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REVIEW

Wnts as ligands: processing, secretion and reception

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Cell to cell communication is vital throughout the development of multicellular organisms and during adult homeostasis. One way in which communication is achieved is through the secretion of signaling molecules that are received by neighboring responding cells. Wnt ligands comprise a large family of secreted, hydrophobic, glycoproteins that control a variety of developmental and adult processes in all metazoan organisms. By binding to various receptors present on receiving cells, Wnts initiate intracellular signaling cascades resulting in changes in gene transcription. Misregulation of Wnt signaling contributes to cancer and other degenerative disorders; thus, much effort has been made to understand the ways in which the pathway is controlled. Although ample research into the regulatory mechanisms that influence *intracellular* signaling events has proved fruitful, a great deal still remains to be elucidated regarding the mechanisms that control Wnt protein processing and secretion from cells, transport through the extracellular space, and protein reception on neighboring cells. This review attempts to consolidate the current data regarding these essential processes.
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Wnt protein overview

During the development of multicellular organisms, evolutionarily conserved signaling pathways are activated in a highly coordinated manner to ensure the proper patterning of the embryo. By releasing signaling molecules such as Wnts, Bone Morphogenic Proteins (BMPs), Hedgehogs (Hh) and so on, distinct cell populations are able to influence the intracellular signaling events of their neighbors from a distance. Research into the mechanisms that control these signaling cascades provides not only a greater understanding of organismal development, but also offers insight as to how one might influence these signals for therapeutic benefit.

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The Wnts comprise a large family of protein ligands that affect diverse processes such as embryonic induction, generation of cell polarity and the specification of cell fate (Logan and Nusse, 2004). In addition to influencing developmental processes, recent studies point to a key role for Wnt signaling during adult homeostasis in the maintenance of stem cell pluripotency (Reya and Clevers, 2005). In the most well understood 'canonical' Wnt signaling pathway, Wnt binding to Frizzled and LRP5/6 co-receptors induces β-Catenin protein stabilization and entry into the nucleus where it affects the transcription of target genes. Because uncontrolled canonical Wnt signaling is a hallmark of cancer and other degenerative diseases, understanding the ways in which the pathway is regulated is of critical importance (Polakis, 2000; Clevers and Batlle, 2006; de Iongh *et al*., 2006; Fox and Dharmarajan, 2006; Krishnan *et al*., 2006).

Historically, the Wnts have been defined by amino-acid sequence rather than by functional properties (Nusse and Varmus, 1992; Miller, 2001). Although the founding member of the family, Wnt1, was originally discovered in a retroviral insertion mutagenesis screen, as many as 19 mammalian Wnt homologs have been cloned to date and are expressed in overlapping, temporal-spatial patterns (www.stanford.edu/~rnusse/wntwindow.html). No crystal structure for a Wnt protein has yet been solved. However, shared features of all Wnts include a signal sequence for secretion, several highly charged amino-acid residues, and many potential glycosylation sites. Wnt proteins also display a characteristic distribution of 22 cysteine residues. The highly conserved spacing of these cysteines suggests that proper protein folding might require the formation of multiple intramolecular disulfide bonds (Mason *et al*., 1992).

Following their synthesis, Wnt proteins are dynamically modified. Upon overexpression in tissue culture cells, several different N-linked glycosylated intermediate Wnt protein products are observed in cell lysates (Mason *et al*., 1992; Reichsman *et al*., 1996; Tanaka *et al*., 2002). By contrast, fewer forms of Wnt are found secreted into media (Kadowaki *et al*., 1996). These data, coupled with the fact that when overexpressed, a large proportion of newly synthesized Wnt protein is found associated with chaperone proteins (Kitajewski *et al*., 1992), suggest that Wnt protein processing and secretion are highly regulated processes.

Wnts are lipid modified

The primary amino-acid sequence of Wnts suggests that they should be quite soluble. However, secreted Wnt proteins are surprisingly hydrophobic and are mostly found associated with cell membranes and the extracellular matrix (ECM) (Reichsman *et al*., 1996). Indeed, experiments have shown that in tissue culture cells stably expressing Wingless (Wg; *Drosophila* ortholog of Wnt1), only a fraction of the protein is actually secreted into the media. With the purification of active Wnt proteins came insight into their hydrophobic character (Willert *et al*., 2003). Mass spectroscopy analyses revealed that Wnt proteins are lipid modified by the attachment of a palmitate moiety on the first absolutely conserved cysteine residue within the protein family.

Lipid modification of Wnt proteins is essential for their function. Treatment of cells with an acyltransferase inhibitor (2-bromopalmitate) or removal of the palmitate moiety enzymatically with acyl protein thioesterase or through mutation of the modified residue results in a protein that is no longer hydrophobic or active (Willert *et al*., 2003; Zhai *et al*., 2004; Schulte *et al*., 2005). Although palmitoylation has been shown to be necessary for canonical Wnt signaling, the role of lipid modification remains unclear.

Palmitate modification of Wnts potentially serves many diverse purposes. One proposed function is the targeting of Wnts to particular domains of the membrane, such as lipid rafts, detergent resistant fractions comprised of sphingolipids and cholesterol that have been implicated as platforms for signal transduction and cell activation (Simons and Toomre, 2000). Mutation of the gene required for Wnt palmitoylation (see below) can be partially rescued by overexpression of Wnts (Noordermeer *et al*., 1995) and Wnt proteins in which the putative palmitoylation site is mutated are still able to signal in an autocrine manner (Willert *et al*., 2003). Thus, the role that palmitate modification plays in targeting Wnts to membranes might be overcome by high protein concentrations (Zhai *et al*., 2004). In addition, palmitoylation of Wnt proteins has been shown to be necessary for their N-linked glycosylation, the lipid moiety perhaps serving to anchor the proteins to the endoplasmic reticulum (ER) membrane in close proximity to the oligosaccharyl transferase complex. (Kadowaki *et al*., 1996; Tanaka *et al*., 2002; Willert *et al*., 2003; Zhai *et al*., 2004). This proposed role for palmitoylation during N-linked glycosylation might also aid in Wnt transport between cells as glycosylation might increase Wnt interactions with heparin sulfate proteoglycans (HSPGs) present on the surface of Wnt responding cells (see below).

Aside from membrane targeting and glycosylation, what other potential role might palmitoylation serve? One exciting idea is that lipid modification might facilitate ligand reception on Wnt-responding cells. It has been observed that mutation of the residue required for Wnt palmitoylation results in decreased Wnt-Frizzled interactions (Cong *et al*., 2004). In addition, when the Wnt binding cysteine-rich domain (CRD) is mutated, Frizzled receptors are still able to produce an attenuated signal, suggesting that there are multiple binding domains present on Frizzled receptors with differing Wnt binding affinities (Chen *et al*., 2004; Povelones and Nusse, 2005). As it has been hypothesized that the function of the CRD is to bring Wnt in close proximity with the membrane portion of the Frizzled receptor, the palmitate moiety could potentially anchor Wnt proteins into the membrane for sustained signaling.

Lastly, we note that various Wnt proteins have been shown to activate multiple diverse signaling cascades (Kuhl *et al*., 2000; Mikels and Nusse, 2006). Thus, whether all Wnt family members are palmitoylated and whether the lipid is crucial for all aspects of Wnt signaling are still open questions. Further structural and biochemical analyses of the Wnt protein family should shed light on the function of this newly discovered Wnt modification.

Porcupine lipid modifies Wnts

By far the most well studied Wnt protein to date is Wg. Wg plays a crucial role in axis patterning and appendage development in *Drosophila*: viable alleles frequently display loss of the wing and duplication of the notum. During germ-band extension in the *Drosophila* embryo, *wg* is expressed in a striped pattern flanking a parasegment border. Reception of the secreted Wg by a neighboring posterior stripe of cells maintains the expression of the homeobox gene *engrailed* (*en*). En expression in these cells positively feeds back to activate Wg signaling. Numerous studies have shown that the segment polarity gene *porcupine* (*porc*) is required for the maintenance of Wg signaling in this tissue. Genetic epistasis analyses demonstrated that *porcupine* acts upstream of intracellular signaling components in a cell non-autonomous manner (van den Heuvel *et al*., 1993; Noordermeer *et al*., 1994; Siegfried *et al*., 1994; Manoukian *et al*., 1995; Tanaka *et al*., 2000). In addition, a genetic screen for maternally expressed genes required for the endoderm induction in *Caenorhabditis elegans* confirmed the cell non-autonomous role of mom-1 (*C. elegans* porcupine ortholog) in Wnt signaling (Thorpe *et al*., 1997).

When the gene was cloned, it was discovered that *porcupine* encodes for a multipass transmembrane, resident ER protein that is conserved across multiple species (Figure 1). In *Drosophila*, *porcupine*-mutant cells accumulate Wg protein suggesting that the protein in unable to be released from the producing cells (van den Heuvel *et al*., 1993; Noordermeer *et al*., 1994; Siegfried *et al*., 1994; Tanaka *et al*., 2000). In *porcupine*-mutant flies, a much smaller fraction of Wg is found associated with cell membranes as compared to wild-type animals (Zhai *et al*., 2004). Porcupine, like Skinny Hh – a transmembrane protein required for the palmitoylation of Hh proteins – shares sequence similarity to *O*-acyltransferase enzymes. Thus, whereas direct biochemical analysis is still lacking, it has been proposed that the

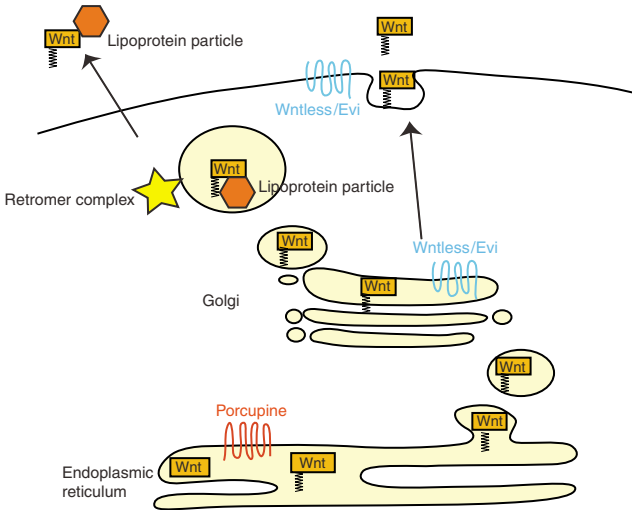

Figure 1 In Wnt-producing cells, the Wnt protein becomes palmitoylated in the ER by the porcupine acyl-transferase. Further transport and secretion of the Wnt protein in secretory vesicles is controlled by the multi-pass transmembrane protein Wntless/Evi, which is present in the Golgi and/or on the plasma membrane. The retromer complex, including VPS35, may act within Wnt-producing cells to generate Wnt forms that can be transported outside cells, possibly in the form of a lipoprotein particle.

Porcupine protein lipid modifies Wnt proteins within the ER (Hofmann, 2000; Tanaka *et al*., 2002; Nusse, 2003).

Wnt secretion

What other proteins might be involved in Wnt protein processing and secretion from producing cells? Two recent genetic screens have identified the multipass transmembrane protein Wntless (Wls)/Evenness interrupted (Evi)/Mom-3 as acting in the secretory pathway to promote the release of Wnts from producing cells (Ching and Nusse, 2006). In *Drosophila* and mammalian tissue culture cells, RNA interference (RNAi) knockdown of *wls/evi/mom-3* in cells overexpressing Wnt inhibits the activation of a Wnt-responsive luciferase reporter in co-cultured responding cells, suggesting that Wls/Evi/Mom-3 acts in a cell non-autonomous manner (Banziger *et al*., 2006). Lack of *wls/evi/mom-3* results in inhibition of Wnt transport to the surface of cells with a concordant lack of Wnt secretion into media. In addition, when clones of cells mutant for *wls/evi/mom-3* are permeabilized, intracellular accumulation of Wnt is observed (Bartscherer *et al*., 2006).

These phenotypes are similar to those observed in *porcupine* mutants. In contrast to Porcupine's function, however, the secretory activity mediated by Wls/Evi/Mom-3 does not depend on the palmitoylation status of Wnt proteins: the secretion of Wnt proteins in which the palmitoylation site is mutated is still impaired in *wls/evi/mom-3* mutants (Banziger *et al*., 2006). In addition, *wls/evi/mom-3* knockdown does not alter the glycosylation status of the retained Wnt proteins and unlike Porcupine protein, Wls/Evi/Mom-3 is not localized to the ER. Future research into understanding the function of Wls/Evi/Mom-3 may yet uncover more interesting proteins required for the secretion and processing of Wnts. Furthermore, it is exciting to consider that Wls/Evi/Mom-3 had previously been discovered in a genetic screen in *C. elegans* (Thorpe *et al*., 1997) and was only recently identified molecularly, opening the door to the possibility that other as yet unknown pathway components are waiting to be uncovered.

Wnts as morphogens

Much data in the literature supports the idea that Wnts serve as secreted morphogens that can act in a long- or short-range manner (Zecca *et al*., 1996; Neumann and Cohen, 1997). During the patterning of *Drosophila* wing imaginal disc, Wg signaling determines distinct regions along the dorsal–ventral (DV) axis. Short-range Wg signaling induces the expression of the proneural gene *achaete* in a distinct striped pattern on either side of the DV boundary. In contrast, long-range Wg targets Vestigial (Vg) and Distalless (Dll) are expressed in broad domains centered on the DV boundary. The boundaries of expression of *vg* and *dll* are not sharp but rather decline in a graded manner as a function of distance from Wg-secreting cells, suggesting a dose-dependent activation by Wg. As Wg protein is able to influence cells as far as 20–30 cell diameters away from producing cells (Neumann and Cohen, 1997), the question arises as to how Wg is able to navigate the aqueous extracellular milieu given its hydrophobic nature.

Increasing evidence suggests that specific interactions with glycosaminoglycan (GAG)-modified proteins might facilitate the extracellular movement of Wnts. Early clues into the role of these proteins in Wnt transport came from studies showing that either injection into wild-type fly embryos (Binari *et al*., 1997) or treatment of cells with exogenous heparin, a sulfated (GAG), results in increased Wnt signaling (Reichsman *et al*., 1996). This is presumably due to the observed increase in Wnt ligand release from the surface of cells following heparin treatment (Bradley and Brown, 1990; Burrus and McMahon, 1995). As Wnt proteins previously had been shown to bind to heparin *in vitro*, these data indicated that Wnts might also bind to GAG-modified proteins in the ECM (Bradley and Brown, 1990). Subsequently, a definitive link between Wnts and GAG-modified proteins was formed when it was discovered that mutations in genes such as *sugarless* (*sgl*) and *sulfateless* (*sfl*), which are required for the biosynthesis of heparin sulfate GAGs, display defects in Wnt signaling in *Drosophila* (Binari *et al*., 1997; Hacker *et al*., 1997; Lin and Perrimon, 1999).

Which GAG-modified proteins influence Wnt protein distribution? HSPGs are ECM and cell surface proteins possessing a protein core to which heparin sulfate GAG chains are attached (Lin and Perrimon, 2002). One family of cell surface HSPGs, the glypicans, are integral membrane proteins linked to the plasma membrane via

a glycophosphatidylinositol (gpi) chain. Mulitple genetic screens in *Drosophila* have shown that two glypican molecules, Dally (division abnormally delayed) and Dally-like (Dly), are required for Wnt signaling. Hypomorphic alleles of *dally* and dsRNA knockdown of *dally* and *dly* results in phenotypes similar to partial loss of Wg activity (Tsuda *et al*., 1999; Baeg *et al*., 2001). In addition, overexpression of *Dly* results in ectopic extracellular Wg accumulation suggesting that Dly plays a role in Wg protein movement between cells (Baeg *et al*., 2001).

How might these cell surface proteins regulate Wnt protein transport? In one model, gpi-linked Dally and Dly might increase the local concentration of Wnts in lipid raft domains present on the cell surface. Interaction of Wnts with these low-affinity binding receptors might bring the ligands into close proximity of Frizzled receptors, expression of which has been shown to stabilize Wg and broaden its range of action (Cadigan *et al*., 1998). Dally and Dly proteins might also be necessary for trapping or stabilizing Wnt protein in neighboring receiving cells thereby restricting its diffusion. In addition, Dally and Dly could facilitate Wnt transport in extracellular structures (see below). Exploring the ways in which Wnts interact with these surface receptors and how they affect intracellular Wnt signaling will greatly enhance our understanding of Wnt action *in vivo*.

Wnt extracellular transport

Recently, large molecules required for lipid transport called lipoprotein particles have been implicated in the facilitated movement of Wnts and other lipid-modified proteins such as Hhs. When overexpressed in the *Drosophila* wing imaginal disc, gpi-linked green fluorescent protein (GFPgpi) is found in non-cellular punctate structures (Greco *et al*., 2001). Because GFPgpi expressed in Wg-producing cells spreads into receiving tissue at the same rate as Wg protein and the two proteins colocalize (Greco *et al*., 2001), it was proposed that Wnts and gpi-linked proteins are transported together in these structures. Although previously postulated to be exosome-like particles, these structures termed 'argosomes' are now thought to be exogenously derived lipoproteins (Panakova *et al*., 2005).

How do argosomes interact with Wnts and affect their movement? Panakova *et al*. (2005) proposed a model wherein palmitoylated proteins associate with lipoprotein particles on the extracellular face of cells. The authors suggest that the movement of Wnt proteins from one cell to the next requires this association as RNAi knockdown of Lipophorin, a *Drosophila* lipoprotein, narrows the range of Wg signaling in the wing disc (Panakova *et al*., 2005). The fact that lipoproteins were also found associated with a variety of gpi-linked proteins raises the possibility that the gpi-linked proteins Dally and Dly may stabilize Wnts throughout their travel in these extracellular particles. It will be interesting to see what mechanism allows these lipoprotein particles to aid in Wnt transport, what other accessory proteins are involved, and whether this mechanism of Wnt transport is conserved across all species.

Lastly, transcytosis has been proposed to regulate Wnt movement. In yeast, a cluster of proteins termed the retromer complex directs endosome-to-Golgi retrieval of proteins and in vertebrate systems the retromer complex functions in basal-to-apical transcytosis (Verges *et al*., 2004). Recently, two groups have independently shown that the *C. elegans* ortholog of the yeast retromer complex subunit Vps35p is required for the long-range signaling capabilities of Egl-20 (*C. elegans* Wnt ortholog) (Coudreuse *et al*., 2006; Prasad and Clark, 2006). Mutation in *vps35* leads to defects in Wnt-mediated posterior lateral microtubule (PLM) mechanosensory neuron polarity as well as Q neuroblast migration. The complex appears to specifically enhance long-range Wnt signaling because mutation of the retromer complex disturbs the long-range signaling capabilities of Egl-20 whereas only mildly affecting the signaling capabilities of other short-range acting Wnts (Coudreuse *et al*., 2006). In addition, when retromer function is abolished by *vps-26*- and *vps-35*-null mutations, the mutant phenotype appears less severe than Wnt-null mutants suggesting that not all of the signaling functions of *C. elegans* Wnts (i.e. short-range) are impaired (Prasad and Clark, 2006).

How might retromer function enhance long-range Wnt signaling? The retromer complex functions in Wnt-producing cells; however, knockdown of Vps35 does not affect Wnt secretion. Thus, it has been hypothesized that the retromer complex promotes the association of secreted Wnts with other proteins required for ligand transport, such as lipoprotein particles, in endosomal trafficking vesicles present in Wnt-producing cells. Time will tell how the retromer complex fits into current models of Wnt transport.

Wnt reception

Wnt signaling complexity is greatly enhanced by the plethora of potential Wnt receptors (Figure 2). The seven-pass transmembrane protein Frizzled (Fz) protein was first receptor found to transduce a Wnt signal (Bhanot *et al*., 1996). In cultured cells normally unresponsive to Wg, overexpression of Fz2 results in cell surface Wg binding and productive canonical Wnt signaling (Bhanot *et al*., 1996). All Frizzled proteins possess a large extracellular domain containing a conserved motif comprised of 10 cysteine residues called the CRD. The CRD domains from various Frizzled receptors have been shown to bind multiple Wnts with high affinity (Hsieh *et al*., 1999b; Wu and Nusse, 2002). However, recent evidence indicates that this domain may be dispensable for Wnt signaling in some contexts suggesting that alternative lower affinity Wnt binding sites might exist (Chen *et al*., 2004; Povelones and Nusse, 2005). The topology of the receptor has lead many to speculate that Frizzleds are coupled to

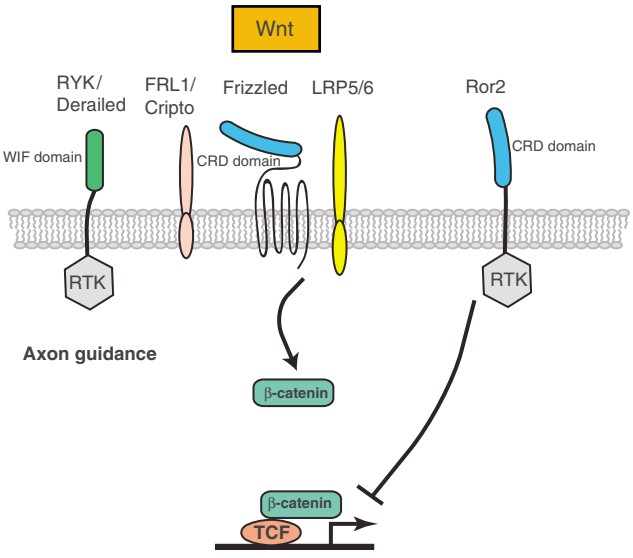

Figure 2 Wnts can interact with multiple receptors. These include the RYK/Derailed-type receptors that have a tyrosine kinase motif and a WIF ligand binding domain. This receptor can mediate signaling during axon guidance in *Drosophila*. During Wnt/β-catenin signaling, the Wnts interact with Frizzleds through the CRD. The LRP5/6 class of transmembrane proteins act as co-receptors, whereas in *Xenopus* axis formation, FRL1/Cripto can acts as an additional co-receptor. Finally, Wnts can also interact with the Ror tyrosine kinases to mediate a signal that can inhibit β-catenin TCF activity in the nucleus.

heterotrimeric G-proteins. Indeed emerging genetic (Katanaev *et al*., 2005) and biochemical analyses (Wang and Malbon, 2004) suggest that G-proteins are activated in response to Frizzled signaling. However, questions such as how heterotrimeric G-proteins are activated in response to Wnt signaling and how they interact with known Wnt pathway components remain largely unanswered. At the cytoplasmic side, Frizzleds may interact directly with the Dishevelled protein, a known mediator of Wnt signaling (Chen *et al*., 2003).

Although many of the players are now known, much remains to be discovered regarding the mechanism by which Wnts initiate productive signals on receiving cells. Some have postulated that Frizzled receptor dimerization induces Wnt signaling (Carron *et al*., 2003). However, that model is complicated by the discovery that single pass transmembrane proteins of the low-density lipoprotein family called LRP5 and -6 (Arrow in *Drosophila*) are required in Wnt responding cells and act upstream of intracellular signaling components (Wehrli *et al*., 2000). Following Wnt binding, it is thought that Frizzleds form a co-receptor complex with LRP proteins to transduce the canonical Wnt signal (Li and Bu, 2005). LRP5 and -6 proteins possess a relatively small intracellular domain and a large extracellular domain containing several potential protein interaction domains (He *et al*., 2004). Truncated proteins that lack the extracellular portion of the protein, but still contain the transmembrane and intracellular domains, produce a constitutively active canonical Wnt signal (Liu *et al*., 2003). By contrast, LRP proteins lacking only the intracellular domain serve as dominant negative proteins (Tamai *et al*., 2000).

Interestingly, the Axin protein, a negative regulator of Wnt signaling, can bind to the cytoplasmic tail of LRP6, providing a mechanism by which Axin is released from beta-catenin (Mao *et al*., 2001). This interaction changes the fate of beta-catenin; instead of being destroyed it accumulates to execute Wnt-induced gene expression. The binding of Axin to the LRP6 tail is promoted by phosphorylation of LRP6. Phosphorylation of LRP6 occurs on several clusters of serines and threonines, with a central proline proline proline serine proline (PPPSP) motif as a hallmark. The serine in the PPPSP motif is modified by GSK3, leading to activation of signaling (Zeng *et al*., 2005). In addition, a member of the CK1 family, CK1 gamma , phosphorylates residues next to the PPPSP motif (Davidson *et al*., 2005). CK1 gamma, interestingly, has a membrane anchor in the form of a palmitoylation domain. Determining how LRP proteins interact with Wnts and Frizzleds on the extracellular face of cells should improve our knowledge regarding how signaling is initiated intracellularly.

Could other proteins containing known Wnt binding domains also serve as receptors for Wnt ligands? The CRD domain is found in two other types of receptor proteins: Smoothened (Smo) and Ror proteins. Although the Smo receptor shares homology to Frizzleds, the Smo CRD does not bind to Wnts or produce productive signals in response to Wnt stimulation (Povelones and Nusse, 2005). By contrast, increasing evidence suggests that the single pass tyrosine kinase Ror2, although structurally distinct from Frizzled receptors, is involved in other forms Wnt signaling (Xu and Nusse, 1998; Matsuda *et al*., 2001; Forrester, 2002; Oishi *et al*., 2003; Mikels and Nusse, 2006). In the mouse, Ror2 and Ror1 knockout phenotypes resemble that of Wnt5a−/− null mice (Yamaguchi *et al*., 1999; Yoda *et al*., 2003). In line with this common phenotype is the finding that Ror2 can mediate the Wnt5a signal that is responsible for inhibition of β-catenin-T-cell factor signaling (Mikels and Nusse, 2006). In *C. elegans*, expression of the Ror2 homolog Cam-1 disrupts Wnt-mediated Q neuroblast migration (Forrester *et al*., 2004). Thus, the CRD domain may enable other alternative signaling cascades to be initiated in response to Wnt ligand stimulation.

Another well-characterized Wnt binding domain is the WIF (Wnt inhibitory factor) module. Although structurally distinct from the CRD domain, the WIF module also binds to Wnt proteins with high affinity (Hsieh *et al*., 1999a) potentially through interactions with the palmitate moiety (Liepinsh *et al*., 2006). The WIF domain was first discovered as a domain present in secreted inhibitors of canonical Wnt signaling called Wifs that act by sequestering Wnts away from Frizzled and other cell surface receptors. Interestingly, the WIF domain is also found in the cell surface atypical receptor tyrosine kinase Ryk (Kroiher *et al*., 2001). In *Drosophila*, the Ryk ortholog Derailed binds to Wnt5 to promote commissural axon guidance in a potentially non-canonical manner (Yoshikawa *et al*., 2003). In

mammalian systems, Ryk is required for Wnt3a-mediated canonical Wnt signaling (Lu *et al*., 2004). Understanding the ways in which alternative Wnt receptors such as Ryk and Ror2 interact with known Wnt signaling components and what intracellular signaling cascades they initiate will lead to exciting advances in the field.

Lastly, we note that receptor availability is crucial for determining what signal a particular Wnt might generate. In *Xenopus*, WNT11 was long thought to solely induce non-canonical convergence extension movements (Du *et al*., 1995; Djiane *et al*., 2000). However, recent evidence shows that maternally contributed WNT11 actually serves as the key canonical Wnt signaling initiating factor during axis formation in the early *Xenopus* embryo (Tao *et al*., 2005). The ability of WNT11 to toggle between these two seemingly diverse forms of signaling might be due to availability of the extracellular epidermal growth factor-like-cripto, Frl-1, cryptic (EGF-CFC) protein family member FRL1, which shares homology to the Cripto protein in mice. FRL1 messenger RNA coinjection with WNT11 enhances WNT11's ability to activate canonical Wnt target genes and FRL1 binds to WNT11 *in vitro* (Tao *et al*., 2005). The ability of one Wnt to signal in two distinct pathways depending on receptor context is not specific to WNT11; Wnt5a can activate or inhibit canonical Wnt signaling depending on whether Ror2 or Frizzled4 and LRP5 are present (Mikels and Nusse, 2006). Although great strides have been made in determining which receptors can bind to Wnts, little is yet known regarding specific receptor-ligand pairings. The isolation and purification of Wnt proteins should allow for more detailed analyses of Wnt ligand specificity. It is becoming more and more apparent, however, that the ability of different Wnts to control different signaling pathways is not intrinsic to the Wnts, but determined by the receptors that they interact with.

Conclusion

Communication between cells is an integral part of development and differentiation. The distribution of specific extracellular ligands such as Wnts provides key positional information to help cells determine their identity and control their fate. Although great strides have been made in understanding Wnt protein function since the first Wnt gene was cloned, additional research into how Wnts are processed and released from secreting cells and are then received in neighboring responding cells should provide new pieces to the ever more complex puzzle that is Wnt signaling.

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