Cell Lineage

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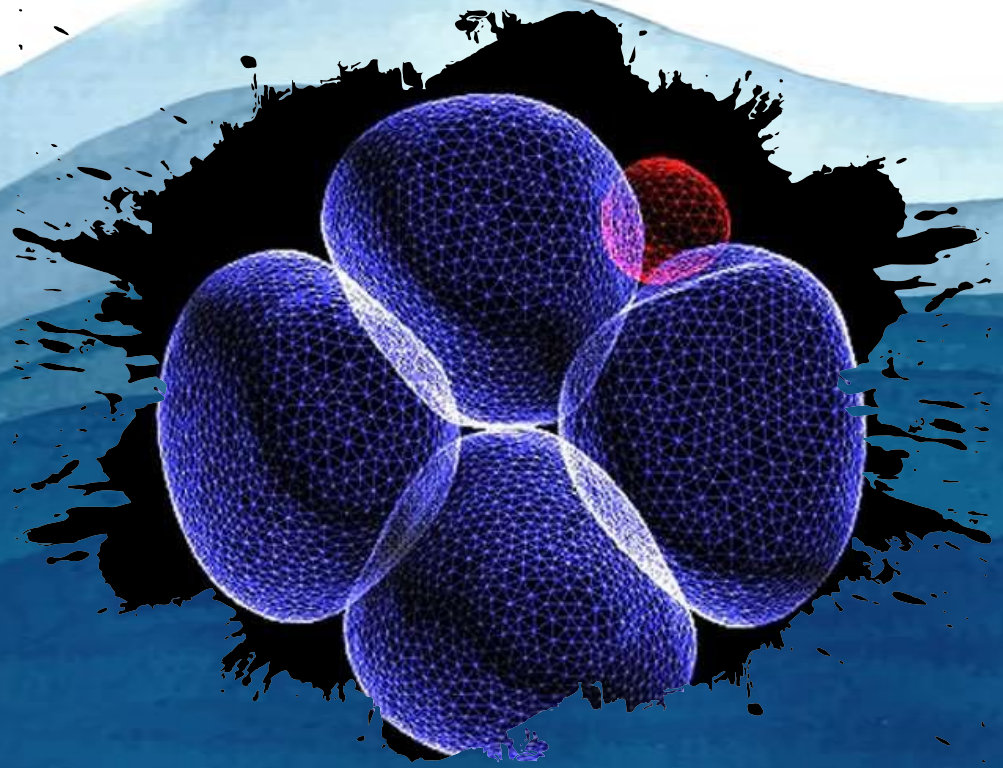
By Dr. Subarna Ghosh

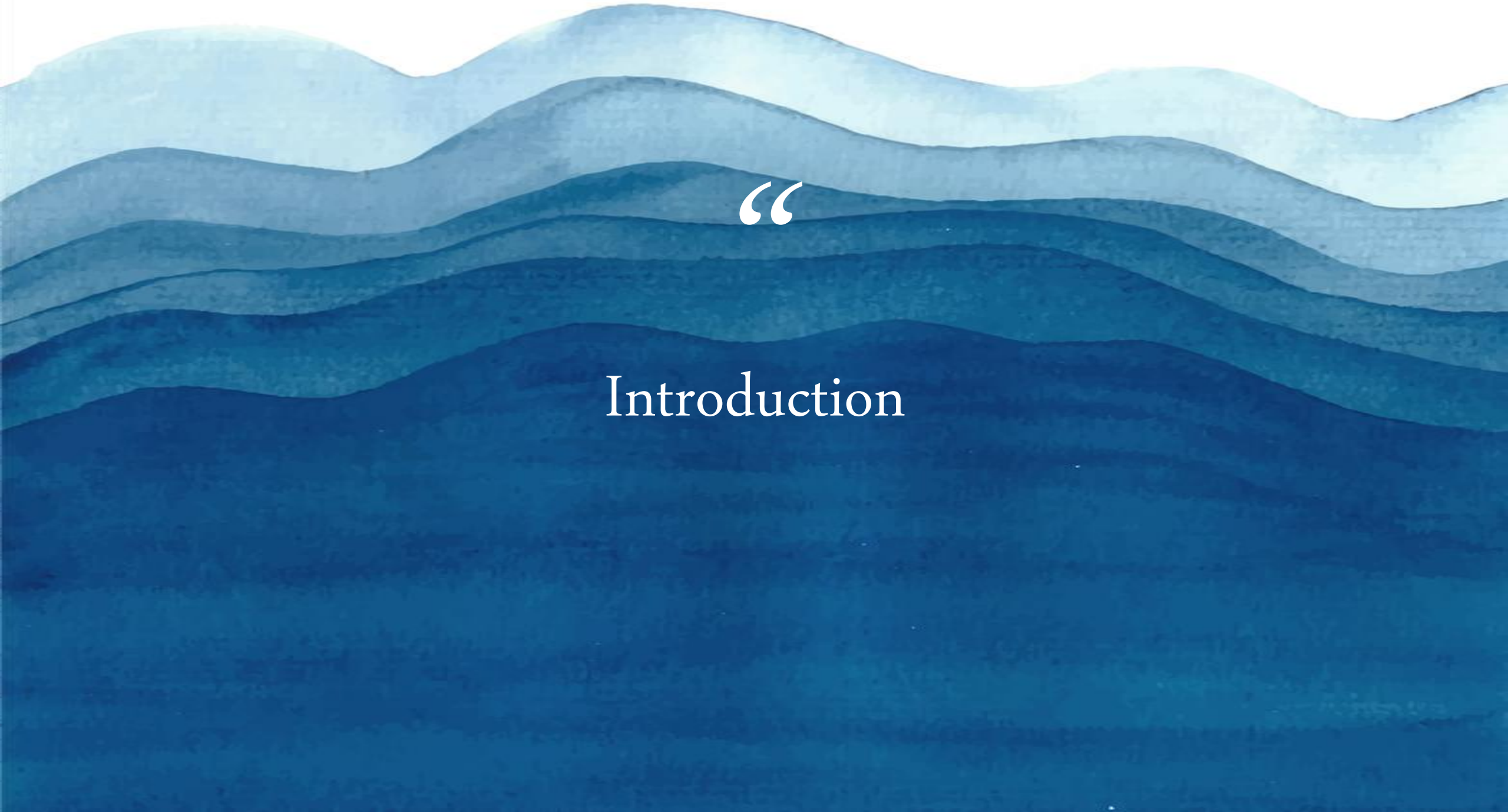


The study of the fate of each blastomere after the first and second cleavage is called cell lineage study or Cytogeny.

1. Facts about Cytogeny

- This study is easier in spirally cleaving mosaic eggs of Flatwoms, Annelids and Molluscs than in radially cleaving regulative eggs of other animals.

- Spirally cleaving eggs exhibit determinate cleavage; that is orientation of each cleavage product or blastomere and its progeny is dependent on its prior division history



Introduction

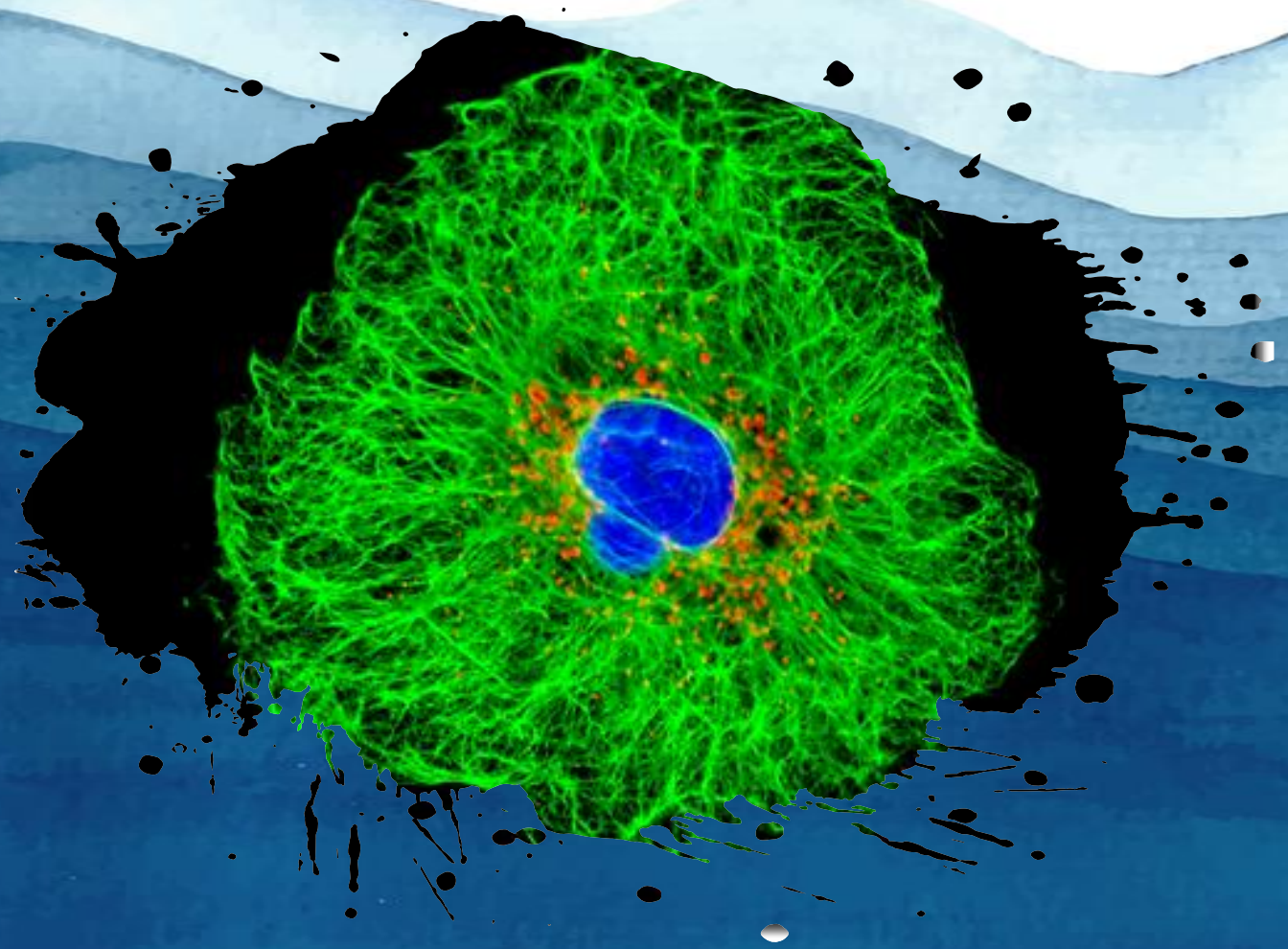
- The cell lineage of an organism is the pattern of cell divisions during its development. Cell lineages are described by following cell divisions in living individuals, or by marking cells and examining their progeny. Some organisms or precursor cells display invariant patterns of cell division, in which specification of cell fates is correlated with cell division patterns; in other organisms, lineage patterns are variable and not correlated with cell fates.

- Invariant cell lineages reflect both cell-autonomous mechanisms of fate determination and highly reproducible cell to cell interactions. Genetic analysis of cell lineages has focused on systems where cell lineage and cell fates are correlated, such as Caenorhabditis elegans or the nervous system of Drosophila.

History of cell lineage study

Cell lineage studies began with Whitman's description of cleavage patterns in leech embryos in the 1870s, and continued with descriptions of lineages in many invertebrate animals, including nematodes, sea urchins, and ascidians. It was found that in some animal groups, such as nematodes and ascidians, the pattern of cell divisions was almost identical from individual to individual. Such `invariant' cell lineages allowed the reconstruction of extensive lineage trees. In other animals, such as leeches and insects, stereotyped patterns of cell division (`sublineages') were seen in the progeny of particular precursor cells. Because of the correlation between cell lineage and cell fate in such invariant lineages, it was assumed that cell fates were determined by factors segregating within the dividing cells (termed `determinate' cleavage). This mode of development was contrasted with the `indeterminate‘ cleavages observed in other animals, in which cell lineages are variable and cell fates are determined by a cell's interaction with its environment. However, as discussed below, invariant cell lineages do not necessarily mean that cell fates are determined by the cell lineage pattern (see Moody, 1999 for examples). Over time, the term `cell lineage' has acquired multiple meanings (Slack, 1991; Price, 1993). Here, cell lineage is defined as the pattern of cell divisions in the development of an organism, whether invariant or not.

Methods involved in the study of Cell lineage



How cell lineages are followed?

1. Direct Observation

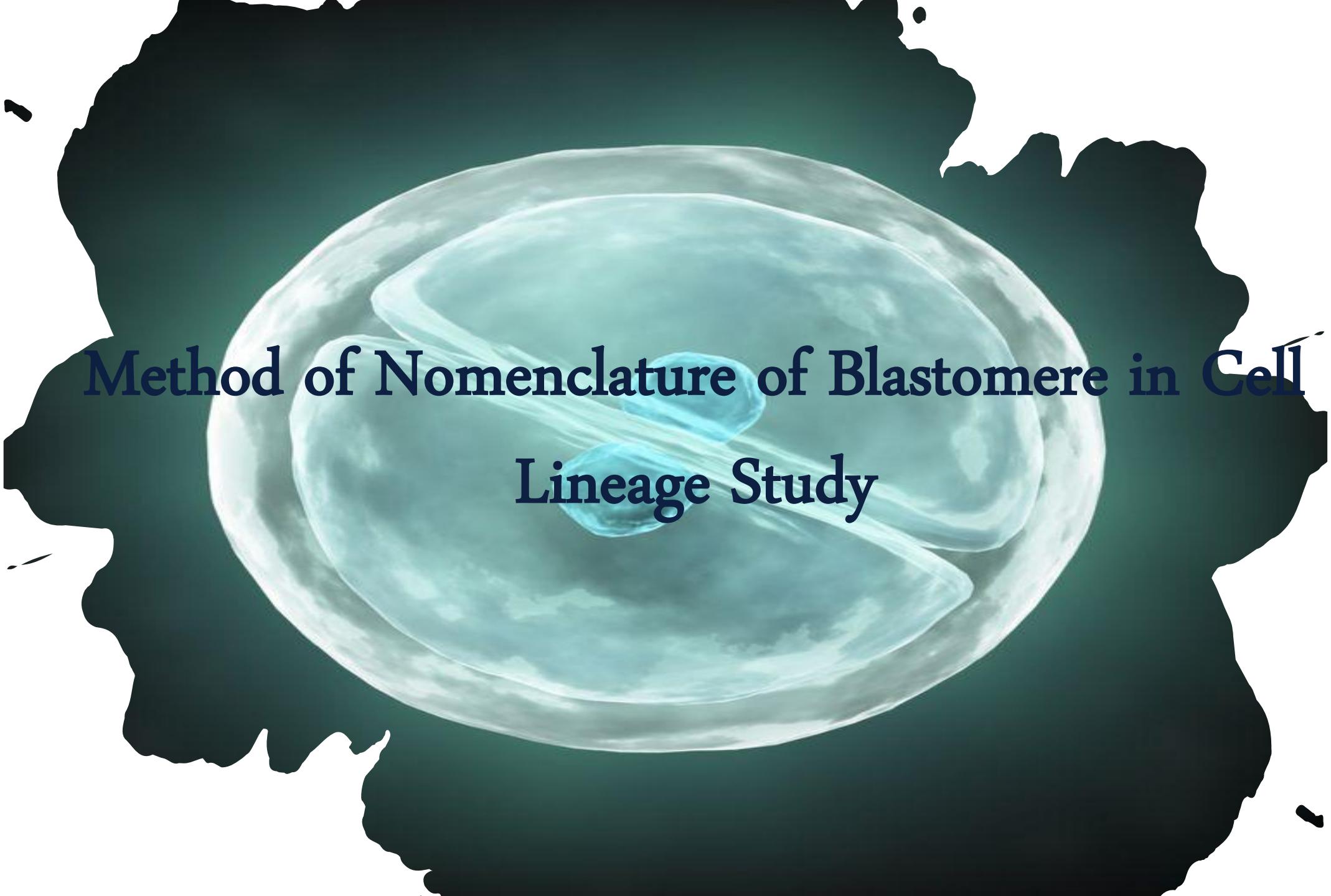
2. Clonal Analysis

1. Direct Observation

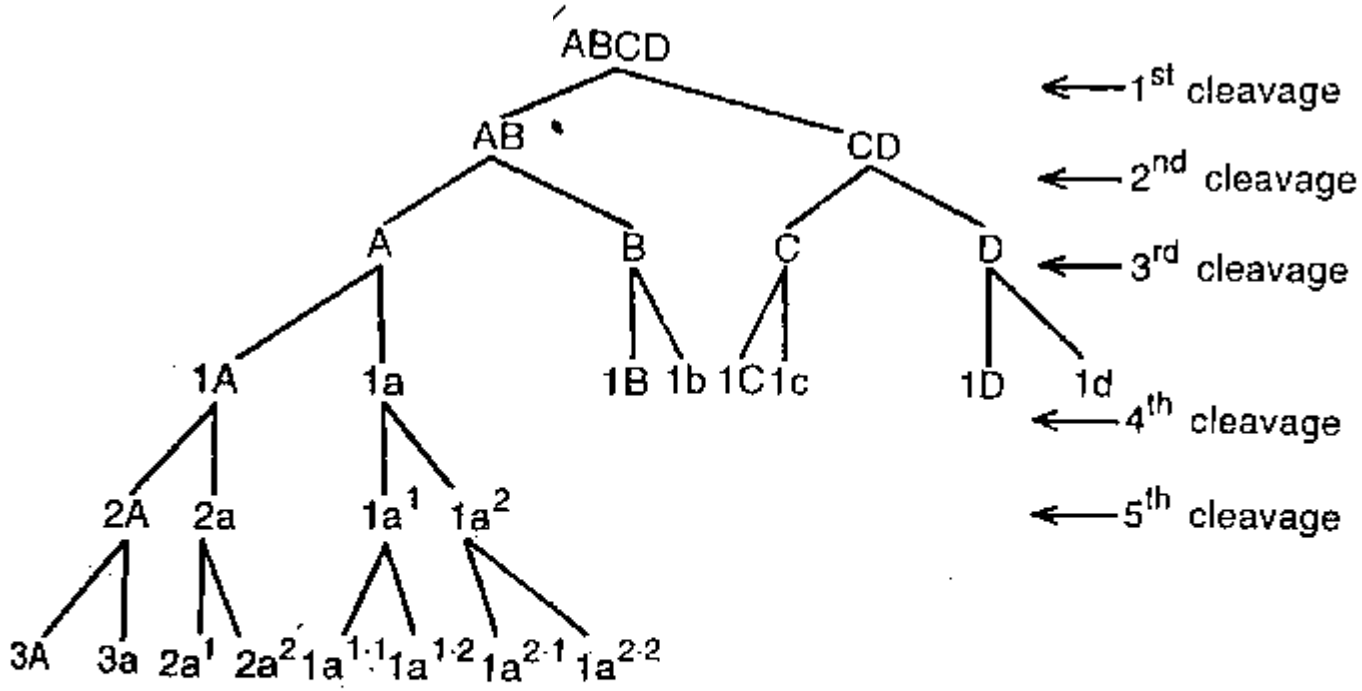
In the nineteenth century, lineages were followed either by direct observation, or by reconstruction from fixed specimens. Such studies required embryos that were small, transparent, and rapidly developing, but were necessarily limited to early embryogenesis where the cells were large and few in number. More extensive observations of cell lineages have been made possible by the development in the 1960s of Nomarski differential interference contrast microscopy, which allows the imaging of transparent specimens. The complete cell lineage of the nematode C. elegans was followed using Nomarski microscopy; cell lineages in the Drosophila central nervous system have also been described by direct observation. More recently, time lapse microscopy in multiple focal planes (`fourdimensional' microscopy) has allowed entire cell lineages of individual animals to be recorded digitally.

2. Clonal Analysis

In large, opaque, or slowly developing embryos, direct observation of cell divisions is not feasible. To analyze cell lineages in such cases, it is necessary to mark individual cells by physical or genetic means, and later to identify their progeny by expression of the marker. Such techniques are known as clonal analysis, because the progeny of a single cell forms a clone. In many animals cells can be labeled by injection with a nondiffusing dye such as fluorescein-conjugated dextran. A problemwith this technique in growing tissues is that the dye can become progressively diluted with each round of cell division. In vertebrates, cells can be marked by infection of an embryo with a replicationdefective retrovirus that expresses a reporter gene such as b-galactosidase or green fluorescent protein (GFP). At low virus concentrations single cells can be infected and their progeny recognized by reporter gene expression; there is no dilution of the marker because each cell in the clone expresses the reporter gene. This technique has been used to analyze cell lineages in chick and mammalian neural development.



Method of Nomenclature of Blastomere in Cell Lineage Study



Similarly the progeny of B will be :

3B, 3b, $2b^1$, $2b^2$, $1b^{1\cdot1}$, $1b^{1\cdot2}$, $1b^{2\cdot1}$, $1b^{2\cdot2}$

The progeny of C will be :

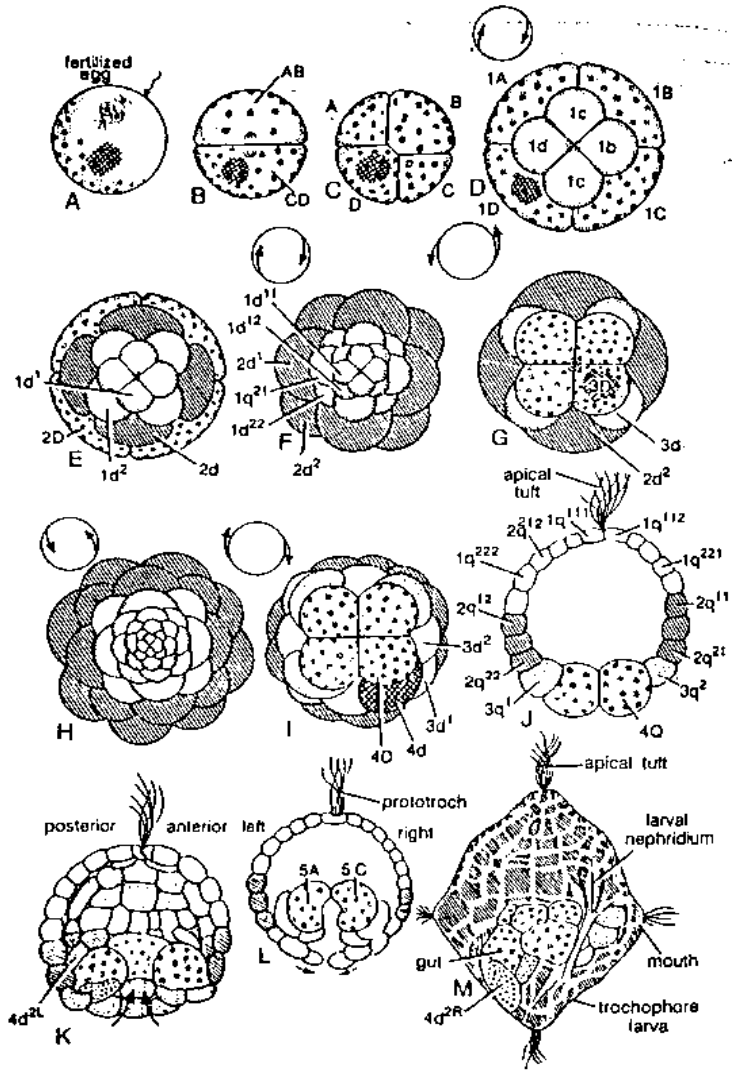
3C, 3c, $2c^1$, $2c^2$, $1c^{1\cdot1}$ $1c^{1\cdot2}$, $1c^{2\cdot1}$, $1c^{2\cdot2}$

The progeny of D will be :

3D, 3d, $2d^1$, $2d^2$, $1d^{1\cdot1}$, $1d^{1\cdot2}$, $1d^{2\cdot1}$, $1d^{2\cdot2}$

Method of nomenclature of blastomeres in cell lineage studies (after Majumdar, 1985).

Example Of Cell Lineage Study



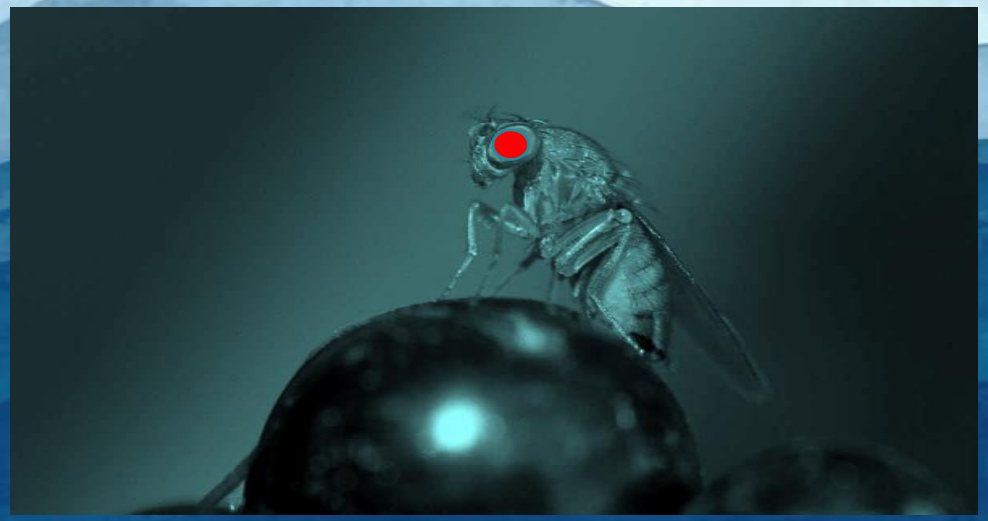
Cell lineage pattern and spiral cleavage stages in *Nereis*. A—Fertilized egg with arrow indicating point of sperm entry; B—2-cell stage with a large CD and a small AB blastomeres; C—4-cell stage; D—8-cell stage (viewed from the animal pole); E—16-cell stage from the animal pole (cell lineage of only D cell has been labelled); F—32-cell stage viewed from the animal pole; G—32-cell stage viewed from the vegetal pole; H—64-cell stage from the animal pole; J—64-cell stage from the vegetal pole; K–Vertical section of a 64-cell stage (from left to right); L—Vertical half (anterior to posterior) of a later stage showing invagination of the micromeres to form gut, *i.e.*, section from B–D quadrant; M—Vertical section of a later stage (taken at right angles to the plane of section in L) showing how the floor of gut is formed, *i.e.*, from A quadrant to C quadrant; N—Trochophore larva with mouth at the site of the front of the 'blastopore' and anus perforated at the opposite end (after Cohen, 1967).

1. An example of cell lineage study is given for the annelid, *Nereis*, a classic work done by **E.B. Wilson** in 1892. In *Nereis*, first four cleavages are synchronous. The **first cleavage** is unequal, giving rise to a small AB blastomere and a large CD. Sixty minutes later, the **second cleavage** furrow produces two equal-sized A, B blastomeres, while the larger CD cell divides into a smaller C and a larger D cell. Cleavage is spiral, alternating dexiotropic to leiotropic with each successive division. At the **third cleavage**, the first quartet is designated 1a–1d, marking the micromere derivative of each A-D macromere. With each succeeding macromere division, the second through fourth micromere quartets are formed, identified as 2a–2d through 4a–4d, respectively. It should be noted that the third cleavage division, in producing the first micromere quartet, segregates yolk and oil droplets into the macromeres, which remain there until the end of cleavage.

At the **fifth cleavage**, the divisions become asynchronous and the 32-cell stage is reached in steps as cell groups divide at different times. The first micromere quartet at the animal pole gives rise to the most apical structures in the larva. It divides into four **central cells** at the animal pole (designated $1a^1$–$1d^1$) and four **trochoblast** cells, $1a^2$–$1d^2$. The latter cells develop into a band of powerful cilia, encircling the larva, known as the **prototroch**. This band develops after only two more divisions, when 12 of the 16 cells produced immediately differentiate cilia and never divide again. The apical tuft of the larva (Fig. 12.8), made up of large cilia extending from the former animal pole, is derived from the four central cells $1a^1$–$1d^1$. These divide unequally into four small cells at the animal pole, designated $1a^{11}$–$1d^{11}$ and four larger cells $1a^{12}$–$1d^{12}$. The former (*i.e.*, $1a^{11}$–$1d^{11}$) immediately differentiate into apical tuft cells with long cilia, while the latter contribute to larval epidermis. In this way, each cell can be followed into the larval organs such as gut, eye spots, ciliary bands and coelomic sacs.

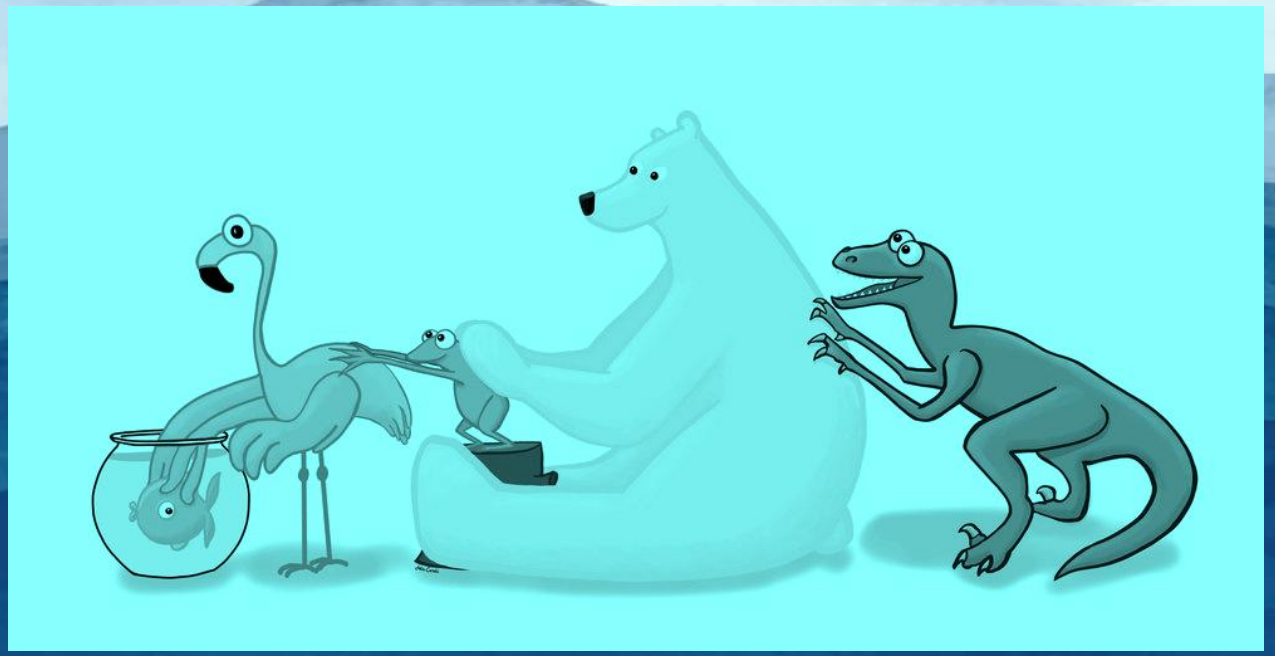
2. When cleavage takes place in the fertilized mosaic egg of *Dentalium* (a marine mollusc), just before the first cleavage, a small part of the cytoplasm bulges out from the vegetal hemisphere of the egg as a **polar lobe**. In the tune of cell lineage studies, the fertilized egg of *Dentalium* can be named as A B C D. The first cleavage furrow forms two blastomeres A B and C D. At this stage the polar lobe hangs from lower side of C D blastomere. However, this two-cell stage embryo has the appearance of three cells, so this stage is called **trefoil** (**Berrill** and **Karp**, 1978).

Before the second cleavage starts, the entire contents of the polar lobe goes into CD blastomere. When the second cleavage starts, the polar lobe again appears. When the second cleavage is completed, four blastomeres are formed, two A and B from AB cell and C and D from CD cell. At the four-cell stage, the polar lobe remains sticking to the D blastomere. If at this stage, the polar lobe is removed, the resulting embryo does not possess any mesoderm. Such an experiment (called **defect experiment**) tends to show that there is specific substance in the polar lobe which is responsible for the formation of the mesoderm in the embryo.



Cell Lineage in Insects

Insects mostly display cell lineages that are variable at the level of individual cell divisions. However, in the central and peripheral nervous systems (CNS and PNS), precursor cells undergo stereotyped sub-lineages giving rise to neurons and neuronal support cells. Analysis of such lineages has involved a combination of direct observation, dye labeling, and examination of lineage-specific molecular markers. Genetic analysis of cell lineages in insects has focused on Drosophila CNS and PNS neuroblast lineages. In the development of a peripheral sensillum such as a bristle, a precursor cell generates one neuron and three support cells. If activity of the Notch signaling pathway is reduced, all cells become neuronal, indicating that Notch signaling normally promotes the non-neuronal fate. Notch signaling appears to operate between sister cells in the lineage. Thus, although fates are specified autonomously within the lineage, they require local interactions between cells in the same lineage.



Cell Lineage in Vertebrates

The size and cell number of most vertebrate embryos make direct observation of cell division patterns difficult, and thus lineage relationships have been largely defined using clonal analysis. Cell marking and transplantation experiments in amphibians and the zebra fish Danio rerio have shown that the early cleavages are not determinative, and that cells do not become committed to specific fates until the blastula stage. Cell lineage studies in the vertebrate CNS and retina showed that individual cells can generate a wide variety of cell fates, even in very small clones. Thus, cell fates in these situations appear to be specified by a cell's environment and not by its lineal ancestry. Evidence suggestive of lineage-autonomous mechanisms of fate determination has come from the analysis of vertebrate homologs of proteins such as numb and Notch, both of which are asymmetrically localized in dividing neuroblasts in the mammalian cerebral cortex. However, the role of these proteins in cell fate specification in vertebrates has not yet been determined.

Cell Lineage in Plants

Stereotyped cell lineages have been observed in the development of many plants. Asymmetric cell divisions occur in the development of colonial algae such as Volvox, in which they segregate somatic versus germline fates. Early cell divisions of flowering plants such as Arabidopsis are highly stereotyped. However, cell interactions appear to be more important than ancestry in specifying fates. Stereotyped cell lineages are also observed during development of Arabidopsis root and floral meristems, and in stomatal development, but again the pattern of cell fates may be determined by interactions rather than ancestry.

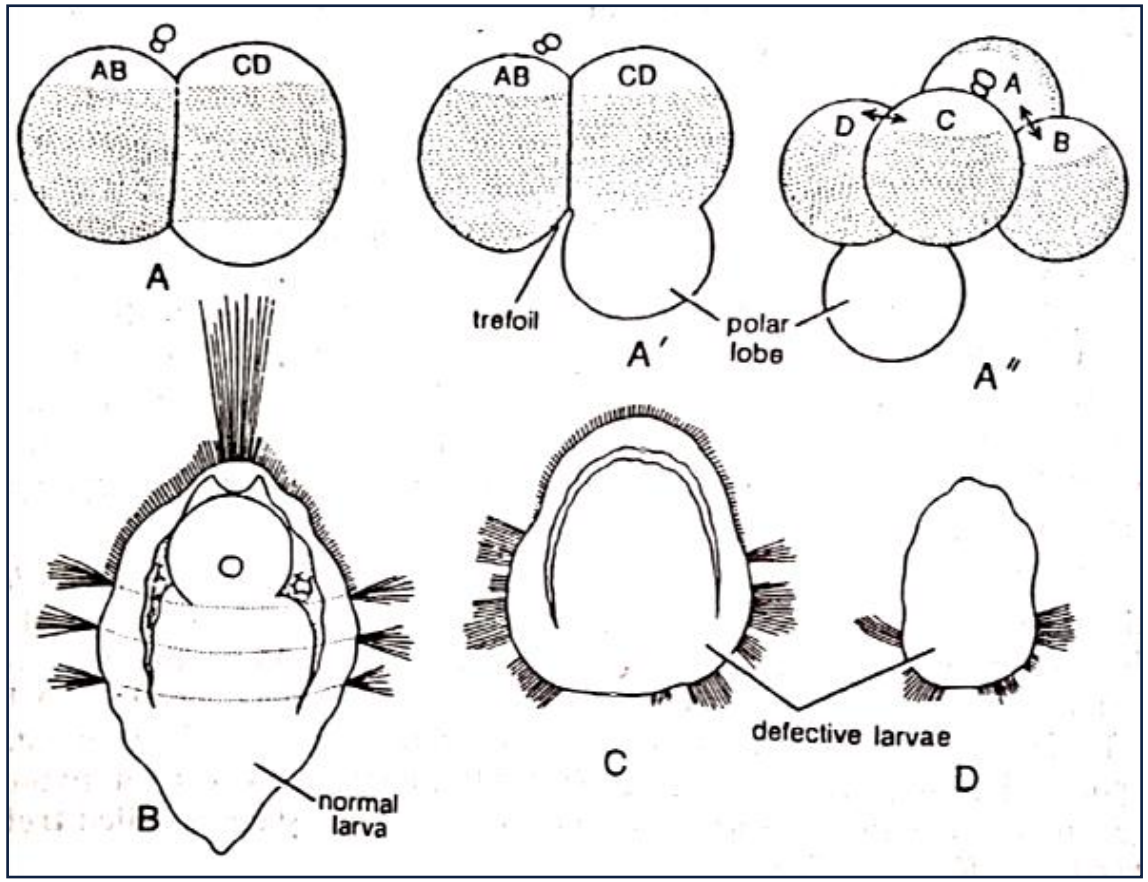
Out Come Of The Cell Lineage Study

The cleavage patterns and lineages in three major phyla—flatworms, annelids and molluscs, are similar. Both annelids and molluscs develop a **trochophore larva** with a common body plan, a sign of close phylogenetic relationship. In each, larval **ectoderm** comes from the first three micromere quartets, the remaining quartets contributing to **mesodermal** derivatives,

while the **endoderm** comes from the macromeres. The ciliated **trochophore bands** in these larvae come from the $1a^2$-$1d^2$ cells just described. Two micromere derivatives, the 2d cell of the second quartet and 4d cell of fourth quartet (both derivative of the D macromere) are called **first** and **second somatoblasts**, respectively. Both of these cells assume a special role in the development of larvae structures in both phyla. Both give rise to such mesodermal derivatives such as **heart**, **muscle bands** and **ventral nerve cord**, as well as other main tissues. The development of each phylum diverges from this common plan later on, as unique structural adaptations appear, such as **shell glands** in mollusc larvae and segmented or **metameric** body plan in annelids.

Cell lineage studies also reveal that each blastomere carries out a prescribed number of divisions before it differentiates into a larval structure. Both its position and division programme seem to be set at the beginning of development, at the first two cleavages. If any of the four macromeres is separated after second cleavage, each divides exactly as it would have in the intact embryo, both in terms of number of divisions and position of progeny.

The cleavage partitions the egg organization according to a predetermined plan and special cytoplasms and morphogenetic substances are precociously separated into different cell lines (*e.g.*, polar plasm in *Dentalium*). More than in radially or bilaterally cleaving eggs, minor perturbations of spirally cleaving eggs upset the developmental fate of blastomeres, and abnormal embryo result. The spiral pattern is a rigid cleavage programme in which blastomere fate may be set as early as the first division. For this reason it has been called **mosaic** ; the progeny of each blastomere contributes one small piece of the "jigsaw puzzle" of the larval body plan. Loss of an early blastomere or a shift in its position produces abnormal larvae.



Cleavage and results of defect experiment in *Dentalium* by **E.B. Wilson.** A—First two cleavage showing appearance of first and second polar lobes (associated with blastomere CD and D); B—Normal trochophore larva; C—Larva of same age developing after removal of polar lobe (post–trochal region and apical tuft missing); D—Another defective larva developing from one of small A, B or C blastomere not containing the yolk lobe (After Berrill and Karp, 1978).

Conclusion

Studies of cell lineages have been critical in our understanding of how cell fates are specified in development and how fates are correlated with cell division patterns. Invariant lineages or sublineages, although initially considered to imply 'lineage-intrinsic' mechanisms of fate determination, are now thought to reflect both intrinsic and extrinsic mechanisms. Thus, animals with invariant cell lineages may not develop in fundamentally different ways from larger animals in which cell lineages are variable. In insects and vertebrates, cells mostly function in groups, within which cell communication specifies fate. In such animals development may be described as a lineage of cell groups. Selection for rapid development and small size might have led to the reduction of such cell groups to individual cells, and thus the appearance of animals with defined cell lineages.

Sources

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2. Chordate EmAbryology by P.S. Verma and V.K. Agarwal


Thanks!