Binding to Cadherins Antagonizes the Signaling Activity of β-Catenin during Axis Formation in *Xenopus*

François Fagotto, Noriko Funayama, Ursula Glück, and Barry M. Gumbiner

Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York 10021

Abstract. β-Catenin, a cytoplasmic protein known for its association with cadherin cell adhesion molecules, is also part of a signaling cascade involved in embryonic patterning processes such as the determination of the dorsoventral axis in *Xenopus* and determination of segment polarity in *Drosophila.* Previous studies suggest that increased cytoplasmic levels of β-catenin correlate with signaling, raising questions about the need for interaction with cadherins in this process. We have tested the role of the β-catenin–cadherin interaction in axis formation. Using β-catenin deletion mutants, we demonstrate that significant binding to cadherins can be eliminated without affecting the signaling activity. Also, depletion of the soluble, cytosolic pool of β-catenin by binding to overexpressed C-cadherin completely inhibited β-catenin–inducing activity. We conclude that binding to cadherins is not required for β-catenin signaling, and therefore the signaling function of β-catenin is independent of its role in cell adhesion. Moreover, because β-catenin signaling is antagonized by binding to cadherins, we suggest that cadherins can act as regulators of the intracellular β-catenin signaling pathway.

CELLS respond to environmental stimuli by generating intracellular signaling cascades, which modify cellular functions including gene expression. While soluble molecules such as growth factors are classical examples of such stimuli, it is obvious that cells also sense direct physical contact, both with the substrate and with neighboring cells. For instance, it is well established that cell–substrate adhesion, mediated by membrane receptors of the integrin family, is at the same time both a source and recipient of intracellular signals (Schwartz et al., 1995). The integration of cell–cell adhesion events and intracellular signals is less well understood. However, cell–cell adhesion also appears to be both regulatable (Brieher and Gumbiner, 1994; Fleming and Johnson, 1988; Williams et al., 1993), and to be able to modulate other cellular functions (Doherty et al., 1991; Gumbiner et al., 1988; Nelson, 1992).

Catenins are cytoplasmic proteins directly associated with the cadherins, which they link to the actin cytoskeleton (Kemler, 1993; Rimm et al., 1995). However, studies on developmental systems have revealed that β-catenin exhibits activities that seem to extend beyond its role as a linking molecule. In *Drosophila*, mutations in the β-catenin homologue, Armadillo, give rise to defects in segment polarity (Peifer, 1995). In *Xenopus*, injection of anti–β-catenin antibodies (McCrea et al., 1993) or of β-catenin mRNA (Funayama et al., 1995) in the ventral side of normal embryos induces the formation of secondary dorsal structures, and similar injections rescue a body axis in UV-treated, ventralized embryos (Funayama et al., 1995; Guger and Gumbiner, 1995). Furthermore, partial depletion of maternal β-catenin by injection of antisense oligonucleotides in oocytes causes eggs to develop into ventralized embryos (Heasman et al., 1994), demonstrating that endogenous β-catenin is required for the establishment of the normal dorsoventral axis. In both *Xenopus* and *Drosophila*, β-catenin/Armadillo appears to be part of a signaling cascade initiated by secreted proteins of the Wnt/wingless family (Gumbiner, 1995; Peifer, 1995).

Because of the association of β-catenin with cadherins, it has been proposed that the primary role of the Wnt/wg–β-catenin/Armadillo pathway is to modulate cell–cell adhesion (Hinck et al., 1994). Wnts appear to increase the cellular levels of β-catenin and plakoglobin, a closely related molecule, and high levels of β-catenin or plakoglobin correlate with tighter adhesion in some cell types (Bradley et al., 1993; Hinck et al., 1994; Guger and Gumbiner, 1995). One then could imagine that changes in cell adhesion may affect other cell–cell interactions, and thus influence the fate of embryonic cells. However, simply increasing cell–cell adhesion does not seem to account for the patterning activity of β-catenin: enhancing cell adhesion in the ventral side of early *Xenopus* embryos by overexpression of C-cadherin, the major cadherin present at these stages, does not cause axis duplication (Guger and Gum-

Please address all correspondence to B. Gumbiner, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 564, New York, 10021. Tel.: (212) 639-6147. Fax: (212) 717-3047.

Drs. F. Fagotto and N. Funayama both contributed equally to this work.

The current address of N. Funayama is Faculty of Science, Kitasato University, Kitasato, Japan.

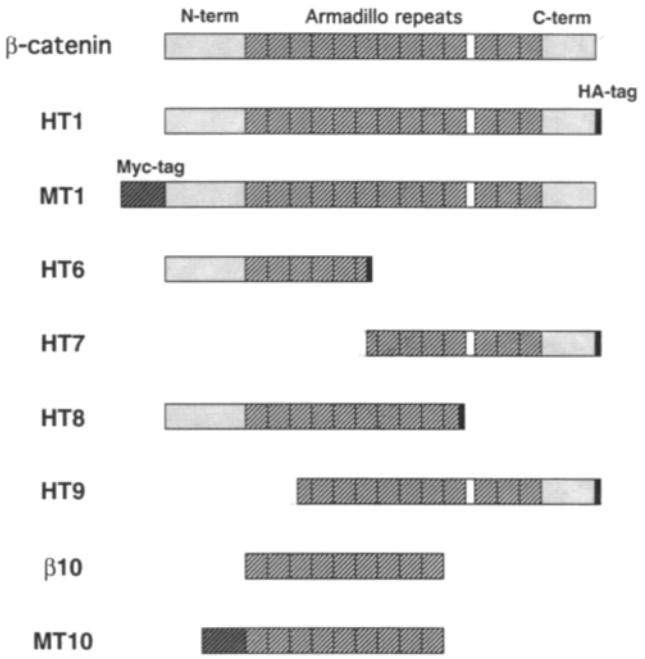

Figure 1. Synopsis of the β-catenin mutants used in this work. Prefix β, untagged; prefix HT, HA-tagged; and prefix MT, myc-tagged. β-Catenin sequence contains a gap (*white case*) between repeats 10 and 11. See Materials and Methods for the precise coding sequence of each construct.

biner, 1995). On the contrary, global overexpression of cadherins in the oocyte inhibits normal axis formation (Heasman et al., 1994). In fact, there is evidence that β-catenin/armadillo has an intracellular signaling activity independent of its role in cell adhesion. In *Drosophila*, mutations of Armadillo reveal that its signaling and adhesive properties of Armadillo are separable (Peifer, 1995). In *Xenopus*, axis formation could be induced by expression of β-catenin variants which are probably not functional in cell adhesion (Funayama et al., 1995). Furthermore, immunofluorescence localization of these β-catenin variants has revealed that they accumulate in the nucleus (Funayama et al., 1995). A similar nuclear accumulation has been observed with plakoglobin, a desmosomal protein highly homologous to β-catenin, which is also able to induce axis duplication in *Xenopus* (Karnovsky and Klymkowsky, 1995). Finally, β-catenin also associates with the Adenomatous Polyposis Coli protein (APC), the product of a tumor suppressor gene, independently of the cadherin-catenin complexes (Rubinfeld et al., 1993; Su et al., 1993; Polakis, 1995). Taken together, these observations suggested that β-catenin has intracellular targets other than cadherins at the plasma membrane.

In the present paper, we address directly the relationship between cadherin-mediated cell–cell adhesion and β-catenin signaling, by analyzing β-catenin mutants with low or no affinity for cadherins, and by examining the effect of sequestering β-catenin to the plasma membrane by coexpressing C-cadherin. The results of these experiments show that binding to cadherins is not required for the inducing activity of β-catenin and, on the contrary, that the association of β-catenin with cadherins inhibits its signaling activity.

Materials and Methods

Plasmids and mRNA Injections

The constructs used in this work are presented in Fig. 1. The coding region of the full-length β-catenin was inserted into two different vectors: into pSP36T (Amaya et al., 1991), with a seven-amino acid hemagglutinin (HA)[1] tag (prefix HT) sequence (DVPDYAS) at its COOH terminus (HT1); or into CS2+ (Rupp et al., 1994; Turner and Weintraub, 1994), containing a sequence coding for five myc epitopes (prefix MT) at the NH_2 terminus (MT1). Mutants HT6 to HT9 were subcloned into pSP36T and contain the β-catenin-coding regions corresponding to amino acids 1 to 366 (HT6), 364 to 781 (HT7), 1 to 541 (HT8), and 232 to 781 (HT9), always with an HA tag sequence at the COOH terminus. β10 (amino acids 149 to 525) lacks the HA tag. MT10 encodes the same fragment as β10 (149-525) but was inserted into the CS2+ vector with an NH_2-terminal five myc tag sequence. The full-length C-cadherin sequence was inserted into pSP36T (Lee and Gumbiner, 1995).

mRNAs were synthesized in vitro using the SP6 RNA polymerase (Promega Corp., Madison, WI) and were dissolved at the appropriate concentration in DEPC-treated water. 10–20 nl mRNA were injected at the 4–8-cell stage. The amounts of mRNA injected in the various experiments are indicated in the corresponding tables and figures. Embryos were allowed to develop at room temperature in 0.1 × MMR (Kay and Peng, 1991). Axis duplication was scored at the neurula stage, and the Dorso-Anterior Index (DAI) (Kao and Elinson, 1988) at tadpole stages. Numbers presented in Tables I, IV, and V are pooled from 2 to 4 experiments. Tables II and III present data of individual experiments.

Immunoprecipitation

Immunoprecipitation of HA-tagged β-catenin variants from *Xenopus* extracts was performed as previously described (Funayama et al., 1995), using the anti-HA-tag mAb 12CA5. Extraction and immunoprecipitation buffer contained 1% NP-40, 150 mM NaCl, 10 mM Hepes, pH 7.4, 2 mM EDTA, but in some experiments, samples were immunoprecipitated in RIPA buffer (0.2% SDS, 1% Triton X-100, 0.5% deoxycholate, 150 mM NaCl, 20 mM Hepes, pH 7.5). Immunoprecipitates were analyzed by immunoblot, using the ECL Western blot development protocol (Amersham Corp., Arlington Heights, IL), after separation by SDS-PAGE (7.5% acrylamide) for detection of β-catenin and C-cadherin, or SDS-agarose gel electrophoresis (1% agarose) for APC (Smith et al., 1993). The antibodies used for blotting were anti–C-cadherin pAb (Brieher and Gumbiner, 1994), anti–human APC pAb α3 (Rubinfeld, 1993), and a mixture of anti–NH_2 terminus β-catenin pAb and of anti-COOH terminus β-catenin pAb (Funayama et al., 1995) (McCrea et al., 1993).

Myc-immunoprecipitation was similarly performed, except that anti–myc-tag mAb 9E10.2 was directly linked to protein G–Sepharose beads (Protein G–Sepharose 4 Fast Flow, Pharmacia Biotech, Uppsala, Sweden) using dimethylsuberimidate as a cross-linker (Pierce Chem. Co., Rockford, IL). Each sample was precipitated using the equivalent of 10 μg of 9E10.2 IgG. Immunoprecipitates were separated on 9% acrylamide SDS-PAGE gels, and C-cadherin was detected using a pAb raised against the extracellular domain of *Xenopus* C-cadherin.

Immunofluorescence

Indirect immunofluorescence on frozen sections was performed as previously described (Fagotto and Gumbiner, 1994). The anti-HA mAb 12CA5 and the anti-myc mouse mAb 9E10.2 (both ascites fluids) were diluted 1:1,000, and the FITC-conjugated goat anti–mouse secondary antibody was diluted to 50 mg/ml (Molecular Probes, Inc., Eugene, OR). Nuclei were stained with HOECHST 33342 (Molecular Probes).

Cell Fractionation

All steps were performed at 4°C. 20 embryos, stage 8 1/2, were homogenized in 500 μl of 250 mM sucrose, 10 mM Hepes, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM EDTA, pH 7.2, containing a cocktail of proteinase inhibitors (Fagotto and Gumbiner, 1994). To separate the soluble from the sedimentable fraction, homogenates were first spun at 750 *g* for 5 min in an

1. *Abbreviations used in this paper*: APC, Adenomatous Polyposis Coli; DAI, dorso-anterior index; HA, hemagglutinin; HT, hemagglutinin tag; MT, myc tag; pAb, polyclonal antibody.

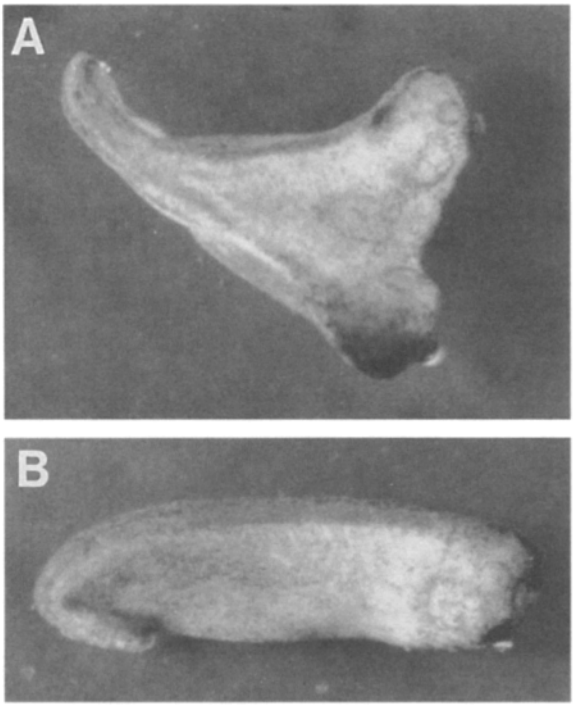

Figure 2. Assay for β-catenin signaling: axis duplication in Xenopus embryos. (*A*) Example of tadpole with two complete body axes, including two heads, obtained by injection of 0.75 ng of a truncated β-catenin mutant mRNA, MT10, in the ventral side of a cleaving embryo. (*B*) Normal tadpole developed from an uninjected embryo.

Eppendorf centrifuge (Netheler and Hinz GmbH, Hamburg, Germany) to remove yolk platelets, and the supernatant was then spun at 100,000 *g* for 50 min in a tabletop ultracentrifuge TL-100 (Beckman Instrs. Inc., Fullerton, CA) using a 100.3 rotor. The final supernatant (fraction S, soluble) was removed and concentrated by acetone precipitation. The two membrane-containing pellets of the first and second spin were extracted in 1% NP-40, 150 mM NaCl, 20 mM Hepes-NaOH, 1 mM EDTA with proteinase inhibitors. These two extracts were combined (total volume 500 μl), and glycoproteins (fraction G) were purified by binding to Con A–Sepharose B (Sigma Chem. Co., St Louis, MO) (1 h incubation with 160 μl Con A–Sepharose slurry, equivalent to ~1 mg Concanavalin). The Con A beads were washed twice, and the bound proteins (fraction G) were solubilized by boiling the beads in SDS-PAGE sample buffer. Fraction G contains the cadherin-associated pool of β-catenin, since cadherins are quantitatively recovered in the Con A–bound fraction. The fraction not bound to Con A (fraction P) contains the particulate, but noncadherin-bound β-catenin, which is probably associated with nonglycosylated membrane proteins or with the cytoskeleton. Fraction P was concentrated by acetone precipitation. Fractions were analyzed by SDS-PAGE (7.5% acrylamide) and immunoblots.

Results

Binding to Cadherin Is Not Required for β-Catenin Inducing Activity

We used deletion mutants of β-catenin to test whether a loss of β-catenin binding to cadherins would have an effect on its signaling activity in early *Xenopus* embryos. Signaling activity was assayed by scoring axis duplication at neurula stage in ventrally injected embryos. An example of a double-axis tadpole obtained by injection of a β-catenin mutant mRNA (MT10) is shown in Fig. 2.

To design β-catenin variants, we took advantage of previous studies that mapped the regions required for interaction with other known proteins (Hülsken et al., 1994; Funayama et al., 1995). β-Catenin can be divided in three regions: the NH_2-terminal domain, a central region containing 13 imperfectly conserved tandem repeats called armadillo repeats (Peifer et al., 1994*b*), and the COOH-terminal domain. The armadillo repeat region binds both cadherins and APC and is also sufficient for axis duplication (Funayama et al., 1995), while the NH_2-terminal region binds to α-catenin. It has also been reported that the cadherin and APC-binding sites in the repeat region overlap but do not coincide (Hülsken et al., 1994) and are separable. A mutant with a truncation of the COOH terminus and the last three armadillo repeats bound to an APC fragment (amino acids 957 to 1211), but not to E-cadherin, whereas a mutant lacking the NH_2 terminus and the first three armadillo repeats bound to E-cadherin but not to the APC fragment. Therefore, we first made similar HA-tagged constructs (called HT8 and HT9, see synopsis of the constructs used in Fig. 1) to test for axis duplication activity, as well as for interaction with endogenous C-cadherin and APC in the *Xenopus* embryo. We also tested two shorter constructs corresponding to both halves of β-catenin (HT6 and HT7) which are not expected to bind to either cadherins or APC (Hülsken et al., 1994).

Table I. Frequency of Axis Duplication by β-Catenin Deletion Mutants

Construct	Duplicated axis embryos (No./total)	Duplicated axis embryos Percent
		%
HT6	0/101	0
HT7	0/175	0
HT8	126/172	73
HT9	85/109	78
β10	36/42	86

Axis duplication was scored at neurula stage. Embryos were injected with 3 ng mRNAs, except for β10, which was 0.6 ng. β10 is the untagged version of the MT10 mutant used in most other experiments.

Both HT8 and HT9 were found to be potent inducers of axis duplication (Table I), demonstrating that a portion of the repeat region of β-catenin is sufficient for signaling. Signaling activity was lost, however, when the armadillo repeat region was cut into half, either NH_2-terminal or COOH-terminal (HT6 and HT7).

From previous reports using E-cadherin in culture cell lines, it was expected that HT8 should not associate with C-cadherin (Hülsken et al., 1994). However, we found that some C-cadherin coimmunoprecipitated with HT8 (Fig. 3 *b*). This association appeared weaker than for HT9 or full-length β-catenin because HT8–C-cadherin complexes could only be immunoprecipitated in buffers containing mild nonionic detergents (1% NP-40, Fig. 3 *b*). HT8–C-cadherin complexes were disrupted under harsher conditions (RIPA buffer, Fig. 3 *c*), which do not affect binding of cadherins to full-length β-catenin (McCrea and Gumbiner, 1991) or to HT9. We do not know whether, in mild detergents, HT8 interacts directly with C-cadherin or indirectly through some other component of the complex. HT6 and HT7 did not associate with C-cadherin under any condition.

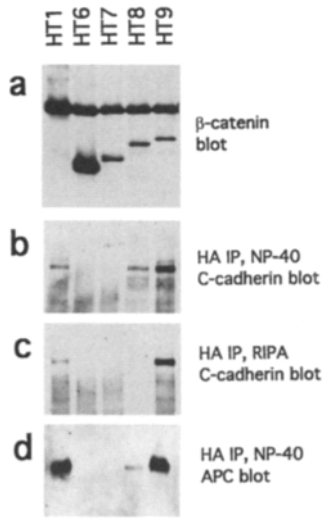

Figure 3. Association of mutants HT6-HT9 with endogenous C-cadherin and APC. (*a*) Expression of full-length β-catenin (*HT1*) and mutants HT6-HT9 in *Xenopus* embryos injected with 3 ng mRNA of each construct. The mutants were detected with a mixture of two pAb raised against the NH_2 and COOH terminus of *Xenopus* β-catenin, respectively. (*b*) Detection of C-cadherin in β-catenin mutant immunoprecipitates under mild conditions (nonionic detergent). Mutants were immunoprecipitated with anti-HA mAb 12CA5 in the presence of 1% NP-40. C-cadherin is associated with HT1, HT8 and HT9, but not HT6 and HT7. (*c*) Detection of C-cadherin in β-catenin mutant immunoprecipitates under harsher conditions (anionic detergent). Mutants were immunoprecipitated with anti-HA mAb 12CA5 in the presence of RIPA buffer, containing 0.2% SDS, 1% Triton X-100 and 0.5% deoxycholate. C-cadherin is associated with HT1 and HT9, but not HT6, HT7, nor HT8. (*d*) Detection of APC in β-catenin mutant immunoprecipitates. Mutants were immunoprecipitated with the anti-HA mAb 12CA5 in the presence of NP-40. C-cadherin is associated with HT1, HT8 and HT9, but not HT6 and HT7.

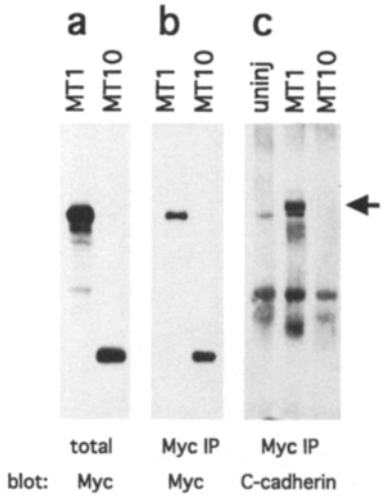

Figure 4. Lack of association between MT10 and C-cadherin. (*a*) Expression of full-length β-catenin (MT1) and MT10, containing only the first nine armadillo repeats, in *Xenopus* embryos injected in the two ventral blastomeres of a 4-cell stage embryo with 2 × 3 ng mRNA. MT1 and MT10 were detected with the anti-myc tag mAb 9E10.2. (*b*) Detection of MT1 and MT10 after immunoprecipitation with anti-myc mAb 9E10.2 in the presence of 1% NP-40. (*c*) Detection of C-cadherin in MT1 and MT10 immunoprecipitates (1% NP-40). The first lane shows a control immunoprecipitation from uninjected embryos. C-cadherin (*arrow*) is associated only with full-length β-catenin, but not MT10.

The weaker association of HT8 with C-cadherin suggested that binding to cadherins is not required for the inducing activity. Nevertheless we continued to screen for mutants that would exhibit no association with C-cadherin. A shorter deletion mutant consisting of only the first nine repeats (β10 or MT10, see Fig. 1) was found to have such characteristics. MT10, the myc-tagged version of β10, did not associate with C-cadherin by immunoprecipitation, even under mild detergent conditions (1% NP-40, Fig. 4). Nevertheless, both β10 (untagged) and MT10 (myc-tagged) caused axis duplication with high efficiency (Tables I and IV). We conclude, therefore, that β-catenin signaling can occur independently of its stable association with cadherin-catenin complexes.

Hülsken et al. (1994) reported that a mutant similar to HT9 did not bind to APC. Since this construct retains axis duplication activity, we analyzed HT8 and HT9 immunoprecipitates for the presence of APC. However, endogenous APC coimmunoprecipitated with both HT8 and HT9 in *Xenopus* embryos (Fig. 3 *d*). This may differ from the previous report because we examined binding to full-length APC rather than to the fragment of APC (amino acids 957 to 1211) that was used in this report. That fragment contains only one of the two known β-catenin–binding sites in the full-length APC molecule (Polakis, 1995). Therefore, the requirement for interaction with APC in β-catenin signaling cannot be evaluated using these mutants.

C-cadherin Overexpression in the Dorsal Side Inhibits Normal Axis Formation

If axis induction results from a molecular interaction of β-catenin independent of its association with cell adhesion complexes, increased binding to cadherins should compete with this signaling activity. Heasman and colleagues (Heasman et al., 1994) found that axis formation could be perturbed by global overexpression of cadherins in oocytes, which suggested that cadherins might indeed compete with β-catenin in this process. We addressed this possibility directly by increasing the levels of C-cadherin in embryos, either at the precise site where the dorsalizing induction normally occurs (this section of Results), or at the site where a secondary axis is experimentally induced by expression of exogenous β-catenin (next section of Results).

To examine the effect of C-cadherin overexpression on normal axis formation, 4–5 ng of C-cadherin mRNA was injected in the most vegetal portions of both the dorsal blastomeres at the 4–8-cell stage. This is the location of the active-inducing region, called the Nieuwkoop center. As a control, the two ventral blastomeres were injected with similar amounts of C-cadherin mRNA. While control injections in the ventral side had no effect on the normal development, embryos injected dorsally developed into tadpoles deficient in anterior and dorsal structures (Fig. 5). The phenotypes observed were typical of so-called ventralized embryos, which can be obtained for example by early UV-treatment (Kao and Elinson, 1988). The extent of dorsal axis formation was quantitatively assessed using the dorso-anterior index (DAI) of Kao and Elinson (1988). On average, C-cadherin–injected embryos had a value of approximately two on the DAI scale (Table II), where zero corresponds to completely ventralized embryos, and five to normal embryos. This result shows that inhibition of axis formation is an immediate effect of in-

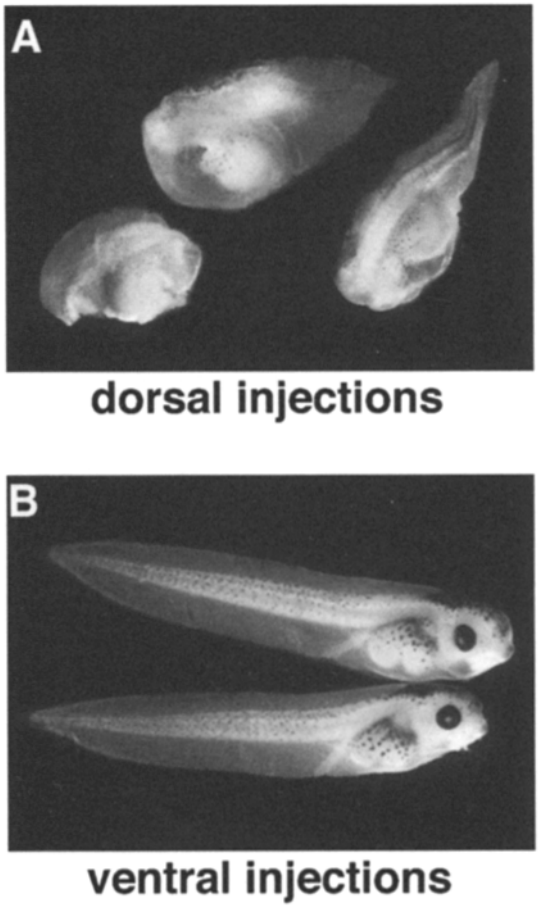

Figure 5. Ventralization of *Xenopus* embryos by overexpression of C-cadherin in the dorsal inducing region. (*A*) When two dorsal blastomeres of 4-cell stage embryos were injected at the vegetal pole with 4–5 ng C-cadherin mRNA, the embryos developed into ventralized tadpoles (no head, diminished dorsal structures, hypertrophy of ventral tissues). (*B*) After injections of the same RNA in the ventral side, the embryos developed normally.

creased levels of C-cadherin, and that inhibition by C-cadherin occurs directly in the known signaling region.

In contrast, when C-cadherin is overexpressed in the marginal zone of the embryo, gastrulation defects are observed (Lee and Gumbiner, 1995), probably due to increased adhesion between these moving cells. In our experiments, however, ventralization was a distinct effect, since C-cadherin–injected embryos gastrulated normally. Injections were targeted to a lower region very close from the vegetal pole, which, unlike the marginal zone, does not play any active role in the gastrulating movements (Keller and Winklbauer, 1992).

Coinjection of C-cadherin mRNA Inhibits β-Catenin-induced Axis Duplication and Depletes the Pool of Cytosolic β-Catenin

To examine whether C-cadherin directly inhibits β-catenin signaling, we tested the effect of coexpressing excess C-cadherin on the capacity of exogenous β-catenin to induce a secondary axis. Injection of full-length, HA-tagged β-catenin (HT1) mRNA (0.3 ng) alone induced a secondary axis at very high frequency. In contrast, coinjection of

Table II. Ventralization of Xenopus Embryos Injected with C-cadherin mRNA

Site of injection	Ventralized embryos (No./total)	Ventralized embryos Percent	Average dorso-anterior index
4 cell-stage			
dorsal	20/20	100	2.2
dorsal	19/20	95	1.9
ventral	0/7	0	5.0
dorsal	16/16	100	2.3
ventral	2/14	14	4.9
		Average	
Dorsal		98	2.2
Ventral		7	5.0
8 cell-stage			
dorsal	36/46	72	3.2
ventral	1/27	4	5.0
dorsal	17/21	81	3.0
ventral	0/23	0	5.0
		Average	
Dorsal		76	3.1
Ventral		2	5.0

4–5 ng C-cadherin mRNA was injected vegetally in each of the two dorsal or ventral blastomeres at either the 4-cell or 8-cell stage. The degree of ventralization (DAI, dorso-anterior index) was scored at early tadpole stages. 5, normal; 0, completely ventralized.

4–5 ng C-cadherin mRNA with the same amount of β-catenin mRNA completely prevented axis duplication (Table III and Fig. 6). Control experiments demonstrated that inhibition of axis duplication was specific: coinjection of 5 ng of β-galactosidase mRNA did not interfere with the inducing activity of β-catenin (Table III). Axis duplication could be rescued by expressing higher levels of β-catenin (1–1.5 ng, Table IV). This demonstrates that inhibition of axis duplication by C-cadherin was due to a specific action on β-catenin signaling rather than to a nonspecific perturbation of the inducibility of the injected cells.

To determine the effect of C-cadherin overexpression on the cellular distribution of the exogenous β-catenin, a myc-tagged β-catenin construct (MT1), expressed either

Table III. Inhibition of β-catenin–induced Axis Duplication by C-cadherin

	β-catenin		β-catenin + C-cadherin	
	Duplicated axis embryos			
	Duplicated axis embryos (No./total)	Percent	(No./total)	Percent
Exp. 1	12/13	92	0/15	0
Exp. 2	42/44	95	0/24	0
Exp. 3	13/16	81	0/17	0
	β-catenin		β-catenin + β-galactosidase	
	17/20	85	18/23	78

Embryos were injected with 0.3 ng β-catenin mRNA (HT1), 0.3 ng β-catenin mRNA and 4–5 ng C-cadherin mRNA, or 0.3 ng β-catenin mRNA and 5 ng β-galactosidase mRNA.

Table IV. High Levels of Expression of β-Catenin Overcome the Inhibition of Axis Duplication by C-cadherin

Amount of mRNA injected	Duplicated axis embryos	
	(No./total)	Percent
0.3 ng β-catenin	21/23	91
0.3 ng β-catenin + 4–5 ng C-cadherin	0/26	0
1 ng β-catenin + 4–5 ng C-cadherin	15/16	94
1.5 ng β-catenin + 4–5 ng C-cadherin	21/28	75

alone or with C-cadherin, was localized in late blastulae-early gastrulae by immunofluorescence microscopy of frozen sections. This construct was used because the detection of the myc tag was more sensitive than for the HA tag used in the previous paper (Funayama et al., 1995) and in the competition experiments described above. Although higher amounts of this construct mRNA (1–1.5 ng) were required to obtain comparable percentages of duplicated axis, axis duplication was also completely inhibited by coinjection of 4–5 ng of C-cadherin mRNA (Table V).

In embryos injected with β-catenin alone, the exogenous protein was found throughout the cytoplasm (i.e., between the large yolk platelets) and enriched at the cell periphery and in the nucleus (Fig. 7 *A*), consistent with our previous data using the HA-tagged construct (Funayama et al., 1995). In embryos injected with both β-catenin and C-cadherin (Fig. 7 *B*), the pattern of myc-tagged β-catenin was drastically different: a very strong signal was detected at the cell membrane, as well as associated with intracellular vesicles, probably belonging to the secretory pathway, while both the cytoplasm and the nucleus were depleted of β-catenin.

Changes in subcellular distribution of exogenous β-catenin in the presence of excess C-cadherin were also analyzed biochemically using a cell fractionation procedure. Homogenates of embryos (prepared without detergent) were first centrifuged to separate the soluble proteins (fraction S) from a sedimentable fraction. The latter was then extracted with a nonionic detergent (1% NP-40) and incubated with Con A beads. Most membrane glycoproteins, and in particular all cadherins, bind to the Con A beads under these conditions (not shown). β-Catenin recovered in the Con A–bound fraction was thus defined as the membrane glycoprotein-associated pool. The fraction not bound to Con A could contain either cytoskeletal-associated or nonglycosylated membrane protein-associated pools of β-catenin. APC–β-catenin complexes are recovered in this fraction (Fagotto, F., and B.M. Gumbiner, unpublished), consistent with their probable association with cytoskeletal elements, such as microtubules (Munemitsu et al., 1994; Smith et al., 1994). Our procedure thus allowed us to estimate the relative distribution of β-catenin into soluble (Fig. 8, *S*), glycoprotein-associated (*G*), and other particulate, noncadherin-bound pools (*P*). A nuclear fraction of β-catenin could not be obtained because nuclei of early embryos are leaky; thus, nuclear β-catenin was probably recovered as part of the soluble fraction. When β-catenin (MT1) mRNA was injected alone,

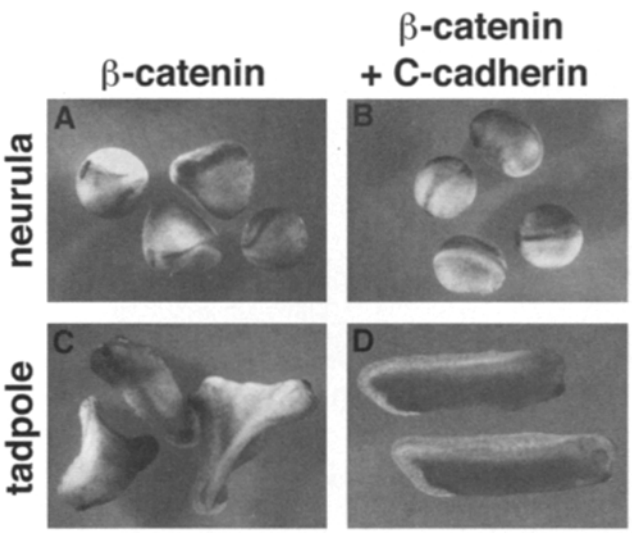

Figure 6. Inhibition of β-catenin–induced axis duplication by coexpression of C-cadherin. (*A* and *C*) Embryos injected with 0.3 ng full-length β-catenin (HT1) mRNA developed duplicated axis, visible at neurula stage by the two pigmented neural tubes (*A*) and at tadpole stage as two complete body axis, including two heads. (*B* and *D*) Embryos coinjected with 0.3 ng HT1 and 5 ng C-cadherin mRNA have only one axis (*B*) and develop into normal tadpoles (*D*).

comparable amounts of exogenous β-catenin were recovered in the three fractions (Fig. 8 *a*). When coexpressed with C-cadherin, β-catenin was much more abundant in the membrane glycoprotein fraction (*G*), while the soluble pool (*S*) was depleted (Fig. 8 *b*). The levels in the particulate noncadherin bound fraction (*P*) did not change detectably. Therefore, overexpression of C-cadherin sequesters β-catenin to a membrane fraction enriched in glycoproteins.

Since MT10 does not associate with C-cadherin in *Xenopus* embryos (Fig. 4), C-cadherin overexpression should not be able to inhibit its signaling activity, nor should it be able to recruit it to the plasma membrane. Therefore, the coinjection experiments described above were also performed with MT10 or β10 (MT10 without tag), and the cellular distribution of MT10 was examined in embryos expressing MT10 alone or coexpressed with C-cadherin. Axis duplication by β10/MT10 was only weakly inhibited

Table V. Axis Duplication by MT10/β10 Is Resistant to Inhibition by C-cadherin

	−C-cadherin		+C-cadherin	
	Duplicated axis embryos			
	(No./total)	Percent	(No./total)	Percent
1.5 ng MT1	48/59	81	0/58	0
0.3 ng HT8	53/64	83	0/76	0
1.5 ng MT10	26/29	90	39/43	91
0.75 ng MT10	25/26	96	23/39	60
0.3 ng β10	27/30	90	19/53	36

Embryos were injected with β-catenin construct mRNAs alone (−C-cadherin) or coinjected with 5 ng C-cadherin mRNA (+C-cadherin).

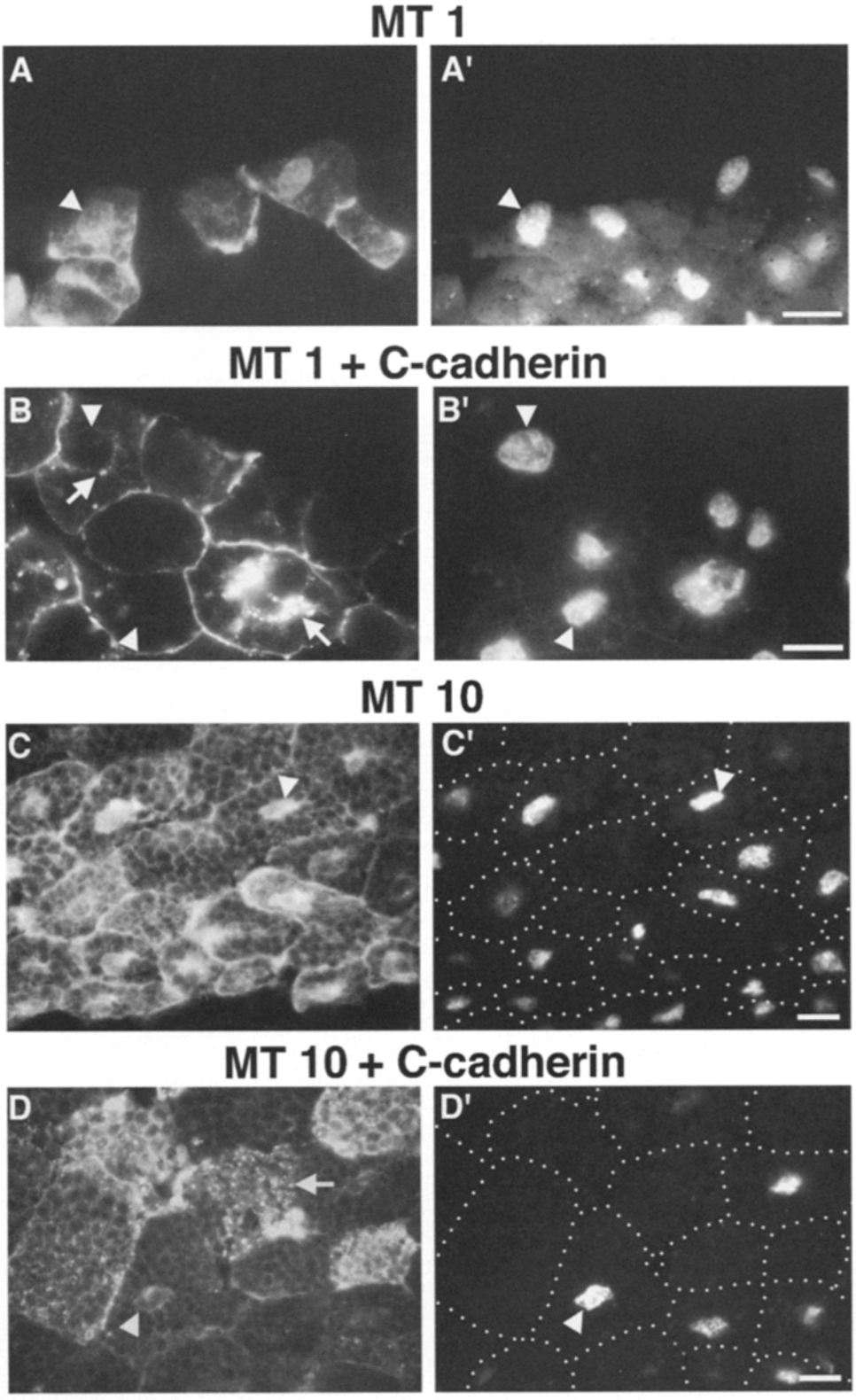

Figure 7. Redistribution of full-length β-catenin (MT1) but not MT10 by coexpression of C-cadherin. Full-length β-catenin (MT1) or MT10 (both 1.5 ng mRNA), expressed alone (*A* and *C*) or coexpressed with excess C-cadherin (5 ng mRNA) (*B* and *D*) were immunolocalized at early gastrula stages by indirect immunofluorescence on frozen sections with anti-myc mAb 9E10.2. (*A'–D'*) HOECHST staining to visualize nuclei on the same sections. Cell–cell boundaries are outlined with dotted lines in panels *C'* and *D'*. (*A*) Full-length MT1 expressed alone is detected in the cytoplasm (i.e., between the yolk platelets, which appear as large dark granules), and accumulates at the plasma membrane and in the nuclei (*arrowheads*). (*B*) In the presence of excess C-cadherin, MT1 is completely redistributed to the plasma membrane and intracellular vesicles (*arrows*). Cytoplasm and nuclei (*arrowheads*) are depleted. (*C*) The mutant MT10 expressed alone is localized in the cytoplasm and the nuclei (*arrowheads*), but not at the membrane. The cell periphery often appears brighter because it contains less yolk. The peripheral cytoplasmic staining closely matches the irregular outline of the yolk platelets (*small arrows*) and can be unambiguously distinguished from the sharper, straight line of membrane staining observed for full-length β-catenin (*A* and *B*), endogenous β-catenin and C-cadherin (not shown, see Fagotto and Gumbiner, 1994). (*D*) MT10 coexpressed with C-cadherin is distributed throughout the cytoplasm, with no enrichment at the cell membrane. Some punctate intracellular staining is observed (*arrow*). Arrowheads point to nuclei. Bar, 20 μm.

by overexpression of C-cadherin (Table V), in clear contrast with the complete inhibition of full-length β-catenin– or HT8-induced axis duplication (Tables III and V). Immunostaining showed that MT10 expressed alone was distributed all over the cytoplasm and accumulated in the nuclei, but there was no detectable enrichment along the plasma membrane (Fig. 7 *C*). The brighter staining for MT10 frequently observed at the cell periphery (*small arrows*) corresponds in most cases to the superficial layer of yolk-free cytoplasm (see legend of Fig. 7), and is a typical

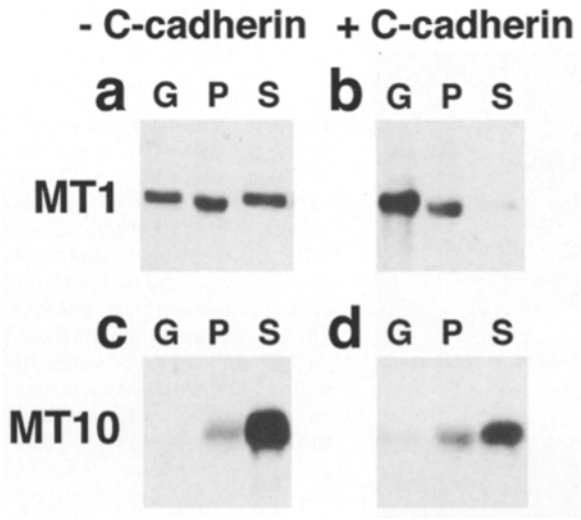

Figure 8. Redistribution of full-length β-catenin (MT1) but not MT10 by coexpression of C-cadherin: cell fractionation. Late blastula embryos expressing MT1 alone (*a*), MT1 with excess C-cadherin (*b*), MT10 alone (*c*), and MT10 with excess C-cadherin (*d*) were fractionated as described in Materials and Methods into a membrane glycoprotein (cadherins) fraction (*G*); a particulate noncadherin-bound fraction (*P*), and a soluble fraction (*S*). MT1 and MT10 were detected on Western blots with anti-myc mAb 9E10.2.

staining pattern for soluble cytosolic proteins (Funayama et al., 1995). When MT10 was coexpressed with C-cadherin, MT10 remained cytoplasmic (Fig. 7 *D*), and we did not observe any redistribution to the plasma membrane, although there was some intracellular punctate staining (Fig. 7 *D*, *arrow*) and reduced nuclear staining (Fig. 7 *D*, *arrowhead*).

These observations were confirmed by cell fractionation: when MT10-expressing embryos were fractionated as described above to compare membrane glycoprotein (cadherin)-bound, particulate noncadherin-bound, and cytosolic pools, most MT10 was found in the cytosolic fraction (Fig. 8 *c*, *S*). Some MT10 was recovered in the particulate noncadherin-bound fraction (*P*), but none was detectable in the membrane glycoprotein fraction (*G*). In embryos coinjected with MT10 and C-cadherin mRNAs (Fig. 8 *d*), most of MT10 was still in the soluble pool (*S*), and very little MT10 appeared in the membrane glycoprotein fraction (*G*). The levels of MT10 in the particulate noncadherin-bound fraction (*P*) were unchanged. These immunofluorescence and cell fractionation experiments demonstrate that MT10 does not significantly associate with plasma membrane glycoproteins, nor does it redistribute to the plasma membrane when C-cadherin is overexpressed, consistent with the finding that C-cadherin overexpression has very little effect on its signaling activity. The partial inhibition observed might be due to some weak residual affinity of β10/MT10 for cadherin complexes. A very weak interaction could partially inhibit signaling under the conditions of low β-catenin mutant expression and high C-cadherin overexpression used in our competition experiments. Nevertheless, β10/MT10 appears to be far less sensitive to C-cadherin competition than full-length β-catenin.

Discussion

In this study we have demonstrated that the inducing activity of β-catenin during embryonic axis formation is not mediated by cell adhesion. This conclusion is supported by two kinds of evidence: (*1*) inducing activity persists in a β-catenin mutant that does not bind to cadherins, and (*2*) binding to cadherins does not stimulate but instead inhibits β-catenin signaling.

A β-catenin deletion mutant, β10/MT10, composed of only nine of the armadillo repeats, could signal very efficiently, but had negligible affinity for C-cadherin, as shown by immunoprecipitation analysis, or for the plasma membrane in general as shown by cell fractionation. In contrast, the association of C-cadherin with HT8, which consists of the NH_2-terminal domain and $9_{1/2}$ armadillo repeats, was reproducible, although clearly weaker and detergent labile compared to full-length β-catenin or HT9. This was surprising, since it had been previously reported that this construct could not interact with E-cadherin (Hülsken et al., 1994). It is unlikely that this discrepancy results from the different cadherins examined because their cytoplasmic tails are very similar. Most likely, cadherin-catenin interactions may be more stable or easier to detect in *Xenopus* embryos. For example, the embryo has preexisting complexes, while the cell line used for the transfection studies was devoid of cadherins and catenins. We do not know why HT8 but not MT10 weakly associates with C-cadherin under mild detergent conditions. The slightly larger armadillo repeat domain may be sufficient for HT8 to bind directly to C-cadherin. Alternatively, HT8 may be incorporated into some higher order complex, possibly via association of its NH_2 terminus with α-catenin.

Global overexpression of cadherins in oocytes was found to lead to axial defects during embryonic development, similar to those obtained by partial depletion of maternal β-catenin (Heasman et al., 1994). The authors hypothesized that increased cadherin levels block signaling by sequestering β-catenin to the membrane. Here we have tested this hypothesis directly, by targeting overexpression of C-cadherin either to the Nieuwkoop center, the site where endogenous β-catenin is thought to act, or at the ectopic site of β-catenin overexpression for axis duplication experiments. We have shown that overexpressed C-cadherin depletes the cytosolic β-catenin pool, and that the shift from soluble to membrane-bound state correlates with loss of β-catenin–signaling activity. Conversely, signaling is restored if β-catenin expression is high enough to overcome C-cadherin competition, demonstrating that inhibition is specific to the signaling activity of β-catenin.

While C-cadherin expression increases cell adhesion (Guger and Gumbiner, 1995), increased adhesion cannot explain the inhibition of axis duplication for the following reasons. (*a*) A small increase in cell adhesion is also observed after β-catenin overexpression (Guger and Gumbiner, 1995), yet β-catenin and cadherins have opposite effects on axis induction. (*b*) Increasing the level of β-catenin expression overcomes the inhibition by C-cadherin, demonstrating that the ratio of the two proteins, rather than increased adhesion, is the crucial variable. (*c*) Mutant forms of β-catenin (β10/MT10) that do not even interact with C-cadherin still possess axis inducing activity, demonstrat-

ing that inducing activity is separable from adhesive function. (*d*) Axis inducing activity of β10/MT10 is resistant to C-cadherin overexpression, showing that the intrinsic signaling activity of β-catenin is not inhibitable by increased cell adhesion.

The demonstration of a signaling function of β-catenin independent of its role in adhesion raises questions about the identity of the target for β-catenin signaling. Signaling activity seems to reside in the soluble pool of β-catenin and not in the particulate nonglycoprotein-associated pool, since the decrease of soluble β-catenin after C-cadherin coexpression correlates with inhibition of the patterning activity, while the levels of particulate nonglycoprotein-associated β-catenin do not appear to be affected. A possible target for the activity of soluble β-catenin may be the nucleus, since this is a major site of accumulation of β-catenin and its active mutants.

A signaling function for β-catenin independent of its role in adhesion is entirely consistent with the well established signaling function of Armadillo in the early *Drosophila* embryo. Armadillo mediates a late step in a signaling cascade initiated by Wingless, and both are required for engrailed expression and the determination of posterior cell fates. In the absence of Wingless virtually all of the Armadillo is membrane bound and associated with cadherins at the zonula adherens junctions, but cells receiving the Wingless signal accumulate high levels of soluble Armadillo protein in the cytosol (Peifer et al., 1994*a*). Wingless acts via Disheveled to inhibit the activity of a protein kinase, called Zeste-White-3, which negatively regulates the cytosolic accumulation of Armadillo. Wingless-dependent Armadillo accumulation in the cytosol results from a very rapid decrease in its turnover rate (van Leeuwen et al., 1994). Thus, the genetic evidence indicates that cytosolic Armadillo accumulation is responsible for the transduction of the Wingless signal leading to changes in the expression of target genes.

Although less is known about the Wnt/β-catenin signaling pathway in *Xenopus* than the Wingless/Armadillo pathway in *Drosophila*, there are striking parallels between the two embryonic signaling systems. An enzymatically inactive mutant form of glycogen synthase kinase-3, which is the vertebrate homologue of *Drosophila* Zeste-white-3-kinase, can also induce axis duplications in *Xenopus* (He et al., 1995; Pierce and Kimelman, 1995). Similar to its inhibitory role in *Drosophila*, expression of the wild-type kinase inhibits both normal axis development and axis induction by Wnt. Also, ventral expression of the *Xenopus* homologue of Disheveled causes axis duplication (Sokol et al., 1995). Thus, the Wnt/β-catenin signaling pathway in the early *Xenopus* embryo and the Wingless/Armadillo pathway in *Drosophila* seem to operate via the same biochemical mechanism. It is therefore not surprising that the signaling activities of Armadillo and β-catenin are both associated with their accumulation in the cytoplasm.

The fact that β-catenin signaling is not mediated by its association with cadherins does not imply that these functions of β-catenin are completely independent of each other. On the contrary, our present results suggest that β-catenin signaling could be regulated by cadherins. Although antagonism of β-catenin by cadherins was observed under experimental conditions, similar situations could occur in vivo. In the simplest model, excess cadherins could act as a sink for β-catenin, and β-catenin levels would have to increase beyond a threshold, determined by the membrane-binding capacity before an intracellular signal could be generated. For instance, the proto-oncogenes Wnts/wg could trigger β-catenin signaling, in spite of the negative control by cadherins, by generating a very large pool of β-catenin which would overwhelm the binding capacity of the membrane.

On the other hand, adhesion might even be directly involved in the control of β-catenin signaling activity, by actively regulating β-catenin intracellular distribution. In such a model, changes in adhesion could be transmitted by cadherins or some other receptor associated with the complex, resulting in the release or the uptake of β-catenin by the cadherin complexes at the plasma membrane. In this way, the degree of cell–cell contact would be reflected intracellularly by the levels of free β-catenin, and consequently by its signaling activity. It is intriguing to consider the possibility that contact inhibition is one process that might use such mechanism. Cadherins are thought to act as tumor suppressors (Hedrick et al., 1993; Vleminckx et al., 1991), activities that have been generally interpreted to result from increased cell–cell adhesion. The present findings raise the possibility that tumor suppression may be due to a direct inhibition of intracellular signaling by cadherins.

Nonetheless, it remains possible that the inhibition by C-cadherin of β-catenin signaling can only occur under experimental conditions. In an alternative model, the adhesive and signaling functions of β-catenin may not be antagonistic, but complementary. The Wnt/wg pathway causes stabilization and accumulation of Armadillo (Peifer, 1995; van Leeuwen et al., 1994), and higher levels of β-catenin and plakoglobin correlate with stronger cell–cell adhesion (Bradley et al., 1993; Hinck et al., 1994). Thus, by increasing the levels of β-catenin/Armadillo, Wnts/wg would act simultaneously on an intracellular target to induce cell fates, and at the membrane, to stimulate cell–cell adhesion. Although it is now clear that adhesion is not the mechanism that mediates the β-catenin inducing event, tighter adhesion may complement the signaling process by delimiting and maintaining the cohesiveness of the inducing tissue.

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References

Amaya, E., T.J. Musci, and M.W. Kirschner. 1991. Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell.* 66:257–270.

Bradley, R.S., P. Cowin, and A.M.C. Brown. 1993. Expression of *wnt*-1 in PC12 cells results in modulation of plakoglobin and E-cadherin and increased cellular adhesion. *J. Cell Biol.* 123:1857–1865.

Brieher, W.M., and B.M. Gumbiner. 1994. Regulation of cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J. Cell Biol.* 126: 519–527.

Doherty, P., S.V. Ashton, S.E. Moore, and F.S. Walsh. 1991. Morphoregulatory activities of NCAM and N-cadherin can be accounted for by G protein-dependent activation of L- and N-type neuronal Ca^{2+} channels. *Cell.* 67:21–33.

Fagotto, F., and B.M. Gumbiner. 1994. β-Catenin localization during *Xenopus* embryogenesis: accumulation at tissue and somite boundaries. *Development.* 120:3667–3679.

Fleming, T.P., and M.H. Johnson. 1988. From egg to epithelium. *Annu. Rev. Cell Biol.* 4:459–485.

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

Guger, K.A., and B.M. Gumbiner. 1995. β-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in Xenopus dorsal-ventral patterning. *Dev. Biol.* 172:115–125.

Gumbiner, B.M. 1995. Signal transduction by β-catenin. *Curr. Opin. Cell Biol.* 7:634–640.

Gumbiner, B., B. Stevenson, and A. Grimaldi. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* 107:1575–1587.

Heasman, J., A. Crawford, K. Goldstone, P. Garner-Hamrick, B. Gumbiner, P. McCrea, C. Kintner, C.Y. Noro, and C. Wylie. 1994. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell.* 79:791–803.

He, X., J.-P. Saint-Jeannet, J.R. Woodgett, H.E. Varmus, and I.B. Dawid. 1995. Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature (Lond.).* 374:617–622.

Hedrick, L., K.R. Cho, and B. Vogelstein. 1993. Cell adhesion molecules as tumour suppressors. *Trends Cell Biol.* 3:36–39.

Hinck, L., W.J. Nelson, and J. Papkoff. 1994. Wnt-1 modulates cell–cell adhesion in mammalian cells by stabilizing β-catenin binding to the cell adhesion protein cadherin. *J. Cell Biol.* 124:729–741.

Hülsken, J., W. Birchmeier, and J. Behrens. 1994. E-cadherin and APC compete for the interaction with β-catenin and the cytoskeleton. *J. Cell Biol.* 127: 2061–2069.

Kao, K.R., and R.P. Elinson. 1988. The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* 127:64–77.

Karnovsky, A., and M.W. Klymkowsky. 1995. Anterior axis duplication in *Xenopus* induced by the overexpression of the cadherin-binding protein plakoglobin. *Proc. Natl. Acad. Sci. USA.* 92:4522–4526.

Kay, B.K., and H.B. Peng. 1991. *Xenopus laevis*: Practical Uses in Cell and Molecular Biology. Academic Press, Inc., NY. 581 pp.

Keller, R., and R. Winklbauer. 1992. Cellular basis of amphibian gastrulation. *In* Current Topics in Developmental Biology. Academic Press, Inc., NY. 39–89 pp.

Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317–321.

Lee, C.H., and B.M. Gumbiner. 1995. Disruption of gastrulation movements in *Xenopus* by a dominant negative mutant for C-cadherin. *Dev. Biol.* 171:363–373.

McCrea, P., and B. Gumbiner. 1991. Purification of a 92-kDa cytoplasmic protein tightly associated with the cell–cell adhesion molecule E-cadherin (uvomorulin): characterization and extractability of the protein complex from the cell cytostructure. *J. Biol. Chem.* 266:4514–4520.

McCrea, P.D., W.M. Brieher, and B.M. Gumbiner. 1993. Induction of a secondary body axis in *Xenopus* by antibodies to β-catenin. *J. Cell Biol.* 123:477–484.

Munemitsu, S., B. Souza, O. Muller, I. Albert, B. Rubinfeld, and P. Polakis. 1994. The APC gene product associates with microtubules *in vivo* and promotes their assembly *in vitro*. *Cancer Res.* 54:3676–3681.

Nelson, W.J. 1992. Regulation of cell surface polarity from bacteria to mammals. *Science (Wash. DC).* 258:948–955.

Peifer, M. 1995. Cell adhesion and signal transduction: the Armadillo connection. *Trends Cell Biol.* 5:224–229.

Peifer M., D. Sweeton, M. Casey, and E. Wieschaus. 1994*a*. *Wingless* signal and zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development.* 120:369–380.

Peifer, M., S. Berg, and A.B. Reynolds. 1994*b*. A repeating amino acid motif shared by proteins with diverse cellular roles. *Cell.* 76:789–791.

Pierce, S.B., and D. Kimelman. 1995. Regulation of Spemann organizer formation by the intracellular kinase Xgsk-3. *Development.* 121:755–765.

Polakis, P. 1995. Mutations in the APC gene and their implications for protein structure and function. *Curr. Opin. Gen. & Dev.* 5:66–71.

Rimm, D.L., E.R. Koslov, P. Kebriaei, C.D. Cianci, and J.S. Morrow. 1995. α_1(E)-Catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc. Natl. Acad. Sci. USA.* 92:8813–8817.

Rubinfeld, B., B. Souza, I. Albert, O. Müller, S.H. Chamberlain, F.R. Masiarz, S. Munemitsu, and P. Polakis. 1993. Association of the APC gene product with β-catenin. *Science (Wash. DC).* 262:1731–1734.

Rupp, R.A., L. Snider, and H. Weintraub. 1994. Xenopus embryos regulate the nuclear localization of XMyoD. *Genes & Dev.* 8:1311–1323.

Schwartz, M.A., M.D. Schaller, and M.H. Ginsberg. 1995. Integrins: emerging paradigms of signal transduction. *Annu. Rev. Cell Dev. Biol.* In press.

Smith, K.J., K.A. Johnson, T.M. Bryan, D.E. Hill, S. Markowitz, J.K.V. Wilson, C. Paraskeva, G.M. Petersen, S.R. Hamiloton, B. Vogelstein, et al. 1993. The APC gene product in normal and tumor cells. *Proc. Natl. Acad. Sci. USA.* 90: 2846–2850.

Smith, K.J., D.B. Levy, P. Maupin, T.D. Pllard, B. Vogelstein, and K.W. Kinzler. 1994. Wild-type but not mutant APC associates with the microtubule cytoskeleton. *Cancer Res.* 54:3672–3675.

Sokol, S.Y., J. Klingensmith, N. Perrimon, and K. Itoh. 1995. Dorsalizing and neuralizing properties of Xdsh, a maternally expressed *Xenopus* homolog of *disheveled. Development.* 121:1637–1647.

Su, L.K., B. Vogelstein, and K.W. Kinzler. 1993. Association of the APC tumor suppressor protein with catenins. *Science (Wash. DC).* 262:1734–1737.

Turner, D.L., and H. Weintraub. 1994. Expression of achaete-scute homolog 3 in Xenopus embryos converts ectodermal cells to a neuronal fate. *Genes & Dev.* 8:1234–1247.

van Leeuwen, F., C.H. Samos, and R. Nusse. 1994. Biological activity of soluble *wingless* protein in cultured *Drosophila* imaginal disc cells. *Nature (Lond.).* 368:342–344.

Vleminckx, K., L. Vakaet, Jr., M. Mareel, W. Fiers, and F. Van Roy. 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell.* 66:107–119.

Williams, C.L., V.Y. Hayes, A.M. Hummel, J.E. Tarara, and T.J. Halsey. 1993. Regulation of E-cadherin–mediated adhesion by muscarinic acetylcholine receptors in small cell lung carcinoma. *J. Cell Biol.* 121:643–654.