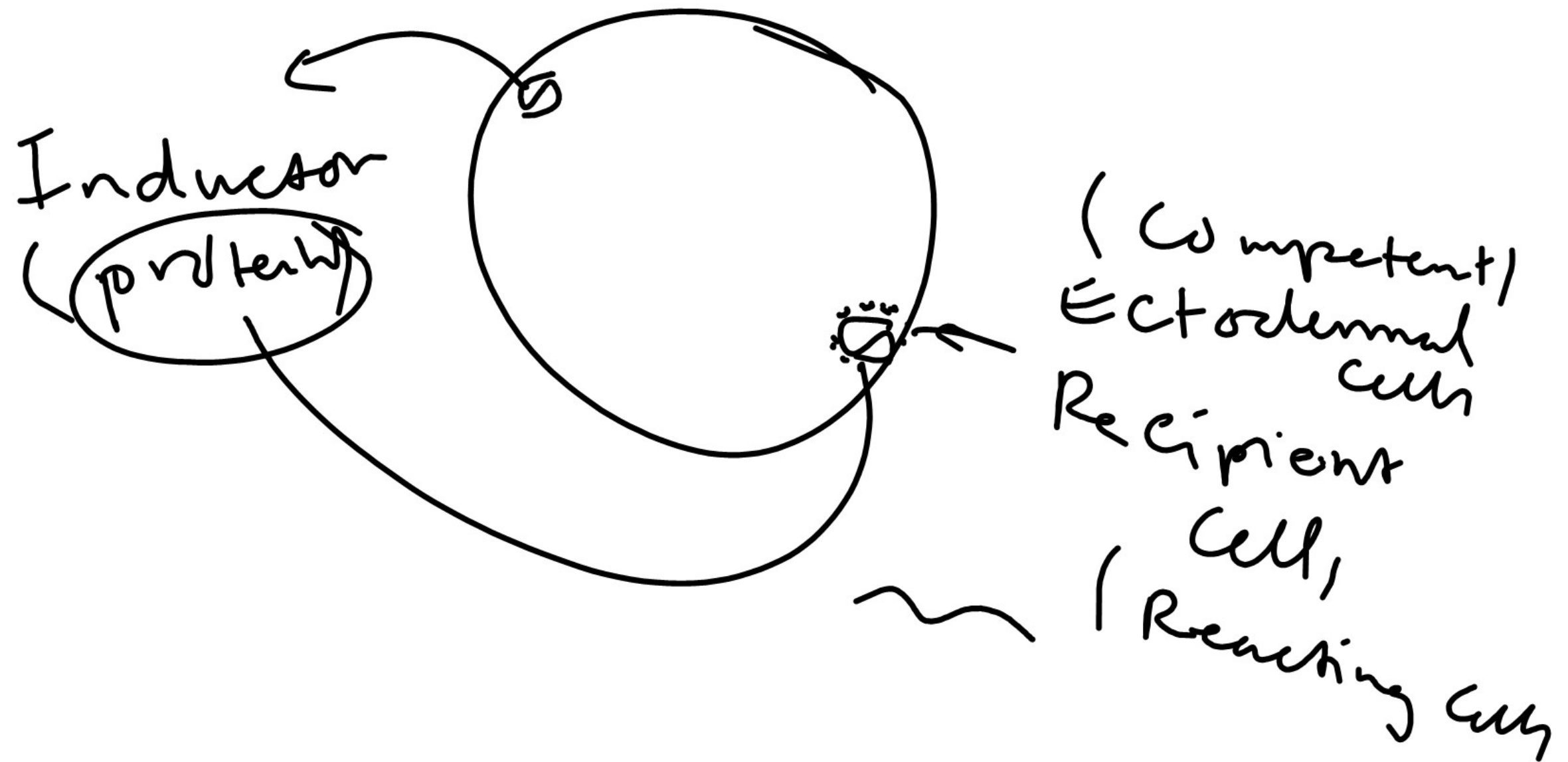
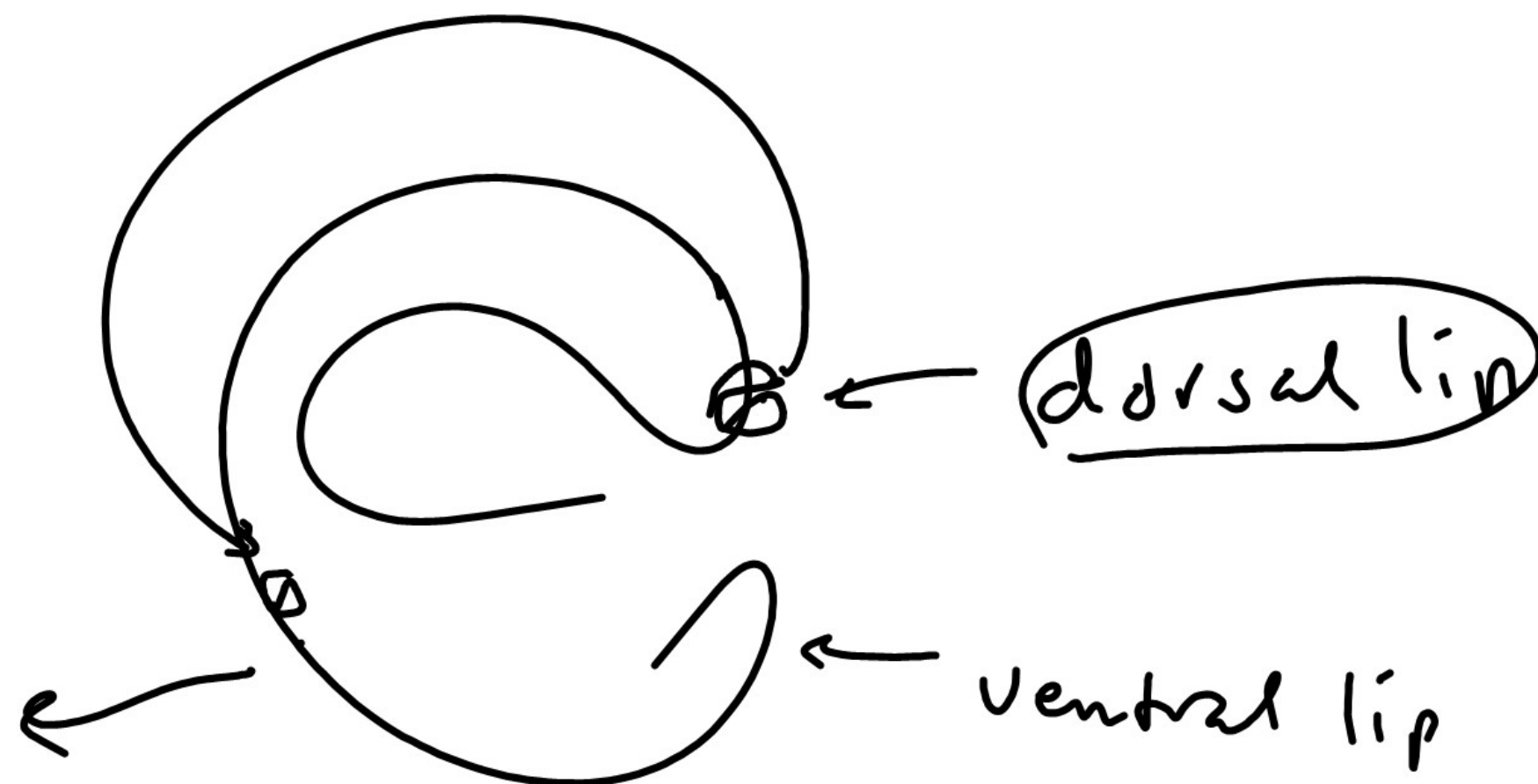
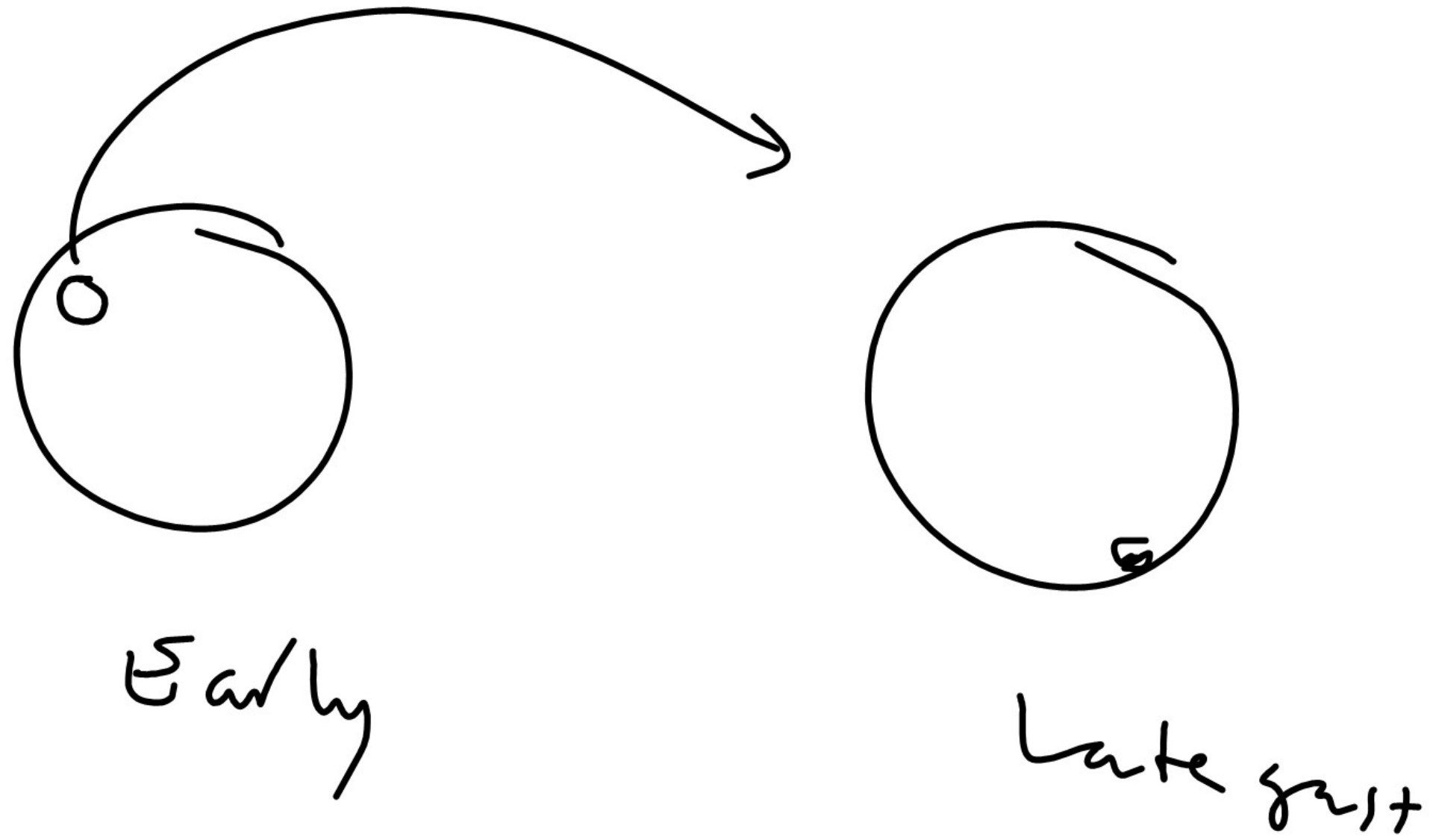
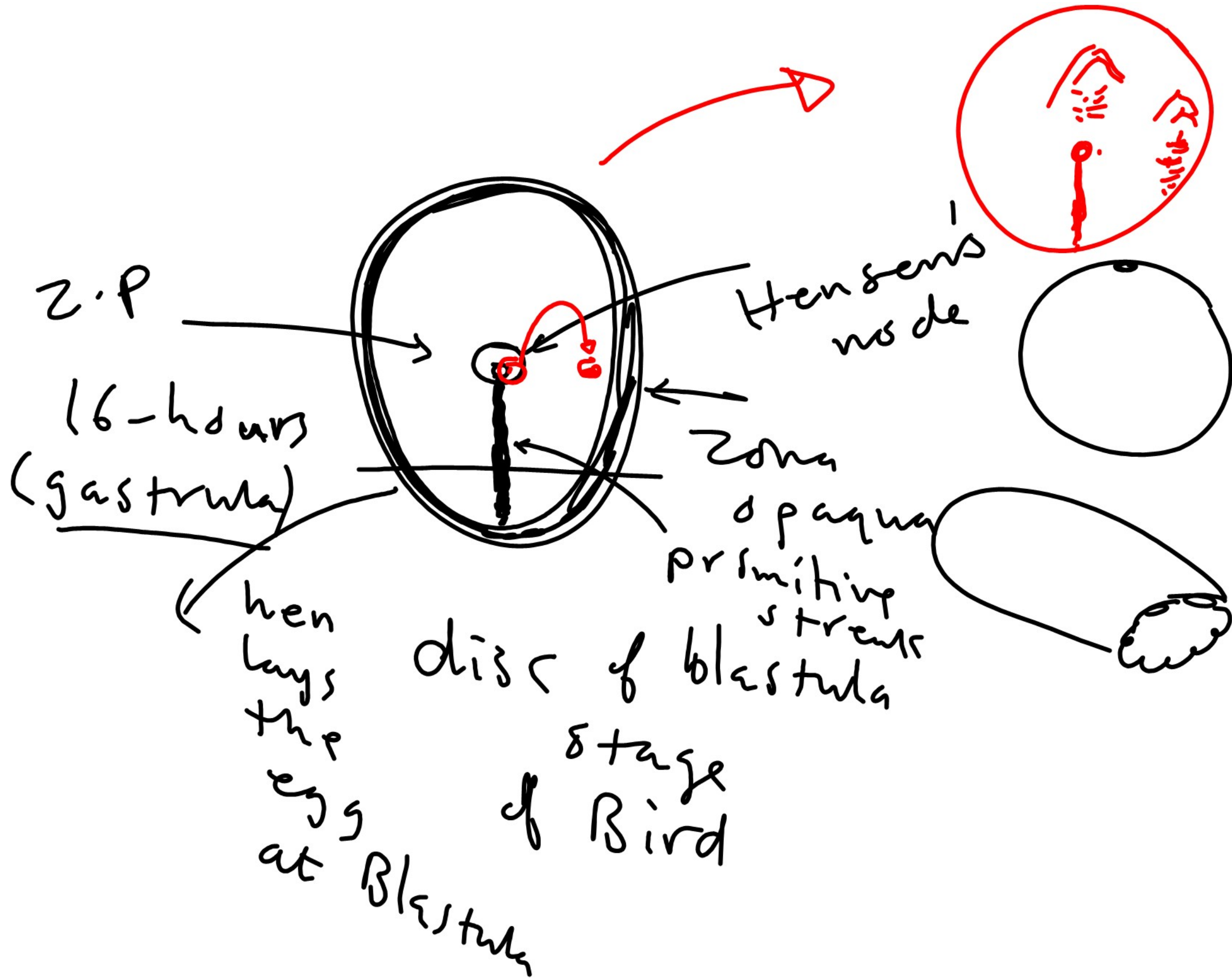


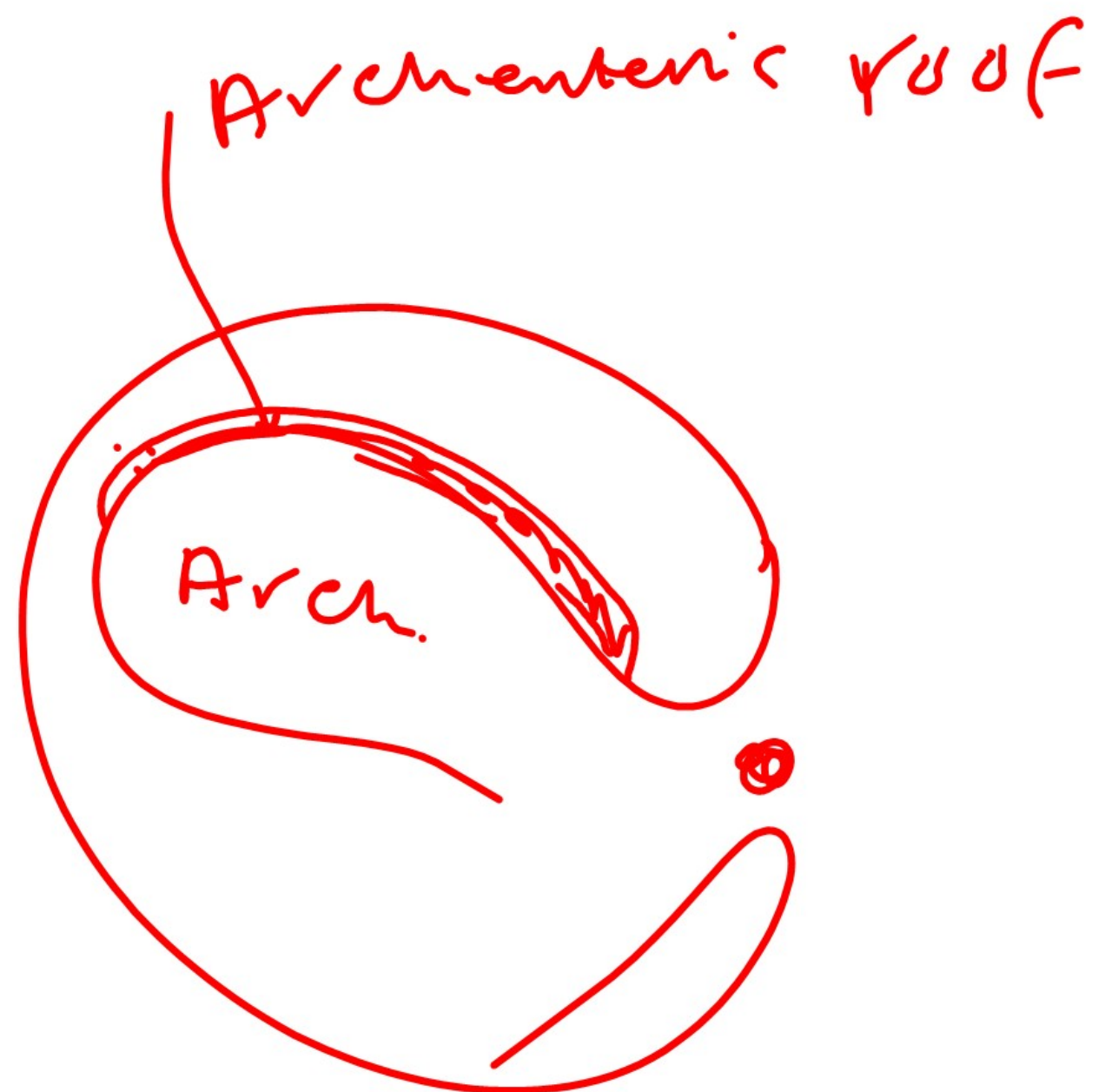
Late Gastrula











GENE ACTIVITY DURING GASTRULATION

- During cleavage the main activity of chromosomes is to duplicate themselves at each cell division. At the completion of cleavage the genes contained in the chromosomes enter a period of new activity, which is the production of large quantities of RNA, particularly mRNA (**new gene expression**).
- This production can be recorded and measured in the embryo by the following methods:

- **1. Using radioactively labeled Uridine which is taken up into RNA not into the DNA:**
- A- Labeling the cells.
- B- RNAs can be separated chemically(the product is a mixture of different RNA's, tRNA, rRNA and mRNA).
- C. Ultracentrifugation to separate the different types of RNA's.
- D. Take mRNA and measure the amount of radioactive material that incorporated in it in an apparatus called a **“scintillation counter”** which records the number of particles emitted by the radioactive sample.

- Using this kind of method shows how the amount of mRNA increase sharply comparing with the amount of mRNA that was produced in the zygote.

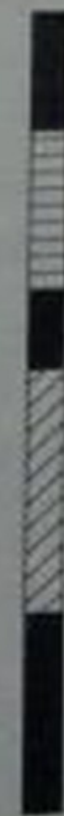
- **2. DNA-RNA Hybridization method:**
- We can determine by this method whether or not the mRNA synthesized just before the onset of gastrulation is the same as the mRNA present in the egg cytoplasm or the mRNA produced immediately after fertilization and during earlier cleavage stages.

Figure 147. Diagrammatic presentation of a DNA-RNA hybridization experiment showing that some messenger RNA is present in a sea urchin gastrula, which was absent in the blastula stage. For the sake of simplicity, only one kind of mRNA is shown as being present in both the blastula and gastrula, and one kind of mRNA as present in the gastrula only. Also, the strands of DNA are shown as lying parallel to each other, instead of being spirally twisted around each other, as they are in reality.

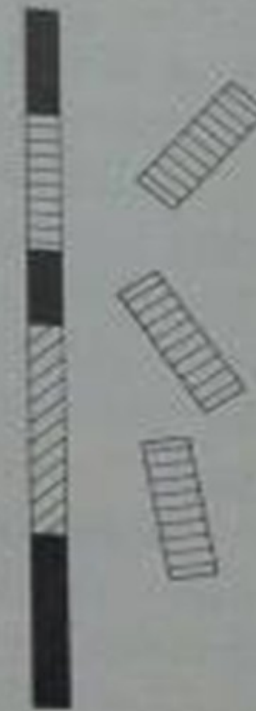
first step:
separate the
double strand of
DNA by heating or
alkali treatment



result:
single-stranded
DNA



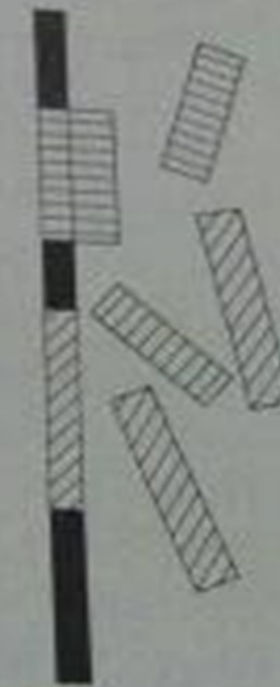
second step:
add blastula mRNA
to single-stranded
DNA



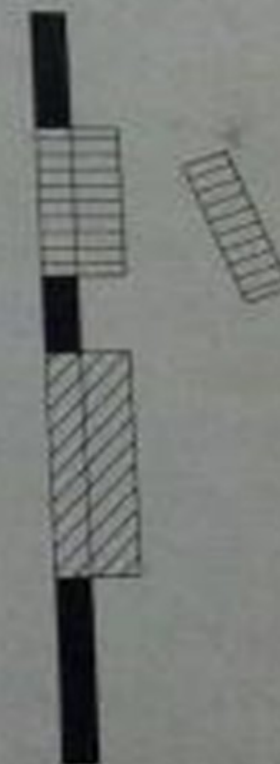
result:
mRNA hybridizes
with corresponding
sections of DNA



third step:
add gastrula/post-
gastrula mRNA



result:
some of mRNA finds its
corresponding section of
DNA occupied, other
mRNA (which was not
present in blastula)
can still hybridize



- **3. Polyacrylamide Gel Electrophoresis(PAGE):**
- A. extract proteins from blastula stage and from gastrula stage,
- B. prepare gel
- C. load protein from blastula in certain well and gastrula proteins in other separated well.
- D. Once electrophoresis finished stain it.
- E. Compare # of bands in each lane
- You will see that some bands that appeared in blastula still produced in gastrula, and new band appeared in gastrula that were not produced in blastula.

- **Conclusion:**
- That means some protein were produced in blastula and still produced in gastrula, this means that their gene still active, and appearing new proteins as new band, this means a new gene activation.

- **4. Immunological method:**
- It has been found that the gastrula contains antigens, capable of causing the formation of antibodies, which were not present before.

- **DETERMINATION OF THE PRIMARY ORGAN RUDIMANTS**

Methods Determination of the primary organ rudiments

- 1, Fate mapping: A chart showing the fate of each part of an early embryo, in particular, a blastula.
- A. Soaking a piece of agar in a vital stain(Nile blue sulfate, neutral red, Bismark brown) and the applying the piece of agar to the surface of the embryo in the necessary position.
- B. wait minutes and remove it(the stain will diffuse from agar to the tissues)
- C. Follow up the stain through the embryo development in the differentiated cells.

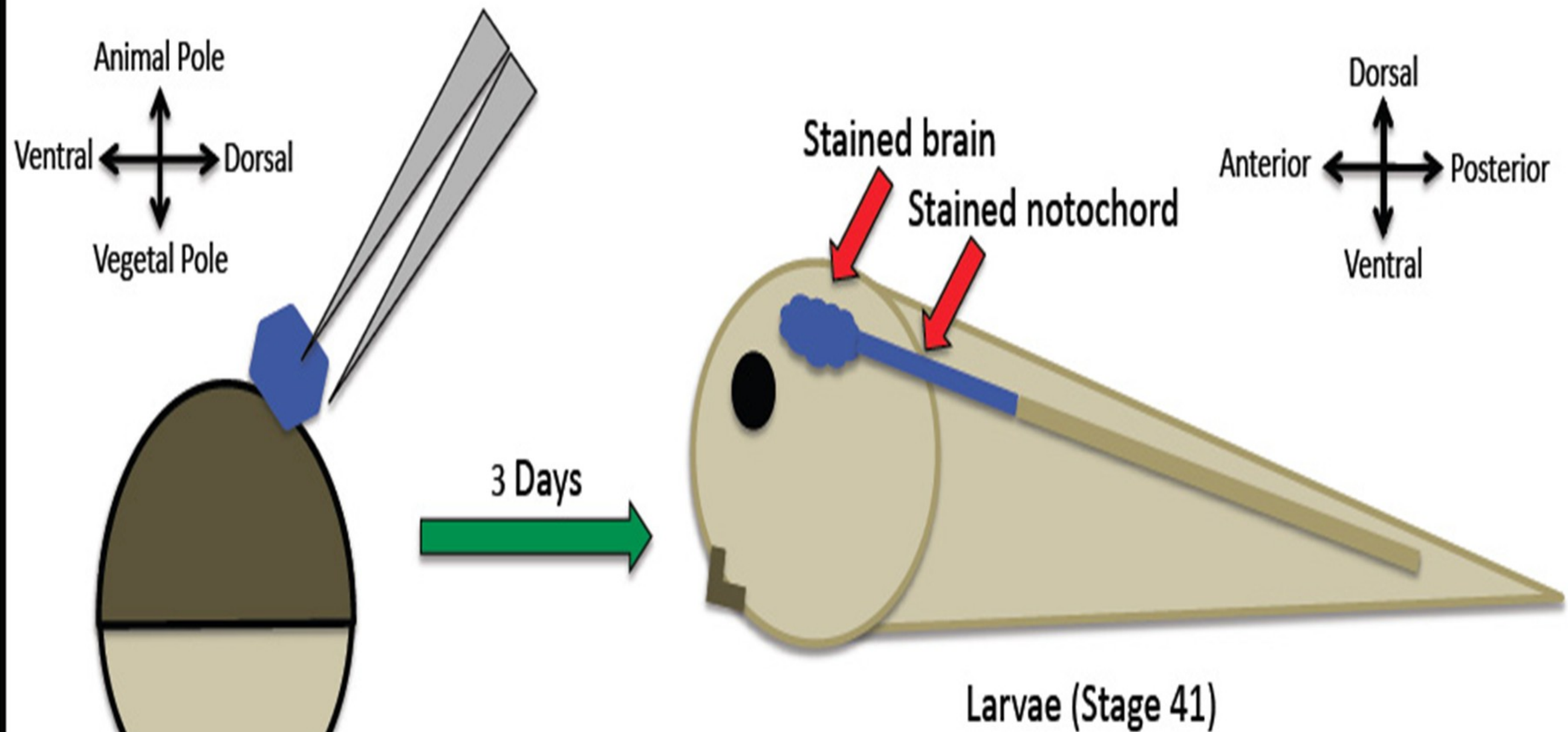
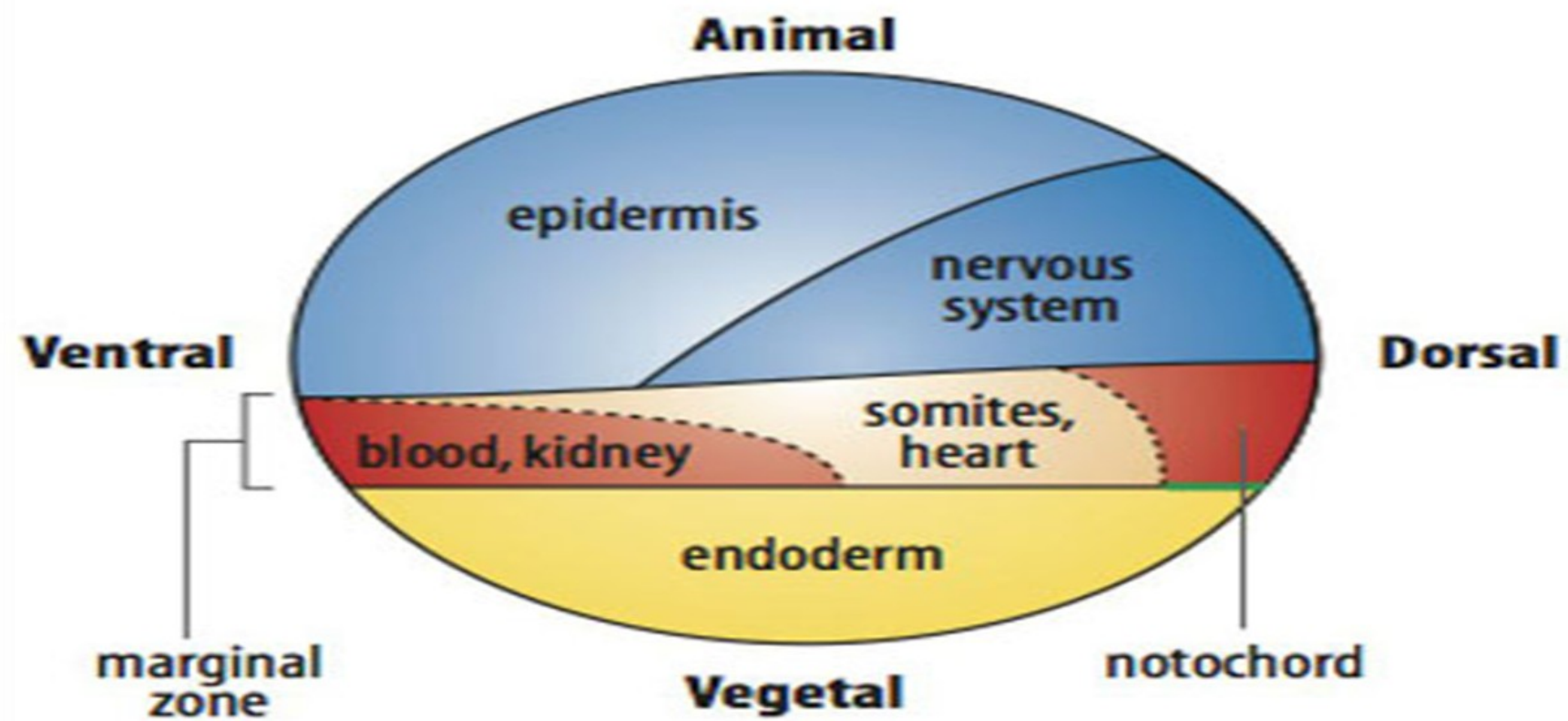
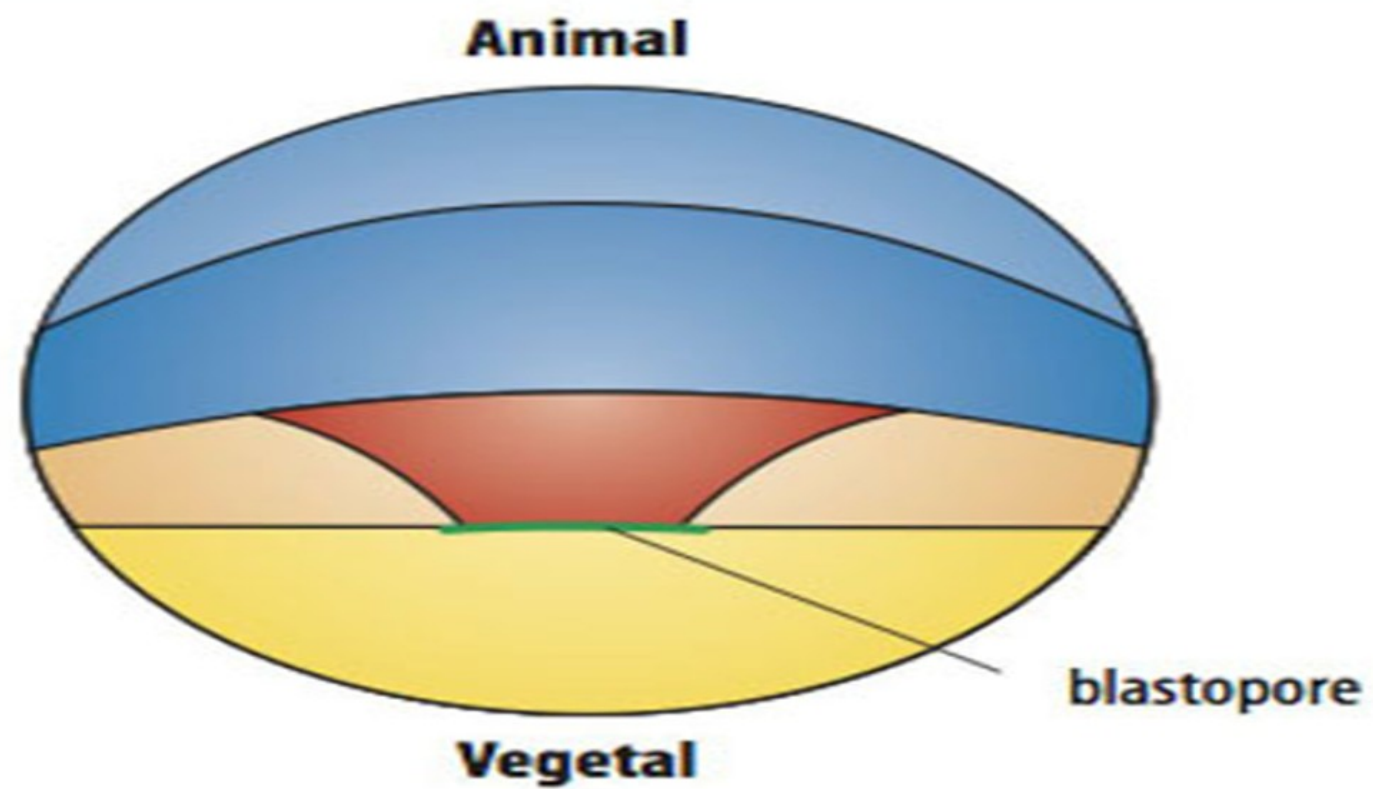


Figure 1: Vogt's Nile Blue Staining of *X. laevis* embryo

Staining the dorsal side of the Animal Pole leads to staining of the brain and the anterior notochord

Fate map: lateral view**Fate map: dorsal view**

- **2. Transplantation:**
- Small pieces may be cut out of embryos in various stages of development and inserted into suitably prepared wounds of the same or another embryo.

Types of Transplantation

- 1. **Autoplastic**: transplantation of a piece of an embryo to another place in the same embryo. (the donor and the host is the same embryo)
- 2. **Homoplastic**: the transplantation is from one individual to another of the same species.
- 3. **Heteroplastic**: the transplantation is to an individual of another species belonging to the same genus,
- 4. **Xenoplastic**: a transplantation to an individual more distantly related than species of one genus.

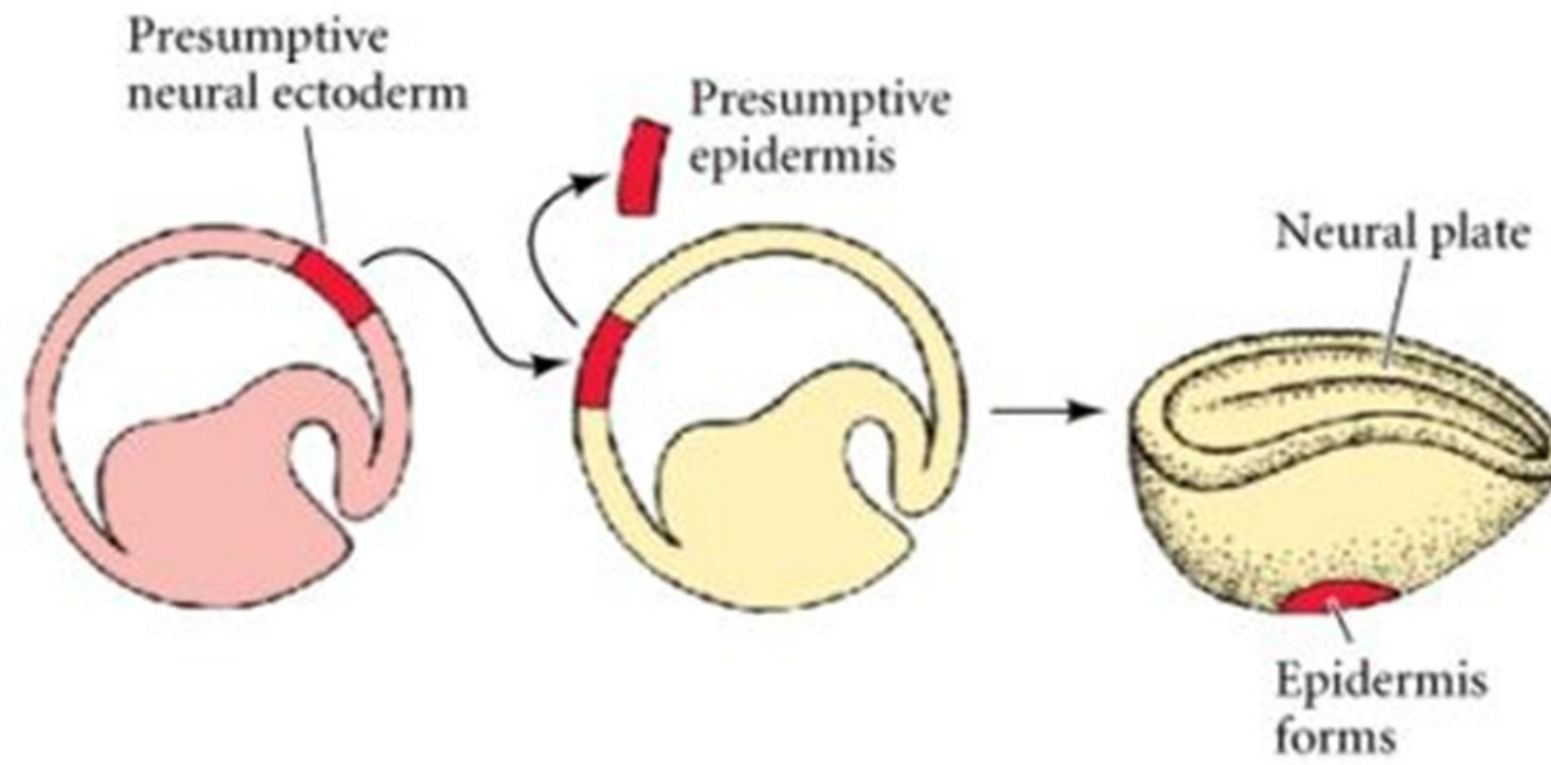
Experiments at early gastrula

- 1. If prospective neural plate cells are moved to an area of prospective epidermis, then the tissue differentiates as epidermis.
- 2. If prospective epidermis is transplanted into the prospective neural plate area then it differentiates into neural structures.
- ***We can conclude that the fate of these tissues is not fixed or not determined (**prospective potency**), in other words tissues have the ability to develop more than one way.***

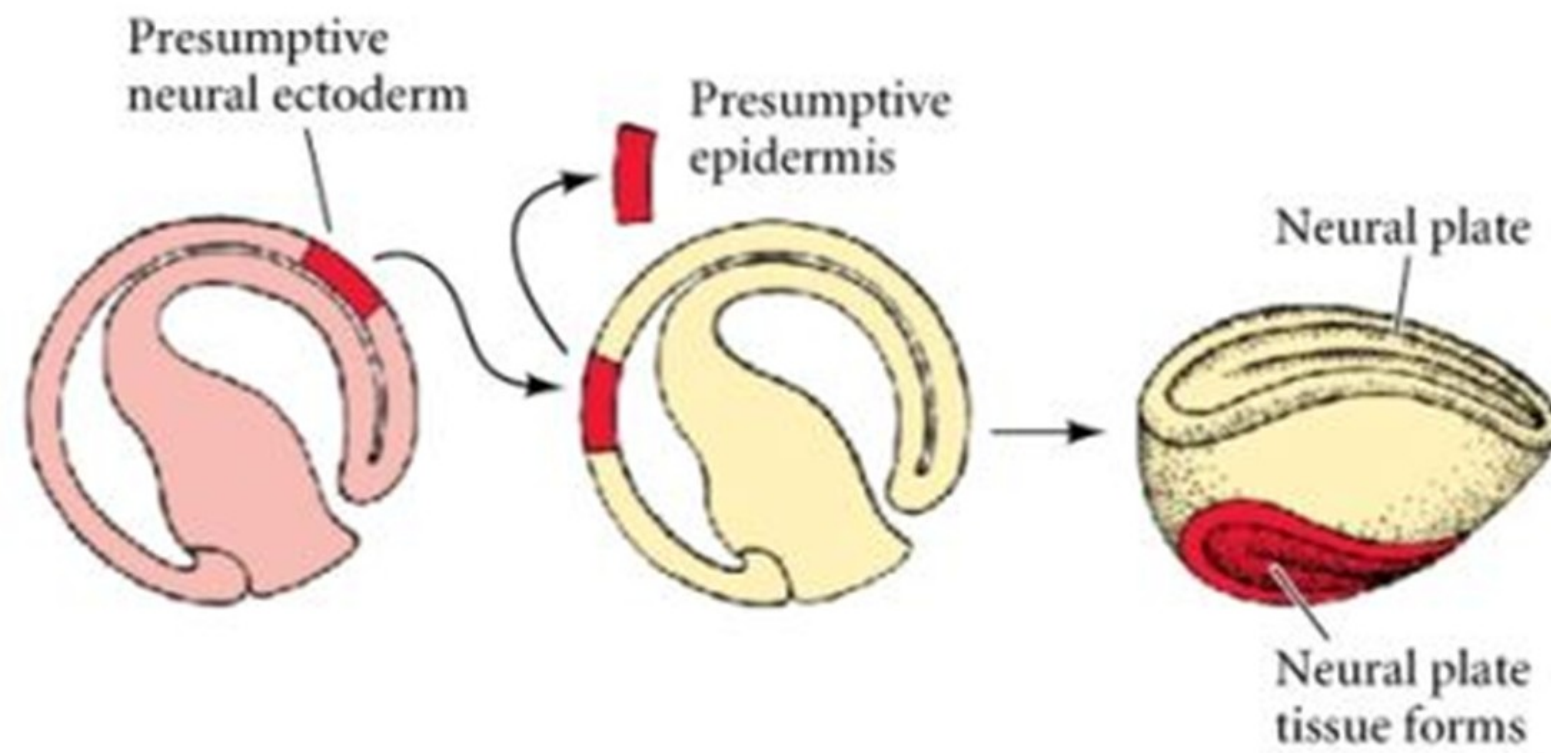
Experiments at Late gastrula

- 1. If prospective neural plate cells are moved to an area of prospective epidermis, then they will differentiate as a neural plate.
- 2. If prospective epidermis is transplanted into the prospective neural plate area then it differentiates into epidermis.
- *We can conclude that the fate of these tissues is fixed or determined (**prospective significance**), in other words tissues have the ability to develop only in one way (narrowing down of the prospective potency of a tissue, so tissue has a single specific fate)*

(A) EARLY GASTRULA



(B) LATE GASTRULA

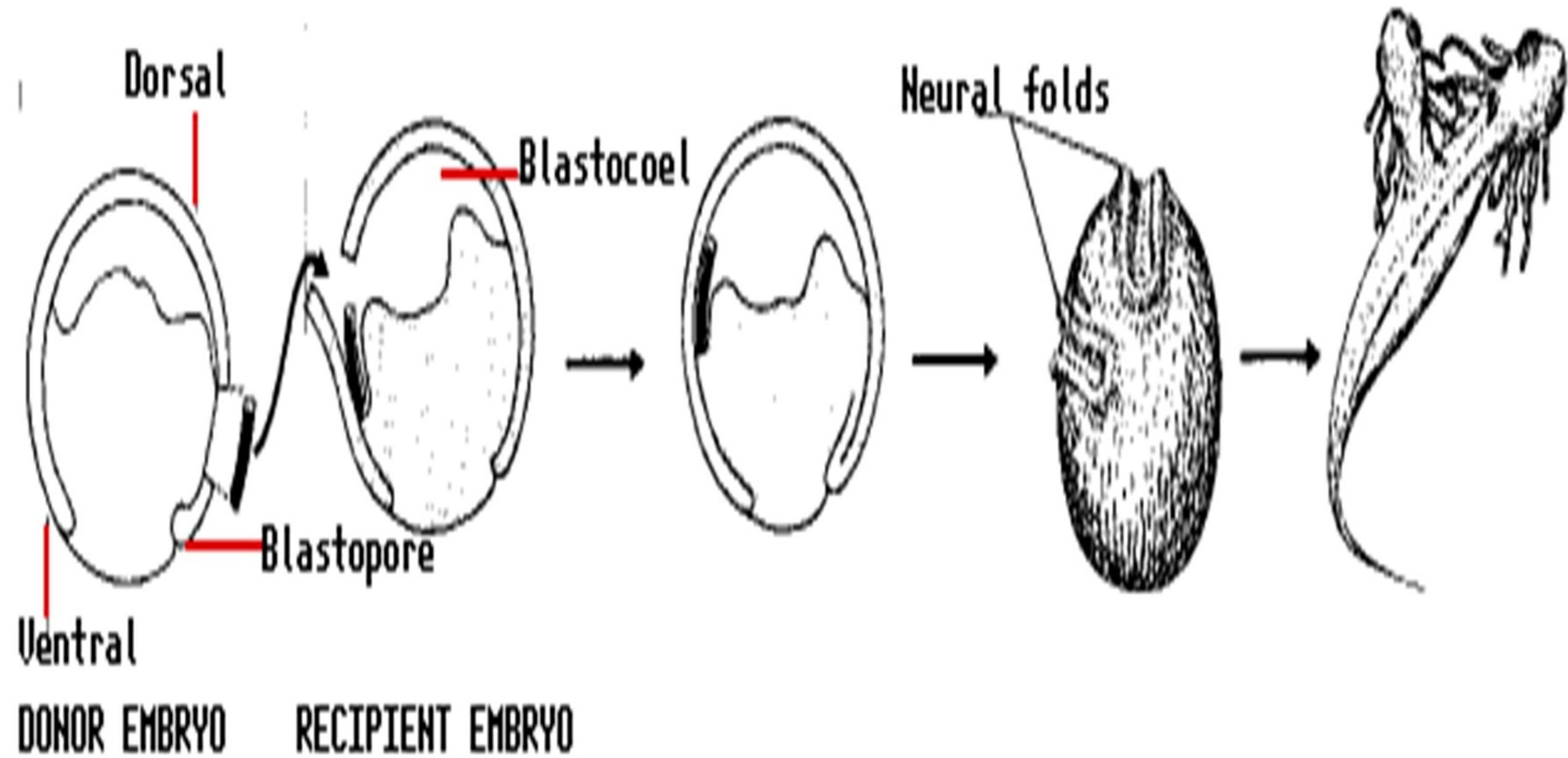


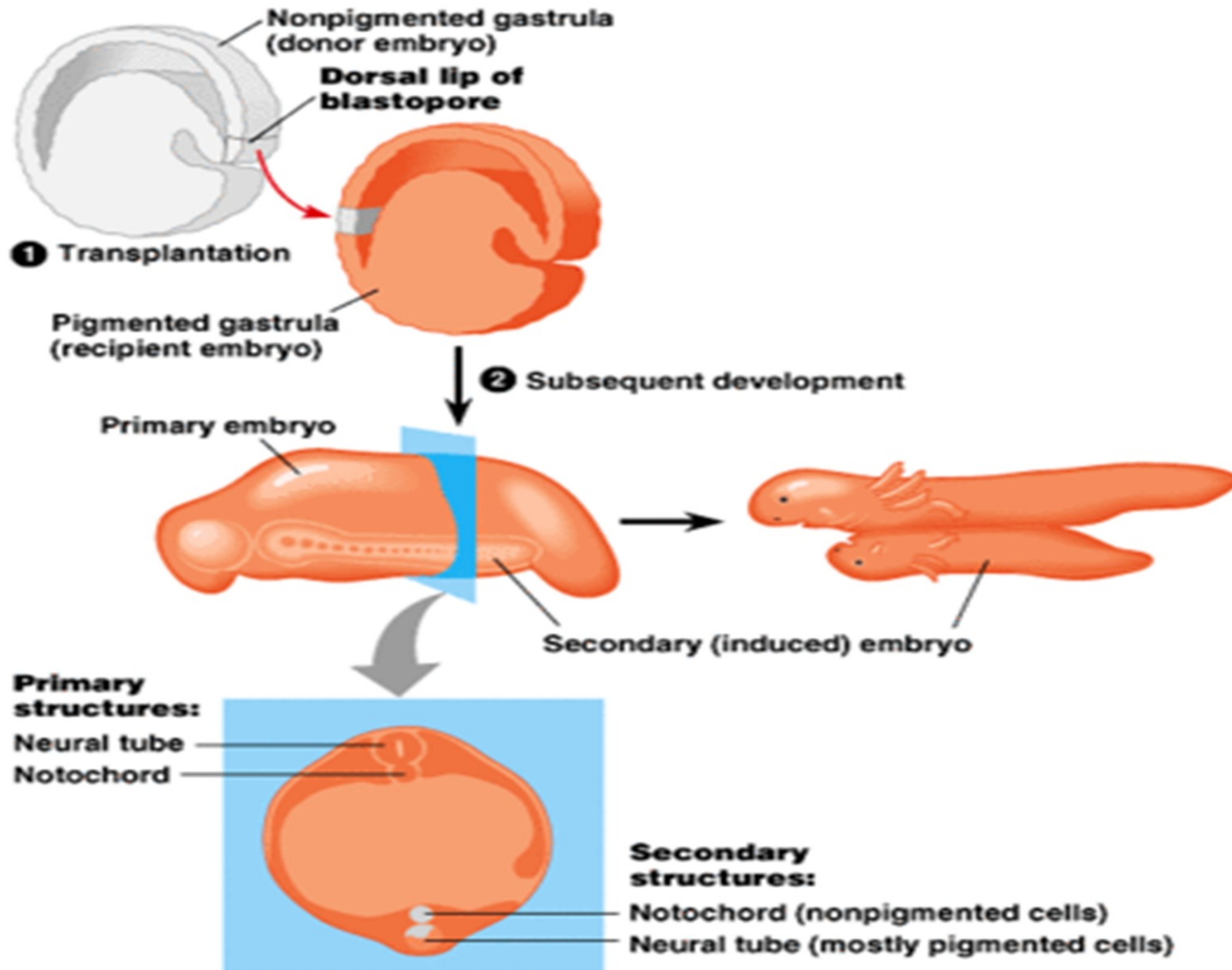
Embryonic Induction

- **Induction** is a mechanism causing cells to form a structure that neither inductor nor reacting cells would form if not combined.
- **An inductor** is a piece of tissue or a substance capable of causing induction.
- **Competence**: Not all tissues would form neural plate in response to inductor, only ectoderm.

Spemann's Experiments in Induction

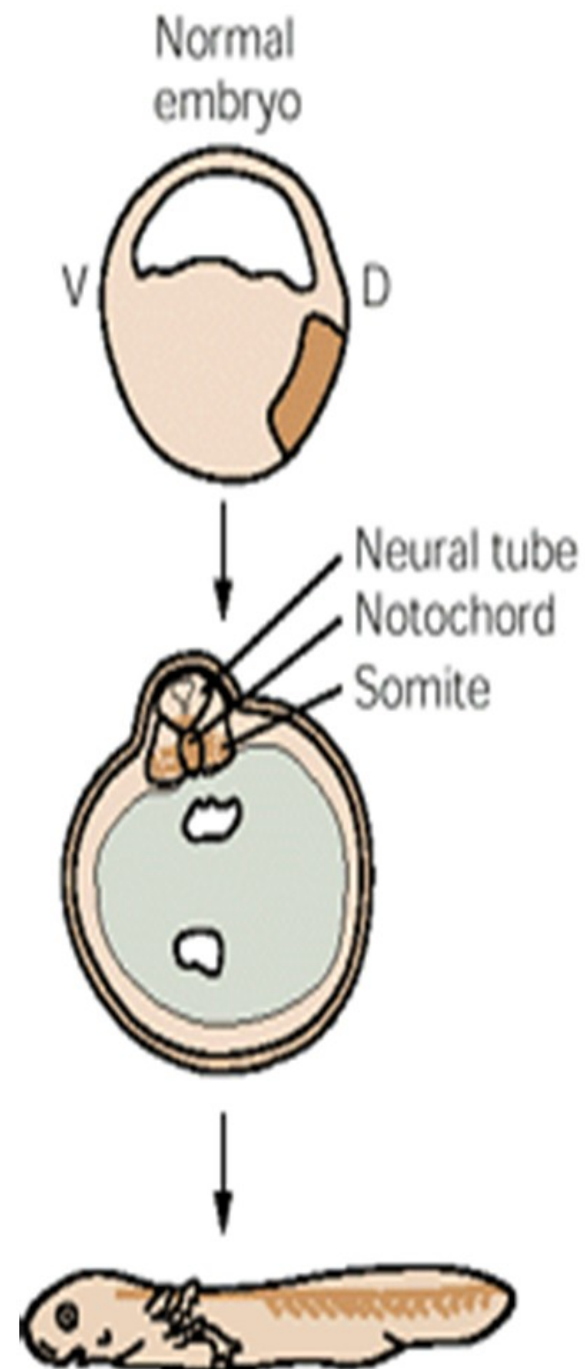
- In Amphibian: Spemann found that only a graft from the dorsal lip of blastopore will form notochord, somites and prechordal plate were capable of causing neural induction, and finally forming an extra head,
- Spemann called that area(dorsal lip) a **Primary organizer**



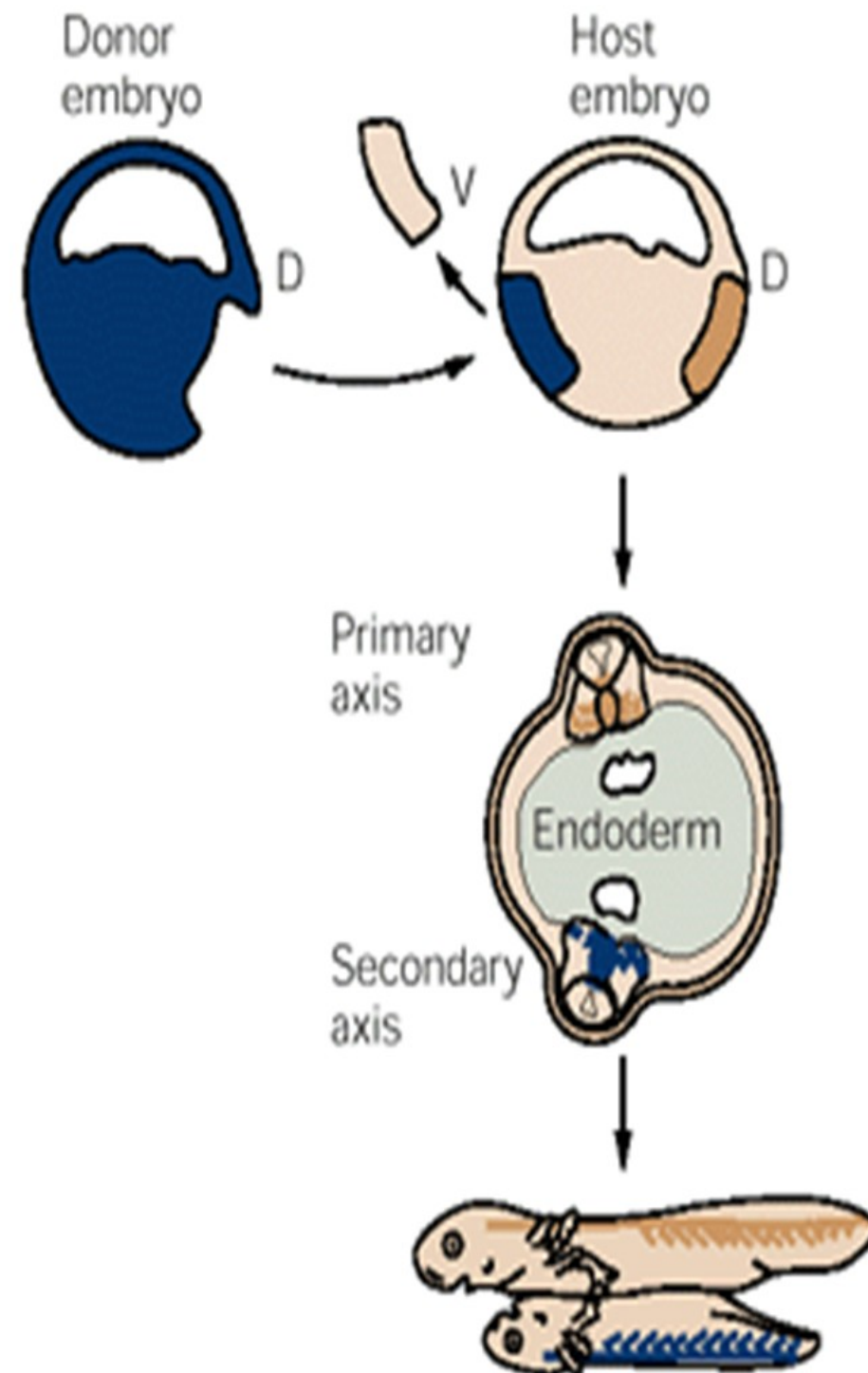


A Organizer grafts induce a twinned axis

The organizer region generates axial mesoderm during normal development



Grafted organizer region induces a secondary axis in host



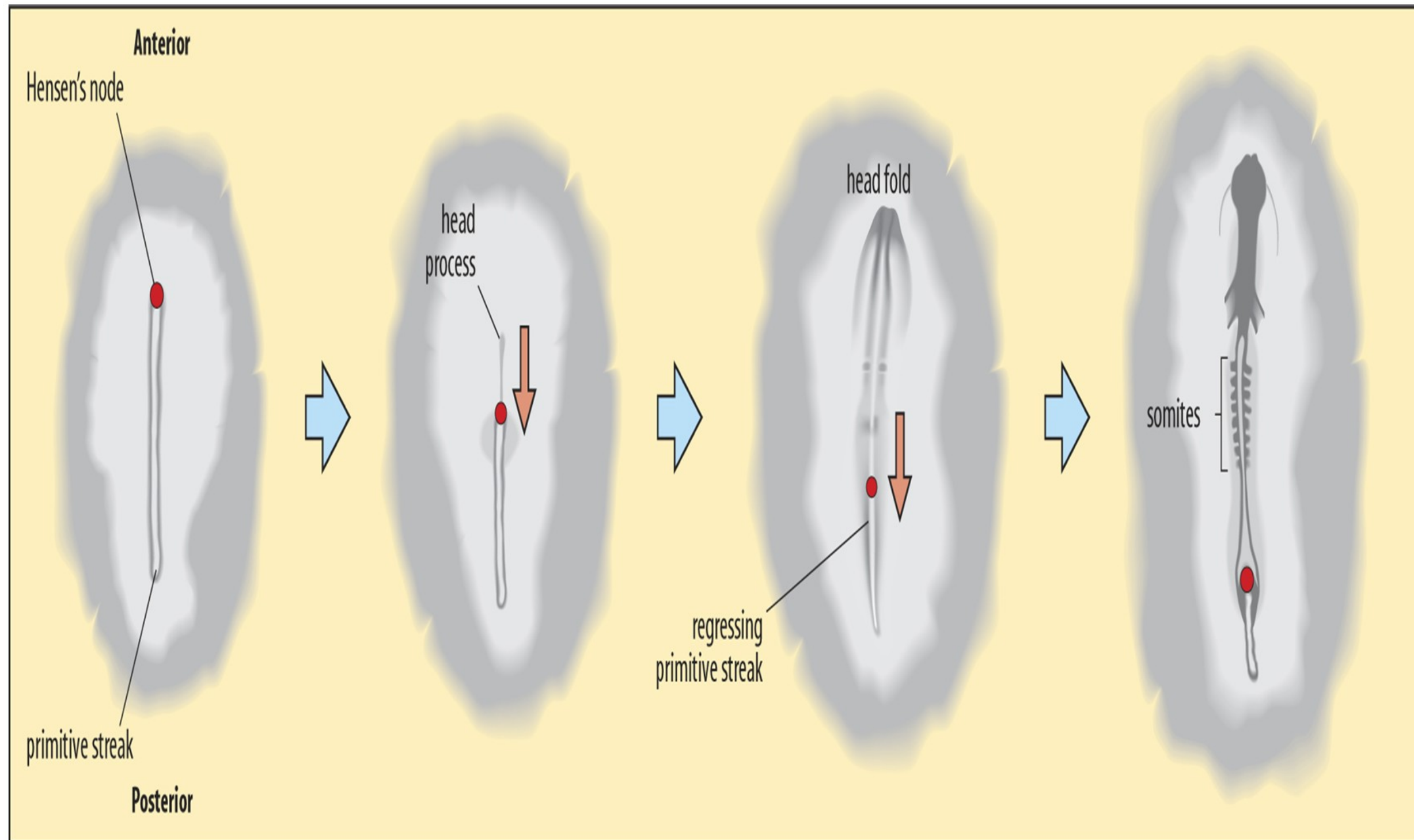
B Frog embryo with twinned axis



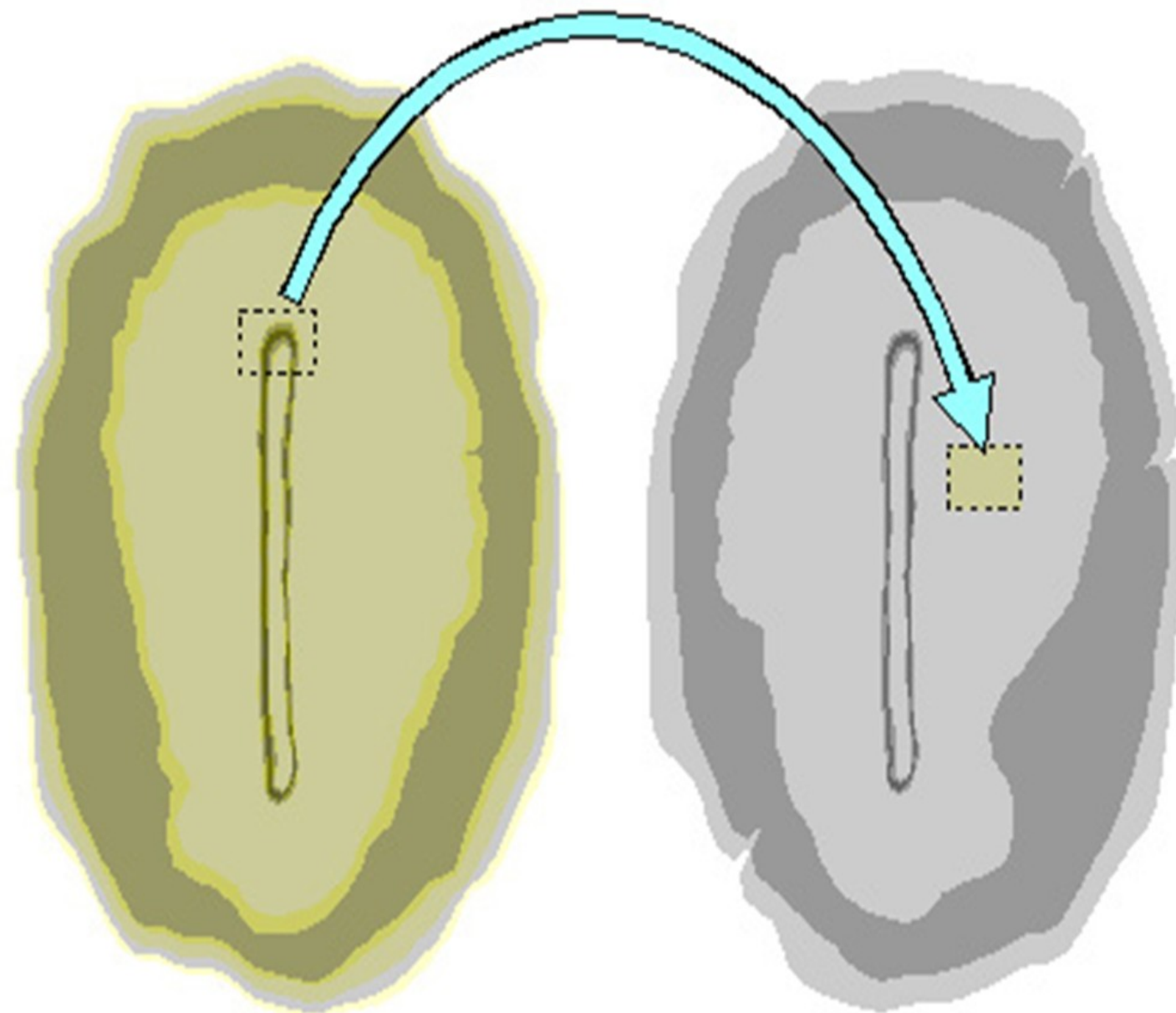
- **Primary organizer** defined as a special region of the embryo that is capable of determining the differentiation of other regions.

- Transplantation of **Hensen's node** in the chick embryo has the same effect in the chick as transplantation of the dorsal lip in amphibian embryo.
- So, the primary organizer in birds is located in the **Hensen's node**

Normal chick embryo development



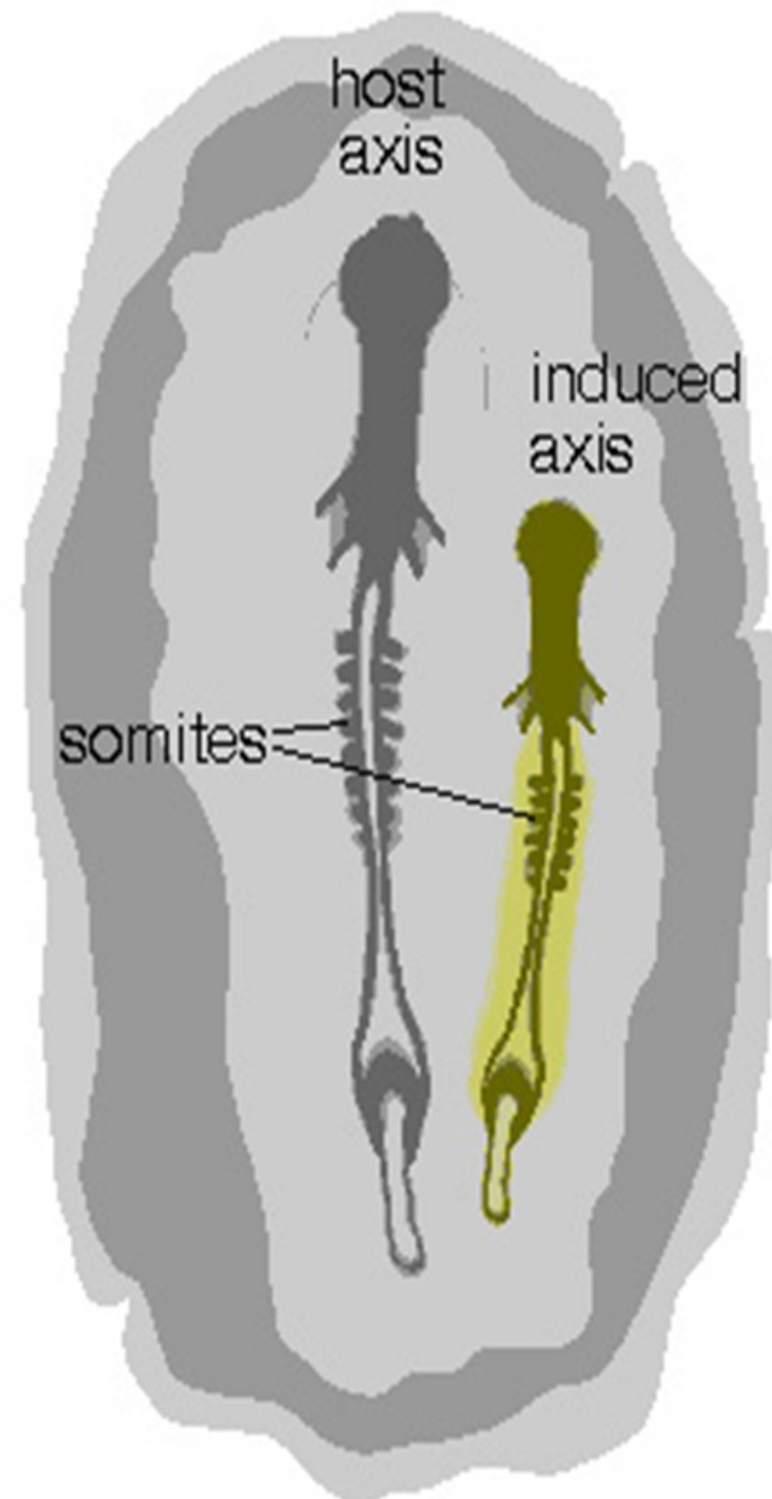
Hensen's node grafted from quail embryo to chick host



Quail embryo

Chick embryo

New axis induced in host



Nature of Inductor

- The normal inductor is a **peptide protein.**
- This was proved by inactivating it by **trypsin**

- The regional specificity may be imposed on the induced organs by the inductor

Archenteric Roof

- I) The anterior part of the archenteric roof called **Head inductor** which differentiated into:
 - **A.** Archencephalic inductor: inducing fore brain, eyes and nose formation.
 - **B.** Deuterencephalic inductor: inducing hind brain and ear vesicles.

- II) The posterior part of archenteric roof called **trunk** or **spinocaudal inductor** induces trunk organs and tail buds.

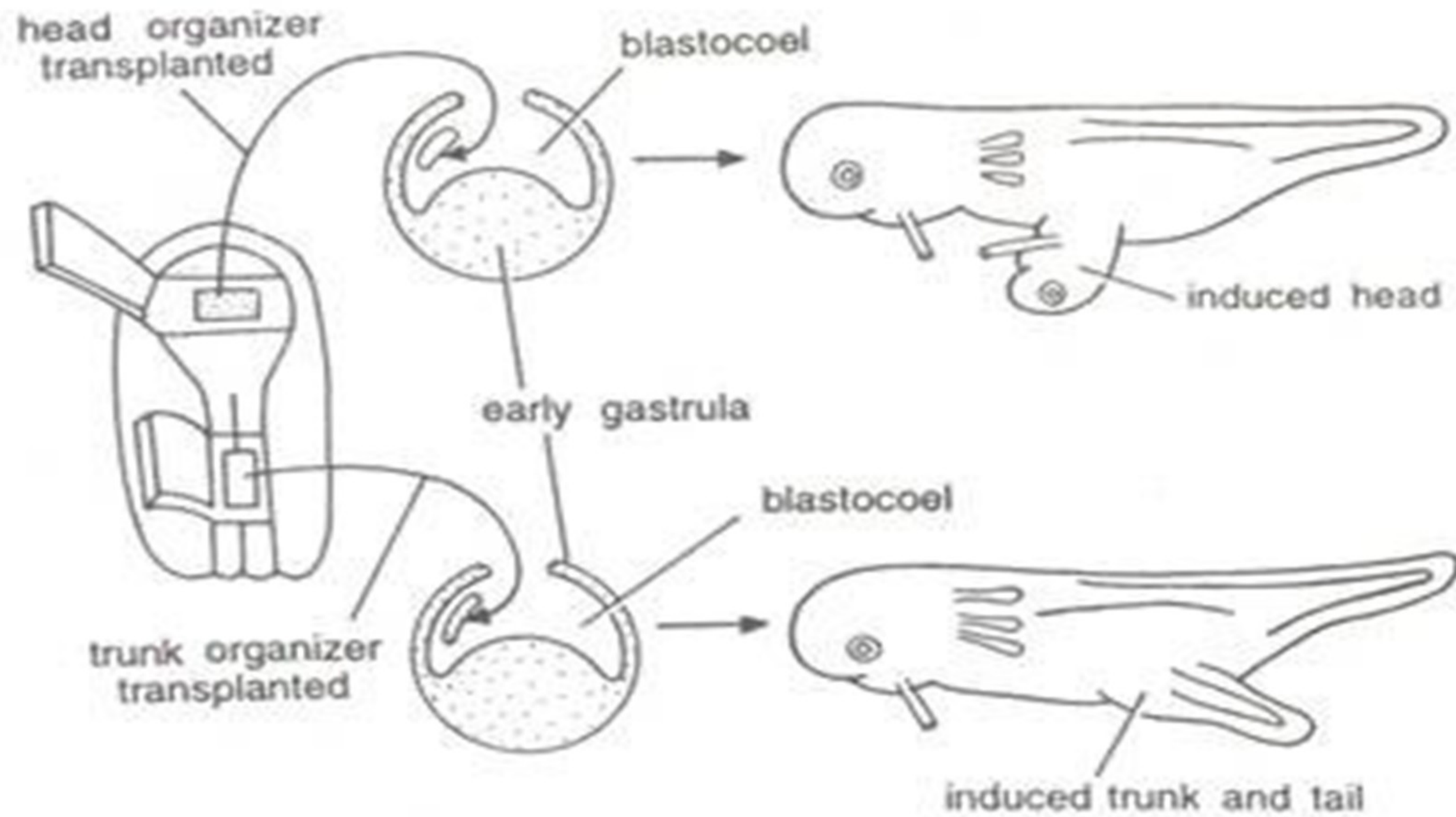
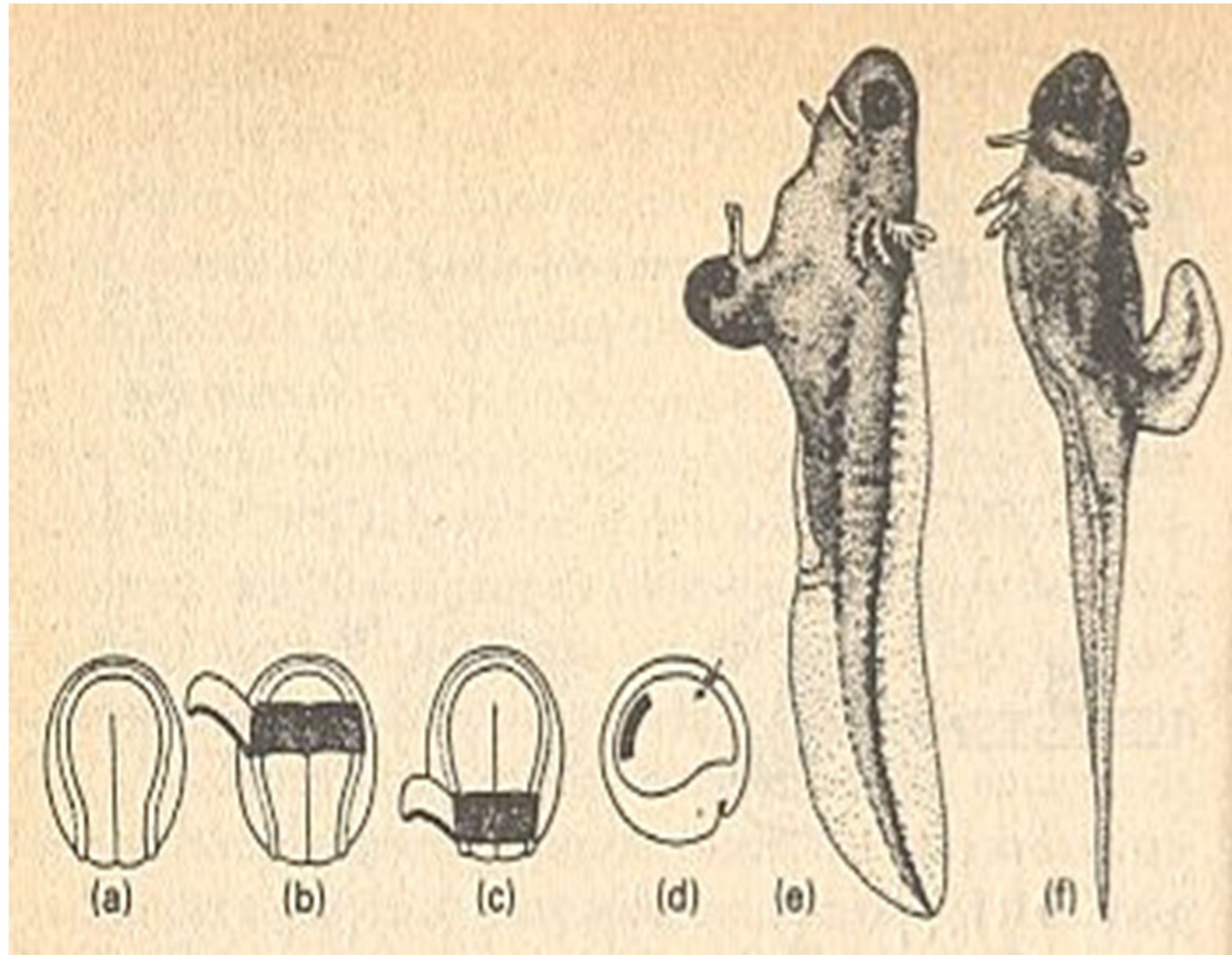


Fig. 2. The separation of the neural inductor into head and trunk organizers.





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