Perspective

Evolution of the bilaterian body plan: What have we learned from annelids?

Marty Shankland* and Elaine C. Seaver†

Section of Molecular Cell and Developmental Biology, and Institute of Cellular and Molecular Biology, University of Texas, Austin, TX 78712

Annelids, unlike their vertebrate or fruit fly cousins, are a bilaterian taxon often overlooked when addressing the question of body plan evolution. However, recent data suggest that annelids offer unique insights on the early evolution of spiral cleavage, anteroposterior axis formation, body axis segmentation, and head versus trunk distinction.

Annelids are one of the largest and most widely distributed animal phyla (1). They were a major subject of embryological investigation in the late 19th century (2), but as 20th century molecular research took the foreground, annelids fell out of favor because of the lack of a model system species amenable to genetics. Now, as we embark on a new millenium, interest in the evolution of animal body plans brings annelids into the research spotlight. Recent phylogenies suggest that the bilaterian animals are divided into three major clades (3), with the well studied model systems coming from either Deuterostomia (e.g., vertebrates) or Ecdysozoa (e.g., fruit flies; nematodes). An understanding of the third clade, Lophotrochozoa, is also required if we are to reconstruct the morphology or embryology of early bilaterians. The goal of this article is to review recent discoveries on the embryology of annelids and related taxa and to discuss the significance of those findings for our understanding of animal evolution.

Spiral Cleavage

Annelids undergo a mode of embryonic development known as spiral cleavage (Fig. 1*A*). This cleavage pattern is thought to have arisen early in bilaterian evolution, because it is conserved between annelids and a number of other lophotrochozoan taxa collectively called "Spiralia" (1). Our basic understanding of spiral cleavage has been handed down from the 19th century, but some of the central tenets of that heritage have been overturned recently. The principal figures in this revision are Henry and Martindale, who have used modern cell-labeling techniques to characterize the embryonic fate map of two less widely studied spiralians, the nemerteans (4) and flatworms (5). It turns out that these embryos have a fate map similar to the maps of annelid and mollusk embryos, indicating a phylogenetic affinity between the groups that is at odds with traditional phylogenies (1) but confirmed by recent molecular data (3).

Martindale, Henry, and coworkers (4, 5) show that the traditional "D is dorsal" view of spiralian embryology (1) is an oversimplification that applies only to even-numbered micromere quartets. In fact, the fate map of the odd-numbered quartets is rotated by 45° such that the dorsal midline falls between quadrants C and D (Fig. 1*B*). A similar discordance of odd- and even-numbered quartets was also noted in the leech *Helobdella* (6, 7) and can be seen in 19th century drawings of polychaete annelids (Fig. 1 *C* and *D*). The only spiralian embryos that deviate significantly are the mollusks, whose first and second quartets do not alternate even though the second and third quartets do (8).

Certain 19th century embryologists noted the alternating symmetry of the spiralian fate map (2), but those observations were effectively forgotten only to be rediscovered a century later. One factor accounting for this oversight is that most of the adult spiralian body plan derives from the second and fourth quartet micromeres of the D quadrant (1, 2), which as a consequence, have received the lion's share of attention. But part of the blame must also lie with the seductive appeal of easy to remember—but overly simplistic—rubrics such as "D is dorsal", which with time, become reified until they attain the status of dogma.

Hox Genes and the Anteroposterior (AP) Axis

One of the great advances in studying the evolution of animal body plans was the discovery that bilaterian animals share a chromosomal array of homeobox genes, the Hox cluster, that functions during development to bring about regionalization of the AP axis (9). Data on annelid Hox genes are generally consistent with this idea. Each gene is expressed in a specific axial domain, and the AP order of Hox expression domains is the same as in other phyla (10, 11). As of yet, there are no gene disruption studies in annelids, but a functional connection between Hox genes and segmental diversification is suggested by the fact that the segment-specific differentiation of identified leech neurons correlates precisely with their expression of particular Hox gene products (12, 13).

Are Hox genes the *primary* determinants of segment identity in annelids? The segmented ectoderm and mesoderm of clitellate annelids (leeches and oligochaetes) arise from embryonic stem cells ("teloblasts") by a stereotyped cell lineage. In the leech *Helobdella*, it is experimentally feasible to frameshift any one of the five teloblastic cell lineages such that it develops out of register with the other four, and cells within the frameshifted lineage differentiate in a manner that is largely consistent with lineal identity regardless of segmental location (14, 15). In the oligochaete *Tubifex*, it is also feasible to transplant teloblasts between embryos, and it seems that the teloblast has an intrinsic segment identity specified by the number of stem cell divisions it has completed (16). Thus, the clitellate embryo seems to establish segment identity as the teloblasts divide to produce new segments, whereas the expression of all seven characterized leech Hox genes begins during organogenesis, long after segments are formed (10). This disparity suggests that clitellate Hox genes may be involved only in late stages of segmental diversification (Fig. 2*A*).

On the contrary, Hox gene expression is closely linked to the process of segment formation in the polychaete annelid *Chaetopterus* (Fig. 2 *B* and *C*). Polychaetes have an indirect life cycle in which the embryo develops into a trochophore larva with little or no segmentation, and the larva then adds segments sequentially from a posterior growth zone (homologous to the teloblasts of clitellates). Five *Chaetopterus*

*To whom reprint requests should be addressed. E-mail: hastypig@mail.utexas.edu.

†Present address: Kewalo Marine Lab, PBRC/University of Hawaii, 41 Ahui Street, Honolulu, HI 96813.

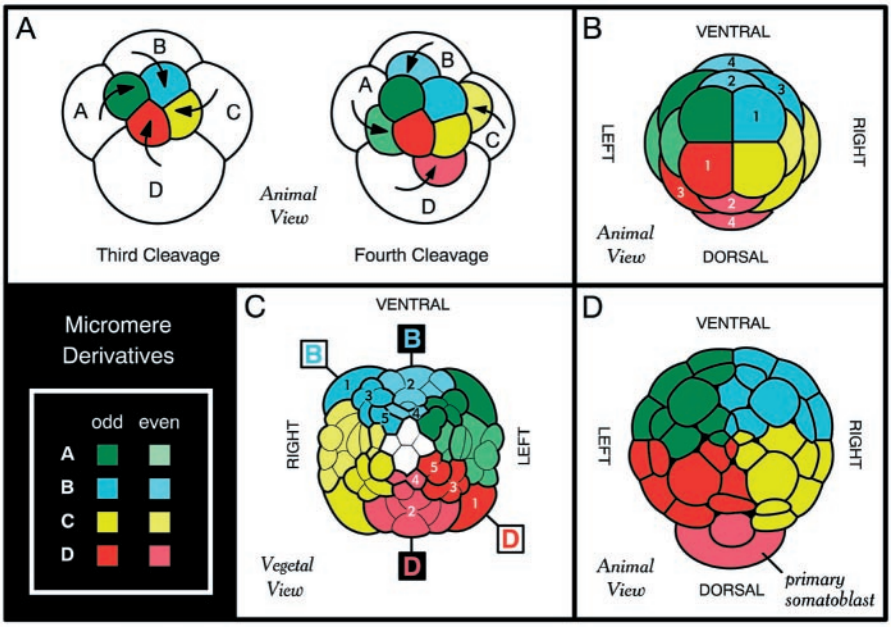

Fig. 1. Annelids and a number of other lophotrochozoans manifest a conserved pattern of early development known as spiral cleavage. (*A*) The first two cleavage planes fall at right angles parallel to the animal–vegetal axis and divide the zygote into the A, B, C, and D quadrants. In some but not all spiralians, the D blastomere is larger than the rest. Beginning with the third round of cleavage, the A, B, C, and D blastomeres cleave off (arrows) quartets of smaller cells called micromeres at the animal pole. Micromeres are colored according to quadrant of origin, with color intensity differing for odd- and even-numbered quartets. In spiral cleavage, each quartet of micromeres is rotated with respect to the parent blastomere, and the chirality of rotation alternates for odd- and even-numbered quartets. (*B*) Embryonic fate map of the nemertean *Cerebratulus* (adapted from ref. 4). Clones derived from the four B quadrant micromeres (cyan) and D quadrant micromeres (red) are numbered. Note that odd- and even-numbered quartets have distinct symmetry properties, with the odd-numbered micromeres being rotated 45° clockwise as viewed from the animal pole. Thus, in the first and third quartets, the A and D quadrants are on the left, bilaterally symmetrical to the B and C quadrants on the right. Only the second and fourth quartets have the ''traditional'' spiralian fate map (1), with D being dorsal and B ventral. (*C* and *D*) Although ignored for many years, the alternating symmetry of the spiralian fate map is readily apparent in the tracings of early annelid embryologists. *C* is an adaptation of R. Woltereck's (30) tracing of the polychaete annelid *Polygordius* nearing the end of gastrulation. Thick outlines demarcate clones derived from single micromeres. Clones derived from the five B quadrant micromeres (cyan) and D quadrant micromeres (red) are numbered, and it can be seen that the plane bisecting the B and D quadrants is rotated by 45° for odd- and even-numbered quartets. Part D is an adaptation of E. B. Wilson's (2) tracing of the polychaete annelid *Nereis* at a similar stage but seen from the animal pole. The animal hemisphere is composed of the four primary micromere clones (same color scheme as other figures), with the D lineage contributing to the left-dorsal quadrant. Also note that the second quartet micromere from the D lineage (primary somatoblast) straddles the dorsal midline. The primary and secondary somatoblasts (second and fourth quartet micromeres from the D lineage) are the main source of ectoderm and mesoderm in the adult spiralian body plan, and in *Nereis*, these cells are larger than the other micromeres. The symmetry properties of these two even-numbered micromeres became an all-encompassing tenet of spiralian embryology (i.e., D is dorsal) for most of the 20th century, and it is only with the advent of modern cell-labeling techniques that the true complexity of the spiralian fate map has been rediscovered (4–8).

Hox genes were characterized recently, and all are initially expressed in the growth zone as segments are first being formed (11). Clitellate annelids most likely evolved from a polychaete-like ancestor, and it is tempting to conclude that the *Chaetopterus* mode of Hox regulation is primitive for the annelids as a whole. However, chaetopterids are highly derived polychaetes with respect to larval development and tagmatization of the AP axis (11), and it seems wise to reserve judgement about the primitive condition until additional polychaetes have been examined.

Onset of Hox gene expression in the *Chaetopterus* growth zone displays a temporal sequence corresponding to the AP order in which segments are generated (11). However, the cessation of Hox gene expression is not so tidy. Two of the ''anterior'' Hox genes continue to be expressed by the growth zone after it has ceased producing segments that will express those same genes. Thus, the differentiating segment does not inherit a ''snapshot'' of the Hox gene expression profile present in the growth zone at the time of its formation. Rather, posterior segments must actively repress the transcription (or increase turnover) of some anterior Hox gene products to acquire their final Hox code.

Body Axis Segmentation

The evolution of segmentation is a hotly debated topic, with various authors relying on diverse data sets to propose one (17), two (1), or three separate origins (18) of segmentation in the phylogenetic lineages that gave rise to annelids, arthropods, and chordates. Given this diversity of opinion, it is not surprising that one major goal of annelid embryologists is to ascertain whether annelids and arthropods (and by extension, ecdysozoans and lophotrochozoans; ref. 3) inherited a homologous mode of segmentation from their last common ancestor.

Annelids generate segments in sequence from a posterior growth zone, and in clitellates, this process involves stereotyped cell lineages arising from a set of teloblastic stem cells. The latter mode of segmentation is not found in the majority of arthropods (e.g., *Drosophila*) but is strikingly similar to certain crustacean embryos (19). This difference serves as a powerful warning to not place excess trust in singular similarities or dissimilarities. It is widely accepted that segmentation is homologous in insects and crustaceans (1), but the general mode of segmentation used between those two arthropod taxa is no less diverse than between arthropods and annelids.

In recent years, the search for potential homologies has focused largely on the molecular mechanisms by which the two phyla generate segments. Gene expression studies suggest that segment polarity specification is one of the most widely conserved steps in arthropod segmentation (19), making it a good choice for comparison with annelids. This process is best understood in *Drosophila*, in which various segment polarity genes are expressed at different AP positions within the nascent segment and thereby specify the normal AP polarity of subsegmental cell fates. Cells expressing the gene *engrailed* (*en*) are pivotal in this process, because they initiate a cascade of intercellular signaling events that pattern cell fates throughout the segment's length (20).

Embryos of the leech *Helobdella* have an outwardly similar pattern of *en* expression—i.e., segmentally repeated transverse stripes that first appear when segment primordia are only a few cells in length (21). Do the *en*-expressing cells of the leech embryo likewise initiate cell interactions that pattern the remainder of the segment?

This question has been put to the test by means of laser cell ablation. In *Helobdella*, the primary blast cell clones behave as segmental repeats, and *en* is first expressed in blast cell clones of the O and P lineages (21). In both cases, selective ablation of the blast cell sublineage that

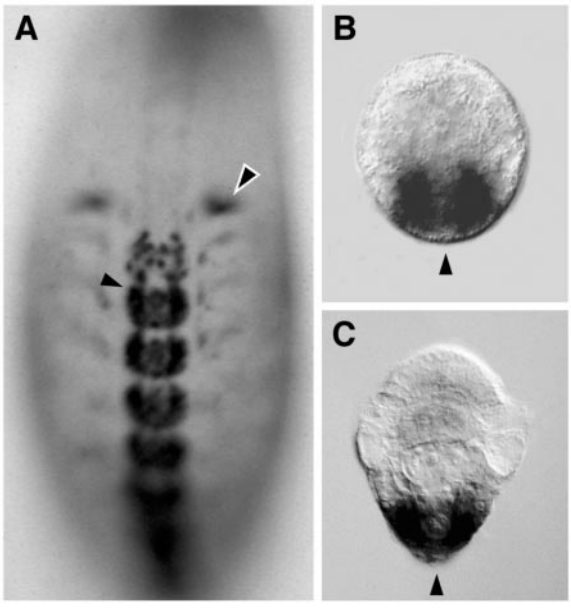

Fig. 2. Expression of Hox genes in developing annelids shown by *in situ* hybridization. (*A*) In embryos of the leech *Helobdella triserialis*, expression of the Hox genes begins during organogenesis, long after the formation of segments and the specification of segment identity (10, 14, 15). Embryo is stained for expression of *Lox2* (Hox paralogue group 7/8) and shown in ventral view with anterior to the top of the page. *Lox2* RNA is detected only in the posterior two-thirds of the body plan, including intense staining in the ganglia of the central nervous system (solid arrow) and reproductive structures (hollow arrow) and faint staining in the segmental mesoderm. (*B* and *C*) The onset of Hox gene expression in larvae of the polychaete annelid *Chaetopterus varieopedatus* is coincident with the formation of segments and first appears in a posterior growth zone from which the differentiating segments emerge (11). Larvae are shown in dorsal view with anterior to the top of the page and an arrowhead marking the posterior pole. Gene expression is restricted to the posterior growth zone, on either side of the pole. *B* shows expression of gene *CHv-Hox2* (Hox paralogue group 2), and *C* shows expression of gene *CHv-Hox3* (Hox paralogue group 3). Images in *B* and *C* are courtesy of Steve Irvine and Mark Martindale (Univ. of Hawaii, Honolulu, HI).

expresses *en* (Fig. 3 *A* and *B*) has no detectable effect on the fate of cells immediately anterior or posterior to the deficit (ref. 22 and E.C.S. and M.S., unpublished results). A comparable result is obtained even if the laser ablation precedes the onset of *en* expression by more than one cell cycle (Fig. 3*C*), suggesting that the unaltered development of adjoining cells could not result from transient *en* expression in the dying cell. This result argues that segment polarity specification in the leech does not depend on intercellular signaling downstream of *en* expression. It should be noted that *en* expression does not seem to play a direct role in the segmental gangliogenesis of the leech nervous system either (23).

Although suggestive, the distinct role of *en*-expressing cells in fly and leech segmentation is alone insufficient to disprove interphyletic homology. Segmentation is unquestionably homologous among insects, but some critical *Drosophila* segmentation genes do not seem to be playing a comparable role in grasshoppers (24). What we can say with certainty is that if annelids and arthropods did share a common segmented ancestor, then the ancestral mechanism of segment polarity specification must have undergone a profound modification in at least one of these two lineages. There is currently no basis to decide whether the mechanism of segment polarity specification in leeches is typical of annelids as a whole, and additional studies of other annelids will be required before we can appreciate the full significance of these results.

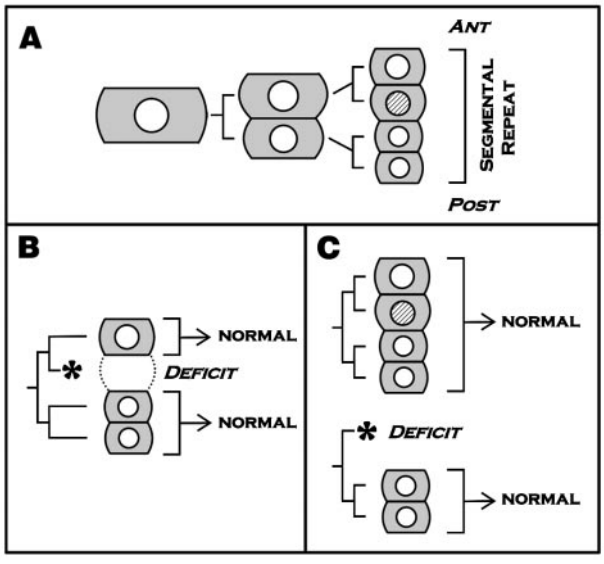

Fig. 3. Expression of the *en* gene does not seem to be required for the establishment of normal segment polarity in the leech *Helobdella* (ref. 22 and E.C.S. and M.S., unpublished results). (*A*) The primary p blast cell gives rise to one segmental repeat of the leech's dorsolateral ectoderm. The en protein (shaded nucleus) is expressed in only one of the four granddaughters of the primary blast cell (21). ANT, anterior; POST, posterior. (*B*) Laser ablation of the *en*-expressing cell has no detectable effect on the specification of more anterior or posterior parts of that same blast clone. (*C*) Laser ablation of the anterior daughter of the primary P blast cell prevents the formation of the *en*-expressing granddaughter. This manipulation has no detectable effect on the specification of the posterior half of that same blast cell clone nor on the segment polarity of the next anterior blast cell clone. These results suggest that *en*-initiated cell interactions are not required for the proper specification of segment polarity in the leech.

Origin of the Head/Trunk Distinction

Hox genes pattern cell fates along most of the AP axis (10), but the extreme anterior region of the embryo relies on a distinct network of patterning genes that are likewise conserved in annelids (25) and other bilaterian phyla (26). This observation has led to the suggestion that the last common ancestor of extant bilaterians already had discrete head and trunk regions whose patterning depended on distinct regulatory genes (27), but how did this head/trunk distinction evolve in the first place? Based on data from annelids, Bruce and Shankland (25) have taken the speculation one step further by putting forward a radial head model, which hypothesizes that the bilaterian head domain is remodeled from the body plan of a radially organized ancestor and that the trunk is a synapomorphic innovation of Bilateria (Fig. 4).

Two lines of evidence lend credence to this model. First is the recognition that

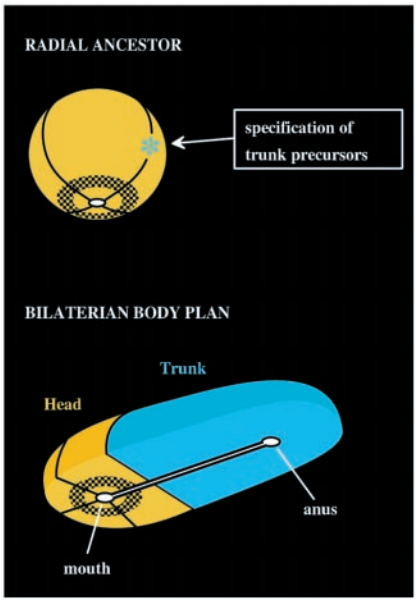

Fig. 4. Radial head model for the origin of the bilaterian body plan. (*A*) This model assumes a prebilaterian ancestor (orange) with a radially organized body plan and a single gut opening, shown here at the bottom. For convenience, the ancestral body plan has been divided into quarters, and a hypothetical gene expression domain is shown by shading. Transition to the modern bilaterian body plan began with the asymmetric specification of a specialized group of ''trunk'' precursor cells (cyan) at only one meridian around the circumference of the ancestral body plan. (*B*) Allometric expansion of the trunk domain produces a body plan typical of most Bilateria. The trunk elongates away from the head domain, carrying with it the anal end of a now bipolar gut. But the head domain retains features of its ancestral radial organization, as noted by a gene expression pattern (shaded) concentric around the mouth. This model proposes that bilaterian ''head genes'' have been relegated to the head domain, because they were not coopted into trunk patterning, and suggests that the AP axis may be an innovation of the Bilateria rather than a modification of a preexisting axis. [Reproduced with permission from ref. 25 (Copyright 1998, Academic Press)].

several features of the annelid head domain are radially organized about the mouth. In *Helobdella*, this organization can be seen both in the organization of cell lineages (6, 7) and in the expression of the head gene *Lox22-Otx* (25). There is also a radial organization of cell lineages in the trochophore larva of polychaetes (Fig. 1*C*), which during metamorphosis, is remodeled to produce the adult's head (in contrast to the trunk, which arises secondarily from the growth zone). The radial features of annelid head development might be derived, or they might reflect a more primitive bilaterian condition that has been obscured to various degrees in other phyla (25).

A second important observation is that spiralians such as annelids convert the radial symmetry of the zygote into the bilateral symmetry of the embryo by specializing the D quadrant. Quadrant specialization distinguishes spiralians from animals such as the ctenophores, whose embryonic quadrants undergo nearly identical cleavage programs and generate domains of largely similar tissue composition (28). The specialization of the D quadrant in spiralians centers around its commitment to produce the ectoderm and mesoderm of the body trunk (2) and the radial head model proposes that this sequence of events is an example of ontogeny recapitulating phylogeny: i.e., the bilaterian trunk originated through the novel specification of a cell population at one meridian of a radially organized ancestral body plan (Fig. 4).

Although speculative, the radial head model makes testable predictions. If the AP axis of Bilateria was modified from a preexisting body axis, then one might expect axial patterning mechanisms to function similarly in both. But if the bilaterian trunk domain arose as a novel outgrowth, then the eventual AP axis of the trunk domain was defined by the vector of outgrowth and may not share axial patterning mechanisms with any part of the prebilaterian body plan. If this latter view is correct, then axial patterning mechanisms associated with the AP axis of Bilateria—e.g., the Hox cluster—may have been coopted secondarily from some other function and hence not serve as axial patterning mechanisms outside the Bilateria. One way to test this prediction is to ask whether the Hox gene cluster serves as an axial patterning mechanism—possibly along the oral-aboral axis—in radially organized animals such as cnidarians or ctenophores (29). Our current knowledge of Hox gene function in these animals is too sketchy to draw a firm conclusion. However, regardless of how the story turns out, it is clear that research into annelids has led to some unique insights and perspectives on the early stages of bilaterian evolution.

1. Brusca, R. C. & Brusca, G. J. (1990) *Invertebrates* (Sinauer, Sunderland, MA).
2. Wilson, E. B. (1892) *J. Morphol.* **6,** 361–480.
3. Adoutte, A., Balavoine, G., Lartillot, N. & de Rosa, R. (1999) *Trends Genet.* **15,** 104–108.
4. Henry, J. J. & Martindale, M. Q. (1998) *Dev. Biol.* **201,** 253–269.
5. Boyer, B. C., Henry, J. J. & Martindale, M. Q. (1998) *Dev. Biol.* **204,** 111–123.
6. Weisblat, D. A., Kim, S. Y. & Stent, G. S. (1984) *Dev. Biol.* **104,** 65–85.
7. Nardelli-Haefliger, D. & Shankland, M. (1993) *Development (Cambridge, U.K.)* **118,** 877–892.
8. Render, J. (1997) *Dev. Biol.* **189,** 301–310.
9. Graham, A., Papalopulu, N. & Krumlauf, R. (1989) *Cell* **57,** 367–378.
10. Kourakis, M. J., Master, V. A., Lokhorst, D. K., Nardelli-Haefliger, D., Wedeen, C. J., Martindale, M. Q. & Shankland, M. (1997) *Dev. Biol.* **190,** 284–300.
11. Irvine, S. Q. & Martindale, M. Q. (2000) *Dev. Biol.* **217,** 333–351.
12. Wong, V. Y., Aisemberg, G. O., Gan, W.-B. & Macagno, E. R. (1995) *J. Neurosci.* **15,** 5551–5559.
13. Berezovskii, V. K. & Shankland, M. (1996) *J. Neurobiol.* **29,** 319–329.
14. Martindale, M. Q. & Shankland, M. (1990) *Nature (London)* **347,** 672–674.
15. Nardelli-Haefliger, D., Bruce, A. E. E. & Shankland, M. (1994) *Development (Cambridge, U.K.)* **120,** 1839–1849.
16. Kitamura, K. & Shimizu T. (2000) *Dev. Biol.*, **219,** 214–223.
17. Kimmel, C. B. (1996) *Trends Genet.* **12,** 391–392.
18. Willmer, P. (1990) *Invertebrate Relationships: Patterns in Animal Evolution* (Cambridge Univ. Press, Cambridge, U.K.).
19. Scholtz, G., Dohle, W., Sandeman, R. E. & Richter, S. (1993) *Int. J. Dev. Biol.* **37,** 299–304.
20. Heemskerk, J. & DiNardo, S. (1994) *Cell* **76,** 449–460.
21. Lans, D., Wedeen, C. J. & Weisblat, D. A. (1993) *Development (Cambridge, U.K.)* **117,** 857–871.
22. Seaver, E. C. & Shankland, M. (1999) *Dev. Biol.* **210,** 207.
23. Shain, D. H., Ramirez-Weber, F.-A., Hsu, J. & Weisblat, D. A. (1998) *Dev. Genes Evol.* **208,** 28–36.
24. Patel, N. H., Ball, E. E. & Goodman, C. S. (1992) *Nature (London)* **357,** 339–342.
25. Bruce, A. E. E. & Shankland, M. (1998) *Dev. Biol.* **201,** 101–112.
26. Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. & Boncinelli, E. (1992) *Nature (London)* **358,** 687–690.
27. Arendt, D. & Nubler-Jung, K. (1996) *BioEssays* **18,** 255–259.
28. Reverberi, G. (1971) in *Experimental Embryology of Marine & Fresh-Water Invertebrates*, ed. Reverberi, G. (North–Holland, Amsterdam), pp. 85–103.
29. Finnerty, J. R. & Martindale M. Q. (1999) *Evol. Dev.* **1,** 16–23.
30. Woltereck, R. (1904) *Arch. Entwicklungsmech. Org.* **18,** 377–403.