh activity by a mechanism largely independent of Mwh protein levels comes from the observation that Mwh overexpression in the wing has no effect on trichome formation (D.S., unpublished), rather than repressing trichome formation as might be predicted if Mwh protein levels were the main determinant of activity.

Our data strongly suggest that Mwh activity is regulated by phosphorylation. Treatment of cell extracts with phosphatase results in increased gel-mobility of Mwh. A similar increase in mobility is observed when *frtz* activity is removed, but not when *stbm* or *fz* activities are removed. Thus, at the least, Mwh phosphorylation correlates with Mwh activity and apical punctate localisation. Hence, we propose that the roles of In, Fy and Frtz might be to activate, or bring into proximity with Mwh, a kinase or kinases responsible for activating Mwh. Similarly, Fz could locally promote the dephosphorylation of Mwh to induce prehair initiation, although any such effect would have to be small, as Mwh phosphorylation is not obviously altered in the absence of Fz.

Definitive proof that phosphorylation of Mwh is important for its activity would require the identification of particular phosphorylation sites required for specific molecular functions and/or the identification of a kinase essential for Mwh activity.

An alternative regulatory mechanism for Mwh, by analogy with Diaphanous family formins, would be via RhoA GTPase activity (Wallar and Alberts, 2003). The FH2 domain of such formins promotes actin nucleation, an activity that is autoinhibited by interaction with the GBD. Upon interaction of the GBD with GTPase-bound Rho GTPases, this autoinhibition is released. Notably, genetic interaction data suggest that Fz/Dsh can activate RhoA activity (Strutt et al., 1997; Winter et al., 2001). This is consistent with a model whereby in the proximal cell, Rho GTPase activity is low and Mwh inhibits prehair initiation, whereas in the distal cell, activated RhoA alleviates the inhibitory activity of Mwh.

Notwithstanding our evidence for post-translational regulation of Mwh activity in the normal context of the pupal wing, in cultured cells we do see an effect of Mwh overexpression on the actin cytoskeleton. This seems likely to be due to the much higher levels of expression that can be achieved in transfected cells as opposed to cells in vivo, and hence the result should be treated with caution, but might nonetheless suggest that S2 cells express a factor able to constitutively activate Mwh.

Our results also indicate that Mwh levels are influenced by temperature, which provides a plausible explanation for why *in*, *fy* and *frtz* phenotypes are stronger at 18°C than at higher temperatures (Adler et al., 1994; Collier and Gubb, 1997; Collier et al., 2005). We suggest that loss of *in*, *fy* and *frtz* reduces Mwh activity and that lower temperatures additively reduce Mwh levels, resulting in lower overall Mwh activity.

What is the molecular function of Mwh?

As already noted, the FH3 domain of conventional formins is thought to be involved in targeting the protein to particular cellular sites, whereas the GBD domain is involved in inhibition of the actin-nucleating function of the FH2 domain (Wallar and Alberts, 2003). A plausible model is that Mwh acts as a dominant-negative by binding via its GBD domain to other FH2-domain-containing formins involved in the nucleation of actin filaments, thereby inhibiting their activity. Notably, this dominant-negative activity of Mwh could then be inhibited distally in the cell by Fz-mediated activation of RhoA GTPase activity.

Electron microscopy studies suggest that prior to prehair initiation, the apical cell surface is covered in electron-dense pimples that are normally only activated at the distal cell edge and serve as foci for actin filament formation (Guild et al., 2005). We propose that at around 32 hours of pupal development, cells receive a general signal for pimple activation that results in actin nucleation, and that Mwh activity is required to inhibit this activation at locations away from the distal cell edge.

The lack of direct vertebrate homologues of Mwh might indicate that in insects, the GBD/FH3 domain of a conventional formin has become separated from the rest of the molecule, but retained its function in inhibiting formin-mediated actin nucleation. Nevertheless, it is also plausible that the core polarity proteins would use similar regulatory mechanisms to promote local changes in

cytoskeletal structure in vertebrate cells as those employed in the *Drosophila* wing. Importantly, vertebrate homologues of both Fuzzy and Inturned have been shown to be involved in regulating apical actin assembly and, thus, in specifying the orientation of cilia (Park et al., 2006). By analogy with our findings, we suggest that core polarity proteins in vertebrates are likely to localise Fuzzy/Inturned activity within cells and to regulate formin activity via phosphorylation and/or Rho GTPase activation.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/18/3103/DC1

References

Adler, P. N., Charlton, J. and Park, W. J. (1994). The *Drosophila* tissue polarity gene *inturned* functions prior to wing hair morphogenesis in the regulation of hair polarity and number. *Genetics* **137**, 829-836.

Adler, P. N., Zhu, C. and Stone, D. (2004). Inturned localizes to the proximal side of wing cells under the instruction of upstream planar polarity proteins. *Curr. Biol.* **14**, 2046-2051.

Axelrod, J. D. (2001). Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signalling. *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.

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.

Collier, S. and Gubb, D. (1997). *Drosophila* tissue polarity requires the cell-autonomous activity of the *fuzzy* gene, which encodes a novel transmembrane protein. *Development* **124**, 4029-4037.

Collier, S., Lee, H., Burgess, R. and Adler, P. (2005). The WD40 repeat protein fritz links cytoskeletal planar polarity to frizzled subcellular localization in the *Drosophila* epidermis. *Genetics* **169**, 2035-2045.

Das, G., Jenny, A., Klein, T. J., Eaton, S. and Mlodzik, M. (2004). Diego interacts with Prickle and Strabismus/Van Gogh to localize planar cell polarity complexes. *Development* **131**, 4467-4476.

Dietzl, G., Chen, D., Schnorrer, F., Su, K. C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S. et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* **448**, 151-156.

Eaton, S., Wepf, R. and Simons, K. (1996). Roles for Rac1 and Cdc42 in planar polarisation and hair outgrowths in the wing of *Drosophila*. *J. Cell Biol.* **135**, 1277-1289.

Emery, G., Hutterer, A., Berdnik, D., Mayer, B., Wirtz-Peitz, F., Gaitan, M. G. and Knoblich, J. A. (2005). Asymmetric Rab 11 endosomes regulate delta recycling and specify cell fate in the *Drosophila* nervous system. *Cell* **122**, 763-773.

Gubb, D. and García-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* **68**, 37-57.

Guild, G. M., Connelly, P. S., Ruggiero, L., Vranich, K. A. and Tilney, L. G. (2005). Actin filament bundles in Drosophila wing hairs: hairs and bristles use different strategies for assembly. *Mol. Biol. Cell* **16**, 3620-3631.

Kato, T., Watanabe, N., Morishima, Y., Fujita, A., Ishizaki, T. and Narumiya, S. (2001). Localization of a mammalian homolog of diaphanous, mDia1, to the mitotic spindle in HeLa cells. *J. Cell Sci.* **114**, 775-784.

Kiger, A. A., Baum, B., Jones, S., Jones, M. R., Coulson, A., Echeverri, C. and Perrimon, N. (2003). A functional genomic analysis of cell morphology using RNA interference. *J. Biol.* **2**, 27.

Klein, T. J. and Mlodzik, M. (2005). Planar cell polarization: an emerging model points in the right direction. *Annu. Rev. Cell Dev. Biol.* **21**, 155-176.

Kunda, P., Craig, G., Dominguez, V. and Baum, B. (2003). Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. *Curr. Biol.* **13**, 1867-1875.

Labarga, A., Valentin, F., Anderson, M. and Lopez, R. (2007). Web services at the European bioinformatics institute. *Nucleic Acids Res.* **35**, W6-W11.

Lawrence, P. A., Casal, J. and Struhl, G. (2004). Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*. *Development* **131**, 4651-4664.

Lee, H. and Adler, P. N. (2002). The function of the *frizzled* pathway in the *Drosophila* wing is dependent on *inturned* and *fuzzy*. *Genetics* **160**, 1535-1547.

Mitchell, H. K., Roach, J. and Petersen, N. S. (1983). The morphogenesis of cell hairs on *Drosophila* wings. *Dev. Biol.* **95**, 387-398.

Park, T. J., Haigo, S. L. and Wallingford, J. B. (2006). Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat. Genet.* **38**, 303-311.

Park, W. J., Liu, J., Sharp, E. J. and Adler, P. N. (1996). The *Drosophila* tissue polarity gene *inturned* acts cell autonomously and encodes a novel protein. *Development* **122**, 961-969.

Petersen, J., Nielsen, O., Egel, R. and Hagan, I. M. (1998). FH3, a domain found in formins, targets the fission yeast formin Fus1 to the projection tip during conjugation. *J. Cell Biol.* **141**, 1217-1228.

Ren, N., He, B., Stone, D., Kirakodu, S. and Adler, P. N. (2006). The *shavenoid* gene of *Drosophila* encodes a novel actin cytoskeleton interacting protein that promotes wing hair morphogenesis. *Genetics* **172**, 1643-1653.

Rivero, F., Muramoto, T., Meyer, A. K., Urushihara, H., Uyeda, T. Q. and Kitayama, C. (2005). A comparative sequence analysis reveals a common GBD/FH3-FH1-FH2-DAD architecture in formins from *Dictyostelium*, fungi and metazoa. *BMC Genomics* **6**, 28.

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

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

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

Strutt, D. and Strutt, H. (2007). Differential activities of the core planar polarity proteins during *Drosophila* wing patterning. *Dev. Biol.* **302**, 181-194.

Strutt, D. I., Weber, U. and Mlodzik, M. (1997). The role of RhoA in tissue polarity and Frizzled signalling. *Nature* **387**, 292-295.

Strutt, H., Price, M. A. and Strutt, D. (2006). Planar polarity is positively regulated by casein kinase Iε in *Drosophila*. *Curr. Biol.* **16**, 1329-1336.

Tree, D. R. P., 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 signalling. *Cell* **109**, 371-381.

Turner, C. M. and Adler, P. N. (1998). Distinct roles for the actin and microtubule cytoskeletons in the morphogenesis of epidermal hairs during wing development in *Drosophila*. *Mech. Dev.* **70**, 181-192.

Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R. W., Schwarz, T. L., Takeichi, M. and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* **98**, 585-595.

Venema, D. R., Zeev-Ben-Mordehai, T. and Auld, V. J. (2004). Transient apical polarization of Gliotactin and Coracle is required for parallel alignment of wing hairs in *Drosophila*. *Dev. Biol.* **275**, 301-314.

Vinson, C. R. and Adler, P. N. (1987). Directional non-cell autonomy and the transmission of polarity information by the *frizzled* gene of *Drosophila*. *Nature* **329**, 549-551.

Wallar, B. J. and Alberts, A. S. (2003). The formins: active scaffolds that remodel the cytoskeleton. *Trends Cell Biol.* **13**, 435-446.

Wang, Y. and Nathans, J. (2007). Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development* **134**, 647-658.

Winter, C. G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J. D. and Luo, L. (2001). *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar polarity signalling to the actin cytoskeleton. *Cell* **105**, 81-91.

Wong, L. L. and Adler, P. N. (1993). Tissue polarity genes of *Drosophila* regulate the subcellular location for prehair initiation in pupal wing cells. *J. Cell Biol.* **123**, 209-221.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.