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CLONAL ORGANIZATION OF THE CENTRAL NERVOUS SYSTEM OF THE FROG

III. Clones Stemming from Individual Blastomeres of the 128-, 256-, and 512-Cell Stages[1]

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Abstract

Horseradish peroxidase injected into individual blastomeres of 128-, 256-, and 512-cell embyros of *Xenopus laevis* was identified in cells of the central nervous system (CNS) at early to middle larval stages. Labeled cells were dispersed, mingled with unlabeled cells. Four boundaries in the CNS could be defined by the behavior of clones of labeled cells: in the transverse plane at the level of the isthmus; in the horizontal plane between dorsal and ventral regions extending the entire length of the CNS; in the dorsal midline extending the entire length; and in the ventral midline of rhombencephalon and spinal cord but absent more rostrally. Cells injected with HRP at the 512-cell stage produced clones that, with rare exceptions, did not cross any boundary, whereas labeled clones initiated at earlier stages frequently crossed boundaries. Axons and dendrites were not restricted by these boundaries. These boundaries subdivided the CNS into seven compartments, each of which was occupied exclusively by descendants of a group of 14 to 26 blastomeres in the 512-cell embryo. These groups of blastomeres formed a bilaterally symmetrical pattern composed of a single anterior median group straddling the dorsal midline near the animal pole and three groups on each side. Because cells mingled in each compartment but not across compartmental boundaries, there was a one-to-one relationship between individual blastomeres and CNS compartments but one-many and many-one relationships between individual blastomeres and neuroanatomical subdivisions smaller than a compartment. There was no constant relationship between phenotypes of nerve cells and their ancestry from individual blastomeres of the 512-cell or earlier stages.

Previous work in which horseradish peroxidase (HRP) was injected into individual blastomeres of *Xenopus* embryos at 2- to 64-cell stages showed the usefulness of this method for tracing cell fates and for clonal analysis of a vertebrate central nervous system (Jacobson and Hirose, 1978, 1981; Hirose and Jacobson, 1979; Jacobson, 1981a, b). The properties of HRP that have made it such an excellent cell lineage tracer are that it is relatively nontoxic and does not alter normal development if injected into cells in nanoliter quantities; it does not diffuse out of the injected cell, it is transferred at mitosis to the daughter cells and can be identified at later stages in all the descendants of the initially injected cell after they have differentiated into many cell types including neurons.

After injection of HRP into a particular blastomere at the 64-cell stage, we found that the labeled descendants at larval stages were confined to a region called a *clonal domain* in which labeled and unlabeled cells were mingled. The observed systematic mapping relationships between the position of a blastomere and the position of its clonal domain in the central nervous system enabled us to deduce a fate map of the central nervous system at the level of resolution of individual clonal domains and their ancestral blastomeres in the 64-cell embryo (Jacobson and Hirose, 1981), that is, at a higher level of resolution and at an earlier stage than had previously been obtained by means of vital staining (Keller, 1975).

Some progress was also made toward understanding the relationship between ancestral cells and types of differentiated nerve cells. After labeling any single blastomere at the 64-cell stage or earlier, we observed that many types of differentiated cells were labeled in the larval central nervous system. Each cell type, such as

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Rohon-Beard neurons, originated from more than one ancestral cell. In general, the relationship between ancestry and cellular phenotype was never found to be one-to-one but always many-to-many (Jacobson, 1981a). Even the Mauthner neuron, only one of which differentiates on each side of the brain, did not originate from the same lineage in all cases but could originate from any one of a group of blastomeres in the 64-cell embryo (Jacobson and Hirose, 1981).

Such observations raised many problems whose solutions require further analysis of clones originating at progressively later cleavage stages. One class of problems is concerned with the relationships between individual ancestral cells and their clonal domains, for example, the factors affecting position and size of the domains and mingling of cells in and between clonal domains. Another class of problems deals with the relationships between cell ancestry and phenotype, for example, the stages of development at which lineages of nerve cells diverge from those of other types of cells and the ways by which lineages of different types of nerve cells diverge from one another.

As a contribution to the solution of those problems, this paper describes the clones in the central nervous system originating from single blastomeres labeled with HRP at 128-, 256-, and 512-cell stages. Distribution of labeled cells outside the central nervous system occurred in all cases, but those are dealt with elsewhere (M. Jacobson, submitted for publication). This is a prospective analysis of clones initiated at the aforementioned cleavage stages and terminated at early larval stages when the central nervous system and many of its constituent neurons are well differentiated. For the most part this is a qualitative study, leaving to succeeding papers the start of a quantitative clonal analysis of specific types of neurons (M. Jacobson and S. Moody, submitted for publication).

Materials and Methods

Embryo production and treatment. *Xenopus laevis* embryos were obtained from matings induced by injection of human chorionic gonadotropin. When embryos reached the two- or four-cell stage their jelly coat was removed by immersion for 4 min in 9 mM dithiothreitol, pH 8.9 (Kirschner et al., 1980), followed by three washes in 100% Steinberg solution. Embryos were selected with well marked pigment gradients in animal-vegetal and dorsal-ventral axes and with regular and symmetrical patterns of cleavage (Jacobson, 1981a). Further selection of embryos with well defined pigment gradients was made when they reached 128-, 256-, and 512-cell stages, and some of these were sectioned serially to count the cells and see how many were at the surface (see "*Controls*").

HRP injection. An intracellular injection of horseradish peroxidase solution (type IX, Sigma; about 10% in distilled water, 1 to 2 nl) was given by pressure lasting a few seconds into a selected blastomere by means of a glass micropipette with a tip diameter of 1 to 5 μm. The position, size, and shape of each injected blastomere on the surface of the spherical blastula was mapped on a plane by using Lambert's azimuthal equal area projection (see below). The embryo was immersed in 100% Steinberg solution during the injection and for about 1 hr later, after which the solution was gradually diluted to 20% with distilled water over a period of several hours. Embryos were kept in 20% Steinberg solution until they reached larval stages 30 to 39 (Nieuwkoop and Faber, 1967) and were then processed as described below.

Histochemistry. Embryos were fixed for 6 hr at 4°C in 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, and washed twice in sucrose solution (5% for 6 to 24 hr and 15% for 1 to 2 hr, both in 0.1 M phosphate buffer, pH 7.4, at 4°C). Frozen sections were cut at 24 μm, mounted on subbed slides, and soaked for 10 min in 0.1 M phosphate buffer, pH 7.4, containing 12.5 mg of diaminobenzidine tetrahydrochloride/100 ml of solution, after which hydrogen peroxide (0.15%, 0.25 ml/100 ml of soaking solution) was added. The reaction was monitored at intervals until a brown reaction product, which will be referred to as the "label," became visible under the microscope (5 to 10 min); the slides were then washed, dehydrated, and coverslipped for further study.

Controls. These were the same as previously reported (Jacobson and Hirose, 1978, 1981; Hirose and Jacobson, 1979). Embryos processed shortly after the intracellular injection of HRP showed that the label was confined to the injected blastomere or its daughter cells. In cases in which all blastomeres had been injected with 1 to 3 nl of HRP at two- or four-cell stages, the label was present in all cells at larval stages 30 to 39. This showed that the enzyme activity of the HRP was sufficiently high to be detectable in all descendants of the injected blastomere. Such fully labeled larvae developed normally when compared with uninjected controls. In addition, 24 embryos were fixed at intervals, starting immediately after the injection at the 128-cell stage and continuing to the onset of gastrulation (stage 10 of Nieuwkoop and Faber, 1967) to determine the positions and numbers of labeled cells.

Choice of map projection. It is impossible to preserve all spatial relationships in a projection of a sphere on a flat surface so that, for constructing a fate map, a projection should be chosen in which the important spatial relationships are preserved, namely relative positions, areas, and distance from some recognizable landmark, such as the animal pole. A photograph, which is nearly the same as an orthographic projection, is not a good choice because it results in area deformation which increases with eccentricity from the center point. I have used Lambert's equal area azimuthal projection (Fig. 1; Lambert, 1772) because, although angular relations are distorted, areas are preserved in all parts of the map and distances are accurately represented along lines radiating from the center (Robinson and Sale, 1969).

Topological analysis. All embryos were studied histologically and 16 cases were selected for making camera lucida drawings of the spatial distribution of all labeled cells in a complete series of sections through the CNS, although the most caudal part of the spinal cord was not included because of its undifferentiated state. These drawings were used for making three-dimensional reconstructions and for the graphical reconstruction procedure known as topological analysis (Nieuwenhuys, 1974; Opdam et al., 1976). Details of the method are given in Jacobson and Hirose (1981). The main value of the

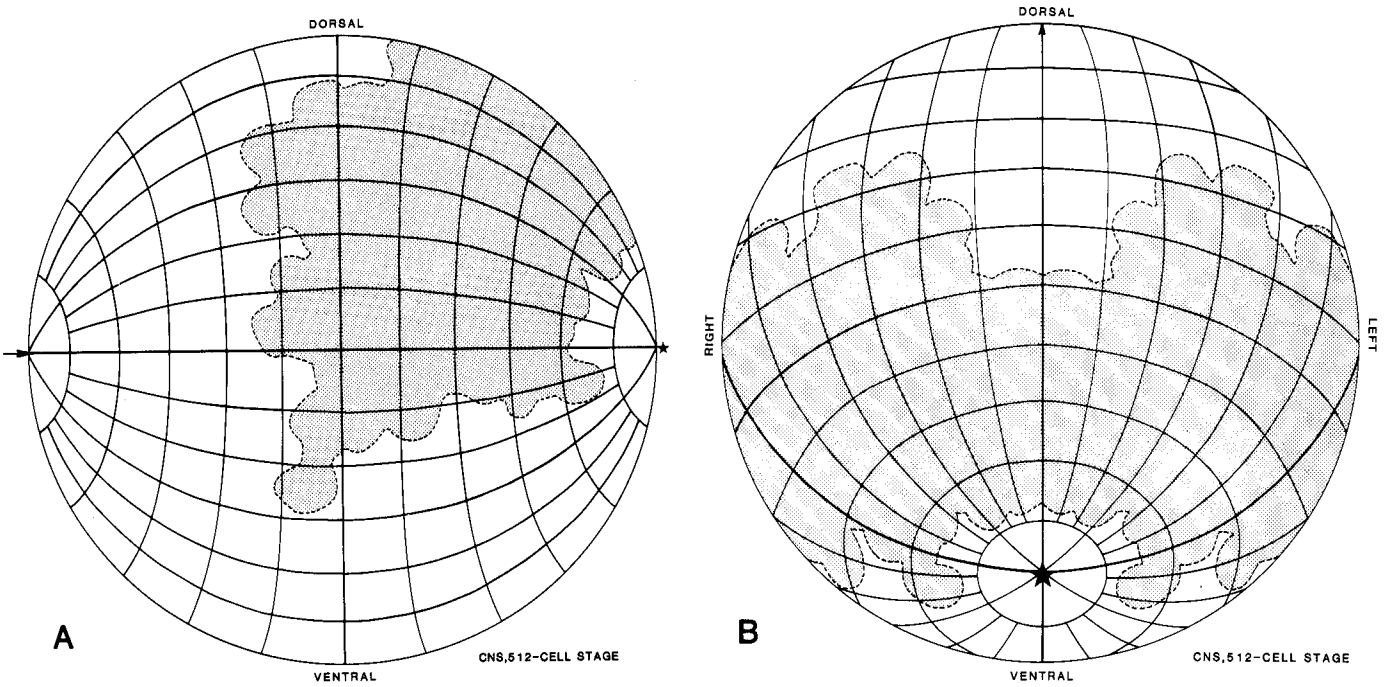


Figure 1. Lambert's equal area azimuthal projection grid, right lateral case (*A*) and anterior oblique case centered on the midline 60° above the horizontal meridian (*B*). *Stippling* shows the extent of the region in the 512-cell blastula that contributes to the central nervous system. The *star* is at the animal pole, the *arrow* points to the vegetal pole. *Right, left, dorsal,* and *ventral* refer to the axes of the blastula.

topological maps is that they depict labeled cells projected onto the plane of the opened-out ventricular surface. Each labeled cell is thus shown at or close to the position from which it had originated in the ventricular germinal zone.

Results

Serial sections of embryos killed before gastrulation showed that at the 128-cell stage all cells were accessible at the surface. Starting at the 128-cell stage, cleavages tangential to the surface resulted in an increasing number of cells completely below the surface and, therefore, not directly accessible to the injection pipette. At the 512-cell stage about half the cells were visible at the surface. The descendants of a single cell at the surface, injected at the 128-cell stage, formed a coherent group extending from the surface to deeper layers at the 1024-cell stage. After that stage increasing mingling of labeled and unlabeled cells and movement of groups of cells and individual cells away from the surface was seen before the onset of gastrulation; this was also observed in earlier experiments (Jacobson and Hirose, 1981; Jacobson, 1982).

Of 301 embryos injected with HRP at 128-, 256-, and 512-cell stages and allowed to develop beyond gastrulation, 32 died or developed abnormally, another 27 developed well but were processed badly, and the remaining 242 embryos (66 at 128 cells; 85 at 256 cells; 91 at 512 cells) were normal and were successfully processed. Labeled cells were distributed in various tissues outside the nervous system in all cases, but only the pattern of labeling in the central nervous system and some neural crest derivatives will be described here. However, it should be noted that there was a high measure of concordance between the spatial distribution of cells of a clone in the CNS and cells belonging to the same clone in other parts of the organism.

No significant differences were found between the left and right sides so that figures will show the blastomere sizes and positions projected on the right side of the embryo, using Lambert's equal area azimuthal projection.

Results that were independent of stage and position of the injected blastomere

Before describing the results that were related to the position of the injected cell and to the stage at which the labeling was started, a number of results will be described that were independent of those two factors. Injection of any single blastomere at the 128-, 256-, or 512-cell stages always resulted in labeling of many cells of many types in many organ systems at the larval stages. The types of cells that developed from a particular blastomere at the 128-cell stage were the same as the types of cells that developed from blastomeres at the same positions in the embryo at the 256- or 512-cell stages. These observations showed that no phenotypic restrictions had occurred in any of the lineages that were examined. The blastomeres that gave rise to parts of the central nervous system were multipotential cells that also gave rise to cells in ectoderm, endoderm, and mesoderm.

These observations apply only to cells at the surface of the embryo. However, in spite of the fact that an increasing proportion of the cells at the 256- and 512-cell stages took up positions beneath the surface of the embryo, there seemed to be no loss of differentiated phenotypes in the clones that arose from the surface cells at the 512-cell stage as compared with those that arose from the

cells at the 128-cell stage. The label persisted and could be recognized in many types of differentiated cells, including nerve cells. Although a relatively insensitive HRP technique was used, the label could be detected after an estimated 10 to 15 cell divisions and after dilution of the label must have occurred as a result of its transport into outgrowing axons and dendrites.

Some labeled neurons had developed an axon and dendrites which were also filled with the label. Such labeled axons could be traced for considerable distances in the CNS as well as into peripheral tissues (Fig. 2). This shows that the intracellular HRP did not affect normal neuronal differentiation.

Another feature that was independent of the stage of initial labeling or of the position of the labeled ancestral cell was the dispersal of the labeled descendant cells into an extensive region of the organism. After labeling an individual blastomere, its descendants were dispersed, mingled with unlabeled cells, in a defined region which we have called a clonal domain (Hirose and Jacobson, 1979).

A major feature of the fate maps was that each type of neuron, for example, Rohon-Beard neurons or spinal motoneurons, was found to originate from a group of blastomeres. Each blastomere in the group gave rise to a small fraction of the total population of that type of neuron (Figs. 3, 4*B*, 11*B*, and 12*B*). Moreover each group of blastomeres gave rise to many different types of neurons all within the same area of the nervous system. Therefore, one may refer to the group of Rohon-Beard ancestral cells as being the same as the group of ancestral cells that gave rise to the dorsal half of the spinal cord. Similarly, a group of ancestral cells located near the animal pole close to the dorsal midline of the 512-cell embryo gave rise to the structures in the ventral parts of the telencephalon, diencephalon, mesencephalon, and retina. Injection of any individual blastomere within this group resulted in labeling of cells of many phenotypes distributed in all the structures that originate from the group as a whole. Organization of the fate map on the basis of groups of blastomeres, the descendants of each group populating a separate region of the nervous system, was incomplete at the 128- and 256-cell stages and only became well established at the 512-cell stage.

Results that were dependent on the stage and position of the injected blastomere

Changes were observed in the fate maps during the 128- to 512-cell stages. During that time, lasting about 2 hr, there was a coherent movement of blastomeres away from the animal pole. For example, blastomeres that gave rise to Rohon-Beard neurons moved from their positions at the 64-cell stage ventral to the horizontal meridian extending from animal pole to the equator (Jacobson and Hirose, 1981) to take up positions midway between animal and vegetal poles at the 128-cell stage and to move progressively more dorsally during the 256- and 512-cell stages. This movement can be seen by comparing Figures 4*B*, 11*B*, and 12*B*, which show the number of Rohon-Beard neurons that descended from individual blastomeres.

The position of the clonal domain in the larval CNS was related in a regular manner to the positions of the injected blastomeres, so that a fate map could be deduced from these results (Figs. 3, 4, 11, and 12). The conventions used for constructing the fate maps shown in these figures were the same as those used by Jacobson and Hirose (1981). A record was made of the spatial distribution of labeled cells in the CNS and the types of cells, where they could be identified. The regions of CNS in which labeled cells were located were indicated by symbols on the profiles of the ancestral blastomeres. Because of the high density of these symbols, it was not feasible to indicate the relative numbers of labeled cells by using symbols of different sizes as was done by Jacobson and Hirose (1981). All the labeled cells were in the indicated regions in most cases. In a few cases a single labeled cell or a few cells were not in the same region as the large majority. When hundreds or thousands of labeled cells were located in one region of the CNS and a few isolated cells were located at considerable distances from the large body of labeled cells, only the positions of the majority of the cells were represented on the map. The question of such "aberrant" cells will be discussed later.

A striking feature of these maps was that the descendants of groups of blastomeres shared the same regions of CNS. This did not change over the dimensions of these groups of blastomeres but changed abruptly when moving from one group of blastomeres to another. These transitions were very abrupt, over the distance of a single cell diameter or less, at the 512-cell stage (Figs. 3, 4*B*, 5, and 6). The transitions were more gradual, over several cell diameters, at the 128- and 256-cell stages (Figs. 11*B* and 12*B*).

The abrupt transitions are illustrated in Figures 5 and 6, which show the distribution of CNS descendants of blastomeres that were neighbors but were in different blastomere groups. Figure 5 shows the clones that descended from blastomeres 13 and 24 (Fig. 4*A*) in the anterior-median (AM) and anterior-lateral (AL) groups, respectively. Blastomere 13 contributed descendants to ventral regions of both sides of the telencephalon, diencephalon, retinae, and mesencephalon, but entirely different regions were occupied by descendants of blastomere 24. The latter contributed its descendants only to the same side of the CNS, namely to dorsal regions of the telencephalon, diencephalon, retina, and mesencephalon. These regions were also populated by the descendants of all the other blastomeres of the AL group, for example, blastomere 47 (Figs 4*A* and 6). Figure 6 shows the spatial distribution of CNS descendants of blastomere 47 in the AL group and its neighbor, blastomere 66 in the posterior-lateral (PL) group. The descendants of these two blastomeres occupied entirely different regions of the CNS: descendants of 47 were distributed in the dorsal telencephalon, diencephalon, retina, and mesencephalon ipsilaterally, and descendants of 66 were in the dorsal regions of rhombencephalon and spinal cord on the same side (Fig. 6).

512-Cell stage. Ninety-one blastomeres were injected at the 512-cell stage and successfully processed. Of these, 58 contributed some descendants to the central nervous system as well as to other systems, and 33 did not contribute to the CNS. Blastomeres that contributed to the CNS occupied most of the dorsal half of the animal hemisphere and they also extended a short distance

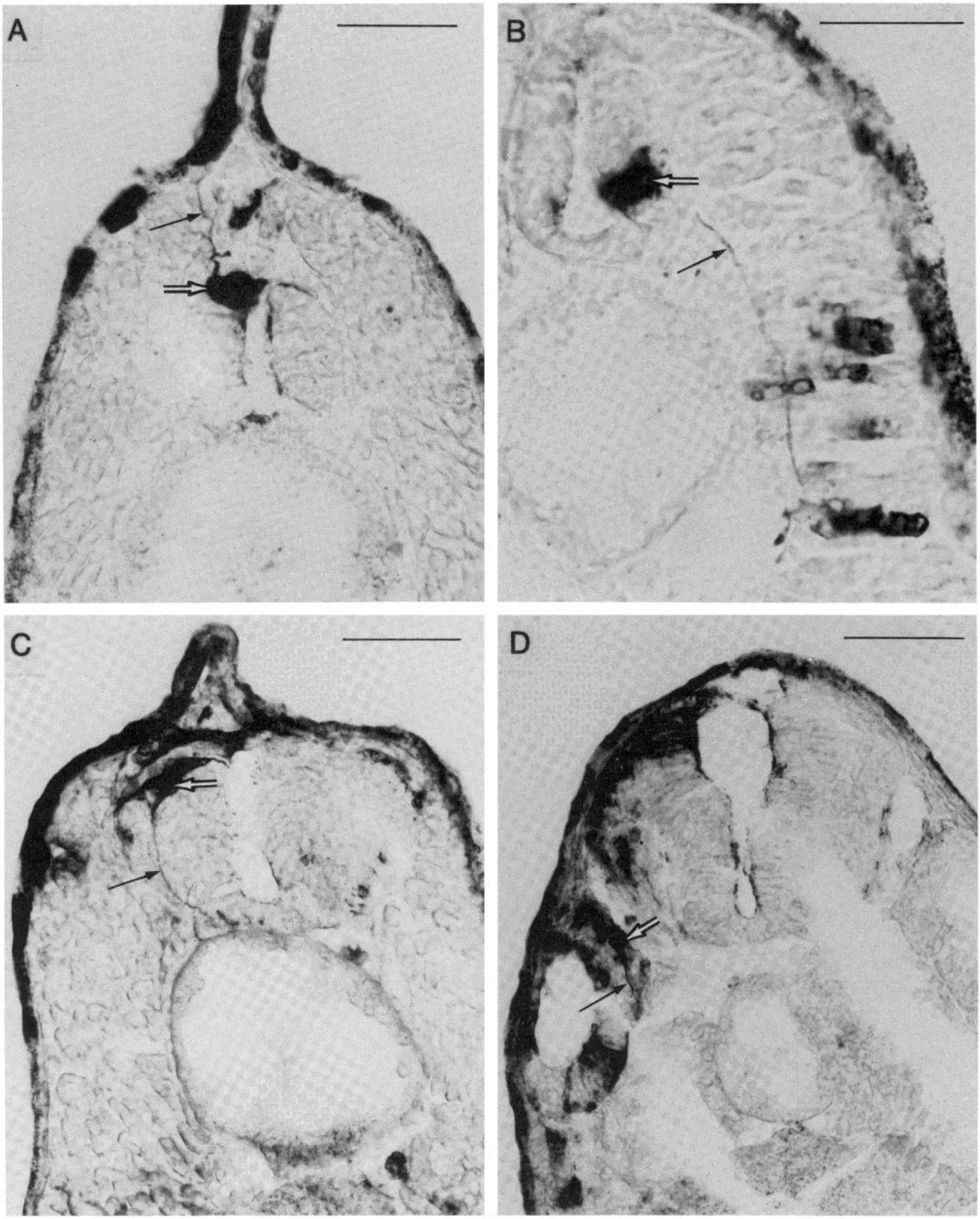
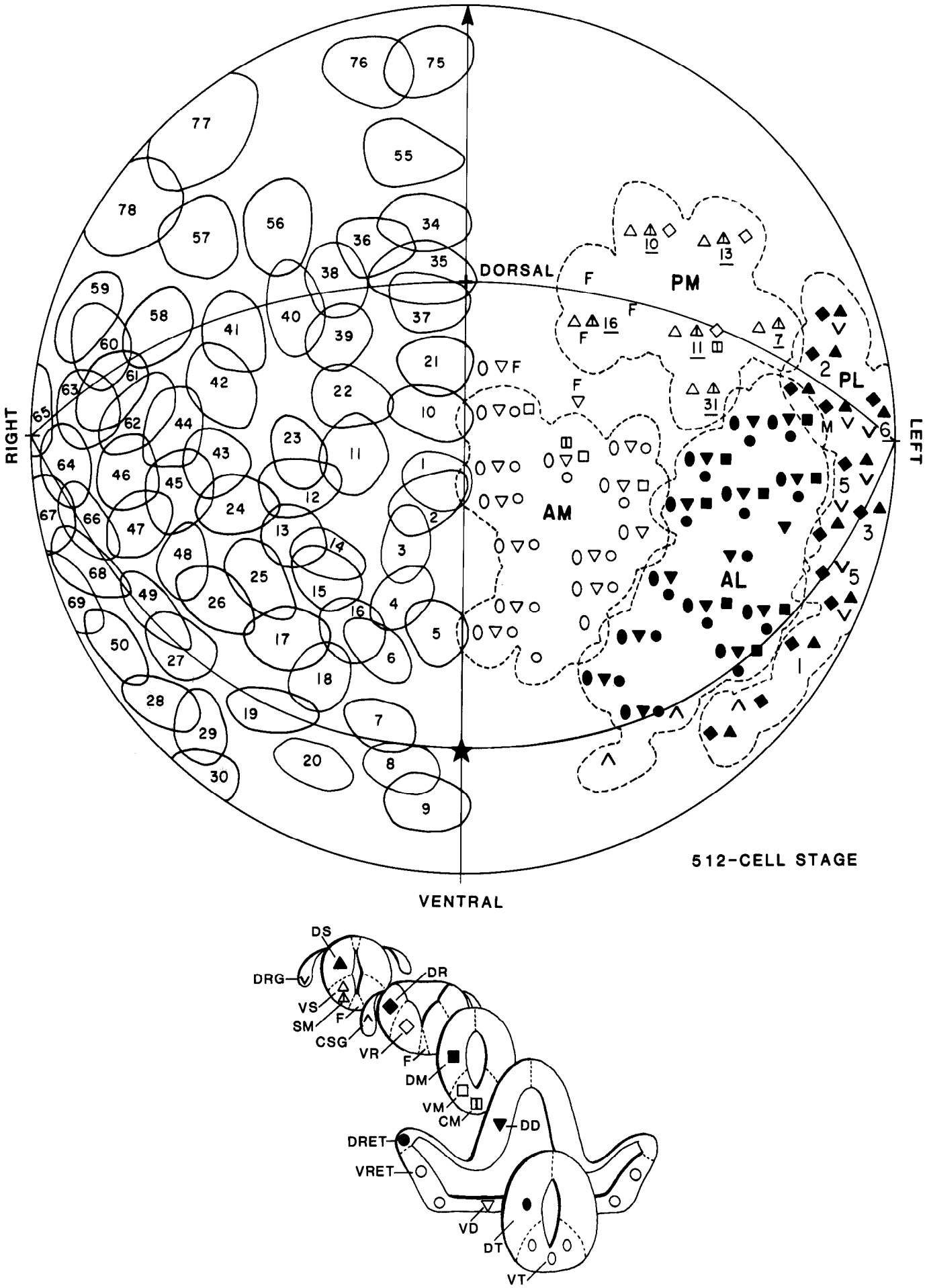


Figure 2. Labeling of axons growing out of neurons that had inherited the label from their ancestral cells. *White arrows* show neuron cell bodies, and *black arrows* show axons. *A*, Labeled Rohon-Beard neuron and its peripheral neurite at larval stage 32 after injection of HRP into blastomere 138 at the 256-cell stage. *B*, Labeled spinal motoneuron and its axon at larval stage 31 after injection of HRP into blastomere 57 at the 512-cell stage. *C*, Labeled commissural neuron in the spinal cord at larval stage 32 after injection of HRP into blastomere 69 at the 512-cell stage. *D*, Labeled neurons of the VIII nerve ganglion and their axons at larval stage 32 after injection of HRP into blastomere 136 at the 256-cell stage. *Bars* are 50 μm. Blastomere numbers refer to Figures 4*A* and 11*A*.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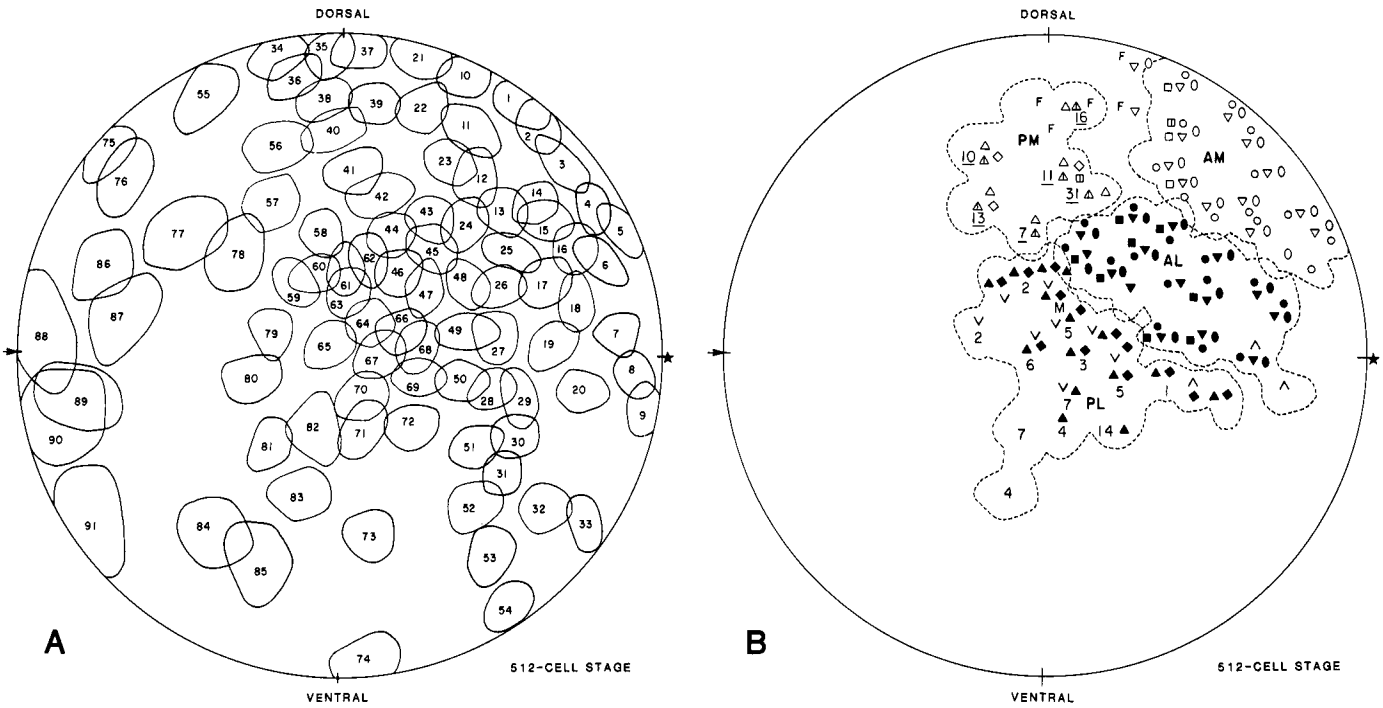

Figure 4. Fate map (right lateral case) of blastomeres of the 512-cell stage that contributed descendants to the CNS. Positions of all blastomeres injected at the 512-cell stage are shown in *A*. Each blastomere is identified by a number. The fate map (*B*) shows the regions of CNS populated by descendants of blastomeres that contributed to the CNS. Symbols for CNS regions are shown in the *bottom part* and legend of Figure 3. Groups of blastomeres that all contributed to the same CNS regions are enclosed by *dashed lines*. Underlined numbers in the PM group are numbers of primary spinal motoneurons that originated from individual blastomeres at the positions occupied by the numbers. *Numbers* in the PL group are the numbers of Rohon-Beard neurons that originated from individual blastomeres at the indicated positions.

ventral to the horizontal meridian and across the equator into the vegetal hemisphere (Figs. 1, 3, and 4).

Blastomeres that contributed descendants to certain regions of the CNS were grouped together in the same part of the 512-cell blastula. Seven such ancestral cell groups were seen. These groups of blastomeres formed a bilaterally symmetrical pattern with three groups on each side of the dorsal midline and one in the anterior median position near to the animal pole (Figs. 3 and 4).

The unpaired *anterior-median group* of blastomeres was located in the roof of the blastocele dorsal to the animal pole. This ancestral cell group consisted of about 26 blastomeres visible at the surface of the blastula. However, it should be recognized that at the 512-cell stage the ectodermal layer is several cells thick in *Xenopus*, and the total number of blastomeres belonging to this or any of the other groups cannot be determined from these results which deal only with the blastomeres that were visible at the surface of the embryo. The unique feature of the anterior-median group was that every blastomere contributed to both sides of the brain. After injecting a single blastomere in this group, the labeled cells were mingled with unlabeled cells in an extensive region extending bilaterally and including ventral retina, septal area, striatum, hypothalamus, and ventral mesencephalon (Figs. 5 and 8). No descendants of this group were found caudal to the third nerve nucleus or in the dorsal regions of the brain.

Figure 3. Fate map (anterior oblique case) of blastomeres of the 512-cell stage that contributed descendants to the CNS. Blastomere positions are shown on the right side of the embryo and CNS regions to which each blastomere contributed are shown on the left side of the embryo and in the lower diagram of a series of transverse sections through the larval CNS. Groups of blastomeres that all contributed descendants to the same CNS regions are enclosed by *dashed lines*. Blastomere groups are: *AM*, anterior-median; *AL*, anterior-lateral; *PM*, posterior-median; *PL*, posterior-lateral. *Numbers* on the right half of the embryo identify each blastomere (see Fig. 4*A*). *Numbers* on the left half of the embryo are numbers of Rohon-Beard neurons that originated from each blastomere in the PL group and the numbers of primary spinal motoneurons that originated from each blastomere in the PM group (*underlined numbers*). *CM*, cranial motoneurons; *CSG*, cranial sensory ganglia; *DD*, dorsal diencephalon; *DM*, dorsal mesencephalon; *DRG*, dorsal root ganglia; *DR*, dorsal rhombencephalon; *DRET*, dorsal retina; *DS*, dorsal spinal cord; *DT*, dorsal telencephalon; *F*, floor plate; *M*, Mauthner neuron; *SM*, spinal motoneurons; *VD*, ventral diencephalon; *VM*, ventral mesencephalon; *VR*, ventral rhombencephalon; *VRET*, ventral retina; *VS*, ventral spinal cord; *VT*, ventral telencephalon. The *star* is at the animal pole, and the *arrow* points to the vegetal pole. Diameter of the blastula is approximately 1.2 mm. *Open ellipse*, ventral telencephalon; *solid ellipse*, dorsal telencephalon; *open circle*, ventral retina; *solid circle*, dorsal retina; *open inverted triangle*, ventral diencephalon; *solid inverted triangle*, dorsal diencephalon; *open square*, ventral mesencephalon; *solid square*, dorsal mesencephalon; *open diamond*, ventral rhombencephalon; *solid diamond*, dorsal rhombencephalon; *open triangle*, ventral spinal cord; *solid triangle*, dorsal spinal cord; *V*, trunk neural crest; Λ, cranial neural crest.

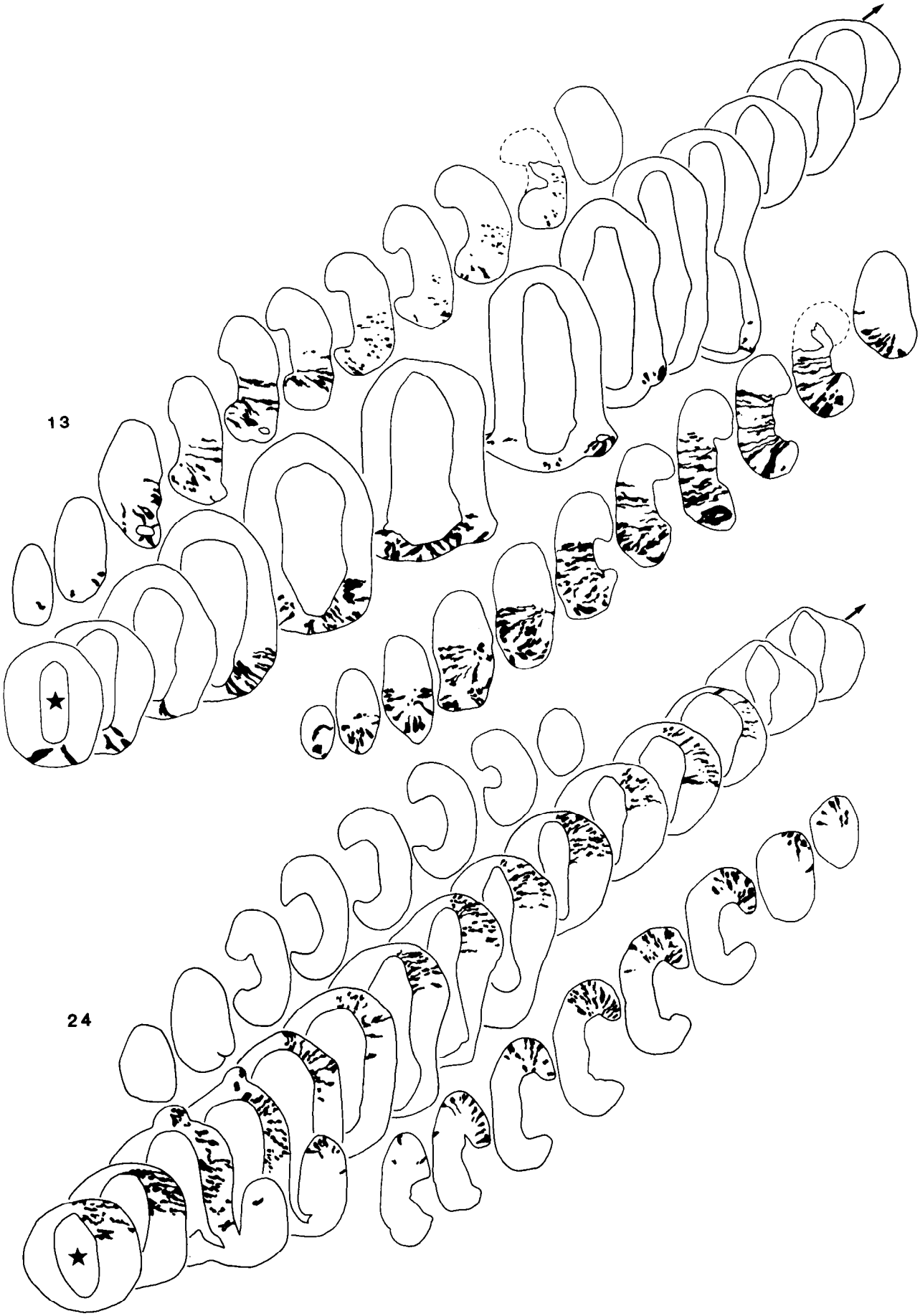

Figure 5. Reconstructions of every coronal section through the retinae and CNS (except caudal spinal cord) of two specimens after labeling individual blastomeres at the 512-cell stage. In the *upper specimen* the injection was into blastomere 13 and in the *lower specimen* into blastomere 24 (Fig. 4*A*). Labeled descendants are shown in *black*. The *star* is at the rostral telencephalon, and the *arrow* points to the caudal part of the spinal cord.

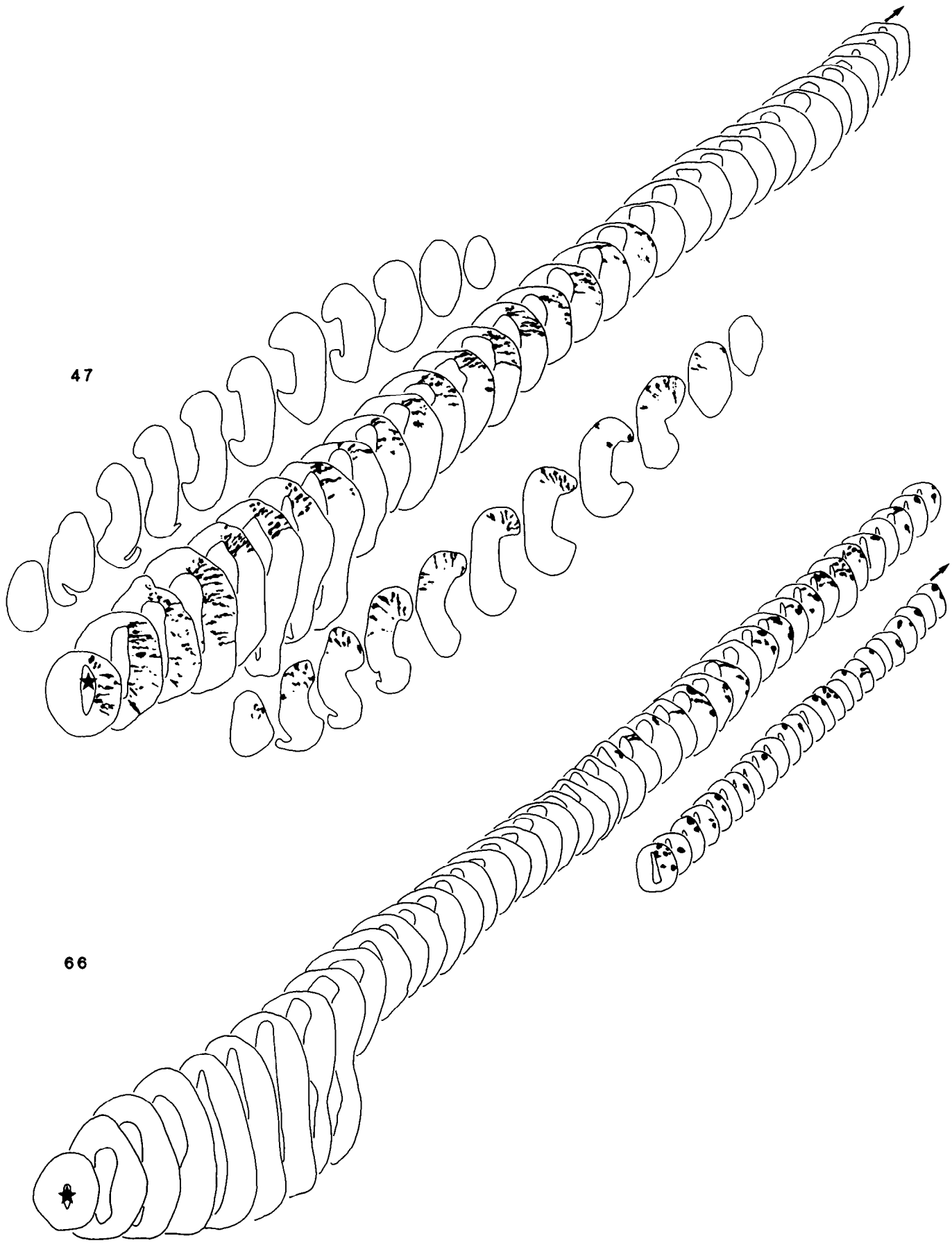

Figure 6. Reconstruction of every coronal section through the CNS (except caudal spinal cord) of two specimens after labeling individual blastomeres at the 512-cell stage. In the *upper specimen* the injection was into blastomere 47 and in the *lower specimen* into blastomere 66 (Fig. 4*A*). Labeled cells are shown in *black*. The retina containing labeled cells is shown in the upper specimen but not in the lower one because the latter contained no labeled cells. The *star* is at the rostral telencephalon, and the *arrow* points to the caudal part of the spinal cord.

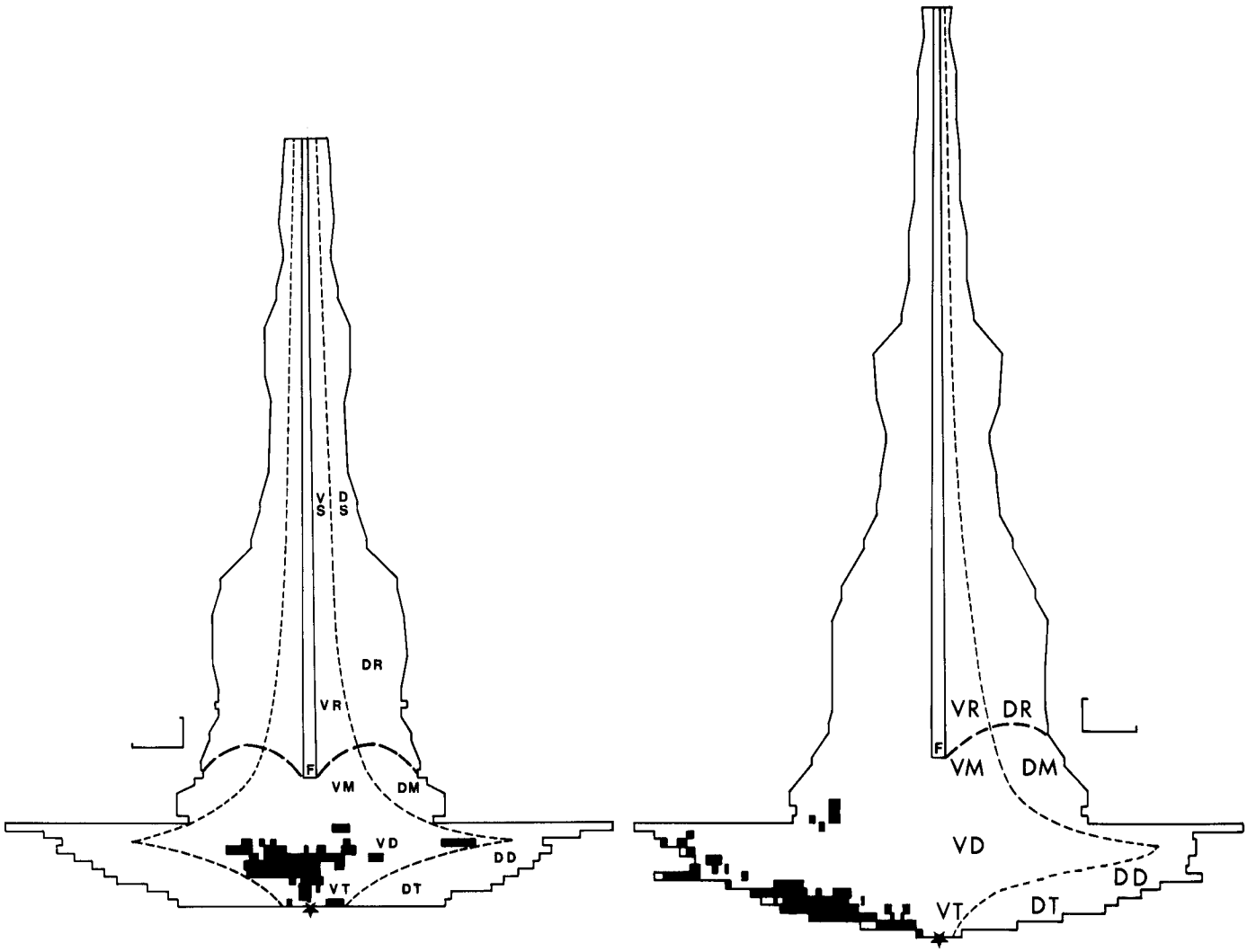

Figure 7. Topological maps showing the distribution of labeled cells (*black*) in the CNS of *Xenopus* at stage 39 after injection of blastomere 5 (*left map*) and blastomere 26 (*right map*), both at the 512-cell stage. The CNS is shown as if opened out like a book along the dorsal midline with the ventral midline forming the axis of symmetry. The *star* is at the lamina terminalis of the CNS. The *light dashed line* shows the location of the boundary zone between dorsal and ventral CNS regions. The *heavy dashed line* shows the boundary zone between mesencephalon and rhombencephalon. *Scale*, 100 μm. Abbreviations are as in Figure 3.

Another two groups, consisting of about 16 surface blastomeres in each group, were located on each side in bilaterally symmetrical positions in the dorsal lateral wall of the blastocele extending from near the animal pole to the equator (Figs. 3 and 4). These were called the *anterior-lateral ancestral cell groups.* Each of these groups contributed descendants exclusively to structures on the same side of the brain. After labeling any one of the blastomeres in either the left or right anterior-lateral groups, labeled descendants were scattered and mingled with unlabeled cells in the dorsal telencephalon, olfactory bulb, accessory olfactory bulb, dorsal diencephalon (including epiphysis, epithalamus, frontal organ, thalamus), dorsal mesencephalon, and the dorsal part of the retina (Figs. 5, 6, 9, and 10). All these labeled descendants were on the same side as the originally injected blastomere. In addition, labeled cells were seen in cranial neural crest derivatives, including cranial sensory ganglia, mainly on the same side but with a few labeled cells on the opposite side. The blastomeres of the anterior lateral group also contributed cells to the olfactory placode, frontal organ, ventral half of the otic vesicle, and the lens. The thin outer wall of the optic cup, which forms pigmented epithelium, as well as the dorsal part of the retinal margin and the lens, were derived from the anterior-lateral blastomere group on the same side. By contrast, the ventral two-thirds of the retina, the ventral wall of the optic stalk, and chiasma ridge were derived from the anterior-median blastomere group, which never contributed descendants to the lens.

Another two groups of blastomeres, each of which contributed descendants exclusively to the dorsal rhombencephalon and dorsal spinal cord on the same side, were situated on each side of the blastula on the equator mostly below the horizontal meridian. These were called the *posterior-lateral ancestral cell groups* (Figs. 3, 4, and 6). Each of these groups consisted of about 16 blastomeres visible at the surface. After labeling any of those blastomeres at the 512-cell stage the labeled descendants were distributed, mingled with unlabeled cells, in an

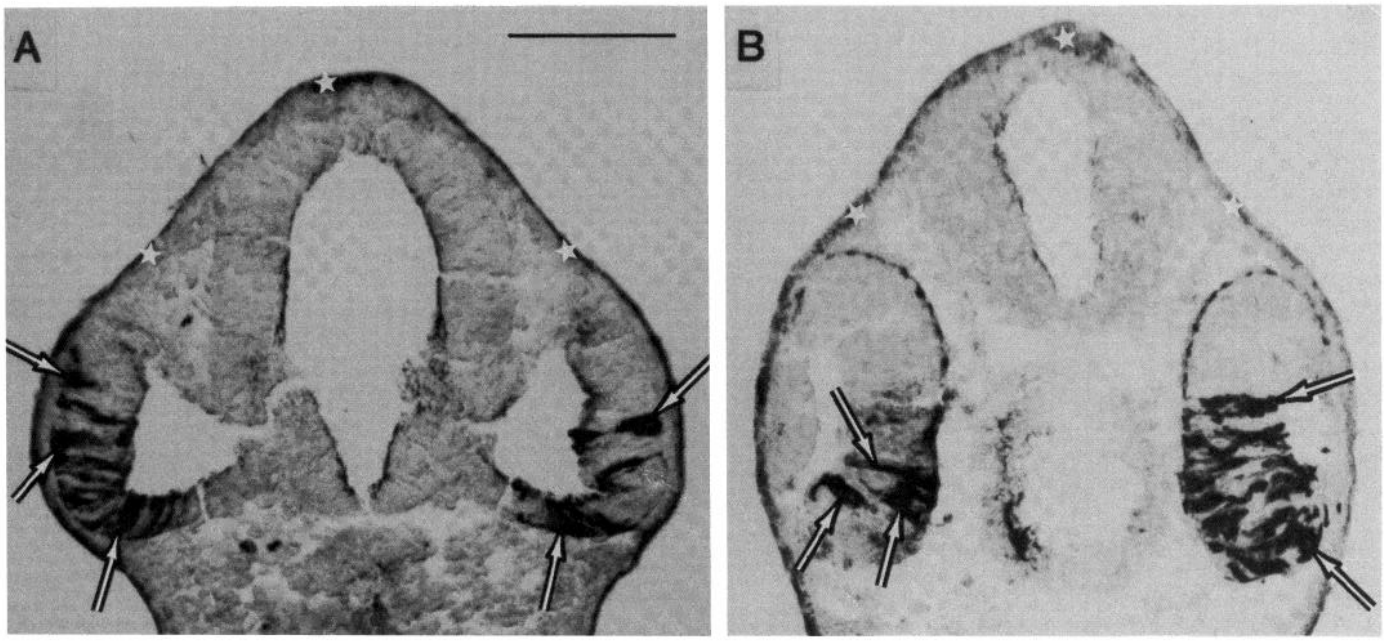

Figure 8. Labeling of cells in the ventral regions of both retinae at larval stages after injection of HRP into a single blastomere of the anterior-median group. *White arrows* indicate some of the labeled cells. *Stars* indicate some unlabeled cells containing melanin. *A*, At larval stage 30 after injection into blastomere 93 (Fig. 11) at the 256-cell stage. This shows that even at the 256-cell stage certain blastomeres gave rise to clones that were restricted to a single compartment. *B*, At larval stage 34 after injection into blastomere 4 (Fig. 3) at the 512-cell stage. Coronal section. *Scale*, 250 μm.

extensive region of dorsal metencephalon, myelencephalon, spinal cord, and the associated neural crest. Many types of cells were labeled (Fig. 2, *A*, *C*, and *D*), including commissural neurons with the cell body located in the dorsal lateral rhombencephalon and spinal cord and the axon projecting across the ventral midline to the ventral lateral funiculus on the opposite side (Fig. 2*C*), Rohon-Beard neurons (Fig. 2*A*) and other neurons in the dorsal part of the spinal cord, neurons of the spinal dorsal root ganglia, and ganglia of cranial nerves (Fig. 2*D*).

Different types of labeled neurons could be identified and counted, and the results of such a quantitative clonal analysis will be published separately (M. Jacobson and S. Moody, submitted for publication). For the present purposes the counts of the numbers of labeled Rohon-Beard neurons and labeled spinal motoneurons that originated from individual blastomeres are shown in Figures 3, 4*B*, 11*B*, and 12*B*. It was found that the average number of Rohon-Beard neurons and spinal motoneurons that originated from individual labeled blastomeres of any two successive generations, such as 256- and 512-cell stages, diminished by less than one-third. These results showed that most of the descendants of each successive generation remained in the Rohon-Beard neuron lineage and motoneuron lineage. It should also be noted that these two lineages were completely separate at the 512-cell stage, that is, they originated from different ancestral cell groups (Fig. 4*B*).

The Mauthner neuron was labeled in only one case, namely after injecting blastomere 63 belonging to the posterior-lateral blastomere group. Most of the cells of the otic vesicle were derived from the descendants of the posterior-lateral group in the same side (Fig. 2*D*). Each posterior-lateral blastomere group contributed to its own side of the central nervous system, but some cells of neural crest origin were distributed to the opposite side.

Another two blastomere groups were located on the equator close to the dorsal midline. These were called the *posterior-medial ancestral cell groups* and consisted of about 15 surface blastomeres in each group (Figs. 3 and 4*B*). Labeling of any single blastomere in this cell group resulted in labeled descendants scattered through a large part of the ventral metencephalon, ventral myelencephalon, and ventral spinal cord, including the floor plate and primary spinal motoneurons (Fig. 2*B*). All the labeled descendants were on the same side as their ancestral cell.

Blastomeres belonging to the same group had large overlaps of their clonal domains but, with two exceptions, did not overlap with the clonal domains of blastomeres belonging to neighboring cell groups (Figs. 5 and 6). Of the 58 blastomeres of the 512-cell stage that contributed descendants to the CNS, there were only two cases (numbers 21 and 22, Fig. 4) in which the individual clonal domains extended into the region of CNS occupied by descendants of the neighboring ancestral cell group. This was in contrast with the results obtained after labeling was started at the 256-cell stage.

256-Cell stage. Of the 85 blastomeres that were successfully injected at the 256-cell stage, 56 contributed descendants to the CNS (Fig. 10). These occupied the same region of the embryo as the blastomeres at the 512-cell stage that contributed to the CNS. The same types of cells differentiated in clones originating at the 256-cell stage as at the 512-cell stage, and it was possible to do a quantitative clonal analysis of Rohon-Beard neurons and primary spinal motoneurons that descended from individual blastomeres of the 256-cell stage (M. Jacobson

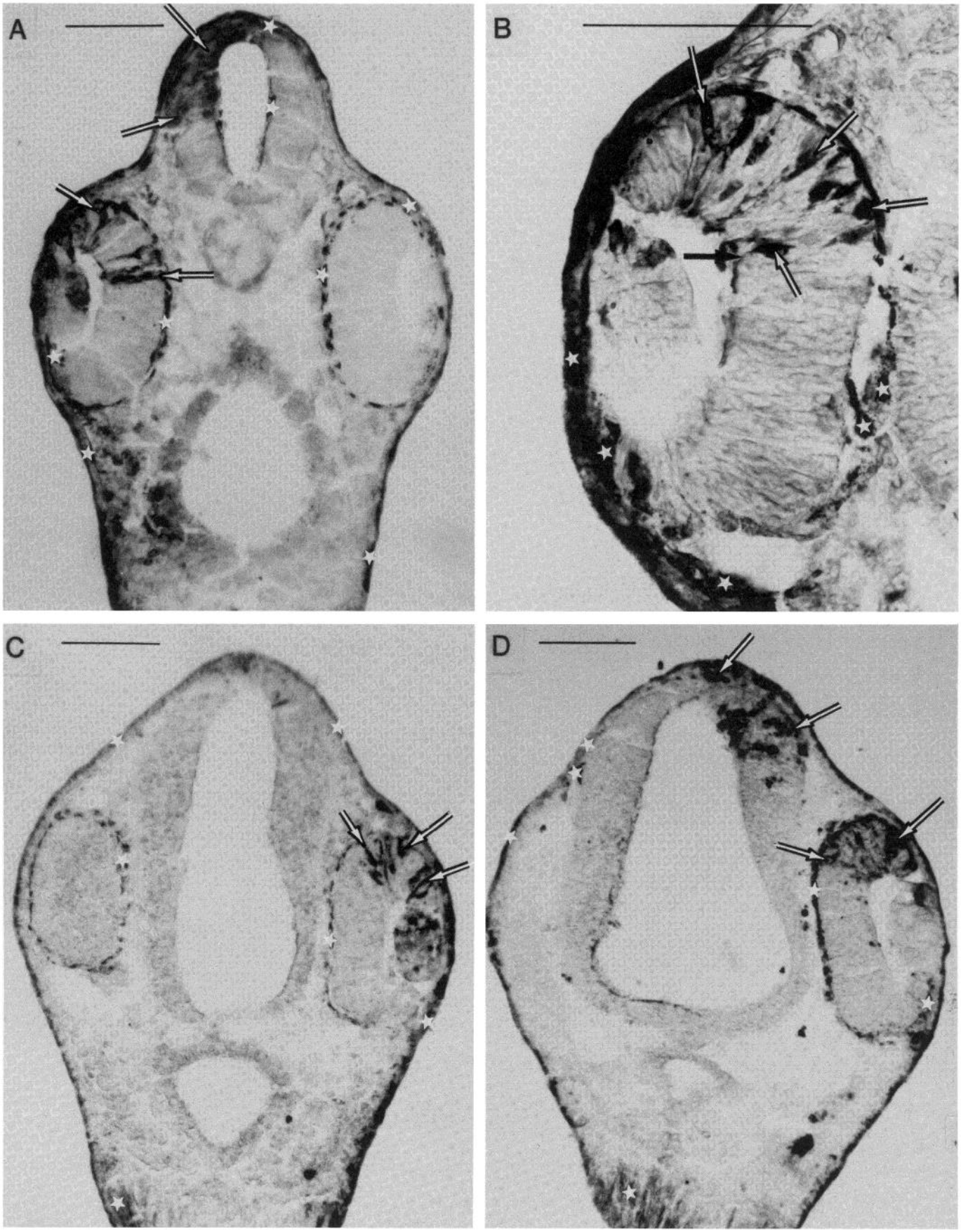

Figure 9. Labeling of cells in the dorsal retina and CNS ipsilaterally after injection of HRP into a single blastomere of the anterior-lateral group at the 512-cell stage. Some labeled cells are indicated by *white arrows*. *Black arrow* points to labeled retinal ganglion cell and optic axon in *B*. *Stars* show some unlabeled cells containing melanin, which was easily distinguishable

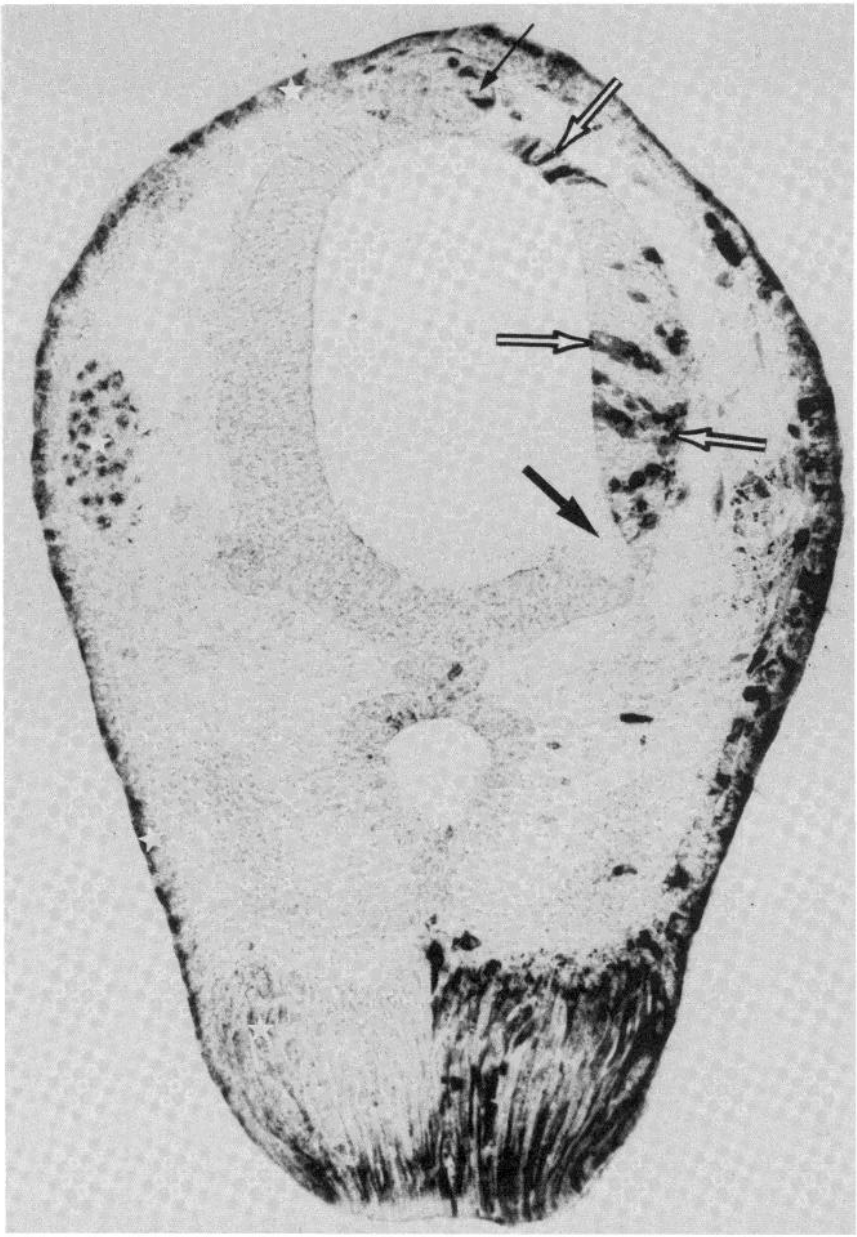

Figure 10. Coronal section through the head at the level of the diencephalon of a larval *Xenopus* at stage 35 that had received an injection of HRP into blastomere 18 at the 512-cell stage. Labeled and unlabeled cells are mingled in the right side of the diencephalon dorsal to the beginning of the optic stalk (*large black arrow*) and in the right half of the epiphysis (*small black arrow*). *White arrows* indicate some labeled cells. *White stars* indicate some unlabeled cell containing melanin. *Scale*, 200 μm.

and S. Moody, submitted for publication). The Mauthner neuron was labeled in two specimens (numbers 136 and 147, Fig. 11*A*).

Distribution of the clones originating from blastomeres at the 256-cell stage was different, in many cases, from the spatial distribution of clones that descended from blastomeres at the same position in the 512-cell embryo. There were few blastomeres of the 256-cell stage (shown enclosed by *dashed lines* in Fig. 11*B*) whose descendants were restricted to the same regions of the CNS as the descendants of similarly positioned blastomeres of the 512-cell stage. Surrounding those blastomeres at the 256-

from the label in the preparation. Labeled and unlabeled cells were mingled in the dorsal part of the retina, whereas the ventral part of the same retina was completely unlabeled. *A*, Coronal section through the head at dimesencephalic level. Dorsal mesencephalon, dorsal retina, and lens are labeled on the left side. Another view of this specimen (27 in Fig. 4) at higher magnification is shown in *B*. *C* and *D*, Labeling of dorsal retina and lens on the same side as the injected blastomere in two different specimens (18 and 19 in Fig. 4) at stage 35 after injecting a single blastomere in each case at the 512-cell stage. *Bars* are 150 μm.

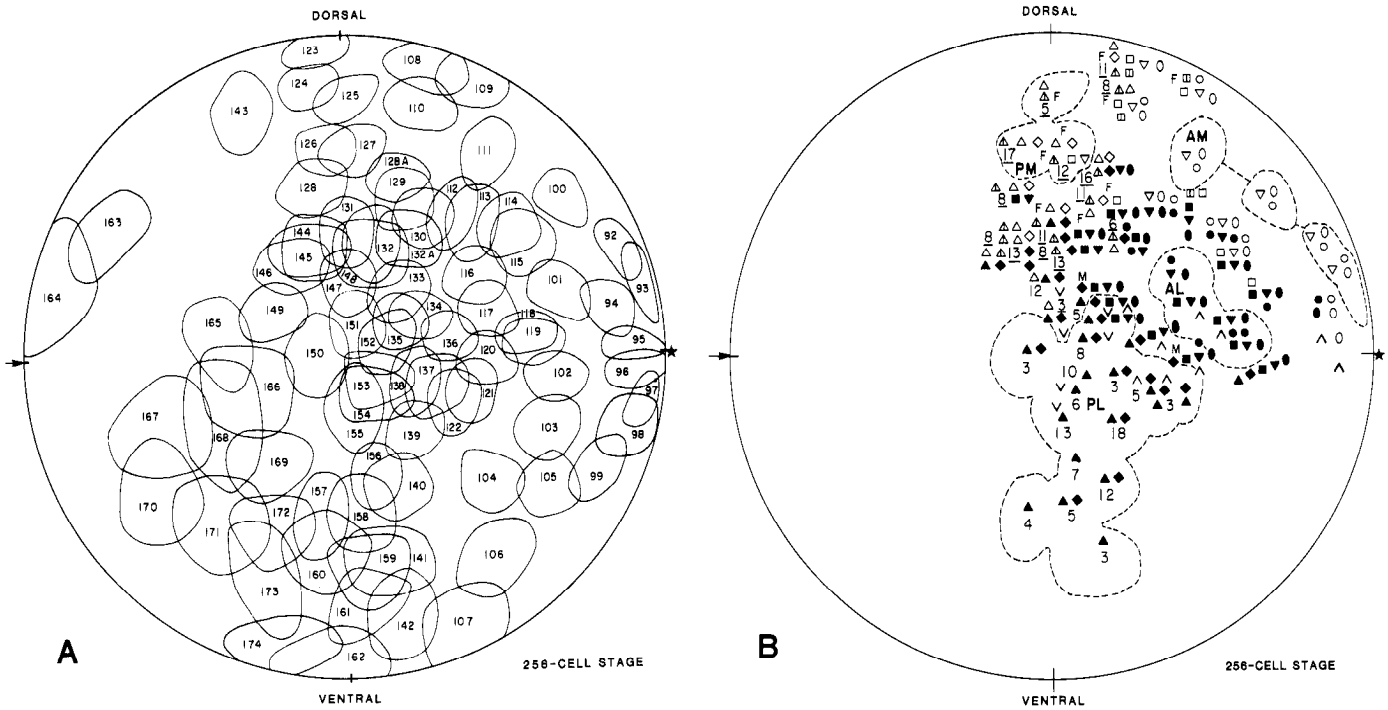

Figure 11. Fate map (right lateral case) of blastomeres at the 256-cell stage that contributed descendants to the CNS. Positions of all blastomeres injected with HRP at the 256-cell stage are shown in *A*, and the CNS regions to which blastomeres contributed are symbolized in the fate map (*B*). Symbols and other conventions are the same as in Figure 3.

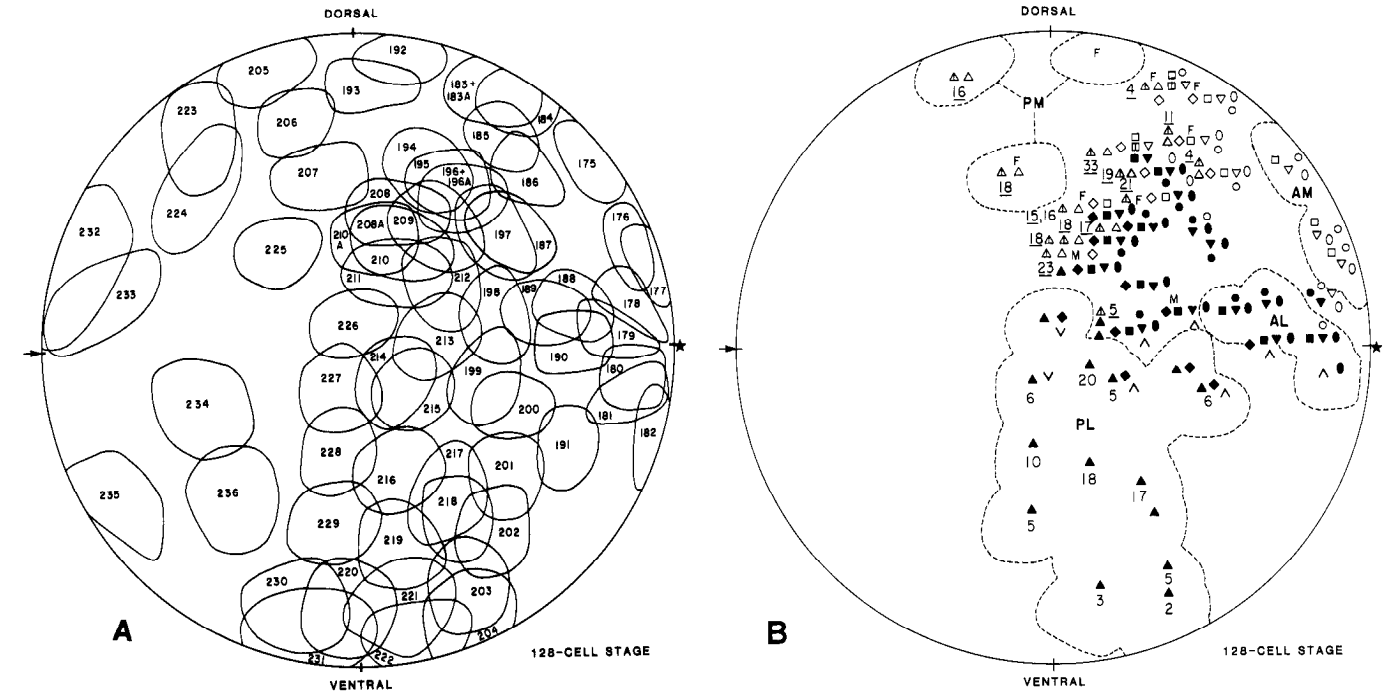

Figure 12. Fate map (right lateral case) of blastomeres at the 128-cell stage that contributed descendants to the CNS. Positions of all blastomeres injected with HRP are shown in *A*. The fate map (*B*) shows the regions of CNS populated by descendants of blastomeres that contributed to the CNS. Symbols and other conventions are the same as in Figure 3.

cell stage was a broad zone of blastomeres whose descendants were widely distributed in a region of CNS equal to that occupied by descendants of more than one ancestral cell group of the 512-cell stage. For example, blastomere 115 (Fig. 11*A*) contributed descendants to dorsal and ventral telencephalon, dorsal and ventral diencephalon and retina, dorsal and ventral mesencephalon—that is, to regions populated by descendants of anterior-median and anterior-lateral ancestral cell groups of the 512-cell stage. Blastomere 102 (Fig. 11*A*) contributed to

dorsal regions of the entire brain and spinal cord, regions that were populated by descendants of anterior-lateral and posterior-lateral ancestral cell groups of the 512-cell stage. It was clear that such blastomere groups had not formed at the 256-cell stage.

128-Cell stage. Of the 66 blastomeres injected successfully at the 128-cell stage, 52 contributed descendants to the CNS. The clonal organization of CNS cells derived from blastomeres of the 128-cell stage was quite similar to that derived from the 64-cell stage (Jacobson and Hirose, 1981) except for the location of blastomeres that gave rise to Rohon-Beard neurons. These had moved from their positions at the 64-cell stage just below the horizontal meridian, extending from the animal pole to the equator on each side, to positions along the ventral part of the equator at the 128-cell stage (Fig. 12*B*). Direct observations showed that blastomeres moved coherently without any apparent movement of individual blastomeres relative to others. This movement of blastomeres away from the animal pole occurred at the same time as rapid enlargement of the blastocele, and the two events may be related.

The large majority of labeled clones initiated at the 128-cell stage were widely distributed across boundaries that were respected by clones initiated at the 512-cell stage (Fig. 12*B*). Restricted distribution of descendants to a single region in the CNS was observed only after labeling blastomeres near the periphery of the embryonic region that gave rise to CNS. Those blastomeres are enclosed by *dashed lines* in Figure 12*B*. Many types of cells in the CNS were labeled after injecting any single blastomere of the 128-cell stage. Although 20 blastomeres contributed cells to the dorsal rhombencephalon, only two of those (numbers 211 and 198, Fig. 12*A*) gave rise to the Mauthner neuron. By contrast, Rohon-Beard neurons originated from 11 blastomeres and spinal motoneurons from 15.

Discussion

A remarkable difference was observed when the spatial organization of clones initiated at the 512-cell stage was compared with the organization of clones initiated at earlier stages. Blastomeres injected at the 512-cell stage contributed all their descendants to a single one of seven regions in the CNS, whereas the large majority of blastomeres injected at the 256- or 128-cell stages distributed their descendants in more than one of those regions. Each of those CNS regions was populated exclusively by the descendants of 14 to 26 blastomeres that formed a coherent blastomere group visible at the surface of the 512-cell embryo. Each blastomere group contributed descendants to a single region of the CNS but did not contribute to the regions populated by neighboring blastomere groups (Figs. 5 and 6). It appeared as if the space of the CNS was partitioned off into compartment-like regions, each region being populated exclusively by the descendants of one group of blastomeres of the 512-cell stage (Figs. 3 and 4).

There are three alternative interpretations of these results that merit discussion. Firstly, the clonal restrictions may have arisen after the 512-cell stage because on average, clones contain fewer cells at later than at earlier stages. Secondly, boundaries develop between clones because cells may have migrated away from the places at which those boundaries are formed. Thirdly, compartments may have arisen as domains of clonal restriction because, beginning at the 512-cell stage, blastomeres may have formed groups arranged in a regular pattern and the descendants of any blastomere may have strongly preferred to associate and mingle with descendants of the same blastomere group rather than with descendants of other blastomere groups. This has been called the compartment hypothesis (Jacobson, 1980, 1982). It is the preferred explanation of these observations because it is consistent with all the evidence and because the alternative explanations cannot account for many of the findings.

The first alternative can be eliminated because clonal restrictions almost identical to those seen after the 512-cell stage were also seen after labeling was started at the 256- and 128-cell stages (Figs. 4, 9*B*, 11, and 12) as well as at the 64-cell stage (Jacobson and Hirose, 1981). Therefore, the restriction of spatial distribution of cells was not determined by the number of cells in the clone. Cases in which all descendants of a single blastomere at the 64-cell stage were restricted within the same morphological boundaries as clones initiated at the 512-cell stage are illustrated in Figure 4, 6, 8, 10, and 14 to 16 of Jacobson and Hirose (1981). I shall presently discuss why clonal restriction occurred in some cases labeled before the 512-cell stage.

The second alternative, that boundaries developed as a result of directionally selective cell movements, can also be dismissed because it is inconsistent with the evidence. Labeled cells were observed mingling extensively with unlabeled cells, and many labeled cells reached the boundaries of the clonal domain. There were no signs of migration of cells away from the boundaries of the clonal domain. Those boundaries do not seem to be related to patterns of morphogenetic movements and cell migrations in the neural plate (Karfunkel, 1974; Jacobson and Gordon, 1976), neural tube, or central nervous system (Sidman and Rakic, 1973). This alternative can also be eliminated because any events that occurred after the 512-cell stage, such as cell migration or cell death, could not account for the difference in behavior of clones initiated at the 512-cell stage compared with those initiated at the 256-cell stage. Therefore, the causes of the great increase in spatial restriction of clones labeled at the 512-cell stage must be found at that stage and cannot be accounted for by mechanisms that start to operate at much later stages.

The best explanation of the evidence is that restriction of cell dispersal and mingling was determined by the ancestry of those cells from one of seven groups of blastomeres at the 512-cell stage: an anterior-median (AM) group straddling the dorsal midline near the animal pole and three groups on each side of the midline, namely, anterior-lateral (AL), posterior-medial (PM), and posterior-lateral (PL). Descendants of an individual blastomere mingled with descendants of other blastomeres in the same group (e.g., blastomeres 24 and 47, Figs. 5 and 6) but did not mingle with descendants of other groups (e.g., blastomeres 13 and 24, Fig. 5, and blastomeres 47

and 66, Fig. 6). Descendants of the AM blastomere group populated the ventral parts of the telencephalon, diencephalon, retinae, and mesencephalon bilaterally (Figs. 5, 7, and 8). Descendants of the AL group populated the dorsal part of the telencephalon, diencephalon, retina, and mesencephalon on the same side (Figs. 6, 7, and 9). Descendants of the PM group populated the ventral parts of the rhombencephalon and spinal cord (Fig. 2*B*), whereas descendants of the PL group populated the dorsal parts of the rhombencephalon and spinal cord (Figs. 2, *A*, *C*, and *D* and 6).

The restrictions to cell dispersal were seen as boundaries at the dorsal midline along the entire length of the CNS (Figs. 5, 6, 7, and 9), at the ventral midline of the rhombencephalon and spinal cord and in the horizontal plane between dorsal and ventral regions of the entire CNS and retina (Figs. 5 to 9). The boundaries between the blastomere groups at the 512-cell stage appeared to be preserved although topologically transformed as a result of morphogenetic cell movements into the boundaries between compartments in the CNS. These boundaries, in the same regions of the CNS in all cases, were respected by virtually all descendants of blastomeres labeled at the 512-cell stage. In a small minority of cases the boundaries were not respected by a few cells, always less than 1% of the clone.

In 8 of the 91 cases labeled at the 512-cell stage a few "aberrant" or "ectopic" labeled cells were found outside the compartment in which the remainder of the labeled clone was confined. The small minority of displaced cells may be considered to be a form of "developmental noise" (Waddington, 1957) that did not alter the main pattern of morphogenesis and did not seem to have had any functional effect. It is possible that irregularities in timing of the events that resulted in recruitment of individual blastomeres to form groups might have left some blastomeres uncommitted to any group at the 512-cell stage. If such a blastomere was labeled at the 512-cell stage but its daughter cells joined different blastomere groups, then its descendants would be expected to be divided almost equally between two compartments. This was observed in only two cases labeled at the 512-cell stage (blastomeres 21 and 22) but was found in many cases labeled at the 256-cell stage. The presence of less than 1% of the labeled cells in a compartment outside the one in which all other cells of the clone were confined was found in eight cases labeled at the 512-cell stage (blastomeres 6, 17, 25, 44, 46, 61, 63, and 66). This indicates that either the majority of "aberrant" cells had died or the ancestors of the "aberrant" cells had proliferated much more slowly than normal or had split away some time after the 512-cell stage. For less than 1% of the cells to be "aberrant," the ancestors of the "aberrant" cells would have had to depart from other cells of their clone no earlier than seven cell generations after the 512-cell stage. From this evidence there is no way of deciding which of the three possible mechanisms resulted in the presence of a very small percentage of ectopically located cells in a few cases.

Although groups of ectopically located cells in the CNS have often been reported, such cells have been recognized only when their modes of differentiation were not congruent with their positions (Jacobson, 1979, p. 305). Single isolated ectopic cells would be very difficult or impossible to detect by means of conventional histological methods. However, isolated labeled cells were easily seen in these specimens because all descendants of any blastomere were labeled by the HRP technique. Therefore, it may be possible in the future to see whether such ectopic cells differentiate in harmony with their final positions or whether their differentiation is determined by their ancestry.

The occasional "aberrant" cell is not surprising when one considers all the changes in cell numbers, sizes, shapes, and positions that occur from the 512-cell stage to midlarval stages. It is difficult to understand how the restriction of cell mingling at those boundaries occurred throughout such a long period of morphogenetic cell movements except in terms of the compartment hypothesis (Jacobson, 1982). There seems to be no other way of interpreting the observations that considerable mingling occurred between descendants of blastomeres belonging to the same group but that mingling was greatly restricted or absent between descendants of different blastomere groups (Figs. 5 to 7).

The observation (Jacobson and Hirose, 1981) that coherent clonal growth occurs up to and including the 512-cell stage provides an explanation of the cases in which clonal restrictions similar to those of descendants of 512-cell blastomeres were seen when clones were initiated at earlier stages. The clonal restriction that was observed in such cases may have occurred because such blastomeres, labeled before the 512-cell stage, contributed all their descendants to a single blastomere group at the 512-cell and thus to a single compartment at later stages. By contrast, those cases, labeled before the 512-cell stage, in which descendants were found in more than one compartment, originated from blastomeres which contributed descendants to more than one blastomere group at the 512-cell stage. In the latter cases the blastomeres at the 128-cell and 256-cell stages were located close to or straddling the positions at which the boundaries between blastomere groups developed at the 512-cell stage (compare Fig. 11*B* and 12*B* with Fig. 3*B*).

There are four factors to be taken into account when interpreting these results. First, that coherent clonal growth occurs during early cleavage stages. Videotape observations showed that the egg becomes divided into progressively smaller cells which do not move individually relative to one other until after the 512-cell stage, at least as seen at the surface of the embryo (Jacobson and Hirose, 1981). Secondly, the patterns of cleavages varied between individuals; even when embryos were selected with regular and symmetrical cleavage patterns at the 64-cell stage, they were individually variable at the 512-cell stage. Moreover, cleavages that appeared to be regular at the surface may have been irregular below the surface. Because the patterns of cleavage after the 64-cell stage became progressively more irregular, resulting in great individual variability of blastomere size and shape at the 512-cell stage, the boundaries of blastomere groups, which necessarily follow cell boundaries, would be expected to show individual variability. The third factor to take into consideration is that variability was observed

in the numbers of differentiated cells that descended from individual blastomeres of the same blastomere group (M. Jacobson and S. Moody, submitted for publication). This is shown in Figures 3*B*, 10*B*, and 11*B* for the numbers of Rohon-Beard cells and primary spinal motoneurons that descended from individual blastomeres of the 512-, 256-, and 128-cell stages. A fourth factor to be taken into consideration is that descendants of any single blastomere were distributed in other systems in addition to the CNS, and it is not known whether the fraction of descendants in the CNS is constant or variable.

The first two factors could give rise to individual variability of the positions of boundaries between blastomere groups and thus to variability in positions of compartment boundaries. The third and fourth factors could give rise to variability in the number of neural descendants of any individual blastomere and, therefore, to the precentage of labeled cells in the CNS compartment as well as at the compartment boundaries. It must be emphasized that the observed individual variability in the numbers of labeled cells and in the absolute positions of the compartment boundaries did not change the compartmental pattern. This remained topologically invariant in spite of metrical variability resulting from differences in the sizes of individual embryos, differences in the numbers of descendants of individual blastomeres, and individual differences in the planes of section and distortions of shape produced by histological processing.

The labeled descendants of any single blastomere of the 512-cell stage mingled with unlabeled cells and dispersed widely in the CNS, often to the boundaries of the compartment-like region. That the mingling was not complete was apparent in many specimens. This can be seen in Figures 5 and 7 where there were more labeled cells on the side of the injected blastomere than on the opposite side of the brain and retina. Figures 5, 7, and 10 also show how few labeled cells had reached the putative boundary. However, these observations are consistent with the prediction that no more than a small fraction of the cells at the boundary will be descendants of any single blastomere of the 512-cell stage. These boundaries were well defined in some individual cases and could be defined more clearly by comparing the patterns of distribution of labeled cells in many specimens. This was done by superimposing several transparencies on each of which the labeled descendants of one blastomere were drawn with the aid of a camera lucida. However, it was not possible to show the results in a single diagram because of the differences in sizes of the specimens and differences in their planes of section. Comparisons of the maps of distribution of descendants derived from different blastomere groups showed that often there was no overlap or only a narrow boundary zone of overlap between the descendants of any two neighboring blastomere groups of the 512-cell stage (Figs. 5 and 6). The vast majority of descendants of any single blastomere group were well segregated from the descendants of the neighboring blastomere cell groups of the 512-cell stage (Figs. 5 to 9). A more direct experimental method of testing the amount of segregation of descendants of different blastomere groups would be to use two different intracellular tracers in the same embryo to label two blastomeres in different blastomere groups. The amount of mingling of the two clones, each labeled with a different tracer, might be seen directly. This method has not proved feasible, so that the available evidence must be used to compare the distribution of clones labeled with HRP that approach one another from opposite sides of a putative boundary in two different specimens. The precise position of such boundaries could be seen in some cases because of the high density of labeled cells at the boundary region. However, in other cases the density of labeled cells was too low to form a continuous front, and in those cases one could not define the precise position of a boundary. I shall refer to "boundary zones" or "putative boundaries" when discussing such evidence.

Boundaries could be defined by the behavior of labeled clones. The large majority of clones initiated at the 512-cell stage were located on one or the other side of the following boundaries in the CNS: (*1*) a dorsal midline boundary between left and right sides. This was respected by all clones in the CNS but not by all neural crest cells. The dorsal midline boundary was most convincingly seen in cases of dorsal midline structures derived from both sides, such as the epiphysis; cells approached but did not cross the dorsal midline (Figs. 5, 6, and 10). (*2*) The floor plate of the rhombencephalon and spinal cord formed a ventral midline boundary. The floor plate extends the full length of the spinal cord and rhombencephalon, but it ends behind the third nerve nuclei (Kingsbury, 1920, 1922, 1930). There was no ventral midline boundary in front of the rostral end of the floor plate, and cells mingled across the ventral midline of the telencephalon, diencephalon, and mesencephalon (Jacobson and Hirose, 1978, 1981; Hirose and Jacobson, 1979). The absence of a boundary across the rostral part of the ventral midline is shown by the repeated observation that labeling any blastomere in the anterior-median blastomere group resulted in the distribution of labeled cells to both sides of the ventral retina and ventral parts of telencephalon, diencephalon, and mesencephalon. In those cases the labeled descendants, approaching from the ventral side, respected a boundary between ventral and dorsal parts of the retina, telencephalon, diencephalon, and mesencephalon (Figs. 5, 7, and 8). When cells were labeled on the dorsal side of that boundary, they approached from the dorsal region of the retina or brain but did not cross into the ventral region (Figs. 5, 6, 8, and 9). This boundary separating dorsal from ventral parts of the CNS extended the entire length of the neural axis. This boundary corresponded with the sulcus limitans in the rhombencephalon and spinal cord. There is a difference of opinion about where the sulcus limitans ends rostrally (Kuhlenbeck, 1973), but these results show that the boundary separating dorsal and ventral parts of the brain extends to the optic stalk and then extends rostrally to the lamina terminalis in agreement with the scheme first proposed by His (1892, 1893).

Finally, a boundary zone was observed in the transverse plane in the isthmus region. The evidence for the existence of this boundary zone was the same as that for the others: after labeling any blastomere at the 512-cell stage all the descendants were located either rostral to

the isthmus or caudal to it (Figs. 5 to 7). The sparseness of cells in the amphibian isthmus at larval stages made it difficult to define the precise position of the putative boundary in this region. However, it has long been recognized that: "In all vertebrate brains the most fundamental structural landmark is the transverse plane separating the spinal cord and rhombencephalon below from the cerebrum (as defined in the BNA) above" (Herrick, 1948, p. 116). The existence of a boundary preventing cells from crossing between mesencephalon and rhombencephalon was clearly evident in all but two cases injected at the 512-cell stage. Clones initiated at the 512-cell stage approached this putative boundary from either side but did not cross it. By contrast, clones initiated at the 256-cell stage or earlier frequently crossed this and other boundary zones (Figs. 11 and 12).

The possible causes of variability of the boundaries have already been discussed. Boundaries need not be related to identifiable anatomical landmarks, as the boundary between dorsal and ventral parts of the retina shows most clearly (Figs. 5, 6, 8, and 9). The significant features in all cases were the farthest extent to which labeled cells had dispersed and the topological relationship between the domain containing all the labeled cells on the one hand and the totally unlabeled regions on the other. These relationships were invariant, namely that clones descended from blastomeres in the AM or AL groups were always more rostrally situated in the CNS than those derived from PM or PL groups, and clones descended from AM or PM groups always occupied more ventral regions of CNS than clones descended from AL or PL groups. All the clones descended from all blastomeres of the same group were restricted to the same region of CNS, and this polyclonal domain is called a compartment. Therefore, in this paper the word compartment is used in the sense of a separate section of the overall structural pattern that has developed from a particular embryonic rudiment, the blastomere group. With respect to the fate map one can think of compartments as topological neighborhoods within the overall morphological pattern. The concept that such neighborhoods can be mapped ontogenetically on corresponding embryonic rudiments is derived ultimately from Wilhelm His (1874, 1892, 1893). Wilhelm His originated the concept that large regions of the adult morphology could be mapped one to one on small multicellular regions of the early embryo which he called organ-forming germinal regions (*organbildende Keimbezirke*; His, 1874, p. 19). This is a term that could justifiably be used to denote the seven blastomere groups in the 512-cell frog embryo. His also used the word "boundary" as the technical term to describe the sulci that he observed between structures that developed from neighboring *Keimbezirke*. He showed that the sulcus limitans was such a boundary sulcus (*Grenzfurche*; His, 1874, 1895).

There is a one-to-one developmental correspondence between groups of blastomeres in the 512-cell embryo and compartments in the larval CNS. This isomorphism breaks down with regard to differentiation of finer subdivisions of structure so that there were one-to-many and many-to-one relationships between individual blastomeres of the 512-cell stage and individual types of nerve cells or between individual blastomeres and neuroanatomical subdivisions, such as the cerebellum or thalamus, which are smaller than a compartment.

Because extensive cell mingling occurred within each compartment, a fate map cannot be made at a higher level of resolution than the entire compartment and the corresponding ancestral cell group at the 512-cell stage. A fate map at the level of resolution of differentiated nerve cells may be made at a later stage, only after the time of commitment of cells to specific programs of differentiation. Before the time of commitment the lineage relationship between any ancestral cell and any cellular phenotype can be expressed as a probability that, following each mitosis, the daughter cells re-enter the lineage of that phenotype (Jacobson and Hirose, 1981; M. Jacobson and S. Moody, submitted for publication). Some types of neurons, including Rohon-Beard neurons and spinal motoneurons, originate as postmitotic cells near the end of gastrulation in *Xenopus* (Lamborghini, 1980). It would seem to be necessary to limit dispersion of such cells that have become committed to specific programs of differentiation early in development while morphogenetic movements are in progress.

The growth of axons and dendrites in the CNS was not restricted by compartmental boundaries. However, we have found evidence of clonal restriction of formation of connections by pioneer axons growing out of the CNS. Primary spinal motoneurons sent their axons first to myotubes belonging to the same clone as themselves, although they later formed connections with other myotubes (S. Moody and M. Jacobson, submitted for publication).

This evidence makes a small contribution to the problem of the relationship between ancestry and phenotypes of nerve cells in *Xenopus*. At stages 30 to 39, when these experiments were terminated, the populations of most types of nerve cells had not been completely generated and many types had not differentiated to the stage of easy recognition. The problem can be approached only for those types of neurons that had completed their final divisions and were well differentiated at the end point of these experiments. Some of these were Mauthner's neurons, Rohon-Beard neurons, and primary spinal motoneurons. The paucity of cases in which Mauthner's neurons were well labeled precludes a quantitative analysis of those results. Nevertheless, the results are consistent with the concept (Jacobson and Hirose, 1981) that a single Mauthner's neuron on each side can be produced with equal probability by any of the blastomeres in the PL group but that only one blastomere can be the actual ancestor in each individual. If the PL group contains about 20 blastomeres at the 512-cell stage, only one-twentieth of the injected blastomeres would be expected to produce a labeled Mauthner's neuron. In fact, 19 blastomeres were labeled in the PL group (Fig. 4) and a labeled Mauthner's neuron was found in one case only. However, from that evidence it is not possible to conclude that the origin of Mauthner's neuron is linked uniquely to the PL ancestral cell group.

The association of a specific type of neuron with a particular ancestral cell group can be made in the cases of the primary spinal motoneurons and the Rohon-Beard

neurons; the former were descended exclusively from blastomeres of the PM group and the latter exclusively from the PL group (Fig. 4*B*). Because those types of neurons were easily identified at larval stages 30 to 39, it was possible to obtain accurate counts of the numbers of Rohon-Beard neurons or primary spinal motoneurons that descended from individual blastomeres. Those results will be published elsewhere (M. Jacobson and S. Moody, submitted for publication), but there are three observations that are relevant to this discussion. These apply to primary spinal motoneurons as well as to Rohon-Beard neurons, although to simplify the discussion the following will refer only to the latter cells. First, 13 of 19 blastomeres of the PL group each gave rise to some of the total population of Rohon-Beard neurons. Second, there was great variability of the numbers of Rohon-Beard neurons that originated from any individual blastomere. Finally, the Rohon-Beard neurons that descended from a single blastomere were scattered widely and irregularly along the length of the spinal cord. Therefore, it was not possible to predict the numbers and positions of Rohon-Beard neurons or spinal motoneurons that would originate from any individual ancestral cell at the 512-cell stage, although there was a very high probability that some Rohon-Beard neurons would descend from any individual blastomere of the PL group and that some primary spinal motoneurons would differentiate in the clone that descended from any single blastomere of the PM group. The relationship between any individual blastomere and any individual Rohon-Beard neuron or primary spinal motoneuron was probabilistic.

These blastomere groups and the compartments of the CNS which they populated invite comparison with the founder cell groups in the imaginal discs of *Drosophila* and with the compartments that originate from such founder cell groups in organs that develop from imaginal discs (Garcia-Bellido, 1975; Morata and Lawrence, 1977). Like the compartments in *Drosophila*, each of the compartments in the frog CNS is a polyclone (Crick and Lawrence, 1975) derived from one group of ancestral cells. Genetic analysis of development of the frog CNS is precluded for the time being, so that the evidence provided here for compartmentation of the frog's CNS falls short of a causal analysis. The evidence is not sufficient to show whether genetic and cellular mechanisms of development of compartments are the same in *Drosophila* and *Xenopus*. One of the differences that may be of importance is that considerable cell dispersal and mingling occurs during development following the foundation of ancestral cell groups in *Xenopus*, whereas coherent clonal growth of founder cell groups results in development of compartments in *Drosophila*. The evidence presented here indicates that clonal restriction is the basis of development of the primary morphological pattern in the CNS of *Xenopus*. Some problems that require further investigation are how the differences between blastomere groups develop initially, by what mechanisms and for how long those differences are maintained over many cell generations, how they limit mingling between descendants of different blastomere groups, and how they may affect outgrowth of neuron processes and the formation of neuronal connections.

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