

Ectodermal Interactions during Neurogenesis in the Glossiphoniid Leech *Helobdella triserialis*

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Morphogenetic cell interactions during development were studied by combining cell ablation and cell lineage tracing techniques in embryos of the leech *Helobdella triserialis*. Ablation of an identified ectodermal teloblast, or teloblast precursor blastomere, on one side of an early embryo was often found to result in the later abnormal migration of the progeny cells of the corresponding contralateral, nonablated teloblast to the ablated side of the embryo; such abnormal migration was termed "midline violation." Two different kinds of midline violation were observed. Crossover: after ablation of an N teloblast individual stem cell progeny of the contralateral N teloblast sometimes cross the ventral midline of the germinal plate of the embryo. Switching: after ablation of an OPQ teloblast precursor bandlets of stem cells produced by the contralateral O, P, or Q teloblasts sometimes switch to the germinal band of the ablated side at the site of origin of the germinal bands. The occurrence of crossover and switching shows that the eventual site occupied by a progeny cell of a particular teloblast is not automatically determined by its lineage, but also depends on interactions with other cells. Midline violation in the leech embryo CNS does not constitute true regulation, however, since the restoration of neurons to the ablated side is accompanied by a neuron deficit on the nonablated side. The occurrence of the two distinct kinds of midline violation, crossover and switching, may be explained by the relative position of the stem cell bandlets within the germinal bands, and by the geometrical features of the formation of the germinal plate from the germinal bands.

INTRODUCTION

It is possible to ascertain the developmental lines of descent of neurons in the segmental ganglia of the leech nerve cord by injection of a tracer into an identified blastomere of the early embryo (Weisblat *et al.*, 1978, 1980b,c). After tracer injection, embryonic development is allowed to progress to a later stage, whereupon the distribution pattern of the tracer within the tissue is visualized. In this way it has been shown that each of the four bilaterally paired ectodermal teloblasts, designated N, O, P, and Q, contributes a topographically stereotyped set of neurons to the ipsilateral hemiganglion (Weisblat and Kim, in preparation). These studies led to the conclusion that neurogenesis in the leech is highly determinate, in the sense that a particular ectodermal teloblast regularly gives rise to specific components of the nervous system.

This paper presents the results of experiments that were carried out to investigate the role of cell interactions within the ectoderm during neurogenesis in the leech. For this purpose, the lineage tracing technique of labeling one blastomere and its descendant cells by tracer injection was combined with ablation of a second blastomere by lethal enzyme injection (Weisblat *et al.*, 1980a,c; Blair, 1982). In such experiments, the extent to which the fate of descendants of the labeled blastomere deviates from normal reveals the extent to which the

normal determinate fate of those cells depends on interactions with the missing cells. These experiments were carried out with embryos of the glossiphoniid leech *Helobdella triserialis*. The early development of the glossiphoniid leech has been previously described, using the same nomenclature and embryonic staging system as is used in this paper (Weisblat *et al.*, 1980b; Fernandez, 1980; Stent *et al.*, 1982), and is summarized schematically in Fig. 1.

Previous application of the combined cell lineage tracer and blastomere ablation technique to *Helobdella* embryogenesis has shown that interactions between cells descended from the mesodermal teloblast, cell M, and the cells descended from the ectodermal precursor cell, NOPQ, are required for the segmental organization of either mesodermal or ectodermal tissue layers (Blair, 1982). The results presented here indicate that morphogenetic interactions also occur during neurogenesis between the descendants of the different ectodermal teloblasts.

MATERIALS AND METHODS

The embryos of *H. triserialis* used in these experiments were obtained from the breeding colony maintained in this laboratory (Weisblat *et al.*, 1979). The embryos are cultured at 25°C in HL saline (Weisblat

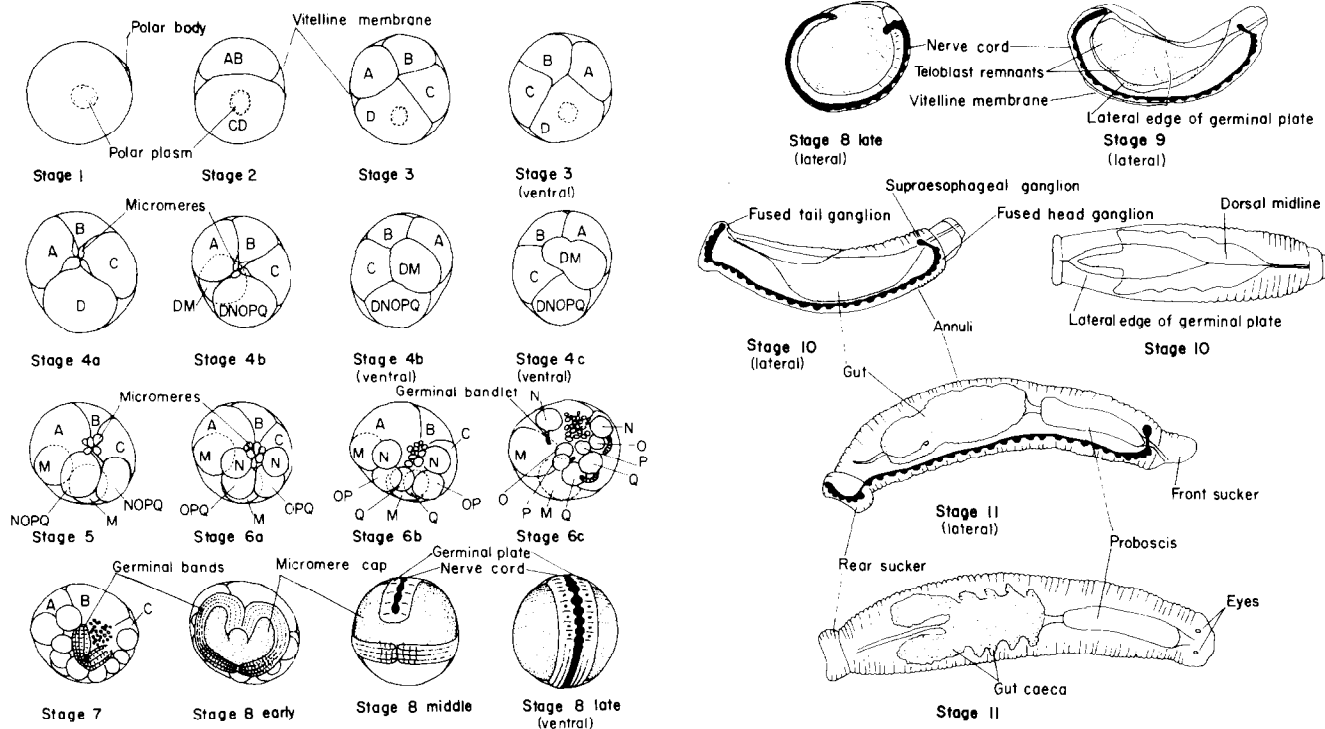


FIG. 1. The 11 stages of development of glossiphoniid leeches, beginning with the uncleaved egg (stage 1). The egg undergoes a series of stereotyped cleavages (stages 2-6) that eventually yield one bilateral pair of mesodermal precursor teloblasts (M) and four bilateral pairs of ectodermal precursor teloblasts (N, O, P, and Q). The teloblasts undergo several dozen unequal cleavages each to produce columns of stem cells which merge to form left and right germinal bands (stage 7). The germinal bands elongate, migrate over the dorsal surface of the embryo to meet at the future head, then coalesce zipper-like along the future ventral midline to form the germinal plate (stages 7 and 8). The segmental structures of the adult leech, including the 32 segmental ganglia of the ventral nerve cord, arise by proliferation and differentiation of the stem cells from which the germinal bands (and hence the germinal plate) were derived (stages 9-11). Unless otherwise noted, all drawings depict the future dorsal aspect.

et al., 1980b) supplemented after ablation with 4 mg garomycin, 10,000 units penicillin, and 10,000 μ g streptomycin per 100 ml solution, which is changed daily. For lineage tracing, blastomeres of early embryos were injected either with horseradish peroxidase (HRP) or with rhodamine-conjugated dodecapeptide (RDP), as previously described (Weisblat *et al.*, 1978, 1980b,c). For blastomere ablations either a 0.2% solution of the proteolytic enzyme Pronase (Sigma Type VI) in 0.05 N KCl and 2% fast green (Parnas and Bowling, 1977), or a 1% solution of DNase I (Sigma type III) in 0.15 N NaCl (Blair, 1982) was injected into the cell.

At the appropriate stages, HRP-injected embryos were stained for HRP, and RDP-injected embryos were counterstained with the DNA-specific fluorescent stain Hoechst 33258. For observation and photography, the embryos were treated as described previously (Weisblat *et al.*, 1978, 1980b,c). Embryos containing no lineage tracers were stained with Hoechst 33258. Some of the photographs presented here were taken originally on color film as double exposures. For the figures used here, these photographs were rendered into black-and-white

double exposures in which the RDP fluorescence appears brighter than the Hoechst 33258 fluorescence.

Pronase injection results in cell death only after several hours, often after the injected cell has gone through several rounds of division. The amount of Pronase injected must be carefully monitored by coinjecting fast green because overinjection results in the death of the entire embryo. Fast green spreads from the injected cell to other cells within the embryo; since it fluoresces in the red, the use of fast green raises the background fluorescence for detecting the distribution of RDP tracer. The use of DNase I avoids these problems. Death of the injection cell after DNase I injection is swift (within 2 hr) and the amount of material injected appears to be less critical (Blair, 1982); this obviates the need for fast green. Furthermore, the ablation of the injected cell can be confirmed by visually observing its lysis. Pronase ablation was used in the initial experiments of this series. Identical results were obtained when these experiments were repeated using DNase ablation, and in later experiments DNase ablation was used exclusively.

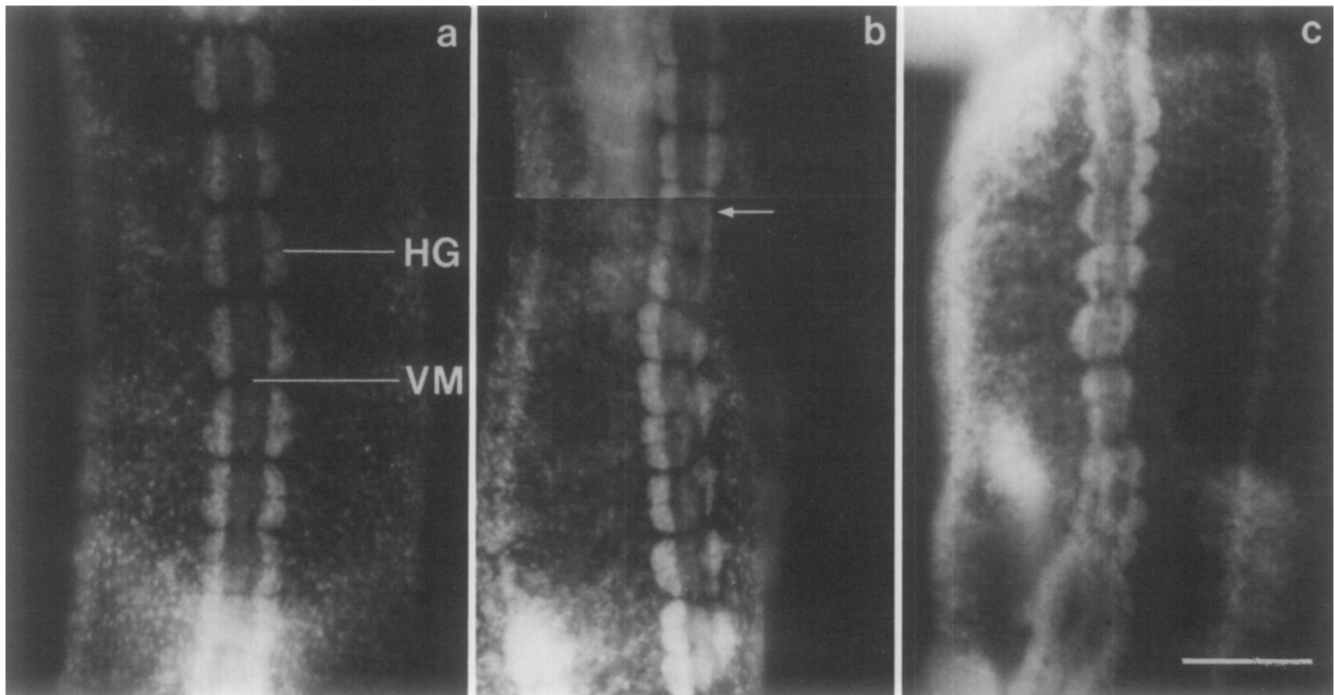


FIG. 2. Ventral aspects of stage 10 *Helobdella* embryos stained with Hoechst 33258. (a) Normal embryo. A bilaterally symmetric chain of segmental hemiganglia (HG) lies along the ventral midline (VM). (b) Embryo in which the left N teloblast was ablated at stage 7. (In this and all subsequent figures, ablation was performed using DNase I.) In the anterior zone (above the arrow), the ganglia appear normal. In the posterior zone (below the arrow), however, the left (apparent right) segmental hemiganglia show variable deficits in size. (c) Embryo in which the left OPQ cell was ablated at stage 6a. Both right and left segmental hemiganglia appear abnormal. Scale bar, 100 μ m.

In some cases, ablation or dye injection adversely affects the general health of the embryo; such cases are not included in the present data. The criteria by which an embryo is judged sufficiently healthy for inclusion in the data were described previously (Blair, 1982). In some experiments, all the embryos died. In successful experiments, an average of 25% of operated embryos were included in the data.

RESULTS

Ganglion Morphology after Ablation of an N Teloblast

In one series of embryos, the left N teloblast was ablated during stage 6 or 7, after production of the n stem cell bandlet by the N teloblast is already underway. Development was allowed to proceed to body closure (stage 10), when all ganglia of the nerve cord and their connectives are normally present. The embryos were then fixed and stained with Hoechst 33258. Comparisons with normal stage 10 embryos (Fig. 2a) showed that the nerve cord of the ablated embryos consisted of an anterior, normal zone and a posterior, abnormal zone (Fig. 2b). Since the anterior normal zone is derived from stem cells produced prior to ablation, the relative length of the abnormal zone was greater the earlier the N teloblast had been ablated. In the abnormal zone the

left hemiganglia were of reduced size and irregular shape. This was to be expected on the bases of the previous finding that a fraction of the neurons of each hemiganglion is descended from the ipsilateral N teloblast (Weisblat *et al.*, 1978, 1980b). However, the severity of the abnormality varied from ganglion to ganglion and from embryo to embryo: some hemiganglia were only slightly reduced in size while others were completely absent. In some segments, moreover, abnormalities were found also in the right hemiganglion. Over 60 such embryos have been studied; as yet, no consistent pattern has been found for the interganglionic variability of the abnormalities observed in the abnormal zones of these embryos.

To test whether the variability in ganglionic morphology in the abnormal zone is due to continued but erratic production of stem cells by the Pronase- or DNase-injected N teloblast, this experiment was repeated, except that the left N teloblast was labeled with tracer prior to being ablated (Weisblat *et al.*, 1980c). As was to be expected, in the resulting embryos the distribution of labeled cells was completely normal within the intact ganglia of the anterior, normal zone of the nerve cord. Moreover, the posterior, abnormal zone did not contain any labeled cells at all, demonstrating that the ablated N teloblast had indeed ceased stem cell pro-

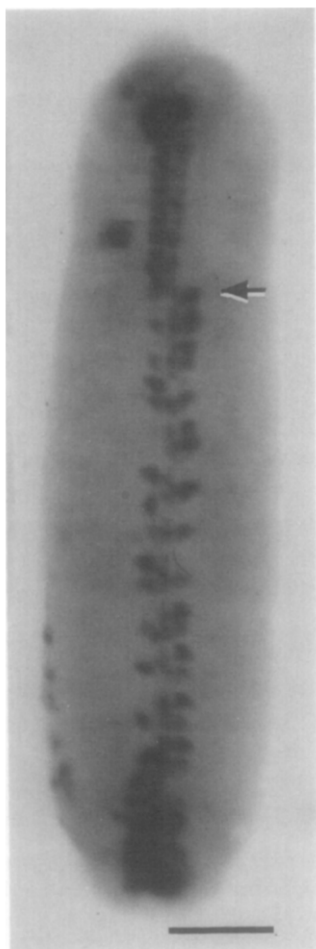


FIG. 3. Ventral aspect of a stage 10 *Helobdella* embryo in which the right N teloblast was injected with HRP at stage 6a and the left N teloblast was ablated at stage 7. The anterior zone (above the arrow) appears normal, showing the segmentally reiterated, ipsilateral staining pattern typical of N teloblast progeny. In the posterior zone, however (below the arrow), the right N progeny are now found on both right (apparent left) and left (apparent right) sides of the ventral midline. Scale bar, 100 μ m.

duction. Hence the variable morphology of ganglia in the abnormal zone cannot be accounted for by a variable, residual stem cell contribution from the ablated N teloblast. [It should be noted, however, that the front-most abnormal ganglia lying at the border between the anterior, normal and the posterior, abnormal zone do contain a few labeled cells. The presence of such cells may be attributable either to a brief residual stem cell production by the ablated N teloblast or to a limited rearward migration of labeled cells from the normal into the abnormal zone. These labeled cells in the abnormal zone are usually confined to within three ganglia of the interzone border (11 of 14 embryos) but are occasionally more extensively distributed (3 of 14 embryos).]

Source of Remaining Neurons on Ablated Side

It seemed likely that most of the cells in the ganglia formed on the ablated left side of the abnormal zone are derived from the unablated left O, P, and Q teloblasts which normally contribute progeny to the left hemiganglia (Weisblat *et al.*, 1980b). To test this possibility, the left OPQ cell, precursor of the left O, P, and Q teloblasts, was injected with tracer at stage 6a, the left N teloblast was ablated at stage 7, and development was allowed to proceed to stage 10. Six such embryos have been obtained; upon examining the distribution of the labeled progeny of the O, P, and Q teloblasts ipsilateral to the ablated N teloblast, it was found that in the anterior, normal zone, the ganglia exhibited the labeling pattern typical of OPQ cell progeny in normal embryos. As for the posterior, abnormal zone, some ganglia showed the normal pattern of OPQ-derived cells, whereas others contained fewer than, or even none of, the normal complement of OPQ-derived cells. This result was to be expected, since some hemiganglia on the ablated side are of nearly normal size and others are smaller or completely missing. However, an unexpected result was found also, namely, that in the left hemiganglia of the abnormal zone, there were some patches of cells that did not contain any label, apparently these cells were derived from a source other than either the left N teloblast or the left OPQ cell.

To test the possibility that these unlabeled cells derived from a contralateral teloblast, experiments were carried out in which the right N teloblast was injected with tracer at stage 6a, the left N teloblast was ablated at stage 6b, and development was allowed to proceed to the stage of body closure. Thirteen such embryos have been obtained. In each, within the anterior, normal zone, the labeled N teloblast progeny formed the normal pattern of regular, segmentally repeating cell clusters along the right side of the embryo, with all labeled cells lying on the same side of the ventral midline as the labeled N teloblast. But in the posterior, abnormal zone the pattern was markedly abnormal (Fig. 3). Here labeled cells were found in both right and left hemiganglia. We refer to any such bilateral distribution of progeny of a single teloblast as a *midline violation*. The extent of the midline violation by progeny of the right N teloblast following ablation of the left N teloblast was variable. In some ganglia, no midline violation occurred, and the labeling pattern seemed normal. In others, all the labeled cells were on the "wrong" side of the midline, in a pattern which would have been normal for the progeny of the contralateral teloblast. In most ganglia the distribution pattern was intermediate between these extremes, in that label was present on both sides of the ventral midline. In all cases, however, there was

nearly the same total amount of stain per ganglion, suggesting that ablation of one N teloblast does not change the number of cells contributed to each ganglion by the other N teloblast, but only the position of these cells with respect to the midline.

In contrast to the N teloblast, the O, P, and Q teloblasts do not contribute any of their progeny to the deficient hemiganglia on the "wrong" side of the embryo following ablation of the contralateral N teloblast. This was shown by injecting the right OPQ cell with tracer at stage 6a, ablating the left N teloblast at stage 6b, confirming the ablation, and allowing development to proceed to stage 9 or 10. In none of the 14 resulting embryos did any labeled cells appear in the hemiganglia of the left (ablated) side.

Mechanism of Midline Violation

There are two mechanisms by which cells might violate the midline of an abnormal embryo. First, upon cessation of stem cell formation by the ablated teloblast, part or all of a contralateral germinal bandlet might switch from the normal to the deficient germinal band at its point of origin. This mechanism of midline violation will be referred to as *switching*. Second, at a later stage, after coalescence of the germinal bands, cells might migrate across the midline of the germinal plate, from the normal to the abnormal side. This mechanism of midline violation will be referred to as *crossover*. To decide which of these mechanisms, switching or crossover, is responsible for the midline violation observed after N teloblast ablation, embryos in which one N teloblast had been ablated and the other injected with tracer at stage 6b were examined late in stage 8, part of the way through germinal band coalescence. In the rostral, developmentally most advanced portion of the embryo containing a completely formed germinal plate, sporadic midline violation by labeled N teloblast progeny was observed (Fig. 4). In the middle portion of the embryo, where the germinal bands had only recently coalesced, no midline violation was seen; here the label was confined to the half of the germinal plate ipsilateral to the labeled teloblast. Finally, in the caudal, least advanced portion of the embryo, where the germinal bands had not yet coalesced, labeled cells were found exclusively within the germinal band ipsilateral to the labeled teloblast, as would be the case in a normal embryo. This result shows that the midline violation by N teloblast progeny results from crossover in the germinal plate after coalescence of the germinal bands. If, by contrast, the midline violation had resulted from switching of germinal bandlets, labeled cells should have been observed in the "wrong" germinal band prior to coalescence.

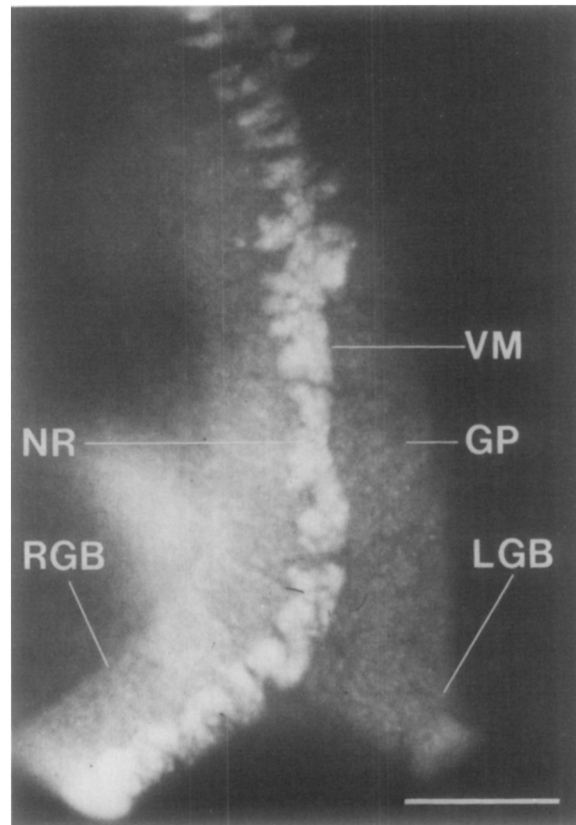


FIG. 4. Ventral aspect of a middle stage 8 *Helobdella* embryo in which the right N teloblast was injected with RDP at stage 6a and the left N teloblast was ablated at stage 6b. This double exposure shows two different types of fluorescence; the less bright Hoechst 33258 staining and the brighter RDP staining. The Hoechst 33258 stains the nuclei of the right and left germinal bands (RGB, LGB), which coalesce anteriorly to form the germinal plate (GP). The RDP-containing progeny of the right N teloblast (NR) are found largely in the right germinal band, and violate the midline only in the anterior region of the coalesced germinal plate. Scale bar, 100 μ m.

The Role of N Teloblast Progeny in Organizing Ganglia

The finding that following ablation of an N teloblast, the nonablated, ipsilateral O, P, and Q teloblasts do not always make their normal contribution to the hemiganglia on their side of the embryo suggests that during the formation of the ganglion, the N teloblast-derived cells play some role in the organization of cells derived from the O, P, and Q teloblasts. The sporadic contribution by the O, P, and Q teloblasts following ablation of an ipsilateral N teloblast might then be explained by the variability in the crossover by the progeny of the nonablated N teloblast. That is to say, in those segments in which a large number of N teloblast-derived cells had managed to cross the midline, many cells derived from the O, P, and Q teloblasts of the ablated side would be able to contribute to their ipsilateral hemi-

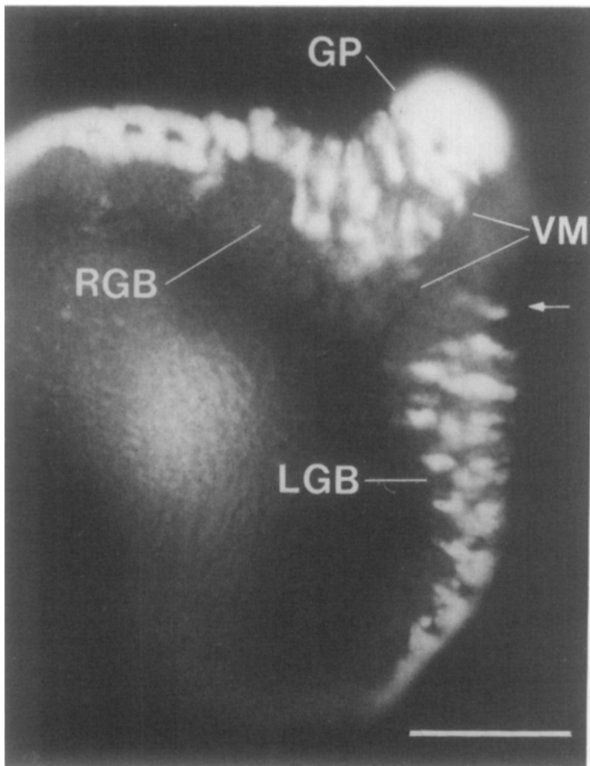


FIG. 5. Oblique, ventroposterior view of a middle stage 8 *Helobdella* embryo in which the right OPQ cell was filled with RDP and the left OPQ cell ablated at stage 6a. This double exposure shows two different types of fluorescence: the less bright Hoechst 33258 staining and the brighter RDP staining. The Hoechst 33258 stains the nuclei of the right and left germinal bands, which coalesce anteriorly to form the germinal plate. The RDP-containing progeny of the right OPQ cell do not violate the midline in the coalesced region of the germinal plate (upper right), but do appear within an uncoalesced portion of the left germinal band posterior to the arrow. Scale bar, 100 μ m.

ganglion. By contrast, in segments in which there had occurred little or no crossover by N teloblast-derived cells, the O, P, and Q teloblasts could contribute only very few cells to the hemiganglion. To test the importance of the inferred role of N teloblast progeny in organizing the genesis of the ganglion, an experiment was carried out in which *both* N teloblasts were first injected with tracer at stage 6a, and later ablated at stage 7. The viability of such embryos is poor; only four embryos were successfully reared to stage 10. The nerve cord of these embryos consisted of an anterior, normal zone with morphologically intact ganglia containing a bilaterally symmetric pattern of labeled cells, and a posterior, abnormal zone containing no labeled (i.e., N teloblast-derived) cells at all. Moreover, there were no normal-sized ganglia in the posterior zone, and in some regions no ganglia whatsoever were discernible. However, it was evident that *some* abnormal ganglia, severely deficient in size, did form in the absence of N teloblast-derived cells. Thus, the organization into ganglia of the progeny of the O, P, and Q teloblasts appears

to be facilitated by the presence of N teloblast-derived cells, but is not strictly dependent on it.

Ganglion Morphology after Ablation of an OPQ Teloblast Precursor Cell

Ablation of the left OPQ teloblast precursor cell at stage 6a resulted in embryos of diverse abnormal morphologies (Fig. 2c). Often the left, and sometimes also the right, body wall appeared deformed. Furthermore, in most cases the two germinal bands failed to coalesce completely. All of these embryos showed abnormalities in the size and shape of the left ganglia, and in some embryos the right hemiganglia were also abnormal. The extent of these abnormalities varied from segment to segment in a given embryo and also between embryos. The OPQ cell-ablated embryos contained no anterior, normal zone, such as that seen in some N teloblast-ablated embryos. This was to be expected, since ablating the OPQ cell before it has cleaved to yield the Q teloblast and cell OP aborts the formation of all ectodermal cells except those descended from the N teloblast.

The variable and bilateral character of the abnormalities observed upon OPQ cell ablation raised the possibility that they too entail sporadic midline violations. To investigate this possibility the right OPQ cell was labeled by tracer injection, the left OPQ cell ablated at stage 6a, and the ablation confirmed. Upon subsequent development to middle or late stage 8, i.e., part-way through the coalescence of the germinal bands, the resulting embryos could be divided into two morphologically distinct groups, referred to here as "switched" and "unswitched" classes. In the unswitched class (30 of 43 embryos), the labeled progeny of the nonablated OPQ cell were present only on the right side of the embryo. And the left germinal band and left half of the germinal plate were substantially narrower than their right counterparts, reflecting the absence from the left side of the cells normally descended from the ablated OPQ cell. Thus, no midline violations occurred in the unswitched class.

In the switched class, however (13 of 43 embryos), labeled progeny of the nonablated OPQ cell were present on both sides of the embryo (Fig. 5). In the anterior zone of these embryos the morphology of the germinal plate and the distribution pattern of labeled cells was similar to that of the unswitched class. But at some posterior position, usually in the caudal half of the embryo, there was an abrupt reduction in the number of labeled cells on the right side and a coincident, abrupt appearance of a segmentally repeated pattern of labeled cells on the left ("wrong") side. This suggests that caudal to that point, all the progeny of one of the labeled teloblasts had switched from the normal to the ablated side. Moreover, on the ablated side, this labeling pattern

extended smoothly from the left half of the germinal plate into the left germinal band in the region where the bands had not yet coalesced. Examination of the point of origin of the germinal bands revealed that one or two of the right o, p and q bandlets (descended respectively from the O, P, and Q teloblasts) had indeed switched from the right to the left germinal band, so that both uncoalesced germinal bands contained progeny of the right OPQ cell. The relative abundance of the switched class appears to increase with time after OPQ ablation, being relatively rare in middle stage 8 embryos (2 out of 21), but more common in late stage 8 embryos (11 out of 22). Only 1 out of 17 embryos examined showed signs of crossover in the CNS; this occurred within a single segmental ganglion. Therefore, the mechanism by which midline violation by o, p, or q neural precursors usually occurs after OPQ cell ablation is switching, in contrast to the extensive crossover of n bandlets following N teloblast ablation.

To examine whether the n bandlet also switches from one germinal band to the other following OPQ cell ablation, late stage 8 embryos were examined in which the left OPQ cell had been ablated, the ablation confirmed and the right N teloblast injected with tracer. Of 19 such embryos, none showed any evidence of switching. Moreover, of 10 such embryos observed, only 2 showed signs of crossover; as above, such crossover only occurred within a single ganglion, and is thus not a common result of OPQ cell ablation. Hence midline violation appears to be usually a specific process in the CNS, since cells that switch or cross over the midline are contralateral homologues of missing cells.

Fate of Neuropil Glial Cell following Ablation of an N Teloblast

The inference that neurogenesis in the leech is a highly determinate process is supported by the observation that the morphologically identifiable neuropil glial cell present in each hemiganglion regularly arises from the ipsilateral N teloblast (Weisblat *et al.*, 1980b). Does this determinancy also obtain under the abnormal conditions of midline violation, i.e., is the fate of a teloblast's progeny preserved despite their having crossed to the "wrong" side of the embryo? To answer this question the development of neuropil glia was followed in embryos whose right N teloblast was injected with tracer at stage 6a, whose left N teloblast was ablated at stage 7, and which were examined at stage 10. Two such embryos were serially sectioned in the horizontal plane, to secure sections containing the nerve cord in the region lying on either side of the border between the anterior, normal and posterior, abnormal zones (Fig. 6). In every ganglion within the normal zone two neuropil glia could be identified, of which one was la-

beled and the other was not. This confirms the previous observations that in normal development each N teloblast contributes one neuropil glial cell per ganglion. However, in ganglia within the abnormal zone, one and only one neuropil glia could be identified, even when progeny of the nonablated N teloblast were present on both sides of the ganglion, i.e., had crossed over the midline. Moreover, in every case that single glial cell was labeled.

DISCUSSION

During the normal development of the leech, each teloblast contributes its progeny to specific portions of the germinal plate, and especially to the ventral nerve cord (Weisblat *et al.*, 1980b; Weisblat and Kim, in preparation). This stereotyped relationship between cell lineage and cell fate may be attributable to factors either intrinsic or extrinsic to the embryonic cells. In the former case, cells would be destined to follow a specific pattern of migration and differentiation because of the orderly segregation of a set of specific intracellular determinants in successive cell divisions. In the latter case, these processes would be governed by cell position, with cell lineage governing cell fate by determining cell position via a highly determinate cleavage pattern. It is of course possible, even likely, that cell fate is governed by a combination of extrinsic and intrinsic factors. The results of the present study, in which the normal relationship between ectodermal cells within the germinal bands and germinal plate was altered by specific cell ablation, emphasize the important role cell lineage plays in determining cell fate.

It may be concluded that cell lineage does not intrinsically assign a cell to a given side of the embryo; rather the fixed, generally ipsilateral, relation between descent and sidedness found in normal embryos depends on intercellular interactions. Thus in the absence of a particular stem cell bandlet, caused by ablation of its progenitor teloblast or teloblast precursor cell, the progeny of a nonablated teloblast may violate the midline, apparently as a result of the absence of the normal confrontation of right and left germinal bands. There are two times in development during which cells of the left and right germinal bands can interact directly across the midline of the embryo. The first opportunity comes during the formation of the germinal bands. At the point of origin of the bands, the most medially located ectodermal bandlets, the right and left q bandlets, lie closely apposed. Thus, here the q bandlet destined for one germinal band has an opportunity to switch to the other germinal band. Whereas such switching occurs rarely, if ever, in a normal embryo, it does occur often following ablation of an OPQ teloblast precursor cell. Thus it is the interaction between apposed q bandlets

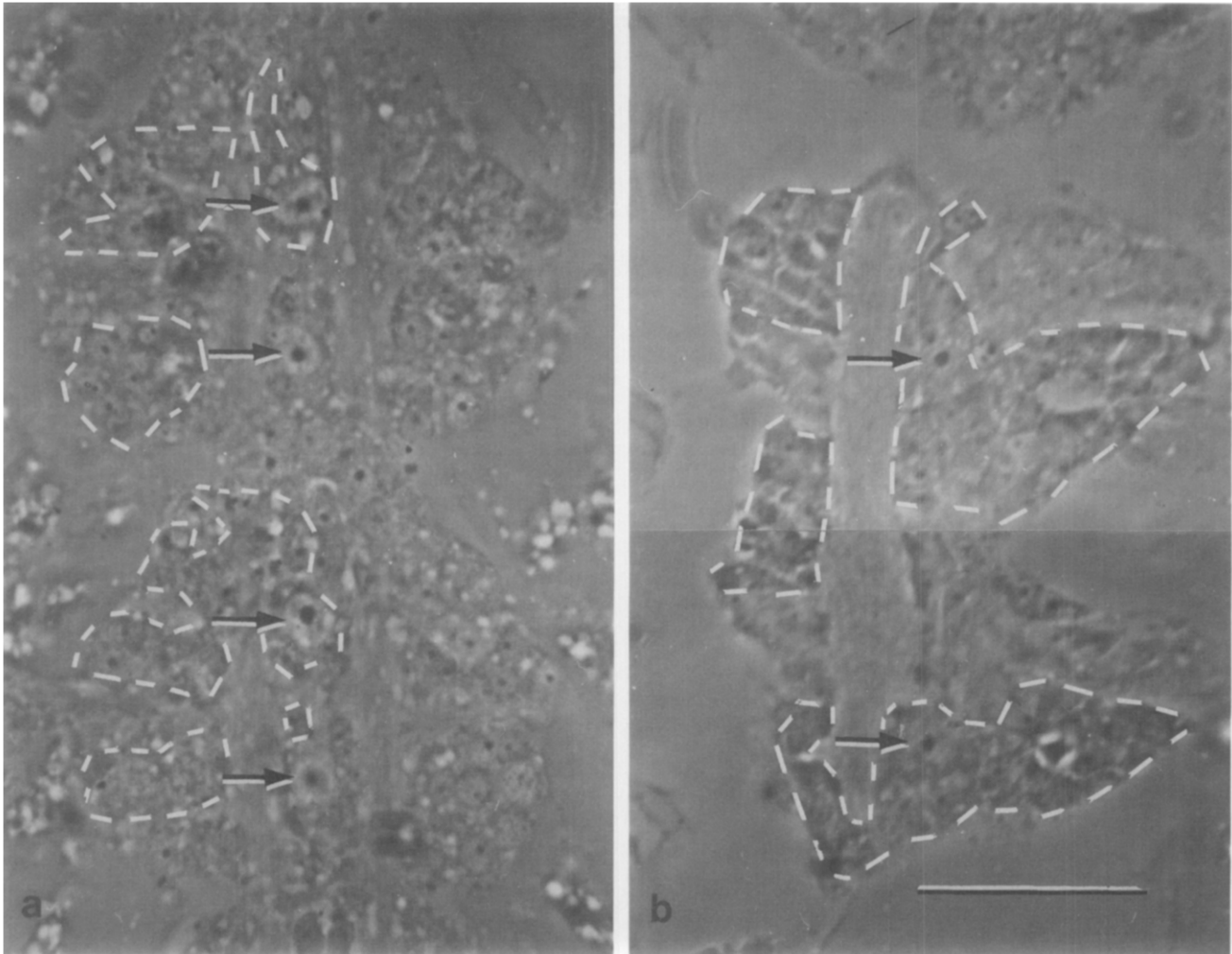


FIG. 6. Effects of teloblast ablation. (a) Horizontal section through two normal ganglia of an embryo in which an N teloblast was labeled with HRP (cf. Fig. 2b). Dotted outlines indicate the regions of HRP stain visible in the original section. Arrows indicate the nuclei of the two giant neuropil glia in each ganglion. (b) A similar section through two ganglia of an embryo in which one N teloblast was labeled with HRP and the other was ablated by DNase injection. Note that, as in Fig. 3, the HRP stain is on both sides of the midline. Also, only one neuropil glia can be seen in each ganglion. No further neuropil glia could be found in other sections through these ganglia. Anterior is up; scale bar, 25 μ m.

that normally prevents them from switching sides at the point of origin of the germinal bands.

A second opportunity for midline violation comes after right and left germinal bands have coalesced to form the germinal plate. After the germinal bands migrate to form the germinal plate it is the two n bandlets which lie medially, in apposition across the ventral midline. In a normal embryo the cells of one half of the germinal plate rarely, if ever, cross the midline into the contralateral half. However, after the ablation of one N teloblast, the progeny of the other N teloblast cross over to the ablated side. Thus also in the germinal plate the integrity of the embryonic midline is maintained by interactions between contralaterally apposed ectodermal bandlets.

The apparent specificity of this midline violation process can be accounted for in purely topographic terms

and does not require invoking any other aspect of the homology between the missing cells and those that take their place by crossover or switching. Thus we can envisage that bandlets violate the midline only if: (a) they are close to the midline; and (b) a vacancy exists close to the midline on the other side. Thus, the q bandlet violates the midline by switching when it is at the point of origin of the germinal bands and the contralateral q bandlet is missing. The n bandlet, by contrast, violates the midline by crossover when it is within the coalesced germinal plate and the normally directly apposed, contralateral n bandlet is missing. According to this explanation of midline violation, the o and p bandlets, which are topographically isolated from the midline—in the germinal band by the q bandlet and in the developing CNS by the n bandlet—would not be able to violate the midline by either switching or crossover fol-

lowing ablation of the O and P teloblast precursor cell OP. (Switching of o or p was sometimes seen when the contralateral OPQ cell had been ablated; this occurred presumably after the q bandlet had switched, leaving p adjacent to the still-deficient contralateral germinal band.) This prediction was tested by labeling the right OP cell, ablating the left OP cell, and confirming the ablation of stage 6b. As predicted, neither switching nor crossover of any CNS cells was observed in 26 embryos examined at late stage 8 or stage 9. It should be noted, however, that as previously reported (Blair, 1981), switching by the mesodermal, or m bandlet can occur also as a result of ablation, not of the contralateral mesodermal M teloblast, but of the ipsilateral NOPQ cell, precursor of the ectodermal N, O, P, and Q teloblasts. In this case, the right and left m bandlets come to lie side-by-side under the ectodermal layer of the germinal band on the nonablated side.

These midline violations cannot be considered instances of regulative development, since there appears to be no net replacement of the neurons absent because of cell ablation in the leech embryo. Rather, ablation invariably leads to deficits in the nerve cord, whether or not midline violation has taken place. For instance, when a q stem cell bandlet switches from the normal to the ablated side, its side of origin is left with a corresponding cell deficit. Furthermore, there is no evidence that the switched q bandlet produces more than the normal number of neurons, even though it may lie in a germinal band containing fewer than the normal number of cells. Similarly, when progeny of the n bandlet cross over the midline they leave corresponding gaps on their side of origin. Thus the number of neurons produced by a single n bandlet appears fixed; it is only the location of these cells that may change upon ablation. The absence of any regulative restoration of cell deficits is also indicated by the finding that following ablation of one N teloblast there arises only the one neuropil glial cell per ganglion that would be normally produced by the other N teloblast. This finding suggests, moreover, that in each n stem cell bandlet there arises one cell per segment that is fated to become a neuropil glial cell, regardless of its position at the time of midline violation. In other words, crossover by n bandlet cells merely results in a change in cell position, not in cell fate. It should be noted that after ablation of an OP cell the *epidermis* derived from the contralateral OP cell does cross over the midline of the germinal plate. Furthermore, it is possible that such crossover constitutes regulative development (Weisblat and Blair, in preparation).

Whereas the present work provides no evidence for regulative restoration in leech CNS of ablated tissues, it does show that cell ablation can disrupt the normal morphogenesis of nonablated tissues. For instance, af-

ter ablation of an N teloblast the contribution to segmental ganglia by the ipsilateral OPQ teloblast precursor cell is sporadically abnormal. This indicates that cells derived from the N teloblast play a role in the organization of the cells derived from the O, P, and Q teloblasts into the segmental hemiganglia. But the formation of *some* ganglia even in the absence of *both* N teloblasts' progeny indicates that other factors, as yet unknown, are also important. Thus, just as formation of segmental structures by mesodermal and ectodermal tissues is the result of an interaction between both germinal tissue layers (Blair, 1982), so is determinate neurogenesis in the leech an interactive, though apparently not regulative, process. Moreover, after the ablation of an N teloblast, the variability in the contribution by both the ipsilateral OPQ cell and the contralateral N teloblast to the deficient hemiganglia occurs even under what we take to be constant conditions, that is from ganglion to ganglion within a single animal and between homologous ganglia in siblings raised together. This variability might thus be ascribed to developmental "noise."

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