# Terminating Wnt signals: a novel nuclear export mechanism targets activated β-catenin

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Nuclear targeting of β-catenin is an obligatory step in Wnt signal transduction, but the factors that control import and export remain to be clarified. In this issue, Hendriksen et al. (p. 785) show that the RanBP3 export factor antagonizes β-catenin/T cell factor (TCF) transcription by targeting the signaling-competent form of β-catenin. We speculate that cells may use multiple export mechanisms to inhibit β-catenin signaling in different ways.

The Wnt/β-catenin signaling cascade is one of the core signal transduction pathways driving tissue morphogenesis during both development and tumor initiation and progression in human cancers. The multifunctional protein β-catenin is a central component in this pathway, transducing extracellular Wnt signals by entering the nucleus and converting TCF-type DNA-binding factors into activators of specific genes. How the nuclear signaling form of β-catenin is regulated by Wnts has been a major focus in the field.

The most conspicuous consequence of Wnt signaling is the inhibition of a constitutive degradation mechanism that serves to keep the cytosolic pool of β-catenin at low levels. In the absence of Wnt, the $NH_2$ terminus of β-catenin is constitutively phosphorylated by casein kinase 1α at serine 45 and, subsequently, by glycogen synthase kinase 3β (GSK3β) at residues 41, 37, and 33 (Yost et al., 1996; Liu et al., 2002). These phosphorylation events are coordinated by the scaffold protein Axin and by the adenomatous polyposis coli (APC) tumor suppressor gene product (Ikeda et al., 1998). β-catenin that is phosphorylated at residues 37 and 33 is ultimately recognized by the β-TrCP E3 ligase, ubiquitylated, and rapidly degraded (Hart et al., 1999). During Wnt activation, GSK3β activity is inhibited, allowing β-catenin to escape degradation and to accumulate in both cytoplasmic and nuclear compartments. Elevated levels alone, however, fail to explain β-catenin/TCF transcriptional activation (Guger and Gumbiner, 2000), and it is now understood that Wnt signaling is specifically mediated through molecular forms of β-catenin that remain unphosphorylated at residues 37 and 41 (Staal et al., 2002).

How does phosphorylation of these residues modulate β-catenin signaling? Although some evidence suggests that phosphorylation can alter β-catenin signaling activity at the level of DNA binding (Sadot et al., 2002), other evidence suggests that phosphorylation may affect nuclear localization (Staal et al., 2002). By designing an antibody that specifically recognizes β-catenin dephosphorylated at residues 37 and 41, Staal et al. (2002) discovered that this unphosphorylated form specifically accumulated within the nuclei of cells activated by Wnt. This important finding demonstrated that β-catenin's nuclear localization is clearly regulated by an active, signal-dependent process. Whether this nuclear accumulation is caused by enhanced import, reduced export, or selective retention, and which factors target this unphosphorylated form of β-catenin, have remained unknown.

In this issue, Hendriksen et al. (2005) set out to identify these elusive regulators of β-catenin import and export. Affinity chromatography was used to uncover proteins from *Xenopus laevis* extracts that interact with β-catenin. Two phenylalanine-glycine repeat–containing proteins were identified as the RanBP3 isoforms a and b. The Ran binding protein 3 (RanBP3) was originally identified as an exportin chromosome region maintenance 1 (CRM1)–dependent export factor, where it binds directly to the nuclear exporting receptor CRM1 and stimulates the export of CRM1 substrates that contain a leucine-rich nuclear export signal (Englmeier et al., 2001). Distinct from this mechanism, however, RanBP3's effects on β-catenin appear to be independent of CRM1. In this regard, RanBP3 b, and to a lesser extent RanBP3 a, was shown to bind directly to its β-catenin substrate (rather than indirectly through CRM1). Moreover, this interaction was enhanced by Ran-GTP, whereas a mutant form of RanBP3 unable to bind RanGTP exhibited reduced affinity for β-catenin, consistent with RanBP3 export function being coupled to a cycle of RanGTP binding and hydrolysis (Fig. 1). Overexpression and loss-of-function (small interfering RNA) approaches demonstrate that RanBP3 is a negative regulator of β-catenin signaling in human cell lines and in *X. laevis* and *Drosophila melanogaster* embryos. Thus, the mechanism by which RanBP3 inhibits β-catenin signaling has remained highly conserved throughout evolution.

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Abbreviations used in this paper: APC, adenomatous polyposis coli; CRM1, exportin chromosome region maintenance 1; GSK3β, glycogen synthase kinase 3β; RanBP3, Ran binding protein 3; TCF, T cell factor.


The Journal of Cell Biology, Vol. 171, No. 5, December 5, 2005 761–763
http://www.jcb.org/cgi/doi/10.1083/jcb.200510127

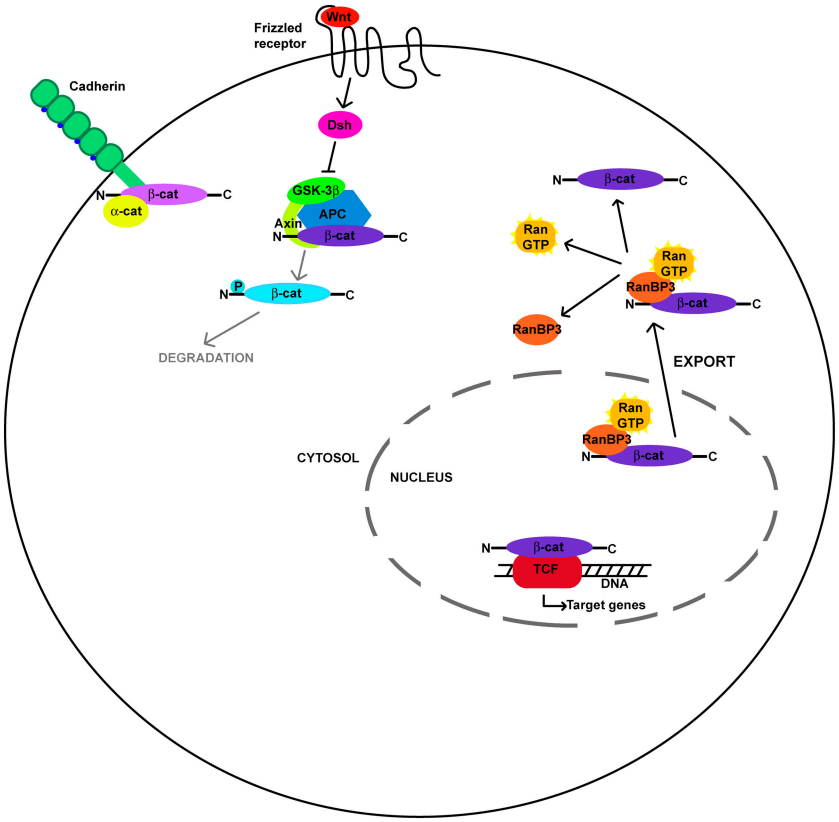


Figure 1. **RanBP3 promotes export of activated β-catenin.** During Wnt signaling, the Axin–GSK3β–APC phosphorylation/predestruction complex is inactivated and leads to cytosolic accumulation of a form of β-catenin that is unphosphorylated at GSK3β-dependent serines 37 and 41. This "activated" form is then available to enter the nucleus and participate as a coactivator for the transcription of TCF target genes. Data presented by Hendriksen et al. (2005) supports a model in which this activated form of β-catenin is exported from the nucleus by RanBP3 in a RanGTP-dependent manner. Cytosolic Ran GTPase-activating proteins promote GTP hydrolysis and presumably release the active form of β-catenin in the cytosol.

Distinct from negative regulators of Wnt signaling, like APC and Axin, which control the posttranslational stability of β-catenin, RanBP3 antagonizes the nuclear accumulation of β-catenin. Importantly, the inhibitory function of RanBP3 does not target all forms of β-catenin, but instead targets the nuclear-signaling form. Specifically, depletion of RanBP3 in Wnt-expressing cells leads to an increase of the $NH_2$-terminally unphosphorylated form of β-catenin detected in a nuclear fraction (using the antibody of Staal et al. [2002]). Conversely, an antibody that is considered to recognize all forms of β-catenin shows no enhanced nuclear detection upon RanBP3 depletion, indicating that there are forms of β-catenin that are not regulated by RanBP3. Although it was challenging to confirm this mechanism using immunofluorescence methods in Wnt-expressing cell lines because of low amounts of the signaling-active β-catenin, SW480 cells (which are mutant for APC and have very high levels of cytoplasmic and nuclear β-catenin) revealed specific nuclear accumulation of the $NH_2$-terminally unphosphorylated β-catenin. Remarkably, this nuclear signal was specifically diminished by RanBP3 overexpression, suggesting that RanBP3 targets the transcriptionally active form of β-catenin for export into the cytoplasmic compartment.

How RanBP3 distinguishes between phosphorylated and unphosphorylated forms of β-catenin remains to be clarified. Although Hendriksen et al. (2005) show that RanBP3 exhibits better binding to full-length β-catenin than the central armadillo repeat region and other studies have shown that both $NH_2$- and COOH-terminal regions of β-catenin can promote nuclear export from *X. laevis* nuclei (Wiechens and Fagotto, 2001), further studies are required to determine whether RanBP3 directly recognizes the unphosphorylated $NH_2$ terminus of β-catenin. Moreover, although this mechanism does not explain how the unphosphorylated forms of β-catenin become nuclear localized in the first place, evidence that the nuclear export machinery can recognize distinct molecular forms of β-catenin opens up the possibility that nuclear import factors may similarly distinguish between these forms. In this regard, a genome-wide RNA interference screen in flies for activators and inhibitors of Wnt signaling has uncovered factors that may collaborate with RanBP3 to regulate the import and export of Wnt pathway components (DasGupta et al., 2005).

Currently there are two models for nuclear export of β-catenin, one in which β-catenin directly engages the nuclear pore complex for export, and the other a CRM1-dependent pathway that relies on nuclear export sequences provided by another carrier, such as APC (for review see Henderson and Fagotto, 2002). Because Hendriksen et al. (2005) demonstrate that RanBP3 exports β-catenin independent of APC and CRM1, this suggests a third mechanism for nuclear export. Why might the cell use so many different modes of β-catenin export? Perhaps different modes of export could allow for different degrees of signaling inhibition (Fig. 2). For example, APC-mediated export may couple nuclear exit with degradation, irreversibly inhibiting the pathway. This export mechanism may predominate in the absence of Wnts or under conditions where a pulse of Wnt signaling must be rapidly diminished. On the other hand, RanBP3-dependent export may be less coordinated with cytoplasmic degradation, and thus this mechanism may predominate during times of more sustained Wnt activation. How these different export mechanisms are calibrated to generate

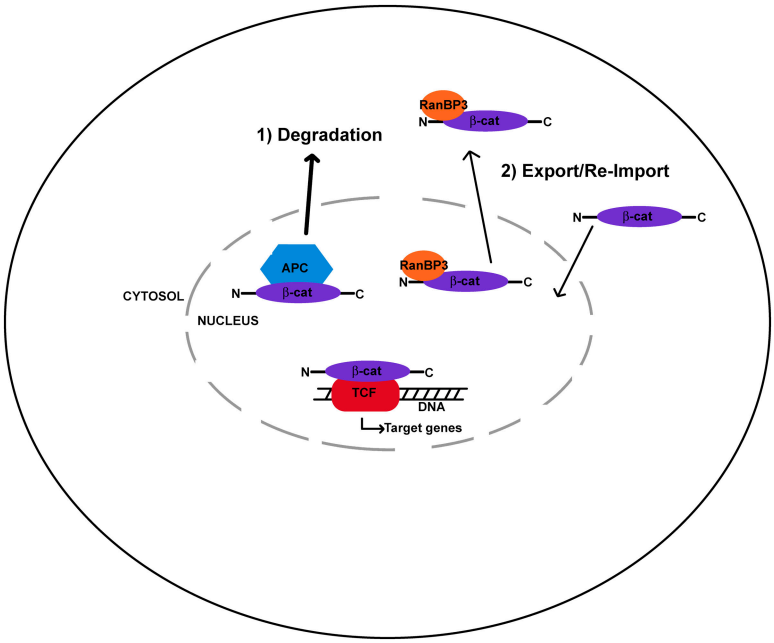


Figure 2. **Distinct modes of β-catenin nuclear export.** APC-directed export of β-catenin may be coupled to degradation, whereas RanBP3-directed export may allow for cycles of export and reimport. See text for details.

different levels and durations of Wnt signals is currently unclear, but Hendriksen et al. (2005) have provided us with new insights into the regulation of β-catenin nuclear export.

Submitted: 24 October 2005
Accepted: 1 November 2005

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