REVIEW ARTICLE
Wnt/β-catenin signalling: from plasma membrane to nucleus

Wantae KIM[1], Minseong KIM[1] and Eek-hoon JHO[2]

Department of Life Science, The University of Seoul, 90 Jeonnong-dong, Dongdaemun-gu, Seoul 130-743, Korea

Wnt/β-catenin signalling plays essential roles in embryonic development as well as tissue homoeostasis in adults. Thus abnormal regulation of Wnt/β-catenin signalling is linked to a variety of human diseases, including cancer, osteoporosis and Alzheimer's disease. Owing to the importance of Wnt signalling in a wide range of biological fields, a better understanding of its precise mechanisms could provide fundamental insights for therapeutic applications. Although many studies have investigated the regulation of Wnt/β-catenin signalling, our knowledge remains insufficient due to the complexity and diversity of Wnt signalling. It is generally accepted that the identification of novel regulators and their functions is a prerequisite to fully elucidating the regulation of Wnt/β-catenin signalling. Recently, several novel modulators of Wnt signalling have been determined through multiple genetic and proteomic approaches. In the present review, we discuss the mechanistic regulation of Wnt/β-catenin signalling by focusing on the roles of these novel regulators.

Key words: axin, β-catenin, Dishevelled, Wnt.

INTRODUCTION

Wnt signalling regulates cell proliferation, differentiation and fate decisions during embryonic development and tissue homoeostasis in adults. Dysregulation of Wnt signalling due to the mutation of main components such as APC (adenomatous polyposis coli), LRPs [LDL (low-density lipoprotein) receptor-related proteins] 5/6 and β-catenin is linked to a variety of human diseases, including cancer, neurodegeneration and osteoporosis [1,2]. Owing to the important role of Wnt signalling in human diseases, further understanding of its intracellular signal transduction mechanisms could provide fundamental insights for clinical applications.
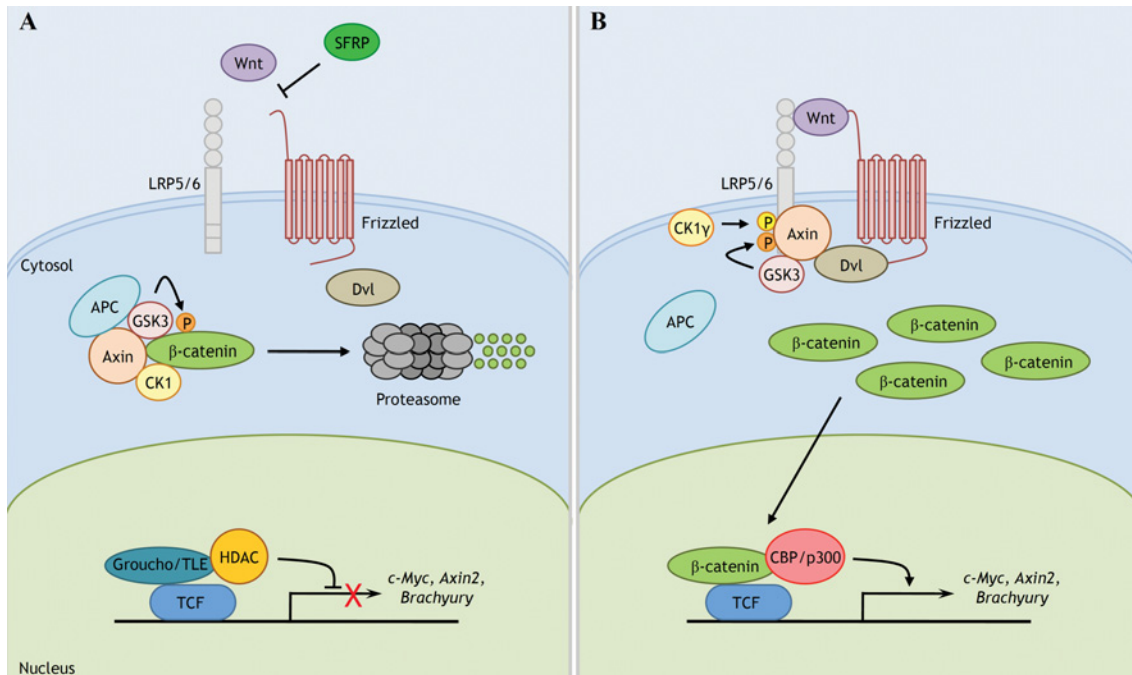
Wnt signalling is transduced through either the β-catenin-dependent pathway (canonical Wnt signalling) or the β-catenin-independent pathway (non-canonical Wnt signalling), which are composed of different types of Wnt ligands or receptors [3]. Non-canonical Wnt signalling is transduced either via small G-proteins such as Rho/Rac, which in turn control planar cell polarity via actin cytoskeletal remodelling, or regulation of the intracellular calcium level, which in turn affects diverse biological processes. In the present review we focus on the transduction of canonical Wnt signalling, as excellent reviews on non-canonical Wnt signalling can be found elsewhere [4,5]. Wnts are secreted as glycosylated/lipid-modified proteins [6–10]. In the canonical Wnt pathway, β-catenin acts as a key transcriptional co-activator and transmits extracellular signals for the activation of target genes. In the absence of Wnt, β-catenin is constitutively phosphorylated by a destruction complex composed of Axin, APC and GSK3β (glycogen synthase kinase 3β), after which it is targeted for ubiquitin-dependent degradation by the E3 ligase β-TrCP (β-transducin repeat-containing protein), resulting in low cellular levels of β-catenin (Figure 1A). However, in the presence of Wnt, Wnt ligand binds to its receptor Fz (Frizzled) and co-receptor LRP6, subsequently leading to GSK3β- or CK1 (casein kinase 1)-mediated phosphorylation of the intracellular region of LRP6. Phosphorylated LRP6 then acts as a docking site for Axin, resulting in the dissociation of β-catenin from the destruction complex in a Dvl (Dishevelled)-dependent manner. This sequential process induces the accumulation of cytosolic β-catenin, which enters into the nucleus and binds to TCF (T-cell factor)/LEF (lymphoid enhancer factor), thereby activating the Wnt transcriptional programme (Figure 1B) [2,11]. Although many studies have investigated the regulation of Wnt/β-catenin signalling, our knowledge remains insufficient owing to the complexity and diversity of the regulatory mechanism. Thus the identification of novel regulators and their functions is a prerequisite to fully elucidating the regulation of Wnt/β-catenin signalling. Recently, several novel modulators of Wnt signalling have been determined through multiple genetic and proteomic

Figure 1   Wnt/β-catenin signalling pathway

(**A**) In the absence of Wnt ligands, cytosolic β-catenin is continuously phosphorylated within the β-catenin destruction complex, which consists of APC, GSK3β, CK1 and Axin. β-TrCP E3 ligase recognizes phosphorylated β-catenin and targets it for ubiquitin-dependent proteasomal degradation in order to keep the level of β-catenin low. In the nucleus, TCF/LEF1 associates with the transcriptional repressor Groucho/TLE. (**B**) After binding of Wnt to Fz and LRP6, GSK3β and CK1γ phosphorylate LRP5/6, after which Axin is recruited to the phospho-LRP5/6 tail. These processes induce dissociation of the β-catenin destruction complex and subsequent accumulation of cytosolic β-catenin. Accumulated β-catenin enters into the nucleus and interacts with TCF/LEF1 for activation of Wnt-responsive genes. SFRP, secreted Fz-related protein. An animated version of the Figure is available at http://www.biochemj.org/bj/450/0009/bj4500009add.htm.

approaches. In the present review, we discuss the mechanistic regulation of key regulators of Wnt/β-catenin signalling from the plasma membrane to the nucleus, including LRP5/6, Dvl, Axin, β-catenin and TCF/LEF1.

PATHS TO LRP6 PHOSPHORYLATION

LRP5/6 was originally characterized as LDL receptor-related protein 5/6 [12,13]. Later, several groups identified LRP5/6 as an essential co-receptor in the canonical Wnt signalling pathway in *Drosophila*, *Xenopus* and mice [14–16]. Yeast two-hybrid experiments have revealed that the intracellular region of LRP5/6 is sufficient to interact with Axin, which is a well-known negative regulator of canonical Wnt/β-catenin signalling [17]. Further investigations have shown that the phosphorylation of five PPPS/TP motifs within the LRP5/6 cytosolic tail creates a docking site for Axin binding [18], and that GSK3β mediates LRP5/6 phosphorylation at serine/threonine residues within the five PPPS/TP motifs [19]. On the basis of these data, an 'initiation-amplification' model was proposed wherein Axin-bound GSK3β mediates LRP6 phosphorylation, which promotes the recruitment of additional Axin–GSK3β complexes resulting in amplification of signal transduction [20]. On the other hand, Niehrs and co-workers used protein modification screening to show that membrane-bound CK1γ phosphorylates Thr[1479], but not Ser[1490], in the PPPSP motif, which is again phosphorylated by an unknown proline-directed kinase for the recruitment of Axin [21]. In addition to GSK3β and CK1γ, several studies have identified additional kinases responsible for LRP6 phosphorylation. Specifically, GRK (G-protein-coupled

receptor kinase) 5/6 phosphorylates PPPS/TP motifs within the LRP6 cytosolic tail [22]. Moreover, kinome siRNA (small interfering RNA) screening revealed that cyclin-Y-dependent PFTK (PFTAIRE kinase) or MAPKs (mitogen-activated protein kinases) [such as ERK (extracellular-signal-regulated kinase) 1/2 and JNK (c-Jun N-terminal kinase)] are sufficient to induce LRP6 phosphorylation (Figures 2A and 2B) [23,24]. These studies suggest that different kinases phosphorylate PPPSP motifs in a context-dependent manner and that LRP6 phosphorylation is a critical regulatory point in the cross-talk between Wnt signalling and other pathways. Therefore it will be of interest to determine how these kinases compete or co-operate for LRP6 phosphorylation.

Regulation of LRP6 phosphorylation is a critical step in Wnt/β-catenin signalling. However, despite extensive investigation, it still remains unclear how LRP6 phosphorylation is regulated by Wnt ligands [25]. Several studies have revealed the occurrence of primary event(s) between binding of Wnt to its receptor and LRP6 phosphorylation. Wnt3a treatment induces the formation of PtdIns(4,5)P_2 via PI4KII (phosphatidylinositol 4-kinase type 2) and PIP5KI (PtdIns4P 5-kinase type 1). Production of PtdIns(4,5)P_2 leads to phosphorylation of LRP6 at Thr[1479] and Ser[1490], followed by the formation of Wnt-induced LRP6 aggregates known as signalosomes [26,27]. Although the mechanism of action of PtdIns(4,5)P_2 for LRP6 phosphorylation has not been fully elucidated, it may function as a docking mediator for membrane recruitment of Amer1 (APC membrane recruitment 1; also called WTX), which in turn increases the local concentrations of GSK3β and CK1γ in the vicinity of LRP6 (Figure 2C). Amer1 is essential for Wnt-induced LRP6 phosphorylation at the membrane [28]. However, Amer1 was
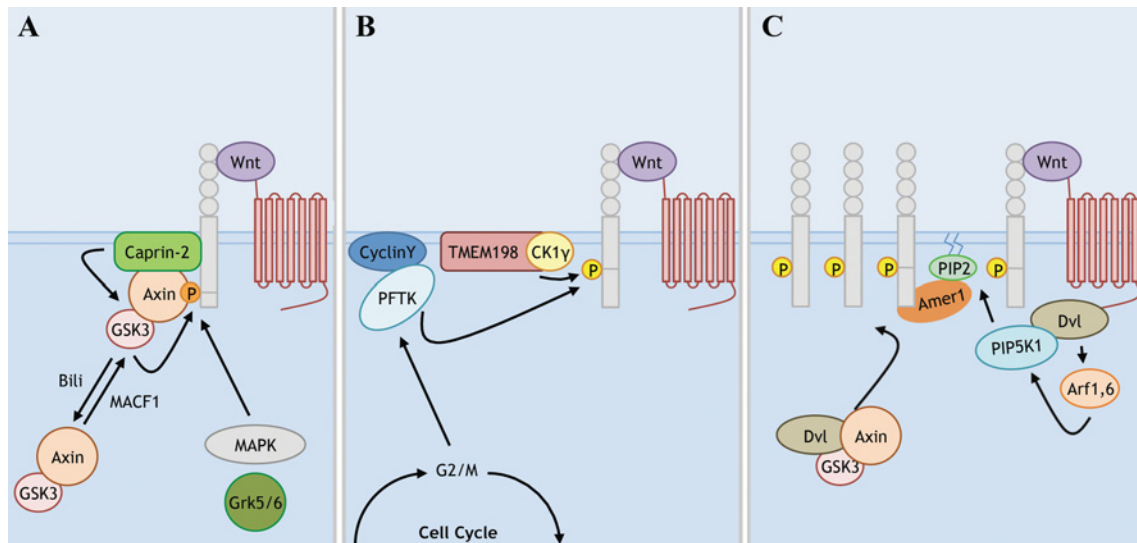
Figure 2   Control of LRP5/6 activation

Under Wnt stimulation, serine/threonine clusters and PPPS/TP motifs within the intracellular regions of LRP5/6 are phosphorylated by CK1γ and GSK3β respectively. (**A**) MACF1 and Bili regulate the recruitment of Axin–GSK3β to the membrane positively or negatively respectively, whereas Caprin-2 stabilizes the LRP6 and Axin–GSK3β complex. MAPKs (such as ERK and JNK) or GRK5/6 are capable of phosphorylating the PPPS/TP motifs of LRP6. (**B**) PFTK/cyclin Y is able to phosphorylate the PPPS/TP motifs in a cell-cycle-dependent manner in order to sensitize the receptor for Wnt stimuli. TMEM198 promotes the recruitment of CK1γ to LRP5/6 in order to enhance phosphorylation of serine/threonine clusters. (**C**) Under Wnt stimulation, Dvl- and ARF1,6-mediated PIP5K1 activation lead to production of PtdIns(4,5)P_2. PtdIns(4,5)P_2-dependent interaction of Amer1 with LRP6 facilitates translocation of the destruction complex to the membrane for phosphorylation of LRP6.

originally identified as a negative regulator of Wnt signalling as it forms a complex with components of the β-catenin destruction complex, resulting in β-catenin degradation in the cytoplasm [29]. These results suggest that Amer1 acts as a dual regulator having both positive and negative functions in Wnt signalling depending on its cellular localization. This is reminiscent of the dual roles of GSK3β and DP1 (dimerization partner 1) (see below and [30,31]) in Wnt signalling, and such dual functionality in a single transduction molecule may represent a common theme in cell signalling. Previously, our group showed that activation of ARFs (ADP-ribosylation factors) 1 and 6, which are known to activate and recruit PIP5KI to the plasma membrane, is necessary for LRP6 phosphorylation [32,33]. Wnt treatment activates ARF1/6 at a very early time point (10–30 min) before activation of LRP6 (30–60 min) in a Dvl-dependent manner, although exactly which factors are involved in activation of ARF1/6 in response to Wnt treatment are not yet known [32]. Taken together, activation of Dvl by Wnt treatment triggers ARF-PIP5KI to produce PtdIns(4,5)P_2, followed by aggregation and phosphorylation of LRP6 (Figure 2C).

Although the detailed mechanism is not fully understood, the recruitment of Axin to phosphorylated LRP6 seems to be tightly regulated. Deletion of the *Macf1* (microtubule-actin cross-linking factor 1) gene in mice has been shown to induce similar phenotypes as those of Wnt-3 and LRP5/6 double-knockout embryos. Extensive biochemical analysis has further revealed that MACF1 directly interacts with Axin and is required for translocation of the Axin–GSK3β complex from the cytoplasm to the plasma membrane in a Wnt3a-dependent manner [34]. Furthermore, knockdown of MACF1 has been shown to inhibit Axin translocation as well as reduce β-catenin/TCF transcriptional activation, suggesting that MACF1 is a positive regulator of Wnt/β-catenin signalling. In contrast with MACF1, Bili, a Band 4.1-domain-containing protein, was identified as a negative regulator of Wnt/β-catenin signalling

that inhibits the recruitment of Axin to LRP6 (Figure 2A) [35].

In contrast with the membrane-associated regulation of LRP6 phosphorylation, Cruciat et al. [36] reported that the preferential formation of acidic endocytic vesicles is crucial for LRP6 phosphorylation. In this case, the Wnt receptor complex including PRR (prorenin receptor) and V-ATPase (vacuolar H$^+$-ATPase), which generates an acidic luminal compartment, is endocytosed upon Wnt stimulation. This acidification event is required for LRP6 phosphorylation, as well as subsequent Wnt activation [36,37]. More recently, TMEM198 (transmembrane protein 198), G$_{\beta\gamma}$ or Caprin-2 was shown to promote the phosphorylation and activation of LRP6 by recruiting CK1 or GSK3β (Figure 2A) [38–40]. More novel regulators involved in LRP6 phosphorylation are expected to be discovered, which will aid our understanding of the mechanisms behind the initial activation of Wnt/β-catenin signalling.

REGULATION OF DISHEVELLED IN Wnt SIGNALLING

Dvl (in mammals; Dsh in *Drosophila*) has been shown to be involved in both the canonical and non-canonical Wnt signalling pathways. Three Dvls (Dvl1, Dvl2 and Dvl3) or one Dsh have been identified in mammals or *Drosophila* respectively. Dvl consists of three well-established domains, the N-terminal DIX (Dvl and Axin) domain, the central PDZ (postsynaptic density 95, discs large and zonula occludens-1) domain and the C-terminal DEP (Dvl, Egl-10 and pleckstrin) domain, and Dvl is able to interact with approximately 80 proteins through these domains. Dvl is considered as a branching point for the differential interpretation of external distinct Wnt ligands, canonical compared with non-canonical Wnts, and the relay of various signals to downstream effectors, although the exact mechanisms still remain elusive [41,42]. In the canonical Wnt pathway, Dvl mediates clustering of

Fz/LRP6 receptors to form signalosomes at the plasma membrane [20,26] as well as transmits input signals received from the Wnt receptor to the β-catenin destruction complex in the cytosol, especially Axin [43–45]. In addition to its function in the cytosol or plasma membrane, nuclear Dvl can regulate Wnt signalling through stabilization of the β-catenin–TCF complex on Wnt target gene promoters via interaction with c-Jun [46,47]. Furthermore, Dvl has been detected at kinetochores, where it plays a crucial role in mitotic progression [48]. These studies imply that Dvl functions differently depending on the context, and a comprehensive understanding of Dvl regulation is important. Dvl activity is regulated by its post-translational modifications, stability and localization, and these regulatory mechanisms will be discussed.

Post-translational modification of Dvl

The activity of Dvl is tightly regulated by various types of post-translational modifications. Among these modifications, phosphorylation of Dvl has been investigated extensively, although its precise role remains unclear. Both canonical and non-canonical Wnt stimuli induce hyperphosphorylation of Dvl at multiple sites, including Ser^{139} and Ser^{142}. Several studies have shown that CKs (CK1δ, CK1ε and CK2) are involved in such phosphorylation events [49–54]. For example, among 25 pharmacological compounds that inhibit heterotrimeric G-proteins or other kinases, only treatment with CK1 inhibitor (D4476) was shown to be able to interfere with Wnt5a-induced Dvl hyperphosphorylation and dopaminergic neuron differentiation, suggesting that CK1 is a main kinase for Dvl phosphorylation [55]. Inhibition of CK1-dependent phosphorylation of Dvl by Kif3a, which is required for ciliogenesis, is one way to restrain Wnt/β-catenin signalling in ciliated cells [56]. Loss of Kif3a results in constitutive phosphorylation of Dvl, which in turn leads to hyperactivation of Wnt/β-catenin signalling, and these effects can be severely blocked by CK1 inhibitor. These results further suggest that CK1-mediated phosphorylation of Dvl is essential for the activation of Wnt/β-catenin signalling [56]. Wnt/β-catenin signalling can undergo cross-talk with other signalling pathways through regulation of Dvl phosphorylation. For example, density-dependent activation of Hippo signalling, which is known to be an essential signalling pathway in organ size control, induces cytoplasmic localization of TAZ (transcriptional co-activator with PDZ-binding motif), which binds to the PY motif and PDZ domain of Dvl and interferes with Dvl–CK1 interactions, thereby inhibiting Dvl phosphorylation and Wnt/β-catenin signalling (Figure 3) [57]. Interestingly, it has been shown that both Wnt3a and Wnt5a, which have opposite effects on β-catenin activation, induce CK1-mediated phosphorylation of Dvl, implying that Wnt-3a or Wnt-5a can induce different signalling outputs through the activation of unknown pathways, in addition to phosphorylation of Dvl by CK1 [55].

In addition to CK1/2, other kinases are known to phosphorylate Dvl. Sun et al. [58] showed previously that Wnt treatment enhances endogenous PAR1 (partitioning defective 1), a determinant of polarity, and PAR1-mediated phosphorylation of Dvl potentiates β-catenin signalling (Figure 3) [58]. Besides serine/threonine phosphorylation of Dvl, Wnt was previously shown to stimulate both Src–Dvl interactions as well as Src-mediated tyrosine phosphorylation of Dvl [59]. In detail, a Src kinase inhibitor, siRNA-mediated Src knockdown and a Dvl tyrosine mutant (Y18F) all impeded TCF/LEF-responsive activation, suggesting that Src acts as a positive regulator
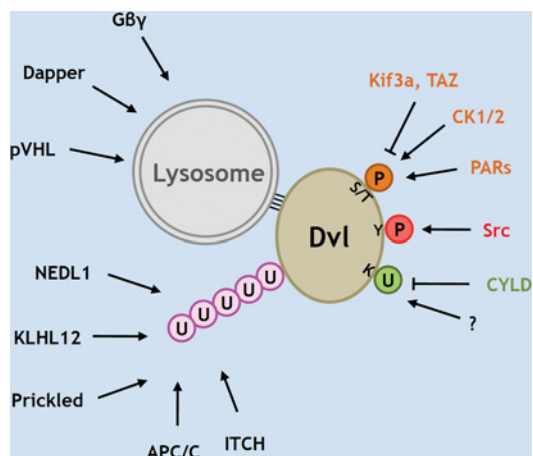
Figure 3   Dvl modification and degradation

Factors that are responsible for the modification of Dvl, such as serine/threonine phosphorylation, tyrosine phosphorylation and monoubiquitination, are depicted. Factors that promote Dvl proteasomal degradation via polyubiquitination and/or lysosomal degradation are presented. Note that ITCH is only responsible for the degradation of phospho-Dvl. pVHL, von Hippel–Lindau protein.

of canonical Wnt signalling (Figure 3) [59]. In addition to phosphorylation, a recent study using proteomic analysis revealed that Dvl3 is methylated at arginine residues in a Wnt3a-dependent manner [60]. Therefore identification of additional modification(s) would provide a better understanding of the multi-functionality of Dvl. A previous study revealed that Lys^{63}-linked ubiquitination of Dvl, which may enhance polymerization of Dvl, is required for the activation of Wnt/β-catenin signalling, although the identity of the E3 ligase remains unknown [61]. CYLD, a DUB (deubiquitinating enzyme) also known as the familial cylindromatosis tumour suppressor, can interact with and inhibit Lys^{63}-linked ubiquitination of Dvl, thereby attenuating Wnt/β-catenin signalling [61]. These findings demonstrate the possibility that Dvl is tightly regulated by various enzymes, and mutations in proteins responsible for these modifications may be implicated in human diseases such as cancer.

Regulation of Dvl turnover

Regulation of Dvl stability is important, and Dvl turnover is mainly mediated by proteosomal or lysosomal pathways (Figure 3) [42,62]. Using yeast two-hybrid screening, a HECT (homologous with E6-associated protein C-terminus)-type ubiquitin ligase, NEDL1 (NEDD4-like ubiquitin protein ligase-1), was identified as the first protein responsible for Dvl turnover. NEDL1 interacts with the C-terminal region of Dvl harbouring three proline-rich clusters, leading to its polyubiquitination and degradation [62]. Angers et al. [63] identified KLHL12 (kelch-like 12) as a Dvl-interacting protein using tandem-affinity purification/MS and showed that the KLHL12–Cullin-3 ubiquitin ligase complex promotes ubiquitination and degradation of Dvl [63]. However, since KLHL12 was shown to interact with Dvl in a Wnt-dependent manner, and Dvl stability is not regulated by Wnt treatment, although a sub-pool of Dvl could be affected, further investigation into its biological role (such as negative feedback or further fine-tuned regulation) in Wnt signalling is required [63]. Recently, another HECT-containing NEDD4-like E3 ligase, ITCH, was identified as a binding partner that promotes ubiquitin-dependent proteasomal degradation of Dvl. ITCH binds to and

degrades the phosphorylated form of Dvl, resulting in inhibition of Wnt/β-catenin signalling [64].

Several reports have shown that ubiquitination and degradation of Dvl by E3 ligases can be indirectly modulated [65–70]. Inversin, a ciliary protein that is responsible for the autosomal recessive cystic kidney disease nephronophthisis type II, can antagonize both the Wnt/β-catenin and Wnt/PCP (planar cell polarity) pathways, a type of non-canonical Wnt signalling pathway, by facilitating ubiquitin-dependent degradation of Dvl [65]. Further studies have shown that ANAPC2 [cullin-domain-containing subunit of the APC/C (anaphase-promoting complex)] interacts with inversin, and APC/C stimulates degradation of Dvl by recognizing the highly conserved D-box (destruction box) within the DEP domain. However, whether or not APC/C contains a genuine E3 ligase for inversin-mediated Dvl degradation needs to be proven [66]. Similar to the action of APC/C, Prickle-1 and Naked Cuticle, an inducible antagonist of Wnt/β-catenin, have been shown to facilitate ubiquitin-dependent proteasomal degradation of Dvl by recruiting an unknown E3 ligase [67–70].

In addition to ubiquitination, Dvl stability is controlled by lysosomal degradation. Dapper1 or $G_{\beta\gamma}$ has been identified as a modulator of this process [71,72]. Dapper1 or $G_{\beta\gamma}$-mediated Dvl turnover can be blocked by lysosomal inhibitors, but not proteasomal inhibitors. Besides lysosomal degradation, a previous study has reported that autophagy negatively modulates Wnt/β-catenin signalling by promoting Dvl instability [49]. In this case, pVHL (von Hippel–Lindau protein) leads to ubiquitination of Dvl under starvation, followed by binding of ubiquitinated Dvl2 to p62, resulting in degradation of Dvl via the autophagy-lysosome pathway.

Stabilization of Dvl is another way Wnt signalling is regulated. It has been demonstrated previously that the ATDC (ataxia-telangiectasia group D complementing gene, also called TRIM29), which is highly expressed in several cancers, as well as SIRT1 [Sirtuin 1, a class III HDAC (histone deacetylase)] promote Wnt/β-catenin signalling by binding to and stabilizing Dvl protein [73,74].

Control of Dvl localization

Asymmetric cortical localization or Wnt-dependent translocation of Dvl to the Fz receptor is a key event in Fz/PCP or Wnt/β-catenin signalling respectively [42]. However, the molecular mechanisms underlying these processes remain poorly understood. Using genome-wide RNAi (RNA interference) screening in *Drosophila* cells, Simons et al. [75] showed that the recruitment of Dsh to Fz receptor at the membrane is dependent on the Na^+/H^+ exchange activity of NHE2 (Na^+/H^+ exchanger 2). NHE2 displays gain- and loss-of-function of PCP phenotypes and genetically interacts with Fz. Furthermore, they have shown that polybasic amino acid clusters within the DEP domain are crucial for binding to negatively charged phospholipids, and interaction with NHE2 stabilizes the association between Fz and Dvl [75]. Interestingly, the Dsh–lipid interaction stabilized by NHE2 is particularly important for activation of the PCP branch. It remains unknown how Dvl is recruited to Fz upon Wnt signalling, although it was shown that translocation of the Axin–GSK3β complex from the cytoplasm to the membrane is mediated by MACF1 [34].

REGULATION OF AXIN STABILITY

Since Axin acts as a scaffold protein for formation of the β-catenin destruction complex, it is accepted as the most important negative regulator of Wnt/β-catenin signalling [76]. Loss-of-
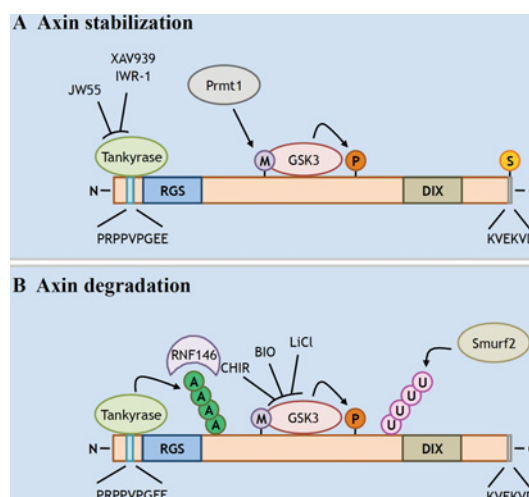
Figure 4   Axin stabilization and degradation

(**A**) GSK3β phosphorylates and stabilizes Axin, and the interaction between GSK3β and Axin is strengthened by the methylation of arginine residues in Axin by PRMT1. Chemical inhibitors of GSK3β, CHIR, LiCl and BIO are shown in (**B**). The C-terminal-conserved motif of Axin, KVEVKD, is SUMOylated and protected from ubiquitin-mediated-proteasomal degradation. (**B**) Tankyrase interacts with the N-terminal-conserved motif of Axin, PRPPVPGEE, and PARsylates Axin. The E3 ligase RNF146 recognizes PARsylated Axin and degrades it via ubiquitin-dependent proteasomal degradation. Chemical inhibitors of tankyrase, JW55, XAV939 and IWR, are shown in (**A**). Smurf2 induces polyubiquitination of Axin at Lys^{505}, followed by degradation.

function mutations in Axin have been identified in various types of cancers, and Axin is considered as a tumour suppressor [77]. Axin is a concentration-limiting component as its cellular level is extremely low compared with those of other Wnt components ($<\sim$1000 fold). Therefore regulation of its level is a critical step for the modulation of Wnt/β-catenin signalling [11,78]. Since Axin is a good therapeutic target for the treatment of cancers caused by aberrant activation of Wnt/β-catenin signalling, identification of small molecules or proteins that regulate Axin stability has become more important (Figure 4).

Previous results have demonstrated that Axin is phosphorylated and stabilized by GSK3β [79,80]. GSK3β inhibitors such as LiCl, BIO or CHIR decrease the stability of Axin, confirming that GSK3β-mediated Axin phosphorylation is crucial for Axin stabilization. Additionally, Willert et al. [81] showed that Axin is dephosphorylated and destabilized in response to Wnt stimulation. These results suggest a molecular mechanism wherein Wnt signalling regulates the β-catenin destruction complex through destabilization of Axin. However, since LRP6 phosphorylation by GSK3β is enhanced upon Wnt signalling, it is controversial whether or not Axin degradation upon Wnt signalling is mediated by reduction of GSK3β activity [82]. To address this, Taelman et al. [83] reported that Wnt signalling induces the sequestration of GSK3β from the cytosol into MVBs (multivesicular bodies), thereby decreasing the available cytosolic pool of GSK3β. However, Clevers and co-workers [84] recently provided a provocative model of Wnt signalling by examining the endogenous Axin complex. According to their data, neither reduction of β-catenin phosphorylation by GSK3β in the Axin complex nor sequestration of GSK3β in MVBs was observed upon Wnt signalling, at least before a noticeable decrease in the Axin level. They claimed that Wnt causes saturation of the Axin complex with phosphorylated β-catenin by inducing detachment of β-TrCP, after which newly synthesized β-catenin accumulates and enters into the nucleus for the regulation of downstream target

gene expression [84]. Further studies to examine this new model are expected.

Axin stability can be regulated by other modifications as well as phosphorylation. The six C-terminal amino acids (C6 motif, KVEKVD) in Axin are SUMOylated, which is required for protein stability, but not for the regulation of Wnt/β-catenin signalling [81]. Deletion of the C6 motif or non-SUMO-targeting mutation results in increased polyubiquitination of Axin, suggesting that the C6 motif protects Axin from polyubiquitin-dependent proteasomal degradation. SUMOylation of the C6 motif, together with phosphorylation of Axin by GSK3β were shown to be crucial in protecting Axin from polyubiquitination [85]. However, it remains unknown how these modifications affect ubiquitin-dependent degradation of Axin as well as which E3 ligase is involved. Cha et al. [86] have shown that PRMT1 (protein arginine N-methyltransferase 1) interacts with and methylates Axin, which then enhances the stability of Axin [86]. Knockdown of PRMT1 or mutation of a methylation site in Axin leads to reduction of Axin stability. PRMT1-mediated methylation of Axin seems to reduce polyubiquitination, thereby increasing the half-life of Axin [86].

Chemical genetic screening for the identification of small molecules capable of targeting the Wnt-mediated response has enhanced our understanding of the regulation of Axin stability. These approaches have provided important insights into the control of Axin and may present new clinical therapeutic options. Chen et al. [87] have shown that IWR (inhibitor of Wnt response) compounds induce stabilization of Axin1/2. Specifically, IWR compounds directly interact with and significantly increase the level of Axin2, resulting in β-catenin destruction in colorectal cancer cell lines. Moreover, IWR compounds lead to the inhibition of Wnt/β-catenin signalling during tissue regeneration in zebrafish [87]. Thereafter, Huang et al. [88] discovered XAV939, which efficiently inhibits Wnt/β-catenin signalling by increasing the level of Axin protein. Using a quantitative chemical proteomics approach, it was found that XAV939 targets and inhibits the PARsylation [poly(ADP-ribosyl)ation] enzyme tankyrase. Furthermore, tankyrase interacts with and promotes PARsylation of the evolutionarily conserved N-terminal region of Axin, thereby stimulating Axin degradation through the ubiquitin–proteasome system [88]. In addition, Waller et al. [89] demonstrated that the compound JW55 acts as a specific tankyrase inhibitor. JW55 stabilizes Axin2 and inhibits Wnt/β-catenin signalling in colon carcinoma cells. Furthermore, they have shown that JW55 reduces tumour growth in conditional APC mutant mice.

In an effort to identify proteins affecting Axin turnover, Smurf2, a critical regulator of TGFβ (transforming growth factor β) signalling, was found to act as an E3 ligase for Axin. Smurf2 interacts with multiple regions (amino acids 228–354, 508–711 and 745–826) of Axin, as well as promotes the polyubiquitination of Lys^{505}, resulting in decreased Axin stability [90]. Although Smurf2 acts as an E3 ligase for Axin, it is not yet known whether or not Smurf2 is responsible for Wnt-induced ubiquitination on Axin. Recently, Cong and co-workers [91] reported that RNF146 (RING finger protein 146), a RING-domain E3 ligase, mediates ubiquitination of PARsylated Axin by tankyrase, which in turn causes proteosomal degradation of Axin [91]. This suggests that RNF146 acts as a positive regulator of Wnt/β-catenin signalling. RNF146 recognizes and directly interacts with PARsylated substrates, such as Axin, resulting in degradation [91]. However, it remains unknown whether or not RNF146 is responsible for Wnt-mediated Axin destabilization. Thus it should be determined whether or not the activity or stability of Smurf2 or RNF146 is regulated by Wnt signalling, and Wnt-mediated down-regulation of Axin can occur in the absence of these E3 ligases.

Since Axin is considered as a negative regulator of Wnt/β-catenin signalling, methods that stabilize Axin are being extensively studied for therapeutic purposes, such as the use of drugs that stabilize Axin for cancer treatment. However, recent findings have led us to consider other functions of Axin/Axin2. Lui et al. [92] used proteomic analysis to identify additional novel Axin-binding partners and found that USP34 (ubiquitin-specific protease 34), an ubiquitin-specific protease, interacts with endogenous Axin and inhibits its ubiquitination, thereby increasing Axin stability by blocking the ubiquitin-dependent proteasomal degradation pathway. Unexpectedly, knockdown of USP34, which lowered the level of Axin, results in the inhibition of Wnt-mediated reporter activity, suggesting that Axin has a positive function in Wnt/β-catenin signalling. Similar to the study by Major et al. [93], Liu et al. [92] have found that depletion of the *AXIN1/2* gene in colon cancer cells harbouring constitutively active β-catenin inhibits β-catenin-dependent transcription, further confirming that Axin1/2 play a positive role in Wnt/β-catenin signalling, although the mechanism is unknown. They further showed that USP34 acts downstream of the β-catenin destruction complex. On the basis of these findings, it could be interpreted that inhibition of Wnt signalling via USP34 knockdown is due to the destabilization and subsequent decreased nuclear accumulation of Axin [92]. In agreement with the above findings, it has been shown that knockdown of Axin2 in colon cancer cells promotes colon carcinoma oncogenic activity via stabilization of nuclear Snail1 levels and activity [94]. The authors [94] have claimed that Axin2 acts as a potent tumour promoter at the invasive front of human colorectal cancers.

REGULATION OF β-CATENIN TRANSCRIPTIONAL ACTIVITY

As described in the Introduction, the transcriptional activator β-catenin is stabilized upon Wnt stimulation, after which it enters into the nucleus, binds to TCF/LEF1 and mediates activation of Wnt-responsive target genes. Since these processes are the final steps of a pathway that relays extracellular signals to the nucleus, multiple regulatory factors operating on β-catenin and the β-catenin–TCF complex are involved. He and co-workers [2] previously published an excellent review article on Wnt/β-catenin signalling; in particular, major strategies for the regulation of TCF–β-catenin transcription were described concretely and inclusively. In the present review, we focus on important emerging factors that regulate the subcellular localization and stability of β-catenin and the β-catenin–TCF complex.

Subcellular localization of β-catenin

From membrane to nucleus and vice versa

β-Catenin is known to play an essential role in cell-to-cell junctions as well as Wnt signalling [95–97]. Since β-catenin forms adherens junctions through association with α-catenin and E-cadherin in the membrane, this interaction seems to be a target for the canonical Wnt signalling pathway. It has been shown that BCL9-2 (B-cell lymphoma 9-like protein) leads to nuclear localization of β-catenin by interacting with phosphorylated β-catenin at Tyr^{142}, which precludes interaction with α-catenin. Therefore BCL9-2 acts as a switch between the adhesive and transcriptional functions of β-catenin in a particular developmental context [98]. Similar to BCL9, Ji et al. [99] previously demonstrated that EGF (epidermal growth factor)-induced
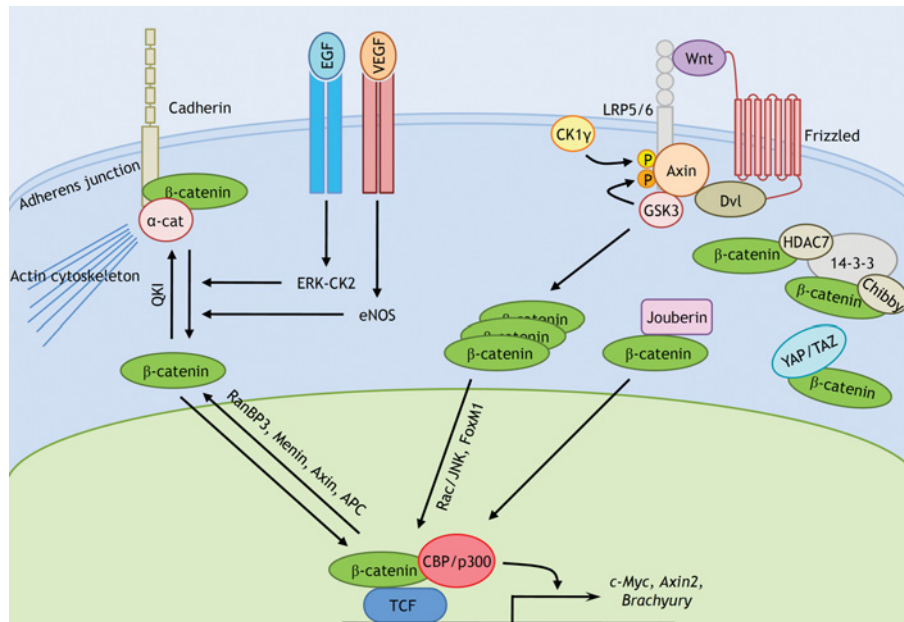
Figure 5   Regulation of β-catenin transactivation

EGF-induced ERK/CK2 activation for phosphorylation of β-catenin as well as VEGF-induced S-nitrosylation of β-catenin due to eNOS activation dissociates β-catenin from α-catenin or E-cadherin within adherens junctions, inducing its translocation into the nucleus and activation of Wnt target genes. Conversely, the RNA-binding protein quaking (QKI) can translocate β-catenin to the membrane. Wnt signalling induces sequential activation of Rac and JNK and/or increases the FoxM1 level to enhance its nuclear localization. Jbn can also promote nuclear accumulation of β-catenin, leading to increased Wnt target gene expression. RanBP3 specifically interacts with unphosphorylated nuclear β-catenin, removing it from the nucleus. Like Axin or APC, Menin acts as a shuttling modulator for β-catenin for the suppression of Wnt/β-catenin signalling. 14-3-3 and YAP/TAZ can anchor β-catenin to the cytoplasm. Retention ability of 14-3-3 requires HDAC7 or Chibby. CBP, CREB (cAMP-response-element-binding protein)-binding protein.

ERK-CK2 activation leads to the phosphorylation of β-catenin, subsequently impeding adherens junction formation between α-catenin and β-catenin, and resulting in activation of β-catenin transcriptional activity and tumour cell invasion (Figure 5) [99]. Furthermore, it was shown that VEGF (vascular endothelial growth factor) induces S-nitrosylation of β-catenin by eNOS (endothelial NO synthase) within adherens junctions, and S-nitrosylation at Cys[619] abrogates the interaction with VE-cadherin (vascular endothelial cadherin), leading to the disruption of adherens junction complexes and resulting in VEGF-induced vascular permeability [100]. It is possible that the released β-catenin may influence the transcriptional activity of β-catenin, although this possibility has not been examined [100]. As shown in the cases described above, translocation of β-catenin from the membrane to the cytoplasm or nuclei, which in turn enhances transcriptional activity of β-catenin, can be regulated by other signalling pathways. However, there is no conclusive evidence supporting that Wnt signalling itself controls the level of cytoplasmic β-catenin by releasing β-catenin from the membrane.

Shuttling between the nucleus and cytoplasm

After β-catenin accumulates in the cytoplasm in response to Wnt stimulation, nuclear localization of accumulated β-catenin is required for canonical Wnt signalling. However, the molecular mechanisms underlying β-catenin nuclear localization are not well understood. Recently, two excellent reports revealed the molecular mechanism for nuclear accumulation of β-catenin in response to Wnt stimulation (Figure 5). First, Wu et al. [101] presented evidence that Rac1/JNK2 activation is necessary for the nuclear localization of β-catenin in response to Wnt

stimulation. Specifically, treatment with Wnt3a leads to an increase in Rac1 activation within 30 or 60 min followed by activation of JNK2, which then phosphorylates Ser[191] and Ser[605] of β-catenin. β-Catenin phosphorylated by JNK2 is crucial to the Wnt-mediated nuclear localization of β-catenin. Identification of the Wnt/Rac1/JNK axis provides mechanistic insight into how canonical Wnt signalling induces nuclear translocation of β-catenin. Secondly, since it has been reported that both the FoxM1 (forkhead box M1) transcription factor and β-catenin are overexpressed and implicated in the pathogenesis of various human cancers, including gliomas, Zhang et al. [102] studied potential links between these two oncogenic pathways. Interestingly, they found that Wnt induces an increase in the FoxM1 level within a short time (15–30 min), and elevated FoxM1 efficiently promotes nuclear localization of β-catenin via its functional NLS (nuclear localization sequence), which may contribute to the induction of glioma tumorigenesis [103]. Since alteration of FoxM1 leads to a dramatic effect on β-catenin localization, further investigation into the molecular mechanism of how Wnt increases the abundance of FoxM1 protein will aid the development of therapeutic drugs for the treatment of Wnt/β-catenin-mediated cancers.

Besides having functions in the β-catenin destruction complex, Axin/APC harbour a functional NLS and NES (nuclear export sequence), and they shuttle between the cytoplasm and nucleus, subsequently modulating the dynamic subcellular distribution of β-catenin [104–106]. The tumour suppressor activities of Axin/APC can be attributed to these regulatory functions, as well as the ability to down-regulate β-catenin. Similar to Axin/APC, RanBP3 (Ran-binding protein 3), a cofactor of the CRM1 (chromosome region maintenance 1)-dependent nuclear export complex, specifically interacts with unphosphorylated β-catenin

(active β-catenin) in the nucleus, promoting the export of active β-catenin to the cytoplasm. However, RanBP3-mediated modulation of β-catenin localization is independent of APC and CRM1 [107]. In addition to Axin/APC or RanBP3, several modulators participating in the intracellular localization of β-catenin have been identified. Menin, which controls proliferation and apoptosis especially in pancreatic β-cells, functions as a tumour suppressor by directly interacting with β-catenin and removing it from the nucleus via nuclear–cytoplasmic shuttling [108]. HDAC7 is also known to control endothelial cell proliferation via prevention of nuclear translocation of β-catenin in conjugation with 14-3-3 (Figure 5) [109].

Several lines of evidence have suggested that cilia or cilia-associated proteins function to regulate canonical Wnt/β-catenin signalling by controlling β-catenin localization. Jbn (jouberin), the protein product of the *AHI1* gene, is mutated in Joubert syndrome, which is characterized by cystic kidney ciliopathy [110]. It is expected that *AHI1* $^{-/-}$ kidneys have primary cilia defects; however, the cilia of *AHI1* $^{-/-}$ kidneys are indistinguishable from those of control kidneys. On the other hand, Lancaster et al. [110] found that *Ahi1* $^{-/-}$ mice show reduced renal Wnt activity, and a subsequent study [111] showed that Jbn plays a positive role in Wnt/β-catenin signalling by facilitating nuclear translocation of β-catenin via its three NLS motifs. In contrast with the positive role of Jbn in the regulation of β-catenin localization, cilia-associated proteins can suppress canonical Wnt signalling by blocking translocalization of β-catenin into the nucleus. Chibby has been reported to be localized at the base of cilia as well as the nucleus, thereby inhibiting Wnt signalling by preventing nuclear entry of β-catenin [112,113]. Recently, Imajo et al. [114] revealed that YAP (Yes-associated protein)/TAZ, a transcriptional co-activator of the Hippo signalling pathway, inhibits Wnt signalling by interacting with and blocking nuclear translocation of β-catenin [114], which is a different way to inhibit Wnt signalling by Hippo signalling as we discussed in the regulation of Dvl [57].

Regulation of β-catenin abundance

In the β-catenin destruction complex, β-catenin undergoes sequential phosphorylation by CK1 and GSK3β [53,82,115], after which β-TrCP E3 ligase recognizes and ubiquitinates phosphorylated β-catenin, targeting it for proteasomal degradation [116–118]. This event is a well-known mechanism for the regulation of β-catenin turnover in the absence of Wnt stimulation. In contrast, Chitalia et al. [119] suggested that Jade1, which acts as a single-subunit E3 ligase and may be correlated with renal cancer, is capable of interacting with and ubiquitinating β-catenin regardless of phosphorylation status. These results indicate that Jade1 regulates β-catenin in both Wnt-on and Wnt-off phases, unlike β-TrCP which only ubiquitinates phosphorylated β-catenin [119]. However, ubiquitination does not always promote degradation of β-catenin. EDD, an E3 ligase identified as a GSK3β-binding partner, directly interacts with and ubiquitinates β-catenin using Lys^{11}- or Lys^{29}-linked ubiquitin chains, which in turn promotes its nuclear accumulation and stability rather than degradation [120].

A few lines of evidence have suggested that β-catenin can be degraded in the nucleus. As proteasomes have been shown to exist abundantly in both the cytoplasm and nucleus [121–123], Sox9 is able to induce β-catenin degradation by mediating nuclear translocation of the β-catenin destruction complex consisting of Axin, GSK3β, APC, CK1α and β-TrCP. These functions of Sox9 explain how it negatively regulates the anti-chondrogenic

activity of Wnt/β-catenin signalling [124]. Similarly, Oct3/4 specifically binds to nuclear β-catenin and Axin, which in turn promotes its proteasomal degradation, resulting in maintenance of undifferentiated ESCs (embryonic stem cells) through inhibition of β-catenin-mediated target gene expression, although it remains unknown how Oct3/4 negatively regulates β-catenin stability [125]. However, Kelly et al. [126] presented another way in which ESCs maintain an undifferentiated state using Oct4 and β-catenin. They showed that β-catenin forms a complex with Oct4 to enhance expression of Oct4-activated pluripotency-related genes, thereby maintaining ESCs in an undifferentiated state [126]. Since enhanced Oct4 activity by β-catenin does not require TCF-dependent transcriptional activity, this result provides evidence for the TCF-independent role of β-catenin in the regulation of ESC fate. These seemingly contradictory data are not mutually exclusive. These results showed that the pluripotency of ESCs is regulated either by reducing β-catenin transcriptional activity [125] or enhancing the expression of pluripotency-related genes by Oct4 [126].

In addition to the regulation of β-catenin turnover, it has been reported that several regulators modulate Wnt/β-catenin signalling by controlling mRNA expression of *CTNNB1*, the gene encoding β-catenin [127,128]. *CTNNB1* mRNA is unstable due to destabilizing elements located in the 3′ UTR (untranslated region). In addition, KSRP (KH-type splicing regulatory protein), an RNA-binding protein and mRNA-decay-promoting factor, was identified as a major regulator of *CTNNB1* mRNA decay. The half-life of mRNA encoding β-catenin can be prolonged by Wnt or PI3K (phosphoinositide 3-kinase)-Akt signalling through Akt-mediated KSRP phosphorylation, which induces interaction with 14-3-3, resulting in abrogation of mRNA destabilization function [127]. Moreover, it has been reported that Dvl3 forms a complex with KSRP for the negative regulation of β-catenin mRNA stability [128].

Unexpectedly, it has been reported that the β-catenin level is regulated in a proteasome- and lysosome-independent manner. CD9 and CD82, tetraspanin proteins containing four membrane-spanning domains that suppress tumour metastasis, were shown to reduce the cellular abundance of β-catenin by inducing its exosome-mediated export [129]. In another study, it was shown that 90K, a tumour-associated glycoprotein, interacts with CD9/CD82 and suppresses Wnt/β-catenin signalling through ISGylation (ISG15 modification) of β-catenin, followed by ubiquitin-dependent proteasomal degradation, but in a GSK3β- and Siah 1-independent manner. Involvement of CD9/CD82 is critical to the regulatory function of 90K [130]. However, it should be further confirmed that 90K also negatively regulates β-catenin stability by enhancing CD9/CD82-mediated exosome formation, and that 90K is an E3 ligase capable of regulating degradation of ISGylated β-catenin.

Novel regulators acting on the β-catenin–TCF complex

In the absence of Wnt, transcription of Wnt target genes is kept inactive by direct promoter binding of TCF/LEF1, which is associated with Groucho/TLE (transducin-like enhancer of split) transcriptional co-repressors that recruit HDACs to silence chromatin [131–133]. In the presence of Wnt, however, stabilized β-catenin enhances Wnt/β-catenin target gene expression by displacing the Groucho/TLE repressor complex from TCF/LEF1 and recruiting transcriptional co-activators, including the histone acetyltransferase CBP [CREB (cAMP-response-element-binding protein)-binding protein]/p300 [134]. Pygopus, which associates with nuclear β-catenin via the adapter protein Legless (or BCL9),
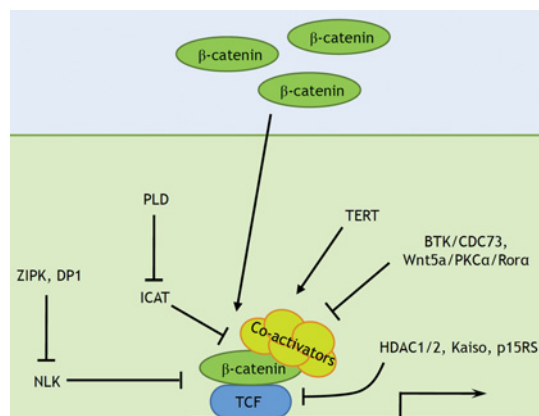
Figure 6   Modulation of β-catenin/TCF transcription factors in the nucleus

ICAT, Kaiso, HDAC1/2 or p15RS can attenuate Wnt/β-catenin signalling by inhibiting the interaction of β-catenin with TCF/LEF1. PLD is a direct Wnt target gene and can inhibit mRNA expression of *ICAT*, resulting in the formation of a positive-feedback loop. TERT can interact with the chromatin remodelling protein BRG1 to occupy Wnt-responsive gene promoters or with the β-catenin/TCF complex to increase Wnt target gene expression. ZIPK or DP1 antagonizes the suppressive effect of NLK on Wnt/β-catenin signalling. The BTK or Wnt5a/PKCα pathway alleviates canonical Wnt signalling though the transcriptional repressor CDC73 or RORα respectively.

is essential for the transcriptional activity of β-catenin during *Drosophila* development [2]. It has been suggested that Pygopus functions as an anti-repressor by overcoming the activities of Groucho/TLE and other repressors [135].

Several groups have reported the presence of novel regulators operating on the β-catenin–TCF complex for modulation of Wnt/β-catenin signalling (Figure 6). Similar to the antagonistic effect of ICAT (inhibitor of β-catenin and TCF4) or Chibby on the β-catenin–TCF complex [136,137], several novel regulators have been shown to inhibit the interaction of β-catenin with TCF, thus interfering with the formation of β-catenin/TCF. For example, deletion of HDACs 1 and 2, chromatin remodelling enzymes, resulted in stabilization and nuclear localization of β-catenin, which negatively regulates oligodendrocyte differentiation, suggesting that inhibition of Wnt/β-catenin signalling is necessary for oligodendrocyte differentiation and that HDAC1/2 inhibits Wnt/β-catenin signalling. Since ectopic expression of *Tcf7l2-enR*, which encodes a dominant-repressive form of TCF7L2, was shown to enhance the expression of oligodendrocytic markers, it was suggested that the TCF7L2-mediated repressor interaction promotes oligodendrocytic differentiation. A subsequent study showed that HDACs 1and 2 inhibit complex formation between β-catenin and TCF7L2, thereby allowing oligodendrocyte differentiation [138]. Kaiso, a p120-catenin/β-catenin-interacting protein and member of the BTB (BR-C, ttk and bab)/POZ (Pox virus and zinc finger) protein family, which generally acts as transcriptional repressors by recruiting the macromolecular HDAC complex, inhibits Wnt/β-catenin signalling by antagonizing β-catenin and xTCF3 (x is *Xenopus*) complex formation [139,140]. Alternatively, it has been suggested that xKaiso inhibits the expression of Wnt target genes by displacing xTCF3 from its target promoters [141]. p15RS, a negative regulator of the cell cycle, can interact with β-catenin and interrupt the β-catenin–TCF interaction, thereby decreasing the expression of Wnt target genes associated with cancer growth [142]. On the other hand, PLD (phospholipase D), which is a transcriptional target gene of Wnt/β-catenin signalling, enhances formation of the β-catenin–

TCF4 complex via PA (phosphatidic acid) generated by PLD. Therefore PLD/PA contributes to a positive-feedback loop in the Wnt/β-catenin signalling pathway [143,144]. E2F1 is known to repress canonical Wnt signalling by elevating mRNA expression of *ICAT*, *AXIN* and *SIAH1*. Subsequent studies have shown that ICAT is a direct transcriptional target of E2F1, providing a mechanism for the inhibition of β-catenin signalling by E2F1 [145,146]. In addition, the positive role of PLD/PA is partly mediated by inhibition of ICAT expression [147].

In addition to the regulation of β-catenin–TCF complex formation, it has been reported that NLK [NEMO (NF-κB essential modulator)-like kinase] phosphorylates TCF and interferes with binding of the β-catenin–TCF complex to DNA, resulting in suppression of Wnt/β-catenin signalling [148]. It has been shown that ZIPK (zipper-interacting protein kinase) serves as an NLK-interacting partner. Ectopic expression or knockdown of ZIPK disrupts or enhances NLK–TCF4 complex formation respectively. These results indicate that ZIPK serves as a positive regulator of Wnt/β-catenin signalling via interaction with NLK [149]. In addition, we recently reported that DP1, which binds to E2F and enhances E2F activity for regulation of the cell cycle, plays a positive role in Wnt/β-catenin signalling by binding to NLK and suppressing its kinase activity [30,31]. Apart from NLK, Sokol and co-workers [150,151] showed that HIPK2 (homeobox-interacting protein kinase 2) mediates xWnt8-induced TCF3 phosphorylation, which enables phosphorylated TCF3 to dissociate from the target gene promoter. LEF1 and TCF4, but not TCF1, also contain conserved HIPK2 phosphorylation sites and are phosphorylated by HIPK2 upon Wnt stimulation. TCF3 is known to function as a transcriptional repressor unlike other TCF family members. Subsequent investigation has revealed that HIPK2-mediated phosphorylation leads to the dissociation of LEF1 and TCF4 from a target promoter, as well as TCF3. These results indicate the context-dependent role of HIPK2 in Wnt/β-catenin signalling during embryonic development.

Upon Wnt stimulation, β-catenin prevents the interaction of TCF with the TLE repressor, which promotes the formation of a transcriptional co-activator complex for the activation of Wnt-responsive gene expression [2]. More recently new players have been reported for controlling the transcriptional ability of the β-catenin–TCF complex. Park et al. [152] presented an unexpected finding that TERT (telomerase reverse transcriptase) directly activates Wnt/β-catenin signalling by co-operating with BRG1 (brahma-related gene 1), an SWI/SNF-related chromatin remodelling protein [152]. They showed that TERT occupies TCF-binding elements of the Wnt target gene promoter and enhances target gene expression by forming a complex with BRG1, TCF3 and β-catenin [152]. Since alternatively spliced forms of human and chicken TERT, which do not have telomerase activity, still activate Wnt signalling and promote cell proliferation, it is suggested that complex formation through physical interaction, but not enzyme activity, is important [153]. More surprisingly, in a knockout and knockin mouse model system, Hoffmeyer et al. [154] showed that Wnt signalling induces TERT expression via binding of the β-catenin–Klf4 complex to the *TERT* promoter in stem cells and cancer cells. These results, mainly potentiation of TERT expression by Wnt/β-catenin signalling and vice versa, provide a mechanistic link between the self-renewal of stem cells and oncogenic potential of cancer cells [152–155].

Non-canonical Wnt signalling can inhibit canonical Wnt/β-catenin signalling [148,156,157]. Lee et al. [156] found that non-canonical Wnt signalling stimulates PKC (protein kinase C) α-mediated RORα (retinoic-acid-receptor-related orphan receptor

α) phosphorylation. Phosphorylated RORα can then interact with β-catenin at Wnt target gene promoters, thereby inhibiting the ability of the β-catenin–TCF complex to recruit polymerase II due to competition with co-activators for interaction with β-catenin [156]. These results suggest an additional way in which non-canonical Wnt signalling antagonizes canonical Wnt signalling through RORα, in addition to Siah2 induction or NLK activation [148,157]. The Wnt5a/PKC/RORα axis may present a clinical applicable pathway for antagonizing Wnt/β-catenin signalling due to the fact that RORα phosphorylation is frequently inhibited in human colorectal cancers.

CONCLUSIONS AND PERSPECTIVE

On the basis of 30 years of research, Wnt/β-catenin signalling has been established as a key regulatory pathway in most fields of biology. As inactivation or aberrant activation of Wnt/β-catenin signalling results in a wide range of human diseases, understanding of the precise molecular mechanisms of Wnt/β-catenin signalling is the first step in developing clinical therapeutic applications as well as drugs targeting human diseases. There has been great progress thanks to the devotion of many researchers. However, we have yet to fully elucidate the mechanisms of Wnt/β-catenin signalling due to its own complexity and cross-talk with other signalling pathways. Activation or inactivation of Wnt/β-catenin signalling always has pros and cons when we consider therapeutic applications. Therefore it is necessary to identify novel regulators that can control specific pathways in a context-dependent manner. Owing to technical advances obtained by high-throughput chemical library screening and whole genome-wide proteomic and genomic approaches, novel regulators of Wnt/β-catenin signalling have been continuously identified. In particular, combinatorial and integrative approaches have not only contributed to the discovery of novel modulators, but also revealed novel binding partners and regulators of Wnt/β-catenin signalling. Identification of novel modulators of Wnt/β-catenin signalling and extensive mechanistic studies will promote the development of clinical therapies for diverse human diseases.

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