Alternative Wnt Signaling Is Initiated by Distinct Receptors
Renée van Amerongen, Amanda Mikels and Roel Nusse (2 September 2008)
Science Signaling **1** (35), re9. [DOI: 10.1126/scisignal.135re9]

CELL BIOLOGY

Alternative Wnt Signaling Is Initiated by Distinct Receptors

Renée van Amerongen,* Amanda Mikels,* Roel Nusse†

Published 2 September 2008

An unanswered question in the field of signal transduction research is how different signaling pathways are activated with strict specificity in a temporally and spatially controlled manner. Because extracellular ligands and membrane receptors constitute the first signaling modalities for most pathways, selectivity in ligand-receptor binding likely dictates the outcome of downstream signaling events. Unfortunately, possible complexities underlying ligand-receptor interactions are often overlooked. Here, we discuss basic principles of signal transduction initiated at the cell membrane, with the Wnt pathway, which harbors a multitude of ligands and receptors, as an example.

Owing to gene duplication events throughout evolution, mammalian genes often have one or more closely related relatives. On the basis of sequence similarity, such genes are grouped into families. Although the proteins encoded by different family members can share biochemical activities, they often also display quite divergent functions. Such differences can be either intrinsic, which are related to specific-sequence variations in the protein, or context-dependent, which are related to the presence or absence of specific interaction partners in a given tissue or at a given time.

Because signal transduction is often initiated at the level of extracellular ligands and their cell-surface receptors, any intrinsic differences between proteins from the same ligand or receptor family would be a failsafe way to ensure pathway specificity. Alternatively, context-dependent ligand-receptor pairings would greatly increase the possible number of combinations by which pathway activation is achieved, allowing for greater sensitivity and fine-tuning of the response.

Thus, an outstanding question in the signal transduction field is whether individual ligands or receptors within a family activate different pathways, culminating in the recruitment of different downstream effector molecules. If so, do different classes of protein ligands, membrane receptors, or both exist? Are specific ligand-receptor combinations favored over others? Answering these and related questions remains a daunting task. However, addressing them will eventually lead to understanding more precisely how different signaling pathways can be activated reliably in a temporally and spatially controlled manner. With the Wnt pathway as our example, we discuss how these fundamental questions affect our view of signal transduction in general.

Wnt Proteins

Wnt genes encode lipid-modified, secreted signaling molecules that constitute a large family. They are highly conserved across the metazoan kingdom, with orthologs of individual Wnts found in animal species ranging from Cnidaria and Porifera (sponges) to flies and vertebrates—thus spanning 600 million years of evolution. In this diverse range of organisms, Wnts play fundamental roles in controlling cell proliferation, cell-fate determination, and differentiation during embryonic development and adult homeostasis (*1*–*4*). The high degree of conservation and evolutionary constraint of individual family members suggests that particular Wnts are likely to have specific functions.

The mammalian genome encodes 19 Wnt proteins and 10 Frizzled (Fz) seven-pass transmembrane receptors, which suggests that, in theory, 190 potential Wnt-Frizzled combinations exist. It has been proposed that Wnts activate a number of different signaling pathways (*5*–*7*), each of which has been shown to intersect with numerous other intracellular signal transduction pathways (*8*). That activation of some, but not all, Wnt pathways requires co-receptors such as low-density lipoprotein receptor–related protein 5 (LRP5) and LRP6 further complicates matters. Thus, an ongoing question in the Wnt field is whether all Wnt family members signal in the same manner or whether intrinsic differences between Wnt family members dictate their signaling capabilities.

The biochemical behavior of Wnts has been difficult to study in detail because these proteins have been notoriously tricky to manipulate. Consequently, initial research designed to study the effects of Wnt proteins usually involved performing gene-transfer experiments in animal models or in cell culture systems. From these studies, it appeared that Wnt family members could be divided into two distinct classes (Fig. 1). For example, overexpression of some Wnts, such as Wnt1, Wnt3a, and Wnt8, is sufficient to induce a secondary dorsal-ventral axis in *Xenopus* embryos and to morphologically transform C57MG mouse mammary epithelial cells, whereas expression of other Wnts, such as Wnt4, Wnt5a, and Wnt11, is not (*9*–*13*). Both of these activities correlate with the ability of these Wnts to induce an increase in the abundance of the cytoplasmic protein β-catenin. In contrast, overexpression of Wnt5a and Wnt11 is associated with the convergence and extension (the developmental process that causes tissues to undergo simultaneous narrowing and lengthening as a result of inter-

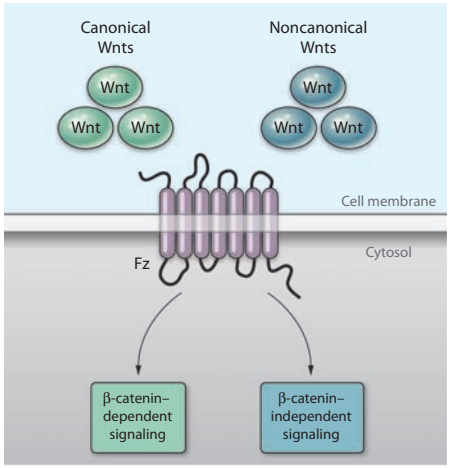

Fig. 1. Previous view of Wnt signaling. Historically, Wnt proteins have been divided into "canonical" and "noncanonical" classes, which activate β-catenin–dependent and –independent signaling pathways, respectively. However, in recent years, the lines between the different Wnts have begun to blur.

Howard Hughes Medical Institute, Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA.

***These authors contributed equally to this work.**

†Corresponding author. E-mail: rnusse@stanford.edu

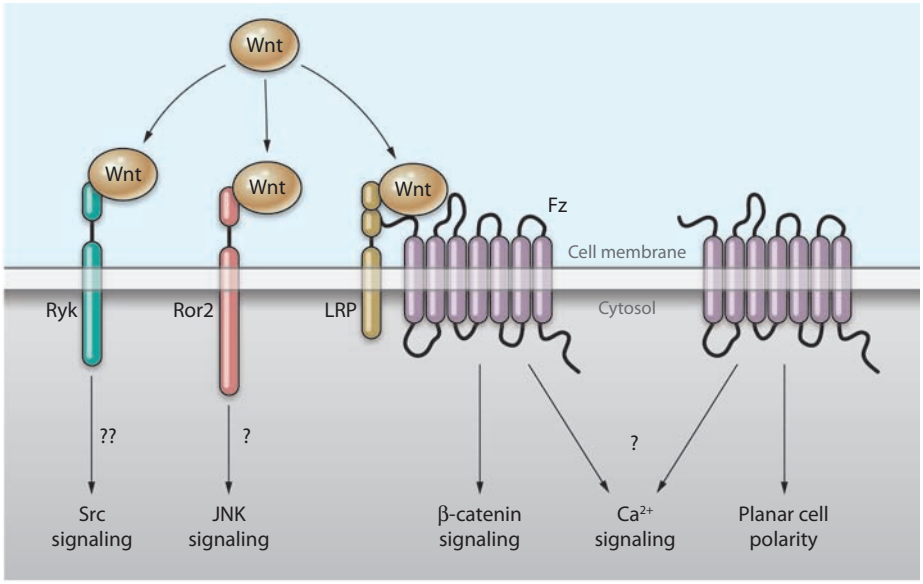

Fig. 2. Current model of receptor-dependent Wnt signaling. Different lines of evidence support a model in which the receptors present at the cell surface determine the outcome of Wnt activity. For most β-catenin–independent pathways, the downstream signaling events following pathway activation are still unclear. For instance, although Src has been implicated in the Wnt-Ryk pathway, its true involvement remains to be established (see text for further details).

calated cell movements) of the *Xenopus* and zebrafish body axes in a manner that is not phenocopied by the other Wnts and that seems to be independent of β-catenin signaling (*8*, *14*—*16*).

From these early studies, a view has emerged of the existence of so-called "canonical" Wnts (including Wnt1, Wnt3A, and Wnt8) and "noncanonical" Wnts (including Wnt5A and Wnt11), which activate "canonical" and "noncanonical" signaling pathways, respectively. However, a more careful inspection of the literature suggests that the subdivision of Wnts into these two categories does not hold up to scrutiny and that the organization of Wnt signaling pathways into canonical or noncanonical categories is not very useful. Instead, we and others have proposed that Wnts themselves are not intrinsically canonical or noncanonical but that the multiple pathways that these ligands initiate are determined by distinct sets of receptors (*17*–*22*) (Fig. 2).

Although differing signaling capabilities are often considered to be specific to distinct Wnts, most notably Wnt5a, the experimental evidence suggests otherwise. First, even though Wnt5a is the Wnt most often associated with noncanonical Wnt signaling, Wnt5a also activates β-catenin signaling. Coinjection of Wnt5a RNA with Frizzled 5 results in axis duplication in *Xenopus* embryos, and treatment of tissue culture cells with Wnt5a following coexpression of Frizzled 4 and LRP5 induces a β-catenin–responsive luciferase reporter construct (*17*, *19*). Moreover, Wnt-Frizzled fusion constructs of presumed noncanonical Wnts do the same (*22*). Likewise, Wnt1 signals to β-catenin in various contexts, causing the activation of T cell factor (TCF)–dependent transcription, and acts as an oncogene (*17*, *19*, *23*). However, from overexpression experiments in various cell lines, Smit *et al*. presented evidence that Wnt1 can also inhibit the activity of TCF (*24*, *25*). Another example of a Wnt with dual signaling capabilities is Wnt11, which is most often implicated in the noncanonical convergence-extension pathway in zebrafish (*24*). In contrast, recent work demonstrated that Wnt11 is the long-sought ligand that activates the β-catenin signaling cascade in the early *Xenopus* embryo, which confirms the idea that a single Wnt protein can activate multiple pathways, most likely by activating different receptors (*26*).

Wnt Signaling Pathways

Since the discovery of Wnts as a conserved class of signaling molecules in the early 1980s, the activation of β-catenin downstream of Wnt-receptor binding is the cellular response that has received the most attention. Historically called "canonical Wnt signaling," this response will hereafter be referred to by us as "Wnt–β-catenin signaling." Briefly, Wnt–β-catenin signaling involves the binding of Wnts to two receptors: Frizzled, through the receptor's cysteine-rich domain (CRD), and LRP5 or LRP6 (*27*–*29*). Through a cascade of events involving the Dishevelled protein, and the inhibition of a multiprotein complex containing axin, glycogen synthase kinase 3 (GSK-3), and adenomatous polyposis coli (APC), the β-catenin protein is stabilized, enabling it to interact with nuclear TCF/Lymphoid Enhancer Factor (LEF) proteins, resulting in changes in gene transcription (30). Remarkable progress has been made on this pathway, which has revealed the importance of Wnt–β-catenin signaling not only in metazoan development, but also in degenerative diseases and cancer (*31*–*35*). Whereas extensive study of Wnt–β-catenin signaling has resulted in its understanding in great (though incomplete) detail, noncanonical Wnt signaling has remained less well defined, with multiple β-catenin–independent pathways potentially existing side by side.

The best-understood variant Wnt pathway was first described in *Drosophila*, where it was shown to be instrumental for the establishment of planar cell polarity (PCP), a process in which fields of cells orient themselves relative to the plane of the tissue in which they reside. However, whereas there are clear roles for *frizzled* (which was discovered because of its polarity phenotype) and *dishevelled* in this pathway, neither LRP, β-catenin, nor TCF is involved. Most strikingly, current evidence in *Drosophila* strongly suggests that no Wnt protein operates in the PCP pathway (*36*–*38*). Thus, it would be a misnomer to call the PCP pathway a noncanonical Wnt-signaling pathway; instead, it might be referred to as "Frizzled-PCP" signaling.

Conserved components that are specific to the PCP pathway, such as Frizzled, Van Gogh–like (Vangl), Prickle, and Celsr (epidermal growth factor–like laminin A G-repeat homology domain–like EGF LAG seven-pass G-type receptor), have revealed a common role for this pathway in the regulation of tissue polarity as well as convergent extension movements in vertebrates (*39*, *40*). In contrast to *Drosophila,* however, it appears that Wnt proteins may be involved in this pathway in higher

vertebrates. For instance, both Wnt5a knockout mice and Wnt11 zebrafish mutants have defects in convergence extension movements (*14*, *41*). Furthermore, secreted Wnt inhibitors disrupt PCP in the neurosensory epithelium of the mouse inner ear by affecting the orientation of cochlear hair cells (*41–43*). In spite of these findings, however, experimental approaches have so far failed to reveal an instructive role for any of the Wnt proteins in this process. We might therefore face a situation in which a variant Wnt-pathway (see below) collaborates with a conserved Frizzled-PCP pathway in the establishment of PCP in mammals.

Wnt–β-catenin and Frizzled-PCP signaling are not the only proposed signaling pathways that involve Frizzled. Experiments performed largely in *Xenopus* and zebrafish embryonic systems have suggested the existence of a "Wnt-Ca^{2+}" signaling pathway (*44–46*), in which the binding of Wnt promotes Frizzled-mediated activation of pertussis toxin–sensitive heterotrimeric guanine nucleotide–binding proteins (G proteins) (*12*, *45*, *47*, *48*). This, in turn, stimulates the release of Ca^{2+} from intracellular stores, which leads to the activation of Ca^{2+}-dependent effector molecules such as the transcription factor nuclear factor associated with T cells (NFAT). However, because both Ca^{2+} flux and activation of Ca^{2+}-dependent effector proteins have seldom been observed in direct response to stimulation of cells with Wnts, and because certain Frizzleds modulate protein kinase C and Ca^{2+}/calmodulin-dependent kinase II activity in the absence of exogenous Wnt (*45*, *47*), it remains to be seen whether, like Frizzled-PCP signaling, Wnt-Ca^{2+} signaling indeed requires Wnts. Given that Frizzled 4 also binds to Norrin, a protein ligand wholly unrelated to Wnts, non-Wnt ligands should also not be excluded from playing a potential role in the activation of any of the pathways mentioned (*5*, *49*, *50*).

Receptors Dictate Wnt Activity

Despite the role that Frizzled receptors play in both β-catenin–dependent and –independent Wnt signaling, increasing evidence demonstrates that other membrane proteins that contain known Wnt-binding domains serve as equally important receptors for Wnts. The atypical tyrosine kinase Ryk, for instance, possesses a Wnt-inhibitory factor (WIF) domain, which was first discovered as a module present in secreted inhibitors of Wnt–β-catenin signaling that act by sequestering Wnts away from Frizzled and other cell-surface receptors (*51*). Although structurally distinct from the Frizzled CRD domain, the Ryk WIF module also binds to Wnt proteins with high affinity (*52*). In *Drosophila*, the Ryk ortholog Derailed binds to Wnt5 to promote commissural axon guidance and proper salivary gland migration, possibly through the activation of members of the Src family of tyrosine kinases (*53–55*). Currently, there is no evidence indicating that Wnt–β-catenin pathway components, other than the Wnts themselves, are involved in Wnt-Ryk signaling, which suggests that this truly is an independent signal transduction pathway.

Lastly, yet another alternative Wnt signaling pathway is mediated by the single-pass receptor tyrosine kinase Ror2. Unlike Ryk, the Ror2 receptor, although structurally distinct from Frizzled receptors, contains a CRD and induces intracellular signaling in response to Wnt5a (*19*, *56–59*). In *Xenopus* embryos and mouse tissue culture cells, Ror2 influences convergence and extension movements and inhibits Wnt–β-catenin signaling, possibly through the activation of the mitogen-activated protein kinase c-Jun N-terminal kinase (JNK). Moreover, a recent study showed clear defects in PCP in the inner ear of mice deficient in Ror2 (*60*). Hopefully, we will gain more insight into the signaling pathway responsible for these phenotypes as more research into the Ror2 receptor is performed.

When these data are examined together, a picture emerges of different pathways eliciting unique responses, which are initiated by distinct receptor-ligand pairings: Wnt-Frizzled-LRP leading to β-catenin signaling; Frizzled-mediated initiation of the PCP pathway and potentially Ca^{2+} signaling; Wnt-Ryk–mediated direction of axon guidance, possibly involving Src proteins; and Wnt-Ror2 signaling through the potential activation of JNKs. When compared in this way, it becomes clear that it is receptor configuration and not properties intrinsic to various Wnt family members that dictates which pathway is activated (Fig. 2).

Once this model is taken into account, many of our old observations and hypotheses require reexamination. For instance, given that Wnt5-Derailed directs axon guidance, studies in vertebrate systems might also have to examine Ryk before concluding that the effects of Wnts on axon guidance are mediated through Frizzled receptor signaling (*61*). Similarly, recent studies regarding a role for Wnt5a in cell migration cannot simply postulate that this is mediated through Frizzled-PCP signaling, because the requirement for either Frizzled or other receptors, such as Ror2, has not been fully investigated (*62*). Lastly, we suggest to researchers that they not assume that certain pharmacological inhibitors, such as those that inhibit G protein–coupled receptors, are acting on Frizzled receptors in the absence of supporting evidence (*63–65*).

Conclusion

In summary, we believe that the unfortunate historic division of Wnts into canonical and noncanonical classes has led to a distorted view of signaling, in which the binding of Wnt ligands to Frizzled transmembrane receptor complexes results in either canonical or noncanonical signaling. Research over the past decade suggests that Wnts from either class are able to elicit β-catenin–dependent and –independent responses and that the outcome is determined by the receptor context on the cell surface. Moreover, the discovery of non-Frizzled proteins as genuine Wnt receptors suggests that pathway activation is determined at the level of Wnt-receptor binding and not at a branching point such as the activation of Dishevelled further downstream. In this regard, Wnts and Frizzleds might be akin to interleukins and interleukin receptors, which also have distinct high-affinity pairings, resulting in individual, yet often related, intracellular responses (*66*). Future studies will have to determine to what degree recruitment of specific intracellular signaling proteins also contributes to the specificity of the different receptors.

Because both Ryk (*67*) and Ror2 (*60*) have been found to interact with Frizzled proteins, the possibility remains that in a given context, these proteins function as coreceptors for Frizzled in β-catenin–independent signaling, similar to the way in which LRP serves as a coreceptor in Wnt–β-catenin signaling. However, owing to the complexity of these diverse ligand-receptor interactions, especially when studied in vivo, it is often difficult to determine whether certain proteins function as coreceptors in the same cell or as independent receptors on different cells. Greater understanding of these complexities should help us to improve our models of how Wnts function.

Given the highly regulated expression patterns of different Wnts in different tissues

in both mammals and lower organisms [for example, during development of the murine gut (*68*) and in distinct domains in Cnidarians (*69*)], their specific functions in vivo might be influenced by their expression domain. In addition, intrinsic properties of the Wnts such as binding affinities might also still contribute to the outcome of signaling. Moreover, at distinct developmental stages or at different sites, Wnt-receptor pairing may have different outcomes depending on the intracellular signaling competence of the responding cell or the tissue environment. Finally, more detailed insight into Wnt-receptor affinities can only be truly gained following intensive biochemical analyses, which will have to wait until all Wnt proteins are purified and analyzed individually. In the meantime, as our understanding of Wnt-Frizzled interactions increases, we urge others to revisit some of the old assumptions regarding Wnt-pathway activation. As similar principles will likely apply to other signaling pathways in which large families of ligands and receptors are present, we hope that the Wnt signaling pathway will serve as an example to underscore the complexities in studying signal transduction.

References and Notes

1. C. Y. Logan, R. Nusse, The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* **20**, 781–810 (2004).
2. C. J. Neumann, S. M. Cohen, Long-range action of Wingless organizes the dorsal-ventral axis of the Drosophila wing. *Development* **124**, 871–880 (1997).
3. T. Reya, H. Clevers, Wnt signalling in stem cells and cancer. *Nature* **434**, 843–850 (2005).
4. M. Zecca, K. Basler, G. Struhl, Direct and long-range action of a wingless morphogen gradient. *Cell* **87**, 833–844 (1996).
5. M. Hendrickx, L. Leyns, Non-conventional Frizzled ligands and Wnt receptors. *Dev. Growth Differ.* **50**, 229–243 (2008).
6. B. T. Macdonald, M. V. Semenov, X. He, SnapShot: Wnt/beta-catenin signaling. *Cell* **131**, 1204.e1–1204.e2 (2007).
7. M. V. Semenov, R. Habas, B. T. Macdonald, X. He, SnapShot: Noncanonical Wnt signaling pathways. *Cell* **131**, 1378.e1–1378.e2 (2007).
8. M. T. Veeman, J. D. Axelrod, R. T. Moon, A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev. Cell* **5**, 367–377 (2003).
9. S. J. Du, S. M. Purcell, J. L. Christian, L. L. McGrew, R. T. Moon, Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in Xenopus embryos. *Mol. Cell. Biol.* **15**, 2625–2634 (1995).
10. D. J. Olson, J. Papkoff, Regulated expression of Wnt family members during proliferation of C57mg mammary cells. *Cell Growth Differ.* **5**, 197–206 (1994).
11. H. Shimizu, M. A. Julius, M. Giarre, Z. Zheng, A. M. Brown, J. Kitajewski, Transformation by Wnt family proteins correlates with regulation of beta-catenin. *Cell Growth Differ.* **8**, 1349–1358 (1997).
12. D. C. Slusarski, V. G. Corces, R. T. Moon, Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* **390**, 410–413 (1997).
13. G. T. Wong, B. J. Gavin, A. P. McMahon, Differential transformation of mammary epithelial cells by Wnt genes. *Mol. Cell. Biol.* **14**, 6278–6286 (1994).
14. C. P. Heisenberg, M. Tada, G. J. Rauch, L. Saude, M. L. Concha, R. Geisler, D. L. Stemple, J. C. Smith, S. W. Wilson, Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76–81 (2000).
15. B. Kilian, H. Mansukoski, F. C. Barbosa, F. Ulrich, M. Tada, C. P. Heisenberg, The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. *Mech. Dev.* **120**, 467–476 (2003).
16. J. B. Wallingford, K. M. Vogeli, R. M. Harland, Regulation of convergent extension in Xenopus by Wnt5a and Frizzled-8 is independent of the canonical Wnt pathway. *Int. J. Dev. Biol.* **45**, 225–227 (2001).
17. X. He, J. P. Saint-Jeannet, Y. Wang, J. Nathans, I. Dawid, H. Varmus, A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* **275**, 1652–1654 (1997).
18. G. Liu, A. Bafico, V. K. Harris, S. A. Aaronson, A novel mechanism for Wnt activation of canonical signaling through the LRP6 receptor. *Mol. Cell. Biol.* **23**, 5825–5835 (2003).
19. A. J. Mikels, R. Nusse, Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol.* **4**, e115 (2006).
20. K. Tamai, X. Zeng, C. Liu, X. Zhang, Y. Harada, Z. Chang, X. He, A mechanism for Wnt coreceptor activation. *Mol. Cell* **13**, 149–156 (2004).
21. G. Liu, A. Bafico, S. A. Aaronson, The mechanism of endogenous receptor activation functionally distinguishes prototype canonical and noncanonical wnts. *Mol. Cell. Biol.* **25**, 3475–3482 (2005).
22. S. L. Holmen, A. Salic, C. R. Zylstra, M. W. Kirschner, B. O. Williams, A novel set of Wnt-Frizzled fusion proteins identifies receptor components that activate beta-catenin-dependent signaling. *J. Biol. Chem.* **277**, 34727–34735 (2002).
23. R. Nusse, A. van Ooyen, D. Cox, Y. K. Fung, H. Varmus, Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature* **307**, 131–136 (1984).
24. F. Marlow, J. Topczewski, D. Sepich, L. Solnica-Krezel, Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr. Biol.* **12**, 876–884 (2002).
25. L. Smit, A. Baas, J. Kuipers, H. Korswagen, M. van de Wetering, H. Clevers, Wnt activates the Tak1/Nemo-like kinase pathway. *J. Biol. Chem.* **279**, 17232–17240 (2004).
26. Q. Tao, C. Yokota, H. Puck, M. Kofron, B. Birsoy, D. Yan, M. Asashima, C. C. Wylie, X. Lin, J. Heasman, Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in Xenopus embryos. *Cell* **120**, 857–871 (2005).
27. P. Bhanot, M. Brink, C. H. Samos, J. C. Hsieh, Y. Wang, J. P. Macke, D. Andrew, J. Nathans, R. Nusse, A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* **382**, 225–230 (1996).
28. M. Wehrli, S. T. Dougan, K. Caldwell, L. O'Keefe, S. Schwartz, D. Vaizel-Ohayon, E. Schejter, A. Tomlinson, S. DiNardo, arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* **407**, 527–530 (2000).
29. J. Yang-Snyder, J. R. Miller, J. D. Brown, C. J. Lai, R. T. Moon, A frizzled homolog functions in a vertebrate Wnt signaling pathway. *Curr. Biol.* **6**, 1302–1306 (1996).
30. M. D. Gordon, R. Nusse, Wnt signaling: Multiple pathways, multiple receptors, and multiple transcription factors. *J. Biol. Chem.* **281**, 22429–22433 (2006).
31. H. Clevers, Wnt/beta-catenin signaling in development and disease. *Cell* **127**, 469–480 (2006).
32. R. U. de Iongh, H. E. Abud, G. R. Hime, WNT/Frizzled signaling in eye development and disease. *Front. Biosci.* **11**, 2442–2464 (2006).
33. S. Fox, A. Dharmarajan, WNT signaling in malignant mesothelioma. *Front. Biosci.* **11**, 2106–2112 (2006).
34. V. Krishnan, H. U. Bryant, O. A. Macdougald, Regulation of bone mass by Wnt signaling. *J. Clin. Invest.* **116**, 1202–1209 (2006).
35. P. Polakis, Wnt signaling and cancer. *Genes Dev.* **14**, 1837–1851 (2000).
36. W. S. Chen, D. Antic, M. Matis, C. Y. Logan, M. Povelones, G. A. Anderson, R. Nusse, J. D. Axelrod, Asymmetric homotypic interactions of the atypical cadherin flamingo mediate intercellular polarity signaling. *Cell* **133**, 1093–1105 (2008).
37. P. A. Lawrence, J. Casal, G. Struhl, Towards a model of the organisation of planar polarity and pattern in the Drosophila abdomen. *Development* **129**, 2749–2760 (2002).
38. J. A. Zallen, Planar polarity and tissue morphogenesis. *Cell* **129**, 1051–1063 (2007).
39. J. R. Seifert, M. Mlodzik, Frizzled/PCP signalling: A conserved mechanism regulating cell polarity and directed motility. *Nat. Rev. Genet.* **8**, 126–138 (2007).
40. Y. Wang, J. Nathans, Tissue/planar cell polarity in vertebrates: New insights and new questions. *Development* **134**, 647–658 (2007).
41. D. Qian, C. Jones, A. Rzadzinska, S. Mark, X. Zhang, K. P. Steel, X. Dai, P. Chen, Wnt5a functions in planar cell polarity regulation in mice. *Dev. Biol.* (2007).
42. A. Dabdoub, M. J. Donohue, A. Brennan, V. Wolf, M. Montcouquiol, D. A. Sassoon, J. C. Hseih, J. S. Rubin, P. C. Salinas, M. W. Kelley, Wnt signaling mediates reorientation of outer hair cell stereociliary bundles in the mammalian cochlea. *Development* **130**, 2375–2384 (2003).
43. A. Dabdoub, M. W. Kelley, Planar cell polarity and a potential role for a Wnt morphogen gradient in stereociliary bundle orientation in the mammalian inner ear. *J. Neurobiol.* **64**, 446–457 (2005).
44. A. D. Kohn, R. T. Moon, Wnt and calcium signaling: Beta-catenin-independent pathways. *Cell Calcium* **38**, 439–446 (2005).
45. M. Kuhl, L. C. Sheldahl, C. C. Malbon, R. T. Moon, Ca(2+)/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. *J. Biol. Chem.* **275**, 12701–12711 (2000).
46. P. Pandur, D. Maurus, M. Kuhl, Increasingly complex: New players enter the Wnt signaling network. *Bioessays* **24**, 881–884 (2002).
47. L. C. Sheldahl, M. Park, C. C. Malbon, R. T. Moon, Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr. Biol.* **9**, 695–698 (1999).
48. D. C. Slusarski, J. Yang-Snyder, W. B. Busa, R. T. Moon, Modulation of embryonic intracellular Ca2+ signaling by Wnt-5A. *Dev. Biol.* **182**, 114–120 (1997).
49. H. Clevers, Wnt signaling: Ig-norrin the dogma. *Curr. Biol.* **14**, R436–R437 (2004).
50. Q. Xu, Y. Wang, A. Dabdoub, P. M. Smallwood, J. Williams, C. Woods, M. W. Kelley, L. Jiang, W.

Tasman, K. Zhang, J. Nathans, Vascular development in the retina and inner ear: Control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* **116**, 883–895 (2004).
51. M. Kroiher, M. A. Miller, R. E. Steele, Deceiving appearances: Signaling by "dead" and "fractured" receptor protein-tyrosine kinases. *Bioessays* **23**, 69–76 (2001).
52. J. C. Hsieh, L. Kodjabachian, M. L. Rebbert, A. Rattner, P. M. Smallwood, C. H. Samos, R. Nusse, I. B. Dawid, J. Nathans, A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* **398**, 431–436 (1999).
53. K. E. Harris, S. K. Beckendorf, Different Wnt signals act through the Frizzled and RYK receptors during Drosophila salivary gland migration. *Development* **134**, 2017–2025 (2007).
54. S. Yoshikawa, R. D. McKinnon, M. Kokel, J. B. Thomas, Wnt-mediated axon guidance via the Drosophila Derailed receptor. *Nature* **422**, 583–588 (2003).
55. R. R. Wouda, M. R. Bansraj, A. W. de Jong, J. N. Noordermeer, L. G. Fradkin, Src family kinases are required for WNT5 signaling through the Derailed/RYK receptor in the Drosophila embryonic central nervous system. *Development* **135**, 2277–2287 (2008).
56. W. C. Forrester, The Ror receptor tyrosine kinase family. *Cell. Mol. Life Sci.* **59**, 83–96 (2002).
57. I. Oishi, H. Suzuki, N. Onishi, R. Takada, S. Kani, B. Ohkawara, I. Koshida, K. Suzuki, G. Yamada, G. C. Schwabe, S. Mundlos, H. Shibuya, S. Takada, Y. Minami, The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. *Genes Cells* **8**, 645–654 (2003).
58. A. Schambony, D. Wedlich, Wnt-5A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. *Dev. Cell* **12**, 779–792 (2007).
59. Y. K. Xu, R. Nusse, The Frizzled CRD domain is conserved in diverse proteins including several receptor tyrosine kinases. *Curr. Biol.* **8**, R405–R406 (1998).
60. S. Yamamoto, O. Nishimura, K. Misaki, M. Nishita, Y. Minami, S. Yonemura, H. Tarui, H. Sasaki, Cthrc1 selectively activates the planar cell polarity pathway of Wnt signaling by stabilizing the Wnt-receptor complex. *Dev. Cell* **15**, 23–36 (2008).
61. A. M. Wolf, A. I. Lyuksyutova, A. G. Fenstermaker, B. Shafer, C. G. Lo, Y. Zou, Phosphatidylinositol-3-kinase-atypical protein kinase C signaling is required for Wnt attraction and anterior-posterior axon guidance. *J. Neurosci.* **28**, 3456–3467 (2008).
62. E. S. Witze, E. S. Litman, G. M. Argast, R. T. Moon, N. G. Ahn, Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors. *Science* **320**, 365–369 (2008).
63. M. D. Castellone, H. Teramoto, B. O. Williams, K. M. Druey, J. S. Gutkind, Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* **310**, 1504–1510 (2005).
64. A. E. Chen, D. D. Ginty, C. M. Fan, Protein kinase A signalling via CREB controls myogenesis induced by Wnt proteins. *Nature* **433**, 317–322 (2005).
65. O. Pourquie, Signal transduction: A new canon. *Nature* **433**, 208–209 (2005).
66. S. L. LaPorte, Z. S. Juo, J. Vaclavikova, L. A. Colf, X. Qi, N. M. Heller, A. D. Keegan, K. C. Garcia, Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259–272 (2008).
67. W. Lu, V. Yamamoto, B. Ortega, D. Baltimore, Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. *Cell* **119**, 97–108 (2004).
68. H. Lickert, A. Kispert, S. Kutsch, R. Kemler, Expression patterns of Wnt genes in mouse gut development. *Mech. Dev.* **105**, 181–184 (2001).
69. A. Kusserow, K. Pang, C. Sturm, M. Hrouda, J. Lentfer, H. A. Schmidt, U. Technau, A. von Haeseler, B. Hobmayer, M. Q. Martindale, T. W. Holstein, Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* **433**, 156–160 (2005).
70. R.v.A. was supported by a long-term fellowship from the European Molecular Biology Organization (EMBO) and by a KWF fellowship from the Dutch Cancer Society.

10.1126/scisignal.135re9

Citation: R. van Amerongen, A. Mikels, R. Nusse, Alternative Wnt signaling is initiated by distinct receptors. *Sci. Signal.* **1**, re9 (2008).