The Emergence of Pattern in Embryogenesis: Regulation of beta-Catenin Localization During Early Sea Urchin Development
Charles A. Ettensohn (14 November 2006)
Sci. STKE **2006** (361), pe48. [DOI: 10.1126/stke.3612006pe48]

The Emergence of Pattern in Embryogenesis: Regulation of β-Catenin Localization During Early Sea Urchin Development

Charles A. Ettensohn

(Published 14 November 2006)

Developmental biologists have long sought to understand the mechanisms by which blastomeres of early animal embryos activate different programs of gene expression and acquire distinct fates. In many organisms, the initial regionalization of the embryo is presaged by the polarized distribution of regulatory molecules (such as proteins and mRNAs) in the unfertilized egg. The canonical Wnt signaling pathway, a highly conserved pathway that regulates the development of diverse tissues in many organisms, has a critical role in early embryonic patterning in animals as diverse as sea anemones and frogs. An intriguing question is how this pathway is regulated in early animal embryos and how the polarity of the unfertilized egg might be linked to the activation of the pathway only in certain blastomeres during early cleavage.

β-Catenin and Early Embryo Patterning

β-catenin was originally identified as a protein that participates in cell-cell adhesion, but it was later found to have a second major function in the nucleus, where it regulates the transcription of specific target genes through interactions with DNA binding proteins of the LEF (lymphoid enhancer factor) and TCF (T cell factor) family (*1*). This nuclear, transcription-related function of β-catenin is regulated by the canonical Wnt signaling pathway (*2*). Activation of the canonical Wnt pathway inhibits the continuous, rapid proteolytic degradation of β-catenin that normally takes place in the cytoplasm, leading to the accumulation of β-catenin in the cytoplasm and in the nucleus.

β-catenin is a critical regulator of polarity in early animal embryos. Among deuterostomes, β-catenin becomes localized in the nuclei of blastomeres at one pole of early amphibian, fish, avian, ascidian, and sea urchin embryos (*3*–*7*). In general, the pole of the embryo in which β-catenin is detected in the nucleus later gives rise to the endomesoderm and also produces signals that have an important role in organizing the early embryo (*7*–*12*). A role for β-catenin in axis specification has also been demonstrated in cnidarians (*13*, *14*). Because cnidarians are a basal metazoan clade, polarized nuclear localization of β-catenin may be a very ancient mechanism of early patterning among metazoans.

β-Catenin in the Early Sea Urchin Embryo

In the early sea urchin embryo, β-catenin is associated with cell-cell contacts in all cells, but only in the vegetal blastomeres is it concentrated in the nucleus (*5*, *15*). The accumulation of β-catenin in the nuclei of vegetal blastomeres is essential for the proper development of the embryo. Molecular biological approaches have been used to (i) sequester β-catenin in the cytoplasm of vegetal cells, preventing its accumulation in nuclei (*5*, *16*); (ii) stimulate the degradation of β-catenin in the cytoplasm of vegetal cells (*17*); and (iii) interfere with the interaction between β-catenin and its LEF/TCF binding partner (*18*). All these experimental manipulations have the same effect on embryonic development: The embryo fails to form endoderm and mesoderm, as assessed by morphological criteria and patterns of gene expression. In this organism, β-catenin functions as an early input into complex gene regulatory networks deployed in the endoderm and mesoderm of the embryo. These gene regulatory networks have been extensively characterized and control the zygotic expression of all genes that are known to be expressed selectively in the endoderm and mesoderm (*19*, *20*).

These observations raise an important question: Why does β-catenin accumulate in the nuclei of vegetal, but not animal, blastomeres? A major control point is at the level of protein stability. β-catenin turns over very rapidly in animal blastomeres (half-life $t_{1/2}$ = 15 min) but is much more stable in vegetal cells ($t_{1/2}$ = 96 min) (Fig. 1) (*21*). At present, the sea urchin is the only organism in which such measurements of β-catenin stability in different regions of the early embryo have been made. Building on earlier work with *Drosophila* (*22*), it was shown that degradation of β-catenin during early *Xenopus* embryogenesis is dependent on phosphorylation of β-catenin by a serine-threonine kinase, GSK3β (glycogen synthase kinase 3β) (*23*, *24*). Similarly, in the sea urchin, degradation of β-catenin in animal blastomeres is dependent on GSK3β. If GSK3β-mediated phosphorylation of β-catenin is blocked, β-catenin accumulates in the nuclei of animal cells, causing them to adopt more vegetal fates (*17*, *21*).

Dishevelled: A Key Regulator of β-Catenin Stability in the Sea Urchin Embryo

Analysis of the canonical Wnt signaling pathway has led to the identification of many proteins that regulate β-catenin turnover (*2*). Theoretically, the polarized degradation of β-catenin in the early embryo could be explained by the local sequestration or activation of positive regulators of β-catenin degradation in animal blastomeres, or of negative regulators in vegetal cells, or a combination of both mechanisms. One candidate regulator is Dishevelled (Dsh), a protein that inhibits GSK3β-mediated degradation of β-catenin (*25*, *26*). In *Xenopus* embryos, Dsh is concentrated on the dorsal side of the embryo where β-catenin accumulates in nuclei, and biochemical studies have shown that Dsh is phosphorylated differentially along the dorsal-ventral axis (*27*, *28*).

In the sea urchin, a green fluorescent protein (GFP)–tagged form of the Dsh protein targets specifically to the vegetal cortex of the fertilized egg and cleavage-stage embryo (Fig. 2). In addition, immunostaining studies have shown that endogenous

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA. E-mail, ettensohn@andrew.cmu.edu

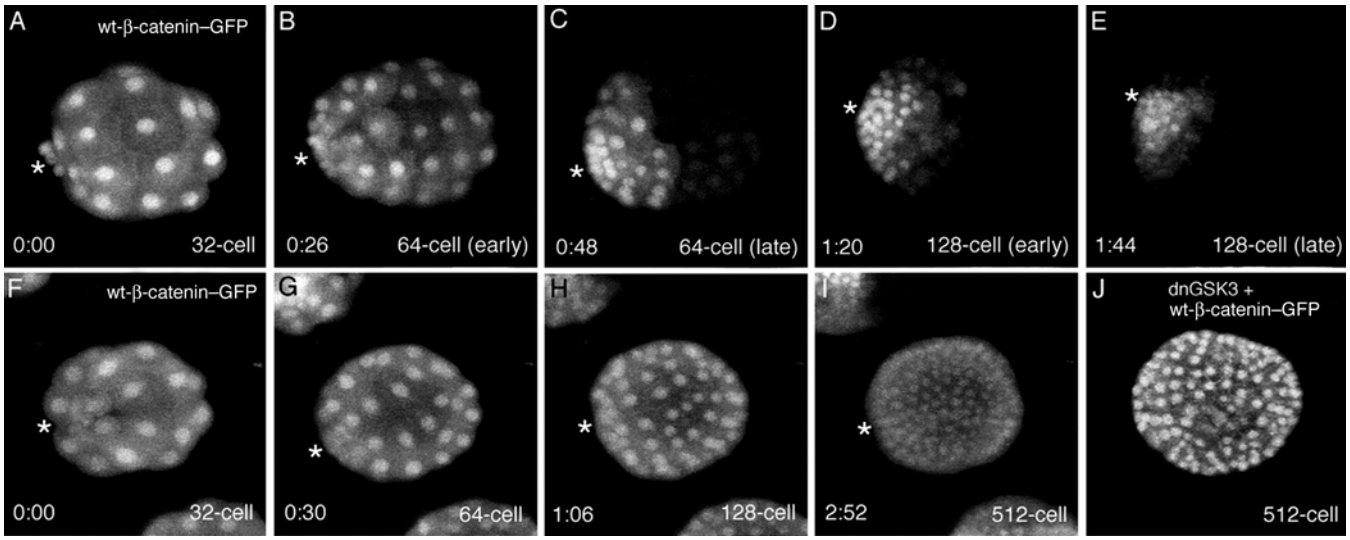

Fig. 1. Time-lapse confocal laser scanning analysis of β-catenin–GFP expression. (**A** to **E**) Frames from a time-lapse sequence after injection of *Xenopus laevis* wild-type (wt) β-catenin–GFP mRNA at the one-cell stage. Times after the start of recording (hr:min) are shown at the lower left of each panel; cell number is shown at the lower right. GFP-tagged β-catenin is initially localized in the nuclei, cytoplasm, and junctional complexes of all blastomeres (A). GFP-tagged β-catenin disappears from the animal region of the embryo over a period of approximately two cell cycles [(B) to (E)]. GFP-tagged β-catenin eventually becomes restricted to a small territory of cells surrounding the vegetal pole (asterisk). (**F** to **I**) Frames from a time-lapse sequence after injection of Xl-pt–β-catenin–GFP at the one-cell stage. This form of β-catenin cannot be phosphorylated by GSK3β. The GFP-tagged protein remains stable in animal blastomeres. The vegetal pole is marked by an asterisk. (**J**) Co-injection of mRNAs encoding Xl-wt–β-catenin–GFP and a kinase-dead, dominant-negative form of GSK3β (Xl-dnGSK3β) at the one-cell stage. Expression of Xl-dnGSK3β stabilizes GFP-tagged β-catenin in animal blastomeres. [*X. laevis* constructs were provided by D. Kimelman.]

Dsh is localized in the vegetal cortex of unfertilized eggs and blastomeres (*29*). Light microscopy shows the protein to be localized in puncta, the nature of which remains uncertain (*25*, *30*). Dsh is a modular protein with several domains, including DIX (Dishevelled-Axin), PDZ (postsynaptic density-95/discs large/zonula occludens-1), and DEP (fly Dishevelled, worm EGL-10, and mammalian pleckstrin) domains. Mutational analysis of Dsh (*21*, *31*) has identified two motifs that are required for vegetal, cortical targeting: (i) a short motif within the DIX domain that has been implicated in binding of the DIX domain to artificial lipid micelles (*32*), and (ii) a 23–amino acid, serine-threonine–rich region immediately upstream of the DEP domain.

When overexpressed, the DIX domain acts as a dominant-negative form of Dsh, blocks accumulation of β-catenin in the nuclei of vegetal blastomeres, and prevents the formation of mesoderm and endoderm (*21*) (Fig. 3). This finding provides strong evidence that Dsh normally plays an essential role in nuclear localization of β-catenin and axis specification in the sea urchin. Similar studies in *Xenopus* have not yet demonstrated such a role for Dsh in endogenous axis formation, although it is possible that dominant-negative Dsh is not expressed at high enough levels in the early *Xenopus* embryo to block the function of maternal Dsh protein (*33*).

One simple hypothesis is that the vegetal, cortical localization of Dsh leads to the partitioning of large amounts of this protein into vegetal blastomeres during cleavage, thereby protecting β-catenin from degradation specifically in these cells. This straightforward sequestration model does not appear to be sufficient, however, at least in the sea urchin. Overexpression of Dsh in animal blastomeres does not stabilize β-catenin in these cells or alter their fates. However, if Dsh function is bypassed by ectopic expression a catalytically inactive, dominant-negative

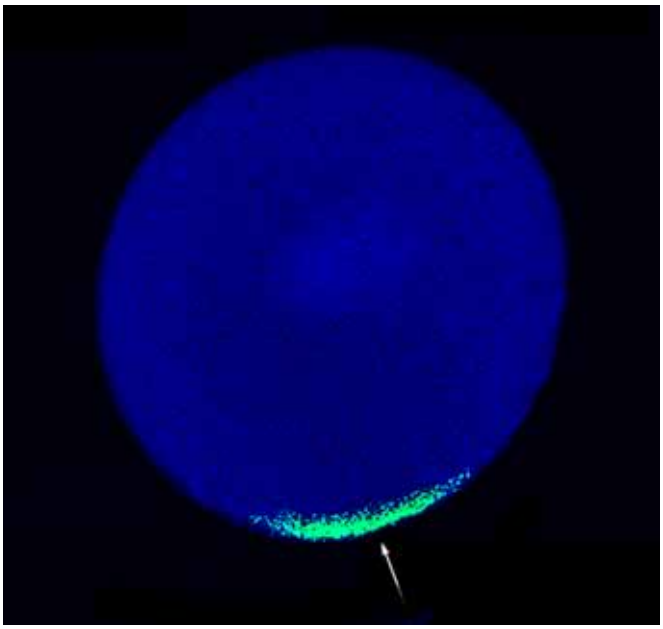

Fig. 2. Vegetal, cortical targeting of GFP-tagged sea urchin (*Lytechinus variegatus*) Dsh (LvDsh-GFP) mRNA encoding LvDsh-GFP (wild-type) was injected into fertilized eggs. This zygote was observed by confocal laser scanning microscopy 1.5 hours after injection, just before first cleavage, which was slightly delayed in this embryo. LvDsh-GFP targets specifically to the cortex at one pole of the fertilized egg (arrow). Continuous observation of such embryos shows that this region corresponds to the vegetal pole [see (*21*)].

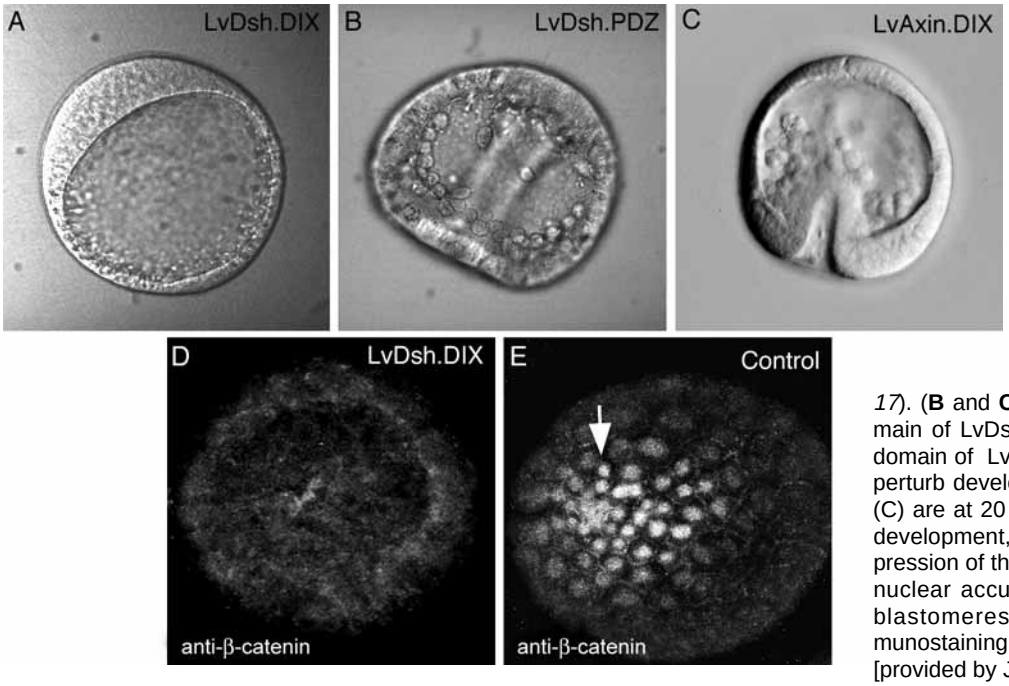

Fig. 3. Dsh function is required for endomesoderm specification and for the accumulation of β-catenin in the nuclei of vegetal blastomeres. (**A**) Injection of mRNA (1.9 mg/ml) encoding the DIX domain of LvDsh results in suppression of endoderm and mesoderm formation and a phenotype indistinguishable from that produced by overexpression of cadherins or GSK3β (*5*, *16*, *17*). (**B** and **C**) Overexpression of the PDZ domain of LvDsh (mRNA, 2.0 mg/ml) or the DIX domain of LvAxin (mRNA, 3.6 mg/ml) does not perturb development. Embryos shown in (A) to (C) are at 20 hours, 20 hours, and 18 hours of development, respectively. (**D** and **E**) Overexpression of the DIX domain of LvDsh blocks the nuclear accumulation of β-catenin in vegetal blastomeres [arrow, (E)], as shown by immunostaining with an antibody to Lv–β-catenin [provided by J. Miller and D. McClay].

form of GSK3β, animal blastomeres adopt vegetal fates (*21*). These findings argue that Dsh is not functional in animal cells, either because an activator of Dsh is missing or inactive in that part of the embryo, or because an inhibitor of Dsh is localized or activated in animal cells. In *Xenopus*, in contrast, ectopic expression of wild-type Dsh in ventral blastomeres alters cell fates and results in secondary axis formation (*33*).

Maternal mRNA encoding Wnt-11 is concentrated in the vegetal region of the unfertilized *Xenopus* egg and translocates to the dorsal side as a consequence of the cortical rotation (*34*). Wnt-11 provides a polarized signal required for asymmetric nuclear localization of β-catenin in this organism, although it remains possible that localized Dsh also has a role, perhaps in a parallel fashion. It is too early to tell whether a localized Wnt signal exists in other organisms. Sea urchins, for example, do not have a homolog of Wnt-11 (*35*), and overexpression of *Xenopus* Wnt-11 in sea urchins does not produce axis defects (*31*). Sea urchin eggs contain mRNAs that encode several Wnt family members, but none of the corresponding maternal mRNAs appear to be concentrated in the vegetal region. These findings, and others highlighted above, suggest that there may be differences in the initial polarizing signal in amphibians and echinoderms. Further work will be needed to identify the upstream regulators of Dsh in early sea urchin embryos and other metazoans. These studies will reveal the extent to which mechanisms that control β-catenin nuclearization in early embryogenesis have been conserved during animal evolution.

References

1. H. Clevers, M. van de Wetering, TCF/LEF factors earn their wings. *Trends Genet.* **13**, 485–489 (1997).
2. C. Y. Logan, R. Nusse, The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* **20**, 781–810 (2004).
3. S. Schneider, H. Steinbeisser, R. M. Warga, P. Hausen, β-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* **57**, 191–198 (1996).
4. B. A. Rowning, J. Wells, M. Wu, J. C. Gerhart, R. T. Moon, C. A. Larabell, Microtubule-mediated transport of organelles and localization of β-catenin to the future dorsal side of *Xenopus* eggs. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1224–1229 (1997).
5. C. Y. Logan, J. R. Miller, M. J. Ferkowicz, D. R. McClay, Nuclear β-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126**, 345–357 (1999).
6. T. Roeser, S. Stein, M. Kessel, Nuclear β-catenin and the development of bilateral symmetry in normal and LiCl-exposed chick embryos. *Development* **126**, 2955–2966 (1999).
7. K. Imai, N. Takada, N. Satoh, Y. Satou, β-catenin mediates the specification of endoderm cells in ascidian embryos. *Development* **127**, 3009–3020 (2000).
8. J. Heasman, A. Crawford, K. Goldstone, P. Garner-Hamrick, B. Gumbiner, P. McCrea, C. Kintner, C. Y. Noro, C. Wylie, Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell* **79**, 791–803 (1994).
9. G. M. Kelly, D. F. Erezyilmaz, R. T. Moon, Induction of a secondary embryonic axis in zebrafish occurs following the overexpression of β-catenin. *Mech. Dev.* **53**, 261–273 (1995).
10. C. Wylie, M. Kofron, C. Payne, R. Anderson, M. Hosobuchi, E. Joseph, J. Heasman, Maternal β-catenin establishes a "dorsal signal" in early *Xenopus* embryos. *Development* **122**, 2987–2996 (1996).
11. F. Pelegri, H. M. Maischein, Function of zebrafish β-catenin and TCF-3 in dorsoventral patterning. *Mech. Dev.* **77**, 63–74 (1998).
12. C. Kelly, A. J. Chin, J. L. Leatherman, D. J. Kozlowski, E. S. Weinberg, Maternally controlled β-catenin-mediated signaling is required for organizer formation in the zebrafish. *Development* **127**, 3899–3911 (2000).
13. B. Hobmayer, F. Rentzsch, K. Kuhn, C. M. Happel, C. C. von Laue, P. Snyder, U. Rothbächer, T. W. Holstein, WNT signaling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* **407**, 186–189 (2000).
14. A. H. Wikramanayake, M. Hong, P. N. Lee, K. Pang, C. A. Byrum, J. M. Bince, R. Xu, M. Q. Martindale, An ancient role for nuclear β-catenin in the evolution of axial polarity and germ layer segregation. *Nature* **426**, 446–450 (2003).
15. J. R. Miller, D. R. McClay, Changes in the pattern of adherens junction-associated β-catenin accompany morphogenesis in the sea urchin embryo. *Dev. Biol.* **192**, 310–322 (1997).
16. A. H. Wikramanayake, L. Huang, W. H. Klein, β-catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9343–9348 (1998).
17. F. Emily-Fenouil, C. Ghiglione, G. Lhomond, T. Lepage, C. Gache, GSK3β/shaggy mediates patterning along the animal-vegetal axis of the sea urchin embryo. *Development* **125**, 2489–2498 (1998).
18. A. Vonica, W. Weng, B. M. Gumbiner, J. M. Venuti, TCF is the nuclear effector of the β-catenin signal that patterns the sea urchin animal-vegetal axis. *Dev. Biol.* **217**, 230–243 (2000).
19. P. Oliveri, E. H. Davidson, Gene regulatory network controlling embryonic specification in the sea urchin. *Curr. Opin. Genet. Dev.* **14**, 351–360 (2004).

20. E. H. Davidson, D. H. Erwin, Gene regulatory networks and the evolution of animal body plans. *Science* **311**, 796–800 (2006).
21. H. E. Weitzel, M. R. Illies, C. A. Byrum, R. Xu, A. H. Wikramanayake, C. A. Ettensohn, Differential stability of β-catenin along the animal-vegetal axis of the sea urchin embryo mediated by dishevelled. *Development* **131**, 2947–2956 (2004).
22. E. Siegfried, T. B. Chou, N. Perrimon, Wingless signaling acts through zeste-white 3, the *Drosophila* homolog of glycogen synthase kinase-3, to regulate engrailed and establish cell fate. *Cell* **71**, 1167–1179 (1992).
23. X. He, J. P. Saint-Jeannet, J. R. Woodgett, H. E. Varmus, I. B. Dawid, Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617–622 (1995).
24. C. Yost, M. Torres, J. R. Miller, E. Huang, D. Kimelman, R. T. Moon, The axis-inducing activity, stability, and subcellular distribution of β-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* **10**, 1443–1454 (1996).
25. J. B. Wallingford, R. Habas, The developmental biology of Dishevelled: An enigmatic protein governing cell fate and cell polarity. *Development* **132**, 4421–4436 (2005).
26. C. C. Malbon, H. Y. Wang, Dishevelled: A mobile scaffold catalyzing development. *Curr. Top. Dev. Biol.* **72**, 153–166 (2006).
27. J. R. Miller, B. A. Rowning, C. A. Larabell, J. A. Yang-Snyder, R. L. Bates, R. T. Moon, Establishment of the dorsal-ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. *J. Cell Biol.* **146**, 427–437 (1999).
28. U. Rothbacher, M. N. Laurent, M. A. Deardorff, P. S. Klein, K. W. Cho, S. E. Fraser, Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. *EMBO J.* **19**, 1010–1022 (2000).
29. A. Wikramanayake, personal communication.
30. T. Schwarz-Romond, C. Merrifield, B. J. Nichols, M. Bienz, The Wnt signalling effector Dishevelled forms dynamic protein assemblies rather than stable associations with cytoplasmic vesicles. *J. Cell Sci.* **118**, 5269–5277 (2005).
31. J. D. Leonard, C. A. Ettensohn, unpublished data.
32. D. G. Capelluto, T. G. Kutateladze, R. Habas, C. V. Finkielstein, X. He, M. M. Overduin, The DIX domain targets dishevelled to actin stress fibres and vesicular membranes. *Nature* **419**, 726–729 (2002).
33. S. Y. Sokol, Analysis of dishevelled signaling pathways during *Xenopus* development. *Curr. Biol.* **6**, 1456–1467 (1996).
34. Q. Tao, C. Yokota, H. Puck, M. Kofron, B. Birsoy, D. Yan, M. Asashima, C. C. Wylie, X. Lin, J. Heasman, Maternal wnt11 activates the canonical Wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* **120**, 857–871 (2005).
35. Sea Urchin Genome Sequencing Consortium, The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* **314**, 941 (2006).

Citation: C. A. Ettensohn, The emergence of pattern in embryogenesis: Regulation of β-catenin localization during early sea urchin development. *Sci. STKE* **2006**, pe48 (2006).