

Developmental cell lineage

GUNTHER S. STENT*

Department of Molecular and Cell Biology, University of California, Berkeley, USA

ABSTRACT Studies of the role of cell lineage in development began in the 1870s, fell into decline in the first half of the 20th century, and were revived in the 1960s. This revival was attended by the introduction of new and powerful analytical techniques. Cell lineage can be inferred to have a causative role in developmental cell fate in embryos in which induced changes in cell division pattern lead to changes in cell fate. Such a causative role of cell lineage is suggested also by cases where homologous cell types characteristic of symmetrical and longitudinally metamerical body plan arise via homologous cell lineages. The developmental pathways of commitment to particular cell fates proceed according to a mixed typologic and topographic hierarchy, which appears to reflect an evolutionary compromise between maximizing the ease of ordering the spatial distribution of determinants of commitment and minimizing the need for migration of differentially committed embryonic cells.

KEY WORDS: *intracellular lineage tracers, cell division modes, commitment, equivalence group, typologic and topographic hierarchies*

Origins of cell lineage studies

Studies of developmental cell lineage were begun in the 1870s, in the context of the controversy then raging about Ernst Haeckel's "biogenetic law." That law seemed to imply that in animal development the cells of early embryos recapitulate the non-differentiated tissues of a remote, sponge-like ancestor. For instance, the 19th century physiologist Eduard Pflueger categorized early cleavage as a process by which the fertilized egg splits into indifferent cells, which, he thought, have no more of a fixed relation to the postembryonic body than have snow flakes to an avalanche. Only after gastrulation would the germ layers -ectoderm, mesoderm and endoderm- be destined to take on the tissue differentiation characteristic of more recent metazoan ancestors.

To test this implication, Charles O. Whitman (1878, 1887) observed the cleavage pattern of early *leech* embryos and followed the fate of individual cells from the egg to the germ-layer stage. He concluded that contrary to the implication of the biogenetic law, a definite developmental fate can be assigned to each identified embryonic cell and to the clone of its descendant cells. These findings not only argued against Pflueger's snowflake theory of embryogenesis, but they also suggested that the differentiated properties that characterize a given cell of the post-embryonic animal are causally linked with that cell's developmental line of descent (Wilson, 1898; Maienschein, 1972).

The first theory of governance of developmental cell fate by cell lineage was put forward by August Weismann in the 1890s. Weismann

proposed that every type of cell fate is represented in the egg by a separate *determinant*, so that the set of determinants amounts to a representation of the whole postembryonic animal. During the cleavage of the egg, various determinants become segregated into different cells. Weismann identified the postulated developmental determinants with chromosomal units of heredity (that is, with the, to him still unknown, Mendelian genes). Before long, however, the study of developmental cell lineage went into decline. It remained a biological backwater for the next 50 years, probably because the discovery of regulative and inductive phenomena in the development of echinoderms and chordates focused the attention of embryologists on cell interactions rather than cell lineage as causal factors in cell differentiation. Hans Driesch's finding in 1891 that upon separation of the two blastomeres produced by the cleavage of a sea urchin egg, each cell is capable of developing into a whole, albeit smaller embryo showed that, in accord with Pflueger but contrary to Weismann, individual blastomeres contain the entire developmental potential of the uncleaved egg, that is, are *totipotent*. Thus the early embryo came to be regarded as a *regulative* system, meaning that each cell has the capacity to restore the tissues normally produced by the missing cells when portions of the embryo are removed.

Cell lineage finally seemed to be deprived of any significant role in the governance of cell fate upon the demonstration by Hans Spemann and Hilde Mangold (1924) that grafting an exogenous blastoporal dorsal lip on the ventral aspect of an amphibian gastrula induces the development of a second, supernumerary

*Address for reprints: Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA. FAX: (510) 643-6791. e-mail: stent@uclink4.berkeley.edu

central nervous system. Attention of embryologists now came to be focused on the mechanism by which one part of the embryo induces the developmental fate of another part. Since such induction was generally attributed to the action of specific chemical inducers, the search for them and their attempted identification came to dominate experimental embryology for the next 30 years. Alas, despite intensive efforts to uncover the chemical basis of embryonic induction, no single substance was identified for which the role of a specific inducer could be convincingly demonstrated. In retrospect, the reason for this failure is readily apparent: In the 1930s, 1940s, and 1950s, embryologists still lacked the latter-day molecular-biological insights which we now know to be necessary to account for the chemical basis of the induction process.

Novel techniques

Interest in the developmental role of cell lineage finally revived in the 1970s and resulted in the introduction of novel analytical techniques more precise and far-reaching than those that were available to the pioneers of the late 19th century.

1. Extension of the range and precision of the direct observation method pioneered by Whitman, by application of advanced microscopic techniques, such as differential interference contrast optics and video time-lapse recording, as well as computer-aided confocal imaging. (White *et al.*, 1987).

2. Generation of embryos whose tissues are mosaics of clones of genetically different cells. One way to produce such clonal mosaics is to induce genetic recombination between homologous chromosomes during the mitotic nuclear division of a somatic embryonic cell (García-Bellido and Merriam, 1969). Another way is to mix embryonic cells derived from genetically different morulas (Mintz, 1965). A third way is to infect single embryonic cells with a non-lethal virus genetically engineered to express a histologically detectable product of a reporter gene. (Cepko *et al.*, 1995).

3. Labeling of embryonic cells with intracellular lineage tracers (Weisblat *et al.*, 1978, 1980a). Under this technique, an identified embryonic cell is microinjected with a tracer molecule that is passed on to all, and only to, the lineal descendants of the injected cell. These descendants can then be identified at a later embryonic stage, by observing the distribution pattern of the tracer within the embryonic or postembryonic tissues. One kind of cell lineage tracer consists of an adduct of a fluorescent dye, such as fluorescein or rhodamine, with an inert carrier molecule, such as dextran (Weisblat *et al.*, 1980b). The cellular distribution of the fluorescent tracers can be observed in living tissues under the fluorescence microscope. The fluorescent tracers can also serve as specific photosensitizers, because the exposure of labeled cells or tissues to light of a particular wavelength leads to death by photo-oxidation of all illuminated descendants of the tracer-injected clonal founder cell, but not of any other, genealogically unrelated cells with which the ablated cells may be intermingled. Fluorescent tracers make it possible, therefore, to determine the developmental effects of selective ablation of cells of particular lines of descent (Shankland, 1984; Shankland and Weisblat, 1984).

Modes of cell division

There are three principal modes of cell division by which a cell may give rise to its clone of descendants: the *proliferative mode*,

under which a cell of type A divides symmetrically to produce two equal daughter cells of type A, both of which also divide symmetrically; 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 the maternal type A and the other is of another type B; and the *diversifying mode*, of which a cell of type A divides into two unequal daughters of type B and C, neither of which ever gives rise again to a cell of type A. In the line of ancestry of any differentiated cell there has usually occurred more than one of these modes of cell division. For instance, embryogenesis in protostomes usually begins with division in the diversification mode and then switches to the proliferative mode in some lines of descent and to the stem cell mode in others. Embryogenesis in deuterostomes, by contrast, usually begins with division in the proliferative mode and then switches to the diversification mode in some lines of descent and to the stem cell mode in others.

The three principal modes do not, by any means, exhaust all the known ways in which cells arise in embryonic development. For instance, the insect egg begins its development with a series of synchronous mitotic divisions of the zygote nucleus, unaccompanied by cell division. There thus arises an embryonic syncytium containing thousands of nuclei. Eventually most of these nuclei migrate to the periphery of the syncytium, where each nucleus becomes cellularized by an infolding of the embryonic cell membrane. In this way, the insect embryo comes to consist of a uniform sheet of several thousand cells, the *cellular blastoderm*, none of which has actually arisen by *cell* division. It is only in subsequent embryogenesis that cells of the blastoderm proceed to divide according to one or more of the three principal modes. Hence the concept of cell lineage is not applicable to insect embryogenesis prior to the cellular blastoderm stage. A yet different mode of cell generation is presented by the skeletal muscles of vertebrates. These are syncytial cells which also do not arise by cell division, but rather by end-to-end fusion of many muscle precursor cells. Thus here the outer branches of the cell lineage tree converge, rather than diverge.

Determinate development

Embryonic development of a species can be said to be *determinate* when the normal division pattern of the early embryo is sufficiently stereotyped to permit identification of individual cells and their developmental fate as the same in specimen after specimen. As shown by use of modern techniques for cell lineage analysis, development is highly determinate in invertebrates such as nematodes and leeches (Stent *et al.*, 1982; Sulston *et al.*, 1983). By contrast, in mammalian embryogenesis, the fate of the cells in the earliest, or morula, stage is *indeterminate*. Any cell of the mammalian morula may be destined either for the extraembryonic feeder layer or for the inner cell mass, which gives rise to the embryo, with the alternative fates being apparently governed by chance factors (Gardner, 1978). There exists a spectrum of intermediate situations between the extremes of wholly determinate and wholly indeterminate development, in which a particular (non-random) probability of realization can be assigned to each of several alternative possible fates of a cell.

That cell lineage is capable of causing cell fate is indicated by the finding that in some types of embryos changes in cell lineage patterns also lead to changes in cell fate. Such changes in the cell

lineage pattern can be induced by mutation of certain genes (Sulston and Horwitz, 1981) and by changes in cell position (Weisblat and Blair, 1984). A causative role of cell lineage in cell fate is suggested also by the finding that in the embryos of nematodes and leeches bilaterally and serially homologous cell types are, on the whole, generated via homologous genealogical pathways (Sulston and Horwitz, 1977; Sulston *et al.*, 1983; Weisblat *et al.*, 1984; Zackson, 1984). Indeed, this generative homology might be thought to account for the evolution of the bilaterally and longitudinally segmented body structure of some higher metazoa.

Commitment

How does the genealogical origin of a given cell of the mature animal determine its characteristic differentiated properties? This question confronts us with *cell commitment*, a fundamental, and yet elusive concept of developmental biology. Commitment refers to the somatically heritable process by which an embryonic precursor cell causes its descendants to differentiate at a much later developmental stage into one cell type (or set of cell types) rather than into another. The concept of commitment is based on the idea that an embryonic cell is initially *pluripotent*, that is, capable of giving rise to descendants of either type A or type B. Commitment of the cell to either of these alternative fates is said to have taken place once its developmental potential has become restricted to generating descendants of one type only, say A. The eventual phenotype of any given cell would therefore depend on a series of commitments made by pluripotent cells in its line of ancestry.

Equivalence groups

That there is a need for a conceptual distinction between the determinate fate of a cell and its actual commitment to that fate is shown by the existence of *equivalence groups*, of which many instances have been brought to light by cell lineage studies in nematodes, leeches, and insects (Kimble *et al.*, 1979). The simplest case of an equivalence group consists of two equally pluripotent cells, **a** and **b**, of which under *normal* conditions **a** follows a pathway leading to cell type A, and **b** follows a different pathway leading to cell type B. Under some *abnormal conditions*, however, one of the two cells of the equivalence group may follow a pathway normally characteristic of the other cell, or both cells may undergo a reciprocal exchange of their fates. In many equivalence groups, the response of the lone survivor to ablation of the other member of the group is not symmetric. For instance, upon ablation of cell **a**, cell **b** may take on fate A, while upon ablation of cell **b**, cell **a** may continue to take on fate A. The preferential fate A taken on by the lone survivor in such cases is referred to as the *primary fate* of the equivalence group (Kimble, 1981).

Agents of commitment

There are two kinds of commonly considered agents that may commit embryonic cells to their fate. One of these is a set of *intracellular determinants* that would account for the differential commitment of sister cells in terms of an unequal partition of the set's elements in successive cell divisions. This mechanism resembles Weismann's original theory of development, except that the intracellular determinants are not necessarily located on the

chromosomes, that is, need not correspond to genes. For instance, a pluripotent cell might possess two determinants, alpha and beta, necessary for producing cell types A and B respectively. Commitment to fate A (and loss of pluripotency) would occur at an asymmetrical cell division at which at least one of the daughter cells receives only alpha but not beta. Under this mechanism cell lineage would play a crucial role in cell commitment by consigning particular subsets of intracellular determinants to particular cells (see review by Fuerstenberg *et al.*, this issue).

The other commonly considered kind of determinant consists of a set of intercellular *inducers* whose elements would be anisotropically distributed over the volume of the embryo. A pluripotent cell would be capable of responding to either of two inducers, *aleph* and *beth*, necessary for producing cell types A and B, respectively. Commitment of the cell to fate A (and loss of its pluripotency) would occur upon having responded to *aleph* at some crucial stage of development. Under this mechanism cell lineage would play a crucial role in cell commitment by placing particular cells at particular sites within the inductive field, and hence govern the pattern of their exposure to inducers. This is the mechanism that evidently operates in the commitment of ectodermal cells of the amphibian embryo to take on a neuronal fate (Nieuwkoop, 1952).

Comparative cell lineage studies carried out under normal and abnormal developmental conditions have shown that in some cases cell lineage plays its determinative role in cell commitment by bringing about the orderly, unequal partitioning of intracellular determinants over daughter cells in successive cell divisions (Whittaker, 1973, 1979) and in other cases by bringing about an orderly topographic cell placement relative to anisotropically distributed intercellular inducers (Shankland, 1984).

Typologic and topographic developmental hierarchies

The conceptual discrimination of two differentiated cell phenotypes A and B usually implies a difference in several distinct characters. And so it seemed plausible that the developmental pathway of a pluripotent cell line to eventual differentiation into various cell phenotypes would proceed according to a stepwise, typologically hierarchic commitment sequence (Slack, 1983). For instance, a cholinergic motor neuron would arise along the following pathway: from a cell clone committed to expression of the characters that distinguish ectoderm from mesoderm to a neural subclone committed to expression of characters that distinguish nervous tissue from epidermis; from the neural subclone, to a neuronal subclone committed to expression of the characters that distinguish neurons from glia; from the neuronal subclone to a motor neuron subclone committed to expression of the characters that distinguish motor neurons from interneurons; and finally from the motor neuron subclone to a cholinergic subclone committed to expression of the gene encoding cholineacetyl transferase. This typologically hierarchical pathway could be implemented by hierarchically structured one-, two-, or three-dimensional arrays of intracellular determinants or fields of intercellular inducers. In invertebrates, such as nematodes, leeches or insects, some of the determinant arrays could be thought to exist in the egg before the first cleavage.

Such a typologically hierarchic pathway of clonal commitments has one grave theoretical drawback, however. If, as envisaged, all

cholinergic motor neurons were generated as a clone, they would arise as a coherent local cluster of differentiated postmitotic cells. But inasmuch as these cells are needed at many different places in the body, they would have to migrate eventually to their ultimate destinations. In other words, at later stages of embryogenesis the topologically hierarchic pathway would entail a horrendous cross-traffic of differentially committed cells. By contrast, if the pathway were to follow a topologically arbitrary but topographically hierarchic scheme, the spatially ordered sequence of cell divisions could be arranged so that each differentially committed postmitotic cell arises at, or very close to, the site where its presence is actually needed. It should not be surprising, therefore, that the overall developmental pathways present a mixture of topologically and topographically hierarchic schemes. Any particular developmental pathway probably represents an evolutionary compromise between maximizing the ease of ordering the spatial distribution of the determinants of commitment and minimizing the need for migration of differentially committed embryonic cells.

In the development of nematodes and leeches, where cell migration plays a relatively minor (though definitely present) role, the commitment sequence appears to be largely topologically arbitrary rather than hierarchic (Sulston *et al.*, 1983; Shankland and Weisblat, 1984; Weisblat *et al.*, 1984). In these invertebrate taxa, where the total number of somatic cells ranges from the hundreds to the hundreds of thousands, the cell lineage trees show little correlation between the phenotypic similarity of two differentiated cells and the closeness of their genealogical relation. For instance, of two differentiated sister cells, one may be a neuron and the other an epidermal cell, whereas of two anatomically similar neurons, one may have arisen on the ectodermal branch and the other on the lineally very remote mesodermal branch of the lineage tree. Here, the developmental pathways are in the main topographically hierarchic, in that it is the position of two cells rather than their phenotype which tends to be correlated with the closeness of their genealogical relation.

In the development of vertebrates, where somatic cell numbers range in the millions and billions and cell migration plays a much more prominent role than in invertebrates, topologically hierarchic commitment schemes do appear to operate, at least on the outer branches of the cell lineage tree. The thousands of vertebrate lymphocytes which are committed to the production of a particular species of antibody molecule arise as a single subclone from one committed member of a pluripotent lymphocyte precursor clone, to be subsequently dispersed over the whole lymphatic system. Or, by way of another example, the, as yet, uncommitted precursors of neurons of the autonomic nervous system, of glial cells of the peripheral nervous system, and of a variety of non-neuronal cell types, such as pigment cells and skull bones, arise in the vertebrate embryo as clusters of genealogically related pluripotent cells in the neural crest of the neurula. These precursor cells later migrate from the neural crest along several specific pathways to a variety of distant sites and become committed to differentiation into cell types appropriate for their ultimate destination, under the local influence of intercellular inductive signals (Le Douarin, 1980).

Conclusion

Niels Bohr consigned true statements to two categories: ordinary truths, whose opposites are false, and deep truths, whose

opposites are also deep truths. As we saw, statements about the role of cell lineage in development represent mainly deep truths, since, more often than not, their opposites are also true. As E. B. Wilson (1898) had already noted, a counter-example can usually be found for any generalization regarding the connection between cell lineage and developmental fate. As we now know, the role played by cell lineage varies greatly in the embryogenesis of different taxa, and even for different aspects of embryogenesis within the same species. Cell lineage is evidently a more important developmental determinant in simple worms than in the much more complex insects and vertebrates. But even in simple worms, cell lineages share the governance of developmental cell fate with cell interactions.

This patchwork of developmental mechanisms, which achieves what appear to be essentially similar ends by a diversity of means, supports the notion set forth by Francois Jacob (1982) that ontogeny is related to phylogeny by "tinkering" –that is, that evolution changed the course of embryogenesis by resort to any tool or trick that may have been handy when it was needed. In fact, the results of cell lineage studies suggest that by the time evolution put the pseudocoelomate nematode 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 likely that during subsequent metazoan evolution there have emerged many novel developmental mechanisms at the cellular level. Rather what does seem likely is that the insects and vertebrates evolved from their humbler ancestors by opportunistic variations in the timing, iteration, and spatial localization of the cell commitment processes that were already at work in the embryos of worms.

References

- CEPKO, C., RYDER, E.F., AUSTIN, C.P., WALSH, C. and FEKETE, D.M. (1995). Lineage analysis using retrovirus vectors. *Methods Enzymol.* 254: 387-419.
- FUERSTENBERG, A., BROADUS, J. and DOE, C. (1998). Assymetry and cell fate in the embryonic *Drosophila* CNS. *Int. J. Dev. Biol.* 42: 379-383.
- GARDNER, R.L. (1978). The relations between cell lineage and differentiation in the early mouse embryo. In *Genetic mosaics and cell differentiation. Results and problems in cell differentiation.* (ed. W.J. Gehring) vol 9. Springer Verlag, Berlin. pp 205-241.
- GARCIA-BELLIDO, A. and MERRIAM, J.R. (1969). Cell lineage of the imaginal disk in *Drosophila* gynandromorphs. *J. Exp. Zool.* 170: 61-76.
- JACOB, F. (1982). *The possible and the Actual.* University of Washington Press, Seattle and London, p. 71.
- KIMBLE, J. (1981). Alternations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Dev. Biol.* 87: 286-300.
- KIMBLE, J., SULSTON, J. and WHITE, J. (1979). In *Cell Lineage, Stem Cells and Cell Determination.* INSERM Symposium No. 10 (Ed. N. le Douarin). Elsevier, Amsterdam, pp. 59-68.
- LE DOUARIN, N. (1980). Migration and differentiation of neural crest cells. In *Current topics in developmental biology* (Ed. R.K. Hunt), Vol. 16, part II. Academic Press, New York, pp. 31-85.
- MAIENSCHNEIN, J. (1972). Cell lineage, ancestral reminiscence, and the biogenetic law. *J. Hist. Biol.* 11: 129-158.
- MINTZ, B. (1965). Experimental genetic mosaicism in the mouse. In *Preimplantation Stages of Pregnancy*, Ciba Found. Symp. (Eds. C.W. Wolstenholme and M. O'Connor) J. and A. Churchill. London. pp. 194-226
- NIEUWKOOP, P.D. (1952). Activation and organization of the amphibian central nervous system. *J. Exp. Zool.* 120:1-130.
- SHANKLAND, M. (1984). Positional control of supernumerary blast cells death in the leech embryo. *Nature* 307: 541-543.

- SHANKLAND, M. and WEISBLAT, D.A. (1984). Stepwise commitment of blast cell fates during the positional specification of the O and P lines in the leech embryo. *Dev. Biol.* 106: 326-342.
- SLACK, J.M.W. (1983). *From Egg to Embryo*. Cambridge University Press Cambridge.
- SPEMANN, H. and MANGOLD, H. (1924). Über Induktion von Embryoanlagen durch Implantation artfremder Organismen. *Arch. Mikrosk. Anat. Entwmech.* 100: 599-638.
- STENT, G.S., WEISBLAT, D.A., BLAIR, S.S. and ZACKSON, S.L. (1982). Cell lineage in the development of the leech nervous system. In *Neuronal Development* (Ed. N. Spitzer). Plenum, New York, pp. 1-44.
- SULSTON, J.E. and HORWITZ, H.R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 56:110-156.
- SULSTON, J.E. and HORWITZ, H.R. (1981). Abnormal cell lineages in mutants of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 82: 41-55.
- SULSTON, J.E., SCHIERENBERG, E., WHITE, J.G. and THOMSON, J.N. (1983). The embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100: 64-119.
- WEISBLAT, D.A. and BLAIR, S.S. (1984). Developmental indeterminacy in embryos of the leech *Helobdella triserialis*. *Dev. Biol.* 101: 326-335.
- WEISBLAT, D.A., HARPER, G., STENT, G.S. and SAWYER, R.T. (1980a). Embryonic cell lineages in the glossiphoniid leech *Helobdella triserialis*. *Dev. Biol.* 76: 58-78.
- WEISBLAT, D.A., KIM, S.Y. and STENT, G.S. (1984). Embryonic origin of cells in the leech *Helobdella triserialis*. *Dev. Biol.* 104: 65-85.
- WEISBLAT, D.A., SAWYER, R.T. and STENT, G.S. (1978). Cell lineage analysis by intracellular injection of a tracer enzyme. *Science* 202: 1295-1298.
- WEISBLAT, D.A., ZACKSON, S.L., BLAIR, S.S. and YOUNG, J.D. (1980b). Cell lineage analysis by intracellular injection of fluorescent tracers. *Science* 209: 1538-1541.
- WHITE, J.G., AMOS, W.B. and FORDHAM, M. (1987). An evaluation of confocal versus conventional imaging of biological structures by fluorescence light microscopy. *J. Cell Biol.* 105: pp. 41-48.
- WHITMAN, C.O. (1878). The embryology of Clepsine. *Q. J. Morphol. (N.S.)* 18: 215-315.
- WHITMAN, C.O. (1887). A contribution to the history of germ layers in Clepsine. *J. Morphol.* 1: 105-182.
- WHITTAKER, J.R. (1973). Segregation during ascidian embryogenesis of egg cytoplasmic information for tissue specific enzyme development. *Proc. Natl. Acad. Sci. USA* 70: 2096-2100.
- WHITTAKER, J.R. (1979). Cytoplasmic determinants of tissue determination in the ascidian egg. In *Determinants of spatial organization* (Eds. S. Subtelny and I.R. Koenigsberg.). Academic Press, New York. pp. 29-51.
- WILSON, E.B. (1898). Cell lineage and ancestral reminiscence. In *Biological Lectures*. Woods Hole Marine Biological Lab., pp. 21-42. [Reprinted in *Foundations of Experimental biology*, (Eds., B.H. Willier and J.M. Oppenheimer) 2nd edn., Hafner Press, New York pp. 52-72].
- ZACKSON, S.L. (1984). Cell lineage, cell-cell interactions, and segment formation in the ectoderm of a glossiphoniid leech embryo. *Dev. Biol.* 104: 143-160.