**β-catenin localization during *Xenopus* embryogenesis: accumulation at tissue and somite boundaries**

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**SUMMARY**

β-catenin is a cytoplasmic protein associated with cadherin adhesion molecules and has been implicated in axis formation in *Xenopus* (McCrea, P. D., Brieher, W. M. and Gumbiner, B. M. (1993) J. Cell Biol. 127, 477-484). We have studied its distribution in *Xenopus* embryos by immunofluorescence on frozen sections. Consistent with its function in cell-cell adhesion, β-catenin is present in every cell. However, high levels are expressed in certain regions and different tissues of the embryo. No simple correlation appears to exist between the levels of β-catenin with the expected strength of adhesion. High levels of β-catenin were found in regions undergoing active morphogenetic movements, such as the marginal zone of blastulae and gastrulae. This suggests that high expression of β-catenin could be involved in dynamic adhesion events. Surprisingly, β-catenin also accumulates on plasma membranes that probably do not establish direct or strong contacts with other cells. In particular, high amounts of β-catenin are found transiently at boundaries between tissue anlagen and at the intersomitic boundaries. This unexpected pattern of β-catenin expression raises the possibility that this molecule participates in developmental processes, perhaps independently of its classical role in cell-cell adhesion.

Key words: cell adhesion, cadherins, catenins, amphibian development, gastrulation, pattern formation, somitogenesis, immunohistochemistry

**INTRODUCTION**

The goal of early embryonic development is to shape the general body pattern by organizing the cells in distinct anlagen, which will later differentiate into functional tissues. Cell adhesion is an essential property that allows embryonic cells to achieve this objective, as they aggregate, sort from neighbors, move, change their shape and migrate. Calcium-dependent adhesion molecules (cadherins) appear to be largely responsible for cell adhesion during early development (Takeichi, 1991). They are complexed to cytoplasmic proteins called α-, β-, and γ-catenin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989). β-catenin appears tightly associated with cadherins (McCrea and Gumbiner, 1991), and α-catenin binds to β-catenin (Ozawa and Kemler, 1992). γ-catenin (plakoglobin), which is related to β-catenin, is mainly found in desmosomes, but also interacts with cadherins (Gumbiner and McCrea, 1993). The function of catenins is not completely understood, but it is commonly assumed that these proteins link cadherins to the actin cytoskeleton. It is becoming increasingly clear that cell adhesion is regulated, and catenins are obvious candidates for modulation of cadherin function (Gumbiner and McCrea, 1993).

Surprisingly, β-catenin has been found to be involved in pattern formation during embryonic development. In *Drosophila*, mutation of the segment polarity gene *Armadillo*, the β-catenin homologue (McCrea et al., 1991; Peifer et al., 1993), causes respecification of cell fate (Peifer et al., 1993). Armadillo acts downstream along a pathway initiated by Wingless, a secreted protein homologous to the vertebrate Wnt proteins (Peifer et al., 1993). Early *Xenopus* embryos injected ventrally with anti-β-catenin antibodies form duplicated axes (McCrea et al., 1993), which is a typical effect obtained by overexpression of some members of the Wnt family (Moon, 1993). Injection of β-catenin mRNA has yielded the same result (Funayama, Guger and Gumbiner, unpublished results). Thus similar inductive pathways, both involving a secreted growth factor of the Wnt family and a cytoplasmic protein associated with cell-adhesion complexes, appear to regulate anterior-posterior polarity in insect segments and dorsoventral pattern formation in amphibians. β-catenin could act in pattern development in different ways: it may modulate cell-cell adhesion, which in turn may influence other cell-cell interactions or, alternatively, the cadherin-catenin complex may be directly involved in cell signalling, in a way similar to signalling by growth factor receptors and integrins. Finally, β-catenin may have a function independent of the cell adhesion complexes. In this context, β-catenin appears to be associated
with the protein encoded by the tumor-suppressor gene APC (Rubinfeld et al., 1993; Su et al., 1993).

To address β-catenin functions during embryogenesis, one must know the pattern of its distribution. In *Xenopus*, several other cadherins and catenins have been characterized and immunolocalized: α-catenin, found in all cells (Schneider et al., 1993), C-cadherin (or EP-cadherin), the major cadherin present during early development (Choi et al., 1990, Ginsberg et al., 1991; Levi et al., 1991a), U-cadherin (Angres et al., 1991), also found in early embryos, but whose relation with C-cadherin is unclear, and the epithelia-specific E-cadherin, expressed from gastrulation on (Choi and Gumbiner, 1989; Angres et al., 1991; Levi et al., 1991b). N-cadherin is expressed after gastrulation in the nervous system and in several mesodermal derivatives (Detrick et al., 1990, Simmoneau et al., 1992). The cellular and tissue distribution of β-catenin has never been studied carefully in vertebrate embryos, although β-catenin and plakoglobin mRNAs have been detected by whole-mount in situ hybridization in dorsal structures of *Xenopus* embryos, and β-catenin mRNA was also found in the midbrain (DeMaraïs and Moon, 1992). Here, we describe the distribution of β-catenin protein during *Xenopus* development, and we show that this molecule, although present in all cells, accumulates at specific sites, and in particular along forming tissue boundaries.

**MATERIAL AND METHODS**

**Antibodies**

Anti-β-catenin polyclonal antibodies were generated against a fusion protein composed of the amino terminus of *Xenopus* β-catenin and the carboxyl terminus of glutathione-S-transferase (GST) (McCrea et al., 1993). Anti-α-catenin polyclonal antibodies were raised against a synthetic peptide corresponding to residues 890 to 906 of the amino-terminus of the mouse α-catenin. Anti-C-cadherin polyclonal antibodies were raised against a bacterial fusion protein containing part of the extracellular domain of C-cadherin (Brieher and Gumbiner, 1994).

**Immunofluorescence**

*Xenopus* eggs were obtained from gonadotropin-injected females and were fertilized artificially (Kay and Peng, 1991). Embryos were allowed to develop in 1:10 MMR at room temperature or at 13-15°C. MMR was 100 mM NaCl, 2 mM KCl, 5 mM Hepes-NaOH, 2 mM CaCl₂, 1 mM MgSO₄, pH 7.4. Embryos were staged according to Nieuwkoop and Faber (1967). Animal caps were isolated from stage 8 embryos in MMR and incubated with or without 5 ng/ml human recombinant activin A (gift of Genentech, South San Francisco, CA, USA).

Embryos were fixed in 80% methanol/20% dimethylsulfoxide (Dent et al., 1989) overnight at −20°C. They were then rinsed in phosphate buffer saline (PBS), pH 7.4 and embedded in 15% cold water fish gelatin (Fluka, Ronkonkoma, NY, USA), 15% sucrose at room temperature for 24 hours. In some cases, the samples were embedded in 7.5% porcine gelatin (Bloom 300, Sigma chemical Co., St. Louis, MO, USA), 15% sucrose at 37°C for 6-10 hours (Sechrist et al., 1993), with similar results. The samples were stored up to two weeks at 4°C in gelatin, then frozen on dry ice. 10 μm sections were cut at −17°C, collected on precoated glass slides (Fisher Scientific Co., Springfield, NY, USA) and stored at −80°C.

The slides were dried at room temperature, dipped 2 minutes in acetone, rehydrated in PBS, incubated at 37°C with the blocking buffer (5% non fat milk in PBS) for 30 minutes, then 2 hours at 37°C with the primary antibody (rabbit serum diluted 1:1000 in PBS containing 2.5% milk). Control sections were incubated in the presence of normal rabbit serum (Sigma) diluted 1:1000. After 3 rinses in 1% milk/PBS, the section were further incubated for 1 hour at 37°C with an FITC-conjugated goat anti-rabbit antibody (0.1 mg/ml, Molecular Probes, Inc., Eugene, OR, USA), then rinsed once in 1% milk/PBS, twice in PBS, once in PBS containing 0.1% Eriochrome Black (Aldrich Chemical Co., Milwaukee, WI, USA) to mask the autofluorescence of the yolk (Torpey et al., 1992) and mounted in a glycerin-PBS medium containing an autofading reagent (SlowFade medium, Molecular Probes, Inc., Eugene, OR, USA). The mouse monoclonal antibody Tor 70, a marker for the notochord, (Bolce et al., 1992) was obtained from Dr R. Harland, University of California Berkeley, Berkeley, CA, USA. An ascites fluid was used at a 1/5000 dilution, in conjuction with an FITC-conjugated goat anti-mouse secondary antibody (0.1 mg/ml, Molecular Probes, Inc., Eugene, OR, USA).

The sections were observed under an Axiosplan microscope (Carl Zeiss, Inc., Thornwood, NY, USA) using a fluorescein filter block. Microphotographs were taken using TMax 400 ASA films (Eastman Kodak Co., Rochester, NY, USA). Photographs of control sections were shot with the same light intensity and exposure time as for the correspondent positive sections.

**Cell fractionation**

All steps were performed at 4°C. Ten embryos were homogenized in 1 ml 250 mM sucrose, 10 mM Hepes-KOH pH 7.4, 2 mM MgCl₂, 1 mM NaNEDTA, 0.5 mM NaEDTA, supplemented with a cocktail of protease inhibitors (1 mM PMSF, 0.5 mM iodoacetamide, 1 μg/ml pepstatin A, 2 μg/ml leupeptin, 4 μg/ml aprotinin, 10 μg/ml antipain, 50 μg/ml benzamidine). The homogenate was centrifuged for 5 minutes at 750 g in a table top Eppendorf centrifuge (Netheler & Hinz GmbH, Hamburg, Germany). The pellet, containing the yolk, some pigment, and large pieces of plasma membrane, was resuspended in 25 μl 0.5% NP-40, 150 mM NaCl, 10 mM Hepes-NaOH, 1.5 mM NaEDTA, with protease inhibitors, spun for 10 minutes at 16,000 g, and the yolk-free supernatant collected (fraction Y). The supernatant of the first centrifugation was layered on top of a 100 μl cushion of 2 M sucrose and spun for 30 minutes at 400,000 g in a table top ultracentrifuge (Beckman Instruments Inc., Columbia, MD, USA). The supernatant (fraction S, soluble) and the sucrose cushion, including the pellet (fraction M, membranes) were concentrated by aceton precipitation. The fractions were analyzed by SDS-PAGE (7% acrylamide) and western blot as previously described (McCrea and Gumbiner, 1991). For each fraction, the amount loaded on the gels corresponded to half an embryo for β-catenin detection, one embryo for α-catenin, and two embryos for C-cadherin. Rabbit anti-β-catenin and anti-α-catenin sera were used at a 1/1000 dilution. C-cadherin was detected with a hybridoma supernatant of the monoclonal mouse antibody 66B diluted 1:1 (Brieher and Gumbiner, 1994). Detection of the antibody was done by chemiluminescence using peroxidase-conjugated secondary antibodies (BioRad Laboratories, Richmond, CA, USA) and the ECL kit (Amersham, Arlington Heights, IL, USA).

**RESULTS**

The immunolocalization of β-catenin during embryonic development was studied and compared to the distribution of α-catenin and C-cadherin. For this purpose, embryo sections were prepared according to a new protocol that combined mild fixation (Dent et al., 1989), gelatin embedding (modified from Sechrist et al., 1993) and sectioning of frozen samples. Using this method, we were able to obtain good sections, even for the earliest embryonic stages, and excellent preservation of the antigens. About 100 embryos from fertilized eggs to advanced tadpole stages were sectioned and immunolabeled in this study. β-catenin staining was intense and could be analyzed in detail. However, β-catenin staining was so much stronger than the signals for α-catenin and C-cadherin, that a precise compari-
son of the relative concentrations of these antigens was not always possible. To verify the specificity of β-catenin staining, in some experiments the anti-β-catenin serum was preincubated with an excess of the GST-β-catenin fusion protein to which the antiserum was raised before staining. The staining was completely blocked by this treatment (Figs 4J, 6E and 8B, higher insert), but was not affected by the GST protein alone (Figs 4K, 6F and 8B, lower insert).

Early stages: cleavage and gastrulation

Xenopus eggs contain large amounts of maternal β-catenin, as well as α-catenin and C-cadherin (Schneider et al., 1993; Choi et al., 1990). Immunofluorescence of the uncleaved fertilized egg detected both β-catenin and C-cadherin in the cytoplasm, but not at the plasma membrane (not shown). This staining was more intense in the animal hemisphere, and could be observed throughout cleavage (Fig. 1A,B). α-catenin seemed to be dis-

![Fig. 1. β-catenin distribution in cleavage stages and early gastrula. (A) Morula (stage 32 cells); (B) late blastula (stage 9), and (C,D) early gastrula (stage 10). β-catenin is present along the blastocoel roof (large arrows), but not the blastocoel floor (small arrows). The arrowhead in panel C shows the apex of the bottle cells on the dorsal side. (D) Detail of the dorsal marginal zone and of the bottle cells of the dorsal blastoporal lip from figure C. Small arrowheads show the superficial cells of the marginal zone. The large arrowhead points at the apex of the bottle cells, and the arrows show their swollen bases. (E) Control section from the marginal zone of a late blastula embryo incubated with a non-immune rabbit serum. Bars = 200 μm (A–C, E) and 100 μm (D). an, animal pole; vg, vegetal pole; mz, marginal zone.](image)
tributed according to the same pattern, but the signal was barely above the background fluorescence. A similar distribution of α-catenin has been reported by Schneider et al. (1993).

Cell fractionation experiments have shown that both α- and β-catenin are mostly membrane-bound in early Xenopus embryos, though small amounts of soluble β-catenin (Fig. 2A) and α-catenin (Fig. 2B) were detectable. These experiments indicate that the observed intracellular staining was largely due to catenins associated with intracellular organelles. Cytoplasmic staining apparently decreased at later stages, but could not be readily observed, due to the decrease in size of the cells and an increase in background fluorescence. Schneider et al. (1993) found larger pools of detergent-soluble catenins in Xenopus embryos, but they did not examine their distribution in the soluble and membrane-bound fractions.

From the first cleavage on, all the newly formed, internal plasma membranes were stained for β-catenin. The only exception was the free surface bordering the blastocoel floor of morulae and blastulae which was negative for β-catenin (small arrows in Fig. 1A-C). On the other hand, the membranes facing the blastocoel roof were clearly stained (large arrows in Fig. 1A-C). β-catenin was never detected on the outer plasma membrane, which is derived from the egg membrane and constitutes the apical membrane domain of the blastomeres. The intensity of the β-catenin signal on the plasma membrane was significantly stronger in the animal cells than in the vegetal cells, but the brightest staining was found around the equatorial zone of morulae (Fig. 1A), and the descendent cells which form the marginal zone of blastulae and early gastrulae (Fig. 1B,C), the region where mesoderm induction occurs. The lateral membranes appeared more intensely stained deep in the embryo than at the periphery (Fig. 1A).

β-catenin appears to be implicated in the determination of the dorsoventral axis, since ventral injection of anti-β-catenin Fab fragments (McCrea et al., 1993) or β-catenin mRNA (Funayama, Guger and Gumbiner, unpublished results) in early Xenopus cleaving embryos causes axis duplication. β-catenin overexpression results in the formation of a second Nieuwkoop center (Guger and Gumbiner, unpublished results). Therefore, sagittal sections from several embryos at morula (32 cells) stage were examined for a possible dorsoventral asymmetry in β-catenin distribution. No obvious difference in the intensity of the β-catenin staining could be detected between dorsal and ventral regions (data not shown). Also, when dorsal and ventral halves dissected from 32- to 126-cell-stage embryos were analyzed for β-catenin by immunoblotting, β-catenin was found equally distributed between dorsal and ventral halves (Fig. 2D). β-catenin was more abundant in the animal hemisphere than in the vegetal hemisphere (Fig. 2E).

During gastrulation, the non-involuting superficial cells showed an increased staining, probably in correlation with expression of E-cadherin in this tissue, while the labeling decreased for the superficial involving cells of the marginal zone (Fig. 3A-C). The involuting deep cells of the marginal zone were intensely labeled for β-catenin (Fig. 3A,B). β-catenin was particularly concentrated in the region of the dorsal blastoporal lip, also known as the Spemann organizer (large arrows, Fig. 3A,B), which will form the axial mesodermal structures of the embryo and will induce the overlying ectoderm to become neural tissue. The staining appeared to fade towards the anterior part of the involuted mesoderm.

Although staining for α-catenin and C-cadherin were less intense, we could not see any obvious difference in the distribution pattern of β-catenin, α-catenin and C-cadherin during cleavage and gastrulation (data not shown).

Neurulation and boundary formation

Starting soon after gastrulation, the embryonic anlagen (ectoderm, neuroderm, notochord, axial and ventrolateral mesoderms) individualize from the mass of as yet undifferentiated cells. Although β-catenin was present at the surface of all cells of neurulae, there was a gradation in the β-catenin distribution, the ectoderm and the neuroderm being more stained than the mesoderm, which in turn was labeled more intensely than the endoderm (Fig. 3E). The most conspicuous feature of β-catenin distribution in neurula stages was that all the major boundaries appearing between these tissues were found to be accentuated by a very bright staining (Fig. 3E). For example, at the end of gastrulation (stage 13), the mesoderm separates from the subjacent endoderm. This boundary was clearly

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Fig. 2. Distribution of β-catenin in membrane and soluble fractions and in dorsal vs. ventral and animal vs. vegetal hemispheres. (A-C) 32-cell-stage embryos were fractionated by differential centrifugation as described in Materials and Methods. Three fractions, a low speed pellet containing the yolk and some plasma membranes (y), a high speed pellet containing all the membranes (m), and the corresponding supernatant (s) were analyzed by Western blot for (A) β-catenin, (B) α-catenin and (C) C-cadherin. Most of these three proteins were recovered in the membrane fraction. The supernatant contained small amounts of both catenins, but no C-cadherin. (D,E) 48-cell-stage embryos were dissected into halves and analyzed for β-catenin by Western blot. Aliquots equivalent of 2 halves of embryo were loaded per lane. an, animal; d, dorsal; v, ventral; vg, vegetal.
detected on our sections (Fig. 3B, arrows), both morphologically (the cells have reorganized in an orderly fashion and aligned along the boundary) and by increased β-catenin staining between the two tissues. The β-catenin-labeled boundary later spread laterally, and then ventrally (Fig. 3E). Fig. 4G shows a detail of this boundary at a midneurula stage. It appears as a bright, continuous line between the two tissues.

In late gastrula stages, the interface between the newly individualized neural plate and the paraxial mesoderm was underlined by an intense β-catenin labeling (not shown), which was maintained during the whole process of neurulation (Figs 3E, 4A,B). The notochord and the floor plate of the neural tube are not separated in early stages. Instead, their cells maintain intimate contacts (Keller et al., 1989). No strong labeling of β-catenin was observed between these two tissues (Fig. 4A,B). In late neurula stages, however, the notochord eventually becomes separated from the neural tube (Keller et al., 1989). A stronger stripe of β-catenin fluorescence appeared transiently at this boundary (stage 19, not shown).

The notochord-paraxial mesoderm boundary forms during

Fig. 3. β-catenin localization in gastrula and neurula stages. (A) Sagittal section, stage 12; the large arrow shows the strong labeling in the deep involuting cells of the dorsal marginal zone. (B) Sagittal section, stage 13; large arrow: dorsal involuting marginal zone; small arrows: mesoderm-archenteron roof boundary. (C) Detail of the dorsal involuting marginal zone at stage 12½, before involution (arrowheads) and after involution (arrows). (D) Detail of the dorsal and ventral blastoporal lips at stage 13; the arrows show the apical staining of the superficial involuting cells. (E) Transverse section, stage 17; the arrows indicate the mesoderm-endoderm boundary. ar, archenteron roof; bc, bottle cells; dl, dorsal lip of the blastopore; ec, ectoderm; en, endoderm; lm, lateral mesoderm; no, notochord; nu, neural plate; vl, ventral lip of the blastopore; yp, yolk plug. Bars = 200 µm (A,B,E) and 100 µm (C,D).
gastrulation (Keller et al., 1989). Initially, no particularly strong labeling was observed along the boundary (not shown). However, a brighter stripe separating these two mesodermal regions soon appeared (stage 14+, Fig. 4A) and persisted during neurulation (Fig. 4B).

α-catenin was also present along the boundaries (Fig. 4E). C-cadherin was expressed in all cells at gastrula stages. It then disappeared from the neural tissue, but was still detected in all other tissues, including at boundaries such as between the notochord and the somitic mesoderm (Fig. 4D).

Another striking pattern observed from late neurula stages on is the enrichment of β-catenin staining in the inner layer of the ectoderm compared to the outer layer (Figs 5, 7 and 8B). β-catenin was detected at high levels all around the inner cells, including at the interface with the mesoderm (Fig. 8B, arrowhead).

![Fig. 4. Distribution of catenins and C-cadherin in axial structures.](image)
β-catenin distribution in *Xenopus* embryos

**Tailbud stages and somitogenesis**

The strong staining at all the tissue boundaries mentioned above was transient. From the end of neurulation to hatching, it progressively disappeared. The notochord lost its peripheral staining quite soon (stage 21, Fig. 4C) and the whole notochord was soon almost devoid of β-catenin staining (Figs 4H, 7A). The β-catenin staining decreased all along the mesoderm-endoderm boundary (Figs 5, 7A), except posteriorly, between the posterior presomitic mesoderm and the archenteron roof (Fig. 5, small arrows). The posterior mesoderm itself remained strongly β-catenin positive throughout the tailbud stages. This region is considered to be a site of active morphogenesis (Gont et al., 1993). α-catenin similarly disappeared from the boundaries, except in the peculiar case of the notochord, where it was highly enriched (Fig. 4I), as already described (Schneider et al., 1993).

A major morphogenetic event occurring at tailbud stages is the segmentation of the paraxial mesoderm into somites. Our sections stained for β-catenin showed that the somites were separated by bright stripes (Fig. 5, short arrows and Fig. 6A, large arrows). The accumulation of β-catenin at the intersomitic boundary is an early event during somitogenesis, since it was detected at the anterior limit of newly formed somites (Fig. 5, long arrow), in continuity with the presomitic mesoderm-endoderm boundary (Fig. 5, small arrows). β-catenin staining increased further in fully separated somites, both at their anterior and posterior limits (Fig. 5, short arrows). The somite boundaries did not display a strong signal for α-catenin (Fig. 6B).

**Late development**

Similar to the tissue boundaries, β-catenin staining between the somites was only transient. Having reached a peak a few hours after somite formation, the intersomitic stripes subsequently decreased in intensity. By stage 35, the staining was weak at all but the most posterior somitic boundaries. At stages 41 and 45, we could barely detect any labeling above background between the somites, while the lateral membranes of the myocytes were clearly stained (Fig. 6C).

In tailbud stages, beside the intersomitic stripes, β-catenin was abundant in the ectoderm, the posterior mesoderm and the neuroderm (Figs 5, 7A). After hatching, however, the staining decreased dramatically in most tissues (Fig. 7B), except the epidermis.

**β-catenin in cell junctions and cell polarity during embryogenesis**

In early *Xenopus* embryos, polarized expression of β-catenin is readily apparent in the cleaving embryo, and later in the ectoderm and the endoderm, where it is excluded from the apical membranes. Cell polarity, however, is transiently lost in the superficial marginal zone during involution, where β-catenin was found at the apical membrane, which makes contact with the underlying vegetal cells of the yolk plug (Fig. 3D). However, a typical epithelial organization, with adhesion molecules concentrated in apical junctions, occurs only progressively during development. Initially, β-catenin was distributed rather homogeneously all along the basolateral membranes. Accumulation of β-catenin at apical junctions...
appeared first in the bottle cells of gastrulae (Fig. 1D) and neurulae (Fig. 3E), then in the cement gland (Fig. 8D, arrow), which is the first truly differentiated organ, and finally, in most epithelial cells (epidermis, Fig. 8C, arrows, neural tube, Fig. 7B, optic and otic vesicles, not shown, cardiac tissue, Fig. 7B, and the intestine, not shown). The distribution of α-catenin was similar, except that its concentration in the apex of ectodermal cells appeared earlier and stronger in many instances (not shown).

Unlike in adult epithelia, β-catenin was clearly present, and even in several cases enriched along the basal membranes of early embryonic epithelia, especially at tissue boundaries. All basal membranes eventually became β-catenin-negative in differentiated tissues. The basal staining in the inner ectoderm disappeared in tadpoles (Fig. 8C, arrowheads), but the interface between the inner and the outer layer remained brightly labeled (Fig. 8D). At least in the case of the epidermis and of the notochord, the loss of basal staining appeared to correlate with their separation from adjacent tissues by a thick extracellular matrix.

**β-catenin distribution in activin-treated animal caps**

We wished to see whether β-catenin staining at boundaries could be experimentally induced in tissue explants. Animal caps isolated from blastula stages develop into balls of ectodermal tissue. However, when treated with activin, mesoderm...
is induced, the caps elongate and axial structures are formed (Symes and Smith, 1987). In uninduced caps, β-catenin staining was relatively uniform throughout the tissue. In activin-induced caps, in contrast, a strong signal was found along the tissue boundaries formed (neuroderm-mesoderm and notochord-axial mesoderm, Fig. 9A), as well as in the inner ectodermal layer (Fig. 9C). Therefore elongated animal caps displayed a pattern for β-catenin very similar to the pattern in normal embryos.

DISCUSSION

In this paper, we have presented a detailed localization of β-catenin in the developing Xenopus embryo. High levels of β-catenin were found during early development at sites of active morphogenesis, particularly in the involuting marginal zone of gastrulae, and later along the boundaries that delimit newly formed tissues, as well as between the somites. In several cases, higher levels of β-catenin clearly correlated with stronger adhesion, for instance in the junctional complexes in the epidermis. In contrast, the accumulation of β-catenin at the tissue boundaries was surprising, since cell-cell adhesion between separating tissues is expected to be low. We will discuss below the possibility that the presence of high levels of β-catenin might, at least in some cases, serve functions other than solely enhancing adhesion.

Though α- and β-catenin distributions were generally similar, there were notable exceptions where either α- or β-catenin were differentially enriched. For instance, in the notochord of tailbud stages, α-catenin was enriched compared to β-catenin. A similar enrichment for α-catenin was also frequently observed at apical junctions of the ectoderm (not shown). α-catenin in these cases may be part of another protein complex, for example containing plakoglobin instead of β-catenin. On the contrary, β-catenin staining was clearly enriched over α-catenin staining at somite boundaries. Although we cannot absolutely exclude the colocalization of an isoform of α-catenin not recognized by our antibody, this antibody labelled both epithelial and neural tissues, and thus

**Fig. 7.** β-catenin distribution in transverse sections of late tailbud and early tadpole stages. (A) Stage 28; (B) stage 35. ca, cardiac tissue; ec, ectoderm; en, endoderm; no, notochord; nu, neural tube; so, somites; arrowheads, inner layer of the ectoderm. Bars, 200 µm.
probably recognizes both known α-catenin subtypes (Hirano et al., 1992).

In Drosophila, a soluble cytosolic pool of Armadillo seems to be involved in pattern formation. In early Xenopus embryos, the majority of both α- and β-catenin is bound to intracellular vesicles, which accounts for the cytoplasmic staining observed on our sections. However, a small soluble pool was detected biochemically both for α- and β-catenin. We could not determine whether soluble catenins are concentrated in some particular region of the embryo. Only membrane-bound antigens could be reliably analyzed in the present immunofluorescence study, since soluble proteins were probably washed out during sample preparation. Also, the amount of soluble catenins is low compared to the large membrane-bound pools present in the cytoplasm of early Xenopus embryos, and would not be discerned by immunofluorescence.

The presence of adhesion complexes is not necessarily indicative of sites of strong cell-cell adhesion. Indeed, C-cadherin and catenins were present all around the blastomeres, while junctional structures are present mostly close to the surface of the cleaving embryo (Sanders and Zalik, 1972). The free surface along the blastocoel roof and the interface between the blastoporal lips and the yolk plug are clear examples where the presence of catenins and cadherins does not correlate with adhesion. This may be consistent with the observation of non-functional cadherin-catenin complexes in other systems (Behrens et al., 1993, Vestweber et al., 1987).

Catenins (and C-cadherin) were found to accumulate in the equatorial zone of morulae, the deep cells of the marginal zone of blastulae and the involuting dorsal mesoderm in gastrulae. These are sites of crucial patterning (inductive) activities during early development (mesoderm induction, axis formation and neural induction). Interestingly, high levels of β-catenin are still present much later in the mesoderm of the tailbud, which is a remnant of the dorsal marginal zone, and continues to be morphogenetically active (Gont et al., 1993). Whether β-catenin or cell-cell adhesion is involved in potentiating inductive events is not known.

The marginal zone undergoes extensive cell rearrangement (involution, convergence and extension), and its cells must maintain a balance between motility and adhesion (Keller and Winklbauer, 1992; Shih and Keller, 1992a). Experiments using the expression of a dominant-negative mutant form of C-cadherin in embryos implicate C-cadherin adhesion in gastrulation movements.
β-catenin distribution in Xenopus embryos

β-catenin distribution in Xenopus embryos (Lee and Gumbiner, unpublished data). Also, adhesion between animal cap cells was found to decrease after treatment with activin, which induces animal caps to elongate (and thus cells to move) and to form mesoderm (Brieher and Gumbiner, 1994). We speculate that high levels of catenins and cadherins might be required to balance cohesion and dynamic movements in this tissue.

A very striking, but unexpected finding is the presence of large amounts of β-catenin at the forming boundaries between tissue anlagen. α-catenin and C-cadherin were also present at the boundaries. The fact that β-catenin also accumulates at tissue boundaries in activin-treated animal caps, which do not form these tissues in normal embryos, suggest that this pattern is not coincidental, but rather reflects a characteristic property of these boundaries. It has been proposed that boundaries would form by withdrawal of adhesion molecules from the periphery of the anlage (Angres et al., 1991). This is clearly not occurring here. One possibility is that the transient presence of high levels of β-catenin could reflect an increase in lateral cell-cell adhesion between cells that line the boundaries (Keller et al., 1989, Shih and Keller, 1992b). Another possibility is that β-catenin, or some other part of the adhesion complex might be regulated so as to function in inhibiting adhesion across the boundaries. Finally, it is also possible that β-catenin may have a function independent of adhesion, such as cell signalling, consistent with the function of Armadillo in Drosophila (Peifer et al., 1993) and of β-catenin during axis formation in Xenopus (McCrea et al., 1993; Funayama, Guger and Gumbiner, unpublished results).

We have observed that β-catenin eventually disappears from the tissue boundaries, but long after the individualization of the tissues. This seems to occur in correlation with the increased deposition of basal lamina. Some extracellular matrix is present all over the embryo early during development (Johnson, 1977), and laminin has also been localized along boundaries (Fey and Hausen, 1990), but typical basement membranes are detected only after separation of the anlagen (Keller et al., 1989). It is tempting to speculate that the early and transient accumulation of β-catenin is involved in creating or provisionally stabilizing the tissue boundaries. Subsequently, the extracellular matrix would definitively delimit the tissues.

At their formation, the somites are separated by a wide gap, as observed by light and electron microscopy (Cary and Klymkowski, 1994, and our unpublished observations). However, the gap soon becomes narrower and contacts are reestablished across the boundary: an extensive system of myotendinous-like cell-matrix junctions is formed (Nakao, 1976, Cary and Klymkowski, 1994). It is unlikely that the intense β-catenin at the intersomitic boundaries is associated with myotendinous junctions, since β-catenin has never been found in cell-matrix junctions. Furthermore, β-catenin accumulation at intersomitic boundaries is transient, while myotendinous junctions will remain an essential structure of developing muscles at later stages. The accumulation of β-catenin, in large excess over detectable α-catenin, raises the possibility that it does not even play a role in cell-cell adhesion, because α-catenin is necessary for the adhesive function of cadherin-catenin complexes (Hirano et al., 1992). It is possible that excess β-catenin at the boundaries is bound to molecules other than cadherins. A candidate for such a molecule is the APC gene product, a tumor-suppressor that has been found to be associated with β-catenin in intestinal cells (Rubinfeld et al., 1993; Su et al., 1993), and in Xenopus embryos (Vleminckx and Gumbiner, unpublished results).

Preliminary immunofluorescence data suggest...

**Fig. 9.** β-catenin distribution in activin-treated animal caps. (A) β-catenin staining of the elongated portion of an activin-treated animal cap fixed at stage 18 (staged from control embryos). β-catenin accumulates along the notochord-paraxial mesoderm (arrowheads) and the neuroderm-mesoderm (arrows) boundaries. (B) Consecutive section stained with Tor 70, a marker of the notochord. (C) β-catenin staining of a larger region of an activin-treated animal cap (stage 18) showing an accumulation of β-catenin in the inner ectodermal layer (arrows). (D) Section of an untreated animal cap (stage 18) stained for β-catenin. β-catenin is distributed homogeneously over the whole tissue. ec, ectoderm; me, mesoderm; no, notochord; nu, neuroderm. Bar, 100 µm.
indeed that APC might also accumulate at the boundaries between the somites (Fagotto and Gumbiner, unpublished).

In Drosophila, Armadillo functions along a signaling cascade initiated by the growth factor Wingless (Peifer et al., 1993, 1994). β-catenin may function in Xenopus like Armadillo in Drosophila. Indeed, injection of β-catenin antibodies (McCrea et al., 1993) or β-catenin mRNA (Funayama, Guger and Gumbiner, unpublished results) in early Xenopus embryos induces axis duplication, similar to the effect of overexpression of Wnt-1, the vertebrate Wingless homologue. Multiple forms of Wnt proteins are expressed in various tissues at different times throughout early Xenopus embryogenesis (Moon, 1993). Their patterns of expression are complex and not completely known, and therefore difficult to compare with β-catenin distribution. Nevertheless, β-catenin is clearly abundant in certain tissues expressing Wnts, e.g. head and tail ectoderm and mesoderm of tailbud, which express Wnt 5A. At least one of these molecules, Xwnt-11, localizes in somites (Ku and Melton, 1993). The complex pattern of β-catenin accumulation could reflect multiple events in early development, perhaps related to the activities of various Wnt molecules.

Overexpression of Wnt-1 in mammalian culture cells has been reported to lead to an increased in β-catenin and plakoglobin pools, and to an increased cell-cell adhesion (Bradley et al., 1993; Hinck et al., 1994). It has been inferred from these data that β-catenin and plakoglobin may act as positive regulators of cell adhesion. Our present results, however, suggest that high expression of β-catenin does not necessarily correlate with strong, stable adhesion, but might in some cases be associated with dynamic systems, or even with sites that form non-adhesive boundaries. The accumulation of β-catenin at boundaries, and especially at the intersomitic furrows, together with the effect of β-catenin overexpression in early embryos on axis formation (Funayama, Guger and Gumbiner, unpublished results), indicate that this molecule may have an important, yet unknown function in tissue patterning, perhaps independent of its role in adhesion.

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