Unit 16 · Development of the Frog

STUDY NOTES

WHAT NEB'S GRADE 12 EXAM ASKS

- **CDC's grid (2078):** Developmental Biology (Units 15–16 together): 4 of 75 marks; 1 short question (4 marks: understanding 1, application 2, higher ability 1); no MCQ by the plan
- **The paper:** Botany 37 + Zoology 38; each part: MCQs × 1 (5 Botany, 6 Zoology) · 4 short × 4 · 2 long × 8; one OR in each short and long group; 3 hours; 1 mark per expected point
- **CDC's model paper:** MCQ on frog gastrulation; short (OR): fertilization in a frog
- **Our model papers:** Papers 2 and 3's short questions are on this unit (cleavage; germ layers and the organizer)
- **MCQ (1 mark)**
  - the gray crescent marks the dorsal lip of the blastopore
  - blastocoel: fluid-filled, room for cells to move
  - epiboly: ectoderm spreads over the embryo
  - no mesoderm → no coelom
- **Short (in parts)**
  - unfertilized vs fertilized egg (figure A, B)
  - cleavage with diagrams; cells after the fifth cleavage
  - why vegetal cells are larger; where the embryo's food comes from
  - blastula: name, formation; label micromeres, megameres, blastocoel
  - gastrulation: events; importance
  - germ layer of brain, gut lining, notochord, muscles
  - neural tube formation; schizocoel formation
  - what Mangold's graft shows (the organizer)

Key terms

Amplexus: male clasps the female's back with **nuptial pads** on his thumbs; eggs laid as she clasps.

Insemination: male sheds sperms in seminal fluid over the eggs; then **fertilization**: sperm fuses with egg.

Cone of reception: small cone the egg's surface raises where the sperm touches.

Fertilization membrane: vitelline membrane lifted, thickened, toughened; keeps out other sperms.

Perivitelline space: fluid-filled space under the fertilization membrane.

Monospermy: one sperm per egg; **polyspermy** prevented.

Sperm (penetration) path: dark streak of pigment the male pronucleus drags inwards; then the **copulation path** to the female pronucleus.

Cleavage: rapid mitotic divisions of the zygote into **blastomeres**; embryo doesn't grow.

Holoblastic: each furrow cuts the whole egg; **unequal**: vegetal cells larger.

Micromeres: small, pigmented, little yolk (above); **megameres** (macromeres): large, yolky (below).

Morula: 32-celled mulberry-like ball; **blastula**: hollow ball with a **blastocoel**.

Coeloblastula: blastula with a large cavity; **monoblastic**: one ball of cells, no germ layers.

Fate map: drawing of a blastula's **presumptive** (prospective) areas.

Gastrulation: blastula → **gastrula** by **morphogenetic movements**; three germ layers.

Archenteron: cavity made in gastrulation; the future gut; opens by the **blastopore**.

Organizer: the **dorsal lip of the blastopore** (Spemann).

Organogenesis: forming organs from the germ layers.

Schizocoel: true coelom formed by **splitting of the mesoderm**.

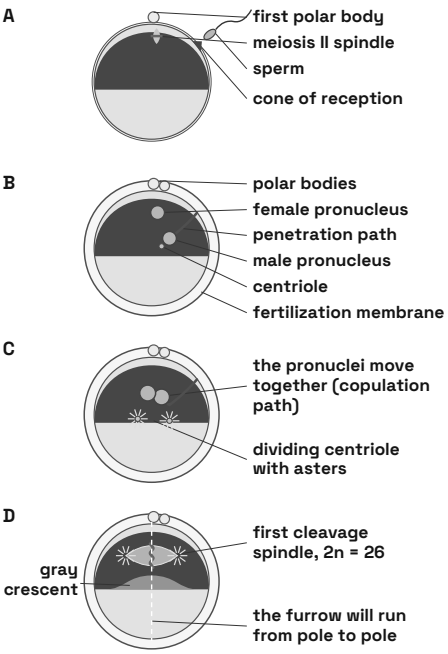
Fertilization

- rainy season; male's **breeding call** → female comes → **amplexus** (hours)
- eggs laid as **secondary oocytes** through the cloaca; sperms shed over them

- **external**: in water

Steps

1. sperm enters the **animal hemisphere**; only **head and middle piece** (tail left outside)
2. **cone of reception** rises to meet it
3. acrosome's **sperm lysins** dissolve jelly and vitelline membrane
4. fluid released under the vitelline membrane → **fertilization membrane**; **perivitelline space**; no other sperm gets in
5. **meiosis II completed** → second polar body; 13 chromosomes → **female pronucleus**
6. sperm nucleus swells → **male pronucleus**; **sperm path**, then copulation path
7. pronuclei **fuse** → **2n = 26**: diploid number restored
8. sperm's **centriole** (egg lacks one) divides → spindle; chromosomes line up for the first cleavage



Fertilization in a frog's egg.

Effects of fertilization

- secondary oocyte completes meiosis II; second polar body
- cytoplasm contracts, gives fluid to the perivitelline space → fertilization membrane
- **monospermy** ensured (sperm lysins can't dissolve the fertilization membrane)
- **centriole** brought in
- **gray crescent** opposite the sperm's entry (cortex turns ~30° towards the entry point)
- gray crescent → future **dorsal lip of the blastopore**, **dorsal side**
- radially → **bilaterally symmetrical**
- body plan marked: gray crescent = **dorsal**; animal pole = **anterior** (head); vegetal pole = **posterior**

Significance

- forms a **diploid zygote** → embryo
- brings the **centriole** (centrosome)
- **prevents polyspermy**
- stimulates the **second maturation division**
- brings **variation**: genes from two parents

Unfertilized vs fertilized egg (activity; figures in Unit 15)

Unfertilized	Fertilized (zygote)
secondary oocyte, stopped at metaphase II	meiosis II completed
haploid, n = 13	diploid, 2n = 26
one polar body	two (or three)
vitelline membrane close to the egg	fertilization membrane raised; perivitelline space
no centriole	the sperm's centriole
radially symmetrical	bilateral: gray crescent
no sperm path	dark sperm path
lies any way round	animal pole up
can't divide	starts cleavage

- **alike**: one cell, 1.5–2 mm; dark animal and yolky vegetal halves; jelly; mesolecithal, telolecithal

Cleavage

- starts **2–3 hours** after fertilization; **holoblastic** and **unequal**
- embryo's size the same; cells more, smaller; cells don't grow, only copy DNA and divide
- yolk slows the furrows, pushes spindles up → vegetal cells larger

Cleavage	Plane	Result
1st	**meridional** (vertical), pole to pole, through the gray crescent	2 equal blastomeres
2nd	meridional, at right angles to the 1st	4 equal
3rd	**latitudinal** (horizontal), **above the equator**	8: 4 micromeres + 4 megameres
4th	meridional, two furrows at once	16: 8 + 8
5th	latitudinal, two at once	32: 16 micromeres + 16 megameres

- then irregular: micromeres divide faster

- energy and building material: the **yolk inside the cells**; no food from outside → big yolky egg
- first furrow usually splits the gray crescent → left and right halves (Spemann: each half of a 2-celled newt can make a whole larva)

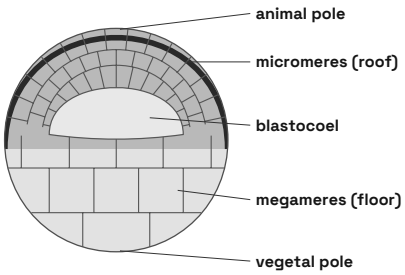
Morula and blastula

Morula

- 32-celled ball after the 5th cleavage, like a mulberry; **morulation**
- textbook definition: **solid**, no blastocoel
- frog: a cavity already starts at the 8-celled stage → morula is a short stage at the start of blastulation

Blastulation

- from 8 cells: **segmentation cavity** among the cells, nearer the animal pole
- micromeres divide fast; megameres slowly (yolk in the way)
- cavity grows → **blastocoel**: fluid pumped in by the cells lining it (water, salts, some proteins and sugars)
- full blastula: **dome-shaped (hemispherical) blastocoel in the animal half**
- **roof**: 2–3 layers of small cells, black at the surface; **floor**: large yolky megameres
- blastocoel: keeps roof and floor cells apart; room to move in gastrulation; **does not feed** the embryo (food = yolk in the cells)
- **coeloblastula**; **monoblastic**



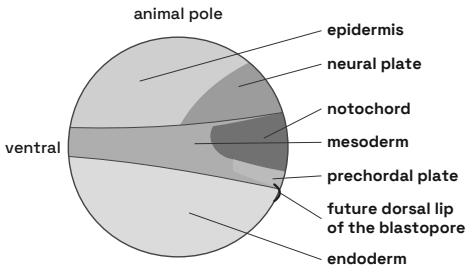
A frog's late blastula, cut vertically.

Micromeres vs megameres

- small vs large
- roof (animal half) vs floor (vegetal half)
- little yolk, pigmented vs full of yolk, unpigmented
- divide fast vs slowly
- become ectoderm vs endoderm

Fate map (presumptive areas): found by marking patches with harmless dyes (**Vogt**, 1920s, **vital staining**; first fate maps 1929), microsurgery

- **animal half**: presumptive **ectoderm**: epidermis; **neural plate** on the dorsal side
- **marginal zone** (belt round the middle): **mesoderm**; widest at the **gray crescent**: **notochord** (chorda-mesoderm)
- **vegetal half**: **endoderm**
- **prechordal plate** (just above the future dorsal lip): pharynx endoderm, head mesenchyme



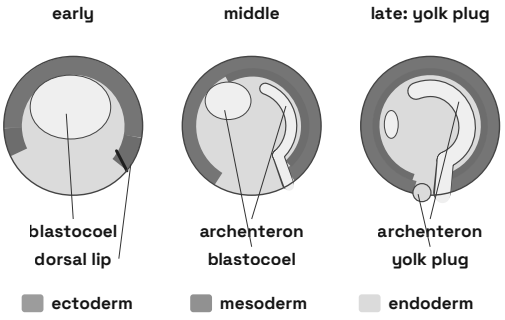
Fate map of a late blastula.

Gastrulation

- blastula → **gastrula**; sheets of cells spread, fold in, slide (**morphogenetic movements**)
- outside cells (future gut, muscles) move in; those left outside → skin, nervous system

Events

1. **Epiboly**: micromeres divide fast, spread down over the megameres like a cap; cover all except the **yolk plug**
2. **Emboly** (invagination): dorsal side, a little **below the gray crescent**: cells sink in → **crescent-shaped groove = blastopore** → deepens into the **archenteron**
   - lips: **dorsal**, **lateral**, **ventral**
   - archenteron grows → **blastocoel squeezed, disappears**
   - archenteron floor and sides **endoderm**; roof **chorda-mesoderm**
3. **Lips close in**: groove → ring → narrows from all sides; megameres show through: **yolk plug stage**
4. **Involution**: marginal-zone cells roll in over the dorsal lip (→ chorda-mesoderm, archenteron roof), then lateral and ventral lips (→ rest of the mesoderm); spread forwards under the ectoderm
5. **Rotation**: center of gravity shifts → embryo turns **~90°** in its fertilization membrane; long axis parallel to the water; animal pole region in front (head), blastopore at the back



Gastrulation, cut down the middle, the future back on the right.

- blastopore → **anus**; mouth later: **deuterostomes**
- **organizer**: Hilde **Mangold** and Hans **Spemann** (1924): dorsal lip grafted into another gastrula's ventral side → **second body axis** (neural tube, notochord, somites) made mostly of host cells → the dorsal lip **tells** cells what to become; Spemann's Nobel 1935

Germ layers

- **ectoderm**: outer, from micromeres
- **endoderm**: inner, round the archenteron, from megameres
- **mesoderm**: between, from chorda-mesoderm and cells rolled in over the lips
- many books: gastrula **diploblastic** (ectoderm + **meso-endoderm**) → mesoderm separates → **triploblastic**

Ectoderm	Mesoderm	Endoderm
epidermis, skin glands	notochord	lining (mucosa) of the gut
conjunctiva epithelium	pericardium, peritoneum linings	gastric, intestinal glands
olfactory, auditory organs	muscles, voluntary and involuntary	liver, pancreas, bile and pancreatic ducts
lining of mouth, cloaca's opening	cartilage, bone	pharynx, Eustachian tube, tonsils, thymus, thyroid
lens, cornea, retina	all connective tissue	larynx, trachea, lungs (linings)
brain, spinal cord	blood, vessels, heart	urinary bladder lining
ganglion cells, nerves	dermis	
neuroglia, myelin sheath, neurilemma	kidneys, ducts, gonads	
infundibulum, pineal, pituitary	spleen	
enamel of teeth	sclera, choroid	

- myelin sheath, neurilemma, neuroglia: **ectoderm** (neural tube, neural crest's Schwann cells), not mesoderm as some books list

Importance of gastrulation

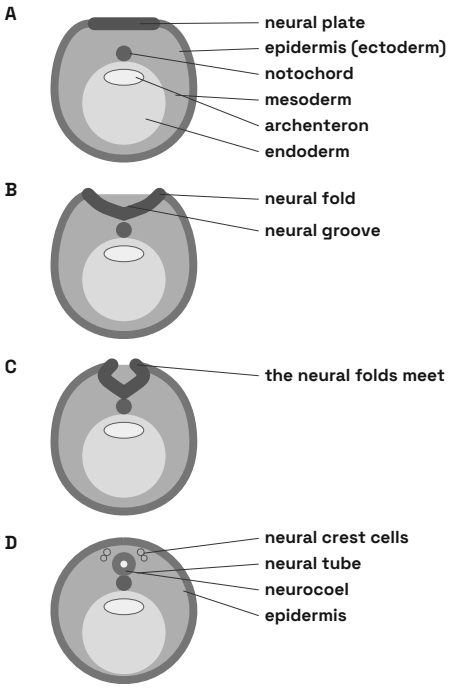
1. forms the **three germ layers**
2. sets the **body axes**: anterior–posterior, dorsal–ventral, left–right
3. moves cells (**morphogenesis**): tissues that work together lie together
4. layers in contact → **induction** (chorda-mesoderm induces the neural plate)
5. lays down the **notochord**; starts the **neural tube**
6. **fixes cell fate**

Organogenesis

1. Neurulation (neurogenesis): the neural tube

- embryo = **neurula**; from **dorsal ectoderm**

1. presumptive neural plate cells tall, thick → **neural plate** (induced by chorda-mesoderm)
2. **neural groove** along its middle
3. edges rise as **neural folds**
4. groove sinks deeper
5. folds meet and **fuse** in the middle
6. closed **neural tube**, separates, lies under the ectoderm (epidermis closes over it)
7. ends (**neuropores**) open briefly, then close
8. front, wider part → **brain**; rest → **spinal cord**
9. cavity **neurocoel** → brain ventricles, central canal



Neurulation, cut across, the back on top.

- **neural crest**: band between neural tube and epidermis → nerves and ganglia, Schwann cells, pigment cells, adrenal medulla, face bone and cartilage ("fourth germ layer", part of ectoderm)

2. Notogenesis: the notochord

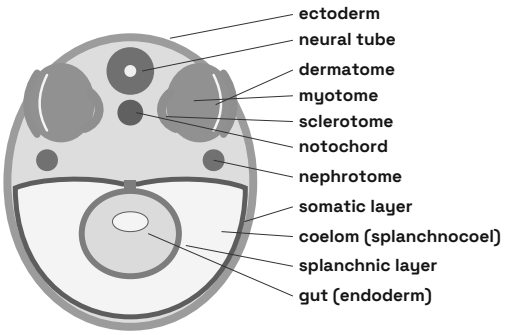
- flexible rod along the back of every chordate embryo; replaced by the **vertebral column** in vertebrates; **mesodermal**

1. **chorda cells** in the middle of the archenteron roof separate from the mesoderm beside them
2. form a solid cylindrical rod under the neural plate
3. cells large, **vacuolated** → firm yet bendable
4. two-layered **notochordal sheath**
5. first **axial support**; signals to neural tube and somites

3. Coelom formation

- frog: **schizocoel**, a **true coelom** (mesoderm on both sides)
- each side's mesoderm: **epimere** (dorsal, thick, beside notochord) · **mesomere** (intermediate, **nephrotome** → kidney tubules) · **hypomere** (lateral plate, thin)

1. hypomere splits: outer **somatic (parietal)** + inner **splanchnic (visceral)** layer
2. space between = **splanchnocoel** (start of coelom)
3. spreads downwards
4. both sides meet **below the gut** → **U-shaped** coelom; above the gut: **dorsal mesentery**
5. somatic layer + ectoderm = **somatopleure** (body wall)
6. splanchnic layer + endoderm = **splanchnopleure** (gut wall)
7. epimere → row of **somites**, each with a **myocoel**
8. somite → **dermatome** (outer, thin → **dermis**) · **myotome** (middle, thick → **muscles**) · **sclerotome** (inner, thin → **vertebrae**)



Coelom formation, cut across, the back on top.

From embryo to frog

- neurula → **tailbud embryo** (head, tail bud, gill swellings, suckers)
- days to two weeks (sooner in warm water) → **tadpole** hatches
- **indirect** development: gills, tail, plant-scraping mouth → **metamorphosis**: hind legs, then front legs; lungs; gut shortens; tail absorbed → froglet
- metamorphosis set off by **thyroxine** (Gudernatsch, 1912: tadpoles fed thyroid changed early)
- **Gurdon** (1962): tadpole gut-cell nucleus in an enucleated egg → frogs; Nobel 2012

COMMON MISTAKES

✗ The sperm enters the vegetal hemisphere
✓ it enters the **animal hemisphere**.

✗ The whole sperm enters the egg
✓ only **head and middle piece**; the tail stays out.

✗ The third cleavage is at the equator
✓ **above** the equator (yolk pushes spindles up).

✗ Megameres divide faster
✓ **micromeres** divide faster; yolk slows megameres.

✗ The blastocoel is full of yolk and feeds the embryo
✓ full of **fluid**; food is the yolk inside the cells.

✗ The blastocoel becomes the gut
✓ the **archenteron** does; the blastocoel disappears.

✗ The blastopore becomes the mouth
✓ in the frog it becomes the **anus**.

✗ The notochord is ectodermal
✓ **mesodermal** (chorda-mesoderm); the neural tube is ectodermal.

✗ The sclerotome forms muscles
✓ **myotome** muscles, **sclerotome** vertebrae, **dermatome** dermis.

✗ Myelin and neuroglia come from mesoderm
✓ **ectoderm**.