

Induction of a Secondary Body Axis in *Xenopus* by Antibodies to β -Catenin

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Abstract. We have obtained evidence that a known intracellular component of the cadherin cell-cell adhesion machinery, β -catenin, contributes to the development of the body axis in the frog *Xenopus laevis*. Vertebrate β -catenin is homologous to the *Drosophila* segment polarity gene product *armadillo*, and to vertebrate plakoglobin (McCrea, P. D., C. W. Turck, and B. Gumbiner. 1991. *Science (Wash. DC)*. 254: 1359-1361.). β -Catenin was found present in all *Xenopus* embryonic stages examined, and associated with C-cadherin, the major cadherin present in early

Xenopus embryos. To test β -catenin's function, affinity purified Fab fragments were injected into ventral blastomeres of developing four-cell *Xenopus* embryos. A dramatic phenotype, the duplication of the dorsoanterior embryonic axis, was observed. Furthermore, Fab injections were capable of rescuing dorsal features in UV-ventralized embryos. Similar phenotypes have been observed in misexpression studies of the *Wnt* and other gene products, suggesting that β -catenin participates in a signaling pathway which specifies embryonic patterning.

THE catenins are cytoplasmic proteins that form protein complexes with the cadherin cell-cell adhesion molecules. They interact with a discrete domain in the cadherin cytoplasmic tail that has been mapped by deletion analysis (25, 26, 34). Catenins seem to be required for cadherin function, since mutant forms of E-cadherin having cytoplasmic tail deletions fail to mediate effective cell-cell adhesion in cultured cells (25, 26, 34). The catenins have been proposed to be responsible for linking the cadherins to the actin cytoskeleton, especially at the *zonula adhaerens* of epithelial cells. Only recently have their individual functions begun to be analyzed.

α -Catenin may fulfill the role of a cytoskeletal linker protein. It shares overall structural similarity and primary amino acid sequence homology with vinculin (14, 27), a protein known to be involved in actin filament attachment to the plasma membrane at sites of adhesive junctions (2, 9, 33). Also, there is some evidence that α -catenin is responsible for a minor association between E-cadherin and actin (34). Moreover, E-cadherin and α -catenin have been found to be required for the tight adhesion, or "compaction," of epithelial cells (15). A carcinoma cell line expressing E-cadherin and β -catenin, but lacking α -catenin forms only loose aggregates, but expression of an α -catenin cDNA in these cells

restored a compact epithelial morphology. Epithelial compaction is thought to depend on the actin cytoskeleton (15), consistent with a cytoskeletal linker function for α -catenin.

The function of β -catenin in the cadherin complex is less clear. β -Catenin binds directly to the cadherin cytoplasmic tail, because it copurifies with E-cadherin under conditions that deplete the other catenins from the complex (22, 35). There is not yet any evidence that β -catenin interacts with actin or is required for epithelial compaction. It shares 63% amino acid identity with plakoglobin (23), a protein of unknown function that is associated with the cytoplasmic surface of desmosomes and *zonula adhaerens* junctions (8a). Perhaps more informative is its striking similarity (70% amino acid identity) to the *Drosophila* segment polarity gene *armadillo* (37). In *armadillo* mutants, the posterior part of each segment of the *Drosophila* embryo is deleted and replaced by a mirror-image duplication of the anterior part of the segment (38, 39). The *armadillo* phenotype is very similar to that produced by the growth factor gene *wingless*, and the genetic analysis suggests that *armadillo* participates in receiving and/or interpreting the *wingless* developmental signal (15a). This sequence homology raises the possibility, therefore, that β -catenin (and thereby cadherins) could have a role in intercellular communication in vertebrate tissues.

The early *Xenopus* embryo is a good experimental system for analyzing the roles of cadherins and catenins in developmental processes. C-cadherin (also called EP- and U-cadherin) is maternally encoded, recruited to the cell surface during cleavage, and is primarily responsible for the Ca^{2+} -

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dependent adhesion between early blastomeres (16, 5, 10a). C-cadherin is also expressed during gastrulation and neurulation and later stages of development (20a). E-cadherin is first expressed at the time of gastrulation in the ectoderm (4), and N-cadherin is expressed first in the neural tube (8). Furthermore, the early *Xenopus* embryo has been used very effectively to analyze the roles of growth factors and their receptors during mesoderm induction and other early patterning events (1a, 13, 20b). Of particular interest in this regard is the observation that certain of the *Wnt* growth factors, which are homologues of the *wingless* segment polarity gene in *Drosophila*, have very potent effects on the patterning of the dorsoanterior body axis in *Xenopus* (41, 43). Thus, the biology and experimental accessibility of the early *Xenopus* embryo can be used to analyze the functions of catenins and cadherins both in the mechanisms of cell adhesion and in intercellular signaling and embryonic patterning.

To address the role of β -catenin in early *Xenopus* development, we needed to perturb its function in vivo. To attempt to do so, we microinjected Fab fragments derived from affinity-purified antibodies that were raised to *Xenopus* β -catenin into early blastomeres of the embryo. Rather than an obvious adhesion defect, which we expected to observe, we found that the anti- β -catenin Fabs greatly influenced patterning of the embryonic body axis.

Materials and Methods

Frogs, Cell Lines, Extractions, and Immunoprecipitations

Adult female *Xenopus laevis* frogs (*Xenopus* I, Ann Arbor, MI) were primed with PMSG, and egg laying induced with hCG (28). Embryos were obtained by artificial fertilization, dejellied in 2% cysteine hydrochloride (pH 7.8–8.0), and cultured in 0.1X MMR as described (17). The *X. laevis* kidney epithelial cell line A6 was maintained as described (4, 11). A6 and embryo extractions in buffers containing (respectively) 0.5% TX-100 or 1% NP-40, and subsequent immunoprecipitations, were carried out as described (5, 22).

Microinjections and Treatment of Embryos

Microinjection of anti- β -catenin Fabs were carried out using a microprocessor controlled oil-driven microinjector (Sutter Instruments, Novato, CA; model NA-1). Injections were performed by piercing a drawn out capillary pipette (~ 10 μ m tip diameter) through the embryo's vitelline envelope and plasma membrane, which was otherwise left undisturbed. The Fabs (25 nl of ~ 5 ng/nl) were delivered just above the equatorial region into the animal (upper) pole of one prospective ventral cell of a four-cell embryo. Prospective ventral cells were identified by their darker pigmentation relative to prospective dorsal cells. The absolute quantity of Fabs delivered into the embryo was likely to be less than 125 ng (25–100 ng) because the sample was spun immediately before injection to remove precipitates (5 min 10,000 g). A two to fivefold molar excess of β -catenin fusion protein over Fabs was used to neutralize the Fabs in control injections, as determined by titrating the fusion protein against the Fabs in a Western blot assay. UV-ventralized embryos were produced according to Smith and Harland using an inverted Stratolinker 1200 (41), except that the UV dose was 100 mJoules/cm². Normal embryos were staged as described (30). Scoring of the dorsoanterior index (DAI)¹ of UV-ventralized embryos was as described (16).

Antibodies and Fab Fragments

To generate anti- β -catenin antibodies, the NH₂ terminus (amino acids

6-138) of *Xenopus* β -catenin (23) was fused to the COOH terminus of glutathione-S-transferase (GST), and the product produced from the bacterial expression vector pGEX1 (1); it was then affinity purified on a CNBr-coupled (Pharmacia LKB Biotechnology, Piscataway, NJ) anti-GST-Sepharose 4B column, and injected into rabbits for the production of polyclonal antibodies (BAbCo, Berkeley, CA). Anti-GST antibodies were removed from the sera using a CNBr-coupled GST-Sepharose 4B column, and the anti- β -catenin polyclonal antibodies were then affinity purified on a CNBr-coupled GST- β -catenin (fusion protein)-Sepharose 4B column using a described protocol (1). Antibodies were concentrated to ~ 5 ng/nl (Centricon-10 Microconcentrator, Amicon), Fab fragments generated using papain as described (12), followed by exhaustively dialyzing the Fabs against injection buffer (17). Immediately before injection Fab fragments were centrifuged to remove precipitates (5 min at 10,000 g). Monoclonal antibody 19A2 (4) was used in the anti-E-cadherin immunoprecipitations shown in Fig. 1. Anti-C-cadherin monoclonal antibody 6B6 was raised to a bacterial fusion protein containing a portion of the extracellular sequence of C-cadherin (unpublished).

Metabolic Labeling, Immunoprecipitation, SDS-PAGE, and Western Blotting

Metabolic labeling, immunoprecipitation, immunoprecipitate washing, SDS-PAGE, and alkaline phosphatase color development of Western blots (BioRad Labs., Hercules, CA), were done as described (22, 23). The ECL Western blot development protocol was also used (Amersham Corp., Arlington Heights, IL).

Sectioning

Embryos were fixed for 48 h at 4°C in 3% paraformaldehyde/0.25% glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.4). Embryos were then dehydrated over 2 h via a graded ethanol solution series terminating at 100% absolute ethanol, moved through 100% propylene oxide, 50% propylene oxide/50% Poly-Bed 812, and finally into 100% Poly-Bed 812. Blocks were hardened 48 h at 60°C, and sectioned at 0.5–1 μ m with a diamond knife perpendicular to the embryo's dorsoanterior axis. Sections were mounted on glass slides and stained with 1% methylene blue.

Results

Specificity of the Anti- β -Catenin Antibodies

The immunochemical specificity of the β -catenin antibodies is shown in Fig. 1. Western blots of whole cell extracts and of E-cadherin immunoprecipitates from the *Xenopus* A6 cell line (31) demonstrate that the anti- β -catenin antibodies react strongly with *Xenopus* β -catenin (92 kD) (lanes 1 and 5) with no or very little cross-reactivity with any other polypeptide (see also Fig. 2, lanes 1–8). The antibodies do not react with plakoglobin (~ 85 kD), which shares primary sequence homology with β -catenin (63% identity) (23). Plakoglobin was first shown to associate with the cytoplasmic domain of desmoglein-1, itself a member of the cadherin superfamily (19, 20). Additionally, plakoglobin was later shown to be a constituent of the E-cadherin complex (23), migrating at the position of γ -catenin (lane 3) (18, 36), but it is not recognized by our anti- β -catenin antibodies (lane 5).

Presence of β -Catenin in the Embryo

The levels of the β -catenin polypeptide expression during early *Xenopus* development was determined by immunoblotting (Fig. 2; lanes 1–8). β -Catenin was present in the egg and at the time at which the microinjection experiments were carried out. A small but reproducible increase in the levels of the β -catenin polypeptide was observed after the mid-blastula stage (Fig. 2; lanes 5–8), at which point zygotic mRNA synthesis ensues (28). Because C-cadherin is the ma-

1. Abbreviations used in this paper: DAI, dorsoanterior index; GST, glutathione-S-transferase.

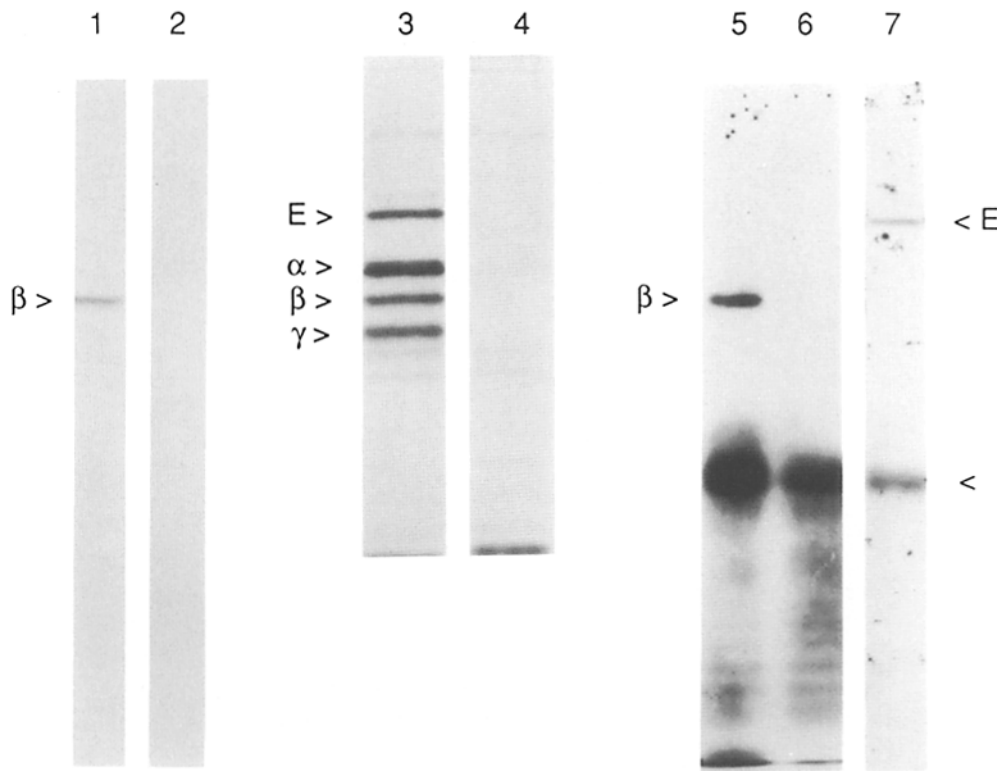


Figure 1. Immunoblot and autoradiograph analysis of the specificity of the anti- β -catenin polyclonal antibody. Western blots and ^{35}S autoradiographs. (Lane 1) Anti- β -catenin Western blot of whole-cell SDS extract of the *Xenopus* kidney epithelial cell line A6. (Lane 2) Same as lane 1 except antibody preincubated with β -catenin fusion protein before blotting. (Lane 3) Autoradiograph of anti-E-cadherin immunoprecipitate from [^{35}S]methionine-labeled A6 cells. (Lane 4) Nonimmune control for lane 3. (Lane 5) Anti- β -catenin Western blot of the sample shown in lane 3. (Lane 6) Nonimmune control for lane 5. (Lane 7) Anti-E-cadherin Western blot of an anti- β -catenin immunoprecipitate. Symbols: E, E-cadherin (120 kD); α , α -catenin (102 kD); β , β -catenin (92 kD); γ , γ -catenin (85 kD). Lone open pointer indicates the mobility position of reduced heavy-chains of immunoprecipitating antibodies (~55 kD).

for if not only *Xenopus* embryonic cadherin present up to the gastrula stage (E- and N-cadherin are later expressed) (4, 5, 8), we wished to test if β -catenin was associated with C-cadherin at early developmental stages. β -Catenin is indeed found specifically in C-cadherin immunoprecipitates from blastula-stage embryos (stage 8) (Fig. 2; lane 10). Moreover, C-cadherin is specifically recovered in β -catenin immunoprecipitates (Fig. 2; lane 12). A very significant fraction of the β -catenin immunoprecipitable from stage 8 embryo extracts is associated with C-cadherin (compare lanes 9 and 10; Fig. 2). Thus perturbation of β -catenin at early developmental stages may also have an impact upon C-cadherin function.

The Duplicate Axis Phenotype in Microinjected Embryos

The developmental phenotype observed after injection of anti- β -catenin Fabs into one ventral blastomere of the *Xenopus* four-cell embryo is shown in Fig. 3. The embryo shown in Fig. 3A displays the most striking phenotype observed at a late stage of development; a duplication of the entire dorsoanterior axis, including the generation of two distinct head structures, each complete with its own pair of eyes and cement glands. However, only ~2% of the embryos develop such a complete secondary dorsoanterior axis. Usually, one of the two head structures does not possess well formed eye pairs or a cement gland (not shown). The phenotype most easily observed at earlier embryonic stages is the duplication of the neural tube, which appears as a pair of darkly pigmented lines of tissue (Fig. 3C). Within a single experi-

ment, 90% of the ventrally injected embryos display clear evidence of a duplication of the dorsoanterior axis, as compared to 0% of control embryos (Table I). To examine the patterning of tissues internally, embryos with apparent duplicated axes were sectioned. At appropriate anterior positions two completely independent notochords, each associated with an overlying neural tube and separated laterally from one another by intervening somitic mesodermal tissue, were observed (Fig. 3B).

Two different controls were used to evaluate the specificity of the anti- β -catenin Fabs. Microinjection of Fabs generated from preimmune sera did not generate duplicated axis embryos (not shown). Furthermore, neutralization of the anti- β -catenin Fabs by preincubation with a saturating quantity of the β -catenin-GST fusion protein before microinjection resulted in no embryos with duplicated axes, despite the injection of a greater total amount of foreign protein (Fig. 3D and Table I). Therefore, nonspecific effects due to the microinjection of this amount of antibody cannot account for the striking effect produced by anti- β -catenin Fabs.

The duplicated axis phenotype was observed predominantly when Fabs were injected into ventral blastomeres of the four-cell embryo. Table I shows the outcome of an experiment in which injections were made into either one dorsal or one ventral cell of a four-cell embryo. 90% of the embryos injected ventrally displayed the duplicated axis phenotype. In contrast, only 13% of the embryos injected dorsally formed duplicated neural tubes; given the small degree of uncertainty in assessing prospective ventral versus dorsal cells, it is possible that these embryos were in fact injected

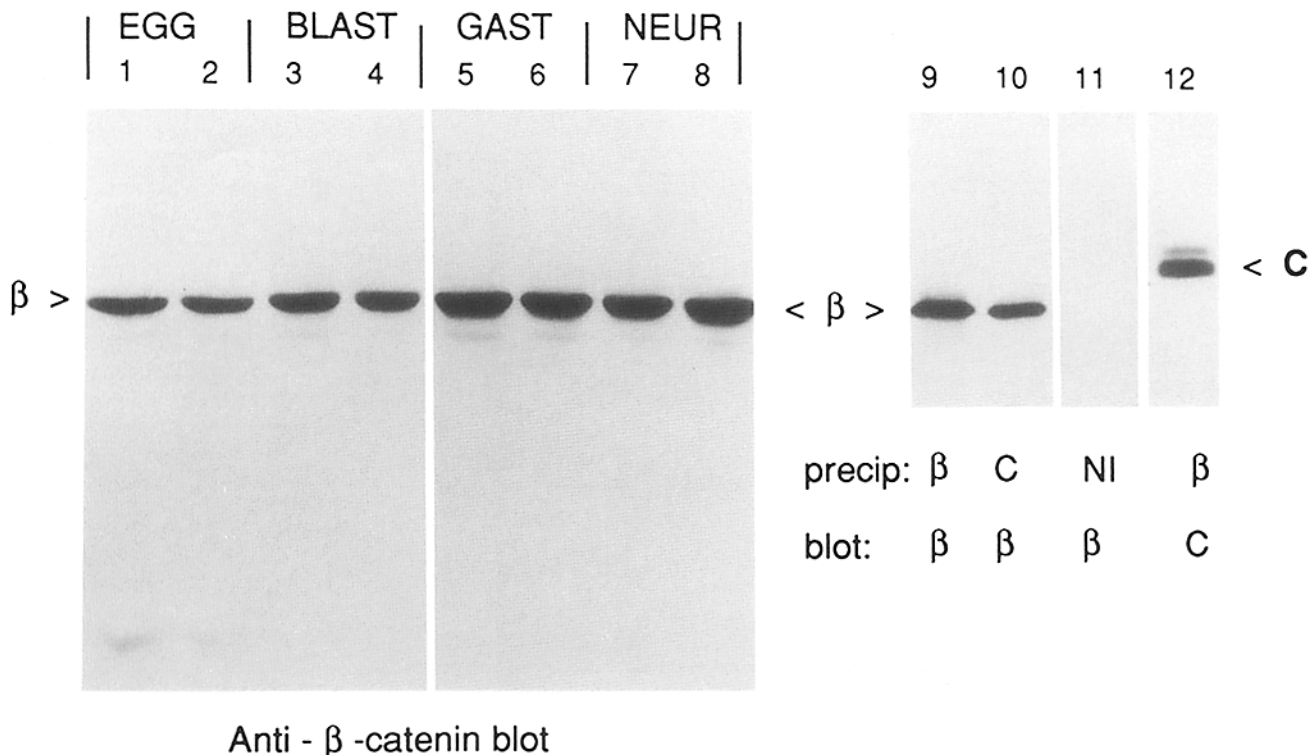


Figure 2. β -Catenin protein expression in *Xenopus* embryos. (Lanes 1–8) Duplicate samples of detergent extracts from *Xenopus* embryos at four developmental stages were subjected to SDS-PAGE and Western blotted for β -catenin. (Lanes 9–11) Anti- β -catenin Western blots of anti- β -catenin, anti-C-cadherin, and nonimmune immunoprecipitates (respectively) from solubilized *Xenopus* blastula embryos (stage 8). (Lane 12) Anti-C-cadherin Western blot of an anti- β -catenin immunoprecipitate from detergent solubilized *Xenopus* blastula embryos. All blots were developed using the ECL horseradish peroxidase protocol (Amersham Corp.). Embryo solubilizations carried out in buffers containing 1% NP-40 (lanes 1–8, extracts of 2.5 embryos per lane loaded), or 0.5% TX-100 (lanes 9–12, immunoprecipitates of five embryos per lane loaded). Symbols: β , β -catenin; C, C-cadherin; NI, nonimmune; EGG, 1-cell stage 1; BLAST, blastula stage 8; GAST, gastrula stage 12; NEUR, neurula stage 20.

ventrally. While the position of the embryo's first two cleavage planes and the asymmetric distribution of its pigment provides a good indicator of dorsal-ventral cell fate, a small uncertainty exists because they are not perfectly predictive (6). Another possibility is that dorsal injections at the dorsal-ventral border (i.e., lateral injections), may have been capable of generating a duplicated axis phenotype.

Rescue of Dorsal Characteristics in UV-Ventralized Embryos

In addition to duplicating the dorsal axis of normal embryos, injection of *Wnt* mRNAs rescues a primary dorsoanterior axis structure in embryos that have been ventralized by treatment with UV light (41, 43). We therefore tested whether the anti- β -catenin Fabs could rescue UV-ventralized embryos. Anti- β -catenin Fab injected embryos showed definite evidence of rescue from UV-treatment (Fig. 4, A and C), compared to no rescue as a result of preimmune Fab injection (Fig. 4, B and D). The dorsoanterior index scoring system for evaluating the extent of dorsal rescue in *Xenopus* embryos (16) was employed (Table II). Anti- β -catenin Fab injected embryos displayed a $DAI = 2.1$ – 3.1 , while preimmune Fab injected embryos had a $DAI = 0.2$ – 1.0 (normal development = 5.0). Therefore, the anti- β -catenin Fabs were also capable of inducing a primary dorsoanterior axis.

Discussion

The findings presented here demonstrate that antibodies (Fab fragments) directed against a known intracellular component of a cell–cell adhesion complex, β -catenin, are capable of influencing developmental patterning in *Xenopus* embryos. Microinjection of Fabs into a ventral blastomere of normal embryos causes the formation of a secondary body axis, while their injection into embryos ventralized by UV irradiation partially rescues the primary dorsoanterior axis. The evidence indicates that this effect of the Fabs is specific. Neither the process of microinjection nor the introduction of this amount of foreign protein can account for the effect, because injection of Fabs from preimmune sera never gave rise to a duplicated axis, nor did it interfere with normal development. Furthermore, only very specific treatments of early *Xenopus* embryos can induce a secondary dorsoanterior axis. In fact, axis induction has been used successfully as an assay to screen for endogenous molecules that participate in normal embryonic patterning (42). The effects of the Fabs are very likely to be specific for β -catenin. The antibodies from which they were derived were affinity-purified, and recognize only β -catenin in immunoblots of extracts from the *Xenopus* A6 epithelial cell line and from *Xenopus* embryos. Moreover, the developmental effects of the Fabs were completely neutralized by preincubation with the β -catenin-GST

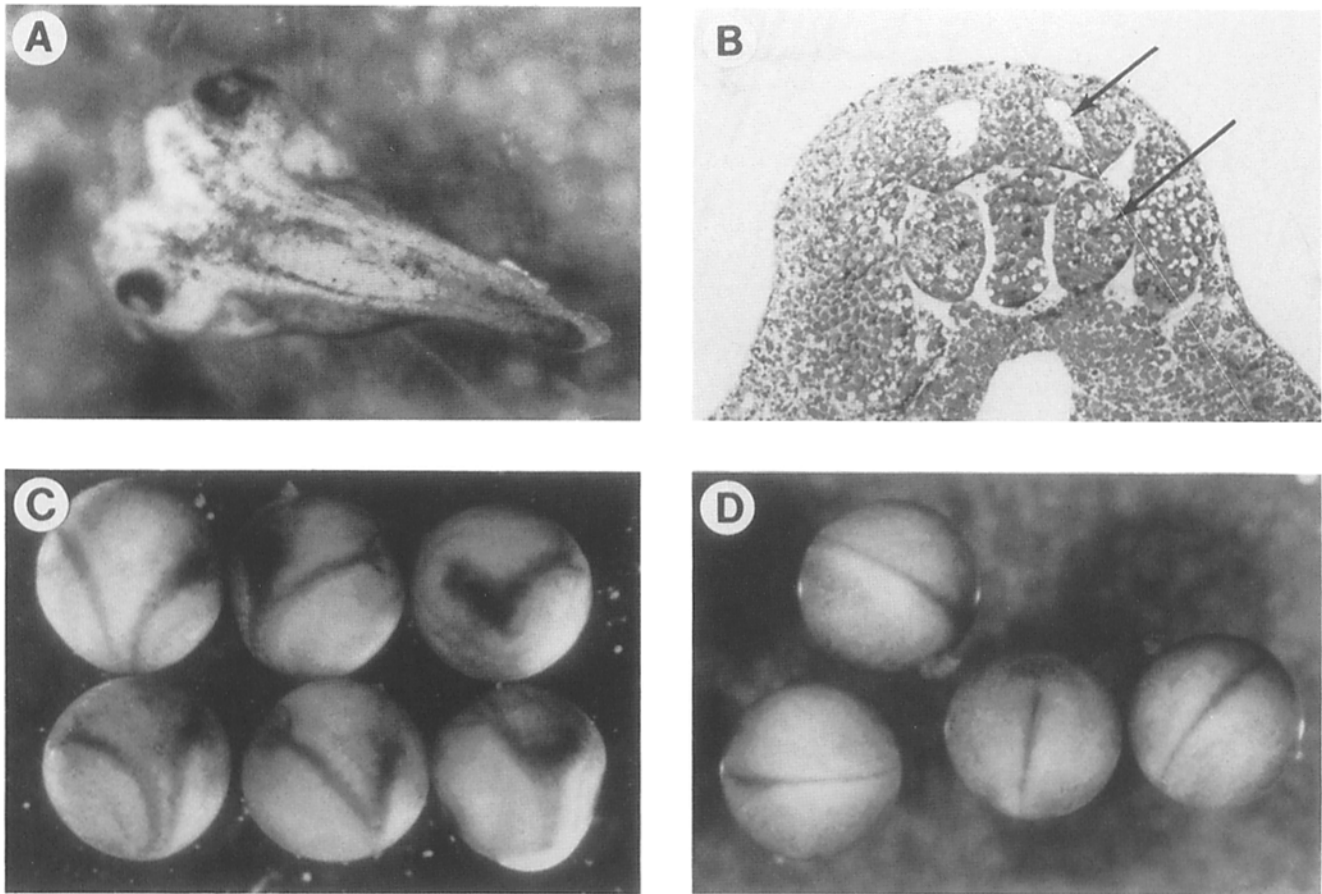


Figure 3. Duplicate-axis phenotype arising after the injection of anti- β -catenin Fab fragments into one prospective ventral cell of a four-cell *Xenopus laevis* embryo. (A) Single embryo displaying complete duplication of the dorso-anterior axis including eye-pairs and cement gland (noninjected control stage 45–50). (B) Section taken from a duplicated-axis *Xenopus* embryo. Note the presence of two distinct neural tubes (upper arrow), and two distinct notochords (lower arrow). (C) Six embryos displaying duplication of the neural tube (noninjected control stage \sim 20). Note the presence of a single neural tube in controls (D). Small “blebs” attached to the side of some embryos is an injection-site artifact. (D) Four embryos injected with a mix of anti- β -catenin Fab fragments, and a two to fivefold molar excess of the anti- β -catenin fusion protein (noninjected control stage \sim 20).

Table I. Duplicate Axis Generation—Ventral Vs Dorsal Injections

Injected sample	Site of injection	DA	no DA	DA %
Experimental				
Fab	ventral	76	8	90%
Fab	dorsal	4	26	13%
Control				
non-injected	---	0	97	0%
Fab + fusion	ventral	0	58	0%
Fab + fusion	dorsal	0	8	0%

Anti- β -catenin Fab injections (25–100 ng) were into ventral or dorsal blastomeres of four cell embryos, and duplicate axis presence determined visually at neurulation (stage 20). Each line above represents data combined from two or three experiments (excepting “Fab + fusion/dorsal”—one experiment), and survival of injected embryos at scoring-stage was $>90\%$ in each case. A two to fivefold molar excess of β -catenin fusion protein over Fabs was used in control samples.

fusion protein to which the antiserum was raised. Therefore, perturbation of β -catenin can influence axis formation, suggesting that β -catenin (or the entire cadherin-catenin complex) may have a role in some of the intercellular signaling events that underlie embryonic patterning.

We do not yet know how the binding of Fab fragments to β -catenin perturbs its function. It is not yet clear whether the antibody acts in an inhibitory or a stimulatory fashion in causing the duplicated body axis. We know, however, that although the injected Fabs bind β -catenin in the C-cadherin complex, the relative stoichiometry of the components in the complex components is not altered by Fab binding (unpublished observations). Other biochemical assays may reveal the functional consequences of Fab binding.

The developmental effects of anti- β -catenin Fabs occurred without any obvious observed change in the adhesiveness between embryonic blastomeres. There was no evidence of cell dissociation, at least when observed from the external sur-

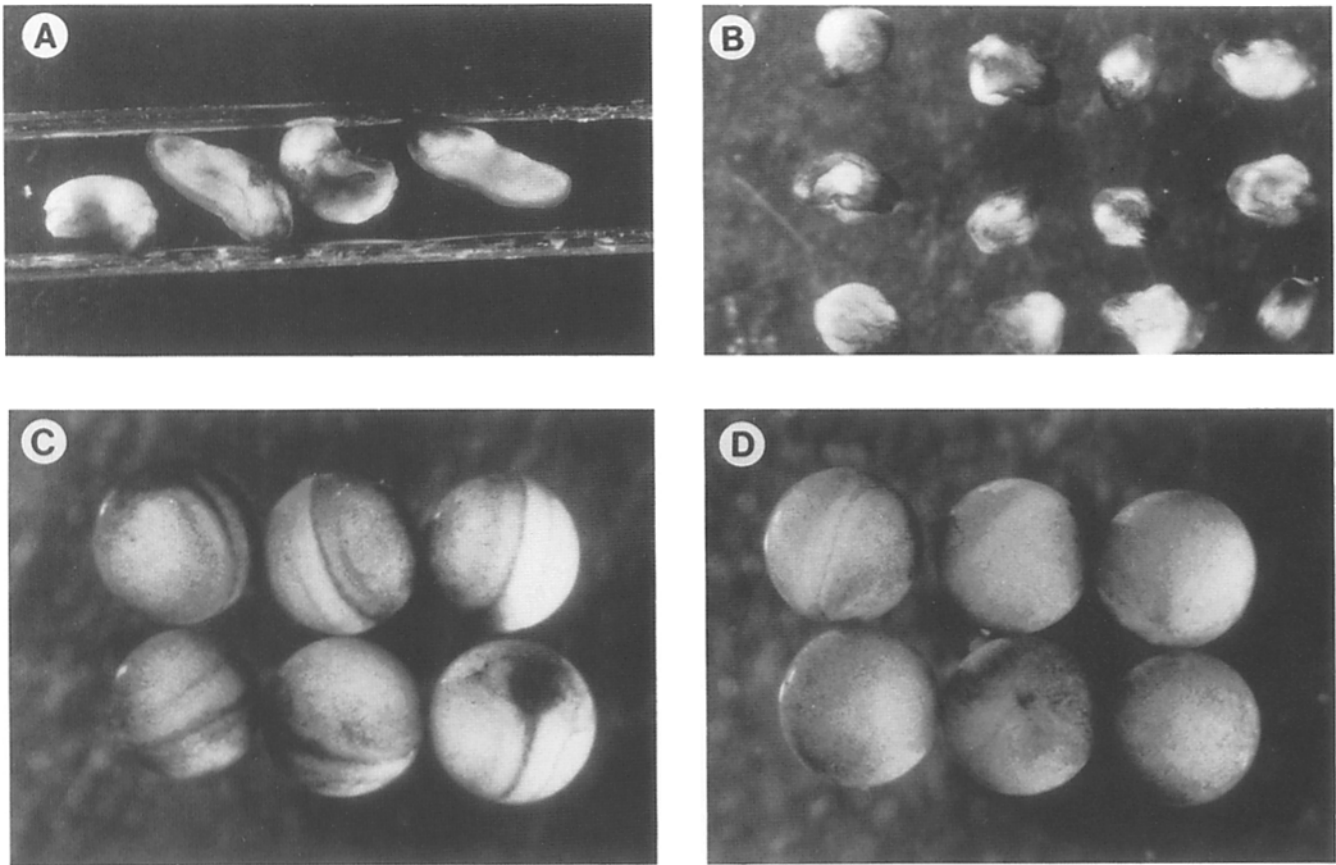


Figure 4. Anti- β -catenin Fab rescue of dorsal features in UV-ventralized *Xenopus* embryos. Embryos were UV-ventralized (46), and then injected at the four-cell stage with either anti- β -catenin Fabs or preimmune Fabs. (A) Rescued embryos that had been injected with anti- β -catenin Fabs (photo taken at noninjected control stage 35). (B) Nonrescued embryos that had been injected with preimmune Fabs. Note that preimmune Fab-injected embryos appear to have remained ventralized (less elongated) relative to those injected with anti- β -catenin Fabs. (C) Rescued embryos that had been injected with anti- β -catenin Fabs as in A but photographed at an earlier stage (control stage 20). (D) Nonrescued embryos which had been injected with preimmune Fabs as in B, but photographed at an earlier stage (control stage 20). Compare the rescue of neural tube structures in C versus the lack of such rescue in D. The upper left-most embryo in D displays weak neural tube features, which likely resulted from incomplete UV-induced ventralization. Embryos in A and B were from the same batch, as were those in C and D. See Table II for quantitation.

Table II. Fab Rescue of Dorsal Structures in UV Ventralized Embryos

Injected sample	n	Injected					Score average
		0	1	2	3	4	
Experimental							
Fabs	16	0	1	1	9	4	3.1
Fabs	69	9	13	16	20	3	2.1
Fabs	55	2	6	16	17	0	2.2
Control							
non-injected	44	37	4	3	0	0	0.2
non-injected	33	16	4	2	7	0	1.0
preimmune Fabs	57	44	6	1	4	0	0.4

UV irradiated embryos were injected with anti- β -catenin Fabs, with preimmune Fabs, or were not injected at the four cell stage (excepting first data line—two cell stage). Scoring of stage 40 embryos was according to Kao and Elinson (16), where $DAI=0$ indicates a completely ventralized embryo, and $DAI=5$ a completely normal one. "n" indicates the number of embryos initially injected; some embryos died before scoring. Data from two experiments are represented.

face, and the morphogenetic movements of gastrulation occurred normally. It is possible that adhesive changes, or other changes in the blastomeres' motility, cell shape, and packing do occur, but are subtle in nature and/or highly localized in the embryo. Alternatively, it's worth considering that β -catenin acts in mediating the cellular response to cadherin-dependent adhesion, rather than regulating cadherin function per se. These alternate possibilities will ultimately be resolved by using more sophisticated assays capable of measuring subtle changes in cell-cell adhesion, and by directly manipulating β -catenin's expression and function.

The molecules *Wnt*, *noggin*, and *gooseoid* are each capable of eliciting the duplicated-axis phenotype after microinjection of their respective mRNAs into early *Xenopus* ventral blastomeres. *Wnt* is a secreted growth factor implicated in many developmental events (41, 43). *Noggin* is secreted and is not homologous to any known protein (42), while *gooseoid* shows homology with homeobox proteins and is likely

a transcription factor regulating developmental patterning events (3). The axis-duplicating activities of *Wnt*, *noggin*, and *gooseoid* suggest that these molecules have roles in the function of either of the two embryonic regions responsible for patterning the dorsoanterior axis: the Nieuwkoop center (10, 29), and the Spemann organizer (3, 42). The Nieuwkoop center and the Spemann organizer are localized at the dorsal side of the embryo. The normal time and pattern of expression of endogenous *noggin* and *gooseoid* are consistent with activities in these regions. Although *Wnts* are very potent axis inducers, no endogenous *Wnt* with the appropriate expression pattern has yet been found. Axis duplicating activity is elicited only when their mRNAs are delivered to ventral blastomeres, because ventral injections induce a new organizing center, leading to the development of novel dorsal structures. Dorsal injections of these molecules are thought to provide a redundant signal. Similarly, only ventral injections of anti- β -catenin Fab fragments appear to be capable of inducing secondary axes.

The temporal and spatial pattern of β -catenin expression does not correlate with the formation of the Nieuwkoop center or Spemann organizer. β -Catenin is present in the *Xenopus* embryo throughout early development. Furthermore in another study of β -catenin expression in *Xenopus* embryos using cross-reacting anti-*armadillo* antibodies, β -catenin was found to be present in most if not all tissues in the early embryo (7). β -Catenin is known to bind to both E- and N-cadherins (18, 22), and we observe that it also binds to C-cadherin, the major and perhaps only cadherin present in *Xenopus* embryos until the gastrula stage (5). Thus, β -catenin is more likely to be a general nonlocalized component of the machinery involved in the induction of dorsal structures rather than a unique or highly localized element defining the organizing centers.

It is intriguing to consider the possibility that β -catenin participates in the same developmental signaling pathway as the secreted *Wnt* growth factor. The β -catenin and *Wnt* homologs in *Drosophila*, *armadillo* and *wingless*, respectively, are both segment polarity genes involved in the determination of cell fates, and mutations in them give rise to very similar phenotypes (37, 38). Moreover, *wingless* regulates the pattern of *armadillo* expression in *Drosophila* embryos (39). Thus, our finding that anti- β -catenin Fabs elicit a similar phenotype as *Wnt* mRNA in the *Xenopus* embryo suggests that a similar relationship may hold for these two molecules in vertebrates. The anti- β -catenin Fabs are neither as efficacious as *Wnt* mRNA in inducing a complete secondary axis, nor in their ability to rescue a primary axis from UV-ventralized embryos, but this is not unexpected. *Wnt* is a secreted factor that has the potential to act on a number of cells, while β -catenin is intracellular and can act only within the cell. Furthermore, perturbation by a microinjected antibody may not be as efficient as the production and secretion of a growth factor by its normal intracellular pathway. So far there is no evidence that *Wnt* expression regulates the levels of β -catenin in *Xenopus* as *wingless* regulates *armadillo* in *Drosophila*, or that *Wnt* alters the association of β -catenin with cadherins (unpublished observations). Further experiments will be needed to determine whether the phenotypic similarities between the effects of *Wnt* mRNA and anti- β -catenin Fabs are due to a mechanistic relationship between these two proteins.

How might β -catenin participate in a developmental signaling event? Unfortunately, its amino acid sequence reveals no obvious biochemical mechanism to be considered at this time. β -Catenin may act directly in an intracellular signal transduction pathway in response to homotypic binding of cadherins at the cell surface. In this model, β -catenin would act directly upstream of the cytoplasmic signal transducing machinery. Alternatively, β -catenin could regulate cadherin adhesion activity (perhaps in response to the reception of a *Wnt* signal) and thereby the extent of specific cell recognition. In this case, the extent of physical cell contact would determine which cells in a tissue are capable of communicating with each other via other juxtacrine signaling systems, such as gap junctions or various cell surface-bound ligand receptor pairs. In this regard, cadherin expression has been found to induce the formation of gap junctions between cultured fibroblasts (24), and to determine the pattern of communication between groups of cells in culture (21). Interestingly, the *Wnts* that induce the formation of a duplicate axis in *Xenopus* have been found to stimulate the extent of gap junction communication between embryonic blastomeres (32). Further experiments will be required to determine whether catenins and cadherins participate directly in cell signaling or influence cell communication by a cosignaling mechanism (40).

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