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Cell fates in leech embryos with duplicated lineages

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ABSTRACT **We have examined the fates of the progeny of supernumerary embryonic stem cells (O/P teloblasts) generated by microinjecting polyadenylic acid into newborn O/P teloblasts in embryos of the leech, *Helobdella triserialis*. In normal development, each O/P teloblast generates a rostrocaudal column of daughter cells (primary blast cells) that contribute distinct segmentally iterated O or P sets of epidermal and neural progeny to the mature leech. Previous results suggest that primary blast cells derived from ipsilateral pairs of O/P teloblasts are equipotent and equivalent at birth; that they and their progeny assume distinct O or P fates according to hierarchical and position-dependent interactions; and that the P fate is the primary, or default, fate and the O fate is the secondary fate. In the work presented here, one O/P teloblast was experimentally induced to undergo a supernumerary equal division, and the developmental fates of the progeny of the three (two "duplicate" and one "nonduplicate") ipsilateral O/P teloblasts were determined at stages 8 and 10. We find that some supernumerary O/P teloblasts produce supernumerary P progeny, whereas others generate supernumerary O progeny. When three O/P-derived bandlets are present, bandlets derived from the duplicate O/P teloblasts give rise to progeny of the same (O *or* P) fate. When the nonduplicate bandlet is absent, the duplicate bandlets assume distinct O *and* P fates. These results suggest that ipsilateral sister O/P teloblasts, while equipotent, might not be equivalent.**

Groups of seemingly equivalent, multipotent embryonic cells whose fates are determined by hierarchical and position-dependent cell interactions (equivalence groups) have been identified in a number of organisms (1–6). In the work presented here, we analyze further the developmental plasticity (i.e., capacity to change fate in response to a developmental perturbation) of the progeny (primary blast cells) of an ipsilateral set of embryonic stem cells, called O/P teloblasts (Fig. 1), that are thought to constitute an equivalence group in embryos of *Helobdella triserialis*, a glossiphoniid leech.

Previous studies suggest that primary blast cells derived from these O/P teloblasts are initially equivalent and that they assume distinct O or the P fates (Fig. 2) as a result of hierarchical, position-dependent interactions. In *H. triserialis*, blast cells in the o bandlet "transfate" (i.e., give rise to progeny characteristic of the P kinship group) to various degrees under several experimental conditions, including the absence of neighboring p blast cells due to the ablation of the ipsilateral generative P teloblast (2) and the photolesioning of the neighboring p blast cells before the third or fourth mitosis in the presumptive o blast cell clone (7, 12, 13). Transfating is not symmetric; the p blast cells do not change fate when generative O teloblasts or neighboring cells in the o bandlet are ablated. Thus, the P fate is defined as the primary or default fate. The o and p blast cells in germinal bands lacking m, n, and q bandlets exhibit normal mitotic patterns (8), indicating that interactions with these other bandlets are not required for at least the early fate distinction in the O–P equivalence group. The fates of o and p blast cells are not determined solely by interactions among themselves, however. In *Theromyzon rude*, for example, o blast cells transfate after ablation of the p bandlet only if they assume the position previously occupied by the p bandlet (14). In addition, when cells in the epithelium overlying the germinal band are photolesioned in *H. triserialis*, o blast cells can transfate, even though the p bandlet is still present (15).

In the present experiments, one O/P teloblast was induced to undergo a supernumerary equal division before it had produced any primary blast cells, and the fates of the progeny of the three (two "duplicate" and one "nonduplicate") ipsilateral O/P teloblasts were determined by their early mitotic patterns or by the phenotypes of their definitive progeny.

MATERIALS AND METHODS

Methods were as described (16–18). The embryonic stages and cell lineage nomenclature are those of Stent *et al.* (19).

RESULTS

Teloblast Duplication. O/P teloblast lineages in *H. triserialis* embryos were duplicated by microinjecting polyadenylic acid [poly(A)] into nascent teloblasts (17). At stage 10, definitive progeny of supernumerary teloblasts are phenotypically indistinguishable from those of a homologous, nonduplicated teloblast. The mechanism by which poly(A) induces supernumerary cleavages of teloblasts is not understood. In the 17 experiments reported here, the percentage of poly(A)-injected teloblasts that underwent a supernumerary division ranged from 10% to 44%. Overall, a total of 165 duplicated O/P teloblasts that generated bandlets were obtained in 842 poly(A)-injected embryos (20%). O/P teloblasts that were injected with poly(A) but failed to subsequently undergo a supernumerary division (n = 456; 54%) gave rise to healthy bandlets and normal sets of either O or P descendants at stage 10. In the remaining embryos (26%), the injected teloblast died or failed to make a healthy bandlet.

Fates of Duplicated Bandlets at Stage 8. The mitotic patterns of bandlets derived from poly(A)-injected O/P teloblasts were those characteristic of o or p, rather than m, n, or q, bandlets. In addition, the relative positions of these putative o and p bandlets were consistent with those of o and p bandlets in normal development; bandlets with cells whose mitotic patterns resembled p blast cells were positioned capward of bandlets with cells whose mitotic patterns resembled o blast cells. Bandlets derived from duplicate O/P teloblasts that lie next to the q bandlet shall be referred to as duplicate capward bandlets and those lying next to the n bandlet shall be referred to as duplicate anticapward bandlets (Fig. 1).

Contrary to the expectation that all supernumerary bandlets generated from O/P teloblasts would exhibit only

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

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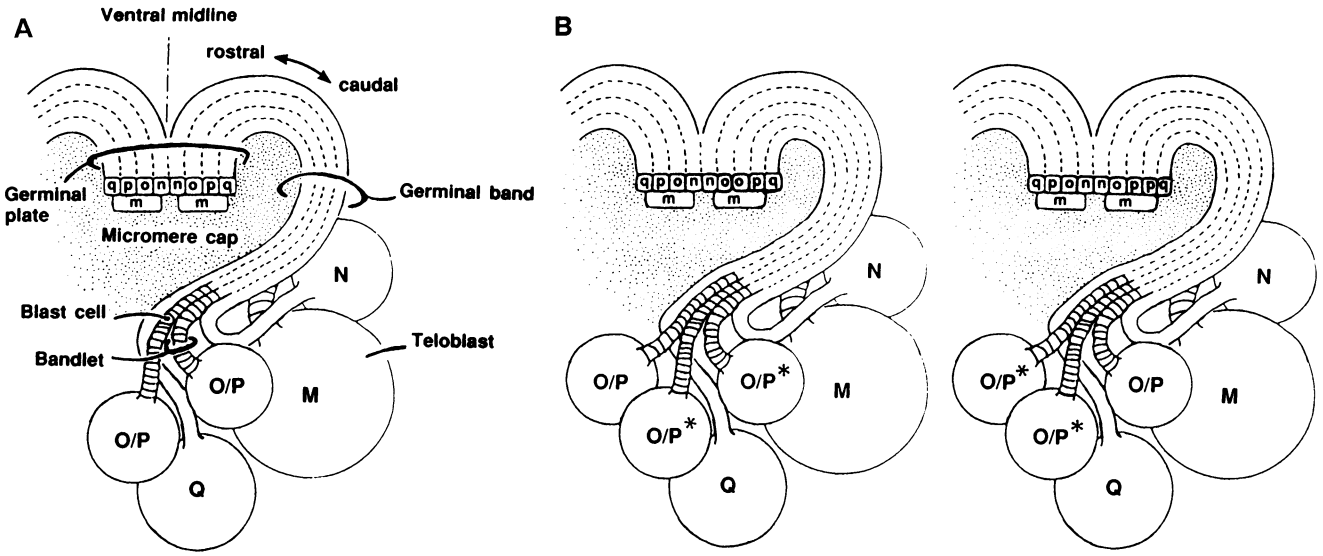


FIG. 1. Development of the germinal plate from the teloblasts; relative positions of the bandlets within the right germinal band. *Helobdella* embryos undergo stereotyped cleavages (stages 1–6) that generate five bilateral pairs of embryonic stem cells (*teloblasts*), designated M, N, O/P, O/P, and Q, that are progenitors of the segmental tissues. (*A*) During normal development, each teloblast undergoes a series of several dozen highly unequal cell divisions (stages 6–8), producing a bandlet of segmental founder cells (primary blast cells). Ipsilateral bandlets merge to form left and right germinal bands that coalesce along the ventral midline to form the germinal plate. A squamous epithelium derived from a cluster of micromere cells (the micromere cap) covers the area between the germinal bands (stippling) and the germinal bands themselves. A cross section of the germinal plate shows the relative positions of the ectodermal (n, o, p, and q) and mesodermal bandlets (m). In normal development, the p bandlet is closer to the micromere cap (capward) than is the o bandlet (anticapward), and the teloblasts from which they arise are designated as generative O and P teloblasts. Bandlets can be distinguished from one another on the basis of the stereotyped mitotic patterns of their blast cell clones (7–10), and each gives rise to a distinct subset of segmentally iterated definitive progeny, which can be identified as pattern elements in the juvenile leech (11). The term kinship group refers to the set of definitive progeny derived from a teloblast lineage that is distributed over one segment. (*B*) Poly(A) injection into an O/P teloblast can induce it to undergo an additional equal cleavage, resulting in an embryo with three ipsilateral OP-derived teloblasts and bandlets. Duplicate bandlets may occupy either the capward position (*Left*) or the anticapward position (*Right*) relative to the nonduplicated bandlet. Asterisks denote the O/P teloblasts derived from the poly(A)-injected O/P teloblast.

the o mitotic pattern, some supernumerary bandlets exhibiting the p type pattern were observed. Moreover, there was a strong correlation between the generative identity of the O/P teloblast that was duplicated and the mitotic pattern of the primary blast cells in the supernumerary bandlet. In

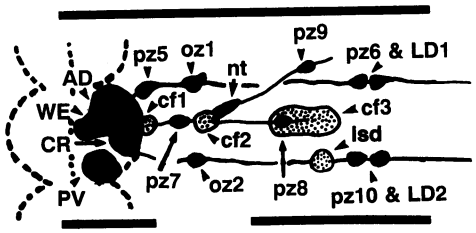


FIG. 2. Schematic showing definitive pattern elements in the O and P kinship groups in a stage 10 embryo that has been cut along its dorsal midline and laid out flat. Rostral is up. One segmental ganglion (and portions of two others) is indicated by dashed curved outlines at left. The ventral midline is indicated by a vertical dashed line; the dorsal midline (not indicated) lies to the right. Cell bodies of neurons and glia are shown in black; nerves are indicated by black lines. Stippled areas indicate domains of specialized epidermal cells. The bar above the schematic indicates the approximate extent of the field shown in Fig. 4 *A* and *B*; bars below the schematic indicate the fields shown in Fig. 4 *C*, *D* and *E*, *F*. Abbreviations of O kinship group pattern elements are as follows: CR, crescent-shaped cluster of neurons; AD, anterodorsal cluster of neurons; LD2, lateral dopamine-containing neuron; nt, nephridial tubule; lsd, lateral skin dot; oz1 and oz2, identified neurons arising from o blast cells; cf1–3, epidermal specializations termed cell florets 1–3. Abbreviations of P kinship group pattern elements are as follows: PV, posterodorsal cluster of neurons; WE, wedge-shaped cluster of neurons; LD1, lateral dopamine-containing neuron; pz 5–10, identified neurons arising from p blast cells.

embryos with duplicate anticapward bandlets, the mitotic pattern in both duplicate bandlets was always (25/25 cases) of the o type (Fig. 3*A*). In embryos with duplicate capward bandlets, the mitotic pattern in both bandlets was of the p type in 69% (22/32) of the cases (Fig. 3*B*). [In a separate experiment (20), we confirmed that both duplicate capward bandlets contained regions of secondary p blast cells, as opposed to premitotic primary p blast cells, by employing an immunohistochemical label that marks S phase nuclei in daughters of cells that have recently passed through mitosis (9).] Of the remaining 10 embryos with duplicate capward bandlets, 7 (i.e., 22% of the total) showed the p type mitotic pattern in the most capward bandlet of the pair and the o type in the other bandlet. In the 3 remaining embryos with apparently healthy duplicate capward bandlets, the mitotic pattern of the bandlet lying between identifiable p and o bandlets was unclassifiable.

Fates of Duplicated Bandlets at Stage 10. The different division patterns of the first mitoses of primary o and p blast cells do not indicate strong commitment to definitive O or P fates (7, 10, 12, 15). Consequently, the fates of primary blast cells in bandlets derived from duplicated O/P teloblasts were also characterized in stage 10 embryos.

In normal development, o and p primary blast cells make segmentally iterated sets of definitive neural and epidermal progeny (Fig. 2), a number of which can serve as diagnostic pattern elements to assess the O or P character of a clone. Moreover, in embryos stained with the dye Hoechst 33258, the number of nuclei associated with a patch of tissue that has been labeled with lineage tracer can be determined, so that the duplication of a given pattern element can be assessed.

To assess the definitive fates of cells in duplicate bandlets, one O/P teloblast was injected with poly(A) and tetramethylrhodamine dextran-amine (RDA) ≈30 hr after egg deposi-

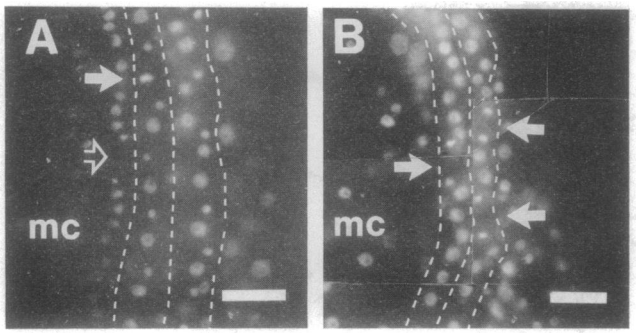


FIG. 3. Mitotic patterns in duplicate bandlets at stage 8. Rostral is up. The right germinal band is shown. Embryos were coinjected at early stage 7 with RDA lineage tracer and poly(A), and fixed at midstage 8 and counterstained with Hoechst 33258. Epifluorescence views of Hoechst-stained nuclei (bright spots). (*A*) Duplicate anticapward bandlets. The pattern of alternating large and small nuclei in both bandlets indicates that primary blast cells have undergone an unequal division characteristic of the O fate. Solid arrow points to a secondary blast cell undergoing mitosis; open arrow points to a mitotic figure in an unlabeled p bandlet that lies to the left of the anticapward bandlets. (*B*) Duplicate capward bandlets. Solid arrows point to mitotic figures in both bandlets. The mitotic pattern of approximately equally sized nuclei indicates that primary blast cells have undergone divisions characteristic of the P fate. The unlabeled o bandlet to the right of the duplicate bandlets is out of the plane of focus. Dashed lines correspond to the approximate borders of the duplicate bandlets. mc, Micromere cap. (Bars = 20 μm.)

tion (early stage 7); the other O/P teloblast was injected with fluorescein dextran-amine (FDA). Two days later, stage 8 embryos were briefly examined by fluorescence microscopy so that embryos with duplicate capward bandlets could be separated from embryos with duplicate anticapward bandlets. The sorted embryos were cultured individually for 3 more days to stage 10, then fixed, and scored for O and P pattern elements derived from duplicate (RDA-labeled) and nonduplicated (FDA-labeled) bandlets. In stage 10 embryos the position of cells derived from the three ipsilateral O/P teloblasts were characteristic of O and P, rather than M, N, or Q, pattern elements. Moreover, the number of RDA- and FDA-labeled cells derived from these teloblasts was far greater than is produced by the two ipsilateral O/P teloblasts during normal development, indicating that substantial numbers of supernumerary definitive progeny were produced.

Two categories of stage 10 embryos were observed. Of 43 embryos with duplicate anticapward bandlets at stage 8, 16 were successfully cultured to stage 10, dissected, and found to contain adequately labeled definitive progeny. In each of these embryos, an apparently complete complement of O kinship group pattern elements, many of which were associated with supernumerary cells, was labeled with RDA only (Fig. 4 *A* and *B*), and an apparently normal complement of P kinship group pattern elements was labeled with FDA. Of 23 embryos with duplicate capward bandlets at stage 8, 10 could be scored at stage 10; in each of these embryos, an apparently complete set of pattern elements in the P kinship group, many of which were associated with supernumerary cells, was labeled with RDA only (Fig. 4 *C–F*), and an apparently normal complement of pattern elements in the O kinship group was labeled with FDA. Thus, we conclude that the definitive progeny derived from supernumerary bandlets assumed the fate characteristic of the generative identity of the duplicated O/P teloblast.

The duplication of O and P pattern elements in stage 10 embryos was not complete, in the sense that not all of the diagnostic pattern elements in the kinship group were associated with supernumerary RDA-labeled cells (Table 1). Certain cells—namely, pz5, pz7, and LD1/pz6 in the P kinship group and oz1, oz2, and LD2 in the O kinship group—were duplicated most regularly. In segments with duplicated peripheral elements, the ganglia also appeared to have many more cells than normal, but such cells could not be unequivocally identified as O or P pattern elements because cells from both kinship groups are found in some of the same areas of the ganglia. Because supernumerary cells were scored only if they were unambiguous, it is possible that all pattern elements were duplicated and that some merely failed to assume their characteristic position. Alternatively, there might be a variable reduction of supernumerary pattern elements due to alterations in cell lineage patterns.

Identifiable definitive progeny of duplicated capward bandlets were exclusively of the P type at stage 10, even though capward bandlets occasionally exhibit o type divisions in stage 8 embryos. From these observations, we infer that primary blast cells in duplicate capward bandlets are capable of giving rise to progeny of the P fate, even after having undergone unequal divisions, and in the presence of an o and a p bandlet. These results are consistent with the notion that the different geometries of the first mitoses of primary o and p blast cells, while strongly correlated with distinct O or P final fates in normal development, do not indicate strong commitment to those fates (10, 12, 15).

Plasticity of Cell Fate. Evidence for the developmental plasticity of blast cells in duplicated bandlets was obtained from a number of embryos in which the FDA injection had temporarily interrupted the production of primary blast cells by the nonduplicated teloblast. The anterior part of the germinal band in these embryos contained only the two RDA-labeled duplicate O/P-derived bandlets, while the posterior part of the germinal band, generated after the FDA-injected teloblast had resumed blast cell production, contained the third FDA-labeled bandlet as well. Some of these embryos had duplicate anticapward (and delayed capward) bandlets, and others had duplicate capward (and delayed anticapward) bandlets.

An examination of the fates of the definitive progeny of the labeled bandlets revealed that duplicate anticapward as well as duplicate capward bandlets are capable of transfating. The rostral segments of these embryos contained the normal number of diagnostic pattern elements of both the O and the P kinship groups labeled with RDA and none labeled with FDA. In the more caudal segments, where FDA-labeled cells derived from the nonduplicated bandlet were present, the identifiable diagnostic cells labeled with RDA in individual specimens were either exclusively O pattern elements or exclusively P pattern elements, and some of these were associated with supernumerary RDA-labeled cells. Pattern elements labeled with FDA were of the complementary type.

Additional evidence for the developmental plasticity of duplicated capward (p) bandlets was obtained from a single embryo that exhibited a spontaneous transposition of the nonduplicate bandlet and one of the duplicate bandlets. In the rostral part of the germinal band in this embryo at stage 8, the nonduplicated (FDA-labeled) bandlet lay capward to the duplicate (RDA-labeled) bandlets, but more caudally, the nonduplicated bandlet lay between the duplicate bandlets. By stage 10, the nonduplicated bandlet had given rise to P pattern elements in the most rostral domain of the embryo and to O pattern elements in a more caudal region (Table 2). In addition, whereas the duplicated anticapward bandlets gave rise to exclusively O pattern elements in the rostral domain of the embryo, they gave rise to both O and P pattern elements in the caudal domain, indicating that one of the duplicate capward bandlets had transfated.

DISCUSSION

Previous investigations of fate plasticity in the O–P equivalence group have relied on cell ablations to perturb normal

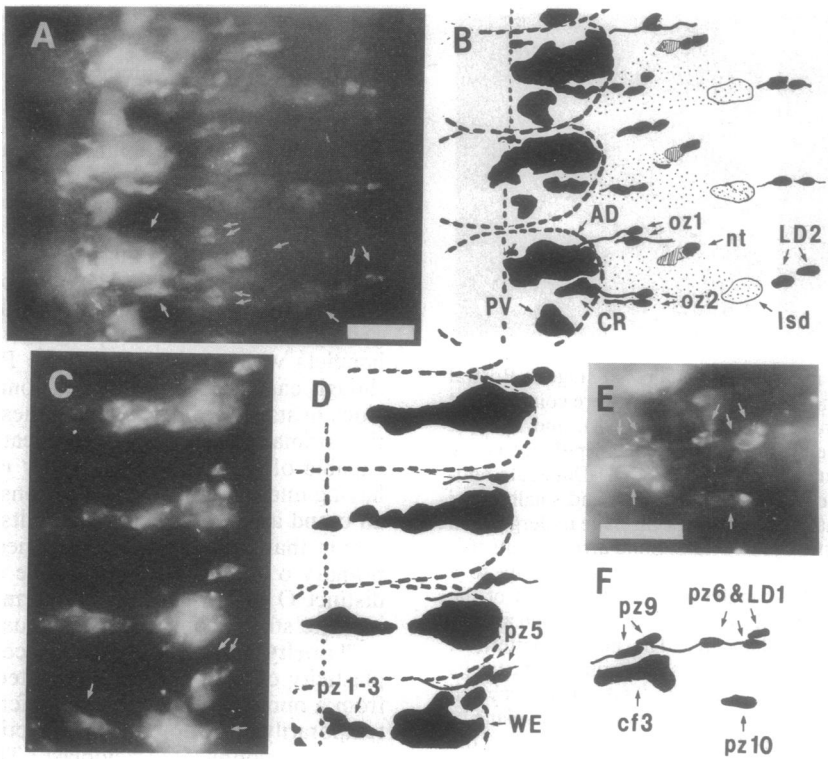


FIG. 4. Definitive progeny derived from duplicate anticapward (o) bandlets and duplicate capward (p) bandlets. Rostral is up. Three different embryos are shown in A, C, and E. Poly(A) and RDA were coinjected at early stage 7 into an O/P teloblast, and the embryos were fixed at stage 10, counterstained with Hoechst 33258, cut along the dorsal midline, and mounted ventral side up under a coverslip. (*A*) Epifluorescence photomicrograph (ventral focal plane) of RDA-labeled definitive progeny derived from duplicate anticapward bandlets in three segmental ganglia and adjoining body wall to the right of the ventral midline. Some dorsal-pattern elements, such as the AD cluster, are not clearly visible because they are out of the plane of focus. A subset of RDA-labeled O pattern elements (alone or in association with supernumerary cells) are indicated in one segment (arrows). No RDA-labeled P pattern elements are present. (*B*) Drawing of the same field as in *A*; identities of a subset of the RDA-labeled cells in one segment are indicated (arrows). (*C*) Epifluorescence photomicrograph of RDA-labeled P definitive progeny derived from duplicate capward bandlets in four segmental hemiganglia. Supernumerary pz5 neurons can be clearly seen in three of the four segments. Note that the PV cluster, an O pattern element, is not RDA-labeled. (*D*) Drawing of the same field as in *C*; identities of a subset of the RDA-labeled cells in one segmental hemiganglion are indicated (arrows). (*E*) Epifluorescence photomicrograph of RDA-labeled P definitive progeny derived from duplicate capward bandlets. A portion of the lateral body wall in one segment is shown. RDA-labeled P pattern elements, pz6 and LD1, and an associated supernumerary cell (top right; three arrows); pz9 and an associated supernumerary cell (top left; two arrows); and cf3 and pz10 (bottom; arrows) are indicated. (*F*) Drawing of the same field as shown in *E*; identities of a subset of the RDA-labeled cells are indicated (arrows). Abbreviations used are the same as those in Fig. 2. Segmental ganglia are indicated by dashed curved lines at left. The dashed vertical lines in *B* and *D* correspond to the approximate location of the ventral midline. (Bars = 20 μm; *A* and *B* are at the same magnification; and *C–F* are at the same magnification.)

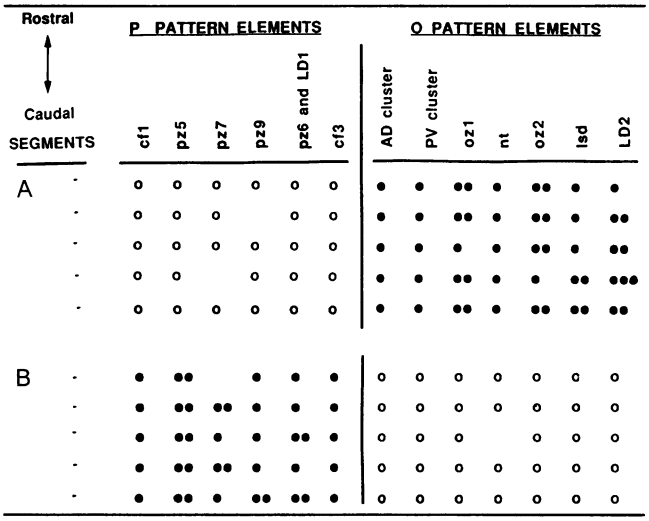
development. Results from these studies favor a model in which the P fate is determined by a factor that is accessible (for example, due to position or quantity) to only one O/P-derived bandlet and that the second O/P-derived bandlet assumes the O fate because it has insufficient access to the determinant. According to this model, any supernumerary O/P-derived bandlet would lack access to the determinant and, thus, should invariably give rise to exclusively O pattern elements. Contrary to such expectations, our results show that blast cell clones in supernumerary bandlets can assume either the P or the O fate and that the fate of a supernumerary bandlet depends, in part, on its teloblast of origin.

If these results are not to be interpreted as contradicting the model under which O/P teloblasts and their primary blast cell progeny are initially equivalent, then it is necessary to postulate that the fates of duplicate bandlets resulting from poly(A) injection are coupled in a way in which normal bandlets are not. Although no physical evidence for such coupling of duplicate bandlets has been obtained, we cannot yet rule out this possibility.

An alternative interpretation of our results is that sister O/P teloblasts and their primary blast cell progeny are initially equipotent but are *not* equivalent—that is, they may share the same range of possible developmental fates and yet respond differently to a given set of environmental conditions. According to this alternative model, both O/P teloblasts would be capable of generating either O or P progeny, but each teloblast would have a predilection for either the O or the P fate. (This distinction will be denoted by referring to the two sister teloblasts as O/P and P/O, respectively.) A bias for either fate could be inherited from the OP proteloblast or could be the outcome of a stochastic process that would bias initially equivalent sister O/P teloblasts and, hence, their primary blast cell progeny. This inherited bias would then influence cell fate decisions made by cells in O/P-derived bandlets by affecting their responses to fate determinants with which they later come in contact. For example, the primary blast cells in bandlets derived from P/O (or O/P) teloblasts might inherit a bias for the capward (or anticapward) position in the germinal band, and once in position, blast cells in the two bandlets would have access to different sets of fate-determining cues.

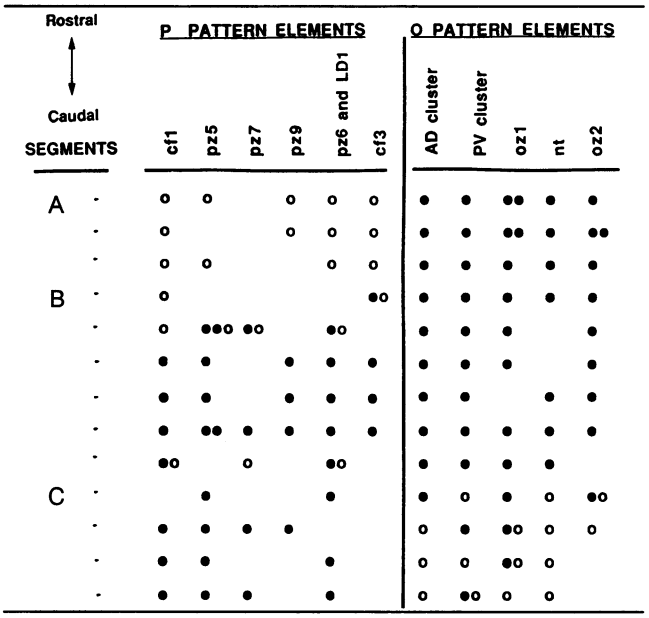
This model would also account for our finding that a third OP proteloblast-derived bandlet lying between the normal capward and anticapward positions has a different fate in embryos with duplicate capward (P/O-derived) bandlets than in embryos with duplicate anticapward (O/P-derived) bandlets. According to the revised model, a centrally located bandlet would be predisposed toward the O or P fate, depending on its teloblast of origin. It should be pointed out

Table 1. Labeled O and P pattern elements in five segments of two representative stage 10 embryos, one (A) with duplicate anticapward bandlets and the other (B) with duplicate capward bandlets



●, RDA-labeled pattern elements; ○, FDA-labeled pattern elements. An extra circle(s) at any one position denotes a supernumerary pattern element. The absence of any circle denotes that the pattern element could not be scored. Abbreviations are the same as for Fig. 2.

Table 2. Plasticity of cell fate in an embryo in which there occurred a spontaneous transposition of a nonduplicated O/P bandlet from its original capward position in the rostral part of the germinal plate to a position between the two duplicated bandlets in the caudal part of the germinal band



RDA-labeled (●) and FDA-labeled (○) pattern elements were scored at stage 10. Labeled pattern elements divide the germinal plate into three distinct zones, A, B, and C. The nonduplicated bandlet gave rise to exclusively P pattern elements in region A, in accord with its capward position, and transfated to give rise to O pattern elements in the caudal region C. The RDA-labeled duplicated bandlets gave rise to exclusively O pattern elements in region A and to both O and P pattern elements in region C, indicating that one of the duplicated bandlets transfated upon its transposition to the capward position. Region B, the transition zone, contained few FDA-labeled cells, most likely due to a short break in the bandlet. Abbreviations are the same as for Fig. 2.

that the one embryo in which a nonduplicate p bandlet lay between the normal capward and anticapward positions and yet assumed the O fate is inconsistent with this aspect of the model. Thus, it is not yet possible to choose definitively between the two alternative interpretations of our results.

Under the revised model proposed above, the primary blast cells in the o and p bandlets, although not equivalent, are still equipotent, and thus both o/p and p/o blast cells would be able to transfate. To date, the only evidence for plasticity in p blast cells was obtained from experiments in which a nominal p bandlet from one germinal band was induced to enter the contralateral germinal band and occupy a position anticapward to one or both of the native o/p bandlets (13). The early mitotic pattern of the switched p bandlets indicated that they had been redirected toward the O fate. In such embryos, only one of the three ipsilateral O/P-derived bandlets exhibited the P mitotic pattern, as if the fate-determining interactions permit only one bandlet to assume the P fate. The present results, based on observations of both early mitotic patterns and definitive progeny, clearly demonstrate that two bandlets within one germinal band can assume the P fate and that the less favored direction of transfating from P to O can, in fact, occur. In addition, these results further support the idea that position-dependent interactions with cells outside of the equivalence group contribute significantly to the o/p cell fate decision.

The observation that both duplicate anticapward bandlets assume the O fate indicates that direct contact with p blast cells is not required for cells to assume the O fate, consistent with the result obtained in *Theromyzon* embryos that blast cells in the o bandlet do not transfate unless they assume the position previously occupied by the p blast cells (14). In addition, the observation that both duplicate capward bandlets can assume the P fate suggests that direct contact with p blast cells may not be a sufficient condition for cells to assume the O fate, consistent with the result, obtained in *Helobdella*, that after ablation of cells in the overlying squamous epithelium, nominal o blast cells transfate even in the presence of the p bandlet (15).

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