

# Wnt/ $\beta$ -Catenin Signaling in Development and Disease

Hans Clevers<sup>1,\*</sup>

<sup>1</sup>Hubrecht Laboratory and Utrecht University, Uppsalalaan 8, 3584CT, Utrecht, the Netherlands

\*Contact: clevers@niob.knaw.nl

DOI 10.1016/j.cell.2006.10.018

A remarkable interdisciplinary effort has unraveled the WNT (*Wingless* and *INT-1*) signal transduction cascade over the last two decades. *Wnt* genes encode small secreted proteins that are found in all animal genomes. Wnt signaling is involved in virtually every aspect of embryonic development and also controls homeostatic self-renewal in a number of adult tissues. Germline mutations in the Wnt pathway cause several hereditary diseases, and somatic mutations are associated with cancer of the intestine and a variety of other tissues.

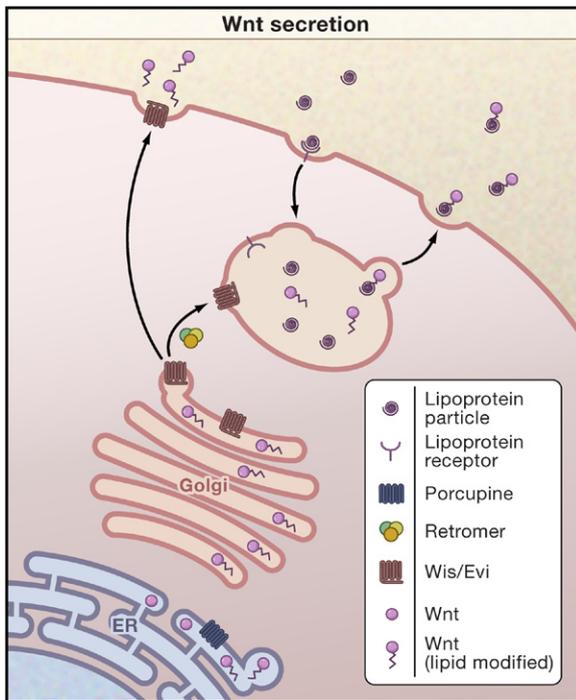
The mouse *wnt1* gene, originally named *Int-1*, was identified in 1982 by Nusse and Varmus as a preferential integration site for the Mouse Mammary Tumor Virus in virally induced breast tumors (Nusse and Varmus, 1982). When sequenced, the *Wnt1* proto-oncogene was seen to encode a secreted protein that is cysteine rich. Subsequently, *Drosophila wingless* (*wg*), which controls segment polarity during larval development (Nüsslein-Volhard and Wieschaus, 1980), was shown to be a fly homolog of *Wnt1* (Rijsewijk et al., 1987). Segmentation of the epidermis of *wg* mutant fly embryos is severely impaired as evidenced by abnormalities in the overlying ventral cuticle. In contrast to the wild-type cuticle, which exhibits alternating denticle and naked belts, the *wg* cuticle is completely covered with denticles. Fly embryos carrying mutations in the *porcupine*, *dishevelled*, and *armadillo* genes display similar cuticle abnormalities to *wg* mutant embryos, whereas mutations in *shaggy/zeste-white 3* cause the opposite phenotype, a naked cuticle. Epistatic analysis of cuticle structure in double mutants indicated that these genes constituted the core of a new signal transduction cascade (Siegfried et al., 1992; Noordermeer et al., 1994; Peifer et al., 1994).

In 1989, McMahon and Moon (McMahon and Moon, 1989) observed a duplication of the body axis in *Xenopus* following injection of mouse *Wnt1* mRNA into ventral blastomeres of embryos at the 4-cell stage. This observation supported the notion that Wnt signaling was shared between vertebrates and invertebrates and, moreover, provided a rapid and convenient assay to study components of the Wnt pathway in vertebrates. Axis duplication was also induced by Dishevelled (Dsh),  $\beta$ -catenin (the vertebrate homolog of *armadillo*), and a dominant-negative version of glycogen synthase kinase 3 (GSK3), the vertebrate homolog of *shaggy/zeste-white 3* (Dominguez et al., 1995; Guger and Gumbiner, 1995; He et al., 1995). Although long elusive, the specific Wnt signal that triggers axis induction in *Xenopus* was identified as Wnt11 by Heasman and colleagues last year (Tao et al., 2005).

The combined observations made in *Drosophila* and *Xenopus* delineated a highly conserved signaling pathway, activated by secreted Wnt proteins. Independent of these studies, the *adenomatous polyposis coli* (*APC*) gene was discovered in a hereditary cancer syndrome termed familial adenomatous polyposis (FAP) (Kinzler et al., 1991; Nishisho et al., 1991). Soon after, the large cytoplasmic APC protein was found to interact with  $\beta$ -catenin (Rubinfeld et al., 1993; Su et al., 1993). This observation provided the first connection between the Wnt pathway and human cancer.

Genome sequencing has since revealed that mammalian species have roughly 20 secreted Wnt proteins, which can be divided into 12 conserved Wnt subfamilies. Of these, only 6 subfamilies have counterparts in ecdysozoan animals such as *Drosophila* and *Caenorhabditis*. In contrast, at least 11 of the Wnt subfamilies occur in the genome of a cnidarian (the sea anemone *Nematostella vectensis*). This finding suggests that some Wnt subfamilies were lost during the evolution of the ecdysozoan lineage but more importantly reveals that a complex inventory of Wnt factors was present in multicellular animals well before the Cambrian explosion (550 million years ago). Thus, comparative genomic analysis underscores the crucial role that *Wnt* genes play in organismal patterning throughout the animal kingdom (Kusserow et al., 2005).

Currently, three different pathways are believed to be activated upon Wnt receptor activation: the canonical Wnt/ $\beta$ -catenin cascade, the noncanonical planar cell polarity (PCP) pathway, and the Wnt/ $\text{Ca}^{2+}$  pathway. Of these three, the canonical pathway is best understood and is the primary subject of this review. For recent comprehensive overviews on the other Wnt signaling pathways, the reader is referred to Katoh (2005) and Kohn and Moon (2005). This review discusses how Wnt proteins are produced and secreted and how they activate the canonical Wnt signaling pathway in recipient cells. Further, the review examines the roles of the canonical Wnt pathway in development, tissue self-renewal, and cancer.



**Figure 1. Wnt Secretion**

To be secreted, Wnt proteins in the endoplasmic reticulum (ER) need to be palmitoylated by the action of Porcupine. Wnt proteins also require Wntless (Wls/Evi) in order to be routed to the outside of the cell. Loading onto lipoprotein particles may occur in a dedicated endo/exocytic compartment. The retromer complex may shuttle Wls between the Golgi and the endo/exocytic compartment.

### Wnt Protein Secretion

Wnt proteins are characterized by a high number of conserved cysteine residues. Although Wnt proteins carry an N-terminal signal peptide and are secreted, they are relatively insoluble. This insolubility has been attributed to a particular protein modification, cysteine palmitoylation, which is essential for Wnt function (Willert et al., 2003). Hofmann (2000) reported that a *Drosophila* gene required in the Wnt-secreting cell, termed *porcupine*, displays homology to acyl-transferases, enzymes that acylate a variety of substrates in the endoplasmic reticulum. Thus, *porcupine* and its worm homolog *mom-1* are believed to encode the enzyme that is responsible for Wnt palmitoylation (Zhai et al., 2004).

Recently, Banziger et al. (2006) and Bartscherer et al. (2006) uncovered in *Drosophila* another conserved gene that is essential for Wnt secretion, named *wntless* (*wls*) and *evenness interrupted* (*evi*), respectively. The gene encodes a seven-pass transmembrane protein that is conserved from worms (*mom-3*) to man (*hWLS*). In the absence of *Wls/evi*, Wnts are retained inside the cell that produces them. The Wntless protein resides primarily in the Golgi apparatus, where it colocalizes and physically interacts with Wnts. A genetic screen in *C. elegans* revealed that the retromer, a multiprotein complex involved in intracellular trafficking and conserved

from yeast to man, is also essential for Wnt secretion and for the generation of a Wnt gradient (Coudreuse et al., 2006). An attractive hypothesis is that the retromer complex is involved in recycling a Wnt cargo receptor (such as Wntless) between the default secretory pathway and a compartment dedicated to Wnt secretion (see Figure 1).

Wnt is thought to act as a morphogen (that is, a long-range signal whose activity is concentration dependent) (reviewed in Logan and Nusse, 2004). However, it is unclear how these long-range gradients are generated. It is conceivable that the palmitoyl moiety constrains movement away from membranes or lipid particles. Thus, Wnts may be tethered to intercellular transport vesicles or lipoprotein particles (Panakova et al., 2005). Alternatively, Wnts may be transported by cytonemes, which are long, thin filopodial processes. Additionally, studies in *Drosophila* suggest a role for extracellular heparan sulfate proteoglycans (HSPG) in the transport or stabilization of Wnt proteins. For instance, flies carrying mutations in Dally, a GPI-anchored HSPG, or in genes encoding enzymes that modify HSPGs resemble *wingless* mutants (reviewed in Lin, 2004).

### Receptors, Agonists, and Antagonists for Wnt

Wnts bind Frizzled (Fz) proteins, which are seven-pass transmembrane receptors with an extracellular N-terminal cysteine-rich domain (CRD) (Bhanot et al., 1996). The Wnt-Fz interaction appears promiscuous, in that a single Wnt can bind multiple Frizzled proteins (e.g., Bhanot et al., 1996) and vice versa. In binding Wnt, Fzs cooperate with a single-pass transmembrane molecule of the LRP family known as *Arrow* in *Drosophila* (Wehrli et al., 2000) and LRP5 and -6 in vertebrates (Pinson et al., 2000; Tamai et al., 2000). The transport of *Arrow*/LRP5/6 to the cell surface is dependent on a chaperone called *Boca* in *Drosophila* and *Mesd* in mice (Culi and Mann, 2003; Hsieh et al., 2003). And consistent with a role of the *Boca*/*Mesd* chaperone in the transport of *Arrow*/LRP5/6 transport, mutations in *Boca* and *Mesd* resemble loss of *Arrow*/LRP5/6. Although it has not been formally demonstrated that Wnt molecules form trimeric complexes with LRP5/6 and Frizzled, surface expression of both receptors is required to initiate the Wnt signal.

Derailed, a transmembrane tyrosine kinase receptor from the RYK subfamily, is an unusual Wnt receptor. *Drosophila* Wnt5 controls axon guidance in the central nervous system. Embryos lacking *Dwnt-5* resemble those lacking *Derailed*, that is, they generate aberrant neuronal projections across the midline (Yoshikawa et al., 2003). *Derailed* binds DWnt-5 through its extracellular WIF (Wnt inhibitory factor) domain. Signaling events downstream of this alternative Wnt receptor remain unclear. Somewhat unexpectedly, the *Derailed* kinase domain may be dispensable for signaling. Lu et al. (2004) propose that, unlike the *Drosophila* Ryk homolog *Derailed*, mammalian Ryk functions as a coreceptor along with Fz. Mammalian

Ryk binds Dishevelled to activate the canonical Wnt/ $\beta$ -catenin signaling pathway. Another tyrosine kinase receptor, Ror2, harbors a Wnt binding CRD motif. Wnt5a can engage Ror2 to inhibit the canonical Wnt signaling pathway, although paradoxically Wnt5a can also activate the canonical pathway by directly engaging Fz4 (Mikels and Nusse, 2006) and Fz5 (He et al., 1997).

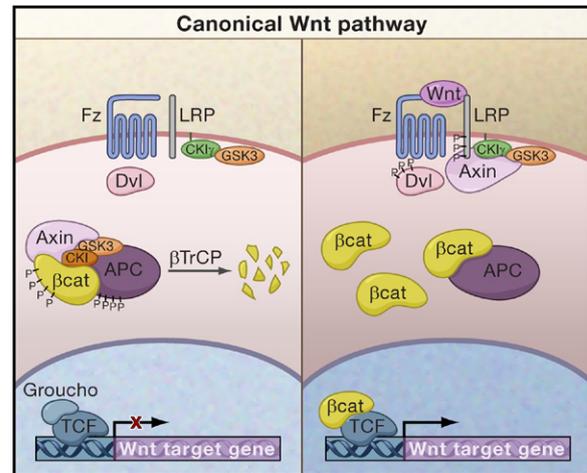
At least two types of proteins that are unrelated to Wnt factors activate the Frizzled/LRP receptors. One of these factors is the cysteine-knot protein Norrin, which is mutated in Norrie disease, a developmental disorder characterized by vascular abnormalities in the eye and blindness. Norrin binds with high affinity to Frizzled-4 and activates the canonical signaling pathway in an LRP5/6-dependent fashion (Xu et al., 2004). Other factors that activate the canonical Wnt signaling pathway are R-spondins, which are thrombospondin domain-containing proteins. In *Xenopus*, R-spondin-2 is a Wnt agonist that synergizes with Wnts to activate  $\beta$ -catenin (Kazanskaya et al., 2004). Human R-spondin-1 has been found to strongly promote the proliferation of intestinal crypt cells, a process which involves the stabilization of  $\beta$ -catenin (Kim et al., 2005). Indeed, studies in cultured cells demonstrate that R-spondins can physically interact with the extracellular domains of LRP6 and Fzd8 and activate Wnt reporter genes (Nam et al., 2006).

The secreted Dickkopf (Dkk) proteins inhibit Wnt signaling by direct binding to LRP5/6 (Glinka et al., 1998). Through this interaction, Dkk1 crosslinks LRP6 to another class of transmembrane molecules, the Kremens (Mao et al., 2002), thus promoting the internalization and inactivation of LRP6. An unrelated secreted Wnt inhibitor, Wise, also acts by binding to LRP (Itasaki et al., 2003), as does the WISE family member SOST (Li et al., 2005; Semenov et al., 2005).

Soluble Frizzled-Related Proteins (SFRPs) resemble the ligand-binding CRD domain of the Frizzled family of Wnt receptors (Hoang et al., 1996). WIF proteins are secreted molecules with similarity to the extracellular portion of the Derailed/RYK class of transmembrane Wnt receptors (Hsieh et al., 1999). SFRPs and WIFs are believed to function as extracellular Wnt inhibitors (reviewed in Logan and Nusse, 2004) but, depending on context, may also promote signaling by Wnt stabilization or by facilitating Wnt secretion or transport.

### Canonical Wnt Signaling

Once bound by their cognate ligands, the Fz/LRP coreceptor complex activates the canonical signaling pathway (Figure 2). Fz can physically interact with Dsh, a cytoplasmic protein that functions upstream of  $\beta$ -catenin and the kinase GSK-3. Wnt signaling controls phosphorylation of Dsh (reviewed in Wallingford and Habas, 2005). However, it remains unclear whether the binding of Wnt to Fz regulates a direct Fz-Dsh interaction, nor is it known how Dsh phosphorylation is controlled or how phosphorylated Dsh functions in Wnt signal transduction.



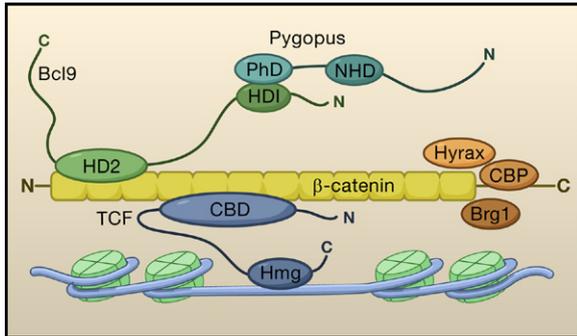
**Figure 2. Canonical Wnt Signaling**

(Left panel) When Wnt receptor complexes are not bound by ligand, the serine/threonine kinases, CK1 and GSK3 $\alpha/\beta$ , phosphorylate  $\beta$ -catenin. Phosphorylated  $\beta$ -catenin is recognized by the F box/WDF repeat protein  $\beta$ -TrCP, a component of a dedicated E3 ubiquitin ligase complex. Following ubiquitination,  $\beta$ -catenin is targeted for rapid destruction by the proteasome. In the nucleus, the binding of Groucho to TCF (T cell factor) inhibits the transcription of Wnt target genes. (Right panel) Once bound by Wnt, the Frizzled(Fz)/LRP coreceptor complex activates the canonical signaling pathway. Fz interacts with Dsh, a cytoplasmic protein that functions upstream of  $\beta$ -catenin and the kinase GSK3 $\beta$ . Wnt signaling controls phosphorylation of Dishevelled (Dsh). Wnts are thought to induce the phosphorylation of LRP by GSK3 $\beta$  and casein kinase I- $\gamma$  (CK1 $\gamma$ ), thus regulating the docking of Axin. The recruitment of Axin away from the destruction complex leads to the stabilization of  $\beta$ -catenin. In the nucleus,  $\beta$ -catenin displaces Groucho from Tcf/Lef to promote the transcription of Wnt target genes.

Recent studies have indicated that the coreceptor LRP5/6 interacts with Axin through five phosphorylated PPP(S/T)P repeats in the cytoplasmic tail of LRP (Davidson et al., 2005; Zeng et al., 2005). Wnts are thought to induce the phosphorylation of the cytoplasmic tail of LRP, thus regulating the docking of Axin. GSK3 phosphorylates the PPP(S/T)P motif, whereas casein kinase I- $\gamma$  (CK1 $\gamma$ ) phosphorylates multiple motifs close to the GSK3 sites. CK1 $\gamma$  is unique within the CK1 family in that it is anchored in the membrane through C-terminal palmitoylation. Both kinases are essential for signal initiation. It remains presently debated whether Wnt controls GSK3-mediated phosphorylation of LRP5/6 (Zeng et al., 2005) or whether CK1 $\gamma$  is the kinase regulated by Wnt (Davidson et al., 2005). When bound to their respective membrane receptors, Dsh and Axin may cooperatively mediate downstream activation events by heterodimerization through their respective DIX (Dishevelled-Axin) domains.

### The Cytoplasmic Destruction Complex

The central player in the canonical Wnt cascade is  $\beta$ -catenin, a cytoplasmic protein whose stability is regulated by the destruction complex. The tumor suppressor protein Axin acts as the scaffold of this complex as it directly interacts with all other components— $\beta$ -catenin, the tumor suppressor protein APC, and the two kinase



**Figure 3. Transactivation of Wnt Target Genes**

The  $\beta$ -catenin/Tcf complex interacts with a variety of chromatin-remodeling complexes to activate transcription of Wnt target genes. The recruitment of  $\beta$ -catenin to Tcf target genes affects local chromatin in several ways. Bcl9 acts as a bridge between Pygopus and the N terminus of  $\beta$ -catenin. Evidence suggests that this trimeric complex is involved in nuclear import/retention of  $\beta$ -catenin (Townsend et al., 2004), but it may also be involved in the ability of  $\beta$ -catenin to activate transcription (Hoffmans et al., 2005). The C terminus of  $\beta$ -catenin also binds to coactivators such as the histone acetylase CBP, Hyrax, and Brg-1 (a component of the SWI/SNF chromatin-remodeling complex).

families (CK1 $\alpha$ , - $\delta$ , - $\epsilon$  and GSK3 $\alpha$  and - $\beta$  [reviewed in Price, 2006]). When WNT receptor complexes are not engaged, CK1 and GSK3 $\alpha/\beta$  sequentially phosphorylate  $\beta$ -catenin at a series of highly conserved Ser/Thr residues near its N terminus (Figure 2). Phosphorylated  $\beta$ -catenin is then recognized by the F box/WD repeat protein  $\beta$ -TrCP, a component of a dedicated E3 ubiquitin ligase complex. As a consequence,  $\beta$ -catenin is ubiquitinated and targeted for rapid destruction by the proteasome (Aberle et al., 1997). Note that the CK1 and GSK3 kinases perform paradoxical roles in the Wnt pathway. At the level of the LRP coreceptor they act as agonists, whereas in the destruction complex they act as antagonists

Although genetic observations imply an essential role for APC in the destruction complex, there is no consensus on its specific molecular activity. APC has a series of 15 and 20 amino acid repeats with which it interacts with  $\beta$ -catenin. Three Axin-binding motifs are interspersed between these  $\beta$ -catenin-binding motifs. Increasing the expression of Axin in cancer cells that lack APC restores the activity of the destruction complex, implying that APC is only essential when Axin levels are limiting. Quantitatively, Axin indeed appears to be the limiting factor (Lee et al., 2003) and may be the key scaffolding molecule that promotes the rapid assembly and disassembly of the destruction complex.

Given that CK1, Dsh,  $\beta$ -TrCP, and GSK3 participate in other signaling pathways, low levels of Axin may insulate the Wnt pathway from changes in the abundance or activity of these signaling components. It has been proposed that APC is required for efficient shuttling and loading/unloading of  $\beta$ -catenin onto the cytoplasmic destruction complex. Both APC and Axin can themselves be phosphorylated by their associated kinases,

which changes their affinity for other components of the destruction complex. Our understanding of the relevance of these phosphorylation events in the regulation of Wnt signaling remains incomplete. For a comprehensive discussion of the kinases in the Wnt pathway, the reader is referred to a recent review (Price, 2006)

$\beta$ -catenin plays a second role in simple epithelia, that is, as a component of adherens junctions. It is an essential binding partner for the cytoplasmic tail of various cadherins, such as E-cadherin (Peifer et al., 1992). Unlike the signaling pool of  $\beta$ -catenin, the pool that is bound to the adherens junction is highly stable. It is currently unclear whether the adhesive and signaling properties of  $\beta$ -catenin are interconnected. In a likely scenario, newly synthesized  $\beta$ -catenin first saturates the pool that is part of the adhesion junction, which never becomes available for signaling. "Excess," free cytoplasmic  $\beta$ -catenin protein is then efficiently degraded by the APC complex. It is only this second, highly unstable pool that is subject to regulation by Wnt signals. In support of this model, these two functions of  $\beta$ -catenin are separately performed by two different  $\beta$ -catenin homologs in *C. elegans* (Korswagen et al., 2000).

Upon receptor activation by WNT ligands, the intrinsic kinase activity of the APC complex for  $\beta$ -catenin is inhibited. It is unclear how this occurs, but it likely involves the Wnt-induced recruitment of Axin to the phosphorylated tail of LRP and/or to Fz-bound Dsh. As a consequence, stable, nonphosphorylated  $\beta$ -catenin accumulates and translocates into the nucleus, where it binds to the N terminus of LEF/TCF (lymphoid enhancer factor/T cell factor) transcription factors (Behrens et al., 1996; Moleenaar et al., 1996; van de Wetering et al., 1997).

It has been suggested that protein phosphatases may regulate  $\beta$ -catenin stability as antagonists of the serine kinases (reviewed in Price, 2006). For example, heterotrimeric PP2A is required for the elevation of  $\beta$ -catenin levels that is dependent on Wnt. Moreover, PP2A can bind Axin and APC, suggesting that it might function to dephosphorylate GSK3 substrates. If and how PP2A activity is regulated by Wnt signals remains to be resolved.

Crystallographic studies are starting to provide insights into the structure of the destruction complex. The central region of  $\beta$ -catenin (to which most partners bind) was the first component of the pathway to be crystallized. It consists of 12 armadillo repeats, which adopt a superhelical shape with a basic groove running along its length. Subsequently, structural interactions of Axin, APC, E-cadherin, and TCF with  $\beta$ -catenin have been visualized (Choi et al., 2006, and references therein). APC, E-cadherin, and TCF bind the central part of the basic groove in a mutually exclusive fashion. Despite very limited conservation of primary sequence in the respective interaction domains, the modes of binding are structurally very similar. Axin utilizes a helix that occupies the groove formed by the third and fourth armadillo repeats of  $\beta$ -catenin. Axin binding precludes the simultaneous

interaction with other  $\beta$ -catenin partners in this region. Based on this observation, it is suggested that a key function of APC is to remove phosphorylated  $\beta$ -catenin from the active site of the complex (Xing et al., 2003). In a further study, the structure of Axin bound to APC (Spink et al., 2000) was solved. These studies form stepping stones to a better understanding of the dynamics of the destruction complex. Unfortunately, biochemical studies of the destruction complex in its different activation states are sorely lacking.

### Nuclear Events

Upon stabilization by Wnt signals,  $\beta$ -catenin enters the nucleus to reprogram the responding cell (Figure 3). There is no consensus on the mechanism by which  $\beta$ -catenin travels between the cytoplasm and the nucleus. In many cases, cells that undergo Wnt signaling may actually display an overall rise in  $\beta$ -catenin protein without a clear nuclear preference.  $\beta$ -catenin's nuclear import is independent of the Nuclear Localization Signal/importin machinery.  $\beta$ -catenin itself is a close relative of importin/karyopherins and directly interacts with nuclear pore components. Two proteins, Tcf and Pygopus are proposed to anchor  $\beta$ -catenin in the nucleus, although  $\beta$ -catenin can still localize to the nucleus in the absence of either of the two (reviewed in Staedeli et al., 2006).  $\beta$ -catenin can also be actively transported back to the cytoplasm, by either an intrinsic export signal or as cargo of Axin (Cong and Varmus, 2004) or APC (Rosin-Arbesfeld et al., 2000) that shuttle between cytoplasm and nucleus.

Whereas the fly and worm genomes both encode a single Tcf protein, the vertebrate genome harbors four *Tcf/Lef* genes. Tcf factors bind their cognate motif in an unusual fashion, i.e., in the minor groove of the DNA helix, while inducing a dramatic bend of over 90°. Tcf target sites are highly conserved between the four vertebrate Tcf/Lef proteins and *Drosophila* Tcf. These sites resemble AGATCAAAGG (van de Wetering et al., 1997). Wnt/TCF reporter plasmids such as pTOPflash (Korinek et al., 1997), widely used to measure Wnt pathway activation, consist of concatamers of 3–10 of these binding motifs cloned upstream of a minimal promoter. The four vertebrate TCF/LEF differ dramatically in their embryonic and adult expression domains, yet they are highly similar biochemically, explaining the extensive redundancy unveiled in double knockout experiments (as in Galceran et al., 1999).

In the absence of Wnt signals, Tcf acts as a transcriptional repressor by forming a complex with Groucho/Grg/TLE proteins (Cavallo et al., 1998; Roose et al., 1998). The interaction of  $\beta$ -catenin with the N terminus of Tcf (Behrens et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997) transiently converts it into an activator, translating the Wnt signal into the transient transcription of Tcf target genes. To accomplish this,  $\beta$ -catenin physically displaces Groucho from Tcf/Lef (Daniels and Weis, 2005). The recruitment of  $\beta$ -catenin to Tcf target genes affects local chromatin in several

ways. Its C terminus is a potent transcriptional activator in transient reporter gene assays (van de Wetering et al., 1997). It binds coactivators such as the histone acetylase CBP and Brg-1, a component of the SWI/SNF chromatin remodeling complex (reviewed in Staedeli et al., 2006). A recent study implies that the human and fly homologs of yeast Cdc37 (Parafibromin and Hyrax, respectively) also interact with the C-terminal transactivation domain of  $\beta$ -catenin to activate target gene transcription (Mosimann et al., 2006). Cdc37 is a component of the PAF complex. In yeast the PAF complex directly interacts with RNA polymerase II to regulate transcription initiation and elongation.

Two dedicated, nuclear partners of the TCF/ $\beta$ -catenin complex, Legless/Bcl9 and Pygopus, were recently found in genetic screens in *Drosophila* (Kramps et al., 2002; Parker et al., 2002; Thompson et al., 2002). Mutations in these genes result in phenotypes similar to *wingless*, and overexpression of both genes promotes TCF/ $\beta$ -catenin activity in mammalian cells (Thompson et al., 2002). Bcl9 bridges Pygopus to the N terminus of  $\beta$ -catenin. The formation of this trimeric complex has been implicated in nuclear import/retention of  $\beta$ -catenin (Townsend et al., 2004) but may also directly contribute to the ability of  $\beta$ -catenin to transactivate transcription (Hoffmans et al., 2005). Although most if not all Wnt signaling events in *Drosophila* appear to be dependent on Bcl9 and Pygopus, it is currently unclear if this holds true in vertebrate development.

Tcf itself can be regulated by phosphorylation. The MAP kinase-related protein kinase NLK/Nemo (Ishitani et al., 1999) phosphorylates Tcf, thereby decreasing the DNA-binding affinity of the  $\beta$ -catenin/Tcf complex and inhibiting transcriptional regulation of Wnt target genes. In *C. elegans*, LIT-1/NLK-dependent phosphorylation results in PAR-5/14-3-3- and CRM-1-dependent nuclear export of POP-1/Tcf (Meneghini et al., 1999; Lo et al., 2004). And lastly, a recent study utilizing chromatin immunoprecipitations suggests that APC, independent of its role in the cytoplasmic destruction complex, acts on chromatin to facilitate CtBP-mediated repression of Wnt target genes in normal, but not in colorectal cancer cells (Sierra et al., 2006).

### Wnt Target Genes

Loss of components of the Wnt pathway can produce dramatic phenotypes that affect a wide variety of organs and tissues. A popular view equates Wnt signaling with maintenance or activation of stem cells (Reya and Clevers, 2005). It should be realized, however, that Wnt signals ultimately activate transcriptional programs and that there is no intrinsic restriction in the type of biological event that may be controlled by these programs. Thus, Wnt signals may promote cell proliferation and tissue expansion but also control fate determination or terminal differentiation of postmitotic cells. Sometimes, these disparate events, proliferation and terminal differentiation, can be activated by Wnt in different cell types



**Figure 4. Self-Renewing Tissues in the Adult Mammal**

(Left) In the intestinal epithelium, proliferating crypt precursors and differentiated villus cells form a contiguous sheet of cells that is in perpetual upward motion. Stem cells, which produce the transit-amplifying progenitor cells, reside near the bottom of the crypt and escape this flow. Evidence suggests that Wnt signaling is required for the establishment of the progenitor compartment in the intestinal epithelium. Wnt proteins also promote the terminal differentiation of Paneth cells at the base of the crypt. A small adenoma carrying a mutation in APC grows inside the right-hand villus. As a consequence of the loss of APC,  $\beta$ -catenin protein accumulates to high levels in the adenoma. (Inset) A section of a mouse small intestine displaying crypts, villi, and a growing adenoma. Staining for  $\beta$ -catenin (brown) reveals its presence in adhesion junctions as well as a modest accumulation at the bottom of the crypts due to local Wnt signaling. Photo by Daniel Pinto. (Right) Wnt signaling in hair follicles activates bulge stem cells, promotes entry into the hair lineage, and recruits the cells to the transit-amplifying matrix compartment.

within the same structure, such as the hair follicle or the intestinal crypt (Reya and Clevers, 2005).

Numerous Tcf target genes have been identified in diverse biological systems. These studies tend to focus on target genes involved in cancer, as exemplified by the wide interest in the Wnt target genes cMyc and Cyclin D1. For a comprehensive, updated overview of Tcf target genes, the reader is referred to the Wnt homepage (<http://www.stanford.edu/~rnusse/wntwindow.html>). The Wnt pathway has distinct transcriptional outputs, which are determined by the developmental identity of the responding cell, rather than by the nature of the signal. In other words, the majority of Wnt target genes appear to be cell type specific. It is not clear whether “universal” Wnt/Tcf target genes exist. The best current candidates in vertebrates are Axin2/conductin (Jho et al., 2002) and SP5 (Weidinger et al., 2005). As noted (Logan and Nusse, 2004), Wnt signaling is autoregulated at many levels. The expression of a variety of positive and negative regulators of the pathway, such as Frizzleds, LRP and HSPG, Axin2, and TCF/Lef are all controlled by the  $\beta$ -catenin/TCF complex.

#### Wnt Signaling in Self-Renewing Tissues in Adult Mammals

Wnt signaling not only features in many developmental processes; in some self-renewing tissues in mammals it remains essential throughout life. It is this aspect of Wnt

signaling that is intricately connected to the development of disease. The examples discussed below illustrate how the Wnt pathway is involved in adult tissue self-renewal. Mutations in the Wnt pathway tip the homeostatic balance in these tissues to cause pathological conditions such as disturbances in skeletal bone mass or cancer.

#### Gut

The absorptive epithelium of the small intestine is ordered into numerous villi and crypts. It constitutes the most rapidly self-renewing tissue in adult mammals. In the mouse, the epithelium turns over entirely within 3–5 days. A massive rate of cell production by a “transit-amplifying” crypt compartment is compensated by apoptosis at the tip of the villus. The proliferating crypt precursors and differentiated villus cells form a contiguous sheet of cells that is in perpetual upward motion. Stem cells reside near the bottom of the crypt and escape this flow. These slowly cycling cells produce the transit-amplifying progenitor cells that are capable of differentiating toward all epithelial lineages. The stem cells self-renew throughout life and regenerate the epithelium after injury (Figure 4). Committed progenitors arrest their cell cycle and differentiate when they reach the crypt-villus junction (reviewed in Reya and Clevers, 2005).

Current evidence indicates that the Wnt cascade is the dominant force in controlling cell fate along the crypt-villus axis. In neonatal mice lacking Tcf4, the differentiated villus epithelium appears unaffected, but the crypt pro-

genitor compartment is entirely absent (Korinek et al., 1998). This implies that physiological Wnt signaling is required for the establishment of this progenitor compartment. Inhibition of Wnt signaling by the transgenic expression of Dkk-1 in adult mice induces the complete loss of crypts, identifying Wnt as the dominant mitogen for crypt progenitor cells throughout life (reviewed in Reya and Clevers, 2005). Transgenic expression of the Wnt agonist R-spondin-1 results in a massive hyperproliferation of intestinal crypts, confirming this notion (Kim et al., 2005). Wnt proteins are physiologically expressed by the crypt epithelial cells rather than by the surrounding mesenchyme (Gregorieff et al., 2005). These Wnt proteins not only stimulate the proliferation of crypt progenitors but also promote the terminal differentiation of Paneth cells, residing at the bottoms of the crypts (van Es et al., 2005).

### Hair Follicle

Multipotent epidermal stem cells reside in the bulge region of the hair follicle (Figure 4). Bulge stem cells can generate all hair lineages but also the sebocytes and even the stem cells of the interfollicular epidermis (Alonso and Fuchs, 2003). To form a hair, cells migrate downward from the bulge through the outer root sheath. At the base of the hair, the cells enter a transit-amplifying compartment termed the germinative matrix where they undergo terminal differentiation in the precortex compartment of the hair.

Wnt signaling is required for the establishment of the hair follicle. Mice with a mutation in *Lef1* have few hair follicles (van Genderen et al., 1994). Conversely, transgenic overexpression of *Lef1* leads to de novo hair follicle formation in the epidermis. Similarly, transgenic overexpression of a constitutively stable (“oncogenic”) form of  $\beta$ -catenin induces additional hair follicles (reviewed in Alonso and Fuchs, 2003).

Within the established hair follicle, the Wnt cascade remains crucial throughout life. Wnt signals play a key role in the activation of bulge stem cells to progress toward hair formation, and this signal is relayed by  $\beta$ -catenin and *Lef1* (Lowry et al., 2005). Conditional deletion of the  *$\beta$ -catenin* gene or transgenic expression of dominant-negative *Lef1* in established hair follicles disrupts the process by which bulge stem cells provide hair lineage precursors (Huelsen et al., 2001). Instead, the mutant cells adopt an epidermal or sebocyte fate. In sum, Wnt signaling in hair follicles, relayed through *Lef1*, activates bulge stem cells, promotes entry into the hair lineage, and recruits the cells to the transit-amplifying matrix compartment.

Multiple hair keratin genes carry Tcf binding sites in their promoters (Zhou et al., 1995), implying the counterintuitive notion that Wnt signaling in the matrix, transduced through  $\beta$ -catenin and *Lef1*, not only controls precursor cell expansion but also drives terminal differentiation of the hair lineage. This situation resembles the terminal differentiation of Paneth cells by Wnt in the crypt (see above).

### Hematopoietic System

Hematopoietic stem cells (HSCs) are the best studied stem cells in mammals. A number of studies have implicated the Wnt signaling pathway as an important regulator of hematopoietic stem and progenitor cells. HSCs themselves as well as the bone marrow microenvironment can produce Wnt proteins. Indeed, Tcf reporters are active in HSCs in their native microenvironment. In vitro, soluble Wnt proteins promote the proliferation and inhibit the differentiation of murine hematopoietic progenitors. In addition, overexpressed  $\beta$ -catenin as well as stimulation with purified Wnt proteins sustain self-renewal of murine and human HSCs in vitro. Such treated HSCs are enhanced in their ability to reconstitute the hematopoietic system of lethally irradiated mice (Reya et al., 2003). Wnt signaling is also implicated in fate determination within the T lymphoid lineage as mice lacking either *Tcf1* alone or both *Tcf1* and *Lef1* display severe reductions in the earliest thymocyte progenitor compartment (Okamura et al., 1998).

In the light of these observations it was unexpected that conditional deletion of  $\beta$ -catenin in mice did not affect the hematopoietic/lymphoid system (Cobas et al., 2004). However, it remains possible that the loss of  $\beta$ -catenin is compensated for in the hematopoietic system by its homolog  $\gamma$ -catenin/plakoglobin.

### Bone

In postnatal and adult life, osteoblasts produce bone matrix, whereas osteoclasts resorb the matrix. Bone density is determined by the relative activities of these two cell types. Gain-of-function mutations in the human *LRP5* gene occur in bone diseases, indicating that canonical Wnt signaling may regulate bone mass. This observation has motivated genetic studies in mouse models, which generally confirm the importance of this signaling pathway in bone homeostasis, primarily as a positive regulator of the osteoblast lineage. Similar to humans carrying the gain-of-function *LRP5*<sup>G171V</sup> mutation, transgenic mice expressing this allele in osteoblasts display increased bone density and elevated numbers of active osteoblasts (reviewed in Hartmann, 2006). Consistent with this notion, mice lacking *Lrp5* exhibit a reduction in bone mass and a defect in osteoblast proliferation and maturation (Kato et al., 2002). In addition, the loss of the *SOST* gene product sclerostin leads to sclerosteosis, a human disease characterized by high bone mass. Two groups recently demonstrated that *SOST/Sclerostin* is a secreted Wnt inhibitor, which binds and blocks *LRP5/6* (Li et al., 2005; Semenov et al., 2005).

Several other mouse models support the notion that activated Wnt signaling leads to a postnatal increase in bone mass, such as transgenic mice expressing *Wnt10b* in adipose tissue and bone marrow and mice lacking the Wnt antagonist *Sfrp1* (reviewed in Hartmann, 2006). Mice lacking *Axin2* exhibit craniosynostosis as the result of increased proliferation of osteoblast progenitors in the skull sutures (Yu et al., 2005). These genetic studies indicate that osteoblast maturation and activity is induced by the canonical Wnt pathway.

## Wnt Signaling in Cancer

### Colon Cancer

The APC gene was among the first tumor suppressors to be cloned. A germline APC mutation is the genetic cause of a hereditary cancer syndrome termed Familial Adenomatous Polyposis (FAP) (Kinzler et al., 1991; Nishisho et al., 1991). FAP patients inherit one defective APC allele and as a consequence develop large numbers of colon adenomas, or polyps, in early adulthood. Polyps are benign, clonal outgrowths of epithelial cells in which the second APC allele is inactivated. Inevitably, some of these polyps progress into malignant adenocarcinoma. Loss of both APC alleles occurs in the large majority of sporadic colorectal cancers (Kinzler and Vogelstein, 1996). Mutational inactivation of APC leads to the inappropriate stabilization of  $\beta$ -catenin (Rubinfeld et al., 1996; Figure 4). Indeed, Tcf reporter constructs, normally transcribed only upon Wnt signaling, are inappropriately transcribed in APC mutant cancer cells through the action of constitutive complexes between  $\beta$ -catenin and the intestinal TCF family member Tcf4 (Korinek et al., 1997). In rare cases of colorectal cancer where APC is not mutated, Axin2 is mutant (Liu et al., 2000), or activating (oncogenic) point mutations in  $\beta$ -catenin remove its N-terminal Ser/Thr destruction motif (Morin et al., 1997). Of note, patients with hereditary Axin2 mutations display a predisposition to colon cancer (Lammi et al., 2004).

In intestinal epithelial cells in which APC is mutated, the constitutive  $\beta$ -catenin/Tcf4 complex activates a genetic program in crypt stem/progenitor cells (van de Wetering et al., 2002). In the crypt, the Wnt signaling gradient drives expression of this genetic program to maintain progenitor cell proliferation. The Wnt gradient also controls expression of the EphB/EphrinB sorting receptors and ligands (Battle et al., 2002). The resulting EphB/EphrinB countergradients establish crypt-villus boundaries as well as position the Paneth cells at the bottom of the crypt. Several EphB genes are initially upregulated as Wnt/Tcf4 target genes in early adenomas, but their expression is lost upon cancer progression (Battle et al., 2005) apparently as the result of a selection process. Activating Wnt pathway mutations are not restricted to cancer of the intestine. Loss-of-function mutations in Axin have also been found in hepatocellular carcinomas, whereas oncogenic  $\beta$ -catenin mutations occur in a wide variety of solid tumors (reviewed in Reya and Clevers, 2005).

Several animal models exist for FAP. Dove and colleagues first described the *multiple intestinal neoplasia (min)* mouse, which carries a stop codon in APC (*Apc<sup>min</sup>*). Unlike FAP patients, *Apc<sup>min</sup>* mice develop adenomas predominantly in the small intestine (Su et al., 1992). Several additional *Apc* knockout models have been generated in mice. Invariably, these mice develop neoplastic lesions but they may differ in tumor incidence and tissue type in which tumors first appear. In a recent elegant study, the Wnt cascade was mutationally activated in adult mice by conditional deletion of *Apc* (San-

som et al., 2004). Within days, villi were entirely populated by crypt-like cells, demonstrating the direct link between active Wnt signaling and the proliferation of crypt progenitors, which when unrestrained results in cancer. Zebrafish that are mutant in *Apc* resemble the mouse models in that heterozygous mutants develop adenomas in organs of endodermal origin including the intestine. These fish may prove useful for genetic screens for genes that modify cancer risk (Haramis et al., 2006).

### Hair Follicle Tumors

The transit-amplifying matrix compartment of the hair follicle appears to be the target for malignant transformation by mutational activation of the Wnt cascade. A constitutively active, oncogenic  $\beta$ -catenin transgene induces pilomatricoma-like lesions (Gat et al., 1998), tumors whose exterior zone of densely packed cells resembles the hair follicle matrix. A tamoxifen-inducible  $\beta$ -catenin transgene induces another hair follicle tumor, a so-called trichofolliculoma (Lo Celso et al., 2004). Moreover, most spontaneous pilomatricomas in humans carry activating mutations in  $\beta$ -catenin (Chan et al., 1999). Like adenomas in the gut, these pilomatricomas and trichofolliculomas subvert the process by which Wnt drives the physiological expansion of hair precursors in the matrix/precortex region of the hair.

Watt and colleagues recently described the first inactivating mutations in the Wnt pathway in human cancer. One-third of a series of human sebaceous tumors carried *LEF1* mutations, impairing LEF1 binding to  $\beta$ -catenin and transcriptional activation. This observation agrees with the model that entry of early progenitors into a hair fate requires Wnt signals transduced through Lef1. In the absence of such signaling, progenitors may be erroneously redirected toward a sebocyte fate (Takeda et al., 2006).

### Leukemia

Drawing from the parallels between self-renewal and cancer in the gut and hair follicle, the effects of Wnt pathway components on hematopoietic progenitors predict that Wnt deregulation may contribute to hematological malignancies. Indeed, a recent report suggests that leukemic growth of both myeloid and lymphoid lineages is dependent on Wnt signaling. Granulocyte-macrophage progenitors from Chronic Myelogenous Leukemia patients and blast crisis cells from patients resistant to therapy display active Wnt signaling as demonstrated by Tcf reporter activity and the accumulation of nuclear  $\beta$ -catenin (Jamieson et al., 2004). The nature of the aberrant Wnt pathway activity is unresolved; no Wnt pathway mutations have been detected in hematological malignancies.

### Future Challenges

Over the last 20 years, a detailed outline of the canonical Wnt pathway has emerged. Although it is likely that most core components of the pathway have now been identified, much remains to be learned about the biochemi-

cal events that connect these components. Many of the gaps in our knowledge are due to the notorious difficulties in the production of purified Wnt proteins. Few good Wnt antibodies exist and, 25 years after the cloning of Wnt1, its structure remains unknown. The routing and the coincident posttranslational modifications of Wnt proteins in the secreting cell are incompletely understood. And the rules that dictate the movement of Wnt proteins between cells remain uncertain. However, a procedure to produce soluble Wnt has recently been developed (Willert et al., 2003), which creates avenues to address many of these issues.

The components of the destruction complex have been long known, yet the biochemistry of its activity has remained elusive. APC is an essential component of the destruction complex, but what is its biochemical activity? How relevant is Dsh to the coupling of Wnt receptors to the destruction complex? And what mechanism inhibits the phosphorylation of  $\beta$ -catenin by the destruction complex when a Wnt signal is being transduced?

In addition, a multitude of proposed pathway components, not discussed here, may activate, modify, or inhibit Wnt signaling or may be involved in crosstalk to other pathways. An updated, comprehensive list of these putative components and interactions appears on <http://www.stanford.edu/~rnusse/wntwindow.html>. Often based on single studies, these candidate components remain to be independently confirmed.

Wnt signaling ultimately controls developmental fates through the transcription of cell type-specific programs of Tcf target genes. Recent developments in array-based technology allow detailed analysis of the nuclear transcriptional response to Wnt signals. With these technologies, it is expected that the dissection of the gene programs in various developmental or pathological events will provide a wealth of insight into the biology of these processes. Such insights will be crucial for the development of targeted therapies for diseases related to the Wnt pathway. To date, the development of such therapies has unfortunately been lagging behind the rapid progress made in our fundamental understanding of this pathway. Small-molecule inhibitors of the Wnt pathway have proven difficult to develop but should hold great promise for the treatment of cancer. Agonists of the pathway have become available, such as small-molecule inhibitors of GSK-3 and soluble Wnt or R-spondin proteins. These may be used to support renewal or repair of tissues such as bone, hair, or damaged intestinal epithelia.

The rapidly maturing Wnt field unites an unusually broad and effective spectrum of scientific disciplines. It can be expected that many of the outstanding questions in the field will be answered in the foreseeable future.

#### ACKNOWLEDGMENTS

The author thanks Xi He, R. Korswagen, E. Verheyen, and C. Niehrs for comments and J. van Eldik for secretarial help with the manuscript.

#### REFERENCES

- Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997). beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* *16*, 3797–3804.
- Alonso, L., and Fuchs, E. (2003). Stem cells in the skin: waste not, Wnt not. *Genes Dev.* *17*, 1189–1200.
- Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G., and Basler, K. (2006). Wntless, a conserved membrane protein dedicated to the secretion of wnt proteins from signaling cells. *Cell* *125*, 509–522.
- Bartscherer, K., Pelte, N., Ingelfinger, D., and Boutros, M. (2006). Secretion of wnt ligands requires evi, a conserved transmembrane protein. *Cell* *125*, 523–533.
- Battle, E., Bacani, J., Begthel, H., Jonkheer, S., Gregorieff, A., van de Born, M., Malats, N., Sancho, E., Boon, E., Pawson, T., et al. (2005). EphB activity suppresses colorectal cancer progression. *Nature* *435*, 1126–1130.
- Battle, E., Henderson, J.T., Begthel, H., van den Born, M., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T., et al. (2002). Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell* *111*, 251–263.
- Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* *382*, 225–230.
- Behrens, J., von Kries, J.P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* *382*, 638–642.
- Cavallo, R.A., Cox, R.T., Moline, M.M., Roose, J., Polevoy, G.A., Clevers, H., Peifer, M., and Bejsovec, A. (1998). *Drosophila* Tcf and Groucho interact to repress Wingless signaling activity. *Nature* *395*, 604–608.
- Chan, E.F., Gat, U., McNiff, J.M., and Fuchs, E. (1999). A common human skin tumour is caused by activating mutations in beta-catenin. *Nat. Genet.* *21*, 410–413.
- Choi, H.J., Huber, A.H., and Weis, W.I. (2006). Thermodynamics of beta-catenin-ligand interactions: the roles of the N- and C-terminal tails in modulating binding affinity. *J. Biol. Chem.* *281*, 1027–1038.
- Cobas, M., Wilson, A., Ernst, B., Mancini, S.J., MacDonald, H.R., Kemler, R., and Radtke, F. (2004). Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. *J. Exp. Med.* *199*, 221–229.
- Cong, F., and Varmus, H. (2004). Nuclear-cytoplasmic shuttling of Axin regulates subcellular localization of beta-catenin. *Proc. Natl. Acad. Sci. USA* *101*, 2882–2887.
- Coudreuse, D.Y., Roel, G., Betist, M.C., Destree, O., and Korswagen, H.C. (2006). Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* *312*, 921–924.
- Culi, J., and Mann, R.S. (2003). Boca, an endoplasmic reticulum protein required for wingless signaling and trafficking of LDL receptor family members in *Drosophila*. *Cell* *112*, 343–354.
- Daniels, D.L., and Weis, W.I. (2005). Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat. Struct. Mol. Biol.* *12*, 364–371.
- Davidson, G., Wu, W., Shen, J., Bilic, J., Fenger, U., Stanek, P., Glinka, A., and Niehrs, C. (2005). Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. *Nature* *438*, 867–872.
- Dominguez, I., Itoh, K., and Sokol, S.Y. (1995). Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis forma-

- tion in *Xenopus* embryos. *Proc. Natl. Acad. Sci. USA* **92**, 8498–8502.
- Galceran, J., Farinas, I., Depew, M.J., Clevers, H., and Grosschedl, R. (1999). Wnt3a/-like phenotype and limb deficiency in Lef1(-/-)Tcf1(-/-) mice. *Genes Dev.* **13**, 709–717.
- Gat, U., Dasgupta, R., Degenstein, L., and Fuchs, E. (1998). De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* **95**, 605–614.
- Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., and Niehrs, C. (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391**, 357–362.
- Gregorieff, A., Pinto, D., Begthel, H., Destree, O., Kielman, M., and Clevers, H. (2005). Expression pattern of Wnt signaling components in the adult intestine. *Gastroenterology* **129**, 626–638.
- Guger, K.A., and Gumbiner, B.M. (1995). Beta-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in *Xenopus* dorsal-ventral patterning. *Dev. Biol.* **172**, 115–125.
- Haramis, A.P., Hurlstone, A., van der Velden, Y., Begthel, H., van den Born, M., Offerhaus, G.J., and Clevers, H.C. (2006). Adenomatous polyposis coli-deficient zebrafish are susceptible to digestive tract neoplasia. *EMBO Rep.* **7**, 444–449.
- Hartmann, C. (2006). A Wnt canon orchestrating osteoblastogenesis. *Trends Cell Biol.* **16**, 151–158.
- He, X., Saint-Jeannet, J.P., Woodgett, J.R., Varmus, H.E., and Dawid, I.B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617–622.
- He, X., Saint-Jeannet, J.P., Wang, Y., Nathans, J., Dawid, I., and Varmus, H. (1997). A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* **275**, 1652–1654.
- Hoang, B., Moos, M., Jr., Vukicevic, S., and Luyten, F.P. (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**, 26131–26137.
- Hoffmans, R., Stadel, R., and Basler, K. (2005). Pygopus and legless provide essential transcriptional coactivator functions to armadillo/beta-catenin. *Curr. Biol.* **15**, 1207–1211.
- Hofmann, K. (2000). A superfamily of membrane-bound O-acyltransferases with implications for wnt signaling. *Trends Biochem. Sci.* **25**, 111–112.
- Hsieh, J.C., Kodjabachian, L., Rebbert, M.L., Rattner, A., Smallwood, P.M., Samos, C.H., Nusse, R., Dawid, I.B., and Nathans, J. (1999). A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* **398**, 431–436.
- Hsieh, J.C., Lee, L., Zhang, L., Wefer, S., Brown, K., DeRossi, C., Wines, M.E., Rosenquist, T., and Holdener, B.C. (2003). Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* **112**, 355–367.
- Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G., and Birchmeier, W. (2001). beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell* **105**, 533–545.
- Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signaling between beta-catenin and transcription factor TCF. *Nature* **399**, 798–802.
- Itasaki, N., Jones, C.M., Mercurio, S., Rowe, A., Domingos, P.M., Smith, J.C., and Krumlauf, R. (2003). Wise, a context-dependent activator and inhibitor of Wnt signaling. *Development* **130**, 4295–4305.
- Jamieson, C.H., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N. Engl. J. Med.* **351**, 657–667.
- Jho, E.H., Zhang, T., Domon, C., Joo, C.K., Freund, J.N., and Costantini, F. (2002). Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol. Cell. Biol.* **22**, 1172–1183.
- Kato, M., Patel, M.S., Levasseur, R., Lobov, I., Chang, B.H., Glass, D.A., 2nd, Hartmann, C., Li, L., Hwang, T.H., Brayton, C.F., et al. (2002). Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. *J. Cell Biol.* **157**, 303–314.
- Kato, M. (2005). WNT/PCP signaling pathway and human cancer (review). *Oncol. Rep.* **14**, 1583–1588.
- Kazanskaya, O., Glinka, A., del Barco Barrantes, I., Stannek, P., Niehrs, C., and Wu, W. (2004). R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for *Xenopus* myogenesis. *Dev. Cell* **7**, 525–534.
- Kim, K.A., Kakitani, M., Zhao, J., Oshima, T., Tang, T., Binnerts, M., Liu, Y., Boyle, B., Park, E., Emtage, P., et al. (2005). Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science* **309**, 1256–1259.
- Kinzler, K.W., and Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell* **87**, 159–170.
- Kinzler, K.W., Nilbert, M.C., Su, L.K., Vogelstein, B., Bryan, T.M., Levy, D.B., Smith, K.J., Preisinger, A.C., Hedge, P., McKechnie, D., et al. (1991). Identification of FAP locus genes from chromosome 5q21. *Science* **253**, 661–665.
- Kohn, A.D., and Moon, R.T. (2005). Wnt and calcium signaling: beta-Catenin-independent pathways. *Cell Calcium* **38**, 439–446.
- Korswagen, R., Herman, M., and Clevers, H. (2000). Distinct beta-catenins mediate adhesion and signaling functions in *C. elegans*. *Nature* **406**, 527–532.
- Korinek, V., Barker, N., Morin, P.J., van Wichen, D., de Weger, R., Kinzler, K.W., Vogelstein, B., and Clevers, H. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* **275**, 1784–1787.
- Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P.J., and Clevers, H. (1998). Depletion of epithelia stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **19**, 1–5.
- Kramps, T., Peter, O., Brunner, E., Nellen, D., Froesch, B., Chatterjee, S., Murone, M., Zullig, S., and Basler, K. (2002). Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. *Cell* **109**, 47–60.
- Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentferm, J., Schmidt, H.A., Technau, U., von Haeseler, A., Hobmayer, B., Martindale, M.Q., and Holstein, T.W. (2005). Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* **433**, 156–160.
- Lammi, L., Arte, S., Somer, M., Jarvinen, H., Lahermo, P., Thesleff, I., Pirinen, S., and Nieminen, P. (2004). Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am. J. Hum. Genet.* **74**, 1043–1050.
- Lee, E., Salic, A., Kruger, R., Heinrich, R., and Kirschner, M.W. (2003). The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol.* **1**, E10. 10.1371/journal.pbio.0000010.
- Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., Harris, S.E., and Wu, D. (2005). Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J. Biol. Chem.* **280**, 19883–19887.
- Lin, X. (2004). Functions of heparan sulfate proteoglycans in cell signaling during development. *Development* **131**, 6009–6021.
- Liu, W., Dong, X., Mai, M., Seelan, R.S., Taniguchi, K., Krishnadath, K.K., Halling, K.C., Cunningham, J.M., Boardman, L.A., Qian, C., et

- al. (2000). Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signaling. *Nat. Genet.* 26, 146–147.
- Lo Celso, C., Prowse, D.M., and Watt, F.M. (2004). Transient activation of beta-catenin signaling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours. *Development* 131, 1787–1799.
- Lo, M.C., Gay, F., Odom, R., Shi, Y., and Lin, R. (2004). Phosphorylation by the beta-catenin/MAPK complex promotes 14-3-3-mediated nuclear export of TCF/POP-1 in signal-responsive cells in *C. elegans*. *Cell* 117, 95–106.
- Logan, C.Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* 20, 781–810.
- Lowry, W.E., Blanpain, C., Nowak, J.A., Guasch, G., Lewis, L., and Fuchs, E. (2005). Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. *Genes Dev.* 19, 1596–1611.
- Lu, W., Yamamoto, V., Ortega, B., and Baltimore, D. (2004). Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. *Cell* 119, 97–108.
- Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B.M., Delius, H., Hoppe, D., Stannek, P., Walter, C., et al. (2002). Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signaling. *Nature* 417, 664–667.
- McMahon, A.P., and Moon, R.T. (1989). Ectopic expression of the proto-oncogene int-1 in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell* 58, 1075–1084.
- Meneghini, M.D., Ishitani, T., Carter, J.C., Hisamoto, N., Ninomiya-Tsuji, J., Thorpe, C.J., Hamill, D.R., Matsumoto, K., and Bowerman, B. (1999). MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* 399, 793–797.
- Mikels, A.J., and Nusse, R. (2006). Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol.* 4, e115 10.1371/journal.pbio.0040115.
- Molenaar, M., Van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). Xtc-3 Transcription factor mediates beta-catenin-induced axis formation in *xenopus* embryos. *Cell* 86, 391–399.
- Morin, P.J., Sparks, A.B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K.W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 275, 1787–1790.
- Mosimann, C., Hausmann, G., and Basler, K. (2006). Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. *Cell* 125, 327–341.
- Nam, J.S., Turcotte, T.J., Smith, P.F., Choi, S., and Yoon, J.K. (2006). Mouse Cristin/R-spondin family proteins are novel ligands for the frizzled 8 and LRP6 receptors and activate beta-catenin-dependent gene expression. *J. Biol. Chem.* 281, 13247–13257.
- Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S., and Hedge, P. (1991). Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 253, 665–669.
- Noordermeer, J., Klingensmith, J., Perrimon, N., and Nusse, R. (1994). Dishevelled and armadillo act in the wingless signaling pathway in *Drosophila*. *Nature* 367, 80–83.
- Nusse, R., and Varmus, H.E. (1982). Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 31, 99–109.
- Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287, 795–801.
- Okamura, R.M., Sigvardsson, M., Galceran, J., Verbeek, S., Clevers, H., and Grosschedl, R. (1998). Redundant regulation of T cell differentiation and TCRalpha gene expression by the transcription factors LEF-1 and TCF-1. *Immunity* 8, 11–20.
- Panakova, D., Sprong, H., Marois, E., Thiele, C., and Eaton, S. (2005). Lipoprotein particles are required for Hedgehog and Wingless signaling. *Nature* 435, 58–65.
- Parker, D.S., Jemison, J., and Cadigan, K.M. (2002). Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* 129, 2565–2576.
- Peifer, M., McCrea, P.D., Green, K.J., Wieschaus, E., and Gumbiner, B.M. (1992). The vertebrate adhesive junction proteins beta-catenin and plakoglobin and the *Drosophila* segment polarity gene armadillo form a multigene family with similar properties. *J. Cell Biol.* 118, 681–691.
- Peifer, M., Sweeton, D., Casey, M., and Wieschaus, E. (1994). Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* 120, 369–380.
- Pinson, K.I., Brennan, J., Monkley, S., Avery, B.J., and Skarnes, W.C. (2000). An LDL-receptor-related protein mediates Wnt signaling in mice. *Nature* 407, 535–538.
- Price, M.A. (2006). CKI, there's more than one: casein kinase I family members in Wnt and Hedgehog signaling. *Genes Dev.* 20, 399–410.
- Reya, T., Duncan, A.W., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R., and Weissman, I.L. (2003). A role for Wnt signaling in self-renewal of haematopoietic stem cells. *Nature* 423, 409–414.
- Reya, T., and Clevers, H. (2005). Wnt signaling in stem cells and cancer. *Nature* 434, 843–850.
- Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D., and Nusse, R. (1987). The *Drosophila* homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. *Cell* 50, 649–657.
- Roose, J., Molenaar, M., Peterson, J., Hurenkamp, J., Brantjes, H., Moerer, P., van de Wetering, M., Destree, O., and Clevers, H. (1998). The *Xenopus* Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* 395, 608–612.
- Rosin-Arbesfeld, R., Townsley, F., and Bienz, M. (2000). The APC tumour suppressor has a nuclear export function. *Nature* 406, 1009–1012.
- Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S.H., Masiarz, F.R., Munemitsu, S., and Polakis, P. (1993). Association of the APC gene product with beta-catenin. *Science* 262, 1731–1734.
- Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996). Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 272, 1023–1026.
- Sansom, O.J., Reed, K.R., Hayes, A.J., Ireland, H., Brinkmann, H., Newton, I.P., Battle, E., Simon-Assmann, P., Clevers, H., Nathke, I.S., et al. (2004). Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev.* 18, 1385–1390.
- Semenov, M., Tamai, K., and He, X. (2005). SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J. Biol. Chem.* 280, 26770–26775.
- Siegfried, E., Chou, T.B., and Perrimon, N. (1992). Wingless signaling acts through zeste-white 3, the *Drosophila* homolog of glycogen synthase kinase-3, to regulate engrailed and establish cell fate. *Cell* 71, 1167–1179.
- Sierra, J., Yoshida, T., Joazeiro, C.A., and Jones, K.A. (2006). The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev.* 20, 586–600.
- Spink, K.E., Polakis, P., and Weis, W.I. (2000). Structural basis of the Axin-adenomatous polyposis coli interaction. *EMBO J.* 19, 2270–2279.

- Staedeli, R., Hoffmans, R., and Basler, K. (2006). Transcription under the control of nuclear Arm/b-catenin. *Curr. Biol.* *16*, R378–R385.
- Su, L.K., Kinzler, K.W., Vogelstein, B., Preisinger, A.C., Moser, A.R., Luongo, C., Gould, K.A., and Dove, W.F. (1992). Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* *256*, 668–670.
- Su, L.K., Vogelstein, B., and Kinzler, K.W. (1993). Association of the APC tumor suppressor protein with catenins. *Science* *262*, 1734–1737.
- Takeda, H., Lyle, S., Lazar, A.J., Zouboulis, C.C., Smyth, I., and Watt, F.M. (2006). Human sebaceous tumors harbor inactivating mutations in LEF1. *Nat. Med.* *12*, 395–397.
- Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.P., and He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. *Nature* *407*, 530–535.
- Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C.C., Lin, X., and Heasman, J. (2005). Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* *120*, 857–871.
- Thompson, B., Townsley, F., Rosin-Arbesfeld, R., Musisi, H., and Bienz, M. (2002). A new nuclear component of the Wnt signaling pathway. *Nat. Cell Biol.* *4*, 367–373.
- Townsley, F.M., Cliffe, A., and Bienz, M. (2004). Pygopus and Legless target Armadillo/beta-catenin to the nucleus to enable its transcriptional co-activator function. *Nat. Cell Biol.* *6*, 626–633.
- Wallingford, J.B., and Habas, R. (2005). The developmental biology of Dishevelled: an enigmatic protein governing cell fate and cell polarity. *Development* *132*, 4421–4436.
- Wehrli, M., Dougan, S.T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaisel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). Arrow encodes an LDL-receptor-related protein essential for Wingless signaling. *Nature* *407*, 527–530.
- Weidinger, G., Thorpe, C.J., Wuennenberg-Stapleton, K., Ngai, J., and Moon, R.T. (2005). The Sp1-related transcription factors sp5 and sp5-like act downstream of Wnt/beta-catenin signaling in mesoderm and neuroectoderm patterning. *Curr. Biol.* *15*, 489–500.
- van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., et al. (1997). Armadillo co-activates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* *88*, 789–799.
- van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Battle, E., Coudreuse, D., Haramis, A.P., et al. (2002). The beta-catenin/TCF4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* *111*, 241–250.
- van Es, J.H., van Gijn, M.E., Riccio, O., van den Born, M., Vooijs, M., Begthel, H., Cozijnsen, M., Robine, S., Winton, D.J., Radtke, F., and Clevers, H. (2005). Notch pathway/ $\gamma$ -secretase inhibition turns proliferative cells in intestinal crypts and neoplasia into Goblet cells. *Nature* *435*, 959–963.
- van Genderen, C., Okamura, R.M., Farinas, I., Quo, R.G., Parslow, T.G., Bruhn, L., and Grosschedl, R. (1994). Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev.* *8*, 2691–2703.
- Willert, K., Brown, J.D., Danenberg, E., Duncan, A.W., Weissman, I.L., Reya, T., Yates, J.R., 3rd, and Nusse, R. (2003). Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* *423*, 448–452.
- Xing, Y., Clements, W.K., Kimelman, D., and Xu, W. (2003). Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. *Genes Dev.* *17*, 2753–2764.
- Xu, Q., Wang, Y., Dabdoub, A., Smallwood, P.M., Williams, J., Woods, C., Kelley, M.W., Jiang, L., Tasman, W., Zhang, K., and Nathans, J. (2004). Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* *116*, 883–895.
- Yoshikawa, S., McKinnon, R.D., Kokel, M., and Thomas, J.B. (2003). Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. *Nature* *422*, 583–588.
- Yu, H.M., Jerchow, B., Sheu, T.J., Liu, B., Costantini, F., Puzas, J.E., Birchmeier, W., and Hsu, W. (2005). The role of Axin2 in calvarial morphogenesis and craniosynostosis. *Development* *132*, 1995–2005.
- Zeng, X., Tamai, K., Doble, B., Li, S., Huang, H., Habas, R., Okamura, H., Woodgett, J., and He, X. (2005). A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* *438*, 873–877.
- Zhai, L., Chaturvedi, D., and Cumberledge, S. (2004). *Drosophila* wnt-1 undergoes a hydrophobic modification and is targeted to lipid rafts, a process that requires porcupine. *J. Biol. Chem.* *279*, 33220–33227.
- Zhou, P., Byrne, C., Jacobs, J., and Fuchs, E. (1995). Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. *Genes Dev.* *9*, 700–713.