Stepwise Commitment of Blast Cell Fates during the Positional Specification of the O and P Cell Lines in the Leech Embryo

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The o and p bandlets of the leech embryo are parallel columns of ectodermal blast cells which are identified by their relative positions, and which during normal embryogenesis follow distinct developmental pathways. A previous study showed that o blast cells are initially capable of following either the O or P pathway, and suggested that commitment to the O pathway depends upon interaction with the adjacent p bandlet. To better understand the nature and timing of this interaction we examined the fate of o blast cells whose p blast cell neighbors had been selectively ablated by photoexcitation of a fluorescent lineage tracer. If an o blast cell has not yet begun its secondary divisions, its normal commitment to the O pathway can be effectively prevented by ablation of the adjacent p bandlet. Comparing the outcome of progressively later lesions reveals that the progeny of the o blast cell become committed to the O pathway in a series of three discrete steps, and that these steps occur around the time of the first three blast cell divisions. Each of the three events affects a different subset of elements within the blast cell clone, and apparently commits those elements to either the O or P pathway depending upon the presence or absence of the other bandlet. These changes in blast cell fate are coextensive with the lesion along the bandlet's length, suggesting that the interaction of the two bandlets is localized to neighboring cells. © 1984 Academic Press, Inc.

INTRODUCTION

The egg of the glossiphoniid leech undergoes an orderly sequence of embryonic cleavages (Whitman, 1878; Fernández, 1980). By labeling individually identified blastomeres with vital cell lineage tracers and following the fate of their descendant clones, it has been shown that these cleavages subdivide cell fates in a highly determinate fashion (Weisblat *et al.*, 1978, 1980a, 1984). Many of the blastomeres have unique identities which appear to be maintained independent of cell interaction (Blair, 1982, 1983; Zackson, 1984), and which may place constraints upon their developmental fate. However, other groups of blastomeres are born with a common developmental potential, forming an "equivalence group" (Sulston and White, 1980), and are subsequently committed to different developmental pathways on the basis of position (Weisblat and Blair, 1984; Shankland, 1984). The present paper deals with the cellular events which bring about the positional specification of divergent pathways within one such equivalence group.

During stages 4–6 of leech embryogenesis the egg cleaves to produce two bilateral sets of blastomeres called *teloblasts*. The M, N, and Q teloblasts can be uniquely identified by their size, lineage, and position; however, each bilateral set also includes two O/P teloblasts which are sister cells arising from a symmetrical cleavage of variable orientation. These teloblasts bud off several dozen much smaller *primary blast cells*[2] which depart from the teloblast in discrete columns or *bandlets* (Fig. 1A), with both blast cell and bandlet being designated by lower case letters corresponding to the teloblast of origin. The five ipsilateral bandlets then converge in parallel to form a hemilateral *germinal band.* Convergence begins at the anterior end, and proceeds posteriorly by the sequential incorporation of progressively younger cells.

Tracer injections show that each of the five teloblasts gives rise to a specific complement of descendant tissues in the mature leech (Stent *et al.*, 1982; Weisblat *et al.*, 1984). The sister O/P teloblasts give rise to distinct O and P descendant patterns, but there is no clear correlation between the position of an O/P teloblast and its developmental fate (Weisblat and Blair, 1984). However, developmental fate is correlated with the relative position of the two o/p bandlets within the ectodermal monolayer of the germinal band. The o/p bandlet which lies closer to the micromere cap is designated the p bandlet and gives rise to the P descendant pattern, while the o/p bandlet further away from the micromere cap is designated the o bandlet and gives rise to the O pattern (Fig. 1B).

In a normal embryo one can determine the *generative*

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[2] Previously called "stem cells."

A

Germinal Band

Blast Cell Bandlet

Primary Blast Cell

Teloblast

B

Micromere Cap

Ectoderm

q p o

m n

Mesoderm

Endoderm
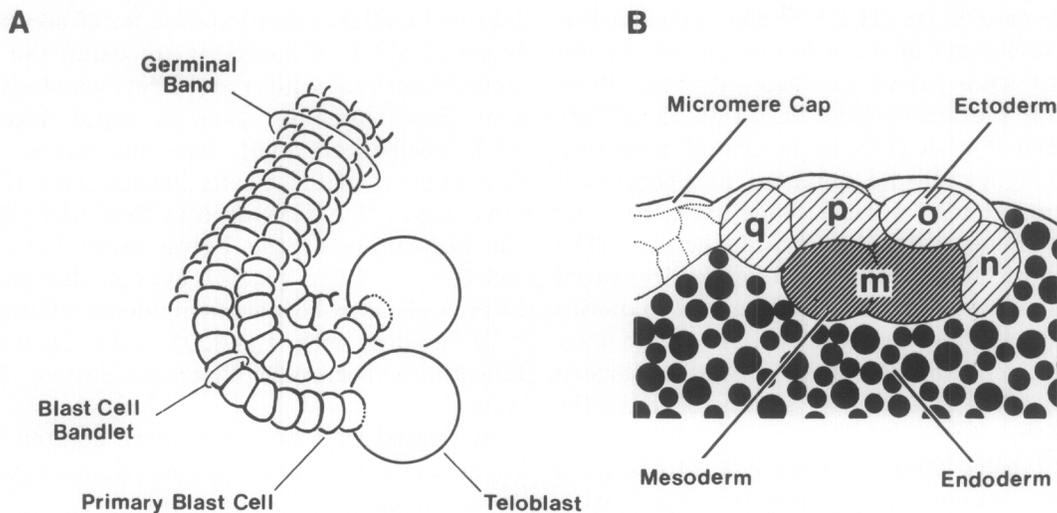
FIG. 1. (A) Germinal band formation in the embryo of the glossiphoniid leech *Helobdella triserialis.* During embryonic stage 7 each of the five teloblasts undergoes a sequence of highly asymmetrical divisions and gives rise to a bandlet consisting of several dozen primary blast cells. Drawing shows the four ectodermal bandlets coming together in parallel to form the outermost layer of the hemilateral germinal band. (B) Transverse section showing the organization of bandlets within the germinal band. The larger m bandlet forms the mesoderm, and is two cells in width at the time it enters the band. The n, o, p, and q bandlets form the ectodermal cell layer, and are arranged in alphabetical order. At this stage the definitive ectoderm is covered by a larval epithelium of micromere origin, and the band as a whole is supported by the yolk-filled endodermal blastomeres.

O or P identity of a given O/P teloblast by either the position or descendant fate of its blast cell bandlet. However, experiments in which one O/P teloblast was labeled with a tracer and the other ablated by toxic enzyme injection have shown that the O and P fates are segregated between the two sister teloblasts without any apparent restriction of developmental potential (Weisblat and Blair, 1984). Ablating the generative O teloblast has relatively little effect on the development of the P cell line, while ablating the generative P teloblast causes the ipsilateral O cell line to change fate and give rise to a P-like descendant pattern. To distinguish this result from fate changes of a purely pathological nature, the term "transfating" was introduced to describe instances in which a cell is experimentally induced to assume the fate of a different cell.

These findings were explained by assuming that (i) o/p blast cells are initially capable of following either the O pathway or the P pathway; (ii) these blast cells become committed to one or the other pathway depending upon the o or p position of their bandlet within the germinal band; and (iii) the two pathways form a developmental hierarchy, with a single o/p bandlet being constrained to the primary P pathway regardless of its original fate (Weisblat and Blair, 1984). This interpretation implies that in a normal embryo the anticapward or o bandlet is diverted from its primary pathway into the secondary O pathway by some form of interaction with the p bandlet that lies beside it (Zackson, 1984).

In this paper we examine the timing and nature of the cellular interactions which bring about the commitment of the o blast cells to the O pathway. Portions of the p bandlet were ablated by photoexcitation of a fluorescent lineage tracer injected into and inherited from the ancestral teloblast (Shankland, 1984). The results show that a primary o blast cell can still be made to follow the P pathway after it has entered the germinal band by the selective ablation of its p blast cell neighbors. By comparing the response of progressively older blast cells we could then reconstruct the sequence of events by which an initially uncommitted o blast cell loses its response to p bandlet ablation and becomes autonomously committed to the O developmental pathway.

METHODS

Eggs were taken from a *Helobdella triserialis* breeding colony maintained at 23.5°C in Black Mountain spring water and fed with physid snails. Embryonic stages and cell lineage nomenclature are those of Stent *et al.* (1982), as amended by Weisblat and Blair (1984).

Lineage tracers. Teloblasts were pressure injected with cell lineage tracers through 5–10 MΩ glass microelectrodes (Weisblat *et al.*, 1978, 1980b). Tracers were dissolved in 0.2 *M* KCl, with 0.1–0.6% fast green FCF added for visual monitoring. Injected embryos were raised in HL saline (Blair and Weisblat, 1984), supplemented with 50 μg/ml tetracycline, 100 units/ml penicillin-streptomycin, and 40 μg/ml gentamycin.

Horseradish peroxidase (HRP; Sigma type IX) was injected into teloblasts at a concentration of 4%. Injected embryos were raised to stage 10, fixed 30–60 min with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and reacted with H_2O_2 in saturated benzidine. After being dehydrated and mounted in Epon, most specimens were cut with a razor blade and viewed as hundred micrometer thick horizontal sections. The overall pattern of labeled cells could be readily traced in these preparations, using Nomarski optics to distinguish the location of labeled cells relative to the skin, nerve cord, and nephridia. The visibility of the brown reaction product was enhanced by placing a blue filter in the light path.

Other HRP-labeled embryos were cut into serial 3 μm transverse sections, lightly counterstained with toluidine blue, and viewed with phase contrast optics in order to verify the identity of cells seen in the handcut sections. Cell counts were obtained by summing the number of labeled profiles with distinct nucleoli over a continuous series of sections.

Fluorescently labeled 10 kDa dextrans were also used as lineage tracers and viewed in living embryos using epifluorescence. Fluorescein-dextran (FDX; peak absorption 490 nm) was obtained from Polysciences, Inc. Teloblasts were injected with 10% FDX, and their labeled progeny visualized with Zeiss filter set 48 77 17.

Rhodamine X-dextran (XDX; peak absorption 580 nm) was synthesized by J. Braun using the isothiocyanate linking technique of de Belder and Granath (1973). Using cyanogen bromide as a linking agent an average of two lysine molecules were added per dextran chain to the XDX employed in this study (J. Braun and R. L. Gimlich, in preparation). Lys-XDX was coinjected with HRP at a concentration of 1.5%, and visualized with filter set 48 77 15. This tracer has two notable advantages over the unlysinated dextrans: (i) it is preferentially concentrated within the nucleus of the labeled cell, and can be used to count tracer-labeled cells in living embryos; and (ii) it is readily fixed with 4% paraformaldehyde in HEPES-buffered saline (pH 7.4). Fixed embryos were counterstained with 2.5 μg/ml of the fluorescent DNA stain Hoechst 33258, and mounted in TRIS-buffered glycerol (pH 9) with 4% n-propyl gallate added to retard fluorescent bleaching. Hoechst fluorescence was viewed with filter set 48 77 02.

Ablations. Blast cells labeled with FDX can be selectively photoablated by exciting the fluorescent dye within the living cell (Shankland, 1984; see also Miller and Selverston, 1979). The injected embryo is held in a pool of saline beneath a Plan-Neofluar 25× water immersion objective (N.A. 0.8), and rotated so that the target cells face the objective. Each sector of the labeled bandlet is then exposed for 60 sec to the focused beam of a 100 W mercury arc using the above-mentioned fluorescein filter set. Previous study has shown that this procedure leads to rapid degeneration of FDX-labeled cells, but does not impair the normal development of blast cells labeled with HRP (Shankland, 1984). The XDX used in this study has <2.5% of the absorption of the Polysciences FDX within the 480–500 nm band passed by our fluorescein exciter filter, and control experiments demonstrated that blast cells labeled with Lys-XDX also develop normally following exposures sufficient to destroy FDX-labeled cells.

Individual O/P teloblasts were ablated by injecting 1% deoxyribonuclease I (DNase; Sigma type III) in 0.15 M NaCl (Blair, 1982).

RESULTS

Anatomy

At stage 10 organogenesis is well underway, and the leech embryo is comprised of 32 largely isomorphic body segments. The O/P teloblasts contribute the vast majority of their descendants to the nervous system, epidermis, and nephridia, the spatial organization of which is depicted in Fig. 2A for a typical midbody segment.

The ventral nerve cord is comprised of segmental ganglia which at this stage already contain close to their mature complement of 400 neurons apiece (Weisblat *et al.*, 1984). On either side of the ganglion there are four separate nerve roots which appear to correspond to the nerve trunks seen in other leech species (Ort *et al.*, 1974; Kramer and Goldman, 1981) and have been named accordingly. The anterior-anterior (AA), medial-anterior (MA), and posterior-posterior (PP) nerves extend directly to the body wall, and there project in parallel around the circumference of the hemisegment (Fig. 2A). The MA nerve courses internal to the body wall musculature, but the AA and PP nerves lie just beneath the skin over most of their length (Fig. 3B). The dorsal-posterior (DP) nerve projects away from the ventral body wall along the dorsoventral muscles, and will receive no further mention.

Early in development the leech embryo has an epidermis of micromere origin, but during stage 10 the definitive, teloblast-derived epidermis replaces this provisional covering and expands to envelop the entire body surface (Stent *et al.*, 1982; Weisblat *et al.*, 1984). For the most part the definitive epidermis consists of a simple squamous epithelium; however, each hemisegment also contains six discrete islands of rounded epithelial cells which have been called *cell florets* due
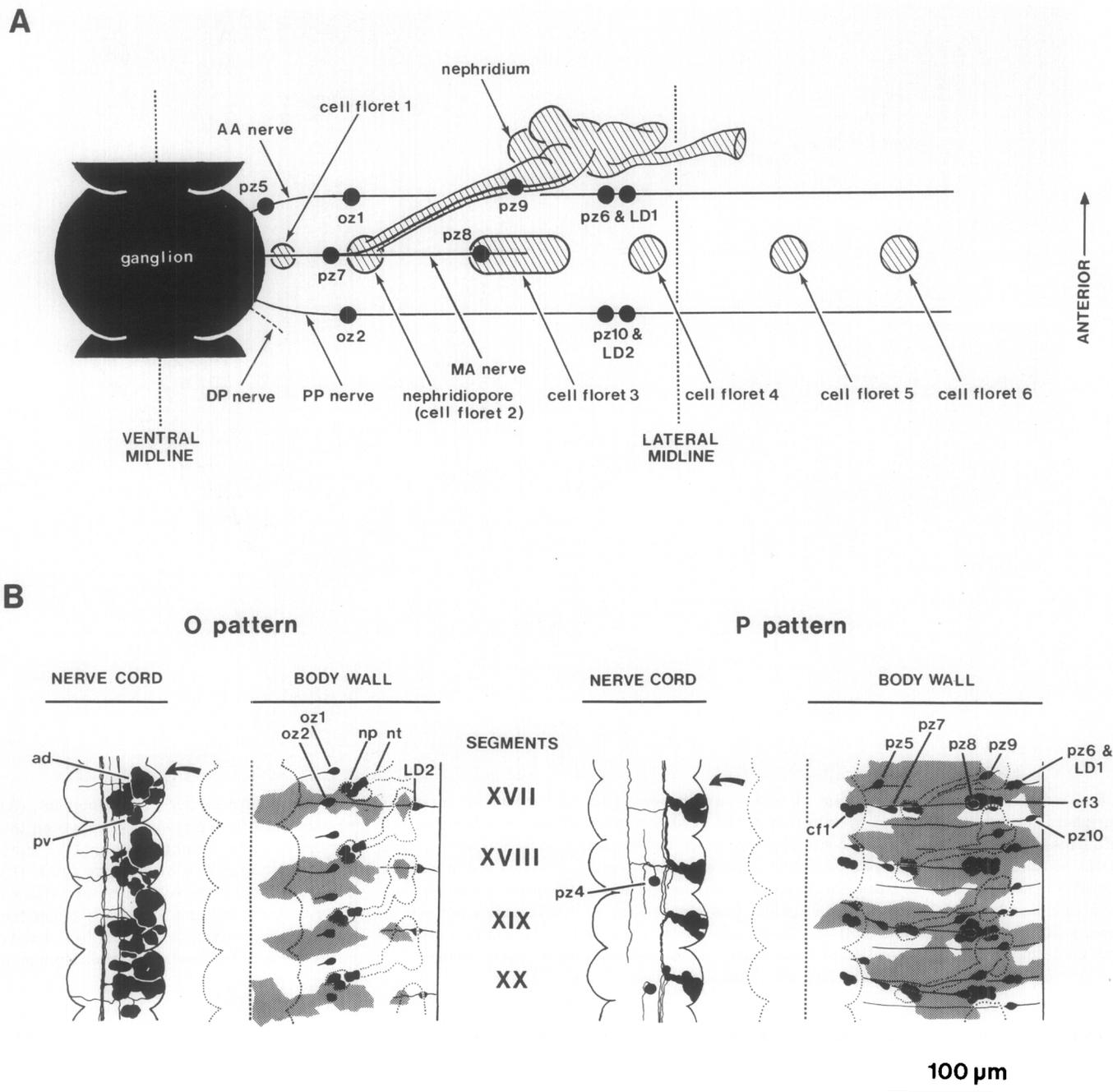
FIG. 2. (A) Diagram depicting the anatomy of a single hemisegment at stage 10 of embryogenesis. The body wall has been unfolded to show the relative positioning of the various peripheral organs. (B) Camera lucida tracings of HRP-labeled O and P descendent patterns in a series of four consecutive midbody segments. Labeled cell bodies and axons are shown in black, with the shaded regions representing labeled portions of the squamous epidermis. These cells were traced from handcut horizontal sections of whole embryos, and—in contrast to A—their distribution has not been corrected for body wall curvature. The ventral nerve cord is shown separately, and its location relative to the body wall is marked by a dotted outline. The nephridia are also shown by a dotted outline on the right side of each segment. Note that the labeled tissues lie on only one side of the midline (dotted line), and that—with the exception of the P-derived neuron pz4— the pattern of labeled tissues is repeated in every segment. The 32 body segments are numbered from head to tail, with the unfused abdominal segments designated V–XXV. ad, anterodorsal cell cluster; pv, posteroventral cell cluster; nt, nephridial tubule; np, nephridiopore; cf1 & cf3, cell florets 1 and 3; peripheral neurons as in the text.

to their bloom-like intrusion into the body cavity (Fig. 4). The cell florets are numbered 1–6 in ventral-to-dorsal order (Fig. 2A), but—with the exception of cell floret 2 (see below)—we do not yet know their ancestral relationship to the epidermal specializations of the adult leech.
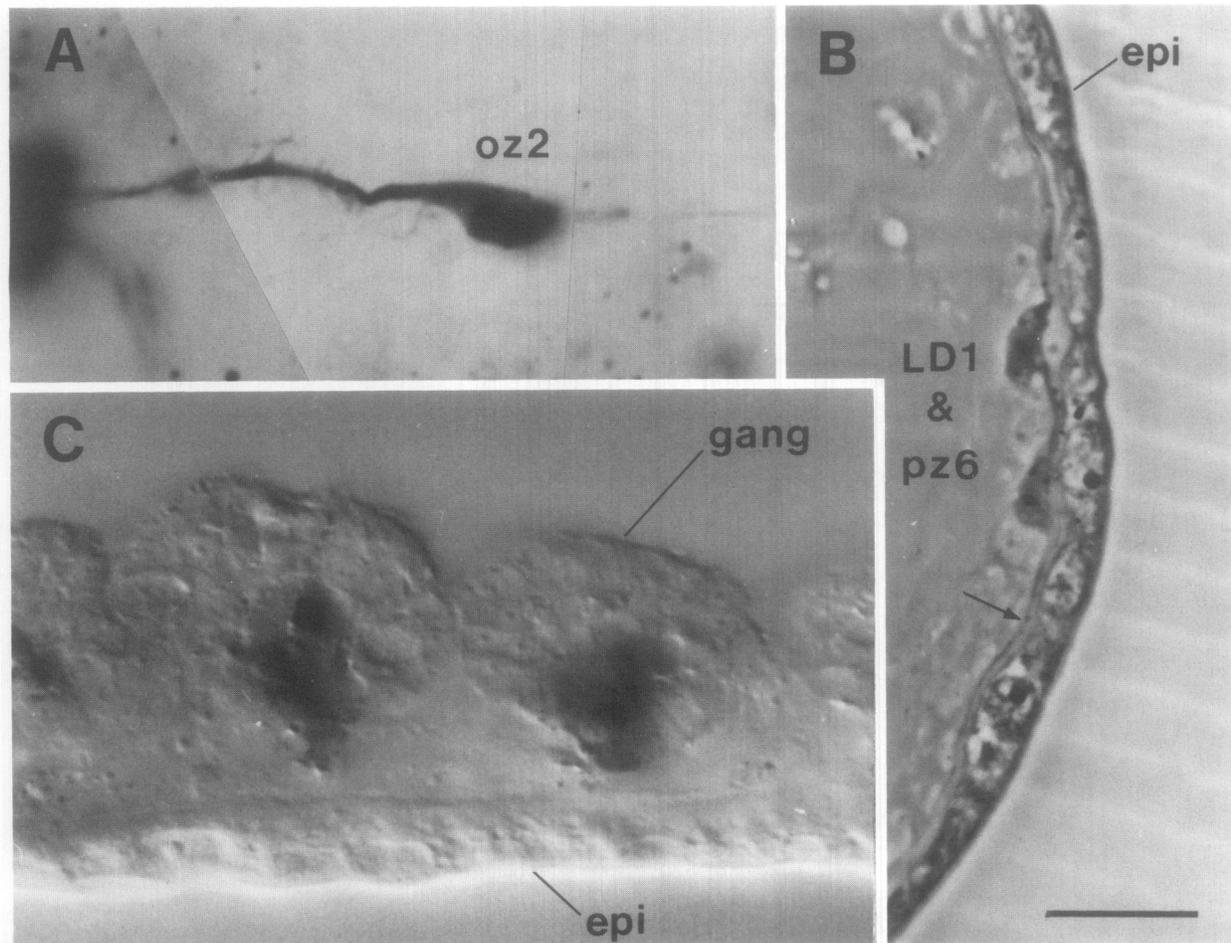
FIG. 3. Labeling of neurons in stage 10 embryos following HRP injection of an ancestral blastomere 6 days earlier in development. (A) Peripheral neuron oz2—labeled by injection of the generative O teloblast—extending an afferent axon into the segmental ganglion on the left. This neuron also has a short distal process, which lies in close association with a much finer labeled axon arising from another more distal neuron. Specimen was photographed from a 100-μm-thick horizontal section using brightfield illumination and a blue filter. (B) Peripheral neurons LD1 and pz6—here labeled by injection of the OP protoblast—as seen in a transverse 3-μm section counterstained with toluidine blue. These neurons lie against the lateral body wall with axons (arrow) projecting both dorsally and ventrally along the inner surface of the epidermis (epi). (C) Thick sagittal section showing the chain of segmental ganglia (gang) lying just dorsal to the body wall epidermis (epi). HRP injection of the generative P teloblast stains a defined wedge of neurons in the middle of each ganglion. Specimen was photographed with Nomarski optics and blue filter. Scale 20 μm.

Cell floret 2 lies at the distal end of the nephridial tubule, and is assumed to be the future site of the excretory nephridiopore (Fig. 2A). The tubule itself consists of a chain of cells linked by a continuous inner canal (Fig. 4), but the canal does not appear to pass through the cell floret at this stage.

The O and P Descendant Patterns

To ascertain the contribution of the O and P cell lines in the mature leech we injected individual O/P teloblasts with HRP in more than 100 otherwise normal stage 7 embryos, and at stage 10 stained their HRP-labeled descendant tissues by the benzidine reaction. The O/P teloblasts give rise to either of two distinct descendant patterns, called the O and P patterns, depending upon the respectively o or p position of the ancestral bandlet within the germinal band (Weisblat and Blair, 1984). Each pattern consists of a segmentally iterated array of cells and cell clusters located on only one side of the body midline (Fig. 2B). The following descriptions compare the complement of O- and P-derived cells in a single midbody segment.

O Pattern. The generative O teloblast contributes approximately 40 central neurons and glia/hemiganglion. The O-derived neurons are distributed in a stereotyped array of cell clusters, the pattern of which has already been described in some detail (Weisblat *et al.*, 1984; Kramer and Weisblat, 1984). Of principal interest here are the anterodorsal and posteroventral
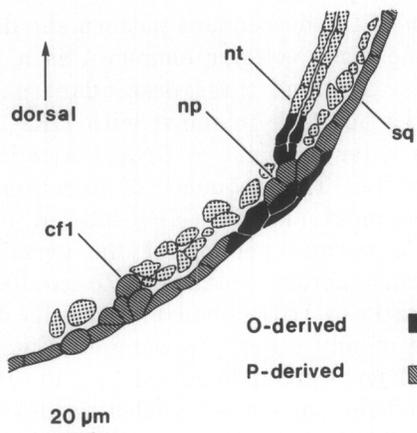
FIG. 4. Camera lucida tracing of a transverse section showing the distal elements of the nephridium at embryonic stage 10. The generative O teloblast was injected with HRP, and its contribution to the body wall is in black. The remainder of the epidermal cells in this region of the body wall are derived from the generative P teloblast, and have been shaded. Cells of other origin are stippled. The nephridial tubule (nt) is a string of cells connected by a continuous central canal. Although most of these cells are of mesodermal origin, the two outermost cells are O-derived. The nephridiopore rudiment (np) is a conglomeration of both O- and P-derived epidermal cells. Unlike the squamous cells (sq) found elsewhere in the epidermis, the cells of this rudiment are rounded and protrude into the body cavity. Cell floret 1 (cf1) is derived from the generative P teloblast, and can be seen as another discrete island of rounded cells.

clusters (Fig. 2B) which occupy regions of the ganglion that are devoid of P-derived cells.

The O pattern also includes three peripheral neurons/hemisegment (Fig. 2B). (i) Neuron oz1 is located at the point where the AA nerve comes into contact with the body wall about 40 μm lateral to the ganglion's edge.[3] (ii) Neuron oz2 is located at a comparable position within the PP nerve. Both of these cells have afferent axons projecting into the ganglion via their respective nerves, and often exhibit short distal processes as well (Fig. 3A). (iii) There is also a single O-derived neuron whose cell body is located in the far lateral portion of the PP nerve with extensive axons projecting both dorsally and ventrally. On the basis of its combined lineage and position this cell is believed to be the previously identified dopamine-containing neuron LD2 (Blair & Stuart, 1982; Weisblat and Blair, 1984).

The O pattern includes two discrete patches of epithelium/hemisegment (Fig. 2B). We counted 2–4 squamous cells in a small patch located just proximal to LD2, and 20–25 squamous cells in a thin strip which

[3] Neurons which are identified with respect to their embryonic origin will, in accordance with the convention of Kramer and Weisblat (1984), be named by the blast cell of origin, the letter z, and a number.

extends laterally from the ventral midline and abuts against the nephridiopore rudiment along its anterior edge. Two or three of the rounded cells that comprise the nephridiopore rudiment are also O-derived, as well as the two most distal cells of the nephridial tubule (Fig. 4). The ectodermal origin of the outermost nephridial elements was previously proposed by Bürger (1891).

P Pattern. The generative P teloblast contributes approximately 15 central neurons and glia/hemiganglion. The P-derived cell bodies form a mediolateral wedge (Fig. 3C), with the exception of a single isolated interneuron, pz4, located posteriorly on the ventral midline in approximately one-half of the midbody segments (Fig. 2B; see also Kramer and Weisblat, 1984).

We found seven P-derived peripheral neurons in most segments examined (Fig. 2B). (i) Neuron pz5 is located in the AA nerve root at the point where it exits from the ventral surface of the ganglion. In one of the three segments examined in serial section a second P-derived neuron was also observed at this same site. (ii, iii) The AA nerve includes a pair of far lateral neurons (Fig. 3B), one of which is presumed to be the previously identified P-derived dopamine-containing neuron LD1 (Weisblat and Blair, 1984). The other cell will be designated pz6. (iv) Neuron pz7 is located just proximal to the nephridiopore rudiment within the MA nerve. (v) In many segments we were able to distinguish what appeared to be a single neuron, pz8, located on the inner surface of cell floret 3. (vi) Neuron pz9 wraps around the main body of the nephridium, and sends an afferent axon along the nephridial tubule to the body wall where it joins the MA nerve. A similar nephridial neuron has been described in the medicinal leech *Hirudo* (Wenning, 1983). (vii) Neuron pz10 lies in the far lateral portion of the PP nerve near neuron LD2.

We serially sectioned one embryo in which the P pattern was labeled with HRP and found a small number of apparently nonneuronal P-derived cells located within the coelum (Fig. 5). Included among these cells is a large, lightly stained, extensively vacuolated cell situated superficial to pz7 in every segment examined.

The P pattern includes a wide swath of ventrolateral epidermis extending unbroken along the body's length (Fig. 2B). We counted 40–60 P-derived squamous cells/hemisegment, as well as the rounded epidermal cells which comprise cell floret 1 (3–4 cells) and cell floret 3 (10–15 cells). The P cell line also contributes 1–4 rounded cells to the nephridiopore rudiment, but has no descendants in the nephridial tubule (Fig. 4).

Thus, at stage 10 of embryogenesis the O and P
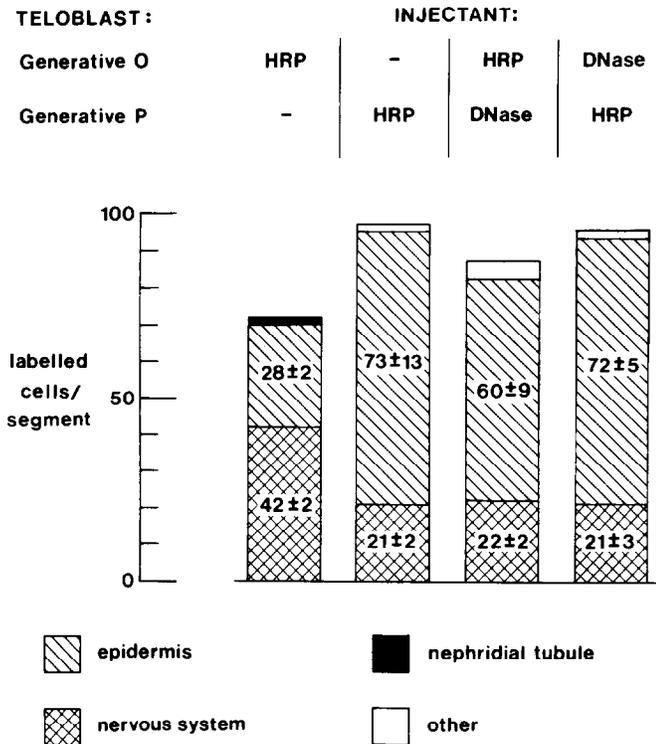
FIG. 5. Number of labeled cells/segment following various combinations of HRP and DNase injection. Counts were taken from series of transverse 3-μm sections, with numbers representing the mean (±SD) obtained from three consecutive midbody segments. In an otherwise normal embryo the injection of HRP into the generative O teloblast labels a relatively large number of neurons and glia, a smaller number of epidermal cells, and two cells per segment within the nephridial tubule. HRP injection of the generative P teloblast labels a relatively small number of neurons and glia, many more epidermal cells, and no cells within the nephridial tubule. The generative P teloblast also gives rise to a small number of nonneuronal cells ("other") located within the coelum or the gut. Ablating the generative P teloblast by DNase injection causes the O cell line to undergo a pronounced change of fate. The generative O teloblast gives rise to a complement of descendant tissues which differs significantly from the normal O pattern (nervous system, $P < 0.0005$; epidermis, $P < 0.005$; Student's t test), and which contains no descendants within the nephridial tubule. The cellular composition of this descendant pattern is quite similar to that of a normal P pattern (n.s., $P > 0.25$; epi., $P > 0.10$). In contrast, ablation of the generative O teloblast has little or no effect on the other cell line.

descendant patterns can be distinguished both by the relative number of neural and epidermal cells (Fig. 5) and by several identified pattern elements.

O/P Teloblast Ablation

The previous study established that a generative O teloblast will forsake the O developmental pathway and give rise to a P-like descendant pattern following ablation of the generative P teloblast on the same side (Weisblat and Blair, 1984). If the o blast cells are actually being transfated into the P pathway, then

that portion of the descendant pattern should be comprised of the same cellular elements as a normal P pattern. We examined these descendant patterns by first injecting one O/P teloblast with HRP, and then ablating its sister teloblast by DNase injection several hours thereafter. This sequence of injections ensures that (i) the most anterior HRP-labeled blast cells develop in a normal portion of the germinal band which contains another, unlabeled o/p bandlet, and (ii) the more posterior HRP-labeled blast cells develop in the absence of any other o/p bandlet (Fig. 6A). The injected embryos were then raised to stage 10, stained for HRP, and the pattern of labeled tissues compared along the body's length.
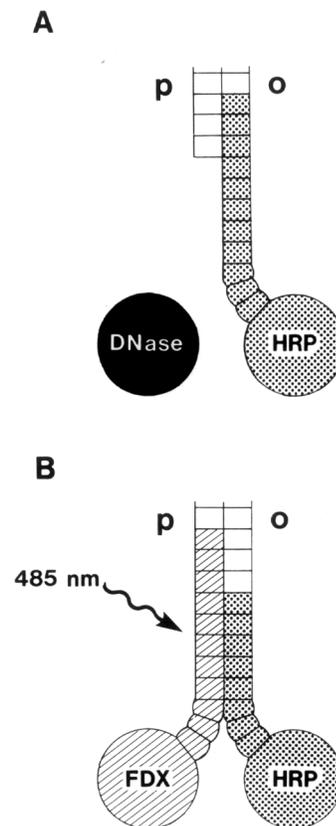
FIG. 6. To study the developmental interaction between the O and P cell lines the generative O teloblast was injected with HRP and the ipsilateral p bandlet eliminated by either of two methods. (A) In one set of experiments the generative P teloblast was ablated by DNase injection, thereby ensuring that the more posterior o blast cells do not come into contact with the p bandlet. Times of injection were routinely staggered so that the first few HRP-labeled o blast cells lie anterior to this point. (B) In a second series of experiments the generative P teloblast was injected with FDX and its fluorescently labeled blast cell progeny photoablated by brief exposure to intense 485 nm illumination. In this way the o blast cells can be deprived of their p blast cell neighbors at various times after they enter the band. Times of injection were routinely staggered so that the first HRP-labeled o blast cells would be unable to interact with any of the more anterior, unlabeled p blast cells.

In 15 out of 20 embryos the most anterior HRP-labeled blast cells gave rise to an O pattern of descendants, indicating that the HRP had been injected into the generative O teloblast. In contrast to a normal embryo the descendant pattern changed dramatically in the posterior segments where it consisted of a mediolateral wedge of central neurons, peripheral neurons in positions characteristic of pz5–10 and LD1, cell florets 1–3 and a relatively large fraction of the ventrolateral epidermis (Fig. 7). Thus, o blast cells located in the deficient portion of the germinal band gave rise to a complement of descendant tissues whose cellular elements were virtually indistinguishable from those comprising a normal P pattern. Cell counts revealed that the total number of descendant neurons and epidermal cells were also much closer to those expected of a P pattern (Fig. 5).

The pattern of tissues descended from a single O/P teloblast is comprised of a longitudinal sequence of essentially isomorphic primary blast cell clones. We subdivided the experimentally induced P patterns into blast cell clones by relying upon the fixed relationship between these clones and the clearly demarcated pattern of segmentation (Weisblat and Shankland, in preparation). Table 1 shows the segmental distribution of O and P pattern elements in one such embryo. Although some clones lack one or more of the expected pattern elements, each primary blast cell clone appears to have given rise exclusively to either O or P pattern elements, with an abrupt transition between the two regions.

In the 5 remaining embryos the labeled bandlet gave rise to an essentially normal P pattern of descendants along the body's length, indicating that in these embryos the HRP had been injected into the generative P teloblast. As previously reported (Weisblat and Blair, 1984), ablation of the O teloblast has relatively little effect on the developmental fate of the P cell line (Fig. 5).
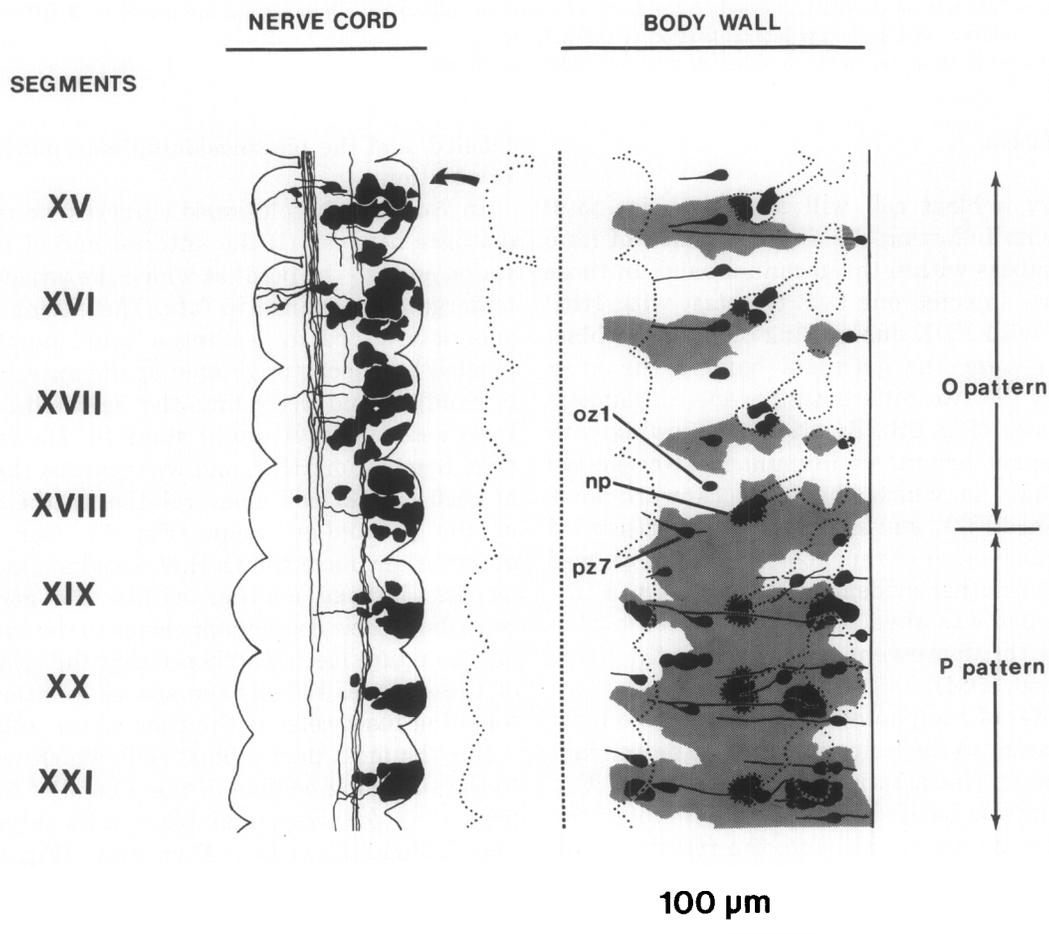
FIG. 7. Camera lucida tracing of a stage 10 embryo in which the HRP-labeled o bandlet changed fate midway along the body's length in response to P teloblast ablation. In the anterior segments, where the p bandlet was present, the o bandlet gave rise to a normal O pattern of descendants. However, the pattern of labeled tissue changes posteriorly to include all of the cellular elements seen in a normal P pattern. The transition between the two regions is abrupt, and the pattern elements at the point of transition have been designated as in Fig. 2.

TABLE 1

TRANSFATING OF THE O CELL LINE FOLLOWING ABLATION OF THE GENERATIVE P TELOBLAST[a]

	Primary blast cell clones													
P pattern elements														
Cell floret 1	+	+		+	+	+								
Neuron pz9	?	+	+	+	+	+								
Neurons pz6 and LD1[b]	+	+	+	+	+	+								
Cell floret 3	+	+	+	+	+	+								
Neuron pz7	+	+	+	+	+	+								
O pattern elements														
Anterodorsal cluster							+	+	+	+	+	+	+	+
Posteroventral cluster							+	+	+	+	+	+	+	+
Neuron oz1							+	+	+	+	+	+	+	+
Nephridial tubule	c								+	+	+	+	+	c
Neuron oz2								+	+	+	+	+	+	+

Transition

Posterior ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯→ Anterior

[a] The presence (+) or absence of 10 different O or P pattern elements was scored for a series of 13 consecutive primary blast cell clones spanning segments X–XXIII of a stage 10 embryo. Question marks represent instances of uncertainty. In this embryo the generative O teloblast was injected with HRP early in stage 7, and the generative P teloblast ablated by DNase injection 10 hr later. The resultant transfating of the posteriormost o blast cells can be seen by the abrupt transition in the cellular composition of their descendant clones.

[b] Neurons pz6 and LD1 can not be distinguished on the basis of position, and we have here scored the presence of HRP-labeled neurons, regardless of their number, within the far lateral portion of the AA nerve.

[c] No nephridia found in these segments in either normal or lesioned embryos.

p Bandlet Ablation

The primary o blast cell will also give rise to P pattern elements following the direct ablation of its p blast cell neighbors within the germinal band. In these experiments we injected one O/P teloblast with HRP and its sister with FDX during stage 7. Labeled blast cells begin to enter the germinal band 15 hr after injection, with the two differentially labeled bandlets lying side-by-side (Fig. 6B). By alternate viewing with epifluorescence and brightfield illumination we selected those individuals in which the more capward or p bandlet contained FDX, and at the same time discarded embryos in which either of the labeled bandlets showed discontinuities or other evidence of trauma. The FDX-labeled p bandlet was then lesioned by intense photoexcitation of the fluorescent tracer within the living cells (Shankland, 1984).

In 95% (42/44) of the photolesioned embryos o blast cells lying adjacent to the lesion gave rise to descendant elements normally characteristic of the P pattern (Fig. 8). If the lesion was located near the point where the blast cells enter the germinal band, the resultant fate change was as dramatic as that obtained by ablation of the generative P teloblast. However, response diminishes as the blast cells grow older and move further into the germinal band (see below). In 2 embryos the o bandlet gave rise to a normal O pattern in all segments—in these cases the p bandlet was faintly labeled, and the photolesioning may not have resulted in ablation.

In 7 of the photolesioned embryos we estimated the distance between (i) the anterior end of the p bandlet lesion and (ii) the point at which the adjacent o bandlet undergoes its change in fate. The extent of the lesion was determined by leaving a small number of FDX-labeled blast cells unablated at the anterior end of the p bandlet, and recording the segmental location of their descendant clones at stage 10. The embryos were then reacted for HRP, and we recorded the O or P fate of each o blast cell clone relative to the anterior end of the p bandlet lesion (Fig. 9). Our unpublished observations have shown that o and p blast cells which lie next to one another within the germinal band contribute their descendant clones to the same segments of the mature leech, and hence that the relative position of these clones reflects the spatial relationship of the founding blast cells at the time of the ablation.

For the most part o blast cells which were adjacent to the surviving portion of the p bandlet followed their normal O pathway, and blast cells adjacent to the photolesion followed the P pathway (Fig. 9). However, there were sometimes 1–2 blast cells near the anterior end of the lesion which followed th O pathway even though they had lost their immediate p blast cell neighbors. These cells may have been influenced by the unablated p blast cells located more anteriorly. This finding suggests that—at least in the posterior direc-
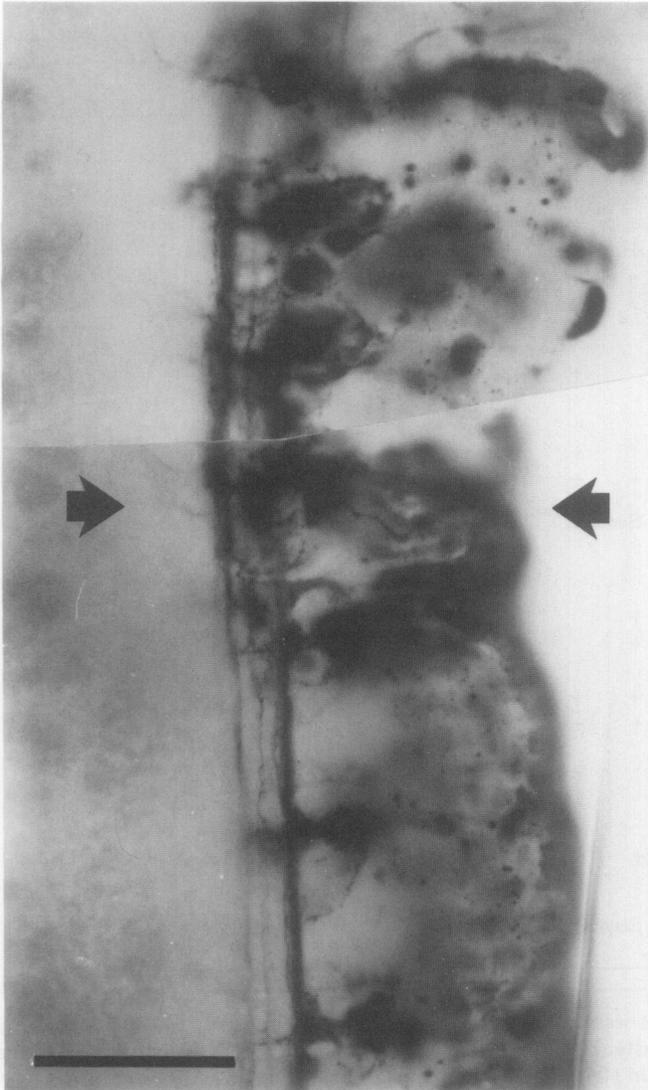
FIG. 8. Change in fate of the o bandlet following photoablation of the adjacent p bandlet. Photomicrograph shows the tissues descended from an HRP-labeled o bandlet near the anterior end of the p bandlet lesion. Arrows mark the abrupt O-to-P transition in the pattern of labeled tissues at this point. Specimen was viewed in thick horizontal section using brightfield illumination and a blue filter. Scale 100 μm.

cells are arranged along the bandlet in the order of their birth. In these experiments the o bandlet was labeled with HRP, and the adjacent, FDX-labeled p bandlet photoablated in its entirety 20–40 hr later. Each blast cell within the o bandlet is at a different stage of maturation at the time of the ablation, and age-related changes in the response of the blast cell could be reconstructed by examining the posterior-to-anterior sequence of HRP-labeled blast cell clones at stage 10.

Table 2 gives the cellular composition of the individual blast cell clones for 2 of 8 embryos in which we allowed a 38 hr interval between teloblast injection and bandlet ablation. The o blast cells appear to lose their response to p bandlet ablation as they grow older and move further into the germinal band. The youngest, i.e., most posterior, labeled blast cells gave rise to a virtually normal P pattern of descendants, as if the p bandlet had had little or no influence on the o blast cell development prior to the ablation. In contrast, the oldest, i.e., most anterior, labeled blast cells gave rise to O pattern elements exclusively, suggesting that they were already committed to the O pathway when the ablation was performed.

The cellular composition of the labeling pattern seen in these embryos changes at three distinct points of transition, with the intervening clones displaying similar—though not always identical—complements of labeled cells (Table 2; Fig. 10). Thus, the pattern of
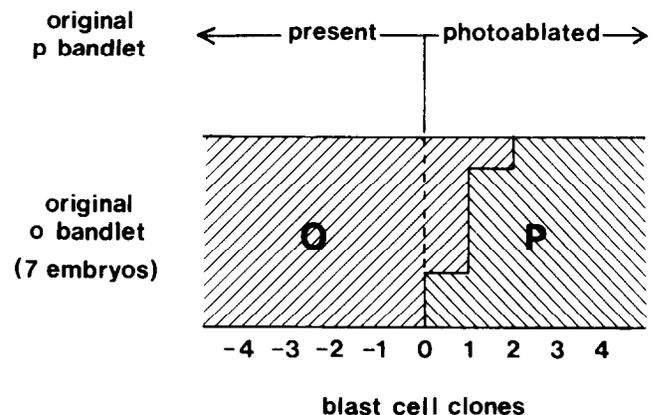
blast cell clones

FIG. 9. Histogram showing the O or P fate of primary o blast cells at the edge of a p bandlet lesion. Blast cell clones are shown in sequence along the longitudinal axis, with anterior at the left. Blast cells which were adjacent to the surviving portion of the p bandlet followed the O pathway, and for the most part blast cells adjacent to the lesion changed fate and followed the P pathway. In some cases there were 1-2 primary o blast cells at the anterior end of the lesion which followed the O pathway despite having lost their immediate p blast cell neighbors, presumably due to the influence of unablated p blast cells located more anteriorly. Thus, at least in the posterior direction, viable p blast cells are able to influence o blast cell development over a distance of no more than 1-2 cell widths.

tion—viable p blast cells are able to exert an influence on o blast cell development over a distance of no more than one or two cell widths. Similar conclusions were reached by Zackson (1984), who used the bandlet-specific geometry of the first blast cell division as a monitor of blast cell fate following teloblast ablations.

Loss of Response to p Bandlet Ablation

We were able to determine the relationship between the developmental age of an o blast cell and its response to p bandlet ablation by noting that blast

TABLE 2

STEPWISE COMMITMENT OF O AND P PATTERN ELEMENTS WITHIN THE o BLAST CELL CLONE FOLLOWING PHOTOABLATION OF THE p BANDLET[a]

Primary blast cell clones																		
Embryo #1																		
P pattern elements																		
Cell floret 1	?	+	+	+	+	+	+	+										
Neuron pz9	?	?	?	+	+	+	+	+										
Neurons pz6 or LD1[b]	?	?	?	+	+	+	+	+				+						
Cell floret 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Neuron pz7		+	+	+	+	+	+	+	+	+	+		+	+	+			
O pattern elements																		
Anterodorsal cluster									+	+	+	+	+	+	+	+	+	+	+
Posteroventral cluster									+	+	+	+	+	+	+	+	+	+	+
Neuron oz1									+	+	+	+	+	+	+	+	+	+	+
Nephridial tubule	c	c	c						+	+	+	?	+	+	c	c	+	+	+
Neuron oz2				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitions		First							Second							Third			
Embryo #2																		
P pattern elements																		
Cell floret 1		+	+	+	+	+	+	+										
Neuron pz9	?	?	?		+	+		+			+				?			
Neurons pz6 or LD1[b]	?	?	+	+	+	+												
Cell floret 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Neuron pz7	+	?	+	+			+	+	+	+	+	+						
O pattern elements																		
Anterodorsal cluster									+	+	+	+	+	+	?	+	+	+	+
Posteroventral cluster									+	+	+	+	+	+	+	+	+	+	+
Neuron oz1									+	+	+	+	+	+	+	+	+	+	+
Nephridial tubule	c	c	c					+	+	+	+	+	?	+	c	c	+	+	+
Neuron oz2			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitions		First							Second							Third			

Posterior ⟶ Anterior

[a] The presence (+) or absence of 10 different O or P pattern elements was scored for 19 consecutive primary blast cell clones spanning segments VI–XXV of two stage 10 embryos. Question marks represent instances of uncertainty. In these embryos the generative O teloblast was injected with HRP and the generative P teloblast with FDX early in stage 7, and the p bandlet photoablated 38 hr later. The change from exclusively p-type clones in the posterior segments to exclusively O-type clones in the anterior segments takes place for the most part as a sequence of three discrete steps. The points of transition have been marked in accordance with the guidelines given in the text.

[b] Neurons pz6 and LD1 can not be distinguished on the basis of position, and we have here scored the presence of HRP-labeled neurons, regardless of their number, within the far lateral portion of the AA nerve.

[c] No nephridia found in these segments in either normal or lesioned embryos.

tissues descended from the injected teloblast is composed of four distinct regions which are comprised of different types of blast cell clone. Shortening the interval between injection and ablation reduces the number of labeled clones anterior to these transitions, but does not alter their longitudinal sequence.

(i) The most posterior clones contain most or all of the pattern elements seen in a normal P pattern, and do not display any of the unambiguous O pattern elements.

(ii) The location of the first transition was defined by the appearance of neuron oz2. Virtually all of the clones anterior to this transition contain a peripheral neuron at the point where the PP nerve contacts the body wall, the site characteristic of this particular O pattern element. Otherwise the clones lying between the first and second transitions display a normal P pattern, except that the majority (88%; 31/38) have fewer than two neurons in the far lateral portion of the AA nerve. The latter finding suggests that either one of two P pattern elements—pz6 or LD1—may be missing from blast cell clones anterior to the first transition (Table 3).

(iii) The location of the second transition was defined by the appearance of the anterodorsal and posteroventral neuron clusters within the segmental ganglion. Clones anterior to this transition display both clusters, and usually also contain the other unambiguous O

second and third transitions also contain two unambiguous P pattern elements—neuron pz7 and cell floret 3—but only rarely (5%; 2/40) contain neurons in the far lateral portion of the AA nerve.

(iv) The third transition was defined by the disappearance of cell floret 3 (Tables 2 and 3). Clones located anterior to this transition contain all of the unambiguous O pattern elements, and none of the unambiguous P pattern elements.

Following p bandlet ablation the contribution of the o bandlet to the squamous portion of the epidermal sheet also varies in a consistent fashion along the body's length (Fig. 10). However, other pattern elements which are normally found in both the O and P descendant patterns (e.g., central neurons in the midportion of the ganglion; peripheral neurons in the far lateral portion of the PP nerve; cell floret 2) did not display any dramatic changes along the body's length.

In addition to the transitions between regions, there was also a small degree of apparently random variation in the cellular composition of individual blast cell clones within each region. This variation can be appreciated by considering the two embryos of Table 2. The presence or absence of 10 different pattern elements was scored for a total of 38 clones, and the actual distribution compared with that which would be predicted if the described transitions were truly invariant. Seven of the clones were clearly missing one of the expected pattern elements, and 1 clone was missing two elements. In addition, there were 3 clones which contained a pattern element not expected given their location relative to the transitions.

The observed transitions would seem to reflect a sequence of events which were occurring at different points along the o bandlet at the time of p bandlet ablation. Each event alters the response of the o blast cell to subsequent ablations, with the apparent effect of committing a specific portion of its descendant clone to the O developmental pathway. The following section

FIG. 10. Camera lucida tracing of an HRP-labeling pattern showing the contribution of the o bandlet to the epidermis of the ventral body wall following p bandlet ablation. Anterior is at the top, and segments are numbered to the left of the thick line representing the ventral midline. Cell florets 1, 2, and 3 are drawn in black and numbered accordingly. In this embryo the o bandlet only gave rise to cell floret 1 posterior to segment XVIII, and only gave rise to cell floret 3 posterior to segment XI. These changes in the cellular composition of the descendant pattern are associated with two of the three transitions (arrows) described in the text. Cell floret 2, distinguishable by its association with the nephridial tubule, was descended from the o bandlet in all labeled segments; however, its mediolateral positioning was clearly abnormal in segments VIII and IX. The labeled squamous contribution is shown by shading and also varies along the body's length, with only the most anterior segments (VI–VIII) exhibiting the small, far lateral patch characteristic of the O pattern. The larger O-derived patch is not present in segments VII and VIII, and appears to have moved posteriorly (cf. Blair and Weisblat, 1984).

pattern elements; including peripheral neurons oz1 and oz2, and the outermost cells of the nephridial tubule (Tables 2 and 3). Most clones located between the

TABLE 3

PATTERN ELEMENTS SHOWING COORDINATE COMMITMENT TO THE O OR P PATHWAY

	O pattern elements	P pattern elements
Transition 1	Neuron oz2	Either neuron pz6 or LD1
Transition 2	Anterodorsal and posteroventral clusters of central neurons Neuron oz1 Nephridial tubule	Neuron pz9 and either pz6 or LD1 Cell floret 1
Transition 3	No identified elements	Neuron pz7 Cell floret 3

considers the location of these three *commitment events* relative to other landmarks within the o bandlet.

Timing of Commitment Events Relative to Blast Cell Divisions

The primary o blast cell undergoes its first division soon after entering the germinal band (Zackson, 1984). We determined the timing of the three commitment events relative to this division by comparing (i) the number of HRP-labeled primary blast cells which had undergone their first division at the time of p bandlet ablation, and (ii) the number which expressed a particular state of commitment in their response to the ablation.

To determine which of the labeled blast cells had divided and which had not, we relied upon the pronounced dimorphism of the primary o blast cell's daughters (Fig. 11; see also Zackson, 1984). In 12 of the embryos described in the preceding section the fluorescent tracer Lys-XDX was coinjected with the HRP. This tracer becomes preferentially concentrated within the nuclei of the labeled cells, and allowed us to count daughter cell pairs in the living embryo just prior to the ablation (Fig. 11B). In another 9 embryos we estimated the number of labeled blast cells which had divided by counts taken from groups of similarly injected sibling embryos which were simultaneously fixed and stained with the nuclear dye Hoechst 33258 (Fig. 11A). Measurements of the variance show that counts taken from siblings accurately predict the group mean within a range of ±2 cells.

To determine how many of the labeled primary blast cells had undergone each of the three commitment events, we raised the photolesioned embryos to stage 10, reacted them for HRP, and counted the number of primary blast cell clones anterior to the first, second, and third transitions. The location of the commitment
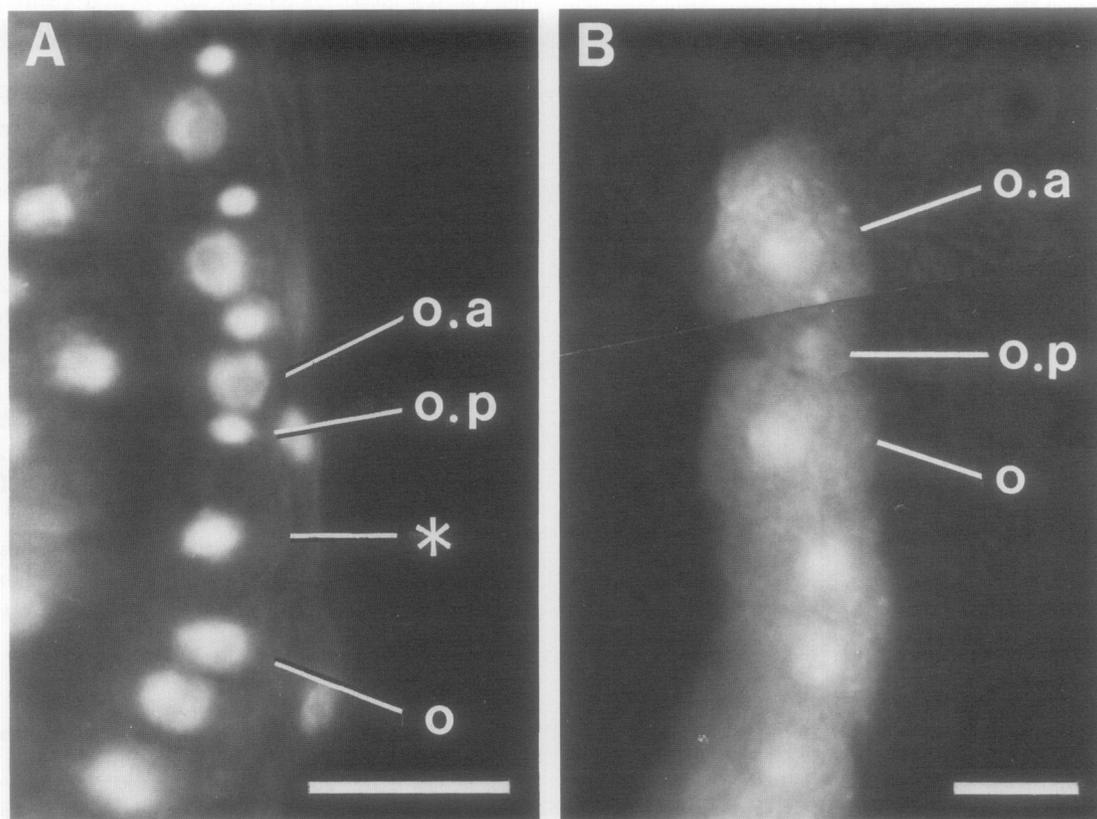
FIG. 11. Fluorescence micrographs showing the point of the first blast cell division within the o bandlet of stage 7 embryos. Blast cells are arranged in the order of their birth, with the oldest, i.e., more anterior, cells at the top. (A) Blast cell nuclei stained with Hoechst 33258 in a fixed specimen. The undivided o blast cells can be distinguished from pairs of daughter cells by the size and staining of their nuclei. Each primary o blast cell divides to produce an anterior daughter, o.a, which has a lightly staining nucleus only slightly smaller than that of the primary blast cell, and a posterior daughter, o.p, which has a much smaller, much more brightly staining nucleus. Blast cell nomenclature is taken from Zackson (1984). In this embryo the most anterior of the undivided o blast cells (*) has entered mitotic prophase for its first division, as seen by its rounded morphology and clumped chromatin. (B) The o bandlet of a living embryo labeled with the fluorescent lineage tracer Lys-XDX. This tracer was injected into the teloblast 25 hr earlier, and has become selectively concentrated within the nuclei of its labeled blast cell progeny. Only one of the labeled primary blast cells has divided. Scales 20 μm.

events relative to the first division was then calculated by subtracting the number of primary blast cells which expressed a particular state of commitment from the number which had divided at the time of the ablation (Fig. 12). This calculation assumes that all of the labeled blast cells gave rise to descendant clones, and that no additional blast cells were committed to the O pathway while the lesion was taking effect. The relative position of these events within the o bandlet has been translated into a developmental time-course by noting that consecutive blast cells within the bandlet are separated by a developmental interval of approximately 1 hr (Wordeman, 1982).

The results show that the primary o blast cell does not become committed to the O pathway until after it has entered the germinal band (Fig. 12). In 7 darkly labeled embryos we were able to clearly define the location of the first transition, and calculated that on

the average the underlying commitment event had taken place 1 ± 1 hr ($\pm$SD) before the primary blast cell entered its first mitosis. Similar calculations reveal that the second and third commitment events had taken place respectively 3 ± 2 hr ($n = 21$ embryos) and 10 ± 1 hr ($n = 5$) after the first division. The calculated timing of the different commitment events showed considerable overlap between embryos (Fig. 12), but within a single embryo the three transitions always exhibited an orderly progression. Counting the number of blast cell clones between transitions showed that there was an average of 5 ± 1 hr ($n = 4$) separating the first and second commitments, and an average of 11 ± 1 hr ($n = 3$) separating the first and third commitments.

The anterior daughter of the first division itself divides several hours later, and the more posterior of its two daughters divides again several hours after
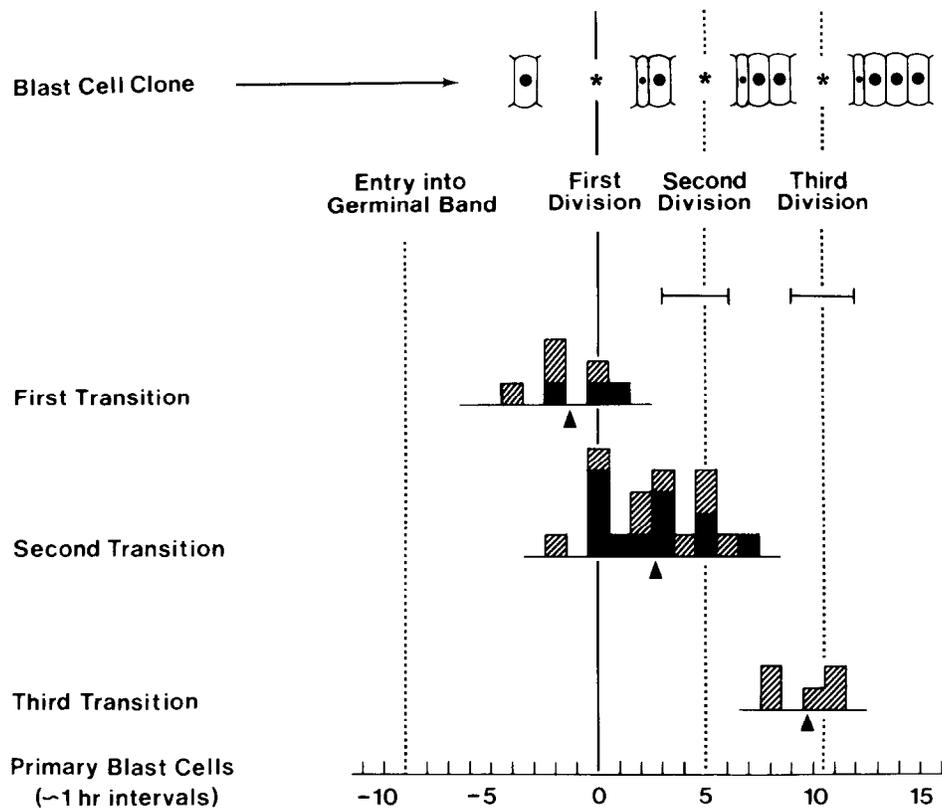
FIG. 12. Histograms showing the location of the three commitment events relative to the first o blast cell division. The primary blast cells are arranged in the order of their birth, with consecutive cells being separated by a developmental interval of approximately 1 hr. Thus, the development of an individual o blast cell is equivalent to the posterior-to-anterior (left-to-right) sequence of cells within the bandlet at a given time. To calculate the locations of the three commitment events relative to the point of first division, we counted the number of tracer-labeled o blast cells which had divided at the time of p bandlet ablation and the number which expressed a particular state of commitment for each experimental embryo (see text). The black squares represent points of commitment calculated using cell counts taken from the experimental embryo, while the shaded squares represent calculations based on estimates taken from sibling embryos. The second and third blast cell divisions are shown relative to the first division by dotted vertical lines, with bars spanning the range of values seen with a frequency > 5%. The mean of each histogram is marked by an arrowhead, and it can be seen that the three commitment events occur in phase with the first three blast cell divisions.

that (Zackson, 1984). The time between successive divisions was estimated by counting the number of intervening primary blast cell clones in embryos stained with Hoechst 33258. The second and third divisions occur respectively 5 ± 1 hr ($n = 94$) and 10 ± 1 hr ($n = 47$) after the first division, which means that the three commitment events occur in phase with the first three blast cell divisions (Fig. 12).

DISCUSSION

The present findings confirm and extend the conclusions of Weisblat and Blair (1984) regarding the positional specification of the O and P cell lines in the leech embryo. These cell lines originate as two parallel bandlets of seemingly equipotent blast cells which, during normal development, give rise to distinct O and P patterns of both neural and epidermal descendants. We have used the cellular composition of the descendant pattern as a means of inferring the developmental commitments made by individual blast cells after experimental manipulations of their environment. We found that the o blast cell is capable of following either the O or P pathway for a period of approximately 20 hr extending from the time of its birth until its first division. At this point the o blast cell has become incorporated into the germinal band, and lies adjacent to the p bandlet. Its subsequent development is influenced by the neighboring p blast cells, and its normal commitment to the O pathway can be prevented by ablating the p bandlet prior to the first division. Commitment to the O pathway occurs around the time of the first three blast cell divisions, and appears to involve three discrete steps which affect different elements of the blast cell clone.

Role of Blast Cell Interactions in Positional Specification

As yet we have no conclusive evidence regarding the cellular basis of the interaction that diverts the o blast cells into the O developmental pathway. Nonetheless, the blast cell ablation experiments demonstrate that p blast cells have a very specific and localized effect on o blast cell development, and the close spatial correlation between the extent of the p bandlet lesion and the extent of the fate change produced in the o bandlet by that lesion suggests that the interaction may depend upon direct physical contact between blast cells or some other form of short range signal. It also seems likely that interaction is maintained as the cells move further into the germinal band, since a given o blast cell is sensitive to the presence of the p bandlet at three different times spanning a period of more than 10 hr.

During normal development the interaction of the two o/p bandlets excludes the o bandlet from following the same pathway as the p bandlet, and thereby establishes a hierarchy in the specification of the O and P fates. Cell lineage studies in the nematode (Sulston and White, 1980; Kimble, 1981; Sulston et al., 1983) and grasshopper (Kuwada and Goodman, 1983; Taghert et al., 1984) have uncovered similar instances in which equipotent cells undergo divergence into different developmental pathways as a result of a nonreciprocal and, at least in some cases, positionally specified exclusion of alternative cell fates. The kind of cellular interactions which govern positional specification among such small groups of lineally identifiable cells may also be involved in the generation of more global body patterns within groups of cells that bear no strict lineal relationship. For instance, it has been proposed that the density of sensory receptors in the insect integument is regulated during growth by a comparable sort of exclusion mechanism (Wigglesworth, 1940; Lawrence, 1973).

During leech embryogenesis the interaction of the o and p bandlets brings about their divergence into separate developmental pathways, but does not by itself explain the positional component of this specification. Initially the two o/p bandlets seem to share a comparable developmental potential, since the o bandlet can be transfated to the P pathway by ablation of the P cell line (Weisblat and Blair, 1984; Zackson, 1984), and the p bandlet can be transfated to the O pathway by transposition into the o position in the contralateral germinal band (Shankland, unpublished). Yet in a normal embryo it is always the capward or p bandlet which follows the P pathway and the anticapward or o bandlet which follows the O pathway (Weisblat and Blair, 1984). It seems likely that at least one of the two bandlets has access to other positional cues from some external source of reference, and that this information serves to bias the direction of bandlet interaction so that the more capward blast cells assume the dominant role within the hierarchy.

Developmental Commitments of the o Blast Cell

As it grows older and moves further into the germinal band the primary o blast cell clone loses its response to p bandlet ablation. This loss of response seems to involve three discrete steps, with each step reducing the number of P pattern elements and, in two out of three cases, increasing the number of O pattern elements produced by the clone following subsequent ablations (Table 3). Within the context of these experiments a clone which has undergone all three events is autonomously committed to the O pathway, and subsequent ablation of the p bandlet has no appreciable

effect on the identity of its descendants. Later ablations did lead to an apparent repositioning of descendants within the body wall epithelium (cf. Fig. 10); however, epidermal spreading is seen following all ectodermal lesions, and probably represents a much later morphogenetic regulation rather than a specification of the ancestral blast cell (Blair and Weisblat, 1984).

The loss of interaction between the o and p bandlets cannot be attributed to physical separation, but rather would seem to imply that the o blast cell clone has lost its ability to either communicate with or respond to the neighboring p blast cells. Thus, one could imagine that each commitment event reflects the end of a different critical period governing the interaction of the two cell lines, and that the presence or absence of neighboring p blast cells during the critical period determines whether a certain portion of the o blast cell clone becomes committed respectively to the O or the P pathway. This interpretation would also seem to imply that the O pattern elements which are added to the descendant pattern at a particular commitment event arise from the same source as the P pattern elements which are lost, and that these two groups of coordinately committed elements represent alternative developmental fates for some part of the blast cell clone.

Based on the calculated times of commitment the primary o blast cell clone is fully committed to the O pathway by the third blast cell division. At this time the clone consists of a total of 4 cells which then proliferate to produce the 70–100 neural and epidermal descendants seen at stage 10. Assuming a geometrical lineage, this comparison would imply that the o and p blast cell clones have completed their divergence into distinct developmental pathways at least four rounds of division prior to terminal differentiation. Hence, the observed differences in the cellular composition of the O and P descendant patterns cannot be attributed to secondary loss or modification of terminal lineage products. Rather, the differences reflect a series of developmental commitments made by the ancestral blast cells at a much earlier stage of embryogenesis. These early commitments would entail a loss of equipotence between the two cell lines, consistent with Zackson's (1984) observation that the o and p blast cell clones begin to manifest bandlet-specific cell morphologies and patterns of mitosis from the very first cell division.

Correlation of Commitment with Blast Cell Divisions

We were able to calculate the timing of the three commitment events relative to the early blast cell divisions by comparing the number of o blast cells which had divided at least once at the time of p bandlet ablation and the number which expressed a particular state of commitment. On the average each commitment event occurs 1–3 hr before one of the first three divisions, suggesting that there may be some functional relationship between the two sequences of events. The degree of correlation between commitment events and cell divisions varied considerably within our sample, but individual embryos contained differing amounts of FDX, and the observed variability might have arisen from differences in the degeneration time necessary for the photolesioned p bandlet to cease having an effect on o blast cell commitment. Differences in degeneration time would not be expected to affect the intervals separating the three commitment events, and measurements taken from single embryos showed that the intervals between commitment events are essentially identical to those separating the first three blast cell divisions.

On the other hand, if the p bandlet degeneration time includes a component which is constant across our sample, all of our calculations would be shifted by that amount. A delay of this sort seems likely, and any such hidden error would mean that the actual commitment events occur *later* in the time-course of o blast cell maturation than our calculations would suggest. Thus, even if the three commitment events occur shortly after each of the first three divisions, a constant error of only a few hours would make it appear that they occur before the respective divisions.

Although our observations are inadequate to determine the exact correlation between commitments and cell divisions, it is nonetheless of interest to consider how the segregation of cellular commitment relates to the segregation of descendant cell fates. Lineage tracer injection of individual blast cells within the germinal band has shown that the fate of the primary o blast cell is segregated between its two daughters in a stereotyped fashion, with the larger, anterior daughter o.a giving rise to all five of the O pattern elements scored in Table 2 (M. Shankland and R. K. Ho, unpublished). In contrast, the first commitment event commits the primary o blast cell clone to give rise to neuron oz2 while leaving the remainder of the unambiguous O pattern elements uncommitted and therefore still sensitive to p bandlet ablation.

If the first commitment event is associated with the first o blast cell division, it would mean that cell o.a manifests a state of commitment which only constrains the fate of certain of its descendants. In this case commitment would not be clonal in nature, as a single blast cell daughter would be expressing a partially committed state. However, at the present time we cannot discount the alternative possibility that commitment is clonal, and that the first commitment event

is actually associated with some later cell division. In the latter case cell o.a or one of its daughters would divide to yield a committed cell giving rise to neuron oz2, and an uncomitted cell which—in the absence of p bandlet ablation—would become committed to give rise to the other unambiguous O pattern elements at a later date.

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