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Trimeric G Protein-Dependent Frizzled Signaling in *Drosophila*

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Summary

Frizzled (Fz) proteins are serpentine receptors that transduce critical cellular signals during development. Serpentine receptors usually signal to downstream effectors through an associated trimeric G protein complex. However, clear evidence for the role of trimeric G protein complexes for the Fz family of receptors has hitherto been lacking. Here, we show roles for the $G\alpha_o$ subunit (Go) in mediating the two distinct pathways transduced by Fz receptors in *Drosophila*: the Wnt and planar polarity pathways. Go is required for transduction of both pathways, and epistasis experiments suggest that it is an immediate transducer of Fz. While overexpression effects of the wild-type form are receptor dependent, the activated form (Go-GTP) can signal when the receptor is removed. Thus, Go is likely part of a trimeric G protein complex that directly tranduces Fz signals from the membrane to downstream components.

Introduction

Two distinct classes of signals are transduced across plasma membranes by Frizzled (Fz) serpentine receptors. One is the Wnt family of secreted glycopeptides that mediate cell-to-cell signaling (Wodarz and Nusse, 1998). The other signal organizes planar cell polarity (PCP), in which epithelial cells are coordinately polarized within the plane of the epithelium (Adler, 2002). The Wnt pathway mediates cell fate-specifying signals during development, and inappropriate activation of this pathway can be oncogenic (Varmus et al., 1986). Thus, from the developmental and medical perspectives, understanding the mechanisms of Wnt signaling is of prime importance. PCP appears similar to chemotaxis or axon growth cone guidance in which cells decode extracellular gradients to direct the appropriate polarizations of their cytoskeletons. Hence, understanding signal transduction in PCP addresses the mechanisms that polarize cells.

Serpentine receptors are heptahelical transmembrane proteins that are typically coupled to trimeric G protein complexes (Gilman, 1987). A G protein complex consists of a GDP bound α subunit and a $\beta\gamma$ dimer and associates with intracellular portions of receptors. Activated receptors catalyze exchange of GDP for GTP on $G\alpha$ subunits, and dissociation of the complexes follow; the released $G\alpha$-GTP and $\beta\gamma$ moieties are then free to engage downstream effectors. With time, the α subunit hydrolyzes GTP to GDP, engendering the reformation of the complex and reassociation with the receptor.

Although Fz proteins are serpentine receptors, roles for trimeric G proteins in their transduction have not yet been clearly shown, and it has been argued that Fz signaling may be G protein independent (Brzostowski and Kimmel, 2001). A body of evidence, however, suggests that Fz signaling is indeed G protein dependent (Malbon et al., 2001). For example, the effects of an activated chimerical β2-adrenergic receptor containing intracellular loops and C terminus of a rat Fz could be blocked by $G\alpha$-inhibiting agents (Liu et al., 2001).

In the fly, Frizzled (Fz) transduces Wnt-1 (Wingless [Wg]) and the unknown PCP ligand, while *Drosophila* Frizzled 2 (Fz2) transduces Wg but not PCP signals (Bhanot et al., 1996; Bhat, 1998; Kennerdell and Carthew, 1998; Chen and Struhl, 1999; Vinson and Adler, 1987). To date, Dishevelled (Dsh) is the only other protein known to be utilized by both pathways (Perrimon and Mahowald, 1987; Gubb and Garcia-Bellido, 1982). Dsh lies high in the pathways and has different parts dedicated to the two pathways. Thus, Dsh can be seen as the branch point in the transduction of the two signals (Axelrod et al., 1998; Boutros et al., 1998).

In the Wg pathway, Shaggy (Sgg, a kinase known as GSK-3 in vertebrates) lies downstream of Dsh (Siegfried et al., 1990). Sgg phosphorylates Armadillo (Arm, the fly β-catenin) and targets it for degradation (Peifer et al., 1994). Binding of Wg to Fz signals through Dsh to inhibit the Sgg phosphorylation of Arm; Arm, now stabilized, translocates to the nucleus, associates with members of the LEF/TCF transcription family, and affects target gene transcription (Brunner et al., 1997; Riese et al., 1997; van de Wetering et al., 1997). In the wing, the target genes include *distalless* (*dll*) and *vestigial* (*vg*), which are used in the paper as meters of Wg transduction.

The PCP pathway downstream of Dsh is less understood; a number of proteins are involved, including Prickle, Strabismus/van Gogh, and Flamingo, but how they interrelate remains unclear (reviewed in Adler [2002]). These PCP proteins redistribute within cells during polarization. In the wing, Fz first accumulates in the apical membrane, but, by 30 hr after puparium formation (APF), it is strikingly localized to the distal end of the cell, at the place where the hair will grow out (Strutt, 2001). Dsh (in the cytoplasm) is first recruited to cortical regions and then localizes with Fz to the distal end

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(Axelrod, 2001), while Strabismus/van Gogh localizes to the proximal end (Bastock et al., 2003). These relocalizations, including the Fz relocalization, are all controlled by Fz signaling (Axelrod, 2001; Strutt, 2001). It appears that there is a two-step process. First, Fz signaling through Dsh decodes an extracellular signal that defines the distal end of the cell. Using this information, Fz and the other PCP proteins then redistribute and adopt polarized localizations within the cells.

Here we examine the role of the fly $G\alpha_o$ subunit (Go) in Fz signaling, and find that it is required for the transduction of both the Wg and PCP pathways. Overexpression of wild-type Go activates signaling in both pathways, as does a constitutively active form (Go-GTP). The overexpression effects in the Wg pathway require the presence of previously identified transduction elements (Dsh, Sgg, Arm), and, in both the Wg and PCP pathways, overexpression effects of the wild-type form are receptor dependent. In contrast, the activated form (Go-GTP) can signal when the receptor is removed. Thus, Go is likely part of a trimeric G protein complex that directly tranduces Fz signals from the membrane to downstream components.

Results

Expression of Pertussis Toxin Modulates Fz Signaling

Pertussis toxin (Ptx) and cholera toxin (Ctx) modulate the activities of different types of α subunits (Moss and Vaughan, 1988). We transformed flies with *UAS-Ptx* and *UAS-Ctx* and crossed them to various Gal4 driver lines. Only Ptx showed phenotypes consistent with modulation of Fz signaling.

In the eye, PCP phenotypes result when *fz* is overexpressed under *sevenless* (*sev*) transcriptional control (*sev-fz*) (Strutt et al., 1997; Tomlinson et al., 1997). Overexpression of Fz (via *sev* transcriptional control) disturbs the cells' abilities to decode the PCP signals that direct the shapes of the ommatidia, and many are incorrectly formed. Ptx suppressed the effects of Fz overexpression: in *UAS-fz, UAS-Ptx* flies, the number of incorrect ommatidia dropped from 30% ($\pm$2.7, n = 1056) to 17% ($\pm$1.4, n = 2337). The ability of Ptx to attenuate Fz signaling suggested that there was a Ptx-sensitive G protein downstream of Fz.

Ptx ADP ribosylates a cysteine residue 4 aa from the C terminus of $G\alpha$ subunits (Moss and Vaughan, 1988), preventing coupling of the $G\alpha$ to its serpentine receptor. The fly genome encodes six $G\alpha$ subunits, of which only the *G-oα47A* (*brokenheart*: hereafter referred to as *Go*) gene encodes a cysteine at position -4. Given that Go is the only potential target for Ptx action, this represents the candidate Ptx-sensitive $G\alpha$.

Go Clones Compromise Wg Transduction

Go mutants are late embryonic lethal (Fremion et al., 1999). We therefore examined clones of *Go* induced during different stages of development. We used a hypomorphic allele (*Go[007]* [Fremion et al., 1999]) and generated a null allele (*Go[0611]* [see Experimental Procedures]). In all tissues examined, only late-induced clones survived into the adult. Thus, Go likely performs a vital function in cells (in addition to any role in Fz signaling).

In wings, *Go[007]* clones induced the loss of the wing margin (Figure 1A), and, in wing discs, clear effects on Wg transduction were observed as evidenced by the effects on target gene expression. *vg* and *dll* (direct targets) and *cut* (indirect) were all downregulated, as evidenced by protein expression (Figures 1B–1J). *Go[0611]* clones were less viable but showed similar phenotypes when recovered (data not shown). Not all clones showed these effects, and, in those that did, not all cells were equally affected. This lack of penetrance was expected; first, because surviving *Go* clones by inference retain some gene function (and, hence, the inferred capacity to transduce Wg); second, cells lacking Wg transduction do not persist in the developing wing blade (Zecca et al., 1996), and so there is a selection for cells maintaining Wg signaling.

Notch (N) activity at the wing disc dorsoventral (D/V) boundary signals local cells to secrete Wg that diffuses to pattern the developing wing. *Go* clones appear deficient in Wg transduction rather than in Wg expression, since Wg protein levels remained unchanged in *Go* clones that invaded the territory of the Wg expression stripe (arrow in Figures 1M–1O). Furthermore, *Go* clones adjacent to the Wg expression stripe (arrowhead) showed the ectopic Wg expression characteristic of cells compromised for Wg signaling in that position (Rulifson et al., 1996). *Go* clones also sometimes showed ectopic expression of *homothorax* (*hth*) (Figures 1I–1L), which can occur in cells lacking Wg transduction (Azpiazu and Morata, 2000). Expression of Wg and Hth suggests that *Go* cells turned down Wg targets because they were compromised for Wg signaling rather than because they were merely dying.

Overexpression of Go Activates Wg Target Genes

The analysis of *Go* clones suggested that Go was critically required for Wg transduction. To test the effects of overexpression, *Go* was expressed in the developing wing under UAS control using *hedgehog-Gal4* (*hh-Gal4*, which drives at high levels exclusively in the posterior wing compartment). Upregulation of Vg and Dll protein levels occurred in the posterior compartment of *hh-Gal4; UAS-Go* wing discs (Figures 2A–2D). Thus, overexpression of Go increased the protein levels of Wg target genes and by inference stimulated Wg transduction above wild-type levels.

Activated serpentine receptors exchange GDP for GTP on $G\alpha$ subunits. Two mutant forms of *UAS-Go* were transformed into flies: one in which Go was maintained in the GTP bound state and the other in the GDP bound state (see Experimental Procedures). *hh-Gal4; UAS-Go-GTP* showed upregulation of Vg (data not shown) and Dll in the posterior compartment at levels similar to *UAS-Go* (Figures 2E and 2F). This suggests that, in Wg/Fz signaling, conversion of Go-GDP to Go-GTP is a key event. In contrast to Go-GTP, *hh-Gal4; UAS-Go-GDP* did not show any effects on the Wg targets (Figures 2G and 2H, see Discussion).

Wg expression remained unchanged when Go was overexpressed (Figures 2I–2L), indicating that the upregulation of the genes was not caused by upregulation of Wg itself.

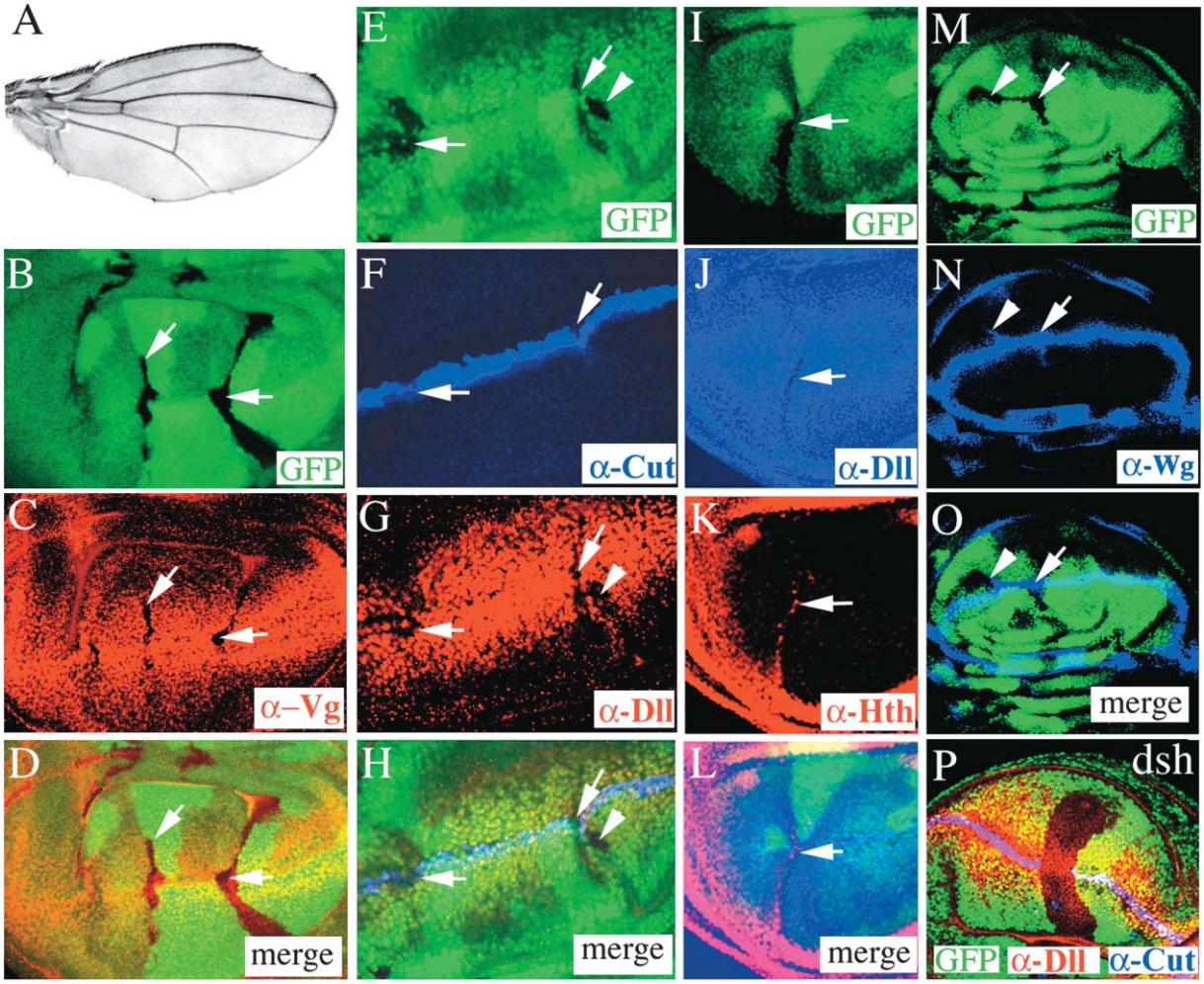

Figure 1. Go[007] Wing Clones Show Loss of Wg Transduction

(A) Clones in the adult wing induce loss of the wing margin.

(B–D) *Go* clones marked by the loss of GFP show associated loss (arrows) of Vg expression (red). (D) is the merge of (B) and (C).

(E–H) *Go* clones (E) show associated loss (arrows) of Cut expression (blue) and Dll (red). (H) is the merge of (E), (F), and (G).

(I–L) *Go* clones show loss of Dll (blue) and associated ectopic expression of Hth (red), indicated by arrows. (L) is the merge of (I)–(K).

(M–O) *Go* clones show no loss of Wg expression (blue). Arrow indicates normal Wg expression in a *Go* clone straddling the Wg stripe. Arrowhead indicates ectopic Wg expression in a clone adjacent to the Wg stripe.

(P) Clones of *dsh[V26]* (absence of GFP) remove Cut expression (blue) and reduce Dll expression (red).

Also, Cut (which requires both Wg and N inputs [Neumann and Cohen, 1996]) also remained unchanged, further arguing that Go activates Wg rather than N signaling.

Positioning Go in the Wg Transduction Cascade

If Go represented an immediate transducer of Fz signaling in the Wg pathway, then it should lie upstream of Dsh, Sgg, and Arm, and epistasis experiments were performed to test this.

Clones of *arm* were induced in *hh-Gal4; UAS-Go-GTP* wing discs, and Dll levels were monitored. In anterior clones, some residual staining was observed, likely reflecting the persistence of *arm* function in surviving cells (data not shown). In posterior clones, where hyperactivation of Dll normally occurred, a downregulation of staining to the level seen in anterior clones was observed (Figures 3A–3D). Thus, *arm* is necessary for the effects of overexpression of Go to be manifest.

Sgg functions negatively in Wg transduction such that its inappropriate expression leads to pathway downregulation. *hh-Gal4; UAS-sgg* was embryonic lethal, so *decapentaplegic-Gal4* (*dpp-Gal4*) was used as the driver line. *dpp-Gal4; UAS-Go-GTP* wing discs show an upregulation of Dll in the *dpp* expression domain (Figure 3E, arrow). *dpp-Gal4; UAS-sgg* wing discs showed a loss of Dll expression in the *dpp* expression domain (Figure 3F, arrow), and, in *dpp-Gal4; UAS-Go-GTP; UAS-sgg* wing discs, no Dll expression was detected in the *dpp* expression domain (Figure 3G). Thus, Wg target gene induction by Go was blocked by inactivation of the Wg pathway, which suggests that Go lies upstream of Sgg.

Dsh lies upstream of Sgg in Wg transduction. Figures 3H–3K show two *dsh* clones induced in a *hh-Gal4; UAS-*

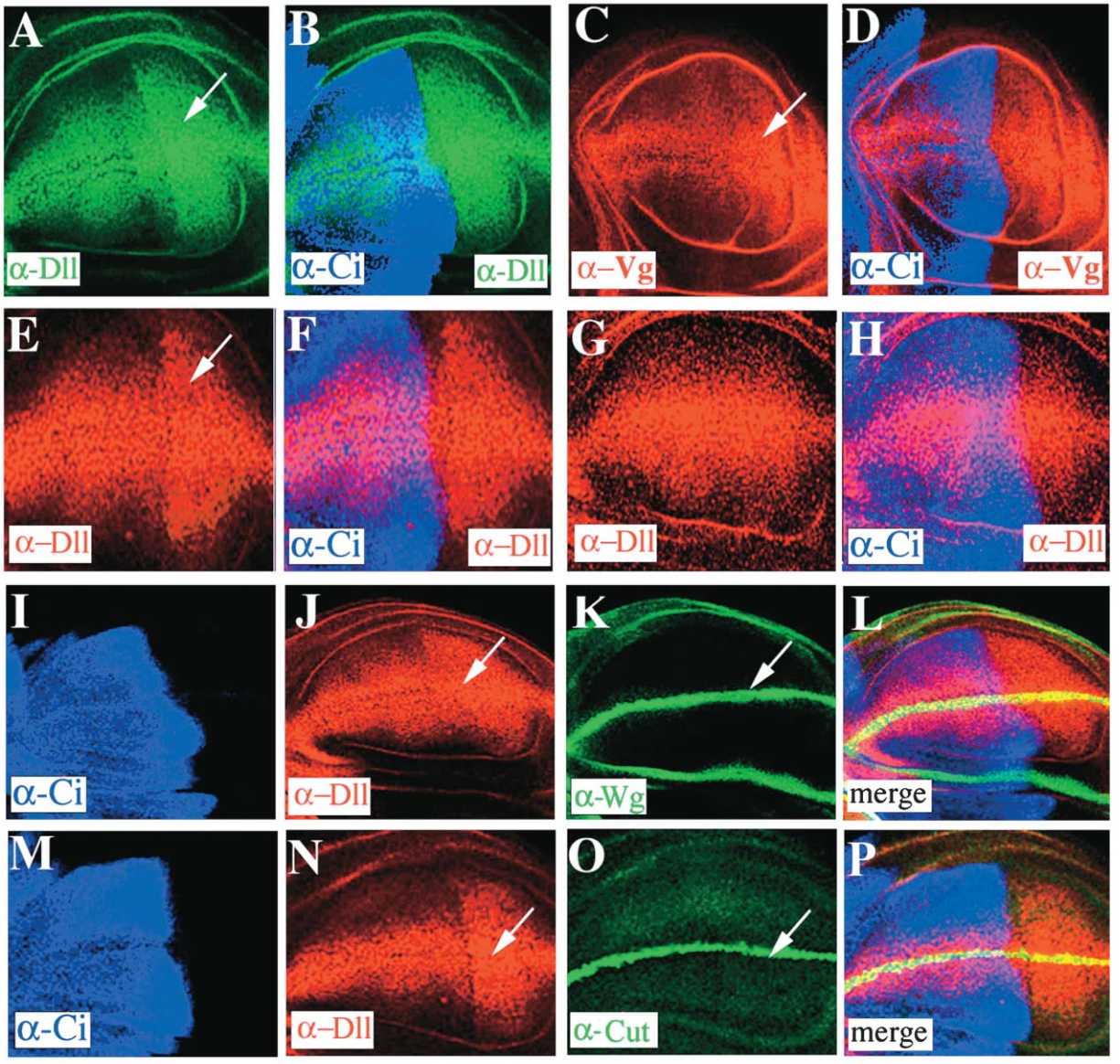

Figure 2. Overexpression of Go Activates Dll and Vg Expression but Does Not Affect Wg or Cut Expression

In all discs, Ci (blue) was used to stain anterior compartments, and *hh-Gal4* was used to drive *UAS* expression in posterior compartments (nonblue regions). (A and B) A *UAS-Go* wing disc stained for Dll (green). In the posterior compartment, Dll expression is upregulated (arrow). (C and D) A *UAS-Go* wing disc stained for Vg (red). In the posterior compartment, Vg expression is upregulated (arrow). (E and F) A *UAS-Go-GTP* wing disc stained for Dll (red). In the posterior compartment, Dll is upregulated (arrow). (G and H) A *UAS-Go-GDP* wing disc stained for Dll (red). In the posterior compartment, there is no change in Dll expression. (I–L) A *UAS-Go* wing disc stained for Dll (red) and Wg (green). Upregulation in of Dll ([J], arrow) is not accompanied by any change in Wg expression ([K], arrow). (L) shows a merge of (I)–(K). (M–P) A *UAS-Go-GTP* wing disc stained for Dll (red) and Cut (green). Upregulation of Dll ([N], arrow) is not accompanied by any change in Cut expression ([O], arrow). (P) shows a merge of (M)–(O).

Go wing disc, one in the anterior (in which Go was not overexpressed) and one in the posterior compartment (where Go was overexpressed). In both clones, there was a reduction in Dll levels, but some signal remained, particularly when close to the source of Wg at the D/V border. The posterior clone (arrow) shows equivalent loss of Dll to the part of the anterior clone (arrowhead) that is similarly distant from the D/V border, indicating that Dsh is required for the overexpression of Go to hyperactivate the Wg targets.

We next examined the dependence of overexpression of Go and Go-GTP on the two Wg receptors, Fz and Fz2. If Go were part of the trimeric complex that transduces these receptors, then the overexpression effects of the wild-type form of Go should require the receptors to catalyze the nucleotide exchange; conversely, the Go-GTP form should be receptor independent. This is what we found. Clones of *fz, fz2* were induced in the Go-overexpression discs, and Dll expression levels were examined. Again, Dll expression was not completely eliminated from clones in the anterior compartment where overexpression did not occur: 19 of 84 clones

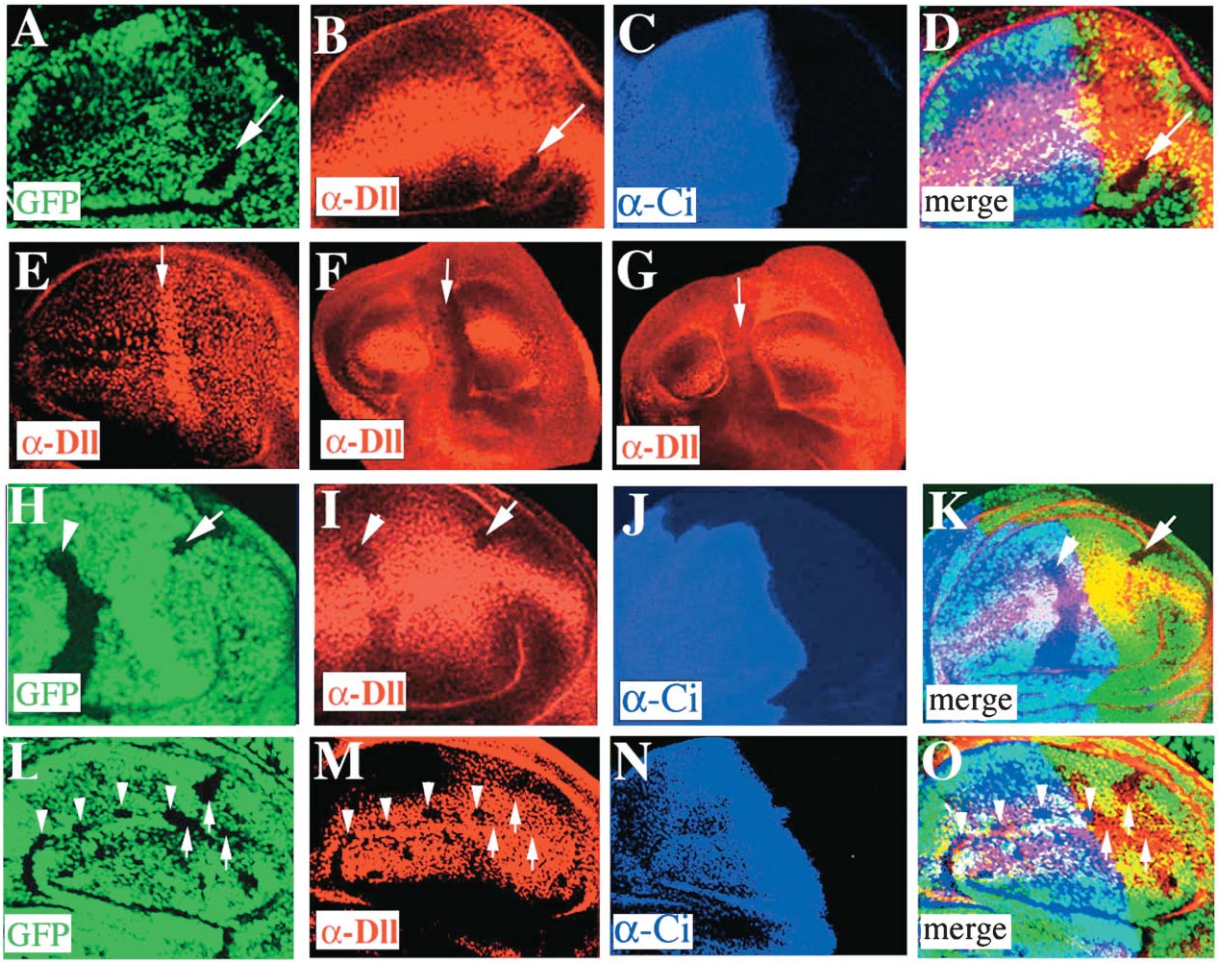

Figure 3. Epistasis Experiments between Overexpressed Forms of Go and Elements of the Wg Pathway

(A–D) Clones of *arm[XM19]* remove the effects of overexpression of Go-GTP. (A) shows an *arm* clone (loss of GFP, arrow) and a corresponding reduction in Dll expression ([B], red, arrow) seen in region of Go-GTP overexpression indicated by the absence of Ci expression (blue) in (C). (D) shows a merge of (A)–(C).

(E–G) The effects of Go-GTP overexpression are negated by cooverexpression of Sgg. (E) shows a *dpp-Gal4, UAS-Go-GTP* wing disc in which Dll expression (red) is upregulated in the *dpp* expression domain (arrow). (F) shows a *dpp-Gal4; UAS-sgg* wing discs with loss of Dll expression in the *dpp* expression domain (arrow). (G) shows a *dpp-Gal4; UAS-Sgg*; *UAS-Go-GTP* wing disc stained for Dll. No Dll expression persists in the *dpp* expression domain (arrow).

(H–K) A *hh-Gal4; UAS-Go* wing disc in which *dsh[V26]* clones are present, stained for Ci (blue), GFP (green), and Dll (red). (H) shows a clone in the anterior (arrowhead) and a clone in the posterior (arrow) marked by loss of GFP. (I) Dll staining. The clone in the posterior (arrow) shows a loss of Dll expression similar to that seen in the equivalent position in the anterior clone (arrowhead). (K) shows a merge of (H–J) at a lower magnification.

(L–O) *fz, fz2* mutant clones are rescued by overexpression of Go-GTP. (L) shows clones of *fz, fz2* mutant clones marked by the loss of GFP in a *hh-Gal4; UAS-Go-GTP* wing disc. The clones in the posterior (absence of Ci, blue in [N]) (arrows) show significant rescue of Dll (red) expression in comparison with clones in the anterior (arrowheads) in which Go-GTP is not overexpressed.

(23%) showed some residual Dll staining. Clones in the posterior of discs in which the wild-type form of Go was overexpressed were similar (five of 22 clones, 23%). But posterior clones in which Go-GTP was overexpressed showed a dramatic rescue of Wg transduction: 27 of 35 clones (77%) showed high levels of Dll expression. The $\chi 2$ p value for the posterior versus anterior clones in the Go-GTP discs is <0.0001, as is the value for posterior clones of the Go-GTP discs versus the Go wild-type discs. Figures 3L–3O show clones double mutant for *fz, fz2* induced in a *hh-Gal4; UAS-Go-GTP* wing disc: anterior clones (arrowheads) show significant loss of Dll expression compared to their posterior equivalents (arrows) (Figure 3M).

Embryonic Phenotypes of Go

Go effects were next examined in the embryo where Wg signaling is well characterized. Since extensive maternal *Go* transcripts prevent the appearance of zygotic phenotypes until late embryogenesis (Fremion et al., 1999), we attempted but failed to generate *Go* germ line clones (suggesting that Go is required for oogenesis). This confounded any examination of complete nulls. Go overexpression, however, phenocopied activation of Wg sig-

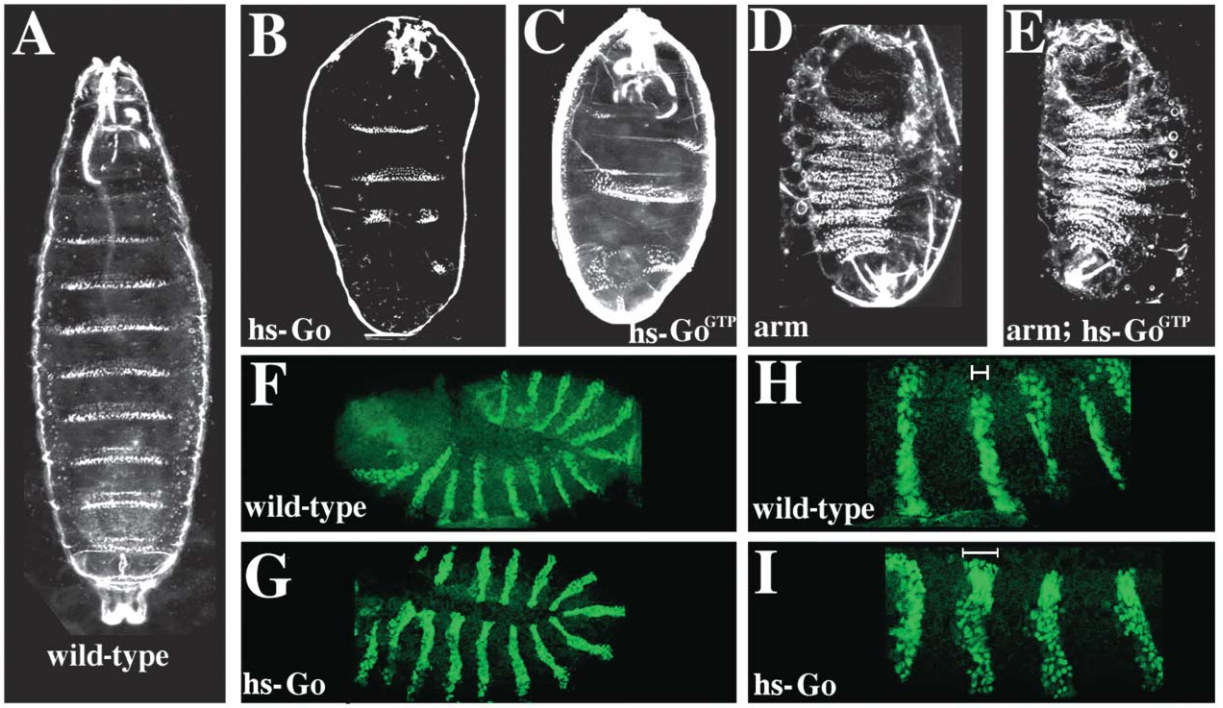

Figure 4. Overexpression of Go in the Embryo Induces Wg Gain-of-Function Phenotypes

(A–E) Embryo cuticle preparations. (A) shows a heat-shocked wild-type control embryo. Overexpression of *Go* (B) or *Go-GTP* (C) by heat shock induces loss of denticle bands. (D) shows an *arm[XM19]/Y* embryo in which Wg signaling is abrogated and a lawn of denticles forms. (E) shows an *arm* embryo in which *Go-GTP* is overexpressed (*arm[XM19]/Y; hs-Go-GTP*).

(F–I) Engrailed-stained embryo preparations. (F) shows a stage 11 embryo in which the 14 En stripes are evident. (H) shows a high-power image of four bands. (G) shows an equivalent embryo in which Go was overexpressed (*hs-Go*). (I) shows a high-power image of four stripes that contain many more En-expressing cells than the wild-type (compare the bars in [H] and [I]).

naling: heat shock-induced expression of Go or Go-GTP produced a loss of denticles (a naked phenotype; Figures 4B and 4C). To demonstrate that the naked phenotypes were caused by ectopic activation of the Wg pathway, Go and Go-GTP were overexpressed in an *arm* mutant, and embryos with lawns of denticles resulted (Figure 4E) that were indistinguishable from the *arm* mutant alone (Figure 4D). Naked phenotypes in *wg* gain-of-function embryos correspond with an increase in the number of Engrailed (En)-expressing cells in each segment. In embryos where Go was overexpressed, a clear increase in En-positive cells was observed (Figures 4F–4I).

Go Clones Induce PCP Phenotypes

To examine effects of Go on Fz-mediated PCP signals, clones of *Go[007]* were induced in developing wings. Early-induced clones did not survive into the adult wing, but clear polarity defects were evident (Figure 5A), which likely resulted from nonautonomous effects of clones that subsequently died. Later clones survived and showed small and slender wing hairs, which was a useful clone marker. These cells frequently secreted multiple wing hairs (mwhs) (Figure 5B), a characteristic phenotype of *multiple wing hair* (*mwh*) and other PCP mutants (Adler, 1992). Unlike *fz* or *dsh* cells in which mwhs usually only number two per cell, *Go* cells showed up to five. In addition, *Go* clones often elicited nonautonomous polarity defects on their proximal side (Figure 5B). In pupal wings, *Go* clones were larger, easier to examine, and showed clear orientation and mwhs defects with associated proximal nonautonomous effects (Figure 5E).

Clones of the null allele (*Go[0611]*, marked with *pawn* [*pwn*, a mutation that affects the shape of the hairs]) were less viable than those of the hypomorphic allele, but, when they survived, they appeared as [*007*] clones with orientation defects, mwhs, and proximal nonautonomous effects (Figures 5C and 5D).

fz clones also show nonautonomous effects but on their distal rather than their proximal side (Vinson and Adler, 1987). The wing margin bristles stereotypically point to the distal end of the wing (Figure 5F), and, when PCP signaling is defective, they point out of the wing (Figure 5G). When *fz* was overexpressed in marked clones, nonautonomous effects occurred proximal to the clone (Figure 5H). Marked *Go[007]* clones induced similar proximal effects (Figure 5I). Thus, Go nonautonomy effects appear as those of overexpression of *fz*, the opposite to *fz* loss of function.

Overexpression of Go Induces PCP Phenotypes

Overexpression of *fz* in pupal wings has different effects (Krasnow and Adler, 1994): early overexpression (before 30 hr APF) leads to defects in hair orientation, but later expression produces mwhs (three to four hairs per cell). Overexpression of Go induced mwhs (Figure 6G), as did

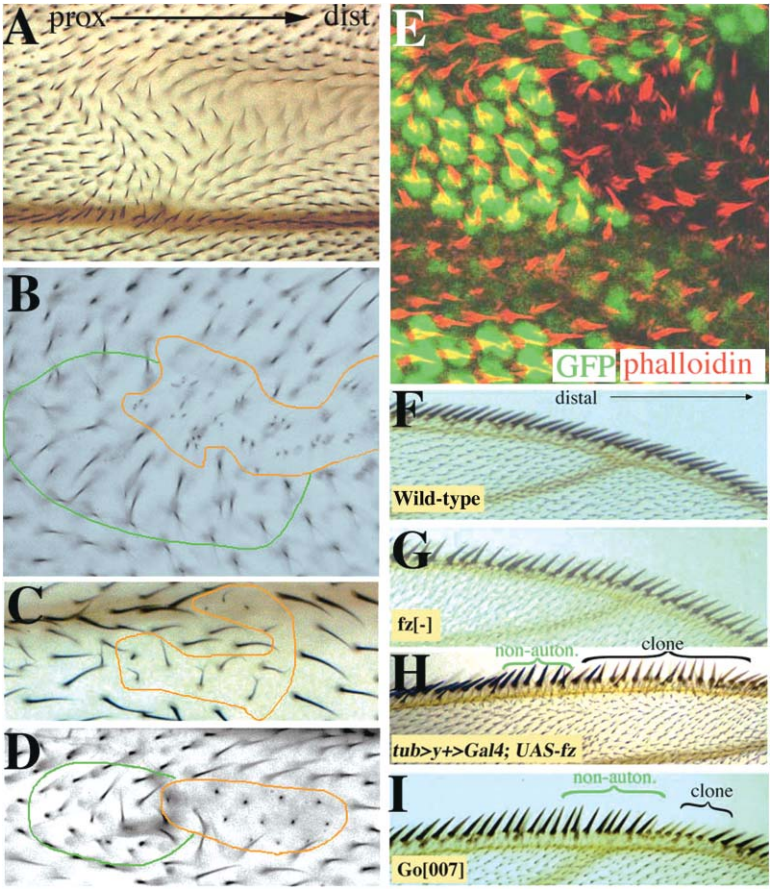

Figure 5. Go Clones Show PCP Phenotypes

Distal is to the right in all panels. (A) A wing in which unmarked *Go[007]* clones have been induced. Clear polarity effects are evident, but the responsible clones likely died. (B) Surviving *Go[007]* clones (outlined in orange) have small and slender wing hairs and frequently show mwhs. They also induce nonautonomous repolarization in the wild-type tissue proximally (outlined in green). (C) *Go[0611]* clones marked by *pwn* are small with deranged polarity (outlined with orange). (D) *Go[0611], pwn* clones show associated nonautonomous effects on their proximal side (outlined in green). (E) A *Go[007]* clone in the pupal wing marked by the loss of GFP. Hairs are highlighted by rhodamine phallodin (red). To the right above the middle is the clone; note that the bright green staining is the wild-type twin spot, and the background tissue has low-level GFP staining. Within the clone hairs show randomized polarity and mwhs, and proximal to the clone, clear nonautonomous polarity effects are evident. (F–I) Nonautonomous effects in the wing margin. (F) A wild-type wing. (G) A *fz[−]* wing. (H) Overexpression of *fz* (marked by *y*) shows nonautonomous effects on the margin bristles on the proximal side. (I) *Go[007]* clones in the margin marked by *y* have small bristles that show nonautonomous effects on their proximal side.

Go-GTP, and this was shown to be cell autonomous (Figure 6D). Go-GDP showed no phenotype.

Synergistic *fz*/*Go* Interactions

Figure 6G shows mwhs on a male wing in which *UAS-Go* was hemizygous (effectively two copies) on the X. In a heterozygous female (effectively one copy), many fewer cells showed mwhs (Figure 6E). Similar overexpression of *fz* induced orientation defects and a few mwhs (Figure 6C), but when *UAS-fz* and *UAS-Go* (heterozygous) were coexpressed, orientation problems became severe, and many cells showed mwhs (Figure 6F). Thus overexpression of *Go* induces mwhs, and it acts synergistically with *fz* to produce extreme PCP defects.

Epistasis between *Go* and *fz*

We next tested whether the effects of Go and Go-GTP were Fz dependent. Figures 6G–6I show the effects of overexpression of Go when *fz* gene dosage was reduced. At normal Fz levels, many mwhs were induced (Figure 6G); many fewer mwhs were induced when one copy of *fz* gene was removed (Figure 6H); and, when both copies were removed (Figure 6I), the number dropped to the few normally found in $fz^{-/-}$ wings (cf. Figure 6B). In contrast, the effects of Go-GTP were Fz independent. At normal Fz levels, a number of mwhs were present (Figure 6J; fewer than in Figure 6G because of dosage compensation effects), but, when one or both copies of *fz* gene were removed, there was no reduction in the number of mwhs (Figures 6K and 6L). Thus, as with the Wg pathway above, the dominant effects of the wild-type form of Go were Fz dependent, whereas those of the Go-GTP form were not.

Go Is Asymmetrically Localized in Pupal Wing Cells

At ~30 hr APF, wing cells show dramatic asymmetry in localization of PCP proteins. For example, Fz, initially found in the membrane circumscribing the cells, relocates to the distal end of the cells, where it appears to organize hair outgrowth (Strutt, 2001). To examine Go localization, we generated an antibody (see Experimental Procedures) and stained developing wings. In early pupae, Go was largely found in the cortical cytoplasm circumscribing the cells (data not shown). At ~30 hr APF, Go was restricted to the proximal and distal ends of the cells (Figure 7A), and by 32 hr APF, the distal localization was lost and Go was restricted to the proximal ends of the cells (Figure 7B). The localization of Go to the proximal and distal ends of the cells occurred before major Fz relocalization, and the restriction of Go to the proximal region was roughly coincident with the strong distal localization of Fz (Figure 7B, note that Fz also deceptively appears to label proximal membranes: only in mosaic patches can the distal localization of Fz be discerned [Strutt, 2001]). Thus, at the critical times of PCP readout, Go showed dynamic expression leading to asymmetric localization.

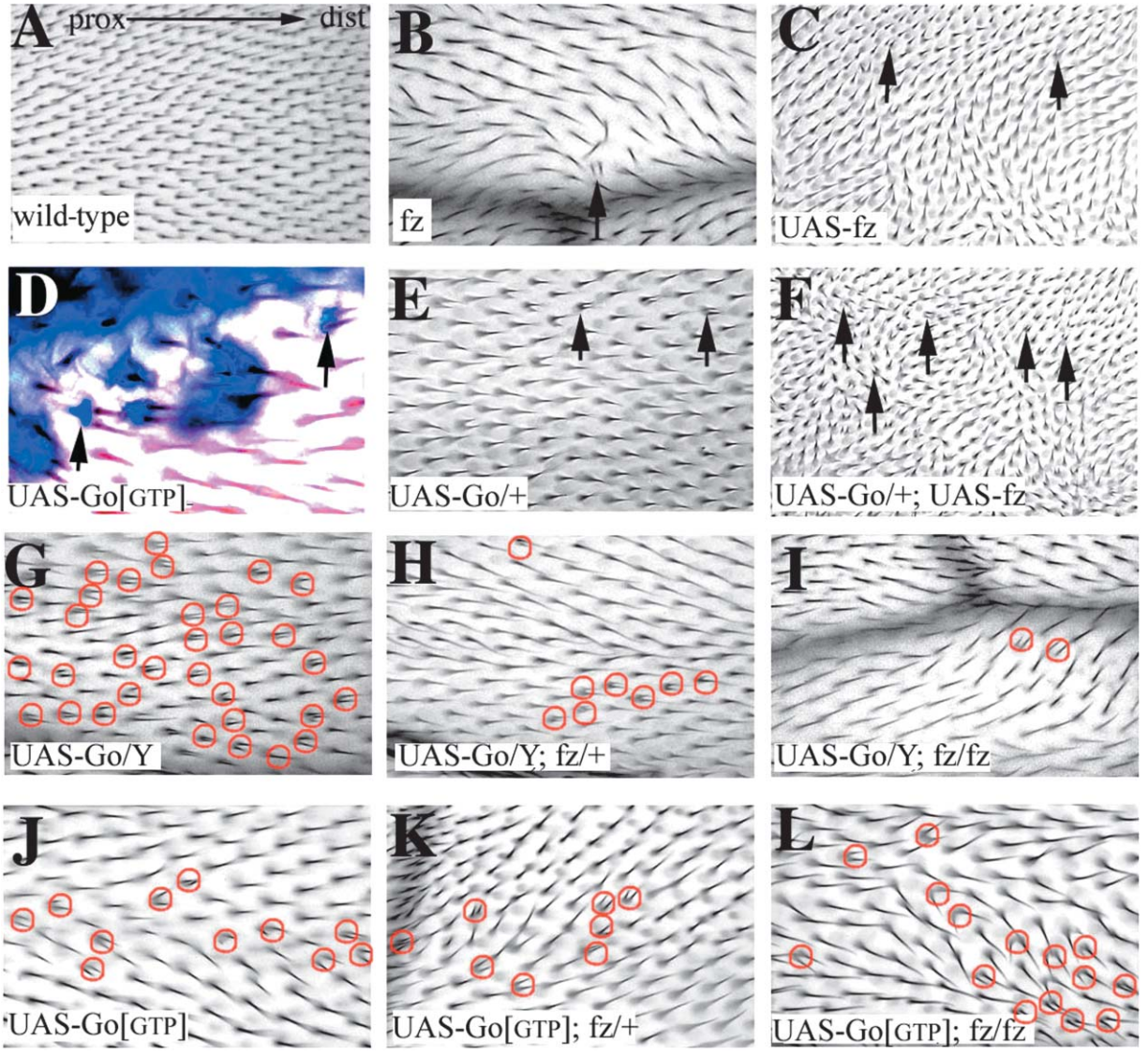

Figure 6. Overexpression of Go Induces PCP Phenotypes

Distal is to the right in all panels. (A) A wild-type wing. (B) In a *fz* mutant, wing polarity disruptions are evident, and occasional multiple mwhs (arrow) are observed. (C) When Fz is overexpressed (*MS1096-Gal4; UAS-fz*), polarity disruptions occur and a few mwhs occur (arrows). (D and E) Overexpression of Go-GTP or Go (MS1096-Gal4) induces mwhs (arrows). (D) Shows a pupal wing in which *tub-Go-GTP* is expressed in clonal patches marked by *lacZ* expression (blue). Only labeled cells show mwhs, indicating a cell autonomous effect. (F) *UAS-Go* and *UAS-fz* act synergistically and produce wings with strong polarity defects and many mwhs (arrows). (G–I) The effects of overexpression of Go are *fz* dependent. (G) shows a male wing of a *UAS-Go* construct on the X chromosome recombined with the *MS1096* driver line. This produces many mwhs (red circles). In *fz* heterozygous (H) or homozygous (I) wings, the number of mwhs drops. (J–L) The effects of overexpression of Go-GTP are *fz* independent. (J) shows a wing with an autosomal insert of *UAS-Go-GTP* driven by *MS1096-Gal4*. Removal of one (K) or both (L) copies of *fz* does not decrease the numbers of mwhs.

Fz Localization Is *Go* Dependent

Fz localization is dependent upon Fz/PCP signaling, so we examined *Go* clones and Go overexpression for effects on Fz distribution. In *Go[007]* clones, Fz-GFP was sometimes reduced on the membrane (with increased cytoplasmic staining), but when present on the membrane, distal localization was lost (Figures 7G–7I). Interestingly, where wild-type cells abutted the clone, increased or inappropriate Fz-GFP membrane staining often occurred at the interface. When Go was overexpressed (with *hh-Gal4*), Fz lost its sharp distal localization and became more broadly localized, including in the cytoplasm (compare Figures 7C and 7D). Thus, when Go levels were modulated up or down, Fz localization was disturbed.

Go Localization Is *fz* Dependent

fz[−] 32 hr APF wings were stained with α-Go to determine if Go localization was *fz* dependent (Figures 7E and 7F). Go no longer tightly localized to the proximal ends of the cells (cf. Figure 7B) but was distributed around subcortical regions. In addition, the earlier (30

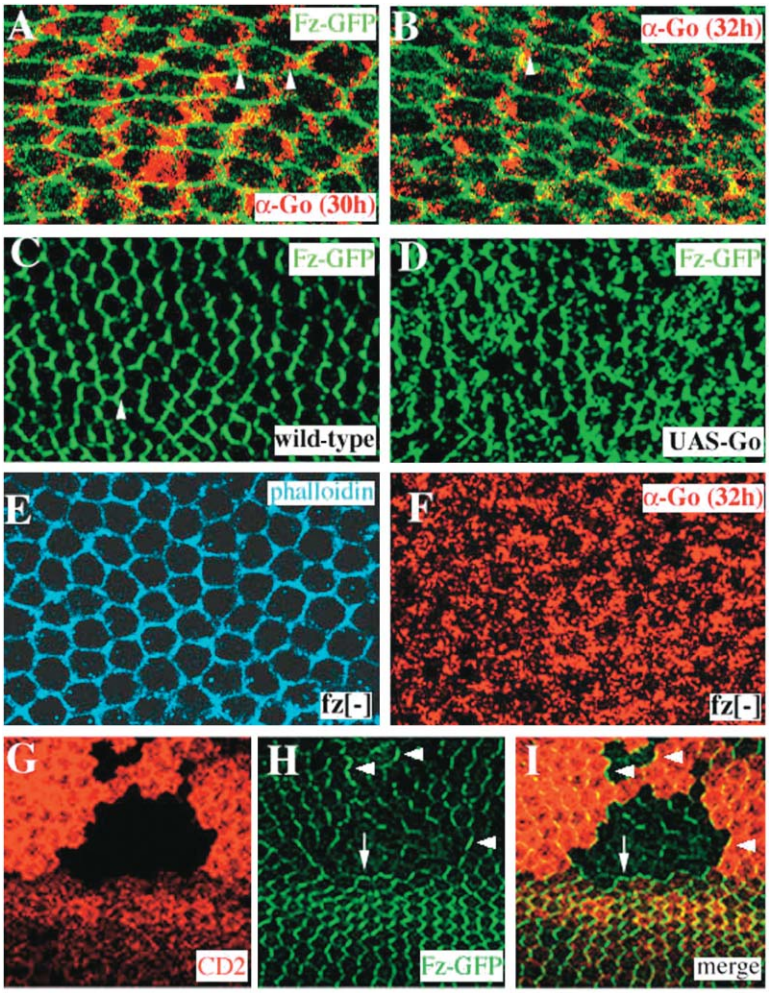

Figure 7. Polarity Features of Go in Pupal Wings

Distal is to the right in all panels. (A and B) Go is distributed asymmetrically within the pupal wing cells. (A) At 30 hr APF, Go (red) localizes to the proximal and distal ends of the cells (arrowheads). Fz-GFP (green) at this stage does not show asymmetric distribution. (B) By 32 hr APF, Go (red) is primarily localized to the proximal ends of the cells (arrowhead) as Fz-GFP (green) localizes to the distal membranes of the cells. (C and D) Overexpression of *Go* changes Fz localization. (C) At 32 hr APF, Fz-GFP is localized to the distal membrane end of the cells that appears as labeling of both proximal and distal ends. (D) When *Go* is overexpressed, Fz-GFP loses its asymmetric distribution and now shows cytoplasmic staining. (E and F) Go localization is *fz* dependent. (E) Shows a 32 hr APF *fz[−]* wing with membranes highlighted by phallodin (blue). (F) Go staining (red) no longer shows asymmetric proximal localization. (G–I) *Go* clones show a deregulation of Fz protein localization. (G) Loss of rCD2 (red) marks *Go[007]* clones. (H) Fz-GFP (green) shows aberrant distribution in the clone. Note that Fz-GFP membrane staining appears stronger (arrowheads) or inappropriately localized (arrow) where mutant and wild-type cells abut. (I) shows a merger of (G) and (H).

hr APF; Figure 7A) distribution of Go to the proximal and distal ends of the cells was not observed (data not shown).

Discussion

Serpentine receptors are typically linked to their intracellular transduction machinery by trimeric G proteins. Evidence presented in this paper suggests that, in *Drosophila*, both the Wnt and PCP Fz pathways can be transduced by a trimeric G protein complex that contains Go as the α subunit.

The Evidence that Go Tranduces Wnt/Fz Signaling

The evidence that Go transduces Wg signaling comes from the analysis of Go mutants, from overexpression studies, and from the epistasis experiments. These are addressed below.

The inherent subviability of *Go* clones prevented a frank assessment of their loss-of-function effects on Wg transduction: surviving cells likely carried perduring wild-type transcripts or protein. This offers a simple explanation for why not all *Go* cells showed effects on Wg targets—many cells still carried enough *Go* function to transduce Wg. However, even given the lack of penetrance of the clones, there was a striking correspondence between *Go* mutant clones and the loss of Wg targets expression, thereby arguing that *Go* gene function is critically required for Wg signal transduction.

Further evidence for the role of Go in transducing Wg comes from the overexpression experiments. When *Go* is overexpressed in the wing disc, clear upregulation of Wg targets is evident. If Go achieves the upregulation of the target genes by hyperactivating the intracellular Wg transduction machinery, then abrogation of transduction downstream of *Go* should nullify its effects. To this end, we showed that the upregulation of Wg targets was *arm* and *dsh* dependent and was abolished by overexpression of *sgg*. Furthermore, Go overexpression in embryos gave gain-of-function *wg* phenotypes that were *arm* dependent.

In *arm* and *dsh* clones (and *fz, fz2* clones described below), residual Dll expression was sometimes found. This occurred in otherwise wild-type tissues and in both anterior and posterior domains of *hh-Gal4; UAS-Go* wing discs and was most noticeable with *dsh* (Figures 1P and 3I) known for strong perdurance (Perrimon and Mahowald, 1987). However, *arm* and *dsh* clones in the regions of Go overexpression lost Dll expression to a level comparable with clones in which Go was not overexpressed.

Thus, we infer that the upregulation of Wg targets induced by overexpression of Go requires the Wg transduction pathway utilizing Dsh, Sgg, and Arm.

Upon activation of serpentine receptors, GDP is exchanged for GTP on Gα, and the complex dissociates, leaving Gα-GTP and βγ free to signal to downstream components. To test whether Go-GTP was able to activate the transduction pathway, we overexpressed a form of Go containing an inactive GTPase (Sprang, 1997). Overexpression of Go-GTP induced Wg targets, indicating that Go-GTP is a positive transducer of the pathway and that one function of Fz activation is to catalyze the release of Go-GTP. Any signaling role of the βγ moiety remains to be investigated. Overexpression of the Go-GDP mutant form did not produce any effect. This form has a low affinity for GTP (Inoue et al., 1995; Slepak et al., 1993) and could be expected to have dominant-negative effects. However, this form may not be sufficiently inactive to allow any effects on Wg transduction (and the PCP pathway, below) to be detected.

The epistasis experiments provide two key indications that Go represents an immediate transducer of Fz signaling. First, Dsh (previously the highest element of the transduction cascade identified downstream of the receptors) is necessary for the effects of Go overexpression. Second, since serpentine receptors act as exchange factors for trimeric G proteins, the effects of overexpression of a wild-type form should require the presence of the exchange factor to load and subsequently reload GTP. Conversely, once loaded with GTP, the form lacking GTPase activity (Go-GTP) will be a long-lived activated subunit. Thus, if Fz acts as the exchange factor for Go, then it would be expected that wild-type Go would require Fz for its overexpression effects but that the activated form would be significantly less dependent. This is what we observed: Wg signaling was significantly rescued in *fz, fz2* cells concomitantly expressing Go-GTP as compared to those expressing wild-type Go.

Given that Go functions in the Wg transduction pathway, given that its overexpression effects require Dsh, and given that its activated form is receptor independent, the simplest explanation is that Go functions in a trimeric G protein complex that relays signals from Fz receptors. These data do not necessarily suggest that Go is the exclusive transducer of Wg signals: other trimeric complexes may be involved, and non-G protein-mediated signaling may also occur.

The Evidence that Go Tranduces PCP-Type Fz Signaling

In the wing, the key molecular events associated with PCP occur by 30 hr APF, when Fz becomes specifically localized to the distal membrane of the cell (Strutt, 2001). The localization of Fz appears to require its own signaling, since, in *dsh* mutants, Fz localization does not occur (Strutt, 2001). A similar effect occurs when Fz is overexpressed: Fz is no longer restricted to the distal membrane. Given this complexity, the following feature of Go can be predicted if it indeed acts as a transducer of Fz signaling. First, loss of Go activity should induce PCP phenotypes. Second, Fz localization should not occur correctly when Go signaling is compromised. In regard to these two predictions, we have shown that (1) reduction of Go function or Go overexpression induce clear PCP defects and (2) Fz localization is aberrant when Go function is down- or upregulated. Furthermore, we have shown that Go itself undergoes a striking asymmetric redistribution in a *fz*-dependent manner.

Go clones can show nonautonomous polarity defects on their proximal side, whereas *fz* clones show effects on their distal sides. This may indicate that Go relays a negative signal in PCP transduction. Go localizes proximally in polarizing cells, as does Strabismus/van Gogh (Bastock et al., 2003), which also shows proximal nonautonomous effects (Taylor et al., 1998). Hence, the proximal nonautonomous effects of *Go* may result from it functioning negatively in the PCP pathway, from it becoming localized proximally, or from some combination of the two. A further aspect of *Go* clones is the inappropriate localization of Fz at the interface of mutant and wild-type cells. It is not clear if this protein is derived from the wild-type cells, the mutant cells, or both. But it implies that the cells are in communication, and again a similar phenomenon has been described for *Strabismus/van Gogh* clones (Strutt, 2001) that may relate to the nonautonomous effects.

Overexpression of either Go or Go-GTP caused PCP defects, suggesting that one function of Fz signaling in the PCP pathway is the generation of free Go-GTP. However, given the difficulty in distinguishing gain-of-function from loss-of-function effects, we cannot say whether Go-GTP acts positively (as in the Wg pathway) or negatively. Any role for the βγ dimer in transducing PCP signals remains to be established. The mwhs produced by overexpression of wild-type Go or Go-GTP show a marked difference: the effects of wild-type Go required the presence of the receptor (Fz), whereas the activated form did not. As for the Wg pathway described above, the most likely explanation of this observation is that Fz functions as an exchange factor for Go.

Experimental Procedures

Histology

Eyes were processed for sectioning and analysis following Tomlinson and Ready (1987). Adult wings were fixed in GMM. Late pupal wings were removed from pupal sacs in PBS, fixed in 3.7% formaldehyde, stained for lacZ, and mounted in Vectashield. Wing imaginal discs from third instar larvae were fixed in formaldehyde and permeabilized in 0.1% Triton X-100 before immunostaining in 0.2% Tween 20, followed by confocal microscopy.

White prepupae were collected and staged at 25°C. Under conditions in our laboratory, prehair growth initiated at 33–34 hr APF. We used a single copy of *arm-Fz-GFP* to visualize Fz through GFP fluorescence. NP-40 (0.5%) was used to permeabilize pupal wings, as the use of Triton X-100 prevented detection of one copy of Fz-GFP. We saw no clear Fz accumulation at distal cell boundaries before ~30 hr APF.

Embryos were treated following Wieshaus and Nusslein-Volhard (1986). Heat shock was performed at 2–4 hr AEL for 1 hr at 37°C. For immunostaining, stage 11 embryos were collected and removed from the vitelline membrane. For cuticle preparations, embryos were collected 24 hr after heat shock and mounted in their vitelline membranes.

The following antibodies were used: rat anti-Go at 1:200 dilution (see below), rat anti-Ci at 1:20 (Motzny and Holmgren, 1995), mouse anti-rCD2 at 1:500 (Serotec), rabbit anti-Dll (gift of G. Struhl) at 1:1000, guinea pig anti-Vg (gift of G. Struhl) at 1:100, and guinea pig anti-Hth (Abu-Shaar et al., 1999) at 1:1000. Mouse antibodies

against Cut (1:30), En (1:10), and Wg (1:100) were from DSHB. FITC-, Cy3-, and Cy5-labeled secondary antibodies were from Jackson ImmunoResearch Laboratories. Rhodamine phalloidin or Alexa Fluor 647 phalloidin (Molecular Probes) were used to stain F-actin.

Generation of a Go Antibody

The peptide ANNLRGCGLY corresponding to the C terminus of Go was synthesized, and a rat antiserum was raised against it by Cocalico Biologicals (Reamstown, PA). Specificity of the antibody was confirmed using Western blots and immunostaining pupal wings with *[Go007]* mutant clones, and with overexpression experiments (data not shown).

Genetics

All transgenes were engineered with a *w[+]* flip-out cassette (Strapps and Tomlinson, 2001) later removed in the germline or mitotically using *hsp70-flp*. *UAS>w[+]>Ctx and UAS>w[+]>Ptx* encode the catalytic subunits of cholera toxin (Burton et al., 1991) and pertussis toxin (Ikeda et al., 1999), respectively. *UAS>w[+]>Go, UAS>w[+]>Go-GTP*, and *UAS>w[+]>Go-GDP* encoded the wild-type, the Q205L (CAG-CTG), and the G203T (GGC-ACC) forms of class II Go (Fremion et al., 1999), respectively. The Go-GTP form was deficient in its GTPase activity (Sprang, 1997), while the Go-GDP form had lowered affinity for GTP (Inoue et al., 1995). Go and Go-GTP constructs under the inducible heat-shock promoter were also independently transformed. Other lines used were *UAS-Fz* (Strapps and Tomlinson, 2001); *arm-Fz-GFP* (Strutt, 2001); *sev-Gal4* (gift of K. Basler); *tub>y[+]>Gal4* (gift of G. Struhl); *hh-Gal4* (Tanimoto et al., 2000); *MS1096-Gal4* (Brand and Perrimon, 1993); *fz[H51]*, *fz[KD4A]*, *fz[P21]* (Jones et al., 1996); and *fz2[C1]* (Chen and Struhl, 1999). *UAS-LacZ*, *UAS-sgg*, and *dpp-Gal4* were from Bloomington Stock Center.

The null *Go[0611]* allele was generated by imprecise P element excision of the P06915 line (Fremion et al., 1999) using Δ2–3 as a source of transposase. The resultant *0611* line was homozygous lethal and lethal over the *Df(2R)47A* deficiency. PCR of genomic *0611/Cyo* DNA using 5′-GCCCATGGTGCGTATTGCCTCGACGC-3′ (specific for the first, ATG-containing exon of the class I Go cDNA) and 5′-GCCCCGTGTAAATCCGAATCCGAATCCA-3′ (specific for the first, ATG-containing exon of the class II Go cDNA) produced a fragment of ~500 bp in addition to the 6 kb wild-type fragment. The intron between these two exons and part of the first exon are deleted in *0611*. *Go[0611]* does not produce either Go cDNAs, as confirmed by in situ hybridization of homozygous embryos. *Go[0611]* was recombined onto an *frt42D*, *pwn* chromosome.

The *Go[007]* chromosome was cleared of the secondary lethal mutation (Fremion et al., 1999) before recombining it onto the *frt42D* chromosome.

Mitotic clones were induced by the Flp-mediated recombination (Golic and Lindquist, 1989) between *frt42D,Go* and *frt42D, hsp70-GFP* or *frt42D, hsp70-rCD2* chromosomes using *hsp70-flp* (Struhl and Basler, 1993). Heat shocks were applied for 1 hr, 37°C at 24–48 hr AEL, except for Figures 5C, 5D, and 5I, where 48–96-hr-old larvae were heat shocked. *dpp-Gal4 UAS-flp* was used to create clones of Figures 5B and 7G–7I. To visualize *hsp70-GFP* or *-rCD2* expression, 1 hr 37°C heat shock/1 hr 25°C recovery was applied before dissecting animals.

For epistasis experiments, *y, w, hsp70-flp; UAS-Go; hsp70-GFP, hsp70-rCD2, frt2A, hhGal4 / fz[P21], fz2[C1], frt2A* larvae were heat shocked at 48–72 hr AEL. We could not recover *fz, fz2* clones of a significant size with earlier induction in otherwise wild-type, *UAS-Go*, or *UAS-Go-GTP* discs, which may suggest that a low amount of Fz receptors is necessary even for loading Go-GTP.

w, dshV26, frt101/w, hsp70-GFP, hsp70-flp, frt101; UAS-Go; hh-Gal4 larvae were heat shocked at 24–48 hr AEL, as were *w, armXM19, frt101/w, hsp70-GFP, hsp70-flp, frt101; UAS-Go; hh-Gal4* larvae.

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References

Abu-Shaar, M., Ryoo, H.D., and Mann, R.S. (1999). Control of the nuclear localization of Extradenticle by competing localization and export signals. Genes Dev. *13*, 935–945.

Adler, P.N. (1992). The genetic control of tissue polarity in *Drosophila*. Bioessays *14*, 735–741.

Adler, P.N. (2002). Planar signaling and morphogenesis in *Drosophila*. Dev. Cell *2*, 525–535.

Axelrod, J.D. (2001). Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. Genes Dev. *15*, 1182–1187.

Axelrod, J.D., Miller, J.R., Shulman, J.M., Moon, R.T., and Perrimon, N. (1998). Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. Genes Dev. *12*, 2610–2622.

Azpiazu, N., and Morata, G. (2000). Function and regulation of homothorax in the wing imaginal disc of *Drosophila*. Development *127*, 2685–2693.

Bastock, R., Strutt, H., and Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. Development *130*, 3007–3014.

Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature *382*, 225–230.

Bhat, K.M. (1998). frizzled and frizzled 2 play a partially redundant role in wingless signaling and have similar requirements to wingless in neurogenesis. Cell *95*, 1027–1036.

Boutros, M., Paricio, N., Strutt, D.I., and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. Cell *94*, 109–118.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Brunner, E., Peter, O., Schweizer, L., and Basler, K. (1997). pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. Nature *385*, 829–833.

Brzostowski, J.A., and Kimmel, A.R. (2001). Signaling at zero G: G-protein-independent functions for 7-TM receptors. Trends Biochem. Sci. *26*, 291–297.

Burton, F.H., Hasel, K.W., Bloom, F.E., and Sutcliffe, J.G. (1991). Pituitary hyperplasia and gigantism in mice caused by a cholera toxin transgene. Nature *350*, 74–77.

Chen, C., and Struhl, G. (1999). Wingless transduction by the Frizzled and Frizzled2 proteins of *Drosophila*. Development *126*, 5441–5452.

Fremion, F., Astier, M., Zaffran, S., Guillen, A., Homburger, V., and Semeriva, M. (1999). The heterotrimeric protein Go is required for the formation of heart epithelium in *Drosophila*. J. Cell Biol. *145*, 1063–1076.

Gilman, A.G. (1987). G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. *56*, 615–649.

Golic, K.G., and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. Cell *59*, 499–509.

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

Ikeda, S.R., Jeong, S.W., Kammermeier, P.J., Ruiz-Velasco, V., and

King, M.M. (1999). Heterologous expression of a green fluorescent protein-pertussis toxin S1 subunit fusion construct disrupts calcium channel modulation in rat superior cervical ganglion neurons. Neurosci. Lett. *271*, 163–166.

Inoue, S., Hoshino, S., Kukimoto, I., Ui, M., and Katada, T. (1995). Purification and characterization of the G203T mutant alpha i-2 subunit of GTP-binding protein expressed in baculovirus-infected Sf9 cells. J. Biochem. (Tokyo) *118*, 650–657.

Jones, K.H., Liu, J., and Adler, P.N. (1996). Molecular analysis of EMS-induced frizzled mutations in *Drosophila melanogaster*. Genetics *142*, 205–215.

Kennerdell, J.R., and Carthew, R.W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. Cell *23*, 1017–1026.

Krasnow, R.E., and Adler, P.N. (1994). A single frizzled protein has a dual function in tissue polarity. Development *120*, 1883–1893.

Liu, T., DeCostanzo, A.J., Liu, X., Wang, H., Hallagan, S., Moon, R.T., and Malbon, C.C. (2001). G protein signaling from activated rat frizzled-1 to the beta-catenin-Lef-Tcf pathway. Science *292*, 1718–1722.

Malbon, C.C., Wang, H., and Moon, R.T. (2001). Wnt signaling and heterotrimeric G-proteins: strange bedfellows or a classic romance? Biochem. Biophys. Res. Commun. *287*, 589–593.

Moss, J., and Vaughan, M. (1988). ADP-ribosylation of guanyl nucleotide-binding regulatory proteins by bacterial toxins. Adv. Enzymol. Relat. Areas Mol. Biol. *61*, 303–379.

Motzny, C.K., and Holmgren, R. (1995). The *Drosophila* cubitus interruptus protein and its role in the wingless and hedgehog signal transduction pathways. Mech. Dev. *52*, 137–150.

Neumann, C.J., and Cohen, S.M. (1996). A hierarchy of cross-regulation involving Notch, wingless, vestigial and cut organizes the dorsal/ventral axis of the *Drosophila* wing. Development *122*, 3477–3485.

Peifer, M., Sweeton, D., Casey, M., and Wieschaus, E. (1994). wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. Development *120*, 369–380.

Perrimon, N., and Mahowald, A.P. (1987). Multiple functions of segment polarity genes in *Drosophila*. Dev. Biol. *119*, 587–600.

Riese, J., Yu, X., Munnerlyn, A., Eresh, S., Hsu, S.C., Grosschedl, R., and Bienz, M. (1997). LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. Cell *88*, 777–787.

Rulifson, E.J., Micchelli, C.A., Axelrod, J.D., Perrimon, N., and Blair, S.S. (1996). wingless refines its own expression domain on the *Drosophila* wing margin. Nature *384*, 72–74.

Siegfried, E., Perkins, L.A., Capaci, T.M., and Perrimon, N. (1990). Putative protein kinase product of the *Drosophila* segment-polarity gene zeste-white3. Nature *345*, 825–829.

Slepak, V.Z., Quick, M.W., Aragay, A.M., Davidson, N., Lester, H.A., and Simon, M.I. (1993). Random mutagenesis of G protein alpha subunit G(o)alpha. Mutations altering nucleotide binding. J. Biol. Chem. *268*, 21889–21894.

Sprang, S.R. (1997). G protein mechanisms: insights from structural analysis. Annu. Rev. Biochem. *66*, 639–678.

Strapps, W.R., and Tomlinson, A. (2001). Transducing properties of *Drosophila* Frizzled proteins. Development *128*, 4829–4835.

Struhl, G., and Basler, K. (1993). Organizing activity of wingless protein in *Drosophila*. Cell *72*, 527–540.

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

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

Tanimoto, H., Itoh, S., ten Dijke, P., and Tabata, T. (2000). Hedgehog creates a gradient of DPP activity in *Drosophila* wing imaginal discs. Mol. Cell *5*, 59–71.

Taylor, J., Abramova, N., Charlton, J., and Adler, P.N. (1998). Van Gogh: a new *Drosophila* tissue polarity gene. Genetics *150*, 199–210.

Tomlinson, A., and Ready, D.F. (1987). Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. *120*, 366–376.

Tomlinson, A., Strapps, W.R., and Heemskerk, J. (1997). Linking Frizzled and Wnt signaling in *Drosophila* development. Development *124*, 4515–4521.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., et al. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. Cell *88*, 789–799.

Varmus, H., Brown, A., Papkoff, J., and Shackleford, G. (1986). The int-1 proto-oncogene. Princess Takamatsu Symp. *17*, 31–41.

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

Wieshaus, E., and Nusslein-Volhard, C. (1986). Looking at embryos. In *Drosophila*: A Practical Approach, D.B. Roberts, ed. (Oxford: IRL Press), pp. 199–227.

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development. Annu. Rev. Cell Dev. Biol. *14*, 59–88.

Zecca, M., Basler, K., and Struhl, G. (1996). Direct and long-range action of a wingless morphogen gradient. Cell *87*, 833–844.