THE WNT SIGNALING PATHWAY IN DEVELOPMENT AND DISEASE

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■ **Abstract**   Tight control of cell-cell communication is essential for the generation of a normally patterned embryo. A critical mediator of key cell-cell signaling events during embryogenesis is the highly conserved Wnt family of secreted proteins. Recent biochemical and genetic analyses have greatly enriched our understanding of how Wnts signal, and the list of canonical Wnt signaling components has exploded. The data reveal that multiple extracellular, cytoplasmic, and nuclear regulators intricately modulate Wnt signaling levels. In addition, receptor-ligand specificity and feedback loops help to determine Wnt signaling outputs. Wnts are required for adult tissue maintenance, and perturbations in Wnt signaling promote both human degenerative diseases and cancer. The next few years are likely to see novel therapeutic reagents aimed at controlling Wnt signaling in order to alleviate these conditions.

CONTENTS

INTRODUCTION

The field of developmental biology is rich with a history of observations predicting the existence of signaling molecules that control key events in embryogenesis (Gilbert 1991). Over the past 20 to 30 years, several families of signaling molecules such as the bone morphogenetic proteins (BMPs), the Hedgehogs, the fibroblast growth factors (FGFs), and the Wnts have been identified, and their signaling mechanisms have been elucidated. These signaling pathways are also often involved in disease, in particular cancer, reinforcing the concept that cancer is a form of development gone awry.

The Wnt family of signaling proteins participates in multiple developmental events during embryogenesis and has also been implicated in adult tissue homeostasis. Wnt signals are pleiotropic, with effects that include mitogenic stimulation, cell fate specification, and differentiation. This review summarizes our current understanding of Wnt signaling in the context of the many developmental roles of this pathway. As the volume of Wnt literature is increasing rapidly, a few aspects of current interest have been selected here, mainly focused on Wnt signaling through its receptors (Frizzleds) to β-catenin, which is often called the canonical pathway. Much work has been done recently on noncanonical pathways, which do not involve β-catenin or Wnt ligands. That topic is not covered here, but we direct readers to some recent reviews (Strutt 2003, Veeman et al. 2003). A continuing update on Wnt signaling, including figures and gene tables, can be found on the Wnt homepage http://www.stanford.edu/~rnusse/wntwindow.html.

WNT SIGNALING: AN OVERVIEW

A simple outline of the current model of Wnt signal transduction is presented in Figure 1. Wnt proteins releasedfrom or presented on the surface of signaling cells act on target cells by binding to the Frizzled (Fz)/low density lipoprotein (LDL) receptor-related protein (LRP) complex at the cell surface. These receptors transduce a signal to several intracellular proteins that include Dishevelled (Dsh), glycogen synthase kinase-3β (GSK-3), Axin, Adenomatous Polyposis Coli (APC), and the transcriptional regulator, β-catenin (Figure 1). Cytoplasmic β-catenin levels are normally kept low through continuous proteasome-mediated degradation, which is controlled by a complex containing GSK-3/APC/Axin. When cells receive Wnt signals, the degradation pathway is inhibited, and consequently β-catenin accumulates in the cytoplasm and nucleus. Nuclear β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1/Tcell-specific transcription factor (LEF/TCF) to affect transcription. A large number of Wnt targets have been identified that include members of the Wnt signal transduction pathway itself, which provide feedback control during Wnt signaling.
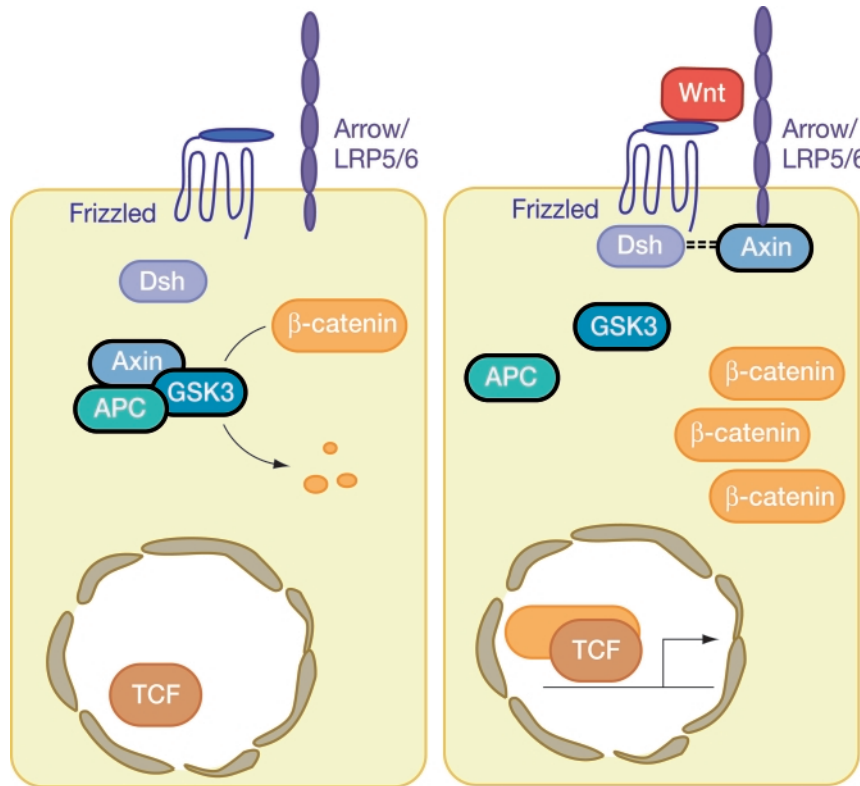
Figure 1   The canonical Wnt signaling pathway. In cells not exposed to a Wnt signal (*left panel*), β-catenin is degraded through interactions with Axin, APC, and the protein kinase GSK-3. Wnt proteins (*right panel*) bind to the Frizzled/LRP receptor complex at the cell surface. These receptors transduce a signal to Dishevelled (Dsh) and to Axin, which may directly interact (*dashed lines*). As a consequence, the degradation of β-catenin is inhibited, and this protein accumulates in the cytoplasm and nucleus. β-catenin then interacts with TCF to control transcription. Negative regulators are outlined in black. Positively acting components are outlined in color.

WNT PROTEINS ARE LIPID MODIFIED

Wnt proteins are defined by sequence rather than by functional properties. They contain a signal sequence followed by a highly conserved distribution of cysteines. Although Wnt proteins are secreted, difficulties in solubilizing active Wnt molecules had hindered attempts to purify the Wnts and precluded a thorough biochemical characterization of this growth factor family. The insoluble nature of Wnts has now been explained by the discovery that these proteins are palmitoylated and are

more hydrophobic than initially predicted from the primary amino acid sequence (Willert et al. 2003). The palmitoylation is found on a conserved cysteine, suggesting that all Wnts carry this modification. Mutant analysis has demonstrated that this cysteine is essential for function, and treating Wnt with the enzyme acyl protein thioesterase results in a form that is no longer hydrophobic or active, providing further evidence that the palmitate is critical for signaling (Willert et al. 2003).

The enzymes that add the palmitate to Wnts are likely to be encoded by the *porcupine* (*por*) gene in *Drosophila* (Kadowaki et al. 1996), called *mom-1* in *Caenorhabditis elegans* (Rocheleau et al. 1997). Phenotypic similarities between *wnt* and *por*/*mom-1* suggest that Porcupine and MOM-1 are enzymes dedicated to Wnt signaling (Kadowaki et al. 1996). These genes are required in Wnt-producing cells rather than in cells receiving the Wnt signal (Figure 2). Hofmann (2000) noticed sequence similarity between Porcupine and membrane-bound acyltransferases, enzymes that are present in the endoplasmic reticulum (ER) membrane and acylate a variety of substrates. Therefore, it is possible that *por* encodes an enzyme that catalyzes the transfer of palmitate onto Wnt. Consistent with this idea, Wingless, like Wnt3a, is also hydrophobic (Zhai et al. 2004). Wingless can associate with membranes, but both its hydrophobicity and membrane localization are lost when *O*-acyltransferase activity is inhibited biochemically or when Porcupine is eliminated genetically. These data support the hypothesis that Porcupine is a key regulator of both Wnt lipidation and membrane targeting. Interestingly, Hedgehog proteins also carry an N-terminal palmitate that is essential for signaling. The addition of this palmitate is thought to be catalyzed by Skinny-hedgehog which, like Porcupine, resembles acyl-tranferases (Chamoun et al. 2001).

Figure 2 Wnt pathway molecules that facilitate secretion or presentation of Wnt proteins or that modulate Wnt signaling levels. Porcupine (Por) is an ER protein required in Wnt-producing cells, and it may attach a palmitate to Wnt. In *C. elegans*, the MOM-3 gene product (not yet identified molecularly) may assist in the production or release of active Wnt. In vertebrates, Wnt proteins are inhibited by direct binding to either secreted frizzled-related protein (SFRP) or Wnt inhibitory factor (WIF). SFRP is similar in sequence to the cysteine-rich domain (CRD) of Frizzled, one of the Wnt receptors. The Wnt inhibitors Dickkopf (Dkk) and Wise bind to the Wnt coreceptors Arrow and LRP. Dkk also interacts with Kremen to down-regulate LRP/Arrow from the cell surface. In *Drosophila*, Wnt can bind to the tyrosine kinase receptor Derailed [related to tyrosine kinases (RYK) in mammals]. This receptor has a domain similar to WIF. Heparin-sulfated forms of proteoglycans (HSPG) are also involved in Wnt reception or transport. Boca/Mesd is specifically required for the transport of Arrow/LRP in the ER. A novel Frizzled ligand, Norrin, has also been identified. Similar to Wnt, Norrin bound to LRP and Frizzled can stimulate the canonical signaling pathway. Negative regulators are outlined in black. Positively acting components are outlined in color.
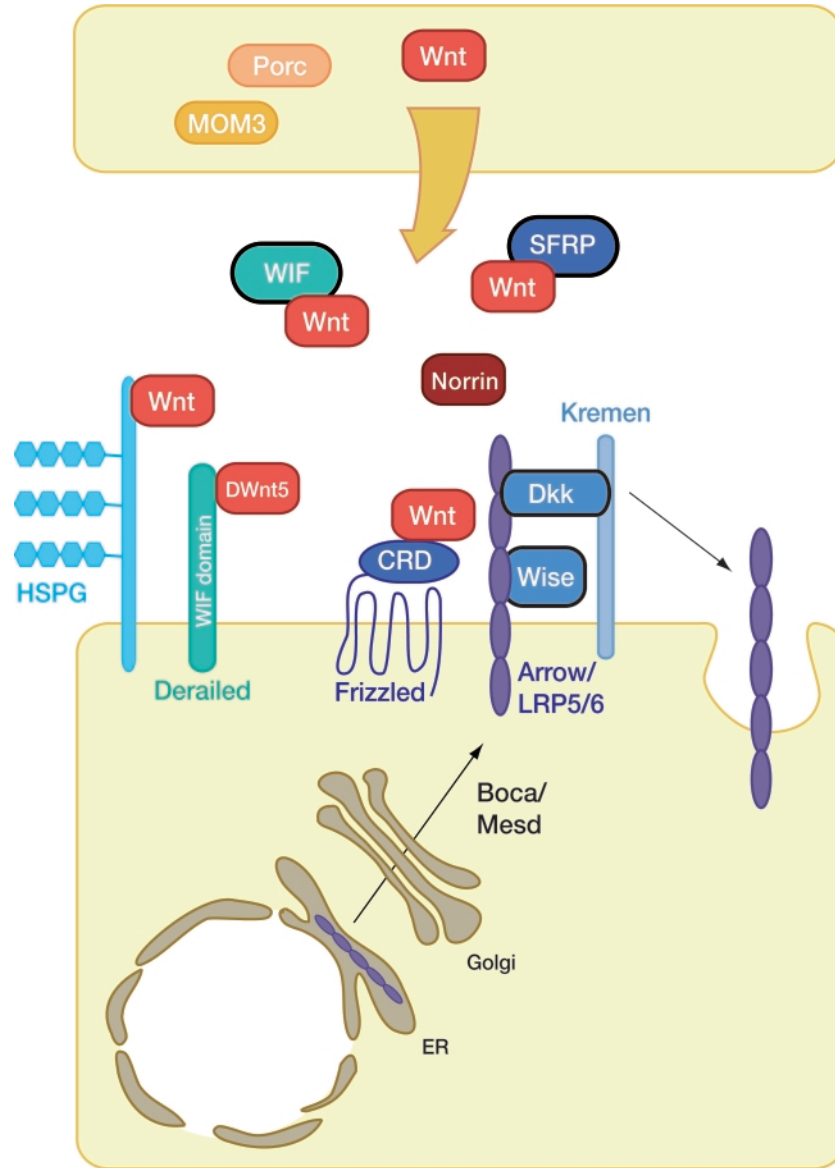
Although palmitoylation is integral to Wnt signaling, its precise function is not known. Overexpression of Wingless in *Drosophila* can partially circumvent the need for *por* function (Noordermeer et al. 1995) and, similarly, Wnt mutant gene constructs lacking the palmitoylation site can produce an attenuated signal when overexpressed in cells (Willert et al. 2003). One explanation for these observations

is that the lipid moiety targets Wnts to membranes but its absence can be overcome by high protein concentrations.

TRANSPORT OF WNT PROTEINS BETWEEN CELLS

The detection of Wnt proteins in many tissues has been problematic owing to lack of suitable antibody reagents, but antibody staining of Wingless has demonstrated significant spread of the protein in *Drosophila* imaginal discs (Cadigan et al. 1998, Strigini & Cohen 2000). These data have indicated that the Wnts, such as Wingless in *Drosophila*, function as concentration-dependent long-range morphogenetic signals that can act on distant neighbors (Cadigan et al. 1998, Strigini & Cohen 2000, Zecca et al. 1996). This raises the questions of whether palmitoylated Wnt molecules are actively transported, how Wnts are released from cells, and how Wnts move over long distances. Are Wnts always tethered to membranes, even when shuttled between cells? Alternatively, are there carrier molecules that bind to the palmitate? Vesicle-based transport outside of cells has been proposed to exist in *Drosophila* wing imaginal discs. The vesicles, termed argosomes, might carry Wg protein as cargo (Greco et al. 2001). Wnts may also be transported by cytonemes, long thin filopodial processes that might carry Wnts and other growth factors away from signaling cells (Ramirez-Weber & Kornberg 1999). There is no evidence for specific exporters of Wnt molecules, although *mom-3*, identified in *C. elegans*, is required in Wnt-producing cells (Figure 2) (Rocheleau et al. 1997). This gene (also called *mig-14*) remains to be characterized molecularly.

Once Wnt proteins are secreted, a number of binding partners can modulate their activity. Emerging evidence suggests a role for HSPGs in the transport or stabilization of Wnt (Figure 2). In *Drosophila*, absence of *Dally*, an HSPG (Lin & Perrimon 1999, Tsuda et al. 1999), and mutations in genes encoding enzymes that modify HSPG (Baeg et al. 2001, Lin & Perrimon 2000) generate phenotypes similar to *wingless* mutants. HSPGs have been postulated to function as coreceptors on target cells (Lin & Perrimon 1999), but cultured cells lacking Dally can respond to Wg (Lum et al. 2003). HSPGs may therefore stabilize Wnt proteins or aid in its presentation or movement between cells.

Secreted Wnts may also bind members of the SFRP family. These are secreted proteins that resemble the ligand-binding domain of the Frizzled family of Wnt receptors (Hoang et al. 1996, Rattner et al. 1997). Alternatively, Wnts may bind WIF proteins, which are secreted molecules resembling the extracellular portion of the Derailed/RYK class of transmembrane Wnt receptors (Hsieh et al. 1999a) (Figure 2). In general, both SFRPs and WIFs are thought to function as extracellular Wnt inhibitors (Bafico et al. 1999, Dennis et al. 1999, Hsieh et al. 1999a, Leyns et al. 1997, Salic et al. 1997, Uren et al. 2000, Wang et al. 1997). However, it has not been ruled out that these proteins, depending on expression levels or cellular context, promote Wnt signaling by protecting Wnts from degradation or by facilitating Wnt secretion or transport (Uren et al. 2000).

WNT RECEPTORS AND THEIR INTERACTIONS WITH
EXTRACELLULAR INHIBITORS

Genetic and biochemical data have demonstrated that the Fz proteins are the primary receptors for the Wnts (Bhanot et al. 1996) (Figure 2). Fzs are seven-transmembrane receptors with a long N-terminal extension called a cysteine-rich domain (CRD). Wnt proteins bind directly to the Fz CRD (Bhanot et al. 1996, Dann et al. 2001, Hsieh et al. 1999b). In *Drosophila* and in cell culture, overexpression of the DFz2 receptor fails to activate Wnt signaling unless its cognate ligand, Wingless, is present (Bhanot et al. 1996, Rulifson et al. 2000), suggesting that Fz activation during canonical signaling is ligand dependent.

In addition to Wnt/Fz interactions, Wnt signaling also requires the presence of a single-pass transmembrane molecule of the LRP family (Figure 2), identified as the gene *arrow* in *Drosophila* (Wehrli et al. 2000) and as *LRP5* or *6* in vertebrates (Pinson et al. 2000, Tamai et al. 2000). The transport of LRP from the ER to the cell surface requires a specific accessory molecule called Boca in *Drosophila* and Mesd in mice (Culi & Mann 2003, Hsieh et al. 2003); mutations in these genes produce phenotypes similar to loss of Arrow/LRP itself. It has been proposed (Tamai et al. 2000) that Wnt molecules bind to LRP and Frizzled to form a receptor trimeric complex, although this has not been observed for Wingless and Arrow in *Drosophila* (Wu & Nusse 2002). Nevertheless, the importance of LRP is underscored by the finding that potent, extracellular inhibitors of Wnt signaling such as Wise (Itasaki et al. 2003) and Dickkopf (Glinka et al. 1998) bind to LRP. The best characterized of the secreted Wnt-signaling inhibitors are the Dickkopf (Dkk) proteins. Dkks have not been found in invertebrates, but mice and humans have multiple Dkk genes (Krupnik et al. 1999, Monaghan et al. 1999). Dkk1, in particular, is a potent Wnt-signaling inhibitor (Glinka et al. 1998). It binds to LRP with high affinity (Bafico et al. 2001, Mao et al. 2001a, Semenov et al. 2001) and to another class of transmembrane molecules, the Kremens (Mao & Niehrs 2003, Mao et al. 2002). By forming a complex with LRP and Kremen, Dkks promote the internalization of LRP, making it unavailable for Wnt reception. The inhibitory function of Dkks depends on the presence of appropriate Kremen proteins. Dkk2 requires Kremen2 in order to inhibit Wnt signaling and cannot function with Kremen1 to down-regulate the Wnt signal (Mao & Niehrs 2003). Likewise, Kremen2 promotes the inhibitory activity of Dkk4 (Mao & Niehrs 2003).

In addition to binding the Wnts, Frizzled can also interact with another ligand. Norrin, a protein with no discernable sequence similarity to the Wnts, binds with high affinity to the Frizzled-4 CRD (Xu et al. 2004). Together with LRP, Frizzled-4 and Norrin can activate the canonical signaling pathway. The ability of Wnt receptors to interact with multiple ligands underscores the pleiotropy of Frizzled activity, and it is possible that there are yet additional ligands for this receptor family.

Recent data show that Derailed, another Wnt receptor, is entirely distinct from the Frizzleds (Figure 2). The Derailed receptor is a transmembrane tyrosine

kinase belonging to the RYK subfamily. Dwnt-5 is a regulator of axon guidance
in the *Drosophila* central nervous system (CNS), and embryos mutant for Dwnt-5
resemble those lacking Derailed, i.e., they display misrouting of neuronal projec-
tions across the midline (Yoshikawa et al. 2003). The Derailed extracellular region
contains a Wnt-interacting WIF domain (Hsieh et al. 1999a) that can bind to the
DWnt-5 protein (Yoshikawa et al. 2003), indicating that Derailed is a DWnt-5
receptor in the CNS. How Derailed signals are transduced is not clear; the De-
railed kinase domain appears dispensable for function (Yoshikawa et al. 2001),
and the possibility that signaling involves a coreceptor has not been excluded. In
vertebrates, both Wnt4 and Wnt5 have been implicated in axon guidance (Hall et al.
2000, Lyuksyutova et al. 2003). Wnt4 appears to signal in this context through a
Frizzled. Whether a RYK binds to Wnt5 is not known because the Wnt5a recep-
tor has not been identified. It will be interesting to determine whether Fz/LRP
and RYK receptors function in the same tissues and cellular processes and, if so,
whether Wnts simultaneously contact Fz/LRP and RYK-like kinases or stimulate
RYK and Fz/LRP receptors in parallel pathways.

HOW DO THE WNT RECEPTORS SIGNAL?

The observation that Fzs contain seven transmembrane regions has led to the
suggestion that Wnt binding might reconfigure the Fz transmembrane domains,
as occurs in other heptahelical receptors. How a reconfigured Fz receptor cou-
ples to downstream effectors is not understood. Dsh, a ubiquitously expressed
cytoplasmic protein (Lee et al. 1999, Yanagawa et al. 1995), functions cell au-
tonomously and genetically upstream of proteins such as β-catenin and GSK-3
(Noordermeer et al. 1994). It has been postulated that Dsh transduces the Wnt
signal into the cell through a direct binding between Dsh and Fz (Figures 2 and
3). Consistent with this idea, Dsh can interact with Fz directly (Chen et al. 2003,
Wong et al. 2003) through a C-terminal cytoplasmic Lys-Thr-X-X-X-Trp motif
in Fz that is required for Fz signaling (Umbhauer et al. 2000). Wnt signaling
also leads to differential phosphorylation of Dsh (Yanagawa et al. 1995), and
this process is mediated by several protein kinases, of which Par1 is the most
likely Wnt-regulated candidate (Sun et al. 2001). Some questions that remain are
whether Wnt binding to Fz regulates a direct Fz-Dsh interaction, how Dsh phos-
phorylation is controlled, and how phosphorylated Dsh functions in Wnt signal
transduction.

Similar to Fz, LRP may also interact with a cytoplasmic component of the
Wnt-signaling pathway. The cytoplasmic tail of LRP contains several Pro-Pro-
Pro-(SerTrp)Pro [PPP(S/T)P] motifs that can become phosphorylated following
Wnt stimulation (Tamai et al. 2004). Because LRP can interact with the cytosolic
protein Axin (Mao et al. 2001b, Tolwinski et al. 2003) (Figures 2 and 3), Wnts are
thought to induce the phosphorylation of LRP on a PPP(S/T)P motif, thus allowing
the docking of Axin to the LRP cytoplasmic tail.

Both Dsh and Axin contain a stretch of amino acids called the DIX domain. DIX domains of Axin can homodimerize (Hedgepeth et al. 1999, Hsu et al. 1999, Sakanaka & Williams 1999), and Dsh, and a *Xenopus* Axin, XARP, can heterodimerize through their DIX domains (Itoh et al. 2000). It is possible, therefore, that Wnt binding of Fz and LRP promotes direct interaction between Axin and Dsh through their DIX domains, reconfiguring the protein complex that regulates β-catenin levels in the cell (Figure 3).

Because most seven-transmembrane receptors signal through heterotrimeric G proteins, it is reasonable to ask whether Fzs interact with G proteins as well.
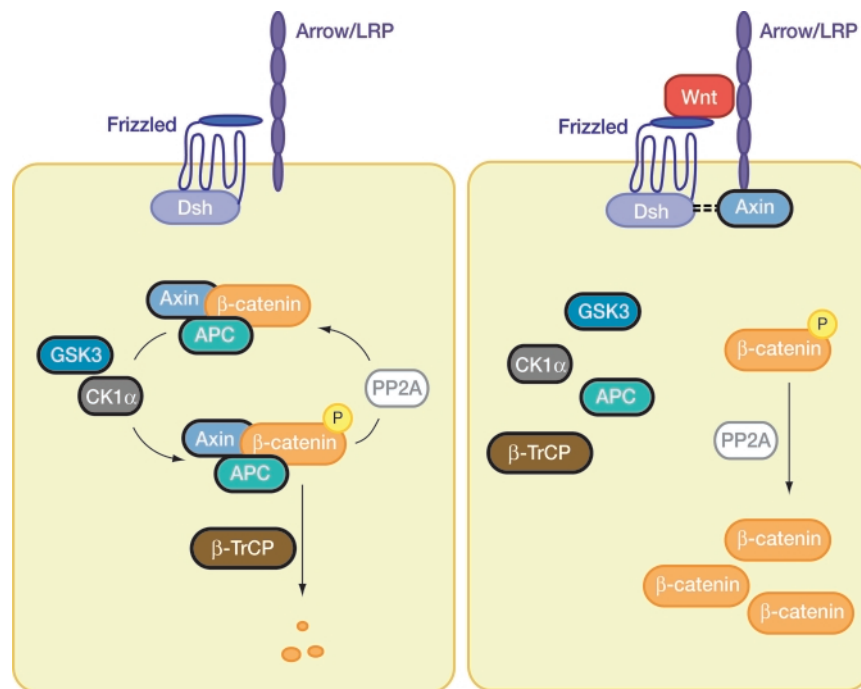

Figure 3 Cytoplasmic components of the Wnt signaling pathway, In naïve cells (*left panel*), β-catenin forms a complex with Axin and APC. Axin acts as a scaffold for the protein kinases CK1a and GSK-3, and the PP2A protein phosphatase. PP2A may act on Axin as well as other on substrates in the Axin/APC complex. After phosphorylation by GSK-3 and CK1a, β-catenin is degraded by ubiquitination involving interactions with Slimb/β-TrCP. After binding of the Wnt ligand (*right panel*), the Fz and LRP receptors recruit Dsh and Axin to the membrane where they may interact with each other (*dashed lines*). Wnt signaling leads to inhibition of β-catenin degradation and its accumulation. As a result, β-catenin is stabilized in the cytoplasm and is no longer degraded. Negative regulators are outlined in black. Positively acting components are outlined in color.

The most direct test of this hypothesis would be to add a known Wnt ligand to cells expressing the cognate Frizzled receptor and to examine the immediate consequences. Because Wnt proteins have been difficult to isolate, experiments along these lines have utilized chimeric Fz receptors that can be activated by a non-Wnt ligand. Chimeric receptors consisting of the intracellular loops of rat Fz1 or rat Fz2 and the transmembrane and exofacial regions of β-adrenergic receptor could be activated by a β-adrenergic agonist and appear to signal through G proteins of the Go, Gq, and Gt classes (X. Liu et al. 1999, Liu et al. 2001). Whether natural Fz molecules can couple directly to heterotrimeric G proteins, however, remains to be tested.

WNT SIGNALING WITHIN THE CYTOPLASM

A hallmark of Wnt pathway activation is the elevation of cytoplasmic β-catenin protein levels. In the absence of Wnt signaling, β-catenin is phosphorylated by the serine/threonine kinases, casein kinase Iα(CKIα) (Amit et al. 2002, Liu et al. 2002, Yanagawa et al. 2002) and GSK-3 (Yost et al. 1996). The interaction between these kinases and β-catenin is facilitated by the scaffolding proteins, Axin and APC (Hart et al. 1998, Kishida et al. 1998). Together, these proteins form a β-catenin degradation complex that allows phosphorylated β-catenin to be recognized by β-TrCP, targeted for ubiquitination, and degraded by the proteosome (Aberle et al. 1997, Latres et al. 1999, C. Liu et al. 1999) (Figure 3).

Activation of Wnt signaling inhibits β-catenin phosphorylation and hence its degradation. The elevation of β-catenin levels leads to its nuclear accumulation (Tolwinski & Wieschaus 2004, Miller & Moon 1997, Cox et al. 1999) and complex formation with LEF/TCF transcription factors (van de Wetering et al. 1997, Behrens et al. 1996, Molenaar et al. 1996). β-catenin mutant forms that lack the phosphorylation sites required for its degradation are Wnt unresponsive and can activate Wnt target genes constitutively (Munemitsu et al. 1996, Yost et al. 1996). β-catenin, APC, and Axin mutations that promote β-catenin stabilization are found in many different cancers, indicating that constitutive Wnt signaling is a common feature in many neoplasms (reviewed in Giles et al. 2003).

Wnt signals might influence the cytoplasmic proteins that regulate β-catenin stability through several mechanisms. Reception of a Wnt signal could trigger the recruitment of Axin either to LRP or to Frizzled-bound Dsh, removing Axin from the destruction complex to promote β-catenin stabilization (Cliffe et al. 2003, Tamai et al. 2004). Protein phosphatases also regulate β-catenin stability. PP2A, for example, is required for the Wnt-dependent elevation of β-catenin levels (J. Yang et al. 2003) and can bind Axin (Hsu et al. 1999), suggesting that it might function to dephosphorylate GSK-3 substrates. How PP2A activity is regulated by Wnt signals is not known. Finally, Dsh can interact with the destruction complex through the GSK-3 binding protein, GBP/Frat (Jonkers et al. 1997, Salic et al. 2000, Yost et al. 1998). Frat may promote the dissociation of GSK-3 from

the degradation complex and prevent the phosphorylation of β-catenin (Li et al. 1999), but how Wnt signals regulate this event is not clear. Current approaches to elucidate the mechanisms of β-catenin regulation include attempts to determine the structure of the degradation complex. There are now crystallographic structures of Axin contacting β-catenin (Xing et al. 2003) and Axin bound to APC (Spink et al. 2000). However, the composition of the destruction complex and the stoichiometry of the various components have not been fully resolved. Recent data suggest that the number of Axin molecules in cells is much lower (5000-fold) than other proteins in the complex (Lee et al. 2003). Axin, therefore, may be a limiting component of the Wnt signaling cascade that, as a key scaffolding molecule, may promote the rapid assembly and disassembly of Wnt pathway components to regulate β-catenin stability in the cell. Given that Dsh, APC, GSK-3, and β-catenin participate in other signaling events, low Axin levels may also help to insulate the Wnt pathway from changes in the abundance of the other Wnt signaling components when they participate in different signaling processes. Further detailed stoichiometric analyses will provide deeper insights into the exact nature of the β-catenin destruction complex and the mechanisms that regulate its function.

SIGNALING IN THE NUCLEUS

The increased stability of β-catenin following Wnt signaling leads to the transcriptional activation of target genes mediated by β-catenin interactions with the TCF/LEF DNA-binding proteins (Figure 4) (van de Wetering et al. 1997, Behrens et al. 1996, Molenaar et al. 1996). In the absence of the Wnt signal, TCF acts as a repressor of Wnt/Wg target genes (Brannon et al. 1997) by forming a complex with Groucho (Cavallo et al. 1998). The repressing effect of Groucho is mediated by interactions with histone deacetylases (HDAC), which are thought to make DNA refractive to transcriptional activation (Chen et al. 1999).

Once in the nucleus, β-catenin is thought to convert the TCF repressor complex into a transcriptional activator complex. This may occur through displacement of Groucho from TCF/LEF and recruitment of the histone acetylase CBP/p300 (cyclic AMP response element-binding protein). CBP may bind to the β-catenin/TCF complex as a coactivator (Hecht et al. 2000, Takemaru & Moon 2000), a hypothesis that remains to be tested directly. Another activator, Brg-1, is a component of the SWI/SNF (switching-defective and sucrose nonfermenting) chromatin remodeling complex which, with CBP, may induce chromatin remodeling that favors target gene transcription (Barker et al. 2001).

Further interactions between the TCF-β-catenin complex and chromatin could be mediated by Legless (Bcl9) and Pygopos (Kramps et al. 2002, Parker et al. 2002, Thompson et al. 2002). Mutations in either of these genes result in *wingless*-like phenotypes in *Drosophila*, and both genes promote Wnt signaling in mammalian cell culture experiments (Thompson et al. 2002).
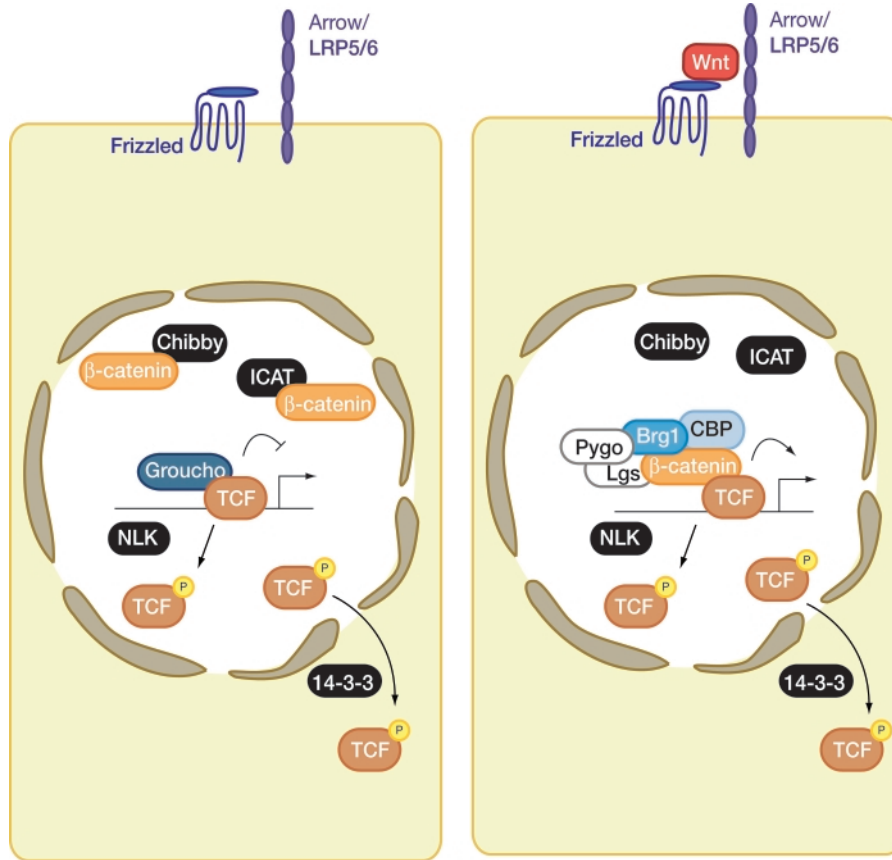
Figure 4   Nuclear factors in Wnt signaling. The interaction between Groucho and TCF is thought to down-regulate transcriptional activation (*left panel*). β-catenin is also negatively regulated by binding to Chibby and Inhibitor of β-catenin and TCF (ICAT). TCF activity in the nucleus can be modulated by phosphorylation by Nemo-like kinase (NLK), and in *C. elegans*, a 14-3-3-like protein has been shown to facilitate nuclear export of TCF (*thin arrow*). β-catenin interferes with the interaction between TCF and Groucho,, and together with TCF, activates gene expression. β-catenin also binds to other components such as Legless (Lgs), Pygopus (Pygo), CREB-binding protein (CBP), and Brg1. Negative regulators are shown in black. Positively acting components are outlined in color.

Wnt signaling events in the nucleus are controlled by a number of protein partners. For example, the protein Chibby is a nuclear antagonist that binds to the C terminus of β-catenin (Takemaru et al. 2003). Another β-catenin-binding protein, ICAT (Tago et al. 2000), not only blocks the binding of β-catenin to TCF (Tago et al. 2000) but also leads to dissociation of complexes between β-catenin, LEF, and CBP/p300 (Daniels & Weis 2002, Graham et al. 2002). TCF is also subject

to regulation, as it can be phosphorylated by the mitogen-activated protein (MAP) kinase–related protein kinase NLK/Nemo (Ishitani et al. 1999). NLK/Nemo itself is activated by the mitogen-activated protein (MAP) kinase kinase, TAK1 (Ishitani et al. 1999). The phosphorylation of TCF/LEF by activated Nemo is thought to diminish the DNA-binding affinity of the β-catenin/TCF/LEF complex, thereby affecting transcriptional regulation of Wnt target genes (Ishitani et al. 1999, 2003). Another consequence of TCF phosphorylation, at least in *C. elegans*, is export of TCF from the nucleus (Meneghini et al. 1999), which is carried out by a 14-3-3 protein, Par5 (Lo et al. 2004). The ability of LEF/TCF to interact with DNA and its other partners is therefore highly regulated and likely plays critical roles in the modulation of Wnt target gene expression. Recently, it was shown that β-catenin can interact with other binding partners in the nucleus, such as Pitx2. β-catenin can convert Pitx2 from a transcriptional repressor into an activator (Kioussi et al. 2002), similar to its interaction with LEF1/TCF. The presence of additional β-catenin-binding partners adds another layer of complexity to the regulation of gene expression by nuclear β-catenin.

WNT TARGET GENES AND FEED BACK LOOPS

Mutant analysis of Wnt genes has shed light on the range of biological processes that Wnts control. In particular, *wingless* in *Drosophila* is involved in numerous developmental events that include embryonic and larval patterning (Cadigan & Nusse 1997) and synaptic differentiation (Packard et al. 2002). In vertebrate development, loss of a single Wnt gene can produce dramatic phenotypes that range from embryonic lethality and CNS abnormalities to kidney and limb defects (Table 1). These diverse phenotypes indicate that the Wnt pathway has distinct transcriptional outputs. In many cases, the cell, rather than the signal, determines the nature of the response, and up- or down-regulation of Wnt target genes is cell-type specific. In other cases, however, the same target genes can be induced in multiple cell and tissue types. Whether these are universal targets of Wnt signaling remains to be shown.

Interestingly, there may be some themes in the types of target genes that are induced by Wnts. Wnt signaling can promote the expression of Wnt pathway components (Table 2). Whether these genes are direct Wnt targets is not known in all cases, but this finding indicates that feedback control is a key feature of Wnt signaling regulation. One class of targets that respond to Wnt signaling is the Frizzleds (Cadigan et al. 1998, Muller et al. 1999, Sato et al. 1999, Willert et al. 2002). Dfz2 in *Drosophila* is down-regulated by *wg* wherever Wg is active, a process that may function to limit the levels of Wnt signaling within the Dfz2-expressing cells. In addition, by reducing the levels of a high-affinity receptor that might otherwise limit Wg distribution, Wg may be allowed to diffuse over longer distances (Cadigan et al. 1998). The levels of LRP and HSPG are also controlled by Wg signaling, providing further fine-tuning of Wg activity at the cell surface

TABLE 1   Wnt mutant phenotypes in the mouse

Gene	Knockout (KO) phenotypes or other functions	Redundancies/ similarities with other KO	References
Wnt1	Deficiency in neural crest derivatives, reduction in dorsolateral neural precursors in the neural tube (with Wnt3A KO) Decrease in thymocyte number (with Wnt-4 KO)	Redundant with Wnt3a and Wnt4; Similar to TCF1	(Ikeya et al. 1997, Mulroy et al. 2002)
Wnt3	Early gastrulation defect, Perturbations in establishment and maintenance of the apical ectodermal ridge (AER) in the limb	In limbs, similar to loss of β-catenin	(Barrow et al. 2003, P. Liu et al. 1999)
Wnt3a	Paraxial mesoderm defects, tailbud defects, deficiency in neural crest derivatives, reduction in dorsolateral neural precursors in the neural tube (with Wnt1 KO) Loss of hippocampus Somitogenesis defects	Redundant with Wnt1, Similar to LEF1/TCF1	(Aulehla et al. 2003; Galceran et al. 1999, 2000; Ikeya et al. 1997; Lee et al. 2000; Yoshikawa et al. 1997)
Wnt4	Defects in female development; absence Mullerian duct, defects in adrenal gland development Decrease in thymocyte number (with Wnt1 KO)	Wnt1	(Heikkila et al. 2002, Mulroy et al. 2002, Vainio et al. 1999)
Wnt5a	Truncated limbs and AP axis Defects in distal lung morphogenesis Chondrocyte differentiation defects, perturbed longitudinal skeletal outgrowth Inhibits B cell proliferation, produces myeloid leukemias and B-cell lymphomas in heterozygotes		(Li et al. 2002, Liang et al. 2003, Yamaguchi et al. 1999, Y. Yang et al. 2003)
Wnt7a	Female infertility; in males, Mullerian duct regression fails Delayed maturation of synapses in cerebellum		(Hall et al. 2000, Parr & McMahon 1998)
Wnt7b	Placental development defects Respiratory failure; defects in early mesenchymal proliferation leading to lung hypoplasia		(Parr et al. 2001, Shu et al. 2002)
Wnt11	Ureteric branching defects and kidney hypoplasia		(Majumdar et al. 2003)

TABLE 2   Wnt signaling components as Wnt pathway targets

Target gene	Effect of Wnt signal on target gene expression	Effect of changes in target gene expression on Wnt pathway	Target gene interacts with	Reference
Fz	Down	Inactivate	Wnt	(Muller et al. 1999)
Dfz2	Down	Inactivate	Wnt	(Cadigan et al. 1998)
Dfz3	Up	Activate	Wnt	(Sato et al. 1999)
Fz7	Up		Wnt	(Willert et al. 2002)
Arrow/*LRP*	Down	Inactivate	Wnt	(Wehrli et al. 2000)
Dally (HSPG)	Down		Wnt	(Baeg et al. 2001)
Wingful/*notum*	Up	Inactivate	HSPG?	(Giraldez et al. 2002)
naked	Up	Inactivate	Dsh	(Rousset et al. 2001)
Axin2	Up	Inactivate	β-catenin	(Jho et al. 2002)
β-TCRP	Up	Inactivate	β-catenin	(Spiegelman et al. 2000)
TCF1 (dn)	Up	Inactivate	TCF	(Roose et al. 1999)
LEF1	Down	Activate	β-catenin	(Hovanes et al. 2001)
Nemo	Up	Inactivate (*Drosophila*) Activate (*Zebrafish*)	β-catenin/ LEF/TCF	(Zeng & Verheyen 2004, Thorpe & Moon 2004)

(Baeg et al. 2001, Wehrli et al. 2000). Two cytoplasmic negative regulators are also induced by Wnt signals. The *naked cuticle* (*naked*) gene is an EF-hand-containing protein that can bind directly to Dsh and inhibit Wnt signaling in both *Drosophila* (Rousset et al. 2001) and vertebrates (Wharton et al. 2001). The *Axin2*[gene is also a direct Wnt target that is expressed in many sites where Wnt signaling is known to occur (Aulehla et al. 2003, Jho et al. 2002). TCF and LEF are transcriptionally responsive to Wnt signaling. In colorectal cancer cells, loss of APC up-regulates LEF1, which may promote increased mis-regulation of Wnt target genes (Hovanes et al. 2001). APC mutations also up-regulate a splice-variant of TCF1 that lacks an N-terminal β-catenin-binding site (Roose et al. 1999). This dominant-negative TCF1 is thought to dampen Wnt signaling and reduce the severity of perturbations that result from loss of β-catenin or APC. Therefore, although APC mutations ultimately induce cancerous lesions in the colon, the induction of both TCF1 and LEF1 expression reveals an exquisite ability of colon cells to modulate Wnt signaling levels through feed-back regulation.

Cell proliferation is commonly regulated by Wnt signaling, and Wnt knockout phenotypes can often be explained by a loss of cell proliferation. For example, limb outgrowth fails in limb buds lacking Wnt5A (Yamaguchi et al. 1999), and expansion of the CNS fails in Wnt1 mutants (Megason & McMahon 2002). A mitogenic effect of *wingless* has also been reported for the *Drosophila* wing imaginal disc (Giraldez & Cohen 2003). The loss of particular cells or tissues in Wnt mutants has often been interpreted as stemming from perturbations in cell fate specification,

but an alternative interpretation may be that in some cases, progenitor cells fail to expand. A general function of Wnt signaling during development may therefore be to regulate the cell proliferation by direct induction of cell cycle regulators. Consistent with this, myc and cyclinD1 are direct Wnt signaling targets in colon cancer cells (He et al. 1998, Shtutman et al. 1999, Tetsu & McCormick 1999).

A recent study in the skin (Jamora et al. 2003) has raised the intriguing possibility that Wnt signaling might also generally regulate cell adhesion, although this must be tested further. Formation of an epithelial bud during hair follicle development requires the repression of E-cadherin transcription. Inputs from both Wnt, which stabilizes β-catenin, and from the BMP inhibitor Noggin, which induces Lef1 expression, directly repress the E-cadherin promoter. If the Wnts can regulate cell-cell adhesion molecules at a transcriptional level, then Wnt signaling may integrate cell fate specification and differentiation with cell behavior changes. It will be interesting to determine whether Wnts regulate cadherin transcription in places such as the teeth and mammary gland where morphogenetic movements similar to those accompanying epithelial bud formation occur (van Genderen et al. 1994). Several components of the Wnt pathway such as β-catenin and APC are also multifunctional, participating in cell-cell adhesion and cytoskeletal rearrangements (reviewed in Bienz 2002, Gumbiner 2000). Therefore, the possibility that Wnt signaling, through changes in β-catenin levels, directly impinges on cell adhesion or cell behavior is tantalizing. and provides exciting avenues for further research. For a discussion of current data that examines connections between cell adhesion and Wnt signaling, we direct the reader to Nelson & Nusse (2004).

WNT PHENOTYPES: REDUNDANCY AND SPECIFICITY

A common approach to understanding the function of a particular gene in a tissue or developmental process is to examine its knockout phenotype. In some cases, the expression patterns and mutant phenotypes correlate closely and clearly demonstrate the requirement for that particular Wnt in a specific developmental event. Wnt3, for example, is expressed in the primitive streak in the early mouse embryo, and Wnt3 mutants display gastrulation defects (P. Liu et al. 1999). There are several cases, however, where mutant phenotypes were not fully revealed until multiple Wnts were removed. One classic example is the Wnt1/Wnt3a double-knockout, which demonstrates a requirement for Wnt signaling in a wider region of the CNS than when only the Wnt1 or Wnt3a gene is eliminated (Table 1) (Ikeya et al. 1997). This is also observed with downstream Wnt pathway components; mutants of Lef1 and TCF1 exhibit defects similar to Wnt3a mutants but only when both Lef1 and TCF1 are missing (Table 3). These examples illustrate that genetic redundancy between Wnt signaling components is likely to greatly influence our ability to discern and interpret Wnt mutant phenotypes.

The analysis of Fz mutants has largely failed to reveal specific Wnt/Fz pairs that interact during development. An exception is Frizzled 4, which affects axonal

TABLE 3 TCF mutant phenotypes in vertebrates

Gene	Knockout (KO) phenotypes or other functions	References
Tcf1 (official name *Tcf7*)	Thymocyte differentiation defects Defects in limb bud development (with Lef1 KO) Mammary and gut tumors, accelerated by loss of Min/APC	(Galceran et al. 1999, Roose et al. 1999, Verbeek et al. 1995)
Tcf3 (official name T*cf7L1*) h*eadless* in Zebrafish	Expanded axial mesoderm in mice, anterior defects in Zebrafish	(Kim et al. 2000, Merrill et al. 2004)
Tcf4 (official name *Tcf7L2*)	Absence of epithelial stem cells in small intestine	(Korinek et al. 1998)
Lef1	Defects in limb bud development (with Tcf1 KO) Defects in pro-B cell proliferation and survival	(Galceran et al. 1999, Reya et al. 2000)

TABLE 4 Frizzled phenotypes in mammals

Gene	Knockout (KO) phenotypes or other functions	References
Fz3 (*Mfz3*)	Defect in fiber tracts in the rostral CNS Perturbed anterior-posterior guidance of commissural axons	(Lyuksyutova et al. 2003, Wang et al. 2002)
Fz4 (*Mfz4*)	Cerebellar, auditory, and esophageal defects In humans, retinal angiogenesis in familial exudative vitreoretinopathy (FEVR)	(Wang et al. 2001, Robitaille et al. 2002, Xu et al. 2004, Toomes et al. 2004)
Fz5 (*Mfz5*)	Essential for yolk sac and placental angiogenesis	(Ishikawa et al. 2001)
Fz6 (*Mfz6*)	Hair patterning defects	(Guo et al. 2004)

guidance in the neural tube by a process that may involve Wnt4 (Lyuksyutova et al. 2003) (Table 4). The lack of a one-to-one correspondence between individual Wnt and Fz mutant phenotypes suggests that a single Frizzled might be activated by multiple Wnts or that a given Wnt might bind multiple Frizzleds. In *Drosophila*, Wg binds to both Fz and Dfz2 and a cuticle patterning defect is observed only when both receptors are mutant (Bhanot et al. 1999, Chen & Struhl 1999, Kennerdell & Carthew 1998, Rulifson et al. 2000). This is a relatively simple example where signaling is mediated primarily by one Wnt and there is genetic redundancy between only two Fzs. In vertebrates, where Wnt and Fz expression patterns may be more elaborate, and where Frizzled may even interact with other ligands such as

Norrin (Guo et al. 2004), the overlapping interactions and relationships between Wnts, other Frizzled binding partners, and Fzs are likely to be far more complex.

In a given cell or tissue, only a subset of Wnts can stimulate the canonical signaling pathway (for examples, see Kispert et al. 1998, Shimizu et al. 1997, Torres et al. 1996). These data likely reflect the ability of different Wnts to bind to the particular receptors that exist on the surfaces of the responding cells. Studies that measure Wnt-Fz binding affinities are only just beginning (Hsieh et al. 1999b, Wu & Nusse 2002). Not much is known about specificity between ligands and receptors in vertebrates, but in *Drosophila*, the affinity between Wingless and its receptors, Fz and Dfz2, is high (Wu & Nusse 2002). As more binding studies are performed, they will provide valuable tools for elucidating physiological interactions between Wnts and Fzs.

WNT SIGNALING IN CANCER AND HUMAN DISEASE

Given the diverse phenotypes produced by Wnt knockouts in mice, it is not surprising that loss of Wnts in humans has dire consequences as well. Recently, Tetra-amelia, a rare human genetic disorder characterized by absence of limbs, has been proposed to result from WNT3 loss-of- function mutations (Niemann et al. 2004).

In adults, mis-regulation of the Wnt pathway also leads to a variety of abnormalities and degenerative diseases (Table 5). An LRP mutation has been identified that causes increased bone density at defined locations such as the jaw and palate (Boyden et al. 2002, Little et al. 2002). The mutation is a single amino-acid substitution that makes LRP5 insensitive to Dkk-mediated Wnt pathway inhibition, indicating that the phenotype results from overactive Wnt signaling in the bone (Boyden et al. 2002). In a different study, mutations in LRP5 were correlated with

TABLE 5   Human genetic diseases and Wnt signaling components

Gene	Disease	References
WNT3	Tetra-amelia	(Niemann et al. 2004)
LRP5	Bone density defects Vascular defects in the eye (osteoperosis-pseudoglioma syndrome; OPPG), familial exudative vitreoretinopathy; FEVR)	(Boyden et al. 2002, Gong et al. 2001, Little et al. 2002, Toomes et al. 2004)
FZD4	Retinal angiogenesis defects (familial exudative vitreoretinopathy; FEVR)	(Robitaille et al. 2002 Xu et al. 2004, Toomes et al. 2004)
Axin2	Tooth agenesis Predisposition to colorectal cancer	(Lammi et al. 2004)
APC	Polyposis coli, colon cancer	(Kinzler et al. 1991, Nishisho et al. 1991)

decreased bone mass (Gong et al. 2001). In this case, frame shift and missense mutations were thought to create loss-of-function LRP5 mutations. These data indicate that Wnt signaling mediated by LRP5 is required for maintenance of normal bone density.

LRP5 mutations (Gong et al. 2001) can also be accompanied by vasculature defects in the eye (osteoperosis-pseudoglioma syndrome or OPPG). In addition, a hereditary disorder, called familial exudative vitreopathy (FEVR), is caused by mutations in both LRP5 and the Fz4 receptor, which results in defective vasculogenesis in the peripheral retina (Toomes et al. 2004, Robitaille et al. 2002). The Fz4 mutation is located in the seventh transmembrane domain, and the LRP5 mutations all create prematurely terminated proteins, suggesting that FEVR results from loss of Fz/LRP signaling. More recently, Norrin, a protein that bears no ressemblance to Wnts, has been identified as the ligand for the Fz4/LRP receptor complex (Xu et al. 2004). Signaling by functional Norrin/Fz/LRP complexes is therefore crucial for proper vasculogenesis and its maintenance in at least some parts of the body.

Mutations in intracellular Wnt pathway components also produce dramatic defects. A nonsense mutation in Axin2 has been shown to produce severe tooth agenesis, or oligodontia, a condition in which multiple permanent teeth are missing (Lammi et al. 2004).

Mutations that promote constitutive activation of the Wnt signaling pathway lead to cancer. In addition to tooth defects, individuals with Axin2 mutations display a prediposition to colon cancer (Lammi et al. 2004). Moreover, the best-known example of a disease involving a Wnt pathway mutation that produces tumors is familial adenomatous polyposis (FAP), an autosomal, dominantly inherited disease in which patients display hundreds or thousands of polyps in the colon and rectum. This disease is caused most frequently by truncations in APC (Kinzler et al. 1991, Nishisho et al. 1991), which promote aberrant activation of the Wnt pathway leading to adenomatous lesions owing to increased cell proliferation. Mutations in β-catenin and APC have also been found in sporadic colon cancers and a large variety of other tumor types (reviewed in Giles et al. 2003). Loss-of-function mutations in Axin have been found in hepatocellular carcinomas (Satoh et al. 2000). These examples demonstrate that the uncoupling of normal β-catenin regulation from Wnt signaling control is an important event in the genesis of many cancers.

It has become increasingly common to view cancer as a stem cell disease (see Taipale & Beachy 2001). In the colon, loss of TCF4 or Dkk overexpression promotes loss of stem cells in the colon crypt, indicating that Wnt signaling is required for maintenance of the stem cell compartment (Korinek et al. 1998, Kuhnert et al. 2004, Pinto et al. 2003). A more extensive discussion of Wnt signaling and colon stem cell control is presented elsewhere in this volume (see Sancho et al. 2004. Wnt signaling may therefore be a fundamental regulator of stem cell choices to proliferate or self-renew. Consistent with this idea, Wnt3a promotes self-renewal of hematopoietic stem cells in vitro (Willert et al. 2003). The use of soluble Wnts to control the proliferation and/or maintenance of stem cells may offer powerful therapeutic reagents for the in vitro manipulation of stem cells and their reintroduction into diseased tissues.

EVOLUTIONARY ORIGIN WNT SIGNALING

Is it possible to trace the evolutionary origins of Wnt signaling? With the completion of several animal genomes and partial sequence information on other organisms, these questions can now be addressed in a systematic manner. The finished genomes of some mammals and invertebrate organisms has led to catalogues of 19 Wnt genes in the human and the mouse, 7 in *Drosophila*, and 5 in *C. elegans*. Between *Drosophila* and mammals, there is fairly extensive conservation of Wnt genes, so that orthologs can readily be recognized. These orthologous relationships suggest similar biological or biochemical activities, the orthologs Wnt1 and wingless, for example, both regulate the expression of their target gene, *engrailed* (Danielian & McMahon 1996). Between *Drosophila* and vertebrates, there is also conservation of clusters of genes. This indicates that there was a common ancestral cluster of Wnt genes containing *WNT1*, *WNT6*, and *WNT10* that predated the last common ancestor of arthropods and deuterostomes (Nusse 2001, Prud'homme et al. 2002).

Members of the Cnidaria, which are primitive diploblasts, contain a bona fide Wnt and a complete set of Wnt pathway genes (Hobmayer et al. 2000). In another member of the Cnidaria family, the Anemone *Nematostella*, a β-catenin homolog has been shown to be involved in Axis specification and the formation of endoderm (Wikramanayake et al. 2003). Sponges have a Fz gene (Adell et al. 2003), providing another striking example of the conservation of Wnt signaling pathway components throughout evolution.

In yet other primitive organisms, components of the pathway are present, but not necessarily regulated by a Wnt signal. *Dictyostelium* has a vestige of a Wnt pathway, as a gene called *aardvark* is not only a β-catenin homolog (Grimson et al. 2000) but is also regulated by GSK-3 phosphorylation (Grimson et al. 2000). However, there is no evidence for Wnt-like genes in this organism, and although several seven transmembrane molecules act as receptors for cyclic AMP and regulate GSK activity (Plyte et al. 1999), there is no significant homology between those receptors and the Frizzleds. There are clearly recognizable homologs of β-catenin in plants (Amador et al. 2001). It is possible, therefore, that an ancient β-catenin-based mechanism existed prior to the evolution of animals. By adding Wnt and Frizzleds, β-catenin activity became subject to control from other cells, a quintessential aspect of organized multicellular life.

CONCLUDING REMARKS

The past few years have been accompanied by an explosion of data that implicates Wnt signaling in development and in adult tissue maintenance. Given the number of Wnt genes and their widely ranging functions, a large fraction of developmental decisions during the lifetime of an animal may be influenced by a Wnt signal. It is not surprising that mis-regulation of such an important pathway leads to disease, and the role of Wnt signaling in cancer is now well established. Whereas we have

an increasingly detailed picture of Wnt signaling as a complex, tightly regulated pathway with many functions, the mechanisms of several outstanding events during Wnt signal transduction still need to be resolved. These include understanding how Wnts are secreted and presented to cells, how Wnt binding to the Fz/LRP complex transduces a signal to Dsh, how proteins within the β-catenin degradation complex are regulated, and how inputs from positive and negative regulators are integrated within the nucleus to effect transcription. In addition, translating our knowledge of Wnt signaling into some form of intervention for disease is a formidable but important challenge. Fortunately, powerful new experimental methods and reagents for the study of Wnt signaling have recently become available. These include purified Wnt ligands (Willert et al. 2003), small molecules that either activate (Meijer et al. 2003) or inhibit Wnt signaling (Lepourcelet et al. 2004), and RNAi screens for components of the Wnt signaling pathway (Boutros et al. 2004, Lum et al. 2003). The quest for additional tools to manipulate this pathway, to a large extent driven by the potential use of these reagents in managing disease, will lead to further insights into the complexity and intricacy of the Wnt signal transduction cascade.

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LITERATURE CITED

Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. 1997. beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* 16:3797–804

Adell T, Nefkens I, Muller WE. 2003. Polarity factor 'Frizzled' in the demosponge *Suberites domuncula*: identification, expression and localization of the receptor in the epithelium/pinacoderm(1). *FEBS Lett.* 554:363–68

Amador V, Monte E, Garcia-Martinez JL, Prat S. 2001. Gibberellins signal nuclear import of PHOR1, a photoperiod-responsive protein with homology to *Drosophila* armadillo. *Cell* 106:343–54

Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, et al. 2002. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev.* 16:1066–76

Aulehla A, Wehrle C, Brand-Saberi B, Kemler R, Gossler A, et al. 2003. Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev. Cell* 4:395–406

Baeg GH, Lin X, Khare N, Baumgartner S, Perrimon N. 2001. Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* 128:87–94

Bafico A, Gazit A, Pramila T, Finch PW, Yaniv

A, Aaronson SA. 1999. Interaction of frizzled related protein (FRP) with Wnt ligands and the frizzled receptor suggests alternative mechanisms for FRP inhibition of Wnt signaling. *J. Biol. Chem.* 274:16180–87

Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. 2001. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nat. Cell Biol.* 3:683–86

Barker N, Hurlstone A, Musisi H, Miles A, Bienz M, Clevers H. 2001. The chromatin remodelling factor Brg-1 interacts with beta-catenin to promote target gene activation. *EMBO J.* 20:4935–43

Barrow JR, Thomas KR, Boussadia-Zahui O, Moore R, Kemler R, et al. 2003. Ectodermal Wnt3/beta-catenin signaling is required for the establishment and maintenance of the apical ectodermal ridge. *Genes Dev.* 17:394–409

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, et al. 1996. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 382:638–42

Bhanot P, Brink M, Harryman Samos C, Hsieh JC, Wang YS, et al. 1996. A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* 382:225–30

Bhanot P, Fish M, Jemison J, Nusse R, Nathans J, Cadigan K. 1999. Frizzled and DFrizzled-2 function as redundant receptors for Wingless during *Drosophila* embryonic development. *Development* 126:4175–86

Bienz M. 2002. The subcellular destinations of APC proteins. *Nat. Rev. Mol. Cell Biol.* 3: 328–38

Boutros M, Kiger AA, Armknecht S, Kerr K, Hild M, et al. 2004. Genome-wide RNAi analysis of growth and viability in *Drosophila* cells. *Science* 303:832–35

Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, et al. 2002. High bone density due to a mutation in LDL-receptor-related protein 5. *N. Engl. J. Med.* 346:1513–21

Brannon M, Gomperts M, Sumoy L, Moon R, Kimelman D. 1997. A beta-catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification. *Genes Dev.* 11:2359–70

Cadigan K, Nusse R. 1997. Wnt signaling: a common theme in animal development. *Genes Dev.* 11:3286–305

Cadigan KM, Fish MP, Rulifson EJ, Nusse R. 1998. Wingless repression of *Drosophila* frizzled 2 expression shapes the Wingless morphogen gradient in the wing. *Cell* 93: 767–77

Cavallo R, Cox R, Moline M, Roose J, Polevoy G, et al. 1998. *Drosophila* TCF and Groucho interact to repress wingless signaling activity. *Nature* 395:604–8

Chamoun Z, Mann RK, Nellen D, von Kessler DP, Bellotto M, et al. 2001. Skinny hedgehog, an acyltransferase required for palmitoylation and activity of the hedgehog signal. *Science* 293:2080–84

Chen CM, Struhl G. 1999. Wingless transduction by the Frizzled and Frizzled2 proteins of *Drosophila*. *Development* 126:5441–52

Chen G, Fernandez J, Mische S, Courey AJ. 1999. A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in *Drosophila* development. *Genes Dev.* 13:2218–30

Chen W, ten Berge D, Brown J, Ahn S, Hu LA, et al. 2003. Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. *Science* 301:1391–94

Cliffe A, Hamada F, Bienz M. 2003. A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling. *Curr. Biol.* 13:960–66

Cox RT, Pai LM, Miller JR, Orsulic S, Stein J, et al. 1999. Membrane-tethered *Drosophila* Armadillo cannot transduce Wingless signal on its own. *Development* 126:1327–35

Culi J, Mann RS. 2003. Boca, an endoplasmic reticulum protein required for wingless signaling and trafficking of LDL receptor family members in *Drosophila*. *Cell* 112:343–54

Danielian PS, McMahon AP. 1996. Engrailed-1 as a target of the Wnt-1 signalling pathway in vertebrate midbrain development. *Nature* 383:332–34

Daniels DL, Weis WI. 2002. ICAT inhibits beta-catenin binding to Tcf/Lef-family transcription factors and the general coactivator p300 using independent structural modules. *Mol. Cell.* 10:573–84

Dann CE, Hsieh JC, Rattner A, Sharma D, Nathans J, Leahy DJ. 2001. Insights into Wnt binding and signalling from the structures of two Frizzled cysteine-rich domains. *Nature* 412:86–90

Dennis S, Aikawa M, Szeto W, d'Amore PA, Papkoff J. 1999. A secreted frizzled related protein, FrzA, selectively associates with Wnt-1 protein and regulates wnt-1 signaling. *J. Cell Sci.* 112:3815–20

Galceran J, Farinas I, Depew MJ, Clevers H, Grosschedl R. 1999. Wnt3a$^{-/-}$ like phenotype and limb deficiency in Lef1$^{-/-}$Tcf1$^{-/-}$ mice. *Genes Dev.* 13:709–17

Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. 2000. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* 127:469–82

Gilbert SF, ed. 1991. *A Conceptual History of Modern Embryology*. New York: Plenum. 280 pp.

Giles RH, van Es JH, Clevers H. 2003. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim. Biophys. Acta* 1653:1–24

Giraldez AJ, Cohen SM. 2003. Wingless and Notch signaling provide cell survival cues and control cell proliferation during wing development. *Development* 130:6533–43

Giraldez AJ, Copley RR, Cohen SM. 2002. HSPG modification by the secreted enzyme Notum shapes the Wingless morphogen gradient. *Dev. Cell.* 2:667–76

Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391:357–62

Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, et al. 2001. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107:513–23

Graham TA, Clements WK, Kimelman D, Xu W. 2002. The crystal structure of the beta-catenin/ICAT complex reveals the inhibitory mechanism of ICAT. *Mol. Cell.* 10:563–71

Greco V, Hannus M, Eaton S. 2001. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* 106:633–45

Grimson MJ, Coates JC, Reynolds JP, Shipman M, Blanton RL, Harwood AJ. 2000. Adherens junctions and beta-catenin-mediated cell signalling in a non-metazoan organism. *Nature* 408:727–31

Gumbiner BM. 2000. Regulation of cadherin adhesive activity. *J. Cell Biol.* 148:399–404

Guo N, Hawkins C, Nathans J. 2004. Frizzled6 controls hair patterning in mice. *Proc. Natl. Acad. Sci. USA*. In press

Hall AC, Lucas FR, Salinas PC. 2000. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* 100:525–35

Hart MJ, de los Santos R, Albert IN, Rubinfeld B, Polakis P. 1998. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. *Curr. Biol.* 8:573–81

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, et al. 1998. Identification of c-MYC as a target of the APC pathway. *Science* 281:1509–12

Hecht A, Vleminckx K, Stemmler MP, van Roy F, Kemler R. 2000. The p300/CBP acetyltransferases function as transcriptional coactivators of beta-catenin in vertebrates. *EMBO J.* 19:1839–50

Hedgepeth CM, Deardorff MA, Rankin K, Klein PS. 1999. Regulation of glycogen synthase kinase 3beta and downstream Wnt signaling by axin. *Mol. Cell. Biol.* 19:7147–57

Heikkila M, Peltoketo H, Leppaluoto J, Ilves M, Vuolteenaho O, Vainio S. 2002. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. *Endocrinology* 143:4358–65

Hoang B, Moos M Jr, Vukicevic S, Luyten FP. 1996. Primary structure and tissue distribution of FRZB, a novel protein related to *Drosophila* frizzled, suggest a role in skeletal

morphogenesis. *J. Biol. Chem.* 271:26131–37

Hobmayer B, Rentzsch F, Kuhn K, Happel CM, von Laue CC, et al. 2000. WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* 407:186–89

Hofmann K. 2000. A superfamily of membrane-bound *O*-acyltransferases with implications for wnt signaling. *Trends Biochem. Sci.* 25:111–12

Hovanes K, Li TW, Munguia JE, Truong T, Milovanovic T, et al. 2001. Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nat. Genet.* 28:53–57

Hsieh JC, Kodjabachian L, Rebbert ML, Rattner A, Smallwood PM, et al. 1999a. A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* 398:431–36

Hsieh JC, Lee L, Zhang L, Wefer S, Brown K, et al. 2003. Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* 112:355–67

Hsieh JC, Rattner A, Smallwood PM, Nathans J. 1999b. Biochemical characterization of Wnt-frizzled interactions using a soluble, biologically active vertebrate Wnt protein. *Proc. Natl. Acad. Sci. USA* 96:3546–51

Hsu W, Zeng L, Costantini F. 1999. Identification of a domain of Axin that binds to the serine/threonine protein phosphatase 2A and a self-binding domain. *J. Biol. Chem.* 274:3439–45

Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. 1997. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 389:966–70

Ishikawa T, Tamai Y, Zorn AM, Yoshida H, Seldin MF, et al. 2001. Mouse Wnt receptor gene Fzd5 is essential for yolk sac and placental angiogenesis. *Development* 128:25–33

Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, et al. 1999. The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. *Nature* 399:798–802

Ishitani T, Ninomiya-Tsuji J, Matsumoto K. 2003. Regulation of lymphoid enhancer factor 1/T-cell factor by mitogen-activated protein kinase-related Nemo-like kinase-dependent phosphorylation in Wnt/β-catenin signaling. *Mol. Cell Biol.* 23:1379–89

Itasaki N, Jones CM, Mercurio S, Rowe A, Domingos PM, et al. 2003. Wise, a context-dependent activator and inhibitor of Wnt signalling. *Development* 130:4295–305

Itoh K, Antipova A, Ratcliffe MJ, Sokol S. 2000. Interaction of Dishevelled and *Xenopus* Axin related protein is required for Wnt signal transduction. *Mol. Cell Biol.* 20:2228–38

Jamora C, DasGupta R, Kocieniewski P, Fuchs E. 2003. Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature* 422:317–22

Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. 2002. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol. Cell Biol.* 22:1172–83

Jonkers J, Korswagen HC, Acton D, Breuer M, Berns A. 1997. Activation of a novel proto-oncogene, Frat1, contributes to progression of mouse T-cell lymphomas. *EMBO J.* 16:441–50

Kadowaki T, Wilder E, Klingensmith J, Zachary K, Perrimon N. 1996. The segment polarity gene porcupine encodes a putative multi-transmembrane protein involved in Wingless processing. *Genes Dev.* 10:3116–28

Kennerdell JR, Carthew RW. 1998. Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* 95:1017–26

Kim CH, Oda T, Itoh M, Jiang D, Artinger KB, et al. 2000. Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* 407:913–16

Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, et al. 1991. Identification of FAP locus genes from chromosome 5q21. *Science* 253:661–65

Kioussi C, Briata P, Baek SH, Rose DW, Hamblet NS, et al. 2002. Identification of a Wnt/Dvl/beta-Catenin → Pitx2 pathway mediating cell-type-specific proliferation during development. *Cell* 111:673–85

Kishida S, Yamamoto H, Ikeda S, Kishida M, Sakamoto I, et al. 1998. Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. *J. Biol. Chem.* 273:10823–26

Kispert A, Vainio S, McMahon AP. 1998. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development* 125:4225–34

Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, et al. 1998. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* 19:379–83

Kramps T, Peter O, Brunner E, Nellen D, Froesch B, et al. 2002. Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. *Cell* 109:47–60

Krupnik VE, Sharp JD, Jiang C, Robison K, Chickering TW, et al. 1999. Functional and structural diversity of the human Dickkopf gene family. *Gene* 238:301–13

Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, et al. 2004. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc. Natl. Acad. Sci. USA* 101:266–71

Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, et al. 2004. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am. J. Hum. Genet.* 74: 1043–50

Latres E, Chiaur DS, Pagano M. 1999. The human F box protein beta-Trcp associates with the Cul1/Skp1 complex and regulates the stability of beta-catenin. *Oncogene* 18:849–54

Lee E, Salic A, Kruger R, Heinrich R, Kirschner MW. 2003. The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol.* 1:E10

Lee J, Ishimoto A, Yanagawa S. 1999. Characterization of mouse dishevelled (Dvl) proteins in Wnt/Wingless signaling pathway. *J. Biol. Chem.* 274:21464–70

Lee SM, Tole S, Grove E, McMahon AP. 2000. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127:457–67

Lepourcelet M, Chen YN, France DS, Wang H, Crews P, et al. 2004. Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex. *Cancer Cell.* 5:91–102

Leyns L, Bouwmeester T, Kim SH, Piccolo S, De Robertis EM. 1997. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88:747–56

Li C, Xiao J, Hormi K, Borok Z, Minoo P. 2002. Wnt5a participates in distal lung morphogenesis. *Dev. Biol.* 248:68–81

Li L, Yuan H, Weaver CD, Mao J, Farr GH 3rd, et al. 1999. Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. *EMBO J.* 18:4233–40

Liang H, Chen Q, Coles AH, Anderson SJ, Pihan G, et al. 2003. Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue. *Cancer Cell* 4:349–60

Lin X, Perrimon N. 1999. Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling. *Nature* 400:281–84

Lin X, Perrimon N. 2000. Role of heparan sulfate proteoglycans in cell-cell signaling in *Drosophila*. *Matrix Biol.* 19:303–7

Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, et al. 2002. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am. J. Hum. Genet.* 70:11–19

Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X. 1999. beta-Trcp couples beta-catenin phosphorylation-degradation and regulates *Xenopus* axis formation. *Proc. Natl. Acad. Sci. USA* 96:6273–78

Liu C, Li Y, Semenov M, Han C, Baeg GH,

et al. 2002. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 108:837–47

Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. 1999. Requirement for Wnt3 in vertebrate axis formation. *Nat. Genet.* 22:361–65

Liu T, DeCostanzo AJ, Liu X, Wang H, Hallagan S, et al. 2001. G protein signaling from activated rat frizzled-1 to the beta-catenin-Lef-Tcf pathway. *Science* 292:1718–22

Liu X, Liu T, Slusarski DC, Yang-Snyder J, Malbon CC, et al. 1999. Activation of a frizzled-2/beta-adrenergic receptor chimera promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via Gαo and Gαt. *Proc. Natl. Acad. Sci. USA* 96:14383–88

Lo M-C, Gay F, Odom R, Shi Y, Lin R. 2004. A 14–3–3 protein, PAR-5, mediates nuclear export of POP-1 in Wnt/MAP kinase responsive cells in *C. elegans* embryos. *Cell* 117:95–106

Lum L, Yao S, Mozer B, Rovescalli A, Von Kessler D, et al. 2003. Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. *Science* 299:2039–45

Lyuksyutova AI, Lu CC, Milanesio N, King LA, Guo N, et al. 2003. Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* 302:1984–88

Majumdar A, Vainio S, Kispert A, McMahon J, McMahon AP. 2003. Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. *Development* 130:3175–85

Mao B, Niehrs C. 2003. Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling. *Gene* 302:179–83

Mao B, Wu W, Davidson G, Marhold J, Li M, et al. 2002. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature* 417:664–67

Mao B, Wu W, Li Y, Hoppe D, Stannek P, et al. 2001a. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 411:321–25

Mao J, Wang J, Liu B, Pan W, Farr GH 3rd, et al. 2001b. Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol. Cell* 7:801–9

Megason SG, McMahon AP. 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* 129:2087–98

Meijer L, Skaltsounis AL, Magiatis P, Polychronopoulos P, Knockaert M, et al. 2003. GSK-3-selective inhibitors derived from Tyrian purple indirubins. *Chem. Biol.* 10:1255–66

Meneghini MD, Ishitani T, Carter JC, Hisamoto N, Ninomiya-Tsuji J, et al. 1999. MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* 399:793–97

Merrill BJ, Pasolli HA, Polak L, Rendl M, Garcia-Garcia MJ, et al. 2004. Tcf3: a transcriptional regulator of axis induction in the early embryo. *Development* 131:263–74

Miller JR, Moon RT. 1997. Analysis of the signaling activities of localization mutants of beta-catenin during axis specification in *Xenopus*. *J. Cell Biol.* 139:229–43

Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, et al. 1996. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell* 86:391–99

Monaghan AP, Kioschis P, Wu W, Zuniga A, Bock D, et al. 1999. Dickkopf genes are co-ordinately expressed in mesodermal lineages. *Mech. Dev.* 87:45–56

Muller H, Samanta R, Wieschaus E. 1999. Wingless signaling in the *Drosophila* embryo: zygotic requirements and the role of the frizzled genes. *Development* 126:577–86

Mulroy T, McMahon JA, Burakoff SJ, McMahon AP, Sen J. 2002. Wnt-1 and Wnt-4 regulate thymic cellularity. *Eur. J. Immunol.* 32:967–71

Munemitsu S, Albert I, Rubinfeld B, Polakis P. 1996. Deletion of an amino-terminal sequence beta-catenin in vivo and promotes hyperphosporylation of the adenomatous

polyposis coli tumor suppressor protein. *Mol. Cell Biol.* 16:4088–94

Nelson W, Nusse R. 2004. Convergence of Wnt, β-catenin and cadherin pathways. *Science* 303:1483–87

Niemann S, Zhao C, Pascu F, Stahl U, Aulepp U, et al. 2004. Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. *Am. J. Hum. Genet.* 74:558–63

Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, et al. 1991. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 253:665–69

Noordermeer J, Klingensmith J, Nusse R. 1995. Differential requirements for segment polarity genes in wingless signaling. *Mech. Dev.* 51:145–55

Noordermeer J, Klingensmith J, Perrimon N, Nusse R. 1994. dishevelled and armadillo act in the wingless signalling pathway in *Drosophila*. *Nature* 367:80–83

Nusse R. 2001. An ancient cluster of Wnt paralogues. *Trends Genet.* 17:443

Packard M, Koo ES, Gorczyca M, Sharpe J, Cumberledge S, Budnik V. 2002. The *Drosophila* Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. *Cell* 111:319–30

Parker DS, Jemison J, Cadigan KM. 2002. Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* 129:2565–76

Parr BA, Cornish VA, Cybulsky MI, McMahon AP. 2001. Wnt7b regulates placental development in mice. *Dev. Biol.* 237:324–32

Parr BA, McMahon AP. 1998. Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. *Nature* 395:707–10

Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. 2000. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* 407:535–38

Pinto D, Gregorieff A, Begthel H, Clevers H. 2003. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev.* 17:1709–13

Plyte SE, O'Donovan E, Woodgett JR, Harwood AJ. 1999. Glycogen synthase kinase-3 (GSK-3) is regulated during *Dictyostelium* development via the serpentine receptor cAR3. *Development* 126:325–33

Prud'homme B, Lartillot N, Balavoine G, Adoutte A, Vervoort M. 2002. Phylogenetic analysis of the Wnt gene family. Insights from lophotrochozoan members. *Curr. Biol.* 12:1395

Ramirez-Weber FA, Kornberg TB. 1999. Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* 97:599–607

Rattner A, Hsieh JC, Smallwood PM, Gilbert DJ, Copeland NG, et al. 1997. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc. Natl. Acad. Sci. USA* 94: 2859–63

Reya T, O'Riordan M, Okamura R, Devaney E, Willert K, et al. 2000. Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* 13:15–24

Robitaille J, MacDonald ML, Kaykas A, Sheldahl LC, Zeisler J, et al. 2002. Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat. Genet.* 32:326–30

Rocheleau CE, Downs WD, Lin R, Wittmann C, Bei Y, et al. 1997. Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* 90:707–16

Roose J, Huls G, van Beest M, Moerer P, van der Horn K, et al. 1999. Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. *Science* 285:1923–26

Rousset R, Mack JA, Wharton KA Jr, Axelrod JD, Cadigan KM, et al. 2001. Naked cuticle targets dishevelled to antagonize Wnt signal transduction. *Genes Dev.* 15:658–71

Rulifson E, Wu C-H, Nusse R. 2000. Pathway specificity by the bifunctional receptor Frizzled is determined by affinity for Wingless. *Mol. Cell* 6:117–26

Sakanaka C, Williams LT. 1999. Functional domains of axin. *J. Biol. Chem.* 274:14090–93

Salic A, Lee E, Mayer L, Kirschner MW. 2000. Control of beta-catenin stability: reconstitution of the cytoplasmic steps of the wnt pathway in *Xenopus* egg extracts. *Mol. Cell.* 5:523–32

Salic AN, Kroll KL, Evans LM, Kirschner MW. 1997. Sizzled: a secreted Xwnt8 antagonist expressed in the ventral marginal zone of *Xenopus* embryos. *Development* 124:4739–48

Sancho E, Batlle E, Clevers H. 2004. Signaling pathways in intestinal development in cancer. *Annu. Rev. Cell Dev. Biol.* 20: In press

Sato A, Kojima T, Ui-Tei K, Miyata Y, Saigo K. 1999. Dfrizzled-3, a new *Drosophila* Wnt receptor, acting as an attenuator of Wingless signaling in wingless hypomorphic mutants. *Development* 126:4421–30

Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, et al. 2000. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat. Genet.* 24:245–50

Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X. 2001. Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr. Biol.* 11:951–61

Shimizu H, Julius MA, Giarre M, Zheng Z, Brown AM, Kitajewski J. 1997. Transformation by Wnt family proteins correlates with regulation of beta-catenin. *Cell Growth Differ.* 8:1349–58

Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, et al. 1999. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc. Natl. Acad. Sci. USA* 96: 5522–27

Shu W, Jiang YQ, Lu MM, Morrisey EE. 2002. Wnt7b regulates mesenchymal proliferation and vascular development in the lung. *Development* 129:4831–42

Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY. 2000. Wnt/beta-catenin signaling induces the expression and activity of betaTrCP ubiquitin ligase receptor. *Mol. Cell.* 5:877–82

Spink KE, Polakis P, Weis WI. 2000. Structural basis of the Axin-adenomatous polyposis coli interaction. *EMBO J.* 19:2270–79

Strigini M, Cohen SM. 2000. Wingless gradient formation in the *Drosophila* wing. *Curr. Biol.* 10:293–300

Strutt D. 2003. Frizzled signalling and cell polarisation in *Drosophila* and vertebrates. *Development* 130:4501–13

Sun TQ, Lu B, Feng JJ, Reinhard C, Jan YN, et al. 2001. PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. *Nat. Cell Biol.* 3:628–36

Tago K, Nakamura T, Nishita M, Hyodo J, Nagai S, et al. 2000. Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. *Genes Dev.* 14:1741–49

Taipale J, Beachy PA. 2001. The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411:349–54

Takemaru K, Yamaguchi S, Lee YS, Zhang Y, Carthew RW, Moon RT. 2003. Chibby, a nuclear beta-catenin-associated antagonist of the Wnt/Wingless pathway. *Nature* 422:905–9

Takemaru KI, Moon RT. 2000. The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. *J. Cell Biol.* 149:249–54

Tamai K, Semenov M, Kato Y, Spokony R, Liu C, et al. 2000. LDL-receptor-related proteins in Wnt signal transduction. *Nature* 407:530–35

Tamai K, Zeng X, Liu C, Zhang X, Harada Y, et al. 2004. A mechanism for Wnt coreceptor activation. *Mol. Cell* 13:149–56

Tetsu O, McCormick F. 1999. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398:422–26

Thompson B, Townsley F, Rosin-Arbesfeld R, Musisi H, Bienz M. 2002. A new nuclear component of the Wnt signalling pathway. *Nat. Cell Biol.* 4:367–73

Tolwinski NS, Wehrli M, Rives A, Erdeniz N, DiNardo S, Wieschaus E. 2003. Wg/Wnt signal can be transmitted through arrow/LRP5,6 and Axin independently of Zw3/Gsk3beta activity. *Dev. Cell* 4:407–18

Tolwinski NS, Wieschaus E. 2004. A nuclear

function for Armadillo/beta-Catenin. *PLoS Biol* 2:486–93

Torres MA, Yang-Snyder JA, Purcell SM, De-Marais AA, McGrew LL, Moon RT. 1996. Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. *J. Cell Biol.* 133:1123–37

Toomes C, Bottomley, HM, Jackson, RM, Towns KV, Scott S, et al. 2004. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am. J. Hum. Genet.* 74:721–30

Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, et al. 1999. The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. *Nature* 400:276–80

Umbhauer M, Djiane A, Goisset C, Penzo-Mendez A, Riou JF, et al. 2000. The C-terminal cytoplasmic Lys-thr-X-X-X-Trp motif in frizzled receptors mediates Wnt/betacatenin signalling. *EMBO J.* 19:4944–54

Uren A, Reichsman F, Anest V, Taylor WG, Muraiso K, et al. 2000. Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J. Biol. Chem.* 275:4374–82

Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. 1999. Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397:405–9

van de Wetering M, Cavallo R, Dooijes D, van Beest M, van Es J, et al. 1997. Armadillo coactivates transcription driven by the product of the *Drosophila*segment polarity gene *dTCF*. *Cell* 88:789–99

van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, et al. 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev.* 8:2691–703

Veeman MT, Axelrod JD, Moon RT. 2003. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev. Cell* 5:367–77

Verbeek S, Izon D, Hofhuis F, Robanus-Maandag E, te Riele H, et al. 1995. An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* 374:70–74

Waltzer L, Bienz M. 1998. *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* 395: 521–25

Wang S, Krinks M, Lin K, Luyten FP, Moos M Jr. 1997. Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* 88:757–66

Wang Y, Huso D, Cahill H, Ryugo D, Nathans J. 2001. Progressive cerebellar, auditory, and esophageal dysfunction caused by targeted disruption of the frizzled-4 gene. *J. Neurosci.* 21:4761–71

Wang Y, Thekdi N, Smallwood PM, Macke JP, Nathans J. 2002. Frizzled-3 is required for the development of major fiber tracts in the rostral CNS. *J. Neurosci.* 22:8563–73

Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, et al. 2000. arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 407:527–30

Wharton KA, Jr., Zimmermann G, Rousset R, Scott MP. 2001. Vertebrate proteins related to *Drosophila* Naked Cuticle bind Dishevelled and antagonize Wnt signaling. *Dev. Biol.* 234:93–106

Wikramanayake AH, Hong M, Lee PN, Pang K, Byrum CA, et al. 2003. An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. *Nature* 426:446–50

Willert J, Epping M, Pollack J, Brown P, Nusse R. 2002. A transcriptional response to Wnt protein in human embryonic carcinoma cells. *Dev. Biol.* 2:8

Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, et al. 2003. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 423:448–52

Wong HC, Bourdelas A, Krauss A, Lee HJ, Shao Y, et al. 2003. Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. *Mol. Cell.* 12:1251–60

Wu CH, Nusse R. 2002. Ligand receptor interactions in the WNT signaling pathway in *Drosophila*. *J. Biol. Chem.* 277:41762–69

Xing Y, Clements WK, Kimelman D, Xu W. 2003. Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. *Genes Dev.* 17:2753–64

Xu Q, Wang Y, Dabdoub A, Smallwood PM, Williams J, et al. 2004. Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* 116:883–95

Yamaguchi TP, Bradley A, McMahon AP, Jones S. 1999. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 126:1211–23

Yanagawa S, Matsuda Y, Lee JS, Matsubayashi H, Sese S, et al. 2002. Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*. *EMBO J.* 21:1733–42

Yanagawa S, van Leeuwen F, Wodarz A, Klingensmith J, Nusse R. 1995. The dishevelled protein is modified by wingless signaling in *Drosophila*. *Genes Dev.* 1:1087–97

Yang J, Wu J, Tan C, Klein PS. 2003. PP2A:B56epsilon is required for Wnt/beta-catenin signaling during embryonic development. *Development* 130:5569–78

Yang Y, Topol L, Lee H, Wu J. 2003. Wnt5a and Wnt5b exhibit distinct activities in coordinating chondrocyte proliferation and differentiation. *Development* 130:1003–15

Yoshikawa S, Bonkowsky JL, Kokel M, Shyn S, Thomas JB. 2001. The derailed guidance receptor does not require kinase activity in vivo. *J. Neurosci.* 21:RC119

Yoshikawa S, McKinnon RD, Kokel M, Thomas JB. 2003. Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. *Nature* 422:583–88

Yoshikawa Y, Fujimori T, McMahon AP, Takada S. 1997. Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev. Biol.* 183:234–42

Yost C, Farr GH 3rd, Pierce SB, Ferkey DM, Chen MM, Kimelman D. 1998. GBP, an inhibitor of GSK-3, is implicated in *Xenopus* development and oncogenesis. *Cell* 93:1031–41

Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT. 1996. The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* 10:1443–54

Zecca M, Basler K, Struhl G. 1996. Direct and long-range action of a wingless morphogen gradient. *Cell* 87:833–44

Zhai L, Chaturvedi D, Cumberledge S. 2004. *Drosophila* Wnt-1 undergoes a hydrophobic modification and is targeted to lipid rafts; a process that requires Porcupine. *J. Biol. Cell.* In press