Signal transduction through β-catenin and specification of cell fate during embryogenesis

Jeffrey R. Miller and Randall T. Moon

Howard Hughes Medical Institute and Department of Pharmacology, University of Washington School of Medicine, Seattle, Washington 98195-7370 USA

The development and maintenance of multicellular tissues requires that cells interact with their neighbors. These interactions include the adhesion of cells to one another, which induces the acquisition of cell polarity and promotes the assembly of cell–cell junctions. The modulation of these junctions is involved in the coordination of cell-shape changes and movements during morphogenesis. In addition, cells interact with their neighbors via cell-surface and secreted signaling molecules that may, among other things, specify responding cells to assume distinct developmental fates. The theoretical synthesis of cell-junction function and signal transduction has increasingly focused on the product of the *Drosophila* segment polarity gene *armadillo*, and its vertebrate homolog β-catenin, which are necessary for both cadherin-mediated cell–cell adhesion and for the transduction of signals involved in specifying cell fate (Gumbiner and McCrea 1993; Heasman et al. 1994; Gumbiner 1995; Haegel et al. 1995; Klymkowsky and Parr 1995; Peifer 1995).

The roles of β-catenin in both cell–cell adhesion and in signal transduction in embryos involve the association of β-catenin with different protein partners in distinct subcellular compartments (Fig. 1). Consistent with these observations, endogenous Armadillo/β-catenin is present at the plasma membrane, in the cytoplasm, and in the nucleus (Riggleman et al. 1990; Fagotto and Gumbiner 1994; Peifer et al. 1994c; Schneider et al. 1996; Yost et al. 1996). This review of β-catenin emphasizes the different protein partners with which it interacts, the likely functions of β-catenin in distinct subcellular compartments, and how dynamic regulatory processes, such as the responses to the Wnt-1 signal transduction pathway, may lead to β-catenin entering the nucleus and participating in regulating gene expression and cell fate.

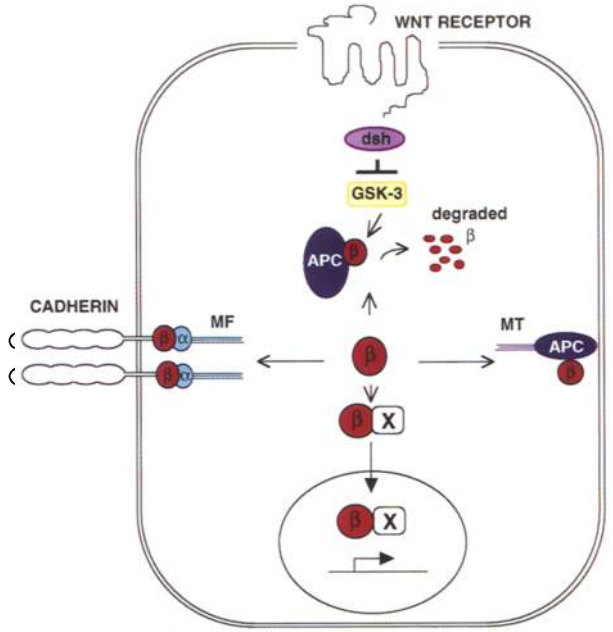

Figure 1. Relationships between the interaction of β-catenin with different protein partners, the intracellular localization of β-catenin and the function of β-catenin in cell adhesion, motility, and signaling. Cytoplasmic β-catenin (β) can interact with membrane-associated cadherin, α-catenin (α), and the adenomatous polyposis coli tumor suppressor protein (APC). In the cytoplasm β-catenin may interact with both glycogen synthase kinase-3 (GSK-3) and APC to regulate its stability, as well as downstream effectors of Wnt-1 signaling (X), resulting in translocation to the nucleus. As shown in greater detail in Fig. 3, the accumulation of β-catenin in the cytosol and nucleus is regulated by Wnt-1 signaling, which increases the steady-state levels of β-catenin. (MF) Microfilaments; (MT) microtubules; (dsh) dishevelled.

Adhesion roles of β-catenin at the plasma membrane

Cell–cell junctions are specialized macromolecular structures that are essential for both intercellular adhesion and communication (Woods and Bryant 1993; Kirkpatrick and Peifer 1995; Gumbiner 1996). Several distinct types of junctions are present in vertebrate cells: tight junctions, adherens junctions, desmosomes, and gap junctions, each possessing a different array of protein components with unique functional characteristics. Tight junctions form seals that prevent solutes from

freely passing across epithelial tissues, whereas gap junctions allow for transport of ions and small molecules between cells. Adherens junctions and desmosomes play an adhesive as well as an architectural role in epithelia by providing a link between cell-surface adhesion molecules and the cytoskeleton. In addition to a role in cell adhesion, adherens junctions are also communication centers necessary for transducing signals from neighboring cells and the environment (for review, see Gumbiner 1996). The importance of cell–cell junctions, and especially adherens junctions, in regulating cellular physiology is underscored by the high number of tumor suppressor gene products that are components of these junctions (Tsukita et al. 1991; Woods and Bryant 1993; Kirkpatrick and Peifer 1995).

The adhesive function of adherens junctions is dependent on members of the cadherin superfamily and the cytoplasmic adaptor proteins α-, β-, and γ-catenin/plakoglobin (Fig. 2; for review, see Kemler 1993; Gumbiner 1996). Cadherins are Ca^{2+}-dependent adhesion molecules that bind homotypically to identical cadherin molecules on apposing cells (Takeichi 1995). β-Catenin interacts directly with the cytoplasmic domain of cadherins (Nagafuchi and Takeichi 1989; Ozawa and Kemler 1992; Aberle et al. 1994; Jou et al. 1995) and forms a complex with α-catenin, a cytoplasmic protein similar to vinculin (Aberle et al. 1994; Hinck et al. 1994a; Jou et al. 1995), which links actin filaments to the adherens junction (Hirano et al. 1987; Rimm et al. 1995). γ-Catenin/plakoglobin is structurally related to β-catenin and is likely to play a similar role to that of β-catenin in regulating cadherin adhesive activity in adherens junctions, as well as modulating the activity of desmosomal cadherins (Klymkowsky and Parr 1995; Gumbiner 1996). Another catenin, p120, which was originally identified as a substrate of Src and several receptor tyrosine kinases (Reynolds et al. 1989, 1992; Kanner et al. 1990, 1991; Downing and Reynolds 1991) and a related protein, p100, also interact with the cadherin–β-catenin complex (Reynolds et al. 1994; Aghib and McCrea 1995; Shibamoto et al. 1995; Staddon et al. 1995) and may participate in regulating the adhesive function of cadherins. Similarly, a p120-related protein (plakophilin) is associated with desmosomes (Hatzfeld et al. 1994).

That β-catenin is important for the adhesive functions of adherens junctions is evident from a range of studies. For example, Armadillo/β-catenin is required for adherens junction assembly during oogenesis (Peifer et al. 1993) and normal embryonic development in *Drosophila* (Cox et al. 1996). Second, the disruption of β-catenin genes in mouse embryos results in detachment of ectodermal cells in the embryo, consistent with reduced cell adhesion (Haegel et al. 1995). Moreover, expression of a truncated β-catenin leads to a loss of cell adhesion in human cancer cell lines (Oyama et al. 1994).

In addition to the aforementioned interaction of β-catenin and γ-catenin/plakoglobin with cadherins at the plasma membrane, it has been suggested that discrete regions of the cell cortex may exhibit high levels of the adenomatous polyposis coli (APC) tumor suppressor protein (Nathke et al. 1996), which binds β-catenin (Munemitsu et al. 1995), γ-catenin/plakoglobin (Rubinfeld et al. 1995), and microtubules (Smith et al. 1994; Munemitsu et al. 1994). These interactions with APC may modulate catenin stability (Munemitsu et al. 1995) and, speculatively, reduce the availability of catenins for interaction with cadherins, thereby affecting cell adhesion and migration (Nathke et al. 1996).

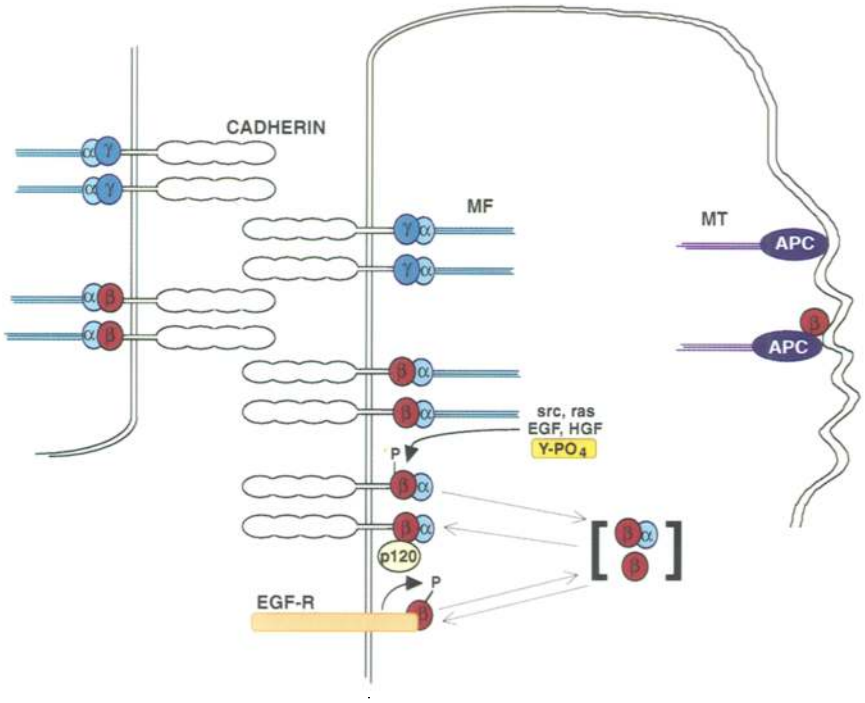

Figure 2. β-Catenin associates with cell-surface proteins involved in cell adhesion, signaling, and migration. Both β-catenin and γ-catenin/plakoglobin bind cadherin and α-catenin, linking transmembrane cadherin cell adhesion molecules to the actin cytoskeleton (MF). Altered tyrosine phosphorylation (Y-PO_4) of β-catenin often coincides with the disassembly of adherens junctions and diminished cell adhesion in response to growth factors and cell transformation. β-Catenin also colocalizes with some membrane-associated APC in migrating cells (ruffled membrane on right) suggesting that localized degradation of β-catenin could play a role in controlling the protrusive behavior of motile cells (Nathke et al. 1996).

Phosphorylation of β-catenin at the plasma membrane

Recent evidence demonstrates the importance of β-catenin–cadherin interactions in modulating cell adhesion, cell migration, and epithelial phenotype in embryonic development and tumorigenesis (Birchmeier et al. 1993; Kirkpatrick and Peifer 1995; Klymkowsky and Parr 1995; Drubin and Nelson 1996). These interactions of β-catenin and cadherin, and consequently cell adhesion, may be regulated in part through phosphorylation of β-catenin (Fig. 2; Matsuyoshi et al. 1992; Behrens et al. 1993; Hamaguchi et al. 1993; Hoschuetzky et al. 1994; Peifer et al. 1994b; Shibamoto et al. 1994; Kinch et al. 1995; Yost et al. 1996). Specifically, transformation of cells via the expression of activated Src and Ras results in tyrosine phosphorylation of β-catenin and a coincident decrease in cell adhesion, disassembly of adherens junctions, and conversion of epithelial cells to a mesenchymal phenotype (Matsuyoshi et al. 1992; Behrens et al. 1993; Hamaguchi et al. 1993; Shibamoto et al. 1994; Kinch et al. 1995; Takeda et al. 1995). Furthermore, β-catenin is also tyrosine phosphorylated in response to secreted signaling molecules including hepatocyte growth factor (HGF) and epidermal growth factor (EGF; Shibamoto et al. 1994; Hoschuetzky et al. 1994). The phosphorylation of β-catenin in response to EGF appears to involve the association of the β-catenin–cadherin complex with the EGF-receptor and the direct phosphorylation of β-catenin by the EGF-receptor upon EGF stimulation (Hoschuetzky et al. 1994).

Although circumstantial evidence suggests that tyrosine phosphorylation of β-catenin plays an important role in modulating cadherin-dependent cell adhesion, the mechanism by which phosphorylation affects the competence of the β-catenin–cadherin complex to promote cell adhesion is unresolved. For example, tyrosine phosphorylation of β-catenin often does not affect β-catenin–cadherin interactions in cultured cells (Matsuyoshi et al. 1992; Behrens et al. 1993; Hamaguchi et al. 1993; Shibamoto et al. 1994). On the other hand, tyrosine phosphorylation of β-catenin in response to *Ras* transformation results in a decrease in β-catenin–cadherin interactions and an accumulation of phosphorylated β-catenin in a detergent extract of cells, which presumably reflects an increase in cytoplasmic β-catenin (Kinch et al. 1995). Similarly, accumulation of tyrosine-phosphorylated β-catenin in the detergent-soluble pool also occurs in response to EGF (Hoschuetzky et al. 1994), suggesting that tyrosine phosphorylation decreases the pool of β-catenin associated with the cytoskeleton—an idea that is consistent with the observed disassembly of cell junctions upon oncogenic transformation (Warren and Nelson 1987; Matsuyoshi et al. 1992; Behrens et al. 1993; Hamaguchi et al. 1993; Shibamoto et al. 1994; Kinch et al. 1995). The movement of β-catenin into a detergent-soluble pool may involve interactions with additional factors such as p120 which, upon *Ras* transformation, becomes tyrosine phosphorylated and displays increased interaction with the catenin–cadherin complex (Kinch et al. 1995). Thus, although a number of studies demonstrate a correlation between altered tyrosine phosphorylation of β-catenin and the regulation of cell adhesion, further studies are necessary to understand this relationship. Finally, phosphorylation of Armadillo/β-catenin at the plasma membrane also occurs at non-tyrosine residues in *Drosophila* embryos. Specifically, membrane-associated Armadillo is more highly phosphorylated on both Ser/Thr and on Tyr residues than soluble Armadillo (Peifer et al. 1994b), though it is somewhat unclear which kinases are responsible for this phosphorylation.

From membrane to cytoplasm: involvement of β-catenin in the wingless/Wnt-1 signaling pathway

A role for β-catenin in signal transduction was suggested by the finding that β-catenin, as well as γ-catenin/plakoglobin, shares a high degree of sequence identity with the product of *Drosophila* segment polarity gene *armadillo* (Peifer and Wieschaus 1990; McCrea et al. 1991). Segment polarity genes are responsible for establishing appropriate anterior–posterior pattern within each segment of the *Drosophila* embryo (Nüsslein-Volhard and Wieschaus 1980). Mutations in *armadillo* disrupt normal patterning (Wieschaus et al. 1984) and yield a phenotype similar to mutations in the *wingless* gene (Nüsslein-Volhard and Wieschaus 1980), which encodes a secreted glycoprotein homologous to the product of the murine proto-oncogene *Wnt*-1 (for review, see McMahon 1992; Nusse and Varmus 1992). These observations place Armadillo/β-catenin in the wingless/Wnt-1 pathway, as summarized and then discussed in detail below.

Genetic and biochemical studies support the following model for the wingless/Wnt-1 pathway (Fig. 3; Klingensmith and Nusse 1994; Siegfried et al. 1994). In the absence of wingless/Wnt-1 signaling (Fig. 3, left panel), the serine/threonine kinase encoded by *zeste white 3* [zw3, homologous to vertebrate glycogen synthase kinase-3 (GSK-3); Bourouis et al. 1990; Siegfried et al. 1992, 1994; Dominguez et al. 1995; He et al. 1995; Pierce and Kimelman 1995] is active and promotes the degradation of β-catenin through a ubiquitination and proteosome pathway. The net result is to maintain low levels of free cytoplasmic β-catenin, while cadherin-associated β-catenin is reasonably stable. In response to wingless/Wnt-1 signaling (Fig. 3, right panel) proteins encoded by the *frizzled* gene family act directly or indirectly as receptors (Bhanot et al. 1996; Yang-Snyder et al. 1996) to promote activation of the novel cytoplasmic phosphoprotein dishevelled (dsh) (Klingensmith et al. 1994; Theisen et al. 1994; Sokol et al. 1995). Through unknown mechanisms, dsh then antagonizes the function of zw3/GSK-3, as well as Notch (Axelrod et al. 1996). Because zw3/GSK-3 had been repressing armadillo/β-catenin function, the inhibition of this kinase in response to the wingless/Wnt-1 signal leads to activation of Armadillo/β-catenin function (Peifer et al. 1994b,c; Siegfried et al. 1994; van Leeuwen et al. 1994; Yost et al. 1996). Biochemically, this activation of β-catenin entails a suppression of its degradation, its accumulation to greater steady-state levels, its interaction with HMG-box tran-

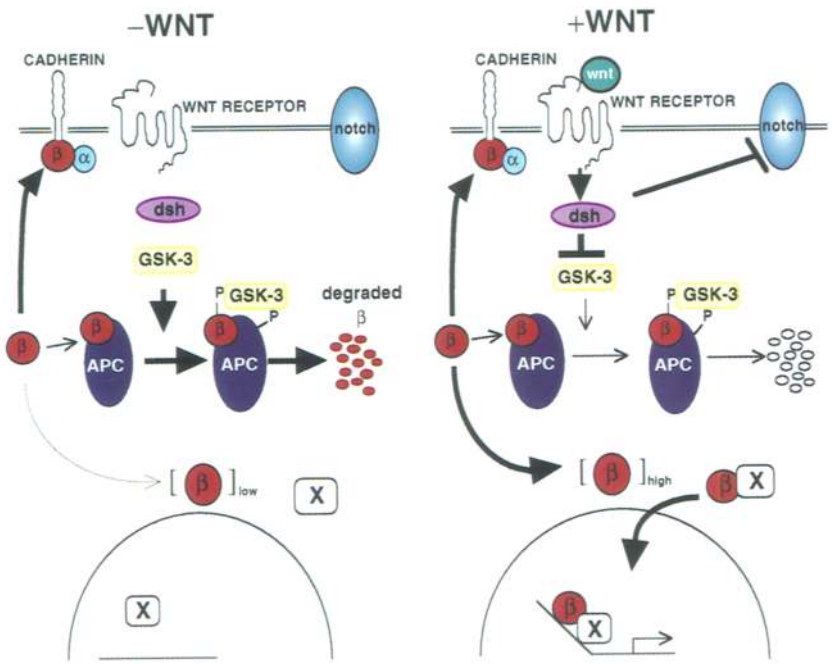

Figure 3. Mechanism for regulating the steady-state levels and intracellular distribution of β-catenin by components of the Wnt-1 signaling pathway (see text for details). In the absence of Wnt-1 signaling (*left*), GSK-3 is active and directly phosphorylates β-catenin and APC (denoted by large downward arrow), leading to the rapid degradation of β-catenin, likely through a ubiquitination and proteosome pathway. Thus, GSK-3 is a repressor of β-catenin activity. Low levels of free, cytosolic β-catenin reduce the extent of interaction with downstream effectors of Wnt-1 signaling (X). In the presence of a Wnt-1 signal (*right*), GSK-3 function is repressed in response to activation of the phosphoprotein dishevelled (dsh), decreasing the phosphorylation of β-catenin and APC (small downward arrow). This results in an increase in the half-life of β-catenin/APC complexes and a coincident decrease in the degradation of β-catenin, promoting increases in cytoplasmic β-catenin. Notch is also antagonized by dsh (Axelrod et al. 1996) though not considered in detail here. Accumulation of β-catenin in the cytosol promotes interactions with downstream effectors (X), the translocation of β-catenin to the nucleus and changes in gene expression. X includes, but may not be restricted to, the HMG box transcription factors LEF-1 and XTcf-3. The transcription factor/β-catenin complex forms ternary complexes with DNA that alter DNA bending and transcription (Behrens et al. 1996; Molenaar et al. 1996).

scription factors, and its translocation to the nucleus, leading to changes in gene expression (Fig. 3, right, and discussed in detail below).

Contribution of cadherins to the regulation of the signaling activity of β-catenin

As discussed previously, β-catenin is found at the plasma membrane, in the cytoplasm, and in the nucleus, and it is involved in transducing the wingless/Wnt-1 signal, raising the obvious question of which subcellular pool of β-catenin is required for transmitting the Wnt-1 signal. Focusing on the plasma membrane, analyses of mutant forms of β-catenin in both *Drosophila* and *Xenopus* have demonstrated that cadherin binding and β-catenin signaling activity are separable (Cox et al. 1996; Fagotto et al. 1996; Orsulic and Peifer 1996), with the signaling activity in *Xenopus* defined as the ability of β-catenin to induce a secondary embryonic axis like Wnt-1. However, cadherin can modulate the pools of β-catenin available for signaling, as ectopic expression of high levels of cadherin in *Xenopus* embryos antagonizes the axis duplicating activity of ectopic β-catenin (Heasman et al. 1994; Fagotto et al. 1996; Torres et al. 1996), and levels of cadherin expression also affect *armadillo* signaling activity in *Drosophila* (Cox et al. 1996). Although it is possible that cadherin-associated β-catenin serves as a pool that can engage in Wnt-1 signaling, it seems unlikely that pre-existing membrane-associated β-catenin exists in a simple equilibrium with cytoplasmic and nuclear pools. Supporting this statement, the targeted depletion of β-catenin transcripts in *Xenopus* oocytes and eggs blocks both the formation of the embryonic axes and responsiveness to ectopic *Xwnt-8*, despite the presence of a residual pool of maternal β-catenin protein (Heasman et al. 1994). Therefore, the connection between β-catenin–cadherin interactions and Wnt-1 signaling requires further study of whether β-catenin readily exchanges between cadherin-bound and free cytoplasmic pools, and which subcellular pool of β-catenin is primarily involved in mediating responses to Wnt-1 signals. Nevertheless, present data support the conclusion that increasing the level of β-catenin at the plasma membrane, in association with cadherins, does not mimic or stimulate Wnt-1 signaling, and likely antagonizes Wnt-1 signaling by reducing levels of β-catenin that needs to be free of its cadherin association to participate in Wnt-1 signaling.

In considering whether β-catenin participates in Wnt-1 signaling at the plasma membrane, it is noteworthy that zw3, a component of the wingless pathway, does not appear to play a crucial role in the function of Armadillo at the adherens junction. Specifically, homozygous mutant *armadillo* germ cells display severe adhesion defects, whereas *zw3* mutant germ cells undergo normal development (Peifer et al. 1993). In addition, although *zw3* mutants have dramatic patterning defects, neither intercellular adhesion nor epithelial polarity appears to be affected (Perrimon and Smouse 1989; Simpson and Cateret 1989; Peifer et al. 1994b). Thus, while only stable Ser/Thr phosphorylated Armadillo/β-catenin is present in adherens junctions (Peifer et al. 1994b), the evidence is consistent with the idea that zw3/GSK-3

does not play a major role in regulating the levels or function of Armadillo/β-catenin associated with the plasma membrane. Taken with the data on cadherins antagonizing embryonic responses to β-catenin, it is reasonable to conclude that cadherin-free Armadillo/β-catenin participates in wingless/Wnt-1-mediated changes in gene expression and cell fate. However, cellular responses to functionally distinct Wnts do involve distinct effects on cell adhesion (Bradley et al. 1993; Hinck et al. 1994b; Torres et al. 1996), thus it is possible that cadherin-associated β-catenin is involved in cell adhesive changes, whereas, as described below, cytoplasmic and nuclear β-catenin are involved in transducing the Wnt signal to the nucleus.

Interaction of β-catenin with GSK-3

On the basis of the above considerations, the involvement of β-catenin in Wnt-1 signaling may depend on post-translational modifications and its interactions with protein partners localized in the cytoplasm and/or nucleus. Post-translational modifications include phosphorylation of β-catenin, which correlates with both its stability and its signaling activity (Peifer et al. 1994b; Yost et al. 1996). With regard to the Wnt-1 pathway (Fig. 3), GSK-3 phosphorylates β-catenin primarily, though not exclusively, at a conserved amino-terminal site in vitro, and this site is required for maximal phosphorylation of β-catenin in vivo (Yost et al. 1996). Elimination of this site by either truncation or by amino-acid substitution significantly increases the stability of β-catenin in *Xenopus* embryos (Yost et al. 1996) and in cultured cells (Munemitsu et al. 1996). This increased stability leads to greater signaling activity, as monitored by its increased ability to duplicate the embryonic axes in *Xenopus* embryos (Yost et al. 1996). Furthermore, inhibition of GSK-3 by overexpression of a kinase-inactive form of GSK-3 results in a decrease in the phosphorylation of β-catenin and an increase in its stability in vivo, resulting in elevated levels of nuclear β-catenin (Yost et al. 1996). An unresolved issue is why mammalian GSK-3 does not phosphorylate β-catenin in vitro (Rubinfeld et al. 1996), though further work in vivo may address this issue. However, both in vitro and in vivo data are consistent with the idea that, in the absence of a Wnt-1 signal, GSK-3 targets β-catenin for a degradative pathway. Signaling by Wnt-1 inactivates GSK-3 [likely via the phosphoprotein dsh (Klingensmith et al. 1994)], and thus stabilizes β-catenin and increases its availability for signaling (Fig. 3, right panel). Though not as intensively studied, in some instances Wnt-1 signaling also increases steady-state levels of γ-catenin/plakoglobin (Bradley et al. 1993).

Interaction of β-catenin with APC

As discussed above, the signaling activity of β-catenin correlates with an increase in its stability. Recent biochemical studies demonstrate that the tumor-suppressor protein APC plays a role in regulating intracellular levels of β-catenin (Munemitsu et al. 1995), thus implicating APC as a modulator of β-catenin signaling (Figs. 1 and 3). In considering the potential roles of APC in modulating β-catenin function, it is important to consider the subcellular localization and levels of APC in cells. In a recent study of MDCK epithelial cells, Nathke et al. (1996) showed that although α- and β-catenin coimmunoprecipitate with APC, much of the APC does not colocalize with β-catenin in cells and intriguingly is clustered around microtubule ends in protruding membranes, consistent with reports of APC binding microtubules (Fig. 2; Smith et al. 1994; Munemitsu et al. 1994).

How does APC function to regulate intracellular levels of β-catenin? APC interacts physically with β-catenin through two distinct sets of binding sites (Rubinfeld et al. 1993, 1995; Su et al. 1993) that differentially affect the steady-state levels of β-catenin in the cell (Munemitsu et al. 1995). Colon cancer cells expressing mutant APC lacking one set of these sites possess an abnormally large pool of free, cytosolic β-catenin that is eliminated by the addition of full-length APC (Munemitsu et al. 1995). These results suggest that APC functions to maintain low levels of cytoplasmic β-catenin in the cell, thereby suppressing its signaling activity. Recent studies extend this idea by demonstrating that APC-dependent degradation of β-catenin can be regulated by Wnt-1 signaling. Papkoff and coworkers (Papkoff et al. 1996) show that the expression of Wnt-1 in cells stabilizes APC–β-catenin complexes and results in an increase in the steady-state levels of free β-catenin. Further, Rubinfeld and colleagues (Rubinfeld et al. 1996) demonstrate that GSK-3 directly interacts with APC–β-catenin complexes and can phosphorylate APC in vitro, regulating its interactions with β-catenin. Together, these data are consistent with a model in which APC and GSK-3 function to antagonize Wnt-1 signaling by eliminating free, cytoplasmic β-catenin (Fig. 3). However, available data do not fully resolve whether GSK-3 can regulate β-catenin stability by phosphorylating it directly (Yost et al. 1996) or whether GSK-3 phosphorylation of APC is equally or primarily responsible for regulating β-catenin stability (Rubinfeld et al. 1996).

Potential involvement of β-catenin in other signaling pathways

As described above, Wnt-1 signaling can increase steady-state levels of both β-catenin and γ-catenin/plakoglobin, and increased levels of either result in their accumulation in the cytoplasm and nucleus. Despite this focus on Wnt-1 signaling it is likely that catenins participate in other signaling pathways, though this has been less studied. As noted above, EGF signaling promotes tyrosine phosphorylation and increases in detergent soluble levels of β-catenin (Hoschuetzky et al. 1994). Moreover, recent data show that dsh and zw3, upstream of armadillo/β-catenin in wingless signaling, are also involved in Notch signaling (Ruel et al. 1993; Axelrod et al. 1996). Lastly, β-catenin stability can increase in response to

retinoids, though this may be an indirect effect (Byers et al. 1996).

From cytoplasm to nucleus: does interaction of nuclear β-catenin with HMG box transcription factors link the wingless/Wnt-1 signal to changes in gene expression?

In *Xenopus* embryos, inhibition of GSK-3, which mimics the Wnt-1 signal, increases steady-state levels of endogenous β-catenin, which then accumulates in nuclei (Yost et al. 1996). Similarly, raising steady-state levels of β-catenin (Funayama et al. 1995) or γ-catenin/plakoglobin (Karnovsky and Klymkowsky 1995) through transient overexpression leads to elevated levels of nuclear catenins. These results raise the questions of whether β-catenin interacts with specific nuclear proteins and whether such interactions are important for some or all of the role of β-catenin in transducing the Wnt-1 signal in general, or in its demonstrable role in inducing an embryonic axis in *Xenopus* (Heasman et al. 1994) in particular.

Two recent studies directly address the issue of the protein partners of nuclear β-catenin and provide compelling evidence of the mechanisms linking a Wnt signal to changes in chromatin structure and gene expression. Behrens et al. (1996) show that the architectural transcription factor LEF-1, a mammalian HMG box factor, binds directly to β-catenin and translocates β-catenin to the nucleus, where it alters the DNA bending properties of LEF-1 (Fig. 3, factor X on right). A *Xenopus* factor related to LEF-1, XTcf-3, similarly binds to β-catenin and translocates it to the nucleus (Molenaar et al. 1996). Furthermore, β-catenin binding to XTcf-3 is necessary for the transactivation of a reporter gene in cultured cells. Finally, expression in embryos of a mutant form of XTcf-3 blocks the ability of ectopic β-catenin to induce a duplication of the embryonic axes and, when expressed on the dorsal side of the embryo, this mutant transcription factor blocks the formation of the endogenous axis. These two studies support the hypothesis that at least some of the ability of β-catenin to alter gene expression and cell fate can be explained through the direct interaction of β-catenin with an architectural transcription factor. The extent to which Wnt-responsive genes contain functionally important binding sites for such architectural transcription factors is clearly an area of current research.

While the exciting data discussed above suggest that nuclear β-catenin may have functionally important protein partners, it is worth noting that Wnt-1 (Olson et al. 1991) and β-catenin (Guger and Gumbiner 1995) promote rapid increases in gap junctional permeability in cleavage stage *Xenopus* embryos, in the absence of transcription. This strongly suggests that all cell physiological responses to Wnt-1 signaling do not depend on nuclear activities of β-catenin.

Do the roles of β-catenin in adhesion or in Wnt-1 signaling involve distinct functional domains?

Dissecting the mechanism of action of Armadillo/β-catenin in mediating a wingless/Wnt-1 signal will ultimately require an understanding of its functional domains. Armadillo/β-catenin contains three distinct domains (Fig. 4A). The amino-terminal domain contains putative sites for phosphorylation by GSK-3 (Peifer et al.

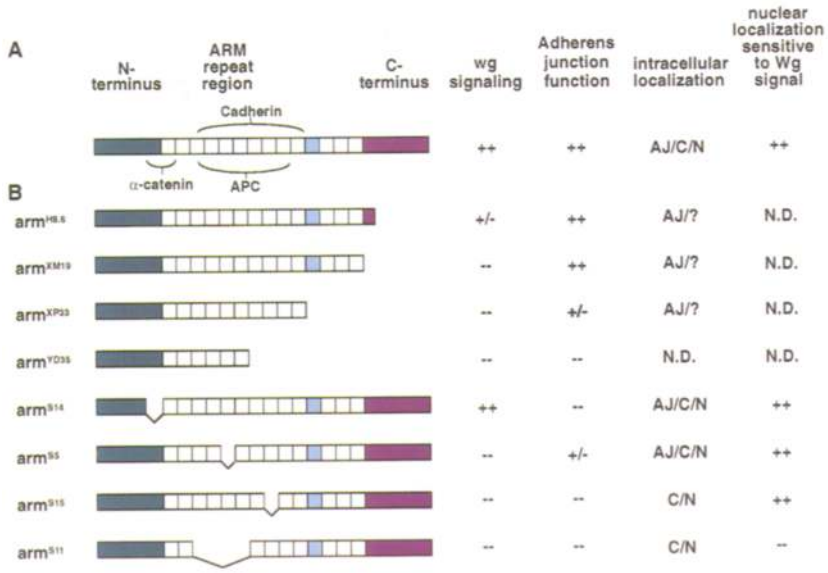

Figure 4. Structure of β-catenin and its relationship to binding of distinct protein partners and function in Wnt-1 signaling. (*A*) Diagram depicting structural features of β-catenin and domains necessary for interactions with α-catenin, cadherin, and APC. Although not shown, LEF-1/XTcf-3 interactions are mediated by the Arm repeat region of β-catenin (Behrens et al. 1996; Molenaar et al. 1996). (*B*) Genetic studies in *Drosophila* have defined domains necessary for the transduction of wingless/Wnt-1 signal and adherens junction function and demonstrate that distinct domains mediate the roles of β-catenin in signal transduction and cell adhesion (Peifer and Wieschaus 1990). Mutant proteins lacking the carboxy-terminal domain are sufficient for adherens junction function but are deficient in wingless signaling. Larger truncations of the Arm repeat domain severely affect both cell adhesion and signaling activity. Expression of specific deletion mutants of Armadillo in *Drosophila* further define regions of the protein essential for adherens-junction function, wingless signal transduction, and intracellular localization (see Orsulic and Peifer 1996, for details). (AJ) Adherens junction; (C) cytoplasm; (N) nucleus; (N.D.) not determined.

1994b; Yost et al. 1996) and is necessary for the binding of α-catenin (Aberle et al. 1994; Hülsken et al. 1994; Oyama et al. 1994; Rubinfeld et al. 1995; Orsulic and Peifer 1996). The central domain is characterized by the presence of 13 imperfect 42-amino-acid repeats, defined as Arm repeats (Peifer et al. 1994a). Arm repeats are found in a number of proteins with diverse intracellular distributions and functions including APC (Groden et al. 1991; Kinzler et al. 1991), p120 (Reynolds et al. 1992), desmosomal band 6/plakophilin 1 (Hatzfeld et al. 1994; Heid et al. 1994), the nuclear pore associated protein SRP1/RCH1 (Cuomo et al. 1994; Yano et al. 1994), and smgGDS (Kikuchi et al. 1992). Arm repeats may act as sites for protein–protein interactions and function similar to SH2/SH3 domains (for review, see Pawson 1994). In support of this idea, interactions with APC, cadherins, HMG-box transcription factors, and the EGF-receptor are mediated by the Arm repeats of β-catenin (Aberle et al. 1994; Hoschuetzky et al. 1994; Hülsken et al. 1994; Funayama et al. 1995; Rubinfeld et al. 1995; Behrens et al. 1996; Fagotto et al. 1996; Molenaar et al. 1996). The potential function of the carboxy-terminal domain is unclear. Though this domain is essential for *wingless* function in *Drosophila* (Peifer and Wieschaus 1990), it is rather divergent between Armadillo and β-catenin.

Genetic studies in *Drosophila* and overexpression studies in *Xenopus* have defined regions of Armadillo/β-catenin that are necessary for its wingless/Wnt-1 signaling activity (Fig. 4B). Characterized *armadillo* mutations in *Drosophila* result in premature stop codons that produce truncated protein products and the extent of the deletion differentially affects the ability of Armadillo to function in cell adhesion and signal transduction. For example, deletions that result in embryos with a severe segment polarity phenotype are still capable of promoting cell adhesion (Peifer et al. 1993). Recent studies by Orsulic and Peifer (1996) have extended these observations by defining the biochemical, cell biological, and genetic properties of various mutant Armadillo proteins to determine their roles in adherens junction function and wingless signaling (summarized in Fig. 4B). Expression of mutant β-catenins in *Xenopus* embryos demonstrates that Arm repeats 1–9 are sufficient to mimic Wnt signals and induce a secondary embryonic axis (Fagotto et al. 1996). However, one should consider that in *Xenopus* studies, it is likely that ectopic β-catenin may compete with, and thus functionally activate, the endogenous, wild-type β-catenin (J.R. Miller and R.T. Moon, unpubl.), or even γ-catenin. Similarly, overexpression of the central Arm repeats of γ-catenin/plakoglobin induce formation of a secondary axis (Karnovsky and Klymkowsky 1995), with the same cautionary note that ectopic γ-catenin/plakoglobin may displace and thus activate the signaling function of endogenous β-catenin.

From cell biology to embryology: distinct roles for maternal and zygotic β-catenin

The first indication that Armadillo/β-catenin or its homologs may participate in the establishment of cell fate during embryogenesis came from noting that *armadillo* mutants phenocopy *wingless* mutations, and the abundance of intracellular Armadillo within each segment of *Drosophila* embryos is enhanced by wingless signaling (Fig. 5D; Riggleman et al. 1990; Peifer et al. 1991, 1994c). Subsequent studies have revealed a role for β-catenin in modulating cell fate in vertebrates, with many recent studies defining an essential role for β-catenin in specifying the embryonic axes.

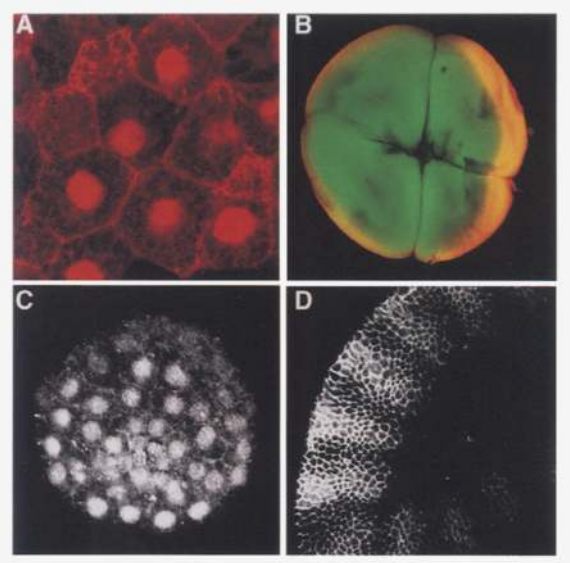

Figure 5. Regional accumulation of β-catenin/Armadillo in the cytoplasm and nucleus predicts the specification of cell fates in vertebrate and invertebrate embryos. (*A*) Confocal micrograph of stage 9 animal cap cells of a *Xenopus* embryo. β-Catenin localizes to several intracellular compartments including the plasma membrane, cytosol, and nucleus (Yost et al. 1996). The accumulation of β-catenin in the cytosol and nucleus in response to Wnt signaling is thought to play a role in the control of gene expression during development. (*B*) Confocal micrograph of a 4-cell-stage *Xenopus* embryo stained with an anti-β-catenin antibody, with β-catenin in orange, and auto-fluorescence in green. β-Catenin accumulates at higher levels in the cytoplasm of dorsal cells (*right*) compared to ventral cells (*left*) and presages the transcription of dorsal-specific genes in early *Xenopus* embryos (C. Larabell and R. Moon, unpubl.). Membrane staining of β-catenin is observed in cleaving *Xenopus* embryos (data not shown), but is not evident under microscopical conditions that show the dorsal–ventral cytoplasmic differences. (*C*) Confocal micrograph of a seventh cleavage stage sea urchin embryo (~108 cells) stained with anti-β-catenin antibody. β-Catenin transiently localizes to nuclei of the micromere and macromere descendants from the fourth to seventh cleavages and predicts the development of endoderm and mesoderm in sea urchin embryos (J. Miller and D. McClay, unpubl.). (*D*) Confocal micrograph of the *Drosophila* embryonic ectoderm at stage 9 stained with an anti-Armadillo antibody (image kindly provided by M. Peifer). Armadillo protein accumulates in the cytoplasm and nucleus in response to *wingless* signal, forming segmentally repeated stripes (Riggleman et al. 1990; Peifer et al. 1994b; Orsulic and Peifer 1996).

Maternal β-catenin is required for establishment of the embryonic axes

In *Xenopus* embryos, β-catenin is maternally expressed at the RNA and protein levels (DeMarais and Moon 1992) and is broadly expressed in many cells of the developing embryo (Fagotto and Gumbiner 1994; Schneider et al. 1996). Similarly, mouse embryos express maternal β-catenin protein, whereas γ-catenin/plakoglobin is expressed later in cleavage-stage embryos (Ohsugi et al. 1996). In a key set of experiments with *Xenopus* embryos, Heasman et al. (1994) showed that depletion of maternal β-catenin RNA blocks formation of the embryonic axis and blocks responses to ectopic Wnt. This indicates that newly synthesized β-catenin is required for axis formation and that the existing pool of β-catenin polypeptides cannot compensate for this loss of de novo synthesis. β-Catenin is also sufficient for the establishment of the dorsal–ventral axis in *Xenopus* (Funayama et al. 1995; Guger and Gumbiner 1995) and in zebrafish (Kelly et al. 1995), working in a cell non-autonomous manner to initiate formation of an ectopic gastrula organizer. It has been suggested that γ-catenin/plakoglobin may also play a role in axis formation (Karnovsky and Klymkowsky 1995); however, loss-of-function experiments showing it to be required remain to be reported.

How might β-catenin function in axis formation? Axis formation in *Xenopus* embryos is known to require a post-fertilization rotation of the cytoplasm relative to the cortex, which activates a transplantable maternal dorsal determining activity in dorso-vegetal blastomeres (for review, see Larabell et al. 1996). Recent observations in *Xenopus* embryos demonstrate that by the 2- to 4-cell stages there are elevated levels of β-catenin on the prospective dorsal side of the embryo, monitored by confocal microscopy (Fig. 5B) and by Western blot analysis (M. Torres and R.T. Moon, unpubl.). By the 16-cell stage, prior to any zygotic transcription, β-catenin accumulates to a greater extent in the nuclei on the dorsal side (C.A. Larabell, M. Torres, B.A. Rowning, J.R. Miller, C. Yost, M. Wu, D. Kimelman, and R.T. Moon, in prep.), and this dorsal bias in nuclear β-catenin persists at the blastula stage (Schneider et al. 1996). These dorso-ventral differences in the distribution of β-catenin are blocked by treatments that prevent the post-fertilization cortical rotation and the subsequent formation of the dorsal determining activity, further linking asymmetries in β-catenin to the establishment of dorsal cell fates in *Xenopus* embryos (Schneider et al. 1996). It is plausible that the observed increase in β-catenin in dorsal nuclei in the early *Xenopus* embryo influences pretranscriptional nuclei such that at the onset of transcription at mid-blastula stage, specific dorsal genes will be expressed. Supporting this claim, localized increases in ectopic and endogenous β-catenin correlate with induction of dorsal-specific transcription factors such as *siamois* and *goosecoid* at the late blastula stage, and these and other transcription factors are then involved in establishing the functions of the gastrula organizer.

This effect on dorsal gene expression likely involves β-catenin interaction with members of the HMG box transcription factors because XTcf-3 (Molenaar et al. 1996) and LEF-1 (Behrens et al. 1996) are involved in binding and translocating β-catenin to the nucleus and because a mutant form of XTcf-3 inhibits formation of the endogenous axis, as well as secondary axes induced by ectopic β-catenin (Molenaar et al. 1996). Thus, in a remarkably short period of time, it has been shown that endogenous β-catenin is required for axis formation and is expressed at the right place and time to play a role in axis formation, and details on how β-catenin functions to specify the axes in concert with architectural transcription factors are being elucidated quickly. Most of this vertebrate work was conducted in *Xenopus* embryos, a system amenable to dissecting complex phenomena with biochemical and cellular approaches, as well as approaches that manipulate protein function.

The nuclear accumulation of endogenous β-catenin in early *Xenopus* embryos is responsive to manipulation of the Wnt-1 signaling cascade (Schneider et al. 1996; Yost et al. 1996), indicating that increased nuclear β-catenin may arise in response to Wnt signaling activity during development. Although there are no loss-of-function data demonstrating that a maternal Wnt ligand is required for the observed dorso-ventral asymmetries in cytoplasmic and nuclear β-catenin, such a requirement remains a formal possibility because maternal Wnts have been reported in *Xenopus* and zebrafish (for review, see Cui et al. 1995; Du et al. 1995; Kelly et al. 1995). An alternative model proposed by Yost et al. (1996) is that dorso-ventral differences in GSK-3 activities may fully or partially arise in response to Wnt-independent mechanisms and thereby regulate the stability of β-catenin. We currently suspect that Wnt ligands, though maternally provided and likely functional, are not required for axis formation because a dominant-negative Wnt ligand blocks formation of ectopic axes induced by some Wnts, but does not interfere with formation of the endogenous dorsal–ventral axis (Hoppler et al. 1996).

Spatial differences in the nuclear accumulation of β-catenin have also been observed in zebrafish embryos (Schneider et al. 1996) and in embryos of the sea urchin *Lytechinus variegatus* (Fig. 5C) in patterns consistent with an involvement for β-catenin in the patterning of the embryonic axes. The localization of β-catenin in nuclei is sensitive to lithium chloride (Schneider et al. 1996), a treatment that mimics embryonic responses to some Wnts (Christian and Moon 1993). Interestingly, the effects of lithium chloride on β-catenin and on development may be attributable to its ability to inhibit GSK-3 (Klein and Melton 1996). The parallels between the spatial patterns of β-catenin distribution in *Xenopus*, zebrafish, and sea urchin embryos is suggestive of the evolutionary conservation of maternally derived signaling cascades required for axial patterning.

Zygotic β-catenin may function as a transducer of Wnt signals in the germ layers to regulate gene expression

There are at least 15 different vertebrate Wnt genes (for review, see McMahon 1992; Nusse and Varmus 1992),

and gene disruption studies in the mouse have rigorously established that some of these Wnts are required at several developmental stages, and in distinct tissues. *Wnt-1* is required for formation of elements of the central nervous system (McMahon and Bradley 1990; Thomas and Capecchi 1990), *Wnt-3a* regulates somite and tailbud formation (Takada et al. 1994), *Wnt-4* is involved in the mesenchyme to epithelial transition within developing kidney (Stark et al. 1994), and *Wnt-7a* is a dorsalizing signal providing polarity in the mouse limb (Parr and McMahon 1995). Loss-of-function through a dominant-negative Wnt ligand has implicated *Xwnt-8* signaling in the expression of *MyoD* and formation of skeletal muscle in *Xenopus* embryos (Hoppler et al. 1996). Thus, Wnts are involved in the formation and patterning of at least mesodermal and ectodermal derivatives. It is possible that β-catenin functions downstream of some or all of these Wnts, but evidence for functionally distinct Wnts (Du et al. 1995; Torres et al. 1996) suggests this cannot be assumed.

The issue of whether β-catenin is an integral component of Wnt signaling in diverse processes of vertebrate embryogenesis, while largely unresolved, has support from studies in *Xenopus*. Ablation of maternal β-catenin transcripts blocks the ability of ectopic *Xwnt*-8 to induce a secondary axis, suggesting that responses to this Wnt requires β-catenin (Heasman et al. 1994). Regarding the potential functions of β-catenin in Wnt signaling in the nervous system, *Xwnt*-3A can function in a combinatorial manner with noggin to regulate neural gene expression along the antero-posterior axis, and β-catenin can substitute fully for *Xwnt*-3A in this respect (McGrew et al. 1995). With the caveat that there are functionally distinct Wnts, which implies functional differences in their signaling pathways (Du et al. 1995; Torres et al. 1996; Yang-Snyder et al. 1996), these studies, coupled with the widespread expression of β-catenin in different embryos (DeMarais and Moon 1992; Fagotto and Gumbiner 1994; Peifer et al. 1994c; Kelly et al. 1995; Ohsugi et al. 1996), strongly suggest that β-catenin is indeed exploited in Wnt signaling throughout embryogenesis.

Conclusion

β-Catenin is a multifunctional protein involved in cell adhesion at adherens junctions and in transduction of receptor-mediated intercellular signals. The importance of the signaling role of β-catenin is underscored by the observations that β-catenin is required for proper formation of the embryonic axes in vertebrates and is sufficient for eliciting changes in gene expression in tissues arising from the mesoderm and ectoderm of embryos. Recent discoveries of how β-catenin levels and subcellular distribution is regulated post-transcriptionally in response to stimulation of receptor mediated signaling pathways, and the various protein partners with which β-catenin interacts in distinct cellular compartments, further demonstrate that complex processes in developmental biology are amenable to dissection and definition in terms of cell biological, biochemical, and genetic interactions. Although much remains to be understood, there has been remarkable progress in identifying the specific mechanisms by which elevated levels of β-catenin lead to changes in gene expression and cell fate during embryogenesis.

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J R Miller and R T Moon

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