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Wnt/ β -catenin signaling: new (and old) players and new insights

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Abstract

Wnt/ β -catenin signaling has central roles in embryogenesis and human diseases including cancer. A central scheme of the Wnt pathway is to stabilize the transcription coactivator β -catenin by preventing its phosphorylation-dependent degradation. Significant progress has been made towards the understanding of this critical regulatory pathway, including the protein complex that promotes β -catenin phosphorylation-degradation, and the mechanism by which the extracellular Wnt ligand engages cell surface receptors to inhibit β -catenin phosphorylation-degradation. Here we review some recent discoveries in these two areas, and highlight some critical questions that remain to be resolved.

Introduction

Signaling by the Wnt family of secreted lipoproteins has central roles in embryogenesis and in adult tissue homeostasis [1,2]. The most well known Wnt pathways is the highly conserved canonical Wnt/ β -catenin signaling, which regulates the stability of the transcription cofactor β -catenin and therefore β -catenin-dependent gene expression [1,2]. Abnormal Wnt/ β -catenin signaling is associated with many human diseases, including cancer, osteoporosis, aging and degenerative disorders [2,3]. Therefore the study of Wnt/ β -catenin signaling not only helps to understand human pathogenesis, but may also provide novel therapeutic targets for treatment. This review will focus on some recent advances in the mechanism of β -catenin destruction complex and Wnt receptor activation. For a more comprehensive discussion on Wnt/ β -catenin signaling we recommend the following reviews for further reading [1-7].

A general outline of Wnt/ β -catenin signaling has been established. In the absence of the Wnt ligand, the cytosolic β -catenin protein level is kept low due to phosphorylation-dependent ubiquitination and degradation. β -catenin phosphorylation is performed by the sequential action of casein kinase I (CK1) and glycogen synthase kinase 3 (GSK3), which reside in a protein complex assembled by the scaffolding protein Axin and the *adenomatous polyposis coli* gene product (APC), a tumor suppressor protein [1,2]. Phosphorylated β -catenin is in turn recognized by β -Trcp, an E3 ubiquitin ligase subunit, and is ubiquitinated and degraded (Figure 1). Upon Wnt stimulation, two distinct cell surface receptors act together to initiate Wnt signaling. One is a member of the Frizzled (Fz) family of serpentine proteins, and the other is low-density-lipoprotein receptor related protein 6 (LRP6) or the closely related LRP5 [4]. Wnt may induce a Fz-LRP6 complex formation, which recruits Axin to the plasma membrane [8], resulting in the inhibition of β -catenin phosphorylation/degradation (Figure 2). Stabilized β -catenin protein accumulates in the nucleus and complexes with the TCF/LEF (T cell factor/

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lymphoid enhancer factor) family of DNA-binding transcription factors to enhance gene expression.

New components and logic of the Axin- β -catenin degradation complex

PP1 has an activating role in Wnt/ β -catenin signaling

Multiple key steps in Wnt signaling involve phosphorylation. GSK3 and CK1 phosphorylate not only β -catenin, but also Axin [9,10] and APC[11]. Thus dephosphorylation by protein phosphatases may also play critical roles in Wnt signaling. The ubiquitous protein phosphatase 1 (PP1) was recently identified as a conserved positive component in Wnt/ β -catenin signaling [12]. PP1 interacts with Axin and dephosphorylates Axin at several CKI-phosphorylated serine residues; Axin dephosphorylation in turn reduces Axin-GSK3 interaction, resulting in inhibition of β -catenin phosphorylation/degradation (Figure 1) [12]. Of note, Axin residues that are phosphorylated by CK1 and dephosphorylated by PP1 are scattered outside the GSK3-binding domain of Axin [12], suggesting that Axin undergoes a phosphorylation-dependent conformational change that regulates Axin-GSK3 interaction. PP1 is a major serine/threonine phosphatase that regulates a variety of cellular processes and signaling pathways, and its functional specificity is largely controlled through the interaction of catalytic subunit PP1C along with many different regulatory subunits [13]. It remains to be examined whether PP1 constitutively antagonizes the assembly of the Axin-GSK3 complex, or whether Wnt regulates PP1C phosphatase activity or interaction with specific regulatory subunits.

Tumor suppressor WTX antagonizes Wnt/ β -catenin signaling

The tumor suppressor WTX was identified as a new component of the β -catenin destruction complex [14]. WTX (Wilms Tumor gene on the X chromosome) is mutated in a significant number of Wilms tumor cases, a pediatric kidney cancer syndrome [15]. WTX is found to complex with Axin, APC, β -catenin, and β -Trcp in cells; and WTX interacts directly with β -catenin and β -Trcp and promotes β -catenin ubiquitination and degradation [14] (Figure 1). Thus WTX acts to antagonize Wnt/ β -catenin signaling. Activating β -catenin mutations are also found in many Wilms tumor cases, but interestingly only in those that do not have mutations in WTX [15]. This is consistent with the notion that WTX and β -catenin act in a common molecular pathway. How WTX promotes β -catenin ubiquitination/degradation is unknown. WTX (also called AMER1) was independently identified as an APC-interacting protein, and can recruit APC to the plasma membrane away from microtubules via the ability of WTX to bind to phosphatidylinositol(4,5)-bisphosphate (PIP2) [16]. Depletion of WTX via siRNA reduces APC protein level in the cell and promotes APC distribution to microtubule ends [16]. WTX regulation of APC may underlie the mechanism by which WTX promotes β -catenin degradation, given that APC has a role in β -catenin phosphorylation and ubiquitination [7, 17], or may be related to the role of APC in regulation of microtubules and cell polarity and adhesion [18-21]. It should be noted that the *Drosophila* genome does not seem to encode an identifiable WTX homolog, and thus the role of WTX in suppressing Wnt/ β -catenin signaling appears to be vertebrate-specific.

Tumor suppressor APC is a double agent

It has been established that APC antagonizes Wnt/ β -catenin signaling [5]. The molecular nature of APC function, however, has not been fully resolved. Because APC binds to both Axin and β -catenin, it likely has a role in facilitating Axin- β -catenin interaction and thus β -catenin phosphorylation [7] and ubiquitination [17]. Additional roles for APC have also been proposed, including exporting β -catenin out of the nucleus [22,23] and antagonizing TCF/ β -catenin transcriptional activation on chromatin [24].

In *Drosophila*, two APC homologs function redundantly to antagonize Wingless (Wg)/Wnt signaling [25,26]. Unexpectedly, these *Drosophila* APC homologs were recently shown to also have an activating role in both physiological and ectopic Wg signaling [27]. Elimination of APC results in elevated Axin protein levels, which is independent of increased β -catenin signaling [27], suggesting that the activating role of APC in Wg signaling may be mediated via its promotion of Axin degradation (Figure 1). This is consistent with an earlier experimental and modeling study using *Xenopus* embryo extracts, which suggested that APC-mediated Axin degradation is an important regulatory feature that buffers β -catenin level alterations when the APC level changes [28]. The Wg signaling activating function resides in the amino terminal region of *Drosophila* APC homologs, thus is distinct from the central domain of APC that is required for β -catenin degradation [27]. These results may have significant implications for understanding colon cancers due to APC mutations, which almost always result in the retention of the APC amino terminal domain [5,29]. It should be noted that a previous study of APC overexpression in *Xenopus* embryos has hinted at a positive role of APC in Wnt signaling [30]. But abundant truncated APC proteins produced in these experiments have raised concerns about the potential dominant negative effect on the endogenous APC function [31]. The molecular nature for the “ying-yang” relationship between APC and Axin in β -catenin degradation and signaling remains to be elucidated.

New insights into Wnt receptor activation and signaling

LRP6 phosphorylation and activation by GSK3 and CK1

Wnt coreceptor LRP6 and its *Drosophila* ortholog Arrow are indispensable for Wnt/ β -catenin signaling [4]. Recent studies indicate that Wnt-induced LRP6 phosphorylation is critical for LRP6 activation [32]. The intracellular domain of LRP6, LRP5 and Arrow share five reiterated PPPSPxS motifs (P, proline; S, serine or threonine; x, any residue) that are essential for signaling [32-35]. Strikingly, transfer of each of these individual PPPSPxS motifs into a heterologous receptor (a truncated LDL receptor) is sufficient to activate β -catenin signaling [32,34] (B. MacDonald and X. He, submitted). In response to Wnt stimulation, the PPPSPxS motif becomes dually phosphorylated in a sequential manner: Wnt induces PPPSP phosphorylation, which primes and is required for the subsequent xS phosphorylation [34]. The dually phosphorylated PPPSPxS motif becomes a docking site for Axin [32,34,35], consistent with an earlier finding on LRP5-Axin interaction [8]. Mutation of the S residues (to alanine) in all five PPPSPxS motifs renders LRP6 completely inactive and unable to bind to Axin [32,34].

The kinases that mediate Wnt-induced PPPSPxS phosphorylation were identified, unexpectedly, as GSK3 and CK1 [34,35]. These results suggest that GSK3 also plays a key positive role in Wnt/ β -catenin signaling, in addition to its established negative role in antagonizing Wnt/ β -catenin signaling via β -catenin phosphorylation/degradation. The positive role of GSK3 has been experimentally demonstrated [34,36]. Thus a membrane-tethered form of GSK3 activates Wnt/ β -catenin signaling in a manner that depends on LRP6 PPPSP motifs [34], whereas inhibition of GSK3 at the plasma membrane via a membrane-tethered inhibitory peptide blocks Wnt signaling [36]. Therefore it appears that membrane associated GSK3 is required for LRP6 phosphorylation to activate Wnt/ β -catenin signaling, whereas the cytosolic GSK3 antagonizes Wnt/ β -catenin signaling through β -catenin phosphorylation. The mouse Gsk3a and Gsk3b have redundant functions in β -catenin phosphorylation/degradation [37] and LRP6 phosphorylation [36]. The 7 CK1 genes in the mouse genome may also have overlapping roles. CK1 γ (γ 1, γ 2, and γ 3), CK1 α and CK1 ϵ/δ may each participate in xS (in PPPSPxS) phosphorylation and perhaps other phosphorylation outside these motifs [34,35], either through direct membrane association (CK1 γ) or via association with other proteins such as Dishevelled (CK1 ϵ/δ) and Axin (CK1 α) (see below). Results from genetic experiments in *Drosophila* are consistent with this view [38].

Control of LRP6 phosphorylation by Fz, Dishevelled and Axin: initiation and amplification

Genetic studies have established essential roles of Fz, and its downstream cytoplasmic partner Dishevelled (Dvl) in Wnt/ β -catenin signaling. Recent studies suggest that Fz and Dvl functions are critical for Wnt-induced Lrp6 phosphorylation [36] [39]. Artificially induced Fz-LRP6 proximity is sufficient to induce PPPSP phosphorylation in the absence of Wnt stimulation [36]. Furthermore, the Fz intracellular domains, which have been implicated in association with Dvl [40–42], are required for the promotion of LRP6 phosphorylation, suggesting that Fz acts via Dvl to control LRP6 activation [36]. Interestingly Axin is also required for Lrp6 phosphorylation in a manner that depends on its ability to bind to GSK3 [36], and Axin is recruited to the plasma membrane by Fz through Dvl [36] [43]. These results suggest a model that upon Wnt-induced Fz-LRP6 complex formation, Fz recruitment of Dvl in turn engages the Axin-GSK3 complex, thereby promoting LRP6 PPPSP phosphorylation by GSK3 [36] (Figure 2). Therefore the Axin-GSK3 complex has both positive (via LRP6 phosphorylation) and negative (via β -catenin phosphorylation) roles in Wnt signaling, and a key feature of the Wnt pathway may be to control the balance of these dual roles through Fz and Dvl.

A recent study in *Drosophila*, using transgenes for a chimeric Fz-Arrow fusion receptor and an artificially dimerized Arrow protein, suggested that Wg signaling involves an initiation step that depends on both Fz and Arrow and an amplification step that is Arrow-dependent [44]. Fz/Dvl promotion of LRP6 phosphorylation by Axin-GSK3 may correspond to the initiation step (Figure 2a). LRP6-Axin interactions, which include both Axin binding to phosphorylated PPPSPxS motifs and Axin promotion of PPPSPxS phosphorylation, may constitute the amplification step (Figure 2a). The observations that the multiplicity of the PPPSPxS motif within LRP6 is essential for LRP6 function, and that phosphorylation of one PPPSP motif critically depends on the presence of other PPPSP motifs are consistent with this notion [45] (B. MacDonald and X. H., submitted). Thus the five PPPSPxS motifs conserved from *Drosophila* to human may represent a built-in amplifier for Wnt signaling (B. MacDonald and X. H., submitted).

Aggregation and clustering of Dvl and Wnt receptors

Two recent studies [43,46] showed that Dvl has an unusual property of dynamic polymerization, which to some extent correlates with the ability of Dvl to activate Wnt/ β -catenin signaling. This property is due to the DIX domain found in both Dvl and Axin. Indeed Axin may also have a polymerization capability, although it is not as dynamic as that observed for Dvl [43]. The crystal structure of Axin DIX domain reveals multiple β -strands that engage in head-to-tail self-interaction [46]. Dvl polymerization is proposed to provide a highly concentrated platform for dynamic recruitment of other Wnt signaling partners, such as Axin [43] (Figure 2b). Another study [39] reported that Wnt induces, in a Dvl-dependent manner, LRP6 aggregation and phosphorylation by CKI (at a site outside the PPPSPxS motifs). Dvl mutants that do not polymerize prevent such LRP6 aggregates. Therefore Dvl polymerization may help to aggregate Wnt-induced Fz-LRP6 complex, Axin and associated GSK3 and CK1 to trigger LRP6 phosphorylation (Figure 2b). One cautionary note is that these aggregations were best demonstrated when all the above (five or six) components were co-overexpressed, and that Dvl was often not detected in the LRP6 aggregates [39]. It also remains to be examined whether these LRP6 aggregates are similar to or distinct from the LRP6 endocytic vesicles [47] (see below). Better analytic tools for endogenous Wnt signaling components will be required to substantiate these observations. Nonetheless, the initiation-amplification model aforementioned and the aggregation model may be complementary in providing temporal and spatial views for Wnt receptor activation.

Other factors proximal to Wnt receptor activation: Macf1 and Caveolin

Macf1 (microtubule actin cross-linking factor 1), also called ACF7 (actin cross-linking factor 7), is a giant protein (600 kD) and belongs to the spectraplakins family of molecules that link the cytoskeletal network to membrane-associated junctional protein complexes [48]. A role of Macf1 in Wnt/ β -catenin signaling was suggested via genetic studies, which show that *Macf1*^{-/-} mouse embryos have similar phenotypes to that of *Wnt3*^{-/-} and *Lrp5/6*^{-/-} double knockout mice [49]. In Wnt-expressing cells Macf1 complexes with Axin, β -catenin, GSK3, and APC in the cytosol, but with LRP6, Axin, GSK3, and β -catenin, but not APC, in the membrane fraction. RNAi depletion of Macf1 inhibits LRP6-Axin association and TCF/ β -catenin-dependent transcription. Macf1 directly interacts with Axin and LRP6, and is suggested to have a role in the translocation and subsequent binding of the Axin complex to LRP6 [49] (Figure 2), although no direct evidence for such a translocation is yet available. One puzzling issue is that Wg-related phenotypes have not been described for *Drosophila* mutants in the Macf1 (*shortstop*) locus, including a possible null allele [50].

Caveolin-1 is another factor that is implicated in LRP6-mediated signaling [47]. Caveolin-1 is a major protein constituent of caveolae, which are lipid-raft enriched invaginations involved in clathrin-independent endocytosis. LRP6 when overexpressed is internalized upon Wnt stimulation via Caveolin-1, which forms a complex with phosphorylated LRP6 and Axin, thereby resulting in less β -catenin-Axin association [47]. This effect was suggested to have a positive role in β -catenin stabilization. While these results imply that Caveolin-mediated LRP6 internalization has a role in Wnt signaling, there is disagreement whether Caveolin- or clathrin-mediated endocytosis is important for Wnt signaling [47,51], and genetic evidence for caveolin involvement in this pathway remains yet to be established.

Conclusions

Because of the space constraints we have focused this review primarily on limited new findings on the β -catenin degradation complex and Wnt receptor activation. Despite sharing common components such as Axin and GSK3, the mechanistic link between these two processes remains far from clear. The fact that APC, Axin, and GSK3 have been shown to exhibit dual positive and negative functions in Wnt/ β -catenin signaling, and the dynamic nature of Dvl and Axin proteins and likely of other components and their associations reveal significant complexity and kinetic nature of this regulatory network. Post-translational modifications, such as phosphorylation and dephosphorylation, likely regulate many critical aspects of the Wnt signaling network. Further investigations via biochemical, genetic and systems approaches will likely yield a better understanding of Wnt/ β -catenin signaling and its involvement in development and diseases.

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Figure 1.

The β -catenin degradation complex. This complex is assembled by Axin, and contains β -catenin, APC, GSK3 and CK1. PP1 and WTX may also associate with the Axin complex. β -catenin phosphorylation by CK1 and GSK3 results in β -catenin recognition by β -Trcp, thereby triggering its degradation (1). Note that CK1 and GSK3 also phosphorylate Axin and APC, and in general these phosphorylation events result in tighter association of Axin and APC with β -catenin. PP1 dephosphorylation of Axin (at sites phosphorylated by CK1) reduces/releases GSK3-binding to Axin, which should not only result in less β -catenin phosphorylation by GSK3, but also less Axin phosphorylation by GSK3, thereby releasing β -catenin from Axin as well (2). APC has a role in Axin degradation (3) in addition to β -catenin degradation (1), thus plays dual functions in the Wnt pathway.

Figure 2.

Activation of the Wnt receptor complex. (A) The initiation-amplification model. In the Wnt-induced Fz-LRP6 complex Fz recruits Dvl, which in turn recruits the Axin-GSK3 complex to initiate LRP6 phosphorylation on PPPSPxS motifs (initiation). Phosphorylated PPPSPxS motifs recruit more Axin-GSK3 complex to promote further PPPSPxS phosphorylation (amplification). Dvl or Axin-associated CK1 (α , δ , or ϵ) and membrane-associated CK1 γ may phosphorylate LRP6 and are omitted from the model for clarity. MACF1 associates with the cytosolic Axin complex and may have a role in promotion of Axin recruitment to the receptor complex. (B) Receptor aggregation model. Dvl polymerization promotes both the clustering of the Fz-LRP6 complex and the recruitment of the Axin complex, resulting in LRP6 phosphorylation.