Volume 215, number 1, 1–8 FEB 04592 May 1987

Review Letter

Cell lineage in development

G.S. Stent

Department of Molecular Biology, University of California, Berkeley, CA 94720, USA

Received 2 February 1987

1. ORIGINS OF CELL LINEAGE STUDIES

Studies of developmental cell lineage – i.e. of the fate of individual cells that arise in an early embryo – were begun in the 1870's, 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 evolutionary ancestor. Only after gastrulation would the germ layers – ectoderm, mesoderm, endoderm – be destined to take on the tissue differentiation characteristic of more recent metazoan ancestors. To test this implication Charles Whitman [1] 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 suggested that the differentiated properties which characterize a given cell of the mature animal are causally linked with that cell's developmental line of descent.

Despite these highly promising beginnings, the study of cell lineage went into decline after the turn of this century. It remained a biological backwater for the next 50 years, probably because the discovery of regulative and inductive phenomena in embryos of sea urchins and frogs focussed the attention of embryologists on cell interactions rather than on cell lineage as causal factors in cell differentiation, especially on the mechanism by which one part of the embryo induces the developmental fate of another part. Since in this induction specific chemical inducers were generally thought to play a key role, the search for and attempted identification of such inducers came to dominate experimental embryology for the next 30 years. By the end of this period, however, no single substance had been identified for which the role of a specific inducer could be convincingly demonstrated. In retrospect, the reason for this failure is quite apparent: prior to the 1960's embryologists lacked the molecular biological insights which we now know to be necessary for understanding the chemical basis of the induction process.

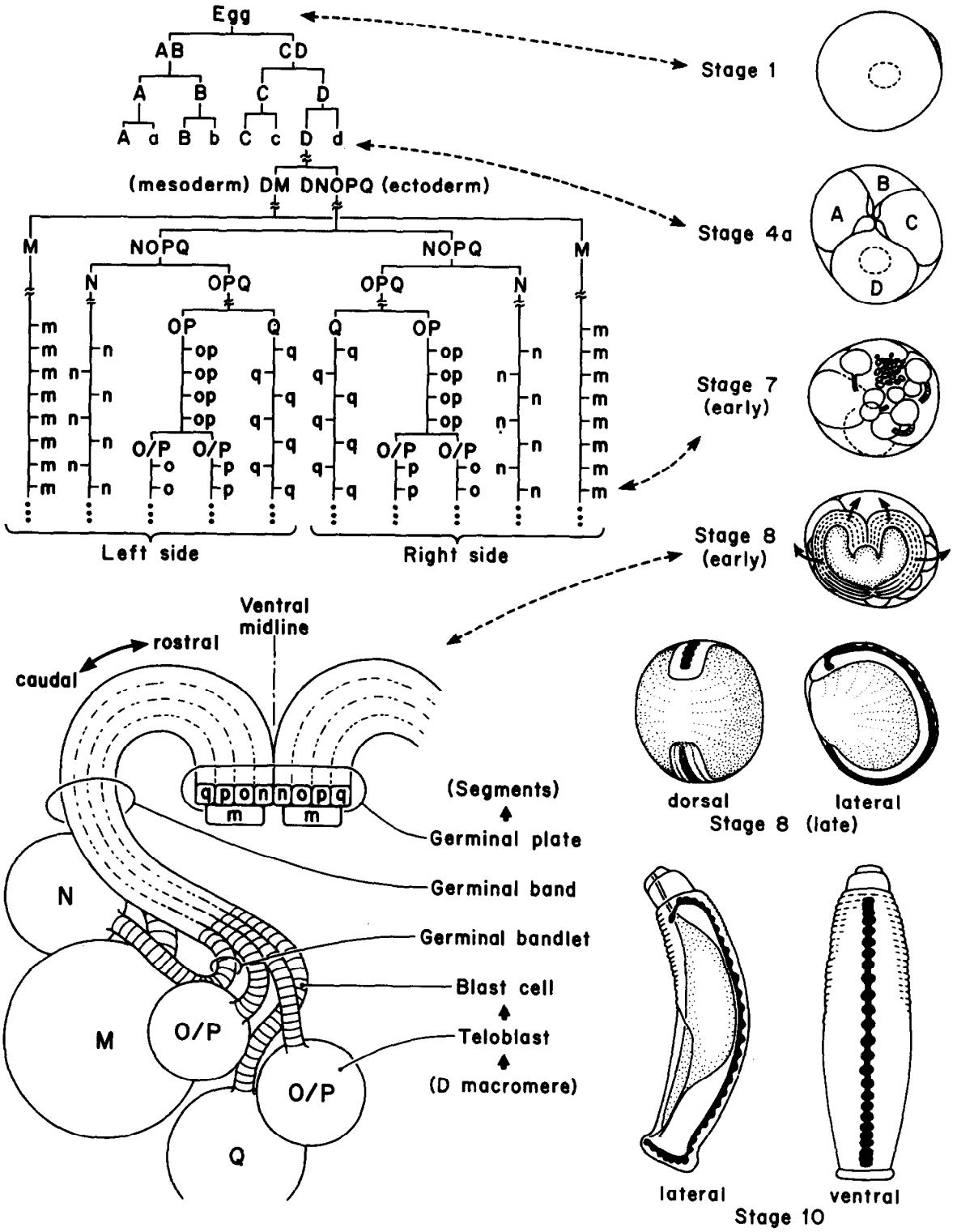
2. CELL LINEAGE TRACERS

About 20 years ago interest revived in the developmental role of cell lineage*. Novel techniques, capable of revealing the line of descent of single, identified cells came into use, such as direct observation of embryogenesis under differential interference contrast optics [2], photoablation of specified precursor cells by laser microbeams [3], generation of embryos whose tissues are mosaics of genetically different cells [4], and cell labelling

Correspondence address: G.S. Stent, Dept of Molecular Biology, University of California, Berkeley, CA 94720, USA

* An excellent overview of modern cell lineage studies is provided in Single Cell Marking and Cell Lineage in Animal Development (Gardner, R.L. and Lawrence, P.A. eds) London: The Royal Society, 1986 [(1985) Phil. Trans. R. Soc. Lond. B312, 1–187]

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50


Egg
AB
CD
A
B
C
D
A a B b C c D d
(mesoderm) DM DNOPQ (ectoderm)
M
NOPQ
NOPQ
M
N
OPQ
OPQ
N
OP
Q
Q
OP
O/P O/P
O/P O/P
Left side
Right side
Stage 1
Stage 4a
Stage 7
(early)
Stage 8
(early)
A
B
C
D
Ventral
midline
rostral
caudal
q p o n n o p q
m m
(Segments)
Germinal plate
Germinal band
Germinal bandlet
Blast cell
Teloblast
(D macromere)
N
M
O/P
O/P
Q
dorsal
lateral
Stage 8 (late)
lateral
ventral
Stage 10

with intracellular lineage tracers [5]. The last of these techniques consists of microinjecting into an identified embryonic cell a tracer molecule that is passed on to all, and only to, the lineal descendants of the injected cell. These descendants can be identified at a later developmental stage, by observing the distribution pattern of the tracer within the embryonic or postembryonic tissues. One such lineage tracer is the enzyme horseradish peroxidase (HRP), whose intracellular presence can be detected by treatment of labeled tissues with a suitable histochemical reagent which causes formation of a black precipitate in any cell containing the enzyme [6]. Another kind of lineage tracer consists of an adduct of a fluorescent dye, such as fluorescein or rhodamine, with an inert carrier molecule, such as dextran [7]. The cellular distribution of fluorescent tracers can be observed in living tissues under the fluorescence microscope, in contrast to HRP, which can be detected only in dead tissues killed by the reagent treatment. The fluorescein-based tracer has an additional, very useful property: it can also serve as a specific photosensitizer. Thus, exposure of fluorescein-labeled embryonic tissues to light of a particular wavelength leads to death by photo-oxidation of all illuminated descendants of the tracer-injected precursor cell, but not of any other, genealogically unrelated cells with which the labeled cells may be intermingled [8]. Fluorescein-based tracers make it possible, therefore, to examine the developmental effects of the selective ablation of cells of particular lines of descent.

3. DETERMINATE AND INDETERMINATE DEVELOPMENT

Use of these novel techniques showed that development is highly *determinate* in simple invertebrates, such as nematodes, where the division pattern of the early embryo is sufficiently stereotyped to permit identification of each cell and where the developmental fate of an identified cell is the same in specimen after specimen [2]. By contrast, in complex vertebrates, such as mammals, the fate of the cells in the earliest developmental stages is *indeterminate*. Here any cell may be destined for any developmental fate, with alternative fates being apparently governed by chance factors [9]. Between nematodes and mammals there exists a broad spectrum of intermediate situations, ranging from the extreme of wholly determinate to that of wholly indeterminate development, in which a particular (non-random) probability of realization can be assigned to each of several alternative possible fates.

4. LEECH DEVELOPMENT

The intracellular lineage tracers found their first extensive application in a resumption of the study of cell lineage relations in the development of leeches, which Whitman had begun a century ago. The bilaterally symmetric, tubular body of leeches is built of 32 segments. Each segment contains a set of serially iterated morphological elements, such as the segmental ganglion of the central nervous system with its approx. 200 pairs of identifiable neurons, sets of a few dozen identifiable neurons of the peripheral nervous system and of identifiable longitudinal and circular muscles, and three circumferential subdivisions of the skin, or annuli. One of these three annuli lies in register with the segmental ganglion and includes circumferentially distributed sensory organs, or sensilla. Traditionally, the sensillar annulus and its in-register ganglion have been considered to mark the middle of the segment and the furrow separating two adjacent non-sensillar annuli to mark the border between two successive segments.

The embryonic development of leeches (fig.1), onward from the fertilized egg, proceeds via a stereotyped sequence of divisions which give rise to an embryo whose cells can be individually iden-

Fig.1. Schematic summary of the development of the leech. Upper left: cell pedigree leading from the uncleaved egg to the teloblast pairs M, N, O/P, O/P, and Q; and the paired primary blast cell bandlets. 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. 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. In the stage 8 (late) embryo the germinal plate is shown to lie on the ventral midline, with the nascent central nervous system and its ganglia indicated in black. In the stage 10 embryo shown, body closure is nearly complete [10].

tified. The segmental tissues arise from bilateral sets of five large embryonic cells, designated *teloblasts*. By a series of iterated, highly unequal divisions, each teloblast generates a bandlet of several dozen much smaller *primary blast cells*.

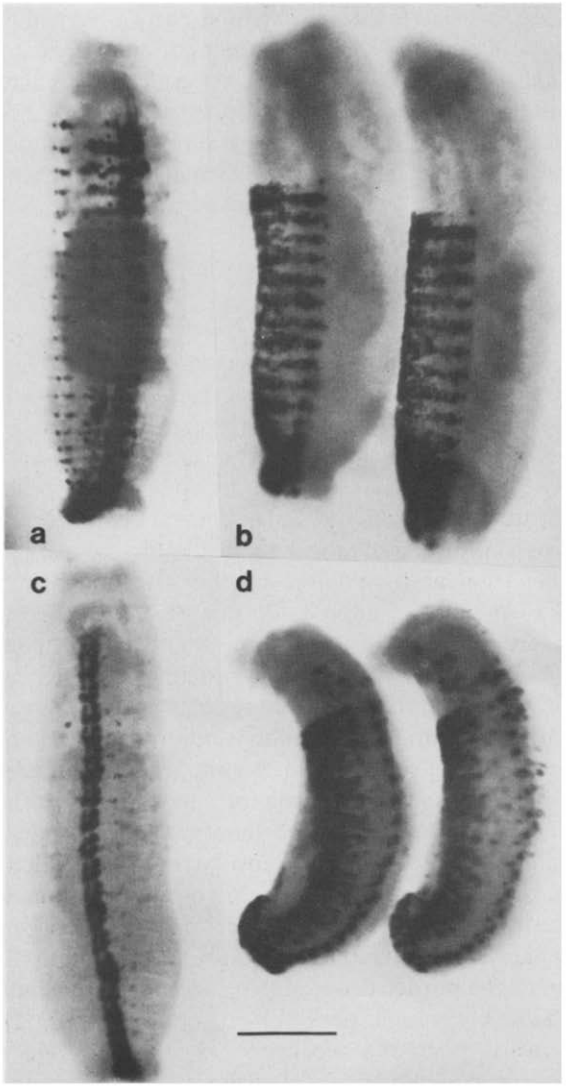


Fig.2. Segmentally iterated contributions of the ectodermal teloblasts to nervous system and epidermis. Labeling patterns of stage 10 leech embryos in which one teloblast had been injected with HRP at stage 7. Since prior to injection the teloblast had already produced some blast cells, the frontmost body segments are unlabeled. All labeled cell bodies lie on the same side of the embryo as the injected teloblast. Anterior is up; scale bar 200 μm. (a) Right O/P teloblast injected; O pattern obtained. Labeled cell processes can be seen to project frontward through the connective nerves from the anteriormost labeled ganglion. (b) Right O/P teloblast injected (in two replicate embryos); P pattern obtained. (c) Left N teloblast injected. (d) Right Q teloblast injected (in two replicate embryos). (a–c) Ventral views, (d) lateral views. Ventral midline of embryo is marked by apparent right edge of labeling pattern in (a,b,d) and by apparent left edge in (c). In (d), leading edge of expanding germinal plate is marked by apparent left edge of labeling pattern [10].

The bandlet designated m will give rise to the mesodermal and the bandlets designated n, o, p and q to the ectodermal cell complements of the 32 segments. On either side, bandlets merge to form left and right germinal bands, and the two bands migrate over the surface of the embryo and eventually coalesce in front-to-rear sequence to form a sheet of cells, the *germinal plate*, along the future ventral midline.

Proliferation and differentiation of the m, n, o, p and q blast cell clones gives rise to a morphological periodicity of the germinal plate, reflecting formation of the 32 body segments. Cell proliferation also causes the germinal plate to expand laterally around the circumference of the embryo, until the right and left leading margins of the plate meet along the dorsal midline. At this point formation of the body tube of the leech is complete.

5. BLAST CELL FATE

To ascertain the developmental fate of each of the five kinds of blast cell bandlets, their teloblasts of origin were individually injected with cell lineage tracers. Subsequent observation at later developmental stages of the tracer distribution among the differentiated cells revealed that each bandlet gives rise to a particular hemilateral complement of segmentally iterated, identifiable cells [10] (figs 2,3). These findings thus extended Whitman's century-old inference of a determinate fate of early embryonic cells from the germ layers to the ultimate level of identified, post-mitotic cells.

6. SEGMENTAL FOUNDER CELLS

The tracer injection technique also made it possible to ascertain the number of primary blast

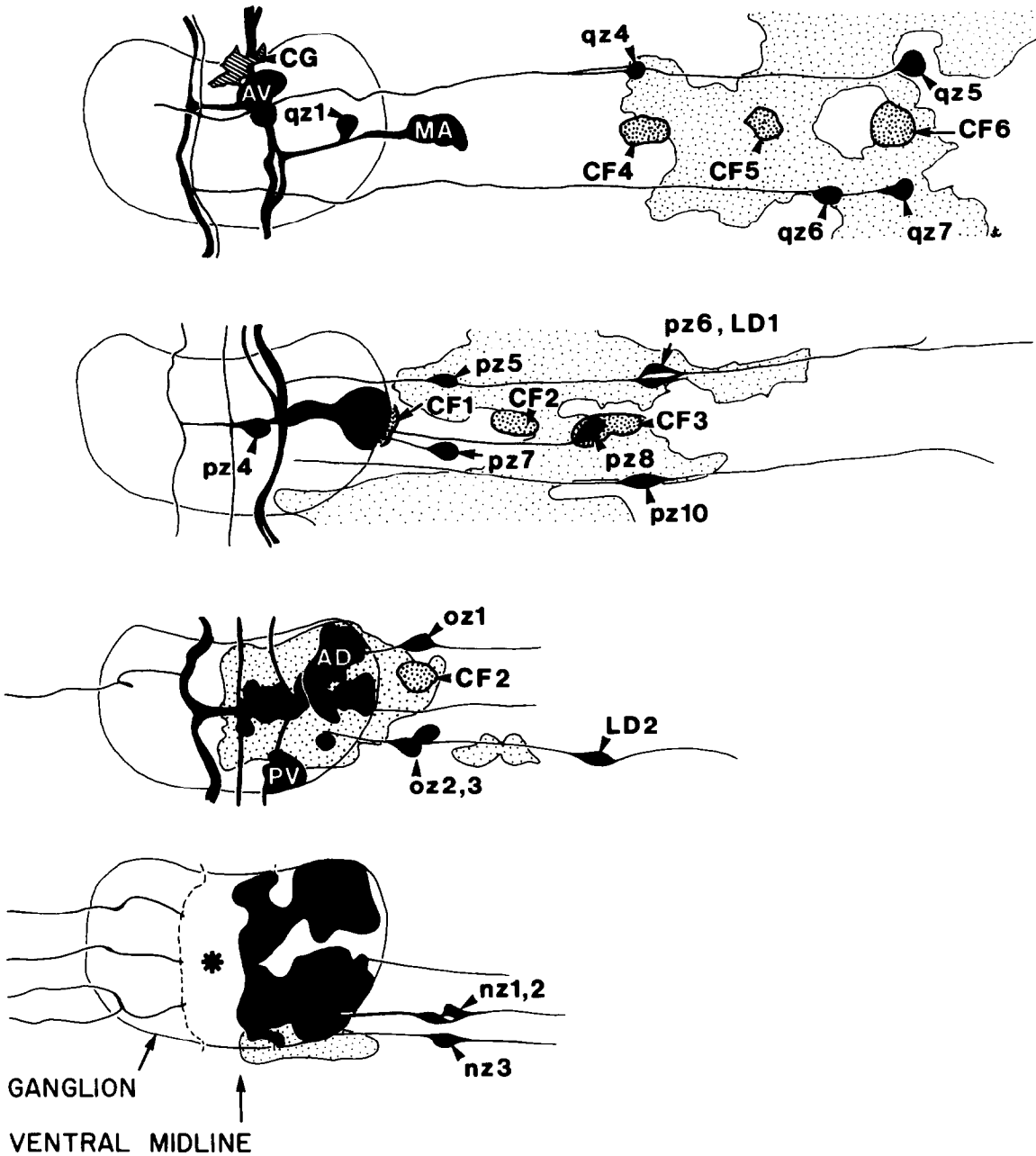


Fig.3. Ectodermal segmental cell complements in early stage 10 leech embryos, traced from photomicrographs of rhodamine tracer-labeled germinal plates. The right edge of the figure corresponds to the leading margin of the expanding germinal plate, and thus to the future dorsal midline. Anterior is up. Labeled epidermis is indicated by light stippling; epidermal cell florets (CF), numbered 1–6, medial to lateral, are more densely stippled. Names of neurons (e.g. gz1) include a letter indicating the blast cell type of origin (n, o, p, or q). LD1 and LD2 designate identified dopamine-containing neurons. Top, q-derived complement. AV, anteroventral, central neuron cluster; CG, connective glioblast; MA, peripheral neuron cluster along the MA nerve. Second from top, p-derived complement. Third from top, o-derived complement. AD, anterodorsal, central neuron cluster; PV, posteroventral, central neuron cluster. Bottom, n-derived complement [11].

cells in the m, n, o, p and q bandlets from which each of the hemilateral cell complements shown in fig.3 to lie in each morphologically identified segment is descended [12]. Each hemilateral segmental o, p or m cell complement was found to be a clone derived from a single o, p or m blast cell. However, for each of these segmental complements the boundary between successive clonal domains turned out not to coincide with the segment border, as marked by the furrow separating two adjacent non-sensillar annuli, but to course midway under the sensillar annulus and *across* the segmental ganglion. By contrast, each hemilateral segmental n or q cell complement was found to be derived from two serially successive n or q primary blast cells. The domains of the two segmental complement cell clones alternate along the longitudinal body axis, with the boundary between every other such domain coinciding with a segment border. Thus it transpired that in the leech there exists a 180° phase shift between the traditional, morphologically defined segments borders and the borders of the cell clones that give rise to the serially iterated tissues, i.e. the *generatively* defined segment borders. A similar 180° phase shift between morphological and generative segment borders has been brought to light recently by cell lineage studies in insects, where the generatively defined segment has been designated 'parasegment', to distinguish it from the traditional, morphologically defined, ganglion-centered segment [13].

Hence, on either side of the leech, each segment (or parasegment) arises as seven distinct cell clones. Each of these seven clones comprises a few dozen characteristic cells, which, as more detailed cell lineage observations have revealed, are derived from their founder blast cell via a clone-specific, stereotyped cell division pattern. By contrast, bilaterally and serially homologous primary blast cells generate homologous cell lineage patterns, thus accounting for the bilaterally symmetrical and segmentally iterated morphological features of the animal.

7. COMMITMENT

How does a given cell of the mature animal come to take on its characteristic phenotype? Cell lineage studies have revealed the existence of a process by which an embryonic precursor cell is committed to impose on its descendants a particular pattern of differential gene expression that turns them into one cell type rather than into another. The commitment concept is based on the idea that an embryonic cell is initially *pluripotent*, i.e. capable of giving rise to differentiated descendants of either of two alternative cell types. Commitment of the embryonic 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. The differentiated phenotype of any given postembryonic cell would therefore depend on a series of commitments made by pluripotent cells in its line of ancestry.

Does the process of commitment of initially pluripotent embryonic cells, which plays an obvious role in the indeterminate development of mammals, have any relevance for the determinate development of nematodes and leeches, where individual embryonic cells seem to have pre-destined fates? Yes, because there is a conceptual as well as empirical distinction between determinate fate and commitment to that fate [14]. One example of the need for making that distinction is provided in leech development by the two equivalently pluripotent ectodermal sister teloblasts, both designated as O/P (fig.1). Upon birth by cleavage of their mother cell, both sister teloblasts 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). Here O and P fates refer to two different cell sets, whose elements comprise identifiable nerve and skin cells. 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 [15]. However, if prior to the first division of an o blast cell, the directly apposed p blast cell is specifically photoablated by use of the photosensitizing cell lineage tracer, then the o blast cell 'transfates' from its normal O fate to the abnormal P fate [16]. Hence, despite its determinate O fate, the o blast cell still remains pluripotent – i.e. capable of taking on either the O or the P fate.

Experiments in which p blast cells were photoablated 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 cell. However, commitment of the o cell to the O fate does not occur 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 the generation of a particular subset of the elements of the P fate. Since for every particular subset of elements of the O fate committed in each of the three steps, the potency for transfating to a particular subset of P fate elements is lost, it can be inferred that there is a complementary relation between the gain of commitment to one subset of elements of the O fate and the loss of developmental potency for a corresponding subset of elements of the P fate. Thus each commitment step seems to pertain to a different set of paired determinants of mutually exclusive fates [16,17].

8. AGENTS OF COMMITMENT

There are two kinds of commonly considered agents which may commit embryonic cells to their fate. One kind is represented by *intracellular determinants* of cell differentiation, which account for the commitment of sister cells to different fates in terms of their unequal partition in successive cell divisions. Here cell lineage would play a crucial role in cell commitment by consigning particular subsets of intracellular determinants to particular cells. The other commonly considered kind of agent consists of a set of *intercellular inducers*, which are anisotropically distributed over the volume of the embryo. Here 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. In some cases cell lineage was found to play its determinative role in cell commitment by bringing about the orderly, unequal partitioning of intracellular determinants over daughter cells in successive cell dividions [18] and in other cases, by bringing about an orderly topographic cell placement [8].

9. TYPOLOGICAL AND TOPOGRAPHIC COMMITMENT HIERARCHIES

Regardless of which of these alternative agents might happen to be responsible for commitment to developmental cell fate, it had been generally expected that commitment to a particular cell type proceeds stepwise, according to a *typologically* hierarchic sequence. For instance, it was thought that in the developmental line of ancestry of a cholinergic motor neuron there would occur a commitment first to ectoderm rather than to mesoderm, then to nervous tissue rather than to skin, then to neuron rather than to glial cell, then to motor neuron rather than to sensory neuron, and finally to synthesis of choline acetyltransferase rather than glutamic acid decarboxylase.

Cell lineage studies have shown that development does not necessarily proceed according to such a typologically hierarchic commitment sequence. For instance, in nematodes and leeches, 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 genealogically very remote mesodermal branch of the lineage tree. Instead of being typologically hierarchic, in these invertebrate embryos the commitment sequence turns out to be *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. Here the spatially ordered sequence of cell divisions represented by that genealogical relation is so structured that most differentially committed postmitotic cells arise at, or very close to, the sites where their presence is actually needed [14].

10. SUMMING UP

Modern cell lineage studies have shown that in animal development the fate of embryonic cells is governed by a diversity of mechanisms. Thus, the development of cell fate ranges from a high degree of indeterminacy in complex vertebrates, such as mammals, to a high degree of determinacy in simple invertebrates, such as nematodes and leeches. And the genealogical process by which an initially pluripotent cell commits a daughter cell to a given phenotype may entail either the transmission to the

daughter of a particular subset of intracellular determinants or the placement of the daughter at a particular site in a field of intercellular inducers. Moreover, the commitment to expressing the ensemble of properties characteristic of that phenotype may proceed according to a sequence which is either typologically or topographically hierarchic. In this connection, the generation of the serially iterated and bilaterally symmetrical morphology of segmented animals via serially homologous cell lineages, each constituting a clone descended from one of a fixed number of segmental founder cells, is a dramatic example of the topographic commitment hierarchy. The phenomenological description of these developmental processes by cell lineage studies has set the stage for the elucidation of their molecular bases.

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