Fate Map

Introduction

For more than 2000 years, embryologists, biologists, and philosophers have studied and detailed the processes that follow fertilization. The fertilized egg proliferates into cells that begin to separate into distinct, identifiable zones that will eventually become adult structures through the process of morphogenesis. As the cells continue to multiply, patterns form and cells begin to differentiate, and eventually commit to their fate. This progression of events can be examined by following the developmental path of each progenitor cell and creating a two-dimensional representation (a fate map) where cell location and fate can be labeled and marked. Fate mapping is a method for tracing cell lineages and a fundamental tool of developmental biology and embryology.

In 1905 Edwin G. Conklin, a biologist and zoologist, conducted the first cell lineage experiments, which involved following the progenitor cells of the embryo of the tunicate Styela partita. This tunicate, or sea squirt, was an ideal organism to study because as its cells differentiate, they become differently colored, a fact that allowed Conklin to follow their developmental pathway. Because of Conklin's work, the developmental history of the tunicate is commonly found in embryology textbooks. However, the development of most organisms is not as easy to visualize as it is in the tunicate embryo, necessitating the invention of alternative fate mapping techniques.

In 1929 Walter Vogt, an embryologist, invented a process in which vital dye and agar chips are used to stain a specific region of a developing amphibian embryo. The dyed cells could then be traced through the developmental phases of the embryo. To do this, Vogt spread dye and agar on a microscope plate and allowed it to dry. He then cut small pieces of the dried agar and applied it to a desired part of the embryo. As the amphibian embryo developed, Vogt continued applying the agar chips to different regions, allowing him to study the movement of the cells. Through this process, he was able to create accurate fate maps and to introduce embryologists to a new approach to studying morphogenesis.

Embryologists continued to make advances in mapping technologies. For example, **radioactive labeling** and **fluorescent dyes** are both relatively simple experimental tools that use a **donor** and a **host** embryo to follow cell migration. The donor embryo is treated with dye or irradiated and a graft from the donor is removed and placed onto the host embryo where it joins the developmental process. The host embryo

continues to develop normally. If at any point in development the embryo is sectioned with a microtome, prepared appropriately, and examined under a microscope, the cells from the donor embryo are then clearly distinguishable from those of the host embryo. Another process for fate mapping was invented by Nicole Le Douarin, a developmental biologist who created chimeras, or animals with two or more sets of genetically distinct cells. Le Douarin removed a portion of neural tube and neural crest from a chick embryo and replaced it with an identical portion of neural tube and neural crest from a quail embryo at the same stage of development. Le Douarin also discovered that Feulgen stain distinguishes quail cells from chick cells, which allowed her to trace the migration of quail cells. Her work with chick quail **chimeric fate maps** led to critical knowledge on the development of nervous systems in higher order organisms. Genetic information can also be used to generate a fate map. **Genetic fate mapping (GFM)** uses two genetically engineered alleles, one of which expresses a site-specific recombinase, such as cyclization (Cre) or flipase (Flp), while the other contains a reporter allele such as green fluorescent protein (GFP). Cre and Flp both splice the DNA in specific locations and are capable of activating the reporter gene, which is used as a cell marker. Cre recombinase works via the Cre-Lox recombination system, in which the Cre protein catalyzes recombination between sites that contain the loxP sequence. The Flp enzyme works via the Flp- FRT recombination system, which is analogous to the Cre-Lox system; however, this system differs by using the enzyme flipase to recombine the sequences at the flipase recognition target (FRT) sites. In both cases, responsive cells are forced to express the reporter allele to generate a genetically distinct and labeled cell lineage. Together, these processes have revolutionized the way that fate maps are generated.

An advance in the technology of genetic fate mapping is **genetically inducible fate mapping (GIFM).** This technique generates the **Cre** fusion proteins used in GFM with a tamoxifen-responsive estrogen receptor ligand binding domain (CreER). CreER is removed from the cytoplasm of the cell via heat shock protein 90 (Hsp90). Administering tamoxifen, an antagonist of the estrogen receptor, leads to a conformational change in the CreER that allows it to escape from Hsp90. Once it is released, it can induce recombination between the loxP sites as in standard GFM. The fate of the progenitor cells can then be determined at any later point during development.

Fate maps are essential for understanding structural developments and processes of formation. As the methods of fate mapping continually change, the accuracy of each fate map is improved. Fate mapping technologies allow embryologists to follow an individual cell through morphogenesis and have led to the ability to manipulate organisms through development. This potential to intervene during embryological development may make room for advances in preventive medicine and stem cell research.

Fate Map Construction

The fate of a cell describes what it will become in the course of normal development. The fate of a particular cell can be discovered by labelling that cell and observing what structures it becomes a part of. When the fate of all cells of an embryo has been discovered, we can build a fate map, which is a diagram of that organism at an early stage of development that indicates the fate of each cell or region at a later stage of development. A fate map tells us which parts of the egg or early embryo contributes to specific tissues or structures at some later, advanced stage of development. Some animals may have a very strict fate map, in which particular parts of the egg or cleavage stage blastomeres always contribute to particular parts of the larva or adult. In a blastula the cells are located near the surface of the embryo and do not exist in layers, so the area of the future germ layer during the blastula stage is called **presumptive** and that area of the blastula designed to form neural ectoderm is called **presumptive neural ectoderm**. Examples include the nematode *C. elegans*, or the urochordate tunicates (sea squirts). Or some animals do not have a very predictable fate map (such as mammals), or other animals have fate maps.

Methods of Construction of Fate Map

Various techniques have been devised for the construction of fate map. Of these tracing the course of natural colours and artificial markings are most important. In practice one makes some sort of "mark" on, or inside, the egg or embryo, with any number of agents: charcoal, dye, soot (old fashioned ways), or modern agents, like fluorescent molecules (rhodamineconjugated dextran) or proteins like green fluorescent protein (GFP), or enzymes encoded by injected genes or mRNAs. Whichever way you choose, the principal is the same: Make your mark at some time "0" and score where the mark is at a later time(s) "x". It is also important to be able to orient your marks on an embryo with respect to some asymmetric feature, such as a pigment difference or a unique structure.

1. **Natural markings**

The cytoplasm of certain eggs such as those of ascidians has natural pigments. Thus in eggs of Styela four coloured centres have been recognized, an upper hemisphere of light protoplasm, an yellow crescent postereo-ventrally, a grey crescent antero-dorsally and a vegetal area of dark grey yolky substance. The fate of these areas can be followed very easily. It has been revealed that the upper clear cytoplasm contains the material for epidermal ectoderm. The grey crescent area differentiates into the prospective neurectodem and notochord. The yellow crescent becomes the prospective mesoderm and the dark grey yolky area forms prospective endoderm.

2. Artificial markings

There are three methods to mark or label the early blastomeres by which their fate can be traced out.

They are:

Vital staining

Early embryologists used "vital dyes" (which would stain but not harm the cells) to follow movements of individual cells or groups of cells. The tissues to which the cells contribute would thus be labeled and visible in the adult organism. The first person to develop and use this technique to study cell fate was embryologist Walter Vogt in 1929. Vogt used small chips of agar impregnated with a vital dye, (such as Nile Blue or Nile Red) which he placed on a particular cell or population of cells in *Xenopus* embryos until the dye absorbed into the yolk platelets within the desired cells. Once the cells were effectively labeled, the agar chip could be removed and the embryo was allowed to develop normally.

With this method, Vogt was able to distinguish movements of particular cell populations and the ultimate organ or tissue into which they integrated. Vital stains are mild blue, sulphate, neutral fed, Jenus green etc.

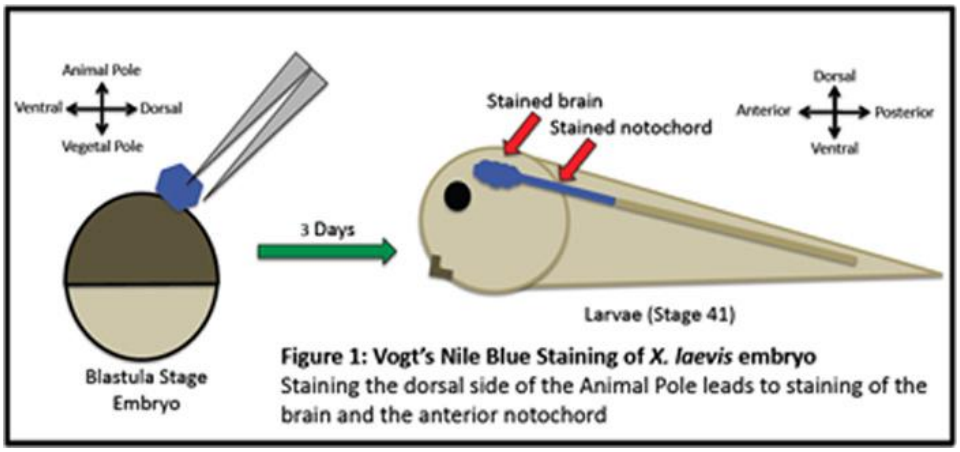


Figure 1: Vogt's Nile Blue Staining of *X. laevis* embryo
Staining the dorsal side of the Animal Pole leads to staining of the brain and the anterior notochord

Carbon particle marking

This technique was introduced by Spratt (1946) to demonstrate the process involved in primitive streak formation in chick. This consists of applying tiny particles of carbon over the surface of blastomeres. They stick to the cell surface and enable to follow the movements of the cells and to determine the fate of these blastomeres.

Radioactive isotope labeling

The radioactive isotope such as C14 and P are used to label the early blastomeres. By carefully following the course of these radioactive isotopes the fate of blastomeres can be determined. A variation of the dye marking technique is to make one area of the embryo radioactive. A donor embryo is taken and grown in a solution containing radioactive thymidine. This thymidine base is subsequently incorporated into the DNA of the dividing embryo.

A second embryo, acting as the host embryo, is grown under normal conditions. The region of interest is cut off from the host embryo and is replaced by a radioactive graft from the donor embryo. The cells that are radioactive will be the descendants of the cells of the graft, and are distinguished by autoradiography.

3. **Histochemical and cytological methods**

A variety of natural markers i.e. the specific molecules of the cell have been used successfully in construction of fate maps. To name some are **melanin** granules, **glycogen** and **alkaline phosphatase.** However their activity depends on metabolic activity of the cell and might change during the course of observation and thus limited to particular kinds of cell.

4. **Genetic Marking**

Radioactive and vital dye marking have their own drawbacks such as dilution over many cell divisions and the laborious preparation of slides. One permanent way of cell marking is to create mosaic embryos and was used by Hotta and Benzar in 1972 to construct fate maps of the early embryo *of Drosophila melanogaster* having different genetic constitutions. Other best example of such a marking is to graft quail cells inside a chick embryo. By doing so, fine-structure maps of the chick brain and skeletal system can be made. In this method the advantage was that **ring- X chromosome (X^R)** is unstable in other mutant strain i.e in (**w^{vc})** of *Drosophila melanogaster* and is lost in 35 percent of female embryo **(X^RX)** during the 1st division of zygote nucleus. One cleavage nucleus thus becomes X0 and the cells containing mitotic products of this nucleus determine male phenotype and will express the recessive genes present in X chromosome, the wild type allele located on ring X chromosome **(X^R). The resultant gynandromorph flies have some genetically male cells (**X0) **and genetically female cells (X^RX). In this type of cases the orientation of mitotic spindle at first nuclear division is random and no mixing between clones of nuclei, derived from the cleavage nuclei, at the time they populates the blastoderm. One half of the blastoderm is thus populated by descendents of one cleavage nucleus (X^RX) and other half from that of the donar (**X0). Fig. 2. It is thus possible to measure the distance between two presumptive sites by counting the percentage of mosaic flies where two structures are of different phenotypes in strut units.

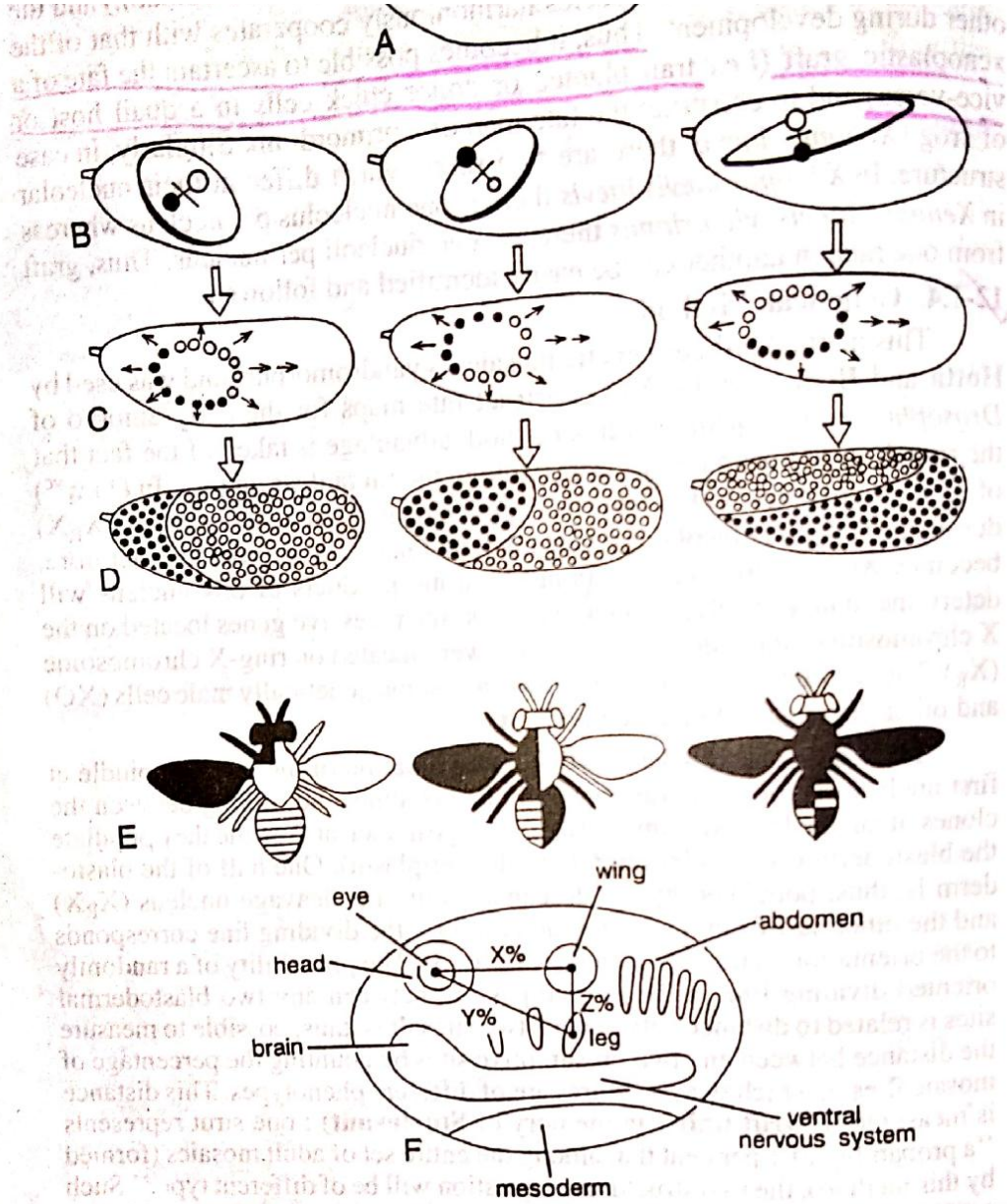


Fig. 12.3. Fate map of *Drosophila melanogaster*. A—Typical mosaic gynandromorphs of *D. melanogaster*. Body parts of each fly derived from female nucleus are shaded, while body parts derived from male nucleus are unshaded. The plane of spindle at first cleavage determines that which body part will be male or female; B—Mechanism of formation of a mosaic fly. 1. The zygote nucleus (female X_RX) divides into two. One daughter nucleus (open circle) loses its one X (*i.e.*, X_R) to become XO male; 2. Clones of nuclei derived from two cleavage nuclei; there is no mixing up; 3. Section of blastoderm; and 4. Surface view of blastoderm. Nuclei have migrated to egg cortex or periplasm to populate periplasm. Distribution of XX and XO nuclei corresponds to plane of first mitotic spindle; C—Fate map of blastoderm for external body parts of adult; distances between body parts projected over the blastoderm; D—Pictorial sketch of external parts of an adult fly on fate map of right half of blastoderm (after Goel, 1984).

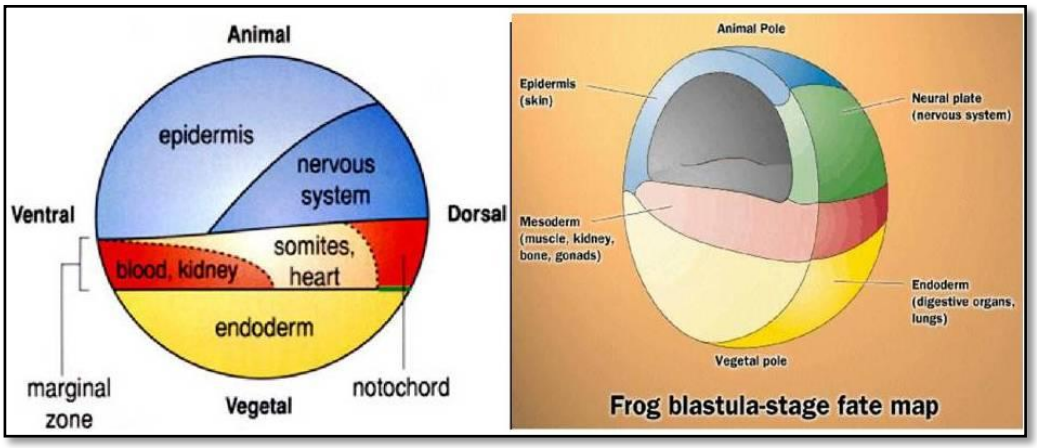
Fate map of typical chordate blastula

The following presumptive areas are discernible in chordate blastula.

1. There is a broad ectodermal area in the animal hemisphere which forms the epidermal layer of the skin. This is known as epidermal ectoderm.

2. A relatively smaller ectodermal area lies below the epidermal ectoderm. This area is known as neurectodem since it contributes to the formation of neural tube and nervous system.

3. A crescentic area below the neurectoderm is designated as notochordal area which gives rise to the notochord of the embryo.



4. On either side of the notochordal area are two lateral areas. These constitute the prospective mesoderm.

5. Most of the yolky vegetal hemisphere blastomeres collectively form the prospective endoderm.

6. At the caudal margin of the notochordal area is a small strip of blastomeres called prechordal plate region. This region mainly gives rise to some of the head mesoderm.

Fate Map of Vertebrates

1. Fate Map of Amphioxus

The fate map of Amphioxus can be traced at an early stage prior to the onset of cleavage. The pre-sumptive organ forming areas in the un-cleaved egg is given in Fig. 5.32. The future endodermal cells lie at the vegetal pole and would subsequently form the floor or hypoblast of the blastula. The area at the animal pole would form the presumptive ectodermal cells.

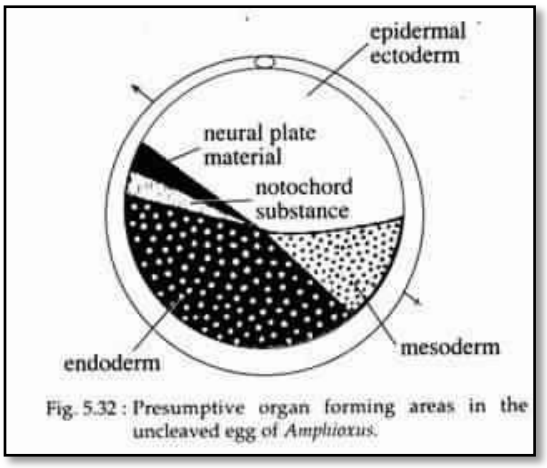


Fig. 5.32 : Presumptive organ forming areas in the uncleaved egg of *Amphioxus*.

The ventral grey crescent area at the future posterior end of the blastula, in between the future ectoderm and endoderm, forms the future mesoderm. Another area, the dorsal crescent, in between the ectoderm and endoderm on the anterior side, gives rise to the notochord and neural cells. The presumptive ectodermal, mesodermal, notochordal and neural cells would subsequently form the epiblast of the blastula.

2. Fate Map of Frog:

The blastula of Xenopus at the 32 cell stage gives no indication as to how the different regions will develop. However, by following the fate of individual cell, or group of cells, the fate map of the blastula can be made. One way of making the fate map is by staining the various parts of the early embryo with a lipophilic dye such as dil and observes where the labelled regions end up.

Another sophisticated way of labelling the blastomeres is by injection of high molecular weight molecules such as rhodamine-labelled dextran, which cannot pass through cell membrane and are, therefore, restricted to the injected cell and its progeny. These cells can be easily detected later, under a UV microscope.

The fate map of the Xenopus blastula (Fig. 5.33) shows the presence of yolky macromeres at the vegetal pole which gives rise to the endoderm. Depending upon the position of the blastopore, the endodermal area can be divided into the sub-blastoporal and supra-blastoporal endoderm.

The cells toward the animal pole gives rise to the ectoderm, which becomes further subdivided into epidermis and the future nervous tissue. The epidermal ectoderm forms at the ventral side of the animal hemisphere, while the neural ectoderm forms at the dorsal side. The mesoderm forms a belt-like region, known as the marginal zone, around the equator of the blastula.

The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord. From this ventrally, the- mesoderm is differentiated by the somites (which gives rise to muscle tissue), lateral plate (which contains heart and kidney mesoderm) and blood islands. In Xenopus, a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone.

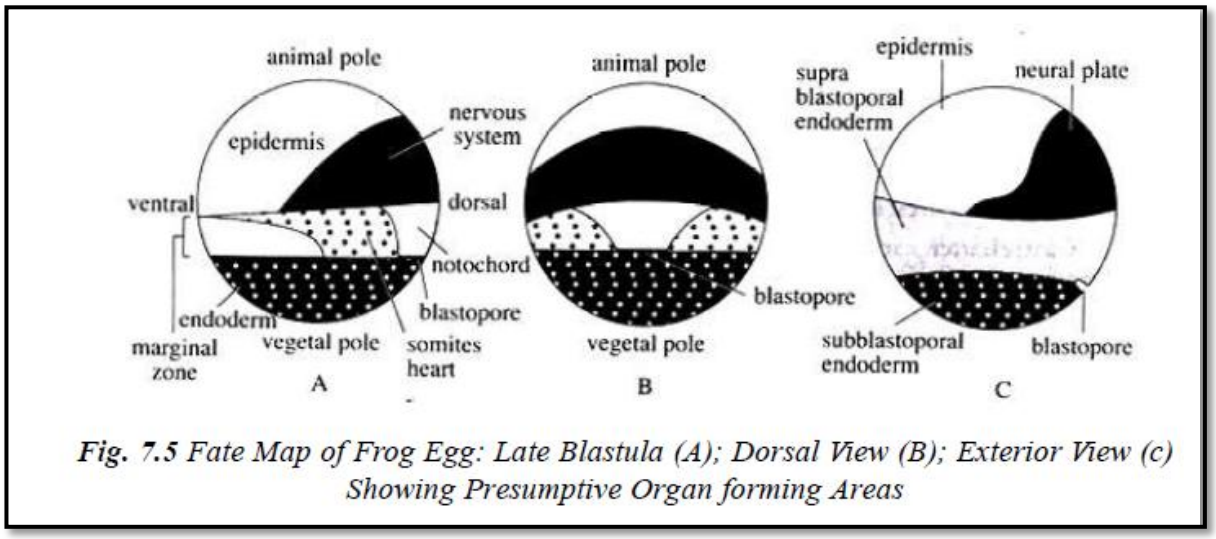


Fig. 7.5 Fate Map of Frog Egg: Late Blastula (A); Dorsal View (B); Exterior View (c) Showing Presumptive Organ forming Areas

3. Fate Map of Chick

Before going through the fate map of chick one should go through the formation of area pellucida and area opaca, and also through the format iod of hypoblast and epiblast. From the study of the above formations, it becomes clear that the hypoblast does not contribute any cells to the formation of the embryo proper, rather they contribute to the formation of a portion of the external membranes.

Recent studies with cell adhesion molecules (CAMs), it has become possible to construct the fate map of chick epiblast (Fig. 5.34). All the three germ layers of the embryo proper is formed by the epiblastic cells. The epiblast also forms a considerable amount of extra-embryonic (mesoderm) membrane.

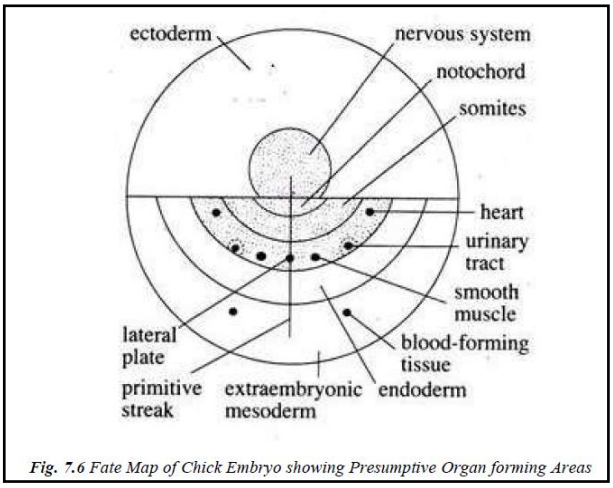


Fig. 7.6 Fate Map of Chick Embryo showing Presumptive Organ forming Areas

The fate map of chick (Fig. 5.34) reveals that the cells of the epiblast are organised around the notochord and nervous system. The neural ectoderm is present as a knob-like structure facing towards the anterior side. The cells at the anterior part of the epiblast form the ectoderm, while the cells at the posterior side gives rise to mesoderm (body proper), endoderm and extra-embryonic mesoderm.

Usefulness of Fate Map

The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis.

Sources

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2. http://www.notesonzoology.com/articles/fate-map-meaning-construction-and-usefulness/2660

Further Reading

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