PERSPECTIVE

Wnt signaling: the β -cat(enin)'s meow

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In a study in the December 15, 2011, issue of *Genes & Development*, Valenta and colleagues (pp. 2631–2643) constructed a series of β -catenin mutants that allowed them to separate β -catenin's activity as a mediator of Wnt signaling from its activity as cell adhesion component. In doing so, they uncovered some surprising properties of Wnt signaling.

Wnt proteins and their downstream signaling cascades have long been at center stage of countless developmental studies in organisms ranging from hydra to humans. In adult organisms, Wnt signaling is essential for tissue homeostasis, as highlighted, for example, by its role in maintaining intestinal integrity. With Wnt's indispensable roles in embryogenesis and adulthood, it is not surprising that deregulation and inappropriate activation are frequently found to be associated with disease, including cancer initiation and progression.

While many extremely exciting findings have been made on Wnt in recent years, it is also this diversity of biological processes that has greatly complicated the analysis of the mechanism of Wnt action. With the mammalian genome harboring as many as 19 *Wnt* genes, some of which act redundantly, individual *Wnt* knockout phenotypes have not uncovered the effect of ablating Wnt signaling completely. The downstream signaling components, such as receptors, Dishevelled, and TCFs, are similarly redundant, making it extremely difficult to discern the role of Wnt signaling in development and disease.

A recent study from the Basler laboratory (Valenta et al. 2011) tackles precisely this problem using an elegant genetic approach in mice and flies and complementing it with biochemical studies. For this study, they examined the function of β -catenin, a nonredundant downstream mediator of the Wnt signaling pathway. However, to perform this analysis properly, Valenta et al. (2011) had to contend with a separate issue: β -catenin is a bifunctional molecule, acting both as a key mediator of Wnt signaling and as an integral component of adherens junction. Therefore,

characterization of β -catenin function in Wnt signaling required that these two functions be separated, which was achieved by generating specific mutations that affect only Wnt signaling but not cell adhesion.

β -Catenin was first isolated as a protein tightly associated with the intracellular domain of E-cadherin, along with two other catenins: α -catenin and γ -catenin (aka plakoglobin). This complex of catenins couples the intracellular domain of E-cadherin to the actin cytoskeleton, thereby forming adherens junctions that lend structural integrity to an epithelium. Molecular cloning of the gene encoding β -catenin revealed that it was a homolog of the *Drosophila armadillo* (*arm*) gene. As a member of the so-called segment polarity genes, *arm*, along with *wingless* (*wg*, a fly *Wnt* gene), *porcupine*, *dishevelled*, and others, acts to define the anterior–posterior polarities of each parasegment within the developing fly embryo. Genetic epistasis experiments of these segment polarity genes established the framework of what we today refer to as Wnt/Wg signaling.

With Armadillo acting in both Wg signaling and cell–cell adhesion, several studies have attempted to integrate these processes. However, Armadillo's functions as a component of adherens junctions are clearly separable from those in Wg signaling; for example, Orsulic and Peifer (1996) showed that two mutant *arm* alleles—one defective in adherens junctions, and one defective in Wg signaling—complemented each other and rescued embryonic lethality.

A wealth of data shifted the focus away from β -catenin's role as a component of adherens junctions to its role in Wnt signaling. For example, *Xenopus* embryos injected with purified Fab fragments to β -catenin produced a secondary axis (McCrea et al. 1993), an activity residing in the Spemann organizer and mediated by the canonical Wnt signaling pathway. With the discovery that β -catenin binds to the Lef/TCF class of DNA-binding proteins (Molenaar et al. 1996), β -catenin became appreciated as a transcription factor acting downstream from Wnt signaling and controlling the expression of a large number of *Wnt* target genes.

Structure–function analysis identified two regions with transcriptional activities within β -catenin: one near the N terminus, where BCL9/Legless binds and recruits the nuclear PHD finger protein Pygopus (Kramps et al. 2002; Parker et al. 2002; Thompson et al. 2002), and another near the C terminus, where several known cofactors bind

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(e.g., TATA-binding protein [TBP], Brahma/Brahma-related gene-1 [BRG1], CREB-binding protein [CBP/p300], etc.) (Hecht et al. 1999; Vleminckx et al. 1999; Takemaru and Moon 2000; Barker et al. 2001). The central domain of β -catenin consists of 12 copies of a 42-amino-acid sequence motif known as an armadillo repeat, which collectively form a long positively charged groove (Huber et al. 1997) where several proteins associate, including E-cadherin and Lef/TCF (see Fig. 1).

Despite β -catenin's critical function as mediator of Wnt signaling, its importance in regulating cell adhesion cannot be understated. Mouse embryos carrying a complete loss-of-function allele of β -catenin fail to develop beyond the implanted egg cylinder stage, with cells of the ectodermal cell layer dispersed into the proamniotic cavity and no detectable signs of mesoderm formation, phenotypes that could be as much a consequence of defective cell adhesion as disrupted Wnt signaling (Haegel et al. 1995; Huelsken et al. 2000). To more specifically examine β -catenin's roles in development and adult tissue homeostasis, many laboratories have used a "floxed" allele for β -catenin (β -catenin^{fllox}), originally developed in the Birchmeier laboratory (Huelsen et al. 2001), to inactivate β -catenin conditionally in specific cell types or tissues. However, because the "floxed" allele could not produce a functional protein, these experiments failed to distinguish β -catenin's function in Wnt signaling from that in cell adhesion.

Building on our understanding of the molecular structure and interactions of β -catenin and Armadillo, Valenta et al. (2011) designed β -catenin alleles that were specifically disrupted in their transcriptional functions without affecting their activities as a component of the adherens junctions. In total, three alleles of β -catenin were evaluated: one lacking the C terminus (Δ C), where several known cofactors bind; one with a mutation that disrupts the interaction with its N-terminal coactivator, BCL9/Legless

(D164A in mouse and D172A in fly); and one that combines both of these mutations (DM, for double mutant) (Fig. 1).

Importantly, the investigators analyzed the phenotypes of these genes in two model organisms, flies and mice, observing largely similar effects in both organisms. Furthermore, to avoid differences in phenotypes produced by overexpression, the investigators ensured that the mutant alleles were expressed at levels equivalent to that of endogenous β -catenin or Armadillo and that cells were devoid of the endogenous β -catenin and *arm* gene products.

The three mutant β -catenin alleles were shown to produce no defects in cell–cell adhesion. In rescue experiments in the fly wing discs, localization of E-cadherin, the binding partner of β -catenin in adherens junctions, was similar for both wild-type and DM Armadillo, indicating that the mutant proteins were able to fulfill their adhesive functions. Likewise, in mouse neural tubes, the mutant β -catenin forms rescued epithelial integrity observed for β -catenin-null mutations.

Having established that the mutant β -catenin proteins acted normally as components of adhesion complexes, the investigators turned their attention to β -catenin's function in Wnt signaling. As brief background, Wnt signaling has been divided into two general categories: canonical and noncanonical. β -Catenin is the key mediator of canonical Wnt signaling (Fig. 2). For the lack of a singular theme, noncanonical Wnt signaling can be generalized as β -catenin-independent. For the purposes of these studies, the investigators addressed only the canonical or Wnt/ β -catenin pathway.

To explore the Wnt/ β -catenin signaling activity of the mutant alleles, Valenta et al. (2011) employed well-established cell culture-based Wnt reporter assays in which a luciferase reporter gene is linked to a Wnt-responsive promoter. Consistently, the DM version of β -catenin produced no transcriptional output in either fly or mouse cell

Figure 1. Diagram of wild-type and mutant versions of β -catenin. Wild-type β -catenin (WT) can be divided into three distinct domains: an N-terminal region, which binds BCL-9/Legless; a central Armadillo (Arm) repeat region (colored ovals), which binds Lef/TCF and E-cadherin; and a C-terminal region that binds several transcriptional coactivators, including TBP, CBP/p300, BRG1, and Mediator subunit 12 (MED12). In addition, a host of other binding proteins have been identified (see http://www.stanford.edu/group/nusselab/cgi-bin/wnt/protein_interactions for a current tally). A mutation in the first Arm repeat (D164A in mouse β -catenin, and D172A in fly Armadillo, designated D>A) disrupts interaction with BCL9/Legless, which recruits Pygopus to β -catenin. Deletion of the C terminus (Δ C) abrogates binding of multiple transcription cofactors. The double mutant (DM) combines both mutations and yields a protein completely devoid of all Wnt-mediated transcriptional activity. Importantly, all three mutant alleles do not affect β -catenin's interactions with adherens junctions, which require the binding of E-cadherin and α -catenin.

Figure 2. Wnt/ β -catenin signaling. (*Left panel*) In the absence of Wnt signaling input, β -catenin is targeted for proteasomal degradation by a complex of proteins, including Axin, APC, and GSK3 β . Ubiquitination (Ub) and subsequent proteasomal degradation requires initial phosphorylation of N-terminal residues by GSK3 β and binding of the F-box/WD40 repeat protein β -TrCP. In the nucleus, transcriptional repressors such as Groucho/TLE (Gro/TLE) are bound to TCF, thereby blocking expression of target genes. (*Right panel*) Upon Wnt binding to its receptor complex, comprised of Frizzled (FZD) and the coreceptor low-density lipoprotein receptor-related protein 5/6 (LRP 5/6), a signal is transduced through Dishevelled (DVL), leading to the disassembly of the complex that mediates β -catenin degradation, with some components, such as Axin and GSK3 β , relocating to the membrane. Consequently, β -catenin is no longer targeted for degradation, accumulates in the cytoplasm, and translocates to the nucleus, where it displaces transcriptional repressors from TCF and recruits transcriptional activators BCL-9/Legless (BCL9/Lgs) and Pygopus (Pyg) and

C-terminal coactivators (CTA), resulting in expression of Wnt target genes. In a separate function, β -catenin associates with the intracellular domain of E-cadherin and α -catenin to form adherens junctions. Coupling of E-cadherin to the actin cytoskeleton via the α -catenin and β -catenin complex lends structural integrity necessary to form an epithelium.

culture systems. Interestingly, in the mouse system, the signaling activity of ΔC was nearly as diminished as that of DM, while D164A (the version that fails to interact with BCL9) had intermediate signaling activity, suggesting that these two domains of β -catenin independently affect the transcriptional potency of β -catenin. Likewise, in the fly system, the single mutants also exhibited diminished signaling activity. However, in contrast to mouse cell culture, the version lacking the interaction site for BCL9/Legless (D172A) resembled the DM version of Armadillo, with ΔC retaining some, albeit reduced, signaling activity. The reason for the difference in signaling potencies of the β -catenin alleles between the two model organisms is unclear. Irrespective, in both systems, the DM proteins were completely transcriptionally inert while retaining their adhesive functions.

Subsequently, the investigators examined the activity of the β -catenin alleles *in vivo*. As expected, the phenotypes observed for the DM proteins were consistent with a complete defect in canonical Wnt signaling. While wild-type armadillo was able to rescue expression of the Wg target gene *senseless* in *arm* mutant clones, the DM version failed to do so, clearly demonstrating that the signaling and adhesive functions of Armadillo can be separated from each other *in vivo*.

In an impressive set of experiments, Valenta et al. (2011) replaced the wild-type β -catenin allele with one of the three mutant alleles by homologous recombination in mouse embryonic stem cells and generated three heterozygous strains carrying the mutant β -catenin alleles: β -catenin^{D164A}/ β -catenin^{wt}, β -catenin ^{ΔC} / β -catenin^{wt}, and β -catenin^{dm}/ β -catenin^{wt}. In a homozygous setting, none of these β -catenin mutants yielded live births. The ΔC and

DM produced similar effects, consistent with the cell culture experiments, and were comparable with those observed for a complete loss of β -catenin: The embryos failed to undergo gastrulation and, consequently, formed no mesoderm. The decreased Wnt signaling activity in these mutants was clearly revealed by using a Wnt-responsive reporter transgene called BAT-gal (Maretto et al. 2003) and monitoring expression of the Wnt target gene *Axin-2* (Lustig et al. 2002).

In contrast to ΔC and DM, the D146A mutant developed normally until embryonic day 10 (E10), at which point developmental defects associated with reduced Wnt signaling were uncovered. Therefore, coactivators associated with the N-terminal portion of β -catenin via BCL9/Legless act independently of the C-terminal coactivators and are important later in development.

By combining these knock-in alleles of β -catenin with a conditional knockout allele (β -catenin^{flox}) (Huelsen et al. 2001) and a tissue-specific Cre driver, Valenta et al. (2011) were able to move past the early embryonic lethality and explore β -catenin's function in subpopulations of cells and tissues during development. Previous experiments in which β -catenin was conditionally deleted in specific tissues, such as the skin, lung, pancreas, blood, and limbs, suffered from the problem that the resulting phenotype could be due to β -catenin's defective function in either Wnt signaling or cell adhesion. The genetic strategy described in this study yields cells and tissues where the only source of β -catenin is from one of the three mutant alleles.

For these experiments, the *Wnt1*-Cre driver (Danielian et al. 1998) was used so that the developing neural tube was devoid of all canonical Wnt signaling activity asso-

ciated with β -catenin. Interestingly, in this setting, the phenotypes of *Wnt1*-Cre; β -catenin^{dm/flox} embryos were milder than those observed for *Wnt1*-Cre; β -catenin^{flox/flox}, presumably because the DM version was able to rescue any defects resulting from compromised epithelial integrity. Furthermore, the single-mutant versions of β -catenin produced less severe phenotypes than that observed for DM, suggesting that the N-terminal- and C-terminal-associating coactivators both contribute in critical ways to the transcriptional output of β -catenin downstream from Wnt signaling.

Finally, by depriving the dorsal neural tube of all Wnt signaling-competent β -catenin, the investigators were able to demonstrate that Wnt plays an essential role in the maintenance of neural tube precursors, a finding consistent with Wnt's critical role in stem cell maintenance and self-renewal. Interestingly, no effects on cell proliferation were observed when Wnt/ β -catenin signaling was blocked with expression of the DM β -catenin version in the CNS. This finding is particularly surprising given the many connections between Wnt/ β -catenin signaling, proliferation (e.g., two prominent Wnt target genes are *c-Myc* and *CyclinD1*), and cancer.

This work by Valenta et al. (2011) provides the community with a new set of tools to dissect and interrogate Wnt signaling in vivo. It will be interesting to explore the function of each of these β -catenin mutant alleles in specific tissues by combining them with one of the many Cre drivers. Such studies will indicate to what extent either the N-terminal or C-terminal coactivators regulate tissue homeostasis and stem cell self-renewal.

The reason why β -catenin evolved to incorporate two distinct functions—Wnt-regulated transcription and cell–cell adhesion—has long been debated. Interestingly, while Wnt signaling is only found among metazoans, a polarized epithelium organized by α -catenin and β -catenin (but lacking cadherin) exists in the nonmetazoan *Dictyostelium discoideum* (Dickinson et al. 2011). Wnt signaling and cadherins subsequently evolved to separately regulate the catenin complex in both transcription and cell adhesion. By being at the nexus of gene regulation and cell adhesion, β -catenin can coordinate these processes. Such coordination is important for processes like gastrulation, neural tube closure, and neural crest cell migration, where cells activate specific gene expression programs and enter a migratory phase by delaminating from the epithelial cell layer through an epithelial-to-mesenchymal transition.

The cross-talk between Wnt signaling and cell–cell adhesion has been examined in cell culture systems where Wnt signaling leads to redistribution of β -catenin from cell adherens junctions to the cytoplasm, a loss in E-cadherin at the cell surface, and a consequent reduction in cell–cell adhesion (Wodarz et al. 2006)—processes that cumulatively will permit cells to dissociate from their neighbors and enter a migratory phase. In addition, in the fly wing disc pouch, a single-layered epithelium, Wg signaling, and E-cadherin expression are critical in regulating cell shape, with reduction in either component leading to extrusion of the mutant cells from the epithelium. In contrast, ectopic activation of Wg signaling leads to elongation of

the cells within the epithelium (Widmann and Dahmann 2009), again underscoring the close association between Wnt signaling and cell–cell adhesion.

Taken together, such experiments point to the importance of coordinating cell signaling processes with cell adhesion to achieve the complex morphogenetic processes associated with development. With these new tools generated by Valenta et al. (2011), it will be exciting to dissect how the distinct Wnt signaling functions of β -catenin affect embryonic development.

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