Gastrulation and Neurulation; the amphibian as a model system

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Rana (pipiens)

Axolotl (Ambystoma mexicanum)

Xenopus (laevis)

Triturus (alpestris)

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First some basic concepts of experimental embryology

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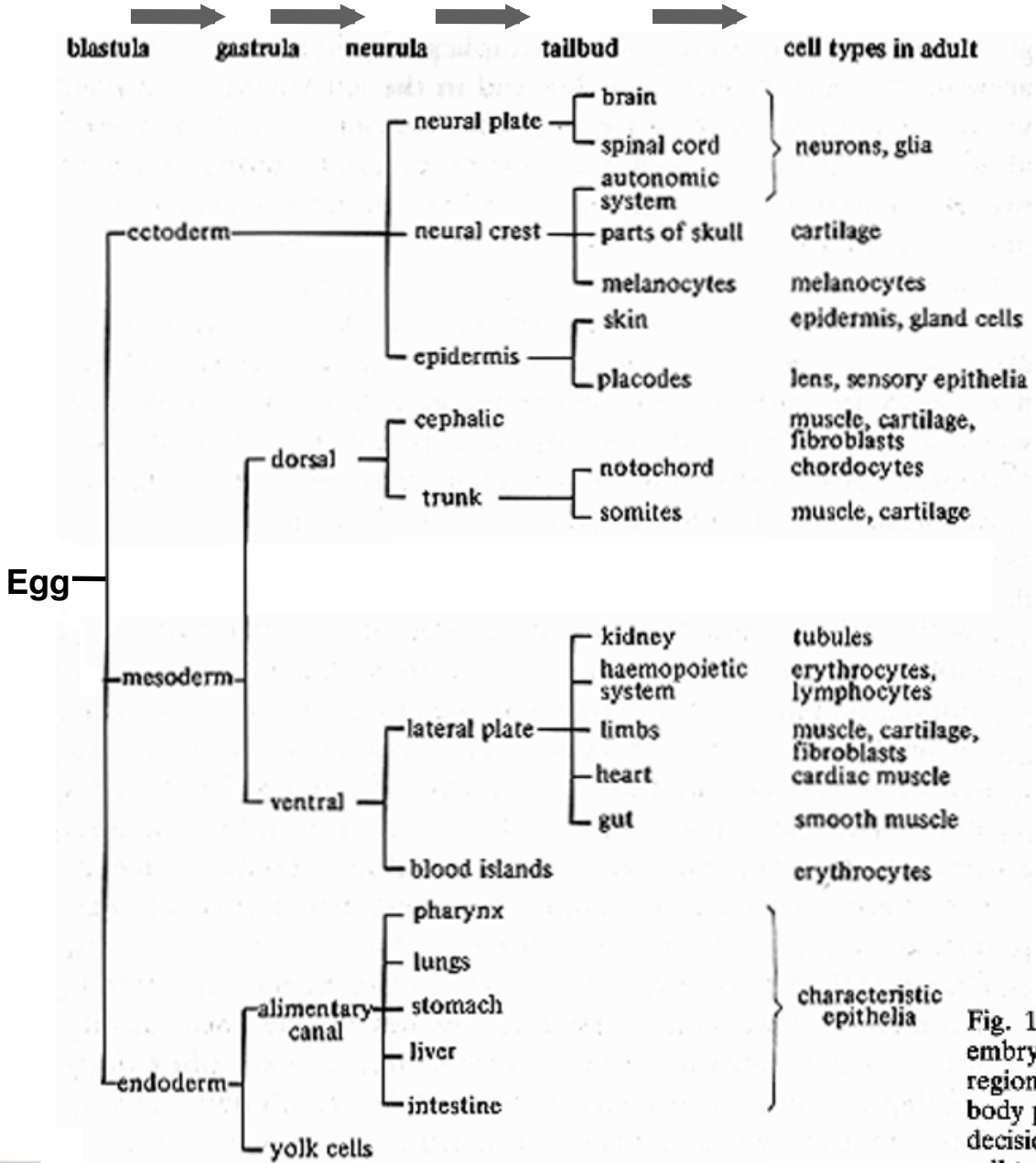
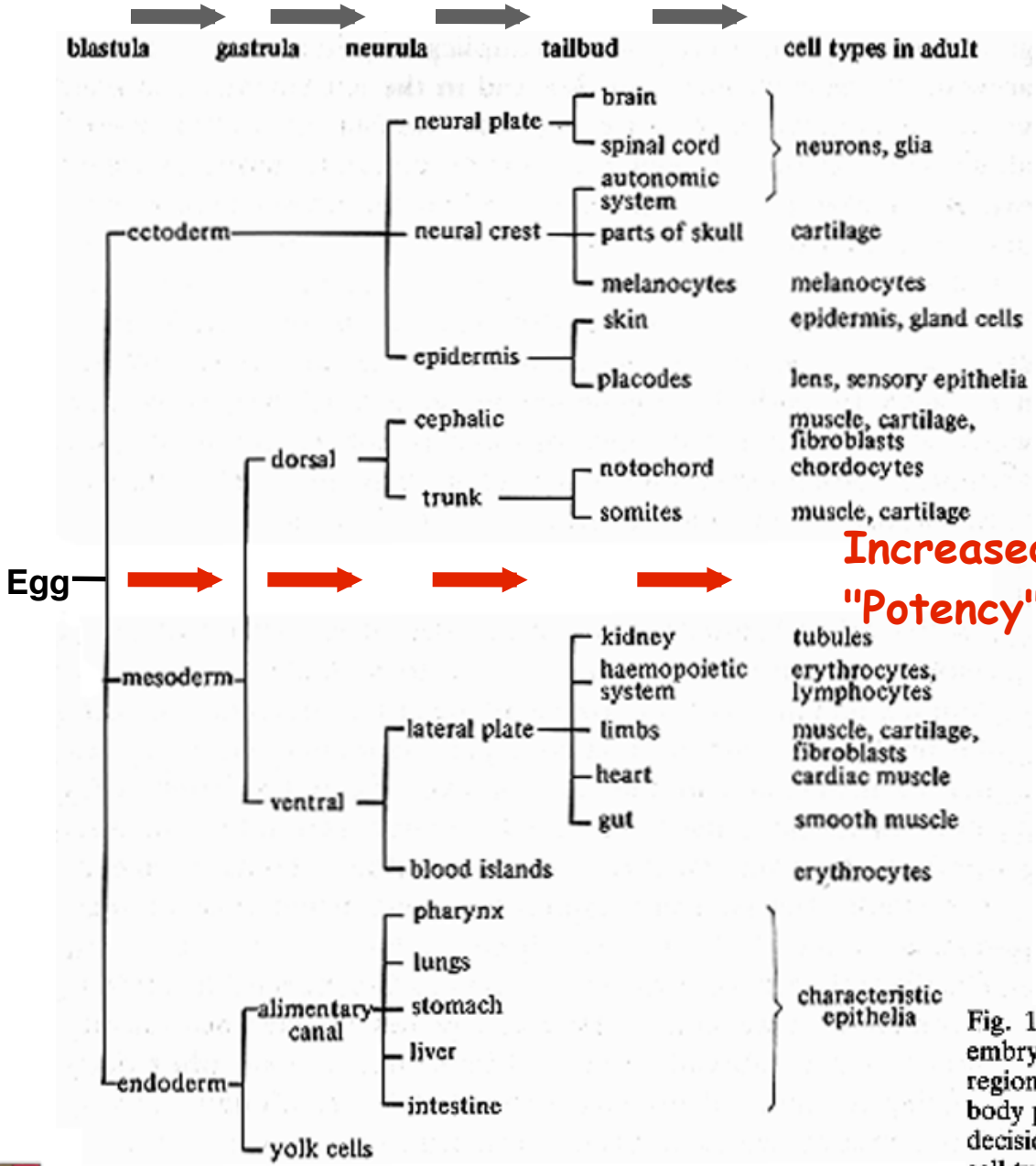


In Xenopus, by tailbud stage, commitment to the major organ types is complete.

Fig. 1.1. Formation of the basic body plan in a vertebrate (excluding extra-embryonic regions). By the early tailbud stage the embryo consists of a mosaic of regions determined to form the principal organs and structures of the body. This body plan is built up as a result of a hierarchy of decisions, and several further decisions will in most cases be taken before the cells differentiate into the terminal cell types shown on the right-hand side. It should be noted that some cell types, such as cartilage, arise from more than one lineage.



In Xenopus, by tailbud stage, commitment to the major organ types is complete.

Increased "Determination"-/-Reduction in "Potency"

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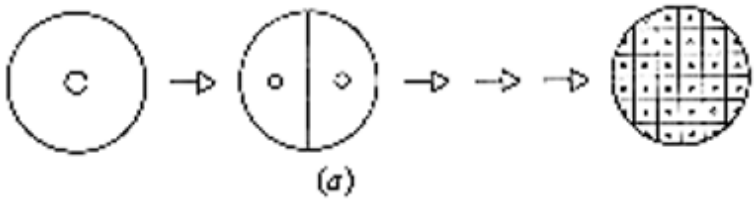
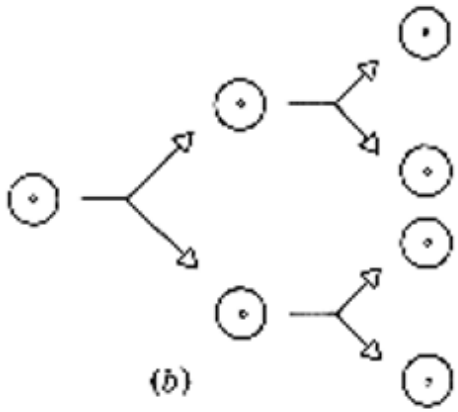
Types of early cell division

Increase in cell number without growth

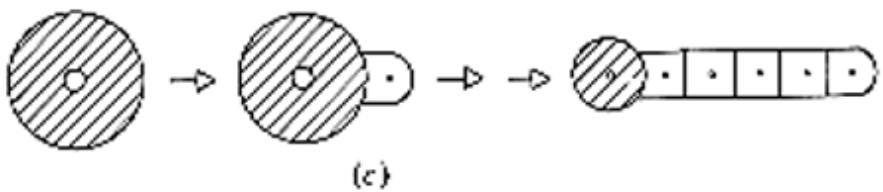


Increase in cell number with growth



Stem cell division producing dissimilar daughter cells:-

Without growth



With growth

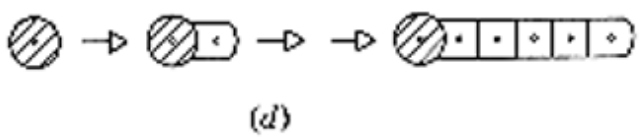


Fig. 2.3. Types of cell division found in embryos. (*a*) Cleavage divisions unaccompanied by growth. (*b*) Exponential proliferation supported by an external nutrient supply such as a yolk mass or a placenta. (*c*) and (*d*) Unequal divisions of a stem cell produce a string of progeny which are not themselves stem cells. In (*c*) there is no growth and in (*d*) there is growth.

The Fate Map:-

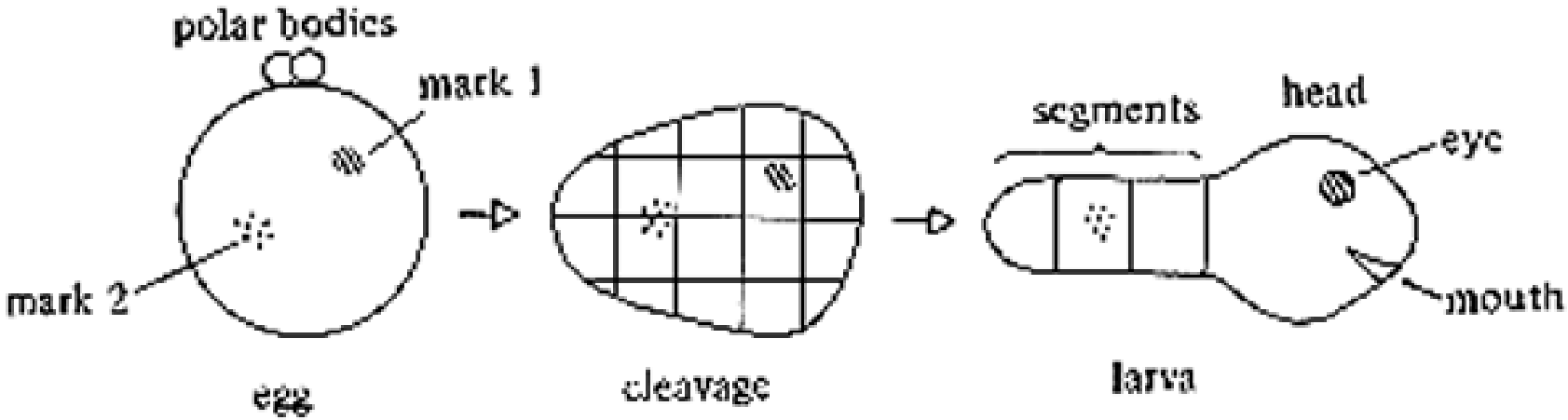
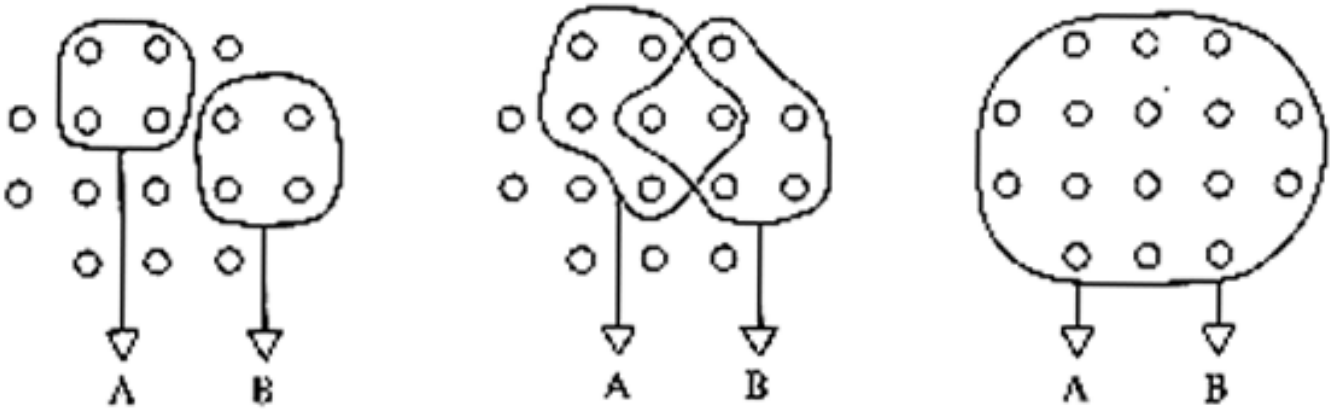


Fig. 2.1. The principle of fate mapping. In the absence of random cell mixing, marks placed on the egg will label particular regions of the larva, in this case the eye and the second abdominal segment.

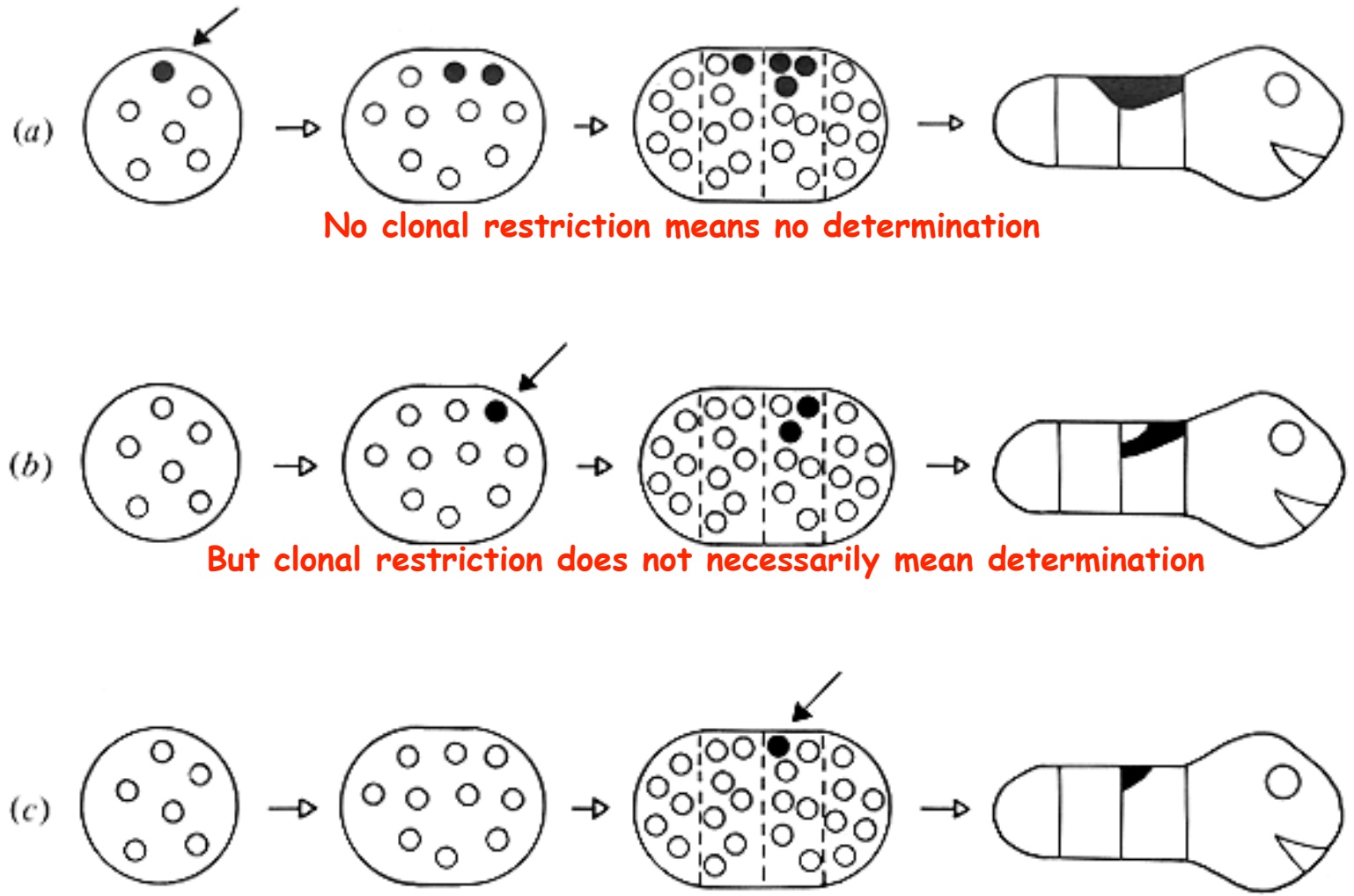
Mixing during development means that some cells have multiple, i.e. less precisely defined, fates



E.g. mosaic development (a) (b) **No fate map definable** (c)

Fig. 2.2. Dependence of the 'primordial cell number' on the amount of mixing which occurs after marking. (*a*) If there is no mixing at all then the prospective regions are the same size as the later structures A and B. (*b*) If there is some mixing then the prospective regions are larger than the later structures because they include all cells which have some probability of contributing to them. (*c*) If there is complete randomisation of position then any cell can contribute to any structure.

Clonal Analysis



Cell Labelled:-

Before determination

No clonal restriction means no determination

Before determination

But clonal restriction does not necessarily mean determination

After determination

Fig. 2.4. No clonal restriction means no determination but the converse is not true. In (*a*) a cell is labelled in the early embryo and its progeny span the boundary between two segments in the adult. In (*b*) a cell is labelled before determination but because of its position its progeny do not span the segment boundary. In (*c*) a cell is labelled after determination and its progeny are also confined to one segment.

1. Mosaic Development; Isolated cells do what they would do in normal development. Each cell forms a specific part of organism. Hence, all cells are necessary for a complete organism.

2. Regulative Development; Isolated cells do not follow the course they take in normal development. Cells may be removed without affecting development, though size may be affected.

Vertebrate development is a combination of early regulative phases and later mosaic phases

Determinant distribution
unchanged,
normal development results

Determinant distribution
changed,
abnormal development results

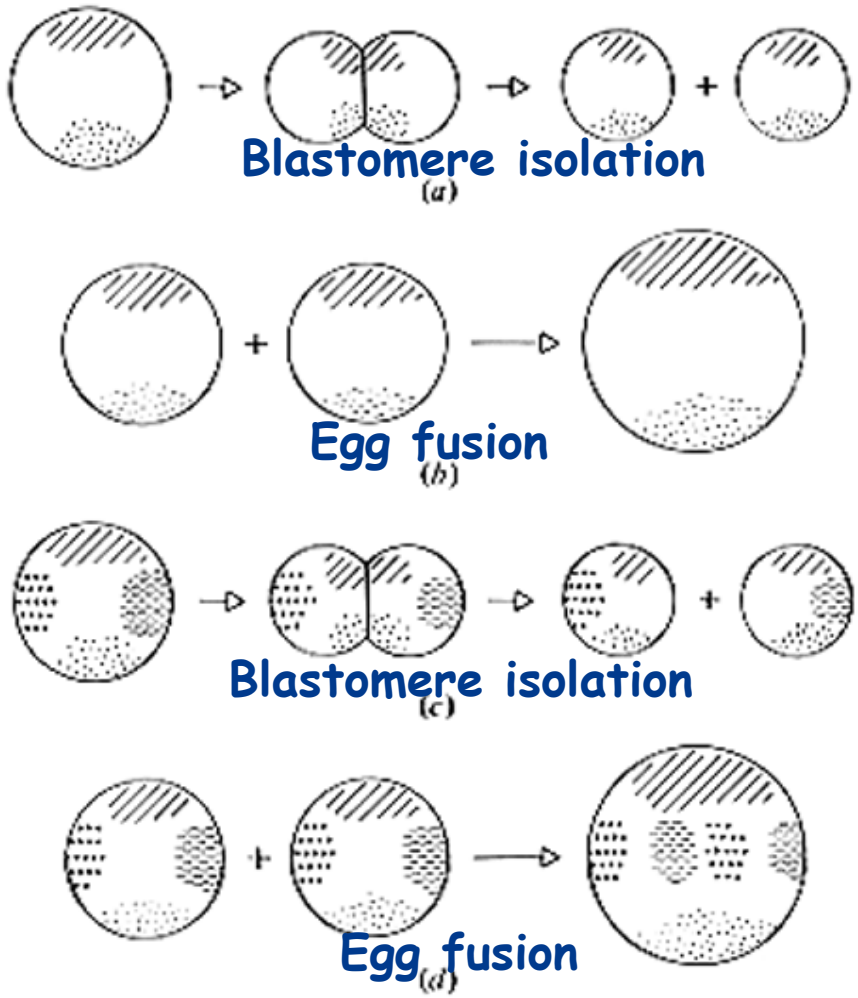


Fig. 2.5. Embryonic regulation and cytoplasmic determinants. (*a*) and(*c*) show blastomere isolation experiments and (*b*) and(*d*) show the fusion of two eggs to form a giant embryo. In either case individuals with a normally proportioned anatomy can only arise if the determinants are symmetrically disposed along the plane of separation or joining. In (*a*) and (*b*) this is the case and in (*c*) and (*d*) it is not.

An "Instructive" signal

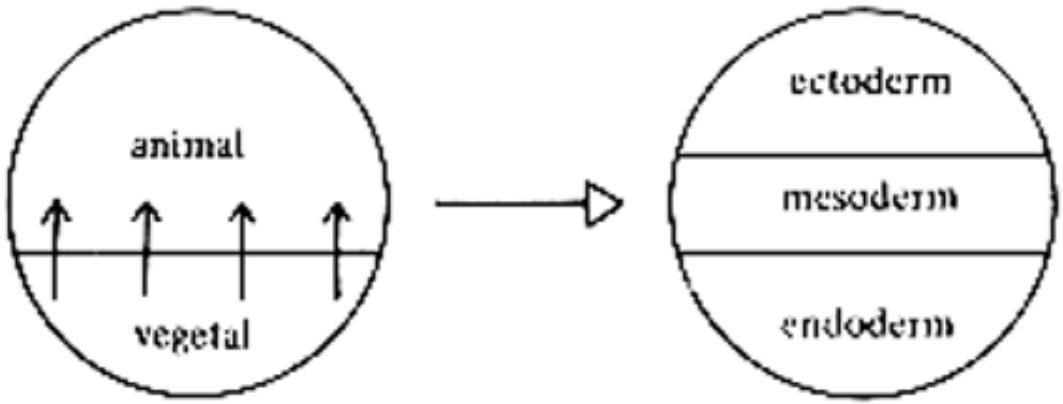


Fig. 2.6. An example of an instructive signal is provided by the induction of the mesoderm from the animal cap in response to a signal from the vegetal region in the early amphibian embryo.

1. Regional specification (of the embryo) comes first, cell differentiation and movement are consequences

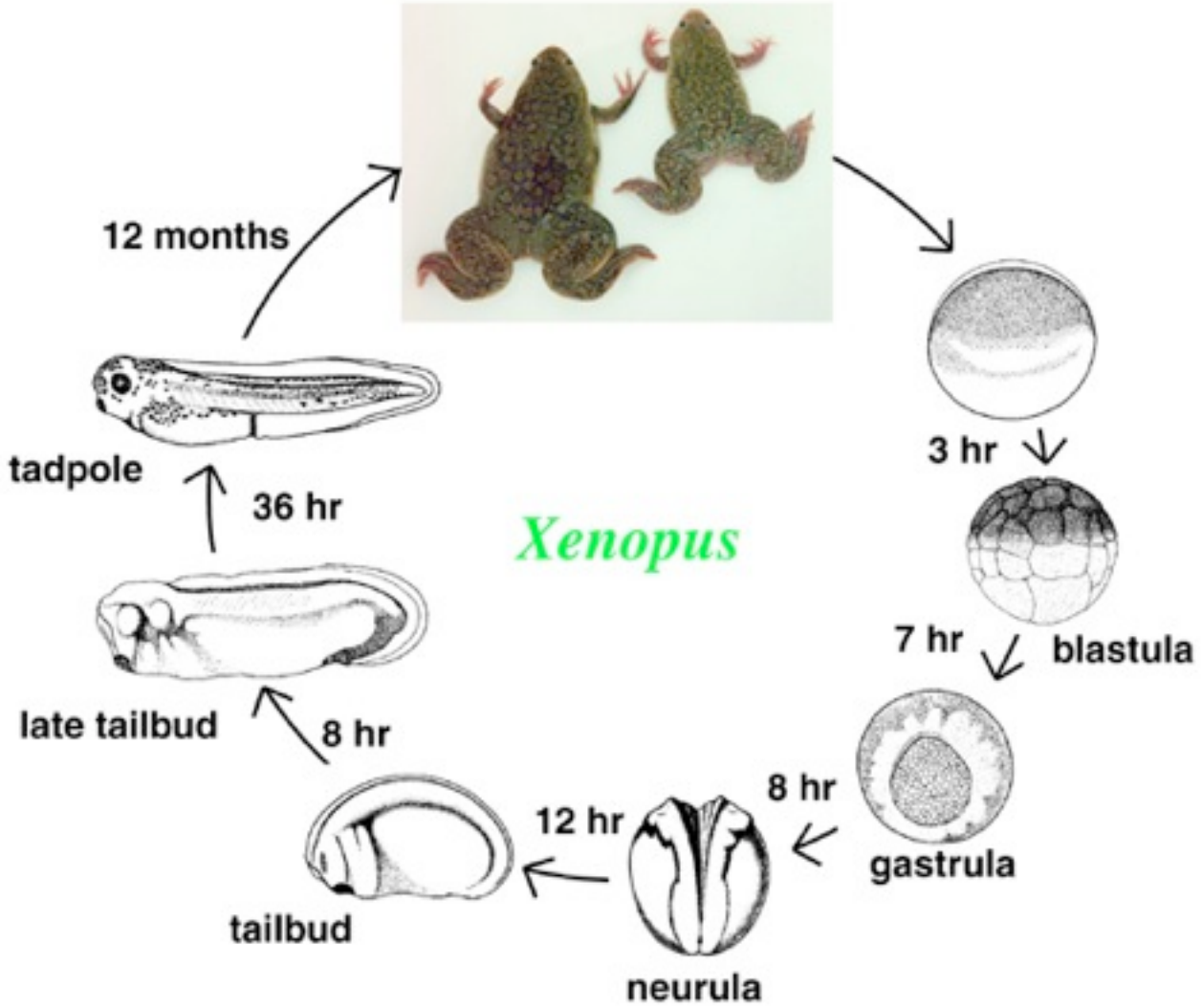
2. Regional specification can always be broken down into two independent processes: an "instructive process" during which positional information is imparted, and an initial response by the competent tissue called "interpretation".

3. The biochemical mechanism underlying positional information (inductive signal) is similar in all animals. The mechanisms of interpretation differs according to the particular anatomy being formed.

4. Cells which end up with the same histological type, but which are of different embryological provenance, are at least transiently 'non-equivalent", i.e. exist in different states of determination.

Slack 1983, after Wolpert 1969/1971

Advantages of studies in amphibia



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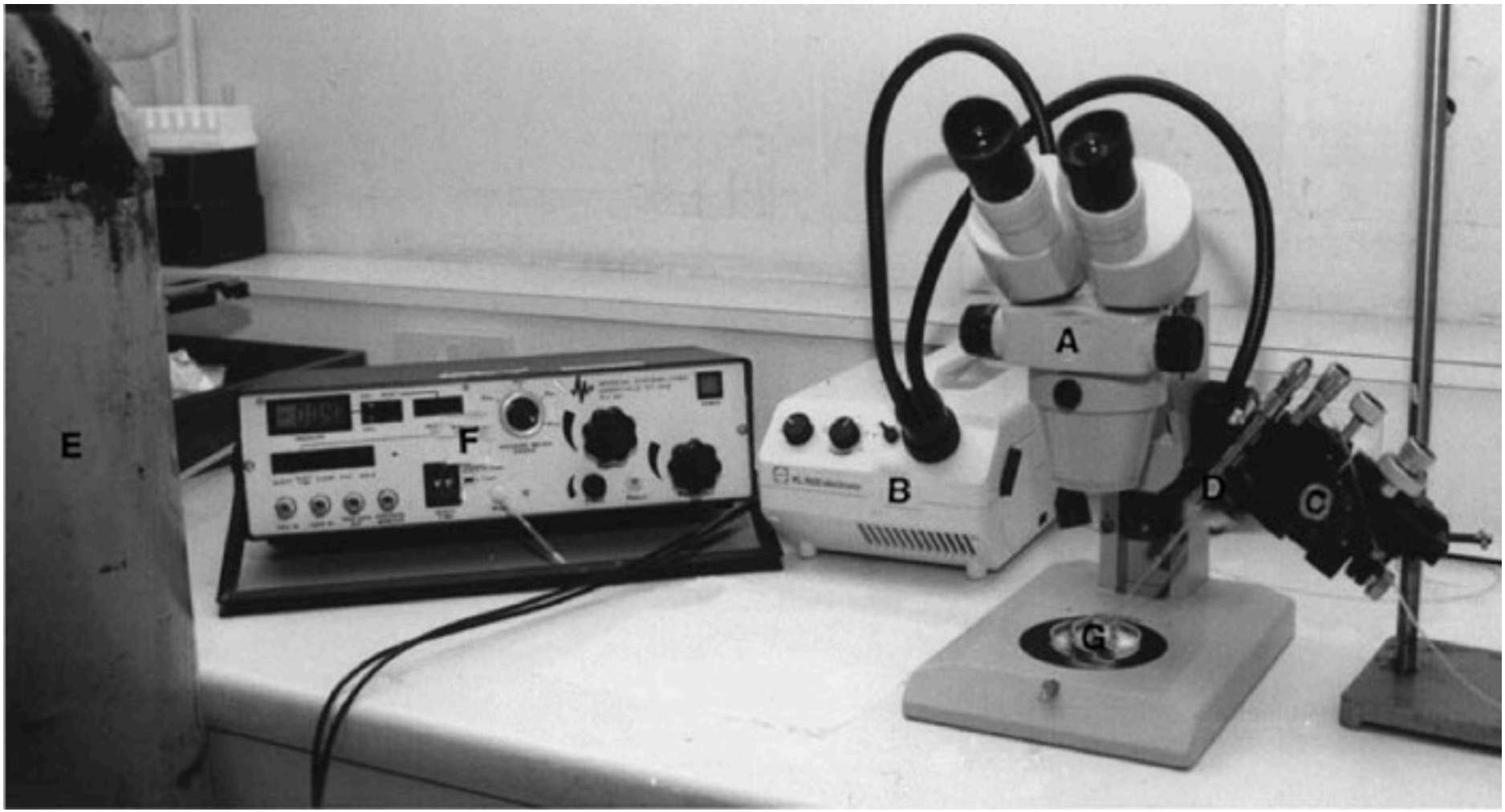

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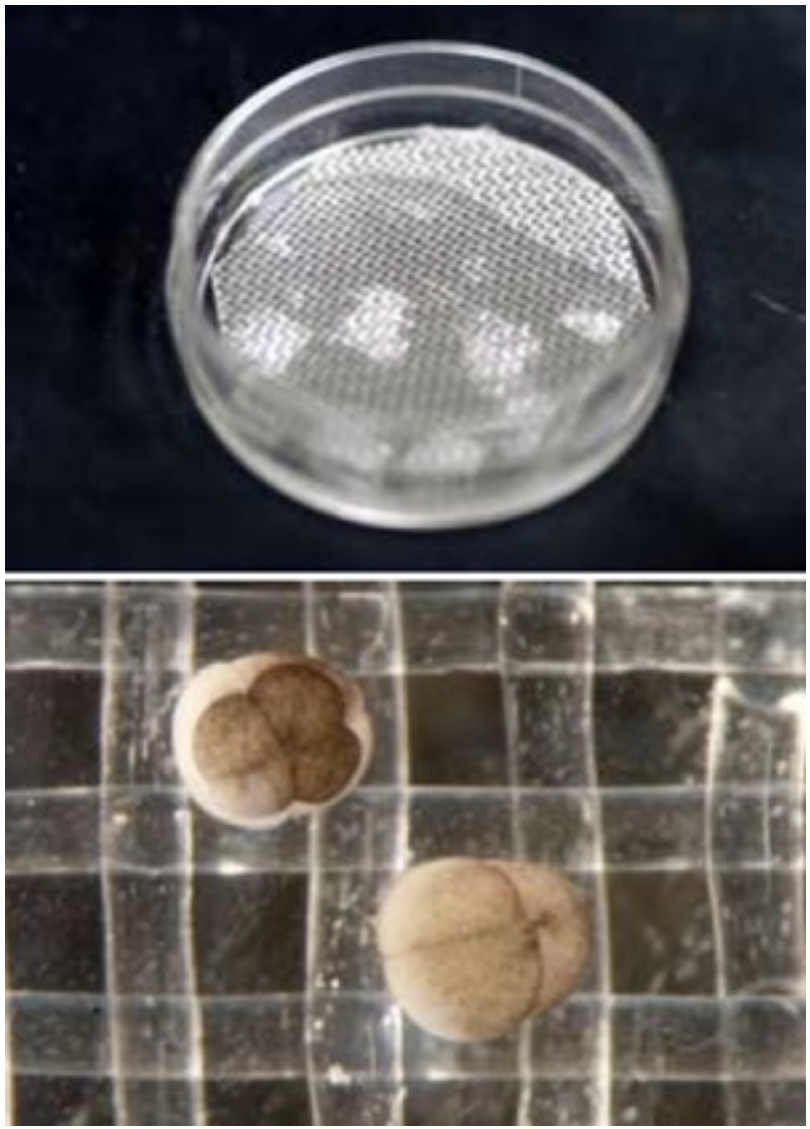

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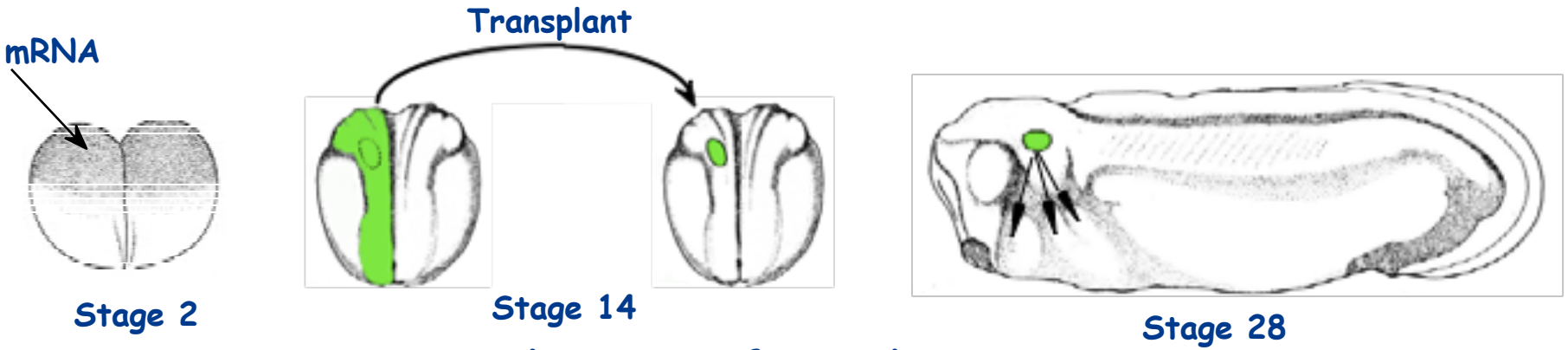
Ease of tissue transplantation



Transplantation of neural crest

Migration of transplanted neural crest

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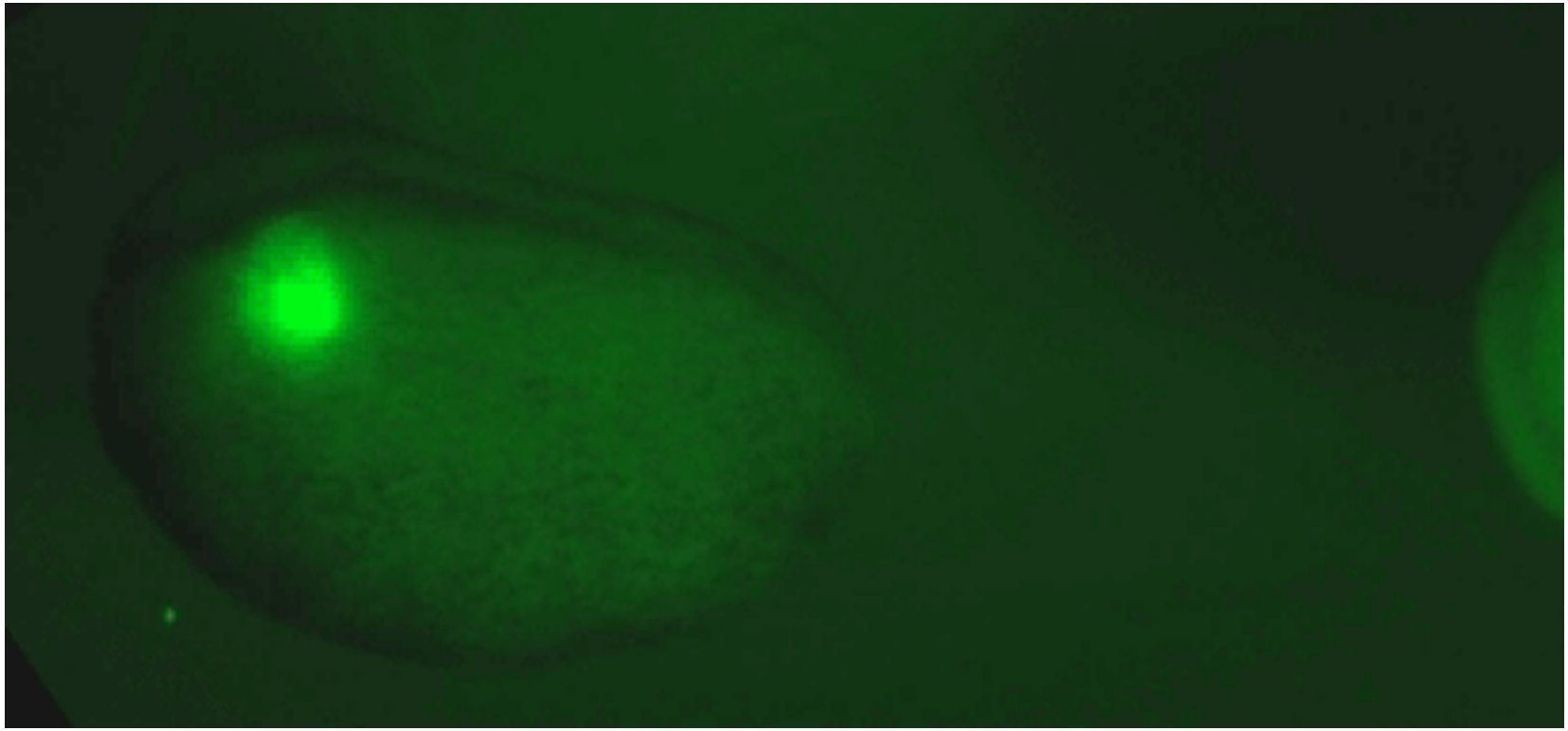

Migration of transplanted neural crest

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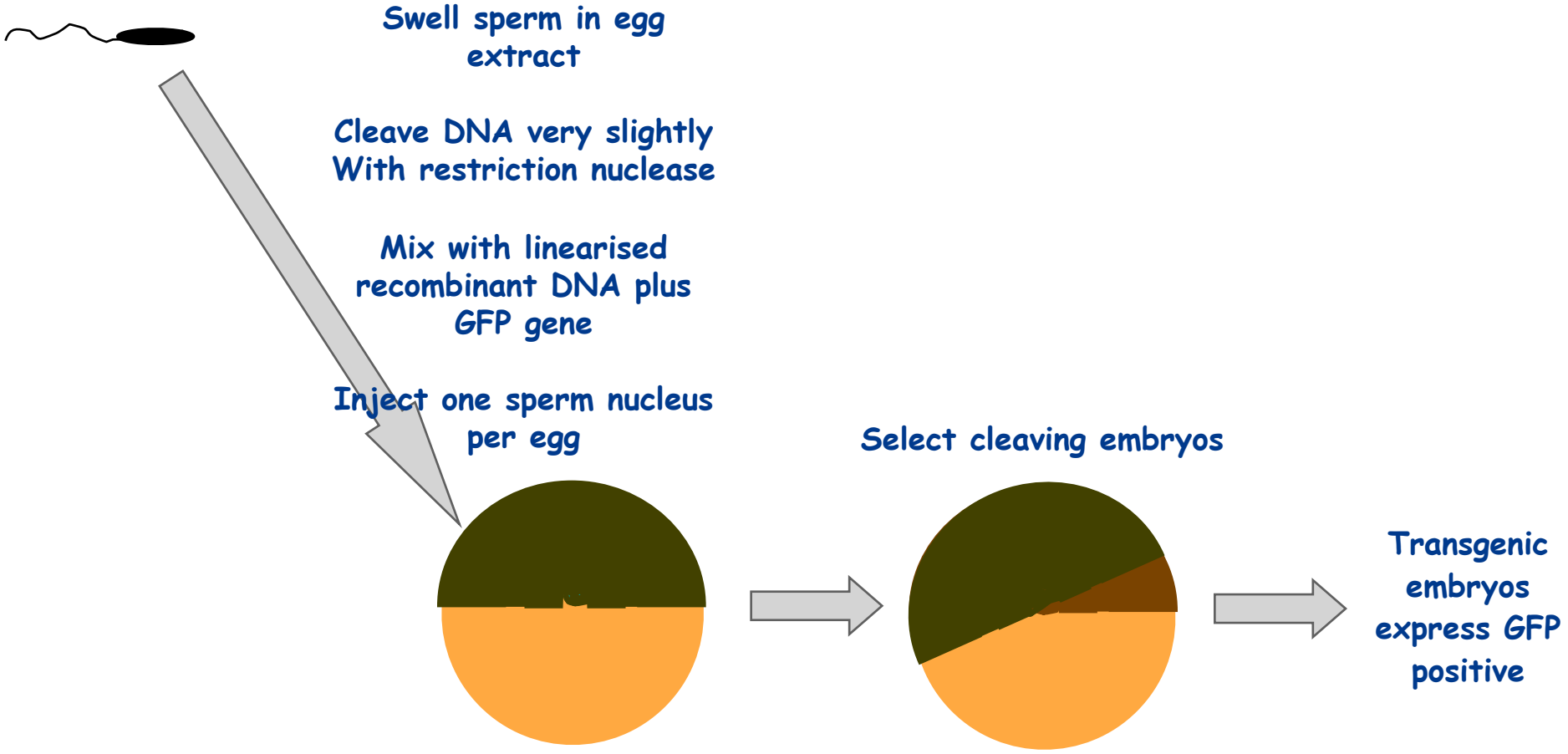

Typically 10% or more of injected eggs give transgenic tadpoles

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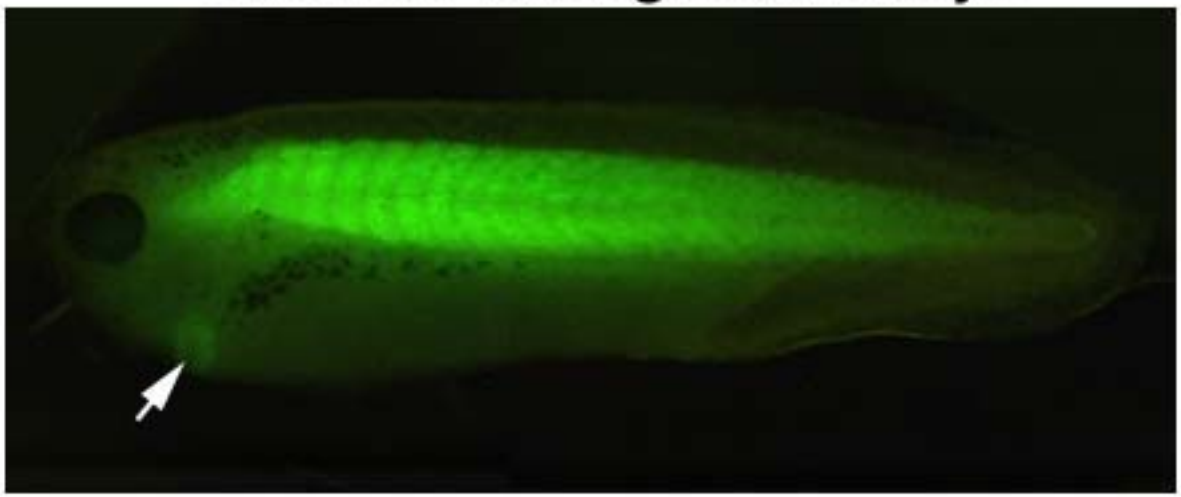
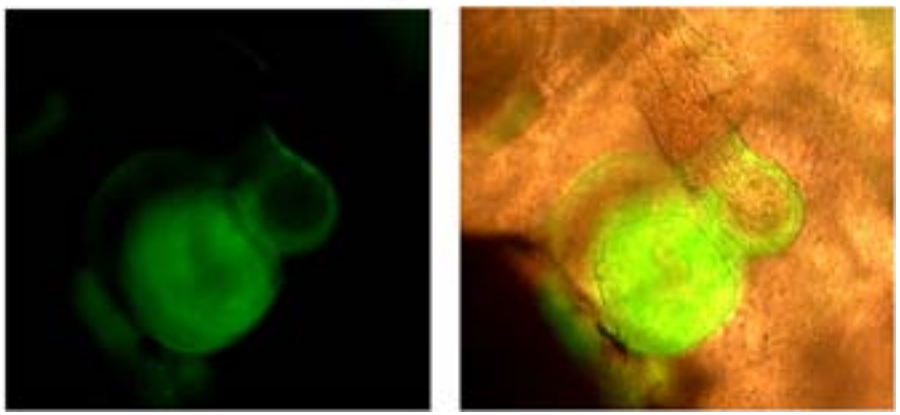
CarGFP3 Transgenic Embryo



Heart Expressing GFP



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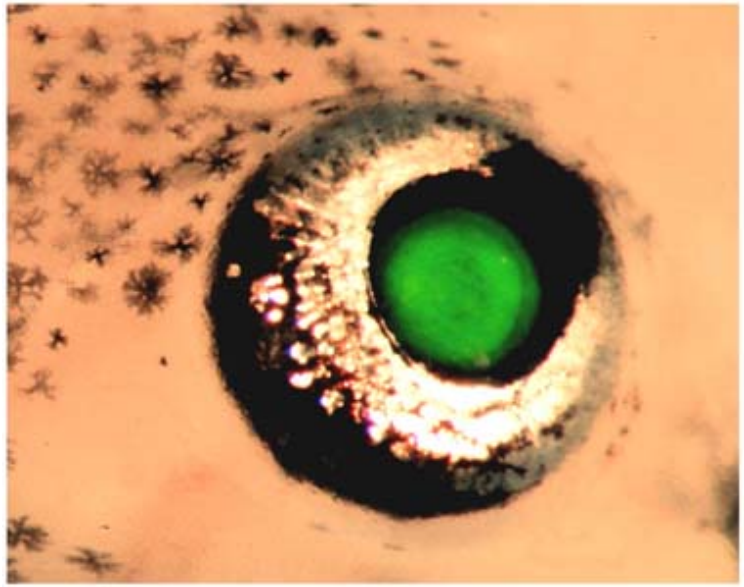
Gamma-crystallin promoter-GFP transgenic embryos



Brief comparison of embryological systems; mammals

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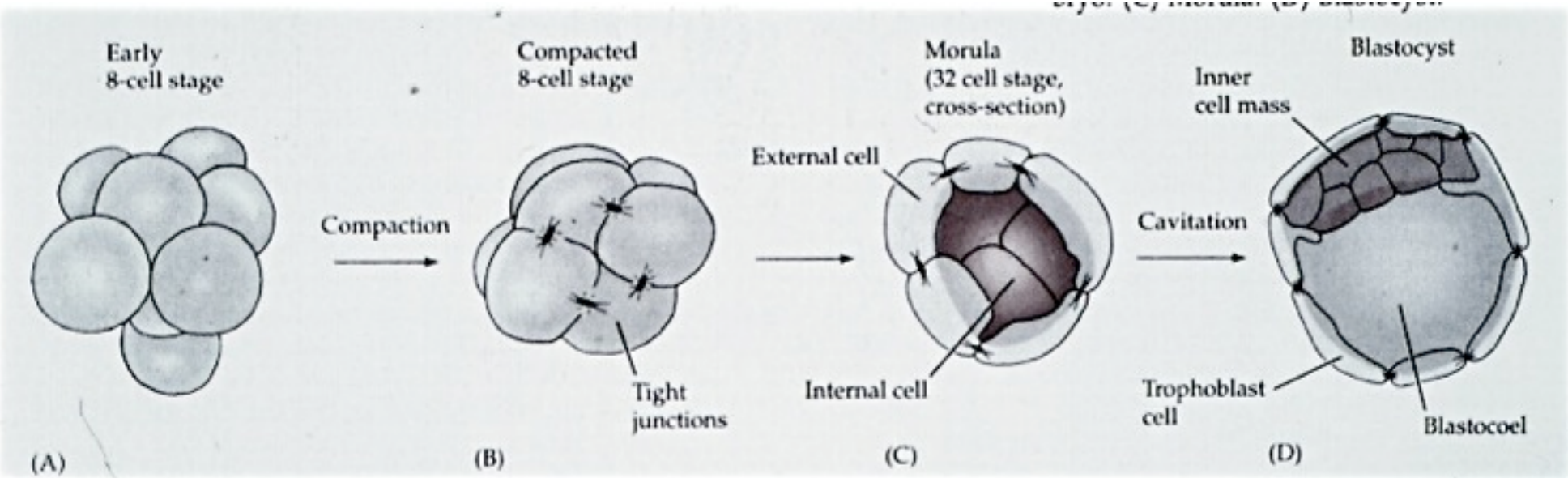

Mammalian cleavage divisions

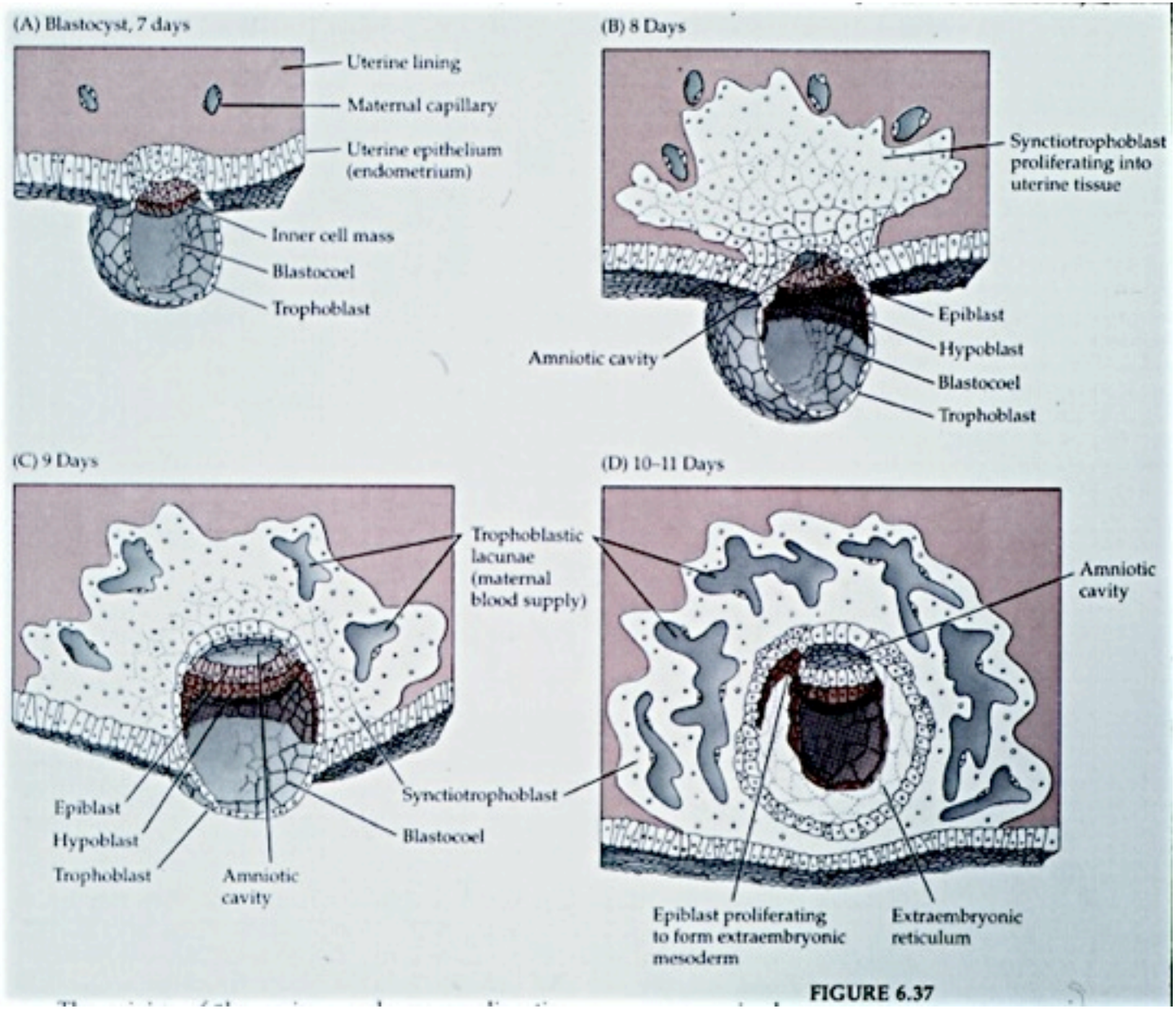

FIGURE 6.37

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Human blastula and implantation

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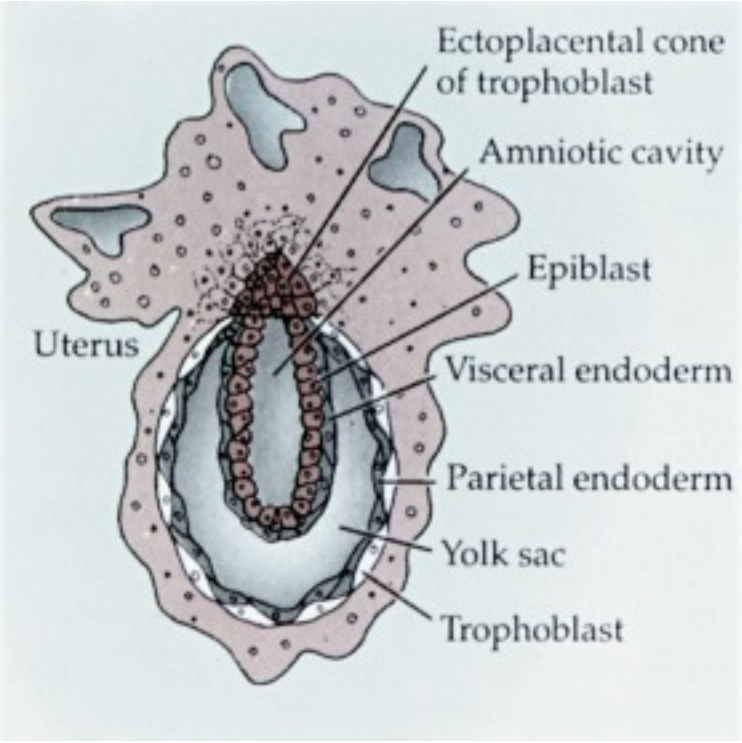

Mouse

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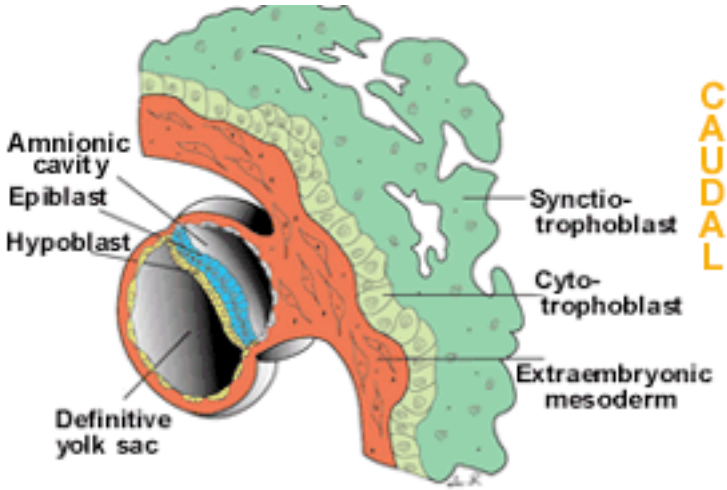
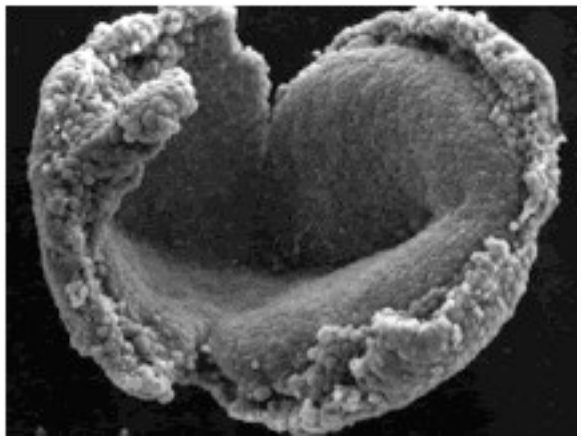
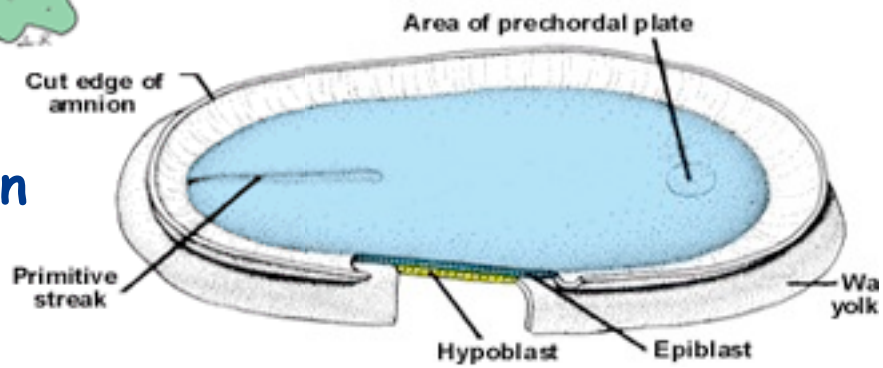




mouse

Human



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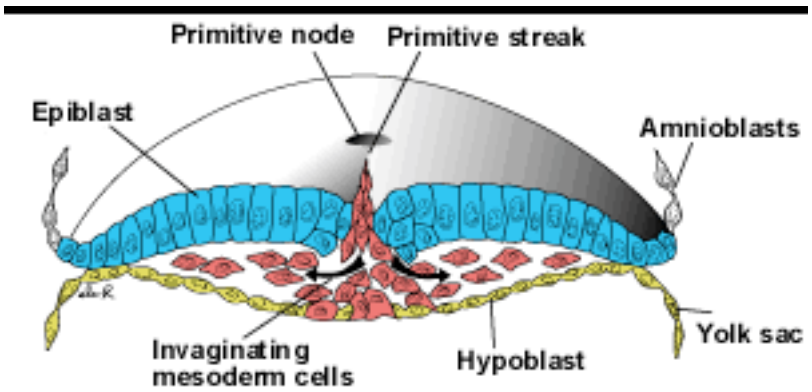
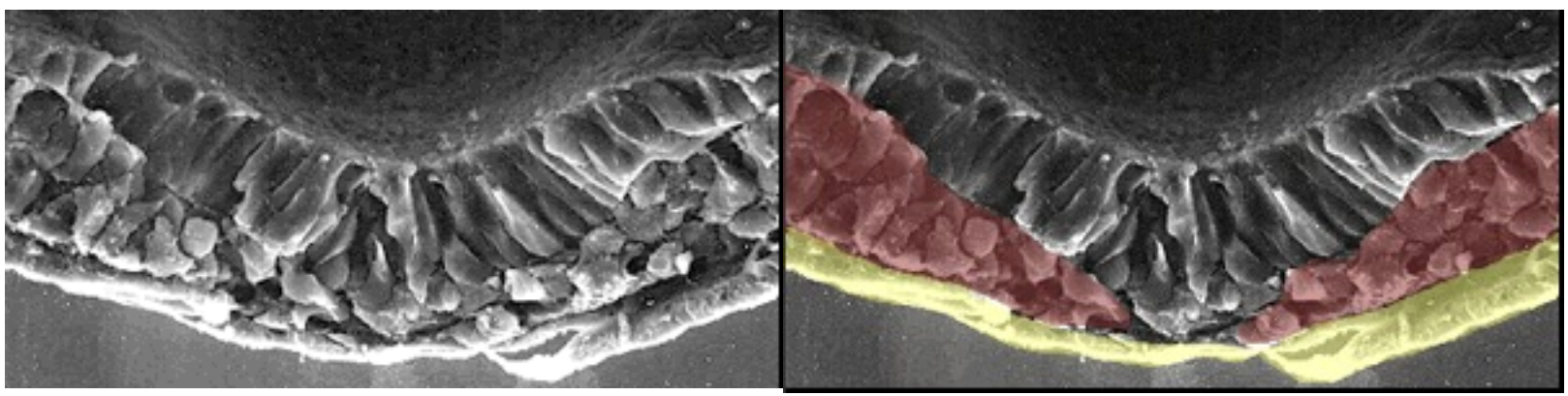
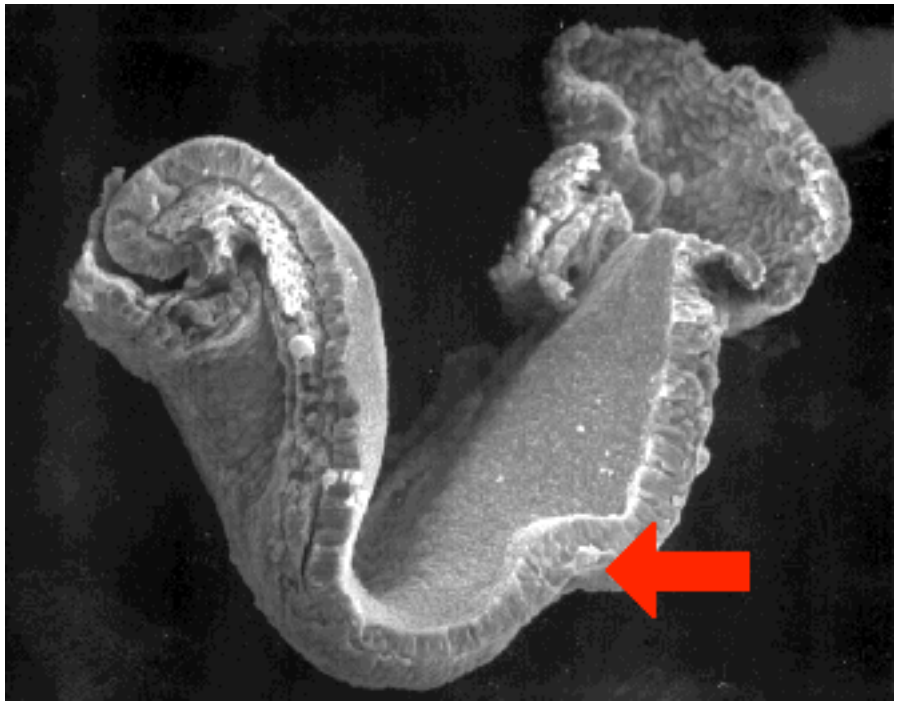
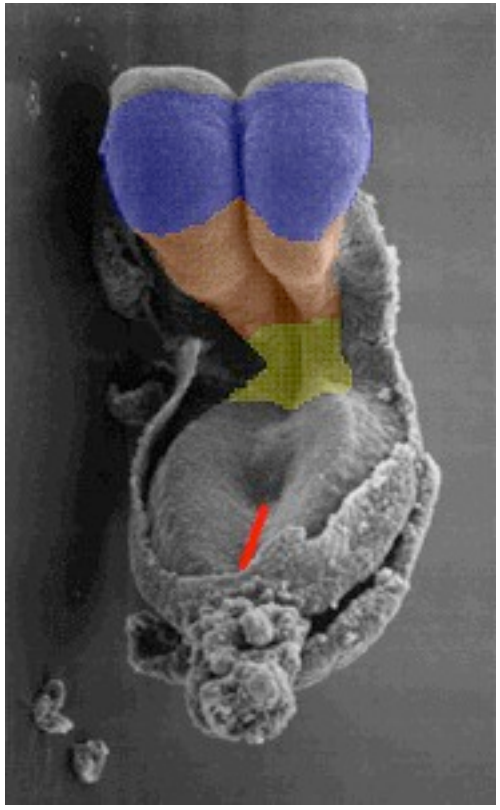

Primitive node
Primitive streak
Epiblast
Amnioblasts
Yolk sac
Invaginating
mesoderm cells
Hypoblast







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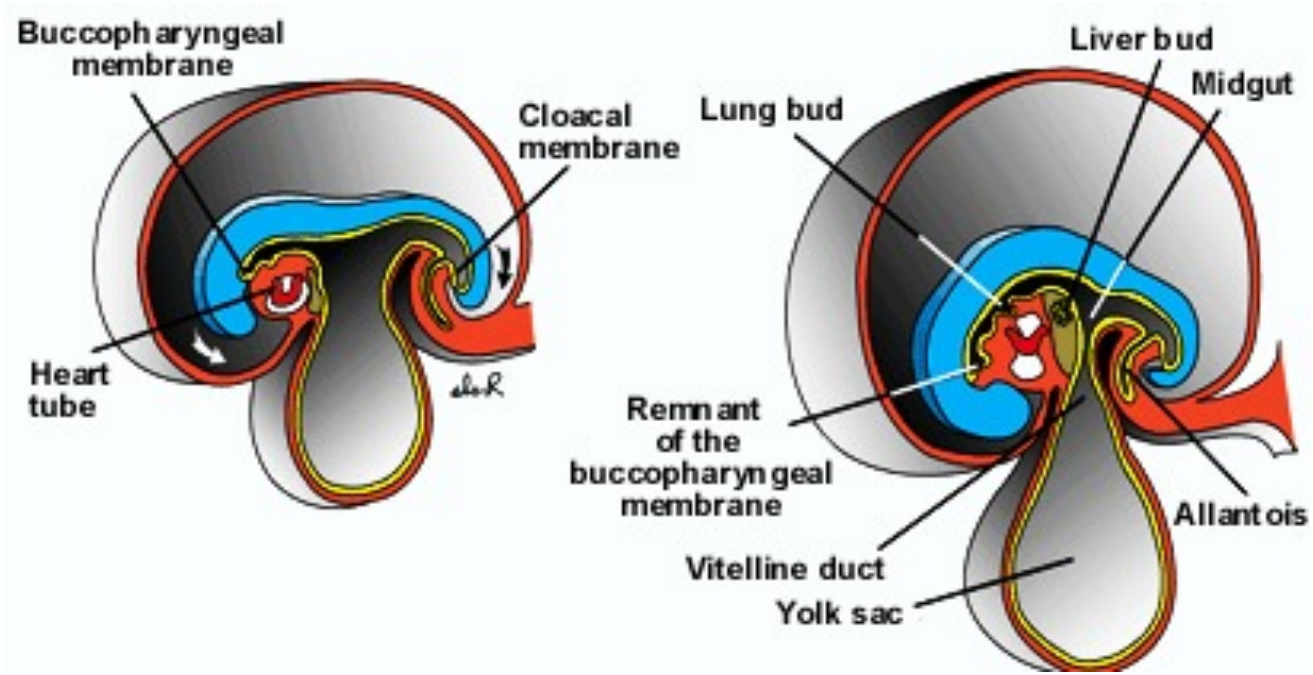

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Brief comparison of embryological systems;
Birds

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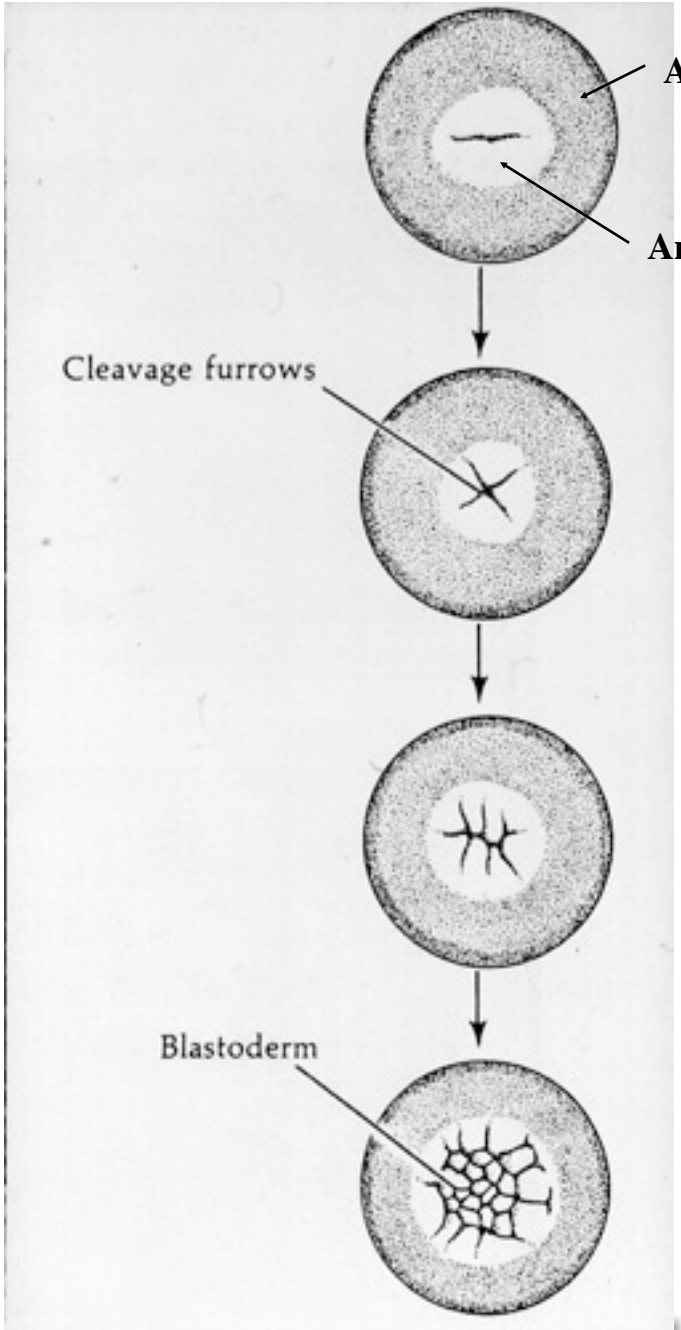

Cleavage divisions in chicken occur within a monolayer

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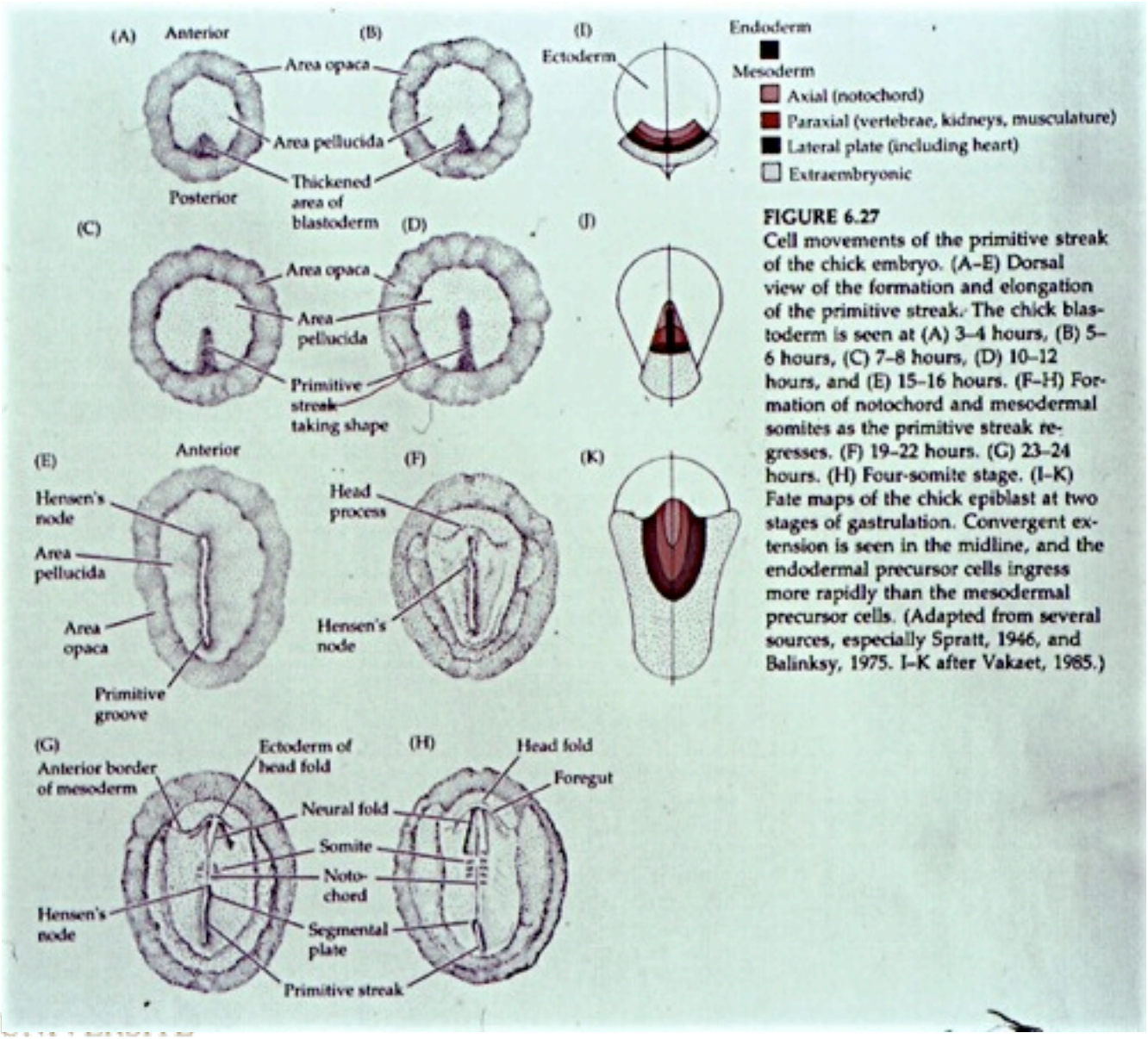

FIGURE 6.27
Cell movements of the primitive streak of the chick embryo. (A–E) Dorsal view of the formation and elongation of the primitive streak. The chick blastoderm is seen at (A) 3–4 hours, (B) 5–6 hours, (C) 7–8 hours, (D) 10–12 hours, and (E) 15–16 hours. (F–H) Formation of notochord and mesodermal somites as the primitive streak regresses. (F) 19–22 hours. (G) 23–24 hours. (H) Four-somite stage. (I–K) Fate maps of the chick epiblast at two stages of gastrulation. Convergent extension is seen in the midline, and the endodermal precursor cells ingress more rapidly than the mesodermal precursor cells. (Adapted from several sources, especially Spratt, 1946, and Balinksy, 1975. I–K after Vakaet, 1985.)

Chicken gastrulation

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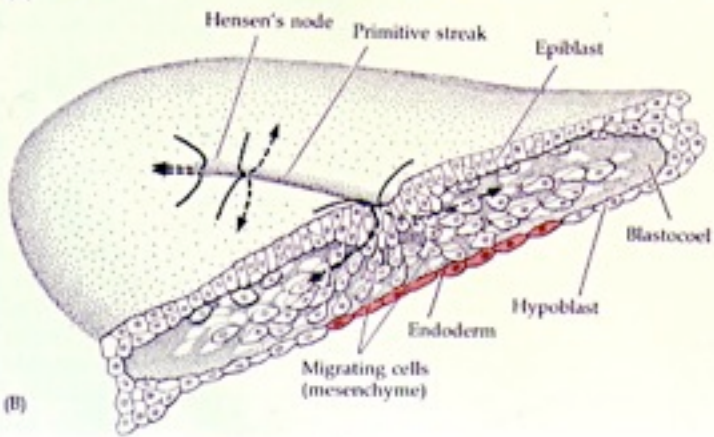
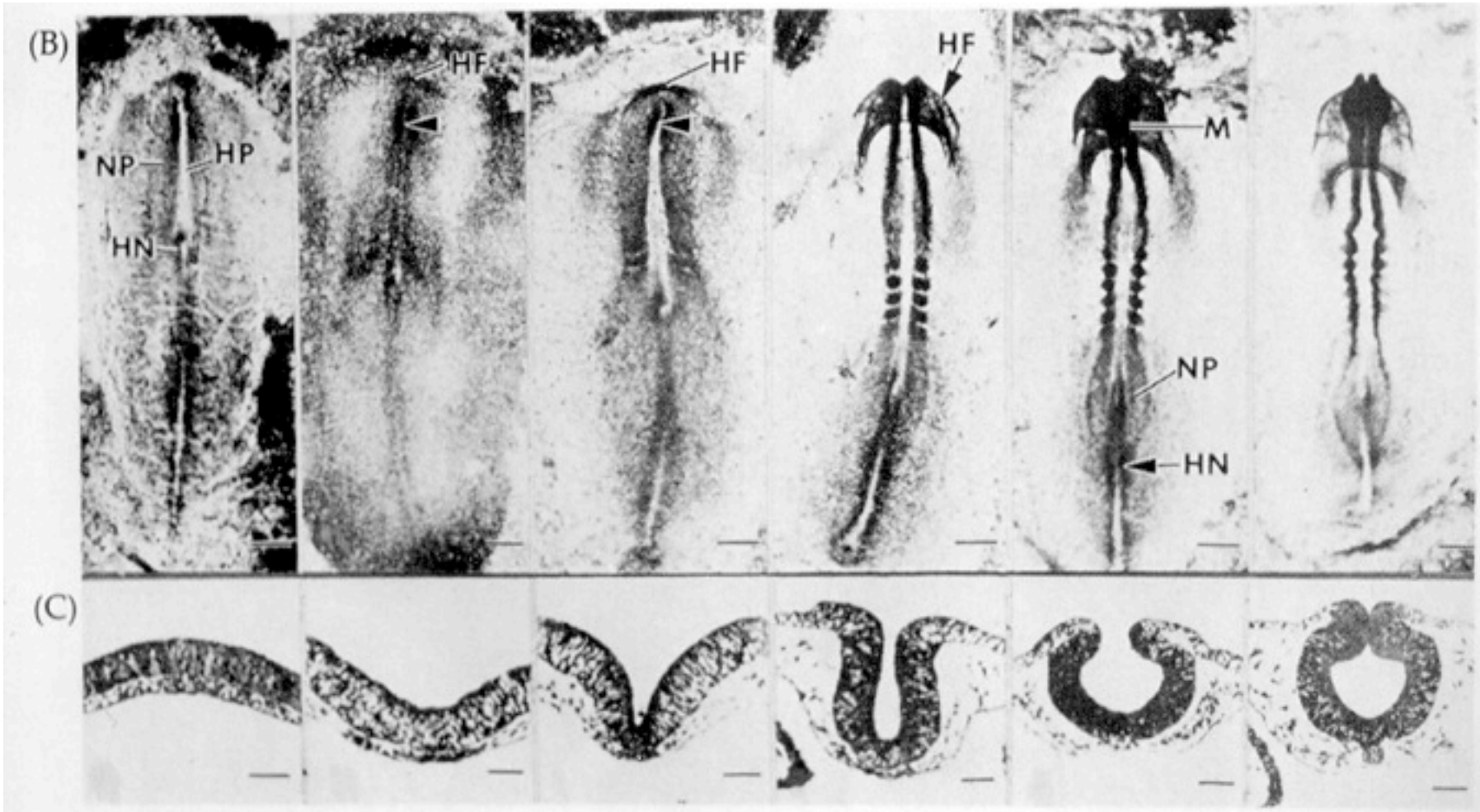
(A)



(B)

FIGURE 6.29
Migration of endodermal and mesodermal cells through the primitive streak. (A) Scanning electron micrograph shows epiblast cells passing into the blastocoel and extending their apical ends to become bottle cells. (B) Stereogram of a gastrulating chick embryo, showing the relationship of the primitive streak, the migrating cells, and the two original layers of the blastoderm. The lower layer becomes a mosaic of hypoblast and endodermal cells; but the hypoblast cells eventually sort out to form a layer beneath that of the endoderm and eventually contribute to the yolk sac. (A from Solursh and Revel, 1978, courtesy of M. Solursh; B after Balinsky, 1975.)



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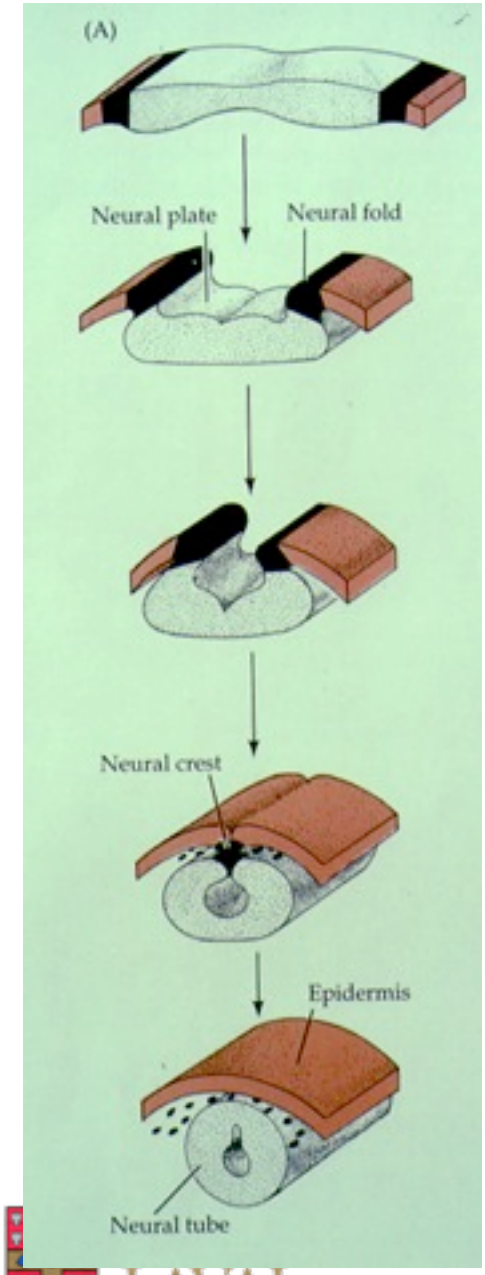
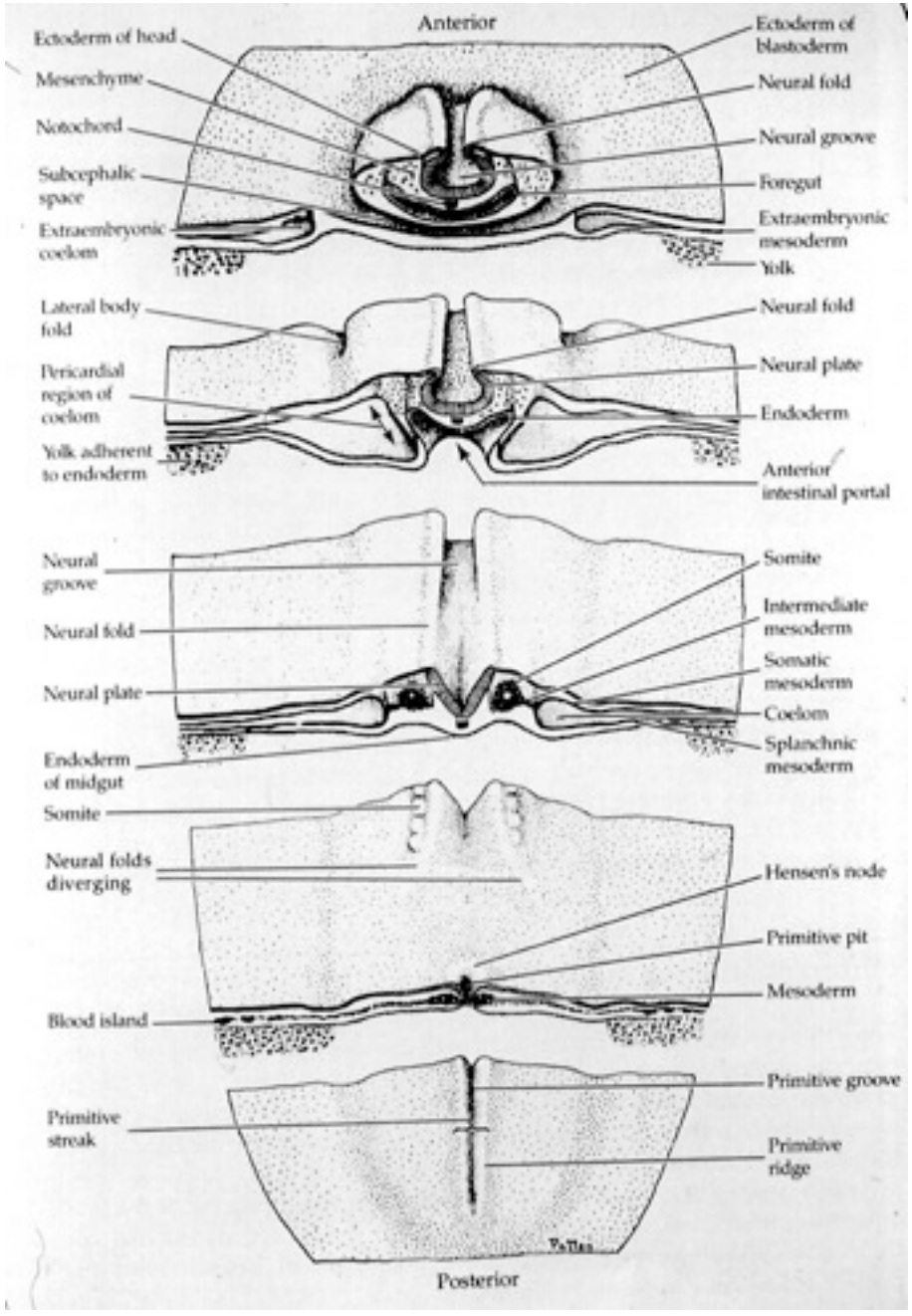
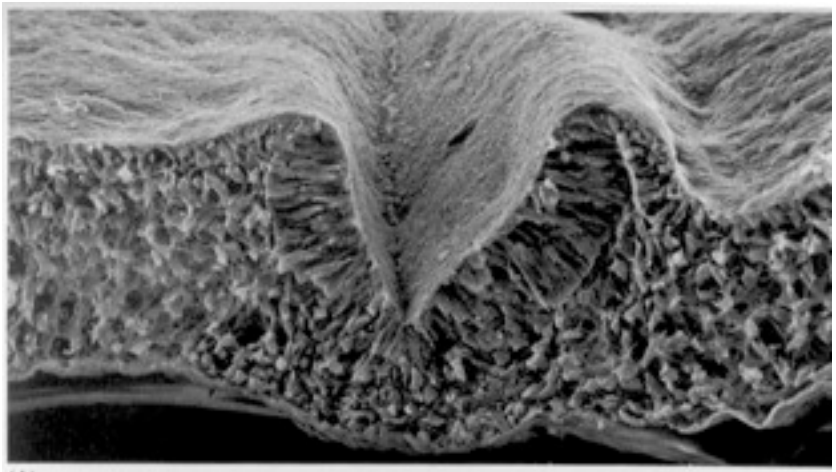
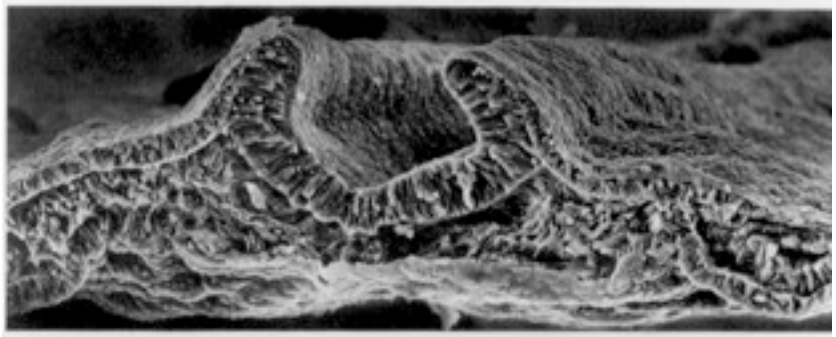

(A)
Neural plate
Neural fold
Neural crest
Epidermis
Neural tube

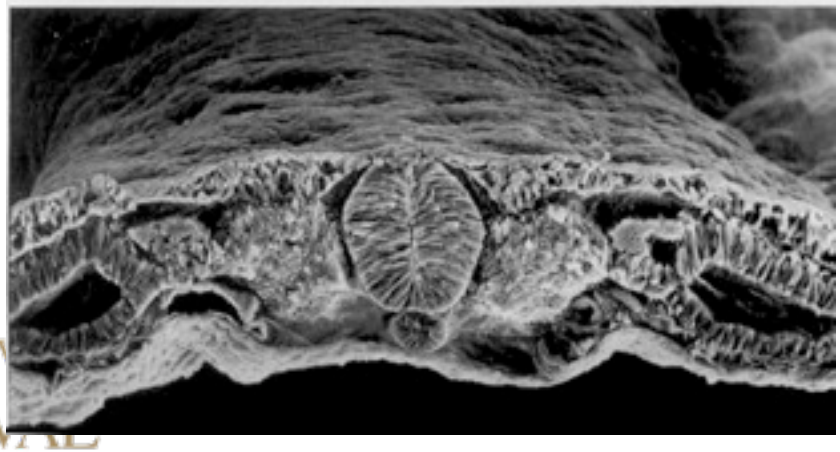

Anterior
Ectoderm of head
Mesenchyme
Notochord
Subcephalic space
Extraembryonic coelom
Ectoderm of blastoderm
Neural fold
Neural groove
Foregut
Extraembryonic mesoderm
Yolk
Lateral body fold
Pericardial region of coelom
Yolk adherent to endoderm
Neural fold
Neural plate
Endoderm
Anterior intestinal portal
Neural groove
Neural fold
Neural plate
Endoderm of midgut
Somite
Intermediate mesoderm
Somatic mesoderm
Coelom
Splanchnic mesoderm
Somite
Neural folds diverging
Blood island
Hensen's node
Primitive pit
Mesoderm
Primitive streak
Primitive groove
Primitive ridge
Posterior

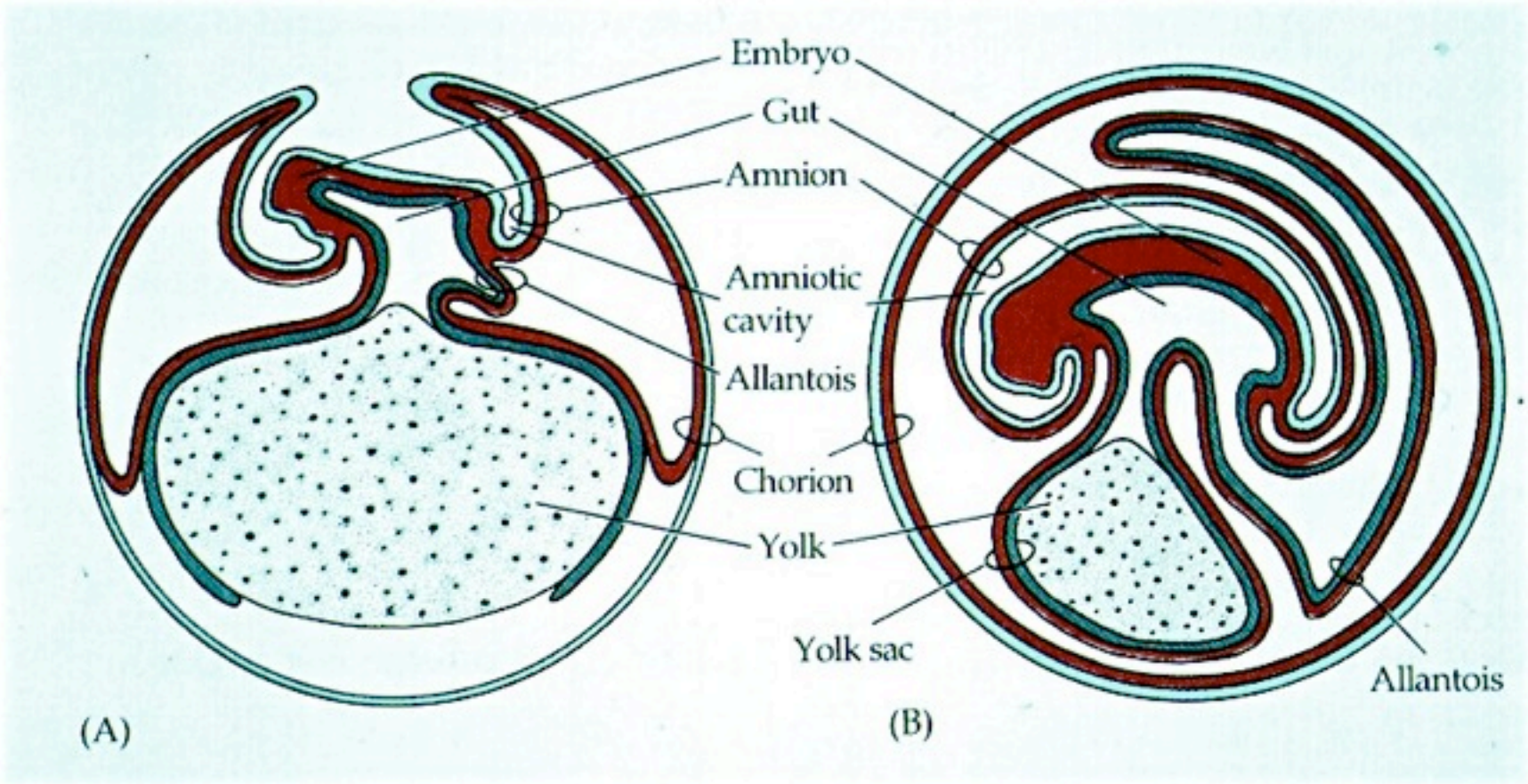


(A)



(B)





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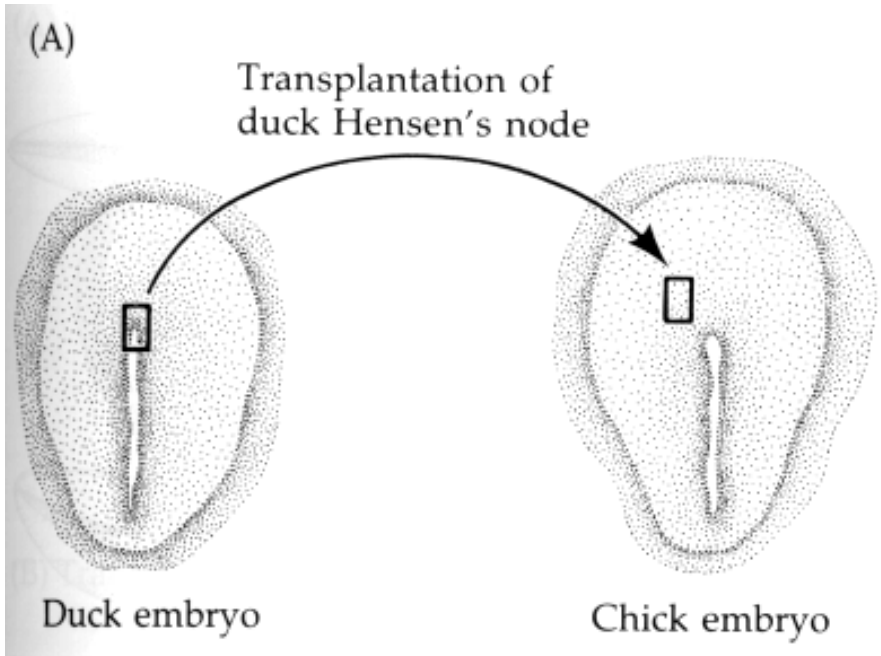

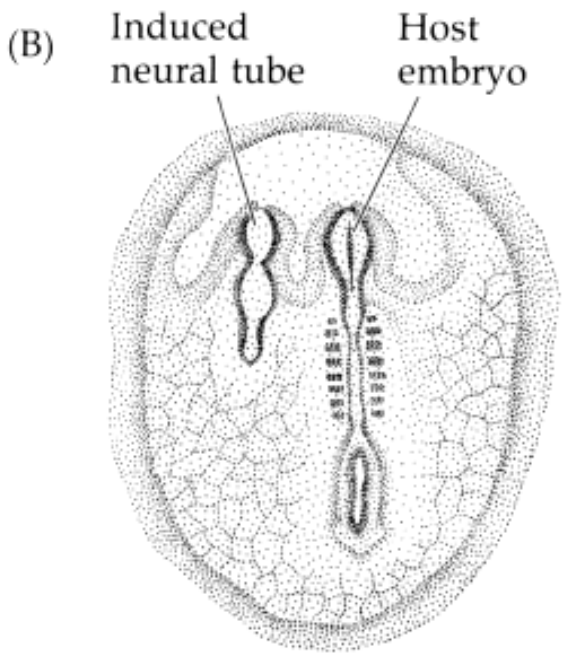

Induction of a new embryonic axis by Hensen's node. (A) Hensen's node tissue is removed from a duck embryo and implanted into a host chick embryo. (B) An accessory neural tube is induced at the graft site. (After Waddington, 1933.)

Brief comparison of embryological systems;
Fish

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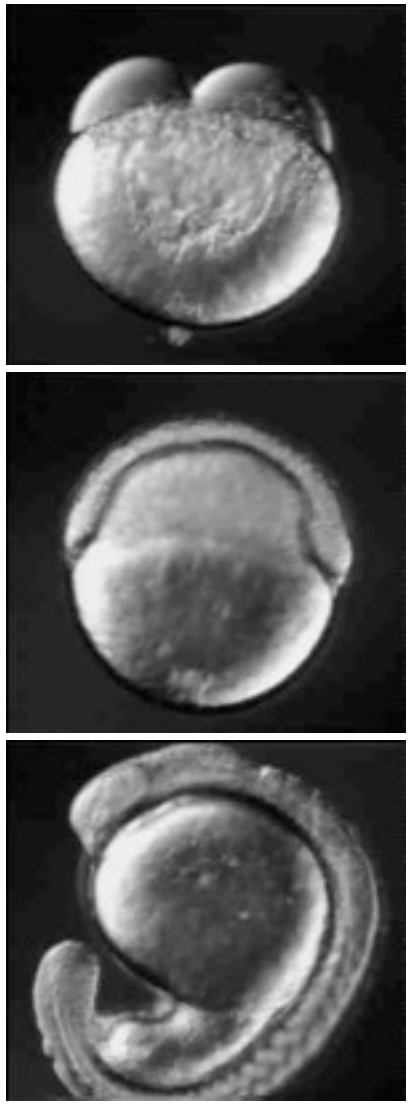

Zebrafish cleavage, epiboly/gastrulation and neurulation

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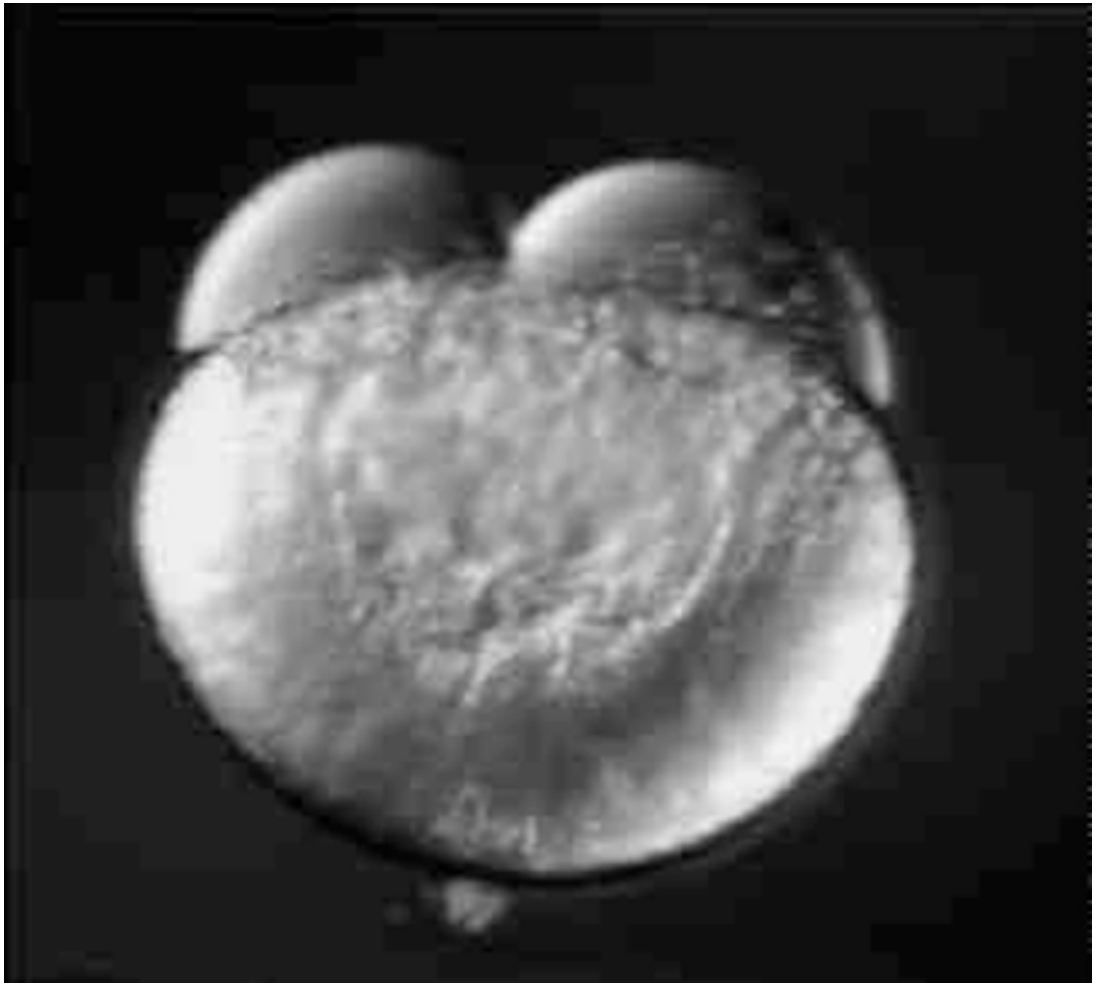

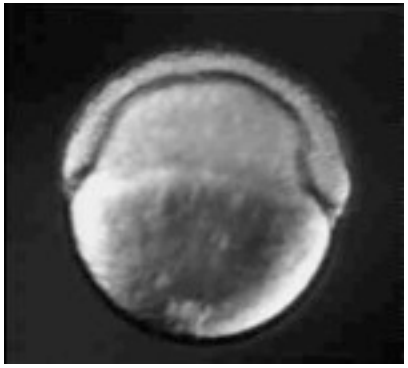

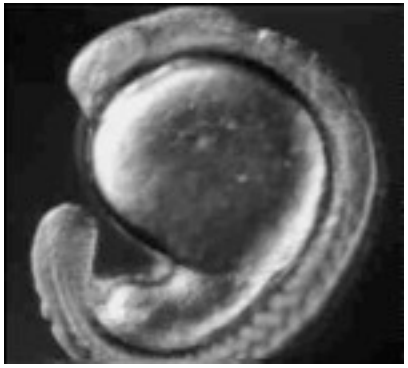

Zebrafish cleavage, epiboly/gastrulation and neurulation

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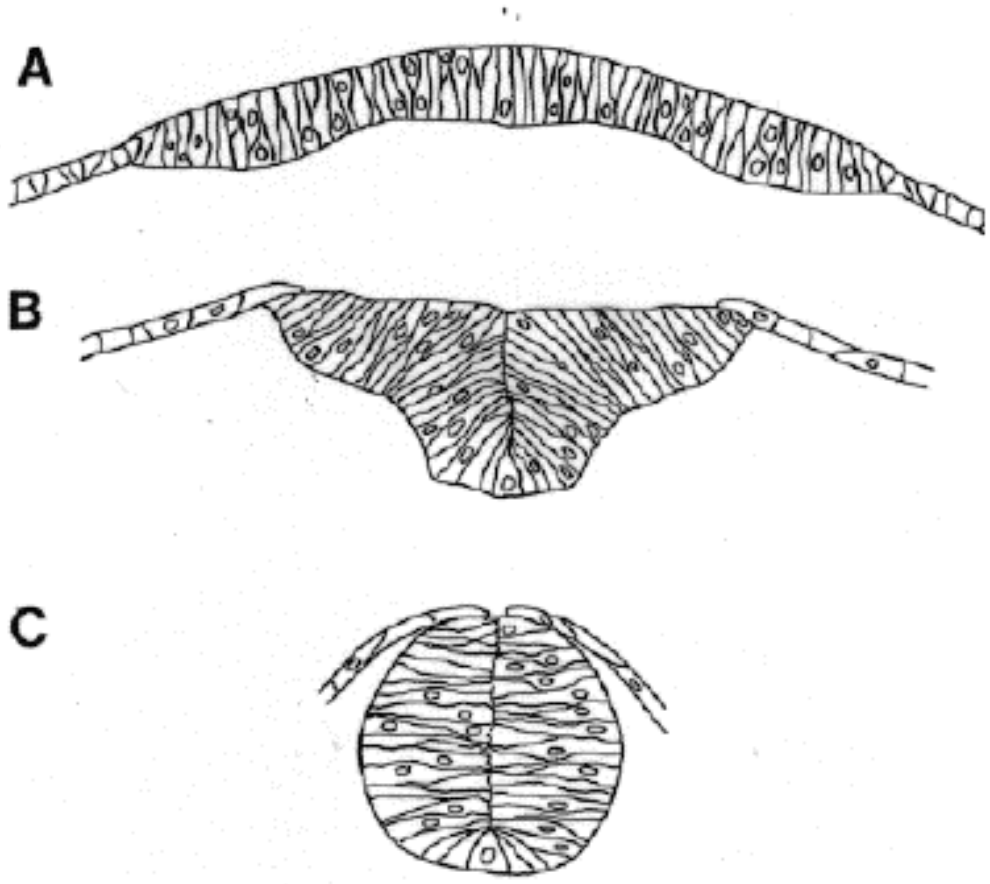
Zebrafish neurulation

Early morphogenesis of the neural primordium. Diagramatic transverse sections, redrawn from Papan and Campos-Ortega (1994). The neural plate (**A**, ca. 10 h) develops into the neural keel (**B**, ca. 13 h) by infolding at the midline. The keel in turn rounds into the cylindrical neural rod (**C**, ca. 16 h).



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Brief comparison of embryological systems; Amphibia (Xenopus)

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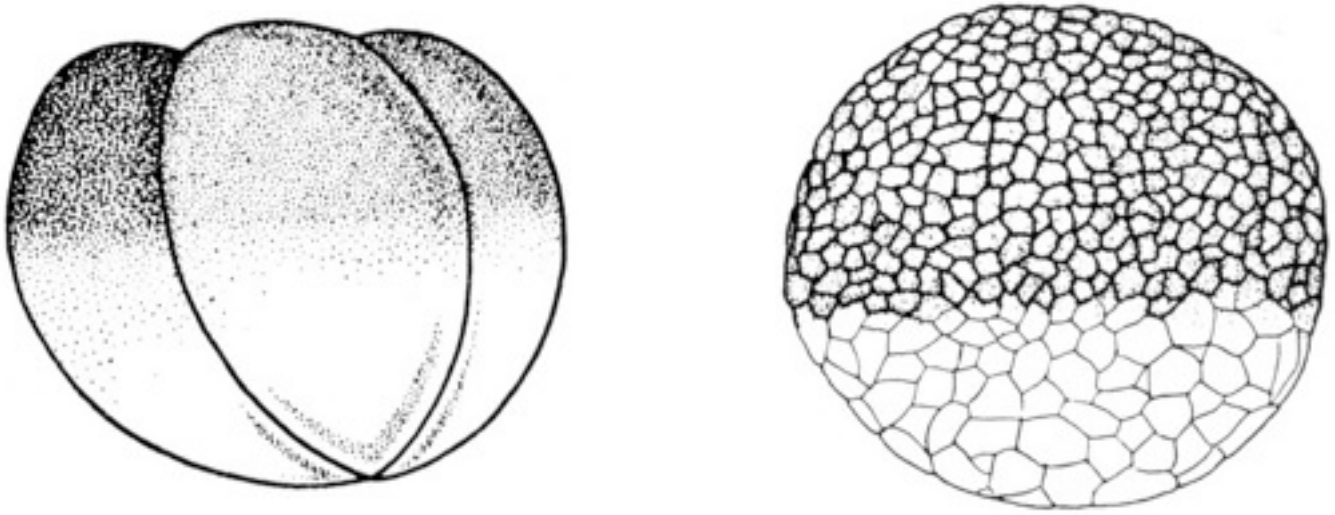

Reduction cleavages


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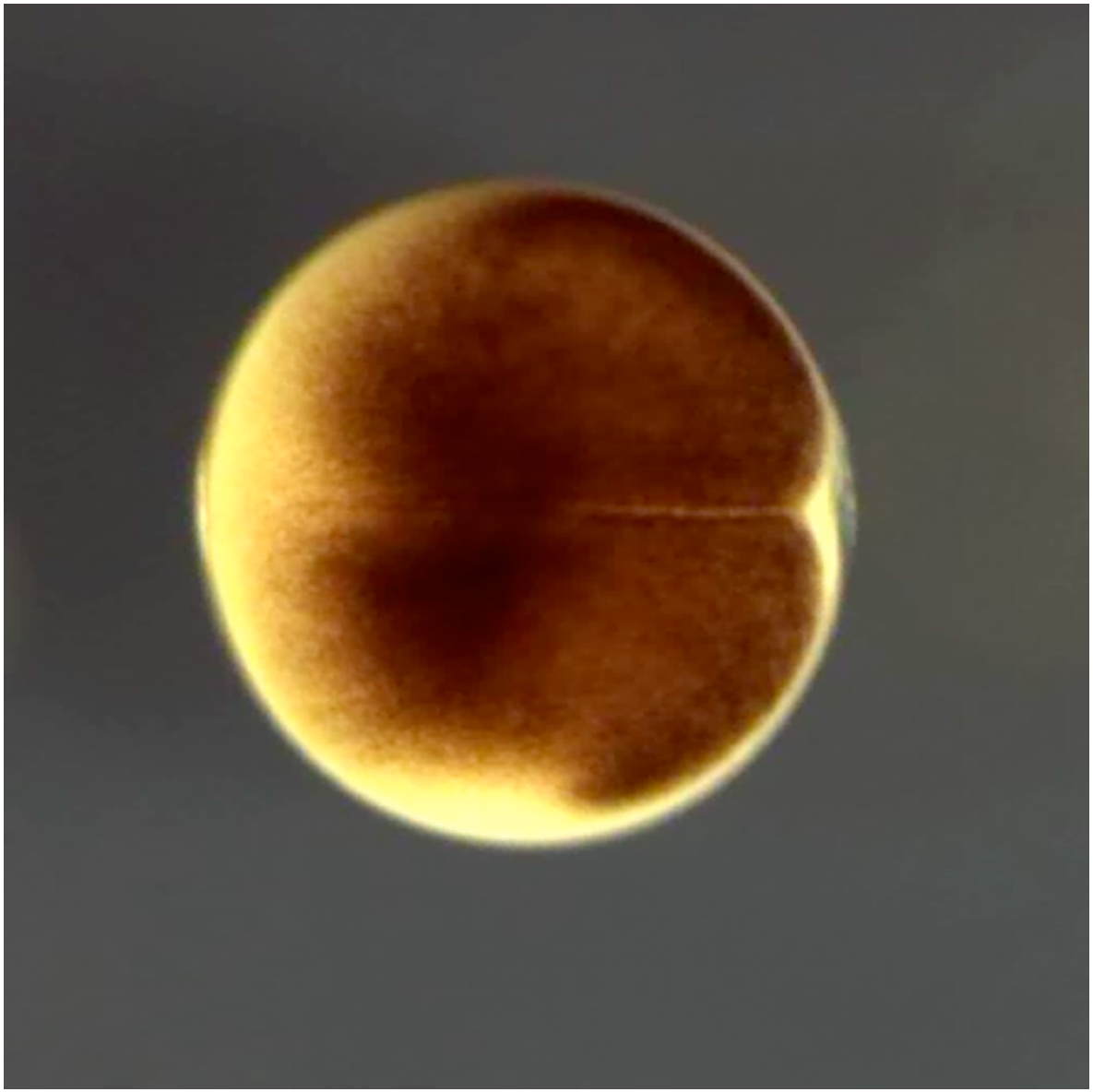

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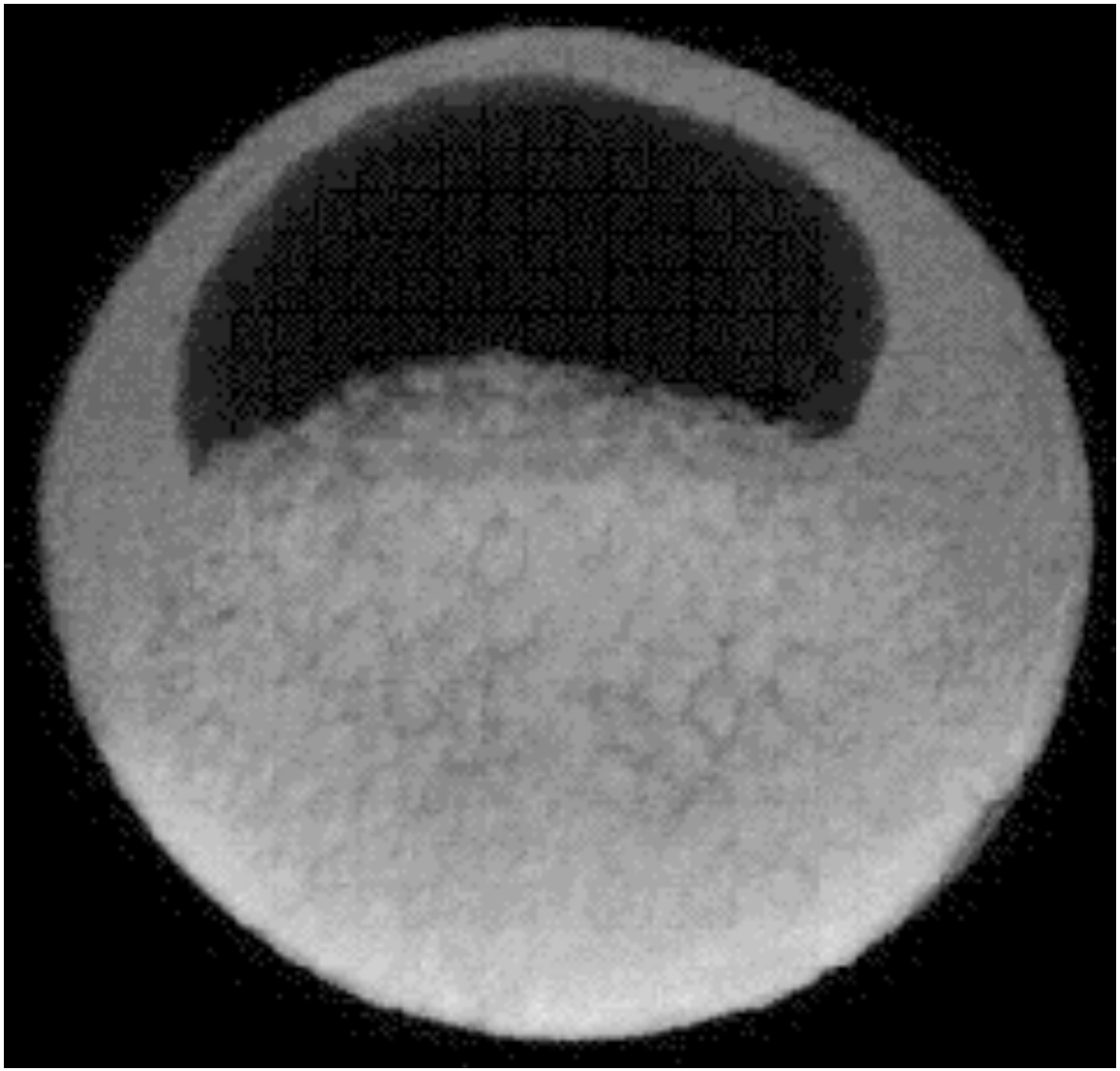

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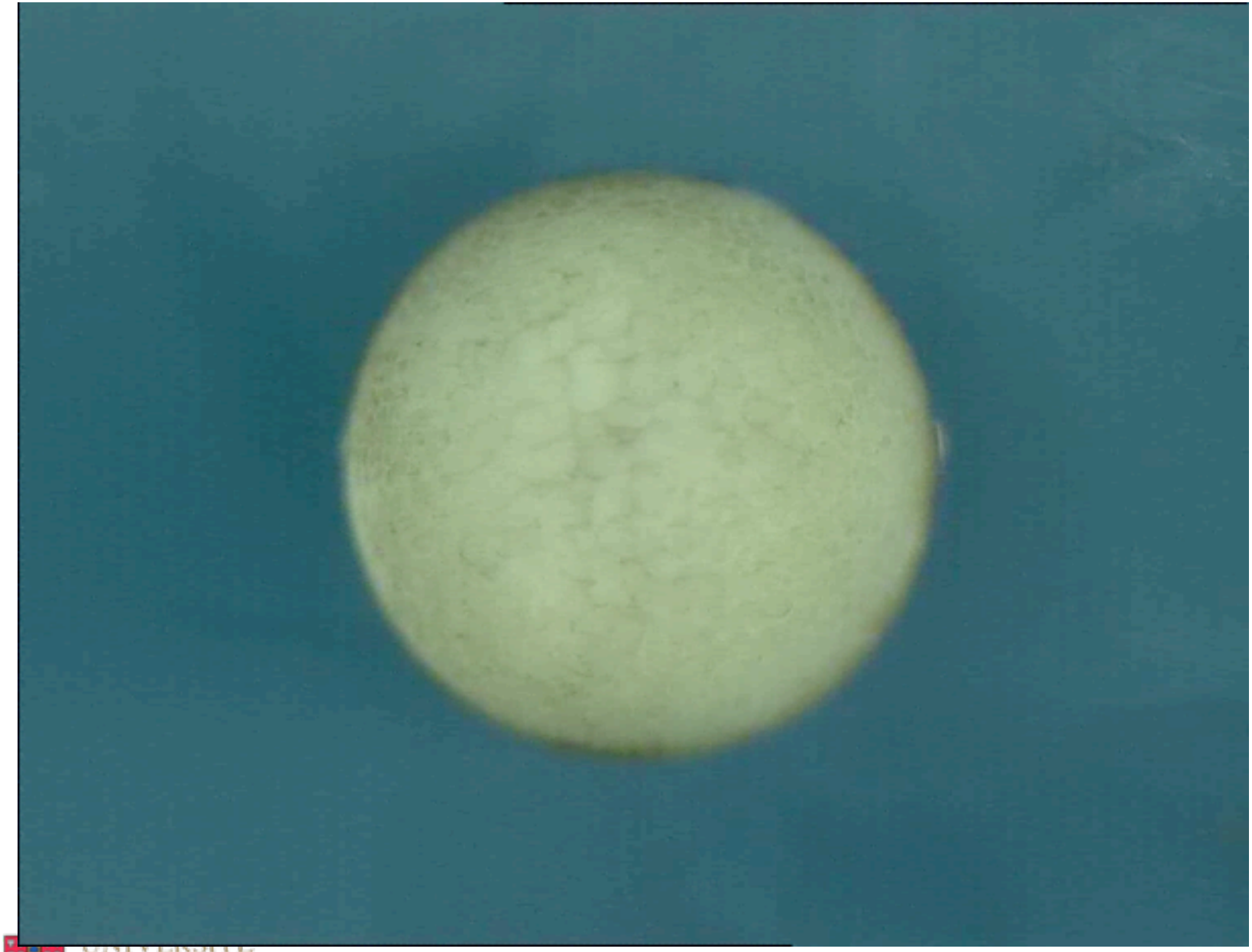

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Development of Xenopus laevis as seen by Hausen and Riebesell

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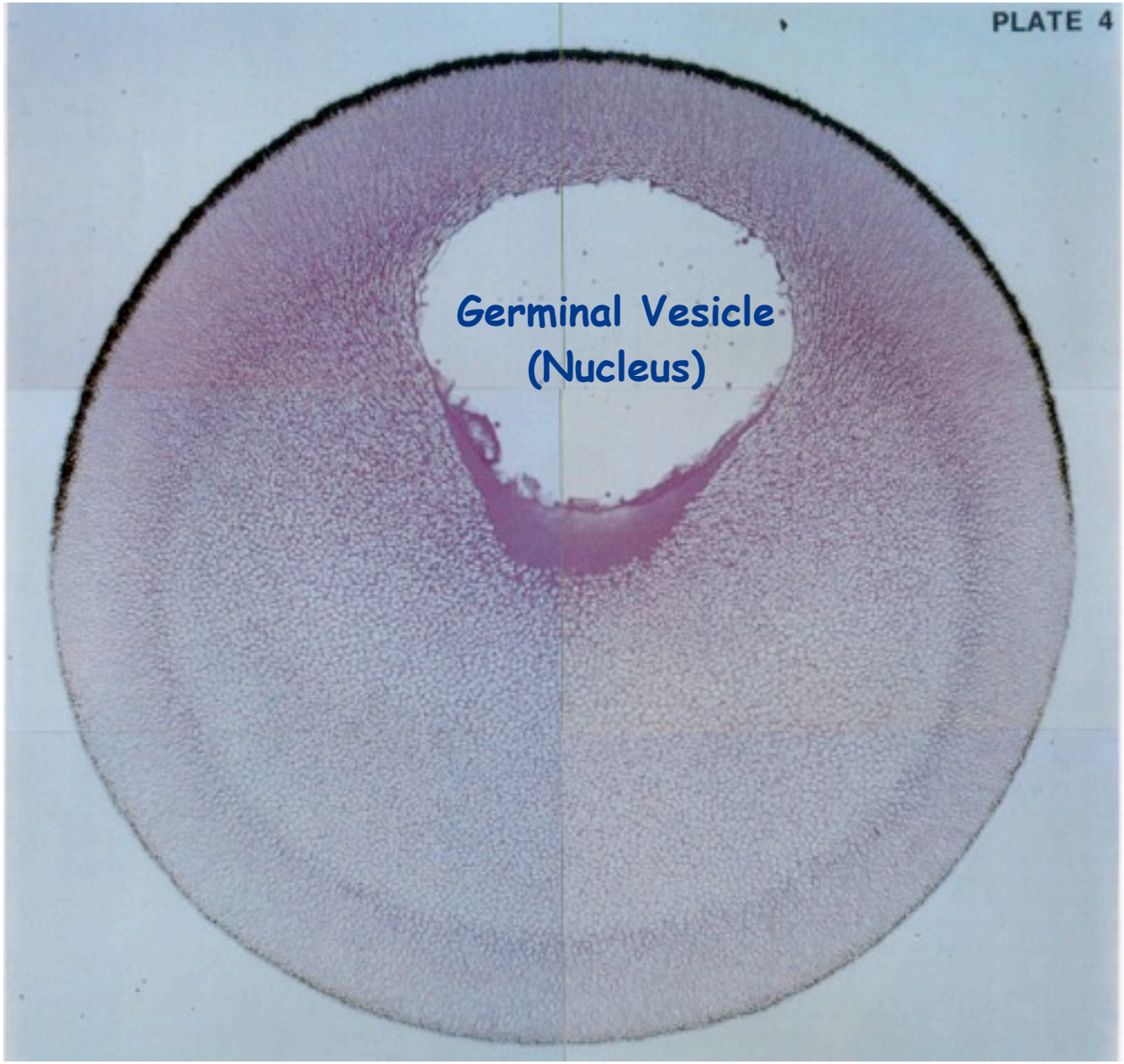
The Oocyte



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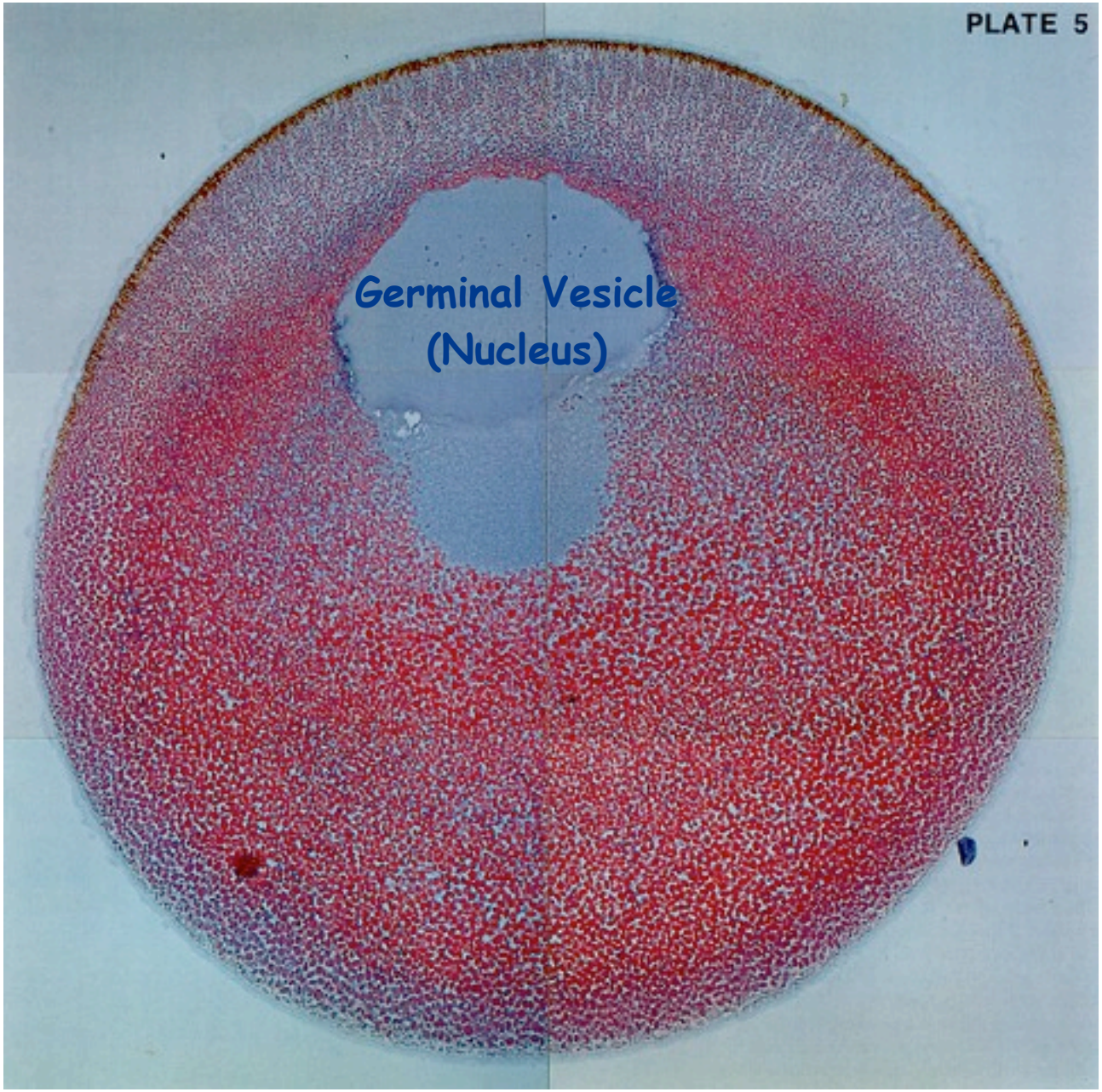
Nuclear Breakdown



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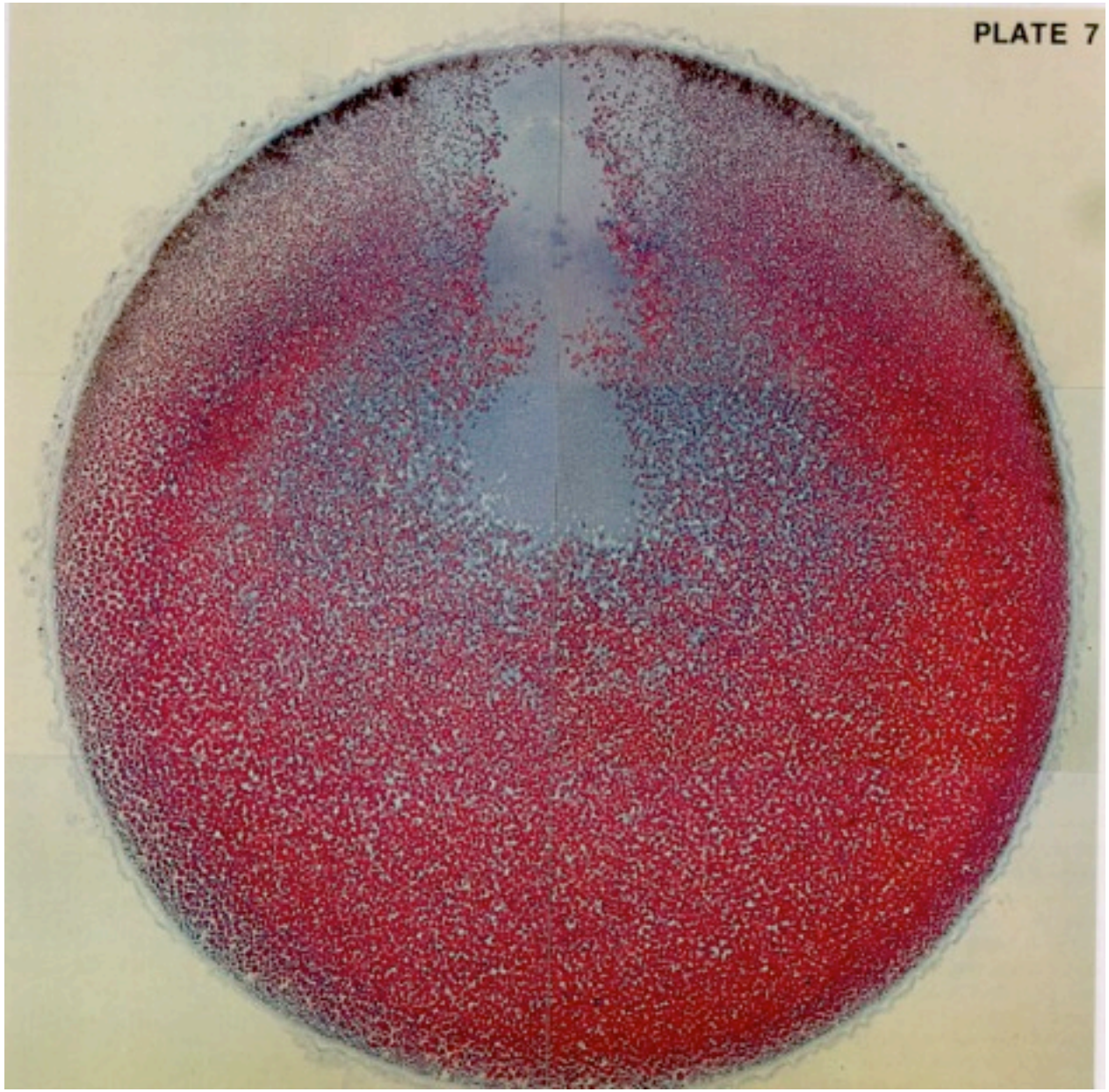
Nuclear Breakdown



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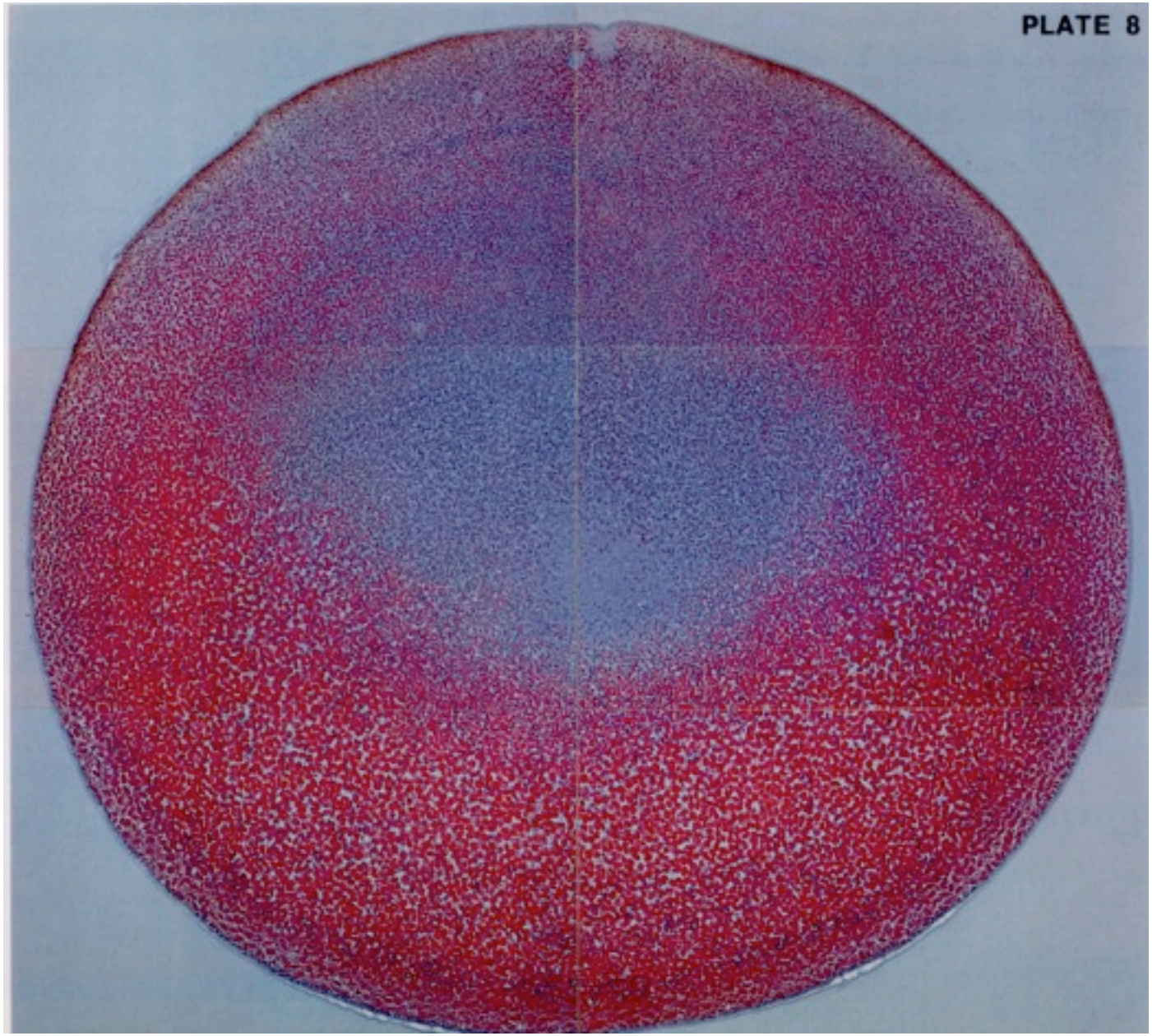
Nuclear Breakdown Complete



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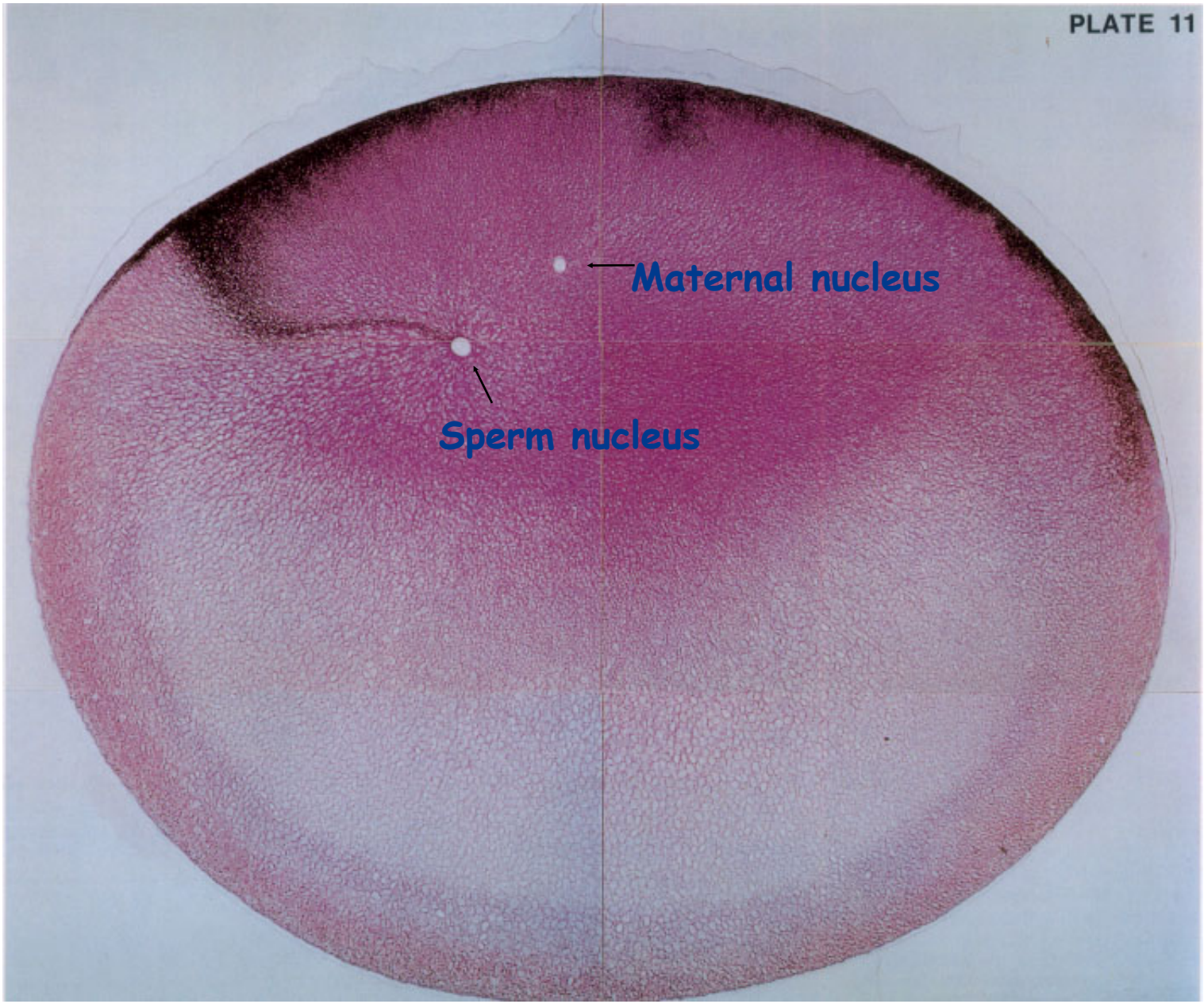
Fertilisation



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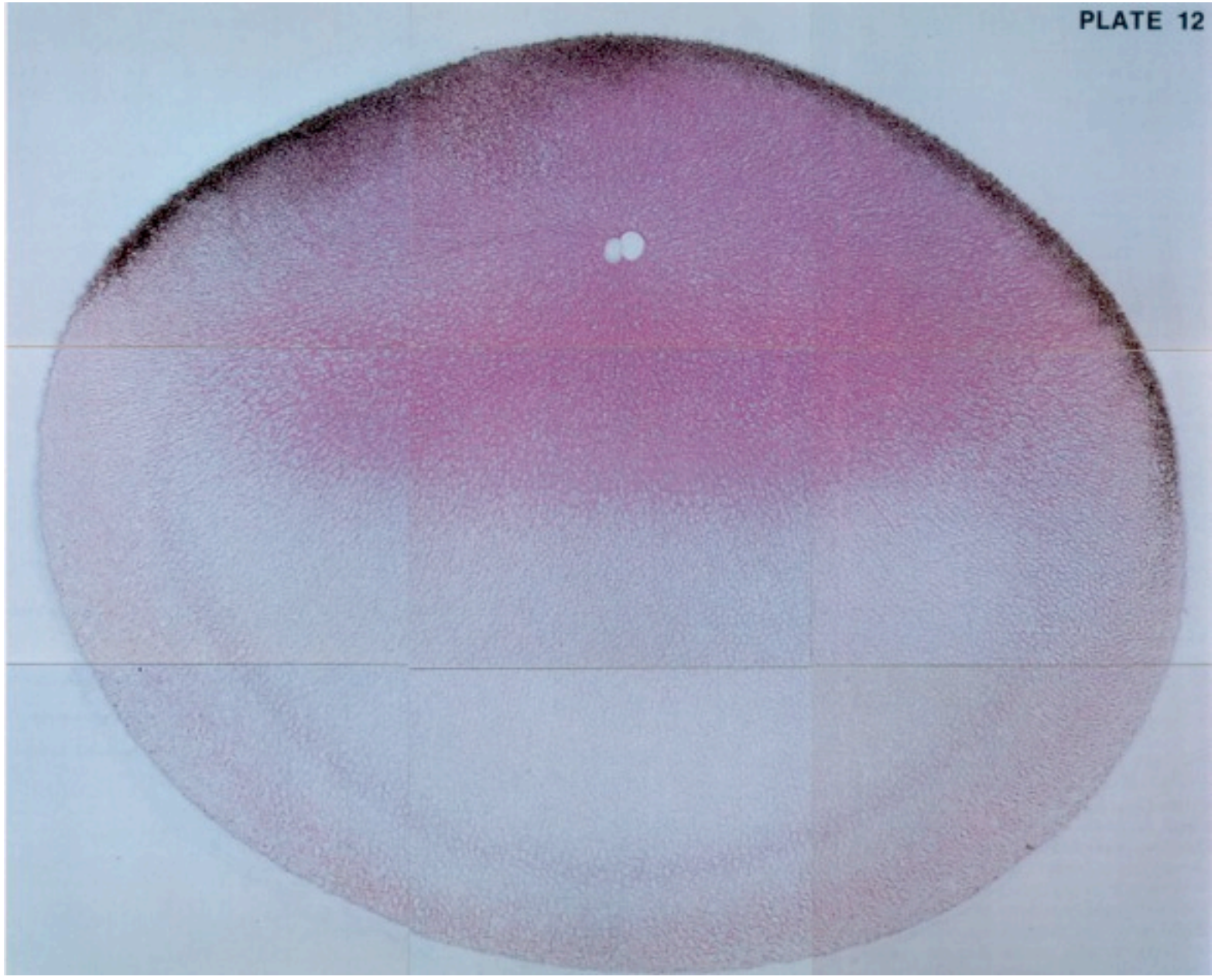
Formation of zygote
Stage 1



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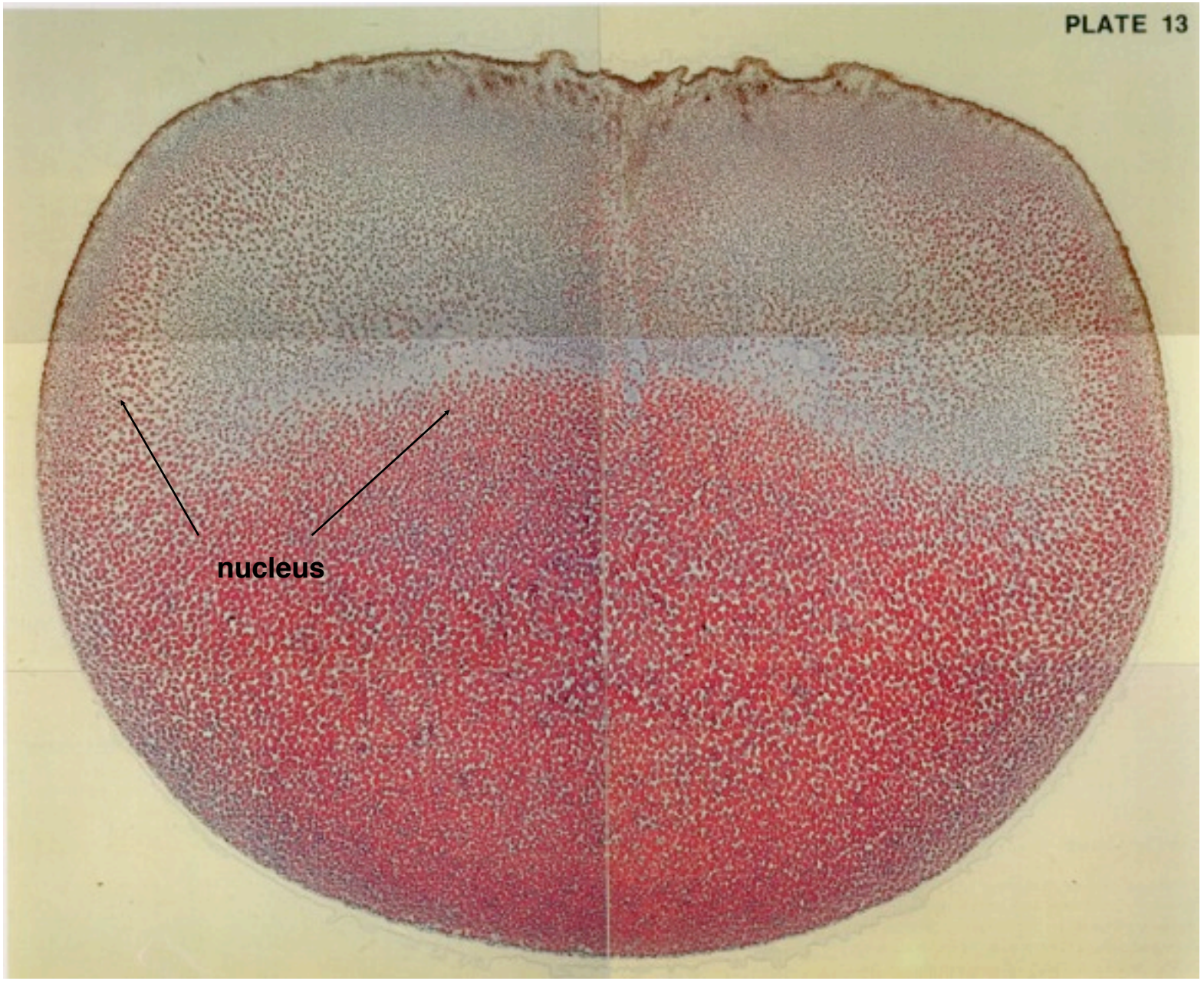
Preparation for division one



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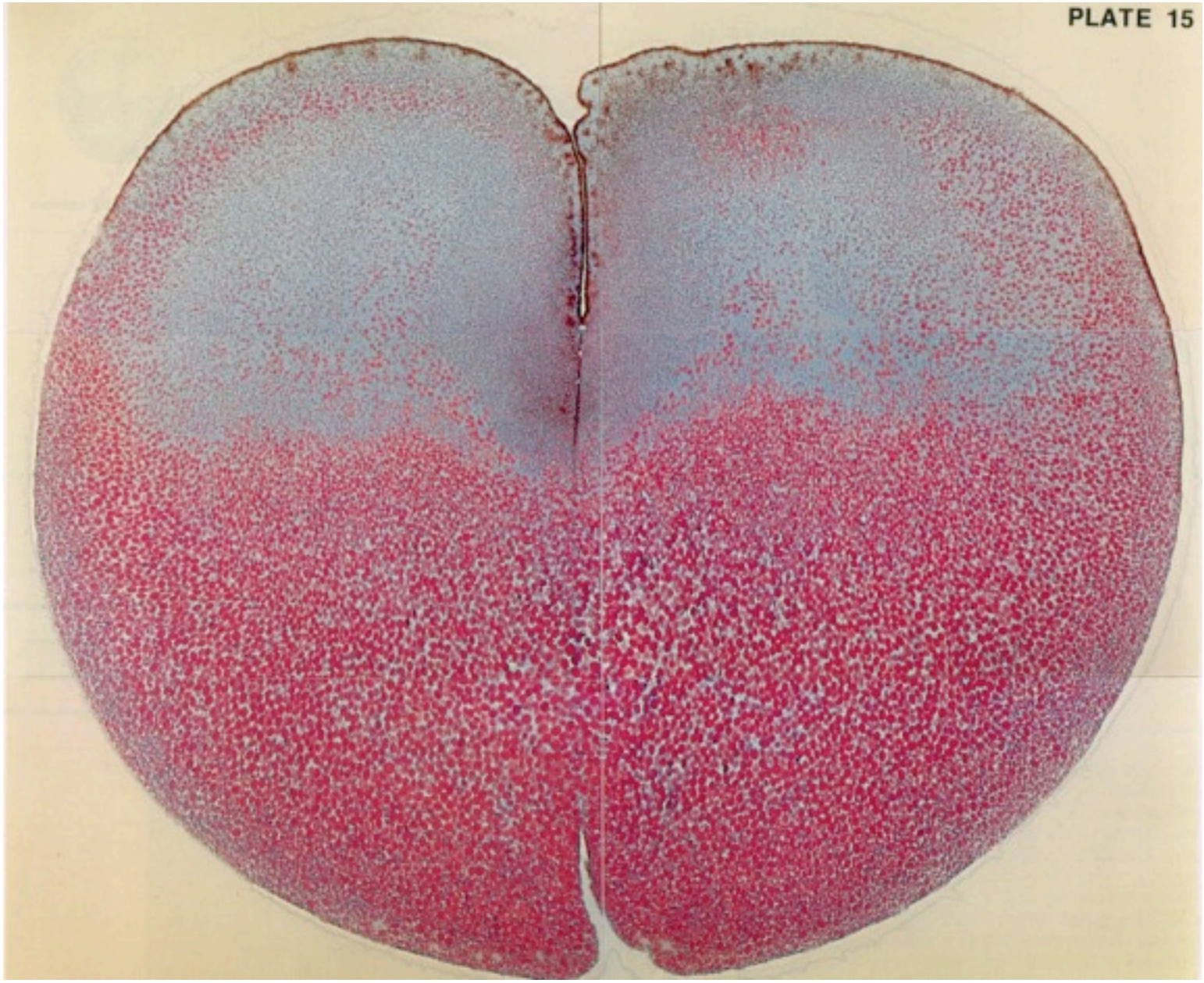
First division
Stage 2



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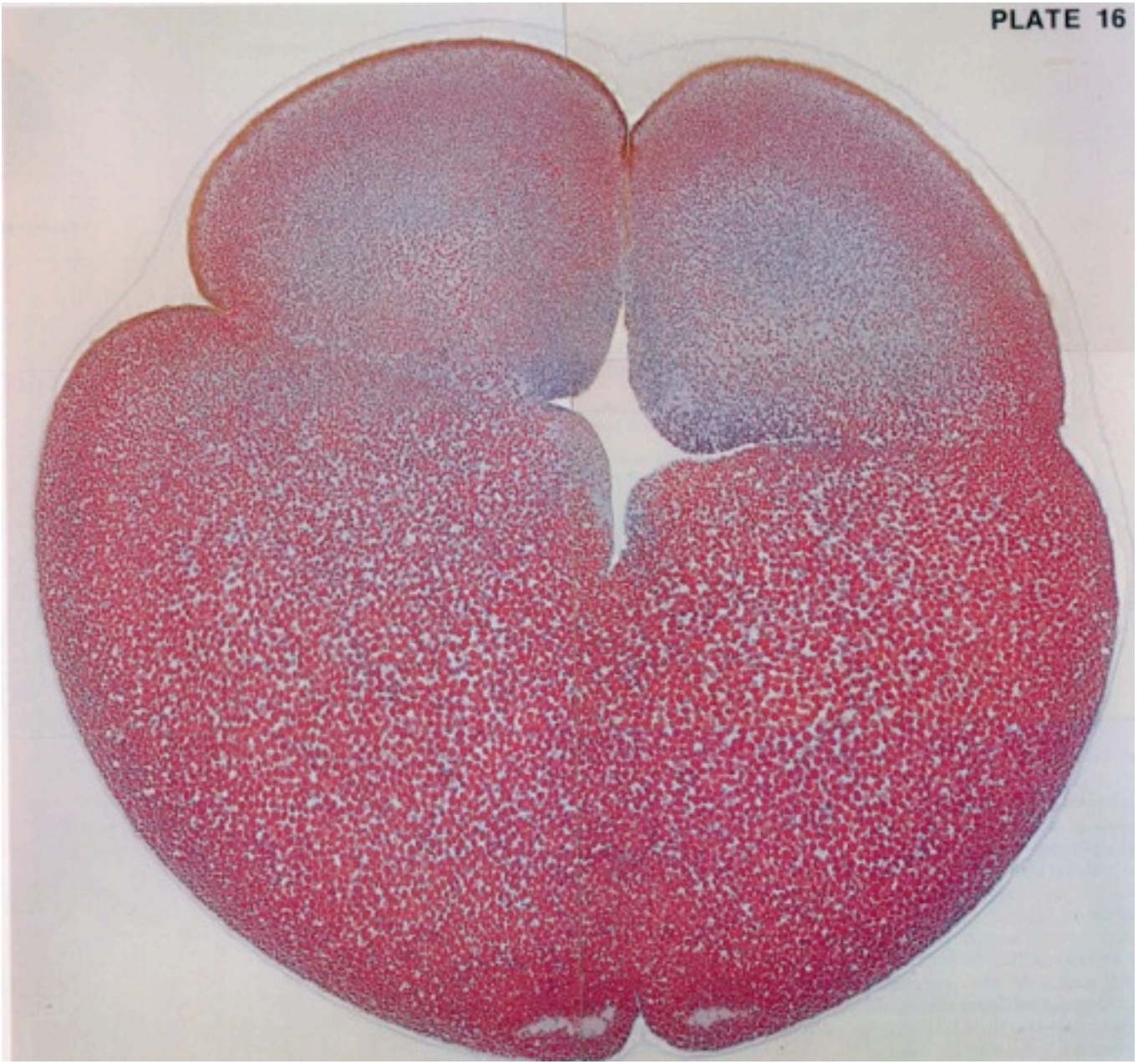
Division 3 complete
Stage 4



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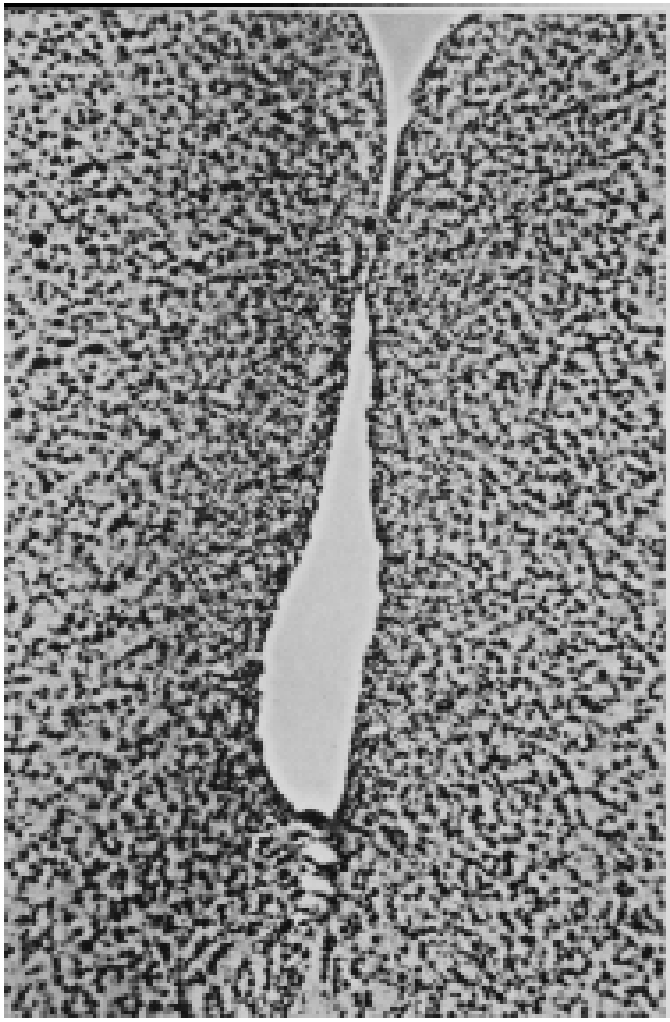
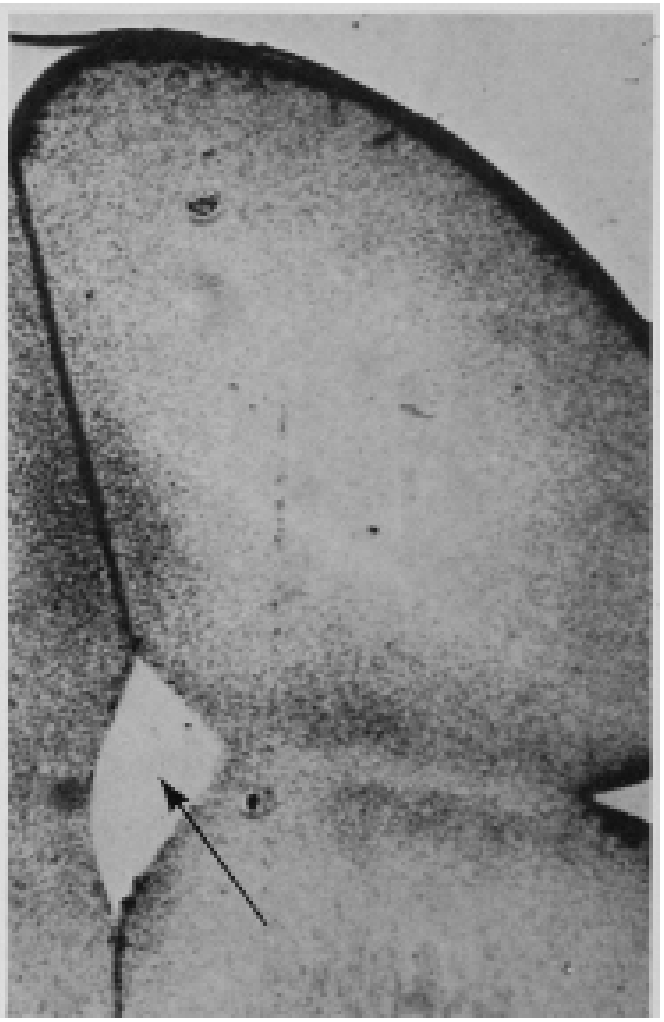


Two cell



Eight cell

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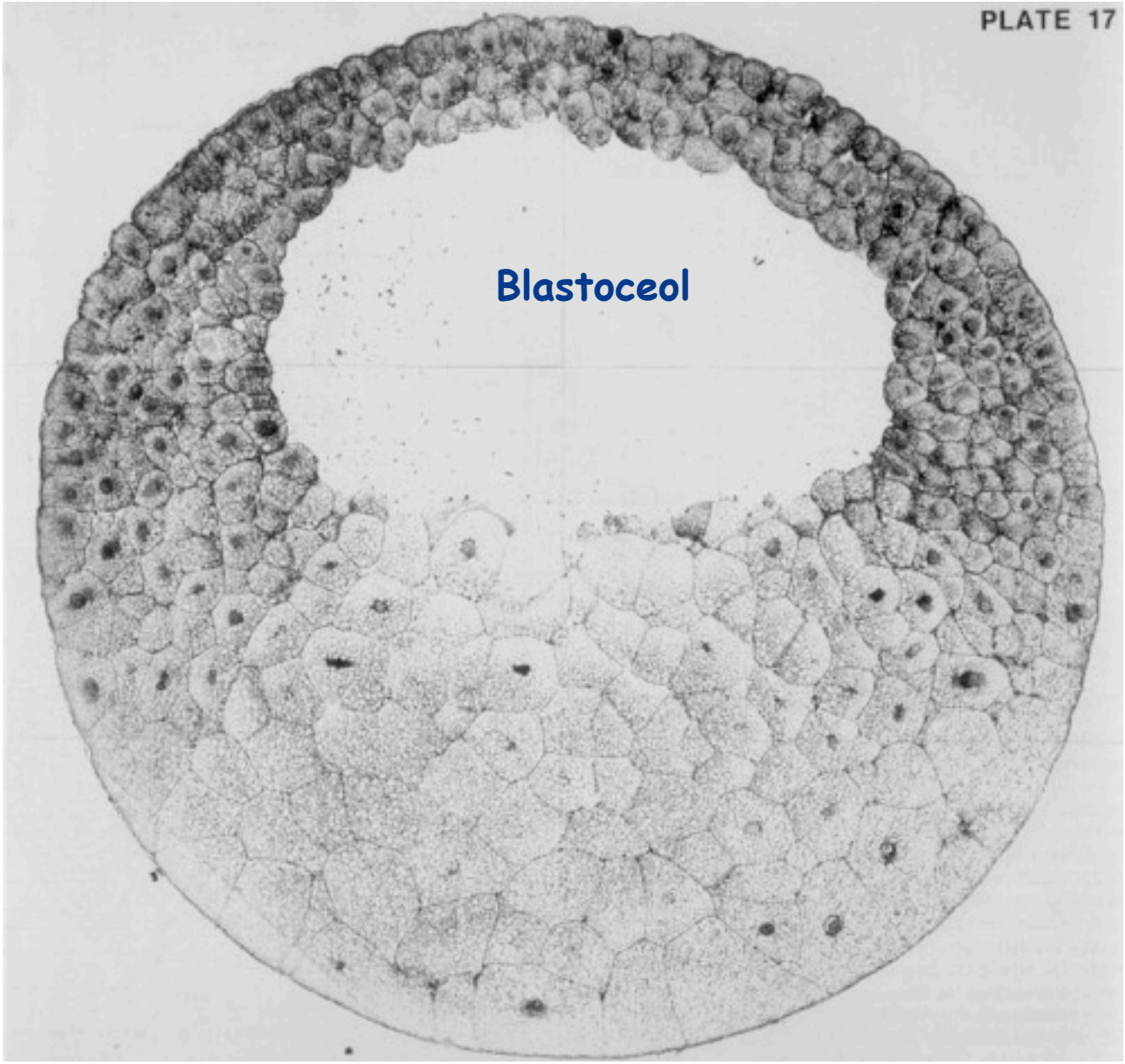

Blastula
Stage 9

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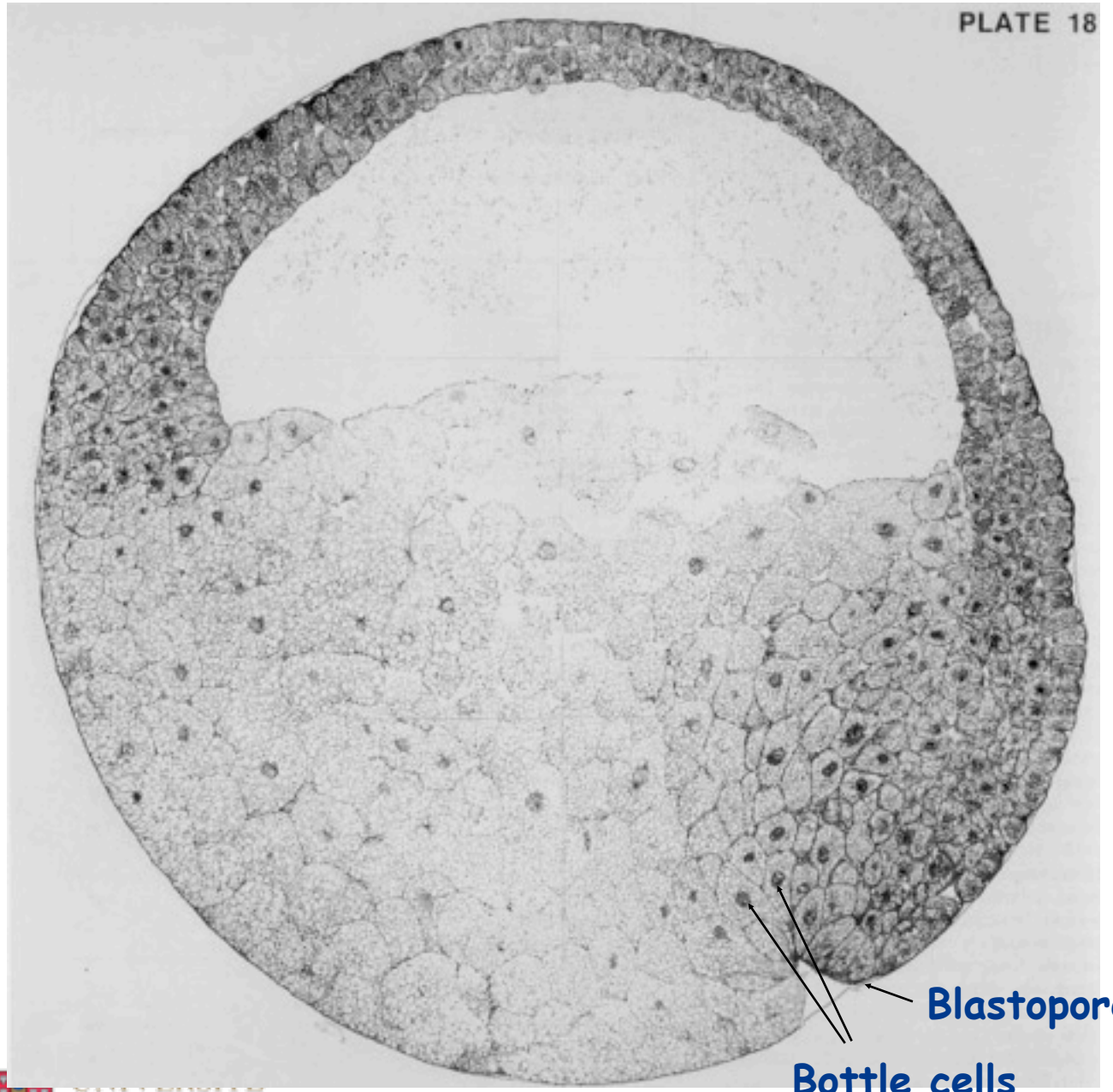

Early gastrula
Stage 10

Dorsal

Blastopore lip

Bottle cells

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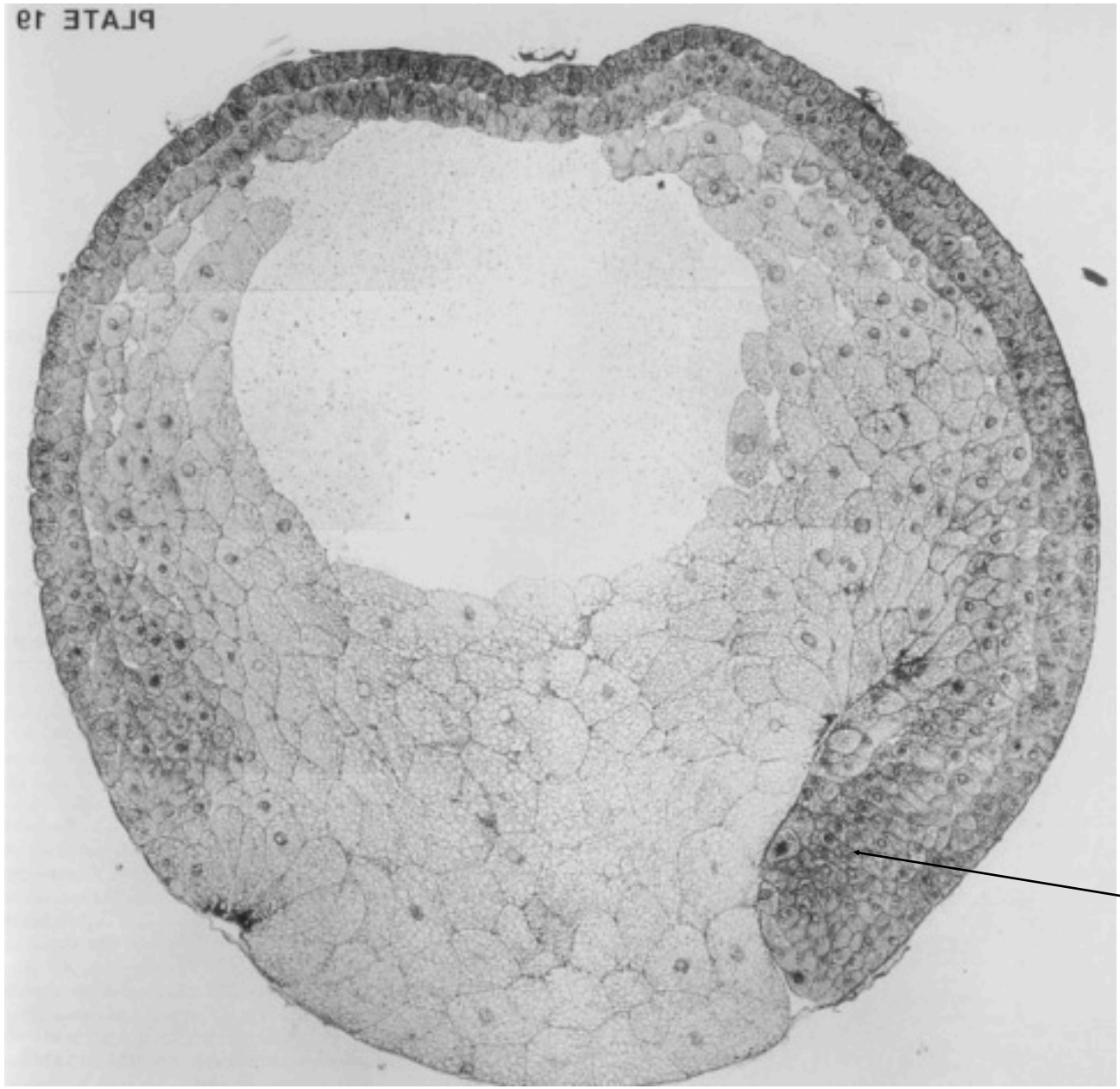
Mid gastrula
Stage 11 1/2



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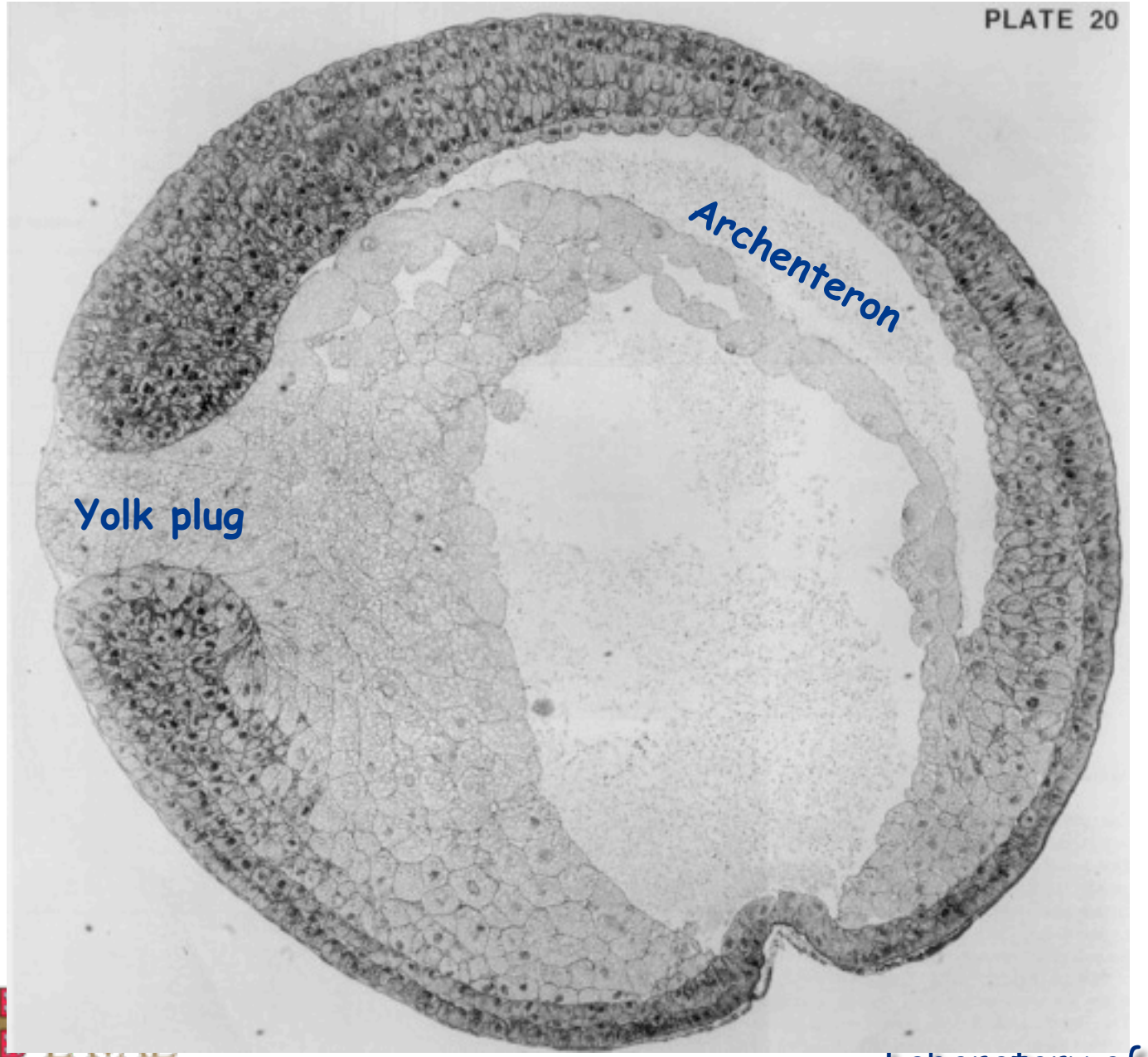
Dorsal



Late gastrula
Stage 13

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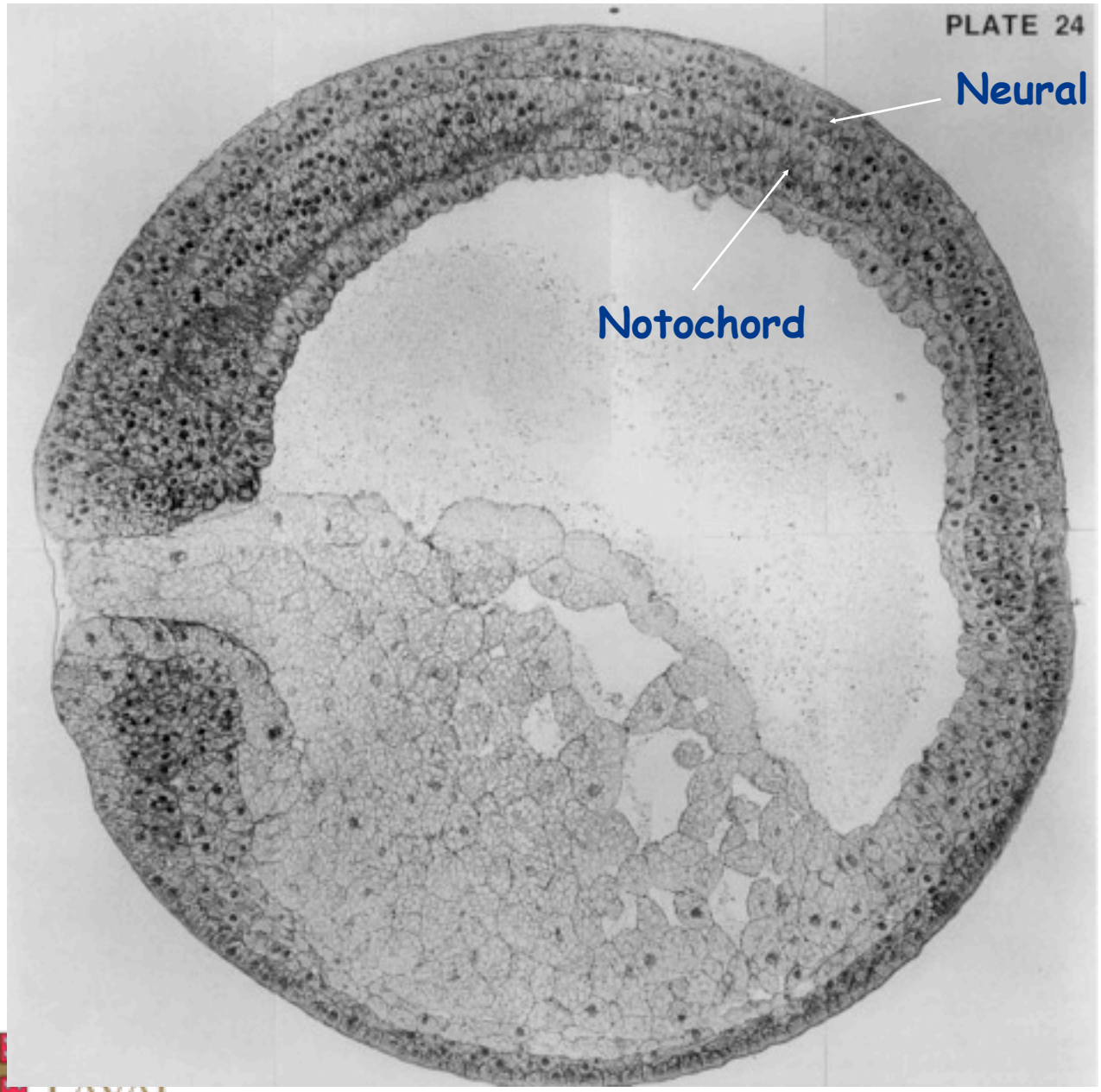
Dorsal



Mid neural fold
Stage 15

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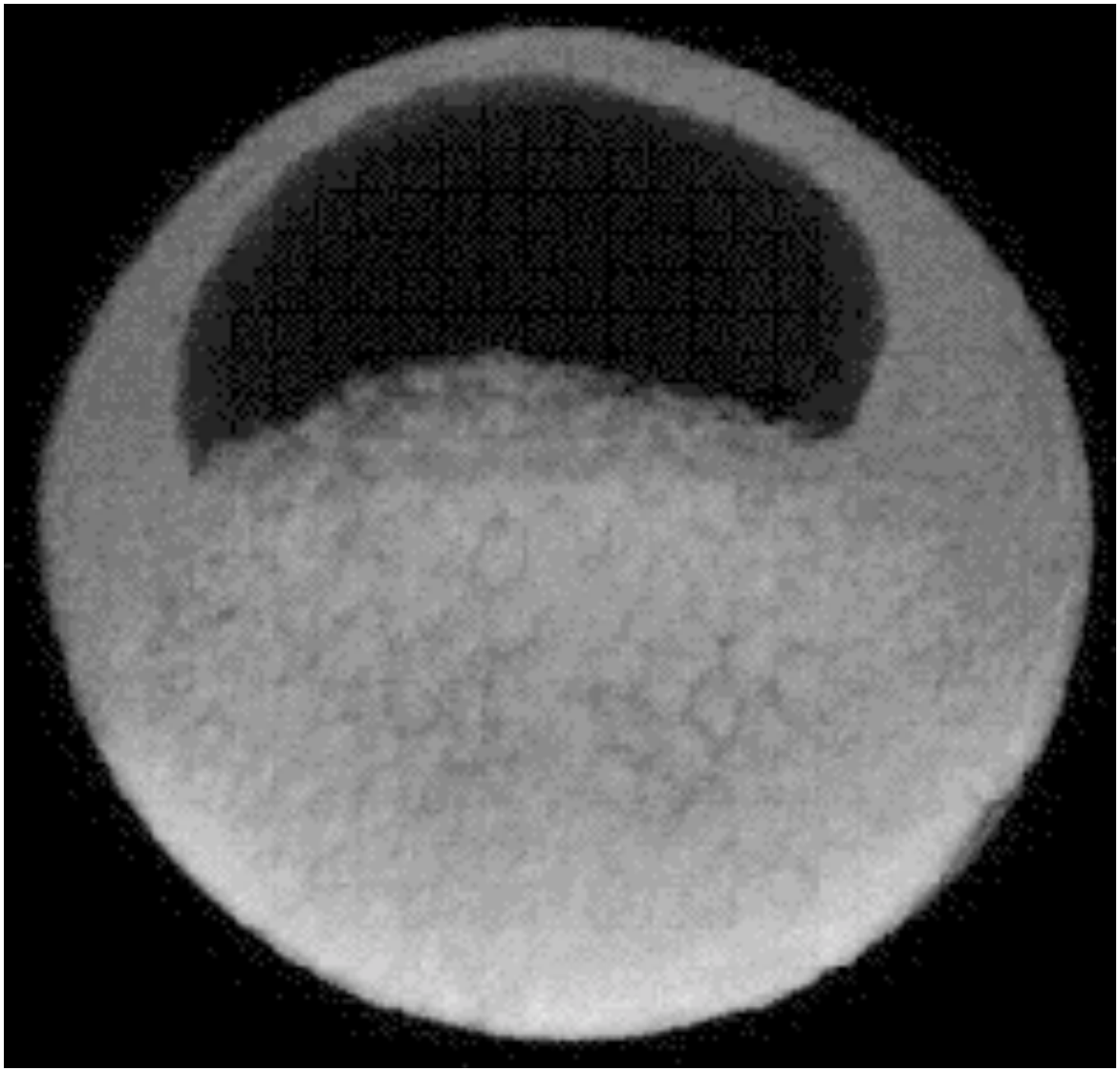

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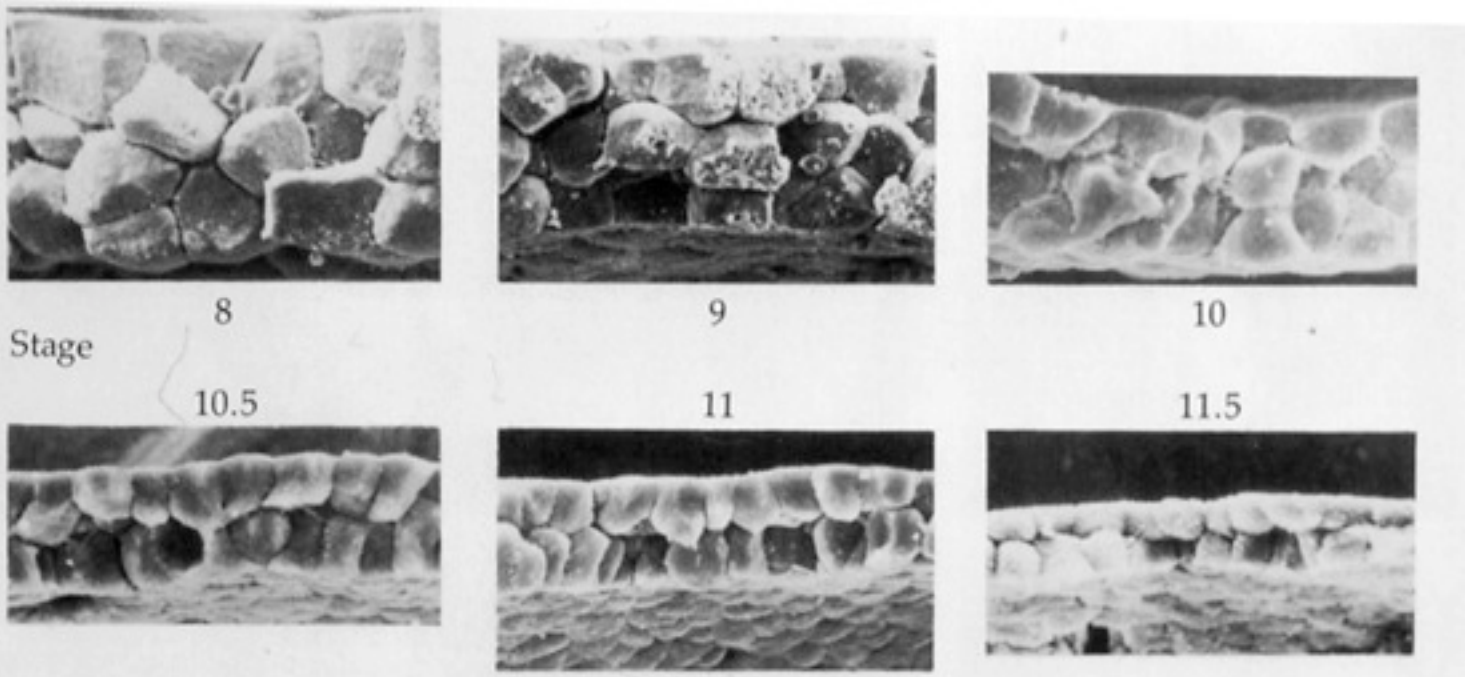
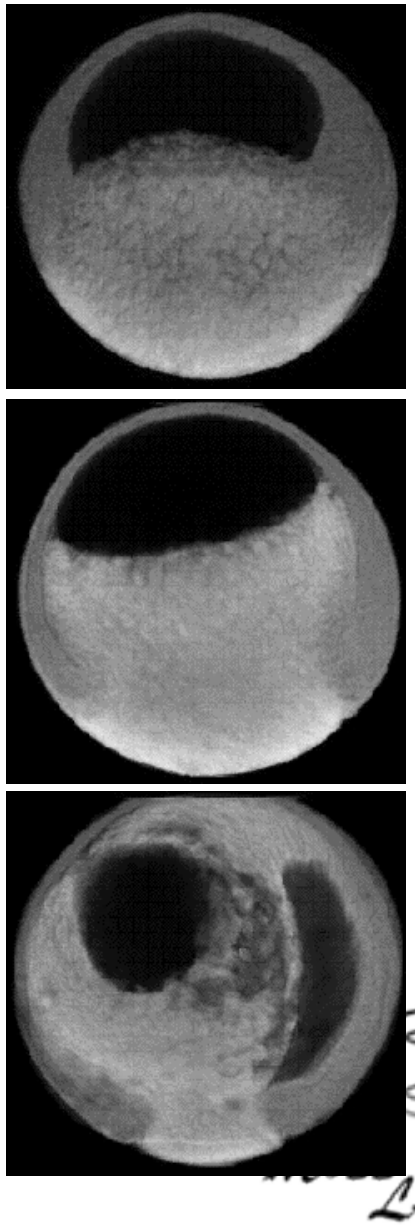


FIGURE 6.24
Scanning electron micrographs of the *Xenopus* blastocoel roof showing the changes in cell shape and arrangement. Stages 8 and 9 are blastulae; stages 10 through 11.5 represent progressively later gastrulae. (From Keller, 1980, courtesy of R. E. Keller.)



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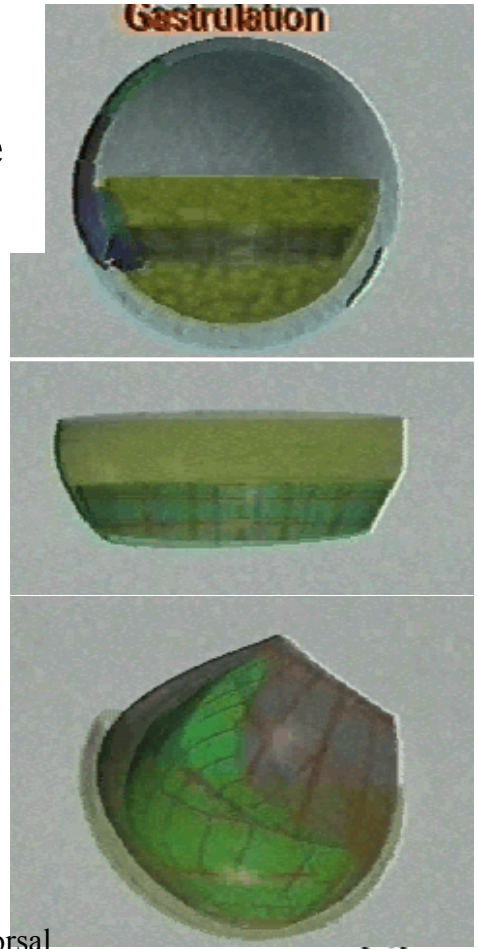
Prospective Dorsal

Marginal zone (MZ) and Involuting Marginal Zone (IMZ)



The video sequence below provides a computer simulation of the behavior of the deep cells of the dorsal involuting marginal zone (DIMZ) in relation to surrounding tissues during Xenopus gastrulation. First, note that the IMZ is a torus (doughnut) of tissue containing deep, non-epithelial cells and superficial epithelial cells. The deep cells of the DIMZ undergo massive convergence and extension to extend dramatically on the dorsal side, forming axial mesoderm, including notochord and somites. At the same time, the superficial cells of the DIMZ form the roof of the archenteron (shown here in green), and are therefore endoderm. The non-involuting marginal zone (NIMZ) remains on the exterior, and forms ectoderm. **By R. Keller**

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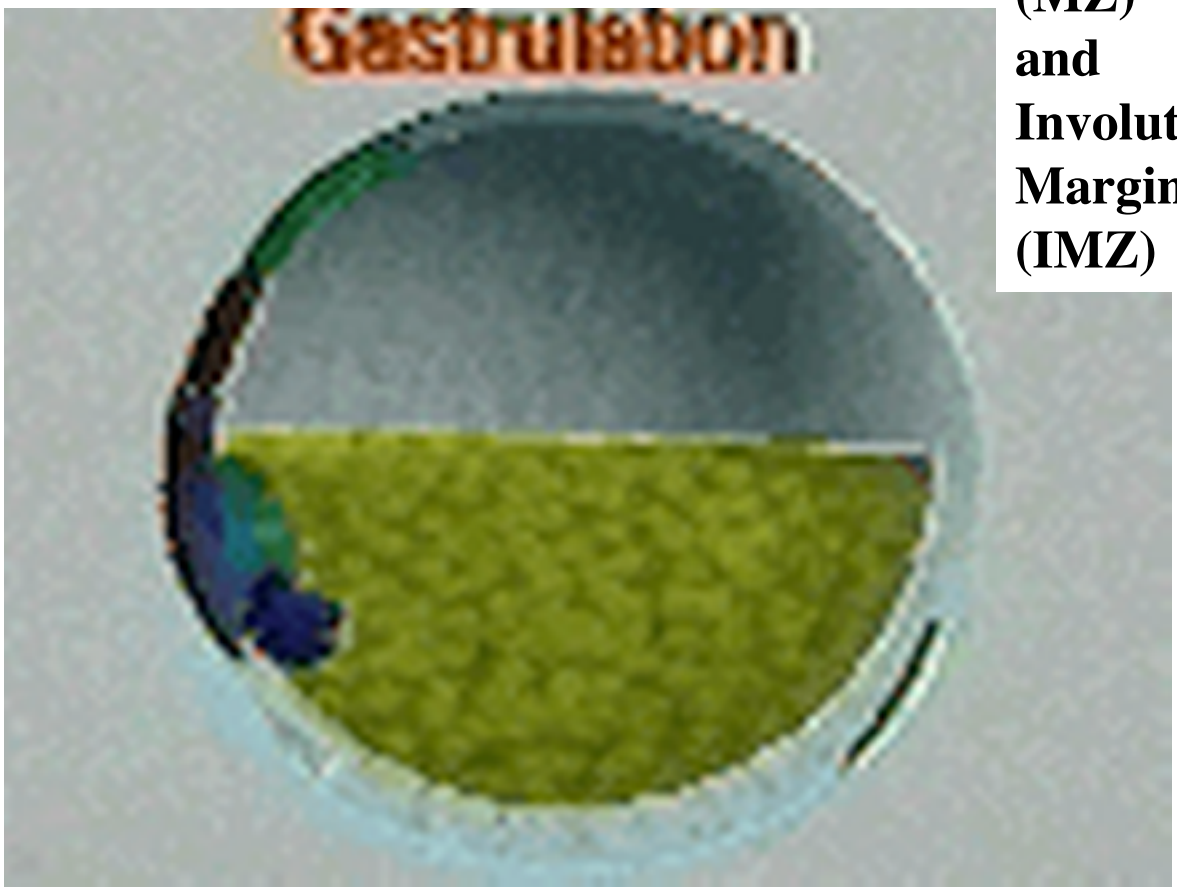
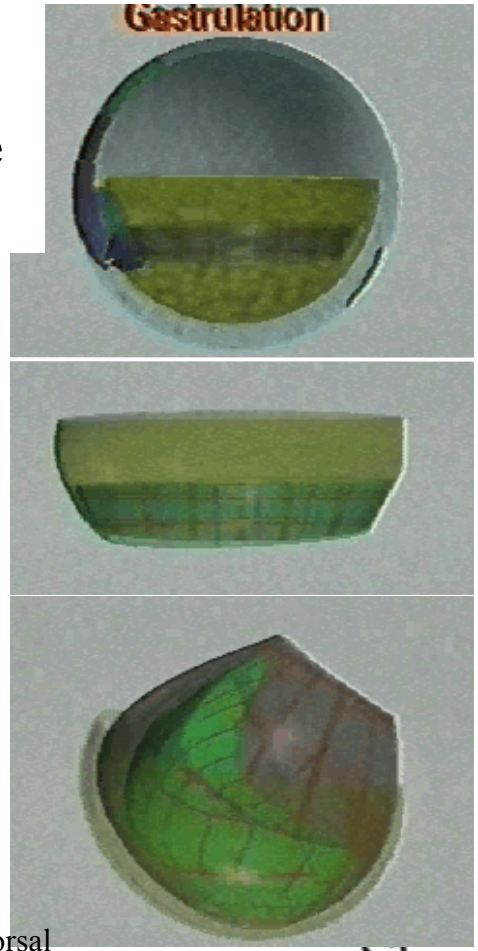
Prospective Dorsal



Marginal zone (MZ) and Involuting Marginal Zone (IMZ)



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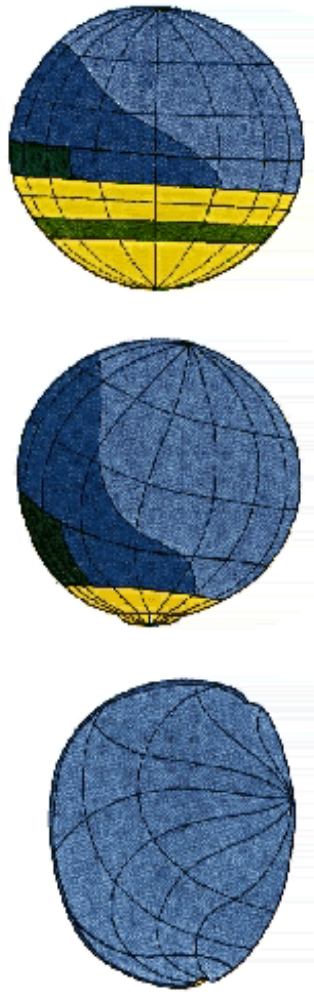
Prospective Dorsal

Surface Fate Map

The animated sequence below provides an overview of the changes in position of various tissues on the embryo's surface during gastrulation in Xenopus. These pictures are adapted directly from pictures hand-drawn by Dr. Ray Keller, Univ. of Virginia, and are based on his fate maps of superficial cells in Xenopus. Note the dramatic spreading of the light blue material (presumptive epidermis), and the convergence and extension of the dark blue material (presumptive neural ectoderm) as gastrulation proceeds. Also note that the yellow material (endoderm) becomes completely covered by ectodermal tissue by the end of gastrulation. The green material is material that will form part of the archenteron. The dorsal non-involuting marginal zone, which gives rise to the tissue of the neural plate during neurulation, undergoes convergence and extension, converging towards the dorsal side of the embryo (on the left here) and extending along the forming anterior-posterior axis.



Superficial cell movement

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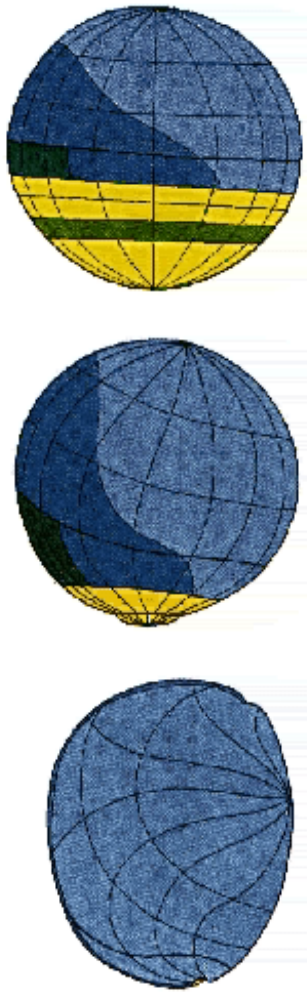
Prospective Dorsal

Superficial cell movement

Surface Fate Map

The animated sequence below provides an overview of the changes in position of various tissues on the embryo's surface during gastrulation in Xenopus. These pictures are adapted directly from pictures hand-drawn by Dr. Ray Keller, Univ. of Virginia, and are based on his fate maps of superficial cells in Xenopus. Note the dramatic spreading of the light blue material (presumptive epidermis), and the convergence and extension of the dark blue material (presumptive neural ectoderm) as gastrulation proceeds. Also note that the yellow material (endoderm) becomes completely covered by ectodermal tissue by the end of gastrulation. The green material is material that will form part of the archenteron. The dorsal non-involuting marginal zone, which gives rise to the tissue of the neural plate during neurulation, undergoes convergence and extension, converging towards the dorsal side of the embryo (on the left here) and extending along the forming anterior-posterior axis.



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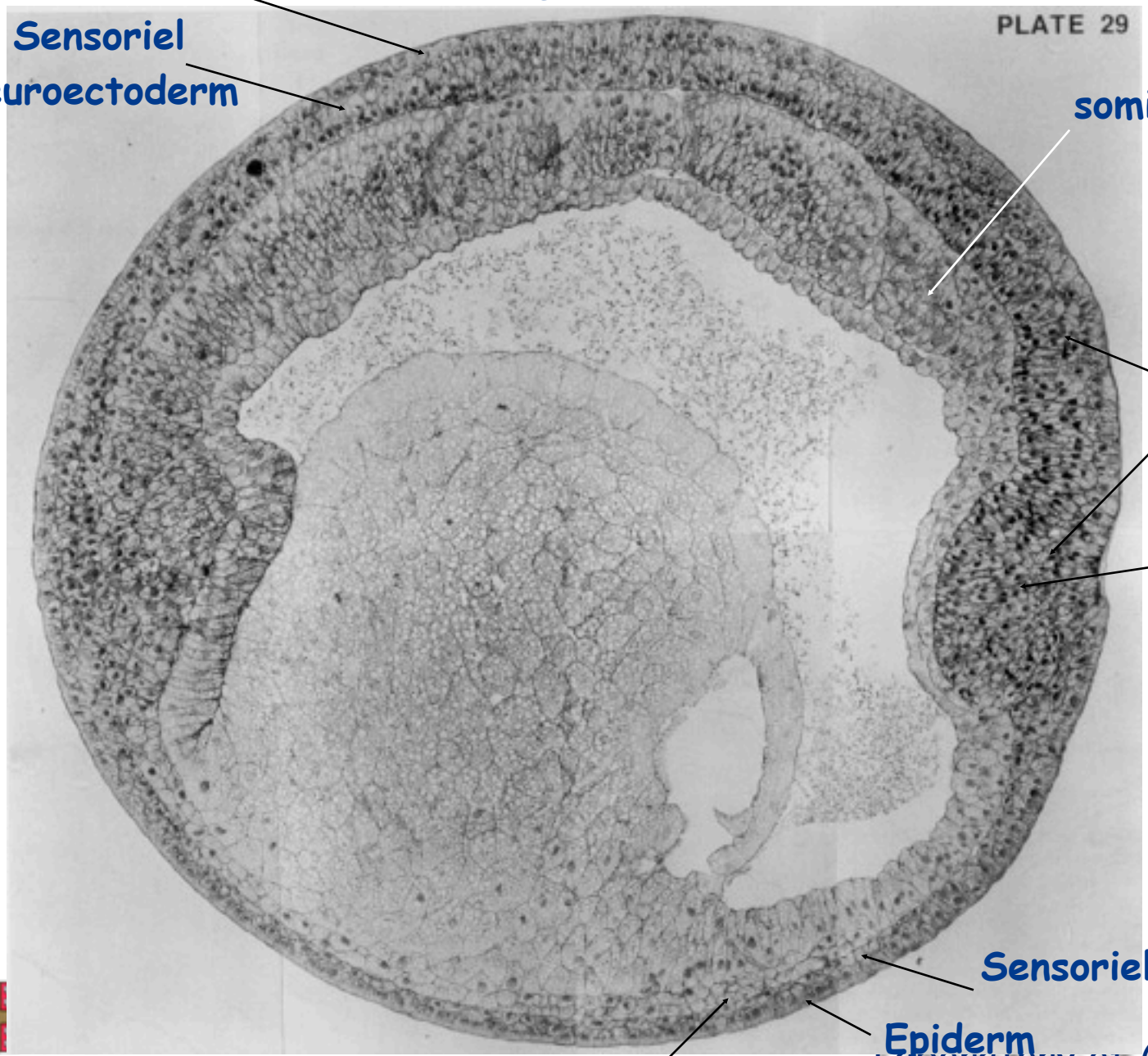


neuroectoderm

Dorsal

Sensoriel neuroectoderm

Late neural fold
Stage 17

somites

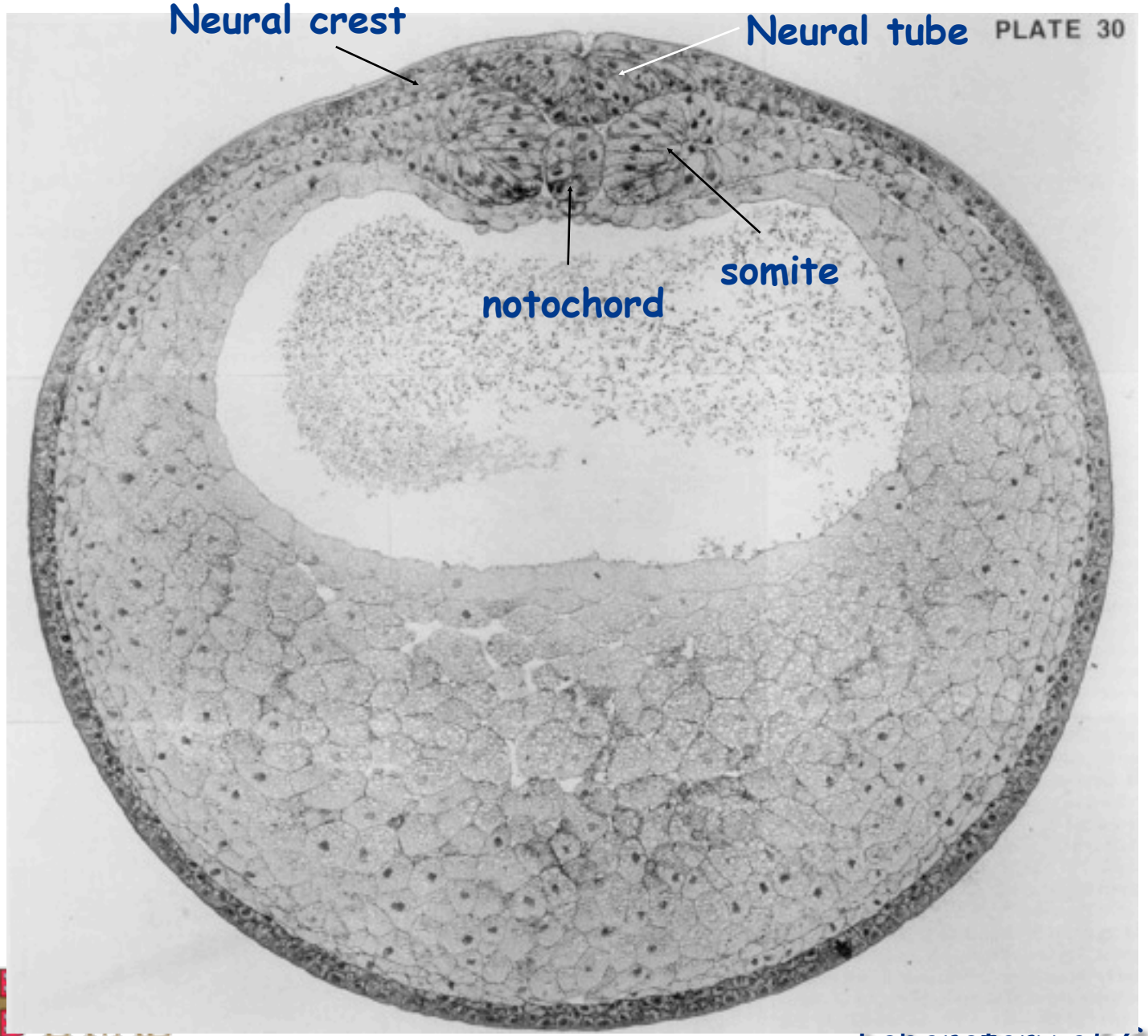
Brain anlage

Eye anlage

Sensoriel epiderm

Epiderm

Ventral mesoderm



Neural groove
Stage 18

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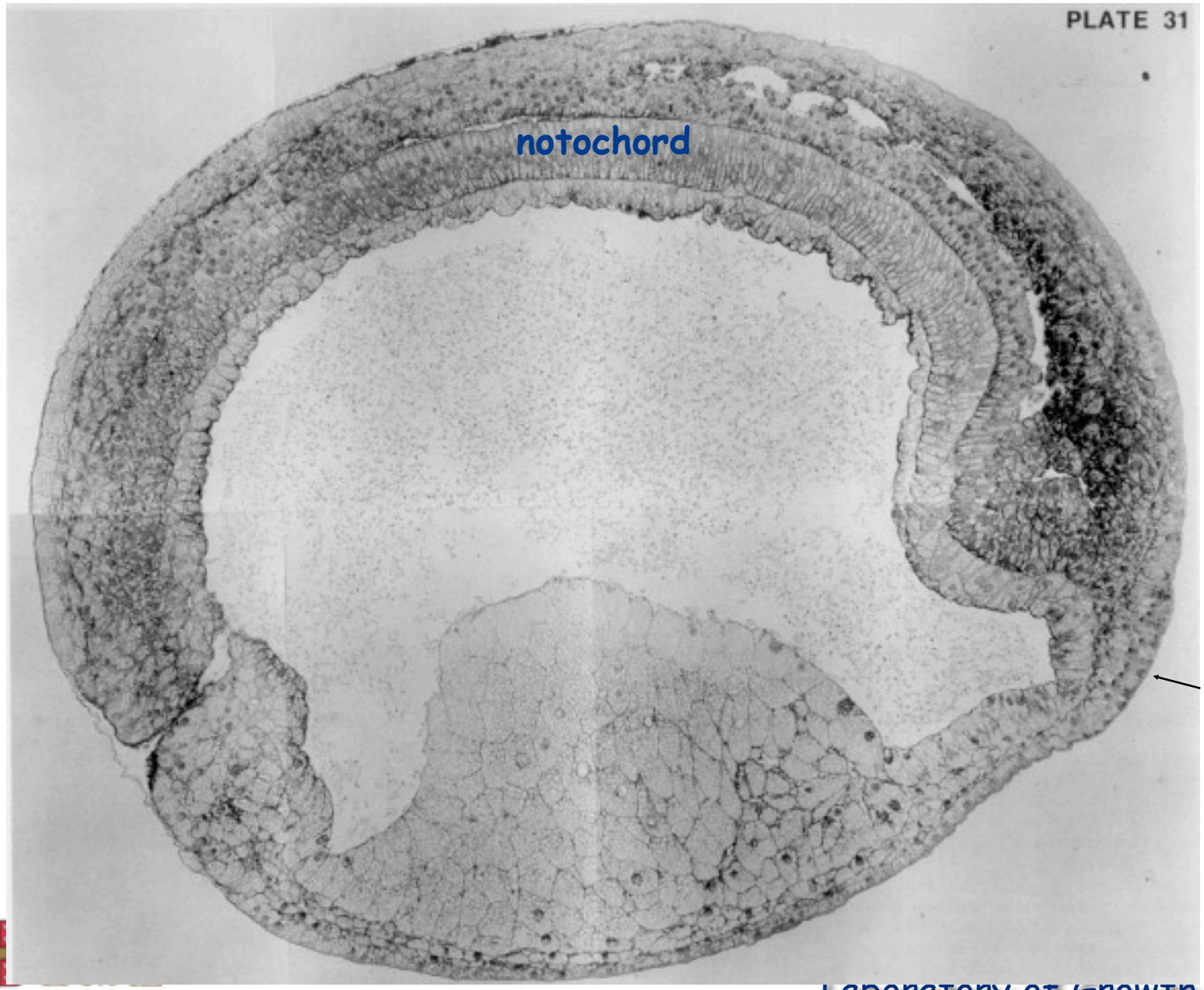
Dorsal



Neural tube
Stage 20

Cement gland

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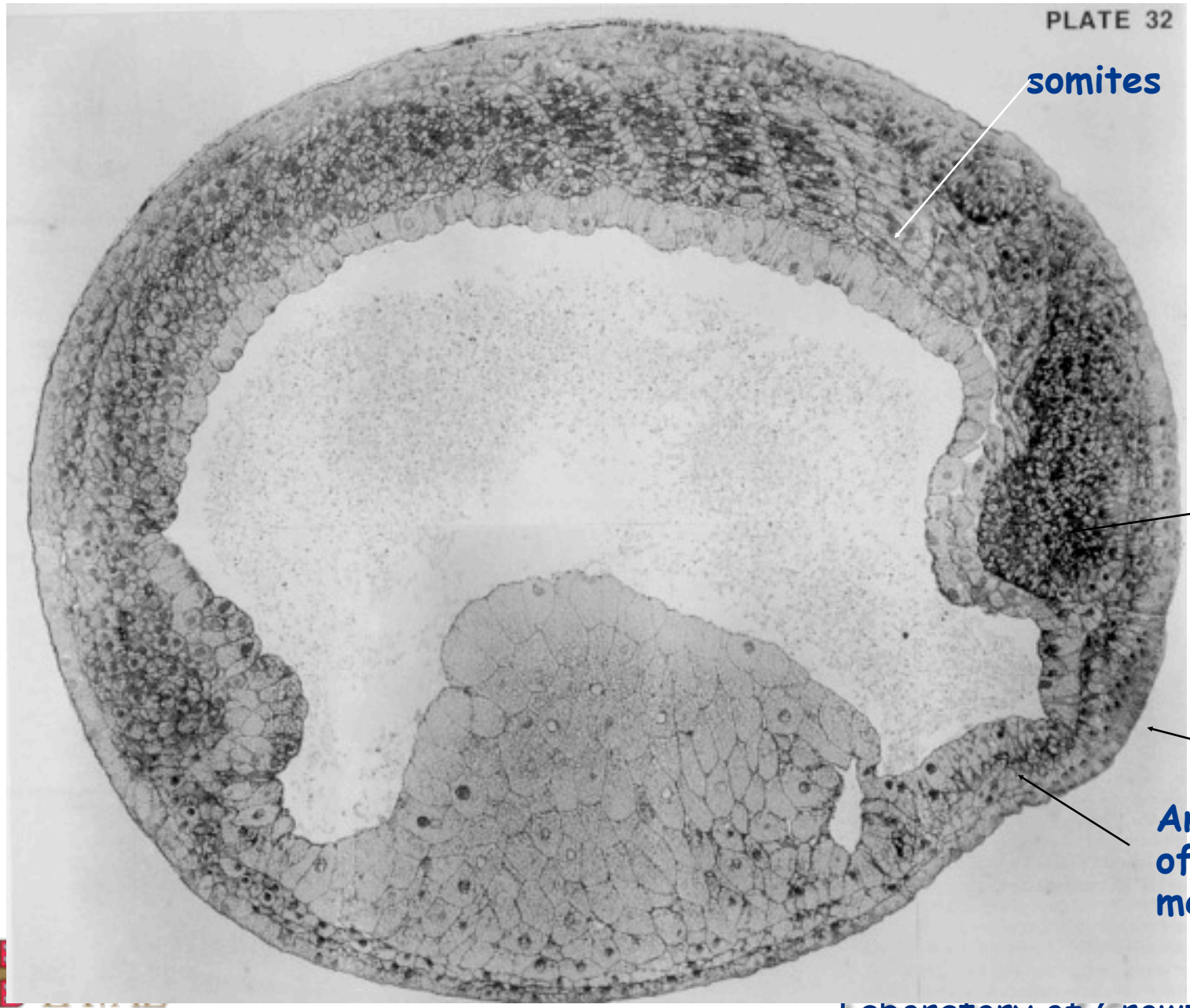
Dorsal



somites

Neural tube, Stage 20 Slightly off longitudinal axis

Eye anlage

Cement gland

Anterior border of ventral mesoderm

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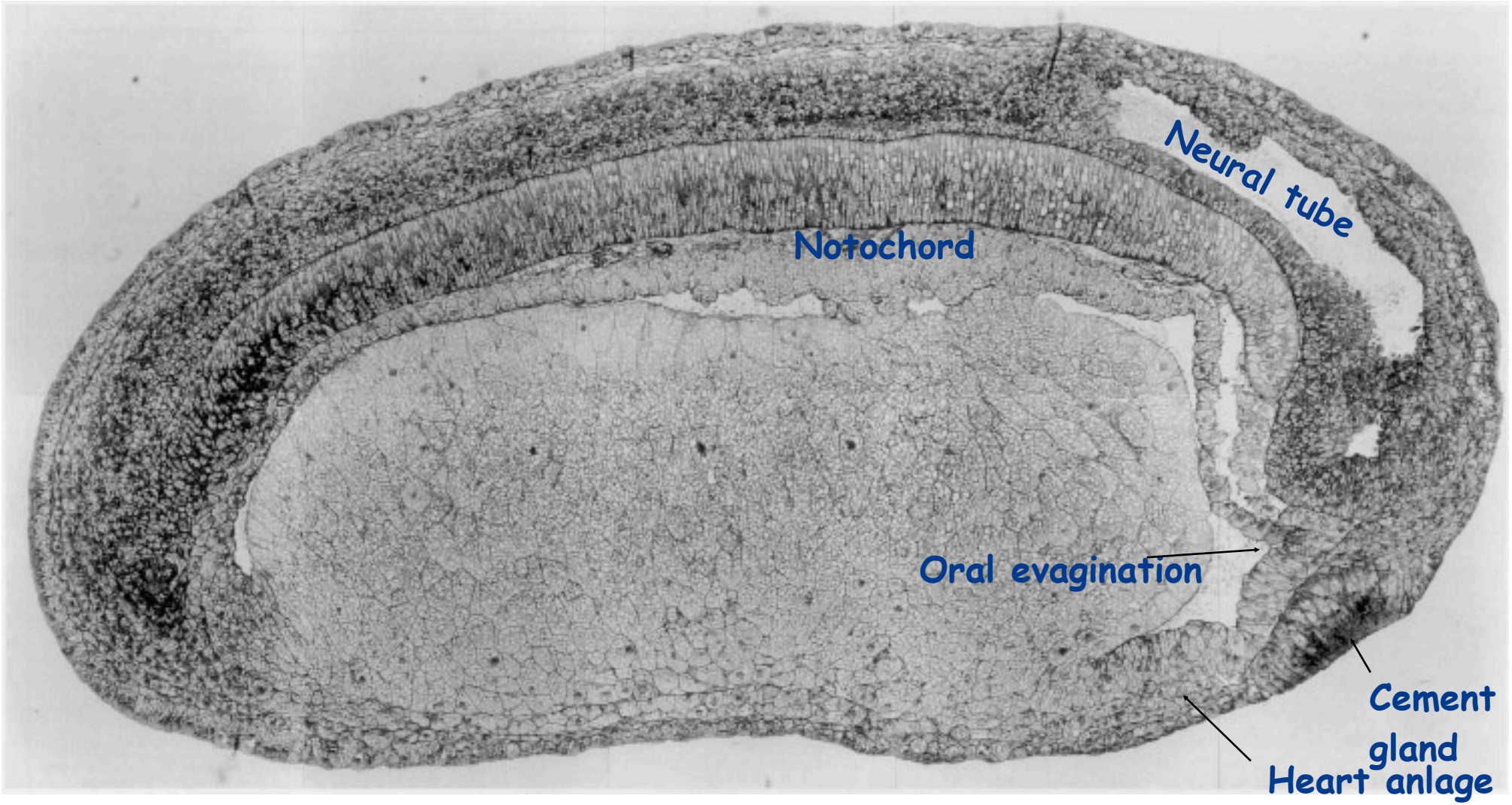
Early tailbud, Stage 23
(1 day after fertilisation)

Dorsal



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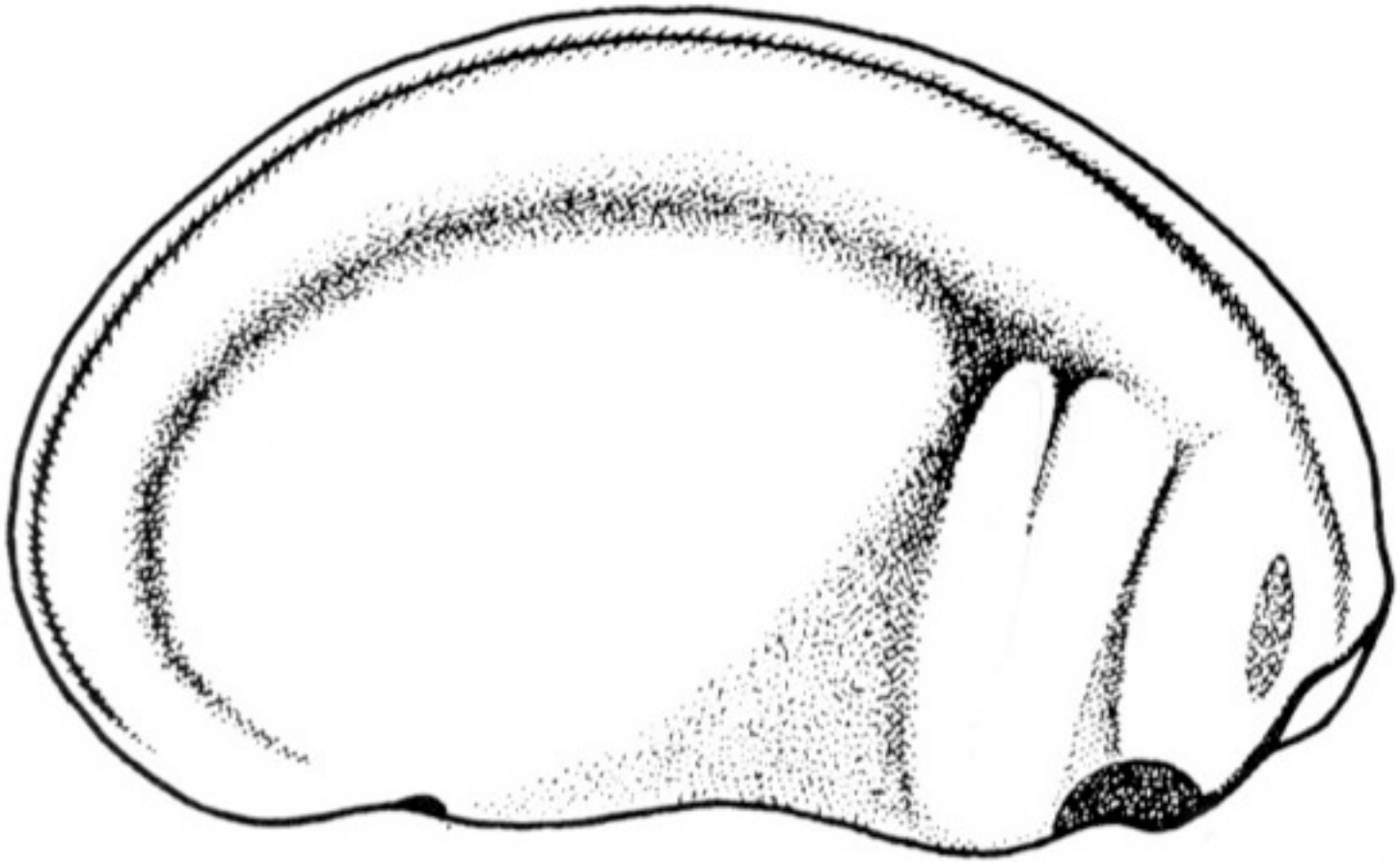

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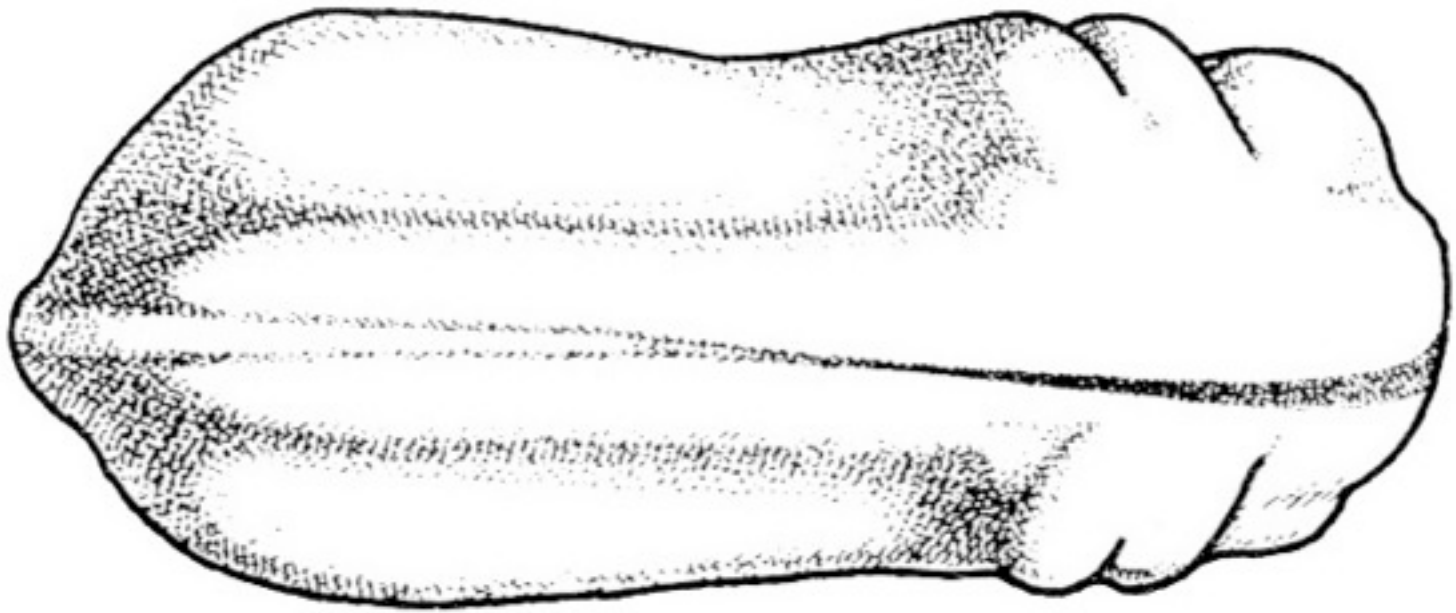

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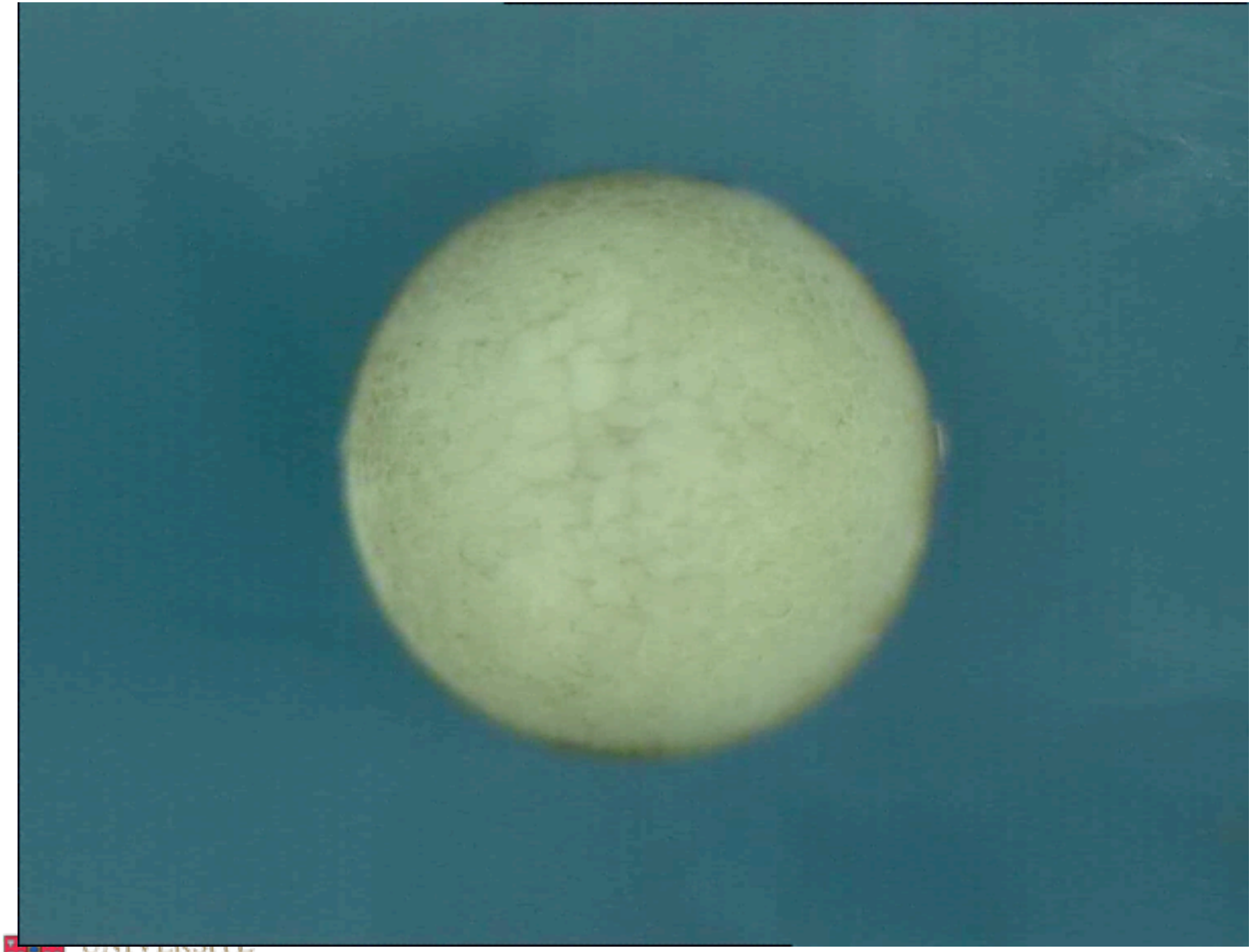
Laboratory of Growth and Development



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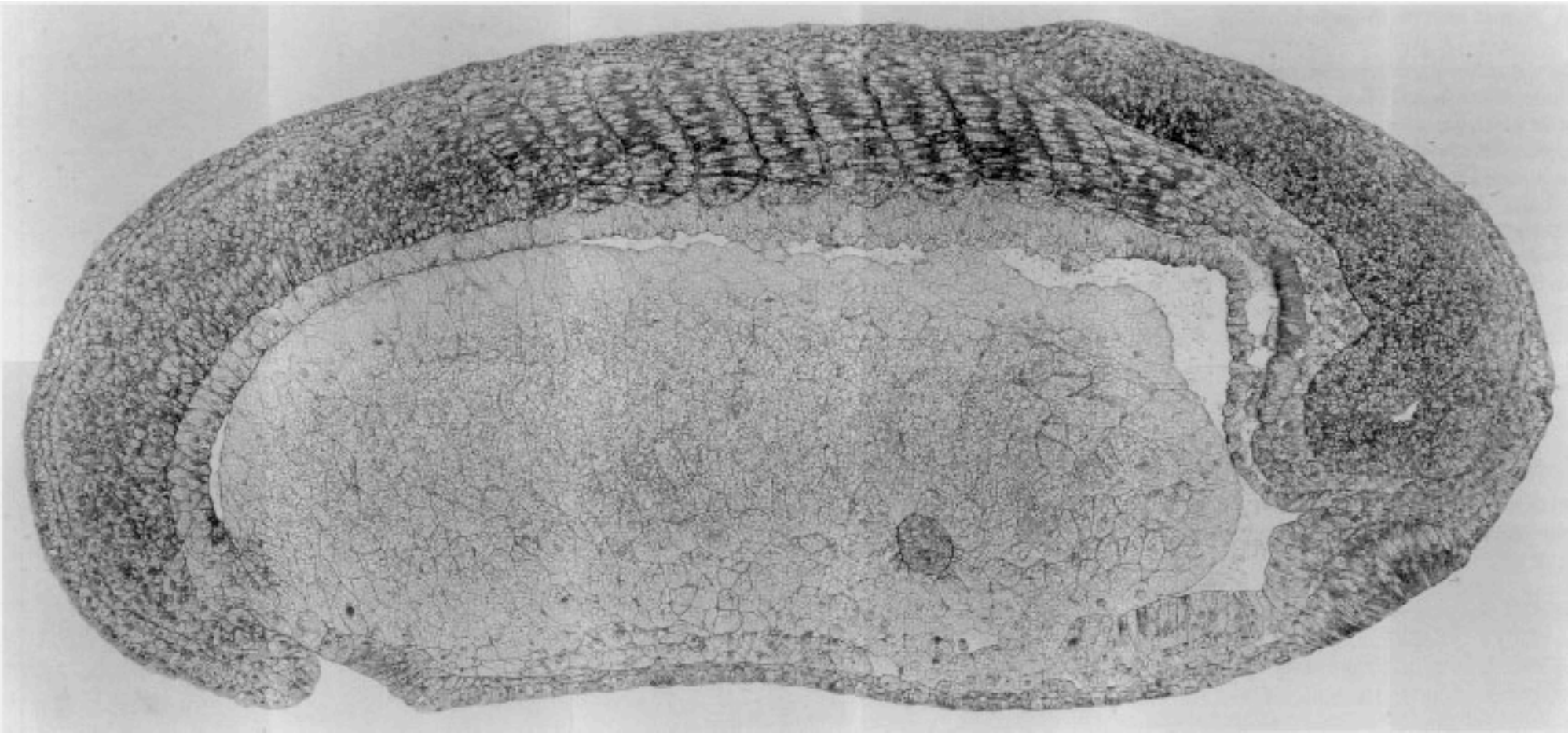
Early tailbud, Stage 23

Off axis

Dorsal



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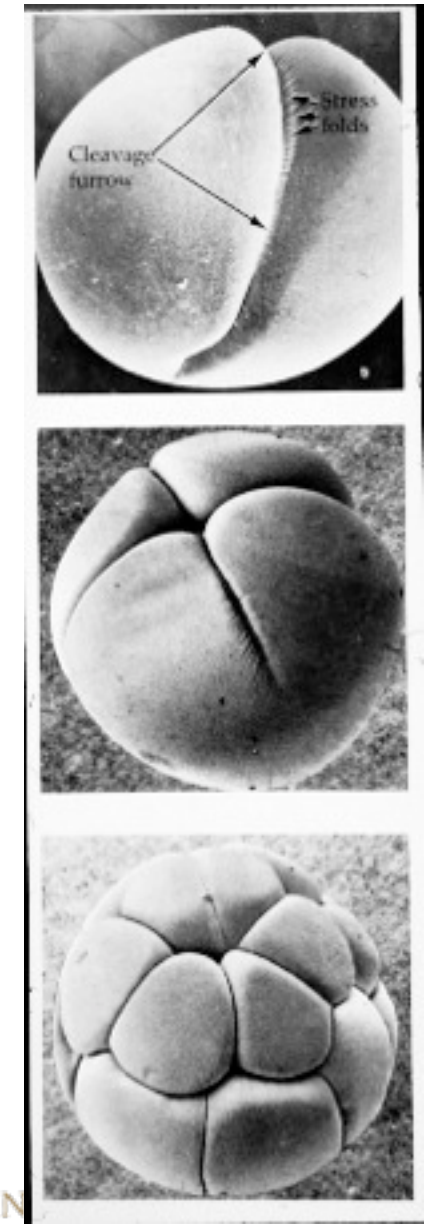
Early cleavage (reduction) divisions



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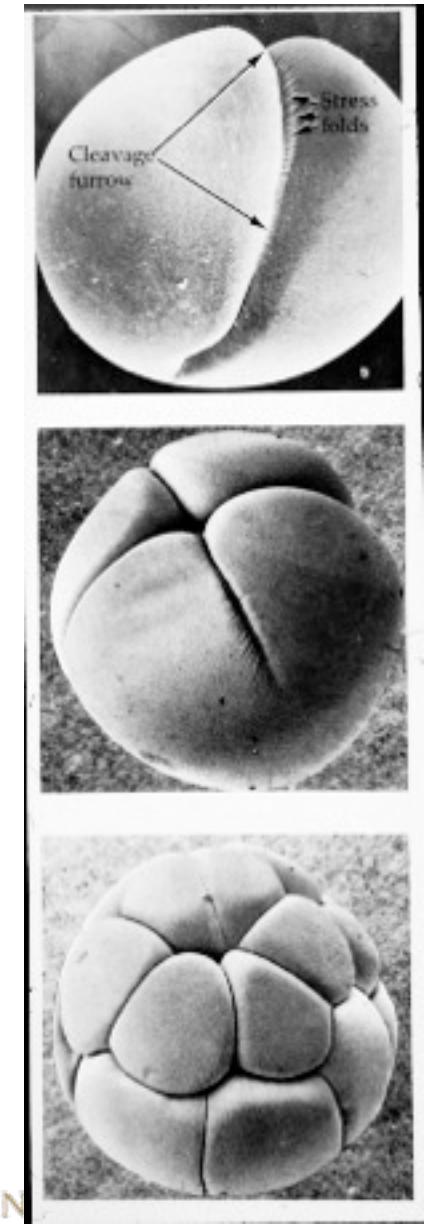

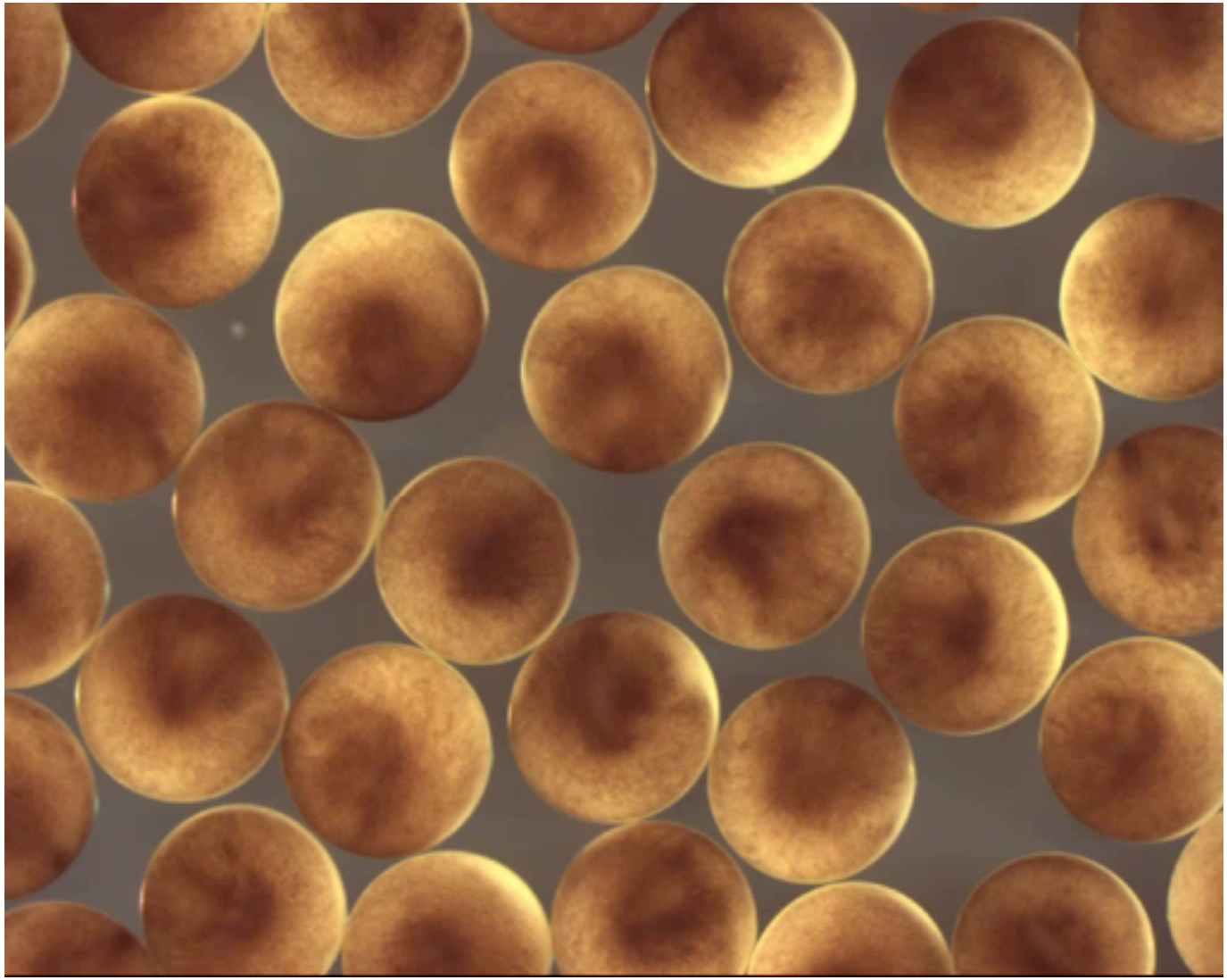

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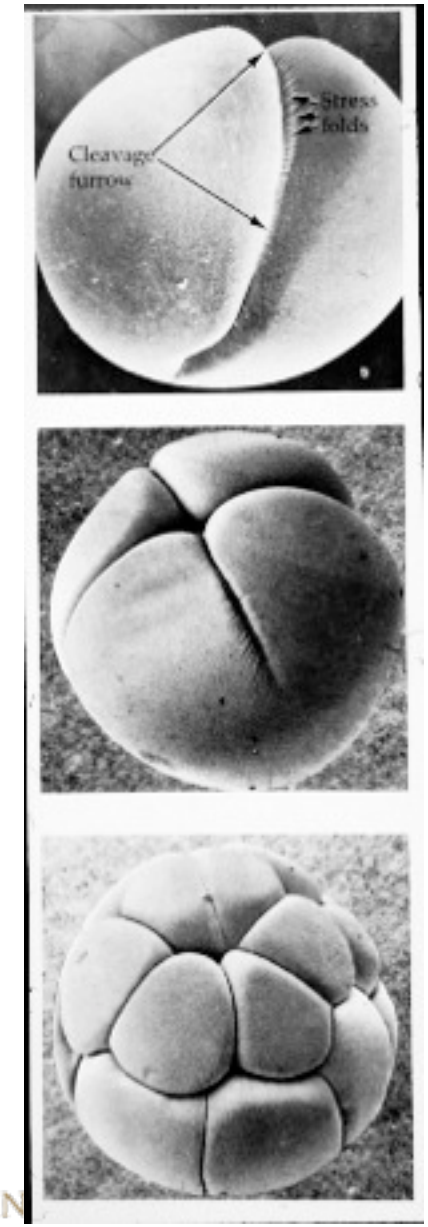

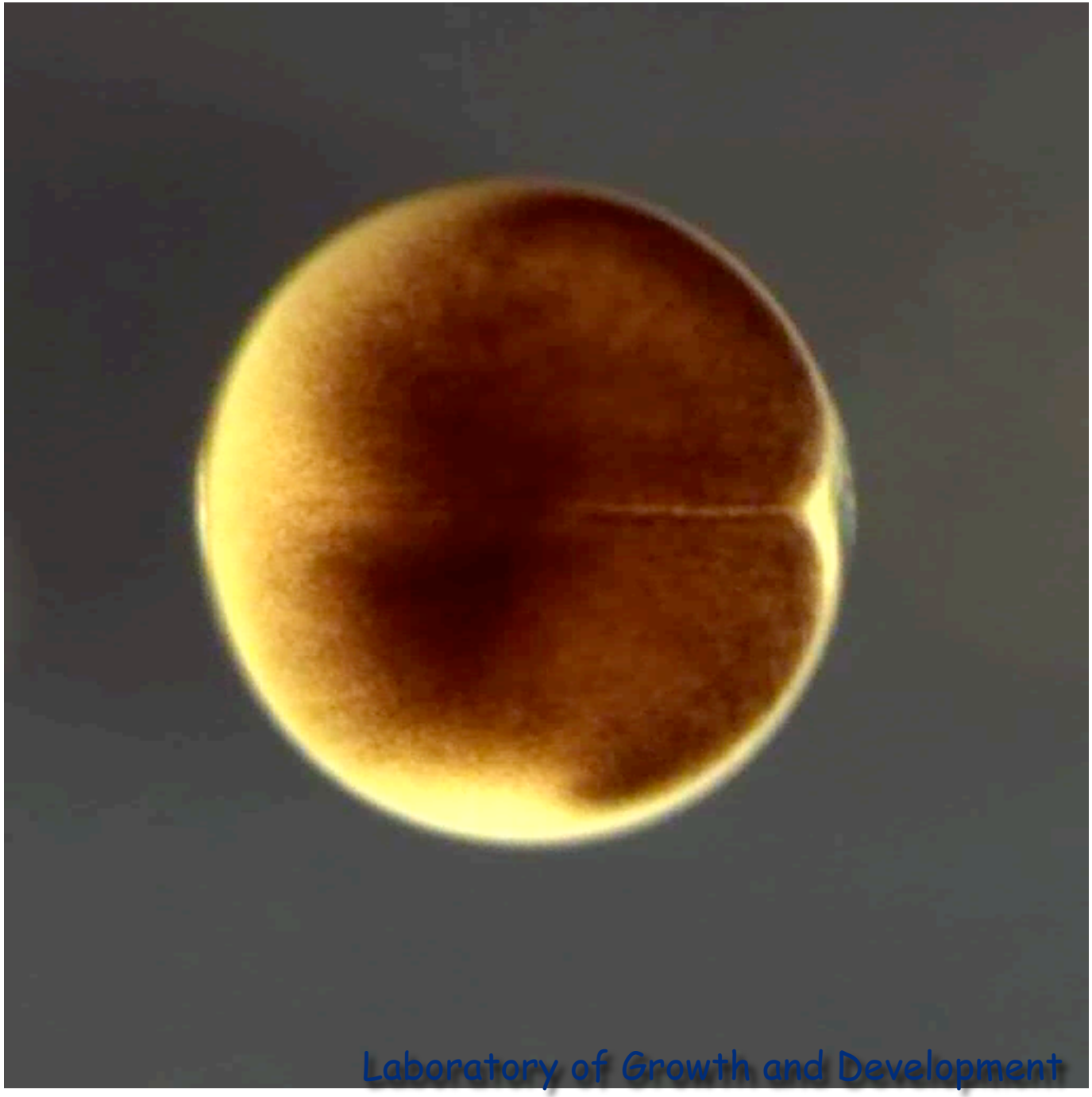

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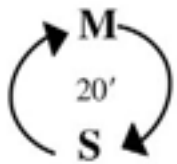
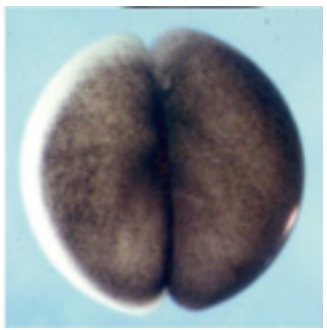
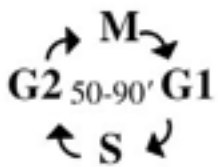
a Fertilized egg

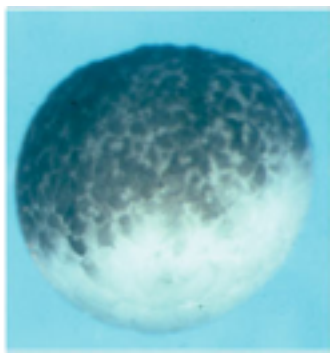
b Two-cell stage



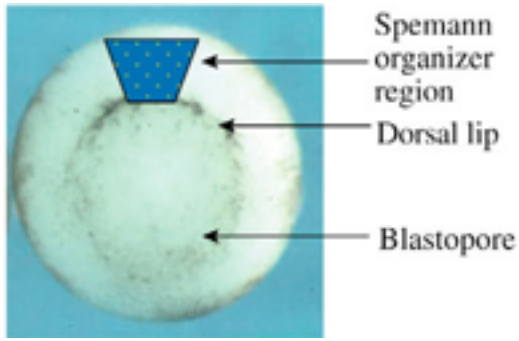


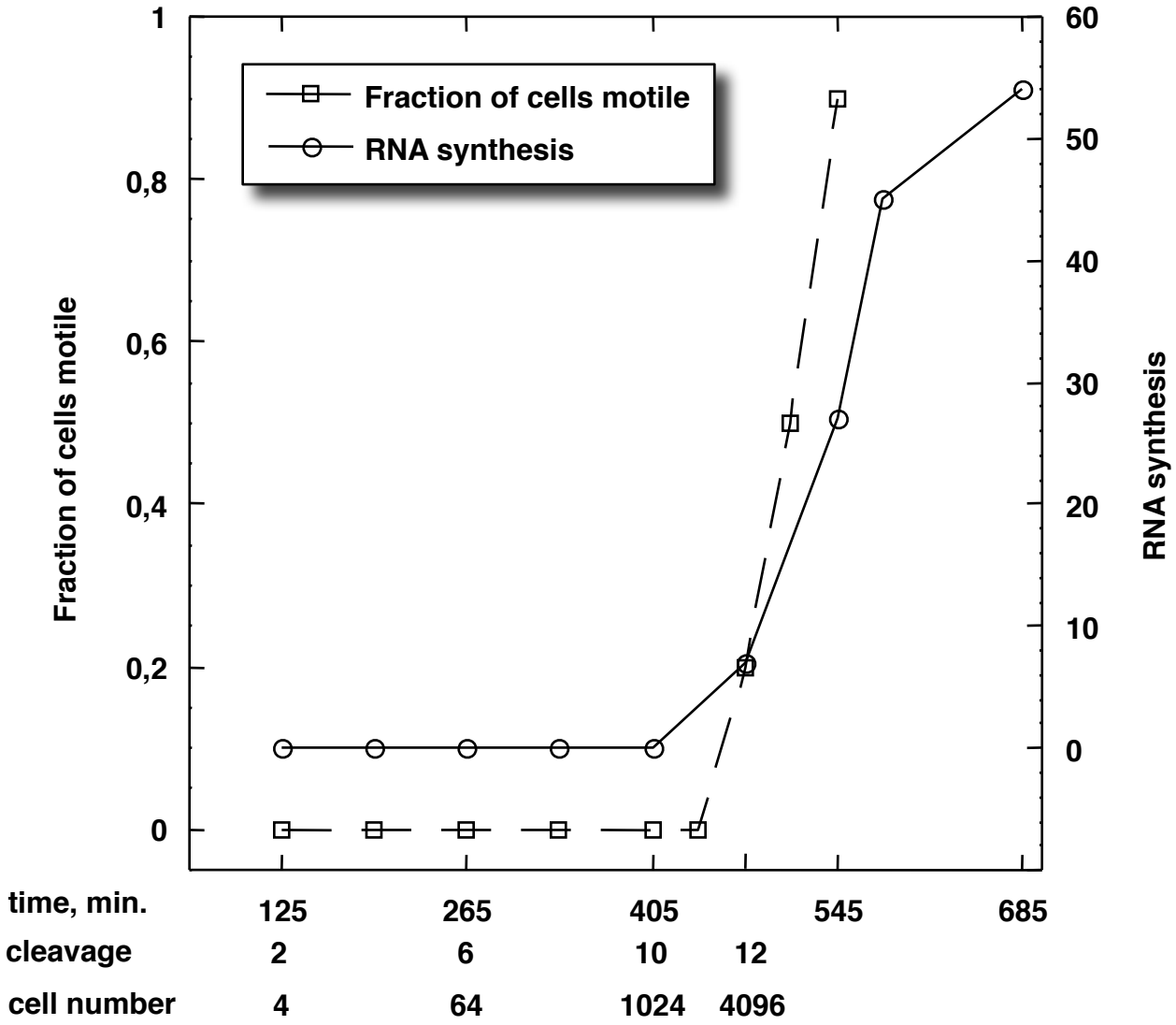
c Mid-blastula stage





d Early gastrula stage





Commitment in the early embryo precedes gene expression;

The Mid Blastula Transition

- mRNA and tRNA/5S synthesis is first to be detected
- Cell division becomes asynchronous

From Newport & Kirschner '82

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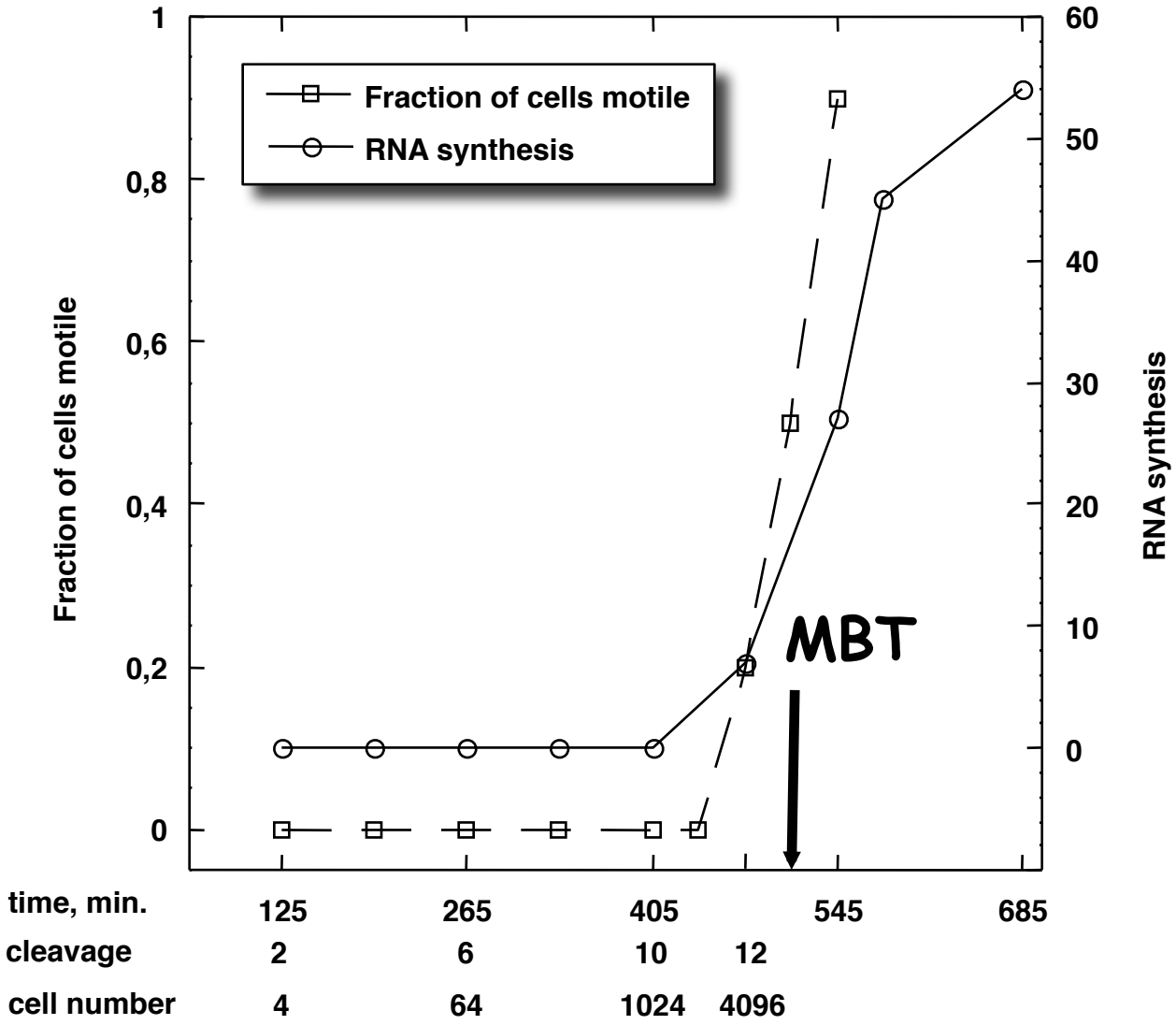

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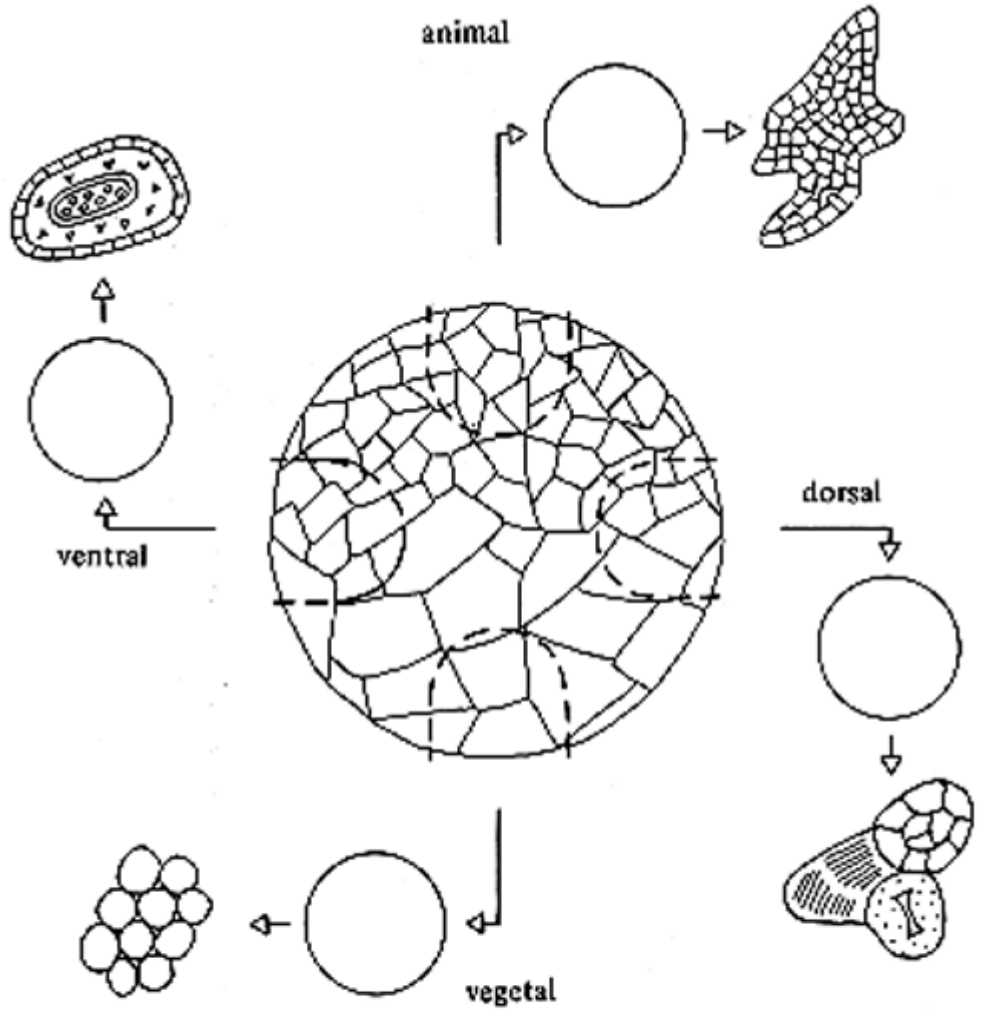
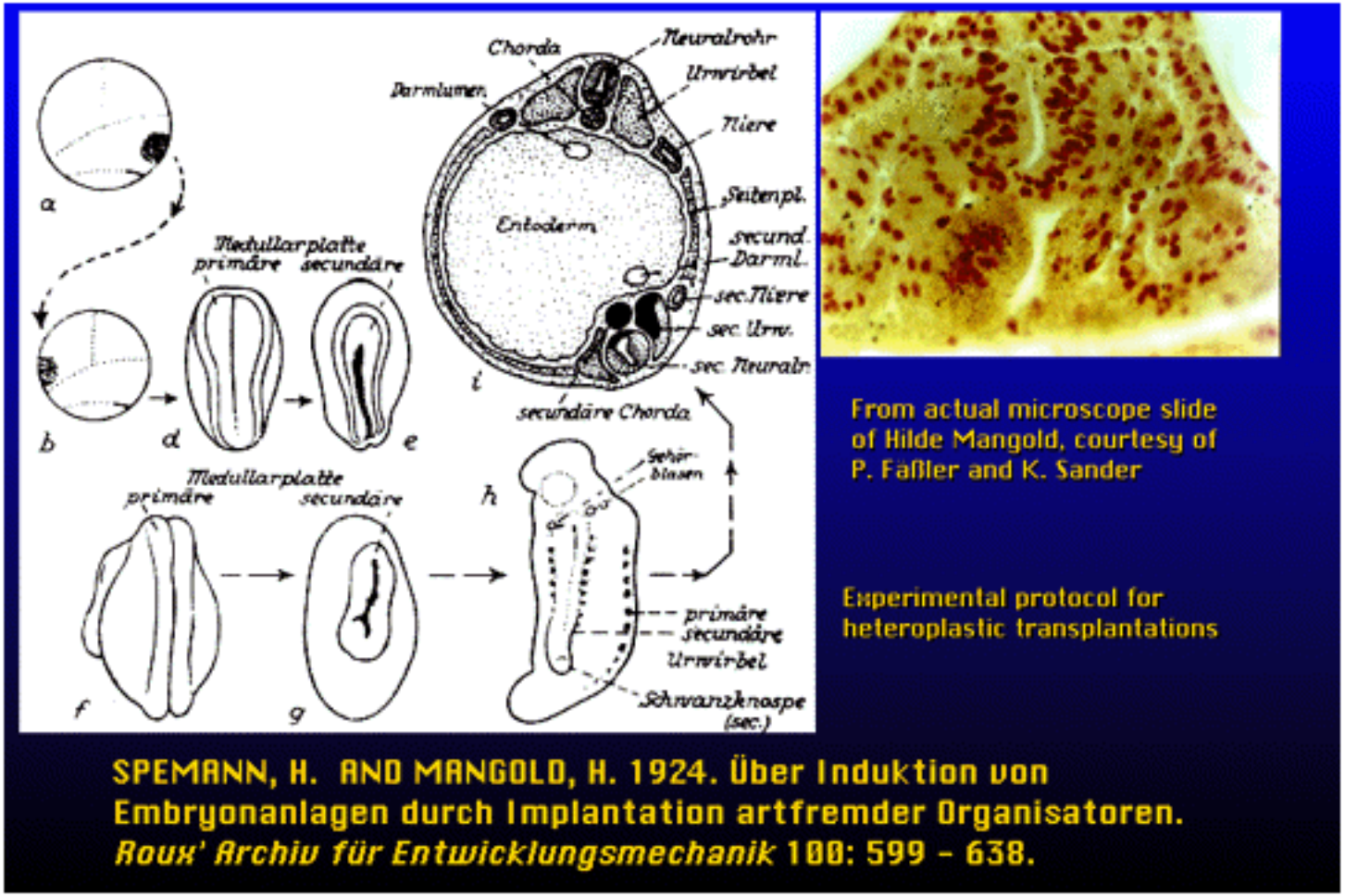


Fig. 3.11. Self-differentiation of isolates from different regions of the amphibian blastula. Isolates from the animal cap produce only epidermis, isolates from the vegetal region fail to differentiate, isolates from the dorsal marginal zone produce notochord, muscle and neuroepithelium, while isolates from the ventral marginal zone produce epidermis, mesenchyme and erythrocytes.

Commitment in the early embryo

Axis determination

The Mangold-Spemann Organiser



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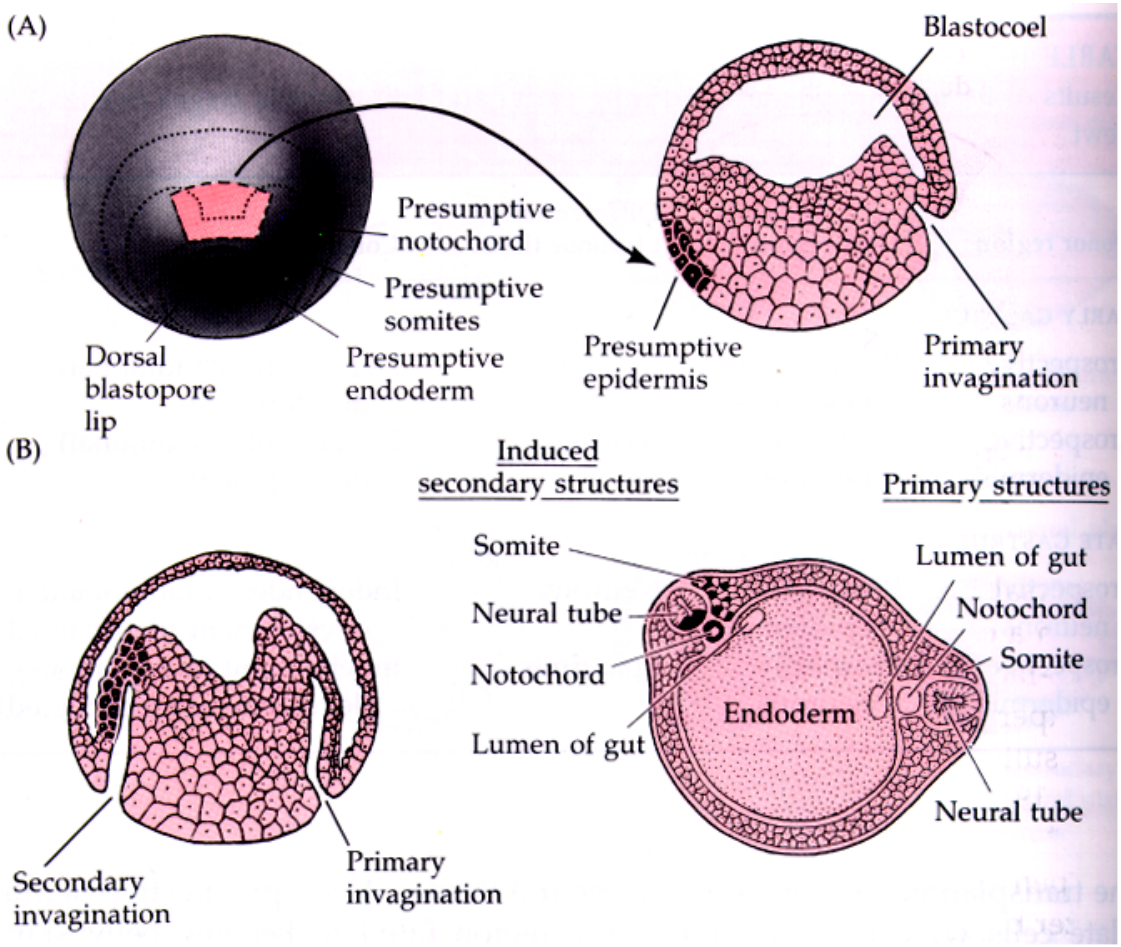
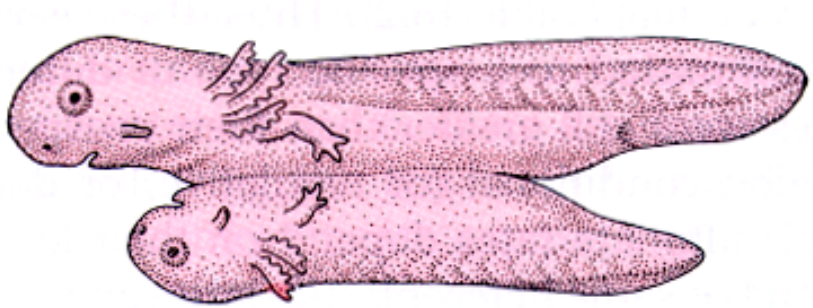




Self-differentiation of the dorsal blastopore lip tissue. (A) Dorsal blastopore lip from early gastrula is transplanted into another early gastrula in the region that normally becomes ventral epidermis. (B) Tissue invaginates and forms a second archenteron and then a second embryonic axis. Both donor and host tissues are seen in the new neural tube, notochord, and somites. (C) Eventually, a second embryo forms that is joined to host.

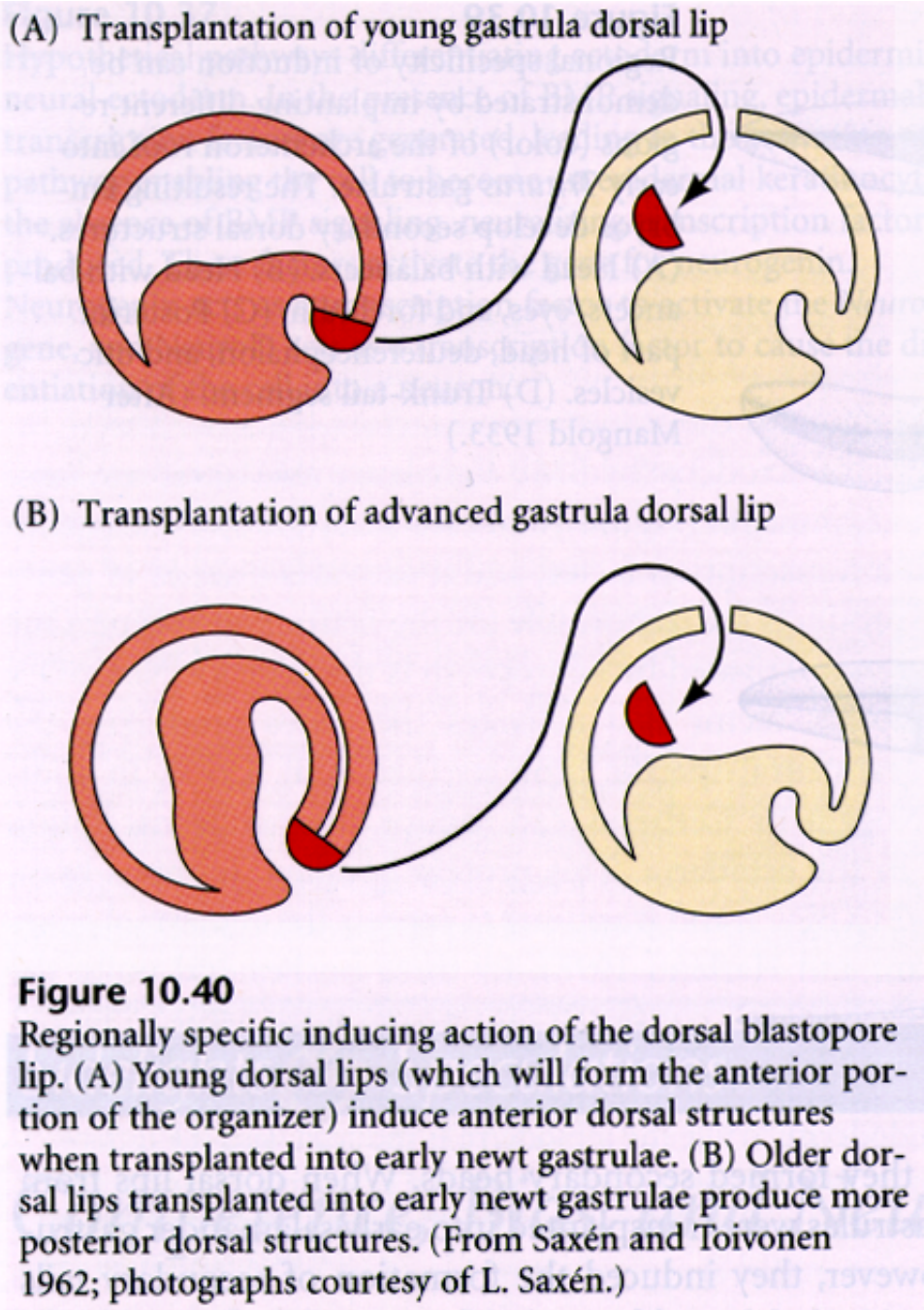
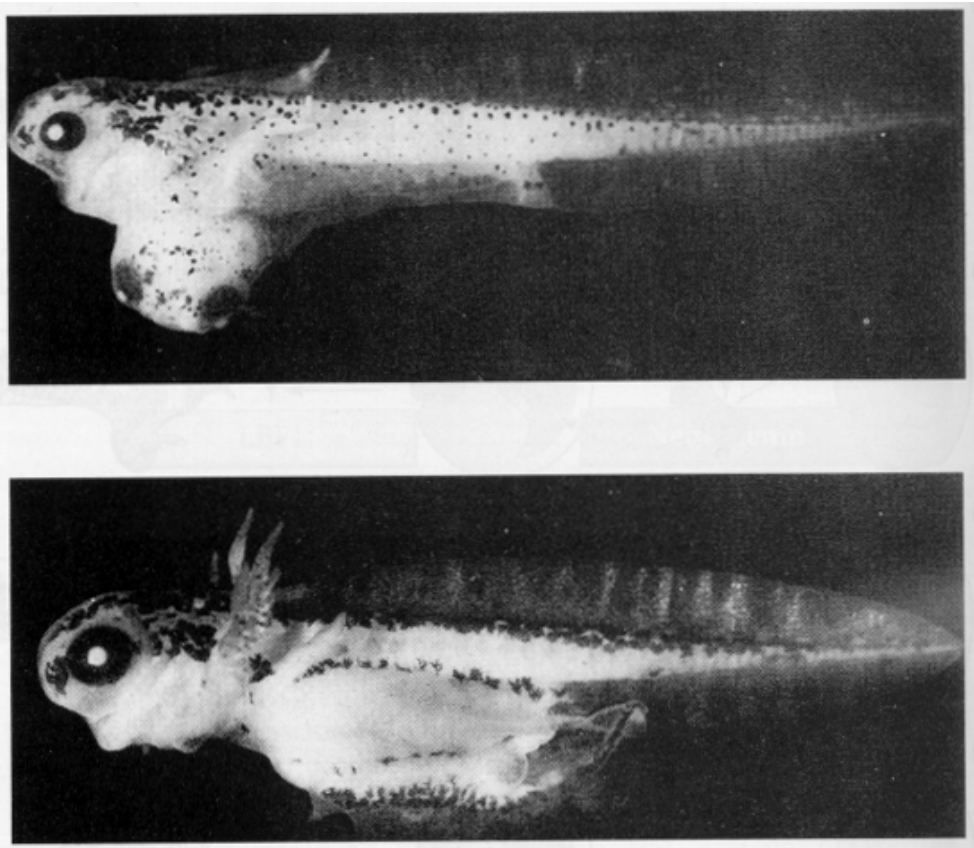


Figure 10.40
Regionally specific inducing action of the dorsal blastopore lip. (A) Young dorsal lips (which will form the anterior portion of the organizer) induce anterior dorsal structures when transplanted into early newt gastrulae. (B) Older dorsal lips transplanted into early newt gastrulae produce more posterior dorsal structures. (From Saxén and Toivonen 1962; photographs courtesy of L. Saxén.)



"Einsteck" technique determines changes in the inducing capacity of the dorsal blastopore lip.

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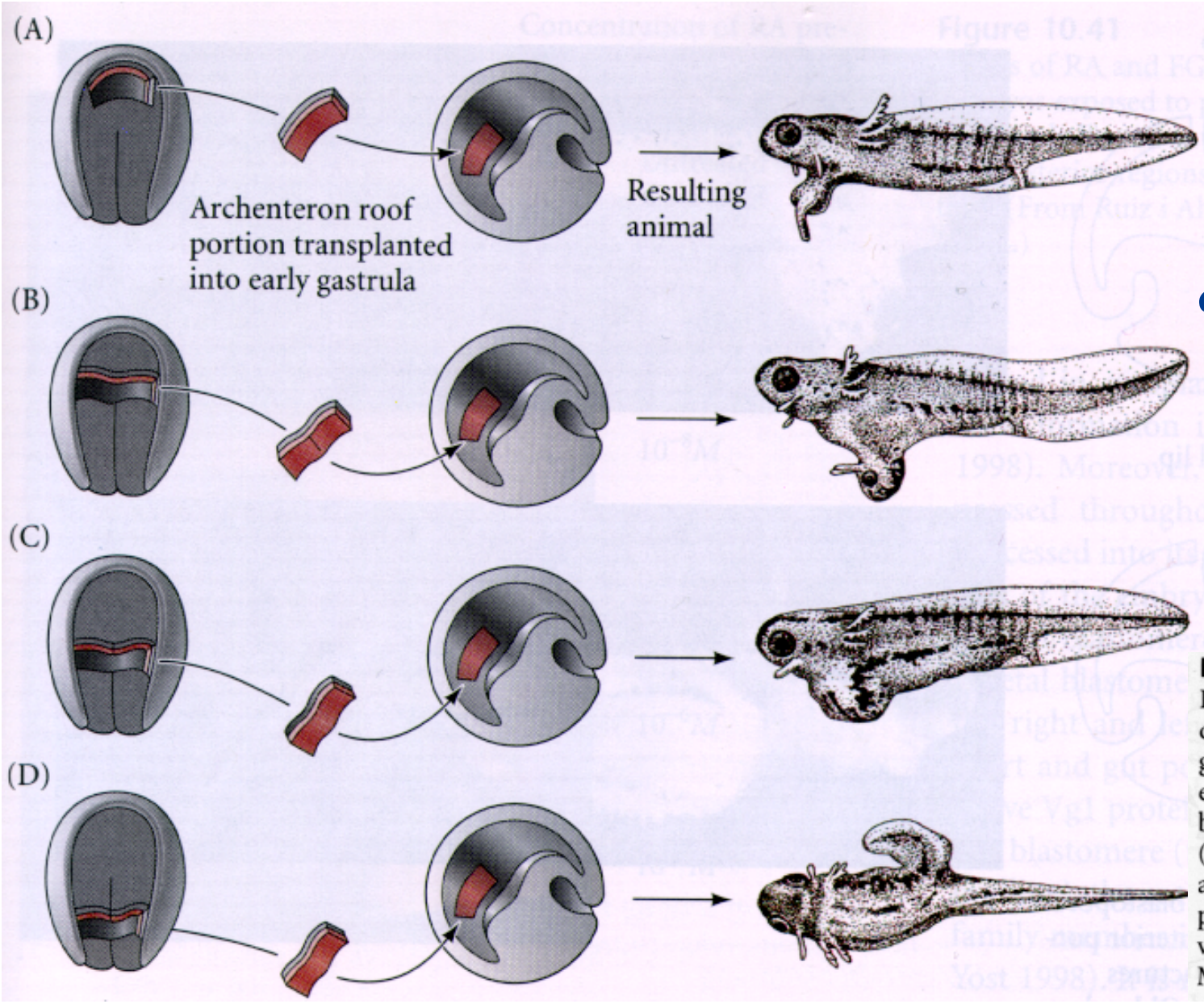

Einsteck shows the inductive capacity of early neuroectoderm varies along the anterior-posterior axis

Figure 10.39
Regional specificity of induction can be demonstrated by implanting different regions (color) of the archenteron roof into early *Triturus* gastrulae. The resulting embryos develop secondary dorsal structures. (A) Head with balancers. (B) Head with balancers, eyes, and forebrain. (C) Posterior part of head, deuterencephalon, and otic vesicles. (D) Trunk-tail segment. (After Mangold 1933.)

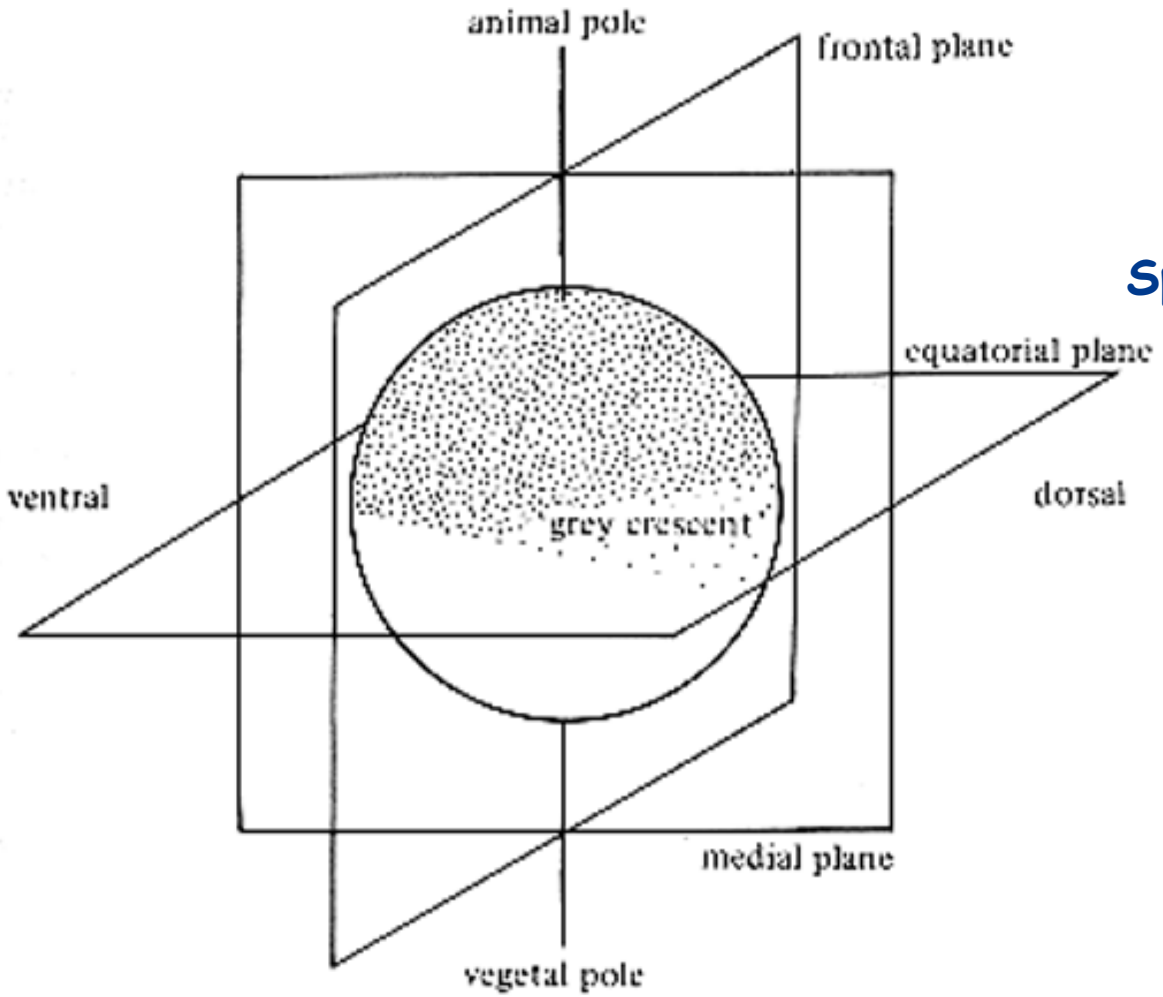

Fig. 3.1. Axes of the amphibian egg after fertilisation.

Sperm entry

Prospective dorsal

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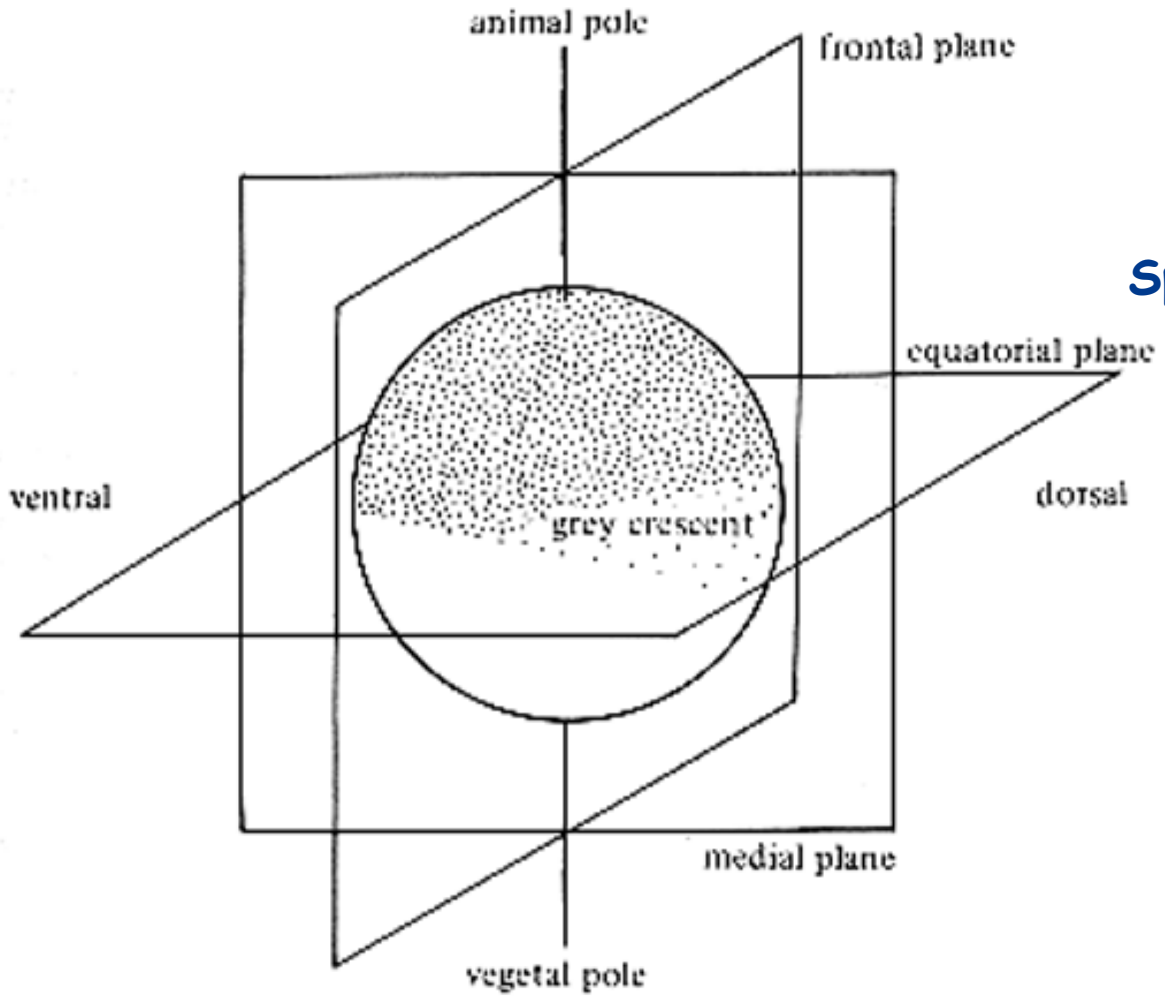
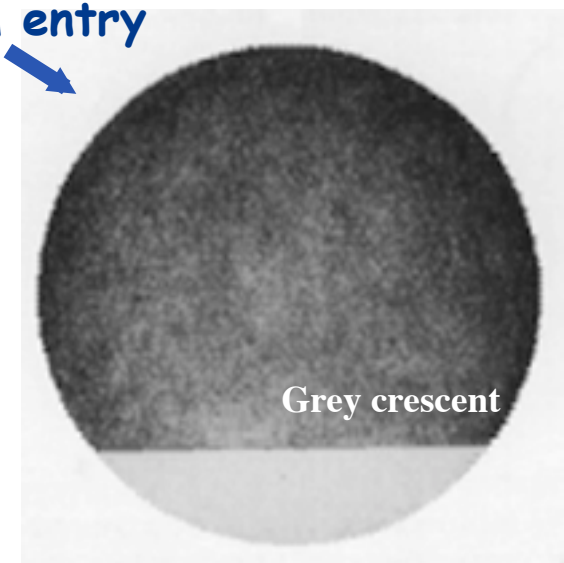


Fig. 3.1. Axes of the amphibian egg after fertilisation.

Sperm entry



Prospective dorsal

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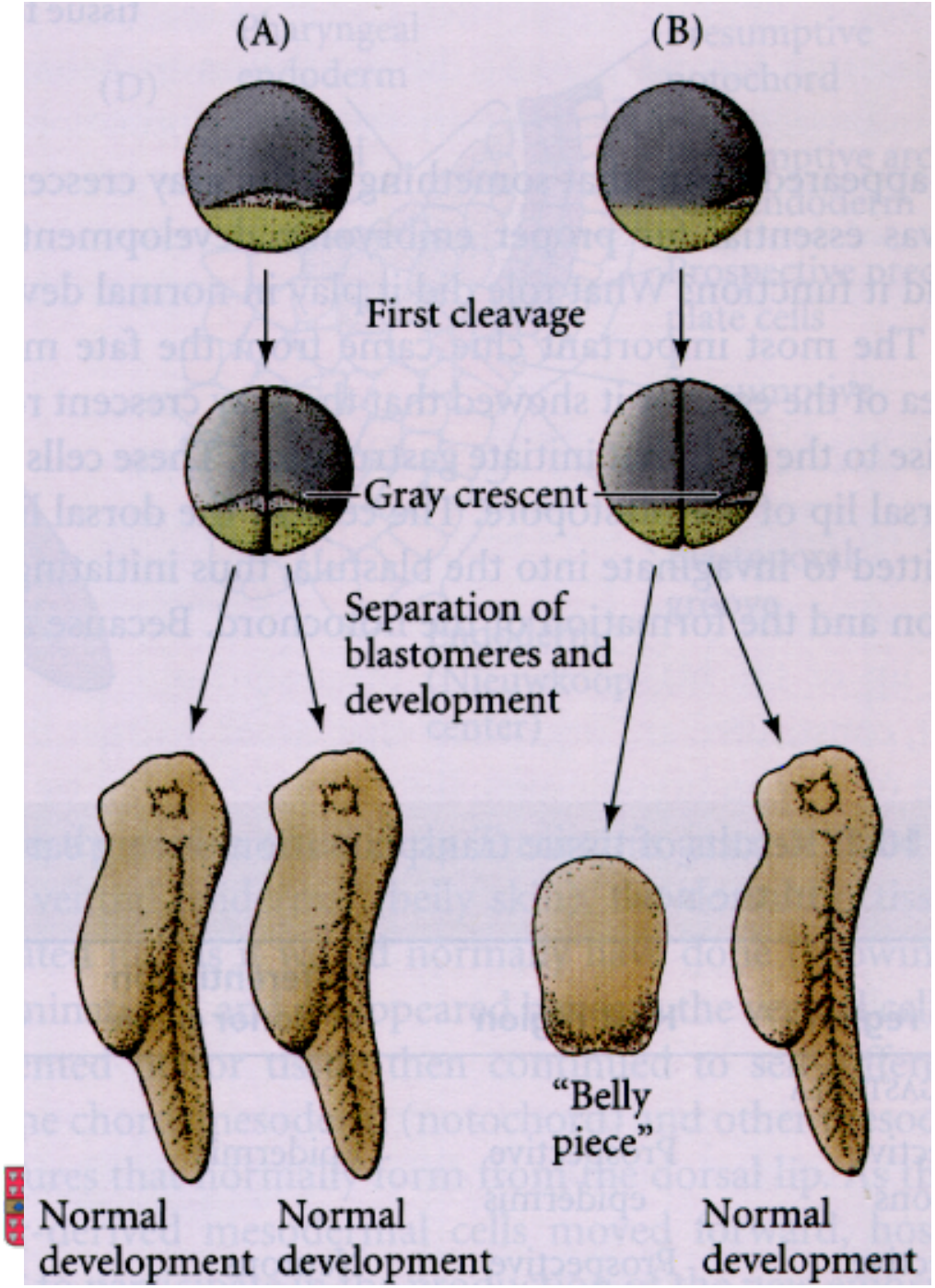


Importance of the Grey Crescent to axis formation

Figure 10.18
Asymmetry in the amphibian egg. (A) When the egg is divided along the plane of first cleavage into two blastomeres, each of which gets one-half of the gray crescent, each experimentally separated cell develops into a normal embryo. (B) When only one of the two blastomeres receives the entire gray crescent, it alone forms a normal embryo. The other half produces a mass of unorganized tissue lacking dorsal structures. (After Spemann 1938.)

Determination of Dorsal-Ventral polarity

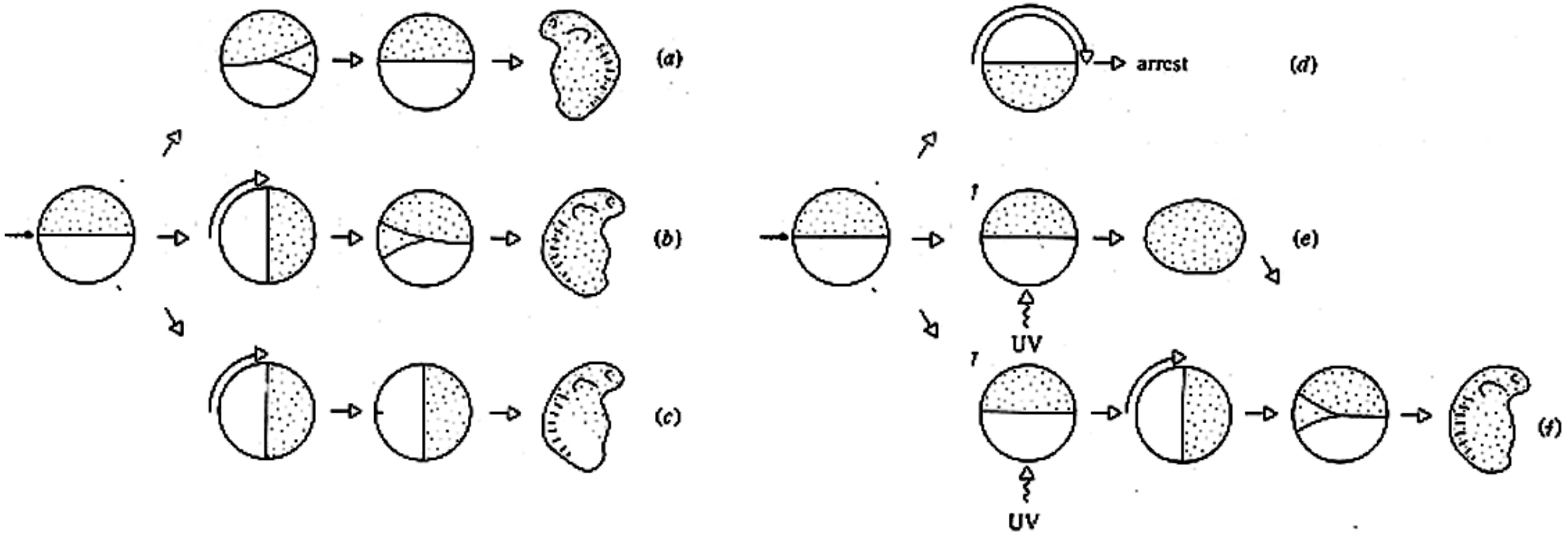
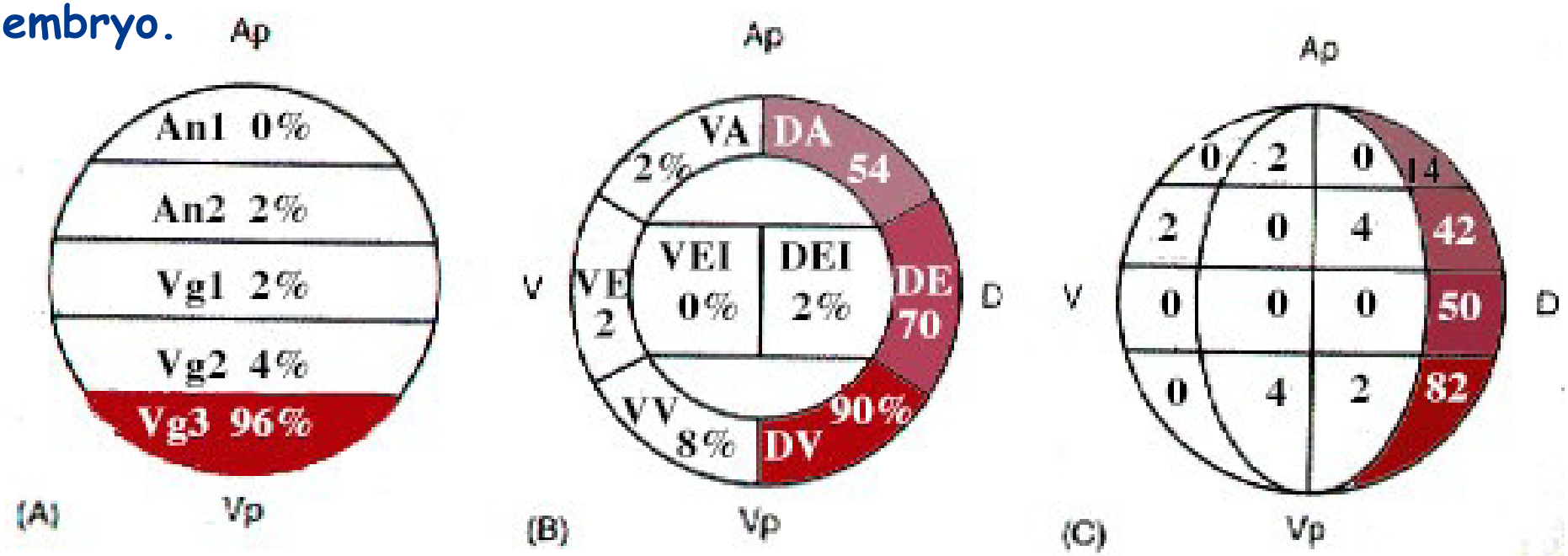


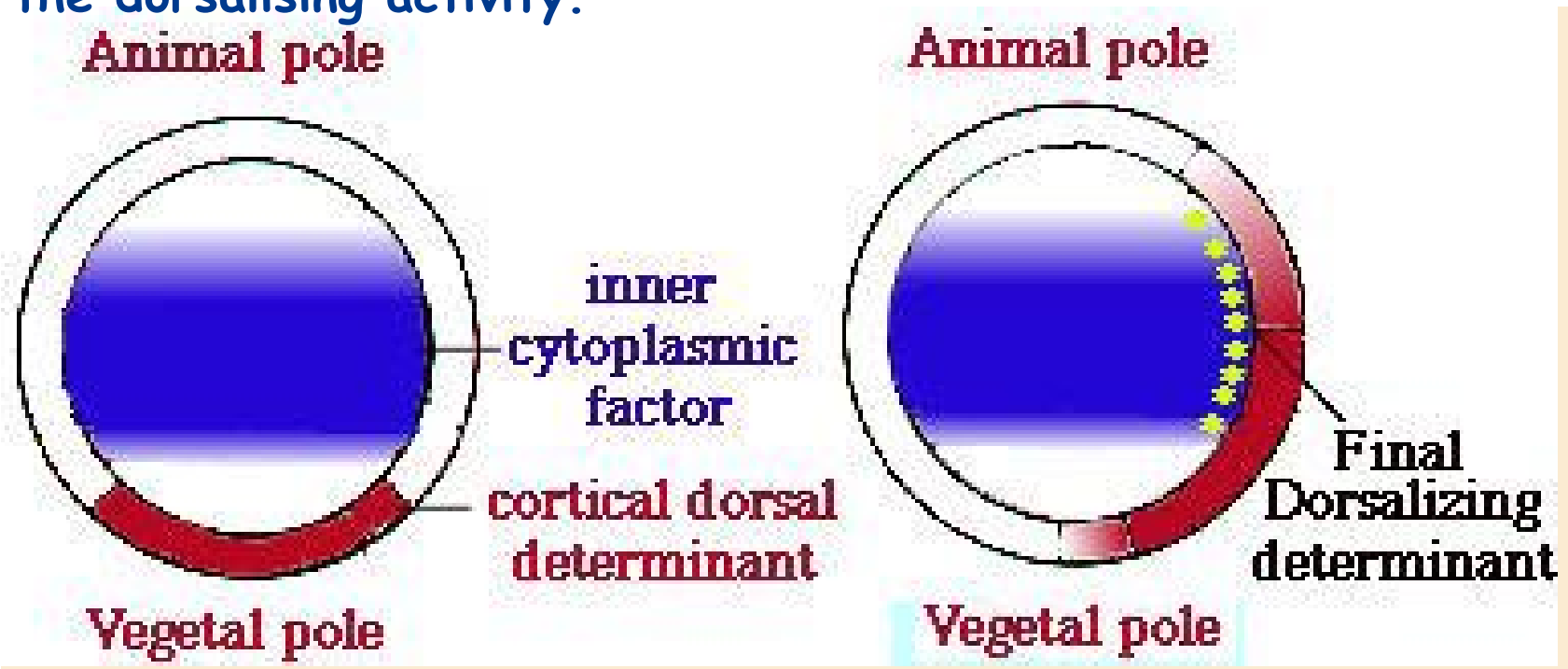
Fig. 3.9. Experiments on determination of dorsoventral polarity in the early amphibian embryo. (*a*) Normal development in which the dorsal side develops opposite the point of sperm entry. (*b*) Inversion of the axis by a 90 degree rotation after fertilisation. (*c*) Alteration of the animal–vegetal axis of the embryo by sustained rotation through 90 degrees. (*d*) Arrest of development following sustained rotation through 180 degrees. (*e*) Symmetrisation of the embryo by irradiation of the vegetal pole with ultraviolet (UV) light shortly after fertilisation. (*f*) 'Rescue' of UV-irradiated embryo by subsequent 90 degree rotation.

Localization of dorsalising (axis inducing) activity of the egg and very early embryo.



Isolated cortical regions (around 0.012 cubic mm) were placed into the ventroequatorial region of a ventrovegetal cell of an 8-cell embryo. Fifty attempts were made for each region of cortex. The figures written as percentages indicate the percentage of operated embryos that produced a secondary axis. (A) Unfertilized egg showing two animal (An) and three vegetal (Vg) cortical regions. (B) 2-cell embryo showing ventral (V), dorsal (D), animal (a), vegetal (v), equatorial (e) cortex as well as inner (i) cytoplasm. (C) 32-cell embryo. (After Kageura, 1997.)

Rotation of the vegetal cortex after fertilisation displaces the dorsalising activity.



(A) Unrotated egg, where the competent inner cytoplasm at the equator is separated from the dorsal determinant in the vegetal cortex. (B) Early embryo after cortical rotation, where the dorsal determinant in the cortex has been rotated (and spreads somewhat) and can interact with the unrotated inner cytoplasm. (After Kageura, 1997.)

Inductive properties of embryonic tissue

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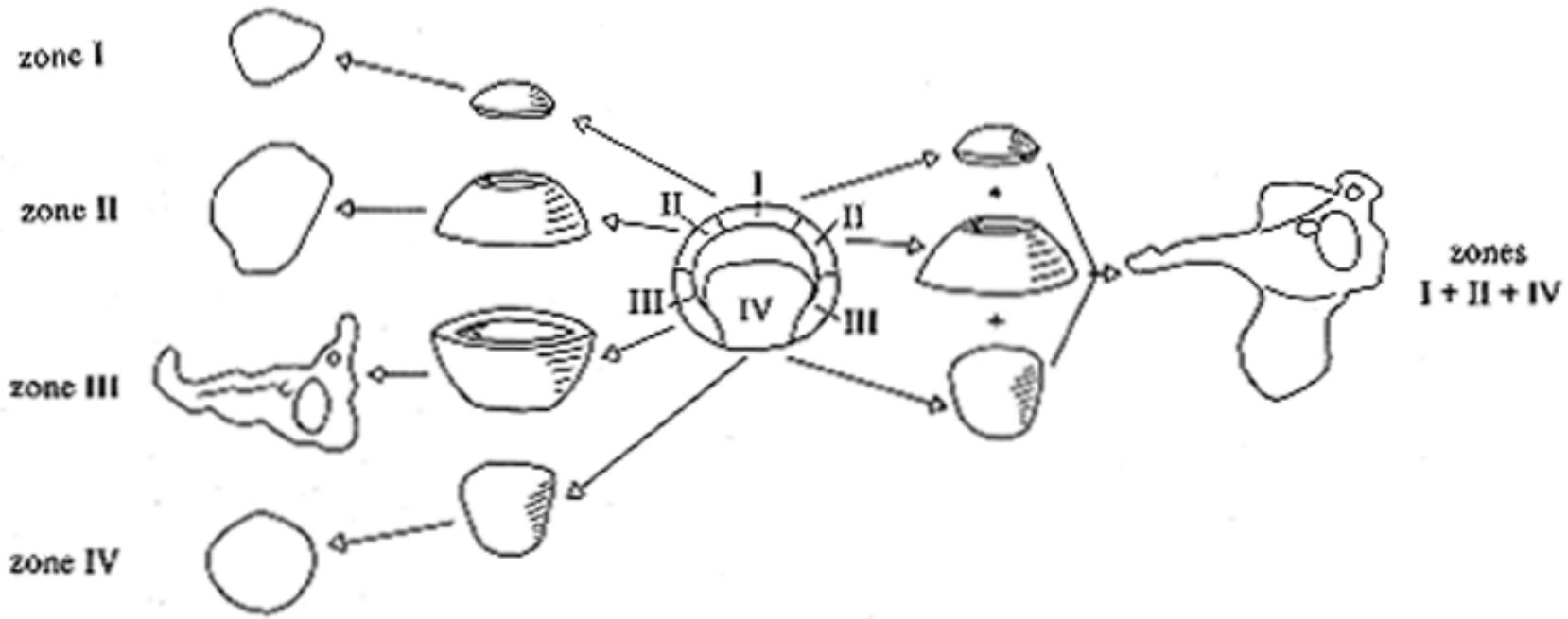


Fig. 3.12. Development of annular fragments from the axolotl blastula. Only zone III can produce a reasonably complete embryo in isolation, but a similar embryo can be produced from zones I, II and IV in combination.

Regulative/inductive properties of the blastula

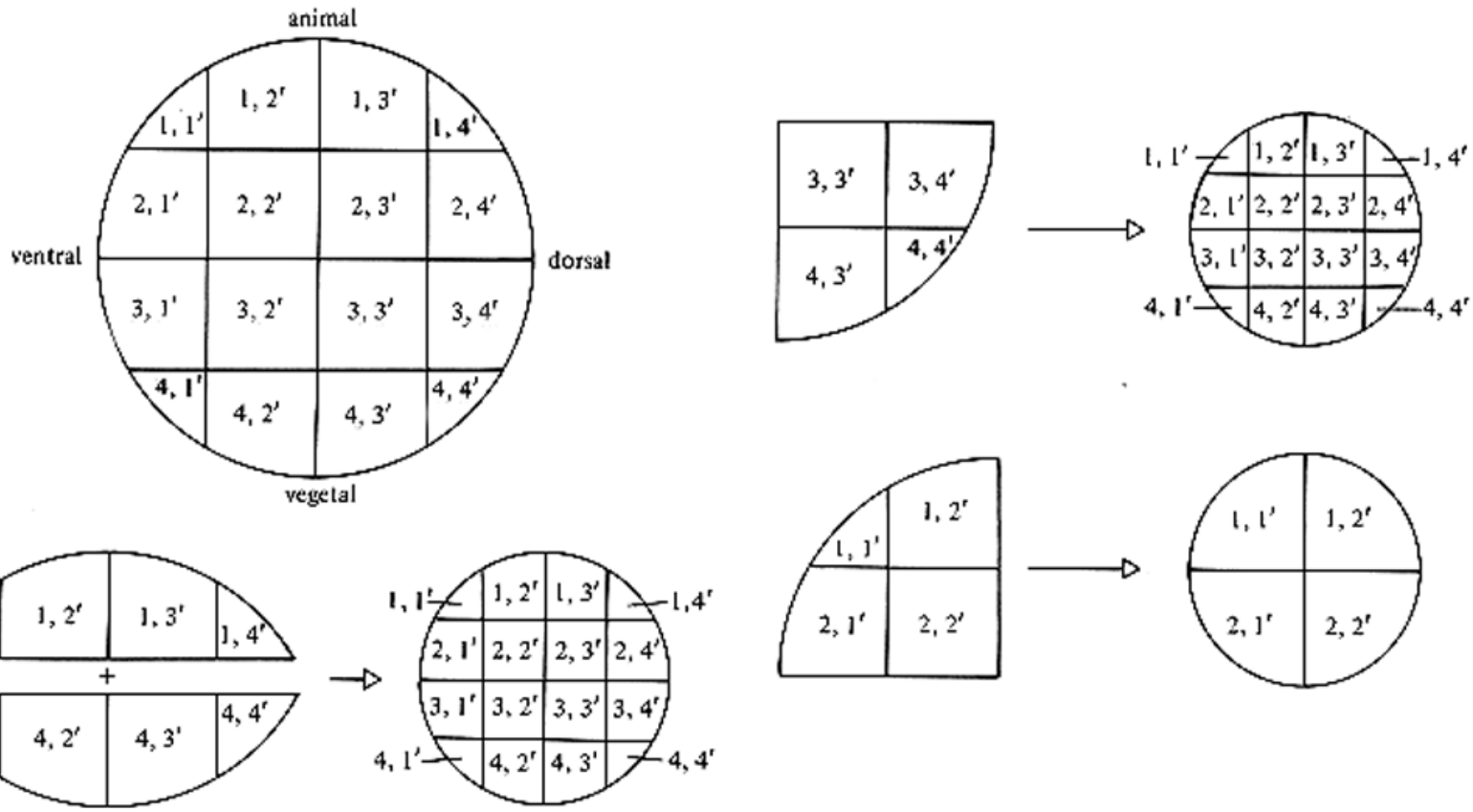
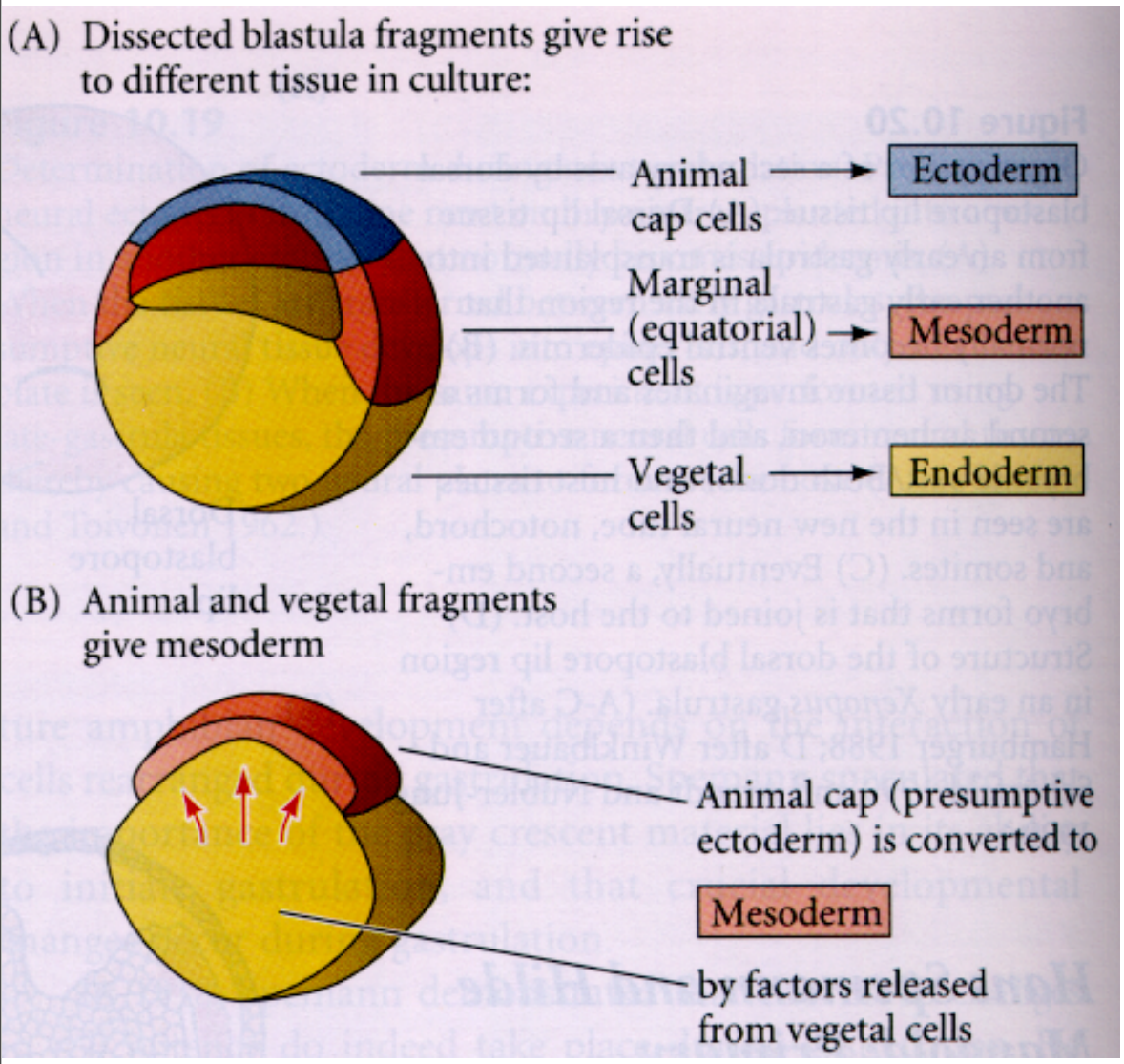
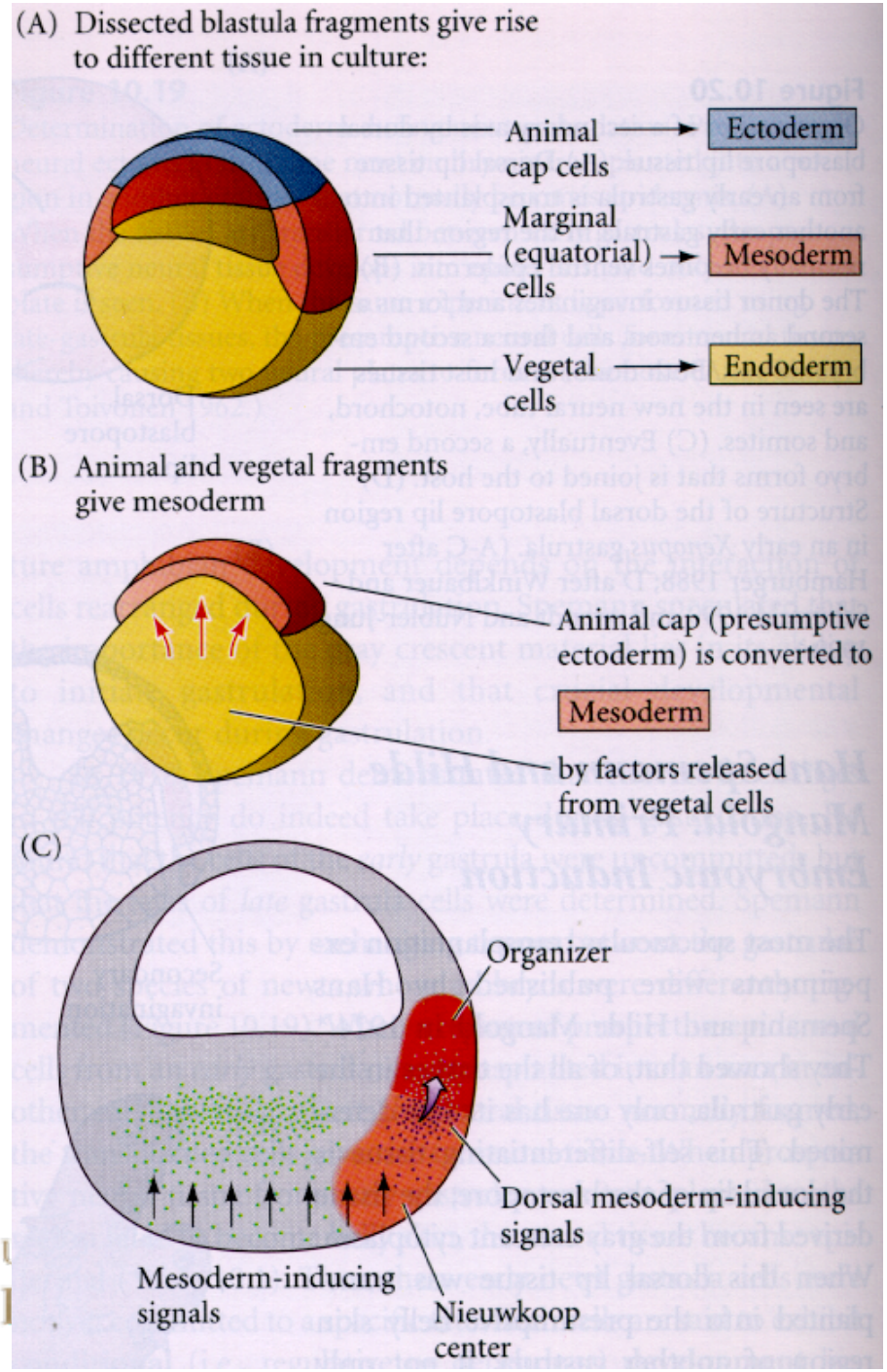


Fig. 3.13. Formal description of the regulative properties of the amphibian blastula. High codings can generate lower ones but not vice versa. A twofold reduction in linear dimensions is allowed for the size of each territory.



Nieuwkoop and Nakamura showed mesoderm is induced in animal cap (prospective epidermis) by the vegetal pole cells

Figure 10.21
Summary of experiments by Nieuwkoop and by Nakamura and Takasaki (1970), showing mesodermal induction by vegetal endoderm. (A) Isolated animal cap cells become a mass of ciliated epidermis, isolated vegetal cells generate gutlike tissue, and isolated equatorial (marginal zone) cells become mesoderm. (B) If animal cap cells are combined with vegetal cap cells, many of the animal cells generate mesodermal tissue.



The Nieuwkoop/Nakamura Centre

Figure 10.21
Summary of experiments by Nieuwkoop and by Nakamura and Takasaki (1970), showing mesodermal induction by vegetal endoderm. (A) Isolated animal cap cells become a mass of ciliated epidermis, isolated vegetal cells generate gutlike tissue, and isolated equatorial (marginal zone) cells become mesoderm. (B) If animal cap cells are combined with vegetal cap cells, many of the animal cells generate mesodermal tissue. (C) Model for mesoderm induction in *Xenopus*. A ventral signal (probably FGF2 or BMP4) is released throughout the vegetal region of the embryo. This induces the marginal cells to become mesoderm. On the dorsal side (away from the point of sperm entry), a signal is released by the vegetal cells of the Nieuwkoop center. This dorsal signal induces the formation of the Spemann organizer in the overlying marginal zone cells. The possible identity of this signal will be discussed later in this chapter. (C after De Robertis et al. 1992.)

Early Xenopus fate map

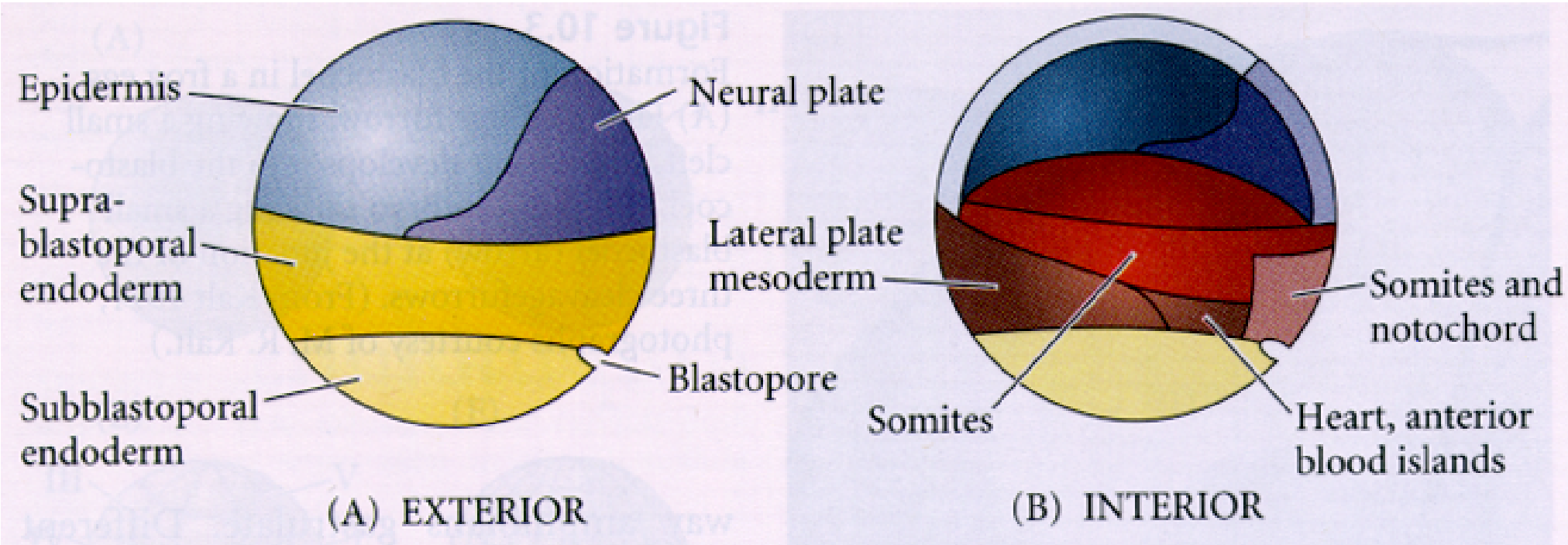
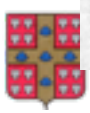


Figure 10.5
Fate maps of the blastula of the frog *Xenopus laevis*: (A) exterior; (B) interior. Most of the mesodermal derivatives are formed from the interior cells. (After Lane and Smith 1999; Newman and Krieg 1999.)



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Molecular determinants of induction and axis formation

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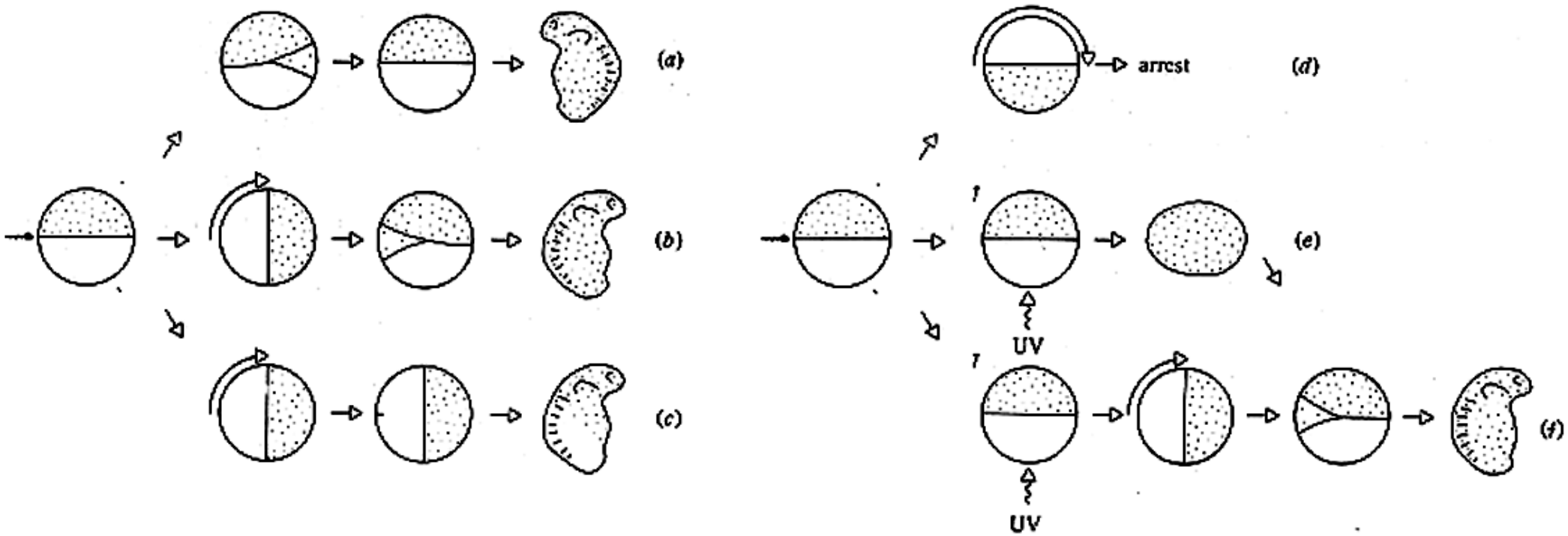
In the search for the molecules which define the amphibian dorsal axis, two basic assays have been applied;

1) The induction of a secondary axis or the recovery of axis forming ability after UV irradiation,

2) The induction of mesodermal and neural molecular markers (gene expression).

Determination of Dorsal-Ventral polarity



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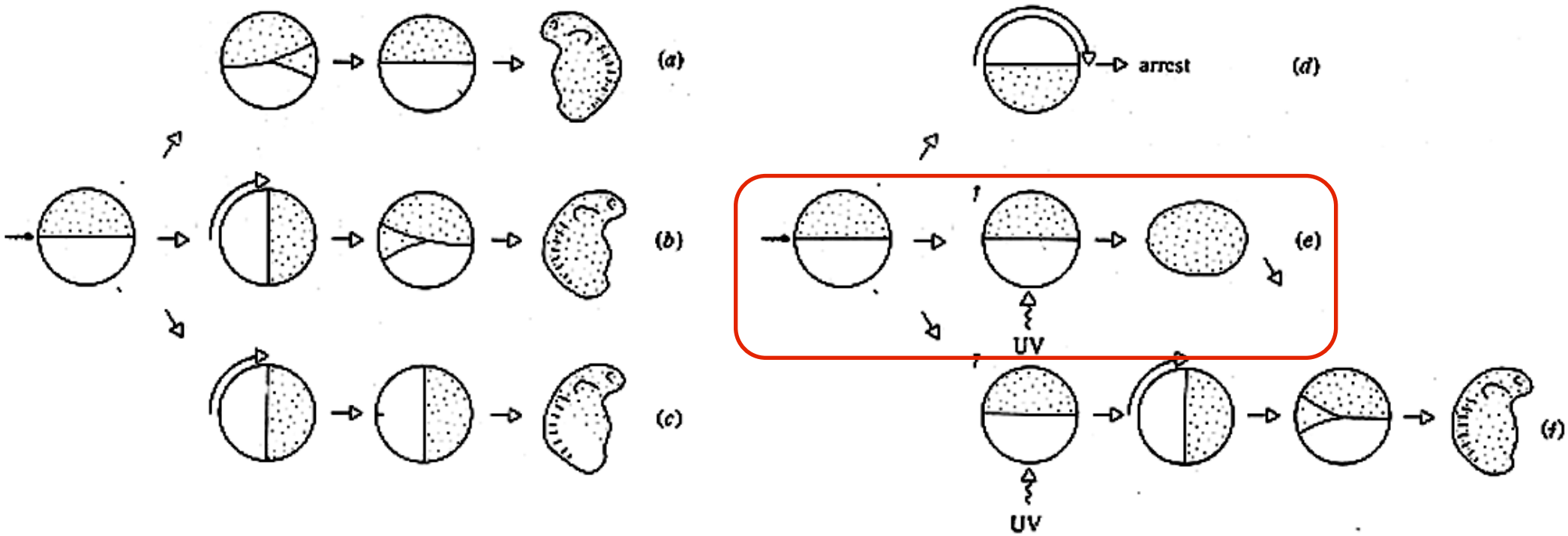

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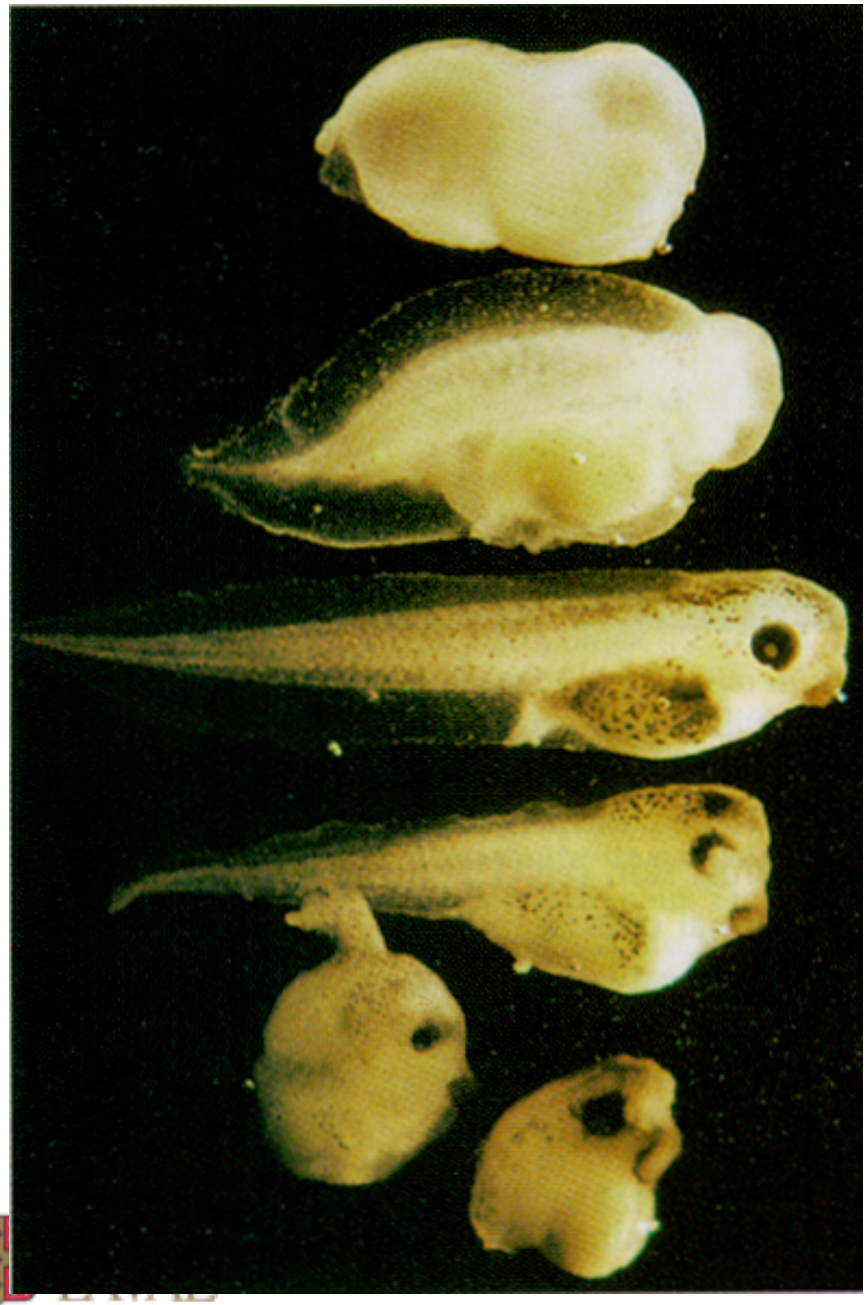
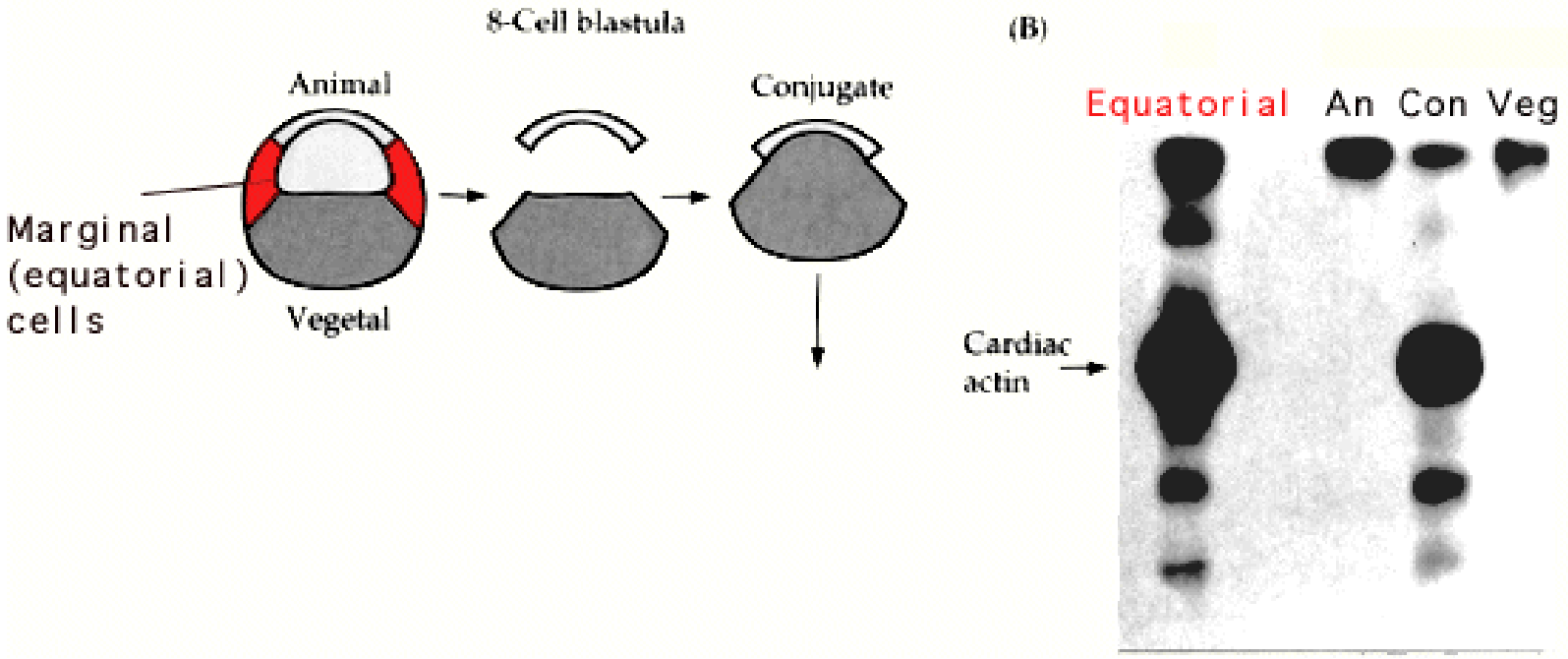


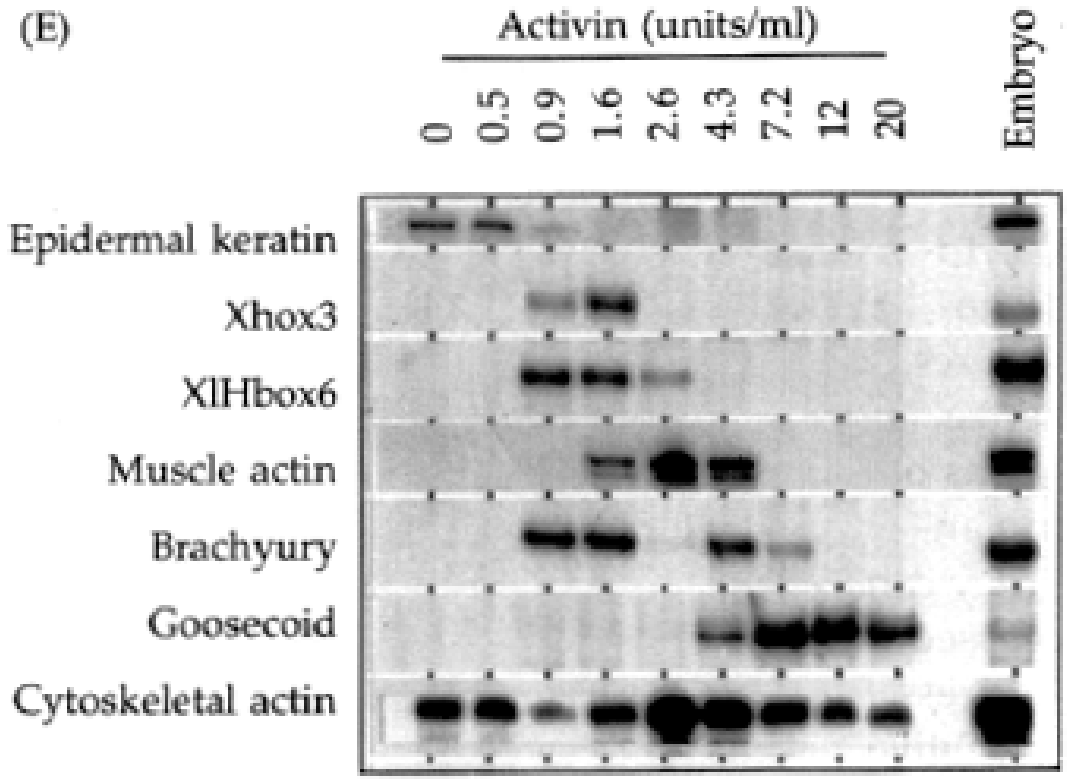
Increasing
Noggin mRNA (BMP/TGFß inhibitor)

Axis rescue experiment

Rescue of dorsal structures by Noggin protein. When *Xenopus* eggs are exposed to ultraviolet radiation, cortical rotation fails to occur, and the embryos lack dorsal structures (top). If such an embryo is injected with *noggin* mRNA, it develops dorsal structures in a dosage-related fashion (top to bottom). If too much *noggin* message is injected, the embryo produces dorsal anterior tissue at the expense of ventral and posterior tissue, becoming little more than a head (bottom). (Photograph courtesy of R. M. Harland.)



The animal cap assay using a molecular marker of mesoderm induction



Activin, Nodal and Vg1, TGFβ-like factors, and bFGF, all present in the ventral hemisphere, can induce mesodermal markers in animal caps

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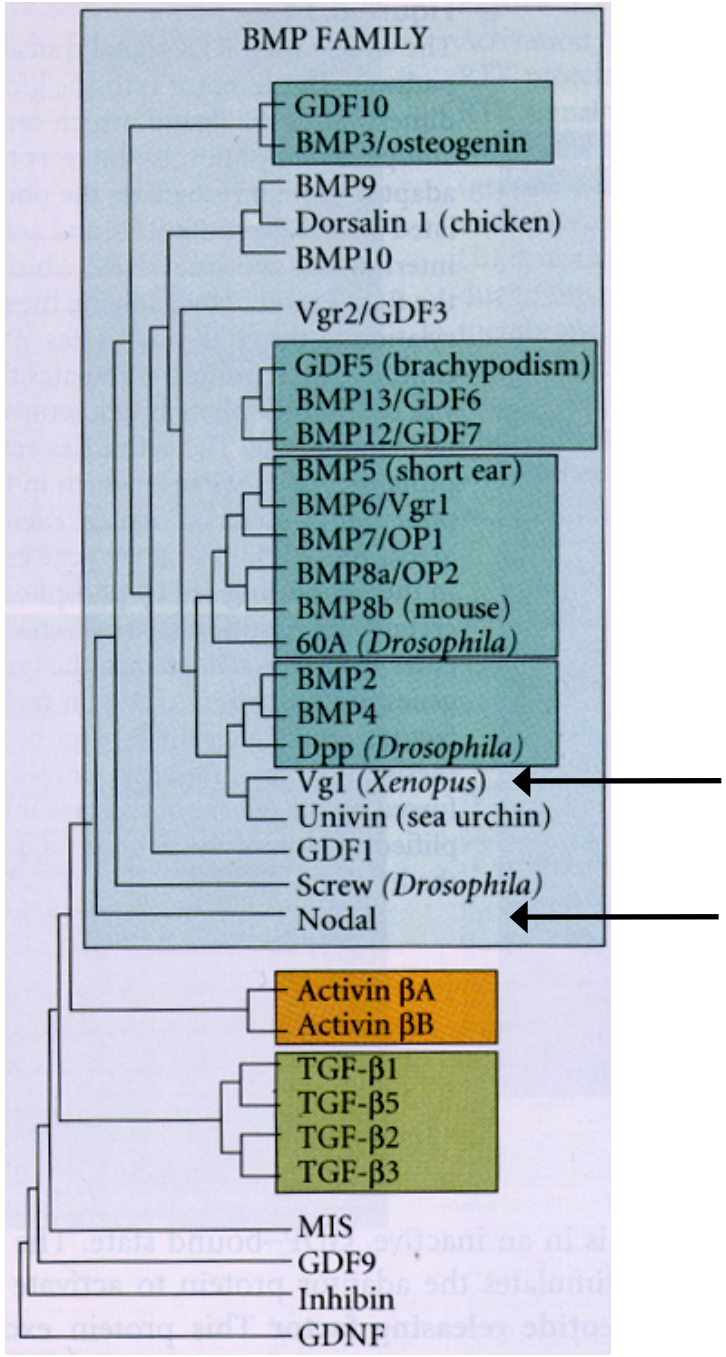
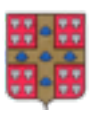


The TGF-beta superfamily



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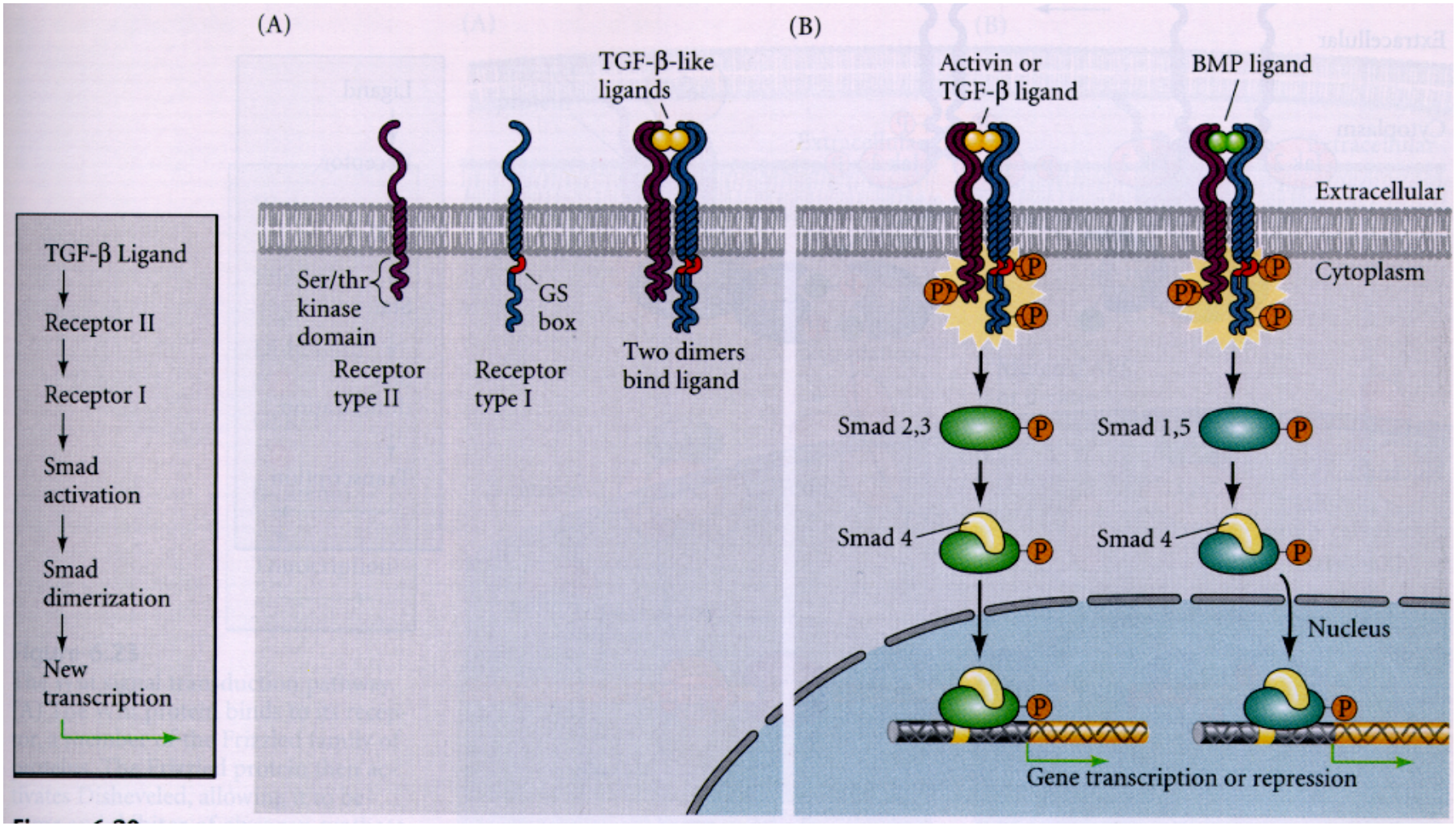
The TGFbeta receptor pathway



Dorsalizing and Axis Inducing Activities

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Discovery of mouse Int-1 (mWnt1) as an axis inducer; implication of the Wnt signal transduction pathway. (McMahon and Moon 1989)

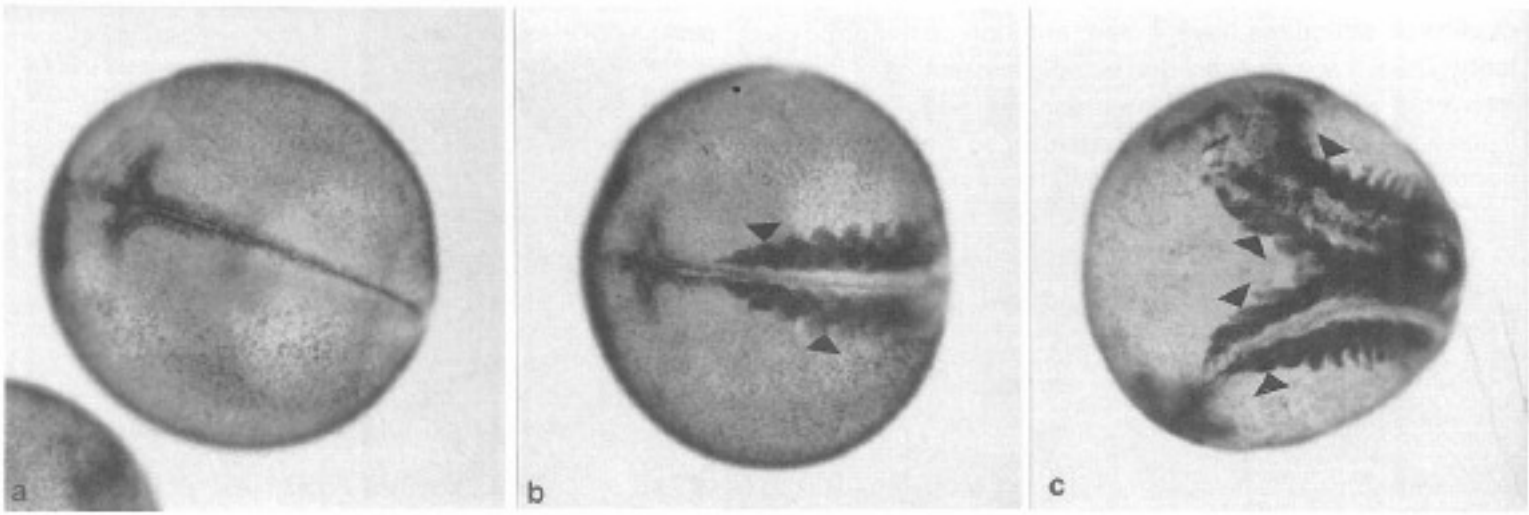
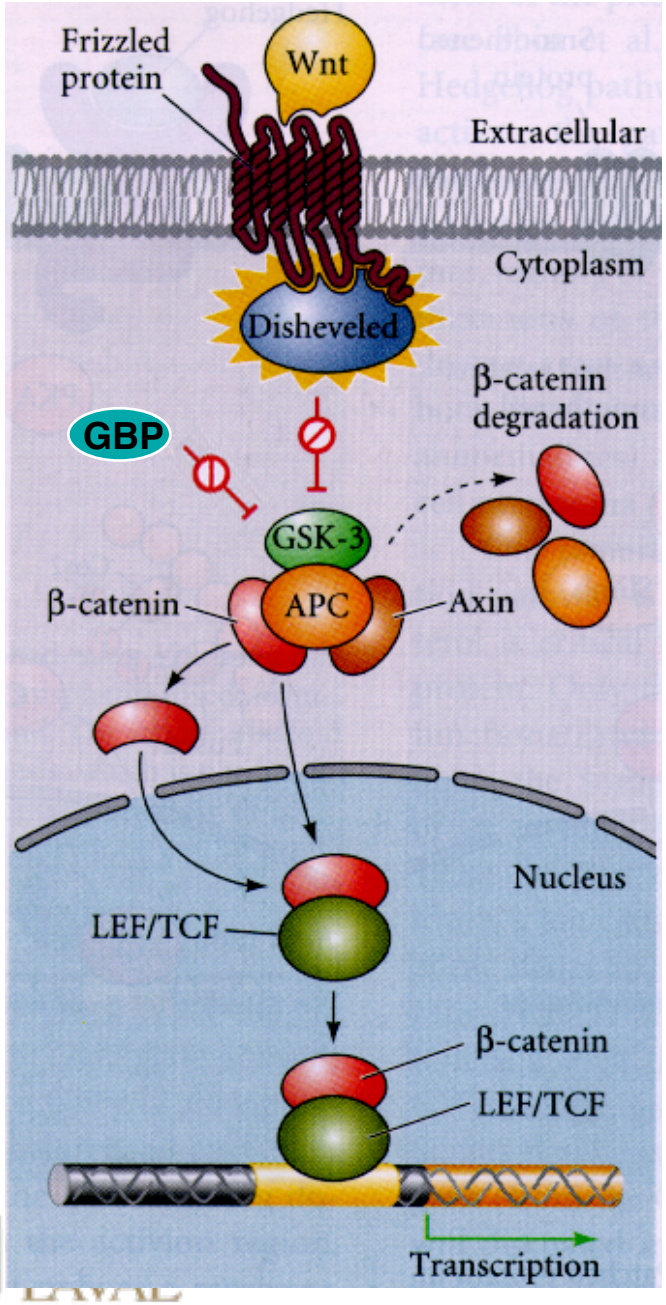
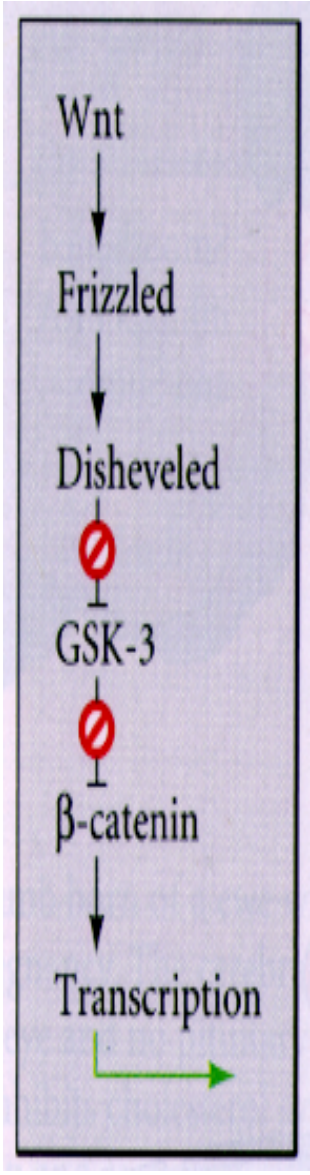


Figure 9. Somite Formation in 323- and 322-Injected Neurulae

Whole-mount immunohistochemistry was performed on 323- and 322-injected neurulae using an antibody, 12/101, which detects somitic derivatives. (a) Staining in the absence of 12/101. (b) staining of 323-injected neurula; (c) staining of 322-injected neurula. Somites in 323-injected embryos are arranged as normal in pairs about the mid-line (arrows). In 322-injected embryos, pairs of somites surround each axis (arrows). Anterior is to the left.





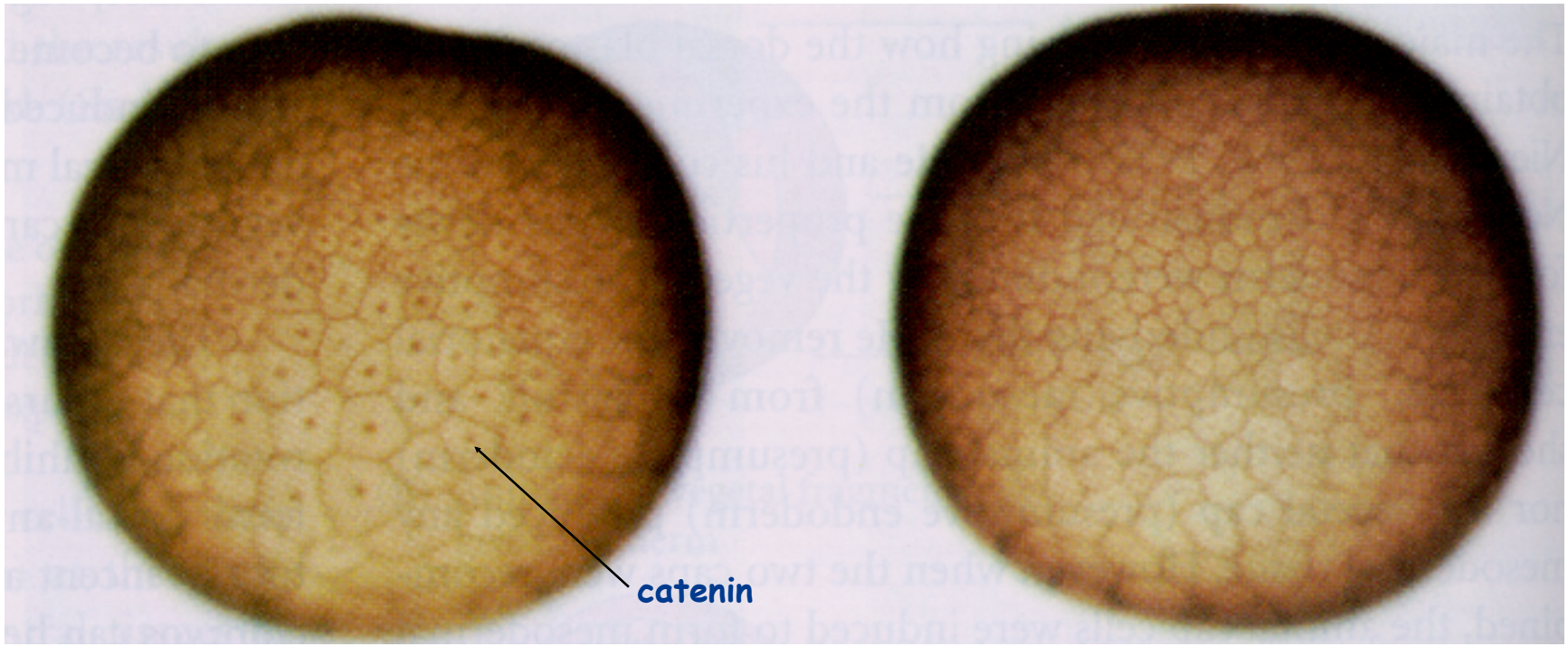
The canonical Wnt/Catenin pathway

GSK-3; glycogen synthases kinase-3

APC; adenomatous poliposis coli, (a tumour suppressor)

GBP; GSK-3 Binding Protein

Catenin is nuclear on the dorsal but not the ventral side of the blastula embryo



dorsal

ventral

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Inhibition of GSK activity or the overexpression of catenin on the prospective ventral side of the embryo induces axis duplication

Conversely, the elimination of maternal catenin using antisense oligonucleotides (Morpholinos) prevents dorsal tissue formation

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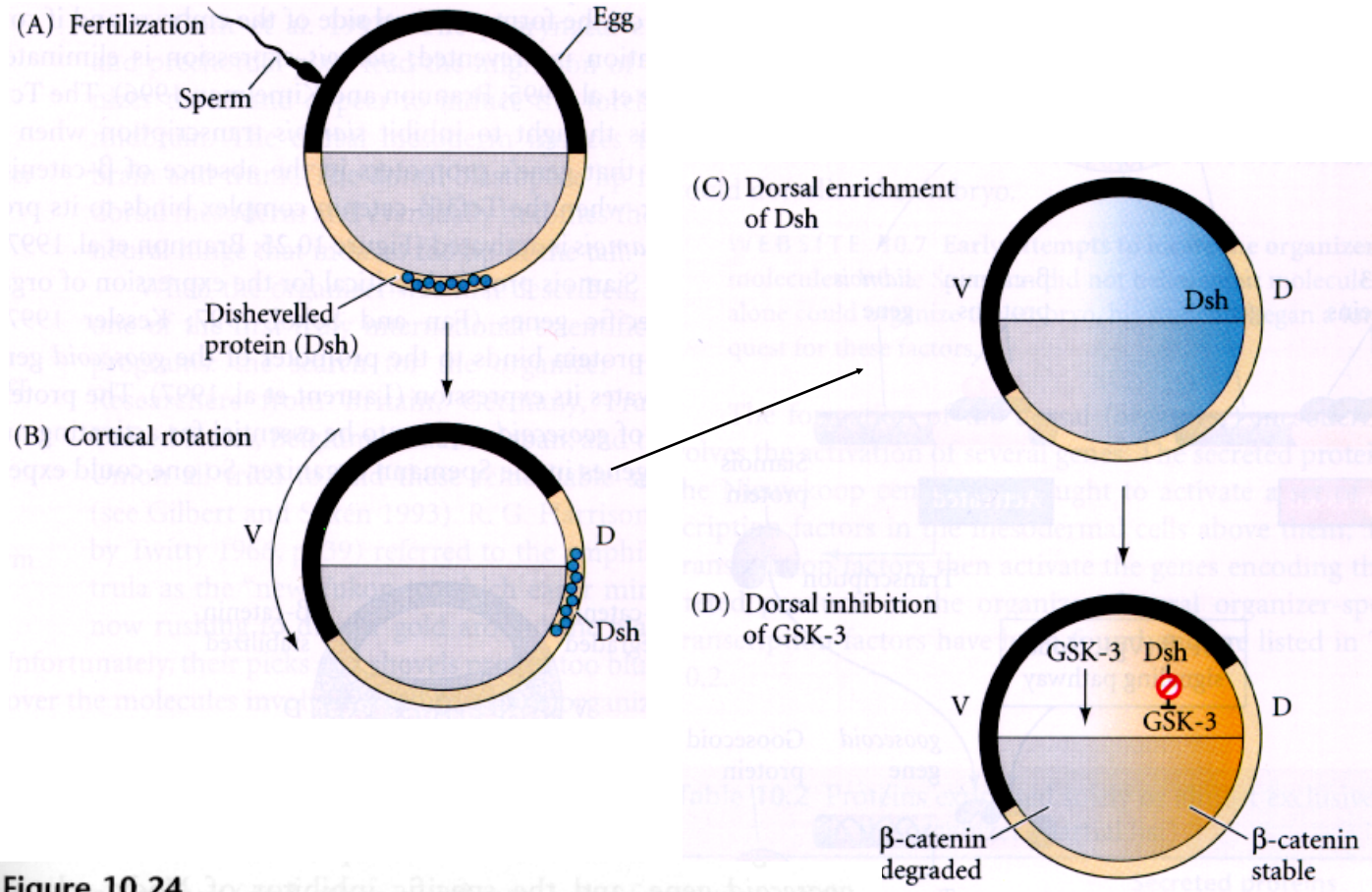

Figure 10.24
Model of the mechanism by which the Disheveled protein stabilizes β-catenin in the dorsal portion of the amphibian egg. (A) Disheveled (Dsh) associates with a particular set of proteins at the vegetal pole of the unfertilized egg. (B) Upon fertilization, these protein vesicles are translocated dorsally along subcortical microtubule tracks. (C) Disheveled is then released from its vesicles and is distributed in the future dorsal third of the 1-cell embryo. (D) Disheveled binds to and blocks the action of GSK-3, thereby preventing the degradation of β-catenin on the dorsal side of the embryo. (E) The nuclei of the blastomeres in the dorsal region of the embryo receive β-catenin, while the nuclei of those in the ventral region do not.

Dishevelled (Dsh) is displaced by cortical rotation at fertilisation.

A similar arguement can be made for GSK-3 Binding Protein (GBP).

Both events potentially induce nuclear catenin accumulation

However, maternal Wnt11 is now believed to be the axis inducing signal.

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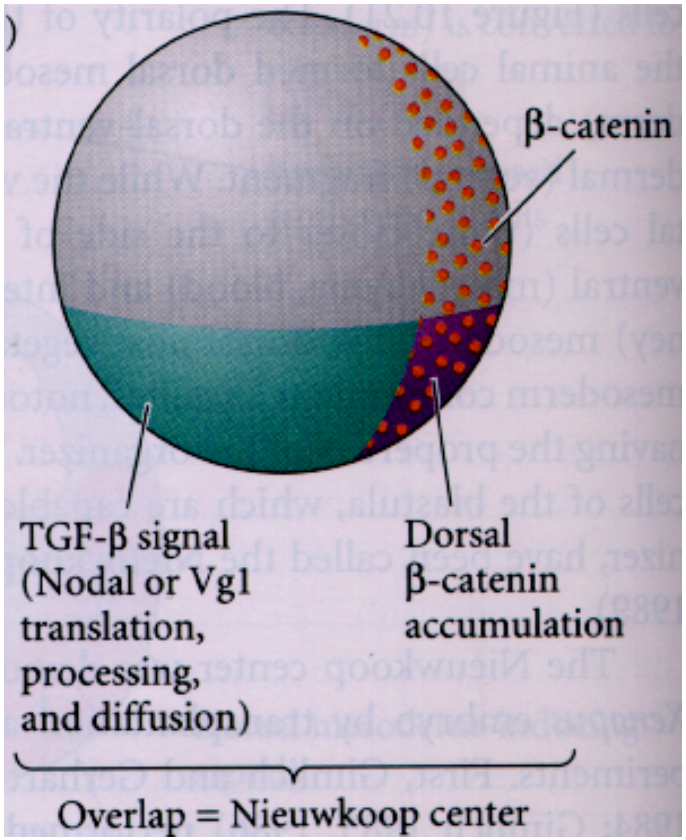
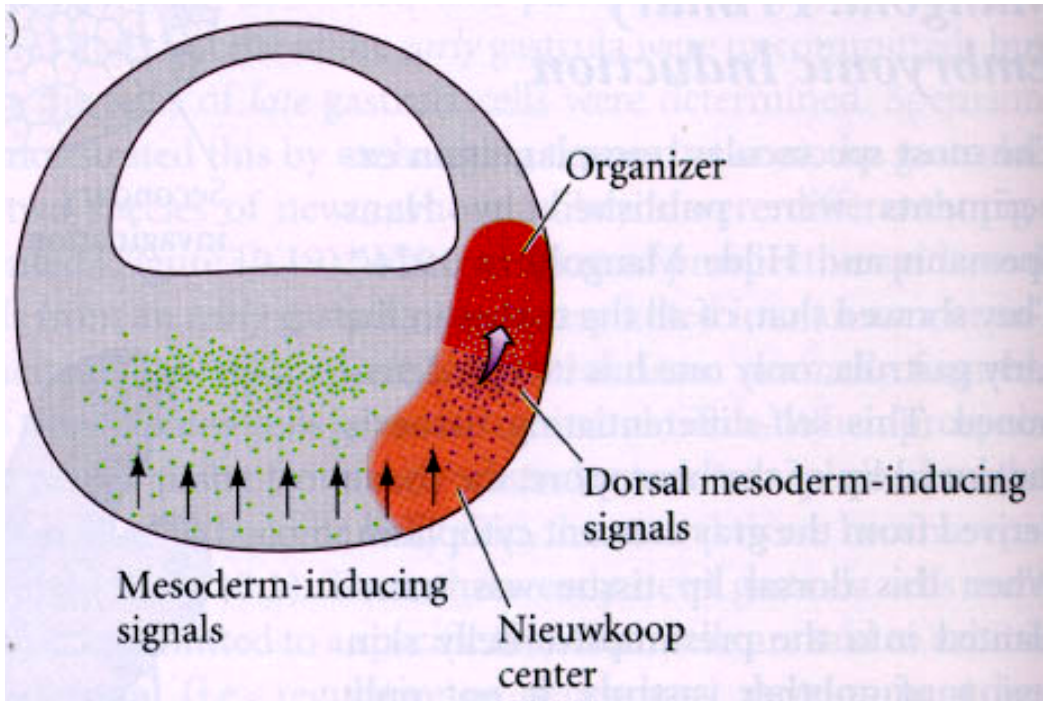
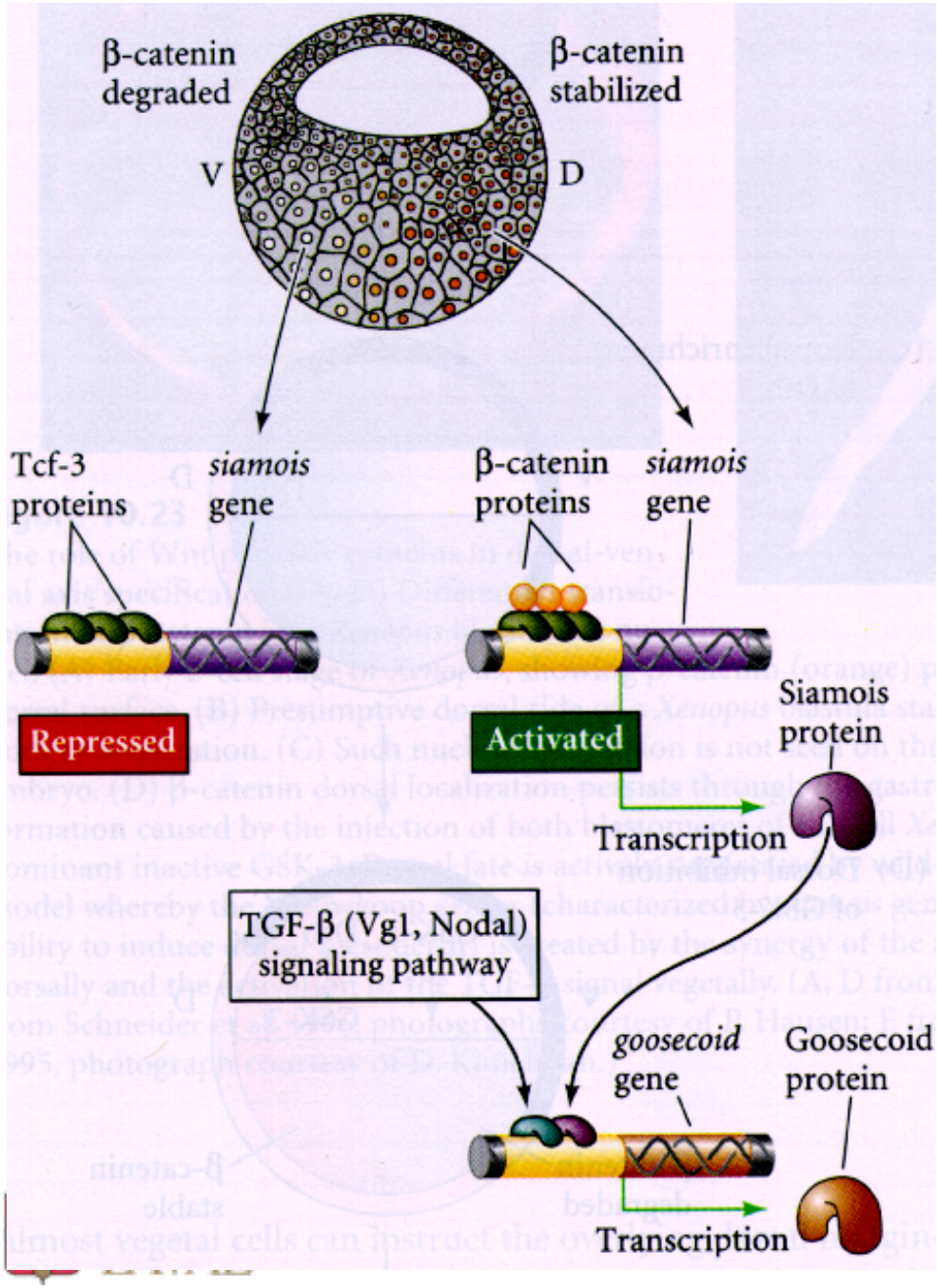


Overlap of the dorsal zone of beta-catenin nuclear accumulation and ventral TGF-beta signal equates with the Nieuwkoop Centre.

What about the Spemann Organiser?





Catenin signalling is responsible for expression of two key genes:-

Siamois and Goosecoid, both transcription factors, are regulated in a cascade.

Figure 10.25
Summary of events hypothesized to bring about the induction of the organizer in the dorsal mesoderm. Cortical rotation causes the translocation of Disheveled protein to the dorsal side of the embryo. Dsh binds GSK-3, thereby allowing β-catenin to accumulate in the future dorsal portion of the embryo. During cleavage, β-catenin enters the nuclei and binds with Tcf3 to form a transcription factor that activates genes encoding proteins such as Siamois. Siamois and Lim-1, a transcription factor activated by the TGF-β pathway, function together to activate the *goosecoid* gene in the organizer. Goosecoid is a transcription factor that can activate genes whose proteins are responsible for the organizer's activities. (After Moon and Kimelman 1998).

The initial stages in defining the Spemann Organiser

Note VegT is a transcription factor, of the "T" box family, found in the vegetal hemisphere of the egg.

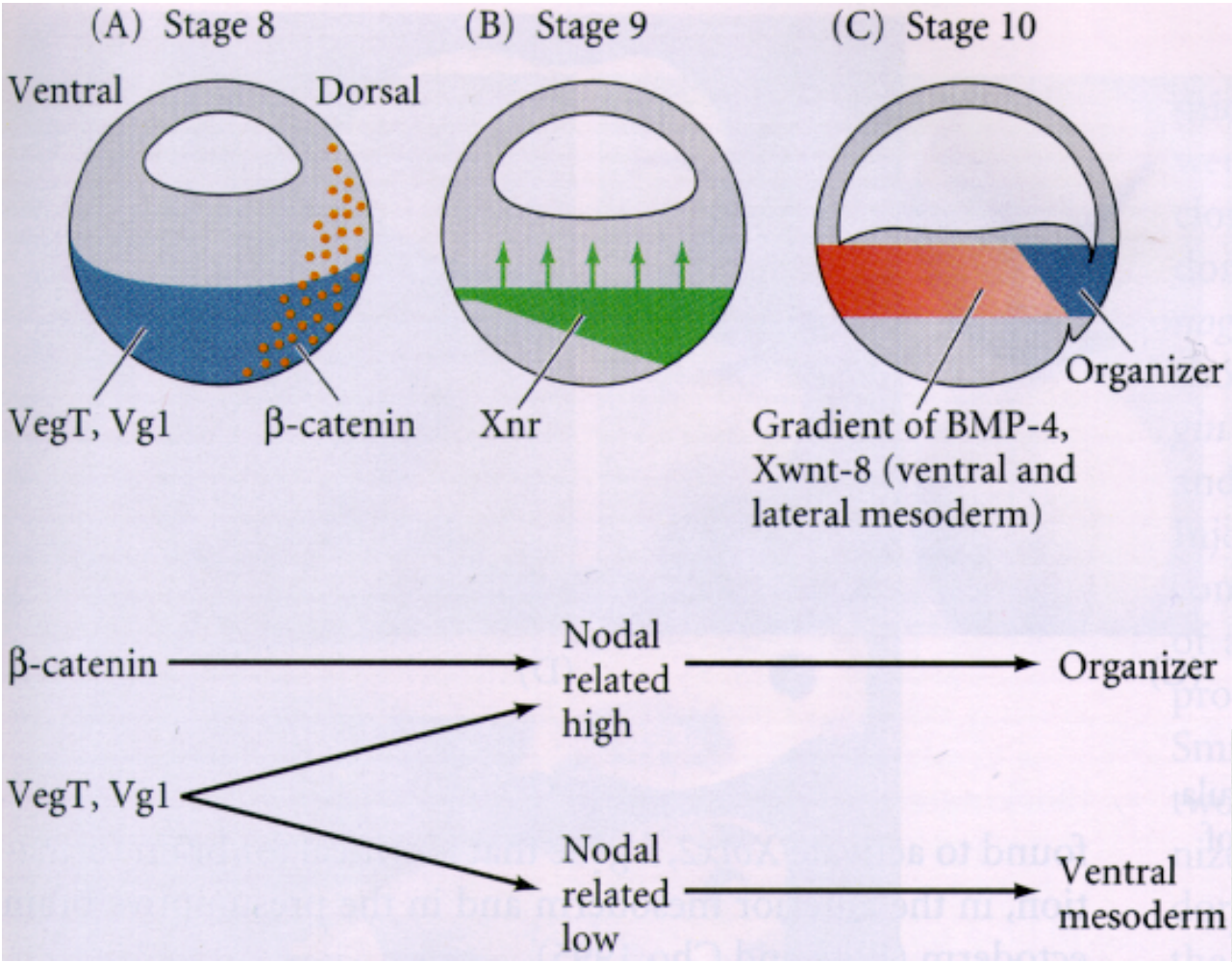


Figure 10.26
Model for mesoderm induction and organizer formation by the interaction of β-catenin and TGF-β proteins. (A) At late blastula stages, Vg1 and VegT are found in the vegetal hemisphere, while β-catenin is located in the dorsal region. (B) β-catenin acts synergistically with Veg1 and VegT to activate the *Xenopus Nodal-related (Xnr)* genes. This creates a gradient of Xnr proteins across the endoderm, highest in the dorsal region. (C) The mesoderm is specified by the gradient of Xnr proteins. Mesodermal regions with little or no Xnr proteins have high levels of BMP-4 and Xwnt-8; they become ventral mesoderm. Those having intermediate concentrations of Xnrs become lateral mesoderm. Where there is a high concentration of Xnrs, the *goosecoid* gene and other dorsal mesodermal genes are activated, and the mesodermal tissue becomes the organizer. (These results may explain the activity concentration experiments mentioned in Chapter 3.) (After Agius et al. 2000.)

Table 10.2 Proteins expressed solely or almost exclusively in the organizer (partial list)

Nuclear proteins	Secreted proteins
XLim1	Chordin
Xnot	Dickkopf
Otx2	ADMP
XFD1	Frzb
XANF1	Noggin
Goosecoid	Follistatin
HNF3β-related proteins (e.g., Forkhead, Pintallavis)	Sonic hedgehog
	Cerberus
	Nodal-related proteins (several)

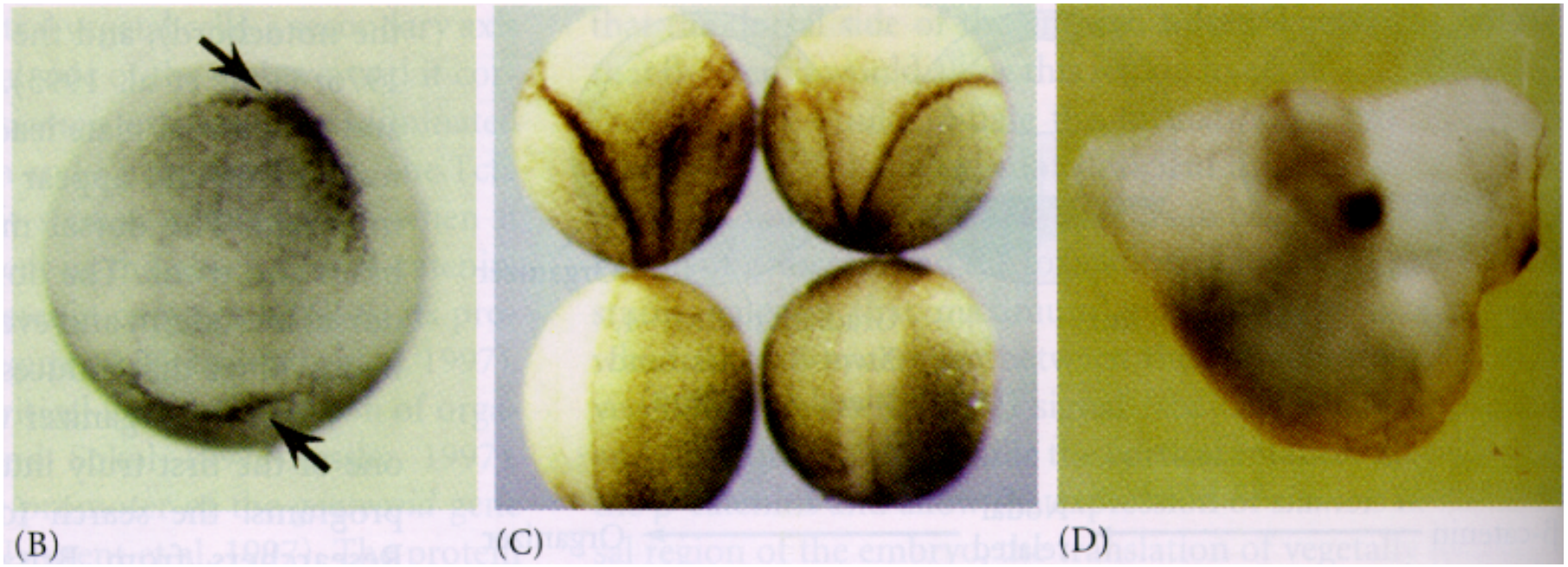


Figure 10.27
Ability of *goosecoid* mRNA to induce a new axis. (A) At the gastrula stage, a control embryo (either uninjected or given an injection of *goosecoid*-like mRNA but lacking the homeobox) has one dorsal blastopore lip. (B) An embryo whose ventral vegetal blastomeres were injected at the 16-cell stage with *goosecoid* message. Note the secondary dorsal lip. (C) The top two embryos, which were injected with *goosecoid* mRNA, show two axes; the bottom two control embryos do not. In the upper embryos, two dorsal axes are seen. (D) Twinned embryo produced by *goosecoid* injection. Two complete sets of head structures have been induced. (After Cho et al. 1991a; Niehrs et al. 1993; photographs courtesy of E. De Robertis.)

Goosecoid induces secondary axes

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Chordin, a BMP2,4 inhibitor is expressed in the dorsal blastopore lip and later in the mesoderm underlying the neural plate

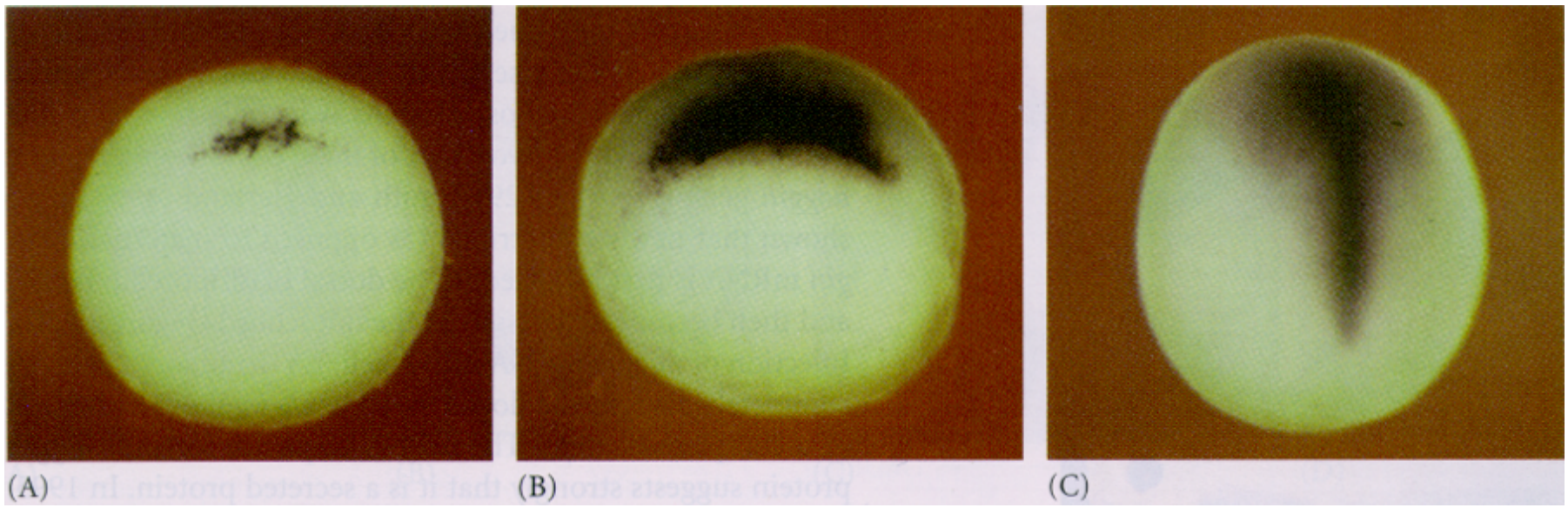
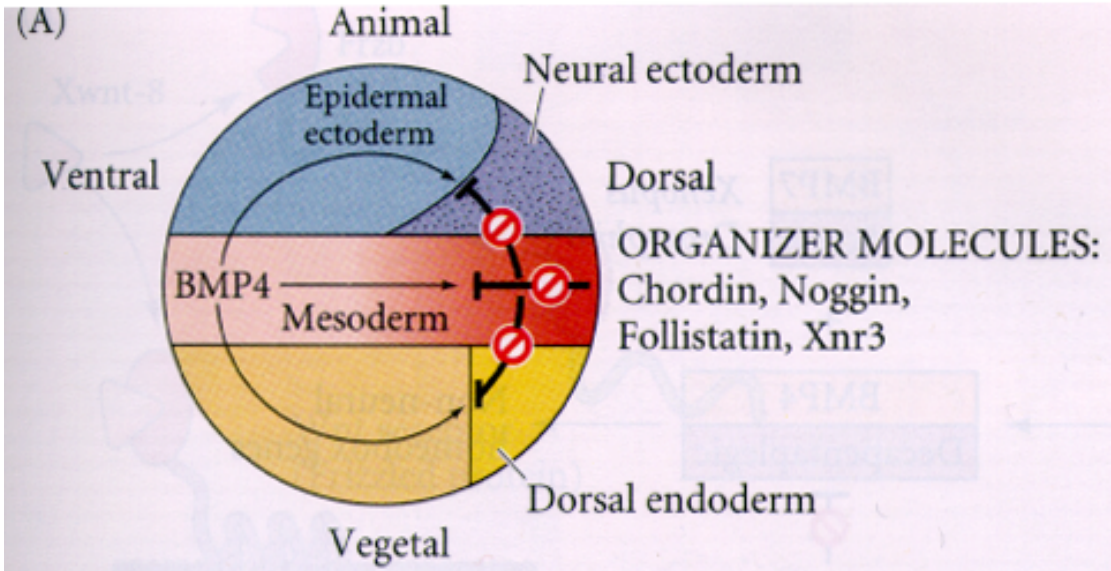


Figure 10.31
Chordin mRNA localization. (A) Whole-mount in situ hybridization shows that just prior to gastrulation, chordin message (dark area) is expressed in the region that will become the dorsal blastopore lip. (B) As gastrulation begins, Chordin is expressed at the dorsal blastopore lip. (C) In later stages of gastrulation, Chordin message is seen in the organizer tissues. (From Sasai et al. 1994; photographs courtesy of E. De Robertis.)



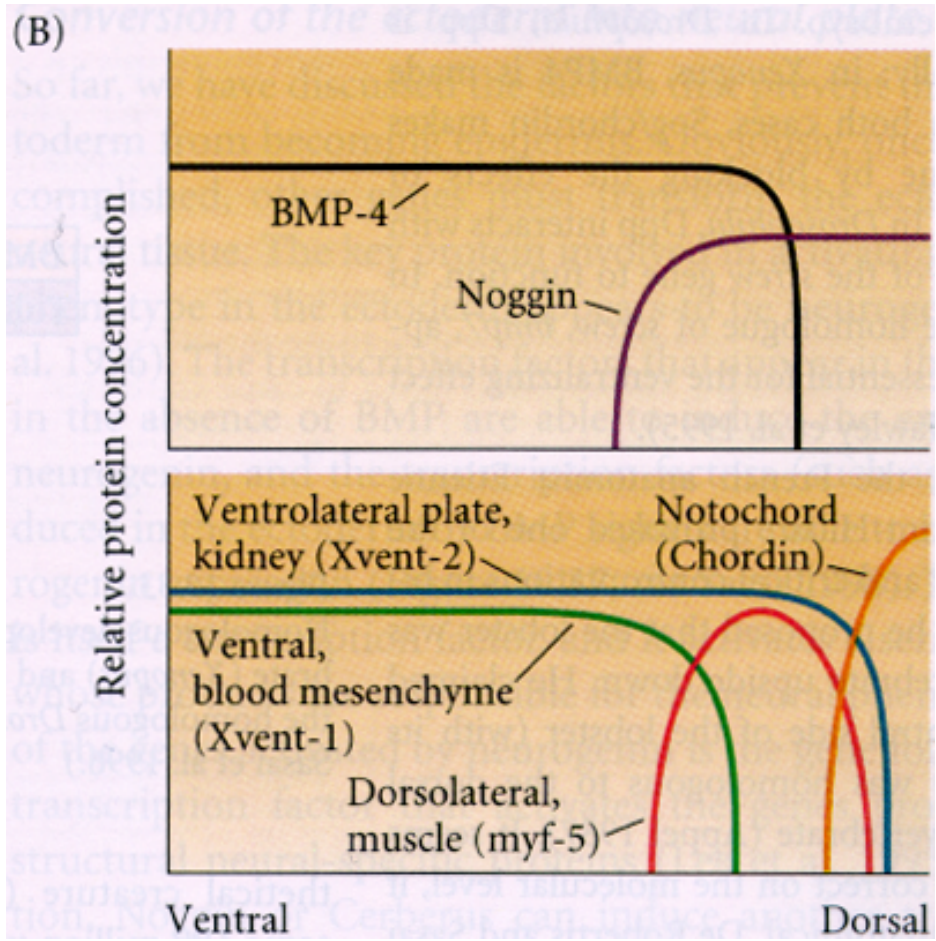


Figure 10.32
Model for the action of the organizer. (A) BMP4 (and certain other molecules) is a powerful ventralizing factor. Organizer proteins such as Chordin, Noggin, and Follistatin block the action of BMP4. The antagonistic effects of these proteins can be seen in all three germ layers. (B) BMP4 may elicit the expression of different genes in a concentration-dependent fashion; in that way, the mesoderm could be patterned. BMP4 is expressed throughout the marginal zone (prospective mesoderm) except in the dorsal domain. Noggin and Chordin are expressed in the dorsal domain. These proteins bind to BMP4 and prevent it from reaching the mesodermal cells. In the regions of noggin and chordin expression, BMP4 is totally prevented from binding, and these tissues become notochord (organizer) tissue. Slightly farther away from the organizer, *myf5*, a marker for the dorsolateral muscles, is activated. As more and more BMP4 molecules are allowed to bind to the cells, *Xvent2* (ventrolateral) and *Xvent1* (ventral) genes become expressed. (After Dosch et al. 1997.)

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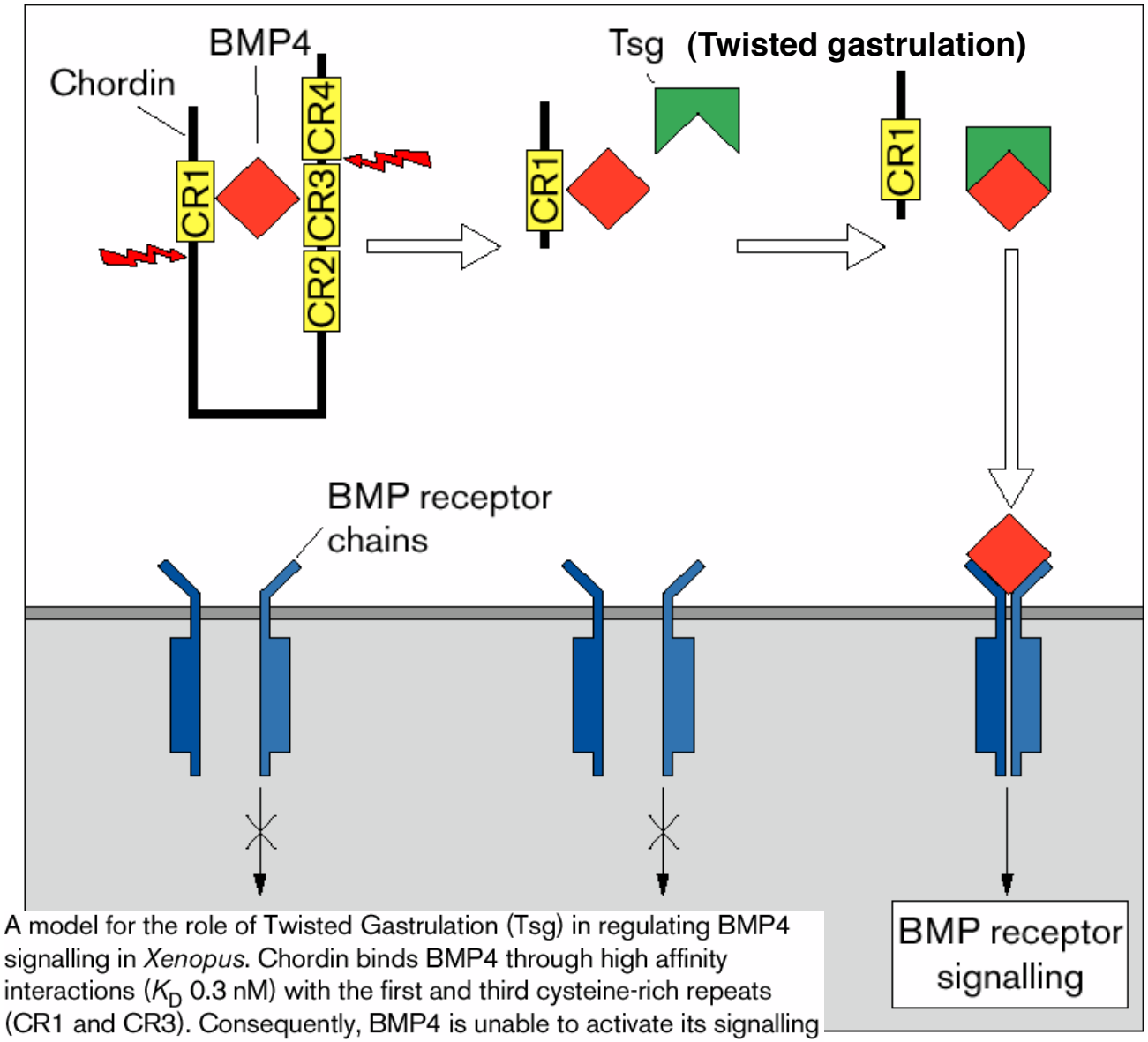
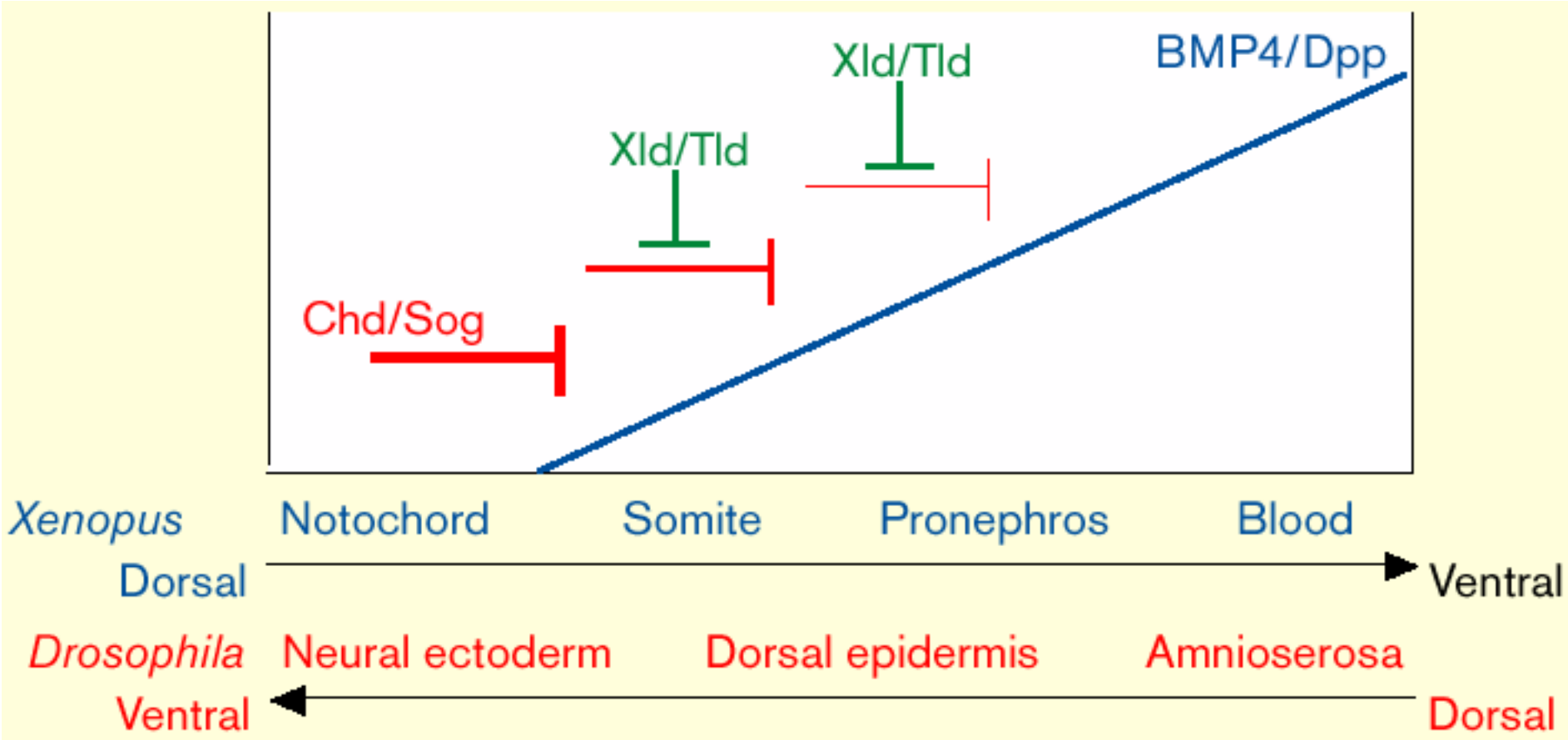


A model for the role of Twisted Gastrulation (Tsg) in regulating BMP4 signalling in *Xenopus*. Chordin binds BMP4 through high affinity interactions (K_D 0.3 nM) with the first and third cysteine-rich repeats (CR1 and CR3). Consequently, BMP4 is unable to activate its signalling receptors. Xolloid cleaves Chordin at two locations (red arrows), just downstream of CR1 and CR3, releasing fragments with reduced affinity (K_D 2.5 nM) for BMP4. Finally, Tsg, which has an affinity for BMP4 (K_D 2.5 nM) similar to that of CR1 and CR3 alone, dislodges BMP4 from the cleaved Chordin fragments, and by a mechanism as yet unknown allows BMP4 to activate its signalling receptors. Modified from [2,3].

Xolloid/Tolloid metalloproteases releave BMP4 inhibition to create a BMP4 gradient

From Dale 2000



Xolloid/Tolloid metalloproteases releave BMP4 inhibition to create a BMP4 gradient

From Dale 2000

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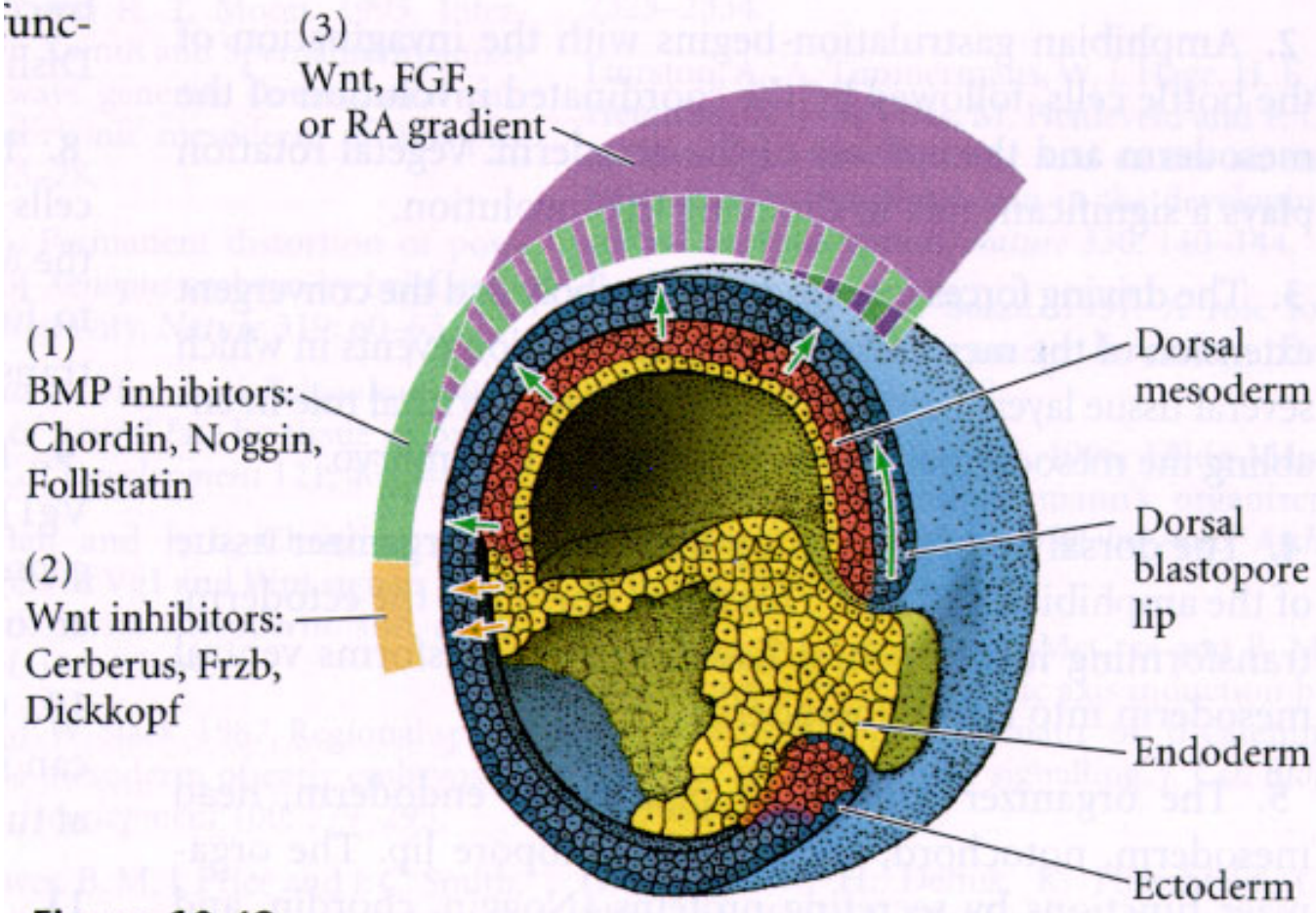

Figure 10.42
Model for the organizer function and axis specification in *Xenopus* gastrula. (1) BMP inhibitors from organizer tissue (dorsal mesoderm and pharyngeal mesendoderm) block the formation of epidermis, ventrolateral mesoderm, and ventrolateral endoderm. (2) Wnt inhibitors in the anterior of the organizer (pharyngeal mesendoderm) allow the induction of head structures. (3) A gradient of caudalizing factors (eFGF, retinoic acid, and/or Wnt3a) specify the regional expression of Hox genes.

Subsequent patterning of the anterior-posterior axis relies on the differential inhibition and increased of signalling.