Wnt Signaling from Development to Disease: Insights from Model Systems

Ken M. Cadigan[1] and Mark Peifer[2]

[1]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109-1048

[2]Department of Biology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3280

Correspondence: peifer@unc.edu

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One of the early surprises in the study of cell adhesion was the discovery that β-catenin plays dual roles, serving as an essential component of cadherin-based cell–cell adherens junctions and also serving as the key regulated effector of the Wnt signaling pathway. Here, we review our current model of Wnt signaling and discuss how recent work using model organisms has advanced our understanding of the roles Wnt signaling plays in both normal development and in disease. These data help flesh out the mechanisms of signaling from the membrane to the nucleus, revealing new protein players and providing novel information about known components of the pathway.

Modern biomedical science is a partnership between scientists studying basic cell and developmental processes in model systems and clinicians exploring the basis of human disease. Few fields exemplify this better than Wnt signaling, born 22 years ago with the realization that the oncogene *int1* and the *Drosophila* developmental patterning gene *wingless (wg)* are homologs (Cabrera et al. 1987; Rijsewijk et al. 1987). Additional connections further fueled research. *Drosophila* Armadillo (Arm), a component of the Wg pathway, is the homolog of the cell junction proteins β-catenin (βcat) and plakoglobin (McCrea et al. 1991; Peifer et al. 1992; Peifer and Wieschaus 1990) joining Wnt signaling and cadherin-based cell adhesion, a connection we still do not fully understand (see Heuberger and Birchmeier 2009). Adenomatous polyposis coli (APC), the tumor suppressor mutated in most colon cancers, binds βcat and is a key regulator of Wnt signaling (Rubinfeld et al. 1993; Su et al. 1993), putting the Wnt field even more squarely in the center of cancer research. Here, we outline recent advances in understanding Wnt signaling, casting new light on these critical regulators of development, homeostasis, and disease.

THE CURRENT MODEL OF WNT SIGNALING

We first outline the reigning model for Wnt signaling, focusing on canonical signaling involving βcat (for reviews of alternate Wnt

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pathways, see e.g., Fanto and McNeill 2004; Kohn and Moon 2005; Semenov et al. 2007; van Amerongen et al. 2008; Veeman et al. 2003). The key regulated Wnt effector is βcat. In cells not receiving signal, βcat accumulates in adherens junctions, but outside junctions βcat levels are low, because of its short half-life (Fig. 1, left). Free βcat is bound by the multiprotein "destruction complex" (Clevers 2006), in which the tumor suppressors APC and Axin present βcat to the kinases glycogen synthase kinase 3 (GSK3) and casein kinase I (CKI), facilitating sequential phosphorylation of sites in βcat's amino terminus. An SCF-class E3-ubiquitin ligase containing the F-box protein Slimb/βTrCP recognizes correctly phosphorylated βcat, and targets it for polyubiqitination and proteasomal destruction. Meanwhile, TCF/LEF proteins bound to Groucho-family corepressors keep Wnt target genes tightly off (Arce et al. 2006).

Wnts bind a two-part receptor: a seven-transmembrane Frizzled (Fz) and LRP5/6 (Fig. 1, right; Clevers 2006). Both are required for canonical signaling. Ligand binding triggers phosphorylation of LRP5/6's cytoplasmic tail, creating an Axin-binding site. Axin recruitment inactivates the destruction complex, in a process requiring Disheveled (Dvl). This stabilizes βcat, and it enters nuclei. βcat displaces Groucho from TCF, nucleating formation of a multiprotein activator complex including Pygopus and Legless/Bcl9, activating Wnt target genes (Arce et al. 2006). The last 5 years have tested this proposed model, revealing new mechanistic insights and further complexity. In the following section, we examine different steps in Wnt signaling in turn and describe these new insights.

PREPARING FOR DEPARTURE—POSTTRANSLATIONAL WNT MODIFICATIONS

After signal sequence cleavage and translocation into the endoplasmic reticulum (ER), Wnts are

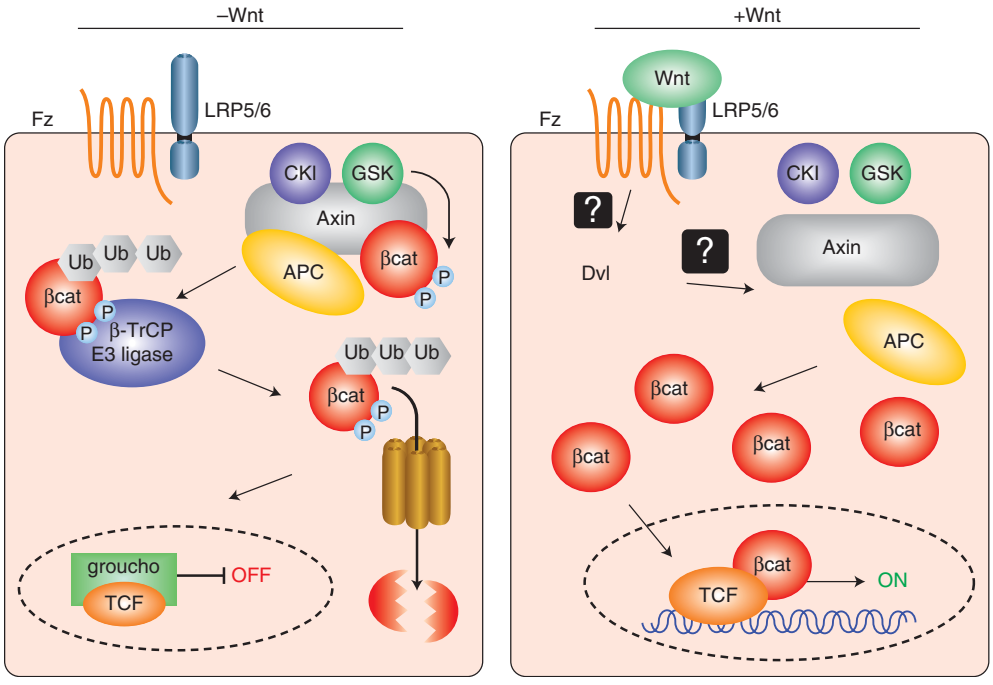

Figure 1. A simplified current view of Wnt signaling. See text for details.

transported through the endomembrane system to the cell surface and undergo several modifications. Wnts undergo N-linked glycosylation (Burrus and McMahon 1995; Kadowaki et al. 1996; Komekado et al. 2007; Kurayoshi et al. 2007; Mason et al. 1992; Smolich et al. 1993; Tanaka et al. 2002). Several Wnts also are palmitoylated at the first conserved cysteine (Galli et al. 2007; Kadowaki et al. 1996; Komekado et al. 2007; Willert et al. 2003). In addition, Wnt3a is modified with palmitoleic acid at a conserved serine (Takada et al. 2006). These acylations likely cause the notoriously hydrophobic nature of secreted Wnts (Willert et al. 2003).

Posttranslational lipidation of mammalian Wnts is clearly important for function. Mutating the conserved cysteine of Wnt1, Wnt3a, or Wnt5a prevents palmitoylation in cell culture. These mutant Wnts are secreted but have little or no signaling activity (Galli et al. 2007; Komekado et al. 2007; Kurayoshi et al. 2007; Willert et al. 2003), and unpalmitoylated Wnts cannot bind Fz receptors (Komekado et al. 2007; Kurayoshi et al. 2007). Mutating the conserved serine in Wnt3a prevents palmitoleic acid addition and blocks secretion (Takada et al. 2006). These studies suggest a model in which palmitoleic acid-modification is required for secretion, and palmitate for Fz binding.

Research on *Drosophila* Wg confirms the importance of acylation, but results differ from those in mammals. For example, mutating the conserved serine in Wg to which palmitoleic acid is added in mouse Wnt3a does not prevent secretion, though it reduces signaling activity (Franch-Marro et al. 2008a). Wg is palmitoylated (Willert et al. 2003), and mutating this amino-terminal cysteine results in secreted but inactive Wg in cell culture (Franch-Marro et al. 2008a). However, the same mutant Wg accumulates in the ER in fly tissues (Franch-Marro et al. 2008a), and mutating this cysteine in the endogenous *wg* gene prevents secretion (Nusse 2003). These data fit well with the *porcupine (porc)* phenotype, a strong loss of Wg signaling (van den Heuvel et al. 1993). Porc is an ER-localized integral membrane O-acyl transferase (Kadowaki et al. 1996) required for Wg palmitoylation (Zhai et al., 2004), and for Wg ER exit (Tanaka et al. 2002). Vertebrate Porc also promotes Wnt lipidation and is required for signaling (Galli et al. 2007). This suggests Porc acylates Wnts, though this has not yet been shown.

Apparent differences in palmitoylation's importance in Wnt signaling may reflect differences in levels of Wnt expression. In mammalian and fly cell culture, overexpression may overcome lack of palmitoylation. Consistent with this, whereas Porc is required for endogenous Wg signaling, it is not required when Wg is overexpressed (Noordermeer et al. 1994). In exploring relationships between Wnt glycosylation and lipidation, conflicting results were also obtained in culture versus in flies. Site-directed mutagenesis of Wnt3a and Wnt5a in cell culture suggested glycosylation precedes acylation and is required for ER export (Komekado et al. 2007; Kurayoshi et al. 2007). However, *porc* mutant fly embryos lacking acylation have defects in Wg glycosylation (Tanaka et al. 2002), suggesting acylation precedes glycosylation. While species or Wnt-specific differences may explain these discrepancies, care must be taken in interpreting experiments in which Wnts are expressed at nonphysiological levels.

GETTING OUT AND ABOUT—WNT SECRETION AND TRANSPORT

For several Wnts, reaching the cell surface also requires Wntless (Wls) and Retromer. Wls (also known as Evi or Sprinter in flies and MIG-14 in *Caenorhabditis elegans*) is an integral membrane protein found in the Golgi, plasma membrane, and endosomes (Banziger et al. 2006; Belenkaya et al. 2008; Franch-Marro et al. 2008b; Port et al. 2008; Yang et al. 2008). Retromer is a multiprotein complex that shuttles cargo from endosomes to the trans-Golgi (Seaman 2005). Several recent reviews covered these proteins in detail (Bartscherer and Boutros 2008; Ching and Nusse 2006; Eaton 2008; Hardin and King 2008). In short, the data suggest Wls is a "Wnt chaperone," guiding Wnts from the Golgi to the cell surface.

Retromer mutants block Wnt secretion because Wls is missorted to lysosomes (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Pan et al. 2008a; Port et al. 2008; Yang et al. 2008). Although this secretion mechanism is highly conserved, it is not universal. *Drosophila* WntD is secreted independently of lipidation, Porc, and Wls (Ching et al. 2008).

On secretion, Wnts move away from producing cells to influence neighbors (Cadigan 2002; Capdevila and Izpisua Belmonte 2001; Cayuso and Marti 2005; Strigini and Cohen 1999). One superb model for this is the *Drosophila* larval wing imaginal disc, a flat columnar epithelium. Wg is expressed along the dorsoventral (D/V) boundary and moves in both directions, forming a morphogen gradient and regulating short- and long-range targets in a concentration-dependent manner (Cadigan 2002; Strigini and Cohen 1999). Wg can directly act up to 20 cell diameters from its synthesis site (Zecca et al. 1996).

Several factors influence Wg movement. Glypicans are heparin sulfate proteoglycans anchored to cell membranes via a glycerol phosphatidylinositol (GPI) linkage (Blair 2005). Two glypicans, Dally and Dally-like (Dly), influence Wg signaling in wing discs (Fig. 2). Dally promotes signaling and is suggested to facilitate Wg movement or act as a coreceptor (Franch-Marro et al. 2005; Han et al. 2005). Interestingly, Dly has different functions than Dally. Loss of Dly increases expression of short-range targets but decreases long-range Wg signaling (Franch-Marro et al. 2005; Han et al. 2005; Kirkpatrick et al. 2004; Kreuger et al. 2004). Consistent with this, *dly* mutants have reduced spread of Wg from its synthesis site (Han et al. 2005; Marois et al. 2006). A recent study suggests Dly mediates transcytosis of apically secreted

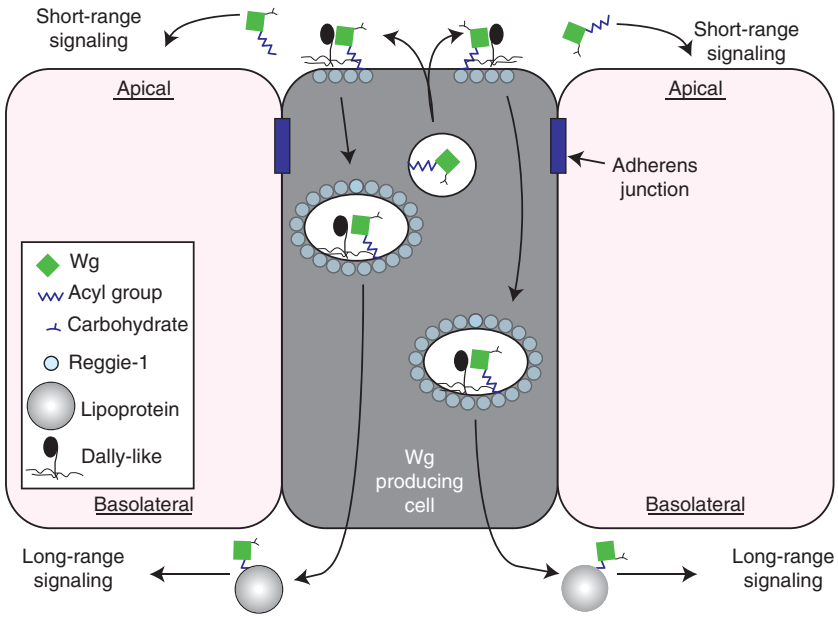

Figure 2. Model depicting Wg transcytosis and how this process could influence short- and long-range Wg signaling in the wing imaginal disc. Wg is secreted apically from producing cells, where association with extracellular glypican Dally-like directs it into endocytic vesicles. These Reggie-1-associated vesicles undergo transcytosis and fuse with the plasma membrane to release Wg on the basolateral side of the epithelial sheet. There, it can undergo long-range diffusion and signaling by associating with lipoproteins. See text for further explanation.

Wg, transporting it to the basolateral compartment (Gallet et al. 2008) where it then is presumed to diffuse to activate long-range targets.

Membrane microdomains also play roles in Wg secretion/diffusion (Katanaev et al. 2008). Reggie-1 (Flotillin-2) is a cytoplasmic component of noncaveolin lipid microdomains (Babuke and Tikkanen 2007; Langhorst et al. 2005). Reggie-1 loss strongly reduces long-range Wg targets but has no effect on short-range targets (Katanaev et al. 2008). Conversely, Reggie-1 overexpression inhibits short-range and activates long-range targets, even when Reggie-1 expression is restricted to Wg-expressing cells. As Reggie-1 is implicated in vesicular trafficking (Babuke and Tikkanen 2007; Langhorst et al. 2008) and Wg can associate with lipid rafts (Zhai et al. 2004), these data suggest Reggie-1 works with Dly to promote Wg transcytosis and subsequent long-range diffusion (Fig. 2).

After transcytosis, how does Wg travel across wing discs? Several proteins participate. Wg can associate with lipoprotein particles, which are required for long-range signaling (Panakova et al. 2005). Endocytosis is proposed to be less efficient basolaterally, facilitating Wg diffusion (Marois et al. 2006). The Wg receptors Fz, Fz-2, and Arrow can promote Wg degradation (Han et al. 2005; Piddini et al. 2005), while the secreted hydrolase Notum can inhibit Wg signaling by modifying Dally and Dly (Giraldez et al. 2002; Han et al. 2005; Kirkpatrick et al. 2004; Kreuger et al. 2004).

It is not clear whether wing imaginal discs provide a general paradigm for Wnt transport. Even in the fly embryonic epidermis, where Wg signaling regulates cell fates (DiNardo et al. 1994), things may be different. Wg is internalized by secreting cells and recycled (Pfeiffer et al. 2002), consistent with transcytosis. However, while Dly is required for Wg signaling in this tissue (Franch-Marro et al. 2005), the inhibitory role evident in wing discs is not observed. Distinguishing general from tissue-specific mechanisms for transport of Wg and other Wnts remains an important goal.

ACROSS THE PLASMA MEMBRANE—ASSEMBLING WNT SIGNALOSOMES

Wnt/βcat signaling is generally mediated by two families of cell surface proteins: Fz family serpentine receptors, and lipoprotein receptor-related proteins (LRP) mammalian LRP5 and LRP6 and fly Arrow (Arr) (Cadigan and Liu 2006; Gordon and Nusse 2006; He et al. 2004). Forced association of Fz and LRP/Arr activates Wnt/βcat signaling (Cong et al. 2004; Holmen et al. 2005; Tolwinski et al. 2003), consistent with reports that Wnt promotes Fz-LRP6 association in vitro (Tamai et al. 2000).

Wnt signaling also promotes phosphorylation of PPPSPxS motifs in LRP6's cytoplasmic tail by GSK3 and CKI (Tamai et al. 2004; Davidson et al. 2005; Zeng et al. 2005). LRP6 contains five PPPSPxP motifs and systematic mutagenesis revealed that all five contribute to signaling activity (MacDonald et al. 2008). Phosphorylated LRP6 recruits Axin to the plasma membrane, presumably inactivating the Axin-APC βcat-destruction complex (Fig. 3) (Tamai et al. 2004; Zeng et al. 2005).

Wnt stimulation also induces LRP6 oligomerization into a large ribosome-size structure, termed the LRP6 signalosome (Fig. 3) (Bilic et al. 2007). Wnt induces phospho-LRP6 aggregates at the cell surface that colocalize with Dvls (Bilic et al. 2007), cytosolic proteins containing PDZ and DIX domains that are required for Wnt/βcat signaling (Wallingford and Habas 2005; Wharton 2003). Intriguingly, Dvl forms microscopic puncta in cells (Roberts et al. 2007; Wharton 2003) that are recruited to the plasma membrane by Wnt signaling by interaction with Fzs (Cong et al. 2004; Wong et al. 2003). Dvl puncta are dynamic and form through interactions between two portions of Dvl DIX domains (Schwarz-Romond et al. 2007a). Dvl dimerization is not sufficient to activate Wnt/βcat signaling; instead, higher order oligomers are required (Schwarz-Romond et al. 2007a). Although Dvl could not be detected in LRP6 signalosomes (Bilic et al. 2007), this may be because of its dynamic properties and instability in vitro.

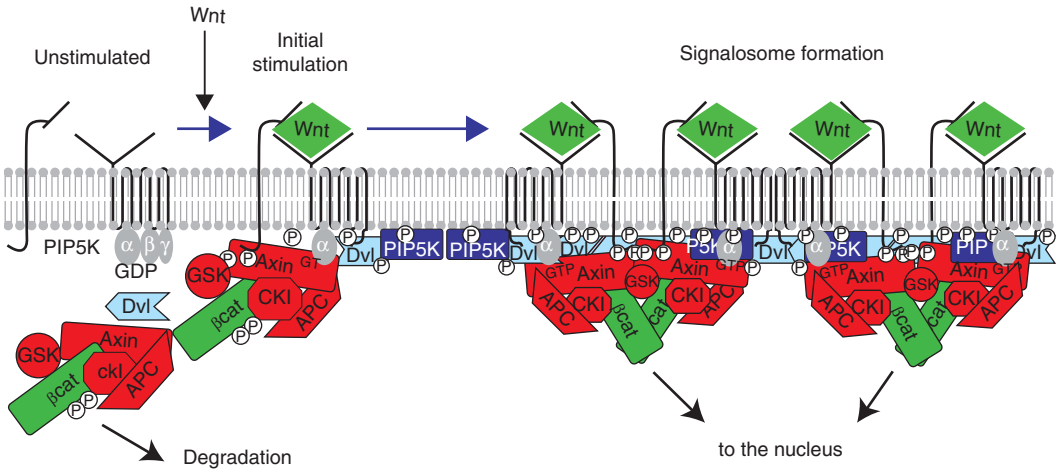

Figure 3. Model for the Wnt-induced formation of LRP5/6 signalosomes. In the absence of Wnt (at the *left*), LRP and Fz receptors are unassociated in the plasma membrane. Some data suggest that Fz may be coupled to trimeric G proteins, with the Gαo subunit in the inactive GDP-bound form. PIPKIIα (not shown for simplicity) and PIP5KI are membrane associated but inactive. The destruction complex (Axin, APC, GSK, and CKI) phosphorylates βcat, tagging the protein for ubiquitination and proteosomal degradation (see text for further explanation). Initially, Wnt promotes association of LRP5/6 and Fz receptor, leading to phosphorylation of the cytoplasmic tail of LRP5/6 by CKI and GSK3 and recruitment of the destruction complex by binding to Axin. In addition, Gαo is converted to its active GTP-bound form. One potential target of Gαo-GTP is Dvl, which is recruited to the receptor complex, where it can bind and activate PIP5KI, causing an increase in PIP2. All of these modifications appear to reinforce each other in a complex positive feedback loop, leading to a large complex of Wnt, LRP5/6, Fz, Dvl, and degradation complex at the cell surface. GSK activity is inhibited by interaction with the LRP phosphorylated tail, resulting in the accumulation of hypophosphorylated βcat, which can then translocate to the nucleus. See text for more details.

However, imaging studies in living cells support a model in which Wnt induces a large plasma membrane complex of phospho-LRP, Fz, and Dvl.

Axin is found in LRP signalosomes (Bilic et al. 2007), consistent with phospho-LRP binding Axin (Tamai et al. 2004; Zeng et al. 2005). GSK3 is also in signalosomes (Bilic et al. 2007) and Axin is required for LRP phosphorylation (Zeng et al. 2008). This suggests signalosome formation is not strictly linear. Rather, LRP phosphorylation initially recruits Axin, bringing additional GSK3 to promote more LRP phosphorylation. This positive feedback loop may be important in signalosome formation (Fig. 3); consistent with this, Dvl puncta can recruit Axin and CKI (Schwarz-Romond et al. 2007b). In *Drosophila*, biochemical evidence for Arrow signalosomes has not yet been reported. Analysis of chimeric Arrow receptors argues for distinct initiation and amplification steps in Wg signaling (Baig-Lewis et al. 2007), which could be consistent with the vertebrate model (Fig. 3).

A recent report added to the complexity of LRP6 signalosomes (Pan et al. 2008b). siRNA screening revealed that phosphatidylinositol 4-kinase type IIα (PI4KIIα) and phosphatidylinositol-4-phosphate 5-kinase type I (PIP5KI) are required for Wnt-induced LRP-phosphorylation and βcat accumulation in culture, and Wnt/βcat signaling in *Xenopus* (Pan et al. 2008b). PI4KII and PIP5KI produce phosphatidylinositol (4,5)bis-phosphate (PIP2) (Doughman et al. 2003), and delivery of PIP2 to cells stimulated Wnt signaling, whereas PIP2 depletion reduced LRP-phosphorylation by Wnt (Pan et al. 2008b). Wnt stimulation also increased PIP2 formation. This was Dvl-dependent, and recombinant Dvl activates

PIPKI in vitro, suggesting a new role for Dvl. PIP2 is required for LRP6 signalosome formation, and PIP2 is enriched in fractions containing signalosomes (Pan et al. 2008b). This provides strong evidence for PI kinases and PIP2 in Wnt receptor function (Fig. 3).

If Dvl binds and activates PIP5KI, how is Dvl activated by Wnt signaling? One possibility is through Fz-mediated G protein signaling. Studies in both mammalian cell culture (Liu et al. 2005) and *Drosophila* (Katanaev et al. 2005) suggest Gαo is required for Wnt/βcat signaling. This requirement is upstream of Dvl (Katanaev et al. 2005), suggesting a model in which information is passed from Fz to Gαo to Dvl (Fig. 3).

How do LRP signalosomes promote βcat accumulation? Wnt stimulation or LRP overexpression decrease Axin protein levels in several systems (Kofron et al. 2007; Mao et al. 2001; Tolwinski et al. 2003), which should compromise the destruction complex. LRP-mediated Axin down-regulation and inhibition of βcat degradation can be recapitulated in vitro, but LRP still stabilizes βcat when endogenous Axin is replaced with a nondegradable version (Cselenyi et al. 2008). LRP's phosphorylated tail can directly inhibit GSK3 activity (Cselenyi et al. 2008; Piao et al. 2008), which may contribute to destruction complex inhibition. Consistent with this, dephosphorylated βcat and APC are recruited to the plasma membrane on Wnt signaling (Hendriksen et al. 2008). Interestingly, dephosphorylated βcat generated by LRP6 activation is much more potent (molecule for molecule) at activating Wnt target genes than overexpressed nonphosphorylatable βcat (Hendriksen et al. 2008); thus, additional βcat activation events may occur on receptor activation.

LRP signalosomes also colocalize with Caveolin-1, a caveolae marker (Bilic et al. 2007). Functional connections between Wnt/βcat signaling and Caveolin-1 were reported, suggesting that caveolae-induced endocytosis of LRP6 is required for signaling (Yamamoto et al. 2006). However, caveolin-1, caveolin-2, and caveolin-3 knockouts are all viable, as are caveolin-1 caveolin-3 double knockouts (Le Lay and Kurzchalia 2005), suggesting that they do not play essential roles. In fact, caveolin-1 mutants were reported to have increased Wnt/βcat signaling (Sotgia et al. 2005). *Drosophila* lacks recognizable Caveolins (Le Lay and Kurzchalia 2005), suggesting that caveolae are not essential for Wg signaling.

IF IT IS IN THE TEXTBOOK, WHY ARE YOU STILL STUDYING IT?

The canonical model for Wnt regulation was an important advance, and is widely accepted and incorporated into cell biology textbooks. However, this overstates our knowledge of how things actually work: Many key questions remain. Given that Axin, which binds numerous partners, is likely the scaffold for destruction-complex assembly, APC's mechanistic role remains mysterious. Second, it is not clear where the destruction complex resides, or whether its localization changes on Wnt signaling to modulate inactivation. Third, the detailed biochemical interactions modulating βcat phosphorylation and release to the E3 ubiquitin-ligase remain to be elucidated. Finally, the multiple APC and Axin family members in many animals raise questions about differential function or redundancy.

With the realization that Axin is the scaffold for destruction-complex assembly, the search began for other APC mechanisms of action. One model is that APC helps localize the destruction complex to the correct location (Fig. 4A). There has been substantial difficulty in localizing endogenous Axin, though if overexpressed it forms large cytoplasmic puncta. GSK3 and CKI both accumulate throughout cells, reflecting their many substrates. Thus, the clearest picture came from studying APC. Both mammalian APC (Näthke et al. 1996) and *Drosophila* APC2 (McCartney et al. 1999; Yu et al. 1999) are enriched at the cortex of epithelial cells, suggesting this may be the normal location of the destruction complex. Consistent with this, missense mutations in fly *APC2* exhibit a strong correlation between loss of cortical protein localization and loss of function in Wnt regulation (McCartney et al. 2006).

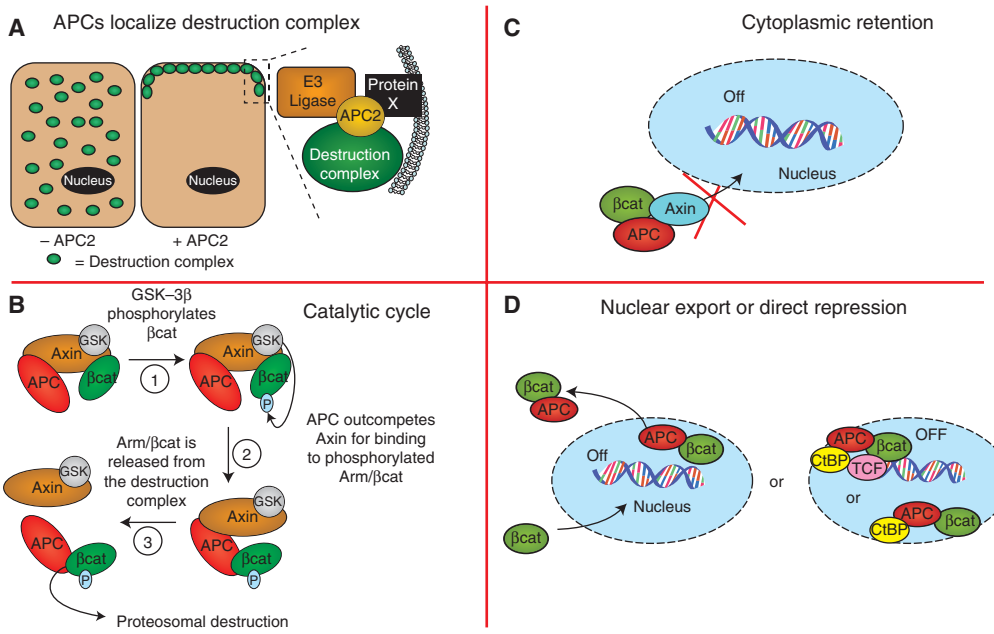

Figure 4. Four proposed models for the role of APC in the destruction complex. (*A*) APC binds the destruction complex and unknown protein X, recruiting the destruction complex to the apical cell cortex and connecting it to the E3 ubiquitin ligase. (*B*) The catalytic cycle model in which βcat is sequentially bound to Axin and then APC before transfer to the E3 ligase. (*C*) Cytoplasmic retention model. Axin and APC bind cytoplasmic βcat and tether it there. (*D*) APC in nucleus. APC exports βcat from nucleus or acts directly at promotors in transcriptional repression. See text for more details.

The apical cortex is attractive, as it brings APC into proximity of at least a subset of Fz receptors (Wu et al. 2004).

However, other data is less consistent with this model. Although fly APC1 and APC2 are redundant in many tissues (Ahmed et al. 2002; Akong et al. 2002a), their intracellular localizations are quite distinct. APC1 localizes to axons in neurons, and to centrosomes and microtubules when overexpressed (Akong et al. 2002a; Akong et al. 2002b; Hayashi et al. 1997). APC1 and APC2 have similar differential localization in male germline stem cells (Yamashita et al. 2003). How do we explain this? Possibilities include: (1) Each APC localizes to the other location at lower but still functional levels, (2) The destruction complex can function at several locations, or (3) APC does not localize the destruction complex. Overexpressed Axin recruits APC to cytoplasmic puncta (e.g., Faux et al. 2008), perhaps consistent with the latter conclusion, but colocalization of endogenous APC and Axin is difficult to detect. It is also important to remember that APC proteins have Wnt independent cytoskeletal functions (Näthke 2006), and thus many of the sites where APC proteins localize, including their predominant localization sites, may reflect these cytoskeletal functions rather than the localization of the destruction complex.

Strikingly, in *C. elegans*, localization of Wnt signaling proteins during Wnt-mediated asymmetric divisions suggests a different way of wiring the pathway (Mizumoto and Sawa 2007; Takeshita and Sawa 2005). Apr-1 (APC), Pry-1 (Axin), and Dvl homologs all localize to the cortex, and do so asymmetrically. Surprisingly, Wrm-1/βcat acts at the cortex to inhibit Wnt signaling, a striking reversal of canonical signaling, whereas cortical Apr-1 mediates nuclear "export" of Wrm-1 (these data might also be explained by cytoplasmic Wrm-1 retention). Thus, natural selection can

reshape even this highly conserved pathway; similar asymmetries may occur in other animals (Schneider and Bowerman 2007).

REGULATING βCAT DESTRUCTION IS A COMPLEX TOPIC

Other models for APC function arose from structural/biochemical studies. The Weis and Xu laboratories examined affinities of βcat for both APC and Axin (Choi et al. 2006; Ha et al. 2004; Liu et al. 2006; Xing et al. 2003). The results were intriguing. Under basal conditions, βcat's affinity for Axin is higher than that for APC. However, if APC is phosphorylated by GSK3 within the βcat-binding 20-amino acid (aa) repeats, APC has a higher affinity for βcat than Axin. This led Kimelman and Xu (2006) to propose that the destruction complex goes through a cycle of structural rearrangements (Fig. 4B). It assembles with Axin bound to βcat, because of its higher affinity. βcat and APC are both phosphorylated by GSK3, triggering transfer of βcat to APC. This is suggested to facilitate βcat transfer to the E3 ubiquitin ligase, with presumed APC dephosphorylation by PP2A resetting the system. This is an intriguing model, which can be tested by site-directed mutations in APC.

In contrast, Weis' laboratory interpreted their data differently. They directly showed that Axin can be a scaffold for substrate phosphorylation (Ha et al. 2004). However, phosphorylated APC bound to βcat is not accessible to dephosphorylation (albeit by PP1, not PP2), inconsistent with the catalytic cycle model. They suggest that altered APC affinity for βcat allows it to accommodate drastically different βcat levels in the presence or absence of Wnt signal.

Recent data support a role for APC in facilitating transfer of phospho-βcat to the E3 ubiquitin ligase (Su et al. 2008). In a cell-free system, extracts from *APC* mutant colon cancer cells can phosphorylate but not ubiquitinate βcat, consistent with Axin playing the primary scaffolding role. Adding tagged APC restores ubiquitination. Added APC also restores the ability of phospho-βcat to coIP with βTrCP. Finally, APC can protect phospho-βcat from dephosphorylation. These results are quite interesting, and suggest transferring βcat from the destruction complex to the E3 ligase is an important APC role.

DECONSTRUCTING THE DESTRUCTION COMPLEX

Full mechanistic understanding requires dissecting the moving parts of the destruction machine. Several laboratories did so. Wehrli's laboratory examined Axin's mechanism of action by site-directed mutagenesis and analysis in vivo in *Drosophila* (Peterson-Nedry et al. 2008). Their results were quite surprising. Substantial Axin function was retained by proteins lacking binding sites for APC (the RGS domain) or for βcat. Even Axin lacking the GSK3 binding site retained some function. These data suggest that the destruction complex is stabilized by multiple interactions, with individual protein contacts somewhat dispensable. Strikingly, mutants lacking the PP2A binding site or Dix domain had phenotypes suggesting that these mutant proteins could not be turned off by Wnt signaling, consistent with proposed roles for PP2A and Dvl in inactivating the destruction complex. Finally, an Axin mutant lacking the βcat binding site was complemented in trans by one lacking the APC binding site, suggesting that the functional destruction complex contains multiple copies of Axin.

APCs are also complex mosaics (Näthke 2006; Polakis 2007). All share core domains that together are sufficient for Wnt regulation: Arm repeats, 15- and 20-aa repeats that bind βcat, and SAMP repeats that bind Axin. Many APCs also carry carboxy-terminal extensions, allowing interactions with microtubules, the microtubule plus-end binding protein EB1, and other partners, presumably modulating cytoskeletal functions. Colon tumors carry truncated APC proteins lacking a subset of βcat binding sites and all the Axin-binding SAMP repeats (Polakis 1995). In mice, a truncated APC lacking the cytoskeletal-interacting carboxyl terminus but retaining one SAMP

motif still regulates Wnt signaling (it is homozygous viable) (Smits et al. 1999). In contrast, loss of 65 additional amino acids abrogated Wnt signaling. This complemented earlier work in cultured colon cancer cells, which also suggested key roles for 20-aa and SAMP repeats (Munemitsu et al. 1995; Rubinfeld et al. 1997).

All APCs share amino-terminal Arm repeats (Peifer et al. 1994), known binding sites for several protein partners (Näthke 2006). In *Drosophila* APC2, the Arm repeats play key roles in Wnt signaling (McCartney et al. 2006; Hamada and Bienz 2002; McCartney et al. 1999). Both the Arm repeats and the region including the 20-aa and SAMP repeats are important for APC2's cortical localization (McCartney et al. 2006), supporting the hypothesis that APC2 acts at the cortex in Wnt regulation and suggesting the existence of an unidentified cortical partner.

Two *APC2* alleles precisely mimic truncations in human tumors (Polakis 2007), allowing testing of hypotheses concerning roles of truncated proteins. Some suggested that they have dominant-negative effects on Wnt signaling or chromosome segregation, and it remained unclear whether they are null for Wnt regulation. Phenotypic comparisons with the null allele revealed that truncated APCs are severely impaired but not null for Wnt signaling. They do not have dominant-negative effects on Wnt signaling, although they can have dominant-negative effects on cytoskeletal events (McCartney et al. 2006). These data support the "just-right" hypothesis (Albuquerque et al. 2002), suggesting that selection during development of colon polyps favors mutations that reduce but do not eliminate APC regulation, producing just the right level of Wnt signal.

Behrens' laboratory explored possible roles of another conserved sequence they term the CID (Kohler et al. 2009), using a cultured cell assay pioneered by Polakis (Munemitsu et al. 1995; Rubinfeld et al. 1997). To their surprise, truncated APC lacking all SAMP repeats substantially rescued βcat regulation, in contrast to what is seen in mice (Smits et al. 1999). Instead, they saw a sharp drop in rescuing ability when they removed the CID, a sequence conserved in both insect and both mammalian APCs. As the authors point out, these data are somewhat paradoxical, because some colon tumors carry truncated APCs retaining CID. They find that different colon cancer cell lines differ in their ability to be rescued by SAMP-less fragments, suggesting that differences at other loci are important. These data also reinforce the idea that multiple protein interactions are likely important to assemble a functional destruction complex.

APC IN AND OUT OF NUCLEI

Although regulating βcat stability is critical for Wnt signaling, some data suggest additional roles for APC and Axin in Wnt regulation. One early, plausible suggestion was that the destruction complex is also a cytoplasmic anchor for βcat, reducing activation of Wnt target genes (Fig. 4C) (Tolwinski and Wieschaus 2001). Wieschaus' laboratory found that Wnt signaling alters Axin stability, thus regulating its cytoplasmic anchoring function (Tolwinski et al. 2003). Others provided evidence for an anchoring role for APCs (Ahmed et al. 2002; Akong et al. 2002a; Krieghoff et al. 2006). It seems likely that cytoplasmic retention plays an important modulatory role, working together with targeted destruction.

Others suggested APC acts in other cellular compartments. Nuclear localization and export signals on APC led to the suggestion that it regulates βcat nuclear export (Fig. 4D) (reviewed in Brocardo and Henderson 2008). Two papers support an even more direct role for APCs in nuclei (Fig. 4D). Hamada and Bienz (Hamada and Bienz 2004) identified interactions between APC and the transcriptional repressor (carboxy-terminal binding protein [CtBP]) by proteomics. They argue that nuclear CtBP, by binding APC and thus indirectly recruiting βcat, is a nuclear βcat sink (see Fig. 5), reducing TCF association and thus reducing Wnt-regulated transcription. Even more direct action is envisioned by Jones' laboratory (Sierra et al. 2006). APC can be ChIPed at the Wnt target gene c-*myc*.

Strikingly, it binds there with βTrCP and CtBP. They suggest that transient APC recruitment leads to long-term repression, and argue that carboxy-terminally truncated APCs in colon tumors, which no longer interact with CtBP, are impaired in this. These possible direct roles for APC in repressing βcat-driven transcription are intriguing. However, activating βcat mutants, lacking single phosphorylation sites and thus not targeted for destruction, can cause colorectal and other cancers (Polakis 2007), suggesting that regulating βcat stability is APC's primary role, with other roles likely to be modulatory.

DOUBLE THE FUN

Questions about APC function are further complicated by the two family members in mammals (APC and APC2) and *Drosophila* (APC1 and APC2). All four regulate βcat stability, so their shared core domains must be sufficient for this. Fly APC1 negatively regulates Wnt signaling in the eye (Ahmed et al. 1998), whereas fly APC2, which is more broadly expressed, regulates Wnt signaling in the embryonic epidermis (McCartney et al. 1999). However, in many tissues, they are functionally redundant (Ahmed et al. 2002; Akong et al. 2002a). Further, even in tissues in which one has the predominant role, removing the other enhances Wnt activation (Akong et al. 2002a; Benchabane et al. 2008). This argues that even low levels of APC, near the detection threshold, confer residual function. Redundancy among mammalian APCs could explain why *APC* mutants are found only in colorectal tumors and not other tumors in which Wnt activation is implicated (Polakis 2007); however, the lack of published mouse *APC2* mutants precludes this analysis.

Exploring functions of the two fly APCs led to further insights (Takacs et al. 2008). In screening for mutations suppressing the *APC1* eye phenotype, Ahmed's laboratory obtained *APC2* alleles. This was surprising, because in other contexts the two APCs act redundantly, and suggests that APCs play positive as well as negative roles in Wnt signaling. Their data further suggest that APC can promote Axin turnover, likely in response to Wnt signals, and suggest that this requires the Arm repeats, while the carboxy-terminal region is dispensable. These striking findings remind us that feedback regulation is likely to play a role in signaling. The mechanism for targeting Axin for proteolysis remains to be determined. The HECT domain E3 ubiquitin-ligase Edd binds to APC, but stabilizes rather than destabilizes Axin and APC (Ohshima et al. 2007) suggesting even more complexity in feedback regulation.

There are also two mammalian Axins, with presumed functional overlap. Axin loss leads to early embryonic lethality (Zeng et al. 1997). Axin2/Conductin mutants are viable with craniofacial defects in mice (Yu et al. 2005) and human patients (Lammi et al. 2004). Chia and Constantini (2005) tested functional equivalence by knocking *Axin2* into the *Axin* locus; this resulted in a viable, normal mouse. Flies have only a single Axin, which is essential for Wnt regulation (Hamada et al. 1999; Willert et al. 1999). In contrast, *C. elegans* also has two Axins. Both diverged dramatically from mammalian and fly Axins, retaining only recognizable RGS and Dix domains (Korswagen et al. 2002; Oosterveen et al. 2007). The two worm Axins functionally overlap; *axl-1* mutations enhance many *pry-1* phenotypes, while Axl-1 has phenotypes in some tissues unaffected by Pry-1 loss. The reduced sequence similarity and altered protein interactions call into question the universality of mechanisms of regulating βcat.

AND IF THAT WAS NOT COMPLEX ENOUGH, LET US ADD NEW PLAYERS

Recent work identified several other potential Wnt regulators. One is Wtx/Amer, originally identified as a gene mutated in renal Wilms tumors (Rivera et al. 2007). Other Wilms tumors have activating mutations in βcat (Koesters et al. 1999), suggesting that Wnt signaling plays a role in pathogenesis. Two recent papers independently connected Wtx to Wnt signaling. Moon's laboratory used proteomics to identify new partners of βcat, APC, and

Axin (Major et al. 2007). Among them was Wtx, which bound all three TAP-tagged baits. Wtx can also interact directly with βTrCP, suggesting that it might help bridge the destruction complex and E3 ligase. In vivo assays in *Xenopus* supported a role in negative Wnt regulation. However, changes in βcat stability and Wnt pathway activation were milder than those caused by loss of core components of the destruction machinery.

In parallel, Behrens' laboratory identified Wtx (they refer to it as Amer1) in a two-hybrid screen for proteins interacting with APC's Arm repeats (Grohmann et al. 2007). Wtx can recruit APC to the plasma membrane, perhaps by binding PIP2, and Wtx positively regulates APC stability. These two datasets are distinct but consistent—stabilizing APC would promote βcat destruction. However, much more remains to be learned about Wtx function. Wtx has a second vertebrate paralog, which might explain the relatively modest effects of knockdown in *Xenopus* and the presence of Wtx mutations in Wilms' but not other tumors (Yoo et al. 2008). Surprisingly, however, there are no Wtx homologs in flies or worms, suggesting that it is not a universal part of the pathway.

Another possible vertebrate-specific Wnt regulator is the cytoskeletal protein MCAF, which cross-links actin, intermediate filaments, and microtubules. *MACF1* mutant mice are embryonic lethal (unlike its paralog BPAG, which has postnatal neural defects) (Chen et al. 2006). Although *MACF* embryos do not die until E11.5, they arrest at E7.5, and lack mesoderm and the primitive streak. The phenotype is similar to that of Wnt3a or LRP 5 + 6 knockouts, though marker analysis in the *MACF* study is relatively limited. MACF1 can coIP with Axin, APC, βcat, and GSK3, suggesting that it associates with the destruction complex, and that *MACF1* siRNA blunts Wnt3-induced transcription and reduces βcat levels. MACF1 also coIPs with LRP5/6, suggesting a possible role in destruction complex inactivation. Consistent with this, MACF1 knockdown reduces Axin recruitment to the membrane after Wnt treatment. It will be interesting to further examine phenotypic similarities and differences between *MACF1* and other key Wnt pathway proteins. The lack of reported Wnt phenotypes of mutations in Shortstop, the fly homolog, suggest that MACF1's role in Wnt signaling may also be vertebrate-specific.

Bejsovec's laboratory discovered another novel negative regulator, Rac-GTPase-activating-protein RacGAP50C, in screening for modifiers of *Drosophila wg* mutants (Jones and Bejsovec 2005). It may act in synergy with Naked cuticle (Nkd). RacGAP50C has a known role in cytokinesis (Gregory et al. 2008; Zavortink et al. 2005), but its role in Wnt signaling is independent of this, and, surprisingly, independent of GAP activity. The mechanism by which RacGAP50C acts, and whether its mammalian homolog regulates Wnt signaling remain to be determined.

Nkd and its mammalian homologs also may differ in their importance in flies and mammals. Both bind Dvl (e.g., Rousset et al. 2001; Wharton et al. 2001), but their function is unknown. Fly Nkd is a key negative regulator (Zeng et al. 2000), although signaling is not activated to quite the same degree as is seen on complete destruction complex inactivation. Overexpressing zebrafish Nkd homologs suppresses both canonical and noncanonical Wnt signaling (Van Raay et al. 2007). Surprisingly, however, *Nkd1* and *Nkd2* single mutant mice and even *Nkd1 Nkd2* double mutants are viable and relatively normal (Zhang et al. 2007). Double mutants have subtle cranial abnormalities like those of *Axin2*. Thus, Nkd is a relatively minor modulator of mammalian Wnt signaling.

INTO THE NUCLEUS—TARGET GENE REGULATION BY βCAT/ARM

On entry into the nucleus, stabilized βcat/Arm transcriptionally regulates Wnt target genes. However, βcat/Arm cannot bind DNA. Rather, it is a coregulator, binding to transcription factors. Members of the TCF family of high mobility group (HMG) DNA-binding proteins are responsible for much of this regulation. Flies and worms have only one TCF (TCF/Pangolin

and POP-1, respectively). Mammals have four TCFs (TCF1, Lef-1, TCF-3, and TCF-4). In addition to TCFs, several other transcription factors can recruit βcat to target genes, including mouse PitX1 (Kioussi et al. 2002), *Xenopus* SOX17 (Sinner et al. 2004), and *C. elegans* FOXO-1 (Essers et al. 2005). Here, we focus on the mechanism by which βcat/Arm regulates transcriptional activity of TCFs.

TCFs are thought to be bimodal regulators of Wnt targets (Fig. 5). In the absence of signal, they act with corepressors to keep Wnt target genes silenced. βcat/Arm binding to TCF antagonizes this repression and recruits additional coactivators, inducing target gene expression. Thus, TCF is a transcriptional switch, with βcat/Arm converting repression to activation (reviewed in Parker et al. 2007; Stadeli et al. 2006; Willert and Jones 2006).

TCFs recognize specific DNA sequences through their HMG domains (Laudet et al. 1993). Several studies of different TCFs largely agree that CCTTTGAT is a high affinity site for the HMG domain (Barolo 2006). However,

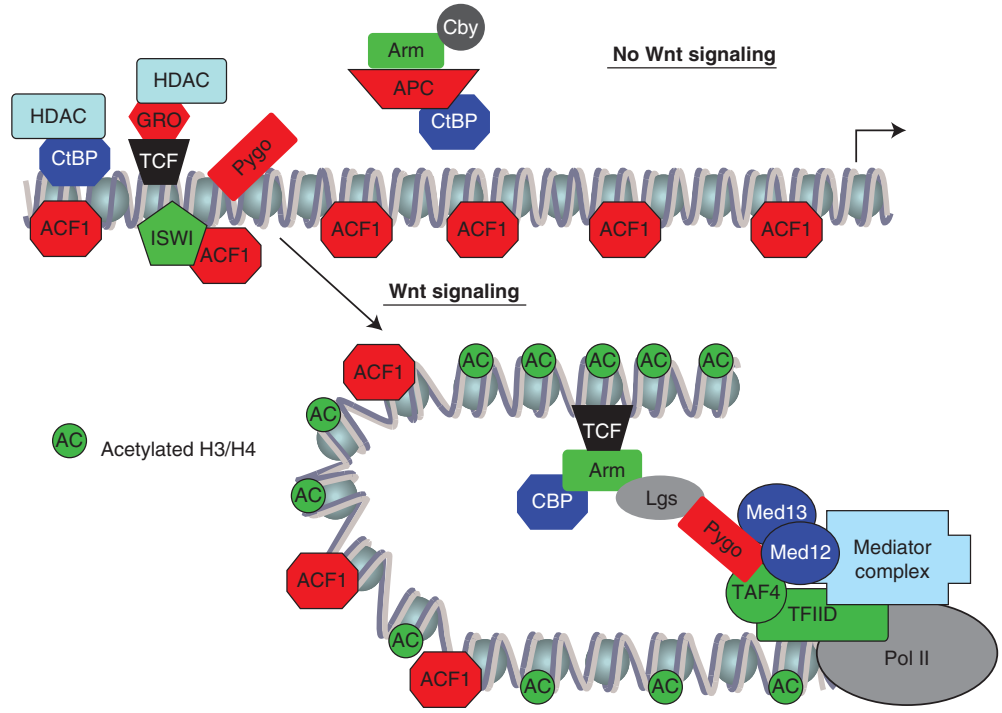

Figure 5. Model for target gene regulation by Wg signaling in *Drosophila*. The top part of the figure outlines some of the mechanisms by which target genes are repressed in the absence of Wg signaling. TCF recruits Gro through direct binding, while CtBP is recruited to WREs in an TCF-independent manner. Both corepressors may act by recruiting HDACs. In addition, the ISWI-ACF1 is recruited to the WRE in a TCF-independent way. Although ISWI remains near the WRE, ACF1 spreads across the loci. Low levels of nuclear Armadillo (Arm) are diverted away from TCF by an APC-CtBP complex or Cby. When high levels of Arm accumulate in the nucleus after Wg signaling, Arm binds to TCF, displacing Gro and recruiting additional coactivators. For simplicity, only two are shown in the bottom part of the figure. The histone acetyltransferase CBP is recruited by the carboxy-terminal Arm transactivation domain and causes a widespread increase in histone acetylation at Wg target loci. ISWI and ACF1 are bound less efficiently to acetylated histone chromatin. The amino-terminal transactivation domain of Arm recruits Lgs and Pygo. Pygo then contacts the Med12 and Med13 subunits of the mediator complex, as well as the TAF4 subunit of the TFIID basal transcription complex. Thus, Pygo acts as an adaptor, connecting the TCF activation complex with the basal transcription machinery to activate target gene expression.

TCF can also bind motifs differing from this consensus (Hallikas et al. 2006). In addition, several Wnt response elements (WREs) in flies have functional TCF-binding sites varying significantly from the consensus (Chang et al. 2008a; Lee and Frasch 2000; Riese et al. 1997). Given the degeneracy of DNA recognition by TCF, potential binding sites are found with high frequency throughout the genome. This suggests that additional sequence information may exist to specify which TCF sites can act as WREs.

For some TCFs, an additional DNA-binding domain was recently shown to provide greater DNA-binding specificity. Some mammalian TCF isoforms (TCF-1E and TCF-4E) contain an additional motif carboxy-terminal to the HMG domain, allowing them to bind an extended DNA sequence (Atcha et al. 2007). This 30-residue motif—the C-clamp—is highly conserved in invertebrate TCFs (Atcha et al. 2007). The extended sequence it binds (RCCG; R=A or G) resembles the sequence of "TCF helper sites" (GCCGCCR), recently discovered in several fly WREs, where they are as essential for Wnt responsiveness as classic TCF-binding sites (Chang et al. 2008b). These WREs require the TCF C-clamp for activation, and in vitro DNA binding studies suggest fly TCF binds in a bipartite manner, with the HMG domain binding the classic site and the C-clamp binding the Helper site (Chang et al. 2008b). This mechanism appears to be essential in flies (where all major TCF isoforms contain a C-clamp) and raises the question of how vertebrate TCFs lacking a C-clamp efficiently locate their targets.

In the absence of Wnt stimulation, TCF is thought to silence target gene expression by recruiting corepressors (Fig. 5). TCFs can bind TLE/Groucho/Grg family corepressors (Cavallo et al. 1998; Daniels and Weis 2005; Roose et al. 1998). Flies only contain one family member (Groucho), which clearly contributes to silencing in the absence of Wnt signaling (Cavallo et al. 1998; Fang et al. 2006; Mieszczanek et al. 2008). TLE and βcat compete for binding to TCF (Daniels and Weis 2005). Consistent with this, TLE1 can occupy Wnt target gene chromatin in a reciprocal manner as βcat (Sierra et al. 2006). Like TLEs, mouse myeloid translocation gene related-1 (Mtgr1) was recently reported to bind to TCF4 and contribute to target gene silencing (Moore et al. 2008).

In addition to this corepressor mechanism, other factors contribute to silencing Wnt target genes in flies by acting in parallel to TCF (Fig. 5). CtBP is required for silencing Wnt targets, and is at WREs in a pattern similar to fly TCF (Fang et al. 2006). However, depleting TCF by RNAi had no effect on CtBP occupancy and CtBP appears to repress target gene expression in parallel to TCF/Groucho (Fang et al. 2006). The DNA-dependent ATPase ISWI, part of the ACF chromatin remodeling complex, is required for repression of Wnt targets in flies (Liu et al. 2008). Like CtBP, it is found at WREs and its localization is TCF-independent (Liu et al. 2008). Presumably, ISWI silences Wnt targets by repositioning nucleosomes at WREs. Intriguingly, although ISWI is localized to WREs, the ACF1 subunit of ACF is broadly distributed across Wnt target loci (Liu et al. 2008) (Fig. 5).

Repression of Wnt targets is relieved when sufficient βcat enters nuclei and binds TCF. TCF's amino terminus directly binds βcat's Arm repeats in vitro (Daniels and Weis 2002; Graham et al. 2000), but several other factors regulate this association in cells. Chibby and ICAT bind to βcat's carboxy-terminal region, preventing TCF-βcat interaction (Tago et al. 2000; Takemaru et al. 2003). Sox 9 can also compete with TCFs for βcat binding (Akiyama et al. 2004), and a complex of APC and CtBP can bind nuclear βcat and divert it from TCF binding (Hamada and Bienz 2004). These proteins act as TCF-βcat "buffers," ensuring the proper threshold of βcat is achieved before it complexes with TCFs (Fig. 5).

In addition to proteins antagonizing TCF–βcat interactions, recent reports identified factors required to stabilize the complex on target gene chromatin. The related transducin beta-like proteins TBL1 and TBLR1 are required to stabilize TCF-βcat on target genes in mammalian and fly cell culture. They appear

to do so through direct interactions with both TCF and βcat (Li and Wang 2008). In mammalian cells and zebrafish embryos, nuclear Dvl and c-Jun form a complex with TCF and βcat to promote target gene expression (Gan et al. 2008). These studies suggest that TCF-βcat interaction on its own is not sufficient under physiological conditions, and needs additional protein-protein contacts to remain on target chromatin long enough to activate gene expression.

After recruitment to TCFs at target gene loci, βcat/Arm acts as a platform for recruiting additional coactivators (Fig. 5). Many βcat-binding factors are implicated in activating Wnt targets (reviewed in Parker et al. 2007; Stadeli et al. 2006; Willert and Jones 2006). Many, such as the histone acetyltransferases CBP and p300 (Hecht et al. 2000; Li et al. 2007b; Sun et al. 2000; Takemaru and Moon 2000) and Parafibromin/Hyrax (Mosimann et al. 2006), bind the last two Arm repeats and carboxyl terminus of βcat and promote target gene activation. In addition, the amino-terminal portion of βcat/Arm recruits Legless (Lgl) in flies (Hoffmans and Basler 2004; Kramps et al. 2002) and BCL9 or BCL9-2 in vertebrates (Brembeck et al. 2004; Hoffmans and Basler 2007; Kramps et al. 2002; Sustmann et al. 2008). Lgl/BCL9 in turn recruits the PHD-finger protein Pygopus (Pygo) to the activation complex (Kramps et al. 2002; Stadeli and Basler 2005). These studies suggest that βcat/Arm's amino and carboxyl termini are both required to recruit distinct coactivators for activation of Wnt targets.

Intensive study of fly Pygo offers a glimpse of the complexities of transcriptional activation. In addition to Pygo's PHD domain, which binds Lgs, Pygo also contains a conserved amino-terminal homology domain (NHD). This is proposed to promote Wnt target activation by binding the mediator complex subunits Med12 and Med13 (Fig. 5) (Carrera et al. 2008), as well as the TFIID complex subunit TAF4 (Wright and Tjian 2009). In addition, Pygo's PHD domain can bind histone H3 methylated at lysine 4 (H3K4me). This is dependent on Lgs and is important for Pygo function in vivo (Fiedler et al. 2008). In addition to a direct role in connecting the TCF-Arm-Lgs complex to Mediator and basal transcription machinery, Pygo and Lgs are also reported to regulate Arm nuclear translocation (Townsley et al. 2004). Furthermore, Pygo is found at WREs in the absence of signaling (de la Roche and Bienz 2007) and functions as an antirepressor, counteracting Groucho (Mieszczanek et al. 2008). Thus, Pygo acts at multiple levels to promote activation of Wnt target genes in flies.

Given the several roles it plays in Wnt signaling, it is perhaps not surprising that Pygo is required for Wnt signaling throughout fly development (Belenkaya et al. 2002; Kramps et al. 2002; Parker et al. 2002; Thompson et al. 2002). However, even though Pygo's biochemical properties are conserved in mammalian Pygo1 and Pygo2 (Fiedler et al. 2008; Kramps et al. 2002), *pygo1*, *pygo2* or double knockouts in mice have a surprisingly mild decrease in Wnt signaling (Li et al. 2007a; Schwab et al. 2007; Song et al. 2007). Perhaps this is because mouse BCL9 contains its own transactivation domain (Sustmann et al. 2008). As the field becomes more sophisticated in understanding how Wnt target genes are activated, discrepancies between different phyla may become more commonplace.

Although extensive protein–protein interactions are clearly critical for TCF-βcat to activate target gene expression, chromatin modifications also play a key role. Several studies in mammals showed an increase in acetylation of the histone H3 and H4 N-termini at WREs in response to pathway activation (Fig. 5) (Feng et al. 2003; Kioussi et al. 2002; Sierra et al. 2006), consistent with correlation between histone acetylation and gene activation (Grewal and Moazed 2003; Robyr et al. 2002). Increased H3 and H4 acetylation was also observed in fly cells, but here the increase was observed over the entire target gene, up to 30 kB away from the WREs (Parker et al. 2008). This increased histone acetylation still occurred when transcription was blocked, but was dependent on CBP. Interestingly, CBP histone acetyltransferase was restricted to the WRE (Parker et al. 2008). The purpose of widespread

histone acetylation is not clear, though it appears to be needed to displace ACF1, which is broadly distributed across silenced Wnt targets (Liu et al. 2008).

Finally, TCF-βcat can also directly repress transcription (Hoverter and Waterman 2008); targets include *decapentaplegic* in fly imaginal discs (Theisen et al. 2007), *E-cadherin* in mouse keratinocytes (Jamora et al. 2003), and *p16INK4a* in melanomas (Delmas et al. 2007). In these cases, TCF acts through traditional sites, but TCF-Arm repression of *Ugt36Bc* in fly hemocytes occurs through highly divergent sites (Blauwkamp et al. 2008). Converting these divergent sites to classic ones results in Wnt signaling activating this WRE, arguing that DNA allosterically regulates the TCF-Arm complex in a profound manner. The prevalence of Wnt-mediated direct repression relative to the more commonly recognized transcriptional activation is one of many important questions requiring further study.

CONCLUSIONS

The past 5 years provided dramatic new insights into the mechanisms of Wnt signaling and its roles in development and disease. However, they also raised many new questions that promise to make the next 5 years equally exciting. For example, the roles of lipid modifications and the mechanisms of Wnt transport remain to be clarified. Signaling by Fz and LRP/Arrow, including possible roles for G-proteins, the function of Dvl, and the mechanisms by which the destruction complex is inactivated, are active areas of research. Interesting questions remain about the function of APC in the destruction complex, the location at which βcat regulation occurs, and the nature of the catalytic cycle. Within the nucleus, hot topics include how TCF selects sites from the entire genome, how TCF complexes mediate both activation and repression, and which transcriptional partners are general and which are target gene or tissue specific. Despite the size of the Wnt community, we will have our hands full addressing these issues.

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