**Drosophila** tissue polarity requires the cell-autonomous activity of the **fuzzy**
gene, which encodes a novel transmembrane protein

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**SUMMARY**

The tissue polarity gene **fuzzy** (*fy*) has two roles in the development of *Drosophila* wing hairs. One is to specify the correct orientation of the hair by limiting the site of prehair initiation to the distal vertex of the wing cell. The other is to control wing cell hair number by maintaining the integrity of the cytoskeletal components that direct hair development. The requirement for *fy* in these processes is temperature dependent, as the amorphic *fy* phenotype is cold sensitive. Analysis of mosaic wings has shown that the *fy* gene product functions cell autonomously. We have cloned the *fy* transcript, which encodes a novel four-pass transmembrane protein that shares significant homology with proteins encoded by vertebrate cDNAs. The fourth putative transmembrane domain does not appear to play a significant role in tissue polarity as it is deleted in a weak *fy* hypomorph. Expression of the *fy* transcript is developmentally regulated and peaks sharply at the time of wing cell pre-hair initiation.

Key words: *Drosophila*, *fuzzy*, tissue polarity, cold-sensitivity

**INTRODUCTION**

The regular orientation of pattern elements on an epithelium is a common feature of animal morphology. One striking example is the organisation into parallel arrays of the bristles and hairs on the cuticle of adult insects. To produce such regular arrays, the cells of the cuticular epithelium must be polarised within the plane of the epithelium and this planar polarity must be aligned with respect to the body axes. Mutations in the *Drosophila* tissue polarity genes result in novel patterns of bristle and hair orientation (Gubb and Garcia-Bellido, 1982; Adler, 1992; Gubb, 1993). These patterns indicate that epithelial cells in tissue polarity mutants still acquire a planar polarity, although the bristle and hair alignments differ from the wild-type pattern. The study of tissue polarity genes should, therefore, provide some understanding of the mechanism by which polarised epithelial cells are aligned with the body axes. In this report, we present the phenotypic and molecular characterisation of the tissue polarity gene *fuzzy* (*fy*).

All tissue polarity mutations affect the development of wing hairs. In the wild-type wing, each cell secretes a single distally pointing hair, the initiation of which is preceded by the accumulation of F-actin at the distal vertex of the hexagonal pupal wing cell. In the pupal wing of tissue polarity mutants, the subcellular distribution of F-actin is altered and, as a direct consequence, the final orientation of cell hairs is changed (Wong and Adler, 1993). On the basis of differences in development of their wing hairs, the tissue polarity genes have been placed in three classes. In the pupal wing of group I mutants (*frizzled* (*fc*), *dishevelled* (*dsh*) and *prickle-spiny legs* (*pk-sple*)), the F-actin bundle is frequently displaced towards the apical centre of the cell and a single hair with aberrant polarity results. In group II mutants (*inturned* (*in*) and *fy*) up to three actin bundles form at the cell periphery, so that many cells secrete extra hairs as well as displaying mutant hair polarity. Group III consists of a single gene, *multiple wing hairs* (*mwh*). The pupal wing cells of *mwh* show a similar F-actin distribution to the group II mutants, but have additional late-forming actin bundles that produce small secondary hairs. The extra hairs formed in *fy* and *in* mutants suggest a role for these genes in maintaining the integrity of cytoskeletal components required for wing hair development, as well as in hair polarity. A similar function has been proposed for Rac1, a member of the rho family of small GTPases, as the expression of a dominant negative form (RacN17) in the wing phenocopies the multiple hair phenotype of the group II genes (Eaton et al., 1995, 1996).

The wing hair phenotypes of combinations of the tissue polarity mutations have suggested epistatic relationships between the three classes of genes (Wong and Adler, 1993). These interpretations are based upon the multiple hair phenotypes. The possible epistatic relationships between these genes based on the polarity phenotypes are less clear cut, although both *mwh* and *in* are close to being epistatic to *fy* and *pk* (Gubb and Garcia-Bellido, 1982; Wong and Adler, 1993; Coulson, 1994). Flies mutant for *fy* or *in*, in combination with the group I mutations, have a wing hair phenotype that more closely resembles the *fy* or *in* single mutant. In contrast, the double mutant combination of *fy* or *in* with *mwh* more closely resembles *mwh*. These observations have led to the proposal of a model in which *fy* and *in* function down-
stream of the group I genes and upstream of mwh in a regulatory pathway that controls the site of hair initiation (Wong and Adler, 1993).

The fz gene encodes a seven-pass transmembrane protein that functions both non-cell autonomously, to transmit tissue polarity information, and cell autonomously, to direct the site of prehair initiation (Vinson et al., 1989; Krasnow and Adler, 1994). The cell-autonomous function of Fz is proposed to act through the product of the dsh gene (Krasnow et al., 1995), which encodes an evolutionary conserved protein that is also part of the wingless (wg) signaling pathway (Theisen et al., 1994; Klingensmith et al., 1994; Noordermeer et al., 1994). Both Fz and an homologous Drosophila protein, Dfz2, have been shown to mediate wg signaling in a cell culture assay (Bhanot et al., 1996). On the basis of its in vivo expression pattern Fz2 has been proposed to be a receptor for wg, although an analogous Wnt ligand for Fz has not been identified. Therefore, the wg and tissue polarity signaling pathways act through Fz-like receptors and converge on dsh. Although the downstream effectors of wg signaling in Drosophila have been well characterised (see, for example, Klingensmith and Nusse, 1994), little is known about the genes proposed to act downstream of Fz and dsh in the tissue polarity pathway. To date, only the in gene has been molecularly characterised and found to encode a novel protein containing two putative membrane-spanning domains (Park et al., 1996). Here we show that fy, a second downstream component of this pathway, also encodes a novel transmembrane protein.

MATERIALS AND METHODS

Phenotypic analysis
Flies were cultured at 25°C on yeast-scamone agar unless otherwise stated. Second chromosomes used for mapping and for phenotypic analysis were 
fy b, 
fy c, 
fy b, 
fy c, T(Y;2) 
fy b, TACAGTTGGA TC-3

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<tr>
<th>Nucleotide sequence analysis of cDNA and genomic clones</th>
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| EcoRI restriction fragments from the genomic phage clone W5-p9 (Neumann-Silberberg and Schuepbach, 1993) that flank the site of the 
fy d deletion were used to screen 5x10^5 clones of an Oregon R imaginal disc plasmid cDNA library by the protocols described in Brown and Kafatos (1988). The longest 
fy c cDNA recovered (fcJ) was subcloned into pBluescript SK+ (Stratagene Ltd.) and deletion constructs from 5’ to 3’ of the sense strand made using exonuclease III. Single-stranded templates were produced by using the R408 helper phage (Stratagene Ltd.) and were sequenced from the M13-20 primer following the Sequenase protocol (US Biochemical Corp.), Sequence data from these clones were assembled into a contig using the GCG (Genetics Computer Group, Inc.) GELASSEMBLE program. The fcJ cDNA was subcloned into pBluescript KS+ and the antisense strand sequenced from oligonucleotide primers complementary to the sense strand by the same protocol. The same primers were used to characterise subclones of a second 
fy c DNA (FcH) and the 3.2 kb BamHI fragment from phage W5-p9 containing the corresponding region of genomic DNA. The best open reading frame within the composite cDNA sequence was identified by the Positional Base Preference method in the Standard Staden Programs for Nucleotide Interpretation. The encoded polypeptide was compared to protein database entries using the Blastp and Fasta programs and to DNA databases using the tblastn program (Altschul et al., 1990; Pearson and Lipman, 1988). Prediction of helical transmembrane domains was performed by the PHDhtm program of the Predictprotein server (Rost et al., 1995) and refined by alignment with the peptide sequence encoded by a homologous D. viridis locus (S. C., unpublished results).

Rescue of 
fy z phenotype
The 3.2 kb BamHI restriction fragment spanning the 
fy transcription unit (see Fig. 4) was subcloned from phage W5-p9 into the pWhite Rabbit transformation vector (Dunin-Borkowski and Brown, 1995). A solution of 1 µg/µl pWhiteRabbit/fyB3.2 and 0.25µg/µl 

| Reciprocal assay of the regulatory pathway for each genotype was drawn after examining at least six mounted wings. Mitotic clones of 
Drosophila phenotype, Dfz2, have been shown to mediate wg signaling in a cell culture assay (Bhanot et al., 1996). On the basis of its in vivo expression pattern Fz2 has been proposed to be a receptor for wg, although an analogous Wnt ligand for Fz has not been identified. Therefore, the wg and tissue polarity signaling pathways act through Fz-like receptors and converge on dsh. Although the downstream effectors of wg signaling in Drosophila have been well characterised (see, for example, Klingensmith and Nusse, 1994), little is known about the genes proposed to act downstream of Fz and dsh in the tissue polarity pathway. To date, only the in gene has been molecularly characterised and found to encode a novel protein containing two putative membrane-spanning domains (Park et al., 1996). Here we show that fy, a second downstream component of this pathway, also encodes a novel transmembrane protein.

MATERIALS AND METHODS

Phenotypic analysis
Flies were cultured at 25°C on yeast-scamone agar unless otherwise stated. Second chromosomes used for mapping and for phenotypic analysis were 
fy b, 
fy c, 
fy c, T(Y;2) 
fy c, cn bw sp, T(Y;2) 
fy c cn bw sp, cn bw sp, 
fy b pr, 
fy c pr, cn, Df(2L)N22-14 and Df(2L)N22-5. Wings for phenotypic analysis were dissected from flies that had been stored at ~7°C or in a 1:1 solution of 70% ethanol:glycerol at room temperature and were mounted in aquamount (BDH Ltd) with isopropanol. A representative hair polarity phenotype for each genotype was drawn after examining at least six mounted wings. Mitotic clones of 
fy a were generated by crossing 
fy a to 
fy a b progeny crossed to a w; Sco/In(2LR)L0 Cy; In(3LR)/TM2, Ubx; 10/10; In(3LR)/TM6B, His stock to balance the inserts. To test for rescue of the 
fy z phenotype, w; In(2LR)L0 Cy/+; 
fy B/In(3LR)/TM2 Ubx; 10/10; males were crossed to 
fy a b homozygous females and the 
fy B/In(2LR)L0 Cy; 
fy B/+ progeny crossed to each other. Wings from w; Cy+ and 
fy B progeny were mounted and hair polarity patterns compared.

Characterisation of 
fy mutants alleles
The 
fy z deletion was characterised by amplifying a DNA fragment spanning the deleted EcoRI restriction site from 
fy 3' homologyte and progenitor genomic DNA using the oligonucleotide primers 5'-GCTACACGGACTGTCTGCTG-3' and 5'-GCACCTCATGCGATGT- GC ATG-3’ following the PCR protocol described in Collier et al. (1992). PCR products were purified using the Prep-a-Gene kit (Bio-Rad), heat-denatured and sequenced from the same primers following the standard Sequenase protocols (US Biochemical Corp.). To identify the 
fy z and 
fy a mutations, the 3.1 kb BamHI/Xhol fragment spanning the 
fy locus (see Fig. 4) was amplified from 
fy z homologyte genomic DNA using the flanking oligonucleotide primers 5’-CTGACACCATACTCTCGT-3’ and 5’-CACACTCAC- TACAGTTGATC-3’. The PCR product was digested with 
BamHI and 
Xhol, cloned into pBluescript KS+ and characterised by single-stranded sequencing from 
fy a specific oligonucleotide primers as described above. Clones from three independent PCR reactions were sequenced to eliminate the possibility that base changes had arisen from nucleotide misincorporation by Taq polymerase. The 
fy a mutation also introduces a novel ByII restriction site into the 
fy coding region, the presence of which has been confirmed by comparative Southern blot analysis.

Northern and in situ analyses of 
fy expression
For northern analysis, developmentally staged wild-type (Canton-S) animals were homogenised in a guanidine isothiocyanate solution and total RNA isolated according to the method of Chomczynski and Sacchi (1987). Poly(A)+ RNA was prepared from the total RNA using the oligo(dT)-cellulose by the method of Aviv and Leder (1972). Approximate 0.5 µg of poly(A)+ RNA from each developmental stage was separated on an agarose-formaldehyde gel, blotted to a nylon membrane and hybridised with radiolabelled 
FcH cDNA and Rp49 probes following standard procedures. For whole-mount in situ hybridisation, sense and antisense digoxigenin-labelled RNA probes were prepared using the FcH 
fc cDNA as a template. Hybridisation
of the riboprobes to pupal wings was performed as described in Sturtevant et al. (1993) but with hybridisation and washing steps increased to 55°C.

RESULTS

The fy gene is not easily mutated

The original fy allele (fy1) has been lost. A mutant with a similar phenotype was recovered in an EMS screen and designated fy2 due to its similar map position (Grell, 1969), although a test of allelism was not possible. An X-ray mutagenesis screen has been undertaken in this laboratory to create new fy alleles. Of 35,000 chromosomes screened, however, only two (fy3 and fy4) showed a fy phenotype in combination with the fy2 allele (Clare Henchcliffe, unpublished results). Polytene chromosome analysis has shown that the fy3 chromosome is cytologically normal and the fy4 mutation is associated with a T(Y:2) translocation that breaks in the interval 29B4-C2. We have recently acquired two EMS-induced fy alleles fy5 (previously fy JN11) and fy6 (previously fy JN12) isolated in Paul Adler’s laboratory (University of Virginia, USA).

The fy3 and fy4 alleles behave as amorphs as they do not give appreciably stronger phenotypes in combination with a deletion of the region (Df(2L)N22-14) than when homozygous. The fy5 and fy6 alleles are weak hypomorphs displaying only mild deviations from wild-type wing hair polarity and a low incidence of wing cells displaying extra hairs. Flies of the genotypes fy2/fy5 and fy2/fy6 have significantly stronger phenotypes than fy3/Df(2L)N22-14 and fy6/Df(2L)N22-14, respectively, implying that the fy2 allele displays some antimorphic character in combination with hypomorphic fy alleles. Since stocks homozygous for the fy2 or fy3 alleles can be maintained, the fy gene appears not to be required for viability or fertility. In contrast, the fy4 translocation is lethal in combination with a deletion of the region (Df(2L)N22-14), although this lethality maps to an adjacent locus (Glynnis Johnson and S. C., unpublished results).

The amorphic fy phenotype is cold sensitive

Mutant fy flies display an altered pattern of bristle and hair orientation. The microchaetae and macrochaetae of the notum and abdomen are turned towards the midline, rather than pointing posteriorly, and the bristles of the anterior wing margin stands more erect than wild type (Grell, 1969). The cuticular hairs, which usually point distally on the appendages and posteriorly on the rest of the body, are arranged into novel patterns of polarity. The wing hair patterns of fy mutant flies of the same genotype that have been cultured at the same temperature are almost identical. Diagrammatic representations of the hair polarity pattern on the dorsal wing surface of fy2/Df(2L)N22-14 mutant flies cultured at 18°C and 29°C and also the wild-type polarity of cn bw sp progenitor flies are shown in Fig. 1. The hair polarity pattern on the ventral surface of the mutant wing is similar to the dorsal surface at the margins, but shows a more pronounced deviation from wild type towards the interior of the wing blade.

Flies carrying either the fy2 or the fy3 allele display a sig-

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Fig. 1. Diagrammatic representation of hair polarity patterns on the dorsal wing surface of fy mutant and progenitor flies. Vectors delineate the direction of hair polarity for each region of the wing blade. Shaded areas represent regions for which a polarity could not be assigned either because of variation between individual wings, or because all wings show close to random orientation. Each diagram represents an average phenotype from six wings of flies raised contemporaneously. The erect triple row of the anterior wing margin associated with the fy phenotype is not represented. Next to each diagram is a photograph of the C region of a wing of the same genotype immediately anterior to the posterior cross vein. Phenotypes represented are; (A) cn bw sp (fy3 progenitor) homozygous flies cultured at 18°C, (B) fy2/Df(2L)N22-14 flies cultured at 29°C and (C) fy3/Df(2L)N22-14 flies cultured at 18°C.
significantly stronger phenotype when cultured at 18°C than at 29°C (Fig. 1B,C). The phenotype at 25°C is intermediate but much closer to that at 29°C. The stronger phenotype is characterised by an increase in the overall deviation from wild-type hair polarity and the proportion of the wing for which a specific polarity cannot be assigned. These changes are more evident towards the interior of the wing blade than at the margins (Fig. 1B,C). As both the fy2 and fy3 alleles are amorphs, this cold-sensitivity appears to derive from a temperature-dependent requirement for the fy gene product, rather than from the products of these alleles being inherently cold sensitive.

A proportion of fy wing cells secrete up to four hairs instead of the single hair produced by wild-type cells. This phenotype is not uniform across the wing blade. Cells close to the wing margin still secrete a single hair, with the exception of cells of the first two rows next to the posterior margin, between the alula and the distal tip of the L4 vein, which frequently produce two (Fig. 2A). There is also a region of wild-type hair number a few cell diameters either side of the distal region of the wing veins, particularly L3 and L5 on the dorsal surface (Fig. 2B). This possibly correlates with the fact that L3 and L5 are dorsal veins, although a reciprocal phenotype with respect to the ventral veins is less apparent on the ventral wing surface. In general, cell hair number increases with the deviation of hair polarity from wild type (e.g. Fig. 1B,C). An exception to this rule is the behavior of cells with reversed (distal-to-proximal) polarity, which usually retain wild-type hair number (Fig. 2C).

Previous studies have found that fy flies do not have the ectopic inverted joints in the tarsi of the leg that are associated with some tissue polarity mutants (Held et al., 1986; Coulson, 1994). However, double mutant combinations of the fy2 allele with either pk or mwh show ectopic tarsal joints at a low frequency, which are never observed in pk or mwh mutants themselves (Coulson, 1994). These results suggest that fy has a redundant role in tarsal joint development.

**Clones of fy2 tissue mimic polarity in homozygous fy2 wings**

Clones of fy2 mutant tissue marked with forked36a (f36a) were induced in a f+30B background by X-ray-induced mitotic recombination. 20 clones displaying the f36a wing hair phenotype ranging from 20 cells to approximately one third of the dorsal wing surface were examined for changes in hair orientation. In each case the changes in planar polarity exhibited by cells within these clones resembles that shown by cells at an equivalent position within a fy2 homozygous wing (Fig. 3). The exceptions were the cells towards the borders of the clones that adopt a polarity that is intermediate between mutant and wild type. It was evident that fy2 clones do not cause dominating non-cell autonomy of the degree shown by clones of the fz gene (Gubb and García-Bellido, 1982; Vinson and Adler, 1987), as hair polarity and hair number of fy+ cells surrounding the clones appeared normal. However, the rescue of the f36a wing hair phenotype by f+30B construct is not complete, which made it difficult to be confident about the precise boundaries of some clones. Therefore it was not possible to rule out a limited degree of non-cell autonomy.

**The fy locus maps close to gurken**

The fy2 allele has a genetic map location of 2-24.1 (Grell, 1969) and had previously been localised to the interval 29C2-29D1.2 using deficiencies of this region recovered by Wustmann et al. (1989; Clare Henchcliffe, personal communication). The cytology of the fy4 translocation (29B4-C2) places the gene towards the distal end of this interval. We have refined this mapping by showing that fy is uncovered by Df(2L)N22-14 (29C1.2;30C8.9), but not by Df(2L)N22-5 (29C3.5;30C8.9). This places the fy locus between the distal endpoints of these two deficiencies (29C1.2;29C3.4), the same interval to which the female-sterile mutation gurken (grk) had previously been mapped. A 48 kb phage walk, initiated from the distal end of YAC clone DY51 and spanning this region, had been undertaken to clone grk (Neumann-Silberberg and Schuepbach, 1993) and was used to assist with the identification of the fy transcript. The fy locus was subsequently localised to the distal
sequences of the *fcH* and *fcJ* clones are identical in their overlapping region and the 1880 bp composite cDNA sequence is indicated in Fig. 5. The site of polyadenylation of the shorter cDNAs indicates that the 5' to 3' orientation of the transcript is from proximal to distal on the chromosome (Fig. 4).

The longest open reading frame (ORF) within the composite cDNA sequence is 416 amino acids in length (Fig. 5). The putative start codon is not embedded within a good *Drosophila* consensus sequence (Cavener and Ray, 1991), but alignment with the ORF encoded by a homologous *Drosophila viridis* locus suggests that it is indeed the start site for translation (S. C., unpublished results). The encoded polypeptide is predicted by the PHDhtm neural network system to have four membrane-spanning helices (Rost et al., 1995, see Fig. 5), but otherwise contains no recognisable functional motifs. The primary sequence of the *fy* gene product has been compared to protein databases using BLAST (Altschul et al., 1990) and FASTA (Pearson and Lipman, 1988) homology searches, but no significant matches were found. However, the conceptual translation of a human-expressed sequence tag (EST, I.M.A.G.E. clone 45228) from an infant brain cDNA library gives a peptide with 32% identity to a 119 amino acid portion of the *fy* sequence between the first (TM1) and the third (TM3) putative membrane-spanning regions (Fig. 6). A mouse embryonic cDNA (Life Tech clone 555546) encodes a peptide with similar degree of homology. We conclude that the *fy* gene product is a novel transmembrane protein that contains at least one domain that is conserved in vertebrate proteins.

The 1.9 kb *fy* transcript maps entirely within a 2.3 kb *BamHI/HindIII* genomic restriction fragment. The alignment of the nucleotide sequence of this fragment with that of the composite cDNA has identified two small introns, of 58 and 39 bp, that lie towards the 5' end of the open reading frame, respectively. The nucleotide sequence of the *BamHI/HindIII* fragment is presented in Fig. 5.

**The *fy* transcript encodes a novel transmembrane protein**

We used the genomic *EcoRI* restriction fragments from the *grk* phage walk that flank the site of the *fy* deletion to screen approximately 5x10^5 plasmid clones from an imaginal disc cDNA library (Brown and Kafatos, 1988). Three cDNA clones spanning the deletion were isolated (*fcH*, *fcI* and *fcJ*) and also clones representing the transcripts lying immediately proximal and distal (Fig. 4). The two shorter clones *fcH* (1.6 kb) and *fcI* (1.1 kb) are polyadenylated at almost identical positions. The longer clone *fcJ* (1.9 kb) is shorter at the 3' end and has no poly(A) tail but has 344 bp of additional 5' sequence. The nucleotide end of the *grk* walk by the identification of a 70 bp deletion on the *fy* chromosome (see below).

**A small genomic region completely rescues the *fy*^2^ phenotype**

The 3.2 kb genomic *BamHI* fragment that spans the *fy* tran-

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**Fig. 3.** Hair polarity of *fy*^2^ clone compared to the equivalent region of a *fy*^2^ homozygous wing. The proximal to distal axis of both wings is from left to right. (B) *fy*^2^ clone from a fly cultured at 25°C. The approximate borders of the clone are marked. (A) Equivalent region of the wing of a *fy*^2^ homozygous fly cultured at 25°C. Hair polarity within the clone mimics that of the mutant wing with the exception of cells near to the clone boundary which display a polarity intermediate between the mutant and the wild-type orientation.

**Fig. 4.** Molecular map of the *fy* locus at 29C1.2. Transcripts are indicated by hatched boxes. The size of the *fy* introns is exaggerated for clarity. The intron/exon structure of the flanking genes is not shown. The *Crml* transcripts encode a peptide with high homology to the Chromosomal Region Maintenance protein of yeast (Adachi and Yanagida., 1989; Toda et al., 1992, Collier et al. unpublished data). *fcG* is an uncharacterised cDNA clone from an imaginal disc library. The 3.2 kb *BamHI* fragment sufficient to rescue the *fy*^2^ phenotype is indicated by the lightly shaded box. The regions deleted on the *fy*^2^ chromosome and by *Df(2L)N22-14* are represented by black boxes. The unshaded portion of *Df(2L)N22-14* box indicates the degree of uncertainty concerning the distal breakpoint of the deletion. Restriction sites are B: *BamHI*, E: *EcoRI*, H: *HindIII* and X: *XhoI*. The lower case ‘e’ is a polymorphic *EcoRI* site present on *cn bw sp* and derivative chromosomes.
script (Fig. 4) was cloned into the wRabbit transformation vector and introduced into flies by P-element-mediated transposition. A single copy of the construct on the third chromo-

mosome (2fB1) has proved sufficient to rescue completely the 2fR homozygous mutant phenotype. Individual rescued flies, most noticeably those cultured at 18°C, can display occasional wing cells with double hairs, but this is not consistent from animal to animal.

For the fy gene function not being dependent upon wild type expression.

Both strong and weak fy alleles encode truncated proteins Phenotypically the 2fR and 2fY alleles behave as amorphs as they are not appreciably stronger in combination with a deficiency of the region that had been amplified from homozygote genomic DNA. by sequencing clones of PCR products spanning the RI site. Lesions were identified in a protein lacking the 220 C-terminal amino acids. Consequently the 2fR allele has a C to T transition at codon 331 that introduces a

expression is also detectable with the length of the composite cDNA sequence presented in

Both strong and weak fy alleles encode truncated proteins. The 2fR allele has a C to T transition at codon 331 that introduces a stop codon at position 334, subsequently the 2fY allele has a C to T transition at codon 1015 that introduces a stop codon at position 1018.

Phenotypically the 2fR and 2fY alleles encode similar truncated proteins that lack the two putative C-terminal membrane-spanning domains and interrupt the region of homology shared with proteins encoded by human and mouse ESTs (Fig. 6). The

The expression of the fy transcript is developmentally regulated. Hybridization of the longest fy cDNA (4J) to a northern blot of poly(A)+ RNA isolated from wild-type (Canton-S) animals at a series of developmental stages is shown in Fig. 7. An mRNA species of just under 1.9 kb was identified consistent with the length of the composite cDNA sequence presented in Fig. 5. A low level of expression of this transcript is detectable at all stages of development, with a relatively strong peak of expression in the 2-day-old pupa that coincides with the period of prehair initiation. Significant expression is also detectable

Fig. 5. Nucleotide sequence of the 2.3 kb HindIII/BamHI genomic fragment that spans the fy locus (see Fig. 4). Sequence present in cDNA clones is shown in upper case. The G to A transition introducing the fyR nonsense mutation, the 70 bp deleted on the 3′ chromosomal and the C to T transition introducing the fyR nonsense mutation are shown in bold type. A one-letter translation of the fy open reading frame is shown below the nucleotide sequence, numbers refer to amino acid positions within the coding sequence. Amino acids highlighted with bold type and underlined are within the putative membrane-spanning regions TM1 to TM4. GenBank accession no. for the cDNA sequence is AF022891.
in the embryo and female, but not male, adult flies suggesting that the embryonic expression has a maternally derived component. The maternal and embryonic transcripts appear slightly shorter than the pupal transcript (Fig. 7). As no evidence has been found for alternatively spliced transcripts, this size difference may reflect differences in polyadenylation.

We have hybridised pupal wings at the time of highest \( fy \) expression with sense and antisense riboprobes. The pattern shown by the antisense strand is shown in Fig. 7B, the sense strand did not produce significant hybridisation. At this stage the \( fy \) transcript is present in all wing cells consistent with the \( fy \) gene having a cell-autonomous phenotype that affects all regions of the wing blade. The transcript is relatively abundant adjacent to the wing margin and in bands of cells flanking each of the wing veins with the exception of L2. This pattern may simply reflect cell densities in the pupal wing at this stage. The lack of staining surrounding L2, however, is reminiscent of the BrdU-staining patterns at 15-20 hours after pupation (Schuebiger and Palka, 1987; Milan et al., 1996), raising the possibility that \( fy \) expression is related to the exit of pupal wing cells from their last cell division.

**DISCUSSION**

**The \( fy \) wing hair polarity phenotype**

Mutations in the \( fy \) gene result in new patterns of wing hair polarity (see Fig. 1B,C). As a rule, hairs in the anterior third of the \( fy \) mutant wing point more anteriorly than wild type and those in the remainder of the wing point more posteriorly than normal. Similar patterns are produced by the other tissue polarity mutations (with the exception of the \( pk \) single mutant where the pattern is almost reversed) and also by the double mutant combinations of tissue polarity genes (Gubb and Garcia-Bellido, 1982; Wong and Adler, 1993; Coulson, 1994).

It seems, therefore, that there is an underlying pattern that results from reduced tissue polarity gene activity. The existence of such a ‘default’ pattern would imply that mutant cells still express a planar polarity that depends upon their position within the wing blade, even without information from the tissue polarity gene pathway. It is not clear, however, what information the mutant cells are responding to. Tissue polarity mutant hair patterns do not appear to be influenced by the anterior-posterior compartment boundary of the wing and the dominant non-cell-autonomy associated with \( fz \) clones can cross the compartment boundary (Gubb and Garcia-Bellido, 1982). These observations suggest that the orientation of a wing cell’s planar polarity is independent of its lineage.

It is clear that the \( fy \) phenotype is influenced by the presence of wing veins. Veins often correspond to discontinuities in the polarity pattern (see Fig. 1B,C) and mutations that remove wing veins also remove the associated discontinuities in the \( fy \) wing hair pattern (S. C., unpublished observations). Cells surrounding the wing veins often show close to wild-type polarity (see Fig. 2B), implying that veins can direct polarity in the absence of \( fy \) gene function. However, wing vein differentiation is not required to align cell polarity in the wing as the
veinlet (ve) vein (vn) double mutant combination, which eliminates all veins but L1, displays normal hair polarity.

The *fy* wing hair number phenotype

One property of the *fy* wing phenotype that is shared by *in* and *mwh*, is a high incidence of cells producing two or more hairs. As a rule, the farther a mutant cell’s planar polarity is from wild type, the more likely it is to produce multiple hairs (see for instance Fig. 1B). There are, however, two pieces of evidence from the *fy* mutant wing that suggest that mutant cell hair number is separable from hair polarity. First, the cells that are most strongly mutant for polarity, i.e. those producing hairs pointing from distal to proximal, usually retain wild-type hair number (Fig. 2C). Second, although the cells close to the posterior wing margin show a comparable hair polarity, only those in the two rows immediately adjacent to the margin display an abnormal hair number (Fig. 2A). Indeed, these are the cells that are most likely to show multiple hairs on *fy* mutant wings that retain close to wild-type hair polarity. In addition, some combinations of *fy* alleles display an incidence of split, rather than doubled, wing hairs. Since wing hairs have been proposed to elongate from the tip (Eaton et al., 1996), the formation of split hairs suggests that *fy* has a role in maintaining the integrity of the hair as well as controlling its initiation. Papal wings treated with microtubule antagonists and a role in establishing planar polarity in the embryo and have no associated phenotype (Adler et al., 1990; Park et al., 1996). This suggests either that the putative tissue polarity signal transduction pathway will be a transcription factor that promotes *fy* expression. None of the tissue polarity genes characterised so far encodes a transcription factor, but it has been reported that the *fc* protein is required for normal expression of the *nemo* gene and in the eye (Zheng et al., 1995). A simple model of *fy* activating *fy* transcription seems unlikely, however, as data on epistatic interactions have suggested that the *fc* gene regulates the activity of *fy* and *in* (Wong and Adler, 1993). It is possible, therefore, that *fy* expression is activated by a mechanism that promotes cell differentiation at the time the cell has completed division and that the *fy* gene product is regulated by *fc* through protein-protein interactions rather than transcriptional control.

The embryonic expression of the *fy* transcript is intriguing, as *fy* mutants have no known embryonic phenotype. However, the tissue polarity genes *fc* and *in* are also expressed in the embryo and have no associated phenotype (Adler et al., 1990; Park et al., 1996). This suggests that the putative tissue polarity signaling pathway is redundant during embryogenesis, or that it plays a role in establishing planar polarity in the embryo that is reflected in a less conspicuous way than the orientation of bristles and hairs on the adult cuticle.

Two roles for the *fuzzy* gene in wing hair development

In summary, the *fy* gene encodes a novel four-pass transmembrane protein that plays two roles in the development of hairs on the *Drosophila* wing. The first is to specify the correct orientation of the hair by restricting its initiation to the distal vertex of the cell. This activity is proposed to be directed by polarity information received by the Fz receptor, possibly through Wnt signaling, and transmitted by the Dsh protein. The second role of *fy* is to permit the development of just a single cell hair by maintaining the integrity of the F-actin and microtubule arrays that are required for hair development, a process that may also require the activity of the small GTPase Rac1.

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