Differential molecular interactions of β-catenin and plakoglobin in adhesion, signaling and cancer

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Plakoglobin and β-catenin are homologous proteins functioning in cell adhesion and transactivation. Their activities are controlled by three types of interactions: those with cadherins in adherens junctions, linking them to the actin cytoskeleton; interactions in the nucleus, where they bind to transcription factors and stimulate gene expression; interactions of free cytoplasmic β-catenin with axin and adenomatous polyposis coli (APC) protein which target it for degradation. Studies in the past year have demonstrated the complex interplay between these three types of interactions and the different behavior of β-catenin and plakoglobin in their involvement in morphogenesis and tumorigenesis strongly suggesting that catenins play key roles in adhesion-mediated signaling.

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Current Opinion in Cell Biology 1998, 10:629–639

http://biomednet.com/elecref/0955067401000629

Abbreviations

APC	adenomatous polyposis coli
GBP	GSK binding protein
GSK	glycogen synthase kinase 3β
HMG	high mobility group
LEF	lymphoid enhancer binding factor
NLS	nuclear localization sequence
TCF	T-cell-specific factor
Wg	Wingless

Introduction

Adhesive interactions of cells exert major long-term effects on cell shape, organization of the cytoskeleton and cell fate. The most obvious effect of adhesion is on morphogenesis, that is the assembly of individual cells into highly ordered tissues through cell–cell and cell–matrix interactions ([1,2] and Figure 1). These adhesions are mediated by transmembrane cell adhesion receptors of the cadherin and integrin families, which link cells to each other or to the matrix [1,3,4]. In cell–cell junctions, effective adhesion also depends on the association of the relevant membrane receptors with the cytoskeleton through specific 'anchor proteins', including α-, β- and γ-catenin (plakoglobin) [5,6,7•]. Recent studies have provided compelling evidence that, besides their direct role as physical linkers of the actin cytoskeleton to cadherins, catenins can also play a central role in signal transduction and the regulation of gene expression (Figure 1). This has opened the way for very rapid and dramatic progress in our understanding of the interplay between cell adhesion, cytoskeletal structure and gene expression, and has also significantly contributed to the unraveling of mechanisms that govern the concerted behavior of cell populations in embryonic and adult tissues. We will address here the localization, function and fate of β-catenin and plakoglobin, members of a group of 'moonshining proteins' that can interact with different partners in distinct subcellular compartments, and which have a dual role: one in the assembly of adherens junctions and the other in the Wingless (Wg)/Wnt signaling pathway, affecting embryonal axis specification in both insects and vertebrates (for recent reviews see [8•–10•]). We will describe the recent developments in our knowledge of the involvement of β-catenin and plakoglobin in this interplay between cell adhesion and signal transduction and discuss their role in tumor development in humans.

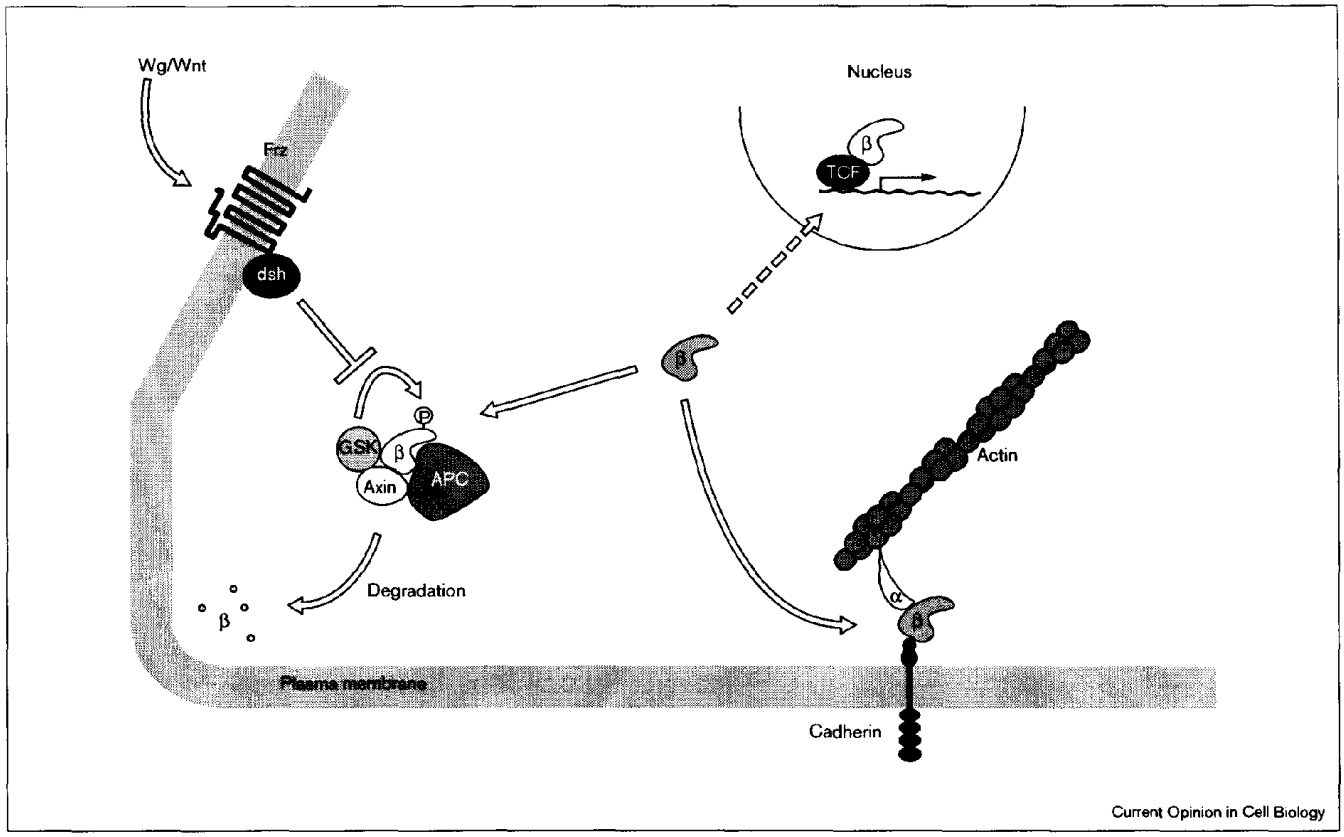
Membrane association of β-catenin and plakoglobin: the junctional connection

Immunofluorescence analysis of cultured cells and of intact epithelial and endothelial tissues has revealed a distinct submembranal staining for β-catenin, similar to that of other adherens junction components, including the classical cadherins. At these submembranal sites β-catenin is anchored to the cytoplasmic tail of cadherin and, via α-catenin, to the actin cytoskeleton. The association with the cytoskeleton is mediated via either α-actinin [11] or vinculin [12•,13•]. In some cases, diffuse cytoplasmic staining for β-catenin can also be seen, which is compatible with the observation that a significant fraction of this protein is associated with the soluble cytoplasmic pool that can be extracted with nonionic detergent [14•,15] (see below). Plakoglobin is also associated with adherens-type junctions and, in addition, it is present in desmosomal junctions of epithelial cells. The 'free' cytoplasmic pool of plakoglobin is usually small. Cadherin–β-catenin interaction is critical for junction formation, since mutant cadherin with a deleted catenin-binding domain is unable to mediate cell–cell adhesion (for review see [5]).

Regulation of β-catenin degradation: the APC/axin/GSK connection

The cellular levels of β-catenin are constitutively down-regulated by a component of the Wnt signaling pathway, glycogen synthase kinase 3β (GSK). This enzyme phosphorylates β-catenin on specific serine and threonine residues [16], preparing it for degradation by the ubiquitin-proteasome system (Figure 1). Another essential component directly involved in regulating β-catenin stability, adenomatous polyposis coli (APC) protein, was first identified as a major tumor suppressor protein involved in predisposition to colon carcinoma in humans (reviewed in [17•]). APC and GSK can form a complex that also

Figure 1



The different molecular interactions of β-catenin in Wg/Wnt signaling and cell adhesion. When Wnt signaling is activated, by binding of Wnt to the Frizzled (Frz) family of receptors, the dishevelled (dsh) protein is hyperphosphorylated and recruited to the membrane area. Activated dsh inhibits glycogen synthase kinase 3β (GSK) action, which normally phosphorylates (P) β-catenin (β) and directs it together with adenomatous polyposis coli (APC) and axin family members to degradation by the ubiquitin-proteosome system. Decreased degradation of β-catenin leads (dashed arrow) to its accumulation, nuclear translocation and association with lymphoid enhancer binding factor/T-cell-specific factor (TCF) transcription factors, leading to the activation of gene expression. This 'linear model' can vary, as suggested by recent studies in *Drosophila*, *Xenopus* and *Caenorhabditis elegans* (see [33•] for review). In addition to its role in Wg/Wnt signaling, β-catenin is a major component of the cadherin–catenin adhesion system that links cadherins via α-catenin (α) to the actin cytoskeleton. By varying the level of β-catenin partners in the adhesion system, the role of β-catenin (β) in signaling can be significantly affected. Wg/Wnt can also apparently influence cell adhesion by mechanisms that are unknown yet.

includes β-catenin and possibly additional proteins of the axin family (see below). Phosphorylation of APC and β-catenin is involved in their binding to each other [18] and in the degradation of β-catenin by the ubiquitin-proteasome system [19••,20,21•]. Mutations in the amino-terminal region of β-catenin, which contains consensus sites for serine phosphorylation by GSK, can stabilize β-catenin against this degradation [16,19••,22] in an APC and Wnt signaling-independent manner. Accumulation of β-catenin can also result from mutations in the β-catenin-binding site of APC which block β-catenin degradation [17•,23].

Additional components of the β-catenin–APC complex which also negatively regulate the level of β-catenin are members of the recently described axin family [24••,25•–31•]. Axin interacts with β-catenin, APC and GSK, stimulating GSK-dependent phosphorylation and degradation of β-catenin. Axin is believed to act as a scaffold for this four-part complex, facilitating APC and β-catenin phosphorylation by GSK. The involvement of G proteins in this process is also implicated, since the regulator of G-protein signaling domain of axin directly interacts with APC [29•]. In addition, a recently identified new component, GSK binding protein (GBP), was shown to inhibit GSK phosphorylation when injected into *Xenopus* embryos, bringing about an elevation in β-catenin levels that results in axis duplication [32••]. Interestingly, GBP has high homology to proto-oncogenes of the FRAT family described in T-cell lymphoma [32••], suggesting that GSK may also play a role in tumorigenesis.

β-Catenin and Wnt signaling: the basic facts

The intricate negative regulation of β-catenin levels by the Wnt/Wg signaling pathway is highly conserved throughout evolution from insects to vertebrates and is

primarily effective during embryonic development (for recent reviews see [8•,33•]). The main events during this signaling include association between members of the Wg/Wnt family of secreted glycoproteins and their transmembrane receptors, the Frizzled family (Figure 1). This, in turn, leads to phosphorylation and activation of the Dishevelled (Dsh) protein, which is recruited to the cell membrane [34,35]. Activated Dsh can inhibit GSK activity, stabilizing and elevating the levels of extrajunctional (soluble) β-catenin. Increased levels of β-catenin lead to its nuclear translocation and the activation of Wg/Wnt responsive genes by a bipartite transcription factor consisting of β-catenin and members of the lymphoid enhancer binding factor (LEF)/T-cell-specific factor (TCF) family. This sequence of events was recently shown to be also followed in cultured 3T3 cells transfected with Wnt-1 [36•].

Nuclear translocation of β-catenin and its role in transactivation

The presence of nuclear β-catenin, especially in transfected cells expressing large amounts of the protein, was noticed some time ago, and usually dismissed as 'nonspecific labeling'. It is now established that the nuclear translocation of β-catenin is a highly specific process which is essential for its transcriptional activity. Using a permeabilized cell system [37••] it was demonstrated that β-catenin can dock onto the nuclear envelope in the absence of other cytoplasmic factors, and this process is not inhibited by classic nuclear localization sequence (NLS)-containing peptides and does not require either importins or karyopherins that serve as receptors for NLS. It appears that β-catenin and importin-β/β-karyopherin interact via a homologous domain with common nuclear pore components in an NLS-independent manner [37••]. It remains to be determined how the activation of Wnt signaling induces the specific nuclear import of β-catenin.

The accumulation of cytoplasmic β-catenin is an important step in its nuclear translocation and complex formation with members of the family of the high mobility group (HMG) architectural LEF/TCF transcription factors [38–40]. This discovery was a major advance in our understanding of the role of β-catenin in the downstream steps of Wnt/Wg signaling. Since LEF/TCF overexpression was shown to drive some of the endogenous β-catenin into the nuclei of cultured cells and of two cell mouse embryos [14•,38–40], and since TCF overexpression mimics Wg hyperstimulation [41••], it was originally thought that β-catenin, which lacks a consensus NLS, could translocate into the nucleus in a complex with LEF/TCF. It was postulated that such a complex between LEF/TCF and β-catenin was formed in the cytoplasm. This, however, does not appear to be the favored mechanism for β-catenin nuclear translocation, because the majority of this protein still accumulates in the nuclei of cells expressing mutated β-catenin that cannot bind LEF/TCF. Furthermore, in cells expressing β-catenin at very high levels with only small amounts of LEF/TCF, β-catenin still accumulates in the nucleus [14•,41••,42].

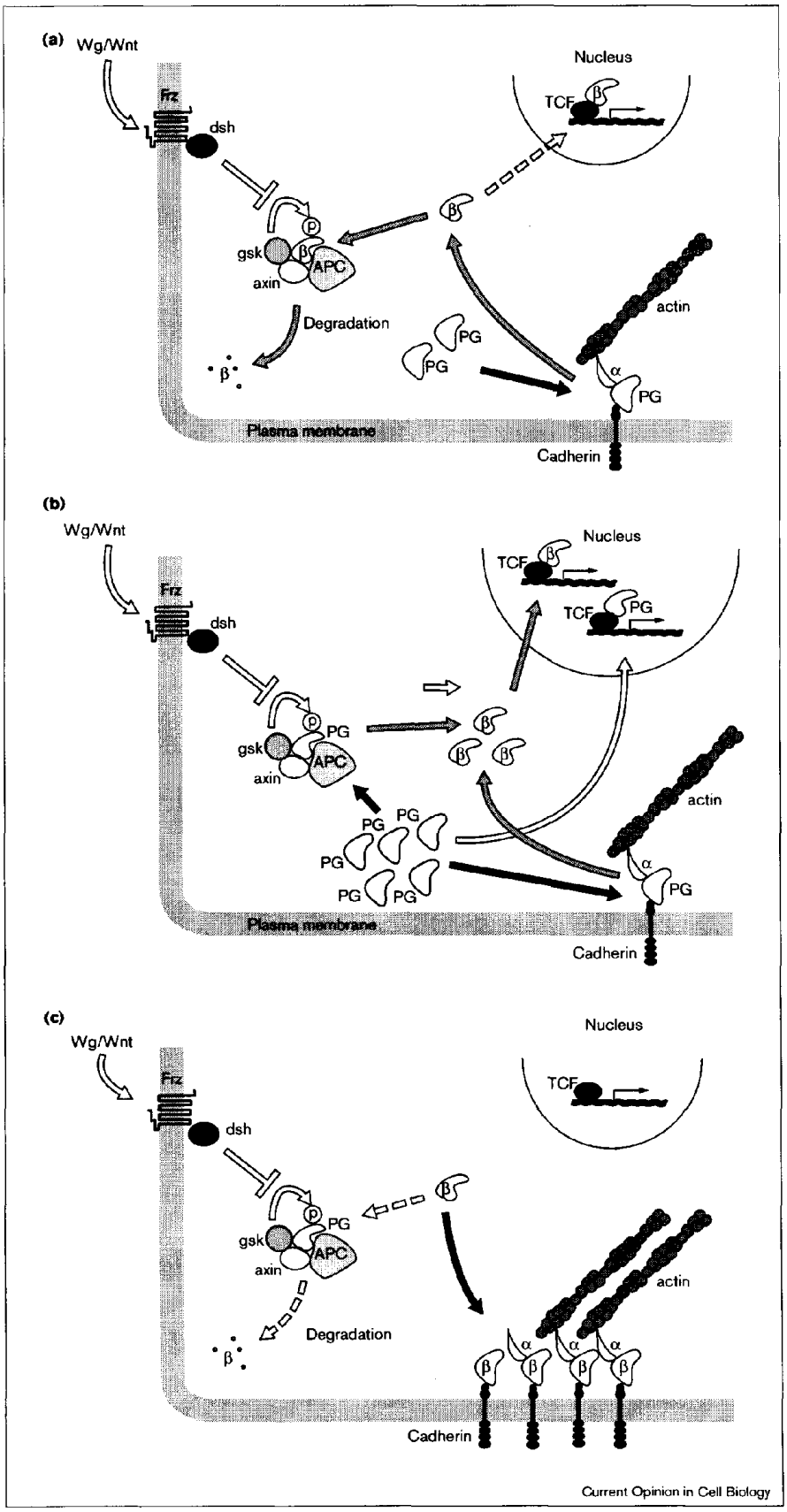
Association between β-catenin and members of the LEF/TCF family members in the nucleus is sufficient to activate the expression of synthetic reporter genes containing a LEF/TCF binding site [14•,39,40], and of several genes containing such sequences in their promoter region [41••,43••]. In *Drosophila*, and in some cases in *Xenopus*, these LEF/TCF binding sequences are required for Wg/Wnt signaling. In addition, Wnt overexpression in mammalian cells was demonstrated to lead to an increase in cytosolic β-catenin levels [44], an induction of serum-independent cellular proliferation, and activation of a synthetic LEF/TCF element [45•]. LEF/TCF family members can bind to this consensus DNA motif by their carboxyl terminus and induce a bend in the DNA, but cannot by themselves activate transcription. The binding between the amino terminus of LEF/TCF members and the armadillo repeat domain (see below) of β-catenin was shown to relieve this bending [39], and the carboxyl terminus of β-catenin was shown to act as the transcriptional activator of this bipartite transcription factor [14•,43••].

The candidate genes for regulation by the LEF/TCF–β-catenin complex are, first of all, those that are known to be influenced by the Wg/Wnt pathway. The Wnt target gene *Siamois* in *Xenopus* was shown to contain TCF binding sites in its promoter [46•,47] and in *Drosophila*, *engrailed* and *ultrabithorax* were show to contain such motifs in their promoter region [41••]. In mammalian cells, E-cadherin has been shown to contain a LEF-1 consensus sequence in its promoter [38], but its significance for the regulation of E-cadherin expression and function is unclear.

Plakoglobin and β-catenin: nonidentical twins

In vertebrates, two proteins closely related to the *Drosophila* armadillo protein are expressed: β-catenin and γ-catenin (plakoglobin) [48,49]. The three proteins are highly homologous, especially in their central part, which consists of so-called armadillo repeats [49,50]. Plakoglobin, like β-catenin, interacts with a multitude of proteins, including classical cadherins [51], α-catenin [52], the actin bundling protein fascin [53], axin [25•–31•], APC [54] and LEF/TCF transcription factors [14•,38]. The levels of both plakoglobin and β-catenin were shown to be elevated when the ubiquitin-proteasome degradation system was inhibited [14•,19••], and the rise was followed by nuclear accumulation of both proteins [21•]. It was noted, however, that plakoglobin is less sensitive to this proteolytic regulation, and in contrast to β-catenin, stable overexpression of plakoglobin in cells lacking cadherins and catenins can readily be obtained [14•,55]. In addition, although LEF-1 overexpression in MDCK cells was shown to result in nuclear translocation of endogenous β-catenin [14•,39], plakoglobin remained junctional under these conditions [14•], suggesting that the two proteins differ in their transcription factor specificity. The phenotypes in knockout

Figure 2



The effects of plakoglobin, cadherin and α-catenin on β-catenin level and function. **(a)** A moderate increase in plakoglobin (PG) level could lead to the displacement of β-catenin (β) from cell–cell junctions by competition for cadherin binding (black arrow), leading to an enhanced turnover of β-catenin in cells containing an APC/GSK/axin-competent degradation system (solid gray arrows) [19••]. **(b)** Massive overexpression of plakoglobin may result in effective competition with β-catenin for both cadherin and APC binding (black arrows), leading to the accumulation and nuclear translocation of β-catenin (gray arrows) [14•,63•]. In the nucleus, plakoglobin may either displace β-catenin from complexing with TCF, or allow transactivation by β-catenin, if β-catenin complexes more effectively than plakoglobin with TCF. Transactivation studies with plakoglobin in β-catenin-null cells will show if plakoglobin, in addition, has TCF-responsive transactivation potential on its own. **(c)** Overexpression of cadherin or α-catenin (α) results in the recruitment and stabilization of 'free' cytoplasmic and nuclear β-catenin (black arrow) [14•] by cell–cell junctions and inhibits transactivation and signaling [14•,72,76,80•].

mice are consistent with differences in the properties of β-catenin and plakoglobin: β-catenin null mutations result in very early defects in the embryo [56], while plakoglobin knockout embryos progress normally through early stages of development, but die at latter stages as a result of failure in heart development [57,58].

Overexpressed plakoglobin was shown to be able to induce a Wnt-like phenotype in *Xenopus* embryos (i.e. axis duplication) [59,60] that was similar to that observed with β-catenin overexpression [61]. It was therefore surprising when membrane-tethered constructs of both plakoglobin [62•] and β-catenin ([63•]; Figure 2) were found to be active in Wnt signaling in *Xenopus*, in contrast to the intuitive expectation that they would not be capable of signaling without entering the nucleus. Tethered plakoglobin and β-catenin, however, were shown to bind and block the APC degradation system and/or release the endogenous wild-type β-catenin from cell–cell junctions, allowing it to enter the nucleus and act in signaling [63•]. Results consistent with this notion were also obtained in transactivation studies in mammalian cells using a LEF/TCF consensus binding site synthetic DNA construct together with mutant plakoglobin and β-catenin proteins lacking the carboxy-terminal transactivation domains [14•]. Interestingly, a recent study has identified a transactivating domain also in the amino terminus of β-catenin [36•].

While the transactivation capacities of the carboxy-terminal domains of either plakoglobin or β-catenin fused to the Gal4 DNA-binding domain were similar, LEF-1-responsive transactivation was significantly lower with full-length plakoglobin than with β-catenin [14•]. The abilities of human plakoglobin and β-catenin in signaling were also examined using *Drosophila* armadillo mutants [64•]. A comparison of the two molecules' ability to rescue the segment polarity phenotype of *armadillo*, showed that both proteins could rescue *armadillo* adhesion, but β-catenin had only a weakly detectable signaling activity and plakoglobin had none [64•]. It will be necessary to use β-catenin-null cells in future studies to directly determine if plakoglobin can induce transactivation or signaling in mammalian cells on its own.

The Wg/Wnt signaling model: not so perfect after all

The Wg/Wnt signaling model presented in Figure 1 is consistent with numerous studies in different species including *Drosophila* and *Xenopus* and in mammalian cells. A number of studies in the past year have shown that, while the components in this signaling system were highly conserved in evolution, their function diverges largely between different organisms (see for reviews [33•,65•]). For example, in addition to the distinct signals that can be induced by the binding of Wg/Wnt to different members of the Frizzled receptor family, the role of APC also appears to be more complex than expected. In *Caenorhabditis elegans*, for example, the APC homolog has a positive rather than a negative role in Wnt signaling [66••]. In *Xenopus* development APC can function to activate Wnt signaling and induce axis duplication [67•], a process dependent on increased β-catenin levels, in contrast to the role of APC in stimulating β-catenin degradation observed in mammalian cells [17•,23]. In addition, *Drosophila* APC null mutants also do not appear to display the same phenotypes as those seen with null mutations of components that act in the Wg signaling pathway [68•]. Furthermore, a recent study has demonstrated that inactivation of the *Drosophila* APC$^{-/-}$ homologue causes retinal neuronal degeneration similar to germline mutations in humans [69••]. This disease in APC$^{-/-}$ *Drosophila* was shown to result from apoptotic cell death and could be rescued by reducing the levels of *Drosophila* β-catenin (armadillo), or by mutations in d'TCF [69••]. This suggests that the armadillo/TCF complex may also be involved in the activation of apoptosis, in addition to tumor progression (see below [70]).

The role of the GSK/APC/axin complex and the kinases involved in the phosphorylation of the β-catenin amino terminus and the subsequent targeting of β-catenin for degradation by the ubiquitin proteasome system are also incompletely understood. The degradation of plakoglobin was also shown to be directed, at least in part, by the ubiquitin-proteasome system [14•,19••], and it has been suggested [62•,63•] that an overexpressed plakoglobin in *Xenopus* could bind to and saturate the degradation system, therefore resulting in stabilization and nuclear translocation of endogenous β-caterin, followed by signaling [63•]. In mammalian cells, however, induction of plakoglobin expression could result in displacement of β-catenin from adherens junctions and its enhanced degradation by the ubiquitin-proteasome system ([21•]; Figure 2). In a cell type that does not express either cadherins or catenins [55], stable overexpression of plakoglobin that accumulated in the cytoplasm and the nucleus was inefficient in blocking the rapid degradation of β-catenin by the proteasome degradation system [14•]. Nevertheless, very high overexpression of plakoglobin, as obtained in transient transfections, could lead to the accumulation of β-catenin in the nucleus, further pointing to the importance of the degradation system in the fine-tuning of both the level and function of β-catenin and the possible role of plakoglobin in regulating β-catenin activities.

The positive role of the β-catenin–LEF/TCF complex in Wg/Wnt signaling via transcriptional activation is supported by genetic analyses in *Drosophila*, but is not supported by studies in *C. elegans*, where Wnt signaling and LEF/TCF activities are antagonistic to each other [66••,71••]. In addition, expression of different members of the LEF/TCF family may [39,41••] or may not [40,43••] result in the activation of the Wnt pathway. The possibility that β-catenin acts to relieve repression of Wnt-responsive genes imposed by LEF/TCF, by its binding to and sequestering LEF/TCF, is still another possibility supported by several studies [62•,72,73•]. Taken together, these studies further

emphasize the divergence in the mode of action of the various components in the Wg/Wnt signaling system in different cell types and species.

Closing the circle: β-catenin, plakoglobin and the relationships between cell adhesion, signaling and tumorigenesis

Several of the binding partners of β-catenin, such as E-cadherin, LEF/TCF, and APC, interact with β-catenin, in a mutually exclusive fashion [54,74], via the armadillo repeat region of β-catenin. The armadillo repeat region, whose three-dimensional structure was recently determined [75••], was shown to generate a groove enriched in basic amino acids that confer a positively charged domain to which highly acidic areas in the binding sites of E-cadherin, APC or LEF/TCF bind. By blocking the groove, these proteins may exclude the association of the other partners with β-catenin.

Mutational analysis has indicated that the cell-adhesive effects of β-catenin and those regulating gene expression (signaling) can be separated [42,76,77]. Wnt signaling, however, was shown to influence cell–cell adhesion and cell morphology in mammalian cells [45•,78,79] and recent reports indicate that adhesive interactions of β-catenin that sequester it to junctions can strongly antagonize its signaling activity in *Xenopus* by reducing the level of free β-catenin [76,80•]. In addition, Wg signaling induced by Dsh overexpression in cultured cells was shown to transcriptionally activate the expression of the E-cadherin gene [81•] which has a LEF-1 binding site within its promoter [38]. Interestingly, an integrin linked kinase that modulates integrin-dependent cell–matrix adhesion was shown to influence cell–cell adhesion by downregulating E-cadherin expression, followed by β-catenin translocation into the nucleus, complex formation of β-catenin with LEF-1 and activation of LEF-responsive transcription [82•]. Finally, the discovery of a new isoform of *Drosophila* armadillo with a different carboxyl terminus indicates that in *Drosophila*, armadillo plays an important role in both Wnt signaling and cell–cell adhesion at different stages during neural development [83•], further implying the existence of an interaction between Wg/Wnt signaling and cell adhesion.

Modulation of the expression of components in the molecular link between the cadherin–catenin system and other junctional plaque proteins with the actin cytoskeleton has often been observed in cancer cells (see [7•] for review). A significant advance in understanding the link between β-catenin-mediated regulation of gene expression and tumorigenesis was provided by the demonstration that transfection of APC can decrease the level of β-catenin [23] and subsequently, decrease β-catenin-mediated transactivation in colon cancer cells that express inactive, mutant APC [84••,85••]. These studies suggest that the tumor suppressor activity of APC is related to its ability to target β-catenin for proteolysis, and thus suppress the oncogenic transcription driven by β-catenin. Mutations in the APC gene appear to be common in colon cancer and other malignancies, leading to elevated β-catenin content and constitutive transcription of genes downstream in the Wnt signaling pathway; this transactivation can be demonstrated with a synthetic LEF/TCF reporter gene [85••]. In addition to APC mutations, in certain colorectal cancers and melanoma, mutations were discovered in β-catenin that result in its accumulation and the enhanced formation of β-catenin–LEF-1 complexes [86••] and enhanced transactivation [84••]. These mutations are of serine residues at the amino terminus of β-catenin, phosphorylation of which is involved in the regulation of β-catenin turnover [16,19••].

A common current view is that β-catenin can act as an oncogene by excessively activating gene(s) that directly contribute to tumor progression. Consequently, mutations in APC lead to accumulation of β-catenin and hence activation of the oncogenic process [70,87•]. This view is supported by studies showing that expression of int-1, the mammalian homolog of Wnt-1, by viral insertional activation in mice promotes tumor formation [88], and by the finding that a fragment of β-catenin lacking its amino terminus can transform NIH 3T3 cells [89]. Furthermore, the majority of the somatic mutations in APC that are found in colon cancers are in an area that regulates the binding and degradation of β-catenin [90•]. Mutations in APC and the N-terminus of β-catenin were recently found to be mutually exclusive in a large number of colorectal tumors, which is consistent with their equivalent effect on β-catenin stability [91•]. In addition to melanoma and colorectal cancers, mutations in the amino terminus of β-catenin were also discovered in sporadic medulloblastomas [92•] and ovarian carcinomas in humans [93•], and in azoxymethane-induced colon cancer in rats [94•].

The possible involvement of cadherin in the oncogenic activity of β-catenin raises some interesting points. On the one hand, cadherin binding may stabilize β-catenin and increase its level in cells (similar to mutations in APC). Yet, the cadherin-associated β-catenin is largely membrane-bound and thus unavailable for transcriptional activation. Indeed, it was recently demonstrated that the overexpression of cadherin or of α-catenin in a colon carcinoma cell line that accumulated high levels of β-catenin (due to mutations in APC) resulted in the inhibition of LEF/TCF-driven transactivation by the cytoplasmic sequestration of the nuclear β-catenin ([14•]; Figure 2c). Key unresolved questions are, first, what are the physiological mechanism(s) that regulate(s) the dissociation of the cadherin–catenin complex and the release of free, transactivation-competent β-catenin in normal cells, and second, is excessive dissociation of this complex also involved in β-catenin-induced tumorigenesis.

In contrast to β-catenin, its closely related homolog, plakoglobin, which also has a carboxy- terminal domain that can

function in transactivation [14•], was shown to suppress the tumorigenesis of cells that either possess or lack a cadherin–catenin system [55]. Moreover, the plakoglobin gene displays loss of heterozygosity in some sporadic breast and ovarian cancers [95] and its expression is often lost in tumor cells (for review, see [7•]). While the exact role of plakoglobin in Wg/Wnt signaling and its influence on tumorigenesis are unknown, possible mechanisms whereby plakoglobin affects the function of β-catenin can be suggested. The first involves competition of plakoglobin with β-catenin for cytoplasmic partners participating in the formation of adherens junctions, such as α-catenin and cadherin (Figure 2b). The release of β-catenin from this complex may lead to its rapid degradation in a cell harboring an APC-dependent degradation system ([14•,21•]; Figure 2a). Another possibility could involve the transcriptional activation, by a plakoglobin/LEF complex, of genes which, contrary to those activated by β-catenin, are tumor suppressive (Figure 2b). Finally, the possibility that plakoglobin can displace β-catenin from its association with LEF/TCF to form an inactive (plakoglobin–LEF/TCF) complex, and thus inhibiting transactivation, cannot be excluded at present.

While the activity of catenins in signaling events may indeed play a major role in their effect on tumorigenesis, β-catenin could affect tumorigenesis indirectly, by interacting with E-cadherin [96], vinculin, α-actinin, and α-catenin [97] (see [7•] for review). All these proteins were shown to have tumor suppressive effects when expressed in tumor cells that are deficient in these proteins, and to affect the organization of cell–cell adhesion in these cells (reviewed in [7•]). It is possible that such effects are attributable to the capacity of these junctional molecules to bind β-catenin and other junctional molecules, thus affecting both the structure and organization of cell adhesions and the expression of specific target genes which, together, are responsible for the various manifestations of the cancer cell phenotype.

Conclusion

The network of molecular interactions involving β-catenin and plakoglobin displays an intriguing complexity which, at present, is only partially understood. Major dilemmas and unresolved issues in this area are listed below.

The differential transcriptional specificity of β-catenin and plakoglobin has yet to be fully understood. While both proteins appear to be potent transactivators interacting with members of the LEF/TCF family of transcription factors, their specificities in binding and regulating target genes in mammalian cells are unknown. As signaling by β-catenin and plakoglobin is thought to result from their transactivation potential, unraveling the nature of their target genes is of utmost importance.

Cadherin could play a dual role in regulating catenin-driven signaling. On the one hand, it can stabilize these proteins (especially β-catenin), yet most of such stabilized molecules are transcriptionally inactive, being membrane-bound with their LEF/TCF-binding site blocked. A related key question is what are the physiological conditions which confer both a regulated release of catenins from the junctional sites and their controlled transport into the nucleus.

Plakoglobin–β-catenin relationships have yet to be fully characterized. Being so similar and sharing molecular partners, changes in the relative levels of the two proteins can trigger a variety of indirect responses. For example, overexpression of plakoglobin can release β-catenin from junctions and target it either for degradation (in cells containing a competent APC-related turnover system) or for transactivation (when the APC-degradation pathway is defective or inhibited by a Wnt signal). Excess plakoglobin may compete with β-catenin for APC binding and thus block β-catenin degradation. Other joint molecular partners are α-catenin, which can bind to both molecules in the cytoplasm, and transcription factors of the LEF/TCF family. Competition between the two molecules for α-catenin and LEF/TCF binding may also have dramatic, and very different, effects on catenin signaling. Better understanding of these aspects and their physiological relevance depends on direct characterization not only of the affinities of the two proteins towards their different cytoplasmic and nuclear partners, but also of the cellular mechanisms that may selectively modulate such interactions.

Finally, we should be aware of the diverse roles of the Wg/Wnt signaling components in the various organisms studied and of the complex interactions between them as demonstrated in the past year. A combination of genetic and biochemical characterization and microscopic studies will be necessary to shed light on this complex network of molecular interactions and their role in adhesion-mediated signaling.

Acknowledgements

We thank J Zhurinsky and M Shtutman for preparing the figures. Studies from the authors' laboratories were supported by grants from the USA-Israel Binational Foundation, the German-Israeli Foundation for Scientific Research and Development, by the Forchheimer Center for Molecular Genetics, and by the Cooperation Program in Cancer Research between The German Cancer Research Center and The Israeli Ministry of Science and Arts. A Ben-Ze'ev holds the Lunenfeld-Kunin Chair in Cell Biology and Genetics, and B Geiger holds the E Neter Chair in Cell and Tumor Biology.

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See annotation to [13•].

13. • Weiss EE, Kroemker M, Rudiger AH, Jockusch BM, Rudiger M: **Vinculin is part of the cadherin-catenin junctional complex: complex formation between α-catenin and vinculin.** *J Cell Biol* 1998, **141**:755-764.
This study and [12•] demonstrate that in addition to α-actinin, which was shown to be involved in linking the cadherin-catenin system to the actin cytoskeleton [11], vinculin can also fulfill this function. In one study [12•] this role of vinculin was observed in cells that lack α-catenin which is partially homologous to vinculin, suggesting that vinculin may substitute for α-catenin. In the second study [13•], the amino terminus of vinculin is shown to form complexes with the carboxyl terminus of α-catenin and both proteins are shown to be part of the cell–cell junctional complex.

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This study shows that vertebrate plakoglobin and β-catenin, the two homologs of *Drosophila* armadillo, differ in their nuclear translocation in response to lymphoid enhancer binding factor (LEF)-1 overexpression, and that β-catenin, when overexpressed, can also translocate vinculin into the nucleus. This suggests that vinculin and β-catenin can complex with each other (see also [12•,13•]). Plakoglobin was not translocated into the nucleus by LEF-1 overexpression and could not complex with vinculin. Plakoglobin has a carboxy-terminal transactivation domain that can act when fused to the Gal4 DNA binding domain, but is less efficient than β-catenin in LEF-responsive transactivation. Overexpressed plakoglobin can drive endogeous β-catenin into the nucleus, but not vice versa (see also [63•]). In colon carcinoma cells displaying constitutive LEF-responsive transactivation, the overexpression of N-cadherin or α-catenin could antagonize this transactivation by sequestering β-catenin to the cytoplasm.

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This paper demonstrates the involvement of the ubiquitin proteasome system in the regulation of β-catenin and plakoglobin degradation and that mutations in the glycogen synthase kinase 3β (GSK) phosphorylation sites stabilize β-catenin. It also shows that Wnt overexpression greatly reduces wild-type β-catenin ubiquitination.

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This study demonstrates that the induction of plakoglobin expression in cells lacking this protein results in the displacement of endogenous β-catenin from cell–cell junctions by competition for N-cadherin binding, and leads to an accelerated degradation of β-catenin by the ubiquitin-proteasome system. This study also shows that when the ubiquitin-proteasome pathway is blocked both plakoglobin and β-catenin accumulate in the nuclei of cells.

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This was the first report of the identification of axin, a new player in the Wnt signaling pathway. This study shows that deletion of the regulator of G-protein signaling (RGS)-domain of the molecule results in the activation of the Wnt pathway that is otherwise negatively regulated by axin, and also implies the involvement of G proteins in its action.

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See annotation to [31•].

27. • Kishida S, Yamamoto H, Ikeda S, Kishida M, Sakamoto I, Koyama S, Kikuchi A: **Axin, a negative regulator of the Wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of β-catenin.** *J Biol Chem* 1998, **273**:10823-10826.
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to form a quaternary complex involved in directing β-catenin to degradation. Axin is a negative regulator of the Wnt pathway by stimulating β-catenin turnover. The binding sites on axin for β-catenin, GSK and APC appear to be different and GSK-dependent phosphorylation of β-catenin is enhanced in this complex. Conductin, a homolog of axin, appears to act downstream of APC [29•], and like other members of the axin family has a negative effect on Wnt signaling. Axin was also shown to inhibit β-catenin-mediated transcription [25•].

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This paper describes the identification by a two hybrid screen of a new glycogen synthase kinase 3β (GSK) binding protein (GBP) that is highly homologous to members of the FRAT proto-oncogene family which are involved in T-cell lymphoma. When microinjected into *Xenopus* blastomers GBP inhibited GSK phosphorylation, elevated β-catenin levels and caused axis duplication. A highly conserved domain in GBP and FRAT is suggested to inhibit GSK and stimulate T-cell lymphoma proliferation. Inhibition of GSK by FRAT overexpression in T-cell lymphoma is suggested to induce IL-2 production and enhanced proliferation, providing another link between β-catenin and tumorigenesis.

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• **regulation by LEF-1 in response to Wnt-1 signaling and association with β-catenin.** *Mol Cell Biol* 1998, **18**:4807-4818.
This study identified an additional domain in mammalian β-catenin at the amino-terminus of the molecule, which is active in transactivation in cooperation with LEF-1. In addition, the authors show that in contrast to the findings in Madin-Darby canine kidney cells where LEF-1 transfection translocated the endogenous β-catenin into the nucleus [14•,39], in 3T3 cells the co-expression of Wnt-1 is necessary for translocation, suggesting the involvement of Wnt-1 in the control of β-catenin translocation in mammalian cells.

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The Wg responsive element in the *ultrabithorax* gene of *Drosophila* is shown to contain a lymphoid enhancer binding factor (LEF)-responsive element that binds both β-catenin and LEF-1 to activate its expression. This study also places LEF-1 in the Wg signaling pathway of *Drosophila*. In addition, it is shown that Wg and decapentaplegic signaling act synergistically to influence this process.

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•• Loureiro J, Ypma A, Hursh D, Jones T, Bejsovec A *et al.*: **Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*.** *Cell* 1997, **88**:789-799.
This study identified the *Drosophila* lymphoid enhancer binding factor (LEF)/T-cell factor (TCF) homolog, mapped its transactivation domain at the carboxyl terminus of the molecule, and demonstrated its necessity for transducing armadillo signaling. Interestingly, a mutant armadillo protein that could not bind *Drosophila* TCF was also capable of entering the nucleus, implying that armadillo can translocate into the nucleus independently of *Drosophila* TCF (see also [37••]).

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• **cytosolic β-catenin, and Tcf/Lef transcriptional activation in Rat-1 fibroblasts.** *Mol Cell Biol* 1998, **18**:2474-2485.
This study demonstrates a direct link between Wnt signaling and lymphoid enhancer binding factor (LEF)/T-cell-specific factor (TCF) by showing that Wnt overexpression in mammalian cells induces growth activation, changes in cell shape, an increase in β-catenin levels and LEF/TCF-induced transcription. Interestingly, overexpression of a 'stable' mutant β-catenin was unable to induce these effects, implying that Wnt induction of β-catenin elevation may function also by a LEF/TCF-independent pathway.

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• ***LEF-1* homologue that acts downstream of *armadillo* to transduce the Wingless signal in *Drosophila*.** *Nature* 1997, **385**:829-833.
This study identifies the *Drosophila* homolog of lymphoid enhancer binding factor (LEF)/T-cell-specific factor (TCF) demonstrates that it is part of the Wg signaling system and places it downstream of armadillo (see also [43••]).

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This study demonstrated that plakoglobin, similar to β-catenin, is capable of inducing Wnt signaling when overexpressed in *Xenopus* oocytes, even when it is tethered to the plasma membrane. It was suggested that plakoglobin could antagonize the negative effect of the *Xenopus* T-cell-specific factor homolog (XTCF) on Wnt signaling by sequestering XTCF and relieving its negative action on transcriptional activation of Wnt-responsive genes (for an alternative explanation, see [63•]).

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• **localization mutants of β-catenin during axis specification in *Xenopus*.** *J Cell Biol* 1997, **139**:229-243.
This study demonstrated that Wnt signaling ability in *Xenopus* oocytes can be induced by cytoplasmically localized constructs of β-catenin that could not accumulate in the nucleus. This was apparently achieved by competition and displacement of the endogenous β-catenin from its association with the APC-degradation system, followed by stabilization and nuclear translocation of the endogenous molecule. This is also suggested to explain the indirect way by which plakoglobin could induce signaling in similar experiments, in contrast to the conclusion reached in [62•].

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• **mammalian β-catenin and plakoglobin in *Drosophila*.** *J Cell Biol* 1998, **140**:183-195.
In this study human β-catenin and plakoglobin were compared for their ability to rescue the segment polarity phenotype of armadillo in *Drosophila*. While both proteins could rescue the mutant armadillo's adhesion properties, β-catenin had only a weak signaling activity and plakoglobin had none, suggesting that the two proteins differ in their signaling capacity.

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This review describes the divergence from the 'dogma' in the functions of components in the Wnt pathway, as revealed from studies in *Caenorhabditis elegans* development (see [66••,71••]) and some other studies.

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•• Priess JR, Mello CC: **Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos.** *Cell* 1997, **90**:707-716.
This study uses a novel technique, 'RNA-mediated interference', in *Caenorhabditis elegans* and identified homologues of components in the Wnt signaling system that operate in *C. elegans* development. Interestingly, the lymphoid enhancer binding factor (LEF)/T-cell-specific factor (TCF) components of the *C. elegans* Wnt system appear to act as antagonists to Wnt signaling as revealed in both this study and in the one described in [71••]. In addition, mutations in this pathway in *C. elegans* are associated with abnormalities in the mitotic spindle, implying a relationship between Wnt signaling and the cytoskeleton.

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• **Adenomatous polyposis 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.
Xenopus adenomatous polyposis coli (APC) is shown to induce Wnt signaling in *Xenopus* oocytes (including activation of the Wnt-responsive gene *Siamois*), contrary to its negative role in β-catenin-induced signaling in mammalian cells. This positive role of *Xenopus* APC in Wnt signaling is inhibited by depletion of β-catenin, suggesting that β-catenin is necessary for this effect of APC. The cytoplasmic level of β-catenin, however, is not altered by this activity of APC.

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• **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.
This paper described the identification of the *Drosophila* homolog of adenomatous polyposis coli (APC) that is shown to be able to bind β-catenin and direct it for degradation. Depletion of *Drosophila* APC, however, (including zygotic depletion) did not lead to changes in the level or distribution of *Drosophila* armadillo.

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•• **armadillo by *Drosophila* APC inhibits neuronal apoptosis during retinal development.** *Cell* 1998 **93**:1171-1182.
The authors demonstrate that a genetic inactivation of *Drosophila* APC results in degeneration of neuronal pigment cells in the *Drosophila* eye, similar to a known human disease associated with germline mutations in the APC gene. This degeneration results from apoptotic cell death. The phenotype could be rescued by reducing the level of *Drosophila* armadillo (by overexpression of Zw3), and partially by mutating *Drosophila* TCF. This suggests that elevated armadillo activates apoptosis by the armadillo/TCF complex. These studies also provide genetic evidence for the negative regulation of armadillo by APC as suggested for colon cancer [84••–86••].

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See annotation to [66••].

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• **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 homeobox gene *Siamois* that regulates axis specification in *Xenopus* is shown to contain a *Xenopus* T-cell-specific factor (TCF)-3 responsive sequence in its promoter and is a direct target for the β-catenin–TCF complex. This study also shows that in the absence of Wnt signaling (without β-catenin elevation) XTCF-3 can act as a repressor of transcription, in agreement with the views presented in [62•,72].

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This study determines the three-dimensional (crystal) structure of the armadillo repeat domain of β-catenin. The tightly packed right-handed α-helical structure forms a basic charged groove suggested to represent the binding site for acidic regions in the binding site of β-catenin partners such as E-cadherin, adenomatous polyposis coli (APC) and lymphoid enhancer binding factor (LEF)/T-cell factor (TCF).

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• **adhesion by an α-catenin mutant, and of the Wnt-signaling pathway by α-catenin in *Xenopus* embryos.** *J Cell Biol* 1997, **139**:1033-1046.
This study demonstrates that the amino-terminal domain of α-catenin (an adhesive partner of β-catenin) that harbors the β-catenin binding site can antagonize Wnt signaling in *Xenopus* embryos, probably by sequestering β-catenin from its interaction with its Wnt signaling partners.

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The notion that the E-cadherin promoter contains a lymphoid enhancer binding factor (LEF)-1 binding domain [38] prompted these investigators to activate the Wg signaling pathway in cultured *Drosophila* cells by overexpressing Dsh or its *Drosophila* homolog. They demonstrated that this leads to transcriptional activation of the *Drosophila* E-cadherin gene. The results support the view that Wg signaling can affect cell adhesion.

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The involvement of an integrin-linked kinase (ILK) in the regulation of cell–cell adhesion is reported in this study. ILK overexpression in epithelial cells results in downregulation of E-cadherin expression, nuclear translocation of β-catenin and transcriptional activation by the β-catenin/LEF-1 complex. Thus, a relationship between molecules regulating cell–extracellular matrix and cell–cell contact and gene expression is demonstrated.

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This study demonstrates that in certain colon carcinoma cell lines the level of β-catenin is elevated owing to mutations on amino-terminal serine residues of β-catenin which are important for regulating its degradation. Such mutant β-catenin molecules are insensitive to adenomatous polyposis coli (APC)-directed degradation of β-catenin and the transactivation driven by the lymphoid enhancer binding factor (LEF)/T-cell specific factor (TCF)–β-catenin complex in such cell lines is not inhibited by transfected APC.

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This study shows that there is constitutive β-catenin-driven LEF/TCF-dependent transactivation in adenomatous polyposis coli (APC)$^{-/-}$ colon carcinoma cell lines and transfection of wild-type APC can block this transactivation.

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