SPECIFICITY OF WNT-RECEPTOR INTERACTIONS

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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/Ca2+ 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/Ca2+ 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/Ca2+ pathway regulates cell adhesion and motility (20), and is mediated through release of intracellular Ca2+ upon Wnt stimulation, and
Interaction of Wnt and receptors

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 LRPS/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.

One feature common to these three Wnt signaling pathways is that they all act through the serpentine 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 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 uncouoperative 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 invariably 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 glycosphatidylinositol (GPI) anchor (49). Interestingly, the Frizzled CRD is also found in a number of other proteins, such as the soluble Flz-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 LRPS 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 Wnt signaling cascade (67). Mouse embryos with an insertion 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. Wnt 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 importance of LRP5/6 in the cell surface to transduce 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 Wnt and the receptors in 93, 94, 1335. 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 and using mouse Wg fusion protein (93). They found a 10-fold difference in the affinities of the two Frizzled CRDs for Wg (Kd of Wg for DFz = 46 nM; Kd of Wg for DFz = 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 synergetic 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


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