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Conservation of the Spiralian Developmental Program: Cell Lineage of the Nemertean, *Cerebratulus lacteus*

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Lineage tracers were injected into individual blastomeres in embryos of the indirect-developing nemertean *Cerebratulus lacteus* through the formation of the fourth quartet of micromeres. Subsequent development was followed to the formation of feeding pilidium larvae to establish their ultimate fates. Results showed that these blastomeres have unique fates, and their clones give rise to highly predictable regions of the larval body. As in other spiralians, four discrete cell quadrants can be identified. For the most part, their identities are homologous to the typical spiralian A, B, C, and D cell quadrants. In some respects their fates differ from the typical spiralian fate map; however, these can be understood in terms of simple modifications of the early cleavage program. Unlike most spiralians, the first quartet micromeres in the eight-celled embryo are larger than the corresponding vegetal macromeres, and generate most of the larval ectoderm. All four of these micromeres contribute to the apical organ and generate four bilaterally situated domains of ectoderm, where the progeny of the 1a and 1d micromeres lie to the left of the median plane while those of 1b and 1c lie to the right. Unlike the progeny of the first quartet, those of the second quartet are situated in left (2a), ventral (2b), right (2c), and dorsal (2d) positions. The third quartet micromeres generate clones situated in a bilaterally symmetrical fashion similar to those of the first quartet. The alternating axial relationships exhibited by successive micromere quartets are a characteristic of spiralian development. Unlike other spiralian larvae possessing a ciliary band, the pilidium larval ciliary band is formed by all blastomeres of the first and second micromere quartets, as well as 3c and 3d. Ectomesoderm is derived from two blastomeres (3a and 3b), which give rise to the extensive array of the larval muscle cells. *C. lacteus* also possesses a true mesentoblast (4d) which gives rise to a pair of mesodermal bandlets, and scattered mesenchymal cells. The dual origin of the mesoderm, as both ectomesoderm and endomesoderm, appears to be a condition present in all spiralians. The gut is formed by all the fourth quartet micromeres as well as the vegetal macromeres (4A, 4B, 4C, 4D). Despite differences in the determination of axial properties and some modifications in quadrant fates, nemerteans appear to be constructed on the typical spiralian developmental platform.

Key Words: **cell lineage; cell specification; dorsoventral axis; evolution; Nemertea; spiralians.**

INTRODUCTION

Almost one quarter of the extant metazoan phyla exhibit features of a highly conserved developmental program that involves spiral cleavage, including the turbellarian platyhelminthes, gnathostomulids, rotifers, mesozoans, nemerteans, sipunculids, echiurans, pogonophorans, vestimentiferans, annelids, and molluscs (Wilmer, 1990). Previous investigators who examined the cell lineages of different spiralian embryos (predominantly those of annelids and molluscs) noted tremendous similarities in cell cleavage pattern, cell fates and even certain larval forms (Wilson, 1898; Costello and Henely, 1976; Verdonk and van den Biggelaar, 1983; Wilmer, 1990; Freeman and Lundelius, 1992; Henry and Martindale, 1994a). These similarities were thought to reflect the common evolutionary origin of these animals. Recent studies utilizing more precise intracellular lineage tracers have revealed interesting differences between the contributions of some embryonic cells to the larval and adult body plans among these organisms (Damen,

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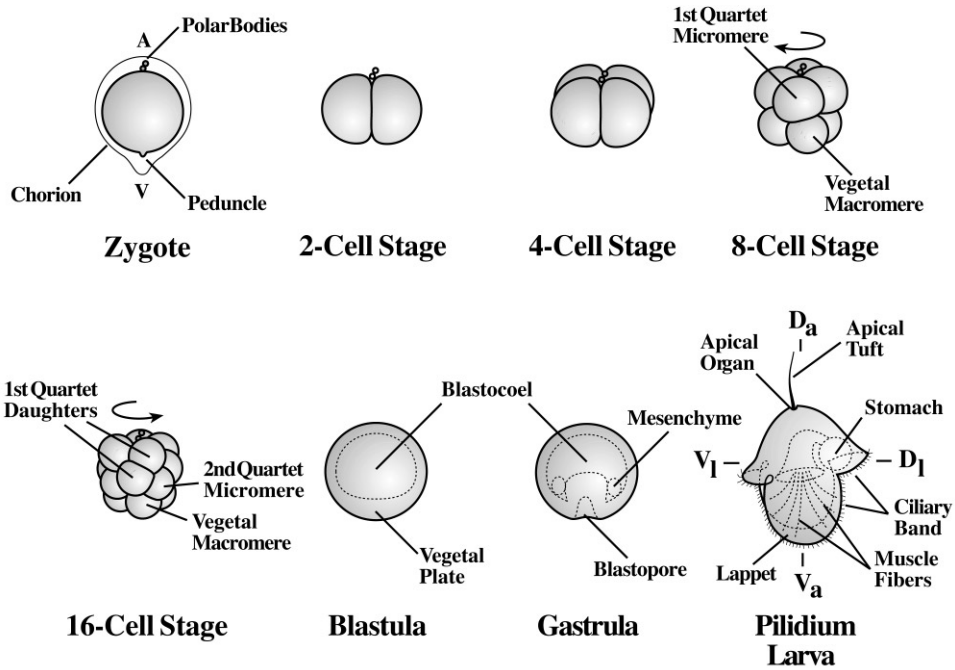

FIG. 1. Diagram summarizing development from the fertilized egg to the formation of the feeding pilidium larva of *Cerebratulus lacteus*. All embryonic stages are illustrated with the animal pole directed upward, which is also indicated by the position of the polar bodies, (A) animal, (V) vegetal. The first two cell divisions are equal. Note that the first quartet micromeres of the 8-celled embryo are larger than the vegetal macromeres due to the subequatorial plane of the third cleavage division. Also note the staggered (oblique) arrangement of the blastomeres in the 8- and 16-celled embryos that results from the shifting relationship of the cleavage spindles. The first quartet micromeres are given off in a clockwise direction, while the second quartet is formed in a counterclockwise direction (see arrows), as viewed from the animal pole. Bilaterally situated populations of mesenchyme lie within the blastocoel during the early gastrula stage along the sides of the invaginating archenteron. Subsequent development leads to the formation of the swimming pilidium larva with a blind digestive tract, numerous muscle fibers, lappets, ciliary band, and an anterior apical organ and ciliary tuft (located at the animal pole). The pilidium larva is shown as a left lateral view with the apical ends directed upward (animal pole). Ultimately the pilidium larva will undergo a radical metamorphosis to give rise to the juvenile worm. The larval dorsoventral axis is labeled V_l–D_l, while the future adult dorsoventral axis is labeled V_a–D_a.

1994; Damen and Dictus, 1994; Henry and Martindale, 1994a). These findings indicate that closer examination of the normal cell lineages of other members of the Spiralia may reveal significant differences in their early development that reflect evolutionary changes in these related forms. An understanding of this fundamental information is essential for experimental investigations into the processes that determine cell fates and axial properties in these organisms, and for understanding the evolution of diverse larval and adult body plans that arise from highly conserved early developmental programs.

First cleavage in spiralian embryos yields two cells referred to as the AB and CD blastomeres, where the CD cell is typically larger then the AB cell in species that display unequal cleavage. The next division of these cells gives rise to the A, B, C, and D cells, which establish the four embryonic cell quadrants. The D cell can usually be distinguished by its larger size in unequal-cleaving forms. Generally, it is claimed that the A, B, and C quadrants give rise to the left, ventral, and right regions of the head, respectively; while the D cell and its progeny give rise to the dorsal region of the larval/adult head, much of the adult mesoderm (endomesoderm), and the majority of the postrochal (posterior) region of the body (Costello and Henley, 1976; Verdonk and van den Biggelaar, 1983). Subsequent cleavage divisions generate successive quartets of micromere cells which are positioned in clockwise and counterclockwise orientations with respect to the vegetal macromeres. This arrangement is generated by the alternating oblique orientation of the cleavage spindles, which defines the spiral cleavage pattern. A similar situation is seen in molluscs displaying equal first and second cleavage divisions.

Nemertean embryos exhibit an equal, spiral cleavage pattern in which the blastomeres are all of the same size at the four-cell stage (illustrated in Figs. 1 and 2). Preliminary lineage analyses performed by Henry and Martindale (1994a) suggested that the contributions of the four nemertean cell quadrants differ from those of other spiralians studied to date. These analyses examined the external ectodermal labeling

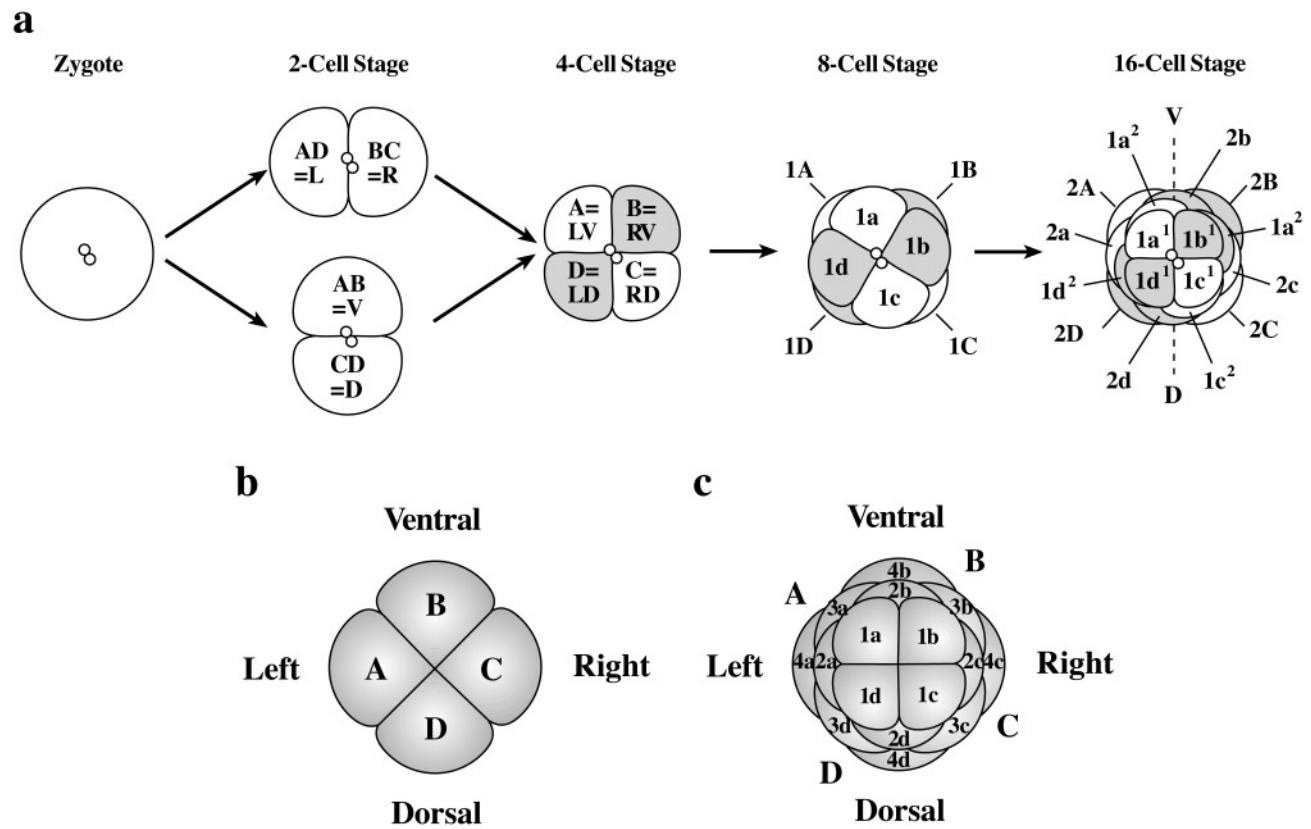

FIG. 2. (a). Diagram of early cleavage in *C. lacteus* that shows the fates and nomenclature used to designate individual cells in the embryos through the 16-cell stage. All stages are viewed from the animal pole, which is indicated by the presence of the polar bodies (small open circles). Note that the first cleavage plane may bear one of two different relationships relative to the future dorsoventral axis (labeled D and V, as a dashed line at the 16-cell stage). This generates what we formally referred to as "left" (L = AD) and "right" (R = BC) or "dorsal" (D = CD) and "ventral" (V = AB) blastomeres. In either case, the second cleavage plane is orthogonal to the first plane of division and subdivides the embryo into four discrete blastomeres which were formally called the left– and right–ventral (LV, RV) and the right– and left–dorsal (RD, LD) cell quadrants. The result presented in this study indicate that these correspond in their ultimate fates to the A, B, C, and D blastomeres, respectively, which have been identified in other spiralians (standard nomenclature follows that of Wilson, 1892). The first quartet micromeres (1a, 1b, 1c, 1d) of the 8-celled embryo are normally larger than the vegetal macromeres (1A, 1B, 1C, 1D); however, the micromeres are shown smaller at this and subsequent stages for the sake of clarity in these animal pole views. The first quartet is generated in a clockwise direction while subsequent quartets are thrown off in alternating counterclockwise (e.g., second quartet) and clockwise directions. The B and D cell quadrants are stippled so that one can more easily follow their progeny. (b). Overgeneralized interpretation describing the relationships between the four basic spiralian quadrants and the various embryonic and larval axial properties in spiralians. (c). More accurate representation of the positions of the four quadrants (A, B, C, D) and their four-micromere daughters (first through fourth quartets) relative to the dorsoventral and bilateral axes. See text for further details.

patterns generated by injecting individual cells at the two- and four-cell stages. Based on these patterns, it appeared that the first two cleavage planes correspond closely to the bilateral and frontal planes, and are not oblique to the dorsoventral axis, as is claimed for other spiralians (Render, 1991; Henry and Martindale, 1994a, 1995). Four discrete cell quadrants are generated as a result of the first two cell divisions, which were referred to as the left- and right-ventral and left- and right-dorsal cell quadrants (Fig. 2).

If the first and second cleavage planes are, in fact, shifted toward the median and frontal planes, one might predict that there are other significant alterations in the ultimate fates of the resulting blastomeres. For instance, one could predict that the developmental potential to give rise to a region of the larva characteristic of the single D quadrant present in molluscs and annelids may be shared by two-cell quadrants in nemerteans. In fact, experiments suggest that both the left and right dorsal quadrants possess inductive potential with regard to the formation of the lateral ocelli in the nemertean *Nemertopsis bivittata* (Martindale and Henry, 1995), while typically the single D quadrant possesses this capacity in

virtually all other spiralian embryos (Verdonk and Cather, 1983). Mesodermal origins may also be different in the nemerteans. Previous investigations with annelids and molluscs revealed that there are two distinct sources of mesoderm (Verdonk and van den Biggelaar, 1983; Boyer *et al.*, 1996). In these forms the D quadrant gives rise to the mesentoblast (4d), which generates most of the adult visceral mesoderm, while additional mesoderm is derived from second and/or third quartet micromeres, referred to as the ectomesoderm. Recent investigations on the origins of mesoderm in a basal spiralian, the polyclad flatworm *Hoploplana inquilina*, revealed that the mesoderm of the Müller's larva is derived as ectomesoderm from the second quartet micromere of the ventral quadrant, 2b, and as endomesoderm from the fourth quartet micromere of the dorsal quadrant, 4d (Boyer *et al.*, 1996). The exact origin of the mesoderm in nemerteans is poorly understood (Henry and Martindale, 1996a, 1997). Some claimed that the mesoderm in *C. lacteus* arises solely as ectomesoderm (Lebedinsky, 1897; Wilson, 1900; Iwata, 1957). Furthermore, there has been some argument regarding whether the ciliated band of the nemertean pilidium larva is homologous with the ciliated prototroch of mollusc and annelid larvae (van den Biggelaar *et al.*, 1997). Careful cell lineage analyses can help settle these arguments for nemerteans and other spiralians.

In this paper we characterize the developmental fates of individual cells through the time of fourth micromere quartet formation in the nemertean *C. lacteus*. The progeny of the first quartet of micromeres generates extensive larval ectodermal territories, including the apical organ and ciliated band. The second quartet also generates ectoderm, including the ciliated band. The larval musculature is generated as ectomesoderm, in a bilaterally symmetrical fashion, by the third quartet micromeres of the A and B cell quadrants (3a, 3b). The other third quartet micromeres (3c, 3d) generate ectoderm and portions of the ciliated band. Endomesoderm (the mesentoblast bands) arises from the fourth quartet micromere of the D quadrant (4d). The other fourth quartet micromeres (4a, 4b, 4c) and all of the remaining vegetal macromeres (4A, 4B, 4C, 4D) contribute solely to gut formation. The larval nervous system is formed by dorsolateral cells that also generate the ciliated band. Despite differences in the mechanisms deployed to establish the embryonic axes, and specific quadrant fates (Martindale and Henry, 1995; Henry and Martindale 1997), the findings presented here clearly show that the four nemertean cell quadrants are homologous to those of other spiralians studied to date.

MATERIALS AND METHODS

Collection of Adults and Embryos

Adult specimens of the nemertean worm *C. lacteus* were obtained by the Marine Resources Department of the Marine Biological Laboratory (Woods Hole, MA). Gametes were prepared as described by Martindale and Henry (1995).

Cell Lineage Analysis via Microinjected Fluorescent Tracer

In some cases, embryos were immobilized for microinjection by affixing them to the bottoms of plastic petri dishes previously treated with a solution of 0.025% poly-L-lysine (Sigma, St. Louis, MO) made up in filtered seawater (FSW). To ensure that this treatment did not bias the plane of cell division, the embryos were applied to the poly-L-lysine-coated dishes after they had reached the appropriate cleavage stages.

Individual blastomeres were injected at all embryonic stages through the formation of the fourth quartet of micromeres. Specific cells were pressure injected with Fluoro Ruby (D-1817, Molecular Probes, Inc., Eugene, OR) dissolved in 0.2 M KCl at a concentration of 50 mg/ml. The amount of dye injected represented approximately 1–5% of the cell's volume. Prior to injection, the dye solution was passed through a Spin-X microcentrifuge filter (CoStar, Cambridge, MA) to remove small particulate matter, which might clog the microinjection needle. Immediately following injection, the embryos were detached from the bottoms of the dishes with a gentle stream of FSW.

In other cases the chorion was removed by gently passing the unfertilized eggs through Nitex mesh (170 μm). These eggs were then placed in small petri dishes which had been coated with gelatin to prevent the denuded cells from sticking to the charged plastic (Zalokar and Sardet, 1984). This treatment facilitated the injection of late stage embryos when the individual cells are much smaller in size. Following fertilization, the denuded zygotes were placed into fresh dishes where small shallow grooves had been etched into the bottoms using a small piece of broken glass. The embryos were rolled into the shallow grooves prior to injection, which helped immobilize them for injection. These cases were injected directly with the fluorescent lipophilic dye, DiI (Molecular Probes Inc.) dissolved in vegetable oil (Terasaki and Jaffe, 1991). A 100 mg/ml DiI stock was made in ethanol and diluted 20-fold in soybean oil.

Embryos that survived microinjection continued to cleave and developed normally, at the same rate as uninjected controls. Thus, neither injection procedure appeared to adversely affect development. Likewise, no differences were detected in the lineage patterns displayed by embryos injected with Fluoro-Ruby vs DiI.

Culture and Preparation of Embryonic and Larval Specimens

The embryos were raised at 19°C in FSW for a period of 48–72 h. Pilidium larvae were fixed briefly in 0.1% formaldehyde in FSW (pH 8.3) at 19°C and photographed immediately with a Zeiss Axioplan equipped for DIC and fluorescence photomicroscopy. In order to clearly visualize the labeled structures, the larvae were lightly compressed under a siliconized coverslip suspended by clay feet. Since the larva is highly transparent, all the internal cell types could be clearly visualized in these whole mounts.

Assignment of Cell Lineage Nomenclature

The cleavage pattern in *C. lacteus* proceeds in a typical spiral fashion where both the first and second cleavage divisions are equal (refer to Figs. 1 and 2). The first and second cleavage planes are orthogonal to one another and include the animal–vegetal axis. As is the case in the direct-developing nemertean *N. bivittata* (Henry and Martindale, 1994a), there are typically no animal or vegetal cross-furrows separating the blastomeres in the four-celled embryo.

TABLE 1
Cerebratulus lacteus Division Chronology

Division	Time (h:min @ 20°C)
Fertilization	0:00
First cleavage	1:40
Second cleavage	2:15
First quartet	2:55
Second quartet	3:40
Third quartet	4:25
Fourth quartet	5:25

Note. Approximate times taken from the average of three different batches of embryos.

The oblique third division is subequatorial, thus the animal "micromeres" are larger then the four vegetal "macromeres." All of these four micromeres appear to be of identical size. Due to the oblique orientation of the cleavage spindles, which is characteristic of the spiral cleavage pattern, the first quartet of micromeres is formed in a dexiotropic (clockwise) manner. The second quartet of micromeres is given off in a laeotropic (counterclockwise) direction relative to the vegetal macromeres. This pattern of divisions continues to alternate in this manner during the formation of the third and fourth micromere quartets. Within each quartet, the micromeres all appear to be of the same size, and they are all generated in a synchronous fashion. Thus, there are no outward signs pointing to differences between the four cell quadrants up through the time of fourth quartet formation. Therefore, individual blastomeres within a quartet were randomly injected at each stage and their labeling patterns analyzed at larval stages. No additional micromeres are formed following production of the fourth quartet. Early cleavage is diagrammed in Figs. 1 and 2. As justified under Results and Discussion below, careful analysis of a large number of samples indicated that the four nemertean cell quadrants correspond to those present in other spiralian embryos (i.e., A. B, C, and D). Thus, we have used the same standard nomenclature to designate each of the blastomeres within the nemertean embryo (Wilson, 1892). A time course of the early divisions, including the formation of each of the four micromere quartets is shown in Table 1. The lineage nomenclature and fates of each quartet of micromeres and the vegetal macromeres are shown in Figs. 2 and 3.

RESULTS

The Fates of Cells in the Two-Celled Embryo

Individual blastomeres were injected at the two-cell stage. Four different labeling patterns were observed as a result of this series of injections. These corresponded to the four patterns previously described by Henry and Martindale (1994a); however, use of the fluorescent lineage tracers afforded a much clearer assessment of the exact contributions of the injected cells. Labeled ectodermal domains included a left lateral pattern, and a complementary right lateral pattern, a dorsal labeling pattern, and a complementary ventral pattern. The number of cases displaying each of these labeled domains is recorded in Table 2. No other patterns were observed. In each case, the labeled domain included surface ectoderm, half of the apical organ/tuft, a portion of the esophagus, and the gut. The distribution of label in deeper structures (mesoderm and endoderm) is obscured somewhat by the overlying labeled ectodermal domains, but these contributions can be clearly discerned from the progeny of these blastomeres labeled at subsequent stages (see below). The boundaries between left and right labeling patterns lie close to the plane of bilateral symmetry. The ectodermal boundaries that lie along the frontal plane, however, are actually skewed slightly such that the right edge is positioned slightly ventral of the left edge. Other oblique relationships were never observed in these embryos; thus, the first cleavage plane does not assume a random orientation relative to the dorsoventral axis. The occurrence of complementary, "left and right," and "dorsal and ventral" labeling patterns indicates that the first cleavage plane may occur along one of two different planes. While the labeled larval ectodermal domains suggest that these planes correspond to either the plane of bilateral symmetry or nearly the frontal plane, the situation is actually more complex (see below).

Interestingly, larval musculature was found to be labeled in all cases except those exhibiting the "dorsal" pattern (labeling of the larval musculature is more obvious in the case of injections carried out at later stages of development, as described below). This finding suggest that the larval musculature is derived solely from progeny of the "ventral" cell.

The Fates of the Cells in the Four-Celled Embryo

A total of 47 larvae with sufficient label for detailed examination were recovered from embryos injected at the four-cell stage (Table 2). Four different, reproducible ectodermal labeling patterns were observed as a result of this series of injections. These included a left–ventral pattern (LV), and a right–ventral pattern (RV), a right–dorsal labeling pattern (RD), and a left–dorsal pattern (LD). These patterns define four embryonic cell quadrants. As will become clear below, these four quadrants correspond closely to the A, B, C, and D cell quadrants, respectively, of other spiralian embryos. Each of these patterns represents a subset of the four patterns observed in embryos injected at the two-cell stage. In other words, each pattern comprises one half of two of the four domains defined by injections at the two-cell stage, above. Regardless of which one of the two planes the first and second cleavage divisions assume, the second cleavage division is orthogonal to the first, and the net result is always the same at the four-cell stage (see Fig. 2). The number of cases displaying each of these four patterns is recorded in Table 2. Each of these labeling patterns is shown in Figs. 4a–4d. All four of these cell quadrants contribute to the formation of the surface ectoderm, including the apical organ, and tuft, the ciliated band, a

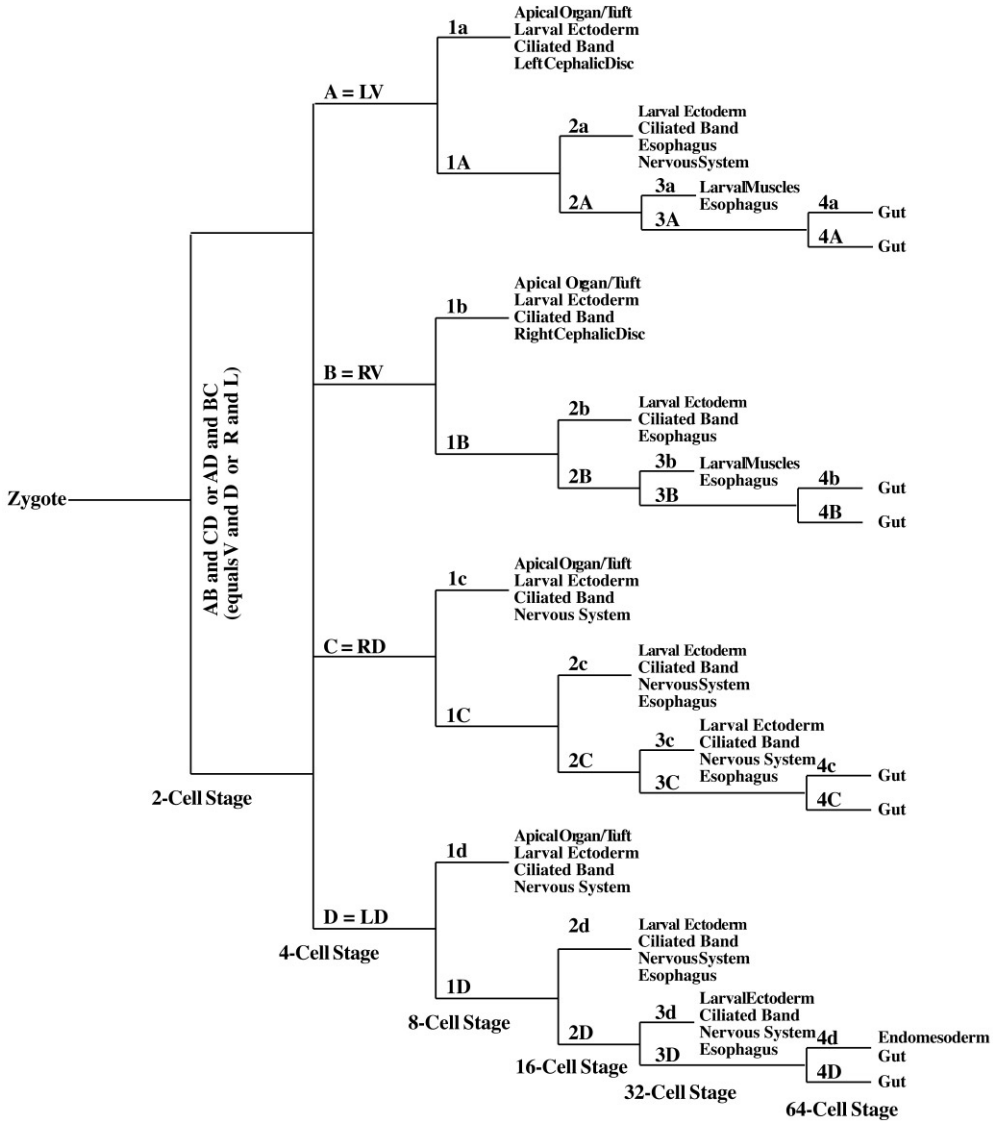

FIG. 3. *C. lacteus* lineage diagram summarizing the lineage relationships and larval fates of each cell through the 64-cell stage, as determined by the distribution of microinjected fluorescent lineage tracer in pilidium larvae. The identities of the four basic cell quadrants are the same in all embryos examined. A continuous line is shown connecting the blastomeres at the four-cell stage since their exact origins relate back to the plane of the first cleavage division. Depending on the orientation of the first cleavage spindle, as discussed in the text and illustrated in Fig. 2, the two-cell stage embryo contains either an AB and CD or an AD and BC pair of blastomeres. This cell lineage fate map is similar to those of other spiralians, and includes the presence of a true mesentoblast, 4d.

portion of the esophagus, and the gut. The exact contributions of these four-cell quadrants are described in greater detail below, since it is easier to appreciate this by following their descendent micromere progeny. Labeled larval muscle fibers were only observed as the progeny of the A and B cell quadrants (Figs. 4a and 4b). Some scattered labeled mesenchyme could be seen in those cases in which the left–dorsal (D) blastomere had been injected (Fig. 4d).

The Fates of the Cells in the Eight-Celled Embryo, the First Quartet Micromeres, and Macromeres

Single animal micromeres or macromeres were injected at the eight-cell stage. Following the conventional spiralian lineage nomenclature we refer to the animal micromeres as 1a, 1b, 1c, and 1d (see Figs. 2 and 3). The corresponding macromeres are referred to as the 1A, 1B, 1C, and 1D. Each of these cells gives rise to discrete, reproducible domains of

TABLE 2

Occurrence of Cell Lineage Labeling Patterns in *Cerebratulus lacteus* Embryos

Two-cell blastomeres	Left (AD)	Right (BC)	Ventral (AB)	Dorsal (CD)
Number observed (64 cases examined)	7	27	15	15
Four-cell blastomeres	**LV (A)**	**RV (B)**	**RD (C)**	**LD (D)**
Number observed (47 cases examined)	5	22	14	6

Eight-cell blastomeres	1a	1b	1c	1d	1A	1B	1C	1D
Number observed (68 cases examined)	9	8	4	9	13	9	8	8

Second quartet micromeres	2a	2b	2c	2d
Number Observed (109) cases examined)	29	43	15	22
Third quartet micromeres	**3a**	**3b**	**3c**	**3d**
Number observed (37 cases examined)	6	8	12	11
4th quartet micromeres	**4a**	**4b**	**4c**	**4d**
Number observed (40 cases examined)	8	14	8	10

4th quartet macromere patterns	4A	4B	4C	4D
Number observed (68 cases examined)	16	14	24	14

Note. See text and figures for specific details regarding the appearance of these labeling patterns.

Nomenclature conversions are listed for the patterns observed at the two- and four-cell stages, following the earlier designations of Henry and Martindale (1994a, 1995.)

labeled larval tissues. Eight discrete labeling patterns were observed. The number of cases displaying each of these different patterns is recorded in Table 2. These patterns are shown in Figs. 5a–5h. These labeled domains are highly consistent from embryo to embryo, and no other labeling patterns were ever observed. The fates of these cells are clearly subsets of the four-cell quadrants described above.

All of the first quartet micromeres contribute to the formation of large ectodermal domains of the outer larval epidermis, including the apical organ and tuft, the outer surface of the lappets, and portions of the ciliated band. Close examination of the ectodermal labeling domains reveals that the individual cells interdigitate along their boundaries, which have a "zigzag" shape (refer to Figs. 5a–5d, and also see Figs. 4a–4d). This may be a remnant of the alternating spiral cleavage divisions. Generally there is no mixing of labeled and unlabeled cells beyond these ectodermal boundaries. There are some slight asymmetries present in the contributions of these four micromeres to the ectoderm. The "frontal" boundary separating the ventral quadrants from dorsal quadrants is slightly skewed, such that the 1b micromere appears to form a somewhat smaller domain of surface ectoderm than does the 1a micromere (compare Figs. 5a and 5b). Likewise, the 1d micromere contributes slightly less to the formation of the surface ectoderm than does the 1c micromere (compare Figs. 5c and 5d). The left and right cephalic imaginal disks, which are the first to form in the larva and contribute to the formation of anterior adult ectoderm, were observed in a number of cases to be formed by la and lb, respectively (Figs. 5a and 5b).

The four vegetal macromeres (1A, 1B, 1C, 1D) contribute to the formation of the ciliated band, as well as ectoderm on the inner surface of the ciliated lappets, and surrounding the stomodeum. They also contribute to the formation of the esophagus and the gut (see Figs. 5e–5h). Larval muscle fiber cells are formed only by the progeny of the 1A, and 1B macromeres as seen in Figs. 5c and 5d). Scattered labeled mesenchyme can be seen in those cases in which the 1D blastomere had been injected (Fig. 5h).

The Fates of the Second Quartet Micromeres

Four discrete reproducible labeling patterns were observed following the injection of individual micromeres of the second quartet (Table 2 and Figs 6a–6d). Unlike the clones derived from the first quartet micromeres, those of the second quartet occupy left (2a), ventral (2b), right (2c), and dorsal (2d) positions. The second quartet micromere of the A quadrant (2a) gives rise to a part of the ciliated band located on the left lappet, ectoderm located on the inner surface of the left lappet, and the left side of the esophagus. 2b gives rise to the ventral-most portion of the ciliated band, the ventral ectoderm adjacent to the stomodeum, and the ventral side of the esophagus. 2c gives rise to the same fates as 2a but on the right side of the larva in a bilaterally symmetrical fashion. Finally, 2d gives rise to the dorsal part of ciliated band extending slightly into both the left and right lappets, dorsal ectoderm adjacent to the stomodeum, and the dorsal side of the esophagus.

The Fates of the Third Quartet Micromeres

Individual micromeres of the third quartet were injected with lineage tracer (Table 2, and Figs. 6e–6h). Four discrete reproducible labeling patterns were observed. Contributions are made in a bilaterally symmetrical fashion, where the contributions of 3a mirror those of 3b, and those of 3c mirror those of 3d, because these domains are located on opposite sides of the plane of bilateral symmetry. All of these micromeres contribute to the formation of the esophagus. However, only two micromeres (3c and 3d) contributed to the formation of small portions of the ciliated band located in the right and left lappets, while the other two (3a

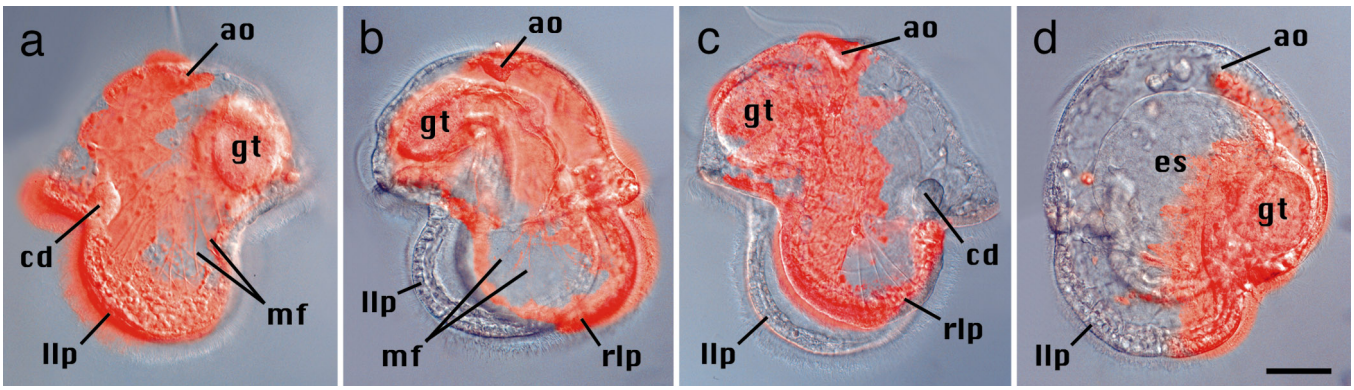

FIG. 4. Superimposed DIC and fluorescence photomicrographs depicting the larval progeny of individual blastomeres from four-celled embryos of *C. lacteus*. The red color depicts the presence of microinjected fluorescent lineage tracer within these cells. The larvae appear to be different sizes due to the fact that they have been slightly compressed to visualize internal cell fates. In these embryos only four different patterns were observed, which correspond to each of the four cell quadrants. While most of the labeled progeny can be visualized in these images, some may not be visible due to the single plane of focus. All larvae are oriented with the apical ends facing upward. (a). Example in which the A (left–ventral) blastomere had been injected. The larva is viewed from the left side with the ventral surface located to the left. Note the patch of labeled left–ventral surface ectoderm. One quarter of the apical organ also contains fluorescent label. A "zig-zag" alternating pattern of labeled cells is apparent at the boundaries of the labeled ectodermal territory. This reflects the alternating arrangement of micromere daughter cells that results from the spiral cleavage pattern (see Figs. 1 and 2). Note also the presence of labeled muscle fiber cells extending into the left lappet. A number of other labeled muscle fibers are not visible due to the plane of focus. Portions of the esophagus and the gut are also labeled. The left cephalic imaginal disc is labeled. This structure will give rise to anterior portions of the juvenile worm. (b). Example in which the B (right–ventral) blastomere had been labeled. This larva is viewed from its right side with the ventral surface located toward the right side of the page. The labeling pattern is very similar to that seen in a; however, the domain of labeled surface ectoderm is somewhat smaller and there is a more extensive domain of labeled cells within the esophagus. A number of the labeled muscle fiber cells extend into the right lappet. Many other labeled muscle fibers are not visible due to the plan of focus. (c). Example in which the C (right–dorsal) blastomere had been injected. While muscle fibers are clearly seen, they are unlabeled since they are not derived from the progeny of this blastomere. Note the larger size of the labeled domain of external ectoderm compared to that derived from either the right–ventral or left–dorsal quadrants. Labeled cells are also located within the apical organ, esophagus, and the gut. Small round labeled structures are actually the nuclei of labeled ectodermal cells located in the inner surface of the lappet, which are often clearly visible. This case is viewed from the right side. (d) Example of a larva in which the D (left–dorsal) blastomere had been labeled. This larva is viewed from the left side. Both lappets can be seen. A small patch of left–dorsal surface ectoderm is labeled. One-quarter of the apical organ is also labeled. No labeled muscle fiber cells can be seen within the larva; however, scattered labeled mesenchyme cells are present. Labeled cells are also located in and adjacent to the esophagus and the gut. (ao) apical organ, (rlp) right lappet, (llp) left lappet, (gt) gut, (mf) muscle fibers, (es) esophagus, (cd) cephalic imaginal disc. Scale bar, 50 μm.

and 3b) generated all of the larval muscle cells. For the most part, 3a and 3b contribute mainly to muscle fibers located on their respective sides of the larva, though a small number of labeled fibers could be observed on the opposite sides (see Figs. 6e and 6f). The extent to which these latter two cells contributed to muscle cell formation on the left vs right sides varied slightly between cases. The only consistent difference that seemed to break mirror symmetry appeared to be that the two long muscles fibers that extend from the apical organ to the left and right cephalic discs were formed only by 3a (data not shown).

The Fates of the Fourth Quartet Micromeres

Individual micromeres of the fourth quartet were injected with lineage tracer (Table 2 and Fig. 7). Four discrete reproducible labeling patterns were observed. Three of the four micromeres contribute only to the formation of endoderm (see Figs. 7a–7c). The fourth micromere forms endoderm as well as a pair of bilaterally paired mesodermal bandlets, which appear to contain from 6 to 8 cells each, and a small number of "loose" mesenchymal cells (Fig. 7d). This cell is the 4d micromere. This determination is readily made by following the progeny of the D, 1D (see Figs. 3d and 4h) and 2D blastomeres (not shown), which also generates the mesodermal bandlets, and whose ectodermal contribution can be clearly discerned from those of the other quadrants. Thus, 4d represents a true mesentoblast just as is the case in other spiralians.

The Fates of the Fourth Quartet Macromeres

Following the production of the fourth quartet of micromeres, individual macromeres were injected with lineage tracer (Table 2 and Fig. 7). Four discrete, reproducible labeling

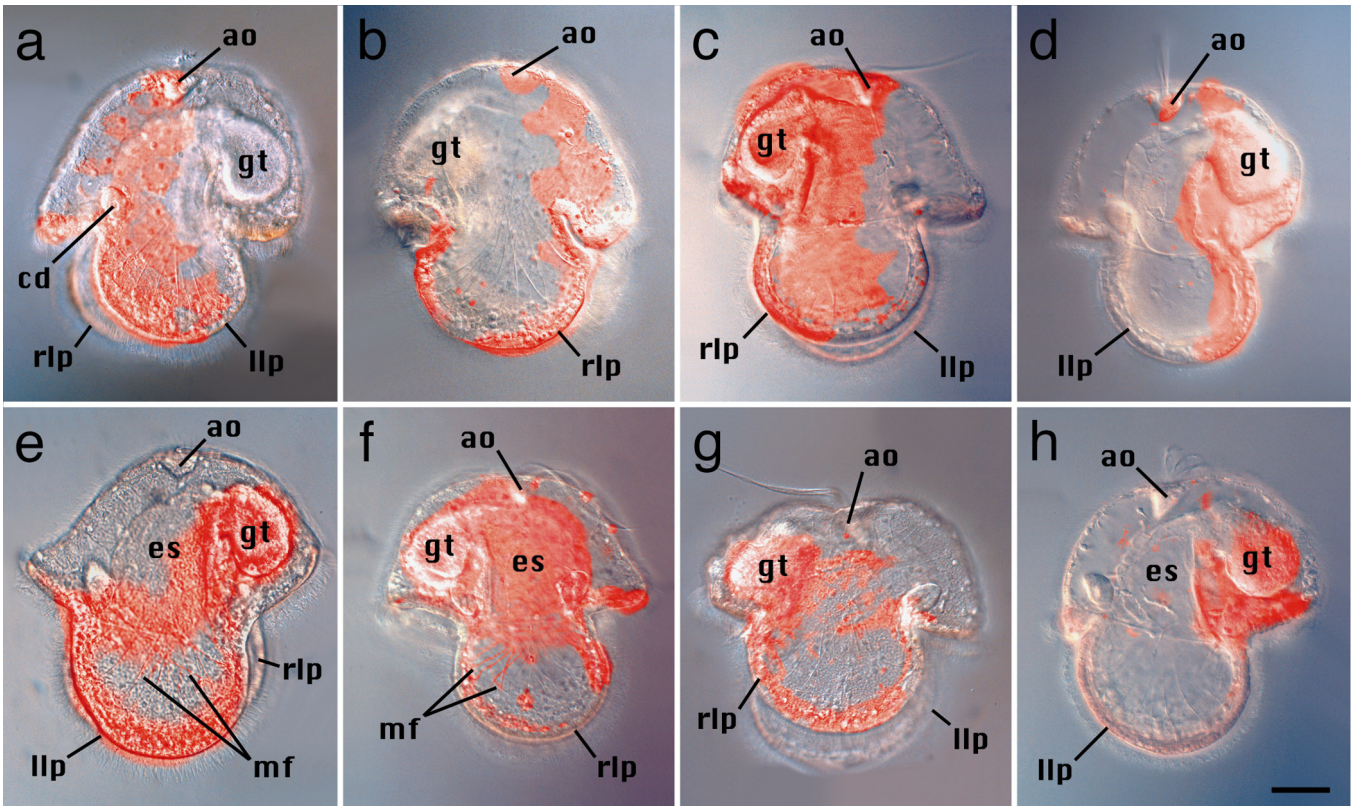

FIG. 5. Superimposed DIC and fluorescence photomicrographs depicting the larval progeny of individual blastomeres from eight-celled embryos of *C. lacteus*. In these embryos four different micromere and four different macromere patterns were observed, which represent subsets of each of the four cell quadrants. All larvae are oriented with the apical ends facing upward. Images in a, d, e, and h are left lateral views, while those shown in b, c, f, and g are right lateral views. While most of the labeled progeny can be visualized in these images, some may not be visible due to the single plane of focus. (a) Example in which the first quartet derivative of A quadrant (1a) had been labeled. Note the patch of labeled left–ventral ectoderm. One-quarter of the apical organ also contains labeled dextran. The label is restricted to ectodermal progeny located on the outer surface of the larva, the apical organ, the left cephalic disc, and a portion of the ciliated band to the ventral edge of the right lappet. No labeled mesodermal, esophageal, or endodermal cells are present. (b) Example in which the first quartet micromere of the B quadrant (1b) had been injected. The labeling pattern is very similar to that seen in a, except the domain of labeled surface ectoderm is smaller. Note that a small band of labeled cells also extends dorsally along the ciliated band of the right lappet. This micromere also gives rise to the right cephalic disc. (c) Example in which the first-quartet micromere of the C quadrant micromere (1c) was labeled. Note the large size of the labeled domain of surface ectoderm. This micromere also contributes to the formation of the apical organ. (d) Example of a larva in which the first quartet micromere of the D quadrant (1d) had been injected. A patch of labeled left–dorsal surface ectoderm is present, which extends superiorly to the apical organ. No labeled muscle fiber cells or other mesenchyme cells can be seen within the larva. There is no label contained in either the esophagus or the gut. (e) Case resulting from an embryo in which the 1A macromere had been injected. Labeled cells are found on the inner surface of the left lappet and in the ciliated band. Note the presence of labeled muscle fibers extending into the left lappet. Only a portion of the labeled muscle fibers appear to be labeled due to the plane of focus. Label is also located in the esophagus and the gut. (f) Larva derived from an embryo in which the 1B macromere had been injected. Note the presence of labeled muscle fibers. Due to the plane of focus not all of the muscle fibers derived from this quadrant appear to be labeled. Label is present in the cells located on the inner surface of the lappet though they are not clearly seen in this photo. These cells tend to be weakly labeled due to the greater degree of stretching that takes place in these cells. A large area of the esophagus and part of the gut are also labeled. (g) Example illustrating the progeny of the 1C macromere. Cells located in the ciliated band, on the inner surface of the right lappet, the esophagus, and in the gut are labeled. The cells located on the inner surface of the right lappet are more faintly labeled, however, one can see many of their labeled nuclei. There are no labeled muscle fibers. (h) Case resulting from the injection of the 1D macromere. A small region of dorsal ectoderm is labeled, as well as the dorsal portion of the esophagus and the gut. Labeled ectoderm on the inner surface of the left lappet is not clearly visible in this photo due to the deeper plan of focus used to reveal a number of internal labeled mesenchyme cells (however, see Fig. 6h). There are no labeled muscle fibers. (ao) apical organ, (rlp) right lappet, (llp) left lappet, (gt) gut, (mf) muscle fibers, (es) esophagus, (cd) cephalic imaginal disc. Scale bar, 50 μm.

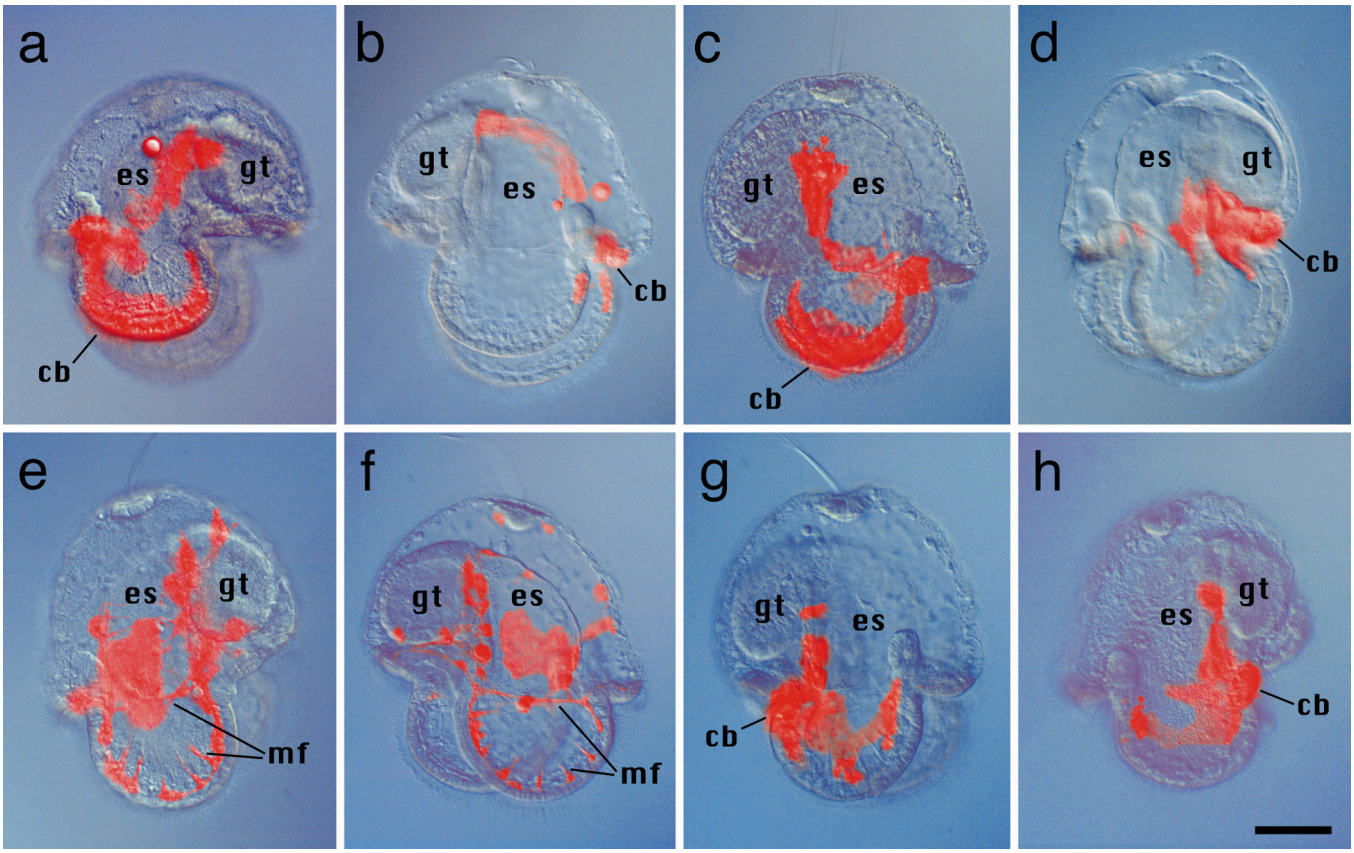

FIG. 6. Superimposed DIC and fluorescence photomicrographs depicting the larval progeny of the second and third quartet micromeres of *C. lacteus*. The different micromere patterns are subsets of each of the four macromere patterns shown in Figs. 5e–5h. All larvae are oriented with the apical ends facing upward. Images in a, d, e, and h are left lateral views, while those shown in b, c, f, and g are right lateral views. (a) Example in which the second-quartet derivative of A quadrant (2a) had been labeled. Part of the ciliated band associated with the left lappet, and the inner surface ectoderm of the left lappet and the esophagus are labeled. (b) Example in which 2b had been injected. The ventral portion of the ciliated band located in the ventral fore lobe is labeled. Tiny ventral portions of the ciliated band extending into both the left and right lappets, and the ventral edge of the esophagus are also labeled. (c) Example in which 2c had been injected. Note that the labeling pattern is bilaterally symmetrical to that seen for the 2a micromere shown in a. (d) Labeling pattern of the 2d micromere. This dorsal labeling pattern is nearly symmetrical to the ventral pattern exhibited by 2b shown in b. Dorsal ectoderm, and the part of the ciliated band located in the dorsal hind lobe are labeled. (e) Example of the 3a labeling pattern. A patch of ectoderm located on the left side of the esophagus is labeled. A number of mesenchyme cells and muscle fibers are also labeled. (f) Example of the 3b labeling pattern. Note that this pattern shows good bilateral symmetry to that exhibited by the 3a micromere as shown in (e). g). Example of the 3c pattern. A small dorsal portion of the ciliated band located in the right lappet is labeled as is ectoderm located on the inner surface of the lappet which extends into the esophagus. (h) Example of the 3d pattern. Note that this pattern shows good bilateral symmetry to that exhibited by the 3c micromere shown in g. (cb) ciliated band, other labels are the same as those used in Fig. 5. Scale bar, 50 μm.

patterns were observed. These cells contributed only to the formation of the endoderm. Embryos were carefully followed for a period of several hours following fourth quartet formation, but no additional micromere quartets were observed to have formed despite the claims of Wilson (1900).

Large numbers of each of the second and third quartet macromeres were also injected at earlier stages of development. Their labeling patterns were perfectly consistent with all the lineage assignments described above. For the sake of brevity, these results are not being shown here.

The Formation of the Larval Nervous System

Labeled components of what must represent the larval nervous system could be observed in the embryos injected with DiI. The distribution and morphology of these labeled cells appear to be identical to those described by Laccalli and West (1985), which were identified on the basis of electron microscopic reconstructions. A composite diagram of these fiber tracts, and one example are shown in Fig. 8. Labeled neurons have been clearly detected in the progeny

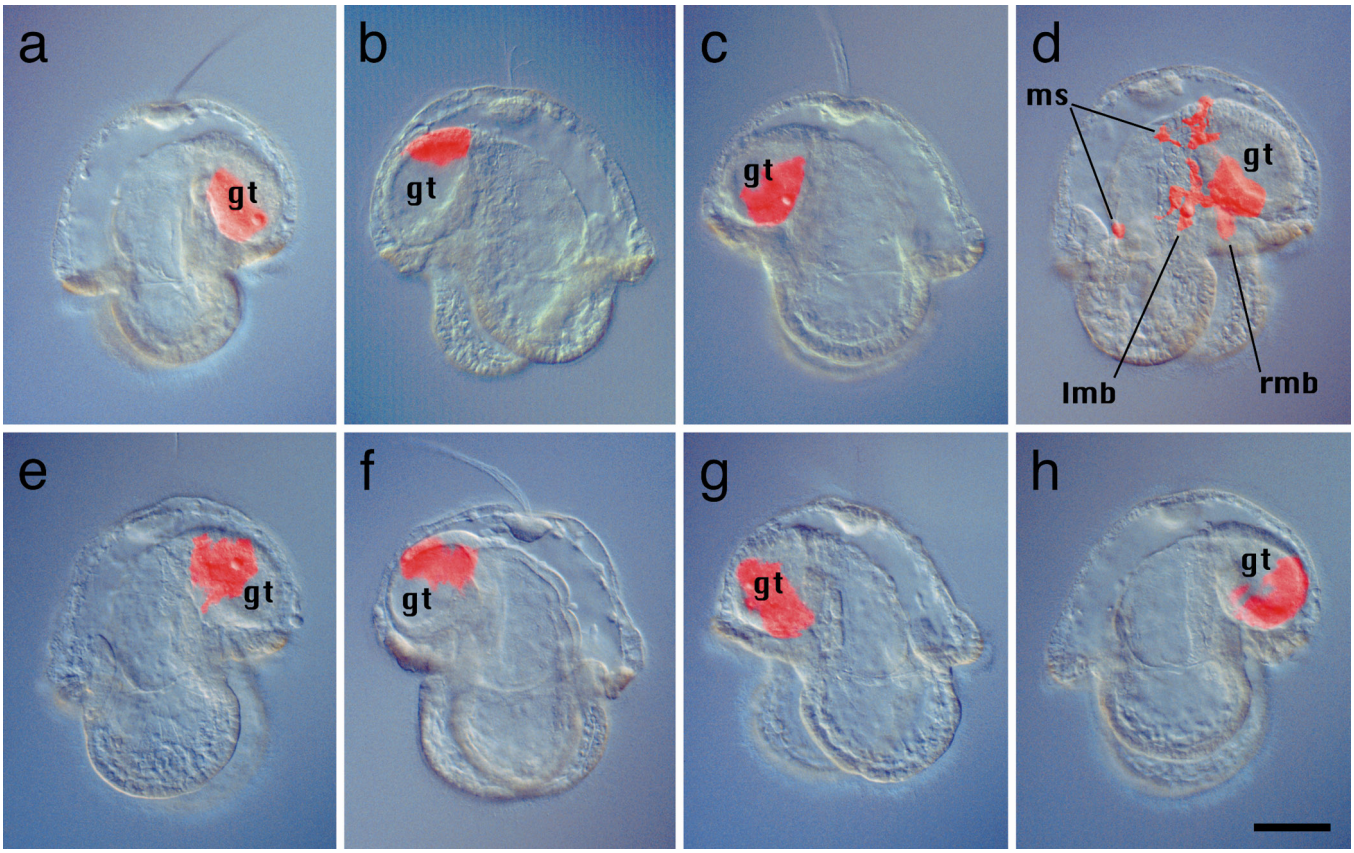

FIG. 7. Superimposed DIC and fluorescence photomicrographs depicting the larval progeny of the fourth quartet micromeres and macromeres. All larvae are oriented with the apical ends facing upward. Images in a, d, e, and h are left lateral views, while those shown in b, c, f, and g are right lateral views. (a) Example in which the 4a micromere had been injected. Label is located only in the left–inferior side of the gut. (b) Example of the 4b labeling pattern. Label is located only in the superior (apical) surface of the gut. (c) Example of the 4c pattern. Label is situated in the right–inferior portion of the gut. (d) Example of the 4d labeling pattern. Notice that label is found in the inferior endoderm, as well as in a number of mesenchyme cells, and in two loosely organized mesodermal bandlets. These bandlets each contain approximately 6–8 cells and are situated in a bilaterally symmetrical fashion within a plane that includes the junction between the esophagus and gut. (e) 4A macromere pattern. Label is restricted to the left side of the gut. (f) 4B pattern. Label is restricted to the right–superior side of the gut. g). 4C pattern. Label is found in the right side of the gut. h). 4D pattern. label is restricted to the dorsal–inferior region of the gut. (lmb, rmb) left and right mesodermal bandlets, (ms) mesenchyme, other labels are the same as those used in Fig. 5. Scale bar, 50 μm.

derived from the 1c, 1d, 2a, 2c, 2d, 3c, and 3d micromeres. To a large extent, these fiber tracts parallel the larger caliber muscle fibers present in the pilidium larva.

DISCUSSION

The Nemertean Fate Map

Hörstadius (1937) was the first to perform cell labeling experiments in nemertean embryos. His limited observations, based on the use of Nile Blue sulfate, suggested that there was no consistent relationship between the first cleavage plane and the plane of bilateral symmetry. He also claimed that all four cell quadrants made equal contributions to the formation of the pilidium during development in *C. lacteus*. It is now clear that these conclusions are incorrect. Instead, we have found that quadrant identities are organized in a highly reproducible and discrete fashion. In previous studies we referred to these quadrants as the LV, RV, RD, and LD quadrants. These assignments were based on observations of the surface ectodermal labeling patterns resulting from embryos injected at the two- and four-cell stages. As discussed below, the results presented here show that these quadrants are, in fact, similar to the A, B, C, and D, cell quadrants found in other spiralians. Hörstadius (1937) also reported on the general fates of the four tiers of cells present in the 16-celled embryo of *C. lacteus*. According to Hörstadius, the animal-most tier of four cells (actually the animal progeny of the first quartet of micromeres,

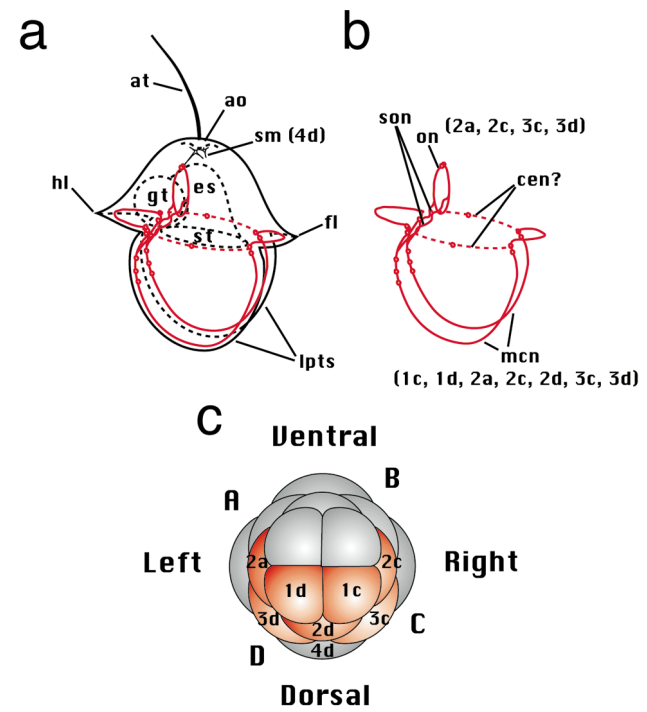

FIG. 8. Diagram illustrating the larval nervous system and the embryonic cells that contribute to its formation, based on observations of labeled embryos examined in this study. (a) Nervous system shown both within the pilidium larva, and removed from the larva for greater clarity (b). Various components of the nervous system are labeled following the terminology used by Schmidt (1937) and Lacalli and West (1985): on, oral nerve tract; son, suboral nerve tracts; mcn, marginal ciliary nerve tracts; sm, stellate mesenchyme observed in the present study which often runs between the apical organ and cell bodies located in the oral nerve tract. Collections of cell bodies (loose ganglia) are also shown associated with the various nerve tracts. In some preparations, putative nerve fibers were seen to lie to the left and right sides of the stomodeum. These nerves were not described by Lacalli and West (1985). Since their existence in all cases is somewhat tenuous, they have been illustrated here with dotted lines and are labeled as "circumesophageal nerves" (cen). (See text for further details.) (c) Simplified diagram showing micromeres that contribute to the formation of the larval nervous system. Note that these cells are located toward the dorsal side of the embryo. (d). Example showing labeled marginal ciliary nerves derived from the 2d blastomere. While these nerves are labeled on both the left and right sides of the larva, only those on the far left side of the larva are visible due to the plane of focus. (e). Example showing labeled stellate mesenchyme derived from 4d that extend between the apical organ and unlabeled cell bodies of neurons located in the oral fiber tract. It is doubtful that these mesenchymal cells are neuronal in function. Labels are the same as those used in Figs. 5 and 7. Scale bar, 50 μm.

equal to $1a^1$, $1b^1$, $1c^1$, $1d^1$) gives rise to the apical organ and tuft, as well as a portion of the external ectoderm. The vegetal progeny of the first quartet ($1a^2$, $1b^2$, $1c^2$, $1d^2$) gives rise to external ectoderm and to a portion of the ciliated band. The second quartet micromeres (2a, 2b, 2c, and 2d) give rise to a portion of the ciliated band, the ectoderm on the inner surface of the lappets, the esophagus, and to a portion of the gut. According to Hörstadius (1937) the fourth tier, the vegetal macromeres (2A, 2B, 2C, and 2D), gives rise primarily to the endoderm. While these rudimentary findings are fairly consistent with ours, they fall short of defining the specific contributions of these blastomeres, and Hörstadius was unable to ascertain the exact origins of the ciliated band, the nervous system, and the mesoderm in nemerteans.

Embryonic Axial Properties and Quadrant Identities

Earlier, we argued that the first and second cleavage planes are shifted toward the median and frontal planes in nemerteans compared to the condition present in other spiralians (Henry and Martindale, 1994a), and assigned nemertean quadrant identities as LV, RV, RD, and LD. The more extensive cell lineage analyses described here have, in fact, allowed us to equate the four quadrants of nemerteans to the ancestral quadrant system seen in the majority of other spiralians (Henry *et al.*, 1995; Boyer *et al.*, 1996). The most compelling features that allow us to make these assignments are the alternating placements of the micromere quartets and the presence of a single endomesodermal precursor cell represented as the fourth-quartet micromere of the dorsal quadrant (4d). Thus, the former LV, RV, RD, and LD quadrants of *C. lacteus* are actually homologous to the typical A, B, C, and D spiralian quadrants, respectively.

A characteristic feature of the spiralian cleavage program is the alternation in the orientation of cleavage spindles that give rise to successive micromere quartets. In dexiotropically cleaving spiralians the first and third quartets of micromeres are shifted toward the median plane, while second and fourth quartets assume interradial positions. Much discussion of this point was raised by early investigators, including Wilson (1892), Lillie (1895), Mead (1897), Conklin (1897), and Treadwell (1901). Since then, many investigators have oversimpli-

fied the axial relationships of the four spiralian quadrants and often refer to the A, B, C, and D quadrants as generating left, ventral, right, and dorsal fates (see Fig. 2b). Our lineage analysis has shown that virtually all of the outer-surface ectoderm of the pilidium larva, including the left and right lappets, is derived from the first quartet of micromeres. These cells are larger than the vegetal macromeres at the eight-cell stage, and they are generated in a dexiotropic fashion. As one views the extensive ectodermal domains generated by these four micromeres, the planes separating the four ectodermal domains (e.g., the first and second cleavage planes) are closely aligned along the plane of bilateral symmetry and the frontal plane. On the basis of preliminary studies in which we injected lineage tracers at the two- and four-cell stages, we concluded that the first and second cleavage planes correspond closely to these two planes. These relationships, however, do not reflect the orientations of the successive micromere quartets. In fact, observations of the labeled domains derived from the second quartet of micromeres (2a, 2b, 2c, 2d) indicate that their progeny are shifted in the opposite (counterclockwise) direction and occupy, left, ventral, right, and dorsal positions, respectively. These two sets of relationships alternate between each of the successive quartets of micromeres which are produced. The alterations in the orientation of the sequential micromere quartets must be considered when identifying the orientation of the early cleavage planes with respect to the larval and/or adult axes (see Fig. 2c). In fact, the distribution of the various micromere quartet derivatives in *C. lacteus* appears to be typical for most spiralians (e.g., *Physa,* see Wierzejski, 1905; *Patella,* see Damen and Dictus, 1994; *Dentalium,* see Van Dongen and Geilenkirchen, 1974; *Lymnaea,* see Verdonk, 1965; *Polygordius* by Woltereck, 1904; Hoploplana, Boyer *et al.,* 1998; see also Henry and Martindale, 1998). Likewise, in the leech *Helobdella* the progeny of the first quartet also comes to lie such that the a′ and d′ micromere clones are separated from the b′ and c′ micromere clones by the median plane (Nardelli-Haefliger and Shankland, 1993), though they subsequently undergo cell rearrangements, which reverses their positions along the dorsoventral axis. A few exceptions to these general relationships have been noted. For instance, both the first- and second-quartet derivatives appear to occupy primarily left, right, dorsal, and ventral positions within the head of the mud snail, *Illyanassa obsoleta* (Render, 1991, 1997).

While a thorough cell lineage analysis has not yet been completed for the direct-developing nemertean, *N. bivittata,* preliminary findings revealed that this species also displays similar axial relationships relative to the first and second cleavage divisions (Henry and Martindale, 1994a). Like *Cerebratulus,* the majority of the ectoderm is derived from the large first-quartet micromeres. Thus, the four quadrants in *N. bivittata* appear to contribute to the formation of the ectoderm in a bilaterally symmetrical fashion. While there is some similarity between these two species, we do not know how the four cell quadrants contribute to other cell fates, such as adult mesoderm, in *N. bivittata.* A significant difference between the two species relates to the fact that *C. lacteus* displays indirect development, while *N. bivittata* displays direct development. Thus, in one case we are considering quadrant identities and axial relationships with respect to the feeding larva, while in the other we are considering adult relationships. It will be interesting to complete a cell lineage analysis of *N. bivittata* to determine whether it exhibits further differences that could be related to its mode of direct development, which have been described for direct-developing vs indirect-developing echinoids (Wray and Raff, 1989, 1990; Henry and Raff, 1990).

The Ciliated Band

Some speculations have been made with regard to the relationship between the ciliated band of the pilidium larva, and similar ciliated structures present in the larvae of other spiralians (Wilmer, 1990). For instance, trochophore larvae possess a prominent prototrochal ciliated band as well as other ciliated bands (e.g., metatroch, telotroch). Typically, as is the case in the mollusc *Patella vulgata,* the prototroch of the trochophore larva is derived from first quartet (1a, 1b, 1c, 1d) and second quartet (2a, 2b, 2c) derivatives. Whether these structures are homologous is unclear because the ciliated band in the pilidium larva is derived from a large number of cells in addition to the first quartet 1a–1d, including, 2a–2d, 3c, and 3d. van den Biggelaar *et al.* (1997) argued that the trochoblasts of nemerteans are not homologous with those of annelids and molluscs on the basis of trochoblast-specific β-tubulin expression. An alternative view posits that these structures are homologous, and that nemertean embryos either recruited third quartet cells to participate in the formation of the ciliated band or else some cell lineages have lost the ability to make "trochal" cilia in other spiralians. A viable homology argument, however, will require additional information describing differences in the origins of ciliated bands among other spiralian larvae.

The Nervous System

The cells that give rise to the ciliated band also appear to contribute to the formation of the larval nervous system, though not all cells that form the ciliated band generate neurons. The shared origins of these structures have been noted in a number of different larvae, and are characteristic of what Jägersten (1972) refers to as "primary" or "primitive" larvae (see also Lacalli, 1982; Lacalli and West, 1985). The larval nervous system runs in close proximity with the ciliated band and encircles the esophagus at its junction with the gut. Lacalli and

West (1985) claim that the nervous system of the pilidium larva consists of approximately 12 neurons. While we did not manage to count the exact number of neurons, it is likely to be somewhat larger than that number, since some blastomeres appear to generate a number of neurons (see Fig. 8d). Because of the extensive contributions of 3a and 3b to the formation of muscle fibers, we could not discern whether these cells also contribute to the formation of neurons within the larva. Lacalli and West (1985) reported that there are no neurons that make connections with the apical organ, which is consistent with our observations. It is possible, however, that neurons are concealed in definitive (contractile) muscle fibers that extend from the lappets and cephalic discs to the apical organ. However, the absence of any definitive neuronal connections with the apical organ supports the argument that this structure does not serve a sensory role, but rather operates as a steering mechanism (see Lacalli and West, 1985).

The observation that the blastomeres giving rise to the larval nervous system in *C. lacteus* arise from cells that originally occupied dorsolateral positions within the embryo (1c, 1d, 2a, 2c, 2d, 3c, and 3d, see Fig. 8) leads to some interesting speculation. Such an arrangement may suggest that these cells are determined via centralized inductive interactions originating from either the D or the C and D quadrants early during development. As mentioned earlier, there is evidence suggesting that both the C and D quadrants possess inductive effects in nemerteans (Martindale and Henry, 1995).

The Origins of the Mesoderm in Nemerteans

In virtually all mollusc and annelid embryos examined, mesoderm is generated by the progeny of various second and/or third quartet micromeres, the so-called "ectomesoderm", and from the fourth quartet micromere from the dorsal (D) quadrant, the so-called 4d "mesentoblast" (Verdonk and van den Biggelaar, 1983). The literature is filled with varied reports regarding the origins of the mesoderm in the nemerteans; and it is possible that this process may differ depending on the species being examined (see discussions by Hörstadius, 1937; Hyman, 1951; Iwata, 1957; Cantell, 1989; Henry and Martindale, 1994a,b, 1996a). Some investigators claimed that the mesoderm may arise solely as ectomesoderm (Lebedinsky, 1897; Wilson, 1900; Iwata, 1957). Wilson (1900) recorded that a pair of mesoderm progenitor cells enter the blastocoel at the vegetal pole during blastula stages in *C. lacteus,* and was under the impression that these cells had different origins. He claimed that these cells give rise to mesenchyme that lines the blastocoel and eventually contribute to the formation of the musculature of the pilidium larva. Nusbaum and Oxner (1913) claimed that the mesoderm in the heteronemertean *Lineus ruber* arises from the 4d micromere. Our cell lineage results, in fact, indicate that mesoderm arises from both ectomesodermal and endomesodermal sources, as it does in other spiralians. The third quartet micromeres of the A and B cell quadrants (3a, 3b) behave as ectomesodermal progenitors and generate the extensive array of muscle fibers found in the larva of *C. lacteus*. In addition, endomesoderm is generated by the fourth-quartet micromere of the D cell quadrant (4d). These findings provide compelling evidence that the early cleavage program of *C. lacteus* is homologous with that of other Spiralia.

Origins of the Adult Body Plan

In heteronemerteans that form pilidium larvae, the juvenile worm forms from a series of ectodermal imaginal discs over a prolonged period of time (Metschnikoff, 1869; Selensky, 1886, 1912). These include three pairs of bilaterally disposed discs and a single dorsal disc. The imaginal discs contribute to the formation of the adult ectoderm, the proboscis, and the nervous system. The left and right cephalic imaginal disks appear to be formed by la, and lb, respectively; however, we have not yet extended our observations to follow the lengthy development of the juvenile worm to ascertain the exact origins of the other imaginal discs or specific adult structures (however, recall discussion on mesoderm formation above). Both the larva and the adult share the same planes of bilateral symmetry; however, the anterior–posterior and dorsoventral axes are shifted to different degrees depending on the species (Cantell, 1989). In *C. lacteus,* these axes are rotated by approximately 90°, such that the dorsoventral axis of the adult worm is orthogonal to the dorsoventral axis of the larva (the dorsal side of the larva is related to the posterior end of the adult worm, and the apical, anterior end of the larva is related to the dorsal side of the adult worm, see Fig. 1).

The literature reports that "larval" mesenchyme cells represent the source of the adult mesoderm; however, our observations have not been extended long enough to substantiate this claim (see Cantell, 1989; Henry and Martindale, 1997, for reviews). The loose mesenchyme contained in the pilidium larva is derived from 3a, 3b, and 4d, while the mesodermal bandlets are derived from 4d. There is no indication that the endomesoderm plays a role in larval development. In other spiralians, the mesodermal bandlets play a significant role in the generation of adult mesoderm. Thus, it is conceivable that the adult mesoderm in *C. lacteus* is derived from a dormant population of endomesodermal "set aside cells" (Davidson *et al.,* 1995; Peterson *et al.,* 1997).

The adult nemertean nervous system consists of dorsal and ventral ganglia connected to prominent paired lateral nerve cords. There are also mid-dorsal, and mid-ventral cords in some species. In heteronemerteans the adult brain and nervous system are said to arise from paired cerebral imaginal discs which arise in a bilaterally sym-

metrical fashion near the larval/adult stomodeum (in a prospective adult ventrolateral position in *C. lacteus*). We do not know which blastomeres generate these adult structures. It will be interesting to ascertain the origins of the adult nervous system in *C. lacteus,* and in direct-developing species, such as *N. bivittata,* where this may be more readily accomplished. Understanding the exact origins of both the larval and adult nervous system will be of supreme importance for testing arguments regarding the origins and modifications of the dorsoventral axis during metazoan evolution (Arendt and Nübler-Jung, 1997). Given the possibility that larval and adult nervous systems may arise from different populations of cells, possibly from diametrically opposed locations within the embryo, it might be interesting to speculate on the significance of this relative to the postulated inversion of the dorsoventral axis that may have occurred within the metazoan taxa (Holly *et al.*, 1995; Ferguson, 1996; De-Robertis and Sasai, 1996)

The Evolution of the Spiralian Developmental Program

When one considers the diverse array of larval and adult body plans that are exhibited by the various spiralian phyla, it seems remarkable that these differences are not established earlier during development. How then did such a highly conserved developmental platform allow for the evolution of such tremendous diversity? Obviously, one will have to examine later stages of development, and study the deployment of genes involved in these processes.

While there is a tremendous degree of conservation in the spiralian developmental program, a number of interesting changes have occurred during the course of evolution. Specific changes have occurred in the relative contributions of various micromere quartets to the formation of certain larval structures, such as the larval ectoderm and ciliated band, in the allocation of cells that contribute to ectomesoderm formation (Boyer *et al.*, 1996), and also in the mechanisms employed to specify quadrant fates and axial properties (see Martindale and Henry, 1995; Henry and Martindale, 1996b).

The tremendous size of the first quartet of micromeres in *C. lacteus* is unusual compared to most other spiralians. Altering the size of the various micromere quartets may represent a mechanism for the diversification of the spiralian cleavage program. In this fashion, specific axial relationships can be selected or altered by biasing contributions from specific micromere quartets. Sipunculid embryos also posses very large first-quartet micromeres (specifically 1a, 1b, and 1c), and it will be interesting to determine how this increased cell size changes their relative contributions to the larval/adult body plans in this group.

In an earlier paper (Martindale and Henry, 1995), we proposed that differences in the spiralian fate map might be generated by shifting the relationships between early cleavage planes and the various embryonic axes, such as the dorsoventral axis. Such changes have occurred during the evolution of the echinoids (Henry *et al.*, 1992); however, it does not appear that this mechanism has been deployed in nemerteans (or other spiralians) even though the potential for exploiting these changes can be demonstrated experimentally (Henry and Martindale, 1996b). Rather, in many spiralians (excluding cephalopods) changes in early development appear to occur in the context of the highly conserved cleavage program, which includes changes in cell size as mentioned above (see also van den Biggelaar and Guerrier, 1983; Lillie, 1895) and in the recruitment or loss of lineages that generate specific structures, such as the ectomesoderm and the ciliated band. This is different from the changes seen in other taxa. For example, the marine nematode *Enoplus brevis* (Voronov and Panchin, 1997) and some echinoids (Raff, 1996) exhibit highly modified cleavage programs, yet their adult body plans are relatively unchanged. In the case of the spiralians it appears that many of the evolutionary modifications which have defined or refined their different larval and adult body plans have occurred during the later stages of development.

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