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Cell lineage in marine nematode *Enoplus brevis*

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SUMMARY

Early cleavages of the marine nematode *Enoplus brevis* are symmetrical and occur in synchrony. At the 2- to 16-cell stages, blastomeres are indistinguishable. The progeny of blastomeres was investigated by intracellular injections of fluorescent dyes and horse radish peroxidase. One blastomere of the 2-cell embryo gives rise to a compact group of cells occupying about half of an embryo. The border between labeled and unlabeled cells differs in each embryo dividing it to anterior-posterior, left-right or intermediate parts. At the 8-cell stage, one blastomere gives rise to only endoderm, whereas the other blastomeres produce progeny that form multiple cell types, including nerve, muscle and hypoderm cells, in various proportions. Thus the fates of the blastomeres of early *E. brevis* embryos, with the exception of the endoderm precursor, are not determined. The process of gastrulation in *E. brevis* is very similar to that in *Caenorhabditis elegans* and other nematodes. At the beginning of gastrulation, the 2-celled endoderm precursor lies on the surface of embryo and then sinks inwards. After labeling of cells on the ventral side (near endoderm precursor) at the beginning of gastrulation, their progeny differentiate predominantly into body muscles or pharyngeal cells of the first stage larva. Cells that are located more laterally give rise mainly to neurons. The dorsal blastomeres differentiated principally into hypoderm cells. Our study suggests that a precise cell lineage is not a necessary attribute of nematode development.

Key words: nematodes, *Enoplus brevis*, *Caenorhabditis elegans*, cell lineage, cleavage, gastrulation

INTRODUCTION

The phylum Nematoda has traditionally been divided into two classes: the Adenophorea and the Secernentea (Chitwood and Chitwood, 1974). Our understanding of nematode development is based primarily on the work that has been done on *Caenorhabditis elegans* from the class Secernentea. *C. elegans* is the first (and only) animal for which the complete cell lineage from zygote to adult has been followed (Sulston et al., 1983; Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble and Hirsh, 1979; Wood, 1988). The lineage in *C. elegans* is uniform during normal development and the initial experimental work suggested that it could not be changed: when blastomeres were killed, their progeny were missing in the developing embryo and, in most cases, the fates of other blastomeres were not affected (Sulston et al., 1983). It was suggested that the uniform lineage pattern reflects some specific mechanism that is necessary for nematode development. More recent studies demonstrated that, in some circumstances, the lineage pattern can be changed (Priess and Thomson, 1987; Wood, 1991), but it is still not clear how important the lineage pattern is for normal nematode development.

The main approach in investigations of *C. elegans* development is based on genetic and molecular methods (for review see Wood and Edgar, 1994). However, in *C. elegans* almost all mutations concerning the cell lineage disturb the normal patterns of determinant redistribution and cell-to-cell interactions to such an extent that development is arrested. Therefore, the study of the natural diversity of the embryogenesis provided by different nematode species may be very useful in understanding the role of cell lineage in nematode development (Sulston et al., 1983; Skiba and Schierenberg, 1992). For example, the development of members of the order Enoplida is quite different from that in all other nematodes; i.e. the pattern of early cleavage is not consistent, and so it was suggested that cell lineage is not fixed (Cherdantsev et al., 1972; Malakhov and Akimushkina, 1976; Malakhov, 1994), as proved for *Enoplus brevis* by tracing cell fates with intracellular labels (Voronov et al., 1986, Voronov and Panchin, 1995b). We believe that the detailed study of enoplidan development in comparison with *C. elegans* could elucidate the role of cell lineage in nematode development and evolution.

MATERIALS AND METHODS

Free-living marine nematodes *Enoplus brevis* Bastian, 1865 (family Enoplidae, order Enoplida) were collected on the sandy littoral in the vicinity of the Kartesh Marine Biological Station of the Russian Academy Sciences (Kandalaksha Bay, White Sea). Uncleaved eggs

were dissected out of gravid females in filtered sea water. In order to observe both sides of the embryo, in some cases, eggs were placed under the coverslips to which they stuck by their outer mucus sheath. Developing eggs were kept at the room temperature (18-20°C).

Fluorescent dyes were injected in experiments where lineage tracers were visualized in cells of the living developing embryo. Two forms of Lucifer yellow (LY): Lucifer Yellow CH (LYCH), Lucifer yellow VS (LYVS) and fluorescein isothiocyanate-dextran (FD), M_r 17,900 were used. To investigate the distribution of progeny of marked cells in the newly hatched animals (the first stage larvae), horse radish peroxidase (HRP), Type XII was used. All drugs were from Sigma, USA.

Glass microelectrodes used for iontophoresis or pressure injections were made of glass with inner filaments, Clark Electromedical Instruments, GC100F-15, and backfilled with injection solutions.

Single blastomeres were injected using a several seconds pulse of 1-5 nA negative current through 30-50 MegΩ glass microelectrodes filled with 3% LYVS or LYCH solution in distilled water. FD and HRP were injected by pressure. Injections were made under the visual control using a fluorescent microscope (LUMAM I-3, LOMO, Russia) with the filter set optimized for LY or fluorescein. In order to visualize HRP injections, it was mixed with LYCH or FD.

To avoid the staining of sister blastomeres, the injections of labels must be made after the completion of the previous cell division when the midbodies are disrupted. In *E. brevis* and *C. elegans* embryos, low molecular weight dyes can leak into uninjected blastomeres through gap junctions (Voronov et al., 1986; Bossinger and Schierenberg, 1992a, 1996a) and it is known that the low molecular weight luminescent dye LYCH can easily diffuse through gap junctions (Stewart, 1978). In our experiments, LYCH always leaked into uninjected cells after the injection at the 4-cell stage or later. To label blastomeres by LYCH at the 2-cell stage, it was injected at the middle interphase, resulting in binding to the cytoplasm with no leakage from the injected blastomere or its progeny. For the cell tracing experiments, only the embryos without any signs of dye transfer were selected. The binding of LYCH to some components of living cells has been previously described, and can be explained by the presence in LYCH of a free hydrasid group (Spiegel et al., 1983). LYVS binds to cytoplasm quickly (Stewart, 1978) and therefore, in our experiments, after injection at all stages of early cleavage it never leaked into neighboring cells. FD and HRP did not leak through gap junctions due to their high molecular weight (Simpson et al., 1977). LYCH injections were used for labeling 2-celled embryos because a higher percentage of embryos survived (about 50% for LYCH compared to 5-10% for LYVS or FD). For the labeling at the 8-cell stage or later, only LYVS, FD or HRP were used. HRP gives the best resolution in first stage larva as it is not granulated as the fluorescent dyes.

In *C. elegans*, some kinds of tracer molecules can be transferred into uninjected midgut from labeled cells (Bossinger and Schierenberg, 1992b). This phenomenon was never observed in *E. brevis*.

For HRP visualization, first stage larvae were fixed by 2% formaldehyde in a 0.1 M sodium phosphate buffer (PBS), pH 7.4, kept overnight at 4°C in 20% sucrose in PBS and preincubated in 0.05% diaminobenzidine (Sigma) in PBS for 10 minutes. H_2O_2 was added to a final concentration of 0.003-0.01%. When the staining developed (10-20 minutes) larvae were rinsed and cleared in glycerol. As a control for non-specific staining, uninjected larvae were treated by the same protocol.

Drawings were made with a camera lucida. Photomicrographs of embryos labeled by fluorescent dyes were taken on the RF film (Tasma, Russia, about 1000 ASA units) or on high-sensitivity videocamera (DeltaTex, Russia). To avoid damage to embryos and photobleaching of fluorescent labels, the embryos were kept in darkness and photographed only once or twice a day.

RESULTS

General description of *E. brevis* development (see also Malakhov and Akimushkina, 1976)

The zygote diameter in *E. brevis* eggs is 120-150 μm with the shape of the egg shell varying between almost spherical and elongated at a ratio up to 1:3 (Fig. 1). The duration of embryonic development up to the hatching of first stage larva is 16-20 days at 20°C. Development can be divided into two stages: cleavage (about 3 days of development) and morphogenesis. The division of zygote occurs 14-18 hours after egg extraction and the divisions of early cleavage follow at 4-6 hours intervals. The interphase blastomeres are motile; their movements can cause a slight rotation of the embryo relative to the egg shell or a change in contacts between blastomeres.

The furrow of first division is usually perpendicular to the longitudinal egg shell axis (Fig. 1A), resulting in two blastomeres situated as in all other studied nematodes (Fig. 1B). However, sometimes the plane of the first division furrow is not perpendicular to the egg shell axis (Fig. 1C), but, in such cases, the embryo usually rotates within the egg shell until the

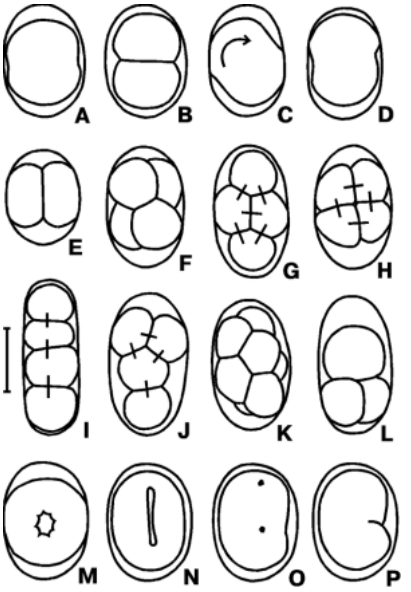

Fig. 1. The general features of *E. brevis* embryonic development. (A-L) The early cleavage. (A,B) Usual sequence of the first division: (A) beginning of furrow formation; (B) 2-cell stage; (C,D) when the first division furrow is not perpendicular to the longitudinal egg shell axis, zygote rotates within the egg shell (the curved arrow in C indicates the direction of the zygote rotation); (E) if the furrow and the egg shell axis are strictly parallel, the two blastomeres are arranged perpendicular to the egg shell axis. (F-J) Blastomeres configurations at the 4-cell stage: tetrahedron (F), rhombus (G), quadrate (H), linear (I) and T-shaped configuration (J). (G-J) The contacts between blastomeres are indicated by the short bars. (K) An example of 8-cell stage; (L) 3-cell-stage embryo arisen as a result of asynchronous division; (M-P) the later stages of development (ventral view, anterior at the top); (M) beginning of the ventral cleft formation; (N) ventral cleft closure; (O) Lima bean stage (mouth and anal opening are depicted as a black spots); (P) comma stage. Scale bar, 100 μm.

normal position of the first two blastomeres is achieved (Fig. 1D). Very rarely, when the furrow and the egg shell axis are strictly parallel, the embryo does not rotate and the first two blastomeres remain perpendicular to the egg shell axis (Fig. 1E).

At the 4-cell stage blastomeres can be arranged in a tetrahedron, rhombus, quadrate, linear or T-shaped configurations, distinguished by topology of their interconnections (Fig. 1F-J). Because of the motility of interphase cells, blastomere configurations can change from one to another.

At the 2- to 16-cell stages, blastomeres are indistinguishable (see, for example, Fig. 1K) being approximately the same size, though sometimes slight irregular differences in their size are visible. Up to the 16-cell stage, the divisions are usually synchronous. However, sporadically before this stage some irregular delays of blastomere divisions can be observed. These delays can last almost half of cell cycle duration, but they do not disturb the normal course of development. We observed such delays at the 2-cell to 4-cell transition (Fig. 1L) in five eggs that demonstrated a clear 3-cell configuration. They were followed up to hatching and all of them produced normal larvae.

During the course of later development (Fig. 1M-P), we can distinguish stages that are similar to those in *C. elegans*. Ventral cleft formation begins at 60 hours of development (Fig. 1M). The ventral cleft is closed in 90-hour old embryos and cleavage is finished at approximately this stage (Fig. 1N). Ventral cleft formation produces the first indication of a longitudinal axis in the embryo though, at this time, we cannot distinguish anterior from posterior. The beginning of the embryo elongation coincides with the ventral cleft development. After the closure of ventral cleft, the mouth and anal openings are formed, and the embryo reaches the Lima bean stage (about 100 hours) (Fig. 1O). The ventral position of the anus is the first visible manifestation of anterior-posterior polarity. Then the rudiment of tail begin to elongate and the embryo achieves the comma stage (120 hours) (Fig. 1P). After elongation of the embryo, cell differentiation and the cuticle formation starts. The first stage larva hatches after 16-20 days of development.

Distribution of descendants of the 2-cell stage blastomeres

Our results are based on the study of 95 embryos in which one blastomere was labeled at the 2-cell stage and the distribution of its progeny was followed at least up to the comma stage (64 embryos were injected by LYCH, 19 by LYVS and 12 by FD).

The distribution of progeny of the first two blastomeres was unique for each studied embryo (Fig. 2). At the Lima bean and comma stages the descendants of the first two blastomeres each formed a rather compact mass. However, some descendants of one of the two blastomeres could be intercalated within the progeny of the other (Fig. 2d,e). Later, when the elongation of embryo begins, additional cell mixing takes place. At the same time, the pattern of label distribution became unclear as the luminescent dyes were sequestered in granules.

At the Lima bean and comma stages, the border between descendants of the first two blastomeres varied from transverse to longitudinal (Fig. 2). Among 95 studied embryos, a transverse or oblique border was observed in 52 cases, and predominantly longitudinal border in 43 cases (in this classification, we considered not only the distribution of label on the surface but also within the embryo). After injection of LY or FD at the 2-cell stage, the midgut can be easily recognized in the Lima-bean- and comma-stage embryos: it was seen as a bright (if it was labeled) or dark (if it was unlabeled) axial mass beneath the dorsal surface in the posterior half of embryo (Fig. 2c,d,e). Only one of first two blastomeres forms the midgut (for additional evidences see below). Thus, in different eggs of *E. brevis*, the first two blastomeres give rise to the different parts of the embryo ranging from anterior-posterior to left-right distribution with a variety of intermediate patterns. Both blastomeres contribute to all embryonic layers, with exception of endoderm, which derives entirely from one of the two blastomeres.

The usual rule characteristic of other nematodes such that one of the first two blastomeres gives rise predominantly to either the anterior or posterior regions of the embryo and larva

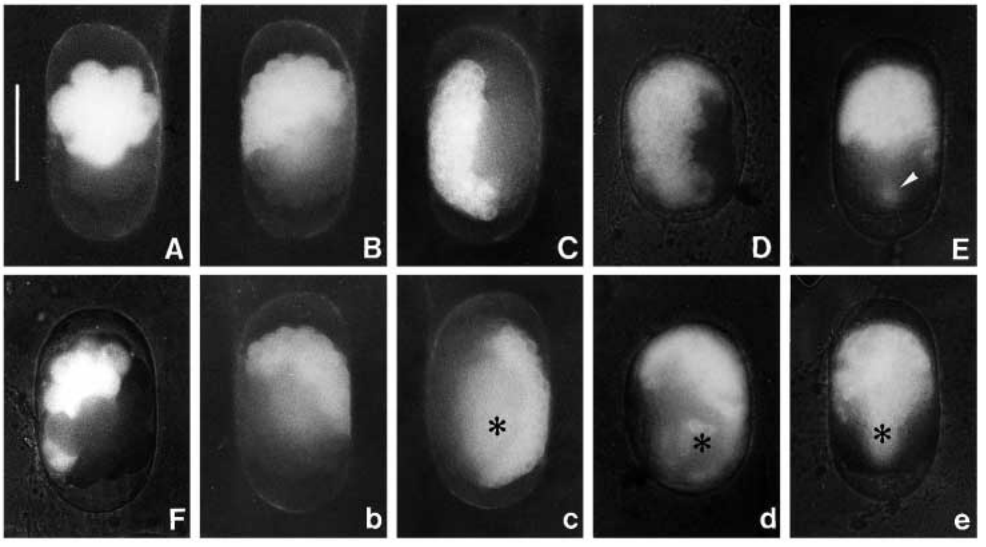

Fig. 2. The progeny distribution of blastomere labeled at the 2-cell stage with LYCH. Anterior at the top, capital letters, ventral view; lowercase letters, dorsal view. Initial position of injected blastomere at the 2-cell stage was at the top. (A-C,b,c) The same embryo: (A) 16-cell stage; (B,b) ventral cleft formation and embryo elongation; (C,c) Lima bean stage. Embryo rotated between stages B,b and C,c, which led to the longitudinal border between descendants of first two blastomeres. (D,d and E,e) Different embryos at the Lima bean stage: (D,d) the oblique border between descendants of 2-cell stage blastomeres; (E,e) the transverse border. (C,c and E,e) The midgut was labeled (c,e, asterisk); (D,d) the midgut was not stained and it was seen as a dark mass under the dorsal surface (d, asterisk). Arrowhead in E, the fluorescence of midgut in the anal opening region. F, a 30-cell-stage embryo, an example of significant rotation at the early cleavage due to interphase motility of blastomeres. Scale bar, 100 μm.

does not apply to *E. brevis* development. Only in about 50% of embryos can we roughly consider the 2-cell stage blastomeres as posterior and anterior. In the 52 embryos with anterior-posterior distribution of first two blastomeres descendants, the midgut precursor was derived from anterior blastomere in 38 cases and from posterior blastomere in 14 cases (Fig. 2).

The first two blastomeres are usually positioned along the egg shell axis. The anterior-posterior axis of the embryo also coincides with the longitudinal egg-shell axis. An example of the translocation of labeled cells during the course of embryogenesis in an embryo with a lateral distribution of the first two blastomeres progeny is shown in Fig. 2A-C,b,c. From the 2-cell stage up to the ventral cleft formation stage, the progeny of the first two blastomeres were positioned along the egg shell (Fig. 2A,B,b). Later, the elongation of the embryo results in embryo rotation within the egg shell and finally the label is distributed perpendicular to the longitudinal egg shell axis (Fig. 2C,c). In some cases, a significant rotation of the embryo occurred during early cleavage due to the interphase motility of blastomeres (Fig. 2F).

Relative to the egg shell, the pattern of dye distribution was determined by two processes, namely, the motility of the early blastomeres (Fig. 2F) and the rotation of embryo during the ventral cleft stage. During the course of early cleavage, the border between labeled and unlabeled material was usually more or less perpendicular to the longitudinal egg shell axis (Fig. 2A). After early cleavage and up to beginning of embryo elongation, the pattern of label distribution relative to the egg shell did not change significantly (Fig. 2B,b). The elongation of embryo started simultaneously with the formation of ventral cleft. The longitudinal axis of embryo initially could be directed along an arbitrary angle relative to the direction of egg shell axis. If the longitudinal axes of embryo and egg shell were not parallel, then the embryo rotated within the egg shell until these axes were coincident (Fig. 2C,c).

Distribution of descendants of the 8-cell-stage blastomeres

The fates of the descendants of the 8-cell-stage blastomeres were studied in the first stage larva after the HRP injections (34 experiments) and showed a wide range of different patterns. In four larvae, the label was detected only in the midgut (Fig. 3A). In all the other animals, the midgut cells were not labeled. Thus, in *E. brevis*, the whole endoderm arises from the single 8-cell-stage precursor (endoderm blastomere).

The progeny of the non-endoderm 8-cell-stage blastomeres was detected in various positions in different tissues in different larvae (Fig. 3B-E). The labeling pattern was unique for all these larvae. Table 1 represents the distribution of the labeled cells in different regions and tissues in 30 larvae after HRP injection of the 8-cell-stage non-endoderm blastomeres. Our data give no evidence for the existence of any blastomere with a fixed lineage fate other than the endodermal blastomere. Even the most similarly stained larvae displayed essential differences and, on the contrary, staining of the same body parts could be found in animals with considerably different labeling patterns.

The temporal pattern of development after the labeling at the 8-cell stage was studied with the LYVS or FD injections (69 eggs). In 12 of such embryos, the label was found only in the endoderm precursor. These embryos were especially useful to follow the gastrulation process (Fig. 4A-F). During the transition from 8- to 16-cell stage, the endoderm blastomere usually divides synchronously with the other blastomeres (Fig. 4A), but later the divisions of endoderm blastomeres are always delayed. At the 30-cell stage, the embryo is organized as a compact cellular mass (morula) with the 2-celled endoderm precursor always lying on the surface of embryo, indicating the future ventral side. Relative to the egg shell, the position of the endoderm precursor is arbitrary: it can be situated near the equator (Fig. 4B) or the pole (not shown). This stage is the onset of gastrulation when the 2-celled endoderm precursor begins to sink into the embryo. The endoderm precursors at this stage are the only visually identifiable cells of the early embryo.

Soon the endoderm primordium (at the 100- to 150-cell

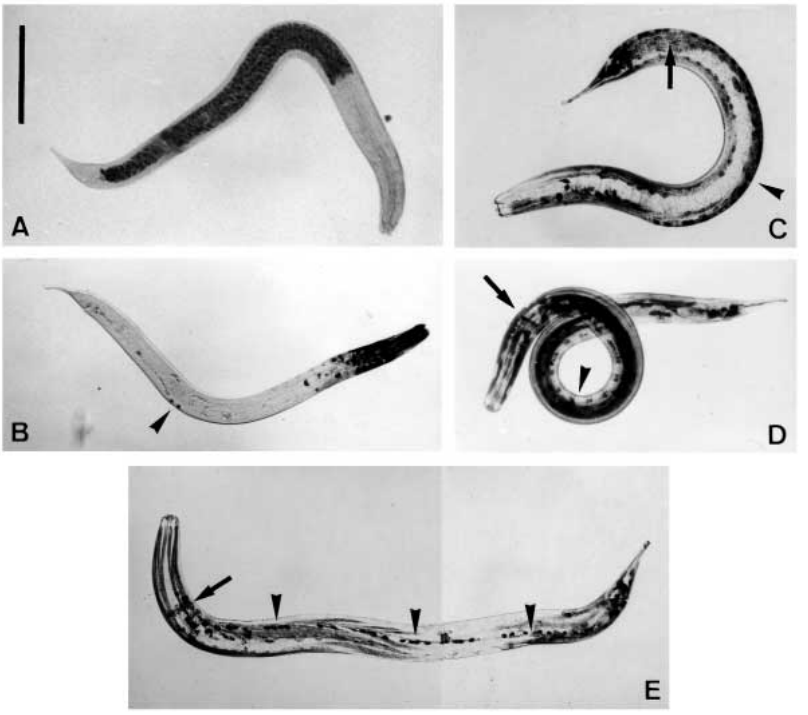

Fig. 3. The progeny distribution of the blastomere labeled with HRP at the 8-cell stage; HRP was developed in the first stage larvae. (A) Midgut only is labeled; (B) anterior body structures (neurons, hypoderm and muscle cells) and 2 neurons of ventral nerve cord (arrowhead) are labeled; (C) left body muscles, neurons in the ventral cord and around the nerve ring, several pharyngeal cells, and hypoderm at the posterior body part (arrow) are labeled; (D) the label is located in the hypoderm and neurons at different positions. (C,D) The ventral side is indicated by arrowhead. (E) The label is located in head and body muscles, in neurons around the nerve ring, along the ventral nerve cord (arrowheads) and the tail region, and in posterior hypoderm. (D,E) The labeled nerve ring is indicated by an arrow. Larvae in B-E are presented in Table 1 by numbers 1,2,3,4, correspondingly. Scale bar, 200 μm.

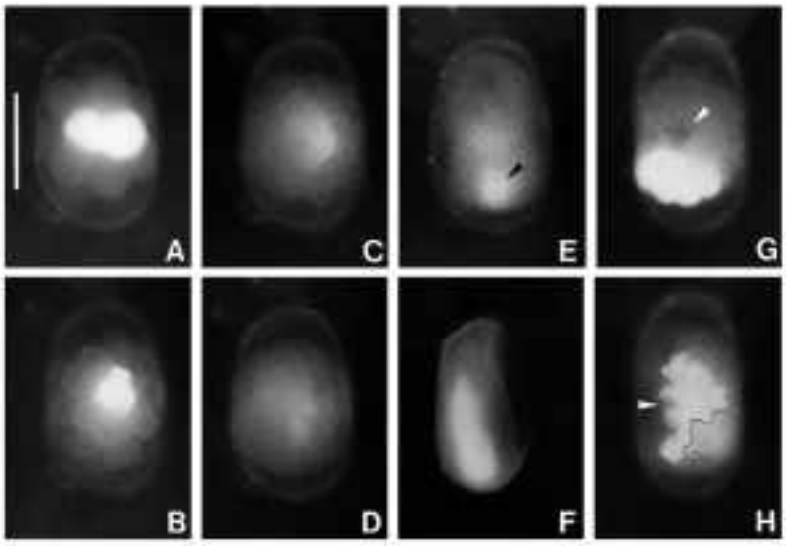

Fig. 4. The gastrulation in the *E. brevis* embryo. LYVS was injected in one blastomere at the 8-cell stage. (A-F) Label redistribution in the same embryo after the injection into the endoderm blastomere; ventral view, anterior at the top. (A) 16-cell stage, 2-celled endoderm precursor is situated at the surface of embryo. (B) 30-cell stage, the beginning of gastrulation, 2-celled endoderm precursor is still visible at the surface of embryo. (C) The stage of approximately 60 blastomeres, the endoderm precursor is almost completely submerged. (D) The endoderm precursor is submerged and blastopore is completely closed (approximately 60 hours after beginning of development). (E) Comma stage: the luminescence of labeled midgut is seen through the surface cells in the posterior body region (arrowhead). (F) The same embryo as E after clearing in methylsalicylate: the fluorescence of the whole midgut is clearly visible. (G,H) An example of the label redistribution after the injection into a non-endoderm blastomere. (G) The beginning of ventral cleft (arrowhead) formation. (H) Lima bean stage; anterior at the top: ventral cleft (arrowhead) is directed along the egg shell, the label is contained in surface and inner cells of the embryo; labeled cells inside the embryo are seen to the right of the dotted line. Scale bar, 100 μm.

Table 1. The distribution of the labeled cells in different regions and tissues in 30 larvae after the HRP-injection of the 8-cell-stage non-endoderm blastomere

N	Body muscles									Pharynx		Neurons				Hypoderm				
	A	Medial				Posterior				A	P	A	P	VC	T	A	Medial		Posterior	
		L		R		L		R									L	R	L	R
		D	V	D	V	D	V	D	V											
1										+++	+	+++	++	+		+++				
2	+	+++	+++			+++	+++			+		+	+	+	++				+	
3												+	+	+	+	+++	+++	+++	++	+
4	+++		+	+	++	+	+	+	+			+	+	++	++				+	++
5				+++	+++			++	++				+	+	+	+		++		+++
6		+	+		++		+		+	+	++	+	+		+	+				
7	+		++		+		+		+	+	++	+	+	+	+			+	+	+
8			+				+			+	++	+	++	++	++	+		+		
9	+			+	+				+	+	++	+	+	+		+	+			
10				++	+++			+++	+++		+	+	+	+	+					
11						+++	+++	+++	+++					+	+		+		++	+
12	+									++	+	++	+	+	+	++	+			
13			+	+	++		+	+	++	+	+	+	+	+	++					
14			+++		+++					+	++	+	+			+				
15	+		+		+		+		+	+	+	++	+	++	++	+	+		+	
16												++	++	+	+	++	+++	++		
17	+									+	+	++	++			++	+	+++	+	+
18	+	+++	+++			+++	+++		+		+	+	+	+	+				+	
19	+									+++	+	++	+	+	+	+	+	+	+	
20	++									++	++	++	+	+	+	+	++		+	
21	++		+		+					++	++	++	++	+		+				
22	+		+				++		+	+		+	+	+	+	+				
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25	++				+				+	+	+++	+	++	+	+	+		+		+
26	+	+++	++		+	+++	++		+				+	+	++		+		+	+
27										++	+	+++	+++	+		+	+	+		
28						+	++	+	++					++	+++			+	++	++
29		+	++			++	+++	+	+								+		++	++
30		+	+							+	+	+	+	+	+	++	++	++	+	

The presence of the label in the body muscles, pharynx, neurons and hypoderm is shown (the germ cells are not indicated as we were not able to reliably detect them). N, the number of the larva. Body muscles: A, anterior to the nerve ring; the rest of the animal was divided into two halves, Medial and Posterior, which were consequently subdivided into L, left; R, right; then D, dorsal; V, ventral regions. Pharynx was divided into two parts: anterior to the nerve ring (A) and posterior (P). Neurons are indicated in four regions: around the pharynx anterior (A) and posterior (P) to the nerve ring, in the ventral cord (VC) and near the anus and in the tail (T). Hypoderm: as for body muscles. Empty cells, no label in the region; +, less then 25% of cells labeled; ++, 25-50% of cells labeled; +++, over 50% labeled.

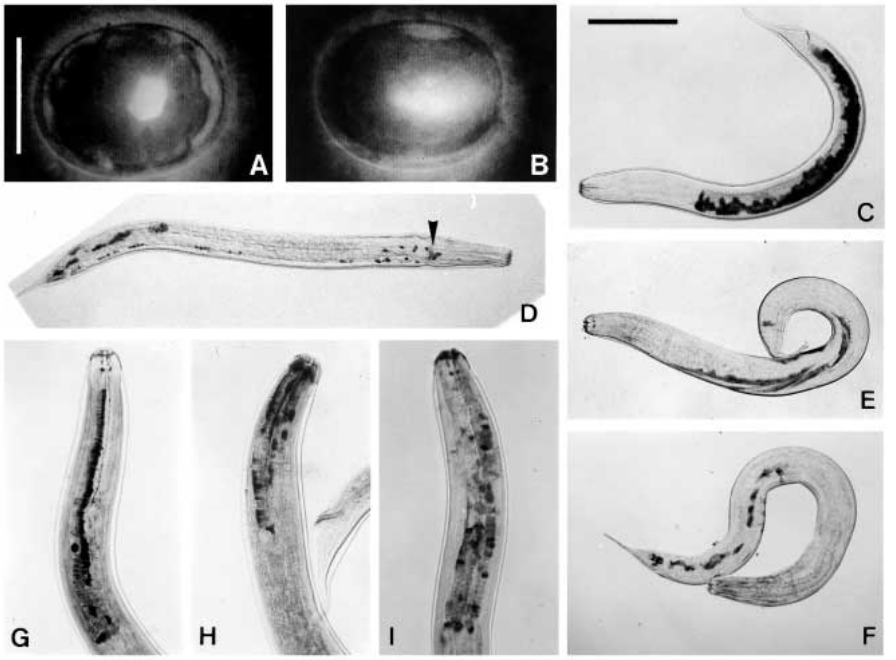

Fig. 5. The labeling of *E. brevis* embryo at the 30- to 60-cell stages. (A-C) The labeling of one cell of the 2-celled endoderm precursor by the FITC-dextran-horse radish peroxidase mixture. (A) The embryo immediately after the labeling at the 30-cell stage; ventral view, anterior to the left. (B) The same embryo at the comma stage; dorsal view, anterior to the left, the luminescence of labeled midgut is seen through the surface cells in the posterior half of the body. (C) The horse radish peroxidase staining in the first stage larva hatched from the same embryo. Approximately one half of the endoderm material is stained. Note the longitudinal distribution of the labeled cells. (D-I) The fates of non-endoderm blastomeres of the 30- to 60-cell-stage embryo in the first stage larva, horse radish peroxidase staining. (D,F) Hypoderm cells are labeled. (D) Lateral view, anterior to the right: neurons around the nerve ring (arrowhead), in the ventral cord and the large hypoderm cells of the posterior part of the larva are labeled. (E) Muscles cells are labeled. (F) Hypoderm cells in the posterior part of the embryo are labeled. (G-I) Pharyngeal cells of different types (muscles, neurons and glandular cells) are labeled: (G) the label is distributed along the pharynx; (H) the label is contained in the anterior part of the pharynx; (I) an example of diffuse distribution of labeled cells. Scale bar in A is 100 μm for A,B; scale bar in C is 200 μm for C-F and 100 μm for G-I.

stage) is covered by other cells (Fig. 4C,D), some of which also migrate into the embryo and form the ventral cleft. This process of cell migration was clearly seen in some embryos with label in cells that did not form endoderm (Fig. 4G,H). Gastrulation occurred through the ventral cleft. No significant migration was observed in other parts of the embryo.

Labeling at the later stages

Additional information concerning the gastrulation process and the fates of blastomeres was obtained by labeling with a HRP-FD mixture at the 30- to 60-cell-stage embryos (31 experiments). FD fluorescence gave us an opportunity to determine the position of the labeled cell descendants relative to the endoderm precursor.

In four eggs, 1 cell of the 2-celled endoderm precursor was successfully labeled. In all larvae hatched from these embryos HRP staining has shown that the labeled cells were distributed along the midgut as a continuous group (Fig. 5A-C). The fate of other blastomeres that did not form endoderm was correlated with their position in the early embryo which was examined at the 30- to 60-cell stage and at the beginning of ventral cleft formation. The blastomeres located near the endoderm precursor gave rise predominantly to muscle and pharyngeal cells (Fig. 5E,G-I). The blastomeres located on the opposite (dorsal) side of the embryo produced mainly hypoderm cells (Fig. 5F). Neurons were often stained along with muscles or hypoderm cells in the same preparation (Fig. 5D). Usually the muscles and the hypoderm were not stained in the same larva. However, if the labeled cells were situated at the head or tail regions, simultaneous staining of hypoderm and muscle or pharyngeal cells was often observed. The labeling pattern in the pharynx did not demonstrate any order: the stained cells could lie as a compact mass or diffusely, and they could be placed along the pharynx or be concentrated in its restricted parts (Fig. 5G-I).

DISCUSSION

In this study, we have demonstrated that an intracellular labeling technique that has already been used in leech (Weisblat et al., 1978), frog (Jacobson and Hirose, 1978) and fish (Kimmel and Warga, 1986) can be successfully applied to the study of the cell lineage of the marine nematode *E. brevis*. It has given us an opportunity to compare the general features of the *E. brevis* cell lineage with the cell lineage of *C. elegans*.

The embryonic cell lineage in *C. elegans* is stereotypical. From the very beginning of development, the blastomeres of *C. elegans* display a regular and reproducible differences in size, position and spatial-temporal pattern of divisions and produce predetermined sets of differentiated descendants (Sulston et al., 1983). Many features of *C. elegans* development are determined by intrinsic factors. The precise cleavage pattern is produced by special mechanisms controlling the movement and positioning of the mitotic apparatus (Hyman and White, 1987). The timing of cleavage is determined by the distribution of some cytoplasmic factor (Schierenberg and Wood, 1985).

In contrast to *C. elegans*, the cell lineage in *E. brevis* is not fixed. At the 2- to 8-cell stage, blastomeres are indistinguishable by their size, position and cleavage pattern (Cherdantsev et al., 1972; Malakhov and Akimushkina, 1976; Malakhov, 1994; Voronov and Panchin, 1995a). Our labeling experiments confirm that blastomeres at these stages have no regular cell lineage pattern. The only exception is the endoderm precursor which segregates from the other

blastomeres at the 8-cell stage. In *E. brevis*, a cleavage pattern when all the blastomeres are the same size is correlated with a changeable pattern of cell contacts. As the fates of blastomeres can not be mapped, we have not denominated designation for them (letters, numbers, etc.) as is usual for nematodes with a constant cell lineage. In fact, we were able to name only the endoderm precursor. Midgut development is probably the most conservative feature in nematodes. In all well-studied species (Sulston et al., 1983; Skiba and Schierenberg, 1992), the entire endoderm derives from the single blastomere at the 8-cell stage.

In *C. elegans* and other related nematodes the two cells of the endoderm precursor give rise to the anterior and posterior halves of midgut. For the *E. brevis* embryo, such a distribution is not observed. In all our experiments, the border between descendants of two endoderm cells divided the midgut more or less longitudinally.

In *C. elegans* and related species, the endoderm precursor derives from the posterior blastomere of the 2-cell stage (Sulston et al., 1983; Skiba and Schierenberg, 1992) while, for representatives of the order Mononchida and some related orders, it has been suggested that the endoderm is produced by the anterior blastomere (Drozdovskii, 1969, 1975; Malakhov, 1994). In *E. brevis*, the first two blastomeres produce different parts of the animal in different embryos, and often cannot be designated as ‘posterior’ or ‘anterior’. The midgut could be produced by the blastomeres that presumably give rise to anterior parts of the body or by blastomeres that give rise mainly to posterior parts. The nature of the positive correlation between the production of anterior parts of the body and the midgut shown in our experiments needs additional study.

As in the midgut, in the pharynx of *C. elegans* two compartments can also be distinguished. The anterior and posterior parts of pharynx are composed of the descendants of AB and MS founder cells, respectively (Sulston et al., 1983). For *E. brevis*, no evidence for such a compartmentalization was found.

In general, the process of gastrulation in *E. brevis* is very similar to that in *C. elegans* and other nematodes (Sulston et al., 1983; Skiba and Schierenberg, 1992). In all species, gastrulation starts after the 24-30 blastomeres stage. At the beginning of gastrulation, the 2-celled endoderm precursor always lies on the surface of embryo and then sinks inwards. The distribution of the primordia that produced specific cell types at this stage in *E. brevis* is also similar to other species. The progeny of ventral cells (near endoderm precursor) labelled at the beginning of gastrulation differentiate predominantly into body muscles or pharyngeal cells of the first stage larva. Cells located more laterally give rise mainly to neurons. The dorsal blastomeres differentiated principally into hypoderm cells. Thus, although the pattern of early development leading to the arrangement of cells at the beginning of gastrulation is substantially different in *E. brevis* and *C. elegans*, the prospective values of blastomeres at this stage appears to be quite similar. From this stage on, all the morphogenetic processes, ventral cleft formation, its subsequent closure, mouth and anus formation and embryo elongation etc. are almost identical.

Thus, the later stages of development in *C. elegans* and *E. brevis* seems to be much more similar then early development. The main difference in early stages is that the fates of *C. elegans* blastomeres are predictable while the fates of *E. brevis* blastomeres appear to be variable. However, in special experimental conditions some plasticity of *C. elegans* development could be observed. The mechanical shift of some blastomeres may result in a dramatic change of their fates (Priess and Thomson, 1987; Wood, 1991). It is possible that the differences in the early development between *C. elegans* and *E. brevis* can be attributed to delay in the timing of many regional specification events in *E. brevis* versus *C. elegans*. Several types of experiments revealed high potential for developmental regulation in *E. brevis*: (1) altering the normal blastomere configuration by squashing or stretching embryo, (2) temporary separation of blastomeres with a glass fiber, (3) removing one blastomere of 4-cell embryo or one or two blastomeres of 8-cell-stage embryo. In all these experiments, embryos were able to produce normal first stage larvae (Voronov and Panchin, 1995a and Voronov and Panchin, unpublished data).

All the nematodes are morphologically similar and present a homogenous taxonomic group (Chitwood and Chitwood, 1974). Although *E. brevis* is considered to be taxonomically very distant from *C. elegans*, it displays many similarities, especially in the first stage larvae (Voronov et al., 1989; Voronov and Nezlin, 1994). The *E. brevis* larva is about 1-1.3 mm long and contains about 1500 cells. Their hypoderm is cellular (not syncytial) and cells are arranged in highly regular pattern. Although the total hypoderm cell number is variable (it contains between 150 and 170 cells), the cell composition of anterior and posterior body regions is constant. The comparison of *E. brevis* and *C. elegans* makes it clear that the general hypoderm structure is very similar in these two species, and it is easy to suggest an exact homology of all nuclei rows and even individual cells. We have shown that the *E. brevis* first stage larva has a strictly constant array of sensory organs, some of which are the same as in *C. elegans*. The same is also true for catecholamine neurons. A one-to-one correspondence could be found for many individual cells of two species.

C. elegans has a constant cell composition that arises as a result of precise and invariant embryonic cell lineage. The first stage larva of *E. brevis* also has a very regular structure, displays a considerable invariance in its cell composition and in many features is very similar to *C. elegans* but its early development is variable. Thus, in nematodes, very similar final products may emerge as a result of different types of development.

The constant cell lineage in *C. elegans* implied that it reflects an important feature of the developmental mechanism in nematodes. The *E. brevis* study suggest that precise cell lineage is not that important. Recent studies in *C. elegans* development have also established an important role for intercellular communications (Priess and Thomson, 1987; Schierenberg, 1987; Wood, 1991; Goldstein, 1992, 1995a,b; Hutter and Schnabel, 1994, 1995a,b; Bossinger and Schierenberg, 1996a,b) and indicate that nematode embryonic development is less stereotyped then it appeared initially.

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