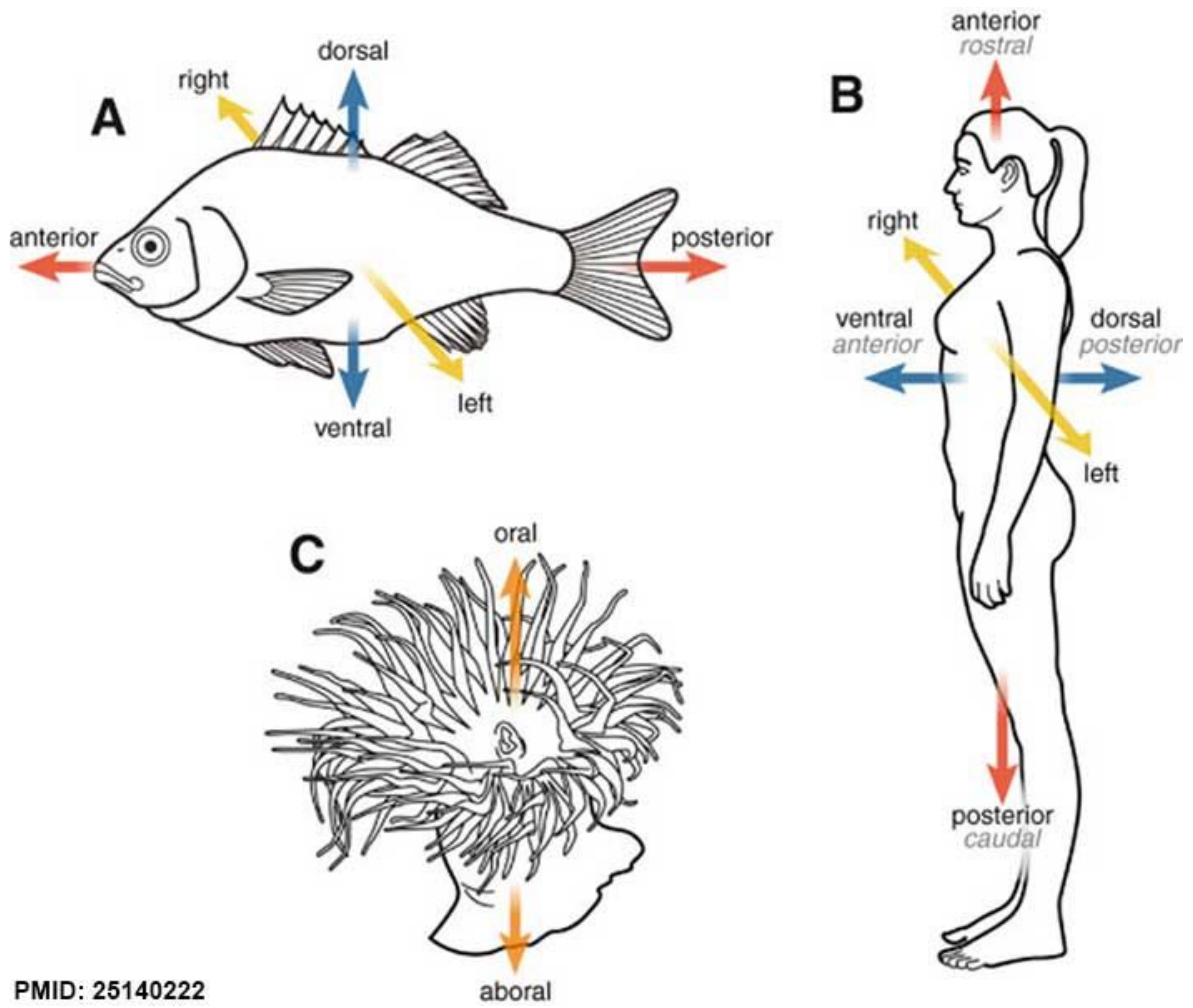
? Big questions ?

Vertebrates+: all 3 spatial axes determined

Anenome, Hydra, jellyfish: radial symmetry



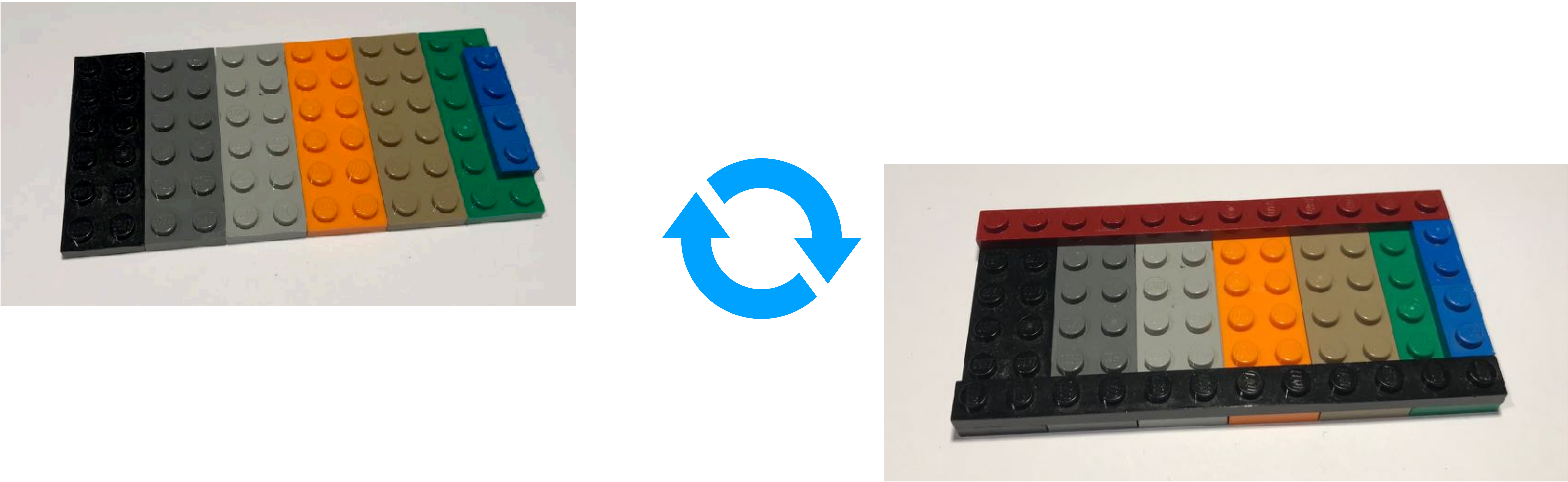
PMID: 25140222

Today's menu - 2 courses

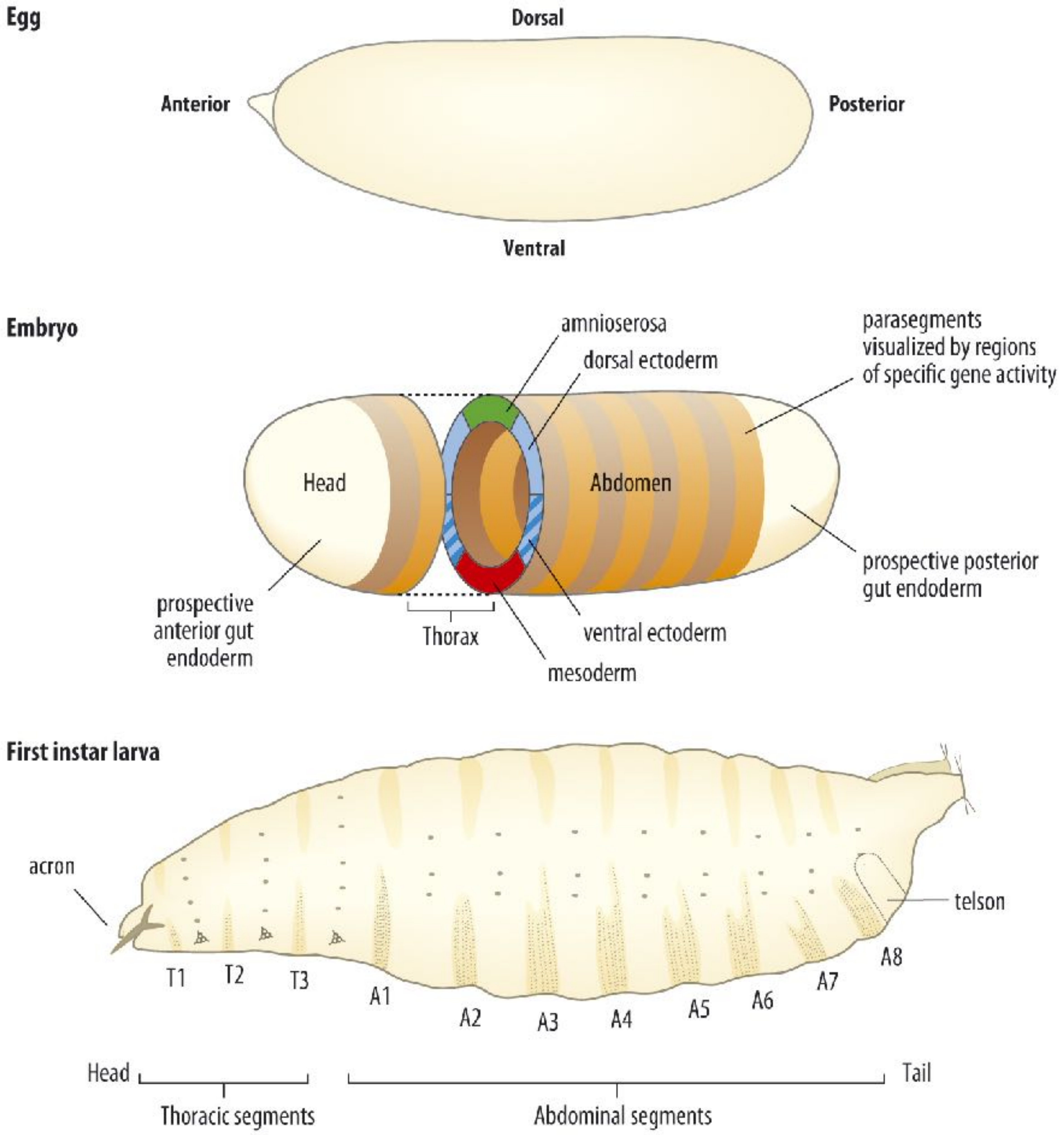
- **Front & Back, Head & Tail**
- Fate maps, specification and committtment
- Gastrulation and patterning of germ layers
- *Nobel* experiment - embryonic induction
- Integrating extracellular gradients
- ***Left and Right***
  - *Situs inversus*
  - *Cilia in the node - directed flow*
  - *Planar polarity*
  - *Nodal and calcium signals to the lateral plate*

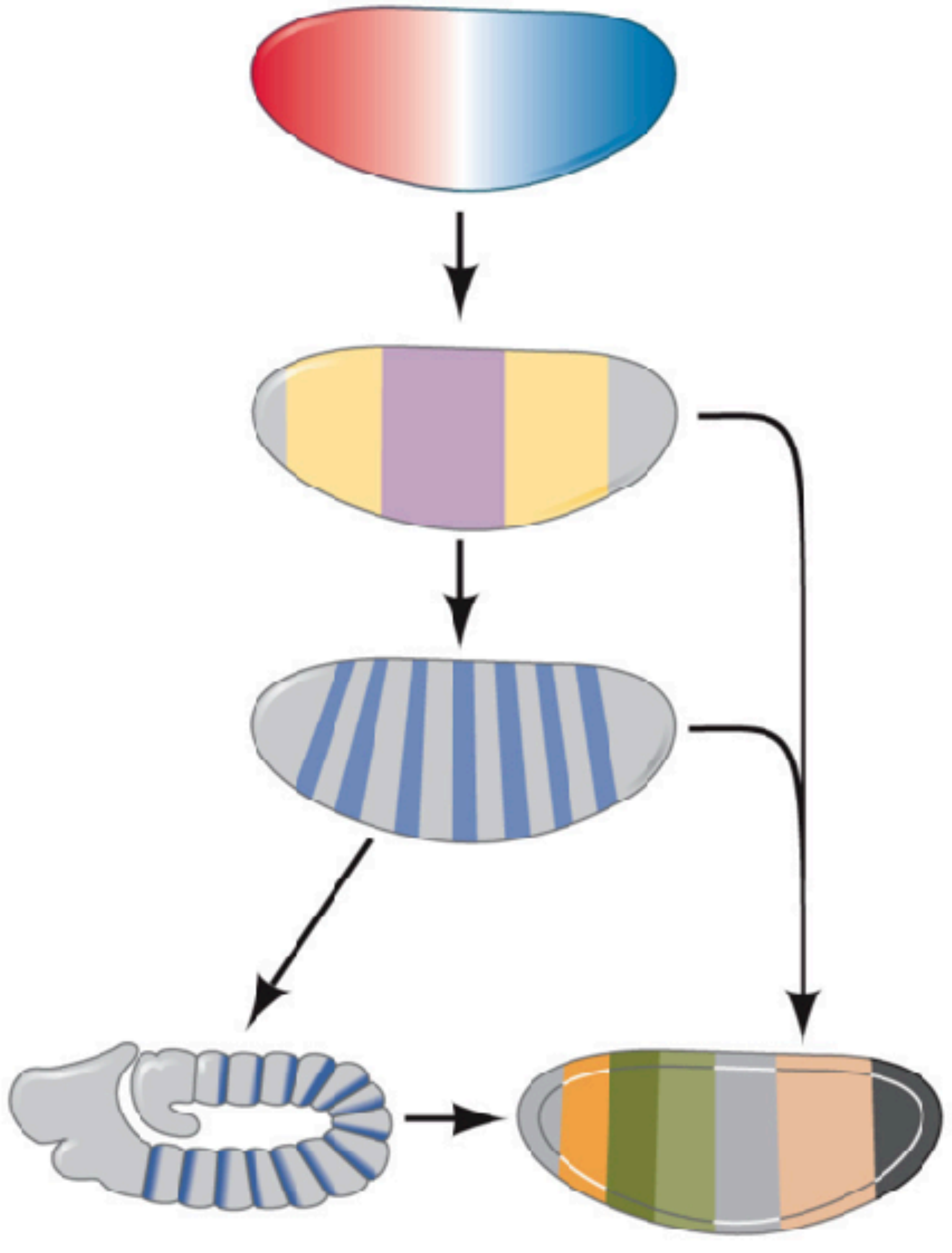
Head (Anterior) - Tail (Posterior) axis

Front (Ventral) - Back (Dorsal) axis

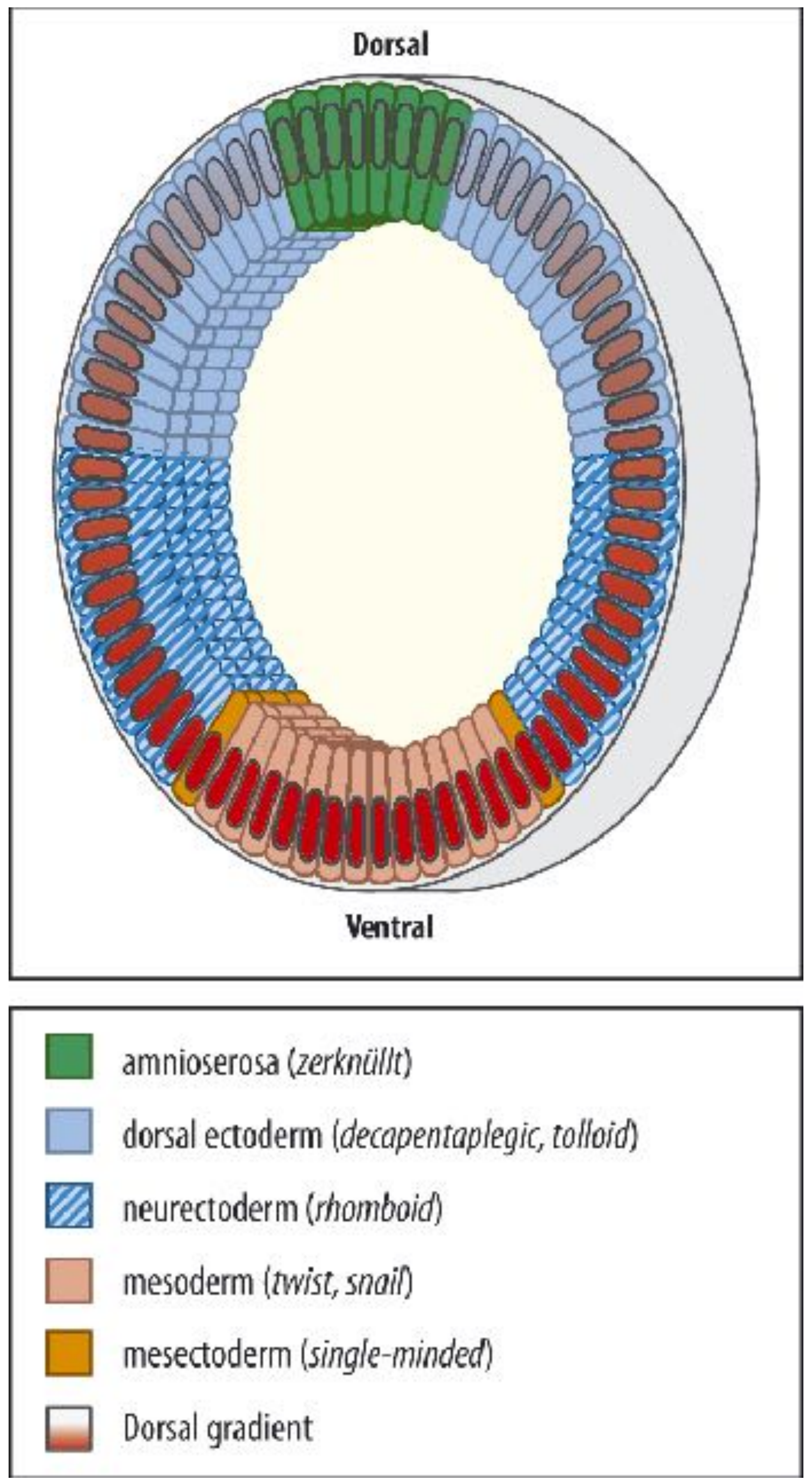


!!! Independent gene systems & orthogonal spatial mapping from egg to embryo for Anterior-Posterior and Dorsal-Ventral axes in fruit fly…





Anterior-Posterior
(Week 4)



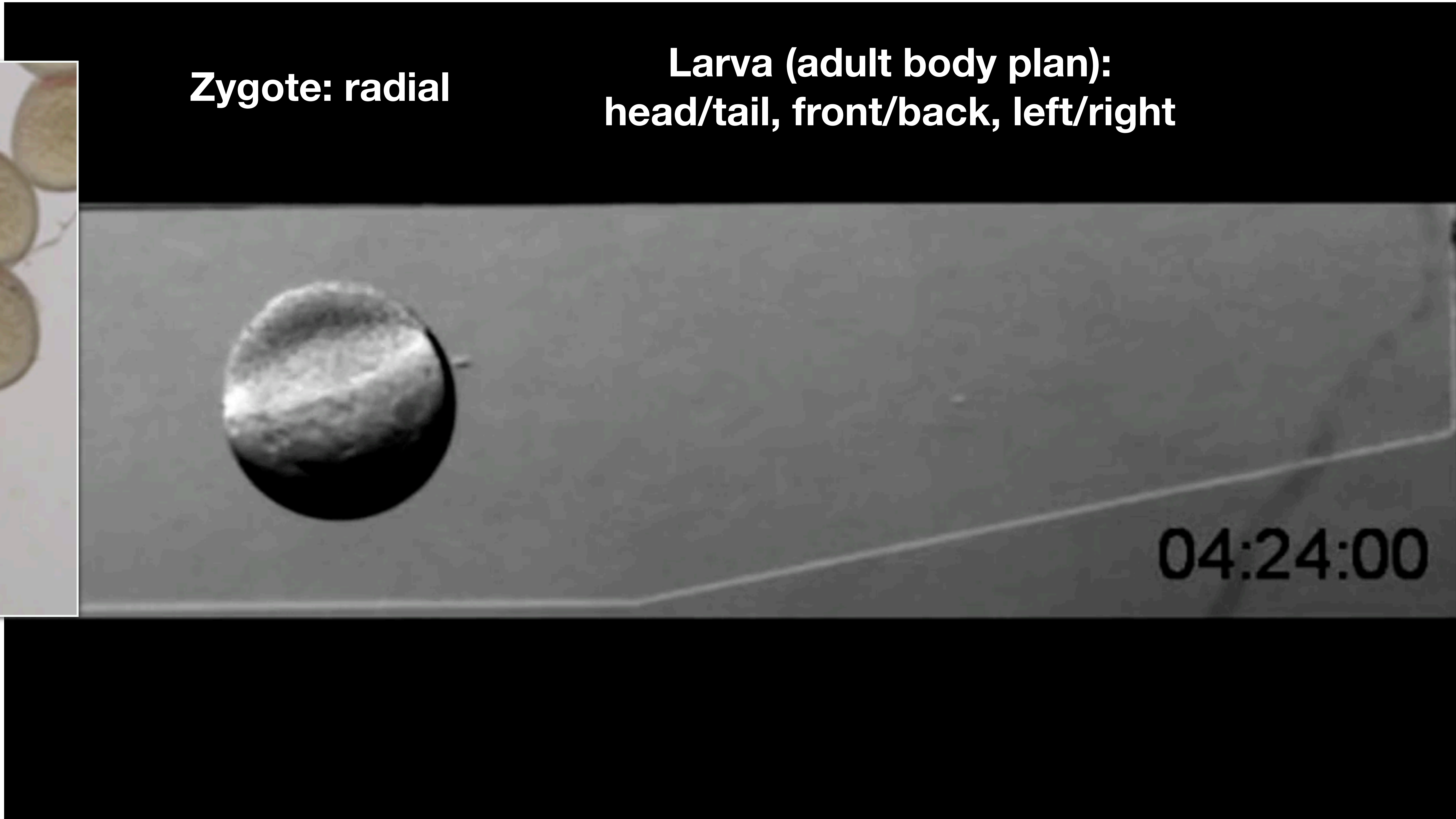
Dorsal-Ventral
(Week 10)

How is symmetry broken?

Connecting the Dots

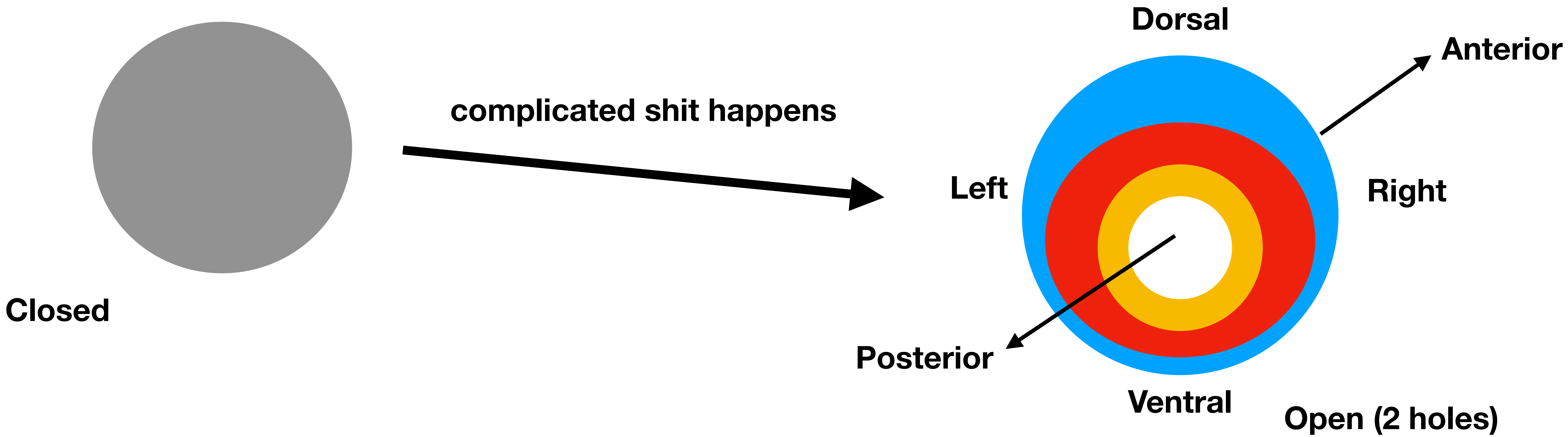
Fertilization (Week 1)

? # How is symmetry broken? ?



1. Differentiation of three* germ layers;
2. Gastrulation moves cells *inside* embryo;
3. Germ layers are patterned along 3 axes

Blastoderm: **compact mass of pluripotent cells**



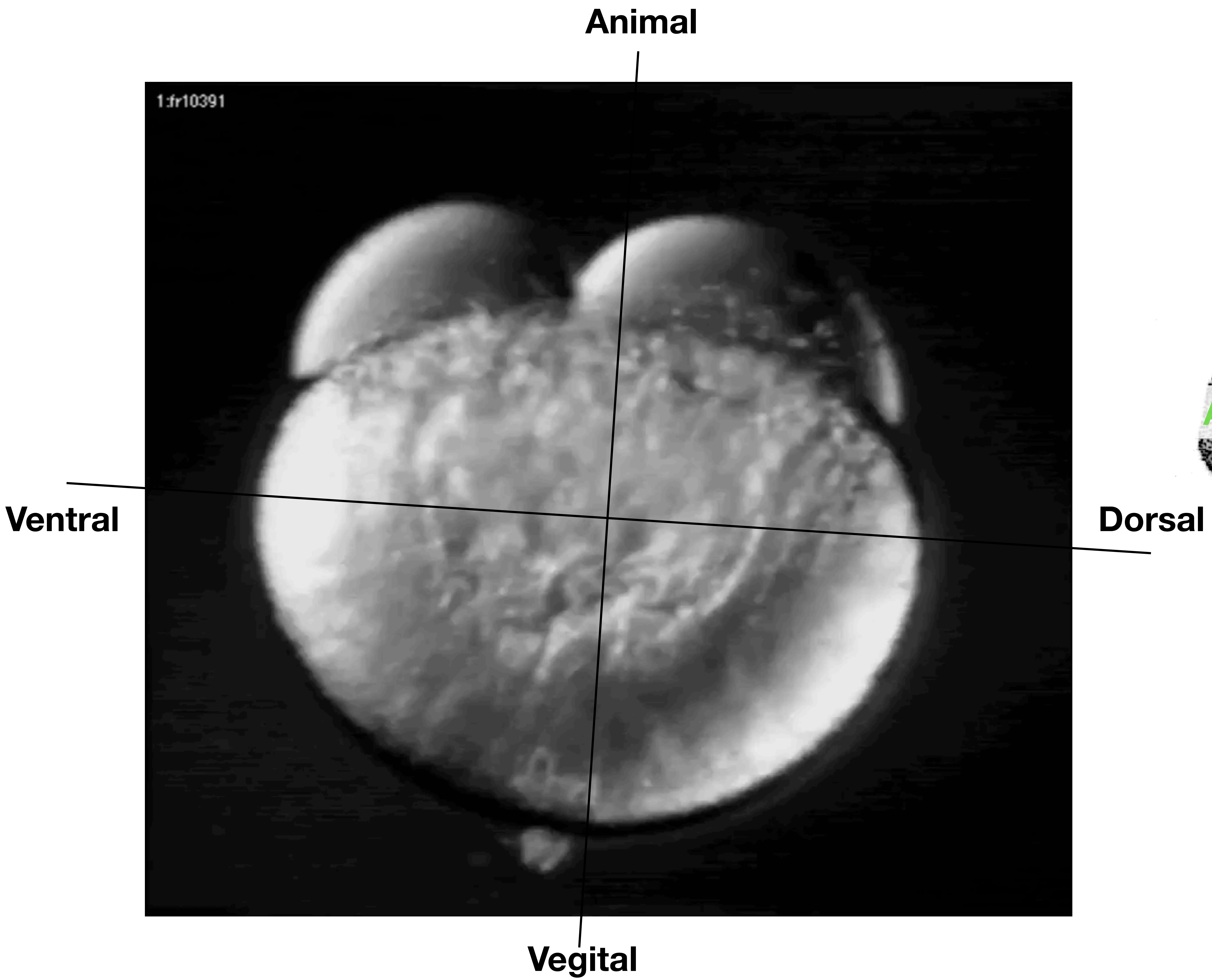
***Ectoderm*: nervous system, skin, sensory organs**

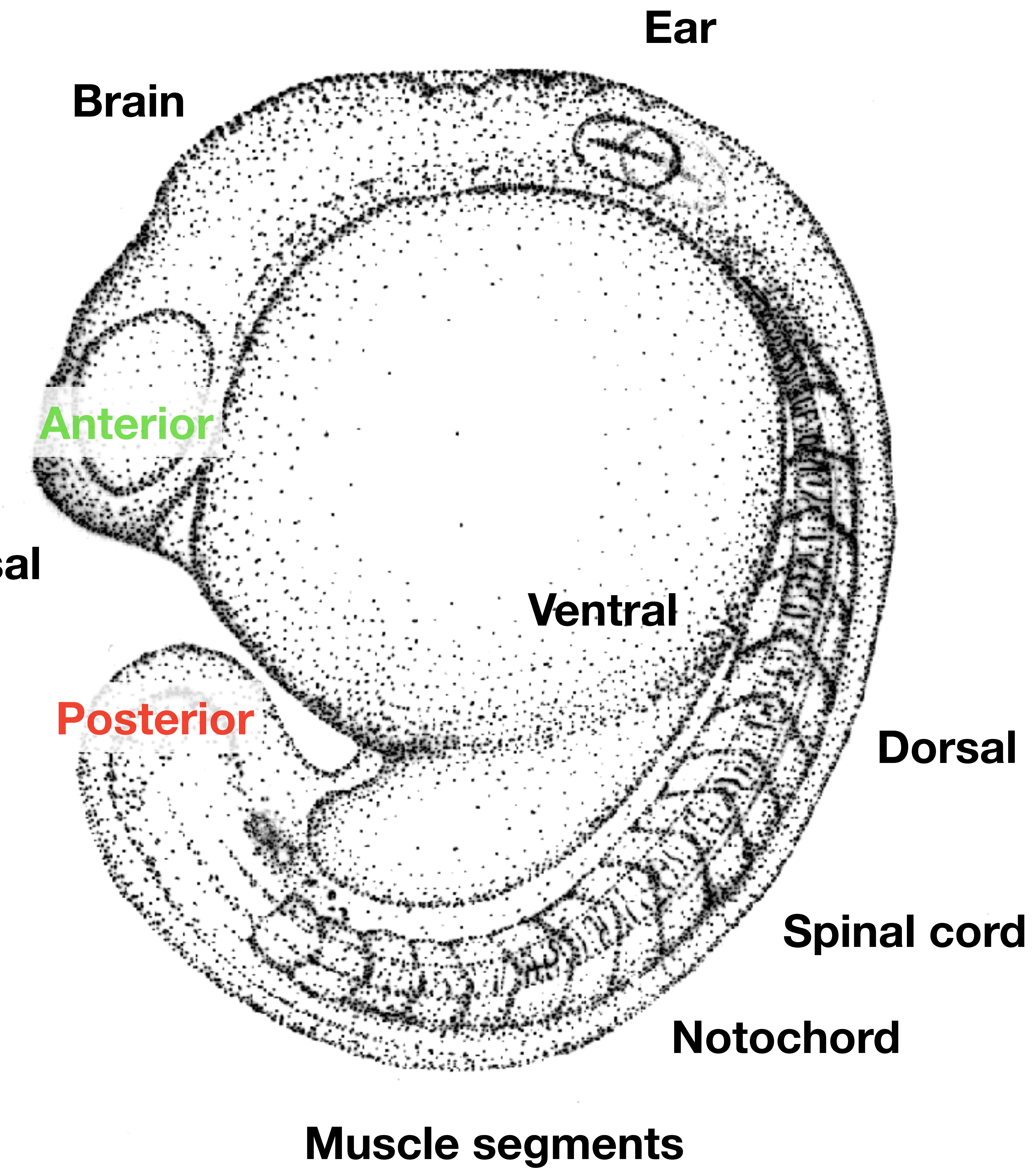
***Mesoderm*: muscle, bone, kidneys, vasculature, blood**

(mes)*Endoderm*: gut, liver, pancreas

Yolk: food!

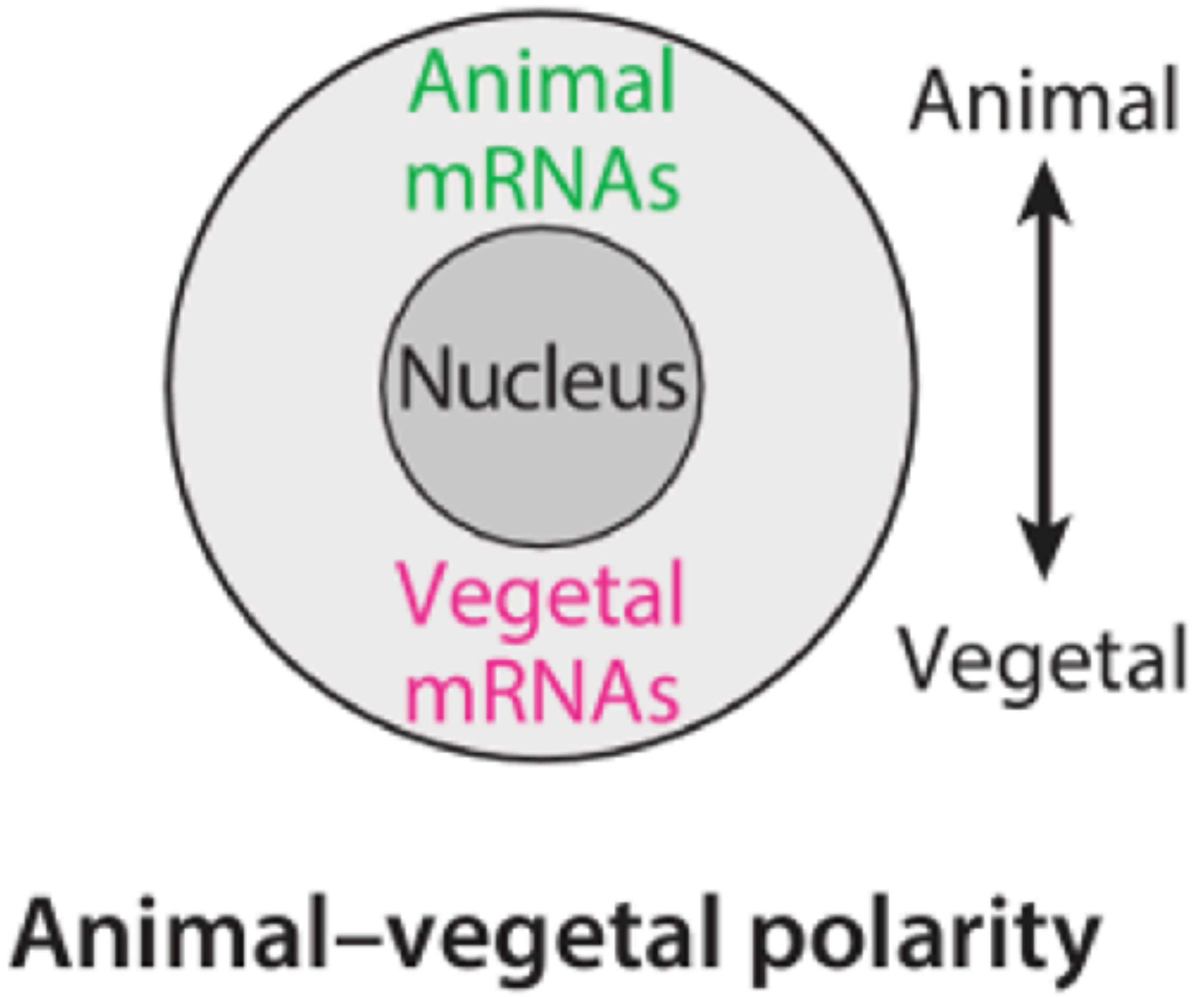
Zebrafish early development: 2-cell to 18 somites, ~18 hours

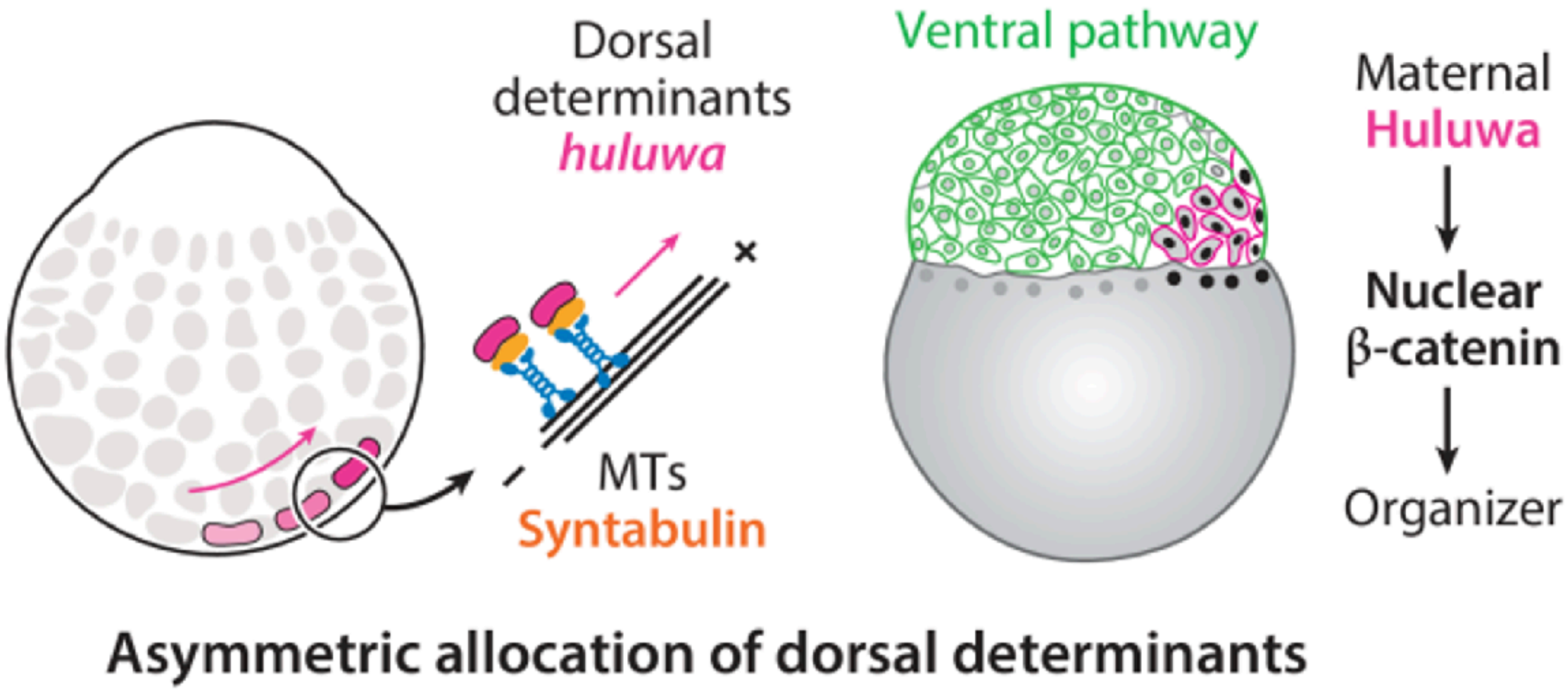




Early symmetry-breaking

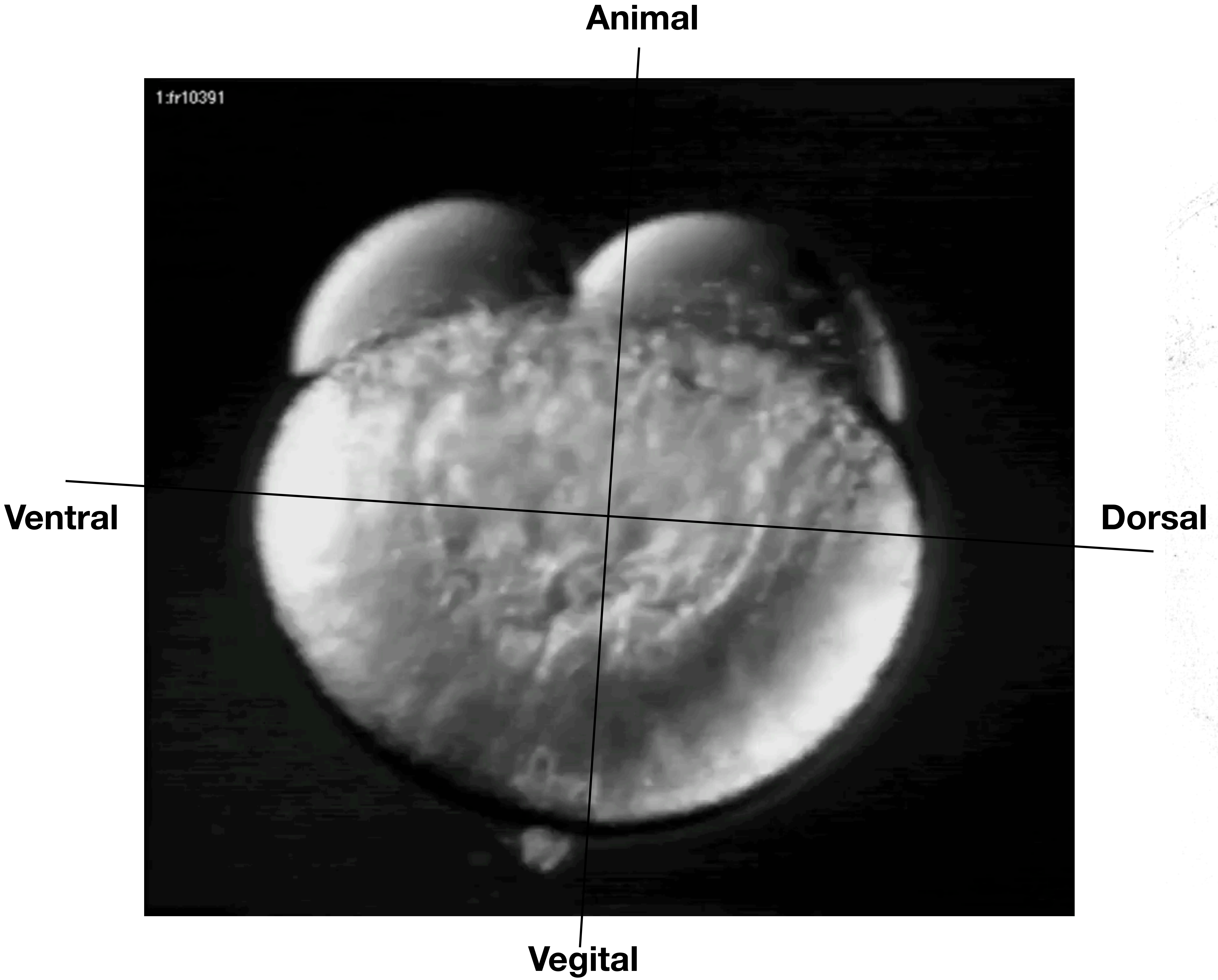
Oocytes:
spherical but patterned

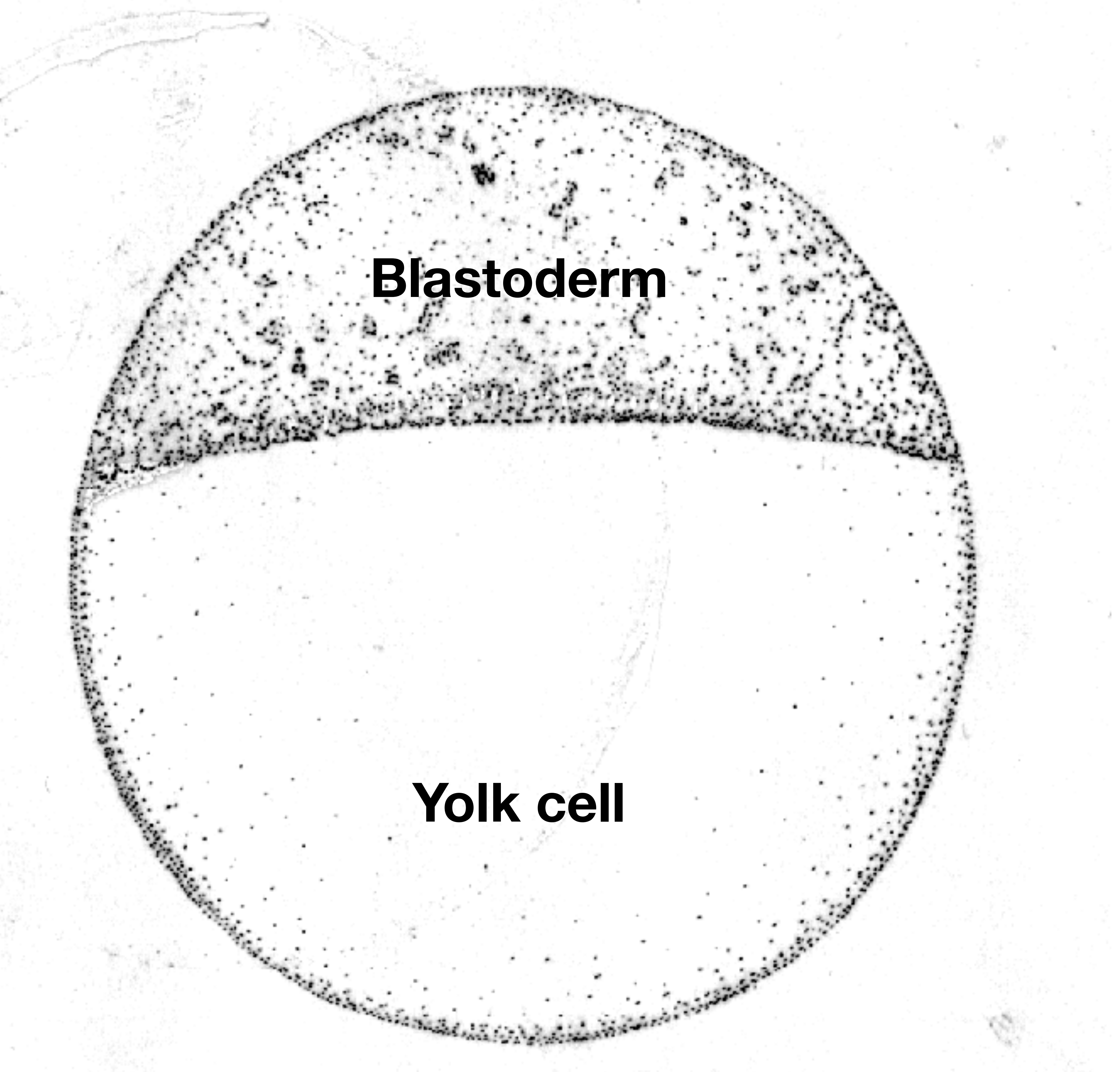




Concha ML. 2025
Annu. Rev. Cell Dev. Biol. 41:89–134

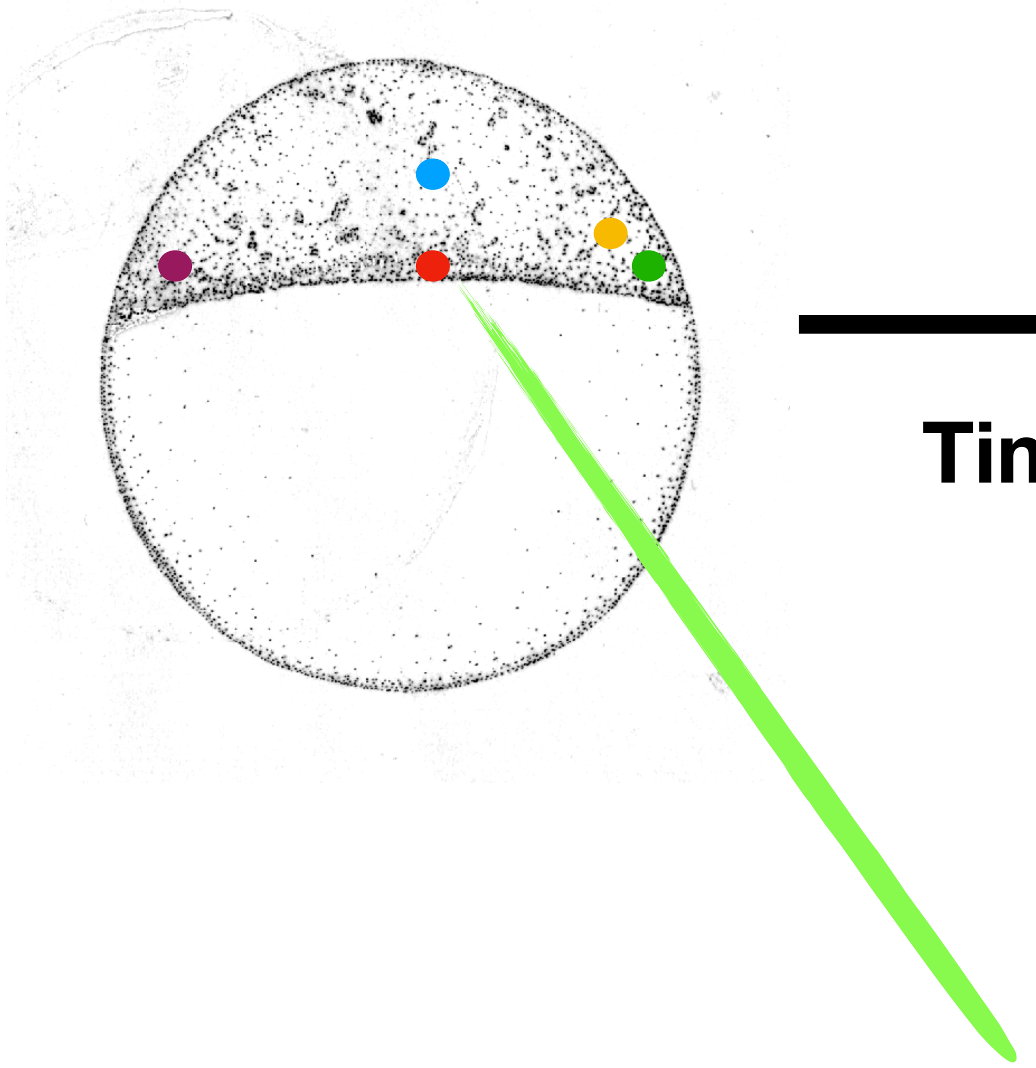
Zebrafish early development: cleavage stage ⇒ sphere



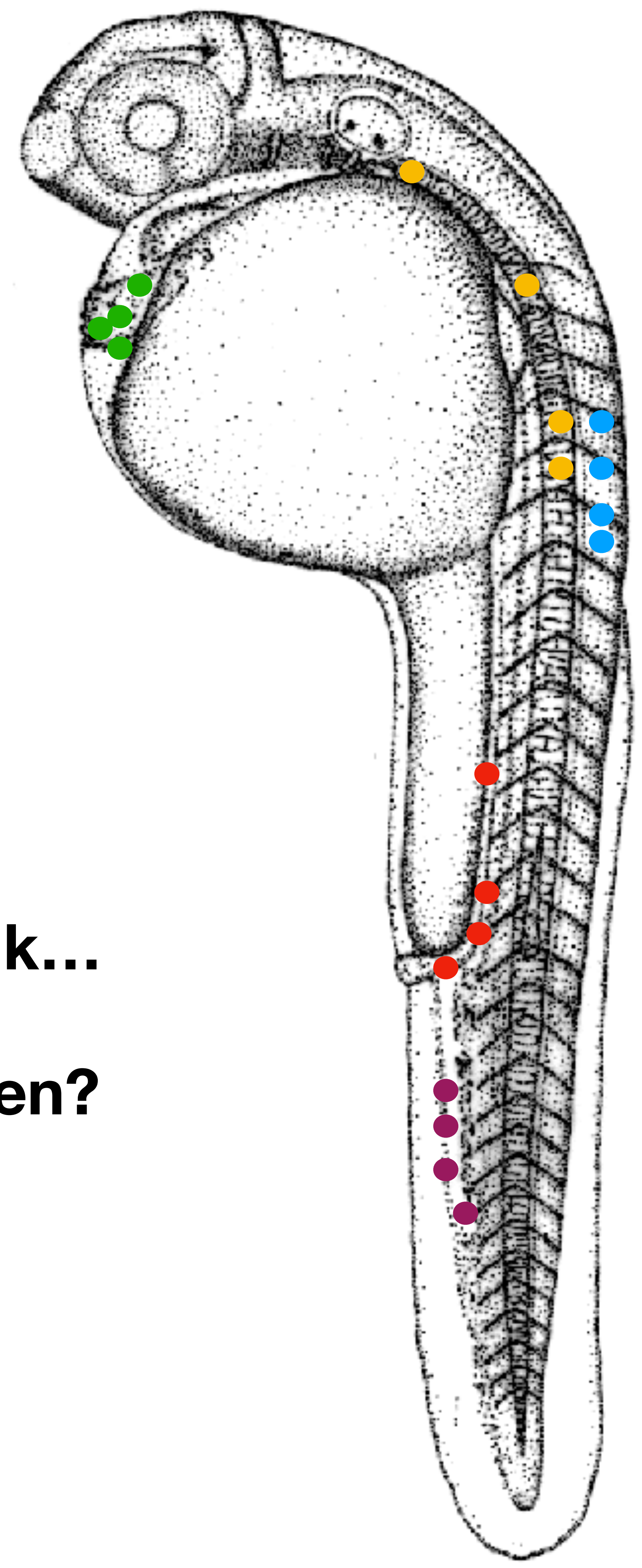


Fate map and lineage of the early zebrafish

Sphere-stage
Pre-gastrulation

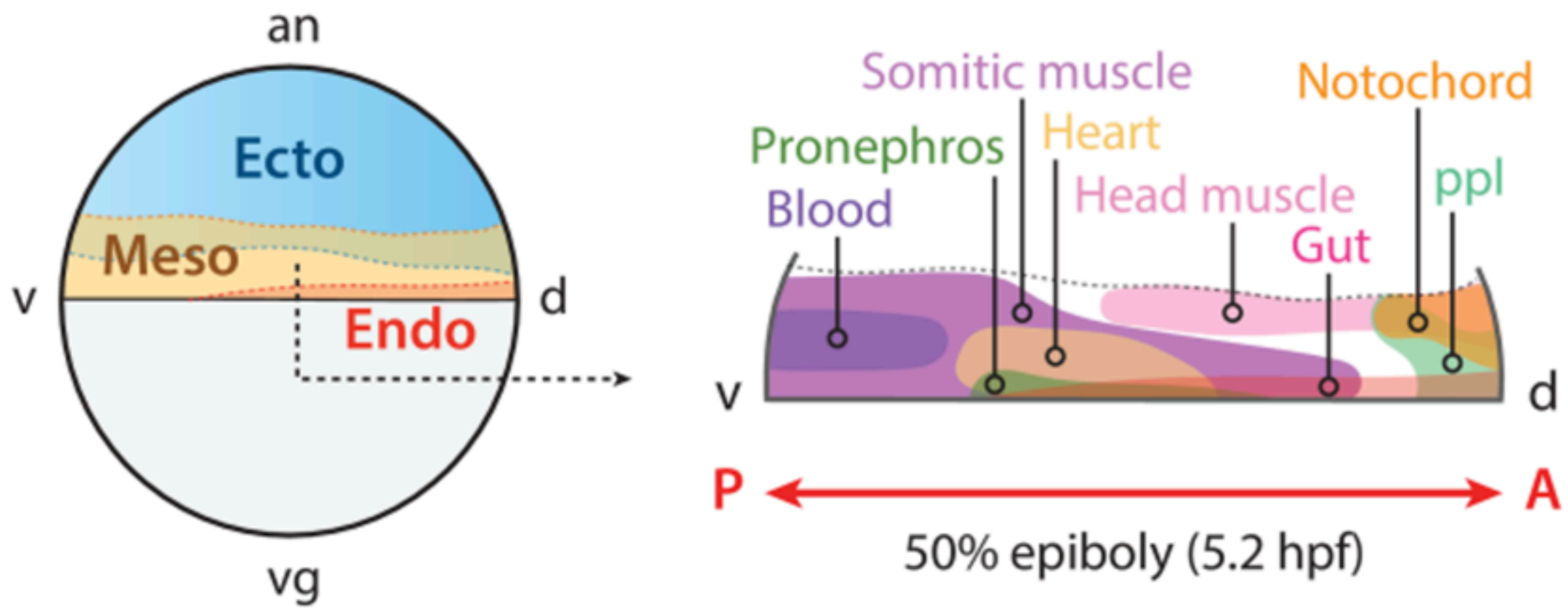


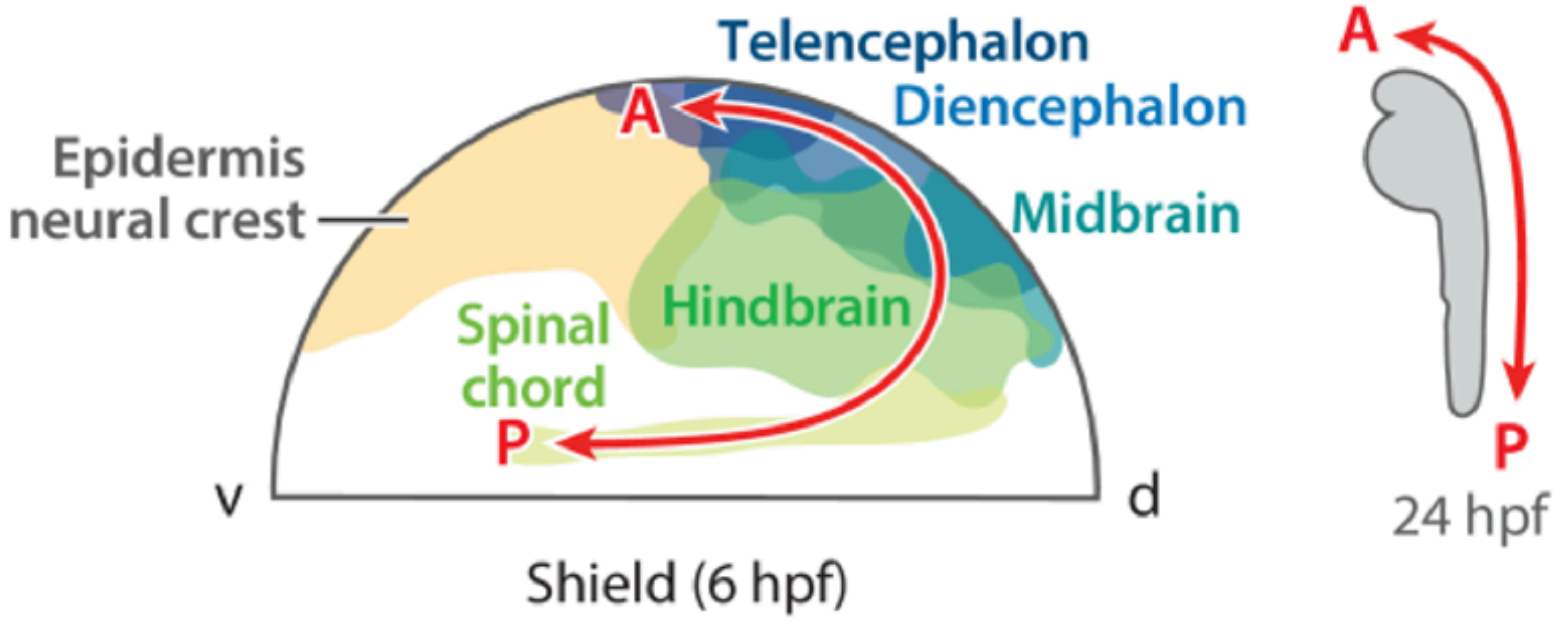
Time



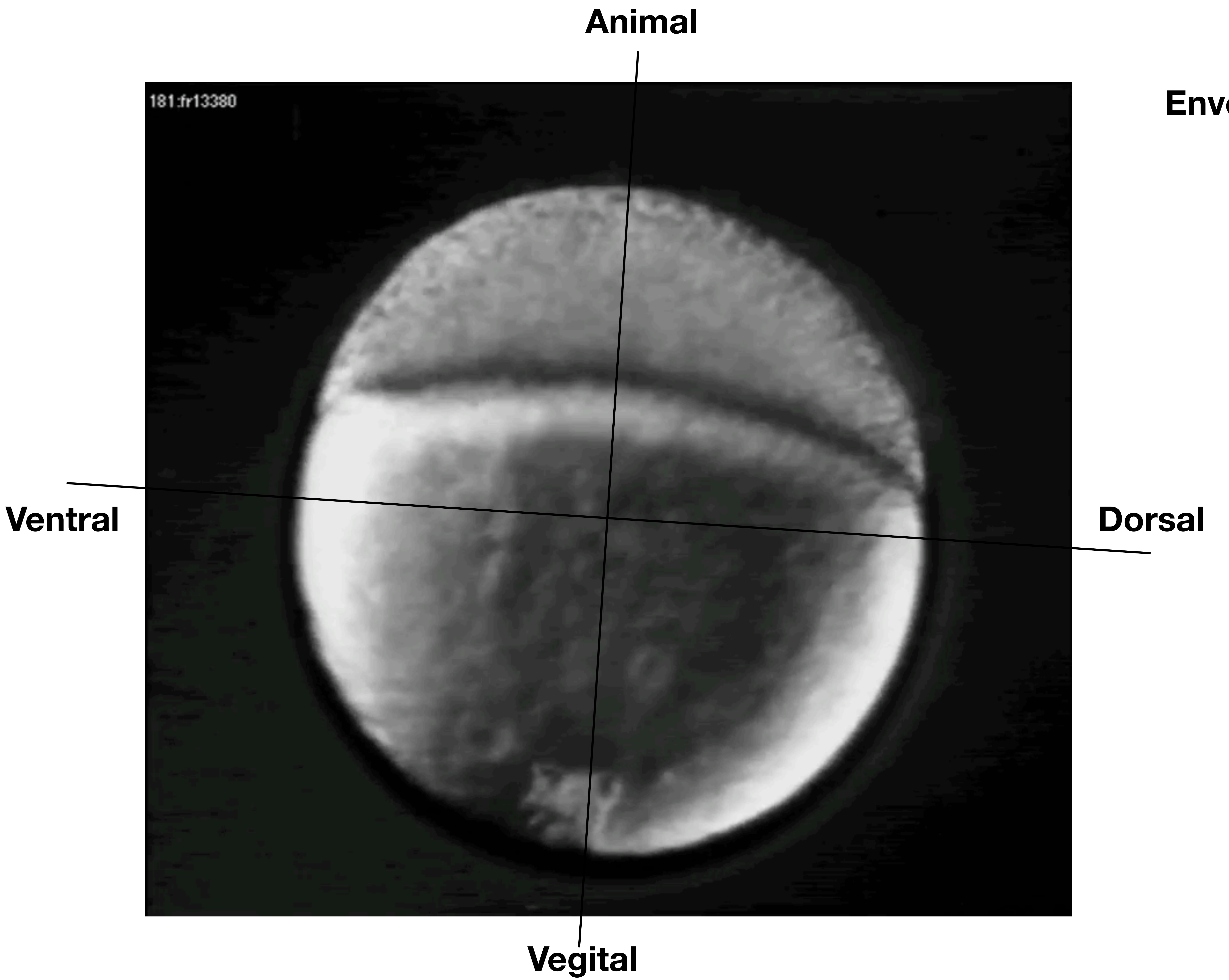
Fluorescent molecule, beads, ink…

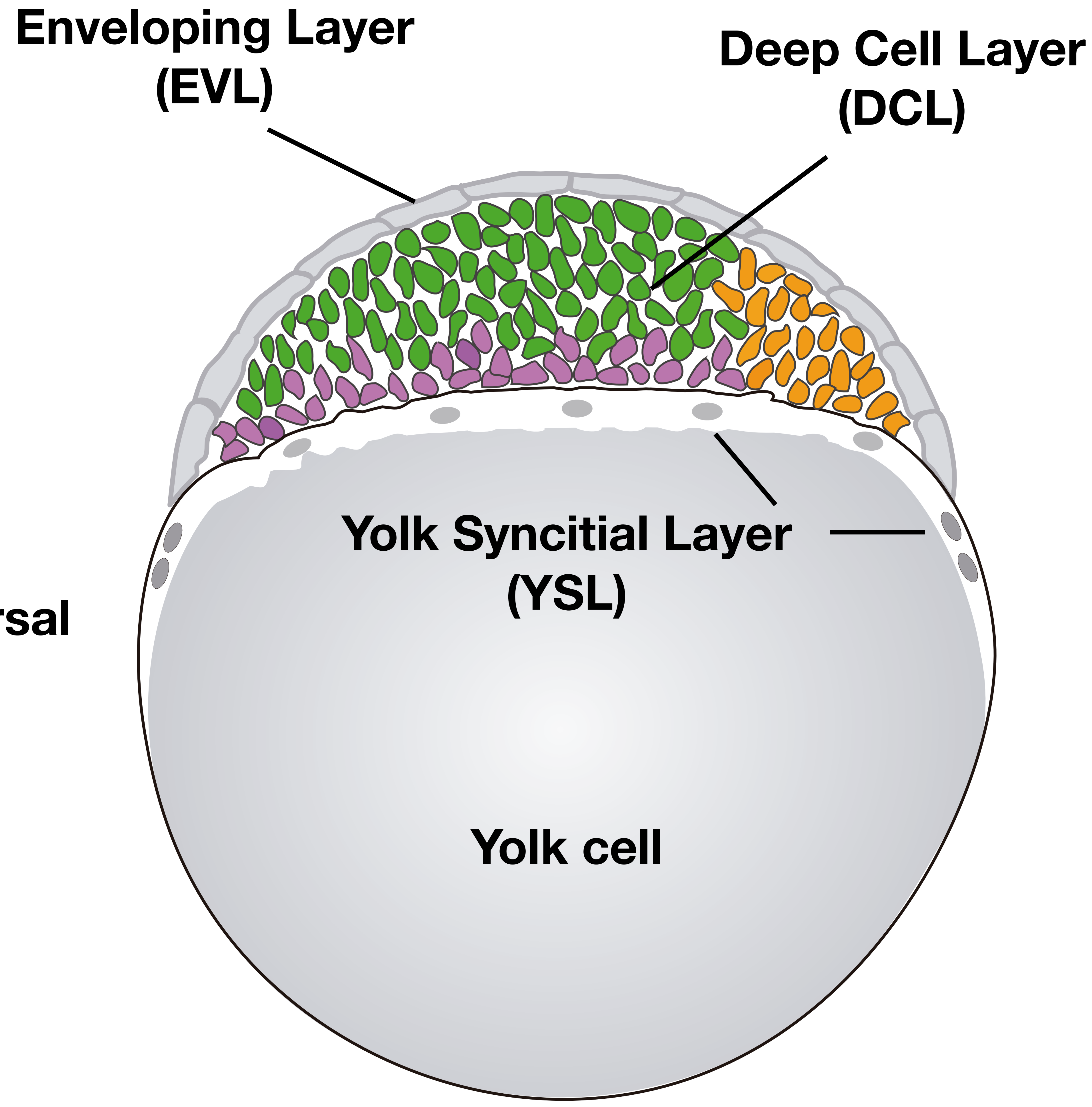
Labelled when? Grown until when?



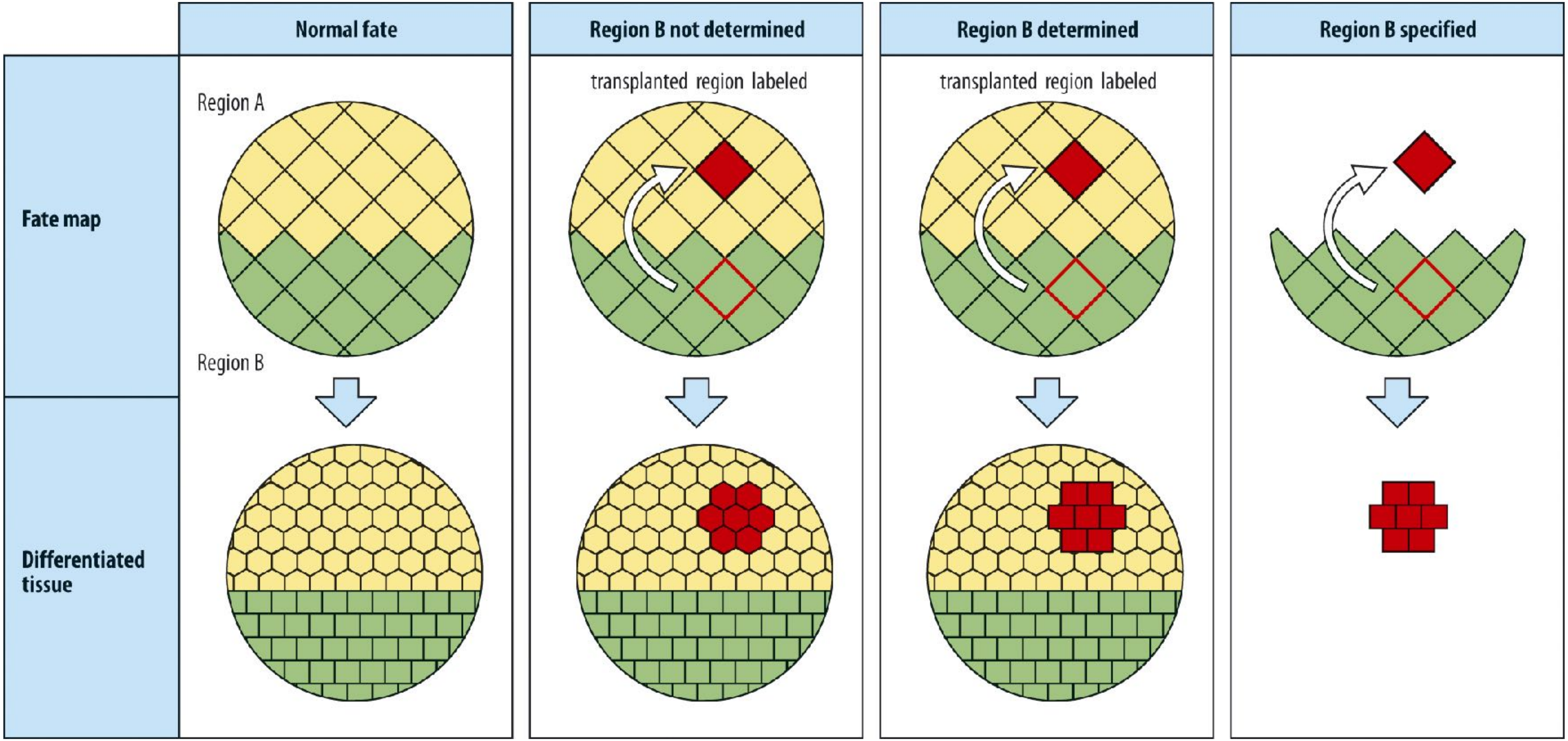


Zebrafish blastoderm well-patterned before gastrulation



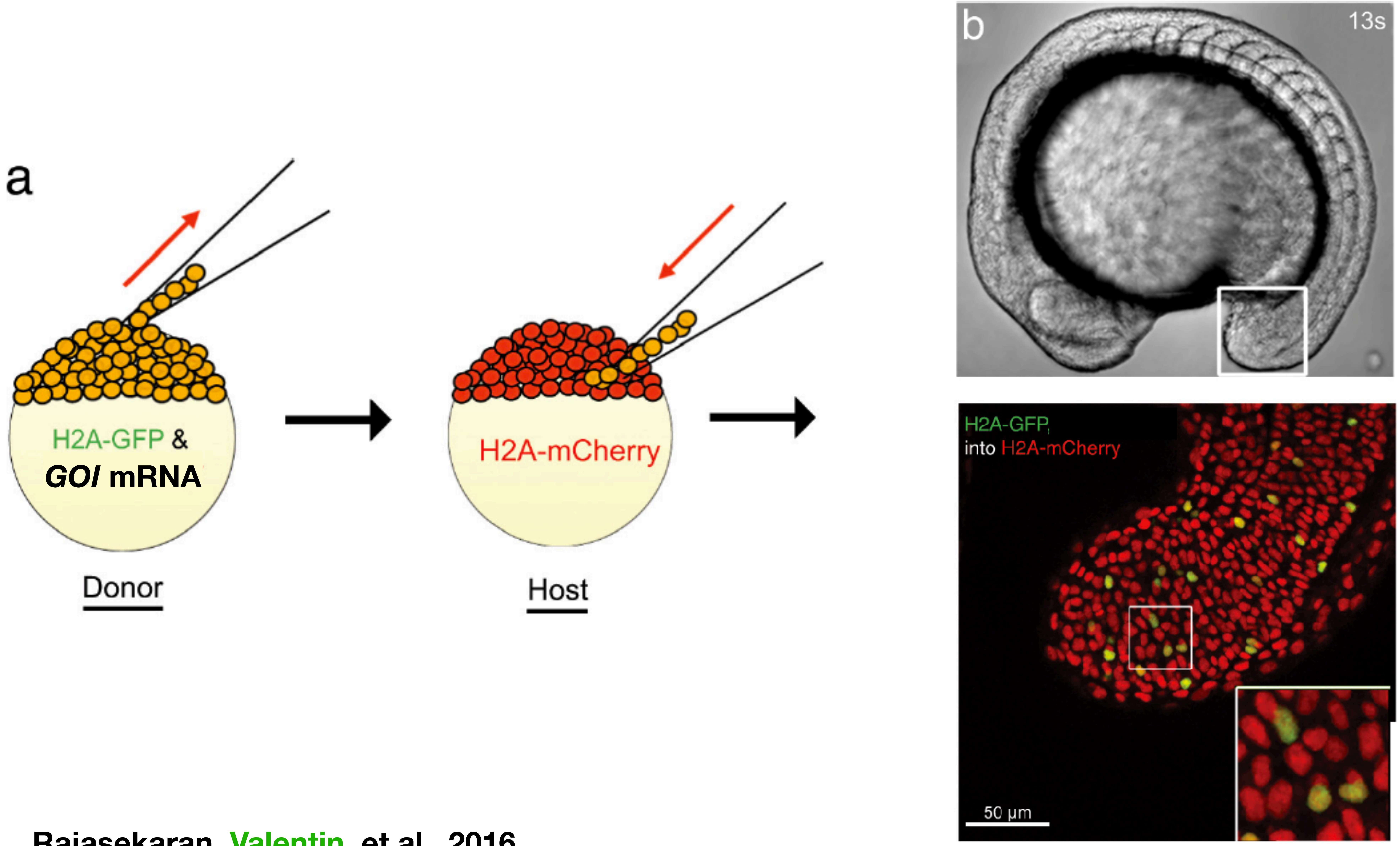


Fate maps, specfication and determination



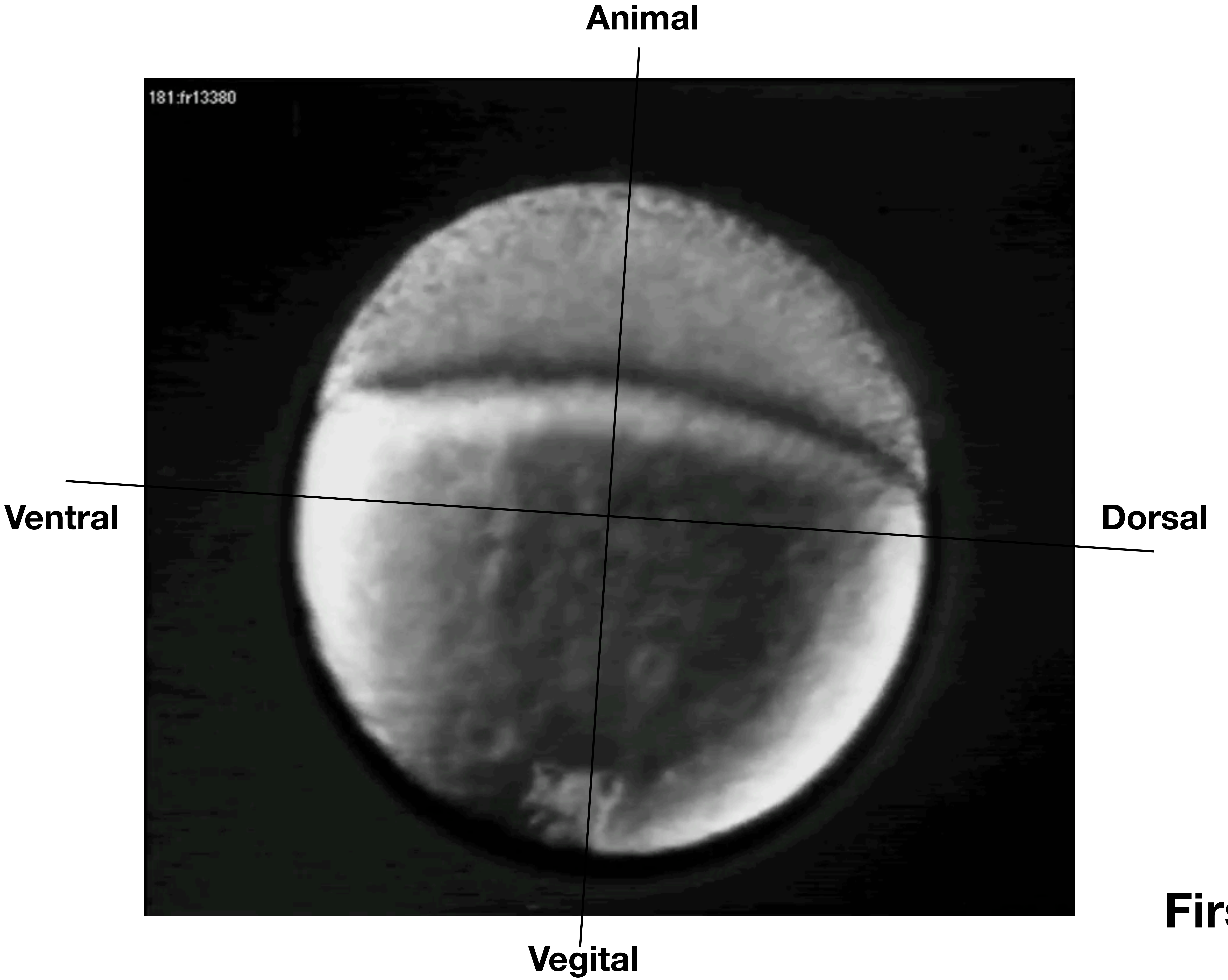
Green/yellow: geographical location; Red: labelled cells; Shape: cell type

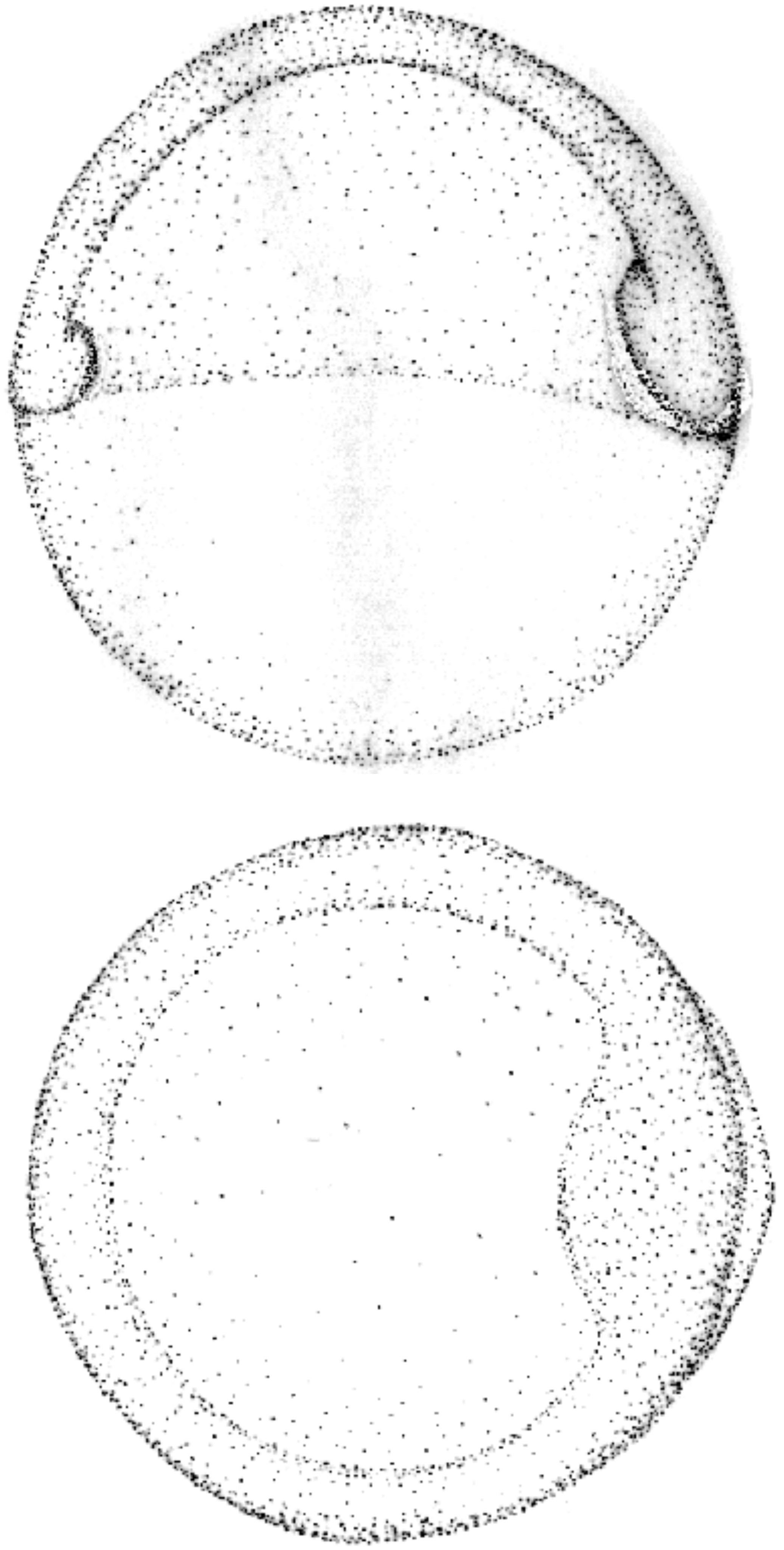
Transplantation of cells at sphere stage



Rajasekaran, Valentin, et al., 2016

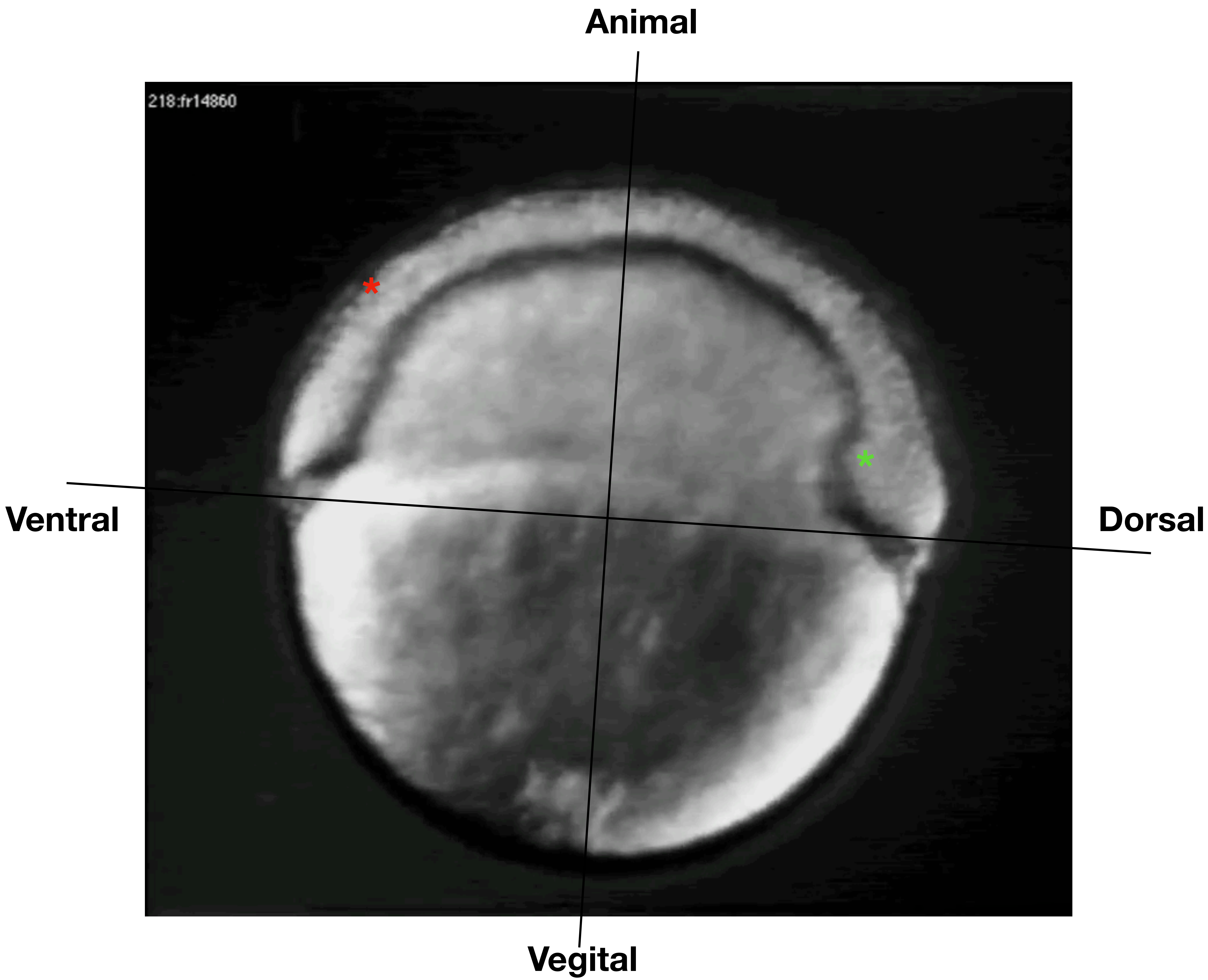
Zebrafish early development: sphere ⇒ sheild

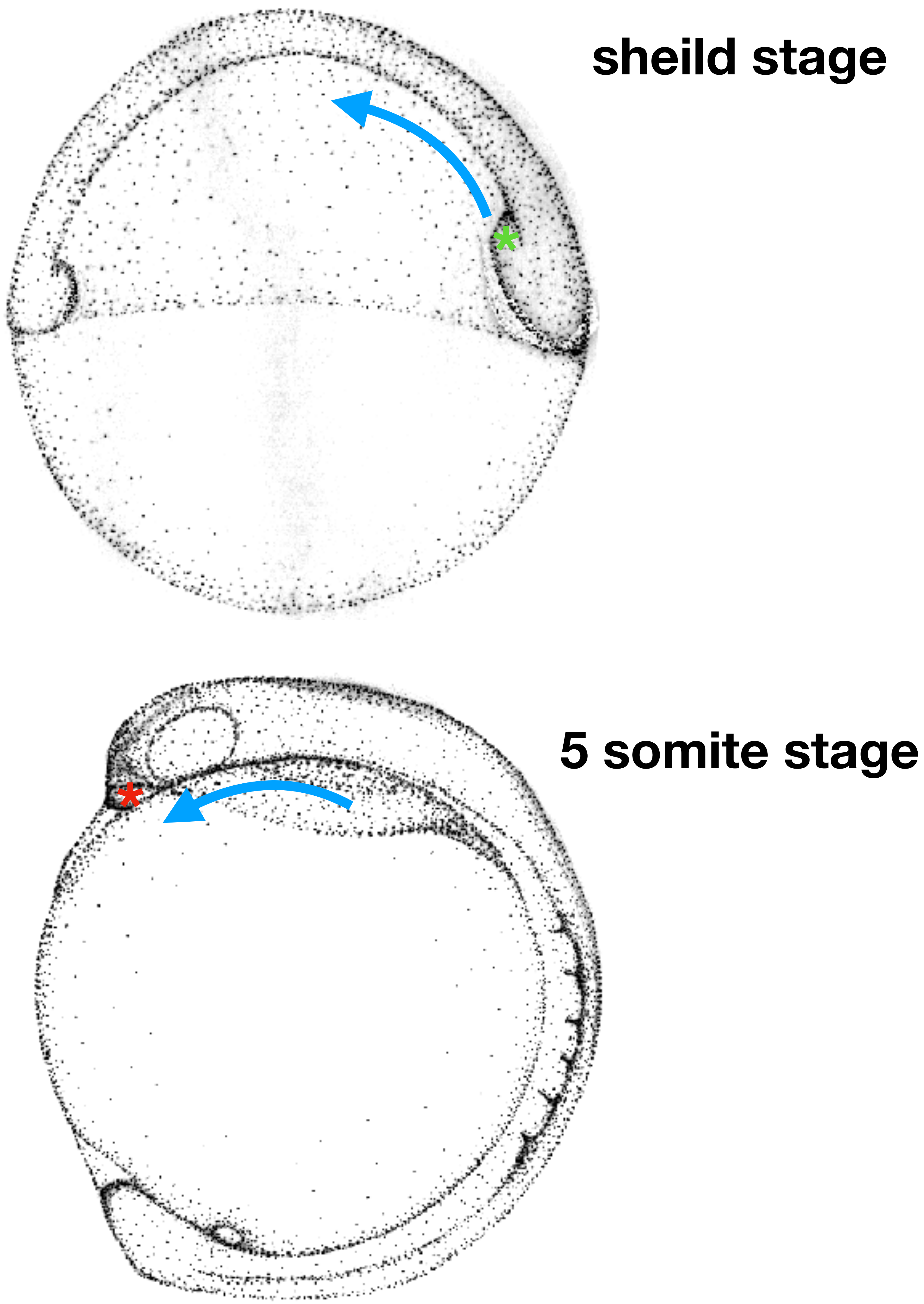




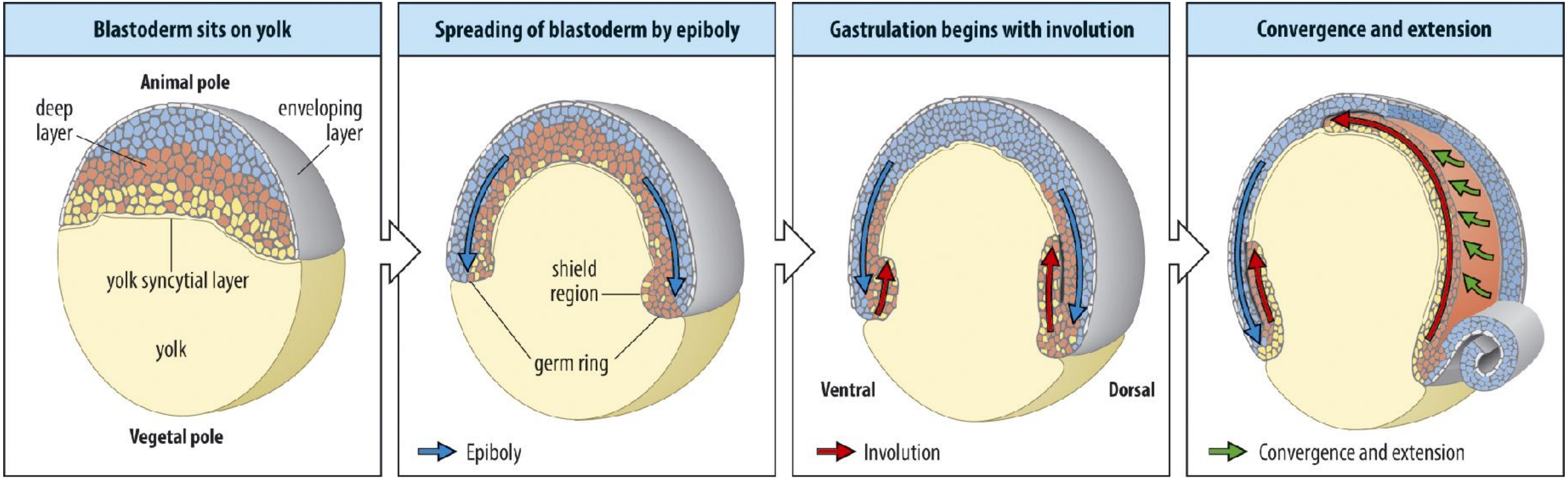
First morphological asymmetry, onset of gastrulation

The dorsal mesoderm moves up to the anterior



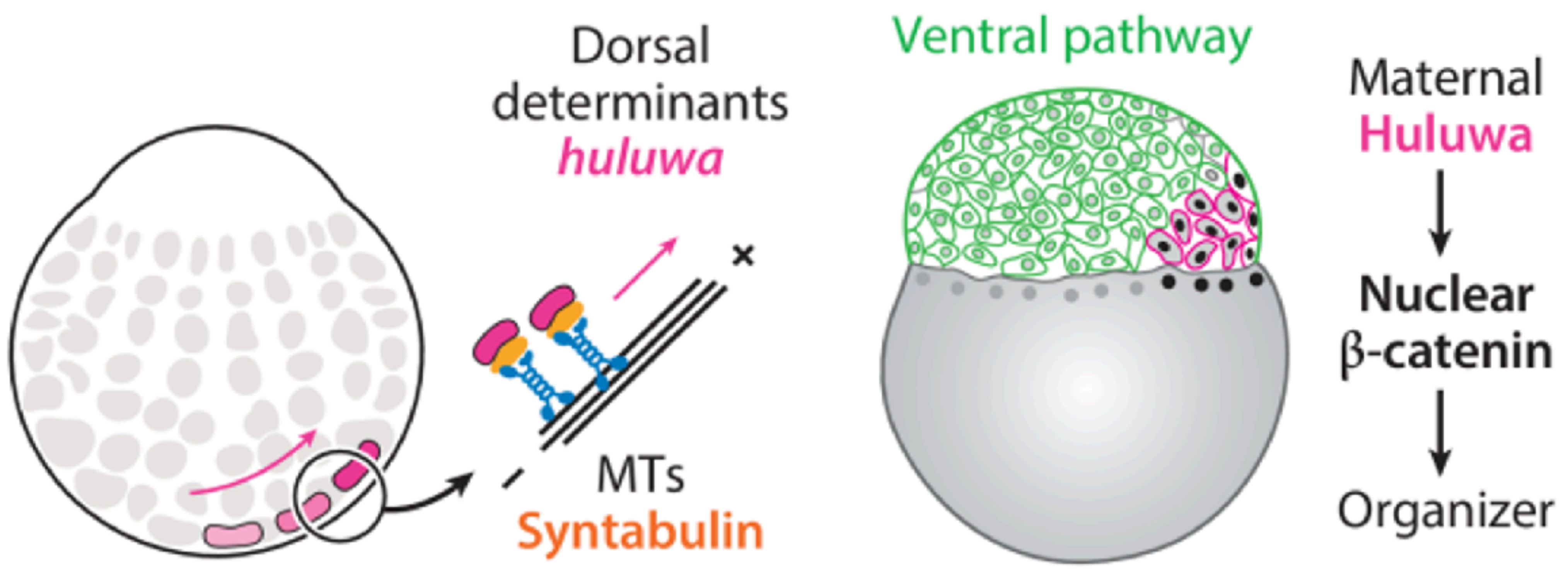


What starts out as Dorsal cells/tissue moves to the Anterior

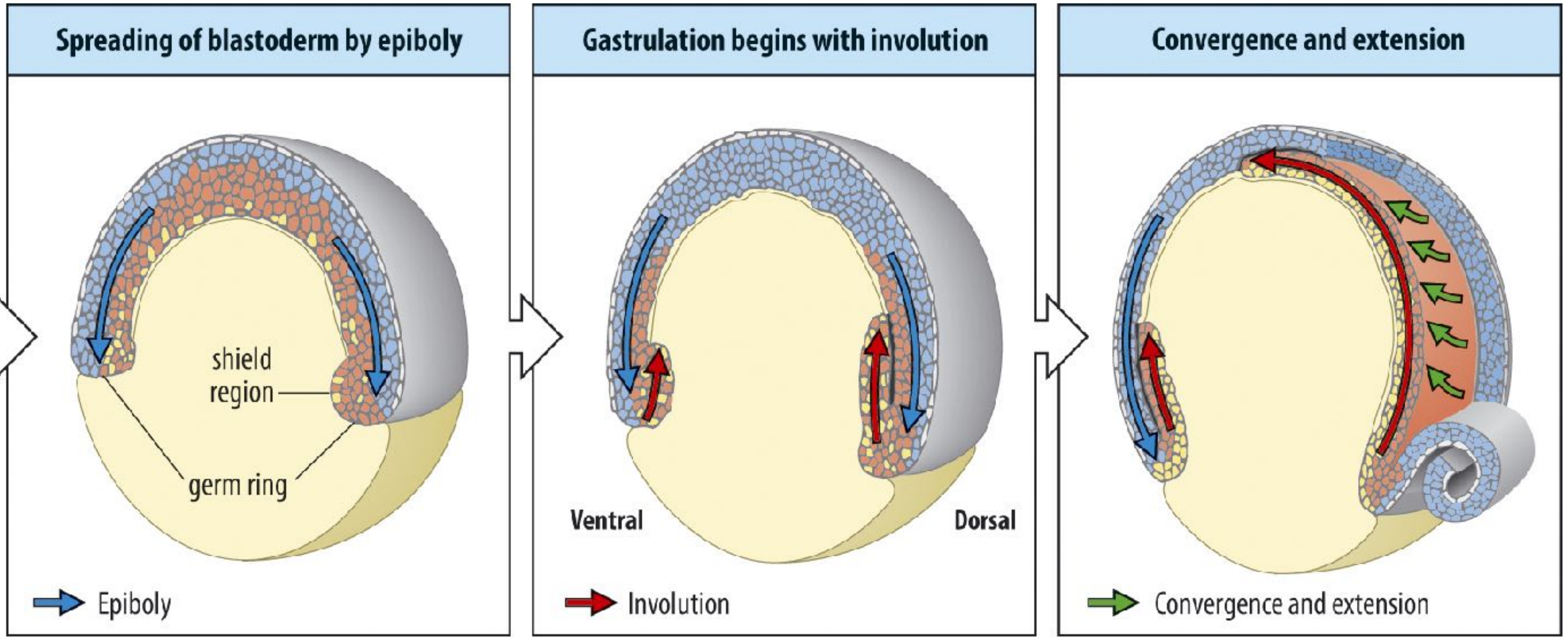


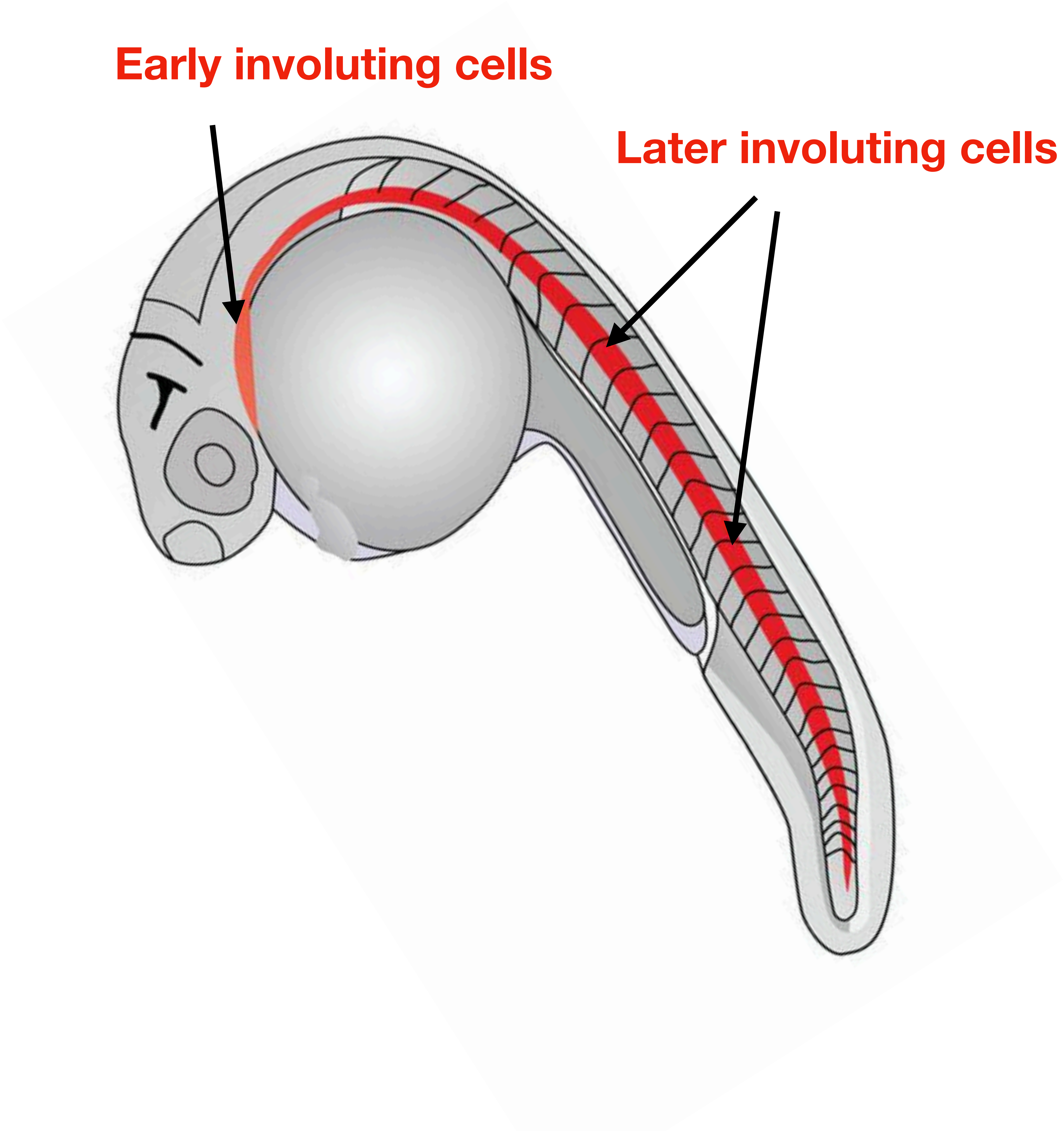
DV and AP axes are thightly coupled

Dorsal maps to Anterior! Sheild makes notochord!

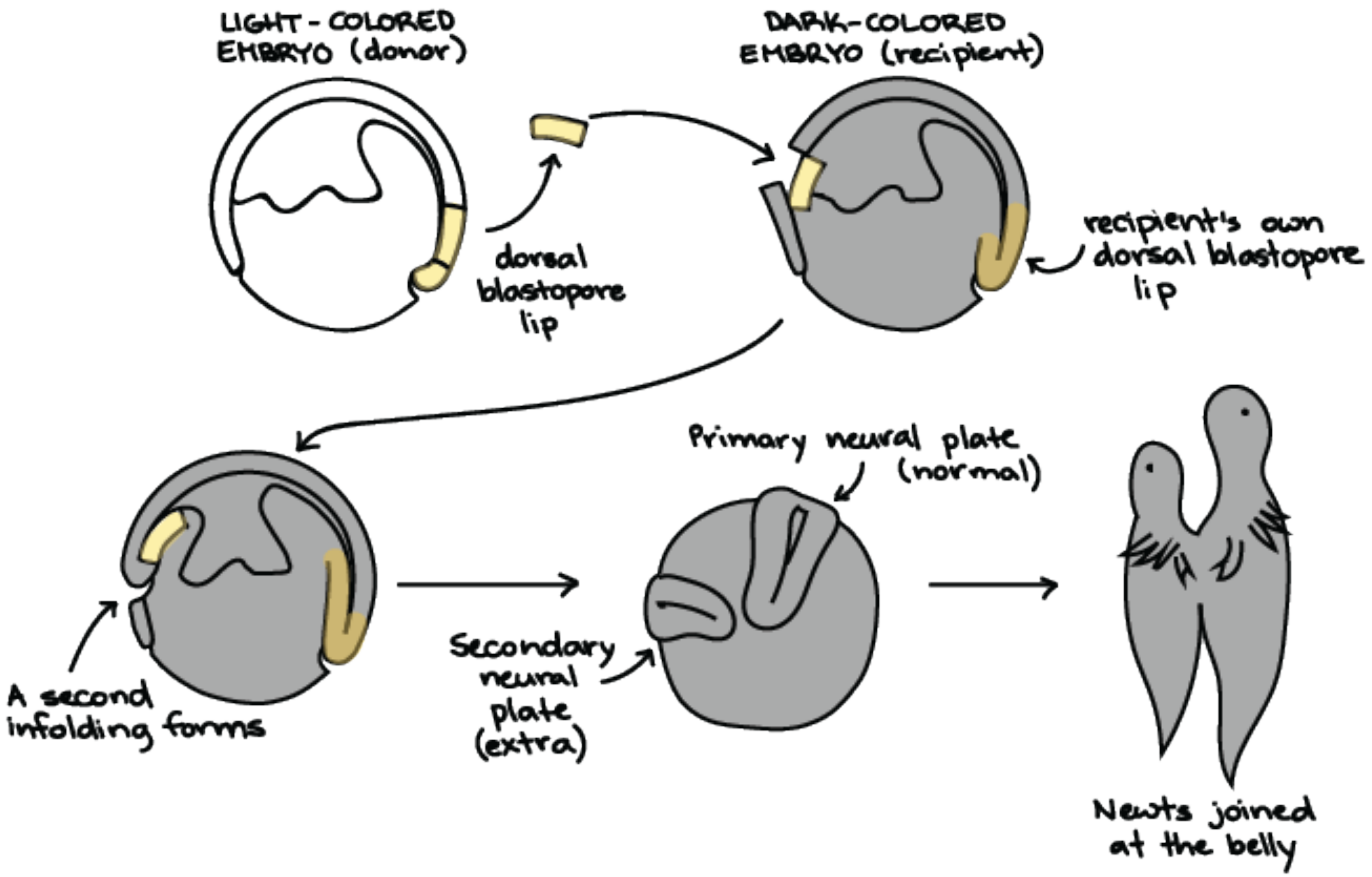


Asymmetric allocation of dorsal determinants

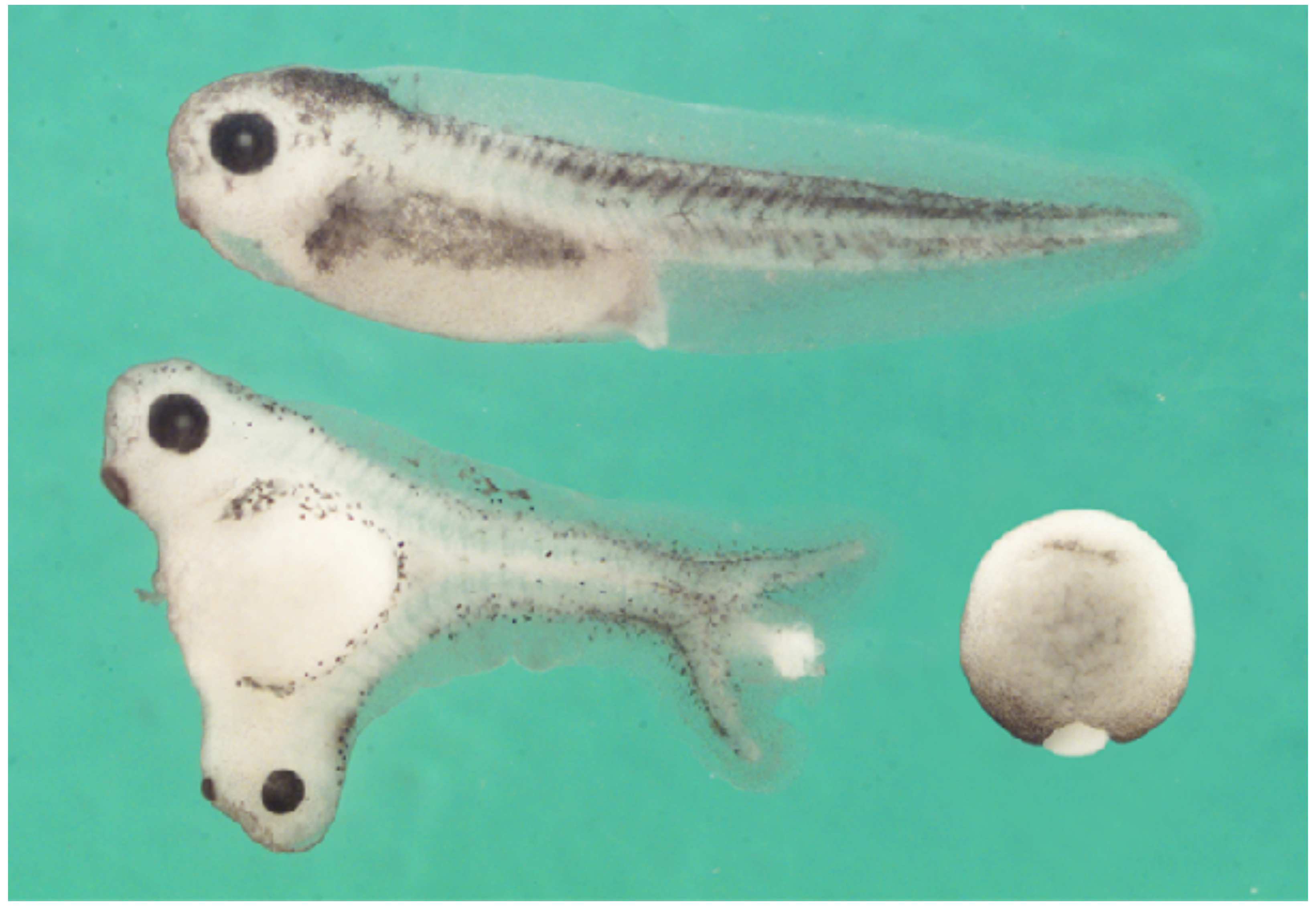




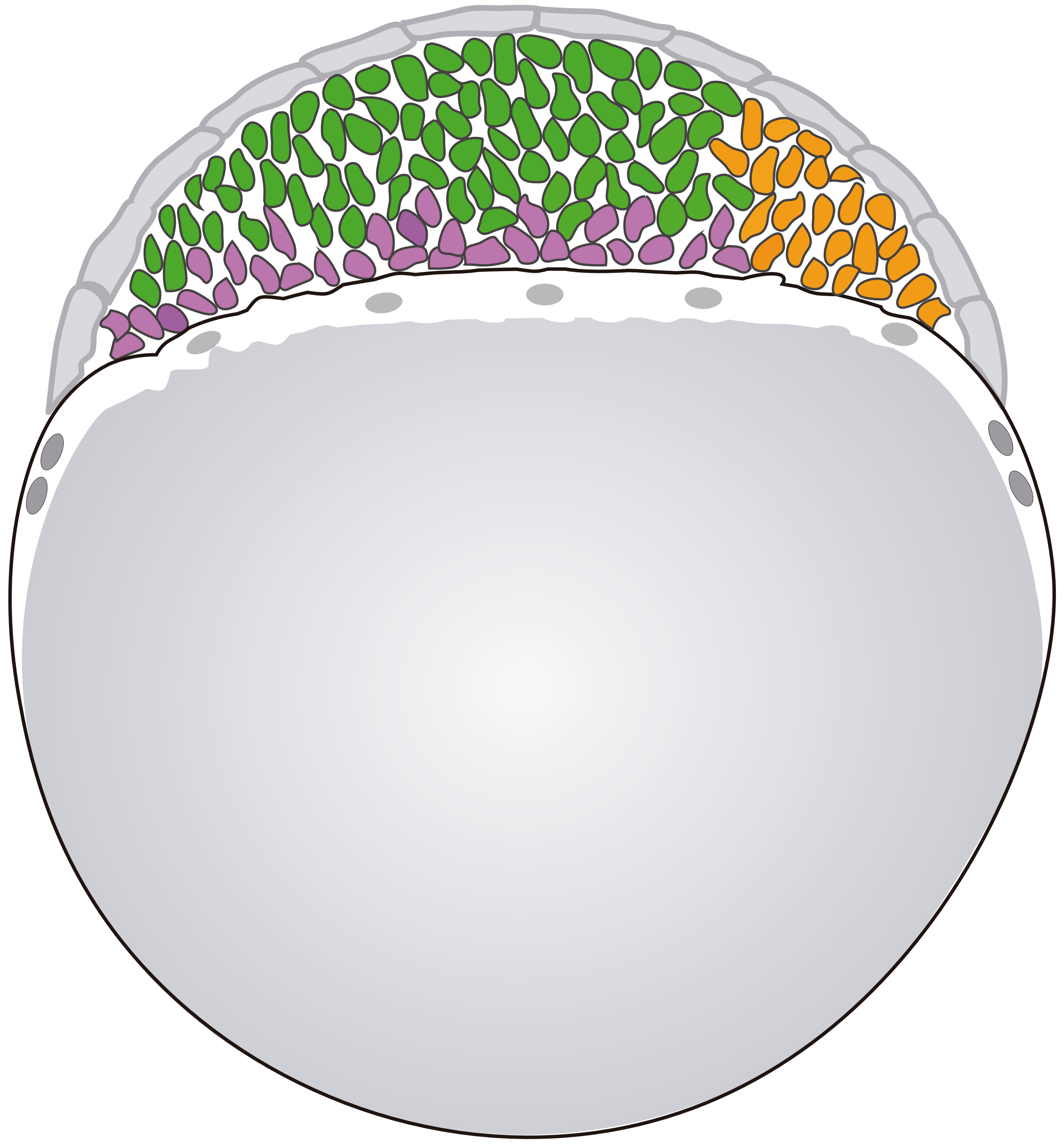
Embryonic Induction: Spemann and Mangold, 1924

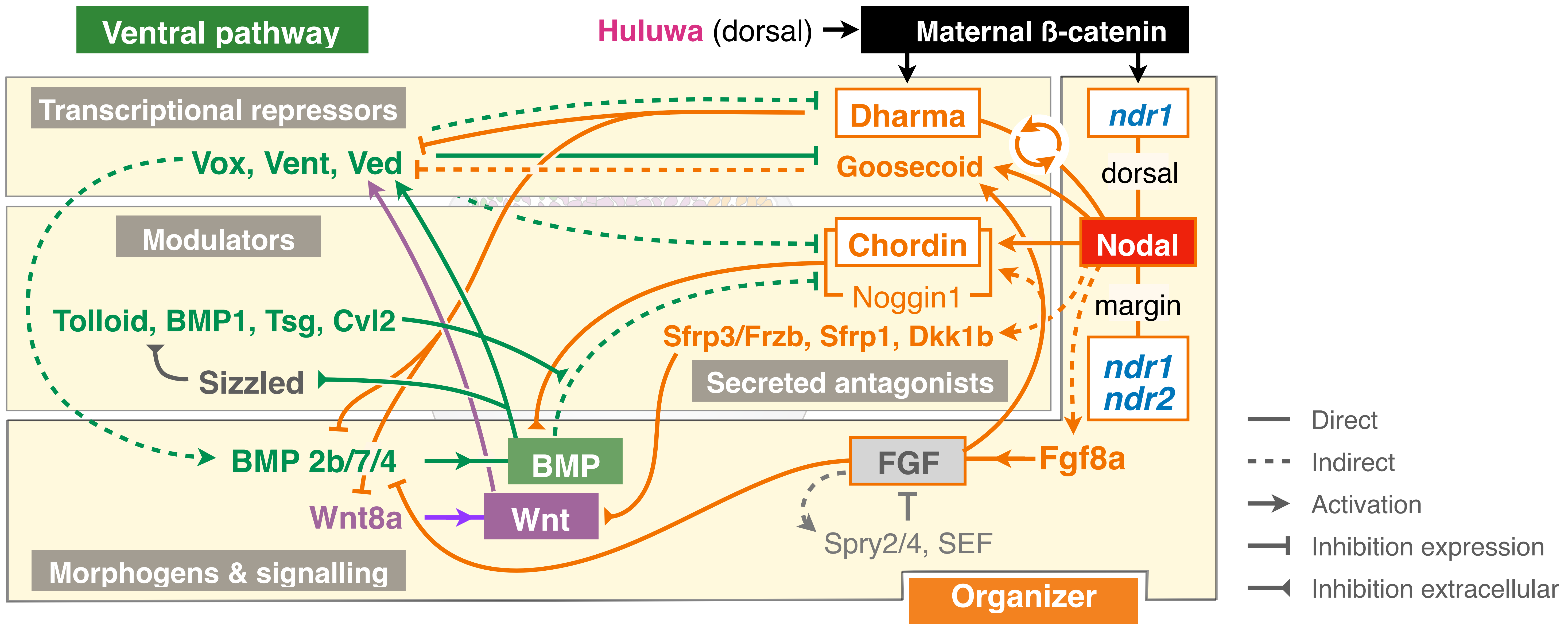


A signal from the light-coloured embryo instructed the dark-coloured embryo's cells to change their behaviour: "organizer" of a new blastopore

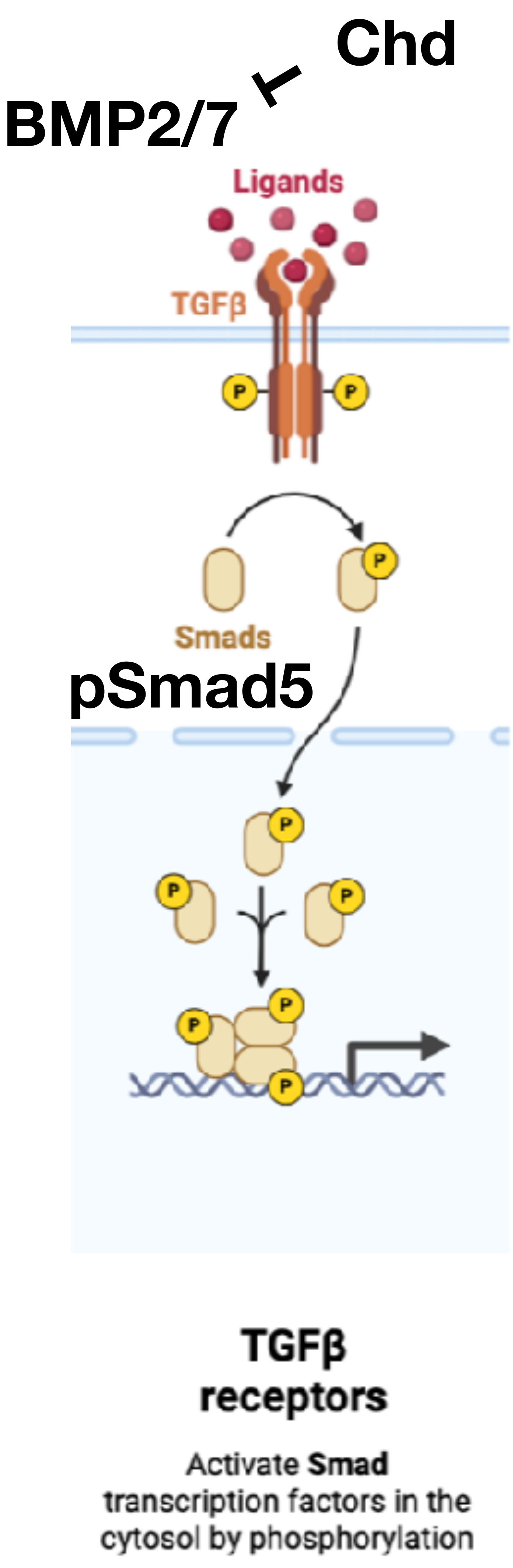


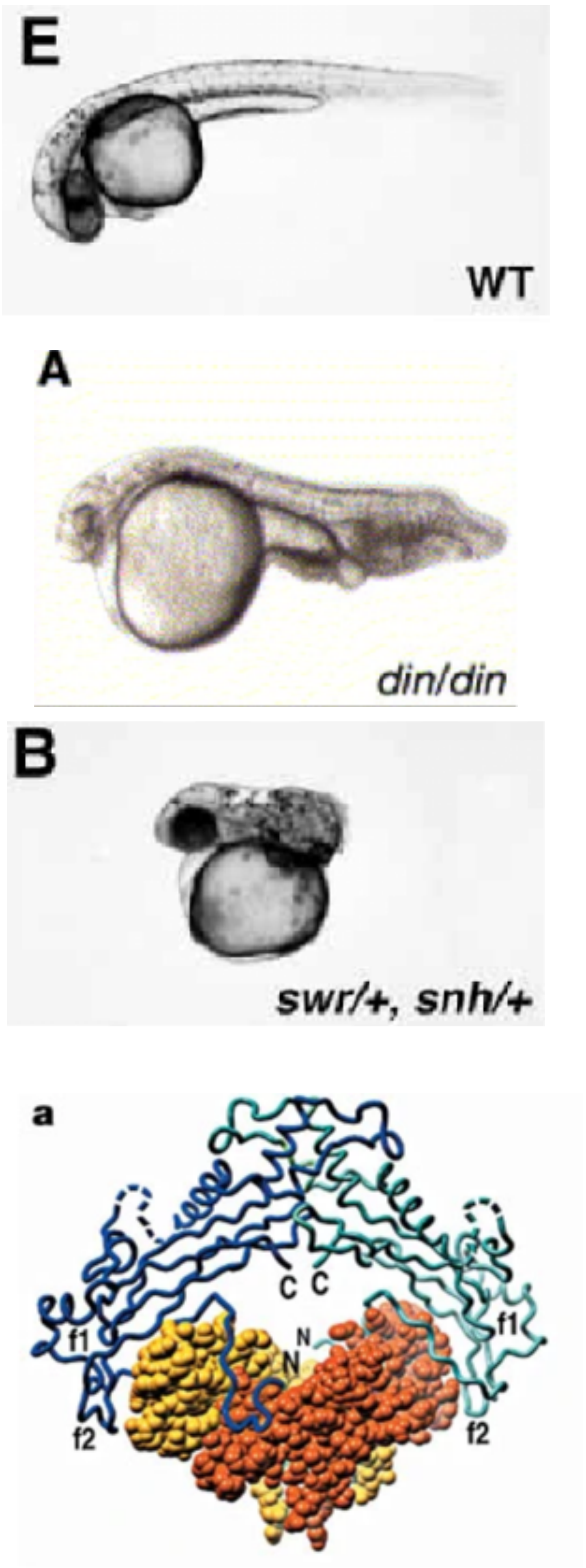
Organizer signaling in the zebrafish

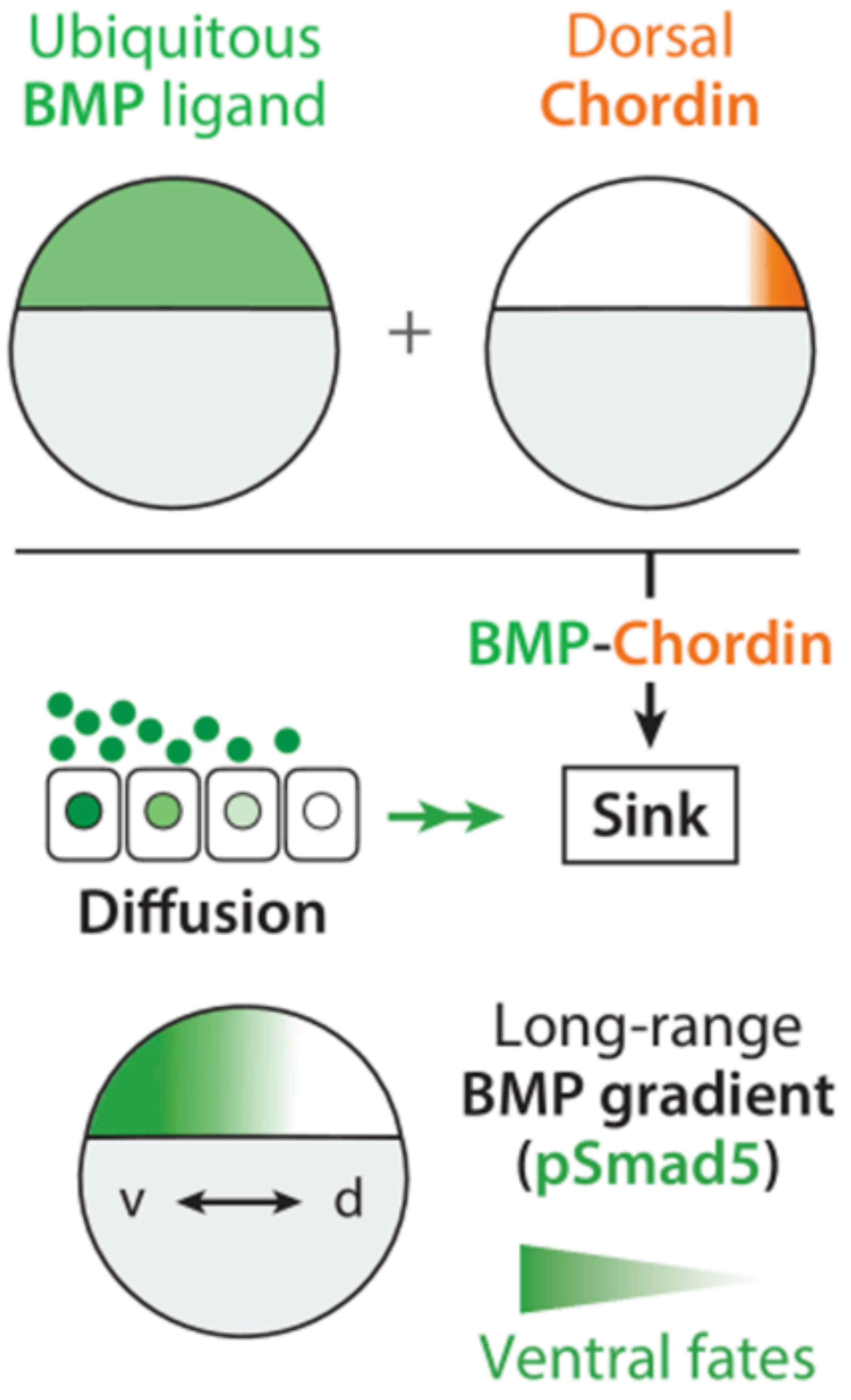


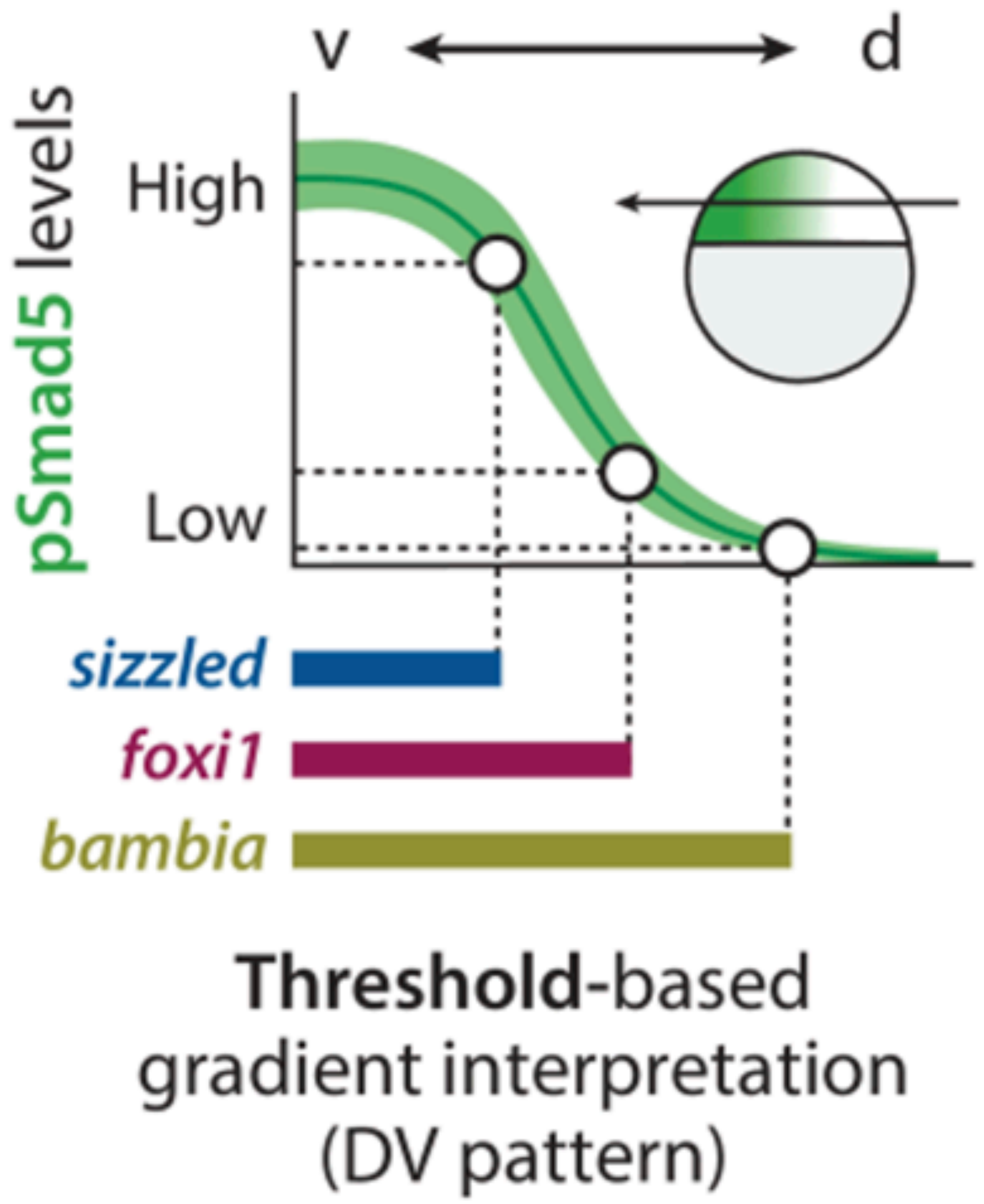


Organizer signaling: dorsal Chordin blocks BMPs





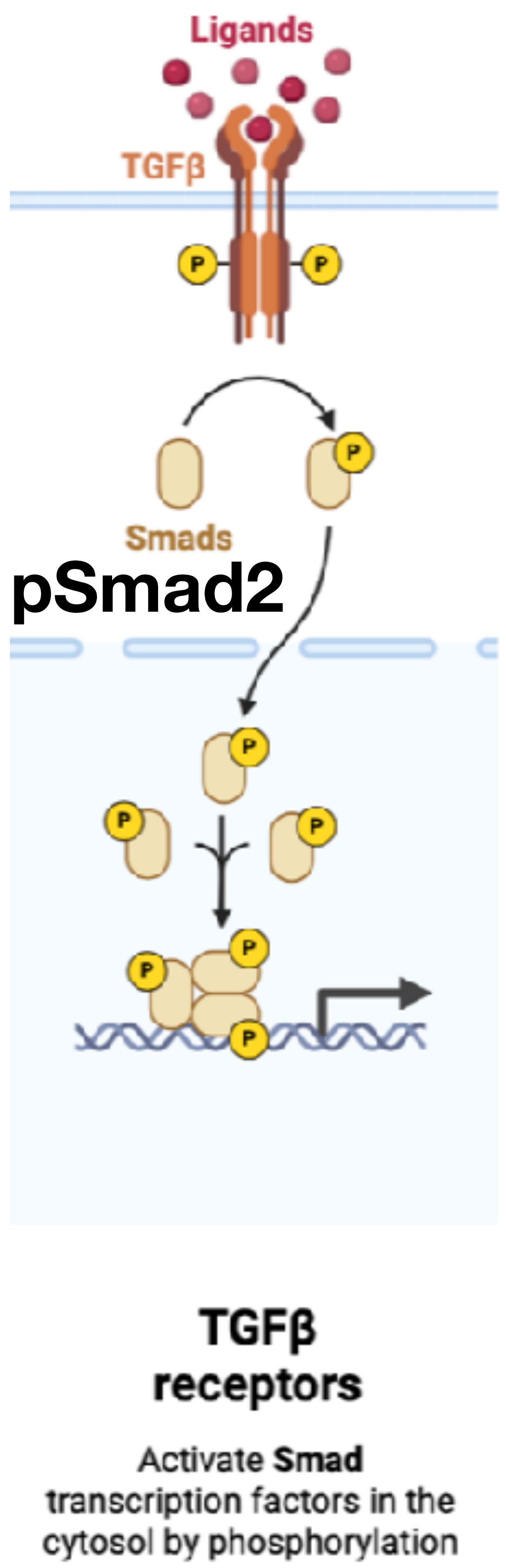


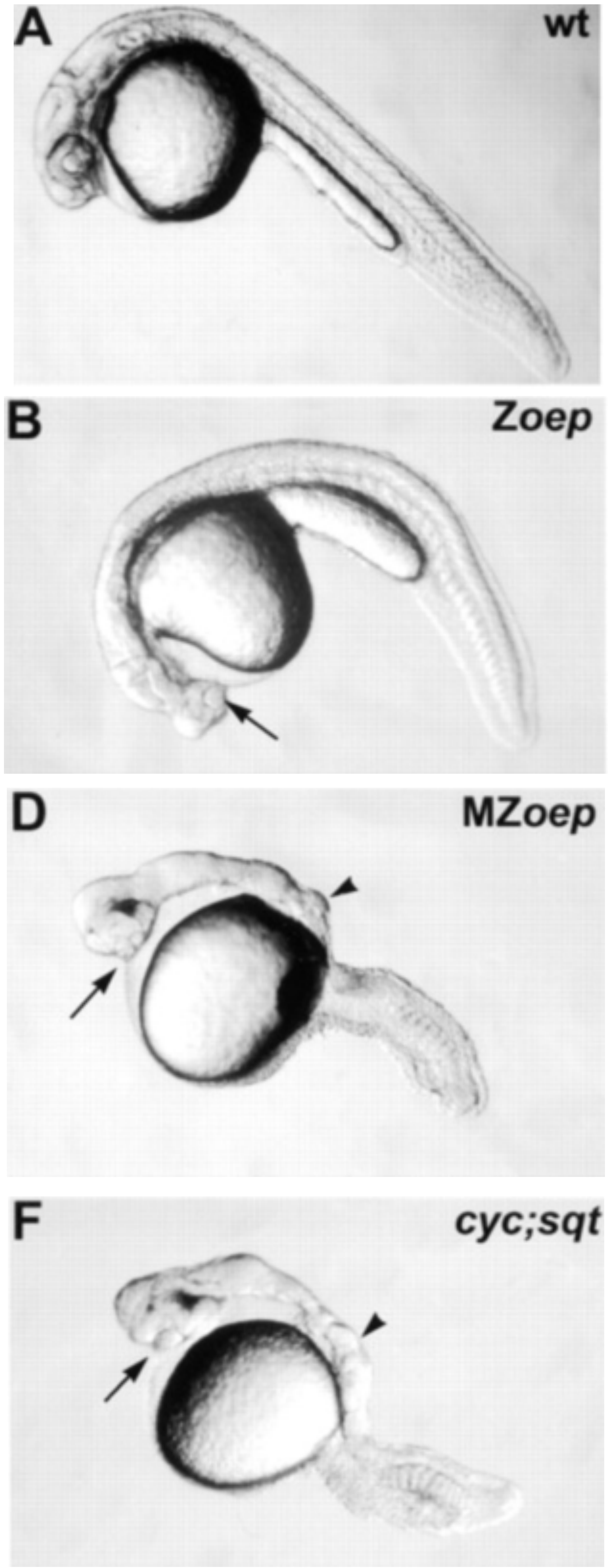


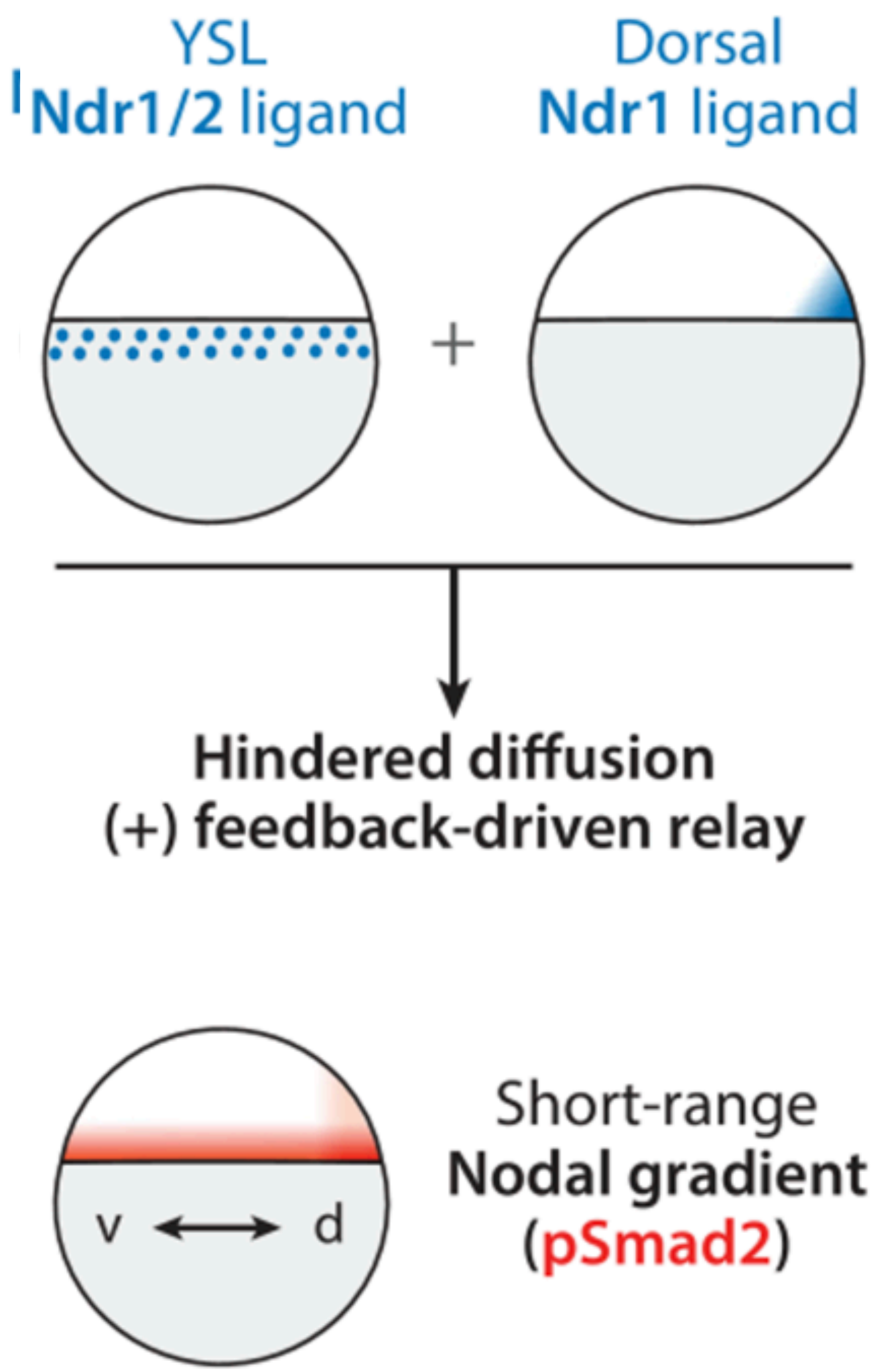
Required for A-P patterning

Organizer signaling in the zebrafish: Nodal gradients

Nodal-related 1/2

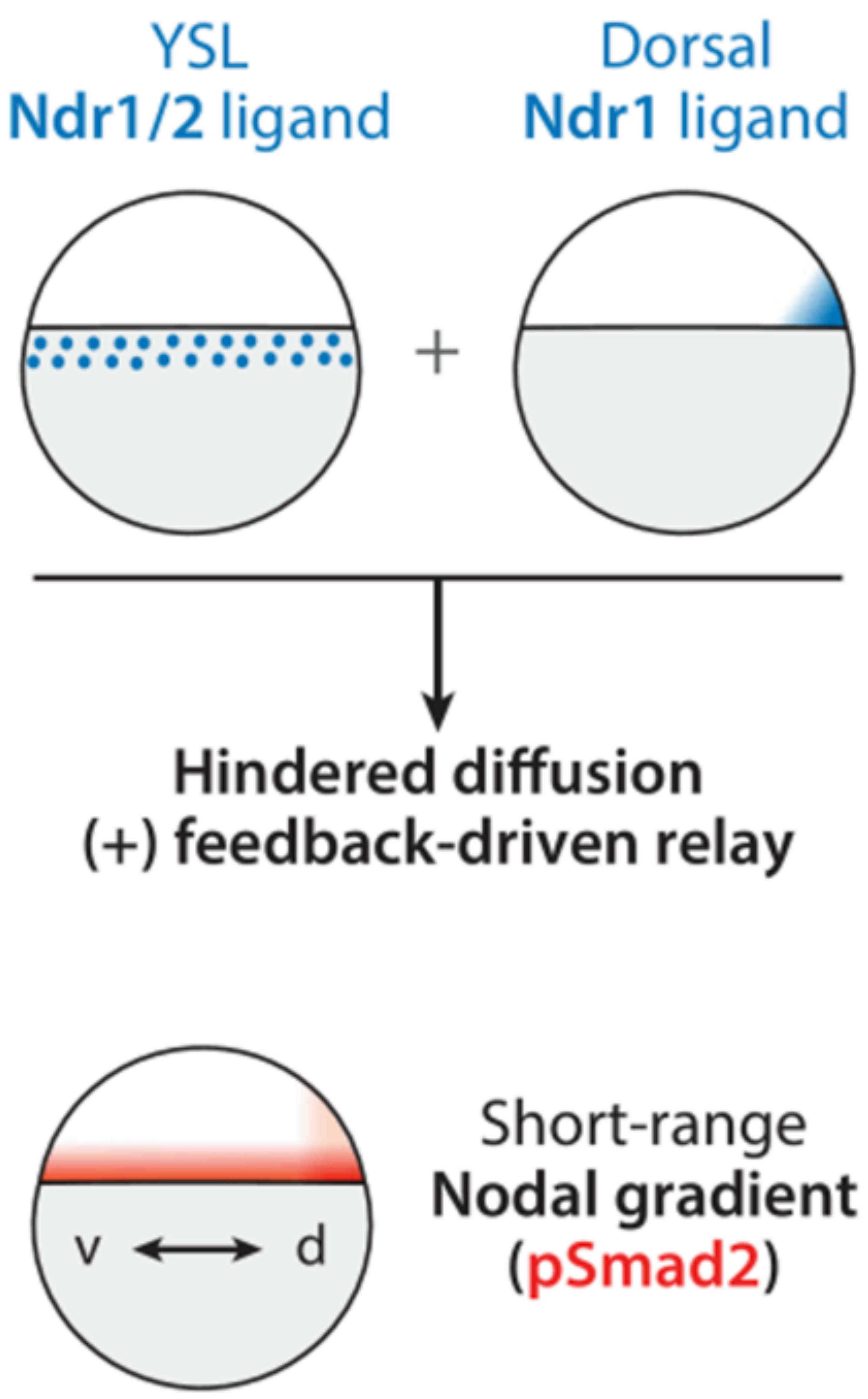


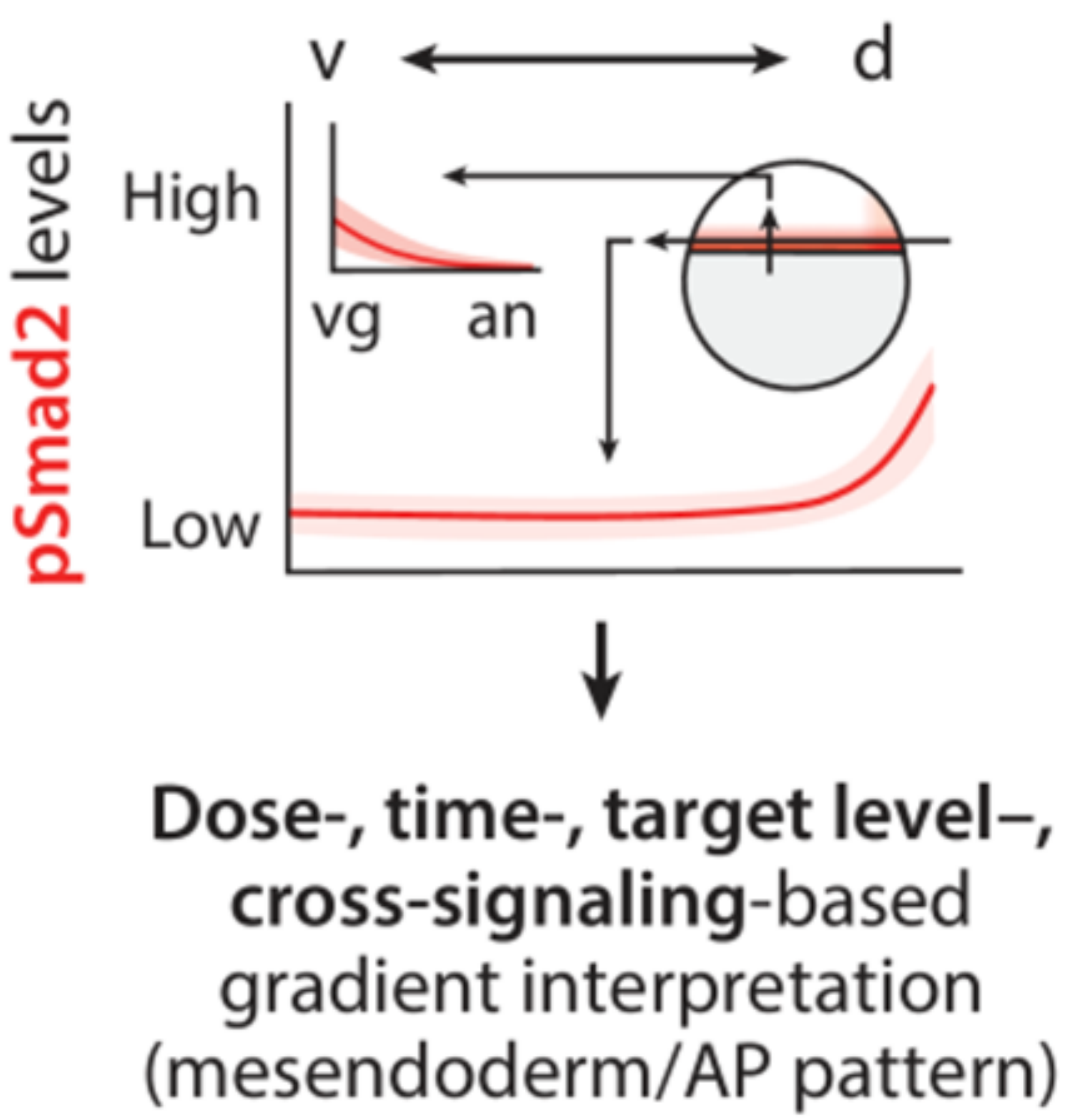


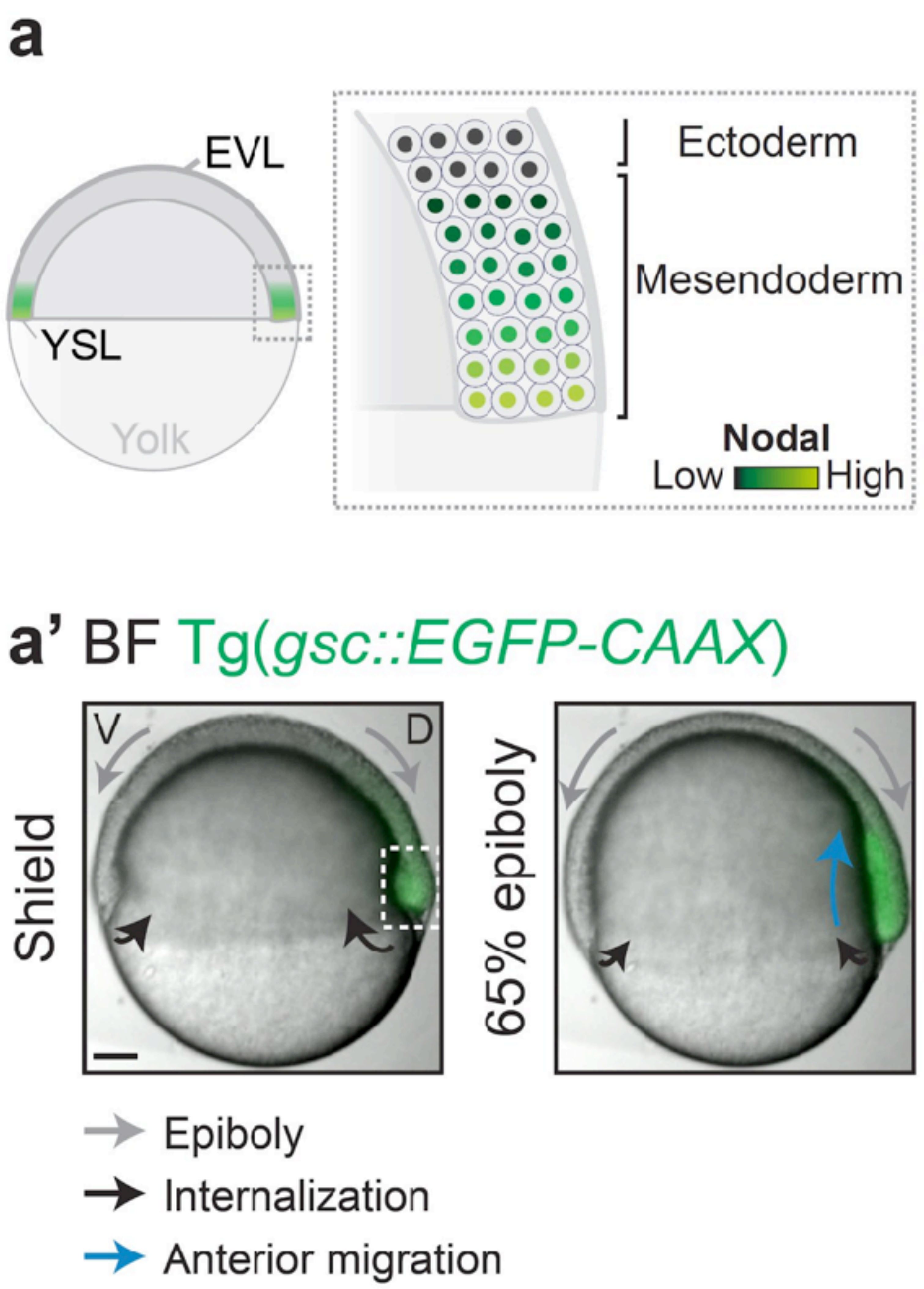


Nodals reqired for Mesoderm and Endoderm, & Chordin induction

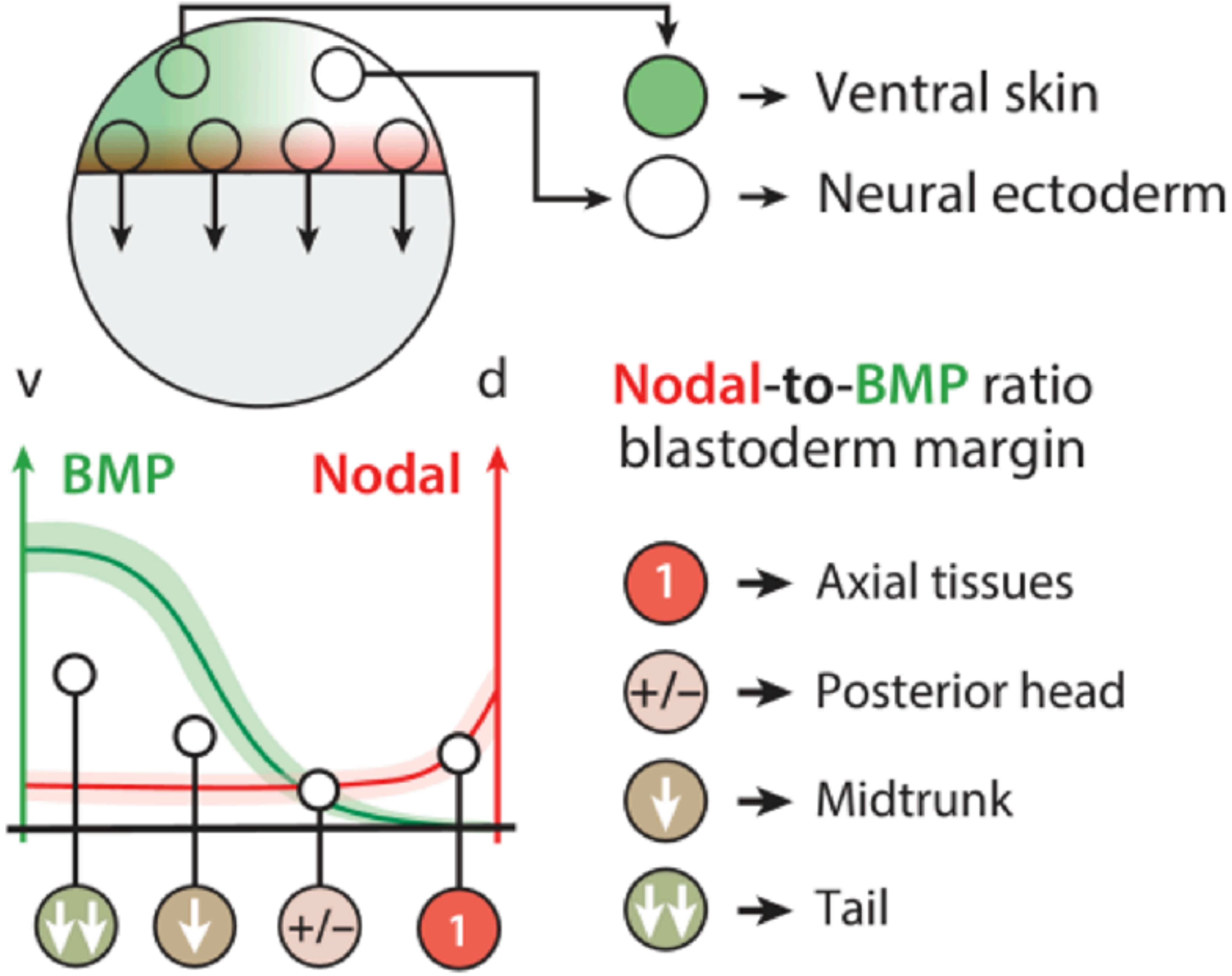
Organizer signaling in the zebrafish: Nodal gradients





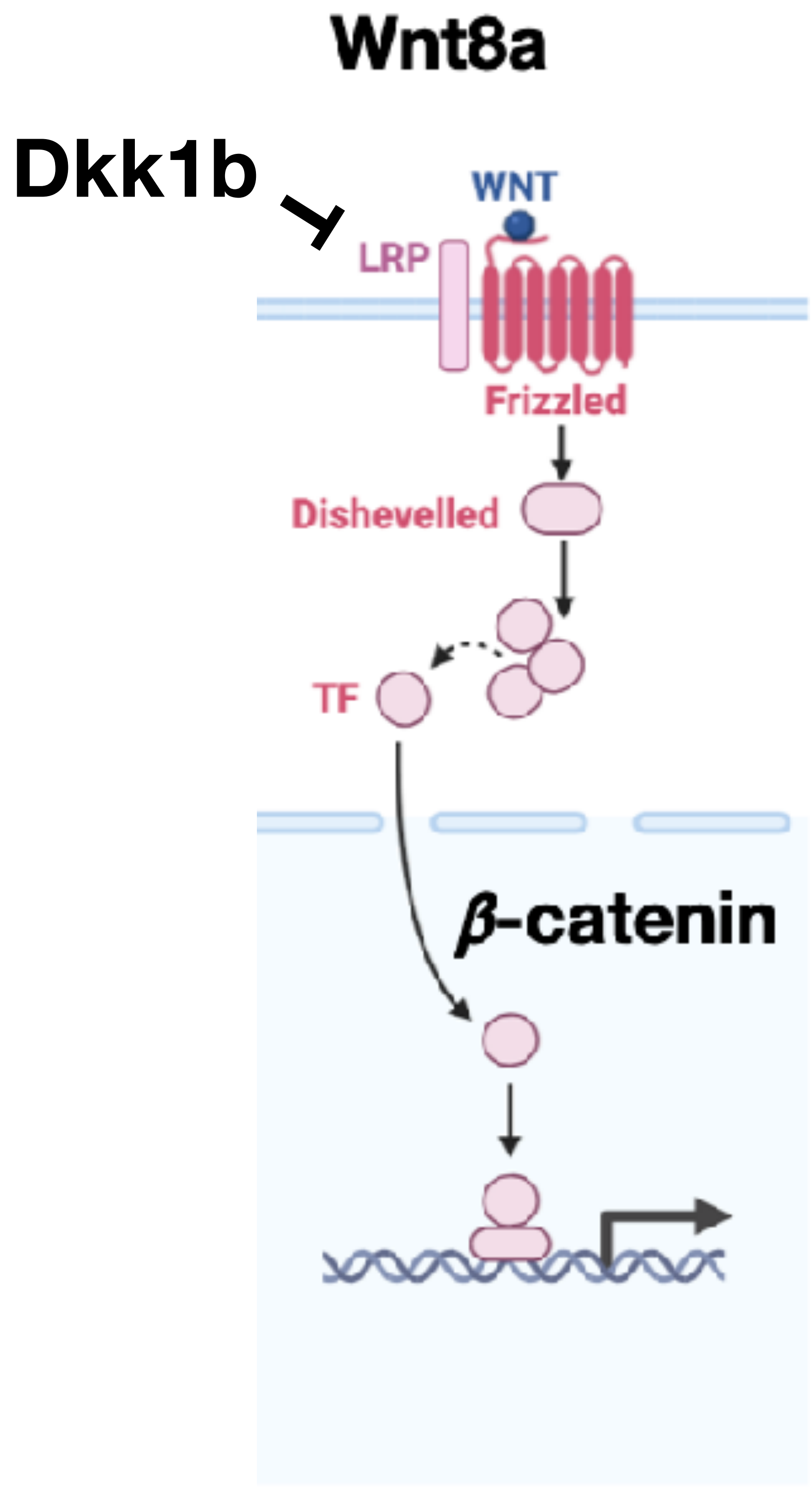


Nodal / BMP ratio sets Anterior-Posterior position



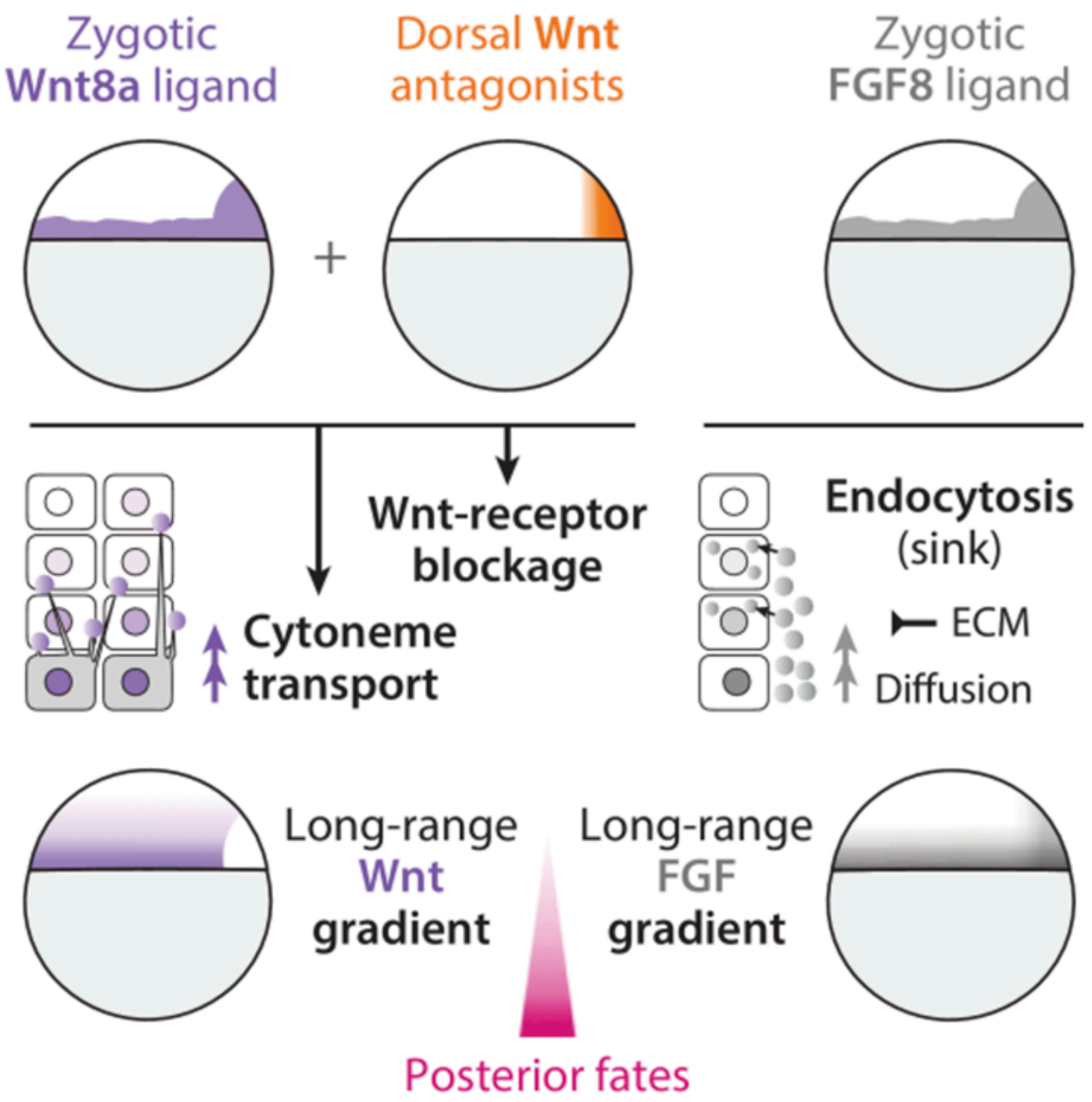
We didn’t cover this in class

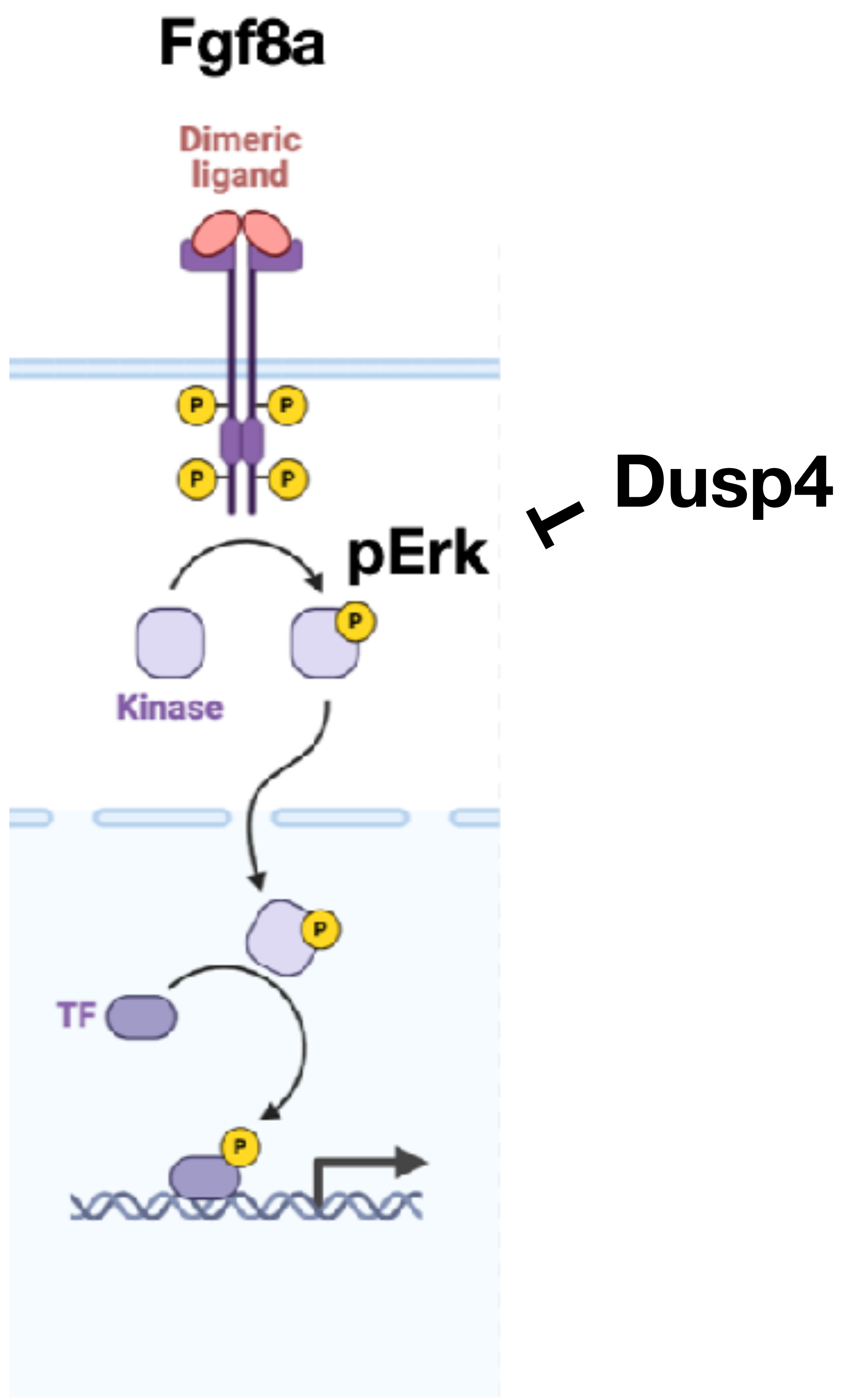
Wnt8a and FGF8 pattern posterior body fates



Wnt receptors

Release an activated **transcription factor** from a multiprotein complex in the cytosol



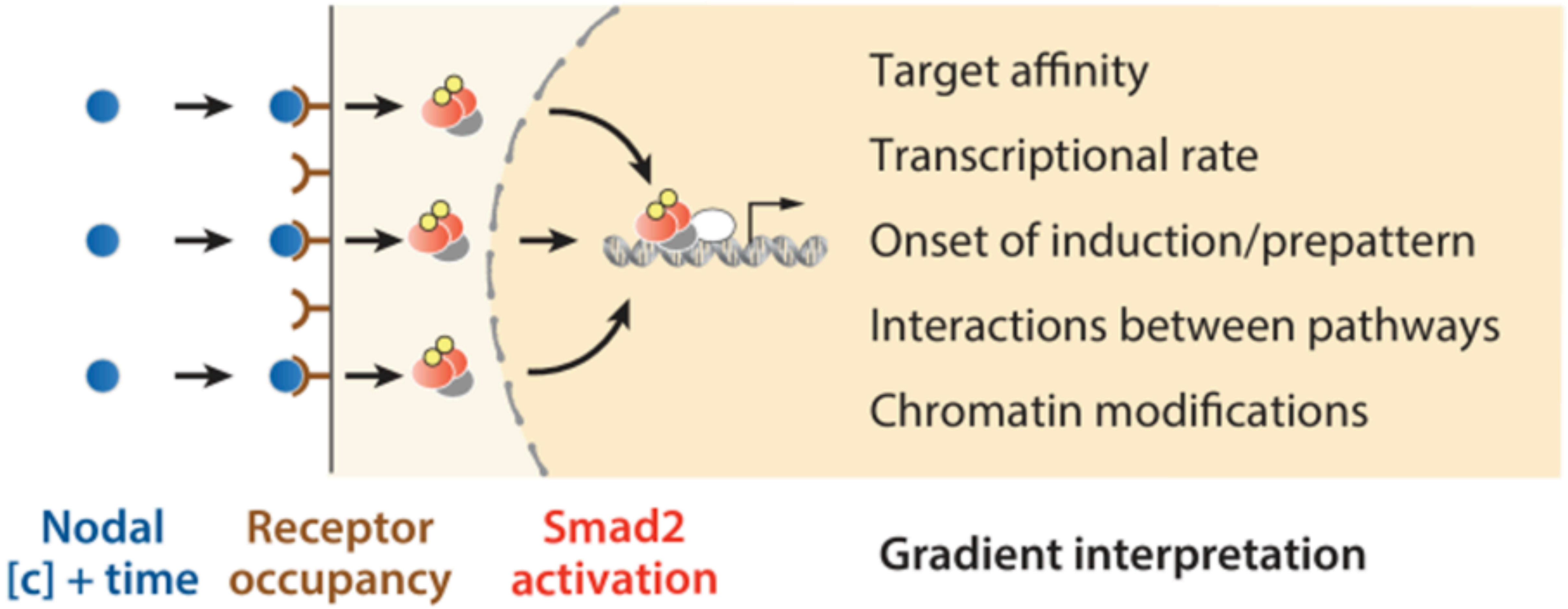


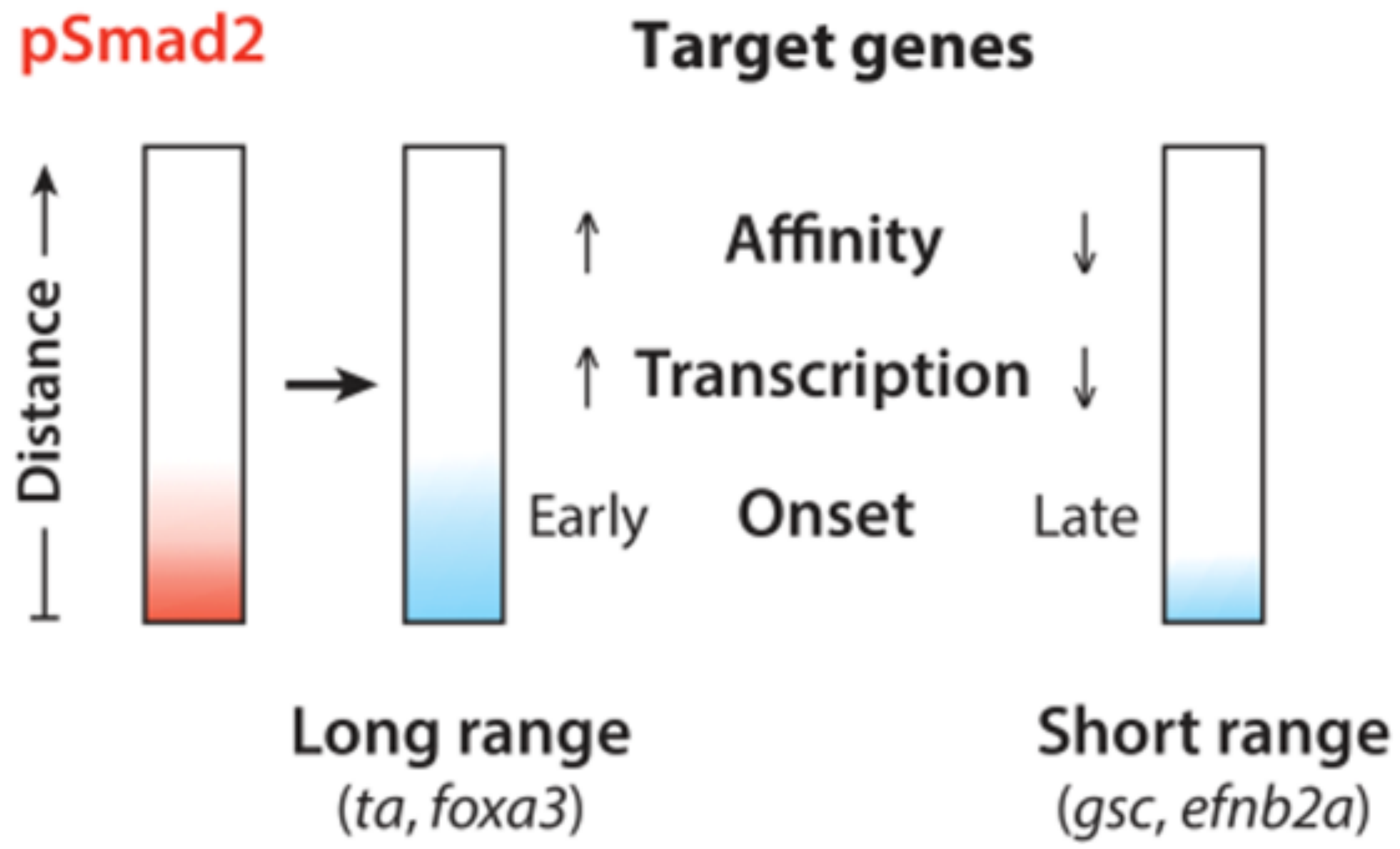
Receptor tyrosine kinases

Activate **cytosolic kinases** that translocate to the nucleus and activate transcription factors by phosphorylation

We didn't cover this in class

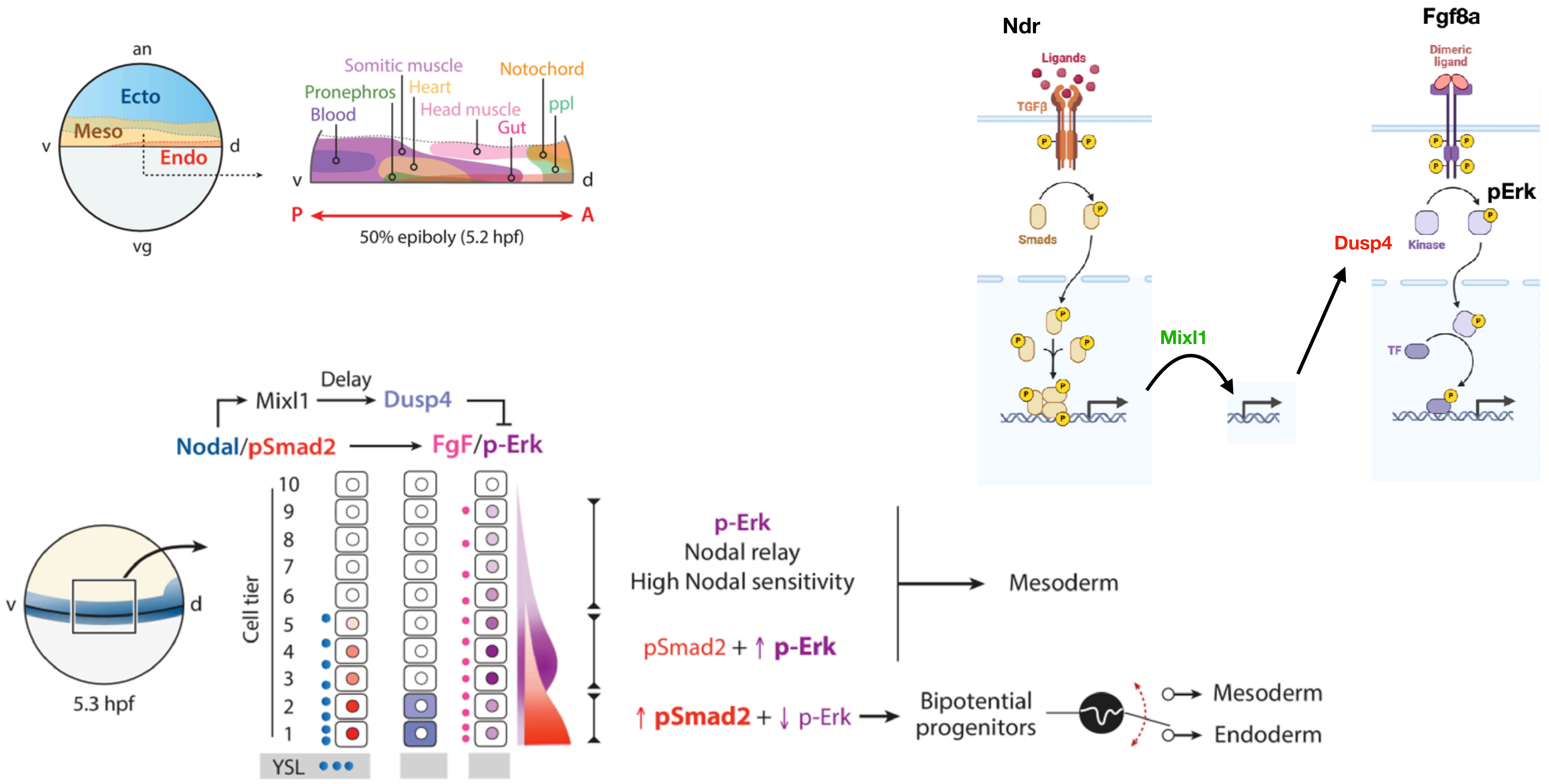
Nodal gradient interpretation - short versus long range



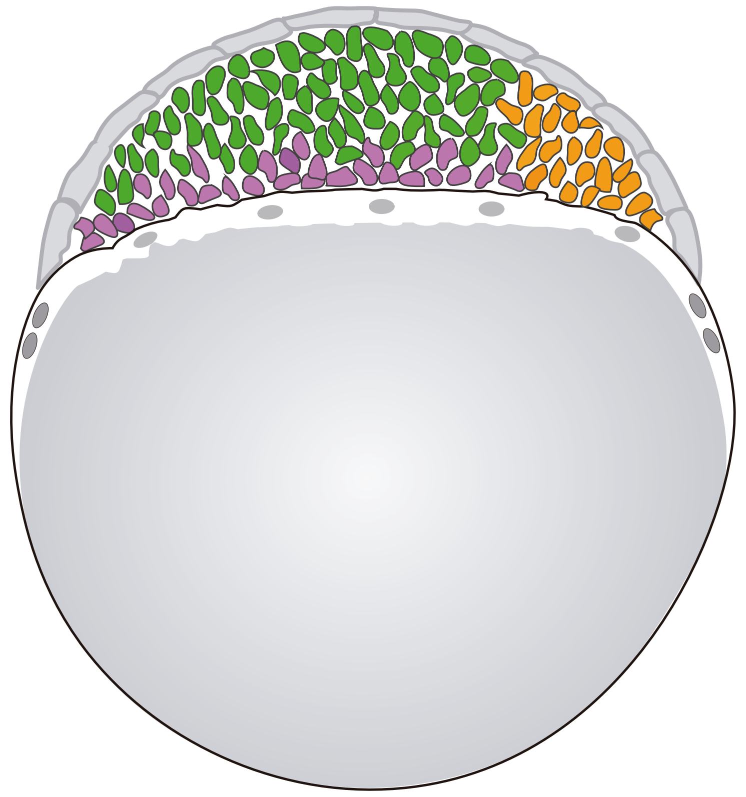


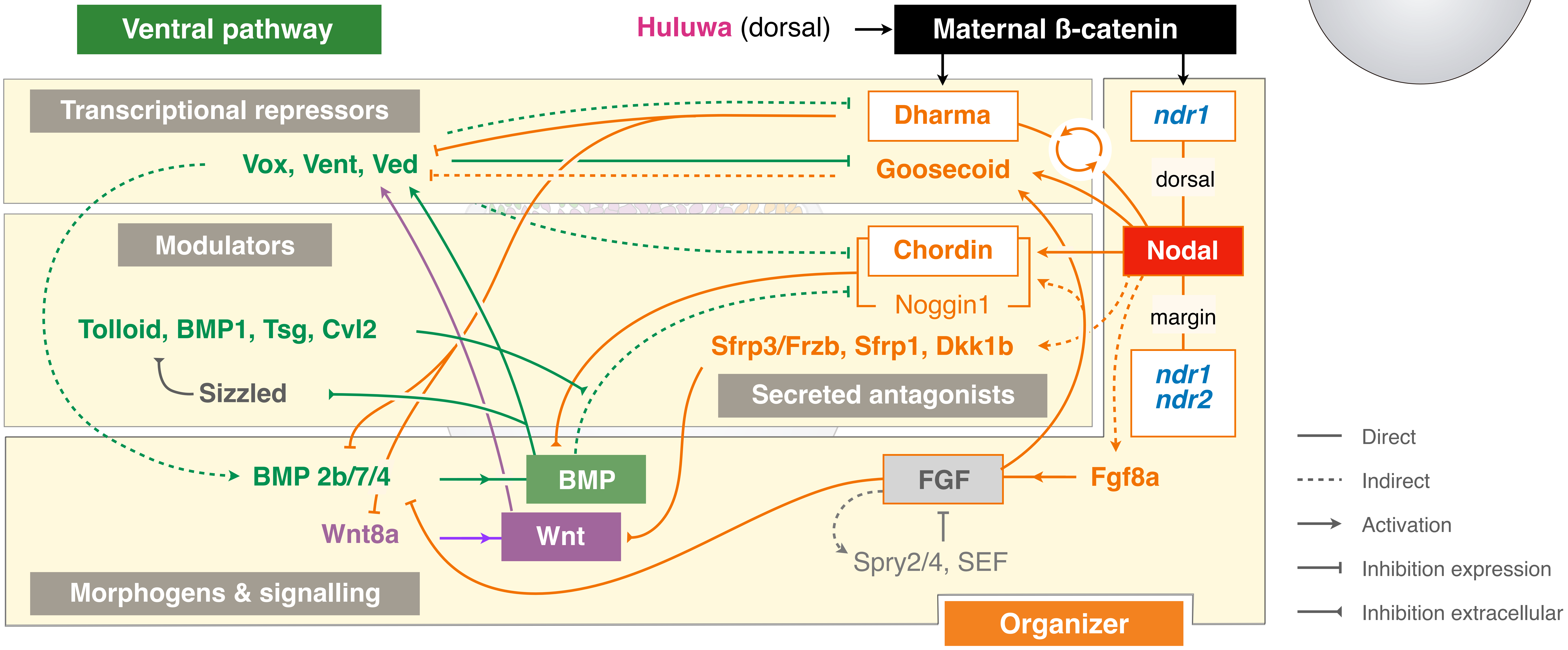
We didn't cover this in class

Nodal and Fgf gradient interpretation - mesoderm versus endoderm



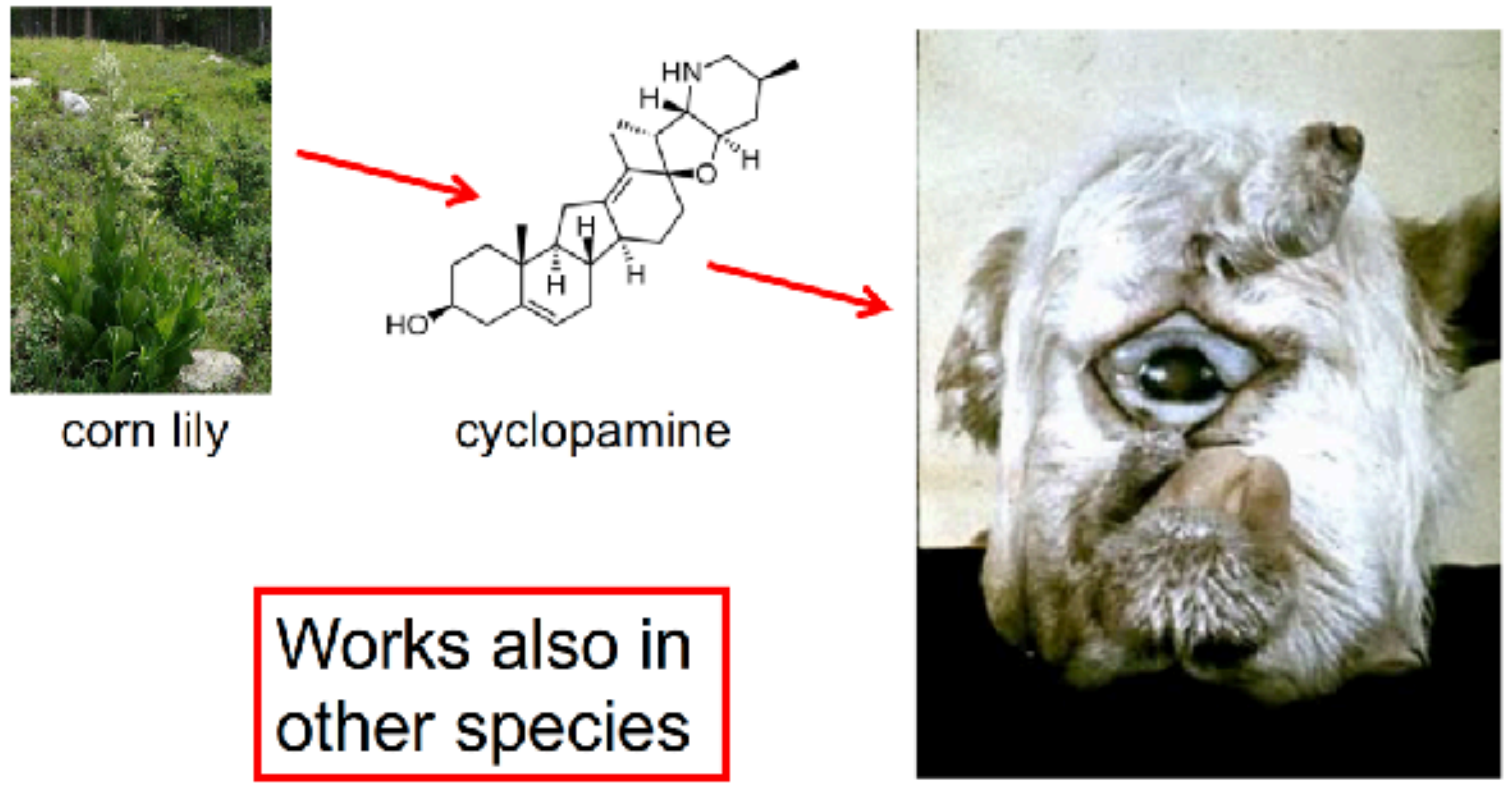
Organizer signaling in the zebrafish





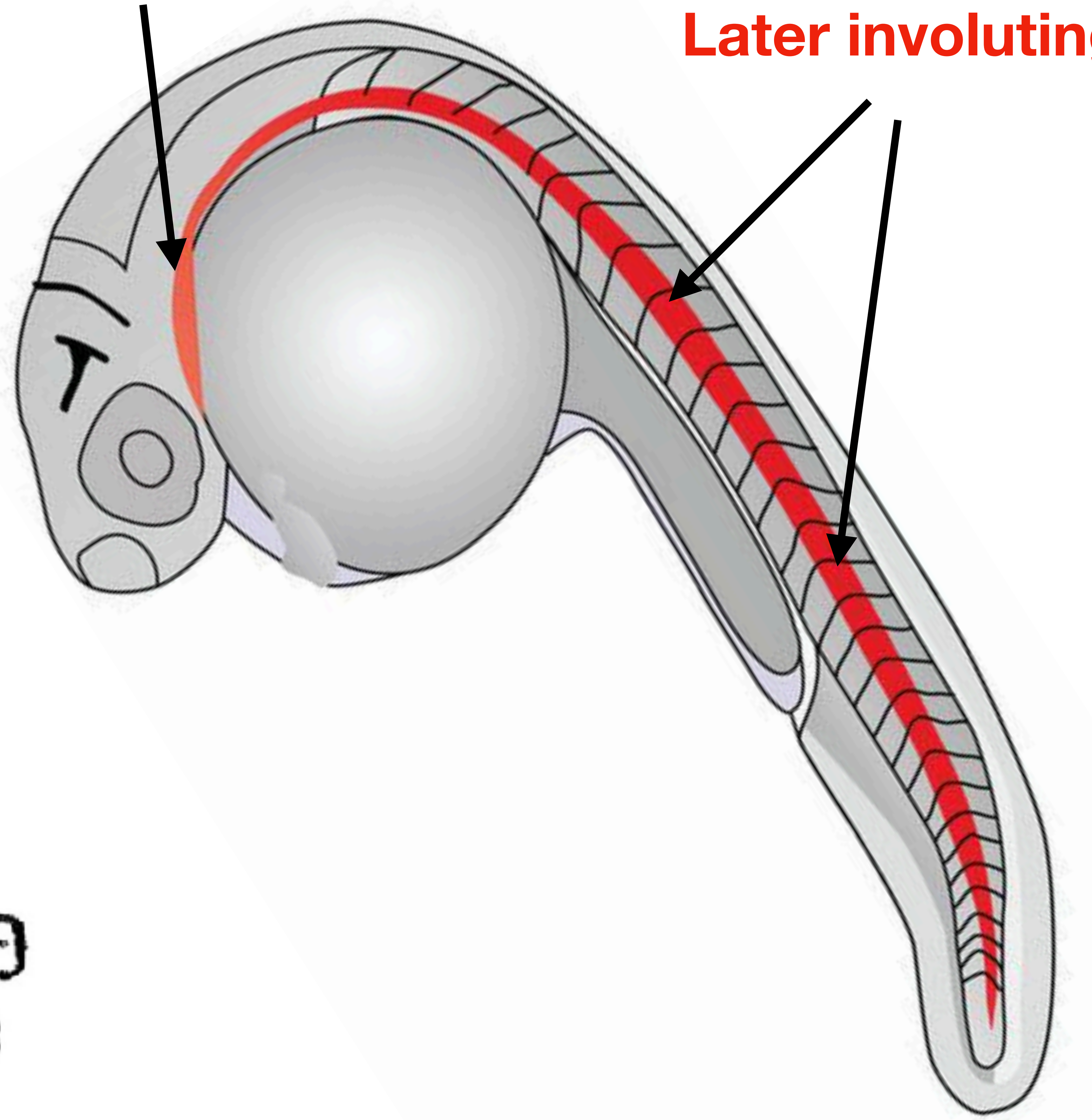
Why does loss of Hh cause cyclopia?

Blocking Hh signaling in mammals



cyclopia (Idaho lamb)

Works also in other species



Connecting the Dots

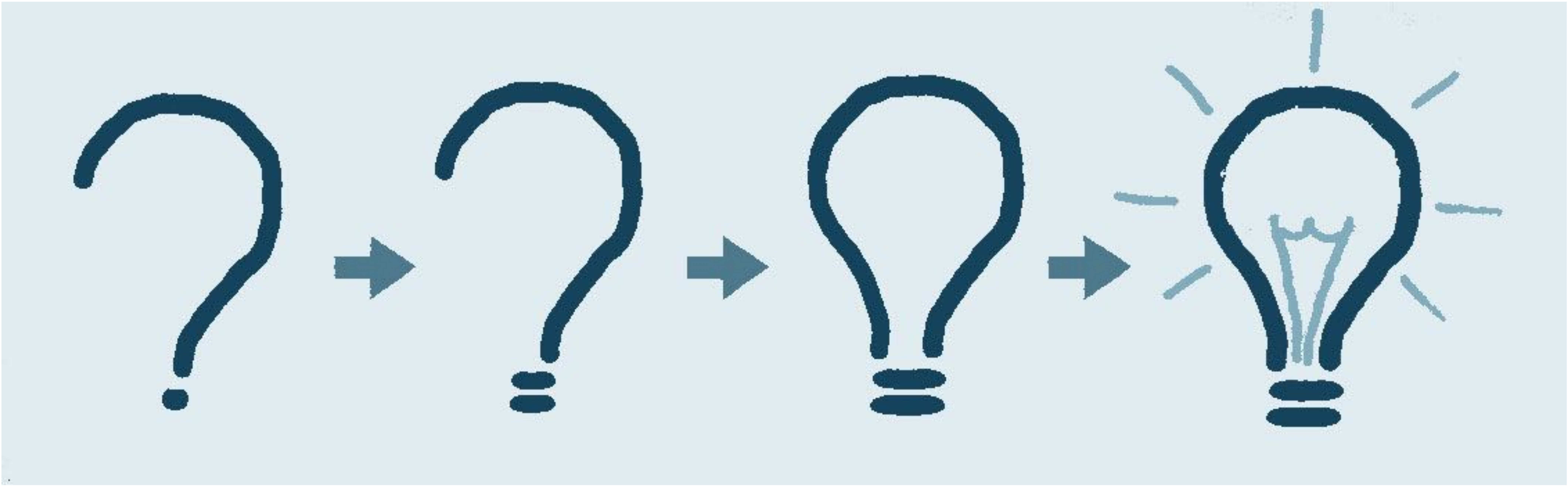
Are these gradients sufficiently precise to account for tissue distributions?

What mechanisms are involved?

How do the gradients scale to regulate smaller or bigger embryos?

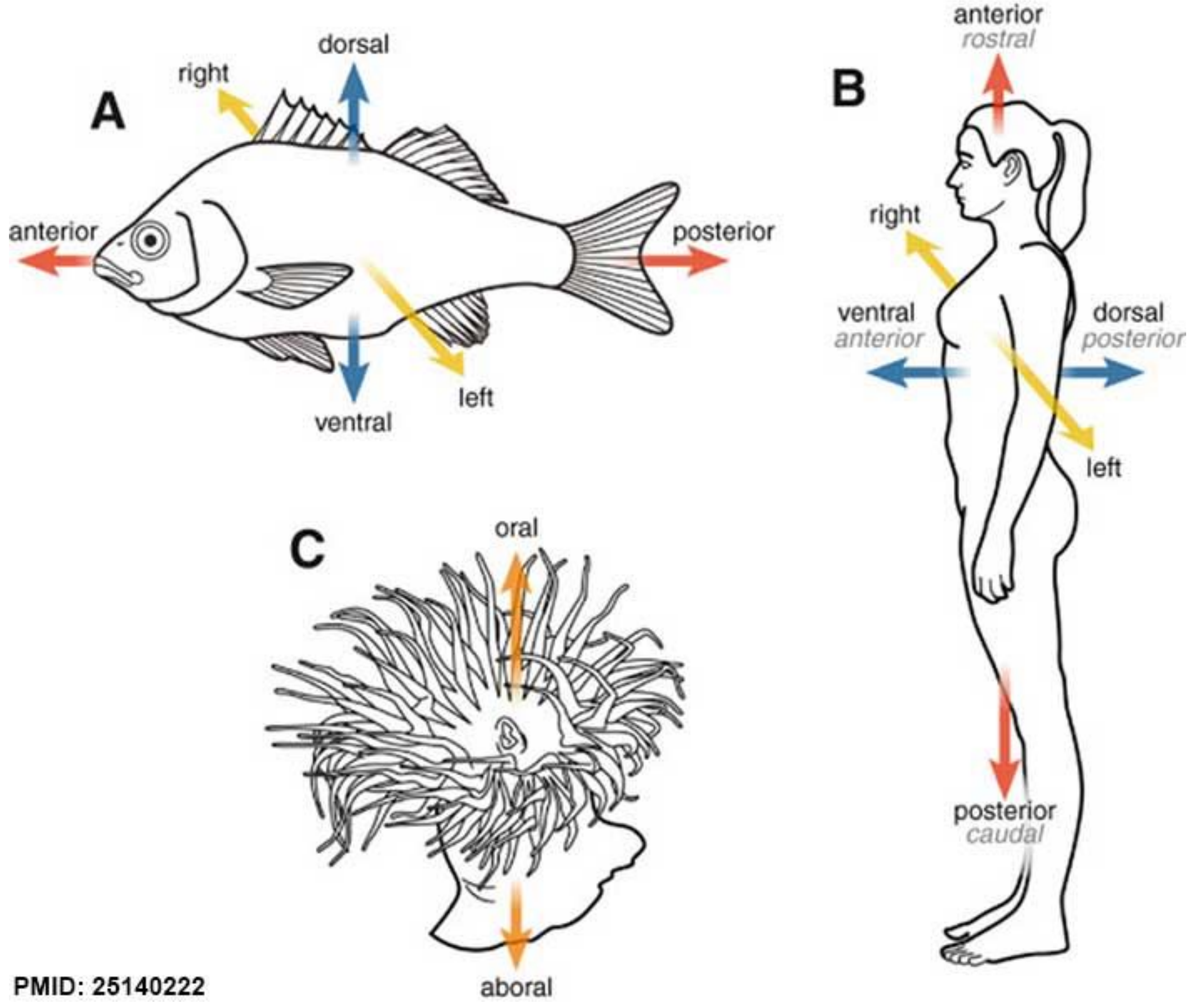
Is there a tail organizer?

Questions?



Take a break

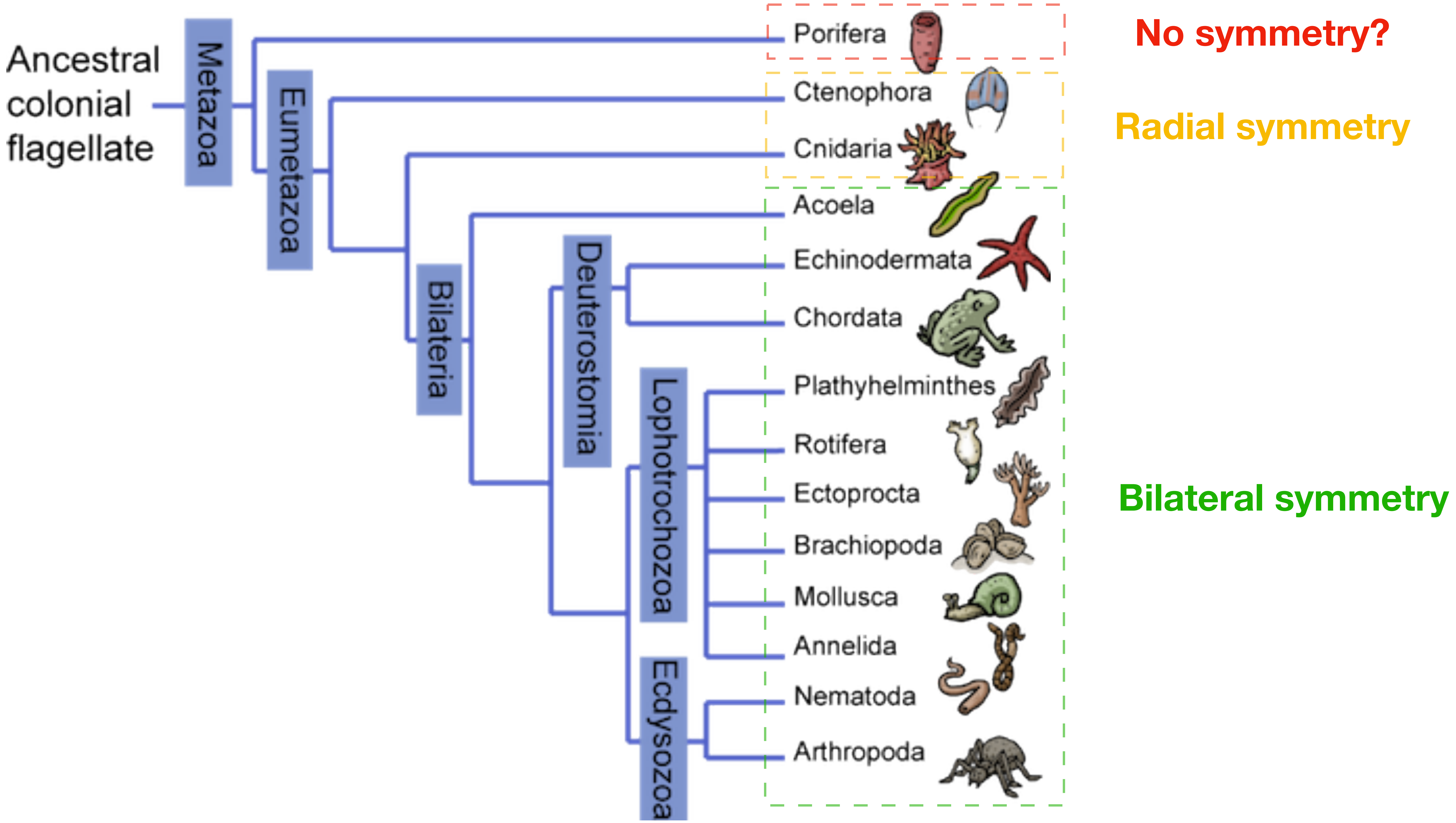
Big questions



Today's menu - 2 courses

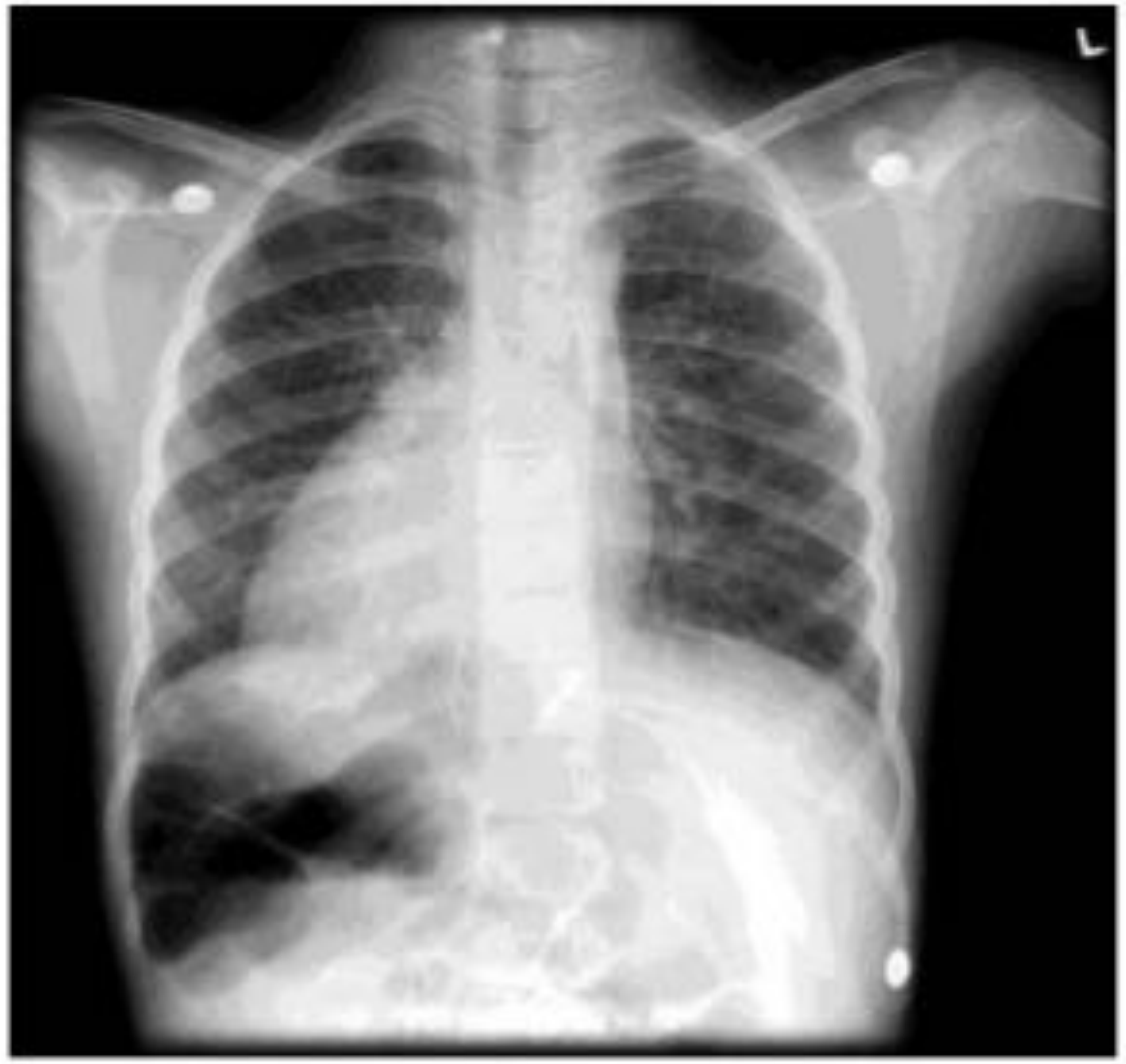
- ***Front and Back***
- *Nobel experiment - embryonic induction*
- *Specification and committment*
- *Gastrulation and patterning of germ layers*
- *Morphogen gradients (again)*
- *Left and Right*
- Situs inversus
- Cilia in the node - directed flow
- Planar polarity
- Nodal and calcium signals to the lateral plate

Body plans across the animal kingdom

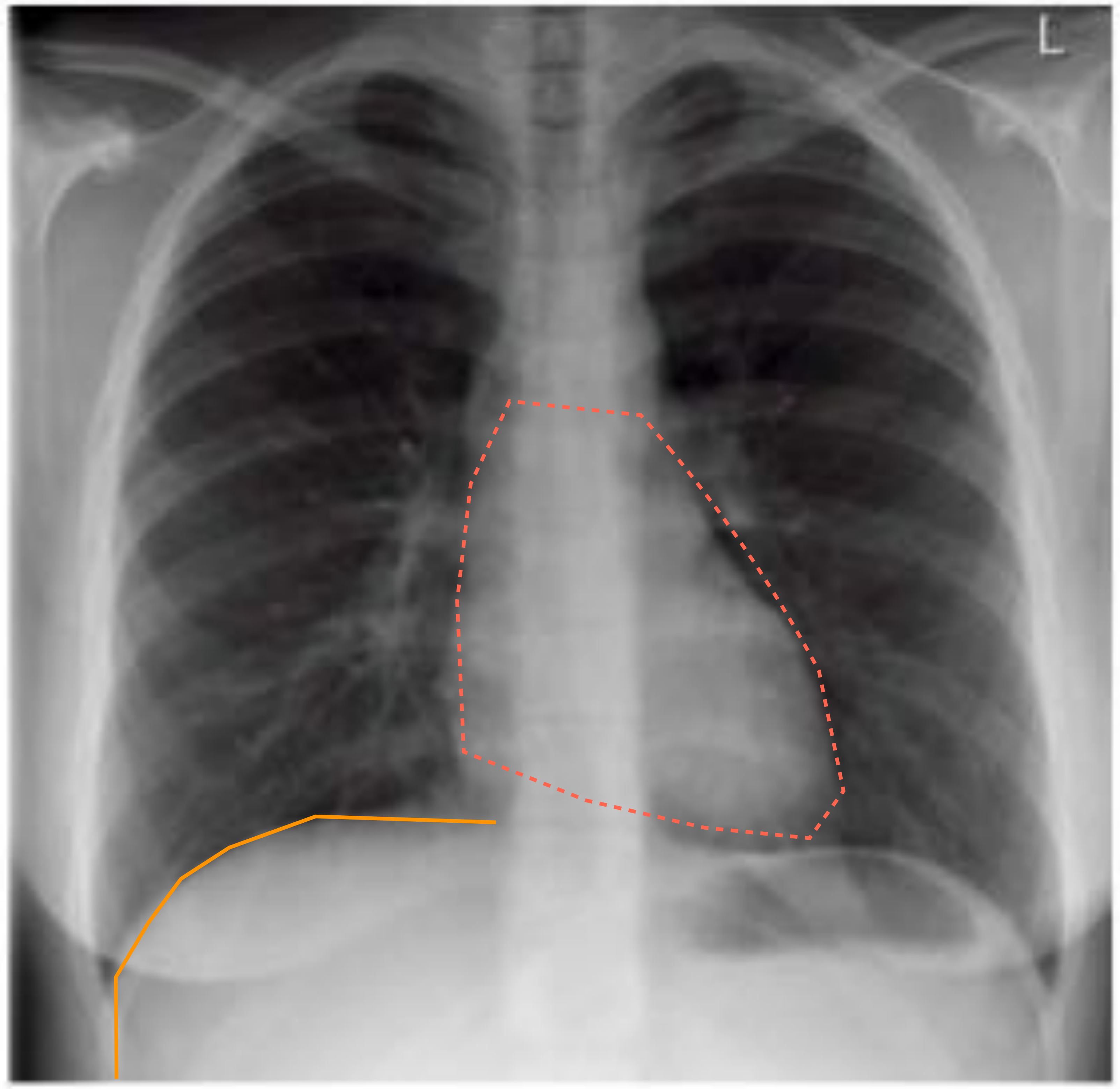


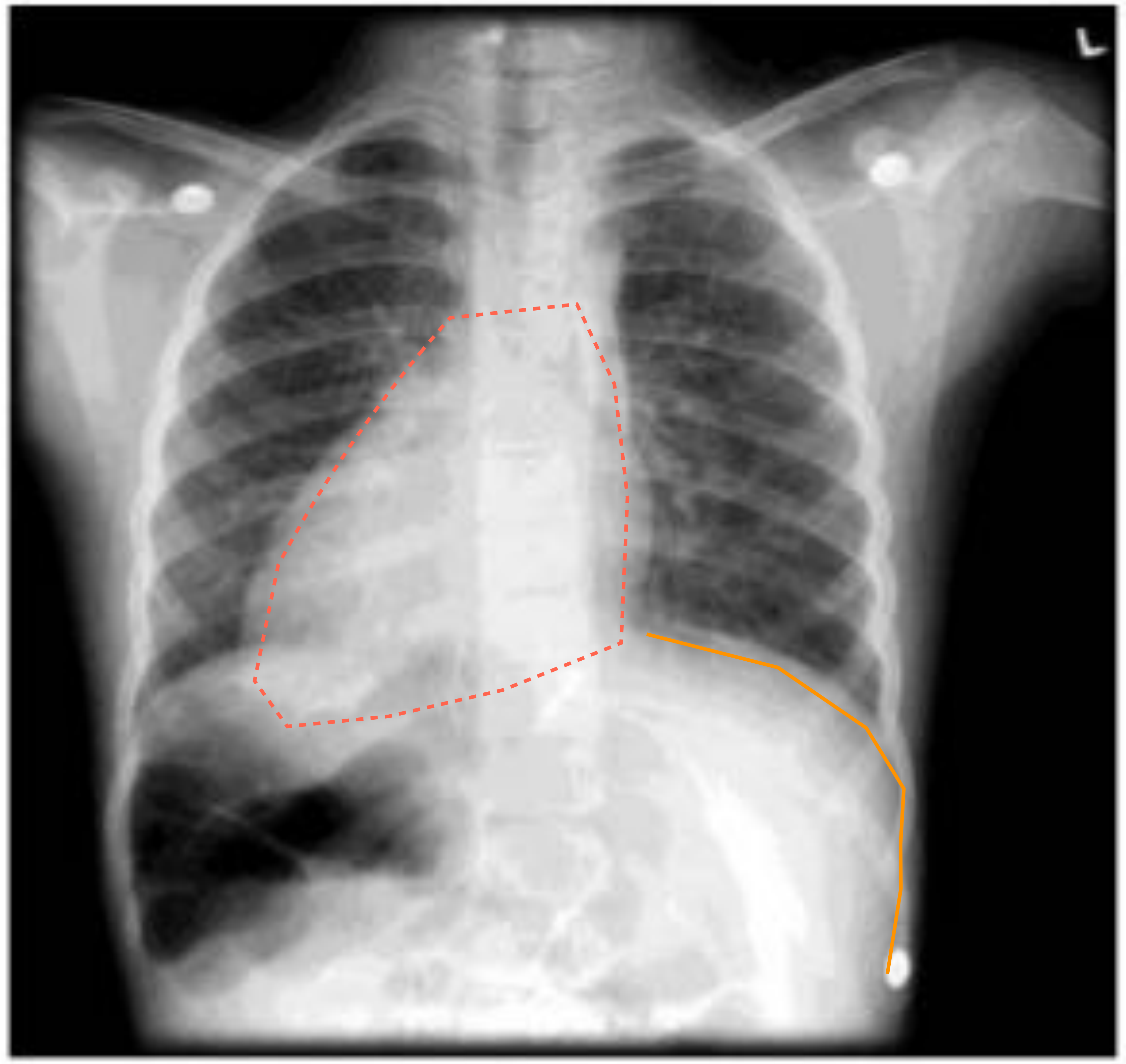
Individual puzzle

Medical Quiz: Spot the difference?



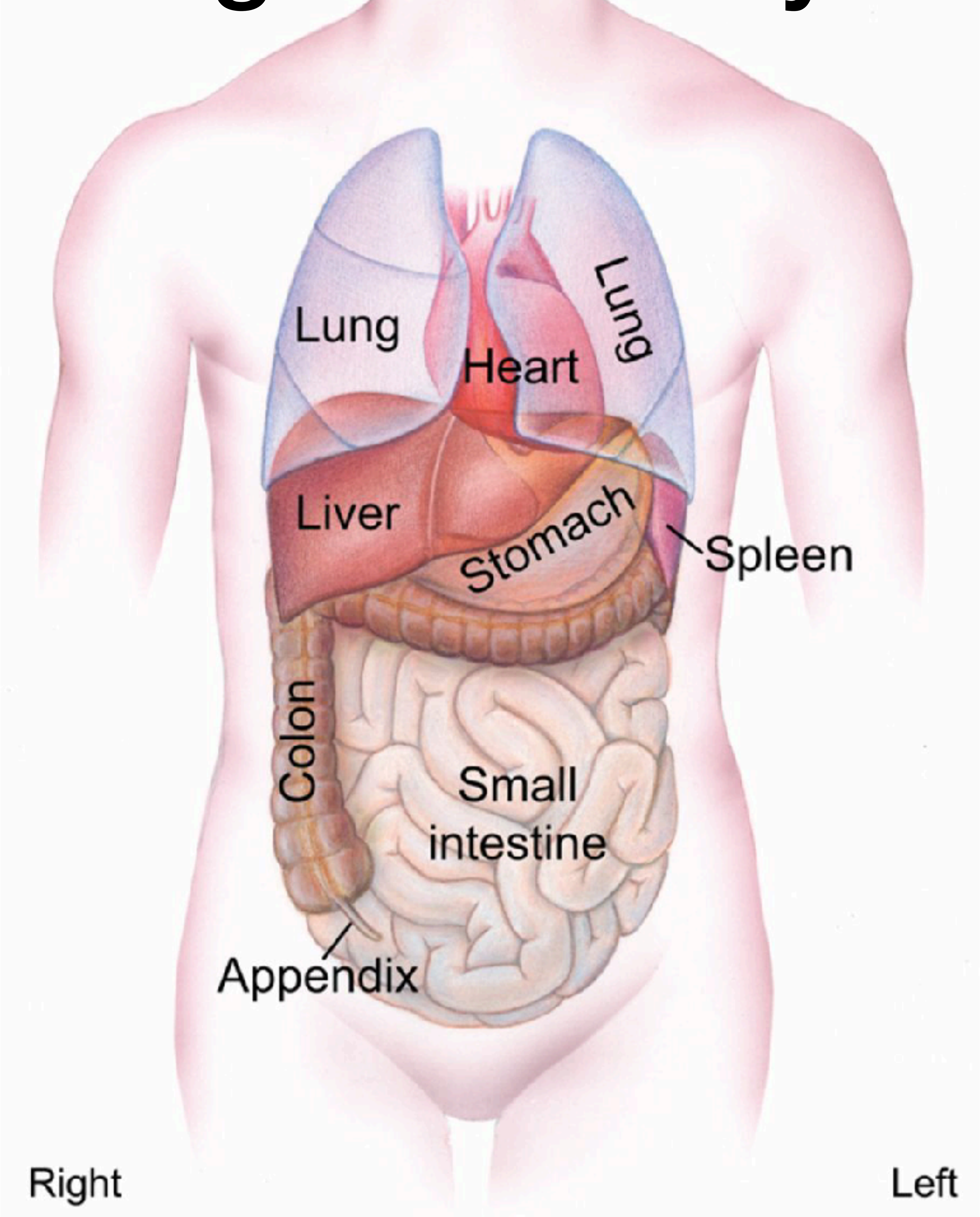
Spot the difference?





Situs inversus

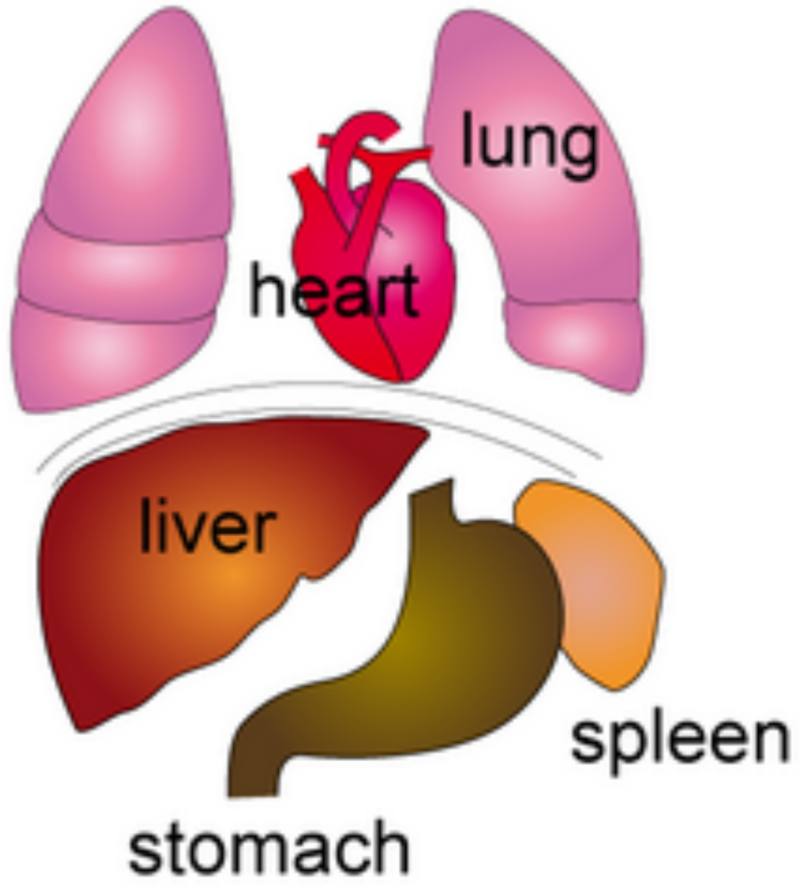
Internal organs are asymmetric



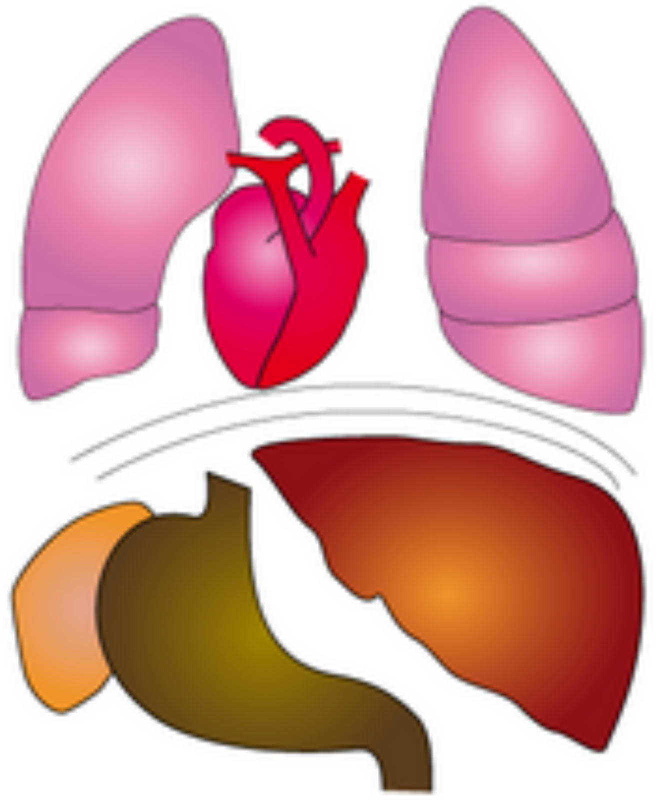
Left-Right axis

Variant internal symmetries in mammals

situs solitus

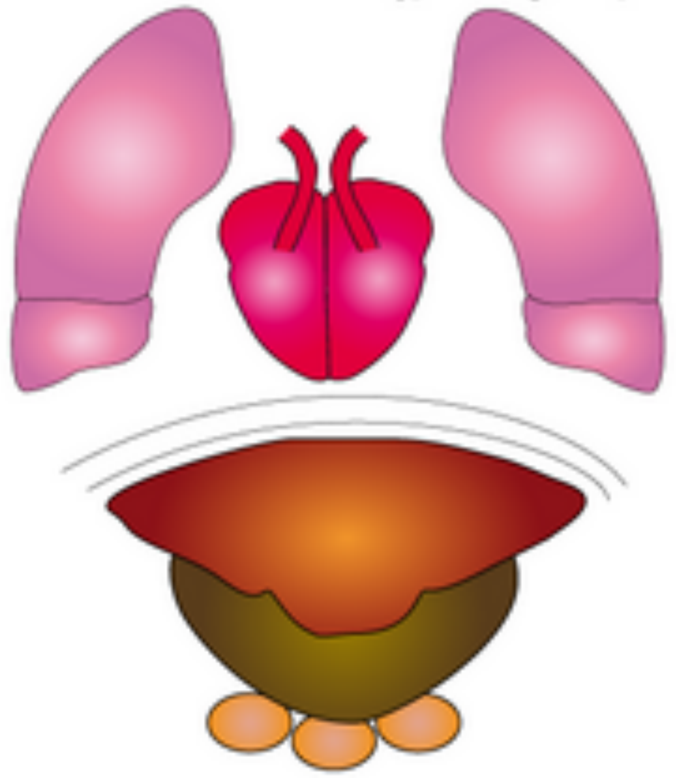


situs inversus totalis

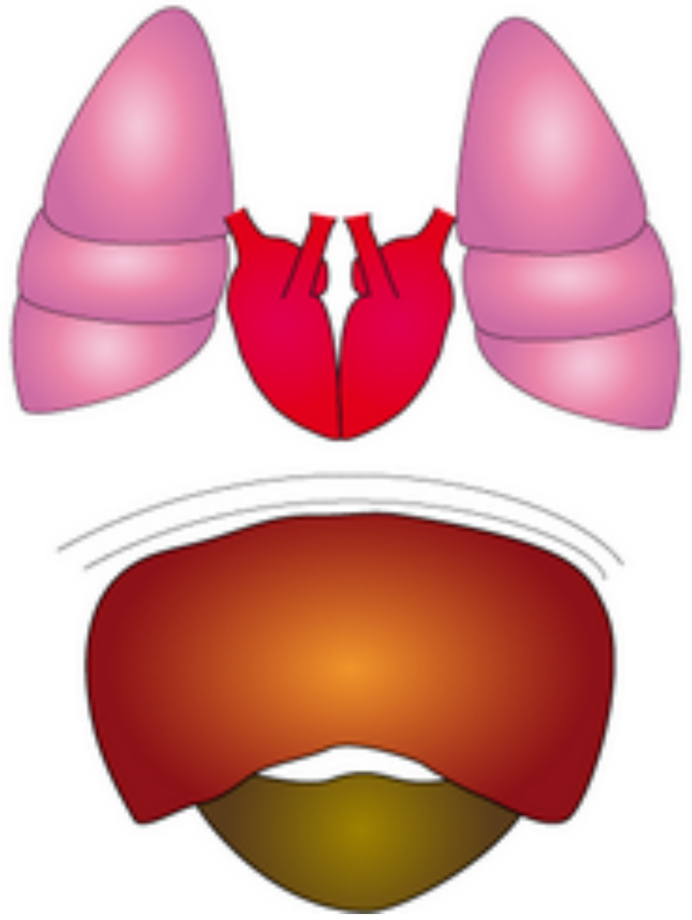


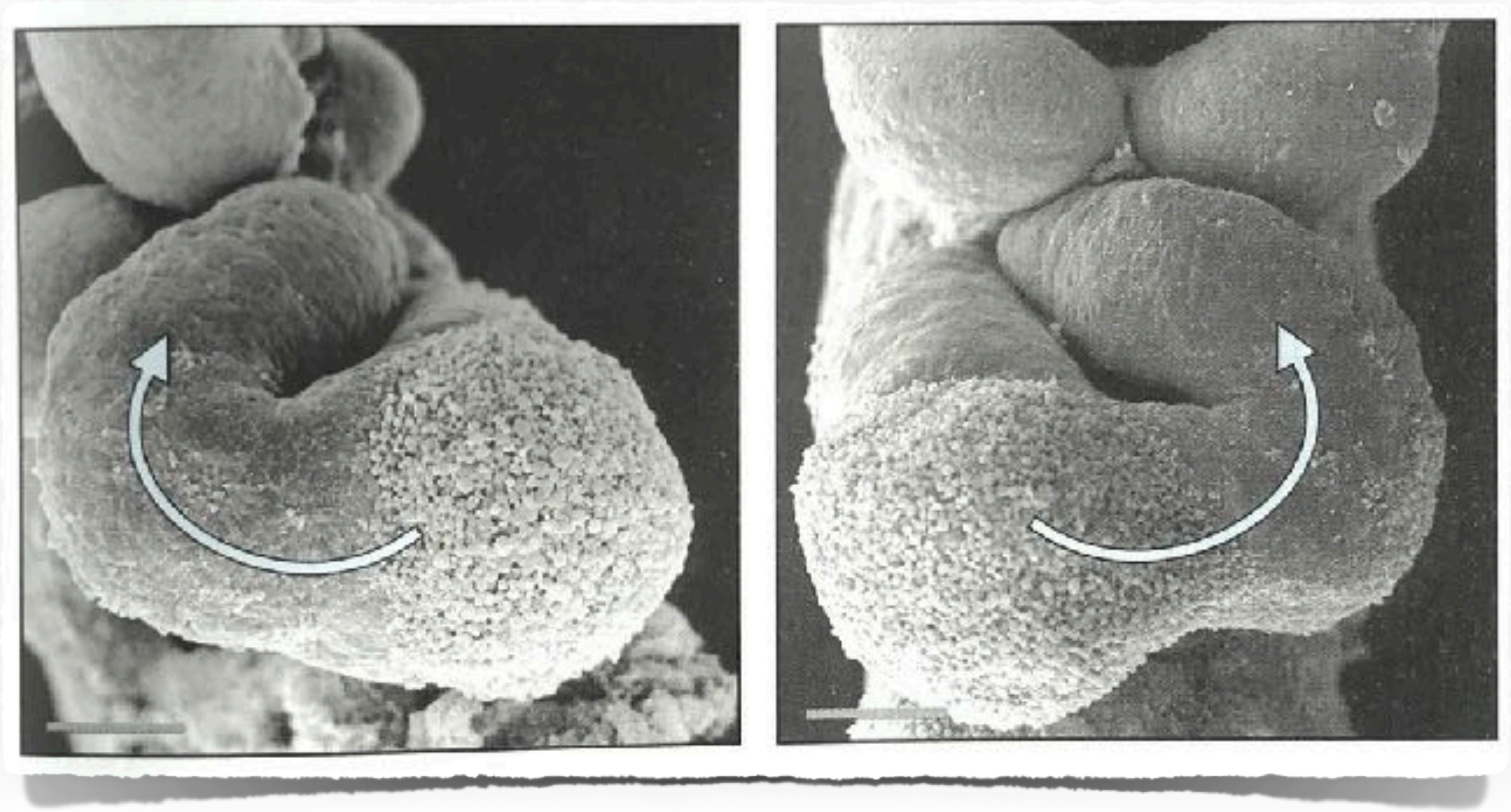
Heterotaxy

left isomerism (polysplenia)



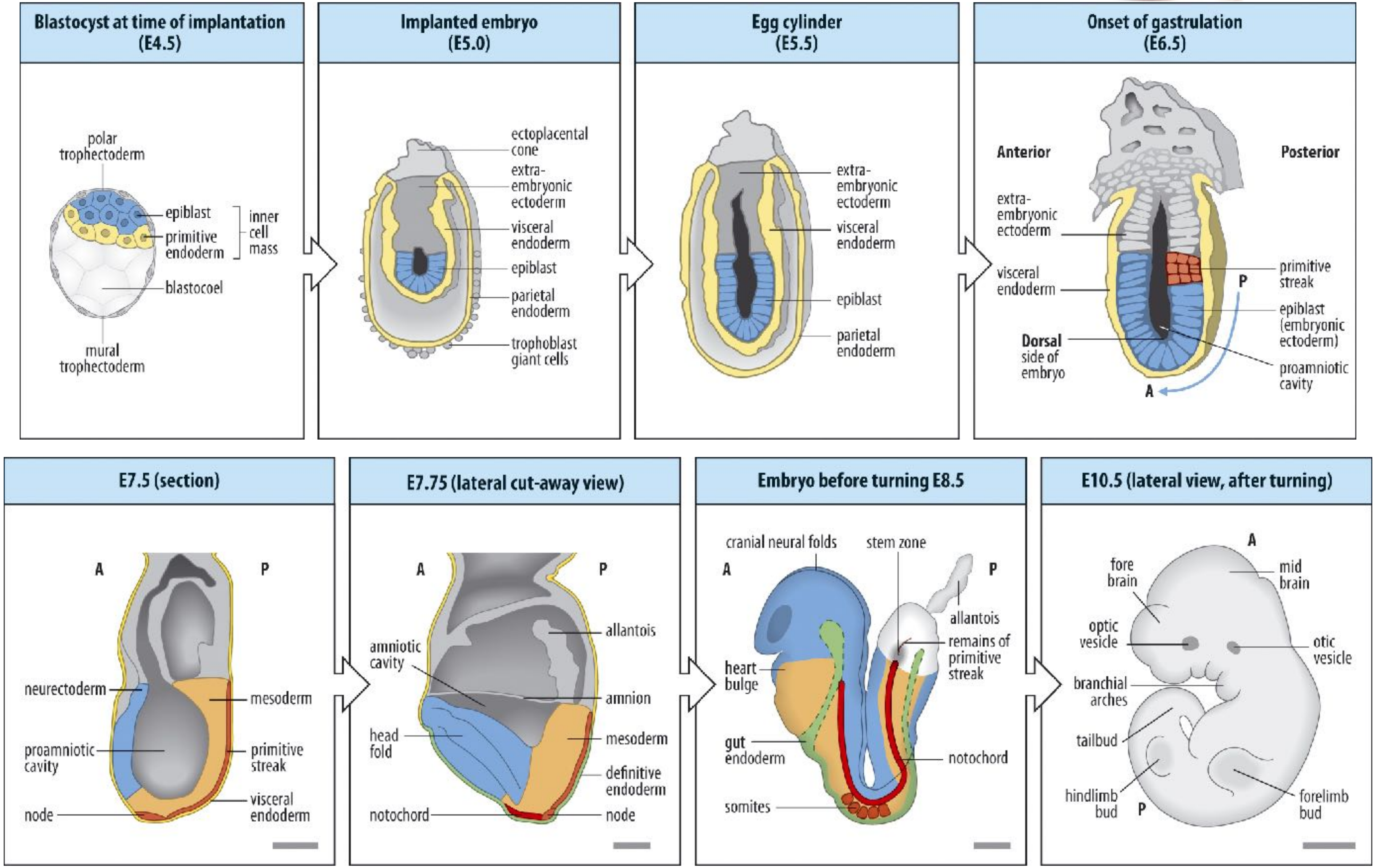
right isomerism (asplenia)



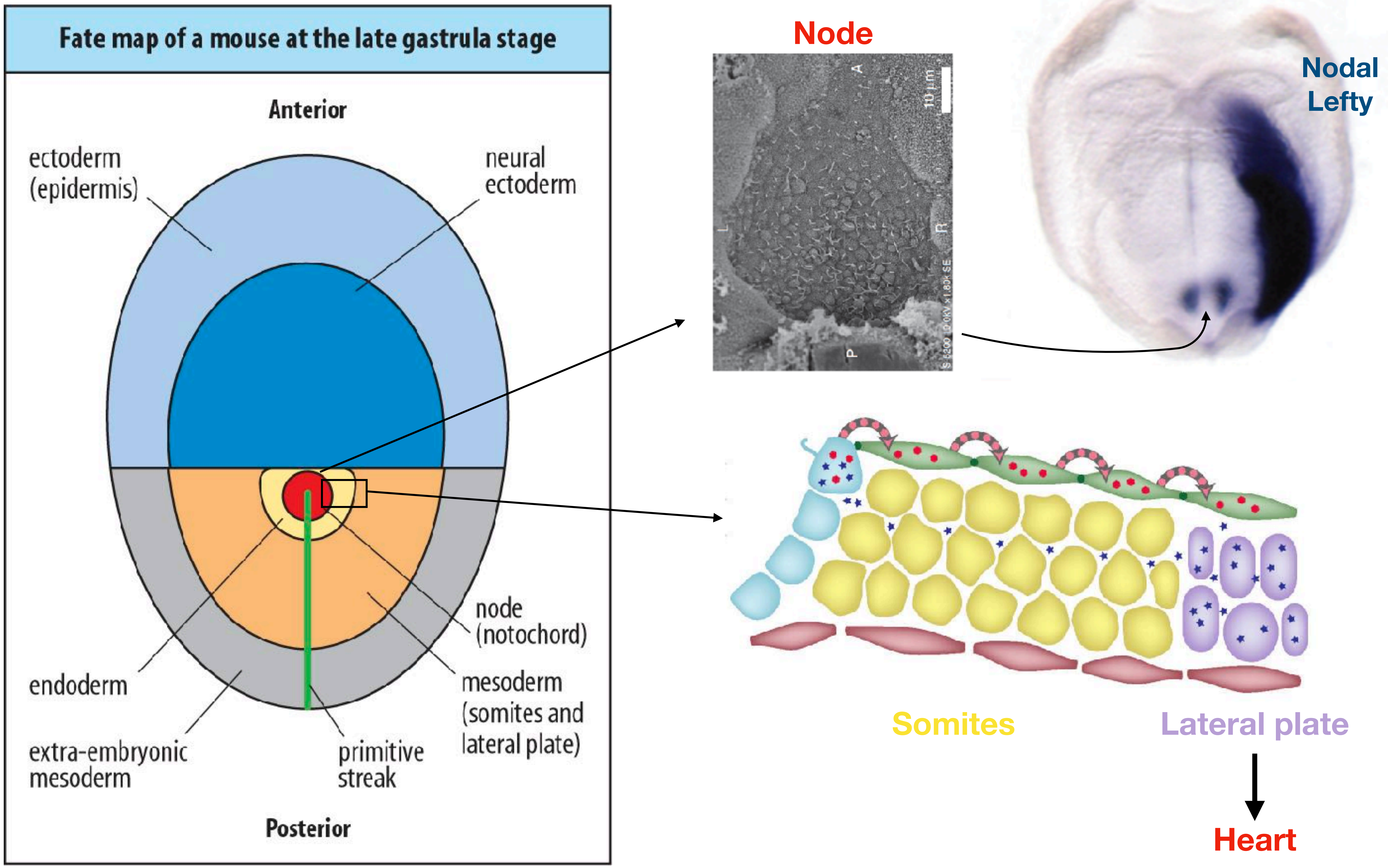


Mouse *inversus viscerum* (*iv)* mutant:
heart looping left-right 50%

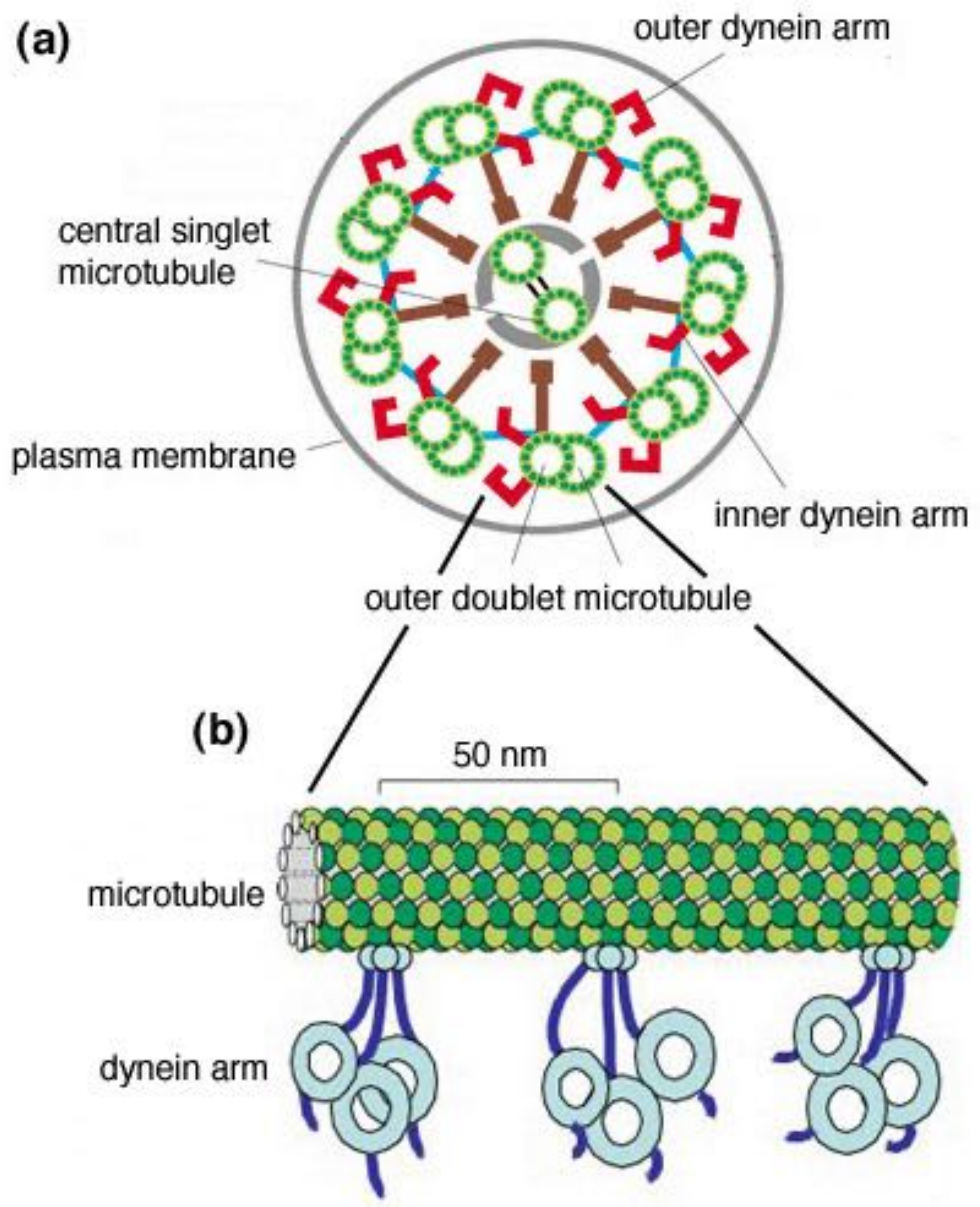
Early mouse development



Embryonic origin of the mouse heart



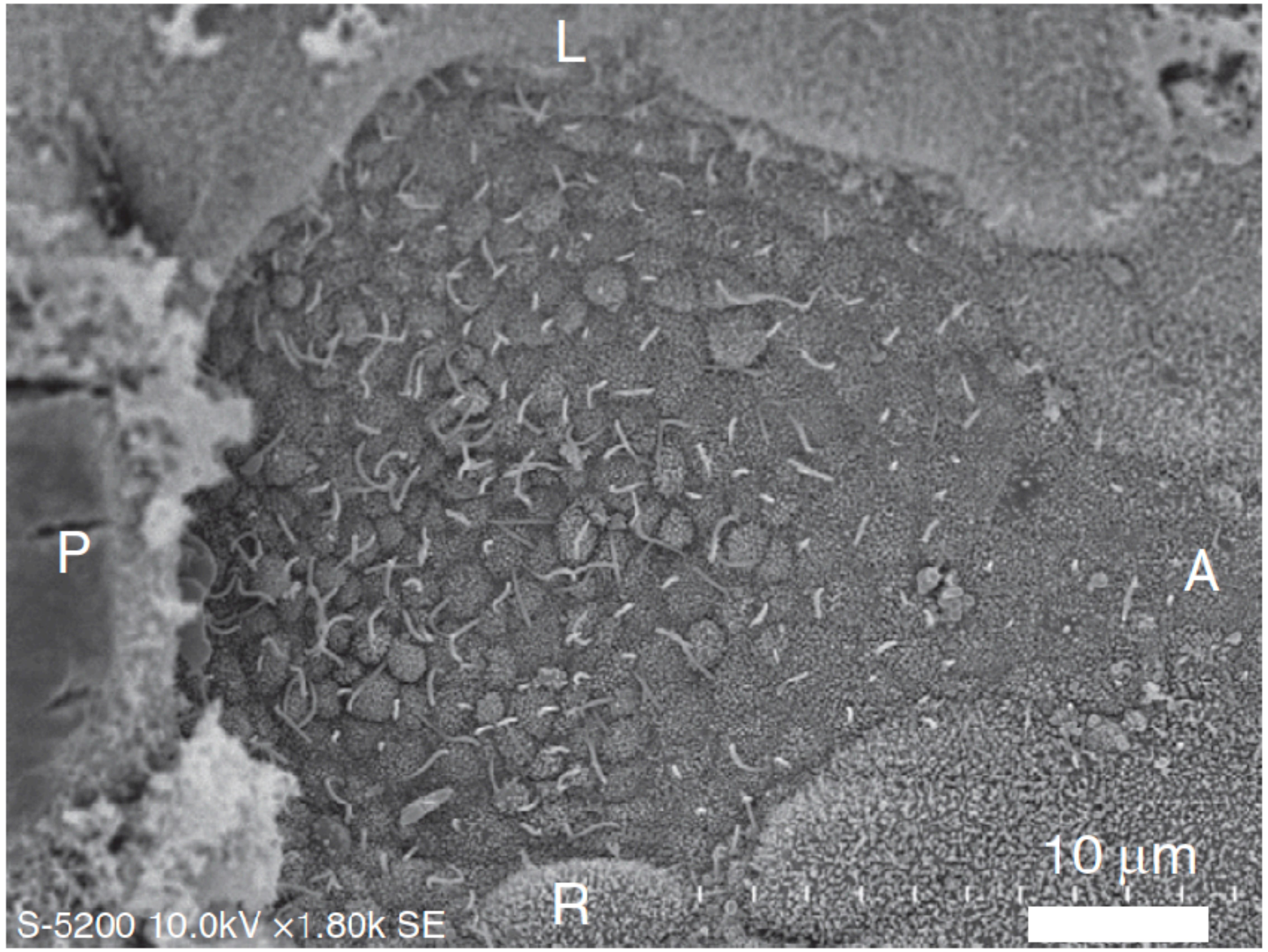
inversus viscerum encodes a Dynein outer arm heavy chain Lrd/Dnah11



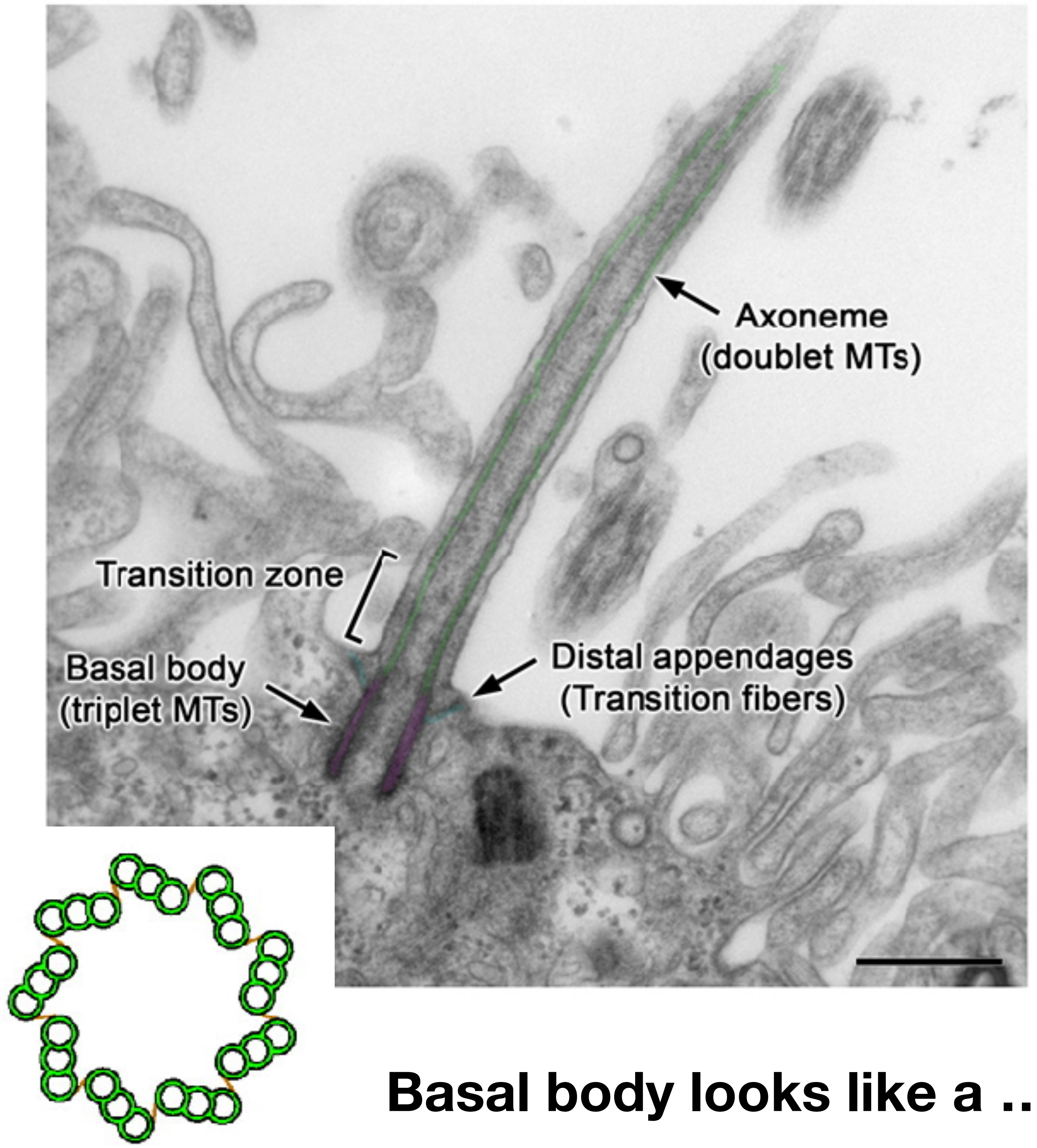
How is axonemal motion generated?

Dnah5 encodes another dynein outer arm heavy chain

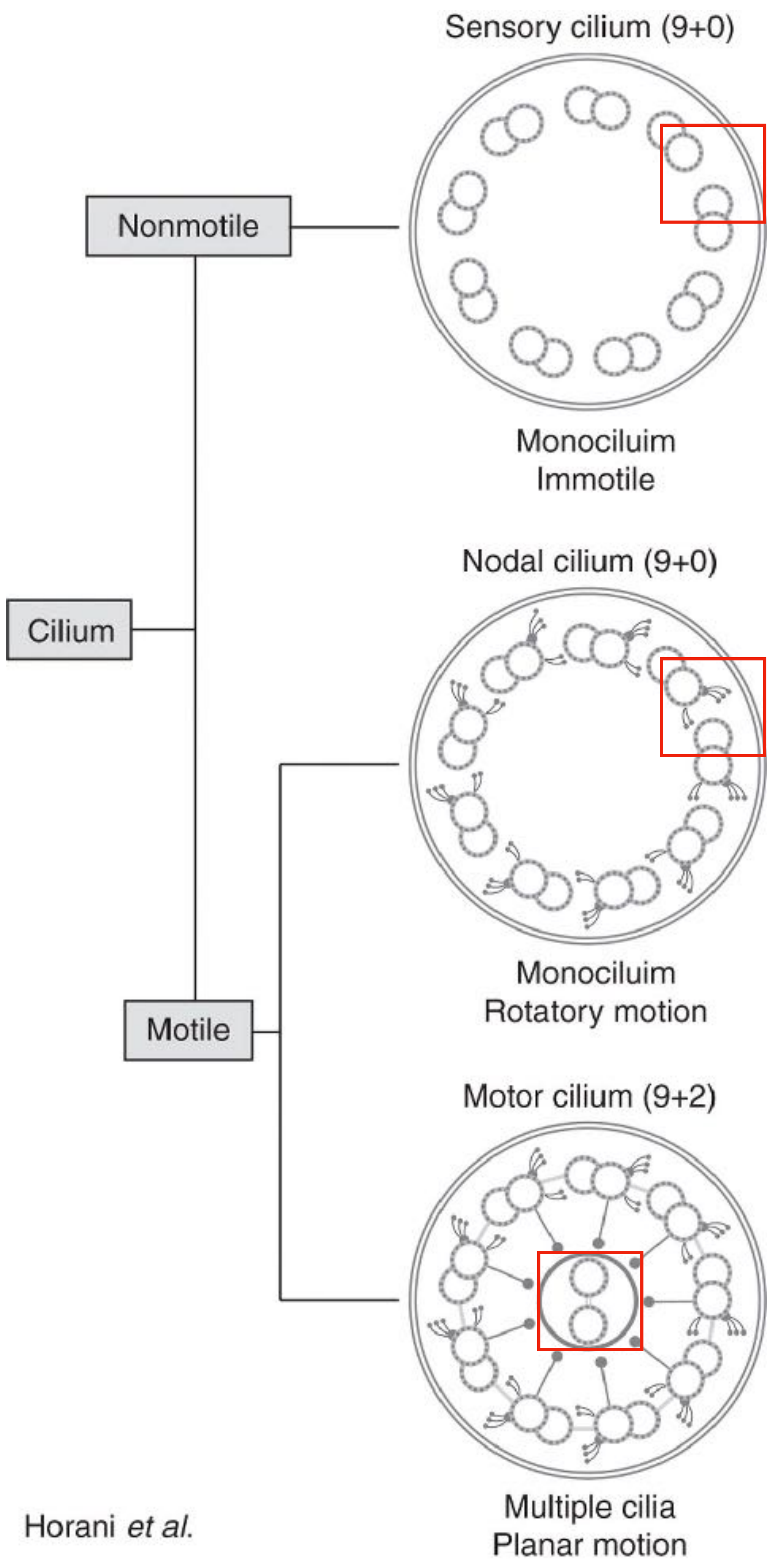
The mouse node is covered in cilia



Different cilia



Basal body looks like a …?



Signaling
Kinocilia
Photoreceptor
Olfactory
Primary

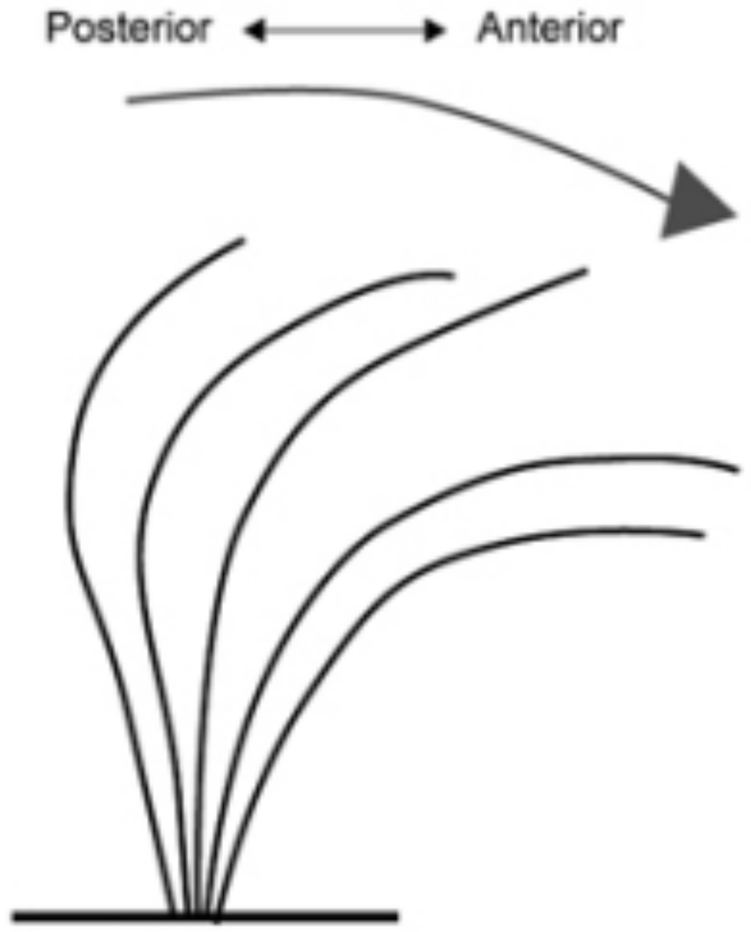
Embryonic node

Transport
Lungs
Fallopian tubes
Testes
CNS ventricles

Sperm

Group puzzle

What motion could a node cilium use to drive persistent fluid flow?

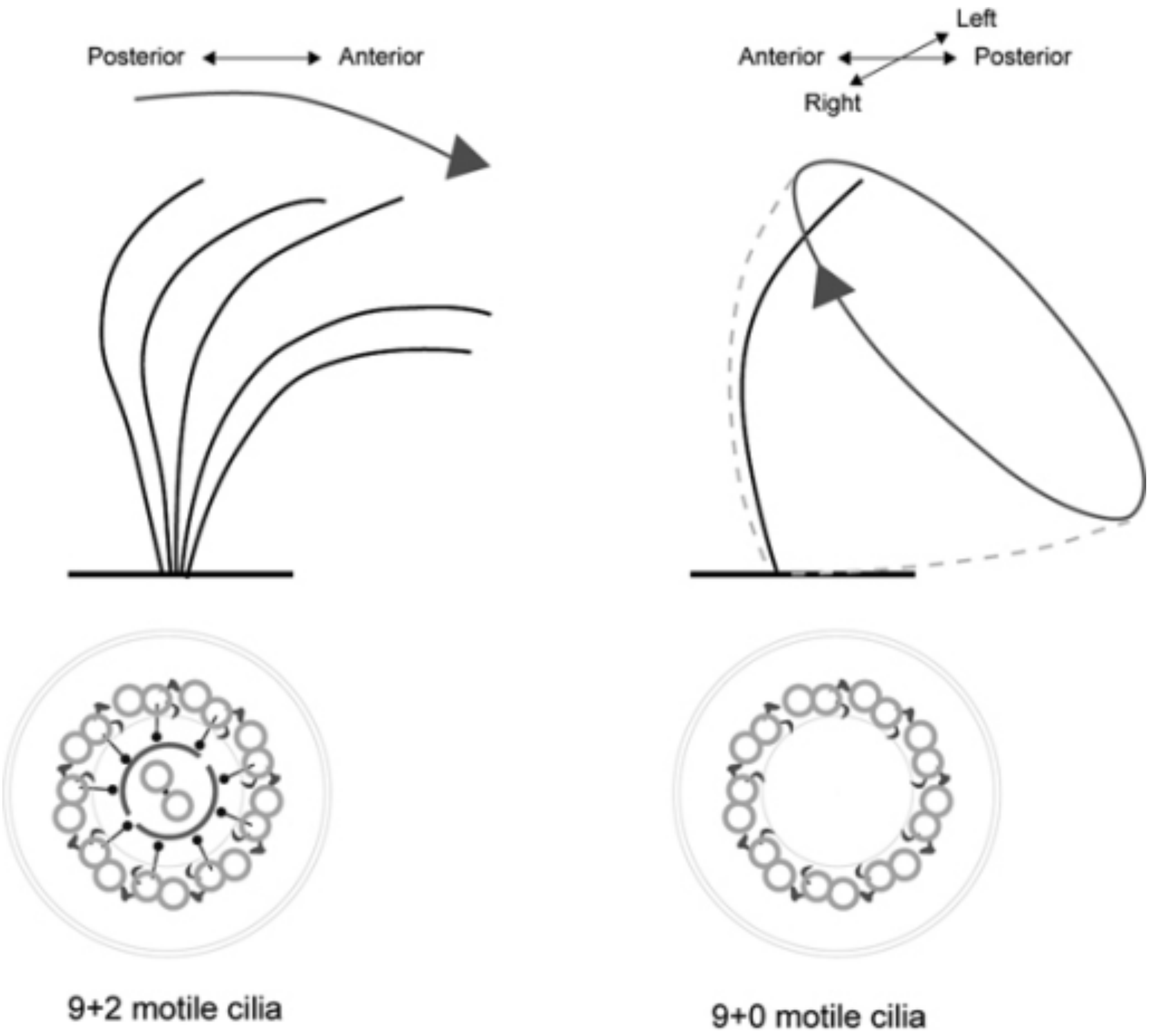


?

9+2 motile cilia

9+0 motile cilia

Paddles and propellors

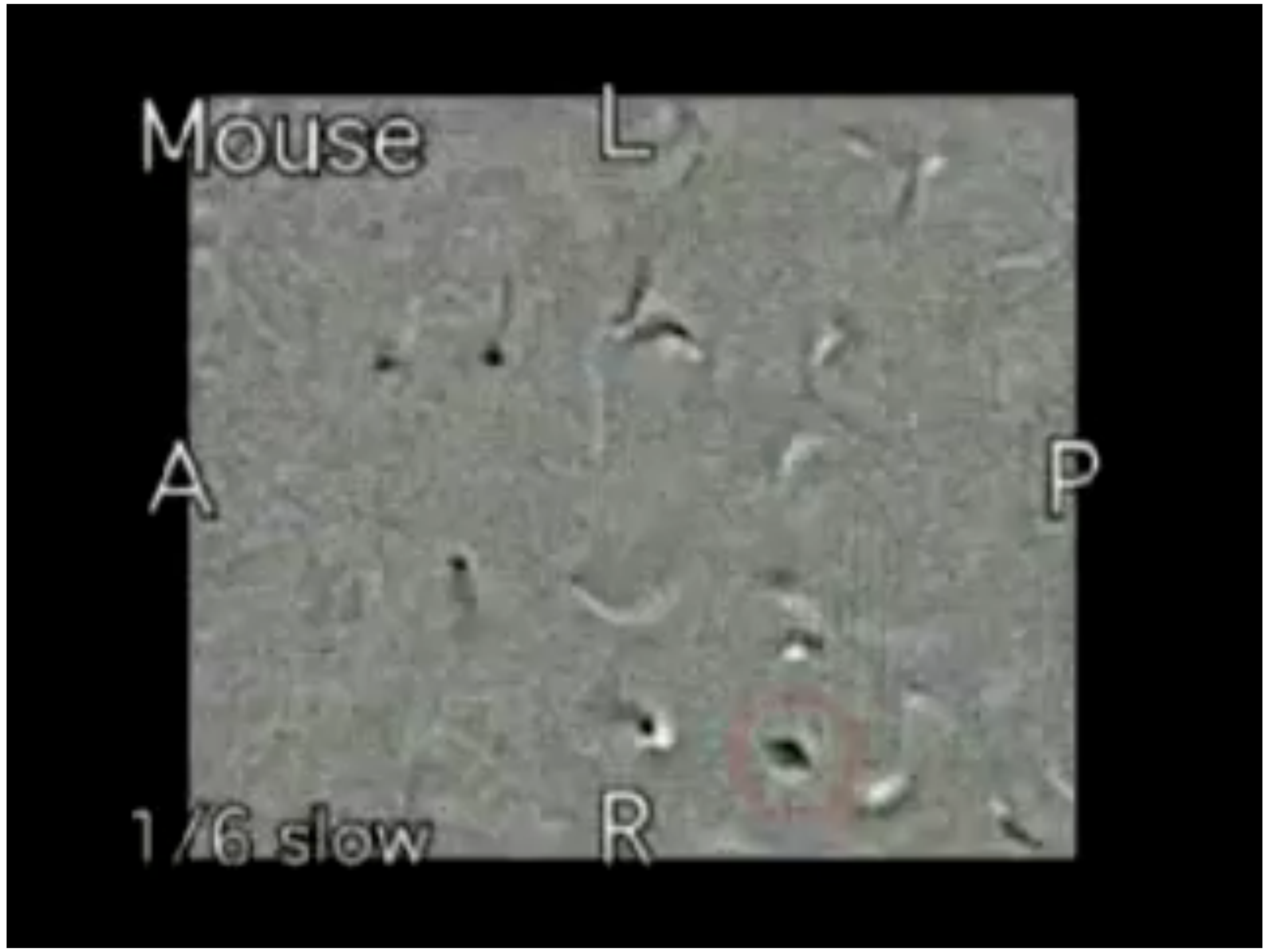


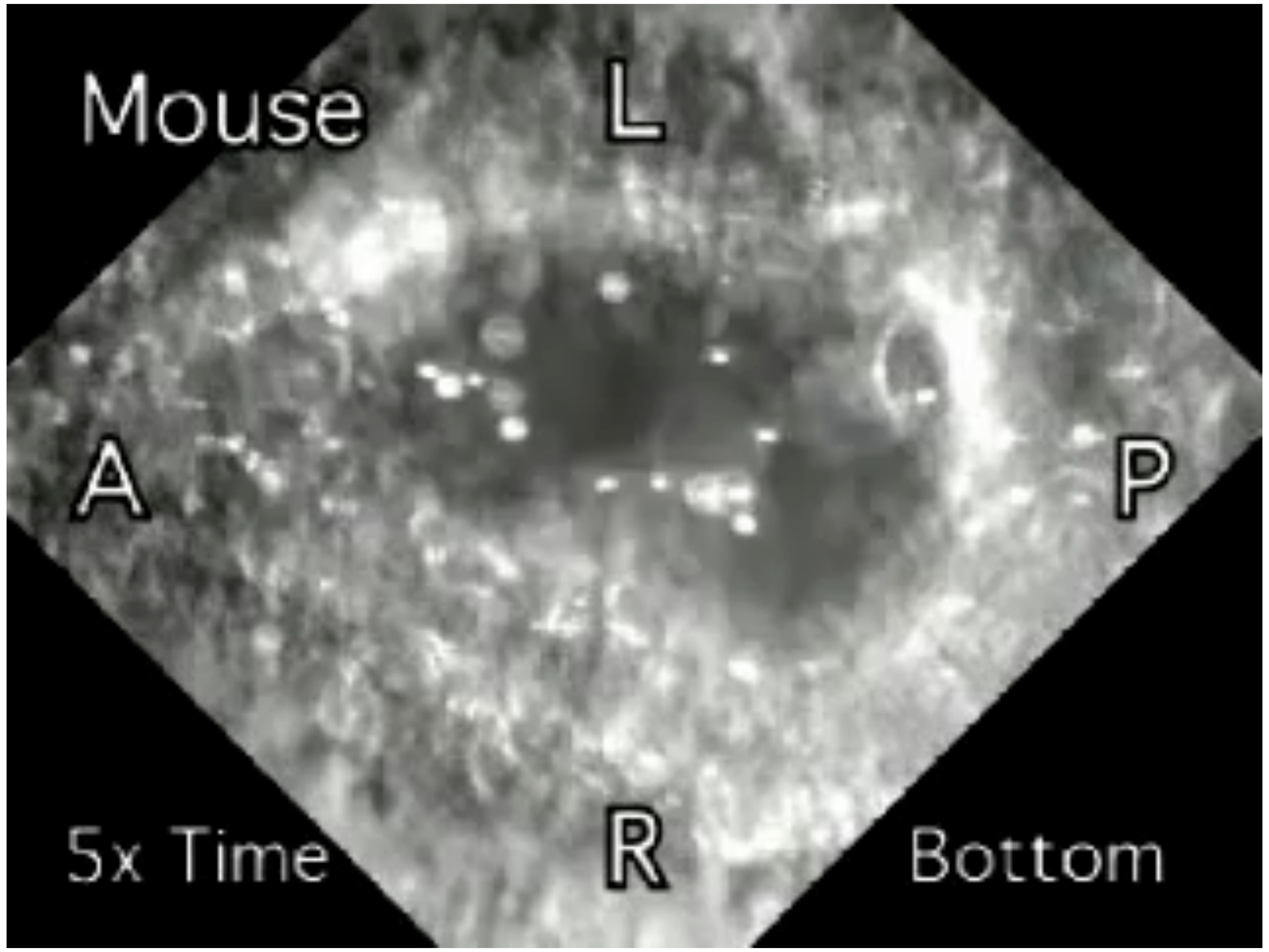
rotation?

tilt?

Rsph4a (radial spoke homolog) mutant mice have rotating cilia in their lungs!

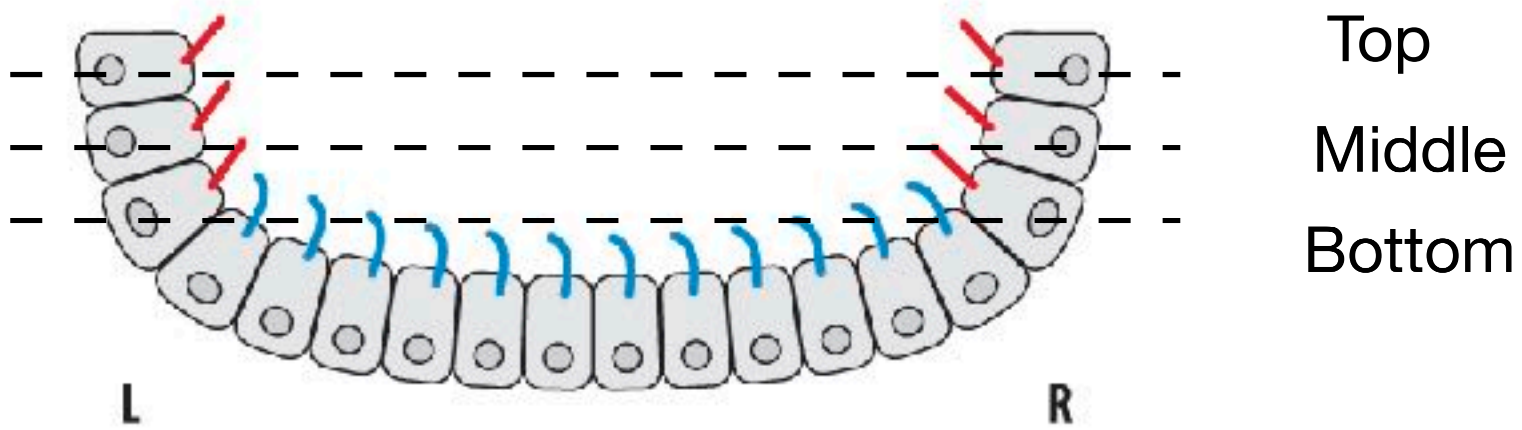
Cilia-induced fluid flow across node





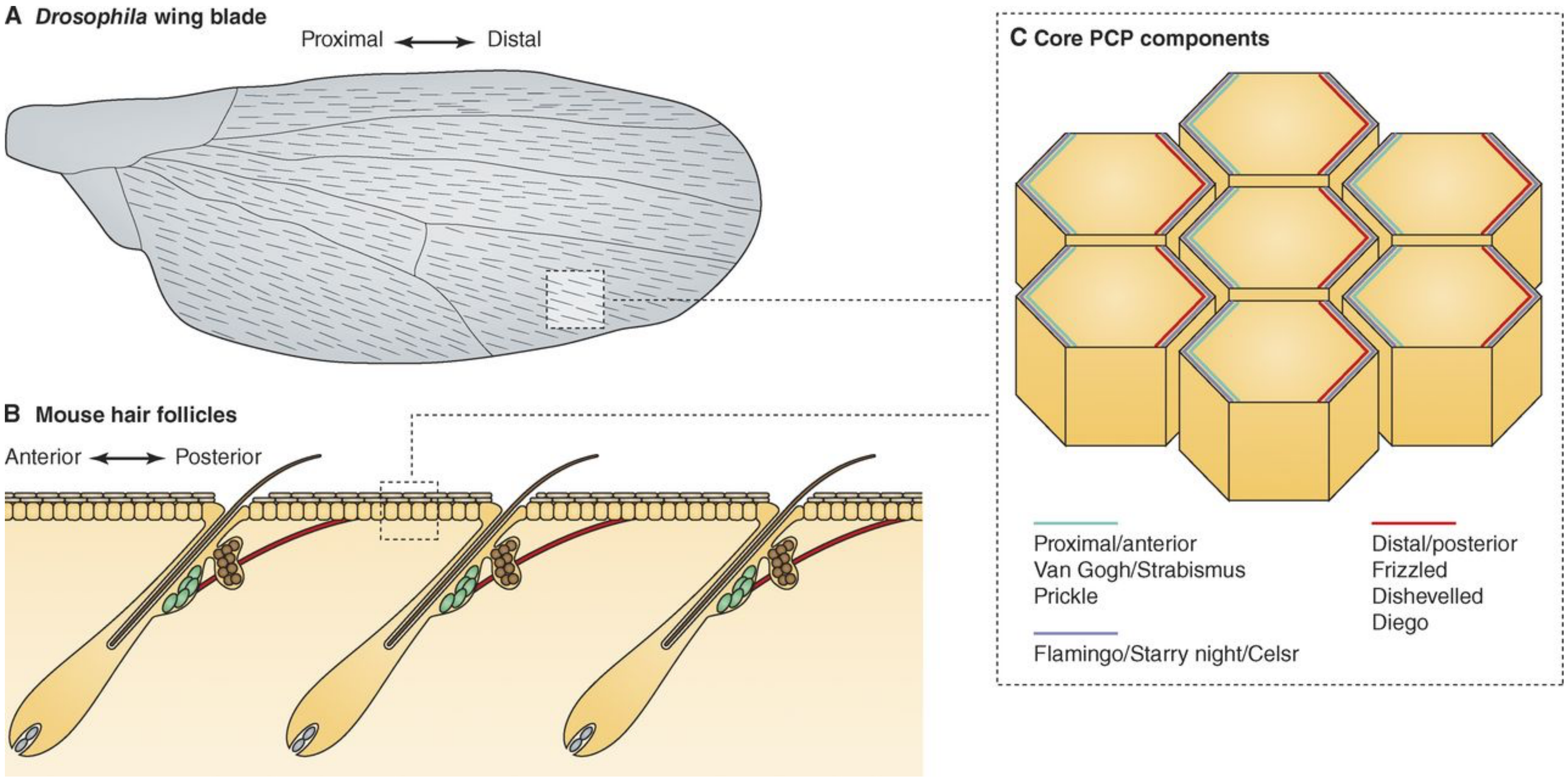
Cilia rotate clockwise,
Elevated power stroke,
Low recovery stroke,
Point posteriorly

Beads in flow
Fast and slow



Okada et al, 2005

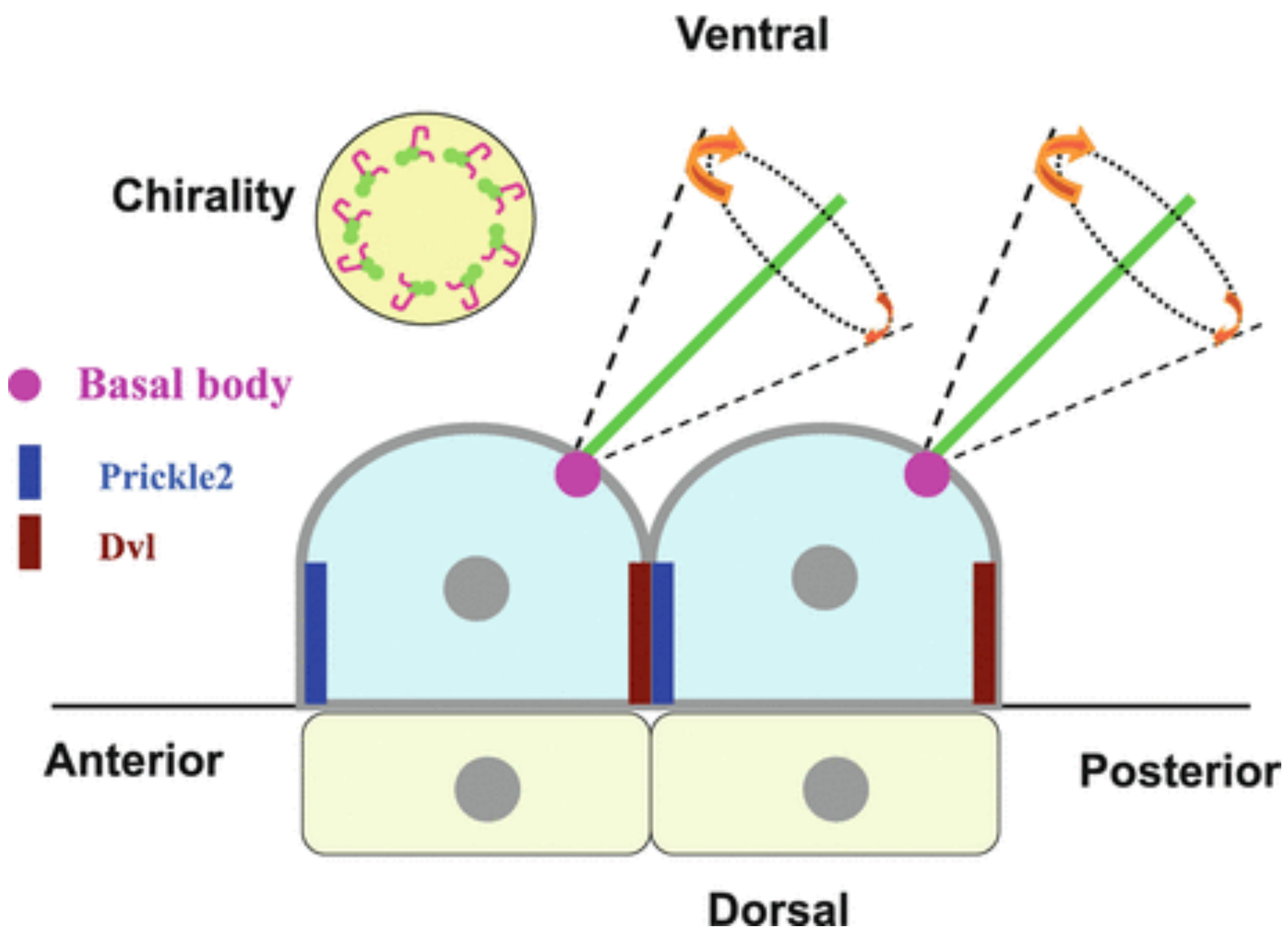
Planar polarity in a 2D epithelial sheet



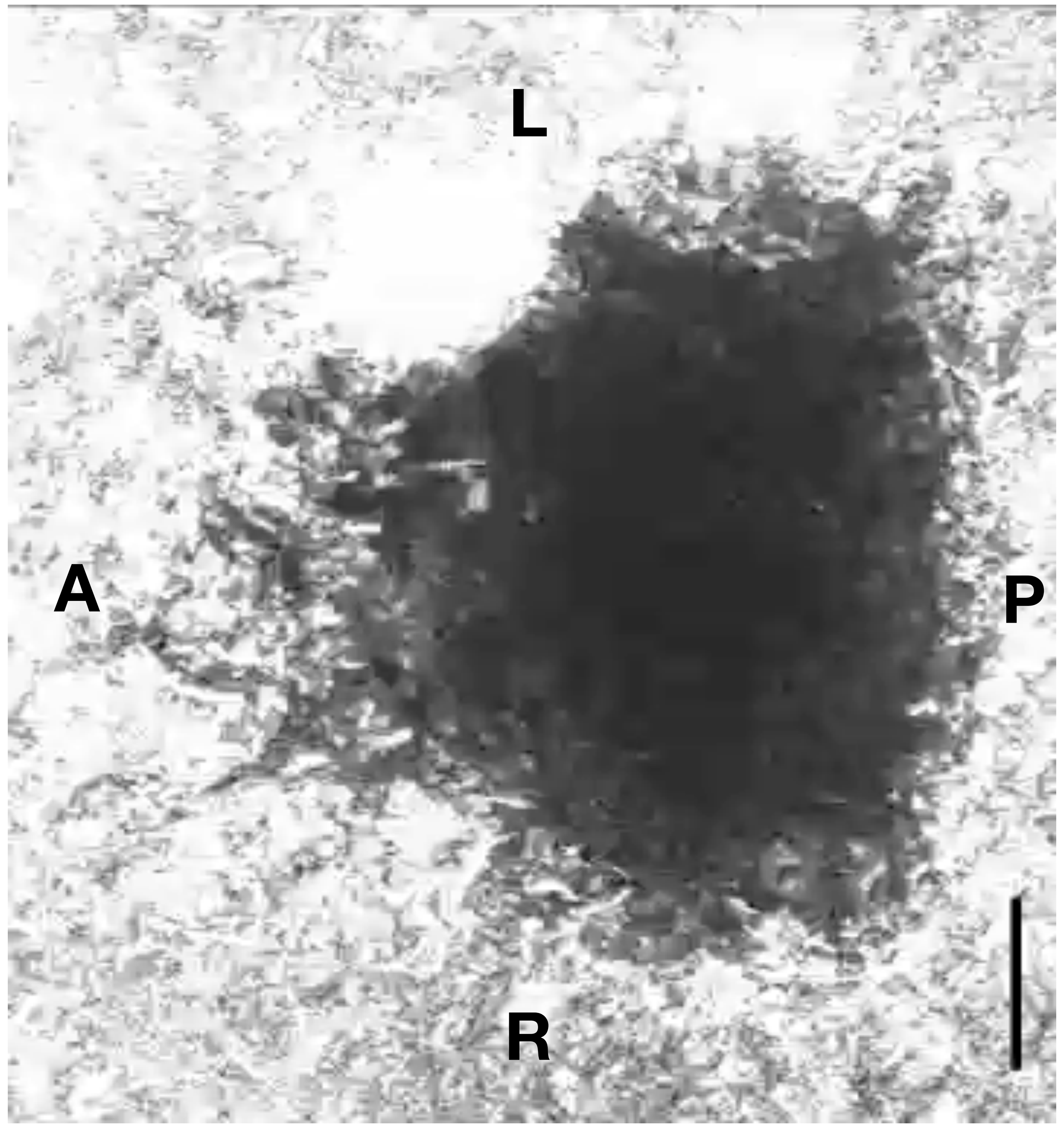
+ many other examples…

Devenport, 2014

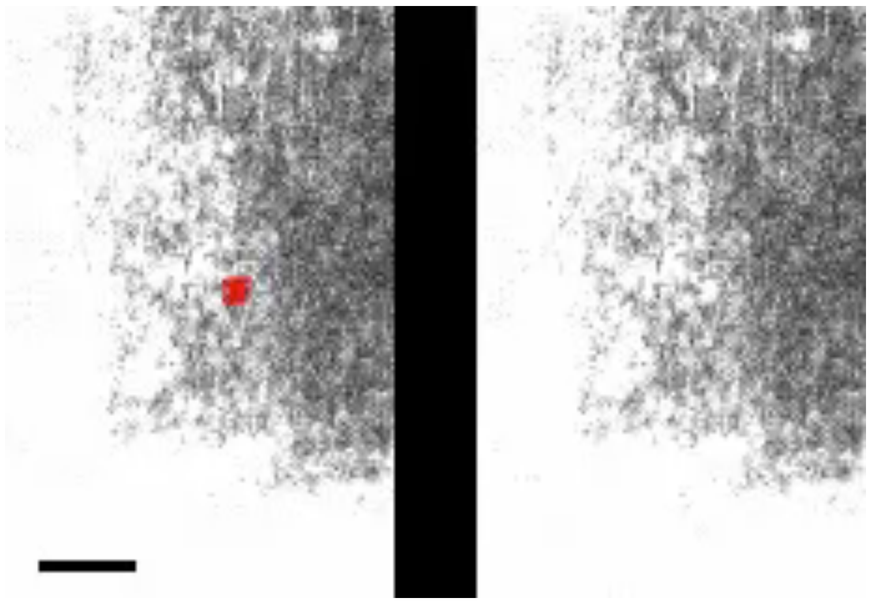
The node epithelium is polarised early along the A-P axis



Membrane particles move across node



DiI labelled membrane

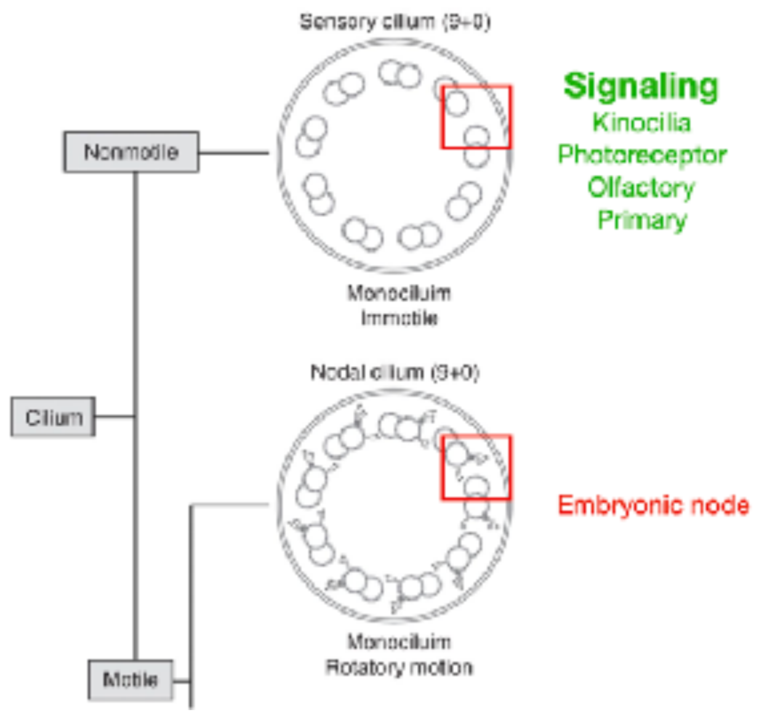


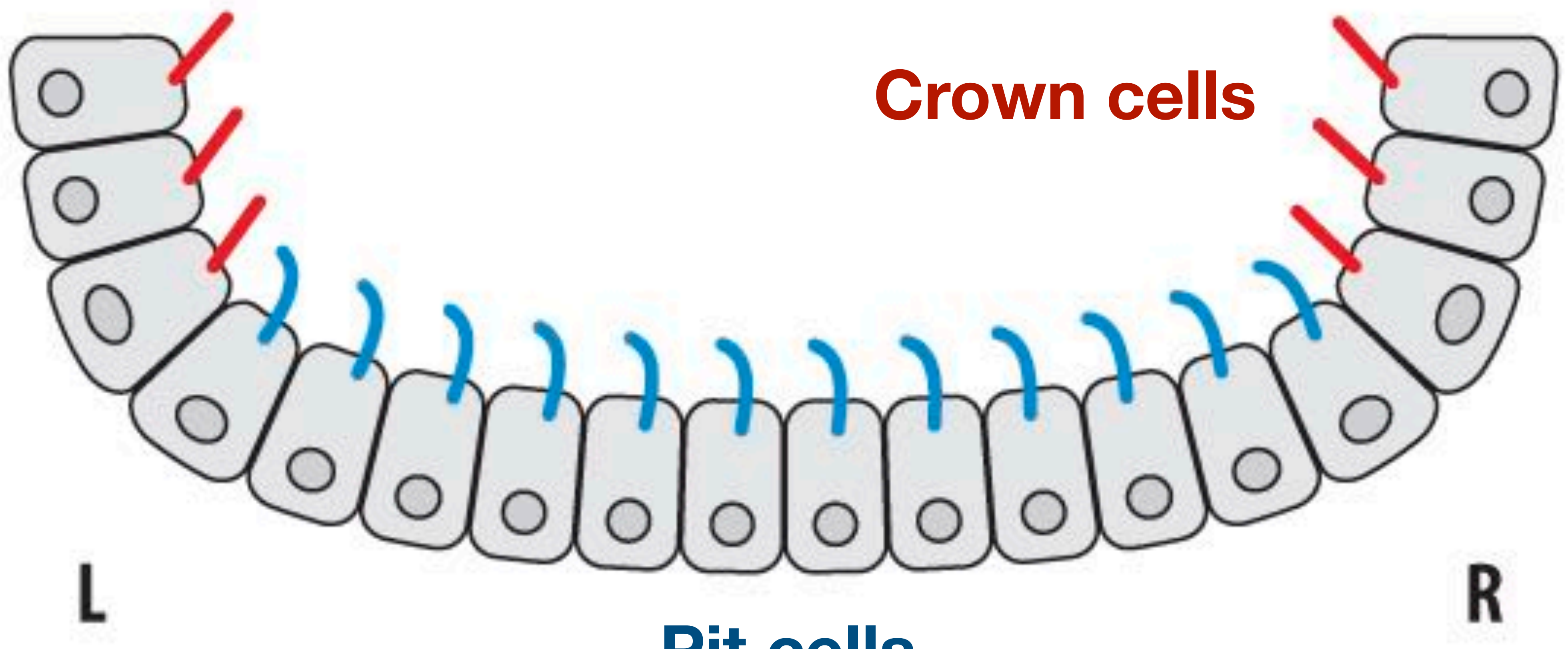
Release from cilium?

Disruption on wall?

Tanaka et al, 2005

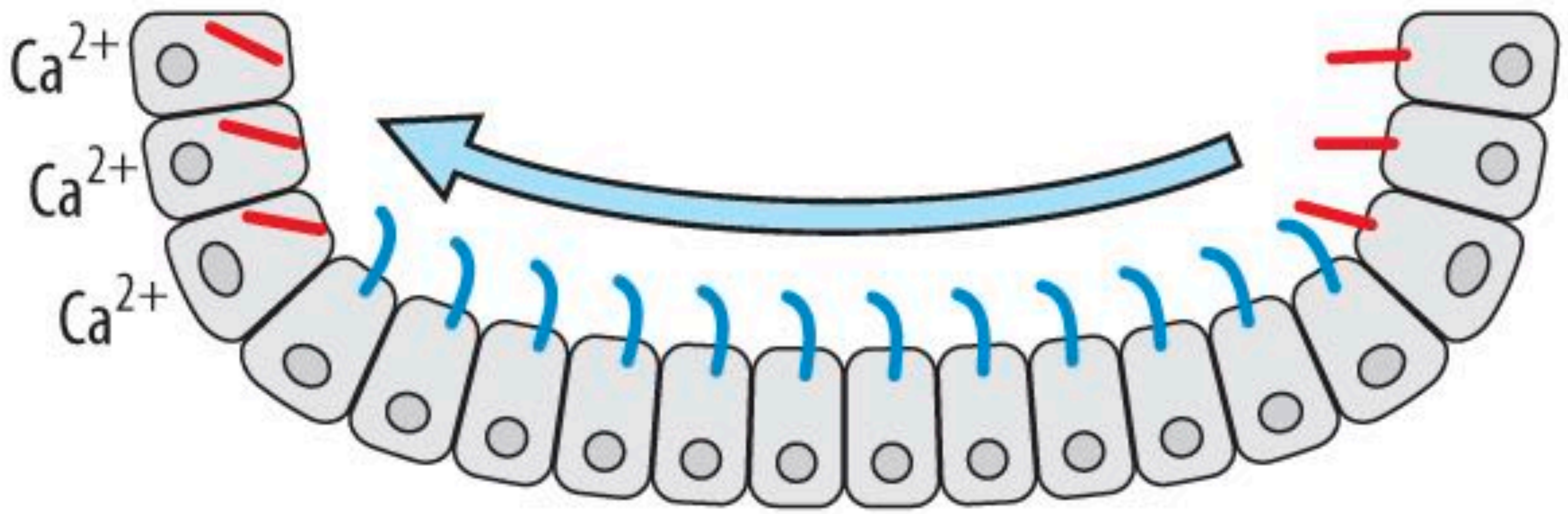
Two types of cilia inhabit the node

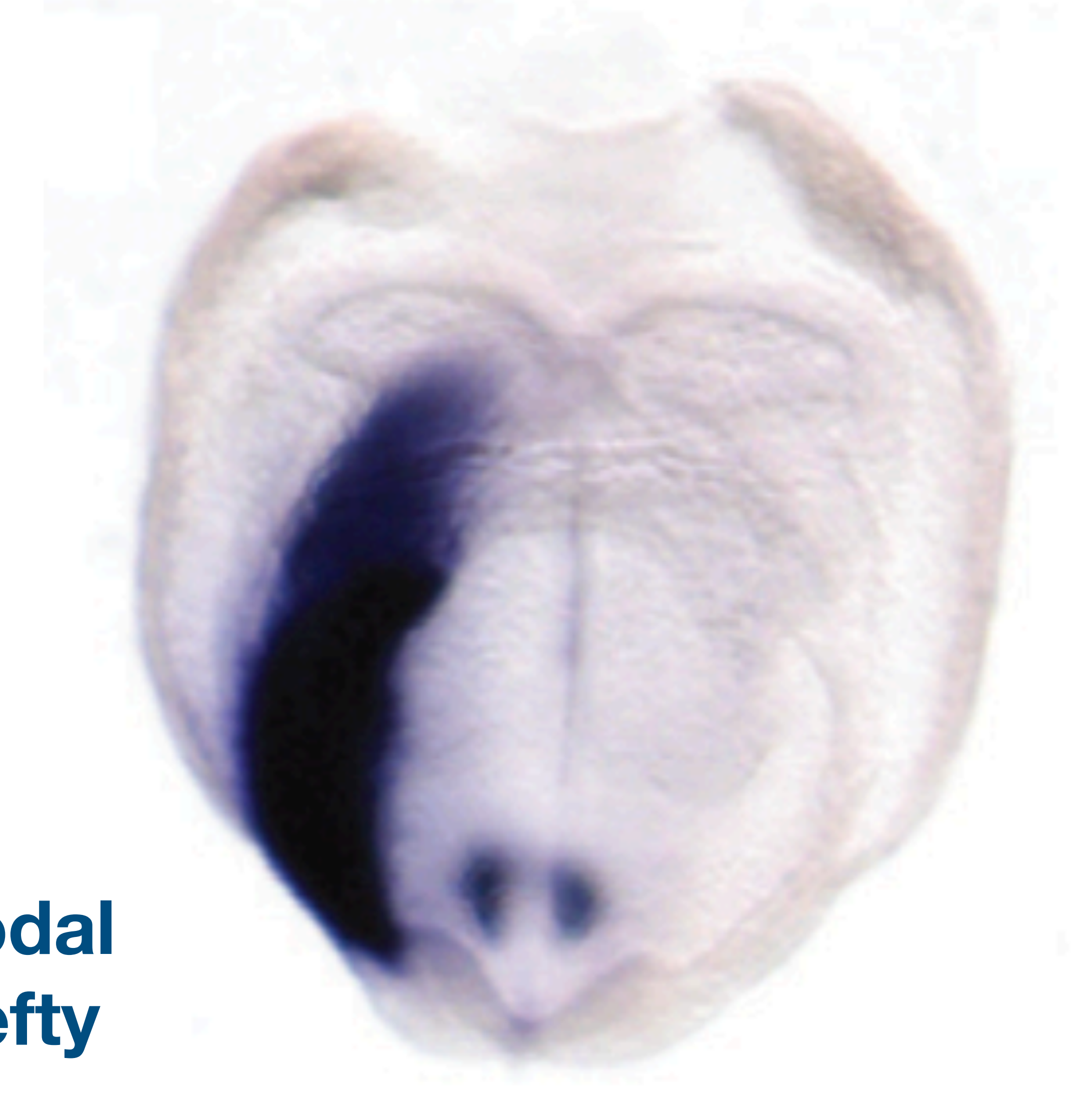




Polycystin-2 (Pkd2,TRPP2),
- Ca^{2+}-permeable cation channel
- polycystic kidney disease in humans
- laterality defects in mouse
- defects rescued by Pkd2 in crown cells

Calcium
- thapsigargin randomises laterality
- Ca^{2+} oscillations observed





Nodal
Lefty

Yoshiba et al. 2012

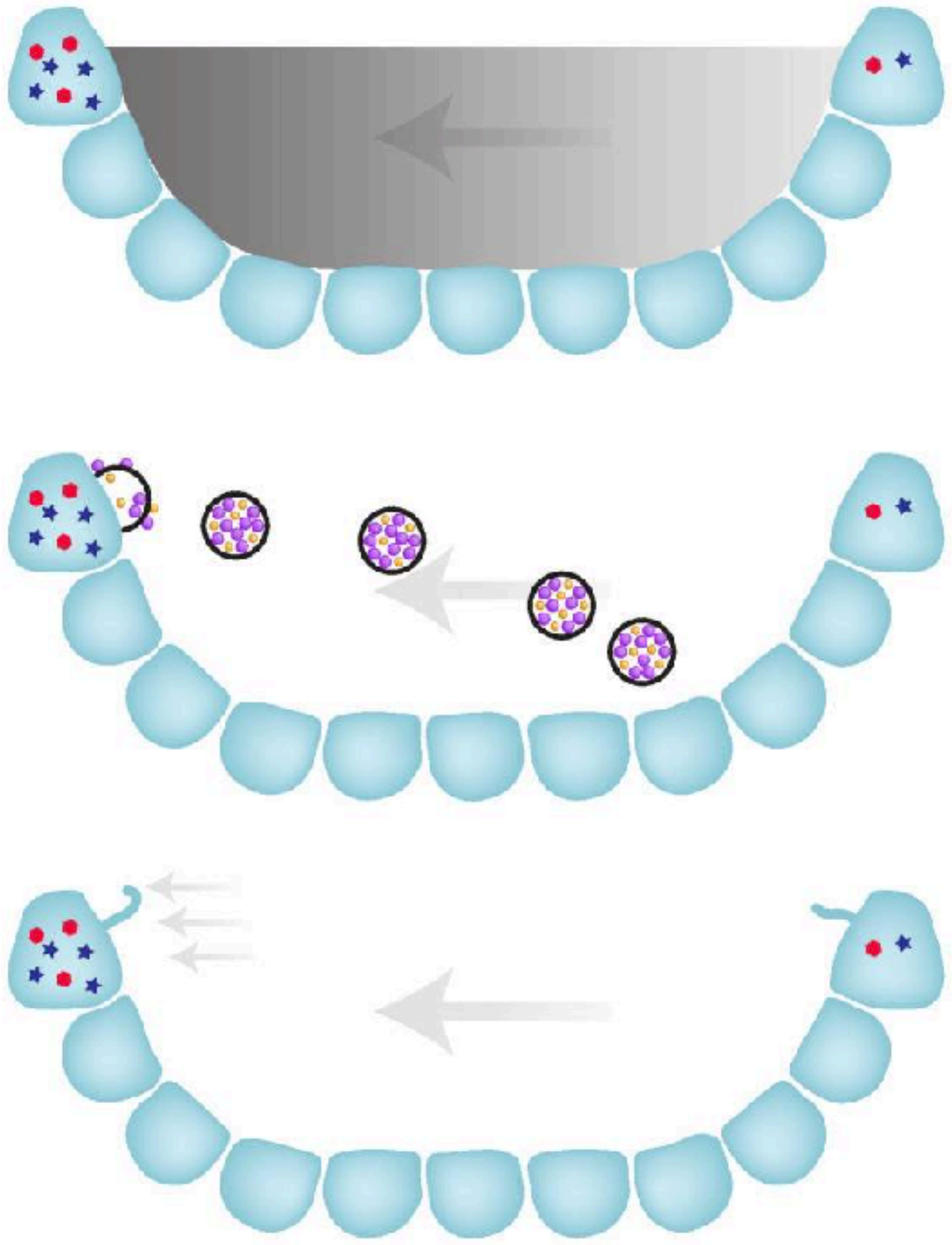
Group puzzle

What is the signal to the cilia in the crown cells?

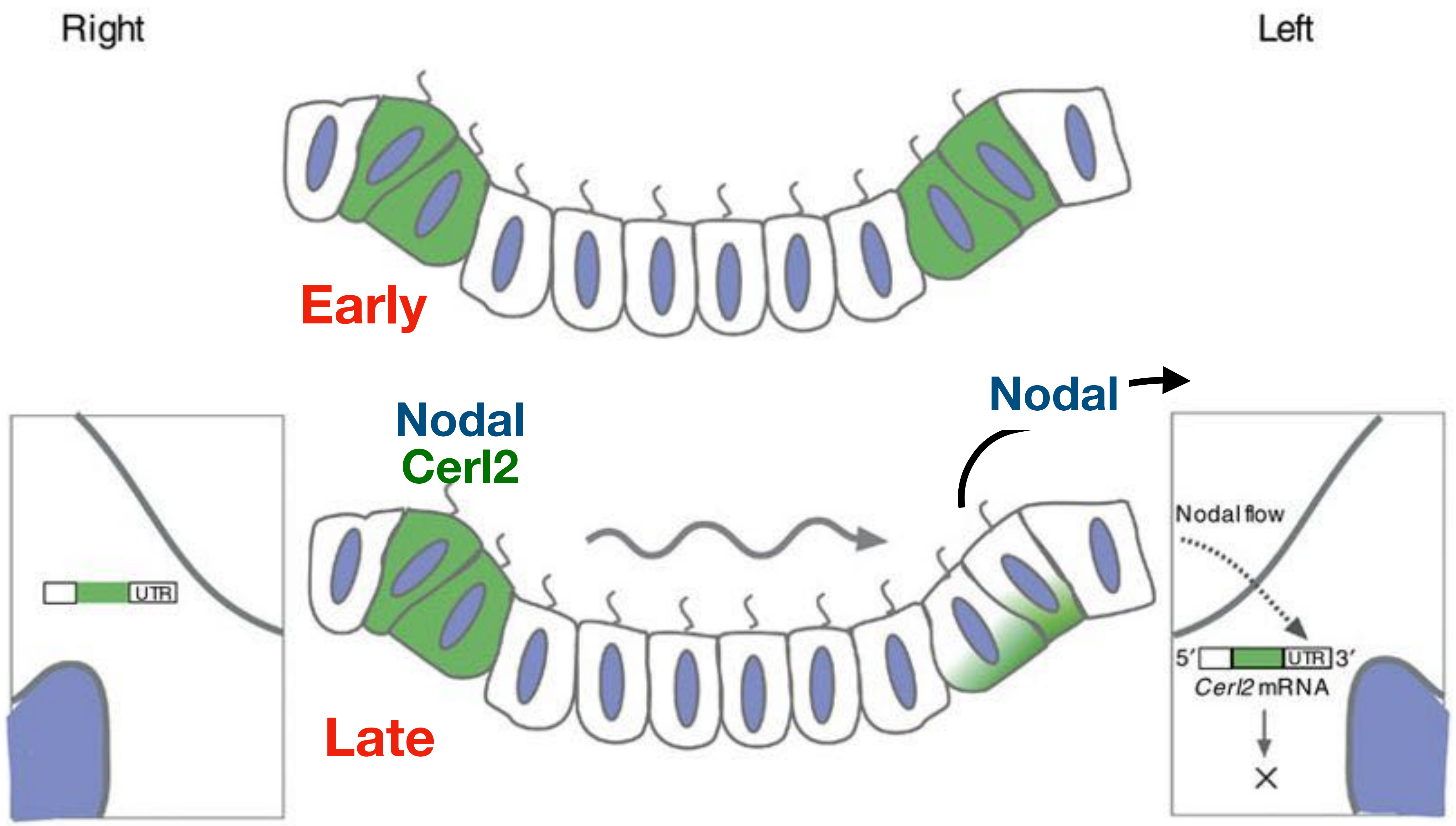
Morphogen gradient

Nodal vesicular parcel (NVP)

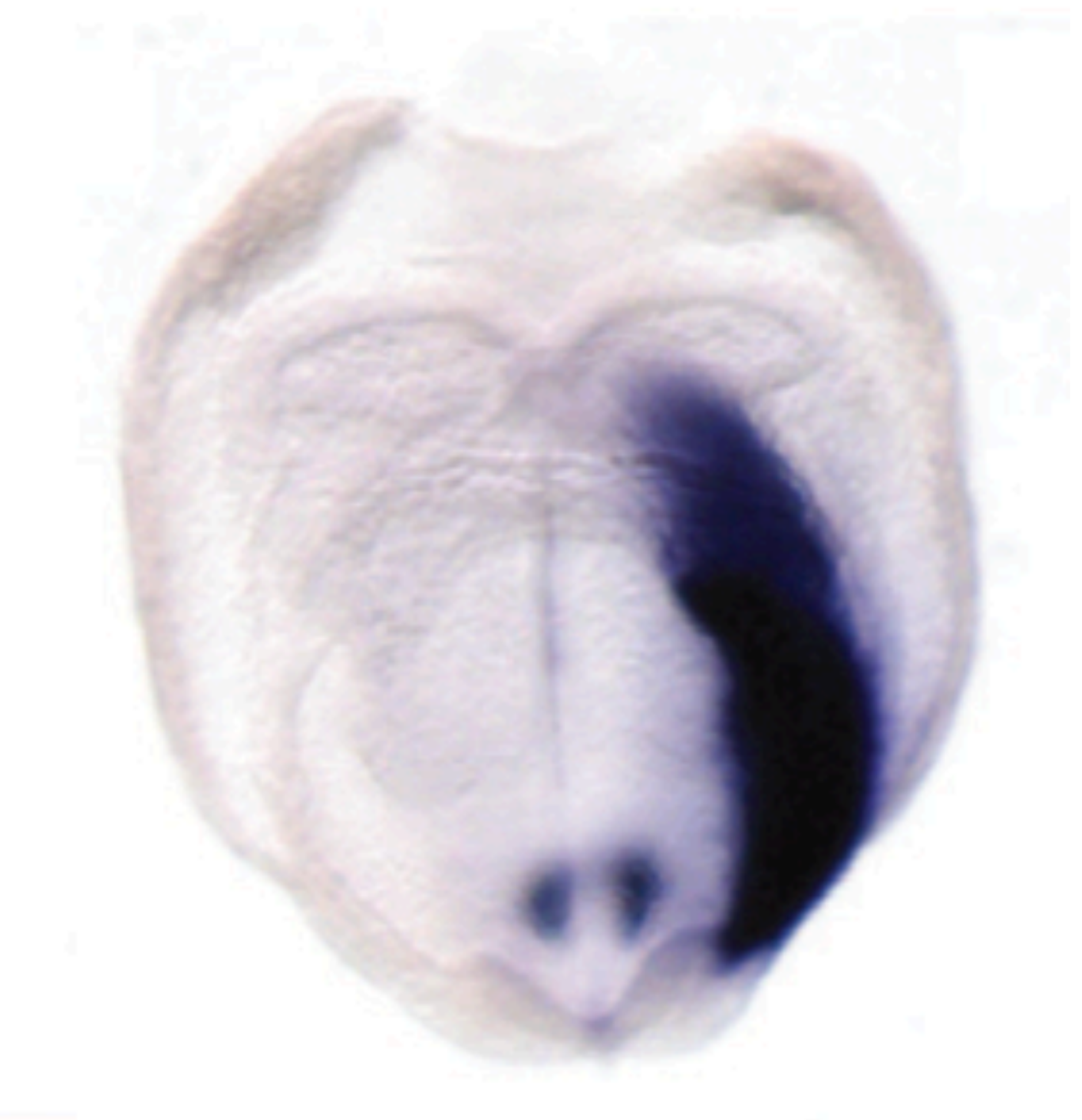
Mechanosensation



Signal processing to release asymmetric Nodal

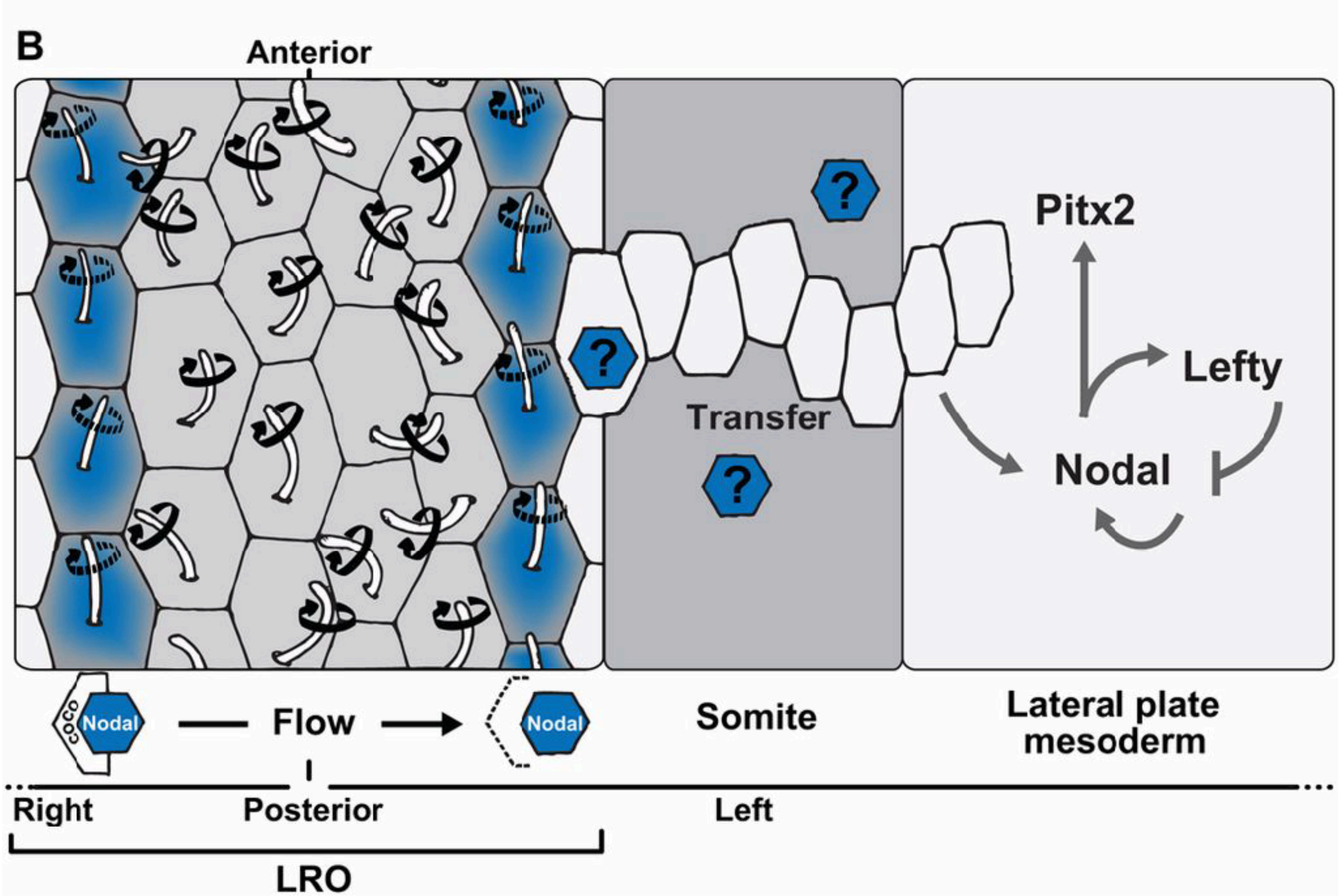


Cerl2 / Dand5
Earliest asymmetric mRNA
symmetric in *iv* mice
Nodal inhibitor(!)
blocks Nodal on RHS
Nodal release on LHS



Nakamura et al., 2012

Nodal signals induce the transcription factor Pitx2, which activates morphogenetic genes in the LPM



How do node cilia rotate in the clockwise direction?

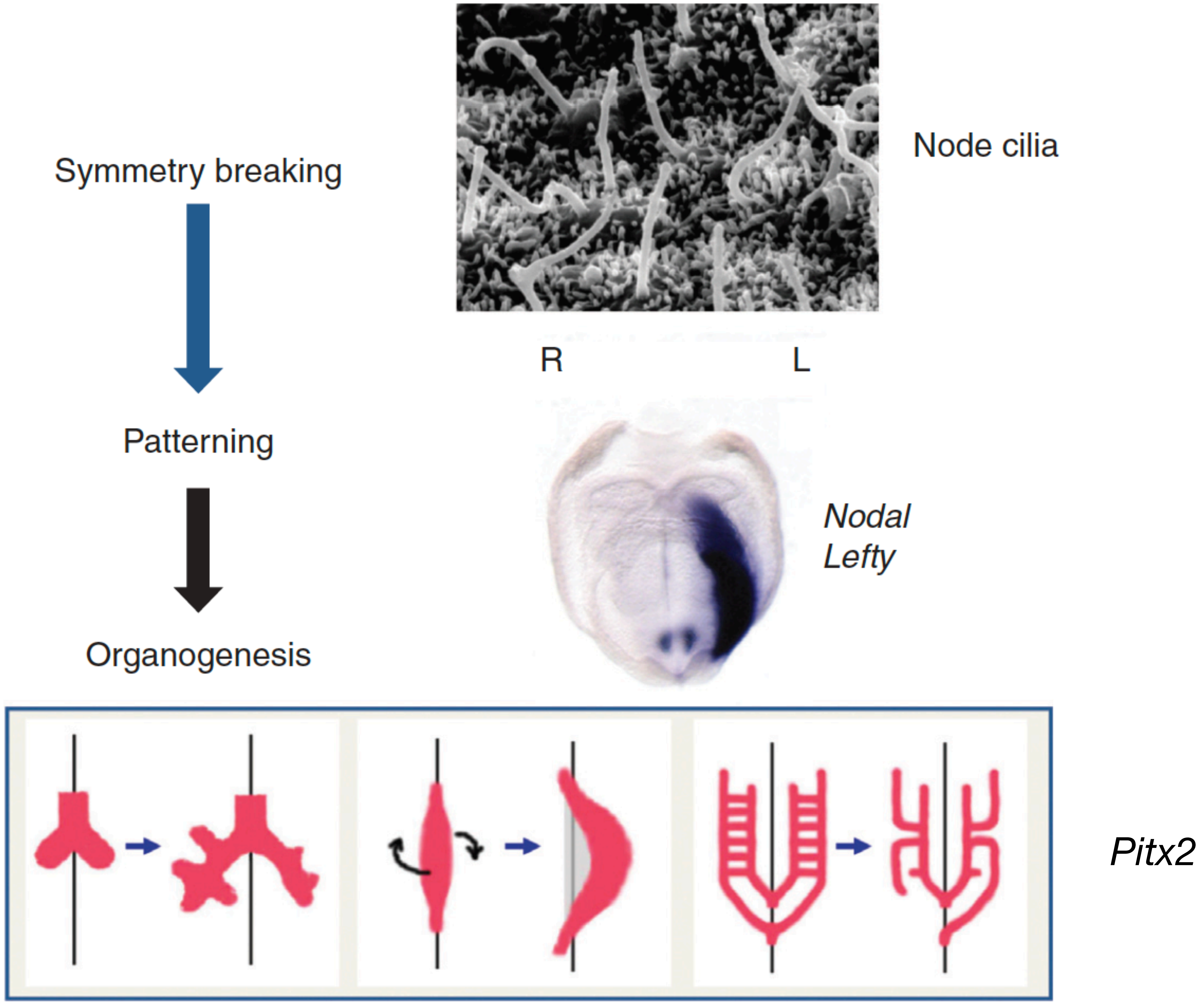
What is the positional cue that polarizes node cells along AP axis?

Do cilia act as mechano-sensors or chemo-sensors?

How is Cerl2 mRNA stability controlled?

How is a small Nodal signal amplified along the LPM?

Summary


Symmetry breaking
Patterning
Organogenesis
Node cilia
R
L
Nodal
Lefty
Pitx2

Questions?