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Review

Signaling through β-catenin and Lef/Tcf

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Abstract. β-Catenin plays a structural role in cell adhesion by binding to cadherins at the intracellular surface of the plasma membrane and a signaling role in the cytoplasm as the penultimate downstream mediator of the wnt signaling pathway. The ultimate mediator of this pathway is a nuclear complex of β-catenin acting as a coactivtor with lymphoid enhancer factor/T cell factor (Lef/Tcf) transcription factors to stimulate transcription of a variety of target genes. Signaling through β-catenin is regulated by modulating its degradation and nuclear translocation. In the absence of an activating signal, phosphorylation of β-catenin by glycogen synthase kinase 3 (GSK3) acting in conjunction with adenomatous polyposis coli and axin/conductin causes β-catenin to interact with the β-transducin repeat-containing protein which results in its ubiquitination and degradation. Signaling from the wnt pathway activates dishevelled which, in an as yet undefined manner, inhibits the activity of GSK3 resulting in an increase in the cytoplasmic free pool of β-catenin, and translocation into the nucleus. The integrin-linked kinase (ILK) pathway also activates β-catenin-Lef/Tcf signaling. ILK phosphorylates GSK3 to inhibit its activity and translocates β-catenin into the nucleus. In addition, ILK downregulates the expression of E-cadherin and upregulates Lef-1 expression. In the final step of the β-catenin-Lef/Tcf signaling pathway, nuclear β-catenin binds pontin52-TATA binding protein and displaces Groucho-related gene or CREB-binding protein corepressors from Lef/Tcf resulting in stimulation of transcription. During development, β-catenin-Lef/Tcf signaling is involved in the formation of dorsal mesoderm and dorsal axis. Furthermore, defects in the β-catenin-Lef/Tcf pathway are involved in the development of several types of cancers.

Key words. β-Catenin; lymphoid enhancer factor/T cell factor (Lef/Tcf); wnt; dishevelled (dsh); glycogen synthase kinase 3 (GSK3); adenomatous polyposis coli (APC); integrin-linked kinase (ILK); cadherin.

Introduction

β-Catenin and its homologue, plakoglobin (also called γ-catenin), were originally described in 1989 as proteins at the intracellular surface of the plasma membrane linked to transmembrane cadherins [1, 2]. This implicated them in cell adhesion because neighboring cells bind each other through the extracellular domains of cadherins [reviewed in ref. 3]. Cadherins interact directly with β-catenin or γ-catenin, which bind α-catenin, which in turn is linked to the cytoskeleton [4, 5]. The finding that β-catenin and plakoglobin were the

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mammalian homologues of *Drosophila* armadillo protein [6, 7] suggested that they were potential signaling molecules because armadillo was genetically identified to be a mediator in the wingless (wg) pathway. Signaling by the wg ligand in *Drosophila* determined segment polarity along the anterior-posterior axis of embryos [8] and increased the stability of armadillo causing it to accumulate in the cytoplasm and nucleus of cells [9] in an underphosphorylated form [10].

Genetic epistatic experiments in *Drosophila* indicated that the wg signal acts through dishevelled (dsh) which inactivates zeste-white 3 (zw3) kinase (also known as shaggy), resulting in increased intracellular concentrations of armadillo [11–14]. dsh is normally phosphorylated and wg signaling causes it to become hyperphosphorylated [15]. A member of the frizzled (frz) family of transmembrane proteins (Dfrz2) was identified as a candidate receptor for wg ligand in 1996 [16].

The mammalian homologues of wg are the wnt family of ligands [17], a name that combined *wg* with the homologous mouse *int-1* proto-oncogene [18]. Ectopic wnt signaling in *Xenopus* embryos caused axis duplication resulting in two-headed tadpoles [19]. Ectopic expression of β-catenin also induced axis duplication in *Xenopus* embryos and the β-catenin localized to the nucleus, leading to the proposal that mammalian β-catenin acts in a signaling pathway similar to that of armadillo in *Drosophila* [20]. The mammalian homologues of the zw3 kinase are the glycogen synthase kinase 3 (GSK3) enzymes [21]. In support of the hypothesis that wg and wnt signaling were similar, *Xenopus*-GSK3 (XGSK3) was found to phosphorylate the amino-terminal domain of β-catenin and inhibition of this phosphorylation increased the steady-state level and nuclear concentration of β-catenin. These results suggested that phosphorylation by GSK3 leads to β-catenin degradation and inhibition of GSK-3 leads to higher cytoplasmic and nuclear concentrations of β-catenin [22]. Degradation of β-catenin occurs through the ubiquitin-proteasome pathway [23].

Another molecule entered as an important component of this pathway when β-catenin was shown to complex with the adenomatous polyposis coli (APC) protein, via a central region of APC containing three repeats of 15 amino acids [24, 25]. APC downregulates β-catenin and this activity was mapped to its central region containing seven repeats of 20 amino acids which are immediately C-terminal to the three repeats of 15 amino acids [26]. APC was placed in the wnt pathway when it was shown that GSK3 phosphorylated APC which enhanced binding of β-catenin to APC [27], and wnt-1 signaling increased the stability of APC [28].

Events downstream of β-catenin were defined by experiments where β-catenin, or plakoglobin, were found to interact with the lymphoid enhancer factor/T cell factor (Lef/Tcf) transcription factors. β-Catenin does not have a nuclear localization signal but coexpression of β-catenin with Lef-1 or XTcf-3 caused the complex to be translocated to the nucleus where it activated transcription, and ectopic expression of Lef/Tcf in *Xenopus* induced axis duplication. These results suggested that the increased free β-catenin induced by wnt signaling binds to Lef/Tcf in the cytoplasm to be translocated into the nucleus [29–31].

It had been found that wnt signaling increased the steady-state concentration of plakoglobin and β-catenin, stabilized catenin-cadherin complexes at the plasma membrane, and increased the strength of cellular adhesion [32, 33]. This raised the question as to whether the effects of wnt signaling resulted from enhanced cellular adhesion. But Funayama et al. [20] outlined four reasons why increased cellular adhesion cannot account for β-catenin signaling: (i) amino-terminal deletions of β-catenin that do not bind α-catenin still induce axis duplication in *Xenopus*, and α-catenin is required for cadherin-mediated adhesion [20]; (ii) ectopic expression of cadherins in *Xenopus* embryos does not induce axis duplication [34, 35]; (iii) in fact, overexpression of cadherins in *Xenopus* oocytes inhibits dorsal axis development [36], and (iv) the adhesion and signaling functions of armadillo in *Drosophila* seem to be genetically separated [37]. Furthermore, (v) both wild-type E-cadherin and E-cadherin without its extracellular domain titrate β-catenin signaling in *Drosophila*, demonstrating that the adhesive function of cadherin is not linked to β-catenin signaling [38]; (vi) a β-catenin mutant for binding α-catenin, which disrupts functioning in adherens junctions, and a mutant for wg signaling are both lethal in *Drosophila* but the two mutants complement each other to produce viable adults [9]; (vii) the increased steady-state concentration of β-catenin resulting from wnt signaling is primarily an increase in the free cytoplasmic pool of β-catenin [28] and this increase occurs in the absence of cadherin [39].

The 1996/1997 working model for wnt signaling through β-catenin-Lef/Tcf

These results led to a consensus model dating to 1996 which has provided the basis from which to view signaling through β-catenin-Lef/Tcf (fig. 1). β-Catenin is normally found in three intracellular locations: (i) at the plasma membrane bound to cadherins and forming a link to the cytoskeleton through α-catenin; (ii) in the cytoplasm, and (iii) in small amounts in the nucleus. In the cytoplasm, β-catenin is either free or in a complex

with APC. GSK3 normally phosphorylates APC and β-catenin which causes β-catenin degradation by ubiquitination and the proteosome pathway. When wnt signals through a frizzled receptor, dsh is hyperphosphorylated and activated, resulting in inhibition of GSK3 through an unknown mechanism. The inhibition of phosphorylation of β-catenin and APC increases the stability of β-catenin causing the free cytoplasmic concentration of β-catenin to rise. Some of this β-catenin interacts with Lef/Tcf and is translocated into the nucleus to stimulate gene transcription as a complex of β-catenin-Lef/Tcf. Cadherins can modulate the signaling of β-catenin by titrating free cytoplasmic β-catenin to the plasma membrane [40–43].

Developmental effects of β-catenin-Lef/Tcf signaling

In vertebrates, β-catenin functions in dorsal mesoderm formation in which epithelial cell precursors differentiate into mesenchymal cells [36, 44]. Duplication of the dorsal axis in *Xenopus* has been achieved not only by ectopic expression of wnt and β-catenin but also by other molecules in the wnt pathway: dsh [45], plakoglobin [46], and Lef-1 in the presence of β-catenin [29, 30]. Formation of dorsal axis is prevented by injection of antisense oligonucleotides to maternal β-catenin mRNA [36], dominant negative XTcf3 mRNA [31], and dominant negative Lef-1 mRNA [29], and injecting GSK3 mRNA along with β-catenin mRNA blocks axis duplication [22]. wnt proteins can be separated into two categories based on their ability to induce axis duplication in *Xenopus* embryos. The wnt-1 class can cause axis duplication and includes wnt-1, -2, -3a, -7b, 8, and wnt-8b while the wnt-5a class cannot and includes wnt-4, -5a, and wnt-11 [47]. However, dorsal axis specification need not arise from wnt signaling but perhaps occurs from dorsal-ventral differences in GSK3 activity by wnt-independent mechanisms [40]. The maternal

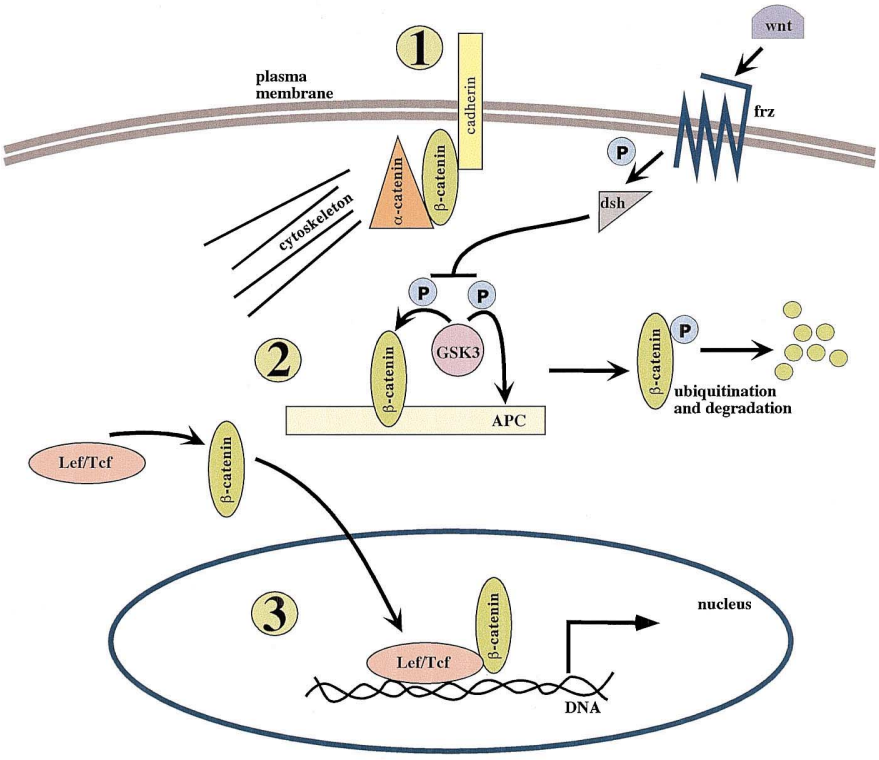

Figure 1. The 1996/1997 model for signaling through β-catenin-Lef/Tcf. β-Catenin is found in three cellular locations. (1) On the inner side of the cell membrane linking transmembrane cadherins to α-catenin and the cytoskeleton. (2) In the cytoplasm interacting with APC or as free monomers. (3) In the nucleus acting as a coactivator for Lef/Tcf. Normally, the catabolism of β-catenin is mediated by GSK3 which phosphorylates APC and β-catenin and the phosphorylation of β-catenin results in its ubiquitination and degradation. The translocation of β-catenin from the cytoplasm into the nucleus is exclusively from wnt signaling. The wnt ligand binds to frz which mediates the hyperphosphorylation of dsh which in turn inactivates GSK3. β-catenin is not phosphorylated and its free intracellular concentration increases; some of it interacts with Lef/Tcf and the complex is transported into the nucleus.

Xenopus GSK-binding protein (GBP) may mediate wnt-independent specification of dorsal axis because it inhibits phosphorylation of GSK3 resulting in an elevation of β-catenin and axis duplication [48].

While Lef-1$^{-/-}$ or Tcf-1$^{-/-}$ knockout mice do not resemble mice with any wnt-targeted gene inactivation, mice with null mutations in both Lef-1$^{-/-}$ and Tcf-1$^{-/-}$ match the phenotype of defective differentiation of paraxial mesoderm found in wnt-3a$^{-/-}$ knockout mice. This shows that Lef-1 and Tcf-1 are redundant in early mouse development [49]. β-Catenin and Lef/Tcf are also involved in the formation of hair and teeth. Ectopic expression of Lef-1 in mice produces abnormal hair patterning and inappropriate hair and tooth growth [50]. Lef-1 is required for tooth and hair development in mice and its expression can be induced by bone morphogenetic protein 4 [51]. Ectopic expression of constitutively active β-catenin in the epidermis produces more hair in newborn mice and as adults these transgenic mice continued to produce more hair follicles [52].

In the sea urchin embryo, β-catenin is found in the vegetal cells and specifies the pattern of cells on either side of the animal-vegetal axis [53]. GSK3β is also involved in this patterning [54]. In *Dictyostelium*, modulation of GSK3 activity is involved in anterior-posterior axis formation [55]. Genetic inactivation of *Drosophila* APC induces apoptosis of neuronal pigment cells of the eye which is rescued by overexpressing zw3 or by down-regulating Tcf, suggesting that β-catenin-Lef/Tcf signaling can be involved in apoptosis [56].

Tumorigenic effects of β-catenin-Lef/Tcf signaling

Activation of β-catenin is involved in many types of cancers. Most patients with colon carcinoma have early mutations in the tumor suppressor APC [57] and are homozygous for truncations which delete the central seven repeats of 20 amino acids necessary to downregulate β-catenin [43]. Cells from such cases have a stable pool of β-catenin which interacts with Lef/Tcf and gives greatly increased constitutive transcription of Lef/Tcf responsive promoters [58]. Some patients with colon carcinoma are heterozygous for activating mutations of β-catenin in amino-terminal residues where GSK3 phosphorylates β-catenin and their cells have constitutively active Lef/Tcf [59]. Mutations at the GSK3 phosphorylation sites of β-catenin as well as stabilizing amino-terminal truncations of β-catenin have been identified in some melanoma cells, while other melanoma cell lines had mutant APC [60].

Activating amino-terminal mutations of β-catenin are also frequently found in hepatocellular carcinoma [61]. Ectopic expression of N-terminal-deleted constitutively active β-catenin in mouse keratinocytes produced hair follicle tumors [52]. Some wnt proteins can cause transformation of mammary epithelial cells. The wnt ligands can be divided into three groups: those that promote strong transformation (e.g., wnt-1), weak transformation (e.g., wnt-6), and no transformation (e.g., wnt-4). The strongly transforming wnts showed increased cellular concentrations of β-catenin [62]. It was demonstrated that the transforming effect of activated β-catenin can be mediated through Lef/Tcf by transforming cells with Lef-1 fused to either β-catenin or to the transcription activation domain of VP16 [63]. C-myc, as well as cyclin D1, have been identified as targets of the β-catenin-Lef/Tcf signaling pathway in colon carcinoma cells, and may be important mediators of tumorigenesis for this pathway [64, 65].

β-Catenin and Alzheimer disease

After translation, most of the newly formed β-catenin and plakoglobin associate with E-cadherin before being transported to the plasma membrane [66]. The trafficking of catenins may be controlled by presenilins (PS) and defects at this stage may contribute to Alzheimer disease. PS1 and PS2 are transmembrane proteins found in the endoplasmic reticulum and Golgi apparatus which may have roles in protein processing and/or intracellular transport. The amyloid precursor protein is processed by a mechanism involving PS1 and PS2. Mutations in PS1 and PS2 lead to increased levels of amyloid-β peptide 42 (Aβ42) which is implicated as the cause of Alzheimer disease [reviewed in ref. 67]. One group has reported that PS1 formed a complex with β-catenin and that wild-type PS1 stabilized β-catenin whereas mutant PS1 destabilized it [68]. Another group reported that both wild-type and mutant PS1 destabilized β-catenin and decreased β-catenin-Lef/Tcf transcription activity, but that the mutant PS1-decreased transcription activity to a greater extent than wild type [69]. A third group found that PS1 and PS2 mutants decreased the nuclear localization of β-catenin but not the total cellular β-catenin after stimulation of wnt signaling by lithium [70]. Results from a fourth group also suggest that lower levels of β-catenin arise from PS mutations. Hyperphosphorylated forms of tau are associated with Alzheimer disease and this group found tau and GSK3β complexed with PS1. Mutant PS1 proteins bound more GSK3β than wild-type PS1 and phosphorylated tau to a much greater extent [71]. Higher concentrations of GSK3β in a complex with mutated PS1 and β-catenin would increase phosphorylation and degradation of β-catenin. The postulated mechanism by which decreased levels of β-catenin contribute to Alzheimer disease is that they render neurons more susceptible to apoptosis by Aβ42 [68].

Lef/Tcf elements in promoters

Several Lef/Tcf-responsive sites in gene promoters have been implicated or identified. Functional sites for c-myc and cyclin D1 have already been mentioned and there are consensus binding sites for Lef/Tcf in the promoters of cyclin A, cdc 2, and cdc 25 [65]. Engrailed is a transcription factor specifying segment polarity and is a target for the wg signaling pathway in *Drosophila* [12]. There is a Lef-1 site in the enhancer of *Drosophila* ultrabithorax which induces endoderm formation [72]. Siamois in *Xenopus* is required for formation of the Spemann organizer. It is activated in the Nieuwkoop center by wnt and ectopic expression of siamois induces a secondary axis [73]. The promoter of siamois has three Lef/Tcf-binding sites and its expression is regulated by β-catenin-Tcf3 [74]. The expression of noggin in somites is controlled by wnt signaling [75]. Lef/Tcf binds to sites in the promoter of c-jun and fra-1 which are elements of the AP-1 transcription complex. Overexpression of β-catenin increases the expression of c-jun, fra-1, and urokinase-type plasminogen activator receptor and downregulates zonula occludens-1, a protein involved in epithelial polarization [76]. The distal region of the enhancer for human immunodefeciency virus 1 contains a functional Lef-1-binding site [77]. Lef/Tcf motifs are found in the keratin promoter [50]. *Xenopus nodal-related 3* gene, which is a member of the transforming growth factor β family expressed in the Spemann organizer, is wnt inducible and has a Lef1-responsive element in its promoter [78]. *Xenopus twin* is a homeobox gene that is a target for Lef/Tcf and which in turn activates the Spemann organizer homeobox gene *goosecoid* [79]. β-Catenin-Lef-1 also binds to a Lef/Tcf consensus site in the promoter for E-cadherin [30] although the functional consequences of this interaction are presently unclear.

Components of the β-catenin-Lef/Tcf signaling pathway

wnts and frizzled

wnts are a large family of secreted glycoproteins that have a multitude of signaling effects including axis formation, myogenesis, neural development and organogenesis [reviewed in refs 47, 80]. The frz family of cell surface proteins are the putative receptors for wnts. These molecules have an extracellular cysteine-rich domain, seven transmembrane domains, and cytoplasmic tails of variable lengths that can contain a PDZ protein-binding domain [81]. Besides Dfrz2 acting as a receptor for wg, homologues of frz from rat [82] and zebrafish [83] transduce the signal from wnt8. Dfrz2 and frz are functionaly redundant for wg signaling in *Drosophila* embryos [84, 85]. Proteoglycans act as coreceptors for wnts [86–88].

frzb is a secreted protein homologous to the cysteine-rich extracellular domain of frizzled receptors and antagonizes wnt signaling by binding to wnts extracellularly [89–91]. Four murine secreted frizzled-related proteins (sFRPs) containing the cysteine-rich domain have been identified, with sFRP-3 identical to human frzb [92]. Another group found a human frizzled-related protein which is equivalent to sFRP-1 and showed that it antagonizes wnt-induced axis duplication in early *Xenopus* embryos [93]. There are two secreted wnt antagonists without a cysteine-rich domain, Dickkopf-1 [94], and wnt-inhibtory factor-1 [95].

dsh

dsh is a phosphorylated cytoplasmic protein with three domains: a DIX domain similar to one in axin (see below), a PDZ domain which recognizes short protein sequences, and a DEP domain that regulates GTPases [96]. Casein kinase 2 phosphoryaltes dsh and may be one of the kinases involved in its wnt-mediated hyperphosphorylation. Although increased phosphorylation of dsh seems to be required for wnt signaling, it is not sufficient; therefore, other wg-induced effects on dsh are required to transduce the signal to β-catenin [97].

Besides being involved in the wnt pathway, dsh also has a role in specifying cell planar polarity. An example of a defect in this pathway is abnormal hair orientation in *Drosophila* wings [98]. Cell planar polarity effects are through Dfrz which signals dsh through its C-terminal DEP domain to translocate to the plasma membrane [96]. The cell planar polarity pathway is independent of armadillo and activates a Jun-N-terminal kinase (JNK) cascade again via the DEP domain of dsh [99]. dsh also interacts with Notch and blocks its signaling [100] while in the same cell, dsh activates Delta (a Notch ligand) to activate Notch signaling in a neighboring cell [101, 102].

GSK3

Signaling from four different pathways results in the phosphorylation of a serine residue in GSK3 and the consequent inhibition of its activity: (i) from growth factors to promote growth and differentiation, through mitogen-activated protein (MAP) kinase with phosphorylation by $p90^{rsk}$; (ii) from insulin to increase glycogen synthesis, invoking phosphoinositide 3 kinase (PI3K) and activation of protein kinase B (PKB)/Akt; (iii) presumably from wnt to specify differentiation, invoking dsh and protein kinase C (PKC) [103], and (iv) from integrin-linked kinase (ILK) to promote growth and differentiation [104] (see below). Only the wnt and ILK pathways activate transcription through β-catenin-Lef/Tcf.

APC

The APC polypeptide is a very large protein of 2843 amino acids. It can oligomerize and is found associated with microtubules [reviewed in ref. 43]. APC2 is a homologue expressed in the central nervous system that can also function to downregulate β-catenin-Lef/Tcf signaling [105, 106]. The effects of ectopic expression of APC do not fit its role as a negative regulator of β-catenin signaling because APC injected into *Xenopus* embryos in the presence of β-catenin induces axis duplication [107] and disruption of APC in *Caenorhabditis elegans* blocks wnt signaling [108].

β-Catenin

Besides associating with cadherins, β-catenin can associate with another molecule involved in cellular adhesion. DF3/MUC1 is a mucin-like glycoprotein [109] which can titrate β-catenin from E-cadherin and also interacts with GSK3β. Phosphorylation of DF3/MUC1 by GSK3 decreases its binding to β-catenin. The resulting free β-catenin binds to E-cadherin but it does not seem to increase β-catenin-mediated transcriptional activation [110].

An additional coactivator has been described for β-catenin-Lef/Tcf. The amino-terminal armadillo repeat of β-catenin interacts with a nuclear protein, pontin52, which is a TATA-binding protein. Lef/Tcf also interacts to form a ternary complex, which suggests that pontin52 may link β-catenin-Lef/Tcf to the basic transcriptional machinery [111].

Although it was first thought that β-catenin had to be transported into the nucleus through its association with Lef/Tcf, mutant forms of β-catenin that were missing their binding sites for Lef/Tcf could localize to the nucleus [9, 112]. It has now been shown that β-catenin naturally enters the nucleus via its armadillo repeats, which are also found in the nuclear importin molecules. β-Catenin first docks to the nuclear envelope through its armadillo domain by binding directly to the nuclear pores and then, in an energy-dependent manner, translocates into the nucleus in a manner similar to that of importin β. The cytoplasm has an inhibitor of β-catenin docking that is not importin β, suggesting that wnt and/or other signaling pathways may alleviate this inhibition by modifying β-catenin to prevent its interaction with the inhibitor [113].

One mechanism of β-catenin nuclear translocation that has not yet been explored is its nuclear export. NF-ATc is exported from the nucleus when it is phosphorylated by GSK3 in the nucleus [114], and nuclear export of cyclin D1 may also be mediated through phosphorylation by nuclear GSK3 [115]. Such a mechanism for β-catenin would provide additional control of its signaling pathway.

Lef/Tcf

Lef/Tcf transcription factors were originally discovered independently by three groups. T-cell-specific transcription factor-1α (Tcf-1α) recognized a motif in the human T cell receptor α enhancer [116] and contained a high-mobility group (HMG) box DNA-binding domain, named after chromosomal nonhistone high-mobility-group protein 1 [117]. A second group discovered the identical molecule in mouse pre B and T lymphocytes and named it lymphoid enhancer-binding factor 1 (Lef-1) [118]. A third group found T-cell-specific transcription factor (Tcf-1), a human homologue of Lef-1/Tcf-1α that activated the T lymphocyte enhancer of the CD3-ε gene [119] and also found several isoforms of Tcf-1 [120]. Two additional human homologues have been found, Tcf3 and Tcf4 [121]. The *Drosophila* homologue of Lef/Tcf is dTcf, or pangolin [122]. There are also chicken, *Xenopus* and *C. elegans* homologues [123]. Lef/Tcf is widely expressed in embryos, although Lef-1 and Tcf-1 show different expression patterns during mouse embryogenesis [124].

HMG boxes induce sharp bends in DNA and Lef-1 induces a 130° bend. This suggests that such transcription factors function as architectural factors which bring into proximity distant transcription factors on a promoter [125, 126]. It was initially thought that the amino domain of Lef/Tcf functioned as a transactivator domain only in a context-dependent manner, i.e., dependent on the binding of neighboring transcription factors [127, 128]. Such context-dependent activation does apply for Lef-1 stimulating the T cell receptor enhancer and this activity of Lef-1 is dependent on the coactivator ALY [129]. However, context-dependent activation and the architectural function of Lef-1 do not apply for β-catenin-Lef/Tcf transcriptional activation [130].

The HMG domain of Lef/Tcf alone is insufficient to stimulate transcription [77, 127] and β-catenin is required as a transcription cofactor for Lef/Tcf [72, 112] which interacts with β-catenin through its amino-terminal (not its HMG) domain [20, 30]. β-Catenin decreased the Lef-1-induced DNA bend by 40° [29].

In 1997, results suggested that Lef/Tcf may be functioning as a transcriptional repressor. Expression in *Xenopus* of plakoglobin or β-catenin anchored to the plasma membrane activated the wnt pathway [131]. The authors hypothesized that the tethered catenins were sequestering Lef/Tcf which normally acted as a repressor. Similar results were interpreted by another group as the tethered β-catenin competing for APC and increasing the concentration of free cytoplasmic β-catenin which then activated Lef/Tcf transcription [132]. This latter interpretation seems to be correct since membrane-tethered β-catenin cannot signal in mutant *Drosophila* embryos which lack endogenous β-catenin [133]. However,

that Lef/Tcf functions as a repressor was shown when elimination of Lef/Tcf sites elevated expression of the target gene *siamois* [74].

In 1998, four independent groups showed that Lef/Tcf normally interacts with corepressors and therefore the dynamic interactions between corepressors and β-catenin coactivator determine the transcriptional activity of Lef/Tcf. The *Drosophila* cAMP-response-element-binding protein (CREB)-binding protein (CBP) usually functions as a transcriptional activator. CBP interacts with dTcf, acetylates a conserved lysine residue in the armadillo-binding domain, thus lowering the affinity of armadillo for dTcf [134]. Groucho (Gro) repressors also act as Lef/Tcf corepressors. The protein product of the *Xenopus* Gro-related gene (Grg), XGrg, binds hTcf-1 in a region carboxyl to the β-catenin-binding region and inhibits transcriptional activation by XTcf-3 [135]. Gro is a corepressor for dTcf [136] and human Grg interacts with Lef-1 to inhibit its transcriptional activation by β-catenin [137].

Until it was shown that Lef/Tcf can act as a repressor, the wnt signaling paradigm in early *C. elegans* embryogenesis appeared opposed to wnt signaling in other organisms, with mutations in β-catenin and Lef/Tcf giving opposite not similar phenotypes. In *C. elegans* blastomeres, levels of the Lef/Tcf homologue, pop-1, are linked to anterior-posterior fate decisions [138]. In the four-cell-stage *C. elegans* embryo, wnt signaling promotes endoderm, not mesoderm, formation, and seems to inhibit Lef/Tcf activity. The EMS cell differentiates into an anterior MS (mesoderm) cell and a posterior E cell (endoderm) and it is the E cell fate that is specified by an inductive signal, otherwise both cells develop into MS cells. The inductive signal is from a wnt homologue (mom-2). The MS cell has higher levels of pop-1 than the E cell and inactivating pop-1 produces two E cells. Suppressing the wnt signal produces two MS cells [139] and suppressing the β-catenin homologue (wrm-1) also produces two MS cells [108, 140]. These results can be explained if pop-1 is acting as a repressor [135], e.g., pop-1 inhibits endodermal gene expression and wnt signaling alleviates this repression in the E cell which has a low concentration of pop-1 but not in the MS cell which has a high concentration of pop-1.

New players in β-catenin-Lef/Tcf signaling

Axin

In 1997, another player was introduced into the wnt pathway. Mouse embryos homozygous for the *Fused* locus (renamed *axin*) have an axis duplication. Axin has a regulation-of-G-protein-signaling (RGS) domain which has potential GTPase-activating activity, and near its C terminus it has homology to dsh (DIX domain). Axin localizes to the inside of the plasma membrane and regulates negatively the wnt signaling pathway [141]. A homologue of axin in mouse is called conductin [142], and in rat, axil [143]. Axin forms a ternary complex with GSK3β and the armadillo repeats of β-catenin, is phosphorylated by GSK3β, and promotes the phosphorylation of β-catenin by GSK3β. There is no significant phosphorylation of β-catenin by GSK3 in the absence of axin [144] and axin inhibits transcriptional activation by β-catenin-Lef-1 [145]. Axin binds APC through its RGS domain and enhances the phosphorylation of APC by GSK3β. In the absence of axin, there is no direct binding of GSK3β to either β-catenin or APC. APC binds axin in its central region that is usually deleted in colon carcinomas, which explains the functional defect of APC mutations [146]. Conductin binds to three sites in the central region of APC termed SAMP domains which are within the seven repeats of 20 amino acids [142]. Contrary to expectations, axin or conductin do not need to interact with APC to downregulate intracellular β-catenin [142, 146]. This led one group to suggest that in vivo both APC and axin are needed to downregulate intracellular β-catenin where APC may derepress axin and to hypothesize that axin acts as a scaffold protein for APC, GSK3β, and β-catenin [146].

Protein phosphatase 2A

Protein phosphatase 2A (PP2A) is a serine-threonine phosphatase composed of three subunits: structural (A), regulatory (B), and catalytic (C), and its structural subunit has been identified as a putative tumor suppressor [147]. Its catalytic subunit binds to the C-terminal domain of axin which suggested that PP2A interacts with the APC-β-catenin-axin-GSK3 complex to modulate the effects of GSK3, e.g., antagonizing the phosphorylation of β-catenin from GSK3 by dephosphorylating β-catenin and increasing its signaling [148]. However, overexpressing a regulatory subunit of PP2A (B56), which binds to the amino-terminal third of APC, reduces the amount and signaling of β-catenin. This suggests that PP2A may dephosphorylate GSK3 thereby increasing its activity, which in turn increases the phosphorylation of β-catenin and its subsequent degradation [149]. The presence of PP2A in the APC-β-catenin-axin-GSK3 complex offers another level of control over β-catenin signaling; however, the pathways modulating PP2A activity, the target of PP2A, and the extent of its involvement in β-catenin-Lef/Tcf signaling are presently unknown.

Supernumeracy limbs protein/β-transducin-repeat-containing protein

In 1998, *Drosophila* supernumerary limbs protein (slimb) was discovered to be an F-box/WD40 repeat protein that targets phosphorylated armadillo and cubitus interruptus of the hedgehog pathway for degradation by the ubiquitin/proteosome pathway [150]. The β-transducin-repeat-containing protein (βTrCP) is the mammalian homologue of slimb and mediates ubiquitin-proteosome degradation of CD4 through its F box binding to the ubiquitin ligase complex [151]. Ectopic expression of βTrCP inhibits dorsal axis formation in *Xenopus* embryos, suggesting that it targets phosphorylated β-catenin for ubiquitination [152]. Phosphorylated serine residues 33 and 37 in β-catenin are recognized by βTrCP as a phosphorylation-dependent destruction motif [153]. Human βTrCP interacts with β-catenin through its WD40 repeats but human βTrCP2 does not interact with β-catenin, and β-catenin with mutations in either serine 37 or serine 45 which are found in human tumors fails to interact with βTrCP [154].

ILK

ILK is a serine-threonine kinase which interacts with β1 and β3 integrins and contains four ankyrin repeats. Overexpression of ILK in epithelial cells induces a fibroblastic phenotype, decreased adhesion to integrin substrates, anchorage-independent growth [155], fibronectin matrix assembly, and tumorigenicity [156]. These ILK-overexpressing cells lose their expression of E-cadherin and without detectable increases in the free pools of β-catenin, which is a hallmark of wnt signaling, translocate most of their β-catenin into the nucleus to form a transcriptionally active complex with Lef-1. At the same time, the level of Lef-1 is increased and stabilized. The nuclear translocation of β-catenin induced by ILK is not solely the result of decreased expression of E-cadherin because cells overexpressing H-ras and v-src also have decreased levels of E-cadherin but no concomitant nuclear translocation of β-catenin [157].

Integrin activation stimulates PI3K to synthesize PtdIns(3,4)P2 and PtdIns (3,4,5)P3 and activate PKB [158, 159]. ILK has a PH domain and its kinase activity is stimulated by PtdIns(3,4,5)P3 and by insulin, growth factors, and extracellular matrix interactions in a PI3K-dependent manner. ILK inhibits the activity of GSK3, directly phosphorylates GSK3, and phosphorylates PKB on serine 473 suggesting it can function as a PDK2 [104]. This raises the question as to whether ILK phosphorylates GSK3 directly or through PKB in vivo. Since wg signaling does not require PI3K [103], it is unlikely that PKB is involved in the ILK-mediated inactivation of GSK3 leading to β-catenin inactivation. However, this still needs to be confirmed. ILK can be found in a ternary complex with PINCH adapter protein and Nck-2 which is an adapter protein associated with growth factors [160].

ILK-overexpressing cells show upregulation of cell cycle proteins that are regulated by cell adhesion to the extracellular surface. There is increased expression of cyclin A and cyclin D1, activation of cyclin D1-Cdk4 and cyclin E-Cdk2, and hyperphosphorylation of the retinoblastoma protein [161]. Recent findings offer two explanantions for some of these effects. First, phosphorylation of cyclin D1 by GSK3β promotes its degradation [115] and since ILK inhibits GSK3 activity, the increased expression of cyclin D1 may be consequent to the reduction in GSK3 activity by ILK. Second, because cyclin D1 is a direct target of β-catenin-Lef/Tcf signaling and cyclin A has a Lef/Tcf-binding site in its promoter [65], the increased expression of cyclin D1 and cyclin A resulting from ILK overexpression may be due to the ILK-induced activation of β-catenin-Lef/Tcf.

Activation of ILK by fibronectin [104] and downregulation of E-cadherin by ILK [157] are consistent with the observation that there is a reciprocal relationship between the expression of integrins and cadherins [162, 163] and suggest that ILK is a mediator in this cross-talk. The downregulation of E-cadherin by ILK is in marked contrast to the increase in steady-state levels of mRNA for E-cadherin in *Drosophila* wing disc cells induced by wg signaling [164]. The decrease in E-cadherin and increase in Lef-1 mediated by ILK is also consistent with the reciprocal relationship between the expression of E-cadherin and Lef-1 in embryonic stem cells [30]. The upregulation of Lef-1 expression by ILK further distinguishes it from wnt activation of β-catenin, demonstrating that there may be alternative means of activating β-catenin-Lef/Tcf-mediated gene transcription.

Current model of β-catenin-Lef/Tcf signaling

Although it has been suggested that axin is a scaffold protein for the complex of β-catenin, APC, axin, and GSK3 [146], an alternative model might make APC the scaffold protein. In this model, axin in a complex with GSK3 binds to APC, followed by β-catenin binding APC in close proximity to axin-GSK3. This need for APC to bring axin-GSK3 into proximity with β-catenin can be circumvented by overexpressing axin. The anomalous results demonstrating that APC overexpression in *Xenopus* embryos activates β-catenin signaling instead of downregulating it [107], and APC underexpression in *C. elegans* embryos inhibiting β-catenin signaling instead of increasing it [108] support this model. A property of scaffold proteins is that when

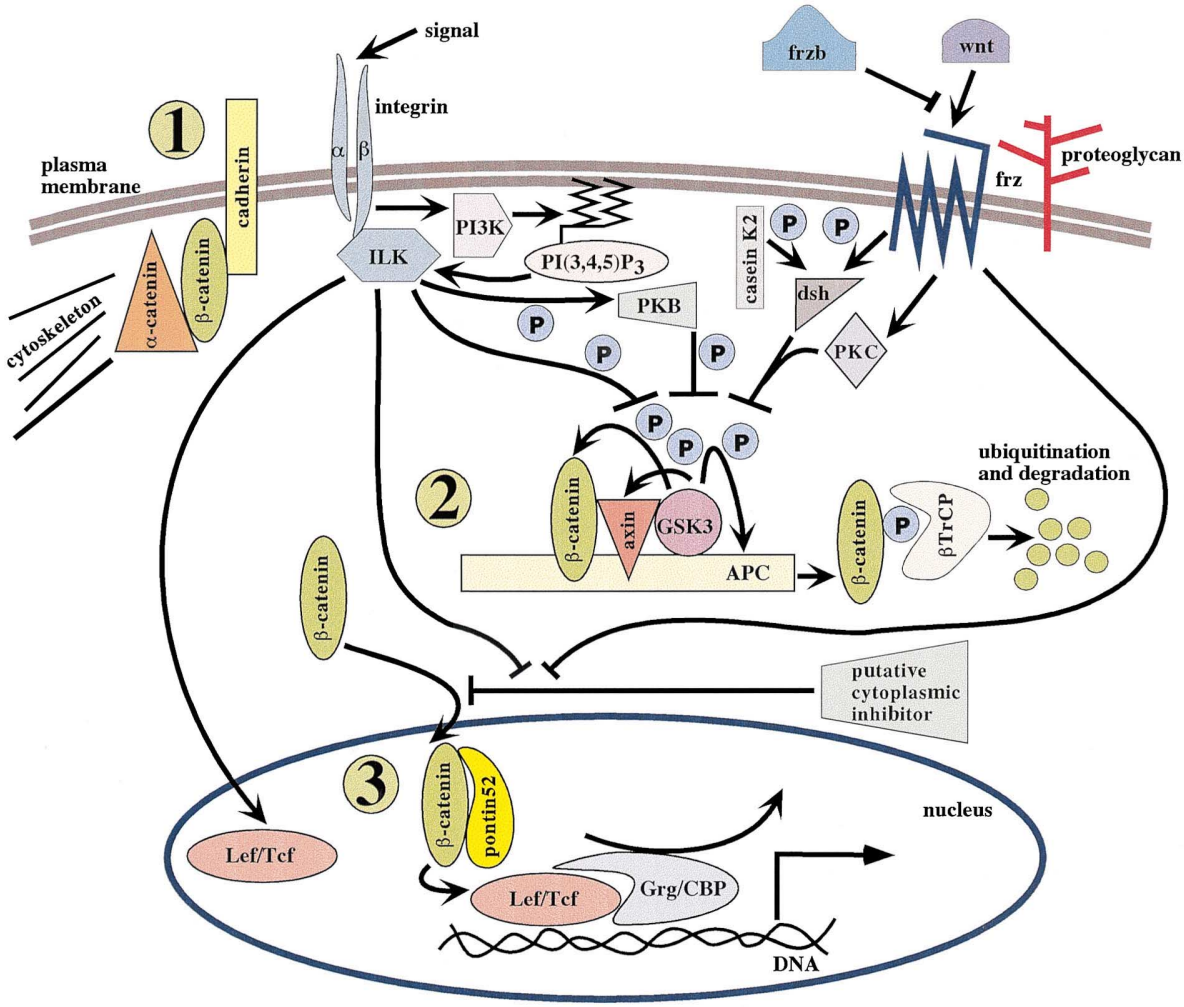

Figure 2. A current model for signaling through β-Catenin-Lef/Tcf. β-Catenin is found in three cellular locations. (1) On the inner side of the cell membrane linking transmembrane cadherins to α-catenin and the cytoskeleton. (2) In the cytoplasm interacting with APC and axin-GSK3 or as free monomers. (3) In the nucleus acting as a coactivator for Lef/Tcf along with pontin52-TATA-binding protein. Normally, the catabolism of β-catenin is mediated on a scaffold of APC which brings β-catenin into proximity with axin-GSK3. GSK3 binds axin and phosphorylates it, and this complex of axin-GSK3 binds to APC which is then phosphorylated by GSK3. β-Catenin binds to the phosphorylated APC, interacts with axin and is phosphorylated by GSK3. Phosphorylated β-catenin is bound by βTrCP which links with a ubiquitin ligase complex to degrade β-catenin. The translocation of β-catenin from the cytoplasm into the nucleus can be mediated by wnt or ILK signaling. In the wnt pathway, wnt ligand binds to frz receptor and its proteoglycan coreceptor. This interaction can be inhibited by frzb binding wnt extracellularly. Engagement of frz activates dsh and PKC. Activation of dsh is at least partly by hyperphosphorylation which includes phosphorylation by casein kinase 2. Dsh and PKC inactivate GSK3 so that β-catenin is not phosphorylated and its free intracellular concentration increases. Some of this β-catenin translocates into the nucleus mediated by its armadillo domain. A putative cytoplasmic inhibitor prevents nuclear translocation and this inhibition is antagonized by wnt signaling which either modifies β-catenin to prevent its interaction with inhibitor or inactivates the inhibitor. In the ILK pathway, engagement of integrins activates PI3K, producing PtdIns(3,4,5)P3 which activates ILK bound to an integrin β subunit. ILK then inhibits GSK3 activity by either phosphorylating it directly or first phosphorylating and activating PKB which then phosphorylates GSK3. This results in the accumulation of free β-catenin, most of which is translocated into the nucleus. ILK, unlike wnt signaling, also upregulates expression of Lef-1. As with wnt signaling, perhaps the ILK pathway downregulates the effect of a putative cytoplasmic inhibitor of nuclear translocation. In the nucleus, Lef/Tcf normally represses gene transcription by binding corepressors Grg or CBP. β-Catenin combines with pontin52-TATA-binding protein and displaces Grg or CBP from Lef/Tcf to activate transcription.

overexpressed, the stoichiometry of the components in the complex is wrong because the scaffold proteins titrate molecules away from their intended partners to assemble incomplete complexes. This downregulates the signaling pathway [165–167]. For example, overexpressing APC would result in complexes not of

{APC, β-catenin, axin-GSK3} but of {APC, β-catenin} and {APC, GSK3-axin} which would stabilize β-catenin and increase its signaling. In the absence of APC but in the presence of excess axin-GSK3, there may be uncontrolled phosphorylation of β-catenin by axin-GSK3 which would destabilize β-catenin and downregulate its signaling. Thus the concentration of APC may control the catabolism of β-catenin. In colon carcinoma, truncations in APC would inhibit the normal catabolism of β-catenin by preventing colocalization of β-catenin with limited concentrations of axin-GSK3.

An updated model of β-catenin-Lef/Tcf signaling is depicted in figure 2. β-Catenin is still in three places: plasma membrane, cytoplasm, and nucleus. In the cytoplasm it is free or in a complex with axin-GSK3 and APC as the scaffold protein. Axin forms a complex with GSK3 which phosphorylates axin; this complex then binds to APC and phosphorylates APC. β-Catenin binds to the phosphorylated APC next to axin-GSK3 and is phosphorylated by GSK3. Phosphorylation of β-catenin leads to its interaction with βTrCP which is linked to a ubiquitin ligase complex and this results in the ubiquitination and degradation of β-catenin. wnt can signal through frizzled in conjunction with proteoglycans if it is not first inhibited by soluble frzb in the extracellular fluid. The wnt signal inactivates GSK3 by activating dsh through a mechanism which includes phosphorylation of dsh by casein kinase 2 and perhaps other kinases and by activating PKC. The free cytoplasmic concentration of β-catenin increases and more of it translocates into the nucleus where it displaces Grg or CBP corepressors from Lef/Tcf. β-Catenin binds to the pontin52-TATA box coactivator, and the ternary complex of β-catenin-pontin52-Lef/Tcf activates transcription. In addition, integrins can signal to ILK via PI3K and PtdIns(3,4,5)P3 to phosphorylate GSK3 directly or indirectly by ILK first phosphorylating PKB, which also downregulates GSK3 activity and releases β-catenin to translocate into the nucleus. ILK also upregulates Lef/Tcf expression.

Another attractive hypothesis for the activation of this pathway is the existence of a cytoplasmic inhibitor for the nuclear translocation of β-catenin which can be circumvented by modifications to β-catenin or to the inhibitor from wnt signaling [113] (fig. 2). Without this level of control, translocation of β-catenin into the nucleus is seen as a mass action effect of increasing concentrations of free β-catenin. The nuclear translocation of β-catenin mediated by ILK is greater than that mediated by wnt because high levels of wnt signaling leave a large free pool of β-catenin in the cytoplasm and increase interactions with cadherins, while a high level of signaling through ILK moves essentially all the β-catenin into the nucleus. This suggests that the effect of ILK signaling on the inhibitor is greater than that of wnt signaling. An indication of an inhibitor operating to keep β-catenin in the cytoplasm could be seen in spc2 cells that were overexpressing ILK [157]. With intermediate amounts of ILK, the β-catenin was perinuclear, while with low amounts of ILK, the β-catenin was cytoplasmic, and with high amounts of ILK, the β-catenin was nuclear. There seemed to be a threshold for nuclear translocation that needed to be overcome. This suggests that once the threshold is exceeded, a feedback loop is set up where the nuclear β-catenin downregulates its inhibition for nuclear translocation and its rate of nuclear transport is further increased. Even newly translated β-catenin may be subject to nuclear translocation and there may be competition for β-catenin to complex with E-cadherin or be imported into the nucleus.

Future directions

The complexity of signaling through β-catenin-Lef/Tcf is now beginning to be appreciated. Of the many details and new avenues that can be explored we would like to highlight four questions that address different levels of the pathway. First, how does dsh inactivate GSK3? Second, what are the other signaling targets of ILK besides GSK3 which would explain the differences between ILK and wng signaling? Third, is there a cytoplasmic inhibitor which prevents β-catenin from translocating into the nucleus, and if so, where does it act? And fourth, while there is a Lef-1-binding site in the promoter for E-cadherin, why is there an inverse relationship between the expression of Lef-1 and E-cadherin in embryonic stem cells [30] and also in ILK-overexpressing cells which have activated β-catenin-Lef/Tcf [157]?

β-Catenin-Lef/Tcf signaling has emerged as a major pathway regulating gene expression, and has also been identified as a central player in development and disease. However, there are sure to be more surprises forthcoming in this exciting area of research.

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