Model organisms and developmental biology

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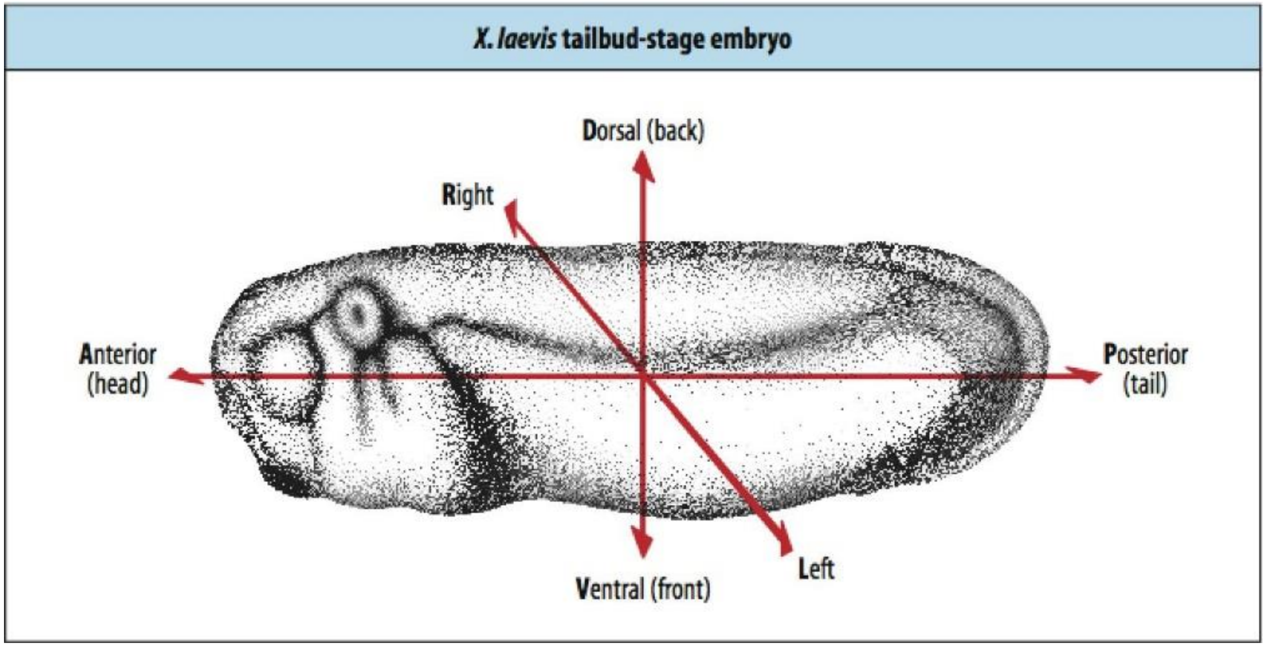
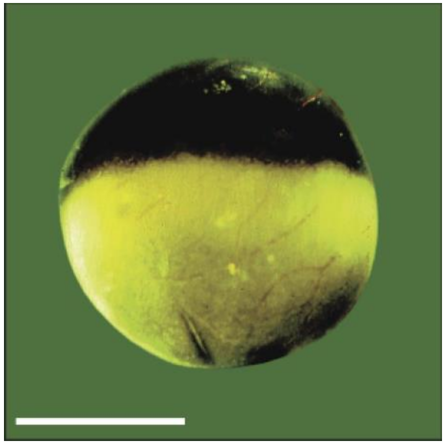
Pattern formation - Organization in time and space in large range

- Body plan, establishment of axes (antero-posterior, dorso-ventral, left-right), and germ layers.



The animal-vegetal axis is maternally determined in *Xenopus* and zebrafish

- 1. The animal pole is uppermost.
- 2. The egg nucleus is located close to animal pole.
- 3. The *Xenopus* egg contains large amounts of maternal mRNAs and proteins. For example, there is sufficient histone protein for the assembly of more the 10,000 nuclei.
- 4. Most of the developmentally important products end up in the vegetal half.

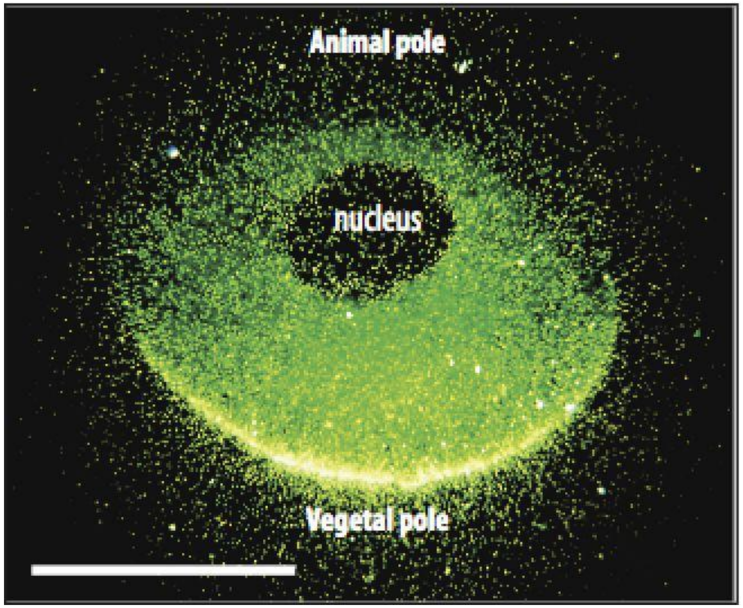


Localization of maternal mRNAs

- Early in oogenesis, via the message transport organizer region (METRO), which is associated with the mitochondrial cloud. The mechanism is not clear. It may involve cytoskeleton and small vesicles of endoplasmic reticulum.

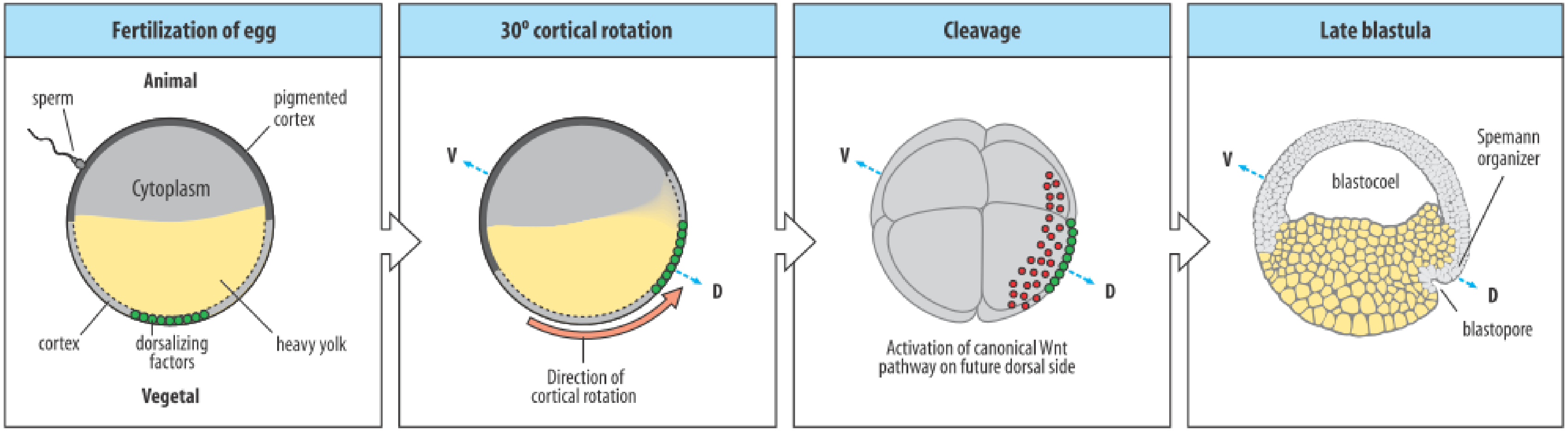
- Later in oogenesis, mRNAs are transported to the vegetal cortex along microtubules by kinesin motor proteins.

Usually the maternal mRNAs are only translated after fertilization

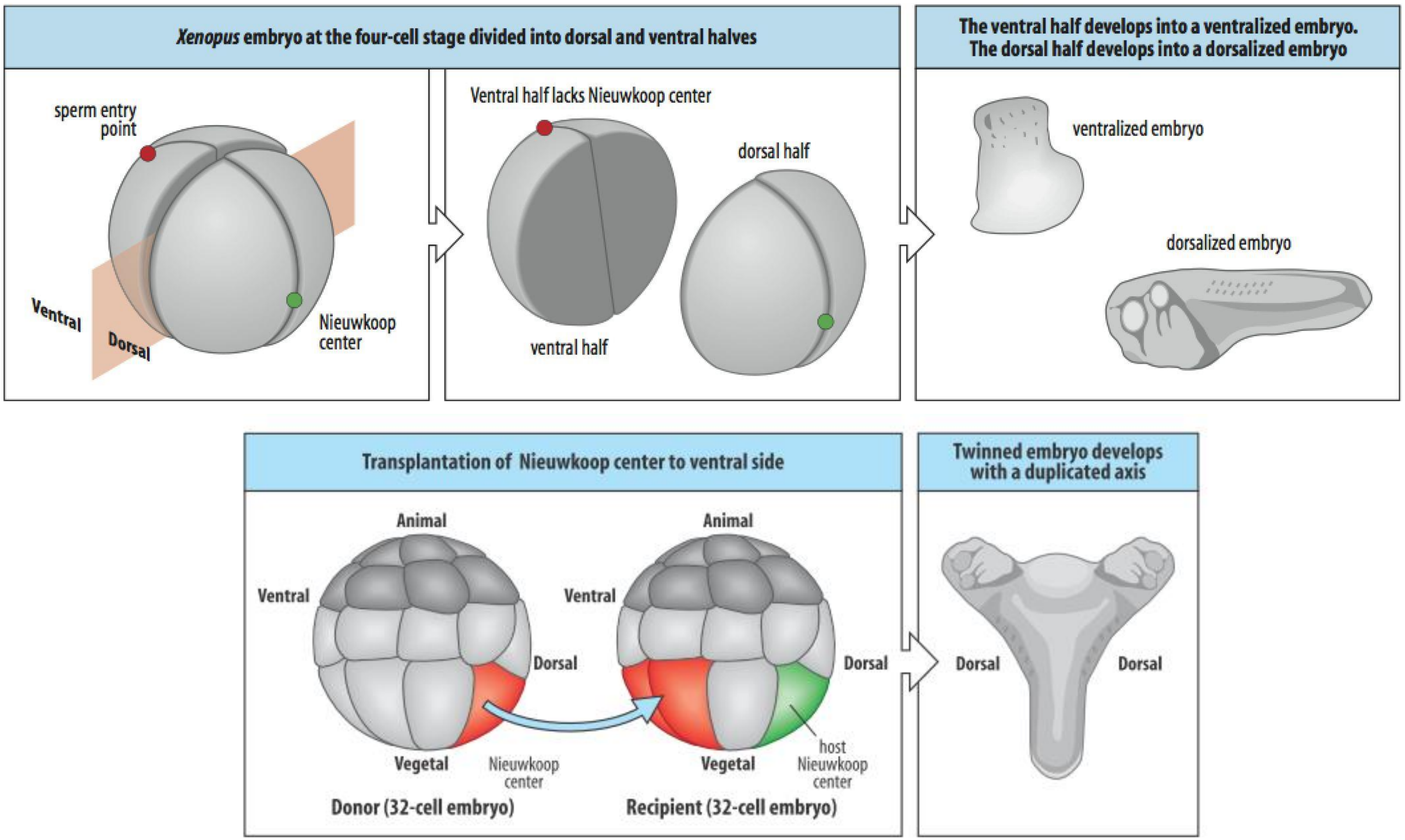


- *Vg-1* (left panel), mesoderm induction.
- *Xwnt-11*, dorso-ventral axis.
- *VegT*, endoderm and mesoderm specification.

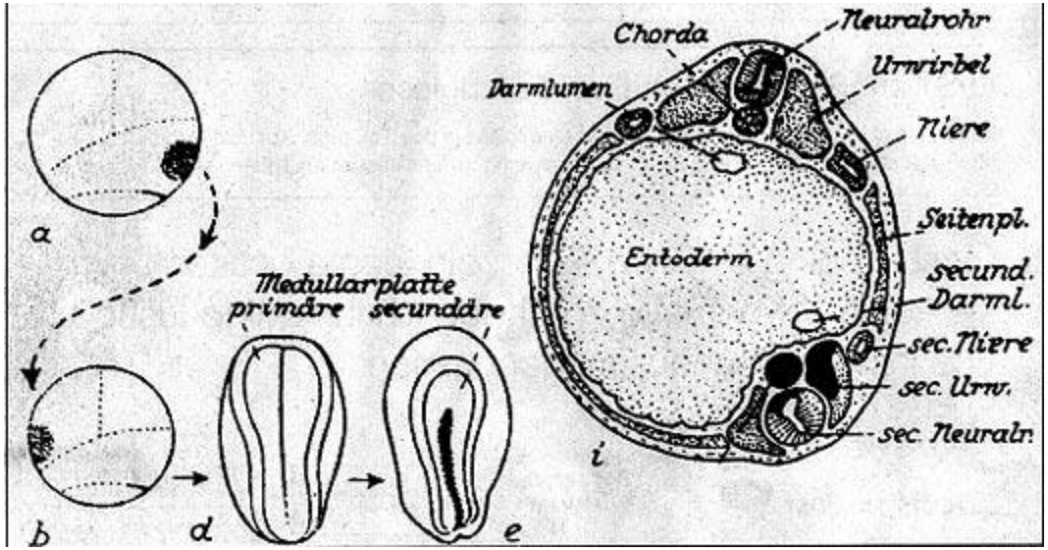
The entry of sperm triggers a series of events, including cortical rotation



Nieuwkoop center is related to dorsalization



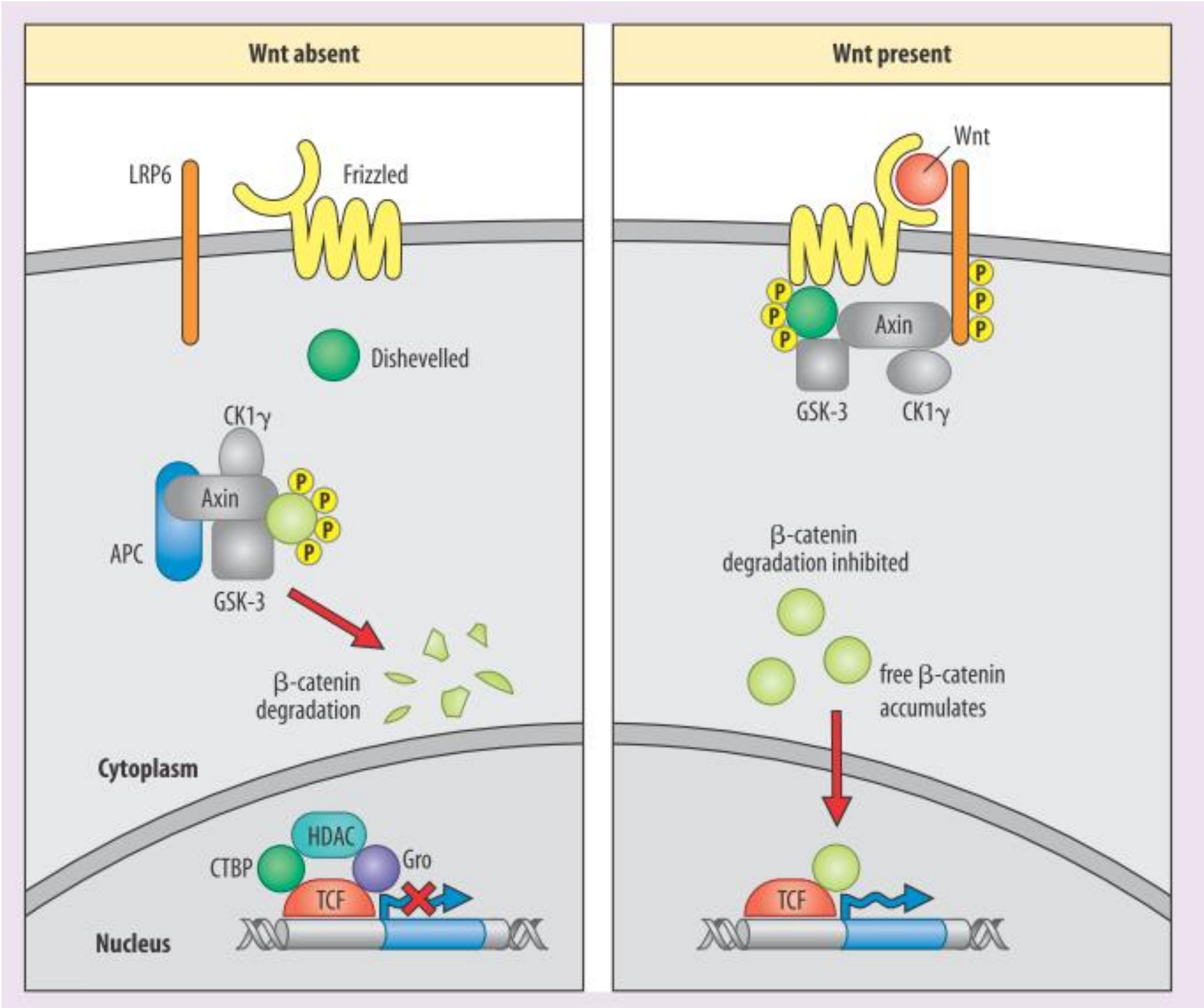
The Discovery of Induction (诱导) (1924)



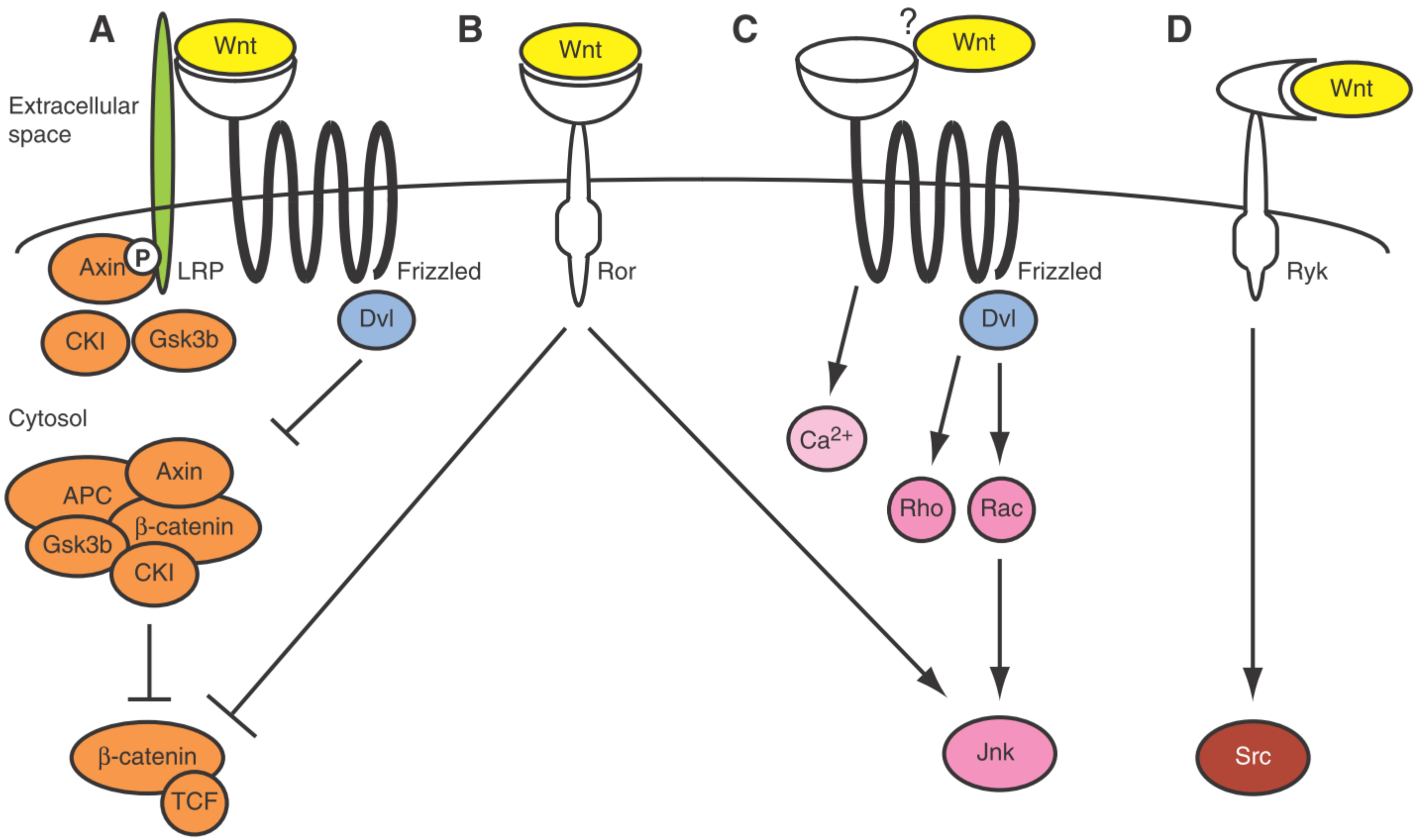
Hans Spemann

Hilde Mangold

Wnt signal pathway

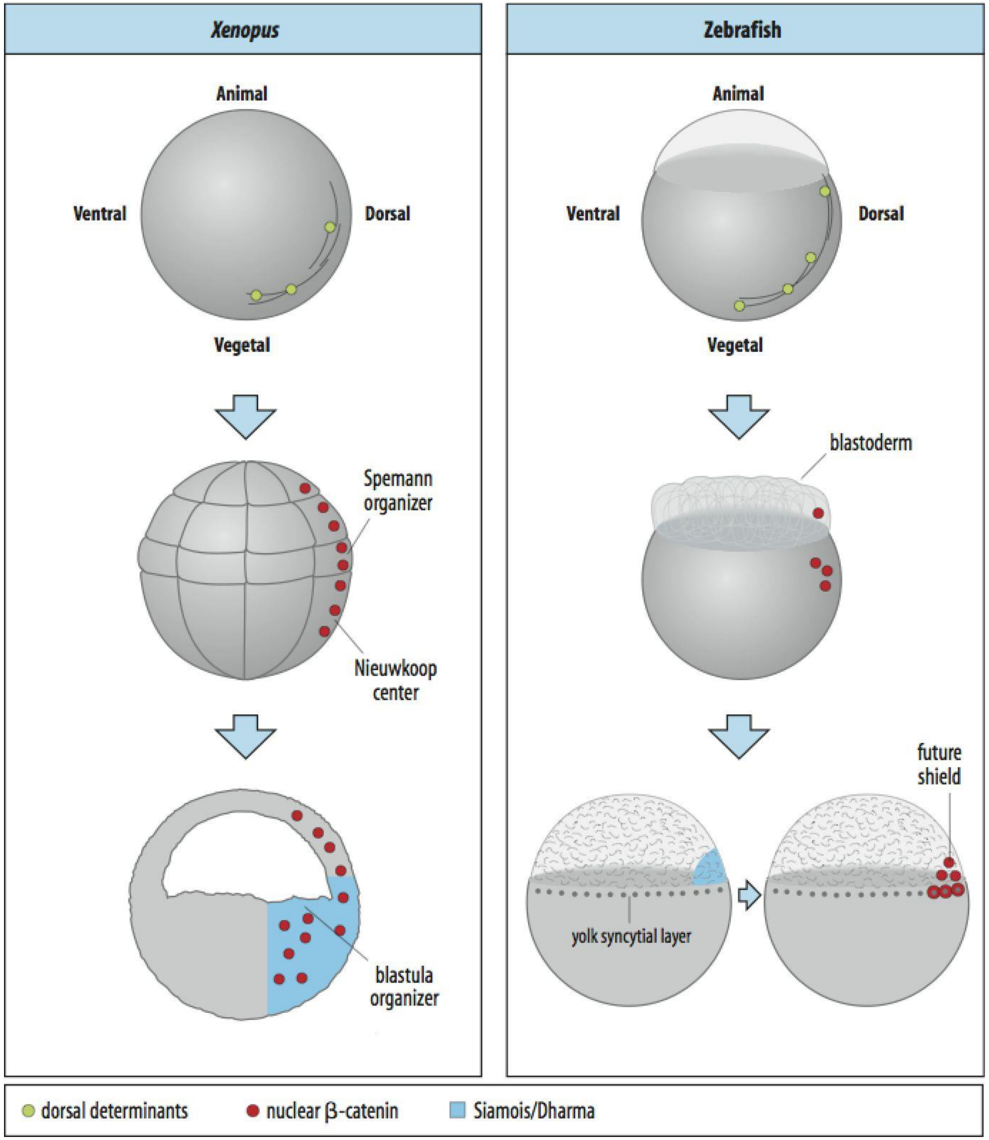


Wnt pathway

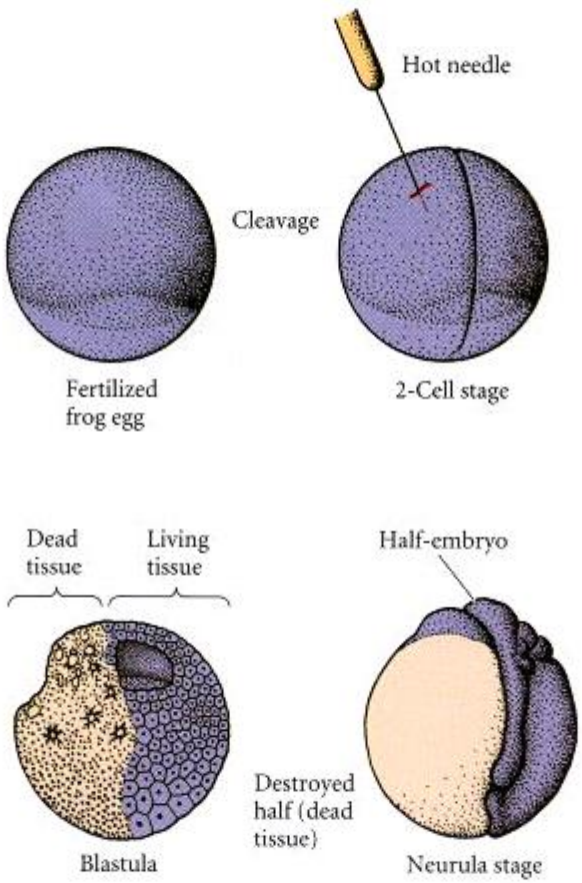


Amerongen and Nusse, *Development*, 2009

A comparison of the establishment of the dorsal organizer in Xenopus and zebrafish embryos

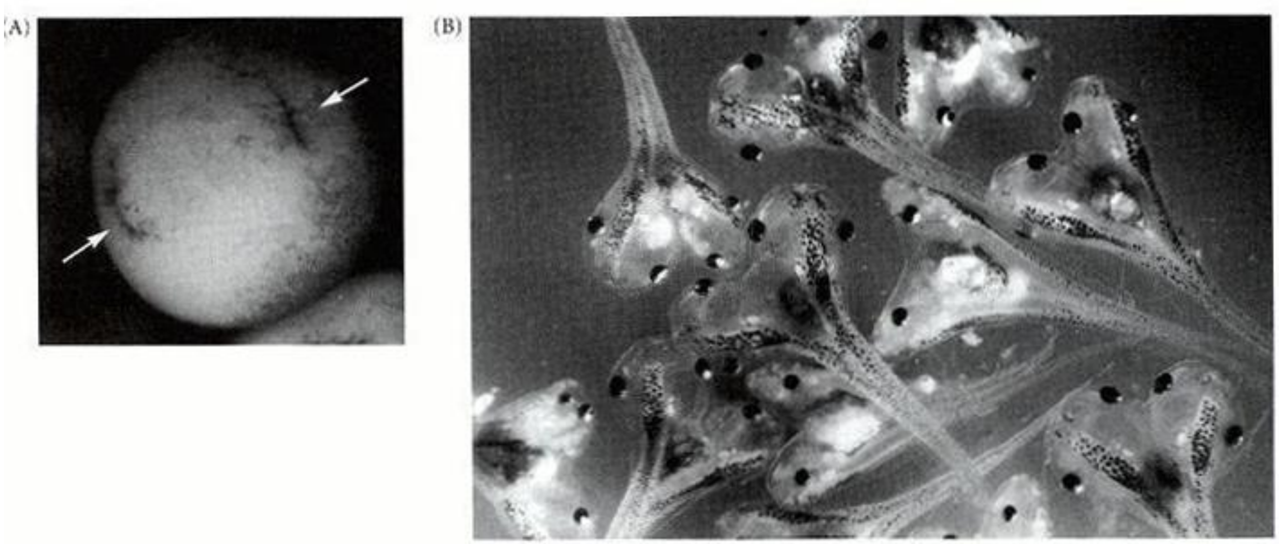


Mosaic Model of Development (1888)



Wilhelm Roux

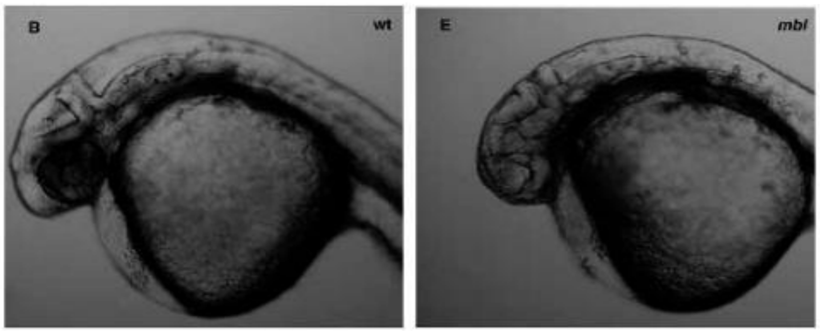
Rearrangement of the Egg Cytoplasm



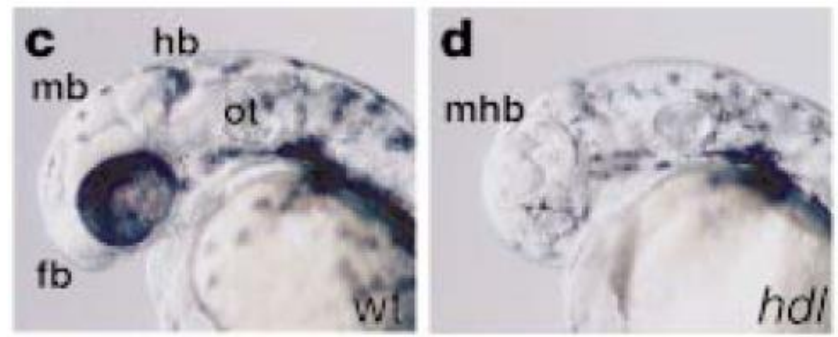
After the initial sperm-directed rotation occur, fertilized eggs were mounted in gelatin and rotated.

Over-activation of Wnt signaling leads to anterior defects

mbl (axin)



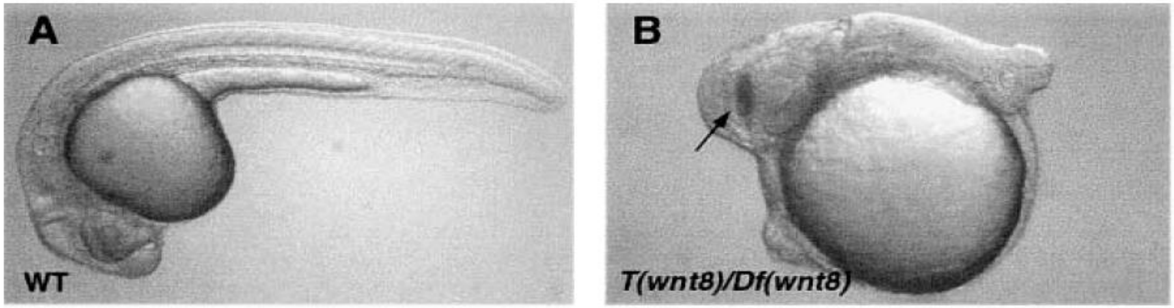
hdl (tcf3)



Heisenberg et al., *Development*, 1996; Heisenberg et al., *Genes Dev*, 2001; van de Water, *Development*, 2001; Kim et al., *Nature,* 2000

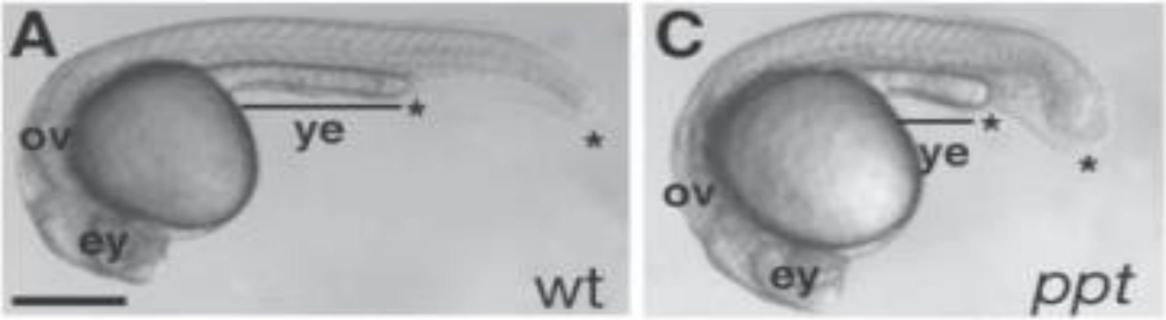
Lack of Wnt signaling results in posterior defects

Df (wnt8), canonical



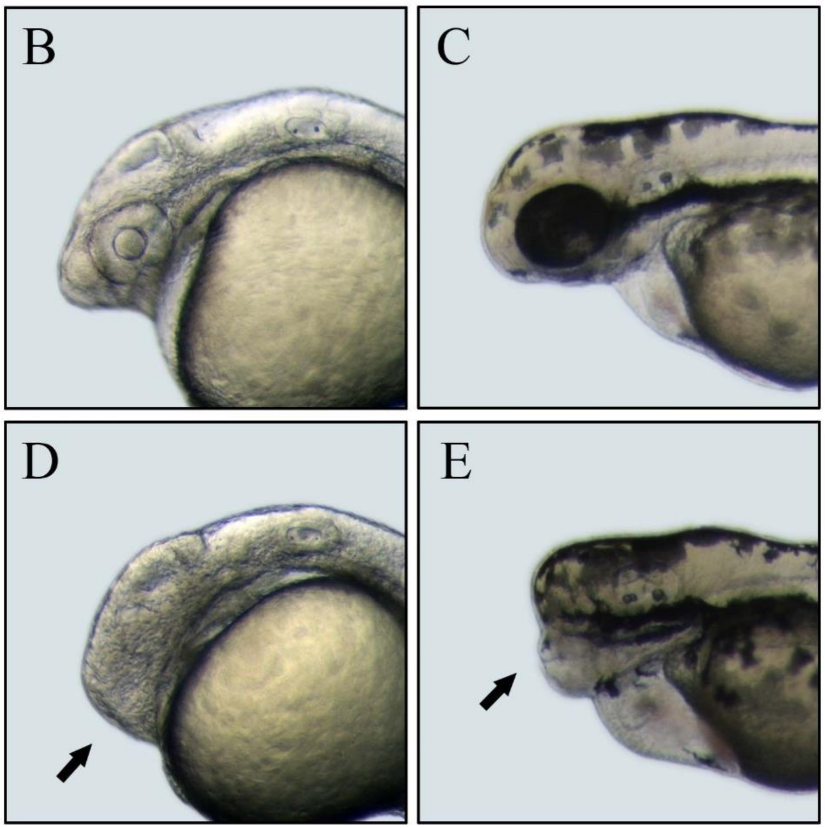
Lekven et al, *Developmental Cell*, 2001

ppt (wnt5), non-canonical

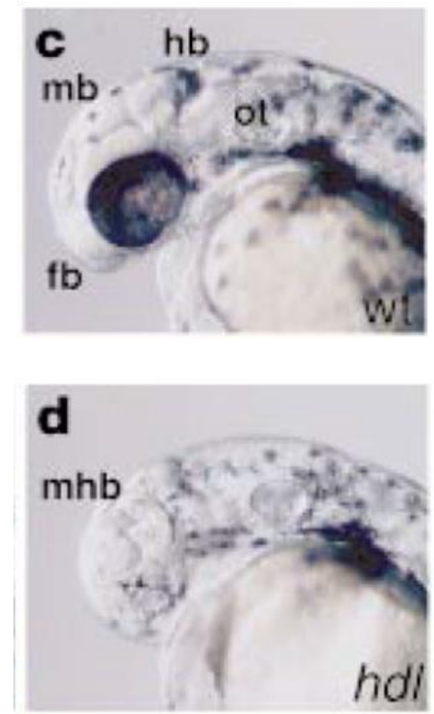


Marlow et al, *Development*, 2004

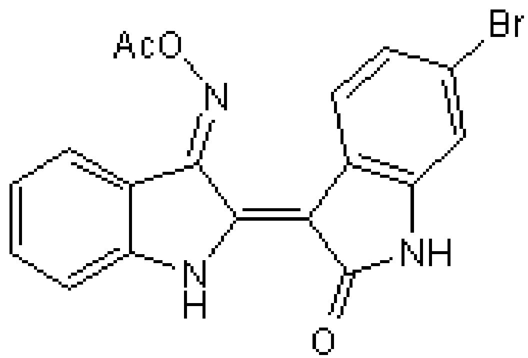
Wnt signaling agonists induces headless embryos



hdl (tcf3)



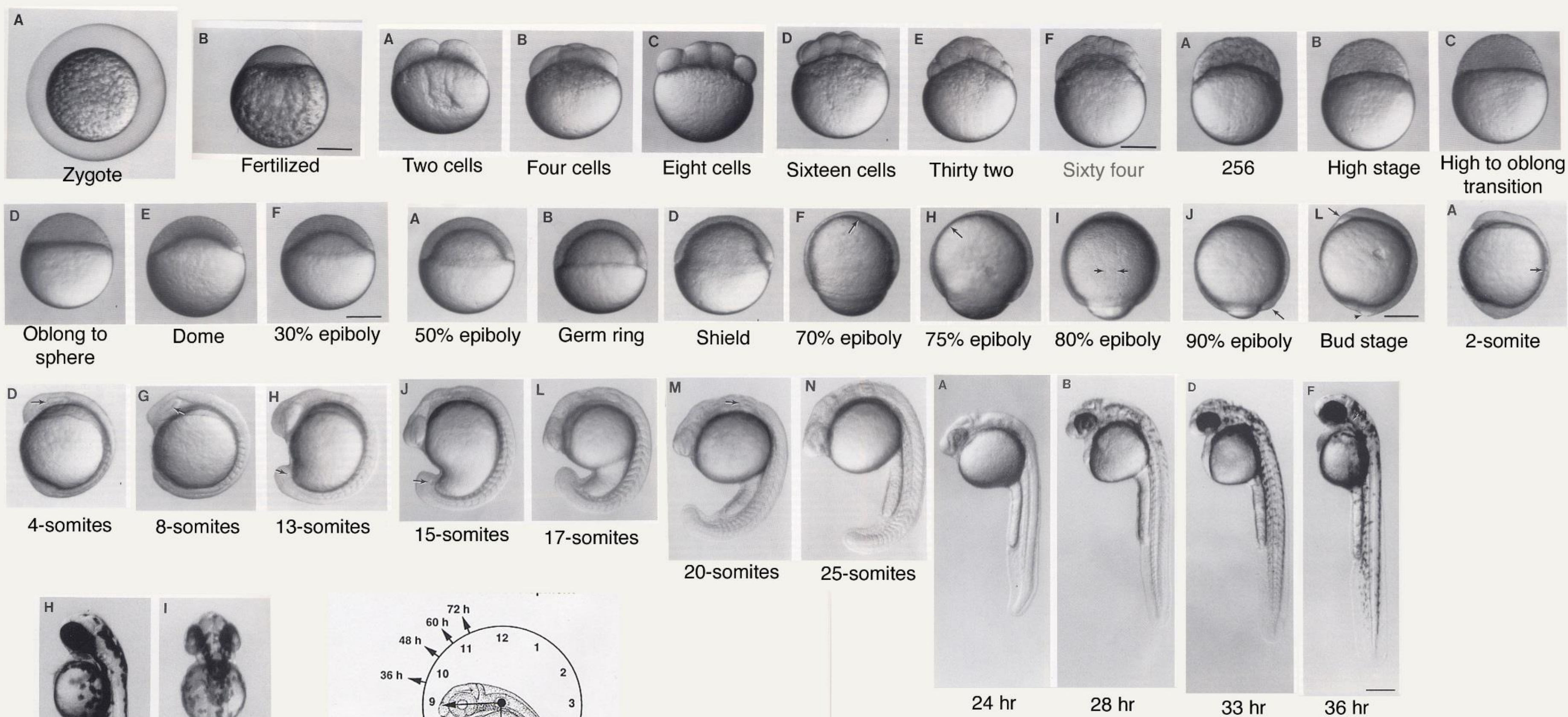
BIO, (2′Z, 3′E)-6-Bromoindirubin-3′ -oxime

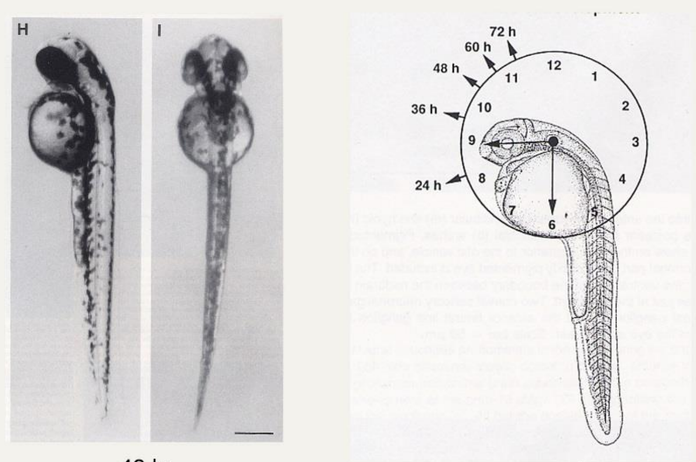


Product information	
Form	Purple solid
Primary Target	GSK-3α/β
Primary Target IC_{50}	5 nM
Secondary target	IC_{50} = 83, 300, 320, and 10,000 nM for Cdk5/p25, Cdk2/A, Cdk1/B, and Cdk4/D1, respectively

From Calbiochem

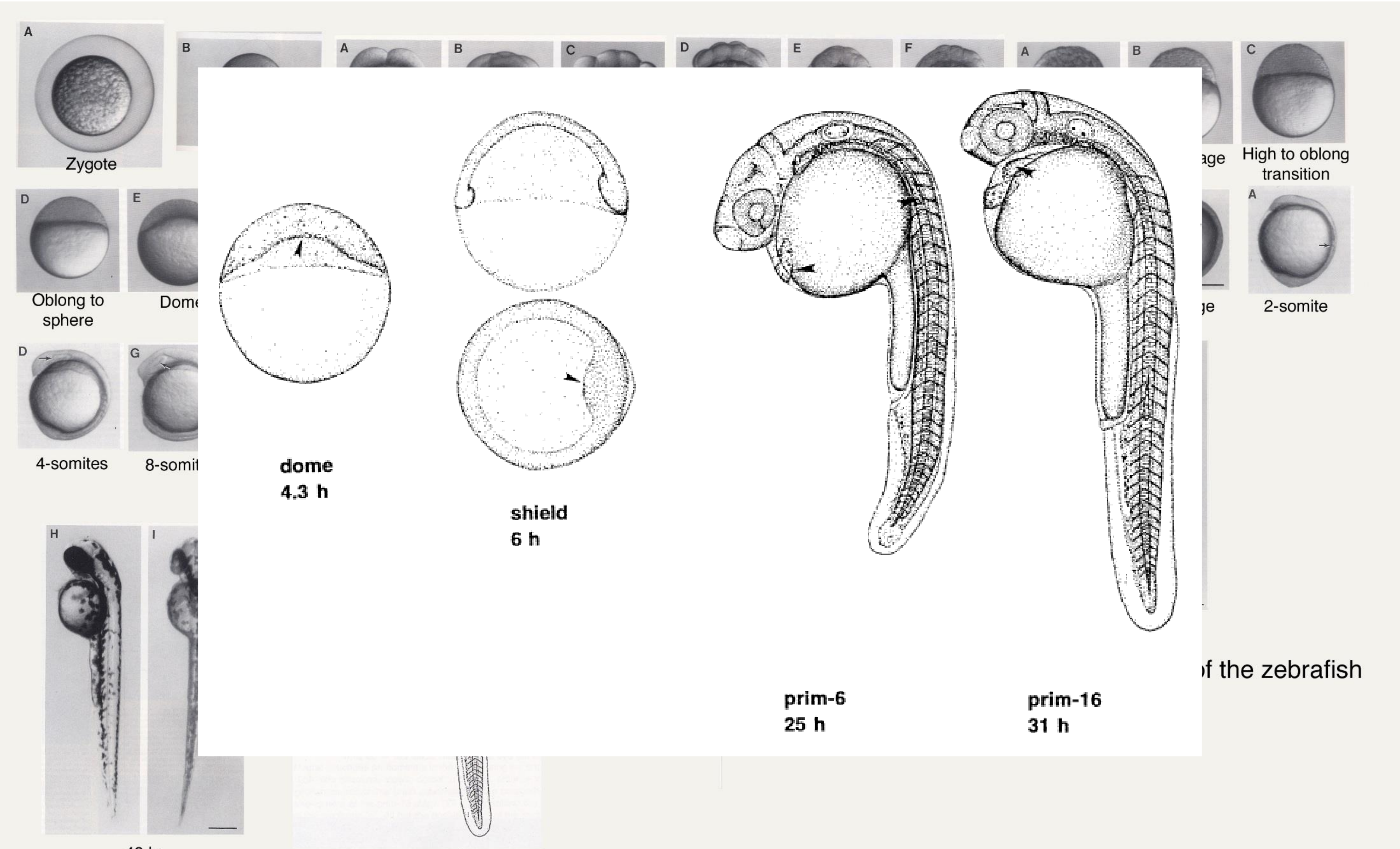
Stages of zebrafish embryo



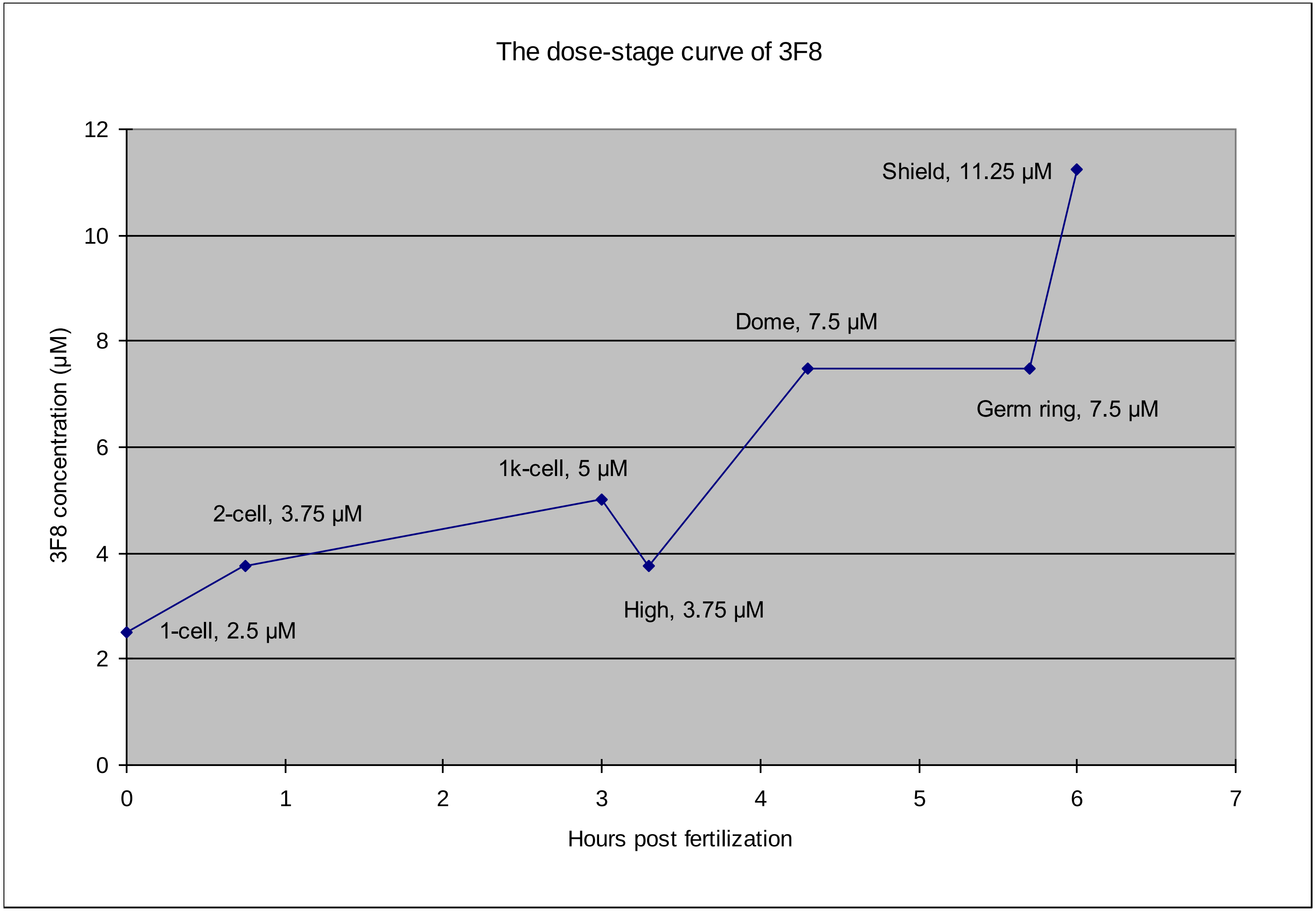


From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

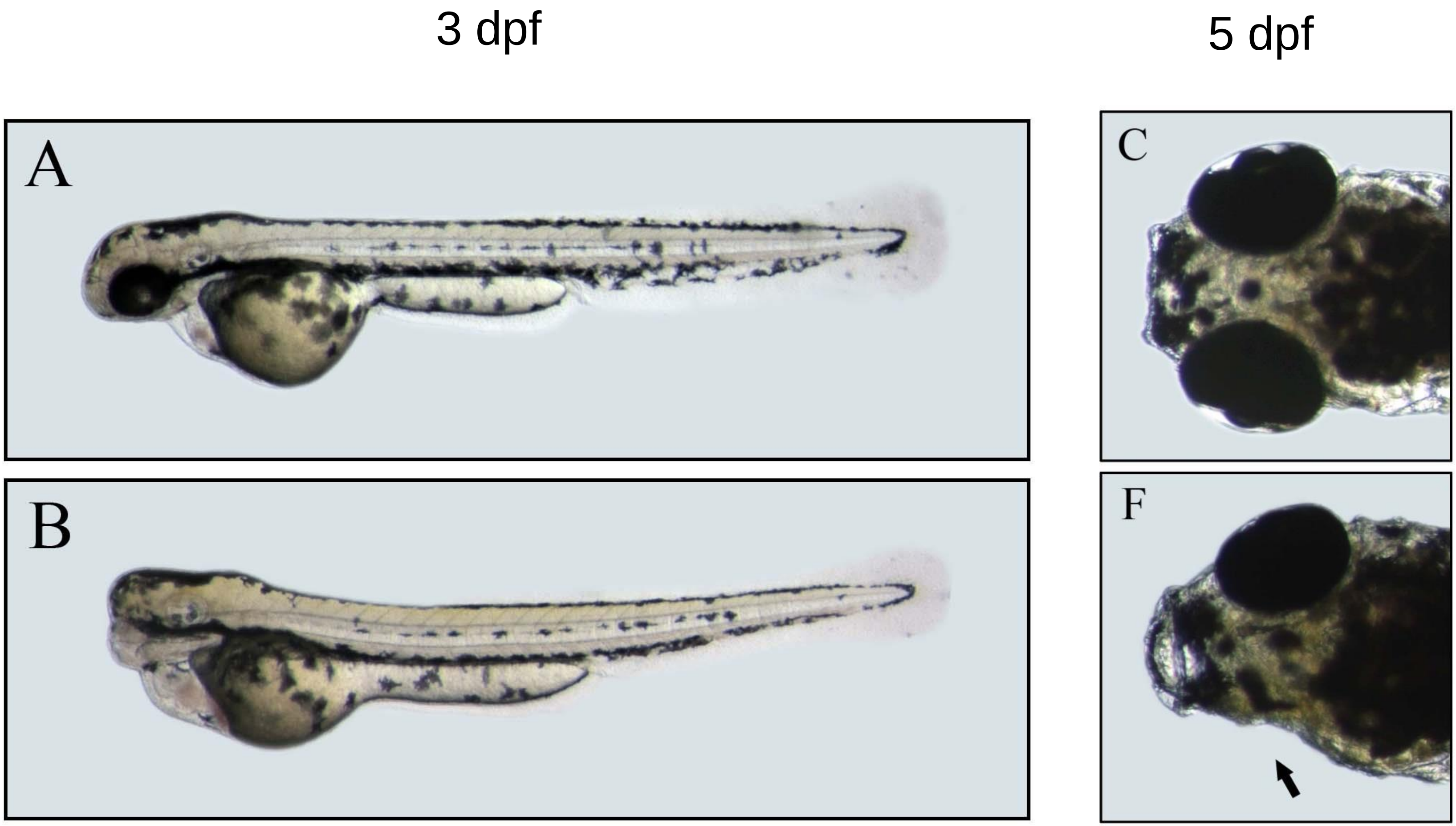
Stages of zebrafish embryo



The dose-stage curve of 3F8, another wnt agonist

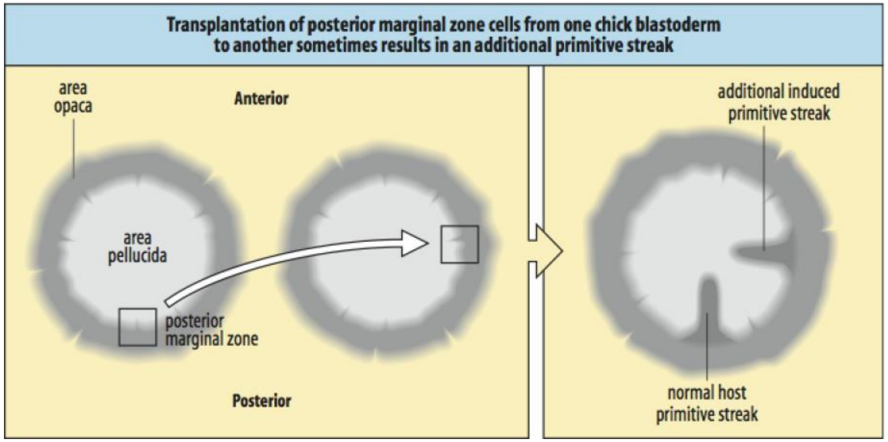


Forebrain recovers after BIO washout

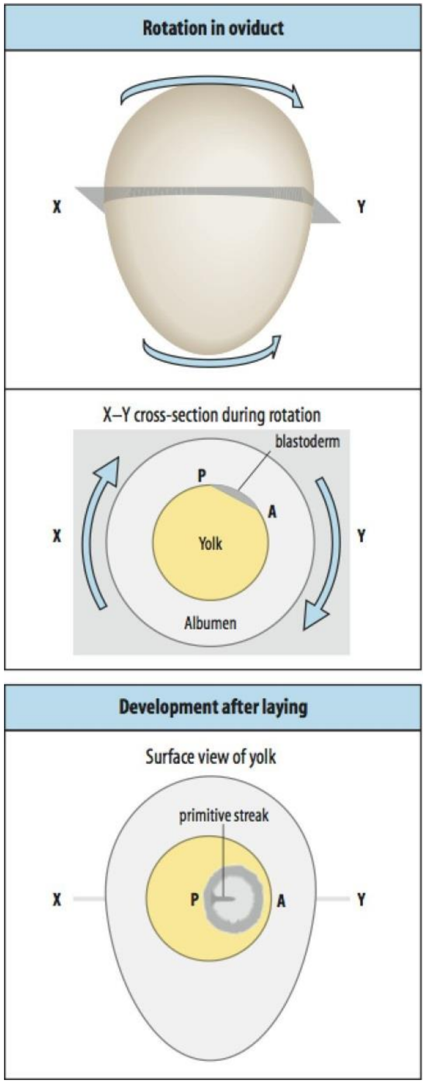


The AP and DV axes of chick embryo are related to the posterior marginal zone

- 1. Koller's sickle is the first recognizable structure.
- 2. Koller's sickle is induced by posterior marginal zone.
- 3. Posterior marginal zone is determined by gravity.

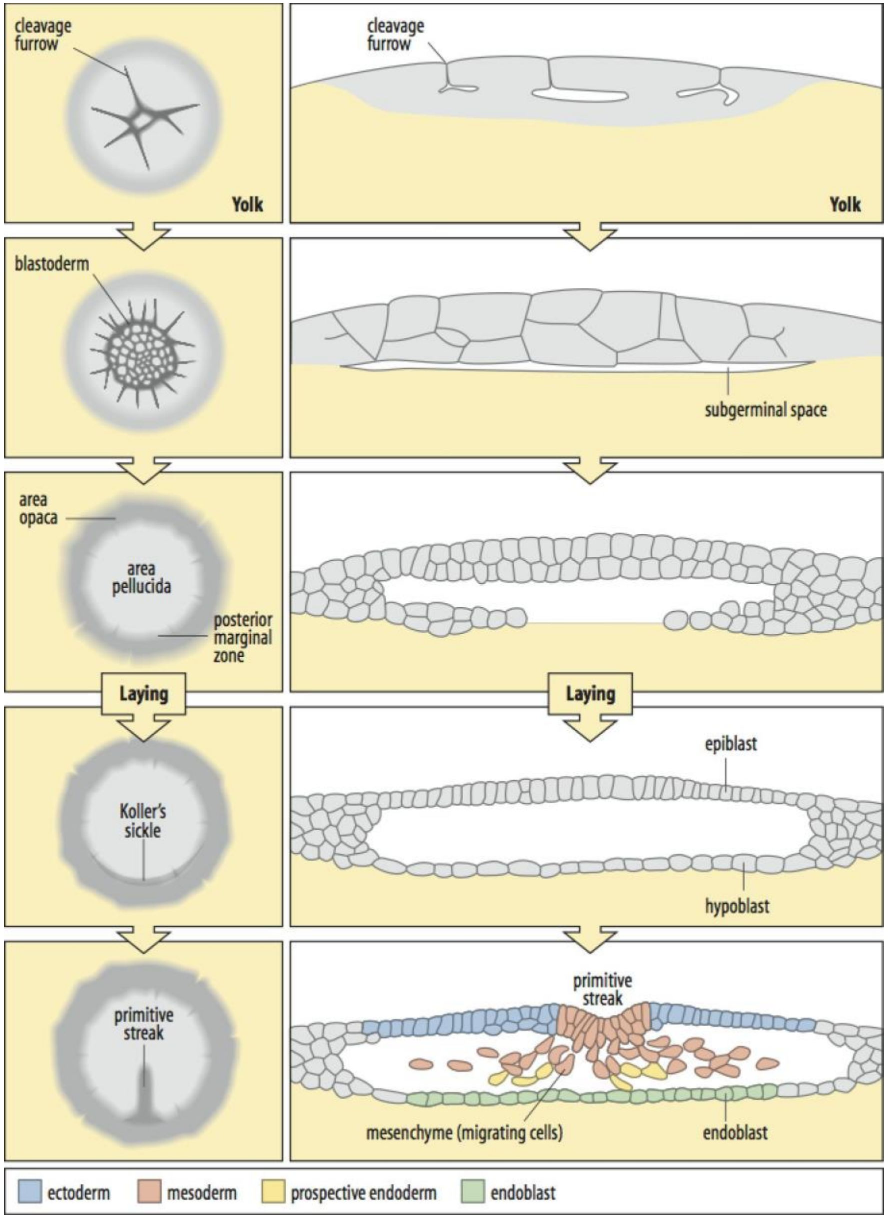


Gravity defines the AP axis of the chick

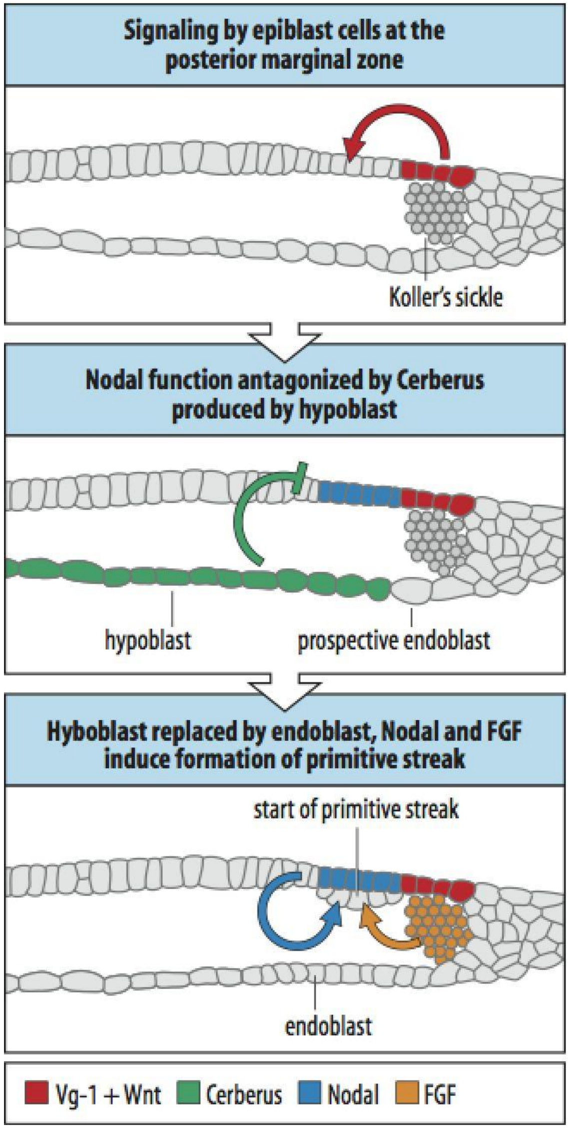


- 1. During the passage through oviduct and uterus (~20 hours), the egg moves pointed end first and rotates slowly, ~ 1/6 rpm.
- 2. The blastoderm remains tilted at an angle of about 45 degree to the vertical.
- 3. The downward pointing edge indicates the future anterior end.
- 4. However, chick blastoderm is highly regulative.

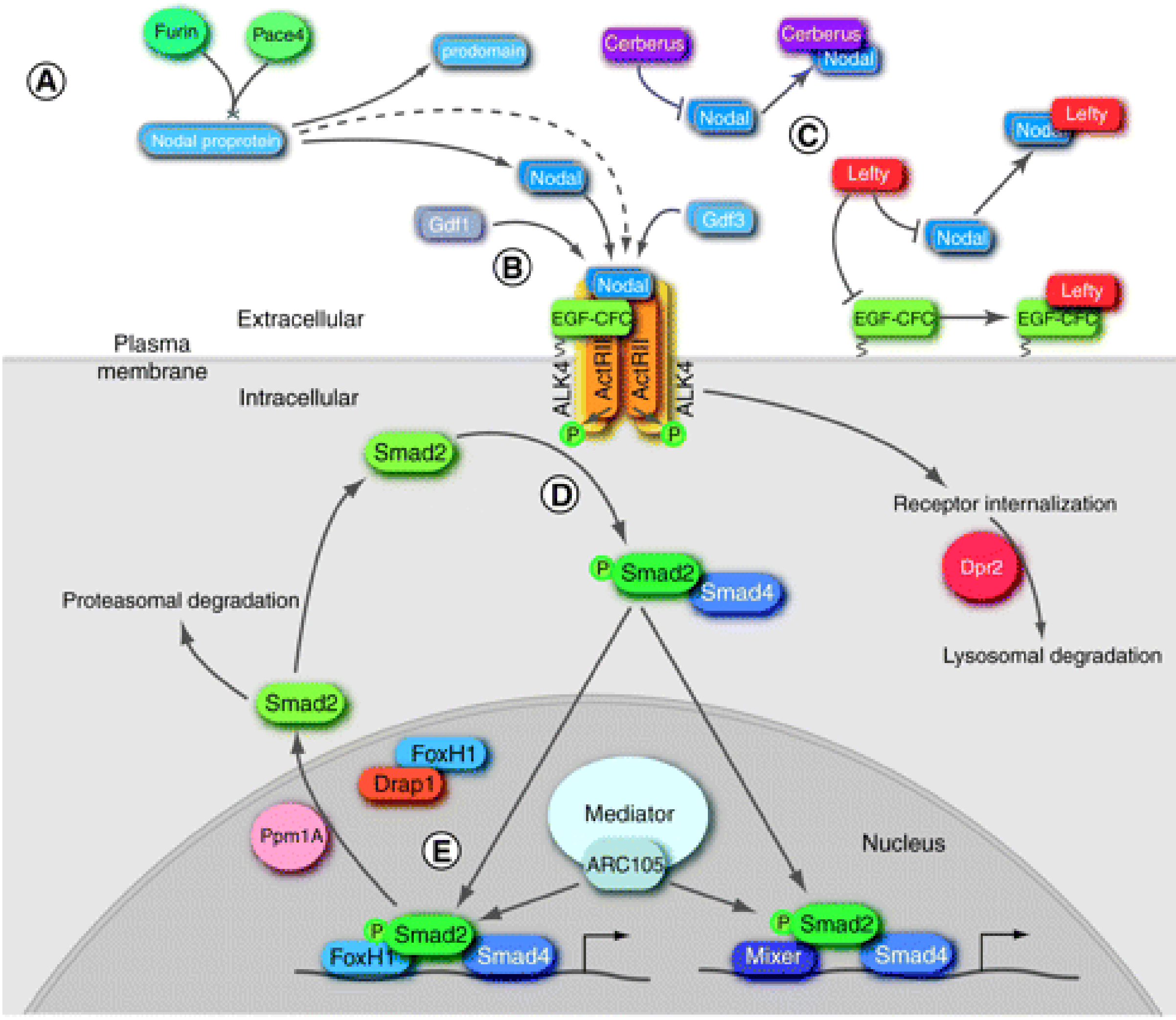
Cleavage and epiblast formation in the chick embryo



Once the hypoblast has been replaced by endoblast, the primitive streak begins to form



Nodal signaling



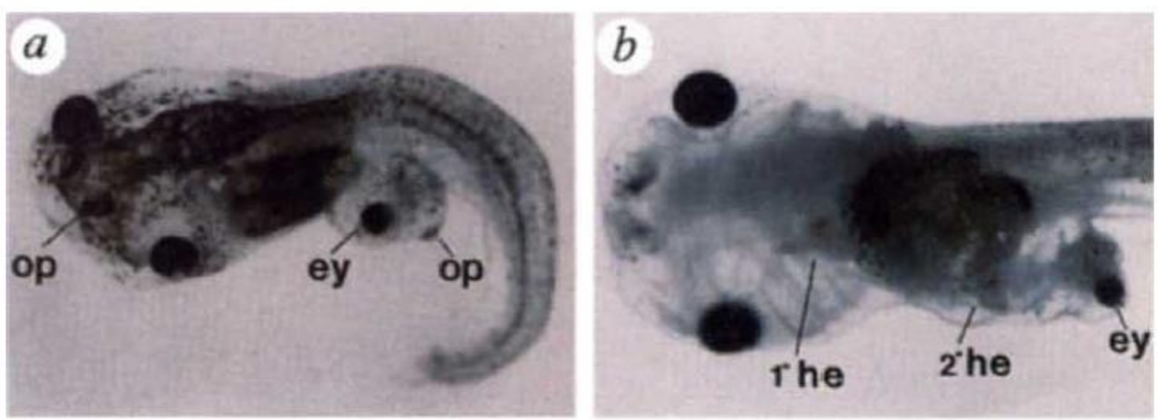
Michael M. Shen, Development, 2007

Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer

Tewis Bouwmeester, Sung-Hyun Kim, Yoshiki Sasai, Bin Lu & Eddy M. De Robertis

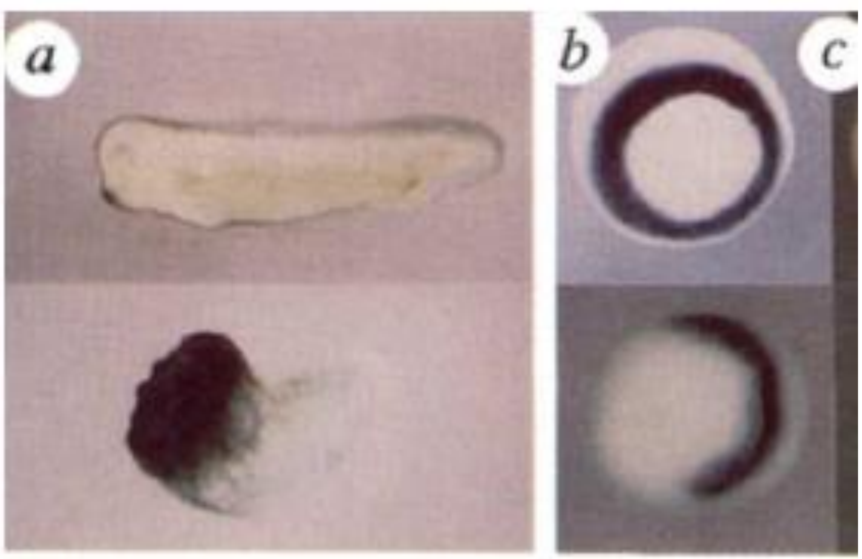
Howard Hughes Medical Institute and Department of Biological Chemistry, University of California, Los Angeles, California 90095–1737, USA

An abundant cDNA enriched in Spemann's organizer, *cerberus*, was isolated by differential screening. It encodes a secreted protein that is expressed in the anterior endomesoderm. Microinjection of *cerberus* mRNA into *Xenopus* embryos induces ectopic heads, and duplicated hearts and livers. The results suggest a role for a molecule expressed in the anterior endoderm in the induction of head structures in the vertebrate embryo.



Suppression of trunk–tail mesoderm formation

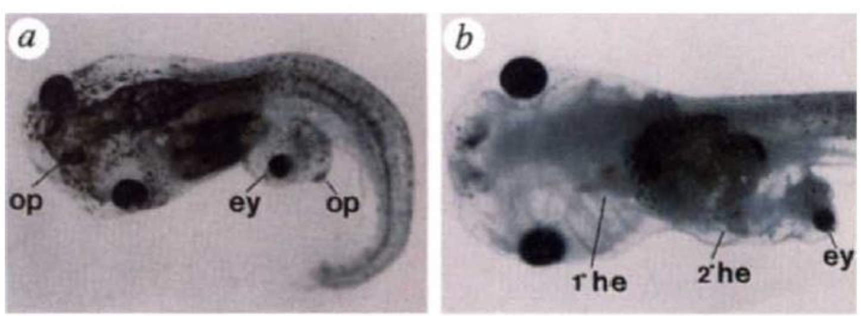
Microinjected *cerberus* mRNA has potent effects on *Xenopus* development. Radial injection of high doses (100 pg per blastomere) of *cerberus* mRNA into each blastomere of the four-cell embryo results in embryos with very large cement glands (Fig. 3*a*). These embryos do not form trunk–tail mesoderm, as indicated by the repression of *Xbra* (Fig. 3*b*), the lack of an external blastopore

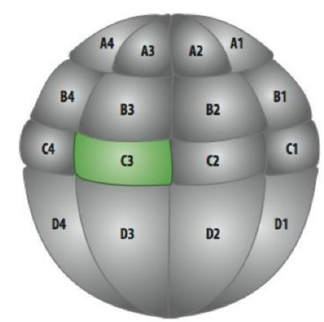


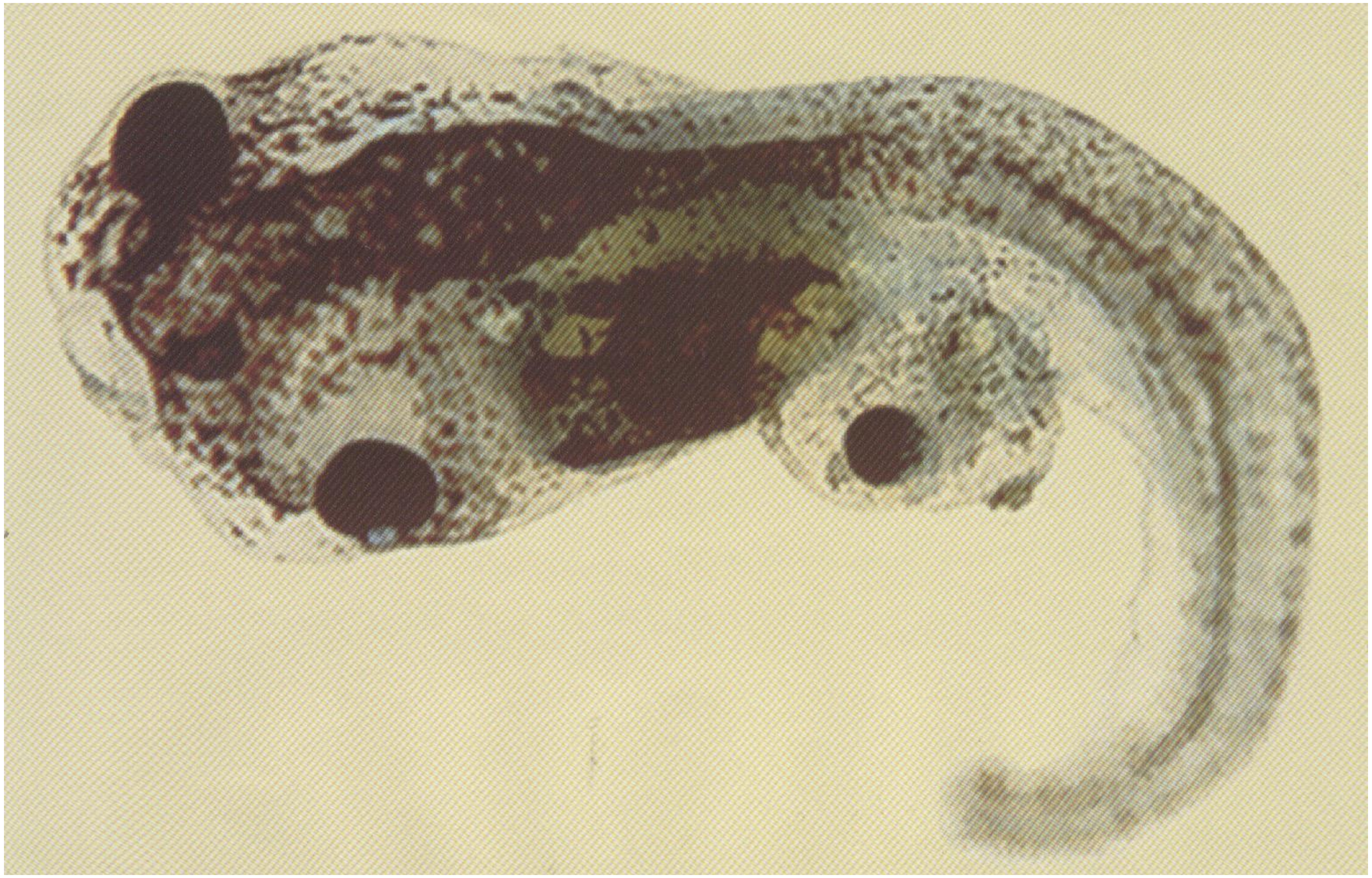
The cement gland is a mucus-secreting organ found at the extreme anterior of frog embryos. It attaches the embryo to a solid support before swimming and feeding begin, and also serves a related sensory function that stops the embryo from moving once it is attached.

Induction of ectopic head structures

To test for more localized effects of *cerberus* mRNA, we injected it systematically into individual blastomeres at the 32-cell state[36]. In dorsal cells (B1, C1 and D1), *cerberus* mRNA resulted in severe gastrulation defects, and in animal cells (A1, A4) it induced ectopic cement glands. However, striking results were found after injection into the D4 (and to a lesser extent into C4) ventral–vegetal blastomere, which led to the formation of small ectopic head structures (Fig. 4*a*). These secondary heads were mirror duplications with respect to the anteroposterior axis, and did not contain any trunk–tail axial structures such as somites or notochord (Fig. 4*a*). In every case in which ectopic eyes developed (scored at 4 days of development), the ectopic head contained only one eye ($n = 45$), suggesting that the secondary heads lack a prechordal plate[35]. In addition, some of these animals ($n = 21$) had a secondary heart that could be seen beating at a rhythm different to that of the primary heart (Fig. 4*b*). Histological

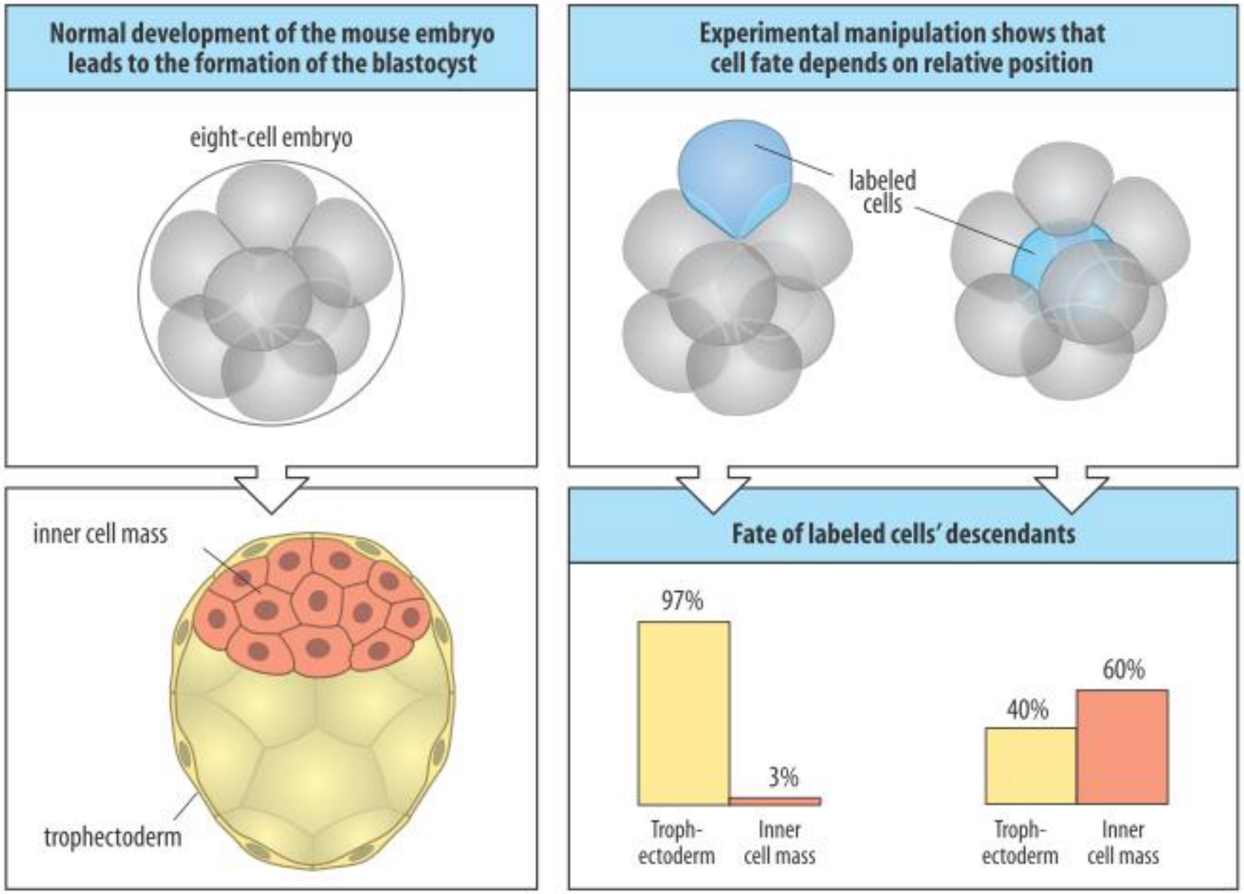




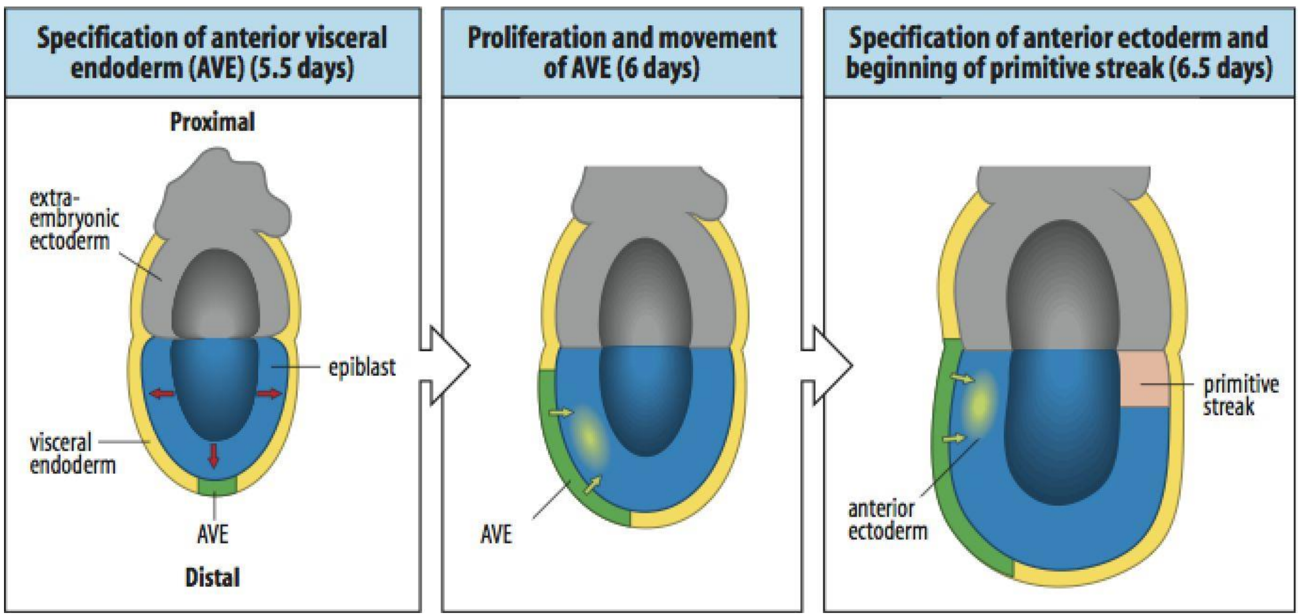


EDDY M. DE ROBERTIS and Ceberus

The specification of the blastomeres that will form the inner cell mass of a mouse embryo depends on whether they are on the outside or the inside of the embryo

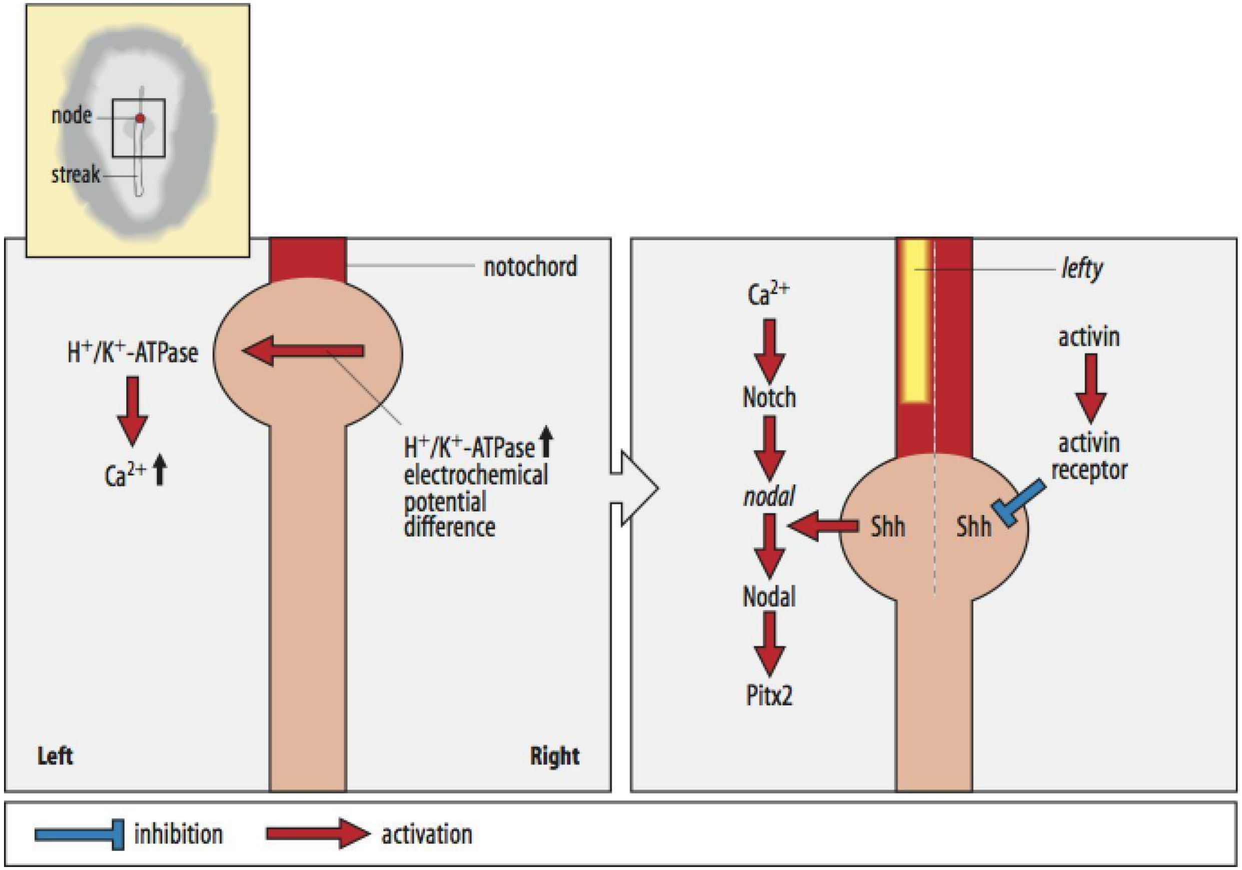


The specification of the distal visceral endoderm is the symmetry-breaking event in the mouse embryogenesis

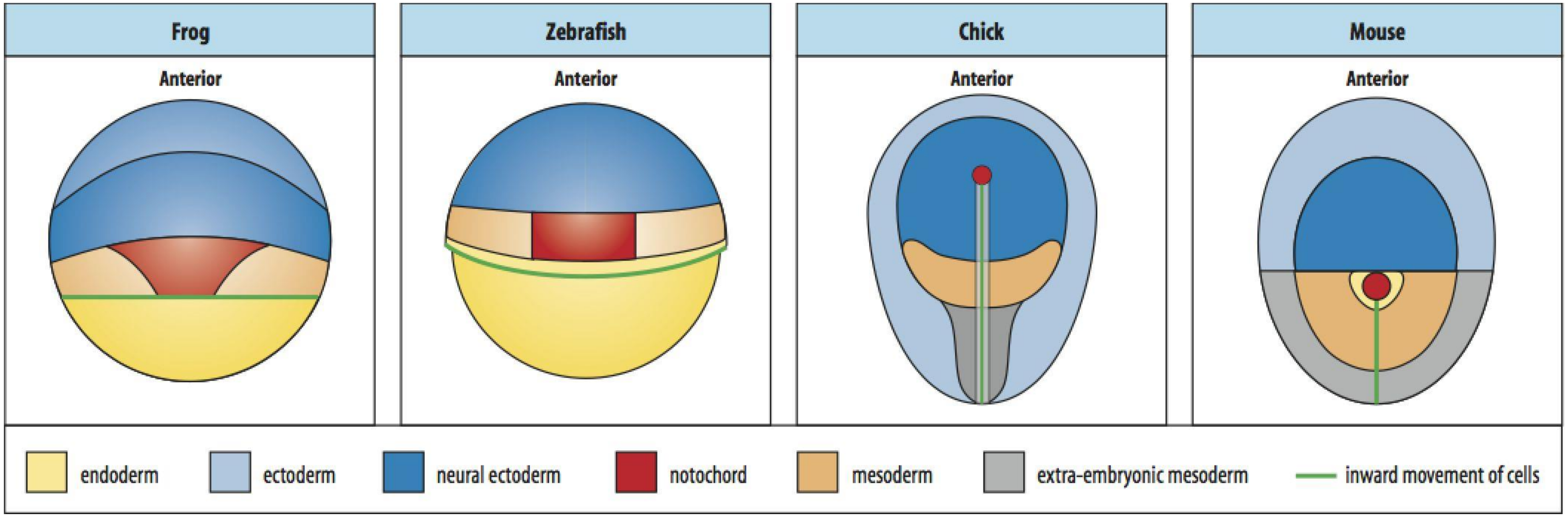


Distal visceral endoderm becomes anterior visceral endoderm (AVE).

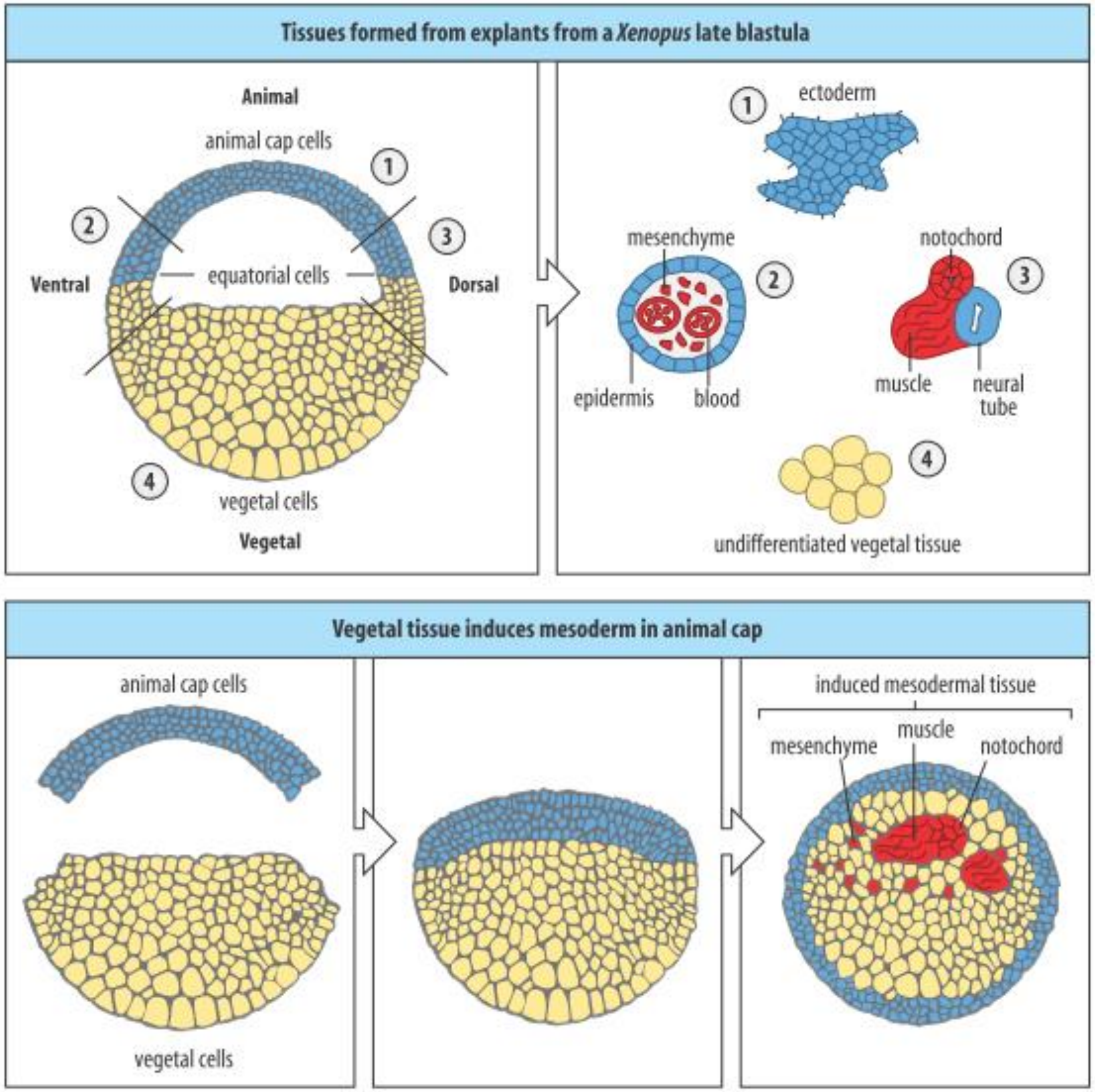
Establishment of the internal left-right asymmetry



The fate map of vertebrate embryos at comparable stages



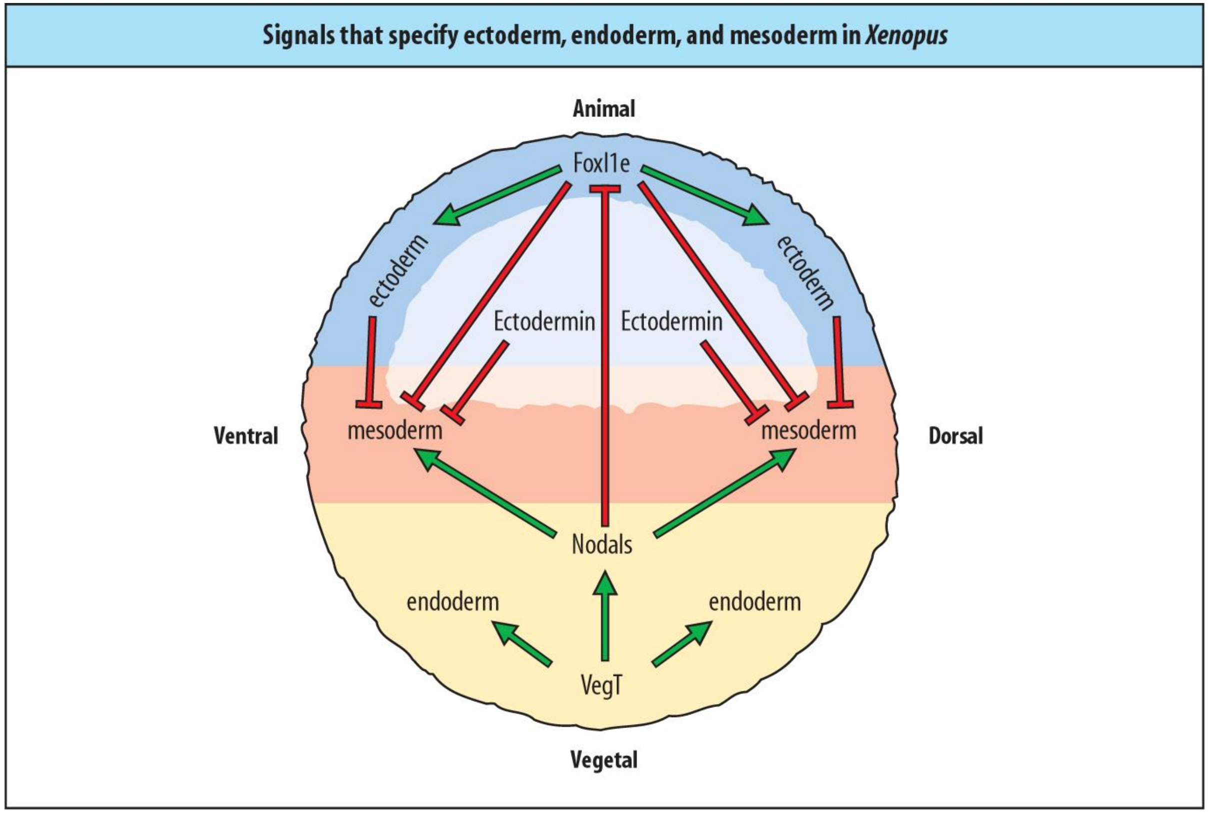
Tissue explant experiments reveal that mesoderm is induced from ectoderm in Xenopus



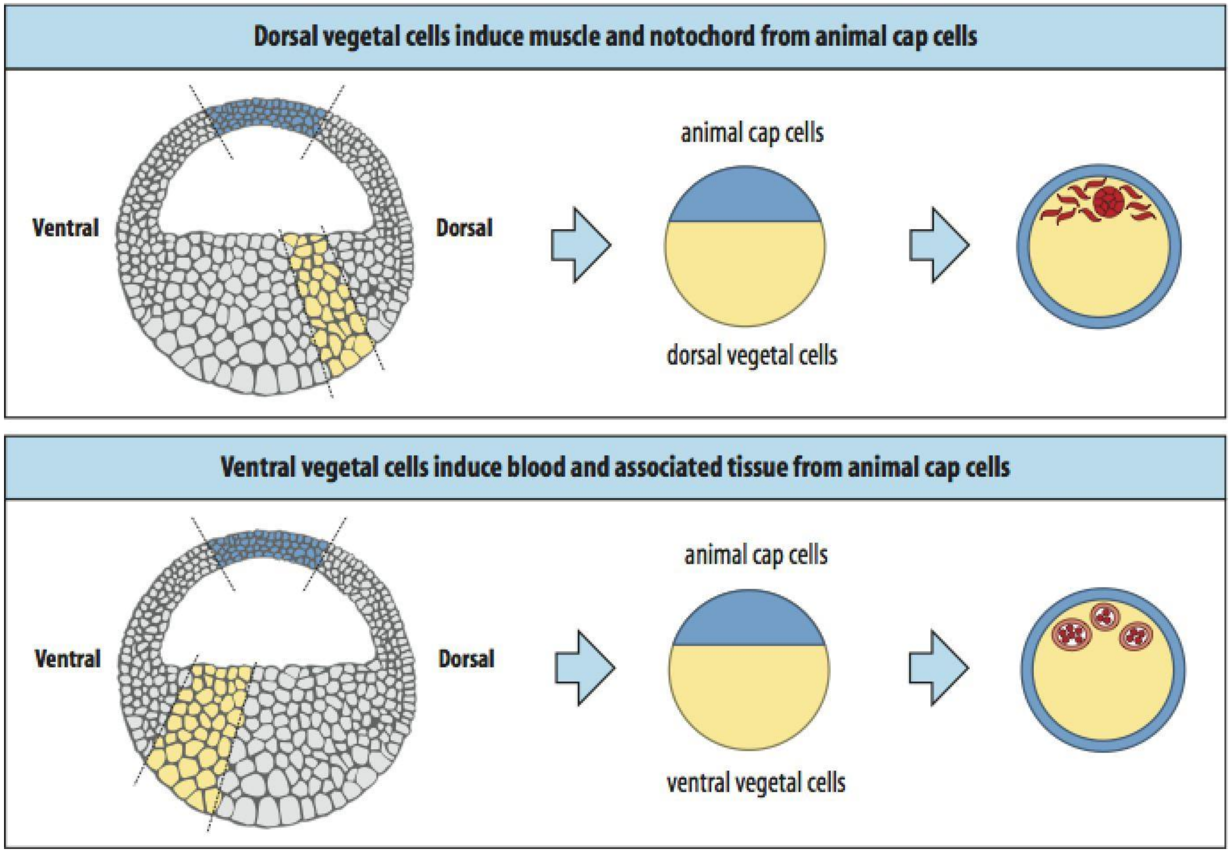
Contact or not -- the filter assay

- A filter with very small pores that do not allow cells contact each other.
- This kind of filter doesn’t block the mesoderm induction.
- It suggests that the signals are in the form of secreted molecules.

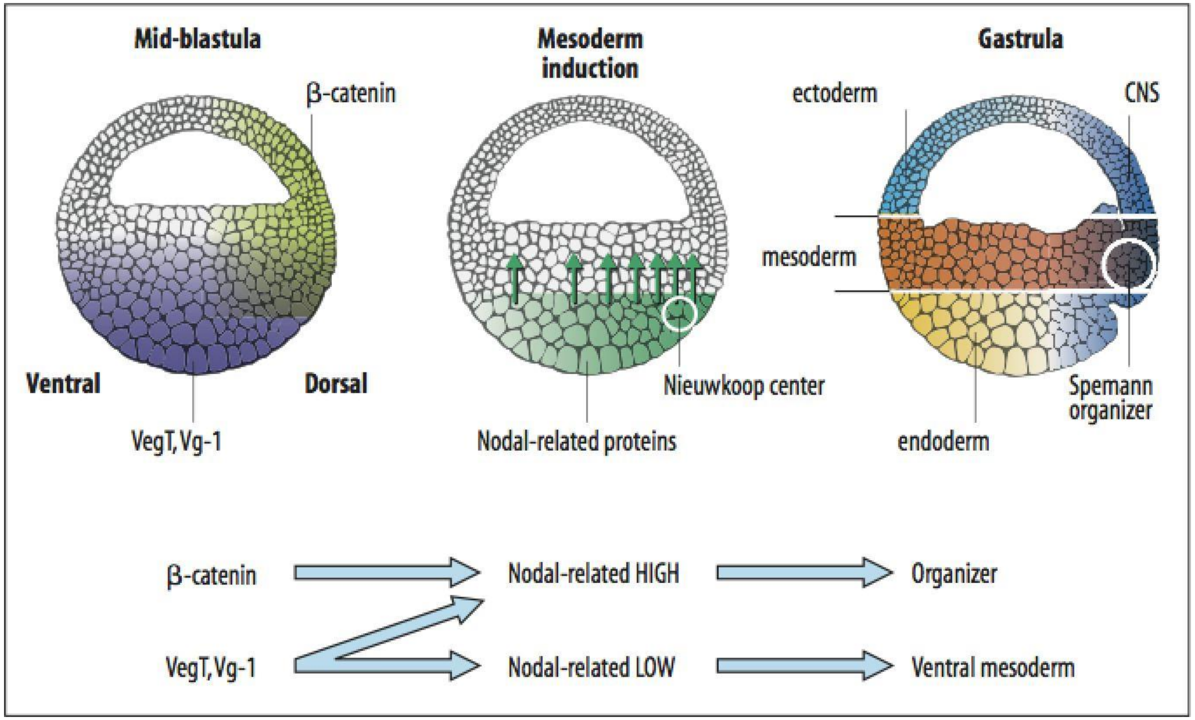
Signals for germ layer specification in *Xenopus*



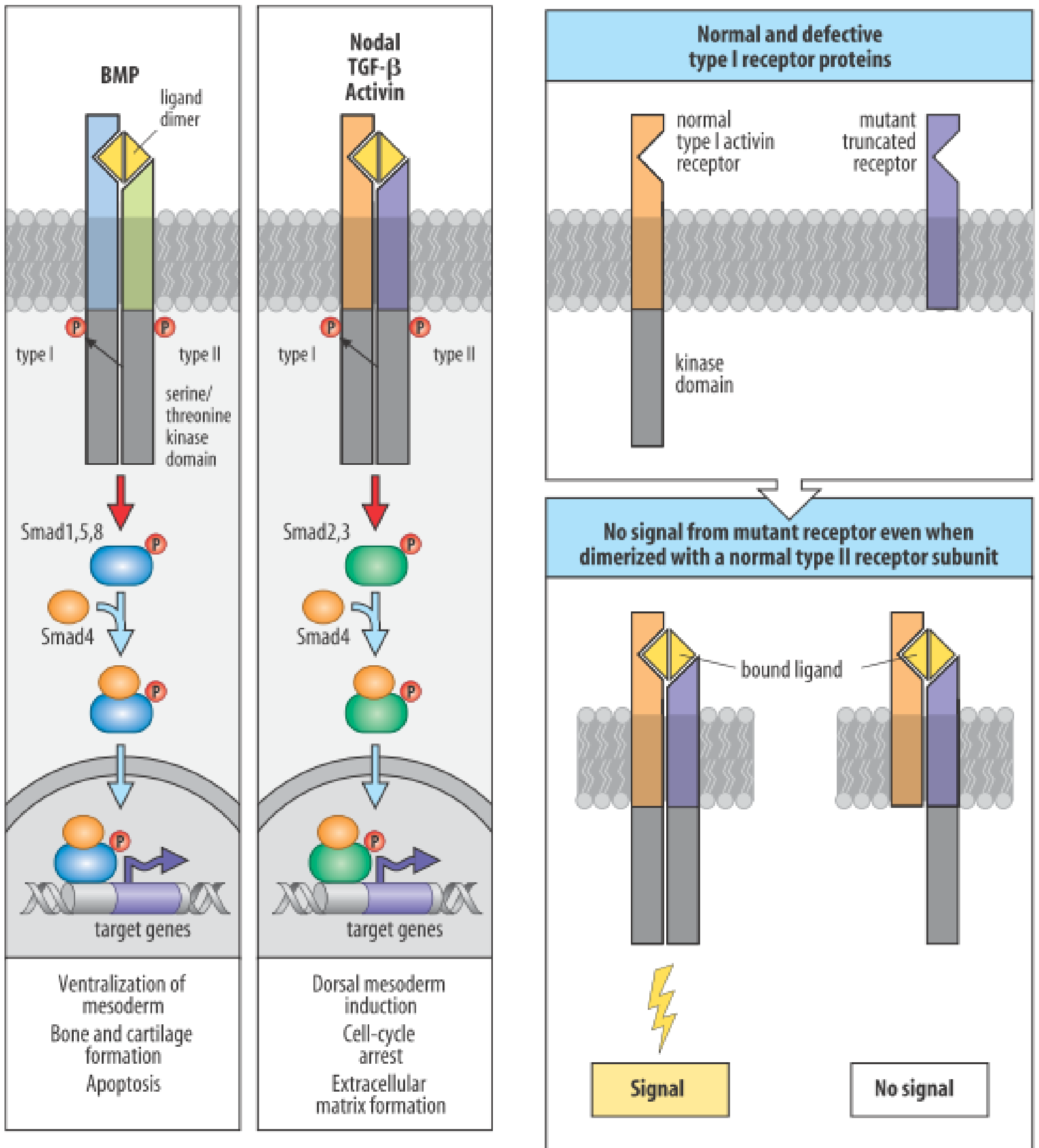
Difference in mesoderm induction by dorsal and ventral regions



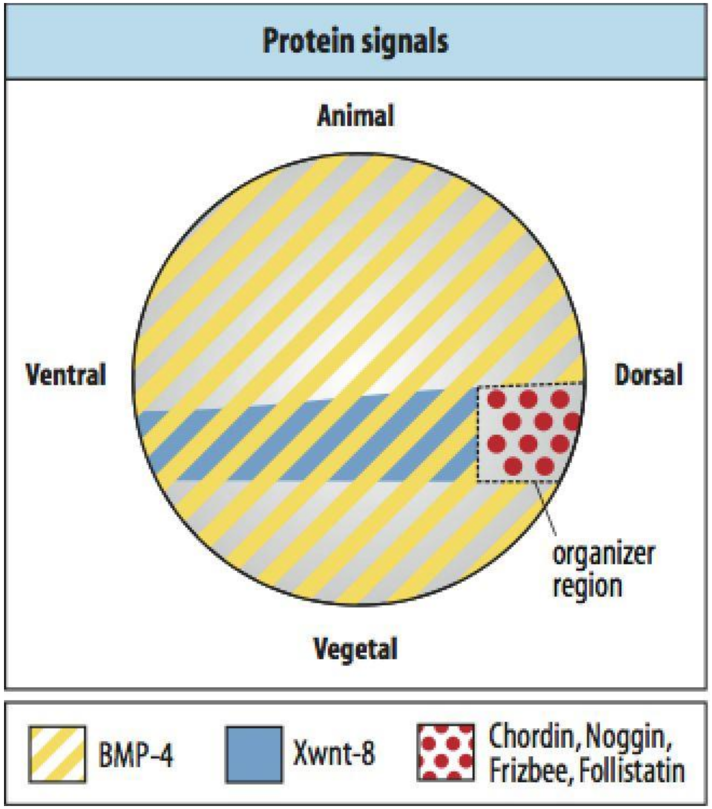
VegT and Wnt signaling set up the gradient of Nodal-related proteins



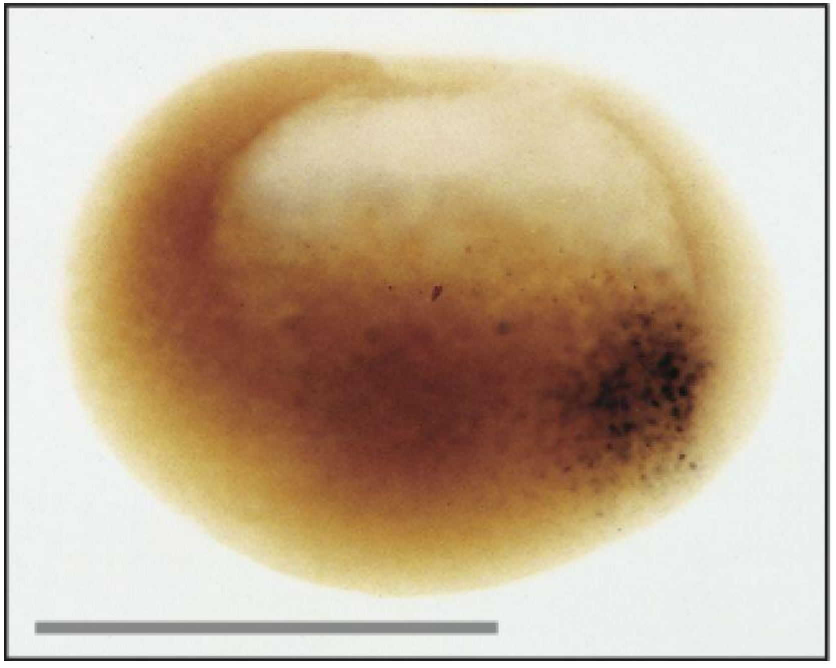
TGF-beta family and dominant negative receptor



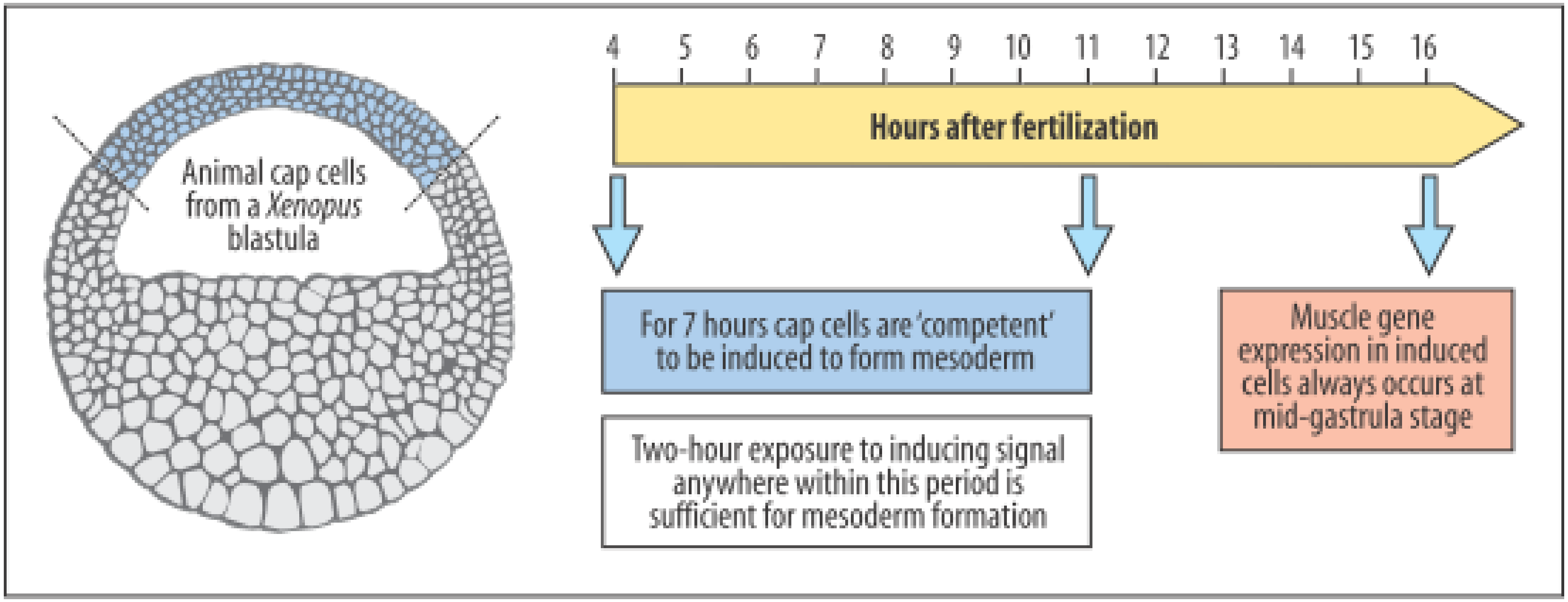
Distribution of mRNAs encoding secreted proteins in *Xenopus*



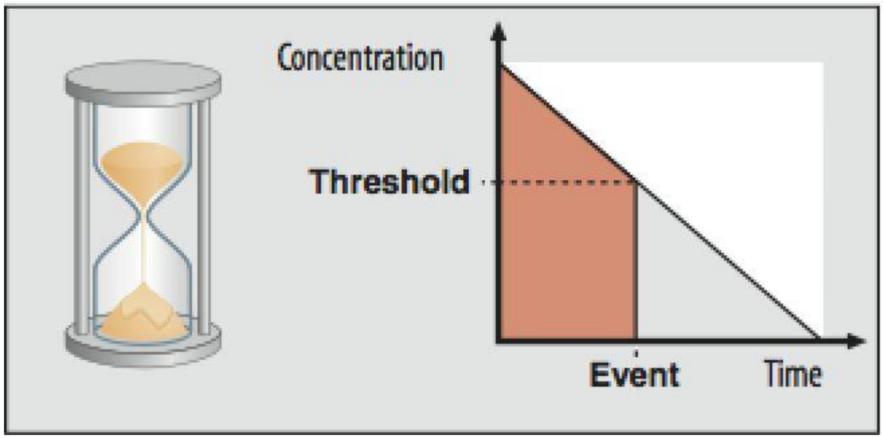
Expression of *noggin* in Xenopus



Time window of mesoderm induction

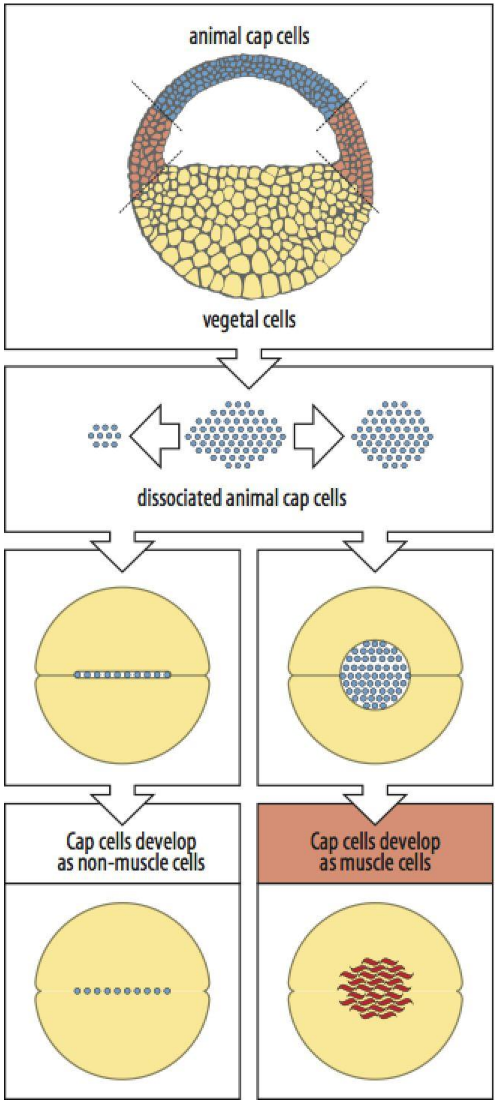


A timing mechanism that could operate in MBT/development



1. Suppression of cleavage but not DNA synthesis does not alter MBT.
2. Nor are cell-cell interactions. Dissociated blastomeres. Seems to be the ratio of DNA to cytoplasm. Chick and mouse.
3. Increase DNA artificially. More sperm. Inject DNA.
4. Some kind of suppressive factors. Like Morphogen.

The community effect



- 1. A single or a small number of animal cap cells are not induced to become mesodermal cells.
- 2. A minimum of about 100/200 animal cap cells must be present for induction.

Saka et al. BMC Systems Biology 2011, 5:54
http://www.biomedcentral.com/1752-0509/5/54

BMC Systems Biology

RESEARCH ARTICLE Open Access

Theoretical basis of the community effect in development

Yasushi Saka[1*], Cédric Lhoussaine[2,3], Celine Kuttler[2,3], Ekkehard Ullner[1,4] and Marco Thiel[4]

Abstract

Background: Genetically identical cells often show significant variation in gene expression profile and behaviour even in the same physiological condition. Notably, embryonic cells destined to the same tissue maintain a uniform transcriptional regulatory state and form a homogeneous cell group. One mechanism to keep the homogeneity within embryonic tissues is the so-called community effect in animal development. The community effect is an interaction among a group of many nearby precursor cells, and is necessary for them to maintain tissue-specific gene expression and differentiate in a coordinated manner. Although it has been shown that the cell-cell communication by a diffusible factor plays a crucial role, it is not immediately obvious why a community effect needs many cells.

Results: In this work, we propose a model of the community effect in development, which consists in a linear gene cascade and cell-cell communication. We examined the properties of the model theoretically using a combination of stochastic and deterministic modelling methods. We have derived the analytical formula for the threshold size of a cell population that is necessary for a community effect, which is in good agreement with stochastic simulation results.

Conclusions: Our theoretical analysis indicates that a simple model with a linear gene cascade and cell-cell communication is sufficient to reproduce the community effect in development. The model explains why a community needs many cells. It suggests that the community's long-term behaviour is independent of the initial induction level, although the initiation of a community effect requires a sufficient amount of inducing signal. The mechanism of the community effect revealed by our theoretical analysis is analogous to that of quorum sensing in bacteria. The community effect may underlie the size control in animal development and also the genesis of autosomal dominant diseases including tumorigenesis.

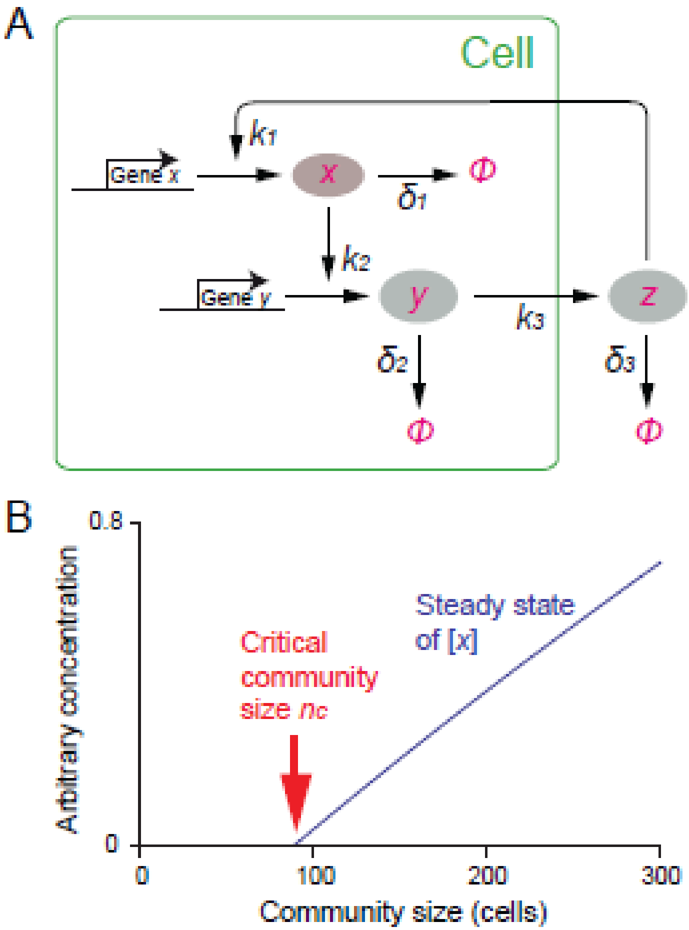


Figure 2 A minimal model of a community effect. (A) A schematic depiction of the model. Each molecule or state is indicated in red, and arrows indicate reactions/transitions between those states with reaction rate parameters as indicated. See text for details. (B) Steady state of [x] plotted as a function of community size n. Parameter values used for the plot are: k_1, k_2, k_3 = 0.02; δ_1, δ_2, δ_3 = 0.01; V_c = 1; V_s = 800.

$$
\begin{aligned}
x'_i &= \frac{k_1 z}{z+1} - \delta_1 x_i \\
y'_i &= \frac{k_2 x_i}{x_i+1} - \delta_2 y_i \\
z' &= \sum_{i=1}^{n}\left(\frac{V_c}{V_s - n\,V_c}\,\frac{k_3 y_i}{y_i+1}\right) - \delta_3 z.
\end{aligned} \tag{1}
$$

Note that V_s - n V_c is the extracellular volume of the system, so V_c /(V_s - n V_c) is the factor of concentration adjustment. k_1, k_2 and k_3 are the rate constants for production of x_i, y_i and z, respectively, and δ_1, δ_2 and δ_3 for degradation. Because cells are identical, Eqs.1 reduce to:

$$
\begin{aligned}
x' &= \frac{k_1 z}{z+1} - \delta_1 x \\
y' &= \frac{k_2 x}{x+1} - \delta_2 y \\
z' &= \frac{\mu y}{y+1} - \delta_3 z,
\end{aligned} \tag{2}
$$

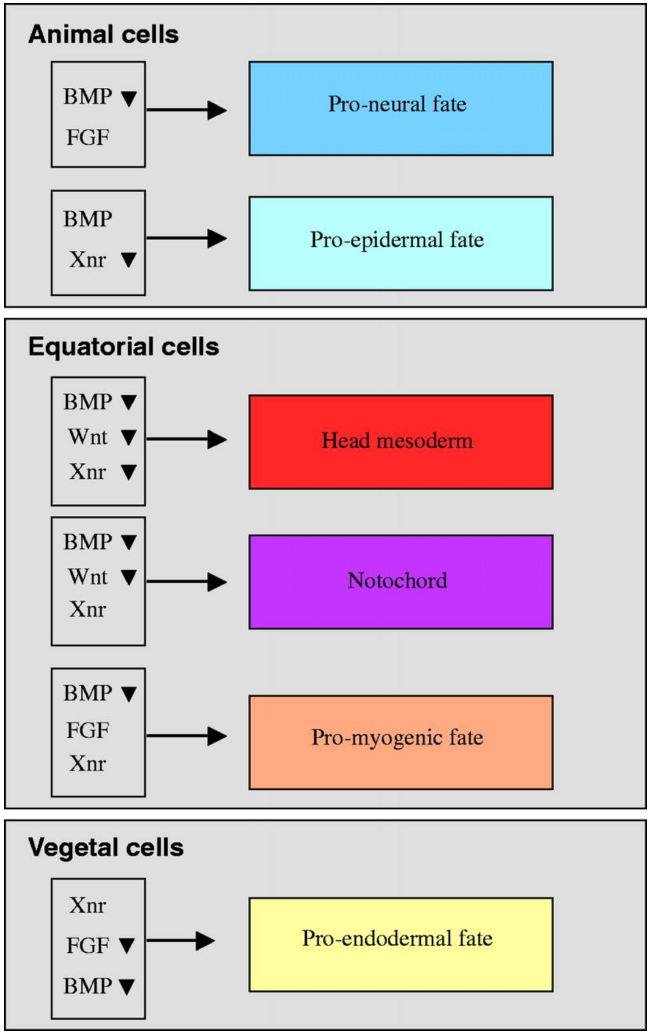
Thanks!

Intercellular protein signals in vertebrate development

Common intercellular signals in vertebrate embryonic development		
Family	**Receptors**	**Examples of roles in development**
Fibroblast growth factor (FGF)		
Twenty-five FGF genes have been identified in vertebrates, not all of which are present in all vertebrates	FGF receptors (receptor tyrosine kinases)	Roles at all stages in development. Maintenance of mesoderm formation. Induction of spinal cord (Chapter 4). Signal from apical ridge in limb bud (Chapter 9)
Epidermal growth factor (EGF)		
Epidermal growth factor	EGF receptors (receptor tyrosine kinases)	Cell proliferation and differentiation. Limb patterning (Chapter 9)
Transforming growth factor-β (TGF-β)		
A large protein family that includes activin, Vg-1, bone morphogenetic proteins (BMPs), Nodal, and Nodal-related proteins	Type I and Type II receptor subunits (receptor serine/threonine kinases) Receptors act as heterodimers	Roles at all stages in development. Mesoderm induction and patterning
Hedgehog		
Sonic hedgehog and other members	Patched	Positional signaling in limb and neural tube (Chapters 9 and 10). Involved in determination of left–right asymmetry
Wingless (Wnt)		
Twenty-one Wnt genes have been identified in vertebrates, not all of which are present in all vertebrates	Frizzled family of receptors (seven-span transmembrane proteins)	Roles at all stages in development. Dorso-ventral axis specification in *Xenopus*. Limb development (Chapter 9)
Delta and Serrate		
Transmembrane signaling proteins	Notch	Roles at all stages in development. Left–right asymmetry
Ephrins		
Transmembrane signaling proteins	Eph receptors (receptor tyrosine kinases)	Guidance of developing blood vessels (Chapter 9). Guidance of developing neurons in the nervous system (Chapter 10)
Retinoic acid		
Small molecule related to vitamin D	Retinoic acid receptors (RARs) Intracellular proteins related to the steroid receptors. They bind to retinoic acid to form a complex that acts as a transcriptional regulator	Somite formation (Chapter 4). Limb patterning (Chapter 9)

Common intercellular signals used in *Drosophila*		
Family and examples	**Receptors**	**Examples of roles in development**
Hedgehog family		
Hedgehog	Patched	Patterning of insect segments Positional signaling in insect leg and wing discs
Wingless (Wnt) family		
Wingless and other Wnt proteins	Frizzled	Insect segment and imaginal disc specification (Wingless) Other Wnts have roles in development
Delta and Serrate		
Transmembrane signaling proteins	Notch	Roles at many stages in development Specification of oocyte polarity
Transforming growth factor-α (TGF-α) family		
Gurken, Spitz, Vein	EGF receptor (receptor tyrosine kinase) One only in *Drosophila*, known as DER or Torpedo.	Polarization of the oocyte Eye development, wing vein differentiation (see Chapter 9)
Transforming growth factor-β (TGF-β) family		
Decapentaplegic	Receptors act as heterodimers of type I (e.g. Thick veins) and type II (e.g. Punt) subunits Serine/threonine kinases	Patterning of the dorso-ventral axis and imaginal discs
Fibroblast growth factor (FGF) family		
A small number of FGF homologs (e.g. Branchless)	FGF receptors (receptor tyrosine kinases) Two in *Drosophila*, e.g. Breathless	Migration of tracheal cells (see Chapter 9)

Signalling combinations that influence cell fate in the early *Xenopus* embryo.



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