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Casein Kinase 1: A Wnt'er of Disconnect

Dispatch

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Phosphorylation of β-catenin, a central downstream component of the Wnt pathway, by glycogen synthase kinase 3 is essential for its targeted degradation by the proteosome. New studies show that casein kinase 1 primes β-catenin for subsequent phophorylation by glycogen synthase kinase 3.

As with any other signaling system, our picture of the Wnt pathway undergoes continuous modification and refinement as new and surprising discoveries are made. Accordingly, a couple of years ago the Wnt field received a jolt when two groups announced that casein kinase 1ε (CKIε) is a positive transducer of the signal [1,2]. This knowledge spawned several additional reports on the regulation of Wnt signaling by CKIε, each advancing a different mechanism by which this regulation is achieved [3–7]. Nevertheless, with the exception of one study to the contrary [7], all were consistent in arguing that CKIε plays a positive role in the Wnt pathway. Now it appears that the pendulum has swung with the release of three new papers [8–10] all proposing a similar mechanism for a negative role of CKI in the Wnt pathway.

A cursory review of Wnt signaling is essential for understanding these studies. The secreted Wnt ligands activate serpentine receptors which transmit signals leading to the stabilization of the β-catenin protein (Figure 1). Once stabilized, β-catenin engages transcription factors of the LEF/TCF family to activate various genes controlling cell growth and differentiation. A complex of proteins united by Axin occupies the space between receptor ligation and β-catenin stabilization. Axin binds to APC, β-catenin, glyocygen synthase kinase 3 (GSK3), protein phosphatase 2Ac (PP2Ac), Dishevelled and CKI. Two of these molecules, Axin and APC, are products of *bona fide* human tumor suppressor genes, and a third, β-catenin, is encoded by a proto-oncogene [11]. APC and Axin are intimately involved in processing of β-catenin for recognition by an E3 ubiquitin ligase containing the F-box protein β-TRCP. Thus, appropriate defects in APC, Axin or β-catenin interfere with the targeted destruction of β-catenin by the proteosome, thereby prolonging its signaling capacity.

At the heart of the matter is the phosphorylation of specific serine and threonine residues in the amino-terminal region of β-catenin. Phosphorylation of these residues is essential for β-catenin binding to β-TRCP, and mutations affecting them are found in a variety of human tumors [11]. It had been presumed for many years that GSK3 catalysed these key phosphorylations, and that they were somehow interfered with by Wnt signaling [12]. The discovery of a GSK3-binding protein (GBP) that disrupted the association of GSK3 with Axin, and also bound to Dishevelled, conformed nicely with the prevailing model [13]. The target serines and threonines in β-catenin, each separated by three intervening residues, conform to consensus motifs for GSK3, and it was recently shown that Wnt signaling indeed modulates their phosphorylation [14]. Lurking in the shadows, however, was the complication that many GSK3 substrates require priming by another kinase that phosphorylates a serine/threonine four residues downstream of the GSK3 site. It was therefore proposed that the simultaneous binding of GSK3 and β-catenin to Axin might obviate the requirement for such priming [15]. After all, addition of Axin to test tubes containing β-catenin and GSK3 accelerated the rate of β-catenin phosphorylation [16].

The three new papers [8–10] on CKI illuminate the once shadowy area and make it clear that GSK3 does not act alone in setting up β-catenin for the kill. Although some differences exist between the results reported in these papers, all concur that serine 45 in β-catenin is phosphorylated by a CKI, and that this likely serves as a priming site for subsequent phos-phorylations by GSK3. Two reagents have made these discoveries possible — antibodies specific to sites of β-catenin phosphorylation, and RNA-mediated interference (RNAi).

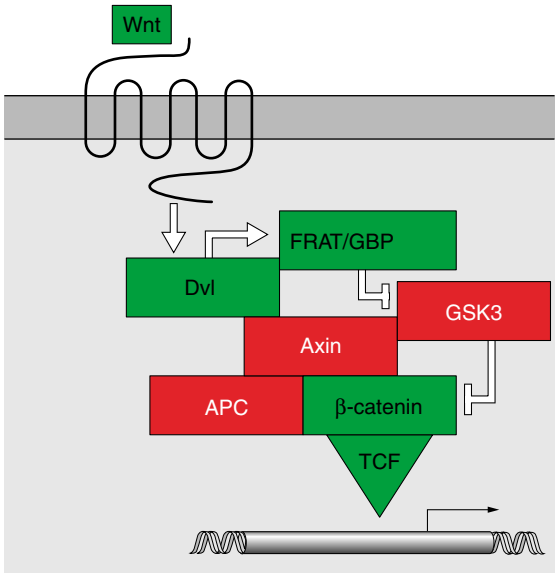

Figure 1. The Wnt signaling pathway.

Signaling is initiated by binding of Wnt to the cell surface receptor Frizzled. Arrowheads and bars indicate activating and inhibitory signals, respectively. Green and red rectangles signify positive and negative acting components in the pathway that ultimately leads to gene activation.

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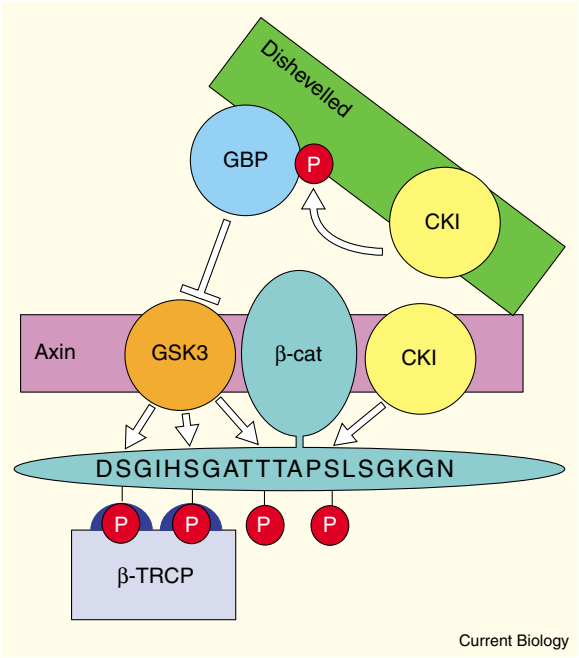

Figure 2. A model with positive and negative roles for CKI in Wnt signaling.

Phosphorylation of β-catenin at serine 45 by CK1 primes it for subsequent phosphorylation by GSK3 at threonine 41, serine 37 and serine 33. The latter two phosphorylations are required for β-TRCP binding that leads to the destruction of β-catenin. CKI also phosphorylates Dishevelled, which is important for GBP inhibition of GSK3 upon receipt of the Wnt signal.

Lui *et al.* [9] generated antiserum specific to β-catenin serine 45 and showed that phosphorylation at this site was stimulated by the expression of an Axin fragment lacking a GSK3 binding site. They then purified the serine 45 kinase, and identified the 37 kDa protein as CKIα. The CK1α binding site on Axin was consistent with previous studies that had mapped the binding of CK1ε to this same region [4,6,7]. Lui *et al*. [9] went on to demonstrate that RNAi depletion of CKIα reduced the level of serine 45 phosphorylation and upregulated the level of β-catenin in 293T cells. Importantly, RNAi depletion of CKIα in *Drosophila* embryos phenocopied the effects of overexpressing the fly Wnt gene *wingless*.

Yanagawa *et al*. [10] used RNAi to explore the function of CKI in *Drosophila* Schneider S2R+ cells. They found that depletion of either CKIε or CKIα led to accumulation of the fly β-catenin Armadillo. Again, RNAi *in vivo* led to a *Drosophila* phenotype consistent with a negative regulatory role for CKI in Wingless signaling. Further investigation, however, demonstrated that stimulation with Wingless did not affect the kinase activity of CKIα recovered from S2R+ cells. The authors mapped the relevant sites of phosphorylation by CKIα using stable cells expressing serine/threonine mutants of Armadillo, as well as using the corresponding peptides *in vitro*. It was apparent that mutation of the Armadillo residue corresponding to serine 45 in β-catenin had a profound effect on its phosphorylation by CKIα. Surprisingly, mutation of acidic residues on the carboxy-terminal side of serine 45 also ablated phosphorylation by CKIα and again stabilized Armadillo. This offers an explanation for some previously inexplicable β-catenin mutations that were identified in a subset of thyroid cancers [17].

Amit *et al.* [8] also used phosphorylation-specific antibodies to identify sites of β-catenin phosphorylation following overexpression of Axin in 293 cells. Expression of Axin alone triggered strong phosphorylation at serine 45, with only minor signals at serine 37 and serine 33, while coexpression of GSK3 greatly enhanced the latter two signals. The precise location of these phosphorylations was determined by mass spectrometry. A point mutant of Axin incapable of binding GSK3 still supported serine 45 phosphorylation, and a mutant GSK3 incapable of recognizing primed substrates was unable to phosphorylate the remaining sites. This agrees with an independent observation [18] that this GSK3 mutant does not induce β-catenin degradation in intact cells. Amit *et al.* [8] also identified GSK3α and GSK3β, as well as CKIα, CKIδ and CKIε, by mass spectrometric analysis of Axin immunoprecipitates. Together, these new papers [8–10] leave little doubt that CKI is instrumental in the phosphorylation and targeted degradation of β-catenin.

These new studies [8–10] turn the role for CKI in Wnt signaling on its head, and have important implications for both basic biology and drug development. Pharmacological inhibitors of CKI have been entertained as anti-cancer prospects, but in the light of the new data one would worry that they might actually induce tumorigenesis. Also, the idea that modulation of insulin signaling, but not of Wnt signaling, might be achieved with GSK3 inhibitors selective for primed substrates appears specious [15].

Finally, how can we reconcile the studies that advance diametrically opposed roles for CKI in the Wnt pathway? Perhaps there are models that accommodate both views. For example, CKI might enforce the assembly of an Axin complex essential for transmitting the Wnt signal (Figure 2). This is consistent with data showing that phosphorylation of Dishevelled by CKI promotes GBP binding [5]. CKI would thus enhance the signal, effectively blocking GSK3, and β-catenin would accumulate, despite its phosphorylation at serine 45. On the other hand, depleting endogenous CKI would preclude phosphorylation at serine 45, and β-catenin would again accumulate, this time in the absence of a Wnt signal. The binding of CKI to both Axin and Dishevelled, as well as its ability to phosphorylate Axin, APC, Dishevelled, TCF3 and β-catenin, have all been reported [1–10,19]. Considering these myriad potential interactions, anything seems possible.

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