Biology 4361

Early Development and Axis Formation in Amphibians

July 9, 2009

The Amphibian Model

Common vertebrate (e.g. *Rana*, *Xenopus*)

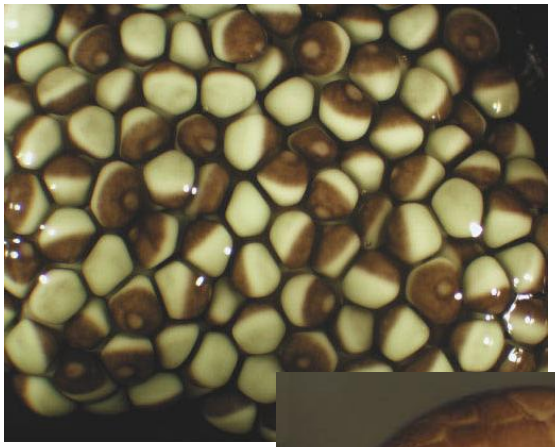
Manipulable, observable (i.e. large eggs and embryos)

1) How are the body axes established?
   - How do relatively homogeneous cells (i.e. zygotes) establish polarity (complexity from simplicity)?

2) How are the germ layers determined?
   - How do zygotes send their cellular progeny (i.e. blastomeres) down pathways toward different fates?

3) How is development organized in a regulative system?

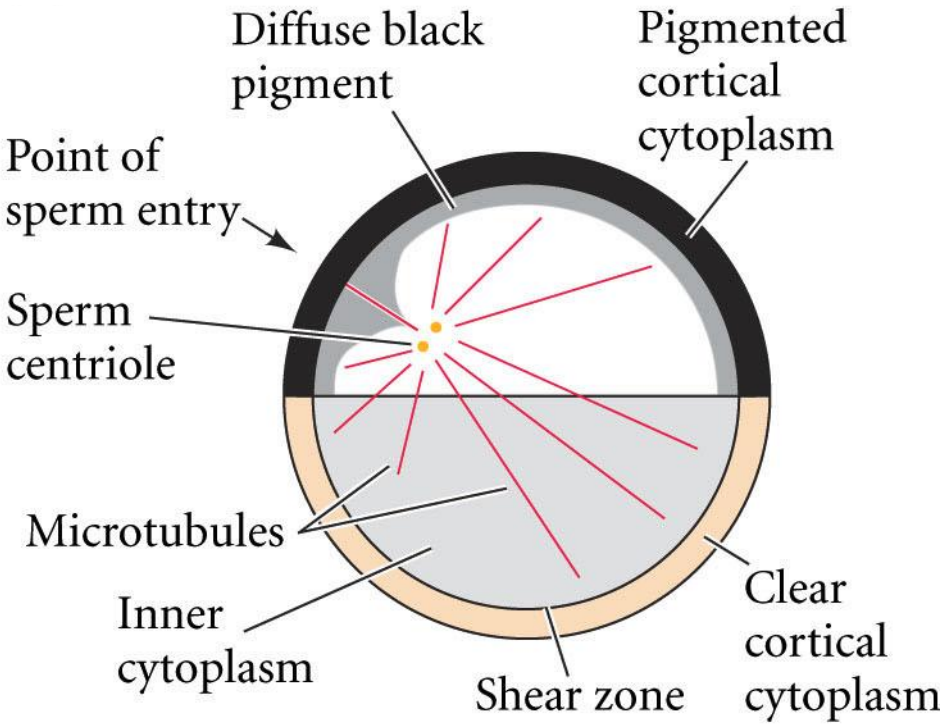
Amphibian Development - Overview

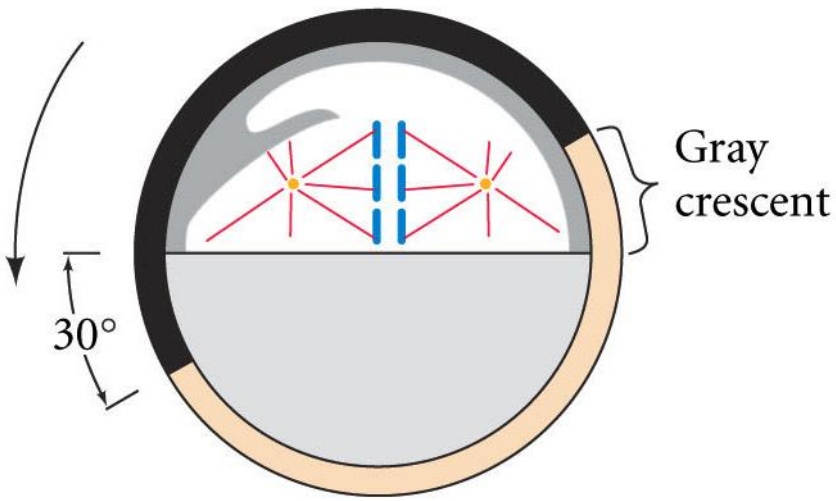


- Fertilization, cortical rotation
- Cleavage
- Gastrulation
- Axis and germ layer determination
  - The “Organizer”
  - Inductions:
    - Mesoderm
    - Dorsal/organizer
    - Ectoderm
  - Axes Summary
  - Left – Right Asymmetry

(Photo by Harland lab/UC Berkeley)

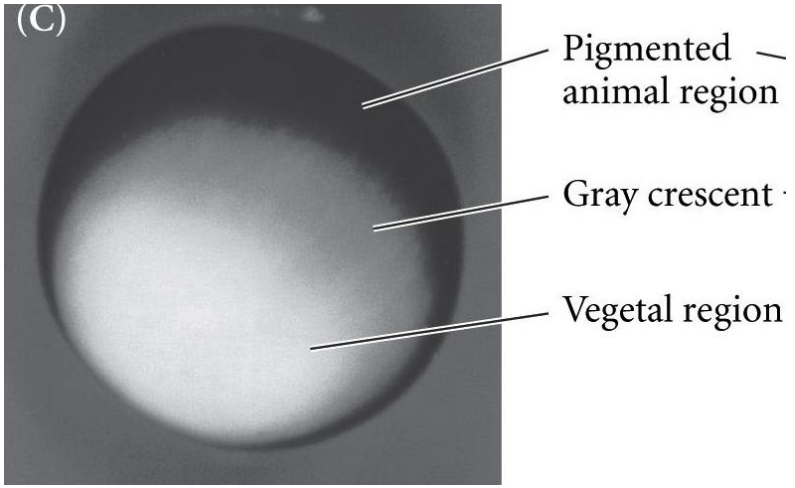
Cortical Rotation





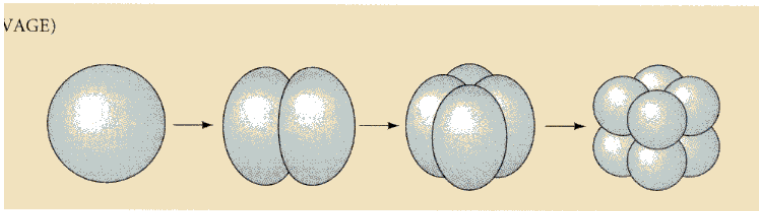
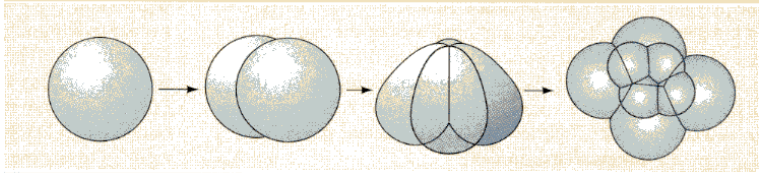
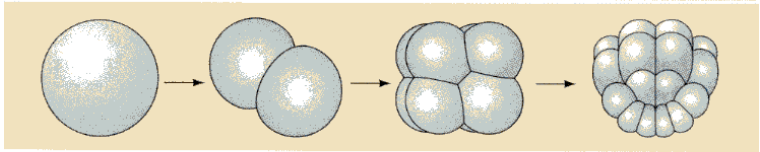
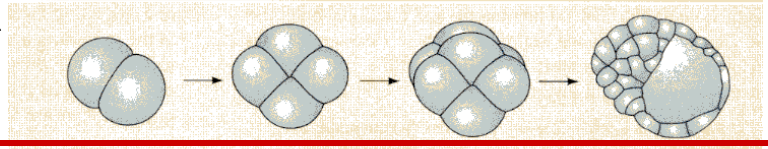
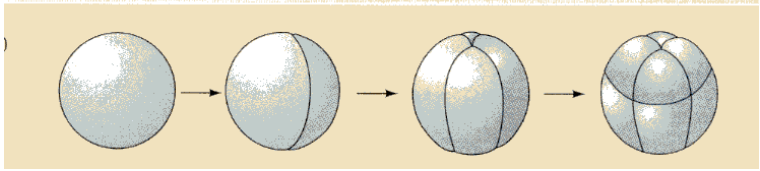
Fertilization – animal hemisphere

Microtubular network originates at fertilization point

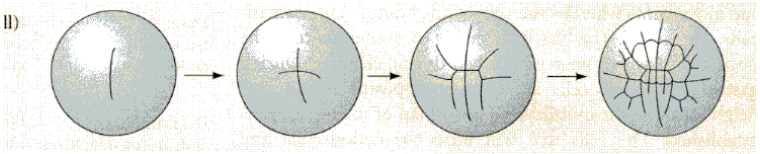
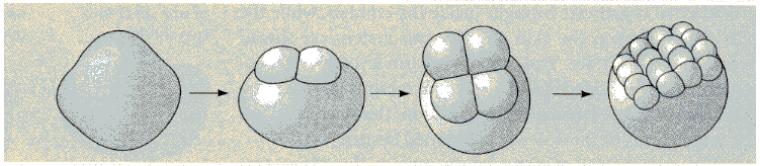
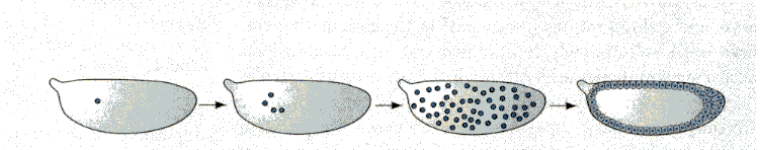


Holoblastic (complete cleavage)

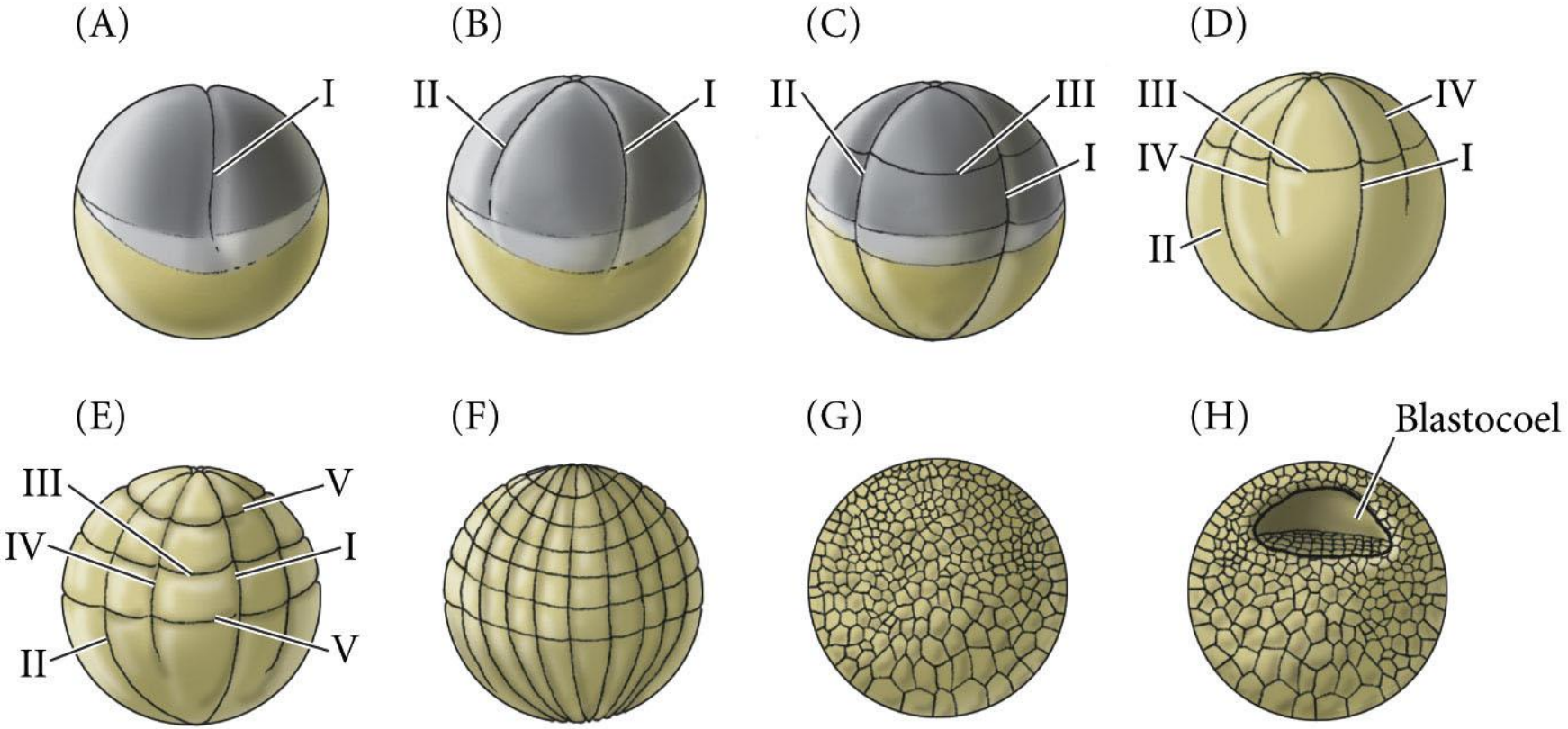
Yolk classification

		Species	Cleavage
Isolecithal		echinoderms, amphioxis	Radial
		annelids, molluscs, flatworms	Spiral
		tunicates	Bilateral
		mammals, nematode	Rotational
Mesolecithal		amphibians	Displaced radial

Meroblastic (incomplete cleavage)

Telolecithal		cephalopod molluscs	Bilateral
		fish, reptiles, birds	Discoidal
Centrolethical		most insects	Superficial

Unequal Radial Holoblastic Cleavage

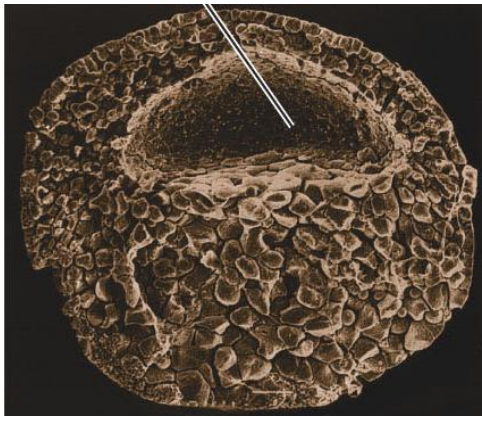


Cell cycles regulated by mitosis-promoting factor (MPF)

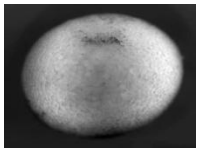
- no G phases

... until **mid-blastula transition**

- promoters demethylated (i.e. de-repressed)
- transcription factors formed in vegetal cytoplasm
- embryonic control of development

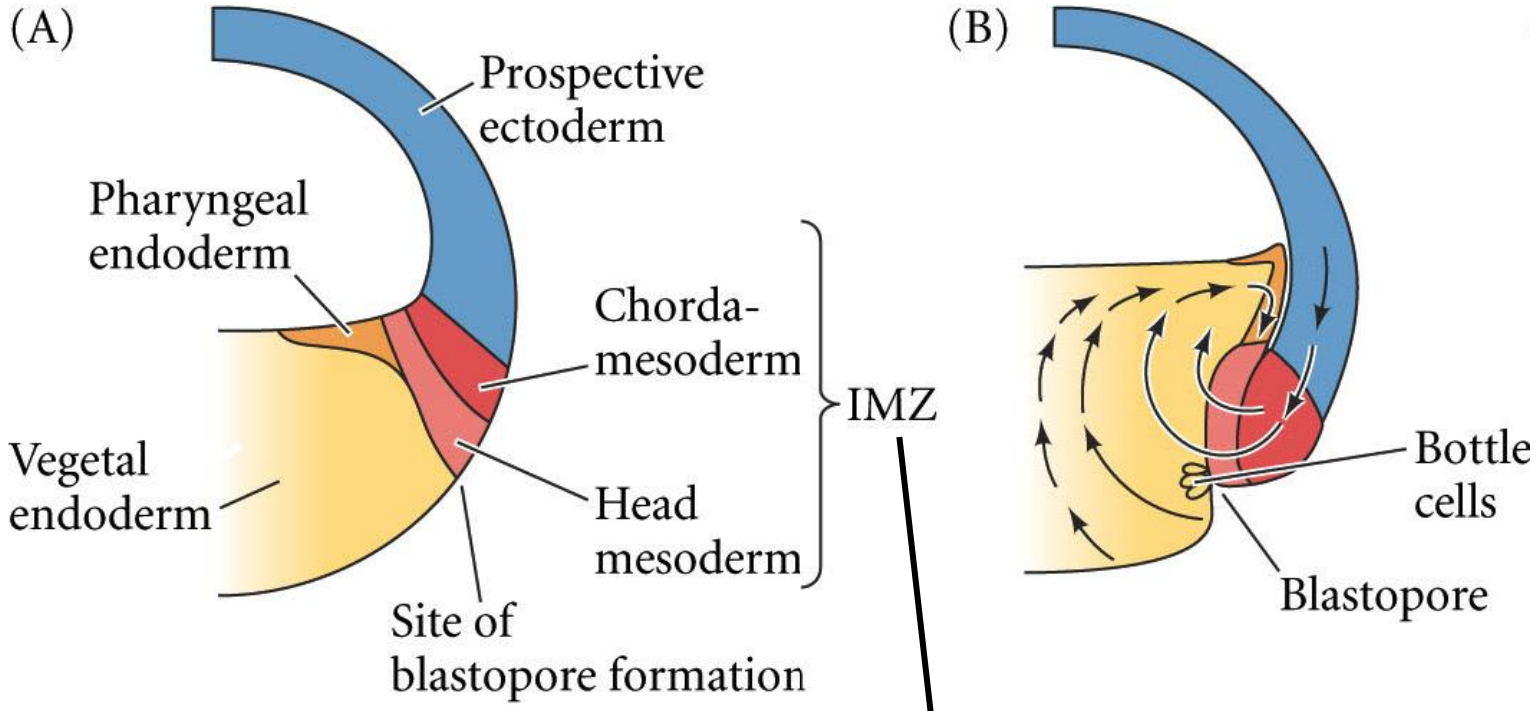


Mechanics of Gastrulation

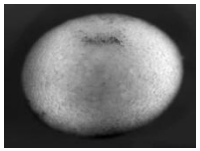


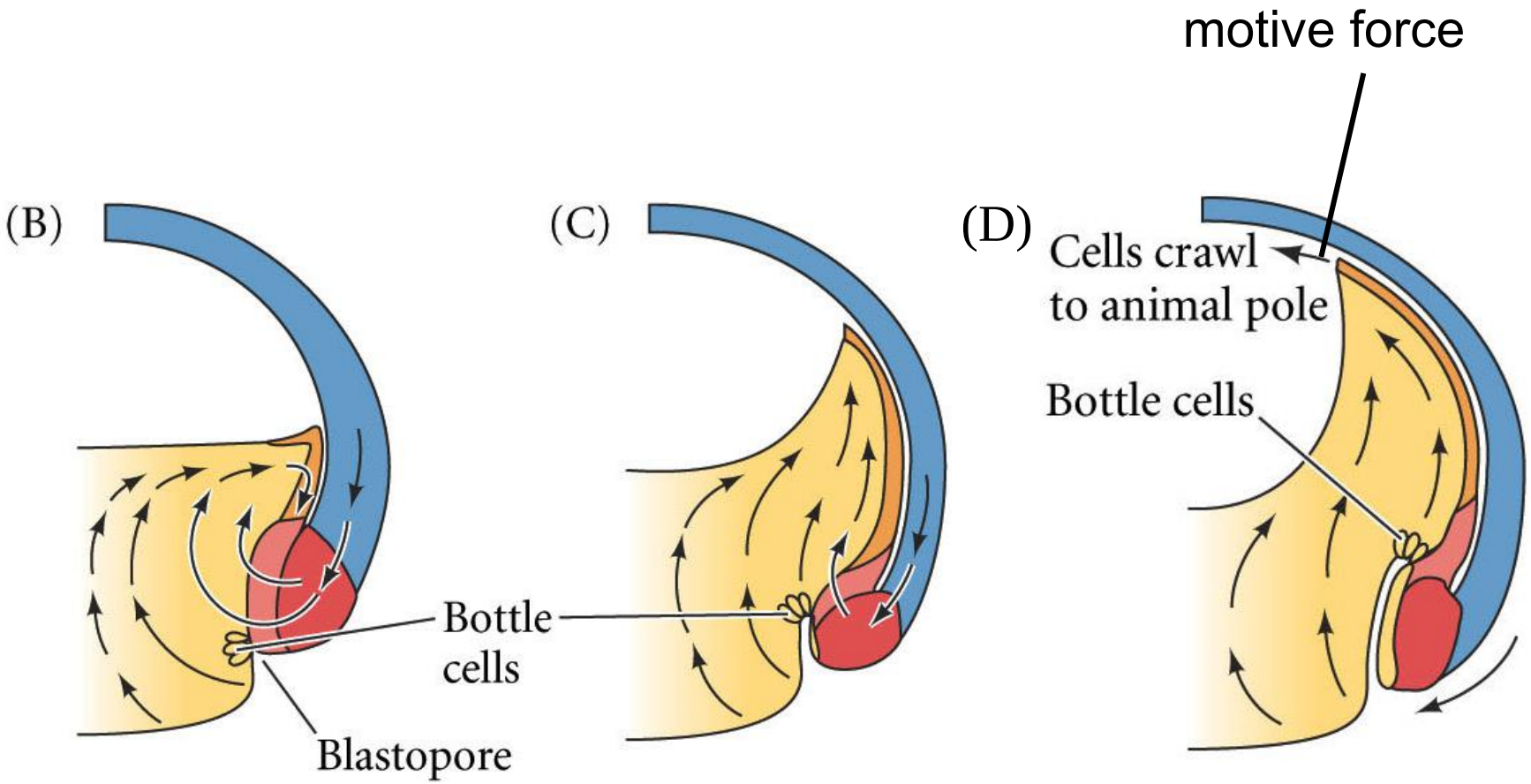
Formation of the dorsal lip

- vegetal rotation
- invagination of bottle cells
- involution of marginal zone cells

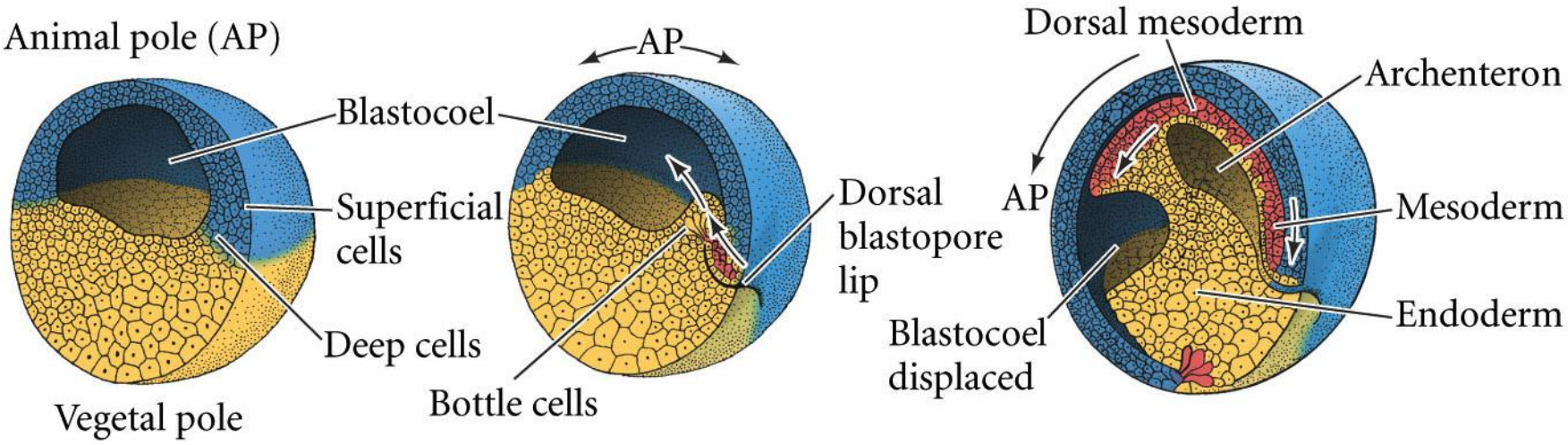


Mechanics of Gastrulation - 2

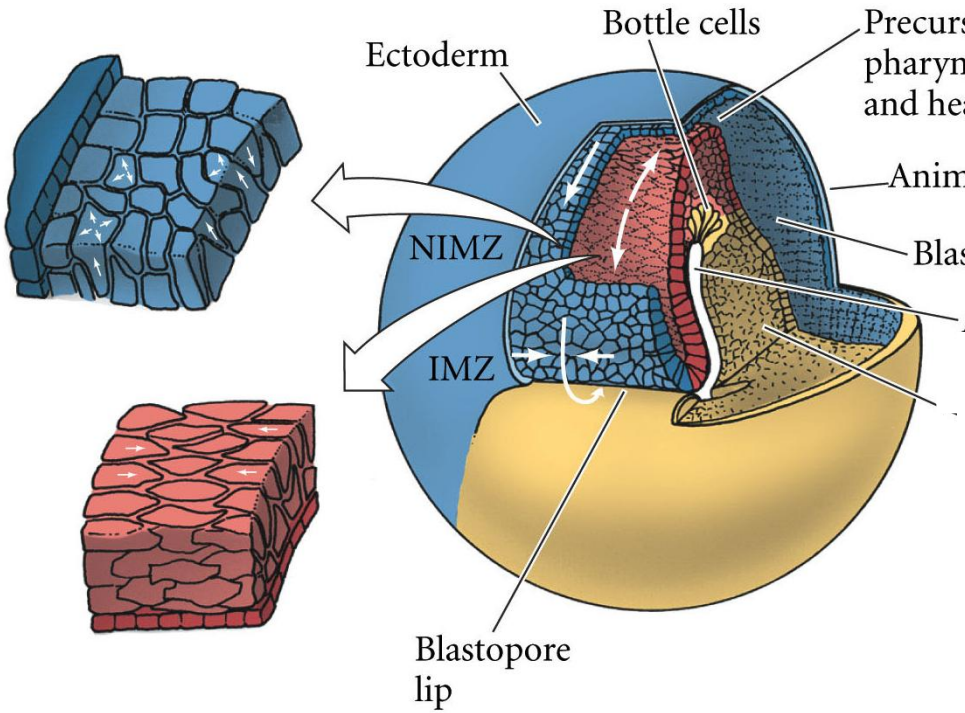


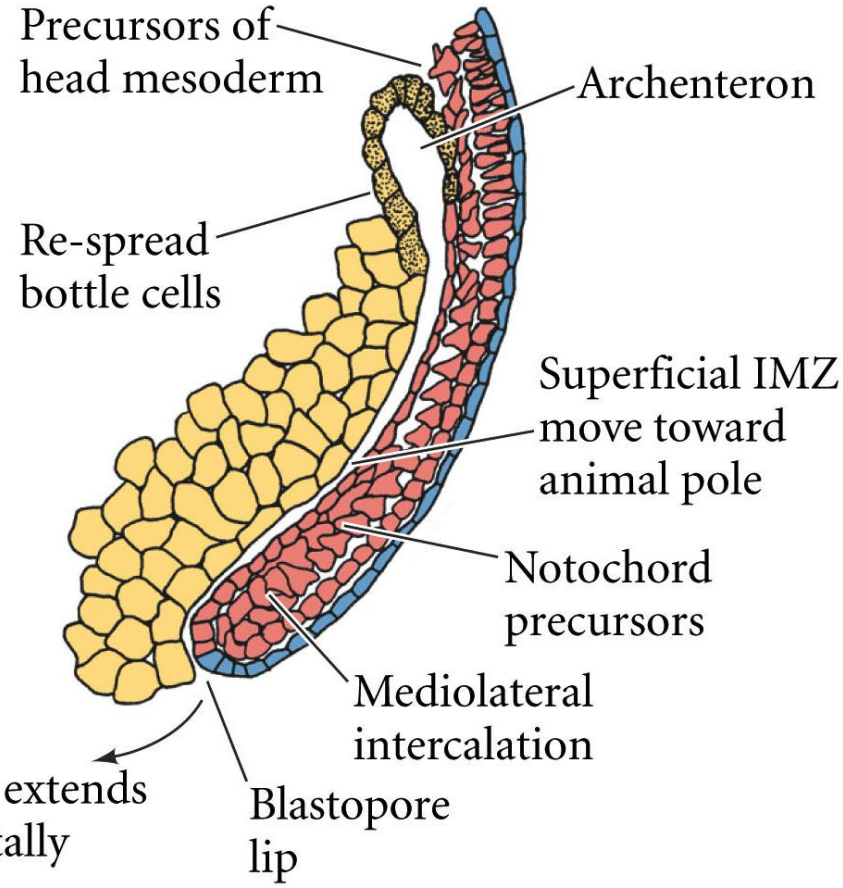


Xenopus Gastrulation



Cell Movements during Gastrulation

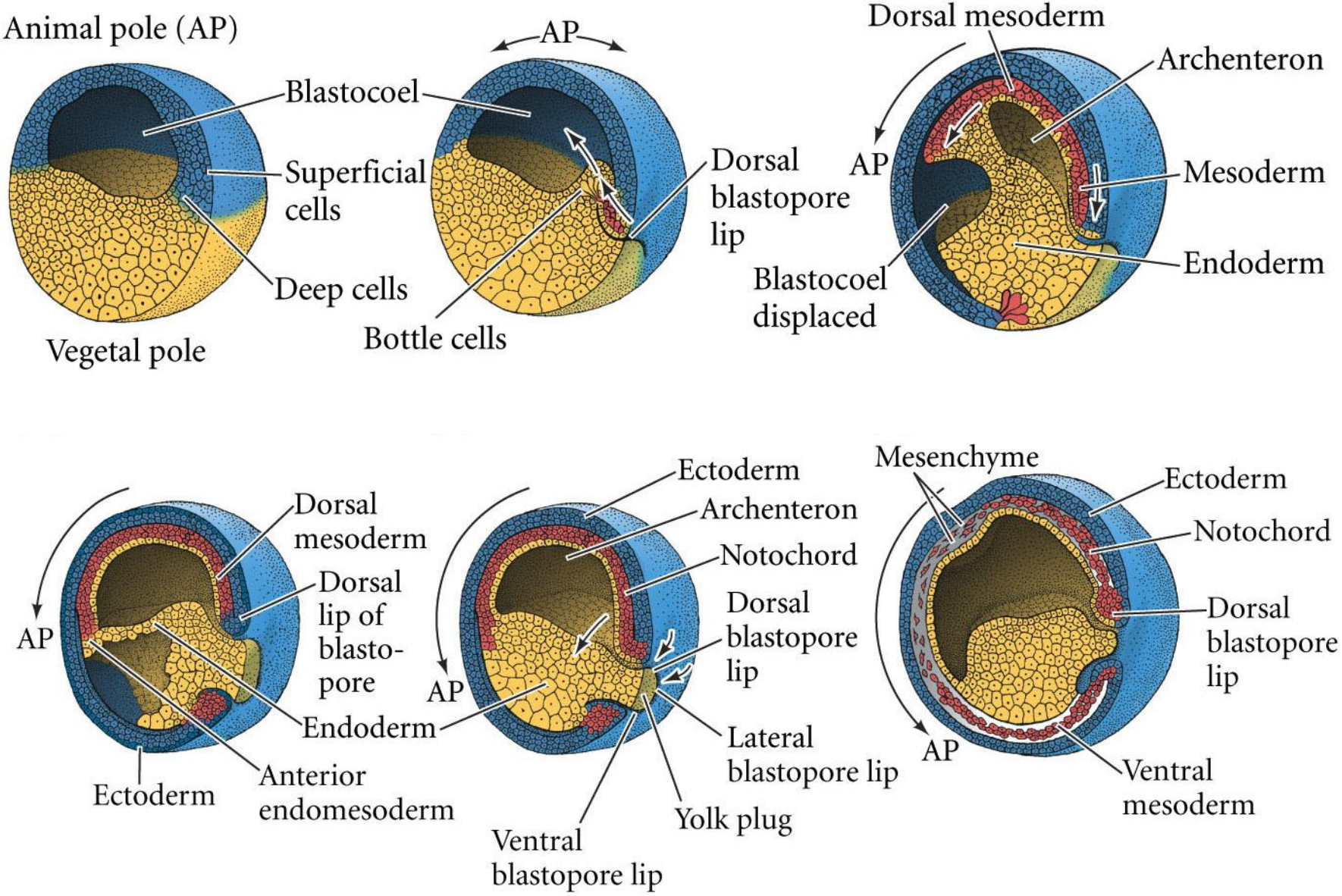




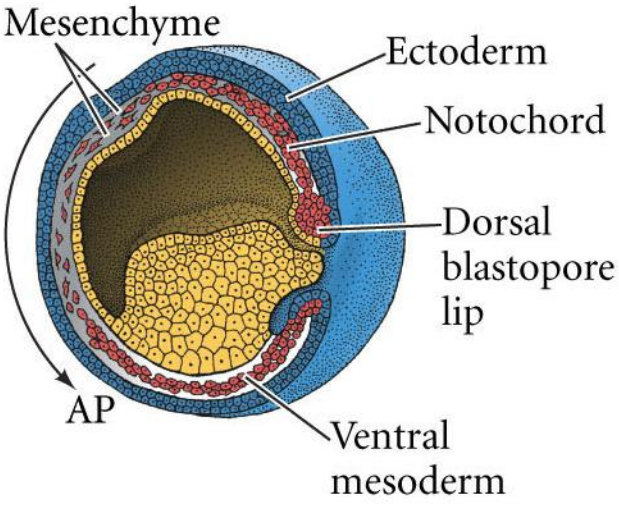
Movements

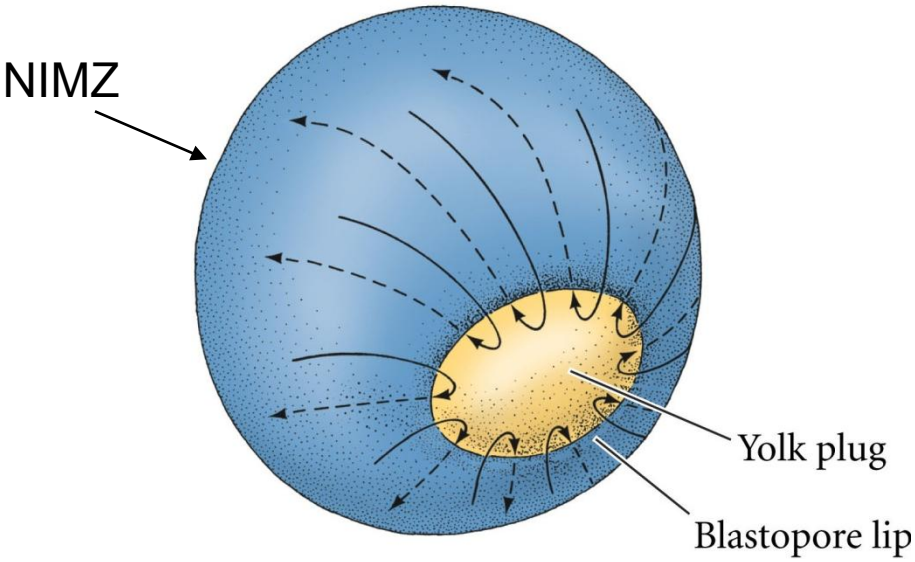
- invagination
- involution
- epiboly (animal cap)
- intercalation and convergent extension
- migration

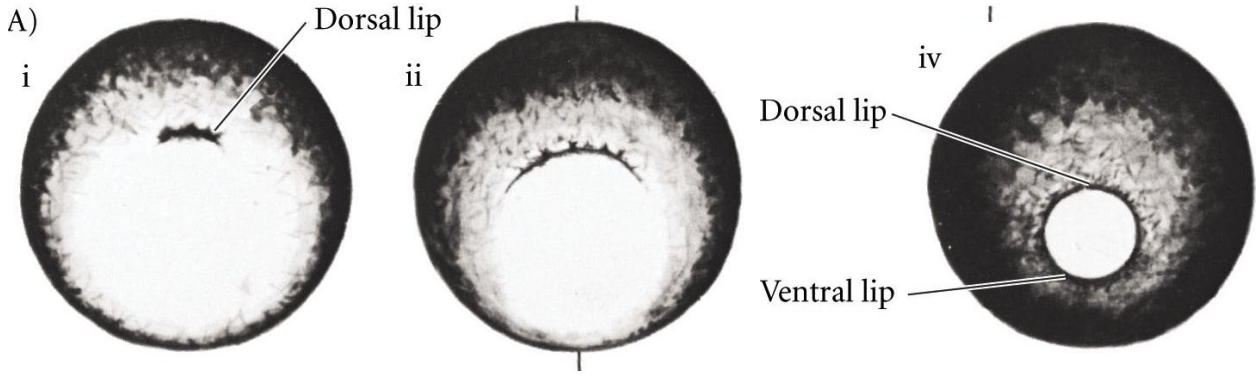
Xenopus Gastrulation

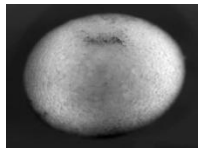


Xenopus Gastrulation - Blastopore









Amphibian Development - Overview

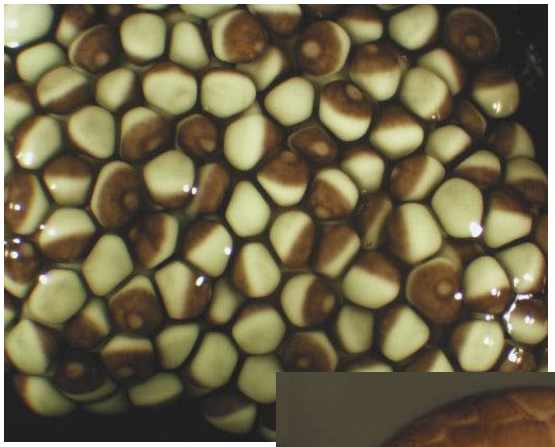
Fertilization, cortical rotation

Cleavage

Gastrulation

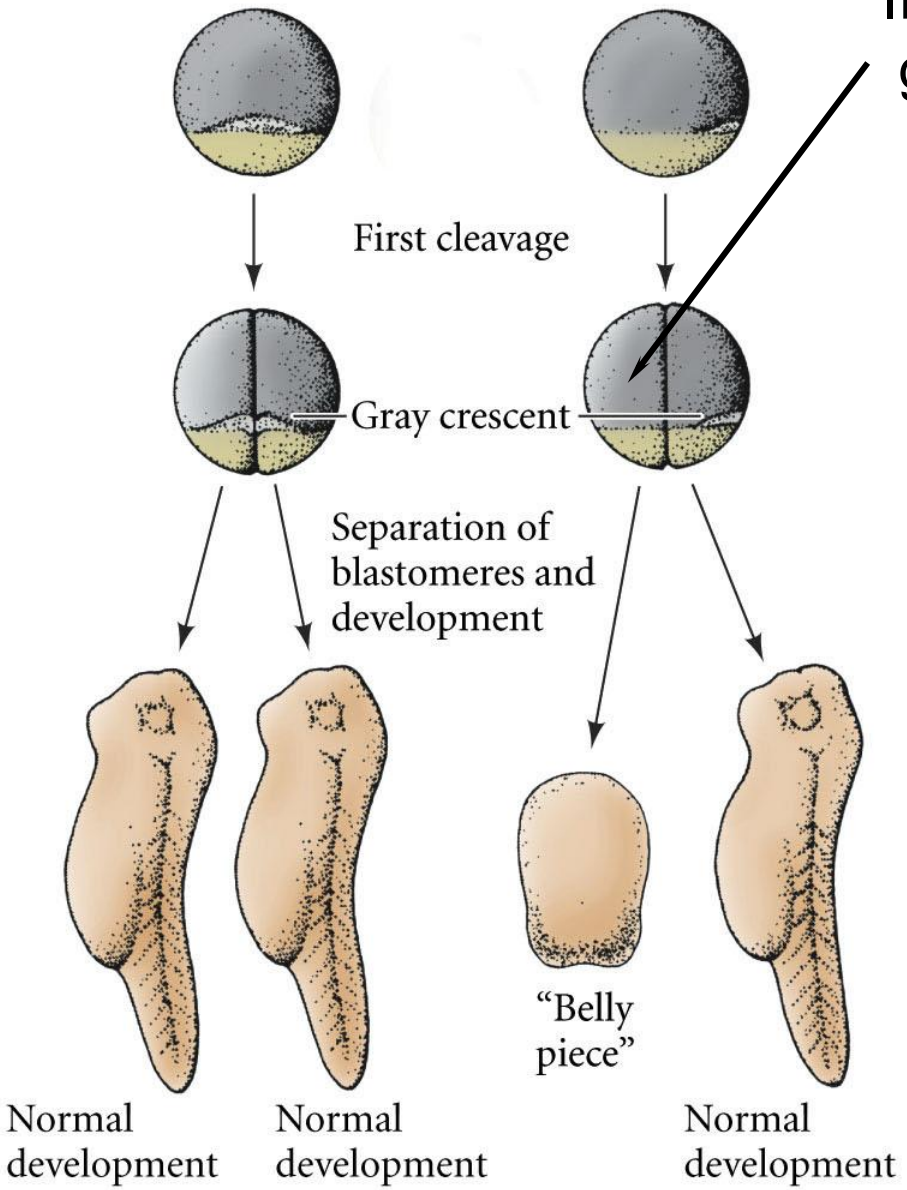
Axis and germ layer determination

- The “Organizer”
- Inductions:
  - Mesoderm
  - Dorsal/organizer
  - Ectoderm
- Axes Summary



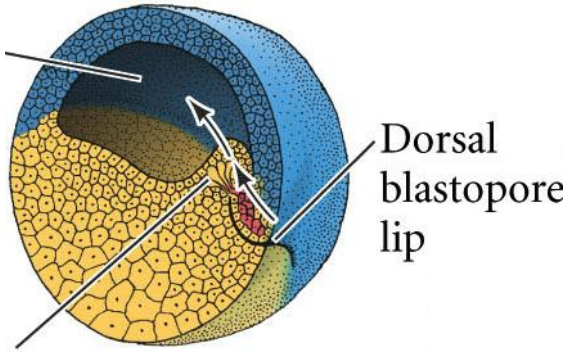
(Photo by Harland lab/UC Berkeley)

Determination of Amphibian Axes



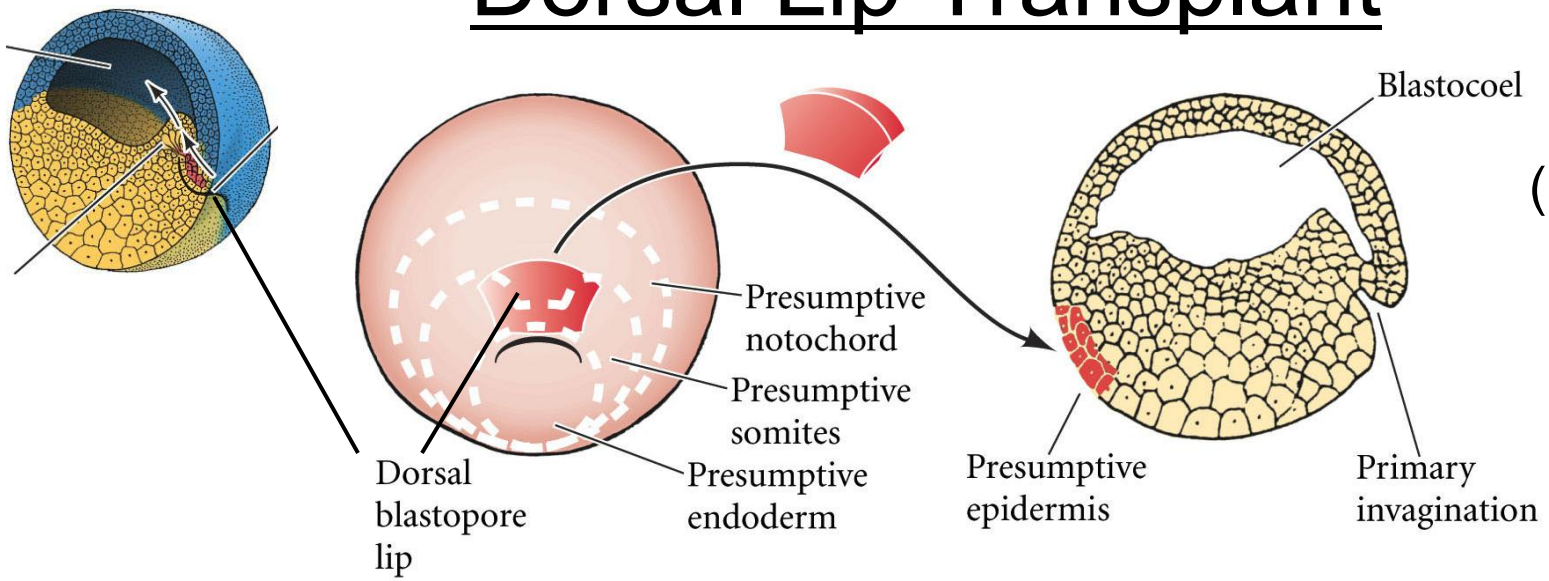
If one blastomere received no gray crescent material, resulted in
- "belly piece" – blood, mesenchyme, gut cells
- *no dorsal structures* (e.g. notochord, somites)

The gray crescent area is critical for proper development

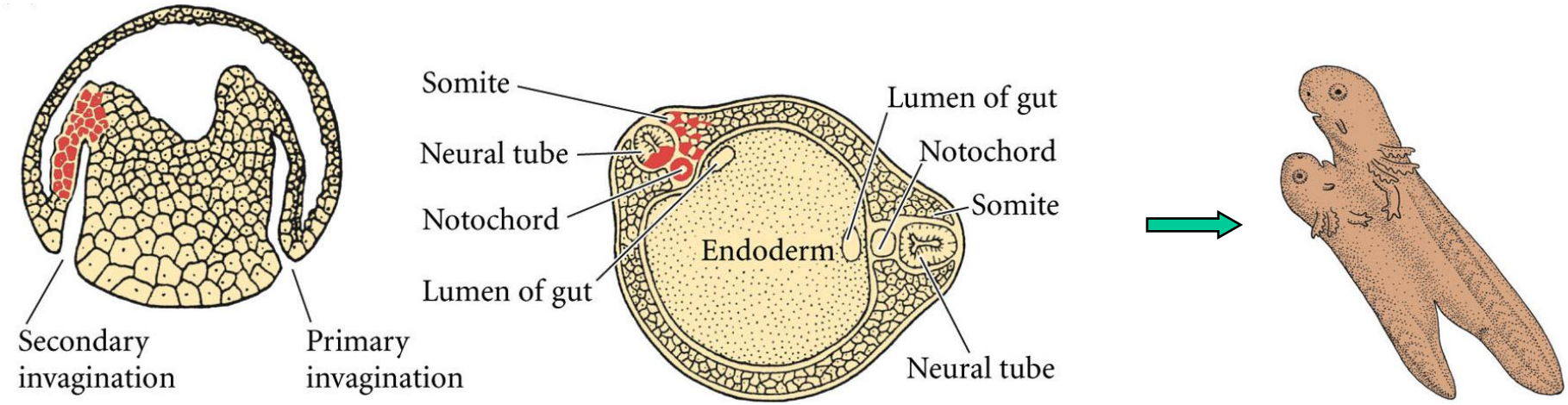


Gray crescent = future **dorsal lip of the blastopore**

Dorsal Lip Transplant



(Spemann, Mangold experiment)



Doral lip = "Organizer" - organizes secondary D-V axis
- induced ventral cells to change fates

Amphibian Development - Overview

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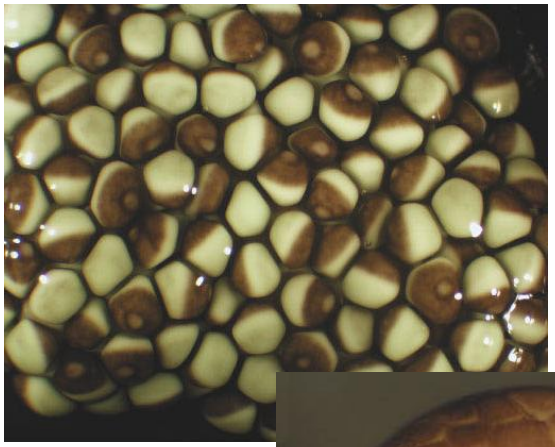

Axis and germ layer determination

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- Axes Summary
- Left – Right Asymmetry

(Photo by Harland lab/UC Berkeley)

The Organizer

Transplantation experiments established the *organizing properties* of the **dorsal blastopore lip**, which ...

1) Self-differentiates (all other tissues conditionally specified)
2) Establishes the dorsal-ventral axis
3) Specifies multiple tissues, including...
    - dorsal mesoderm, which includes...
        - head mesoderm (prechordal plate)
        - chordamesoderm (notochord)
4) Dorsalizes surrounding mesoderm into paraxial mesoderm
5) Induces the neural tube
6) Initiates the movements of gastrulation

How is the dorsal lip specified?

Dorsal Signal: β-Catenin

β-catenin

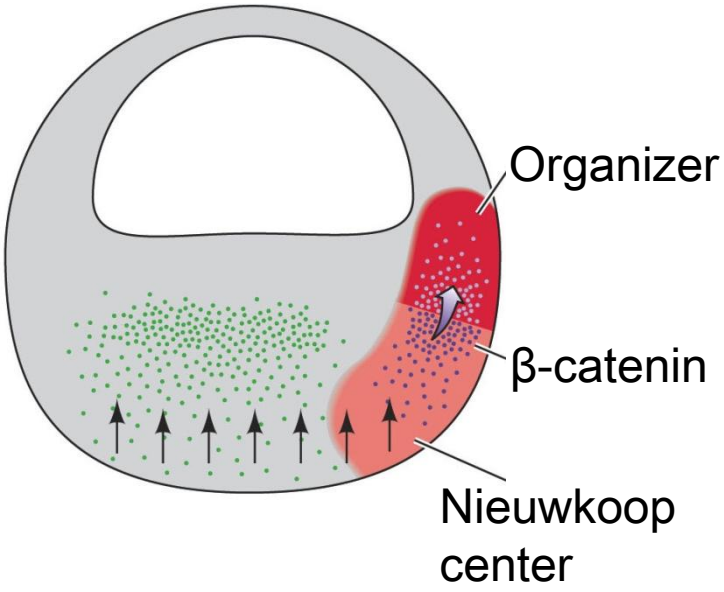
nuclear transcription factor (in Wnt pathway)

(in sea urchins, specifies micromeres, endomesoderm)

(in *Xenopus*, specifies dorsal structures; e.g. organizer)

β-catenin is initially distributed throughout the embryo,

- accumulates only in prospective dorsal cells.
- concentrated in the Nieuwkoop center and organizer



Dorsalization of β-catenin:

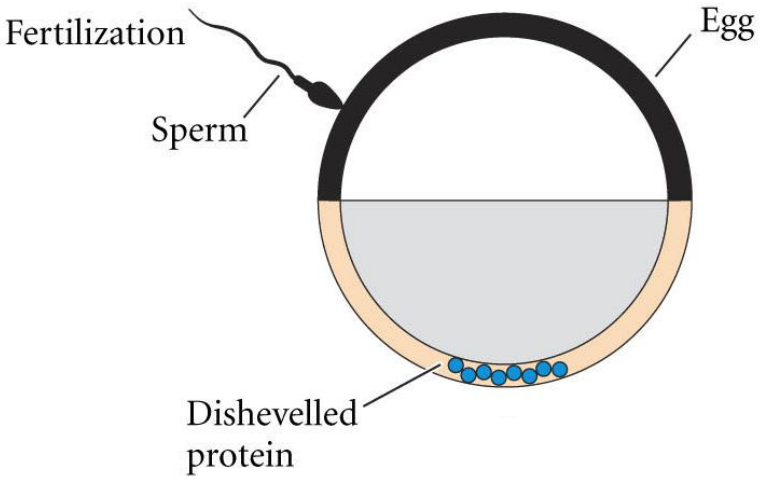
a) protect β-catenin in dorsal area,

b) degrade β-catenin everywhere else.

Mechanism - cortical rotation ...

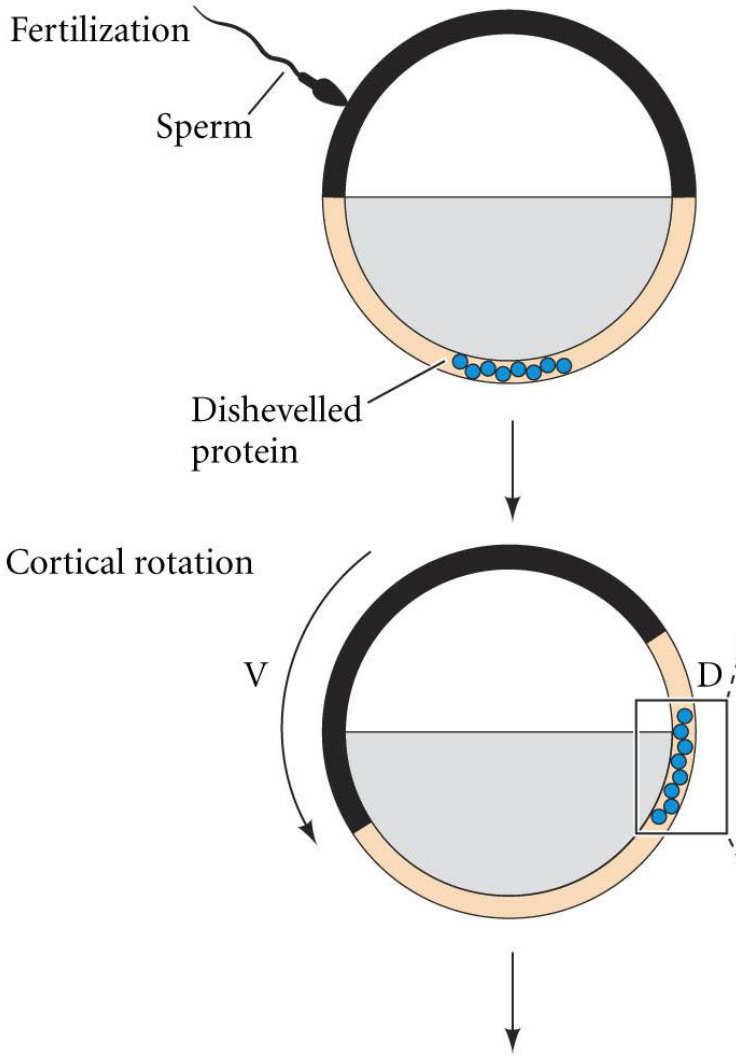
Disheveled/β-Catenin/Cortical Rotation

β-catenin induces cells to dorsal fates



- **β-catenin** is initially distributed throughout the oocyte
- **Glycogen synthase kinase 3** (**GSK3**) is also distributed throughout oocyte
- GSK3 marks β-catenin for degradation
- **Dishevelled** (**Dsh**) blocks GSK3 activity
- Dsh localizes in the cytoplasmic cortex at the vegetal pole

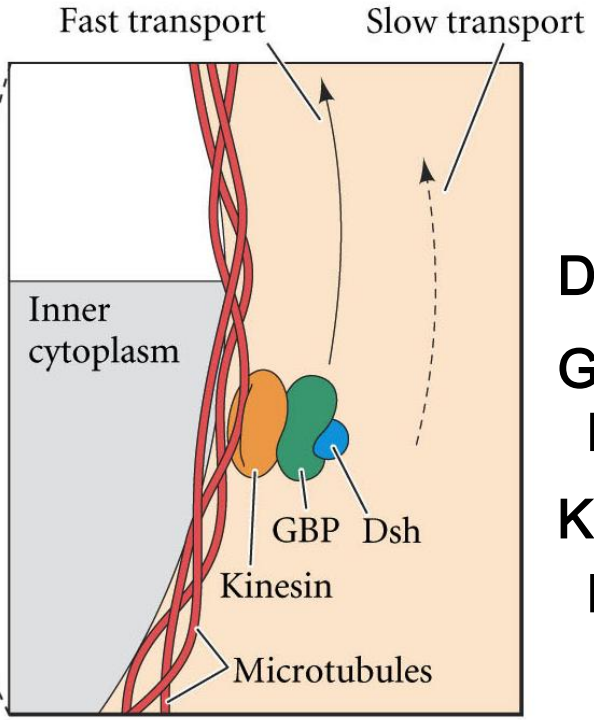
Disheveled/β-Catenin/Cortical Rotation



β-catenin, **GSK3** distributed throughout

Dishevelled - blocks GSK3-mediated β-catenin degradation

- anywhere Dsh exists, β-catenin survives

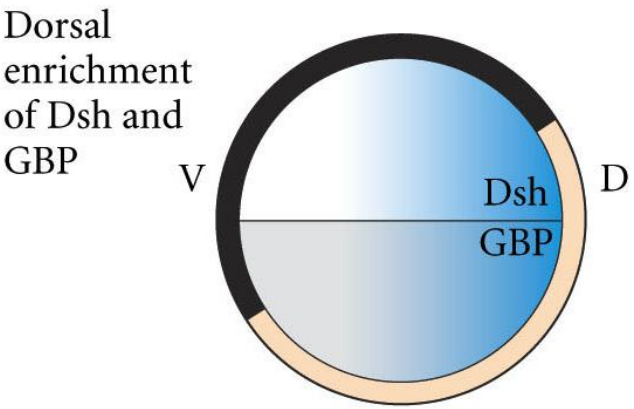


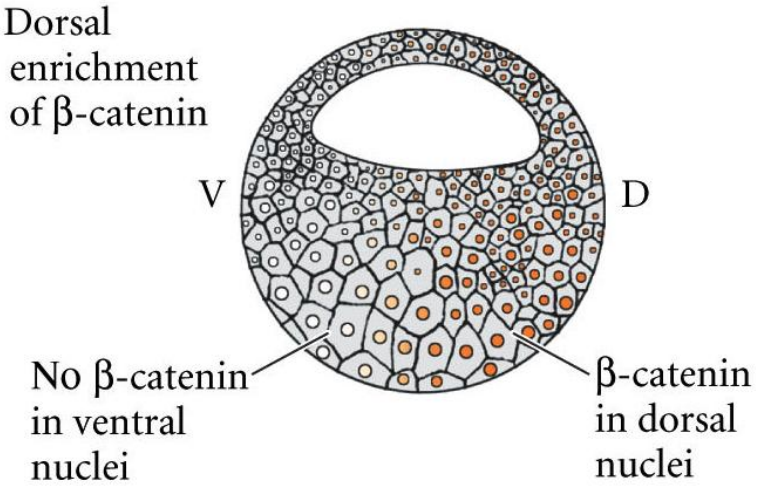
Dsh – Dishevelled

GBP – GSK3 binding protein

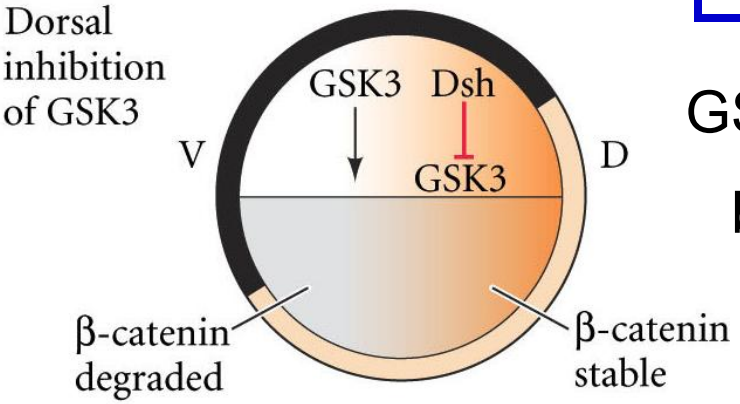
Kinesin - motor protein

Disheveled/GSK3/ β-Catenin





At the blastula stage, β-catenin is located exclusively in the future dorsal region

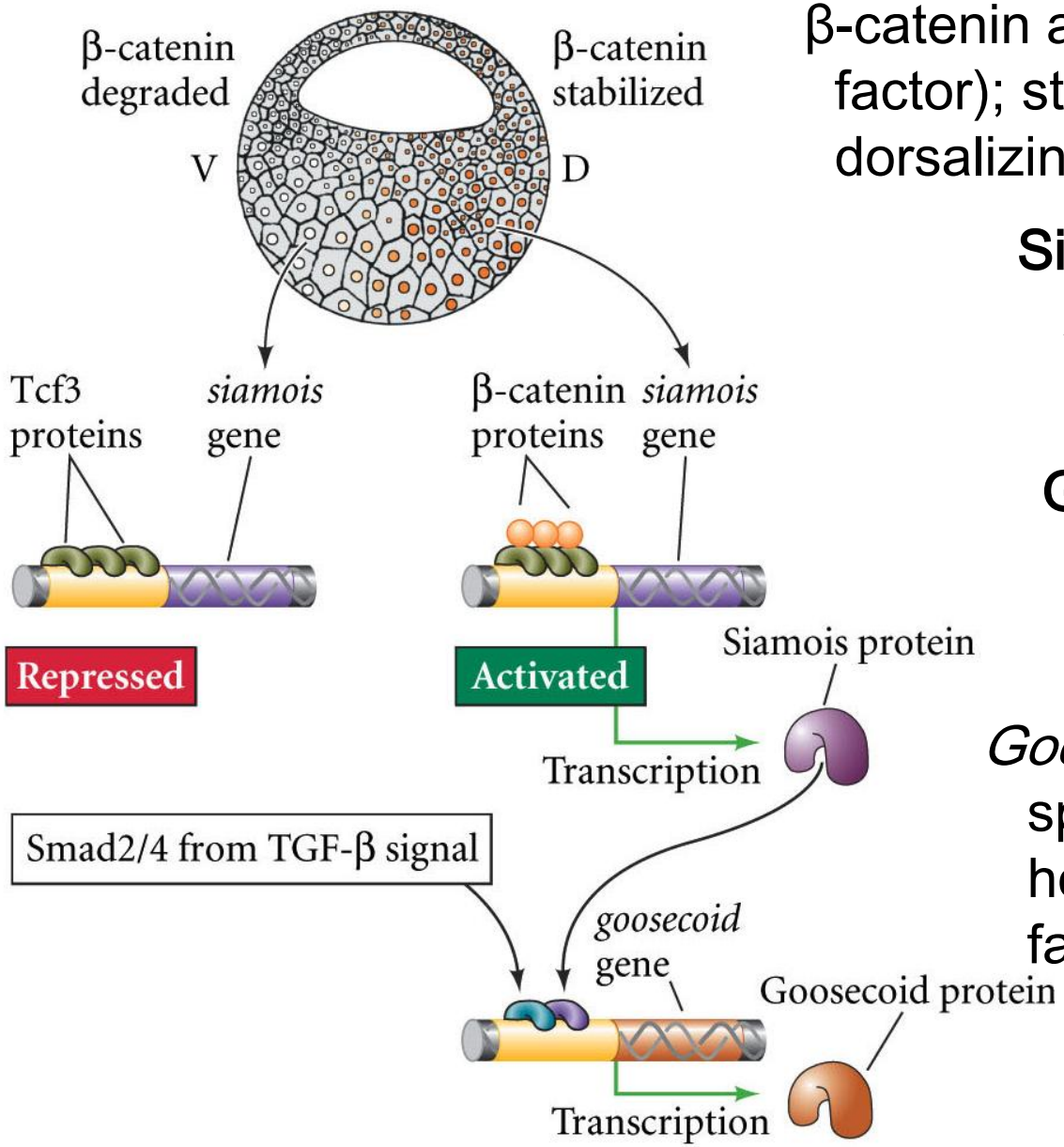


GSK3 mediates β-catenin destruction .....

but Disheveled and GBP block GSK3;

- resulting in β-catenin present *only* in the marginal area opposite the point of sperm entry (i.e. future dorsal lip)

Organizer Induction



β-catenin acts with Tcf3 (transcription factor); stimulates expression of dorsalizing genes:

Siamois – TF; activates *Xlim*, *goosecoid* (dorsal determinants)

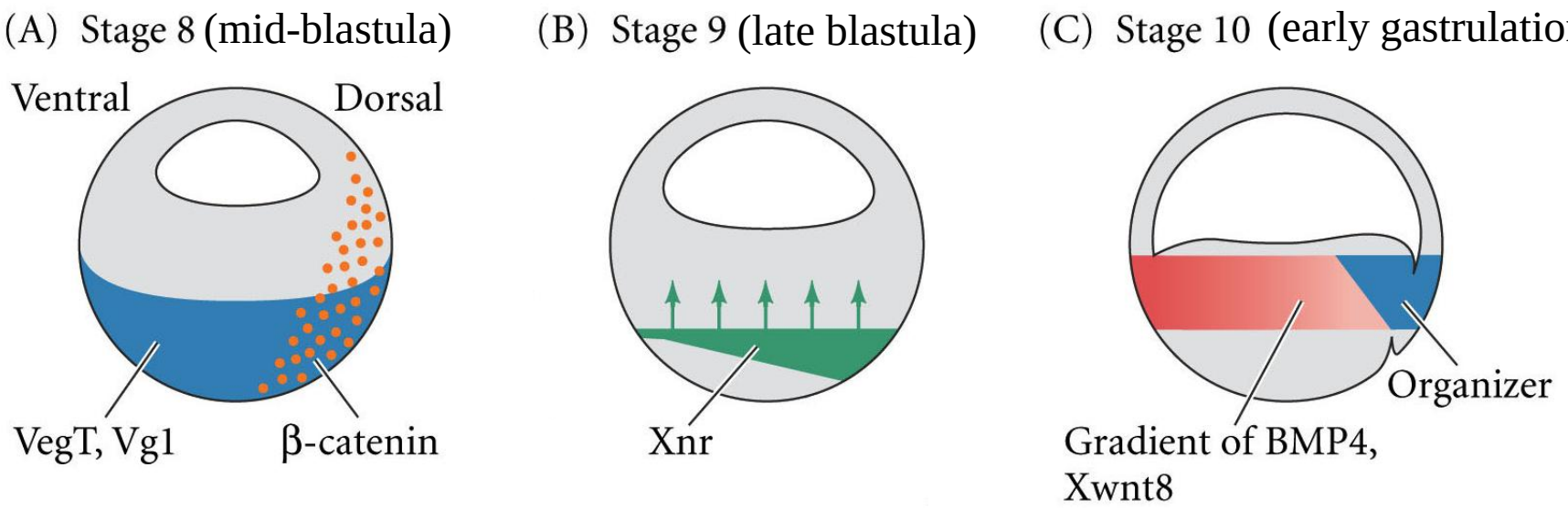
Goosecoid protein – TF responsible for organizer properties

Goosecoid also plays a part in specifying **dorsal mesoderm**; however, additional vegetal factors are needed: e.g.

Vegetal TGF-β signals

Mesoderm/Organizer Induction

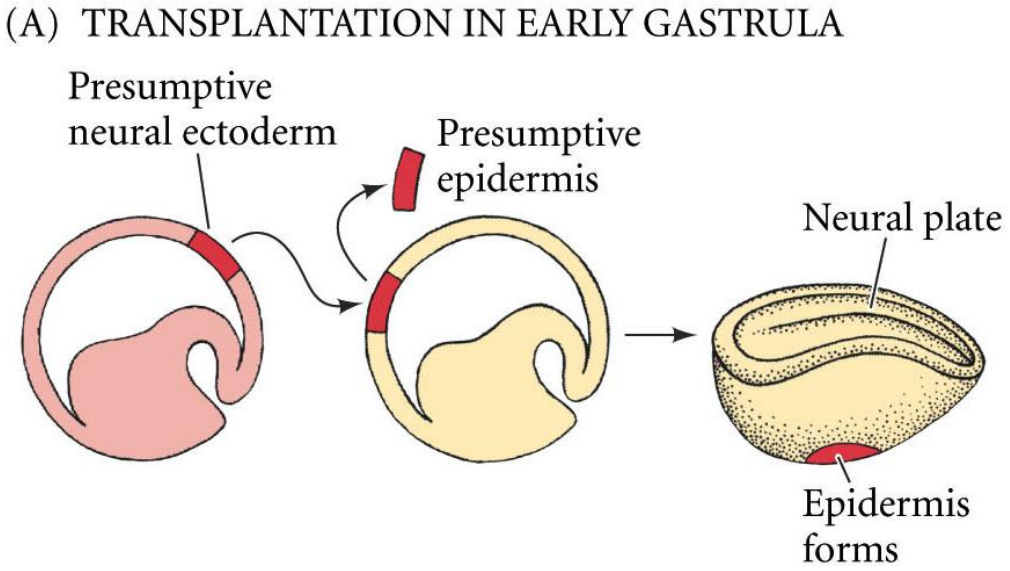
VegT, Vg1, Xnr(s) – *Xenopus* nodal related genes; TGF-β family



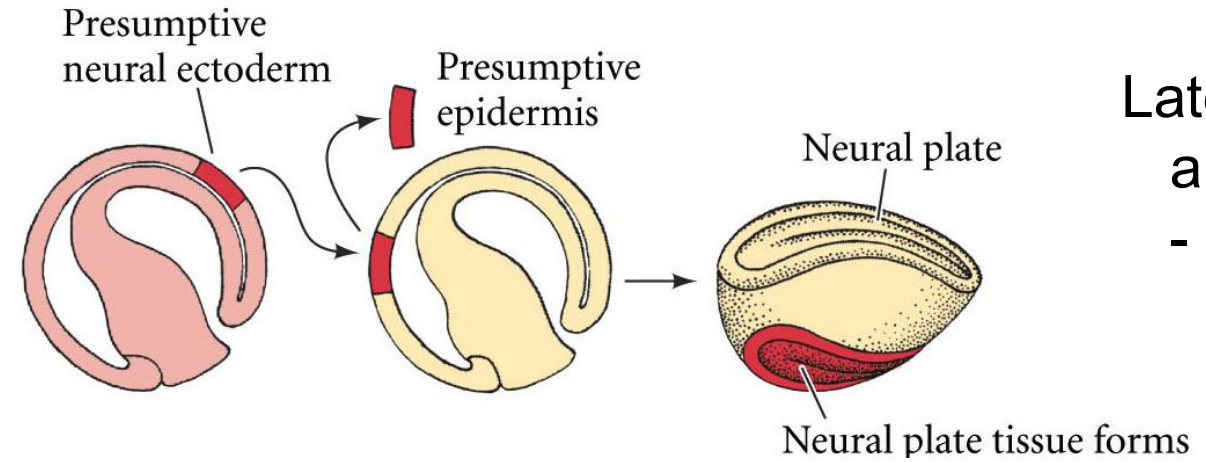
β-catenin → Nodal related high → Organizer, dorsal and lateral mesoderm
- somites, notochord

VegT, Vg1 → Nodal related low → Ventral & intermediate mesoderm
- mesenchyme, blood muscle, kidney

Determination of Ectoderm

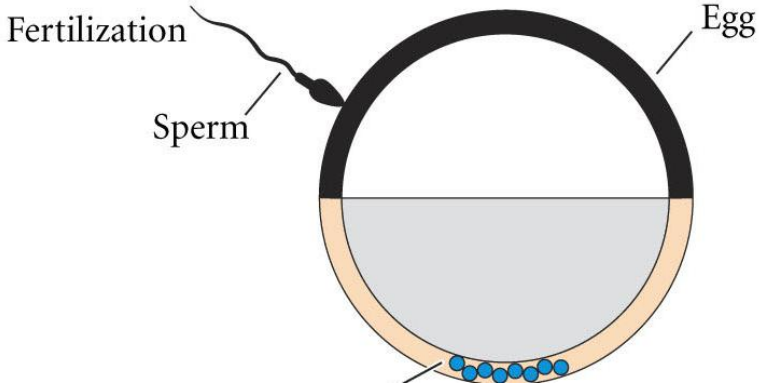


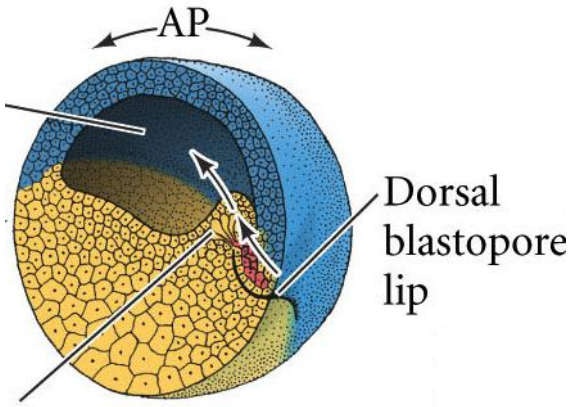
Early gastrulation stage cells are uncommitted
- exhibit regulative development

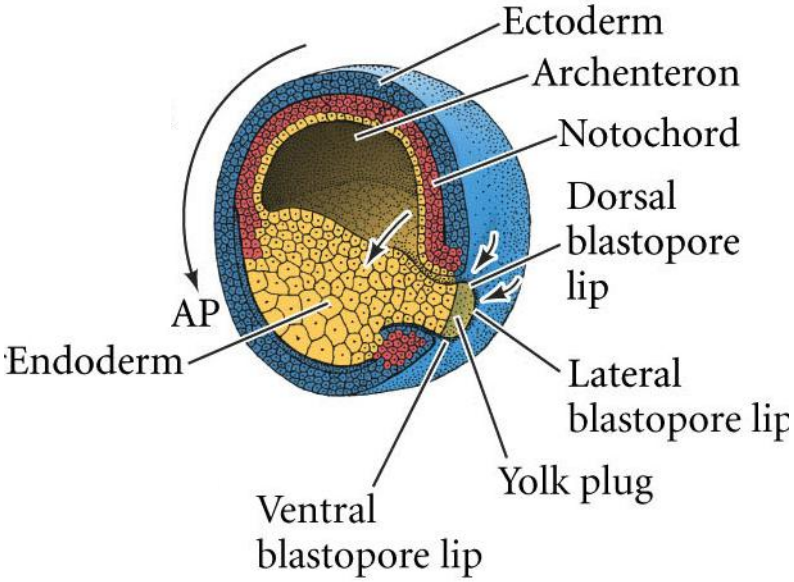


Later gastrula cells are determined
- exhibit autonomous development

Establishment of Axes - Summary







D-V axis – set up at fertilization

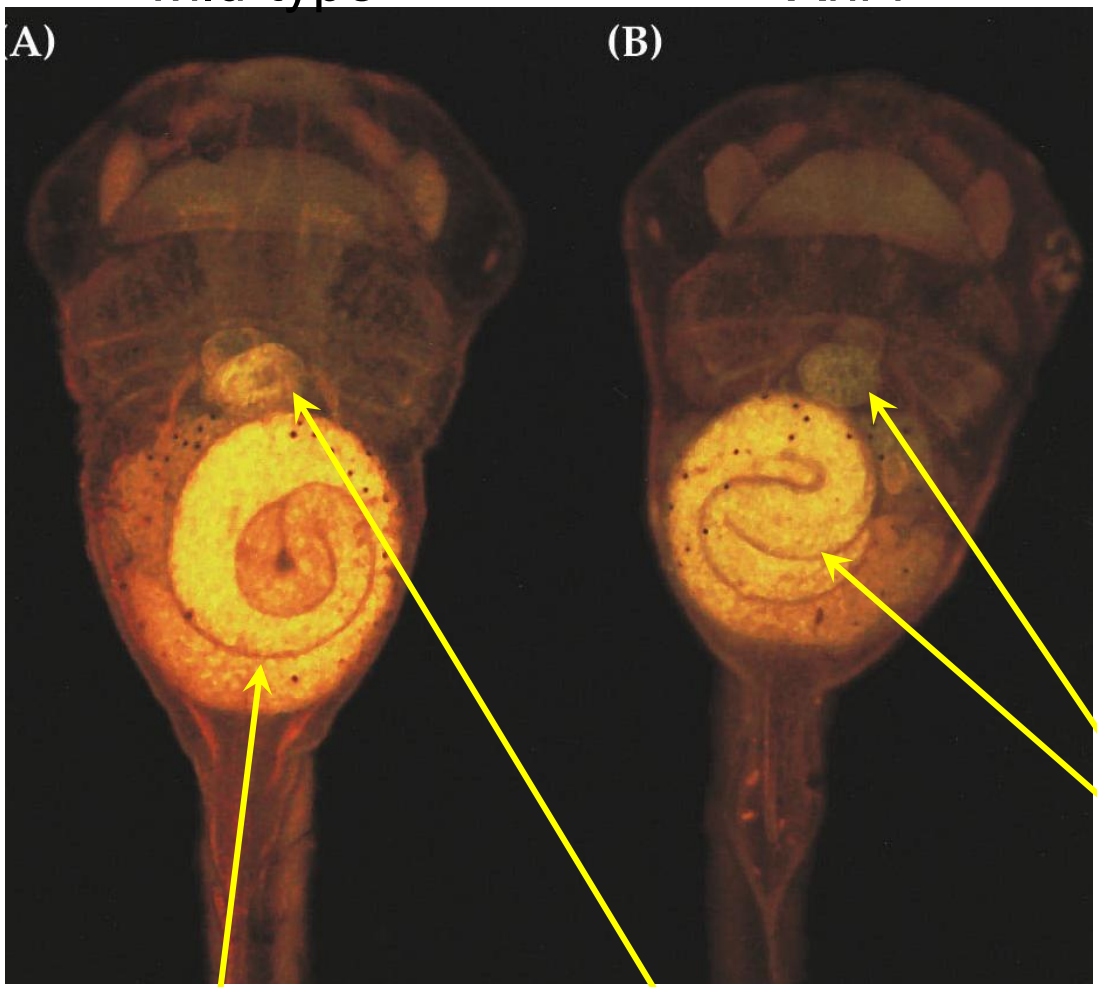
A-P axis – established by gastrulation movements across the dorsal lip of the blastopore

L-R axis – Nodal expression on Left, not Right

Left – Right Asymmetry

wild-type

Xnr1$^{-/-}$



heart loops to the left

gut coiling – counter-clockwise

Left–Right axis established by *Xenopus nodal-related* (Xnr1)

Nodal expression:
- common to all vertebrates
- expressed on the **left side!**

Xnr1 expression is limited to the left side in a process involving cortical rotation and Vg1

Block Xnr1 expression = random gut coiling, heart looping