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CELL LINEAGE IN THE DEVELOPMENT OF INVERTEBRATE NERVOUS SYSTEMS

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Historical Background

Studies of developmental cell lineage, i.e. of the fate of individual blastomeres that arise in an embryo, were begun in the 1870s, in the context of the controversy then raging about the "biogenetic," or "recapitulation," law promulgated by Ernst Haeckel. The biogenetic law seemed to imply that the early, pre-gastrula stages of metazoan embryogenesis recapitulate the nondifferentiated condition of a remote colonial ancestor. Hence, prior to gastrulation, all blastomeres should be of equivalent developmental potency. Only after gastrulation would particular domains of the embryo become committed to the differentiated tissues characteristic of more recent metazoan ancestors. This implication was tested by a group of American biologists, led by Charles O. Whitman (1878, 1887). By observing the cleavage pattern of early leech embryos, Whitman traced the fate of individual blastomeres, from the uncleaved egg to the gastrular germ layer stage and concluded that, contrary to the simplest interpretation of the biogenetic law, even the earliest blastomeres are developmentally distinct and that each identified blastomere, and the clone of its descendant cells, plays a specific role in later development.

Whitman set the pattern for all subsequent lineage studies. His disciples, including such future leaders of American cell biology as E. B. Wilson, E. G. Conklin, and F. R. Lillie, studied the embryos of other annelids, ascidians, and molluscs. Comparisons of their data revealed significant cross-phyletic similarities, as well as differences, in developmental cell lineage relations. Hence they concluded that there must be some relation between ontogeny and

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phylogeny, but that that relation cannot be one of simple recapitulation. [A full account of these origins of cell lineage studies has been provided by Maienschein (1978).] The study of developmental cell lineage went into decline during the subsequent half century. In fact, cell lineage analyses are still not mentioned in most contemporary textbooks of embryology. One notable exception to the eclipse of cell lineage studies occurred in the 1920s, when A. H. Sturtevant (1929) devised a genetic method for mapping the developmental fate of cells of the *Drosophila* embryo. But it was only in the 1960s and 1970s that there occurred a revival of interest in the role of cell lineage in development, accompanied by the invention of more precise and far-reaching analytical techniques.

Conceptual Background

FATE MAPS AND CELL LINEAGE Before reviewing the results of recent cell lineage analyses, we attempt to clarify a few developmental concepts and problems to which these studies pertain. [An excellent explication of the concepts of experimental embryology is given in a recent monograph by Slack (1983), on which part of the following discussion is based.] A *fate map* is a diagram that shows what becomes of each region of an embryo in the course of subsequent normal development to some arbitrarily chosen endpoint of maturity. *Cell lineage* analysis is a form of fate mapping in which a single cell and its clone of progeny is followed. Two principal methods are available for cell lineage analysis. One consists of continuous observation of the entire course of development, following the blastomeres and their progeny visually all the way to the endpoint tissues. This method can be used only as long as the embryo remains transparent to the chosen endpoint and consists of a relatively small number of cells. The other principal method consists of the labeling of a specific blastomere at an early developmental stage and examining the location and distribution of the label at the endpoint. This is the method that must be used when the embryo is insufficiently transparent or the number of cells is too large to permit tracing of their fate by direct visual observation.

ABLATION In addition to these two principal methods of lineage analysis there is a third method, which consists of ablating a specific cell of the embryo and noting which particular organs and tissues are missing at the endpoint. The missing parts might then be inferred to represent the normal fate of the ablated cell. Strictly speaking, this method does not provide a real fate map, since ablation precludes normal development. On the one hand, an organ or tissue might be missing at the endpoint, not because its precursor cell had been ablatcd, but because an interaction with the cell that was ablated, or its

normal progeny, is needed for the precursor cell to express its normal fate. On the other hand, an organ or tissue might be present at the endpoint, even though its normal precursor cell had been ablated, because it arose from another cell among whose progeny it is not normally included. Nevertheless, even though the ablation method cannot yield definitive information regarding the normal fate of embryonic cells, it may provide suggestive data. And in case a fate map has been established by either of the other methods, the ablation method can be used to probe the possible role of interactive or regulative processes in the determination of normal fate.

COMMITMENT A central focus of interest in the study of fate maps, especially at the cell lineage level, is the process by which a cell is *committed,* or commits its descendants, to express some trait *A* rather than another trait *B*. The differential commitment to *A* is said to be *clonal* if a group of cells expresses it that comprises all the descendants of a single ancestor cell. The concept of differential commitment implies that the cell has taken on a state R_A, which persists and which at some later time is bound to lead to another state S_A in some (or all) of its descendants, sufficient for expression of *A*. Suppose that under the conditions of normal development all of the descendants of the cell express *A* and none *B*. Does this mean that the cell had entered state R_A and was thus differentially committed? This question has no empirical answer, unless a set of *abnormal* developmental conditions, such as tissue transplantations or explantations, ablations of neighbors, or perfusion, is specified under which R_A still persists and leads to state S_A. If the cell responds to such abnormal conditions by giving rise to descendants that express *B* rather than *A*, the cell is judged to have been in a reversible state, and hence differentially uncommitted with respect to traits *A* and *B*. But if, despite these interventions, the descendants of the cell still express only *A*, then the cell can be said to be committed under those experimental conditions which did not result in the expression of *B* rather than *A*. (Of course, it is always possible that a new set of conditions of abnormal development can be found under which the cell would not be committed to the expression of *A*.) In this latter case the expression of *A* can be said to be *autonomous,* in the sense that the persistence of state R_A and the path leading from it to state S_A does not require the entire set of conditions to which the cell is exposed in normal development.

MODELS The significance for normal development of empirical tests for commitment under abnormal conditions lies in their use for distinguishing between different models of commitment, of which the two most common are the following. One model envisages that taking on state R_A (differential commitment to *A*) requires an *intracellular determinant, a,* whereas taking on state R_B requires another determinant *b*. A pluripotent cell contains and passes on

to its descendants both determinants *a* and *b*, and a differential commitment to *A* (and a restriction of potency for *B*) occurs at an asymmetric cell division in which at least one of the daughter cells received only *a*. These intracellular determinants could be cytoplasmic structures or molecules that are distributed anisotropically in the egg. The determinants could also be nuclear structures, especially parts of the DNA, that are differentially modified in successive cell divisions and passed on in that modified form. Under this model cell lineage would play a crucial role in differential commitment, because the line of descent of any cell would govern which particular subset of intracellular determinants has been passed on to it.

The other model envisages that taking on states R_A or R_B depends on the anisotropic distribution of *intercellular inductive signals* α and β over the volume of the embryo. A pluripotent cell is capable of responding to either inductive signal, and once having responded to α at some crucial stage of development it has taken on state R_A. These intercellular inductive signals could be electrical potentials, diffusible molecules, or nondiffusible surface structures that signal by direct contact. Here cell lineage would play a role in differential cell commitment, because the line of descent of a cell would govern its position in a determinant field, and hence the set of inductive signals to which it is exposed at the critical stage of normal development. As formulated here, the intracellular determinant model equates differential commitment with restriction of potency. If both models are combined, however, i.e. if taking on state R_B (differential commitment to *B*) requires an interaction of determinant *b* with signal β, the potency for expression of *B* can be independent of the differential commitment to state R_A.

MODES OF CELL DIVISION Another important focus of interest in cell lineage analyses is the mode of division by which a cell gives rise to its clone of descendants. There are three principal modes:

1. The *proliferative* mode, under which a cell divides symmetrically to produce two equal daughter cells, both of which also divide symmetrically.
2. The *stem cell mode*, under which a cell of type A divides asymmetrically to give rise to two unequal daughters, of which one is of type A and the other is of type B. The (regenerative) daughter of type A divides again, as did its mother cell, to yield one daughter of type A and one of type B, and division can be said to proceed according to a *parental reiteration pattern*. Under one variant of the stem cell mode, the regenerative daughter cell is of type A′, different from A but dividing asymmetrically to yield one daughter of type A and another of a fourth type, C. This variant of the stem cell mode is referred to as a *grandparental reiteration pattern* (Chalfie et al 1981).

3. The *diversification mode,* under which a cell of type A divides to yield two unequal daughters of types B and C, neither of which ever gives rise again to a cell of type A. The diversification mode is a characteristic feature of early embryogenesis in invertebrates. Usually it terminates upon the generation of daughter cell types which, if they divide at all, do so according to either the proliferation or stem cell modes. In embryos of annelids and mollusks, where the stem cell division mode plays a prominent role in development, blastomeres that divide according to that mode are designated *teloblasts*.

Nematodes

The entire cell lineage is now known for the nematode *Caenorhabditis elegans,* thus completing a project that was begun late in the last century by another group of pioneering students of cell lineage (Boveri 1887, 1892, zur Strassen 1896; for review cf von Ehrenstein & Schierenberg 1980). Despite being built of only 810 nongonadal (i.e. somatic) cells, *C. elegans* contains the principal metazoan tissue types, such as nerve, muscle, epidermis, and intestine. The number of cells is constant in all somatic tissues, and in the case of the nervous system, the organization of its 302 cells is known also in its ultrastructural details (White et al 1976, 1983). The nervous system includes a cephalic part, with sensory sensilla and their nerves, a circumpharyngeal nerve ring, as well as a dorsal and ventral nerve cord, and a variety of sensory organs and ganglia.

The fertilized nematode egg cleaves asymmetrically in the diversification mode to generate a set of blastomeres designated as *founder cells* AB, MS, E, C, D, and P_4, of which AB is removed by one division, MS, E, and C by three divisions, and D and P_4 by four divisions from the uncleaved egg, respectively. Embryonic development culminates in the hatching of a larva, designated as L1, comprised of 550 nongonadal cells. Postembryonic development continues through three more larval stages (L2–L4) to the sexually mature, adult worm with its 810 nongonadal cells. In postembryonic development, the 260 additional somatic cells, including 61 neurons, are generated according to a stereotyped lineage pattern, as descendants of 55 blast cells carried over from the embryo to the larva.

The complete description of developmental cell lineage in the nematode was accomplished by continuous observations of living embryos and larvae, using time-lapse video recording and Normarski differential interference contrast optics (Deppe et al 1978, Sulston & Horvitz 1977, Kimble & Hirsch 1979, Sulston et al 1983). Of the founder cells, AB is the largest single contributor of somatic cells. During embryogenesis, 214 of the 222 neurons of the newly hatched L1 larva derive from AB, as well as a substantial fraction

of the cells of the hypodermis and of the pharyngeal and trunk muscles. Moreover, all of the neurons formed postembryonically are derived from blast cells descended from the AB founder cell. The next largest single contributor to somatic cells is MS, which gives rise mainly to tissues regarded as mesodermal, including muscles, glands, and coelomocytes, and had been designated as the mesodermal founder cell. However, just as the mainly ectodermal founder AB includes muscles among its descendants, so does the mainly mesodermal founder MS include six neurons among its descendants. A single blast cell descended from MS accounts for all of the mesoderm produced postembryonically. Of the remaining founder cells, C gives rise to muscles and hypodermis, as well as to the remaining two prelarval neurons; D gives rise exclusively to muscles, E exclusively to intestine (i.e. endoderm), and P_4 to the germ line (whose cell lineage is not included in this review).

In *C. elegans,* the sequence of events leading from each founder cell to the differentiated, postmitotic cells of larva and adult is highly invariant with respect to timing and equality or inequality of cell divisions, as well as relative cell positions and movements. This invariant sequence also includes the death of identifiable cells at exactly defined stages of development. There are some exceptional groups of cells, however, whose fates are not invariant. Each such group is called an *equivalence group,* whose members resemble each other closely in structure and function and are usually of similar origin (Kimble et al 1979). Some equivalence groups consist of a bilaterally symmetric pair of cells, which move to the midline and meet; subsequently, one cell (sometimes from the left and sometimes from the right) takes on one particular fate while the other takes on another fate, suggesting the intervention of an element of chance in the alternative commitment of two equally pluripotent cells.

To learn at which stage of the invariant lineage pathway there occurs commitment to the normal fate, ablation experiments have been carried out, in which various identified cells were killed by irradiation with a laser microbeam (Sulston & White 1980, Kimble 1981, Sulston et al 1983). The result of the majority of these ablation experiments was that those cells, and only those cells, failed to develop in the lesioned embryo which, on the basis of the fate map, are known to be the normal descendants of the ablated cell. Thus, commitment to developmental fate appears to proceed autonomously in most cell lineages, there being neither regulative restoration of the ablated cell line from an as yet uncommitted, abnormal source, nor a need for an inductive interaction of another cell line with the ablated line to become committed to its normal fate. However, in a minority of the experiments a different result was obtained. These cases were used to define equivalence groups, in which another cell of the group may abandon its normal fate and take on the fate of the missing cell (designated in this case as the *primary* fate of one group). This result shows that commitment to a particular fate among the initially

equally pluripotent members of an equivalence group is the result of intercellular interactions. The alternative of autonomous versus interactive commitment applies also when the fate in question is cell death: in some cases an identified cell normally destined to die will do so at the normal time, regardless of ablation of any neighboring cell ("suicide"), whereas in other cases the normally moribund cell survives if a particular neighboring cell has been ablated ("murder").

Comparison of the cell lineages of *C. elegans* with lineages that have been partially elucidated in other nematode species, such as *Turbatrix aceti, Panagrellus redivivus,* and *Aphelencoides blastophorus* (Sulston et al 1983, Sternberg & Horvitz 1982), reveals striking similarities in developmental pattern. Moreover, morphological differences by which one species is distinguished taxonomically from another have been traced in several instances to focal differences in otherwise homologous cell lineages, such as the death or terminal differentiation in one species of an identified cell, whose homolog undergoes further divisions in another species.

To apply genetic techniques to the study of cell lineages in *C. elegans,* mutants were isolated in which normal development is disrupted (Sulston & Horvitz 1981, Chalfie et al 1981, Greenwald et al 1983). Many of these mutant strains display widespread abnormalities, which are the likely consequence of some general disturbance of cell function. But some mutant strains were found, in which the mutation causes specific alterations of particular cell lineages, while leaving other lines of descent apparently unaffected. One such group of mutations, which occur at a genetic locus designated *lin*-4, induces three different kinds of supernumerary divisions, or lineage reiterations, in various postembryonic blast cell lines. The first, and simplest type of reiteration, equivalent to the proliferative division mode, consists of supernumerary equal divisions by an ordinarily postmitotic cell, leading to a geometric increase in the number of (presumably) equivalent supernumerary cells. The second and third types of lineage reiterations arise from the conversion of an ordinarily postmitotic cell into a stem cell, dividing either in the parental or grandparental reiteration pattern, respectively. Another group of lineage-specific mutations, mapped to the *unc*-86 locus, induces abnormal reiterative cell lineages in several postembryonic blast cell lineages. The mutations at both the *lin*-4 and the *unc*-86 loci are recessive; thus their mutant phenotype is likely to be the result of a reduction in activity, or loss of a gene product. This finding suggests that the capacity for lineage reiteration is latently present in the normal wild type strain, where its expression is specifically suppressed by the products of the normal alleles of the mutant loci.

Another genetic locus, designated *lin*-12, has also been found relevant for the cell lineage pattern. The effect of mutations within *lin*-12 is analogous at the cellular level to the effect of homeotic mutations at the tissue level, as

known in insects, especially in *Drosophila* (Morata & Lawrence 1977, Ouweneel 1976). Homeotic mutations cause one group of cells to adopt the fate normally associated with another group, resulting in the transformation of one structure into another. Mutations at the *lin*-12 locus effect a number of such transformations throughout the embryo, especially between members of equivalence groups, both within neural and nonneural cell lineages and between neural and nonneural lineages. By shifting a homozygous mutant embryo carrying a temperature-sensitive mutation at the *lin*-12 locus from the permissive to the restrictive temperature at various developmental stages, it was found that the time at which the mutant gene acts to induce the transformation of cells in an equivalence group corresponds just to the stage at which the pluripotent cells of the group become committed to a particular fate, as defined by their lack of response to laser ablation of a fellow-member of their group.

Although most of the cells affected by mutations at the *lin*-12 locus are members of equivalence groups, some of them are not. In fact, one example of the effect of mutations at the *lin*-12 locus on the development of the nematode nervous system is provided by two bilaterally homologous descendants of founder cell AB, whose commitment appears to be autonomous, as judged by laser ablation experiments. Normally, the right homolog becomes a motor neuron, designated PDA, and the left homolog becomes a different motor neuron, designated DA9. But in animals homozygous for a semidominant mutation at *lin*-12, both cells take on the PDA fate. By contrast, in animals homozygous for a recessive null mutation at *lin*-12 (i.e. one that eliminates the gene product altogether), both cells take on the DA9 fate. Such findings led Greenwald et al (1983) to suggest that, in analogy with the proposals made for the function of homeotic mutant loci in *Drosophila* (Morata & Lawrence 1977), "*lin*-12 functions as a binary switch to control decisions between alternative cell fates during *C. elegans* development."

Leeches

The total number of somatic cells in the leech is several orders of magnitude greater than in *C. elegans;* so the kind of total cell lineage analysis now available for the nematode seems out of reach for the leech. Indeed, such an undertaking might not even be meaningful for animals in which, unlike in the nematode, the number of cells making up a particular organ or tissue, such as muscle or epidermis, is variable from specimen to specimen. Nevertheless, the prospect of establishing a very extensive genealogy of the approximately 15,000 cells of the leech CNS is by no means out of sight. Each of the 32 segmental ganglia of the leech ventral nerve cord is composed of about 400 bilaterally paired neurons, eight paired giant glial cells, as well as a few unpaired neurons (Muller et al 1981). Since most of these neurons are serially homologous, not only with respect to their traits, but also, insofar as is pres-

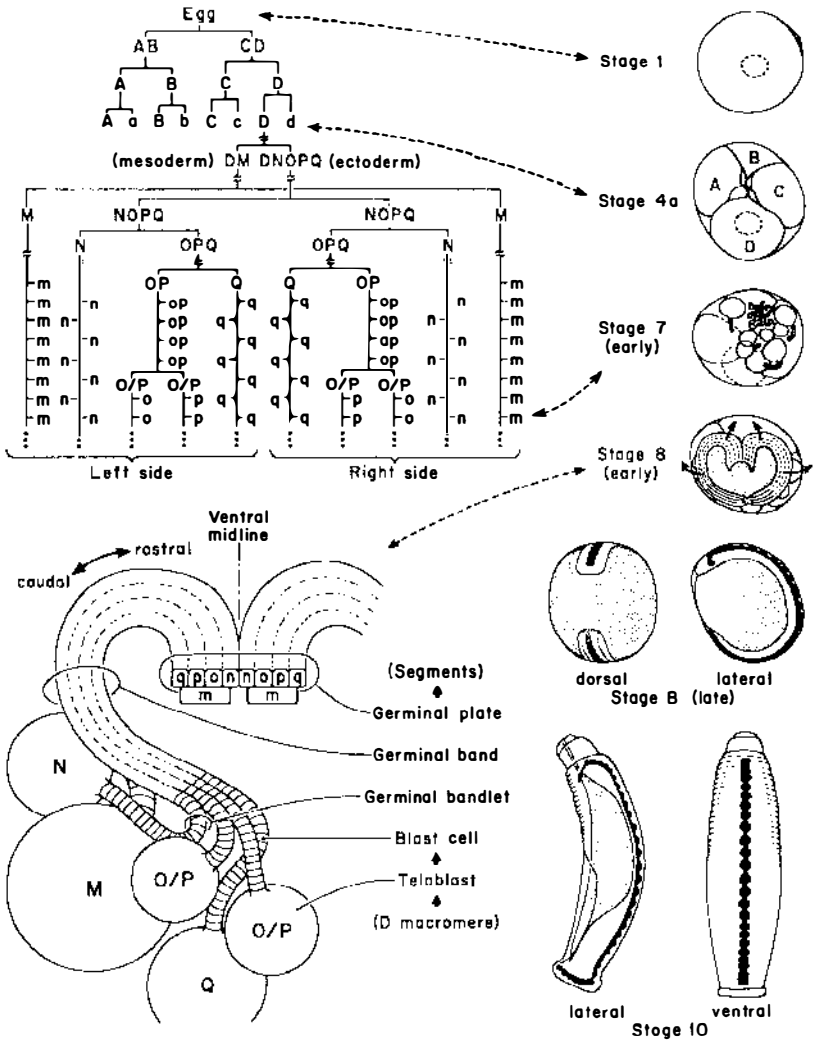
ently known with respect to their embryonic lines of descent, the cell lineage analyst of the leech CNS seeks to account for the origins of about 200 cell types, a task comparable in magnitude to that already achieved for the nematode nervous system. (Reviews of leech development can be found in Weisblat 1981, Stent et al 1982, Fernandez & Olean 1982.)

The initial cleavages divide the leech egg in the diversification mode into four large cells, macromeres A, B, C, and D. Each macromere buds off a micromere at the animal pole, designated by the corresponding lower case letter a, b, c, or d. Cell D continues to divide in the diversification mode to yield five bilateral blastomere pairs, namely M, N, Q, and two sister pairs, both designated O/P. Separation of the embryo into the three germinal layers has now been accomplished: A, B, and C give rise to endoderm, N, Q, and the O/Ps to ectoderm, and M to mesoderm (Whitman 1887). The paired M, N, O/P, O/P, and Q blastomeres divide in the stem cell mode, and hence are designated as teloblasts. Each of them carries out a series of 40–100 highly asymmetric divisions, producing a bandlet of *primary blast cells*. The bandlets merge on either side of the midline to form the right and left *germinal bands*. In either band, the mesodermal bandlet lies under the four ectodermal bandlets. Gastrulation is represented by a movement of the right and left germinal bands over the surface of the embryo and their coalescence on the ventral midline to form the *germinal plate,* in which the superficial ectodermal blast cell bandlets lie in mediolateral order n, o, p, q. (In the case of the M, N, and Q teloblasts, their primary blast cells and bandlets are designated by lower case letters corresponding to their own upper case letter; in the case of the two sister O/P teloblasts, their primary blast cells and bandlets are designated o and p, according to which bandlet lies proximal and which distal, respectively, to the n bandlet.) The germinal plate is partitioned along its length into a series of tissue blocks, each separated from its neighbors by transverse septa. Each block corresponds to a future body segment, including a globular ganglion containing about the same number of cell bodies as an adult ganglion.

To establish the line of descent of identified cells of the leech CNS, Whitman's century-old cell lineage studies were refined and extended, by means of a novel tracer technique (Stent et al 1982). This technique consists of injecting a tracer molecule, such as horseradish peroxidase (HRP) or a fluorescent dye conjugated to a large carrier molecule, into an identified cell of the early embryo, allowing embryonic development to proceed to the endpoint, and then observing the distribution pattern of the tracer within the tissues (Weisblat et al 1978, 1980a, 1980b).

Use of the lineage tracer technique has shown that the leech CNS is derived from all five teloblast pairs. The progeny of the N teloblast are found almost exclusively within the ganglia of the ventral nerve cord. Descendants of the O/P and Q teloblasts, however, give rise to characteristic patterns of cell

clusters, both in the segmental ganglia and in the epidermis. The dorsal aspect of the segmental epidermis is derived from the ipsilateral Q teloblast and the ventral aspect mainly from the ipsilateral O/P sister teloblasts, with the N teloblast providing a few epidermal cells on the ventral midline (Weisblat et al 1984). In addition, three or four paired neurons of the ganglion are derived from the mesodermal teloblast M, whose main contribution is made to tissues and organs to which a mesodermal origin is generally assigned, namely connective tissue, nephridia, and muscle.



The tracer technique showed that individual segments arise as cell clones, each segment being founded by a fixed number of primary blast cells: one primary m, o, and p blast cell and two primary n and q blast cells per half segment on each side. Of the two primary n and q founder blast cells, one has a *mixed* fate, giving rise to descendants in both the CNS and periphery, while the other has a *pure* fate: purely CNS for n and purely peripheral for q (Zackson 1982, 1984, Weisblat & Shankland 1984). To give rise to its segmental complement of descendants, each primary blast cell divides according to a stereotyped and lineage-specific manner. In the underlying m bandlet pair, blast cell divisions occur in rapid succession in three dimensions, so that the clones of m-derived progeny form a chain of isomorphic, protosegmental clusters. In the overlying ectodermal bandlets, each of the four bandlet pairs is characterized by its own unique cell division pattern. In the n and q bandlets, moreover, there occur two alternating types of primary blast cell divisions, corresponding to the two different types of founder blast cells per segment. Later blast cell divisions show a diversity of characteristic patterns as well, including both proliferative and stem cell division modes (Zackson 1984). These findings suggest that the primary blast cells of different bandlets are endowed by their parent teloblasts with different states of commitment that cause them to follow lineage-specific pathways of cell division, and hence to take on different lineage-specific fates. In the case of the N and Q teloblasts, moreover, it would appear that each of them passes on in alternating sequence two different states of commitment to its daughter blast cells, and hence divides according to the grandparental reiteration pattern of the stem cell mode.

Figure 1 Schematic summary of the development of the leech *Helobdella triserialis*. *Upper left:* Cell pedigree leading from the uncleaved egg to the macromeres A, B, and C; the micromeres a, b, c, and d; the teloblast pairs M, N, O/P, O/P, and Q; and the paired primary blast cell bandlets. Breaks in the lineage indicate points where additional micromeres may be produced. The number of op blast cells produced prior to cleavage of proteloblast OP varies from four to seven. *Lower left:* Hemilateral disposition of the teloblasts and their primary blast cell bandlets within the germinal band and germinal plate. *Right margin:* Diagrammatic views of the embryo at various stages. The *dashed circle* in the uncleaved egg (stage 1) signifies the right M teloblast (which is invisible from the dorsal aspect), and the many small, closed contours in the upper midportion indicate the micromere cap. In the stage 8 (early) embryo, the heart-shaped germinal bands migrate over the surface of the embryo in the directions indicated by the *arrows*. The incipient larval integument is shown as a *stippled area* lying in between. In the stage 8 (late) embryo the germinal plate is shown to be on the ventral midline, with the nascent ventral nerve cord and its ganglia and ganglionic primordia indicated in black. The stippled larval integument covers the entire embryo, from one edge of the germinal plate to the other. In the stage 10 embryo shown, body closure is nearly complete. Here, the *stippled areas* signify the yolky remnant of the macromeres and teloblasts, now enclosed in the gut of the embryo. The chain of ganglia linked via connectives, shown in black, already closely resembles the adult nerve cord (from Weisblat et al 1984).

The ganglionic subpopulations of neurons arising from each teloblast pair form five identifiable neuronal *kinship groups:* M, N, O, P, and Q (Stent et al 1982, Weisblat et al 1984). (The kinship groups designated as O and P refer to those descendants of the O/P sister teloblasts which are respectively derived from the o and p bandlets.) Combined use of the fluorescent lineage tracers with electrophysiological, anatomical, and histochemical identification techniques showed that each kinship group invariably contains a particular set of identified neurons and glial cells, along with a number of as yet unidentified neurons (Kramer & Weisblat 1984). Thus far, no unique set of traits has come to light that separates the members of one kinship group from another, such as functional category (glia or sensory, motor or interneuron) or type of neurotransmitter synthesized, except that all serotonergic neurons belong to group N and all dopaminergic neurons to groups O, P, and Q.

In addition to its 32 segmental ganglia, the leech CNS has a supraesophageal ganglion at its rostral end. The origin of that ganglion has long been the subject of controversy (Whitman 1892), but it has now been shown that these frontmost cells of the CNS arise from the a, b, c, and d micromeres, rather than from the blast cells of the germinal bands (Weisblat et al 1980a, 1984). This finding indicates that the supraesophageal ganglion of leeches is developmentally homologous to the much more elaborate supraesophageal ganglion of polychaetes, which is known to arise as the neural tissue of a nonsegmented larva entirely derived from the micromeres (Dawydoff 1959).

Cell lineage analyses thus indicate that in the leech, neurogenesis is as highly determinate as it is in the nematode. Ablation experiments show here, too, that commitment to cell fate is largely, but not wholly, autonomous. Ablation of a teloblast of the early embryo by intracellular injection of a toxic enzyme results in an embryo whose nervous system lacks all those identified neurons which are normally derived from that teloblast (Weisblat et al 1980b, Blair & Weisblat 1982, Blair 1983). (The special cases of the O/P sister teloblasts and of unpaired neurons are considered below.) But in the defective ganglia of such embryos, the positions of the neurons that are present may be highly abnormal, and in some cases even the neurons normally descended from a nonablated teloblast may be missing (Blair & Weisblat 1982, Weisblat et al 1980b). These findings indicate the role of a morphogenetic interaction during gangliogenesis between the blast cells derived from different bandlets. In contrast to the lack of restoration of identified neurons following ablation of the teleoblast from which these neurons are normally derived, some kind of regulative restoration does occur from blast cell progeny derived from other bandlets for the portion of the epidermis normally derived from that very same teleoblast (Blair & Weisblat 1984). Hence the developmental commitment of a given primary blast cell is more autonomous in regard to its neural than to its epidermal fate.

The consequence of direct ablation of individual blast cells rather than of their teloblast progenitors can be studied by focal photoablation of cells labeled with fluorescent lineage tracers (Shankland 1984). This method can provide information regarding cell lineage relations among the descendants of primary blast cells (subject to the usual limitations placed on the inference of fate maps from ablation data). The method can be used also to induce rearward slippage of one bandlet relative to the other bandlets. By means of such slippage the cause of death of the last, supernumerary blast cells produced by each teloblast in excess of the number needed to found the 32 body segments (Fernandez & Stent 1982, Zackson 1982) has been clarified: blast cells that would, on the basis of their birth rank, have survived in a normal embryo and participated in segment formation, degenerate along with the other supernumeraries if they slip rearward into positions behind the caudal end of the germinal band. Hence the birth rank of a primary blast cell does not commit it autonomously to survival or death; rather its fate is decided by an inductive signal received after entrance into or exclusion from the germinal band (Shankland 1984).

Notwithstanding the highly determinate fate of each teloblast in the course of neuronal development, the primary blast cells derived from the O/P sister teloblasts can interchange their fate and thus form an equivalence group. In the leech such interchange has been designated "transfating" (Weisblat & Blair 1984). The underlying cause of O/P transfating is that the bandlets of primary blast cells generated by either of the two sister teloblasts may take either position in the germinal band, and that the blast cells are committed to their specific fates, designated as O fate and P fate, only after the bandlet has come to lie in either the o or p position (Weisblat & Blair 1984, Shankland & Weisblat 1984). But upon ablating either of the O/P sister teloblasts, progeny of the surviving teloblast take on the P fate (Weisblat & Blair 1984). Thus, in the parlance of nematode lineage analysis, the P fate is the primary fate of the O/P equivalence group. Cytological examination of the bandlets has shown that the commitment to these alternative fates occurs within the first few divisions of the blast cell, once it lies in the germinal band (Zackson 1984, Shankland & Weisblat 1984).

The unpaired interneurons of the segmental ganglion provide another example of an equivalence group. Initially, an unpaired interneuron is present as a bilateral pair of cells, of which one member later dies. Lineage tracers show that the teloblast of origin, right or left, of the surviving member of the pair varies randomly from ganglion to ganglion, and from specimen to specimen. Moreover, upon ablating one of the parent teloblasts, none of the unpaired interneurons is missing (Blair & Stuart 1982, Blair 1983). Hence, in the formation of the ganglionic primordia, both right and left primary blast cells give rise to an interneuronal precursor cell, and by random outcome of a

competitive process one interneuronal precursor cell is committed to survival and the other to death.

Insects

The late developmental stages and adult forms of insects and leeches demonstrate homologous segmental body plans. This homology is particularly obvious in the case of the CNS. In insects, as in leeches, the CNS consists of a ventral nerve cord composed of a chain of bilaterally symmetric, metameric ganglia, joined via longitudinal connective nerves. The dorsally situated brain seems homologous to, but very much more elaborate than, the supraesophageal ganglion of leeches. In embryogenesis each ganglion arises as a distinct pair of primordia—one neuromere per segment—but as development proceeds some ganglia fuse to give rise to the rostrocaudally differentiated adult CNS. However, the initial stages of embryogenesis in insects are radically different from those of leeches. The insect egg begins its development with a series of synchronous mitotic divisions of the zygote nucleus without cell cleavage. This process gives rise to an embryonic syncytium containing thousands of nuclei. Eventually most of these syncytial nuclei migrate to the periphery of the egg, where each nucleus becomes enclosed by an infolding of the egg cell membrane. Thus the insect embryo comes to consist of a uniform sheet of several thousand cells, the *blastoderm,* on its outer surface, which encloses an acellular yolky interior. The cells of the blastoderm continue the process of proliferation and differentiation. Along the length of the ventral aspect of the embryo there eventually forms a multilayered cell structure designated as the *germ band,* of which the outer and inner layers correspond to ectoderm and mesoderm, respectively. The germ band shows clear signs of segmentation and already reflects the later body plan. In its general structure and role in subsequent embryogenesis the insect germ band is evidently homologous to the germinal plate of leeches, even though the two structures are generated by radically different processes in early embryogenesis.

GRASSHOPPER Cell lineage analyses have been carried out by visual observation for the final stages of development of the segmental ganglia in the grasshopper, onwards from the germ band stage (for review cf Goodman 1982). As in the case of the germinal plate of the leech, the ectodermal cell layer of the insect germ band that gives rise to the CNS extends longitudinally, flanking the body midline. Within that cell layer, some cells become recognizable as neuronal precursors: they round up and enlarge relative to other ectodermal cells. In the grasshopper embryo two types of such neuronal precursors can be identified: one type, designated *neuroblast,* or NB (Bate 1976), is a stem cell, and the other type, which is not a stem cell, is designated *midline precursor,* or MP (Bate & Grunewald 1981). The number of both types

of neuronal precursors per segment is fixed. There are two bilaterally paired sets of 30 unique NBs each, arranged in seven transverse rows, plus one unpaired NB, designated MNB, lying on the midline at the posterior margin of the segment. And there are seven MPs, lying in a stereotyped arrangement along the midline, of which two, designated MP2L and MP2R, are bilaterally paired, and the remainder, numbered in rostrocaudal order MP1, MP3, MP4, MP5, and MP6, are unpaired. Upon subsequent development each MP divides only once to produce two daughter cells straddling the midline (in the case of the unpaired cells) or a pair of dorsoventral two-cell stacks (in the case of the paired MP2 cells). By contrast, each NB undergoes a series of stem cell divisions, to give rise to a chain of smaller cells designated *ganglion mother cells,* or GMCs, each of which, in turn, divides once to produce a pair of daughter cells designated *ganglion cells,* or GCs, which eventually differentiate into neurons. Thus each NB contributes a clone of prospective neurons to the CNS; ultimately, all NBs die and degenerate, with some of them having contributed as few as 10 and others as many as 100 neuronal progeny to their ganglion. According to recent experiments, each NB_x arises from an equivalence group of a few neuroepithelial cells, of which any one may become NB_x, but no other NB. By the time that NB_x has made its first division, however, other members of its equivalence group can no longer replace it (Taghert et al 1984).

The paired descendants of the MPs are the first to extend neuronal processes from their cell bodies. Each pioneers a characteristic and segmentally stereotyped, central or peripheral, hemilateral or bilateral, axonal pathway (Bate & Grunewald 1981, Goodman et al 1981). Of the MP3 pair, one sister differentiates into a neuron designated as cell H, identifiable on the basis of its characteristic bilateral axonal branching pattern, whereas the other sister projects only hemilaterally (Goodman & Spitzer 1979, Goodman & Bate 1981). As the MNB stem cell carries out its iterated divisions, the string of GMC progeny cells advances anteriorly. Each GMC divides once to produce a GC pair that straddles the midline.

The first six MNB-derived GC pairs differentiated into 12 identified neurons, designated collectively as *dorsal unpaired medial neurons,* or DUM. They project their axons bilaterally, with the axons of each pair initially following the axonal pathway previously laid down by one or another of the MP cell pairs. Any two sister DUM cells initially project their axons along the pathway laid down by the same MP pair, but eventually their axonal branching patterns diverge, resulting in the generation of two identifiably different neurons. However, which of the two DUM sisters, right or left, develops which of the two different branching patterns depends on which sister happens to have been the first to extend an axonal growth cone from its cell body. Thus they form an equivalence group. The DUM neurons share one striking bio-

chemical characteristic: they all contain the neurotransmitter, octopamine (Evans & O'Shea 1977, Goodman et al 1979), and it is likely that they are the only neurons in the segmental ganglia that do.

Cell lineage analyses have also illuminated the problem of how the segmentally iterated sets of neuronal precursor cells in the germ band give rise to adult ganglia that are specialized to function in the regionally differentiated body segments of the adult insect. For example, whereas the ganglia of the three thoracic segments (T1, T2, and T3) each contain about 3000 neurons, the ganglia of the 11 abdominal segments (A1–A11) each contain only about 500 neurons. Much of this difference in cell number per ganglion is attributable to cell death during embryogenesis (Bate 1982, Bate et al 1981): after the NBs have already produced their crop of descendants, the degeneration of hundreds of cells can be observed visually in each embryonic abdominal ganglion. Specifically, in the adjacent segments T3 and A1, the homologous MNB stem cells give rise to about 100 and 90 descendants, respectively. But in segment T3 all 100 descendants survive, while as many as 45 descendants die in segment A1, among them several of the identifiable DUM cells. Death of the abdominal DUM cells occurs only after they have already begun to project axons into their characteristic pathways within the CNS and are about to enter the periphery. Similarly, some of the identified descendants of MPs, such as cell H, die in segments A3 through A6.

Other regional differences in segmental ganglion structure are attributable to specific differences in the pattern of differentiation of the neurons that do survive (Bate et al 1981). For instance, homologous H cells form the characteristic H-shaped axon branching pattern (from which the cell's name is derived) only in segments T1, T2, and T3; in segments A1 and A2 they develop only part of the axonal H pattern.

These neurodevelopmental studies in the grasshopper have thus shown that here, too, as in the nematode and in the leech, specific identified neurons arise by a specific sequence of cell divisions from an identifiable embryonic precursor cell and that serially homologous neurons have corresponding cell pedigrees. This work has revealed, moreover, that the sixfold higher neuron number per ganglion in the adult thoracic segments is attributable mainly to the specific death of particular cells in the abdominal segments, after the cells had already begun to differentiate. However, these studies have not as yet provided much direct information regarding the mechanism—partition of intracellular determinants or positioning in a determinant field—by which the line of descent of a neuron governs its commitment to the expression of one trait rather than another. In the case of the MNB descendants, their common neurotransmitter trait, whose expression depends on the presence (and function) of just a few specific enzymes, seems more plausibly explained by partition of an intracellular determinant, whereas their individual axonal projection patterns seem more likely to be the consequence of their positions relative to

the set of MP descendants to which their rank order of birth from the stem cell has consigned them.

DROSOPHILA Because of the syncytial character of the early insect embryo, it is impossible, indeed meaningless, to trace back the cellular pedigree of any neuron of the insect nervous system to the egg. (Indirect evidence indicates, moreover, that the nuclei of the syncytium have no definable fates.) What has been done, however, is to establish fate maps for various regions of the blastoderm, particularly in the case of the embryo of the *Drosophila*. One such fate map was established by direct histological observation by Poulson (1950). This map shows that the rostrocaudal segmental sequence manifest in the ectodermal and mesodermal layer of the germ band is already presaged in the blastoderm, and that the ventral nerve cord arises from the bilateral bands of cells extending longitudinally on the ventral aspect of the blastoderm, spearated by a band of mesodermal presursor cells lying on the future ventral midline. The brain, by contrast, arises from two paired blastoderm patches that lie front-and dorsalward to the nerve cord precursor bands. The finding of a separate origin for the insect brain is in agreement with the results of the cell lineage analyses of the leech, which assigned to the supraesophageal ganglion a line of descent separate from that of the ventral nerve cord ganglia.

Another fate map of the *Drosophila* blastoderm was established upon revival of Sturtevant's (1929) genetic mapping method (Garcia-Bellido & Meriam 1969, Hotta & Benzer 1972, Janning 1978). This method is based on the experimental generation of gynanders, or flies whose tissues form a mosaic of male and female cells. The gynander map of the embryonic origin of the insect nervous system showed that in accord with Poulson's direct fate map, the precursors of the segmental nerve cord ganglia lie bilaterally on the ventral aspect of the blastoderm in their eventual rostrocaudal sequence, with the precursors of the brain and its optic lobe being more dorsally disposed (Kankel & Hall 1976). It was possible also to estimate that only a few blastoderm cells (from three to ten) are the precursors of each ganglion on the right or left side of the body. If these findings apply also to the grasshopper, then it would follow that the set of NBs and MPs that make up the ganglionic primordium in the germ band arise by multiplication of a much smaller number of blastodermal founder cells.

A further method is available for producing insects with genetically mosaic bodies, which can likewise be used for developmental cell lineage analyses. This method is based on the discovery by Stern (1936, 1968) of genetic recombination between homologous chromosomes during the mitotic nuclear divisions in the somatic tissue of *Drosophila*. The somatic recombination method has been used for cell lineage analysis in the arthropod compound eye. The regular array of ommatidia, each with a fixed number of regularly

arranged photoreceptor cells, had led to the suggestion that each ommatidium arises as a clone, i.e. that its set of photoreceptor cells is descended from a single ommatidial founder cell (Bernard 1937). Later radiological and genetic experiments seemed to support this view of the development of the 700 to 800 ommatidia of the *Drosophila* compound eye (Becker 1957). But more recent findings made with the somatic recombination method, argue against this view (Hofbauer & Campos-Ortega 1976, Ready et al 1976). The eight photoreceptors within a single ommatidium are not all descendants of a single founder cell, and the commitment of eight photoreceptor cells to form a given ommatidium is not determined by their lineage. Moreover, even the possibility that *Drosophila* photoreceptors do arise as ommatidium-sized clones but that the members of a clone are not constrained to take part in the formation of the same ommatidium (Campos-Ortega & Hofbauer 1977, Campos-Ortega et al 1978) was eliminated by statistical analysis of the size distribution of identified clones (Lawrence & Green 1979). Thus, fixed cell lineage does not seem to play a determinative role in ommatidial development.

Ascidians

One of Whitman's disciples, E. G. Conklin (1905), had studied cell lineage in the embryos of ascidians. In their development, these sessile marine animals pass through a free-living tadpole stage whose morphology is very similar to that of the vertebrates: it has a notochord, segmented tail muscles, and a CNS consisting of a brain, brainstem, and a spinal cord. The ascidian egg cleaves meridionally, to yield a bilateral cell pair designated AB2. The second cleavage, also meridional, is orthogonal to the first and results on either side in an anterior blastomere pair designated A3 and a posterior blastomere pair designated B3. The third cleavage is equatorial and results in two cell pairs, a4.2 and b4.2, in the animal hemisphere and two cell pairs, A4.1 and B4.1, in the vegetal hemisphere. A series of further, highly regular cleavages follows, leading to a 64-cell blastula composed of individually identifiable blastomeres. Gastrulation now begins, leading to the formation of neural ectoderm and an underlying mesodermal layer on the dorsal aspect of the embryo. Conklin (1905) managed to establish a fate map for the 64-cell ascidian blastula, which was later refined by Ortolani (1955). On that map the prospective region of the CNS is located near the future dorsal midline in the anterior hemisphere, with the future rostrocaudal array of brain, brainstem, and spinal cord oriented in the animal-vegetal direction.

Although on this map the prospective CNS regions are contiguous, the differential commitment to nervous vs nonnervous tissue is not clonal: at the 16-cell stage, eight cells each give rise to some part of the CNS, as well as to non-CNS tissues, such as notochord, gut, and epidermis. This means that the boundaries between prospective CNS and non-CNS regions do not cor-

respond to cellular boundaries, making it unlikely that differential commitment by segregation of nuclear determinants occurs at this early stage of development. Nishida & Satoh (1983) have recently applied the intracellular cell lineage tracer technique to the ascidian embryo, injecting HRP into identified blastomeres at various early stages of development. They obtained a fate map that generally confirmed Conklin's classical map, except that the HRP label showed that muscles are derived also from blastomeres A4.1 and b4.2, and not only from blastomere B4.1, previously identified as their sole source.

The ascidian embryo has provided one of the few convincing demonstrations of the existence of intracellular determinants that are distributed anisotropically in the egg and later partitioned unequally over daughter cells in the course of asymmetric cell divisions. This demonstration derives from the work of Whittaker (1973, 1979) on the cellular commitment for expression of acetylcholinesterase present in the tail muscles of the tadpole. This enzyme normally makes its first appearance in the tail muscle cell line at the time of formation of the neural tube. Upon inhibiting further cell division in the embryo at various stages of early development by exposing the embryo to cytochalasin B, Whittaker found that acetylcholinesterase still makes its appearance in the arrested embryo after the normal lapse of time in, and only in, those blastomeres which, according to the classical fate map, are precursors of muscle cells. Thus if cleavage is inhibited at either the one- or two-cell stage, acetylcholinesterase eventually appears throughout the arrested embryo. But if cleavage is inhibited at the four- or eight-cell stage, the enzyme appears only in the B3 or B4.1 blastomere pairs, respectively. Moreover, commitment to expression of acetylcholinesterase in the B4.1 cell line, as well to its nonexpression in other cell lines, is *autonomous*: the enzyme will appear after the normal lapse of time in a B4.1 blastomere pair surgically removed from the eight-cell embryo and cultured in isolation, while the remainder of the embryo lacking these blastomeres does not produce the enzyme (Whittaker et al 1977).

To demonstrate that the commitment to differential expression of acetylcholinesterase is, in fact, attributable to the partition of a cytoplasmic determinant, Whittaker (1980) compressed the embryo just prior to its third cleavage. This operation causes transmission to the b4.2 blastomere pair of some of the cytoplasm ordinarily passed on only to the B4.1 pair. Upon inhibition of further cleavage by cytochalasin B in such manipulated embryos, acetylcholinesterase is now expressed in both the B4.1 and the b4.2 blastomere pairs. Whittaker (1982) also apportioned cytoplasm destined for the B4.1 blastomere pair to the b4.2 pair microsurgically. He found upon culturing the cytoplasmically enriched b4.2 blastomeres in isolation that their abnormal expression of acetylcholinesterase develops autonomously, just as does the normal expression in the B4.1 cell line. Unfortunately, the force of these

impressive results is slightly weakened by Nishida & Satoh's later findings by use of the HRP lineage tracer method that blastomere B4.1 is not, in fact, the sole precursor of muscle cells, which are derived also from blastomeres b4.2 and A4.1. Hence, according to Whittaker's argumentation on behalf of the role of cytoplasmic determinants in commitment, acetylcholinesterase should have been expressed not only in the B4.1 blastomere but also in the b4.2 and A4.1 blastomeres upon inhibition of further cell division at the eight-cell stage.

Satoh (1979) and Satoh & Ikegami (1981a,b) have also used another inhibitor of cell division, namely aphidicolin. In contrast to cytochalasin B, which acts by blocking the cytokinesis phase of cell division while permitting the indefinite continuation of successive rounds of DNA replication, aphidicolin stops cell division in ascidian embryos by blocking DNA replication. The effect of blocking cell division by arrest of DNA replication turns out to be dramatically different from that found after blocking cytokinesis; if the embryo is exposed to aphidicolin at any time prior to gastrulation (or about the seventh division), no acetylcholinesterase appears in the cells of the lineage normally destined to express it. However, if aphidicolin is added at about the 76-cell stage (by which time the cell division rhythm has become asynchronous), the enzyme is eventually expressed in some, but not all, of the cells belonging to the known muscle lineage. At this stage there have been seven to nine rounds of DNA replication in the line of ancestry of different muscle precursor cells. Satoh & Ikegami (1981a) were able to show that only those cells whose DNA had undergone eight or nine rounds of replication eventually express the enzyme in the arrested embryo. Hence, they suggest that the rounds of DNA replication provide a developmental clock, and that it is only after the eighth round of its replication that the genome becomes competent to interact with the cytoplasmic determinant to effect the commitment for eventual expression of the enzyme. It is worthy of note that it is also after the eighth round of replication that the muscle cell lineage has finally become clonal, i.e. that there are precursor cells that give rise only to muscle cells and to no other cell types.

Amphibia

Although the focus of this review is on the analysis of cell lineage in the development of the nervous system of invertebrates, we consider finally but briefly one such analysis performed on a vertebrate nervous system. We present this case—M. Jacobson's (1982) application to the frog embryo of the methodology of cell lineage analysis by injection of tracers—only because it has sown confusion among developmental neurobiologists.

Fate maps of the amphibian embryo, obtained by labeling its various regions with externally applied vital stains, became available in the 1920s (Vogt 1929) and were later refined by a succession of workers (Pasteels 1942, Keller 1975, 1976). The fate maps, whose regularity revealed that there is very little random

mixing of surface cells at any stage of amphibian development, indicated that the precursors of the CNS lie in the dorsal quadrant of the animal hemisphere of the amphibian blastula, symmetrically disposed on either side of the meridian of the future body midline. Moreover, the prospective regions of the rostrocaudally sequential subdivisions of the CNS—forebrain, midbrain, hindbrain, spinal cord—lie in that same order, from the animal pole toward the equator of the blastula. To bring the classical amphibian fate map down to the single blastomere, i.e. cell lineage, level, individual blastomeres of frog embryos at various early developmental stages, from the two-cell to the 1024-cell stage, were HRP-injected, and the distribution of label in the CNS was observed in the resulting larva (Jacobson & Hirose 1978, 1981, Hirose & Jacobson 1979). The results of these cell lineage analyses confirmed the classical fate map, and, in addition, showed at a higher level of resolution that deep cells in clones labeled prior to the 512 cell stage mix extensively, and that surface cells mix somewhat also, in the course of development. Nevertheless, it was found that the later the developmental stage at which an individual blastomere is labeled, the smaller is the domain of the larval CNS that contains labeled cells.

These results were interpreted to mean (Jacobson 1980) that, by the 512-cell stage, blastomeres and their descendant clones are already committed to express a neural phenotype. This interpretation was contrary to the accepted view that the descendants of the dorsal quadrant cells identified on the fate map as prospective neural tissue must receive an inductive signal from the underlying dorsal mesoderm at, or after, gastrulation to become differentially committed to develop as neurons rather than epidermis (Nieuwkoop 1952). Moreover, the commitment of blastomeres at the 512-cell stage would be difficult to reconcile with the finding by Spemann & Mangold (1924) that grafting a second dorsal blastoporal lip on the ventral aspect of an amphibian gastrula, containing by then more than 10,000 cells, results in the development of a second, ventrally situated CNS in the host tissue. It was therefore proposed (Jacobson 1982) that, contrary to the usual interpretation, in this experiment the second CNS was not "induced" from previously uncommitted cells on the ventral aspect, whose normal fate is belly and tail epidermis, but that it arose by an abnormal, ventral-ward *migration* from the dorsal aspect of cells committed long ago to a neural fate or by a self-differentiation of the graft.

This radical reinterpretation of one of the classical experiments in the history of embryology was shown to be incorrect by Gimlich & Cooke (1983), by use of a modification (Gimlich & Braun 1984) of one of the fluorescent cell lineage tracers devised for use with leech embryos. In a series of frog morulas at the 32-cell stage, Gimlich & Cooke injected a fluorescent lineage tracer into either of two identified blastomeres, D2 or V3, lying in the prospective CNS or ventral epidermis regions, respectively. At early gastrula, a second

blastoporal lip, from synchronous but unlabeled donor embryos, was implanted on the ventral aspect of the labeled embryos, to induce formation of a second, ventral CNS. The result of this experiment was completely unambiguous. In embryos with a labeled D2 blastomere, only the normal, dorsal CNS contained any labeled cells, whereas the second, ventral CNS was free of label. But in embryos with a labeled V3 blastomere, only the second, ventral CNS contained any labeled cells, whereas the normal, dorsal CNS was free of label. Hence there can be no doubt that Spemann & Mangold (1924) had, in fact, interpreted the result of their experiment correctly and that the second, ventral CNS does arise from cells that would normally have become belly and tail epidermis.

Conclusion

The examples of cell lineages presented here show that in metazoan development, the line of descent of a cell plays a critical role in determining its fate. The strongest indicator of this fact is the finding that in the embryos of nematodes, leeches, and insects, most rostrocaudally homologous neurons arise on rostrocaudally homologous branches of the cell lineage tree. But just how that determinative role is played has been elucidated so far in only a very few cases, despite the fact that for one animal, the nematode *C. elegans,* the exact line of descent of every somatic cell, neuronal and non-neuronal, is now known. One of the most surprising facts to emerge from this superbly detailed pedigree is the bewildering diversity of ontogenetic processes that are at work even at this comparatively modest level of metazoan complexity. For the nematode provides examples of almost any developmental mechanism that can be reasonably put forward to explain how a nerve cell becomes differentially committed to express the set of traits that make it uniquely identifiable. Moreover, the data also provide a counterexample for almost any nontrivial generalization that might be proposed regarding the mechanism of commitment. For instance, although most similar cell types expressing the same trait arise via corresponding branches of homologous sublineages, some arise via proliferative divisions of a common precursor cell, and yet others via seemingly chance interactive recruitment of genealogically unrelated cells that happen to lie in appropriate parts of a morphogenetic field. Or, by way of another example, most bilaterally homologous cells take on the same fate, while some have two different fates. And of those homologous cell pairs that do take on different fates under normal conditions, some pairs belong to an equivalence group, so that whenever either cell is ablated, the survivor takes on the primary fate, while in other pairs each cell is autonomously committed to its fate, even if the precursor cell of another group member has been ablated several generations previously. Finally there are a few equivalence groups whose members, as judged by their lines of descent, are not even homologs.

The much less complete cell lineage analysis of leech development leads to similar conclusions. The leech, more complex than the nematode, yet still far removed from the complexity of the vertebrates, resorts for its development to the same variety of developmental processes. This patchwork of mechanisms, which achieves what appear to be essentially similar ends by a great diversity of means, supports the notion set forth by Francois Jacob (1982) that ontogeny is related to philogeny by "tinkering," i.e. that evolution changed the course of embryogenesis by resort to any tool or trick that may happen to have been handy when it was needed. These findings suggest that by the time evolution had put the pseudoceolomate nematode worm on the scene, it had already tried most of the items in its bag of tools and tricks for determining cell fate. Thus it does not seem very probable that in the relatively brief period of subsequent metazoan evolution there have emerged many novel developmental mechanisms at the cellular level. Rather, what does seem likely is that the vertebrate nervous system arose by opportunistic variations in the timing, in the number of iterations, and in the spatial localization of the determinative processes that were already at work in the embryos of invertebrates.

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