β-catenin: a key mediator of Wnt signaling
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β-catenin is a pivotal player in the signaling pathway initiated by Wnt proteins, mediators of several developmental processes. β-catenin activity is controlled by a large number of binding partners that affect the stability and the localization of β-catenin and is thereby able to participate in such varying processes as gene expression and cell adhesion. Activating mutations in β-catenin and in components regulating its stability can contribute to the formation of certain tumors.

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Abbreviations

APC	adenomatous polyposis coli
APR-1	APC-related protein in *C. elegans*
Arm	Armadillo
Dsh	Dishevelled
E-cad	E-cadherin
Fz	Frizzled
GSK	glycogen synthase kinase
mom	more mesoderm
TCF	T-cell factor
Zw3	Zeste-white 3
Wg	Wingless
WRM-1	worm arm motif protein

Introduction

β-catenin was first isolated as a protein associated with the intracellular domain of E-cadherin, a component of the adherens junction (reviewed in [1]) [2]. The homology between β-catenin and the *Drosophila* segment polarity gene *armadillo* (*arm*) and recent discoveries of a transcriptional role of β-catenin indicated that this protein functions as an unusual signal transducer as well.

β-catenin is an essential component of the Wnt/Wingless (Wg) signaling pathway which is involved in a large variety of developmental processes, including segment polarity in *Drosophila*, axis specification in *Xenopus*, mesoderm induction in *Caenorhabditis elegans*, and the genesis of mammary tumors in mice, to name a few (reviewed in [3–6]). In the past few years, great strides have been made in our understanding of the Wnt signaling pathway with the identification of the *Frizzled* (*Fz*) gene family as a class of Wnt receptors [7•] and the identification of a functional heterodimeric transcriptional complex, consisting of β-catenin and members of the family of transcriptional factors, variably referred to as T-cell factor

(TCF), lymphoid enhancer factor (LEF), or pangolin (for simplicity we will refer to this large family collectively as TCF) [8•–12•].

The Wnt signaling cascade (summarized in Figure 1) initiates at the cell membrane when a Wnt protein interacts with its cognate Fz receptor [7•]. This interaction relays the signal into the interior of the cell where it is further transduced by the protein products of the *dishevelled* (*dsh*) and the *zeste-white 3/glycogen synthase kinase 3*β (*zw3/GSK3*) genes (reviewed in [3]). Genetically, *dsh* becomes activated and this activation precedes the inactivation of *zw3/GSK3* [13–15]. Biochemically, it has been demonstrated that Wg stimulation of mouse fibroblast cells leads to the inactivation of GSK3 kinase activity [16], thus agreeing well with the genetic model. Inactivation of *zw3/GSK3* by the Wnt signal leads to the activation, that is to say the accumulation, of β-catenin/Arm protein in the cytoplasm [17]. β-catenin/Arm then enters the nucleus where it modulates gene expression in concert with TCFs [8•–12•]. As this review centers on the role of β-catenin in this signaling process, we will restrict this discussion to the structure of β-catenin, the regulation of β-catenin stability, its nuclear entry, and the interaction with TCFs.

Structure of β-catenin

The primary structure of the β-catenin protein comprises a 130 amino acid amino-terminal domain, 12 imperfect repeats of 42 amino acids (referred to as arm repeats), and a carboxy-terminal domain of 100 amino acids (Figure 2a). Several somewhat unrelated proteins also contain arm repeats, including the product of the tumor suppressor adenomatous polyposis coli (APC), the src substrate p120, and the nuclear import factor importin [18]. The amino terminus of β-catenin is known to be important for regulating the stability of β-catenin [19–21], whereas the carboxyl terminus functions as a transcriptional activation domain when fused to the GAL4 DNA-binding domain [11•].

Recently, the three dimensional structure of the central arm repeat region of β-catenin has been determined (Figure 2b) [22••]. Unlike other proteins, like the immunoglobulins, in which domains form 'beads-on-a-string', the individual arm repeats do not form separable domains. Rather, the arm repeats form a compact unit as evidenced by its resistance to proteolysis. This is consistent with the observation that arm repeats never appear individually; no protein with fewer than six arm repeats has been described.

The canonical 42 amino acid arm repeat consists of 3 α-helices that are connected by short loops which reorient the polypeptide by 90° [22••]. The final structure is a

Figure 1
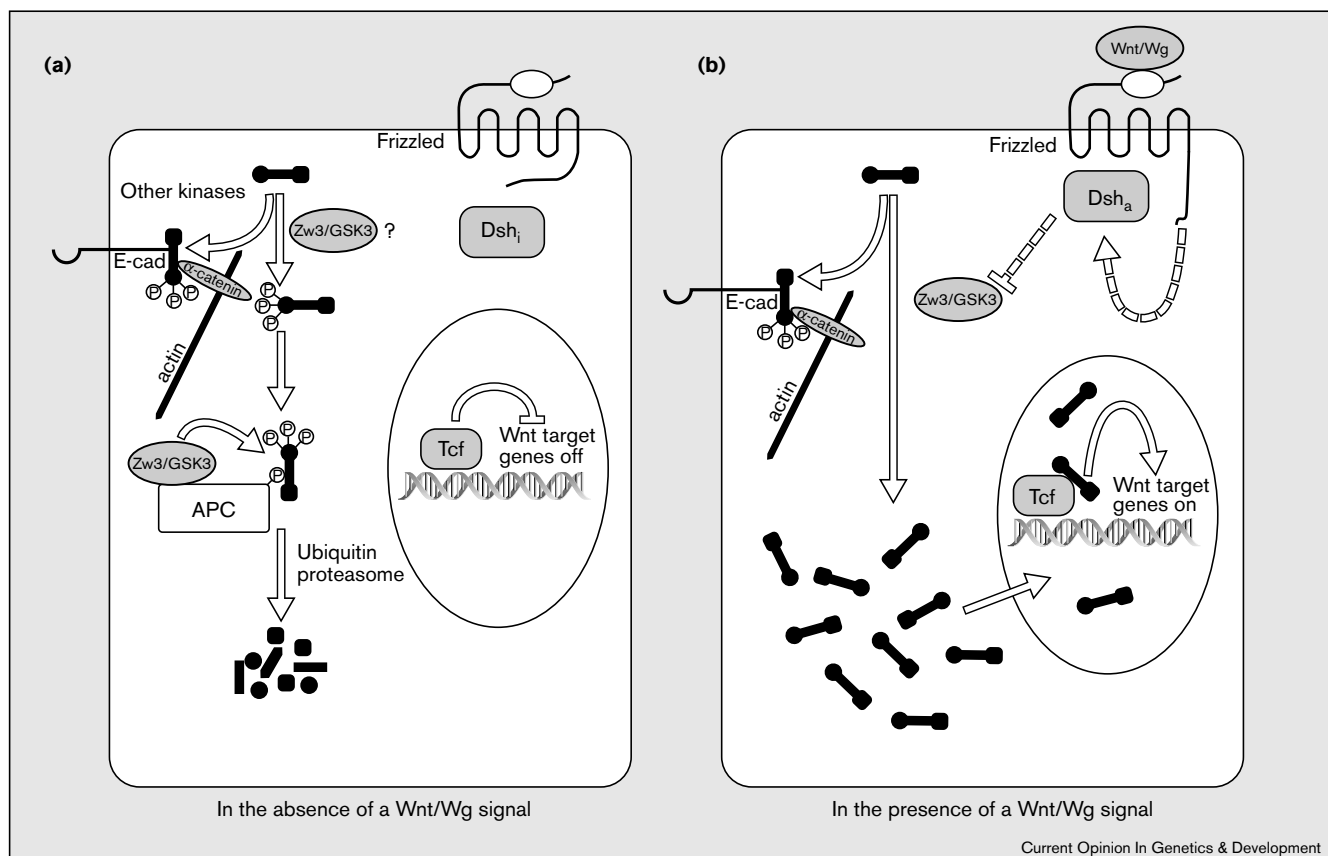
A model for Wnt/wg signaling. β-catenin is represented by the black dumb-bell shape. **(a)** In the absence of a Wnt signal, Dishevelled is inactive (Dsh$_i$) and *Drosophila* Zeste-white 3 or its mammalian homolog glycogen synthase kinase 3 (Zw3/GSK3) is active. Phosphorylation of β-catenin, possibly by Zw3/GSK3, is believed to be a major step in destabilizing β-catenin. Association with the APC–Zw3/GSK3 complex targets β-catenin for degradation by the ubiquitin–proteasome pathway. Meanwhile, TCF is bound to its DNA-binding site in the nucleus where it is able to repress the expression of such genes as *Siamois* in *Xenopus*. **(b)** In the presence of a Wnt signal, Dishevelled becomes activated (Dsh$_a$) which then leads to the inactivation of Zw3/GSK3 by an unknown mechanism. β-catenin fails to be phosphorylated and thus is no longer targeted into the ubiquitin–proteasome pathway. Instead, β-catenin accumulates in the cytoplasm and enters the nucleus by an unknown pathway where it interacts with TCF, alleviates its repression of the downstream genes and provides a transcriptional activation domain.

tightly packed series of interacting α-helices that form a rigid right-handed superhelix. In a simplified view, β-catenin may resemble a dumb-bell: the amino- and carboxy-terminal domains — the structures of which still need to be elucidated — being attached at the ends of a rod-like central arm repeat domain.

Several proteins bind to β-catenin through the arm repeat region and it has been shown that binding partners interact in an overlapping and mutually exclusive fashion [23,24]. The structural model of the arm repeat domain can explain these data: the right-handed superhelical twist of the β-catenin arm repeat region generates a shallow groove lined with basic amino acids that confers on the groove an overall positive charge [22••]. This positively charged groove may serve as a binding surface for several proteins, including TCF [8•–12•], APC [25], and adherens junction protein E-cadherin (E-cad) [23,26•]. Whether binding of the actin bundling protein Fascin [27] also occurs in this basic groove remains to be shown. Binding of one of these

proteins may block the binding site of a second protein, either directly by overlapping with this binding site or indirectly through steric hindrance.

The hypothesis that a basic groove represents the binding surface on β-catenin is supported by the observation that the binding regions in E-cad, APC and TCF — although not conserved in primary structure — are all acidic. Furthermore, as Huber and colleagues point out [22••], the phosphorylation of APC by GSK3 — which increases the negative charge on APC — strengthens the interaction of β-catenin with APC [28]. Mutations in *pangolin*, a *Drosophila TCF* homolog, provide further support for the model that the basic groove represents the binding surface on β-catenin: a substitution of lysine for glutamate in the Arm-binding domain of Pangolin diminishes the interaction of these two proteins [12•].

Interestingly, more distantly related proteins containing arm repeats, such as importin, don't carry an overall

Figure 2
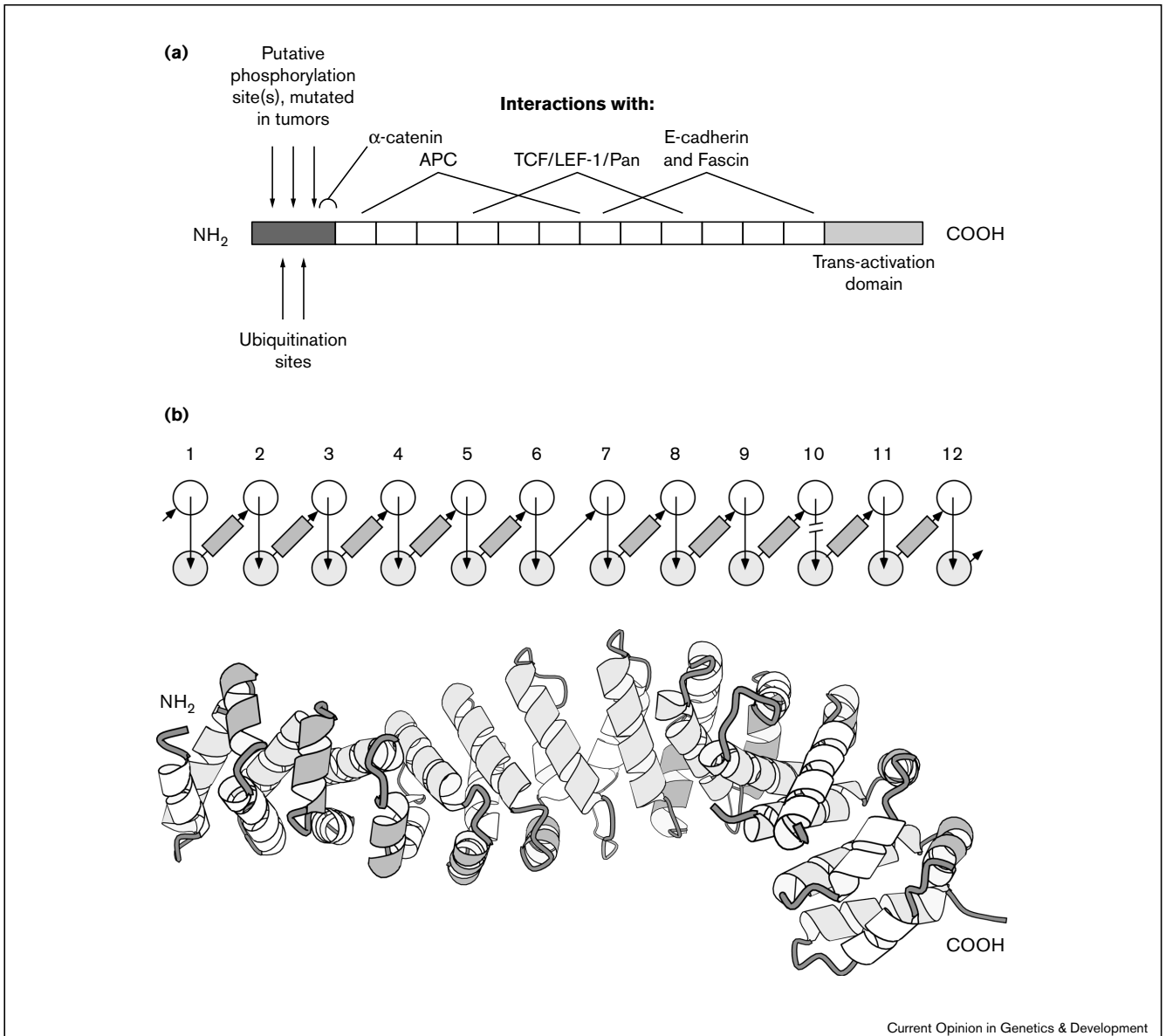
Structure of β-catenin. **(a)** Primary structure of β-catenin. The central domain of β-catenin consists of 12 imperfect repeats, called arm repeats, that interact in an overlapping and mutually exclusive manner with APC, Tcf, E-cad and Fascin. The amino terminus contains multiple phosphorylation sites which are correlated with the downregulation of β-catenin. Mutation of these potential phosphorylation sites or deletion of the amino terminus produces a more stable and hence constitutively active β-catenin protein. The carboxyl terminus contains a transcriptional activation domain. **(b)** Topology (top) and ribbon structure (bottom) of the arm repeat region of β-catenin. Circles and rectangles represent α-helices viewed from the top and the side, respectively. The arrows indicate the direction from amino to carboxyl termini of the polypeptide. Each repeat consists of three α-helices, H1, H2 and H3, connected by short loops.

positive charge in this domain and therefore may not contain a similar basic groove. Thus, a general consensus sequence for arm repeat binding proteins may not be easily identifiable, and post-translational modifications such as phosphorylation may greatly influence these interactions.

Regulation of β-catenin stability

The current model for Wnt signal transduction is that the Wnt signal stabilizes the β-catenin/Arm protein, thus allowing it to accumulate in areas outside of

adherens junctions, namely the cytoplasm and the nucleus. Stabilization of β-catenin is likely to be the key event in the transduction of the Wnt signal. To this end, the cell has evolved an elaborate system of regulating the stability and thus the steady-state levels of β-catenin. The complex system that fine-tunes the amounts of free β-catenin levels involves several proteins and is still poorly understood.

The amino terminus of β-catenin is crucial in regulating its stability; mutant alleles of β-catenin and Arm lacking

the amino terminus or carrying certain point mutations are more stable and are constitutively active [19–21,29•]. This region of β-catenin contains a series of serine and threonine residues, which may be phosphorylated. Phosphorylation of these residues is thought to be a signal for the degradation of β-catenin by the ubiquitin–proteasome pathway [30•]. Interestingly, several colon carcinoma and melanoma cell lines contain elevated levels of β-catenin; in some of these cell lines the increased stability can be attributed to point mutations in β-catenin that change the serine and threonine residues, thereby blocking its phosphorylation and subsequent degradation [31•,32•].

Presently, at least two proteins are known to directly affect the stability of β-catenin: the protein products of the *zw3/GSK3* [13] and *APC* genes (reviewed in [33•]). *zw3/GSK3* encodes a Ser/Thr protein kinase which is an essential negative regulator of the Wnt pathway [13] and whose kinase activity is downregulated by Wg in cell culture [16]. It has been suggested that it directly phosphorylates β-catenin on the amino-terminal serine and threonine residues and thereby targets it for destruction by the ubiquitin–proteasome pathway [30•]. Yost *et al.* [19] have demonstrated that GSK-3 is able to phosphorylate β-catenin, albeit weakly and only *in vitro*. Pai *et al.* [29•] have presented evidence that other kinases in addition to Zw3/GSK3 may be involved in the phosphorylation of Arm: junctional Arm is phosphorylated to equal levels in wild-type and in *zw3* mutants.

APC was first identified as a tumor suppressor as mutations predispose individuals to develop colon carcinomas (reviewed in [33•]). It was shown later that APC binds directly to β-catenin and GSK3 [25]. Several colorectal carcinoma cell lines contain mutant APC and elevated levels of β-catenin; overexpression of wild-type APC in these cell lines significantly reduces the levels of free β-catenin [34]. Furthermore, the phosphorylation of APC by GSK3 enhances the binding of β-catenin to APC [28] and hence the destabilization of β-catenin. These results suggest that APC plays a direct role as negative regulator of the Wnt signaling pathway.

The APC story may not be that simple, however: first, null mutations in a *Drosophila APC* gene do not display the expected phenotypes of mutations in genes involved in the Wg signaling pathway [35•]. This may be either due to the presence of additional redundant *APC* genes or to maternally contributed APC protein that is sufficient to rescue the destruction of Arm. Second, ectopically expressed wild-type APC leads to secondary axis formation in *Xenopus* embryos, consistent with APC playing a positive rather than a negative role in Wnt signaling [36•]. Finally, using an RNA-mediated interference technique (referred to as RNAi), Rocheleau *et al.* [37••] demonstrated that disruption of the function of an APC-related gene, *apr-1*, in *C. elegans* produces a phenotype predicted for a positive regulator of the Wnt pathway [37••]. Clearly, the role of APC in Wnt signaling and the mechanism of its action are relatively poorly understood.

Several models can be proposed for the mechanism by which the stability of β-catenin is regulated. For example, the components that target β-catenin into the degradation pathway may be in limiting supply, so that a small reduction in the kinase activity of ZW3/GSK3, for instance, could greatly affect the steady-state levels of β-catenin. Evidence for this type of fine-tuning comes from the studies of Miller and Moon who found that ectopic expression of a membrane-tethered β-catenin in *Xenopus* embryos leads to the stabilization of the endogenous β-catenin pool which then translocates into the nucleus and initiates the process of axis duplication [38•]. They propose that this overexpressed membrane-tethered β-catenin competes for the limited supply of degradation machinery thus allowing endogenous β-catenin levels to escape the negative regulation by such factors as APC and GSK.

These experiments also shed light on an apparent discrepancy in the analysis of the signaling activity of β-catenin between *Drosophila* and *Xenopus*. A mutant allele of β-catenin lacking the carboxyl terminus (the transactivation domain) is able to induce a secondary axis in *Xenopus* [39], whereas a similar mutant allele of *arm* does not transduce the Wg signal in *Drosophila* embryos [26•,40]. This disparity can be explained by proposing that ectopically expressed β-catenin acts indirectly by saturating the degradation machinery, thus allowing endogenous β-catenin to accumulate, enter the nucleus, and interact with TCF. Levels of overexpressed Arm may not be sufficient to produce this effect in *Drosophila* embryos.

Nuclear entry of β-catenin and interaction with TCF

The mechanism by which β-catenin is translocated into the nucleus is not clear. On the basis of overexpression studies, it has been argued that TCF facilitates the movement of β-catenin into the nucleus: when overexpressed together with TCF, β-catenin is found primarily in the nucleus, whereas in the absence of ectopic TCF, it is evenly distributed between the nucleus and the cytoplasm [8]. Furthermore, overexpression of a *TCF* in *wg* mutants mimics the effects of Wg hyperstimulation, suggesting that TCF may bind Arm in the cytoplasm and carry it into the nucleus [41•]. The overexpression of TCF in both of these studies, however, may override the cell's finely tuned machinery to regulate levels of β-catenin.

Alternatively, β-catenin may enter the nucleus with the assistance of an additional protein or complex. Compelling evidence for this hypothesis is provided by the observation that a mutant allele of *arm* that fails to bind TCF still localizes to the nucleus [11•,26•]. One hint as to the mechanism of this nuclear localization may be provided by the presence of arm repeats in importin, a major

component of the nuclear import machinery [42]. It is conceivable that higher than normal levels of β-catenin, as is the case when the cell receives a Wnt signal, can interact with the nuclear import machinery and thereby become translocated into the nucleus.

In the nucleus, β-catenin associates with TCF and, in the case of *Drosophila*, acts as a transcriptional activator [11•,12•,41•]. In 16 to 32 cell stage *Xenopus* embryos, β-catenin is observed to accumulate in dorsal but not in ventral nuclei, suggesting that asymmetries in β-catenin localization may specify the dorsal–ventral axis [43•]. In cell culture, Korinek *et al.* (personal communication) have demonstrated that *Wnt-1* expression leads to the transcription of a reporter construct carrying a TCF responsive element, thus connecting the extracellular Wnt signal to the nucleus.

In the absence of β-catenin, TCF can bind to its target DNA through its HMG-box but fails to activate transcription. Several groups have suggested that TCF may act as a transcriptional repressor in the absence of β catenin [41•,44••]. Brannon *et al.* [44••], who studied the Wnt target gene *Siamois* in *Xenopus*, found that elimination of the TCF sites in the *Siamois* promoter elevated the normally low levels of ventral *Siamois* expression, supporting the model that TCF can act as a transcriptional repressor in the absence of β-catenin.

The mechanism by which TCF can act both as transcriptional repressor and activator are not clear yet. It has been shown previously that TCF can bend DNA [45], and Behrens *et al.* [8•] have demonstrated that this DNA bending activity is significantly decreased when TCF is bound to β-catenin [8•]. Thus, in the absence of β-catenin, TCF's induced DNA bend may repress the transcription of the downstream genes, whereas in the presence of β-catenin, the DNA bend is altered and a transcriptional activation domain is provided, thus activating transcription.

Recent experiments in *C. elegans* complicate the simplified model for β-catenin as a transcriptional activator when complexed with TCF as depicted in Figure 1. Several groups studied the process by which one cell, called P2, signals to the neighboring E/MS blastomere to polarize it, thereby giving rise to two distinct daughter cells, E and MS [37••,46••]. The E cell lineage gives rise to endoderm while the MS cell lineage gives rise to mesoderm. An inductive signal from the P2 cell specifies the fate of the E cell. Several mutants which form mesoderm at the expense of endoderm (these mutants were called *mom* [more mesoderm]) were isolated and shown to contain mutations in genes of the Wnt signaling pathway, including *porcupine* (*mom-1*), *wnt* (*mom-2*), and *frizzled* (*mom-5*). Mutation in the *C. elegans* TCF homolog *pop-1* results in both cells adopting the E cell fate [47]. Thus, *pop-1* would act to repress the Wnt signal. Levels of POP-1 protein are higher

in the nucleus of the MS cell than in the E cell but, in *mom* mutants, levels of POP-1 are equally high in the nuclei of the E and MS cells. In the simplest model, the Wnt signal would therefore act to lower POP-1 in the E-cell nucleus. The mechanism by which POP-1 levels are decreased in the E nucleus involves a β-catenin-related gene called *wrm-1* and are presently mysterious.

In addition to their role in specifying endoderm, some *mom* genes appear to function in orienting the mitotic spindle during early development [37••,46••], suggesting a role for Wnt signaling in cytoskeletal organization. *wrm-1*, *apr-1*, *mom-4* (uncloned), and *pop-1* do not participate in this process, a finding that suggests that Wnt signaling pathways may branch and have signaling endpoints in the cytoplasm as well as in the nucleus. Such a model is consistent with experiments in the tissue polarity determination in *Drosophila*: *frizzled*, *dsh* [48] and *rhoA* [49•] but not *arm* participate in this pathway. A Wnt signal in tissue polarity determination remains to be identified.

β-catenin and cell adhesion revisited

β-catenin is a multi-functional protein that can affect both cell adhesion and gene expression. By binding to both E-cad and αcatenin simultaneously, β-catenin links the adherens junction to the cytoskeletal polymer actin; however, β-catenin's role in cell adhesion and gene expression are separable as mutants of β-catenin that fail to interact with α-catenin and thus fail to function in cell adhesion, are still capable of transducing a Wnt signal [26•,39]. In addition, Orsulic and Peifer [26•] demonstrated that two mutant alleles of *arm* — one defective in cell adhesion because of the deletion of its α-catenin-binding site, and one impaired in Wg signaling because of the deletion of its carboxyl terminus (the transactivation domain) — complement each other. The ability of β-catenin to participate in both Wnt signaling and cell adhesion is most likely the result of β-catenin interacting with a variety of proteins in a mutually exclusive fashion [23,24], thus giving rise to pools of β-catenin molecules that are restricted to participating in a single process. Whether Wnt signaling has a direct effect on cell adhesion still remains to be determined.

The mouse *E-cad* promoter contains a consensus TCF site [10•], and Yanagawa *et al.* [50•] have shown that activation of the Wg signaling pathway in cell culture by overexpression of *dsh*, dominant negative *zw3*, or activated *arm* leads to the transcriptional activation of E-cad. Whether this transcriptional activation is mediated directly through the TCF/β-catenin complex remains to be shown. Thus, in an indirect fashion Wnt signaling appears to modulate cell adhesion. More importantly though, the activation of E-cad expression and subsequent increased levels of E-cad protein may serve as a means to inactivate the Wnt signal input, as it is able to soak up excess amounts of free β-catenin [51].

Figure 3
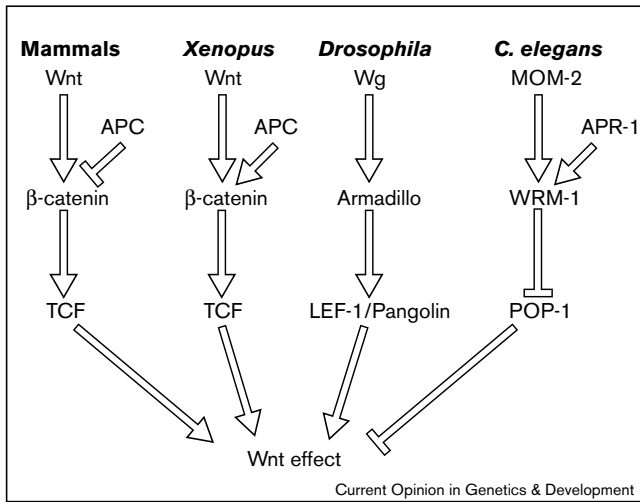
Comparison of the Wnt signaling pathway across species. The components involved in the Wnt signaling pathways are remarkably well conserved. The regulation of each component's activity in response to a Wnt signal is not yet fully established and some notable differences are apparent. APC in mammalian cell culture appears to inhibit the Wnt signal by destabilizing β-catenin whereas, in *Xenopus*, APC appears to promote the Wnt effect on axis formation. The effect of Wnt signaling on TCFs in the various systems also appears to be different: the TCF homolog POP-1 in *C. elegans* is downregulated in response to the Wnt signal encoded by the *mom-2* gene whereas, in other systems, Wnt activates transcription through TCF.

Conclusion

Several components of the Wnt signaling pathway are members of fairly extensive gene families, the Wnt family leading the way with at least 14 different genes in the mouse. Cloning of novel *Frizzled* and *TCF* genes may reveal an equally large complexity. The function of β-catenin and TCFs may vary from signaling cascade to signaling cascade: β-catenin is able to act as a transcriptional activator but, as evidenced in *C. elegans*, can act negatively by lowering nuclear levels of POP-1. TCFs in turn are able to act as transcriptional activators and repressors, and β-catenin may play a role in both of these processes. Some differences in Wnt signaling pathways in various species are highlighted in Figure 3.

One consequence of this diversity in signaling components and their actions may be to allow a highly conserved signaling pathway to participate in a variety of biological processes, ranging from altering gene expression to altering cytoskeletal polarity. In the coming years, the challenges are to connect β-catenin to the upstream signaling components and to identify the crucial downstream transcriptional targets that participate in the vast array of biological processes regulated by Wnt genes.

Acknowledgements

References and recommended reading

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This paper presents a very thorough *in vivo* mutational analysis of *arm* in *Drosophila*. Among this vast amount of data is an experiment in which two embryonic lethal alleles of *arm* − one being defective in Wg signaling and the other being defective in cell adhesion − complement each other, thus demonstrating that the functions of Arm in Wg signaling and cell adhesion are truly separable and independent. One mutant Arm protein, which does not interact with dTCF and is inactive in Wg signaling, still localizes to the nucleus, thus arguing that a mechanism not involving dTCF is required for Arm's nuclear localization.

27. Tao YS, Edwards RA, Tubb B, Wang S, Bryan J, McCrea PD: **β-catenin associates with the actin-bundling protein fascin in a noncadherin complex.** *J Cell Biol* 1996, **134**:1271-1281.

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When complexed with β-catenin, APC also associates with GSK3, another component of the Wnt signaling pathway. GSK3 phosphorylates APC *in vitro* and this phosphorylation greatly enhances the binding of β-catenin to APC.

29. Pai LM, Orsulic S, Bejsovec A, Peifer M: **Negative regulation of**
• *armadillo*, a *wingless* effector in *Drosophila*. *Development* 1997, **124**:2255-2266.
The authors characterize an *arm* allele lacking 54 amino acids in its amino terminus and show that it is constitutively active in Wg signaling. This allele is still phosphorylated even in a *zw3* mutant background, suggesting that additional kinases other than Zw3 are involved in its phosphorylation.

30. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R: **β-catenin is**
• **a target for the ubiquitin–proteasome pathway.** *EMBO J* 1997, **16**:3797-3804.
This paper demonstrates that β-catenin is degraded by the ubiquitin–proteasome pathway. Mutation of putative phosphorylation sites (speculated GSK3 phosphorylation sites) stabilizes β-catenin and blocks ubiquitination. A remaining issue is whether phosphorylation at these sites is a prerequisite for degradation by the ubiquitin-proteasome pathway.

31. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein
• B, Kinzler KW: **Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC.** *Science* 1997, **275**:1787-1790.
Certain colorectal tumor cell lines contain mutations in β-catenin that render the β-catenin protein insensitive to APC-mediated destabilization. Many of the mutations in β-catenin are in serine residues which are implicated in downregulation of β-catenin by GSK3 phosphorylation. These cell lines contain constitutive β-catenin/TCF-regulated transcription which was not inhibited by exogenous APC.

32. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P:
• **Stabilization of β-catenin by genetic defects in melanoma cell lines.** *Science* 1997, **275**:1790-1792.
In this paper, the authors demonstrate that a number of human melanoma cell lines contained elevated levels of β-catenin. This increase was attributed to mutations in the β-catenin gene that result in the stabilization of the protein and to mutations in APC. Elevated levels of β-catenin also correlated with the presence of a constitutive β-catenin/LEF-1 complex. Interestingly, several of the mutations in β-catenin, that resulted in increased stability of the protein, changed conserved serine residues in the amino terminus, a finding that along with other data suggests that these residues are phosphorylated and that this phosphorylation is involved in the destabilization of β-catenin.

33. Polakis P: **The adenomatous polyposis coli (APC) tumor**
• **suppressor.** *Biochim Biophys Acta* 1997, **1332**:F127-F147.
A comprehensive review of the literature on APC.

34. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P: **Regulation of intracellular β-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein.** *Proc Natl Acad Sci USA* 1995, **92**:3046-3050.

35. Hayashi S, Rubinfeld B, Souza B, Polakis P, Wieschaus E,
• Levine AJ: **A *Drosophila* homolog of the tumor suppressor gene adenomatous polyposis coli down-regulates β-catenin but its zygotic expression is not essential for the regulation of Armadillo.** *Proc Natl Acad Sci USA* 1997, **94**:242-247.
By screening a *Drosophila* expression library with an antibody against human APC, the authors isolate an APC homolog, called D-APC. Even though D-APC interacts directly with Arm *in vitro* and reduces β-catenin levels when overexpressed in cells lacking wild-type APC, removal of zygotic D-APC did not alter Arm protein distribution and no phenotypes suggesting an inhibitory role in Wg signaling were observed. These results, in combination with the results in *C. elegans* [37••] and the results in *Xenopus* [36•], suggest a more complex function for APC in Wnt signaling than was previously thought.

36. Vleminckx K, Wong E, Guger K, Rubinfeld B, Polakis P,
• Gumbiner BM: **Adenomatous polyposis coli tumor suppressor protein has signaling activity in *Xenopus laevis* embryos resulting in the induction of an ectopic dorsoanterior axis.** *J Cell Biol* 1997, **136**:411-420.
Ectopic expression of APC in *Xenopus* embryos induces a secondary axis, suggesting a positive rather than a negative role for APC in Wnt signaling. This axis induction by APC is dependent on the availability of free β-catenin. These results in conjunction with the work in [37••] suggest a more complex role for APC in the Wnt signaling pathway than simply targeting β-catenin for degradation.

37. Rocheleau C, Downs W, Lin R, Wittmann C, Bei Y, Cha Y-H, Ali
•• M, Priess J, Mello C: **Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos.** *Cell* 1997, **90**:707-716.

The authors use both a genetic screen and an RNA-mediated interference (RNAi) technique to identify genes involved in the specification of endoderm in early *C. elegans* embryos. The genes described are related to *wnt/wingless* (*mom-2*), *porcupine* (*mom-1*), *frizzled* (*mom-5*), β-catenin/armadillo (*wrm-1*), and *APC* (*apr-1*), all of which have been implicated in the transduction of the Wnt signal in other systems. Unlike in previously studied systems, *wrm-1* acts to inhibit the activity of *pop-1*, a *TCF* homolog, presumably by facilitating the movement of POP-1 out of the nucleus. Several of these so-called *mom* mutants also display an abnormal orientation of certain mitotic spindles. *pop-1* mutants do not display this effect, thus suggesting that the Wnt signaling pathway affects spindle orientation in a *pop-1* independent pathway.

38. Miller J, Moon R: **Analysis of the signaling activities of**
•   **localization mutants of** β-**catenin during axis specification in**
      Xenopus. J Cell Biol 1997, **139**:229-243.
A membrane-tethered β-catenin is still able to induce a secondary axis in *Xenopus* even though it fails to enter the nucleus itself. The authors argue that overexpression of this mislocalized β-catenin competes with the endogenous degradation machinery thus permitting accumulation and subsequent nuclear entry of the endogenous β-catenin.

39. Funayama N, Fagotto F, McCrea P, Gumbiner BM: **Embryonic**
    axis induction by the armadillo repeat domain of β-**catenin:**
    evidence for intracellular signaling. J Cell Biol 1995, **128**:959-968.

40. Peifer M, Wieschaus E: **The segment polarity gene** *armadillo*
    encodes a functionally modular protein that is the *Drosophila*
    homolog of human *plakoglobin.* Cell 1990, **63**:1167-1176.

41. Riese J, Yu X, Munnerlyn A, Eresh S, Hsu S-C, Grosschedl R,
•   Bienz M: **LEF-1, a nuclear factor coordinating signaling inputs**
      from *wingless* **and** *decapentaplegic.* Cell 1997, **88**:777-787.
LEF-1 activates transcription of Ubx in the midgut by binding to a TCF-binding site in the Wingless-responsive element of the Ubx promoter. Arm forms a stable ternary complex with LEF-1 and DNA and is required for LEF-1-dependent activation. Overexpression of LEF-1 displays similar phenotypes as ubiquitously expressed *wingless*, demonstrating that LEF-1 overexpression bypasses the Wingless signal. Furthermore, *wingless* and *decapentaplegic* signaling work synergistically to affect this process.

42. Goerlich D, Prehn S, Laskey RA, Harmann E: **Isolation of a**
    protein that is essential for the first step of nuclear import.
    Cell 1994, **79**:767-778.

43. Larabell CA, Torres M, Rowning BA, Yost C, Miller JR, Wu M,
•   Kimelman D, Moon RT: **Establishment of the dorso-ventral axis**
      in *Xenopus* **embryos is presaged by early asymmetries in**
      beta-catenin that are modulated by the Wnt signaling pathway.
      J Cell Biol 1997, **136**:1123-1136.
By careful confocal microscopy, the authors argue that at the two cell stage more cytoplasmic β-catenin has accumulated on the future dorsal side of the *Xenopus* embryo. By later stages, β-catenin accumulates in dorsal but not ventral nuclei. Based on these results, the authors suggest that β-catenin asymmetries presage and may specify dorsal–ventral differences in gene expression.

44. Brannon M, Gomperts M, Sumoy L, Moon R, Kimelman D: **A**
••   β-**catenin/XTcf-3 complex binds to the** *Siamois* **promoter to**
      regulate dorsal axis specification in *Xenopus.* Genes Dev 1997,
      11:2359-2370.
The promoter of the dorsalizing homeobox gene *siamois* in *Xenopus* is shown to be a direct target of the β-catenin/XTCF-3 complex, thus establishing a link between the Wnt pathway and the activation of genes involved in specifying the dorsal axis. The authors also demonstrate that XTCF-3 in the absence of β-catenin, and hence in the absence of a Wnt signal, can act as a transcriptional repressor. The dorsal activation in combination with ventral repression of the *siamois* promoter is hypothesized to result in a very restricted pattern of *siamois* expression.

45. Giese K, Kingsley C, Kirshner JR, Grosschedl R: **Assembly**
    and function of a TCRα **enhancer complex is dependent on**
    LEF-1-induced DNA bending and multiple protein–protein
    interactions. Genes Dev 1995, **9**:995-1008.

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••   **polarizes an early** *C. elegans* **blastomere to distinguish**
      endoderm from mesoderm. Cell 1997, **90**:695-705.
In a genetic screen in *C. elegans* for mutants that produce more mesoderm at the expense of endoderm, the authors identify five genes, *mom-1* through *mom-5*. *mom-2* encodes a Wnt protein which signals to a neighboring cell to adopt an endoderm fate. In *mom-2* mutants, POP-1 – a TCF-related protein, which normally is at relatively low levels in the nucleus of this neighboring cell – accumulates to higher levels, suggesting that *mom-2*'s role is to reduce nuclear levels of POP-1. This result contradicts findings in other systems where TCF is thought to be activated by Wnt signaling. The authors furthermore characterize a mitotic spindle defect in *mom* mutants and suggest that Wnt signaling regulates cytoskeletal polarity.

47. Lin R, Thompson S, Priess JR: *pop-1* **encodes an HMG box**
    protein required for the specification of a mesoderm precursor
    in early *C. elegans* **embryos.** Cell 1995, **83**:599-609.

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    1995, **121**:4095-4102.

49. Strutt DI, Weber U, Mlodzik M: **The role of** *RhoA* **in tissue**
•   **polarity and** *Frizzled* **signaling.** Nature 1997, **387**:292-295.
The authors demonstrate that hypomorphic alleles of *RhoA* p21 GTPase give rise to phenotypes that are very similar to those of tissue polarity mutants, namely *frizzled* and *dsh*. Genetic evidence is provided that JNK/SAPK-like kinases act in the Fz/RhoA-mediated polarity signaling. This pathway may represent a novel Wnt pathway that does not involve Arm and TCF.

50. Yanagawa S, Lee J-S, Haruna T, Oda H, Uemura T, Takeichi M,
•   Ishimoto A: **Accumulation of Armadillo induced by Wingless,**
      Dishevelled, and dominant negative Zeste-white 3 leads to
      elevated DE-cadherin in *Drosophila* **Clone 8 wing disc cells.**
      J Biol Chem 1997, **272**:25243-25251.
Activation of the Wingless pathway in *Drosophila* cell culture by overexpression of *dsh* or dominant negative *zeste-white 3* leads to the transcriptional activation of the *DE-cadherin* gene. The authors speculate that *DE-cadherin* is a direct transcriptional target of the β-catenin/Arm–TCF complex and hence of the Wnt pathway. These results demonstrate that Wingless signaling, at least indirectly, can modulate cell adhesion.

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    cadherins antagonizes the signaling activity of β-**catenin during**
    axis formation in *Xenopus.* J Cell Biol 1996, **132**:1105-1114.