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Developmental Interdeterminacy in Embryos of the Leech *Helobdella triserialis*

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In embryonic development of the leech *Helobdella triserialis*, each of the four paired *positionally identifiable*, ectodermal teloblasts (N, O, P, and Q) generates a bandlet of blast cell progeny that merges with ipsilateral bandlets into a germinal band. Left and right germinal bands coalesce into the germinal plate which gives rise to the segmental tissues of the leech and wherein the progeny of each teloblast generate a characteristic pattern of epidermal and neuronal cells. Experiments reported here show that the positionally identified O teloblast sometimes generates the P pattern and vice versa. The reversal of these teloblasts' *generative* identities was shown to correspond to the formation of chiasmata by their blast cell bandlets, so that the positions of their bandlets in the germinal band are reversed as well. Thus it is the position of the bandlet in the germinal band, rather than the position of the parent teloblast, which correlates with the fate of o and p blast cells. Moreover, two types of ablation experiments have shown that, in the absence of generative P teloblast progeny, those cells which would normally generate the O pattern take on a new fate and give rise to the P pattern in the nervous system, both at the gross pattern level in the segmental ganglia, and at the level of identified neurons in the peripheral nervous system. If related, these phenomena suggest that the O and P teloblasts, which derive from the symmetric cleavage of the OP proteloblasts, have a common developmental pluripotency. And in that case, the fates of their progeny are determined hierarchically on the basis of relative position in the nascent germinal band, with P-type fate being preferred.

INTRODUCTION

The normal development of the leech is highly determinate. Pairs of identifiable M, N, O, P, and Q teloblasts arise via a sequence of stereotyped cleavages during the first six stages of leech embryogenesis, and the injection of cell lineage tracers into these cells in embryos of the glossiphoniid species *Helobdella triserialis* and *Haementeria ghilianii* has shown that each teloblast contributes a characteristic and identifiable population of descendant cells in subsequent development. For instance, individual identifiable neurons and glia in the segmental ganglia of the ventral nerve cord normally arise from specific teloblasts. The same is true for groups of epidermal cells populating particular territories of the segmental skin (Stent *et al.*, 1982; Weisblat *et al.*, 1983). Ablation either of an M teloblast (and thus elimination of half the mesoderm), or of an NOPQ blastomere, the precursor of one set of N, O, P, and Q teloblasts (and thus elimination of half the ectoderm) prevents normal development of both ectodermal and mesodermal tissues on that side, and the ablated tissues are not replaced by the remaining cells (Blair, 1982). Similarly the determinancy of neural cell fate within the ectoderm holds after ablation of an N or Q teloblast, or of the OP proteloblast (precursor of the O and P teloblasts). Here the particular neurons and glia normally descended from the ablated blastomere are simply missing, while the remaining blastomeres give rise to their normal complements of progeny cells (Blair and Weisblat, 1982; Blair, 1983). However, cell fate may be less rigidly determined for epidermal cells; following ablation of one ectodermal teloblast (O, P or Q), progeny of the remaining teloblasts cover the territory of the body wall normally populated by progeny of the ablated teloblast (Blair and Weisblat, 1984).

The findings presented in this paper show that there is at least one instance of indeterminancy in the development of the neurons and glia as well: although the set of identified neurons and glia derived from O and P teloblasts taken together is highly stereotyped, that set comprises two stereotyped subsets, each of which can arise from either O or P teloblast. A priori this indeterminacy in the developmental fate of these two teloblasts might reflect merely a spontaneous variability in their relative position, the sole criterion by which they can be identified; in that case the fate of their progeny could be as determinate as that of the N and Q teloblasts. However, ablation experiments show that at least one of the two sister teloblast descendants of

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the OP proteloblast is developmentally pluripotent, in that it can assume the fate of either the O or the P teloblast. Progeny of the pluripotent teloblast assume the fate typical of the O teloblast in normal development, apparently being denied the fate typical of the P teloblast by an interaction with progeny of the sister teloblast.

The nomenclature and developmental staging system used in this paper for the early development of the glossiphoniid leech has been previously described (Weisblat *et al.*, 1980a; Fernandez, 1980; Stent *et al.*, 1982).

MATERIALS AND METHODS

The materials and methods used in these experiments were as described in the preceding paper, with two exceptions.

To improve the resolution with which the HRP labeling pattern can be visualized in whole-mounted embryos, some fixed and stained specimens were immersed in a shallow layer of unpolymerized Epon 812 plastic embedding medium for observation under a compound microscope. The high viscosity of the Epon medium lets one orient the specimens in any attitude for viewing. Cell bodies of presumptive dopaminergic peripheral neurons were identified by their characteristic fluorescence induced by treatment with glyoxylic acid (Blair, 1983; Stuart *et al.*, in preparation).

RESULTS

Variability in Fate of O and P Teloblast Progeny

The M, N, and Q teloblasts, as well as the OP proteloblast, of the *Helobdella* embryo can be identified unambiguously on the basis of the sequence of cleavages that give rise to each of these cells and by their characteristic positions within the embryo. Following injection of any of these identified cells with a cell lineage tracer during stages 6 or 7, a distinct and invariant segmentally repeated pattern of labeled progeny cells is observed later in the stage 10 embryo (At that stage, the leech embryo has been enclosed by a body wall along most of its length and its nervous system is in an advanced state of development.) The identification of the O and P teloblasts is more ambiguous, however. These two teloblasts arise by symmetric cleavage of the OP proteloblast at the end of stage 6c, and hence are not distinguishable from each other on the basis of cleavage patterns. Thus, their designation has been based solely on relative cell position within the embryo; that teloblast of the two which is more anterolateral and thereby closer to the N teloblast is designated as O and the other, more posteromedial, is designated as P. Moreover, following the injection of a cell lineage tracer into a teloblast identified either as O or as P, one of two different patterns of labeled progeny cells may result (Fig. 1). One of these patterns, designated as the "O pattern," is observed in most embryos in which the teloblast identified by its position as O was injected, and the other pattern, designated the "P pattern," is observed in most embryos in which the teloblast identified by its position as P was injected. Both labeling patterns comprise an epidermal and a neuronal (and glial) component.

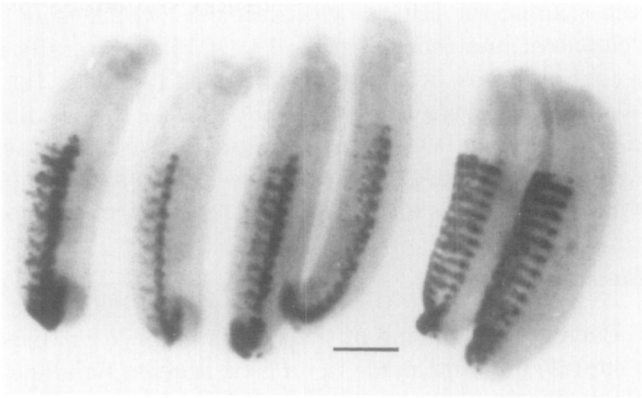

FIG. 1. Variability in fate of a positional O teloblast's progeny. Ventral view of six HRP-stained stage 10 embryos whose right positional O teloblast was injected with HRP early in stage 7 (after the blast cells contributing to the most anterior ganglion had already been produced). The four embryos on the left exhibit the typical O pattern of label, while the two on the right exhibit the pattern typical of P. The epidermal components of both patterns lie in the ventral body wall ipsilateral to the injected teloblast, forming sets of segmentally iterated, interdigitating projections. In the O pattern, discrete transverse fingers extend laterally from the ventral midline. In the P pattern, a continuous strip of epidermis runs longitudinally near the future lateral edge of the embryo, and transverse fingers extend medially, complementary to the O pattern. The neuronal (and glial) components of the O and P patterns consist of both ganglionic and peripheral cells (see Fig. 4 for details); in the four O-pattern embryos some peripheral neurons can be seen as dots outside the labeled epidermis. The variation in stain intensity is presumed to reflect variation in the amount of HRP injected. Anterior is up; scale bar, 200 μm.

In a minority of embryos, the inverse situation obtains. Here the P labeling pattern is observed upon injection of the O teloblast, and the O labeling pattern is observed upon injection of the P teloblast. (The sum of O and P patterns is nevertheless equal to the invariant pattern regularly resulting from injection of the OP proteloblast.) Thus, for the two sister teloblasts descended from the OP proteloblast, it is necessary to distinguish between *positional* identity, which is manifest at stage 7 by virtue of the relative location of the two cells within the embryo, and *generative* identity, which is manifest only later in development, by virtue of the segmental distribution pattern, and hence fate, of their progeny. For instance, in one series of embryos the positionally identified O teloblast was injected with horseradish peroxidase (HRP) at stage 7, and the embryos were fixed

and stained for HRP 1 week later, by which time development had reached stage 10. Of 19 successfully injected embryos in two experiments, 14 showed the O labeling pattern and 5 the P pattern, so that roughly 26% of positional O teloblasts are generative P teloblasts. A similar proportion of generative O teloblasts is found when positionally identified P teloblasts are labeled.

Bandlet Chiasmata

Each germinal band of the stage 7 embryo comprises a superficial layer made up of four aligned bandlets of blast cells (n, o, p, and q) derived from four ectodermal teloblasts (N, O, P, and Q), respectively, lying over the single bandlet of m blast cells derived from the mesodermal teloblast (M). In that superficial ectodermal layer, the n and q bandlets lie at the lateral and medial edges of the germinal band, respectively, separated by the o and p bandlets. It has been generally observed that of the two inner bandlets, the bandlet of the positional O teloblast lies laterally, i.e., next to the n bandlet, and the bandlet of the positional P teloblast lies medially, i.e., next to the q bandlet. However, Fernandez and Stent (1980) found that in embryos of the glossiphoniid leech *Theromyzon*, the positions of these two bandlets may be transposed so that the o bandlet lies next to the q bandlet and the p bandlet lies next to the n bandlet. It seemed possible that the indeterminate relation between positional and generative identities of O and P teloblasts derives from a variability of the relative position of their blast cell bandlets in the germinal band.

To explore this possibility, an OP proteloblast was injected with HRP in a series of stage 6b embryos, before it cleaved to yield the O and P teloblasts. Development was allowed to proceed to late stage 7, at which time the embryos were fixed and stained for HRP. At this stage, it is possible to follow the labeled o and p bandlets from their position in the germinal band back to their teloblasts of origin (Fig. 2). Of 46 such embryos examined in three experiments, 31 showed the typical configuration of germinal bandlet and teloblast of origin; here the anterolateral, positional O teloblast was the source of the bandlet next to n and the posteromedial, positional P teloblast was the source of the bandlet next to q. However, 15 embryos of this series showed the reverse configuration; here the positional O teloblast was the source of the bandlet next to q and the positional P teloblast was the source of the bandlet next to n. In these latter embryos, the two bandlets crossed one another between their teloblast of origin and their point of entrance into the germinal band, forming chiasmata similar to those described by Fernandez and Stent (1980).

The fraction of embryos in which the o and p bandlets form a chiasm prior to their entry into the germinal band (33%) is similar to that (26%) in which the positional and generative identities of the O and P teloblasts are not in concordance. Hence it seems likely that these two phenomena are correlated, in that the embryos in which an O teloblast gives rise to a P labeling pattern are just those in which there is a bandlet chiasm. If this were actually the case, then it would follow that there is no indeterminacy of cell fate as regards the o and p bandlets once their relative positions in the germinal band have been established: the bandlet next to the n bandlet would always give rise to the O pattern and that next to the q bandlet, the P pattern.

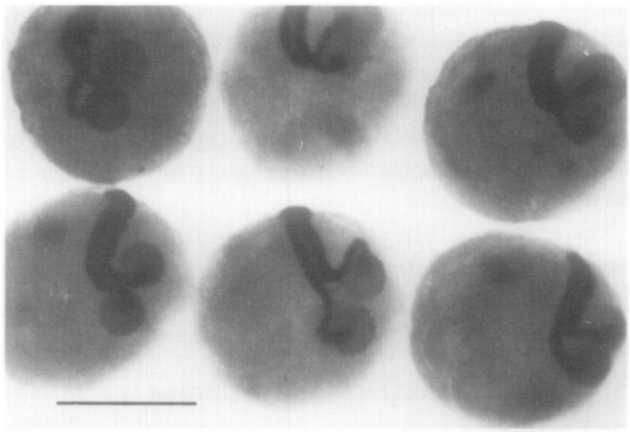

FIG. 2. Bandlet chiasmata. Dorsal view of six HRP stained, late stage 7 embryos, whose right OP proteloblasts were injected with HRP at stage 6b. In each embryo the daughter right O and P teloblasts appear as two lightly stained spheres from which darkly stained blast cell bandlets emerge and meet at the point of origin of the right-hand germinal band. Note that in the bottom three embryos the anterolateral, positional O teloblast gives rise to the more lateral of the two labeled bandlets and the posteromedial, positional P teloblast gives rise to the more medial one. But in the top three embryos, these relations are reversed so that the o and p bandlets cross at their point of entry into the germinal band. Scale bar, 250 μm.

Concordance of Blast Cell Bandlet Position and Cell Fate

To test whether it is indeed the relative position of the O- or P-derived bandlet in the germinal band that is correlated with its developmental fate, an O or P teloblast was injected with HRP in a series of early stage 7 embryos. The embryos were allowed to develop to early stage 9 before being fixed and stained for HRP. At this stage, thanks to the rostrocaudal progression of development in the leech embryo, blast cell proliferation and morphogenesis have progressed far enough in the anterior sectors of the germinal plate to enable one to ascertain whether an O or P labeling pattern is

being produced, whereas in the posterior part of the embryo, where right and left germinal bands have just coalesced to form the germinal plate, the relative position of the labeled blast cell bandlet within the band is still discernible. (It should be noted that the coalescence of the right and left germinal bands along the ventral midline is preceded by their circumferential migration over the surface of the embryo, from dorsal to the ventral aspect. As a result, an inversion of the mediolateral order of the bandlets within the germinal plate has occurred: the initially medial q bandlet now lies at the lateral edge and the initially lateral n bandlet now lies at the ventral midline of the germinal plate. Accordingly, for the two center bandlets, the n-proximal position is medial to the q-proximal position.)

The identification of an O or P labeling pattern in the anterior germinal plate of stage 9 embryos is based on a comparison of that early O or P pattern with the "early" labeling pattern in the *posterior* germinal plate of stage 10 embryos, in which (again thanks to the rostrocaudal progression in development) the anterior germinal plate already manifests the definitive, i.e., "late", O or P labeling pattern. The early O labeling pattern consists of a zig-zagging longitudinal array of clustered, stained cells (Fig. 3a, lower), whereas the early P labeling pattern consists of a longitudinal band of stained cells. The lateral edge of this band is smooth but from its medial edge a finger of stained cells projects toward the ventral midline in each segment (Fig. 3a, upper). In six of the nine embryos examined in this experiment, the labeled blast cell progeny displayed the early O pattern in the anterior germinal plate, and the labeled blast cell bandlet lay in the medial position in the posterior germinal plate. In the remaining three embryos, the early P labeling pattern was manifest in the anterior germinal plate, and the labeled blast cell bandlet lay in the lateral position in the posterior germinal plate. No embryos were encountered in which an anterior early O or P labeling pattern was associated with the inverse bandlet order in the rear. The relative positions of the labeled blast cell bandlets within the posterior germinal plate, as inferred from inspection of the whole-mounted embryos, was confirmed by examination of sectioned embryos (Fig. 3b). Thus it appears that, as far as the blast cell bandlets are concerned, positional and generative identities coincide: regardless of its teloblast of origin, the bandlet next to n gives rise to the O pattern and the bandlet next to q gives rise to the P pattern.

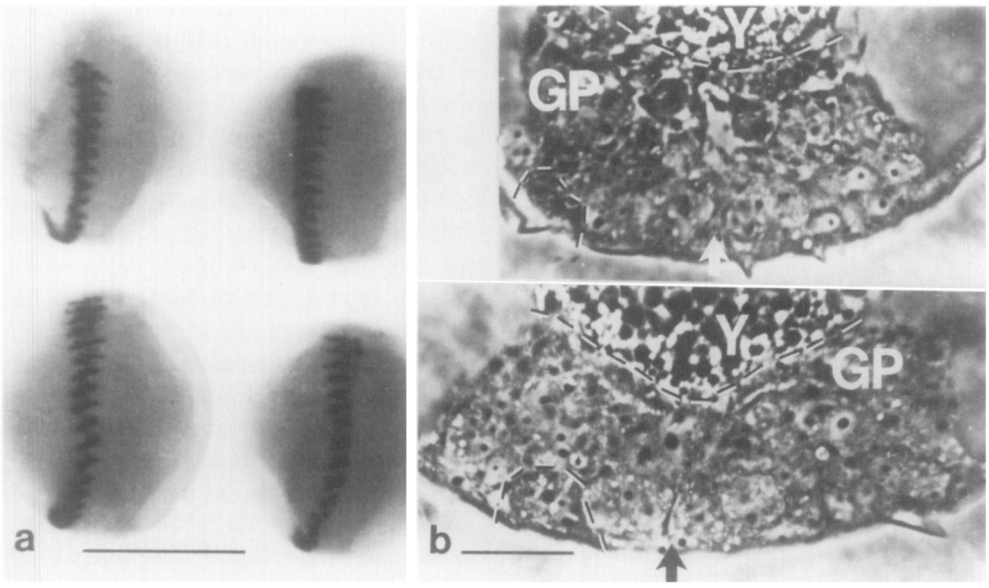

FIG. 3. Concordance of blast cell bandlet position and cell fate. (a) Ventral view of four HRP stained, early stage 9 embryos, of which the right O or P teloblasts were injected with HRP early in stage 7. The two upper embryos exhibit early P-labeling pattern in their rostral, developmentally more advanced segments. This pattern consists of a longitudinal band of stain with a smooth lateral edge and medially (rightward) projecting fingers (cf. Fig. 1, right). The two lower embryos exhibit early O labelling patterns in their rostral segments consisting of a zig-zagging array of clusters, from which laterally (leftward) projecting fingers are just emerging (cf. Fig. 1, left). Anterior is up; scale bar, 250 μm. (b) Sections through the caudal germinal plate of embryos such as those shown in (a). HRP-stained cells, which appear yellow against a blue background in the toluidine blue counterstained sections, are indicated here by a dotted outline at the superficial edge of the germinal plate (GP). The bandlet giving rise to the P pattern (upper section) is more lateral than the bandlet giving rise to the O pattern (lower section). The midline is indicated by an arrow; yolk (Y) in the gut (outlined by upper dashed line in each section) underlies the germinal plate. Scale bar, 25 μm.

Developmental Plasticity of Teloblast Fate

The findings presented thus far can be accounted for by the hypothesis that the two teloblasts produced by symmetric cleavage of the OP proteloblast are of equivalent developmental pluripotency and that their generative identities are not fixed. Instead, under this hypothesis, the O-pattern or P-pattern fate is allotted to their progeny on the basis of the relative position (n-proximal or q-proximal) of their blast cell bandlets in the germinal band. However, an alternative exists, namely that cleavage of the OP proteloblast *does* segregate developmental potential and produces two teloblasts with determinate O and P generative identities, but whose positions in the stage 7 embryo are indeterminate. We shall designate such hypothetical teloblasts of determinate generative identity as O* and P*. Under this alternative hypothesis, the blast cell bandlet generated by either teloblast would invariably seek out its appropriate position (n-proximal for the O*-derived and q-proximal for the P*-derived bandlet) in the germinal band, creating chiasmata in those atypical embryos wherein the positions of the O* and P* teloblasts happen to have been interchanged. A fundamental conceptual distinction between these two hypotheses is that the first requires that the blast cell progeny of both daughter teloblasts of the OP proteloblast be capable of giving rise either to the O or to the P pattern; in other words, it envisages a developmental plasticity of teloblast and blast cell fate. The second hypothesis, by contrast, invokes an indeterminacy of teloblast *position* but does not require the postulation of a plasticity in blast cell fate.

Transfating

To explore the possibility that the developmental fates of sister O and P teloblasts are plastic, three types of experiments were carried out in which effects of ablating one teloblast of an O/P pair on the fate of the other were observed. In the first, an O or a P teloblast of early stage 7 embryos was injected with the usual mixture of HRP and fast green. After 8 to 12 hr of further development (to mid stage 7), the ipsilateral, previously *un*injected P or O teloblast (identified by its lack of green dye) was ablated by injecting it with DNase I (Blair, 1982). The doubly injected embryos were then allowed to develop to late stage 10, at which time they were fixed and stained for HRP. Since the ablated teloblast had already produced a dozen or so blast cells prior to its injection with DNase I at mid-stage 7, the anterior zone of these stage 10 embryos, consisting of segments derived from a full complement of blast cells, would be expected to be of normal morphology. By contrast, the posterior zone of these embryos, consisting of segments derived from an incomplete complement of blast cells (lacking the bandlet normally provided by the ablated O or P teloblast on one side), would be expected to show morphological deficits. Thus in each successfully injected specimen of this series, the generative identity of the HRP-labeled teloblast was ascertained by examining whether the distribution of labeled cells in the anterior, morphologically normal zone of the embryo corresponded to the O or to the P pattern. The generative identity of the ablated sister teloblast was inferred as being of the complementary P or O type. The effects of ablating a teloblast of known P or O generative identity on the fate of the descendants of the surviving sister O or P teloblast were then observed in the posterior, deficient zone of the embryo.

Seventeen embryos of each type were obtained in four experiments. Figure 4b shows one specimen in which the P labeling pattern is manifest in the anterior, normal zone. Hence here a generative P teloblast was HRP-labeled and its generative O sister teloblast was ablated. The labeling pattern of the body wall is clearly different in the posterior, deficient zone, in that here the labeled epidermal cells have expanded from the territory of the medially projecting transverse fingers normally occupied by P teloblast descendants (as manifest in the body wall of the anterior zone of this specimen) into the territory of the discrete transverse fingers normally occupied by the descendants of the ablated O teloblast. This result is consonant with the general finding that following ablation of any ectodermal teloblast, the descendants of the remaining teloblasts spread to cover the resulting epidermal deficiency (Blair and Weisblat, 1984). As for the labeling pattern of the segmental ganglia and connective nerves in the posterior, deficient zone of this specimen, it appears to be essentially the same characteristic P pattern as that seen in the anterior, normal zone. Hence ablation of a generative O teloblast and elimination of its blast cell bandlet descendants appears not to have an obvious effect on the neuronal (and glial) fate of the P teloblast descendants.

Figure 4a shows another specimen from this experiment, in which the O labeling pattern is manifest in the anterior, normal zone. Hence, in contrast to the specimen presented in Fig. 4b, here a generative O teloblast was HRP-labeled and a generative P teloblast was ablated. As was the case in that specimen, the labeling pattern of the body wall is clearly different in the posterior, deficient zone. Again the labeled epidermal cells have expanded, in this case from the territory normally occupied by the O teloblast descendants (as manifest in

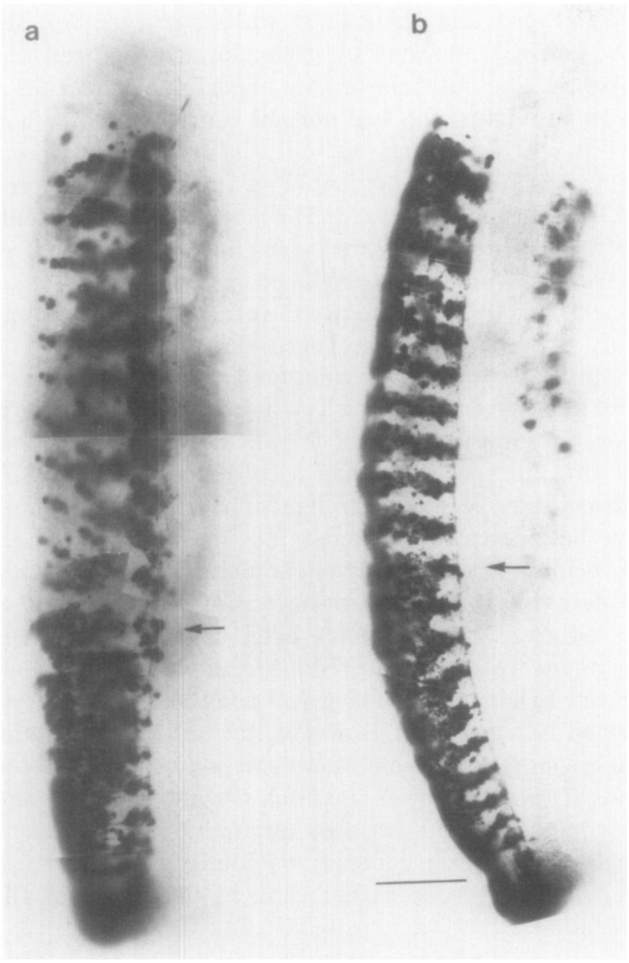

FIG. 4. Transfating. Ventral view of two HRP-stained stage 10 embryos in which either the O or P teloblast was injected with HRP at the beginning of stage 7, and the P or O teloblast was ablated by DNase I injection about 12 hours later. Each embryo is presented as a montage of photographs taken through a compound microscope, so that the staining pattern can be observed in greater detail. (a) Embryo showing a normal O staining pattern anterior to the arrow, focused on the ventral nerve cord. The central neuronal (and glial) component of the O pattern comprises relatively more labeled cells, distributed throughout the ipsilateral half ganglion from the lateral margin to the midline, with processes coursing in both segmental nerves. Transverse fingers of epidermis and medial peripheral neurons are out of focus. Lateral peripheral neurons are in focus. Posterior to the arrow, the staining pattern in the CNS changes to that typical of the P pattern (see b) and the stained epidermis has spread to form a continuum. (b) Embryo showing a normal P pattern anterior to the arrow. Here the central neuronal (and glial) component can be seen to consist of relatively fewer cells (partially obscured by overlying epidermis) in a narrow transverse band extending from the lateral margin in the center of each ipsilateral half ganglion toward (but not reaching) the midline, with visible processes in the ipsilateral but not the contralateral connective nerve. Peripheral neurons are largely obscured by overlying epidermis. Posterior to the arrow, the staining pattern in the CNS remains the same, while the stained epidermis has expanded into a continuum and also moved somewhat laterally, revealing the ganglion more clearly. Anterior is up; scale bar, 100 μm.

the body wall of the anterior sector) into the territory normally occupied by the descendants of the ablated P teloblast, as was expected. But an entirely novel finding emerges upon scrutiny of the labeling pattern of the segmental ganglia and connective nerves in the posterior, deficient zone of this specimen: here the characteristic O labeling pattern manifest in the anterior of the embryo, characterized both by a large number of ganglionic cells and by processes coursing in both sides of the connective nerve, has been transformed into the characteristic P labeling pattern, consisting of a narrow, transverse band of ganglionic cells and by processes visible only in the ipsilateral connective nerve. Evidently, upon ablation of the P teloblast, the fate of the remaining blast cells produced by the O teloblast has changed from the typical O pattern to the typical P pattern. We shall refer to such a change in developmental cell fate as *transfating.* The phenomenon of transfating demonstrates that the generative O teloblast is developmentally pluripotent, as is required by the hypothesis explaining the variability in the fate of progeny of (positionally identified) O and P teloblasts on the basis of their indeterminate generative identity. It should be noted, however, that the transfating phenomenon revealed by this experiment is not reciprocal: whereas upon ablation of the P teloblast the O teloblast takes on the P generative identity, upon ablation of the O teloblast the P teloblast retains its P generative identity. Hence the question of whether or not the P teloblast also possesses the kind of developmental pluripotency required by this hypothesis is left open.

Transfating of Identified Neurons

The whole-mounted embryo of Fig. 4a demonstrates transfating of the O teloblast at the relatively gross level of the labeling pattern of groups of cells and cell processes in the segmental ganglia and connective nerves. A second type of experiment was carried out to demonstrate transfating at the level of identified neurons. For this purpose, attention was focused on three bilaterally paired, segmentally iterated dopaminergic neurons, whose cell bodies lie in the ventral body wall outside the segmental ganglion. These neurons can be identified in dissected body wall preparations of adult leeches by their blue-green fluorescence after treatment with glyoxylic acid (Stuart *et al.*, in preparation) and by their stereotyped positions. Of these three dopaminergic cells, the largest, designated MD, lies medially just outside the segmental ganglion. The two smaller peripheral dopaminergic cells lie more laterally; the larger of these, designated LD_1, lies adjacent to or within one anulus anterior to the MD cell, while the smaller,

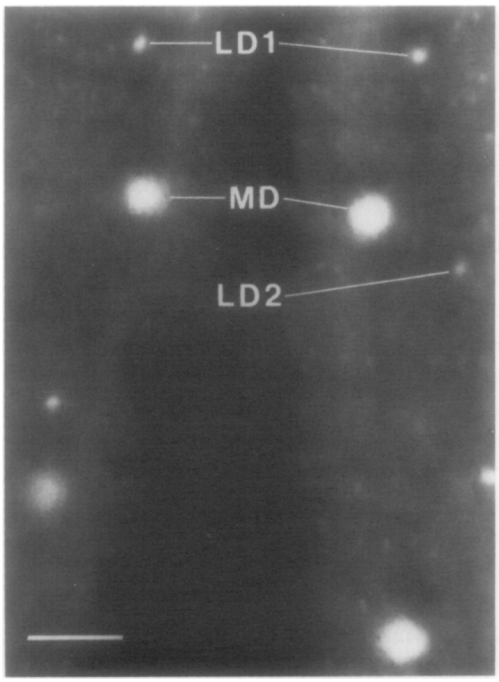

FIG. 5. Transfating of identified neurons. Fluorescence photomicrograph of dissected, glyoxylic acid-treated body wall from a mature *Helobdella* in which either the O or P teloblast was ablated at stage 6c of embryogenesis. The nerve cord has been removed from this preparation. Bilateral pairs of MD (Q-derived) and LD_1 (generative P-derived) neurons are present (two pairs of each are shown), but LD_2 (generative O-derived) neurons are present only on the right (unablated) side. Anterior is up; scale bar, 100 μm.

designated LD_2, lies slightly posterior to the MD cell (Fig. 5). Cell lineage studies using fluorescent tracers have shown that the MD neuron is descended from the Q teloblast, and that the LD_1 and LD_2 are descended from the (generatively identified) P and O teloblasts, respectively (Stuart *et al.*, in preparation). These inferences reached from direct cell lineage tracing studies are supported by results of teloblast ablation experiments: upon ablation of a Q teloblast, the operated embryo develops into an adult leech that lacks half of its normal complement of MD neurons but has its full complement of LD_1 and LD_2 neurons. By contrast, upon ablation of an OP proteloblast, the operated embryo develops into an adult leech that lacks half its normal complement of LD_1 and LD_2 neurons but has its full complement of MD neurons (Blair, 1983).

To demonstrate transfating at the level of identified neurons, either an O or a P teloblast was ablated at random by DNase I injection in a series of early stage 7 embryos. Thus, in about half of these embryos, a generative O teloblast, and in the other half, a generative P teloblast, would be expected to have been ablated. Sixteen such operated embryos were successfully raised to adulthood and their body walls surveyed for MD, LD_1, and LD_2 neurons by glyoxylic acid-induced fluorescence. The outcome of this experiment was that in all 16 specimens the full normal complements of both MD and LD_1 neurons, but only half the normal complement of LD_2 were present (Fig. 5). A full complement of LD_2 neurons was found in the most anterior segments, but this was to be expected since here LD_2 neurons are derived from blast cells produced by the OP proteloblast prior to its cleavage into the O and P teloblasts (Stuart *et al.*, in preparation; see Discussion).

The presence of the full complement of MD neurons in all of these specimens was expected, of course, on the basis of their known line of descent from the Q teloblast. But that all specimens had a full complement of LD_1 neurons and a reduced complement of LD_2 neurons would have been surprising except for the discovery of the nonreciprocal transfating phenomenon. Since it is most unlikely that in all 16 embryos a generative O teloblast was ablated, it would have been expected that some specimens would show a reduced complement of the P-derived LD_1 neurons and a full complement of the O-derived LD_2 neurons. However, by taking transfating into account, the present result can be readily explained: in all of those embryos in which the generative P teloblast happened to have been ablated, the O teloblast's progeny took on the generative P identity and thus gave rise to LD_1 neurons rather than to their normal LD_2 descendants.

Bandlet Switching

A third type of experiment was carried out to further test the hypothesis that the O and P sister teloblasts are developmentally pluripotent. Here the developmental fate of these teloblasts' progeny was examined under conditions in which they have been caused to grow to ectopic positions in the germinal bands and germinal plate. To effect such relocation, advantage was taken of blast cell bandlet "switching," a phenomenon induced by certain ablations during stage 7 of development (Blair and Weisblat, 1982, 1984). For instance, following ablation of an OPQ blastomere (precursor of the O, P, and Q teloblasts) on one side of an early stage 7 embryo, the bandlet being produced by the surviving, contralateral Q teloblast may break at its point of entry into the germinal band so that younger q blast cells produced thereafter are deflected from entering the normal germinal band and switch to the deficient germinal band on the operated side. The stage 10 embryos that develop in the wake of such a bandlet switch consist of an anterior zone in which the nonoperated side has the descendants of q blast cells, while the operated side does

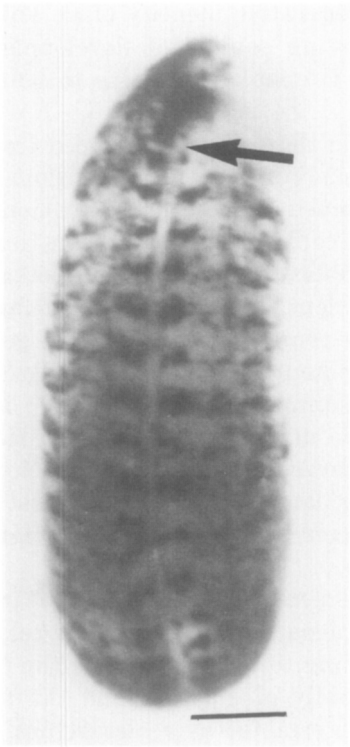

FIG. 6. Bandlet switching. Ventral view of an HRP-stained stage 10 embryo whose left OPQ blastomere was ablated by DNase injection at stage 7a and whose right OP proteloblast was injected with HRP at stage 6b, so that both the surviving o and surviving p bandlets were labeled. The stain pattern consists of an anterior, unswitched zone (above the arrow) formed from germinal bands made while both labeled bandlets were still together on the right (apparent left) side, and a posterior, switched zone (below the arrow) formed from germinal bands made after one of the labeled bandlets had switched to the ablated side. In the anterior zone (partially hidden and out of focus due to the curvature of the embryo) stained neural tissues are confined to the ipsilateral half germinal plate, as are the stained epidermal cells, except for some spread across the ventral midline (see Blair and Weisblat, 1984). In the switched zone, stain is bilaterally distributed in a mirror symmetric pattern. This distribution of neuronal progeny resembles that of the P pattern (cf. posterior zones of embryos shown in Fig. 4). Anterior is up; scale bar, 100 μm.

not, and of a posterior zone in which the reverse is true. The rostrocaudal position of the boundary between the morphologically distinct anterior and posterior zones of such "switched" stage 10 embryos reflects the number of blast cells produced by the surviving Q teloblast prior to the switch of its bandlet from one germinal band to the other. Similarly, in OPQ blastomere-ablated embryos, the blast cell bandlet of a surviving contralateral O or P teloblast may switch to the germinal band on the operated side, independently of the q bandlet switching (Blair, unpublished observations). In that case, the resultant "switched" embryo has on one side a sector of the germinal band (and later of the half-germinal plate) in which O- and not P-derived blast cells are present, whereas on its other side the reverse situation obtains, with P- but not O-derived blast cells being present.

In order to examine the *generative* identity of O and P teloblasts in such "switched" embryos, the left OPQ blastomere was ablated by DNase I injection in a series of stage 6a embryos. At stage 6b, the right OP proteloblast was injected with HRP, so that both the surviving O teloblast and the surviving P teloblast were labeled. Development was allowed to proceed to stage 10, at which time the embryos were fixed and stained for HRP. Following ablation of an OPQ blastomere, the survival rate of the embryos is low and HRP injection of the contralateral OP proteloblast is difficult (because debris from the ablated blastomere is trapped under the vitelline membrane, which makes it hard to identify and impale proteloblast OP); in addition, switching of o or p bandlets is infrequent. Thus, among over a hundred operated specimens in eight experiments only two stage 10 embryos were obtained in which a segmental HRP labeling pattern was observed on both sides of a posterior body sector. Of these two embryos, one was of abnormal morphology, in that the left and right germinal bands had failed to coalesce completely to form an intact germinal plate. (Failure of germinal band coalescence is a common abnormality in leech development which occurs even more frequently following blastomere ablation.) Nevertheless, the segmental labeling pattern of both embryos was sufficiently unambiguous to allow an assignment of generative identities to the surviving O and P teloblasts (Fig. 6). In both embryos there was an anterior zone in which label was present only on the right (unoperated) side. This anterior zone represents those tissues which developed from blast cells produced prior to the bandlet switch, and corresponds to the normal OP (i.e., O plus P) pattern. More importantly, in both embryos there was a posterior zone in which the label was found on both sides; this posterior zone comprises tissues which developed from blast cells produced after an o or p bandlet had switched from the right to the left germinal band. Moreover the labeled cells in this zone occurred in a bilaterally symmetric pattern corresponding to the P pattern in the ventral nerve cord. That is to say, on either side, there is a narrow, transverse band of a few labeled cells on the ventral aspect of each ganglion extending from the lateral margin toward the midline. And on each side processes of the HRP-labeled cells could be seen in the ipsilateral connectives, with no visible processes crossing the midline of the ganglion to enter the contralateral connective nerve. The bilateral symmetry of the labeling pattern in this posterior sector indicates that following the

switch, the surviving positional 0 and P teloblasts shared a single generative identity; thus in this embryo one teloblast had clearly changed its generative identity from the O to the P type.

This result provides additional evidence that at least one of the daughter teloblasts of the OP proteloblast is developmentally pluripotent. For in the single successfully operated specimen, both sister teloblasts took on the generative P identity after one of the blast cell bandlets had switched from one germinal band to the other.

DISCUSSION

This paper presents findings relating to cellular indeterminacy and pluripotency in embryogenesis. In discussing these findings, it is necessary to distinguish between the positional identity of a cell, based on its location in the embryo, and its generative identity, based on the particular set of identifiable progeny to which it ultimately gives rise.

The present findings show that the *positional* and *generative* identities of the ectodermal sister teloblasts O and P, produced by symmetric cleavage of the OP proteloblast, are not necessarily the same: in about one-third of *Helobdella* embryos, the progeny of the teloblast located at O position take on a fate that is typical of the P teloblast's progeny and vice versa. In a similar fraction of embryos, the blast cell bandlets of sister O and P teloblasts cross one another before entering the germinal bands. Furthermore, it was shown in a separate experiment that the positional identities of the bandlets, defined by their relative position within the germinal band, *are* the same as their generative identities. Therefore we conclude that the interconvertibility of the fates of (positionally identified) O and P teloblasts is associated with the transposition of their blast cell bandlets in the germinal band. Whereas the o bandlet typically lies next to the n bandlet, and the p bandlet next to the q bandlet, the positions of o and p bandlets are reversed in just those embryos in which the positional and generative identities of the parent teloblasts differ.

A second series of experiments reported here deals with the phenomenon of trasfating. It was shown that progeny of the generative O teloblast give rise to the P pattern of descendants when developing in isolation from progeny of the generative P teloblast, whether that isolation results from ablation of the generative P teloblast or from bandlet switching following ablation of the contralateral OPQ blastomere. The phenomenon of transfating demonstrates that the O teloblast is developmentally pluripotent. Transfating, as observed here, is nonreciprocal, in that a P teloblast does not take on the generative identity of an ablated O sister teloblast. Thus an equivalent developmental pluripotency for the P teloblast remains to be directly demonstrated.

It is possible that ablation-induced transfating and the indeterminacy of the fate of (positionally identified) O and P teloblasts are unrelated phenomena. In that case one must consider the hypothesis that cleavage of the OP proteloblast produces one unipotent teloblast of generative P identity (whose blast cell bandlet is destined for the q-proximal position in the germinal band) and one pluripotent teloblast, nominally of O type but capable of assuming that P generative identity after ablation of the unipotent P teloblast. Here the indeterminacy observed might be ascribed to a variability in the cleavage plane of the OP proteloblast that consigns the two daughter teloblasts to their relative positions in the embryo.

However, it seems more likely that the indeterminacy of O and P teloblasts is a phenomenon related to transfating. According to this hypothesis the O and P teloblasts are equally pluripotent and the fates of their progeny are determined by a hierarchical, position dependent interaction within the germinal band. [These cells thereby constitute an equivalence group according to the terminology applied to similar cases in nematode development (Kimble *et al.*, 1979)]. Under this hypothesis the o and p bandlets both prefer the position in the germinal band next to q, which is somehow associated with the generative P identity. But whichever bandlet comes to occupy the preferred q-proximal position (usually the bandlet arising from the positional P teloblast), excludes its sister bandlet from that position, thereby restricting it to the generative O identity. Upon ablation of the generative P teloblast, or after switching isolates the generative o bandlet, the q-proximal position becomes vacant. Blast cells produced thereafter by the former generative O teloblast move into the position from which they would have otherwise been excluded and take on the generative P identity.

Just how bandlet position in the germinal band might determine the particular fate of pluripotent blast cells remains to be understood. Interaction with the n bandlet cannot be a necessary condition for preventing expression of the generative P identity, and hence causing expression of the generative O identity, since the O pattern arises even after ablation of an ipsilateral N teloblast (Blair and Weisblat, 1982; Blair, 1983). Nor can interaction with the q bandlet be a necessary condition for determination of the generative P identity, since in the "switched" OPQ-ablated embryos presented here, the generative P identity arose on a side which lacked a q bandlet.

It should be noted that transfating seems to be a special property of the descendants of the OP proteloblast, inasmuch as the M, N, and Q teloblasts neither change their fate in response to ablation of another teloblast nor have their fates taken on by any other cell in any ablation protocol devised so far (Blair, 1983; Blair and Weisblat, 1982). Hence the interchangeability of fate appears to be confined to the only two sister teloblasts that are indistinguishable by their cleavage pedigrees. The O and P teloblasts share another distinguishing feature, namely the delayed entry of their blast cell bandlets into the nascent germinal band as the result of the production by the OP proteloblast itself of a bandlet of op blast cells, beginning soon after it and the Q teloblast have been formed by cleavage of the OPQ blastomere at stage 6b (Muller, 1932; Fernandez and Stent, 1980). The OP proteloblast continues blast cell production until its own symmetric cleavage into the O and P sister teloblasts at stage 6c, by which time the op bandlet is between 5 to 8 blast cells long. The newborn O and P teloblasts then produce their own bandlets of blast cells, which enter the germinal band attached to the end of the op bandlet, broadening the germinal band from a three- to a four-bandlet width. The decision of which bandlet, o or p, comes to lie proximal to the n bandlet and which proximal to the q is evidently made at this point. It is interesting to note that at least a few of the op blast cells give rise to both LD_1 and LD_2 neurons, and thus are equivalent, in at least this respect, to the sum of an o and a p blast cell (Stuart *et al.*, in preparation).

A final point regarding the special relationship between the O and P teloblasts lie in the qualitative homology of some of their segmental progeny. In addition to the fact that each of these sister teloblasts gives rise to one of the two lateral dopaminergic peripheral neurons per segment, it appears that O and P each generates one of the pressure receptive sensory neurons in each half ganglion and that each contributes to the complement of packet glia that envelop neuronal cell bodies in the ganglion (Kramer and Blair, unpublished observations; Kramer and Weisblat, unpublished observations). This raises the possibility that the equivalence in the cell pedigrees of the O and P teloblasts may, in some cases, be extended to the level of their differentiated progeny. In that case transfating may, to some extent, represent the positional reassignment of O-derived cells to the position of their absent P-derived homologs (an instance of lineage regulation according to the terminology of Sulston and White, 1980). Unlike the situation in the epidermis, however, in which surviving epidermal progeny seem to cover the territory normally occupied by ablated cells nonspecifically, transfating of neuronal progeny must be a highly specific process because the O teloblast, which normally contributes many more cells to the ganglion than does P, seems restricted upon transfating to generate only what are normally P-derived neurons.

The results presented thus suggest that the sister teloblasts designated here and previously as O and P are, in fact, of equal developmental potential. Henceforth, except in cases where their generative identities have been verified, the designation O/P will be applied to both of these teloblasts, to indicate their apparent developmental equivalence.

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