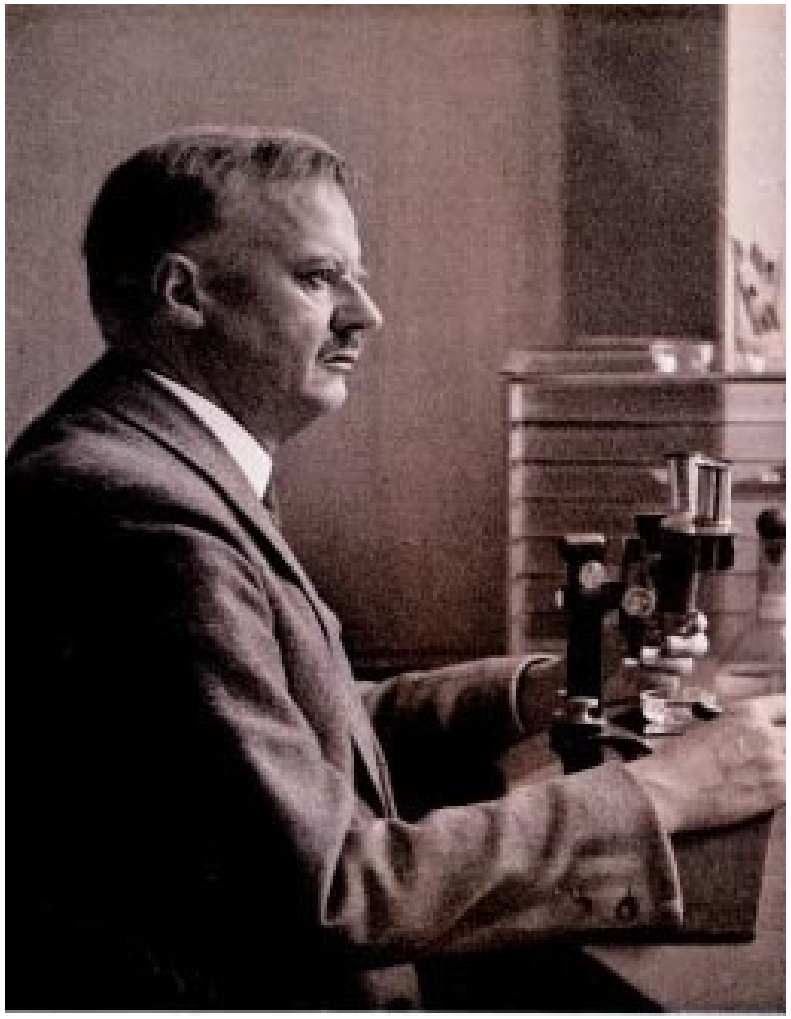


Hans Spemann (1869-1941) ***at his laboratory desk, probably in the*** *1920's in a photograph owned by Otto Mangold (SAF).*

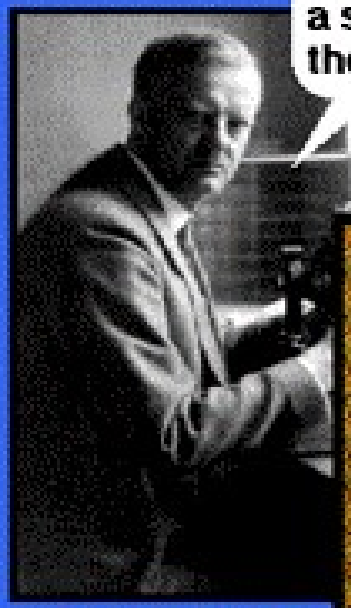
(1869 - 1941) Hans Spemann, a **German embryologist**, is one of the original pioneers of modern embryology

Hans Spemann
Born: 27 June 1869, Stuttgart, Württemberg (now Germany)
Died: 12 September 1941, Freiburg im Breisgau, Germany
Affiliation at the time of the award: University of Freiburg im Breisgau, Breisgau, Germany
Prize motivation: "for his discovery of the organizer effect in embryonic development"
Field: embryology
Prize share: 1/1

In 1935, Spemann received the Nobel Prize in
Physiology or Medicine 1935

The “**organizer paper**” by Hans Spemann and Hilde Mangold (1924) initiated a new epoch in developmental biology. It marked the climax of Spemann’s life-long research, and the “organizer effect” received special mention by the committee that honoured him with the **Nobel Prize for physiology or medicine in 1935**.

A piece of the upper blastopore lip of an amphibian embryo undergoing gastrulation exerts an organizing effect on its environment in such a way that, if transplanted to an indifferent region of another embryo, it causes there the formation of a secondary embryonic anlage. Such a piece can therefore be designated as a Organizer.



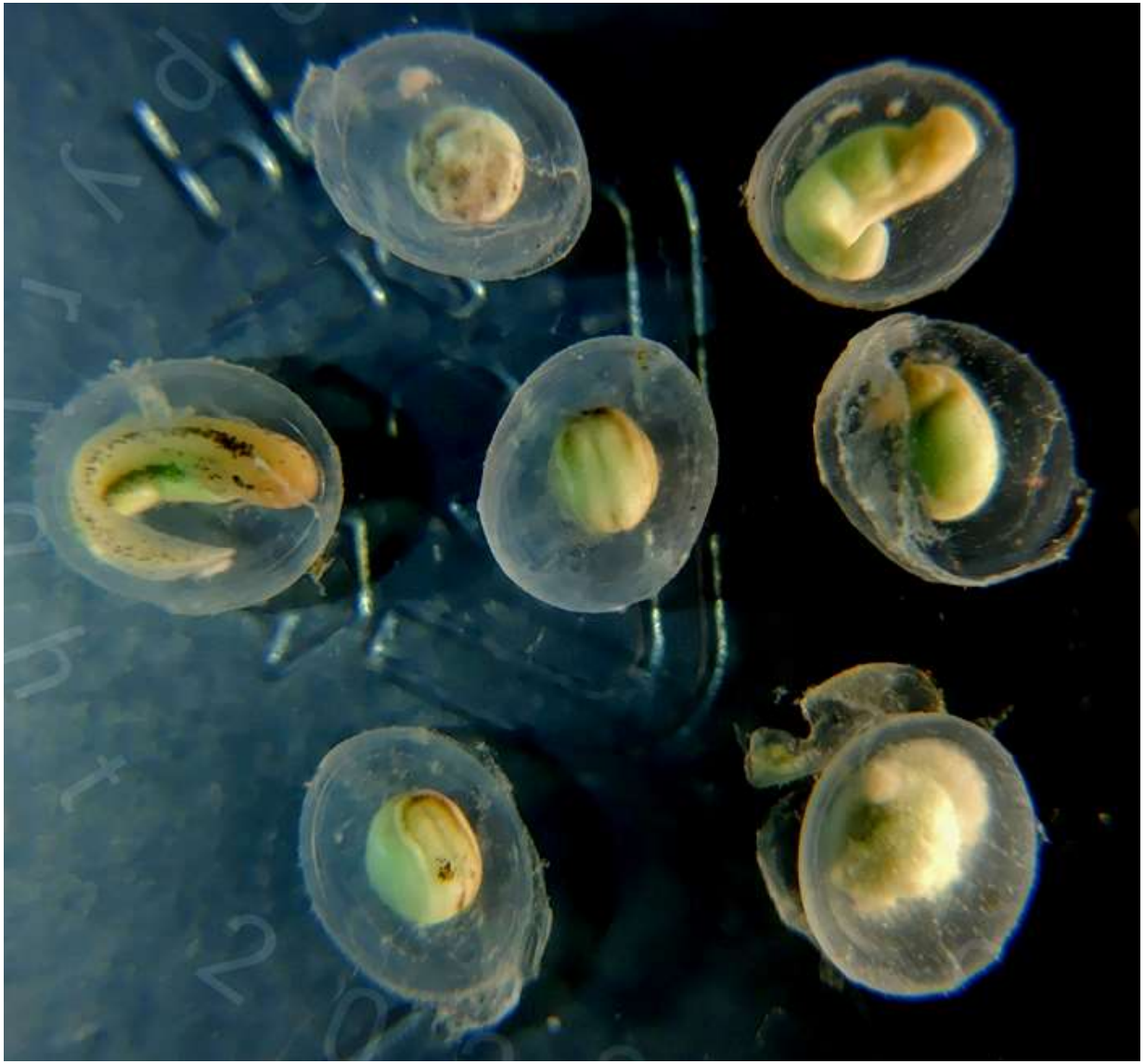
Hans Spemann

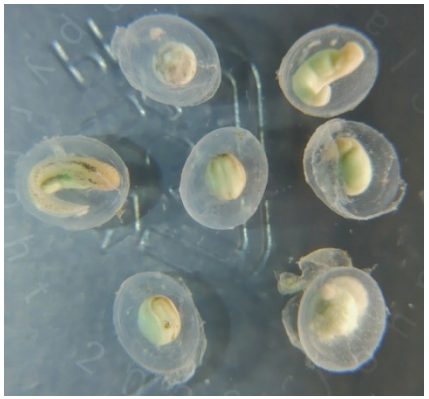
Hilde Mangold

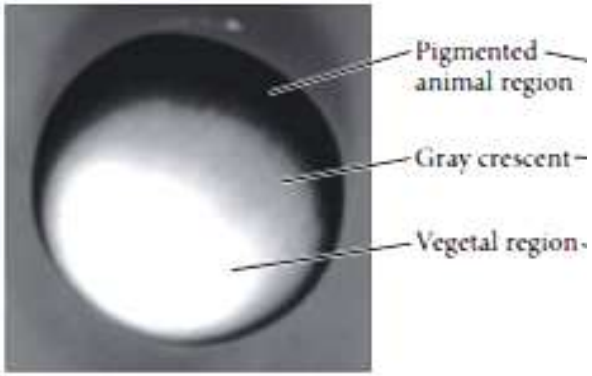
Experiment1.
Early newt blastomeres have identical nuclei

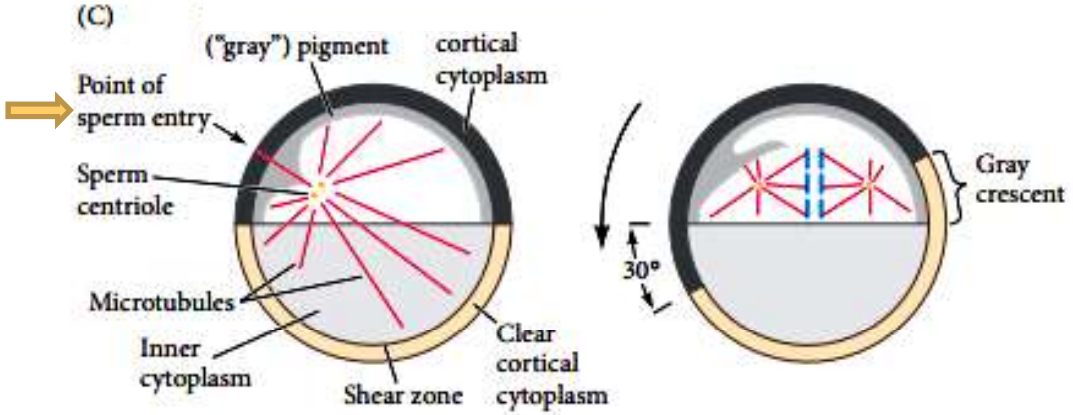
Triturus cristatus

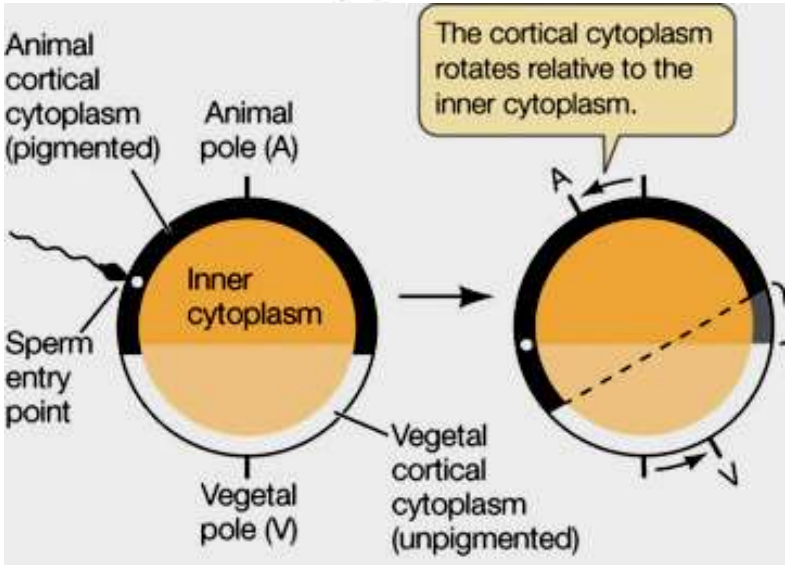
Tritrurus taeniatus

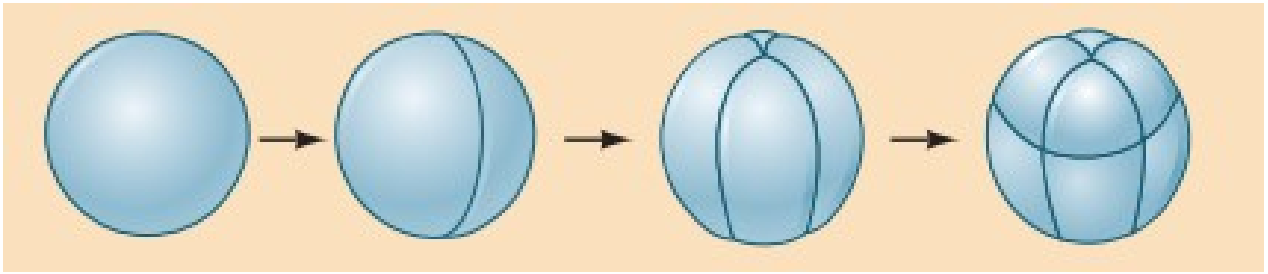


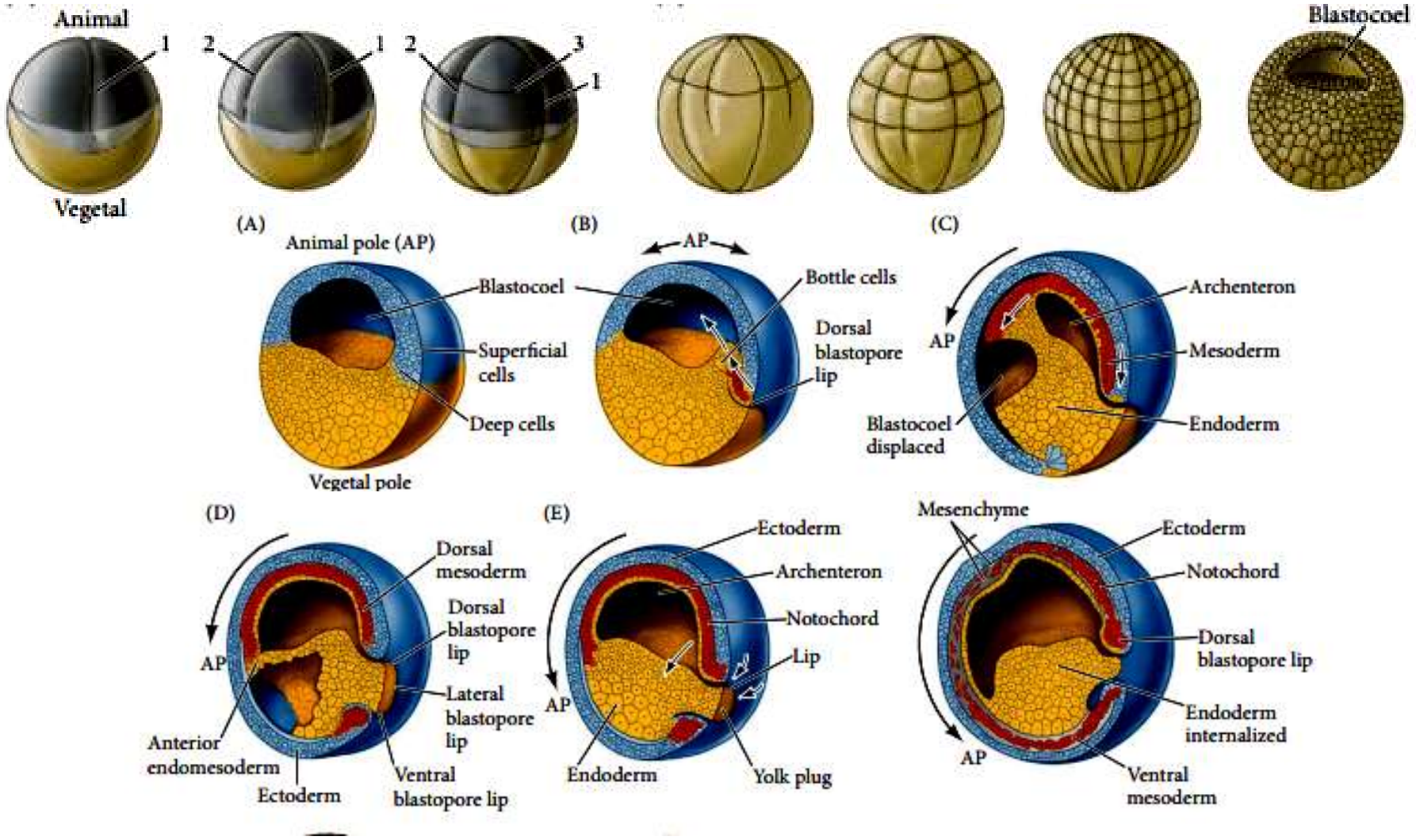



Pigmented animal region
Gray crescent
Vegetal region


(C)
("gray") pigment
cortical cytoplasm
Point of sperm entry
Sperm centriole
Microtubules
Inner cytoplasm
Shear zone
Clear cortical cytoplasm
30°
Gray crescent


Animal cortical cytoplasm (pigmented)
Animal pole (A)
The cortical cytoplasm rotates relative to the inner cytoplasm.
Inner cytoplasm
Sperm entry point
Vegetal pole (V)
Vegetal cortical cytoplasm (unpigmented)
A
V

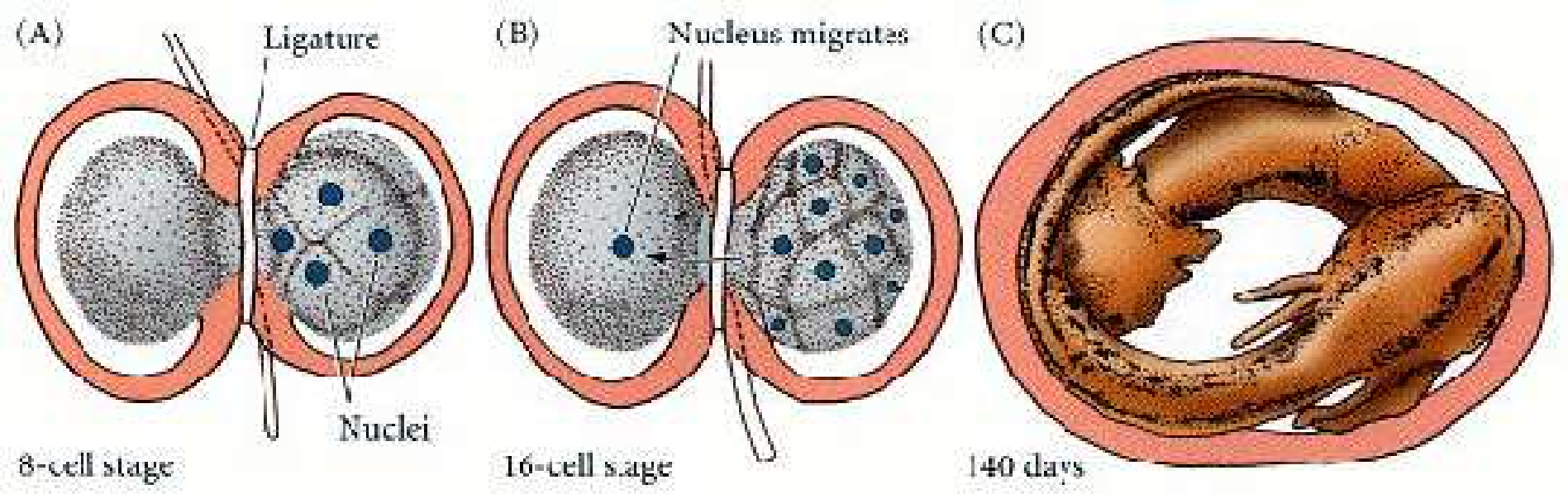



Animal
Vegetal
1
2
1
2
3
1
(A)
(B)
(C)
Blastocoel
Animal pole (AP)
Blastocoel
Superficial cells
Deep cells
Vegetal pole
AP
Bottle cells
Dorsal blastopore lip
AP
Archenteron
Mesoderm
Endoderm
Blastocoel displaced
(D)
(E)
Dorsal mesoderm
Dorsal blastopore lip
AP
Lateral blastopore lip
Anterior endomesoderm
Ectoderm
Ventral blastopore lip
Ectoderm
Archenteron
Notochord
Lip
AP
Endoderm
Yolk plug
Mesenchyme
Ectoderm
Notochord
Dorsal blastopore lip
Endoderm internalized
AP
Ventral mesoderm

Early newt blastomeres have identical nuclei

- Shortly after fertilization of the newt egg , Spemann used a baby's hair to "lasso" the zygote in the plane of the first cleavage.
- Then partially constricted the egg causing all nuclear division to remain on one side of the constriction.
- At 16 cell stage one nucleus would escape across the constriction into the non nucleated side.
- Cleave began in the non nucleated part following which he tightened the lasso until the two halves were completely separated.

Spemann concluded from this experiment that early amphibian nuclei were genetically identical and that each cell was capable of giving rise to an entire organism.



A lock of baby hair, found in Spemann's file holder for 1899, ***within the envelope (top) marked with*** *his daughter's name.*

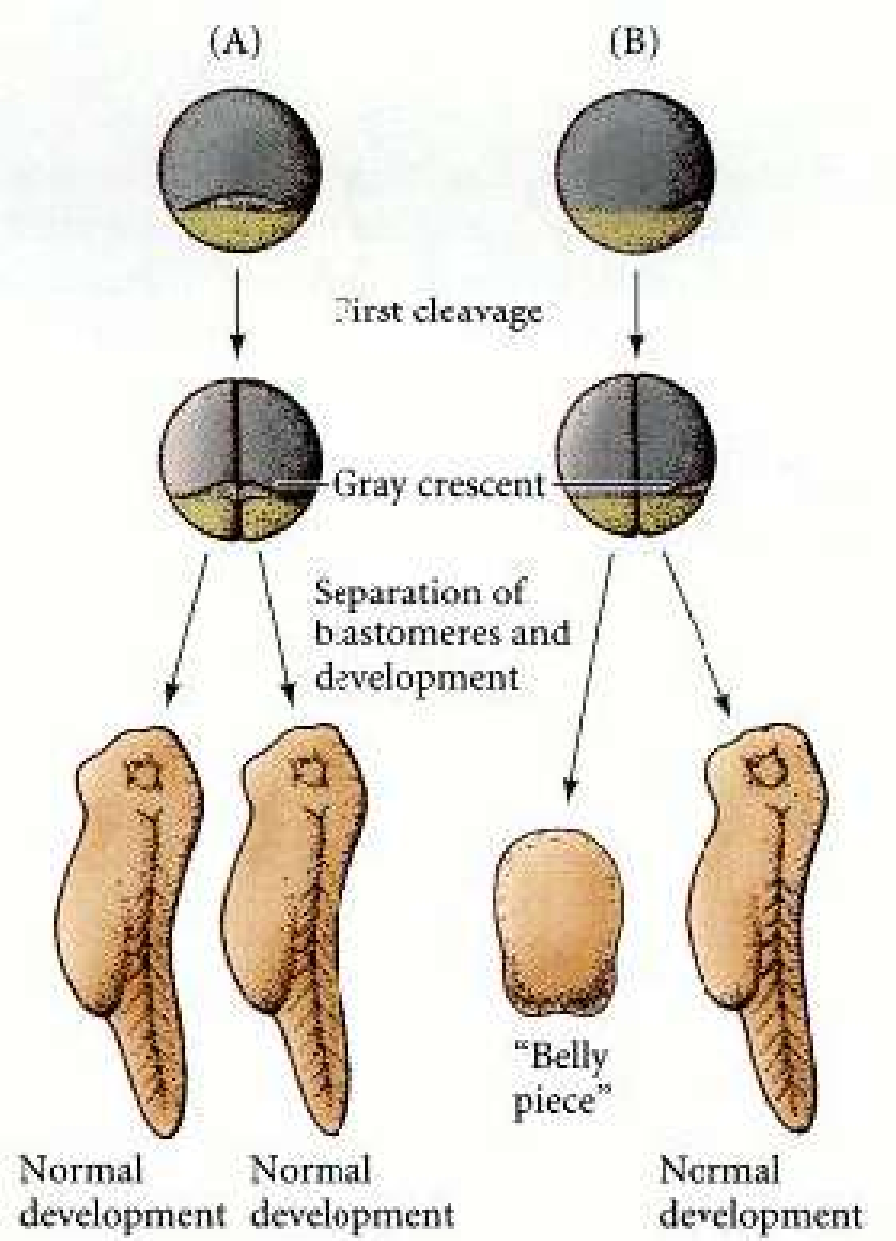
Second Experiment

•The experiments of Hans Spemann in the early 1900s demonstrated the role of certain cytoplasmic *signaling* substances in the amphibian embryo. :

• By cleaving the fertilized egg unnaturally, Spemann showed that an area called the *gray crescent* is essential to embryonic development.

• By the two-cell-layer (blastula) stage, the cells in the embryo have already been "mapped" to the structures they will form in the complete embryo.

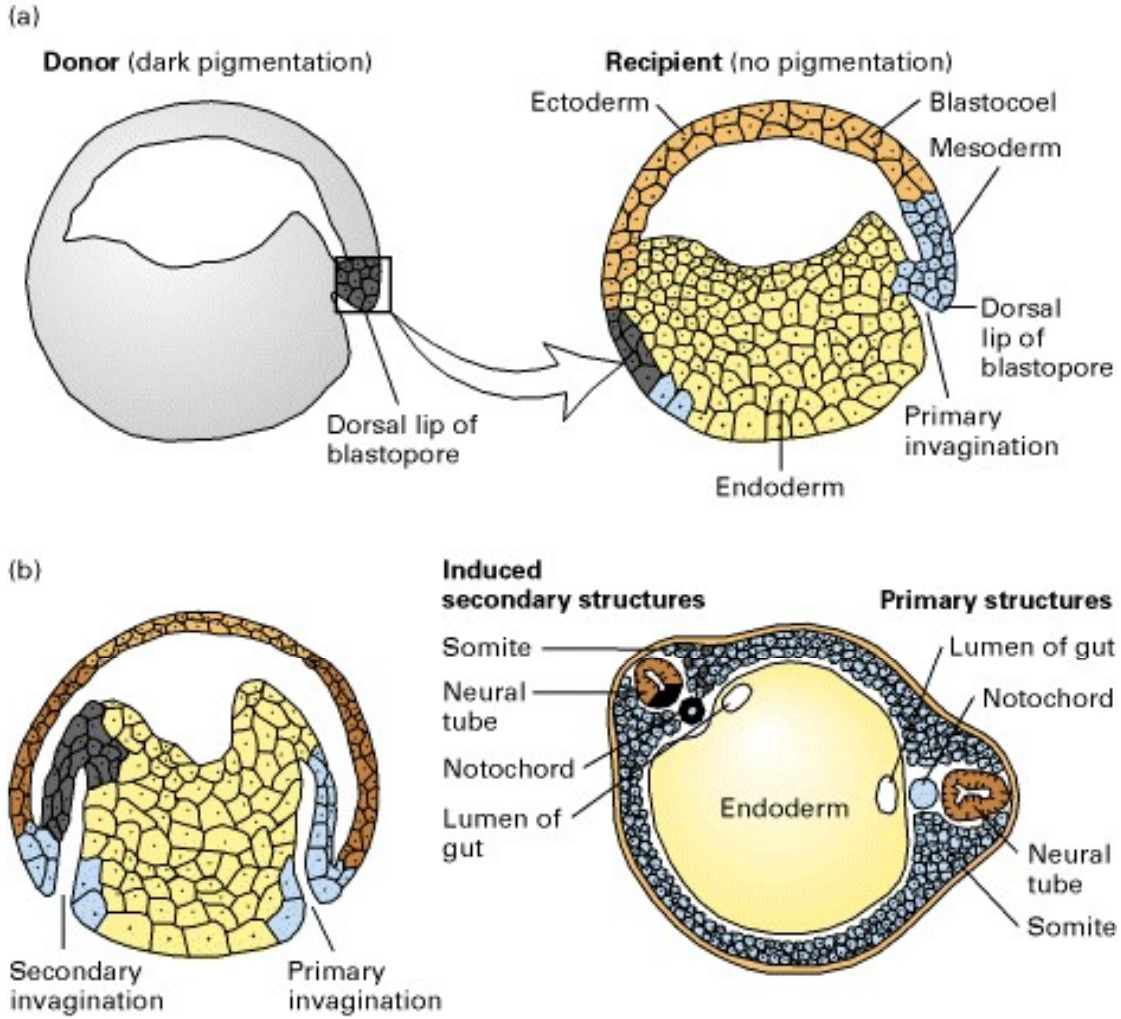
•Spemann showed that if the embryo were cleaved at this point, only the section containing the dorsal lip of the blastopore (once the gray crescent area) would continue to develop.

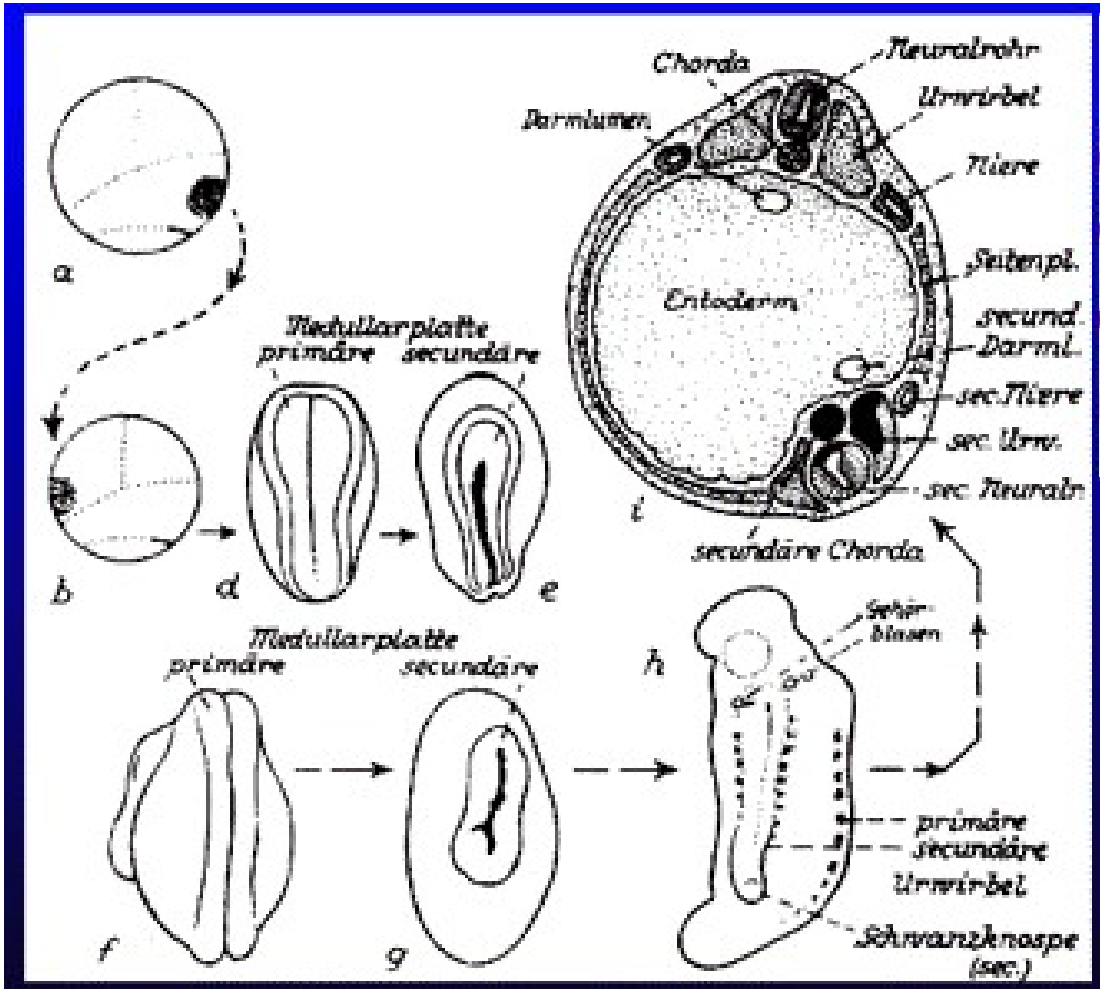


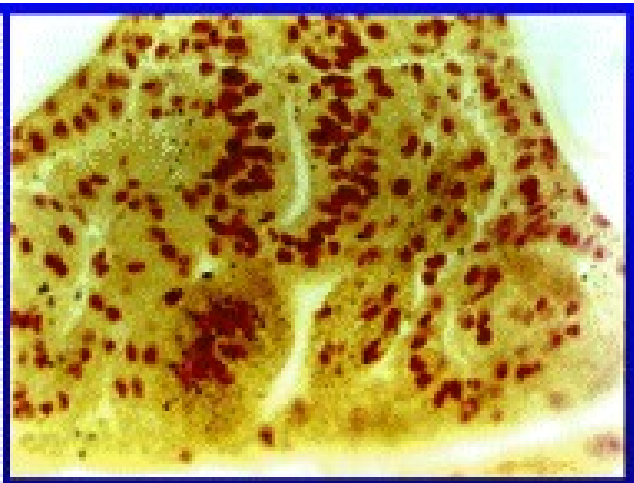
The first cleavage plane normally splits the gray crescent equally into the two blastomeres. If cleavage plane is not through gray crescent,only the blastomere containing the gray crescent develops normally.

Third Experiment

- In 1924, Spemann and Mangold published some of their findings on the dorsal blastopore lip transplantations. They had found
- (a) that the dorsal blastopore lip transplants invaginated almost completely,
- (b) that the transplanted tissue caused the formation of a secondary neural plate composed almost entirely of host tissue,
- (c) that while the notochord was primarily derived from donor tissue, the flanking mesoderm was a combination of donor and host cells. Some somites were chimeric, some completely host, some completely donor .


(a)
Donor (dark pigmentation)
Recipient (no pigmentation)
Ectoderm
Blastocoel
Mesoderm
Dorsal lip of blastopore
Primary invagination
Endoderm
Dorsal lip of blastopore
(b)
Induced secondary structures
Primary structures
Somite
Neural tube
Notochord
Lumen of gut
Endoderm
Lumen of gut
Notochord
Neural tube
Somite
Secondary invagination
Primary invagination
(c)

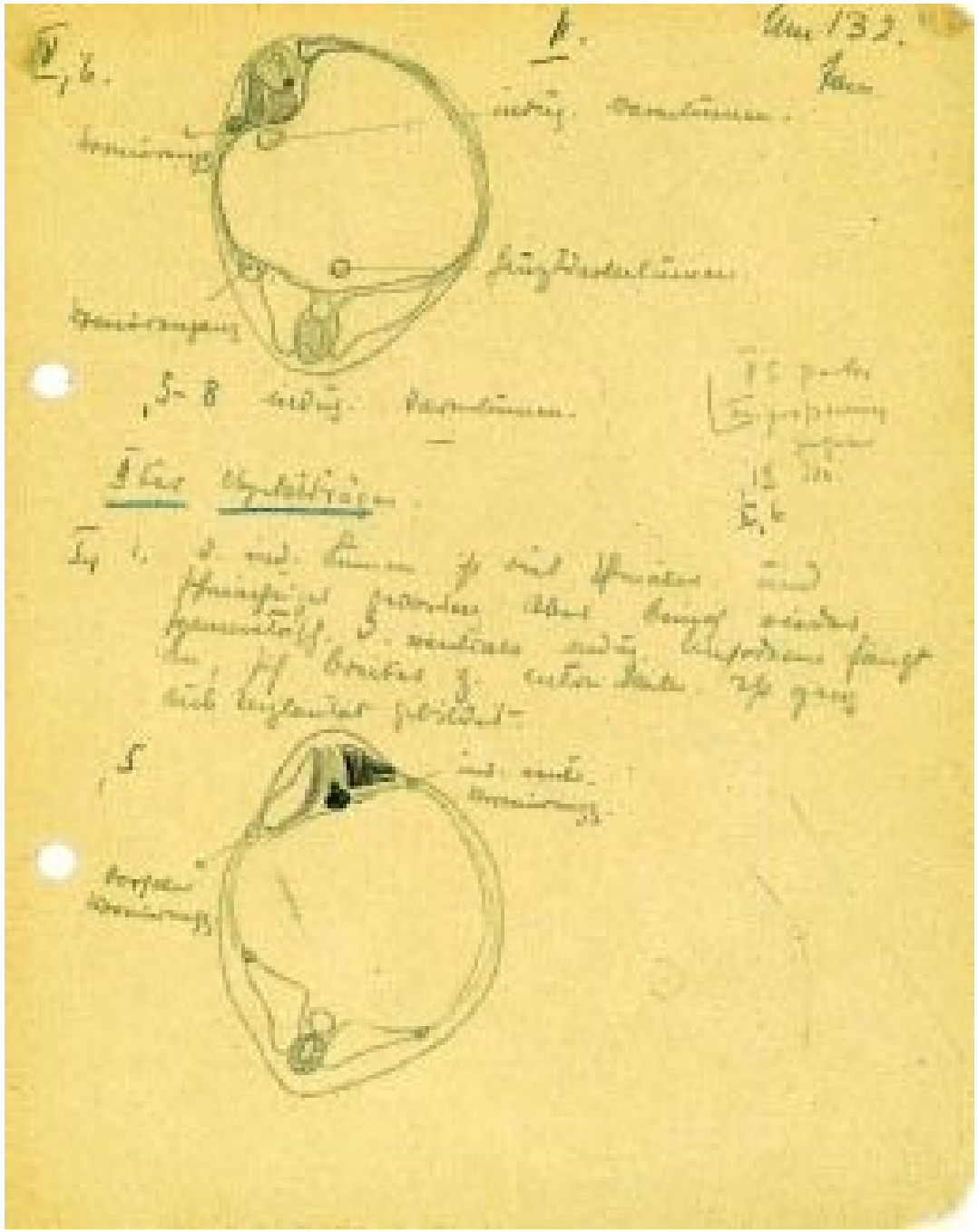




From actual microscope slide of Hilde Mangold, courtesy of P. Fäffler and K. Sander

Experimental protocol for heteroplastic transplantations

SPEMANN, H. AND MANGOLD, H. 1924. Über Induktion von Embryonanlagen durch Implantation artfremder Organisatoren. *Roux' Archiv für Entwicklungsmechanik* 100: 599 - 638.



Hilde Mangold´s Lab notes on the famous heteroplastic lip transplantation (Um. 132) and her own slide boxes *labelled "Mangold"*
by the curator of the Embryological Collection of the Hubrecht Laboratory (Utrecht/The Netherlands).

Tr. Taen. 1909 | 143

x 60

Oc 2 Obj aa Tub 19,6

/ 5 Tina

horizontal
Ansicht von Bauch

Ansicht v. d.
rechten Seite.

Lebers inversum
Cordis normal

Lebers
Cordis inversum

Leber / invers,
aber nach d. linken
Seite verschoben

Gallenblase normal

Darmschlinge ~~angelegt~~
median

17.II.1908.

Bronze plate commemorating Hilde Mangold, unveiled in the new Zoology building at Freiburg University on her 100th birthday. ***It was cast from the plate on Hilde's tombstone at Gotha, Poland. (Photograph K.S., 2000).***

Robert Briggs (1911–1983)
Thomas J. King (1921–2000).

Briggs, R. and King, T. J. “Transplantation of Living Nuclei from Blastula Cells into Enucleated Frogs’ Eggs.” *Proceedings of the National Academy of Sciences* 38 (1952): 455–463.

1952: Briggs and King cloned tadpoles using the method of nuclear transfer

*TRANSPLANTATION OF LIVING NUCLEI FROM BLASTULA CELLS INTO ENUCLEATED FROGS' EGGS**

By Robert Briggs and Thomas J. King

Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pennsylvania

Communicated by C. W. Metz, March 15, 1952

Introduction.—The role of the nucleus in embryonic differentiation has been the subject of investigations dating back to the beginnings of experimental embryology. At first it was supposed by Roux, Weismann and others that differentiation is the result of qualitative nuclear divisions, different blastomeres thereby receiving the different kinds of nuclei which determine their subsequent differentiation. Later on this theory was disproved by numerous experiments showing that, during early cleavage

Experiment

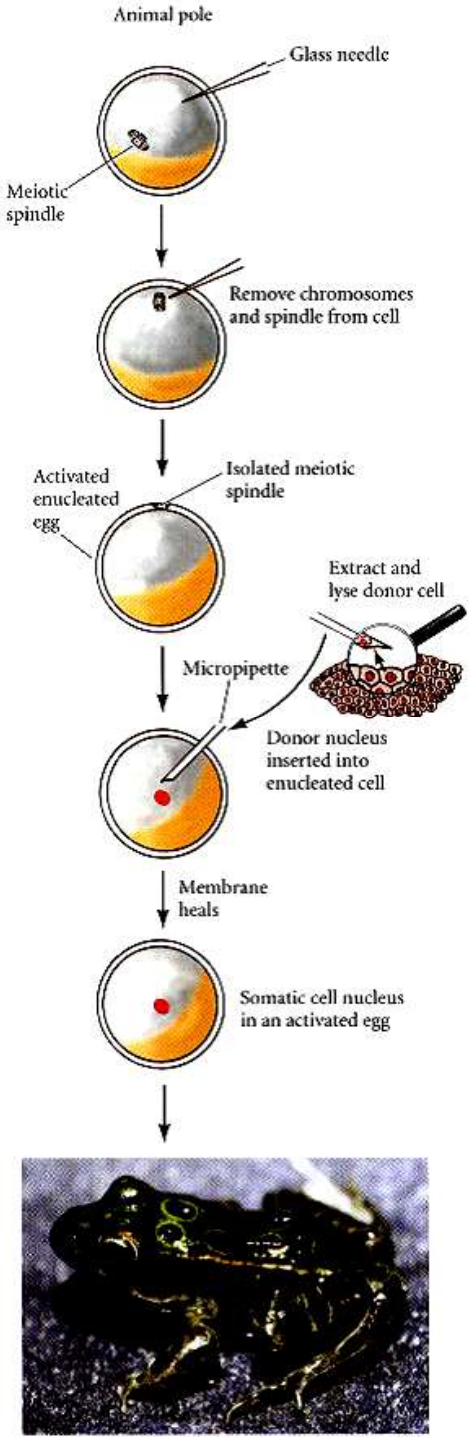
In this paper Briggs and King examined whether nuclei of embryonic cells are differentiated, and by doing so, were the first to conduct a successful nuclear transplantation with amphibian embryos.

During the experiment, they used two different species of frogs, *Rana pipiens* and *Rana catesbeina*, to study and test whether the nucleus is differentiated.

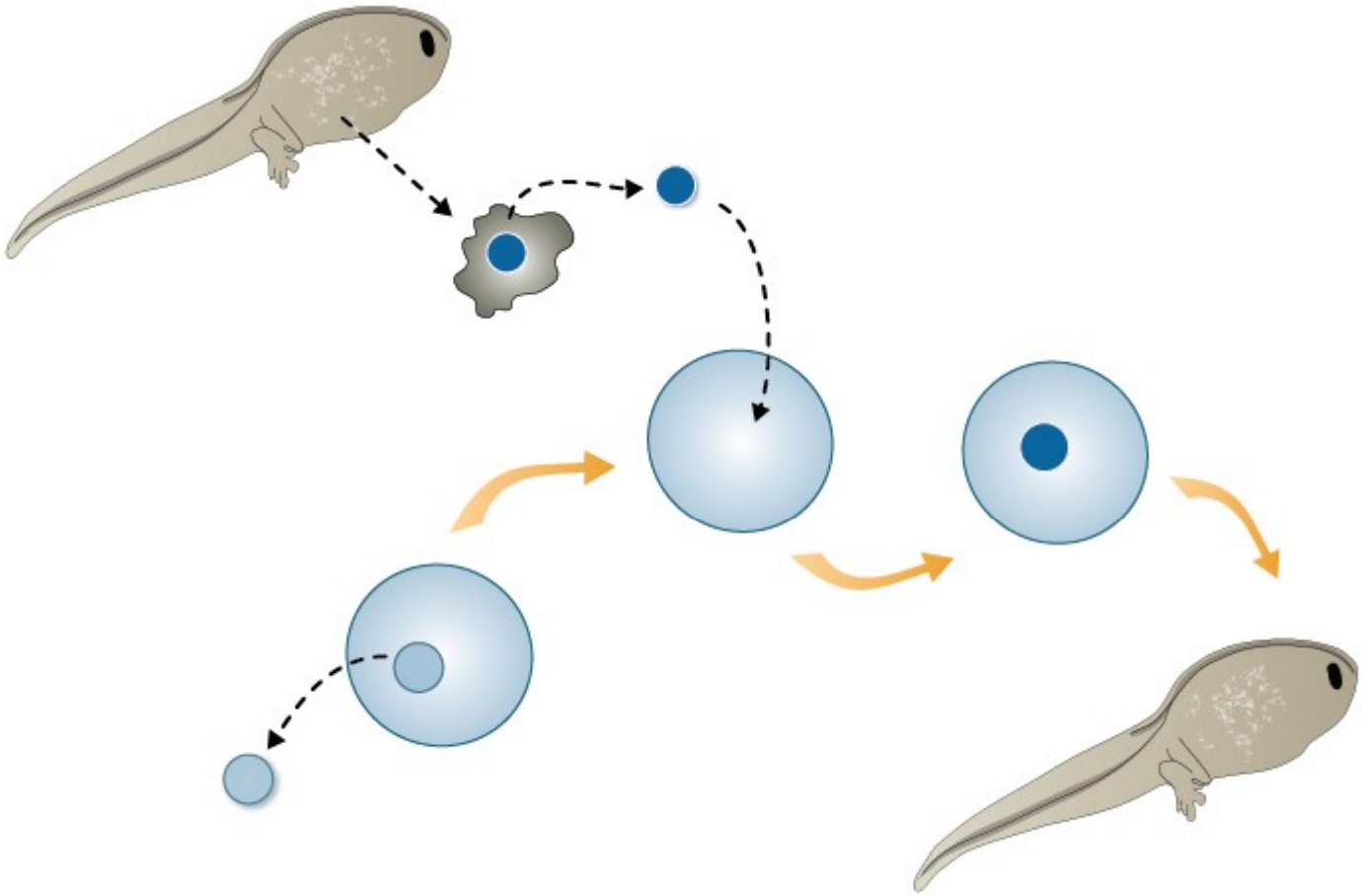
In order to make such tests of nuclear differentiation it is first necessary to develop a method of transplantation that leaves both the transplanted nucleus and the recipient egg cytoplasm in undamaged condition. The only way to determine if this can be done is to work first with nuclei from undifferentiated cells which, if transplanted properly, should give rise to normal embryos. In the Amphibia the cells of choice for this purpose are those of the late blastula. They are almost as small as the differentiated cells of slightly older embryos and so present the same technical problems. At the same time they are, with the exception of the future dorsal lip cells, still undetermined and therefore their nuclei cannot be irreversibly differentiated. For these reasons we have worked out a method for transplanting nuclei from these cells into enucleated frogs' eggs. These eggs cleave and in a significant proportion of the cases develop into complete embryos.

Method.—The transplantation of nuclei is carried out in the following steps: First the recipient egg is pricked with a clean glass needle. This activates the egg and causes it to rotate so that the animal pole is uppermost and the egg nucleus can be taken out with a glass needle by Porter's[9] technique. The outer jelly coats are then removed and the egg is placed in a depression in a wax-bottomed dish in Niu-Twitty's[10] solution. A blastula or early gastrula (St. 8 to 10, Shumway[11]), placed in the same dish, is then opened up and one of the subsurface animal pole cells is dissected free from its neighbors. The cell is now drawn up into the mouth of a thin-walled glass micropipette, the lumen of which is somewhat smaller than the diameter of the cell. The pipette is held in a Leitz-Chambers holder connected via rubber pressure tubing to an ordinary 5-ml. syringe. All of the system except the tip of the needle is filled with air. The tip contains the column of solution drawn up with the cell. Provided the needle is really clean the movements of the column can be controlled accurately. Now, as the cell is drawn up into the needle it is compressed and distorted in such a way as to break the cell surface without dispersing the cell contents. The needle is then inserted into the enucleated egg and the broken cell is injected, thus liberating the nucleus within the egg. The injection can be controlled by watching the meniscus

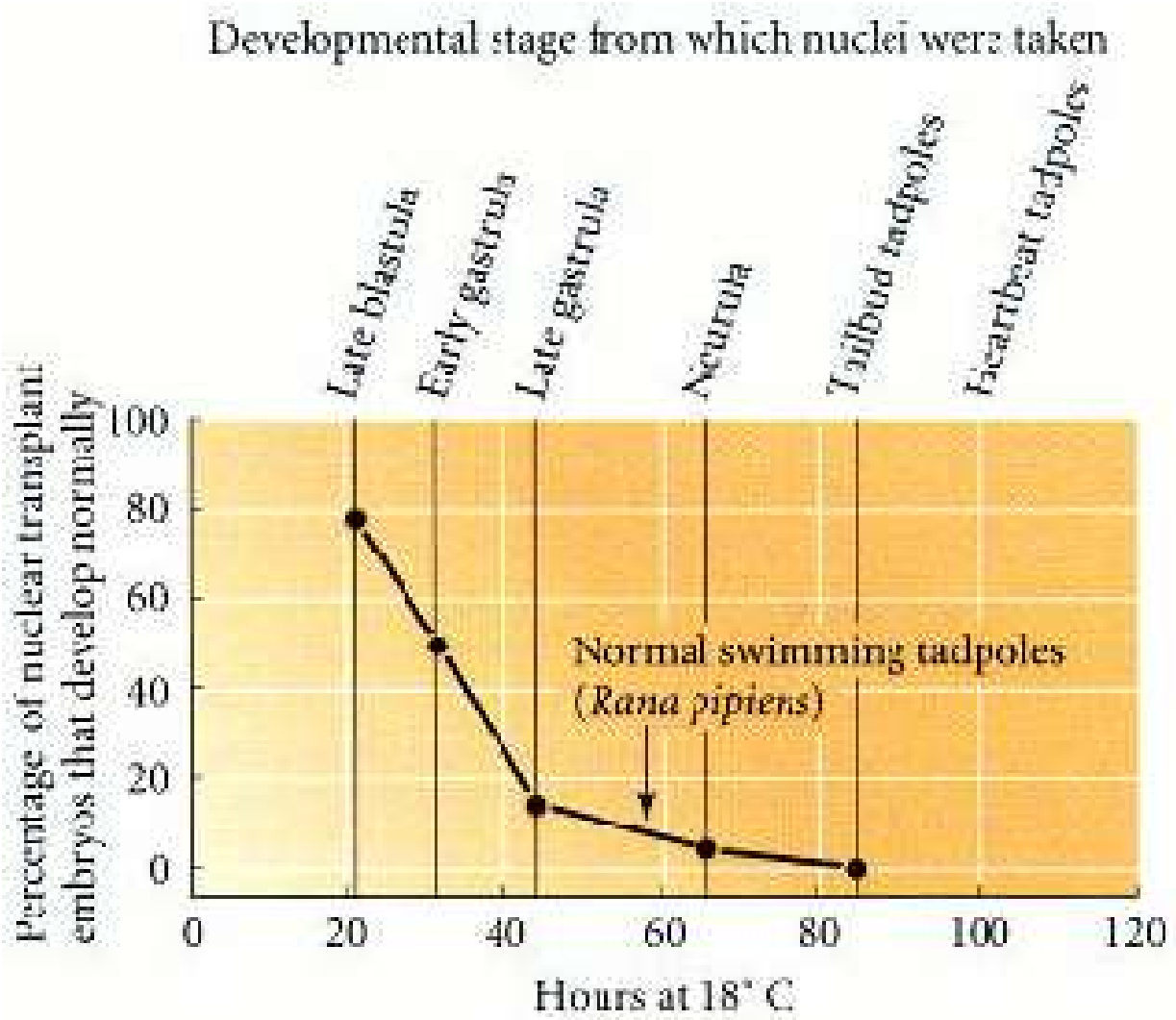
of the fluid column within the needle, things being arranged so that the broken cell is kept near the tip of the needle while the meniscus of the column is higher up but still within the field of the microscope. Following the injection the needle is slowly withdrawn. Usually as it is withdrawn it pulls the surface coat up against the vitelline membrane so that a small canal is formed through which the egg substance may subsequently leak. This can be prevented by cutting the connection between the egg surface and the vitelline membrane with glass needles. The egg is then removed from the operating dish and placed in a small Stender dish in spring water.



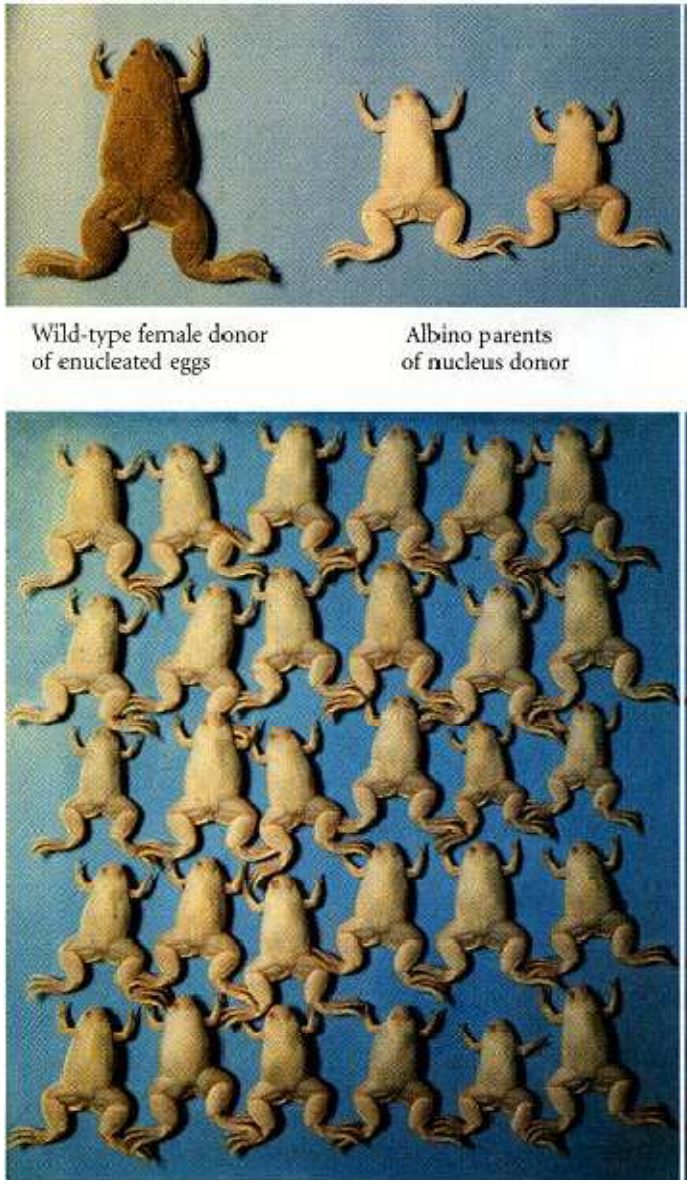
Procedure for transplanting blastula nuclei into activated enucleated *Rana pipiens* eggs. Vitelline envelope is the extracellular matrix surrounding the egg.



Most somatic cells appeared to lose their ability to direct development as they became determined and differentiated.



- Robert Briggs envisioned the cloning experiment as a way to study the activation and deactivation of genes during cell development.
- He intended to transfer the nucleus of a blastula cell, which is an embryo cell during the period in which a young embryo consists of only around eight to sixteen thousand cells, into a fertilized egg whose nucleus had been removed.
- Thomas King was found by Briggs to actually perform the experiment.
- Using a glass pipette wider than the cell's nucleus, but smaller than the width of the cell, King extracted the nucleus of a blastula cell. Because the outer part of the cell was wider than the pipette, it was crushed and broke away from the nucleus as the cell was sucked into the pipette, leaving only the cell's nucleus intact inside the pipette.
- Next, the nucleus of the fertilized egg was extracted using a glass needle. An incision was then cut into the egg's coating and the blastula cell nucleus was inserted into the egg.



A clone of *Xenopus laevis* frogs. The nuclei of all members of this clone came from a single individual (a female tail bud stage tadpole) whose parents were marked by albino genes.

The nuclei (containing these defective pigmentation genes) were transferred into enucleated eggs from a wild type female.

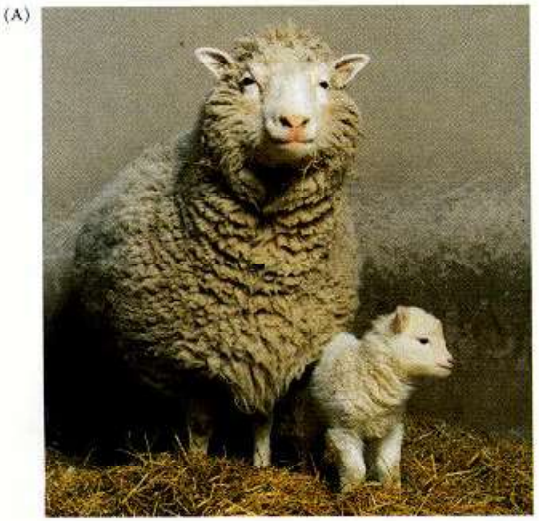
The resulting frogs were all females and albino.

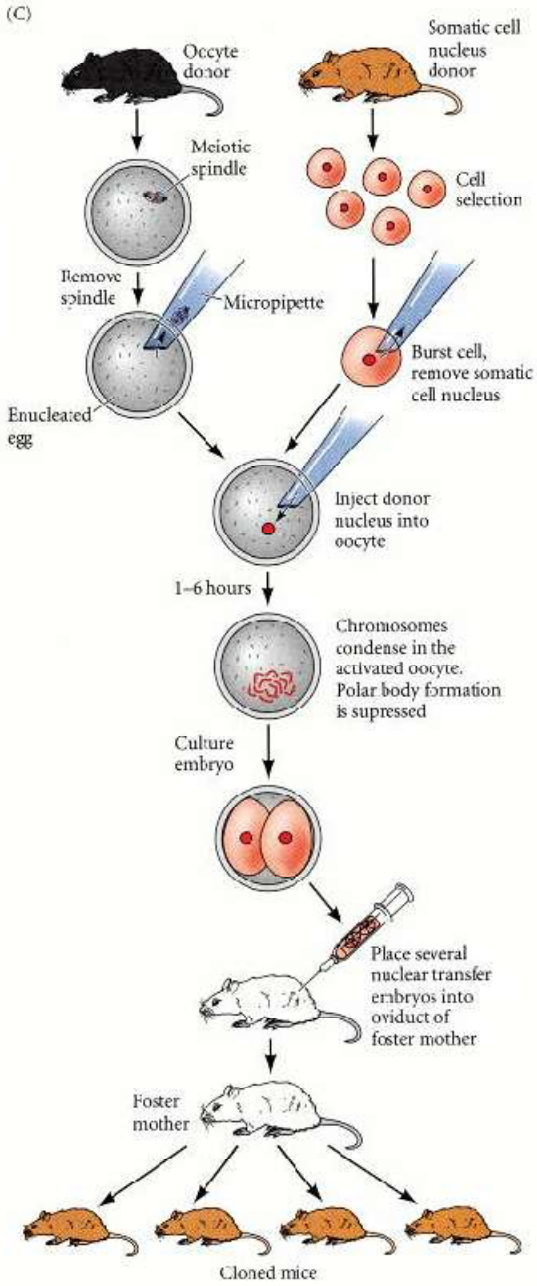
The experiments described here have established two general conclusions.

First, nuclear genes are not necessarily lost or permanently inactivated in the course of cell differentiation.

Second, major changes in chromosome function as well as in different kinds of gene activity can be experimentally induced by normal constituents of living cell cytoplasm.

Cloning mammals (Dolly and Bonnie)





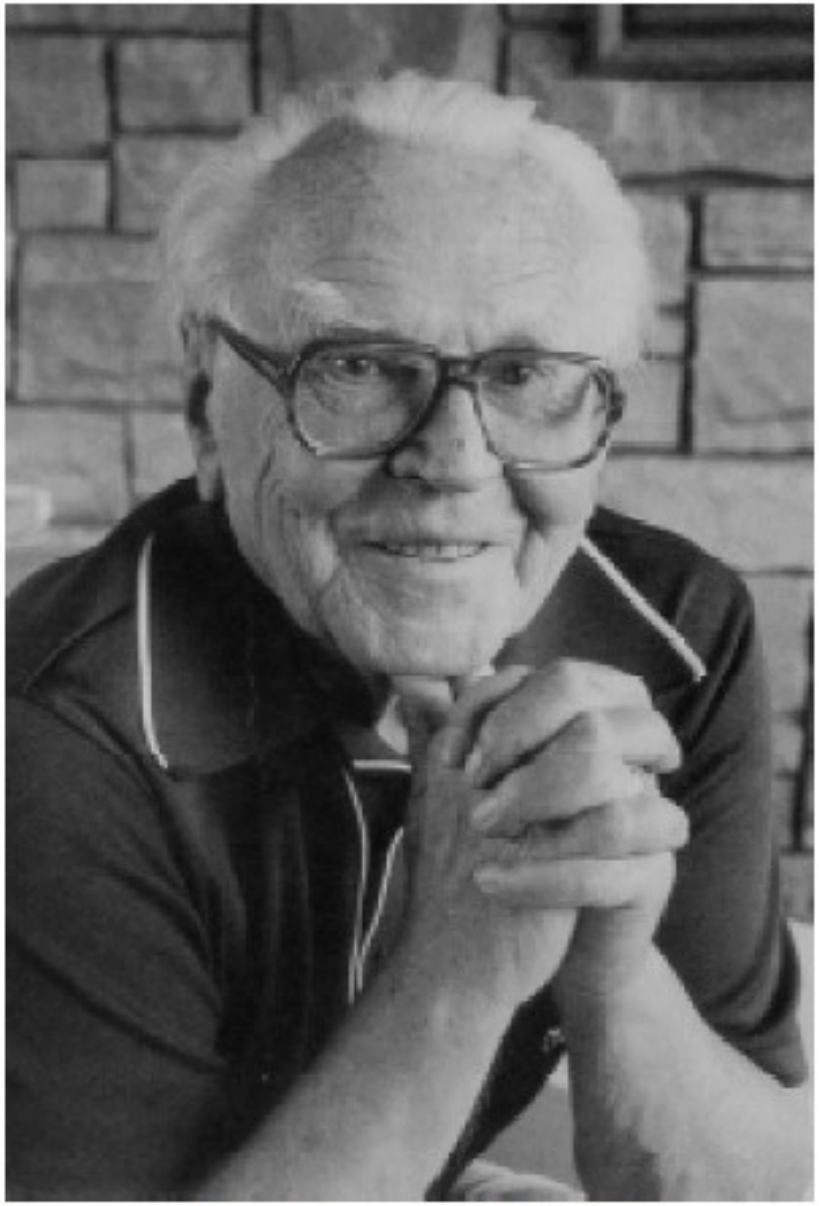
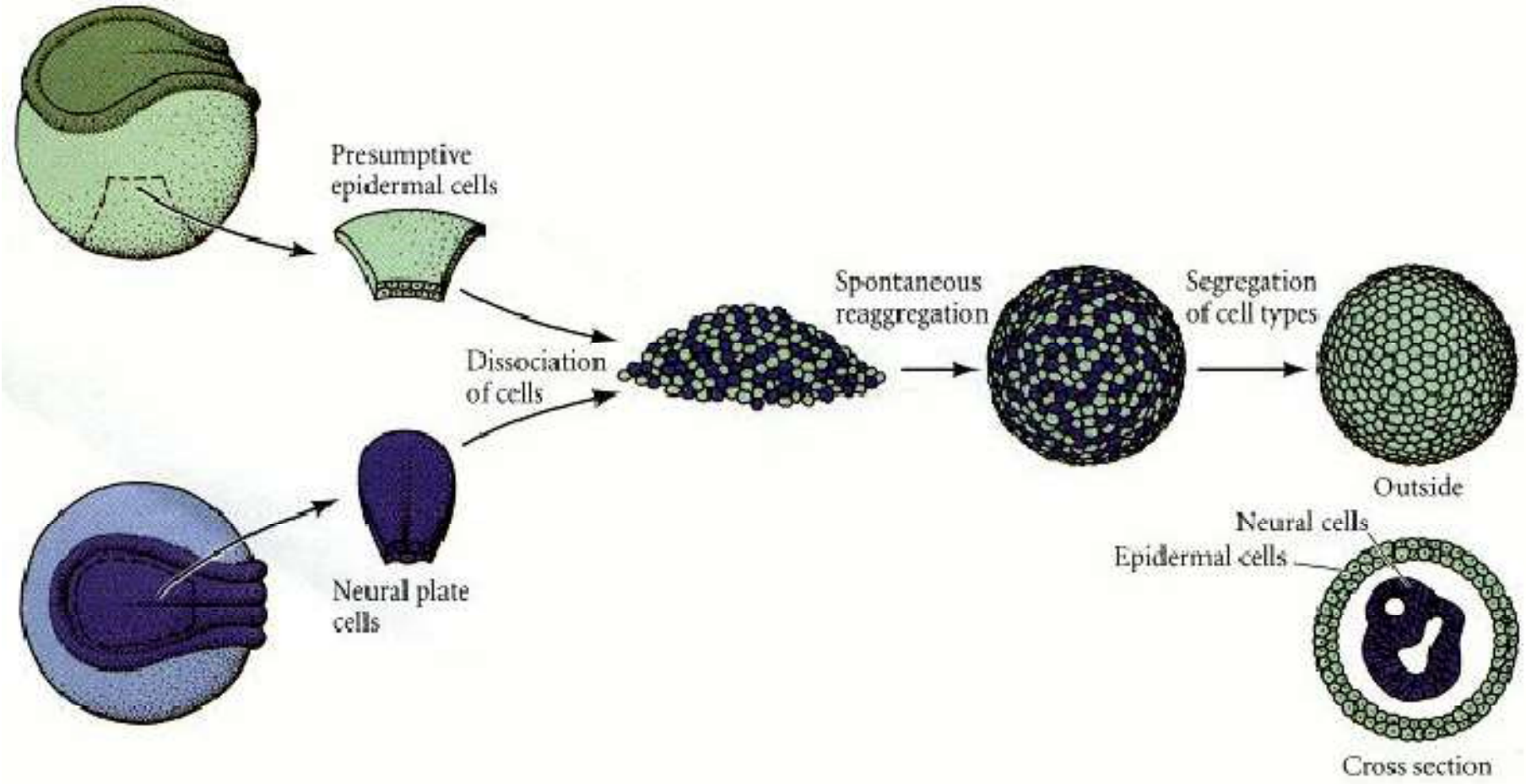
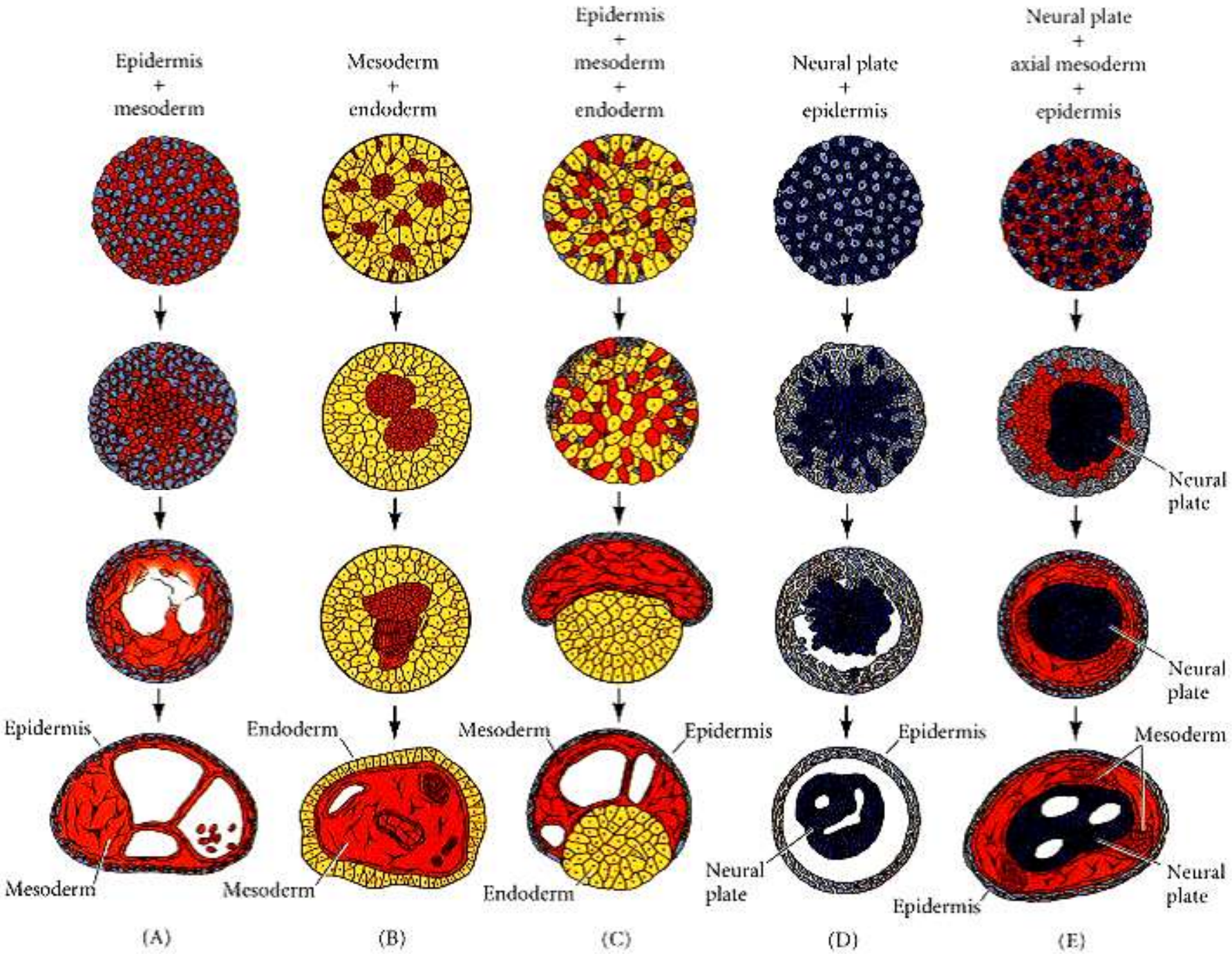


Fig. 7. Johannes F.C. Holtfreter (1901-1992). *Reproduced with permission from Academic Press.*

Directed movement and selective adhesion of embryonic Amphibian cells (Johannes Holtfreter)




Epidermis + mesoderm
Mesoderm + endoderm
Epidermis + mesoderm + endoderm
Neural plate + epidermis
Neural plate + axial mesoderm + epidermis
Neural plate
Neural plate
Epidermis
Mesoderm
Endoderm
Mesoderm
Mesoderm
Epidermis
Epidermis
Neural plate
Epidermis
Mesoderm
Neural plate
Endoderm
Neural plate
(A)
(B)
(C)
(D)
(E)

- Cells exhibit tissue specific tendencies of moving either centrifugally or centripetally with in a composite cell aggrement.
- Directed movements are followed by the phenomenon of cell-specificity of adhesion. The combined effect of these processes necessarily result in segregations and recombination of tissue primordia or individual cells.
- The homologous cells when they meet remain permanently united to form functional tissues when a cleft develops between certain non-homologous tissues.
- The principles of **cell-specific movements**, **selective adhesion** and **cavitation** are factors instrumental in the normal formation of germ layers and their further segregation