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Cell position and developmental fate in leech embryogenesis

(cell lineage/transfating/equivalence group/determination)

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ABSTRACT **The o and p blast cell bandlets of the leech *Theromyzon rude*, which normally produce two different sets of identifiable cells designated the "O" and "P" fates, respectively, form an equivalence group: in embryos experimentally deprived of their p bandlet, the blast cells of the adjacent o bandlet may "transfate" and take on the P fate. Loss of the p bandlet is not, however, a sufficient condition for transfating of the o bandlet. Rather, loss of the p bandlet allows the o bandlet to shift into ectopic positions, and it is the ultimate position of the o bandlet that mandates which fate—O or P—the blast cells will take on. Therefore, the choice of the pluripotent o blast cells to follow either the O or P developmental pathway depends on their perception of positional cues provided by cells outside the equivalence group rather than on a direct interaction with p blast cell equivalence group members.**

Two alternative mechanisms are often proposed to account for cell commitment—the process by which the developmental potential of a pluripotent cell becomes restricted, leading to the appearance of one type of differentiated descendant rather than another. One mechanism is attributed to cell-autonomous processes governed by cell lineage, whereas the other invokes processes governed by cell–cell interactions (1–3). The autonomous mechanism of commitment merits consideration in the development of leeches, which proceeds according to a fixed mitotic pedigree (4–6). However, study of the developmental fate of two equivalently pluripotent sister teloblasts in embryos of the glossiphoniid leech *Helobdella triserialis* has shown that their commitment is governed by cell–cell interactions (7–12).

Both sister teloblasts divide repeatedly producing two adjacent bandlets of several dozen smaller primary blast cells indicated by the lowercase letters o and p (Fig. 1). The developmental fate of the o and p blast cells is determinate (3), in the sense that they normally produce two different sets of cells designated as the "O fate" and the "P fate."

Although their fate is determinate, primary o blast cells remain pluripotent; i.e., they are not yet committed to that fate: Primary o blast cells deprived of the adjacent p bandlet will abandon their normal fate and "transfate" to take on the P fate. Under the reciprocal protocol, however, the primary blast cells of the p bandlet do not change their P fate (7, 9). The o- and p-bandlet cells thus represent one of several known instances of equivalently pluripotent embryonic cells designated as "equivalence groups" in nematodes, leeches, and insects (2, 8, 13, 14). Under some experimental operations, such as the ablation of one member of the equivalence group, another group member may adopt the fate normally assumed by the ablated cell. For many equivalence groups, as for the o- and p-bandlet group, the response to ablation is not symmetric. The preferential fate taken on by the non-ablated member of the equivalence group (for the o and p bandlets, the P fate) is called the primary fate (15). The intercellular interactions that guide the choice of alternative fates by equivalence group members are thought to occur directly among them (1, 2, 13–17). Accordingly, transfating in the leech has been interpreted in terms of the hypothesis that commitment to the O rather than the P fate is the result of a signal that o blast cells receive from the blast cells of the adjacent p bandlet. In the absence of the p bandlet, this interaction would not occur and the o blast cells would assume the P fate.

Our results show that, contrary to this hypothesis, in the glossiphoniid leech *Theromyzon rude*, the absence of the p bandlet is not a sufficient condition for transfating to occur. Rather, the absence of the p bandlet allows the o bandlet to shift into ectopic positions, and it is the ultimate position of the o bandlet that mandates which future fate—O or P—the blast cells will assume. Thus, the restriction of the initial pluripotency of o blast cells is attributable to an interaction with positional cues provided by cells outside the equivalence group.

MATERIALS AND METHODS

All experiments were carried out with embryos of the glossiphoniid leech *T. rude* (18) collected in Golden Gate Park in San Francisco. The methods were as described (19, 20). The embryonic staging system is that of Fernandez (18), as modified by Weisblat *et al.* (21).

RESULTS

Differences Between *H. triserialis* and *T. rude*. The development of *T. rude* is very similar to that of *H. triserialis*. However, there is a significant difference in the determinacy of the fates produced by the parent teloblasts of the o and p bandlets. These teloblasts are sister cells generated by the division of a stage 6 blastomere designated OP, which lies between two flanking teloblasts named N and Q. In *H. triserialis* either daughter of OP can give rise to either the o or p bandlet and, therefore, to either the O or P fate (7). Consequently, the designation "O/P" has been used to indicate the indeterminate fate of these two sister teloblasts in *H. triserialis*. In *T. rude*, by contrast, the fate of either sister is determinate, as shown by the results of the following experiment.

Cell lineage tracer was injected into either one or the other OP daughter of stage 6 *T. rude* embryos, before the onset of blast cell production. The fate of the descendants from each labeled teloblast was ascertained by examination of the labeled progeny at stage 10–11 (Fig. 2). In 88 of 89 cases in which the OP daughter next to the N teloblast had been injected, the labeled cells took on the O fate. Conversely, the OP daughter adjacent to the Q teloblast produced labeled cells that took on the P fate in all (31/31) cases in which it was

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Abbreviations: RDA, tetramethylrhodamine-dextran-amine; FDA, fluorescein-dextran-amine.

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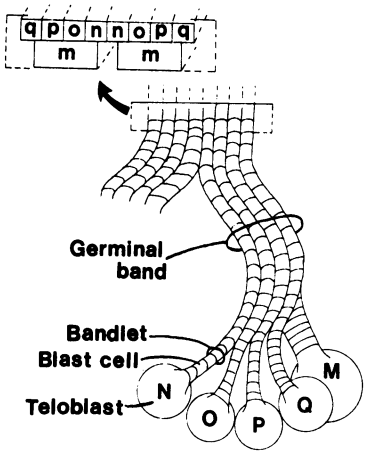

FIG. 1. Schematic diagram of an early stage 8 *T. rude* embryo. Each of the five (M, N, O, P, and Q) teloblasts divides repeatedly to produce a bandlet of several dozen smaller primary blast cells designated m, n, o, p, and q, respectively. Each primary blast cell continues to divide forming a clone of cells. Primary (undivided) blast cells are located posteriorly and clones of progressively higher-order blast cells are located ever more anteriorly. The bandlets unite forming a germinal band composed of both the ectodermal n, o, p, and q bandlets, superficially, and the mesodermal m bandlet underneath them. Right and left germinal bands join along the future ventral midline to form the bilaterally symmetric germinal plate (shown in transverse section; arrow), which will give rise to the nervous system and body wall of the leech. Anterior is up in all figures.

injected. Therefore, in work using *T. rude* we will refer to the OP daughter next to the N teloblast as the O teloblast and the OP daughter next to the Q teloblast as the P teloblast (Fig. 1).

A second difference between the two species concerns the frequency of transfating in response to experimental deprivation of the adjacent p bandlet. In *H. triserialis* embryos deprived of their p bandlets by ablation of the precursor P teloblast, more than 95% of the o bandlets transfate to the P fate (7, 8). The same procedure leads to a much more variable outcome in *T. rude*, as shown by the results of the following experiment.

The left O and P teloblasts were injected with tetramethylrhodamine-dextran-amine (RDA) and fluorescein-dextran-amine (FDA) lineage tracers, respectively, at stage 6c before the onset of blast-cell production. After the P teloblast had produced at least one primary p blast cell, it was ablated by injection with DNase, aborting further p-bandlet formation. Embryos developed until stage 10–11, when they were fixed and examined for the fate of the labeled cells in each of the 21 abdominal segments. This protocol yields embryos with an anterior control zone, whose segments contain both the RDA-labeled descendants of the o bandlet and the FDA-labeled descendants of the p bandlet. The control zone is separated by an ablation border from a posterior experimental zone, in whose segments only the RDA-labeled descendants of the o bandlet are present. In this and all subsequent experiments, the fate of the RDA-labeled cells in the experimental zone was scored only in embryos in which the control zone exhibited normal O- and P-fate patterns; 56 embryos deprived of their left p bandlet satisfied this criterion. In 17 of these embryos (30%), the RDA-labeled cells in the experimental zone displayed a normal O fate in all segments, in 12 embryos (22%), they displayed a mixture of O and P fates, and, in 27 embryos (48%), they displayed a P fate in the majority of segments. These variable results show that the absence of the p bandlet is not a sufficient condition for the transfating of o blast cells to the P fate.

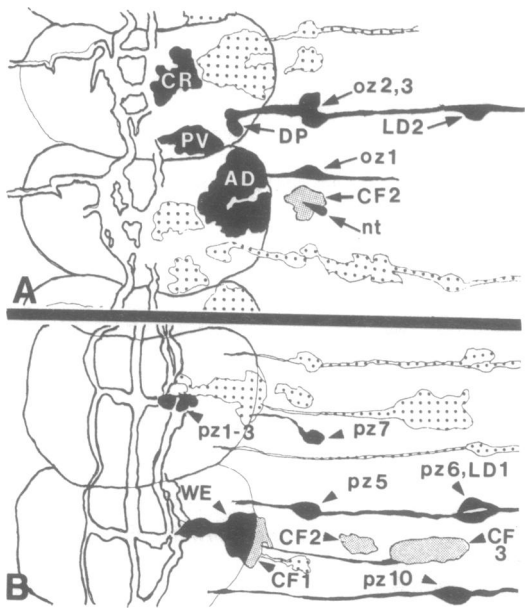

FIG. 2. Camera lucida drawings showing the normal stereotyped distribution of cells (or "elements") of the O and P fate in the segmental ganglia and body wall of stage 10–11 *T. rude* embryos. Solid or finely stippled cells represent, respectively, neuronal or epidermal elements descended from a single o or p primary blast cell. Coarsely stippled cells represent elements derived from the neighboring o or p primary blast cells. Central axon tracts labeled under these conditions are shown in outline. Elements of the O or P fate derived from a single primary blast cell are distributed over two consecutive abdominal segments so that neighboring clones overlap along the length of the 21 abdominal segments that make up the midbody of the leech. (*A*) *O fate*. Axon tract projects into the contralateral side in each segment. In the ganglion of the anterior segment: a crescent-shaped cluster of neurons (CR) along the dorsal ganglionic midline, a smaller discrete clump of cells (PV) at the posteroventral margin of the ganglion, and two adjacent neuronal cell bodies (DP) apposed to the edge of the dorsal ganglionic margin. In the body wall of the anterior segment: three peripheral neurons (oz2, oz3, and LD2). (Neuron oz3 is present in *T. rude* but not in *H. triserialis*.) In the ganglion of the posterior segment: a cluster of neurons (AD) on the dorsal aspect of the anterior margin. In the body wall of the posterior segment: one peripheral neuron (oz1), a cluster of specialized epidermal cells (CF2), and the distal cell of the nephridial tubule (nt). (*B*) *P fate*. No axon tracts project contralaterally. In the ganglion of the anterior segment: a cluster of three neurons (pz1, pz2, and pz3) on the ventral aspect near the midline. In the body wall of the anterior segment: a peripheral neuron (pz7). In the ganglion of the posterior segment: a cone-shaped group of cells (WE) on the ventral aspect whose base lies at the lateral ganglionic margin and whose apex points toward the midline. In the body wall of the posterior segment: two clusters of specialized epidermal cells (CF1 and CF3) and a single cell contributed to CF2; three peripheral neurons (pz5, pz6, and LD1) and one peripheral neuron (pz10).

o-Bandlet Position After Ablation of the P Teloblast. To explain this variability, we hypothesize that transfating, in this case, requires the o bandlet to move from its normal position in the germinal band into the position of the missing p bandlet. If ablation of the P teloblast provokes this movement in only some but not all embryos of *T. rude*, then variable transfating results would be observed. To test this hypothesis, the preceding experiment was repeated, except that, additionally, the left N and Q teloblasts were both injected with tetramethylrhodamine-dextran so that their labeled n and q bandlets would serve as topographic landmarks within the germinal band. Unlike the RDA and FDA labels, which are retained in fixed embryos, the tetramethylrhodamine-dextran label is eliminated during fixation at stage 10–11.

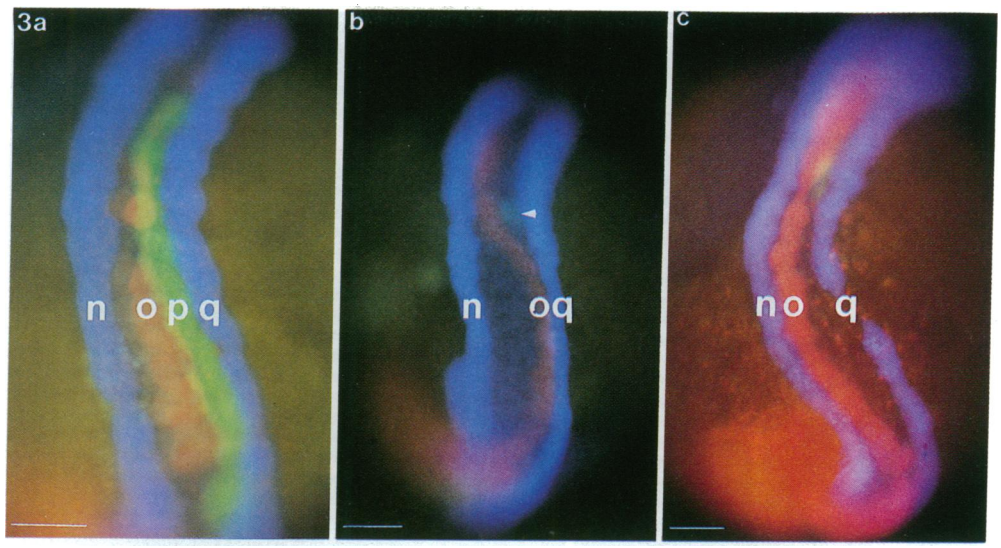

FIG. 3. Fluorescence photomicrographs of stage 8 *T. rude* embryos with differentially labeled ectodermal bandlets within the left germinal band are n [blue = Cascade blue-dextran (CdBDX)], o (red = RDA), p (green = FDA), q (blue = CdBDX). The n and q bandlets were labeled with the blue-fluorescing dye CdBDX (Molecular Probes) solely for illustrative purposes; the experimental results were obtained by the use of tetramethylrhodamine-dextran in the n and q bandlets as described in the text. (Bar = 50 μm.) (*a*) Normal embryo displaying the positions of the four ectodermal bandlets. (*b* and *c*) Embryos in which the p-bandlet formation has been aborted by ablation of the parental P teloblast at stage 6 after it had produced a few primary blast cells. (*b*) The o bandlet has shifted and lies next to the q bandlet in the position of the missing p bandlet. The truncated end of the p bandlet (green = FDA label) can be seen at the top (arrowhead). (*c*) The o bandlet has not shifted and lies next to the n bandlet in its normal position.

These living experimental embryos were examined by epifluorescence microscopy at early stage 8 to determine whether the o bandlet, in the p-bandlet-deprived germinal band, lay next to the n bandlet (in the normal o-bandlet position) or next to the q bandlet (in the normal p-bandlet position) (Fig. 3*a*). The ablation border could be readily visualized by the position of the caudal end of the truncated FDA-labeled p bandlet (Fig. 3*b*).

In the experimental zone of 17 of 31 specimens, the o bandlet had shifted to the position of the missing p bandlet (Fig. 3*b*). In 10 of 31 specimens, the o bandlet lay in positions intermediate between the n and q bandlets. And in 4 of 31 specimens, the o bandlet remained in its normal position without shifting (Fig. 3*c*).

After the position of the o bandlet was recorded, the embryos were cultured individually until stage 10–11 and then fixed, and each segment of the experimental zone was scored as before.

The results confirmed the hypothesis that position in the germinal band determines the fate of the o blast cells. Clones in o bandlets classified as lying in the normal o-bandlet position gave rise only to O-pattern elements (Table 1, Fig. 4*a*), whereas those classified as lying in positions intermediate between the n and q bandlets often displayed a mixture of O and P fates. Some embryos of this later class exhibited segmental alteration between fates and in some segments a single o blast cell clone had given rise to a mixture of both O- and P-pattern elements (Fig. 4*b*). The highest frequency of transfating was observed in embryos in which the o bandlet had been classified as lying in the p-bandlet position (Table 1). In most of these embryos complete transfating from the O to the P fate had taken place.

Table 1. Relationship between o-bandlet position at stage 8 and fate at stage 10–11

Position	No. of embryos	No. of segments expressing fates/no. of total segments		
		O	Mixed	P
Normal	4	61/61	0/61	0/61
Intermediate	10	23/115	52/115	40/115
Shifted	17	24/188	42/188	122/188

Thus, the probability that a member of the o blast cell clone transfates increases with its distance from the n bandlet in the germinal band and with its proximity to the q bandlet.

DISCUSSION

To account for the observed features of the o- and p-bandlet equivalence group, it was hypothesized (8–12) that o-bandlet cells are poised to take on the P fate but are normally prevented from doing so by a signal from neighboring p-bandlet cells. Elimination of the p bandlet and loss of that signal would free the o bandlet to adopt the P fate. The P fate would be primary because its realization would not depend on any signal provided by the o-bandlet cells (8–10). This explanation resembles that advanced for equivalence groups found in nematodes and insects, for which it has been similarly hypothesized that cells committed to the primary fate provide a signal to other cells of the equivalence group preventing them from taking on the primary fate for which they are poised and committing them to a secondary fate (1, 2, 13–17, 22).

Our results demonstrate the inadequacy of this hypothesis for the o- and p-bandlet equivalence group in the leech *T. rude*. Commitment to the O fate cannot be attributable to a signal from p-bandlet cells. Transfating requires the o bandlet to shift into the position normally occupied by the p bandlet. The absence of the p bandlet permits—but it does not necessarily result in—such a shift. This shows that there is a difference between the o- and p-bandlet positions with regard to the presence of cues governing blast cell commitment. In the absence of the p bandlet, the o bandlet may shift to the p-bandlet position, where it comes under the influence of different positional cues and takes on the P fate. If the shift is incomplete and the o bandlet lies in an intermediate position, then some clonal members may encounter one set of cues and take on the O fate, while others may encounter another set of cues and take on the P fate.

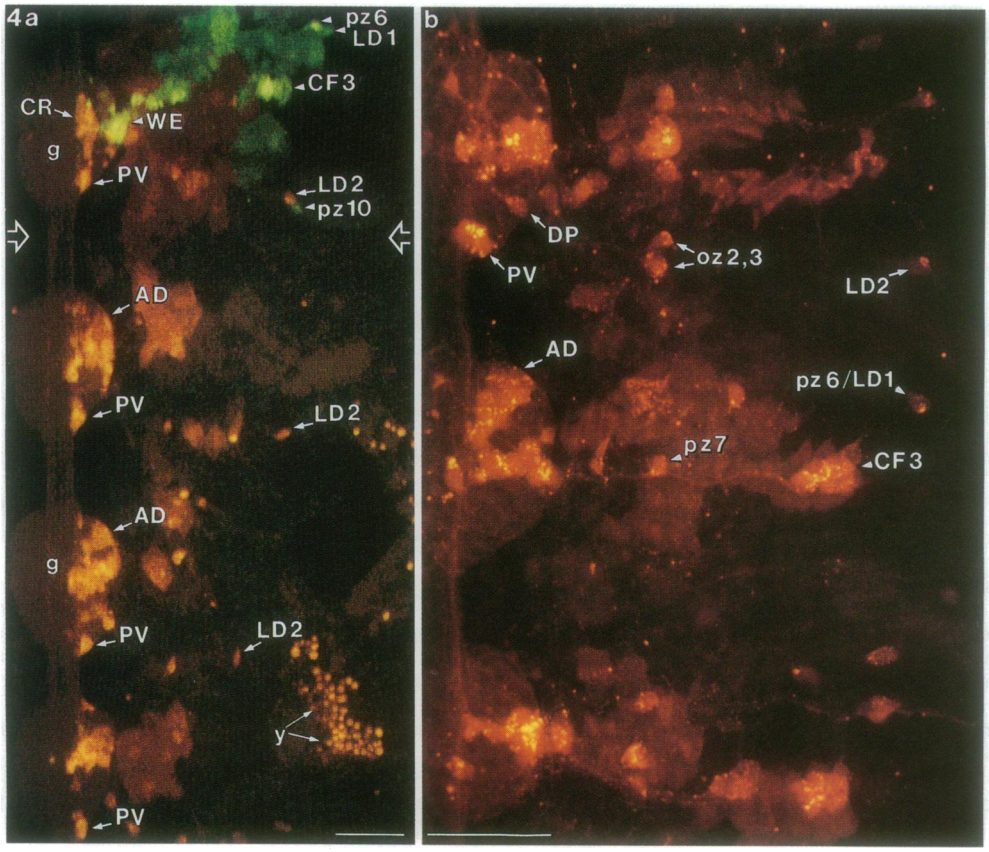

FIG. 4. Fluorescence photomicrographs of stage 10–11 *T. rude* embryos deprived of their p bandlets. Arrowheads identify P-fate elements; arrows identify O-fate elements; g indicates ganglia; y indicates autofluorescent yolk granules. (Bar = 50 μm.) (*a*) An embryo in which the o bandlet did not shift, remaining in its normal position next to the n bandlet. In the control zone (above large open arrows) both the normal O (red = RDA label) and P (green = FDA label) fates are observed. Posterior to the ablation border (between large open arrows) in the experimental zone only the RDA-labeled progeny of the o bandlet are present, which display a normal O fate. (*b*) Embryo in which the o bandlet did shift into an ectopic position intermediate between the n and q bandlets. Three segments display both O and P fates from the progeny of the RDA-labeled o bandlet. The progeny from a single primary o blast cell (marked by open arrows and arrowheads) display both O and P elements.

In this regard, the commitment of o blast cells to their fate resembles that of vulval precursor cells in the nematode *Caenorhabditis elegans*, which has been shown to depend on positional cues provided by a cell external to the equivalence group (22, 23).

The finding that an o blast cell clone can give rise to a mixture of both O- and P-pattern elements demonstrates that it is not the primary clonal founder o blast cell that becomes committed to the O or P fate. Rather, higher-order blast cells are committed separately and independently to producing elements of one or the other fate. This inference agrees with previous findings made in *H. triserialis* (8, 10, 11). The relation of the patterns of mixed O- and P-fate elements produced by our operation as compared with previous experimental operations (8) will be examined in a subsequent report.

In the light of these considerations, it is possible to put forward a simple hypothesis that accounts for the primacy of the P fate in this leech equivalence group: The elimination of the o bandlet would not cause the p bandlet to take on the O fate if, because of a cell-substrate adhesivity gradient or some other structural reason, a shift of a p bandlet from its normal position to the o bandlet position is much less likely after elimination of the o bandlet than a shift of the o bandlet in the opposite direction after elimination of the p bandlet. This hypothesis finds support in our observations (unpublished) that the p bandlet shifts to the o-bandlet position in very few embryos experimentally deprived of their o bandlet.

The most plausible hypotheses regarding the source of these positional cues are that they originate in the m, n, or q bandlets or in the cells of the overlying provisional epidermis—a thin epithelium that lies atop the four ectodermal bandlets as a tight cover of the germinal bands.

The results of experiments (to be published elsewhere) in which embryos were deprived of their n or q bandlets by ablating their parental teloblast have shown that neither the n nor the q bandlet is a source of these putative positional cues. By contrast, the ablation of a section of the provisional epidermis overlying the o and p bandlets in *H. triserialis* results in a partial transfating of the o blast cells and a partial duplication of P-fate elements (12). It is hard to imagine, however, how epithelial cells putatively specialized for the production of different positional cues could be precisely positioned over one but not the other of the o and p bandlets. It seems more probable that the hole created in the provisional epidermis by ablation releases the compression it normally exerts on the underlying o blast cells, thus allowing them to move with respect to the putative positional cues and consequently to change their fate.

The m bandlet, precursor of the mesoderm, appears to be the most likely source of positional cues relevant to the commitment of o blast cells to the O or P fate, especially since the mesoderm of the leech has been shown (20, 24) to supply positional cues to the overlying ectoderm for the morphogenetic movements associated with stationing neural precursor cells within the central and peripheral nervous systems.

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