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Review

Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases

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Signaling by the Wnt family of secreted glycolipoproteins via the transcriptional coactivator β-catenin controls embryonic development and adult homeostasis. Here we review recent progress in this so-called canonical Wnt signaling pathway. We discuss Wnt ligands, agonists, and antagonists, and their interactions with Wnt receptors. We also dissect critical events that regulate β-catenin stability, from Wnt receptors to the cytoplasmic β-catenin destruction complex, and nuclear machinery that mediates β-catenin-dependent transcription. Finally, we highlight some key aspects of Wnt/β-catenin signaling in human diseases including congenital malformations, cancer, and osteoporosis, and discuss potential therapeutic implications.

Introduction

Signaling by the Wnt family of secreted glycolipoproteins is one of the fundamental mechanisms that direct cell proliferation, cell polarity, and cell fate determination during embryonic development and tissue homeostasis (Logan and Nusse, 2004). As a result, mutations in the Wnt pathway are often linked to human birth defects, cancer, and other diseases (Clevers, 2006). A critical and heavily studied Wnt pathway is the canonical Wnt pathway, which functions by regulating the amount of the transcriptional coactivator β-catenin, which controls key developmental gene expression programs. This review focuses on our current understanding of Wnt/β-catenin signaling, drawing mainly from genetic, developmental, and biochemical analyses in *Drosophila*, *Xenopus*, mouse, and human. For a more comprehensive and historic perspective, we refer readers to earlier reviews (Clevers, 2006; Logan and Nusse, 2004) and the Wnt homepage (www.stanford.edu/~rnusse/wntwindow.html). The nematode *Caenorhabditis elegans* exhibits similar but also divergent Wnt/β-catenin pathways, which have been covered previously (Mizumoto and Sawa, 2007) and are covered in the accompanying review by Phillips and Kimble (2009) in this issue of *Developmental Cell*. Wnt also activates a number of noncanonical signaling pathways that are independent of β-catenin and have been recently reviewed as well (Seifert and Mlodzik, 2007; Wang and Nathans, 2007).

The central logic of Wnt/β-catenin signaling has emerged from two decades of studies (Figure 1). In the absence of Wnt, cytoplasmic β-catenin protein is constantly degraded by the action of the Axin complex, which is composed of the scaffolding protein Axin, the tumor suppressor *adenomatous polyposis coli* gene product (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3 (GSK3). CK1 and GSK3 sequentially phosphorylate the amino terminal region of β-catenin, resulting in β-catenin recognition by β-Trcp, an E3 ubiquitin ligase subunit, and subsequent β-catenin ubiquitination and proteasomal degradation (He et al., 2004). This continual elimination of β-catenin prevents β-catenin from reaching the nucleus, and Wnt target genes are thereby repressed by the DNA-bound T cell factor/lymphoid enhancer factor (TCF/LEF) family of proteins (Figure 1A). The Wnt/β-catenin pathway is activated when a Wnt ligand binds to the seven-pass transmembrane Frizzled (Fz or Fzd) receptor and its coreceptor, low-density lipoprotein receptor-related protein 6 (LRP6), or its close relative LRP5. The formation of a likely Wnt-Fz-LRP6 complex, together with the recruitment of the scaffolding protein Dishevelled (Dvl), results in LRP6 phosphorylation and activation and the recruitment of the Axin complex to the receptors. These events lead to inhibition of Axin-mediated β-catenin phosphorylation and thereby to the stabilization of β-catenin, which accumulates and travels to the nucleus to form complexes with TCF/LEF and activates Wnt target gene expression (Figure 1B).

Wnt Ligands and Biogenesis

Wnts are conserved in all metazoan animals. In mammals, complexity and specificity in Wnt signaling are in part achieved through 19 Wnt ligands, which are cysteine-rich proteins of approxiamately 350–400 amino acids that contain an N-terminal signal peptide for secretion. Murine WNT3A was the first purified and biochemically characterized Wnt protein (Willert et al., 2003), owing to its relatively efficient secretion (in contrast to most other Wnt proteins). In addition to N-linked glycosylation, which is required for WNT3A secretion (Komekado et al., 2007), WNT3A undergoes two types of lipid modifications that likely account for the hydrophobicity and poor solubility of Wnt proteins (Hausmann et al., 2007). The first reported lipididation was the addition of palmitate to cysteine 77 (Willert et al., 2003). Its mutation had minimal effect on WNT3A secretion but diminished the ability of WNT3A to activate β-catenin signaling (Galli et al., 2007; Komekado et al., 2007; Willert et al., 2003). The second identified lipididation was a palmitoleoyl attached to serine 209, and its mutation resulted in WNT3A accumulation in the endoplasmic reticulum (ER) and a failure in secretion (Takada et al., 2006).

Drosophila Wingless (Wg) is the Wnt molecule most investigated in vivo (Hausmann et al., 2007). These studies plus work in nematodes have identified genes that regulate Wnt biogenesis and secretion. Porcupine encodes a multipass transmembrane ER protein that contains an O-acyl transferase domain, suggesting a role in Wg lipid modification (Hausmann et al., 2007). Porcupine

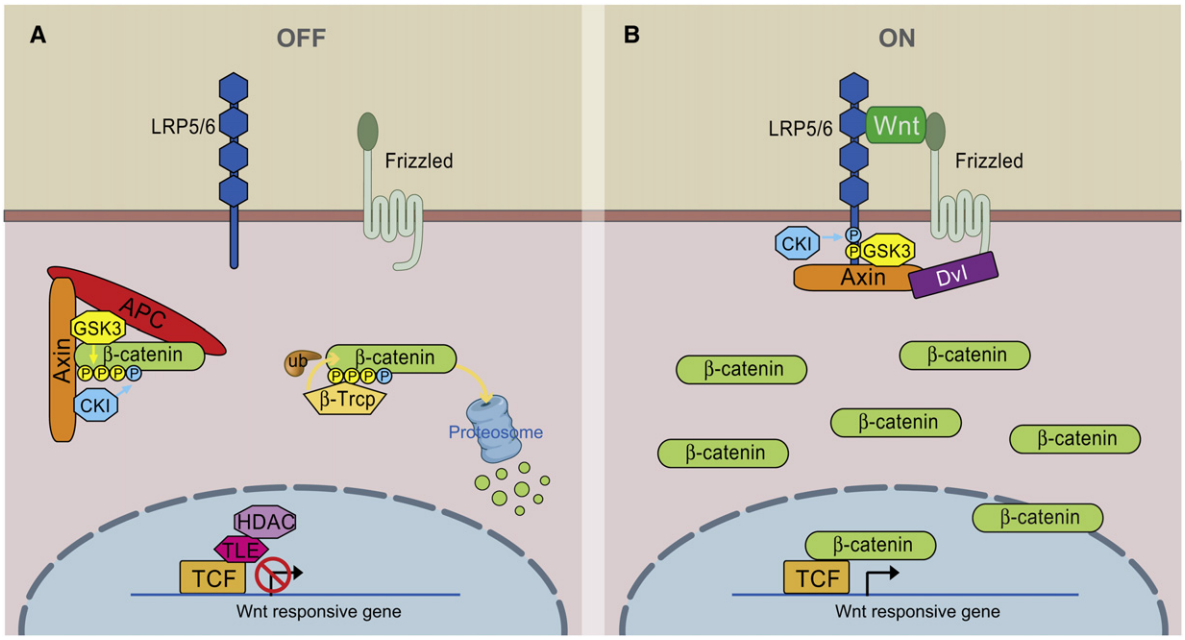

Figure 1. Overview of Wnt/β-Catenin Signaling

(A) In the absence of Wnt, cytoplasmic β-catenin forms a complex with Axin, APC, GSK3, and CK1, and is phosphorylated by CK1 (blue) and subsequently by GSK3 (yellow). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-Trcp, which targets β-catenin for proteosomal degradation. Wnt target genes are repressed by TCF-TLE/Groucho and histone deacetylases (HDAC).

(B) In the presence of Wnt ligand, a receptor complex forms between Fz and LRP5/6. Dvl recruitment by Fz leads to LRP5/6 phosphorylation and Axin recruitment. This disrupts Axin-mediated phosphorylation/degradation of β-catenin, allowing β-catenin to accumulate in the nucleus where it serves as a coactivator for TCF to activate Wnt-responsive genes.

deficiency results in Wg and WNT3A accumulation in the ER and diminished WNT3A palmitoleoylation at serine 209 (Takada et al., 2006), suggesting that Porcupine is responsible for this particular lipidation. Whether Porcupine or a distinct acyltransferase is involved in WNT3A palmitoylation at cysteine 77 remains unknown.

Two additional proteins/protein complexes were identified for Wg/Wnt secretion: Wntless (Wls), also known as Evenness interrupted (Evi) or Sprinter (Srt), in *Drosophila*, and the retromer complex in nematodes (Hausmann et al., 2007). Wls is a multipass transmembrane protein that localizes to the Golgi, endocytic compartments, and the plasma membrane, and is essential for Wg secretion. The retromer complex, which is composed of five subunits, was defined first in yeast. It mediates membrane protein trafficking between endosomes and the Golgi apparatus (Hausmann et al., 2007). Several groups recently reported that the retromer complex is required for retrieval/recycling of Wls from the endosome to the Golgi (Belenkaya et al., 2008; Franch-Marro et al., 2008b; Pan et al., 2008a; Port et al., 2008; Yang et al., 2008), which is likely mediated by direct interaction between Wls and the retromer Vps35 subunit. Loss of retromer function causes Wls to be degraded in the lysosomes and results in reduction of Wls and thus Wnt secretion. These studies led to an emerging picture of Wnt biogenesis (Figure 2). Wnt is glycosylated and lipid modified by Porcupine in the ER, and is escorted by Wls from the Golgi to the plasma membrane for secretion. Wls is recycled by endocytosis and trafficked back to Golgi by the retromer. Note that *porc*, *wls*, and retromer mutants largely phenocopy *wg*/*wnt* mutants in flies and worms, attesting to their dedicated roles in Wnt biogenesis.

Caution is warranted, however, when the above model is extrapolated to individual Wnt proteins. The effects of altering the two lipid-modified residues on Wg secretion and function are different from those on WNT3A (note the caveat that amino acid substitutions may or may not fully mimic the effects of lacking lipid adducts), leading to the suggestion that overall lipidation levels may be more critical (Franch-Marro et al., 2008a). Differences in the order of glycosylation and lipidation also seem to exist between WNT3A and Wg (Komekado et al., 2007; Tanaka et al., 2002; Zhai et al., 2004). Lastly, *Drosophila* WntD neither requires Porcupine and Wls for secretion nor is palmitoylated at the conserved cysteine position (Ching et al., 2008).

Wnt Extracellular Distribution and Movement

Wnt proteins can function as morphogens that are capable of both short- and long-range signaling, as best demonstrated for Wg. Wg lipidation raises the issue of its diffusion and distribution through the aqueous extracellular space. Indeed, purified WNT3A exhibits increased activity via artificial liposomal packaging (Morrell et al., 2008). Two distinct Wg secretory pathways for short- and long-range signaling have been speculated but not fully substantiated. Wg may form multimers to bury lipid modifications inside (Katanaev et al., 2008) or bind to lipoprotein particles that may be involved in Wg long-range signaling (Panakova et al., 2005) (Figure 2). The membrane microdomain protein reggie-1/flotillin-2 specifically promotes Wg long-range secretion (Katanaev et al., 2008). The Wg receptors (see below) and heparan sulfate proteoglycans (HSPGs) such as Dally and Dally-like protein have important roles in the Wg morphogen

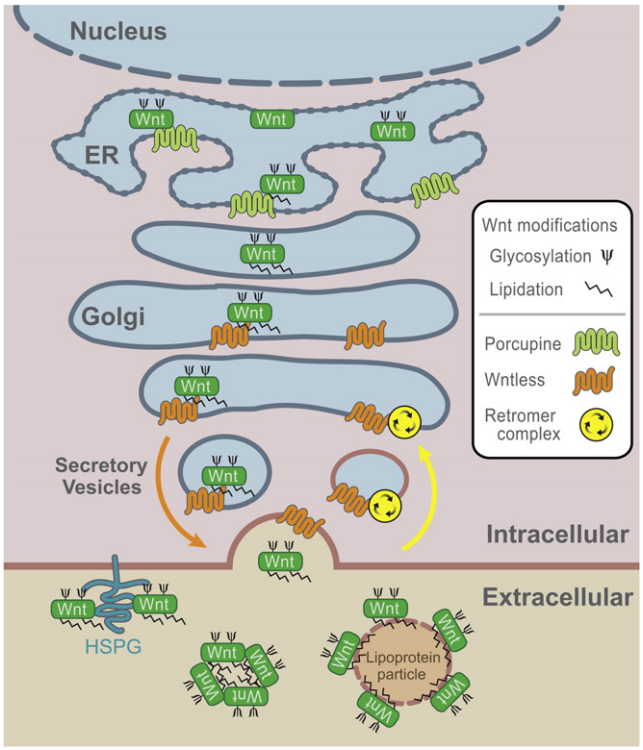

Figure 2. Wnt Biogenesis and Secretion
Wnts are glycosylated and lipid modified in the ER, which involves Porcupine and are escorted by Wntless from the Golgi to the plasma membrane for secretion. Wntless is retrieved from endocytic vescicles and brought back to the Golgi by the retromer complex. After secretion mature Wnts either bind to HSPGs and lipoprotein particles or form multimers, which can modulate Wnt gradients and facilitate long-range Wnt signaling. The numbers of Wnt molecules bound to the lipoprotein particle and composed in multimeric form were drawn arbitrarily.

concentration via regulating Wg degradation, diffusion, and endocytosis/transcytosis, and Dally and Dally-like may also function in Wg signaling as potential low-affinity coreceptors (Lin, 2004). Note that reggie-1/flotillin-2, lipoprotein particles, and Dally and Dally-like protein are important analogously for secreted Hedgehog morphogen, which is also lipid modified (Katanaev et al., 2008; Lin, 2004; Panakova et al., 2005).

Wnt Receptors: Frizzled and LRP5/6

Two distinct receptor families are critical for Wnt/β-catenin signaling (Figure 3): the Fz seven-pass transmembrane receptors (Logan and Nusse, 2004) and LRP5/6 (He et al., 2004). The Wnt-receptor relationship is best illustrated for Wg, which binds to *Drosophila* Fz2 (Dfz2) and Dfz1 with high affinity (1–10 nM) and requires either Fz in a redundant manner (Logan and Nusse, 2004). Wg reception also absolutely depends on Arrow, the LRP5/6 homolog (He et al., 2004). The mammalian genome harbors 10 Fz genes, most of which have variable capacities to activate β-catenin signaling when co-overexpressed with Wnt and LRP5/6 (e.g., Binnerts et al., 2007), and functional redundancy among Fz members is likely prevalent (Logan and Nusse, 2004). Between the two LRPs, LRP6 plays a more dominant role and is essential for embryogenesis, whereas LRP5 is dispensable for embryogenesis but critical for adult bone homeostasis. Nonetheless, LRP5 and LRP6 are partially redundant because their functions together are required for mouse gastrulation (He et al., 2004). Most data, including Wnt binding to LRP5/6, WNT1-FZ8-LRP6 complex formation in vitro, and observations that engineered Fz-LRP5/6 proximity is sufficient to activate β-catenin signaling (Cong et al., 2004; Holmen et al., 2005; Tolwinski et al., 2003), support the model that Wnt induces the formation of Fz-LRP5/6 complex (He et al., 2004) (Figure 1). But unambiguous demonstration of this receptor complex in vivo is lacking. It is noteworthy that WNT3A palmitoylation (at cysteine 77) is important for binding to both Fz and LRP6 (Cong et al., 2004; Komekado et al., 2007), explaining in part the importance of this lipid modification.

A particular Wnt may activate β-catenin and/or noncanonical pathways depending on the receptor complement (van Amerongen et al., 2008). Fz function is involved in β-catenin and noncanonical pathways. The Fz-LRP5/6 coreceptor model stipulates that a Wnt-Fz pair capable of recruiting LRP5/6 activates the β-catenin pathway, consistent with the specific requirement of LRP5/6 in Wnt/β-catenin signaling (He et al., 2004). However, some evidence suggests that LRP6 antagonizes noncanonical Wnt signaling in vivo, possibly via competition for Wnt ligands (Bryja et al., 2009) or an unknown mechanism (Tahinci et al., 2007). Other Wnt receptors exist such as Ryk and ROR2, which are not required for, but in some cases may antagonize, Wnt/β-catenin signaling (van Amerongen et al., 2008).

Wnt Antagonists and Agonists

Several secreted protein families antagonize or modulate Wnt/β-catenin signaling (Figure 3). Secreted Frizzled-related proteins (sFRPs) and Wnt inhibitory protein (WIF) bind to Wnt, and in the case of sFRPs, also to Fz (Figure 3), and thereby function as Wnt antagonists for both β-catenin and noncanonical signaling (Bovolenta et al., 2008). Loss-of-function studies in mice have revealed significant redundancy for the sFRP genes (Satoh et al., 2008). The Wnt-binding property suggests that sFRPs and WIF may also regulate Wnt stability and diffusion/distribution extracellularly beyond just Wnt inhibitors. Some sFRPs have been shown to act in Wnt-independent roles such as regulators of extracellular proteinases (Bovolenta et al., 2008).

Two distinct classes of Wnt inhibitors are the Dickkopf (DKK) family and the WISE/SOST family (Figure 3). DKK proteins, exemplified by DKK1, are LRP5/6 ligands/antagonists and are considered specific inhibitors of Wnt/β-catenin signaling. Although two different models for DKK1 action have been proposed (Mao et al., 2002; Semenov et al., 2001), recent biochemical and genetic studies (Ellwanger et al., 2008; Semenov et al., 2008; Wang et al., 2008) have argued against the model that DKK1 inhibits Wnt signaling via inducing LRP6 internalization/degradation through transmembrane Kremen (Krm) proteins (Mao et al., 2002). DKK1 disruption of Wnt-induced Fz-LRP6 complex remains a more likely mechanism (Semenov et al., 2001), with Krm playing a minor modulatory role in specific tissues (Ellwanger et al., 2008). WISE and SOST constitute another family of LRP5/6 ligands/antagonists (Itasaki et al., 2003; Li et al., 2005; Semenov et al., 2005). Like DKK1, SOST is able to disrupt Wnt-induced Fz-LRP6 complex in vitro (Semenov et al., 2005). Both DKK1 and SOST are strongly implicated in human diseases (see below).

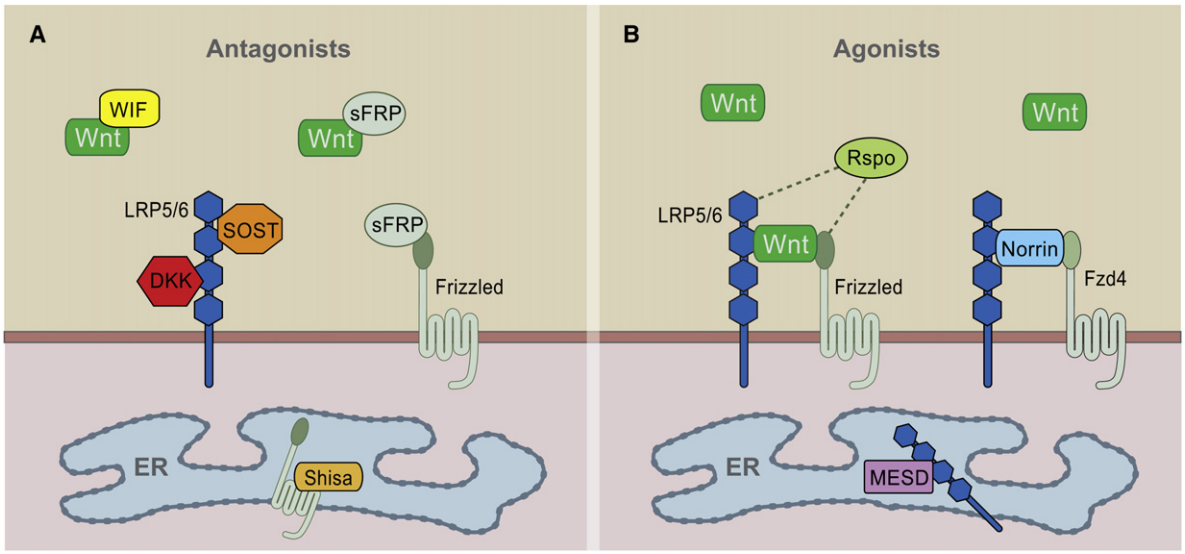

Figure 3. Secreted Wnt Antagonists and Agonists

(A) Antagonists. WIF and sFRP bind directly to secreted Wnts and/or Fz. DKK and SOST/WISE proteins bind LRP5/6 to prevent Fz-LRP6 complex formation. Shisa proteins trap Fz in the ER.

(B) Agonists. Wnts are the primary agonists and form a complex with LRP5/6 and Fz to activate signaling. Norrin acts similarly to Wnt, but binds specifically to FZD4. R-spondin proteins (Rspo) act via and may bind to LRP5/6 and/or Fz receptors. In the ER, the chaperone MESD is needed for LRP5/6 maturation.

Shisa proteins represent a distinct family of Wnt antagonists (Figure 3) that trap Fz proteins in the ER and prevent Fz from reaching the cell surface, thereby inhibiting Wnt signaling cell-autonomously (Yamamoto et al., 2005). Shisa proteins also antagonize fibroblast growth factor (FGF) signaling by trapping FGF receptors in the ER. Other Wnt antagonists with multivalent activities exist. *Xenopus* Cerberus binds to and inhibits Wnt as well as Nodal and bone morphogenetic protein (BMP) (Piccolo et al., 1999), and Insulin-like growth-factor (IGF) binding protein-4 (IGFBP-4) antagonizes Wnt signaling via binding to both Fz and LRP6, in addition to modulating IGF signaling (Zhu et al., 2008).

Norrin and R-spondin (Rspo) proteins are two families of agonists for Wnt/β-catenin signaling (Figure 3). Norrin is a specific ligand for FZD4 and acts through FZD4 and LRP5/6 during retinal vascularization (Xu et al., 2004). Rspo proteins exhibit synergy with Wnt, Fz, and LRP6 (Kazanskaya et al., 2004; Kim et al., 2005; Nam et al., 2006; Wei et al., 2007), and show genetic interaction with LRP6 during embryogenesis (Bell et al., 2008), but their mechanism of action is controversial. Results that Rspo binds to both Fz and LRP6 (Nam et al., 2006), to LRP6 primarily (Wei et al., 2007), or to neither (Kazanskaya et al., 2004) have been reported. Another model suggests that Rspo is a ligand for Krm and antagonizes DKK/Krm-mediated LRP6 internalization (Binnerts et al., 2007), but this seems unlikely given that *Krm1* and *Krm2* double knockout mice are viable and do not exhibit Rspo mutant phenotypes, and that Rspo activates β-catenin signaling in cells lacking both *Krm* genes (Bell et al., 2008; Ellwanger et al., 2008). Rspo genes are often coexpressed with, and depend on, Wnt for expression (Kazanskaya et al., 2004), and may represent a means of positive feedback that reinforces Wnt signaling. Mutations in Norrin and Rspo genes cause distinct hereditary diseases (see below).

Wnt Signaling

Wnt-off State: β-Catenin Phosphorylation/Degradation by the Axin Complex

Cytosolic β-catenin phosphorylation/degradation and its regulation by Wnt are the essence of Wnt signaling (Figure 1). The scaffolding protein Axin uses separate domains to interact with GSK3, CK1α, and β-catenin, and coordinates sequential phosphorylation of β-catenin at serine 45 by CK1α, and then at threonine 41, serine 37, and serine 33 by GSK3 (Kimelman and Xu, 2006). β-catenin phosphorylation at serine 33 and 37 creates a binding site for the E3 ubiquitin ligase β-Trcp, leading to β-catenin ubiquitination and degradation (Figure 4). Mutations of β-catenin at and surrounding these serine and threonine residues are frequently found in cancers, generating mutant β-catenin that escapes phosphorylation and degradation (Table 1). Axin also contains a regulator of G protein signaling (RGS) domain that interacts with APC, a large multifunctional scaffolding protein that binds β-catenin. These core Axin complex components (Kimelman and Xu, 2006) share a common goal of ensuring β-catenin phosphorylation and degradation. Indeed, both APC and Axin are tumor suppressor genes, and APC mutations are particularly prevalent in colorectal cancer (Table 1).

Several aspects of the Axin complex deserve further discussion. (1) In addition to β-catenin, GSK3 and CK1 also phosphorylate Axin and APC, leading to increased association of Axin and APC with β-catenin, and thus, enhanced β-catenin phosphorylation/degradation (Huang and He, 2008; Kimelman and Xu, 2006) (Figure 4). (2) Two abundant serine/threonine phosphatases, PP1 and PP2A, both of which associate with Axin and/or APC, counteract the action of GSK3 and/or CK1 in the Axin complex. Thus PP1 dephosphorylates Axin and promotes the disassembly of the Axin complex (Luo et al., 2007), whereas PP2A dephosphorylates β-catenin (Su et al., 2008), with each

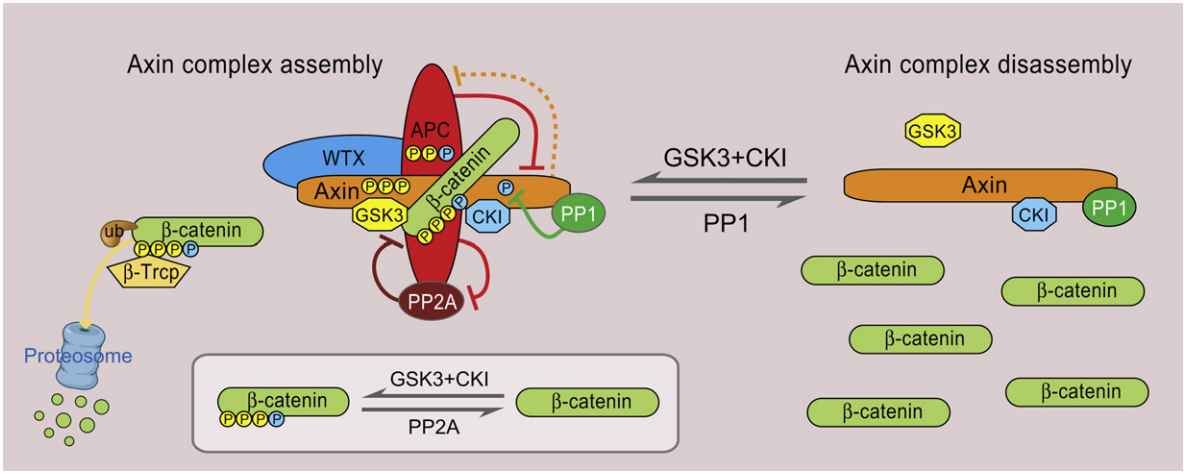

Figure 4. Regulation of Axin Complex Assembly for β-Catenin Degradation

The core components of the Axin complex—Axin, APC, GSK3 and CK1—collectively promote β-catenin phosphorylation for degradation by β-Trcp. In addition to phosphorylating β-catenin, GSK3 (yellow) and CK1 (blue) also phosphorylate Axin and APC and enhance their binding to β-catenin and degradation complex stability, further ensuring β-catenin phosphorylation. The inset illustrates β-catenin phosphorylation (by CK1 and GSK3) and dephosphorylation (by PP2A). APC may also act to prevent PP2A dephosphorylation of β-catenin. APC paradoxically facilitates Axin degradation and possibly vice versa (indicated by dashed line, see text). PP1 dephosphorylates Axin to antagonize CK1 phosphorylation and negatively regulates GSK3-Axin binding, resulting in complex disassembly.

dephosphorylation resulting in reduced β-catenin degradation (Figure 4). One should note that PP2A may have multiple and opposing roles in the Wnt pathway depending on the particular associated regulatory subunits and substrates (Kimelman and Xu, 2006). (3) The assembly of the Axin complex appears to be multivalent and robust. In fly embryos that are null for Axin, expression, at physiological levels, of Axin mutants lacking the APC-, GSK3-, or β-catenin-binding domain restores a significant degree of normal patterning, implying quasifunctional Axin complex assembly via multivalent interactions; furthermore, some of these Axin deletion mutants can complement each other and restore fly viability, possibly via Axin dimerization or multimerization (Peterson-Nedry et al., 2008). Indeed, Axin has multiple potential dimerization domains (Luo et al., 2005) and the Axin DIX domain may form multimeric polymers (Schwarz-Romond et al., 2007a). (4) Axin concentration is exceedingly low compared to other components in *Xenopus* oocytes, indicating that Axin is rate limiting for the complex assembly. This feature may ensure that changes in the Axin protein level will not concomitantly affect the availability of GSK3 (or other components) for non-Wnt functions, thereby further insulating Wnt and other signaling events (Lee et al., 2003). It is unknown, however, whether the drastic difference between the concentration of Axin versus the other components applies universally, and whether different cells employ quantitative differences in the ratio of Axin and other components to shape their unique Wnt response kinetics (such as the speed and level of β-catenin accumulation). Indeed, in *Drosophila* photoreceptors, APC appears to be present at minimal levels such that a 50% reduction alters the graded Wg response (Benchabane et al., 2008).

Other proteins such as Wilms tumor gene on the X chromosome (WTX) may have roles in β-catenin degradation. Loss of WTX and activating β-catenin mutations seem to have nonoverlapping occurrence in Wilms tumor (a pediatric kidney cancer) (Rivera et al., 2007). WTX binds to β-catenin, Axin, APC, and β-Trcp to promote β-catenin ubiquitination, although its biochemical role remains unknown (Major et al., 2007). Another Axin-binding protein, Diversin, can facilitate β-catenin degradation via recruiting CK1ε to phosphorylate β-catenin (Schwarz-Romond et al., 2002).

APC Function and APC-Axin Cross-Regulation

The biochemical nature of APC has been enigmatic. A recent study suggested that APC protects β-catenin from dephosphorylation by PP2A, thereby enhancing β-catenin phosphorylation/degradation (Su et al., 2008) (Figure 4), consistent with the observation that Axin overexpression causes β-catenin degradation even in cells lacking APC function (Behrens et al., 1998). Surprisingly APC (upon phosphorylation by CK1/GSK3) and Axin bind to and compete for the same β-catenin interaction interface, leading to a proposal that APC acts as a "ratchet" to remove phosphorylated β-catenin from Axin for ubiquitination and for an increase in Axin availability for a further round of β-catenin phosphorylation (Kimelman and Xu, 2006; Xing et al., 2003). A different model was proposed based on differential β-catenin binding affinity by unphosphorylated versus phosphorylated APC (Ha et al., 2004). APC has also been shown to promote β-catenin nuclear export and to act as a chromatin-associated suppressor for β-catenin target genes, thus functioning in the nucleus (see below).

Another paradoxical observation is that APC has a positive function in physiological and ectopic Wg/Wnt signaling through the promotion of Axin degradation (Lee et al., 2003; Takacs et al., 2008) (Figure 4). One model suggests that this represents a fail-safe mechanism to buffer dramatic β-catenin fluctuations when APC levels vary (Lee et al., 2003). Thus a decrease in the APC level results in higher Axin amounts, compensating for β-catenin degradation. APC-mediated Axin degradation depends on the APC amino terminal domain that is not involved in β-catenin degradation (Takacs et al., 2008). It is intriguing that colon cancer cells are rarely null for APC but do retain the amino terminal half, suggesting that they may have hijacked a part of this fail-safe regulation for tumorigenesis. Conversely, Axin can also facilitate APC degradation upon overexpression (Choi et al.,

Table 1. Human Diseases Associated with Mutations of the Wnt Signaling Components

Gene	Function	Human Disease	References
PORCN	+ Wnt lipid modification/processing	LOF X-linked Focal dermal hypoplasia	Grzeschik et al., 2007; Wang et al., 2007
WNT3	+ Ligand for Wnt/β-catenin signaling	LOF tetra-amelia	Niemann et al., 2004
WNT4	+ Ligand for Wnt/β-catenin signaling	LOF Mullerian-duct regression and viriliation	Biason-Lauber et al., 2004
WNT5B	+ Ligand for Wnt/β-catenin signaling	(?) type II diabetes	Kanazawa et al., 2004
WNT7A	+ Ligand for Wnt/β-catenin signaling	LOF Fuhrmann syndrome	Woods et al., 2006
WNT10A	+ Ligand for Wnt/β-catenin signaling	LOF odonto-onchyo-dermal hypoplasia	Adaimy et al., 2007
WNT10B	+ Ligand for Wnt/β-catenin signaling	LOF obesity	Christodoulides et al., 2006
RSPO1	+ Wnt agonist	LOF XX sex reversal with palmoplantar hyperkaratosis	Parma et al., 2006
RSPO4	+ Wnt agonist	LOF autosomal recessive anonychia and hyponychia congenita	Bergmann et al., 2006; Blaydon et al., 2006
SOST	- LRP5/6 antagonist predominantly expressed in osteocytes	LOF high bone mass, sclerosteosis, Van Buchem disease	Balemans et al., 2001; Balemans et al., 2002; Brunkow et al., 2001
Norrin (NDP)	+ Specific ligand for FZD4 and LRP5 during eye development	LOF familial exudative vitreoretinopathy	Xu et al., 2004
LRP5	+ Wnt coreceptor	GOF hyperparathyroid tumors (alt. splicing), GOF high bone mass, LOF osteoporosis-pseudoglioma, LOF FEVR eye vascular defects	Bjorklund et al., 2007; Boyden et al., 2002; Gong et al., 2001; Little et al., 2002; Toomes et al., 2004
LRP6	+ Wnt coreceptor	LOF early coronary disease and osteoporosis	Mani et al., 2007
FZD4	+ Wnt receptor	LOF familial exudative vitreoretinopathy	Robitaille et al., 2002
Axin1	− facilitates β-catenin degradation; acts as a tumor suppressor	LOF caudal duplication, cancer	Oates et al., 2006; Satoh et al., 2000
Axin2	− facilitates β-catenin degradation; acts as a tumor suppressor	LOF tooth agenesis, cancer	Lammi et al., 2004; Liu et al., 2000
APC	− facilitates β-catenin degradation; acts as a tumor suppressor	LOF familial adenomatous polyposis, cancer	Kinzler et al., 1991; Nishisho et al., 1991
WTX	− facilitates β-catenin degradation; acts as a tumor suppressor	LOF Wilms tumor	Major et al., 2007; Rivera et al., 2007
β-catenin (CTNNB1)	+ primary Wnt effector; acts as anoncogene	GOF cancer	Korinek et al., 1997; Morin et al., 1997
TCF4 (TCF7L2)	+ β-catenin transcriptional partner	(?) type II diabetes	Florez et al., 2006; Grant et al., 2006

LOF, loss-of-function; GOF, gain-of-function.

2004), constituting perhaps the other side of the Axin-APC regulation circuit (Figure 4). Mechanisms for Axin and APC degradation, which are proteosome-dependent, have not been characterized.

Wnt-on State: Activation of Wnt Receptors

Wnt signaling requires both Fz and LRP6 (or LRP5), likely through a Wnt-induced Fz-LRP6 complex (Figure 1). Wnt-induced LRP6 phosphorylation is a key event in receptor activation (Tamai et al., 2004). LRP6, LRP5, and Arrow each have five reiterated PPPSPxS motifs (P, proline; S, serine or threonine, x, a variable residue), which are essential for LRP6 function and are each transferrable to a heterologous receptor to result in constitutive β-catenin signaling (MacDonald et al., 2008; Tamai et al., 2004; Zeng et al., 2005). These dually phosphorylated PPPSPxS motifs are docking sites for the Axin complex (Davidson et al., 2005; Tamai et al., 2004; Zeng et al., 2005), thereby recruiting Axin to LRP6 upon Wnt stimulation (Mao et al., 2001) (Figure 5).

The kinases responsible for PPPSPxS phosphorylation have been identified unexpectedly as GSK3 and CK1 (Davidson et al., 2005; Zeng et al., 2005). Although one study argued that only CK1 phosphorylation is Wnt induced (Davidson et al., 2005), most available data support that Wnt induces PPPSP phosphorylation (Binnerts et al., 2007; Khan et al., 2007; Pan et al., 2008b; Wei et al., 2007), which is carried out by GSK3 and primes xS phosphorylation by CK1, thereby leading to dually induced phosphorylation (Zeng et al., 2005) (Figure 5). Although potential involvement of additional kinases cannot be ruled out, experiments in GSK3α/β null cells indicate that GSK3 accounts for most, if not all, PPPSP phosphorylation (Zeng et al., 2005, 2008). As in β-catenin phosphorylation, Axin-bound GSK3 appears to mediate LRP6 phosphorylation (Zeng et al., 2008). Thus PPPSPxS phosphorylation exhibits a mirror image of β-catenin phosphorylation in sequential order, in priming requirement, and importantly, in functionality, but apparently by the same Axin-GSK3 complex (Huang and He, 2008) (Figure 5).

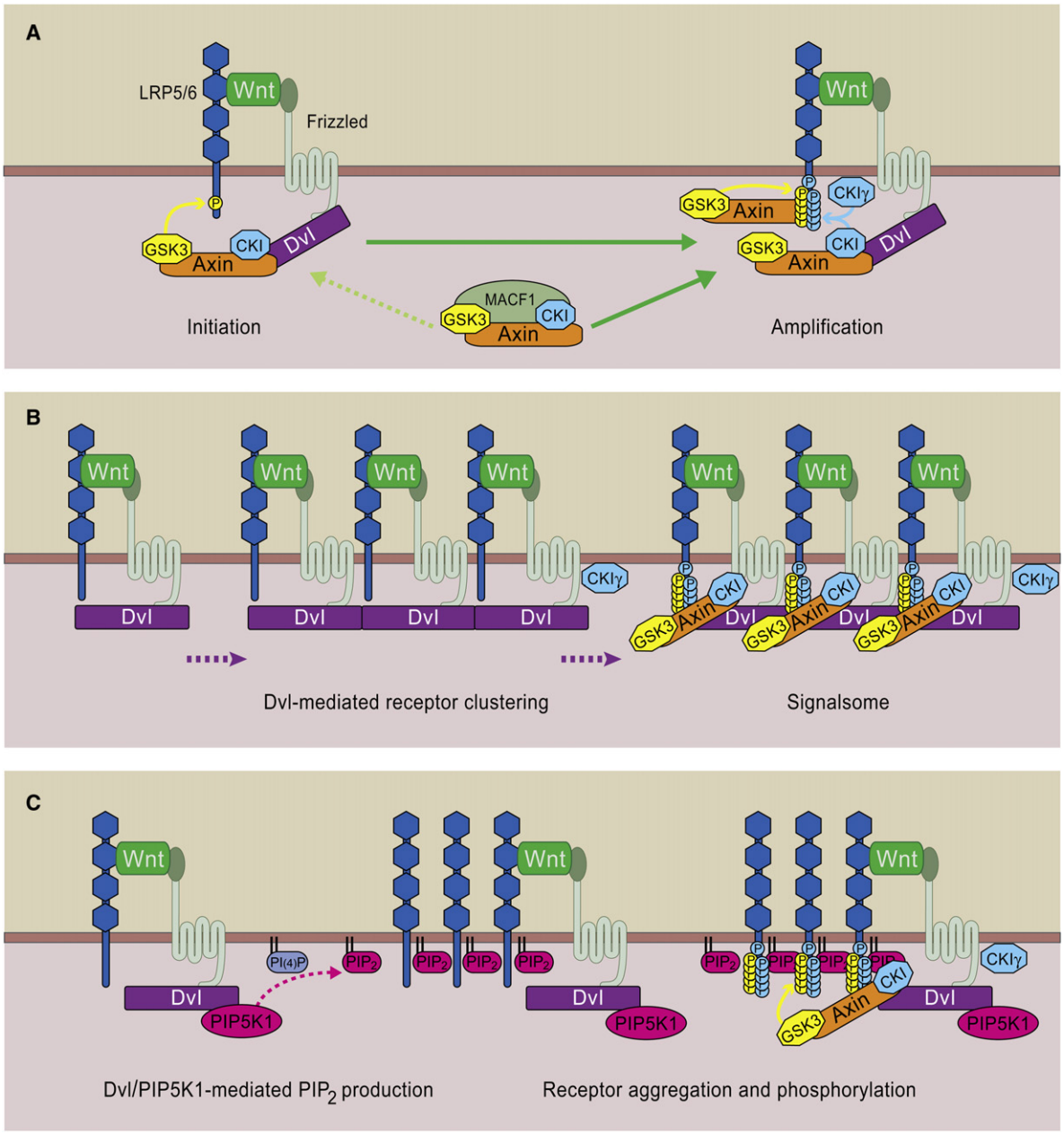

Figure 5. Models of Wnt Receptor Activation

(A) Initiation and amplification. Wnt forms a complex with LRP6 and Fz-Dvl at the membrane. Dvl recruits Axin-GSK3, resulting in the phosphorylation of one or more PPPSP motifs in LRP6 (initiation). Partially phosphorylated LRP6 may be able to recruit and more efficiently bind Axin-GSK3 and promote more PPPSP phosphorylation (amplification).

(B) Signalsome formation via Dvl polymerization and receptor clustering. The oligiomerization property of Dvl promotes the aggregation of individual Wnt-LRP6-Fz complexes, resulting in Axin recruitment to the membrane and LRP6 phosphorylation by GSK3 and CK1.

(C) PI4KIIα and PIP5KI kinases, the latter of which binds directly with Dvl and promote PIP_2 production and receptor clustering/phosphorylation.

The configurations of receptor clustering in (B) and (C) were drawn arbitrarily. In all models, PPPSPxS motifs are sequentially phosphorylated by GSK3 and CK1, probably via CK1γ (membrane-associated) and/or CK1α and CK1ε associated with Axin and Dvl, respectively, and MACF1 may have a role in the translocation of the Axin complex to the receptors.

This unusual mechanism, using the same kinase complex for both positive and negative regulation, is reminiscent of another morphogenetic pathway, Hedgehog signaling in *Drosophila* (Price, 2006), and implies the simple view that Wnt signaling regulates the two opposing activities of the Axin-GSK3 complex.

One caveat is that GSK3 is genetically defined as a negative regulator of β-catenin signaling. The positive requirement of GSK3 in LRP6 activation is demonstrated when a membrane-tethered GSK3 inhibitory peptide blocks Wnt signaling (Zeng et al., 2008).

Fz function is required for Wnt-induced LRP6 phosphorylation, and forced Fz-LRP6 association is sufficient to trigger LRP6 phosphorylation (Zeng et al., 2008). Fz function is usually linked to Dsh/Dvl (Wallingford and Habas, 2005), a cytoplasmic scaffolding protein that may directly interact with Fz (Wong et al., 2003). Indeed, Fz-Dvl interaction and Dvl function are critical for Wnt-induced LRP6 phosphorylation (Bilic et al., 2007; Zeng et al., 2008). Because Dvl interacts with Axin (Wallingford and Habas, 2005), and is required for Axin recruitment to the plasma membrane during Wg signaling (Cliffe et al., 2003) or in Fz overexpression (Zeng et al., 2008), one model stipulates that Fz-Dvl recruitment of the Axin-GSK3 complex initiates LRP6 phosphorylation by GSK3 (Zeng et al., 2008) (Figure 5A).

Several features of Wnt receptor activation deserve further discussion. (1) The observation that Axin is required for LRP6 phosphorylation, and that phosphorylated LRP6 in turn recruits Axin, suggests a positive feedforward loop, potentially amplifying and ensuring the phosphorylation of all five PPPSPxS motifs (Figure 5). Indeed, the phosphorylation of these motifs relies on the presence of one another, and LRP6 activity is particularly sensitive to the PPPSPxS copy number (MacDonald et al., 2008; Wolf et al., 2008). This may explain the distinct roles of Fz and LRP6/Arrow in the "initiation" (which requires both Fz and Arrow) and "amplification" (which requires Arrow only) during Wg signaling (Baig-Lewis et al., 2007) (Figure 5A). (2) It has been reported that Wnt-induced clustering of Fz-LRP6 receptor critically depends on Dvl, Axin, and GSK3 for formation (see below) (Bilic et al., 2007; Schwarz-Romond et al., 2007a). Although unambiguous evidence for such aggregation under physiological conditions without overexpression remains to be demonstrated, this "signalsome" model (Figure 5B) and the "initiation-amplification" model (Figure 5A) together provide a spatial and temporal framework for understanding Wnt receptor activation. (3) Wnt also induces LRP6 phosphorylation by CK1γ outside the PPPSPxS motifs, in particular in a conserved S/T cluster amino-terminal to the first PPPSPxS motif (Davidson et al., 2005). This region upon phosphorylation binds to GSK3 (Piao et al., 2008), potentially accounting for the observed LRP6-GSK3 interaction (Mi et al., 2006; Zeng et al., 2005). The significance of this S/T cluster in LRP6 function has not been investigated in the intact receptor, but these results imply multiple interaction interfaces among LRP6, Axin, and GSK3. (4) Wnt may also "activate" Fz, which is structurally related to G protein-coupled receptors (GPCRs). Some genetic and pharmacological evidence suggests that trimeric G proteins, specifically Gα_o and Gα_q, are required downstream of Fz and probably upstream of Dvl in Wnt/β-catenin signaling (Katanaev et al., 2005; Liu et al., 2001, 2005). Whether G proteins are involved in Wnt/Fz/Dvl-regulated LRP6 phosphorylation is unknown.

Dvl is involved in Wnt/β-catenin and other Wnt/Fz-dependent pathways and has numerous putative binding partners (Wallingford and Habas, 2005). For example, CK1ε (or CK1δ) binds to Dvl and is a potent activator of β-catenin signaling, possibly via phosphorylating Dvl, LRP6, and/or the Axin complex (Price, 2006) (Figure 5). PP2A also associates with Dvl but has a positive or negative influence on Wnt signaling depending on the associated regulatory subunit (Kimelman and Xu, 2006). In addition, Dvl is subjected to proteasomal degradation via distinct ubiquitination pathways (Angers et al., 2006; Simons et al., 2005). Some of these Dvl regulation events have been suggested to switch Dvl between β-catenin and noncanonical pathways. Despite this progress, the mechanism by which Dvl acts in Wnt/β-catenin signaling remains enigmatic. Two recent findings suggest potential new insights. (1) Polymerization/aggregation of Dvl (and Axin). Fz-Dvl and Dvl-Axin interactions are relatively weak (Schwarz-Romond et al., 2007b; Wong et al., 2003). However, Dvl and Axin each harbor a homologous DIX domain that exhibits dynamic polymerization (Schwarz-Romond et al., 2007a). This unusual property is proposed to allow Dvl and Axin to form large aggregates that facilitate weak but dynamic protein interactions (Figure 5B). Indeed, Wnt-induced receptor clustering requires an intact Dvl DIX domain (Bilic et al., 2007; Schwarz-Romond et al., 2007a). It is unclear whether Wnt regulates DIX-dependent polymerization, and perhaps in a related manner, Fz-Dvl or Dvl-Axin interaction. (2) Dvl stimulation of phosphatidylinositol 4,5-bisphosphate [PtdIns (4,5)P_2 or PIP_2] production by sequential actions of phosphatidylinositol 4-kinase type II (PI4KIIα) and phosphatidylinositol-4-phosphate 5-kinase type I (PIP5KI) (Pan et al., 2008b). Wnt induces Dvl, via the DIX domain, to bind to and activate PIP5KI, and the resulting PIP_2 production is suggested to promote LRP6 clustering and phosphorylation, although the underlying mechanism remains unclear (Figure 5C). Given that PIP_2 has pleiotropic functions in cells, including receptor endocytosis (see below), other potential mechanisms for PIP_2 in LRP6 phosphorylation remain to be explored. Nonetheless Dvl DIX polymerization and stimulation of PIP_2 may act in concert to ensure LRP6 clustering/phosphorylation/activation.

Other Regulatory Events at or proximal to Wnt Receptors

A cytoplasmic protein in vertebrates, referred to as Caprin-2, binds to LRP6 and facilitates LRP6 phosphorylation by GSK3 (Ding et al., 2008). Caprin-2 has an oligomerization domain that may enhance LRP6 aggregation, and Caprin-2 additionally may also associate with both GSK3 and Axin and promote LRP6-Axin-GSK3 complex formation (Ding et al., 2008). Besides the requirement of Dvl, recruitment of Axin to the receptor complex may involve a giant protein (600 kD), microtubule actin cross-linking factor 1 (MACF1) (Chen et al., 2006). MACF1 is a member of the spectraplakin family of proteins that link the cytoskeleton to junctional proteins. Defective gastrulation in *Macf1*$^{-/-}$ mouse embryos phenotypically resembles *Lrp5/6*$^{-/-}$ double knockout mutants. Upon Wnt stimulation MACF1 associates with the Axin complex (including APC) in the cytosol and with LRP6 and the Axin complex (but not APC) in the membrane fraction (Chen et al., 2006), and may shuttle Axin to LRP6 (Figure 5A). This MACF1 function may be vertebrate specific because *Drosophila* MACF1 (*shortstop*) mutants do not exhibit *wg*-related phenotypes.

A controversial issue concerns the involvement of receptor endocytosis in Wnt signaling. Contradicting results have been reported regarding the role of clathrin- and caveolin-dependent receptor internalization and endosomal trafficking in Wnt receptor activation and signaling (Gagliardi et al., 2008). For example, one study suggested that caveolin-mediated LRP6 endocytosis promotes Axin recruitment and is required for Wnt stabilization of β-catenin (Yamamoto et al., 2006), whereas another study using *caveolin*$^{-/-}$ mutant mice reached the apparently opposite conclusion (Sotgia et al., 2005). Pharmacological and molecular

tools currently in use to block receptor endocytosis and trafficking have pleiotropic and sometimes nonspecific effects, making interpretations of some of these studies complicated.

Inhibition of β-Catenin Phosphorylation

How receptor activation leads to inhibition of β-catenin phosphorylation remains uncertain, and available data suggest possible parallel mechanisms. In the LRP6-centric view, because constitutively activated forms of LRP6 fully activate β-catenin signaling in an apparently Fz- and Dvl-independent manner (He et al., 2004), LRP6 represents the key output whereas Fz and Dvl act upstream to control LRP6 activation (Bilic et al., 2007; Zeng et al., 2008). On the other hand, Dsh overexpression in *Drosophila* or recombinant Dvl in *Xenopus* egg extracts can activate β-catenin signaling presumably in the absence of Arrow/LRP6 (Salic et al., 2000; Wehrli et al., 2000), and so does a GPCR-Fz chimeric protein in response to the GPCR ligand (Liu et al., 2001). These results argue that Fz/Dvl may activate β-catenin signaling independent of LRP6. The fact that nematodes have a related Wnt/β-catenin pathway (Phillips and Kimble, 2009), but have no LRP6 homolog, may be consistent with this notion. Perhaps in *Drosophila* and vertebrates, Wnt signaling components exist under suboptimal levels and the two parallel branches need to operate together to counteract efficient β-catenin phosphorylation/degradation, whereas overactivation of either branch is sufficient to stabilize β-catenin.

Several biochemical mechanisms by which Wnt inhibits β-catenin phosphorylation have been suggested (Kimelman and Xu, 2006). (1) Wnt-induced and Dvl-(and Gα)-dependent Axin-GSK3 (or β-catenin) dissociation (Liu et al., 2005; Logan and Nusse, 2004). PP1 dephosphorylation of Axin also causes Axin-GSK3 dissociation (Luo et al., 2007) (Figure 4), but whether PP1 counteracts Axin complex assembly constitutively or is regulated by Wnt is unknown. (2) Inhibition of GSK3. Phosphorylated LRP6 cytoplasmic domain or individual phospho-PPPSPxS peptides can directly inhibit GSK3 phosphorylation of β-catenin in vitro (Cselenyi et al., 2008; Piao et al., 2008; Wu et al., 2009), suggesting a potential mechanism for β-catenin stabilization. This is consistent with the observation that upon Wnt stimulation, dephosphorylated β-catenin first appears at the plasma membrane close to activated LRP6 and Axin (Hendriksen et al., 2008). (3) Axin degradation. Overexpression of activated Wnt receptors or recombinant Dvl can lead to Axin degradation (Lee et al., 2003; Mao et al., 2001; Tolwinski et al., 2003), whereas GSK3 phosphorylation stabilizes Axin (Willert et al., 1999; Yamamoto et al., 1999). However, Wnt-induced Axin degradation lags significantly behind β-catenin stabilization (Liu et al., 2005; Willert et al., 1999; Yamamoto et al., 1999), and thus is unlikely to represent a primary response despite its importance. Wnt signaling elevates APC protein levels (Choi et al., 2004; Doble et al., 2007), but whether APC accumulation is responsible for Wnt-induced Axin degradation is unknown.

β-Catenin Nuclear Function

β-Catenin Nuclear/Cytoplasmic Shuttling and Retention

β-catenin stabilization results in its higher nuclear levels, but how β-catenin is shuttled to and retained in the nucleus is not well understood (Henderson and Fagotto, 2002; Stadeli et al., 2006). Earlier studies suggested that β-catenin enters the nucleus in a nuclear localization signal (NLS)- and importin-independent fashion by interacting directly with nuclear pore proteins (Henderson and Fagotto, 2002). β-catenin also exits the nucleus via export involving APC (Henderson and Fagotto, 2002), Axin (Cong and Varmus, 2004), and Ran binding protein 3 (RanBP3), which binds to β-catenin in a Ran-GTP-dependent manner (Hendriksen et al., 2005). Live cell imaging suggests that while Axin and APC can enrich β-catenin in the cytoplasm, and while TCF and β-catenin coactivators (BCL9 and Pygopus [Pygo]; see below) increase nuclear β-catenin, they do not accelerate the export or import rate of β-catenin, thereby arguing for their roles in β-catenin retention rather than shuttling (Krieghoff et al., 2006). Thus β-catenin nuclear and cytoplasmic partitioning is likely the dynamic sum of both shuttling and retention between the two compartments via multiple mechanisms.

A recent study argues that Wnt-induced β-catenin stabilization is not sufficient for its nuclear accumulation, but Wnt activation of the Rac1 GTPase is required in parallel (Wu et al., 2008). Specifically, Rac1, Jun N-terminal kinase 2 (JNK2), and β-catenin form a cytoplasmic complex, and JNK2 phosphorylates β-catenin (at serines 191 and 605) and promotes its nuclear translocation. Other studies have suggested a role of Rac1 and its guanine nucleotide exchange factors in β-catenin signaling, either as components of the TCF/β-catenin transcriptional complex or as an antagonistic partner of the Axin-APC complex (Esufali and Bapat, 2004; Schlessinger et al., 2009). Further studies will be required to clarify and substantiate whether and/or how Rac1, which participates in noncanonical Wnt signaling, is involved in the Wnt/β-catenin pathway.

TCF/LEF

The TCF/LEF family of DNA-bound transcription factors is the main partner for β-catenin in gene regulation (Arce et al., 2006; Hoppler and Kavanagh, 2007). TCF represses gene expression by interacting with the repressor Groucho (TLE1 in human), which promotes histone deacetylation and chromatin compaction; Wnt-induced β-catenin stabilization and nuclear accumulation leads TCF to complex with β-catenin, which appears to displace Groucho (Daniels and Weis, 2005) and recruits other coactivators for gene activation (Figure 1). While a single TCF gene is found in *Drosophila* and worm, four TCF genes, TCF1, LEF1, TCF3, and TCF4, exist in mammals. Alternative splicing and promoter usage produce a large number of TCF variants with distinct properties (Arce et al., 2006; Hoppler and Kavanagh, 2007). TCF proteins are high-mobility group (HMG) DNA-binding factors, and upon binding to a DNA consensus sequence referred to as the Wnt responsive element (WRE), CCTTTGWW (W represents either T or A), they cause significant DNA bending that may alter local chromatin structure. A genome-wide analysis in colon cancer cells suggests that TCF4/β-catenin target genes are frequently "decorated" with multiple WREs, most of which are located at large distances from transcription start sites (Hatzis et al., 2008). Some TCF1 and TCF4 splicing variants harbor a second DNA-binding domain called C-clamp, which recognizes an additional GC element downstream of the typical WRE, allowing regulation of different sets of target genes (Atcha et al., 2007). These similarities and differences, combined with overlapping and unique expression patterns, underlie in part distinct and sometimes redundant functions of vertebrate/mammalian TCF genes. Note however that all major isoforms of the *Drosophila*

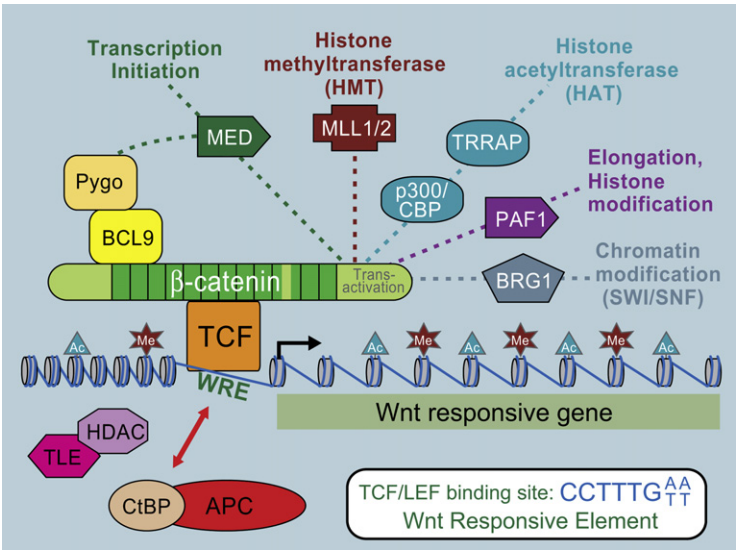

Figure 6. Nuclear TCF/β-Catenin Coactivator Complexes
Upon Wnt/β-catenin signaling activation, WRE-bound TCF/β-catenin recruits many coactivator complexes to Wnt target genes. For simplicity only a few representative complexes are illustrated. Dotted lines represent their interactions with β-catenin or between complexes. During active Wnt target gene transcription, APC promotes the exchange between β-catenin/coactivators with corepressors CtBP, TLE, and HDAC in a cyclic manner (double-headed red arrow) while TCF remains bound to the WRE. Ac and Me symbolize histone modifications, i.e. acetylation and methylation, respectively.

TCF contain the C-clamp domain that binds to a seven-base-pair "helper site" near the classic WRE, both of which together are required to mediate most, if not all, Wg-responsive gene expression (Chang et al., 2008). Some general rules appear to describe TCF/LEF functions approximately: TCF1 and TCF4 act as both repressors and activators, and LEF1 is often an activator whereas TCF3 is mostly a repressor but sometimes an activator (Arce et al., 2006; Hoppler and Kavanagh, 2007).

Three major strategies exist to regulate TCF/β-catenin transcription. (1) Alternative promoter usage in TCF1 and LEF1 genes produces dnTCF1/dnLEF1, which lack the amino-terminal β-catenin-binding domain and thus act as the endogenous dominant-negative TCF/LEF (Arce et al., 2006; Hoppler and Kavanagh, 2007). Indeed, the TCF1 locus acts as an intestinal tumor suppressor primarily due to the production of dnTCF1, which antagonizes TCF4 in stem cell renewal. (2) Nuclear antagonists Chibby and ICAT bind to β-catenin and disrupt β-catenin/TCF and β-catenin/coactivator interactions and promote β-catenin nuclear export (Li et al., 2008; Tago et al., 2000). Besides these devoted inhibitors, many DNA-binding transcription factors interact with β-catenin or TCF and can antagonize TCF/β-catenin-dependent transcription (Table S1). For example, KLF4 inhibition of β-catenin transcriptional activation is important for intestinal homeostasis and tumor suppression (Zhang et al., 2006). (3) Posttranslational modifications of TCF/LEF exist, including phosphorylation, acetylation, sumoylation, and ubiquitination/degradation (Arce et al., 2006; Hoppler and Kavanagh, 2007; Phillips and Kimble, 2009, this issue). For instance, TCF3 phosphorylation by CK1ε and LEF1 phosphorylation by CK2 enhances their binding to β-catenin and diminishes LEF1 binding to Groucho/TLE, whereas LEF1 and TCF4 phosphorylation by Nemo-like kinase (NLK) leads to less LEF/TCF/β-catenin complex binding to DNA and leads to LEF1/TCF4 degradation. LEF1 and TCF4 sumoylation (by the SUMO ligase PIASy) represses LEF1 activity by targeting it to nuclear bodies but enhances TCF4/β-catenin transcription, whereas CBP-mediated acetylation of TCF results in decreased TCF/β-catenin binding in *Drosophila* and increased TCF nuclear retention in nematodes, both leading to transcriptional repression. These diverse modifications are often specific to individual TCF/LEF proteins, conferring differential regulation. It is unclear whether these TCF/LEF modifications are controlled by Wnt or other signaling pathways in vertebrates. Studies in nematodes have demonstrated that Wnt directly regulates TCF phosphorylation (by NLK) and therefore its nuclear export (in addition to β-catenin stability), and the nuclear TCF:β-catenin ratio, intriguingly, may determine transcriptional activation versus repression (Phillips and Kimble, 2009, this issue).

β-Catenin-Associated Coactivators

A plethora of β-catenin-associated coactivators have been identified. These multiprotein complexes include BCL9 and Pygo, Mediator (for transcription initiation), p300/CBP and TRRAP/TIP60 histone acetyltransferases (HATs), MLL1/2 histone methyltransferases (HMTs), the SWI/SNF family of ATPases for chromatin remodeling, and the PAF1 complex for transcription elongation and histone modifications (Mosimann et al., 2009; Willert and Jones, 2006) (Figure 6). While the central Arm-repeats of β-catenin associate with TCF, and the amino-terminal Arm-repeat binds to BCL9, most of the coactivator complexes interact with the β-catenin carboxyl terminal portion (Figure 6), creating a dazzling interplay between β-catenin, the transcriptional apparatus, and the chromatin. Indeed, TCF/β-catenin binding to WREs leads to histone acetylation in a CBP-dependent manner over a significant genomic distance (30 kb), suggesting that local TCF/β-catenin recruitment results in widespread chromatin modifications (Parker et al., 2008). Histone H3K4 (lysine 4) trimethylation, which is indicative of active transcription, is also observed at the WRE at the c-Myc gene, a known Wnt target gene in some mammalian cells (Sierra et al., 2006), but whether this is due directly to the recruitment of HMTs by β-catenin or indirectly to reflect active promoter usage remains to be established (Parker et al., 2008). Whether various β-catenin-recruited coactivator complexes act simultaneously or in a particular sequential order remains unclear.

Intriguingly, under a Wnt-stimulated condition, while LEF1 occupies the WRE in the c-Myc gene continuously, β-catenin and its coactivators cycle on and off the WRE with a 60 min periodicity and are replaced by Groucho/TLE1 (Sierra et al., 2006; Wang and Jones, 2006). Thus Groucho/TLE1 also acts as a repressor during Wnt-responsive transcription. It is unknown how the cyclic occupancy is governed and whether this cyclic pattern between β-catenin/activators and Groucho/repressors is integral to transcription of other Wnt-responsive genes, but this dynamic exchange requires APC recruitment to chromatin (Figure 6). These separate silencing (in the absence of Wnt)

and repressing (during Wnt stimulation) functions of Groucho/TLE1 may apply to other repressors such as CtBP, and may help to explain different modes of CtBP repressor function that have been described (Fang et al., 2006; Hamada and Bienz, 2004; Sierra et al., 2006). Curiously, CtBP (Fang et al., 2006) and two related components of the SMRT/N-CoR corepressor complex, TBL1 and TBLR1 (Li and Wang, 2008), are also β-catenin coactivators for at least some Wg/Wnt-responsive genes. In sum, both coactivator and corepressors appear to be active during β-catenin-mediated transcription, perhaps ensuring a dynamic chromatin environment for precise Wnt responses.

Unlike most coactivators that have general roles in transcription, BCL9 and Pygo in *Drosophila* are specifically required for β-catenin-dependent transcription and their biochemical functions proposed provide a glimpse of the complexity of TCF/β-catenin-coactivator interactions (Mosimann et al., 2009). (1) BCL9 and Pygo function as a "chain of activators" (Hoffmans et al., 2005). β-catenin binding to BCL9 recruits Pygo, which also interacts with Mediator (Carrera et al., 2008) (Figure 6); (2) Pygo is constitutively nuclear and may have a role in recruiting/retaining BCL9/β-catenin in the nucleus upon Wg/Wnt signaling (Brembeck et al., 2004; Townsley et al., 2004); (3) Pygo also co-occupies chromatin loci with and via TCF in the absence of Wg signaling (despite a lack of direct TCF-Pygo interaction), and may help capture BCL9/β-catenin for TCF at the onset of Wg signaling (de la Roche and Bienz, 2007); and (4) Pygo has a plant homology domain (PHD) that binds preferentially to dimethylated H3K4 upon interaction with BCL9 (Fiedler et al., 2008). This "histone code" recognition leads to the speculation that Pygo/BCL9 act during the transition from gene silencing to Wnt-induced transcription by participating in histone methylation changes. Alternatively, Pygo/BCL9 binding to dimethylated H3K4 may provide a separate β-catenin anchor on chromatin, thereby freeing TCF for interaction with Groucho to pause or terminate transcription (Mosimann et al., 2009). Finally, (5) Pygo function is not required when Groucho activity is absent, suggesting that Pygo acts as an antirepressor (Mieszczanek et al., 2008). Therefore, either a single biochemical mechanism of Pygo underlies these diverse observations, or multiple functional properties of Pygo participate in β-catenin signaling. Perplexingly, however, Pygo function is not as critical for Wnt/β-catenin signaling in mice, because *Pygo1* and *Pygo2* double mutants exhibit significantly milder phenotypes than expected (Schwab et al., 2007), possibly reflecting Pygo's redundancy with other coactivators. Mammalian BCL9 function has also diverged compared to that in *Drosophila*, and exhibits cell-type-specific and Pygo-independent roles in modulating Wnt signaling (Sustmann et al., 2008).

Nuclear Functions of "Cytoplasmic" Wnt Signaling Components

APC also acts directly on chromatin/WREs to antagonize β-catenin-mediated gene activation via promoting the exchange of coactivators with corepressors in a stepwise and oscillating manner, because such exchange does not occur in APC mutant cancer cells (Sierra et al., 2006). How APC is recruited to chromatin is a mystery, but it is unlikely due to β-catenin/TCF, because APC and TCF bind to β-catenin in a mutually exclusive manner. GSK3 and β-Trcp also appear to be associated with the WRE in a cyclic fashion that synchronizes with APC but is opposite to that of β-catenin/coactivators, suggesting that they may have negative roles in TCF/β-catenin-mediated transcription (Sierra et al., 2006). Some studies have also suggested that Dvl is observed in the nucleus (Itoh et al., 2005; Torres and Nelson, 2000) and that nuclear Dvl is a component of the TCF/β-catenin complex and facilitates TCF/β-catenin interaction in conjunction with the c-Jun transcription factor (Gan et al., 2008). While provocative, some of these findings require substantiation and, in particular, reconciliation with (the lack of) genetic evidence. Nonetheless, the proposed nuclear functions for Dvl, APC, and perhaps GSK3 and β-Trcp in the TCF/β-catenin complex are in sync with their cytoplasmic functions in regulation of β-catenin stability (i.e., to either promote or inhibit β-catenin signaling).

β-Catenin-Mediated Repression and Other Transcriptional Events

Wnt signaling, via the TCF/β-catenin complex, also represses transcription of certain target genes. Note that this is distinct from TCF-mediated repression in the absence of β-catenin. One mechanism is competitive repression, through which TCF/β-catenin displaces or inhibits other DNA-binding transcription activators (Kahler and Westendorf, 2003; Piepenburg et al., 2000). Another mechanism is direct repression via TCF/β-catenin binding to the canonical WREs by recruiting corepressors (Jamora et al., 2003; Theisen et al., 2007). A third mechanism is revealed by a novel TCF binding element, AGAWAW, which specifically mediates TCF/β-catenin repression in *Drosophila* (Blauwkamp et al., 2008). There is evidence that β-catenin is capable of recruiting corepressors including Groucho/TLE and histone deacetylases (Olson et al., 2006), but the mechanism by which β-catenin recruits coactivators versus corepressors is unknown. The involvement of cofactors (Theisen et al., 2007) or distinct TCF/β-catenin configurations offers potential explanations. A less understood aspect of β-catenin signaling is that many DNA-binding transcription factors, in addition to TCF/LEF, interact with β-catenin to either activate or repress transcription (Table S1B). These β-catenin partners in principle expand significantly the gene expression programs that are regulated by Wnt/β-catenin signaling, but further substantiation of their roles in mediating Wnt signaling is required.

Wnt/β-Catenin Target Genes and Wnt Pathway Self-Regulation

Because Wnt/β-catenin signaling regulates proliferation, fate specification, and differentiation in numerous developmental stages and adult tissue homeostasis, Wnt target genes are diverse (Vlad et al., 2008) and cell and context specific (Logan and Nusse, 2004). An emerging feature is that Wnt signaling components, including Fz, LRP6, Axin2, TCF/LEF, Naked (a Dvl antagonist), DKK1, and Rspo, are often regulated positively or negatively by TCF/β-catenin (Chamorro et al., 2005; Kazanskaya et al., 2004; Khan et al., 2007; Logan and Nusse, 2004). Wnt induction of Axin2, DKK1, and Naked and suppression of Fz and LRP6 constitute negative feedback loops that dampen Wnt signaling, and the suppression of Fz and LRP6 also enhances Wg/Wnt gradient formation over longer distances (Logan and Nusse, 2004). On the contrary, Wnt induction of Rspo and TCF/LEF genes constitutes positive feedforward circuits that reinforce Wnt signaling, a feature that has been exploited during colon carcinogenesis (Arce et al., 2006; Hoppler and Kavanagh, 2007). These various Wnt pathway self-regulatory loops are mostly utilized in a cell-specific manner, affording additional

complexity in the control of amplitude and duration of Wnt responses. However, Axin2 and *Drosophila* Naked genes seem to represent a few "universal" Wnt/Wg-induced target genes, respectively (Logan and Nusse, 2004).

Wnt/β-Catenin Signaling in Diseases and Potential Therapeutics

Given the critical roles of Wnt/β-catenin signaling in development and homeostasis, it is no surprise that mutations of the Wnt pathway components are associated with many hereditary disorders, cancer, and other diseases (Table 1). These include mutations in Porcupine (PORCN in human) and various Wnt ligands, agonists, and antagonists, and have shed light on Wnt regulation of human development. For example, RSPO1 mutations result in XX sex reversal (Parma et al., 2006), a condition with similarities to patients with WNT4 mutations (Biason-Lauber et al., 2004), which is consistent with cooperation between Rspo and Wnt genes. Mutations in either FZD4 or LRP5 are associated with familial exudative vitreoretinopathy (FEVR) (Toomes et al., 2004), which is manifested by defective retinal vascularization that is also seen in Norrie-disease-carrying Norrin mutations, leading to the identification of Norrin as a ligand for FZD4 and LRP5 (Xu et al., 2004).

One fast-growing field involves Wnt/β-catenin regulation of bone mass, because osteoporosis remains a global health problem. This was triggered by the discovery of LRP5 loss-of-function mutations in patients with osteoporosis pseudoglioma syndrome (OPPG), a recessive disorder characterized by low bone mass and abnormal eye vasculature (Gong et al., 2001). Conversely, patients with autosomal dominant high bone mass (HBM) diseases harbor LRP5 missense (gain-of-function) mutations (Boyden et al., 2002; Little et al., 2002), which are clustered in the LRP5 extracellular domain and render LRP5 resistant to binding and inhibition by the antagonist SOST (Ellies et al., 2006; Semenov and He, 2006) and DKK1 (Ai et al., 2005). Interestingly, mutations in the SOST gene cause Sclerosteosis (Table 1), which resembles HBM disorders. Thus LRP5 activity likely correlates with bone mass via regulation of osteoblast (bone-forming cell) proliferation, whereas SOST and DKK1, which are specifically expressed in osteocytes, negatively regulate bone mass by antagonizing LRP5. Given that reduction of sFRP1 or DKK1 also results in increased bone mass in mice, secreted Wnt antagonists have become important drug targets for the treatment of osteoporosis (Williams and Insogna, 2008). Indeed, humanized antibodies against SOST and a small molecule antagonist against sFRP1 are being tested, and both showed promise in increasing bone mass (Bodine et al., 2009). Antibodies against DKK1 have also been used to treat mouse models of multiple myeloma (Yaccoby et al., 2007) and osteoarthritis (Diarra et al., 2007), both of which are associated with bone tissue loss. Because LRP6 also acts as a coreceptor for parathyroid hormone (PTH) (Wan et al., 2008), which is used as a drug for osteoporosis, and because a new model for LRP5 function in bone mass regulation via an endocrine route (through serotonin) has recently been proposed (Yadav et al., 2008), interest in the roles of LRP5, LRP6, and their agonists and antagonists in bone homeostasis will continue to grow. Such interest likely extends to other metabolic disorders because LRP6 mutation is also linked to coronary artery disease (Mani et al., 2007) (Table 1).

TCF7L2 (i.e., TCF4) is strongly implicated in type II diabetes (Grant et al., 2006), echoing the implication of WNT5B in the disease (Table 1). Although the diabetes-associated TCF7L2 gene polymorphism does not alter protein coding (because it occurs in intron 4) and its exact molecular nature (i.e., reduction or increase in TCF7L2 function) is unclear, its association with the disease has been confirmed in numerous populations (Welters and Kulkarni, 2008). Some studies have suggested that the predisposing TCF7L2 variant causes a decrease in insulin secretion in insulin-producing pancreatic β cells, but other pathogenic mechanisms involving additional tissues/organs or endocrine functions remain to be investigated.

Association of deregulated Wnt/β-catenin signaling with cancer has been well documented, particularly with colorectal cancer (Polakis, 2007) (Table 1). Constitutively activated β-catenin signaling, due to APC deficiency or β-catenin mutations that prevent its degradation, leads to excessive stem cell renewal/proliferation that predisposes cells to tumorigenesis. Indeed, APC deletion or β-catenin activation in stem cells is essential for intestinal neoplasia (Fuchs, 2009). Blocking β-catenin signaling as a cancer treatment has therefore generated significant interest. In fact, the beneficial effect of nonsteroidal anti-inflammatory drugs (NSAIDS) in colorectal cancer prevention and therapy has been attributed partially to the perturbation of TCF/β-catenin signaling through the ability of NSAIDS to inhibit Prostaglandin E2 production, which enhances TCF/β-catenin-dependent transcription (Castellone et al., 2005; Shao et al., 2005). Small molecules that disrupt TCF/β-catenin (Lepourcelet et al., 2004) or β-catenin/coactivator (CBP) interaction (Emami et al., 2004) and thereby block TCF/β-catenin signaling have been described. The task of disrupting TCF/β-catenin interaction specifically, however, is a difficult one because β-catenin interacts with TCF and other binding partners such as APC, Axin, and E-cadherin via the same or an overlapping interface (Barker and Clevers, 2006). Another potential therapeutic target is the kinase CDK8, which, as a Mediator subunit, is often amplified in and is required for β-catenin-dependent transcription and proliferation of colon cancer cells (Firestein et al., 2008; Morris et al., 2008). A new class of small molecules that inhibits β-catenin signaling has recently be identified (Chen et al., 2009), which via an unknown mechanism stabilizes the Axin protein, thereby promoting β-catenin degradation even in cancer cells that lack APC function. As discussed above, since Axin protein levels are the rate-limiting step for β-catenin degradation, manipulation of Axin stabilization represents a promising therapeutic strategy.

Many cancers that do not harbor mutations in the Wnt pathway nonetheless rely on autocrine Wnt signaling for proliferation or survival (Barker and Clevers, 2006). In fact APC mutant colon cancer cells maintain their dependence on Wnt and epigenetically silence the expression of secreted Wnt antagonists (He et al., 2005; Suzuki et al., 2004). Therefore targeting Wnt signaling upstream of TCF/β-catenin is also an important therapeutic option. Reagents against Wnt proteins such as antibodies (He et al., 2005) or secreted Fz extracellular domains (DeAlmeida et al., 2007), which act outside the cancer cells to block Wnt-receptor interaction, show promise in certain experimental settings, as do small molecule and peptide inhibitors that antagonize Fz-Dvl interaction (Shan et al., 2005; Zhang et al., 2009). Small molecules have also been identified that inhibit Porcupine

and thus prevent Wnt lipidation and secretion (Chen et al., 2009). We will likely see additional molecular and chemical agents that can interfere with different steps of Wnt/β-catenin signaling, whose complexity presents many potential therapeutic targets. The challenge will be ensuring that these agents target cancer cells without damaging normal tissue homeostasis.

Perspectives

Since the discovery of the WNT1 gene 27 years ago (Nusse and Varmus, 1982), Wnt/β-catenin signaling has cemented its role as a key regulatory system in biology. Studies of different animal models and human diseases have established a complex Wnt signaling network far beyond a linear pathway, with many components having multiple distinct roles and acting in different cellular compartments, and many modulators feeding into and cross-regulating within this network. The patterns of dynamic and kinetic protein phosphorylation/modification and complex assembly/disassembly are beginning to emerge. Challenges and excitement both lie ahead. (1) Novel regulators will likely continue to be identified using classical genetic, molecular, and modern genomic and proteomic approaches. (2) New analytical and imaging technologies should enable us to dissect and visualize the dynamic signaling events in vivo and to shed light on the cell biological aspects of Wnt signaling, including where, when, and how signaling occurs inside the cell. (3) Although we have obtained significant structural information on individual domains and protein interaction interfaces, atomic structures of protein complexes such as the Axin complex and ligand-receptor complexes remain daunting challenges. (4) Additional specific small molecular inhibitors or activators with defined targets and mechanisms would provide not only leads for therapeutics but also research tools to manipulate the Wnt pathway in precise temporal and spatial manners. (5) Integration of vast amounts of information into quantitative models will allow us to predict the behavior and to study the robustness and evolvability of Wnt signaling in various biological contexts. (6) The Wnt-responsive transcriptome remains a gold mine in which we should dig for answers surrounding Wnt-regulated biology. Unfolding examples include Wnt regulation of intestinal and hair follicle development/homeostasis, which has provided significant insights into stem cell biology and cancer pathogenesis (Clevers, 2006; Fuchs, 2009). Because β-catenin is a coactivator for other transcription factors in addition to TCF/LEF, comparative analyses of Wnt-responsive transcription programs that depend on TCF/LEF versus others will likely uncover further complexity in Wnt-regulated gene expression. (7) β-catenin and APC are also key components in the E-cadherin cell adhesion complex and the microtubule network, but how Wnt/β-catenin signaling interacts with these cellular structures remains poorly understood. In addition, the involvement of the primary cilium, a centrosome- and microtubule-based protrusive organelle in vertebrate cells, in Wnt/β-catenin versus noncanonical Wnt signaling remains an intriguing but debated topic (Gerdes et al., 2009).

Finally, the study of Wnt signaling in human diseases, and in stem cell biology and regeneration, holds promises for translational medicine. In addition to cancer and osteoporosis, both of which will likely see Wnt-signaling-based therapeutics moving into clinical trials or even clinics in the near future, potential links between neurological diseases (De Ferrari and Moon, 2006) and a schizophrenia susceptibility gene product (Mao et al., 2009) to Wnt/β-catenin signaling offer new hopes for the treatment of neurological and psychiatric disorders. Manipulation of Wnt signaling for stem cell regulation also offers exciting opportunities for regenerative medicine (Clevers, 2006; Fuchs, 2009; Goessling et al., 2009; Willert et al., 2003). A better understanding of Wnt/β-catenin signaling will have a broad impact on biology and medicine.

SUPPLEMENTAL DATA

Supplemental data include one table and can be found with this article online at http://www.cell.com/developmental-cell/supplemental/S1534-5807(09)00257-3.

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