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The role of cell lineage in development

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Studies of the role of cell lineage in development began in the latter part of the 19th century, fell into decline in the early part of the 20th, and were revived about 20 years ago. This recent revival was accompanied by the introduction of new and powerful analytical techniques. Concepts of importance for cell lineage studies include the principal *division modes* by which a cell may give rise to its descendant clone (proliferative, stem cell and diversifying); developmental *determinacy*, or *indeterminacy*, which refer to the degree to which the normal cleavage pattern of the early embryo and the developmental fate of its individual cells is, or is not, the same in specimen after specimen; *commitment*, which refers to the restriction of the developmental potential of a pluripotent embryonic cell; and *equivalence group*, which refers to two or more equivalently pluripotent cell clones that normally take on different fates but of which under abnormal conditions one clone can take on the fate of another. Cell lineage can be inferred to have a causative role in developmental cell fate in embryos in which induced changes in cell division patterns lead to changes in cell fate. Moreover, such a causative role of cell lineage is suggested by cases where homologous cell types characteristic of a symmetrical and longitudinally metameric 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 the determinants of commitment and minimizing the need for migration of differentially committed embryonic cells. Comparison of the developmental cell lineages in leeches and insects indicates that the early course of embryogenesis is radically different in these phyletically related taxa. This evolutionary divergence of the course of early embryogenesis appears to be attributable to an increasing prevalence of *polyclonal* rather than *monoclonal* commitment in the phylogenetic line leading from an annelid-like ancestor to insects.

Origins of cell lineage studies

Studies of developmental cell lineage – that is, of the fate of individual cells that arise in an early embryo – were begun in the 1870s, in the context of the controversy then raging about Ernst Haeckel's 'biogenetic' law. The biogenetic law seemed to imply that the cells of early metazoan 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 up into indifferent cells, which, he thought, have no more of a fixed relation to the adult body than have snowflakes to an avalanche. Only after gastrulation would the germ layers of the embryo be destined to take on the tissue differentiation 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 cells from the uncleaved egg to the germ-layer stage and concluded that, contrary to the implication of the biogenetic

law, a characteristic fate in the mature organism can be assigned to identified embryonic cells, and to the clone of their descendant cells. These findings not only argued against Pflueger's snowflakes theory of embryogenesis, but they also suggested that the differentiated properties that characterize a given cell of the mature animal are somehow determined by its genealogical line of descent from the egg (Wilson 1898; Maienschein 1972).

The first theory of the 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 adult organism. During cleavage of the egg, various determinants become segregated into different cells. Weismann identified his 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 on cell lineage as causal factors in cell differentiation. Hans Driesch's finding in 1891 that upon separation of the two cells produced by the first 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 cells contain the entire developmental potential of the uncleaved egg – that is, are totipotent. Thus, the early embryo came to be considered 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 & Hilde Mangold (1924) that grafting an exogenous dorsal blastoporal lip on the ventral aspect of an amphibian gastrula induces the development of a second, supernumerary central nervous system. Attention of embryologists now came to be focused on the mechanism by which one part of an embryo induces the developmental fate of another part. Since this induction was generally attributed to the action of specific chemical inducers, the search for and attempted identification of such inducers 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 quite apparent: the embryologists of the 1930s, 1940s and early 1950s 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

It may have been the disappointment over the lack of progress in uncovering the chemical basis of the induction process that brought about a revival of interest in the role of cell lineage in development about 20 years ago. This revival was accompanied by the introduction of analytical techniques more precise and far-reaching than those available to the pioneers of the late 19th century. These techniques pertain to the two principal methods of developmental cell lineage analysis. One method consists of continuous *direct observation* of the entire course of development, following the embryonic cells and their progeny visually all the way from the

uncleaved egg to some arbitrarily chosen endpoint of maturity. This method can be used only as long as the embryo remains transparent and comprises a reasonably small number of cells. The application of modern microscopic techniques, such as differential interference contrast optics, video time-lapse recording and computer-aided image processing, has greatly extended the range and precision of the direct observation method (Sulston *et al.* 1983).

The other principal method consists of labelling a specific cell at an early developmental stage with a cell lineage tracer and surveying distribution of the label at a later stage. This is the method that must be used when the embryo is opaque or comprises too many cells for their fate to be traced by direct visual observation. A genetic technique for labelling embryonic cells of *Drosophila* was devised by A. H. Sturtevant (1929), based on the experimental generation of flies whose tissues form a mosaic of male and female cells. Another genetic technique for labelling *Drosophila* cells is based on the discovery by C. Stern (1936, 1968) that by X-irradiating the embryo one can induce genetic recombination between homologous chromosomes during the mitotic nuclear division of a somatic embryonic cell. It was only in the late 1960s and early 1970s, however, that these techniques were exploited for extensive cell lineage analyses (Garcia-Bellido & Merriam 1969; Hotta & Benzer 1972; Kankel & Hall 1976; Ready *et al.* 1976; Janning 1978). Following the fate of genetically labelled cells became possible in the mouse upon the development of techniques for generating mosaic specimens by direct mixing of cells derived from genotypically different morulas (Mintz 1965; Gardner 1978). A more generally applicable technique for labelling embryonic cells appeared with the development of intracellular lineage tracers, such as horseradish peroxidase or fluorescent dyes conjugated to carrier molecules. Upon their injection into an identified cell of the early embryo, these tracers are passed on to, and can be made visible in, the clone of descendants of the labelled cell (Weisblat *et al.* 1978, 1980*a*, *b*).

In addition to these two principal methods of cell lineage analysis there exists a third method, which consists of ablating a particular cell of the embryo and noting which cells or tissues are absent at a later stage. The missing cells or tissues might then be inferred to represent the normal descendants of the ablated cell. Strictly speaking, ablation does not amount to a genuine method for cell lineage analysis, since ablation precludes *normal* development. On the one hand, a cell or tissue might be missing at the later stage, not because its normal precursor cell had been ablated, but because an inductive 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, a cell or tissue might be present at the later stage, even though its normal precursor had been ablated, because the cell or tissue arose by regulative restoration 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 cell lineage relations have been established by either of the first two methods, the ablation method can be used to probe the role of inductive processes in the governance of normal fate, or, as we shall see, to test a cell for its commitment to one of several alternative developmental pathways. The precision of the ablation method has been greatly improved by the use of novel photoablation techniques, such as focusable laser beams (Sulston & White 1980) and specific photosensitization of intended target cells by labelling them with fluorescent cell-lineage tracers (Shankland 1984; Shankland & Weisblat 1984).

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Modes of cell division

There are three principal modes of 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*, 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 (figure 1). 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 divisions in the proliferative mode and then switches to the diversification mode in some lines of descent and to the stem cell mode in others.

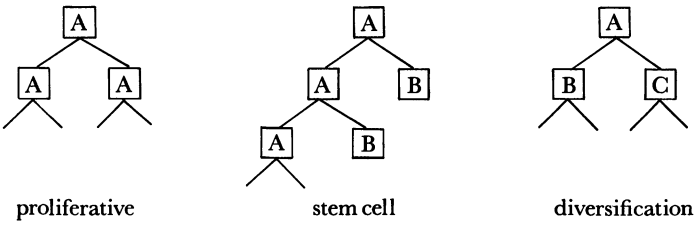

Figure 1. The three principal modes of cell division in development.

These three principal division 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. In this way there 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 before the cellular blastoderm stage. A yet different mode of cell generation is presented by the skeletal muscle fibres 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 the developmental fate of these cells is 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 (Sulston *et al.* 1983; Stent *et al.* 1982). By contrast, in mammalian embryogenesis, the fate of the cells of the earliest, or morula, stage is *indeterminate*. Any cell of the mammalian morula may be destined for either 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.

It is important to distinguish between the concepts of determinate and of mosaic development, which are often conflated. In embryological parlance 'mosaic' is the antonym of 'regulative', and hence refers to developmental situations in which, following cell removal or ablation, there is no regulative restoration of the tissues normally produced by the missing cells. For instance, development of tunicate embryos is said to be mosaic because separation of the two first cells produced by division of the egg results in the formation of two half-embryos, in contrast to the regulative development of sea urchins, where, as Driesch had found, the analogous operation leads to formation of two complete embryos. Since 'determinate' and 'indeterminate' refer to *normal* and 'mosaic' and 'regulative' to *abnormal* development, there is no reason to assume *a priori* (as is sometimes done) that development of a mosaic embryo cannot be indeterminate or that of a regulative embryo cannot be determinate.

That cell lineage is capable of causing cell fate is indicated by the finding that in some types of embryos changes in the cell lineage pattern also lead to changes in cell fate. Such changes in cell lineage pattern can be induced by mutation of certain genes (Sulston & Horwitz 1981) and by changes in cell position (Weisblat & 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 & 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 symmetrical and the longitudinally segmented body structure of some higher metazoa.

Segmentation in leeches

The segment concept is rooted in the plain fact that along their longitudinal axes the bodies of annelids, arthropods and vertebrates show a morphological periodicity. However, despite the central importance of segmentation for modern developmental biology and the many publications devoted to that subject, it is hard to find an explicit definition of the segment concept, or even a reference to one, in the current literature. There is a good reason for this: the segment concept, though seemingly obvious intuitively, is very hard to define unambiguously without loss of general applicability to actual animals. According to Lankester (1926), the segment, or *metamere*, is a module of serially iterated structures, or *meromes*, such as appendages, skin specializations, muscles or ganglia. The 'ground plan' of each half-metamere on either side of the body midline would include one of each kind of merome, with the interface between successive metameres, or segment boundary, being formed by a transverse surface drawn with reference to a more or less arbitrarily chosen landmark merome. The metameres are not exactly alike, however, differing to various extents from the basic ground plan at various positions along

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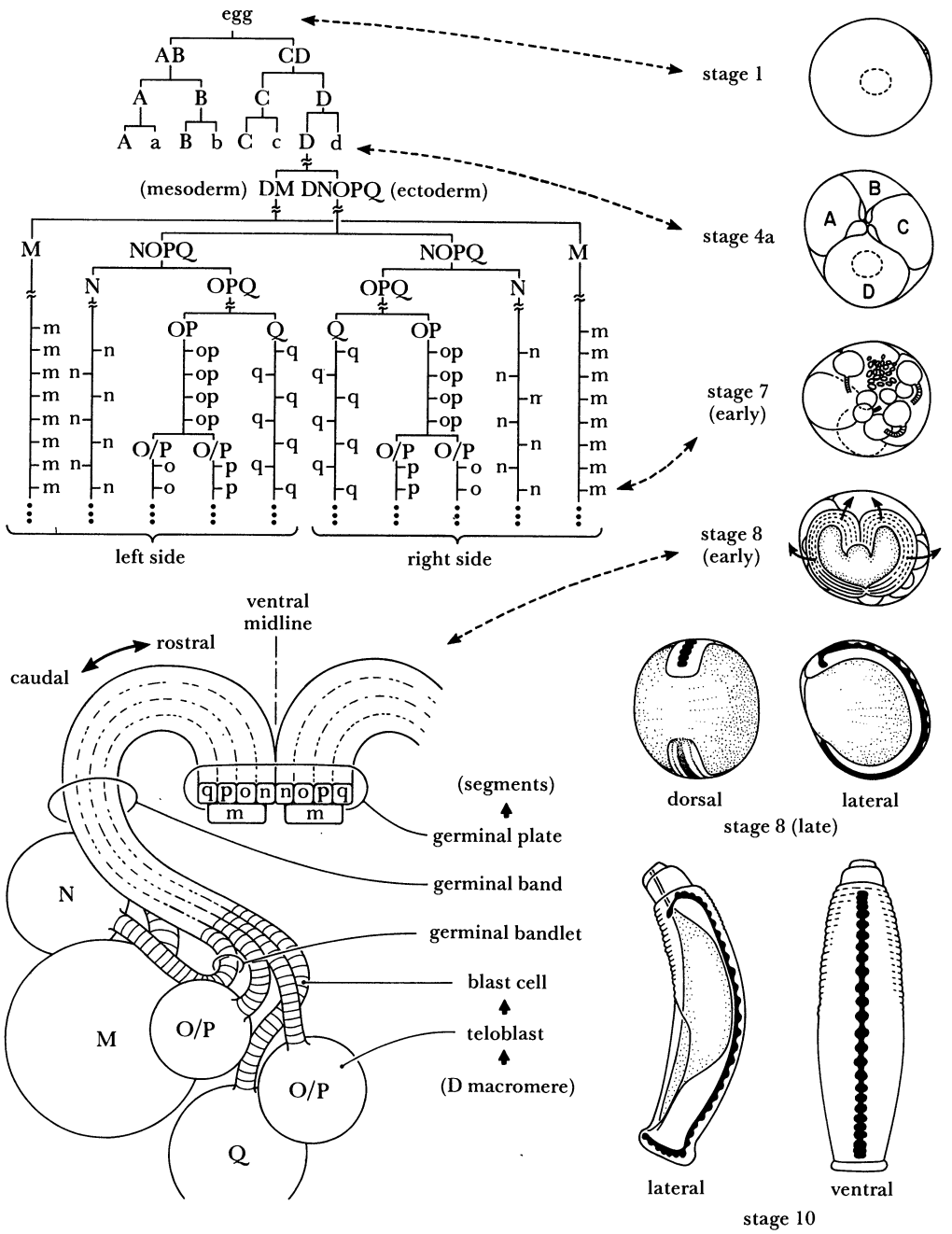

FIGURE 2. Schematic summary of the development of the leech *Helobdella triserialis*. Upper left: cell pedigree leading from the uncleaved egg to the macromeres A, B, and C; the micromeres a, b, c, and d; the teloblast pairs M, N, O/P, O/P, and Q; and the paired primary blast cell bandlets. Breaks in the lineage indicate points where additional micromeres may be produced. The number of op blast cells produced before 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 teloplasm, which is passed on mainly to the D macromere (stage 4a). In the stage 7 embryo the dashed circle 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 longitudinal body axis. These differences arise from *heterosis*, or segment-specific differentiation of meromes, and *dislocation*, or movement of meromes from the metameres to which, according to the ground plan, they ought to belong into the domains of other metameres. Thus the key notions inhering in the segment concept, metameric ground plan and segment boundary, are not free of subjective, and hence ambiguous, elements. These conceptual ambiguities notwithstanding, it seems highly probable on anatomical as well as phyletic grounds that segmentation in annelids and in arthropods is homologous (Anderson 1973; Sawyer 1984). But whether this homology extends also to vertebrates, or whether segmentation in vertebrates and invertebrates are merely anàlogous phenomena, cannot be presently decided.

Application of the modern methods of cell lineage tracing to the problem of the developmental origin of the body segments of the leech and of insects has now made it possible to free the segment concept of its subjective elements, by focusing on modules of periodically iterated cell lineages, or *generative* metameres, rather than on modules of structural meromes, or *morphological* metameres. In the case of the leech, its 32 bilaterally symmetrical segments arise from five, bilateral pairs of one-cell-wide, parallel bandlets of several dozen blast cells, designated m, n, o, p and q, which extend the length of the embryo (figure 2). The m bandlets give rise to mesodermal metameres and the n, o, p and q bandlets to ectodermal metameres. Each bandlet is produced by iterated divisions in the stem cell mode of one of five large, bilaterally paired cells called teloblasts. To each morphological half-metamere there correspond seven distinct cell clones, or clonal meromes, each descended from a primary blast cell: one m, one o, one p, two n and two q primary blast cell clones. The two n primary blast cells (designated n_f and n_s) are serial successors in the bandlet, and the domains of their descendant clones alternate rostrocaudally. The same is true for the two q primary blast cells (designated q_f and q_s) and their descendant clones (Weisblat *et al.* 1980*a*; Zackson 1984; Stent *et al.* 1982; Weisblat & Shankland, this symposium). Each of the seven clonal meromes consists of a few dozen characteristic cells which arise from the primary blast cell by an idiosyncratic, determinate division pattern in a mixed proliferative, stem cell and diversifying mode, so that bilaterally and serially homologous primary blast cells produce homologous cell lineages (Zackson 1984). These seven clonal meromes therefore constitute the generative half-metameres, whose determinate periodically repeated patterns can be readily made visible by use of cell lineage tracers. Analysis of these patterns shows that there are no segment boundaries that can be drawn that would produce topologically coherent generative metameres. Regardless of the anatomical landmark and the surface shape chosen for drawing the segment boundary, the metameric modules would always contain a mixture of cells belonging to two or more clonal meromes of the same type. That is to say, in the leech morphological segment, boundaries do not correspond to borders of clonal restriction (Weisblat & Shankland, this symposium).

Commitment

How does the genealogical origin of a given cell of the adult organism 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

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is initially *pluripotent*, that is, capable of giving rise to differentiated descendants of either type A or type B (where A and B may signify also sets of cell types A_1, A_2, A_3,... and B_1, B_2, B_3,...). Here A and B can be said to be alternative fates of the pluripotent embryonic cell, and in case fates A and B are sets of different cell types, A_1, A_2, A_3,... and B_1, B_2, B_3,... are the *elements* of these fates. Commitment of the cell to one of these 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.

Suppose that under conditions of normal development all the descendants of a particular cell are always of type A and never of type B – that is, that the cell has a determinate single fate. Does this mean that the cell is, in fact, committed to fate A and no longer pluripotent? Except for the few cases for which the molecular basis of the commitment process is already known, this question has no empirical answer unless some *abnormal* developmental condition, C_x, such as tissue transplantation or explantation, ablation of neighbours, or perfusion, is known under which the cell gives rise to descendants of type B rather than of type A. If such a change in normal fate can be induced by C_x at a developmental stage S_1, then the cell is evidently still pluripotent, and hence uncommitted, at S_1. However, if upon implementation of C_x at a later developmental stage, S_2, the cell or its descendant clone gives rise only to descendants of the normal type A, then commitment to fate A can be said to have occurred between stages S_1 and S_2. It is important to note that this empirical test of commitment is critically dependent on the particular abnormal condition, C_x, specified, since it is always conceivable that a different abnormal condition, C_y, could be found which, even if implemented at stage S_2, would still cause the cell to take on fate B. In that case, by stage S_2 commitment would have occurred only with respect to C_x but not with respect to C_y. Moreover, in some cases (especially in tissues capable of regeneration) a cell may retain its pluripotency to give rise to a cell of type B even after it has already differentiated into a cell of the normal type A. In other words, total commitment or loss of pluripotency is not a necessary prelude to or adjunct of cell differentiation.

The need for a conceptual distinction between determinate fate and commitment to that fate is exemplified in leech development by the two equivalently pluripotent ectodermal sister teloblasts, both designated as O/P (figure 2). Upon birth by cleavage of their mother cell, both sister cells have an indeterminate fate: the blast cell bandlet to which each subsequently gives rise may come to lie either next to the n bandlet (in which case the O/P-derived bandlet is designated as o and the fate of its blast cells as O) or next to the q bandlet (in which case the O/P-derived bandlet is designated as p and the fate of its blast cells as P) (Fernandez & Stent 1983; Weisblat & Blair 1984). Thus the initially indeterminate fate of either O/P teloblast becomes determinate after its blast cell bandlet has come to lie in one or the other of these alternative positions. However, if before the first division of an o bandlet blast cell the directly apposed p bandlet blast cell is ablated, then the o bandlet blast cell 'transfates' from its normal O fate to the abnormal P fate (Weisblat & Blair 1984; Zackson 1984). Hence, despite its determinate O fate, the o blast cell still remains pluripotent – that is, uncommitted.

Experiments in which p bandlet blast cells were ablated at progressively later developmental stages have shown that the o blast cell clone does become committed eventually to the O fate, at which time it no longer transfates to the P fate in response to ablation of the apposed p bandlet. However, commitment of the o bandlet cells to the O fate occurs, not in a single event

affecting the fate of its entire descendant clone, but in a sequence of three successive steps. In each of these steps the o blast cell clone becomes committed only to a particular subset of the elements of the O fate and loses its potency to transfate into a particular subset of the elements of the P fate. Accordingly, an o blast cell clone that has undergone only one or two, but not all three, of these commitment steps responds to p bandlet cell ablation by giving rise to a mixture of elements, of which some are characteristic of the O and others of the P fate. Since for every particular set of elements of the O fate committed in each of the three steps the potency for transfating to a particular set of P fate elements is lost, it can be inferred that there is a complementary relation between the gain of commitment to one set of elements of the O fate and the loss of developmental potency for a corresponding set of elements of the P fate. Thus each commitment step seems to pertain to a different set of paired determinants of mutually exclusive fates (Shankland & Weisblat 1984).

The three commitment steps to the O fate, and the progressive losses of pluripotency, appear to be associated with successive divisions of the o blast cell clone. In each of these divisions, one of the two daughter cells is committed to production of a subset of O fate elements (and to loss of potency for the corresponding elements of the P fate) while the other daughter cell remains pluripotent for the remainder of the, as yet uncommitted, elements of the O and P fates (Shankland & Stent 1985). This orderly stepwise sequence of partial, mutually exclusive commitments accompanying asymmetrical cell divisions must reflect some profound aspects of the nature and organization of the determinants which commit embryonic cells to their eventual fates. But just what this profound aspect is still awaits fathoming.

Equivalence groups

The O/P sister teloblasts represent only one of many instances of equivalently pluripotent cell sets meanwhile brought to light by cell lineage studies in nematodes, leeches and insects, which have been designated as 'equivalence groups' (Kimble *et al.* 1979). The simplest case of an equivalence group consists of two cells, of which one normally follows a pathway leading to cell types A and the other follows a different pathway leading to cell types B. Under some abnormal conditions (including the presence in the cell genome of a mutant allele of a particular gene) 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, in the case of the o and p blast cell bandlets, ablation of a p bandlet blast cell induces its apposed o bandlet blast cell to transfate and take on the P fate, but ablation of an o bandlet blast cell does not induce a reciprocal transfating of the apposed p bandlet blast cell, which continues to take on its normal P fate. The preferential fate taken on by the lone survivor in such cases is referred to as the *primary* fate of the equivalence group (Kimble 1981).

In terms of the equivalence group concept, regulative development can be described simply as devolving from the presence of two embryonic cells whose pluripotency consists of the capacity to take on *both* fates A *and* B. Although under normal conditions one cell takes on fate A and the other fate B, upon ablation of one cell before commitment of the other to its normal fate, the surviving cell would transfate and take on both fates, thus providing regulative restoration of the cell types normally provided by the ablated member of the equivalence group.

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Agents of commitment

There are two kinds of commonly considered agents which may commit embryonic cells to their fate. One of these is a set of *intracellular determinants* which would account for the differential commitment of sister cells in terms of an unequal partition of its 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, *a* and *b*, 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 *a*. Under this mechanism cell lineage would play a crucial role in cell commitment by consigning particular subsets of intracellular determinants to particular cells. This mechanism actually operates in one of the few cases in which the molecular basis of cell commitment happens to be understood, namely the commitment of a clone of vertebrate lymphocytes to production of a particular species of antibody molecules. Here commitment consists of an (indeterminate) DNA rearrangement in the genome of a pluripotent lymphocyte precursor stem cell, creating a gene that encodes the primary structure of a particular antibody species and which is passed on and expressed in the descendants of that stem cell.

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 α and β, 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 α 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 governing 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, in case A and B refer to *sets* of cell types, a difference in several distinct kinds of cell phenotypes. 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 the 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 sensory neurons; and finally from the motor neuron subclone to a cholinergic subclone committed to expression of the gene encoding cholineacetyl transferase rather than glutamic acid decarboxylase. The 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 of the body, they would have to migrate eventually to their ultimate destinations. In other words, at later stages of embryogenesis the typologically hierarchic pathway would entail a horrendous cross-traffic of differentially committed cells. By contrast, if the pathway were to follow a typologically 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. The generative metameres underlying the segmental body structure are, of course, a striking example of such a topographically hierarchic pathway of commitment. It should not be surprising, therefore, that the overall developmental pathways present a mixture of typologically 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 typologically arbitrary rather than hierarchic (Sulston *et al.* 1983; Weisblat *et al.* 1984; Shankland & Weisblat 1984). In these invertebrate species, 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, typologically hierarchic commitment schemes do appear to operate, at least on the outer branches of the cell lineage tree. As already mentioned, 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 clone of pluripotent lymphocyte precursor cells, 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

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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 (LeDouarin 1980).

Segmentation in insects

Studies of the developmental genetics of *Drosophila*, coupled with cell lineage studies, have illuminated the role of generative metameres in the segmentation of insects. The rostrocaudal differentiation of morphological meromes, or heterosis, is much more pronounced in flies than in leeches, especially in the head and tail regions, and for that reason it is difficult to make an unambiguous assignment of the total number of morphological metameres in the adult fly. However, the *Drosophila* larva is generally considered to be composed of about 15 metameres, of which three are cephalic, three thoracic and nine or more abdominal. Since the concept of cell lineage is not applicable to insect embryogenesis before the formation of the approximately 6000 cells of the blastoderm, here the origin of the generative metameres cannot be traced back, as it can be in the leech, to the serial production of homologous sets of segmental founder cells by iterated divisions of a few paired teloblasts. Rather, it would appear that, in insects, the generative metameres arise only upon subdivision of the two-dimensional sheet of blastoderm cells into a longitudinal series of 15 or more circumferential bands, of which each comprises a set of a few dozen founder cells committed to the formation of one generative metamere. Individual bands, or segmental primordia, are three cells wide (in the longitudinal direction). There is good evidence that this periodicity of metameric cell commitment depends on the activity of a special set of genes which respond differently to the presence of a caudorostral determinant gradient (Nüsslein-Volhard *et al.* 1982).

Within each segmental primordium the cells are destined for various fates according to a circumferential pattern (Poulson 1950). An arc of founder cells straddling the ventral midline comprises the founder cells of the mesodermal metamere. The arcs lying to either side of the mesodermal founder arc and extending towards the future dorsal midline comprise the founder cells of the ectodermal metamere, with a mixed neural and epidermal fate. The ventral zone of the ectodermal arcs adjacent to the mesodermal arc provides ventral epidermis and neurons of the segmental ganglion. The dorsolateral zone of the ectodermal arcs provides dorsal epidermis and peripheral neurons (Hartenstein & Campos-Ortega 1984). Thus as regards their fate, the arcs of mesodermal and ectodermal segmental founder cells in the *Drosophila* blastoderm appear to correspond to the seven bilaterally paired metameric founder clones, or generative meromes, in the leech embryo derived from the m, n_s, n_f, o, p, q_s and q_f primary blast cells. The neurons of the insect ganglion derived from the ventral zone of the ectodermal arcs are the clonal descendants of 32 bilaterally paired and six unpaired ectodermal blast cells. Each of these blast cells takes on a determinate, segmentally homologous fate, whose elements are a particular set of identifiable neurons (Goodman 1982), with a sixfold lower number of neurons per adult ganglion in abdominal segments than in thoracic segments (that is, heterosis of the neural meromes) being attributable to a determinate pattern of cell death during embryogenesis.

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Polyclonal commitment

How then is one to explain that the course of embryogenesis in these two phyletically related taxa, insects and leeches, is so radically different? How can it be that whereas in leeches determinate cell lineages can be traced back to the uncleaved egg (as they can be in nematodes), in insects cell lineage plays little or no role before the cellular blastoderm stage, at which a 6000-cell embryo suddenly springs forth, full-blown, like Athena from Zeus's head? This evolutionary divergence of the mechanism of embryogenesis can be accounted for by the increasing prevalence of polyclonal commitment in the phylogenetic line leading from an annelid-like ancestor to insects.

The notion of polyclonal commitment was first put forward by Crick & Lawrence (1975) in their explication of Garcia-Bellido's compartment concept designed to account for some findings related to the developmental genetics of the *Drosophila* wing (Garcia-Bellido *et al.* 1973). As epitomized recently by Martinez-Arias & Lawrence (1985), its compartments make up what structuralist philosophers would call the deep structure of an insect, namely its '(objective) internal representation', as distinct from 'our (subjective) external description'. Unfortunately, the compartment concept has been saddled with too many diagnostic criteria (Garcia-Bellido *et al.* 1979), of which only some seem to be the attributes needed for an 'internal representation'. This over-definition has caused confusion among developmental biologists trying to apply the compartment concept to systems other than that for which it had been tailor-made. And so I will confine my attention to polyclonal commitment, which I find to be the heuristically most useful core of the compartment concept.

'Polyclone' refers to an ensemble of cells jointly committed to a common fate and representing all the clonal descendants of a small set of spatially contiguous founder cells located within a given domain of the embryo. For an ensemble to qualify as a polyclone, it is essential that its founder cells do not, in fact, constitute an ordinary (mono-) clone (figure 3). The polyclone shares a common determinate developmental fate, which, in the case of the *Drosophila* compartment, is reflected in its occupancy of a coherent body region with a topographically determinate boundary and in the topological connectedness of its cellular elements. The particular subfates of individual member clones of the polyclone may be indeterminate, however, as reflected, for instance, by topographically indeterminate boundaries separating the subregions occupied by individual clones within the determinate compartment boundary. The most generally applicable feature of the polyclone concept, however, is not the determinate boundary delimiting the area it may occupy, or the topological connectedness of its elements, but the joint commitment of its (non-clonal) set of pluripotent founder cells to a common fate. This fate may still include a set of subfates, with respect to which the founder cells have remained pluripotent. Thus, at a later stage two or more (non-clonal) sets of cells of the polyclone may become committed to take on different subfates. In this way, the polyclone would be split into two or more cellular subsets, or newly constituted sister polyclones.

The list of diagnostic criteria of compartmenthood includes the requirements that these successive polyclonal subdivisions have to be 'binary' (that is, never give rise to more than two sister polyclones), and that the sister polyclones so generated have to be characterized by different 'cell recognition' properties. Neither of these two requirements seems to be an essential ingredient of the polyclone concept, however. For instance, the differential cell recognition requirement had been introduced to account for the formation of sharp boundaries delimiting

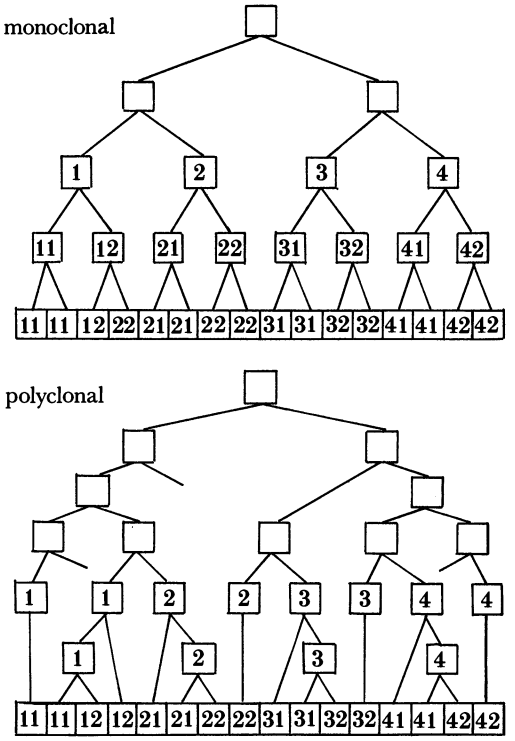

FIGURE 3. Comparison of monoclonal and polyclonal pathways for generating a string of 16 cells committed to forming four different four-cell 'segments' (designated by the first digit 1, 2, 3 or 4), each of which consists of two different, serially homologous, two-cell subsegments (designated by the second digit 1 or 2). Along the monoclonal pathway, each member of a four-cell clone of founder cells is primarily committed to formation of a particular segment. Each segmental founder cell gives rise to a two-cell subclone, of which each member is secondarily committed to formation of one or the other subsegment. Each of the eight subclone founder cells gives rise in turn to a two-cell subclone of unchanged commitment. The final string of 16 committed cells is a clone, all the members of which are removed by four divisions from the original clonal founder cell. Along the polyclonal pathway, non-sister cell pairs of a non-clonal eight-cell ensemble are jointly committed to formation of a particular segment. Some of the segmental founder cells are secondarily committed to formation of one or the other subsegment without further division; other segmental founder cells divide once, yielding one daughter which is secondarily committed and another daughter which does not undergo secondary commitment until it divides once more, yielding two daughters of which one may be committed to formation of one kind of subsegment and the other to formation of another. The final string of 16 committed cells is not a clone; some of the cells are removed by as few as four and others by as many as six divisions from the original founder cell.

the adjacent areas occupied by sister polyclones in the *Drosophila* epidermis. But, as has been shown more recently, the origin of some of these sharp boundaries is attributable to processes other than cell recognition, such as the pattern and timing of mitoses within the sister polyclones (O'Brochta & Bryant 1985). It is in its invocation of a multicellular joint commitment process that polyclonal development in insects differs most significantly from the monoclonal development characteristic of embryogenesis in nematodes and leeches, where a single clonal founder cell is committed to a particular fate. Thus it is the topographic placement of a cell rather than its line of descent which governs its inclusion in the set of founder cells of a particular polyclone.

The notion of the equivalence group can be readily extended from monoclones to polyclones: two polyclones, of which one normally differentiates into a set of cell types A and the other into a set of cell types B, constitute an equivalence group, if under some abnormal condition

one polyclone can take on the fate normally characteristic of the other. As revealed by the developmental effects of the 'homeotic' genes of *Drosophila* (Ouweneel 1976; Lewis 1978), the entire set of metameric primordia of the cellular blastoderm stage forms an equivalence group, consisting of 15 or so equivalently pluripotent polyclones. Ordinarily, each of the polyclonal generative metameres takes on a segment-specific fate, as reflected in the heterosis of their meromes, due to the interaction of the products of rostrocaudally differentially expressed set of genes (Beachy *et al.* 1985; Sanchez-Herrero *et al.* 1985). But upon mutation (or deletion) of one or more of those genes, a particular generative metamere may take on the fate characteristic of another segment. This phenomenon is referred to as 'homeosis', to connote the abnormal reversal, or abolition, of normal heterosis. The genes whose mutation leads to homeosis are designated as 'homeotic' genes, although their normal wild type function is, in fact, the generation of heterosis.

Conclusion

I conclude my brief overview by recalling that 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. Indeed, nearly 80 years ago E. B. Wilson (1898) had already noted that a counter-example can usually be found for any generalization regarding the connection between cell lineage and developmental cell fate. Hence, as exemplified by the recent fate of the compartment concept, one is well advised to eschew over-definitions in the formulation of concepts pertaining to this connection if they are to be of more than parochial significance. As we now know, the role played by cell lineage varies greatly in the embryogenesis of different taxa, and even for different aspects of the 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 very 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 the 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.

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