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# Positional cues governing cell migration in leech neurogenesis

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## Summary

**The stereotyped distribution of identified neurons and glial cells in the leech nervous system is the product of stereotyped cell migrations and rearrangements during embryogenesis. To examine the dependence of long-distance cell migrations on positional cues provided by other tissues, embryos of *Theromyzon rude* were examined for the effects of selective ablation of various embryonic cell lines on the migration and final distribution of neural and glial precursor cells descended from the bilaterally paired ectodermal cell lines designated *q bandlets*. The results suggest that neither the commitment of q-bandlet cells to migrate nor the general lateral-to-medial direction of their migration depend on interactions with any other cell line. However, the ability of the migrating cells to follow their normal pathways and to find their normal destinations does depend on interactions with cells of the mesodermal cell line, which appears to provide positional cues that specify the migration pathways.**

Key words: cell lineage, cell commitment, selective cell ablation, mesoderm, leech, neurogenesis, cell migration, positional cues.

## Introduction

Individually identified neurons and glial cells occupy characteristic positions in the central and peripheral nervous systems of leeches. Although these cells arise *via* determinate cell lineages in the embryos of glossiphoniid leeches, the pattern of cell divisions does not place most cells directly into their final positions. Rather, the definitive pattern of cell positions is the product of stereotyped cell migrations and rearrangements (Weisblat *et al.* 1984; Zackson, 1984; Kramer and Weisblat, 1985; Weisblat and Shankland, 1985; Torrence and Stuart, 1986; Bissen and Weisblat, 1987, 1989; Stuart *et al.* 1987; Martindale and Shankland, 1988; Braun and Stent, 1989*a*). The stereotypy of cell migration routes and of final cell positions in normal development suggests that there are mechanisms that constrain migrating cells to move along appropriate paths and allow them to recognize appropriate destinations. A variety of ectodermal and mesodermal cell types are normally present along the migration paths, and are thus candidates for providing guidance or positional cues (Torrence and Stuart, 1986).

Previous experiments, in which leech embryos were deprived of various major cell lines, revealed that at least some identified glial cells and neurons derived from the bilateral pair of homologous, ectodermal cell lines called the n bandlets (collectively, *n-kinship cells*) can differentiate autonomously in the absence of any other ectodermal or mesodermal cell line (Blair and Weisblat, 1982; Blair, 1982, 1983; Stuart *et al.* 1987, 1989; Torrence *et al.* 1989). Although n-kinship cells can achieve their characteristic final positions in the absence of any other ectodermal cell line, they require the presence of mesodermal tissues to do so (Stuart *et al.* 1989; Torrence *et al.* 1989). These observations suggested that ectodermal cells become committed to give rise to specific cell types before the onset of cell migrations and that, during their subsequent migrations, the committed cells use cues provided by the mesoderm to find the positions appropriate to their individual fates.

Because n-kinship cells arise entirely within the prospective territory of the CNS and undergo most of their rearrangements within large clusters of other n-kinship cells (Weisblat *et al.* 1984; Torrence and Stuart, 1986; Stuart *et al.* 1987; Braun and Stent, 1989*a*), it is difficult to use cell lineage tracers to analyze the paths by which they reach their final positions, either in normal development or following ablations of other cell lines. By contrast, cells of the bilaterally paired ectodermal cell lines designated the *q bandlets* (collectively, *q-kinship cells*) arise completely outside the prospective territory of the CNS, and small groups of q-kinship cells migrate toward the CNS along the longest pathways followed by any ectodermal neural precursor cells (Weisblat *et al.* 1984; Torrence and Stuart, 1986).

This paper reports the results of selective ablation of ectodermal and mesodermal cell lines on the migration and final positions of q-kinship cells. The results indicate that neither the commitment of q-kinship cells to migrate nor the general lateral-to-medial direction of their migration depend on interactions with any other cell line. The ability of the migrating cells to follow their normal pathways, however, does depend on interac-

tions with cells of the mesodermal cell line, which appears to provide the positional cues that define the migration pathways.

*Summary of leech development and definition of terms*

The progeny of the determinate, asymmetrical early cleavage divisions of the leech embryo include five bilateral pairs of large blastomeres called *teloblasts*. Each teloblast acts as a stem cell, budding off several dozen smaller *primary blast cells*, which form a longitudinal row, or *bandlet*, of proliferating cells. The left and right *n*, *o*, *p* and *q* bandlets give rise to the definitive ectoderm, and the left and right *m* bandlets give rise to the mesoderm. The term *kinship group* denotes a set of cells derived from a given bandlet. On either side of the bilaterally symmetrical later embryo, each kinship group gives rise to a unique, segmentally iterated set of identifiable cells whose distribution forms a characteristic *kinship group pattern* (Weisblat *et al.* 1984; Weisblat and Shankland, 1985; Kramer and Weisblat, 1985; Torrence and Stuart, 1986).

The bandlets become aligned on the ventral aspect of the embryo during embryonic stage 8 to form the rudiment of the body wall and nervous system, the *germinal plate*. The first-born and developmentally oldest blast cells of each bandlet contribute their mitotic progeny to the anteriormost segments of the germinal plate. Progressively younger blast cells contribute their progeny to correspondingly more posterior segments. In accord with this gradient of blast cell age, any given developmental event occurs first in the anteriormost segments and progressively later in the more posterior segments. Thus, an embryo's longitudinal axis is equivalent to a developmental time axis. Cell migrations and rearrangements during embryonic stage 9 generate organ primordia, including the ganglia of the CNS. The germinal plate expands circumferentially during stages 9 and 10 until its margins fuse along the dorsal midline. Neuronal differentiation occurs primarily during stages 10 and 11 (Kuwada and Kramer, 1983; Kramer and Kuwada, 1983; Glover *et al.* 1987).

The leech CNS is composed of a supraesophageal ganglion and a ventral nerve cord comprising 32 segmental ganglia, or neuromeres, linked *via* longitudinal connective nerves (Mann, 1962; Muller *et al.* 1981). The rostral four neuromeres, designated S1–S4 in rostrocaudal sequence, fuse to form the adult subesophageal ganglion. The succeeding 21 unfused 'abdominal' neuromeres are designated A1–A21. The caudal seven neuromeres fuse to constitute a caudal ganglion. Segments are referred to by the designation of the neuromere they contain (Mann, 1953; Weisblat and Shankland, 1985). *Hemisegment* and *hemiganglion* refer to the left or right half of a segment or ganglion.

## Materials and methods

*Embryos*

Embryos of *Theromyzon rude* were obtained from gravid adults collected in the lakes of Golden Gate Park, San Francisco, and were cultured as previously described (Torrence and Stuart, 1986; Stuart *et al.* 1987, 1989). Embryos were staged by the system of Fernandez (1980) as modified by Weisblat *et al.* (1980*a*).

*Labelling*

To label an n, q or m bandlet, the red-fluorescing lineage tracer tetramethylrhodamine–dextran–amine (RDA) or the green-fluorescing lineage tracer fluorescein–dextran–amine (FDA) (Gimlich and Braun, 1985), was injected into the bandlet's parental teloblast at stage 6b as previously described (Weisblat *et al.* 1978, 1980*b*; Stuart *et al.* 1990). To co-label the o and p bandlets, a lineage tracer was injected at stage 6b into the common progenitor of their parental teloblasts, the OP blastomere.

Embryonic muscle fibers were stained by indirect immunofluorescence as described by Torrence and Stuart (1986), using a monoclonal antibody provided by Dr B. Zipser (Zipser and McKay, 1981) and a fluorescein- or tetramethylrhodamine-conjugated second antibody. Dopamine-containing neurons were stained by glyoxylic acid-induced fluorescence as described by Stuart *et al.* (1987).

*Ablations*

To abort formation of a bandlet, the bandlet's precursor teloblast was killed at stage 6c or early stage 7 by an intracellular injection of a solution containing 14–17 mg ml$^{-1}$ of the lethal enzyme DNAase I (Sigma type IV) (Blair, 1982), 5–10 mg ml$^{-1}$ fast green FCF and 200 mM KCl.

FDA-sensitized photoablation (Shankland, 1984; Braun and Stent, 1989*b*) of part of a bandlet was performed later in development as described by Stuart *et al.* (1989, 1990).

*Fixation and dissection*

Stage 10 or 11 embryos were relaxed for a few minutes in ice-cold embryo medium containing 80 mM chlorobutanol (Sigma), pinned on Sylgard and fixed for 18–24 h at 4°C in 20 mg ml$^{-1}$ paraformaldehyde in 50 mM sodium Hepes buffer, pH 7.4, supplemented with 5 μg ml$^{-1}$ of the DNA-specific, fluorescent dye Hoechst 33258 (Aldrich). After fixation, the embryos were rinsed several times in a buffered saline (HBS) containing 50 mM sodium Hepes, pH 7.4, 145 mM NaCl and 15 mM sodium azide. A dorsal, longitudinal incision was made in each fixed embryo, still pinned on Sylgard in ice-cold HBS, and the yolk was removed. Stage 9 embryos were treated similarly, but were pinned after fixation rather than before.

*Microscopy*

Fixed and dissected embryos were cleared and whole-mounted in a solution of 80% glycerol and 20% 0.1 M Tris–HCl, pH 9.0, containing 40 mg ml$^{-1}$ *n*-propyl gallate to retard photobleaching of the fluorophores (Giloh and Sedat, 1982).

Whole-mounted embryos were examined by conventional epifluorescence microscopy using Zeiss filter sets 487701, 487715, 487717 and 487705 to visualize the fluorescence of Hoechst 33258, tetramethylrhodamine, fluorescein and glyoxylic acid-stained monoamines, respectively. Embryos were photographed on Ektachrome 160 Professional or Ektachrome 400 film (Figs 3, 6B, 7, 8). Epifluorescence micrographs showing two different labels in the same specimen (Fig. 3) were taken as double exposures, using first the filter set appropriate for one of the labels, then the filter set appropriate for the other label.

Confocal microscopy was performed using a Bio-Rad MRC 500 system attached to a Zeiss Axioplan epifluorescence microscope. A series of digitized optical sections was

collected from each specimen. After contrast enhancement, these sections were reassembled to generate an image of the entire thickness of the specimen (Figs 1, 4, 5, 6A, 9, 10). The images generated by this system are intrinsically monochrome. To illustrate the relationship of two different labels in the same specimen (Figs 4, 6A), paired series of optical sections were collected, one for each label, which were subsequently superimposed and assigned different, computer-generated colors to represent the different original labels. Colored confocal micrographs were photographed from the computer screen on Kodak Ektachrome 200 or Ektar 125 film.

All black and white micrographs are reproduced as negative images, in which fluorescence is rendered dark against a light background. Epifluorescence micrographs (Figs 7, 8) were printed directly from Ektachrome transparencies onto Kodak Panalure or Panalure II Repro panchromatic enlarging papers. Monochrome confocal micrographs (Figs 1, 5, 9, 10) were electronically converted to negative images before being photographed from a high-resolution, flat-screen monitor (Lucius and Baer VM1710) on Kodak Technical Pan film 2415.

## Results

### *Normal q kinship group pattern*

In each hemisegment of a stage 10 or 11 embryo, the q kinship group pattern includes four distinct elements in and near the ventral nerve cord that are derived from migratory precursor cells (Figs 1,2): a glioblast situated in the ipsilateral interganglionic connective nerve; an anteroventral cluster of neurons, AV, within the ganglion; a central interneuron, qz1, situated near the root of the segmental nerve designated MA; and a cluster of six or seven peripheral neurons, MAc, situated a short distance from the lateral margin of the ganglion along the MA nerve (Weisblat *et al.* 1984; Kramer and Weisblat, 1985; Torrence and Stuart, 1986). In some hemisegments, the MAc cluster is divided into medial and lateral subclusters. All other q-kinship cells lie in the lateral part of the germinal plate (Figs 3, 10B). They include four peripheral neurons and most of the epidermis of the future dorsal body surface, including squamous epidermal cells and three clusters of cuboidal cells designated cell florets.

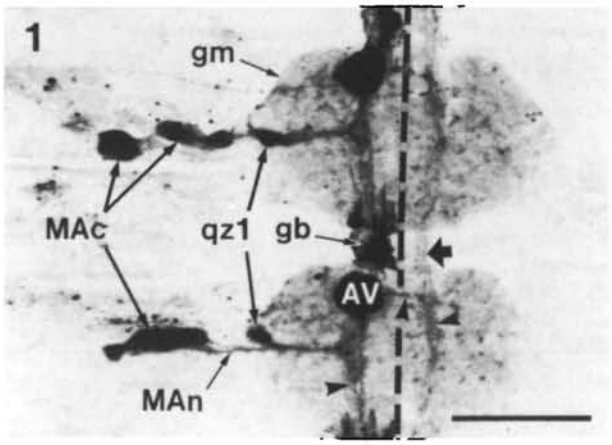


**Fig. 1.** Normal distribution of tracer-labeled, left q-kinship cells in two abdominal segments (A9–A10) of a stage 10 embryo. The fluorescence of the lineage tracer is rendered dark against the light background. In this and all subsequent figures, anterior is up. The ventral midline is indicated by the dashed line. Axons of q-kinship neurons are indicated by arrowheads within the ganglion and by the solid arrow in the right interganglionic connective nerve. Abbreviations: AV, anteroventral cluster of central neurons; gb, glioblast of the ipsilateral interganglionic connective nerve; g, ganglionic margin, visible by background fluorescence; MAc, cluster of peripheral neurons along the MA segmental nerve; MAn, labeled axons of MAc neurons in the MA segmental nerve; qz1, central interneuron. Scale bar, 50 μm.

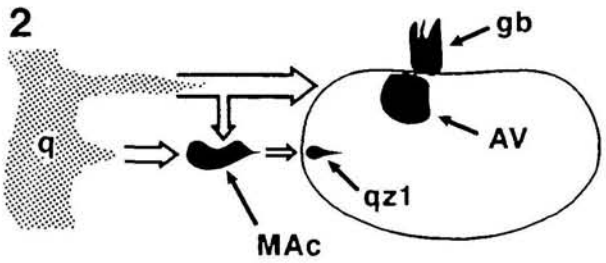


**Fig. 2.** Normal migratory pathways of q-kinship cells. Open arrows indicate the pathways followed by cells of the q bandlet (q, stippled) to contribute to the q-kinship pattern elements (solid black) in and near a segmental ganglion (squashed oval) of the CNS. The lengths of the arrows are not to scale. Abbreviations as in Fig. 1.

The pathways of the cell migrations that generate the q kinship group pattern during stage 9 are illustrated in Fig. 2 and the right side of Fig. 5B. Before migration begins, the q bandlet lies at the lateral margin of the germinal plate. In every hemisegment, two groups of mitotically active cells leave the q bandlet to migrate toward the ventral midline and the presumptive CNS (Weisblat *et al.* 1984; Torrence and Stuart, 1986). The larger and earlier-departing group initially comprises three cells. It migrates mediad along the anterior margin of the segment. Most of the cells of this group enter the ganglionic rudiment, where they give rise to the connective glioblast and the neurons of the AV cluster. A few cells remain outside the ganglionic rudiment and move a short distance posteriad to contribute to the MAc cluster of peripheral neurons. The remainder of the MAc cluster is derived from the smaller and later-departing group of migratory cells, which initially comprises two cells. They migrate mediad along a mid-segmental path, posterior to the path followed by the larger group. After the MAc cluster has formed, one cell leaves it to enter the ganglion and become the central neuron qz1. The q-kinship cells that do not migrate mediad give rise to the dorsal epidermis and peripheral neurons.

### *Effects of mesoderm deprivation on migration and final positioning of q-kinship cells*

#### *Unilateral mesoderm deprivation*

To ascertain the effects of mesoderm deprivation on the migration and final positions of q-kinship cells, the left q bandlet was labeled with the red-fluorescing lineage

tracer RDA and the left m bandlet with the green-fluorescing lineage tracer FDA. After the teloblasts had produced a few labeled blast cells, further formation of the left m bandlet was aborted by ablation of its parent teloblast. The distribution of RDA-labeled q-kinship cells was examined in 45 such embryos raised to early stage 10 or late stage 9, when migration of q-kinship cells in normal embryos is complete, or nearly so (Torrence and Stuart, 1986). After fixation, muscle fibers in these specimens were stained by indirect immunofluorescence using a fluorescein-conjugated second antibody.

The anteriormost segments of a mesoderm-deprived embryo generated by such a protocol constitute a control zone in which the morphology of the nerve cord and body wall are normal (Blair, 1982; Torrence *et al.* 1989). Control zone segments are populated on both the right and left sides by mesodermal progeny of the m primary blast cells produced before the formation of the left m bandlet was aborted. In the present experiment, the bilateral presence of mesoderm in the control zone was demonstrated by the presence of immunolabeled circular and longitudinal muscle fibers (Fig. 3). An embryo was included in the population to be analyzed only if the distributions of muscle fibers and of RDA-labeled q-kinship cells in its control-zone segments resembled those in corresponding segments of normal embryos.

Posterior segments constitute an experimental zone lacking the left mesoderm (Fig. 3). Here, the mesoderm-deprived left side of the germinal plate was narrower than the non-deprived right side, and segmentally iterated structures, including hemiganglia, were not formed on the left side. A normal array of muscle fibers was found on the non-deprived right side of the germinal plate, but not on the mesoderm-deprived left side. Some circular muscle fibers extended into the mesoderm-deprived side from the non-deprived side. The control and experimental zones were separated by a transition zone of about two segments, which contained reduced numbers of muscle fibers. Provisional muscle fibers (Fernandez, 1980; Weisblat *et al.* 1980*a*, 1984), which lie outside the germinal plate, contacted the lateral margins of the plate in all three zones. These observations confirm the results of Blair (1982) and Torrence *et al.* (1989).

The distribution of RDA-labeled q-kinship cells in the mesoderm-deprived experimental zone was severely disorganized (Fig. 3). In the lateral part of the left germinal plate, q-kinship cells formed irregular clumps rather than a confluent epithelium, and cell florets were not evident. RDA-labeled cells were also present in the presumptive ventral body wall and in the morphologically unsegmented left side of the nerve cord, indicating that q-kinship cells had migrated toward the ventral midline in the absence of the normal ipsilateral complement of mesoderm. These cells were not organized into their normal pattern, but were scattered in groups of irregular size, shape and position. The irregularity of this pattern precluded accurate counting of the number of migratory cell groups, but it appeared that fewer than the normal number of q-kinship cells reached the nerve cord.

The distribution of q-kinship cells in the transition zone was also disrupted. In some embryos, the number of q-kinship cells in transition-zone segments appeared to be greater than normal, but this was not observed consistently.

To confirm that q-kinship cells migrated toward the ventral midline in the absence of ipsilateral mesoderm and to examine the pathways of migration, the left q bandlet was labeled with RDA, and the left m bandlet was labeled with FDA and then aborted as before, but the embryos were examined at an earlier stage of development. To permit direct comparison of the migrations of mesoderm-deprived and non-deprived q-kinship cells, the right q bandlet was also labeled with RDA in some embryos. In unilaterally mesoderm-deprived embryos, some mesodermal cells arising on the non-deprived side extend into the mesoderm-deprived side. Most, and probably all, of these crossed mesodermal cells are circular muscle fibers (Torrence *et al.* 1989). To examine the disposition of the surviving mesoderm, the right m bandlet, and therefore the right mesoderm, was labeled with the lineage tracer FDA in 9 such embryos, while in another 17 such embryos, developing muscle fibers were stained after fixation by indirect immunofluorescence. The embryos were fixed and examined at early or middle stage 9, when q-kinship cell migration is still underway in normal embryos (Figs 4, 5).

Within the mesoderm-deprived experimental zones of these embryos, RDA-labeled cells were found extending mediad from the left q bandlet or lying between the q bandlet and the ventral midline (Figs 4, 5B). These cells are interpreted to be migrating away from the q bandlet in the general direction of the ventral midline, since, in more posterior, developmentally less advanced segments, as well as in younger embryos, all RDA-labeled cells were found to lie together within the coherent q bandlet (not illustrated). The migrating cells did not form the regular, segmentally iterated pattern that is seen when mesoderm is present (Fig. 5B). Rather, they were scattered in groups of irregular size, shape and position. This suggests that although the cells were able to migrate in the absence of ipsilateral mesoderm, they failed to follow normal migration pathways. As in the older embryos described above, the irregularity of the pattern precluded accurate counting of the number of migratory cell groups, but here too a comparison of mesoderm-deprived and non-deprived q bandlets gave the impression that fewer than the normal number of q-kinship cells migrated (Fig. 5B).

The extent to which FDA-labeled contralateral mesoderm or immunoreactive circular muscle fibers had extended into the mesoderm-deprived region from the non-deprived side varied considerably among these embryos (Figs 4, 5A) and often along the length of a given specimen. Some of the observed groups of migrating q-kinship cells were apparently not in contact with any lineage tracer-labeled contralateral mesoder-

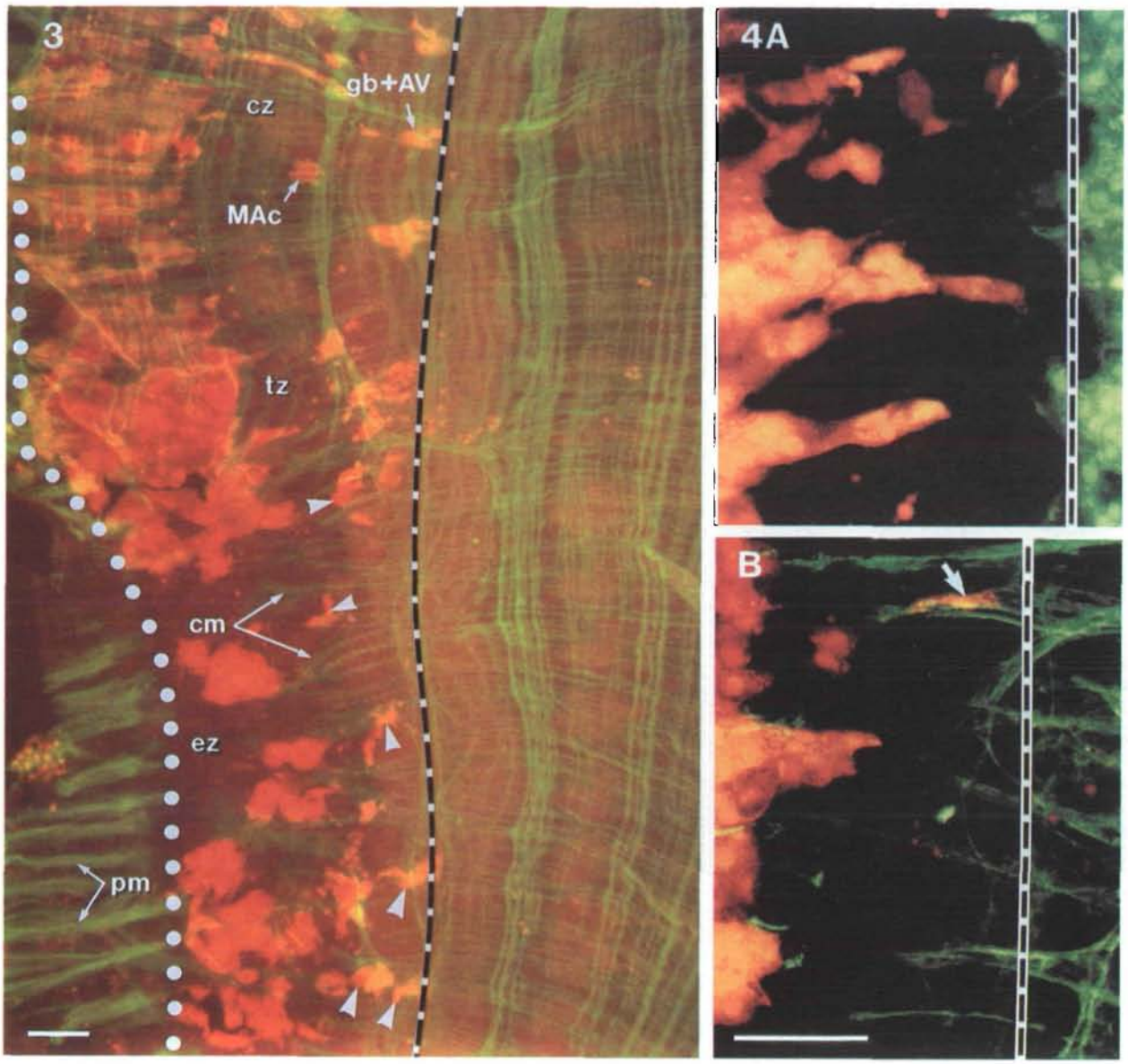


**Fig. 3.** Effects of mesoderm deprivation on positioning of q-kinship cells in approximately 15 segments (A2–A16) at stage 10. The ventral midline is indicated by the dashed line, and the left margin of the germinal plate is indicated by the white dots. RDA-labeled left q-kinship cells appear red; immunolabeled muscle fibers appear green. A normal, bilateral grid of muscle fibers is present in control zone segments (cz, top), and here the distribution of q-kinship cells is normal. A transition zone (tz) separates the control zone from the more posterior experimental zone (ez). Segments in the experimental zone lack mesoderm on the left side, except that some circular muscle fibers (cm) extend across the ventral midline from the non-deprived right side. Here, the distribution of q-kinship cells is abnormal. Arrowheads: mesoderm-deprived q-kinship cells that have migrated toward the ventral midline. pm, provisional muscle fibers outside the germinal plate; other abbreviations as in Fig. 1. Scale bar, 50 $\mu$m.

**Fig. 4.** Mesoderm-deprived q-kinship cells in two stage 9 embryos. The left q bandlet lies at the left edge of either panel, and tracer-labeled left q-kinship cells appear red–orange. The ventral midline is indicated by the dashed line. Scale bar, 50 $\mu$m. (A) Tracer-labeled right mesodermal cells appear green. In this embryo very few right mesodermal cells have extended across the ventral midline into the mesoderm-deprived left side, and q-kinship cells are migrating toward the ventral midline despite lacking detectable contact with mesodermal cells. (B) Immunolabeled muscle fibers appear green. One of the migrating q-kinship cells (arrow) has elongated along a muscle fiber that extends into the mesoderm-deprived left side from the non-deprived right.

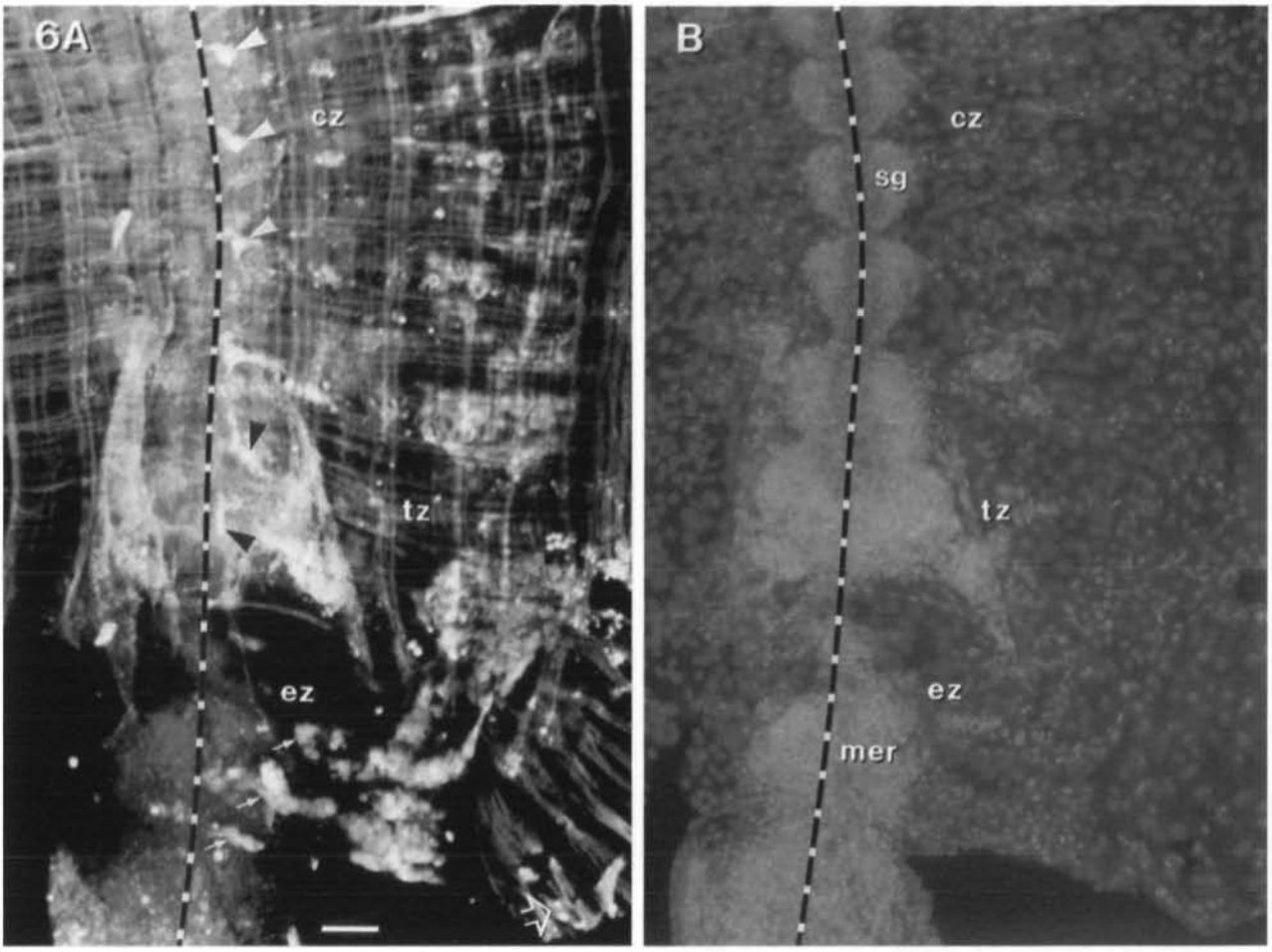


**Fig. 6.** Effects of bilateral mesoderm deprivation. The ventral midline is indicated by the dashed line. cz, control zone; ez, experimental zone; tz, transition between control and experimental zones. Scale bar, 50 $\mu$m. (A) Tracer-labeled left q-kinship cells appear yellow–green; immunolabeled muscle fibers appear red or orange. In the control zone, the distribution of q pattern elements is normal (white arrowheads indicate normal AV neuron clusters). In the bilaterally mesoderm-deprived experimental zone, the few q-kinship cells that have migrated toward the ventral midline (small white arrows) do not form a regular pattern. Black arrowheads: deformed AV neuron clusters in the transition zone; hollow arrow: a single q-kinship cell among provisional muscle fibers. (B) Distribution of Hoechst 33258-stained cell nuclei (blue dots). In the control zone the nerve cord is composed of normal segmental ganglia (sg); in the experimental zone there is only an irregular, medial ridge of ectodermal cells (mer). The apparent aggregations of cells just lateral to the nerve cord in the transition zone are artifactual folds in the specimen.

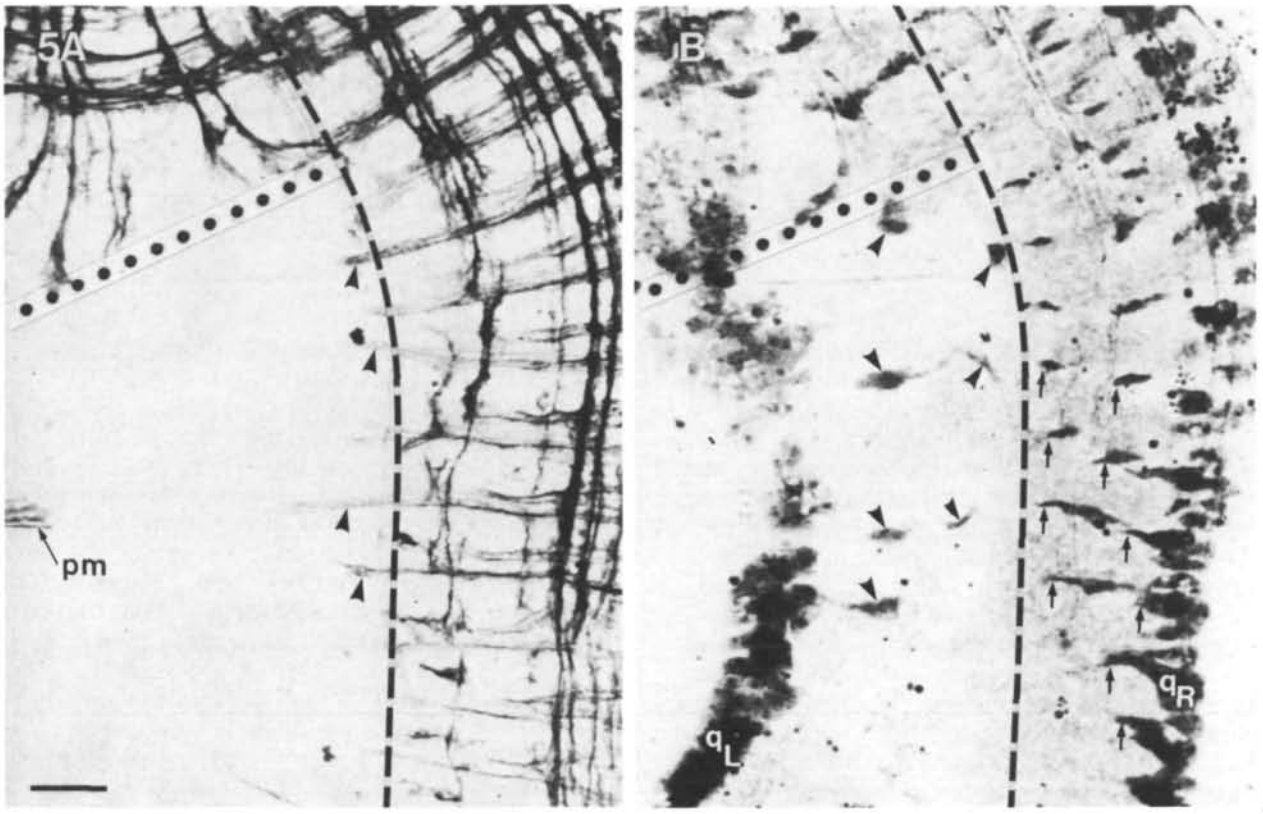


**Fig. 5.** Effects of mesoderm deprivation on migration of q-kinship cells at stage 9. Panels A and B show the same field of view, encompassing approximately 13 segments. The ventral midline is indicated by the dashed line. Scale bar, 50 $\mu$m. (A) Immunolabeled muscle fibers. The mesoderm-deprived experimental zone on the left side (below the dotted line) is devoid of muscle fibers, except for a few circular muscle fibers (arrowheads) extending across the ventral midline from the non-deprived right side. pm, provisional muscle fiber outside the germinal plate. (B) Lineage tracer-labeled left ($q_L$) and right ($q_R$) q bandlets. The non-deprived right side illustrates the normal pattern of early q-cell migration. Here, small groups of cells (arrows) migrating toward the ventral midline from the q bandlet form a regular, segmental pattern. Migration is least advanced in the posteriormost segments (bottom) and progressively more advanced in progressively more anterior segments (Torrence and Stuart, 1986). In the mesoderm-deprived region of the left side, the few q-kinship cells migrating toward the ventral midline (arrowheads) do not form a regular pattern. No cells have moved laterally from either q bandlet.

mal cell (Fig. 4A) or immunolabeled muscle fiber (Figs 4B, 5). Thus, contact with the circular muscle fibers that often extend from the non-deprived into the mesoderm-deprived side of such embryos is not required for q-kinship cells to migrate toward the ventral midline. Other migratory cell groups were in contact with crossed, contralateral muscle fibers. Migratory cells in contact with crossed muscle fibers were typically elongated along the muscle fibers and appeared to be following them (Fig. 4B).

*Bilateral mesoderm deprivation*

To eliminate the possibility that the mesoderm on the non-deprived side of unilaterally mesoderm-deprived embryos somehow promotes the migration of q-kinship cells on the deprived side, the distribution of q-kinship cells was examined in embryos bilaterally deprived of mesoderm. For this purpose, the left q bandlet was labeled with FDA, and formation of both m bandlets was aborted. Nine such embryos were raised to the beginning of stage 10 and, after fixation, the embryonic muscle fibers were stained by indirect immunofluorescence using a rhodamine-conjugated second antibody.

As in unilaterally mesoderm-deprived embryos, the anteriormost segments of these bilaterally mesoderm-deprived embryos constituted a control zone in which mesoderm was present bilaterally, as revealed by a normal pattern of immunoreactive muscle fibers (Fig. 6A), and in which the morphology of the nerve cord (Fig. 6B) and the distribution of FDA-labeled q-kinship cells (Fig. 6A) were normal.

Between the control zone and the posterior, bilaterally mesoderm-deprived experimental zone was a transition zone comprising 2–3 segments. In the transition zone, the number of muscle fibers was reduced (Fig. 6A) and segmentation of the nerve cord was poorly defined (Fig. 6B). In these segments, q-kinship cells had migrated into the nerve cord and had given rise to the connective glioblast and what appeared to be deformed AV clusters, but their overall distribution was abnormal (Fig. 6A). As in unilaterally

mesoderm-deprived embryos, the number of q-kinship cells in transition-zone segments sometimes appeared to be greater than normal, but this was not observed consistently.

The ectoderm in the bilaterally mesoderm-deprived experimental zone formed an irregular medial ridge, presumably corresponding to the nerve cord, flanked by a thin sheet of sparse cells (Fig. 6B). This morphology resembles that previously observed following focal, bilateral mesoderm ablation by another method (Torrence *et al.* 1989).

The experimental zone was much shorter than expected. The control zone encompassed the 4 segments of the head and the anteriormost 7 (7 embryos) or 8 (2 embryos) abdominal segments. The transition between the control and experimental zones extended through an additional 2–3 segments. Thus, the control and transition zones together encompassed about half of the 32 segments normally found in a leech. The experimental zone might have been expected to encompass 17–19 ectodermal segments. Instead, the experimental zone of each embryo extended over a length corresponding to only about 5 control zone segments. Ectodermal cells were not obviously more densely packed in the experimental zone than in the control zone, as judged either from the density of Hoechst 33258-stained cell nuclei or from the mass of FDA-labeled q-kinship cells (Fig. 6). Although this estimate of how much mesoderm-deprived ectoderm was present is imprecise, bilateral mesoderm deprivation evidently reduced the total number of ectodermal segmental complements present in the germinal plates of these embryos. Apparently, mesodermal tissues or their precursor m bandlets play some role in the determination of ectodermal segment number.

Within the short, bilaterally mesoderm-deprived region of these embryos, the distribution of labeled q-kinship cells resembled that in unilaterally mesoderm-deprived embryos. That is, q-kinship cells were present in the presumptive ventral body wall and in the medial ectodermal ridge (Fig. 6A), indicating that q-kinship cells had migrated toward the ventral midline in the absence of germinal plate mesoderm. Furthermore, the migratory cells were not organized into their normal pattern, but instead were scattered in groups of irregular size, shape and position. In the presumptive dorsal part of the germinal plate, non-migratory q-kinship cells formed irregular clumps. In 2/9 embryos, one or two FDA-labeled cells were also observed lateral to the q bandlet, among the provisional muscle fibers outside the germinal plate.

### *Neurochemical differentiation of mesoderm-deprived q-kinship neurons*

In each hemisegment of a normal stage 11 embryo, glyoxylic acid-induced monoamine fluorescence reveals the cell bodies and axons of three neurons that exhibit the blue fluorescence characteristic of dopamine. All three are peripheral neurons that extend axons into the segmental ganglion. One, the neuron MD, is descended from the q bandlet; the others, neurons LD1 and LD2, are descended from the p and o bandlets, respectively (Blair, 1983; Stuart *et al.* 1987). Neuron MD is one of the cells of the MAc cluster, and is therefore normally derived from a migratory precursor cell. To examine the effect of mesoderm deprivation on the differentiation of this neuron, the left q bandlet was labeled with RDA and formation of the left m bandlet was aborted as above. The embryos were raised to mid-stage 11 and subjected to glyoxylic acid staining. Six such embryos were examined, in each of which the mesoderm-deprived experimental zone extended over a length corresponding to 6–11 non-deprived contralateral abdominal hemisegments for a total of 54 experimental zone segments.

Twenty RDA-labeled neurons exhibiting the blue fluorescence characteristic of dopamine were found in these segments. Rather than occupying the normal position of the MAc cluster, a short distance lateral to the nerve cord, all but one of these MD neurons derived from the left q bandlet was abnormally situated within or superficial to the nerve cord (Fig. 7). One MD neuron occupied a peripheral position between the nerve cord and the q bandlet. Seven dopamine-containing cells were found that lacked RDA; these presumably represented neurons LD1 and LD2, derived from the mesoderm-deprived p and o bandlets. Thus, although the precursors of MD neurons are able to migrate and MD neurons are able to develop their neurochemical phenotype in the absence of mesoderm, mesoderm deprivation causes a substantial reduction in their number. That reduction is even more severe in the case of the o- and p-bandlet-derived dopamine-containing neurons.

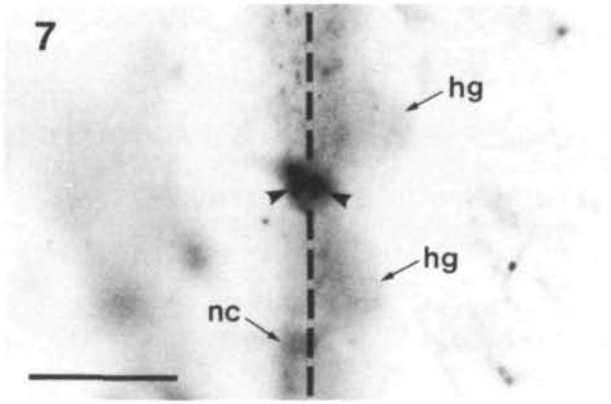


**Fig. 7.** Ectopic dopamine-containing, q-kinship neurons in a stage 11 embryo deprived of the left mesoderm. Two glyoxylic-acid-stained neurons exhibiting the blue fluorescence characteristic of dopamine (arrowheads) are situated beneath the connective nerve between two right hemiganglia (hg), which are visible by background fluorescence. Both of these dopamine neurons also contained RDA lineage tracer inherited from the q bandlet (not illustrated). The ventral midline is indicated by the dashed line. nc: nerve cord. Scale bar, 50 $\mu$m.

*Effects of ectodermal bandlet deprivation on migration and final positioning of q-kinship cells*

*n bandlet deprivation*

Preliminary experiments in which one n bandlet was aborted by ablation of its parent teloblast produced normal q kinship group patterns (not illustrated). However, this ablation protocol induces substantial numbers of n-kinship cells originating on the non-deprived side to cross the ventral midline into the initially n-deprived hemiganglia (Blair and Weisblat, 1982; Blair, 1983; Stuart *et al.* 1987, 1989). Thus, this experiment failed to test the effects of n-kinship deprivation on q-kinship cells, because n-kinship cells are still present in nearly every hemiganglion. Two strategies were employed to circumvent this problem. Under the first, one n bandlet was ablated late enough in development that few or no surviving, contralateral cells crossed the ventral midline. Under the second, formation of both n bandlets was aborted.

Late unilateral n bandlet ablations were performed by the method of FDA-sensitized photoablation (Shankland, 1984; Braun and Stent, 1989*b*). For this purpose, the left q bandlet was intensely labeled with RDA, the right n bandlet was dimly labeled with RDA, and the left n bandlet was labeled with FDA. Although descendants of the left q and right n bandlets were both labeled with RDA, they were distinguishable at stage 10 by the differential brightness of their fluorescence. Shortly before the onset of gangliogenesis, a part of the FDA-labeled left n bandlet containing precursor cells destined for several contiguous midbody segments was photoablated by laser irradiation at the fluorescein excitation wavelength.

The distribution of labeled cells was examined in 11 such embryos raised to late stage 9. In each, FDA-labeled cells were absent from 6 to 14 contiguous abdominal ganglia constituting an n-bandlet-deprived experimental zone. A total of 94 n-deprived segments were examined. The unirradiated parts of the left n bandlet contributed FDA-labeled cells to the ganglia in segments anterior and (in 9/11 embryos) posterior to the experimental zone, constituting non-deprived anterior and posterior control zones. Ganglionic morphology (Fig. 8A) and the distributions of labeled n-kinship and q-kinship cells were normal in control zone segments.

Within the experimental zone, hemiganglia on the non-deprived right side were normal in shape and size. By contrast, hemiganglia on the n-bandlet-deprived side were much smaller than normal (Fig. 8A), reflecting the absence of n-kinship cells, which normally constitute more than half of the cells in a hemiganglion (Kramer and Weisblat, 1985). In the experimental zone, dimly FDA-labeled right n-kinship cells sometimes extended a short distance across the ventral midline, within the ventromedial glial packets where they normally interdigitate slightly with their contralateral homologues (Stuart *et al.* 1989), but they never extended into the lateral region of the n-deprived left hemiganglia (Fig. 8B).

Brightly RDA-labeled left q-kinship cells had migrated centripetally in 93/94 experimental zone segments examined. Furthermore, in the majority (67/94) of these segments, the q-kinship cells succeeded in forming an essentially normal pattern consisting of a glioblast in the interganglionic connective nerve, the AV cluster of central neurons, the central interneuron qz1 near the emergence of the MA nerve root, and a cluster of peripheral neurons along the MA nerve (Fig. 8B).

To examine the fate of q-kinship cells in embryos bilaterally deprived of n-kinship cells, the left q bandlet was labeled with FDA and both n bandlets were aborted. The distribution of q-kinship cells was examined in 266 abdominal segments in 13 such embryos (14–21 segments per embryo) at early stage 10.

As previously reported (Blair and Weisblat, 1982), abdominal ganglia in the experimental zones of bilaterally n-deprived embryos were very much smaller than normal, consistent with the expected absence of more than half of the normal number of neurons (cf. Figs 1,9). Most ganglia examined in the present experiment were of nearly normal mediolateral width, but of only about half the normal anteroposterior length. A few adjacent ganglia were fused into masses nearly as large as a normal ganglion.

In the majority (218/266) of bilaterally n-bandlet-deprived segments examined, the distribution of FDA-labeled q-kinship cells resembled that found in unilaterally n-deprived segments, having formed a complete pattern (Fig. 9). The relative positions of the q pattern elements were judged to be essentially normal, although in some ganglia the AV cluster of central neurons was situated closer to the center of the ganglion than to its anterior margin.

In contrast to the quasi-normal distribution of q-kinship cell bodies, the pattern of axons projected by q-kinship neurons within bilaterally n-deprived ganglia was grossly abnormal. In normal ganglia (Fig. 1; also Fig. 5A of Torrence and Stuart, 1986), many q-kinship axons course in longitudinal tracts near the lateral margin of the ipsilateral neuropil and interganglionic connective nerves; these are joined by the axons of qz1 and the peripheral neurons in the MAc cluster. There are also smaller projections extending across the ventral midline in an anterior commissure and coursing in longitudinal tracts near the lateral margin of the contralateral neuropil and interganglionic connective nerves. In bilaterally n-deprived segments, peripheral q-kinship neurons extended axons into the ganglia normally, and the axon of neuron qz1 fasciculated normally with the axons of the MAc neurons (Fig. 9). Within the neuropil, however, q-kinship axons formed disorganized knots, and very few labeled axons extended to adjacent ganglia *via* the connective nerves.

*o or p bandlet deprivation*

To examine the fate of q-kinship cells deprived of interaction with o- or p-kinship cells, the left q bandlet was labeled with FDA, and the left o and p bandlets

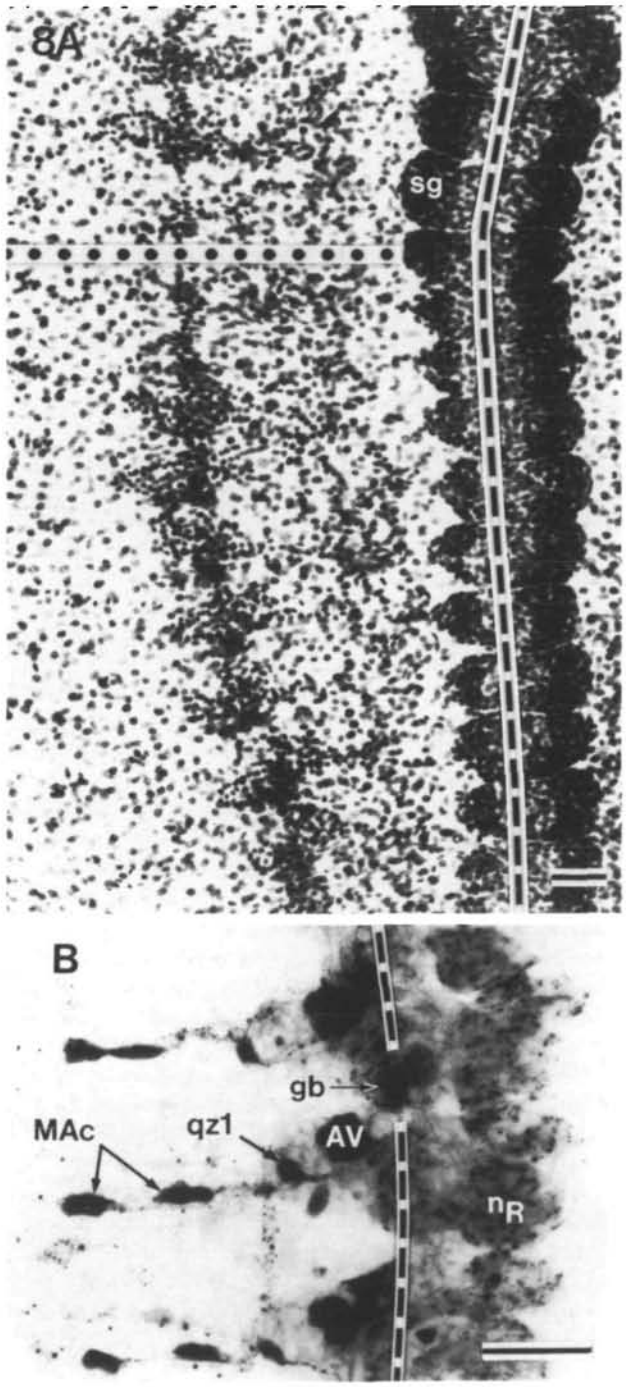


**Fig. 8.** Effects of late unilateral n bandlet ablation. The ventral midline is indicated by the dashed line. Scale bars, 50 μm. (A) Distribution of Hoechst 33258-stained cell nuclei (dots) in approximately 15 segments (A2–A16) of a stage 10 embryo. Segmental ganglia (sg) in the anterior control zone (above the dotted line) are normal. The left half of each ganglion in the n-bandlet-deprived experimental zone (below the dotted line) is much smaller than normal, reflecting the absence of n-kinship cells. The posterior control zone in this embryo is not illustrated. (B) Tracer-labeled left q-kinship cells (black) and right n-kinship cells ($n_R$, mottled gray) in three stage 10 segments from which the left n bandlet had been photoablated. Right n-kinship cells extend a short distance across the ventral midline, but are restricted to the ventromedial glial packets. Although the n-deprived hemiganglia are much smaller, the q kinship group pattern resembles that in normal hemiganglia (c.f. Fig. 1). Abbreviations as in Fig. 1.

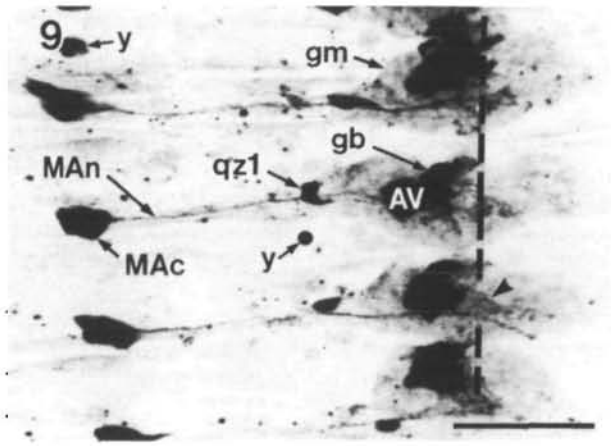


**Fig. 9.** Effect of bilateral n bandlet deprivation on tracer-labeled q-kinship cells (black), illustrated in four segments (A8–A11) of a stage 10 embryo. The ventral midline is indicated by the dashed line. Despite the small size of the ganglia, which are faintly visible by background fluorescence, the distribution of q-kinship cells is essentially normal (c.f. Fig. 1). Within the neuropil, q-kinship axons form disorganized knots (arrowhead). Although connective nerves are present between the ganglia, they are not visible because very few labeled axons extend into them. y, autofluorescent yolk spheres; other abbreviations as in Fig. 1. Scale bar, 50 μm.

were labeled with RDA by injecting that tracer into the common ancestor of their precursor teloblasts, cell OP. Either the left o or the left p bandlet was subsequently aborted after a few primary blast cells had been produced.

The distribution of q-kinship cells was examined in 185 abdominal hemisegments of 12 stage 10 embryos (7–20 segments per embryo) that had been deprived of their left p bandlet and in 133 abdominal hemisegments of 7 stage 10 embryos (18–20 segments per embryo) that had been deprived of their left o bandlet. The pattern of q-kinship cells was normal in 185/185 of the p-bandlet-deprived segments (Fig. 10B) and in 131/133 of the o-bandlet-deprived segments (not illustrated).

Interpretation of the effects of p bandlet deprivation on q-kinship cells is complicated by a phenomenon known as transfating. Depriving an embryo of the leech *Helobdella triserialis* of a p bandlet causes all progeny of the surviving o bandlet to adopt the normal fate of the absent p-kinship cells. The pattern elements normally derived from the o bandlet are then absent, and the resulting embryos resemble o-bandlet-deprived embryos (Weisblat and Blair, 1984; Shankland and Weisblat, 1984; Zackson, 1984). Recent investigations have revealed that transfating also occurs in embryos of

**Table 1.** *Normality of the q-kinship pattern as a function of the fate of o-kinship cells in hemisegments deprived of the p bandlet*

| Pattern elements formed by o-bandlet progeny | Numbers of hemisegments | | |
|---|---|---|---|
| | q pattern | | |
| | normal | abnormal | total |
| p | 116 | 12 | 128 |
| o | 20 | 1 | 21 |
| mixed o & p | 24 | 4 | 28 |
| 1/2 p | 4 | 3 | 7 |
| 1/2 o | 1 | 0 | 1 |
| | 165 | 20 | 185 |

*T. rude*, but that not all o-bandlet cells are affected (Keleher and Stent, 1990). Thus, eliminating a p bandlet from an embryo of *T. rude* results in the development of a mosaic pattern in which some o blast cells give rise to elements normally characteristic of the p kinship group pattern while others give rise to elements of their normal o kinship group pattern. As in *H. triserialis* (Weisblat and Blair, 1984; Shankland and Weisblat, 1984; Zackson, 1984), elimination of an o bandlet from embryos of *T. rude* in the present experiment had no effect on the fate of the surviving, RDA-labeled p bandlet, which formed p kinship group patterns in all segments.

The o and p kinship group patterns each include characteristic elements that allow the patterns to be unambiguously distinguished (Shankland and Weisblat, 1984). In the present experiment, the presence or absence of these elements among the RDA-labeled cells in each p-bandlet-deprived segment was scored to ascertain whether that segment contained o or p pattern elements or a mixture of both. In *T. rude* (G. P. Keleher, personal communication), as in *H. triserialis* (Weisblat and Shankland, 1985), each o or p primary blast cell clone extends from the middle of one segment into the posterior half of the next more posterior segment, and each segment contains specific contributions from two successive primary blast cell clones. Thus, in the mosaic pattern resulting from p-bandlet deprivation, a given segment could contain only o pattern elements (descended from two adjacent, untransfated clones), only p pattern elements (descended from two adjacent, transfated clones), or a mixture of o and p pattern elements (descended from one transfated and one untransfated clone). Instances of all three possibilities were observed in the present experiments (Table 1, Fig. 10A). Indeed, instances of all three possibilities were observed in most individual embryos. In a few cases, a single primary blast cell clone had apparently produced both o and p pattern elements; these were also categorized as mixed. Unmixed segments were also found which lacked the pattern elements (either o or p) that should have been contributed by one of the segment's two constituent o blast cell clones. Such segments were categorized as 1/2o or 1/2p, depending on whether the elements that were present were characteristic of the o or of the p pattern.

The frequency of occurrence of these various segment categories among the 185 p-bandlet-deprived abdominal hemisegments in which the q kinship group pattern was examined is given in Table 1. Regardless of what o or p pattern elements were present, the majority of segments contained normal q patterns (Table 1, Fig. 10B). Thus, the presence of neither the o nor the p kinship group is needed for the formation of a normal pattern by the q kinship group.

## Discussion

### *Cell commitment*

Cells of the q kinship group were found to migrate and generate their normal descendant pattern in hemisegments deprived of cells expressing the normal fates of the n, o or p kinship groups. Previous experiments have shown that the dopamine-containing, q-kinship neuron MD is able to differentiate its neurotransmitter phenotype in the absence of cells derived from the other ectodermal bandlets (Blair, 1983). Thus, commitment of q-kinship cells to migrate and to give rise to their characteristic complement of descendant cells does not require interactions with cells of any other ectodermal kinship group.

Cells of the q kinship group were also found to express migratory phenotypes when deprived of their ipsilateral m kinship group. In unilaterally mesoderm-deprived embryos, some mesodermal cells arising on the non-deprived side extend into the mesoderm-deprived side. Most, and probably all, of these crossed mesodermal cells are circular muscle fibers (Torrence *et al.* 1989). However, migration of q-kinship cells does not depend on contact with such crossed, contralateral mesodermal cells, since many q-kinship cells migrating in mesoderm-deprived regions were not associated with any crossed mesodermal cells. This conclusion was confirmed by the observation that q-kinship cells also migrated in bilaterally mesoderm-deprived regions.

Because most elements of the q kinship group pattern in normal embryos are identified by their characteristic positions and shapes, the disorganization of mesoderm-deprived q-kinship cells prevented their detailed identification. However, the finding that some mesoderm-deprived q-kinship cells differentiated the neurotransmitter phenotype characteristic of neuron MD indicates that cells of the q bandlet do not require interaction with the ipsilateral m kinship group to give rise to this identified neuron. Most mesoderm-deprived MD neurons were abnormally situated within or superficial to the nerve cord, indicating that the precursor of an MD neuron need not occupy its normal position in a hemisegment in order to differentiate its neurochemical phenotype. All of the MD neurons that were observed in mesoderm-deprived regions arose from cells that had migrated: all lay medial to the q bandlet, and most had reached the ventral midline. This result suggests that the cells that became committed to differentiate the

dopamine phenotype were coordinately committed to migration. According to this hypothesis, the abnormal distribution of mesoderm-deprived MD neurons arose from the expression of normal morphogenetic behaviors by q-kinship cells deprived of their normal positional cues.

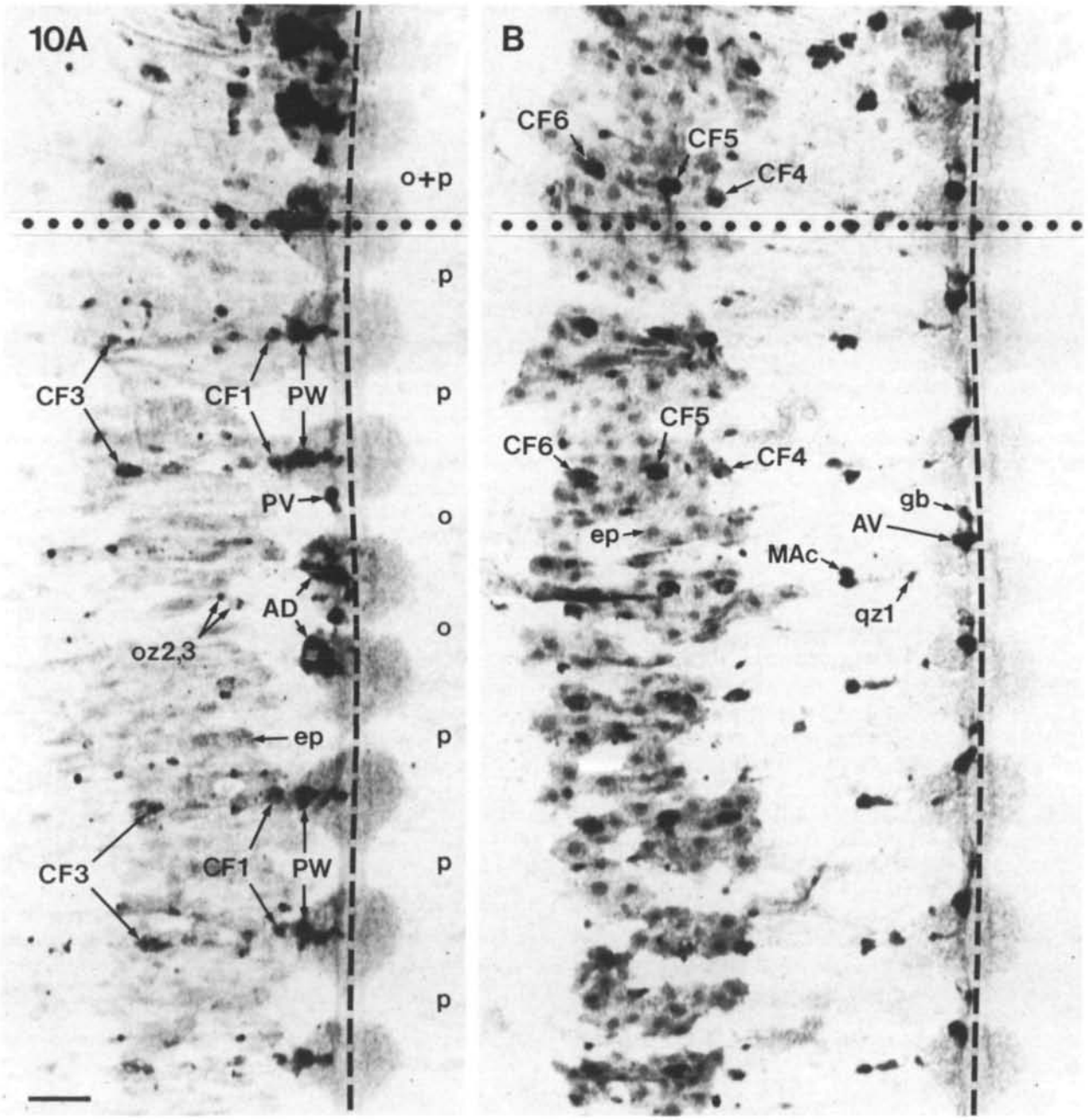


**Fig. 10.** Effects of p bandlet deprivation. Both panels show the same field of view, encompassing 10 segments (S4–A9) of a stage 10 embryo. The dashed line indicates the ventral midline. Scale bar, 50 $\mu$m. (A) RDA tracer-labeled descendants of the left o and p bandlets. In anterior, control zone segments (above the dotted line), progeny of both bandlets are present. Segments in the posterior experimental zone (below the dotted line) lack progeny of the p bandlet, whose precursor teloblast was killed. Whether a given primary blast cell clone in the surviving o bandlet expressed its original, O fate or transfated to express the P fate is indicated along the right side of the figure. Pattern elements diagnostic of the O fate include the AD and PV clusters of central neurons and the peripheral neurons oz2 and oz3. Pattern elements diagnostic of the P fate include two groups of cuboidal epidermal cells, cell florets 1 and 3 (CF1, CF3), and the cluster of central neurons called the P Wedge (PW). Both fates include squamous epidermal cells (ep). (B) FDA tracer-labeled q-kinship cells. Regardless of the fate expressed by the progeny of the consegmental o blast cells, the q-kinship cells formed their normal pattern. Dark spots within labeled squamous epidermal cells (ep) are the cell nuclei, which have preferentially concentrated the lineage tracer. CF4, CF5, CF6: cell florets 4, 5 and 6; other abbreviations as in Fig. 1.

These results resemble the previous finding that neither interactions with mesoderm nor occupancy of appropriate positions is necessary for neurochemical differentiation of 5-hydroxytryptamine-containing neurons descended from the n bandlet (Stuart *et al.* 1989; Torrence *et al.* 1989), and further support the proposal that neural precursor cells in the leech first become committed to give rise to specific cell types and then migrate to find the positions appropriate to their fates. In this regard, leech neural precursors resemble neurons in the visual cortex of the ferret, at least some of which become committed to migrate to particular cortical layers, and to differentiate into cell types appropriate to those layers, before leaving the proliferation zone where they are generated (McConnell, 1988). It is important to note that commitment of a cell to a particular developmental fate need not imply that the cell can give rise to only one cell type (Stent, 1985). Although the committed migratory cells in the ferret are postmitotic, the committed migratory cells in the leech are still mitotically active (Torrence and Stuart, 1986), and the specific fates to which they are committed include more than one cell type.

Although at least some cells descended from a mesoderm-deprived q bandlet migrate toward the prospective ventral midline, comparison of mesoderm-deprived and non-deprived q kinship groups suggested that the deprived kinship groups gave rise to fewer than normal migratory cells. This might reflect a reduced *total* number of q-kinship cells. Mesoderm deprivation could reduce the number of q cells in the germinal plate by slowing cell division or by causing cells that would normally survive and migrate to die instead. Alternatively, mesoderm deprivation could reduce the number of q cells in the germinal plate by reducing the number of q primary blast cell clones incorporated into the germinal plate. Even in normal development, each teloblast produces 5 to 15 more blast cells than are necessary to generate the 32 segments of the leech body (Fernandez and Stent, 1980; Zackson, 1984). These supernumerary blast cells do not enter the germinal plate; instead they degenerate and die. If ectodermal primary blast cells that would normally survive are prevented from entering the germinal plate by experimental manipulations, they die as if they were supernumeraries (Shankland, 1984). Thus, some mesoderm-deprived q blast cells that would normally survive and give rise to segmental tissues might have become stranded outside the germinal plate and died.

Mesoderm deprivation also reduced the number of q-kinship cells that differentiated the neurotransmitter dopamine to less than half the expected number, and the number of dopamine-containing cells originating from the o and p bandlets was reduced even more. Again, these reductions might be simple consequences of reduced total numbers of ectodermal precursor cells incorporated into the mesoderm-deficient germinal plate. Alternatively, although occupancy of a correct final position is not required for the differentiation of dopamine by q-kinship cells, migration or proximity to the ventral nerve cord might be required, so that a cell that failed to migrate would never express the dopamine phenotype to which it might otherwise be committed.

The reduced numbers of dopamine-containing neurons derived from mesoderm-deprived o, p, and q bandlets contrasts with the nearly normal numbers of 5-HT neurons made by mesoderm-deprived n bandlets (Blair, 1982; Torrence *et al.* 1989). This difference might reflect a lesser effect of mesoderm deprivation on the incorporation of n blast cells into the germinal plate than on o, p, and q blast cells. Where it first enters the germinal plate, the n bandlet comes into contact with the contralateral m bandlet, while the o, p, and q bandlets do not. Thus, contact with the contralateral mesoderm might aid the incorporation of n blast cells deprived of their ipsilateral mesoderm into the germinal plate.

### *Control of migration*

A variety of cell types are normally present along the migration paths of q-kinship cells, and are thus candidates for providing pathway guidance to the migrating cells. Among these are peripheral neurons and cell florets descended from the o and p bandlets and a variety of mesodermal cells, including muscle fibers (Torrence and Stuart, 1986). Similarly, cells descended from the m, n, o and p bandlets are present near the final positions of the various elements of the q kinship group pattern, so cells descended from any of these bandlets are candidates to provide positional cues to allow migrating cells to recognize their destinations.

The results reported here indicate that cells of the q bandlet can migrate and find their normal final positions in segments deprived of cells expressing the fates of the ectodermal n, o, or p kinship groups. By contrast, when deprived of the mesodermal m kinship group, q-kinship cells failed to follow normal migration pathways or to find appropriate final positions. Apparently, mesodermal tissues provide pathway guidance for migrating q-kinship cells, and may also provide positional cues that allow migrating cells to recognize their destinations. Previous studies in embryos of the leech *Helobdella triserialis* suggested that the ganglionic organization of o-, p- and q-kinship cells may be facilitated by n-kinship cells, but is not strictly dependent on them (Blair and Weisblat, 1982). The present results, particularly the sporadic posteriorward shift in the position of the AV cluster of q-kinship neurons following bilateral n-bandlet deprivation, are compatible with this interpretation, but indicate that any role of n-derived cues in controlling the positions of neuronal cell bodies is subsidiary to the role of mesoderm-derived cues.

Cells of the n kinship group do appear to play an important role in the organization of the ganglionic neuropil, since the absence of both n bandlets seriously deranged the axonal projections of q-kinship neurons into the neuropil and interganglionic connective nerves. This result may indicate either that q-kinship axons normally navigate through the forming neuropil by following earlier-growing axons of n-kinship neurons or that the neuropil glial cells, which are descended from

the n bandlets (Weisblat *et al.* 1984), play a necessary role in organizing the neuropil.

Many of the migrating q-kinship cells observed in unilaterally mesoderm-deprived stage 9 embryos were not in contact with any mesodermal cell, but some were elongated along circular muscle fibers that extended into the mesoderm-deprived left side of the embryo from the non-deprived right side. Thus, while q-kinship cells apparently do not require contact with germinal plate mesoderm in general, or muscle fibers in particular, to migrate toward the ventral midline, they often appear to follow muscle fibers if they do come into contact with them. This apparent affinity of at least some q-kinship cells for circular muscle fibers may also be important for normal migration, since circular muscle fibers closely parallel the normal migration path of the larger and earlier-appearing group of migratory q-kinship cells (Stuart *et al.* 1982; Torrence and Stuart, 1986), and thus are prime candidates for providing guidance along this pathway.

Between the control and experimental zones of unilaterally or bilaterally mesoderm-deprived embryos lay a transition zone, about two segments long, in which the distribution of q-kinship cells was abnormal even though some mesoderm was present. It seems likely that this transition zone reflects the normal distribution of cells derived from an m primary blast cell. Each m blast cell clone contributes a different, but specific, subset of the mesodermal elements in each of three successive hemisegments (Weisblat and Shankland, 1985). Each hemisegment consequently contains contributions from three successive m primary blast cell clones. The anteriormost segment of a transition zone would be expected to contain the specific mesodermal elements provided by the last two m blast cells produced before their parental teloblast was killed, but to lack the elements that would have been provided by the first blast cell whose formation was aborted by the death of the teloblast. The second segment of a transition zone would be expected to contain the mesodermal elements provided by the very last m blast cell produced, but to lack the elements that normally would have been provided by the first two aborted blast cells. Thus, the abnormality of q kinship group patterns in transition-zone segments probably reflects the absence of specific subsets of mesodermal elements that are necessary for providing positional cues to q-kinship cells. The distribution of individual m blast cell progeny among the three hemisegments to which a clone contributes has not yet been mapped in sufficient detail to allow correlation of specific pattern abnormalities in transition-zone segments with the presence or absence of individual mesodermal cells.

Although mesoderm-deprived q-kinship cells failed to follow normal migration pathways, the general direction of their migration was almost always correct. That is, migratory cells leaving the q bandlet moved toward the ventral midline and the prospective CNS, not away. The only exceptions to this rule were one or two cells in each of two bilaterally mesoderm-deprived embryos. Thus, while mesoderm within the germinal plate is required to provide positional cues governing migratory pathways and destination recognition, it is apparently not required to provide the basic lateral-to-medial polarity of migration. Although this polarity might be intrinsic to the q bandlet, there are also several candidates for q-bandlet-extrinsic sources of polarity. For example, the provisional muscle fibers and the micromere-derived simple squamous epithelium, which together form the provisional integument lying outside the germinal plate, might be inhospitable substrates for migration. Alternatively, q-kinship cells might respond to chemotropic signals, such as have been proposed to play important roles in axon guidance in the developing vertebrate nervous system (Dodd and Jessell, 1988; Jessell, 1988). The lateral-to-medial polarity of q-cell migration might be given either by repulsive signals originating from the provisional integument or by attractive signals originating at the midline of the germinal plate. During the early stages of cell migration, the medialmost cells within the germinal plate are members of the n and m bandlets. Since q-kinship cells continued to migrate preferentially mediad in segments deprived bilaterally of either the n or the m bandlets, these bandlets are unlikely to be sources of a midline chemotropic signal. In some mesoderm-deprived embryos, the number of q-kinship cells in transition-zone segments appeared greater than normal, as if cells had migrated into the transition zone from the experimental zone. While this may indicate that germinal plate mesoderm attracts q-kinship cells, it seems unlikely to explain the lateral-to-medial direction of q-cell migration for two reasons. First, the direction of migration was consistent, while the apparent aggregation of cells into the transition zone was not. Second, when it was observed, such apparent aggregation occurred not only at the midline, as predicted by the chemotropic signal hypothesis, but also at the lateral margin of the germinal plate. Another cell that comes to lie along the midline remains a candidate as a source of a midline chemotropic signal – the B macromere. The surface of the B macromere is broadly exposed on the future ventral surface of the early embryo, but the morphogenetic movements that bring the left and right bandlets together to form the germinal plate also reduce the exposed surface of the B macromere to a narrow strip (author's unpublished observations). This exposed strip contacts the inner surface of the germinal plate only along the ventral midline, in an appropriate position to provide an attractive chemotropic signal.

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