

Cell fate determination in an annelid equivalence group

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SUMMARY

In embryos of glossiphoniid leeches such as *Helobdella triserialis* and *H. robusta*, pairs of adjacent 'o/p' ectodermal blast cells are known to be developmentally equipotent and yet eventually contribute distinct sets of 'O' and 'P' progeny to the nervous system and epidermis of the mature leech. It has been thought that the fate-determining interactions in this 'O-P equivalence group' take place between the equipotent cells themselves. We show here that such

intra-group interactions are neither necessary nor sufficient. Instead, transient contact with cells in another ectodermal lineage is necessary and sufficient to induce o/p blast cells to assume the P fate. In the absence of this contact they assume the O fate.

Key words: equivalence group, leech, annelid, cell interactions, *Helobdella*

INTRODUCTION

That initially equipotent cells can assume distinct fates is a central feature in development. Groups of equipotent cells that interact hierarchically in choosing from two or more possible fates are called 'equivalence groups'. Equivalence groups have been described in diverse phyla, including nematodes (Sulston and White, 1980; Kimble, 1981), chordates (Nishida and Satoh, 1989), arthropods (Kuwada and Goodman, 1985) and annelids (Weisblat and Blair, 1984; Zackson, 1984).

The final fates of cells in equivalence groups often correlate with their relative positions in the embryo. A priori, the fate-determining interactions could occur either among the equipotent cells themselves, or between these cells and one or more external cues. In nematodes, the cellular origin of cues that induce the vulval precursor cells to differentiate from the hypodermal cells have been examined extensively (Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1986; Mango et al, 1991; Herman and Hedgecock, 1990; Hill and Sternberg, 1992; Simske and Kim, 1995) and reviewed (Horvitz and Sternberg, 1991; Hill and Sternberg, 1993). There, the primary fate-determining step originates from outside the equivalence group, but interactions among the cells of the equivalence group are important in refining the fates of the vulval precursors (Simske and Kim, 1995). In the case of the ascidians, grasshopper and leech, the sources of the fate-determining signals are less well studied. Is there a general mechanism by which the cell fates are assigned in equivalence groups? To address this question, we have re-examined the cellular origins of positional information in the O-P equivalence group of leech.

A summary of the relevant aspects of glossiphoniid leech development is provided in Fig. 1. The main feature of gastrulation in these embryos is the rostrocaudal coalescence of bilaterally symmetric germinal bands into a germinal plate, from which segments arise. Each germinal band comprises five

columns (bandlets) of primary blast cells (Fig. 1A) differing in the timing and symmetry of their stereotyped mitoses and in the differentiated cell types to which they ultimately give rise (Zackson, 1984; Weisblat and Shankland, 1985; Shankland, 1987a,b; Bissen and Weisblat, 1989); collectively these phenotypic characteristics serve to define distinct fates for the blast cells in each bandlet. Within each germinal band, four ectodermal bandlets (n, o, p and q from ventral to dorsal) lie side by side between the squamous epithelium of a provisional integument and the underlying mesodermal bandlet (m; see Fig. 1C). Each bandlet arises from an embryonic stem cell called a teloblast. At the onset of coalescence, the germinal bands abut one another proximal to the teloblasts, diverge in their mid-regions beneath the squamous epithelium and finally merge again at their rostral ends, where coalescence begins (Fig. 1B).

Blast cell fates in the m, n and q bandlets are fixed by the teloblast of origin, which are therefore designated unambiguously as M, N and Q teloblasts, respectively. But as will be described below, ipsilateral o and p bandlets are initially equipotent and differentiate from each other according to their position within the germinal band, so their parent teloblasts (which arise as symmetric sister cells) are both designated as O/P teloblasts and their undetermined progeny are designated as o/p blast cells and bandlets. In *Helobdella triserialis*, after ablation of one O/P teloblast (Weisblat and Blair, 1984; Zackson, 1984) or its bandlet (Shankland and Weisblat, 1984), blast cells in the surviving ipsilateral o/p bandlet assume the P fate. The fate thus shown to be preferred by surviving members of experimentally truncated equivalence groups in otherwise normal embryos is called the 'primary fate'. One can also distinguish a 'default fate' for equivalence group cells, defined as the fate they follow in embryos from which putative external cues have been removed, to the extent that this is consistent with continued development. Whether or not external cues are operative in the O-P equivalence group is unclear. In *Helobdella*, ablation of one or more

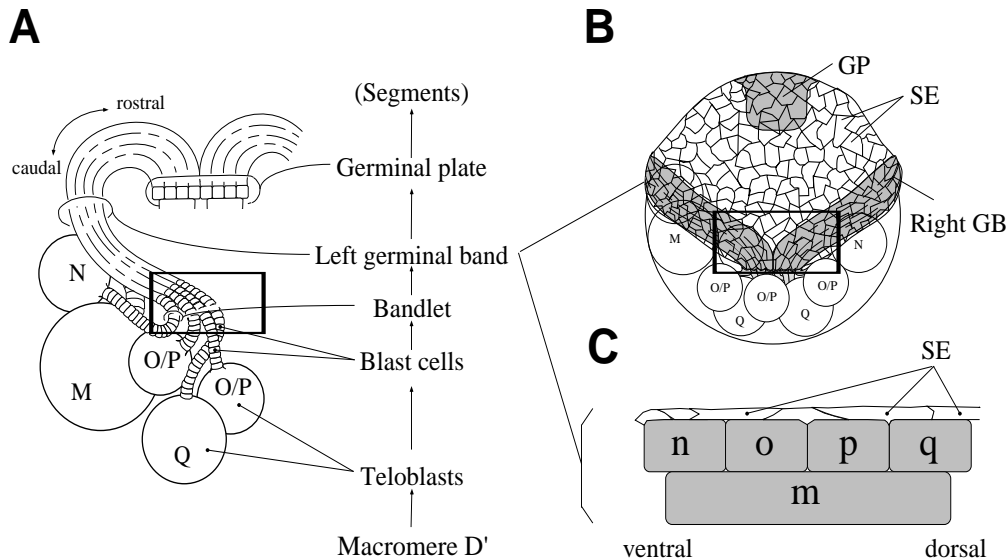


Fig. 1. (A) Schematic representation of germinal band and germinal plate formation. Mesodermal (M) and ectodermal (N, O/P, O/P and Q) teloblasts, blast cells and bandlets are shown only on the left side. (B) Dorsal view of an embryo at mid-stage 8; germinal bands (GB) and germinal plate (GP) are shaded; overlying cells of the squamous epithelium (SE) are outlined; not all teloblasts are shown. The box in A and B indicates the area where the germinal bands abut one another before diverging. (C) Cross section through a left germinal band, showing the relative positions of the squamous epithelium and the five bandlets (m, n, o, p and q).

of the ipsilateral M, N and Q teloblasts (whose progeny adjoin the o and p bandlets within the germinal band; Fig. 1C) fails to affect the differentiation of the O/P-derived bandlets (Zackson, 1984). This result was interpreted as suggesting two things: first, that the P fate is both the primary and the default fate in the O-P equivalence group; and second, that the O fate must therefore be induced by interactions between equivalent o/p blast cells. But other considerations indicate that external cues must play some role in determining the proper differentiation of the o/p blast cells and our findings suggest an alternative interpretation of these previous results.

Two observations lead us to reconsider the issue of cell fate determination in the O-P equivalence group. First, ablation experiments showed that the epithelium overlying o/p blast cells is necessary for normal expression of the O fate (Ho and Weisblat, 1987b). Second, both in normal embryos and after unilateral teloblast ablation experiments, the relative positions of ipsilateral o and p bandlets is never randomized; the O/P-derived bandlet lying closer to the dorsal edge of the germinal band invariably assumes the P fate. This suggests that external cues must impart polarity information to the cells of the O-P equivalence group. We therefore re-examined the O-P equivalence group in *Helobdella*, performing unilateral and bilateral ablations of various combinations of teloblasts, and examining the fates of the o/p blast cells at two different stages of development.

MATERIALS AND METHODS

Embryos

Helobdella robusta were collected in Sacramento, California and bred as a laboratory colony (Shankland et al., 1992). Isolated embryos were cultured at 23°C in HL saline (4.8 mM NaCl, 1.2 mM KCl, 2.0 mM MgCl₂, 8.0 mM CaCl₂ and 1.0 mM maleic acid, pH 6.6). The embryonic staging system and cell nomenclature are those of Stent et al. (1992). Macromeres and teloblasts are designated by capital letters; bandlets and blast cells are designated by the lower case letter corresponding to the teloblast of origin. Within the germinal bands, o/p bandlets and blast cells are also designated as o and p according to their relative ventral and dorsal positions, respectively.

Cell lineage tracing, teloblast ablation and fixation

For cell lineage tracing, a mixture of lysinated, rhodamine-conjugated dextran or fluorescein-conjugated dextran (RDA or FDA; 75 mg/ml; Molecular Probes) and 1% fast green in 0.2 M KCl was pressure-injected into Q or O/P teloblasts soon after their birth (stage 6b or 7, respectively). Teloblast ablation was performed at selected stages by pressure-injecting the teloblast with a mixture of ricin A chain (0.14 mg/ml; Sigma) and fast green (1%) in 0.2 M KCl.

Experimental embryos were allowed to develop until stage 8 or stage 10, then fixed overnight at 4°C (4% formaldehyde in 0.1 M Tris-HCl; pH 7.5) and briefly rinsed, at room temperature, in 0.1 M Tris-HCl (pH 7.5). Nuclei were stained by incubating the embryos at room temperature in a solution of Hoechst 33258 (1 µg/ml in 0.1 M Tris-HCl; pH 7.5). Stage 8 embryos were cleared in 80% glycerol and were examined in whole mount. Stage 10 embryos were cleared in 80% glycerol containing 40 mg/ml n-propyl gallate (Giloh and Sedat, 1982), then dissected along the dorsal midline with insect pins and flattened between slide and cover slip with the ventral surface facing up. The preparation was then sealed with clear nail polish.

In some stage 8 embryos, epithelial cells were visualized by silver staining (Arnolds, 1979; Ho, 1988). For this purpose, embryos were fixed in 0.8% formaldehyde in 0.1 M sodium cacodylic acid (pH 7.4) for 30 minutes at room temperature, rinsed briefly in distilled water, then transferred to silver methenamine solution (0.1% AgNO₃, 1% hexamethylene tetramine, and 0.25 M boric acid, pH 9.4). Embryos were incubated in the dark for 5 to 20 minutes, then exposed to strong white light until the contours of superficial cells were outlined by brownish silver precipitate. Embryos were then rinsed briefly in distilled water and fixed overnight in Carnoy's fixative (100% ethanol:chloroform:acetic acid; 6:3:1). Embryos were stored in 100% ethanol or transferred to 80% glycerol for observation.

Microscopy

Stage 8 embryos were viewed by epifluorescence microscopy (Zeiss Axiophot). Photographs were taken using Ektachrome 400 film. Stage 10 embryos were analyzed with a confocal microscope (BioRad MRC600).

RESULTS

To re-examine the process of cell fate determination in the O-P equivalence group, we examined the fate of o/p blast cells

after unilateral and bilateral ablations of various teloblasts or combinations of teloblasts. In the first series of experiments, blast cell fates were assessed on the basis of their early mitotic patterns in stage 8 embryos. Teloblasts were ablated soon after their birth (stages 4c-7) and the fates of the O/P-derived blast cells were analyzed at mid-stage 8. A bandlet was scored as following the O fate if it contained alternating large and small nuclei in the portion containing the 2-cell clones of primary blast cells (indicative of the fact that the first mitosis of the primary blast cells had been unequal); conversely, it was scored as following the P fate if it contained nuclei of about equal size throughout this region (Zackson, 1984; Shankland, 1987c; Fig. 2A,B). Unilateral ablation experiments are summarized in Table 1; bilateral and combinations of bilateral and unilateral ablation experiments are summarized in Table 2. Bandlets not accounted for in the tables are those in which the mitotic pattern was mixed or uninterpretable.

P is the primary fate in the O–P equivalence group in both *Helobdella triserialis* and *H. robusta*

The initial description and previous analyses on the O–P equivalence group were carried out using embryos of *Helobdella triserialis* (Weisblat and Blair, 1984; Zackson, 1984). More recently, however, a closely related species, *H. robusta* has provided a more reliable supply of embryos. To ensure that the O–P equivalence group is present in *H. robusta*, we first repeated some of the previous experiments using *H. robusta* embryos. Unilateral ablation of one O/P teloblast, or unilateral ablation of combinations of M, N and/or Q teloblasts were performed (Table 1). The results obtained were the same as for *H. triserialis*: in the absence of one o/p bandlet, the surviving ipsilateral o/p bandlet assumes the P fate, confirming that the P fate is the primary fate; and unilateral ablation of M, N and one O/P teloblast (Table 1) or unilateral ablation of one Q teloblast (Table 2, rows i, j) affects neither the differentiation of the o/p bandlets into the O and P fates, nor the relative positioning of o and p bandlets with respect to the dorsoventral axis. Hence, we conclude that the results obtained in *H. robusta* can be extended to *H. triserialis* and vice versa.

O is the default fate in the O–P equivalence group

To determine the fate of equivalence

group cells in a more simplified environment, we examined the mitotic patterns of o/p blast cells in embryos in two series of embryos. In one series, the M, N and Q teloblasts and one O/P teloblast had been ablated on each side of the embryo. Thus, these embryos contained an isolated o/p bandlet on each side, contacted by the overlying micromere-derived epithelium and an underlying macromere. The embryos presumably also contained non-epithelial progeny of the micromeres (Smith

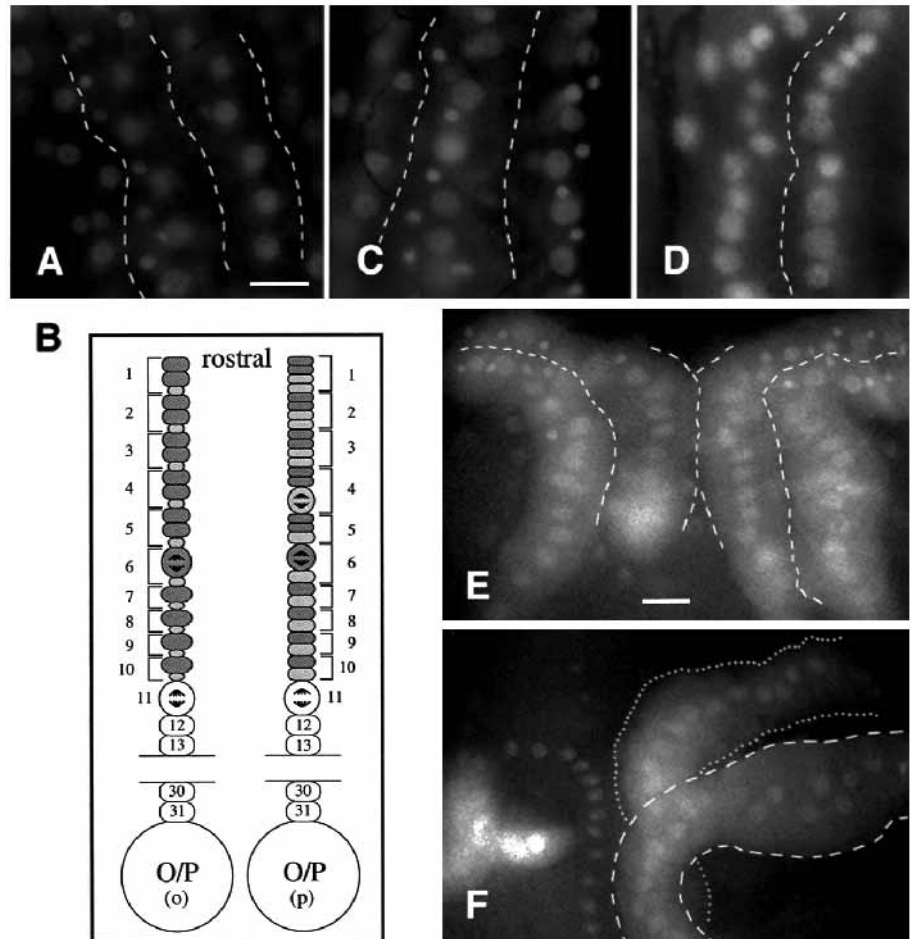


Fig. 2. Fate of o/p bandlets in response to teloblast ablations. In all panels, rostral is up. In A, C and D, ventral is left and the leading edge of the squamous epithelium and some epithelial cells are outlined by silver staining. (A) Left germinal band in a control embryo; n, o, p and q bandlets lie from left to right; approximate boundaries between bandlets are indicated by dotted lines. (B) Schematic representation of the early mitotic pattern in o (left) and p (right) bandlets. Each numbered bracket denotes the clone arising from a single blast cell. Light grey cells and dark grey cells represent the posterior and anterior secondary blast cell clones, respectively. Numbers represent the birth order of the primary blast cells in each bandlet. (C) The n and both o/p bandlets in the left germinal band of an embryo in which both Q teloblasts had been ablated. Both o/p bandlets (center and right) display alternating large and small nuclei, characteristic of the O fate. (D) The o/p (left) and q bandlets in the left germinal band of an embryo in which all teloblasts were ablated except for one O/P teloblast on each side and one Q teloblast. The surviving o/p bandlets both followed the P fate. In E and F, bandlets are viewed in the area where the germinal bands abut one another before diverging; the embryonic midline lies roughly in the middle of these panels. (E) An embryo in which M, N and Q teloblasts have been bilaterally ablated. As a result, all 4 o/p bandlets followed the O fate. (F) An atypical embryo in which both M teloblasts, both N teloblasts, and one Q teloblast were ablated; in this embryo, the right pair of o/p bandlets (upper and lower, labeled with lineage tracer and outlined with dots and dashes, respectively) both contacted the contralateral q bandlet (not labeled with lineage tracer; hence, only the Hoechst-stained nuclei are visible) and both followed the P fate. The proximal ends of the o/p bandlets are superimposed. Scale bar, 10 μ m.

Table 1. Effects of unilateral (u) ablation of teloblasts on the fate of O/P-derived bandlets in mid stage 8 embryos

Teloblasts ablated	No. of embryos	No. O/P-derived bandlets		No. P fate bandlets		No. O fate bandlets	
		left GB	right GB	left GB	right GB	left GB	right GB
(1 O/P) _u	12 (12)	10 (12)	20 (24)	10	11	0	9
(N, 1 O/P and M) _u	9 (10)	9 (9)	18(18)	9	9	0	9

After ablation, some embryos developed abnormally and were not scored; in those that were scored, not all bandlets were sufficiently well labeled to be characterized definitively. Numbers in parentheses indicate the total number of embryos surviving to stage 8 (column 1) or bandlets expected in each category (columns 2 and 3). All unilateral ablations were done on the left side of the embryo. GB, germinal band.

Table 2. Effects of bilateral (b) and unilateral (u) ablations of teloblasts on the fate of O/P-derived bandlets in mid stage 8 embryos

Row	Teloblasts ablated	No. of embryos	No. O/P-derived bandlets		No. P fate bandlets		No. O fate bandlets	
			left GB	right GB	left GB	right GB	left GB	right GB
a	(1 O/P) _b	6 (6)	3 (6)	6 (6)	3	6	0	0
b	(M, N and 1 O/P) _b	22 (22)	20 (22)	17 (22)	20	16	0	1*
c	(N) _b	5 (5)	10 (10)	8 (10)	5	4	5	4
d	(M) _b	20 (32)	40 (40)	40 (40)	15	19	25*	21*
e	(M, N, 1 O/P and Q) _b	8 (9)	8 (8)	8 (8)	0	0	8	8
f	(M, N and Q) _b	5 (7)	10 (14)	10 (14)	0	0	10	10
g	(1 O/P and Q) _b	15 (17)	13 (15)	14 (15)	0	0	13	14
h	(Q) _b	15 (15)	26 (30)	30 (30)	0	0	26	30
i	(M and N) _b and (Q) _u	7 (8)	14 (14)	12 (14)	7	7**	7	5**
j	(M, N and 1 O/P) _b and (Q) _u	4 (5)	4 (4)	4 (4)	4	4	0	0

*In some germinal bands lacking m bandlets, the ectodermal bandlets separated from one another so that the dorsal O/P-derived bandlet did not touch the q bandlet, and assumed the O fate.

**Includes one case (see Fig. 2F) in which both contralateral o/p bandlets assumed the P fate (see text). Numbers in parentheses are as in Table 1; GB, germinal band.

and Weisblat, 1994). The blast cells invariably underwent unequal divisions characteristic of normal o blast cells (Table 2, row e), and we therefore define the O fate as the default fate in this equivalence group.

In another series of embryos, bilateral ablations of the M, N and Q teloblasts were carried out, leaving pairs of O/P-derived bandlets on each side of the embryo. In these embryos, all four bandlets followed the O fate (Fig. 2E; Table 2, row f). This result indicates that interactions between the o/p bandlets are not sufficient to assure differentiation of distinct o and p bandlets. It also suggests that the P fate is induced, either directly or indirectly, by cues originating in the M, N and/or Q bandlets.

A single q bandlet is sufficient to induce the P fate bilaterally

To determine which cell type(s) (presumably m, n, and/or q blast cells) are responsible for inducing the P fate, we analyzed embryos in which different combinations of their parent teloblasts had been bilaterally ablated (Table 2). When only the two N teloblasts were ablated, normal o and p bandlets arose in their correct positions along the dorsoventral axis (Table 2, row c). Therefore the n blast cells are not required either to induce O/P-derived blast cells to follow the P fate or to give polarity information to the o and p bandlets.

In contrast, ablation of the two Q teloblasts yielded embryos in which all o/p bandlets exhibited mitotic patterns corresponding to the O fate (Fig. 2C), regardless of whether one or two o/p bandlets were present in the germinal band (Table 2, rows e-h). This shows that q bandlets are necessary to induce the P fate.

In contrast to the uniform results obtained when ablating

ectodermal (N and Q) teloblasts, bilateral ablation of the M teloblasts gave mixed results (Table 2, rows b, d). In most cases, ipsilateral O/P teloblasts in these embryos gave rise to normal o and p bandlets that occupied their normal dorsoventral positions, but in some germinal bands both ipsilateral o/p bandlets differentiated according to the O phenotype. In these rare cases, we also observed that the q bandlet was clearly separated from the adjacent o/p bandlet, whereas normally they lie in direct apposition. Disorganization of ectodermal bandlets in germinal bands from which the mesodermal bandlet is missing has been observed previously (Zackson, 1984; our unpublished observations). This result suggests that the signal from the q bandlet to the adjacent o/p bandlet operates only over a very short range.

The results obtained with bilateral Q teloblast ablation were surprising, since unilateral Q ablation does not affect the differentiation of any of the o/p bandlets (Zackson, 1984; Blair and Weisblat, 1984; Table 2, rows i, j). One explanation for these results, that the left and right Q teloblasts differ and that only one of them is capable of inducing the P fate, was ruled out by the observations that unilateral ablations of either left or right Q teloblasts fail to perturb p bandlet formation. This leaves the possibility that a single q bandlet, on either side, is sufficient to induce the P fate in both germinal bands. As a test of this hypothesis, we analyzed embryos in which all but three teloblasts were ablated, leaving one O/P teloblast on each side and either the left or the right Q teloblast. In these embryos, the O/P teloblasts gave rise to p bandlets on each side (Table 2, row j; Fig. 2D), showing that one q bandlet is sufficient to induce P fate, both in the ipsilateral and contralateral germinal bands.

In a related series of experiments, we analyzed embryos in

which all but five teloblasts were ablated, leaving all four O/P and one Q teloblast intact (Table 2, row i). In most of these embryos, the o/p bandlets on each side assumed the O and P mitotic patterns as expected. But in one such embryo, we observed that both o/p bandlets on one germinal band had divided according to the P fate. Upon inspection, we found that in this germinal band, the three bandlets formed a strand with the q bandlet contacting both o/p bandlets, rather than the normal planar array in which it only contacts one o/p bandlet (Fig. 2F).

Differences in the adhesive behaviors of q and O/P-derived blast cells

Experiments described above lead us to conclude that one q bandlet can induce the P fate in a contralateral o/p bandlet, and that the q bandlet signals only over a very short range. From these conclusions, it would seem that in embryos with only one q bandlet, the induction of the P fate in the q-less germinal band must take place at the proximal juncture of the two germinal bands, before they diverge. Moreover, since the primary blast cells of the p bandlet divide only after having diverged from the surviving q bandlet, it appears that a transient contact between the q and prospective p blast cells is sufficient to induce and maintain the P fate. It seems likely, therefore, that direct contacts between the q and nominal p bandlets at the point of origin of the germinal bands mediate induction of the P fate.

Consistent with this notion, we have made light microscopic observations suggesting that direct contacts between the q and nominal p bandlets do occur at the point where the germinal bands diverge. Moreover, we note differences in the contact behaviors of blast cells derived from O/P and Q teloblasts. After ablation of one Q teloblast, the surviving q bandlet always (6 out of 6 embryos) contacted both the adjacent ipsilateral o/p bandlet and one of the contralateral o/p bandlets. Moreover, in most cases (4 out of 6 embryos), broad and thin contralateral processes extended from blast cells in the surviving q bandlet to the contralateral p bandlet in the zone of germinal band divergence, as if a special adhesion between these cells were being broken at the point where the contralateral p and q bandlets separate (Fig. 3A). No reciprocal processes were seen from the p bandlet to the q bandlet, nor were any processes observed from the q blast cells in control embryos (0 out of 5 embryos), where the germinal bands separate via q-to-q contacts (Fig. 3B). Finally, in embryos with bilateral Q teloblast ablations, so that the germinal bands contact one another via their o/p bandlets, they frequently failed to separate normally, so that instead of abutting over only a narrow range, they remain in contact over the length of several blast cells (Fig. 2E). This suggests that contacts formed between two o/p bandlets are of significantly higher affinity than those between two q bandlets, with heterologous contacts being of intermediate affinity.

P-to-O fate conversion of nominal p bandlet is maintained throughout development

It has been shown previously that the differences in the symmetry of the initial mitoses of primary o and p blast cells do not indicate an irreversible commitment to the O and P fates (Shankland and Weisblat, 1984; Shankland, 1987c; Ho and Weisblat, 1987b; Lans et al., 1994). To determine whether the

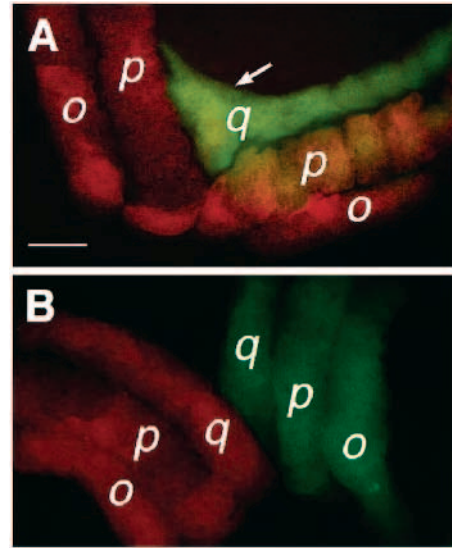


Fig. 3. Interactions between p and q blast cells. Stacked series of confocal images of experimental (A) and control (B) embryos at mid stage 8, viewed in the region where the germinal bands abut one another before diverging (see Fig. 1B). (A) Experimental embryo in which the left Q teloblast had been killed. At the point of germinal band divergence, the surviving q bandlet has formed an extensive process (arrow) where it separates from the contralateral O/P-derived bandlet. (B) Comparable view of a control embryo showing o, p and q bandlets on each side. Scale bar, 30 μ m.

P-to-O fate conversions, seen at mid-stage 8 in response to bilateral Q teloblast ablation, are maintained in the terminally differentiated progeny of the primary blast cells, we examined the progeny of o/p blast cells in domains of stage 10 embryos that lacked one or both q bandlets. For this purpose, we ablated one Q teloblast at birth (stage 6b) and injected an ipsilateral O/P teloblast with lineage tracer at the beginning of stage 7. The contralateral Q teloblast was ablated 10-15 hours later (mid stage 7), by which time it had made progeny destined for the anterior 8-10 segments. The resulting embryos were then allowed to develop to stage 10. In such embryos, the nominal p bandlets consist of anterior domains that had experienced the transient, short range inductive effects of the contralateral q bandlet, and posterior domains that had experienced no such inductive interactions.

In normal development, each of the five types of bandlets (m, n, o, p and q) gives rise to a distinct, segmentally iterated pattern of definitive progeny, comprising individual cells and cell clusters whose shapes or positions can be used to diagnose the identity of the bandlet of origin (Weisblat and Shankland, 1985). Diagnostic patterns elements for the O and P fates are summarized in Fig. 4A,B.

Ablation of the Q teloblasts yields embryos with severe deficits in definitive epidermis (Blair and Weisblat, 1984) and greatly increased mortality. Thus, of 71 experimental embryos, only 26 survived to stage 10. The labeled o/p bandlet was missing or developed abnormally in 18 of these embryos, presumably due to over-injection of lineage tracer. Among the eight embryos suitable for analysis, four exhibited an O pattern of labeled cells that was normal (save for perturbations in the size and position of epidermal cells (Blair and Weisblat, 1984) in both the anterior and posterior domains; this indicates that

the nominal o bandlet had been labeled in these embryos, which were thus of no use except to demonstrate that the fate of the o bandlet is not affected by the absence of one or both q bandlets. The remaining four embryos exhibited the P pattern of labeled progeny in the anterior domain, indicating that the p bandlet had been labeled and that, as expected, the P fate induced by transient contact with the contralateral q bandlet had been maintained through stage 10. But in the posterior domain of these embryos, the labeled o/p bandlet had given rise to the O pattern of differentiated progeny, indicating that

the P-to-O fate conversion seen in the absence of the short range q bandlet signal was maintained throughout the terminal differentiation of the clones.

Fig. 4C shows one such embryo. In the four anteriormost segments, the o/p blast cells have differentiated according to the P fate: a small wedge of ganglionic neurons lies midway along the rostrocaudal axis of the ganglion and there is a large contribution to the epidermis. After two transitional segments, in which a mixture of O and P pattern elements are present, the four posteriormost segments exhibit a distinct O pattern of

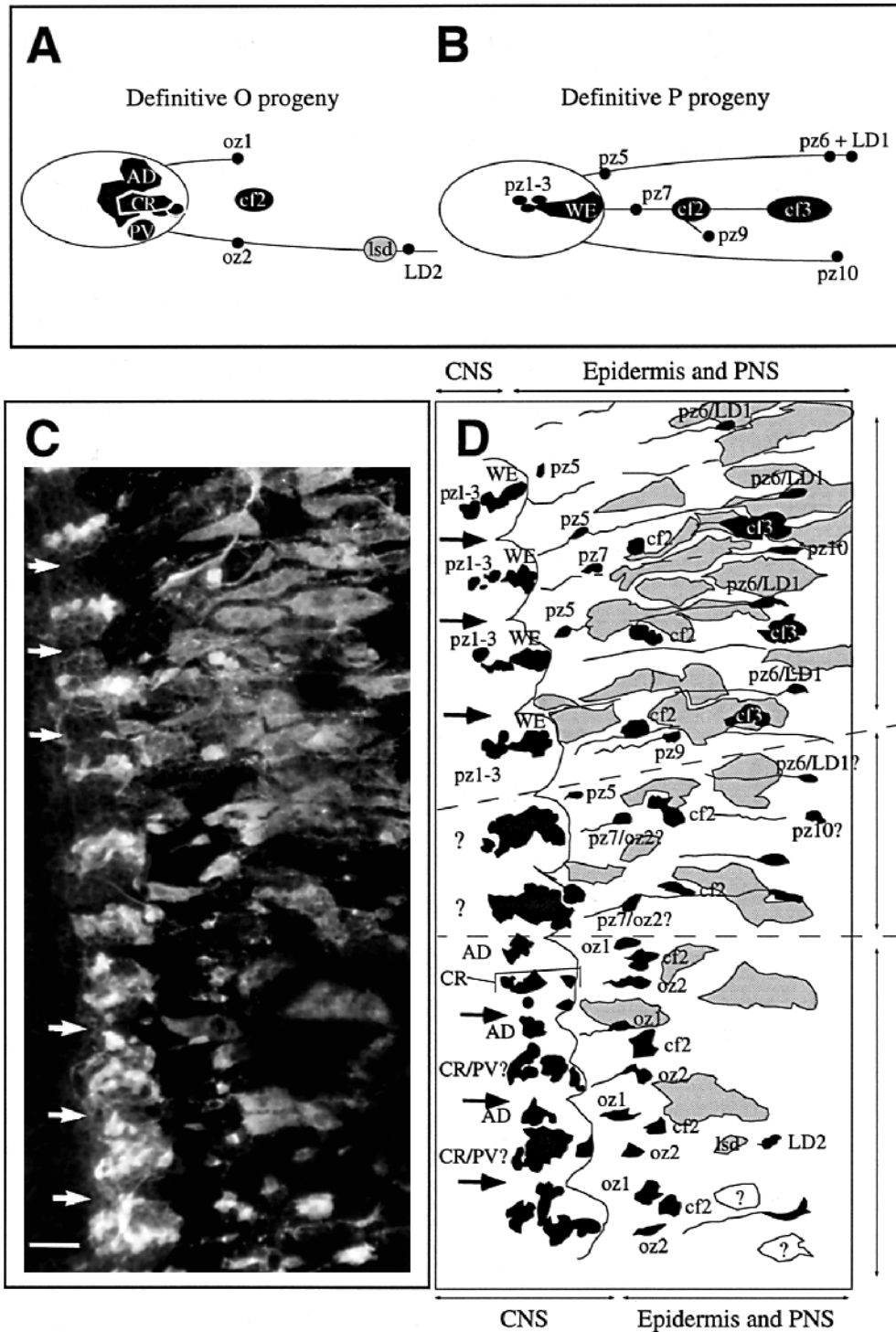


Fig. 4. Fate of o/p bandlets in stage 10 embryos. Rostral is up in all panels. (A,B) Schematic representation of definitive O and P progeny, respectively. In each panel, the large oval represents the segmental ganglion at the ventral midline; individual cells or groups of cells that serve as diagnostic pattern elements are indicated for the right side of the germinal plate. (C) Stacked series of confocal micrographs of a stage 10 embryo, showing the definitive progeny of a nominal p bandlet in the right half of the germinal plate. One Q teloblast had been ablated at its birth and an ipsilateral O/P teloblast was injected with lineage tracer; the contralateral Q teloblast was ablated 10 to 15 hours later. Boundaries between the four most rostral and four most caudal ganglia are indicated by arrows along the ventral midline. In the four rostralmost segments, labeled cells are characteristic of the P fate: a small wedge of ganglionic neurons midway along the rostrocaudal axis of the ganglion and a large contribution to the epidermis. After two transitional segments, labeled cells are characteristic of the O fate: relatively large numbers of neurons in clusters throughout the ipsilateral hemiganglion and relatively few epidermal cells. (D) A tracing of C, showing identified neurons and neuron clusters. Neurons or neuron clusters are shown in black; epidermal cells are shown in grey. AD, anterodorsal cell cluster; cf, cell flocet; CNS, central nervous system; CR, crescent neuron cluster; I, intermediary segments; lsd, lateral skin dot. LD1 and LD2, lateral dopamine-containing neurons; oz and pz, identified neurons characteristic of the O and P fates, respectively; PNS, peripheral nervous system; PV, posteroventral cell cluster; WE, wedge-shaped neuron cluster; ?, ambiguous elements. Scale bar, 20 μ m.

terminally differentiated progeny: there are relatively large numbers of neurons in clusters throughout the ipsilateral hemiganglion and relatively few cells in the epidermis. This result shows both that a *transient* signal from one q bandlet is sufficient to induce the P fate in the contralateral o/p bandlet and also that this early P fate induction is maintained throughout division and differentiation of an o/p primary blast cell clone. In the absence of the signal from the q bandlet, the o/p blast cells assume and maintain the O fate.

DISCUSSION

Embryos in which identifiable cells have predictable fates in normal development are advantageous for separating the roles of intrinsic and extrinsic factors in determining cell fate. In the ectodermal layer of *Helobdella robusta* embryos, nominal o and p primary blast cells are initially equipotent and assume distinct O and P fates on the basis of their relative ventral and dorsal positions within the germinal bands. Here, we have shown that primary q blast cells, the dorsalmost cell line in the ectoderm, break the equipotency of the o and p cells by inducing the P fate through a short range interaction with the p blast cells. In addition, our results reveal several features of the process by which cell fates are determined in the O-P equivalence group of *Helobdella* as follows:

Inductive interactions between o/p blast cells are neither necessary nor sufficient to pattern the O-P equivalence group

Inductive interactions between initially equipotent cells are thought to act in patterning fields of cells, for example in chick limb (Newman, 1996) and in the insect nervous system (Ghysen et al., 1993). It was previously suggested that the initially equipotent o/p cells also differentiate from each other as the result of mutual induction (Zackson, 1984). But we have found that a single o/p bandlet can be reliably directed to assume either the O or P fate, and that when two o/p bandlets are the only component of a germinal band, they can both assume the O fate (Table 2). These results demonstrate that within the context of the selective perturbations carried out here, inductive interactions between cells in the O-P equivalence group are neither necessary nor sufficient to break their equivalence.

A signal from q blast cells is necessary and (usually) sufficient to pattern the O-P equivalence group

Even if inductive interactions between o/p blast cells did determine fates in the O-P equivalence group, an external signal would still be required to account for the polarity of the o and p bandlets, i.e. the fact that the p bandlet always lies dorsal to the o bandlet within the germinal band. In contrast, a fate-determining signal from outside the equivalence group can simultaneously break the equivalence of the cells and account for the polarity of the differentiated cells. This is exemplified by our results showing that q cells are necessary and usually sufficient to break the symmetry of the O-P equivalence group by inducing the P fate in the more dorsal of the two O/P-derived bandlets. We speculate that the roughly planar arrangement of the ectodermal bandlets (in ventrodorsal order n, o/p, o/p, q) within the germinal bands at the time of the fate-deter-

mining interactions (see below) normally allows only one o/p bandlet to receive the postulated short range signal from the q bandlet, and also insures that, after the induction, the bandlets lie in ventrodorsal order n, o, p, q.

That the q bandlet signal is necessary for fate determination in the O-P equivalence group is demonstrated by the results that ablating both Q teloblasts invariably causes all o/p bandlets to assume the O fate. But the evidence that the q bandlet signal is sufficient to provide the fate determining signal for the O-P equivalence group is less clear cut. For one thing, there are practical limitations on our ability to delete some cells in the embryo (macromeres, deep micromeres) and still be able to assess the fate of the o/p bandlets. Moreover, in some embryos, the dorsal o/p bandlet assumes the O fate when only the M teloblasts are ablated; we interpret these cases as resulting secondarily from separations between ectodermal bandlets that sometimes occur in the absence of the underlying m bandlet.

The signal from the q bandlet acts over short distances at the point of germinal band divergence and need only be present for a short time

Converging lines of evidence indicate that: the q blast cells induce o/p blast cells to follow the P fate by direct contact or short range inductive interactions; and even a few hours of interaction, at the point where the germinal bands abut one another proximal to the teloblasts, can induce a permanent fate change. The fact that embryos with intact q bandlets in only the anterior germinal plate exhibit the P-to-O fate change in only the posterior germinal plate indicates that the q-derived signal acts locally. The local nature of the q signal, together with the observation that a single q bandlet can induce the P fate bilaterally, means that the site of the fate-determining interaction in such embryos must be at the point of germinal band divergence, since this is the only site where the q and contralateral o/p bandlets are near one another. Finally, since q and contralateral o/p blast cells are near one another for only a few hours as they pass through the point at which the germinal bands abut, it follows that a transient exposure to the signal has, either directly or indirectly, a permanent effect on the fate of the p bandlet cells.

Two lines of evidence indicate that P fate-determining signals are not limited to the zone of germinal band divergence, however. First, when p bandlet cells are photolesioned in germinal bands well beyond the zone of divergence, the nominal o blast cells are still capable of assuming the P fate (Shankland and Weisblat, 1984); in light of our findings, the most parsimonious explanation for their result is that, upon p bandlet ablation, the nominal o bandlet now has access to P-fate inducing signals from the q bandlet. Second, photolesioning domains of the overlying squamous epithelium, also at a site beyond the zone of germinal band divergence, causes some o blast cell progeny to assume P fates, even in the presence of an intact p bandlet (Ho and Weisblat, 1987b). It could be that damaging the epithelium also affects the access to a P-fate inducing signal again, presumably, from the q bandlet. Alternatively, it could be that in the absence of the epithelium, the P fate is the default fate for the O-P equivalence group.

Cell fate determination after O/P teloblast duplication: equipotency or equivalence?

It has been previously demonstrated that supernumerary

teloblasts result when a newborn teloblast undergoes an extra, equal division before initiating blast cell production (Ho and Weisblat, 1987a). These supernumerary teloblasts retain the character of their lineage and can contribute apparently normal columns of blast cells to the germinal bands and definitive progeny to the germinal plate. In examining the fates of the three ipsilateral o/p bandlets that result when one O/P teloblast is duplicated, Lans and co-workers (1994) found that the three bandlets lie side by side within the germinal band between the n and q bandlets, with the bandlets derived from the duplicated teloblast almost always lying next to each other (i.e. in the dorsal and middle positions if a nominal P teloblast was duplicated or in the ventral and middle positions if a nominal O teloblast was duplicated). In such embryos, it was found that the bandlets derived from the duplicated teloblast assume the same fate, yielding embryos with supernumerary o bandlets and definitive progeny when a nominal O teloblast was duplicated and supernumerary p bandlets and definitive progeny when a nominal P teloblast was duplicated.

Two alternative interpretations were suggested for these results. One is that the two O/P teloblasts are equipotent, but not equivalent (i.e. one O/P teloblast is born with a preference for the O fate and other with a preference for the P fate). The second interpretation is that the O/P teloblasts are indeed equivalent, but that the bandlets derived from the duplicated teloblast are somehow coupled in a way that normal o/p bandlets are not.

Although this question remains open, our present finding, that the P fate can be induced by transient interactions with q blast cells as the o/p bandlets enter the germinal band, is consistent with the notion that the o/p blast cells are equivalent and that slight differences in positional interactions of the duplicated bandlets are responsible for the results reported by Lans et al. For example, Fig. 2F shows an embryo in which one Q teloblast had been ablated, along with both N and both M teloblasts. In most such embryos, the o/p bandlets follow their normal O and P fates (Table 2); but in this exceptional embryo, both o/p bandlets in the right germinal band contacted the surviving contralateral q bandlet as they entered the germinal band. Within the germinal band, the bandlets fall into the normal planar array, but both exhibit the mitotic pattern of the P fate. Assuming that the bandlets arising from duplicate O/P teloblasts lie close together at their point of entry into the germinal bands, it seems reasonable that they would have a high probability of exhibiting similarly linked cell fates, both contacting the q bandlet (and therefore assuming the P fate) if a nominal P teloblast were duplicated, and neither contacting the q bandlet (and therefore assuming the O fate) if a nominal O teloblast were duplicated.

Comparison with other organisms

In addition to *H. triserialis* and *H. robusta*, species of another glossiphoniid leech genus, *Theromyzon*, have also been used for studies of development (Fernandez, 1980; Sandig and Dohle, 1988; Torrence, 1991; Gleizer and Stent, 1993). *Theromyzon* embryos feature cleavage patterns, morphogenetic processes and patterns of definitive progeny that are very similar to those in *Helobdella* (Sandig and Dohle, 1988; Bissen and Weisblat, 1989) but it is not clear whether the O-P equivalence group as such exists in *Theromyzon*. In response to ablation of a nominal P teloblast, some but not all of the

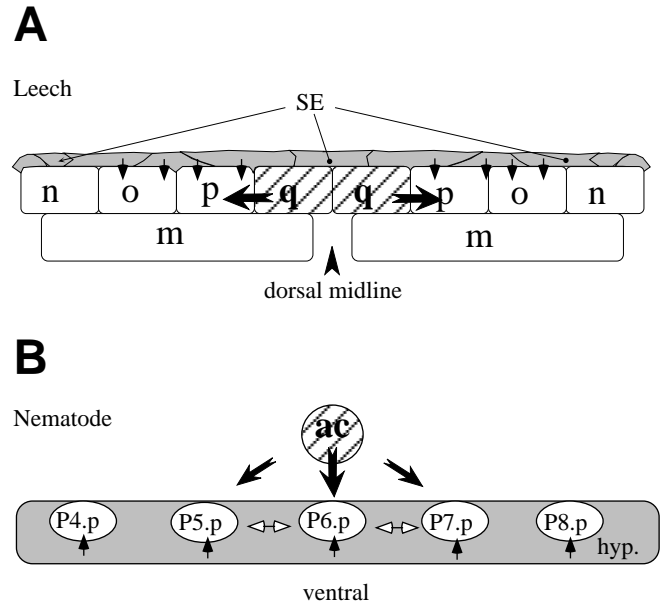


Fig. 5. Diagrams comparing cell interactions in (A) leech and (B) nematode equivalence groups. (A) Transverse view of the area where the germinal bands abut one another in *Helobdella*, showing the initially equipotent o/p bandlets relative to the m, n, and q bandlets. (B) Sagittal view of the ventral mid-region of *Caenorhabditis*, showing the initially equipotent Pn.p cells; anterior is to the left. In both panels, large and medium black arrows represent the localized signal, from the q bandlet or the anchor cell (ac), that breaks the equivalence and defines the polarity of the cell fate hierarchy; small black arrows represent the widespread signal, emanating from the squamous epithelium (SE) or the hypodermis (hyp), that induces the default fate. Double-headed white arrows represent interactions between equivalent cells in nematode.

nominal o blast cells change fate, (Keleher and Stent, 1990). These differences indicate that knowledge of the interactions governing cell fate decisions in *Theromyzon* can be inferred only partially from results obtained with *Helobdella* and merit independent investigation.

We have shown that in the O-P equivalence group of *Helobdella*, induction of the primary (P) fate requires a signal from the q bandlet, which is thus analogous to the anchor cell in the nematode vulval equivalence group (Fig. 5). In embryos lacking q bandlets, all O/P-derived bandlets adopt the default (O) fate, just as all the vulval equivalence group cells adopt the tertiary fate when the anchor cell is killed. But previous work suggests that the default fate in *Helobdella* is itself induced by a broadly distributed signal from the overlying epithelium (Ho and Weisblat, 1987a), analogous to induction of the tertiary fate by hypodermis in nematode (Herman and Hedgecock, 1990). Thus, in both species, a uniform default fate is specified by a widespread epithelial signal, while a local signal simultaneously breaks the equivalence and defines the polarity of the cell fate hierarchy (Fig. 5). Notwithstanding the obvious importance of species-specific aspects of cell fate determination, the similarity of the equivalence groups in annelid and nematode suggests that the same scheme may function in other equivalence groups exhibiting positionally determined cell fate hierarchies. For example, equivalence groups have been described in grasshopper (phylum Arthropoda; Kuwada and

Goodman, 1985) and ascidians (phylum Chordata; Nishida and Satoh, 1989). In each of these systems, as previously in leech, it has been suggested that the equivalence-breaking interaction occurs between the equipotent cells themselves, but this leaves the question of polarity unresolved. Our results suggest that in such systems, external cues that both break equivalence and provide polarity information may yet be found.

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