REVIEW: CANCER

Wnt Signaling in Oncogenesis and Embryogenesis—a Look Outside the Nucleus

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The Wnt cell-cell signaling pathway plays a critical and evolutionarily conserved role in directing cell fates during embryogenesis. In addition, inappropriate activation of the Wnt signal transduction pathway plays a role in a variety of human cancers. Many recent studies of Wnt signaling have provided mechanistic insight into these dual roles. Here we focus on two areas of rapid advance: (i) the machinery that regulates the stability of the key signal transducer, β-catenin, and (ii) the effect of Wnt signaling on cellular targets outside the nucleus, the actin and microtubule cytoskeletons.

Some of the most profound questions in biology were first asked by ordinary people confronting events in their everyday lives. The miracle of a newborn baby raises questions about how an egg assembles itself into an animal. The tragedy of a cancer diagnosis leads to questions about how normal cells go wrong. Although seemingly distinct, biological processes like embryogenesis and carcinogenesis both rely on cell communication via identical signaling pathways. For example, in the larva of the fruit fly *Drosophila*, cells determine their position within each body segment by communicating with one another. One of the key signaling molecules is the secreted Wnt family protein Wingless (Wg), which acts as a "Be posterior" signal [reviewed in (*1*)]. Fate decisions require changes in gene expression; thus cells must transmit information from the cell surface to the nucleus.

Wnt signaling also regulates cell proliferation in adult tissues. The epithelial cells lining the colon provides an excellent illustration of this [reviewed in (*2*)]. Colonic cells proliferate at a rate that perfectly balances the death of cells due to attrition. Cells are sent signals to proliferate when appropriate, and when sufficient cell numbers are attained, proliferation is halted. Colon cancer, like other cancers, results in part from mutations that cause cells to receive a continuous signal to proliferate. Mutations can lock a signal transduction component in an ON position or can inactivate a protein that normally keeps the pathway in an OFF position. For example, in most colon cancers, a negative regulator of Wnt signal transduction, APC [the tumor suppressor protein encoded by the adenomatous polyposis coli (*APC*) gene], is inactivated, and the Wnt pathway is aberrantly turned on.

In this review we will summarize the current model for Wnt signal transduction and then discuss two particularly active areas of investigation: the critical role of regulated protein destruction in determining where the Wnt pathway is ON or OFF and cytoskeletal targets of the pathway. There are many other aspects of Wnt signaling that we will not cover; for a more comprehensive review see (*1*). Detailed information about Wnt signaling can also be found on the Wnt gene homepage (http://www.stanford.edu/~rnusse/wntwindow.html) and at the connections map at *Science*'s STKE Web site (http://www.stke.org).

A Model for Wnt Signaling

Wnt signaling is regulated by the presence or absence of the intracellular protein β-catenin [Fig. 1; reviewed in (*1*)]. A large multiprotein machine that includes proteins of the APC and Axin families normally facilitates the addition of phosphate groups to β-catenin by glycogen synthase kinase-3β (GSK3β). Phosphorylated β-catenin binds to a protein called βTrCP, and is then modified by the covalent addition of a small protein called ubiquitin. Proteins tagged with ubiquitin are degraded by the proteosome, the cell's protein-recycling center. When Wnt signal is absent, the signal transduction pathway is OFF because β-catenin is rapidly destroyed. When cells are exposed to Wnt, it binds to cell surface receptors of the Frizzled family. Receptor activation antagonizes the APC-Axin "destruction complex" by an unknown mechanism that requires Dishevelled protein. This blocks β-catenin phosphorylation and its subsequent ubiquitination. β-Catenin is thus diverted from the proteosome, and it accumulates and enters the nucleus, where it finds a partner, a DNA binding protein of the TCF/LEF family. Together, they activate new gene expression programs. In the embryonic skin of a fruit fly, Wg signal turns on genes conferring "posterior identity," whereas in human colon cancer cells, inappropriate activation of the Wnt pathway drives cell proliferation by turning on genes encoding oncoproteins and cell-cycle regulators. As discussed below, Wnt signals also regulate the polarity and function of both the actin and microtubule cytoskeletons.

The β-Catenin Destruction Complex

The tumor suppressor protein APC is a critical component of the β-catenin destruction machinery. APC is inactivated in most colorectal cancers, resulting in activation of the Wnt pathway. The Wnt pathway also plays a role in other cancers: for example, viral activation of *Wnt-1* causes mammary tumors in mice, and mutations that make β-catenin refractory to destruction are found in a variety of human tumors [reviewed in (*2*)]. Elucidation of the β-catenin destruction machinery may reveal how Wnt signaling regulates embryogenesis, and may uncover new oncogenes and tumor suppressors. Many proteins that work with APC in regulating β-catenin have been identified, including Axin, Dishevelled, GSK3β, β-TrCP, casein kinases 1ε and II, protein phosphatase-2A, and FRAT (frequently rearranged in advanced T cell lymphomas). These proteins all interact functionally or physically, but their specific roles in β-catenin regulation remain to be resolved.

A second key component of the destruction machinery is Axin [reviewed in (*2*)]. Mice lacking functional Axin have defects in development of the dorsal-ventral body axis similar to those elicited by Wnt overexpression in embryos of the frog, *Xenopus laevis*. In both *Xenopus* and mammalian cells, Axin and its paralog Conductin/Axil destabilize β-catenin, thus negatively regulating Wnt signaling (*3*–*6*). Axin and APC physically interact. The binding site in Axin is in its RGS (regulator of G protein signaling) domain. The RGS domain binds to three copies of a short protein motif in APC known as the SAMP repeat (*4*, *6*). The truncated APC proteins found in most colon cancers lack these SAMP repeats and thus cannot bind Axin.

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Likewise, APC protein fragments that destabilize β-catenin in cultured cells contain both β-catenin and Axin binding sites [reviewed in (*2*)]. It has been hypothesized that the truncation of APC observed in cancer may be driven by selection against Axin's ability to bind APC. Consistent with this idea, mice carrying truncating mutations in *APC* that remove the Axin binding sites develop colon tumors at a high frequency [reviewed in (*2*)], whereas mice carrying truncating *APC* mutations that retain an Axin binding site do not develop tumors (*7*).

Why is the physical interaction between APC and Axin so critical to β-catenin regulation? This issue is far from clear, as conflicting data have emerged from studies of different animal models. In *Xenopus*, Axin mutants lacking the RGS domain not only fail to inhibit Wnt signaling but they act positively, promoting dorsal axis duplication. This is consistent with an obligate role of APC binding in Axin-mediated destruction of β-catenin [reviewed in (*2*)]. In contrast, mammalian Axin mutants lacking the RGS domain still destabilize β-catenin in cultured cells (*4*–*6*, *8*, *9*). Similarly, *Drosophila* Axin lacking the RGS domain inhibits Wg signaling when ectopically expressed (*10*). One interpretation is that APC stimulates or localizes Axin by binding to its RGS domain; overexpression of Axin mutants lacking this domain might overcome the requirement for APC. The differences observed in *Xenopus* could relate to relative expression levels. Consistent with the hypothesis that APC family members localize the destruction complex, a mutation in *Drosophila* APC2 that disrupts its cortical localization also disrupts its function (*11*). Whereas most data suggest that APC negatively regulates Wnt signaling, experiments with *Xenopus* and the nematode *Caenorhabditis elegans* suggest that APC may also play an unexpected positive role in Wnt signaling [reviewed in (*2*)].

The APC-Axin complex is thought to modulate activity of the serine/threonine kinase GSK3β. Mutation of the GSK3β homolog in *Drosophila* and expression of dominant-negative GSK3β in *Xenopus* activate Wnt signaling [reviewed in (*1*)], suggesting that Wnt signaling inhibits GSK3β (Fig. 1). One substrate of GSK3β is β-catenin. The GSK3β phosphorylation sites of β-catenin are mutated in tumors; these mutations stabilize β-catenin [reviewed in (*2*)]. Although β-catenin is a poor GSK3β substrate in vitro, β-catenin phosphorylation is significantly enhanced when a fragment of Axin containing β-catenin and GSK3β binding sites is included in the reaction (*3*, *4*, *12*). Thus, Axin may serve as a scaffold on which both GSK3β and β-catenin reside, facilitating their interaction (Fig. 2). Both APC [reviewed in (*2*)] and Axin (*12*) are also GSK3β substrates and their ability to bind β-catenin in vitro is enhanced by phosphorylation. Wnt signaling in mammalian cells leads to Axin dephosphorylation, as does incubation of cells with LiCl, a GSK3β inhibitor (*12*, *13*). The mechanism by which Wnt signaling "turns off" GSK3β remains unclear. In fruit flies, at least, Dishevelled is critical for this. Recent demonstrations that Dishevelled is part of the "destruction complex" (*8*, *9*, *14*) and binds directly to Axin, may provide the first mechanistic clue. Other proteins such as FRAT, which binds GSK3β and Dishevelled and which antagonizes GSK3β function when ectopically expressed (*14*), may also play a role.

Phosphorylation by GSK3β is critical to β-catenin destruction. Other proteins are targeted for ubiquitination—and thus destruction—by phosphorylation. Sites for this phosphorylation have been mapped in some, such as the nuclear factor kappa B (NF-κB) regulator IκB. IκB and β-catenin share sequence similarity within the region required in both proteins for destruction. This is reminiscent of the situation in yeast, where the stability of certain proteins is regulated by E3 ubiquitin ligases that only recognize their substrates when phosphorylated. F-box proteins act as the recognition subunits. Mutation of the *Drosophila* F-box protein Slimb stabilizes the fruit fly β-catenin homolog (*15*), leading to speculation that Slimb and its human homolog β-TrCP form part of an F-box–containing ubiquitin ligase targeting phosphorylated β-catenin. This hypothesis has now been experimentally verified (*16*–*18*). β-Catenin binds β-TrCP only when phosphorylated by GSK3β, and oncogenic β-catenin mutants that lack GSK3β phosphorylation sites do not bind β-TrCP. Further, overexpression of a β-TrCP derivative lacking the F-box activates β-catenin–dependent gene expression. Studies with *Xenopus* embryos are also consistent with a role for β-TrCP as a negative regulator of β-catenin signaling (*19*). Thus β-TrCP is the penultimate stop on β-catenin's trip to the proteosome. These observations explain how oncogenic mutants of β-catenin avoid the proteosome and continue to signal.

Although this may seem complex enough, other protein players continue to be identified. Where there are kinases, there must be phosphatases. Wnt signal triggers Axin dephosphorylation, thus reducing its ability to bind β-catenin and trigger its destruction (*12*). The protein phosphatase PP2A dephosphorylates Axin in vitro, and the PP2A inhibitor okadaic acid blocks Wnt-mediated Axin dephosphorylation in mammalian cells, suggesting that PP2A may regulate β-catenin stability in response to Wnt signaling (*12*).

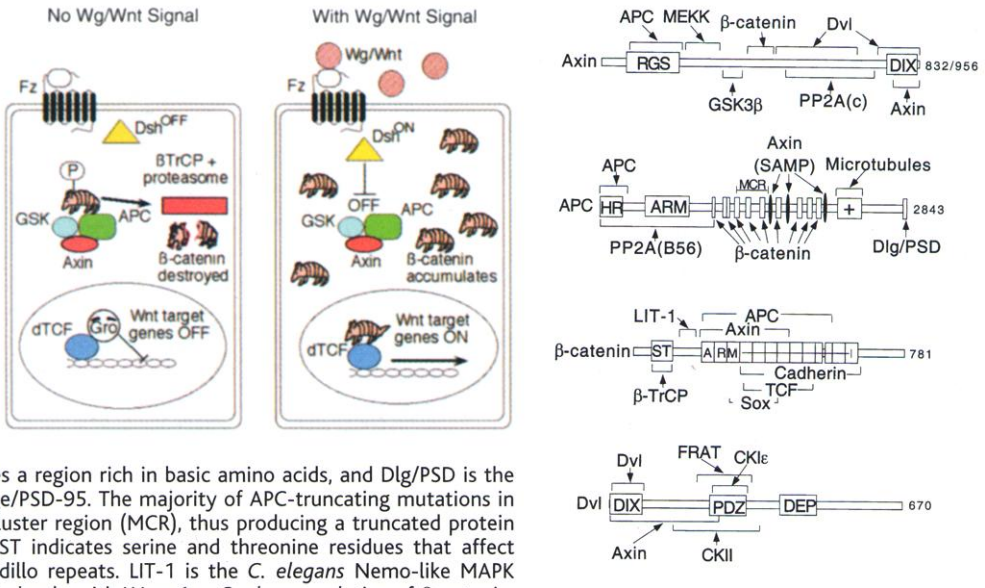

Fig. 1 (left). The Wingless/Wnt signaling pathway. Schematic summary of the response of a cell to Wg signal. See text for details. The icon for β-catenin, an armadillo, is based on the name of its fruit fly homolog. **Fig. 2 (right).** Linear representation of Axin, APC, β-catenin, and Dishevelled (Dvl), with the approximate locations of the binding sites for various protein partners indicated with arrows. The length of each polypeptide in amino acids is shown to the right. Two numbering conventions have been adopted for Axin, as the initiation codon has not been identified. In Axin, the DIX domain is a domain shared with Dishevelled. In APC, HR indicates heptad repeat, ARM indicates seven Armadillo repeats (a motif first found in the fruit fly β-catenin homolog Armadillo), + indicates a region rich in basic amino acids, and Dlg/PSD is the binding site for the PDZ protein Discs large/PSD-95. The majority of APC-truncating mutations in colon cancer occur within the mutation cluster region (MCR), thus producing a truncated protein lacking Axin binding sites. In β-catenin, ST indicates serine and threonine residues that affect protein stability; ARM indicates 12 Armadillo repeats. LIT-1 is the *C. elegans* Nemo-like MAPK homolog; its binding has been demonstrated only with Wrm-1, a *C. elegans* relative of β-catenin. Dishevelled has three recognizable domains: a DIX domain shared with Axin, a PDZ domain, and a DEP (Dishevelled, EGL-10, pleckstrin) domain.

PP2A's catalytic subunit binds directly to Axin; its B56 regulatory subunit interacts with APC (*20, 21*). Overexpression of the B56 subunit downregulates β-catenin protein levels and Wnt signaling (*21*). Finally, the Aβ regulatory subunit of PP2A is mutated in a subset of primary colon tumors (*22*). It remains problematic that PP2A's effect on Wnt-triggered Axin dephosphorylation is consistent with a positive role in regulating the Wnt pathway, whereas the downregulation of β-catenin by the B56 subunit is consistent with a negative role.

More recently, another serine/threonine kinase, casein kinase 1ε (CK1ε), entered the fray. CK1ε can stabilize β-catenin and thus activate Wnt-target genes, and dominant negative forms of CK1ε inhibit Wnt signaling (*23, 24*). CK1ε has been placed downstream of Dishevelled and upstream of GSK3β, although biochemical analysis suggests that it binds directly to Dishevelled's PDZ region (Fig. 2). Dishevelled is phosphorylated when coexpressed with CK1ε, implicating it as a possible target. Thus, CK1ε has the hallmarks of a Wnt signaling molecule and may represent a missing link between the Wnt receptor and β-catenin stabilization. Axin also binds a third serine/threonine kinase, the MAP kinase kinase MEKK1 [Fig. 2 (*25*)].

The APC-Axin Complex—Some Assembly Required

When considering the numerous interactions reported to occur within the APC-Axin complex (Fig. 2), one is confronted with many potential models for complex assembly and mechanisms of action. APC binds directly to itself, β-catenin [reviewed in (*2*)], Axin (*4, 6, 18, 26*), and the PP2A B56 subunit (*21*), whereas Axin binds directly to itself (*5, 8, 20*), APC, β-catenin, GSK3β (*3, 4, 6, 27*), the PP2A catalytic subunit (*20*), and Dishevelled (*8, 9, 14*). In turn, Dishevelled binds to itself (*8*), Axin, CK1ε (*23, 24*), and FRAT (*14*). Moreover, β-catenin can bind β-TrCP while associated with the APC-Axin complex (*16*). Given this complexity, it is clear that linear models for signaling are not appropriate.

How might things work? The binding of APC to the Axin-GSK3β complex could promote APC phosphorylation and thus increase its affinity for β-catenin. This might allow proper presentation of β-catenin to GSK3β, resulting in its efficient phosphorylation and subsequent binding to β-TrCP. Upon activation by Wnt, CK1ε might phosphorylate Dishevelled, resulting in the displacement of FRAT from Dishevelled, thereby positioning FRAT to inhibit the GSK3β that is associated with Axin. These models are consistent with the literature, but so are many others. There are also unsolved mysteries: for example, what is the link between the destruction machinery and Wnt receptors? Perhaps we are still missing a G protein. After all, Frizzled proteins distantly resemble G protein–coupled receptors, Axin harbors an RGS domain, and Dishevelled contains a DEP (Dishevelled, EGL-10, pleckstrin) domain, which is also present in certain RGS proteins. However, researchers looking for a G protein connection to Wnt-1 signaling have so far come up empty-handed, though Wnt-5A may utilize one. A related mystery is the intracellular localization of the destruction complex. Is it assembled adjacent to the Frizzled receptor? Finally, thus far only APC and β-catenin have been found mutated in primary human tumors. Mutations in other destruction complex components may also be uncovered in cancer.

All Roads Do Not Lead to the Nucleus

Extracellular signals clearly affect gene expression, and they also induce changes in cell shape, in cell-cell interactions, and in cell migration. Many of these effects are mediated by the cytoskeleton. Wnt signals affect both the actin and microtubule cytoskeletons. The effect on the actin cytoskeleton occurs in epithelial cells, which possess two sorts of cell polarity. Apical-basal polarity distinguishes the top and bottom surfaces of the cell sheet; planar polarity allows cells to determine directions in the plane of the sheet, providing compass coordinates as on a map. Wnt signaling regulates planar polarity (Fig. 3A) [reviewed in (*28*)]. For example, the hexagonal cells of a fruit fly wing each secrete a single hair, an actin-filled plasma membrane projection. All hairs extend distally from the distal cell vertex. Certain mutations disrupt planar polarity, so that cells polymerize actin at random locations, thereby randomizing the position and direction of wing hairs. Two planar polarity genes encode the most upstream known components of the Wg pathway: Frizzled, a Wg receptor, and Dishevelled, which acts just downstream.

An alternative signal pathway appears to mediate the effects of Wnts on planar polarity (Fig. 3C) [reviewed in (*29*)]. Genetic epistasis experiments suggest that the small guanosine triphosphatase Rho, and a kinase cascade including Misshapen (a Ste20 homolog), JNKK, and JNK (the JNK pathway) act downstream of Frizzled in planar polarity. Furthermore, Dishevelled can activate JNK (*30, 31*), suggesting that there is a branchpoint at Dishevelled, with one branch leading to the canonical Wg pathway and regulating cell fate, and the other leading to the JNK pathway and regulating planar polarity. The discovery that Axin overexpression stimulates JNK signaling (*25*) suggests that Dishevelled may work with Axin in this process. Loss-of-function mutations in JNKK and JNK do not affect planar polarity directly (*30*), suggesting that other MAPK proteins play a redundant role in the process. Candidate genes have been identified in *C. elegans*: one encodes a MAPKKK related to vertebrate TAK1, and the other encodes a divergent MAPK family member related to *Drosophila* Nemo (*32–35*). These kinases are required for Wnt signaling but are postulated to act in a pathway parallel to the traditional Wnt pathway. TAK1 and Nemo thus could be part of an alternative kinase pathway that operates in planar polarity.

In the simplest model, Frizzled receptors could activate the canonical Wnt pathway in some tissues and the JNK pathway in others. This could occur if different Frizzled family receptors used specific signal transduction pathways. However, *Drosophila* Frizzled, originally implicated in planar polarity, acts upstream of Armadillo, the β-catenin homolog, in the embryonic epidermis and central nervous system (*36–40*), implying that individual receptors can act through either

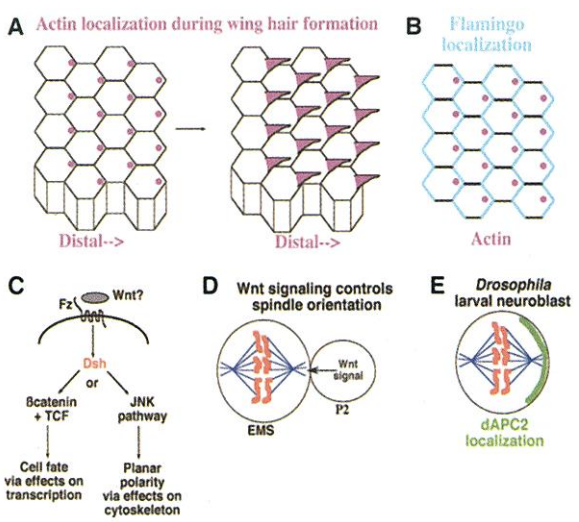

Fig. 3. Wingless/Wnt signaling directly regulates the cytoskeleton. (**A**) In tissue polarity, Wnt signaling directs polymerization of the actin cytoskeleton at particular positions on the cell cortex with respect to the animal's body axes. (**B**) Flamingo protein is enriched on particular sides of the cell and thus may mark the side of the cell where actin polymerization should be directed. (**C**) Model in which Dishevelled acts as a branchpoint, with different signal transduction pathways activated to modulate cell fate choices or to regulate planar polarity. (**D**) Wnt signaling directs the positioning of the cell's mitotic spindle in both early *C. elegans* embryos (this example) and *Drosophila* sensory cells. (**E**) dAPC2 localizes to a crescent in larval neuroblasts undergoing an asymmetric cell division. This crescent localizes with one pole of the spindle.

pathway. One remaining question is which Wnt, if any, is involved in planar polarity.

Other fruit fly proteins required for planar polarity are not obviously connected to Wg signaling. Most encode novel proteins with recognizable motifs but without homologs of known function: for example, Strabismus and Fuzzy are transmembrane proteins [reviewed in (*28*)], and Prickle has multiple Lim domains (*41*). These proteins may be the effector machinery activated by Wnt signaling to alter actin polymerization. The discovery of Flamingo (also known as Starry Night) (*42*–*44*) may reveal new insight. Flamingo's extracellular domain resembles that of the cadherins, a family of cell adhesion molecules, and Flamingo can mediate cell-cell adhesion in culture. COOH-terminal to Flamingo's cadherin repeats is an apparent G protein–coupled receptor domain, distantly resembling Frizzled family receptors. Perhaps most intriguing is Flamingo's subcellular localization. Before the initiation of planar polarity, it is found uniformly around the cell cortex. However, as planar polarity is being established, Flamingo becomes preferentially localized to the faces of the cell where actin polymerization will occur (Fig. 3B). It thus may mark those cell surfaces, distinguishing them from the others. Both genetic and cell biological tests suggest that Flamingo acts downstream of Frizzled. It could couple Wnt signal to cytoskeletal rearrangements, marking a site on the cell cortex where actin should polymerize.

Another possible effector that may couple Wnt signaling to actin rearrangement is the APC relative *Drosophila* APC2 (dAPC2). dAPC2 colocalizes with actin and is found in cell-cell adherens junctions of polarized cells (*11*, *45*). dAPC2 colocalizes with actin during assembly of the embryonic denticle (*11*), an actin-based structure similar to the developing wing hair. Thus dAPC2 is properly positioned to influence actin polymerization. However, a role for it or other APC proteins in planar polarity remains to be tested.

Wnt signaling also affects positioning of the mitotic spindle. Certain cells divide with a fixed orientation relative to the body axes of the animal, requiring that the mitotic spindle be properly oriented. In both *C. elegans* and *Drosophila* this process can be directed by Wnt signaling. During division of an early embryonic cell in *C. elegans* called EMS (Fig. 3D) [reviewed in (*46*)], the mitotic spindle is oriented by contact with a neighboring cell, P2, such that one pole is directed toward the signaling cell. The polarizing signal is thought to be a Wnt molecule acting via a Frizzled receptor. Wnt signaling directly affects the cytoskeleton (*47*): inhibition of transcription does not block spindle positioning, indicating that this effect does not require activation of Wnt target genes. In *C. elegans*, Frizzled and GSK3β homologs are essential for spindle orientation (*47*), whereas in fruit flies Frizzled and Dishevelled are essential (*48*). In contrast, double-stranded RNA inhibition of the *C. elegans* β-catenin relative Wrm-1 or of the distant APC relative APR-1 does not block spindle orientation, nor do mutations in the Wnt *mom-2* or the TCF relative *pop-1*. One interpretation of these data is that there is a branch in the pathway at GSK3β, with one pathway affecting gene regulation and the other influencing spindle orientation. However, differences in the genetic circuitry and divergence in the sequences of Wrm-1 and Apr-1 relative to their homologs suggest that the nematode pathway may be mechanistically different than that in mammals or fruit flies, so this may not apply to other animals. Regardless, because Dishevelled, GSK3β, and APC can all reside in the same protein complex (see above), output from this complex may affect signaling to both the nucleus and to the cytoskeleton.

The mechanism by which Wnt regulates spindle positioning remains to be determined. One possibility is that APC plays a role: mammalian APC binds and bundles microtubules in vitro, and in migrating cultured cells it localizes to spots at the plasma membrane where bundles of microtubules terminate [reviewed in (*49*)]. Further, in dividing stem cells of the *Drosophila* brain, dAPC2 localizes asymmetrically within the cell to a crescent adjacent to one spindle pole (*11*) (Fig. 3E). The functional significance of this localization remains to be tested. The cadherin-relative Flamingo is also required for spindle orientation in neural precursors (*43*), suggesting that it mediates the effects of Wnt signaling on both the actin and the microtubule cytoskeletons.

In summary, investigation of the Wnt signaling pathway has revealed insights into both embryogenesis and oncogenesis. Many challenges remain. We now have an outline of how this critical pathway operates, but fleshing out the precise mechanisms involved will occupy us for years to come.

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