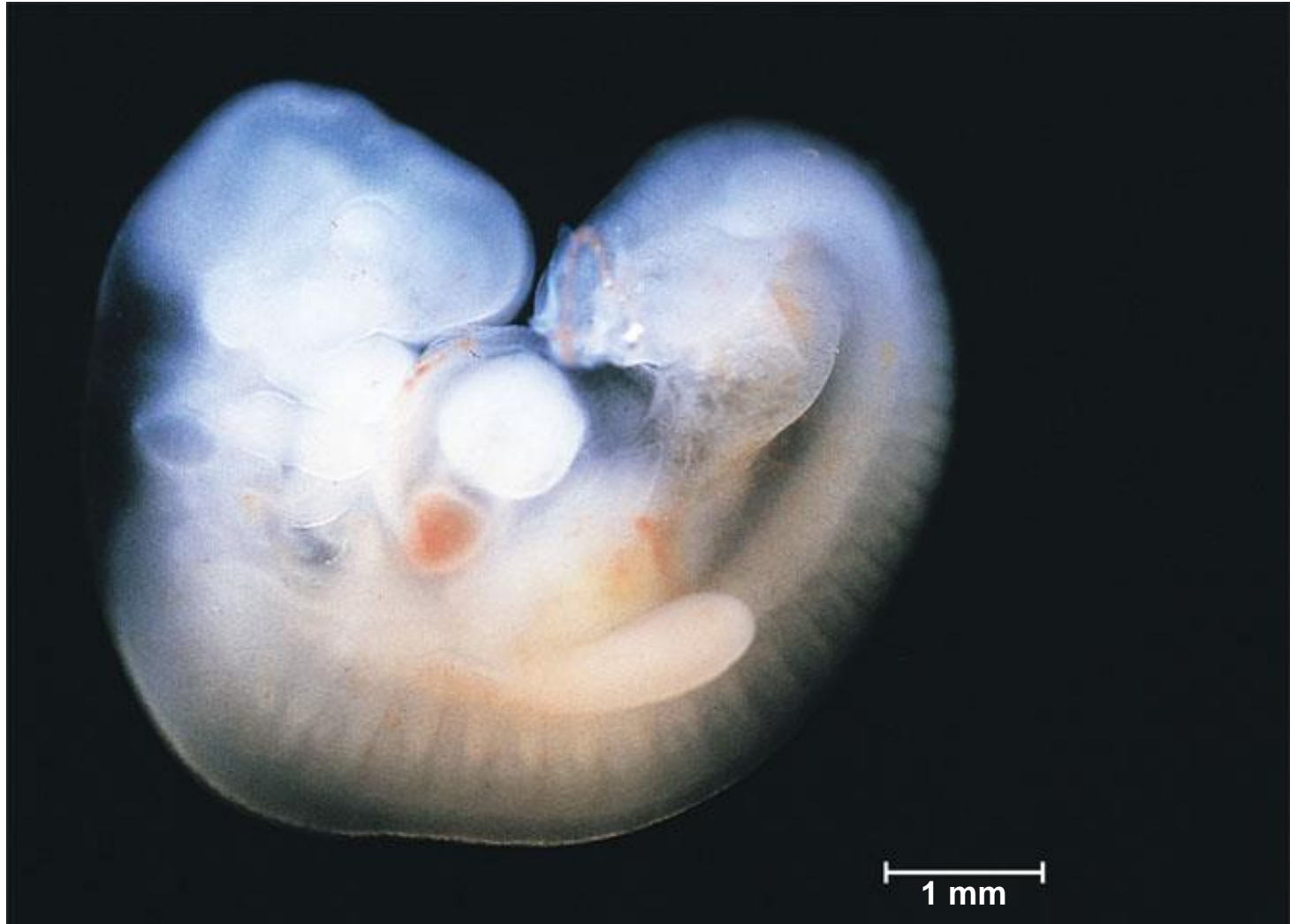
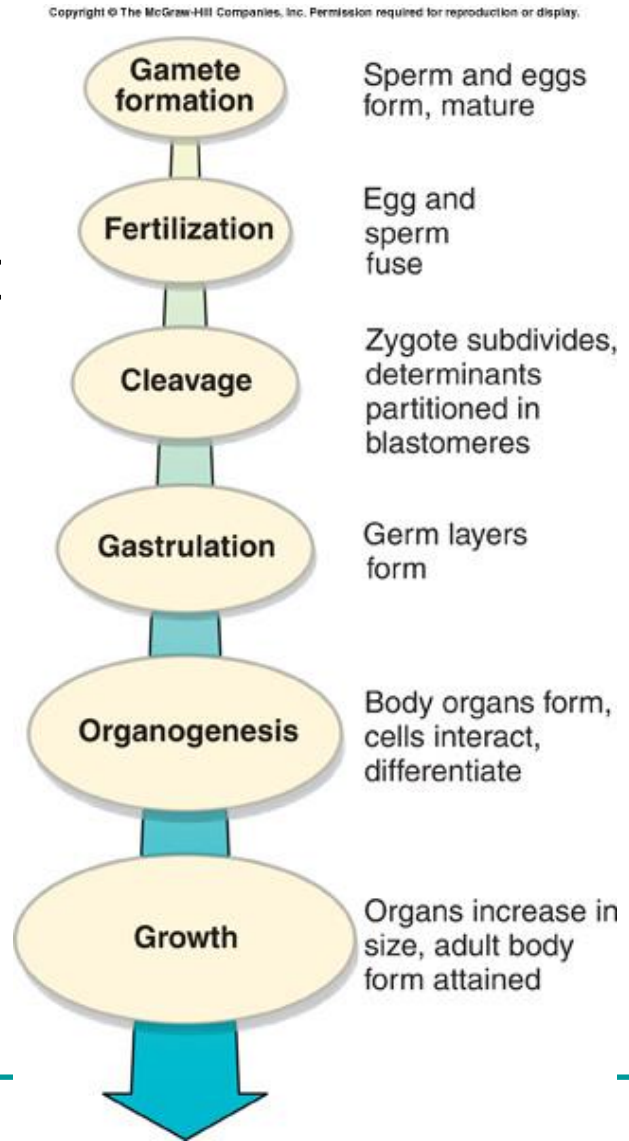


A human embryo about six to eight weeks after conception



Key Events in Development

- **Development** describes the changes in an organism from its earliest beginnings through maturity.



Fertilization

- **Fertilization** is the initial event in development in sexual reproduction.
 - Union of male and female gametes
 - Provides for **recombination** of paternal and maternal genes.
 - Restores the diploid number.
 - **Activates** the egg to begin development.

Fertilization

- **Oocyte Maturation**

- Egg grows in size by accumulating yolk.
 - Contains much mRNA, ribosomes, tRNA and elements for protein synthesis.
- **Morphogenetic determinants** direct the activation and repression of specific genes later in post-fertilization development.
- Egg nucleus grows in size, bloated with RNA.
 - Now called the **germinal vesicle**.

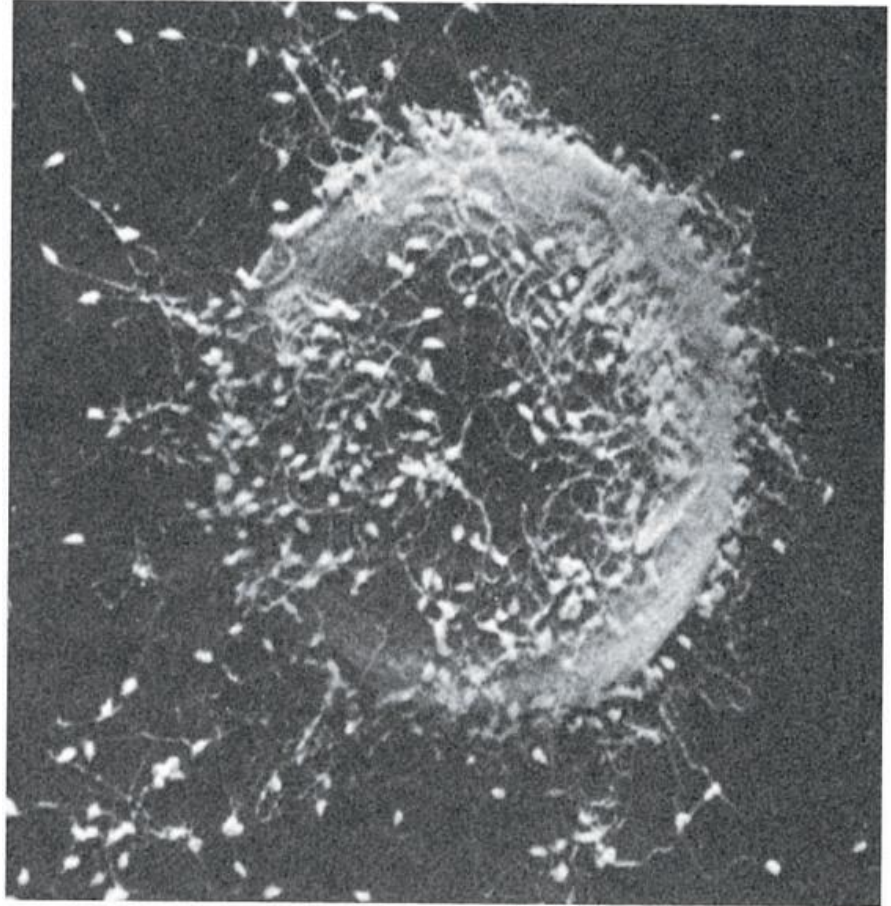
Fertilization

- Most of these preparations in the egg occur during the prolonged prophase I.
 - In mammals
- Oocyte now has a highly structured system.
 - After fertilization it will support nutritional requirements of the embryo and direct its development through cleavage.
- After meiosis resumes, the egg is ready to fuse its nucleus with the sperm nucleus.

Fertilization in Sea Urchins

- Prevention of **polyspermy** – only one sperm can enter.
 - Fast block
 - Depolarization of membrane
 - Slow block
 - Cortical reaction resulting in fertilization membrane

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The acrosomal and cortical reactions during sea urchin fertilization

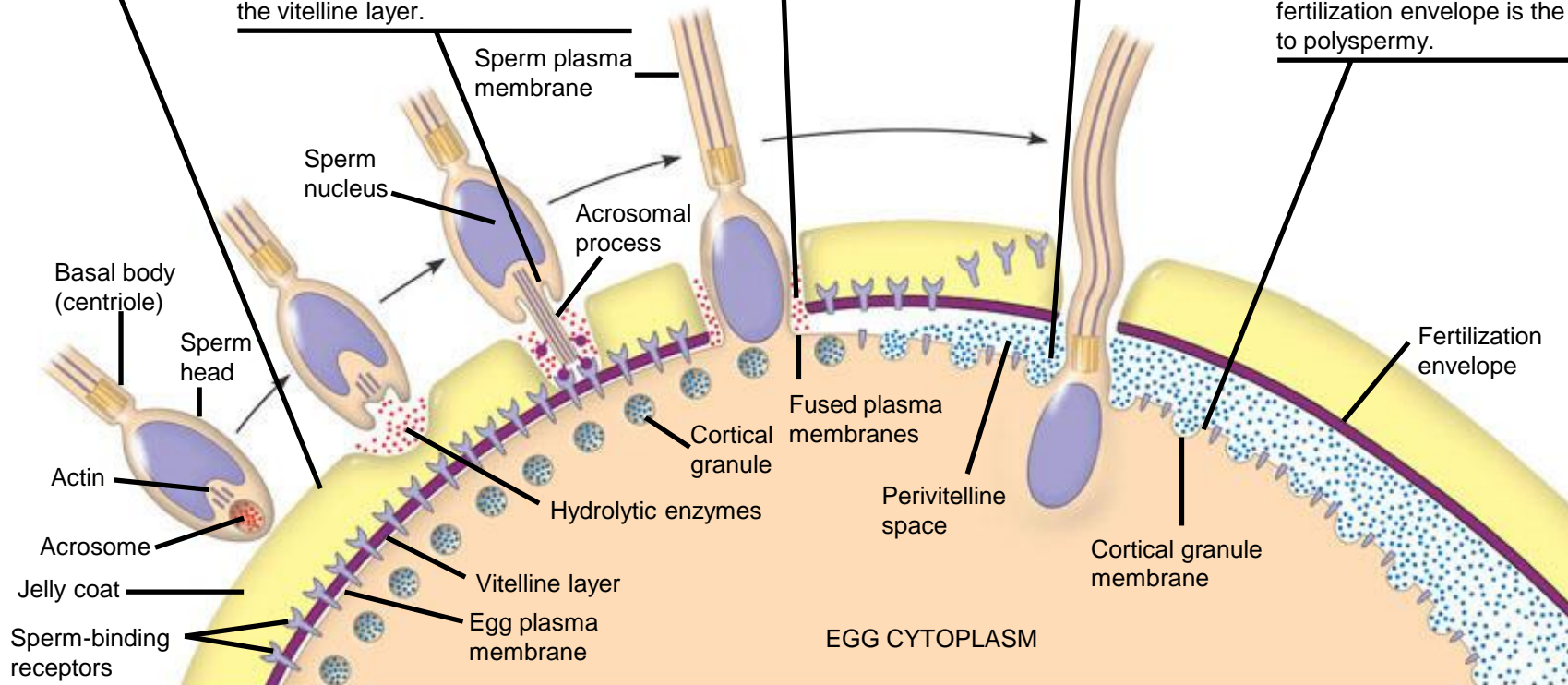
1 Contact. The sperm cell contacts the egg's jelly coat, triggering exocytosis from the sperm's acrosome.

2 Acrosomal reaction. Hydrolytic enzymes released from the acrosome make a hole in the jelly coat, while growing actin filaments form the acrosomal process. This structure protrudes from the sperm head and penetrates the jelly coat, binding to receptors in the egg cell membrane that extend through the vitelline layer.

3 Contact and fusion of sperm and egg membranes. A hole is made in the vitelline layer, allowing contact and fusion of the gamete plasma membranes. The membrane becomes depolarized, resulting in the fast block to polyspermy.

4 Entry of sperm nucleus.

5 Cortical reaction. Fusion of the gamete membranes triggers an increase of Ca^{2+} in the egg's cytosol, causing cortical granules in the egg to fuse with the plasma membrane and discharge their contents. This leads to swelling of the perivitelline space, hardening of the vitelline layer, and clipping of sperm-binding receptors. The resulting fertilization envelope is the slow block to polyspermy.



Early events of fertilization in mammals

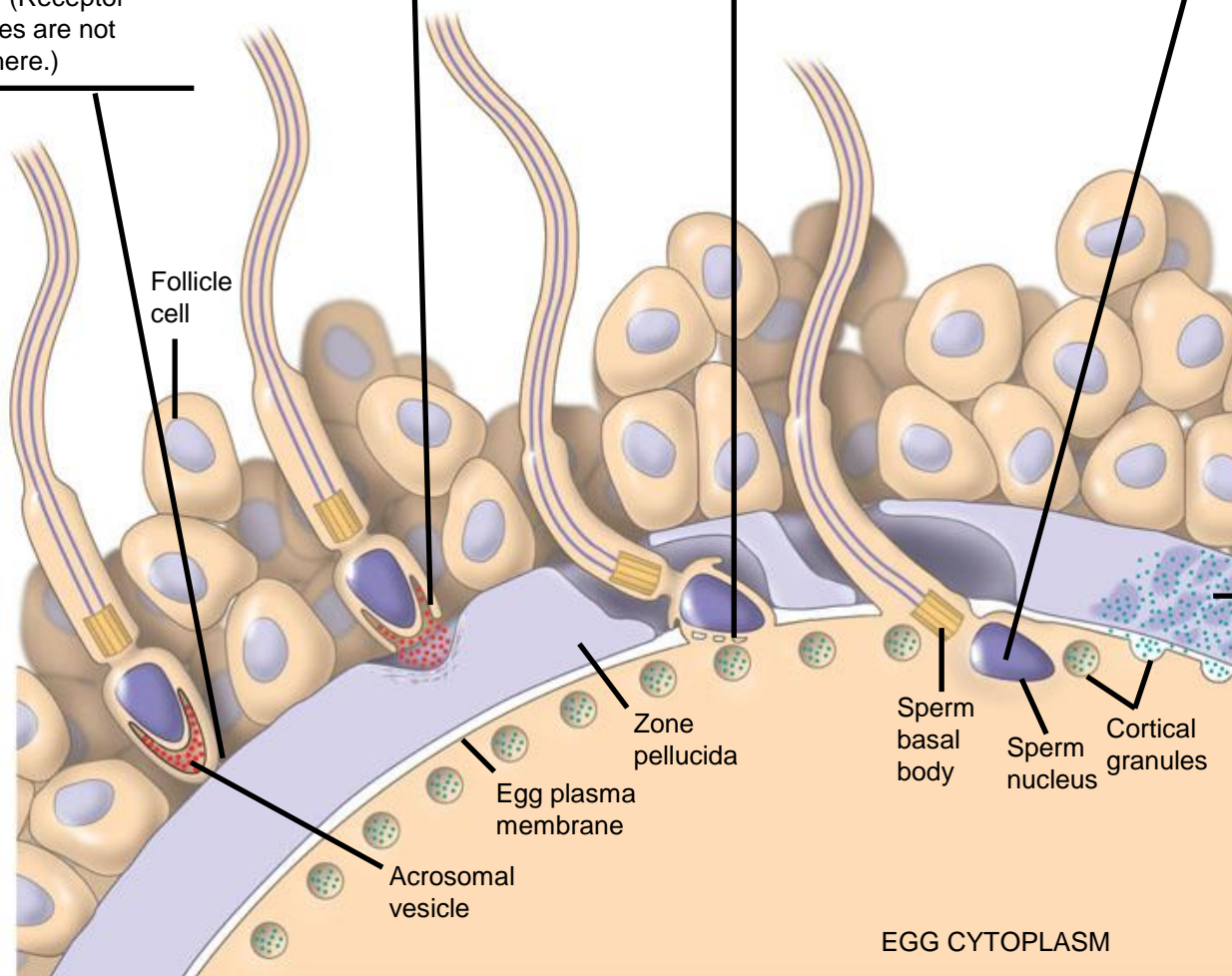
1 The sperm migrates through the coat of follicle cells and binds to receptor molecules in the zona pellucida of the egg. (Receptor molecules are not shown here.)

2 This binding induces the acrosomal reaction, in which the sperm releases hydrolytic enzymes into the zona pellucida.

3 Breakdown of the zona pellucida by these enzymes allows the sperm to reach the plasma membrane of the egg. Membrane proteins of the sperm bind to receptors on the egg membrane, and the two membranes fuse.

4 The nucleus and other components of the sperm cell enter the egg.

5 Enzymes released during the cortical reaction harden the zona pellucida, which now functions as a block to polyspermy.



Fertilization in Sea Urchins

- The **cortical reaction** follows the fusion of thousands of enzyme-rich cortical granules with the egg membrane.
 - Cortical granules release contents between the membrane and vitelline envelope.
 - Creates an osmotic gradient
 - Water rushes into space
 - Elevates the envelope
 - Lifts away all bound sperm except the one sperm that has successfully fused with the egg plasma membrane.

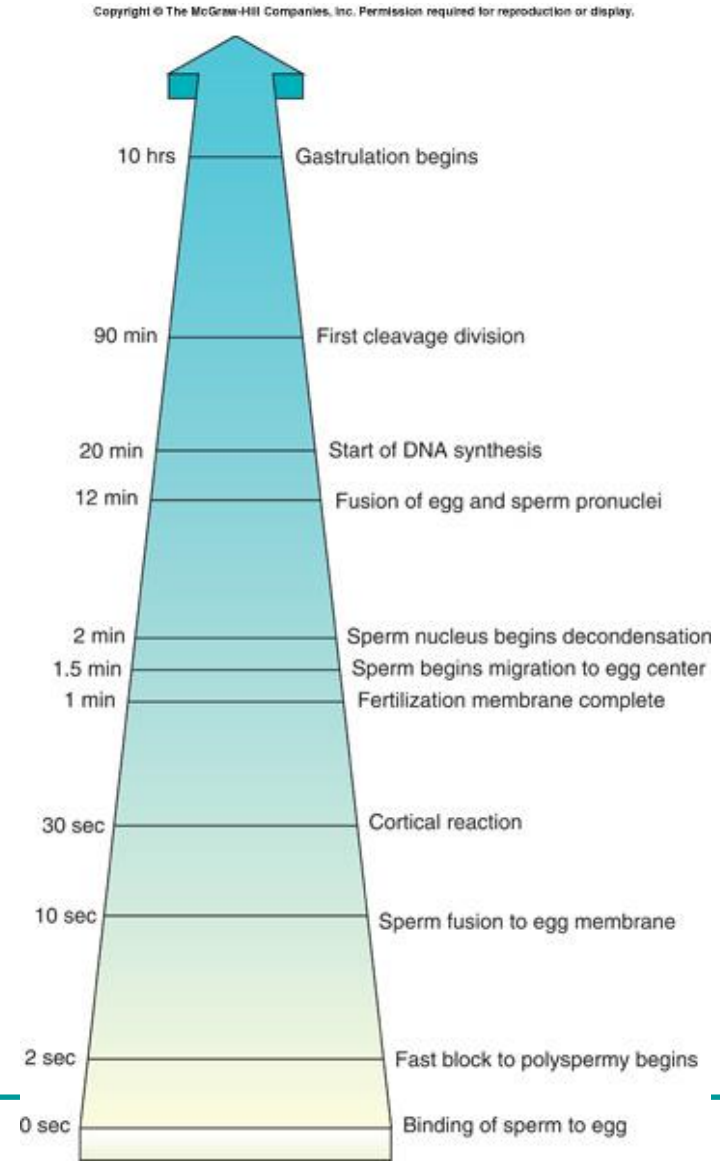
Fertilization in Sea Urchins

- One cortical granule enzyme causes the vitelline envelope to harden.
 - Now called the **fertilization membrane**.
 - Block to polyspermy is now complete.
- Similar process occurs in mammals.



Fertilization in Sea Urchins

- The increased Ca^{2+} concentration in the egg after the cortical reaction results in an increase in the rates of cellular respiration and protein synthesis.
- The egg is **activated**.

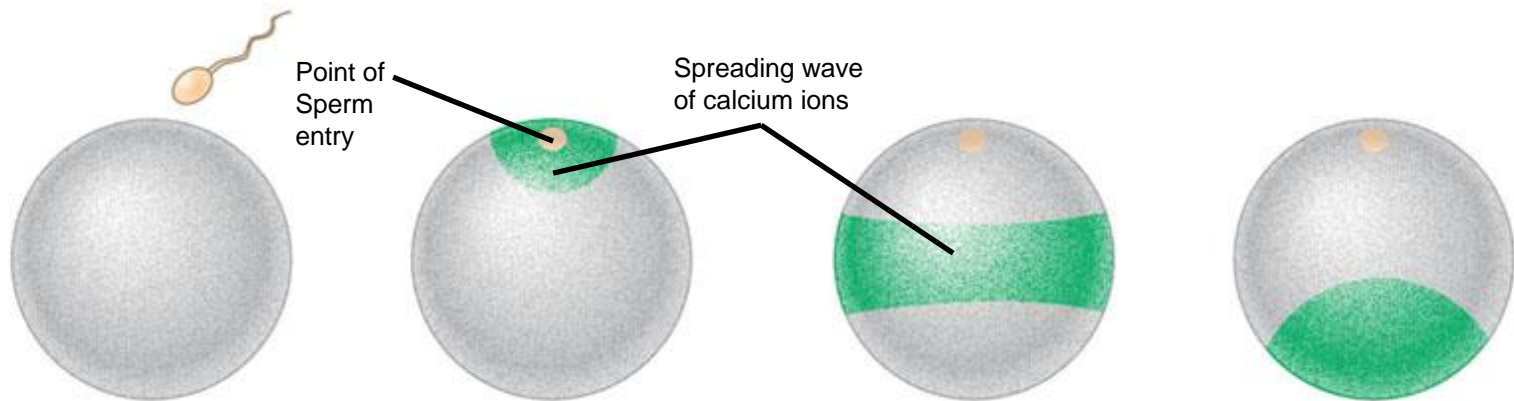
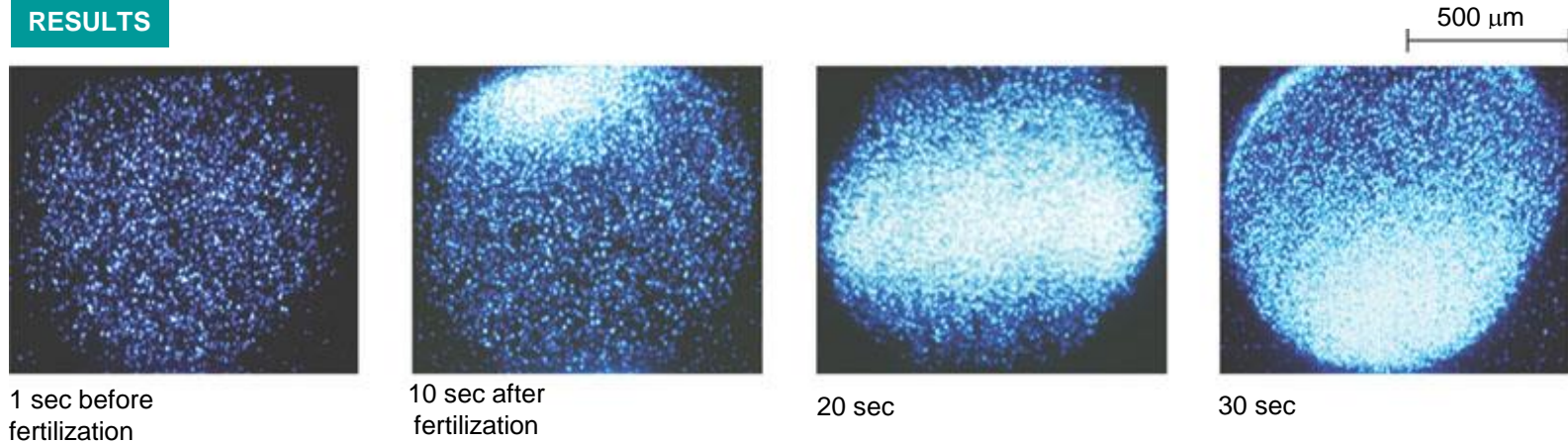


What is the effect of sperm binding on Ca^{2+} distribution in the egg?

EXPERIMENT

A fluorescent dye that glows when it binds free Ca^{2+} was injected into unfertilized sea urchin eggs. After sea urchin sperm were added, researchers observed the eggs in a fluorescence microscope.

RESULTS



CONCLUSION

The release of Ca^{2+} from the endoplasmic reticulum into the cytosol at the site of sperm entry triggers the release of more and more Ca^{2+} in a wave that spreads to the other side of the cell. The entire process takes about 30 seconds.

Key Events in Development

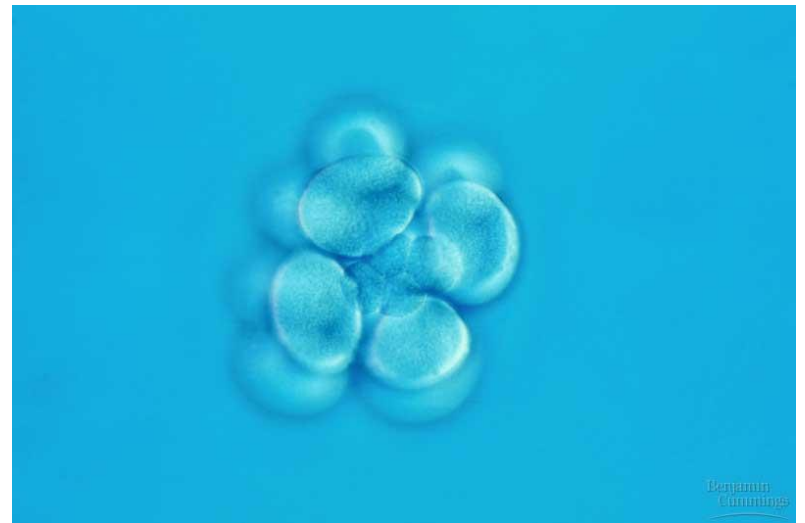
- The two basic processes responsible for this progressive subdivision:
 - **Cytoplasmic localization**
 - **Induction**

Fusion of Pronuclei

- After sperm and egg membranes fuse, the sperm loses its flagellum.
- Fusion of male and female pronuclei forms a **diploid zygote nucleus**.

Cleavage

- **Cleavage** – rapid cell divisions following fertilization.
 - Very little growth occurs.
 - Each cell called a **blastomere**.
 - **Morula** – solid ball of cells. First 5-7 divisions.

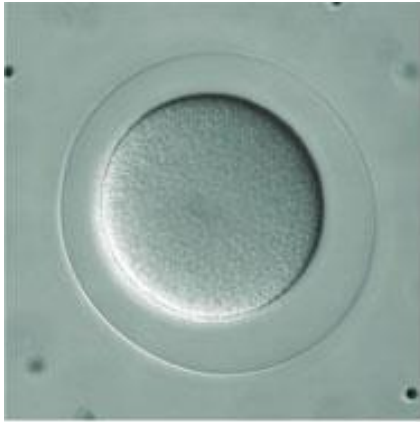


Blastula

- A fluid filled cavity, the **blastocoel**, forms within the embryo – a hollow ball of cells now called a **blastula**.



Cleavage in an echinoderm embryo



(a) Fertilized egg. Shown here is the zygote shortly before the first cleavage division, surrounded by the fertilization envelope. The nucleus is visible in the center.



(b) Four-cell stage. Remnants of the mitotic spindle can be seen between the two cells that have just completed the second cleavage division.

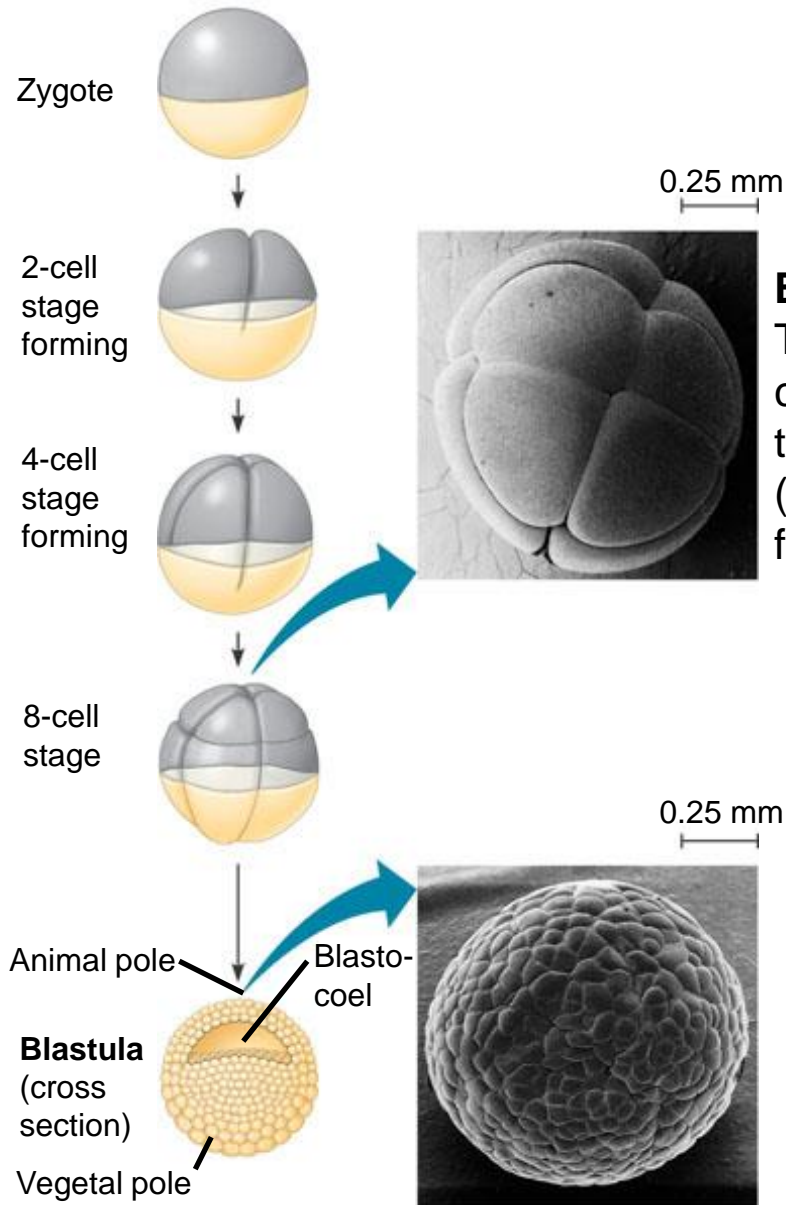


(c) Morula. After further cleavage divisions, the embryo is a multicellular ball that is still surrounded by the fertilization envelope. The blastocoel cavity has begun to form.



(d) Blastula. A single layer of cells surrounds a large blastocoel cavity. Although not visible here, the fertilization envelope is still present; the embryo will soon hatch from it and begin swimming.

Cleavage in a frog embryo



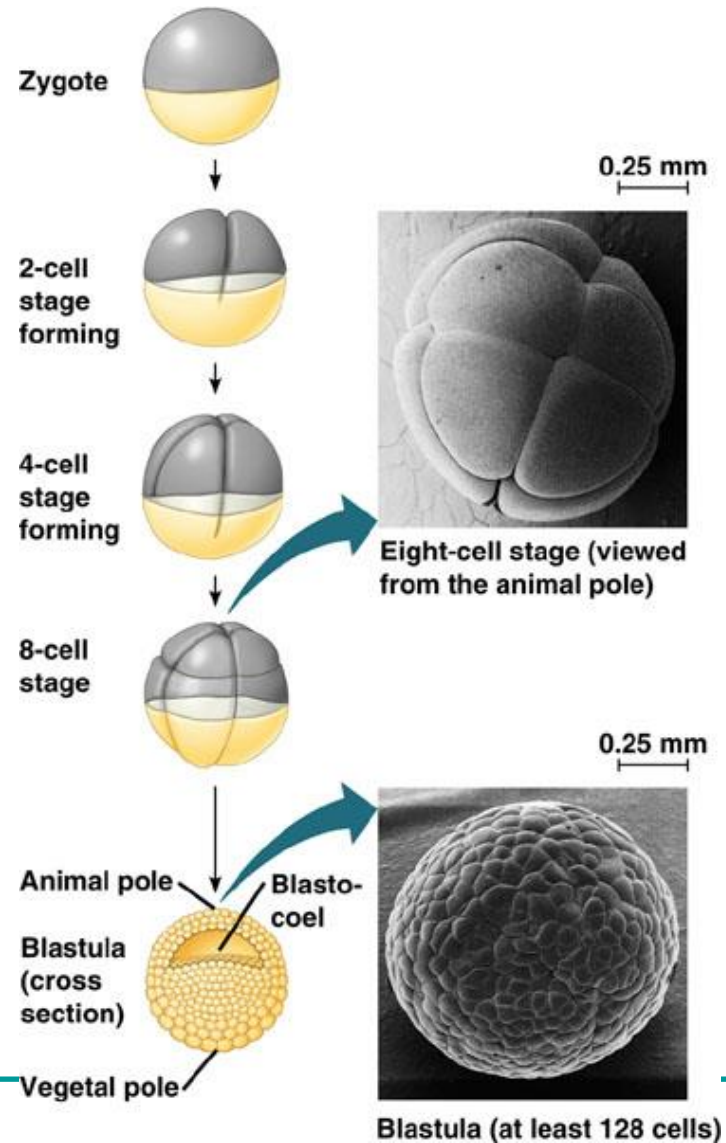
Eight-cell stage (viewed from the animal pole).

The large amount of yolk displaces the third cleavage toward the animal pole, forming two tiers of cells. The four cells near the animal pole (closer, in this view) are smaller than the other four cells (SEM).

Blastula (at least 128 cells). As cleavage continues, a fluid-filled cavity, the blastocoel, forms within the embryo. Because of unequal cell division due to the large amount of yolk in the vegetal hemisphere, the blastocoel is located in the animal hemisphere, as shown in the cross section. The SEM shows the outside of a blastula with about 4,000 cells, looking at the animal pole.

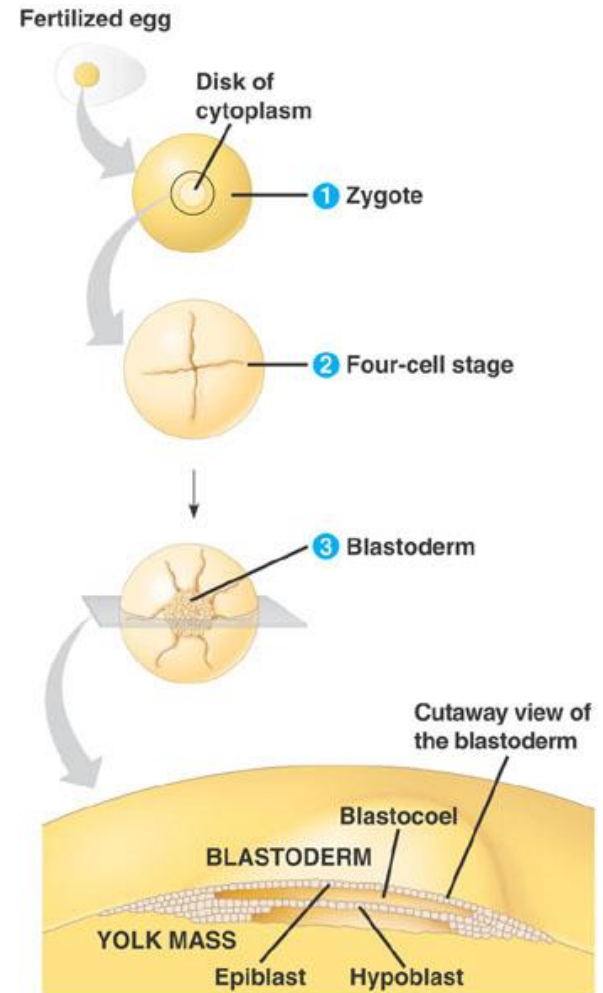
Cleavage in Frogs

- Cleavage planes usually follow a specific pattern that is relative to the animal and vegetal poles of the zygote.
 - Animal pole blastomeres are smaller.
 - Blastocoel in animal hemisphere.
 - Little yolk, cleavage furrows complete.
 - **Holoblastic cleavage**



Cleavage in Birds

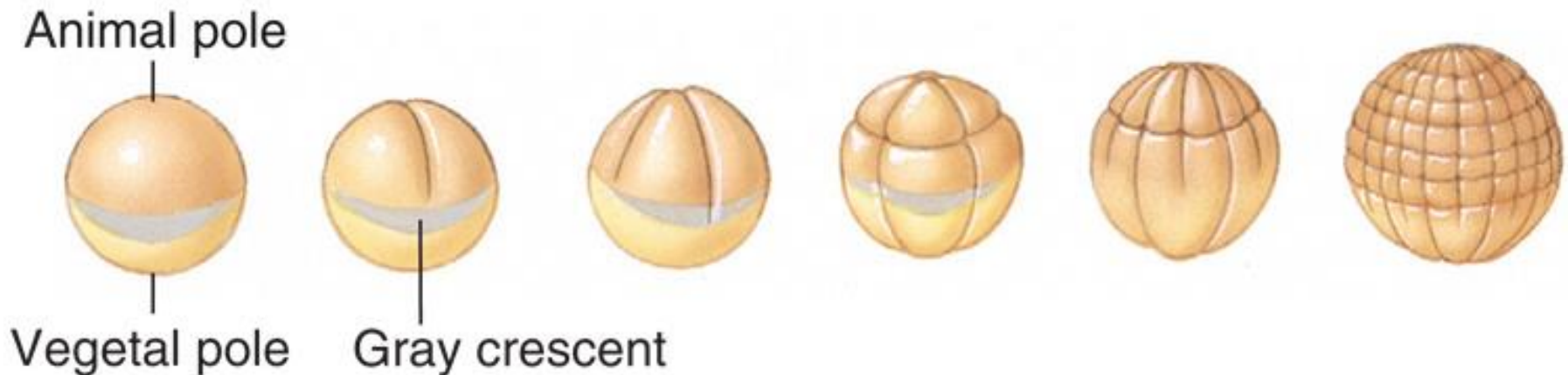
- **Meroblastic cleavage**, incomplete division of the egg.
 - Occurs in species with yolk-rich eggs, such as reptiles and birds.
 - **Blastoderm** – cap of cells on top of yolk.



Polarity

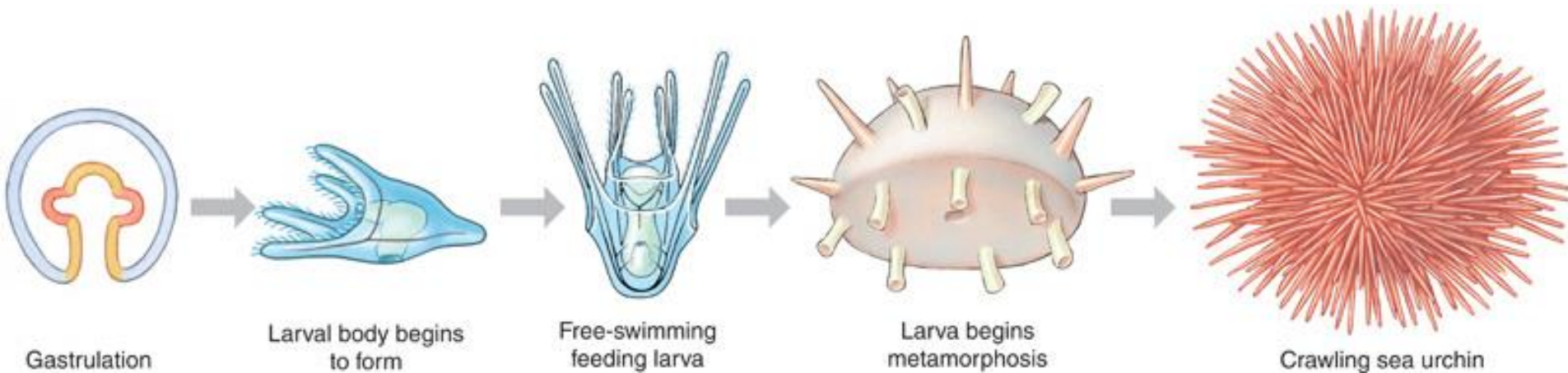
- The eggs and zygotes of many animals (not mammals) have a definite **polarity**.
- The polarity is defined by the distribution of yolk.
 - The **vegetal pole** has the most yolk and the **animal pole** has the least.

B Frog: Mesolecithal egg



Direct vs. Indirect Development

- When lots of nourishing yolk is present, embryos develop into a miniature adult.
 - **Direct development**
- When little yolk is present, young develop into larval stages that can feed.
 - **Indirect development**



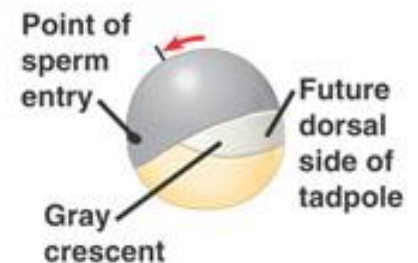
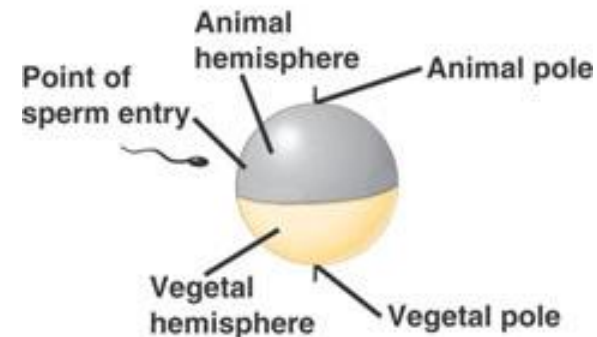
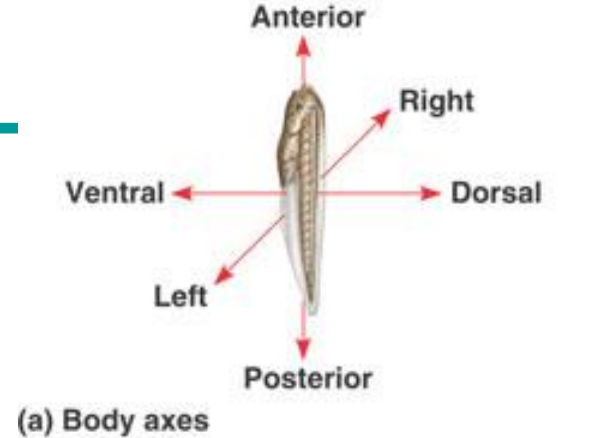
Body Axes

- The development of body axes in frogs is influenced by the polarity of the egg.

The polarity of the egg determines the anterior-posterior axis before fertilization.

At fertilization, the pigmented cortex slides over the underlying cytoplasm toward the point of sperm entry. This rotation (red arrow) exposes a region of lighter-colored cytoplasm, the **gray crescent**, which is a **marker of the dorsal** side.

The first cleavage division bisects the gray crescent. Once the anterior-posterior and dorsal-ventral axes are defined, so is the left-right axis.



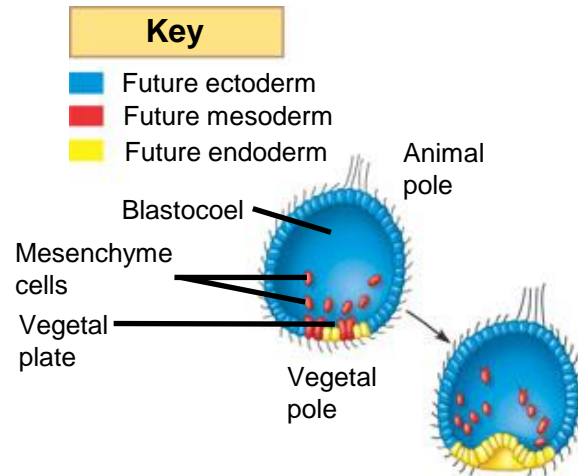
(b) Establishing the axes

Gastrulation

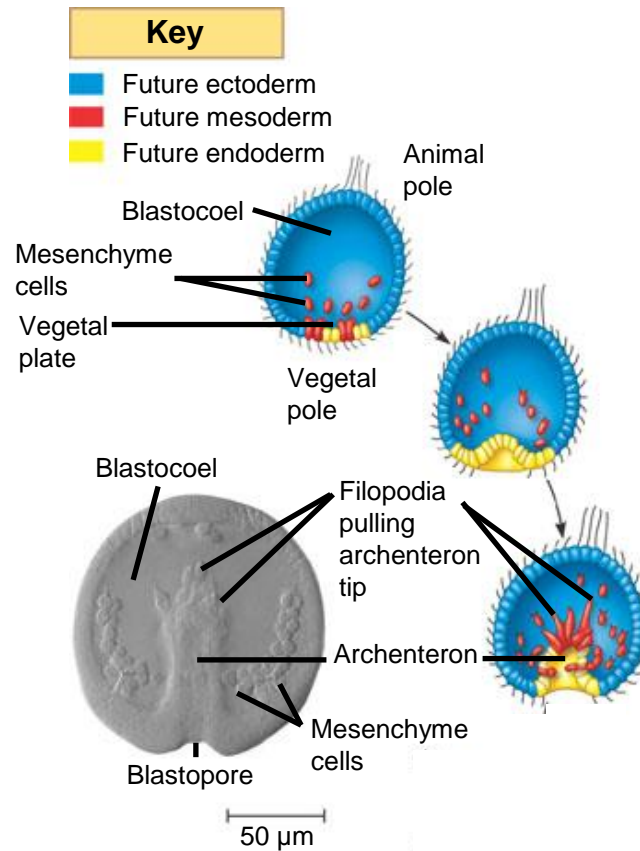
- The morphogenetic process called **gastrulation** rearranges the cells of a blastula into a three-layered (**triploblastic**) embryo, called a **gastrula**, that has a primitive gut.
 - **Diploblastic** organisms have two germ layers.



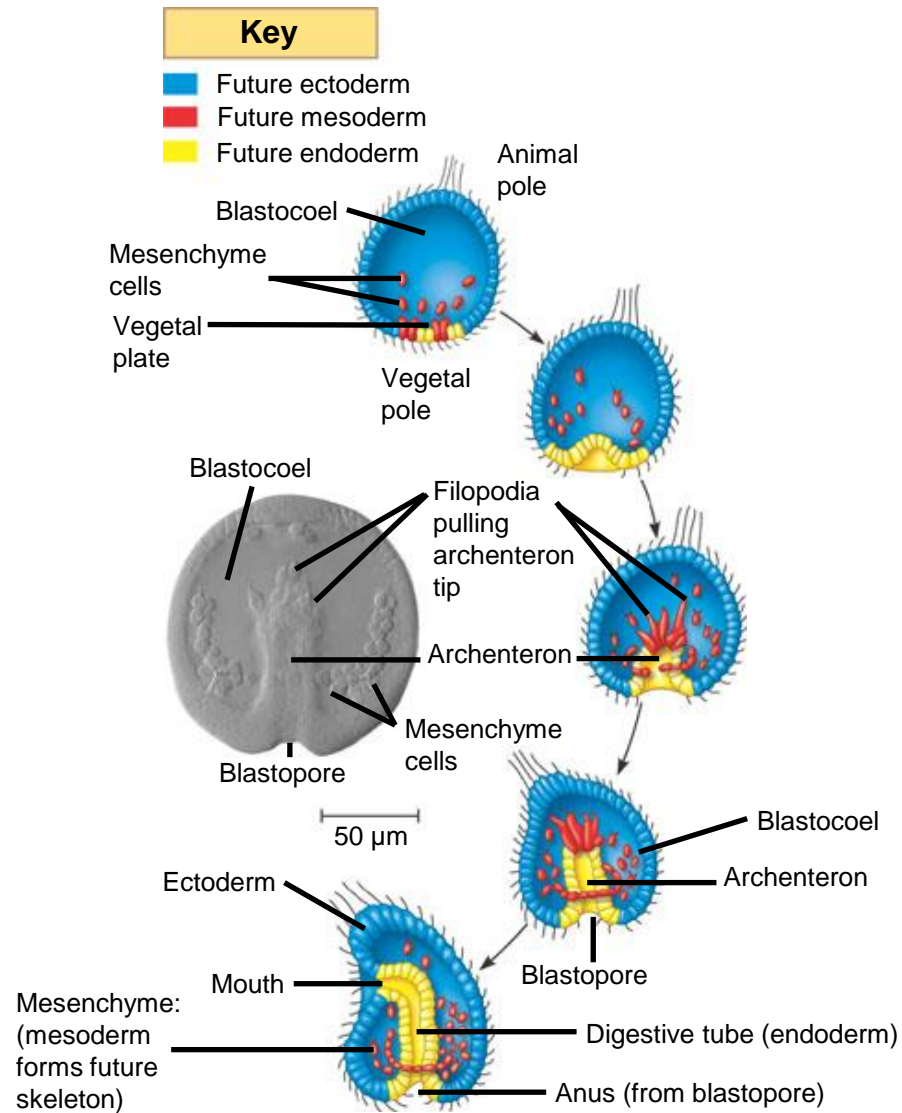
Gastrulation in a sea urchin embryo (layer 1)



Gastrulation in a sea urchin embryo (layer 2)

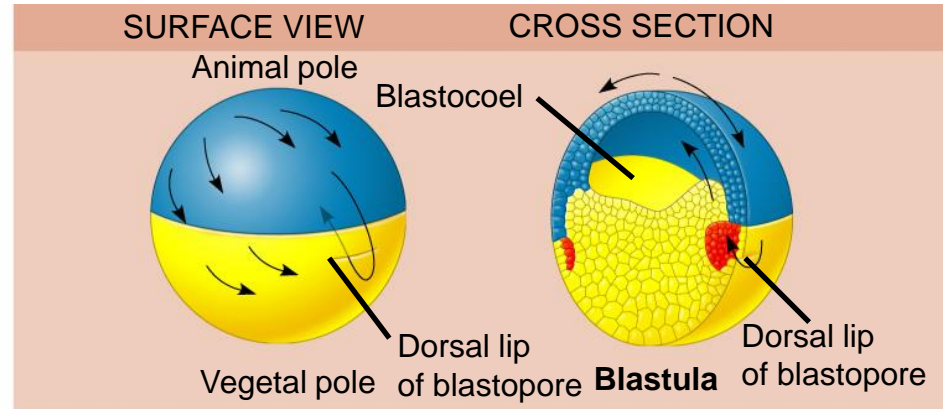


Gastrulation in a sea urchin embryo (layer 3)

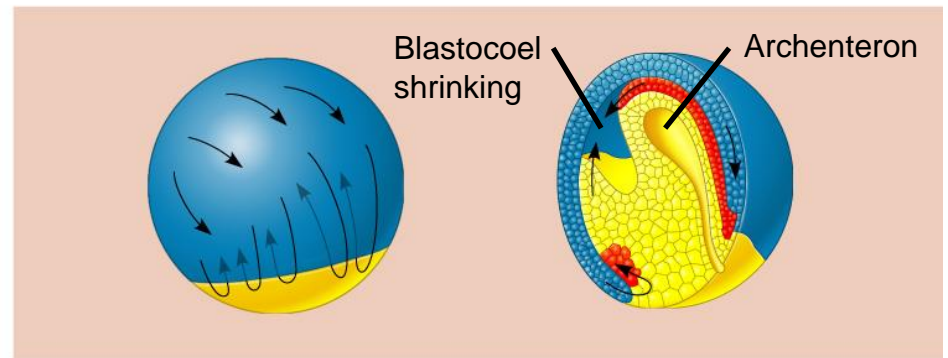


Gastrulation in a frog embryo

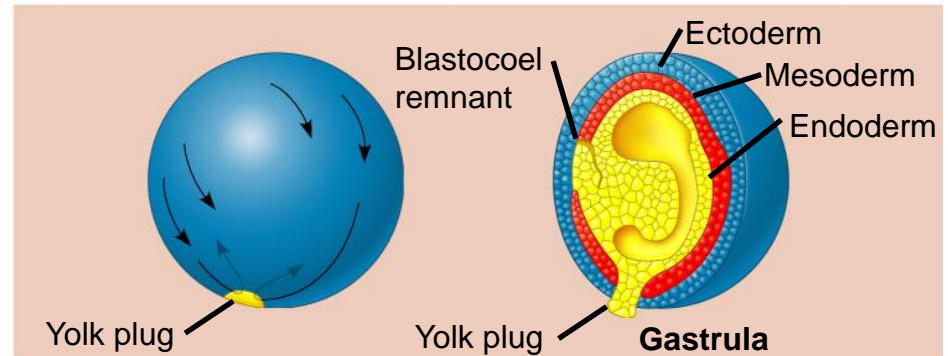
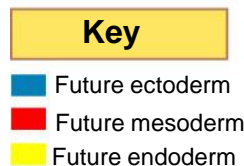
1 Gastrulation begins when a small indented crease, the dorsal lip of the blastopore, appears on one side of the blastula. The crease is formed by cells changing shape and pushing inward from the surface (invagination). Additional cells then roll inward over the dorsal lip (involution) and move into the interior, where they will form endoderm and mesoderm. Meanwhile, cells of the animal pole, the future ectoderm, change shape and begin spreading over the outer surface.



2 The blastopore lip grows on both sides of the embryo, as more cells invaginate. When the sides of the lip meet, the blastopore forms a circle that becomes smaller as ectoderm spreads downward over the surface. Internally, continued involution expands the endoderm and mesoderm, and the archenteron begins to form; as a result, the blastocoel becomes smaller.



3 Late in gastrulation, the endoderm-lined archenteron has completely replaced the blastocoel and the three germ layers are in place. The circular blastopore surrounds a plug of yolk-filled cells.



Cleavage in a chick embryo

Fertilized egg

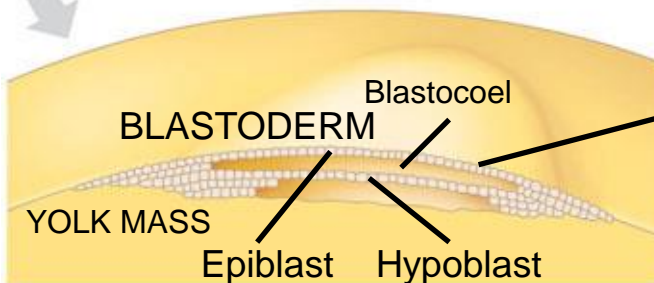
Disk of cytoplasm

1 Zygote. Most of the cell's volume is yolk, with a small disk of cytoplasm located at the animal pole.

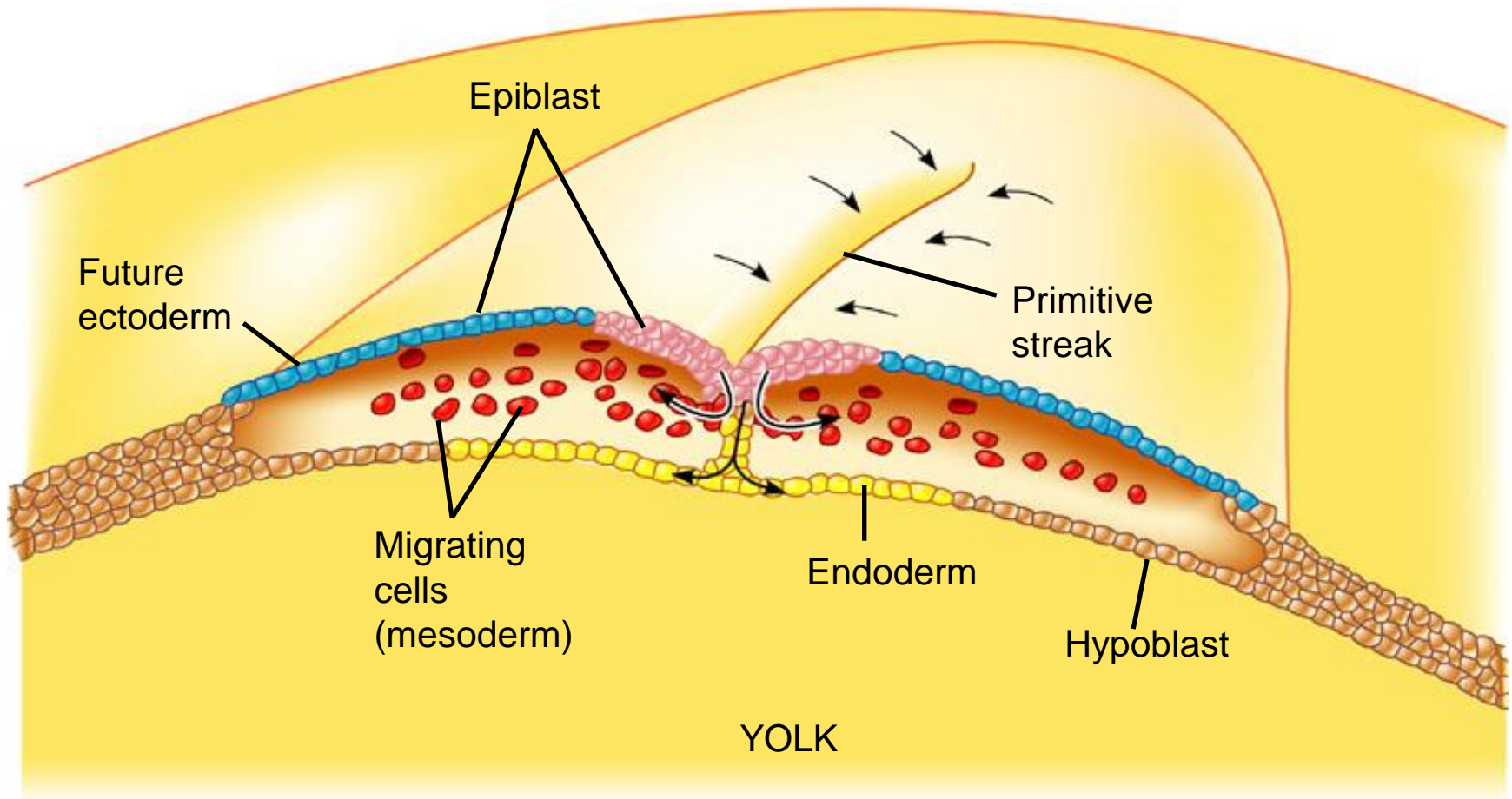
2 Four-cell stage. Early cell divisions are meroblastic (incomplete). The cleavage furrow extends through the cytoplasm but not through the yolk.

3 Blastoderm. The many cleavage divisions produce the blastoderm, a mass of cells that rests on top of the yolk mass.

Cutaway view of the blastoderm. The cells of the blastoderm are arranged in two layers, the epiblast and hypoblast, that enclose a fluid-filled cavity, the blastocoel.

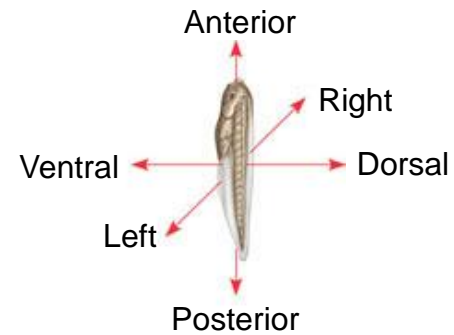


Gastrulation in a chick embryo

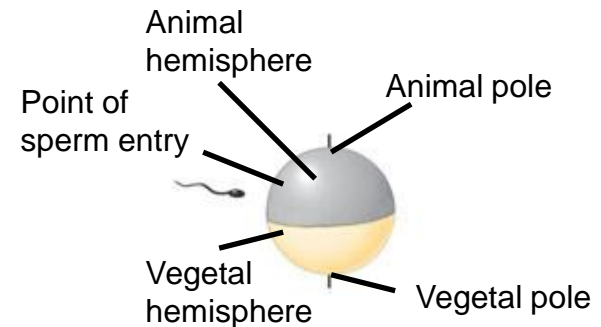


The body axes and their establishment in an amphibian

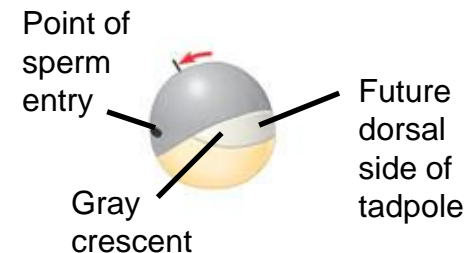
(a) Body axes. The three axes of the fully developed embryo, the tadpole, are shown above.



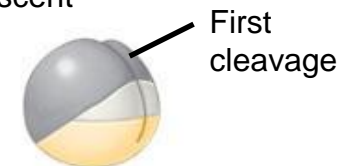
1 The polarity of the egg determines the anterior-posterior axis before fertilization.



2 At fertilization, the pigmented cortex slides over the underlying cytoplasm toward the point of sperm entry. This rotation (red arrow) exposes a region of lighter-colored cytoplasm, the gray crescent, which is a marker of the dorsal side.

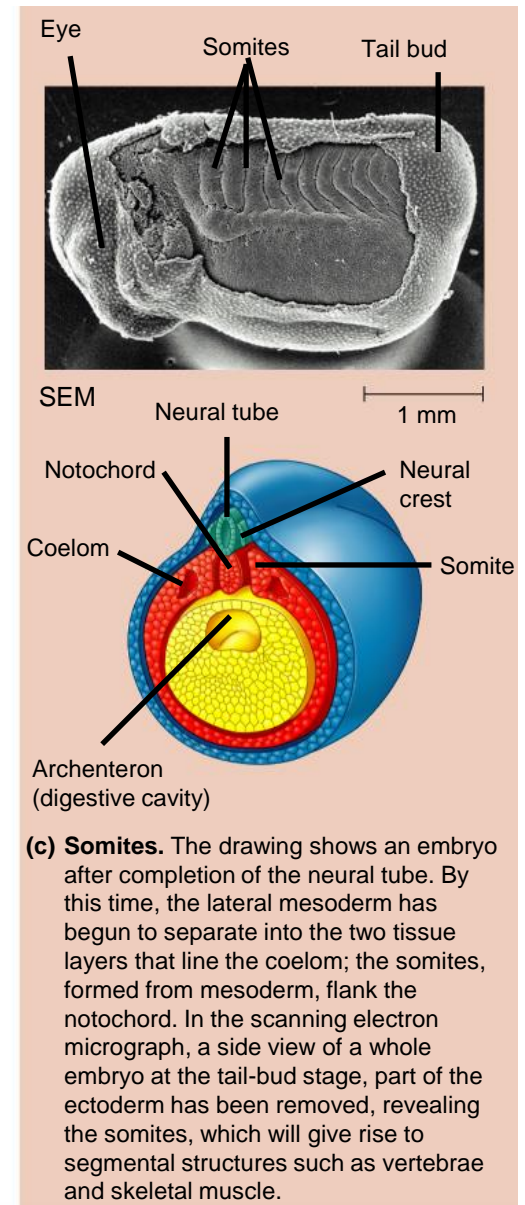
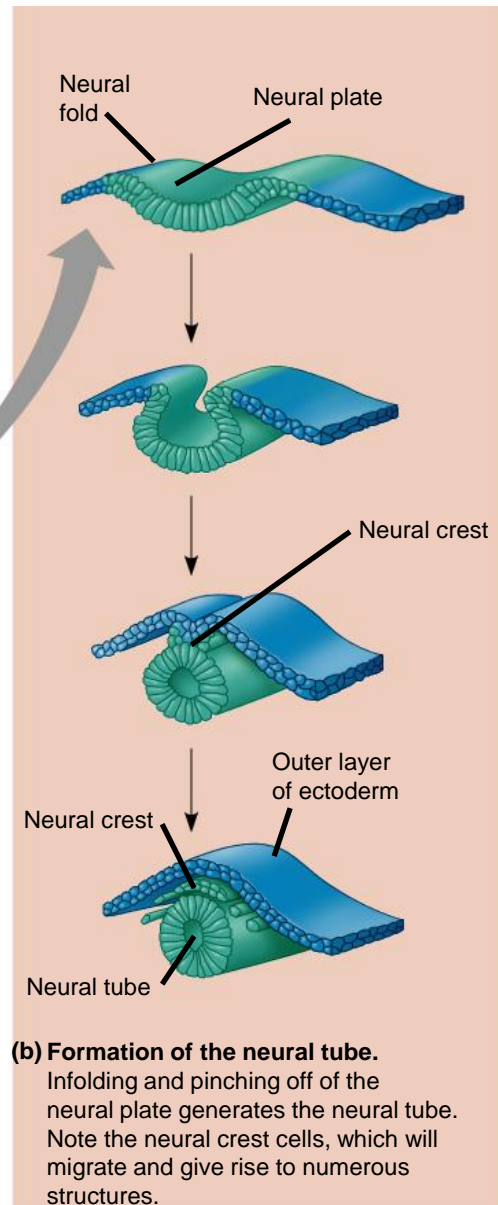
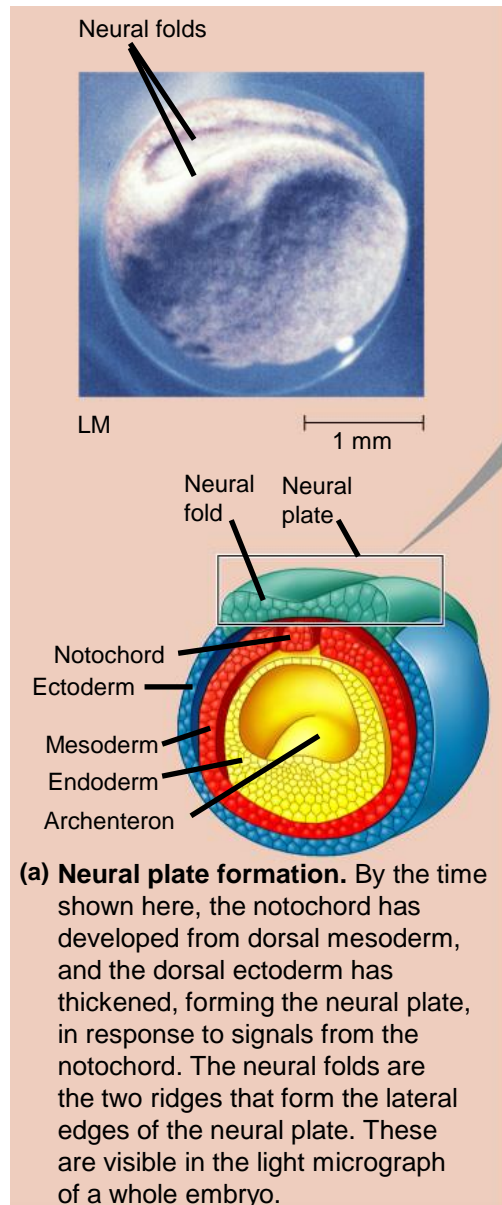


• The first cleavage division bisects the gray crescent. Once the anterior-posterior and dorsal-ventral axes are defined, so is the left-right axis.

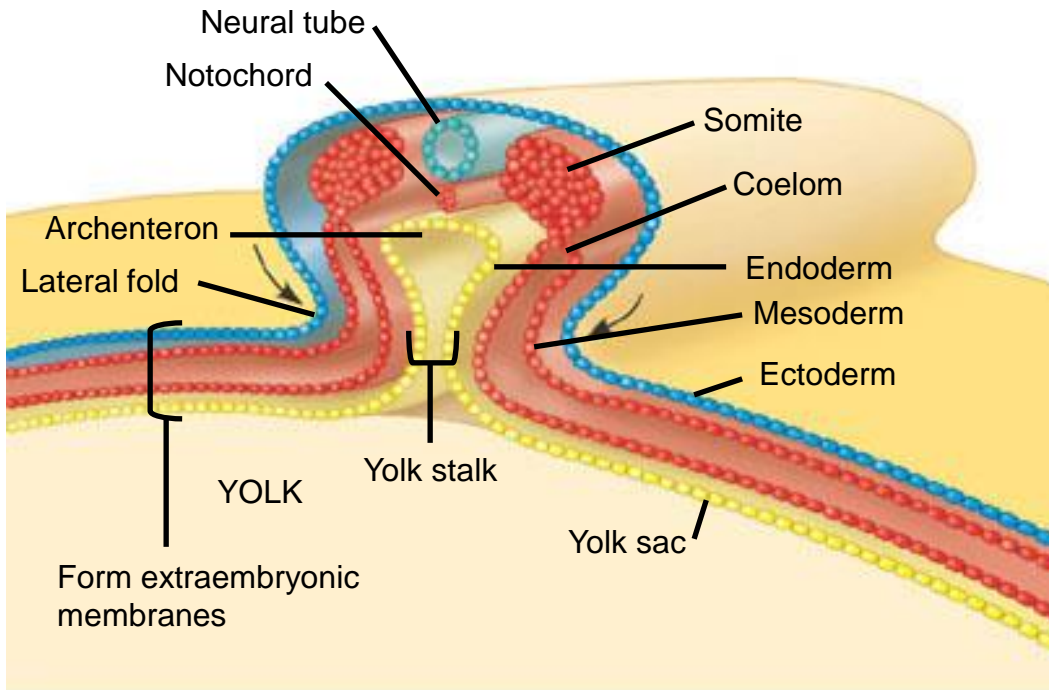


(b) Establishing the axes. The polarity of the egg and cortical rotation are critical in setting up the body axes.

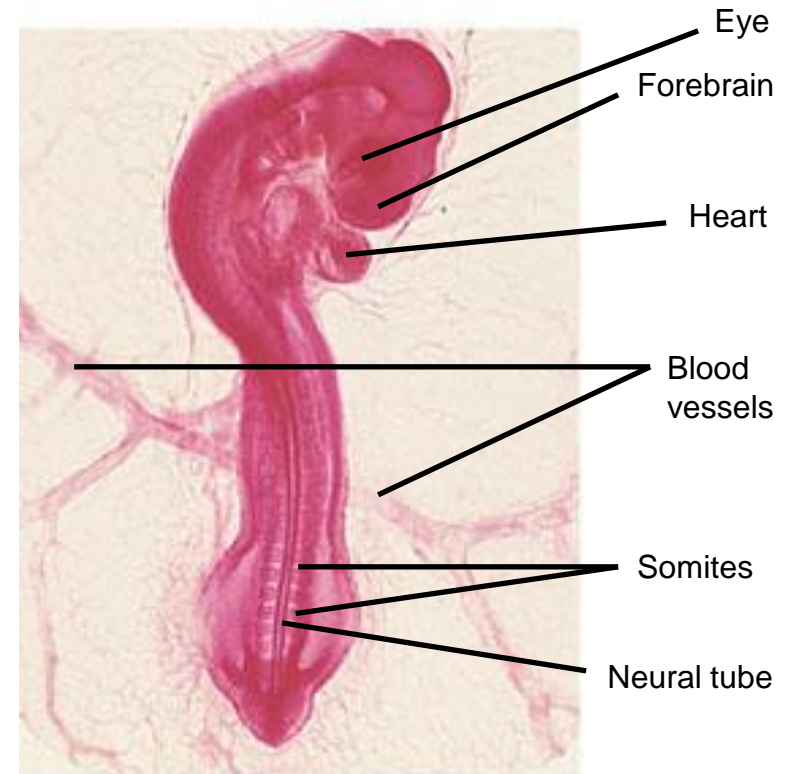
Early organogenesis in a frog embryo



Organogenesis in a chick embryo



(a) Early organogenesis. The archenteron forms when lateral folds pinch the embryo away from the yolk. The embryo remains open to the yolk, attached by the yolk stalk, about midway along its length, as shown in this cross section. The notochord, neural tube, and somites subsequently form much as they do in the frog.



(b) Late organogenesis. Rudiments of most major organs have already formed in this chick embryo, which is about 56 hours old and about 2–3 mm long (LM).

Adult derivatives of the three embryonic germ layers in vertebrates

ECTODERM

- Epidermis of skin and its derivatives (including sweat glands, hair follicles)
- Epithelial lining of mouth and rectum
- Sense receptors in epidermis
- Cornea and lens of eye
- Nervous system
- Adrenal medulla
- Tooth enamel
- Epithelium of pineal and pituitary glands

MESODERM

- Notochord
- Skeletal system
- Muscular system
- Muscular layer of stomach, intestine, etc.
- Excretory system
- Circulatory and lymphatic systems
- Reproductive system (except germ cells)
- Dermis of skin
- Lining of body cavity
- Adrenal cortex

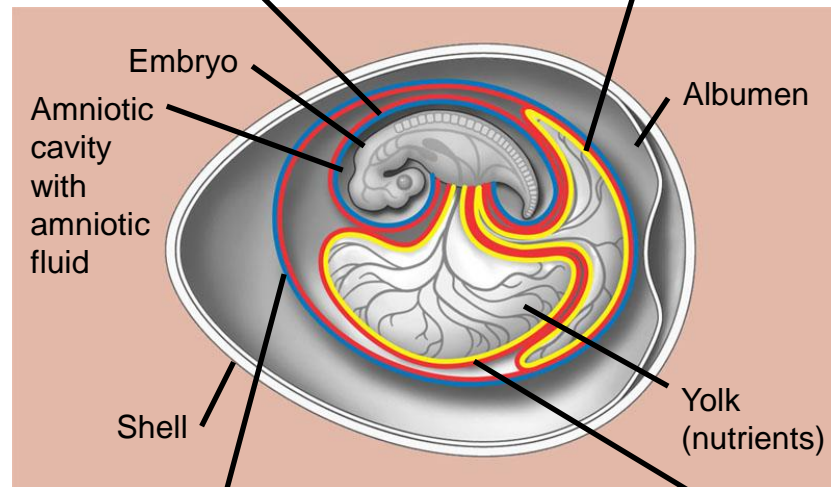
ENDODERM

- Epithelial lining of digestive tract
- Epithelial lining of respiratory system
- Lining of urethra, urinary bladder, and reproductive system
- Liver
- Pancreas
- Thymus
- Thyroid and parathyroid glands

Extraembryonic membranes in birds and other reptiles

Amnion. The amnion protects the embryo in a fluid-filled cavity that prevents dehydration and cushions mechanical shock.

Allantois. The allantois functions as a disposal sac for certain metabolic wastes produced by the embryo. The membrane of the allantois also functions with the chorion as a respiratory organ.



Chorion. The chorion and the membrane of the allantois exchange gases between the embryo and the surrounding air. Oxygen and carbon dioxide diffuse freely across the egg's shell.

Yolk sac. The yolk sac expands over the yolk, a stockpile of nutrients stored in the egg. Blood vessels in the yolk sac membrane transport nutrients from the yolk into the embryo. Other nutrients are stored in the albumen (the "egg white").

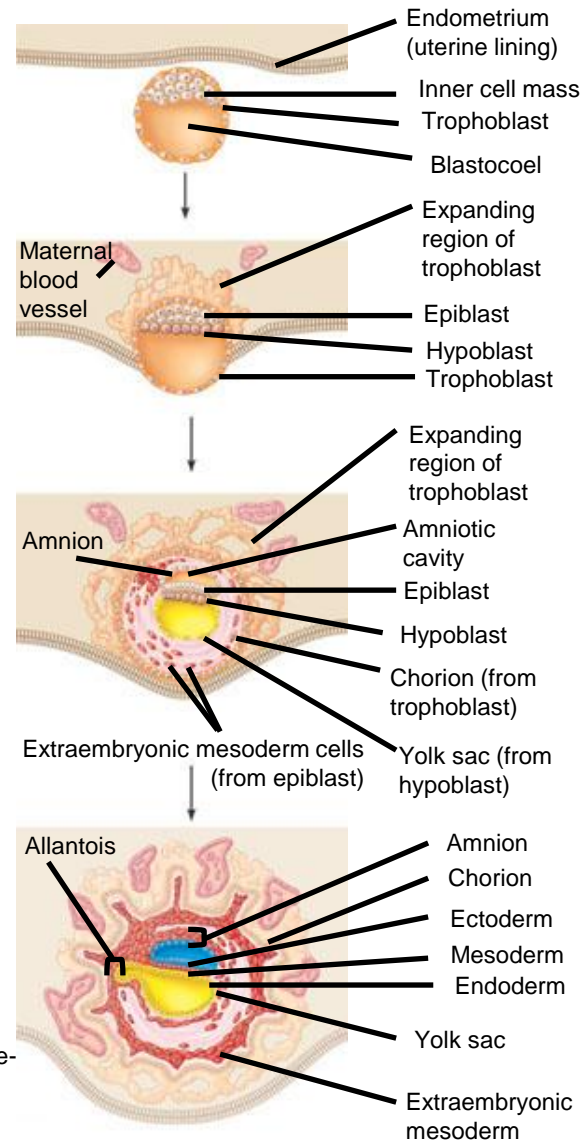
Four stages in early embryonic development of a human

- 1 Blastocyst reaches uterus.

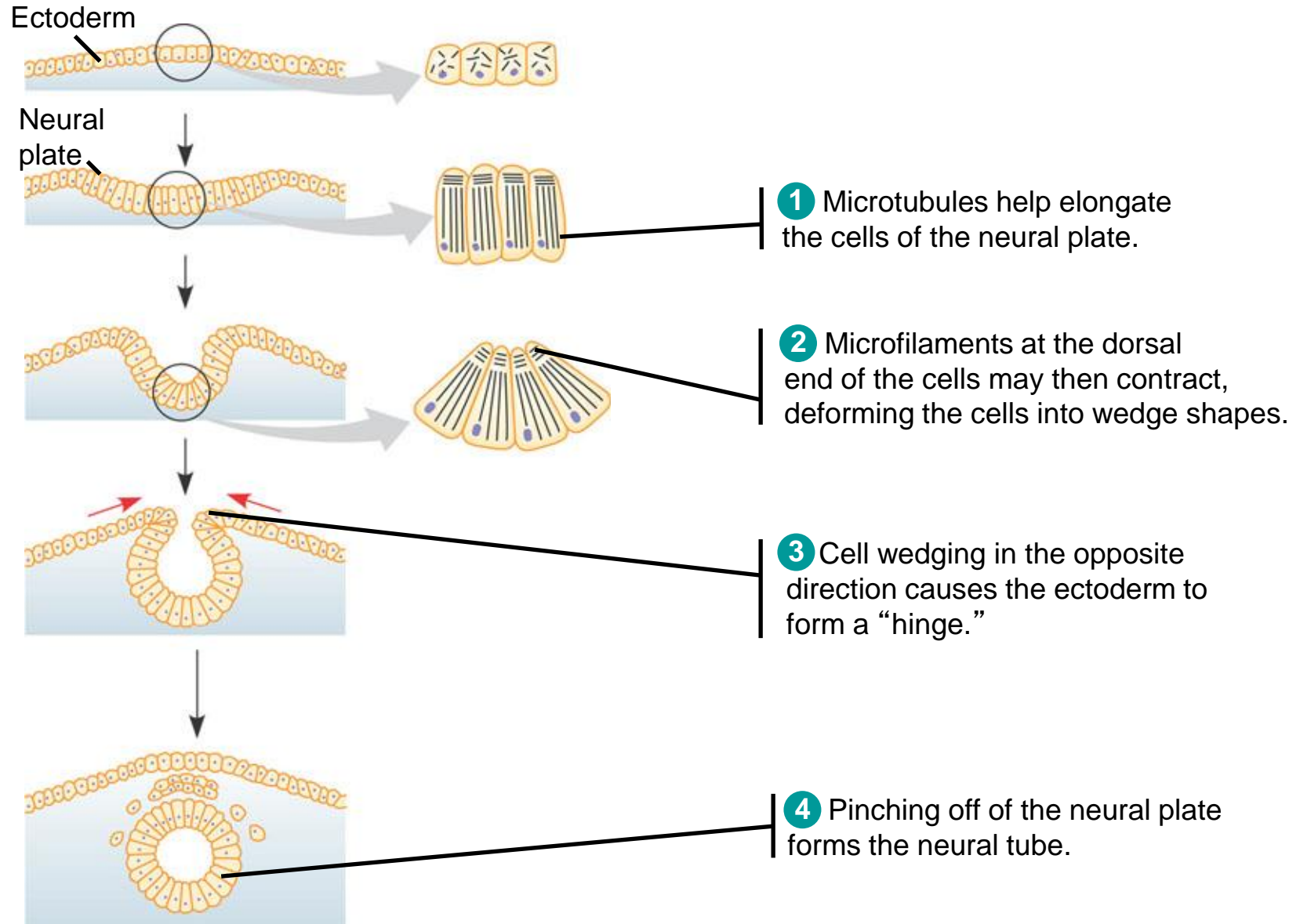
- 2 Blastocyst implants.

- 3 Extraembryonic membranes start to form and gastrulation begins.

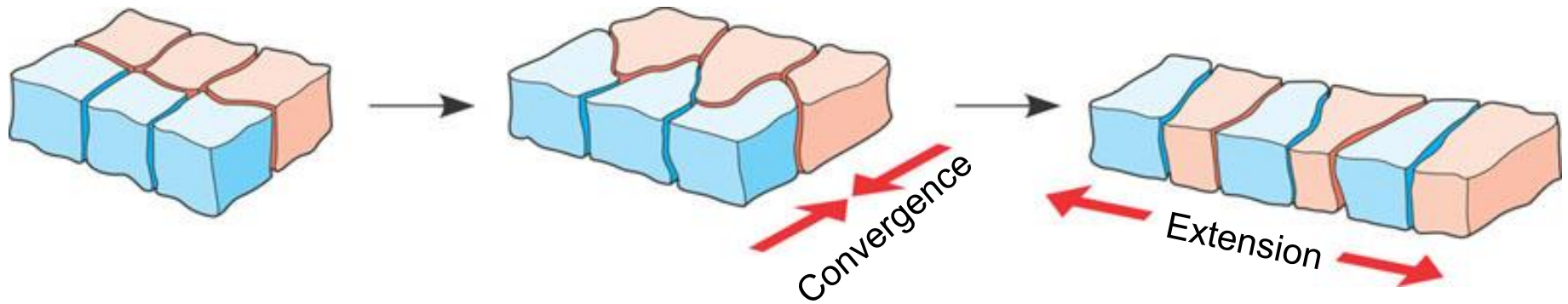
- 4 Gastrulation has produced a three-layered embryo with four extraembryonic membranes.



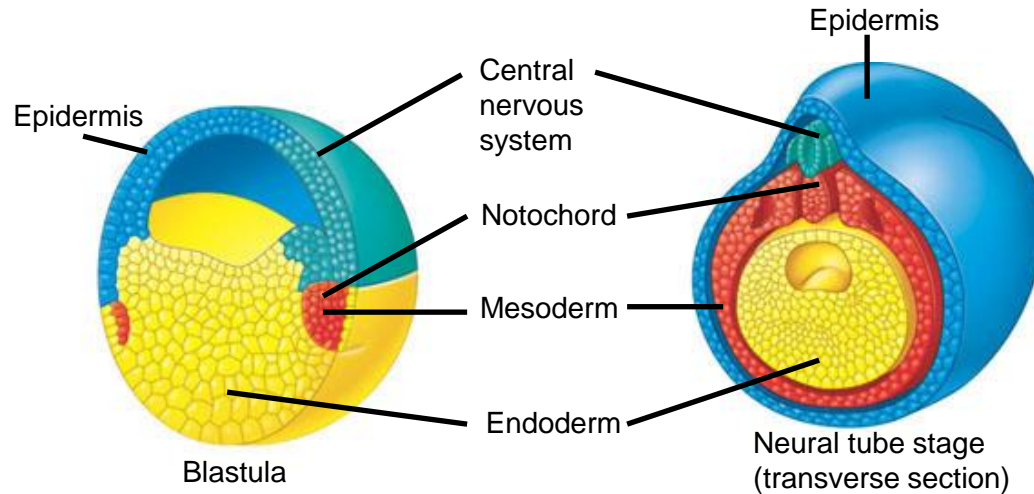
Change in cellular shape during morphogenesis



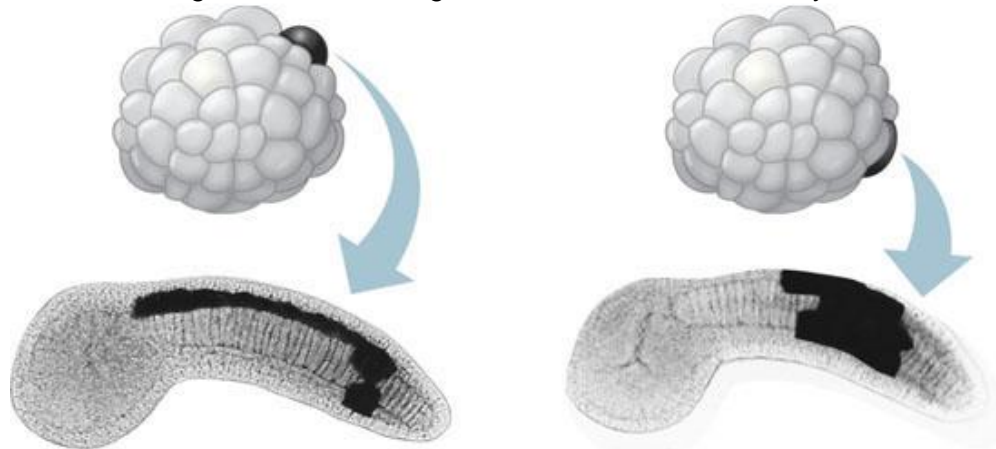
Convergent extension of a sheet of cells



Fate mapping for two chordates



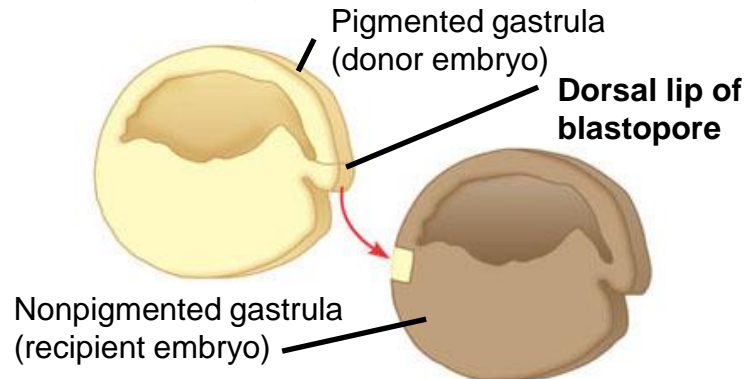
- (a) **Fate map of a frog embryo.** The fates of groups of cells in a frog blastula (left) were determined in part by marking different regions of the blastula surface with nontoxic dyes of various colors. The embryos were sectioned at later stages of development, such as the neural tube stage shown on the right, and the locations of the dyed cells determined.



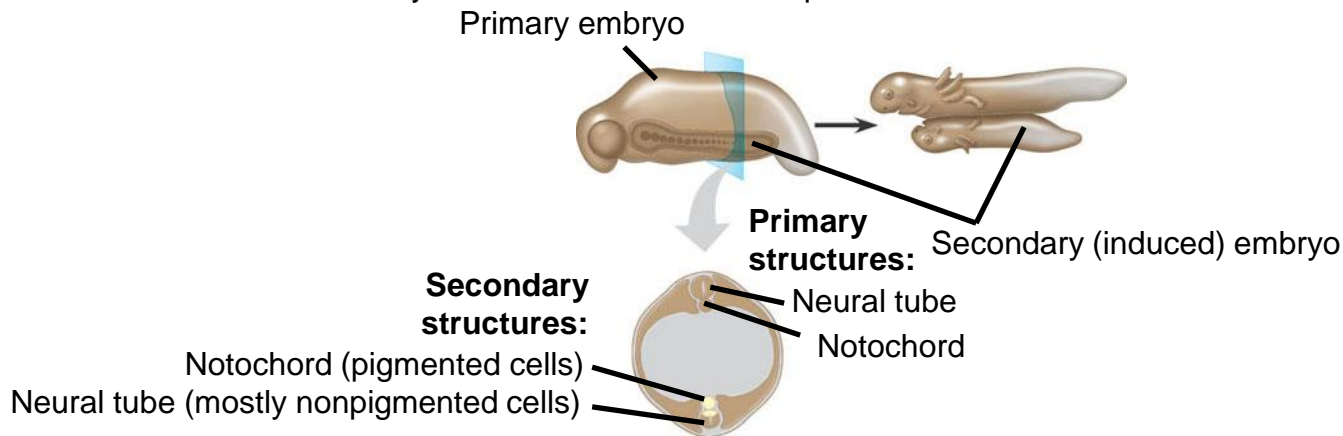
- (b) **Cell lineage analysis in a tunicate.** In lineage analysis, an individual cell is injected with a dye during cleavage, as indicated in the drawings of 64-cell embryos of a tunicate, an invertebrate chordate. The dark regions in the light micrographs of larvae correspond to the cells that developed from the two different blastomeres indicated in the drawings.

Can the dorsal lip of the blastopore induce cells in another part of the amphibian embryo to change their developmental fate?

EXPERIMENT Spemann and Mangold transplanted a piece of the dorsal lip of a pigmented newt gastrula to the ventral side of the early gastrula of a nonpigmented newt.



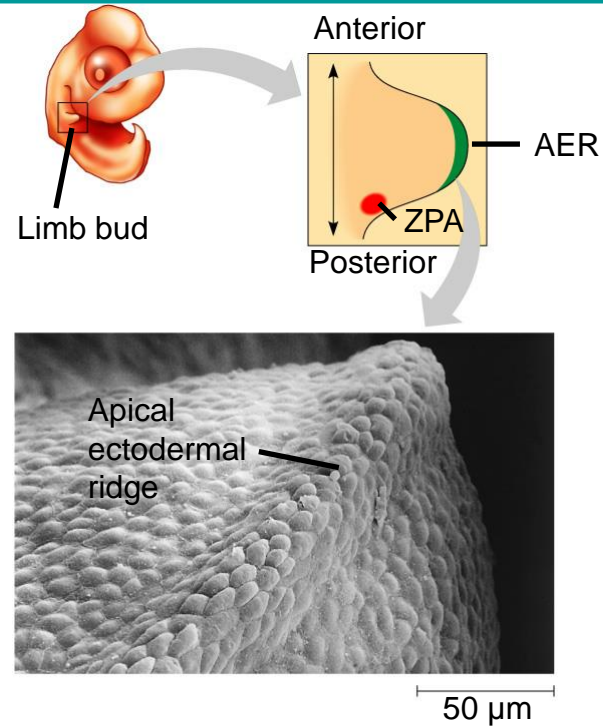
RESULTS During subsequent development, the recipient embryo formed a second notochord and neural tube in the region of the transplant, and eventually most of a second embryo. Examination of the interior of the double embryo revealed that the secondary structures were formed in part from host tissue.



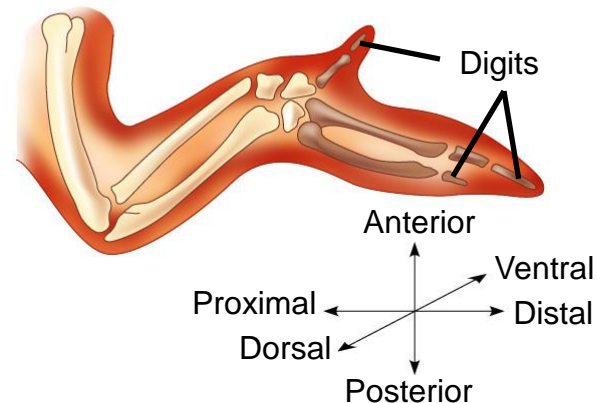
CONCLUSION The transplanted dorsal lip was able to induce cells in a different region of the recipient to form structures different from their normal fate. In effect, the dorsal lip “organized” the later development of an entire embryo.

Vertebrate limb development

(a) Organizer regions. Vertebrate limbs develop from protrusions called limb buds, each consisting of mesoderm cells covered by a layer of ectoderm. Two regions, termed the apical ectodermal ridge (AER, shown in this SEM) and the zone of polarizing activity (ZPA), play key organizer roles in limb pattern formation.

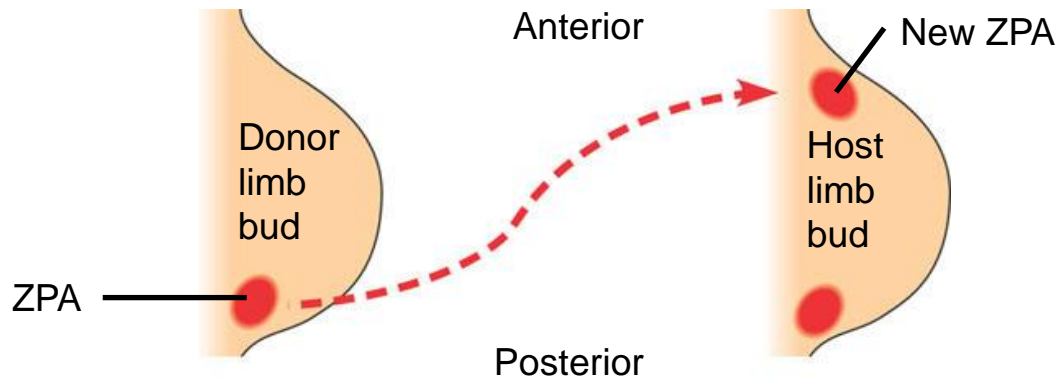


(b) Wing of chick embryo. As the bud develops into a limb, a specific pattern of tissues emerges. In the chick wing, for example, the three digits are always present in the arrangement shown here. Pattern formation requires each embryonic cell to receive some kind of positional information indicating location along the three axes of the limb. The AER and ZPA secrete molecules that help provide this information.



What role does the zone of polarizing activity (ZPA) play in limb pattern formation in vertebrates?

EXPERIMENT ZPA tissue from a donor chick embryo was transplanted under the ectoderm in the anterior margin of a recipient chick limb bud.



RESULTS In the grafted host limb bud, extra digits developed from host tissue in a mirror-image arrangement to the normal digits, which also formed (see Figure 47.26b for a diagram of a normal chick wing).



CONCLUSION The mirror-image duplication observed in this experiment suggests that ZPA cells secrete a signal that diffuses from its source and conveys positional information indicating “posterior.” As the distance from the ZPA increases, the signal concentration decreases and hence more anterior digits develop.

Overview of Development

Development is the successive process of systematic **gene-directed changes** throughout an organism's life cycle

- Can be divided into four sub-processes:
 - Growth (cell division)**
 - Differentiation**
 - Pattern formation**
 - Morphogenesis**

Cell Division

After fertilization, the diploid zygote undergoes a period of rapid mitotic divisions

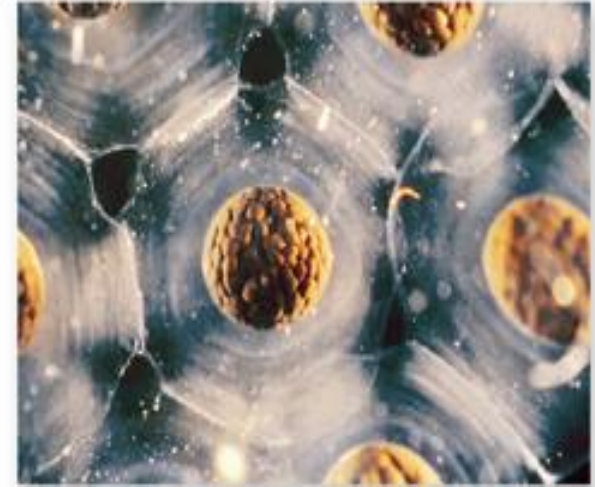
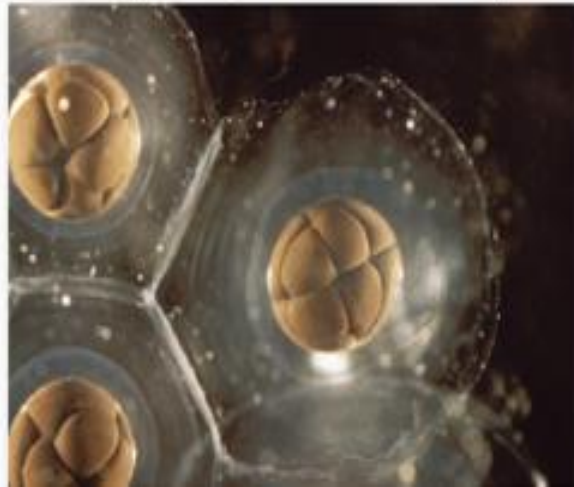
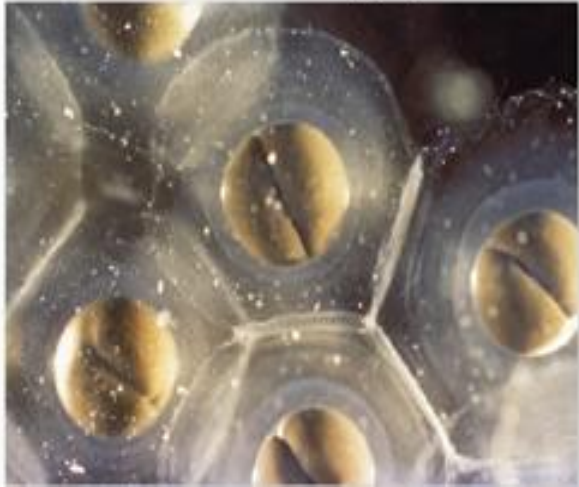
- In animals, this period is called **cleavage**

- Controlled by **cyclins** and **cyclin-dependent kinases (Cdks)**

During cleavage, the zygote is divided into smaller & smaller cells called **blastomeres**

- Moreover, the G1 and G2 phases are shortened or eliminated

Cell Division



Stem cells

Blastomeres are nondifferentiated and can give rise to any tissue

Stem cells are set aside and will continue to divide while remaining undifferentiated

- Tissue-specific**: can give rise to only one tissue

- Pluripotent**: can give rise to multiple different cell types

- Totipotent**: can give rise to any cell type, and whole organism

Stem cells

Cleave in mammals continues for 5-6 days
producing a ball of cells, the **blastocyst**

-Consists of:

-**Outer layer** = Forms the placenta

-**Inner cell mass** = Forms the embryo

-Source of **embryonic stem cells**
(ES cells)

Cell Differentiation









A human body contains more than 210 major types of differentiated cells

Cell determination commits a cell to a particular developmental pathway

- Can only be “seen” by experiment

- Cells are moved** to a different location in the embryo

- If they develop according to their new position, they are not determined**

	Normal	Not Determined (early development)	Determined (later development)
Donor	No donor	 Tail cells are transplanted to head	 Tail cells are transplanted to head
Recipient Before Overt Differentiation	 Tail Head		
Recipient After Overt Differentiation		 Tail cells develop into head cells in head	 Tail cells develop into tail cells in head

Cell Differentiation

Cells initiate developmental changes by using **transcriptional factors to change patterns of gene expression**

Cells become committed to follow a particular developmental pathway in one of two ways:

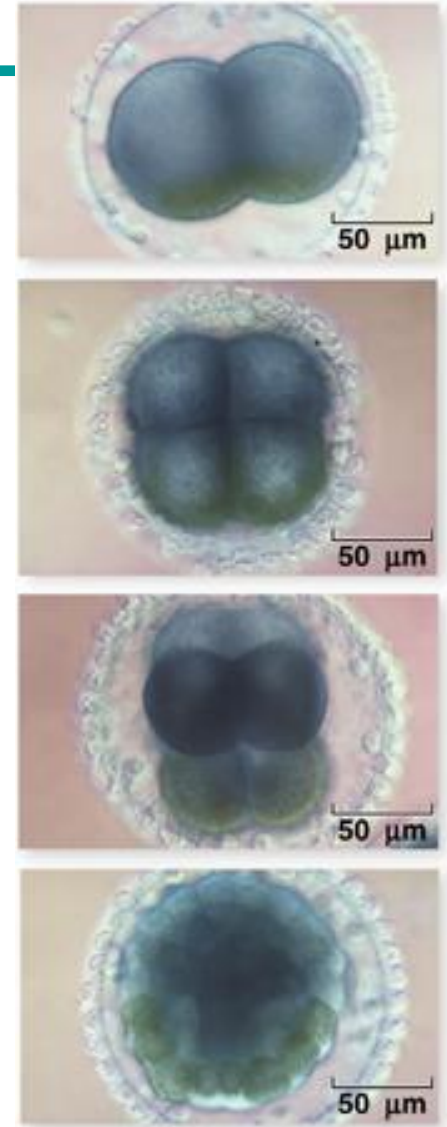
1) via differential inheritance of **cytoplasmic determinants**

2) via **cell-cell interactions**

Cell Differentiation

Cytoplasmic determinants

- Female parent provides egg with *macho-1* mRNA
- Encodes a transcription factor that can activate expression of muscle-specific genes



Cell Differentiation

Induction is the change in the fate of a cell due to *interaction with an adjacent cell*

If cells of a frog embryo are separated:

- One pole (“animal pole”) forms **ectoderm**
- Other pole (“vegetal pole”) forms **endoderm**
- No mesoderm is formed

**If the two pole cells are placed side-by-side,
some animal-pole cells form the mesoderm**

The Nobel Prize in Physiology or Medicine 2012

Sir John B. Gurdon, Shinya Yamanaka

The Nobel Prize in Physiology or Medicine 2012

Nobel Prize Award Ceremony

Sir John B. Gurdon

Shinya Yamanaka

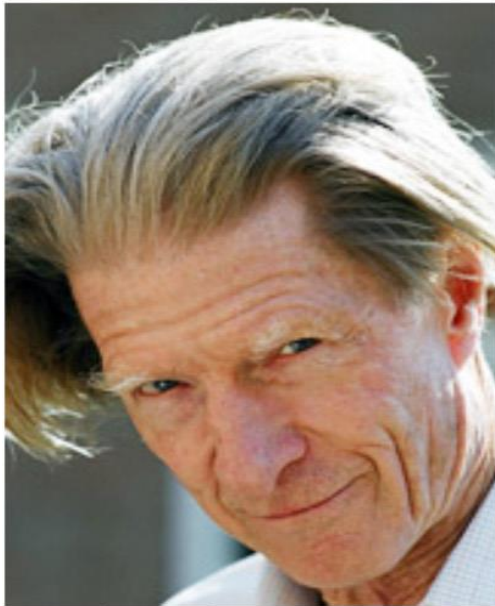


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Sir John B. Gurdon

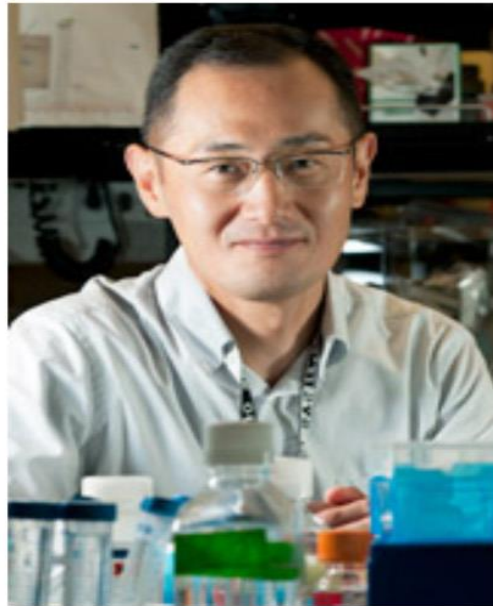
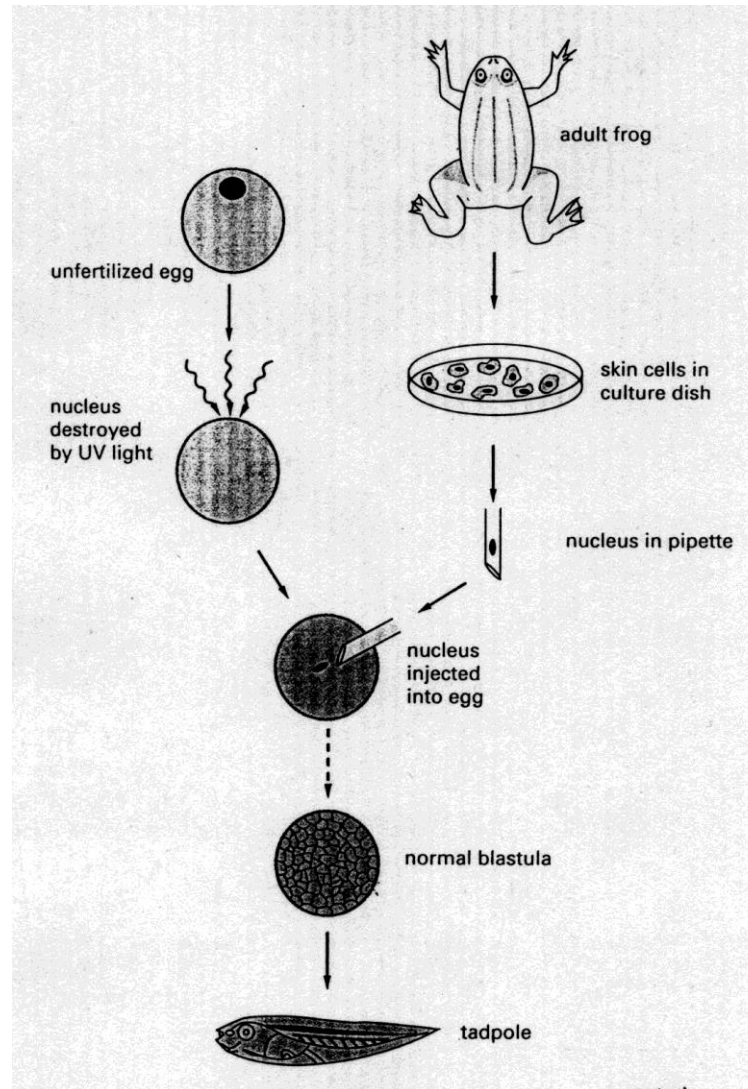


Photo: Gladstone Institutes/Chris
Goodfellow

Shinya Yamanaka

The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka *"for the discovery that mature cells can be reprogrammed to become pluripotent"*

Transplantation experiment



John Gurdon

What does this transplantation experiment tell you?

- A) Localized cytoplasm determinants are important**
- B) The genome stays the same during cell differentiation**
- C) Skin cells have the potential to generate all cell types**
- D) You don't really need nuclei for cell differentiation**
- E) The oocyte without a nucleus did just fine**

Cloning

The nucleus from a skin cell of a diabetic patient is removed.



Diabetic patient

The nucleus from a skin cell of a healthy patient is removed.

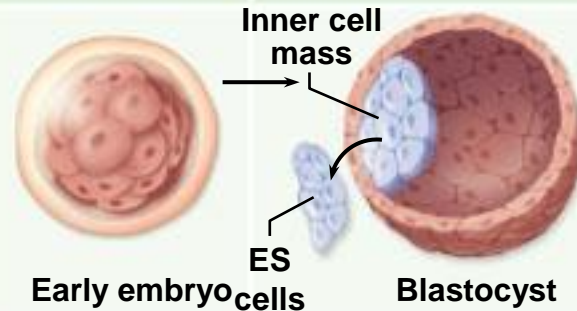


Healthy patient

The skin cell nucleus is inserted into the enucleated human egg cell.

Cell cleavage occurs as the embryo begins to develop in vitro.

The embryo reaches the blastocyst stage



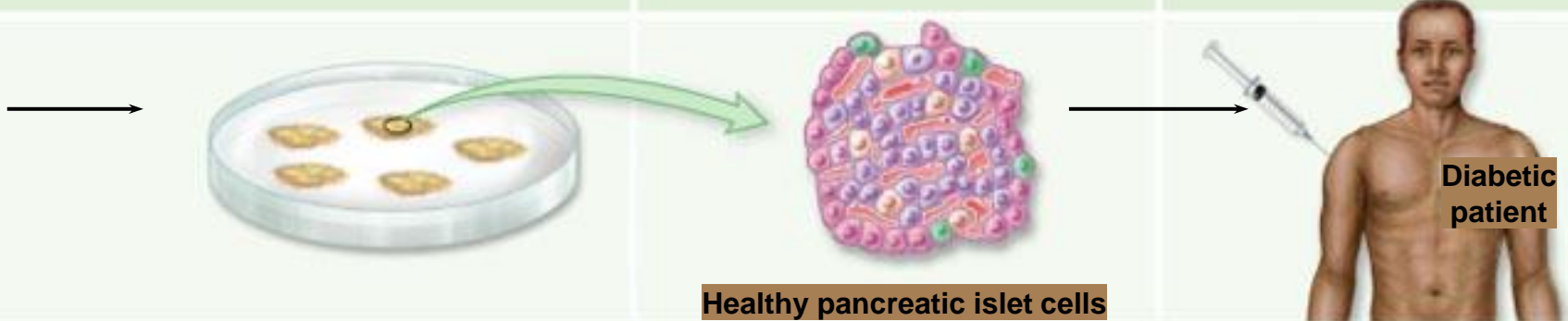
Cloning

Therapeutic Cloning

Embryonic stem cells (ES cells) are extracted and grown in culture.

The stem cells are developed into healthy pancreatic islet cells needed by the patient.

The healthy tissue is injected or transplanted into the diabetic patient.



Reproductive Cloning

The blastocyst is kept intact and is implanted into the uterus of a surrogate mother.

The resulting baby is a clone of the healthy patient.



Cloning

Human embryonic stem cells have enormous promise for treating a wide range of diseases

- However, stem cell research has raised profound ethical issues

Very few countries have permissive policy towards human reproductive cloning

- However, many permit embryonic stem cell research

Pattern Formation

In the early stages of pattern formation, **two perpendicular axes are established**

- Anterior/posterior** (A/P, head-to-tail) axis
- Dorsal/ventral** (D/V, back-to-front) axis

Polarity refers to the acquisition of axial differences in developing structures

Position information leads to changes in gene activity, and thus cells adopt a fate appropriate for their location

Production of Body Plan

Homeotic gene complexes

- The **HOM complex** genes of *Drosophila* are grouped into two clusters
 - Antennapedia complex**, which governs the anterior end of the fly
 - Bithorax complex**, which governs the posterior end of the fly
- Interestingly, the order of genes mirrors the order of the body parts they control

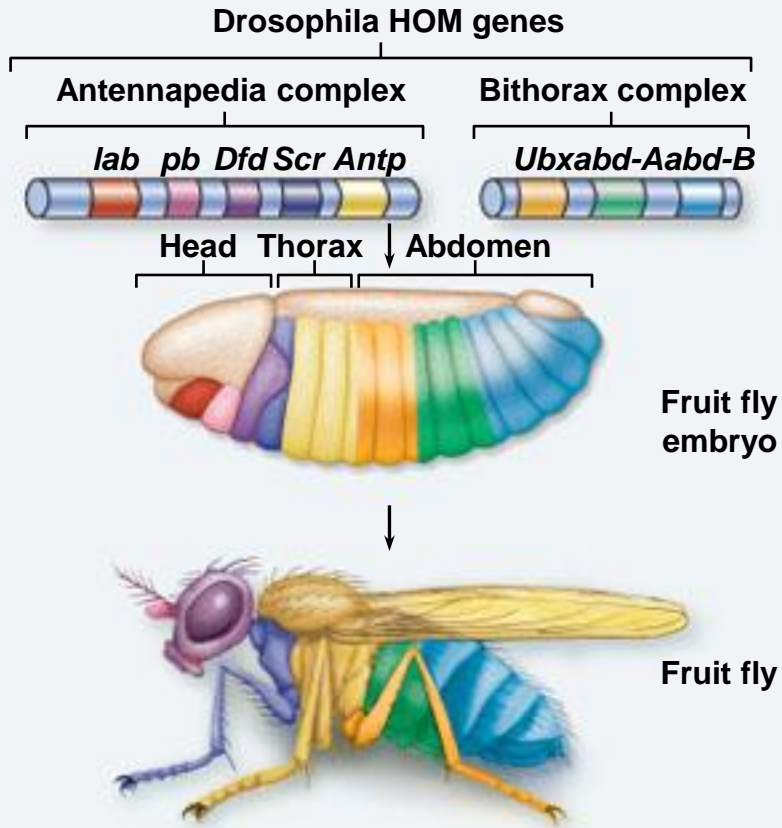
Production of Body Plan

Homeotic gene complexes

- All of these genes contain a conserved 180-base sequence, the **homeobox**
 - Encodes a 60-amino acid DNA-binding domain, the **homeodomain**
- Homeobox-containing genes are termed **Hox genes**
 - Vertebrates have 4 *Hox* gene clusters

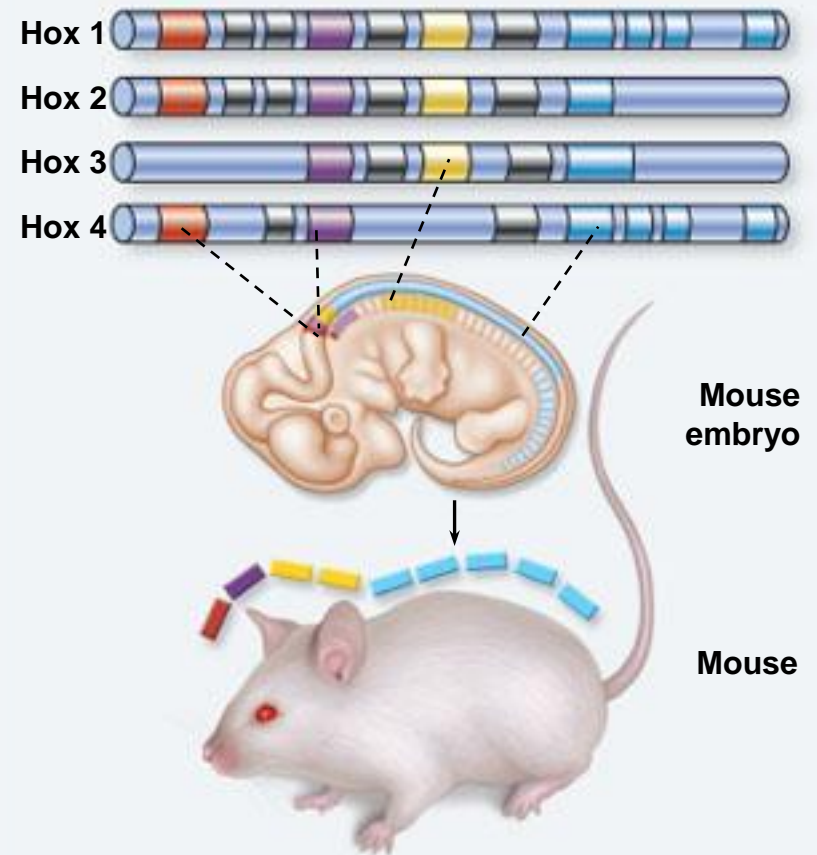
Production of Body Plan

Drosophila HOM Chromosomes



a.

Mouse *Hox* Chromosomes



b.

Morphogenesis

Morphogenesis is the formation of ordered form and structure

-Animals achieve it through changes in:

- Cell division

- Cell shape and size

- Cell death

- Cell migration

-Plants use these except for cell migration

Morphogenesis

Cell division

-The **orientation of the mitotic spindle** determines the plane of cell division in eukaryotic cells

-If spindle is **centrally located**, two equal-sized daughter cells will result

-If spindle is **off to one side**, two unequal daughter cells will result

Morphogenesis

Cell shape and size

-In animals, **cell differentiation is accomplished by profound changes in cell size and shape**

-**Nerve cells** develop long processes called axons

-**Skeletal muscles** cells are large and multinucleated

Morphogenesis

Cell death

- **Necrosis** is accidental cell death
- **Apoptosis** is programmed cell death
 - Is required for normal development in all animals
 - “Death program” pathway consists of:
 - Activator, inhibitor and apoptotic protease

Morphogenesis

Cell migration

-Cell movement involves both adhesion and loss of adhesion between cells and substrate

-Cell-to-cell interactions are often mediated through **cadherins**

-Cell-to-substrate interactions often involve complexes between **integrins** and the **extracellular matrix (ECM)**

Environmental Effects

Both plant and animal development are affected by environmental factors

- Germination** of a dormant seed proceeds only under favorable soil and day conditions

- Reptiles** have a **temperature-dependent sex determination (TSD)** mechanism

- The water flea *Daphnia* changes its shape after encountering a predatory fly larva

Environmental Effects

“Helmet Head” in *Daphnia*

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