TOPIC: FATE MAPS AND FOETAL MEMBRANCES

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FATE MAPS

In developmental biology, fate mapping is a method of understanding the embryonic origin of various tissues in the adult organism by establishing the

correspondence between individual cells (or groups of cells) at one stage of development, and their progeny at later stages of development. When carried out at single-cell resolution, this process is termed cell lineage tracing.

The first attempts at fate mapping were made by inferences based on the examination of embryos that had been fixed, sectioned, and stained at different developmental time points. The disadvantage of this technique was that observation of single points in developmental time provides only snapshots of what cell movements are actually occurring and what fates are being assigned. Early embryologists thus had to infer which cells became what tissues at later stages.

Early embryologists used "vital dyes" (which would stain but not harm the cells) to follow movements of individual cells or groups of cells over time in Xenopus frog embryos. The tissue(s) 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 cell(s). 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 discern movements of particular cell populations and the ultimate organ or tissue into which they integrated. Although innovative for the time, this technique is limiting in that the size of a chip of agar may not accommodate single-cell resolution studies at later stages of development, since successive cell divisions will yield smaller cells (until the embryo develops into a larval form that can eat, and thereby grow larger). Additionally, the cell or cell population of interest must be superficial, since the agar chip with the dye must be placed on the surface of the embryo.

The information Vogt gathered from his tracing experiments of distinct cells and populations of cells in Xenopus was then pooled to construct a fate map. The map

was a representation of an early-stage embryo (such as a blastula) that has particular regions highlighted which are known to give rise to specific tissues in the adult organism. For instance, in Figure 1, Nile blue staining of a 32-cell blastula at the dorsal side of the animal pole yields a blue-stained brain and (depending on the size of the agar chip) may also stain the anterior portion of the notochord.

In 1978, David Weisblat and colleagues in Gunther Stent's lab at Berkeley improved the technique of single-cell resolution fate mapping by injection of horseradish peroxidase (HRP) enzyme, and later fluorescent peptides (1980), into individual cells in Helobdella triserialis (leech) embryos during early development. All progeny of the injected cells could later be discerned by staining for HRP using benzidine substrate or visualized by fluorescence microscopy. This technique allowed the experimenter greater control and selectivity over what cell was labeled and traced. However, the opaque character of the HRP stain prevented use of vital dye nuclear counter-stains such as Hoechst 33258 (blue) to observe the mitotic state of the injected cell's progeny. Also, embryos had to be fixed in order to stain for the HRP, thus allowing only a single time point view of each individual leech embryo injected. The use of fluorescent peptides such as Rhodamine-D-protein (red, RDP) and Fluorescein-D-protein (yellow/green, FDP) conjugated to

large carrier molecules to prevent diffusion through cell gap junctions, alleviated several of the shortcomings of HRP injection. Leech embryos injected with the fluorescent tracers could be visualized, and images collected of the same specimen at multiple time points, without fixation. The fluorescent tracers could also be combined with nuclear Hoechst staining to visualize the mitotic status of the progeny of injected cells. Seth Blair, also in Stent's lab, introduced a novel ablation technique that could be used in tandem with lineage tracing to pursue the questions relating to developmental potential changes in cell fate in experimentally perturbed embryos that were first raised by Roux and Driesch (1980). For this purpose, specific cells were ablated by microinjection with Pronase (an enzyme that digests proteins) to ablate the cell; later modifications of this technique employed DNase or the ricin A chain. The single-cell injection technique is now also in use by researchers studying other model organisms such as Xenopus (frogs), Danio rerio (zebrafish), and Caenorhabditis elegans (worms).

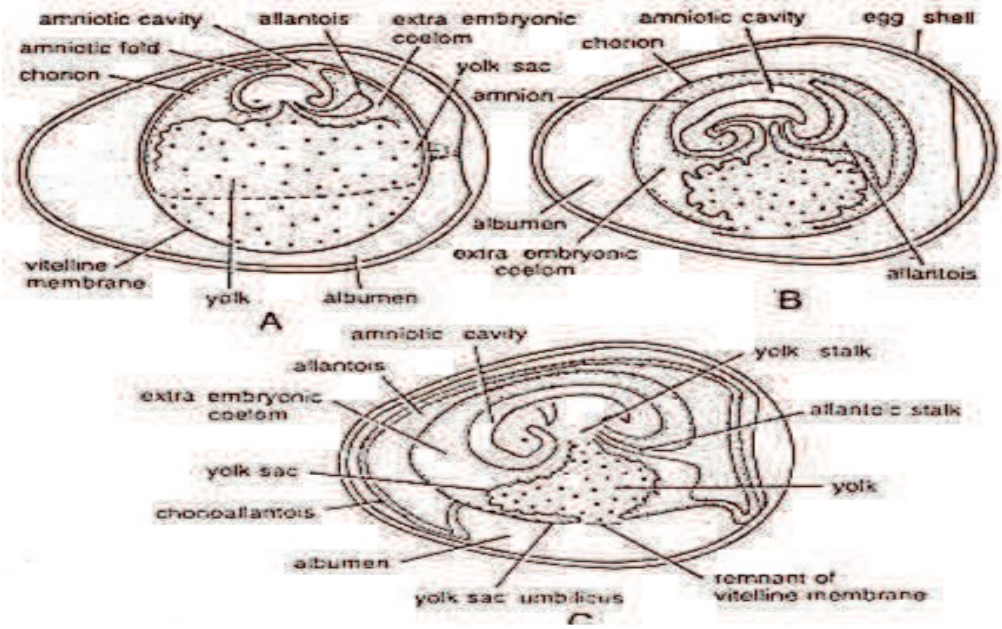
FOETAL MEMBRANCES: THEIR FORMATION AND SIGNIFICANCE

In amniotes certain tissues of the developing embryo do not enter into the formation of embryo proper, but helps in care

and maintenance of developing embryo. These part termed as Foetal membranes or extra embryonic membranes. Developed foetal membrane serve for nutrition, respiration, excretion and protection of the embryo. The extra embryonic membranes are chorion, amnion, yolk sac, and allantois.

Foetal membranes of chick:

In the development of chick these membranes develop from original blastoderm. The central part of blastoderm forms embryo proper, the marginal blastoderm gives extra embryonic membranes. Amnion and chorion develop from somatopleurae, yolk sac and allontois, develop from splanchnopleurae.



Amnion & Chorion : In the development of embryo amnion and chorion are closely associated, Amnion is bag like covering over'the em¬bryo, it separates the embryo from internal environment, Amnion is developed from somatopleuric amniotic folds. These folds are head fold, lateral folds and tail folds.

- At about 30 hours of incubation, in front of the head of embryo a head fold is developed, it is called amniotic head fold.
- At about third day of incubation amniotic tail fold is developed. It grows opposite to head fold.
- Mean while lateral folds will develop, they grow dorsomedially.
- After some time head fold, lateral folds, and tail fold will fuse near posterior end of a embryo.
- At 72 hrs. of incubation they are still not fused. They show an opening called amniotic umblicus, afterwards they unite.
- After their union at the point of union "sero-amniotic raphae" is present. It is a fold.
- Because of this union outer chorion inner amnion will form, because it is developed from somatopleure. In chorion ectoderm is present outside and mesoderm is present inside. In amnion ectoderm is inside, mesoderm is outside. Hence the space between amnion and chorion is called exocoel or extraembryonic coelome.

Functions of chorion:-

The extra embryonic coelome is filled with a fluid. It gives protection to the developing embryo.

This coelome gives space, for developing allantois.

Chorion combines with allantois and acts as a respiratory organ.

Functions of Amnion:

Amnion is sac like structure around embryo. It contains amniotic fluid. It will protect embryo from mechanical shocks and dessications.

It protects the embryo when the egg is laid. It gives artificial aquatic environment for growth of embryo.

Yolk sac:

At 16 hours of incubation, yolk sac makes its appearance
It develops from Splanchanopleurae contains endoderm and mesoderm layers.

The Splanchanopleurae instead of forming a close gut, it will grow over yolk, and becomes yolk sac. The primitive gut is present above the yolk. This yolk region is in contact with midgut. Finally the yolk sac is communicated with midgut through an opening.

Functions of Yolk sac :

It digests the yolk, and the digested food will be circulated through blood to the developing embryo. Hence yolk sac is considered as a nutritive organ of the embryo.

Allantois :

It develops from the ventral part of caudal end of the hindgut at third day of incubation. It develops from Splanchanopleurae This Splanchanopleurae contains endoderm and mesoderm. The allantois grows rapidly, and occupies the entire exocoel. The mesoderm of the chorion and mesoderm of allantois will unite. It forms chrio allantoic membrane. Allantois is connected to the hindgut, and is called as allantoic stalk. As the embryo is growing the allantoic and yolk stalk are brought together. Their mesodermal layers will unite. It is called umblical stalk. It is covered by somatic umblicus.

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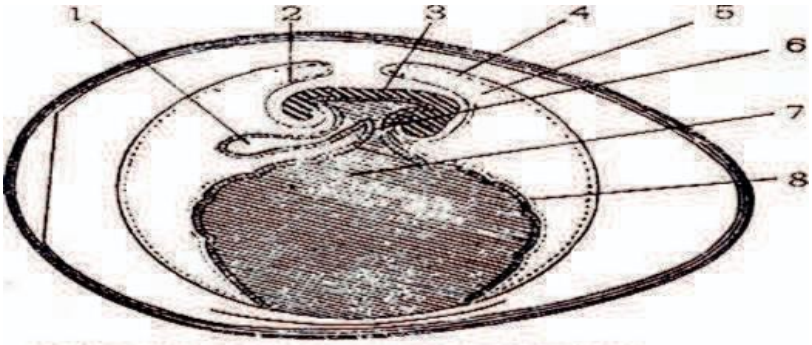


Early stage in development of extra-embryonic membranes in chick

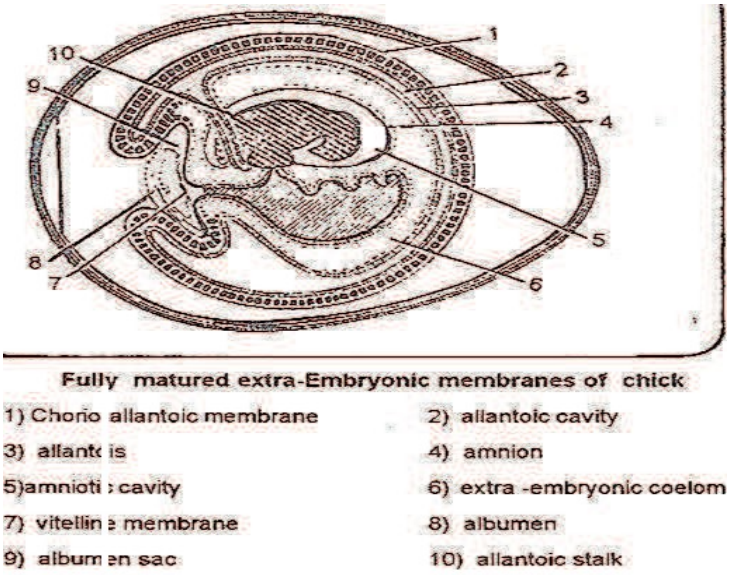
1) allantois 2) prospective amnion
4) amniotic folds 5) prospective chorion
7) yolk8) yolk sac

Fig : Allantois

Function of Allantois:

Allantois is richly vascularised. Hence it works as respiratory organ.

It stores nitrogenous waste material of the embryo.



Fully matured extra-Embryonic membranes of chick

1) Chorio allantoic membrane
2) allantoic cavity
3) allantois
4) amnion
5) amniotic cavity
6) extra -embryonic coelom
7) vitelline membrane
8) albumen
9) albumen sac
10) allantoic stalk

Fig : fully matured extra embryonic membrane of chick

In later development the allantoic circulation will absorb calcicum from the shell. This calcium is used in construction of bones in young ones.Allantois absorbs calcium from shell. Hence the shell becomes thin. It helps in rupturing the shell during hatching. These are the membranes that develop outside the embryo but in close association with it and they carry out certain specific functions. In human beings foetal membranes are amnion, chorion, yolk sac and allantois.

Extra embryonic membranes of mammals:

Amnion: This is formed above the embryo. This consits of a cavity (amniotic cavity) and encloses a fluid called amniotic fluid. The embryo is suspended into the amniotic cavity by the umbilical cord. The aminotic fluid provides a shock absorbing effect to the embryo against bumps infections etc.

The watery fluid around the embryo helps in maintaining constant temperature and pressure and protects the embryo in case the mother has a fall. The aminotic fluid is derived from the mother's blood and contains foetal cells. This is made use of in the prenatal sex test
for the foetus- known as aminocentesis. In aminocentesis the amniotic fluid is drawn out wtih a syringe and the cells are tested for the presence of the sex chromosomes.

Chorion: The chorion completely surrounds the embryo and has small projections all around it during early stages of development. The chorion is composed of trophoblast on the outside and mesoderm on the inside. Chorion protects the embryo and forms placenta for metabolic exchange between the mother and the foetus.

Yolk sac: This is formed below the embryo. In human beings this contains a fluid but no yolk. It is vestigial organ. Its wall is made up of tropho- blast and endoderm. The yolk sac functions as the region of formation of blood

cells upto about 6th week of development when the liver of the foetus takes up this function.

Allantois: This is a small bag like structure that develops from the gut of the embryo and near the yolk sac. This membrane develops around the third week of development. Gradually the allantois shrinks in size and gets enclosed in the umbilical cord. Allantois helps in the formation of umbilical arteries and veins. The allantois also forms blood cells.

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