Origins of Bilateral Symmetry: Hox and Dpp Expression in a Sea Anemone

John R. Finnerty, *et al.*

Science **304**, 1335 (2004);
DOI: 10.1126/science.1091946

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

Origins of Bilateral Symmetry: *Hox* and *Dpp* Expression in a Sea Anemone

John R. Finnerty,[1]* Kevin Pang,[2] Pat Burton,[1]
Dave Paulson,[2] Mark Q. Martindale[2]

Over 99% of modern animals are members of the evolutionary lineage Bilateria. The evolutionary success of Bilateria is credited partly to the origin of bilateral symmetry. Although animals of the phylum Cnidaria are not within the Bilateria, some representatives, such as the sea anemone *Nematostella vectensis*, exhibit bilateral symmetry. We show that *Nematostella* uses homologous genes to achieve bilateral symmetry: Multiple *Hox* genes are expressed in a staggered fashion along its primary body axis, and the transforming growth factor–β gene *decapentaplegic* (*dpp*) is expressed in an asymmetric fashion about its secondary body axis. These data suggest that bilateral symmetry arose before the evolutionary split of Cnidaria and Bilateria.

The Bilateria is an evolutionary lineage that encompasses more than 1.5 million modern-day animal species, including such diverse forms as humans, fruit flies, and soil nematodes (*1*). Bilateral symmetry has been implicated as a "key innovation" of the Bilateria, associated with an evolutionary transition from stationary or drifting planktonic animals to active burrowers and swimmers. In the Bilateria, bilateral symmetry is achieved by the orthogonal intersection of the anterior-posterior (A-P) axis and the dorsal-ventral (D-V) axis. The conserved deployment of homologous developmental regulatory genes argues for the underlying homology of both the A-P axis and the D-V axis (fig. S1). *Hox* genes play a conserved role in patterning the A-P axis (*2*), and *decapentaplegic* (*dpp*) plays a conserved role in patterning the D-V axis (*3*, *4*).

Because bilateral symmetry was already present in the ancestral bilaterian over 500 million years ago, modern-day bilaterians cannot shed much light on the origin of bilateral symmetry. Outgroup taxa, animals that do not fall within the Bilateria, may reveal key steps in the evolution bilateral symmetry. One important outgroup to the Bilateria is the phylum Cnidaria (sea anemones, corals, hydras, and jellyfishes). Modern cnidarians resemble the earliest known fossil animals (*5*), and they may be "representative of a grade of late Precambrian organization from which bilaterians evolved" (*6*). Current textbooks characterize cnidarians as radially symmetrical, like simple cylinders (*7*–*9*). However, it has long been recognized that many cnidarians exhibit bilateral symmetry (*10*). For example, the sea anemone *Nematostella vectensis* possesses two orthogonal body axes (Fig. 1). The primary body axis, the oral-aboral axis, runs from the mouth to the foot (*11*). The secondary body axis, the directive axis, traverses the pharynx at a right angle to the primary body axis (*10*).

The co-occurrence of bilateral symmetry in cnidarians and bilaterians suggests two possibilities: homology or convergence. If bilateral symmetry is homologous in Cnidaria and Bilateria, then homologous molecular mechanisms are expected to pattern their body axes. Gene expression data from *Nematostella* support the hypothesis of homology. During *Nematostella* development, five *Hox* genes are expressed in staggered domains along the primary body axis, and *decapentaplegic* (*dpp*) is expressed asymmetrically about the secondary body axis, a pattern of gene expression reminiscent of bilaterians.

Five *Hox* genes were recovered from *Nematostella*. Several previous studies have reported the recovery of anterior and posterior *Hox* genes from various cnidarians (*12*–*18*). Phylogenetic analyses performed here are consistent with these earlier studies. According to neighbor-joining and maximum-likelihood analyses of homeodomain sequences (Materials and Methods), three genes recovered from *Nematostella* appear related to anterior *Hox* genes (*anthox6*, *anthox7*, and *anthox8*), whereas two appear related to posterior *Hox* genes (*anthox1* and *anthox1a*) (*12*–*14*). Additional evidence consistent with the identification of these genes as *Hox*

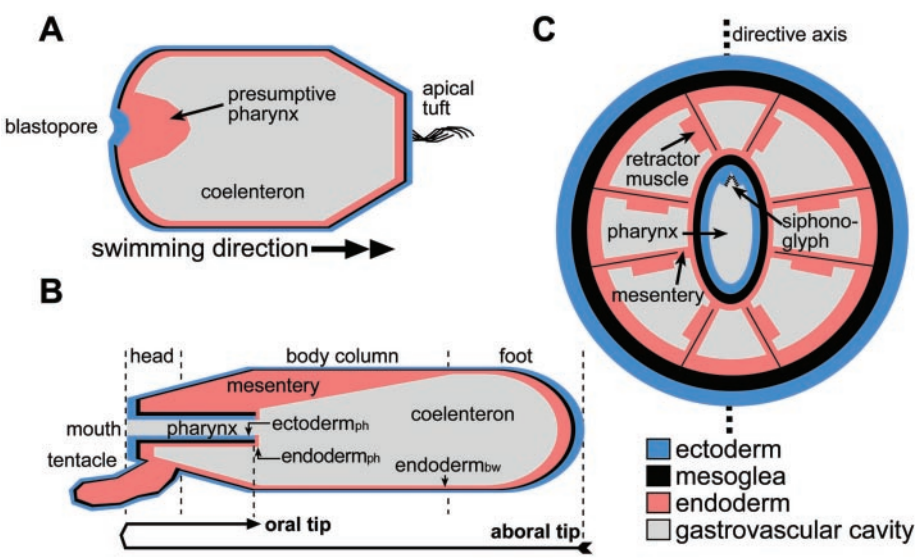

Fig. 1. Anatomy of *Nematostella*. The body consists of an outer ectodermal epithelium and an inner endodermal epithelium, separated by an intervening layer of largely acellular connective tissue known as mesoglea. The mouth connects to the gut via the pharynx, a bilayered invagination of the body wall at the oral pole. (**A** and **B**) Longitudinal section through the oral-aboral axis. (A) Planula larva. The primary body axis of the planula is the apical-blastoporal axis. The animal swims in the direction of the apical tuft (arrow head). The blastopore becomes the mouth of the adult polyp. (B) Adult polyp. The primary body axis is known as the oral-aboral axis. The epithelium lining the lumen of the pharynx (ph) is ectodermal in origin. The oral extremity of the body axis occurs where the pharynx empties into the gut cavity. bw, body wall. (**C**) Cross section through the pharyngeal region. The pharynx is attached to the outer body wall via eight endodermal mesenteries. Each mesentery bears a retractor muscle on one face. The only plane of mirror symmetry passes through the directive axis.

[1]Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA. [2]Kewalo Marine Laboratory, Pacific Biomedical Research Center, University of Hawaii, 41 Ahui Street, Honolulu, HI 96813, USA.

*To whom correspondence should be addressed. E-mail: jrf3@bu.edu

genes includes genomic linkage data (*19*), the presence of *Hox*-specific hexapeptide sequences upstream of the homeodomain in *anthox7* and *anthox8*, and the simultaneous recovery of numerous other members of the extended *Hox* family from *Nematostella* including *Gsx*, *Evx*, *Mox*, and *Gbx* (*20*). Two cnidarian-specific gene duplications appear to have produced two pairs of sister genes, *anthox1-anthox1a* and *anthox7-anthox8*, suggesting that the common ancestor of Cnidaria and Bilateria likely possessed three distinct *Hox* genes [Supporting Online Material (SOM) Text].

Hox gene expression was assayed by in situ hybridization throughout the blastula stage, during gastrulation, in the ciliated swimming larvae (planulae), and in newly settled polyps that are displaying their first four tentacles. *Hox* expression was observed in all regions along the primary body axis and in both body layers, endoderm and ectoderm (Fig. 1). A*nthox1* is first expressed in early cleavage stages at the aboral pole. In the blastula, *anthox1* is expressed over a wide area of the aboral blastoderm (Fig. 2A). After gastrulation in the two-layered planula larva, the expression of *anthox1* is restricted to a small circular patch of ectodermal cells at the extreme aboral pole (Fig. 2, B and C).

Three genes are expressed primarily in the body wall endoderm: *anthox1a*, *anthox7*, and *anthox8*. Each of these genes is first expressed in the endoderm near the blastopore during the gastrula stage (Fig. 2, D, G, and J). Throughout larval development, expression remains restricted to the endoderm, primarily along the outer body wall. Where the outer body wall joins the pharynx, the expression of *anthox1a* and *anthox8* extends into the endoderm of the pharynx (arrowheads in Fig. 2, F and L).

In addition to being restricted along the oral-aboral axis, the expression of *anthox1a*, *anthox7*, and *anthox8* is also restricted along the directive axis (Fig. 2, E, H, and K). *Anthox1a* is expressed in a thin strip of body wall endoderm at one end of the directive axis, flanked on either side by expression of both *anthox7* and *anthox8*. The *anthox8* expression overlaps with the *anthox1a* expression.

Anthox6 is expressed solely in the outer, endodermal layer of the pharynx (Fig. 2, M and N). *Anthox6* expression first becomes apparent in endoderm near the blastoporal pole in the gastrula (Fig. 2M), and its expression remains fairly constant through the early and late larval stages and in the juvenile polyp (Fig. 2N).

Hox expression in *Nematostella*, consisting of staggered domains that collectively span nearly the entire oral-aboral axis, is reminiscent of *Hox* expression in bilaterian

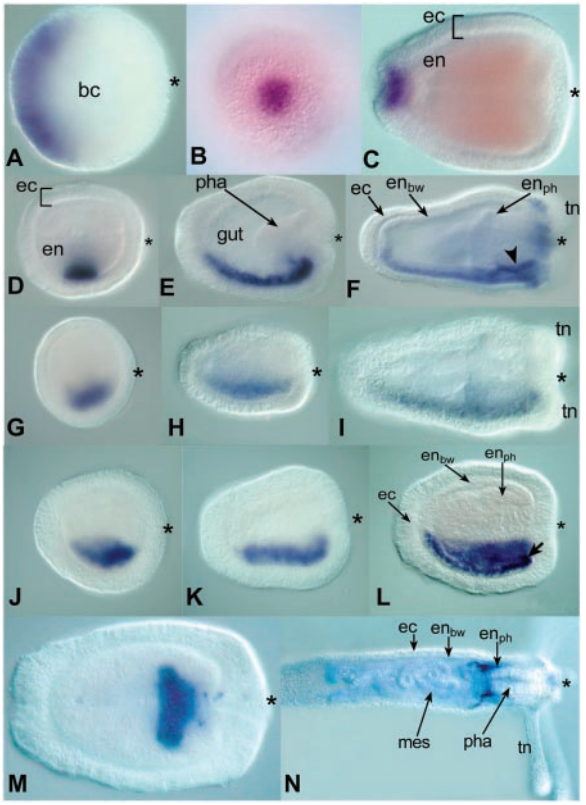

Fig. 2. Developmental expression of *Hox* genes. All images are seen from the lateral aspect, except for (B), which is an aboral view. The apical or aboral pole is toward the left. The asterisks indicates the blastoporal pole, the site of the future mouth. Expression of *anthox1* in (**A**) early blastula stage and (**B** and **C**) planula larva stage. Expression of *anthox1a* in (**D** and **E**) gastrula and (**F**) late larval stages. Expression of *anthox7* in (**G** and **H**) gastrula and (**I**) late larval stages. Expression of *anthox8* in (**J** and **K**) gastrula and (**L**) late larval stages. Expression of *anthox6* in (**M**) early larval stage and (**N**) juvenile polyp. bc, blastocoel; ec, ectoderm; ec_{ph}, pharyngeal (ph) ectoderm; en, endoderm; en_{ph}, pharyngeal (ph) endoderm; en_{bw}, body wall (bw) endoderm; mes, mesentery; and tn, tentacles.

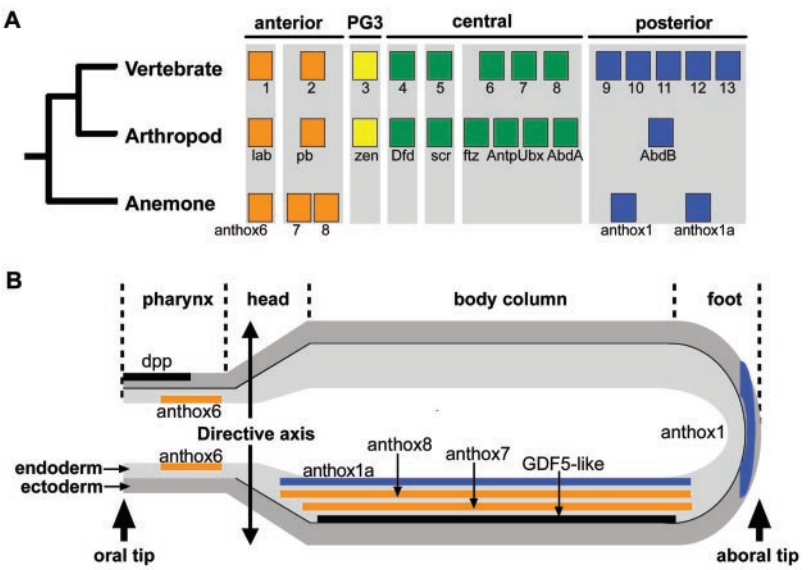

Fig. 3. Summary of *Hox* and TGFβ gene expression. (**A**) Provisional homology of *Nematostella Hox* genes based on phylogenetic analysis of homeodomains (Materials and Methods). Vertebrate *Hox* paralogs are numbered from 1 to 13. Arthropod *Hox* paralogs are named with *Drosophila* gene terminology (lab, *labial*; pb, *proboscipedia*; zen, *zerknullt*; Dfd, *Deformed*; scr, *sex combs reduced*; ftz, *fushi tarazu*; Antp, *Antennapedia*; Ubx, *Ultrabithorax*; abd-A, *abdominalA*; and AbdB, *AbdominalB*). (**B**) Gene expression along the oral-aboral and directive axes. The germ layer composition of *Nematostella* is shown in longitudinal section. To simplify the depiction of the primary body axis, the pharynx has been drawn as though everted. The mesenteries are not shown. Collectively, the five *Hox* expression domains span practically the entire oral-aboral axis. *Anthox1a*, *anthox7*, and *anthox8* are restricted to one side of the directive axis. Likewise, both TGFβ genes, *dpp* and *GDF5-like*, exhibit asymmetric expression about the directive axis. Only the asymmetric aspects of their expression are shown. *Dpp* is expressed in the pharyngeal ectoderm on the side of the directive axis opposite the sector expressing *anthox1a*, *anthox7*, and *anthox8*. *GDF5-like* is expressed in the endoderm on the same side as *anthox1a*, *anthox7*, and *anthox8*.

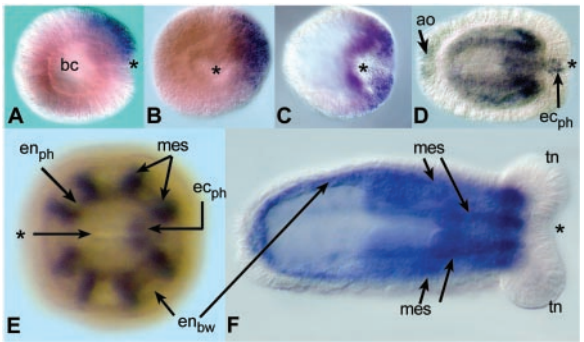

Fig. 4. Developmental expression of *Nv-dpp*. (**A**) Early gastrula in lateral view. (**B**) Early gastrula in blastoporal view. There is asymmetric expression along the blastopore. (**C**) Late gastrula in oblique view. (**D**) Lateral view of planula larva. ao, apical organ. (**E**) Optical section through the oral-aboral axis at the level of the pharynx of a planula larva. There is asymmetric expression in the pharyngeal ectoderm (ec_{ph}). mes, mesentery. (**F**). Lateral view of a later stage planula with tentacle buds (tn). Strong expression is seen throughout the gastrodermis. bc, blastocoel; asterisks, blastopore.

animals (Fig. 3). The interpretation of axial expression in *Nematostella* is complicated by the fact that the pharynx is an inversion of the body wall at the oral end of the animal (*10*). The region of the pharynx that protrudes most deeply into the coelenteron may be regarded as axially equivalent to the oral tip of cnidarians that lack a pharynx, such as *Hydra* (Fig. 1B). Therefore, the expression of *anthox6* deep in the pharynx (Fig. 2N) is closer to the oral extremity than the expression of *anthox1a* or *anthox8* at the junction of the pharynx with the outer body wall (Fig. 2, F and L). Data from the hydrozoan jellyfish *Podocoryne* are consistent with the conclusion that *Hox* genes are involved in patterning the primary body axis of cnidarian larvae, though the axial expression boundaries of specific homologs at the larval stage do not appear to be evolutionarily conserved between *Podocoryne* and *Nematostella* (*17*, *21*).

We next assayed *dpp* expression because *dpp* is implicated in dorsal-ventral patterning in bilaterians. We recovered a single *dpp* ortholog from *Nematostella* (Materials and Methods). Expression of *dpp* commences at gastrulation, initially skewed to one side of the blastopore (Fig. 4, A and B) but later expanding to surround the blastopore (Fig. 4C), as it does in the coral *Acropora* (*22*). In the planula, the entire gastrodermis expresses *dpp*, including the eight mesenteries, the pharyngeal endoderm, and the body wall endoderm (Fig. 4D). Ectodermal expression occurs in the area of the apical organ at the aboral end of the planula, and in a thin strip of ectodermal tissue where the pharynx meets the mouth (Fig. 4, D and E). The expression in the pharynx is highly asymmetrical relative to the directive axis (Fig. 4, D and E). This pharyngeal staining is transient (lasting 48 to 72 hours) and disappears before the polyp stage (Fig. 4F). The pharyngeal expression of *dpp* occurs on the opposite side of the directive axis from the expression of *anthox1a*, *anthox7*, and *anthox8* (SOM Text and fig. S7). In addition to *dpp*, we recovered another member of the transforming growth factor–β (TGFβ) family from *Nematostella*, a *GDF-5-like* gene, which also displays asymmetric expression, although at the opposite pole of the directive axis (SOM Text).

Symmetry is an unresolved issue in metazoan evolution [reviewed in (*23*)]. Were the earliest metazoans radially symmetrical, or were they bilaterally symmetrical? The phylum Cnidaria is central to the debate because it is a closely related outgroup to the Bilateria and because major cnidarian lineages differ with respect to symmetry. Radial symmetry predominates in the class Hydrozoa (hydras and hydromedusae), whereas bilateral symmetry predominates in the class Anthozoa (sea anemones, corals, etc.). Most theories suggest that the cnidarian-bilaterian ancestor was a radially symmetrical animal [e.g., (*11*, *24*–*29*)]. According to this view, extant hydrozoans retain the ancestral radial symmetry. Competing theories propose that the cnidarian-bilaterian ancestor was a bilaterally symmetrical animal [e.g., (*30*–*33*)]. According to this view, the Hydrozoa evolved radial symmetry secondarily, perhaps in conjunction with the evolution of the pelagic medusa phase of their life history.

The data summarized here suggest that bilateral symmetry evolved before the split between Cnidaria and Bilateria. Both taxa exhibit bilateral symmetry. Both taxa exhibit staggered *Hox* expression domains along the primary body axis and asymmetric *dpp* expression along the secondary body axis. Homology is the most parsimonious explanation for the shared possession of these morphological and molecular traits. If we invoke homoplasy as an explanation, we must presume that one or both of these complex axial patterning systems evolved convergently in two independent evolutionary lineages.

References and Notes

1. A. G. Collins, J. W. Valentine, *Evol. Dev.* **3**, 432 (2001).
2. S. B. Carroll, J. K. Grenier, S. D. Weatherbee, *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design* (Blackwell Science, Malden, MA, 2001).
3. S. A. Holley *et al.*, *Nature* **376**, 249 (1995).
4. E. L. Ferguson, *Curr. Opin. Genet. Dev.* **6**, 424 (1996).
5. M. F. Glaessner, M. Wade, *Palaeontology* **9**, 599 (1966).
6. G. E. Budd, S. Jensen, *Biol. Rev.* **75**, 253 (2000).
7. R. C. Brusca, G. J. Brusca, *Invertebrates* (Sinauer Associates, Sunderland, MA, 2003).
8. N. A. Campbell, J. B. Reece, E. J. Simon, *Essential Biology* (Benjamin Cummings, San Francisco, CA, 2004).
9. G. B. Johnson, *The Living World* (McGraw Hill, New York, ed. 3, 2003).
10. T. A. Stephenson, *British Sea Anemones*, T. R. Society, Ed. (Ray Society, London, 1926), vol. 1.
11. C. Nielsen, *Animal Evolution: Interrelationships of the Living Phyla* (Oxford Univ. Press, Oxford, ed. 2, 2001).
12. J. R. Finnerty, M. Q. Martindale, *Biol. Bull.* **193**, 62 (1997).
13. J. R. Finnerty, M. Q. Martindale, *Curr. Opin. Genet. Dev.* **8**, 681 (1998).
14. J. R. Finnerty, M. Q. Martindale, *Evol. Dev.* **1**, 16 (1999).
15. D. Gauchat *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4493 (2000).
16. D. E. Martinez, D. Bridge, L. M. Masuda-Nakagawa, P. Cartwright, *Nature* **393**, 748 (1998).
17. N. Yanze, J. Spring, C. Schmidli, V. Schmid, *Dev. Biol.* **236**, 89 (2001).
18. M. J. Kourakis, M. Q. Martindale, *J. Exp. Zool.* **288**, 175 (2000).
19. J. R. Finnerty, *Am. Zool.* **41**, 608 (2001).
20. S. Banerjee-Basu, A. D. Baxevanis, *Nucleic Acids Res.* **29**, 3258 (2001).
21. L. M. Masuda-Nakagawa, H. Gröger, B. L. Aerne, V. Schmid, *Dev. Genes Evol.* **210**, 151 (2000).
22. D. C. Hayward *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8106 (2002).
23. P. Willmer, *Invertebrate Relationships: Patterns in Animal Evolution* (Cambridge Univ. Press, Cambridge, 1990).
24. B. Werner, *Publ. Seto Mar. Biol. Lab.* **20**, 35 (1973).
25. E. Haeckel, *Q. J. Microsc. Sci.* **14**, 142 (1874).
26. R. Siewing, *Zool. Jahrb. Abt. Syst. Oekol. Geogr. Tiere* **103**, 439 (1980).
27. B. Hatschek, *Lehrbuch der Zoologie* (Fischer, Jena, Germany, 1891).
28. V. N. Beklemishev, *Principles of Comparative Anatomy of Invertebrates* (Univ. of Chicago Press, Chicago, IL, 1969).
29. L. v. Salvini-Plawen, *Z. Zool. Syst. Evolutionsforsch.* **16**, 40 (1978).
30. J. Hadzi, *The Evolution of the Metazoa* (Pergammon, Oxford, 1963).
31. E. D. Hanson, in *The Lower Metazoa*, E. C. Dougherty, Ed. (Univ. of California Press, Berkeley, CA, 1963), pp. 7–22.
32. L. H. Hyman, *The Invertebrates; Protozoa through Ctenophora* (McGraw Hill, New York, 1940).
33. G. Jägersten, *Zool. Bidr. Uppsala* **30**, 321 (1955).

34. We gratefully acknowledge the constructive comments of E. Seaver and three anonymous reviewers. This research was supported by NSF (grant DEB-9727244 to M.Q.M. and J.R.F. and grant IBN-0212773 to J.R.F.) and NASA (grant NAG2-1374 and NAG2-1519s to M.Q.M.)

Supporting Online Material
www.sciencemag.org/cgi/content/full/1091946/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
References and Notes

26 September 2003; accepted 2 April 2004
Published online 6 May 2004;
10.1126/science.1091946
Include this information when citing this paper.