Tissue-Specific Cell Lineages Originate in the Gastrula of the Zebrafish

CHARLES B. KIMMEL AND RACHEL M. WARGA

In the zebrafish, cells of a clone derived from a single blastomere migrate away from one another during gastrulation. Later in development their descendants are usually found scattered within several different types of tissues of embryo. The divisions and migrations of individual cells were monitored during early development, revealing that in most cases the lineal descendants of single cells present at gastrula stage exclusively populate only single tissues, and may have stereotyped positional relationships within these tissues. Thus the gastrula stage is the first stage when heritable restrictions in cell type might arise in the zebrafish.

LITTLE IS KNOWN ABOUT POSSIBLE ROLES OF CELL LINEAGE in determining cell fate in vertebrate embryos. Clonal analyses of zebrafish development by use of genetic mosaics (*1*) and lineage tracer molecules (*2*) have shown that clonal descendants of blastomeres scatter in the embryo and mix extensively with clonally unrelated cells. Furthermore, the early lineages do not appear to influence cell position at later (gastrula) stages (*2*). These observations suggest that cell fate is not specified during cleavage stages, even though the pattern of divisions is often stereotyped (*3*). We now have made an analysis of vertebrate cell lineage by directly tracking cells of a single labeled clone in vivo as the cells divide, migrate, and populate the primary organ systems of the zebrafish embryo. We find that progeny of single cells, present through the stage when the blastoderm has about 2000 cells, migrate to diverse locations in the embryo and populate several tissues. In contrast, most clonal lineages that arise subsequently, during gastrula stage, populate single tissues, and cells that share the same lineage migrate apart in regular patterns. Thus developmental restrictions may first appear during gastrulation (*4*).

Scatter of clonally related cells. We first surveyed the distribution of the clonal progeny of single blastomeres that were labeled by intracellular injection with a lineage tracer dye (*5*). We previously showed (*2*) that initially, during blastula stage, the clone has the form of a compact cluster of cells. The cluster disperses as the cells begin the spreading movements of blastoderm epiboly that precede and accompany gastrulation (*6*). For example, the clone shown in the mid-gastrula in Fig. 1, a and b, stretched along one side of the embryo from near the animal pole (top) to the blastoderm margin (arrow). Most of the individually discernible cells are so-called deep cells, present in layers beneath an outer flattened enveloping layer (evl) of epithelial cells (*6*). Except for close apposition of pairs of recently divided cells, order in the arrangements of the labeled cells was not apparent in clones examined at this stage.

By late gastrula stage, cells within some labeled clones were distributed preferentially along the elongating axis of the embryo, and many cells had converged toward the embryonic shield at the embryo's dorsal surface. There was often regular spacing of labeled cells along the embryonic axis (for example, Fig. 1, c and d; where the period length is approximately 40 μm, the same as somite length when somites first appear 3 hours later).

Still later, when the organ rudiments had formed, clusters of labeled cells were present in more than one tissue, such as skin, myotomal muscle, and the central nervous system (CNS). In a few exceptional cases, clones derived from blastomeres injected at late blastula stages populated only the enveloping layer, but usually more than a single type of labeled cell was observed (see below).

In the CNS, cells descended from the same blastomere were present in clusters along the axis. Adjacent clusters sometimes included morphologically similar kinds of neurons. For example, Fig. 1, e and f, show two spinal motoneurons (*7, 8*) that were present one segment length apart from one another. In addition, in the CNS specifically, we frequently observed labeled cells or clusters of cells directly across the midline from one another (Fig. 2). These bilateral pairs also sometimes comprised similar neurons. In six embryos analyzed quantitatively, 60 percent or more of the labeled cells on one side of the spinal cord had labeled partners on the other side; this distribution was not due to chance alone (Table 1). The spread of clonally related cells across the midline occurred at any longitudinal level of the CNS, including brain and spinal cord.

Tissue-specific cell lineages. To learn how these clonal distributions arise we traced, in the live embryo, lineages that arise during gastrulation within clones marked by injections of blastomeres. The study was facilitated by the small size, optical clarity, and rapid development of the zebrafish embryo. Repeated observations can be made on a single embryo for at least several days; however, in most of the cases reported here the observations were confined to the first day after fertilization. We selectively followed cells that were converging rapidly toward the embryonic shield in the early gastrula. Most of these cells eventually populated dorsal regions of the head and trunk of the embryo.

We were able to unambiguously follow some individual cell lineages during gastrulation and primary organogenesis (Table 2). In nearly all of these cases, the progeny of individual gastrula cells exclusively populated a single type of tissue. The large majority of examples were of cells that formed the enveloping layer of the gastrula and then populated exclusively the enveloping layer of the 24- to 30-hour embryo (Table 2, row 1). Enveloping layer cells were technically easy to follow because of their distinctive position and flat shape, and because labeled deep-lying cells often migrated away from the enveloping layer cells of the same clone. All other tissue-types (Table 2) (*9*) arose from deep cells of the gastrula. Most of these cells founded clones restricted to neural (Table 2, row 2) or

Charles B. Kimmel is a professor in the Department of Biology and is a member of the Institute of Neuroscience and Rachel M. Warga is a research assistant in the Institute of Neuroscience, University of Oregon, Eugene 97403.

muscle (Table 2, row 3) tissue; this bias reflects the relatively large number of cells present in these two tissues in the embryo, and probably also the fact that we selected dorsally located clones for study. During the interval of development examined the clonal expansion averaged about sixfold (Table 2), and in the case of the deep cells there was considerable cell migration (see below). Thus there must have been ample opportunity for clonally related cells to disperse into different tissues. Yet usually they did not.

In a small number of cases (five clones in three embryos, Table 2) the clones populated two or more tissue types. In two of these atypical cases (observed in one embryo) the lineage included Rohon-Beard neurons, extramedullary neurons, pigment cells, and small mesenchymal cells adjacent to the somites. The occurrence of these phenotypes suggested that the cells arose from the neural crest, whose derivatives are known to be diverse in other vertebrates (*10*), but have not yet been studied extensively in teleosts. In the other exceptional cases, one or a few unspecialized looking cells were located near, but not within tissue (muscle in two examples and neural in the other) populated by the majority of cells in the clone.

Cell lineages arising in one embryo that were restricted to single tissues are shown in Fig. 3. The labeled clone developed from a 512-cell stage blastomere injected about 3 hours after fertilization (*11*). Beginning at the midgastrula stage (7 hours) lineages of selected cells of the clone were followed until organ anlage had appeared (14 hours). Later, at 24 hours, the detailed morphology was examined

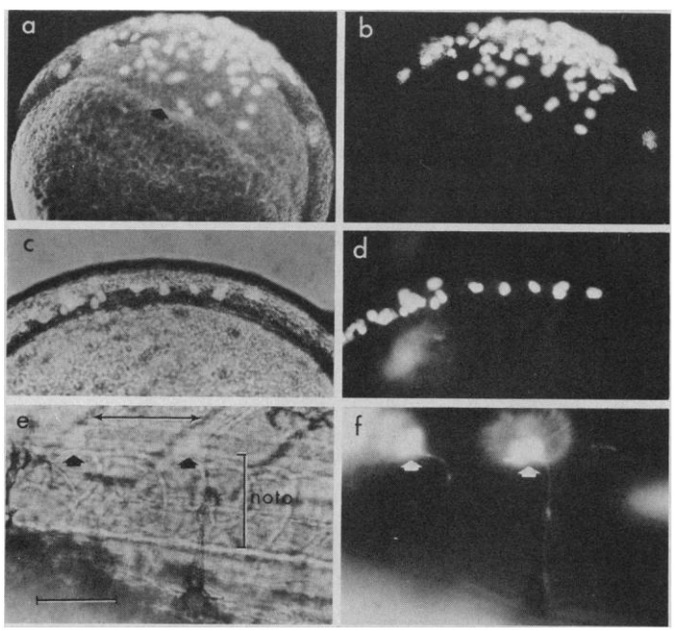

Fig. 1. Labeled clonal descendants of single blastomeres injected with a fluorescent lineage-tracer dye. The micrographs are of live zebrafish embryos. The left panels show a mixture of bright-field illumination and epifluorescence and the right panels show epifluorescence alone. (a and b) A clone at midgastrula stage (50 percent epiboly, 7 hours after fertilization). The clone originated from a blastomere at the 64-cell stage of development. (c and d) A side view of the developing axis in another embryo at a late gastrula stage (70 percent epiboly, 9 hours). Dorsal is up and anterior is to the left. Some of the descendants of a 256-cell stage blastomore lie in a row parallel to the embryonic axis. (e and f) Two labeled cell clusters (arrowheads) that contain motoneurons in a left-side view of the spinal cord at about 30 hours. Dorsal is up and anterior is to the left. Somite boundaries (double-headed arrow) are visible in (e). The motor axons are in the periphery adjacent to the notochord (noto). The small growth cone of the more anterior axon is visible. The blastomere was injected at the 128-cell stage. The scale bar in (e) represents 200 μm for (a) and (b), 130 μm for (c) and (d), and 50 μm for (e) and (f).

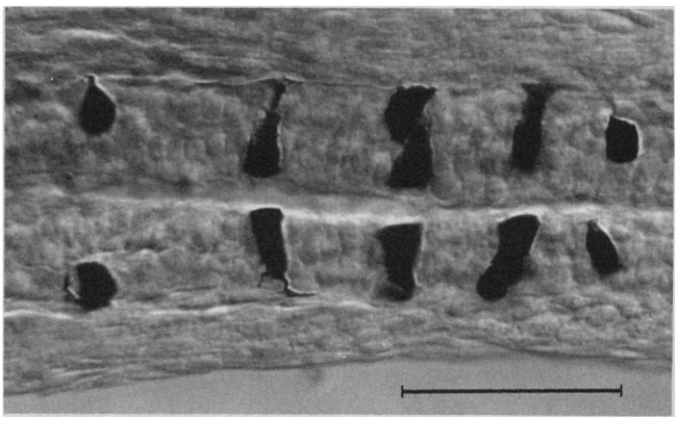

Fig. 2. Clonally related neural cells in the caudal spinal cord at about 27 hours. Horizontal 50-μm section, with rostral to the left and the midline in the center. A blastomere in a 512-cell embryo was injected with a conjugate of rhodamine and horseradish peroxidase (*2*), and peroxidase activity is histochemically displayed (*20*). Nomarski optics. The scale bar is 50 μm.

Table 1. Bilateral association of clonally related neural cells. Single blastomeres in embryos between the 256-cell and the approximately 1000-cell stages were injected with a conjugate of rhodamine isothiocyanate and dextran (*21*). At 30 hours the embryos were immobilized in a methyl cellulose gel (*22*), and the three-dimensional coordinates of each labeled cell in the rostral spinal cord were digitized with the aid of a computer (*2*). These columns show the total numbers of cells observed on each side, irrespective of their longitudinal positions. The observed data express the percentage of labeled cells on the left side that had labeled right-side counterparts present within a 10-μm window centered at the same longitudinal level.

Animal	Labeled cells		Percent of left-right associations			
	Left	Right	Observed	Expected*	χ^2	*P*
1	24	26	62.5	29.2	12.3	<0.001
2	24	18	66.7	41.7	6.0	<0.02
3	46	38	87.0	43.5	33.6	<0.001
4	25	20	60.0	24.0	16.8	<0.001
5	25	20	84.0	24.0	47.6	<0.001
6	11	14	100.0	36.4	18.6	<0.001

*The null hypothesis is that the labeled cells are randomly distributed along the axis. To obtain the expected percentages, cell positions were randomized by a computer algorithm that preserved the rostrocaudal limits of the clone and the numbers of left and right cells.

Table 2. Clonal restrictions to single types of tissues.

Tissue	Embryos (No.)	Cells		Mean expansion*
		In gastrula (No.)	At 24 to 30 hours	
Enveloping layer	62	287	1748	6.1
Neural	11	56	446	8.0
Muscle†	9	23	115	5.0
Kidney	1	1	13	13.0
Epidermis	1	1	7	7.0
Mixed‡	3	5	32	6.4
Totals	66§	373	2361	6.3

*The mean expansion is the number of cells present at 24 to 30 hours divided by the number of gastrula stage founder cells. †Most of the labeled cells at 24 to 30 hours were differentiated muscle fibers, and in many of these cases the cells had more than a single nucleus, as expected from the fusion of myoblasts. Fusion of two or more labeled cells with one another would lead to an underestimate of the expansion of the clone. In 4 of the 23 clones, labeled undifferentiated cells were present in myotomes that also contained labeled fibers. We assume that these cells were myoblasts. ‡These clones included cells of more than a single tissue type. §The total number of embryos examined is less than the sum of the figures in this column. This is because in many cases clones founding different tissues were followed in the same embryo (that is, as in the example in Fig. 3).

in sectioned material. Each of the cells indicated in the first panel of Fig. 3a gave rise to subclones that eventually populated deep layers of the embryonic epidermis (E), myotomal muscle (M), and neural tube (N), respectively. The other cells (those unlettered) were part of the clone originating from the injected blastomere, but their lineages were not followed. As shown for the neural lineages diagrammed in Fig. 3b, cells within a particular subclone can migrate quite far away from one another even though they will later occupy the same tissue anlagen. Extensive migrations were often observed of deep cells, including those that develop into muscle (*9*). On the other hand, cells of lineages that develop into different tissues can come to lie geographically near one another (for example, Fig. 3c, where labeled E, M, and N cells are near neighbors). These observations suggest that the observed restriction to single tissue types are not simply due to spatial constraints.

In the neural lineage shown in Fig. 3b, two progenitors in the midgastrula (which we suspect were siblings because of their proximity) eventually produced four groups of undifferentiated neuroepithelial cells in the spinal cord. In each group, cells populated both sides of the spinal cord (Fig. 3c). Each one of the four groups is a clone, the founders arising at 7.5 hours (the second stage shown in Fig. 3, a and b). Thus the first cell divisions within the neural lineage produced cells that dispersed along the embryonic axis. Later, lineal descendants of these four cells migrated across the midline.

The same sequence shown in Fig. 3b (longitudinally migrating cells giving rise to cells that migrated across the midline) was observed without exception in neural lineages from 13 other embryos. In 18 of these lineages, from five embryos, bilateral pairs of cells arose from the division that immediately followed the period of longitudinal dispersion. One of these cases is shown in Fig. 4. At the initial observation in the midgastrula (Fig. 4a) five prospective hindbrain cells (A to E), descended from the injected blastomere, were present in a single cluster. Cell E divided first, 40 minutes later, and the pair of daughter cells (Ea and Ep) separated longitudinally, such that by the late gastrula stage (Fig. 4b) Ea and Ep were about

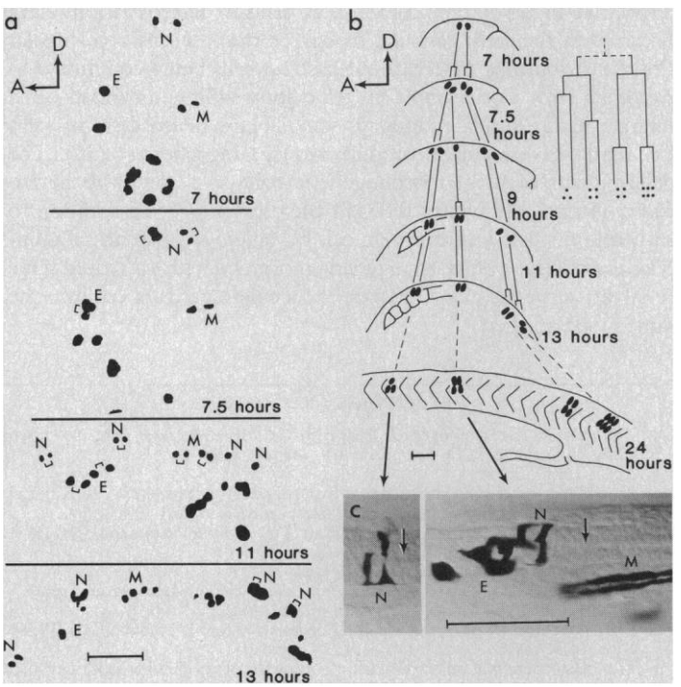

Fig. 3. Partial lineage analysis of lineages arising at the gastrula stage of development in a single embryo. The orientation of the embryo is approximately the same in all of the panels; the view (slightly oblique) is of the left side with anterior (A) to the left and dorsal (D) up. (a) Selected stages from an extensive series of observations beginning with the midgastrula (7 hours) through eight somites (13 hours). Each panel in (a) is a montage, representing several planes of focus as photographed from the face of the video monitor. The images are negative, such that the fluorescent cells are dark. Lineages of deep cells are indicated that eventually populated the neural tube (N), mytotomal muscles (M), and deep epidermis (E). Some cell divisions are indicated (ɾ). Although only one cell of the E lineage is visible in the last panel, three others were present in a plane of focus above that of the nearby N cells. (b) Schematic drawing of the same neural (N) lineage. The dashed lines indicate that after 13 hours we no longer kept track of the individual cell identities within the cell clusters. (c) Peroxidase labeling of some of the same cells in the sectioned 24-hours embryo. The oblique section includes the midline of the spinal cord (arrows), and labeled muscle fibers (M) and deep epidermal cells (E) on the animal's left side. Labeled neuroepithelial cells (N) are present in the spinal cord in two groups that cross the midline. One of these groups is partially hidden beneath the epidermal cells. There is a gap of about 300 μm between the two photographs. Compare with (a) to see the precursors of these cells earlier in development. The scale bars all represent 100 μm.

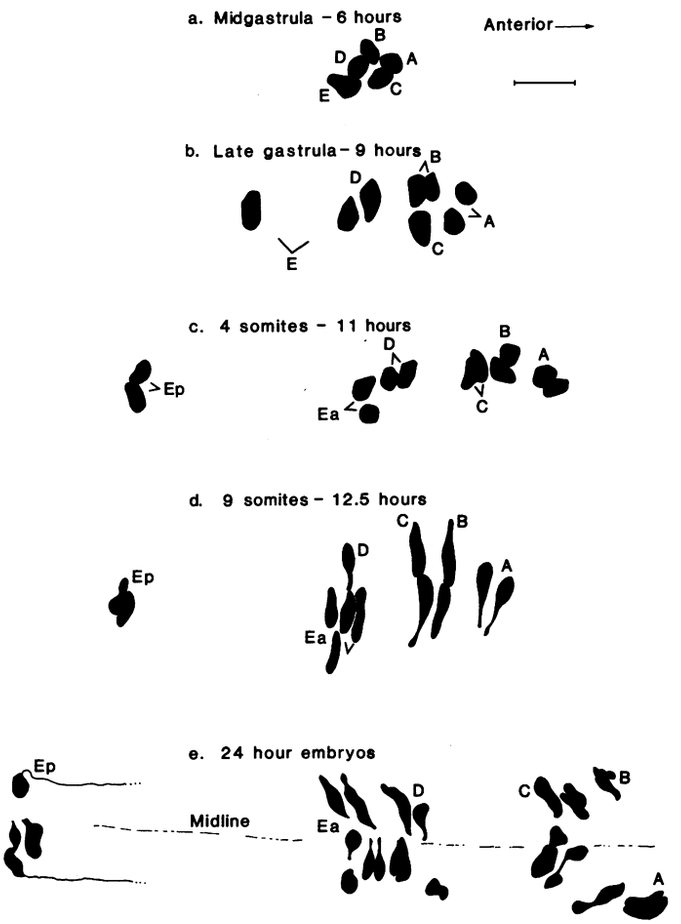

Fig. 4 Dispersal of sibling neural progenitor cells across the midline. Cells arising from divisions between 9 and 12.5 hours (b, c, and d) migrated apart in the transverse plane (d), and eventually founded bilateral clusters in the hindbrain (e). This migration was observed in the cases of the daughters of cells B, C, D, and Ea (presumably the daughters of cell Ep did the same). The Ep subclone includes a bilateral pair of interneurons whose axons were growing anteriorly (e). In (d) there is foreshortening due to the angle of viewing. The cells were descended from a blastomere that had been injected with a conjugate of rhodamine isothiocyanate and dextran. The injection was made in a blastula of about 4000 cells, 3.5 hours after fertilization. The cells shown include all of the labeled deep cells of the clone. There was also a single evl cell labeled at midgastrula stage that produced four evl descendants by 24 hours; the evl subclone is not included in the drawings. The scale bar is 50 μm.

four cell diameters apart. Divisions of more anterior cells produced daughters (Fig. 4, b and c) that did not separate longitudinally. In each case, except cell A, the pairs spread apart perpendicularly to the embryo's long axis, in the transverse plane (Fig. 4d). Eventually the descendants of these cell pairs, except for the A daughters, formed clusters on both sides of the midline (Fig. 4e). The observed stretching of the cells in the transverse plane (Fig. 4d) suggests that the migration that eventually separated the pair of siblings at the midline was an active process. Furthermore the division plane did not predict the subsequent morphogenetic behavior: cells B and D divided longitudinally yet their daughters migrated transversely. Cell A divided transversely, yet both daughters remained on the same side of the midline.

Periodic cell arrangements. As a consequence of the early longitudinal migrations, clonally related cells come to lie in different, often adjacent body segments (Figs. 1e and 2). In the leech, founder cells termed teloblasts each produce a clonal chain of blast cells that populate successive body segments (*12*). We looked quantitatively for periodic longitudinal arrangement of labeled CNS clusters within zebrafish clones. The two examples in Fig. 5 show pronounced periodicity, as was evident in nine out of ten embryos examined this way. However, the period length was variable, ranging from 1.0 to 3.2 segment lengths. In Fig. 5a the period was about 2, and in Fig. 5b about 2.7 segment lengths. From these results, it is clear that any possible relationship between cell lineage and body segments is more complex than in the leech.

Clonal restrictions. We have described restrictions in the types and positions of cells that make up the clonal progeny of single cells present at the gastrula stage of development. It is interesting that the restrictions appear to arise not long after cells begin the active spreading movements of epiboly that eventually scatter the clonal progeny of blastomeres to diverse locations across the blastoderm, and mix together at these locations cells derived from more than one blastomere.

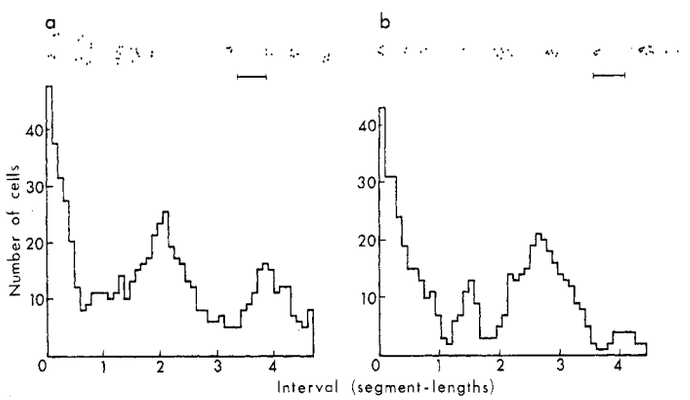

Fig. 5. Autocorrelation analyses of the distribution of clonally related neural cells along the embryonic axis in two embryos (a and b). Single blastomeres were injected in embryos between the 256-cell and the approximately 1000-cell stage, and at 30 hours the positions of clonally derived cells in the rostral spinal cord were digitized (legend to Table 1). The dot plots in both (a) and (b) show computer-generated dorsal views of the distributions of the labeled cells. The scale bars indicate two segment lengths, as determined from the digitized positions of somite boundaries; in (a) segment length is 73 μm and in (b) it is 77 μm. The histograms were constructed from an algorithm that determines, for each cell, whether another cell is present within a more caudally located window 10 μm wide that is centered at given distances, as plotted along the *x*-axis of the histograms. Major peaks are present at an interval of 0, showing that the cells are present in clusters. Major peaks also occur at about (a) two segment lengths and (b) 1.4 and 2.7 segment lengths, showing that the clusters are periodically distributed in both embryos, but that the period length varies among embryos.

Our observations suggest that with respect to cell fate there may be a general sequence in the pattern of diverging lineages during normal development: The first divisions that occur in the early gastrula produce tissue-specific lineages. The next divisions produce cells that populate different axial levels, and finally (in the CNS specifically) divisions occur that separate the two sides of the organ. This sequence is unlike most of the embryonic cell lineages in *Caenorhabditis elegans* (*13*), and the embryonic cell lineages in the leech (*12*), and an ascidian (*14*). In these cases, lineages forming the left and right sides separate during early cleavage. In the fish CNS, the cells separate relatively later, during or after gastrulation. More similar to the fish CNS, hyp7 cells in *C. elegans* migrate across the midline after gastrulation (*13*), and in *Xenopus* progeny of single blastomeres populate both sides of the midline in the CNS, but only in the forebrain (*15*). Futhermore, in both *C. elegans* and the leech many but not all embryonic cell lineages include a number of diverse tissues, while in the fish, divisions that occur shortly after the blastula stage appear usually to give rise to founders of tissue-specific lineages. Since we have carried out no tests of the developmental potencies of cells at the gastrula stage we do not know whether the restriction in tissue-type means that cellular determinations have occurred in the early gastrula. It may be that the observed regular clonal distributions arise without gastrula cells being committed to particular fates. For example, in cell culture sibling fibroblasts often migrate along similar pathways and sibling neuroblastoma cells frequently have identical or mirror-image morphology (*16*). The determinants of these morphogenetic behaviors appear to be encoded intrinsically within the cells themselves (*17*). In addition to such cell-intrinsic influences on cell behavior, environmentally imposed restrictions of morphogenetic movements (*6, 18*) could serve to sweep all of the clonal progeny of a single gastrula cell into the same tissue anlagen.

REFERENCES AND NOTES

1. D. Grunwald, G. Streisinger, C. Walker, *J. Cell. Biochem. (suppl).* **9b**, 270 (1985).
2. C. B. Kimmel and R. D. Law, *Dev. Biol.* **108**, 94 (1985).
3. ———, *ibid.*, p. 78.
4. An abstract describing some of this work has been published: C. B. Kimmel, R. Warga, R. D. Law, *Soc. Neurosci. Abstr.* **10**, 1044 (1984).
5. D. A. Weisblat, S. L. Zackson, S. S. Blair, J. D. Young, *Science* **209**, 1538 (1980).
6. J. P. Trinkaus, *Am. Zool.* **24**, 673 (1984).
7. P. Z. Myers, *J. Comp. Neurol.* **236**, 555 (1985).
8. J. S. Eisen, P. Z. Myers, M. Westerfield, *Soc. Neurosci. Abstr.* **10**, 371 (1984).
9. C. B. Kimmel and R. M. Warga, in preparation.
10. J. A. Weston, in *Cell Interaction and Development*, K. M. Yamada, Ed. (Wiley, New York, 1983).
11. The blastomere was injected with a conjugate of rhodamine-isothiocyanate and horseradish peroxidase (*2*). Later the fluorescent cells were viewed with low illumination at 10- to 30-minute intervals with a General Electric silicon-intensified target video camera. This procedure minimized photodynamic damage to the labeled cells [S. B. Kater and R. D. Hadley, in *Cytochemical Methods in Neuroanatomy*, V. Chan-Palay, Ed. (Liss, New York, 1982); J. P. Miller and A. I. Silverstein, *Science* **206**, 702 (1979)]. The appearance of the clone was recorded on video tape at each viewing. At 24 hours, the embryo was fixed, serially sectioned at 50 μm, and processed histochemically (*20*) to reveal peroxidase labeling of the cells.
12. G. S. Stent, D. A. Weisblat, S. S. Blair, S. L. Zackson, in *Neuronal Development*, N. Spitzer, Ed. (Plenum, New York, 1982).
13. J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomson, *Dev. Biol.* **100**, 64 (1983).
14. H. Nishida and N. Satoh, *ibid.* **99**, 382 (1983).
15. M. Jacobson, *J. Neurosci.* **3**, 1019 (1983).
16. G. Albrect-Buehler, *J. Cell Biol.* **72**, 595 (1977); F. Solomon, *Cell* **16**, 165 (1979).
17. F. Solomon, *J. Cell Biol.* **90**, 547 (1981).
18. W. L. Long, *BioScience* **34**, 84 (1984).
19. In each of the examples in Fig. 1 the founding blastomere was injected intracellularly with the tracer fluoresecein isothiocyanate dextran (Sigma) as described (*3*), under conditions that were expected (*2*) to yield specific labeling of a normally developing clone of cells. The embryos were maintained at about 28°C.
20. W. K. Metcalfe, C. B. Kimmel, E. Schabtach, *J. Comp. Neurol.* **233**, 377 (1985).
21. A. N. DeBelder and K. Granath, *Carbohydrate Res.* **30**, 375 (1973).
22. G. M. King, K. Karmali, L. J. Biberman, *J. Exp. Zool.* **220**, 147 (1982).

23. We thank P. Sternberg, J. Trinkaus, D. Weisblat, and our Oregon colleagues for their comments and criticisms. Supported by NSF grant BNS-811247.

26 April 1985; accepted 27 November 1985