Hydrobiologia **402:** 285–290, 1999.
A.W.C. Dorresteijn & W. Westheide (eds), Reproductive Strategies and Developmental Patterns in Annelids.

Cellular origins of bilateral symmetry in glossiphoniid leech embryos

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Key words: Annelida, leeches, spiral cleavage, bilateral symmetry

Abstract

In the embryos of glossiphoniid leeches, as in other spirally cleaving embryos, there is ambiguity as to how the early cleavages in the embryo relate to the bilateral symmetry of the adult. Traditionally, we have aligned the B–D axis of the 4-cell stage with the anterior–posterior (A–P) axis of the adult. This requires that the first cleavage be skewed with respect to the A–P axis. Here, we re-examine the fates and behaviours of early blastomeres and conclude that a more accurate representation of the embryo is to have the first cleavage plane transverse to the A–P axis.

Introduction

The aim of this contribution is to reconsider the question of how bilaterally symmetric animals arise from spirally cleaving embryos, focussing on the embryos of *Helobdella robusta*, a glossiphoniid leech. In the early embryos of glossiphoniid leeches, as in other unequally cleaving spiralians, stereotyped cleavages lead to segregation of yolk-deficient cytoplasm (teloplasm) into one blastomere at the 4-cell stage (Figure 1, top). This cell, designated as cell D, is fated in leeches to give rise to precursors of segmental ectoderm and mesoderm, along with some other, non-segmental tissues. Cells A, B and C, by contrast, are fated to give rise primarily to the definitive gut. At third cleavage, blastomeres A–D cleave to form quartets of animal micromeres (1a–1d) and vegetal macromeres (1A–1D). In certain labs, including ours, a modified cell terminology is used in which these cell quartets are named a'–d' and A'–D', respectively (Stent et al., 1992; Figure 2).

Beginning at fourth cleavage, macromere D' undergoes a unique series of cleavages that end up by producing 5 bilateral pairs of embryonic stem cells, the M, N, O/P, O/P and Q teloblasts (Figure 1, middle). As described elsewhere (e.g., Weisblat & Shankland, 1985; Shankland & Savage, 1997), teloblasts give rise to bilaterally paired, coherent columns of progeny arrayed in left and right germinal bands, comprising the founder cells for segmental mesoderm and ectoderm (Figure 1 middle, bottom). In addition to the teloblasts and macromeres, a total of 25 micromeres are produced during cleavage, 3 each from the A–C quadrants and 16 from the D quadrant (Sandig & Dohle, 1988; Bissen & Weisblat, 1989). We deviate again from classical spiralian terminology in designating as micromeres all the small cells arising during cleavage that are not blast cells, without respect to the orientation of the cleavage by which they arise. In the embryos we study, this designation makes sense also in terms of the distinct fates of these cells (Smith & Weisblat, 1994; Huang et al., in prep.).

The ideas presented here spring from efforts to resolve a paradox regarding the establishment of the second embryonic axis during zygotic development. The first, animal–vegetal (A–V) axis is established during oogenesis. Neither of these embryonic axes corresponds exactly to either the anterior–posterior (A–P) or the dorsal–ventral (D–V) axis of the mature leech, but together they define the plane of bilateral symmetry for the animal.

Paradoxical aspects of the establishment of the second axis

The paradox is this. The five bilateral pairs of teloblasts constitute a posterior growth zone that gives rise to bilaterally symmetric, segmentally iterated complements of ectodermal and mesodermal progeny

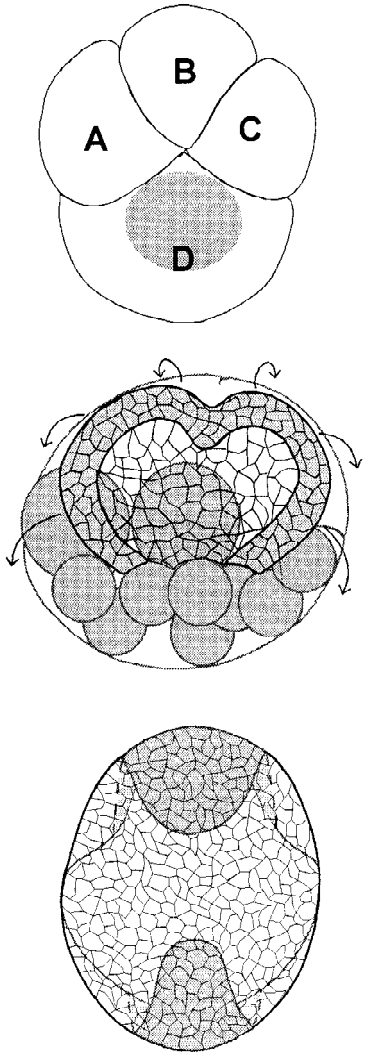

Figure 1. Selected stages of glossiphoniid leech development. *Top*. Animal pole view of 4-cell embryo, corresponding roughly to dorsal view. Teloplasm (gray) arises at the animal and vegetal poles prior to first cleavage and is segregated by two unequal cleavages to cell D, progenitor of the teloblasts. *Middle.* Animal pole (roughly dorsal) view of embryo at the onset of the epibolic gastrulation movements. By iterated series of highly unequal divisions, the teloblasts (gray circles) have given rise to left and germinal bands (gray heart shaped bands) joined to one another at their distal ends (anterior; top). Individual cells within the germinal bands are not indicated. The germinal bands and the area between them in the prospective dorsal region of the embryo are covered by a squamous epithelium (irregular gridwork) derived from micromeres that arise during cleavage (Figure 2). During gastrulation, as the teloblasts add more cells to the posterior ends of the germinal bands, the bands move ventrovegetally (arrows) over the surface of the embryo, accompanied by the epiboly of the micromere-derived epithelium. *Bottom*. By the end of gastrulation the epithelium covers the entire embryo and the germinal bands have coalesced along the ventral midline to form the germinal plate (gray). In this animal pole (roughly dorsal) view, only the anterior and posterior ends of the germinal plate are visible; the middle region lies on the far side of the embryo, hidden by the syncytial yolk cell (faint contour).

(Whitman, 1887; Weisblat & Shankland, 1985) and therefore it seems natural that at the 4-cell stage and beyond, the embryo should be oriented with the D quadrant at the future posterior end of the embryo (Figure 3A, B, second row). In this orientation, the future A–P axis bisects blastomeres B and D, and blastomeres A and C are situated bilaterally across the midline. But if this orientation is projected backwards to the 2-cell stage, it means that the first cleavage, which breaks the cylindrical symmetry of the zygote and establishes the second embryonic axis, lies oblique with respect to the future A–P axis rather than transverse or parallel to it (Figure 3A, B, top row). This discrepancy certainly does not violate any physical laws, but it does seem to be a puzzle that merits investigation.

A resolution of this paradox has been suggested by recent studies in which we used microinjected lineage tracers, chiefly ß-galactosidase, to follow the shapes and positions of the A, B and C quadrant macromeres during early development of *Helobdella robusta* (Liu et al., 1998). [Note that in the nomenclature we favor, macromeres that have produced one, two or three micromeres are designated by one, two or three 'primes', e.g. A', A" and A"' (Figure 2).] We have been able to document these changes more accurately using ß-galactosidase as a microinjected lineage tracer. This enzyme acts on a chromogenic substrate to form an intensely colored precipitate that remains insoluble even when the embryos are cleared in organic solvents such as benzyl benzoate/benzyl alcohol. This technique allows us to examine the shapes of the cells using transmitted light under the dissecting or compound microscope. Moreover, it also provides a sensitive assay for detecting macromere fusion (an early step in the formation of the syncytial yolk cell from which the gut epithelium arises) by observing the diffusion of the tracer enzyme from one cell to another (Liu et al., 1998).

In the 8-cell embryo, the macromeres have relatively simple shapes resembling curved wedges. During cleavage, however, as the teloblasts arise in the D quadrant, the three remaining macromeres change shape and position, as if to compensate for changes in the geometry of the D quadrant derivatives. The A, B and C quadrant macromeres do not participate equally in this process. As teloblasts arise, the space around and between them in the embryo is taken up chiefly by macromere C"'. By the end of cleavage in *Helobdella*, macromere C"' has assumed a highly complex shape, contacting all ten of the teloblasts and completely enveloping most of them in the posterior

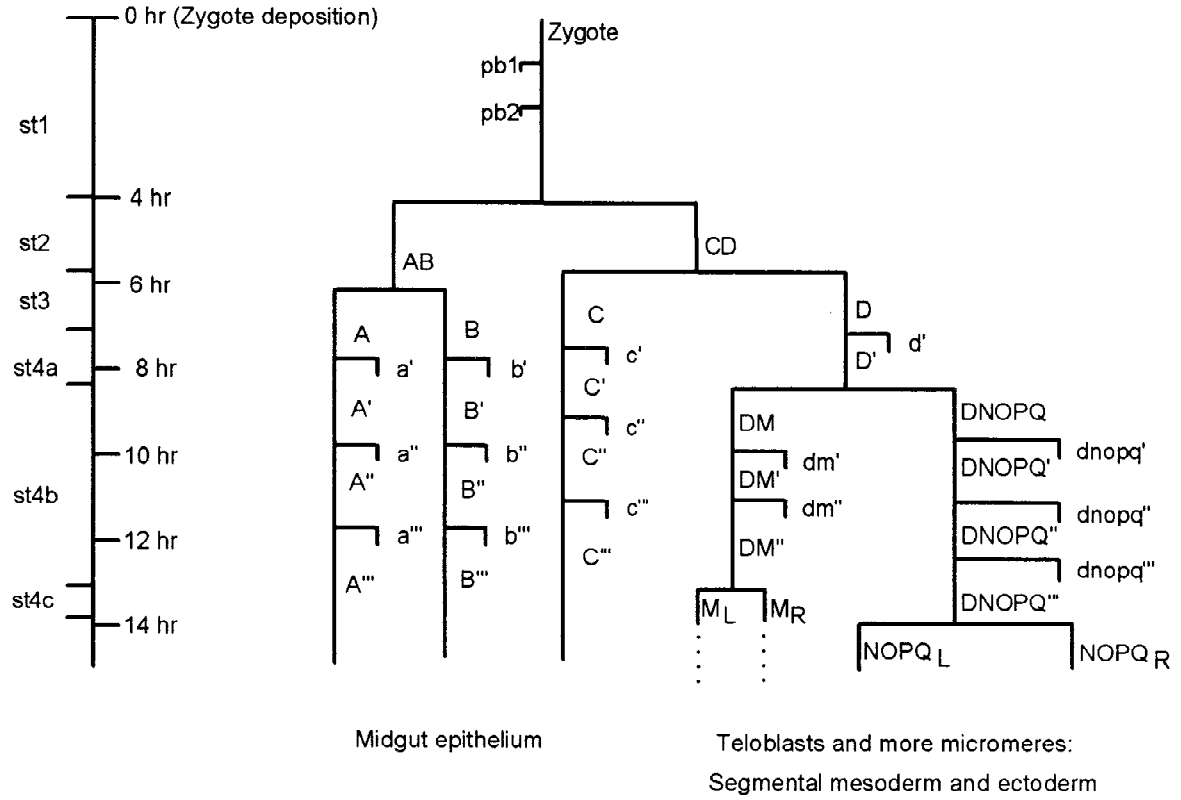

Figure 2. Partial cell lineage diagram for glossiphoniid leech embryos, up to the point at which cleavages in the D quadrant derivatives have formed left and right precursors of segmental mesoderm and ectoderm. The corresponding times of development after zygote deposition for *Helobdella robusta* at 23 ° C are indicated at left.

part of the embryo, while macromeres A"' and B"' lie across the midline from one another in the anterior part (Liu et al., 1998; Figure 3, fourth row). Another important consequence of this process is that the C"' macromere and the D-derived teloblasts shift positions relative to one another, clockwise in the case of the C"' macromere and counterclockwise for the teloblasts when the embryo is viewed from the animal pole (Figure 3, third row). This effectively restores the situation in the 2-cell embryo, in which the prospective C and D quadrants also share the same space in the embryo, in the form of the single cell CD. In which case, why not consider the first cleavage as transverse to the A–P axis, with cells A and B as a left-right pair from their birth at second cleavage (Figure 3C)? In this representation, the second cleavage in the CD line generates only a temporary lateral displacement of both the C and D lineages, while they complete their separate cleavage divisions, after which each of these lineages resumes a posterior position, straddling the midline.

While this representation seems inherently appealing (at least to me!), one could of course achieve the identical result maintaining the classical representation of the embryo, in which the D quadrant derivatives remain fixed at the posterior of the embryo. In this case, the first cleavage would be skewed with respect to the prospective A–P axis, and all three of the A"', B"' and C"' macromeres must spread and move clockwise to varying degrees during teloblast formation (Figure 3A, B, third row). Is there any meaningful distinction to be drawn between the two representations? Several lines of evidence support the notion that the A and B quadrant macromeres are truly a bilaterally situated pair of cells that resemble each other more than either resembles the C quadrant cell:

Animal–vegetal rearrangements

During teloblast formation, in addition to enveloping the nascent teloblasts, macromere C"' also shifts to occupy more territory at the vegetal pole of the embryo

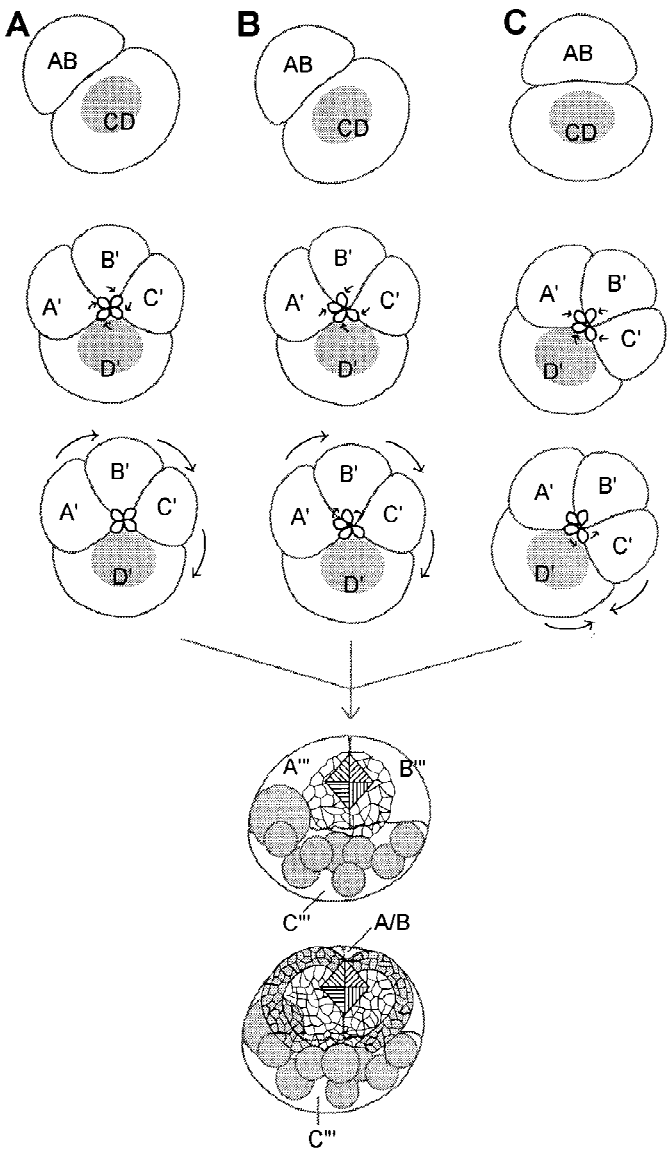

Figure 3. Origins of bilateral symmetry in the *Helobdella* embryo. All embryos are depicted as viewed from the animal pole (with anterior up, according to each representation). Grey shading indicates teloplasm in top three rows, and teloblasts and germinal bands in the bottom two rows (compare with Figure 1). A. 'D-centric' view with classical spiral third cleavage. B. 'D-centric' view with modified spiral third cleavage. C. 'AB-centric' view with modified spiral third cleavage. In the 'D-centric' representations, the first cleavage (top row) is oblique to the A-P axis, so that the D quadrant lies at the posterior pole. If the spiral third cleavage was completely dextrorotatory (A, second row), then the primary quartet micromeres (small circles) would arise with a' and b' as one left-right pair of cells, and d' and c' as another, with respect to the germinal bands, which indicate the bilateral plane of the adult (bottom row). This orientation is consistent with the distribution of their definitive progeny, as indicated schematically by the hatched triangles in the bottom two rows (a' and b' progeny, left and right diagonal hatching, respectively; d' and c', horizontal and vertical hatching, respectively; for more accurate representations on the positions of these cells, see Nardelli-Haefliger & Shankland, 1993; Smith & Weisblat, 1994). In this representation, the three macromeres must spread and shift clockwise to reach the positions they hold by the end of cleavage (Liu et al., 1998). But since the B quadrant cleaves with reverse handedness (B, second row; Sandig & Dohle, 1988), maintaining the 'D-centric' representation requires additional positional shifts from the a' and b' micromeres (B, third row). In the 'AB-centric' representation (C), the first cleavage is transverse to the A–P axis of the embryo, and the lateral displacements of the C and D quadrant cells are corrected when C"' envelopes the nascent teloblasts during late cleavage (C, third row). In this representation, micromeres a' and b' arise as a left-right pair and only cells c' and d' (or their progeny) must shift to reach their definitive positions (C, third row). By the time cleavage is complete (fourth row) most of the teloblasts are completely enveloped by macromere C"', and macromeres A"' and B"' occupy portions near the animal pole of the embryo that were originally occupied by macromere C"'. This symmetry is maintained through germinal band formation (fifth row), by which point macromeres A"' and B"' have fused, forming cell A/B.

and less at the animal pole. Concurrently, the A"' and B"' macromeres occupy more territory at the animal pole, beneath the micromeres and less at the vegetal pole (Figure 3, fourth row; Liu et al., 1998).

Mirror symmetry of A and B quadrant cleavages

Sandig & Dohle (1988) reported that *Theromyzon* embryos deviate from the classical spiralian cleavage pattern in that micromeres arising from the B quadrant arise with the opposite handedness to those in the A and C (and D quadrants), i.e. levorotatory at third and fifth cleavage and dextrorotatory at fourth cleavage. This has the effect of making the divisions in the A and B quadrants mirror symmetric with respect to the AB cleavage plane (Figure 3B, C second row); similar observations have been made for the smaller embryos of *Helobdella* (F. Z. Huang, personal communication) and for more distantly related annelids as well (Dohle, 1999). Thus, if the A and B quadrants are not regarded as a left–right pair, it would result in a puzzling asymmetry for the early embryo and would require that the micromeres or their progeny shift clockwise along with the A"' and B"' macromeres (Figure 3B, third row).

Micromere cell fates

In addition to the cleavage patterns by which they arise, available evidence suggests that the definitive progeny of the A and B quadrant micromeres distributed in mirror symmetric patterns with respect to the ventral midline. The present micromere fate maps (Weisblat et al., 1984; Nardelli-Haefliger & Shankland, 1993; Smith & Weisblat, 1994) are of relatively low resolution and precision compared to those for the segmentally iterated progeny of the teloblasts. Still, it seems clear that prostomial neurons descended from the a' and d' micromeres of the primary quartet lie on the left side of the supraoesophageal ganglion and are mirror symmetrically disposed with respect to those descended from the b' and c' micromeres, respectively (Figure 3, fifth row).

Macromere fusion

In glossiphoniid leeches, the midgut epithelium forms around the yolky remnants of the macromeres and teloblasts. This epithelium arises from a multinucleate syncytial yolk cell by cellularization of cortical nuclei within the yolk cell (Nardelli-Haefliger & Shankland, 1993) in response to mesodermally derived cues (Wedeen & Shankland, 1997). The syncytial yolk cell is formed by cell-cell fusions of the macromeres and spent teloblasts themselves (Liu et al., 1998), along with supernumerary blast cells (Shankland, 1999). The cell fusions do not occur all at once, nor do they occur randomly. Instead, the A"' and B"' macromeres, which are in constant contact from the 4-cell stage onwards (∼6 h after zygote deposition at 23 °) fuse with each other, and with no other cells, beginning ∼51 h after zygote deposition (Figure 3, fifth row). The resultant A/B cell fuses with macromere C"' to form cell A/B/C , beginning only ∼87 h after zygote deposition, despite the fact that the C quadrant cell is in constant contact with the A and B quadrant cells for all that time. It is unlikely that the involvement of macromere C"' in enveloping the teloblasts prevents it from fusing earlier; in embryos from which cell C is microsurgically removed, A"'–B"' fusion occurs on schedule, despite the fact that A"' and B"' envelop the teloblasts in such embryos. Moreover, the A"'–B"' fusion does not occur autonomously, but requires a signal from one or more of the D quadrant derivatives (Isaksen, 1997; Isaksen et al., 1999). While the details of this fusion process remain to be elucidated, its stepwise nature provides further evidence for the notion that the A and B quadrant cells are in some ways set apart from the C quadrant cell.

Conclusions

The question of how bilaterally symmetric animals (such as mollusks and annelids) arise from spirally cleaving embryos is not one on which civilizations stand or fall (the latter issue being more pertinent to students of deuterostome development). But it is an interesting problem nonetheless. Moreover, the answer(s) to this question should contribute to our understanding of how the distinct body plans of the modern phyla arose by changes in the developmental processes that operated in their common ancestor.

Our standard approach to this question, based on the uniqueness and developmental significance of the D quadrant, has been to align the B–D axis with the prospective A–P axis of the animal (e.g., Whitman, 1878, 1887; Weisblat et al., 1984; Shankland & Savage, 1997; Figure 3A). In this representation, a classical dextrorotatory spiral cleavage would bring the primary quartet of micromeres into alignment

with the symmetry of their prospective clones within the prostomium (Figure 3A, second row; Nardelli-Haefliger & Shankland, 1993). This representation is awkward in that it entails having the first cleavage oriented obliquely with respect to the A–P axis. In addition, we now know that the primary quartet arises by a modified version of spiral cleavage, so that micromeres a'–a"' and b'–b"' maintain the same spatial relationships as their parent macromeres. Thus, adhering to the standard representation (Figure 3B) puts *all* the A and B quadrant cells, both micromeres and macromeres off axis, requiring that they move and spread in a clockwise direction to assume their definitive positions, in apposition across the embryonic midline from one another, by the beginning of germinal band coalescence Figure 3B, third row). It is not clear what the substrate for such motions would be if all these cells are moving together in the same direction.

The thesis of this review is that the standard representation is inappropriate and that the appropriate representation for the early embryo is that derived from orienting the first cleavage plane transverse to the A–P axis of the embryo (Figure 3C). This representation puts the all A and B quadrant cells in the proper left–right positions from the beginning. Moreover, it is easy to envision mechanisms for the movements of the C and D quadrant derivatives back past one another toward the midline, because we see that the C quadrant cell selectively spreads over the D-derived proteloblasts and teloblasts as they form (Liu et al., 1998). This representation not only has the first cleavage oriented transverse to the body axis, it also is self-consistent in that the A and B quadrant macromeres resemble each other and differ from the C quadrant macromere in cell behaviours involved in cell rearrangements and cell–cell fusion. Indeed, the only ad hoc requirement consequent to this representation of the early embryo is that the c' and d' micromeres of the primary quartet (or their progeny) must shift positions at some point to become symmetrically disposed with respect to the embryonic midline (Figure 3C, third row).

Leeches as a group and glossiphoniid leeches in particular are specialized relative to most other annelids in various ways. For example, they undergo direct development, exhibit an invariant number of segments, terminal suckers and lack regenerative capacity. The process by which they generate bilateral symmetry from spirally cleaving embryos may be equally specialized. Studies of how other spiralian embryos (annelids, mollusks and flatworms) achieve bilateral symmetry should reveal whether the scheme proposed here is ancestral or derived.

Acknowledgements

I thank Françoise Z. Huang for assistance in preparing the figures, and FZH and Bob Goldstein for helpful discussions. Research in our laboratory reported here was supported by grants from the National Science Foundation.

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