综述与专论

β-Catenin and The β-Catenin Destruction Complex: From Basic Science to Drug Design

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Abstract The canonical Wnt/β-catenin signaling pathway plays critical roles in both embryonic development and tumorigenesis. Central to the pathway is the turnover of β-catenin, a protein that functions in both cell adhesion and transcription. In the absence of a Wnt signal, free cytosolic β-catenin is phosphorylated by a large protein complex called the "β-catenin destruction complex" that targets β-catenin for degradation by an ubiquitin ligase/proteasome system. In the presence of a Wnt signal, the binding of Wnt to its receptor Frizzled and co-receptor LRP leads to the inhibition of β-catenin phosphorylation in the β-catenin destruction complex through an unknown mechanism. Inhibition of the β-catenin destruction complex leads to the accumulation of nuclear β-catenin, which in turn forms a complex with Tcf and BCL9. Recent studies have provided important clues regarding the molecular mechanism of the β-catenin destruction complex as well as an explanation for how β-catenin switches between its roles in cell adhesion and transcription.

Key words Wnt pathway, β-catenin, β-catenin destruction complex, APC, cancer, stem cell, intercellular signaling

Wnts constitute a family of secreted glycoproteins that mainly act on target cells in a paracrine fashion. While the original Wnt gene was identified as an oncogene that causes mammary tumors, they have since been recognized as critical regulators of embryonic development, stem cell growth and as a key agent in a wide variety of tumors[1~3]. In embryos, Wnts play critical regulatory roles in many processes including the initial formation of the embryonic axes, the development of the heart, gut and nervous system, and the formation of the limbs[4]. The Wnts have been subdivided into two categories: the canonical Wnt pathway, which works through β-catenin, and the non-canonical Wnt pathway, which includes the planar cell polarity and Ca^{2+} signaling pathways. The focus of this review is the canonical Wnt/β-catenin pathway that controls the degradation of cytosolic β-catenin. Deregulation of the canonical Wnt/β-catenin pathway is tightly associated with cancer and other diseases[4~8].

The Wnt/β-catenin pathway is highly conserved. The central regulatory step for the turnover of β-catenin is the phosphorylation of β-catenin by the two serine/threonine protein kinases Casein kinase Iα (CKIα) and glycogen synthase kinase 3β (GSK-3β), with CKIα acting to prime β-catenin for phosphorylation by GSK-3β[3,9,10]. In the absence of a Wnt signal, a cytoplasmic protein complex called the β-catenin destruction complex containing CKIα, GSK-3β, the tumor suppressor APC protein, and the scaffolding protein Axin among others, catalyzes the phosphorylation of β-catenin.

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Phosphorylated β-catenin is recognized by a ubiquitin ligase complex, which ubiquitinates β-catenin and targets it for degradation by the proteosome. In the presence of a Wnt signal, through an as of yet unknown mechanism, the phosphorylation of β-catenin is inhibited, which leads to the accumulation of β-catenin in the cytosol. Accumulated β-catenin migrates into the nucleus and binds to a DNA-binding protein of the Tcf/LEF-1 family, BCL9/BCL9-2 and p300/CBP[11~23], and together they turn on the transcription of specific target genes. Depending on the context, these can be embryonic genes including transcription and signaling factors[24,25], or genes involved in cell growth, including c-MYC[26] and cyclin D1[27] (Figure 1).

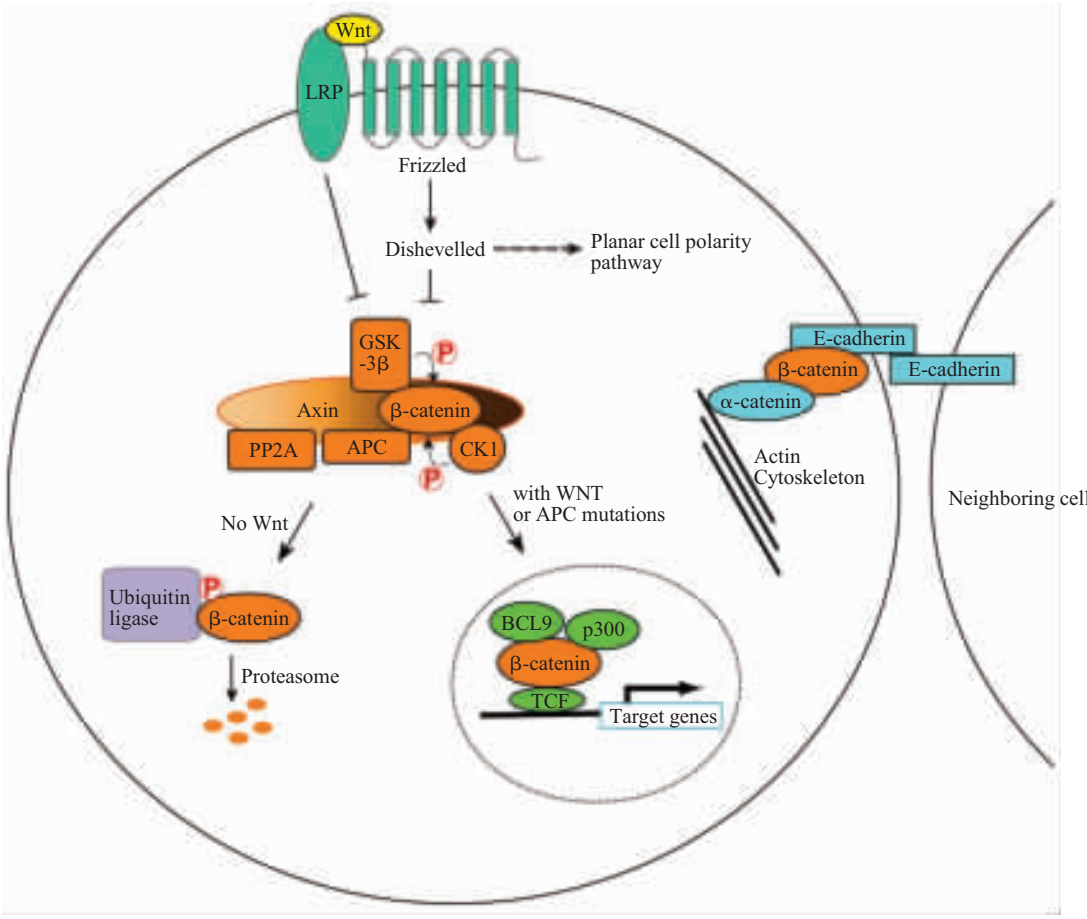

Fig. 1 Overview of the Wnt signaling pathway

Central to the canonical Wnt pathway is β-catenin, which is a dual function protein that not only activates the transcription of Wnt target genes, but also connects cell adherens junctions to the actin cytoskeleton. In the absence of a Wnt signal, β-catenin is constitutively phosphorylated by a β-catenin destruction complex that contains Axin, CKIα, GSK-3β and APC along with other proteins not shown. β-catenin is targeted for degradation by the proteasome by a ubiquitin ligase that recognizes the phosphorylated form of β-catenin. The Wnt protein binds to Frizzled and co-receptor LRP, and through an unidentified mechanism involving Dishevelled protein, phosphorylation of β-catenin by GSK-3β is inhibited. β-catenin accumulates and migrates into the nucleus to form the β-catenin/Tcf/BCL9/p300 complex, which activates the transcription of Wnt target genes.

Abnormal activation of the Wnt/β-catenin pathway through loss-of-function mutations in the tumor suppressors APC and Axin, or through gain-of-function mutations in β-catenin itself, is linked to various human cancers[3,8]. In particular, constitutive up-regulation of β-catenin transcriptional activity is a uniform feature of almost all colon cancers. APC mutations that lead to β-catenin accumulation were found in more than 80% of colon cancers[7,28]. In addition, β-catenin has been found mutationally activated in 90% of hepatoblastomas and 75% of pilomatricomas, among other cancers[6,7,29~33]. The connection between the Wnt/β-catenin pathway and cancer has fueled a search for Wnt/β-catenin pathway

antagonists, which may become lead compounds for anticancer drugs[34-37]. A better understanding of the Wnt/β-catenin pathway may benefit patients with other diseases or conditions as well, because this pathway is also involved in regulating angiogenesis, adipogenesis, and stem cell proliferation[3,8]. More human diseases related to Wnt pathway components are listed at http: // www. stanford. edu/ ~ rnusse / diseases / Humangeneticdis.htm.

1 Drug targets for inhibiting the Wnt signaling pathway: the β-catenin transcriptional complex

Transcription of Wnt target genes is the most important readout of the canonical Wnt signaling pathway, and is the basis of Wnt-induced changes in normal and malignant development. There are many ways to inhibit Wnt signaling in cultured cells. For example, the Wnt signal can be inhibited through the addition of secreted Wnt inhibitors such as WIF-1, DKK (Dickkopf) or sFRP proteins[38]. However, since most mutations observed in cancers occur in the proteins of the β-catenin destruction complex, it is generally accepted that the best targets for inhibiting the Wnt pathway in cancer treatment lie downstream of the β-catenin destruction complex. The formation of the nuclear β-catenin/Tcf (or β-catenin/LEF-1) complex is essential for Wnt target gene transcription and has been a key target for drug development. Compounds that disrupt this complex may suppress the transcription of Wnt target genes and be useful for cancer treatment. In fact, several lead compounds have been selected from libraries of natural compounds in a high-throughput assay for immunoenzymatic detection of the β-catenin/Tcf interaction[37]. Importantly, these selected compounds that disrupt β-catenin/Tcf complexes potently antagonize the cellular effects of β-catenin-dependent activities, including reporter gene activation, *c-myc* or *cyclin D1* expression, cell proliferation, and duplication of the *Xenopus* embryonic dorsal axis, thus confirming that the β-catenin transcriptional complex is a valid drug target.

While Tcf is necessary to bring β-catenin to the promoters of target genes, CBP and the highly related protein p300 play important roles in activating Wnt target genes[14-17]. A small molecule (ICG-001) that inhibits the CBP/β-catenin interaction, but not the p300/β-catenin binding [39], was shown to block in cancer cells the expression of *survivin*, an inhibitor of apoptosis that is upregulated in many cancers[40]. This study not only demonstrates the possibility of interfering with the transcription of Wnt target genes using small molecules, but indicates that it will be possible to design inhibitors that block subsets of Wnt target genes, which may be very useful in designing therapies that are less toxic than would be obtained with an inhibitor that completely blocks the response to Wnt pathway activation.

In addition to p300/CBP, BCL9 and its *Drosophila* ortholog named Legless interact with β-catenin in the nucleus, and are necessary for expression of Wnt responsive genes[20]. Human *BCL9* is a previously defined oncogene that was found over-expressed in a patient with precursor B-cell acute lymphoblastic leukemia[41]. *BCL9* genes share three short regions of homology, termed HD1, HD2, and HD3. It has been shown that the HD2 region of BCL9 interacts directly with the first 4 armadillo repeats of β-catenin, and that this interaction is required for Wnt/Wg signal transduction[20,42]. It has been proposed that one of the main functions of BCL9/Legless is to tether Pygopus to the β-catenin/TCF complex[20,22,43,44]. Since BCL9 is specific and essential for the transactivation of Wnt responsive genes, the BCL9/β-catenin interaction has been proposed as an important drug target for the canonical Wnt pathway[20,23,42].

BCL9-2, a homologue of BCL9, contains about 60% sequence identity among vertebrates and 35% identity to the human BCL9[21]. There are seven domains of homology among BCL9-2 proteins, two of which include the original HD1 and HD2 domains that are homologous between BCL9 and Legless. In addition to its role as a co-activator of β-catenin transcription in the nucleus, BCL9-2 has also been proposed to have an essential role in switching β-catenin between its roles in adhesion and transcription (see below)[21].Importantly, transcriptional activation of Wnt target genes by BCL9-2 does not involve Pygopus, indicating that a drug that targets the BCL9/Pygopus interaction may not be very effective[21]. If BCL9 and BCL9-2 bind β-catenin in very similar ways, it may be possible to find a drug that disrupts the interaction of β-catenin with both BCL9 proteins, which would have more general applicability.

2 Switching between β-catenin's dual functions: cell-cell adhesion and transcription of Wnt target genes

In the canonical Wnt pathway, β-catenin is the central effector and is required for the transcription of

Wnt target genes. In addition to transcription, β-catenin is also required for cell-cell adhesion[5,45-47]. In fact, β-catenin was originally isolated as a protein associated with the cytoplasmic region of cadherin, a transmembrane protein involved in homotypic cell-cell contacts[48]. Only when associated with β-catenin does cadherin exhibit its normal adhesive function. In adherens junctions, β-catenin is the bridge between cadherins and α-catenin, which in turn interact with the actin cytoskeleton (Figure 1). While β-catenin bound to cadherin is apparently quite stable, the cytosolic pool of catenin has a short half-life. In *C. elegans*, the different functions of β-catenin are carried out by different β-catenin-family proteins[49,50]. In vertebrates, a single β-catenin protein carries out both the transcriptional and cell adhesion functions. An important question is whether β-catenin intercellular adhesion and the activation of Wnt target genes is coordinated by shuttling β-catenin between the cell membrane and the nucleus[5,45,51]. While the constitutive formation of the β-catenin/Tcf/BCL9 complex leads to tumorigenesis, the loss of cell adhesion (the cadherin/β-catenin/α-catenin link) has also been shown to play a critical role in cancer formation and metastasis, suggesting a possible connection[52,53].

A critical clue suggesting how the balance of cell adhesion and Wnt signaling is regulated came from a study of BCL9-2. Using overexpression and RNAi studies, Brembeck *et al*. (2004)[21] presented clear evidence that BCL9-2 regulates whether β-catenin is at the membrane involved in cell adhesion, or in the nucleus activating Wnt target genes. The important regulator of these functions is residue Tyr142 in β-catenin. When β-catenin Y142 was changed to alanine, BCL9-2 binding was abolished, whereas mutation of Y142 to the phospho-mimic glutamate dramatically increased BCL9-2 binding. Intriguingly, Y142 must remain de-phosphorylated for the proper binding of α-catenin, a critical interaction that maintains the integrity of adherens junctions. These results lead to a model in which, in the absence of Y142 phosphorylation, β-catenin is bound to α-catenin and cadherin at the membrane, promoting cell adhesion. When β-catenin Y142 is phosphorylated, BCL9-2 binds β-catenin strongly, and β-catenin binding to cadherin is disrupted. The β-catenin/BCL9-2 complex translocates to the nucleus and activates Wnt target genes. In addition to Y142, β-catenin can be phosphorylated at Y654, which also disrupts the β-catenin/cadherin interactions[54]. However, unlike Y142 phosphorylation that results in BCL9-2 binding and nuclear localization, β-catenin proteins phosphorylated at Y654 can be captured by the β-catenin destruction complex and may not reach the nucleus.

A second level of switching between the different roles of β-catenin may be regulated by intramolecular interactions within β-catenin. Structural and molecular studies have shown that β-catenin contains three regions (Figure 2a). The central core region consists of twelve armadillo repeats and is responsible for interacting with Tcf, APC, Axin and cadherin[55,56]. Each armadillo repeat consists of three helices. The 12 armadillo repeats form a superhelix of helices that features a long, positively charged groove. The N-terminal region contains the GSK-3β phosphorylation sites. Both the N- and C-terminal regions contain transcriptional activation domains that are required for gene expression. It has been shown *in vitro* that the C-terminal domain of β-catenin, and possibly the N-terminal domain as well, can interact with the armadillo repeat domain[54,57-59]. Moreover, the C-terminus prevents β-catenin binding to cadherin, but does not inhibit Tcf binding to β-catenin[54,57-59]. This intramolecular interaction also prevents the β-catenin C-terminus from binding to other β-catenin partners, such as the PDZ domain of Lin7, a scaffold protein involved in cell adhesion[59,60]. These results suggest that intramolecular interactions within β-catenin could play an important role in regulating its function. In support of this, a recent study demonstrated that in the presence of a Wnt signal, a form of β-catenin is generated that binds Tcf but not cadherin. This Wnt-stimulated, Tcf-selective form of β-catenin is regulated by the C-terminus of β-catenin selectively preventing cadherin binding through an intramolecular "fold-back" interaction as suggested by *in vitro* studies[59]. It was thus proposed that the Wnt signal not only inhibits the degradation of β-catenin, but also switches the β-catenin binding activity towards Tcf by stabilizing this intramolecular interaction. How does a Wnt signal stabilize the β-catenin intramolecular interaction? One obvious possibility is posttranscriptional modification. While Y654 phosphorylation may disrupt the interaction between the armadillo repeats and the C-terminal domains[54], it remains to be tested whether or not Y142 phosphorylation, or the binding of BCL9-2, also affects the intramolecular interactions of β-catenin. Future studies are needed to search for the potential β-catenin modification that stabilizes the fold-back conformation.

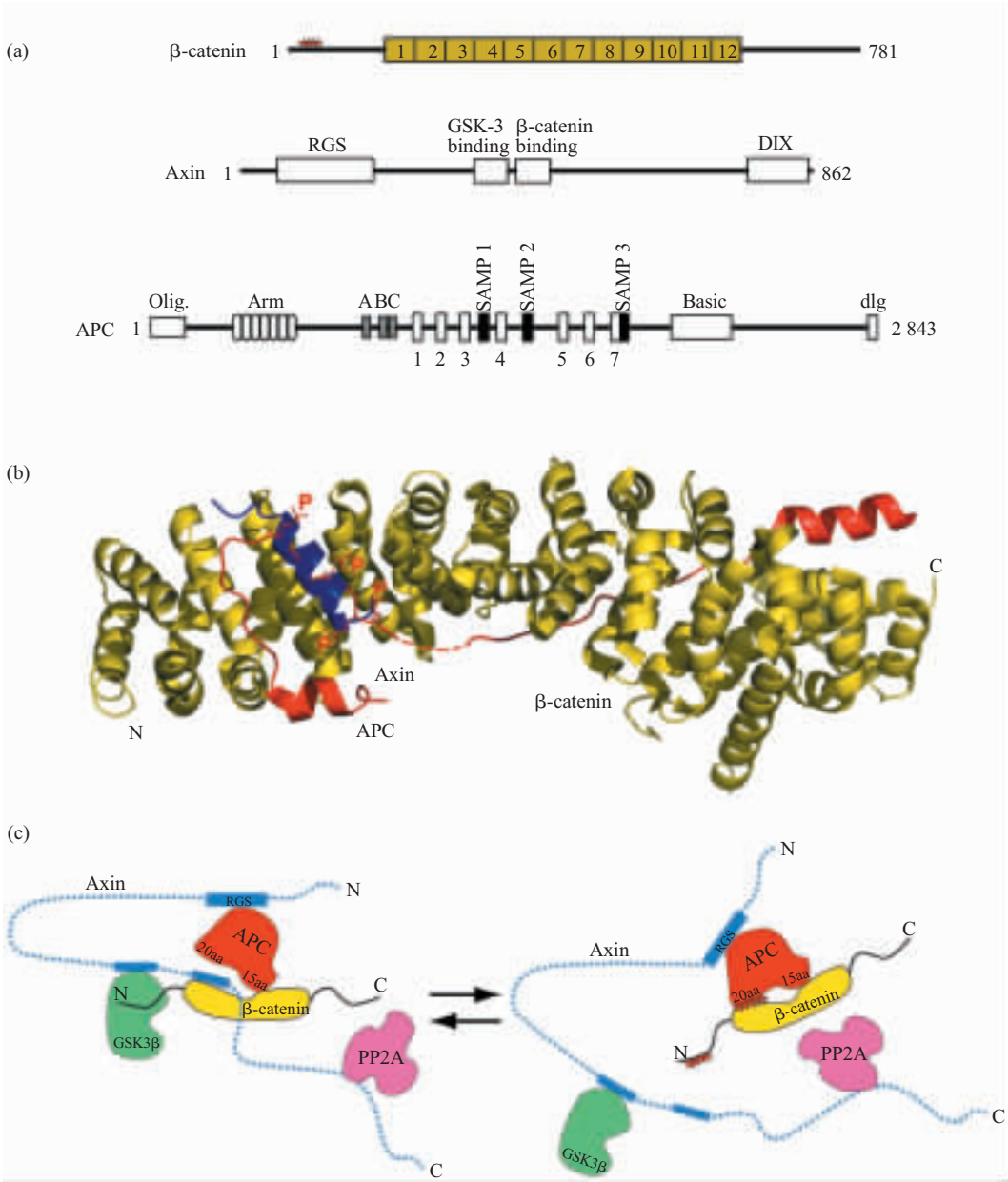

Fig. 2 β-catenin and the β-catenin destruction complex components

(a) Domain structures of β-catenin, Axin and APC. β-catenin contains a central armadillo repeat domain consisting of 12 repeats. β-catenin is subjected to ubiquitination and degradation upon the phosphorylation of four conserved Ser/Thr residues in the N-terminal domain (indicated by red stars). Axin consists of an N-terminal "regulator of G-protein signaling" (RGS)-like domain that binds APC, separate binding domains for GSK-3 and β-catenin located in the center of the protein, and a C-terminal DIX domain related to a segment of Dishevelled, which mediates dimerization of Axin and binding to Dishevelled. The DIX domain is also required for the binding of the cytoplasmic domain of the Wnt co-receptor LRP5/6. The C-terminal half of Axin also contains the binding site for CK1α. APC contains heptad repeats at its N-terminus that mediate homo-oligomerization, and seven armadillo repeats (arm) of unclear function. The C-terminal region contains a basic domain that binds microtubules. The central part of APC (~1 000 amino acids) contains ten β-catenin binding sites which includes three 15 amino acid repeats (A-C), and seven 20 amino acid repeats (1~7). Interspersed within the 20 amino acid repeat region of APC are three so-called SAMP repeats that have been shown to mediate the interaction with Axin/Conductin[24].

(b) Crystal structures of β-catenin armadillo repeat domain (yellow), in complex with the β-catenin-binding domain of Axin (Axin-CBD; blue) or the phosphorylated third 20aa repeat of APC (red). Four phosphorylated Ser residues in the APC 20aa repeat are also shown in red sticks and labeled with a red P. Each armadillo repeat contains three helices, and the twelve armadillo repeats form a superhelix with a positively charged structural groove, which forms the binding site for APC, Axin and Tcf. Note that the binding site on β-catenin for the phosphorylated APC 20aa repeat overlaps the binding site for the Axin-CBD.

(c) A structural model for the role of APC in the β-catenin destruction complex. (Left side) Axin recruits unphosphorylated β-catenin to the destruction complex, via its interaction with β-catenin armadillo repeats 3 and 4. The GSK-3β binding site of Axin is N-terminal to the β-catenin binding site, positioning GSK-3β near the N-terminus of β-catenin, which can then be phosphorylated. APC is recruited to the complex through an interaction between its SAMP repeats and the RGS domain of Axin. (Right side) APC and β-catenin are phosphorylated by GSK-3β and CKI (red stars). The phosphorylated APC 20aa repeats compete β-catenin away from the Axin binding site, and open up Axin's β-catenin binding site for the next β-catenin substrate molecule.

3 APC: the rejuvenator of the β-catenin destruction complex?

One of the most critical questions in Wnt signaling is how APC, which is mutated in the large majority of colon cancers, plays a critical role in β-catenin turnover[61]. APC is a large protein with 2 843 residues in a single peptide chain, which interacts with both β-catenin and Axin. The N-terminal region of APC contains an oligomerization domain and seven armadillo repeats of unclear function (Figure 2a). The C-terminal region contains a basic domain that binds microtubules. The central part of APC (~1 000 amino acids) contains three 15 amino acid (15aa) repeats[62,63], and seven 20 amino acid (20aa) repeats[64] that both bind β-catenin. Interspersed within the 20 amino acid repeat region of APC are three so-called SAMP repeats that have been shown to mediate the interaction with Axin, the scaffold protein of the β-catenin destruction complex[65]. Axin consists of an N-terminal "regulator of G-protein signaling" (RGS)-like domain that binds APC[65,66], separate binding domains for GSK-3β and β-catenin located in the center of the protein[65,67], and a C-terminal DIX oligomerization domain (Figure 2a).

One of the proposed roles for APC is promoting β-catenin nuclear export[68,69]. It is also generally assumed, although it has not been strictly proven, that APC promotes the phosphorylation of β-catenin in the β-catenin destruction complex, the most critical step of β-catenin turnover. Consistent with this concept, APC directly interacts with the scaffolding protein Axin in the β-catenin destruction complex[65]. Moreover, tansfection of the APC central region containing the Axin and β-catenin binding motifs into colon cancer cells, which lack a functional APC, enhanced the degradation of β-catenin[70]. Exactly how APC promotes β-catenin degradation within the destruction complex has been an important mystery.

Recently, based on structural and biochemical studies, we and Ha *et al*. have proposed that APC may play a critical role in the β-catenin destruction complex by removing the catalytic product, phosphorylated β-catenin, from the active site, thus allowing the destruction complex to efficiently recruit the next β-catenin substrate[71~73]. In this way, APC functions as the active site "rejuvenator" of the β-catenin destruction complex. Critical to this model is the proposal that the 15 and 20 amino acid repeats have different roles, and that the 20aa repeats are regulated by phosphorylation. The model is largely based on the following observations. First, the intracellular concentration of Axin is in picomolar range, at least in frog eggs where it was carefully measured[74]. Since Axin is the essential scaffold, the concentration of the β-catenin destruction complex in these frog eggs must be also in this range. Nevertheless, in the presence of Wnt signal, the concentration of cytosolic β-catenin increases from <50 nmol/L range to 100~200 nmol/L range within minutes[74]. Therefore, the β-catenin destruction complex has to be an efficient enzymatic complex. Second, phosphorylated β-catenin, the product of each catalytic cycle, can interact avidly with the single β-catenin binding site in Axin, which is required for recruiting the next β-catenin substrate to the active site[73,75]. Third, structural studies demonstrated that the binding sites on β-catenin for Axin and the phosphorylated APC 20aa repeats overlap (Figure 2b), and that the phosphorylated 20 amino acid repeats can prevent Axin from binding β-catenin[71~73].

It is important to note that the phosphorylation of the APC 20aa repeat dramatically increases its binding affinity for β-catenin. Without phosphorylation, the APC 20aa repeats can not inhibit Axin binding to Axin or Tcf[71~73]. Thus APC phosphorylation is essential for the proposed "rejuvenator" function. In the proposed model, β-catenin enters the complex by binding both Axin and the 15aa APC repeats (Figure 2c). β-catenin is then phosphorylated by CK1α and GSK3, both of which are bound to Axin. The APC 20aa repeats are also phosphorylated, and this causes them to bind β-catenin tightly, displacing Axin from β-catenin. We suggest that this conformation promotes the exit of β-catenin from the destruction complex. β-catenin may be released from the destruction complex through the dephosphorylation of the 20aa repeats by PP2A, which is present in the destruction complex through its direct interactions with both APC and Axin[76,77]. Alternatively, APC and phosphorylated β-catenin may be released from destruction complex as a complex, which are separated when β-catenin is degraded by the proteasome. The overall model is attractive as it provides a mechanism for β-catenin release from the destruction complex, and because it is consistent with many biochemical observations. Future studies will be needed to directly test this model.

4 Regulation of the β-catenin destruction complex

A fundamental question in Wnt signaling is how

the presence of Wnt signal in the extracellular space regulates the activity of the β-catenin destruction complex. It is generally accepted that the binding of certain Wnt proteins to the extracellular domains of Frizzled and LRP5/6 is essential for the inhibition of β-catenin phosphorylation in the β-catenin destruction complex[78](Figure 1). At this point, it is unclear if the Wnt signal leads to the disassembly of the β-catenin destruction complex or if it controls its activity through a subtle conformational change.

A large body of work demonstrates that Axin is a critical regulator of the β-catenin destruction complex. First, Axin mutations cause an increase in β-catenin levels and Wnt gene activation, and are associated with cancers[33,75,79,80]. Second, Axin is the one component of the complex that directly interacts with all of the other proteins in the complex. Third, Axin is the limiting factor for the formation of the complex. The Axin concentration in the cell is much lower than the concentration of the other components in the β-catenin destruction complex[74]. In fact, it was proposed that Wnt regulates the activity of the destruction complex through the degradation of Axin[81]. Finally, Axin is the only known partner for the PPPSP motif of the cytosolic domain of LRP5/6, which is sufficient for activating the Wnt pathway[82] (see below).

Dvl, the mammalian homolog of *Drosophila Dishevelled* (Dsh), was shown to be genetically required for the canionical Wnt signaling pathway[83]. The Dvl N-terminal region, including the DIX domain, interacts with the C-terminal region of Axin that also contains a DIX domain (Figure 2a)[84~88]. Dvl is also a critical regulator of the non-canonical Wnt pathway. It remains unclear how Dvl regulates the activity of the β-catenin destruction complex separately from its non-canonical role[89].

LDL receptor related proteins 5 and 6 (LRP5/6) and their *Drosophila* homolog *Arrow* are single span transmembrane proteins essential for Wnt/β-catenin signaling[90~93]. The cytoplasmic domain of LRP5/6 contains five PPPSP motifs that are necessary and sufficient to trigger canonical Wnt signaling[82]. A single PPPSP motif, attached to the LDL receptor is sufficient to activate the Wnt pathway[94]. Wnt signaling induces, and requires, phosphorylation of the PPPSP motif, which creates a binding site for Axin[94]. Thus the LRP-Axin interaction is another key step for understanding β-catenin destruction complex regulation[78,94,95].

5 Perspectives

The Wnt signaling pathway has been shown to control cell differentiation and proliferation, and is critical for embryonic development, cancer and stem cell maintenance. In recent years, as more and more players were found to play a role in Wnt signaling regulation, this pathway turned out to be very complex. However, the main theme for this pathway remains the regulation of the β-catenin and β-catenin destruction complex, which is still poorly understood. In the coming years, it will be profoundly important to reveal the mechanism of β-catenin destruction complex and to determine how β-catenin controls the transcription of Wnt responsive genes. Unraveling the Wnt signaling mechanism will be key to understanding many aspects of embryonic patterning and stem cell regulation as well as providing the basis for drug design to deal with Wnt-related cancers.

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