[Frontiers in Bioscience 9, 1333-1338, May 1, 2004]

SPECIFICITY OF WNT-RECEPTOR INTERACTIONS

Jen-Chih Hsieh

Department of Biochemistry and Cell Biology, Center for Developmental Genetics, State University of New York at Stony Brook, Stony Brook, New York 11794

TABLE OF CONTENTS

1. ABSTRACT

The highly conserved Wnt signaling proteins play critical roles in guiding pattern formation, cell fate decision, and morphogenetic movement during animal development. They bind to the Frizzled family of seven-pass transmembrane proteins and initiate at least three different intracellular signaling pathways, resulting in regulation of gene expression and/or changes in cell behavior. A single transmembrane protein from the low-density-lipoprotein family functions as a co-receptor in the canonical/beta-catenin pathway. The specificity of Wnt signaling depends in part on the affinities between various Wnt-Frizzled pairs. A Wnt-dependent receptor dimerization or clustering step has been hypothesized as the step that initiates the canonical signaling cascade in cells.

2. WNT SIGNALING IN DEVELOPMENT

The Wnt genes encode a large family of secreted glycoproteins that play essential roles during animal development as well as in the maintenance of tissues (1-5). Signaling by Wnt proteins during embryo development is crucial in guiding basic cellular processes necessary for correct pattern formation, cell-fate determination, and cell polarity. In addition to the important roles in development, more and more studies have connected aberrant Wnt signaling to tumorigenesis (6-8). Given that Wnt signaling affects basic cellular processes, such as proliferation, differentiation, and migration, it is not surprising that inappropriate deregulation of Wnt signaling plays a significant role in tumor progression. Activating mutations in several genes coding for Wnt signaling components have been implicated in human cancers including melanoma (9), colon cancer (10, 11), and hepatocellular carcinoma (12). The field of Wnt signaling has seen a rapid expansion in Wnt-related research. These recent research efforts have not only filled several major knowledge gaps in our understanding of Wnt signaling, but also added extra layers of complexity to the Wnt pathways with the identification of additional components at every level of the Wnt signaling cascades. While new signaling components and new details has been added to the Wnt pathways at an accelerated speed, one major puzzle in our understanding of Wnt signaling has remained unresolved, namely, the mechanisms by which the Wnt signals are transduced by the receptors and the specificity of ligand-receptor interactions is determined. This review focuses on recent studies related to these two aspects of Wnt signaling, with the emphasis on the biochemical aspects of the ligand-receptor interaction.

3. OVERVIEW OF THE WNT SIGNALING PATHWAYS

Functionally, the Wnt proteins act as signaling molecules that interact with receptors on the surface of recipient cells, leading to an intracellular cascade that alters gene expression and/or cell behavior. As many as 19 different Wnt paralogs have been identified in organisms ranging from nematode to human (4, 5). Depending on the specific Wnt-receptor combination, the intracellular pathways that transduce the signal intracellularly diverge into at least three branches (1-5): the canonical/beta-catenin pathway, the planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway.

A great majority of research efforts on Wnt signaling in the last two decades focused on the canonical/beta-catenin pathway, which affects cell fate determination by regulating gene expression. Our current understanding of this pathway in vertebrates can be briefly summarized below. Playing a pivotal role in this signaling pathway is beta-catenin. Beta-catenin is a multifunctional protein that exists as a component of the high molecular weight complex that forms cell-cell adherens junctions and as an unstable monomer in the cytoplasm. Cytoplasmic beta-catenin can function as a transcriptional co-activator upon entering the nucleus (13, 14). However, it is rapidly turned over through the action of a multi-component protein phosphorylation machinery consisting of glycogen synthase kinase-3beta (GSK3beta), Axin, and adenomatous polyposis coli (APC) protein (15, 16). Phosphorylated beta-catenin is targeted for degradation by proteosome (17). Binding of Wnts to the receptors on the cell surface leads to activation of the intracellular protein Dishevelled, which in turn inactivates the GSK3beta/Axin/APC complex, allowing free beta-catenin in the cytoplasm to accumulate and enter the nucleus. In vertebrate, the nuclear beta-catenin interacts with DNA-binding proteins of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family to alter the expression of target genes (10, 11, 13, 14, 18).

The PCP and Wnt/Ca^{2+} pathways are known as the "noncanonical pathways", and our understanding of both pathways has been lacking until recently (2, 19). Signaling through either pathway is independent of beta-catenin. The PCP pathway regulates cell polarity and morphogenetic movements during development, and is mediated by Dishevelled, small GTPases of the Rho family and c-Jun amino-terminal kinase. The Wnt/Ca^{2+} pathway regulates cell adhesion and motility (20), and is mediated through release of intracellular Ca^{2+} upon Wnt stimulation, and

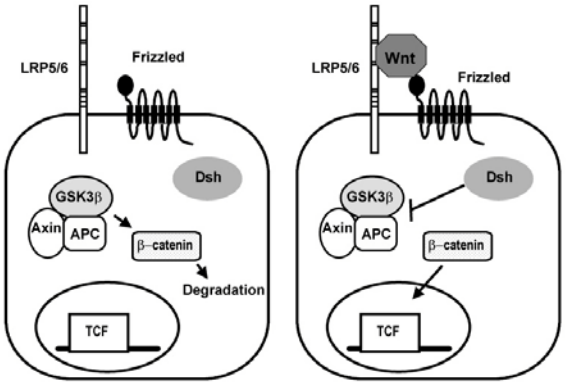

Figure 1. The canonical Wnt signaling pathway. *Left*, In the absence of Wnt, little beta-catenin is present in the cytoplasm because it is degraded as a result of phosphorylation by the Axin/APC/GSK3beta complex; *Right*, Interaction of Wnt with Frizzled and LRP5/6 activates Dsh, which in turn inactivates the Axin/APC/GSK3beta complex, allowing beta-catenin to accumulate and enter the nucleus. Upon entering the nucleus, beta-catenin interacts with the transcription factor of the TCF family to activate gene transcription.

involves activation of protein kinase C and calcium/calmodulin-dependent kinase II (21), leading to the regulation of the Ca^{2+}-responsive transcription factor NF-AT (22).

One feature common to these three Wnt signaling pathways is that they all act through the serpertine receptor of the Frizzled family, which consists of at least 10 different members in human. The founding member of the Frizzled family, the *frizzled* gene of *Drosophila*, is an essential player in the PCP pathway for establishment of the polarized structure throughout adult cuticle (23, 24). Both the *frizzled* and *Frizzled2* genes of *Drosophila* have been shown to function redundantly as receptors for Wingless in the canonical pathway (25-27).

4. STRUCTURES AND FUNCTIONS OF WNT PROTEINS AND RECEPTORS

4.1. Wnt proteins

All Wnts share the following characteristics: they are secreted glycoproteins of 350-400 amino acids, with a conserved pattern of 23-24 cysteine residues and several asparagine-linked glycosylation sites (28). Some Wnt proteins have additional domains. For example, *Drosophila* Wg contains an 85-amino acid domain near the center of the protein (28). One major barrier hampering our understanding of the functions of Wnt proteins has been the difficulty in obtaining soluble and biologically active Wnt preparations. Production of soluble Wnt proteins by ectopic expression in cultured cells has been problematic, as Wnt proteins generally accumulate in the endoplasmic reticulum (ER) (29, 30). With the recent success by Roel Nusse and colleagues in purifying Wnt-3a (31), the chemical basis for this uncooperative property of Wnt proteins was elucidated. The purified Wnt-3a is covalently modified with a palmitate at cysteine 77. Palmitoylation greatly affects the signaling activity of Wnt-3a, and may account for the sticky property of Wnt observed by many groups.

Many Wnt members have been shown to control the development of various tissues. It is not clear how the *in vivo* specificity of Wnt activities is determined. Earlier studies suggest that Wnt proteins fall into subgroups with different activities. For example, transient expression of *Wnt-1, Wnt-2*, *and Wnt-3a* in C57MG mouse mammary epithelial cells causes morphological transformation, whereas the other Wnts have little effect on the cell morphology (32). Furthermore, ectopic expression of various Wnt mRNAs in *Xenopus* embryos leads to two distinct phenotypes. *Xenopus Wnt-1* (*XWnt-1*), *XWnt-3a*, and *XWnt-8* induce duplication of the body axis when injected into the ventral blastomeres of four-cell embryos (33-36). In contrast, overexpression of other *Wnt* genes, including *XWnt-5a*, *XWnt4*, and *XWnt11*, interferes with morphogenetic movement without causing axis duplication (37). More recent results suggest that different subsets of Wnt proteins can trigger distinct intracellular pathways that lead to different physiological changes (38-40).

4.2. Wnt receptors – the Frizzled proteins

Wnts are signaling molecules, acting primarily in a paracrine fashion on target cells. A series of genetic, cell biological, and biochemical studies have provided mounting evidence that members of the Frizzled (**Fz**) family function as Wnt receptors (25-27, 41-47). The Frizzled family consists of at least 10 mammalian members and is named after the first member, the *Drosophila* tissue polarity gene *frizzled* (23, 24). Structurally the Frizzled proteins are similar to other seven-pass transmembrane proteins, such as G protein-coupled receptors, and have the following features: (i) an extracellular domain that consists of a 120-amino acid, cysteine-rich domain (CRD) characterized by 10 invariantly spaced cysteine residues; (ii) a linker region that shows little sequence similarity among family members; (iii) a highly conserved seven-transmembrane domain; and (iv) a cytoplasmic domain of variable size and little sequence homology among family members.

Direct binding with full-length Frizzled has been demonstrated for some Wnt proteins, including Wg and XWnt8 (41, 48). These *in vitro* qualitative binding experiments show that a single Wnt can bind to several Frizzled proteins, including homologous members from a different species. A more comprehensive assessment of the interactions between various Wnt-Frizzled pairs remains to be conducted subject to the availability of suitable Wnt preparations. The Wnt binding activity of Frizzled is mediated primarily through the conserved CRD (41), which has a unique compact structure consisting predominantly of α-helices, with all 10 conserved cysteines forming 5 disulfide bonds (49). The potential Wnt binding sites of the CRD have been mapped out using a binding assay that detects direct binding of XWnt-8 to the CRD tethered to plasma membrane through glycophosphotidylinositol (GPI) anchor (49). Interestingly, the Frizzled CRD is also found in a number of other proteins, such as the soluble Fz-related proteins (sFRP) (50-57), some receptor tyrosine kinases (58-60), carboxypeptidase Z (CPZ) (61), the membrane-anchored serine protease Corin (62), and an isoform of collagen (63). sFRPs have been shown to function as soluble Wnt antagonists when ectopically expressed in *Xenopus* embryos (53, 54, 56, 64, 65). A recent report by Moeller *et. al*. shows that ectopic expression of CPZ, a member of the metallocarboxypeptidase family, in the chick presomitic mesoderm causes skeletal defects, probably by enhancing the signaling activity of Wnt4 (66). A similar ectopic expression of a mutant CPZ lacking a critical active site glutamate fails to interfere with skeletal development. These observations suggest that CPZ functions to modulate Wnt-4a activity through its CRD. Although the precise biological functions of these Frizzled CRD-containing proteins remain to be determined, it is conceivable that the existence of these and other soluble modulating proteins not described here will add another level of regulation to Wnt-Frizzled interactions.

4.3. Co-receptors

Unlike the noncanonical Wnt pathways, which seem to be signaling primarily through the Frizzled protein, the canonical/beta-catenin pathway requires an additional single-pass transmembrane protein, known as LRP5 and LRP6 from the low-density-lipoprotein (LDL) receptor family, to function as an obligate co-receptor for transducing Wnt signal (67-69). The LDL receptor gene family encodes cell surface proteins involved in

receptor-mediated endocytosis, cargo transport and cell signaling (70). Mutants in a *Drosophila* gene *arrow*, which is homologous to mouse *LRP5* and *LRP6*, phenocopy *wg* mutants (67). The function of Arrow is required upstream of Dishevelled in target cells receiving Wg signal (67). Mouse embryos with an insertion mutation in the *lrp6* gene show phenotypes resembling a combination of phenotypes caused by mutations in individual Wnt genes including *Wnt1*, *Wnt3a*, and *Wnt7a* (69). A mutant of *LRP6* lacking the intracellular domain acts as a dominant negative mutant for Wnt signaling when injected into *Xenopus* embryos (68). In a co-immunoprecipitation experiment, the extracellular domain of LRP6 was found in a complex consisting of Frizzled CRD and Wnt-1 (68). The intracellular domain of LRP5 has recently been reported to bind Axin (71, 72), a key component in the GSK3beta complex. For the sake of brevity, we will use "LRP5/6" to represent LRP5, LRP6 and Arrow in the following text.

Because of their cell surface localization, LRP5/6 has been proposed to function as a co-receptor for Wnts. This assertion is further strengthened by the recent identification of a chaperone, MESD/Boca, specific for the folding and/or trafficking of the LDL receptor family of proteins (73, 74). Mouse mutant embryos with the *mesd* gene deleted produce embryonic polarity and mesoderm differentiation defects, resembling those of *wnt3*-deficient mutants (75). A *Drosophila* strain with a mutation in the homologous *boca* gene displays *wg* phenotype (73). The *mesd/boca* gene encodes a resident ER protein whose function is necessary for cell surface localization of proteins of the LDL receptor family, including Arrow, LRP5 and LRP6. Wnt3 signal is required for anterior/posterior polarity and mesoderm development; however, in the absence of a functional MESD, Wnt3 signal can not be transduced through the canonical pathway due to the failure of LRP5/6 to reach the plasma membrane. Although how MESD/BOCA functions to promote proper trafficking of these proteins remains unclear, these observations underscore the important role of LRP5/6 on the cell surface to transducer the Wnt signal through the canonical/beta-catenin pathway.

5. INTERACTION BETWEEN WNTS AND RECEPTORS

Given that both Wnt and Frizzled families consist of multiple members and each member capable of binding to multiple partners, how various Wnts achieve specific interaction with Frizzleds is an important question in our understanding of Wnt signaling. The promiscuous binding pattern observed with some Wnt and Frizzled members raises the possibility that there may be considerable redundancy in ligand-receptor interactions (41, 48), analogous to the cases of the fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-beta) families (76-78). It is conceivable that the highly specific expression patterns observed with many Wnt and Frizzled members provide the first level of restriction in potential ligand-receptor interactions. However, there are cases in which overlapping expression of several *Wnt* or *Frizzled* genes are observed. For example, several *Wnt* genes, including *Wnt7a*, *Wnt5a*, *Wnt2*, and *Wnt10b*, are present in the embryonic cochlea where Wnt signaling is necessary to set up the orientation of the stereociliary bundles (79). Finding the responsible Wnt protein in a case like this would be a daunting effort. While some Wnt and Frizzled proteins may play redundant roles in various occasions, the severe phenotypes observed in mice with targeted disruption in individual *Wnt* or *Frizzled* gene clearly point to the unique *in vivo* roles displayed by each of these genes (75, 80-92). Therefore, other mechanisms are likely operating to further ensure proper interactions between the correct pairs of Wnt and Frizzled at the right time and right place.

Differing affinities among various Wnt-Frizzled pairs would provide a very important filtering mechanism. However, unlike other productive area of Wnt research, there have been less than a handful of studies that examined the interactions between Wnts and the receptors (48, 93, 94). Due to the lack of suitable Wnt preparations, quantitative assessment of Wnt-Frizzled interaction has been difficult to pursue. Successful production of a soluble, active and "well-behaved" XWnt8 fusion protein, tagged at its C-terminus with alkaline phosphatase (AP) catalytic domain, allowed the binding of XWnt8 and mouse Frizzled8 CRD to be examined quantitatively using a solid phase binding assay (48). The dissociation constant for XWnt8 binding to mouse Frizzled8 CRD is ~ 9 nM, which is close to that (16 nM) for XWnt8 binding to a Wnt inhibitor, WIF-1. To overcome the difficulty in obtaining sufficient Wg protein for similar binding assay, Roel Nusse and colleagues developed a "reversed" binding assay in which a soluble fusion protein consisting of the Frizzled CRD and AP (FzCRD-AP) was used to bind membrane-tethered Wg by expressing in S2 cells a Neurotactin-Wg fusion protein. (93). They found a 10-fold difference in the affinities of the two Frizzled CRDs for Wg (K_D of Wg for DFz = 46 nM; K_D of Wg for DFz2 = 5.6 nM), and suggested that this difference may be an important factor in specifying which receptor plays a dominant role in signaling when both are available. This approach was expanded recently to include other *Drosophila* Wnt and Frizzled members except for DWnt3 (94), and provides a comprehensive coverage of the possible interactions among most of the relevant Wnt and Frizzled proteins.

These binding assays do not fully recapitulate the Wnt-Frizzled interactions required for paracrine signaling, since they measure only interactions between Wnt and Frizzled ligand-binding domain. The transmembrane domain of Frizzled, although not playing an obligatory role in Wnt binding, may also contribute to ligand binding. Furthermore, these assays can not detect potential synergistic contribution to ligand binding from other receptor/co-receptor molecules, if Wnt-Frizzled interaction involves receptor dimerization or clustering, as discussed below. Nonetheless, the binding constants obtained from these studies provide a first approximation to the affinity of Wnt-Frizzled interaction as well as a framework for quantitative comparison of different Wnt-Frizzled combinations.

6. HOW DO FRIZZLED AND LRP5/6 TRANSDUCE WNT SIGNAL?

How does Wnt binding engages the two membrane receptors, a seven-pass and a single-pass transmembrane protein, and initiate the signaling cascade? This has been one key question that many Wnt researchers have been trying to answer. The prevailing model hypothesizes that Wnt binding to the two receptors causes dimerization or clustering of the receptor, thus juxtaposing different subsets of downstream components that are associated differentially with the two receptors. Several observations are consistent with this model. First, Axin and Dishevelled, two key intracellular components thought to have opposing effect on the stability of beta-catenin, have been reported to bind LRP5/6 and Frizzled (71, 72, 95), respectively. Dimerization or clustering of receptors upon Wnt binding would allow Dishevelled to be in close proximity to exert its effect on Axin; and second, the artificial juxtaposition of Frizzled cytoplasmic domain and that of LRP5/6, created by fusing the cytoplasmic domain of Arrow to the C-terminus of DFz2, leads to Wnt-independent constitutive activation of the canonical/beta-catenin pathway by this chimeric receptor (72). This dimerization model would also suggest that Wnt forms a ternary complex with Frizzled and LRP5/6 on the extracellular side. It has been reported that Wnt co-immunoprecipitates with soluble extracellular domain (ECD) of LRP6 and soluble Frizzled CRD as a ternary complex (68). However, other groups have also reported the absence of direct Wnt binding to LRP6 ECD alone or in the presence of Frizzled CRD (94, 96). These apparently contradictory results

may be due to the differences in the methods or materials employed, and point to the need to more critically re-examine this issue. Although these observations combined are consistent with and supportive of the receptor dimerization or clustering model, the assembly of the Wnt-Frizzled-LRP5/6 ternary complex remains to be unequivocally established and the signaling activity by such a complex has yet to be demonstrated *in vitro* and/or *in vivo*.

7. SUMMARY AND PERSPECTIVE

Despite the technical difficulty in producing soluble Wnt proteins for binding assay in the past, the recent progress in the purification of Wnts will soon allow direct measurement of Wnt-receptor interaction to include other ligand-receptor combination. Expansion of such studies to include other Wnt-Frizzled pairs, especially the murine ones, will allow more insightful interpretation of the physiological functions of various *Wnt* and *Frizzled* genes. For example, several murine *Wnt* and *Frizzled* genes have been disrupted by gene knockout (75, 80-92), and the resultant null mutant mice show a range of developmental defects. Interestingly, while the phenotypes of some of the Wnt mutants overlap with those of certain Frizzled mutants, none of Wnt mutants phenocopies the phenotypes of the known Frizzled mutants. Interpretation of these results has been difficult without knowing which Wnt-Frizzled pairs interact *in vivo*. Determination of the affinities of Wnt-Frizzled pairs will greatly improve our interpretation of the phenotypes.

If proven correct, the Wnt-stimulated receptor dimerization or clustering model can also account for the dexterity of some Wnt and Frizzled in signaling through either the canonical/beta-catenin pathway or one of the noncanonical pathways, depending on the presence or absence of LRP5/6 in a particular cell type and the relative affinities of Wnts for Frizzled and/or LRP5/6 proteins. Some Wnts may have little or no affinity for LRP5/6, and they would function as obligatory noncanonical Wnt signals in this model, while others capable of binding to both Frizzled and LRP5/6 can signal through both canonical and noncanonical pathways depending on the compositions of the receptor complex and intracellular cascades in a particular cell type.

8. REFERENCES

1. Thorpe, C. J., A. Schlesinger, and B. Bowerman: Wnt signalling in Caenorhabditis elegans: regulating repressors and polarizing the cytoskeleton. *Trends Cell Biol* 10, 10-17 (2000)

2.Strutt, D.: Frizzled signalling and cell polarisation in Drosophila and vertebrates. *Development* 130, 4501-4513 (2003)

3.Wu, J., J. P. Saint-Jeannet, and P. S. Klein: Wnt-frizzled signaling in neural crest formation. *Trends Neurosci* 26, 40-45 (2003)

4.Cadigan, K. M. and R. Nusse: Wnt signaling: a common theme in animal development. *Genes Dev* 11, 3286-3305 (1997)

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

6.Peifer, M. and P. Polakis: Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. *Science* 287, 1606-1609 (2000)

7.Taipale, J. and P. A. Beachy: The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411, 349-354 (2001)

8.Giles, R. H., J. H. Van Es, and H. Clevers: Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* 1653, 1-24 (2003)

9.Rubinfeld, B., P. Robbins, M. El-Gamil, I. Albert, E. Porfiri, and P. Polakis: Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science* 275, 1790-1792 (1997)

10.Morin, P. J., A. B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, and K. W. Kinzler: Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 275, 1787-1790 (1997)

11.Korinek, V., N. Barker, P. J. Morin, D. Van Wichen, R. De Weger, K. W. Kinzler, B. Vogelstein, and H. Clevers: Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* 275, 1784-1787 (1997)

12.Satoh, S., Y. Daigo, Y. Furukawa, T. Kato, N. Miwa, T. Nishiwaki, T. Kawasoe, H. Ishiguro, M. Fujita, T. Tokino, Y. Sasaki, S. Imaoka, M. Murata, T. Shimano, Y. Yamaoka, and Y. Nakamura: AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat Genet* 24, 245-250 (2000)

13.Brannon, M., M. Gomperts, L. Sumoy, R. T. Moon, and D. Kimelman: A beta-catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification in Xenopus. *Genes Dev* 11, 2359-2370 (1997)

14.Carnac, G., L. Kodjabachian, J. B. Gurdon, and P. Lemaire: The homeobox gene Siamois is a target of the Wnt dorsalisation pathway and triggers organiser activity in the absence of mesoderm. *Development* 122, 3055-3065 (1996)

15.Ikeda, S., S. Kishida, H. Yamamoto, H. Murai, S. Koyama, and A. Kikuchi: Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *Embo J* 17, 1371-1384 (1998)

16.Itoh, K., V. E. Krupnik, and S. Y. Sokol: Axis determination in Xenopus involves biochemical interactions of axin, glycogen synthase kinase 3 and beta-catenin. *Curr Biol* 8, 591-594 (1998)

17.Aberle, H., A. Bauer, J. Stappert, A. Kispert, and R. Kemler: beta-catenin is a target for the ubiquitin-proteasome pathway. *Embo J* 16, 3797-3804 (1997)

18.Molenaar, M., M. Van De Wetering, M. Oosterwegel, J. Peterson-Maduro, S. Godsave, V. Korinek, J. Roose, O. Destree, and H. Clevers: XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. *Cell* 86, 391-399 (1996)

19.Veeman, M. T., J. D. Axelrod, and R. T. Moon: A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell* 5, 367-377 (2003)

20.Kuhl, M., L. C. Sheldahl, M. Park, J. R. Miller, and R. T. Moon: The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 16, 279-283 (2000)

21.Kuhl, M., L. C. Sheldahl, C. C. Malbon, and 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, 12701-1211 (2000)

22.Saneyoshi, T., S. Kume, Y. Amasaki, and K. Mikoshiba: The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in Xenopus embryos. *Nature* 417, 295-299 (2002)

23.Adler, P. N., C. Vinson, W. J. Park, S. Conover, and L. Klein: Molecular structure of frizzled, a Drosophila tissue polarity gene. *Genetics* 126, 401-416. (1990)

24.Vinson, C. R., S. Conover, and P. N. Adler: A Drosophila tissue polarity locus encodes a protein containing seven potential transmembrane domains. *Nature* 338, 263-264 (1989)

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

26.Bhanot, P., M. Fish, J. A. Jemison, R. Nusse, J. Nathans, and K. M. Cadigan: Frizzled and Dfrizzled-2 function as redundant receptors for Wingless during Drosophila embryonic development. *Development* 126, 4175-4186 (1999)

27.Kennerdell, J. R. and R. W. Carthew: Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* 95, 1017-1026 (1998)

28.Nusse, R. and H. E. Varmus: Wnt genes. *Cell* 69, 1073-1387 (1992)

29.Kitajewski, J., J. O. Mason, and H. E. Varmus: Interaction of Wnt-1 proteins with the binding protein BiP. *Mol Cell Biol* 12, 784-790 (1992)

30.Burrus, L. W. and A. P. Mcmahon: Biochemical analysis of murine Wnt proteins reveals both shared and distinct properties. *Exp Cell Res* 220, 363-373 (1995)
31.Willert, K., J. D. Brown, E. Danenberg, A. W. Duncan, I. L. Weissman, T. Reya, J. R. Yates, 3rd, and R. Nusse: Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 423, 448-452 (2003)
32.Wong, G. T., B. J. Gavin, and A. P. Mcmahon: Differential transformation of mammary epithelial cells by Wnt genes. *Mol Cell Biol* 14, 6278-86. (1994)
33.Sokol, S., J. L. Christian, R. T. Moon, and D. A. Melton: Injected Wnt RNA induces a complete body axis in Xenopus embryos. *Cell* 67, 741-752 (1991)
34.Wolda, S. L., C. J. Moody, and R. T. Moon: Overlapping expression of Xwnt-3A and Xwnt-1 in neural tissue of Xenopus laevis embryos. *Dev Biol* 155, 46-57 (1993)
35.Smith, W. C. and R. M. Harland: Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing center. *Cell* 67, 753-65 (1991)
36.Christian, J. L., D. J. Olson, and R. T. Moon: Xwnt-8 modifies the character of mesoderm induced by bFGF in isolated Xenopus ectoderm. *Embo J* 11, 33-41 (1992)
37.Moon, R. T., R. M. Campbell, J. L. Christian, L. L. Mcgrew, J. Shih, and S. Fraser: Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of Xenopus laevis. *Development* 119, 97-111 (1993)
38.Tada, M. and J. C. Smith: Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* 127, 2227-2238 (2000)
39.Heisenberg, C. P., M. Tada, G. J. Rauch, L. Saude, M. L. Concha, R. Geisler, D. L. Stemple, J. C. Smith, and S. W. Wilson: Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405, 76-81 (2000)
40.Winklbauer, R., A. Medina, R. K. Swain, and H. Steinbeisser: Frizzled-7 signalling controls tissue separation during Xenopus gastrulation. *Nature* 413, 856-860 (2001)
41.Bhanot, P., M. Brink, C. H. Samos, J. C. Hsieh, Y. Wang, J. P. Macke, D. Andrew, J. Nathans, and R. Nusse: A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* 382, 225-230 (1996)
42.Yang-Snyder, J., J. R. Miller, J. D. Brown, C. J. Lai, and R. T. Moon: A frizzled homolog functions in a vertebrate Wnt signaling pathway. *Curr Biol* 6, 1302-1306 (1996)
43.Sawa, H., L. Lobel, and H. R. Horvitz: The Caenorhabditis elegans gene lin-17, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the Drosophila frizzled protein. *Genes Dev* 10, 2189-2197 (1996)
44.He, X., J. P. Saint-Jeannet, Y. Wang, J. Nathans, I. Dawid, and H. Varmus: A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* 275, 1652-1654 (1997)
45.Rocheleau, C. E., W. D. Downs, R. Lin, C. Wittmann, Y. Bei, Y. H. Cha, M. Ali, J. R. Priess, and C. C. Mello: Wnt signaling and an APC-related gene specify endoderm in early C. elegans embryos. *Cell* 90, 707-716 (1997)
46.Thorpe, C. J., A. Schlesinger, J. C. Carter, and B. Bowerman: Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. *Cell* 90, 695-705 (1997)
47.Muller, H., R. Samanta, and E. Wieschaus: Wingless signaling in the Drosophila embryo: zygotic requirements and the role of the frizzled genes. *Development* 126, 577-586 (1999)
48.Hsieh, J. C., A. Rattner, P. M. Smallwood, and J. Nathans: Biochemical characterization of Wnt-frizzled interactions using a soluble, biologically active vertebrate Wnt protein. *Proc Natl Acad Sci U S A* 96, 3546-3551 (1999)
49.Dann Iii, C.D., J.-C. Hsieh, A. Rattner, D. Sharma, J. Nathans, and D.J. Leahy: Insights into Wnt binding and signaling from the structures of two Frizzled cysteine-rich domains. *Nature* 412, 86-90 (2001)
50.Hoang, B., M. Moos, S. Vukicevic, and F. P. Luyten: Primary structure and tissue distribution of FRZB, a novel protein related to Drosophila frizzled, suggest a role in skeletal morphogenesis. *J Biol Chem* 271, 26131-26137 (1996)
51.Rattner, A., J. C. Hsieh, P. M. Smallwood, D. J. Gilbert, N. G. Copeland, N. A. Jenkins, and J. Nathans: A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc Natl Acad Sci U S A* 94, 2859-2863 (1997)
52.Pfeffer, P. L., E. M. De Robertis, and J. C. Izpisua-Belmonte: Crescent, a novel chick gene encoding a Frizzled-like cysteine-rich domain, is expressed in anterior regions during early embryogenesis. *Int J Dev Biol* 41, 449-458 (1997)
53.Leyns, L., T. Bouwmeester, S. H. Kim, S. Piccolo, and E. M. De Robertis: Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88, 747-756 (1997)
54.Wang, S., M. Krinks, K. Lin, F. P. Luyten, and M. Moos, Jr.: Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* 88, 757-766 (1997)
55.Mayr, T., U. Deutsch, M. Kuhl, H. C. Drexler, F. Lottspeich, R. Deutzmann, D. Wedlich, and W. Risau: Fritz: a secreted frizzled-related protein that inhibits Wnt activity. *Mech Dev* 63, 109-125 (1997)
56.Finch, P. W., X. He, M. J. Kelley, A. Uren, R. P. Schaudies, N. C. Popescu, S. Rudikoff, S. A. Aaronson, H. E. Varmus, and J. S. Rubin: Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc Natl Acad Sci U S A* 94, 6770-6775 (1997)
57.Melkonyan, H. S., W. C. Chang, J. P. Shapiro, M. Mahadevappa, P. A. Fitzpatrick, M. C. Kiefer, L. D. Tomei, and S. R. Umansky: SARPs: a family of secreted apoptosis-related proteins. *Proc Natl Acad Sci U S A* 94, 13636-13641 (1997)
58.Glass, D. J., D. C. Bowen, T. N. Stitt, C. Radziejewski, J. Bruno, T. E. Ryan, D. R. Gies, S. Shah, K. Mattsson, S. J. Burden, P. S. Distefano, D. M. Valenzuela, T. M. Dechiara, and G. D. Yancopoulos: Agrin acts via a MuSK receptor complex. *Cell* 85, 513-523 (1996)
59.Jennings, C. G., S. M. Dyer, and S. J. Burden: Muscle-specific trk-related receptor with a kringle domain defines a distinct class of receptor tyrosine kinases. *Proc Natl Acad Sci U S A* 90, 2895-2899 (1993)
60.Wilson, C., D. C. Goberdhan, and H. Steller: Dror, a potential neurotrophic receptor gene, encodes a Drosophila homolog of the vertebrate Ror family of Trk-related receptor tyrosine kinases. *Proc Natl Acad Sci U S A* 90, 7109-7113 (1993)
61.Song, L. and L. D. Fricker: Cloning and expression of human carboxypeptidase Z, a novel metallocarboxypeptidase. *J Biol Chem* 272, 10543-10550 (1997)
62.Yan, W., N. Sheng, M. Seto, J. Morser, and Q. Wu: Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *J Biol Chem* 274, 14926-14935 (1999)
63.Rehn, M. and T. Pihlajaniemi: Identification of three N-terminal ends of type XVIII collagen chains and tissue-specific differences in the expression of the corresponding transcripts. The longest form contains a novel motif homologous to rat and Drosophila frizzled proteins. *J Biol Chem* 270, 4705-4711 (1995)
64.Wang, S., M. Krinks, and M. Moos, Jr.: Frzb-1, an antagonist of Wnt-1 and Wnt-8, does not block signaling by Wnts -3A, -5A, or -11. *Biochem Biophys Res Commun* 236, 502-504 (1997)
65.Xu, Q., P. A. D'amore, and S. Y. Sokol: Functional and biochemical interactions of Wnts with FrzA, a secreted Wnt antagonist. *Development* 125, 4767-4776 (1998)
66.Moeller, C., E. C. Swindell, A. Kispert, and G. Eichele: Carboxypeptidase Z (CPZ) modulates Wnt signaling and regulates the development of skeletal elements in the chicken. *Development* 130, 5103-11 (2003)
67.Wehrli, M., S. T. Dougan, K. Caldwell, L. O'keefe, S. Schwartz, D. Vaizel-Ohayon, E. Schejter, A. Tomlinson, and S. Dinardo: arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 407, 527-530 (2000)

68.Tamai, K., M. Semenov, Y. Kato, R. Spokony, C. Liu, Y. Katsuyama, F. Hess, J. P. Saint-Jeannet, and X. He: LDL-receptor-related proteins in Wnt signal transduction. *Nature* 407, 530-535 (2000)
69.Pinson, K. I., J. Brennan, S. Monkley, B. J. Avery, and W. C. Skarnes: An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* 407, 535-538 (2000)
70.Strickland, D. K., S. L. Gonias, and W. S. Argraves: Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* 13, 66-74 (2002)
71.Mao, J., J. Wang, B. Liu, W. Pan, G. H. Farr, C. Flynn, H. Yuan, S. Takada, D. Kimelman, L. Li, and D. Wu: Low-density lipoprotein receptor-related protein-5 binds to axin and regulates the canonical wnt signaling pathway. *Mol Cell* 7, 801-809 (2001)
72.Tolwinski, N. S., M. Wehrli, A. Rives, N. Erdeniz, S. Dinardo, and E. Wieschaus: Wg/Wnt signal can be transmitted through arrow/LRP5,6 and Axin independently of Zw3/Gsk3beta activity. *Dev Cell* 4, 407-418 (2003)
73.Culi, J. and R. S. Mann: Boca, an endoplasmic reticulum protein required for wingless signaling and trafficking of LDL receptor family members in Drosophila. *Cell* 112, 343-354 (2003)
74.Hsieh, J. C., L. Lee, L. Zhang, S. Wefer, K. Brown, C. Derossi, M. E. Wines, T. Rosenquist, and B. C. Holdener: Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* 112, 355-367 (2003)
75.Liu, P., M. Wakamiya, M. J. Shea, U. Albrecht, R. R. Behringer, and A. Bradley: Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* 22, 361-5. (1999)
76.Seifert, R. A., C. E. Hart, P. E. Phillips, J. W. Forstrom, R. Ross, M. J. Murray, and D. F. Bowen-Pope: Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. *J Biol Chem* 264, 8771-8. (1989)
77.Ten Dijke, P., K. Miyazono, and C. H. Heldin: Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. *Curr Opin Cell Biol* 8, 139-145 (1996)
78.Johnson, D. E. and L. T. Williams: Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 60, 1-41 (1993)
79.Dabdoub, A., M. J. Donohue, A. Brennan, V. Wolf, M. Montcouquiol, D. A. Sassoon, J. C. Hseih, J. S. Rubin, P. C. Salinas, and M. W. Kelley: Wnt signaling mediates reorientation of outer hair cell stereociliary bundles in the mammalian cochlea. *Development* 130, 2375-2384 (2003)
80.Mcmahon, A. P. and A. Bradley: The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* 62, 1073-1085 (1990)
81.Thomas, K. R. and M. R. Capecchi: Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* 346, 847-850 (1990)
82.Monkley, S. J., S. J. Delaney, D. J. Pennisi, J. H. Christiansen, and B. J. Wainwright: Targeted disruption of the Wnt2 gene results in placentation defects. *Development* 122, 3343-3353 (1996)
83.Takada, S., K. L. Stark, M. J. Shea, G. Vassileva, J. A. Mcmahon, and A. P. Mcmahon: Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev* 8, 174-189 (1994)
84.Greco, T. L., S. Takada, M. M. Newhouse, J. A. Mcmahon, A. P. Mcmahon, and S. A. Camper: Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev* 10, 313-324 (1996)
85.Lee, S. M., S. Tole, E. Grove, and A. P. Mcmahon: A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127, 457-67. (2000)
86.Stark, K., S. Vainio, G. Vassileva, and A. P. Mcmahon: Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 372, 679-83. (1994)
87.Vainio, S., M. Heikkila, A. Kispert, N. Chin, and A. P. Mcmahon: Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397, 405-409 (1999)
88.Yamaguchi, T. P., A. Bradley, A. P. Mcmahon, and S. Jones: A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 126, 1211-1223 (1999)
89.Parr, B. A. and A. P. Mcmahon: Dorsalizing signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb. *Nature* 374, 350-3. (1995)
90.Parr, B. A. and A. P. Mcmahon: Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. *Nature* 395, 707-710 (1998)
91.Ishikawa, T., Y. Tamai, A. M. Zorn, H. Yoshida, M. F. Seldin, S. Nishikawa, and M. M. Taketo: Mouse Wnt receptor gene Fzd5 is essential for yolk sac and placental angiogenesis. *Development* 128, 25-33 (2001)
92.Wang, Y., D. Huso, H. Cahill, D. Ryugo, and J. Nathans: Progressive cerebellar, auditory, and esophageal dysfunction caused by targeted disruption of the frizzled-4 gene. *J Neurosci* 21, 4761-4771. (2001)
93.Rulifson, E. J., C. H. Wu, and R. Nusse: Pathway specificity by the bifunctional receptor frizzled is determined by affinity for wingless. *Mol Cell* 6, 117-1126 (2000)
94.Wu, C. H. and R. Nusse: Ligand receptor interactions in the Wnt signaling pathway in Drosophila. *J Biol Chem* 277, 41762-41769 (2002)
95.Chen, W., D. Ten Berge, J. Brown, S. Ahn, L. A. Hu, W. E. Miller, M. G. Caron, L. S. Barak, R. Nusse, and R. J. Lefkowitz: Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. *Science* 301, 1391-1394 (2003)
96.Mao, B., W. Wu, Y. Li, D. Hoppe, P. Stannek, A. Glinka, and C. Niehrs: LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 411, 321-325 (2001)

Key Words: Wnt, Frizzled, signal transduction, cancer, oncogene, animal development, ligand, receptor, dimerization, Review

Send correspondence to: Jen-Chih Hsieh, Department of Biochemistry and Cell Biology, 422 CMM Bldg. SUNY/Stony Brook, Stony Brook, New York 11794-5140 Tel: 631-632-1163, Fax: 631-632-1692, E-mail: jhsieh@ms.cc.sunysb.edu