Early development in zebrafish and xenopus

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To improve learning in class

- Structure of my teaching

  one phenomena (one concept)

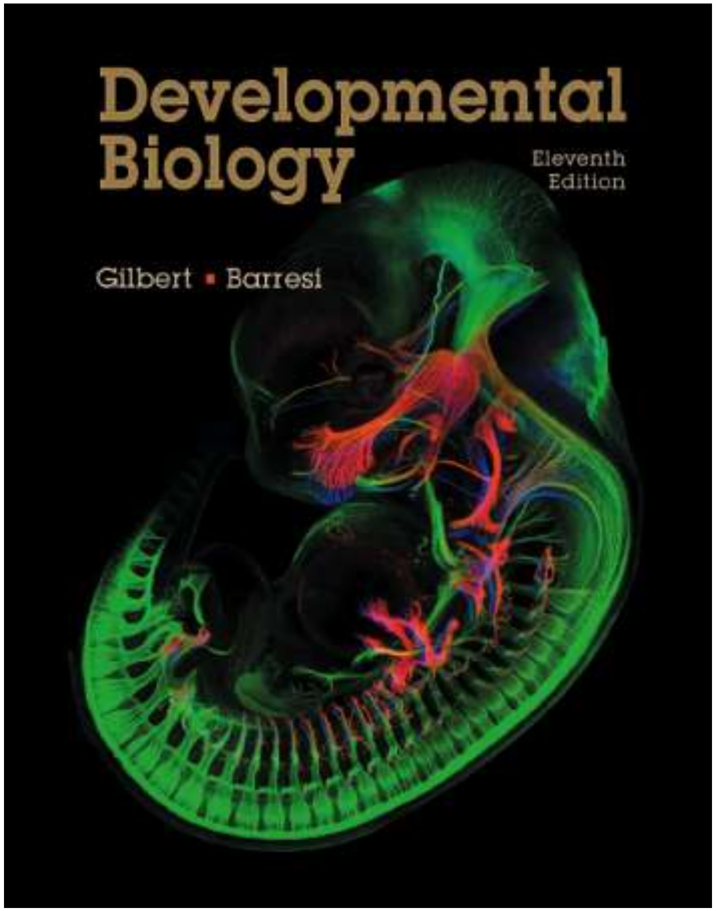
  how is this formed (process)? What's the mechanism?

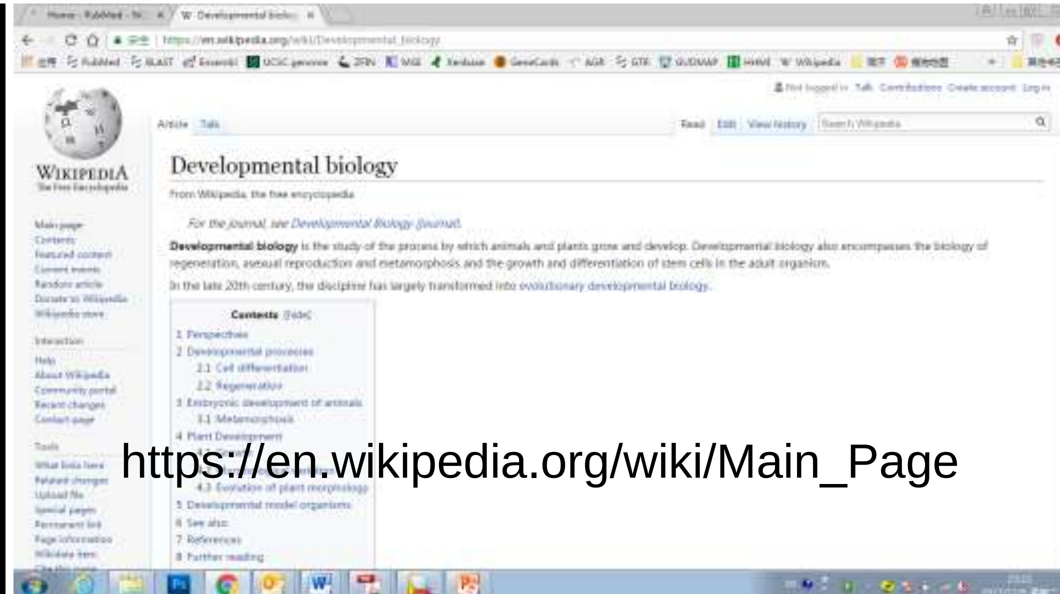
  what's the function/derivatives?
- To remember concepts

  find the connection among different concepts

  read more about one concept (BMP)

References



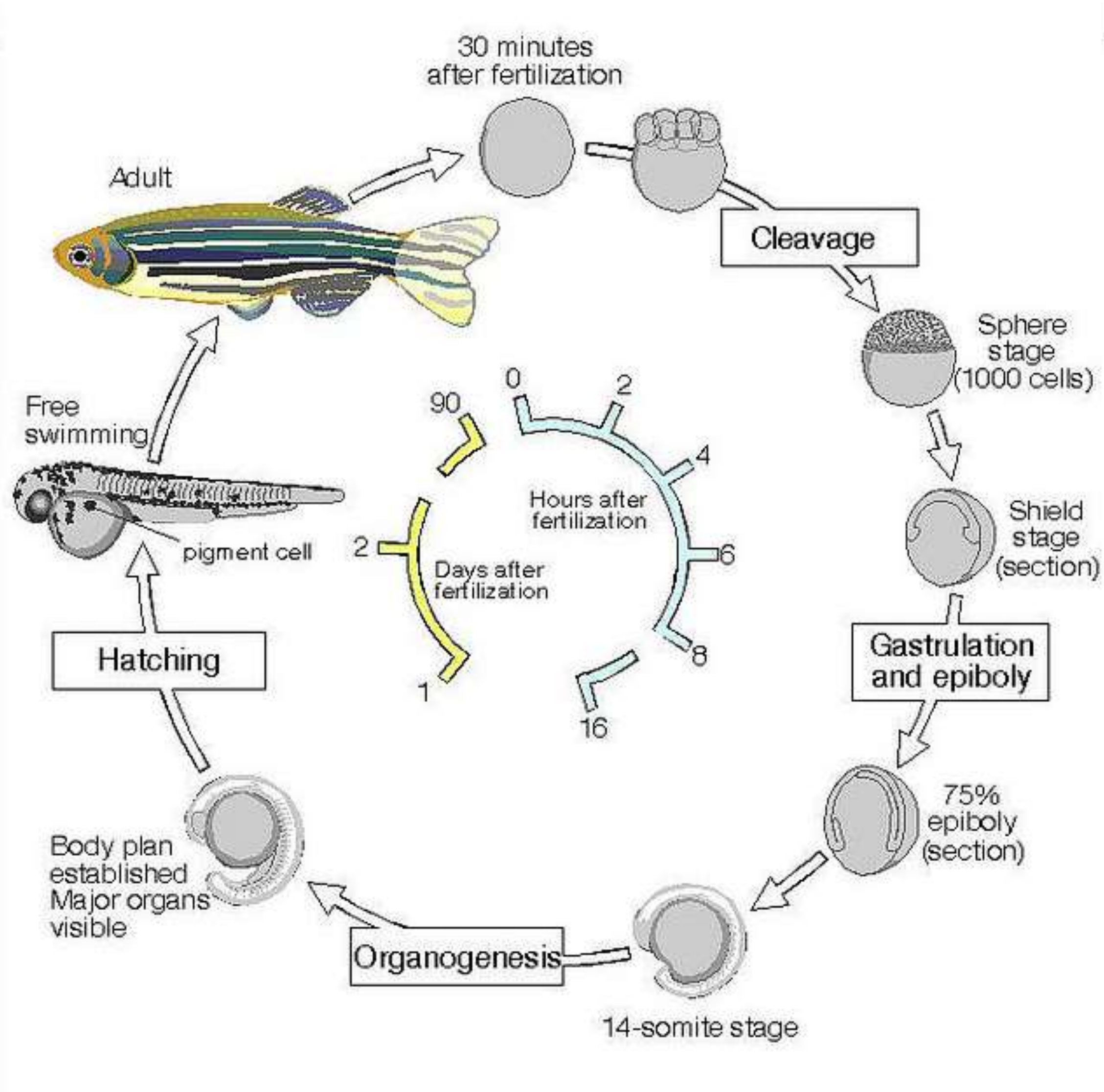


https://en.wikipedia.org/wiki/Main_Page

https://www.ncbi.nlm.nih.gov/pubmed/

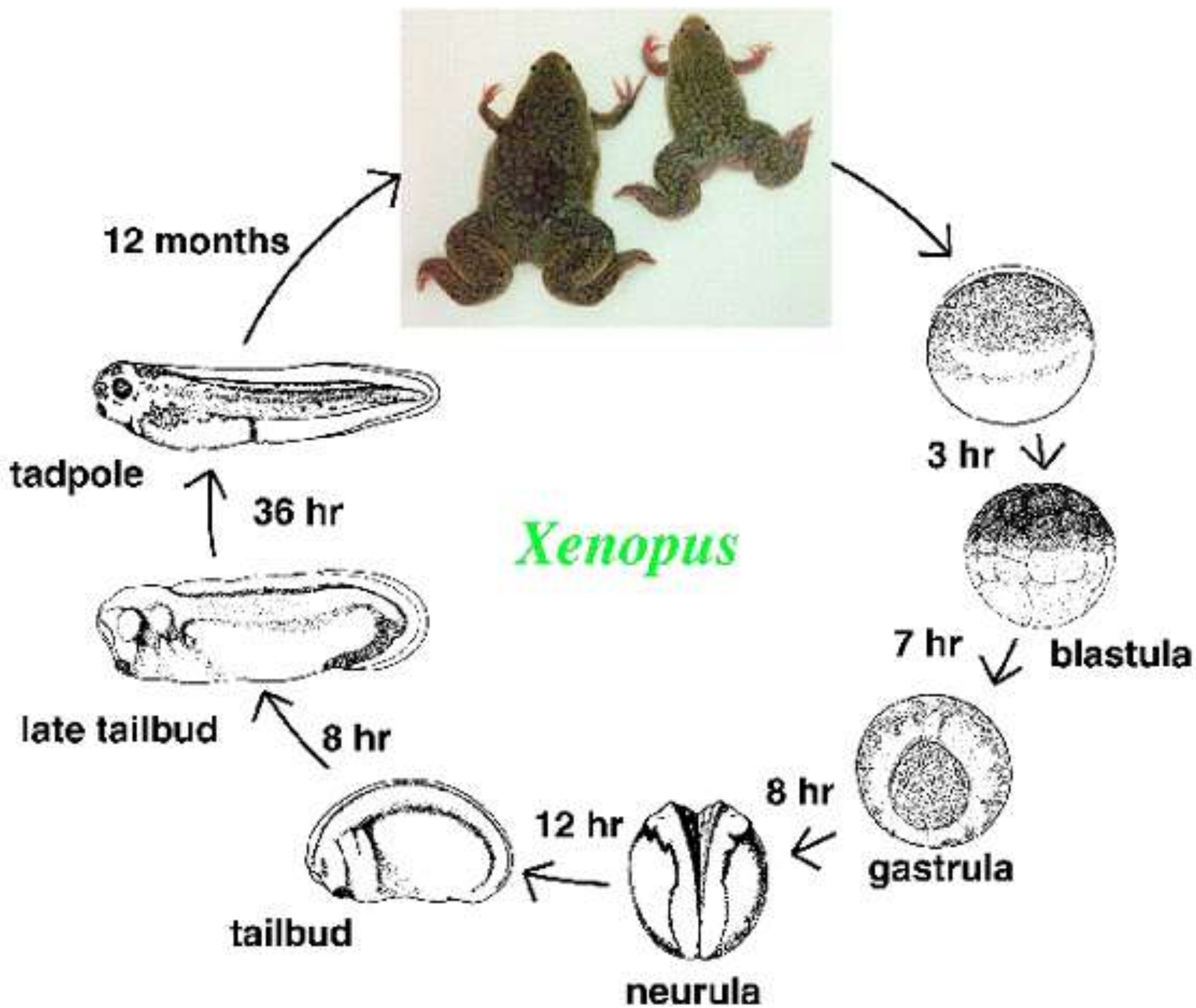
Adult zebrafish

Zebrafish life cycle



Adult Xenopus Lavis

Xenopus life cycle



Why xenopus and zebrafish?

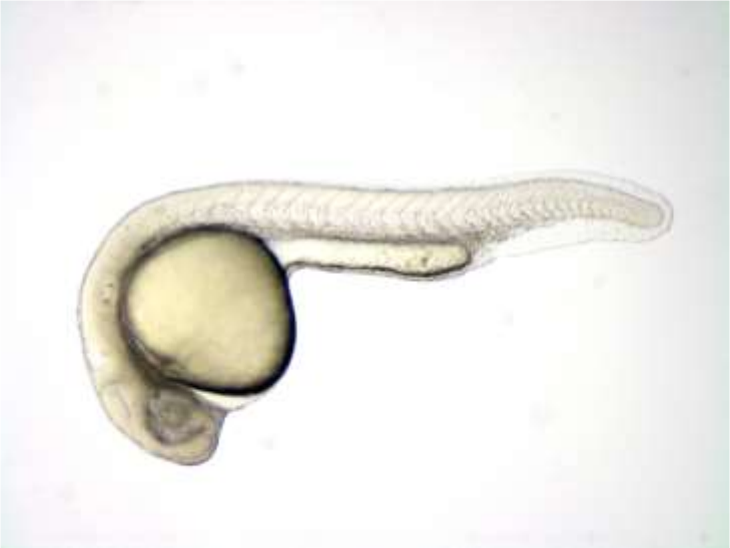
Features of zebrafish and xenopus

- Common feature：

  large cells, develop *ex vivo,* develop very fast, vertebrate

Zebrafish embryonic development

zebrafish



24 hpf

human

week 5

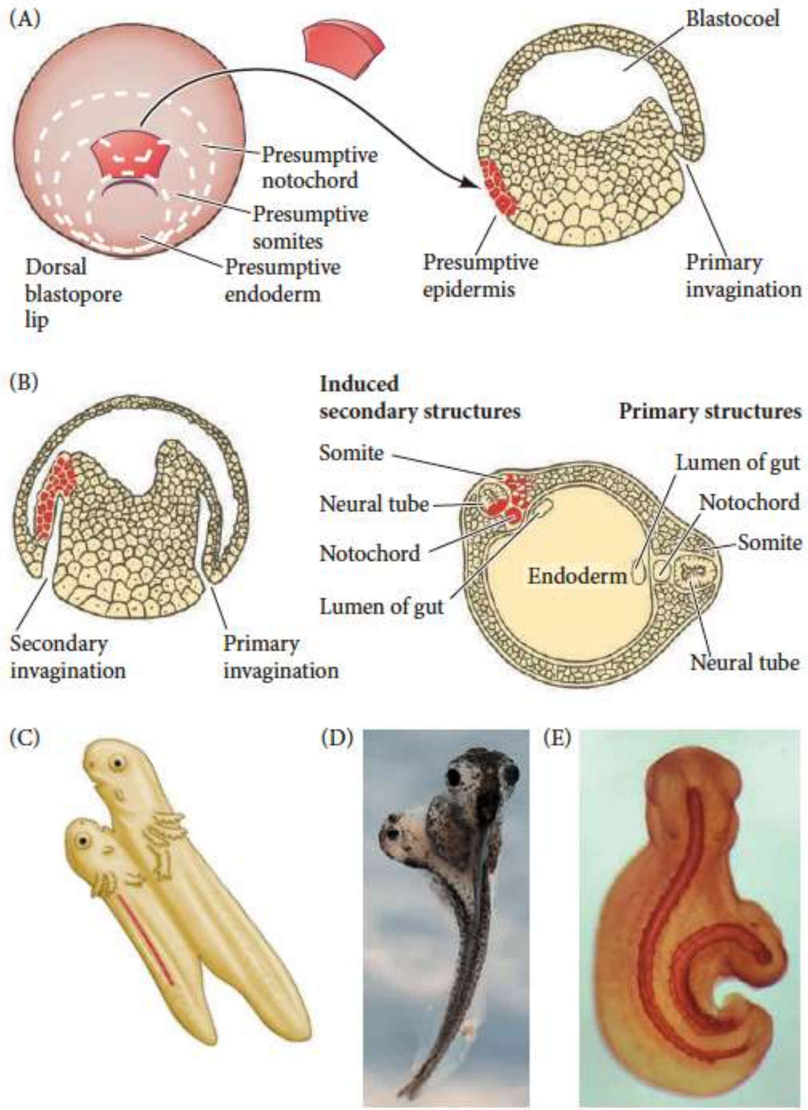
Features of zebrafish and xenopus

- Common feature：

  large cells, develop *ex vivo,* develop very fast

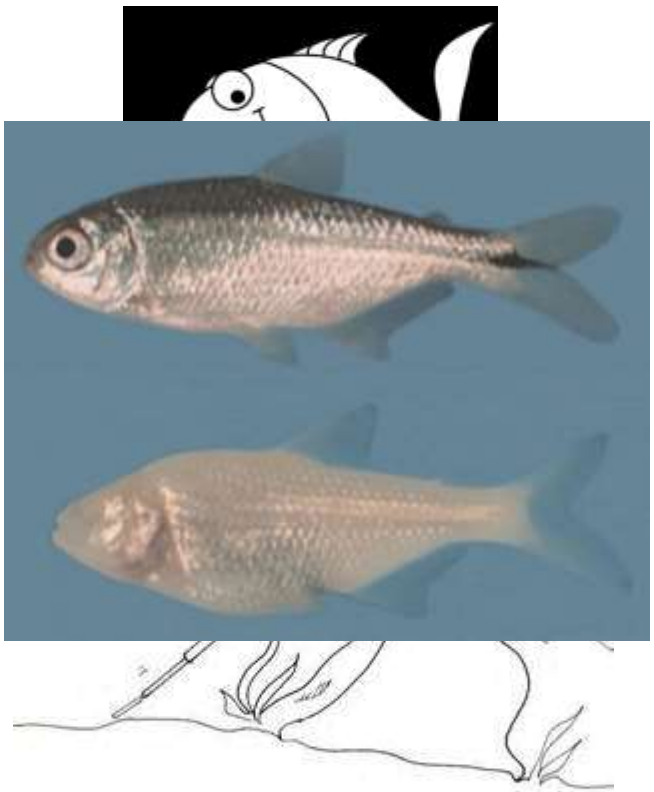
- xenopus：transplantation
- zebrafish：genetic screen

Transplantation in xenopus



(newt)

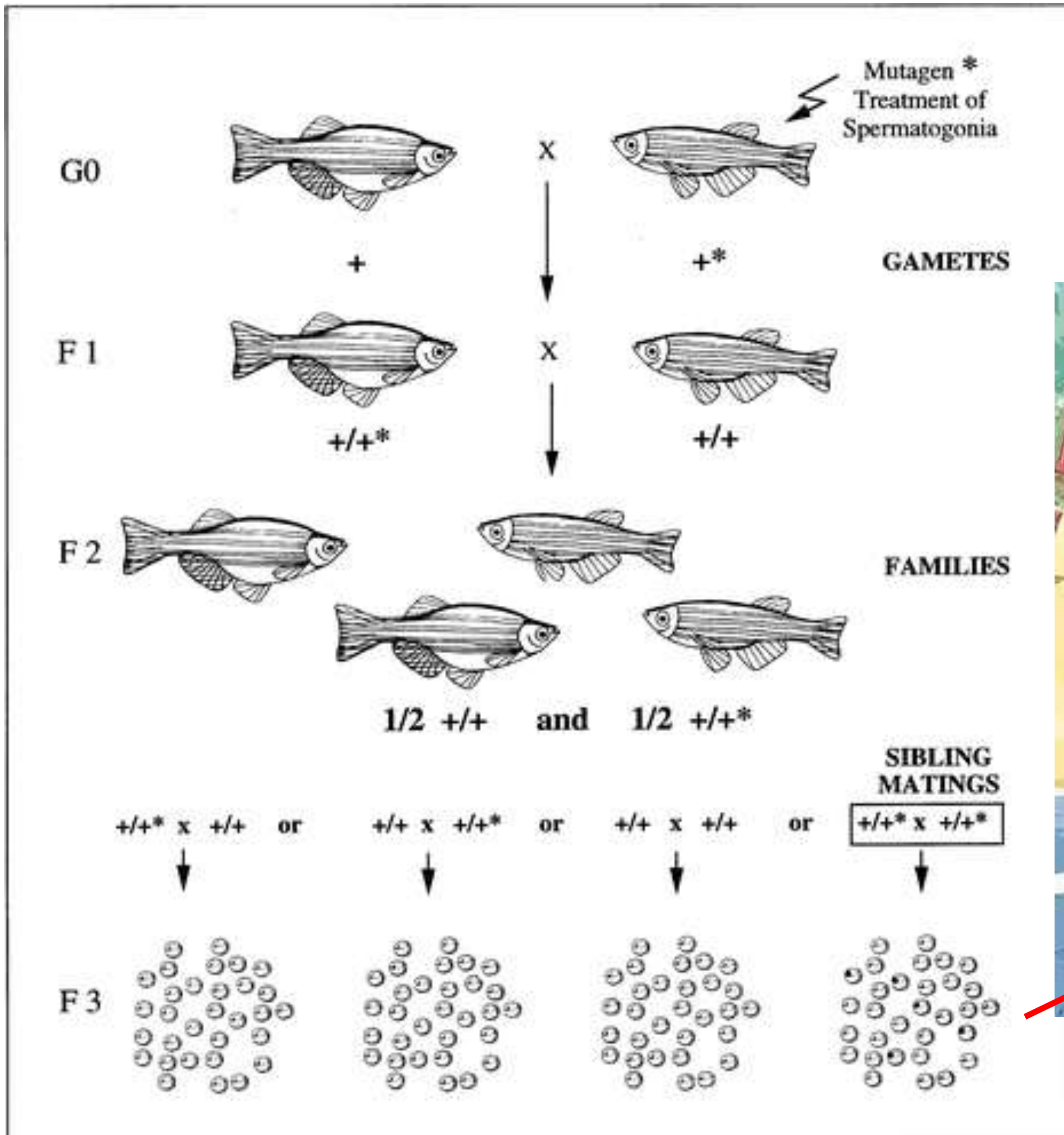
genetics

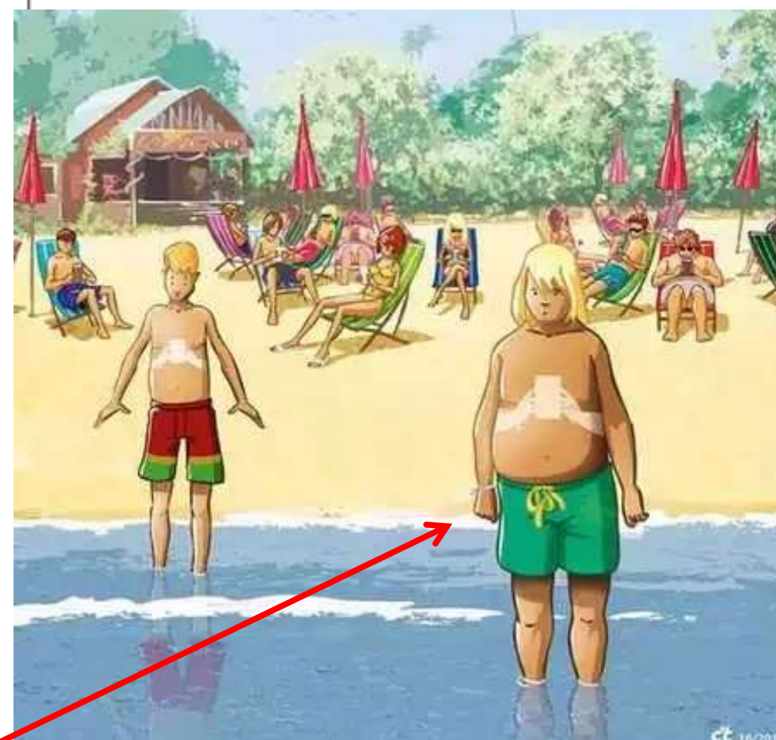


gene A is mutated in blind fish

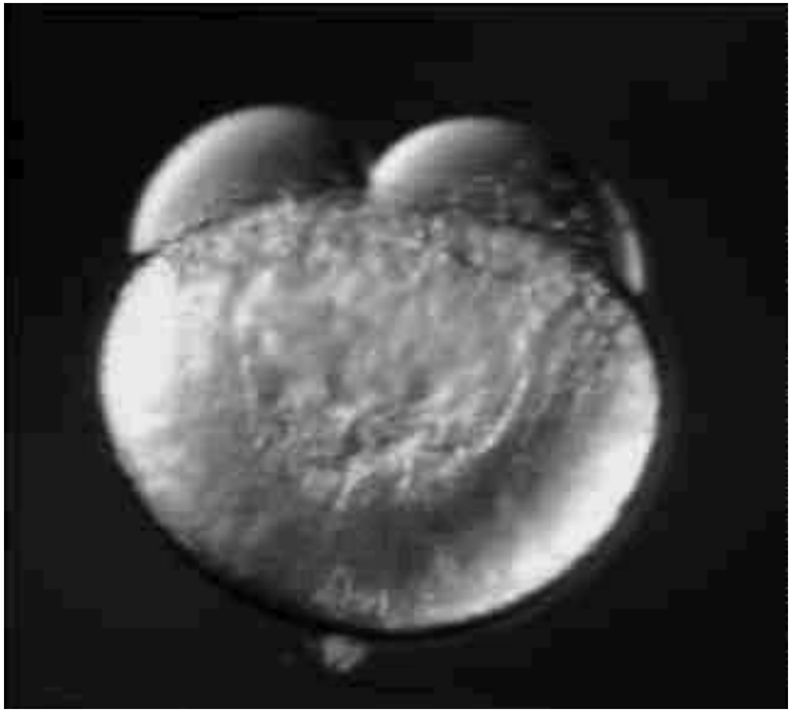
gene A is required for
eye development

Forward genetics (phenotypes → genes): Genetic screen in zebrafish





Zebrafish development



outline

- Fertilization (受精)
- Cleavage and blastula stage (卵裂期和囊胚期)
- Gastrulation (原肠胚期)

1）Cell migration and germ layers formation (细胞运动)

2）Mesoderm induction (中胚层诱导)

3）Specifying body axis (胚轴分化)

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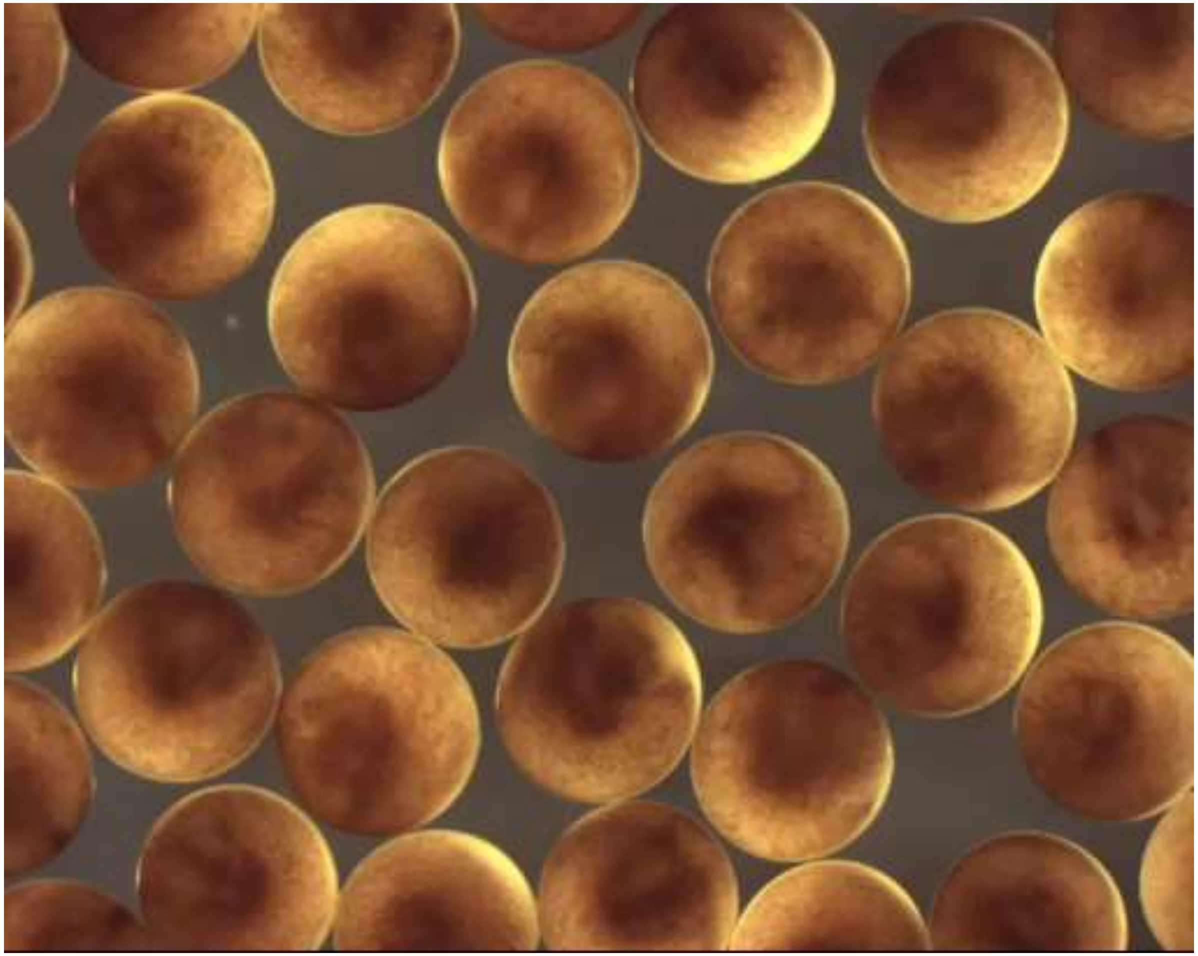
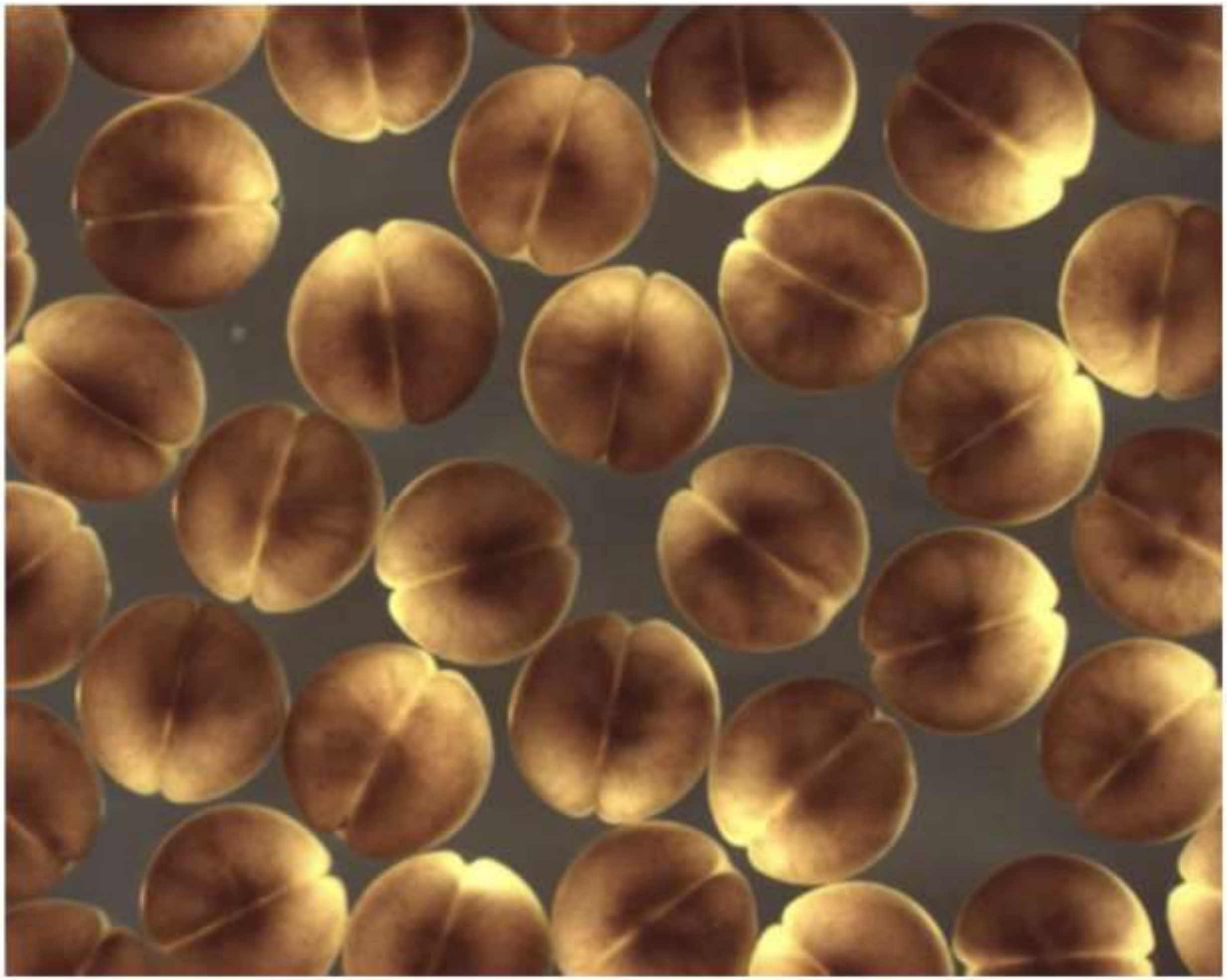
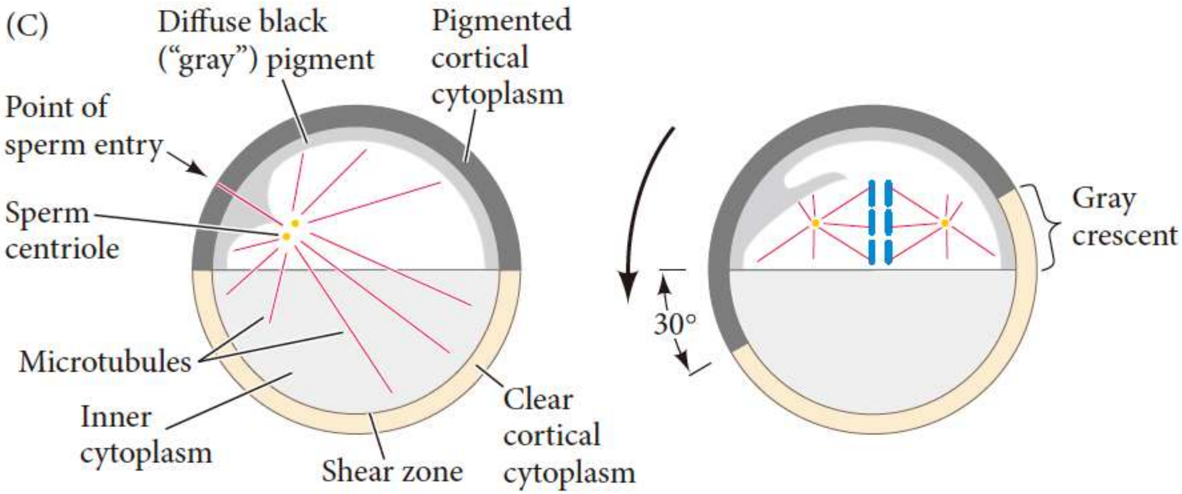
Fertilization (受精)

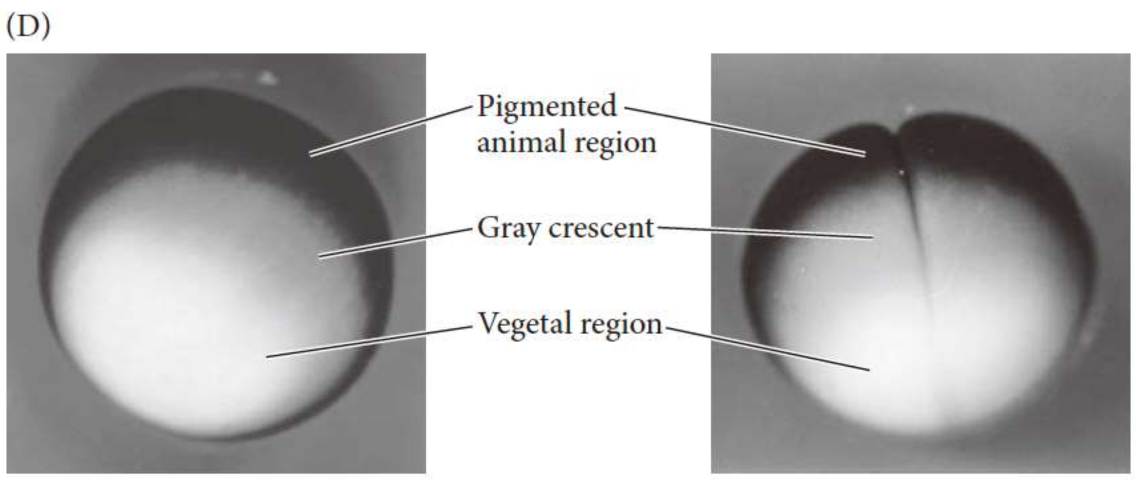


Fertilization

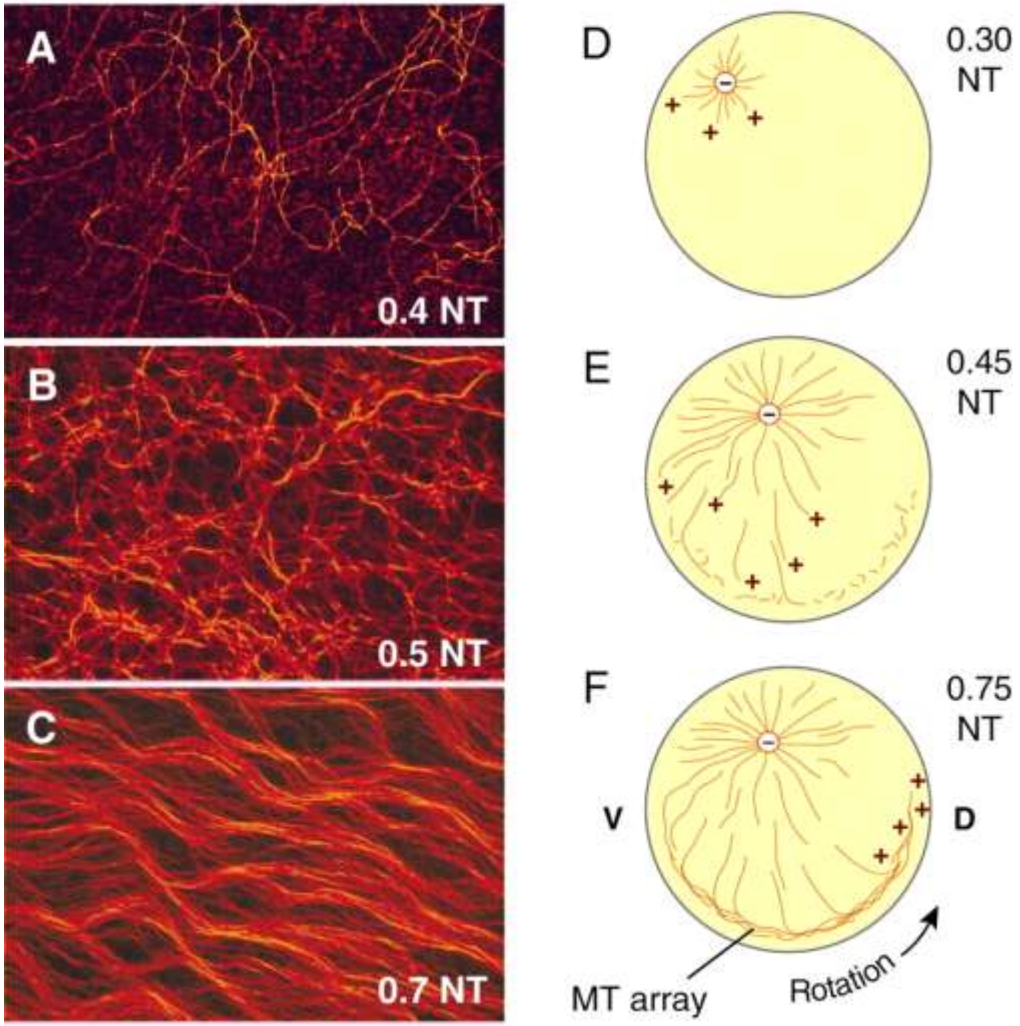


Fertilization (受精)





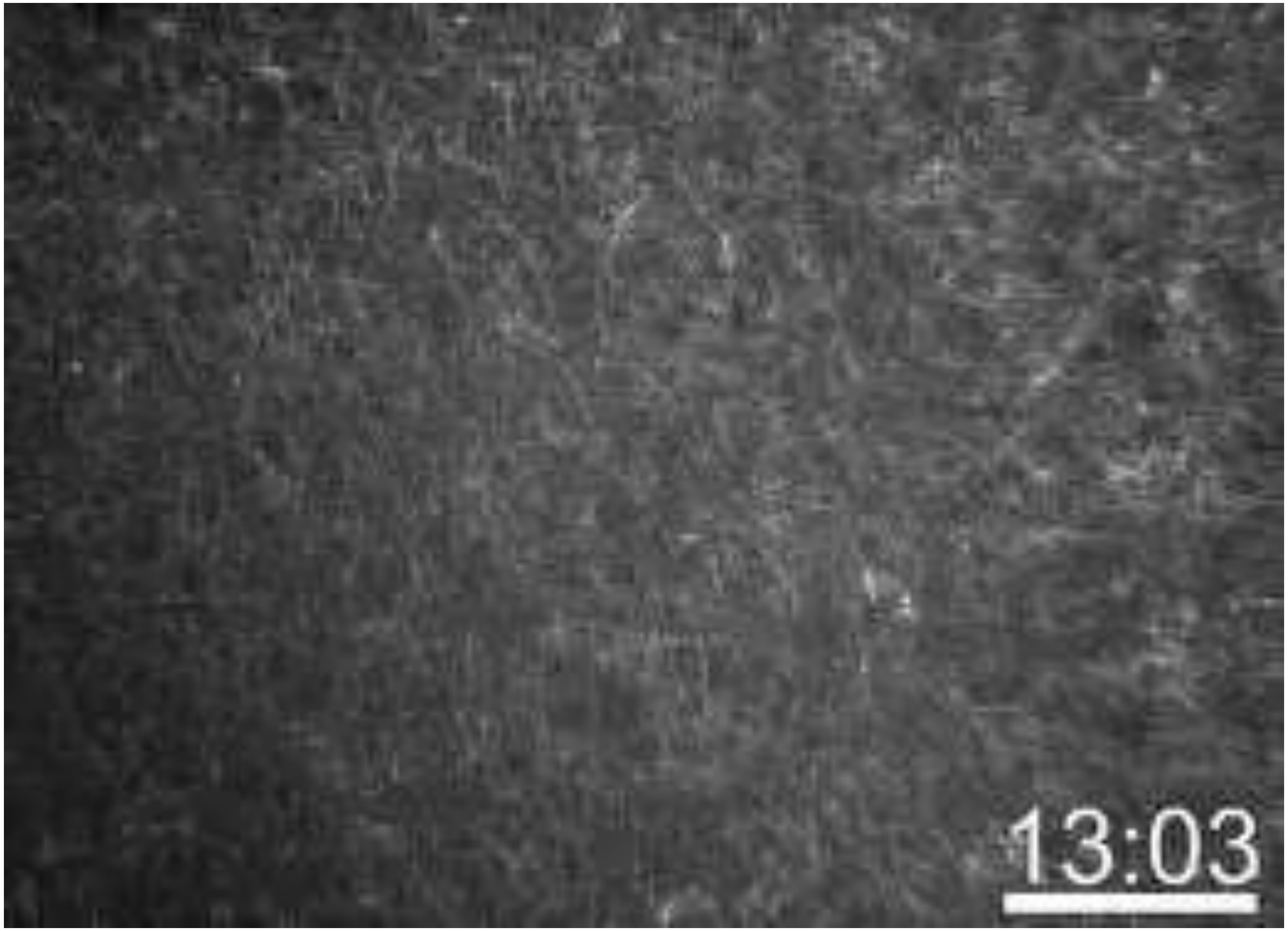
Formation of the microtubule array (微管束）in xenopus egg



(A-C) Vegital view.
NT: normalized time

Weaver, 2004

Similar process in zebrafish



Tran, *et. al*., 2012, Dev

outline

- Fertilization (受精)
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1）Cell migration and germ layers formation (细胞运动)

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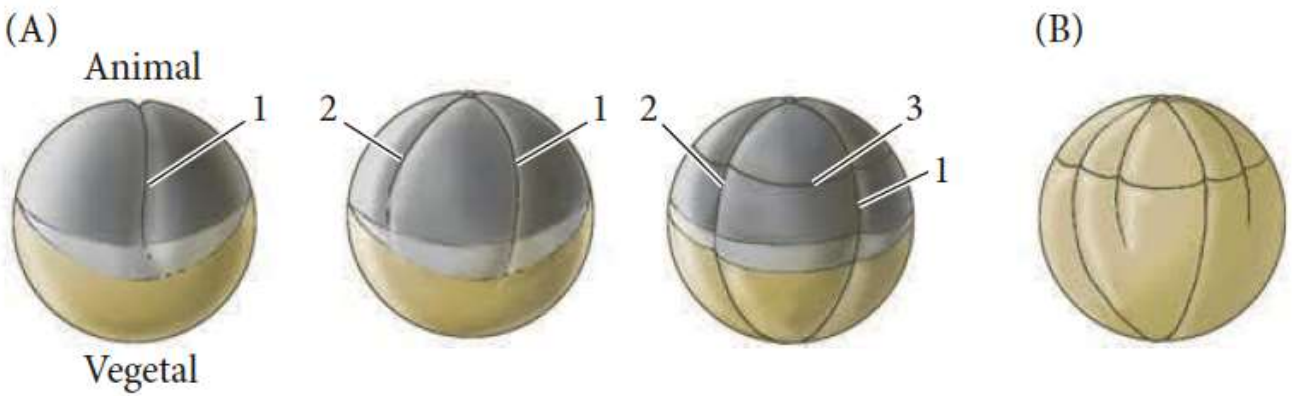
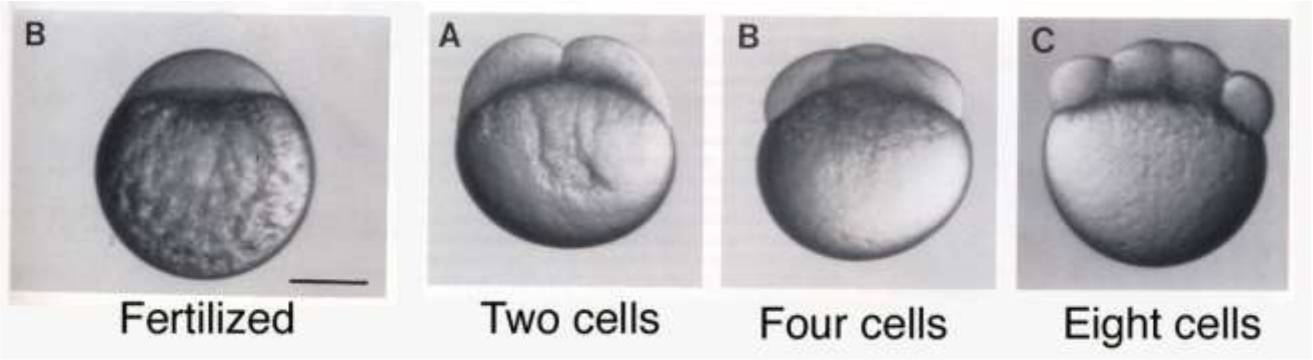
cleavage

- Different ways of cleavage：

Xenopus:
Holoblastic
Cleavage
(全裂)

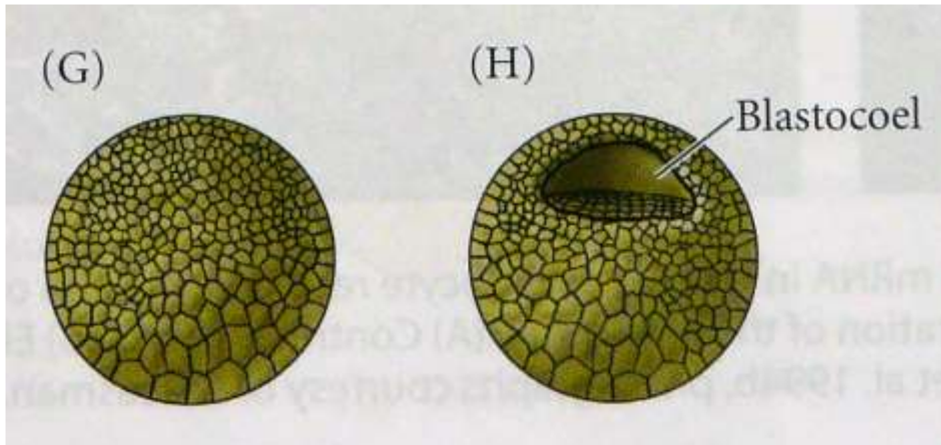


zebrafish：
Meroblastic
Cleavage
(偏裂)

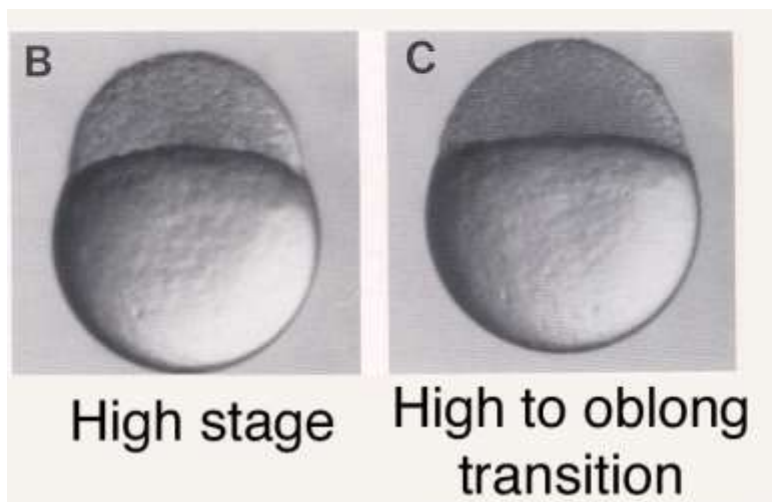


blastula (囊胚期)

xenopus



zebrafish



- MBT：mid-blastula transition (maternal→zygotic)

outline

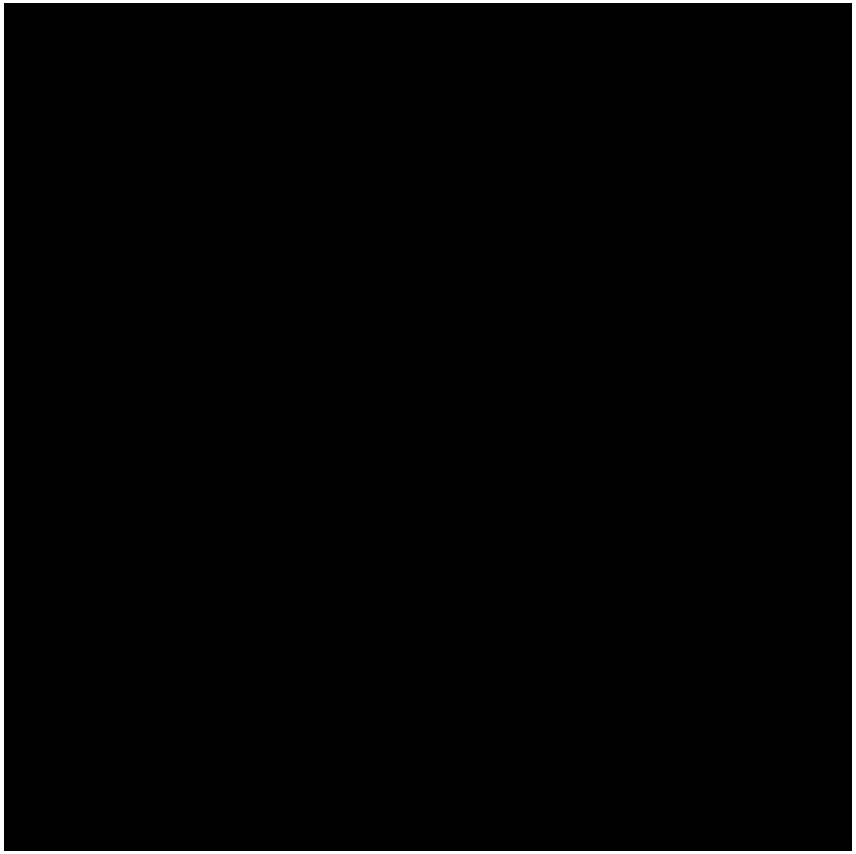
- Fertilization (受精)
- Cleavage and blastula stage (卵裂期和囊胚期)
- Gastrulation (原肠胚期)

1）Cell migration and germ layers formation (细胞运动)

2）Mesoderm induction (中胚层诱导)

3）Specifying body axis(胚轴分化)

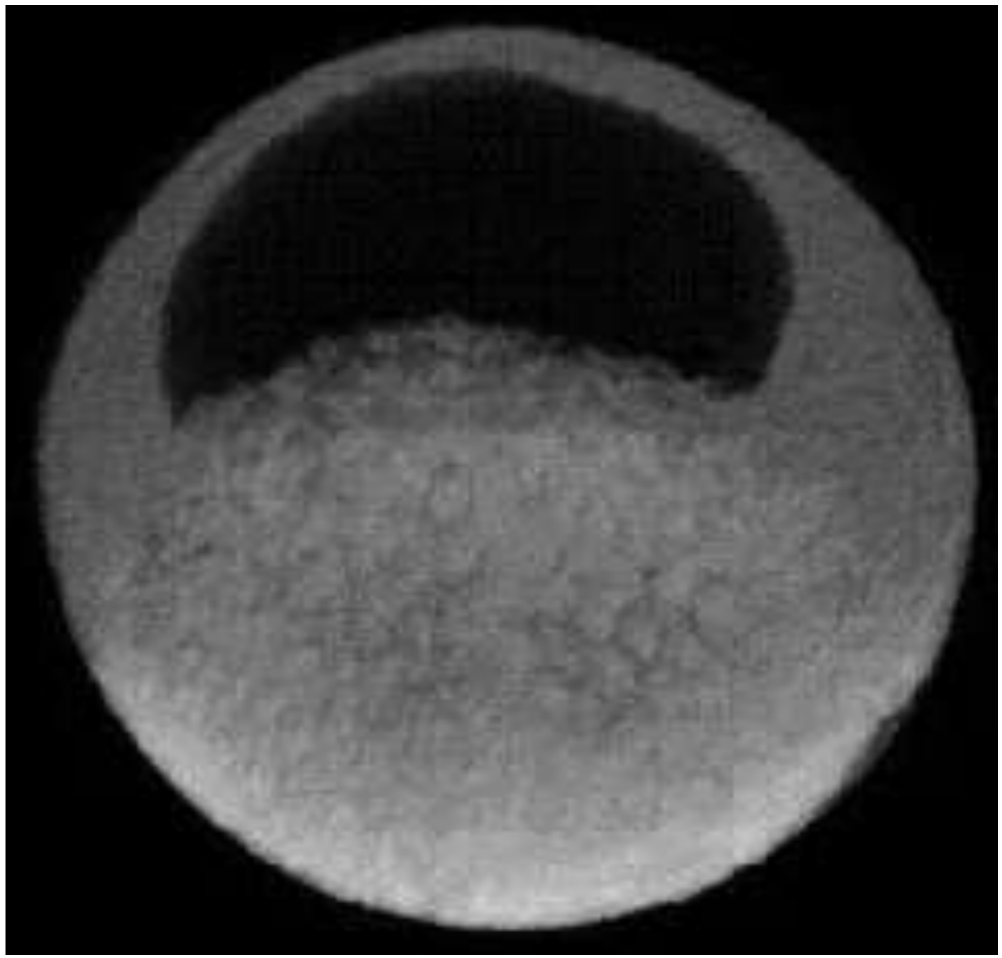
gastrula (原肠胚期)



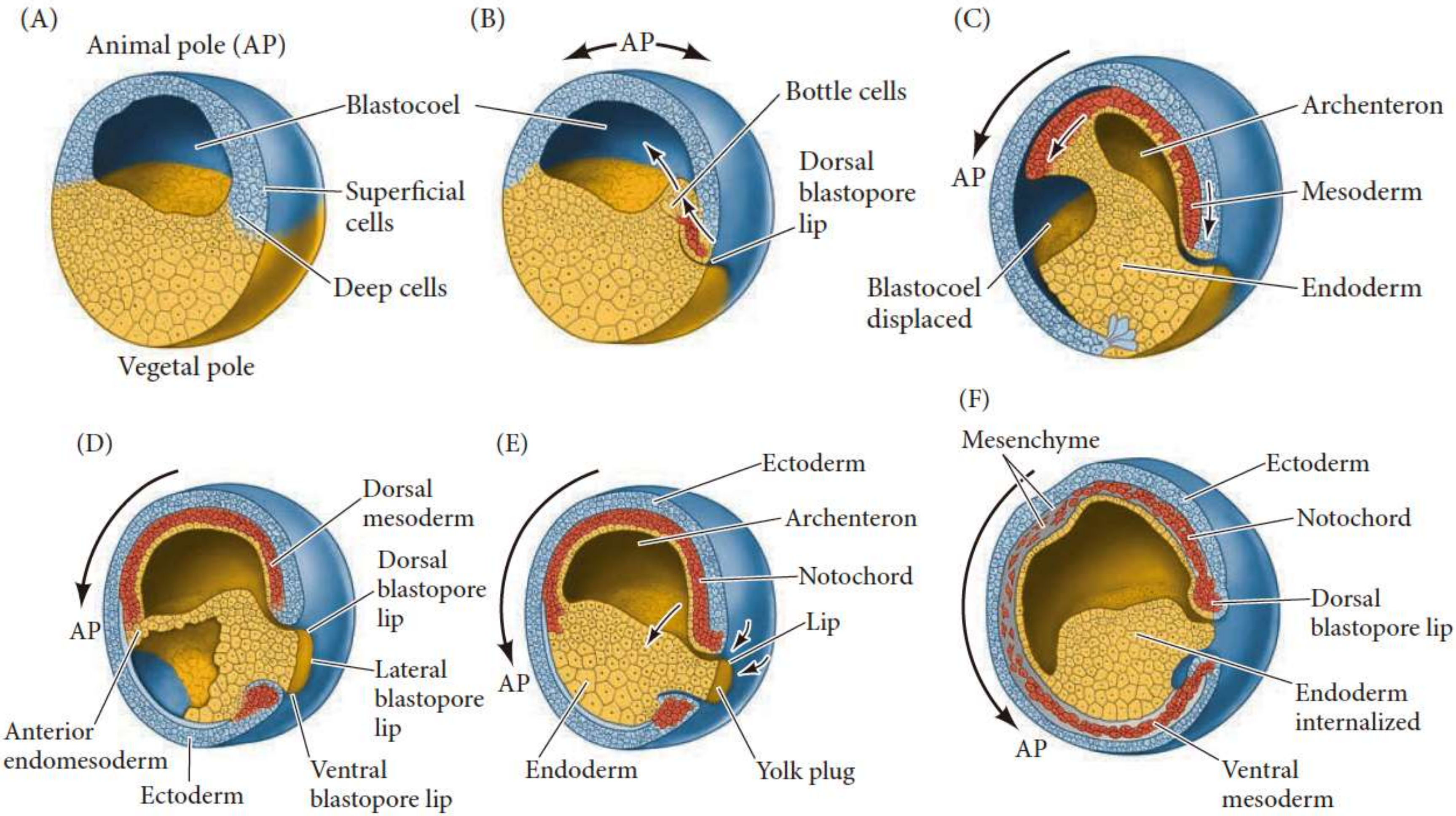
Gastrulation in xenopus
(爪蟾的原肠运动)

Gastrulation in zebrafish
(斑马鱼的原肠运动)

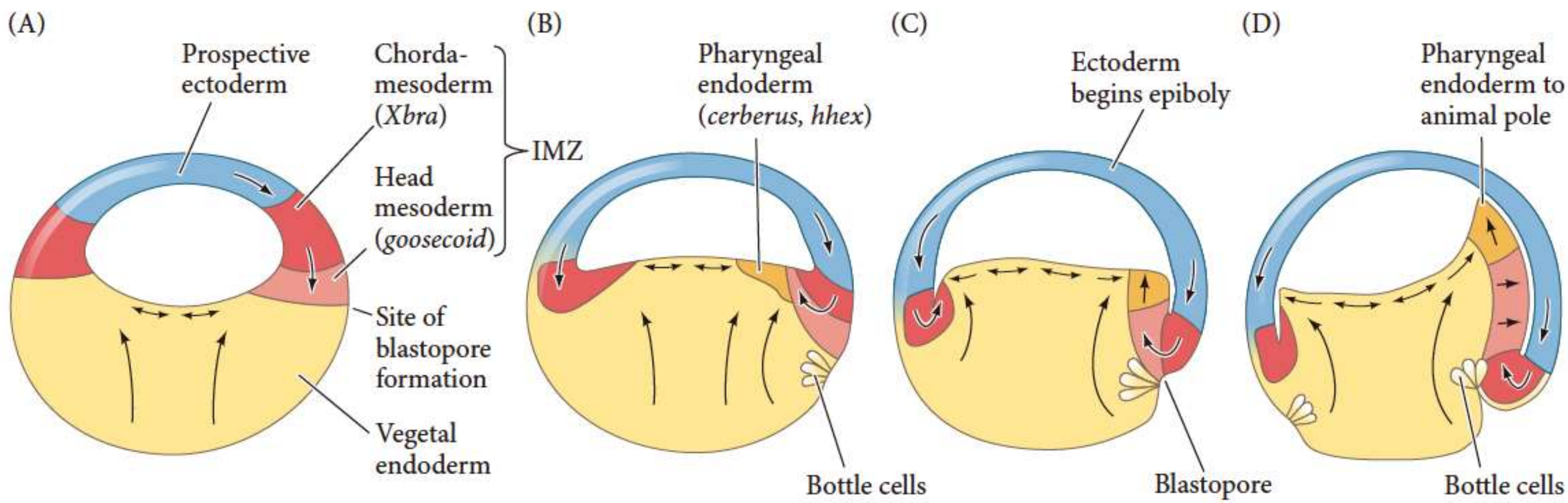
Internal cell movement during epiboly in Xenopus



Cell migration during gastrula: epiboly (外包), involution (内卷)

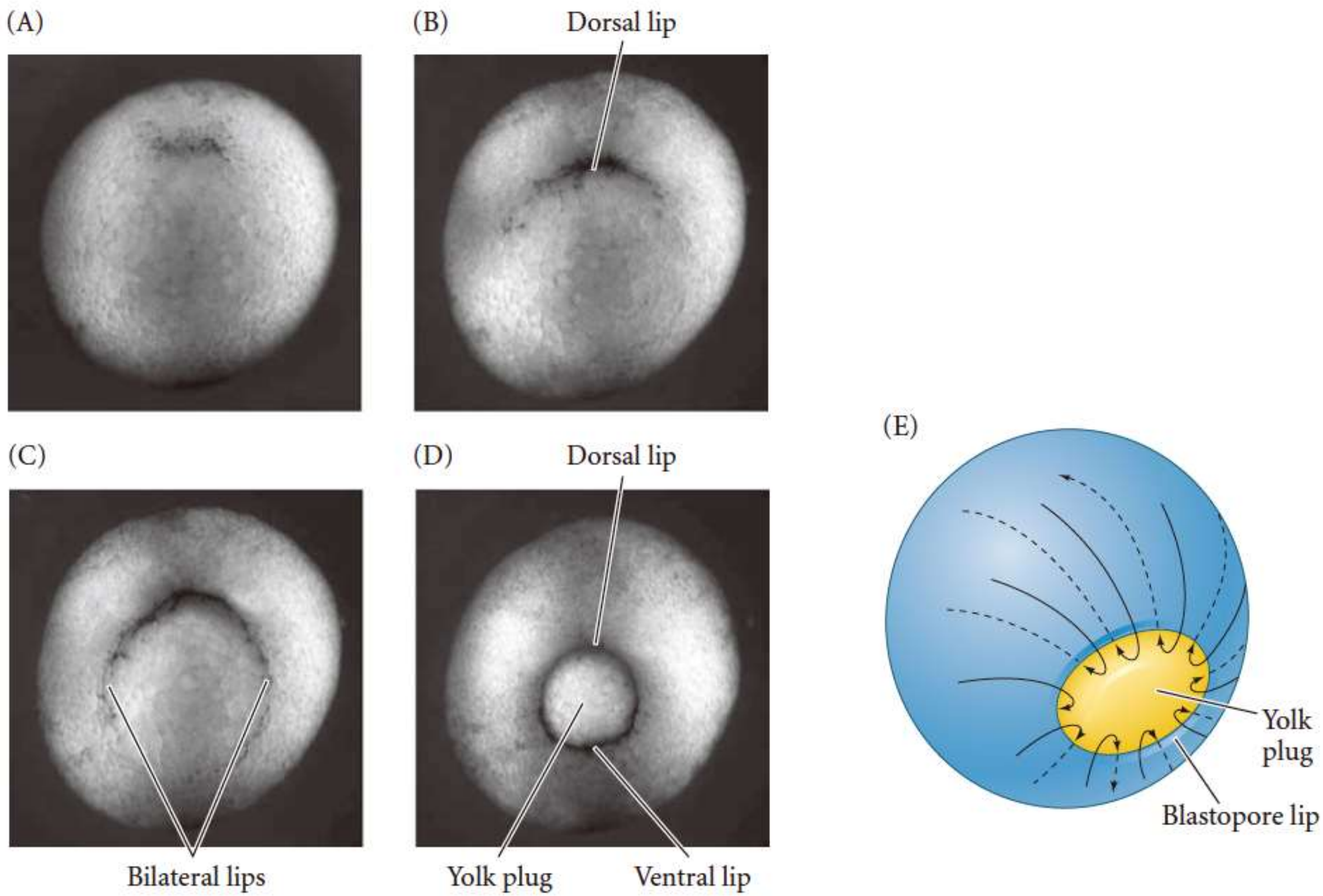


Early movements of Xenopus gastrulation

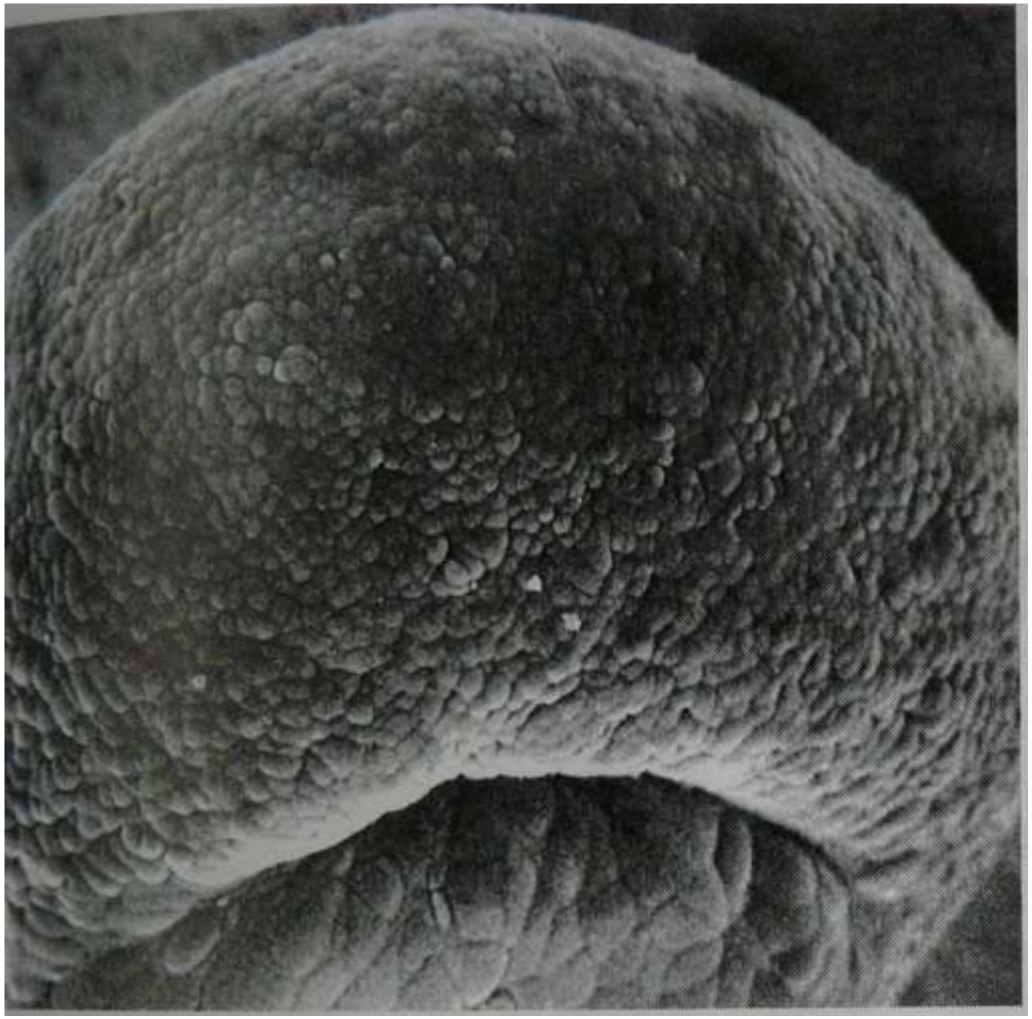


瓶颈细胞对于内卷过程非常重要

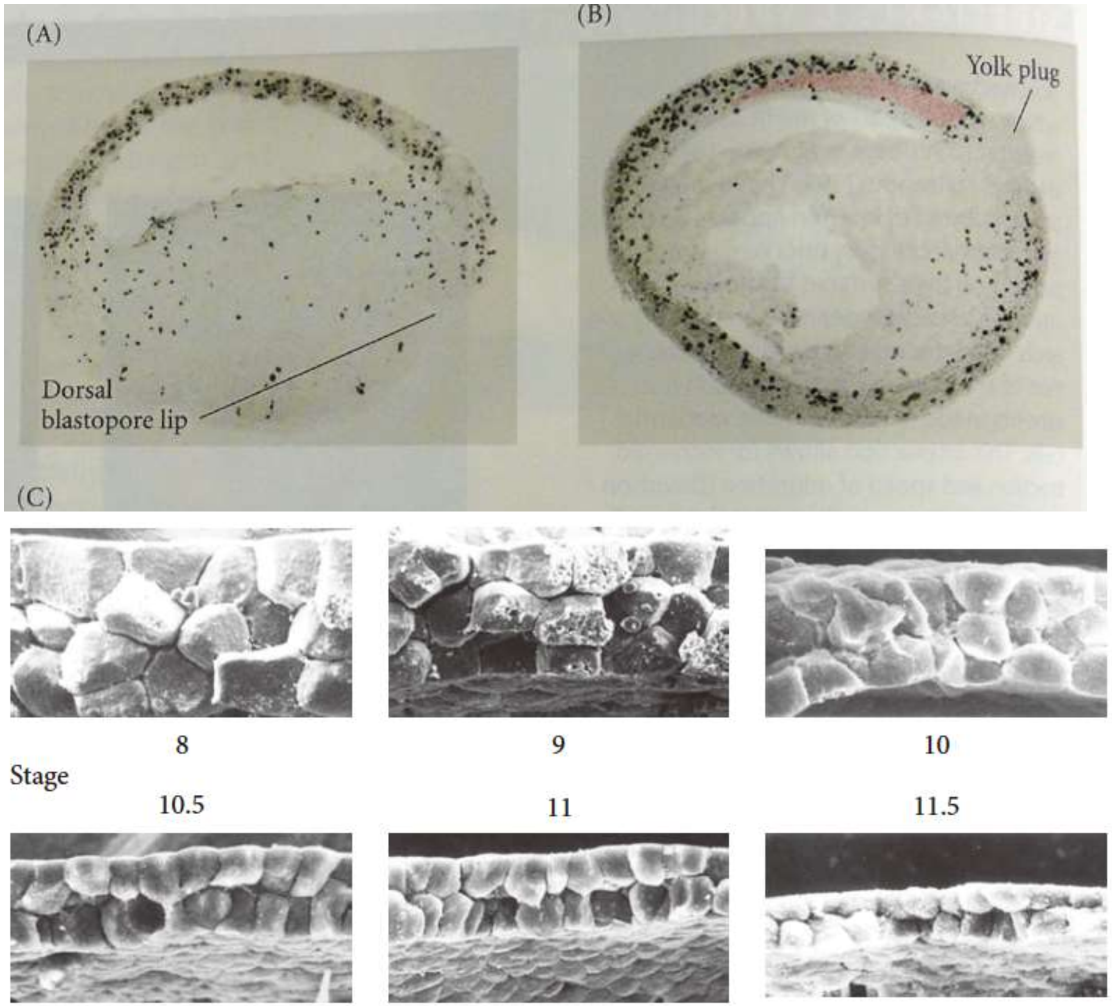
epiboly（外包）initiates at dorsal lip



dorsal lip （背唇）

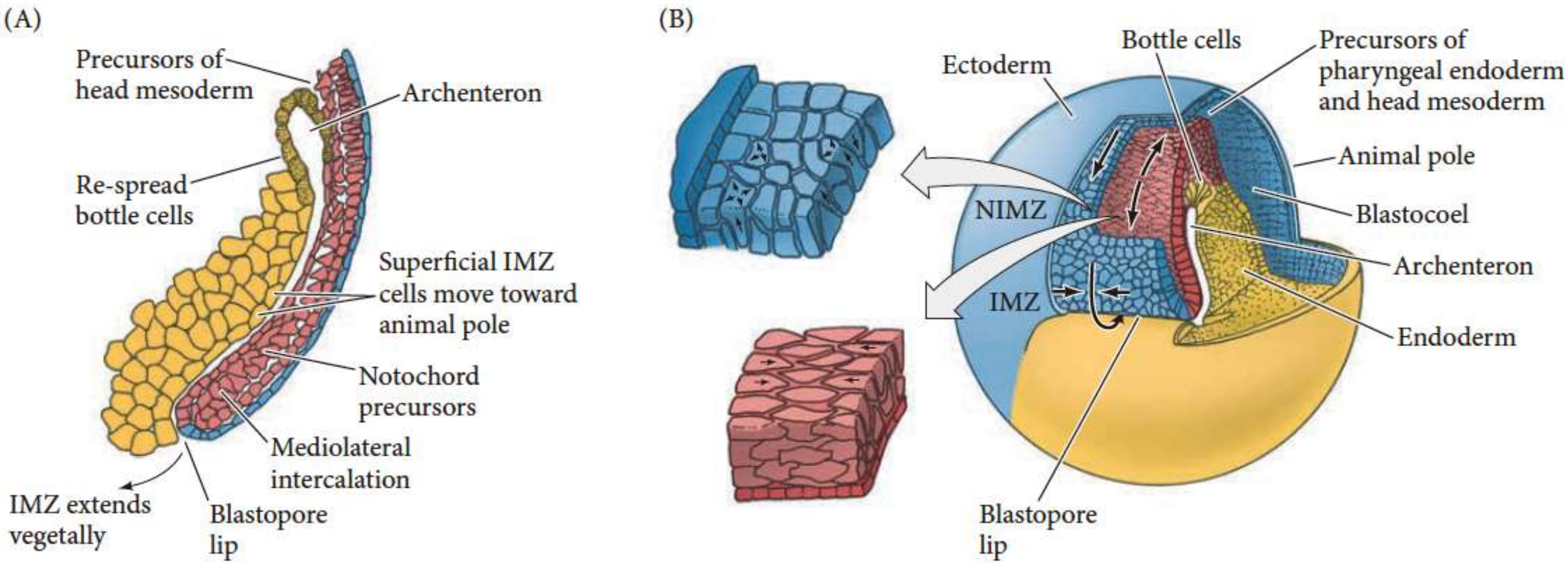


Epiboly is accomplished by cell division and intercalation



外包机制：
细胞分裂
相互置入

Xenopus gastrulation continues



外包→外胚层
内卷→中胚层
汇聚延伸→中轴中胚层

Cell movement during zebrafish epiboly

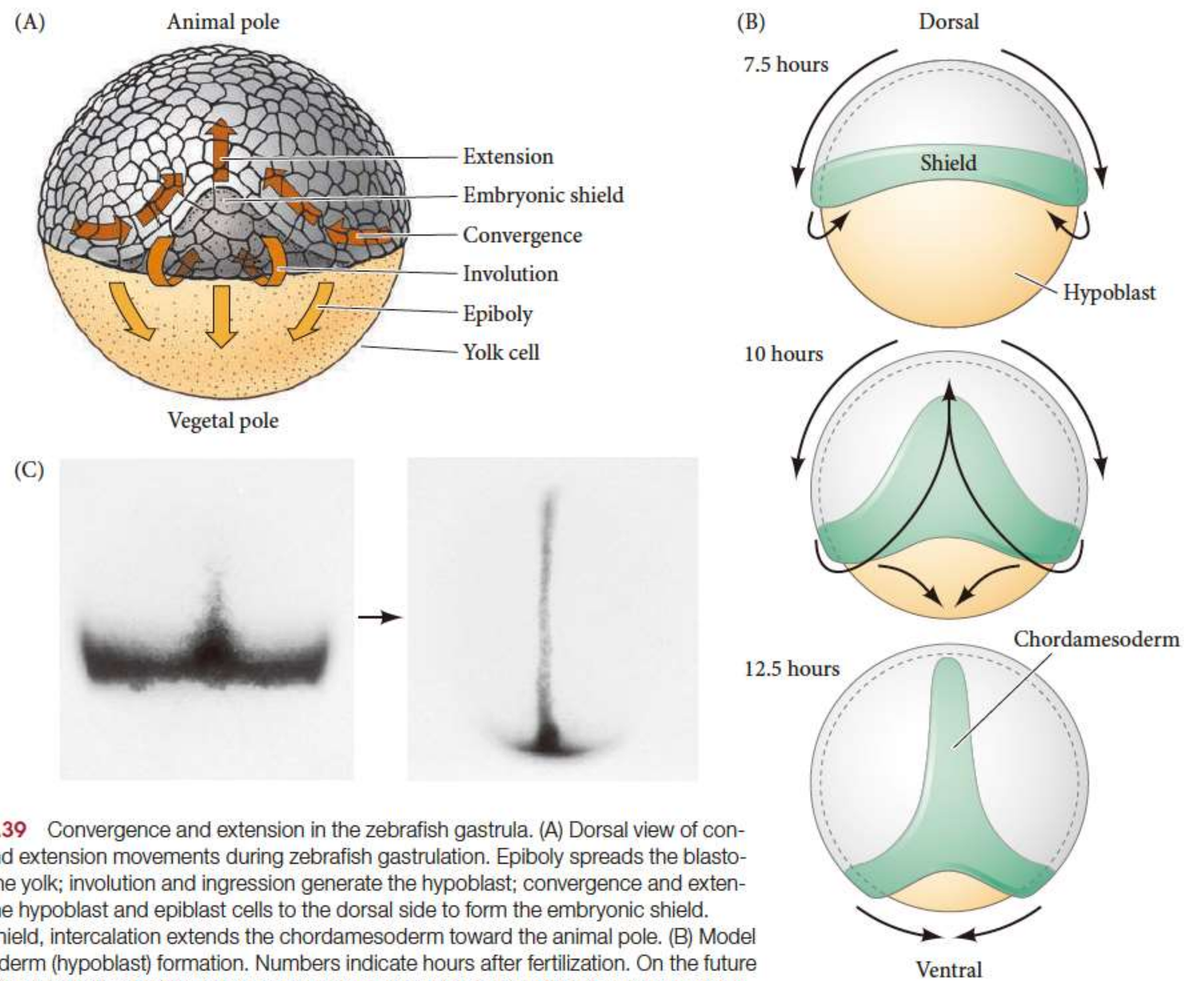


FIGURE 11.39 Convergence and extension in the zebrafish gastrula. (A) Dorsal view of convergence and extension movements during zebrafish gastrulation. Epiboly spreads the blastoderm over the yolk; involution and ingression generate the hypoblast; convergence and extension bring the hypoblast and epiblast cells to the dorsal side to form the embryonic shield. Within the shield, intercalation extends the chordamesoderm toward the animal pole. (B) Model of mesendoderm (hypoblast) formation. Numbers indicate hours after fertilization. On the future dorsal side, the internalized cells undergo convergent extension to form the chordamesoderm.

汇聚延伸

Summary (I)

Key words:

- Grey crescent (灰色新月区),
- Cleavage and blastula (卵裂期和囊胚期),
- Gastrulation (原肠运动)

Event:

- Fertilization
- Cleavage and blastula
- Cell migration during gastrulation

outline

- Fertilization (受精)
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- Gastrulation (原肠胚期)

  1）Cell migration and germ layers formation (细胞运动)

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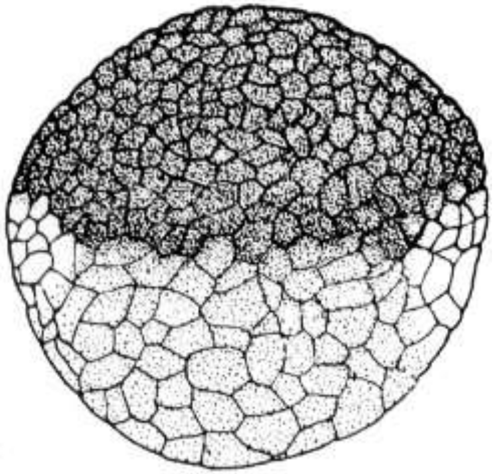
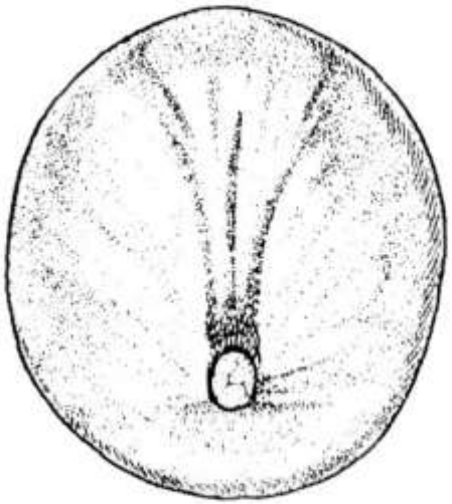
  3）Specifying body axis(胚轴分化)

body axis formation



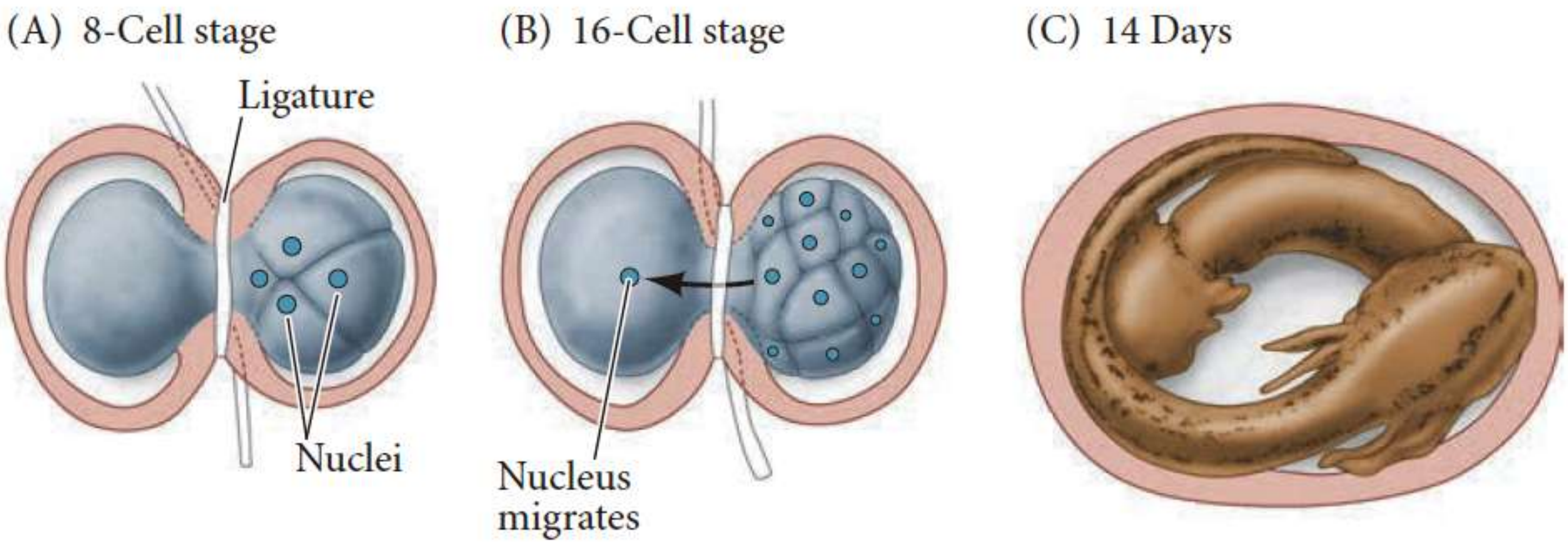
St. 8



St. 12.5

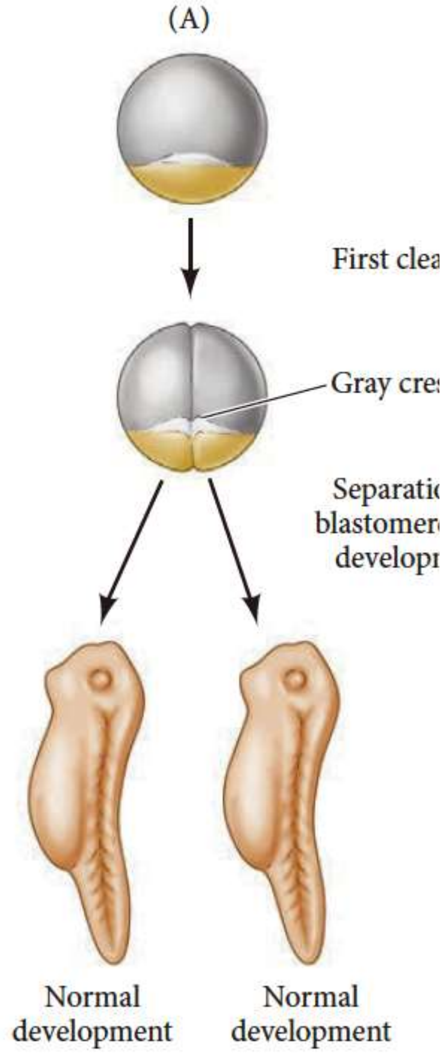
- Anterior-posterior patterning (前后分化)
- Dorso-ventral patterning (背腹分化)
- Left-right patterning (左右分化)

Spemann's demonstration of nuclear equivalence in newt cleavage



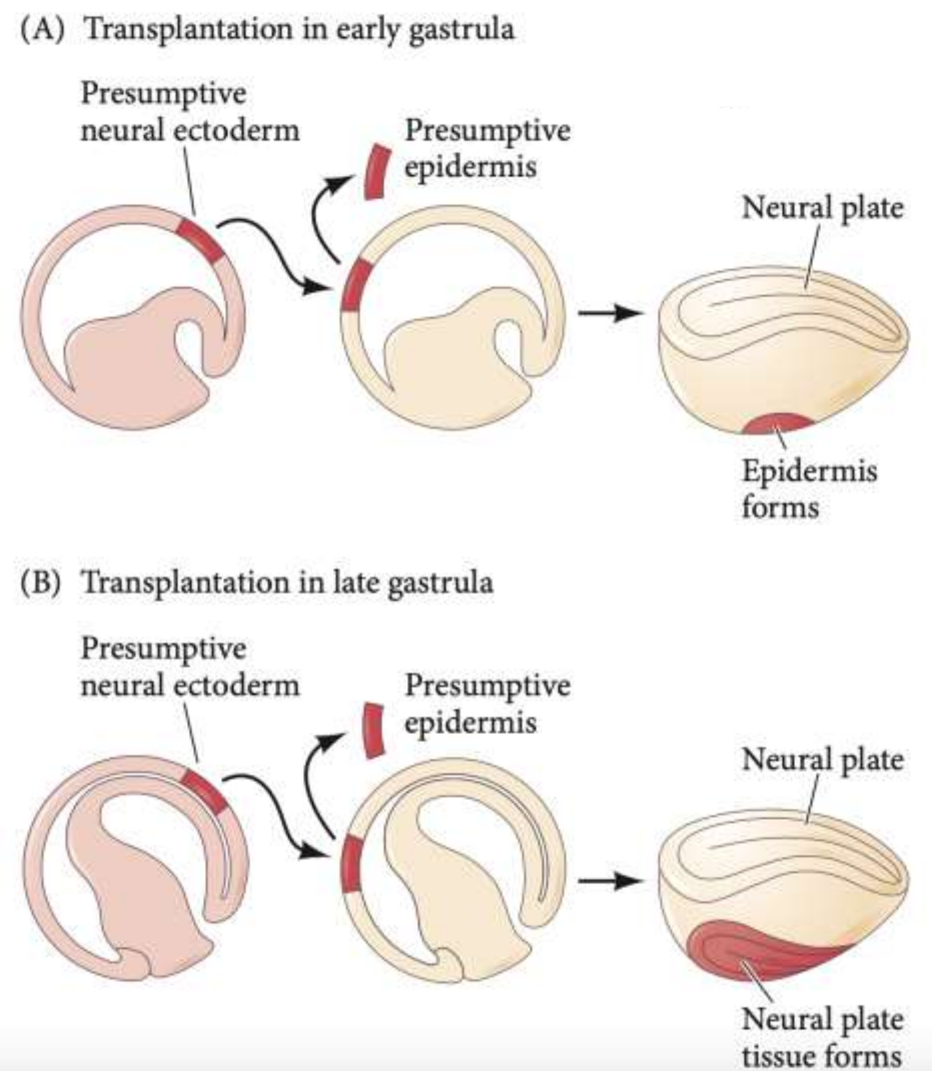
newt： 蝾螈

But…

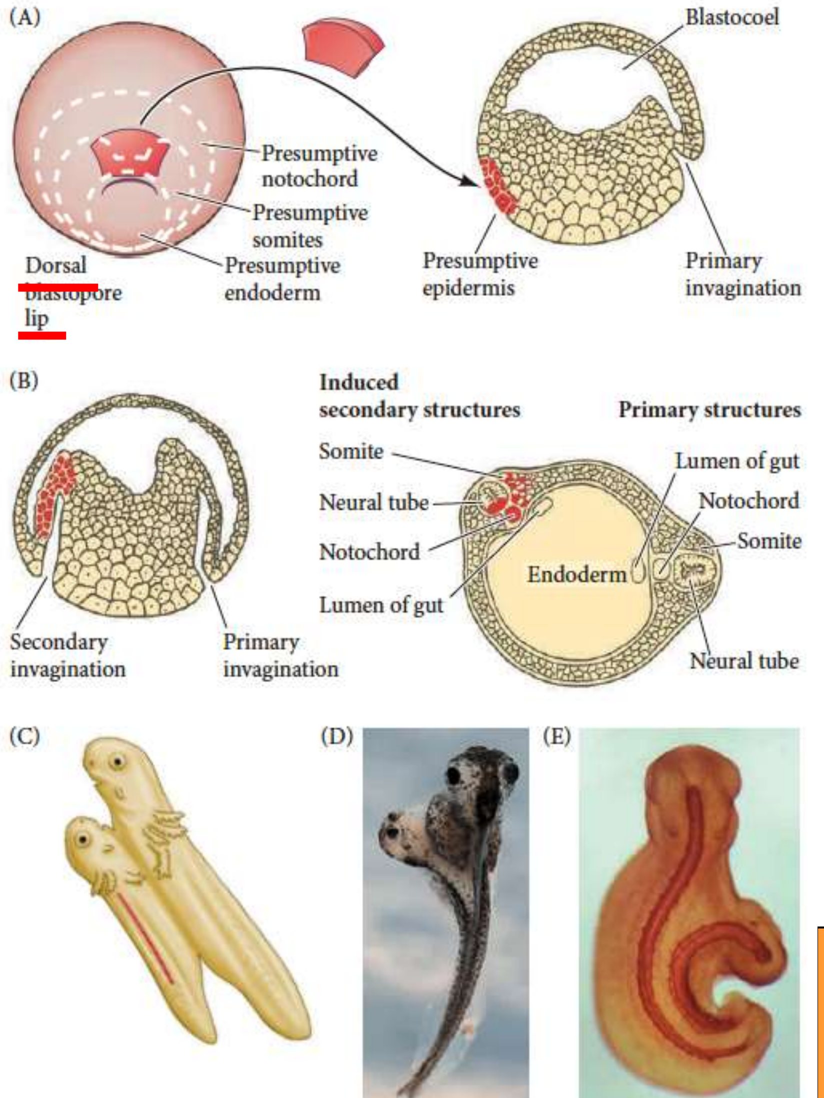


Grey crescent is very important for dorsalization

Determination of ectoderm during newt gastrulation



Hans Spemann and Hilde Mangold: primary embryonic induction

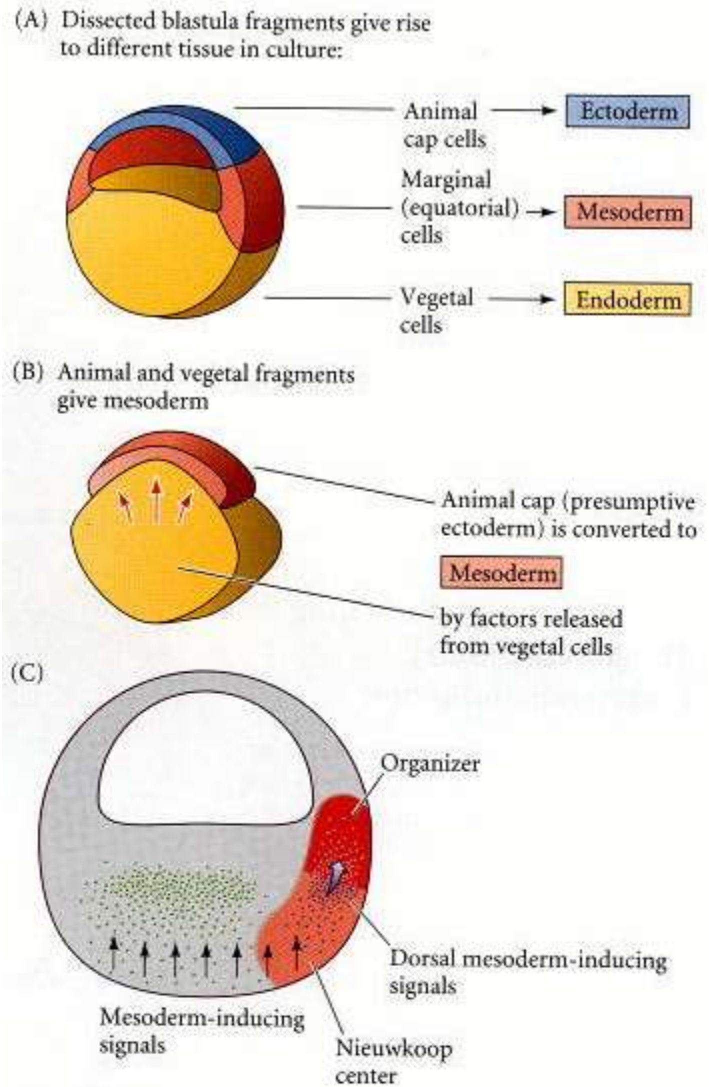


(A-C) newt
(D, E) xenopus

背唇组织能够诱导第二胚轴的形成，因此也称之为组织者（organizer）

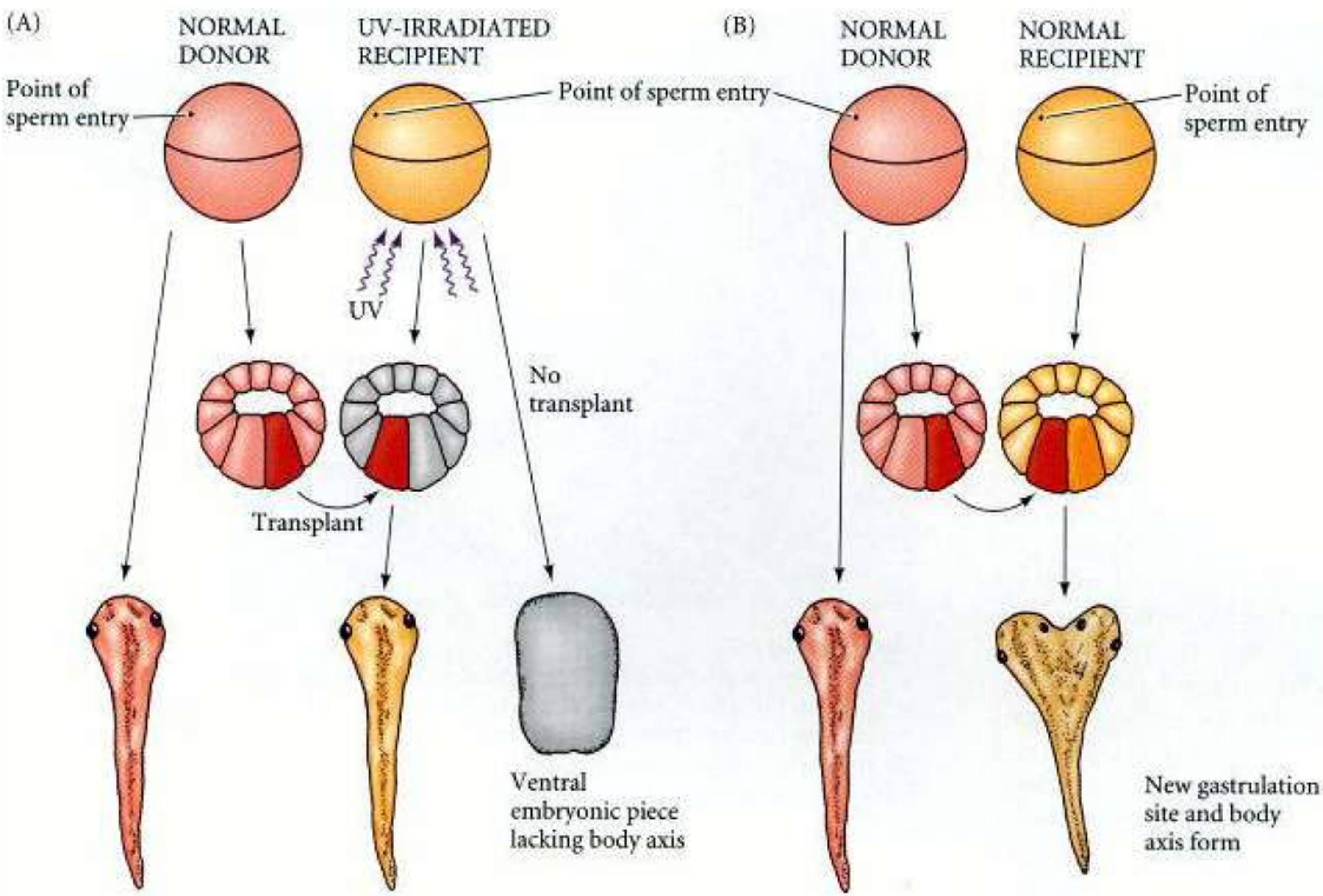
The Nobel Prize in Physiology or Medicine 1935: Hans Spemann

Organizer is induced by Nieuwkoop center



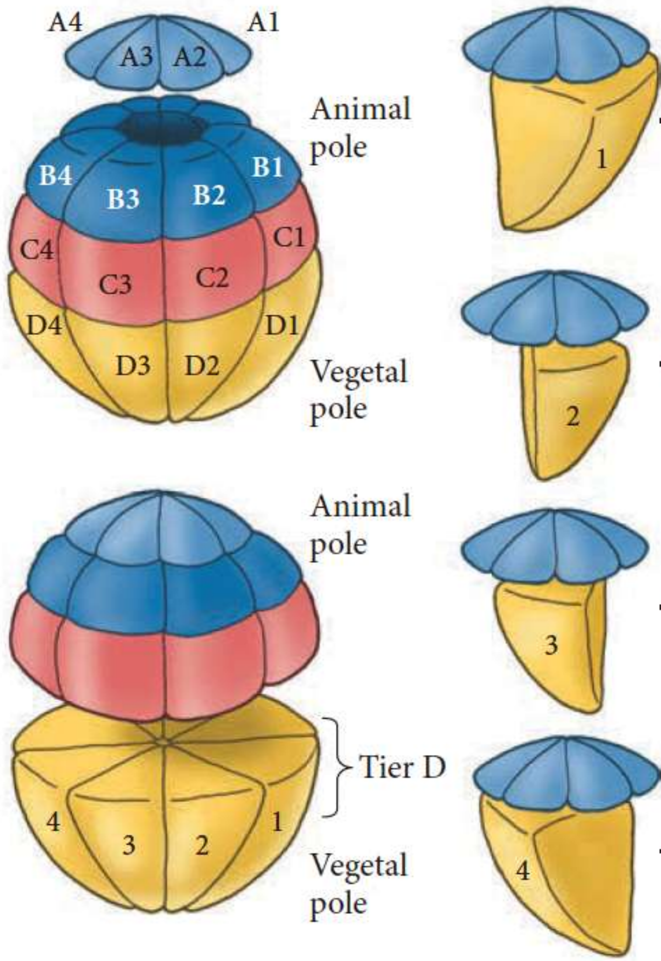
Nieuwkoop center → organizer

Vegetal cells are important for organizer formation



The dorsalmost vegetal blastomere induces dorsal mesoderm

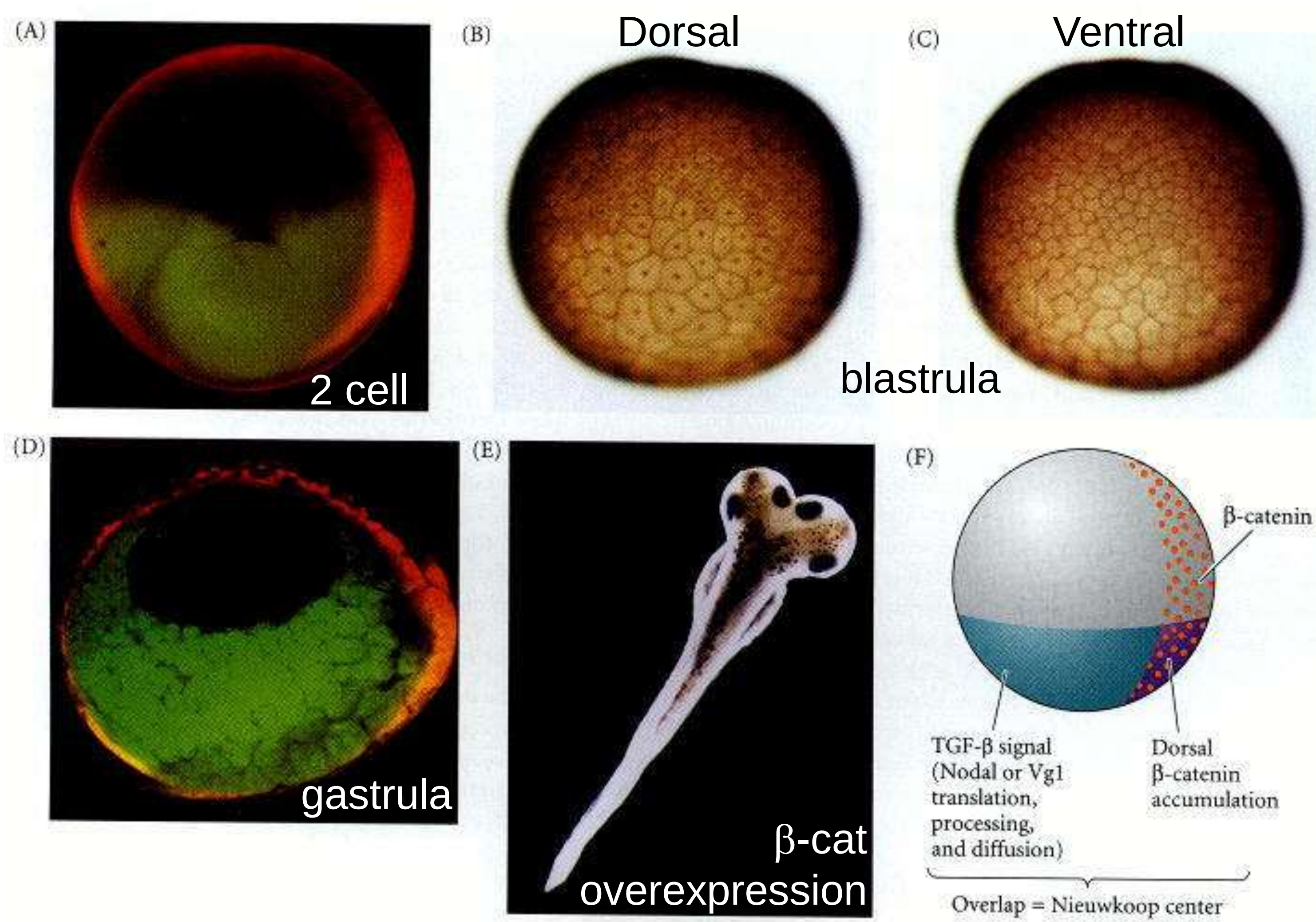
(B)



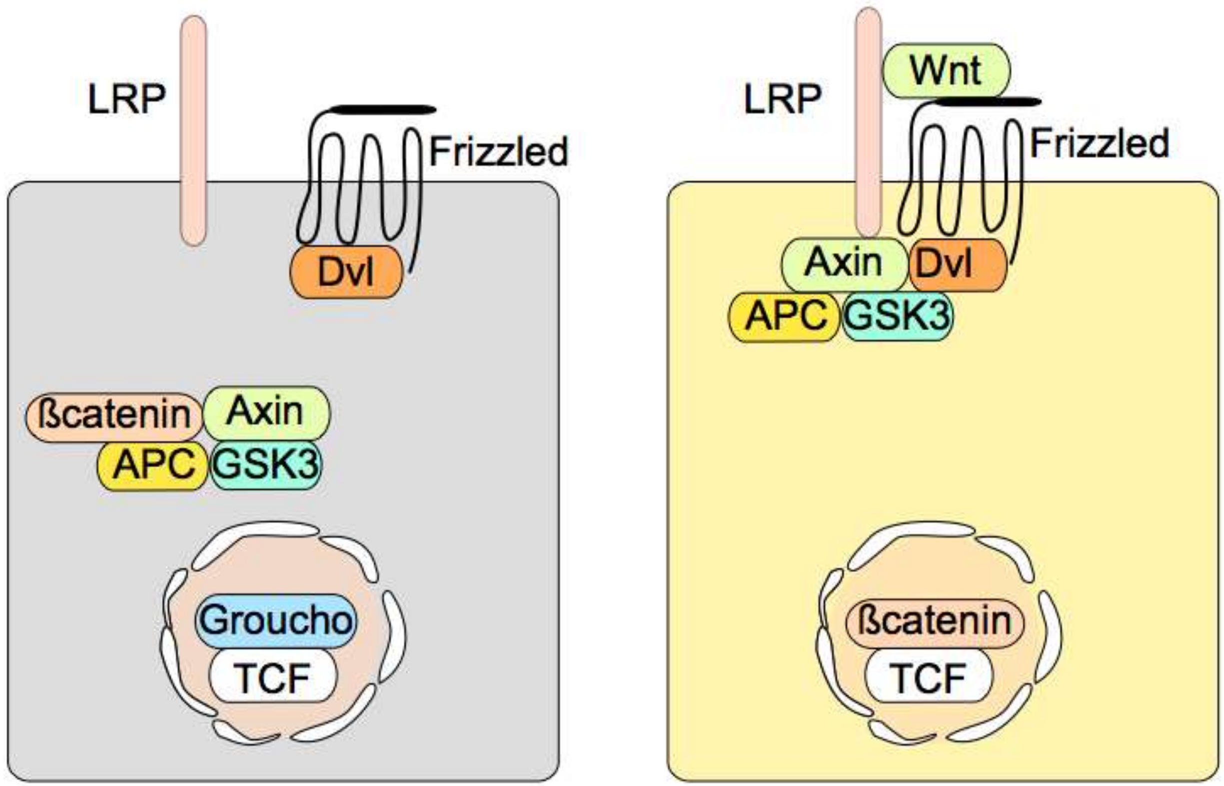
Percentage of total inductions

Dorsal	Intermediate	Ventral
77	23	0
11	61	28
5	45	50
16	42	42

Nieuwkoop center factor: β-catenin is important for DV patterning

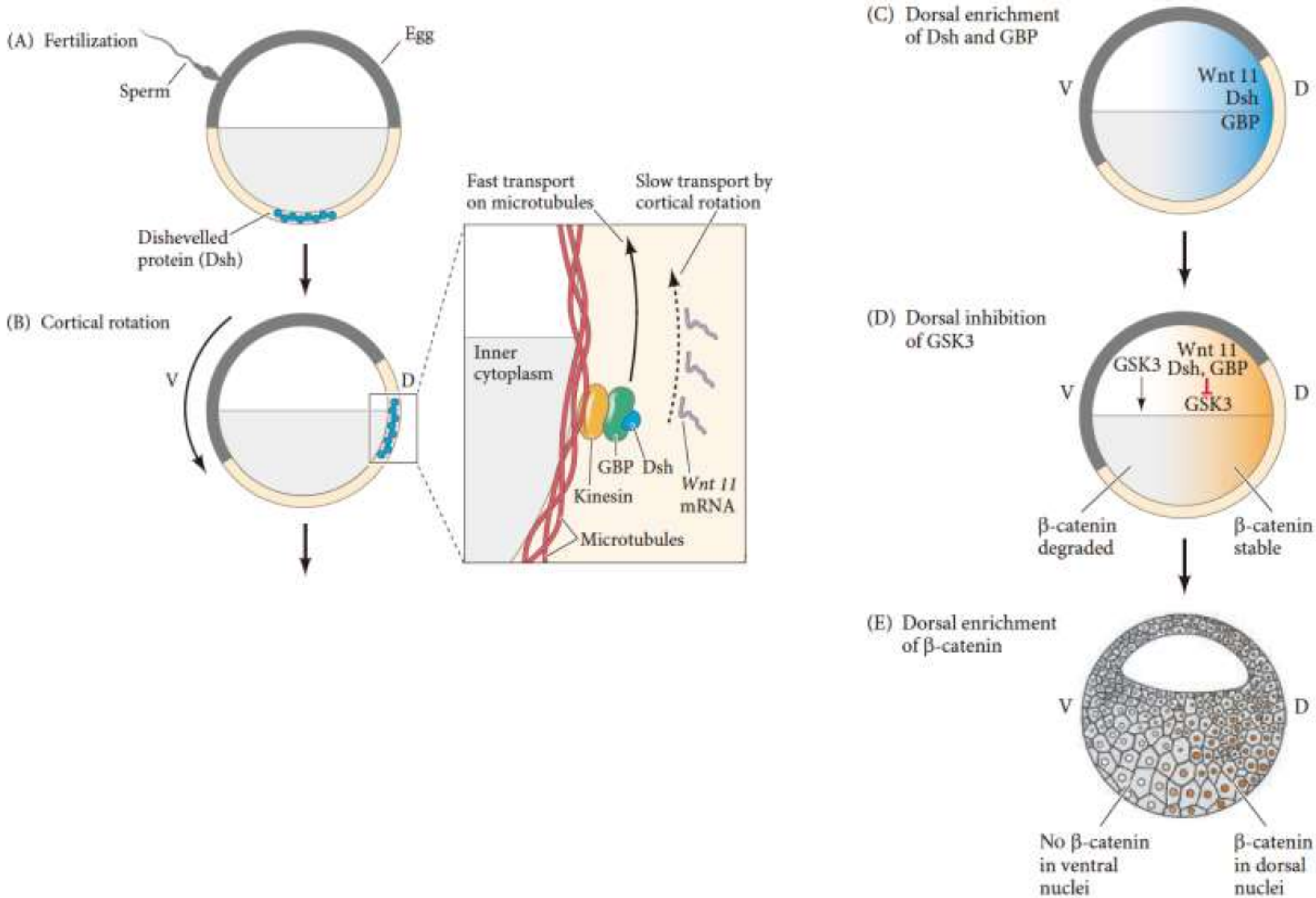


Wnt signaling

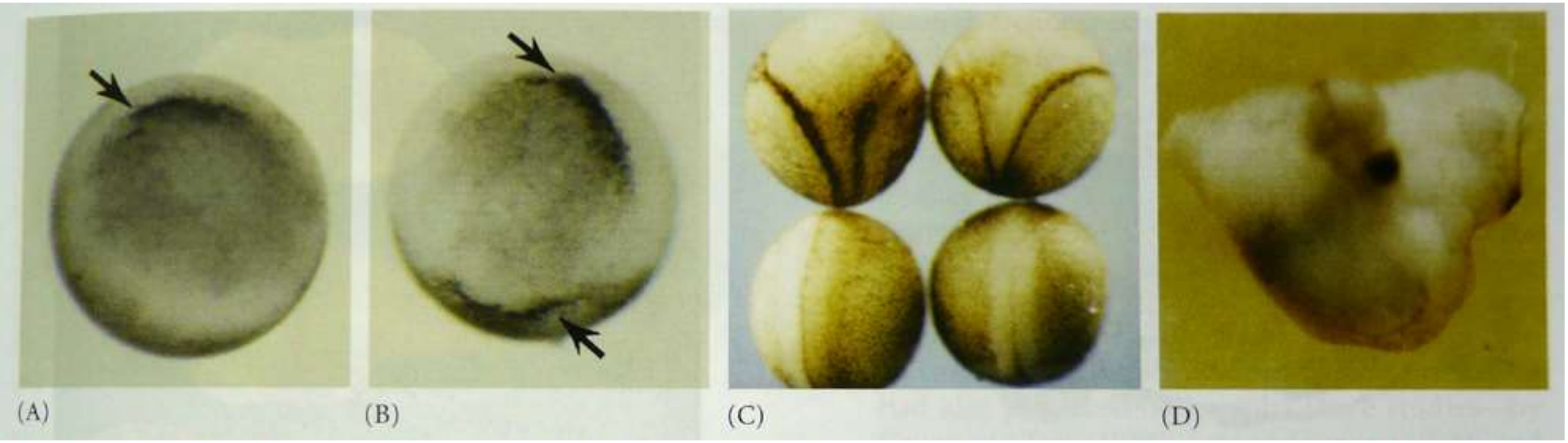


https://web.stanford.edu/group/nusselab/cgi-bin/wnt/node/261

Wnt signaling and DV patterning

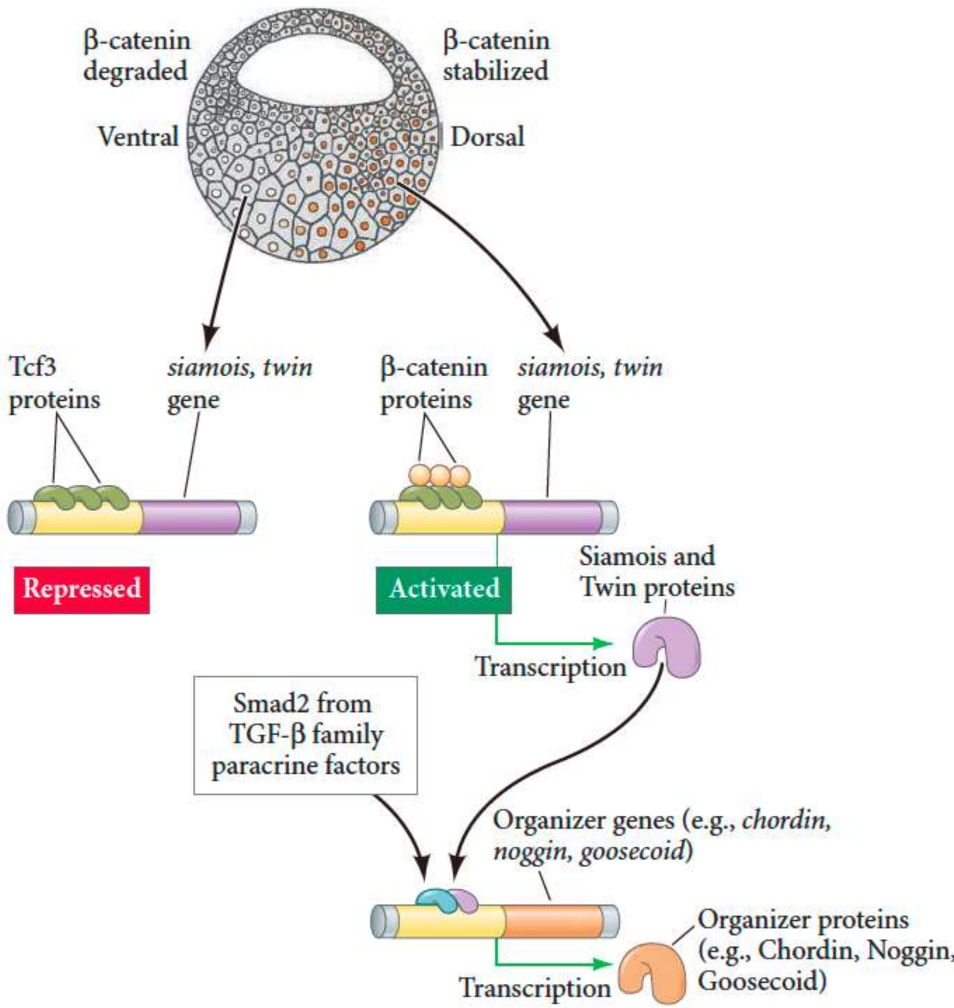


goosecoid, downstream gene of wnt pathway, can induce 2nd axis

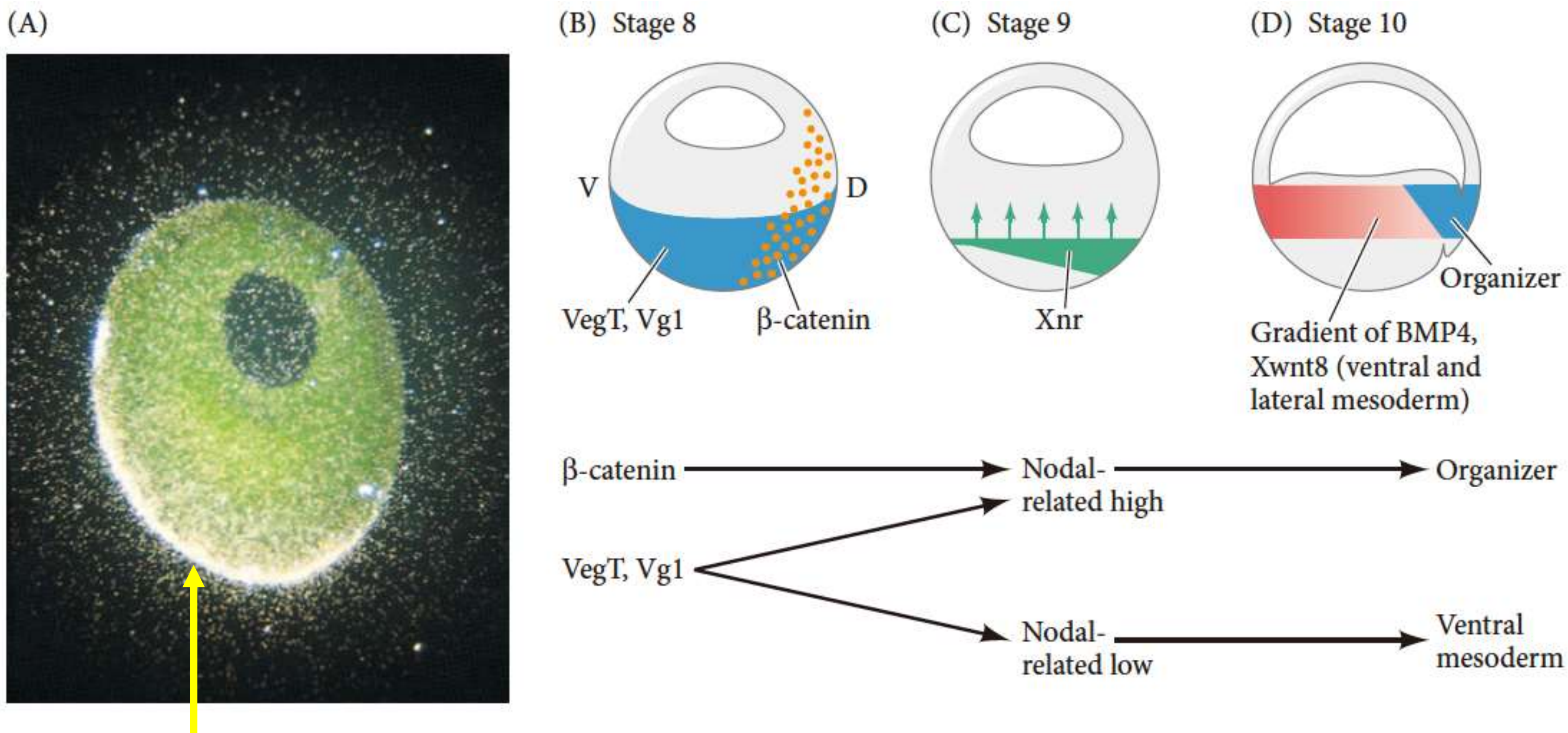


Wnt信号下游基因goosecoid能够诱导第二胚轴的形成

Hypothesis for organizer (dorsal mesoderm) induction



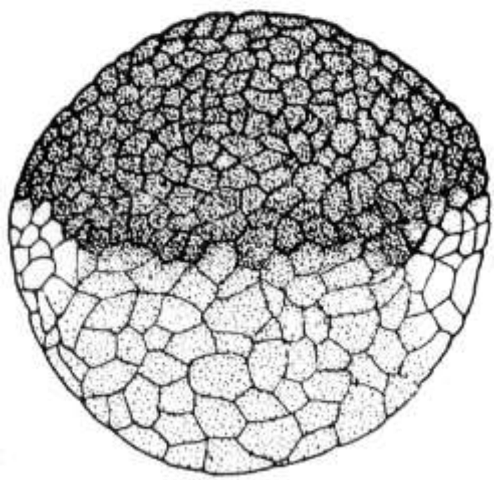
Model for mesoderm induction and organizer formation



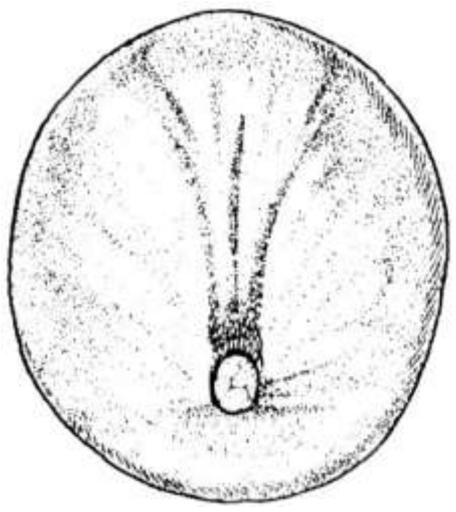
The maternal RNA encoding Vg1 is tethered to the vegetal cortex of a Xenopus oocyte.

How does organizer direct DV patterning (induce neural ectoderm)?

(组织者如何在背腹分化中起作用？)



St. 8



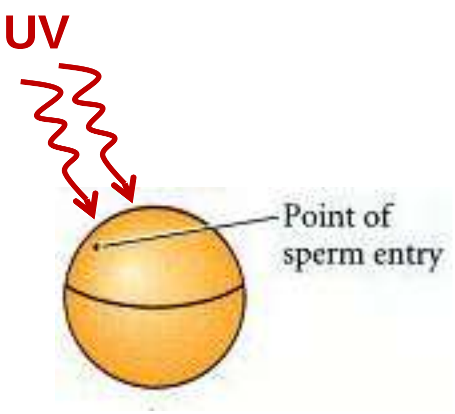
St. 12.5

Working strategy

Some molecular？

↓

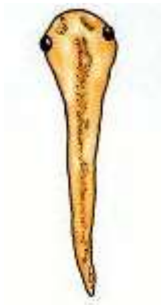
UV treated embryos

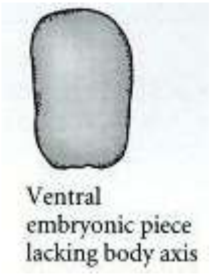


↓ Rescued (回复)

Yes → Dorsalizing factor

No → Not interested

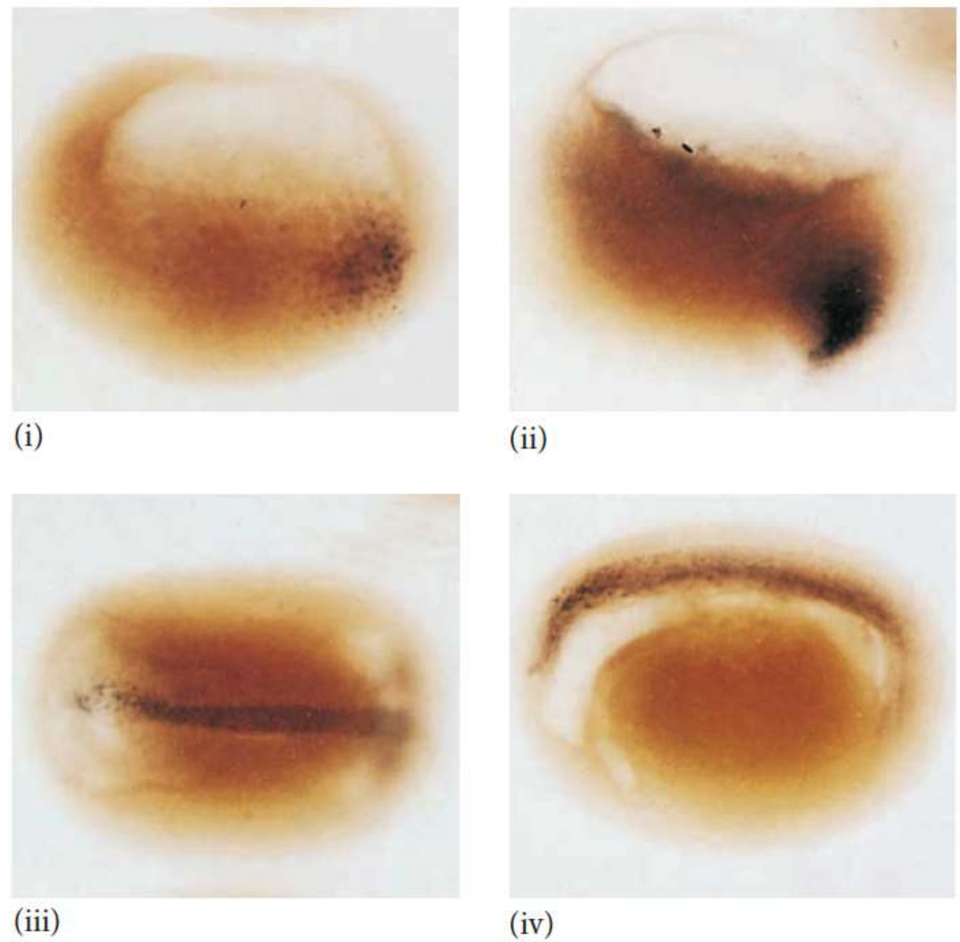




Dosalizing factor: Noggin

(A)

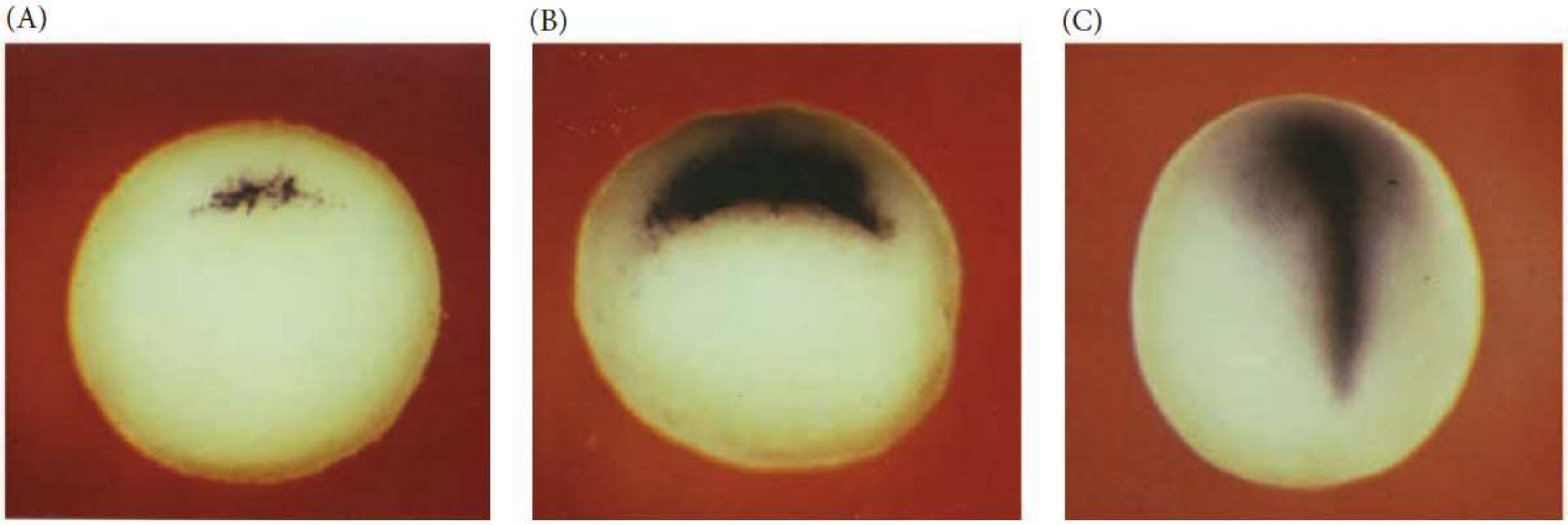
(B)



Rescue of dorsal structures by Noggin protein

Localization of *noggin* mRNA in the organizer tissue

Dosalizing factor: Chordin

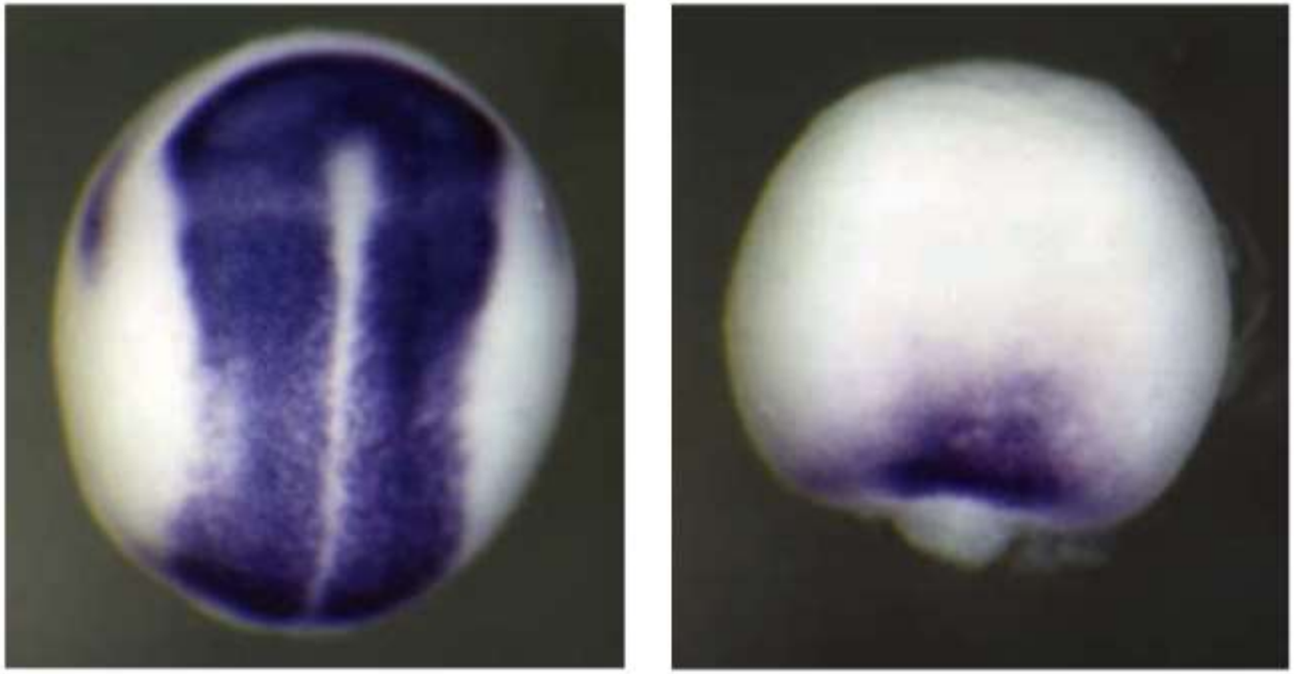


Localization of *chordin* mRNA in the organizer tissue

Knockdown of *chordin*, *noggin* and *follistatin* suppresses dorsal structure

ctrl

chd, *nog* and *fst mor*

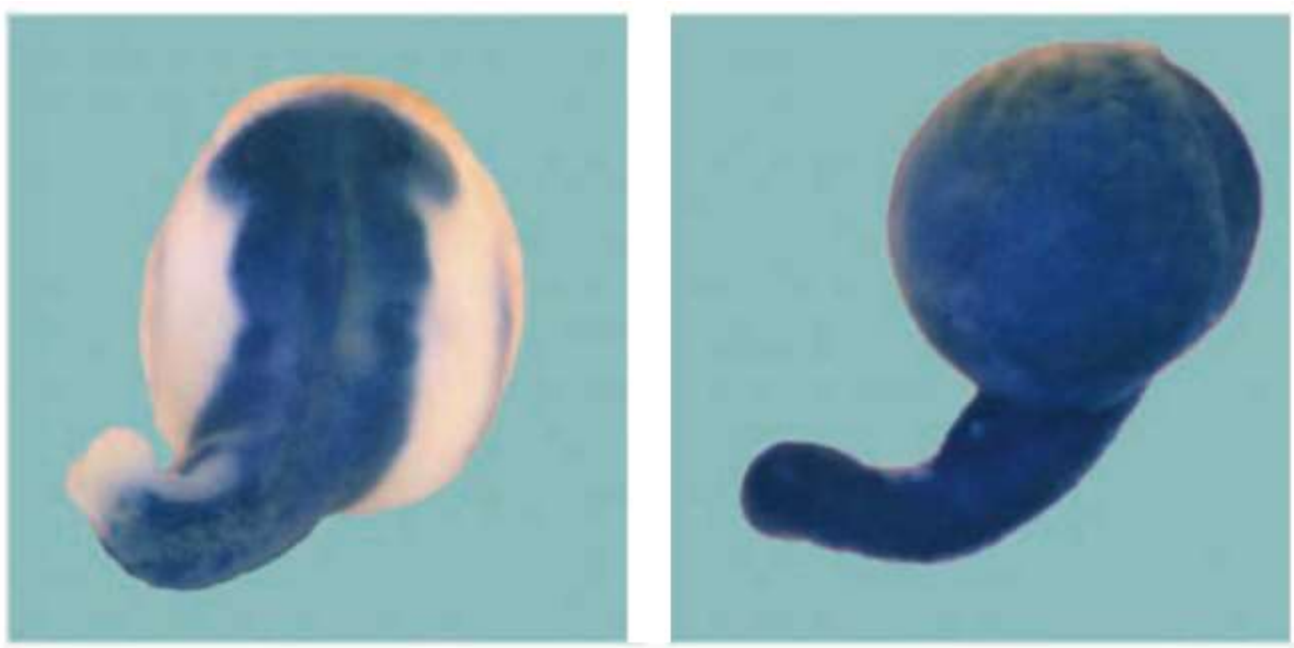


Sox2, marker for neural plate

Knockdown of *bmp2, bmp4* and *bmp7* enhances dorsal structure

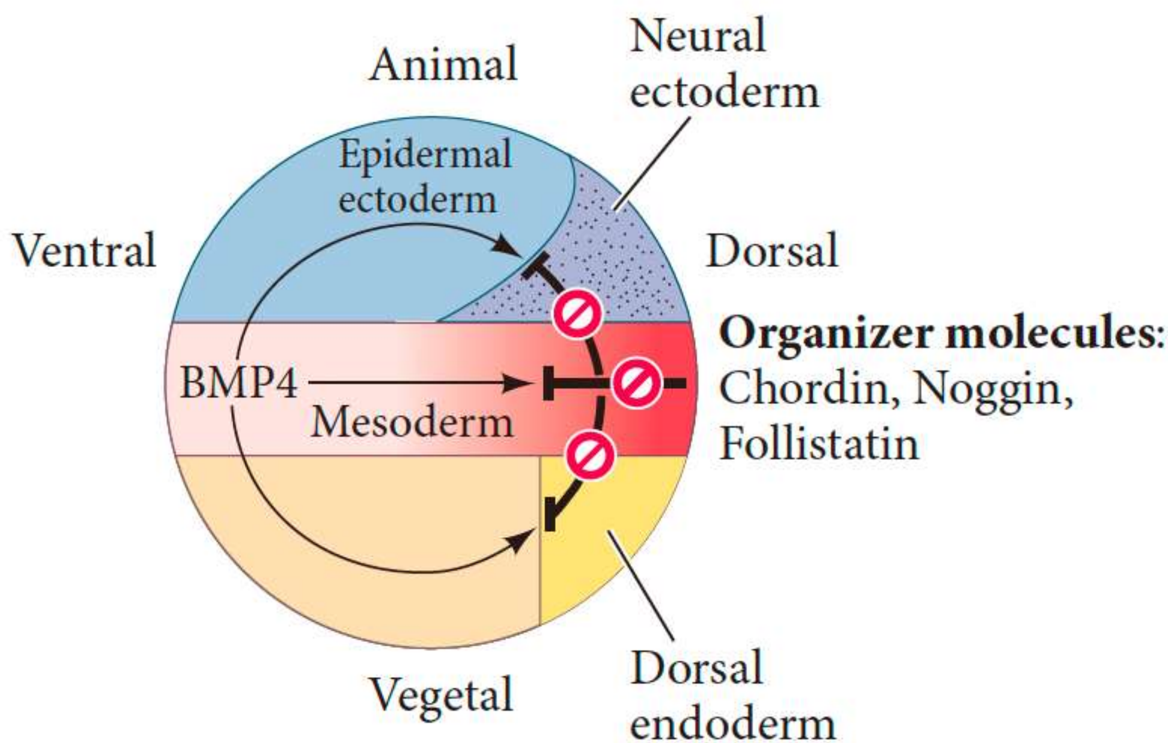
ctrl

bmp2, bmp4 and bmp7 mor

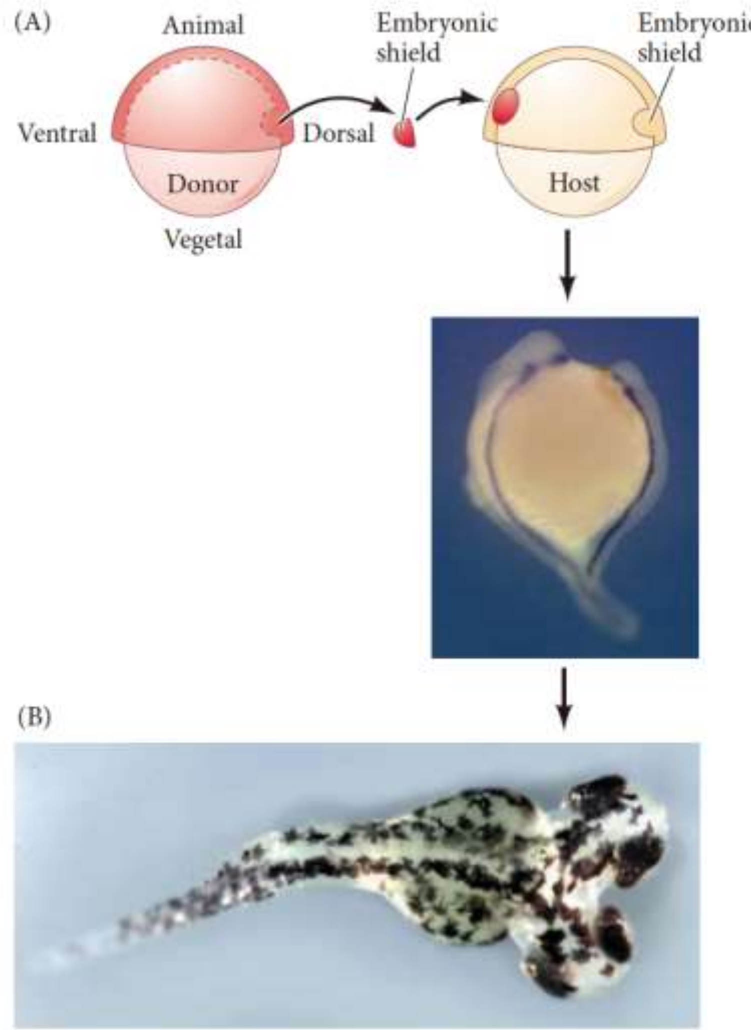


Sox2, marker for neural tissue

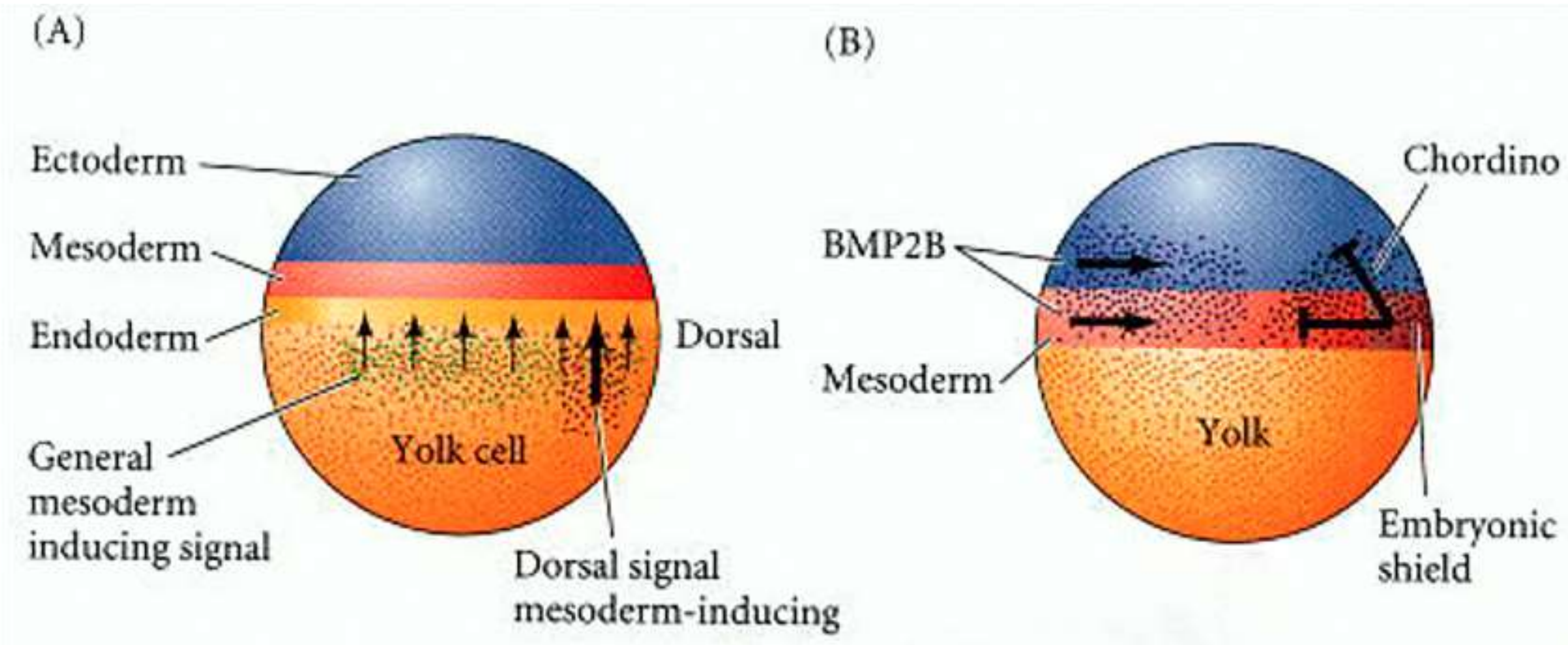
Mechanism of organizer's function in DV patterning



DV patterning in zebrafish embryonic development



Mechanism of DV patterning in zebrafish

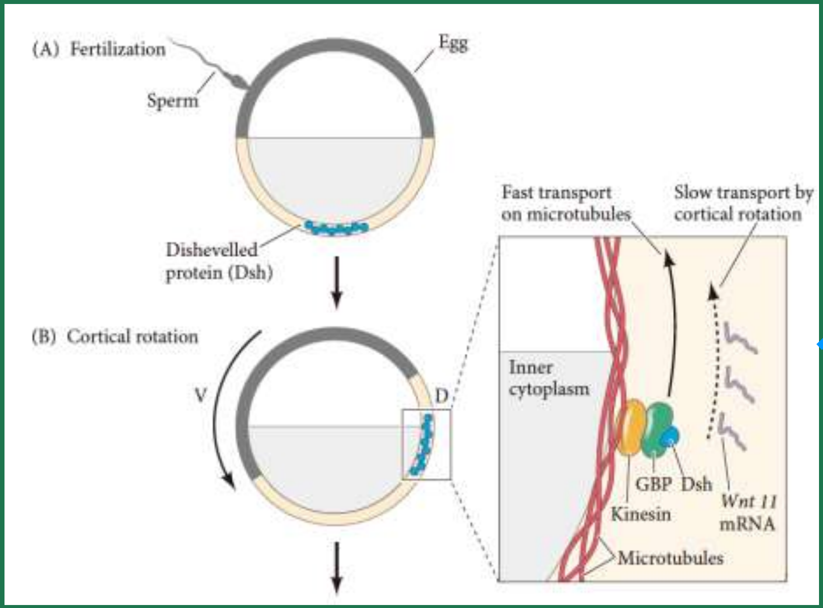


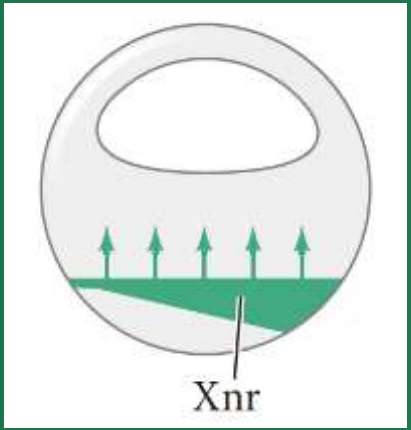
Summary (II)

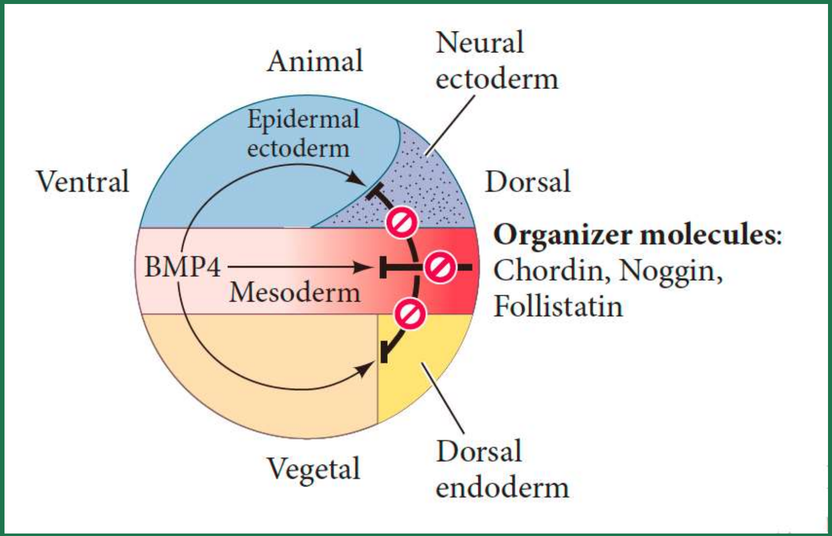
Key words:

- mesoderm induction (中胚层诱导)
- organizer (组织者)
- Dorso-ventral patterning (背腹分化)
- Morphogen (形态素)
- Cell signaling(信号通路): BMP, Nodal, Wnt

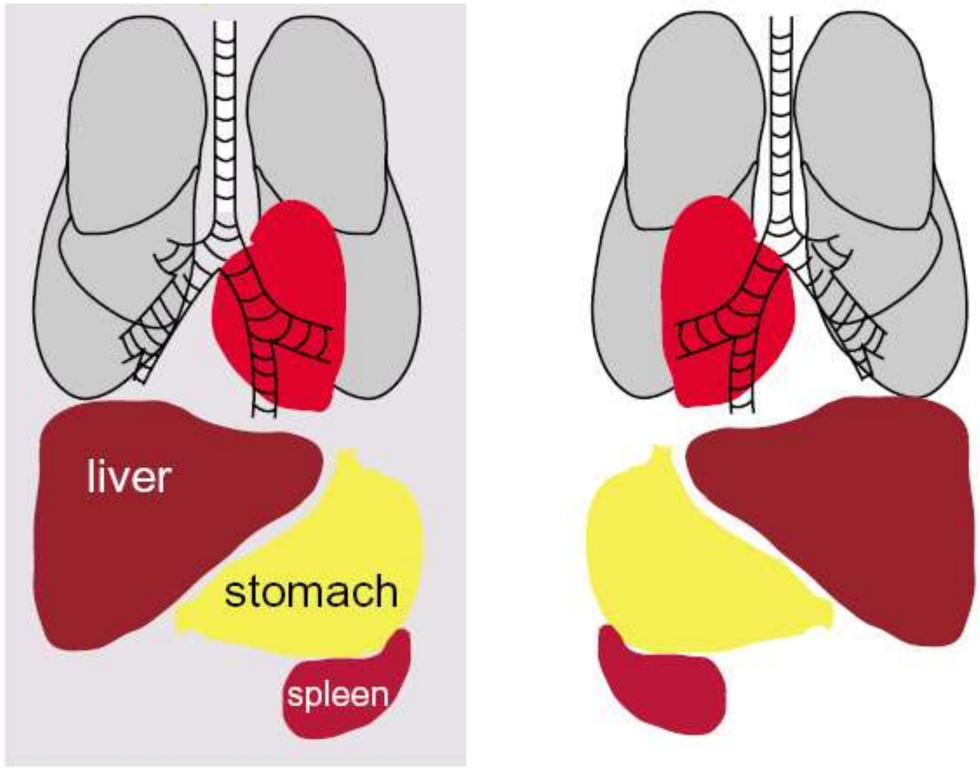
Graph Summary







LR defect and human disease

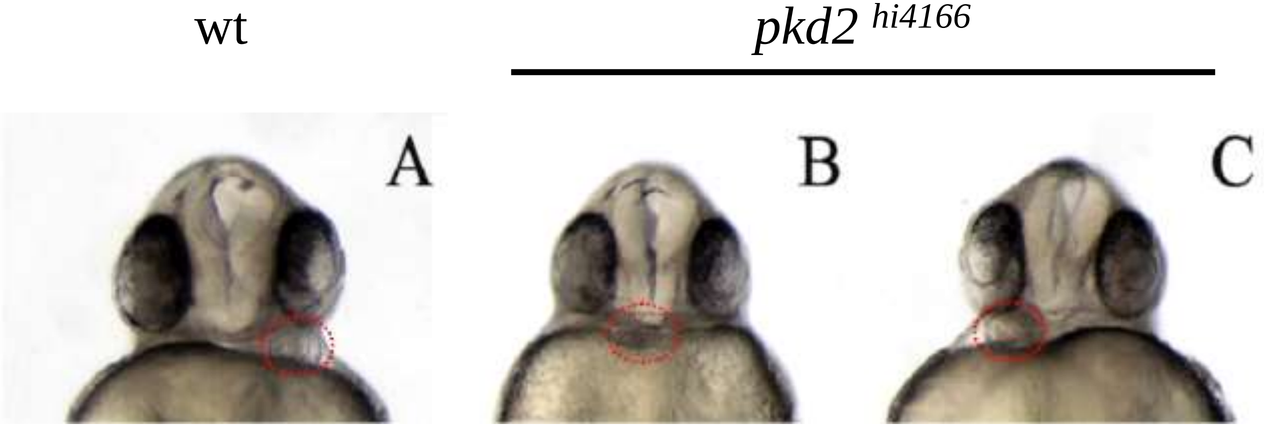


Normal–*situs solitus*

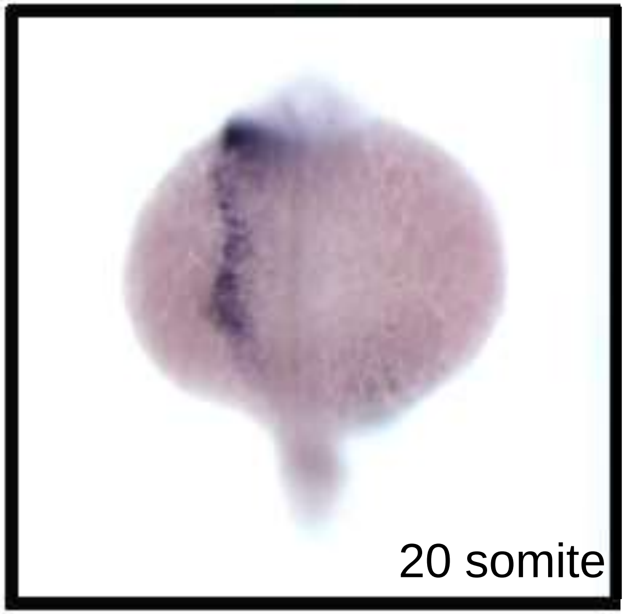
Situs inversus

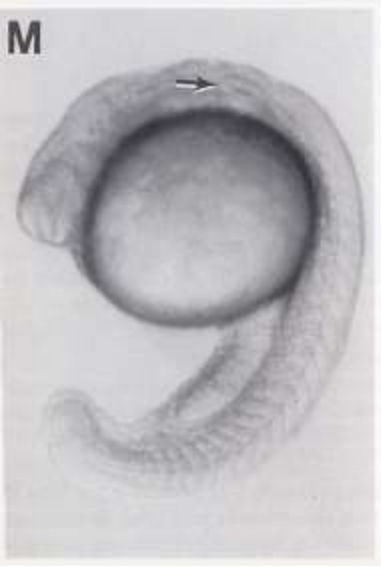
McGrath, 2003

LR patterning



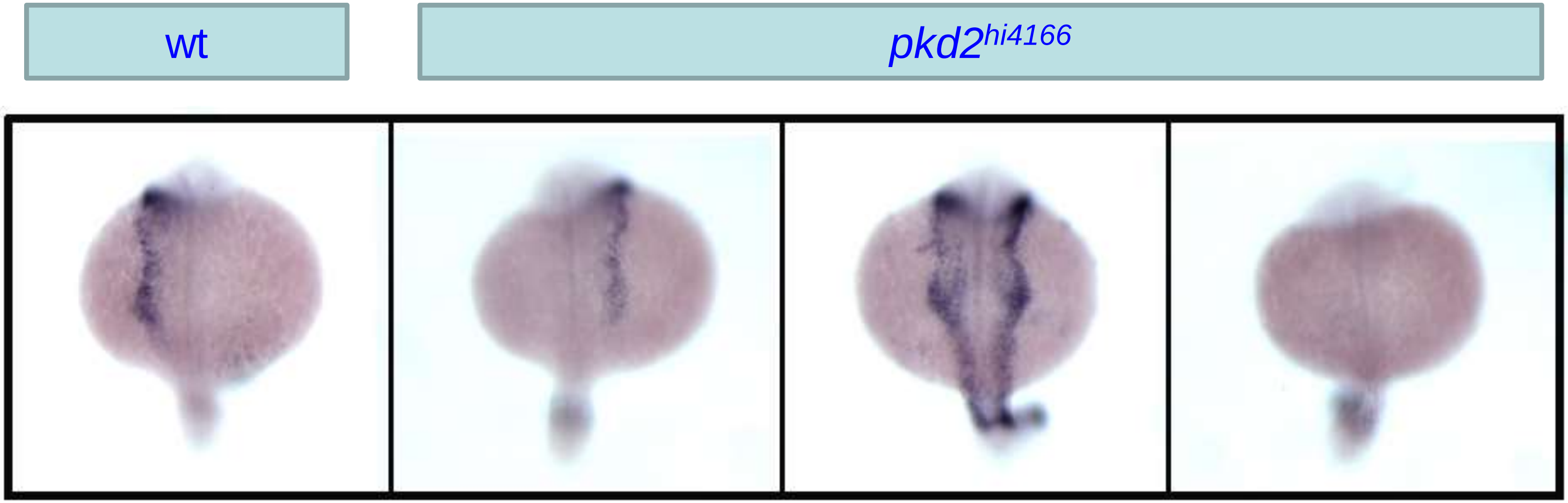
LR patterning is controlled by nodal signaling



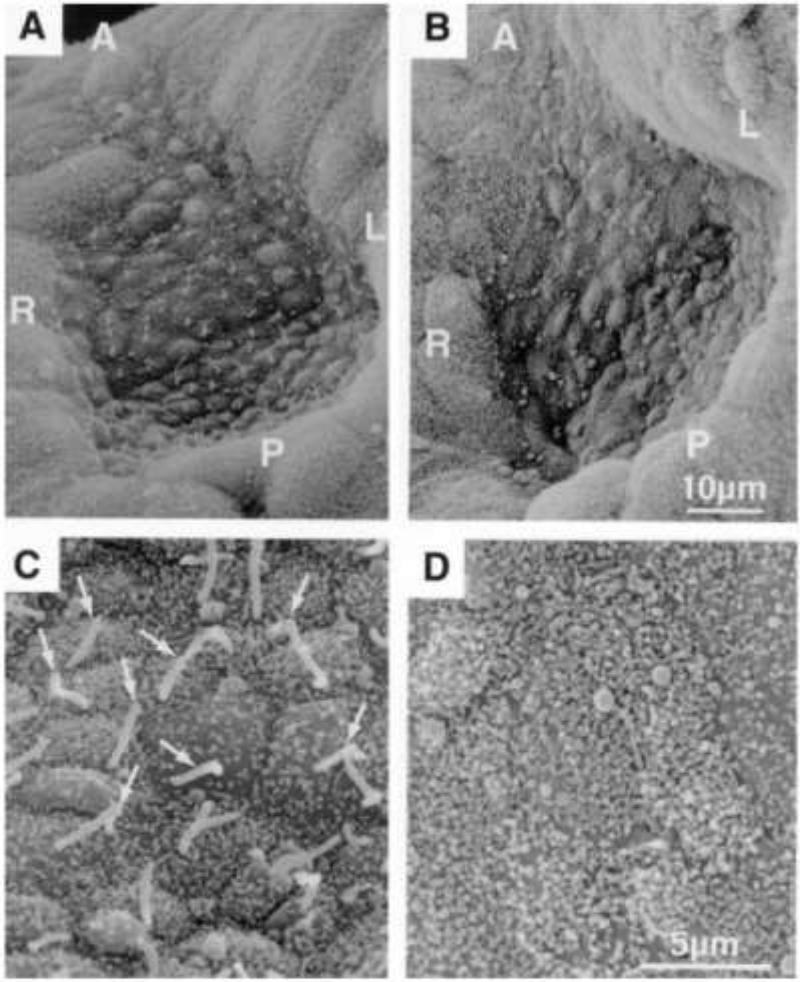


Southpaw, one Nodal related gene,
expresses on left side of zebrafish embryos

spaw expression is radomized in LR mutant

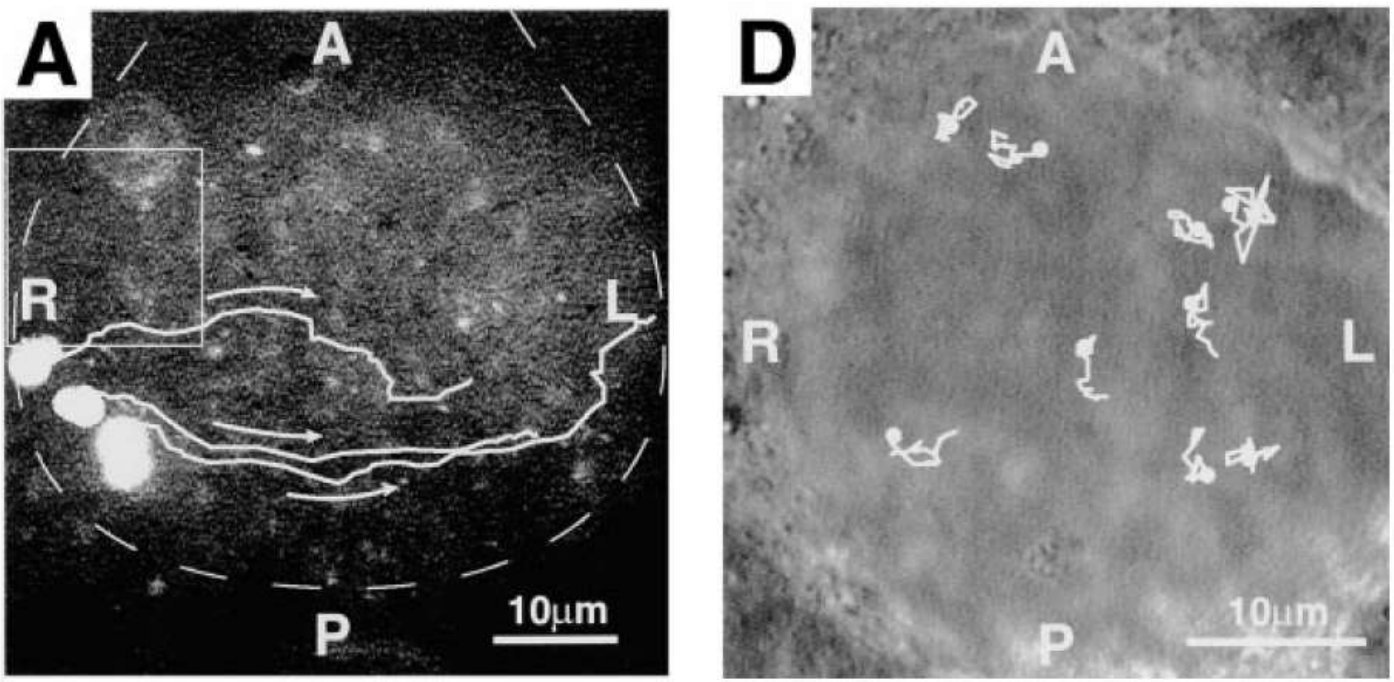


Absence of the Nodal cilia in *Kif3B*$^{-/-}$ mutant



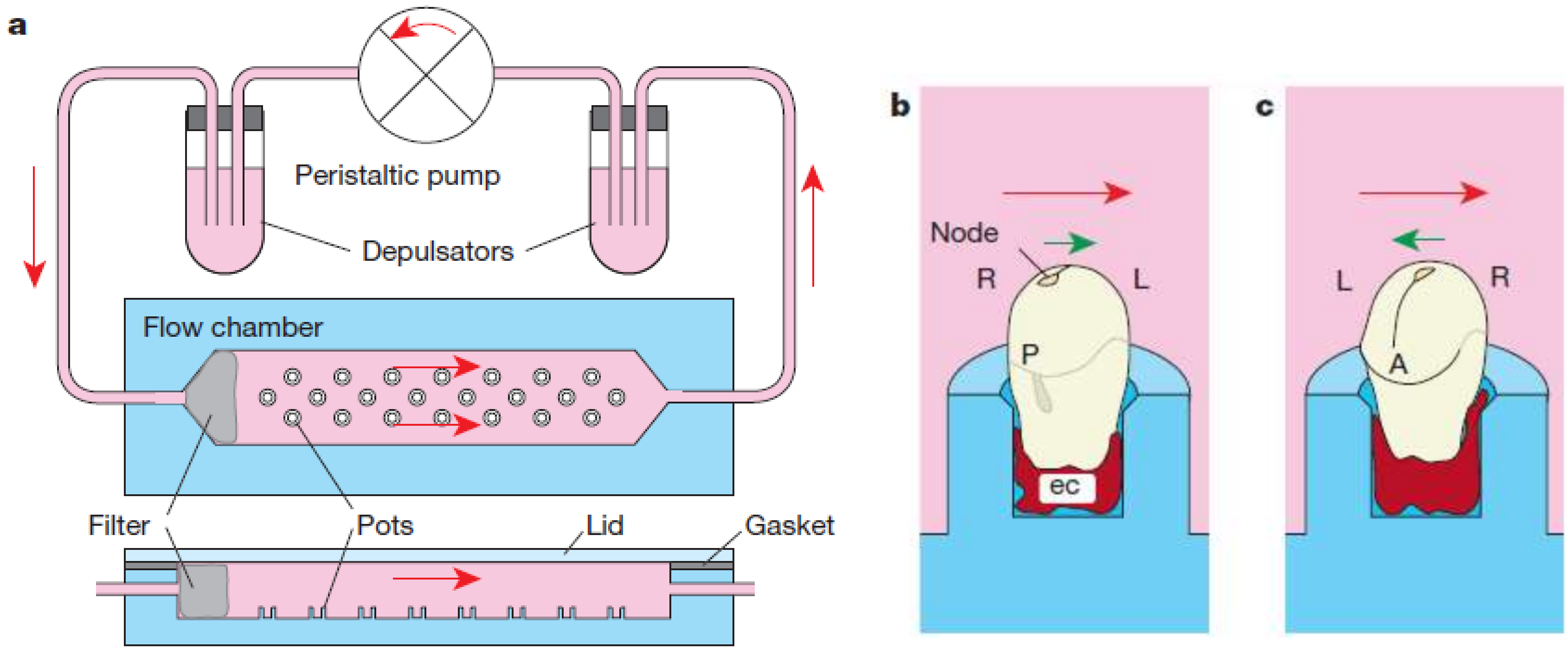
Nonaka *et al.*, 1998 Cell

Leftward flow in wt mouse node while not *Kif3b*$^{-/-}$ node



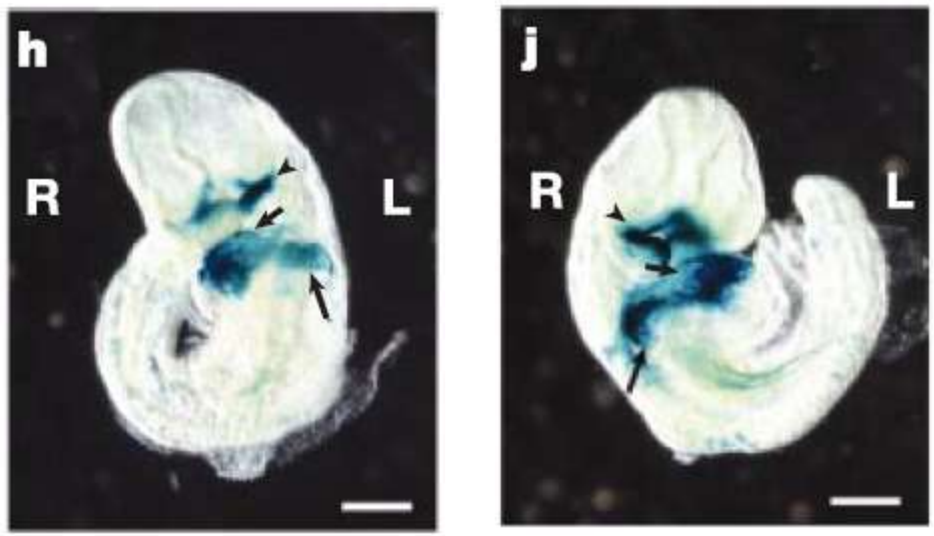
Nonaka *et al.*, 1998 Cell

Devise for artificial nodal flow

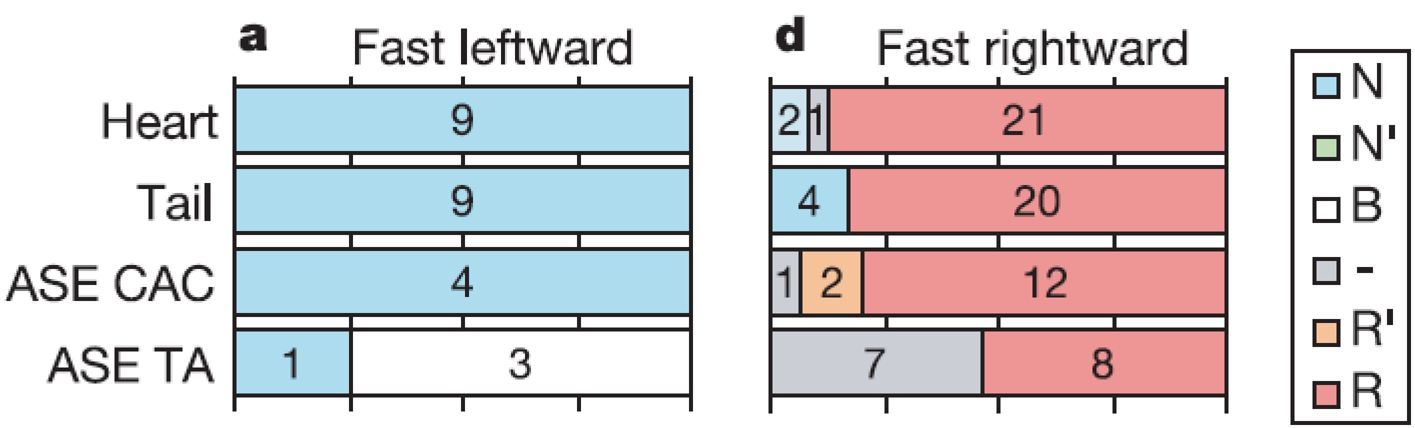


Nonaka *et al.*, 2002 Nat.

LR patterning is reversed by artificial nodal flow

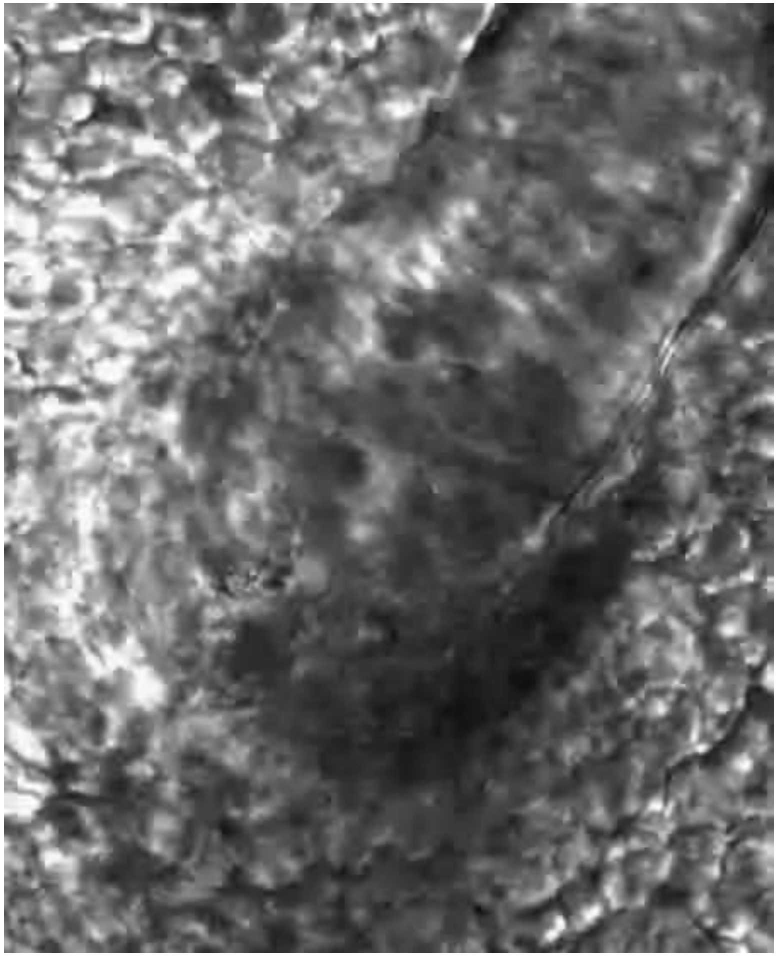


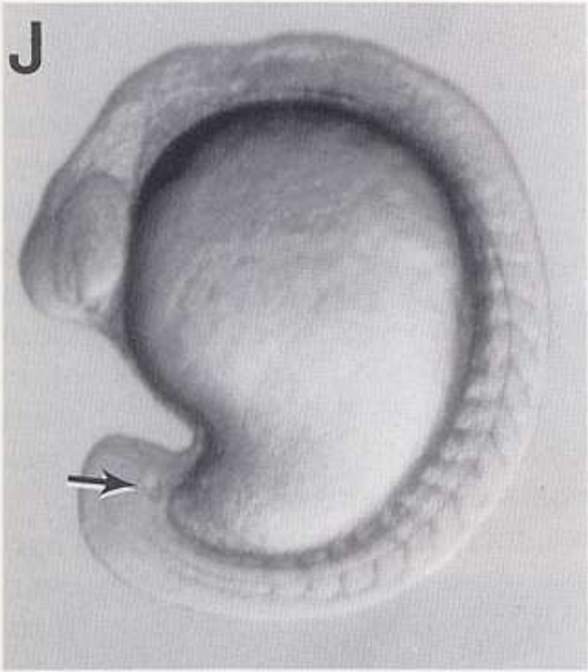
Pitx2-lacZ



Nonaka *et al.*, 2002 Nat.

LR is controled by flow in Kupffer's vesicle





15-somites

Model for LR patterning in zebrafish

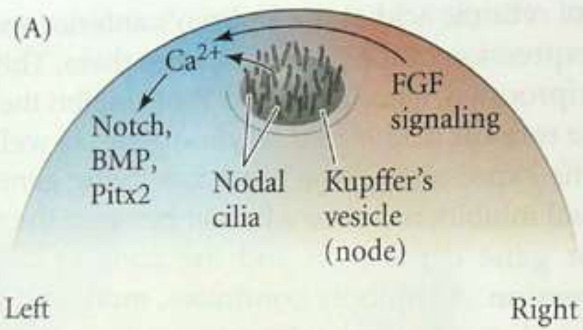
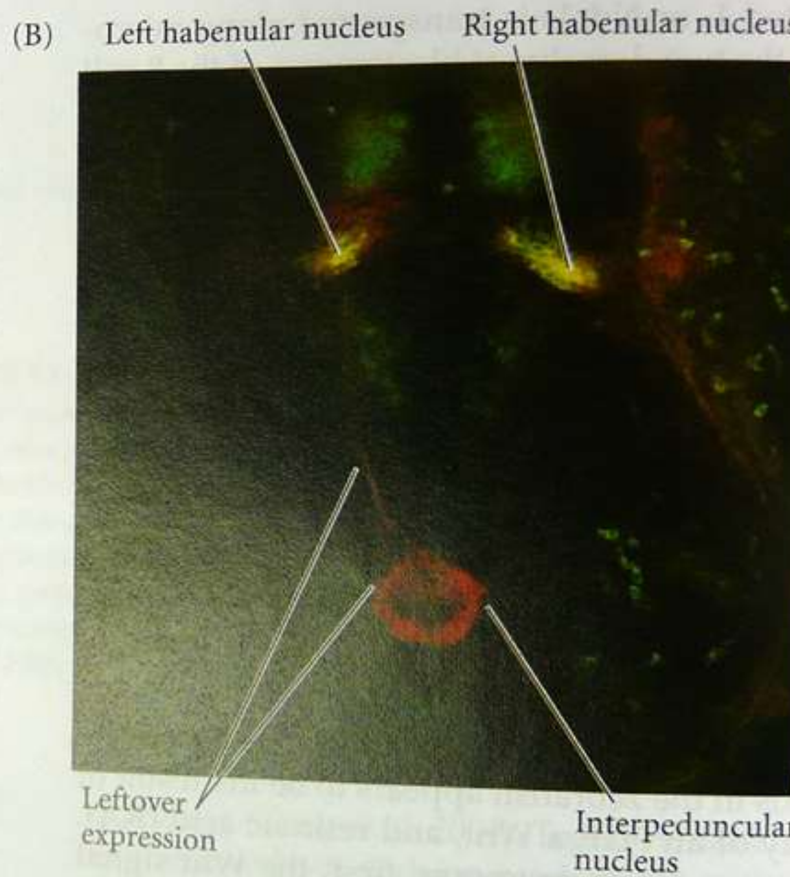


FIGURE 11.13 Left-right asymmetry in the zebrafish embryo. (A) Model for asymmetric gene expression. Nodal cilia in Kupffer's vesicle create a current that causes the release of Ca^{2+} on the embryo's left side. Calcium ions stimulate Notch and BMP4 pathways on the left side and activate the Pitx2 transcription factor in the left-hand mesoderm (blue). FGF expression is seen predominantly on the right-hand side (red). (B) Brain asymmetry in zebrafish. Antibody staining of the Leftover (red) and Right-on (green) proteins in neurons of the habenular nucleus (a behavior-controlling region of the zebrafish forebrain) and the axonal projections to their midbrain target (the interpeduncular nucleus) reveals marked asymmetry. Most Leftover-positive axons emerge from the left habenula to innervate the target. (A after Okada et al. 2005; B from Gamse et al. 2005, photograph courtesy of M. Halpern.)



Model for LR patterning

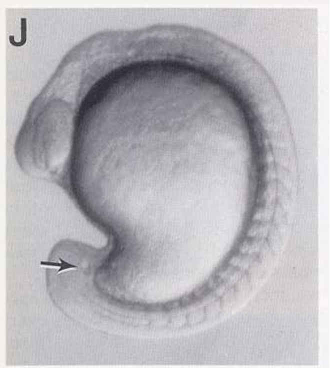
Flow driven by cilia

↓

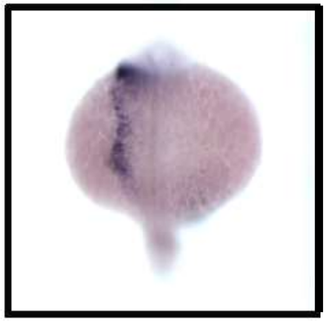
Nodal genes express on left side

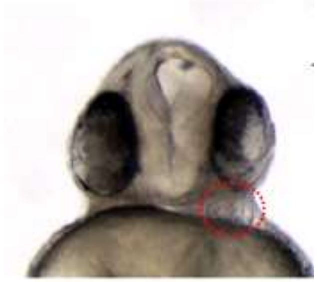
↓

LR patterning



15-somites





Mechanism of AP patterning in zebrafish

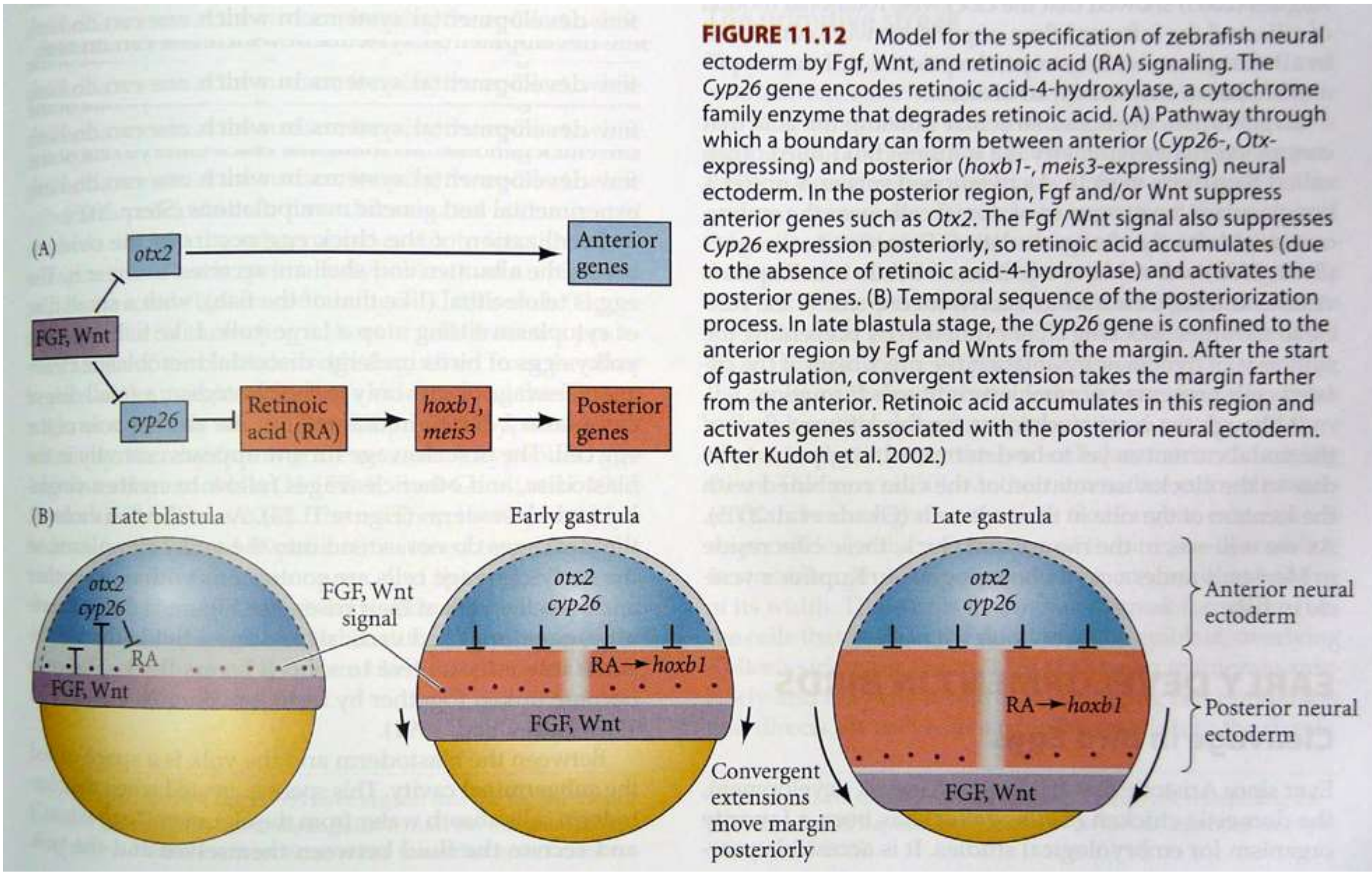


FIGURE 11.12 Model for the specification of zebrafish neural ectoderm by Fgf, Wnt, and retinoic acid (RA) signaling. The *Cyp26* gene encodes retinoic acid-4-hydroxylase, a cytochrome family enzyme that degrades retinoic acid. (A) Pathway through which a boundary can form between anterior (*Cyp26*-, *Otx*-expressing) and posterior (*hoxb1*-, *meis3*-expressing) neural ectoderm. In the posterior region, Fgf and/or Wnt suppress anterior genes such as *Otx2*. The Fgf /Wnt signal also suppresses *Cyp26* expression posteriorly, so retinoic acid accumulates (due to the absence of retinoic acid-4-hydroylase) and activates the posterior genes. (B) Temporal sequence of the posteriorization process. In late blastula stage, the *Cyp26* gene is confined to the anterior region by Fgf and Wnts from the margin. After the start of gastrulation, convergent extension takes the margin farther from the anterior. Retinoic acid accumulates in this region and activates genes associated with the posterior neural ectoderm. (After Kudoh et al. 2002.)

Summary (III)

- Key word:

  LR patterning, Shh Signaling, Nodal signaling, Cilia

- Event and mechanism:

  LR patterning, AP patterning