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Authors: Shimotori, Taishin, and Goto, Taichiro

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Establishment of Axial Properties in the Arrow Worm Embryo, *Paraspadella gotoi* (Chaetognatha): Developmental Fate of the First Two Blastomeres

Taishin Shimotori[1,2] and Taichiro Goto[1]*

[1]*Department of Biology, Faculty of Education, Mie University, Tsu, Mie 514-8507, Japan and*
[2]*Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan*

ABSTRACT—Chaetognaths are bilateral animals totally symmetrical on both sides of their body. To elucidate the manner in which cell fates are established, single blastomeres of the two-cell stage embryos of the benthic arrow worm, *Paraspadella gotoi*, were injected with a fluorescent lineage-tracing dye. The distribution of labels was observed in the hatchlings by using a confocal laser scanning microscope. A total of four different labeling patterns was observed: 1) the dorsal epidermis, the right half of the ventral epidermis, and the right half of the dorsal longitudinal muscles (DR pattern); 2) the dorsal epidermis, the left half of the ventral epidermis, and the left half of the dorsal longitudinal muscles (DL pattern); 3) the right half of the ventral epidermis, the ventral longitudinal muscles, the right half of the dorsal body muscles, and the primordial germ cells (PGCs) (VR pattern); and 4) the left half of the ventral epidermis, the ventral longitudinal muscles, the left half of the dorsal body muscles, and the PGCs (VL pattern). Thus, one blastomere of the two-cell stage largely contributes the dorsal epidermis and the other contributes the ventral longitudinal muscles and the PGCs. Bilateral halves of the dorsal longitudinal muscles and the ventral epidermis were labeled as complementary pairs. These labeling patterns indicate that the first cleavage plane runs oblique to the bilateral and dorsoventral axes. In addition, the occurrence of complementary "DL and VR" and "DR and VL" labeling patterns indicates that the first cleavage plane bears one of two different angular relationships relative to the future body axes.

INTRODUCTION

The Chaetognatha, commonly called arrow worm, is a phylum of bilaterally symmetrical marine carnivores. The embryology, especially the coelom formation, in chaetognaths resembles the enterocoel of the deuterostomes, particularly that of brachiopods, which is the reason so many authors have placed chaetognaths in the Deuterostomia. However, a more specific position or sister group has never been pointed out, and most other characters point to protostomian relationships (cf. Nielsen, 1995).

The cleavage pattern of the zygotes of chaetognaths is total and equal. Elpatiewsky (1909) first described the germinal determinant in the periphery of zygotes near the vegetal pole. The germinal determinant is contained in one of the blastomeres at the two-cell stage, which is a mixed blastomere giving rise to both somatic and germ cells. The other blastomere without the germinal determinant is purely somatic. The germinal determinant remains undivided only in the mixed blastomere from which new somatic blastomeres continue to separate. Elpatiewsky (1909) called the blastomere containing the germinal determinant at the four-cell stage "d", and followed its lineage until the gastrula. The cell containing the germinal determinant at further stages he named d1 at the 8-cell stage, d^{11} at the 16-cell stage, and d^{111} at the 32-cell stage. After the fifth division, the two cells formed from the mixed blastomere are clearly determined: one (d^{111}, also called a "G" cell at this stage), containing the still undivided germinal determinant, is the primordial germ cell (PGC), and the other (d^{112}, also called an "E" cell at this stage) will become the primitive endodermal cell. The G cell does not divide at the next stage forming the 63-cell stage. During the next division, when the PGC divides for the first time, the germinal determinant contained in it also distributes itself into the two daughter-cells. The two PGCs produced at the 126-cell stage do not divide but become attached to the wall of the archenteron in the young gastrula. Four PGCs are visible in a later stage of the gastrula and do not divide further during the subsequent phases of embryonic development. At hatching, two of the PGCs are

* Corresponding author: Tel. +81-592-231-9260;
FAX. +81-592-231-9352.

situated in front of the caudal septum from which they will give rise to the oogonia; the other two, situated behind the septum, will form the male germ line (Hertwig, 1881; Doncaster, 1902; Burfield, 1927; Ghirardelli, 1968). In this way the "d" cell at the four-cell stage, which is a vegetal quadrant containing the germinal determinant, gives rise to the PGCs and endodermal cells.

Previous studies in experimental embryology supporting these observations have major limitations. Ghirardelli (1954) conducted a cell-deletion experiment at the two-cell stage in *Spadella cephaloptera*. When the blastomere containing the germinal determinant was destroyed with a fine glass needle, the remaining blastomere continued to develop into a gastrula without the PGCs. His study is important to show that each blastomere has the same developmental potential except for the production of the PGCs.

In order to resolve the origin of the organ-forming regions of embryos, and to determine the relationship of the first cleavage plane and the future body axes in chaetognaths, we have labeled one of the blastomeres of the two-cell stage and traced its fate in the hatchlings in the present study. The development of chaetognaths is direct. Hatchlings have the same body parts as adults, although they are much simpler than adults in morphological construction. By this experiment, it is also possible to determine when the dorsoventral and/or bilateral axes and attendant cellular patterns are first specified.

MATERIALS AND METHODS

Animals

Specimens of *Paraspadella gotoi* (Casanova, 1990) were originally collected from tide pools near the Amakusa Marine Biological Station of Kyushu University, Kumamoto, Japan in April 1996. Methods for the laboratory culture of this species are described in Goto and Yoshida (1997). They were kept in a constant temperature chamber at 17°C with a 12:12-hr LD cycle. Water used for culture and experiments in the present study was artificial sea water (Marine Art SF, Senju Pharmaceutical Co.). Ten to twenty mature individuals were placed into each plastic vessel (15 cm diameter, 8 cm depth). Sea water was changed at least once a week.

Gamete and embryo preparation

Chaetognath fertilization occurs internally after mating. Mature specimens kept in a mass culture develop to contain fertilized eggs, which are the first meiotic metaphase and remain in the oviducal complex for a few days after ovulation in this species (cf., Goto, 1999). Thus, oocytes after germinal vesicle breakdown (GVBD oocytes) are already fertilized and do not develop further until being laid. The egg stages are easily identified through the body which is relatively transparent. The GVBD oocytes were isolated in sea water by decapitation (about 20 oocytes per one specimen), and were placed in a constant temperature chamber at 17°C until the two-cell stage to prepare for microinjection.

Observation of the germinal determinant was done in the two-cell stage embryos, which were fixed with 4% paraformaldehyde in sea water, rinsed with phosphate buffered saline (PBS), and stained with 0.001% toluidine blue dissolved in PBS.

Lineage-tracing techniques

In order to label cells at the two-cell stage, Fluoro-Ruby (10,000 MW rhodamine-conjugated dextran, Molecular Probes) was injected into the cell. It was diluted to a 4% solution in Ca^{2+}-free 50% sea water and filtered with a Millipore filter (0.45 μm). Before injection, the embryos were put in hypertonic sea water (120%) to prevent cytoplasm leaking after injection of the dye. Then they were held in an egg holder which created a gap (about 200 μm in thickness) between two coverslips to immobilize the eggs. One of the two blastomeres which had completed first cleavage was injected by air-pressure using an Eppendolf microinjection system. Injection was carried out under an epifluorescent microscope (Zeiss Axiovert 135 M) to monitor the injected dye which spread evenly within the injected blastomere in about a minute. The injected embryos were put in Millipore-filtered sea water containing antibiotics (penicillin and streptomycin) and incubated at 17°C until hatching. Juveniles typically hatched in 5 days at this temperature. The hatchlings were fixed with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.4 M sucrose for 1 hr and kept in the same buffer. They were mounted in a PBS-glycerin mixture (1:1), and observed under a confocal laser scanning microscope (CLSM, Zeiss LSM 410) . A complete Z-series of images (optical sections) through the hatchlings was collected at intervals of 2.5 μm at a section thickness of 2.5 μm using a 40×0.75 NA objective, giving about 20 images in each preparation.

Histology of the hatchling

To determine the structure labeled with the injected dye, the gross anatomy of the hatchlings of *P. gotoi* was examined. Hatchlings were fixed with a mixture of 1% OsO_4 and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.4 M sucrose for 1 hr at 0°C. After dehydration with a graded series of ethanol, they were embedded in epoxy resin and sectioned for light and electron microscopy. EM observations were made with a Hitachi H 700 operated at 100 kV.

RESULTS

Two-cell stage embryo

The zygotes of *P. gotoi* are 270 μm in diameter and are relatively opaque. Naturally laid zygotes are surrounded by a tough vitelline membrane and an egg capsule. The egg capsule is produced during egg laying, while the zygotes isolated by our procedure were without egg capsules. Polar bodies are not visible in living embryos under a light microscope because of their small size and opaque egg cytoplasm. Therefore, the animal and vegetal poles are not clear without staining the nuclei. The first cleavage is equal and occurs about 2 hr after isolation (Fig. 1A). It has been reported that germinal determinant is present near the vegetal pole (Elpatiewsky, 1909; Ghirardelli, 1954, 1968). Although not visible in living

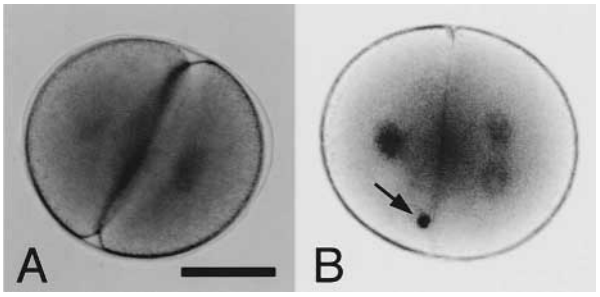

Fig. 1. The two-cell stage embryos of *P. gotoi.* (**A**) A living embryo. (**B**) A fixed embryo stained with toluidine blue. Arrow indicates the germinal determinant. Bar = 100 μm

P. gotoi, the germinal determinant can be made visible by toluidine blue staining (Fig. 1B). Since there was no marker to distinguish the blastomeres in the living two-cell stage embryos, injection of dye into single blastomeres was done without identification.

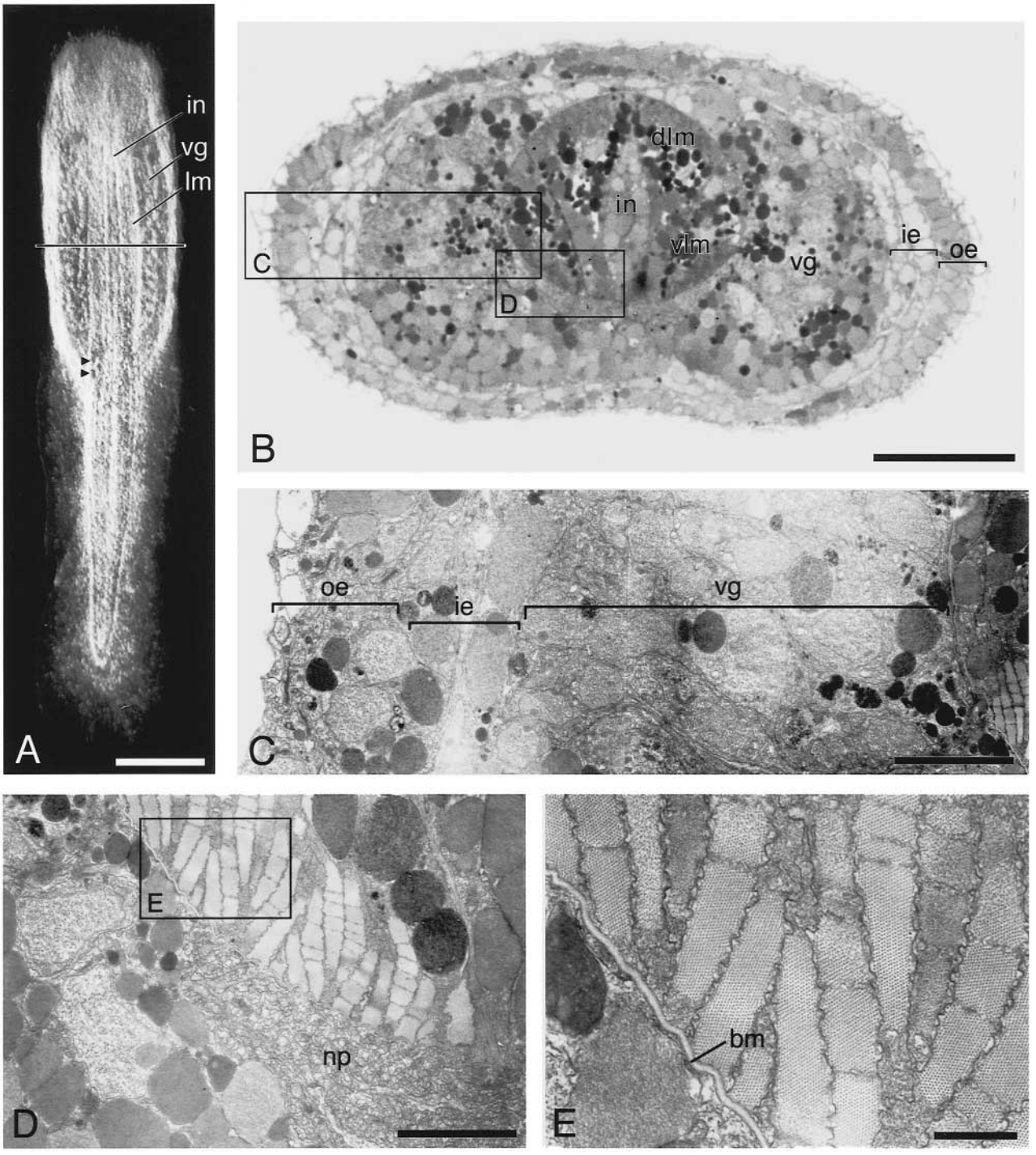

Fig. 2. Morphology of the hatchling. (**A**) Dorsal view of a living specimen. Solid line marks the position of the transverse section in B. Arrowheads indicate the primordial germ cells. (**B**) Light micrograph of a transverse section through the trunk. (**C**) Electron micrograph of the lateral region of the trunk (higher view of the lettered box "**C**" in **B**). (**D**) Electron micrograph of the ventral side of the medial part of the trunk (higher view of the lettered box "**D**" in **B**). (**E**) Electron micrograph of a part of longitudinal muscle (higher view of the lettered box "**E**" in **D**). bm, basement membrane; dlm, dorsal longitudinal muscles; ie, inner epidermis; in, intestine; lm, longitudinal muscles; np, neuropile; oe, outer epidermis; vg, ventral ganglion; vlm, ventral longitudinal muscles. Bars = 100 μm in **A**, 30 μm in **B**, 5 μm in **C**, **D**, 1 μm in **E**.

Morphology of the hatchling

Development of chaetognaths is direct, without larval stage. The general morphology of hatchlings has been reported by Shinn and Roberts (1994). Hatchlings typically lack grasping spines and teeth and have a non-functional intestine. Feeding structures differentiate within several days after hatching.

The hatchling of *P. gotoi* is about 1 mm in body length (Fig. 2A). Gross anatomy of the hatchling was examined by epon sections and optical sections of the dye-injected preparations with the CLSM. A schematic representation of the morphology of the hatchling is shown Fig. 3. In transverse sections, a solid column of mesodermal and endodermal cells occupied the medial part of the trunk (Fig. 2B). There are two layers in the epidermis; outer and inner. This was first described by Duvert *et al.* (1984). The outer layer was stained denser than the inner layer in LM sections stained with toluidine blue (Fig. 2B) and in EM sections (Fig. 2C). These two

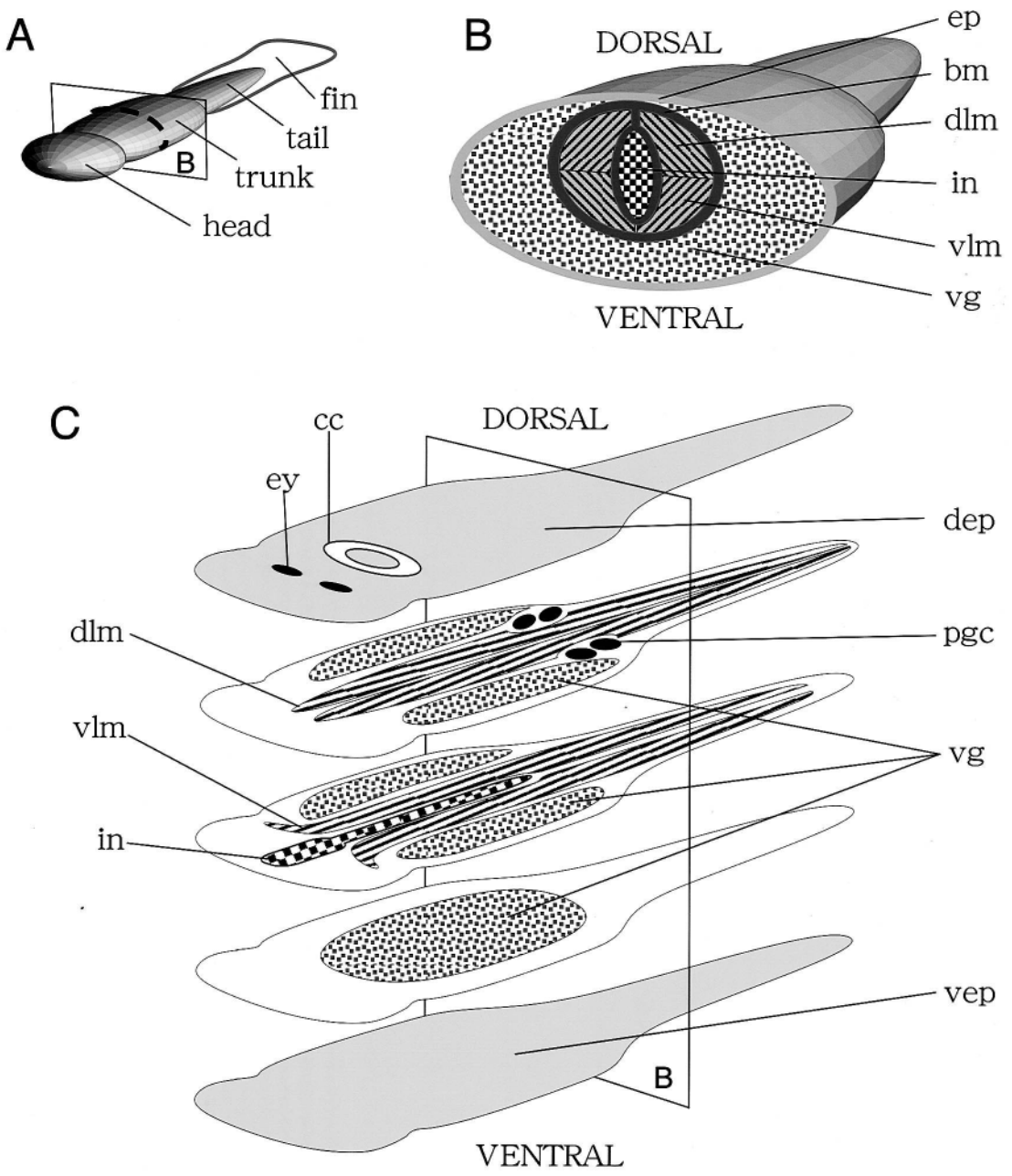

Fig. 3. Diagram of the gross anatomy of the hatchling. (**A**, **B**) 3D diagram of the hatchling. Lettered box "**B**" in **A** is a transverse plane, showing a transverse profile of the trunk (**B**). (**C**) Diagram of a series of frontal sections of the hatchling. bm, basement membrane; cc, corona ciliata, dep, dorsal epidermis; dlm, dorsal longitudinal muscles; ep, epidermis; ey, eyes; in, intestine; pgc, primordial germ cells; vep; ventral epidermis; vg, ventral ganglion; vlm, ventral longitudinal muscles.

layers were also identified by the CLSM as described below. In the present study, only the outer layer is called the epidermis. The body musculature and the intestine were faintly visible near the central axis of the trunk in wholemount preparation (Fig. 2A). The body musculature is composed of quadrants of two bilaterally located longitudinal muscles (Fig. 2B, D, E). The solid column was surrounded dorso-laterally and ventrally by the large ventral ganglion which gives the appearance of a U in transverse sections (Fig. 2B), while the adult ventral ganglion does not extend laterally beyond the ventral surface. The ventral ganglion was composed of mostly somatic regions, and the neuropile region was restricted to a small area just below the solid column (Fig. 2B, D). In a wholemount preparation, both sides of the lateral expanded regions of the trunk comprise the ventral ganglion. Thus, body parts including the epidermis, the ventral ganglion, the longitudinal muscles, and the intestine can be recognized in a wholemount preparation. The head was more complicated, although the rudimentary eyes and corona ciliata could be identified. Two pairs of PGCs were also evident from a wholemount preparation (Fig. 2A).

Distribution of the dye

More than 200 two-cell embryos were injected in single blastomeres at the two-cell stage. The injected dye spread through half of the embryo (Fig. 4A). Most of the embryos injected developed only until the gastrula stage, but no further. Only 44 hatchlings contained a sufficient quantity of fluorescent dye to be visualized. The distribution of dye did not correspond simply to the body axes (Figs. 4B, C, 5, 6). The dye distribution was examined in each structure as follows.

Epidermis—The whole dorsal epidermis of the trunk was either labeled or unlabeled. However, the ventral epidermis was labeled mostly in the left half. The outer layer was easily identified with the CLSM (Fig. 6A, D). However, exact identification of the inner layer was restricted to the lateral side of the trunk (Fig. 5D, E). In some cases these two layers were clearly distinguished, and the labeling patterns were complementary to each other. The dorsal epidermis (outer layer) of the head was either labeled (Fig. 5A) or partially labeled (Fig. 6A) in the whole area. The labeling pattern of the eyes and corona ciliata corresponded with that of the dorsal epidermis of the head (Figs. 5A, B, 6A, B).

Ventral ganglion—The ventral ganglion occupied most of the trunk, i.e., lateral and ventral regions. Two labeling patterns were observed. One was the labeling of both halves of the lateral regions and the left or right half of the ventral region of the ventral ganglion (Fig. 6C, D, E, G, H). In this case the labeling of the ipsilateral half (corresponding to the labeled half of the ventral region) of the lateral region formed in a mosaic fashion. The other was the labeling of the left or right half of the ventral region of the ganglion (Fig. 5D, E, G, H).

Longitudinal muscles—The labeling pattern of the body musculature was different between the dorsal and ventral components. The dorsal side was labeled mostly in the bilateral half (Fig. 6C), but the ventral side was either labeled in both halves (Fig. 5D, H) or unlabeled (Fig. 6D, H). In addition to the longitudinal muscles, mesodermal cells including the mesentery could be detected in a few preparations (Fig. 5A, B, C, G). Usually it was difficult to distinguish the mesodermal cells from the intestine.

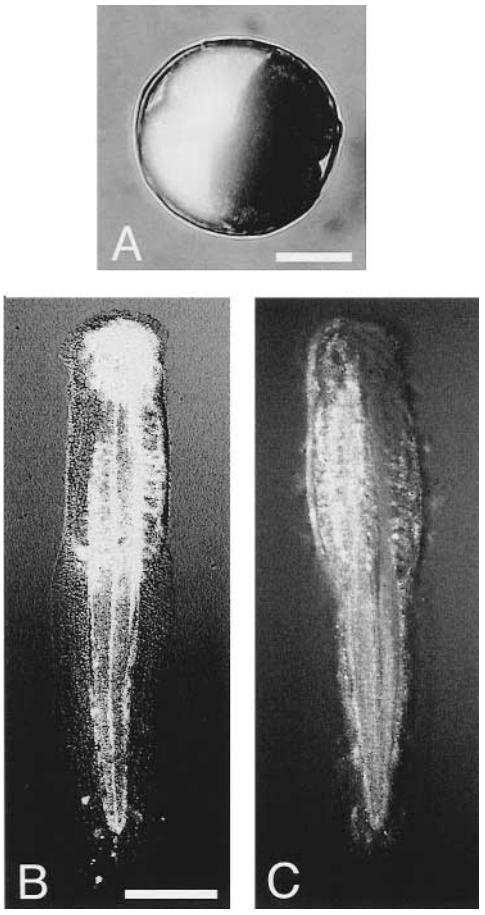

Fig. 4. Lineage tracing of the two-cell embyos observed under a fluorescent microscope. (**A**) The four-cell stage embryo labeled in a single blastomere at the two-cell stage. (**B**) The hatchling with labeled domain in the ventral side. (**C**) The hatchling with labeled domain in the dorsal half. Preparations shown in **B** and **C** are the same ones in Fig. 5 and Fig. 6, respectively. Bar = 100 μm

Intestine—The intestine was composed of mixtures of labeled and unlabeled areas in all the preparation examined. In other words, the labeling of the intestine formed in a mosaic fashion (Figs. 5D, H, 6D, H).

PGCs—All the PGCs (four PGCs in the hatchling) were labeled or unlabeled at the same time (Figs. 5G, 6G).

Fourteen hatchlings were clearly labeled. The dye distributions in the structures of these preparations are recorded in Table 1. From the examinations of each structure, the dye distributions could be divided into four modes; labeled in both halves, labeled in the left or right half, labeled partially, and unlabeled. Two sets of complementary labeling patterns were observed in the injected hatchlings based on the labeling of the PGCs. The PGC-positive preparations had a labeled do-

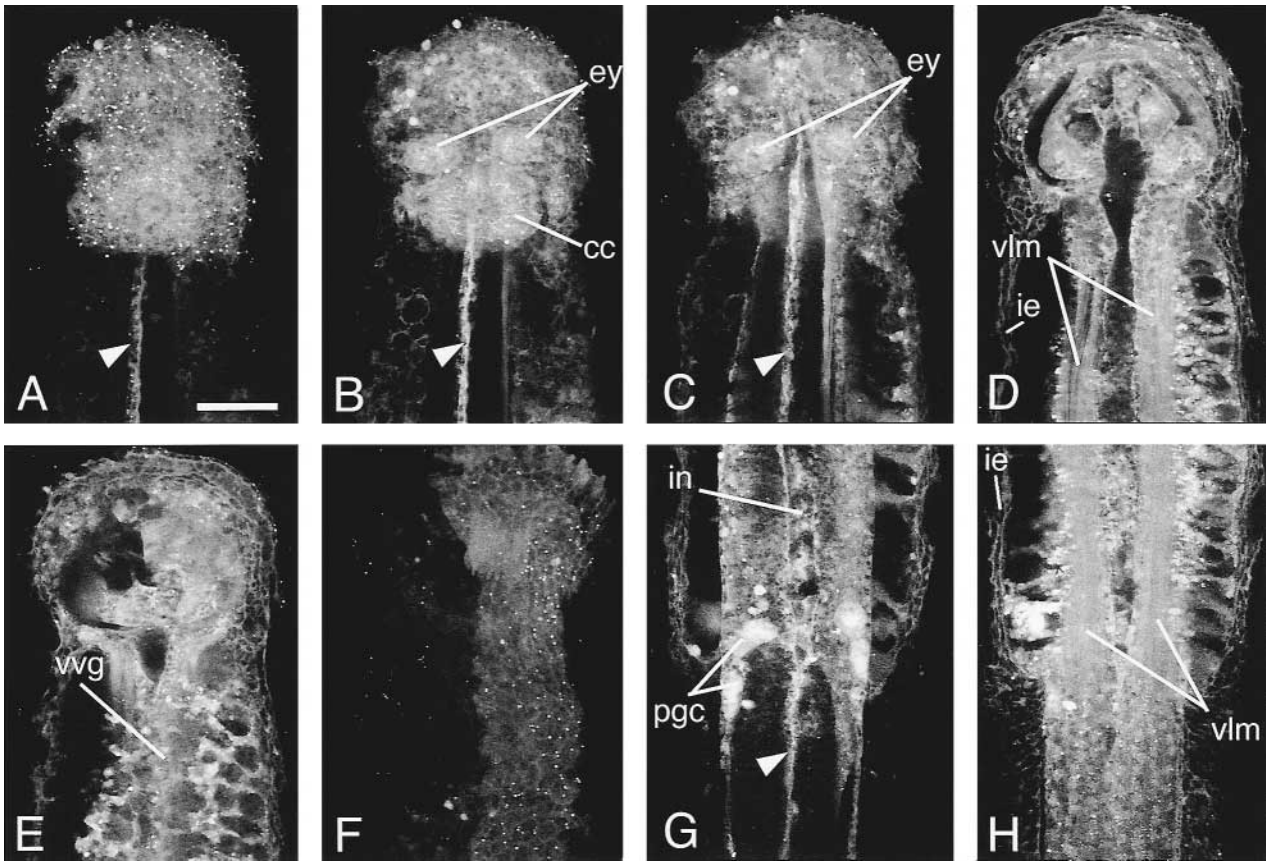

Fig. 5. A series of frontal sections of the hatchling with labeled domain in the ventral side (the same preparation shown in Fig. 4B). The dye distribution of this preparation is shown in Table 1 (preparation No. 7). (**A**–**F**) Head-trunk regions from dorsal surface (**A**) to ventral surface (**F**). Each image shows the planes through the dorsal epidermis (**A**), eyes and corona ciliata (**B**), the dorsal longitudinal muscles and the lateral regions of the ventral ganglion (**C**), the ventral longitudinal muscles, the intestine, and the lateral region of the ventral ganglion (**D**), the ventral region of the ventral ganglion (**E**), and the ventral epidermis (**F**). (**G**, **H**) Trunk-tail regions through the PGCs (**G**) and the ventral longitudinal muscles (**H**). Arrowheads in **A**, **B**, **C**, **G** indicate mesodermal cells. cc, corona ciliata; dlm, dorsal longitudinal muscles; ey, eyes; ie, inner layer of epidermis; in, intestine; pgc, primordial germ cells, vlm, ventral longitudinal muscles; vvg, ventral region of the ventral ganglion. Bar = 50 μm.

main over the entire ventral body muscles, and usually included the left or right half of the dorsal longitudinal muscles, the ventral epidermis, and the ventral region of the ventral ganglion (Figs. 4B, 5). The PGC-positive preparations with labeling on the left halves and the right halves are called the VL and VR patterns, respectively. The PGC-negative preparations had a labeled domain on the dorsal body epidermis and the dorso-lateral regions of the ventral ganglion, and usually included the left or right halves of the ventral region of the ventral ganglion, ventral epidermis, and the dorsal longitudinal muscles (Figs. 4C, 6). In this case, the PGC-negative preparations with labeling on the left and right halves are called the DL and DR patterns, respectively. Thus, a total of four different labeling patterns were observed, all of which are schematically shown in Fig. 7. Table 2 shows the number of preparations in each pattern. 57% of the preparations showed the labeling of the PGCs, and 70% of the preparations were labeled on the left halves (DL and VL).

DISCUSSION

Experimental embryology in chaetognaths

The present study was the first to report a method of injecting the chaetognath embryos. We have established a culture method of *P. gotoi* (Goto and Yoshida, 1997), and have been able to use the eggs all year. Fertilized eggs are arrested in the oviducal complex at the first meiotic metaphase (Goto, 1999), and the metaphase release occurs after laying into sea water. Thus, early development can be followed by the artificial isolation of the fertilized egg. Naturally laid eggs have two layers of egg membranes. One is a vitelline membrane which hardens in contact with sea water, presumably by the exocytosis of cortical granules in an egg. We have not succeeded to remove this membrane. The other is an egg capsule which is presumably produced with material excreted near the genital opening during egg laying. We could obtain the eggs without the egg capsules by artificially isolating eggs. Insertion of the injection capillary could be done in the artificially isolated eggs without the egg capsules, which would prevent injection.

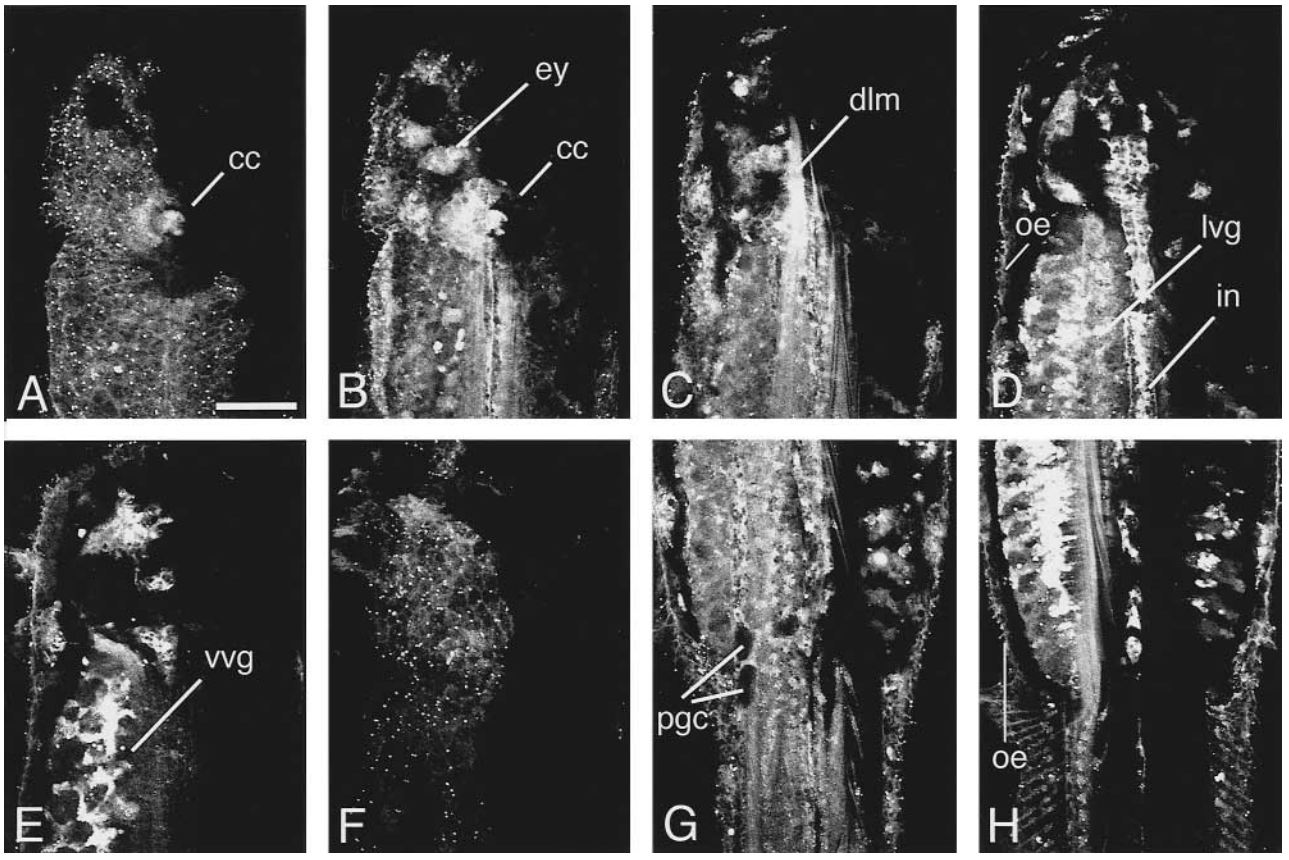

Fig. 6. A series of frontal sections of the hatchling with labeled domain in the dorsal side (the same preparation shown in Fig. 4C). The dye distribution of this preparation is shown in Table 1 (preparation No. 8). (**A**–**F**) Head-trunk regions from dorsal surface (**A**) to ventral surface (**F**). Each image shows the planes through the dorsal epidermis (**A**), eyes and corona ciliata (**B**), the dorsal longitudinal muscles and the lateral regions of the ventral ganglion (**C**), the ventral longitudinal muscles, the intestine, and the lateral region of the ventral ganglion (**D**), the ventral region of the ventral ganglion (**E**), and the ventral epidermis (**F**). (**G**, **H**) Trunk-tail regions through the PGCs (**G**) and the ventral longitudinal muscles (**H**). cc, corona ciliata; dlm, dorsal longitudinal muscles; ey, eyes; in, intestine lvg, lateral region of the ventral ganglion; oe, outer layer of epidermis; pgc, primordial germ cells, vvg, ventral region of the ventral ganglion. Bar = 50 μm.

Table 1. Distribution of dye microinjected into single blastomeres at the two-cell stage in 14 hatchings of *P. gotoi*

Structure	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	VL						VR	DL					DR	
Dorsal epidermis	–	–	–	–	–	±	–	+	+	+	+	+	+	+
Ventral epidermis	L*	L*	±	–	L	L*	R	L	L*	L	L*	L*	R	R*
Dorsal longitudinal muscles	L*	L*	L*	–	L*	–	R*	L	±	±	L	±	R*	R
Ventral longitudinal muscles	+	+	+	+	+	+	+	±	–	–	±	–	±	–
PGCs	+	+	+	+	+	+	+	–	–	–	–	–	–	–
Ventral ganglion	L*	±	L	L	L	R*	R*	L*	L	L	L*	±	R	R
Eye(s)	+	+	–	L	–	R	+	L	+	+	+	+	R	+
Corona ciliata	+	–	–	L	–	+	+	L	+	+	+	+	R	+
Intestine	±	±	±	±	±	±	±	±	±	±	±	±	±	±

+, labeled; –, unlabled; ±, labeled partially; L, labeled in left half of the structure; L* labeled in left half stronger tahn in right half; R, labeled in right half of the structure; R*, labled in right half stronger than in left half.
VL, VR, DL, DR; labeling patterns (see Fig. 7)

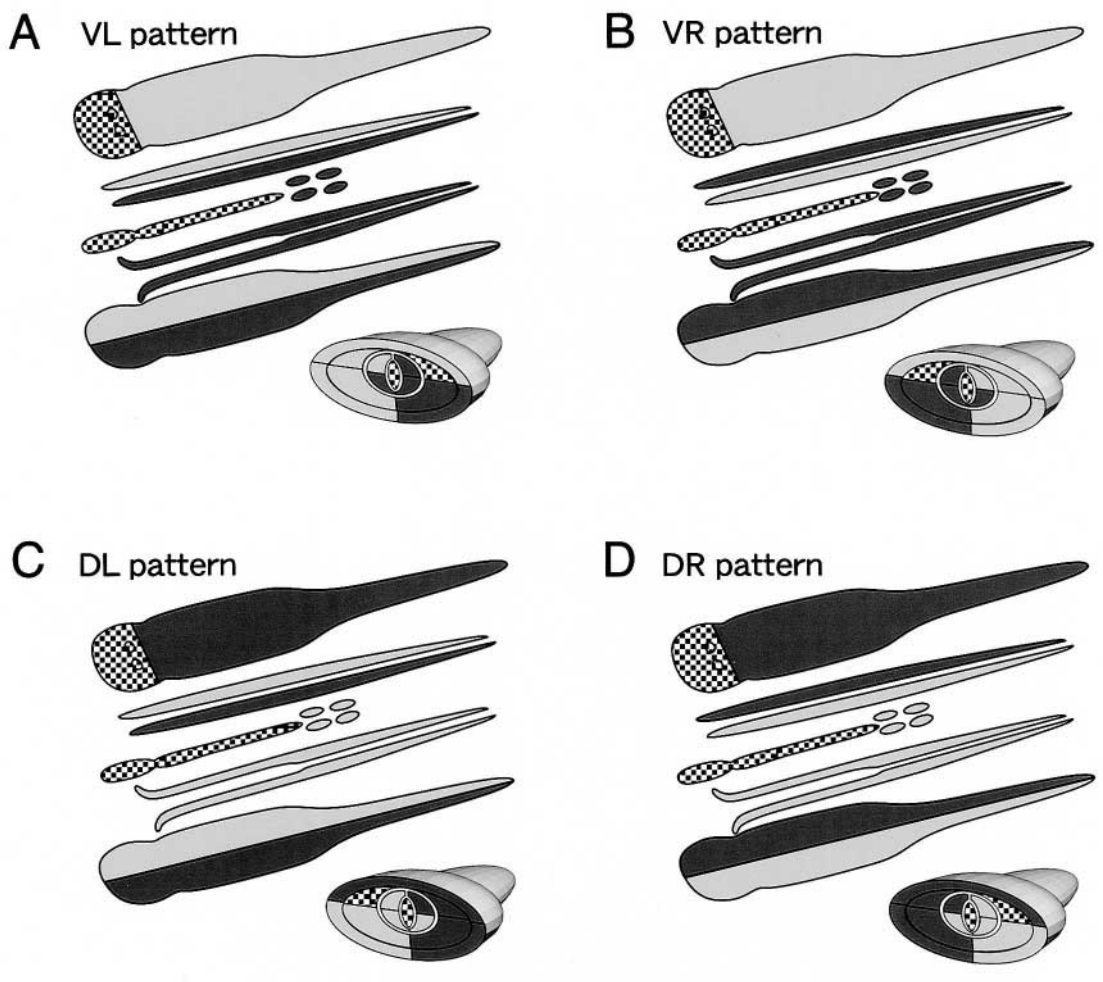

Fig. 7. Diagram showing the four labeling patterns (**A**, VL pattern; **B**, VR pattern; **C**, DL pattern; **D**, DR pattern) found in the hatchlings of *P. gotoi* (a series of frontal sections and a transverse section as shown in Fig. 3C and Fig. 3B, respectively). Dark gray means the labeled regions in whole and hatched gray means the regions labeled partially.

Table 2. Labeling patterns in hatchings of *P. gotoi* injected in single bastmeres at the two-cell stage

	Patterns				total
	VL	VR	DL	DR	
Number observed	21	4	10	9	44

Fates of cells in two-celled embryo

When single blastomeres of *P. gotoi* were injected at the two-cell stage with a dextran-conjugated fluorescent dye, the distribution of dye in hatchlings did not correspond simply to the body axes, and the various labeling patterns observed depended on the structures. The labeling of the PGC was especially useful in understanding the fate of the blastomeres and the relationship between egg axis and body axis. Occurrence of the PGC-positive and PGC-negative labeling patterns supports the existence of the germinal determinant in one blastomere at the two-cell stage. The preparation with labeled PGCs showed labeling of the ventral longitudinal muscles but no labeling on the dorsal epidermis (VL and VR). In contrast, the preparation with unlabeled PGCs showed labeling of the dorsal epidermis and the lateral regions of the ventral ganglion but no labeling in the ventral longitudinal muscles (DL and DR). The blastomere containing the germinal determinant (B cell) and the other blastomere (A cell) mainly develop into the ventral side and the dorsal side of the body, respectively. Considering the existence of the germinal determinant near the vegetal pole in zygotes (Elpatiewsky, 1909; Ghirardelli, 1954, 1968), cell determination may be established early based on an underlying system of axial properties present in the fertilized egg before first cleavage in chaetognaths. Figure 8 is a schematic representation showing the labeling pattern and the two-cell stage embryos with reference to their prospective dorsoventral and bilateral axes.

The ventral ganglion occupies a large portion of the trunk, i.e., the lateral and the ventral regions. The lateral regions of the ventral ganglion extend to the dorsal side. In the ventral ganglion, two sets of complementary labeling patterns were found that are symmetrical to each other (Fig. 8A, B). In the

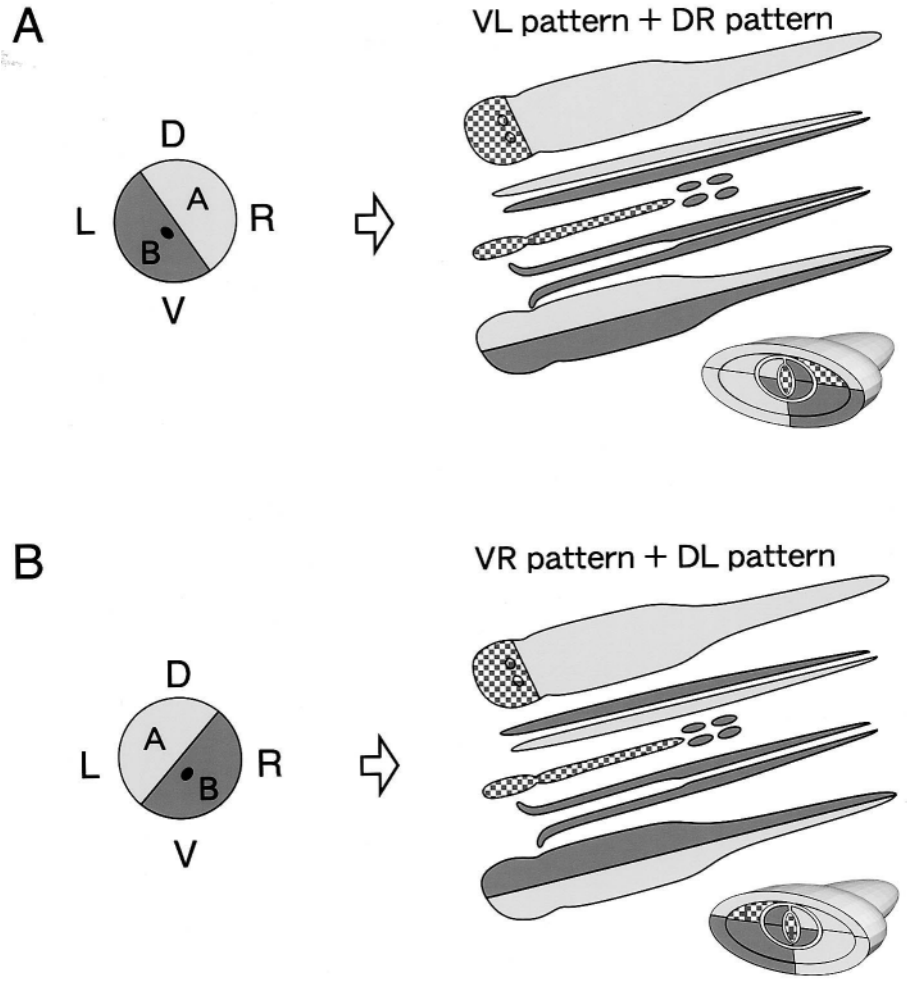

Fig. 8. Diagram summarizing the relationship between the first cleage plane and the body axes of the hatchling of *P. gotoi.* The two-cell stage embryo viewed from vegetal pole (drawn at the left of this figure). The embryo is composed of A and B cells and the B cell contains the germinal determinant (small dot). Future dorsoventral and bilateral axes are shown in DV and LR, respectively. The labeling patterns found in the hatchlings are drawn in right side of the two-cell embryos. Light and dark gray mean the regions derived from the A and b cells, respectively. Hatched gray means the regions derived from both cells. (**A**) A complementary pair of the VL and the DR patterns. A cell generates the DR pattern and B cell generates the VL pattern. (**B**) A complementary pair of the VR and the DR patterns. A cell generates the DL pattern and B cell generates the VR pattern.

DL and DR patterns, the respective bilateral half of the ganglion was strongly labeled, and the labeling pattern of the other half formed in a mosaic fashion. In the VL and VR, however, only the respective ventral half was labeled. The boundary between labeled and unlabeled regions is not distinct in bilateral symmetry. For example, the VL and DR are complementary to each other, but not symmetrical. The blastomere containing the prospective dorsal region at the two-cell stage (A cell in Fig. 8) rather than the other blastomere (B cell in Fig. 8) usually gives rise to the ventral ganglion. Since the A cell contributes largely to the epidermis, the ventral ganglion may be derived from epidermis.

Invagination occurs at the vegetal region, and mesodermal tissues are derived from the archenteron wall in chaetognaths (Kowalevsky, 1871; Hertwig, 1880; Doncaster, 1902; Burfield, 1927). The labeling pattern of the ventral longitudinal muscles indicates that they are derived from the blastomere containing the vegetal region of the zygote. In all the labeling patterns, the respective bilateral half of the dorsal longitudinal muscles was labeled. This result suggests that the two blastomeres contribute to the formation of the dorsal longitudinal muscles, and that the first cleavage plane passes through the antero-posterior axis.

The labeling of the intestine was formed in a mosaic fashion and was found in all the preparations. This result indicates that the two blastomeres contribute to the formation of the intestine. Elpatiewsky (1909) reported that the origin of the endodermal cells is the “d” cell containing the germinal determinant. If the intestine is derived only from the “d” cell, the labeling pattern of the intestine should be the same as that of the PGCs. Therefore, our result may not support this view, but rather indicate that the intestine is derived from both blastomeres at the two-cell stage.

In order to elucidate the manner in which cell fates are

established in chaetognath embryos, we have to conduct cell-isolation and deletion experiments to examine the developmental potential of the early cleavage blastomeres in chaetognaths.

Relationship of first cleavage plane and body axes

When one injected blastomere produced the DR pattern, the other one produced the VL. In contrast, when one injected blastomere produced the DL pattern, the other one produced the VR. Thus, complementary distribution is regarded as the result of the injection of the other blastomere. The existence of two patterns of the complementary label indicates that the bilateral axis is not directly related with the first cleavage plane, and that there are two positional relationships on the first cleavage plane as shown in Fig. 8. The first cleavage plane runs largely through the future antero-posterior axis and bisects the future bilatera and dorsoventra axes. Both the A and B cells have a possibility to contribute the left and right sides, suggesting that the plane of the first cleavage does not play a causal role in establishing the bilateral axis.

The DL and VL patterns were generated at a higher rate than the DR and VR. Though the number of preparations examined in the present study was only 44, this result suggests that the marking of a cell switches the bilateral location of the left/right structures, and that the determination of the bilateral axis is not established at the two-cell stage. In some lineage experiments there was a significant bias towards embryos being labeled on the right side (in sea urchins, Henry *et al*., 1992; McCain and McClay, 1994; in phoronids, Freeman, 1991; in nemerteans, Henry and Martindale, 1998). To understand the inversion of the bilateral axis, further observation using two- and four-cell stage embryos is much needed.

It has been well known that there are variations in the relationship between early cleavage planes and the various developmental axes. The relationship between the first cleavage plane and the axes of symmetry has been investigated with cell-lineage tracing markers in sea urchins, and four different patterns have been reported depending on the species (Cameron *et al*, 1989; Henry *et al*, 1992; McCain and McClay, 1994). In sea urchins, the mechanism of cell determination seems to be tied to an underlying framework of axial systems resident within zygotes and embryos. Recent investigations into the origins of the dorsoventral axis and the specification of cell fate in equal-cleaving spiralian embryos have focused on the characteristics of early cleavage divisions (Henry and Martindale, 1994, 1996). There is a highly consistent relationship between early cleavage and the adult dorsoventral axis in higher Spiralia (molluscs and annelids) with the bilateral organization of the body plan depending on the dorsal lineage, referred to as the “D quadrant” (Martindale *et al*, 1985; Boring, 1989). A labeling study of the two- and four-cell stages in nemerteans revealed that the first cleavage plane can run either through the plane of bilateral symmetry or through the frontal plane (Henry and Martindale, 1994). To reveal the relationship between early cleavage orientation and the body axes in chaetognaths, further cell lineage study, especially of the four-cell stage embryos, is needed.

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