Cell–cell signalling: **Wingless lands at last**

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A novel class of receptor – the Frizzled family – has been identified and the members shown to be receptors for Wingless and its homologs, the Wnts, which mediate key cell–cell interactions during the development of fruitflies and vertebrates, respectively.

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If molecular families can attain celebrity status, the Wingless and Hedgehog families of secreted signaling proteins both qualify. The eponymous Wingless and Hedgehog proteins were first identified in *Drosophila*, where they provide positional information and direct cell fate in many tissues (reviewed in [1]). Wingless and Hedgehog homologs were subsequently identified and shown to mediate key cell-fate decisions in mammals. Some favorite processes of developmental biologists, including embryonic dorsal–ventral patterning, limb formation, and neural-tube patterning, require signaling by Wingless and/or Hedgehog family proteins. If this were not enough, activation of Wnt-1, a mammalian Wingless homolog, causes breast cancer in mice, and Patched, a component of the Hedgehog signaling pathway, is mutated in the most common human skin cancer [2,3]. Despite this notoriety, and intensive efforts by geneticists and biochemists, receptors for both Wingless and Hedgehog remained elusive until the past few months.

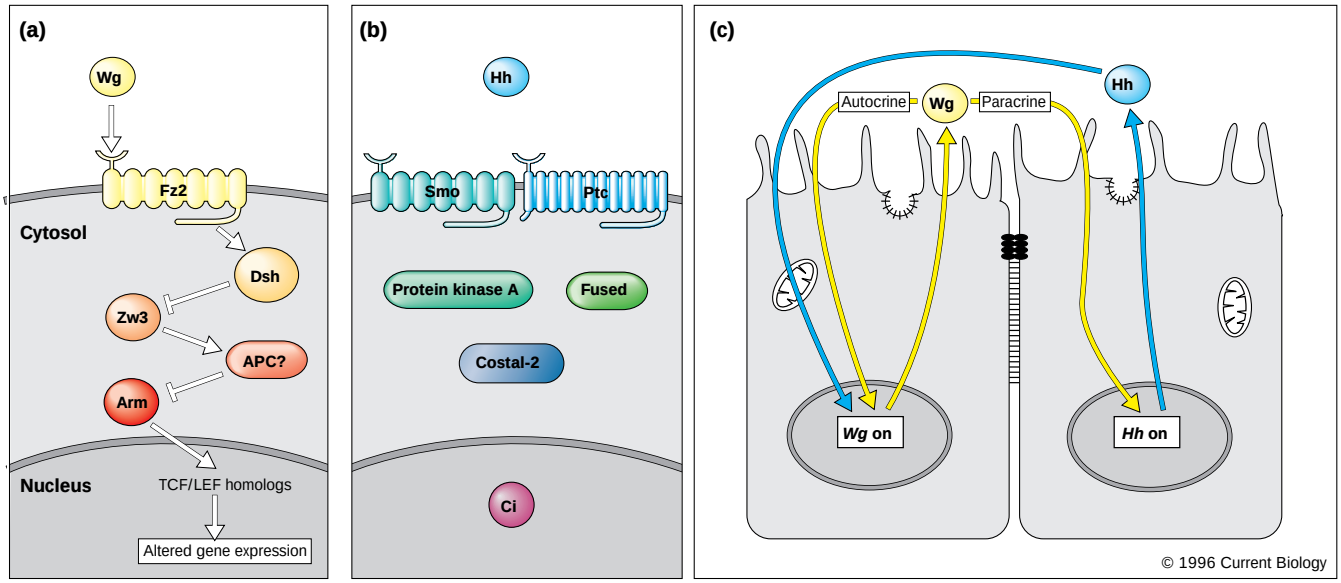
Our introduction to Wingless and Hedgehog came from genetic analysis of patterning in *Drosophila*. In the pioneering genetic screen of Nüsslein-Volhard and Wieschaus, mutations in a number of 'segment polarity' genes were identified that alter cell fates within each embryonic segment. In wild-type embryos, cells in different anterior–posterior positions within each segment make distinctive types of cuticle, reflecting their cell-fate choices along the anterior–posterior axis: cells in anterior positions secrete cuticle decorated with small hairs, whereas cells in posterior positions secrete naked cuticle devoid of hairs. In segment polarity mutants, cells make incorrect cell-fate choices; for example, in *wingless* or *hedgehog* mutant embryos, all cells choose anterior cell fates and secrete hairs, regardless of their actual position.

Other segment polarity genes encode components of the Wingless or Hedgehog signal transduction pathways. A number of components of the Wingless pathway have been identified (reviewed in [4]). In the current model (Fig. 1a), the serine/threonine kinase Zw3 and, at least in mammals, the tumor suppressor protein APC [5] cooperate in the absence of Wingless to target cytoplasmic Armadillo protein for degradation. As a result, cytoplasmic Armadillo levels are low. In the presence of Wingless, Dishevelled is activated, presumably *via* interaction of Wingless with a cell-surface receptor. An, as yet unidentified, kinase phosphorylates Dishevelled, and its activity may be modulated, in turn, by Dishevelled [6]. Dishevelled antagonizes the action of Zw3 kinase, perhaps by modulating its enzymatic activity. Armadillo is no longer degraded and so accumulates in both cytoplasm and nucleus [7], where it has been suggested to act as a co-activator, along with a transcription factor in the TCF/LEF family, and alter gene expression [8,9]. Many elements of the Wingless pathway are shared by all multi-cellular animals [10–12]. In *Xenopus*, Dishevelled, the Zw3 homolog GSK-3 and the Armadillo homolog β-catenin all are involved in transduction of Wnt signals. This view of Wingless signaling is incomplete: other proteins are likely to play roles at a number of places in the pathway; until recently these included the putative Wingless receptor.

Our view of the players thought to be in the Hedgehog signaling pathway has also advanced recently (reviewed in [13]; Fig. 1b). This pathway may be more complex than that of Wingless, as it may have two branches. The transmembrane protein Patched has genetic and molecular properties consistent with a role as a Hedgehog receptor, but the data are also consistent with the possibility that Patched modulates Hedgehog signaling downstream of the true receptor. Other molecules thought to be in the Hedgehog pathway are the protein kinases Fused and protein kinase A, the novel proteins Costal-2 and Su(fu), and the transcription factor Ci. Most, if not all, of these proteins have homologs in other animals, and evidence that they function together in other animals is accumulating [14–16].

The genetic analysis placing these proteins in the Hedgehog pathway has an important caveat, however. Although Wingless and Hedgehog are produced by distinct cells and are thought to be transduced by distinct pathways, they are inextricably linked in the embryonic ectoderm (Fig. 1c). The cells expressing Wingless and Hedgehog are adjacent and talk to one another; each cell continues production of its designated ligand only if it receives the other signal. Thus, in *wingless* mutant embryos production of Hedgehog soon ceases, and conversely, in *hedgehog* mutants Wingless production is not maintained. Furthermore, Wingless is both an autocrine and a paracrine

Figure 1



The Wingless (Wg) and Hedgehog (Hh) signaling pathways. **(a)** A model of the Wingless signaling pathway. Fz2, *Drosophila* Frizzled-2; Dsh, Dishevelled; Arm, Armadillo. (See text for details.) **(b)** The components of the putative Hedgehog pathway, arranged according to their cellular location or probable site of action. Smo, Smoothened; Ptc, Patched (which is thought to have twelve transmembrane domains). **(c)** In the *Drosophila* embryonic ectoderm, cells expressing Wingless and cells expressing Hedgehog are adjacent and signal to one another. Each signal stimulates the maintenance of expression of the other in the adjacent cell. An autocrine signaling pathway is thought to operate in the Wingless-secreting cell.

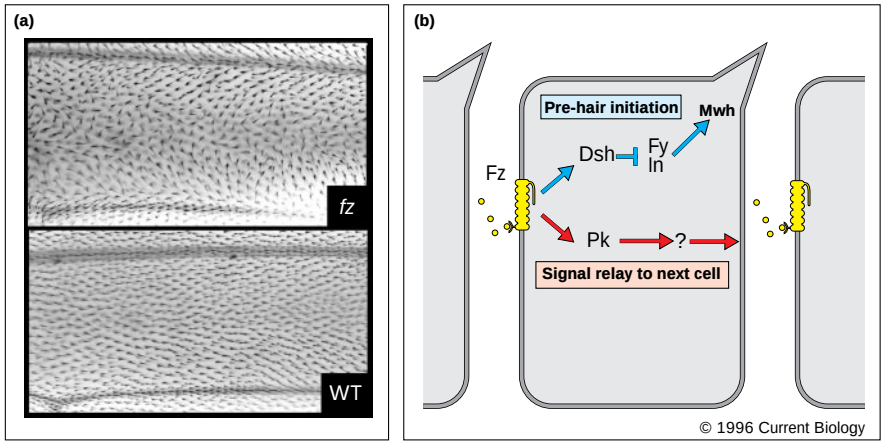
signal, affecting the cell that secretes it (autocrine) as well as its neighbors (paracrine). Therefore, unambiguously placing a protein in one or other pathway using only genetic analysis of embryonic patterning is difficult. The proteins ascribed to the Hedgehog signaling pathway could instead be part of an autocrine Wingless signaling pathway; some have suggested that autocrine and paracrine Wingless signaling involve distinct signal transduction pathways [17,18].

To some thoughtful people, the identity of the Wingless receptor was not a complete surprise. To explain the clues that existed, we must describe an area of research into fly development that previously seemed unrelated, namely tissue polarity (reviewed in [19]). One remarkable property of many cells in flies and other animals is that they not only have a knowledge of their position within the body, allowing them to choose fates, but they also have an internal compass, allowing them to distinguish different cardinal directions. One striking example of this involves the orientation of the hairs decorating the cuticle of adult insects. In the wing, each cell produces a single hair as a projection of its cytoplasm and, remarkably, these hairs are all aligned perfectly, such that all hairs emerge from the corresponding vertices of each cell and thus all point in the same direction (Fig. 2a). Each cell can distinguish direction in the plane of the epithelial sheet, and more importantly, cells do this in a coordinated fashion.

To understand this cellular ability, scientists looked for mutants in which this process is disrupted (Fig. 2a). Genetic and molecular analyses [19] provided the basis for a model of tissue polarity, in which a signal passes across the field of cells (Fig. 2b). This signal is sensed by Frizzled, a transmembrane protein of the serpentine receptor class [20]. The signal is transduced by a pathway including Dishevelled, also part of the Wingless pathway; however, no other component is known to be shared by both the Frizzled and Wingless pathways (Fig. 2b). The signal has two effects: to determine the location of the hair and to stimulate propagation of the signal across the field of cells, presumably by stimulating ligand production. In discussing their evidence that Dishevelled is downstream of Frizzled [21], Adler and colleagues made the telling prediction "that the Wingless receptor could share structural features or sequence homology with Frizzled". As they also pointed out, however, *frizzled* null mutants do not show a *wingless*-like embryonic lethal phenotype, but rather are adult viable with tissue polarity defects. So if Frizzled is a Wingless receptor, it is a redundant one.

The next clues as to the nature of the Wingless receptor(s) came from an unexpected quarter. While sequencing retinal cDNAs, Wang *et al.* [22] identified a mammalian protein related in sequence to fly Frizzled. From their work and that of others, we now know of at least eight *frizzled* homologs in mammals, eleven in zebrafish, several in

Figure 2



Tissue polarity and the Frizzled pathway. **(a)** In a wild-type wing (bottom), all hairs point in a single direction, but in a *frizzled* mutant (top), the hairs are essentially oriented randomly. **(b)** The Frizzled signal is thought to have two effects on target cells, one determining the location of the hair within the cell and the other stimulating further production of ligand. Fz, Frizzled; Fy, Fuzzy; In, Inturned; Mwh, Multiple wing hair; Pk Prickle. For details see [19]. (Adapted from a figure kindly provided by R. Krasnow and P. Adler.)

chickens and sea urchins, and at least two in the nematode *Caenorhabditis elegans* [12,22]. These may not represent the full spectrum of *frizzled* homologs. Each mouse *frizzled* gene has a distinct tissue distribution of transcripts in adult tissues, and all *frizzled* transcripts are found in more than one tissue [22]; some transcripts are also present in embryonic tissues.

The Frizzled homologs range in length from 500 to 700 amino acids, and are all predicted to be serpentine receptors (Fig. 3). All the homologs begin with a conserved cysteine-rich domain, predicted to be extracellular. The central part of each protein consists of the seven putative transmembrane domains, characteristic of serpentine receptors. The carboxy-terminal domains of different Frizzled homologs show little similarity in sequence or length, so they may interact with different effector molecules. Although serpentine receptors are typically G-protein coupled, no Frizzled family protein has yet been shown to exhibit G-protein-coupling and there is little or no sequence similarity between any Frizzled protein and a known G-protein-coupled receptor.

The diversity of mammalian Frizzled homologs suggested flies might also have multiple family members. Bhanot *et al.* [23] searched for and identified a second *Drosophila* Frizzled homolog, Fz2. At certain stages of embryogenesis Fz2, like many segment polarity genes, is expressed in one stripe of cells per segment. To test whether Frizzled relatives might be Wingless/Wnt receptors, Bhanot *et al.* [23] used two *Drosophila* tissue culture cell lines: cl8 cells, which are responsive to Wingless, and S2 cells, which are not [24]. It was possible that S2 cells are unresponsive because they do not express the Wingless receptor. Interestingly, cl8 cells express Fz2 mRNA, but S2 cells do not, and thus provide an *in vitro* system in which to test Fz2. Expression of Fz2 following transfection made S2 cells responsive to Wingless, as assayed by Armadillo accumulation, and also conferred binding of Wingless to the cell surface; Fz2 thus meets both criteria for being a Wingless receptor or an essential component of one.

Evidence for a role of Frizzled homologs in vertebrate Wnt signaling was recently obtained from studies of *Xenopus* [25]. These studies also place Dishevelled and GSK-3, the Zw3 homolog, downstream of a vertebrate Frizzled. Yang-Snyder *et al.* [25] found that Frizzled-1 overexpression in *Xenopus* embryos stabilized Wnt-8 association with the cell surface and recruited Dishevelled to the plasma membrane. Frizzled-1 overexpression also induces expression of two genes, *Xnr3* and *siamois*, which have previously been shown to be induced by Wnt signaling; this effect is antagonized by GSK-3 overexpression. Wnt-8 and Frizzled-1 have synergistic effects on these target genes when both are overexpressed. Yang-Snyder *et al.* [25] also provide some evidence that begins to address the issue of the match between different Wnts and different Frizzled proteins: Wnt-5a is distinct from Wnt-8 in its biological effects on *Xenopus* embryogenesis, and Wnt-5a association with the plasma membrane was not stabilized by Frizzled-1 overexpression.

Analysis of asymmetric cell divisions in *C. elegans* has also revealed a connection between Wnts and Frizzled proteins. Certain nematode cells undergo asymmetric cell division, and mutations in several genes disrupt this asymmetry. Mutations in *lin-44*, which encodes a Wnt family member [11], result in the reversal of polarity, with respect to the body axis, of certain asymmetric cell divisions. Mutations in *lin-17* result in a loss of asymmetry of these same cell divisions. Sawa *et al.* [12] found that *lin-17* encodes a Frizzled homolog expressed in the cells about to undergo the asymmetric cell division, consistent with Lin-17 being a Lin-44 receptor. In contrast, Lin-44 is expressed in nearby cells [11], consistent with its role as a signalling molecule that confers positional cues. The difference in

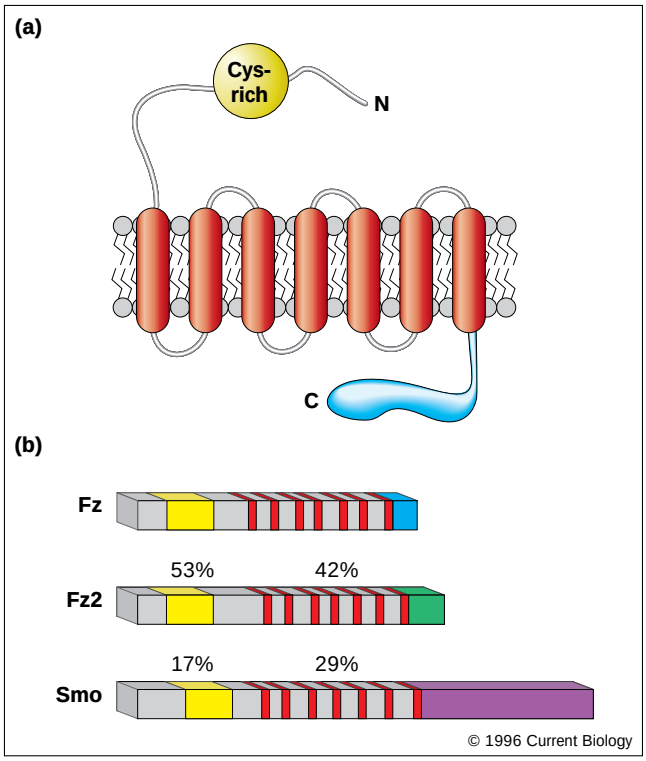
phenotypes of *lin-17* and *lin-44* mutants suggests that there may be other Wnt relatives in *C. elegans*. Perhaps the most interesting aspect of this work is the parallel with Frizzled itself: both Frizzled and Lin-17 confer on cells the ability to distinguish one cell surface from another, allowing them to set up proper cell polarity.

Given these findings on Wingless/Wnt receptors, the cloning of *smoothened*, a gene thought to be required for Hedgehog signaling, provided a substantial surprise [26,27]. Smoothened is also a Frizzled relative, though a more distant one. This discovery has two possible explanations, either of which would be remarkable. One is that Smoothened is a Hedgehog receptor, suggesting that Hedgehog and Wingless share related receptors despite their total lack of sequence similarity and their striking structural differences. Given the key role of Hedgehog and Wnt proteins in animal development, this would elevate the Frizzled family to a position of even greater prominence. The second possibility, even more remarkable, is that Smoothened is the receptor for the Wingless autocrine signal. This possibility, perhaps more consistent with the structure of Smoothened, would require revision of our current thinking about Hedgehog signaling, suggesting that the proteins ascribed to the Hedgehog pathway might in fact be part of the autocrine Wingless pathway (Fig. 1b,c).

If Smoothened is an autocrine Wingless receptor, where does that leave Hedgehog? Hedgehog may act *via* a different receptor, such as Patched, but there is an interesting alternative. Hedgehog is a molecule of many talents: it encodes an autoprotease that simultaneously cleaves itself and adds a lipid moiety [28], generating an amino-terminal fragment with signaling activity, and this amino-terminal domain is similar in structure to zinc hydrolases, so it may also have an enzymatic activity. One heretical possibility is that Hedgehog acts not as a ligand but as an enzyme — it may, for example, modify Wingless, Smoothened, or another protein, regulating Wingless autocrine signaling. Clearly, attention will now focus on biochemical analysis of Wingless, Hedgehog, Fz2, Smoothened and Patched.

Despite the fact that Wingless landed in somewhat familiar territory, the recent discoveries raise many questions about the match between the diverse Wnts, the many Frizzled relatives and the diverse array of possible signal transduction components. How promiscuous are the receptors as to which Wnts they bind? What is the relationship between the flood of Frizzled proteins and the fact that vertebrate Wnts fall into at least two classes differing in their biological activities? What is the ligand for the family prototype, Frizzled itself, Wingless, another Wnt, or perhaps a second Hedgehog? How is expression of different Frizzled relatives regulated, and what spectrum of receptors is expressed in a given tissue? In flies, for example, Fz2 is expressed at higher levels in a subset of Wingless-responsive cells; comparison of Fz2 and Smoothened expression patterns should prove interesting. Finally, do different receptors trigger different responses *via* distinct signal transduction pathways? The differences in the cytoplasmic domains of Frizzled, Fz2 and Smoothened, and the lack of extensive overlap among the molecules thought to be downstream of each, support this possibility. The questions raised and the excitement inherent in obtaining answers mean that the Frizzled family's continued celebrity is assured.

Figure 3



(a) Frizzled proteins are predicted to be serpentine receptors, with seven transmembrane domains. The extracellular amino-terminal region contains a cysteine-rich domain followed by a variable linker. The central part of the protein consists of the transmembrane domains The cytosolic carboxy-terminal region is expected to interact with effector proteins – possibly G proteins, like other serpentine receptors, though there is no direct evidence for this. (b) A comparison of *Drosophila* Frizzled proteins (Fz and Fz2) and Smoothened (Smo). The red segments represent the seven putative transmembrane domains; yellow segments represent the cysteine-rich domains within the extracellular regions; the blue, green and magenta segments in Fz, Fz2 and Smo, respectively, represent their carboxy-terminal cytosolic domains, which are very variable in length and sequence. The percentages above some Fz2 and Smo segments indicate similarity to corresponding segments in Fz.

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