REVIEW

Wnt signaling: is the party in the nucleus?

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The Wnt signaling pathway controls cell proliferation and body patterning throughout development. A surprising number of cytoplasmic Wnt regulators (e.g., β-catenin, Bcl-9/Lgs, APC, Axin) also appear, often transiently, in the nucleus. β-Catenin is an integral component of E-cadherin complexes at intercellular adherens junctions, but also recruits chromatin remodeling complexes to activate transcription in the nucleus. The APC tumor suppressor is a part of the cytoplasmic β-catenin destruction complex, yet also counteracts β-catenin transactivation and histone H3K4 methylation at Wnt target genes. Furthermore, APC coordinates the cyclic exchange of Wnt coregulator complexes at the DNA. These opposing roles of APC and β-catenin enable a rapid coordination of gene expression and cytoskeletal organization throughout the cell in response to signaling.

The canonical Wnt signaling pathway affects cell fate determination (e.g., the decision to proliferate or differentiate) and axis specification in all metazoan organisms. The interplay of these two processes allows a single cell with little discernible polarity to develop the complex structures and morphologies that are found throughout the animal kingdom. Wnt signaling is implicated in a variety of cellular processes, including proliferation, differentiation, survival, apoptosis, and cell motility. The signaling cascades initiating these processes include the widely studied canonical Wnt/β-catenin pathway, as well as a variety of noncanonical pathways. To better understand the complex roles of Wnt signaling in development, organogenesis, and cancer, we refer the reader to several excellent recent reviews (Bienz and Hamada 2004; Moon et al. 2004; Nelson and Nusse 2004; Bienz 2005; Gregorieff and Clevers 2005; Kohn and Moon 2005; Radtke and Clevers 2005; Reya and Clevers 2005). In this review, we address the mechanism of β-catenin-regulated transcription in light of new data supporting a central role for polymerase-associated factor 1 (Paf1) and SET1 (trithorax) complexes that mediate H3K4 methylation at Wnt-responsive genes. New findings also implicate a role for APC, potentially together with other subunits of the cytoplasmic Wnt destruction complex, in the turnover and cycling of Wnt coregulator complexes at target genes. Conversely, we discuss recent evidence indicating that the Polycomb group repressor Ezh2 histone methyltransferase may play a role in the cytoplasm to alter cytoskeletal organization or cell adhesion in response to various signaling pathways. To provide a broader biological perspective, we start by examining the role of β-catenin in the transient activation of adult stem cells as well as during the process of somitogenesis, where Wnt target gene transcripts oscillate in a precise temporal and spatial manner under the control of the vertebrate segmentation clock.

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Wnt signaling in stem cells: an ancient system at the cutting edge

A primitive Wnt signaling pathway regulates axis specification as well as stem cell proliferation in Cnidarians, thus dating these conserved functions of Wnt signaling to a common ancestor that lived 650 million years ago (Teo et al. 2006). For example, ectopic activation of Wnt signaling in *Hydractinia* induces a transient expansion of interstitial stem cells, which then undergo terminal differentiation to nerve cells and nematocytes. This transient expansion of interstitial cells correlates with an increase in the levels of cytoplasmic and nuclear β-catenin, which subsequently decline prior to terminal differentiation, in a manner strikingly reminiscent of the transient stem cell activation induced by Wnt ligands in mammalian stem cells.

Recent studies have shown that canonical Wnt signaling regulates virtually all of the defined human adult stem cell systems, including skin, blood, intestine, and brain (Radtke and Clevers 2005; Reya and Clevers 2005). Wnt signaling in the mammalian epidermis induces proliferation of resting stem cells in the niche, which form a transient amplifying cell population that form new hair follicles (Lowry et al. 2005). Likewise, Wnt signaling induces the proliferation of transient amplifying cells in the intestine and maintenance of the intestinal stem cells residing in the crypt. Inactivation of Wnt signaling

components results in depletion of intestinal crypt stem cells and loss of proliferative epithelial cells (Pinto et al. 2003; Kuhnert et al. 2004). Conversely, constitutive activation of the Wnt pathway causes a hyperproliferation of intestinal epithelial cells and is closely linked with the development and progression of colorectal cancer (Gregorieff and Clevers 2005). As in *Hydractinia*, where high β-catenin levels directly correlate with interstitial-cell activation, the highest levels of β-catenin are observed in the nuclei of intestinal crypt cells, where intestinal stem cells reside (van de Wetering et al. 2002).

The hematopoietic stem cell (HSC) is arguably the best-characterized adult stem cell system in mammals (Reya et al. 2003; Reya and Clevers 2005). Introduction of an activated β-catenin protein (Reya et al. 2003) or stimulation with *Wnt3a* (Willert et al. 2003) leads to ex vivo proliferation and expansion of HSCs. *Wnt-1*, which was first identified as an oncogene in mouse mammary tumorigenesis (Nusse et al. 1984), also regulates the amplification of mammary progenitor cells (Liu et al. 2004). Recently, a discrete population of mammary stem cells was identified and shown to be able to reconstitute a complete mammary gland in vivo (Shackleton et al. 2006; Stingl et al. 2006). Interestingly, this cell population was markedly expanded in premalignant mammary tissue from MMTV-Wnt-1 mice, indicating that Wnt-1 exerts its proliferative effect in the mammary gland by directly influencing the mammary stem cell population.

Unlike adult stem cells, which are resting until they become activated, embryonic stem cells (ESCs) are continuously proliferative. Given the observed function of Wnt in stem cell activation as discussed above, one might expect that Wnt would not be required for ESC proliferation. Unfortunately, recent studies that address the role of Wnt signaling in ESC maintenance and proliferation have arrived at opposite conclusions (Sato et al. 2004; Dravid et al. 2005). Consequently, further approaches will be needed to resolve this question, and in particular it will be important to examine the role of specific Wnt ligands, other than Wnt-3a, that are expressed in early development and might contribute to ESC proliferation and differentiation.

Wnt signaling and the vertebrate segmentation clock

Wnt signaling plays a specialized role in the orderly segmental patterning observed during somite formation, which gives rise to the vertebrae of the spinal column (Aulehla and Herrmann 2004; Dubrulle and Pourquie 2004). This complex process depends upon a molecular oscillator, known as the vertebrate segmentation clock, which induces Notch gene transcripts to cycle with a periodicity of 90–120 min within the presomitic mesoderm (PSM). The periodic expression of Notch target genes depends not only upon Notch signaling but also on canonical Wnt/β-catenin signaling, which induces periodic transcription of many Wnt target genes, including *Axin2* (Aulehla et al. 2003) and *Snail* (Dale et al. 2006). Whereas oscillation of Notch target genes requires ongoing Wnt signaling, the converse is not true, suggesting that Wnt lies upstream of Notch signaling in the segmentation clock (Aulehla et al. 2003). Most remarkably, the cycling expression patterns of the *Axin2* (Wnt-dependent) and *lunatic fringe* (Notch-dependent) genes are out of phase and alternate in the PSM (Aulehla et al. 2003). The periodic expression of *lunatic fringe* mRNA is established by the Notch enhancer (Morales et al. 2002), indicating that segmentation arises from the periodic transcription of genes that encode short-lived mRNAs and proteins. At present, little is known of how the cyclic Wnt and Notch transcription patterns are initiated and integrated to maintain this alternating pattern of transcription. In part, the mechanism may depend upon indirect effects, such as the induction by Wnt of the Notch ligand, Delta-like 1 (Galceran et al. 2004; Hofmann et al. 2004); however, direct cross-talk between the two pathways is also likely (Aulehla et al. 2003; Kawamura et al. 2005; Dale et al. 2006). A broader understanding of this process awaits a more detailed analysis of the mechanisms that control Wnt and Notch transcription.

Wnt signaling in cancer

Misregulation of the Wnt signaling pathway has been linked to various human cancers, including colon and hepatocellular carcinomas, leukemia, and melanoma (Moon et al. 2004; Gregorieff and Clevers 2005; Radtke and Clevers 2005; Reya and Clevers 2005). Most colorectal cancers contain sporadic or inherited truncations of the APC tumor suppressor. The most frequent of these cancer-causing mutations yield truncated APC proteins that are unable to bind Axin or degrade β-catenin (Kinzler and Vogelstein 1996), resulting in the sustained expression of c-Myc and other Wnt target genes. Wild-type, but not mutant, APC also interacts with the microtubule cytoskeleton and can help maintain chromosomal stability through association with the kinetochore of metaphase chromosomes (Bienz and Hamada 2004). A fraction of tumors with wild-type APC contain oncogenic stabilizing mutations that prevent phosphorylation of β-catenin, allowing it to accumulate in the nucleus independently of a Wnt signal. Remarkably, some tumors may arise as a consequence of the selective loss of Wnt/β-catenin signaling. Thus, a recent study found that one-third of human sebaceous gland tumors contain a dual-site mutation in LEF-1 that impairs its ability to bind β-catenin, and causes it to function as a dominant-negative inhibitor of Wnt signaling (Takeda et al. 2006). The loss of Wnt signaling correlates with induced expression of sebaceous marker proteins, mirroring the induction of sebaceous tumors in mice that express an N-terminal truncated dominant-negative form of LEF-1 (Merrill et al. 2001).

Wnt signaling mobilizes β-catenin, an unstable coactivator

In an unstimulated cell, most of the endogenous β-catenin is found at epithelial cell adherens junctions,

where it interacts with E-cadherin and α-catenin to help mediate cell adhesion (Fig. 1). To ensure its rapid turnover, the excess newly synthesized cytoplasmic β-catenin is targeted to a multisubunit destruction complex, which includes Axin, the APC tumor suppressor, protein phosphatase 2A (PP2A), and the protein kinases GSK3β and CK1α. Sequential phosphorylation of β-catenin within this complex—first at Ser-45 by Casein Kinase 1α (CK1α), and then at Ser-33, Ser-37, and Thr-41 by GSK3β—targets it for ubiquitination by the β-transducing repeat-containing protein (β-TrCP) E3 ubiquitin ligase and subsequent proteolytic destruction by the proteosome (Price 2006).

Binding of Wnt ligand to its receptor complex, composed of the seven-span transmembrane receptor Frizzled (Fzd) and the single-pass transmembrane protein LRP5/6, triggers a series of events that ultimately disrupts the APC/Axin/GSK3β complex (Fig. 1). Recent publications have shed new light on the mechanism by which the destruction complex is rendered inactive. Binding of Wnt ligands induces phosphorylation of the LRP5/6 intracellular domain through the combined actions of CK1γ, a membrane-tethered kinase, and GSK3β (Davidson et al. 2005; Zeng et al. 2005). The hyperphosphorylated LRP5/6 domain binds Axin with high affinity, sequestering it away from the destruction complex. Because Axin is present at limiting levels in the cell (Lee et al. 2003), its relocation to the membrane should rapidly halt the degradation of β-catenin. The inactive LRP-bound Axin may then be dephosphorylated and subsequently degraded by an unknown mechanism (Willert et al. 1999; Yamamoto et al. 1999). Thus the stabilization of β-catenin by Wnt signaling is accomplished through the sequestration of scaffolding proteins, rather than by direct inhibition of GSK3β activity.

Wnt signaling also requires the cytoplasmic Dishevelled (Dsh) protein, which, like Axin, is rapidly recruited to the receptor upon Wnt signaling (Fig. 1). Activated Dsh binds Axin, CK1, Par-1, and CK2, and may help titrate these components away from the destruction complex (Cadigan 2002; Bienz and Clevers 2003). The process by which the Wnt signal is transduced from the receptor to Dsh is not known, but one intriguing possibility is that it involves G-protein signaling. Several lines of evidence argue that trimeric G proteins are essential in transmitting the Wnt signal (Ahumada et al. 2002; Katanaev et al. 2005), a possibility first indicated by the serpentine topology of Fzd receptors, which is shared with all G-protein-coupled receptors (GPCRs). Genetic epistasis experiments in *Drosophila* place a Gαo downstream of Fzd and upstream of Dsh (Katanaev et al. 2005). Recent cell culture experiments further support the role for G proteins in the transduction of the Wnt signal from Fzd to β-catenin (Liu et al. 2005). However, direct biochemical data demonstrating that Wnt ligand binding leads to activation of a G protein by GDP–GTP exchange, as is the case for other GPCRs, has not been described.

Phosphorylation of the APC tumor suppressor appears to trigger the release of ubiquitinated β-catenin from the Axin/APC/GSK3β complex (Ha et al. 2004; Xing et al. 2003, 2004). Unphosphorylated β-catenin and APC bind to separate sites on Axin, and both proteins are targets for phosphorylation by CK1α and GSK3β. CK1α phosphorylation of the APC 7 × 20-amino-acid repeat region induces it to bind with high-affinity to β-catenin, displacing Axin, which is then free to rebind a new molecule of unphosphorylated β-catenin. Other post-translational protein modifications are likely to be instrumental in sorting β-catenin and APC to various complexes (Harris and Peifer 2005). For example, Gottardi and Gumbiner (2004) showed that Wnt signaling enhances the relative amount of monomeric β-catenin in the cytoplasm that is free to bind LEF-1/TCF protein. By

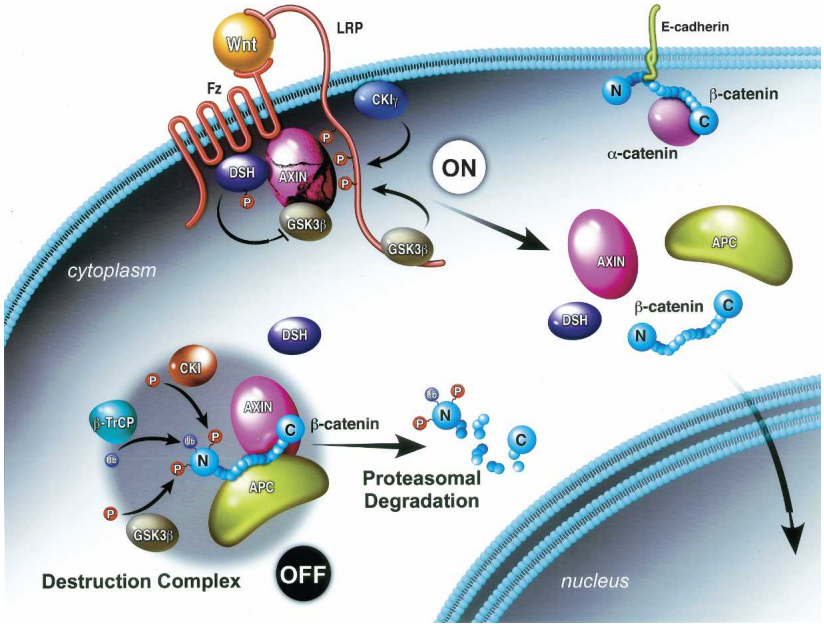

Figure 1. Key Wnt regulators are found at the plasma membrane and in various cytoplasmic complexes. In the absence of Wnt ligands, β-catenin binds to a destruction complex containing APC, Axin, and the CK1 and GSK3 kinases and is marked for proteolytic destruction ("off"). Wnt signaling promotes CK1γ and GSK3β mediated hyperphosphorylation of LRP5/6 and enhances Dsh phosphorylation, which jointly recruit Axin to the receptor complex at the plasma membrane ("on"), where it undergoes proteolytic degradation. Unphosphorylated β-catenin is no longer rapidly degraded and enters the nucleus. Many of the β-catenin destruction complex components are shuttling proteins that distribute both in the cytoplasm and the nucleus, and some of these, such as APC, Axin, and Bcl-9/Lgs, are required for the accumulation or retention of β-catenin in the nucleus.

contrast, α-catenin:β-catenin heterodimers are thought to bind preferentially to E-cadherin. Moreover, tyrosine phosphorylation of β-catenin at Y142 disrupts its binding to α-catenin and may release free β-catenin molecules from E-cadherin complexes at the plasma membrane (Brembeck et al. 2004). Accordingly, activation of cells with the receptor tyrosine kinase c-Met can induce β-catenin to migrate to the nucleus even in the absence of a Wnt signal (Brembeck et al. 2004).

Dynamic nuclear shuttling of Wnt regulators

Several cytoplasmic Wnt regulators, including APC and Axin, have been found to contain nuclear import and export sequences that direct them to shuttle in and out of the nucleus. The nuclear export sequences of APC are important for its function and serve to promote the nuclear export of β-catenin (Bienz 2002). As discussed in greater detail below, nuclear APC also directly counteracts β-catenin activation at Wnt target genes and is necessary for the periodic cycling of coactivator and corepressor complexes at Wnt enhancers (Sierra et al. 2006). Like APC, Axin has also been detected in the nucleus and promotes the accumulation of β-catenin in the cytoplasm (Cong and Varmus 2004; Wiechens et al. 2004). Thus, it is possible that Axin and APC shuttle as part of the same complex and that the observed effects of these proteins on nuclear export of β-catenin may reflect the activity of a larger complex that includes a subset of destruction complex proteins. In principle such a complex could also include the Bcl-9/Lgs adaptor protein, which is cytoplasmic in some cells and can mediate nuclear import of β-catenin by targeting it to the nuclear Pygopus protein (Townsley et al. 2004). An alternative scenario is that some destruction complex proteins enter the nucleus independently but later reassemble at Wnt target genes. Other central Wnt regulators that are known to translocate in and out of the nucleus include Dsh (for review, see Habas and Dawid 2005), α-catenin (Giannini et al. 2000), ICAT (Tago et al. 2000), and the regulatory kinases CK1, CK2, and GSK3β. It will be important to establish how many of these factors are present with β-catenin at the DNA and how they might function in nuclear trafficking or transcription.

The β-catenin activation mechanism

Within the coactivator complex, β-catenin functions as a scaffold to link the LEF-1/TCF proteins to specific chromatin remodeling complexes, as well as to the Wnt coactivators, Bcl-9/Lgs and Pygopus (Fig. 2). The N-terminal armadillo (ARM) repeat of β-catenin interacts directly with Bcl-9/Lgs, which forms part of a "chain of adaptors" (Stadeli and Basler 2005) that connects LEF-1 to the Pygopus (Pygo) PHD finger protein (Belenkaya et al. 2002; Kramps et al. 2002; Parker et al. 2002; Thompson et al. 2002). Bcl-9/Lgs and Pygopus are implicated in nuclear localization of β-catenin (Brembeck et al. 2004; Townsley et al. 2004) as well as transcription (Thompson 2004; Hoffmans et al. 2005). LEF-1/TCF proteins bind to the β-catenin central ARM repeats in a region that largely overlaps the binding sites for APC or E-cadherin (Fig. 2). Other β-catenin-interacting proteins include the DNA ATPase/helicase TIP49a/Pontin52 (Bauer et al. 2000), which is present in mammalian TRRAP/p400/TIP60, INO80, and SWRCAP/SWR1 chromatin remodeling complexes, Brg-1 (Barker et al. 2001), and CBP/p300 (for reviews, see Cadigan 2002; Bienz and Clevers 2003).

The region C-terminal to the β-catenin ARM repeats contains a very strong activation domain, which binds to multiple chromatin remodeling subunits (Fig. 2). A recent biochemical analysis of proteins that associate with this region of β-catenin by MALDI-TOF mass spectrometry identified the *transformation/transactivation domain* TRRAP protein, which is part of TRRAP/p400/TIP60 and TRRAP/GCN5 (SAGA) complexes, an ISWI remodeling complex, and a histone methyltransferase (HMT) complex containing the Trithorax-family *mixed-*

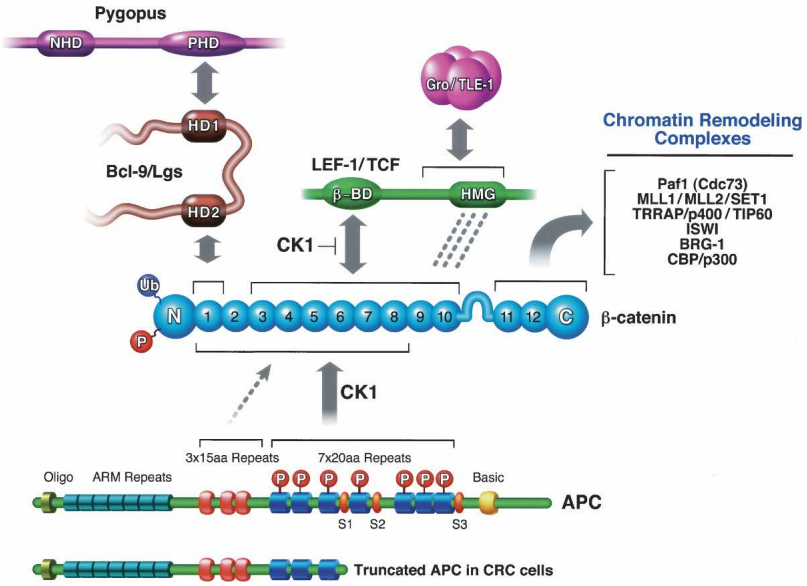

Figure 2. Post-translational modifications govern the interactions between different Wnt transcriptional regulators. The schematic diagrams indicate the overlapping sites within the β-catenin armadillo (ARM) repeats for LEF-1/TCF and APC. Phosphorylation of APC by CK1 dramatically enhances its affinity for β-catenin (Ha et al. 2004; Xing et al. 2004), which would disfavor binding to LEF-1/TCF. Similarly, CK1 phosphorylation of LEF-1 has been reported to disrupt binding to β-catenin (Hammerlein et al. 2005). Other post-translational protein modifications, potentially including β-TrCP-mediated ubiquitination of β-catenin, are likely to regulate the protein interactions within the complex; however, the precise mechanism is unknown. The C terminus of β-catenin interacts, directly or indirectly, with several distinct chromatin remodeling complexes. Sequential interactions with these various complexes is likely to govern transcription initiation and elongation at Wnt target genes.

lineage-leukemia (MLL1/MLL2) SET1-type proteins (Sierra et al. 2006). The mammalian SET1-related HMT proteins form complexes with Ash2, menin, HCF, WRD5, and RbBP5, and are thought to be frequent direct targets of transcriptional regulators (Yokoyama et al. 2004; Dou et al. 2005; Wysocka et al. 2005b). The SET1 proteins mediate trimethylation of histone H3-Lys-4 (H3K4Me3), which is a chromatin modification found in highly active genes and associated with open chromatin structures in vivo (Sims et al. 2004; Tenney and Shilatifard 2005). Chromatin immunoprecipitation (ChIP) data indicate that β-catenin recruits MLL1/2 complex coactivators to the c-Myc gene in vivo and strongly induces H3K4Me3 (Sierra et al. 2006). Another recent study independently identified human and *Drosophila* Parafibromin/Hyrax (Cdc73 in yeast), an integral subunit of the Paf1 complex (Mosimann et al. 2006), as a direct target of the β-catenin C-terminal activation domain. The Paf1 complex (Paf1p, Cdc73, Rtf1, Ctr9p, and Leo-1) associates with RNA polymerase II initiation and elongation complexes to recruit enzymes required for ubiquitination of histone H2B and subsequent trimethylation of H3K4 (Adelman et al. 2006). Thus, these combined studies highlight a key role for H3K4Me3 in Wnt-regulated transcription. Interestingly, genetic studies indicate the Pygopus may also help regulate this step in transcription (Mosimann et al. 2006).

Known transcriptional corepressors for Wnt transactivation include the C-terminal-binding protein (CtBP) corepressor, which influences epigenetic chromatin modifications through interactions with Polycomb corepressors and other factors (Chinnadurai 2002), and Groucho/TLE1, which interacts directly with the LEF-1/TCF DNA-binding proteins (Daniels and Weis 2005). The 9-kDa inhibitor of β-catenin and Tcf-4 (ICAT) protein selectively recognizes the β-catenin ARM repeats and sterically blocks binding to LEF-1/TCF proteins (Tago et al. 2000). Chromatin remodeling complexes that counteract Wnt signaling include a fly Osa-containing Brahma complex (Collins and Treisman 2000). Genetic screens have implicated a role for several other corepressors (Das-Gupta et al. 2005); however, little is known in detail of the repression mechanisms responsible for the low basal activity of Wnt target genes in the absence of signaling.

APC directs the exchange of coactivator and corepressor complexes at Wnt target genes

ChIP studies indicate that LEF-1 binds constitutively to Wnt enhancers, whereas the coactivator and corepressor proteins alternate on and off of the DNA during active transcription (Sierra et al. 2006). The cycling of coregulator complexes has also been observed at other rapidly induced genes, such as those regulated by nuclear receptors and NF-κB (Perissi and Rosenfeld 2005). This cycling of enhancer components appears to be triggered by post-translational modifications, especially ubiquitination, of the enhancer factors during transcription. In contrast, the RNAPII transcription complexes do not cycle, but rather continually accumulate within the transcribed region of the gene. The periodic turnover of coregulator complexes is thought to provide a means for the enhancer complex to assess the environment of the signaling cell and permits active genes to rapidly reset to the repressed state in anticipation of new rounds of signaling (Perrissi and Rosenfeld 2004). Coupled to genes that express short-lived mRNAs and proteins, the intermittent exchange of coactivator and corepressor complexes might contribute to the oscillating gene expression pattern of Wnt target genes observed during somitogenesis. More generally, it is important to understand the sequence of events that governs transcription intiation and elongation during these transcription cycles. Many inducible genes have been shown to recruit the protein chaperone 19S proteosomal subcomplex, including the AAA^+ ATPases Rpt4 and Rpt6, which have been implicated to play a role in the coupling of histone ubiquitination and methylation events and may also facilitate the assembly and disassembly of multisubunit enhancer complexes in vivo (Ezhkova and Tansey 2004).

Remarkably, APC and β-TrCP are recruited to Wnt enhancers together with β-catenin, Bcl-9/Lgs, and Pygopus in C2C12 cells treated with the GSK3β inhibitor, lithium, which leads to the activation of Wnt target genes. These factors displace Gro/TLE1 and GSK3β, which are bound to Wnt enhancers in the repressed state (Sierra et al. 2006). The colocalization of GSK3β with Gro/TLE1 was unexpected because it is not known to regulate any of the Wnt corepressor proteins. Interestingly, GSK3β phosphorylates and stabilizes a circadian gene repressor, RevErb-α (Yin et al. 2006), and consequently it could function similarly to stabilize Wnt gene corepressors. In HT29 CRC cells, which contain a Class II APC mutant protein that is unable to degrade β-catenin, the coactivator complex at the c-Myc gene is stable and does not move on and off of the DNA. Although the mutant APC protein is recruited to the active c-Myc enhancer in these cells, β-TrCP is not recruited to the DNA, which raises the possibility that ubiquitination of β-cat may be required for turnover or cycling of the coactivator proteins. As mentioned above, precedent exists for transcription factor ubiquitination in regulating the cyclic exchange of nuclear receptor complexes at target genes in vivo (Perissi and Rosenfeld 2005). This scenario predicts that Wnt regulatory complexes would also not exchange in HCT116 CRC cells, which express a mutant β-catenin protein that is unable to undergo phosphorylation-dependent ubiquitination. However, if cycling requires ubiquitination of β-catenin, the kinase(s) involved must be distinct from GSK3β because the Wnt coregulator exchange occurs in cells treated with the GSK3β inhibitor lithium and because GSK3β itself cycles with the corepressor proteins, rather than with β-catenin, at the DNA. Additional studies to examine whether Wnt enhancer complexes cycle in cells lacking β-TrCP may help resolve this question. When the full-length APC protein is expressed in HT29 cells, it binds transiently to the enhancer, together with β-TrCP, the CtBP corepressor, and the Polycomb repressor protein, YY1, and is subsequently displaced by the Gro/

TLE1 and HDAC1 corepressors (Fig. 3). These rapid changes at the c-Myc enhancer precede the gradual decline in β-catenin protein levels due to proteolytic degradation, suggesting that APC acts directly in these cells to shut off Wnt-regulated genes.

These findings raise the question of how APC, potentially together with other destruction complex components, including β-TrCP, is recruited to Wnt enhancers. Because APC and LEF-1 compete for overlapping regions of the β-catenin ARM repeats (Fig. 2), β-catenin cannot interact with APC when bound to LEF-1 on DNA. Thus APC must initially interact with other proteins in the enhancer complex, or it may be recruited and function as part of a separate complex. How might APC promote the disassembly of the enhancer complex? One attractive possibility is that the affinity of APC for various proteins in the enhancer complex, as in the destruction complex, is controlled by post-translational protein modifications. Thus, phosphorylation of APC by CK1 would induce it to bind tightly to β-catenin and prevent the latter from binding to LEF-1. Moreover, CK1 may phosphorylate LEF-1 and lower its affinity for β-catenin (Hammerlein et al. 2005). Collectively, these findings indicate that APC recruits β-TrCP and CtBP to mediate the exchange of coactivator and corepressor complexes within transcription cycles at active genes, as well as to shut down transcription as genes reset to await later rounds of signaling.

CtBP: transcriptional corepressor and APC-interacting protein

Hamada and Bienz (2004) originally identified CtBP (Ad-E1A CtBP) as an APC-interacting protein from a two-hybrid screen, and showed that APC binds CtBP in vivo and in vitro and can sequester excess β-catenin in the nucleus. In nuclear extracts, CtBP associates with full-length, but not mutant, APC proteins, which explains the failure of the mutant proteins to recruit CtBP or repress Wnt target gene expression in vivo (Sierra et al. 2006). CtBP repressor is a weak NAD/NADH-regulated dehydrogenase, which frequently binds repressor proteins directly, through a PXDLS motif, in an NADH-dependent manner (Chinnadurai 2002). CtBP complexes also contain CoREST, G9A, EuHMT HDAC1/2, and LSD1 (KIAA0601/NPAO), among other proteins (Shi et al. 2003), and binding of CtBP is correlated with the loss of histone modifications, including H3K4Me3, at the E-cadherin gene in vivo (Shi et al. 2003; Wysocka et al. 2005b). The LSD1 subunit demethylates mono- and dimethylated H3K4 (Wysocka et al. 2005a); however, it is not capable of reversing trimethylation. The ChIP experiments indicate that H3K4Me3 levels do decline upon repression of c-Myc transcription in vivo; however, the enzyme that reverses this modification is currently unknown. It will be important to identify other proteins that function with CtBP in this regulatory step and better evaluate the potential contribution of kinases such as the homeodomain-interacting protein kinase-2 (HIPK2), which is activated by Wnt signaling and phosphorylates CtBP (Zhang et al. 2005) as well as Groucho (Choi et al. 2005) to control repression and apoptosis.

Multiple opposing roles for β-catenin and APC in Wnt signaling

β-Catenin and APC are enigmatic examples of nuclear gene-specific transcription factors that, in epithelial and

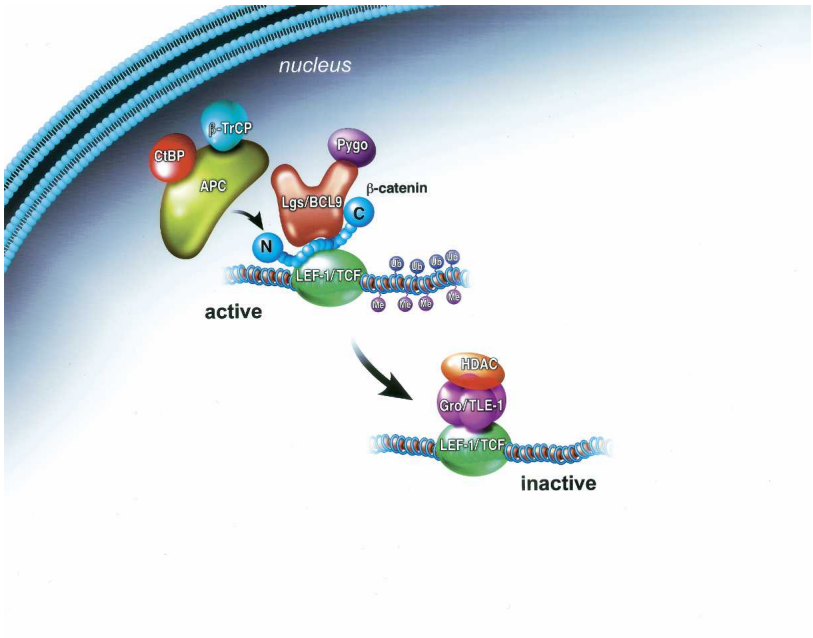

Figure 3. Model for transcriptional regulation of Wnt target genes. In the nucleus, β-catenin assembles a coactivator complex containing Bcl-9/Lgs, Pygopus, and specific chromatin remodeling complexes that are needed to establish the various chromatin modifications commonly found at active genes, including histone H3K4 trimethylation. β-Catenin also recruits nuclear APC, β-TrCP, and CtBP to target genes, potentially as part of a larger complex containing other destruction complex subunits. Whereas LEF-1 binds constitutively to Wnt enhancer, the coactivator and corepressor complexes cycle on and off of the DNA in lithium-treated cells. Cycling is not observed in HT29 CRC cells that contain a mutant APC protein that fails to recruit β-TrCP or CtBP to the gene. Unexpectedly, the GSK3β kinase was found to be present at Wnt enhancers in the absence of signaling and to cycle on and off the template together with the TLE1 corepressor. Expression of the full-length APC protein leads to the rapid repression of the active c-Myc gene and is correlated with the recruitment of β-TrCP and CtBP. Wild-type, but not mutant, APC proteins associate with CtBP in extracts, indicating that the mutant APC proteins in CRC cells are unable to repress Wnt target genes.

endothelial cells, also localize to adherens junctions to control cell adhesion and respond to cellular signaling pathways. Similar properties have been noted for LIM domain proteins, which mediate gene expression, cell adhesion and mobility, and cytoskeletal architecture in response to signaling through integrin receptors and other pathways (Kadrmas and Beckerle 2004). As indicated in Figure 1, α-catenin:β-catenin heterodimers assemble into complexes with membrane-bound E-cadherin to mediate calcium-dependent cell adhesion. In addition, α-catenin forms homodimers that bind to actin filaments and promote actin polymerization (Drees et al. 2005). Although it had been assumed that the adhesion complexes are anchored to actin through α-catenin, recent studies show that the α-catenin:actin complexes interact only transiently, if at all, with the membrane-anchored E-cadherin junctional complexes (Drees et al. 2005; Yamada et al. 2005). Therefore β-catenin is an integral part of the cadherin adhesion complex, but can also influence actin polymerization indirectly, through the binding and release of α-catenin. APC also associates with the cytoskeleton and plasma membrane at cellular junctions, and genetic disruption of APC in flies as well as overexpression in human colorectal cancer cells indicate that it plays a role in cell adhesion (Bienz and Hamada 2004).

It has long been speculated that the competition for limiting pools of available factors, such as β-catenin and APC, could provide an indirect means to integrate cell signaling and adhesion. Wnt signaling induces or stabilizes repressors of E-cadherin, such as Slug, Snail and Twist1, which mediate epithelial–mesenchymal transitions that are accompanied by changes in cell adhesion (Conacci-Sorrell et al. 2003) and mobility (for review, see Huber et al. 2005). The ability of β-catenin and APC to function as scaffolds that bind protein kinases and other enzymes such as histone methyltransferases could provide a rapid means to translate signaling events at the membrane into changes in nuclear gene expression. Moreover, the ability of signaling proteins to bind actin, or actin-related proteins, could have a direct effect on the recruitment or activity of chromatin remodeling complexes required for transcription (Olave et al. 2002; Blessing et al. 2004). Both β-actin and actin-related proteins are integral subunits of chromatin remodeling complexes, including TRRAP/p400/TIP60 and TRRAP/GCN5 (SAGA), INO80, and SWI/SNF-like BAF/Brg-1-containing complexes, several of which are already known to be required for Wnt transcription. The yeast homolog of the β-catenin-interacting protein TIP49a/b, which is found in several different chromatin remodeling complexes, was recently shown to interact directly with the actin-related protein, Arp5p, during the assembly of functional Ino80 complexes (Jonsson et al. 2004). Whether β-catenin simply recruits chromatin remodeling complexes or also regulates remodeling activity through its ability to bind to proteins like TIP49 remains an open question.

Interestingly, H3K4Me3 chromatin has been shown to be recognized by chromatin remodeling complexes, including Chd1 and, in yeast, ISWI, and is strongly linked to transcription elongation (Sims et al. 2004; Tenney and Shilatifard 2005). The events that couple elongation to H3K4Me3 are unknown, but the observation that β-catenin associates with Paf1 (Mosimann et al. 2006), MLL1/2, and ISWI complexes (Sierra et al. 2006) indicates that this is an important step for Wnt transcription. A deeper understanding of the role of chromatin in the regulation of β-catenin activity and the binding of β-catenin:LEF-1 complexes should come from biochemical studies that reconstitute Wnt enhancer activity in vitro. Using a chromatin assembly system from *Drosophila* embryo extracts, we have previously shown that recombinant LEF-1 and β-catenin bind cooperatively to chromatin, but not nonchromatin, templates (Tutter et al. 2001). Truncation of the β-catenin interaction domain at the N terminus of LEF-1 allows it to bind with high affinity to chromatin even in the absence of β-catenin, indicating that the charged N-terminal domain of LEF-1 is inhibitory to binding to chromatin, potentially due to repulsion from the histone tails, and that this inhibition is relieved or masked upon binding to β-catenin. Transcription activation by recombinant β-catenin:LEF-1 complexes requires ubiquitin in vitro (Sierra et al. 2006), presumably for the purposes of chromatin modification, and thus this system provides a useful approach to evaluate the possible roles of Pygopus, Paf1, and other complexes in this process.

The Ezh2 histone methyltransferase controls actin polymerization in receptor signaling cells

The observation that proteins like APC may function in transcription raises the converse question of whether nuclear proteins involved in chromatin remodeling or modification might ever function in the cytoplasm to control cytoskeletal structure or cell adhesion. Whereas transcription activation of inducible genes frequently requires H3K4 trimethylation by the Trithorax-related MLL1/MLL2/SET1 complex, gene silencing is associated with trimethylation of H3-Lys-27, which is mediated by the Polycomb (Pc) group repressor Enhancer of zeste homolog 2 (Ezh2). In the nucleus, Ezh2 functions within a complex containing Embryonic Ectoderm Development (Eed), Suppressor of Zeste 12 (Suz12), and other proteins, to silence homeotic genes, inactivate the X chromosome, and recruit DNA methyltransferase complexes that are required for long-term gene silencing (Cao and Zhang 2004). In fibroblasts and T lymphocytes, the Ezh2/Eed/Suz12 complex shuttles between subcellular compartments and associates with the cytosolic Rho GDP–GTP exchange factor, Vav1 (Su et al. 2005). Vav1 lies downstream of growth factor receptors and the T-cell receptor and functions with the Ezh2/Eed/Suz12 complex to induce T-cell proliferation and antigen-receptor-dependent actin polymerization in a manner that requires Ezh2 methyltransferase activity (for review, see Nolz et al. 2005). The Eed subunit of the Ezh2 complex has also been shown to be recruited to activated integrin or chemokine receptors (Witte et al. 2004). Interestingly,

Vav1 has also been detected in the nucleus at NF-AT and NFκB-regulated genes and may function together with Ezh2 in transcription (Tybulewicz 2005). Activation of the Akt kinase phosphorylates Ezh2 and lowers its affinity for histones, derepressing Ezh2-regulated genes (Cha et al. 2005). Thus, the Ezh2/Eed/Suz12 HMT complex and Vav1 represent an important paradigm for transcriptional regulators that coordinate actin cytoskeletal reorganization with proliferative responses in gene expression in response to multiple signaling pathways.

Perspectives

To date the majority of Wnt/β-catenin signaling studies have focused on events at the plasma membrane and the cytosol, in particular focusing on the mechanism that leads up to β-catenin protein stabilization. However, as highlighted in this review, while cytosolic accumulation of β-catenin in response to a Wnt signal is a crucial signaling event, it is only an intermediate step in the transduction of the signal into the nucleus to its ultimate destinations, the Wnt target genes. A surprising observation has been that many of the central cytosolic Wnt regulators, including APC, Axin, Dsh, and the Wnt regulatory protein kinases, also localize to the nucleus, where they may, directly or indirectly, interact with β-catenin and modulate its transcriptional activity. The recent studies outlined here have strengthened the notion that histone methylation and other epigenetic markers of open or repressed chromatin ultimately control the expression of Wnt gene targets. It will be important to assess whether specific Wnt regulators, like Axin and Dsh, are also recruited to Wnt enhancers, and how these proteins, as well as other coactivators like Pygopus, might interact with chromatin modifying and remodeling complexes to regulate transcription. Further work should elaborate whether these proteins translocate in unison to Wnt target genes or instead enter the nucleus independently and reassemble at Wnt enhancers. The mechanism of transcription regulation by β-catenin deserved much further scrutiny, including the possible role of β-catenin ubiquitination in the cycling of coregulator complexes and the role of β-TrCP or of protein kinases such as CK1. Biochemical studies that define the various protein interactions and protein modifications that mediate β-catenin-induced transcription or APC/CtBP-mediated repression may help uncover useful new targets that might lead to selective inhibitors of the pathway.

Equally important will be to characterize the roles of these factors in the cytoplasm, either in the regulated destruction of β-catenin, cytoskeletal actin reorganization, or effects on cell adhesion and mobility. As exemplified by the histone methyltransferase Ezh2, some of the well-defined Wnt transcription coactivators and corepressors could also have important functions in the cytoplasm. Thus it will be important to investigate the possible roles for CtBP and associated cofactors in the various APC-regulated activities in the cytosol and more generally to understand how the coordination of cytoplasmic (e.g., cell adhesion or actin polymerization) and nuclear events is crucial to execute complex cellular functions such as cell activation, apotosis, or cell mobility and migration.

In the years ahead it will be exciting to learn how the mechanism of Wnt regulation in cancer cells relates to the process of Wnt activation in adult stem cells, which must continually adjust their needs as they migrate from the niche to enter a transient amplifying stage prior to terminally differentiating. Future studies will elaborate the β-catenin transactivation mechanism in stem cells and allow a better understanding of how β-catenin regulates transcription in the absence of a Wnt signal, for example, in response to signaling through Akt or other pathways (Castellone et al. 2005; Chen et al. 2005; Ding et al. 2005). It will also be important to determine whether cycling of Wnt coregulators contributes to the periodic expression of Wnt target genes in the PSM during somitogenesis and how the vertebrate segmentation clock integrates different inputs to control oscillating Wnt and Notch transcription (Aulehla et al. 2003). Looking forward, these studies are certain to provide new and surprising insights that deepen our understanding of Wnt mechanism and biology, along with plenty of excitement to keep the Wnt party going well into the future.

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