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Transducing properties of *Drosophila* Frizzled proteins

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SUMMARY

In *Drosophila*, two closely related serpentine receptors, Frizzled (Fz) and D-Frizzled2 (Fz2) are able to act as receptors for the secreted Wnt peptide, Wingless (Wg). In addition to transducing the Wg signal, Fz (but not Fz2) is able to transduce a second, unidentified signal that mediates planar polarity. Much attention has been focused on the structure of the N-termini of the Fz-class receptors and their role in ligand binding. Experiments using techniques of high-level expression have suggested a role for the C-termini in specifying which of the two second messenger systems the receptors are able to activate (M. Boutros, J. Mihaly, T. Bouwmeeste and M. Mlodzik (2000). *Science* 288, 1825-1828). We argue here that experiments involving high level expression of the receptors cannot be adequately interpreted and we have tested the ability of the receptors and chimeric forms when driven at moderate levels to rescue loss of function of the *fz* and *fz2* genes. Under these conditions we find that all receptors tested will function as Wg receptors, but only a subset show the ability to rescue the polarity pathway. The presence of this subset implies that the N terminus is necessary but not sufficient and suggests that the ability to transduce the polarity signal is widely distributed throughout the protein.

Key words: Wnt, Frizzled, Signal transduction, Polarity, *Drosophila melanogaster*

INTRODUCTION

A few families of secreted peptides mediate much of the intercellular signaling that occurs during animal development. One such family is the Wnt class of secreted glycopeptides [for review see (Cadigan and Nusse, 1997)]. Not only are Wnts critical cell signaling molecules during development, but in adult life the inappropriate activation of their signaling pathway has major oncogenic effects [for review see (Varmus et al., 1987)]. Accordingly, a large body of work has been directed to understanding the mechanisms of Wnt signaling. The plasma membrane receptors for the Wnt molecules have been identified as the Frizzled (Fz) class of serpentine receptors (Bhanot et al., 1996). Understanding how the binding of the Wnt ligand to its Fz receptor elicits signal transduction within the cell constitutes a major goal of Wnt research.

In *Drosophila*, Wingless (Wg) is the functional counterpart of mammalian Wnt1 (Cabrera et al., 1987; Rijsewijk et al., 1987) and two Frizzled proteins, Fz and Fz2, can function redundantly to transduce Wg signals (Chen and Struhl, 1999; Bhanot et al., 1999). Transduction of Wg through these Frizzled proteins results in a cascade of biochemical events within the cell, including the phosphorylation of the Dishevelled (Dsh) glycoprotein, the down-regulation of Shaggy (Sgg – the fly homologue of Gsk3) kinase activity, and the stabilization of cytoplasmic pools of Armadillo (Arm – the fly β-catenin) (Peifer et al., 1994; Yanagawa et al., 1995; Ruel et al., 1999).

Planar polarity is a phenomenon whereby cells within an epithelium are uniformly organized within the plane of that epithelium. The most obvious manifestation of planar polarity is the coordinated orientation of hairs or bristles in animal ectoderms. Planar polarity appears to be organized by the action of a diffusible signal and Fz is the receptor for that signal (Gubb and Garcia-Bellido 1982; Vinson et al., 1989; Wehrli and Tomlinson, 1998). A number of proteins have been implicated in the transduction of the polarity pathway through Fz, most notably Dishevelled (Dsh). Dsh, as described above, also transduces Wg, and discrete domains of the protein appear dedicated to each of the two second messenger pathways (Axelrod et al., 1998; Boutros et al., 2000). A number of other components of the polarity pathway have been described, including Strabismus, Prickle/spiny-legs and members of the small GTPase family (Wolff and Rubin, 1998; Gubb et al., 1999; Strutt et al., 1997). How these various molecular components are interconnected in the transduction of the polarity pathway remains unclear.

Flies mutant at the Fz2 locus (*fz2*) show no aberrant phenotype, those mutant for Fz (*fz*) show polarity defects, but flies mutant for both do not transduce Wg signals (Chen and Struhl, 1999). Thus Fz appears to transduce both the polarity and Wg signals, whereas Fz2 is dedicated to the Wg pathway. Further evidence for this dual role of Fz is presented in this paper where it is shown that *fz* when expressed under the tubulin promoter (*tub-fz*) will rescue Wg signaling in clones mutant for both *fz* and *fz2*.

Since Fz is able to transduce both Wg and the polarity signal and Fz2 can only transduce Wg, then a detailed functional and

structural comparison of the two receptors may reveal where signal transduction specificity lies within this receptor type. Fz-class serpentine receptors can be viewed as consisting of three distinct domains (Fig. 1). (1) The N terminus, from the first amino acid to the beginning of the first transmembrane domain; (2) the transmembrane (TM) domains, from the beginning of the first transmembrane domain to the end of the seventh TM domain including the intra- and extra-cellular loops; and (3) the C-terminal domain, which is measured from the end of the seventh transmembrane domain to the end of the protein. Of these three domains, the most highly conserved is the TM domain region that shows an identity of 41% between the two proteins. The C-terminal regions are relatively divergent. The Fz C terminus is only 28 amino acids in length whereas the Fz2 C terminus contains 89 amino acids. Over the first 28 amino acids the two C termini are approximately 46% identical. The N termini of these two proteins have an overall identity of about 28%. However, the N terminus can be divided into two subdomains; a cysteine-rich domain (CRD), which has been implicated in the binding of Wg (and other Wnts) (Rulifson et al., 2000; Lin et al., 1997; Uren et al., 2000; Wang et al., 1997; Hsieh et al., 1999), in which the two proteins have nearly 50% identity; and the so-called non-conserved region (NCR) where there is approximately 11% identity between the two proteins.

We constructed and expressed in flies, chimeric versions of the proteins containing one domain (N, TM or C) from one receptor and two from the other (Fig. 1). We then investigated whether the chimeric proteins behaved as Wg receptors, polarity receptors or both. Two distinct approaches were employed. First, in mutant rescue experiments, the abilities of the chimeric receptors to rescue Wg signaling in tissue mutant for both *fz2* and *fz*, or to rescue polarity signaling in tissue mutant only for *fz* were tested. Second, in over-expression studies, the effects of the chimeric proteins were compared with those of the wild-type receptors (Fz and Fz2) when expressed at high levels using the Gal4-UAS system.

Two organizational features of the fly wing under the separate control of the Wg and polarity pathways are relevant to these analyses. First, Wg signaling about the margin directs the local elaboration of marginal bristles, which in the anterior part of the wing are particularly distinctive. Second, the polarity pathway organizes the uniform proximal-to-distal orientation of the wing hairs (restricted to the blade) and marginal bristles (Fig. 1B). We used the formation of the margin bristles as an assay of a chimera's ability to transduce the Wg signal. The wing hair organization was not used to assay rescue of the polarity pathway since the required level of expression could not be engineered in this tissue. Instead we used rescue of the Fz-dependent polarized organization of the ommatidia in the eye (Fig. 1C). In the wing we determined that when expressed under tubulin transcriptional control Fz2, Fz and the chimeric forms all rescued the loss of function of both *fz* and *fz2*. Hence all appeared by this assay to be functional Wg receptors. In the eye we tested the ability of the receptors to rescue the polarity phenotypes of *fz* mutants. When expressed under *sevenless* (*sev*) transcriptional control, we found that only those receptors bearing the Fz N terminus were able to rescue *fz* mutant eye tissue. However, the N-terminal domain was not sufficient for *fz*-rescuing function. That is, only chimeric proteins containing the N terminus and one other (the TM domain or the C terminus) Fz domain could rescue. Thus polarity signal transduction cannot be discretely localized to any one of the receptor domains.

The results from the rescue experiments contrasts with those of Boutros et al. (Boutros et al., 2000), which from over-expression studies suggested that the signaling specificities of the two receptors lie in their C-termini; chimeric proteins of the two receptors, when over expressed, were able to differentially activate the Wg or polarity signaling pathways depending on the C terminus present in the receptor. These findings were in contrast to those of Rulifson et al. (Rulifson et al., 2000), who found that the cysteine-rich domain (CRD) of the N-termini was the region of the protein most responsible for signaling specificity. The results that emerged from our own over-expression studies suggested that little could be understood about the signaling abilities of chimeric receptors from the dominant phenotypes that emerge under these conditions. Our major conclusions therefore are drawn from the rescue experiments that suggest that the ability of Fz to transduce the polarity signal from one side of the plasma membrane to the other appears to be distributed throughout the domains of the protein with necessary information residing in the N terminus.

MATERIALS AND METHODS

Construction of chimeras

The chimeric transgenes were made as follows. A *Bsp*120I site in the N terminus of Fz2 at amino acid (aa) position 308 was used to generate the N-terminal chimeras. Specifically, a *Bsp*120I site was inserted in the Fz N terminus at the same position that this site is found in the Fz2 ORF. This allowed the fusing of the two proteins such that the first 242 aa of the Fz protein (in the Fz/Fz2/Fz2 and the Fz/Fz2/Fz chimeras) was added to the remaining 10 aa of the Fz2 N terminus and the Fz2 TM domains. Similarly, the first 308 aa of the Fz2 were appended onto the 10 remaining aa of the Fz N terminus to generate the Fz2/Fz/Fz chimera.

For the C-terminal chimeras, a conserved TS di-amino acid, 5 amino acids from the end of the seventh TM domain in both proteins was edited in such a way that the coding remained the same, but it now formed a *Spe*I site. This site was used to exchange the C termini of the proteins. For more complete details on construction please contact the authors.

Expression systems

All constructs were engineered with a *white*$^+$ flip-out cassette (Wehrli and Tomlinson, 1998) between the promoter elements and the coding sequence. This was used as the transformation marker. Three forms of construct were made; those having the UAS promoter element, those having the alpha-tubulin promoter, and those with the *sev* enhancers-heatshock promoter [modified from Basler et al. (Basler et al., 1989)]. The constructs for the over-expression experiments placed the relevant coding sequence into the standard pCaSpeR3-UAS vector, which includes a *white*$^+$ gene in the backbone of the vector. In a previous publication we reported that Fz when expressed under the control of the *sev* enhancers-heatshock promoter gave a dominant *fz* phenotype in the eye (Tomlinson et al., 1997). These original constructs were engineered in a pW8 transformation vector. However when we switched to a derivative of Carnegie 20 (C2NXT) (Struhl et al., 1993) in which the *ry*$^+$ gene had been removed, the dominant effect of the *sev-fz* was lost. We suspect the presence of eye enhancer sequences in the *white* gene that up-regulate transcription levels in the pW8 versions.

Over-expression in the wing

High levels of expression of the various genes were obtained through the use of the C765 wing-specific) GAL4 driver (Guillan et al., 1995). Wings were removed in 75% ethanol and fixed in GMM as per standard methods.

Clone induction

Eyes

For *sev-fz* clones in *fz* mutant eyes flies of the following genotype were heat shocked at 35°C for 1 hour. *y, w hs-flip; sev > w+> fz; fz^{KD4A}/fz^{H51}*.

Similar genotypes were used for other *sev-fz* constructs, the *sev-fz2* and the *sev*-chimera constructs. When constructs were located on the third chromosome they were recombined with a *fz* allele.

Wings

fz, fz2 mutant clones were induced in flies in a tub-fz background of the following genotype. y,w, hsflip; tub > w+ > fz/+;$fz2^{C1}$,fz^{H5},ri,FRT 2A/hs-CD2,y^+ w^+,M(3L)i55,ri,FRT 2A.

24-48 hour larvae were heat-shocked at 37°C for 1 hour. The *cis* recombination between the FRTS excising the w+ flip-out cassette occurs with significantly higher efficiency than the *trans* recombination required to generate the *M^+, fz, fz2* clones. *yellow* mutant clones were never observed in control wings (identical except lacking the *tub > w+ > fz*). The rescue of the *tub*-chimeras was assayed in the same way. The presence of the *tub* constructs in flies that had *yellow* patches in the wings was verified by PCR. In no cases was there *yellow* tissue in the wings where the constructs were absent.

RESULTS

Generation of chimeric receptors

A detailed description of the generation of the chimeras is given in the Methods. The proteins were divided into three domains – the N terminus (terminating close to the first transmembrane domains); the transmembrane domain region (TM) containing all seven transmembrane helices and associated intra- and extra-cellular loops; the C terminus beginning close to the last transmembrane domain and extending to the C-terminal end of the proteins (Fig. 1A). For simplicity of description we use '1' to signify a domain from Fz and '2' for a domain of Fz2. The regional identity of the domain (N,TM or C) is given by its position in the sequence describing the chimera – N/TM/C. For example **1/2/1** refers to a chimera containing the N terminus of Fz, the TM region of Fz2 and the C terminus of Fz. We made all chimera combinations except **2/1/2**.

Rescuing abilities of the chimeric receptors

Rescue of Wg transduction in the wing

The wing margin is the interface between the dorsal and ventral surfaces of the wing. During development reciprocal signaling between the dorsal and ventral cells about the margin leads to the marginal expression of Wg. From this medial position Wg organizes growth and patterning in the dorsoventral axis of the wing from a very early stage of wing development (Rulifson et al., 1996). The inability of cells to transduce Wg leads to their exclusion from the wing epithelium. For example *fz, fz2* clones fail to survive in the wing (Chen and Struhl, 1999).

Fz2 expressed under the tubulin promoter (*tub-fz2*) rescues

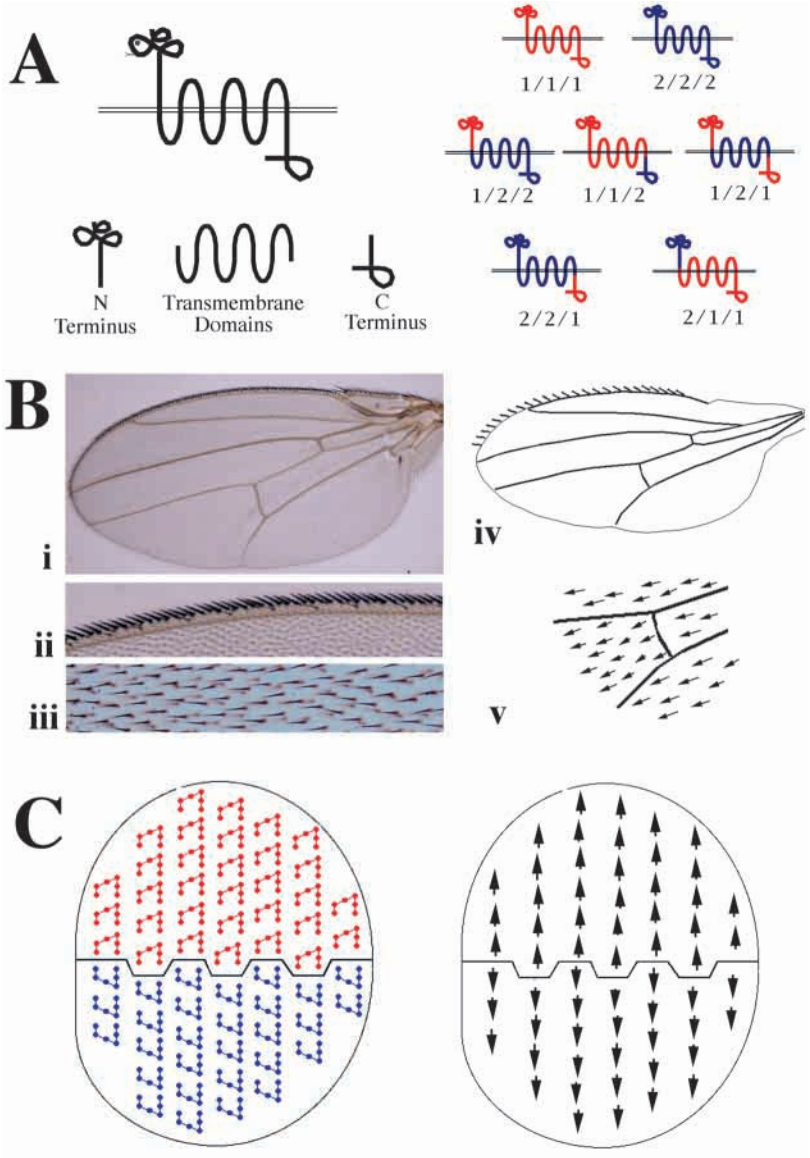

Fig. 1. Frizzled receptors, their chimeras and the phenotype they control. (A) The Frizzled class proteins are serpentine receptors. Each has a large extra cellular domain (N terminus) with a cysteine-rich domain (loops), a transmembrane domain region containing the seven transmembrane helices and the associated intra- and extra-cellular loops, and an intracellular domain (C terminus) of variable length. Chimeras were constructed by splicing the Fz (red) domains to Fz2 (blue) domains. (B) The wing (i) contains two features relevant to the signaling of the two receptors. Wg signaling organizes the wing margin, most notably the large bristles on the anterior margin (ii, iv). On the blade of the wing each cell is decorated with a small hair. Collectively the small hairs point to the distal end of the wing (iii, v). (C) The shape of the ommatidia indicate the polarity in the eye (the different chiral forms are shown as red or blue). In each half of the eye all ommatidia are of the same shape and this indicates polarity running from the midline of the eye towards the dorsal and ventral extremes (arrowheads).

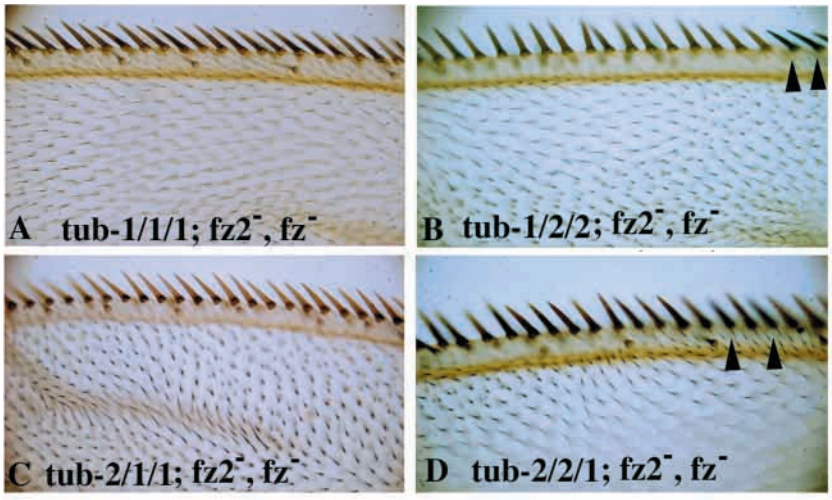

Fig. 2. Rescue of Wg signaling in the wing. *Minute* clones of $fz2^{C1}$,fz^{H5} were induced in wings in the presence of (A) *tub-**1/1/1***, (B) *tub-**1/2/2***, (C) *tub-**2/1/1*** and (D) *tub-**2/2/1***. Clones were marked by the loss of the *yellow* marker. Arrowheads indicate non-mutant (*yellow*) bristles. In the presence of the *tub* constructs large healthy patches of mutant tissue identified by *yellow* bristles are present. Polarity defects are not rescued. The 3 chimeras shown are those that failed to rescue polarity in the eye. All chimeras tested showed rescue.

Wg transduction in *fz, fz2* mutant wing patches (Chen and Struhl, 1999). Similarly *tub-fz* rescues *fz, fz2* clones in the wing (Fig. 2A). In this experiment the *tub-fz* construct was activated concomitantly with the induction of *fz, fz2* clones. The clones were marked by *yellow*, a color marker for the bristles. In the absence of *tub-fz* no *yellow* clones were observed in the wings, but in its presence large patches (the Minute technique was used) of *yellow* margin bristles were observed. Thus Wg transduction in the *fz, fz2* mutant tissue was restored by the presence of the *tub-fz* construct, but polarity defects associated with the clones were not rescued. Whether the levels were too high (hypermorphic condition) or too low (hypomorphic condition) to transduce the polarity signal appropriately remains unclear. *tub-fz2* similarly rescued Wg transduction but not polarity transduction in this assay (data not shown). Five chimeric proteins were then similarly tested (**1/1/2, 1/2/2, 1/2/1, 2/1/1** and **2/2/1**) and all rescued wing morphology (transduced the Wg signal) but failed to rescue wing polarity (3 examples are shown in Fig. 2B,C,D). From this we inferred that all five chimeras were functional Wg receptors.

Rescue of polarity transduction in the eye

The *Drosophila* eye contains many hundred ommatidia. At the core of each ommatidium lie the photoreceptors arranged in a characteristic trapezoidal pattern (Dietrich, 1909). The trapezoids occur in two chiral forms. We color-code these as red and blue. Each dorsal or ventral half of an eye contains only one chiral form and the other half contains the other chiral form. The shape of the ommatidia indicate their polarity in the dorsoventral axis (Fig. 1C). In *fz* mutants, polarity information is lost and red and blue chiral forms are found interspersed (Fig. 3B). In addition a third (black) symmetrical form is also found (Zheng et al., 1995; Tomlinson et al., 1997). Fz appears to be a critical transducer of the polarity signal that directs the ommatidia to their appropriate chiral form. Fz function is only required in the R3 and R4 presumptive photoreceptors for the appropriate chiral choice, and the *sevenless* (*sev*) enhancer drives in these two cells at the appropriate stage (Basler et al., 1989). In earlier work we described the dominant effects of *sev-fz* constructs (Tomlinson et al., 1997). However, when we engineered new *sev-fz* constructs in a different vector backbone (see Materials and Methods) the dominant phenotype did not occur, and when crossed into a *fz* mutant background the polarity phenotype was rescued (Fig. 3C; Table 1). In *fz* mutant eyes, correct ommatidia occur at approximately 50% (Table 1) and in the presence of a *sev-fz* (wild-type gene) the number of correct ommatidia rose to 86% (Fig. 3C; Table 1). In contrast, *sev-fz2* constructs showed no rescue (Table 1). The chimeras were tested in this manner and two of them showed significant rescue of polarity transduction; *sev-**1/1/2*** rescued to 93% and *sev-**1/2/1*** rescued to 76% (Fig. 3C,D; Table 1). In both cases the rescue was statistically significant compared to the *fz* mutant alone, and both rescues were not significantly different from that of *sev-fz*. The three other chimeras *sev-**2/2/1**; sev-**2/1/1*** and *sev-**1/2/2*** showed no rescue of the *fz* phenotype (Table 1). Thus of the five chimeras able to transduce the Wg signal, two (**1/1/2** and **1/2/1**) were also able to transduce the polarity signal. These results suggested that the presence of the Fz N terminus was critical for the transduction of the polarity signal but not sufficient since *sev-**1/2/2*** did not rescue. In addition to the presence of the Fz N terminus one other domain of Fz was required, either the TM domain (as in **1/1/2**) or the C terminus (as in **1/2/1**).

Table 1. The rescue ability of chimeric forms of the Frizzled proteins

Construct in *fz* mutant background	% ommatidia with correct chiral shape
No construct	47.1±8.9 (*n*=240)
1/1/1	86.0±5.1 (*n*=410) (*P*<0.001)
2/2/2	48.3±4.7 (*n*=292) (*P*=0.236)
1/1/2	93.3±5.0 (*n*=587) (*P*<0.001)
2/2/1	50.3±5.4 (*n*=360) (*P*=0.19)
1/2/1	76.5±6.4 (*n*=400) (*P*<0.001)
2/1/1	47.6±6.1 (*n*=370) (*P*=0.40)
1/2/2	51.6±5.7 (*n*=188) (*P*=0.14)

Ommatidia were scored for whether or not they had adopted the correct chiral shape for their location in the eye, when expressing the relevant construct. A standard *t*-test was performed on the data to determine if each genotype resulted in a statistically significant difference from the 'no construct' control. In all cases, these results were scored in a fz^{H51}/fz^{KD4A} transheterozygote background.

Over-expression experiments

The conclusions we drew form the rescue experiment differed from those of Boutros et al. (Boutros et al., 2000) who argued that the C terminus was the critical determinant of which

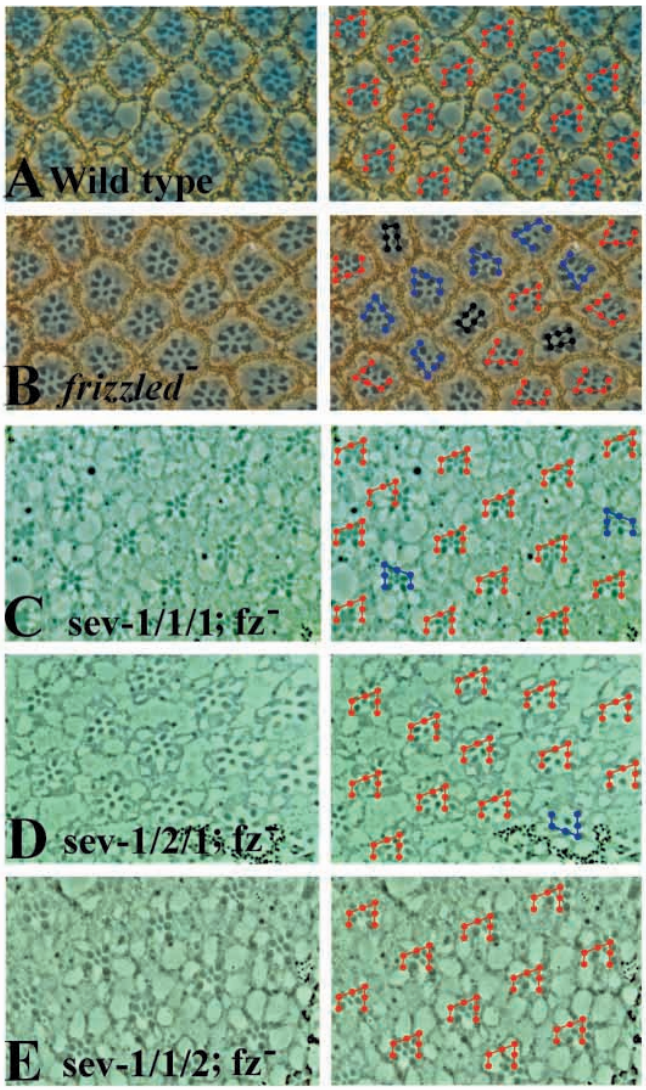

Fig. 3. Rescue of *fz* mutant phenotypes in the eye. (A) Wild-type eyes show ommatidia uniformly organized and of the same chiral form (color). (B) *fz* mutant eyes show deregulation of the shape and orientation of the ommatidia. (C-E) When expressed under *sev* transcriptional control two of the chimeras show clear rescue of the *fz* phenotype. (C) *sev-fz* ***(1/1/1)***; *fz. (D) sev-**1/2/1***; *fz* and (E) *sev-**1/1/2***; *fz*. Only the chimeras that rescue are shown, those that failed are shown in the Wg rescue assay of Fig. 2.

pathway was activated by a receptor. These results were drawn from experiments studying which of the two pathways (Wg or polarity) was activated when chimeric receptors were over-expressed using the UAS/Gal4 system (Brand and Perrimon, 1993). To address the contradictions between the two sets of results, we too performed over-expression studies with chimeras we had constructed.

Over-expression of Fz and Fz2 results in distinct phenotypes

Fz2 and Fz were over-expressed in the developing wing under the control of the C765 Gal4 line that drives expression at high levels in the majority of wing cells (Guillan et al., 1995). Over-expression of Fz2 in this manner induced ectopic marginal bristles usually close to the wing margin (Fig. 4G,H) and over-expression of Fz caused polarity phenotypes throughout the wing blade and margin (Fig. 4E,F). These results are consistent with those previously obtained (Boutros et al., 2000; Rulifson et al., 2000; Krasnow et al., 1995; Zhang and Carthew, 1998).

Over-expression of chimeric proteins results in similar phenotypes

Three chimeric forms of the Fz and Fz2 proteins (**1/1/2**, **1/2/2**, **2/1/1**) were then over-expressed using the same method as the full-length forms of the Fz and Fz2 proteins. All three chimeric forms gave the same basic phenotype of ectopic margin bristles and disturbed polarity (two are shown Fig. 4I,J and K,L). These phenotypes represented the additive effects of the misexpression of wild-type Fz2 (ectopic margin bristles) and wild-type Fz (disturbed polarity). We suspended this approach at this point because of problems with interpretation of the data. For example, when polarity effects occurred in the wings we did not know whether they were caused because the chimeras positively activated the polarity pathway or dominantly suppressed it. This and the other interpretation problems are addressed at more length in the Discussion.

DISCUSSION

Fz2 functions as a Wg receptor and Fz functions both as a Wg receptor and as a receptor for the polarity signal. The experimental goals have been to map critical regions of the receptors required for the two distinct pathways. Experimentally the approach has been to subdivide the receptors into three domains and then create various chimeric forms of the two receptors. The chimeras were then assayed for their signaling properties in the Wg and polarity pathways. First, the ability of the chimeras to rescue tissue mutant for the receptors was tested. Second, the effects of over expression of the chimeras were compared with the over-expression phenotypes of the native receptors.

From the rescue experiments we inferred that all chimeras assayed behaved as functional Wg receptors but only a subset was able to rescue polarity signaling in *fz* mutant tissue. Comparison of the chimeras that rescue polarity signaling with those that do not suggests that the N-terminal domain of Fz is critical for the transduction of the polarity signal but that alone it is not sufficient. In addition to the N terminus, one of the two other Fz domains is required. Thus the specificity for signal transduction appears spread through the three domains of the protein. The simple way we view this is that ligand binding requires the N terminus, and that transmission of the signal to intracellular molecular machinery can be achieved by at least one of two distinct sites in the remainder of the protein. We are currently attempting to map these three signaling sites more finely.

Our interpretations are in contrast to the conclusions of Boutros et al. (Boutros et al., 2000) who argued from over-expression studies that the C terminus was sufficient to determine which pathway was activated. Specifically, we note that *sev-**1/1/2*** which carries the Fz2 C terminus is still able to transduce the polarity signal. However, the chimeras were not constructed in the same manner particularly in the region of the N-terminal replacements (see below), but this is an unlikely explanation for the discrepancy. Rulifson et al. (Rulifson et

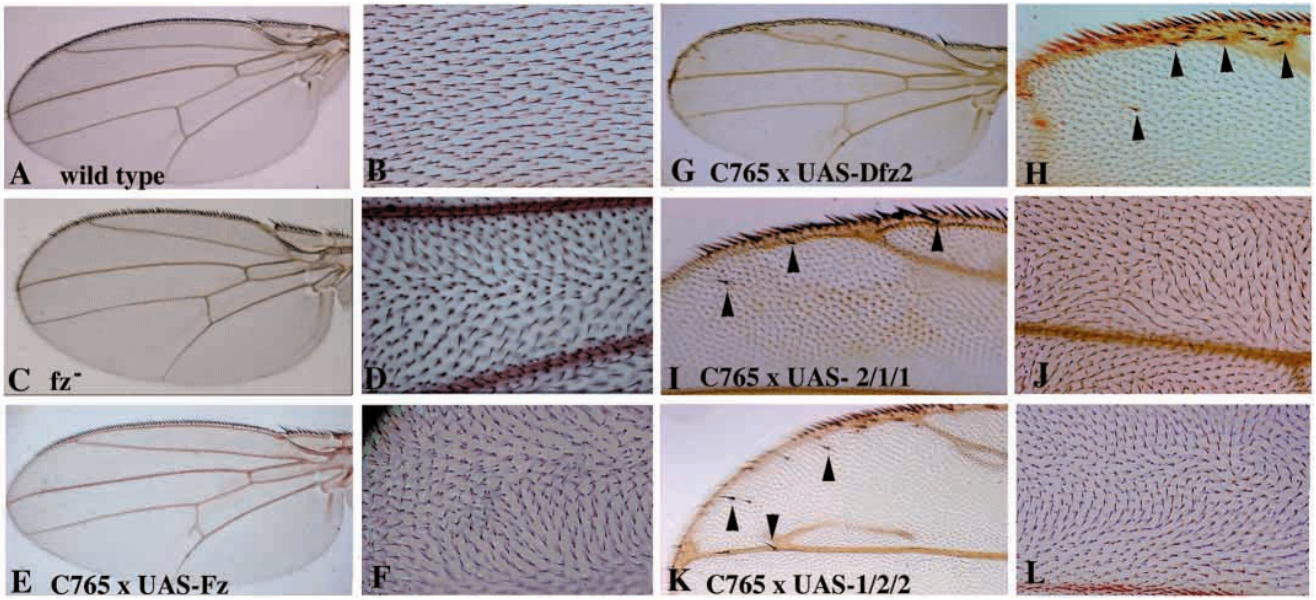

Fig. 4. Over-expression studies in the wing. In a wild-type wing the large bristles are confined to the anterior wing margin (A) and hairs on the wing blade are uniformly organized pointing towards the distal parts of the wing (B). In *fz* wings the margin bristles show polarity defects (C) and a severe polarity disruption is evident in the wing blade hairs (D). Over-expression of UAS-Fz using the C765 Gal4 driver line phenocopies *fz* mutant wings. The margin bristles (E) and wing blade hairs (F) show clear polarity defects. Over-expression of Fz2 does not affect polarity but ectopic margin bristles occur (G,H), usually close to the margin but some can be in the main blade region (arrowheads). Over-expression of the two chimeras **2/1/1** and **1/2/2** induces ectopic margin bristles (I,K) and polarity defects (J,L).

al., 2000), suggested that the CRD of the Fz-class proteins determines the specificity of pathway activation. Our results agree in as much as the Fz N terminus appears critical to activate the polarity pathway, but suggest that it is not in itself sufficient for that purpose.

The rescue assays

The rescue experiments investigate which domains must be present in the chimeras for them to transduce each of the signals? In the case of Wg transduction it appears that all chimeras are functional receptors and the Fz and Fz2 domains are interchangeable. This is not surprising given that both Fz and Fz2 themselves are Wg receptors. But a further value of this result lies in its control for the chimeras that fail to rescue polarity signaling – those that fail to rescue polarity do rescue Wg transduction, which suggests they are at least functional generic receptors. The failure of these receptors to rescue polarity signaling suggests that they lack specific domains required for that polarity signaling. However, the formal possibility remains that their failure to rescue polarity signaling results from other features of the chimeras such as reduced protein levels resulting from protein instability or translational inefficiency or such like. But we note that only low levels of Fz are normally required for polarity transduction and that at least two of the constructs that failed to rescue polarity transduction (**1/2/2** and **2/1/1**) resulted in strong polarity phenotypes when over-expressed in the wing.

Over-expression studies

The N-terminal region of the Fz class receptors contains two distinct domains – the CRD positioned close to the N terminus of the protein and the non-conserved region (NCR) that lies between the CRD and the first transmembrane domain. In our chimeras we cleanly exchanged the N-termini of the two proteins at a site directly adjacent to the plasma membrane. Other studies exchanged only the CRDs of the two receptors, and these exchanges were accompanied by deletions and/or duplications of portions of the NCR of each of the proteins (Rulifson et al., 2000; Boutros et al., 2000).

Over-expression of a chimera in which the Fz2 CRD was exchanged for the Fz CRD in an otherwise Fz2 protein induced ectopic bristles but no polarity defects (Boutros et al., 2000; Rullifson et al., 2000). In contrast the **1/2/2** reported here induced both effects. This suggests a role for the NCR and we are presently investigating the differential functions of the NCR and the CRD.

Although the over-expression studies generated distinct phenotypes we were unable to adequately interpret them in terms of where signaling specificity resides in the Fz-class proteins. The reasons for this are as follows.

(1) Polarity phenotypes. Polarity phenotypes cannot be easily classified into those caused by ectopic or hyperactivation of the polarity pathway from the effects caused by down regulation of the pathway. For example, when the chimera **2/1/1** is over-expressed in the wing, do the polarity phenotypes result from hyperactivation of the polarity pathway because of the abundance of an active Fz C terminus or, does it result from a dominant negative effect such that an impotent (no ligand binding domain on the outside) C terminus sequesters down-stream components from wild-type Fz receptors?

(2) Wg phenotypes. The appearance of ectopic wing margin bristles has long been thought to result from ectopic Wg pathway activation. For example ectopic activation of Armadillo (Arm) or loss of *shaggy* (*sgg*) gene function both result in ectopic margin bristles (Blair, 1992; Zecca et al., 1996). When we observe ectopic bristles resulting from over-

expression of Fz2 or the chimeras we infer therefore the activation of the Wg pathway. The problem arises with Fz. It is equally capable, with Fz2, of transducing Wg in almost all tissues tested (Chen and Struhl, 1999). *fz2* flies show no phenotype, only when *fz* is concomitantly removed does the wg mutant phenotype emerge. Furthermore, *tub-fz2* or *tub-fz* appears to rescue Wg transduction in *fz, fz2* mutant tissue equally well. The problem then is that over-expression of Fz does not lead to the ectopic margin bristles seen with over-expression of Fz2. Thus in over-expression studies the absence of ectopic bristles does not indicate an inability to transduce Wg, and must be viewed as relatively meaningless as an assay for determining differences in signaling specificity.

Conclusions

We report here that all chimeric forms of Fz and Fz2 made were functional Wg receptors but only a subset was polarity receptors. Those that transduced the polarity signal had the Fz N terminus and one of the two other (TM and C) domains. Thus we have mapped a critical role to the N terminus but other sequences required in addition for the signal transduction to occur are redundantly distributed between the two other domains

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