Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 9343–9348, August 1998
Developmental Biology

β-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo

ATHULA H. WIKRAMANAYAKE, LING HUANG, AND WILLIAM H. KLEIN*

Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Communicated by William J. Lennarz, State University of New York, Stony Brook, NY, May 28, 1998 (received for review March 18, 1998)

ABSTRACT **In sea urchin embryos, the animal-vegetal axis is specified during oogenesis. After fertilization, this axis is patterned to produce five distinct territories by the 60-cell stage. Territorial specification is thought to occur by a signal transduction cascade that is initiated by the large micromeres located at the vegetal pole. The molecular mechanisms that mediate the specification events along the animal–vegetal axis in sea urchin embryos are largely unknown. Nuclear β-catenin is seen in vegetal cells of the early embryo, suggesting that this protein plays a role in specifying vegetal cell fates. Here, we test this hypothesis and show that β-catenin is necessary for vegetal plate specification and is also sufficient for endoderm formation. In addition, we show that β-catenin has pronounced effects on animal blastomeres and is critical for specification of aboral ectoderm and for ectoderm patterning, presumably via a noncell-autonomous mechanism. These results support a model in which a Wnt-like signal released by vegetal cells patterns the early embryo along the animal–vegetal axis. Our results also reveal similarities between the sea urchin animal–vegetal axis and the vertebrate dorsal–ventral axis, suggesting that these axes share a common evolutionary origin.**

Axis specification is a prerequisite to initiation of pattern formation and subsequent diversification of pluripotent blastomeres to generate embryonic form. Like eggs of most deuterostomes, the initial axis in the sea urchin egg represents a cytoplasmic polarity along the animal–vegetal pole that is established during oogenesis (1, 2). This polarity can be discerned experimentally by bisecting unfertilized eggs equatorially, fertilizing each half, and observing the developmental outcomes. The animal half develops into a ciliated ball of ectoderm whereas the vegetal half develops into an almost normal pluteus larva (2, 3). Clearly, determinants localized to the vegetal half of the egg cytoplasm play a critical role in the normal development of the embryo, but, despite almost a century of effort, these determinants are yet to be identified.

By around the 60-cell stage, five distinct territories have been established in the embryo, and these territories lie strikingly along the animal–vegetal axis. Specification of the embryonic territories is thought to occur along this axis by a signal transduction cascade initiated by the vegetal pole-located large micromeres (4). Specification is defined here as the "fundamental early developmental processes by which various spatial domains are initially assigned their different fates" (4). It is postulated that the large micromeres inherit vegetally localized determinants that allow these cells to be autonomously specified as a vegetal organizing center (4). Experimental embryology has demonstrated that the large micromeres can indeed act as an organizer and can induce a secondary gut when transplanted to an ectopic location (5). The large micromeres are therefore likely to play a role in normal specification of the vegetal plate territory, which gives rise to endoderm and secondary mesoderm (5, 6). Upwardly directed signaling from vegetal tiers also has been shown to be required for specification of aboral ectoderm and for patterning of ectoderm along the oral–aboral axis (7).

Although the molecular basis for axial patterning in sea urchin embryos is not well understood, classical embryologists identified chemicals that affected axial patterning along the animal–vegetal axis (8). For example, certain agents cause "animalization," in which embryos develop similar to those embryoids developing from isolated animal halves (8). Lithium chloride added to embryos during the cleavage stages leads to "vegetalization" of embryos (8). Embryos vegetalized with lithium are radialized and develop with an excess of endoderm and mesoderm at the expense of ectodermal tissues (2, 9). In addition to its vegetalization effects on embryos, lower concentrations of lithium can pattern animal half-derived ectoderm along the oral–aboral axis (7). Recent reports have demonstrated that, in some species, the developmental effects of lithium can be attributed to it activating the Wnt signaling pathway by inhibiting the serine/threonine protein kinase glycogen synthase kinase 3β (GSK-3β), a negative regulator of β-catenin (10, 11, 12). The striking morphogenetic effects of lithium on sea urchin embryos suggested that β-catenin and the Wnt signaling pathway may play a role in patterning the early sea urchin embryo. This idea has been strengthened by the observation that β-catenin protein appears in the nuclei of vegetal blastomeres as early as the 16-cell stage in *Lytechinus variegatus* embryos (13). In this report, we demonstrate that β-catenin is necessary for specification of the vegetal plate, and, in addition, we demonstrate a direct role for β-catenin for endoderm formation. Our results show that β-catenin is required for specification of aboral ectoderm cells and for patterning ectoderm along the oral–aboral axis, presumably by a noncell-autonomous mechanism. This study also shows that many of the morphogenetic effects of lithium on sea urchin embryos can be mimicked by β-catenin and that blocking β-catenin leads to strong animalization of embryos. These data support the hypothesis that the animalization and vegetalization phenomena induced in sea urchin embryos can be attributed to the modulation of the activity of the Wnt signaling pathway along the animal–vegetal axis. Finally, our results suggest a possible evolutionary relationship between the sea urchin animal–vegetal axis and the vertebrate dorsal–ventral axis.

MATERIALS AND METHODS

Embryo Culture. *L. variegatus* were obtained from Susan Decker Services (Hollywood, FL) or Tracy Andacht (Duke

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GSK-3β; glycogen synthase kinase 3β; ASW, artificial seawater; RT-PCR, reverse transcription–PCR.

*To whom reprint requests should be addressed at: Department of Biochemistry and Molecular Biology, Box 117, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. e-mail: wklein@odin.mdacc.tmc.edu.

University Marine Laboratory, Beaufont, NC). Eggs and sperm were obtained by intracoelomic injection of 0.5 M KCl. Embryos were cultured in filtered artificial seawater (ASW) at 25°C.

RNA Synthesis and Microinjection. The plasmids containing cDNAs were linearized, and capped RNA was transcribed by using the mMessage mMachine kit (Ambion, Austin, TX) as recommended by the manufacturer. The pt β-catenin cDNA (14) was a gift from D. Kimelman (University of Washington); HT-6 and C-cadherin cDNAs (15) were a gift from B. Gumbiner (Memorial Sloan-Kettering Cancer Center). These cDNAs were derived from *Xenopus* and were thus heterologous. RNA was diluted in 40% glycerol before injection. The approximate concentrations of the RNAs injected are as follows: pt β-catenin RNA, 0.05 pg/pl; C-cadherin RNA, 0.5 pg/pl; and HT-6 RNA, 0.05 pg/pl. The HT-6 RNA was injected at a concentration of 0.5 pg/pl in some experiments. Fertilized eggs were injected with 1–2 pl of the RNA solution. Eggs were prepared and microinjected as described (16). For all experiments, 300–500 fertilized eggs were injected, and viability after injection was usually >90%. Embryos were harvested at the prism or early pluteus stage. For reverse transcription–PCR (RT-PCR) analysis, RNA was isolated from 40 embryos whereas 100–150 embryos were collected for antibody staining experiments.

Expressing RNA in Animal Halves. Eggs were fertilized in ASW containing 3-amino 1,2,4-triazole and were injected with a given RNA. For experiments on the induction of endoderm in animal halves by β-catenin, fertilized eggs were injected with 0.05–0.1 pg RNA before isolation of animal halves. In experiments monitoring the effect of β-catenin on ectoderm, fertilized eggs were injected with 0.01–0.02 pg RNA before the isolation of animal halves. Shortly after injection, the zygotes were removed from the injection plates by using a mouth pipette and were placed in fresh ASW. This procedure usually removed the zygotes from the vitelline envelope. Embryos were monitored periodically and, at the third cleavage, were placed in a dish with hyaline extraction medium for 5 min. After this incubation, embryos were placed in calcium-free seawater, where they were bisected by using a drawn-out glass needle. Each half was placed in ASW and was monitored at the fourth cleavage. Animal halves were identified easily by the characteristic cleavage pattern of the mesomeres (see Fig. 2). The injected animal halves were placed in fresh ASW and were cultured at 25°C. The preparation of dishes and compositions of solutions used have been described (17). For each injected RNA, 10–15 animal halves were collected for RT-PCR analysis.

Immunofluorescence and RT-PCR. Immunofluorescence and RT-PCR procedures were done essentially as described (7, 17). The anti-myosin heavy chain antibody was a gift from Gary Wessel (Brown University), and the Endo-1 antibody was a gift from David McClay (Duke University). LvS1 is an aboral ectoderm-specific marker (18) whereas LvN1.2 and LvEndo16 are endoderm markers (19, 20).

RESULTS

Overexpression of β-Catenin Vegetalizes Sea Urchin Embryos and Mimics the Effects of Lithium. A key regulator of β-catenin and the Wnt signaling pathway in cells is GSK-3β. GSK-3β negatively regulates the Wnt signaling pathway by phosphorylating β-catenin on several serine/threonine residues, including a cluster of residues at the amino terminus of the protein, leading to its targeted degradation through the proteosome pathway (14, 21). In pt β-catenin, these amino terminal serine/threonine residues are mutated to alanine. This mutated β-catenin protein escapes regulation by GSK-3β, becomes hyperstable in the cytoplasm, and mimics Wnt signaling, essentially functioning as an "activated" form of the protein (14). Sea urchin embryos overexpressing this activated β-catenin by RNA injection were highly vegetalized (Fig. 1*A*). There were increased numbers of endoderm cells in these embryos as well as in the numbers of secondary mesenchyme-derived pigment cells (Fig. 1*A*) and muscle cells (data not shown). The increase in the number of endoderm cells and muscle cells was apparent by the increase in the number of Endo-1 and myosin heavy chain-positive cells, respectively, in injected embryos (data not shown). Small triradiate spicules were present in some of the vegetalized embryos (data not shown). In addition, the embryos were radialized and lost ectodermal structures such as the stomodeum and the ciliary band. These embryos strikingly resembled embryos exposed to lithium during the cleavage stages (Fig. 1*B*; ref. 9), implying that the morphogenetic effects of lithium on sea urchin embryos were caused by its perturbation of the Wnt/β-catenin signaling pathway. Embryos developed normally when eggs were injected with an mRNA encoding a truncated form of β-catenin (HT-6) lacking the armadillo repeats 5–13 and the carboxy terminus of the protein (Fig. 1*C*). This truncated protein is unable to interact with DNA binding proteins such as LEF1/TCF that allow β-catenin to function as a transcriptional coactivator (22). Normal embryonic development was seen even with the injection of 1 pg of this truncated RNA into fertilized eggs (data not shown). These results indicated that functional β-catenin could vegetalize sea urchin embryos.

β-Catenin Causes Ectodermal Precursors to Adopt Endodermal Fates. β-Catenin could vegetalize sea urchin

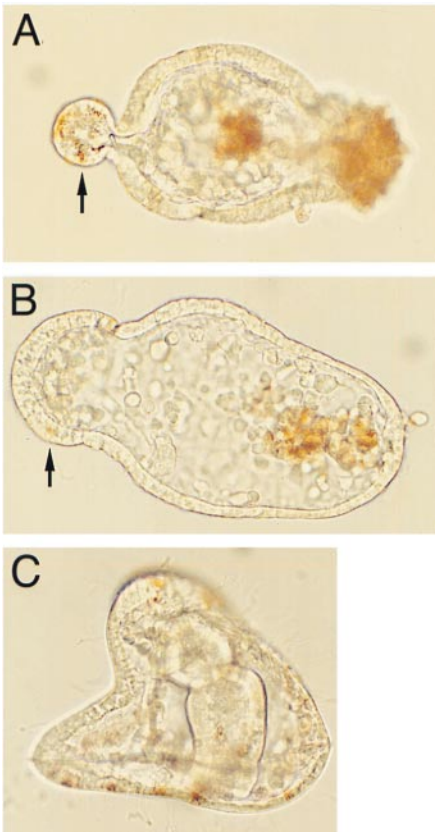

FIG. 1. Vegetalization of embryos by β-catenin and lithium. (*A*) A vegetalized embryo developing from an egg injected with pt β-catenin (amino terminal serine/threonine mutated to alanine) RNA. The arrow points to the remaining ectoderm in this embryo. The increased number of secondary mesoderm-derived pigment cells are seen clearly in this embryo. (*B*) A vegetalized embryo resulting from incubation in 35 mM lithium chloride. The arrow points to the remaining ectoderm in this embryo. (*C*) A pluteus larva developing from an egg injected with an RNA encoding a truncated β-catenin (HT-6) protein (lacking the armadillo repeats 5–13).

embryos in two ways. It could lead to increased endoderm and suppression of ectodermal cell fates, perhaps by increasing signaling from vegetal cells. Alternatively, β-catenin could change directly the fate of ectodermal precursors to endoderm. To test the possibility that pt β-catenin could directly vegetalize mesomeres, we used a protocol to express pt β-catenin in isolated animal halves, which are fated to only form ectoderm (Fig. 2*A*; refs. 2 and 4). Injection of 0.05–0.1 pg of pt β-catenin RNA into fertilized eggs and subsequent culturing of isolated animal halves resulted in induction of endoderm in these explants whereas HT-6 RNA had no effect (Fig. 2 *B* and *C*). In addition to the morphology, RT-PCR analysis demonstrated that both early (Endo 16) as well as late gut markers (LvN1.2) were expressed in pt β-catenin RNA-injected animal halves (data not shown). These results clearly demonstrated that β-catenin could change the fates of ectodermal precursors to endoderm as observed for lithium (23) and that β-catenin was sufficient for endoderm formation. In these experiments, we did not observe spicule formation in animal halves after injection of the pt β-catenin RNA.

Low Concentrations of β-Catenin Pattern Ectoderm Along the Oral–Aboral Axis. When animal halves are made from eight-cell *Lytechinus* embryos, they form polarized embryoids that do not form any endoderm or mesoderm. In addition, these animal halves do not form any aboral ectoderm and lack ectoderm-specific structures such as the stomodeum and the ciliary band (7, 17). We have shown that these animal halves express the oral ectoderm marker Ecto V on the surface of all cells and in addition produce oral ectoderm-derived serotonergic cells (7, 17). When these animal halves are treated with concentrations of lithium that do not induce endoderm, they undergo morphological as well as molecular changes in a manner consistent with patterning along the oral–aboral axis (7). Because it was clear that β-catenin was mimicking many of the effects of lithium, we reasoned that lower doses of β-catenin would mimic this lithium effect and reproduce the range of effects induced by low concentrations of lithium. Injection of 0.01–0.02 pg of pt β-catenin RNA into fertilized eggs and subsequent isolation of animal halves resulted in strong induction of the aboral ectoderm-specific gene LvS1 in animal blastomeres with no expression of endoderm-specific genes (Fig. 3*A*). Furthermore, although HT-6 injected animal halves formed the typical morphologically polarized embryoids (Fig. 3*B*), animal halves injected with the low concentrations of pt β-catenin mRNA developed stomodea and ciliary bands, demonstrating that normal patterning along the oral–aboral axis had occurred in the absence of endoderm and mesoderm (Fig. 3 *C* and *D*). Thus, pt β-catenin mimicked many of the developmental effects of lithium on sea urchin embryos. Moreover, these experiments revealed that, in addition to a role for β-catenin in patterning the animal-vegetal axis, it also had a distinct role in patterning of the oral–aboral axis.

Blocking β-Catenin Signaling Animalizes Sea Urchin Embryos. To ascertain the role of endogenous β-catenin in animal–vegetal axis patterning, we attempted to block endogenous β-catenin signaling by overexpressing the cell adhesion molecule C-cadherin. C-cadherin binds β-catenin via its cytoplasmic domain, and overexpressing C-cadherin leads to the depletion of the signaling pool of β-catenin (15, 24, 25). Fertilized sea urchin eggs were injected with ≈0.5–1.0 pg of C-cadherin RNA and were analyzed when control embryos were at the prism stage. At this stage of development, control uninjected embryos had gastrulated and had begun to form the characteristic larval shape (Fig. 4*A*). In contrast, C-cadherin RNA injected embryos appeared severely animalized (Fig. 4*B*). These embryos lacked archenterons and did not have any

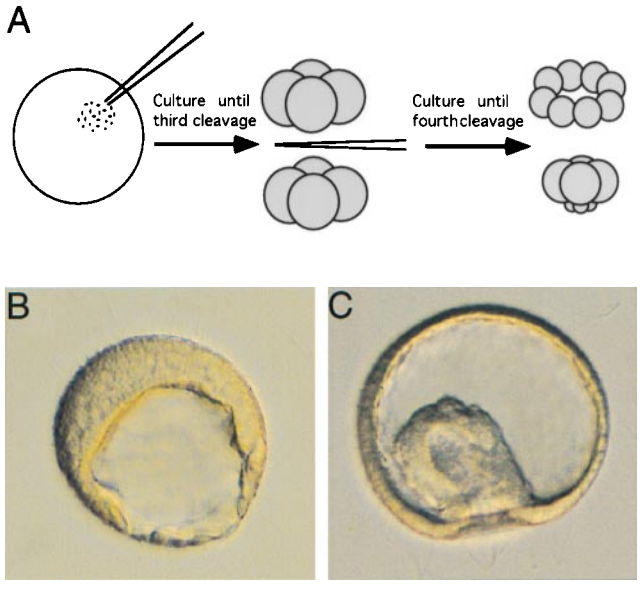

FIG. 2. Induction of endoderm in animal halves by β-catenin. (*A*) Protocol for introducing mRNA into animal halves. (*B*) An animal half made from an embryo that was injected at the one-cell stage with an RNA encoding a truncated β-catenin protein (HT-6). It develops as a polarized embryoid that does not form aboral ectoderm, endoderm, or mesoderm. (*C*) Induction of endoderm and gastrulation in an animal half made from an embryo injected at the one-cell stage with pt β-catenin RNA.

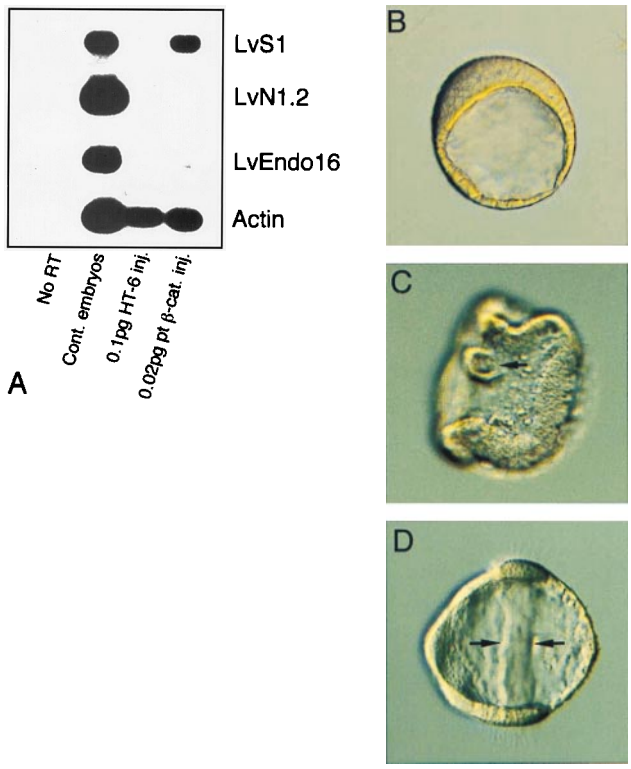

FIG. 3. Patterning of ectoderm by low concentrations of β-catenin. (*A*) Induction of aboral ectoderm in isolated animal halves by β-catenin. RT-PCR was used to monitor the expression of aboral ectoderm and endoderm-specific markers. LvS1 is an aboral ectoderm-specific marker; LvEndo16 and LvN1.2 are endoderm-specific markers. Actin primers were used to monitor input cDNA for each sample. The autoradiograph shows that aboral ectoderm is induced after injection of a low concentration of pt β-catenin RNA into animal halves whereas expression of endodermal markers is not detected. (*B*) Animal half made from an HT-6 RNA-injected embryo. (*C* and *D*) Patterning of ectoderm by low concentrations of pt β-catenin. (*C*) Induction of a stomodeum in a pt β-catenin-injected animal half (arrow). (*D*) Induction of a ciliary band in a pt β-catenin-injected animal half (arrows).

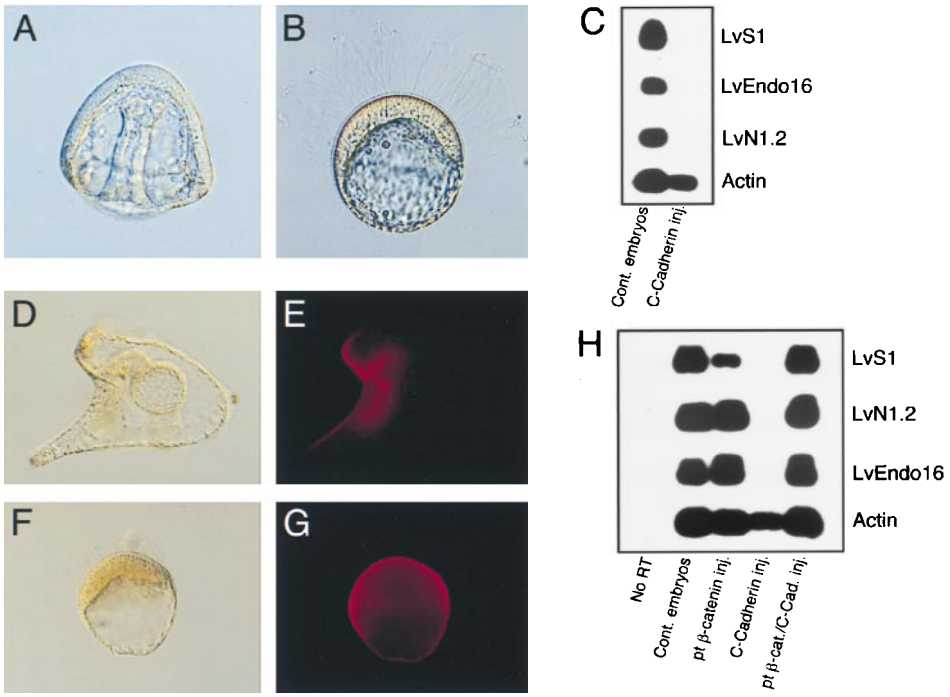

FIG. 4. Animalization of sea urchin embryos by overexpression of C-cadherin. (*A* and *B*) Morphology of control and C-cadherin RNA-injected embryos. (*A*) Uninjected embryo at the early prism stage. (*B*) An embryo animalized by C-cadherin. It is radialized and does not develop endoderm or mesoderm, and long cilia are seen in the cuboidal epithelium. (*C*) RT-PCR analysis of C-cadherin RNA-injected embryos. In addition to the loss of the endodermal markers LvEndo16 and LvN1.2, these embryos do not express the aboral ectoderm-specific marker LvS1. (*D*, *E*, *F*, and *G*) Expression of the oral ectoderm marker Ecto V in control embryos (*D* and *E*) and C-cadherin RNA-injected embryos (*F* and *G*). (*D* and *F*) Differential interference contrast images. (*E* and *G*) Corresponding indirect immunofluorescent images. In control embryos, the Ecto V antigen is localized to the oral ectoderm whereas, in the C-cadherin RNA-injected embryos, the Ecto V antigen is seen on the surface of all blastomeres. (*H*) RT-PCR analysis of C-cadherin RNA and pt β-catenin RNA co-injected embryos. Autoradiograph shows that, although marker genes for endoderm and aboral ectoderm are not expressed in C-cadherin-injected embryos, they are rescued with injection of pt β-catenin RNA.

secondary mesenchyme-derived pigment cells or primary mesenchyme-derived spicules (Fig. 4*B*). In addition, these embryos had a polarized epithelium and a morphology reminiscent of animal half explants. This included the extended cilia seen on the surface of the cuboidal epithelium (Fig. 4*B*). Analysis of these embryos by using RT-PCR showed that they did not express endoderm-specific or aboral ectoderm-specific markers (Fig. 4*C*). Furthermore, similar to animal halves made from eight-cell embryos, the embryos overexpressing C-cadherin expressed the oral ectoderm marker Ecto V in all cells (Fig. 4 *D–G*). The lack of aboral ectoderm-specific gene expression in C-cadherin RNA-injected embryos complemented our observation that aboral ectoderm genes were induced when low doses of β-catenin RNA were injected into animal halves (Fig. 3*A*) and indicated that, in addition to its role in endoderm formation, β-catenin also was required for specification of aboral ectoderm and patterning along the oral–aboral axis. To determine whether the effects of C-cadherin were caused by its sequestering of β-catenin, we attempted to rescue the C-cadherin-induced phenotype in embryos by co-injection of pt β-catenin RNA. Fertilized eggs were injected with 1–2 pl/egg from a mixture of RNA containing C-cadherin and pt β-catenin RNA at concentrations of 0.5 pg/pl and 0.05 pg/pl, respectively. Co-injection of pt β-catenin and C-cadherin RNA resulted in morphologically normal embryos (data not shown) as well as restoration of marker gene expression, indicating that the effect of C-cadherin was caused by its sequestering of β-catenin (Fig. 4*H*).

DISCUSSION

The specification of the animal–vegetal axis in the sea urchin embryo as well as the patterning of this axis during embryogenesis has been a topic of investigation and debate since the beginning of this century. Although models have been proposed to explain how these processes occur, molecular evidence supporting these models has been limited. Our results provide evidence that β-catenin plays a critical role in the patterning of the animal–vegetal axis and raises the possibility that specification of this axis in sea urchin embryos may involve a vegetally localized Wnt-like activity.

***β*-Catenin and Specification of Vegetal Cell Fates.** In the sea urchin embryo, vegetal cells produce three of the five initial territories: the vegetal plate, which gives rise to the endoderm and secondary mesenchyme; the large micromeres, which give rise to the skeletogenic mesenchyme; and the small micromeres (4). Several lines of evidence indicate that β-catenin plays a critical role in the specification of several of these cell types. The first indication of a possible role for β-catenin in this process came from the observation that the protein was strikingly localized to the nuclei of vegetal cells in 16- to 120-cell stage embryos (13). Because specification of the five territories occurs around the 60-cell stage (4), β-catenin was in the right place at the right time to play a role in the establishment of the vegetal territories. In gain of function as well as loss of function experiments, we have provided direct evidence of a role for β-catenin in specification of vegetal cell fates and a direct role for this protein in endoderm formation. Embryos overexpressing β-catenin were highly vegetalized and had a phenotype very much like those vegetalized by lithium. There were increased numbers of endoderm cells in these embryos as well as secondary mesenchyme-derived pigment and muscle cells. The effect of vegetalization by β-catenin on the skeletogenic mesenchyme or small micromeres is harder to assess, mainly because of the lack of early markers for these cells. Vegetalized embryos usually formed only small triradiate spicules, but this may be a secondary effect caused by the reduction in the ectoderm. Ectoderm-derived cues are re-

quired for spicule growth (26), and vegetalized embryos have severe defects in ectoderm patterning. If these ectodermal defects lead to the loss of the ectoderm-derived cues for spicule growth and patterning, then, even if the primary mesenchyme cells were present, they may be unable to secrete the spicule matrix. Blocking the signaling activity of β-catenin by overexpressing C-cadherin completely blocked formation of all vegetal cell types, including the large micromere-derived primary mesenchyme cells, indicating that β-catenin signaling was required for their formation. Because the large micromeres are specified autonomously, this raises the interesting possibility that β-catenin may play a role in this process. Although our data suggest that β-catenin may be important in this autonomous specification, further experiments are needed to test whether the differentiation and inductive activities of the large micromeres are affected by the loss of the signaling pool of β-catenin. For example, it would be interesting to remove large micromeres from C-cadherin overexpressing embryos and transplant them to untreated host embryos or culture them *in vitro* to see whether they could differentiate into skeletogenic mesenchyme. Similarly, it also would be informative to determine whether blocking β-catenin affects the gut-inducing activity of the micromeres by transplanting them to the animal pole of host embryos.

β-Catenin and Ectoderm Patterning. In the sea urchin embryo, there is evidence that specification of aboral ectoderm and patterning of the oral–aboral axis result from upwardly directed signals from vegetal cells (7). The loss of ectoderm patterning and the loss of aboral ectoderm gene expression in embryos animalized with C-cadherin indicate that β-catenin signaling also is required for these events to occur. Remarkably, these animalized embryos resembled animal halves in morphology as well as in the global expression of the oral ectoderm marker Ecto V. Because mesomeres never display nuclear β-catenin (13, 27), the influence of this protein on patterning the oral–aboral axis most likely occurs via a noncell-autonomous mechanism. Horstadius (2, 28) showed that, when animal halves were recombined with the veg1 tier of cells, these embryoids formed ciliary bands and stomodea, suggesting that signals from this tier are necessary for the normal patterning of ectoderm. McClay and colleagues have shown that, although the macromeres have a high level of β-catenin in nuclei, when these cells divide to form the veg1 and veg2 tiers of cells, high levels of nuclear β-catenin are maintained in the veg2 tier whereas the veg1 tier has reduced nuclear levels of the protein (D. R. McClay, personal communication). Low levels of β-catenin expressed in animal halves can induce morphological and molecular alterations consistent with the patterning of ectoderm along the oral–aboral axis. These observations suggest that the β-catenin in veg1 cells may be critical for generating signals that pattern ectoderm. This idea could be tested by recombining animal halves from untreated embryos with veg1 tiers from β-catenin-depleted embryos to see whether these cells retain their patterning activity.

The mechanisms by which β-catenin can effect these changes are not clear at this time, but target genes of β-catenin are likely to play a role in these processes. Perhaps low levels of β-catenin would activate a certain subset of genes that together would specify a "veg1" fate in isolated animal halves, and signaling from these cells would lead to polarization of the ectoderm. We have proposed (7) a similar mechanism for the effect of low concentrations of lithium on animal halves. Zecca *et al*. (29) have shown that, in *Drosophila*, wingless protein can act as a morphogen and elicit patterning of wing imaginal discs in a concentration-dependent manner. This patterning is mediated by armadillo protein, which is the β-catenin homolog in flies. It is possible that a similar mechanism occurs in sea urchin embryos in which a concentration gradient of nuclear β-catenin can pattern cells along the animal–vegetal axis. Consistent with this idea, we have observed that overexpressing high concentrations of β-catenin in animal halves leads to the generation of cells consistent with a secondary mesenchyme phenotype in addition to induction of endoderm (A.H.W., unpublished observations). Our results, therefore, are consistent with a model in which a β-catenin-dependent signal transduction cascade originating in the vegetal cells patterns the sea urchin embryo along the animal–vegetal axis in a concentration-dependent manner.

Regulation of β-Catenin Nuclear Localization in Sea Urchin Embryos. The nuclear localization of β-catenin in vegetal cells is one of the earliest molecular asymmetries identified along the animal–vegetal axis. Identifying the upstream signals mediating this nuclear translocation should provide insights into the specification of the animal–vegetal axis. Given the clear role of β-catenin in endoderm specification and the well known role for the large micromeres in endoderm formation, it would be reasonable to expect that signals from the large micromeres would mediate the nuclear localization of this protein in vegetal cells. However, McClay and colleagues have reported that nuclear localization of β-catenin was not affected in embryos with deleted micromeres. In addition, when the large micromeres were transplanted to the animal pole of a host embryo, β-catenin was not localized to the nucleus in these cells, indicating that nuclear localization of this protein is not necessary for induction of endoderm in animal pole transplants (D. R. McClay, personal communication). It is possible that, in the transplant experiments, the large micromeres activate components of the pathway leading to endoderm formation downstream of β-catenin. The normal role for the micromeres may be to synergize with other signals, such as lateral signals from macromeres, to ultimately specify of the vegetal plate.

A Conserved Role for β-Catenin in Axial Patterning. The pivotal role of β-catenin in patterning the animal–vegetal axis in sea urchins has revealed intriguing similarities between axial patterning in sea urchins and axial patterning in vertebrates. In vertebrates such as *Xenopus*, it has been suggested that a biochemical activity that is displaced to the presumptive dorsal region during the cortical rotation serves to specify dorsal cell fates by selective inhibition of GSK-3β (14, 27, 30). This, in turn, would lead to stabilization of β-catenin and the subsequent nuclear localization of this protein. A role for GSK-3β in patterning the animal–vegetal axis in sea urchins has been shown recently by Gache and colleagues (31). Thus, it appears that this entire pathway may be conserved in axial patterning in vertebrates and sea urchins.

What is unclear in vertebrates is the upstream signals leading to the nuclear localization of β-catenin. Although Wnts have long been thought to be candidates for specifying dorsal cell fates in *Xenopus*, and a Wnt-like activity is associated with the vegetal cortex (30), a dominant negative form of XWnt-8 has no effect on specification of the dorsal–ventral axis (32). In sea urchins, several Wnt genes are expressed maternally, and we have observed that XWnt-8 is a strong inducer of endoderm in these embryos (A.H.W., unpublished observations). Identifying the spatial distribution of these maternal Wnt molecules may give some insight to whether any of these molecules have a localization that would be consistent with their playing a role in animal–vegetal axis patterning. An appealing model is that a maternal Wnt localized to vegetal cells would act as a morphogen to initiate patterning of the animal–vegetal axis by localizing β-catenin to the nucleus. The cells closest to the source may localize higher levels of β-catenin to the nucleus whereas cells more distant localize lower levels, thus producing the distinct cell types along the animal–vegetal axis.

We thank David Kimelman for the pt β-catenin cDNA and Barry Gumbiner for the HT-6 and C-cadherin cDNAs, Javier Capdevila for very helpful suggestions, and Tongyu Cao for assistance with preparation of the figures. We also thank David McClay and Cati Logan for

sharing data before publication. This work was supported by National Institutes of Health Grant HD22619 and Grant G 1210 from the Welch Foundation to W.H.K.

1. Goldstein, B. & Freeman, G. (1997) *BioEssays* **19,** 105–116.
2. Horstadius, S. (1973) *Experimental Embryology of Echinoderms* (Clarendon Press, Oxford).
3. Maruyama, Y. K., Nakaseko, Y. & Yagi, S. (1985) *J. Exp. Zool.* **236,** 155–163.
4. Davidson, E. H. (1989) *Development (Cambridge, U.K.)* **105,** 421–445.
5. Ransick, A. & Davidson, E. H. (1993) *Science* **259,** 1134–1138.
6. Ransick, A. & Davidson, E. H. (1995) *Development (Cambridge, U.K.)* **121,** 3215–3222.
7. Wikramanayake, A. H. & Klein, W. H. (1997) *Development (Cambridge, U.K.)* **124,** 13–20.
8. Lallier, R. (1975) in *The Sea Urchin Embryo: Biochemistry and Morphogenesis*, ed. Czihak, G. (Springer, New York), pp. 473–507.
9. Nocente-McGrath, C., McIsaac, R. & Ernst, S. G. (1991) *Dev. Biol.* **147,** 445–450.
10. Klein, P. S. & Melton, D. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8455–8459.
11. Stambolich, V., Ruel, L. & Woodgett, J. R. (1996) *Curr. Biol.* **6,** 1664–1668.
12. Hedgepeth, C. M., Conrad, L. J., Zhang, J., Huang, H. C., Lee, V. M. & Klein, P. S. (1997) *Dev. Biol.* **185,** 82–91.
13. Logan, C. Y. & McClay, D. R. (1998) in *Cell Fate and Lineage Determination*, ed. Moody, S. A. (Academic, San Diego), in press.
14. Yost, C., Torres, M., Miller, J. R., Hung, E., Kimelman, D. & Moon, R. T. (1996) *Genes Dev.* **10,** 1443–1454.
15. Faggotto, F., Funayama, N., Gluck, U. & Gumbiner, B. M. (1996) *J. Cell Biol.* **132,** 1105–1114.
16. McMahon, A. P., Flytzanis, C. N., Hough-Evans, B. R., Katula, K. S., Britten, R. J. & Davidson, E. H. (1985) *Dev. Biol.* **108,** 420–430.
17. Wikramanayake, A. H., Brandhorst, B. & Klein, W. H. (1995) *Development (Cambridge, U.K.)* **121,** 1497–1505.
18. Wessel, G. M., Zhang, W., Tomlinson, C. R., Lennarz, W. J. & Klein, W. H. (1989) *Development (Cambridge, U.K.)* **106,** 355–365.
19. Wessel, G. M., Goldberg, L., Lennarz, W. J. & Klein, W. H. (1989) *Dev. Biol.* **136,** 526–536.
20. Ransick, A., Ernst, S., Britten, R. J. & Davidson, E. H. (1993) *Mech. Dev.* **42,** 117–124.
21. Aberle, H., Bauer, A., Stappert, J., Kispert, A. & Kemler, R. (1997) *EMBO J.* **16,** 3797–3804.
22. van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hush, D., Jones, T., Bejsovec, A., *et al.* (1997) *Cell* **88,** 789–799.
23. Livingston, B. T. & Wilt, F. H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3669–3673.
24. Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. & Wylie, C. (1994) *Cell* **79,** 791–803.
25. Sanson, B., White, P. & Vincent, J. (1996) *Nature (London)* **383,** 627–630.
26. Guss, K. A. & Ettensohn, C. A. (1997) *Development (Cambridge, U.K.)* **124,** 1899–1908.
27. Miller, J. R. & Moon, R. T. (1996) *Genes Dev.* **10,** 2527–2539.
28. Horstadius, S. (1939) *Biol. Rev. Cambridge Philos. Soc.* **14,** 132–179.
29. Zecca, M., Basler, K. & Struhl, G. (1996) *Cell* **87,** 833–844.
30. Marikawa, Y., Li, Y. & Elinson, R. P. (1997) *Dev. Biol.* **191,** 69–79.
31. Emily-Fenouil, F., Ghiglione, C., Lhomond, G., Lepage, T. & Gache, C. (1998) *Development (Cambridge, U.K.)* **125,** 2489–2498.
32. Hoppler, S., Brown, J. D. & Moon, R. T. (1996) *Genes Dev.* **10,** 2805–2817.