Cell Lineage in the Development of the Leech Nervous System

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INTRODUCTION

Knowledge of the lines of descent of cells that compose the mature organism should help to discern basic mechanisms of development. The leech is well-suited for such cellular investigations of neurodevelopment because both the early embryo and the adult nervous system comprise identifiable cells accessible to experimental manipulation. In fact, the first studies of developmental cell lineage used leech embryos (Whitman, 1878, 1887). We have refined and extended such studies using intracellularly injected cell lineage tracers and ablatants with embryos of the leech *Helobdella triserialis*. A fuller account of the work presented here can be found elsewhere (Weisblat et al., 1978, 1980; Blair and Weisblat, 1982; Stent et al., 1982; Weisblat and Kim, 1982).

The yolky, 0.5 mm egg of *Helobdella* undergoes a series of stereotyped cleavages to produce an early embryo containing (among other blastomeres) four bilateral pairs of ectodermal precursors (the N, O, P and Q *teloblasts*) and one bilateral pair of mesodermal precursors (the M teloblasts). Individual teloblasts can be identified by their size and position within the embryo. Each teloblast undergoes several dozen highly unequal cleavages, producing a one cell wide column of *stem cells* called a *germinal bandlet*. The oldest stem cells (at the front of the bandlet) move away as new ones are produced by the parent teloblast. During gastrulation, the 10 bandlets of stem cells and their progeny come to lie parallel to the midline on the ventral side of the embryo, forming a structure known as the germinal plate. The leading ends of the bandlets are at the future head of the embryo; within each half-germinal plate the ectodermal bandlets are superficial and lie in the order n, o, p, q, from medial to laterial, and the m bandlet lies beneath them.

The situation of the left and right n bandlets (in direct apposition across the ventral midline) led Whitman to suggest that they give rise to the chain of segmental ganglia forming the ventral nerve cord. But the fates of individual stem cells and their progeny cannot be determined by the direct methods used to establish the lineage relations in the early leech embryo because they are small, numerous and lie in multiple layers in the germinal plate. We have pursued the question of the embryonic origins of the nervous system by labeling individual teloblasts early in development by intracellular injection of horseradish peroxidase (HRP), and then visualizing their progeny by histochemical staining later in embryogenesis, when the ganglia have already begun to approach their adult morphology.

RESULTS

Fig. 1a–e shows the pattern of HRP staining observed in late embryos after labeling specific teloblasts early in development. The labeled progeny of each

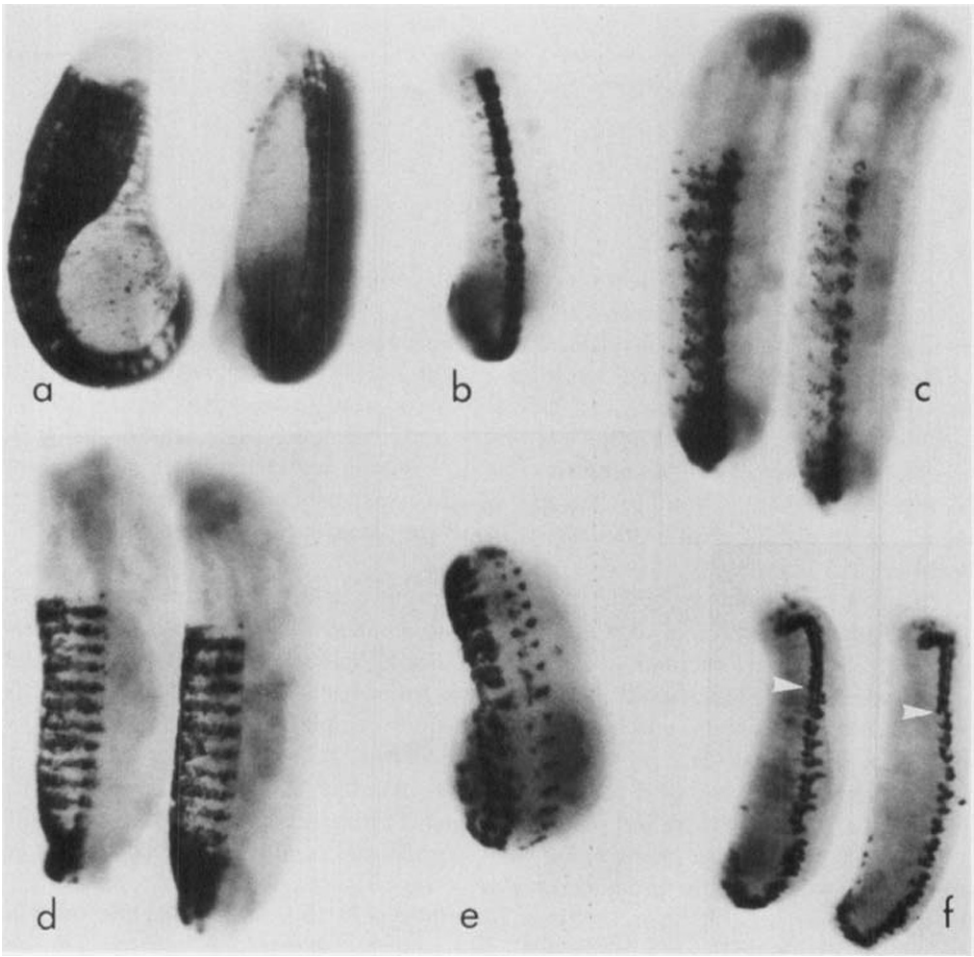

Fig. 1. Distribution of teloblast progeny revealed by HRP labeling. This figure shows embryos that were fixed and stained about six days after HRP had been injected into an identified teloblast. Except for those in panel a, a right hand teloblast in each embryo shown was labeled and, except for the embryos shown in panels e and f, teloblasts were labeled after the initiation of stem cell production, so that the anterior segments, which contain progeny of unlabeled stem cells, are unstained. All views are ventral unless otherwise indicated; anterior is up. a: lateral (left) and ventral (right) view of embryos in which the left hand M teloblast was labeled. Stain extends from the ventral midline to the lateral edge of the germinal plate. b: an N-labeled embryo. c: two O-labeled embryos. d: two P-labeled embryos. e: a Q-labeled embryo. This embryo bent during fixation so that the head is not visible in this photograph. f: two embryos, in each of which the right N teloblast was labeled with HRP soon after formation and the left N teloblast was ablated by DNAase injection after stem cell production was underway. Above the arrows are anterior ganglia formed from the full complement of stem cells; the stain pattern resembles that seen in panel b. Below the arrows are posterior ganglia lacking progeny of the left N teloblast; the normal pattern is disrupted and stain is seen on both sides of the ventral midline.

teloblast form a unique, segmentally repeating pattern within the germinal plate. In each case, the progeny are confined to the same side of the embryo as the labeled teloblast. Furthermore, in embryos in which the teloblast was injected after stem cell production had begun, there is a sharp anterior-posterior boundary to the stain pattern. This boundary corresponds to the boundary between anterior tissue produced from stem cells made prior to HRP injection and posterior tissue derived from stem cells made subsequently. Thus, there appears to be little or no migration of cell bodies across the midline or in the posterior to anterior direction within the germinal plate during normal development.

As predicted from the relative positions of the germinal bandlets within the germinal plate, the progeny of the N teloblast are largely confined to the medial

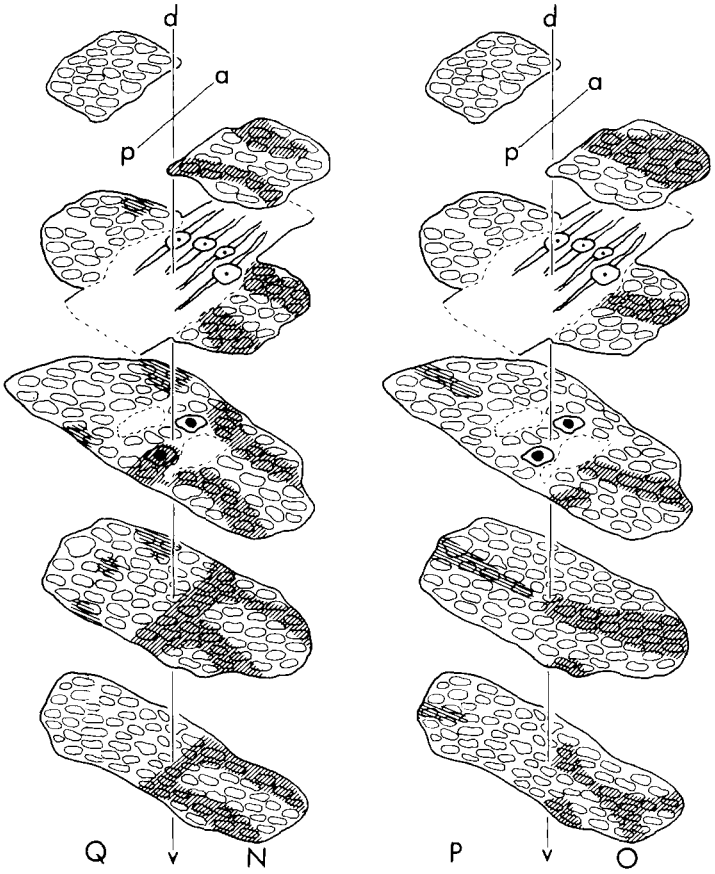

Fig. 2. Embryonic origins of the cells of the *Helobdella* segmental ganglion. The drawing shows 5 horizontal sections through a midbody segmental ganglion of the embryo at mid stage 10. (Dorsal aspect at the top; front edge facing away from the viewer.) The two pairs of dark, elongated contours in the center of the second section from the top represent identifiable muscle cells in the longitudinal nerve tract. They are descendants of the M teloblast pair. The two dark, circular contours in the center of the middle section represent two identifiable glial cells, each of which is a descendant of one N teloblast. The faint contours do not correspond to actual cells but are shown to indicate the approximate size, disposition and number of neurons in the ganglion. In each half-ganglion, cross-hatched domains contain descendants of the teloblast designated at the bottom of the figure.

portion of the germinal plate, in the vicinity of the ventral nerve cord. Although the shape of the repeating pattern of N-teloblast progeny conforms roughly to that of the segmental hemiganglion, there are unstained regions within each hemiganglion. The bulk of the O, P and Q teloblast progeny are progressively more laterally distributed, but there are clear exceptions to this rule. In particular, some progeny from each teloblast can be seen near the ventral midline; the distribution of these cells appears to complement that of the N teloblast progeny within the ganglionic borders.

These observations on intact embryos suggested that the segmental ganglia may have a more complex embryonic origin than was originally supposed. In fact, when labeled embryos were examined in section, it was found that each teloblast contributes a stereotyped, topographically distinct subpopulation of cells to the ipsilateral hemiganglia of the nerve cord. The major groups of cells arising from the ectoteloblasts are distributed as shown schematically in Fig. 2 (Weisblat and Kim, 1982). The N and O teloblasts each contribute many more cells to the ganglion than

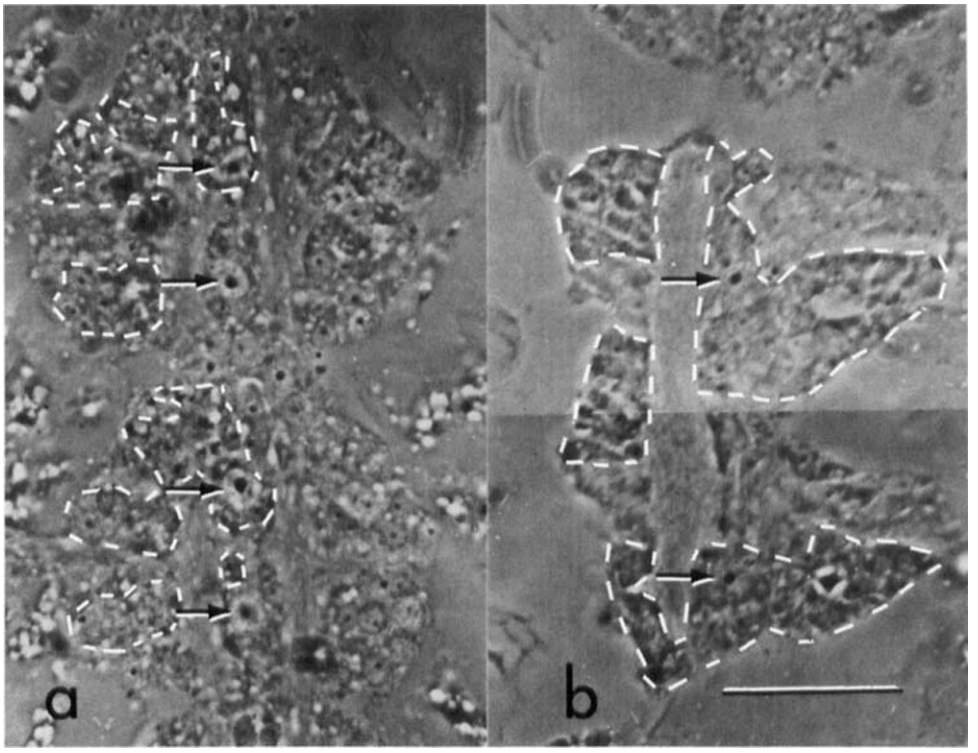

Fig. 3. Effects of N teloblast ablation. a: horizontal section through two normal ganglia of an embryo in which an N teloblast was labeled with HRP (cf. Fig. 1b). Dotted outlines indicate the regions of HRP stain visible in the original section. Arrows indicate the nuclei of the two giant neuropil glia 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 DNAase injection. Note that, as in Fig. 1f, the HRP stain is on both sides of the midline. Also, only one neuropil glia can be seen in each ganglion. No other neuropil glia could be found in other sections through these ganglia. Anterior is up; scale bar = 25 μm.

do P and Q. A small number of additional cells in the ganglion arise from the M teloblast. Some of these are known to be nonneuronal (Weisblat et al., 1980); but some others are (Weisblat and Kim, 1982).

The sterotyped position of each teloblast's progeny in the embryonic ganglion, taken with the fixed position of identified neurons in the adult ganglion (cf. Nicholls and Baylor, 1968; Stuart, 1970), suggests that identified neurons may arise from specific teloblasts during normal development. Of the cells identifiable in the adult nervous system, only three, nonneuronal ones can be identified morphologically at the intermediate stage of development reached in these experiments. These are the two pairs of longitudinal muscle cells in the interganglionic connective nerve, which derive from the M teloblast, and the pair of giant glia associated with the ganglionic neuropil, each of which derives from one N teloblast (Fig. 3a). To probe the limits of the developmental determinacy indicated by these results, further experiments were done in which the right N teloblast was labeled with HRP and the left N teloblast was ablated by DNAase injection (Blair and Weisblat, 1982). Examination of such embryos, fixed, stained and sectioned, revealed that ganglia missing the progeny of one N teloblast also lack one neuropil glia (Fig. 3b). This deficit is seen even when progeny of the surviving N teloblast have aberrantly crossed the ventral midline and are present on both sides of the ganglion (Fig. 1f) (Blair and Weisblat, 1982).

DISCUSSION

The experiments reported here reveal that each teloblast of the early leech embryo, not just the pair of N teloblasts, contributes progeny to the segmental ganglia. There appears to be little or no migration of cell bodies across the midline or from posterior to anterior within the nerve cord during normal development. This implies that neuronal pathways within the leech central nervous system are established by process growth rather than by migration of cell bodies. There is movement of cell bodies during neurogenesis into the ventral nerve cord from laterally situated germinal bandlets, especially the q bandlet, but there is no evidence that any cell processes are left behind during this movement.

The significance of the distinct neuronal kinship groups from each of four ectoteloblast pairs, or why there are four pairs of ectodermal precursors and just one pair of mesodermal precursors, is not clear. Early cleavages in the leech embryo seem to result in the segregation of specific developmental fates. But the possibility implicit in Whitman's analysis, that different ectoteloblasts give rise to different tissue types, has been ruled out by the work reported here. Indeed, each ectoteloblast seems to give rise to both neural and body wall cells (Weisblat and Kim, 1982). A second hypothesis is that the teloblasts are developmentally equipotent, and are replicated to guarantee the production of a complete set of ectodermal structures. But the failure of the *Helobdella* embryo to generate a complete set of neuropil glia after N teloblast ablation argues against this interpretation, too. Alternatives include the segregation of identified neurons among the kinship groups on the basis of branching pattern, biochemical or biophysical traits, or spatial distribution of targets in the periphery. Further analysis of the lineage relations of identified cells in the leech nervous system, using techniques already at hand, should permit us to test these possibilities.

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