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 coactivator 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 z-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 with the homologous mouse int-1 proto-oncogene [18]. Ectopic wg signaling in *Xenopus* embryos caused axis duplication resulting in two-headed tadpoles [19]. Ectopic expression of \(\beta\)-catenin also induced axis duplication in *Xenopus* embryos and the \(\beta\)-catenin localized to the nucleus, leading to the proposal that mammalian \(\beta\)-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 \(\beta\)-catenin and inhibition of this phosphorylation increased the steady-state level and nuclear concentration of \(\beta\)-catenin. These results suggested that phosphorylation by GSK3 leads to \(\beta\)-catenin degradation and inhibition of GSK-3 leads to higher cytoplasmic and nuclear concentrations of \(\beta\)-catenin [22]. Degradation of \(\beta\)-catenin occurs through the ubiquitin-proteasome pathway [23].

Another molecule entered as an important component of this pathway when \(\beta\)-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 \(\beta\)-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 \(\beta\)-catenin to APC [27] and wnt-1 signaling increased the stability of APC [28].

Events downstream of \(\beta\)-catenin were defined by experiments where \(\beta\)-catenin, or plakoglobin, were found to interact with the lymphoid enhancer factor/\(T\) cell factor (Lef/\(T\)cf) transcription factors. \(\beta\)-Catenin does not have a nuclear localization signal but coexpression of \(\beta\)-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/\(T\)cf in *Xenopus* induced axis duplication. These results suggested that the increased free \(\beta\)-catenin induced by wnt signaling binds to Lef/\(T\)cf 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 \(\beta\)-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 \(\beta\)-catenin signaling: (i) amino-terminal deletions of \(\beta\)-catenin that do not bind \(\alpha\)-catenin still induce axis duplication in *Xenopus*, and \(\alpha\)-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 \(\beta\)-catenin signaling in *Drosophila*, demonstrating that the adhesive function of cadherin is not linked to \(\beta\)-catenin signaling [38]; (vi) a \(\beta\)-catenin mutant for binding \(\alpha\)-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 \(\beta\)-catenin resulting from wnt signaling is primarily an increase in the free cytoplasmic pool of \(\beta\)-catenin [28] and this increase occurs in the absence of cadherin [39].

The 1996/1997 working model for wnt signaling through \(\beta\)-catenin-Lef/\(T\)cf

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