The WD40 Repeat Protein Fritz Links Cytoskeletal Planar Polarity to Frizzled Subcellular Localization in the Drosophila Epidermis

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ABSTRACT

Much of our understanding of the genetic mechanisms that control planar cell polarity (PCP) in epithelia has derived from studies of the formation of polarized cell hairs during Drosophila wing development. The correct localization of an F-actin prehair to the distal vertex of the pupal wing cell has been shown to be dependent upon the polarized subcellular localization of Frizzled and other core PCP proteins. However, the core PCP proteins do not organize actin cytoskeletal polarity directly but require PCP effector proteins such as Fuzzy and Inturned to mediate this process. Here we describe the characterization of a new PCP effector gene, fritz, that encodes a novel but evolutionarily conserved coiled-coil WD40 protein. We show that the fritz gene product functions cell-autonomously downstream of the core PCP proteins to regulate both the location and the number of wing cell prehair initiation sites.

During animal development epithelial cells develop molecular and morphological asymmetries that give them a cellular polarity. Conventionally, epithelial cell polarity is defined along two orthogonal axes, one through the thickness of the epithelium (apical-basal cell polarity) and the other along the length of the cell layer (planar cell polarity or PCP). Apical-basal polarization occurs in a single dimension and defines the top and bottom of the cell and consequently the inside and outside of the epithelial layer. In contrast, planar polarization occurs within a two-dimensional field and epithelial cells frequently align their planar polarity with neighboring cells to give the whole epithelium a specific tissue polarity.

Evidence that genetic mechanisms exist specifically to organize PCP in the Drosophila epidermis (Adler 2002; Tree et al. 2002a). A group of Drosophila gene mutations that results in an altered patterning of polarized epidermal structures, such as the sensory bristles (macrochaetes and microchaetes) and cell hairs (trichomes), has been defined (Gubb and Garcia-Bellido 1982). These phenotypes are indicative of changes in the planar polarity of the epithelial cells that produce these structures. Significantly, these cell polarity changes occur largely without affecting cell fate decisions or tissue morphogenesis.

Within this group a set of core PCP genes has been identified that not only controls the orientation of bristles and cell hairs but also controls ommatidial polarity in the eye and tarsal joint specification in the leg. The core PCP genes include frizzled (fz), dishevelled (dsh), prickle (pk), starry night (stan also called flamingo), van gogh (vang also called strabismus), and diego (dgo). There is also a group of planar polarity effector genes that includes fuzzy (fy), inturned (in), and multiple wing hairs (mwh), which are also required for the normal orientation of bristles and cell hairs but do not have substantial roles in ommatidial or tarsal joint development.

Much of our understanding of the genetic control of PCP in Drosophila has come from studies of wing cell hair development. Each wing cell produces a single hair that points toward the distal tip of the wing. The formation of this cell hair is initiated by the accumulation of F-actin at the distal vertex of the hexagonal pupal wing cell to form an actin-rich prehair (Wong and Adler 1993). Recent studies have shown that prior to prehair formation the core PCP proteins localize within the pupal wing cell to the distal edges (Frazzled, Dishevelled, and Diego) (Axelrod 2001; Strutt 2001; Das et al. 2004), the proximal edges (Prickle and Van Gogh) (Tree et al. 2002b; Bastock et al. 2003), or to both the distal and the proximal edges (Starry night) (Usui et al. 1999). In core PCP gene mutants the core PCP proteins fail to localize normally (McNeill 2002; Strutt 2002) and F-actin accumulation and prehair formation occurs at the apical center of the pupal wing cell rather than at the periphery, resulting in an abnormally oriented cell hair (Wong and Adler 1993). Therefore, it appears that the appropriate subcellular localization of...
the core PCP proteins is required to ensure the correct site of prehair initiation and cell hair polarity.

Several experiments have shown that the PCP effector genes act downstream of the core PCP genes in wing hair development. First, an analysis of epistatic interactions placed the PCP effector genes downstream of the core PCP genes in a regulatory pathway controlling the site of prehair initiation (Wong and Adler 1993). Second, temperature-shift experiments have shown that the PCP effector gene in is required later than the core PCP gene fz in wing hair development (Adler et al. 1994). Third, both fy and in gene mutations have been shown to block core PCP gene gain of function phenotypes (Lee and Adler 2002). It has also been found that the core PCP protein Stan localizes normally in a moh mutant (Usui et al. 1999) and that Fz localizes normally in an in mutant background (Strutt 2001), implying that core PCP protein localization is independent of PCP effector gene function. However, despite the normal localization of the core PCP proteins, prehairs in PCP effector gene mutants form at aberrant sites around the apical periphery of the pupal wing cell and abnormal cell hair polarity results (Wong and Adler 1993). It appears, therefore, that the primary role of the PCP effector proteins in wing cell planar polarity is to link the site of F-actin accumulation and prehair formation with the polarized distribution of the core PCP proteins.

In PCP effector gene mutants, F-actin frequently accumulates at multiple sites on the pupal wing cell periphery, resulting in the production of multiple cell hairs (Wong and Adler 1993). This implies that the PCP effector proteins also have a role in restricting the number of sites of prehair initiation within the developing wing cell. This function is largely independent of the core PCP proteins as core PCP mutants display very weak multiple wing cell hair phenotypes. However, cytoskeletal regulators such as the small GTPases RhôA, Rac, and Dcde-42, and Rho kinase also appear to play a role in restricting prehair initiation site number as loss-of-function or dominant-negative phenotypes include multiple wing cell hairs (Eaton et al. 1996; Strutt et al. 1997; Winter et al. 2001). Other genes implicated in the control of wing hair initiation sites include furry (Cong et al. 2001) and the Drosophila NDR kinase tricornered (Geng et al. 2000).

In this article we report the identification and characterization of frtz (frtz), a new PCP effector gene. frtz encodes a novel but evolutionarily conserved coiled-coil WD40 protein that functions cell-autonomously downstream of the core PCP proteins and is required for normal wing cell hair polarity and number.

MATERIALS AND METHODS

Phenotypic analysis: All flies were raised at 25° unless indicated otherwise. Adult wings were washed with isopropanol and mounted in GMM (1:1 Canada balsam:methyl salicylate or Euparol. Adult wing clones were produced by X-ray irradiating f^{iso}[Y; f^{+} 30B] ek^{+} pr^{+} par4/frtz^{1} b pr en ha larvae with 1000 R at 48–72 hr old, and homozygous frtz clones were identified by the forked wing hair phenotype. Cuticle preparations of first instar larvae were made by mounting newly hatched larvae directly in Hoyer’s medium and incubating the slides overnight at 60°.

Molecular characterization of mutant frtz alleles: Homozygous or hemizygous frtz mutant genomic DNA was PCR amplified between frtz gene-specific oligonucleotide primers and the PCR products sequenced from oligonucleotides on the frtz sense strand. Nucleotide sequences were compared to wild type using the BDGP BLASTn server and between differences analyzed for potentially deleterious mutations (Table 1). Putative neutral polymorphisms occurring in two or more independent frtz alleles were Iso411Val (frtz, frtz, and frtz), Asp542Asn (ftz and frtz), Pro720Thr (ftz, ftz, ftz, ftz, frtz, and frtz), and Pro776Thr (ftz, ftz, ftz, ftz, and frtz).

Analysis of pupal wing phenotypes: We examined the frtz pupal wing phenotype in both completely mutant wings and somatic clones. Similar results were obtained by both approaches. Pupal frtz clones marked by a loss of GFP were generated by heat-shocking w hs-flp; + Ubi-GFP FRT40/frtz FRT40 larvae and collecting white prepupae, followed by dissection and fixation at desired times. To determine whether frtz was required for the asymmetric accumulation of Fz, we examined GFP in frtz; arm-fz-GFP pupal wings. To determine whether frtz was required for the asymmetric accumulation of Dsh or Stan, we stained frtz mutant wings using antibodies provided by T. Uemura. Pupal wings of the desired age were fixed in 4% paraformaldehyde PBS and then stained using standard procedures (Lee and Adler 2004). As cytoskeleton probes we used fluorescently labeled phalloidin (actin cytoskeleton) and antitubulin antibody (Sigma, St. Louis). Fluorescently labeled secondary antibodies were purchased from Molecular Probes (Eugene, OR). In most experiments we used direct visualization of GFP but on a few occasions we amplified the GFP signal using a rabbit polyclonal anti-GFP antibody (Molecular Probes). Samples were mounted using Prolong (Molecular Probes) and examined on either a Nikon laser scanning confocal microscope at the Keck Center for Cellular Imaging or a Nikon TE200 microscope equipped with an ATTO-CARV spinning disc confocal run by the Metamorph software package. Some images were deconvolved using AutoDeblur (AQI). Figures for the article were assembled using Adobe Photoshop.

Interaction of core PCP genes with frtz: To determine whether frtz was required for the gain-of-function phenotypes that result from the directed expression of planar polarity genes, we generated flies that were mutant for frtz and that overexpressed one of the planar polarity genes using a Gal4 driver and the relevant UAS transgene. To determine whether frtz was required for cells to respond to the presence of a clone of cells that lacked fz function, we generated w hs-flp; frtz/ftz; fz trc FRT80/FRT80 flies and heat-shocked the larvae to induce clones. The clones could be identified by the strong multiple hair phenotype of tricornered (trc). As a control, we used a similar strategy to induce clones of cells mutant for trc in a frtz mutant background. In this experiment we used the null fz allele fz.

RESULTS

The frtz mutant phenotype is cold sensitive and cell autonomous: The frtz phenotype is strikingly similar to the phenotypes of the PCP effector genes fy and in (Wong and Adler 1993; Adler et al. 1994; Collier...
and Gubb 1997). For this reason we have also classified fritz as a PCP effector gene. The bristles, both macrochaetes and microchaetes, of the adult notum and abdomen of fritz mutants have an altered orientation and usually point toward the midline (Figure 1, B and D) rather then posteriorly as in wild type (Figure 1, A and C). The bristles of the triple row on the anterior wing margin of fritz mutants point more anteriorly than those of wild type and follow the local polarity of cell hairs (see Figure 2, A and B).

The trichomes or cell hairs of the fritz mutant adult cuticle show altered, but reproducible, patterns of orientation. The majority of hairs on fritz mutant wings posterior to the L3 vein point more posteriorly than those of wild type and those anterior to the L3 vein point more anteriorly. A similar pattern has been described

**Figure 1.**—(A–D) Macrochaete and microchaete orientation is altered in fritz mutants. (A) Notum of wild type (Oregon-R) fly. (B) Notum of fritz33/Df fly raised at 18°. (C) Dorsal abdomen of wild type (Oregon-R) fly. (D) Dorsal abdomen of fritz33/Df fly raised at 18°. Anterior is uppermost. fritz mutant bristles point toward the midline rather than posteriorly. (E–H) Cell hair polarity and number are altered on a fritz mutant wing. Photomicrographs are of the “C” cell region of wing immediately anterior to the posterior cross-vein (PCV). The proximal-distal axis of the wings runs from left to right. Dorsal wing surface of (E) wild type (Oregon-R), (F) fritz33/Df raised at 18°, and (G) fritz33/Df raised at 29°. Note that the fritz phenotype is weaker at higher temperature. (H) Ventral surface of fritz33/Df wing at 18°. Most cells displaying reversed polarity have wild-type hair number.

**Figure 2.**—(A and B) Adult wing hairs emerge from the apical center of the cell in both wild-type and fritz mutants. (A) Anterior wing margin of m36/Y mutant. (B) Anterior wing margin of m36/Y; fritz1/fritz1 mutant. The arrowhead indicates a fritz mutant cell producing two centrally located cell hairs. Note that margin bristle polarity follows local cell hair polarity. (C and D) The fritz wing cell hair phenotype is cell autonomous. (C) Small (~60 cells) f36a; fritz33/fritz33 clone in the “D” region of a f36a, P[+]/30B/fritz33 wing. Proximal is to the left, distal to the right. Note the presence of fritz homozygous mutant cells carrying two cell hairs at the edge of the clone and the more posterior orientation of cell hairs within the clone. (E and F) Organization of larval denticles is disrupted in fritz mutants. Dentine belt A7 of cuticle preparations of L1 larvae of (E) wild-type (Oregon-R) and (F) homozygous fritz33 mutants; anterior is at the top. Dentine rows are disrupted in fritz mutants and individual denticles often appear smaller.
for the core PCP mutants and other PCP effector mutants (Gubb and Garcia-Bellido 1982; Wong and Adler 1993; Collier and Gubb 1997; Taylor et al. 1998; Chae et al. 1999) and can be regarded as the “default” PCP mutant pattern. All frtz alleles, with the exception of frtz, have a cold-sensitive phenotype (Table 1) with mutant flies raised at 18° showing more dramatic alterations in wing hair polarity than those cultured at 25° or 29° (Figure 1, F and G). A similar conditional sensitivity has been described for strong alleles of the PCP effector genes fy and in (Adler et al. 1994; Collier and Gubb 1997) and is also true of mwh alleles. Changes in wing cell hair polarity in strong frtz mutants raised at 18° are more profound than those for loss-of-function core PCP gene mutations and display substantial regions of reversed (proximal pointing) hair polarity, especially on the ventral wing surface (Figure 1H).

frtz mutant wings are also characterized by a high degree of cell autonomy with respect to both cell polarity and cell hair number. No substantive changes in hair polarity are seen outside of frtz homozygous mutant clones, but even small frtz clones can display hair polarity phenotypes (Figures 2C and 3E). The change of cell hair polarity seen within frtz clones is similar to that at the same position on a frtz homozygous mutant wing. Cells at the edges of homozygous frtz clones often produce additional hairs, whereas cells surrounding frtz homozygous clones never do (Figures 2C and 3, D and E). Similar cell autonomy is shown by the other mutations in other PCP effector genes (Gubb and Garcia-Bellido 1982; Adler et al. 1994; Collier and Gubb 1997) although weak nonautonomy has described for in clones (Park et al. 1996).

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**TABLE 1**

Molecular and phenotypic characteristics of extant frtz alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Original name</th>
<th>Mutagen</th>
<th>Source</th>
<th>Phenotype</th>
<th>Mutation</th>
<th>Protein product</th>
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<td>frtz1</td>
<td>EMS</td>
<td>Lancaster</td>
<td>Strong</td>
<td>TAT &gt; TAA in exon 3</td>
<td>Tyr506 &gt; STOP</td>
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<td>AAA &gt; TAA in exon 3</td>
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<td>Cambridge</td>
<td>1329-nt deletion upstream from nt -11 (see Figure 5A)</td>
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<td>Virginia</td>
<td>Strong</td>
<td>N-terminal 493 + 7 novel aa</td>
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EMS, ethyl methanesulfonate; nt, nucleotide; ND, not determined.
Our RT-PCR and in situ hybridization experiments have shown that frtz is expressed during embryogenesis and that frtz transcripts are most abundant in the embryonic epidermis (data not shown). To investigate possible roles for frtz in patterning the embryonic epidermis, cuticle preparations of first instar (L1) larvae from homozygous stocks of the strong alleles frtz1 and frtz33 (Table 1) were made. We found that frtz mutant L1 larvae show abnormal patterning of ventral denticles. In wild-type larvae, rows of evenly spaced denticles of a common orientation (either anteriorly or posteriorly pointing) form the denticle belts (Figure 2E). In frtz mutant larvae, both the spacing and the alignment of denticles within the denticle rows is disrupted especially in the three anterior rows of the belts (Figure 2F). Significantly, frtz mutant denticles still point either anteriorly or posteriorly as in wild type. Individual frtz mutant denticles can also appear smaller or stunted compared to wild type. Similar denticle phenotypes have been reported in mah mutant larva (Dickinson and Thatcher 1997). The PCP effector genes fy and in are also known to be expressed in the embryo (Park et al. 1996; Collier and Gubb 1997) and we have seen similar denticle phenotypes in L1 larvae of strong fy and in mutants (data not shown). In contrast, it has been reported that alleles of the core PCP genes, e.g., frizzled1 (Dickinson and Thatcher 1997) and prickleda12 (Gubb et al. 1999), do not affect embryonic denticle structure or organization.

**frtz functions downstream of the core PCP genes:** The similarity of PCP mutant polarity patterns (see above) means that epistatic analysis of double mutants is not conclusive for polarity defects (Wong and Adler 1993). However, the directed expression of core PCP genes can produce polarity patterns that are distinctly different from the loss-of-function patterns. To determine whether frtz was required for the directed expression of the core PCP genes to alter hair polarity, we expressed different core PCP genes in a frtz mutant background using the Gal4 UAS system. When the expression of spiny-legs (sple) is driven relatively evenly across the wing using the actin-GAL4 driver, hair polarity is largely reversed. This dramatic gain-of-function phenotype was blocked in wings simultaneously mutant for frtz (Figure 4). We also used omeg-GAL4 to drive the expression of UAS-stan. In the wing disc, omeg-GAL4 drives expression in a band located centrally along the anterior/posterior axis. In the pupal wing, the expression pattern is more complex and in the distal region of the wing it consists of bands of expression and lack of expression. This leads to bands of polarity changes. This phenotype was also blocked in wings mutant for frtz (Figure 4). Similar results were obtained in analogous experiments using the expression of fz and pk (data not shown). These results are equivalent to those obtained previously for in and fy (Lee and Adler 2002).

The presence of a clone of cells that lacks fz function results in neighboring cells responding and producing hairs that appear to be attracted to the clone (Vinson and Adler 1987). Previous experiments have shown the formation of denticles only weakly affected by frtz located in the mid-distal part of the “C” cell. This region is different core PCP genes in a frtz mutant background due to the asymmetric accumulation of Dsh in the stereotypic zig-zag pattern. Note also that the zig-zag pattern of Fz accumulation is present. (C) A frtz mutant wing stained with an anti-Dsh antibody. Once again note the asymmetric accumulation of Dsh in the stereotypic zig-zag pattern. (D and E) frtz clones marked by the loss of GFP. In D the clone was located in the mid-distal part of the “C” cell. This region is only weakly affected by frtz and the clone shows a corresponding weak phenotype. The clone in E was located more proximal in a region that has a strong frtz phenotype. Note the corresponding strong phenotype in the clone, that no wild-type cells differentiate in an abnormal way, and that mutant clone cells juxtaposed to wild-type cells can show a mutant phenotype.

**frtz is required for embryonic denticle organization:** Our RT-PCR and in situ hybridization experiments have shown that frtz is expressed during embryogenesis and...
that the ability of cells to respond to such a clone requires the function of both planar polarity genes, such as Vang and stan, and PCP effector genes, such as in and fy (Taylor et al. 1998; Chae et al. 1999; Lee and Adler 2002). To determine whether frtz was also required for cells to sense or to respond to a clone of fz mutant cells, we induced clones of fz cells marked with the multiple hair cell marker trc in flies mutant for frtz. As a control we first induced trc clones in a frtz mutant background and found that it was easy to identify the mutant cells on the basis of their hair phenotype (see Figure 4). In regions of frtz mutant wings where hair polarity is reproducible, albeit abnormal, we examined cells surrounding fz trc clones and saw no evidence for the clone acting nonautonomously. Hence, frtz, like in and fy, is required for cells to sense or to respond to a clone of cells lacking fz function (Lee and Adler 2002).

Mutations in other PCP effector genes are known to not block the asymmetric accumulation of core PCP proteins such as Fz, Dsh, Stan, etc. (Usui et al. 1999; Strutt 2001). To determine if this was also the case for frtz, we examined the location of Fz, Dsh, and Stan in frtz mutant wings. All three of these proteins localized asymmetrically (Figure 3, B and C; data not shown for stan) in frtz mutant wing cells as is the case for in and fy mutant wings. This is consistent with frtz functioning downstream of the core PCP genes.

**Mapping of the frtz locus:** There have been three independent isolations of frtz alleles: the frtz allele was recovered from an EMS screen by Allan Shiras at the University of Lancaster, Lancaster, United Kingdom; the frtz allele was identified during a screen for new ph mutants at the University of Cambridge, Cambridge, United Kingdom; and the frtz allele [originally fuzzy’s twin (fyt)] was recovered from an F1 FLP-FRT EMS screen for wing hair phenotypes at the University of Virginia, Charlottesville, Virginia. Seven additional frtz alleles have been recovered from F1 X-ray screens at the University of Cambridge and the University of Virginia (Table 1).

The frtz locus is uncovered by both Df(2L)S2 (21C8-D1;22A8-B1) and Df(2L)dp-79b (22A2-3;22D5-E1), placing it within the cytological region 22A2-3–22A8-B1. This localization is supported by the cytology of deficiencies recovered from an X-ray screen for new frtz mutants (Table 2). frtz is also uncovered by the small deletion Df(2L)F.1. Genetically, the distal breakpoint of Df(2L)F.1 is proximal to the capping protein beta (cph)

**Figure 4.—Distal is to the right in all photomicrographs.** (Top) Micrographs of the dorsal surface of the wing just anterior to the posterior cross-vein. In the frtz mutant, many multiple hair cells tend to point posteriorly. The overexpression of sple driven by actin-Gal4 leads to hairs in this region pointing proximally and anteriorly. This gain-of-function phenotype is blocked in wings simultaneously mutant for frtz. (Middle) Images from the posterior distal region of the wing (“D” cell). In this region of a frtz mutant, wing hairs point posteriorly and a substantial number of cells produce multiple hairs. When omb-Gal4 is used to drive expression of stan, this region of the wing hairs points anteriorly and multiple hair cells are rare. Once again the double mutant shows the frtz mutant phenotype. The requirement for the frtz function of cells to respond to a clone of cells lacking fz function is shown at the bottom. All images are from the posterior region of the wing (“E” cell). A fz trc mutant clone in an otherwise wild-type wing results in the typical fz domineering nonautonomy. In a frtz mutant wing this is not seen. A control trc clone in a frtz mutant wing is also shown. The trc multiple hair phenotype is not suppressed by a frtz mutant.

**TABLE 2**

<table>
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<td>22A2.3;B1</td>
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<td>X ray</td>
<td>Cambridge</td>
<td>22A3.4;B1</td>
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<td>Cambridge</td>
<td>22A3.4;C1.2</td>
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locus (M. Welte, unpublished data) and we have molecularly mapped the proximal endpoint of Df(2L)F.1 to between the P(lacW)k09624 insertion site and the distal break of a transposition event on the frtz chromosome within transcript CG17646 (position 1722500 on the GadFly annotated genome map). We screened frtz homozygous or hemizygous mutant genomic DNA by PCR to identify rearrangements or point mutations within seven of the eight candidate genes in this interval (CG17660, CG17642, CG17657, CG18317, CG17711, CG17652, and CG17646). The gene Eno (CG17654), which encodes a phosphopyruvate hydratase, was not considered a strong candidate for a PCP gene. We have identified putative deleterious mutations at the CG17657 locus on nine independent frtz mutant chromosomes (Table 1). We were able to confirm that these mutations were not present on the progenitor chromosome (or on an independent mutant chromosome from the same screen) for the seven alleles where these chromosomes were available, establishing that CG17657 is the frtz gene.

**frtz encodes an evolutionarily conserved WD40 repeat protein:** The frtz transcript encodes a polypeptide of 951 amino acids with a predicted molecular weight of 106 kDa. A single homologous protein is encoded by the human (Homo sapiens), mouse (Mus musculus), puffer fish (Fugu ruprisses), and mosquito (Anopheles gambiense) genomes. The N-terminal 620 amino acids of fruit fly Frtz shares 30% amino acid identity with the mammalian Frtz proteins, which is evenly distributed over this region (Figure 5B). However, the next 320 amino acids of the fly Frtz protein are not conserved in the mammalian proteins. This unique region of the fly protein contains 14.5% proline and is predicted to fold as a random coil. The equivalent region of the mammalian Frtz proteins is shorter and not significantly proline rich but is remarkably diverged in sequence between mouse and human, showing just 45% identity compared with 82% for the rest of the protein. Despite this high degree of evolutionary variability, the nonconservative substitution of a lysine by a methionine in this region encoded by the frtz allele is associated with a strong frtz phenotype (Table 1). This suggests a functional constraint on this part of the Frtz protein that is either specific to planar polarity in Drosophila or not dependent on overall amino acid sequence conservation. The fly, mouse, and human Frtz proteins share a highly conserved hydrophobic 10-amino-acid peptide at the extreme C terminus of the protein (Figure 6C).

The Frtz protein is predicted by the COILS program (Lupas et al. 1991) to have a short N-terminal coiled-coil region consisting of five heptad repeats. The phase of the Frtz coiled-coil region and its alignment within other Frtz proteins is shown in Figure 6A. Coiled-coil regions are known to mediate protein multimerization. The MultiCoil program (Wolf et al. 1997) predicts that the fly Frtz coiled-coil forms a trimeric structure although the equivalent region in human Frtz is predicted to form a dimer.

A single WD40 repeat is strongly predicted in the fly Frtz protein by the SMART annotation tool (Schultz et al. 1998). SMART also predicts a WD40 repeat at an equivalent position in both human and mouse Frtz proteins with a second WD40 repeat immediately C-terminal to it. WD40 repeats fold together to form a β-propeller structure that provides surfaces for protein-protein interaction (Smith et al. 1999). The alignment of the Frtz WD40a and WD40b repeats from all of the Frtz sequences currently available is shown in Figure 6B. The sequences of the two Frtz WD40 repeats are unusual as
A  Coiled Coil Region

\[
\begin{align*}
\text{Frtz:} & \quad 68-\text{TGLKDLKLELDRQRHHRIBSWQDAQVLLEF-102} \\
\text{aFrtz:} & \quad PWKLYKLLELDRQRHYVQCVNHVSLQCDLML \\
\text{mFrtz:} & \quad PEKLDLLEELRQRHYVQCVNHVSLQCDLML \\
\text{hFrtz:} & \quad PEKLDLLEELRQRHYVQCVNHVSLQCDLML \\
\text{Heptad repeats:} & \quad abcdedfbcdefabcdefgabcdefgabcdedf \\
\end{align*}
\]

\[
\begin{align*}
\text{Frtz WD40a} & \quad 296-\text{QRTAITSIPQGAGIQSCSFAPSFQDKLFLSVDNRLCHD-334} \\
\text{aFrtz WD40a} & \quad RIVAVSTIPLQQCVCAADSDLFEEKLILGDSVVDLD \\
\text{fFrtz WD40a} & \quad HRLSVTVPLSNFSVCRHRPGTALLLGLDSSSLVLD \\
\text{mFrtz WD40a} & \quad HGVSVTTIPRLSKASVRNSTDRELVCDGDSVLYE \\
\text{hFrtz WD40a} & \quad QCVSVTTIPRLSKASVRNSTDRELVCDGDSVLYE \\
\text{Strand} & \quad \text{Strand a} \quad \text{Strand b} \quad \text{Strand c} \\
\end{align*}
\]

\[
\begin{align*}
\text{Frtz WD40b} & \quad 336-\text{QQQSTYANKQIERVPQCAWCDASMCLCVANERSVQLCFD-375} \\
\text{aFrtz WD40b} & \quad GRGZGHLV-KRAAPFTVAWHESSVYMINENKQLCFD \\
\text{fFrtz WD40b} & \quad RRGVSLSLA-SCPVKLWVWAHPAGMNVLGIGGQVLCFD \\
\text{mFrtz WD40b} & \quad HHRVLQSS-QLEPSLISCHSGAQLLGVSNQQLCPFD \\
\text{hFrtz WD40b} & \quad HHRVLQSS-QLEPSLISCHSGAQLLGVSNQQLCPFD \\
\text{Strand} & \quad \text{Strand a} \quad \text{Strand b} \quad \text{Strand c} \\
\end{align*}
\]

\[
\begin{align*}
\text{Frtz} & \quad 942-\text{SIIKUHFGV-COOH} \\
\text{aFrtz} & \quad LRMWHFGVL-COOH \\
\text{hFrtz} & \quad SLWKHHFGV-COOH \\
\end{align*}
\]

B  WD40 Repeats

both lack the highly conserved histidine in the loop between the propeller blades (between strands d and a) and WD40b also lacks the conserved aspartate normally present in the tight turn between strand b and strand c.

A \textit{frtz} WD40b mutation interacts synergistically with hypomorphic \textit{fuzzy} and \textit{inturned} alleles: The importance of the second Frtz WD40 repeat (WD40b) for the stability or function of the Frtz protein is demonstrated by the \textit{frtz}^{29} allele, which encodes a protein that lacks the C-terminal 24 amino acids of WD40b and the adjacent 10 C-terminal amino acids and has a strong \textit{frtz} phenotype (Table 1). At the permissive temperature of 18° the wing hair phenotype of homozygous or hemizygous \textit{frtz}^{2} flies is close to wild type (Figure 7B). Such a weak phenotype is surprising as the terminal Frtz WD40b aspartate is absolutely conserved in both vertebrate and invertebrate Frtz proteins (Figure 6B). However, an alanine is present at the C terminus of Frtz WD40b (Figure 7A), is associated with only a weak hypomorphic temperature-sensitive phenotype. At the permissive temperature of 18° the wing hair phenotype of homozygous or hemizygous \textit{frtz}^{2} flies is close to wild type (Figure 7B). Such a weak phenotype is surprising as the terminal Frtz WD40b aspartate is absolutely conserved in both vertebrate and invertebrate Frtz proteins (Figure 6B). However, an alanine is present at the C terminus of ~2% of WD40 repeats (Yu et al. 2000), suggesting that it does not preclude the formation of a standard WD40 β-propeller structure. At 29° homozygous or hemizygous \textit{frtz}^{2} flies show moderate changes in hair polarity and cell hair number (Figure 7C), similar to strong cold-sensitive \textit{frtz} mutants at this temperature (Figure 1G). It appears, therefore, that the \textit{frtz}^{3} gene product is almost completely active at 18° and almost completely inactive at 29°.

The \textit{frtz}^{3} allele shows strong synergistic interactions with the hypomorphic PCP effector gene alleles \textit{in}^{93} and \textit{fy}^{*}. The \textit{in}^{93} allele has a temperature-sensitive phenotype that is strikingly similar to \textit{frtz}^{3} (Adler et al. 1994) (Figure 7D) and encodes a mutant protein with an additional nine C-terminal amino acids due to a point mutation in the normal termination codon. The \textit{fy}^{*} allele is associated with a mild abdominal bristle phenotype but type and shows temperature sensitivity. The combination of the two alleles at the same temperature appears to completely block PCP effector gene function. The \textit{frtz}^{3}, \textit{fy}^{*} allele combination also shows a strong synergistic interaction at 18° (Figure 7G) although the \textit{frtz}^{3}, \textit{fy}^{*} phenotype is weaker than the \textit{frtz}^{3}; \textit{in}^{93} phenotype and shows temperature sensitivity.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{Evolutionary conservation of Frtz protein domains. (A) Alignment by homology of the predicted N-terminal coiled-coil regions in Frtz proteins from fruit fly (Frtz), mosquito (aFrtz), mouse (mFrtz), and human (hFrtz). The phase of the heptad repeats is indicated below; the a and d (in boldface type) residues are conventionally hydrophobic. (B) Alignment by homology of the Frtz WD40a and WD40b repeats from fruit fly (Frtz), mosquito (aFrtz), puffer fish (fFrtz), mouse (mFrtz), and human (hFrtz) proteins. (C) Alignment by homology of the Frtz C terminus of fruit fly (Frtz), mouse (mFrtz), and human (hFrtz) proteins. All alignments were produced by the T-Coffee algorithm (Notredame et al. 2000). Residues identical to the Drosophila sequence are in boldface type. An asterisk (*) indicates absolute evolutionary conservation; (·) and (·) indicate increasing degrees of conservation as defined by the T-Coffee algorithm. The Frtz proteins cited are aFrtz, \textit{A. gambiae} Frtz accession no. EAA03756; fFrtz, \textit{F. ruprides} Frtz accession no. JGI 12262; mFrtz, \textit{M. musculus} Frtz accession no. AAL24810; hFrtz, \textit{H. sapiens} Frtz accession no. AAD20026.}
\end{figure}
Figure 7.—A fritz WD40 repeat mutation interacts synergistically with other hypomorphic PCP effector gene alleles. (A) The Asp-to-Ala substitution in the WD40b repeat of the fritz gene product. (B–G) Photomicrographs of the “C” region of PCP effector gene mutant wings immediately anterior to the PCV; the proximal-distal axis of the wings runs from left to right. Single mutants at 18° show an almost wild-type wing cell hair phenotype; double mutants show strong polarity and hair number phenotypes.

DISCUSSION

The Frz protein has dual functions in cytoskeletal regulation: The fritz wing phenotype is characterized by altered cell hair polarity and an increase in cell hair number. This is a consequence of the formation of multiple F-actin-rich prehairs at aberrant sites around the apical periphery of the pupal wing cell. It is clear that the formation of multiple hairs is not an inevitable consequence of the loss of normal planar cell polarity since core PCP gene mutant wings display a low incidence of multiple hair cells. This implies that the Ftz protein has two distinct activities. The first is to link prehair localization to the subcellular localization of the core PCP proteins. The second is to restrict prehair initiation to a single site.

In wild-type wings, a prehair forms at the distal periphery of the cell but later translocates to the apical center where the mature hair is found in the adult wing. Prehairs in developing fritz mutant wings initiate at a different location along the apical periphery of the cell than in wild type but are found at the final same location in the adult wing. Therefore, the activity that translocates the developing wing hair from the cell periphery to the apical center must be independent of the position at which the prehair forms. One possibility is that this translocation process is microtubule driven as developing wing cells have webs of microtubules that connect the apical center of the cell to points on the periphery (Eaton et al. 1996). This mechanism would require that the microtubule network is still intact in fritz mutant epithelial cells and consistent with this hypothesis we have not found abnormalities in the microtubule cytoskeleton of fritz pupal wing cells. On a fritz mutant wing, cells that produce hairs with distal or proximal polarity almost invariably produce a single hair, implying that there is little requirement for fritz in restricting the number of sites of prehair initiation at the distal or proximal vertices of the cell. Perhaps the localization of the core PCP proteins at the distal and proximal ends of the pupal wing cell stabilizes hair formation and overcomes the requirement of the Ftz protein for restricting hair number.

The larval denticle phenotypes displayed by fritz and other PCP effector mutants do not appear to involve changes in epithelial cell planar polarity as individual denticles continue to point to either the posterior or the anterior of the embryo as in wild type. As this phenotype does not involve altered polarity and dentine organization does not require the core PCP genes, it is possible that the fritz denticle phenotype has the same origin as the multiple cell hair phenotype shown in fritz mutant wings. The reason that both fritz and mwh mutations
interaction, which appears appropriate given its putative wider evolutionary relevance. 

and that the PCP effector proteins Fy and In have pre-
is within a putative protein-protein interaction domain J. Cell Biol. 

Das, G., A. Jenny, T. J. Klein, S. Eaton Adler 1993). Therefore it might reflect a threshold 

Wong Cong, J., W. Geng, B. He, J. Liu, J. Charlton 

frtz 3 

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Arkhipova, I. R., A. M. Mazo, V. A. Cherkasova, T. V. Gorelova, 

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organizer of the microtubule cytoskeleton (Hartman et al. 1998). It is possible that an association between Fritz and the microtubule cytoskeleton is required to regulate wing cell hair number as a microtubule antagonist mimic the frtz multiple wing hair phenotype (Turner and Adler 1998). The Fritz protein may homodimerize to bring together the four WD40 repeats required to form the archetypal β-propeller structure as has been proposed for proteins with less than four WD40 repeats (Smrhi et al. 1999). However, the homology with p80 katanin extends upstream of the Fritz WD40 repeats and into the second katanin WD40 repeat, suggesting that there is weak homology outside of Fritz WD40 repeats. Therefore, cryptic WD40-like sequences that can fold with the WD40a and b repeats to form a β-propeller-like structure may be present in the Fritz protein. Another potential protein interaction domain in Fritz is the evolutionarily conserved hydrophobic C terminus, which is a potential PDZ domain-binding motif (Harris and Lim 2001). This raises the possibility that Fritz physically interacts with PDZ PCP proteins such as the PCP effector protein Inturned or the core PCP protein Dishevelled.

The strong synergistic interactions among the hypomorphic frtz, Fy, and in alleles are not indicative of a functional redundancy among the PCP effector genes as double mutants of strong PCP effector alleles are not quantitatively stronger than single alleles (Wong and Adler 1993). Therefore it might reflect a threshold effect in which a combination of weak alleles decreases PCP signaling beyond a critical point where little or no signal is transduced. One possibility is that normal PCP signaling requires the PCP effector proteins to form a protein complex. PCP signaling would be lost if a combination of mutant protein components cause this complex to physically dissociate. Supportive evidence for this idea comes from the fact that the frtz mutation is within a putative protein-protein interaction domain and that the PCP effector proteins Fy and In have previously been shown to physically interact in Drosophila cell culture and transgenic flies (Lee 2002).

In both invertebrate and vertebrate genomes the frtz, Fy, and In genes are present in single copy. The simplest assumption is that these genes arose prior to the branching of these evolutionary lines. Without gene duplication to introduce diversity into the function of these genes it is likely that they have maintained essentially the same functions in both invertebrates and vertebrates. If the PCP effector proteins function in a complex to control cytoskeletal integrity and polarity in invertebrates, then they most likely do in vertebrates and so observations made in Drosophila should have a wider evolutionary relevance.

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