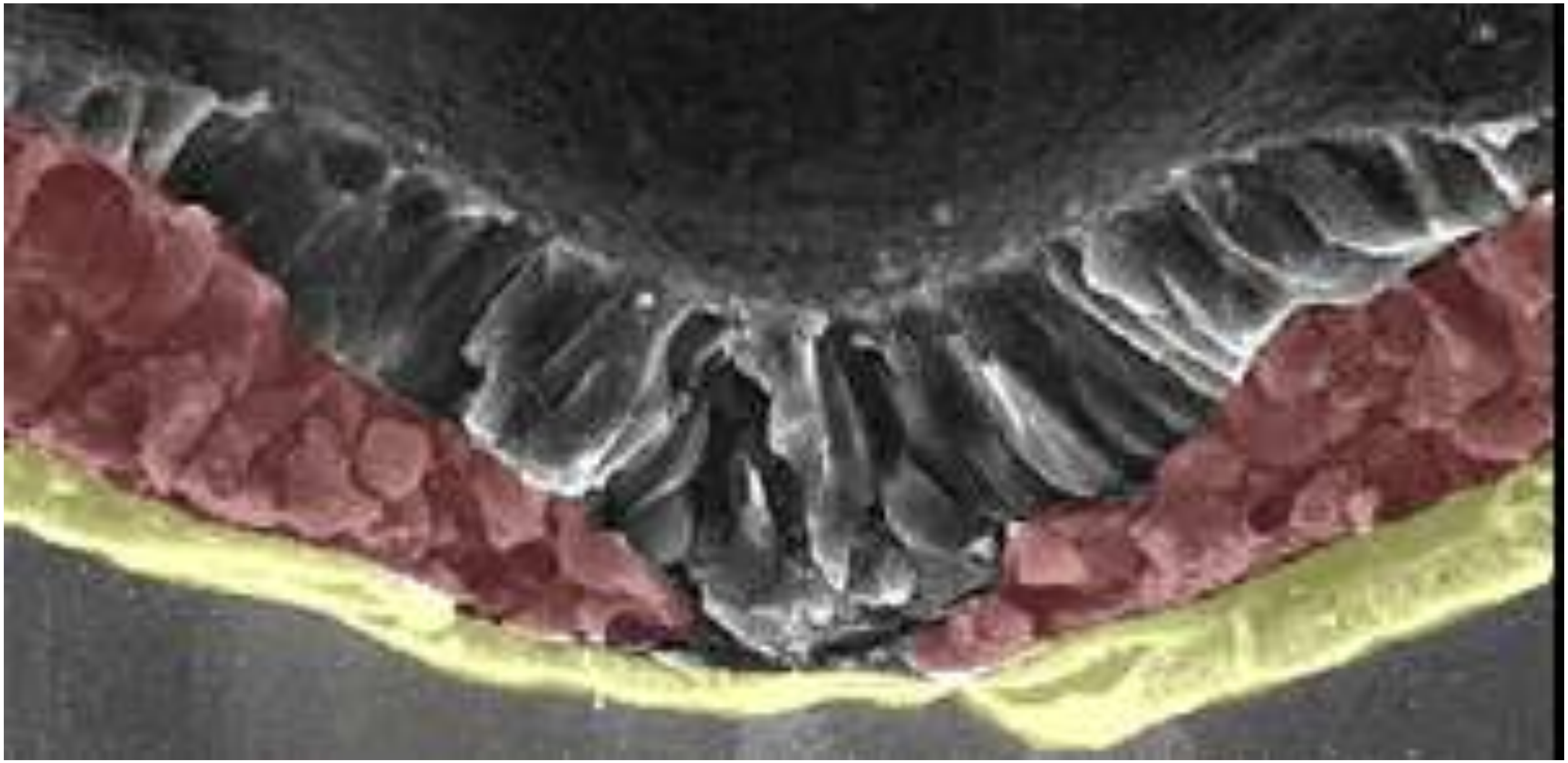
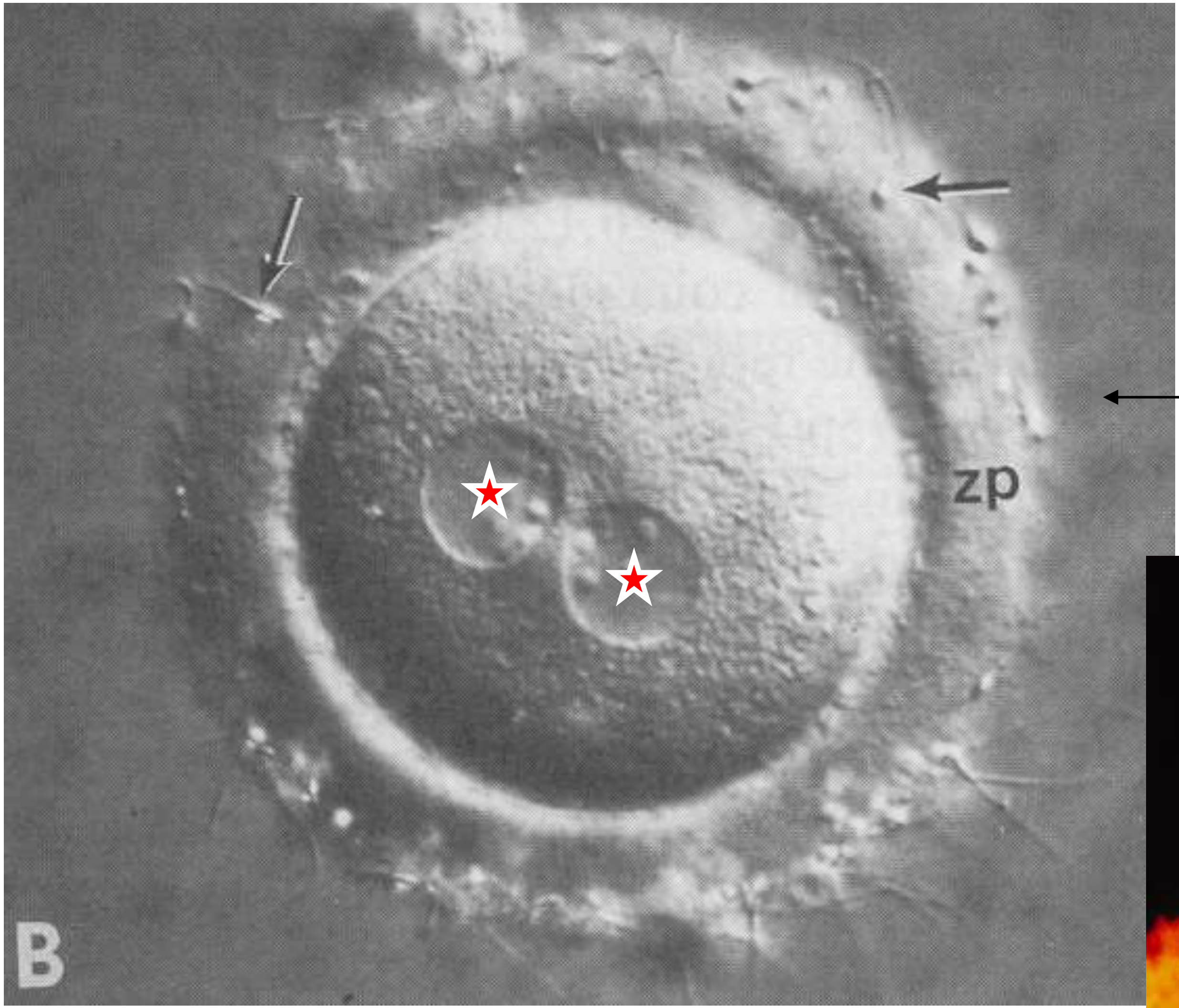
Early development, cleavage, GASTRULATION

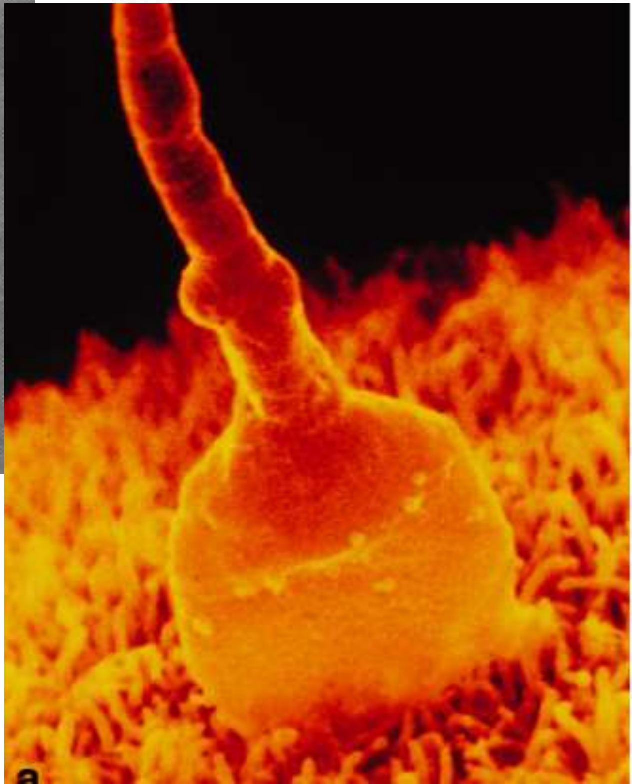


Semmelweis University,
Department of Anatomy, Histology and Embryology

The zygote

=fertilized egg



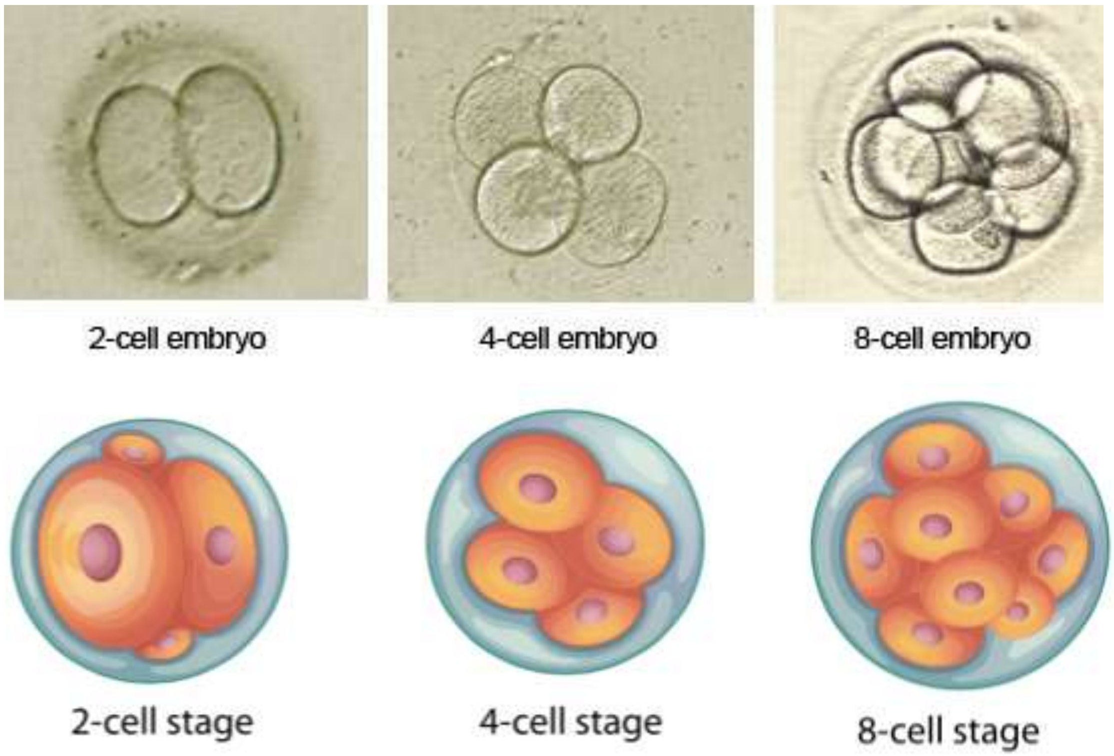


The new diploid cell is created by the fusion of the male and female *pronucleus (★)*

purple dashed double line: probe (mock) test (yellow line: exam test material)

Cleavage

- Mitotic division process
- Cleavage stage cells: blastomeres
- The volume of egg's cytoplasm divides into numerous smaller, nucleated cells → in humans whole cytoplasmatic volume of the egg packs into smaller and smaller blastomeres
- The human cleavage is equal, but asynchronous



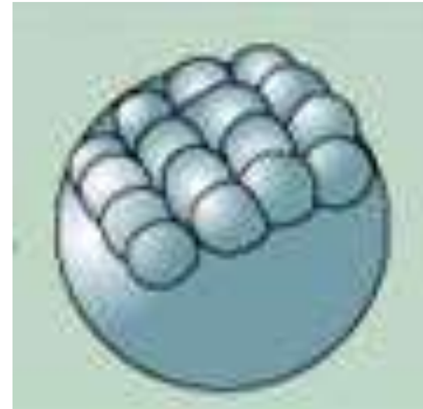
Different type of cleavage

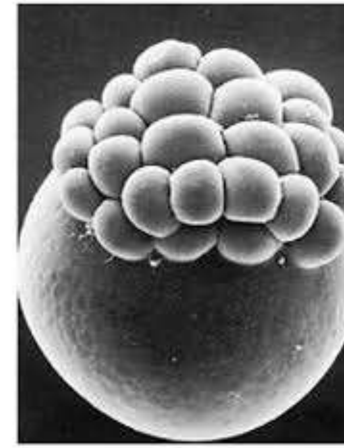
- The cleavage process is influenced by the yolk contents of the egg.
- The egg may contain large or small amount of yolk
- The distribution of the yolk:
  - uniform
  - concentrated on one of pole of the egg.

- Due to the asymmetric yolk distribution:
  - Only a part of the egg is packed into blastomeres
  - blastomeres have different size
- Complete and incomplete types of cleavage can be distinguished

Incomplete (Meroblasticus)

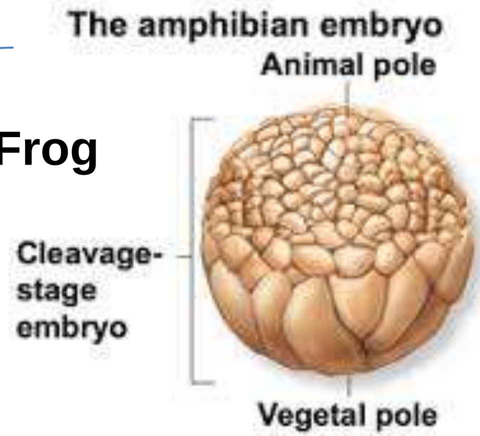
fish, reptiles, birds

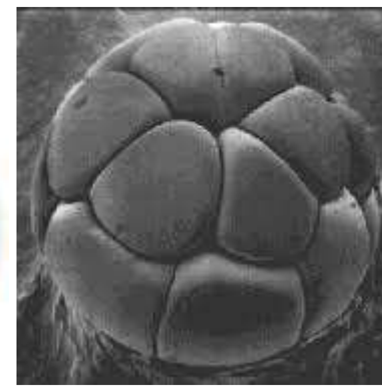




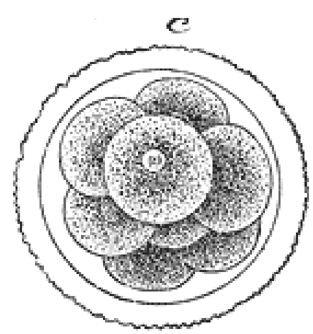
Complete (Holoblasticus)

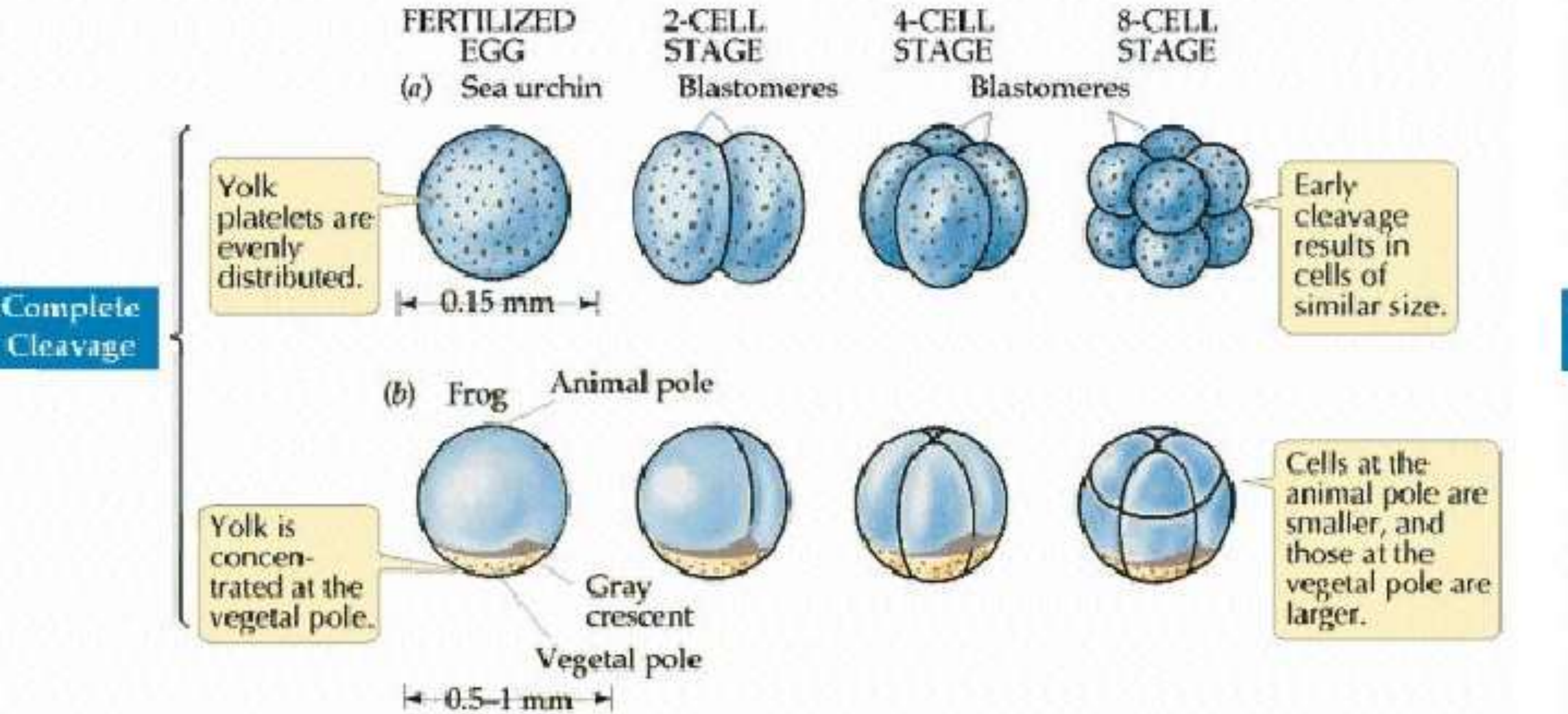
Frog

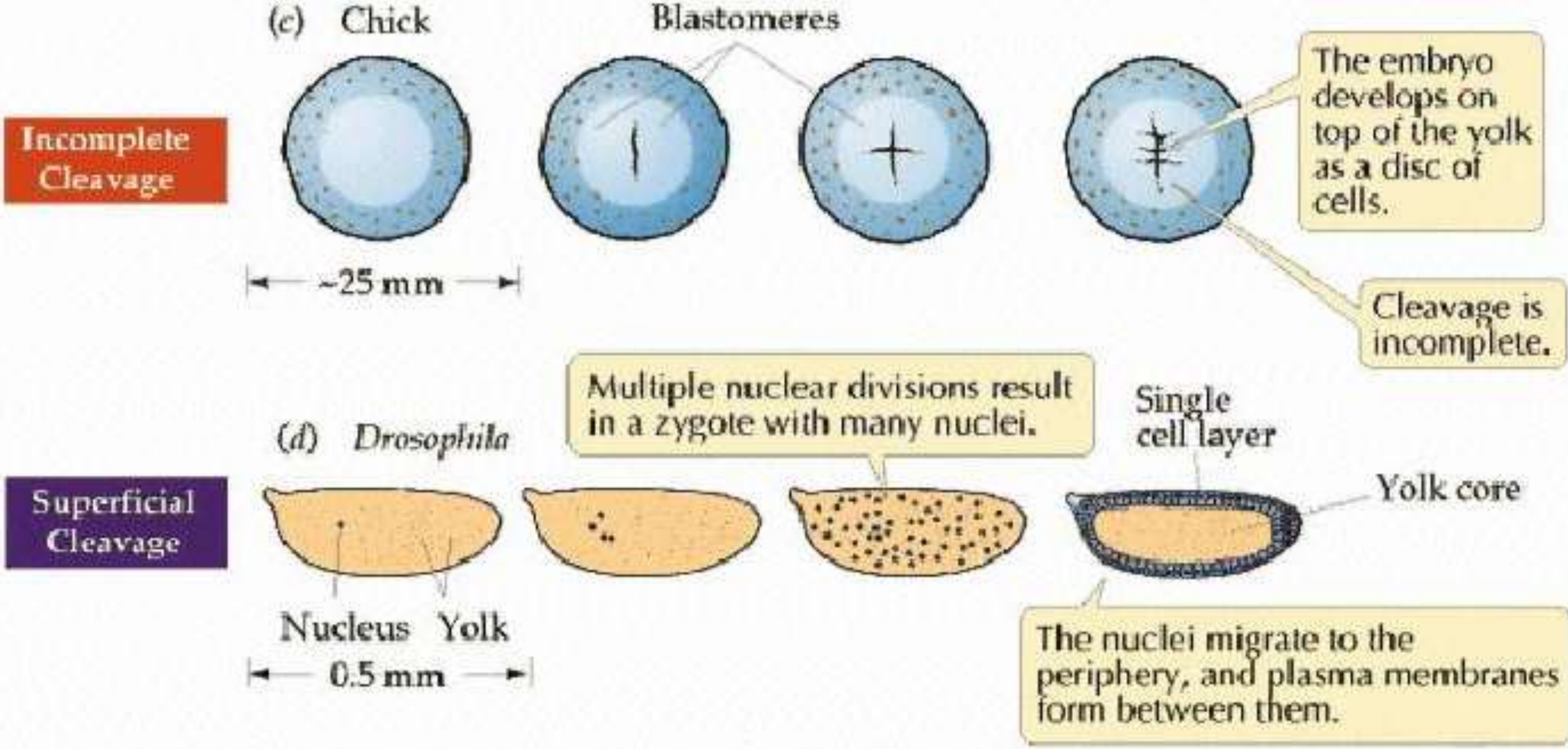




Human




Complete Cleavage
FERTILIZED EGG
2-CELL STAGE
4-CELL STAGE
8-CELL STAGE
(a) Sea urchin
Blastomeres
Blastomeres
Yolk platelets are evenly distributed.
0.15 mm
Early cleavage results in cells of similar size.
(b) Frog
Animal pole
Yolk is concen-trated at the vegetal pole.
Gray crescent
Vegetal pole
0.5–1 mm
Cells at the animal pole are smaller, and those at the vegetal pole are larger.


Incomplete Cleavage
(c) Chick
Blastomeres
The embryo develops on top of the yolk as a disc of cells.
~25 mm
Cleavage is incomplete.
Superficial Cleavage
(d) Drosophila
Multiple nuclear divisions result in a zygote with many nuclei.
Single cell layer
Yolk core
Nucleus Yolk
0.5 mm
The nuclei migrate to the periphery, and plasma membranes form between them.

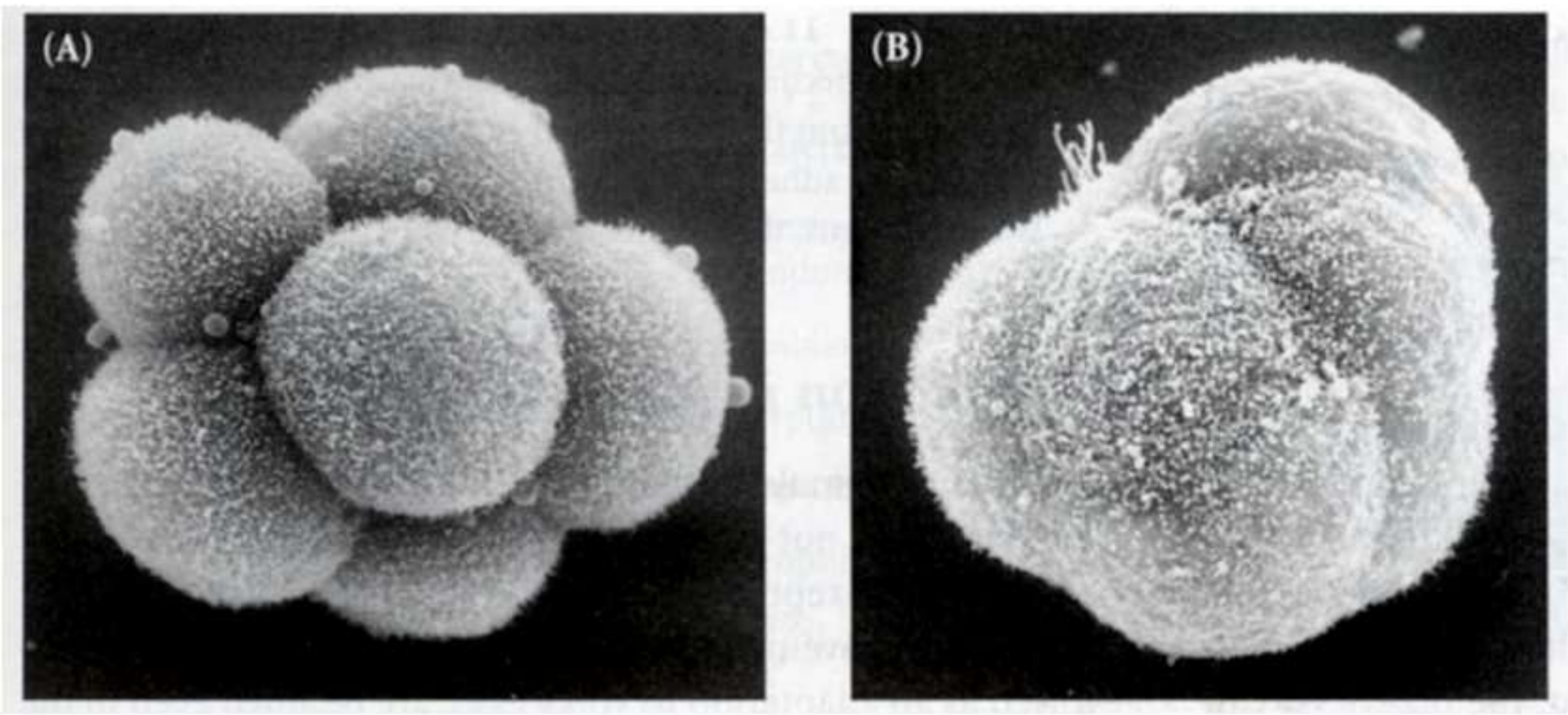
Compaction

-in the 8-cell-stage cleavage.

-The blastomeres, which show loose arrangement, suddenly form a compact ball

-Before the compaction blastomeres loosely adhere to each other by microvilli on their surface.

-After compaction blastomeres tightly adhere with each other through intercellular junctions



before compaction and after compaction

•Compaction is a membrane polarization process → well-defined apical, basal and lateral side is developing.

•Different components of the cell membrane concentrate at different regions of the cell causing the polarization of the cells.

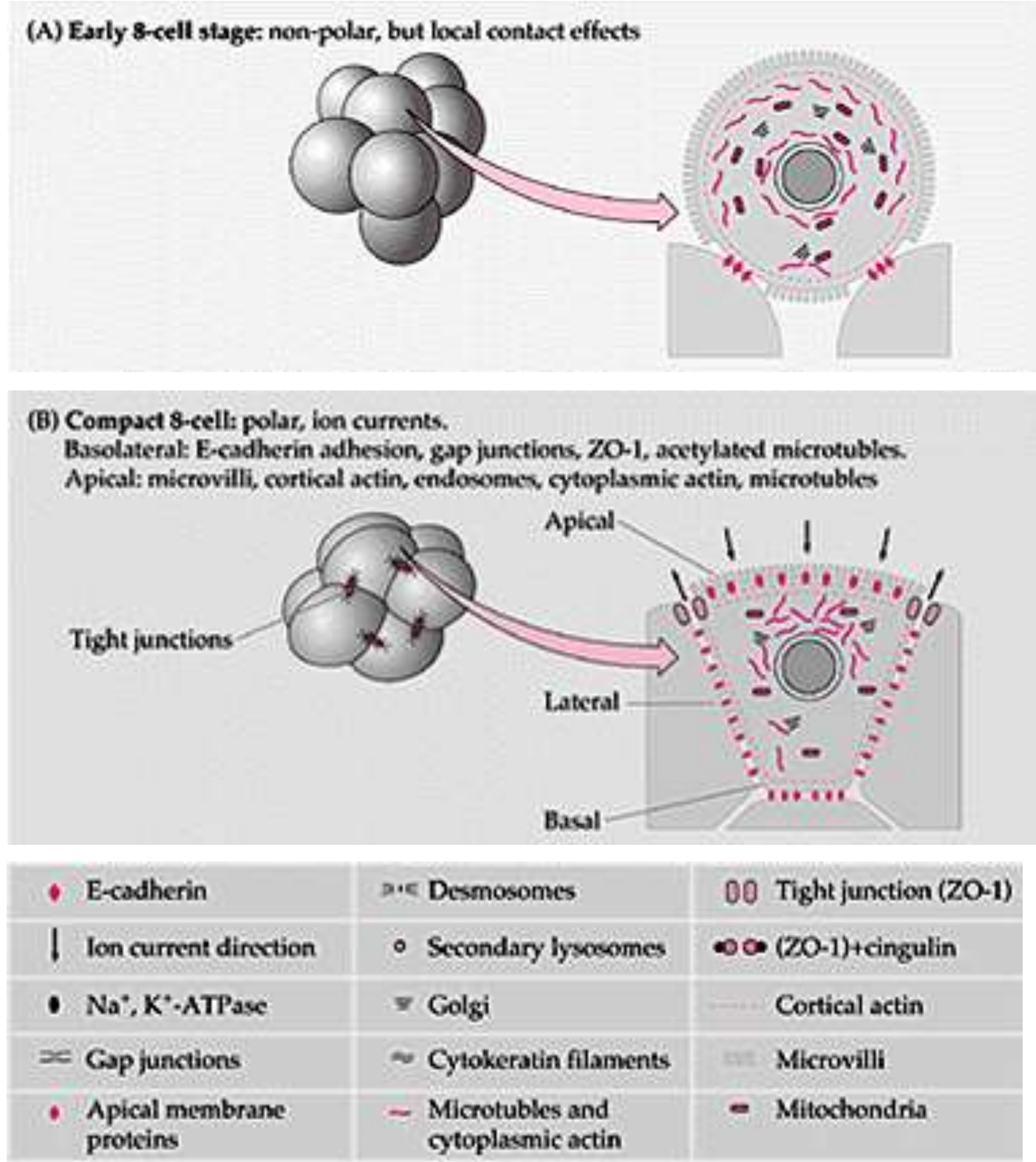
•Membrane polarization is influenced by cell-cell interactions

•This polarization process takes place only in those parts of the cell membrane where the cell is in contact with other blastomeres.

•E-cadherin plays a main role in compaction.

•At 2-cell stage, E-cadherin is uniformly spread throughout the cell membrane. During compaction E-cadherin becomes restricted to those sites of cell membrane where adjacent blastomeres are in contact with each other.

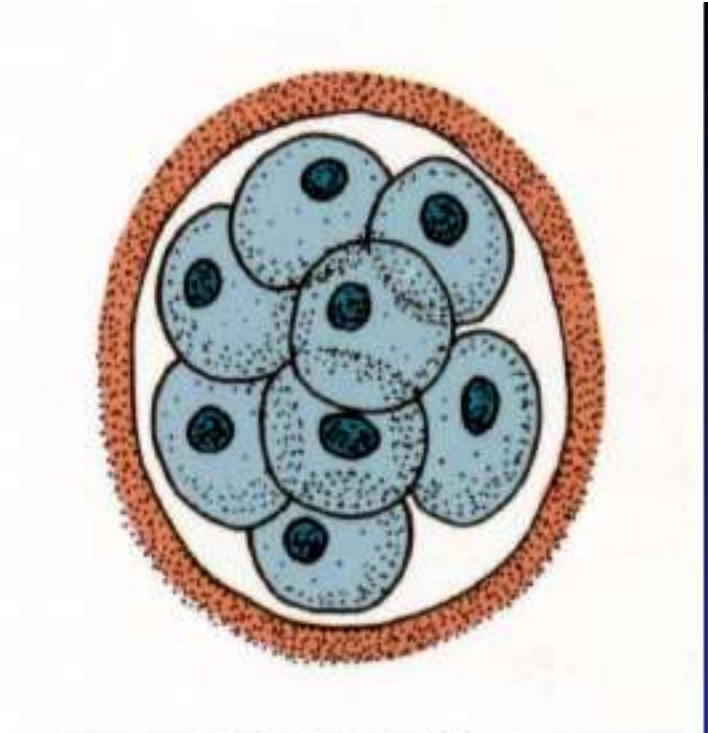
• E-cadherin molecules accumulate and form zonula adherens

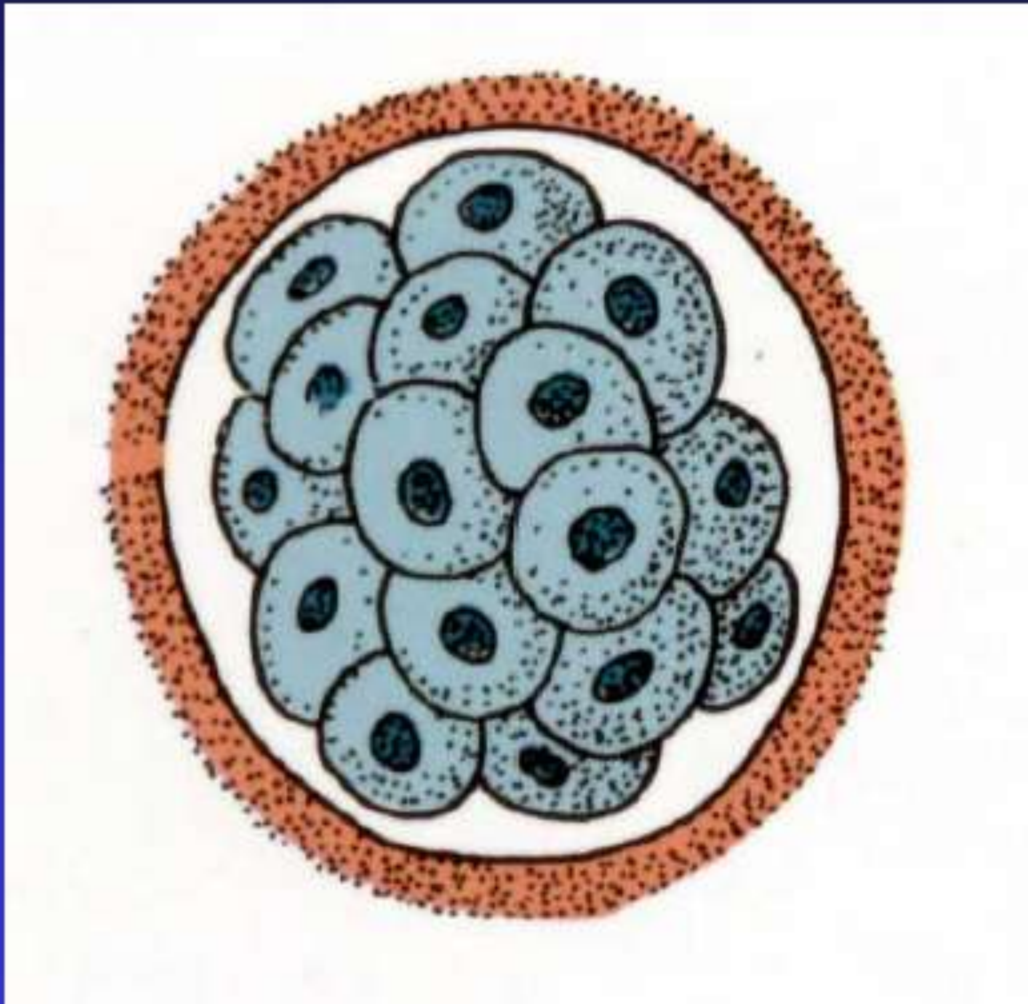


Morula

16-64 cells stage

'Berry' - appearance





Blastula formation

•Newly formed structures between the outer blastomeres:

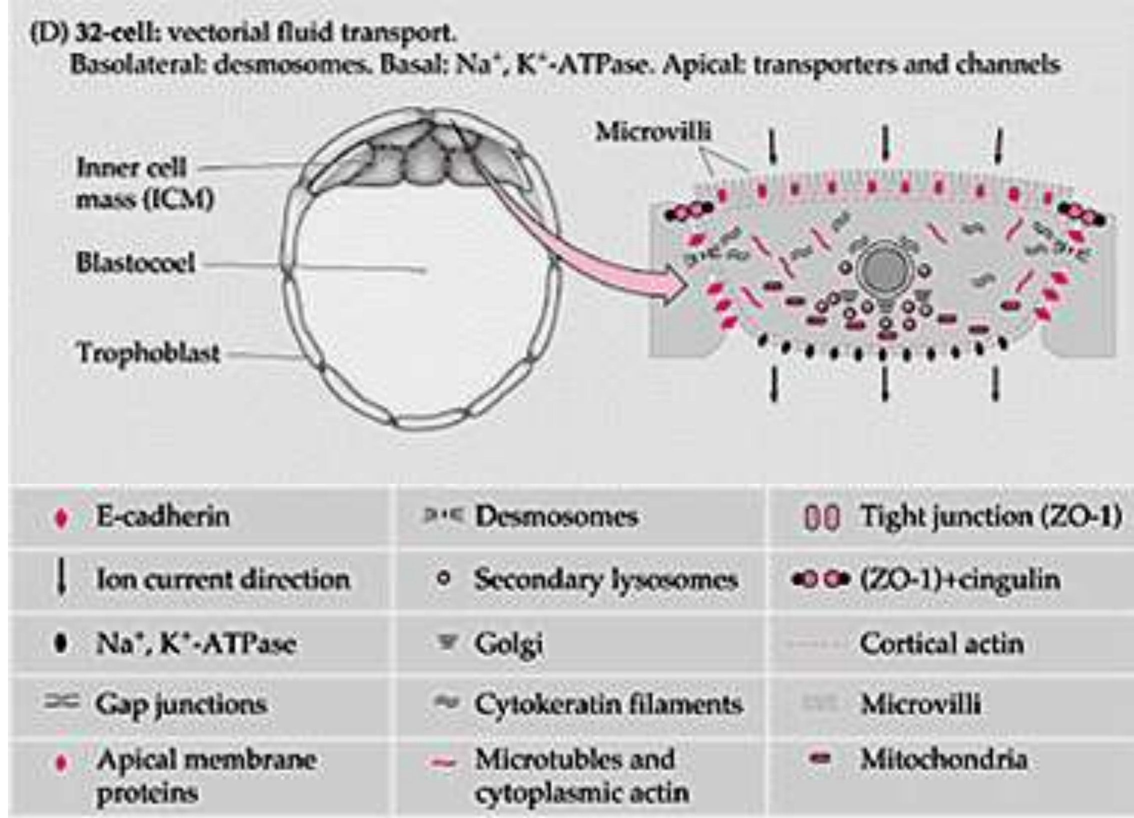
-*tight junctions* (apical part)

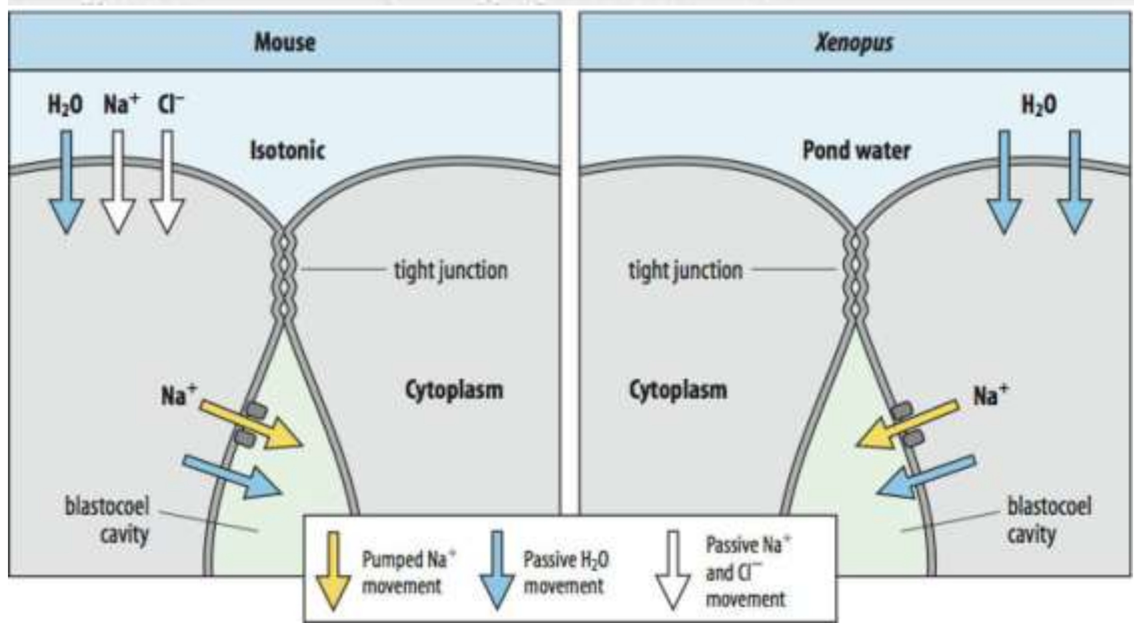
-*gap juction* (lateral part)

-*membrane transport molecules* on basal part: mainly sodium pumps.

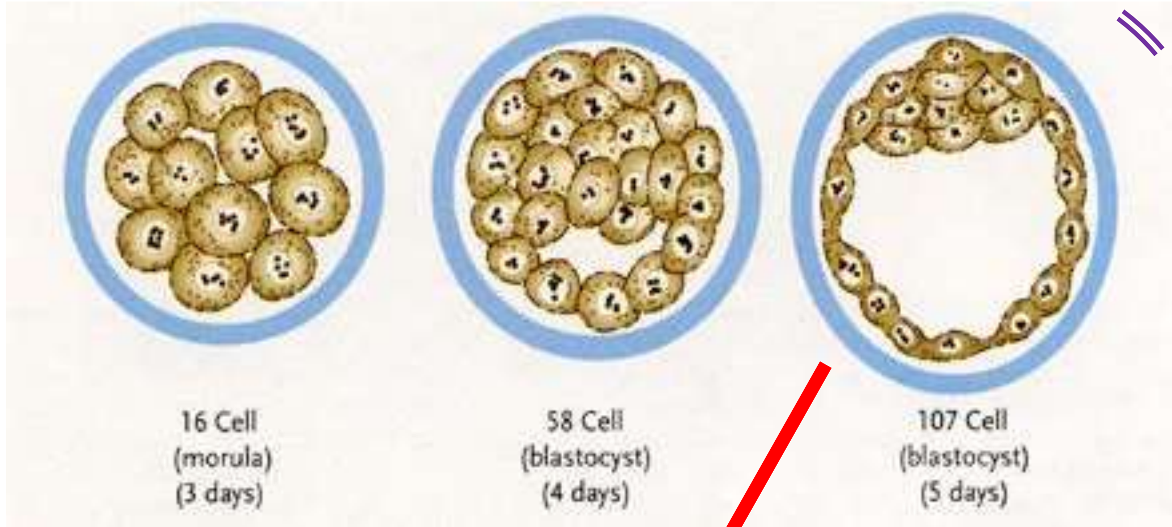
•Due to the activated sodium pumps the sodium concentration increase in the intercellular space and parallel the water flows into the intercelullar space by osmosis → forming fluid filled cavity called blastocoel.

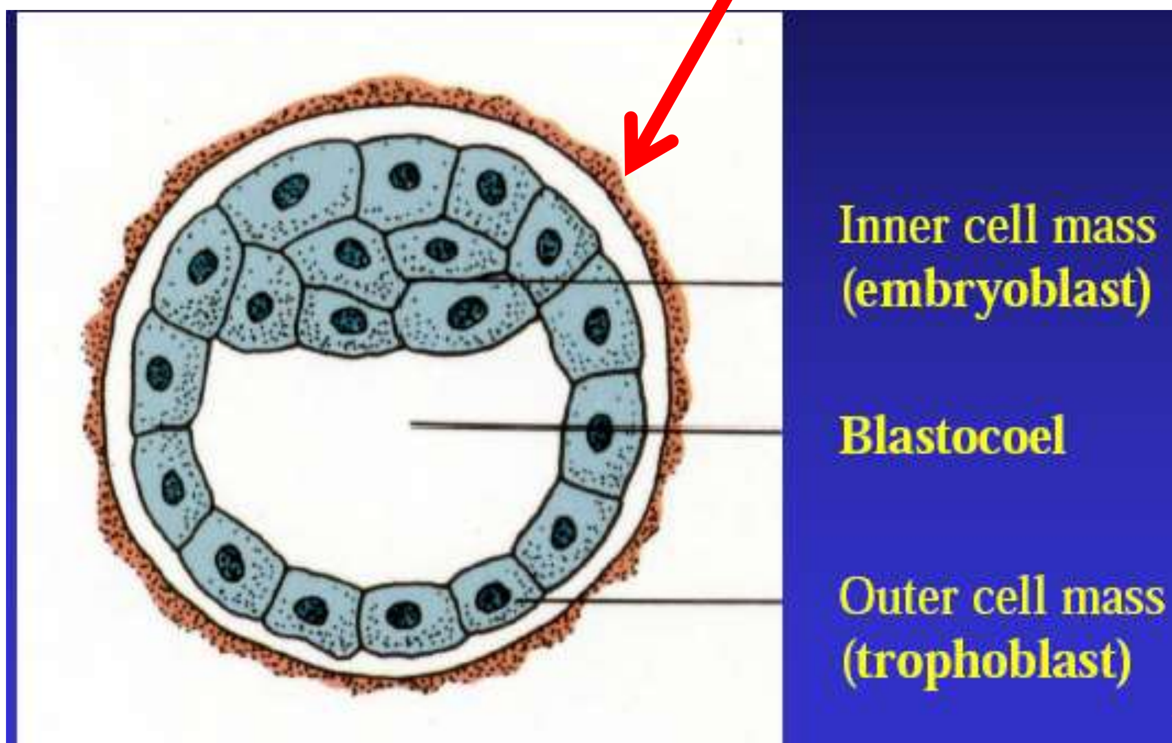
•The blastocoel is expanded gradually by the increasing amount of the fluid





The expanding blastocoel pushes the internal cells to one side of the blastocyst → **inner cell mass (ICM)**





In mice:

- trophoblast cells differentiate into the fetal membrane system

- inner cell mass forms the whole body of the embryo and extraembryonic (primitive) endoderm

Twins

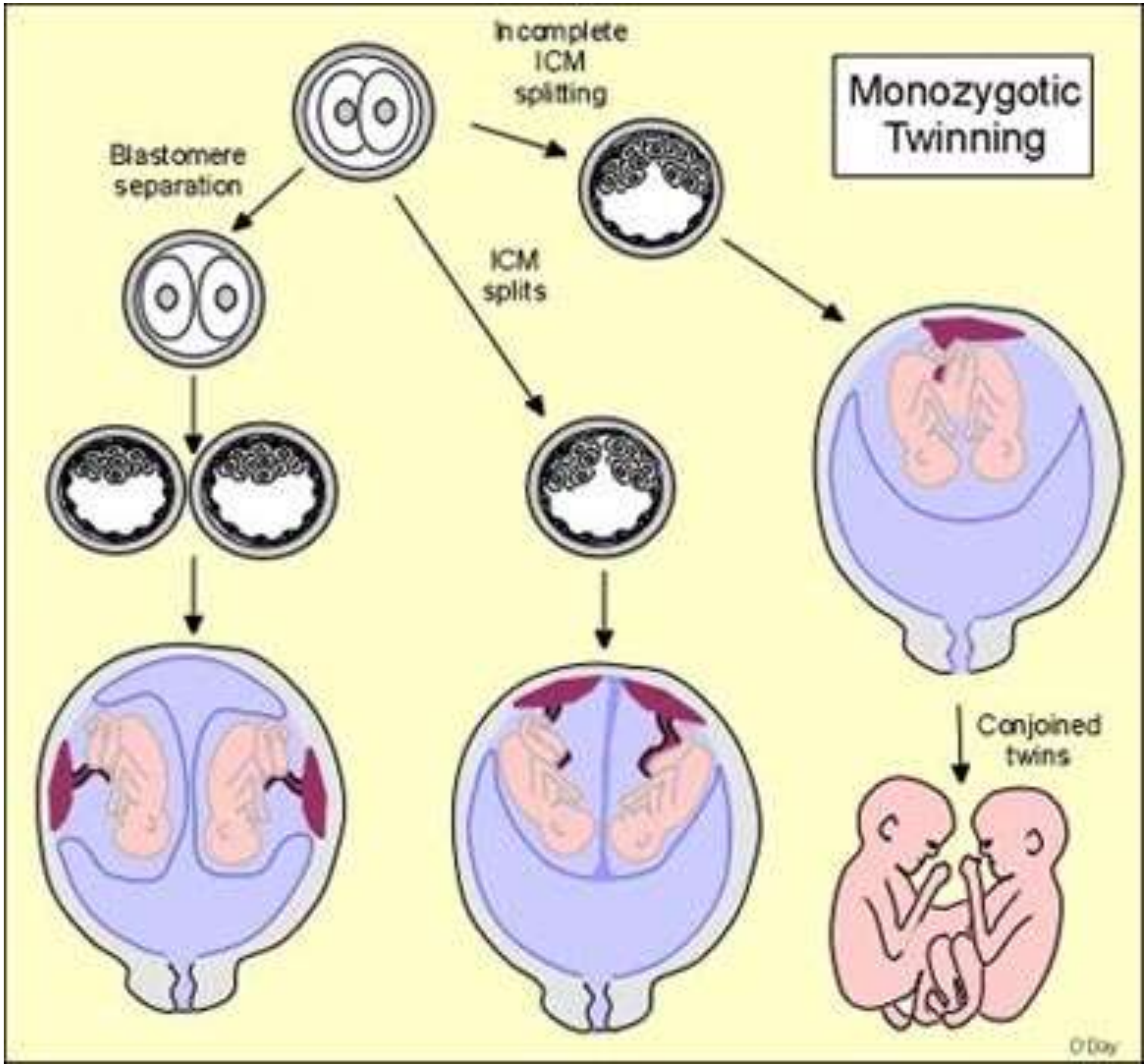
•Monozygotic twins arise from one zygote

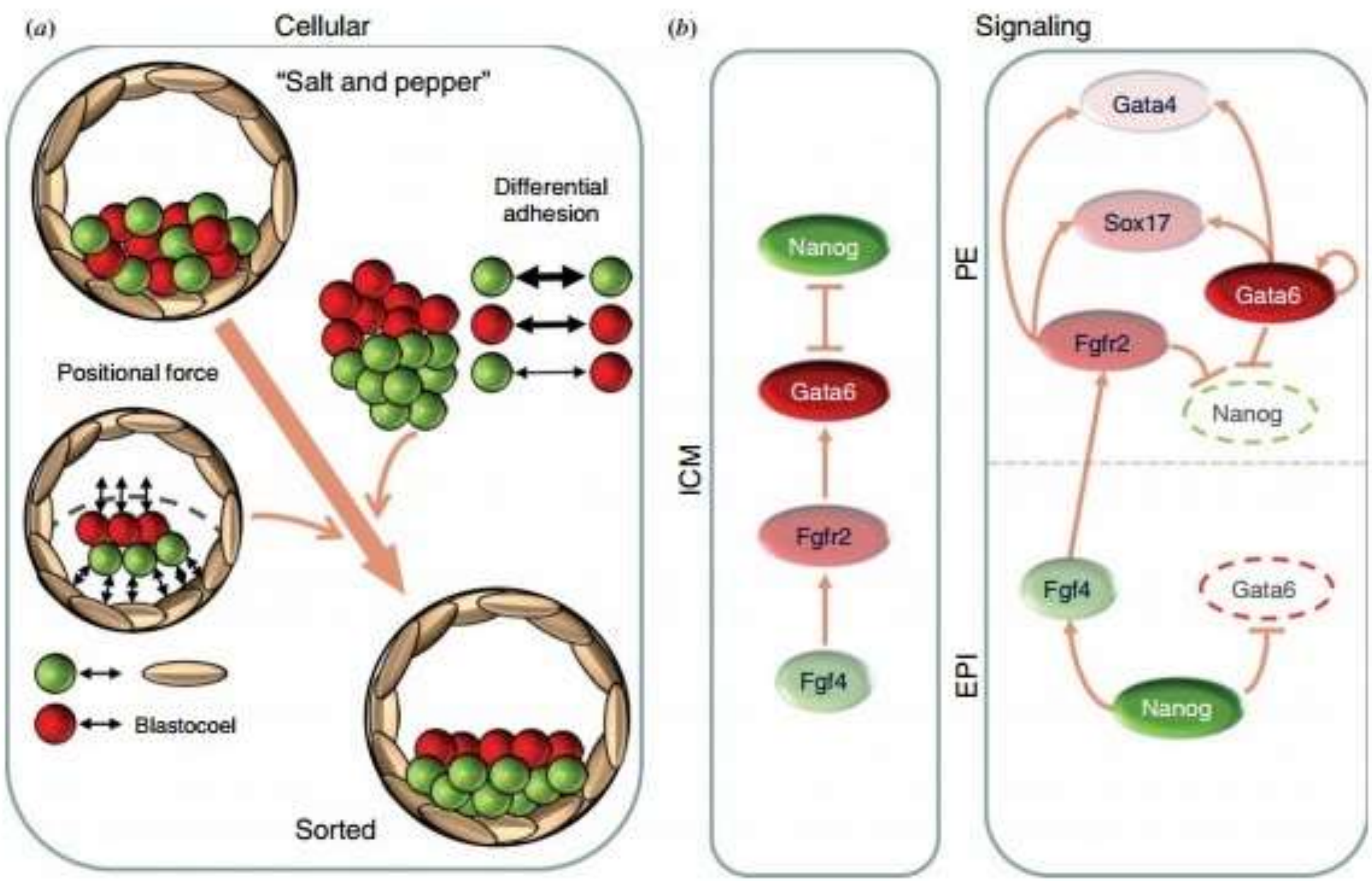
•Dizygote twins arise two different eggs, that are fertilised by two different sperms

.Monozygotic twins

•**At cleavage:** after the first cell division the newly formed two blastomeres completely separete from each other.

•**At blastulation:** the subdivision of the inner cell mass within the blastocyst.



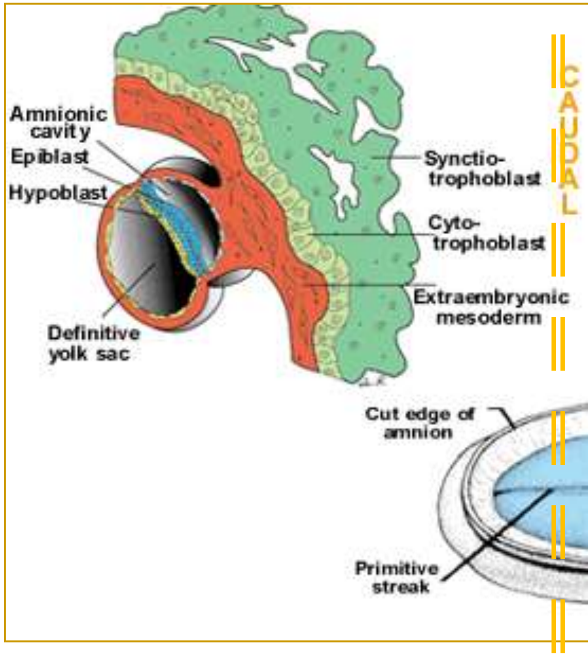


The differentiation of the ICM cells into a bilaminar structure containing epiblast and hypoblast (primitive endoderm) layer.

The sorting model. At the beginning, the epiblast and hypoblast cells distribute within the ICM showing „salt and pepper pattern". The sorting event, in which the bilaminar disc will be developed, is caused by two main ways. 1.: the different strenghts of adhesion between the two cells type (adhesion is stronger between same type cells than different type cells) and 2.: signals coming from either the blastocoel or from trophoblast (the epiblast cells express **nanog** while hypoblast cells express **GATA6** and this expression pattern is caused by the different **FGF signaling**.

Why is gastrulation so important?

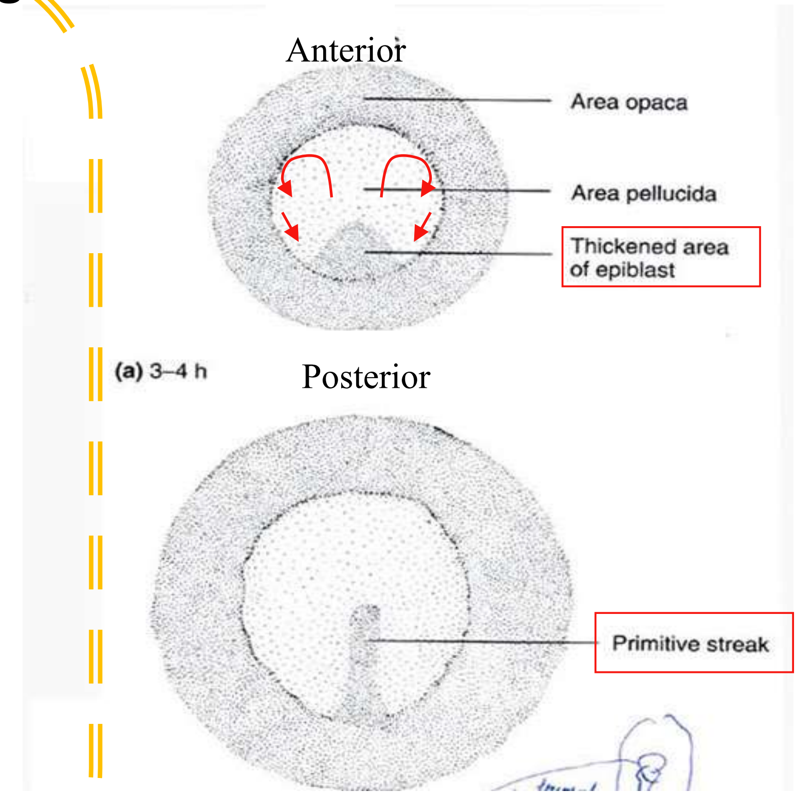
- Generation of the basic body plan.
- Specification of the axes:
  - Anterior and posterior
  - Dorsal and ventral
  - Left and right
- Generation of the three germ layers
  - Ectoderm, mesoderm, and endoderm

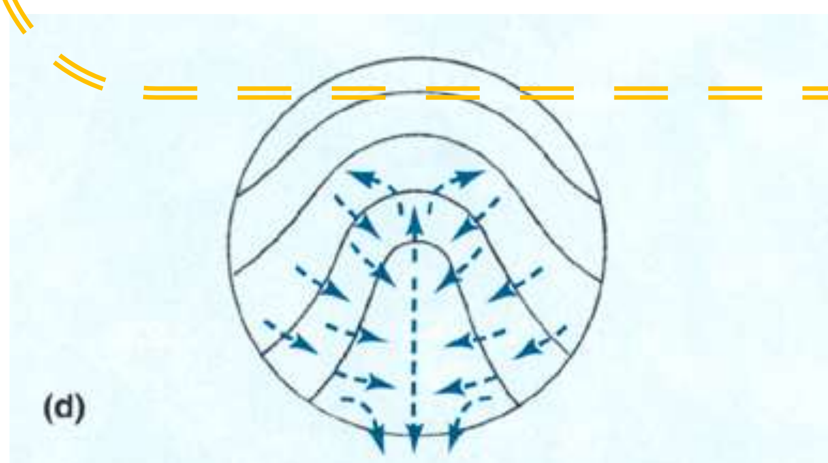


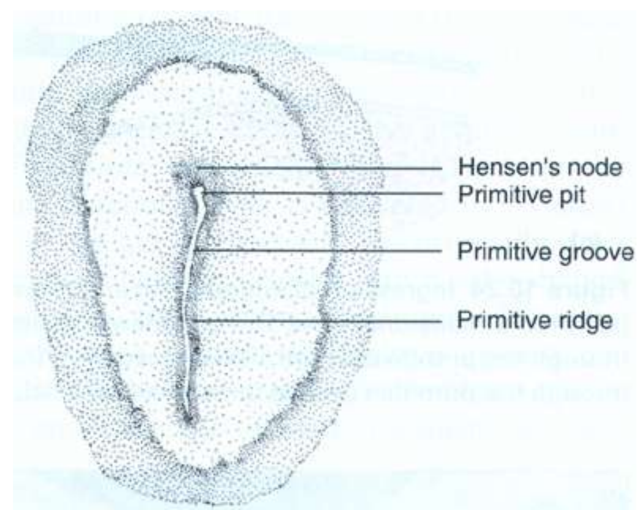
Primitive streak, groove

•Gastrulation begins with the formation of **primitive streak.** Epiblast proliferate and they are pushed toward the midline of the embryo → they are jammed in the midline forming the primitive streak that first appear in the posterior part of the embryo.

•Cells, which are located in the middle of the primitive streak, migrate into the interior of the embryo resulting the formation of **primitive groove** in the middle of the primitive streak.





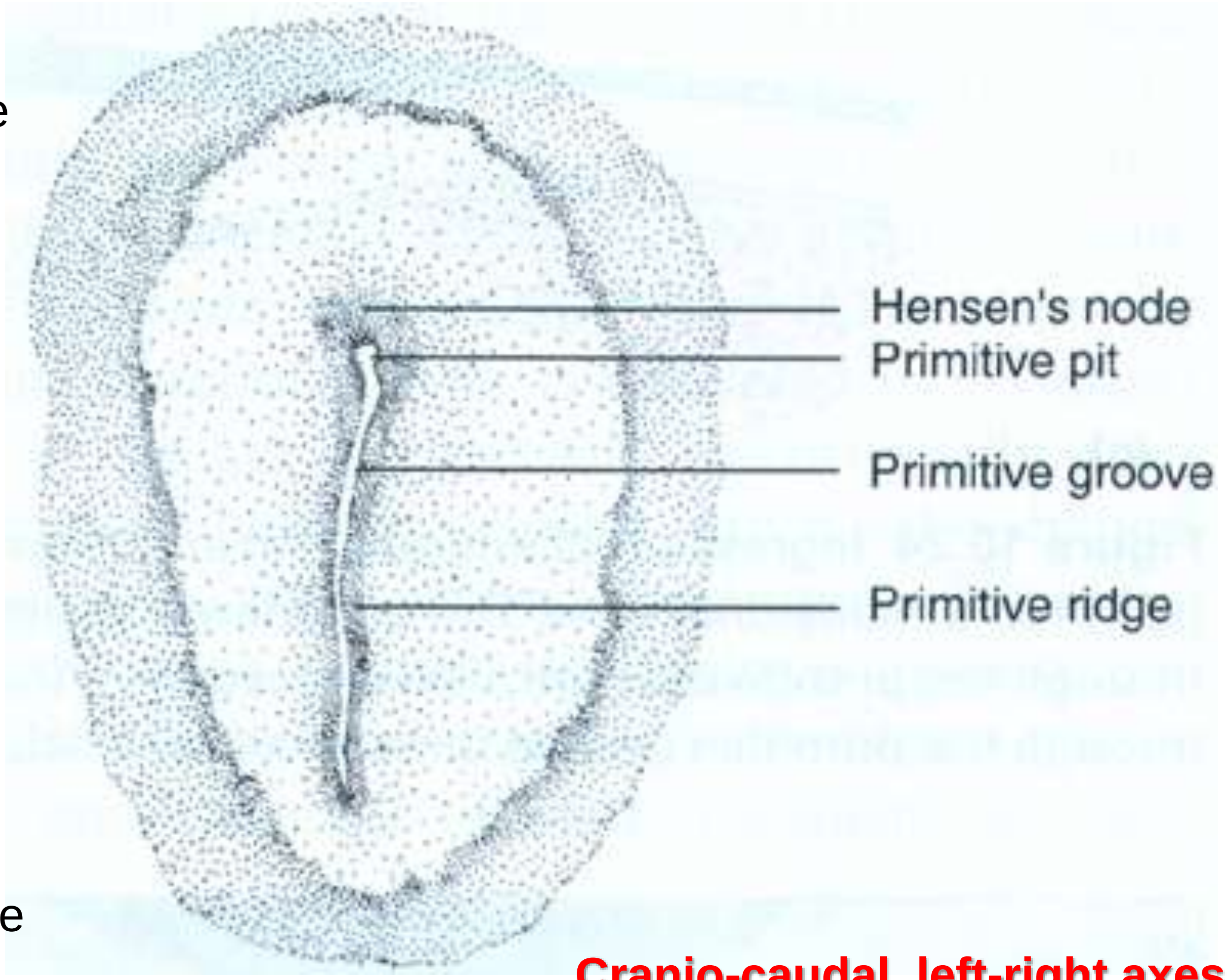


Hensen's node

•The primitive streak with the primitive groove is growing gradually anteriorly

• At the anterior end of the primitive streak there is a small but well-defined accumulation of cells, called primitive node or Hensen's node.

cranial side



caudal side

Cranio-caudal, left-right axes well-defined!

What happens to the cells in the primitive groove?

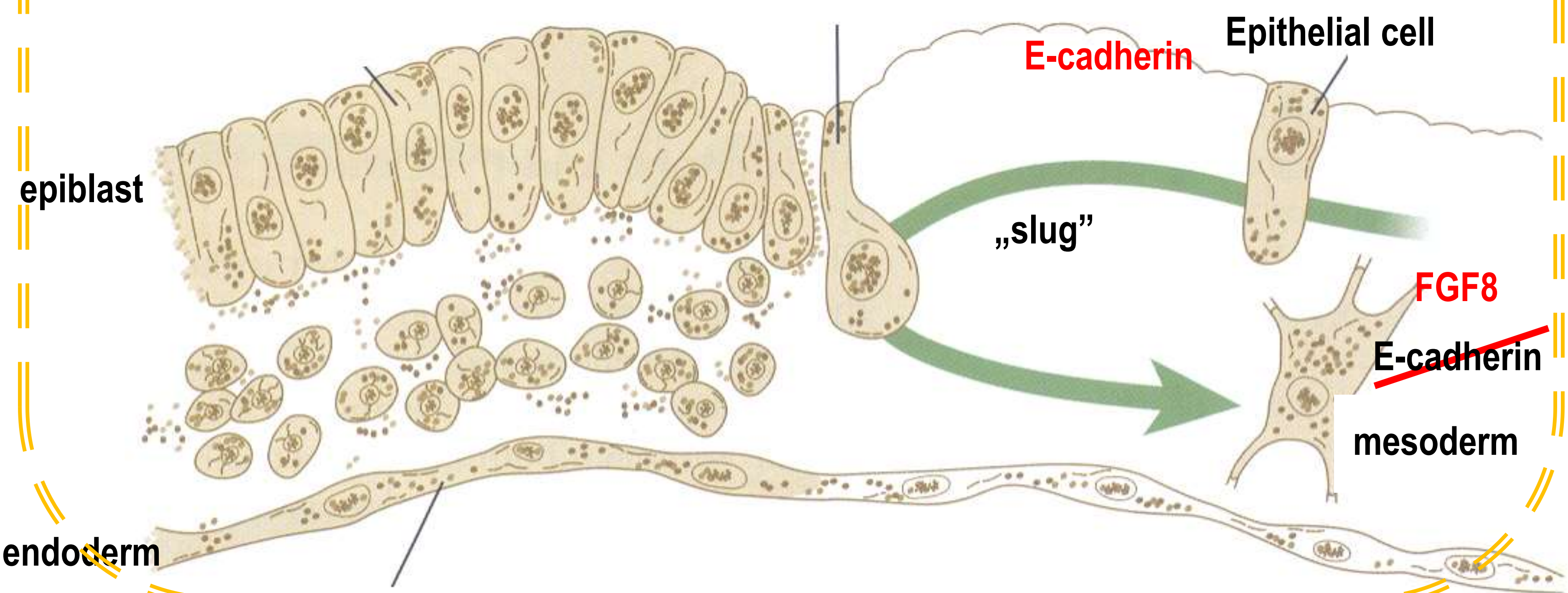
•The movements of the cells are accompanied by major changes in their structure.

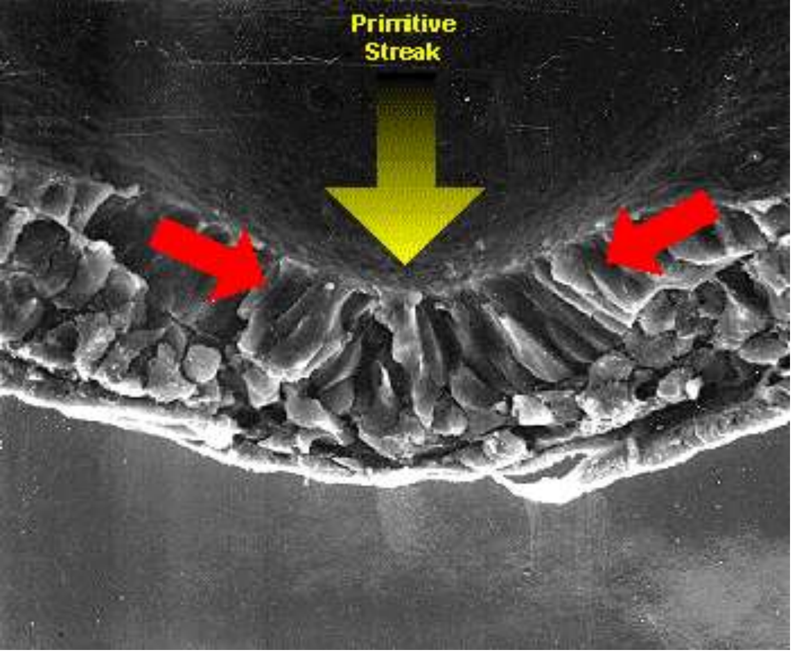
•When epiblast cells enter the primitive streak, they become elongated and lose their connection with the adjacent cells → their morphology change forming bottle cells.

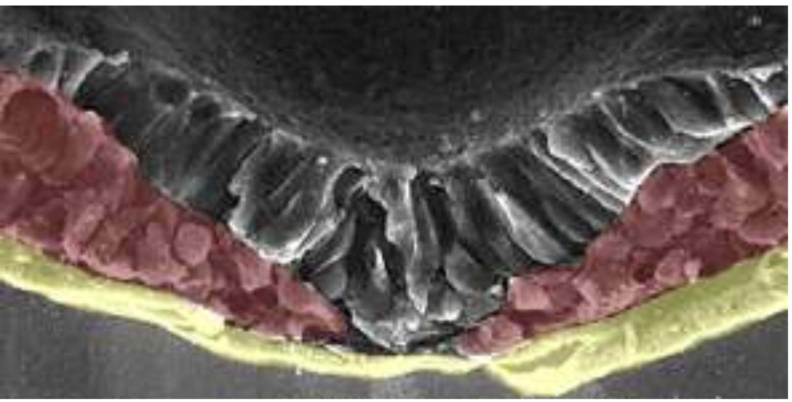
•Within the primitive groove these bottle cells lose their connection with the basal lamina and become free from the epiblast layer.

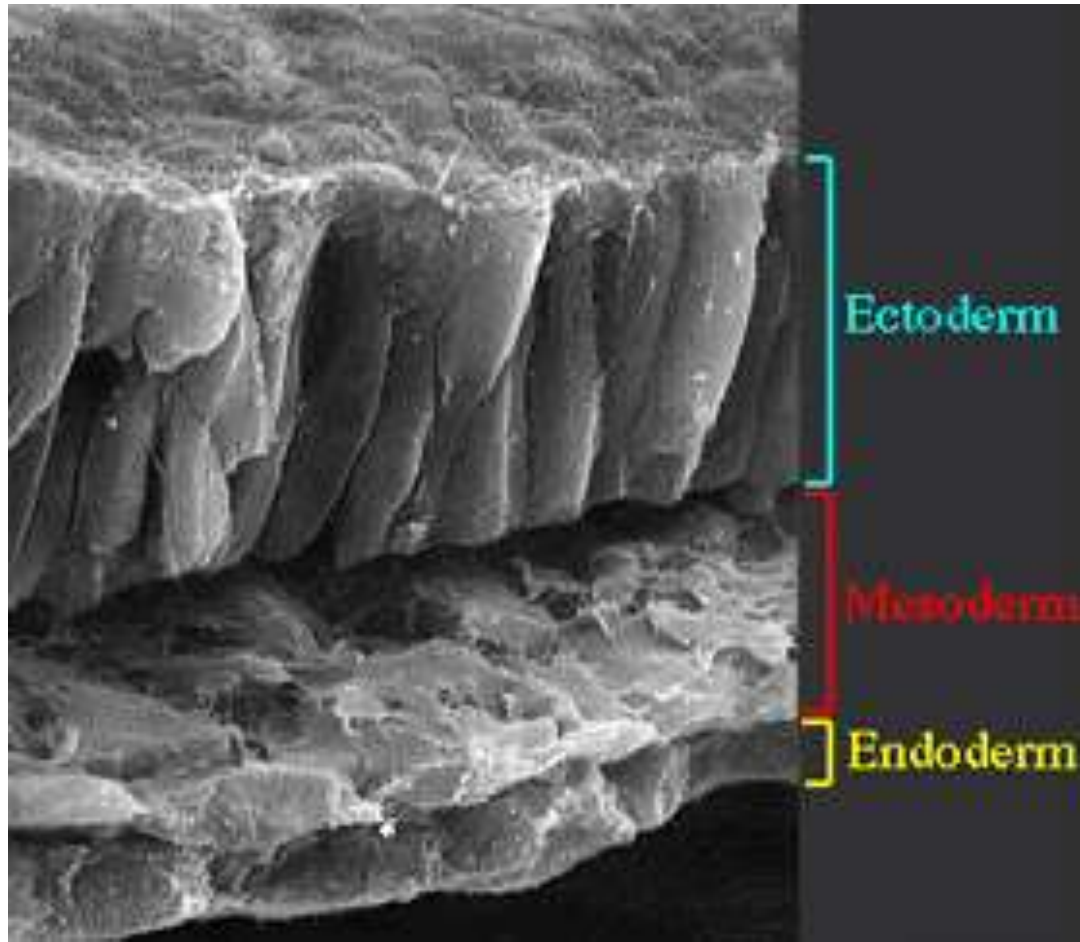
•Bottle cells undergo an epithelio-mesenchymal transformation within the primitive groove and the newly formed mesenchymel cells are able to migrate as individual cells.

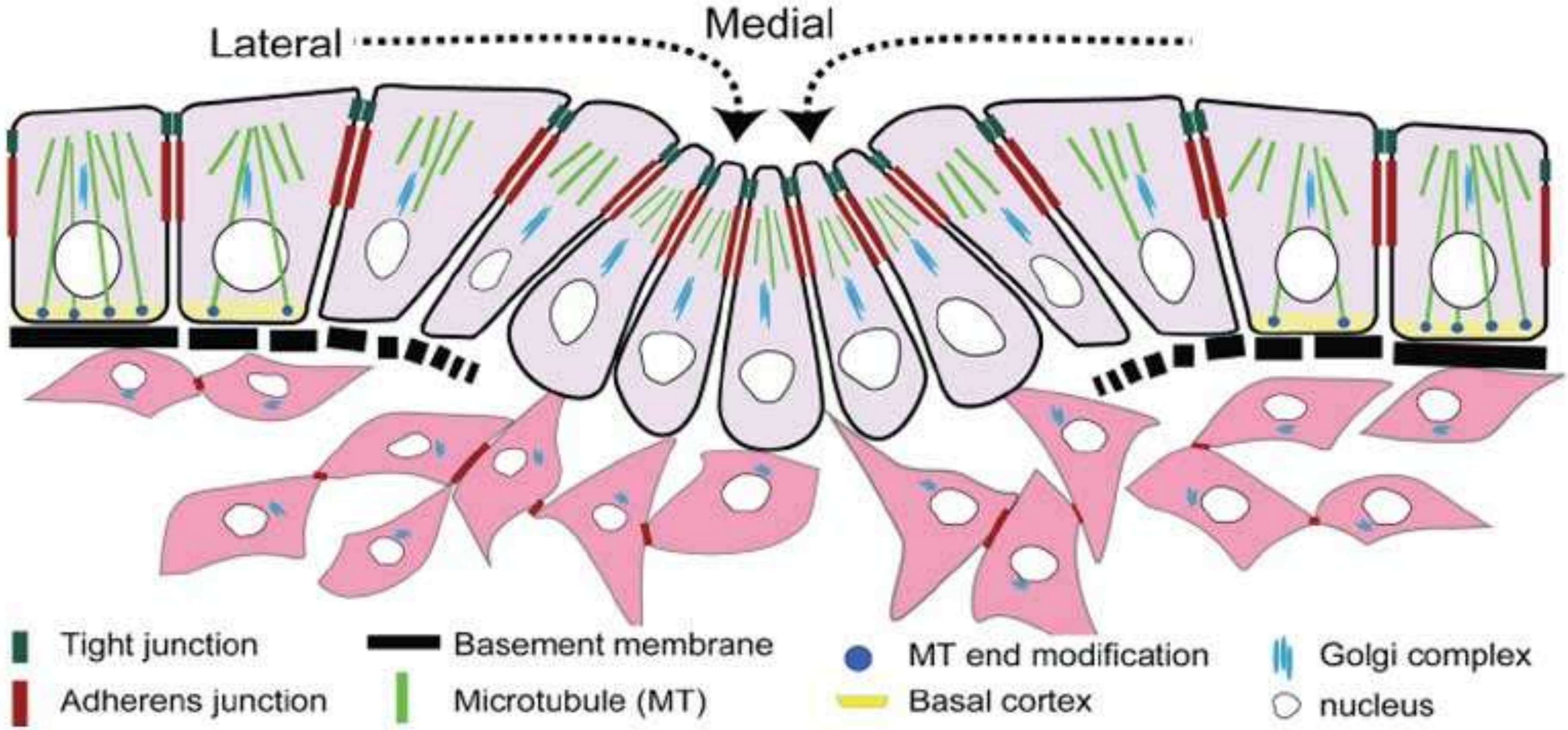
•During the epithelio-mesenchymal transformation the E-cadherin synthesis is downregulated within the bottle cells,



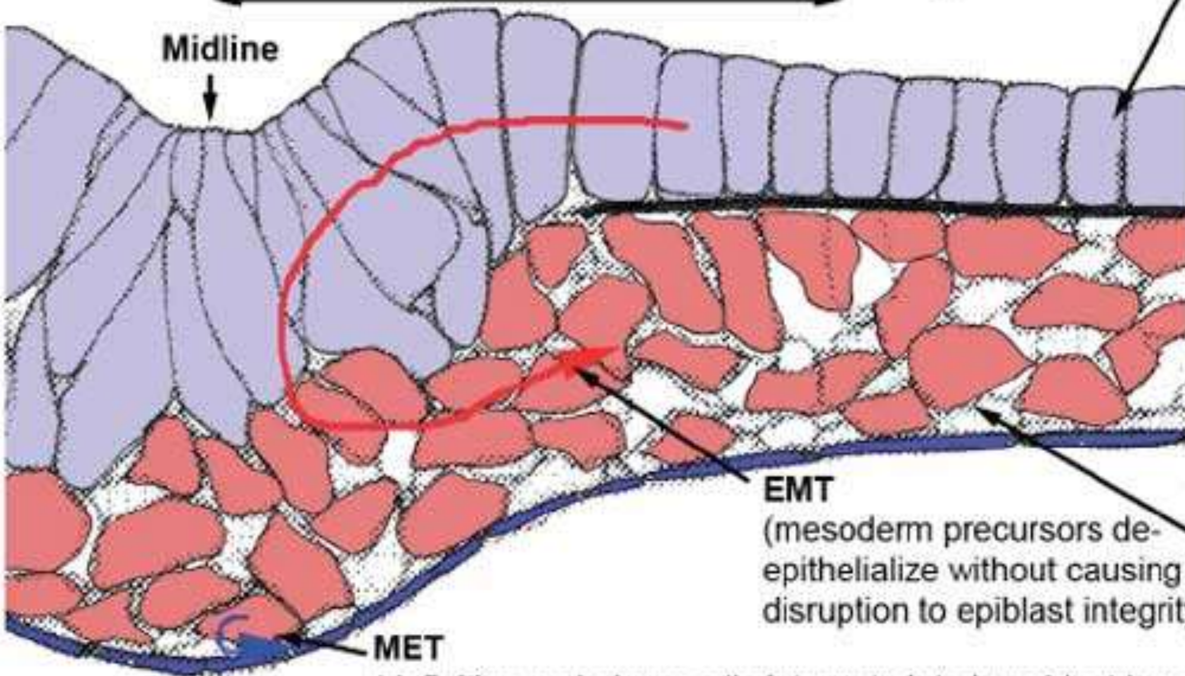

Primitive
Streak




Ectoderm
Endoderm

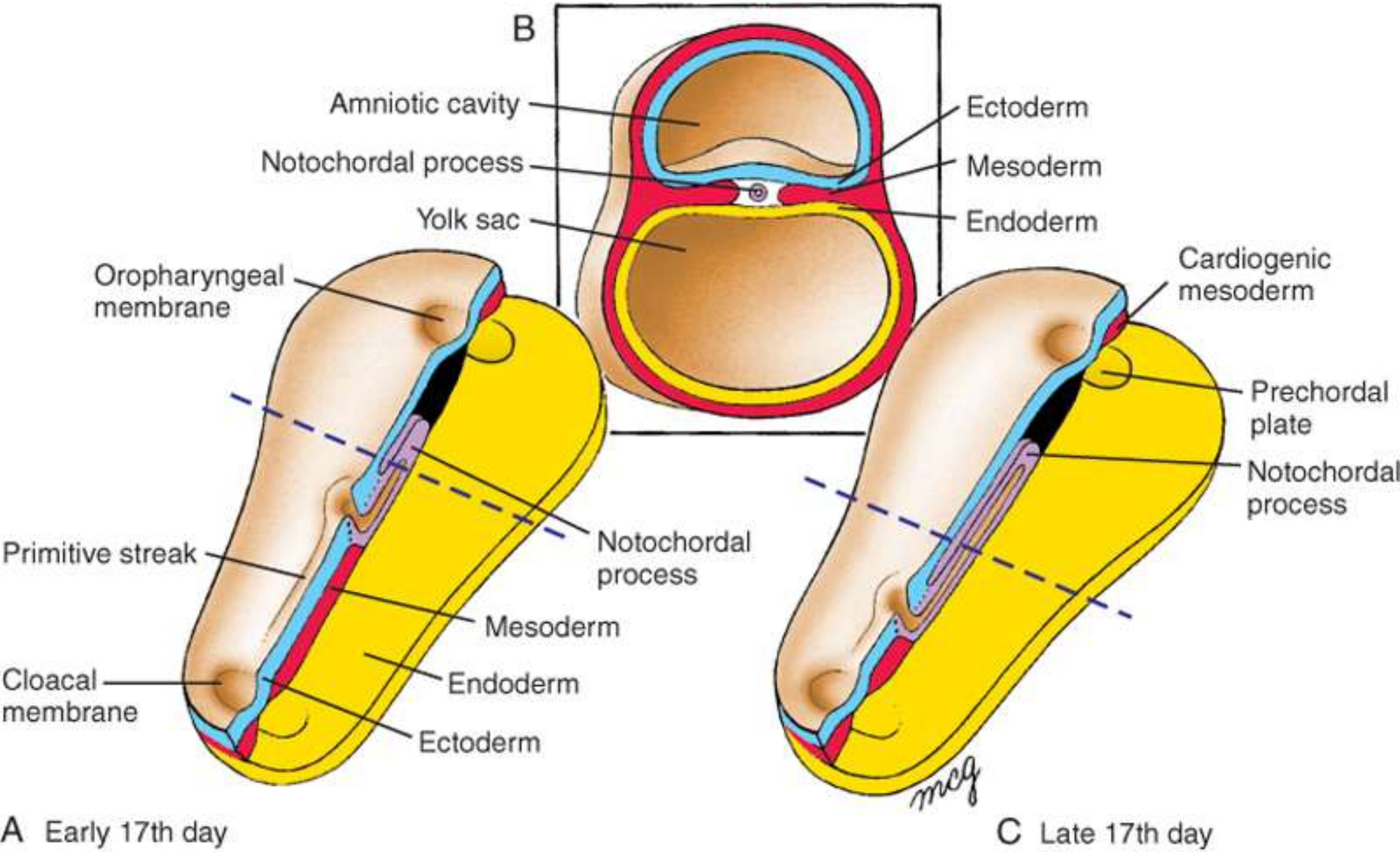


Chicken Gastrulation An amicable separation: Chick's way of doing EMT. Nakaya Y, Sheng G. Cell Adh Migr. 2009 Apr;3(2):160-3. Epub 2009 Apr 10. PMID: 19262172



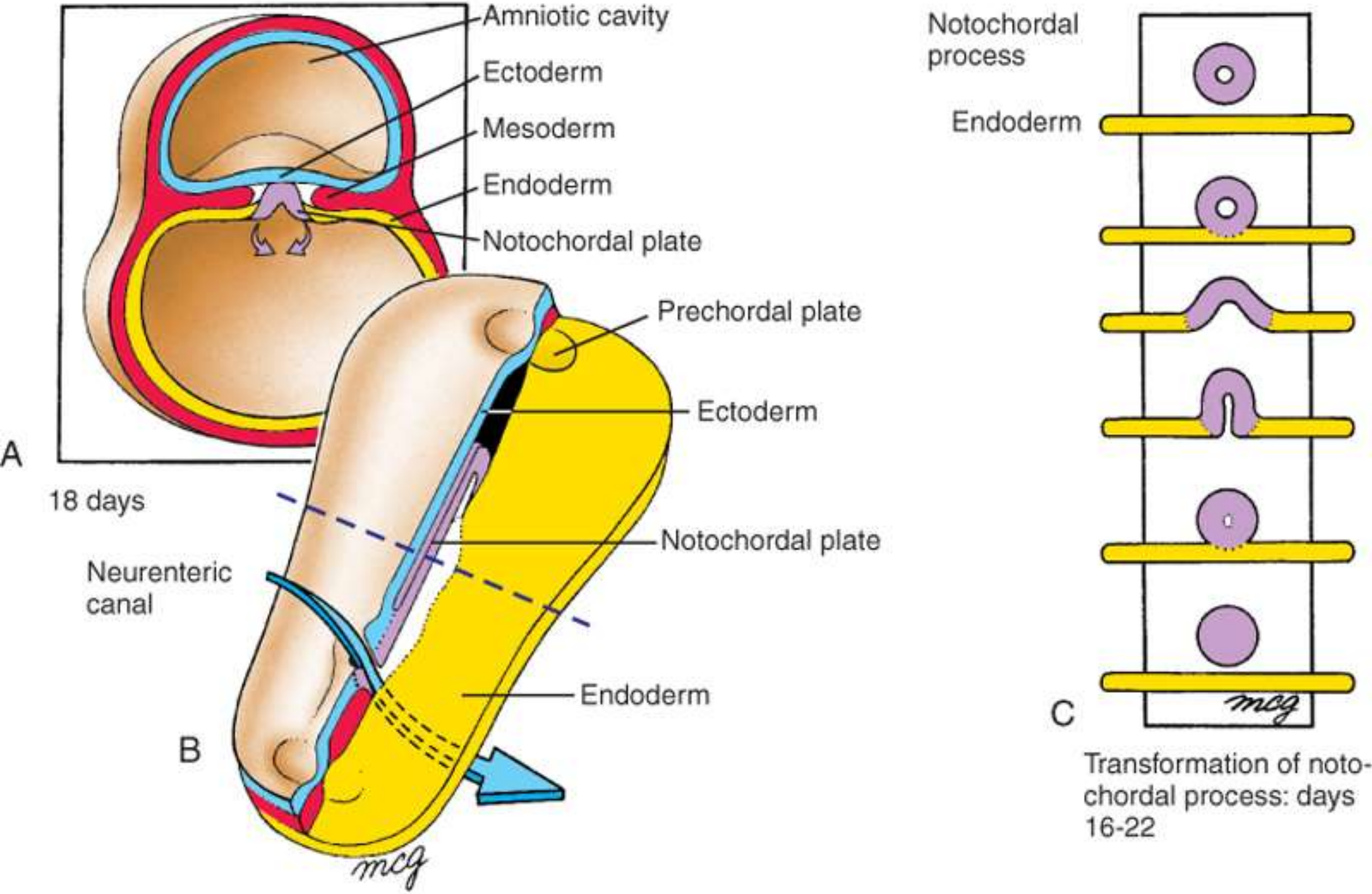
Chicken Gastrulation

An amicable separation: Chick's way of doing EMT. Nakaya Y, Sheng G. Cell Adh Migr. 2009 Apr;3(2):160-3. Epub 2009 Apr 10. PMID: 19262172



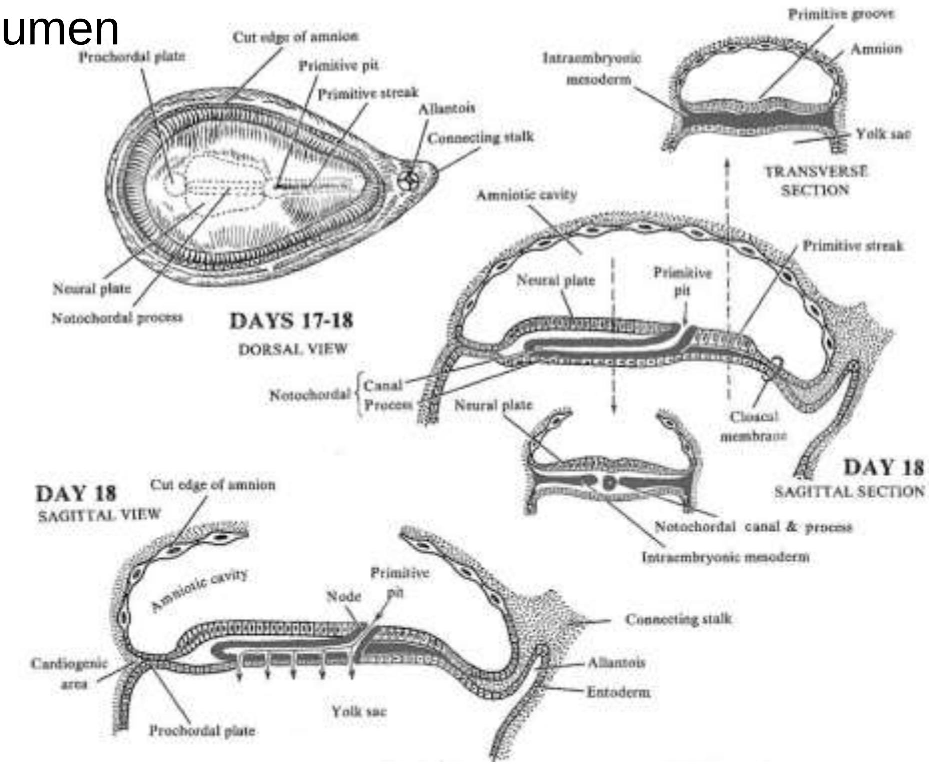
A Early 17th day

C Late 17th day


Amniotic cavity
Ectoderm
Mesoderm
Endoderm
Notochordal plate
A
18 days
Prechordal plate
Ectoderm
Notochordal plate
Neurenteric canal
Endoderm
B
Notochordal process
Endoderm
C
Transformation of noto-chordal process: days 16-22

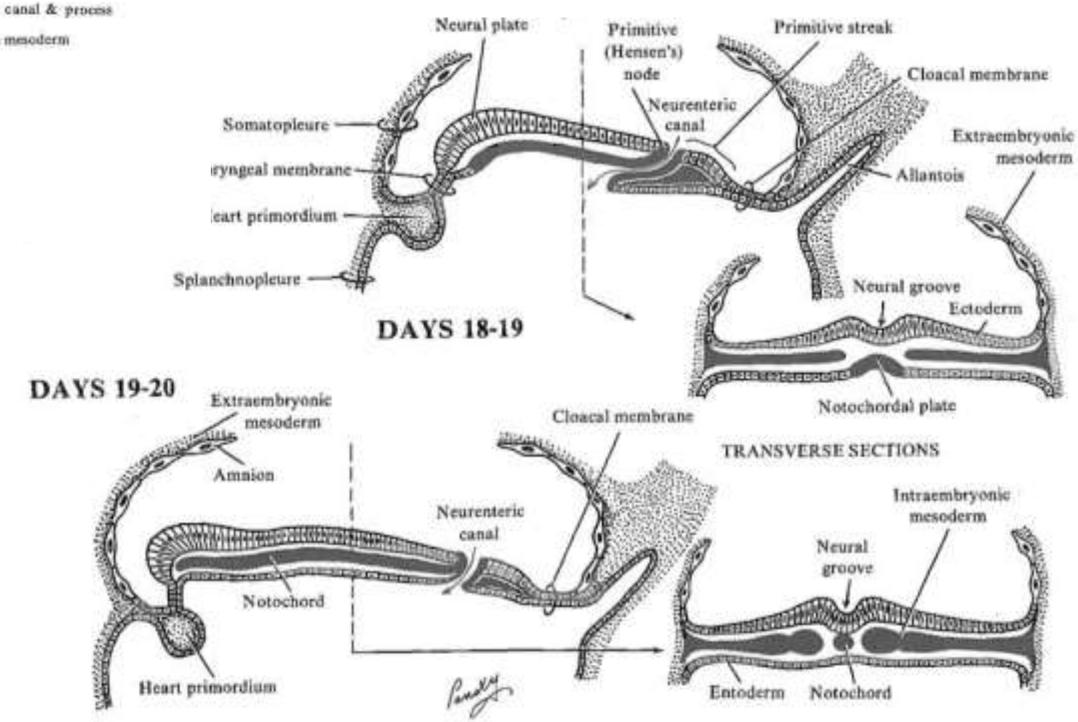
Human notochord

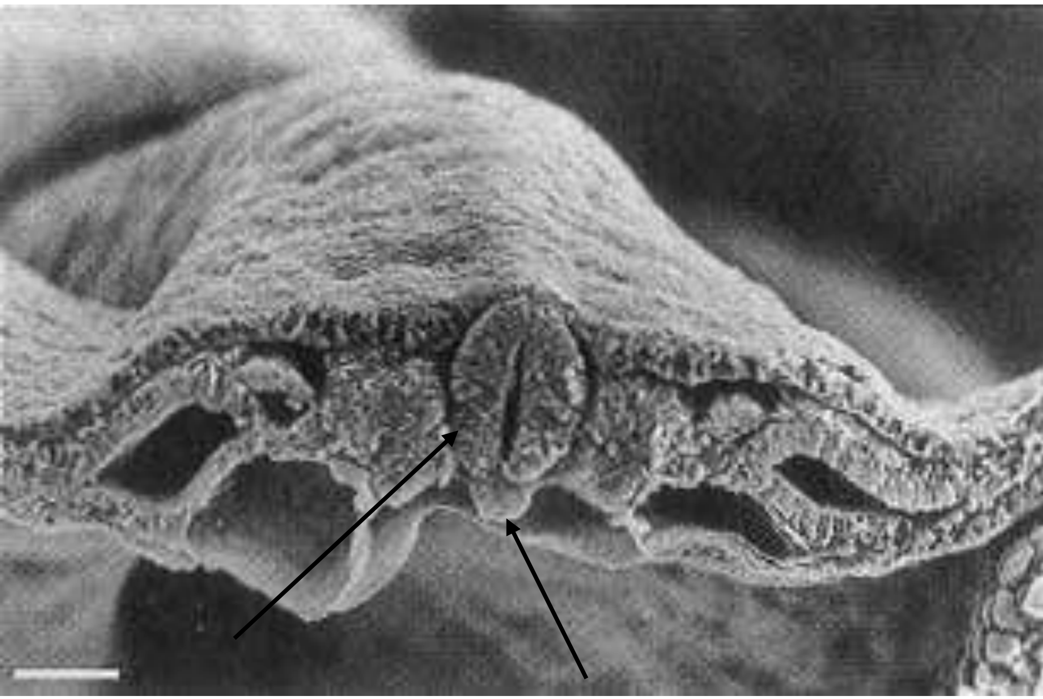
1.: At the biginnig of the notochord process first form a notochord canal with a central lumen

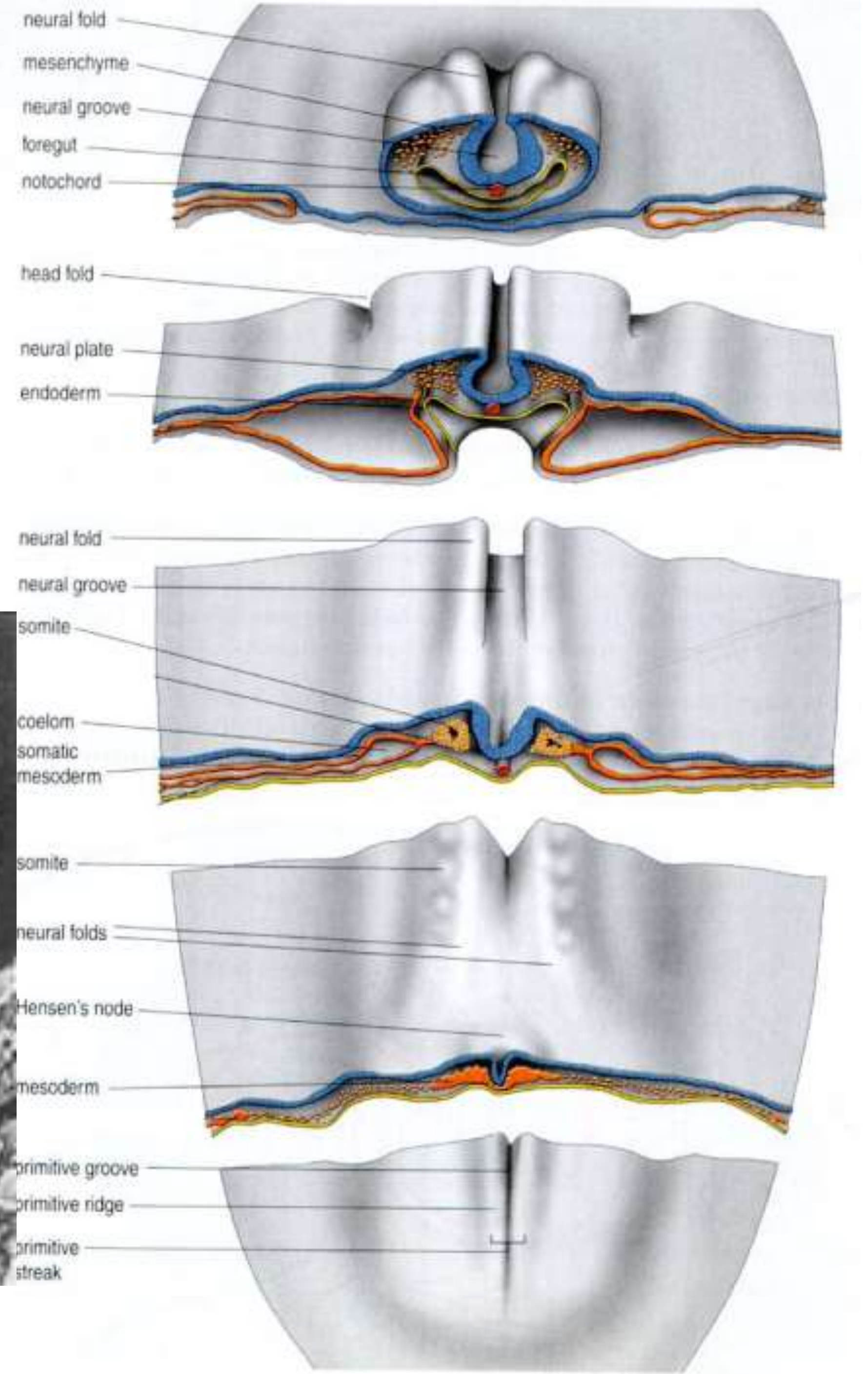


2.:The floor of the notochordal canal disappears remaining a flatten plate (notochordal plate) which incorporate into the definitiv endoderm

3.:The notochord plate starts to infold later form the rope like notochord

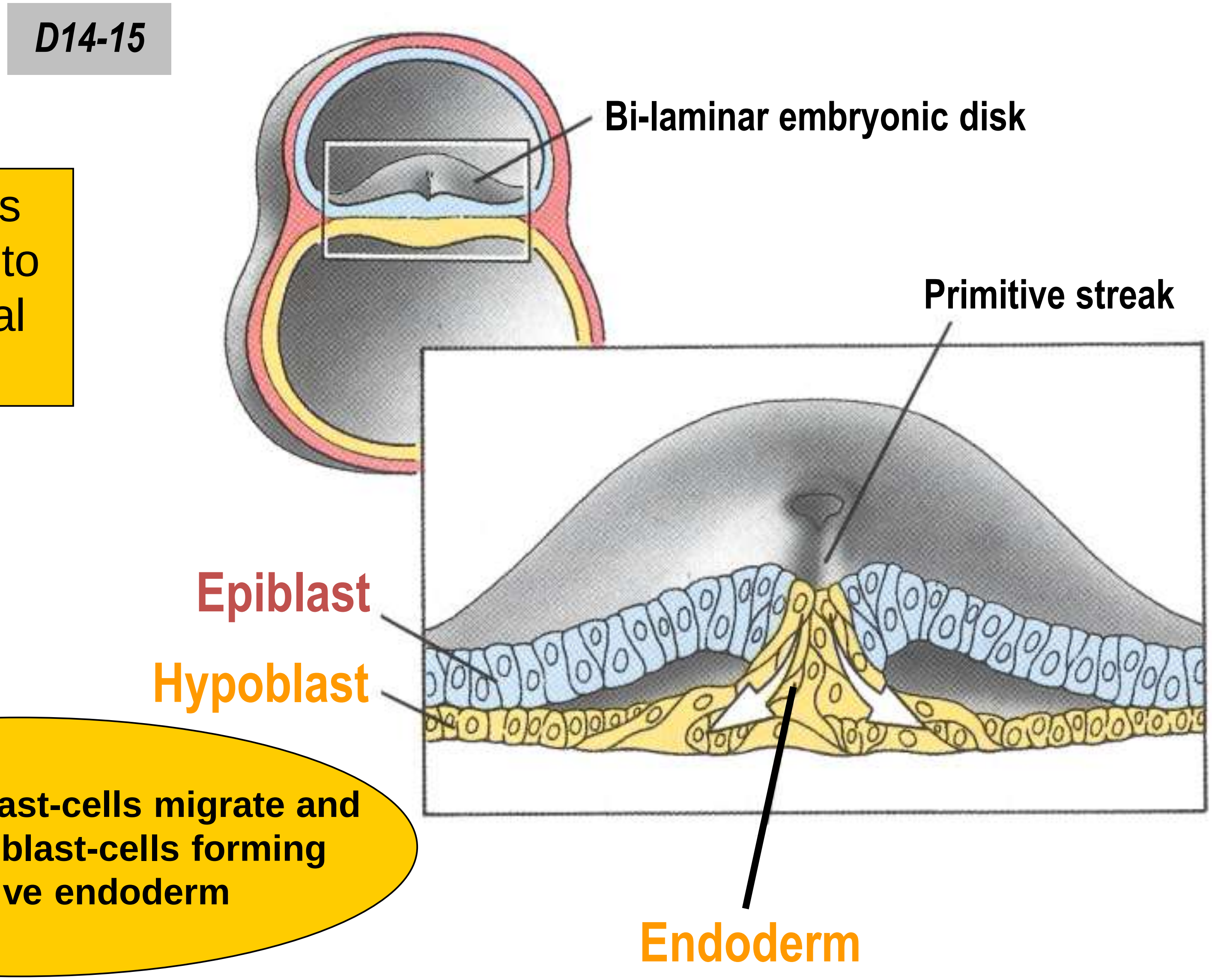






neural fold
mesenchyme
neural groove
foregut
notochord
head fold
neural plate
endoderm
neural fold
neural groove
somite
coelom
somatic
mesoderm
somite
neural folds
Hensen's node
mesoderm
primitive groove
primitive ridge
primitive
streak

Development of the definitive endoderm



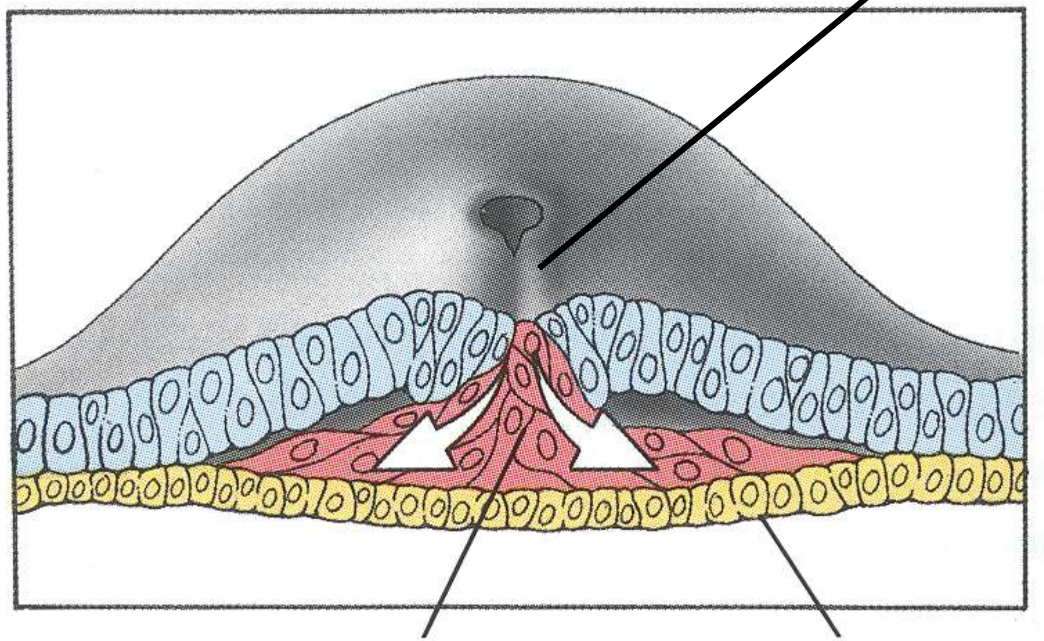
Hypoblast cells develop only into extraembryonal mesoderm

First entering epiblast-cells migrate and replace the hypoblast-cells forming the definitive endoderm

Epiblast cells give rise to the three germ layers of the embryo!!

Development of the Intraembryonal Mesoderm

D16



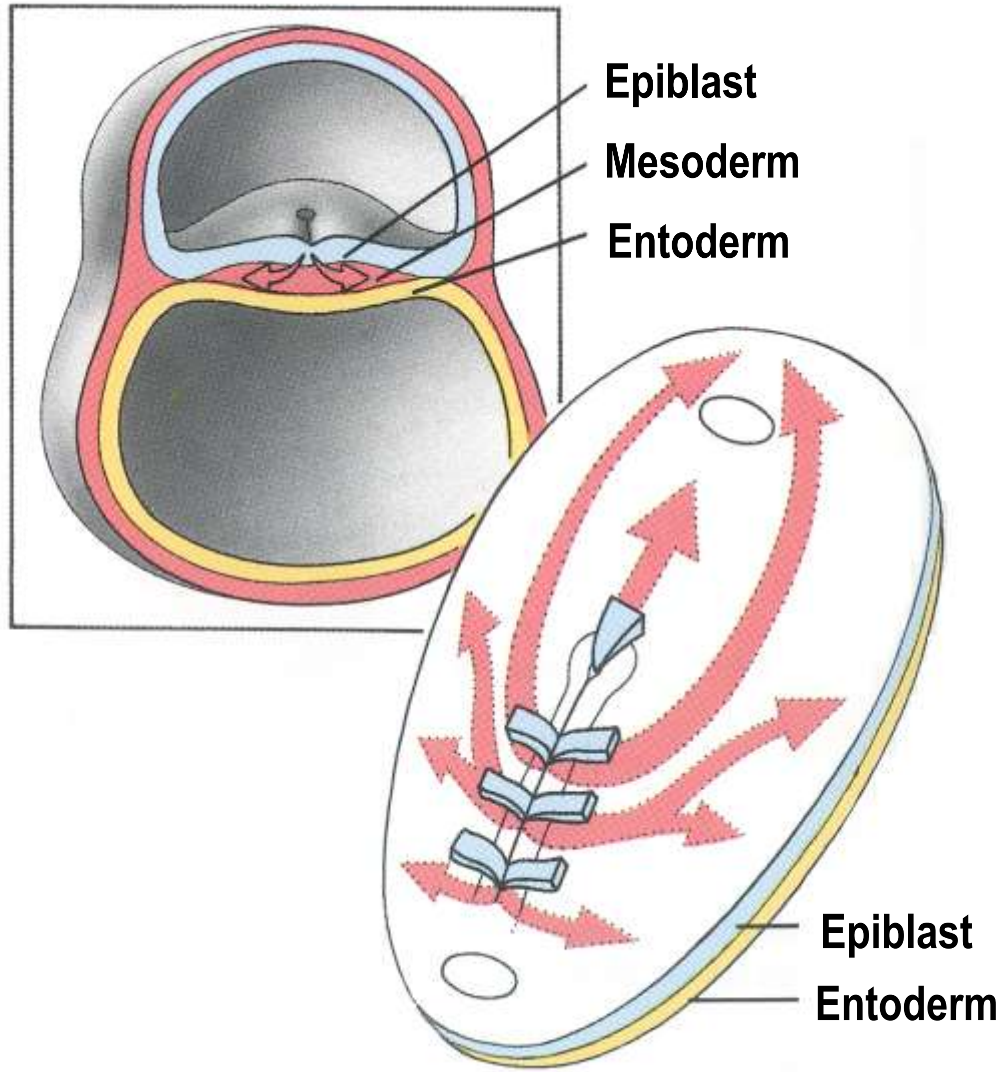
epithelio-mesenchymal transformation

Epiblast-cells migrate in the interlaminar space and forming intraembryonal mesoderm

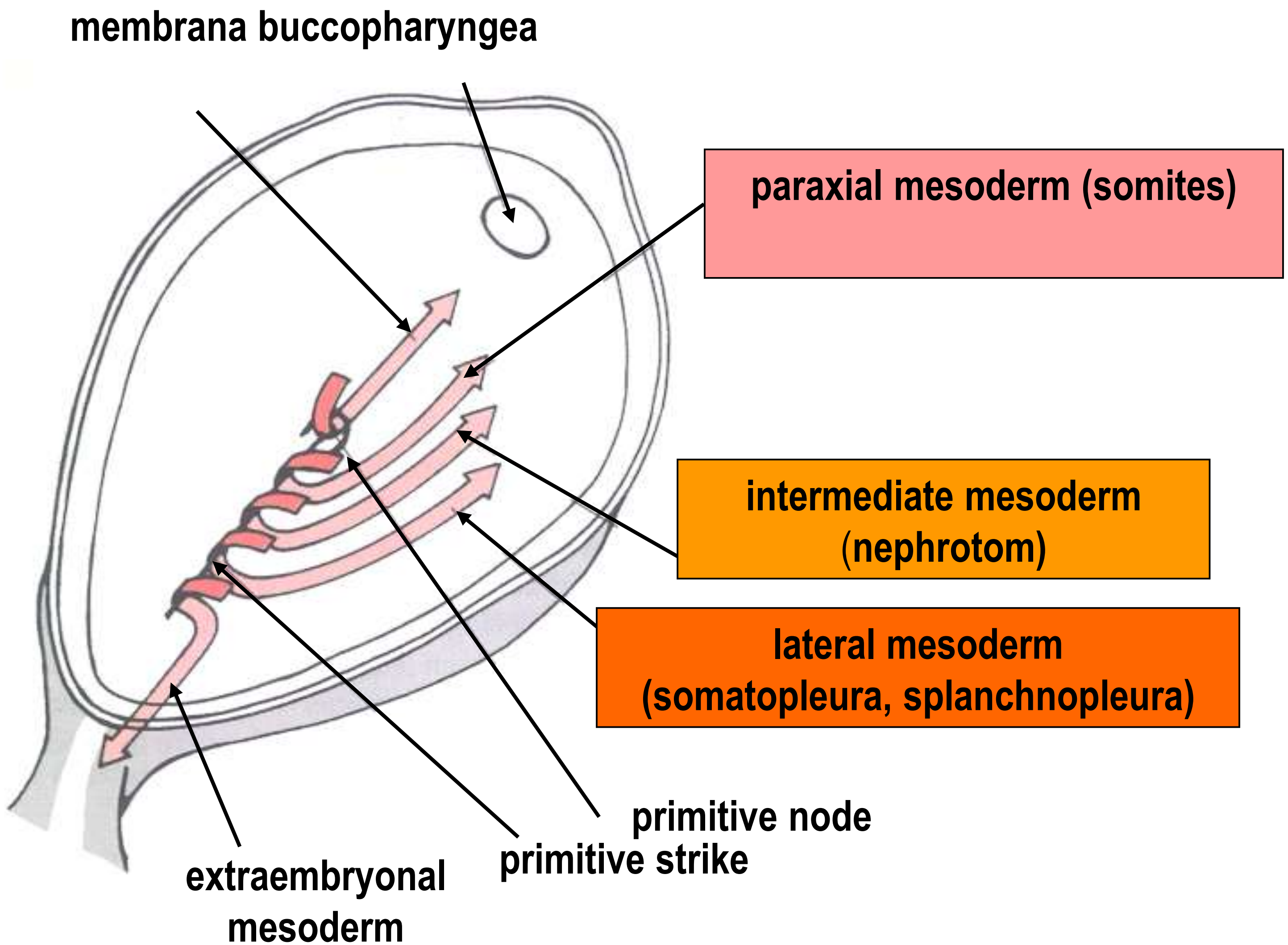
Migration of the Mesodermal Cells

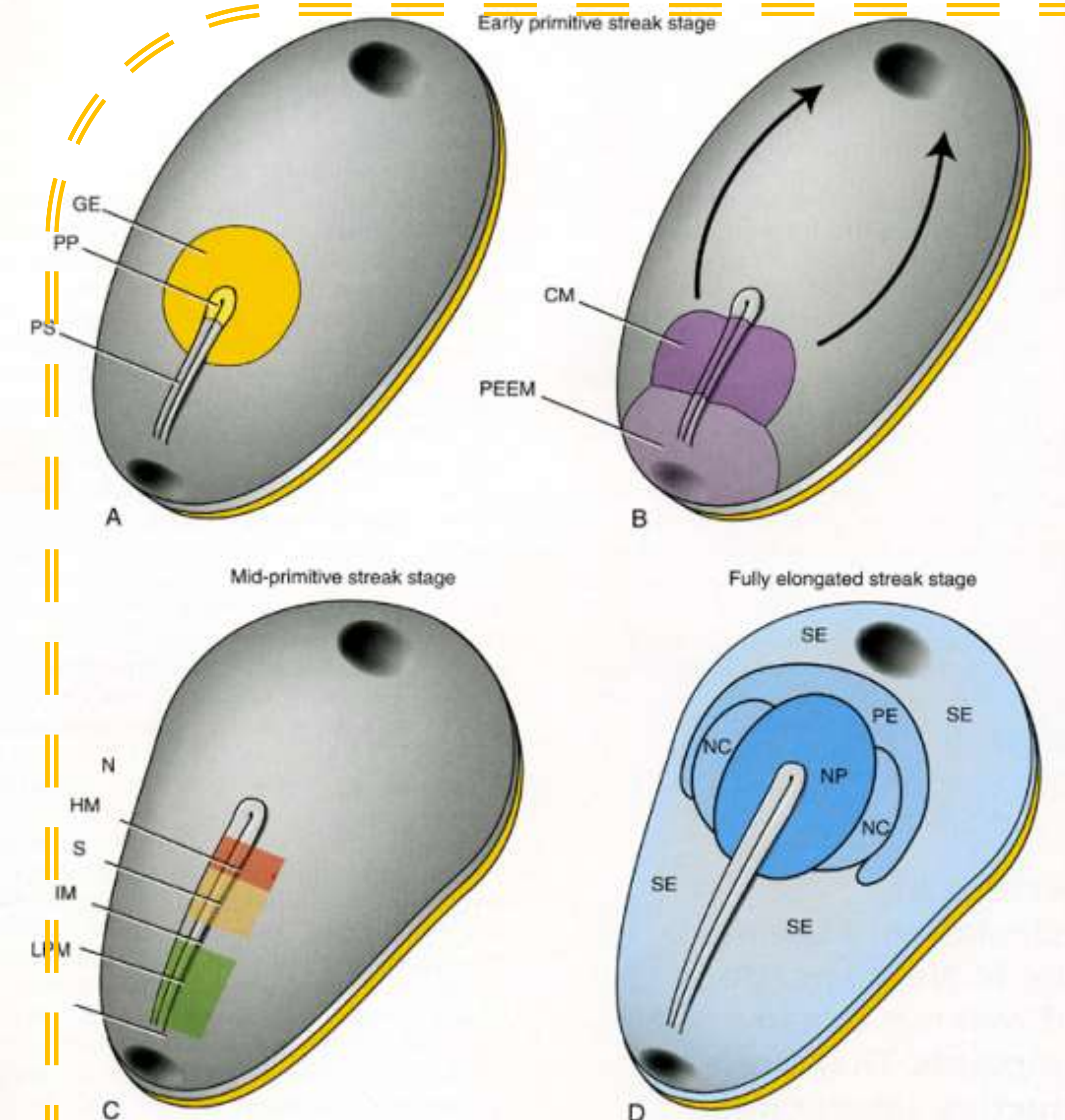
•The newly form mesenchymal cells migrate and spread bilaterally.

•Those cells, which pass through at the level of Hensen's node, migrate directly cranially and form the **precordal plate** and later take part in the formation of **the notochord**.



Differentation of the mesoderm, convergent extension





Fate map

GE: gut endoderm
PP: prechordal plate
PS: primitive streak

CM: cardiac mesoderm
PEEM: extraembryonic mesoderm

HM: head mesoderm
S: somitic mesoderm
IM: intermedier mesoderm
LPM: lateral plate mesoderm

SE: surface ectoderm
NP: neural plate
PE: placod ectoderm
NC: neural crest

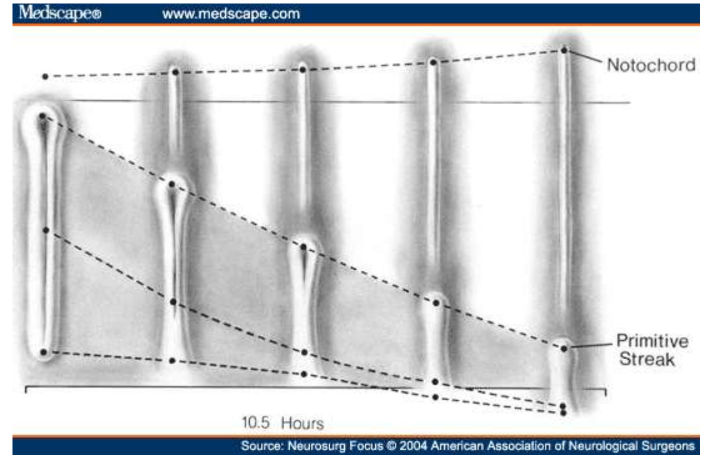
Primitiv streak regression

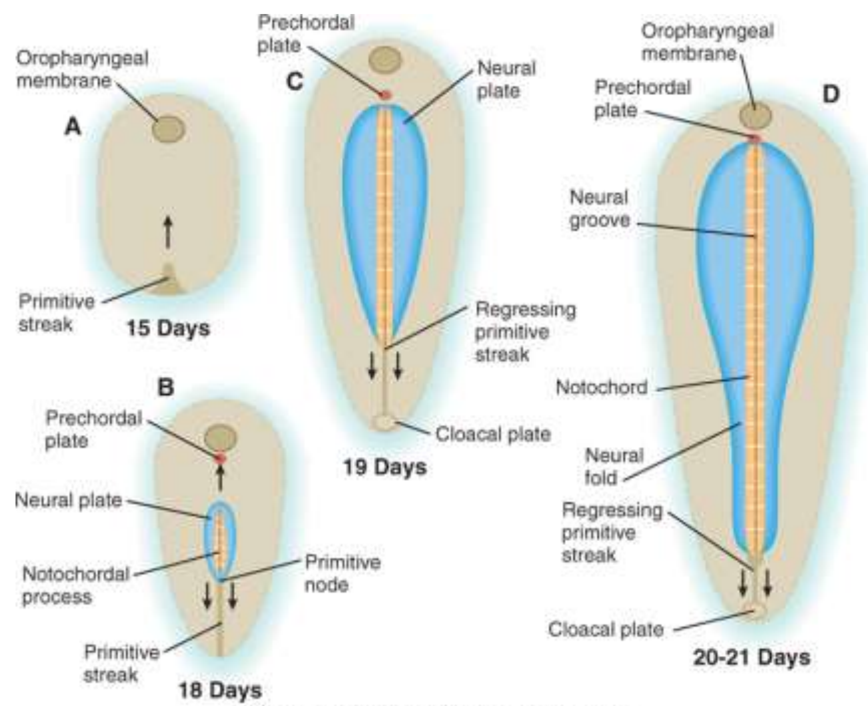
•At the beginning of gastrulation the primitive streak grows cranially

•The primitive streak growing changes for regression. → its length is decreasing toward caudally

•This regression process is related with the elongatoin of the notochord

•The notochord is formed by the addition of cells to its caudal end while the primitive streak becomes shorter and shorter





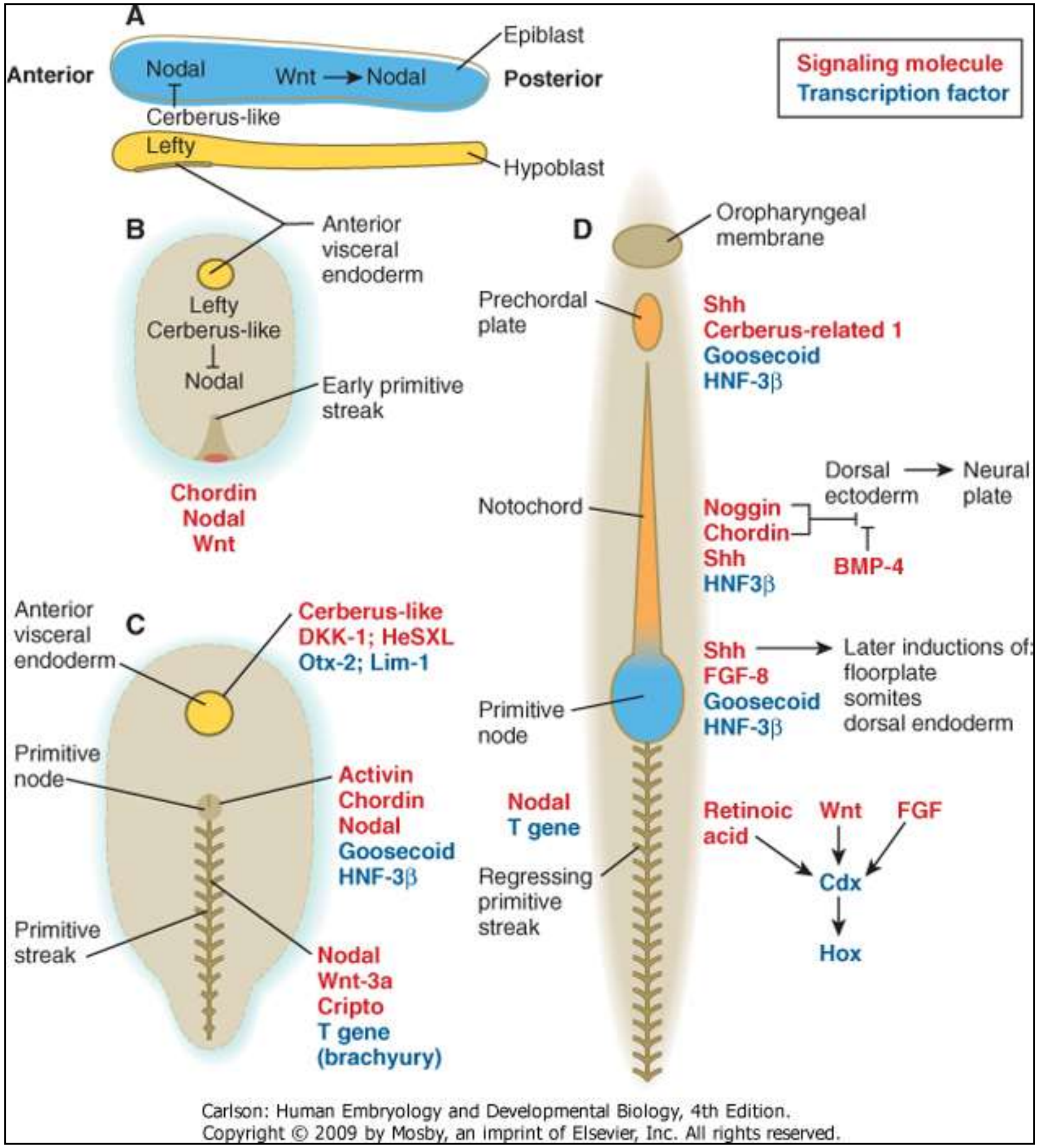
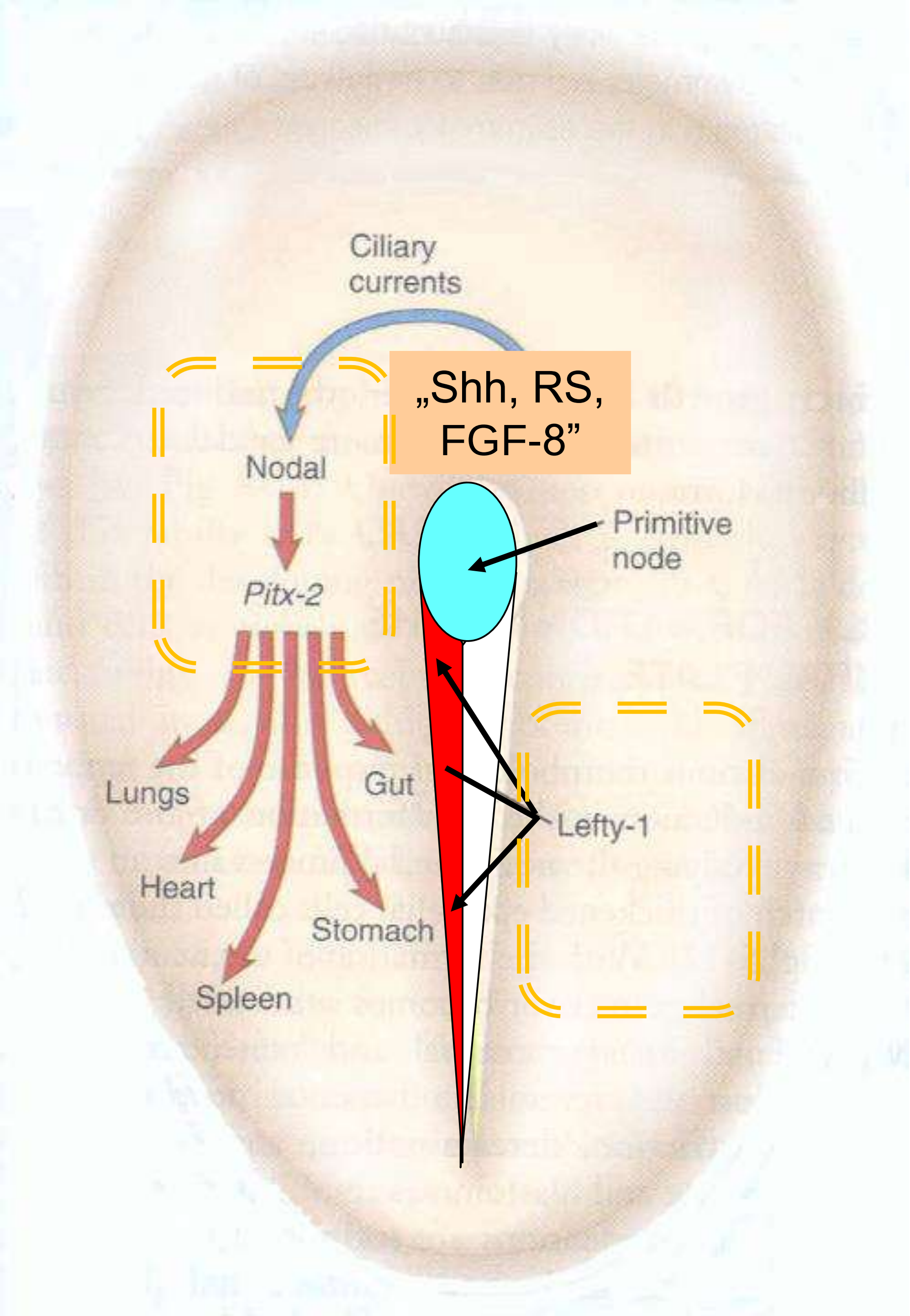
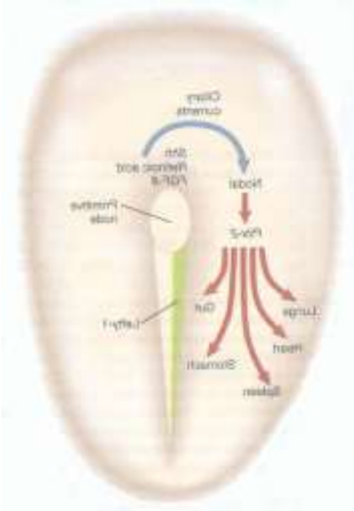


Figure 5-7 Summary of major genes involved in various stages of early embryonic development. A, Preprimitive streak (sagittal section). B, Early formation of the primitive streak. C, Gastrulation (period of germ layer formation). D, Late gastrulation and neural induction. The molecules in red are signaling molecules, and the molecules in blue are transcription factors. Names of specific molecules (bold) are placed by the structures in which they are expressed.


Ciliary currents
„Shh, RS, FGF-8”
Nodal
Pitx-2
Primitive node
Lungs
Gut
Lefty-1
Heart
Stomach
Spleen



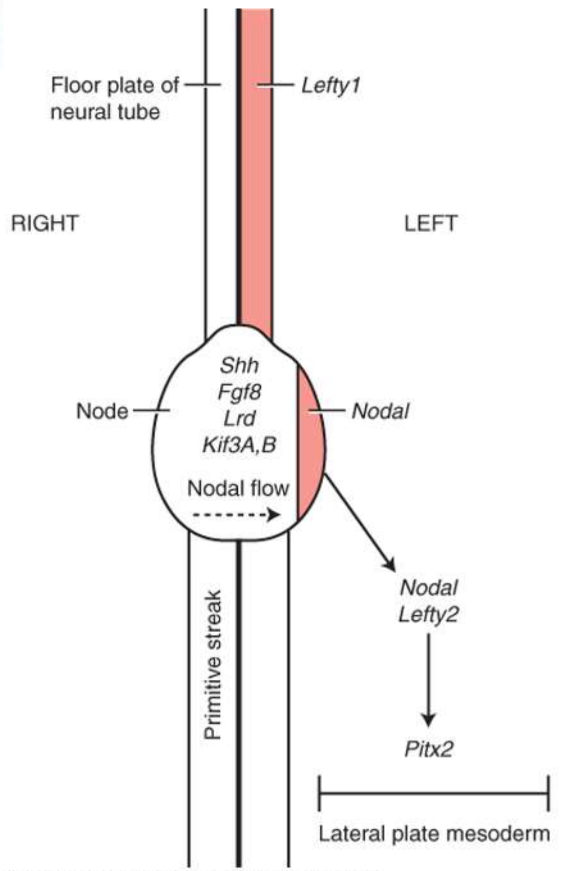
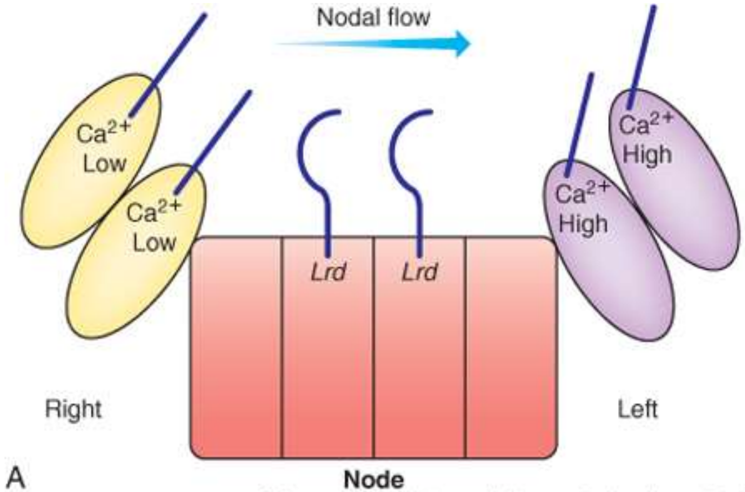


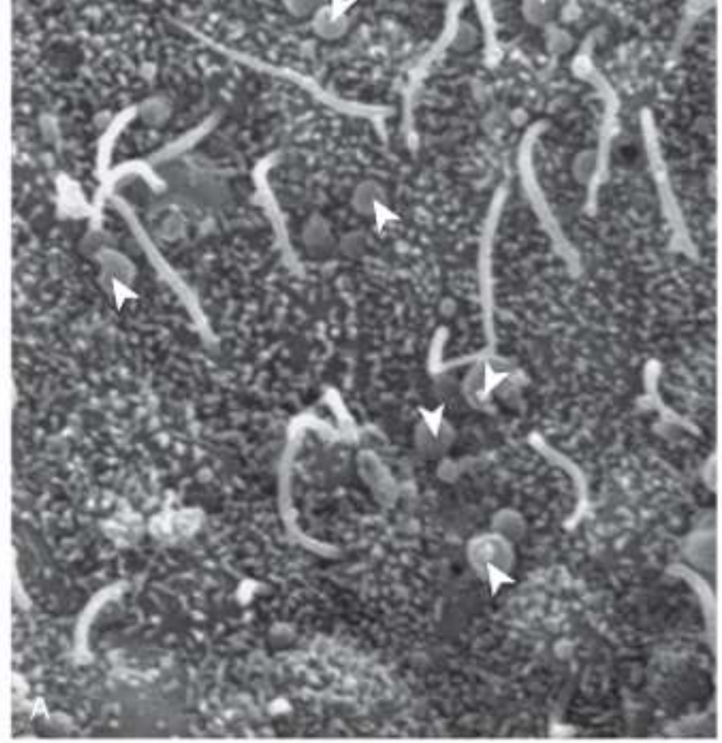
Figure 3-2. Diagram illustrating a simplified scheme of key genes involved in establishing left-right asymmetry. The primitive streak, node, and early floor plate of the neural tube are viewed from the ventral side.

Motor proteins (Lrd, Kif3A, B) expressed by the node regulate leftward (dashed arrow) nodal flow.

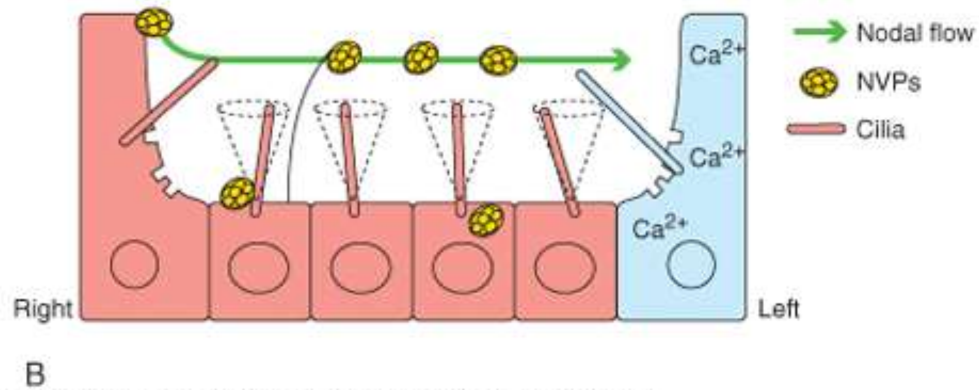
Secreted factors (Shh, Fgf8, Nodal) expressed by the node result in signaling to the lateral plate mesoderm, thereby resulting in asymmetric gene expression in the lateral plate mesoderm (e.g., Nodal, Lefty2 in left lateral plate).

This in turn results in expression of Pitx2 in the left lateral plate and changes in cell behaviors that result in asymmetric morphogenesis. Lefty1 is expressed in the left floor plate of the neural tube. It is believed to serve a barrier function, allowing information that specifies left and right sides to remain separate.

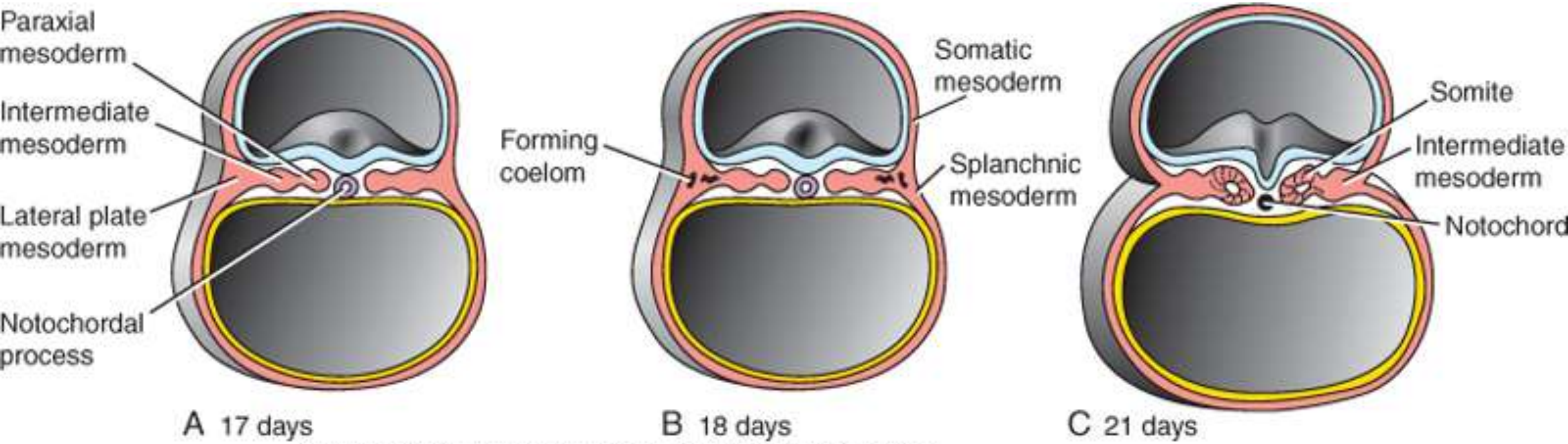




The mechanosensory model of nodal flow.
Model showing that nodal flow, generated by motile monocilia in cells expressing Lrd, stimulates
calcium flux in cells containing nonmotile cilia that sense flow on the left side.



Model showing the transport of nodal vesicular parcels by motile cilia and the stimulation of calcium signaling (blue) at the left side of the node by nonmotile cilia.



Schoenwolf et al: Larsen's Human Embryology, 4th Edition.

Gastrulation