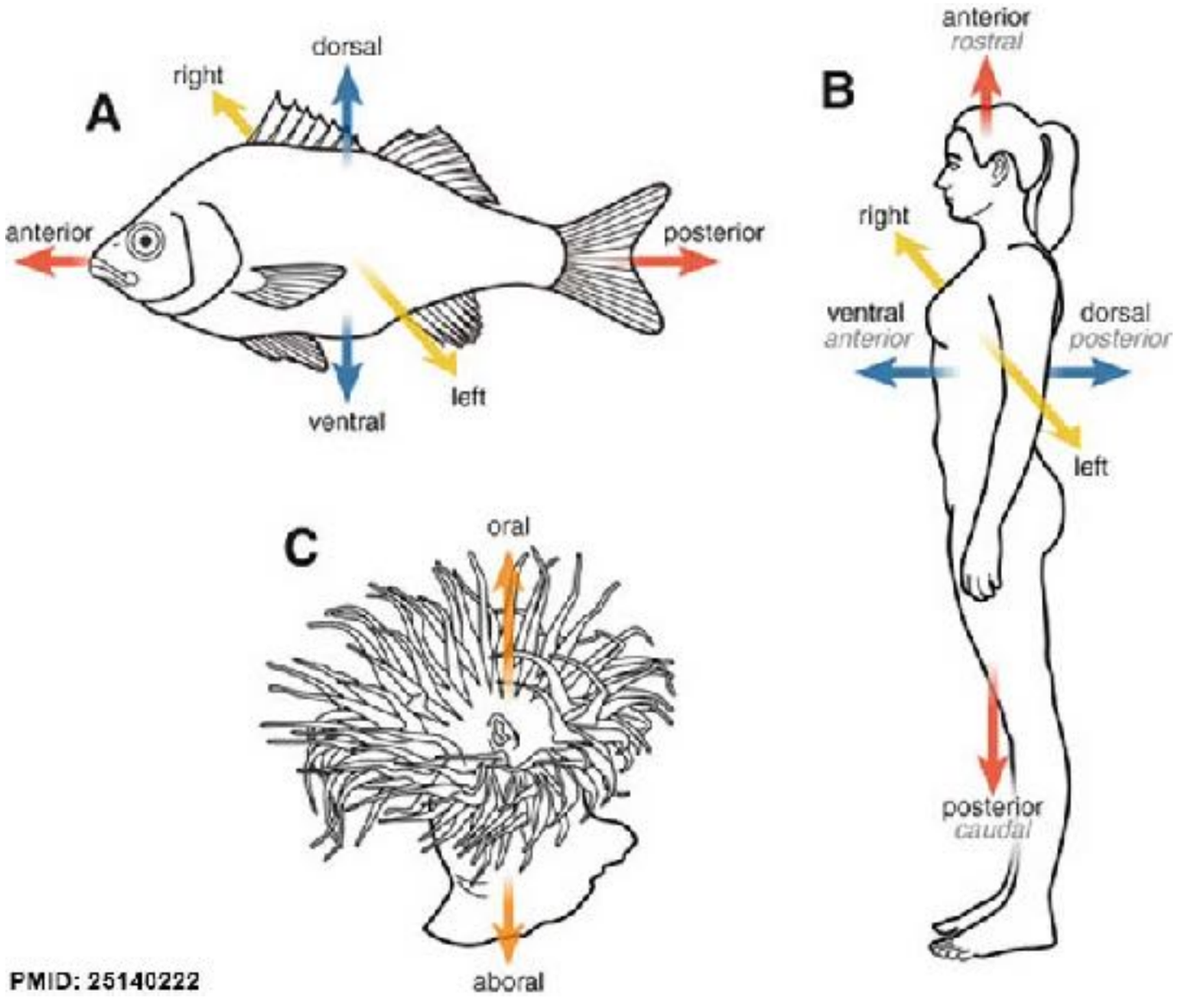
Big questions

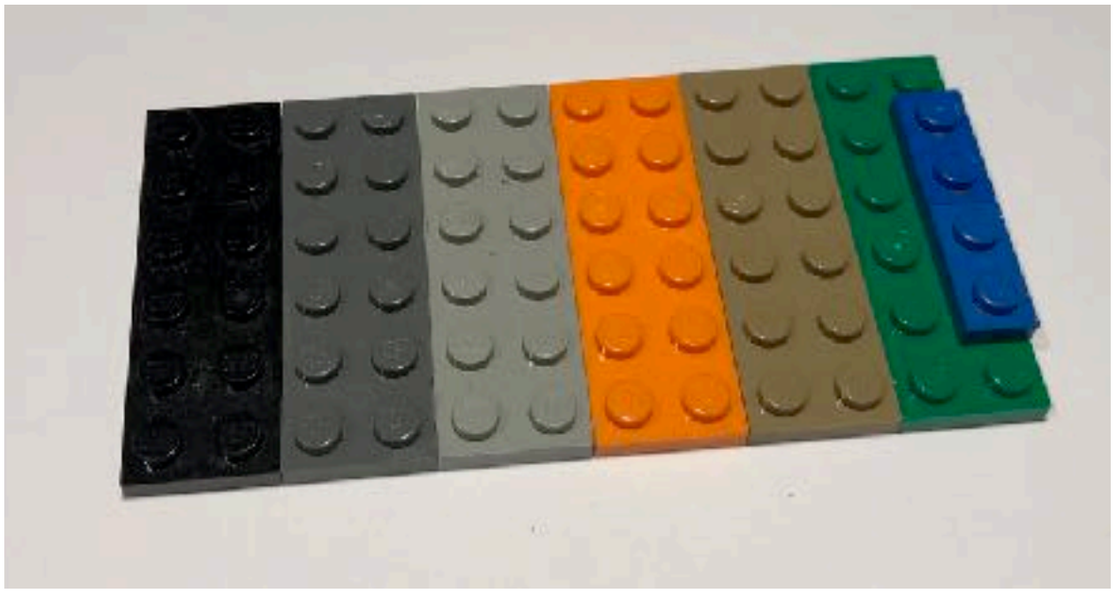


PMID: 25140222

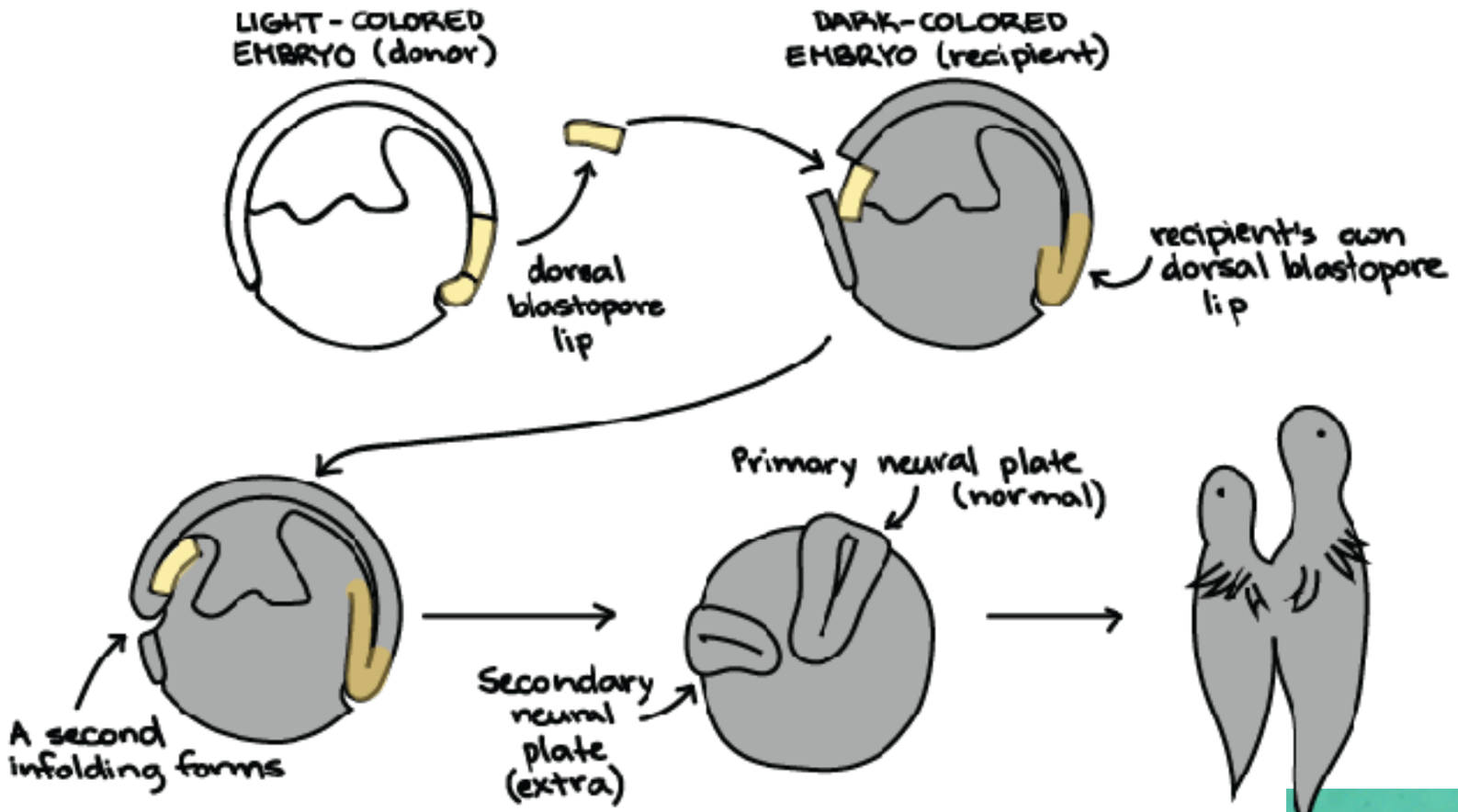
Today's menu - 2 courses

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

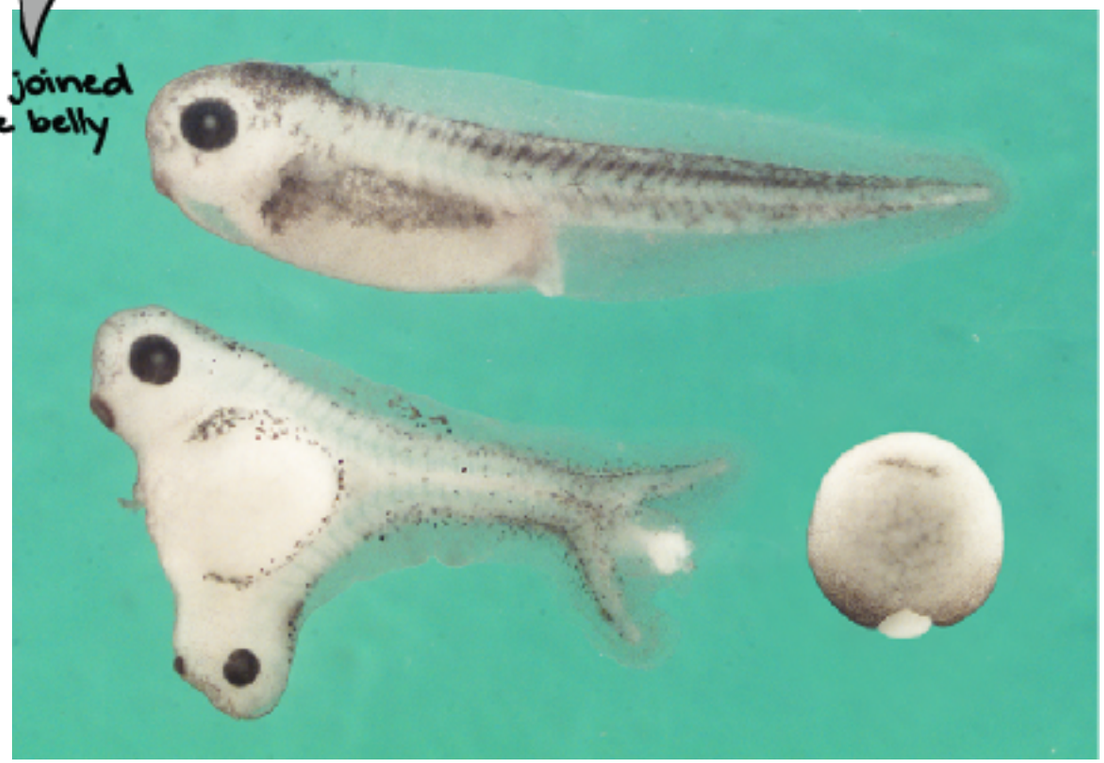
Front - Back / Dorsal - Ventral axis



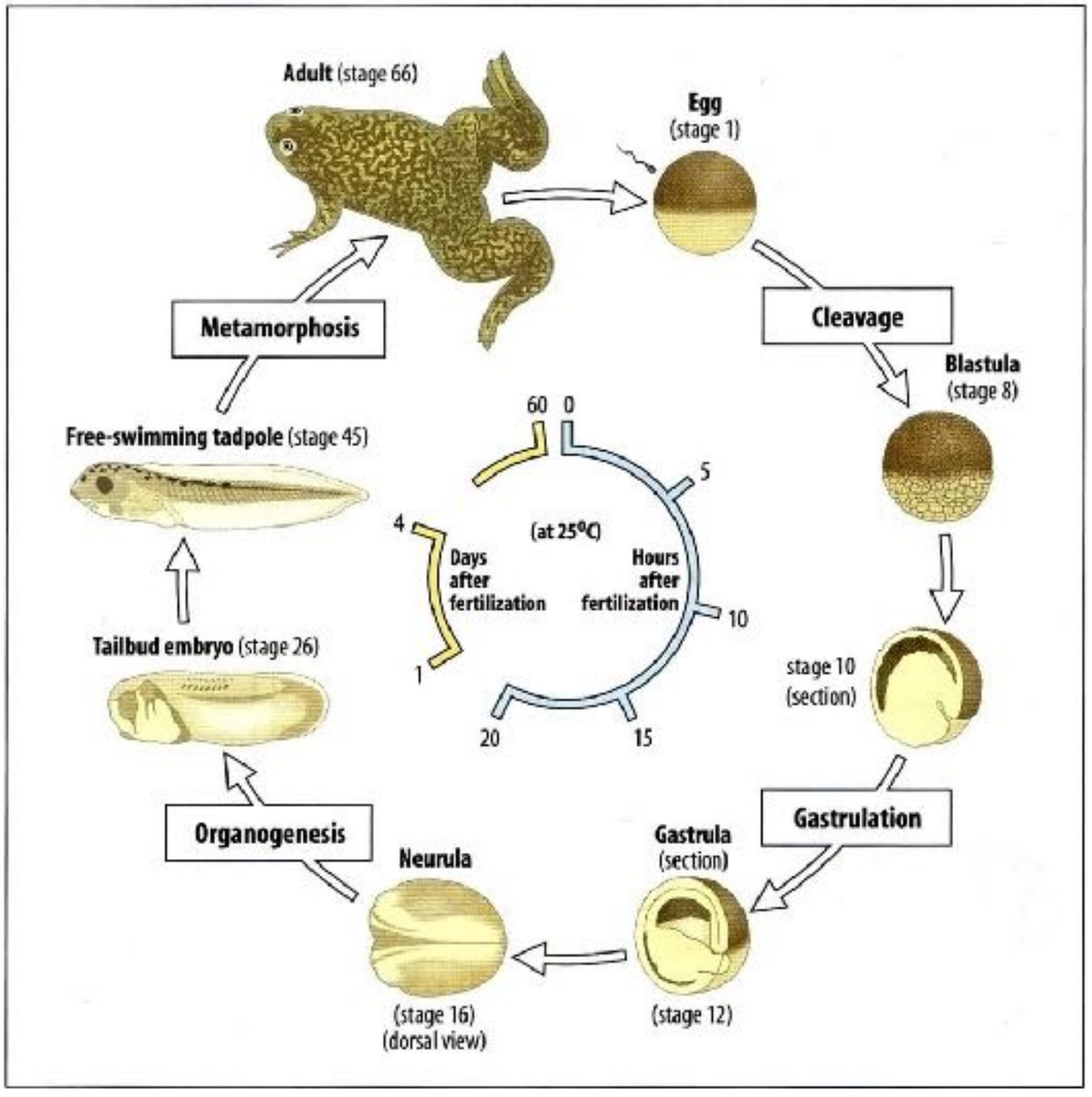
Embryonic Induction: Spemann and Mangold, 1924

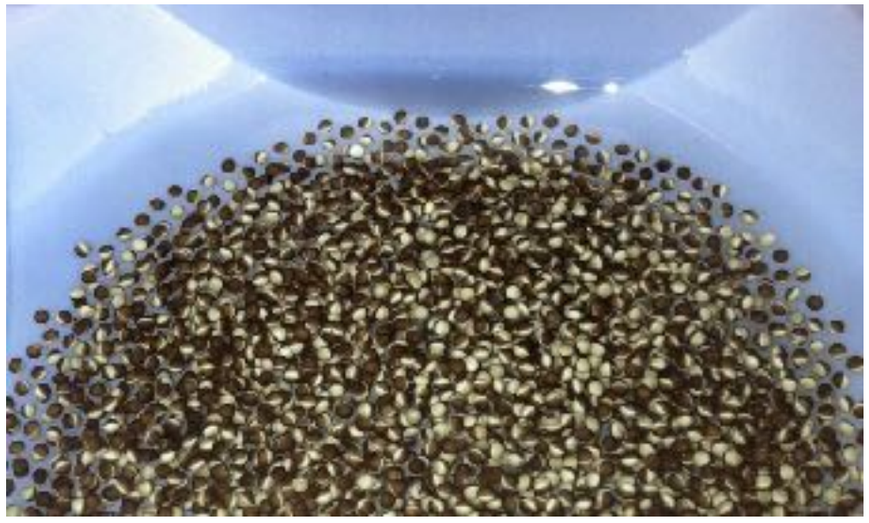


A signal from the light-coloured embryo instructed the dark-coloured embryo's cells to change their behaviour: "organizer" of a new blastopore



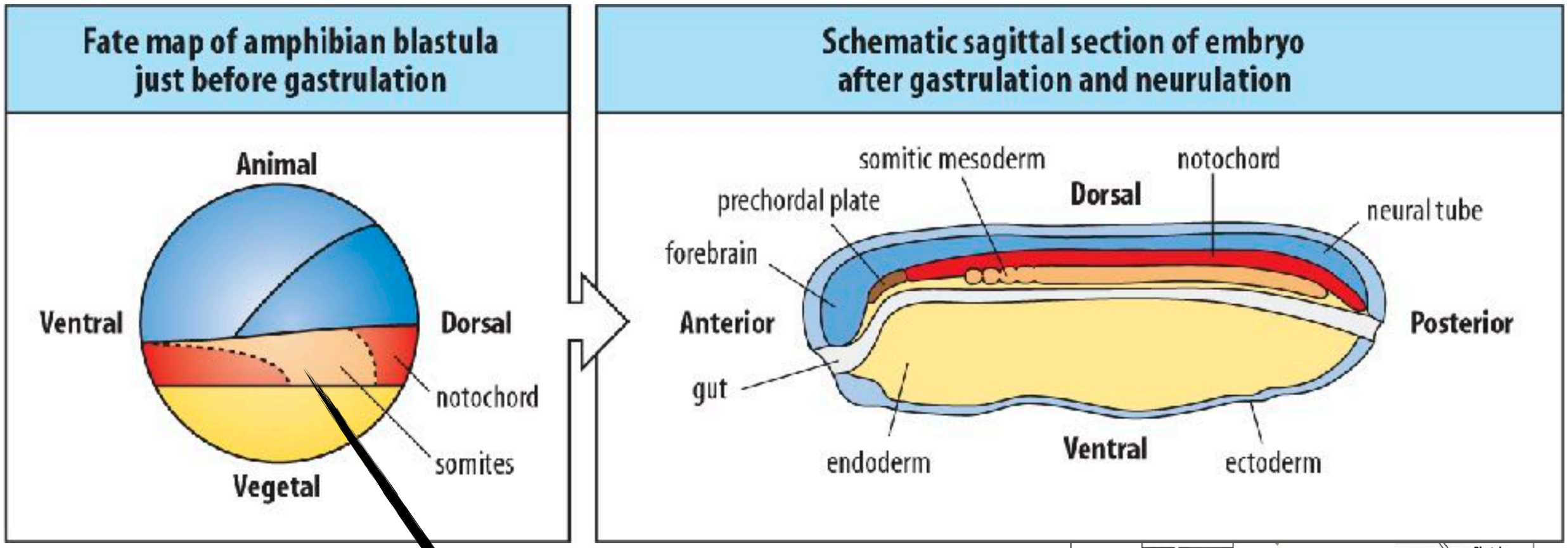
Xenopus laevis - disgusting and useful



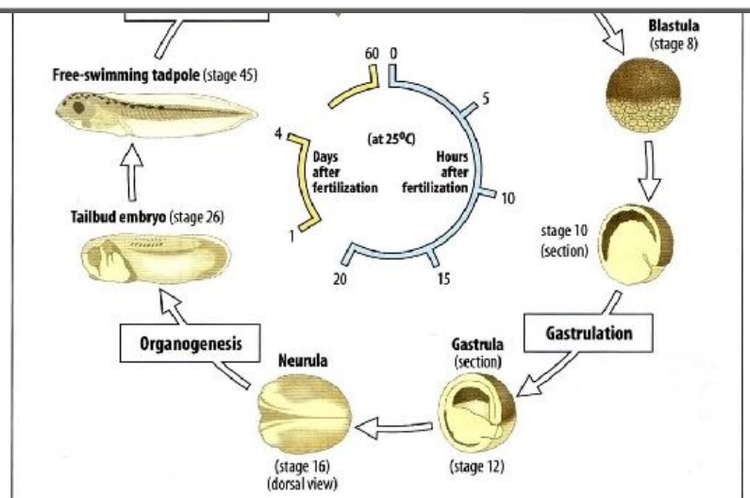


Fate map - the spatial origin of the mature tissues

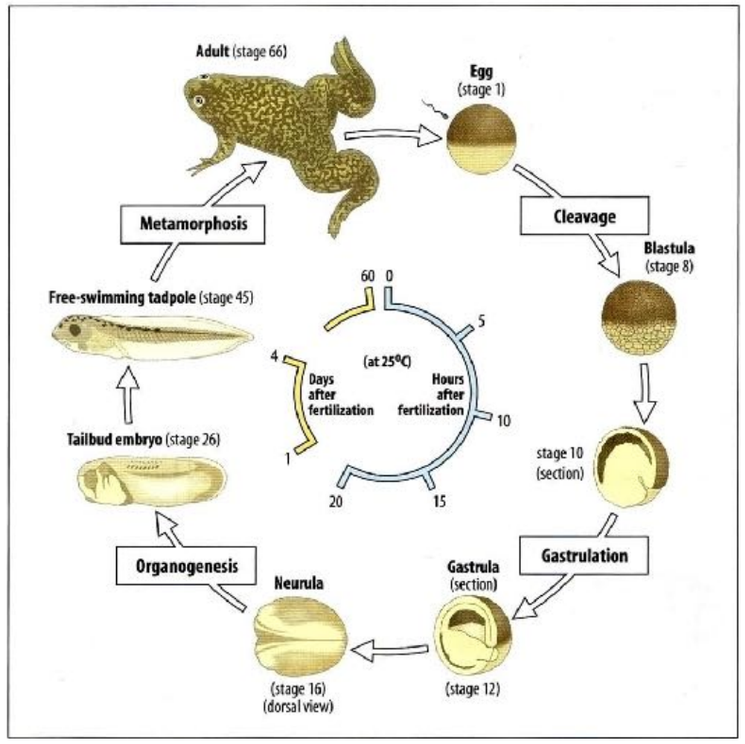
Labelled when? Grown until when?

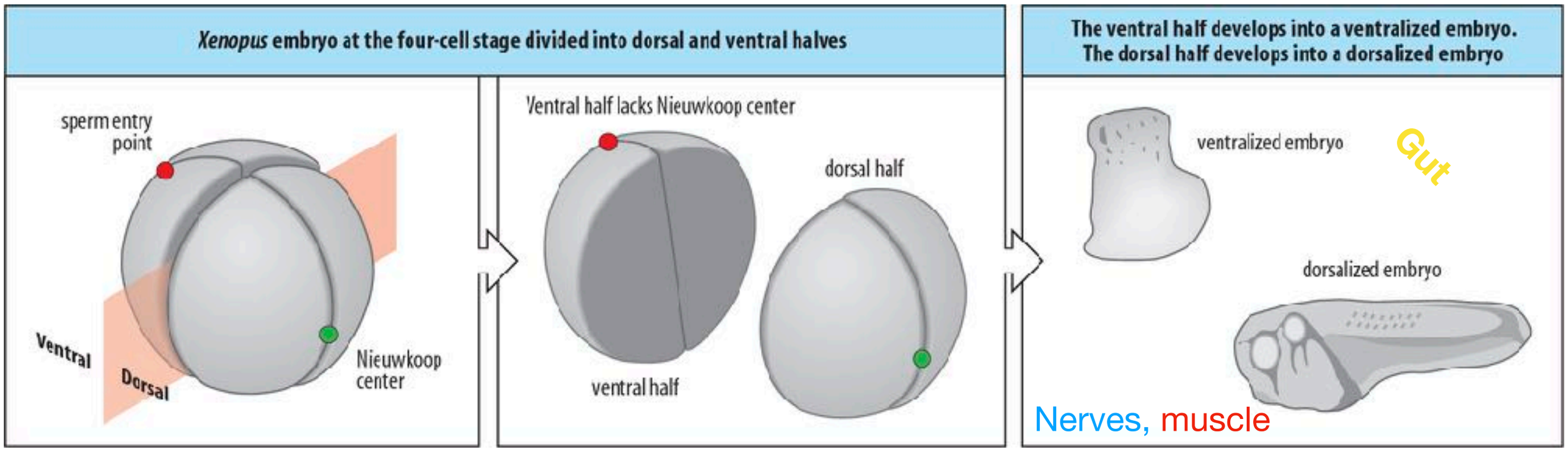


Fluorescent molecule, beads, ink…



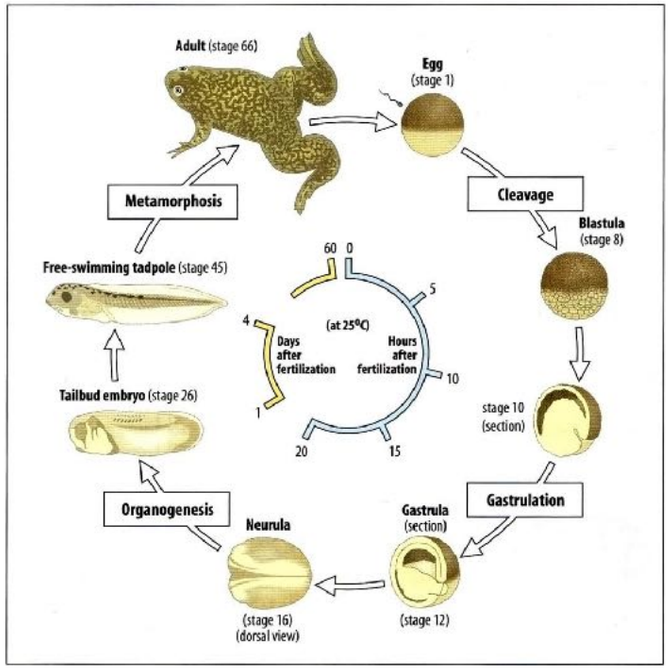
Specification *versus* commitment

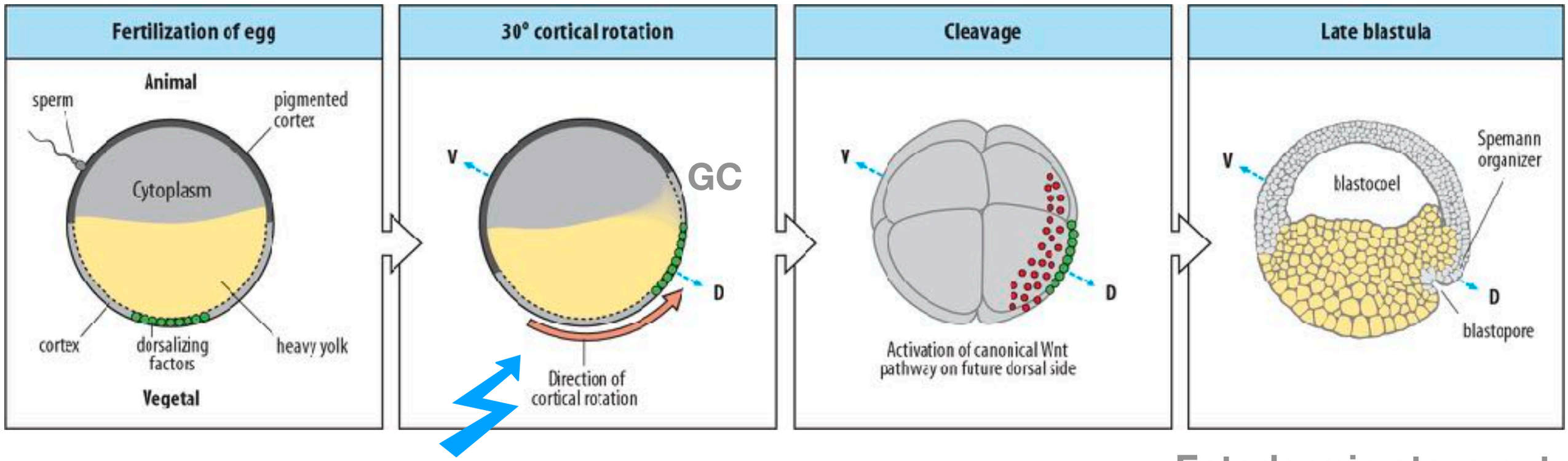




Dorsal and ventral are *specified* early

Radial symmetry broken by movement of *maternal* factors in response to *sperm* entry point





UV ⊣ MTs

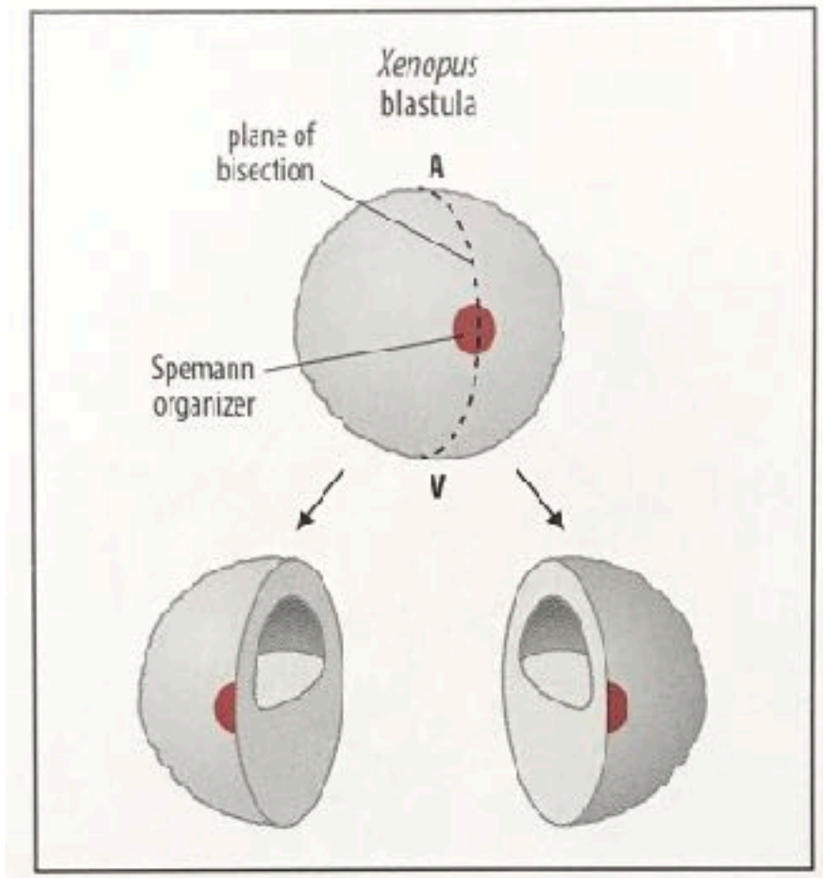
Dkk ⊣ Wnt

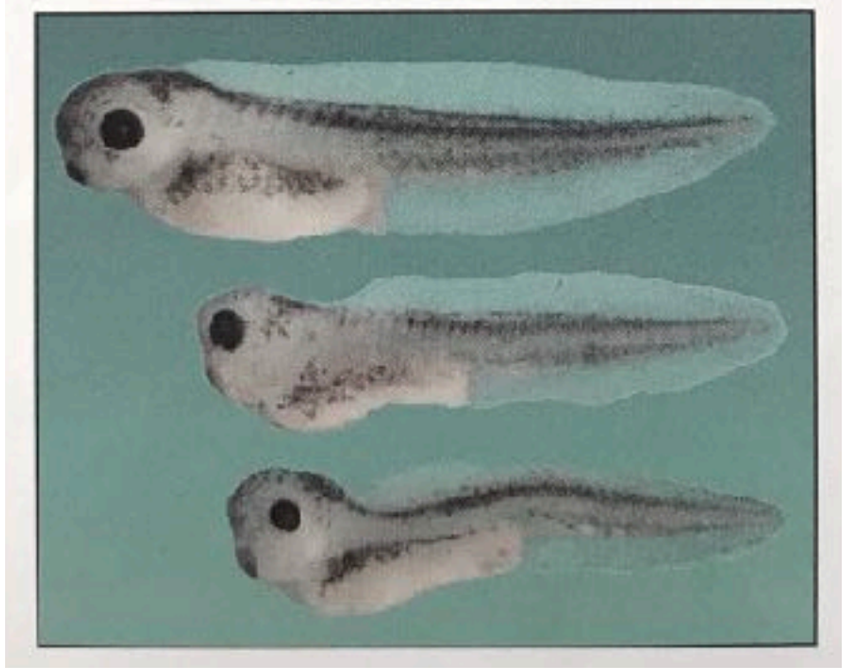
Ectodermin stays put

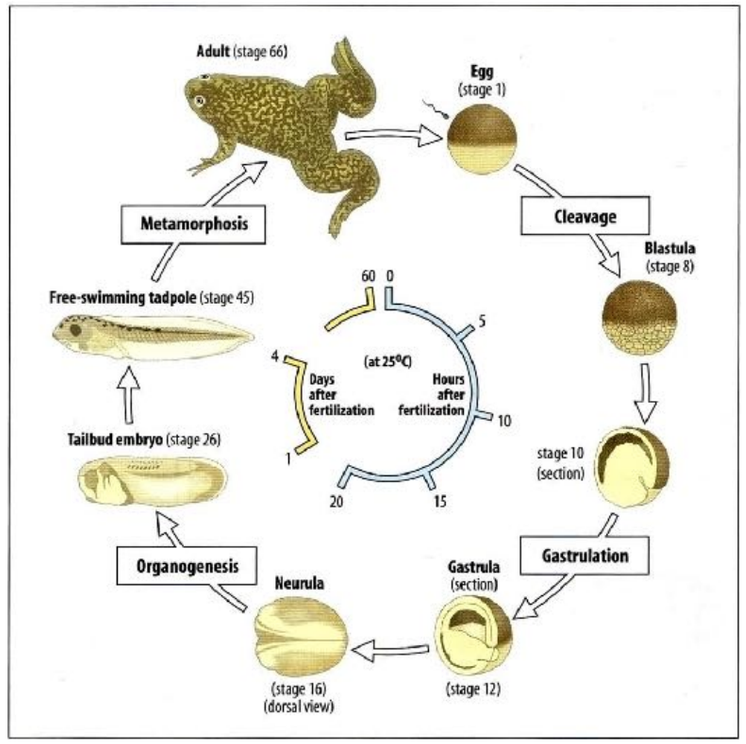
Vg1, VegT stay put

Ectoderm and endoderm are specified by maternal factors

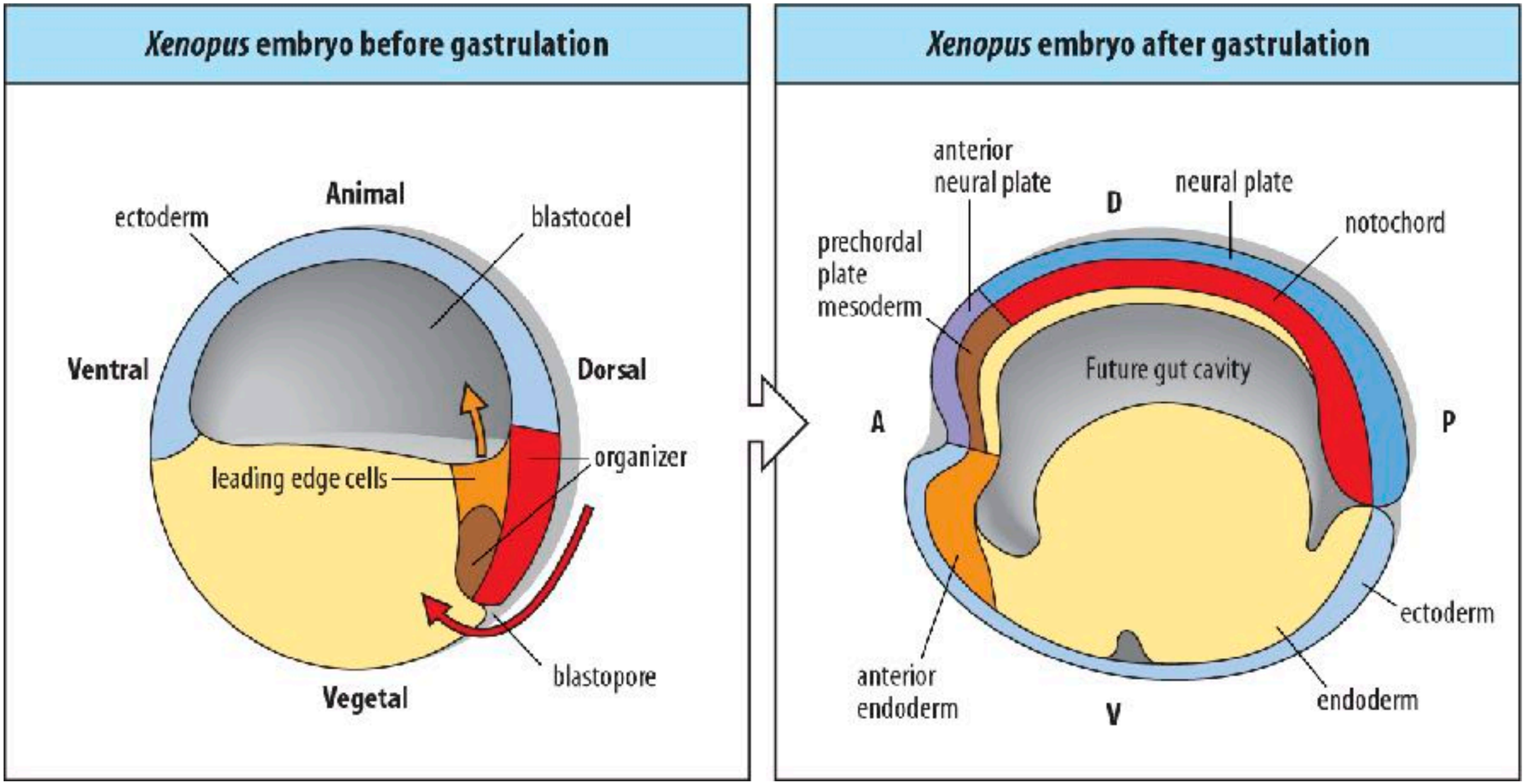
Xenopus embryos *can* regulate their patterns; cells are not yet *committed* before gastrulation







Gastrulation - organisation of the three “germ” layers



Nerves, skin

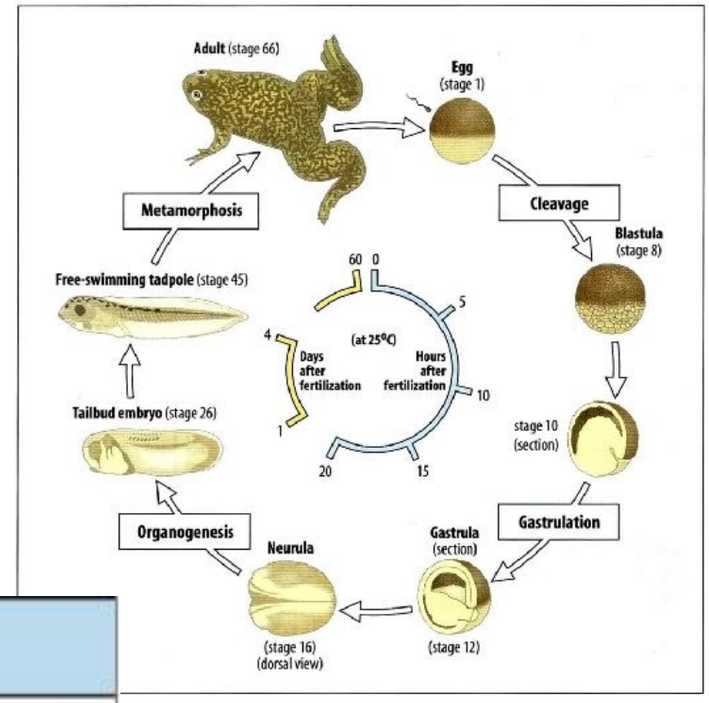
Notochord

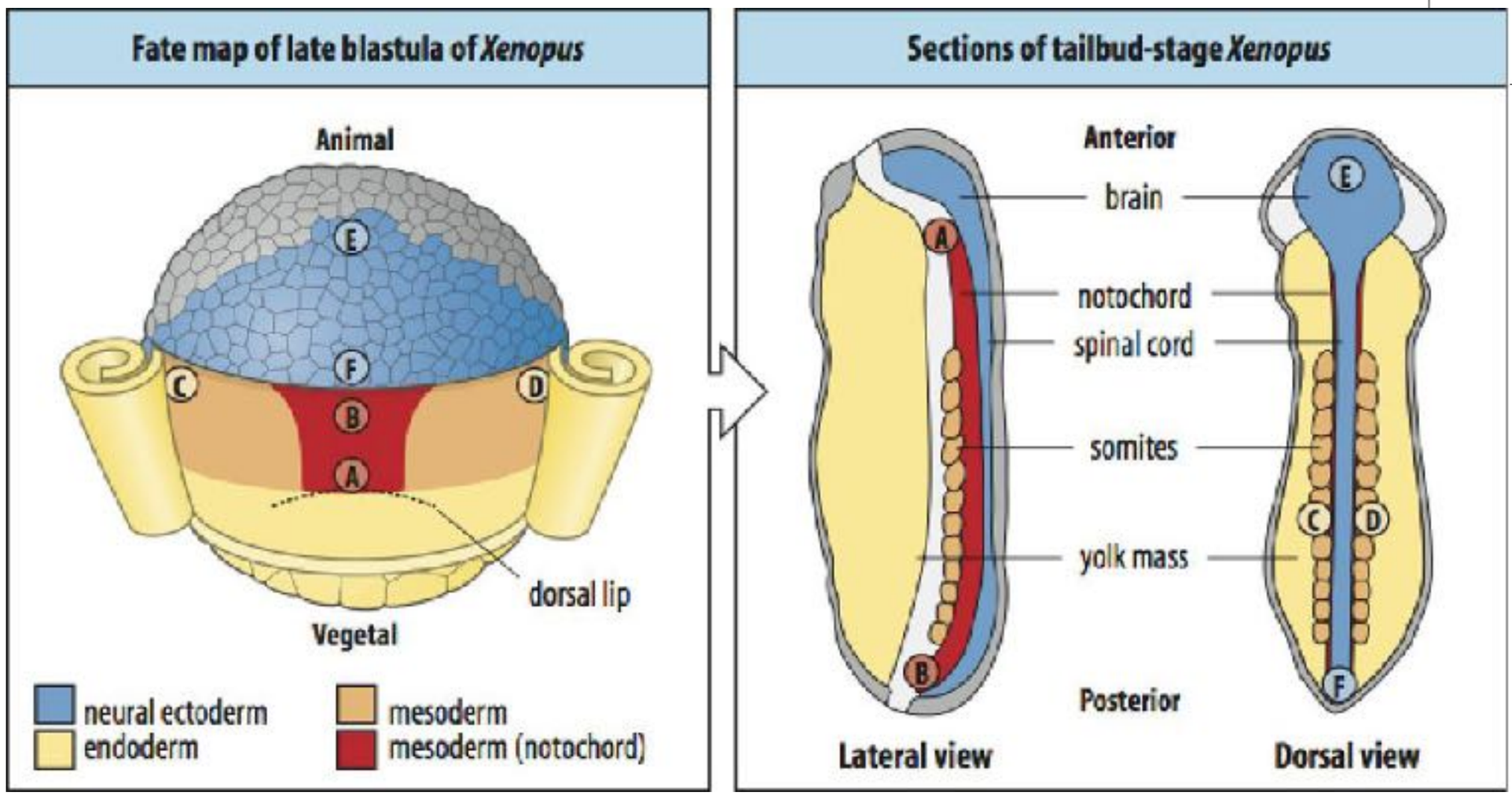
Gut

Gut and mesoderm move inside
Dorsal flows into Anterior

Wolpert, Principles of Development

Gastrulation - patterning along AP *and* DV axes

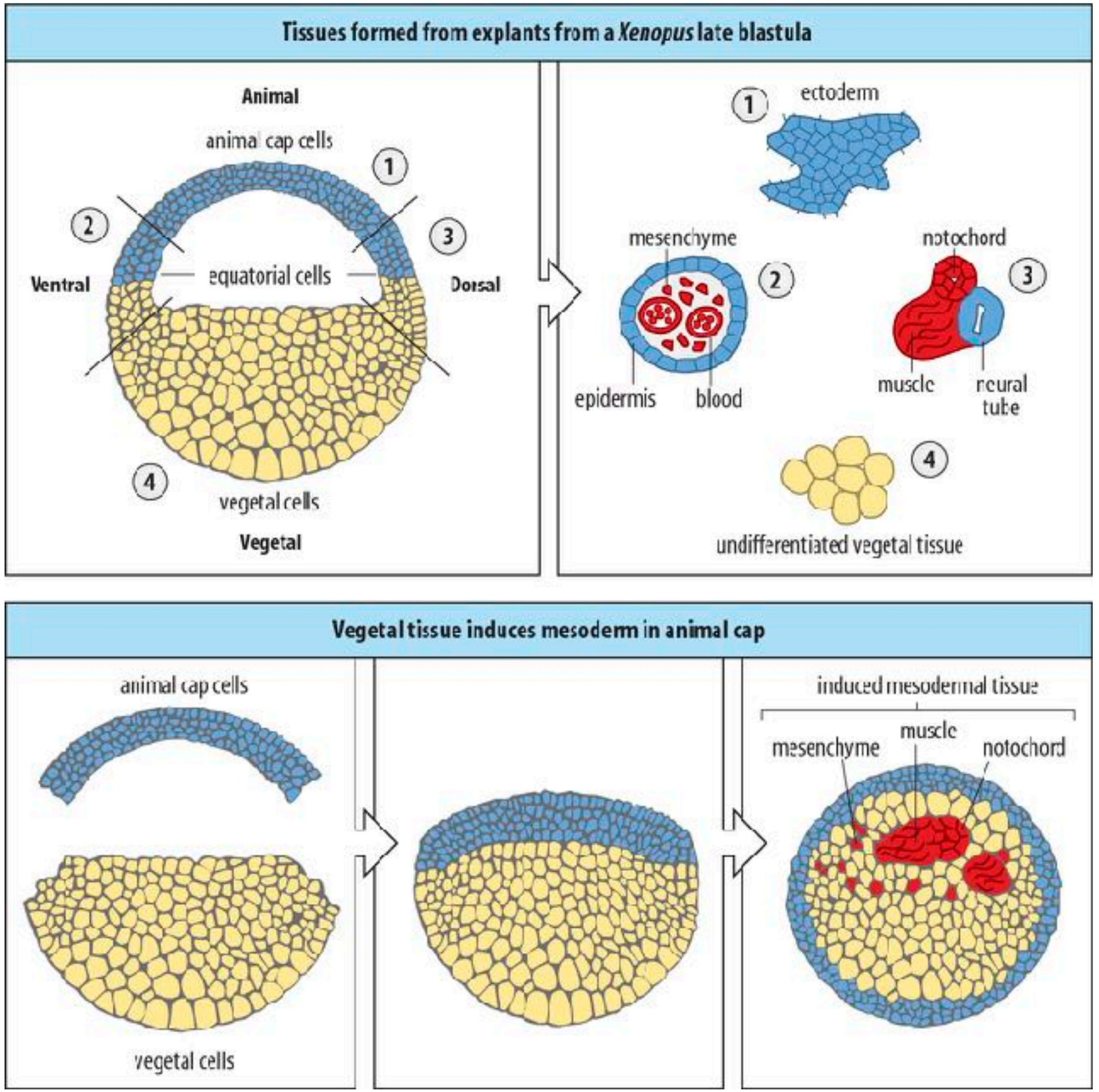


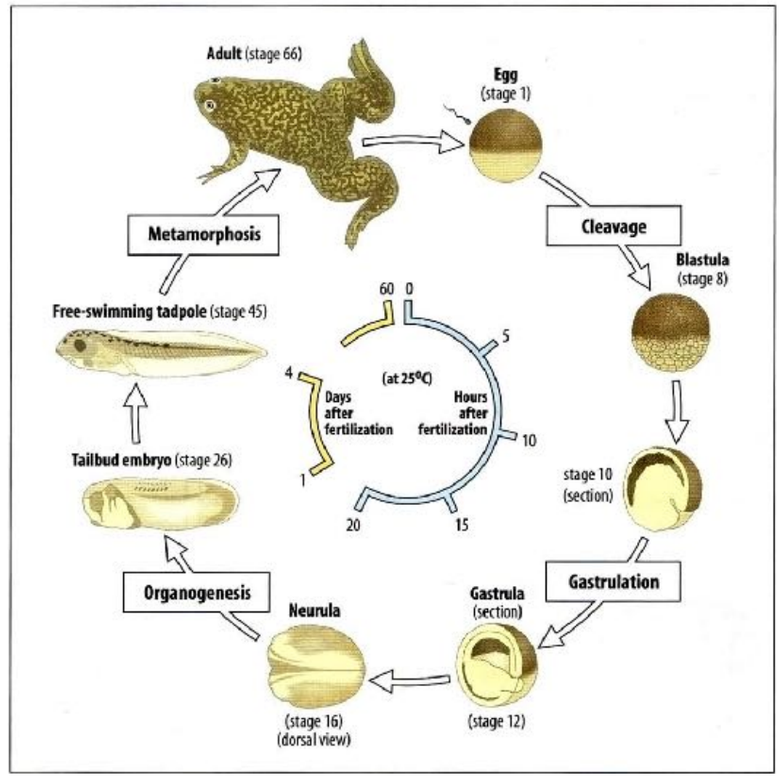


Dorsal flows into Anterior = not independent axes

Wolpert, Principles of Development

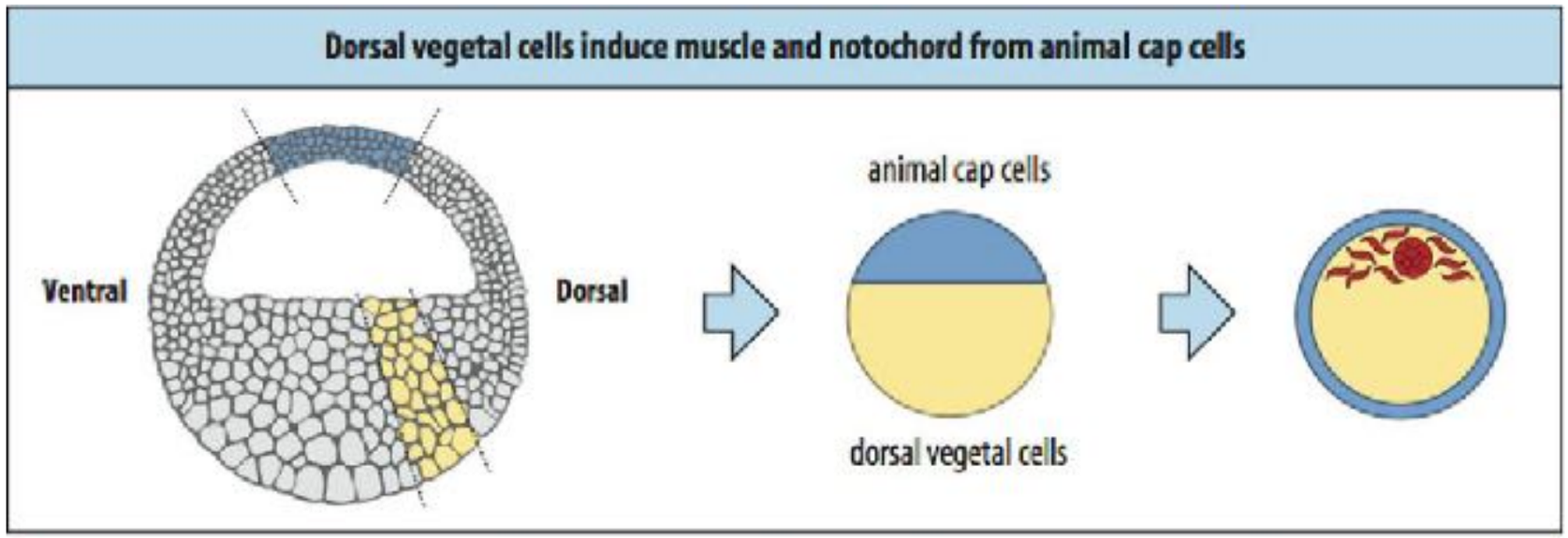
Animal caps - a model for inductive interactions





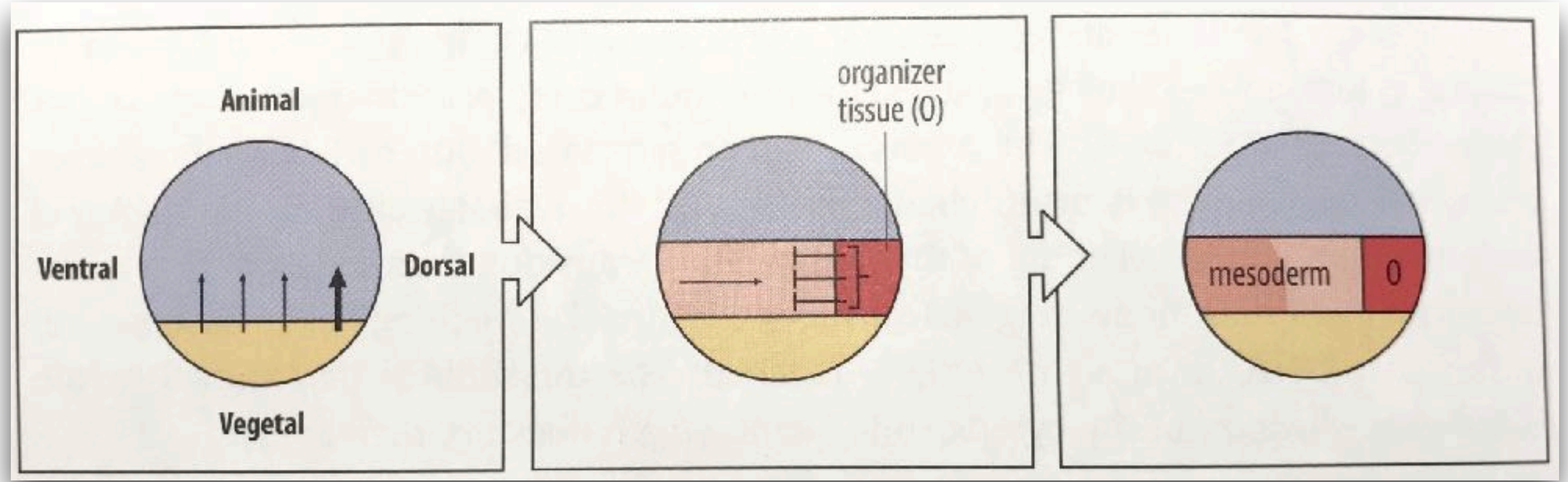
Wolpert, Principles of Development

Different vegetal cells induce different mesoderm



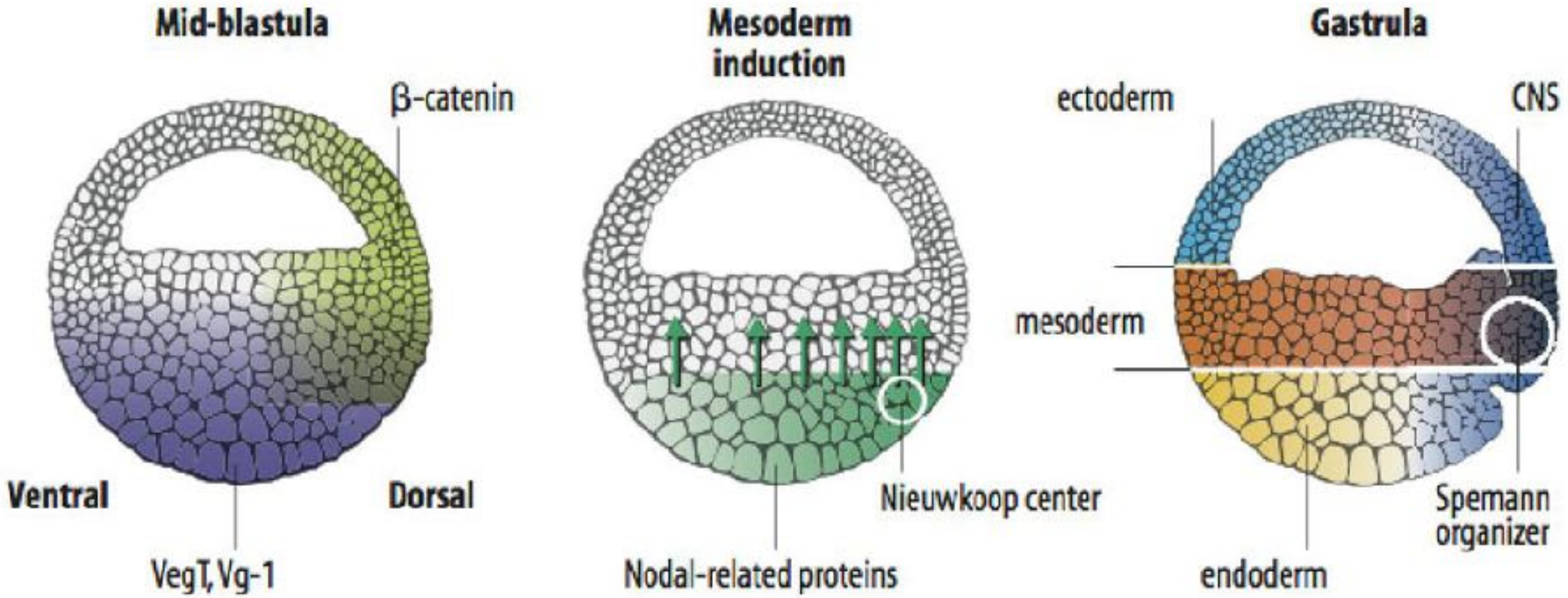
Dorsal - Ventral signals (from 4-cell experiment) are now spread across the embryo?

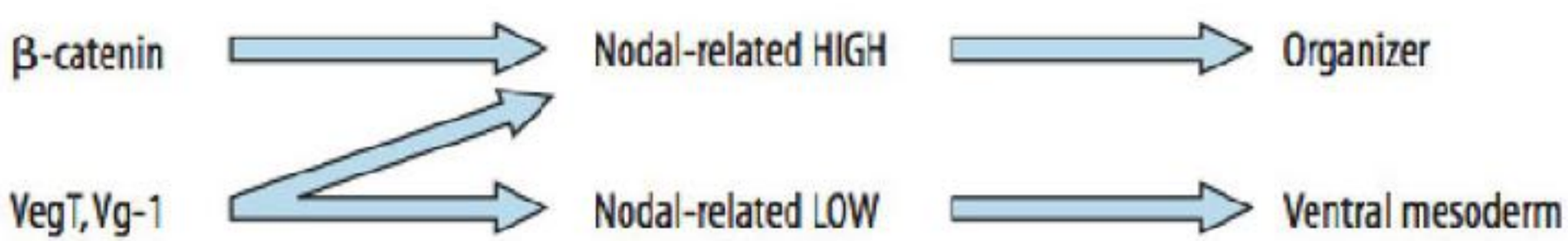
How is germ layer induction coordinated with patterning?



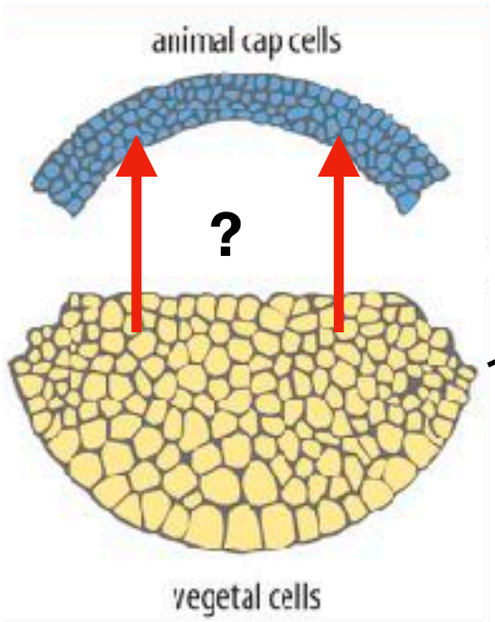
One signal? Many? Simultaneous or sequential?

Where we are going…

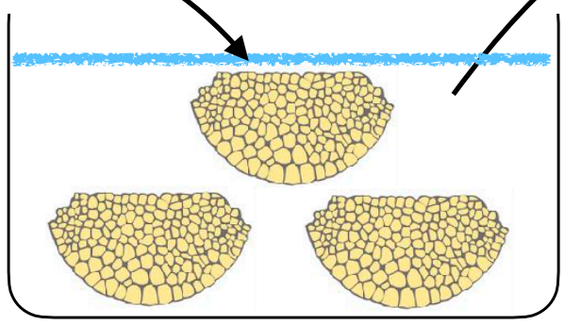


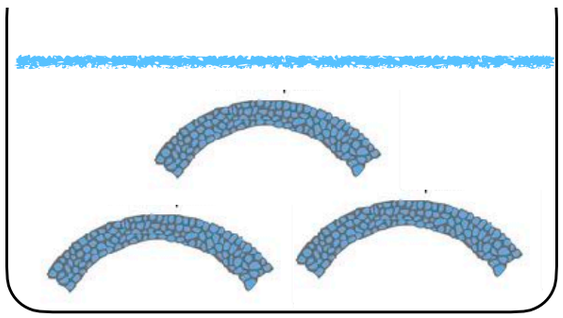


A mesoderm-inducing factor (Activin/Nodal-related)

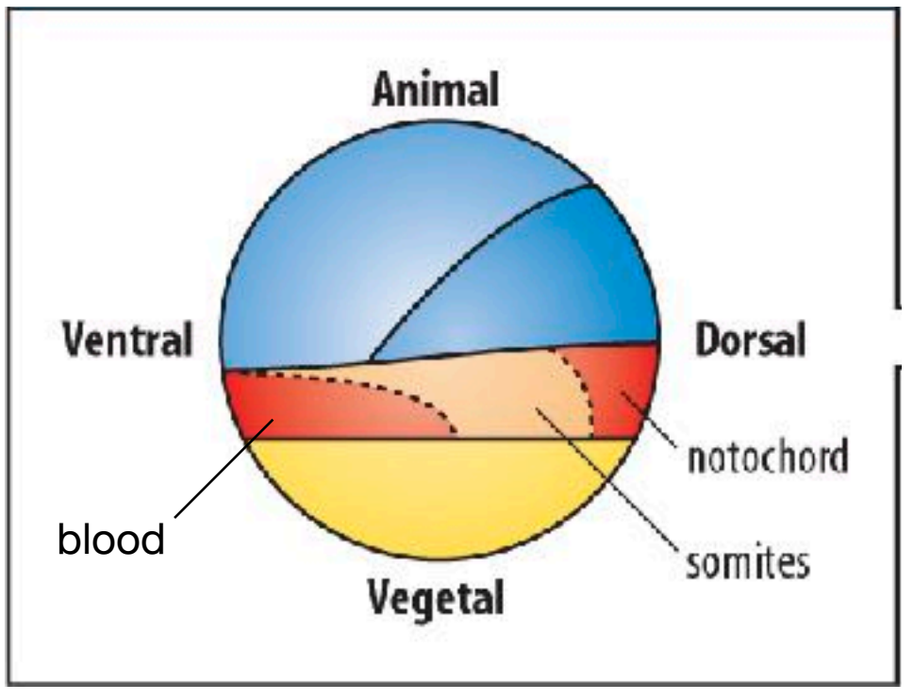


Supernatant





Soluble activity (proteins) could be purified from the supernatant and cell lines



Blood, **muscle**, **notochord**

Dose dependence…

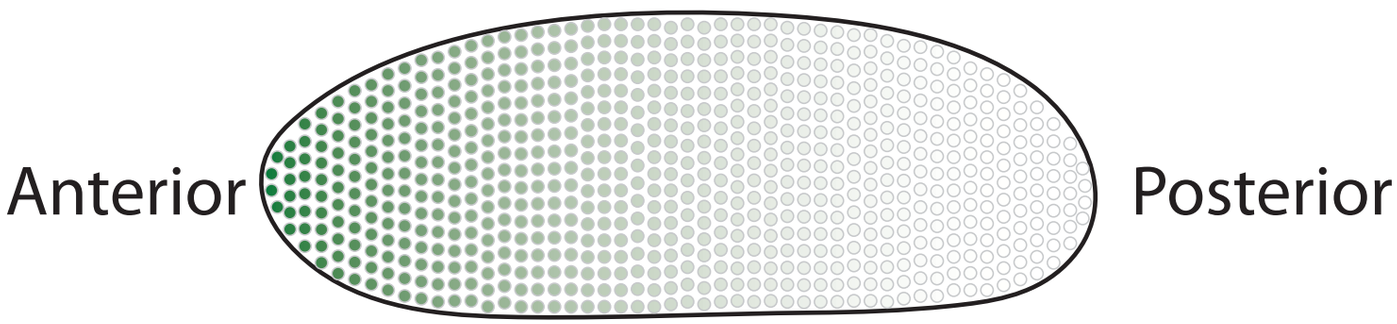
Activin - a morphogen?

Activin: expressed in the vegetal cells, and induces several mesoderm cell-types

Bicoid patterns the fly embryo as a morphogen

Early *Drosophila* embryo

Bicoid protein gradient



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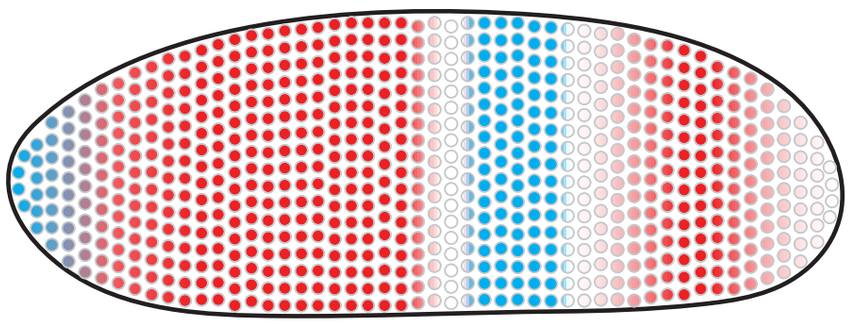
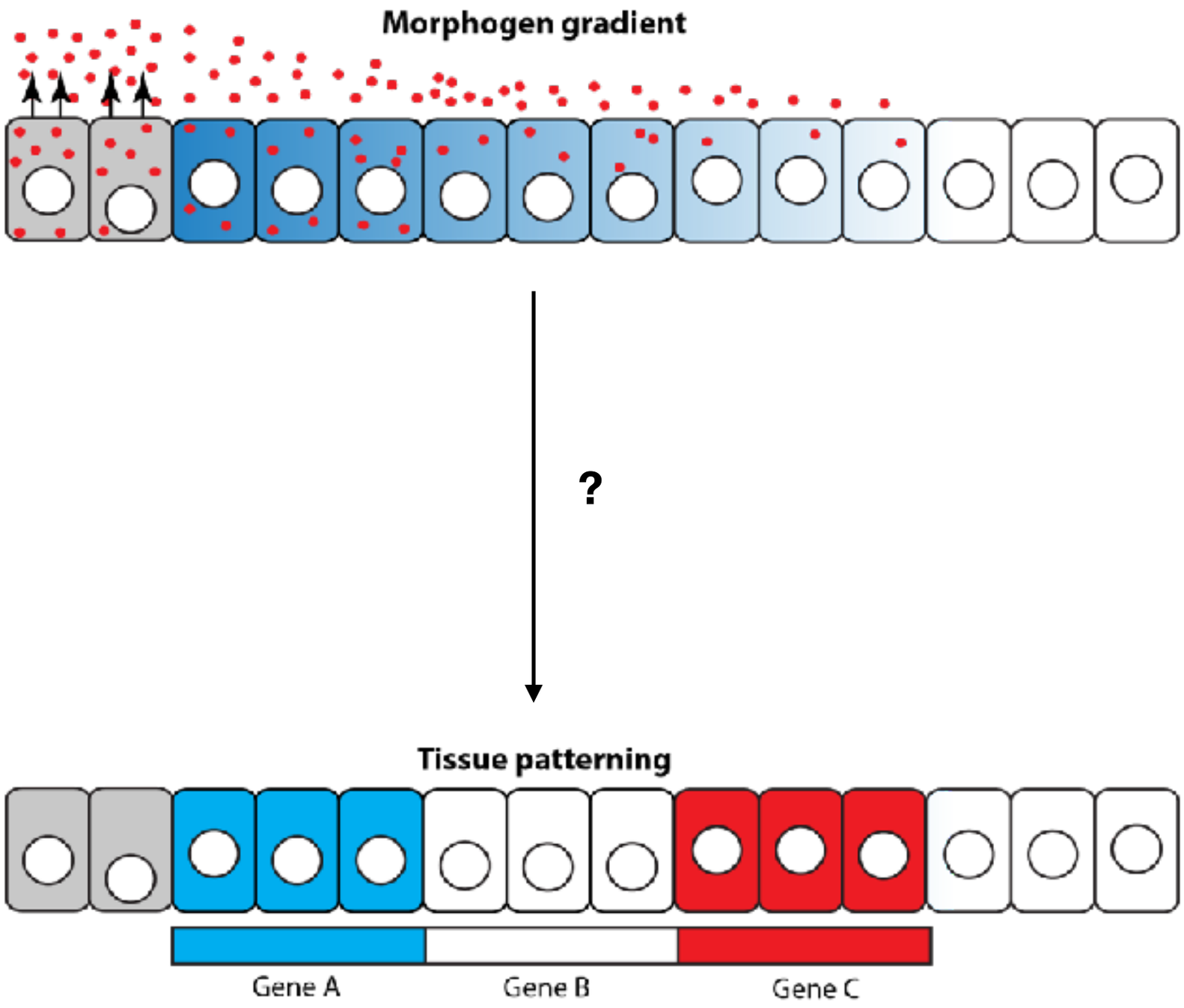
Hunchback and Knirps protein domains



Morelli et al., *Science* 2012.

The French Flag model (classical)



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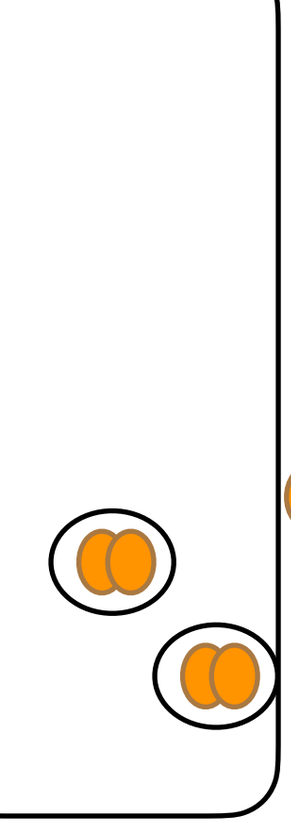
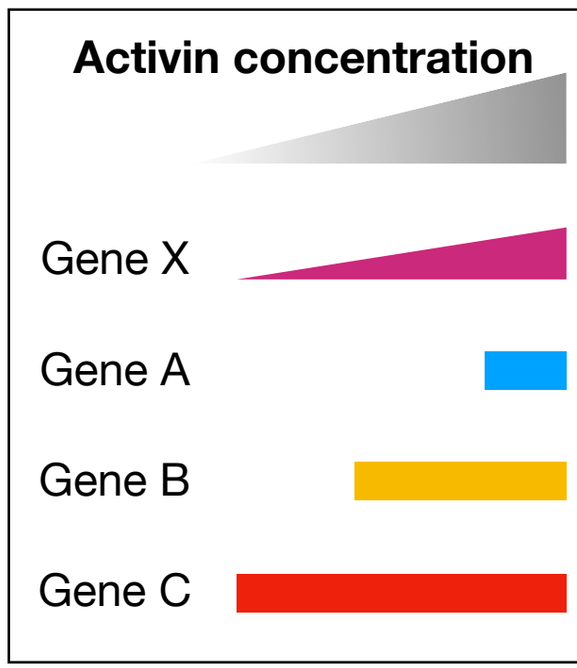
Signal transduction - Activin (TGF-beta family)

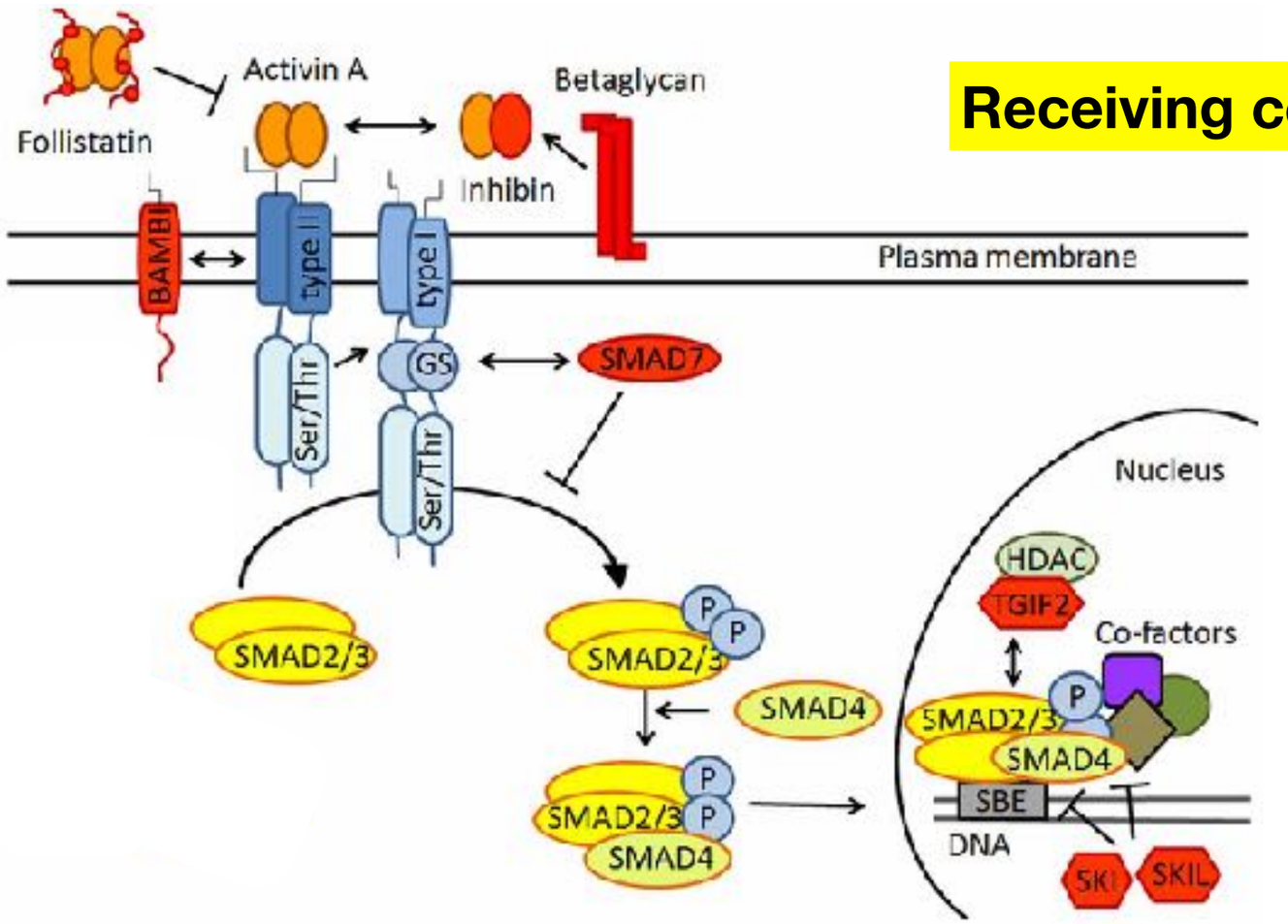
Signals from one cell can cause dose-dependent changes in gene expression in another cell

Sending cell



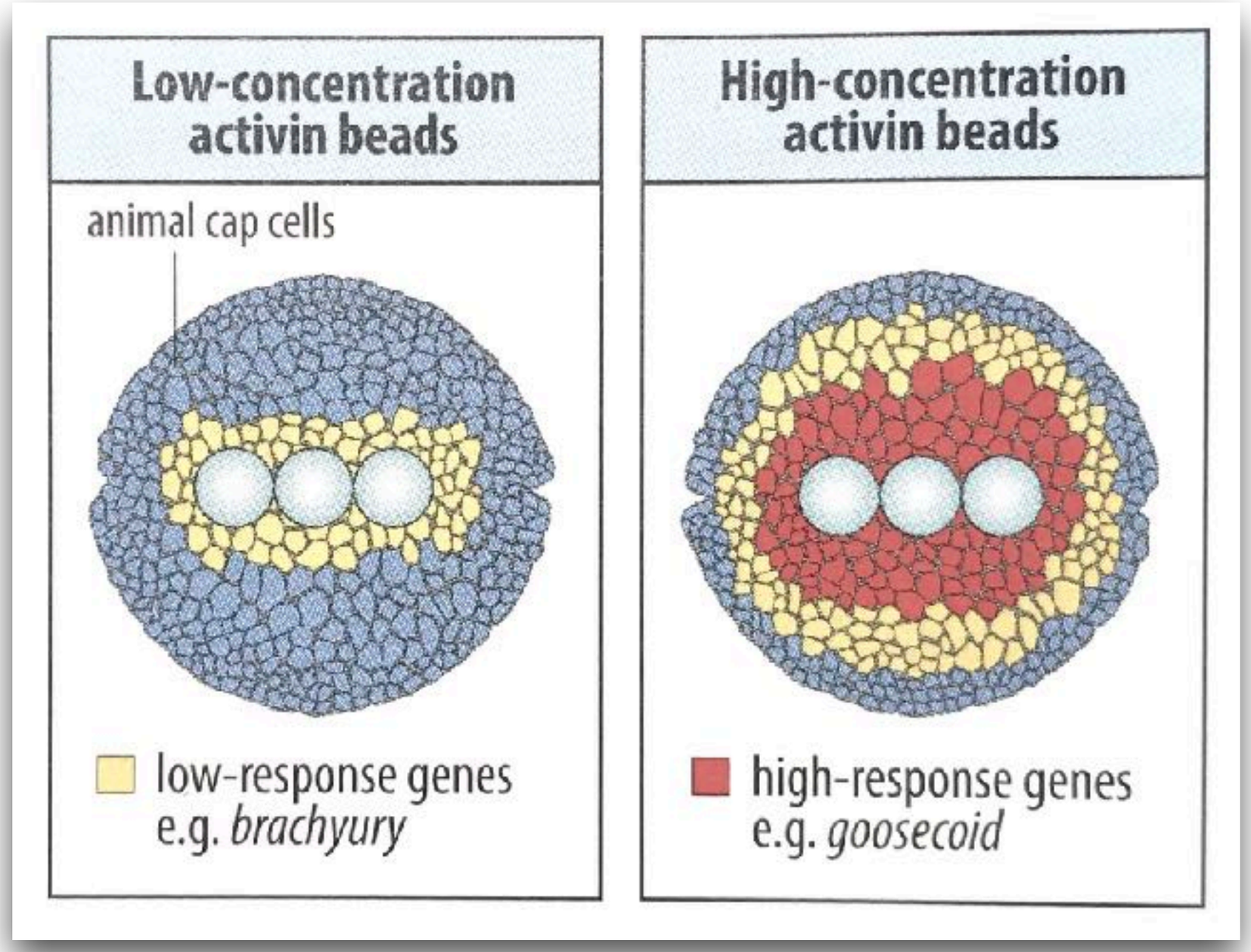


Receiving cell

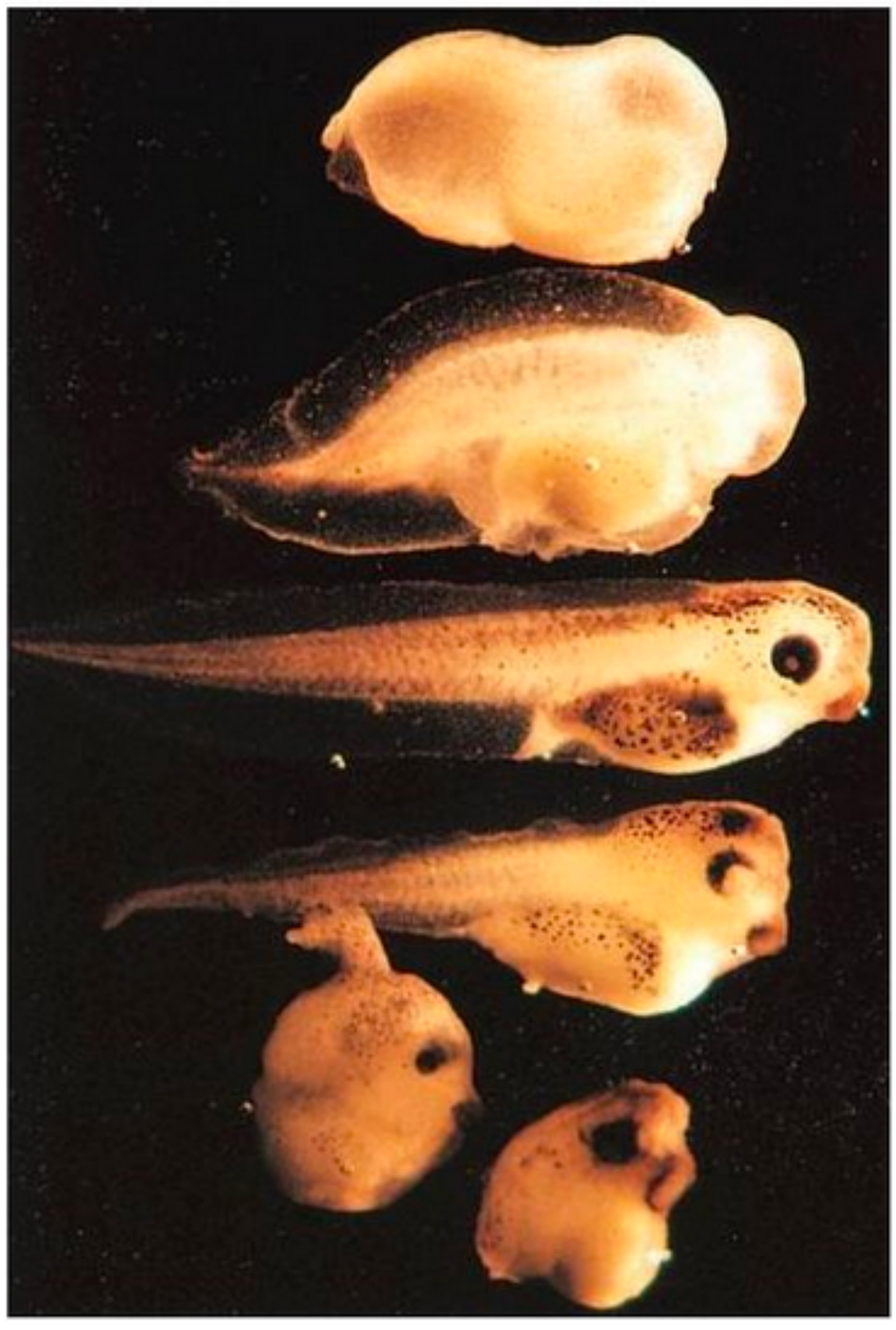


Link, Zheng, Alzheimer, 2016

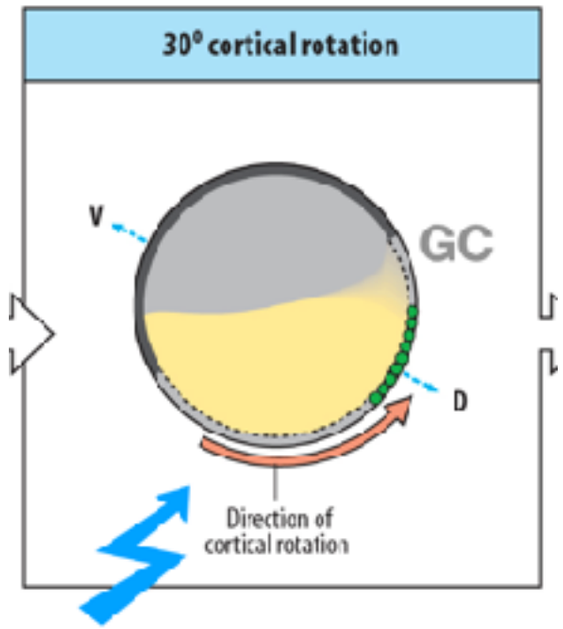
Activin /Nodal-related can act as a morphogen



UV rescue with Noggin mRNA



UV irradiated



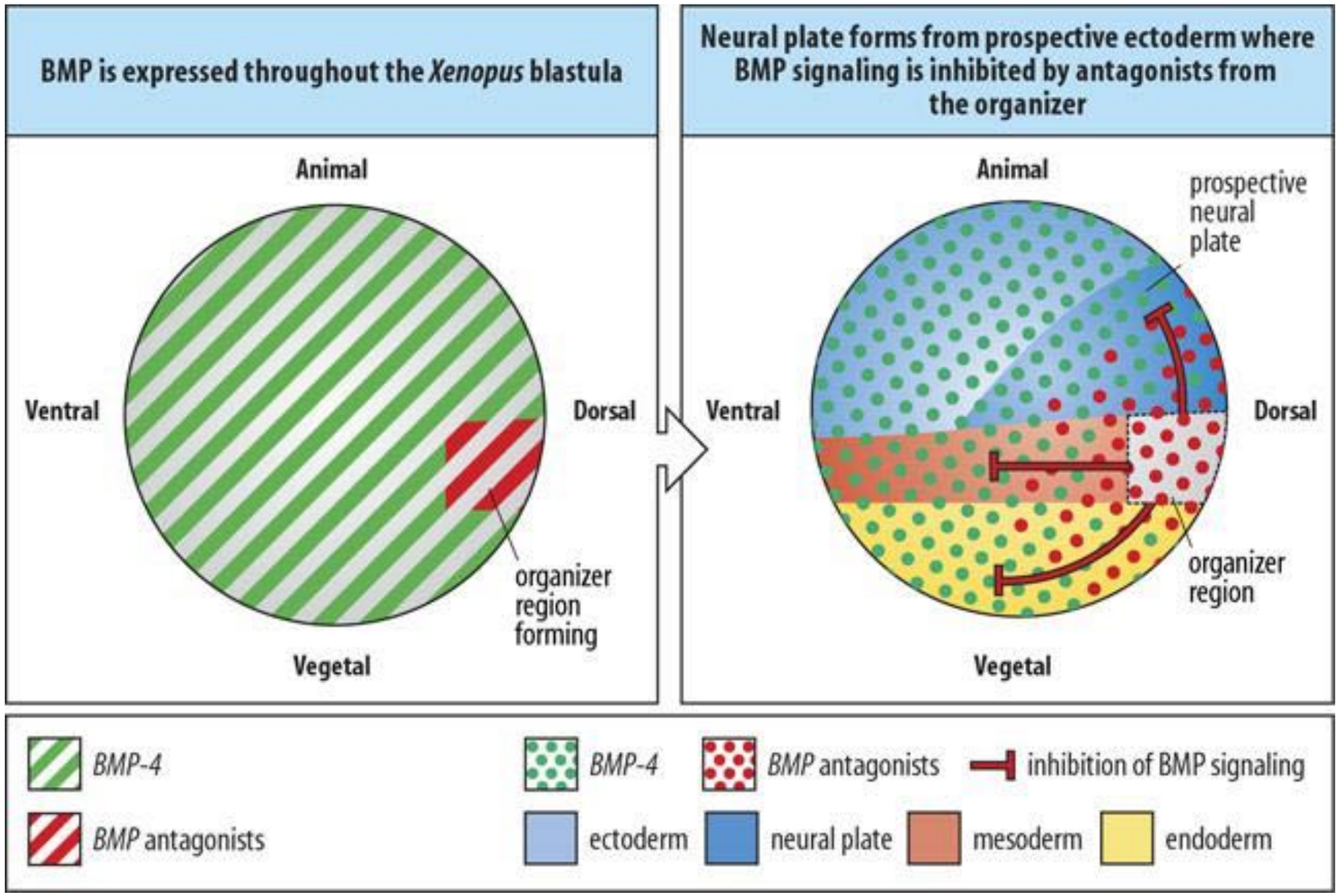
UV ⊣ MTs

Noggin mRNA injection

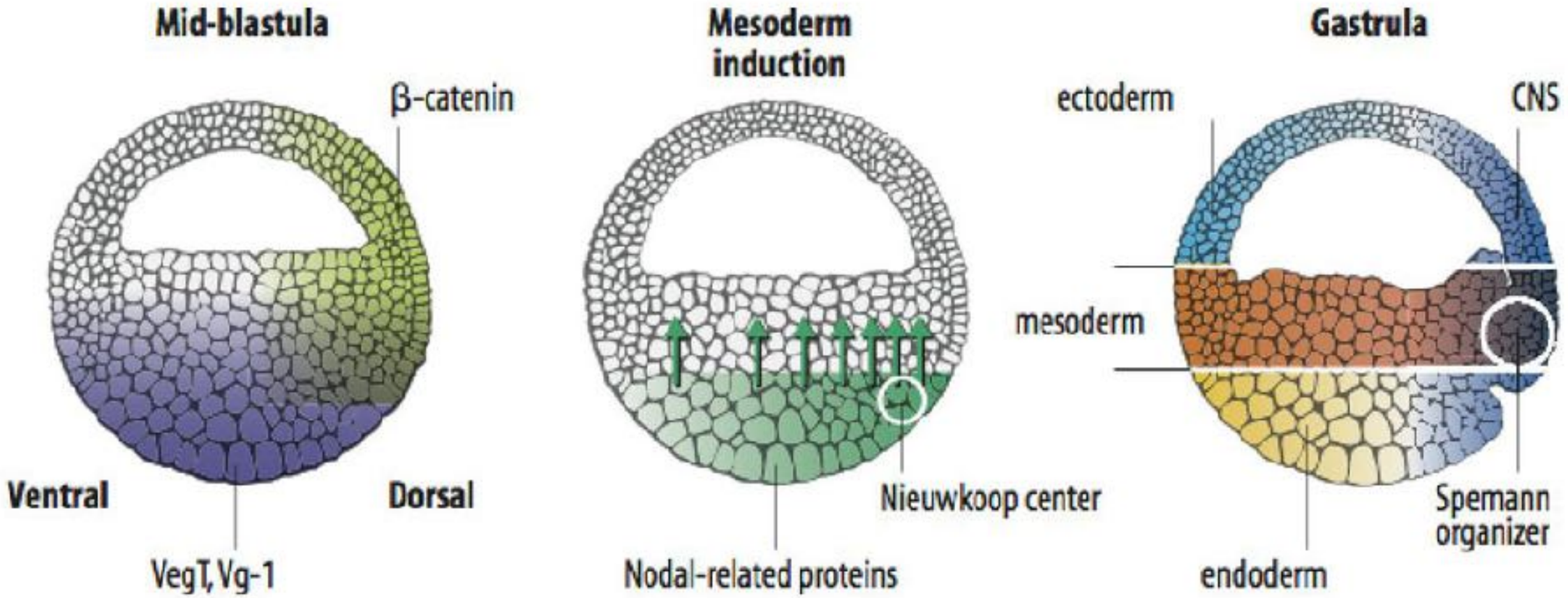
Harland et al.,

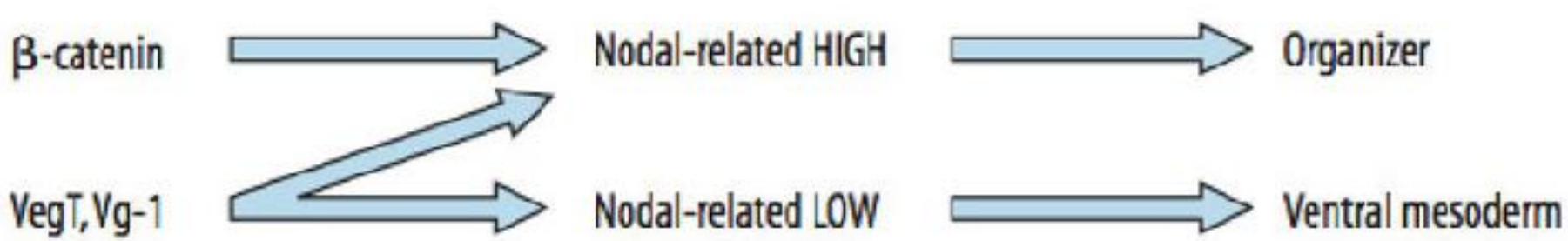
Spemann's Organizer's main role - to secrete BMP *antagonists*

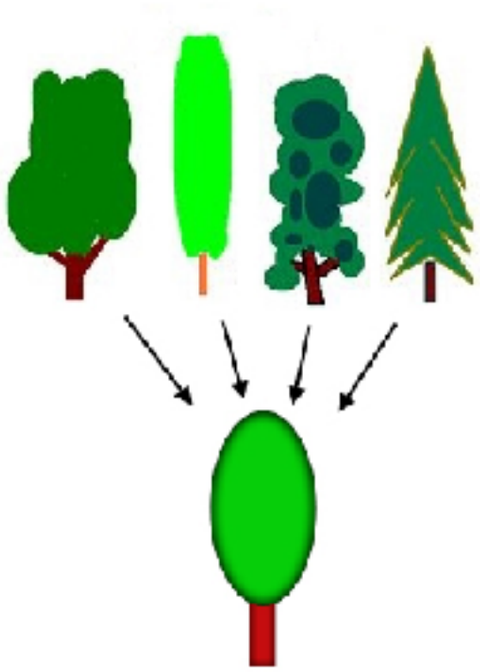
Noggin, Follistatin, Chordin
Do not induce mesoderm



Summary

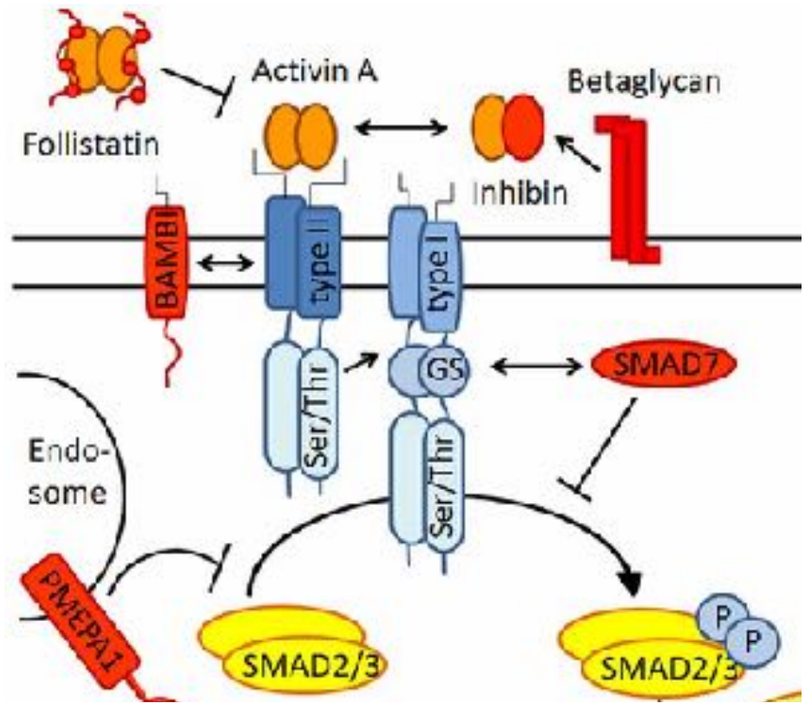

Mid-blastula
β-catenin
Ventral
Dorsal
VegT, Vg-1
Mesoderm induction
Nieuwkoop center
Nodal-related proteins
Gastrula
ectoderm
CNS
mesoderm
Spemann organizer
endoderm

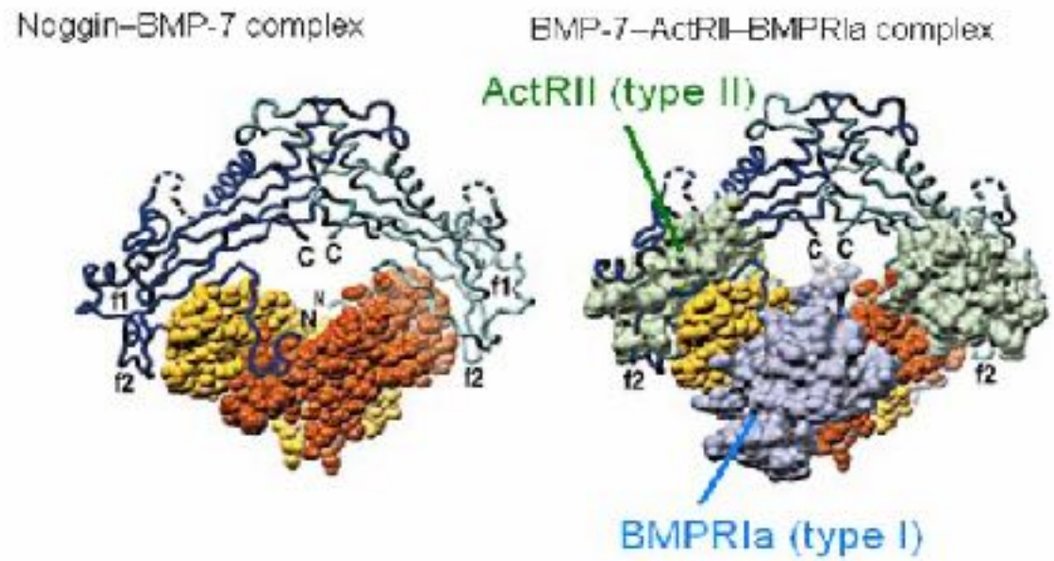

β-catenin
Nodal-related HIGH
Organizer
VegT, Vg-1
Nodal-related LOW
Ventral mesoderm



Opposing gradients

ligand and ligand-sequestration mechanism to limit range and sharpen boundaries



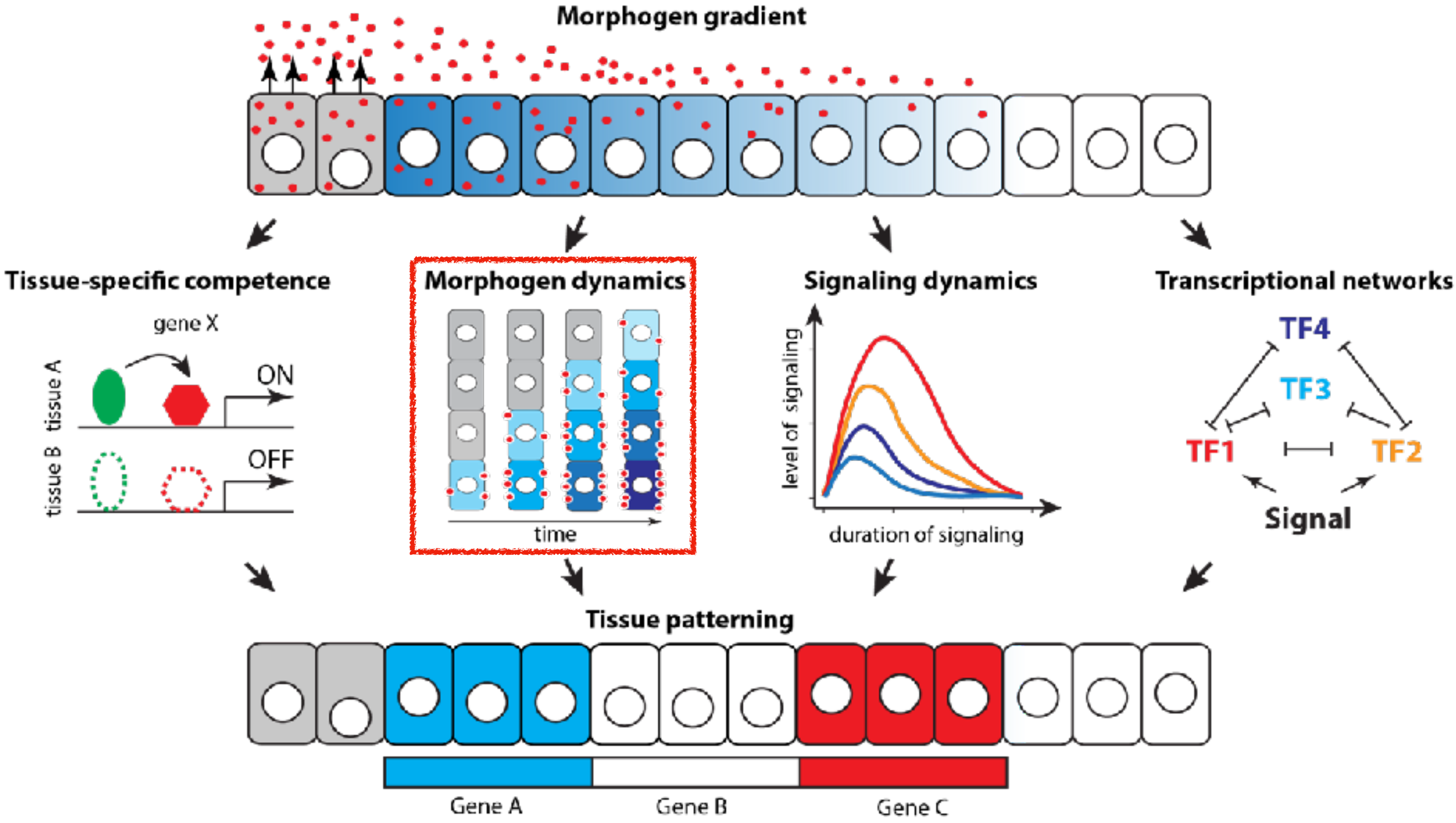


Are these gradients sufficiently precise to account for tissue distributions?

What mechanisms are involved?

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

The French Flag model (modern version)



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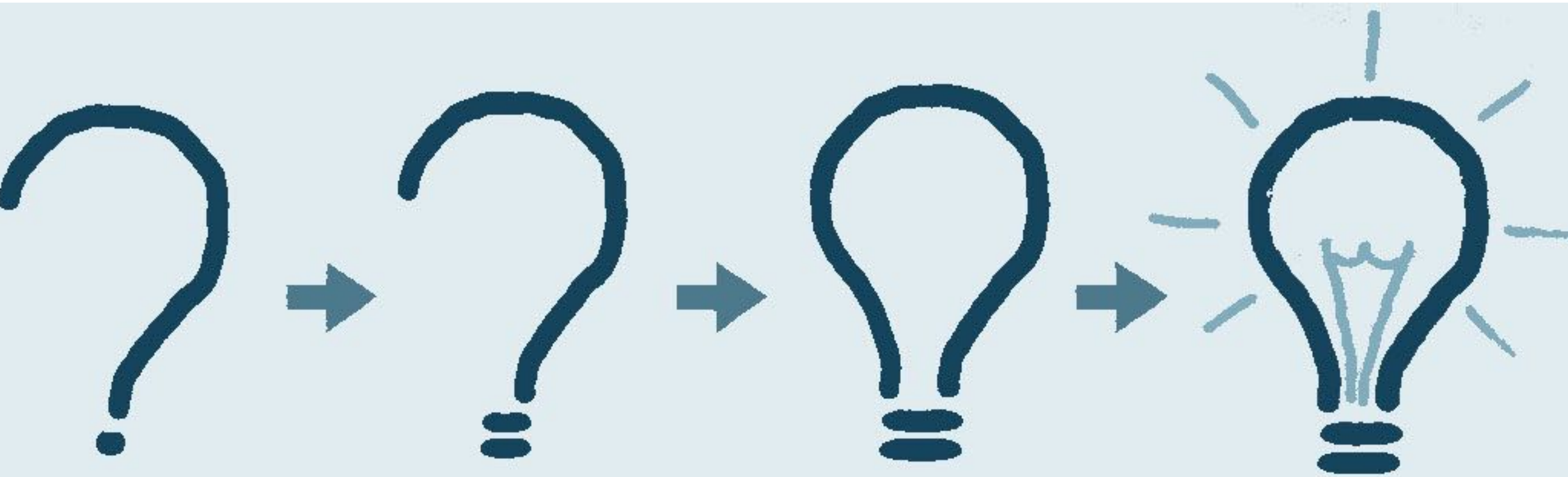
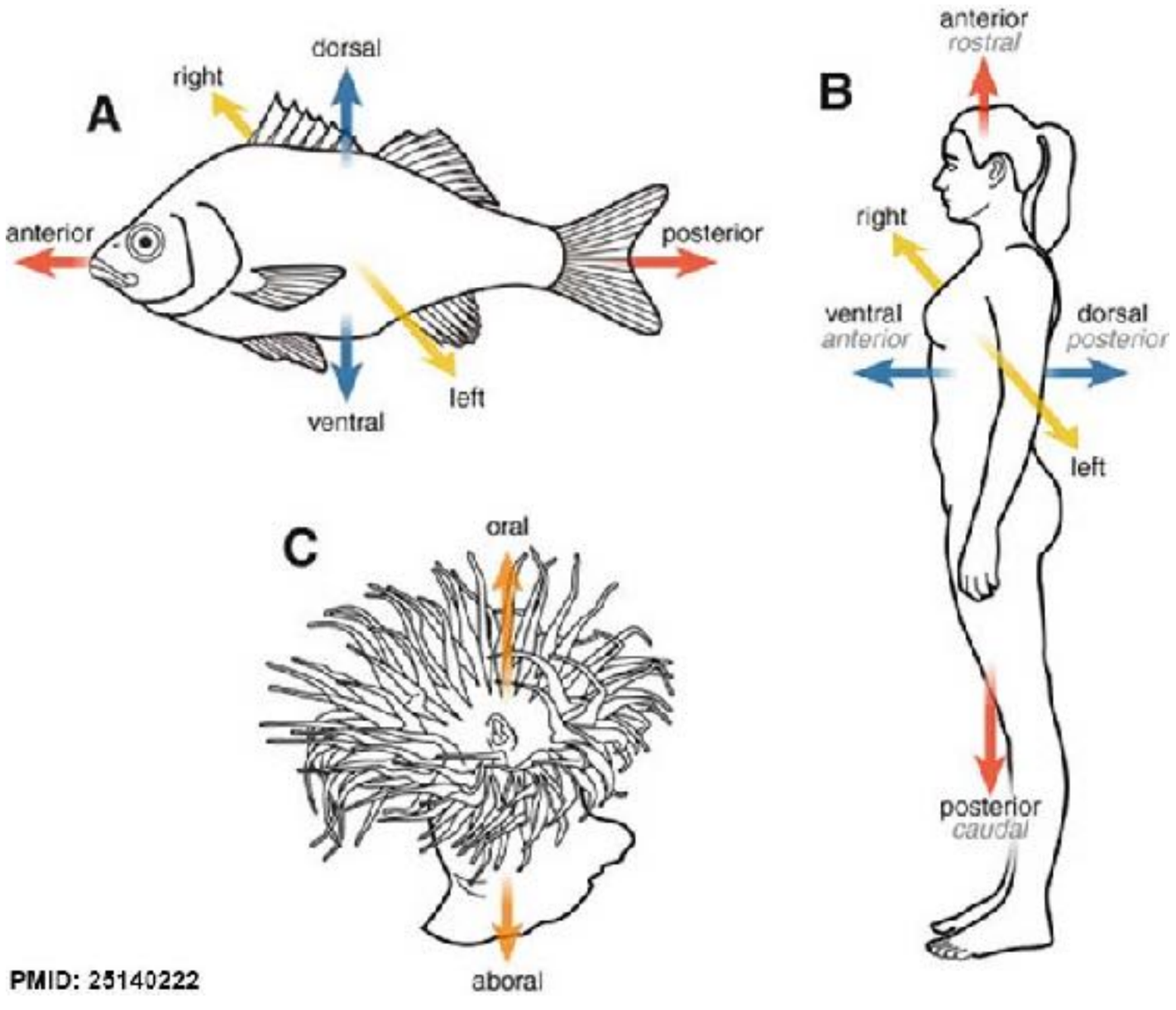
Questions?



Take a break

Big questions

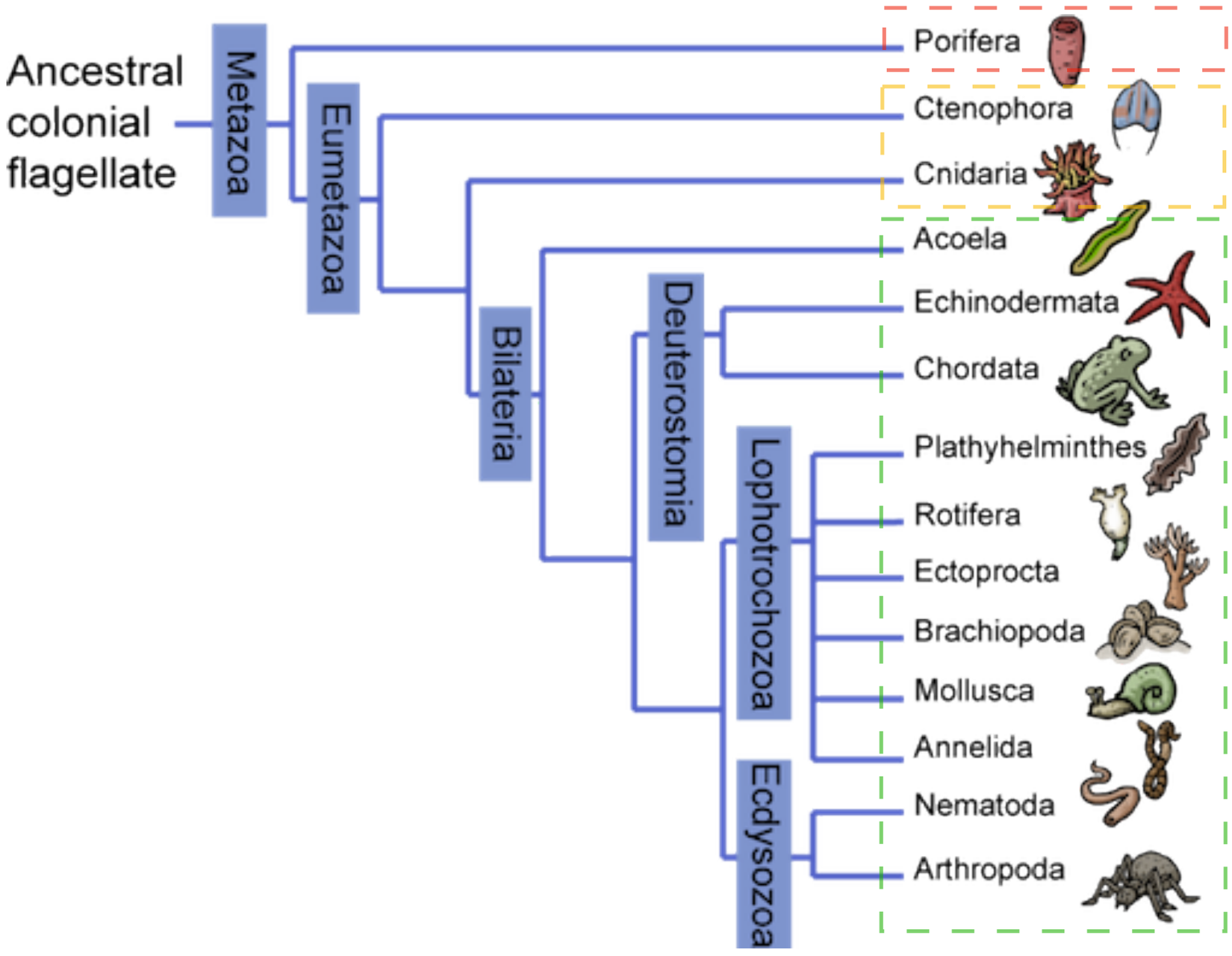


PMID: 25140222

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



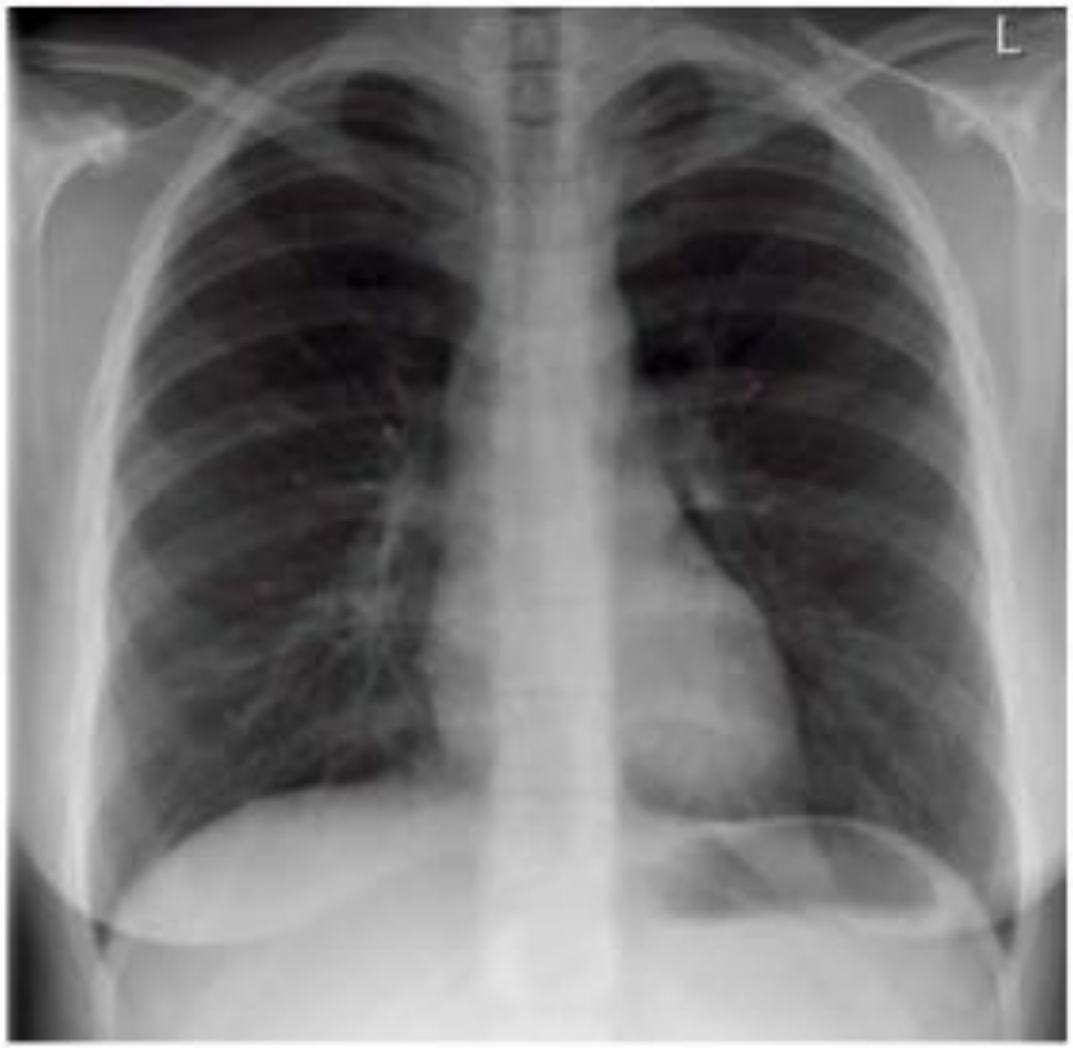
No symmetry?

Radial symmetry

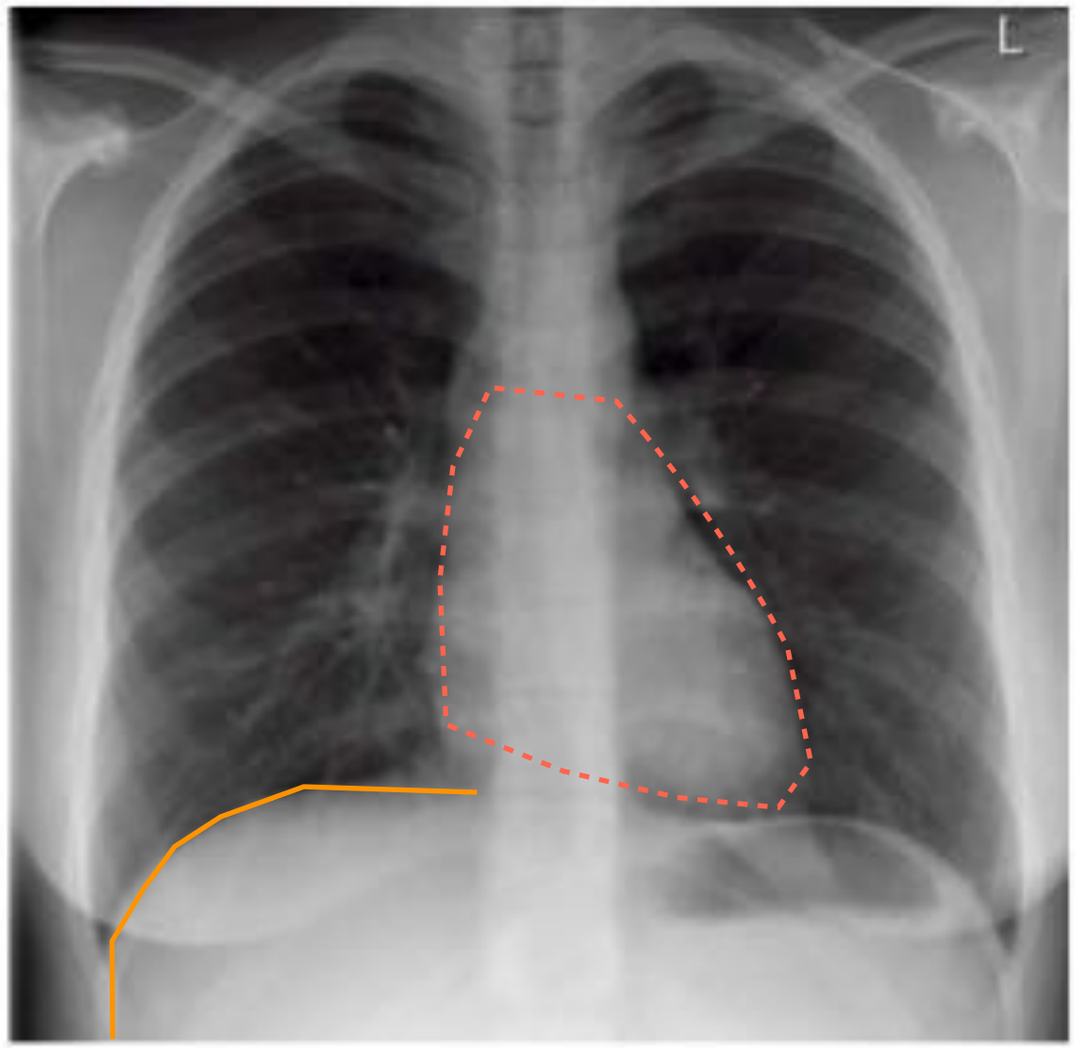
Bilateral symmetry

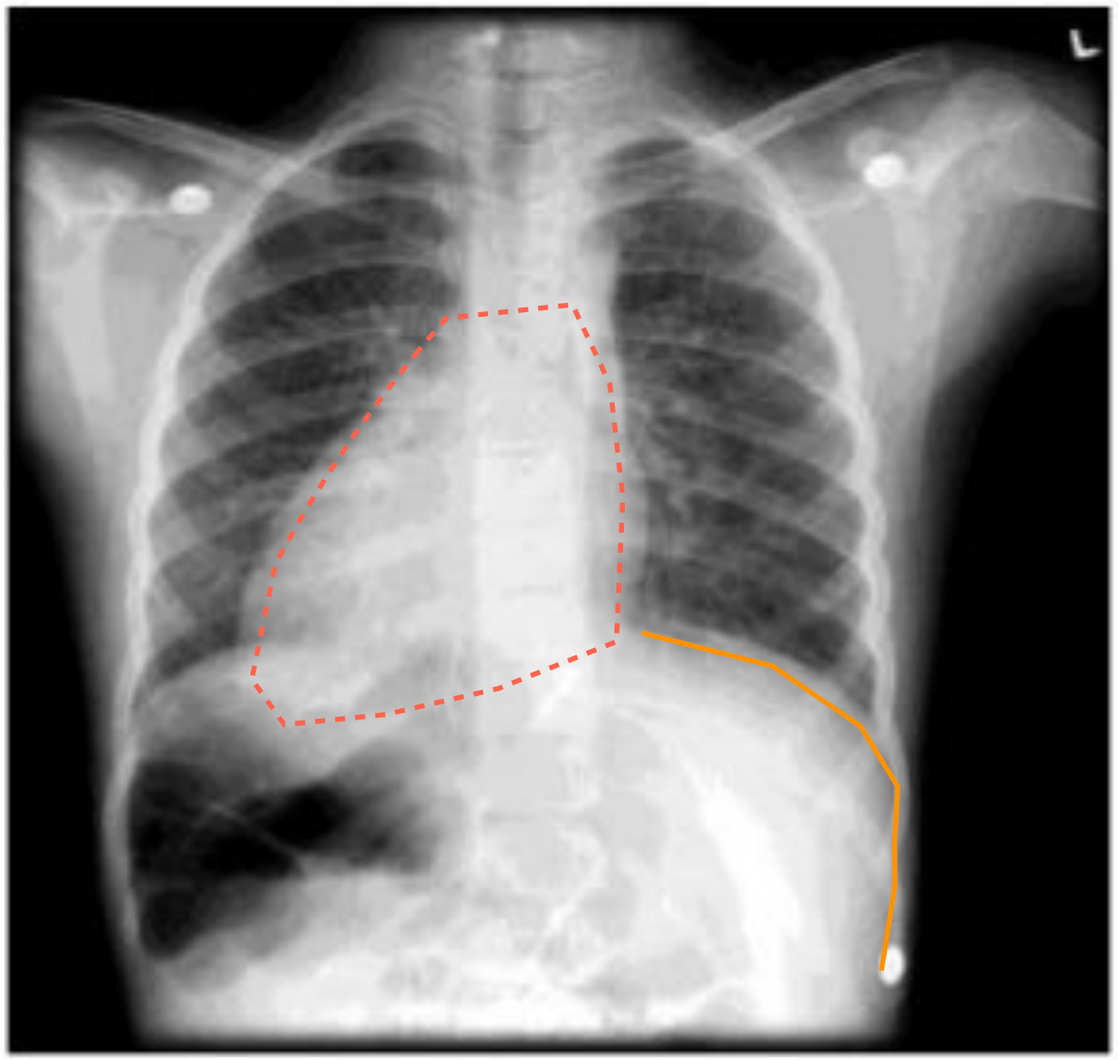
Individual puzzle

Medical Quiz: Spot the difference?



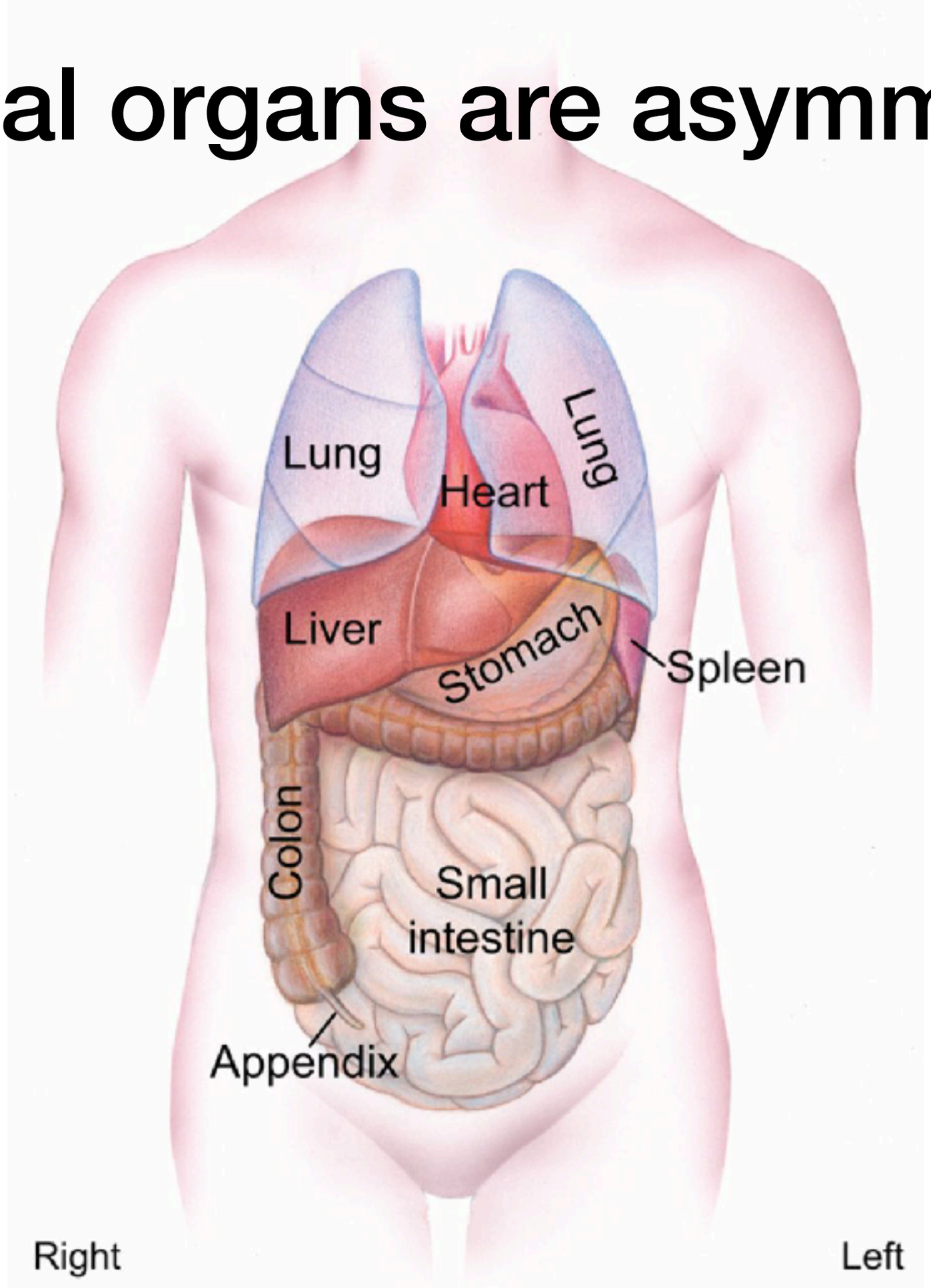
Spot the difference?



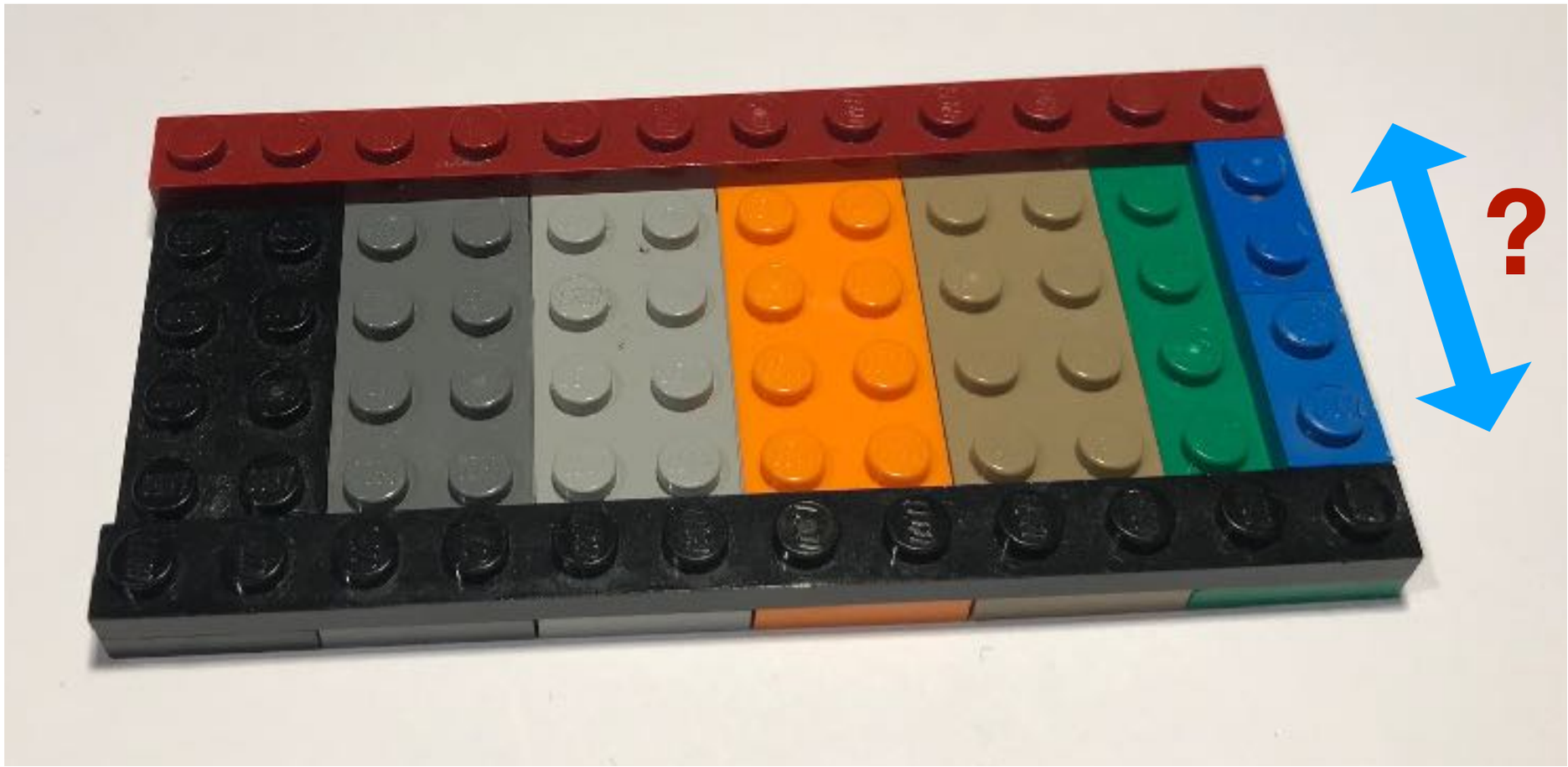


Situs inversus

Internal organs are asymmetric

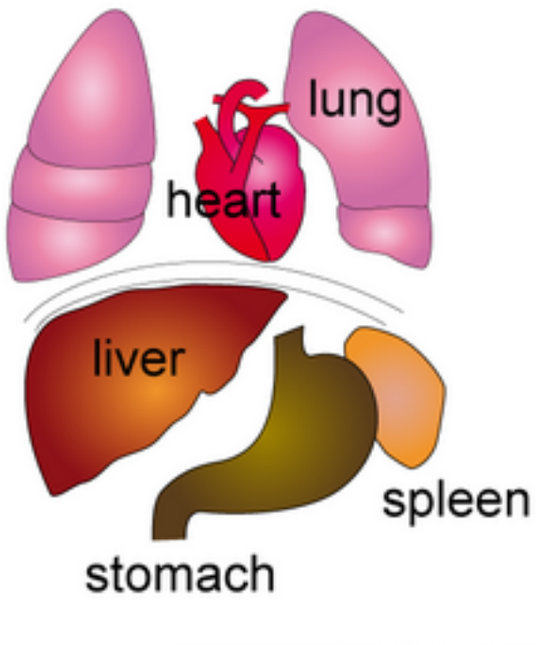


Left-Right axis

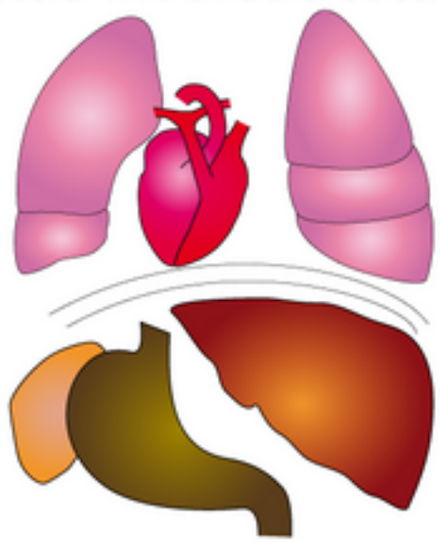


Variant internal symmetries in mammals

situs solitus

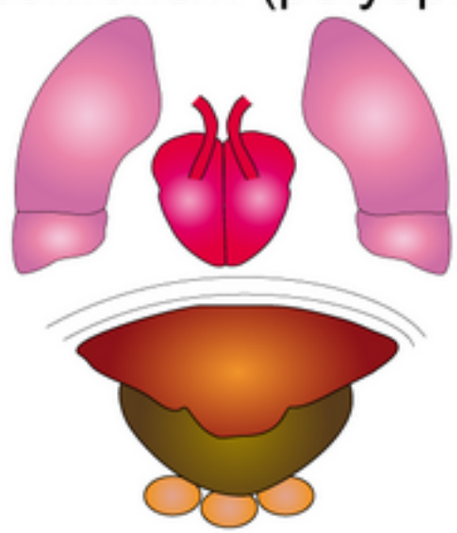


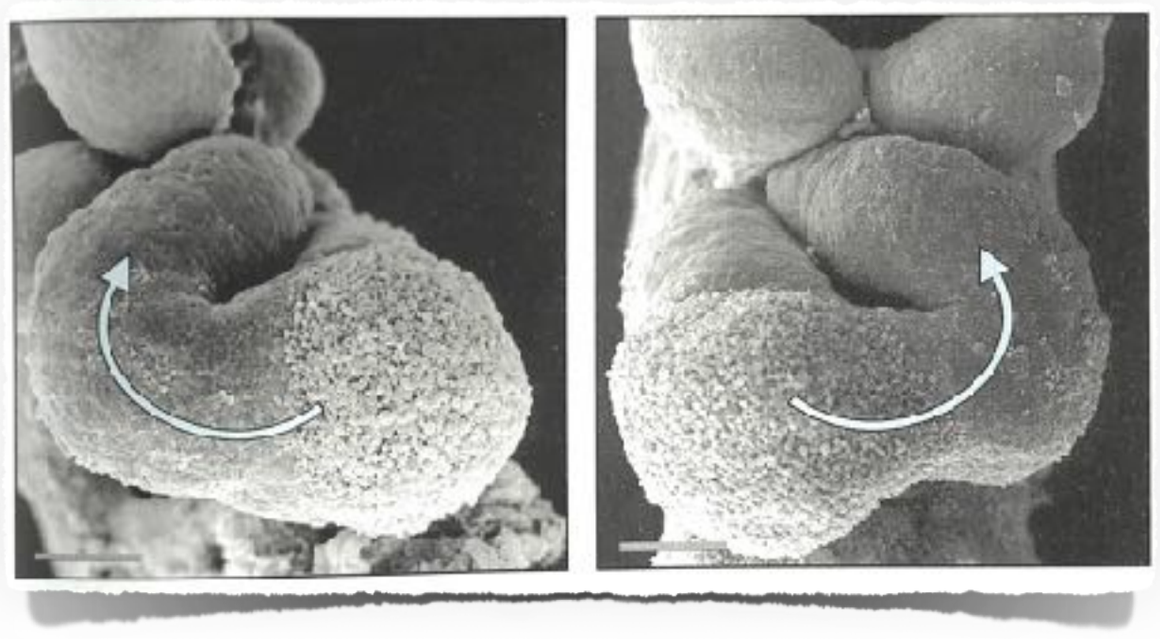
situs inversus totalis



Heterotaxy

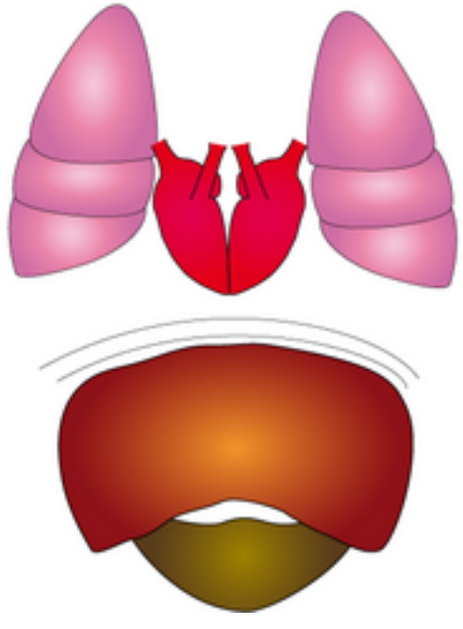
left isomerism (polysplenia)



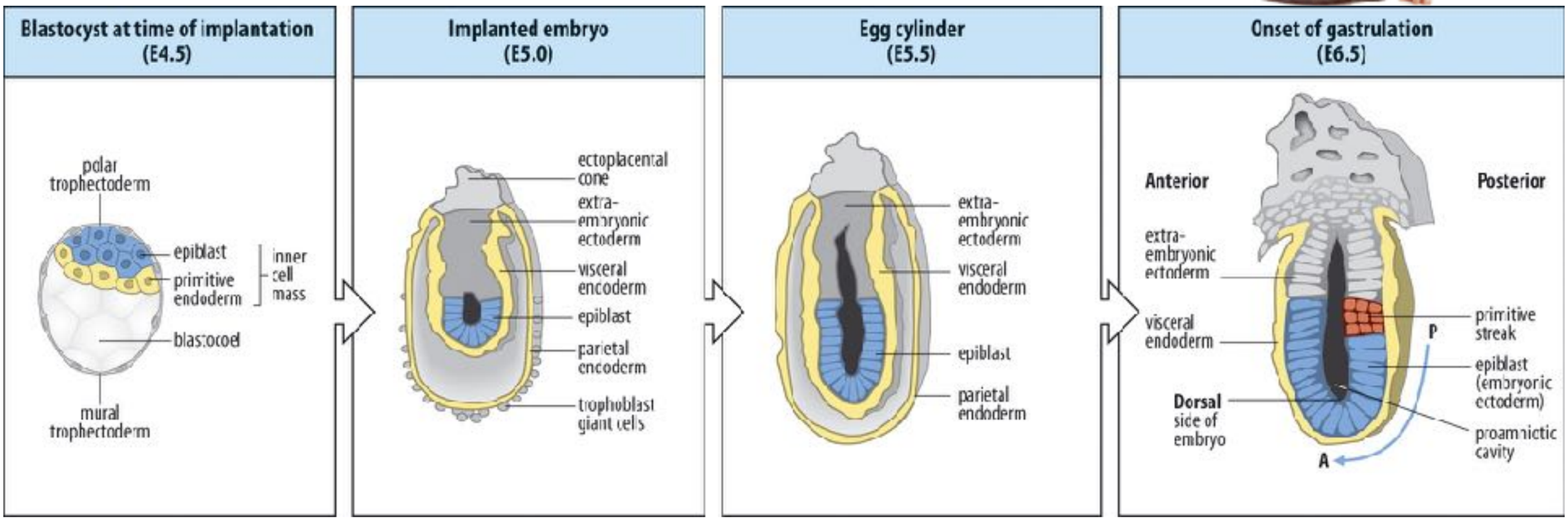


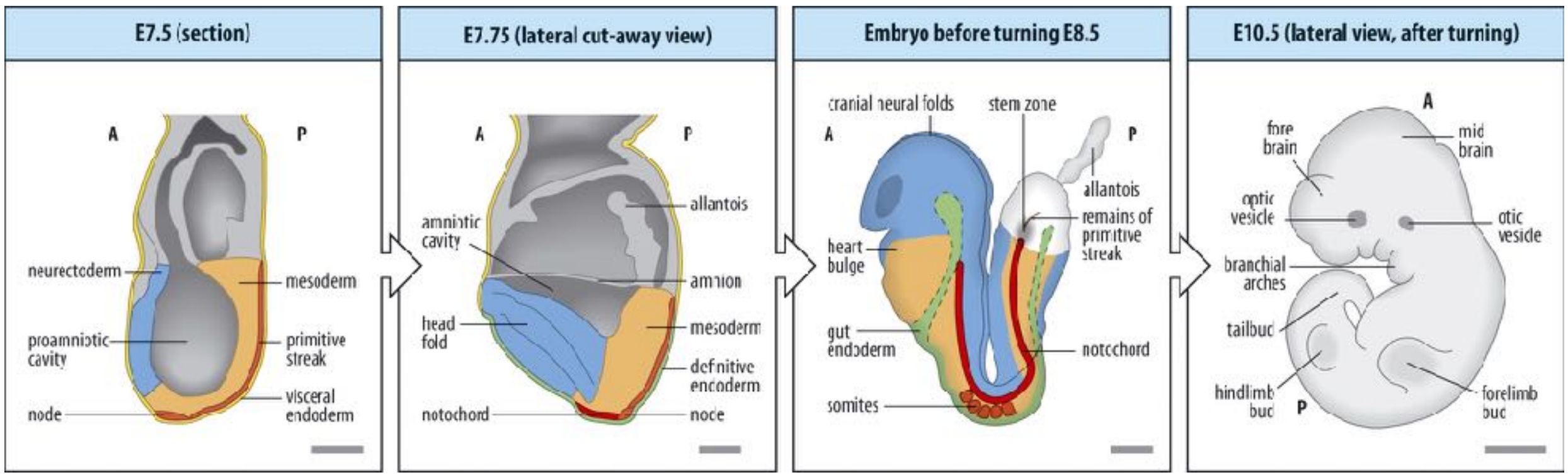
Mouse *inversus viscerum* (*iv)* mutant: heart looping left-right 50%

right isomerism (asplenia)

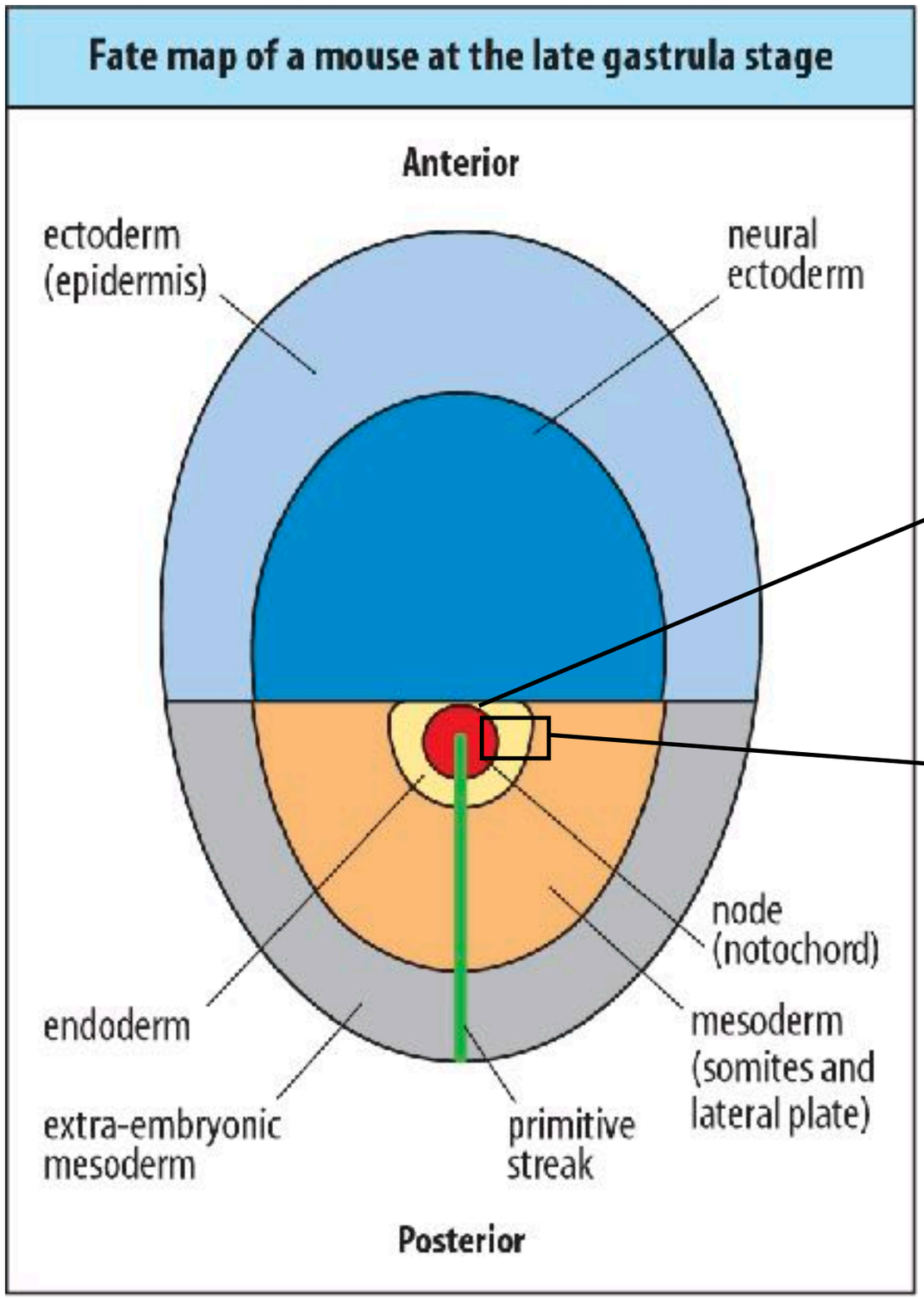


Early mouse development

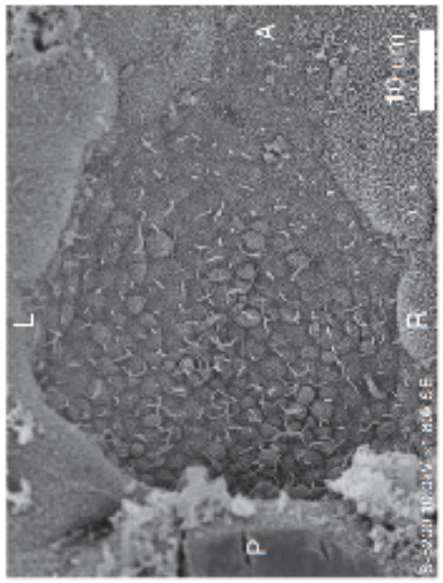


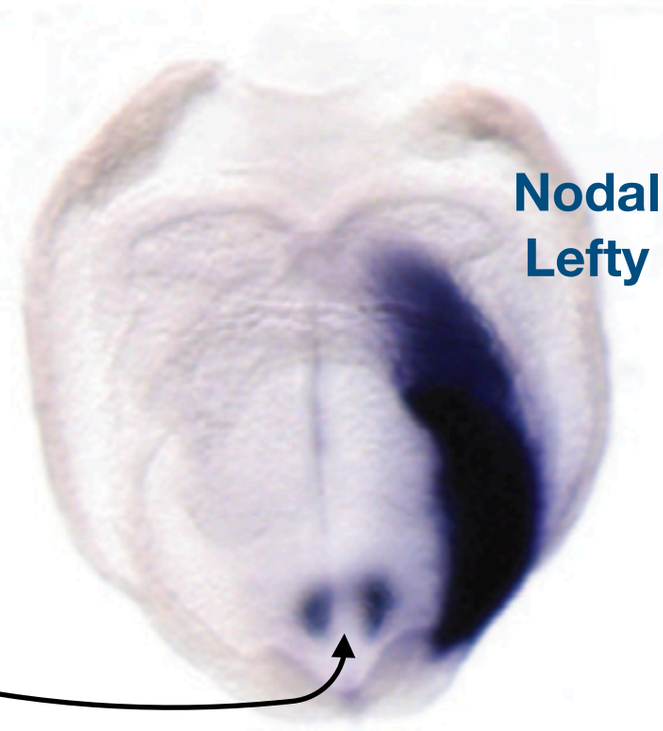


Embryonic origin of the mouse heart

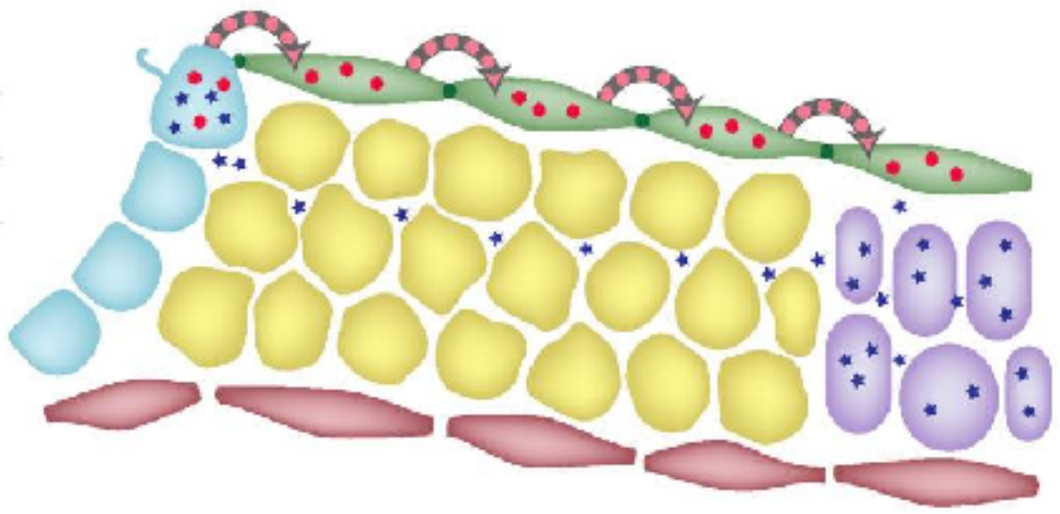


Node





Nodal
Lefty

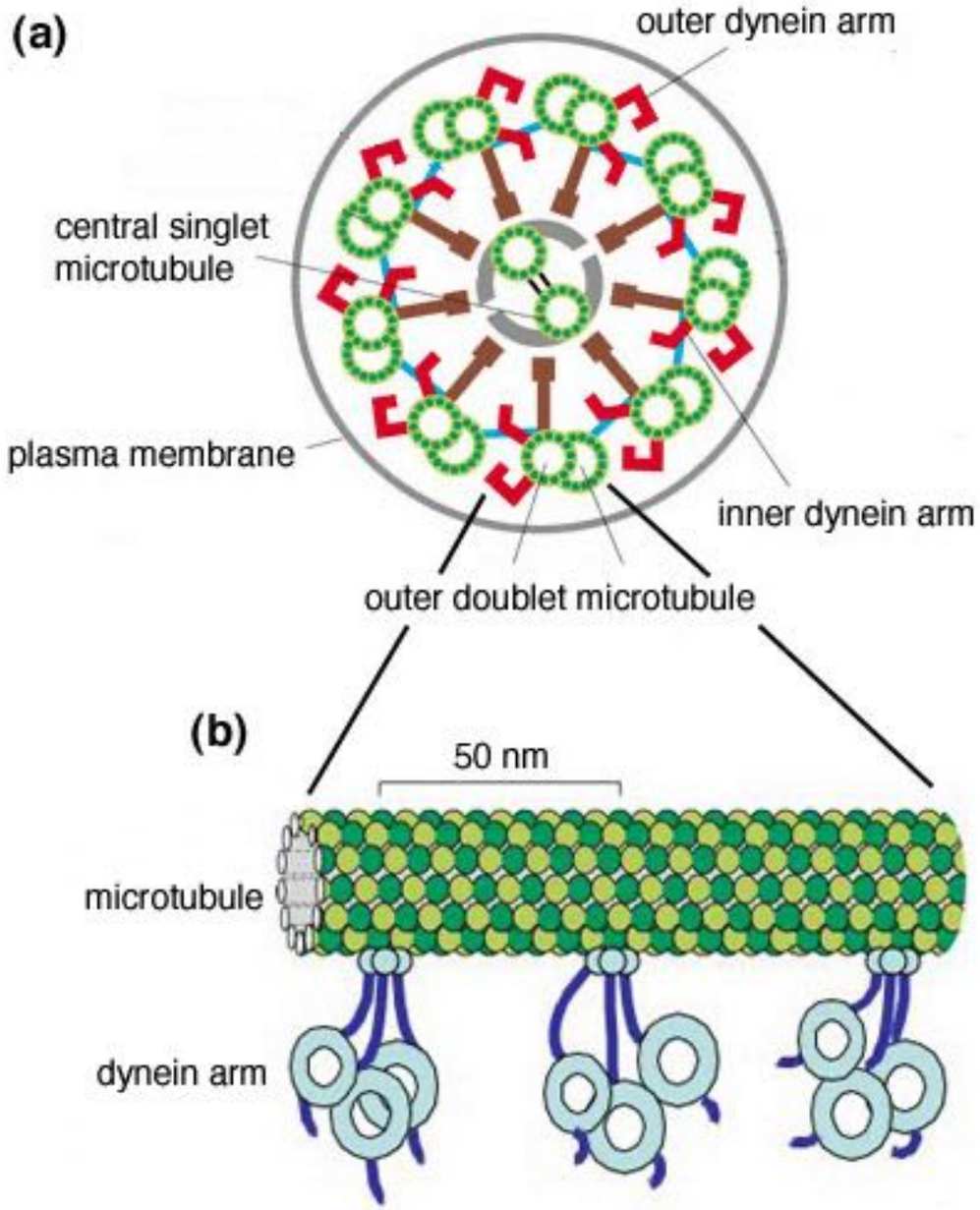


Somites

Lateral plate

Heart

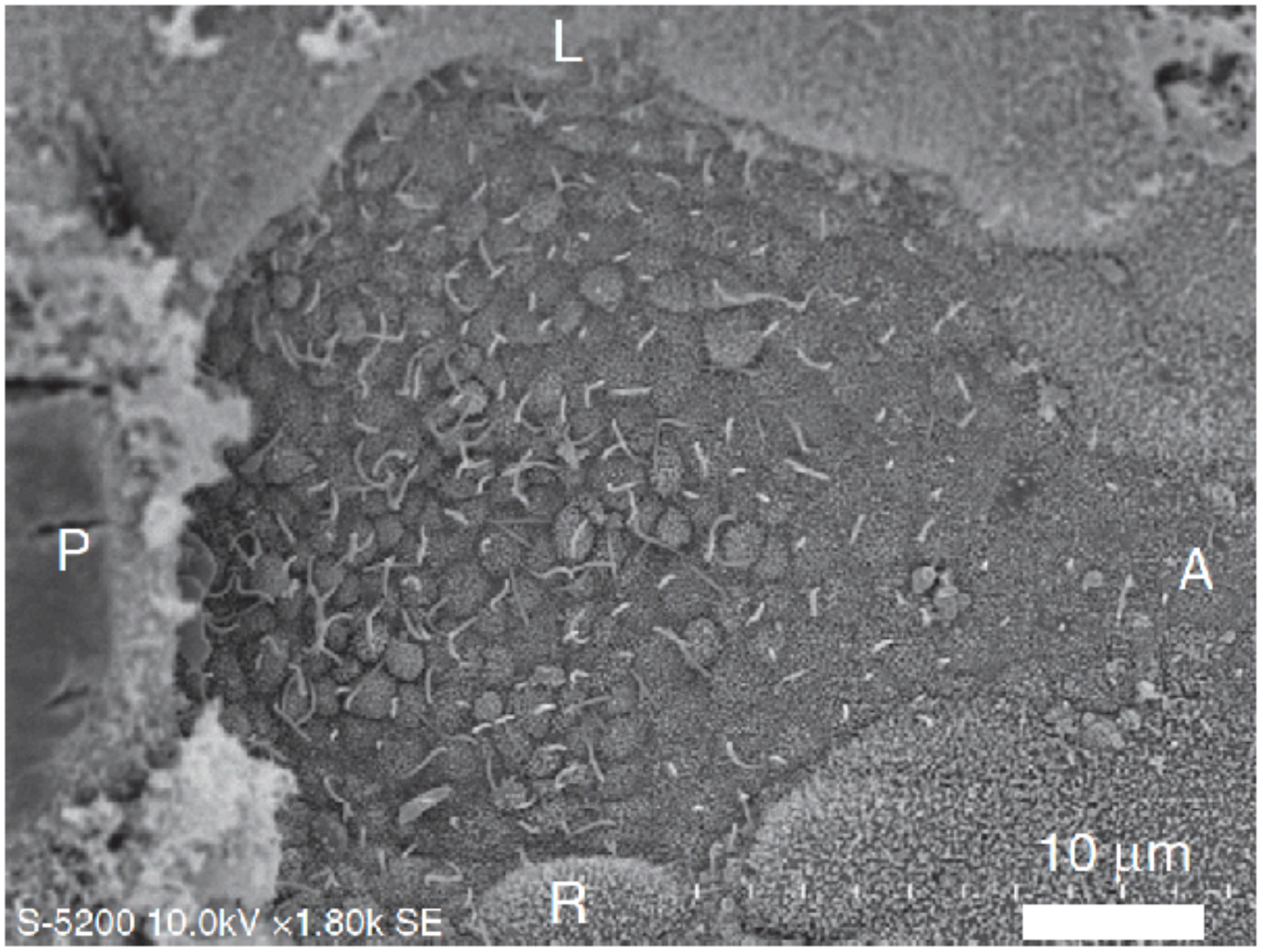
inversus viscerum encodes a Dynein outer arm heavy chain Lrd/Dnah11



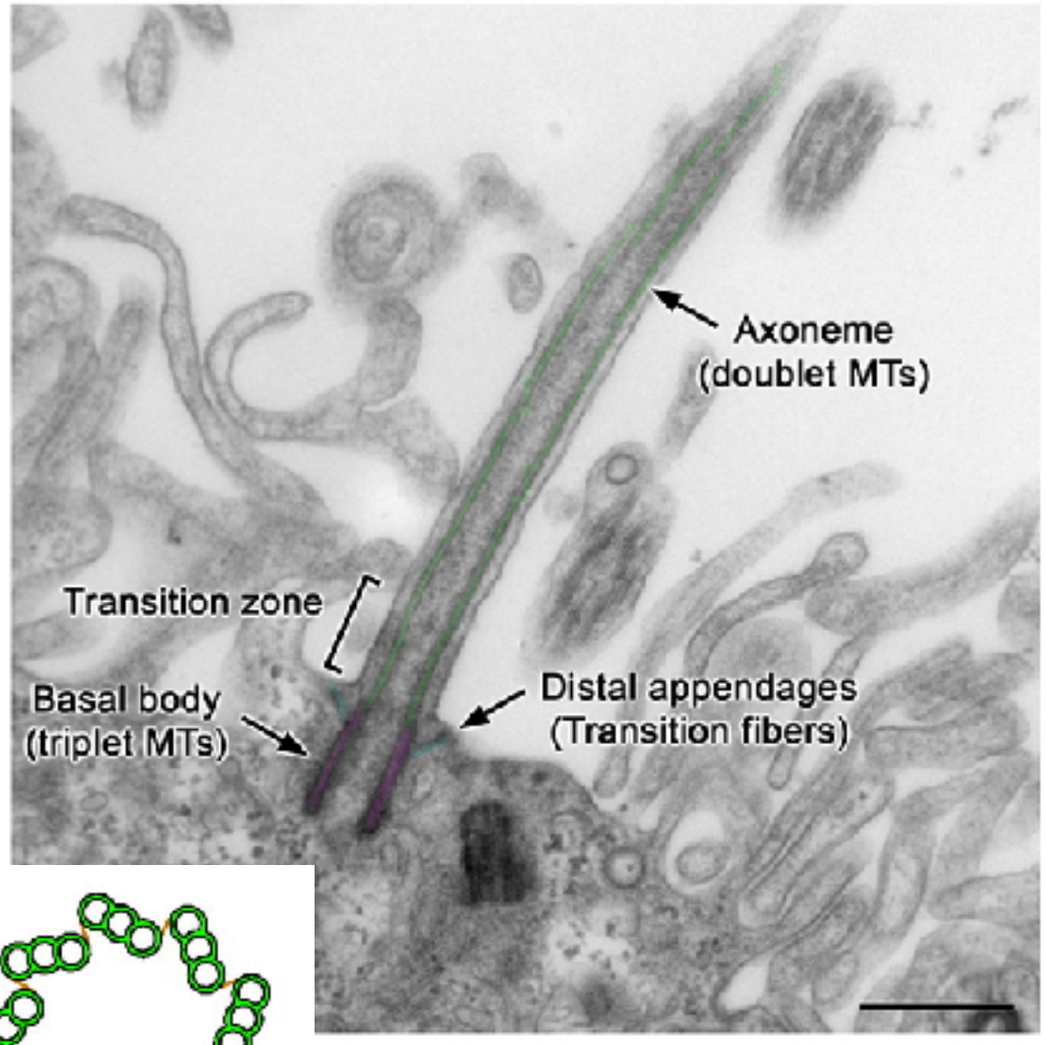
How is axonemal motion generated?

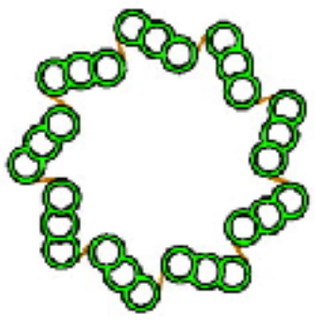
Dnah5 encodes another dynein outer arm heavy chain

The mouse node is covered in cilia

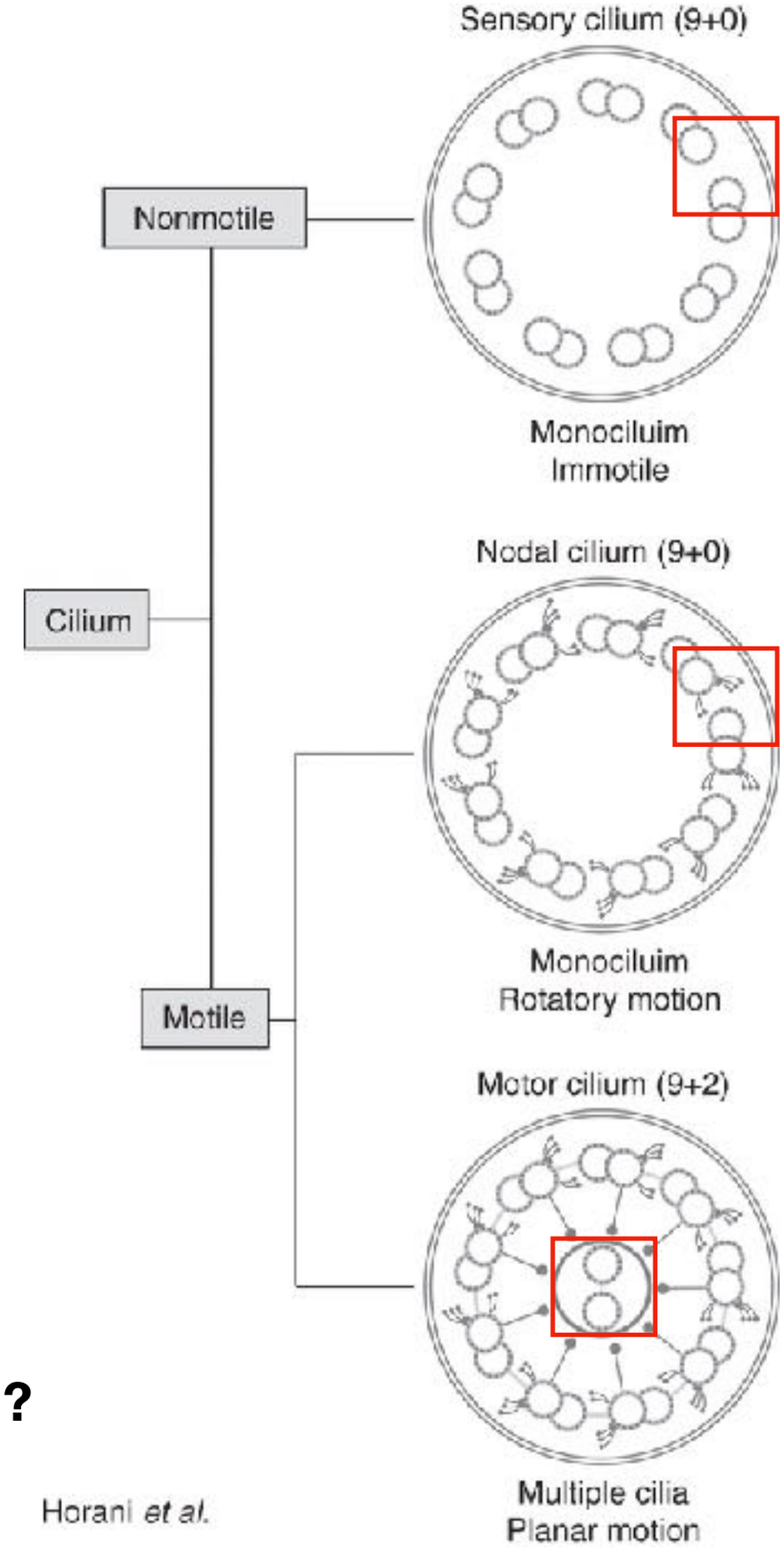


Different cilia





Basal body looks like a …?



Horani *et al.*

Signaling
Kinocilia
Photoreceptor
Olfactory
Primary

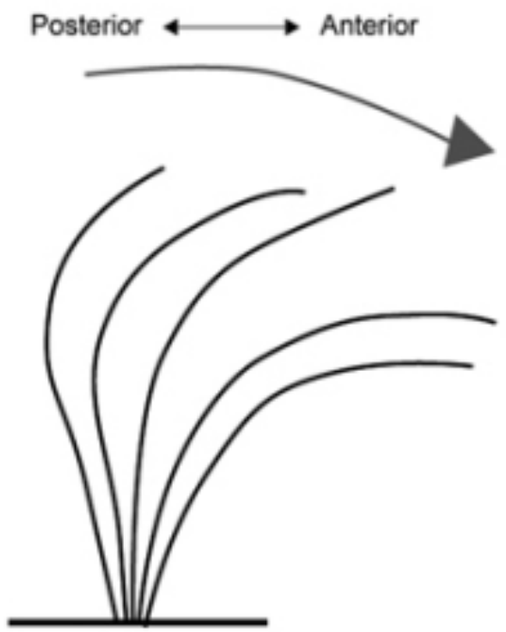
Embryonic node

Transport
Lungs
Fallopian tubes
Testes
CNS ventricles

Sperm

Group puzzle

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

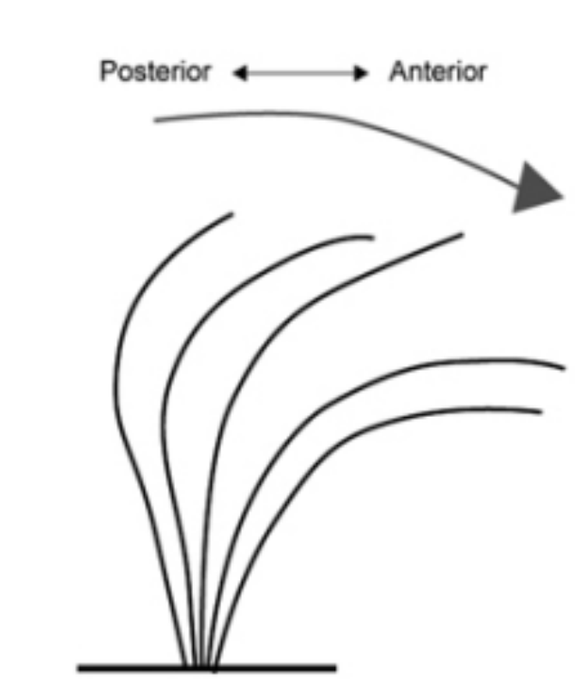


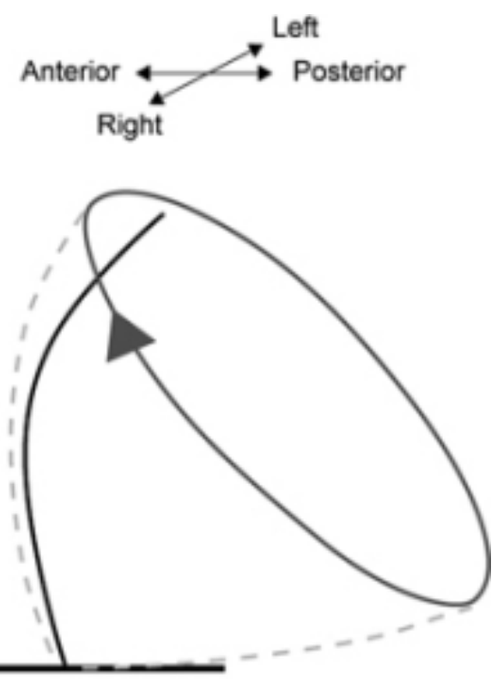
?

9+2 motile cilia

9+0 motile cilia

Paddles and propellors





rotation?

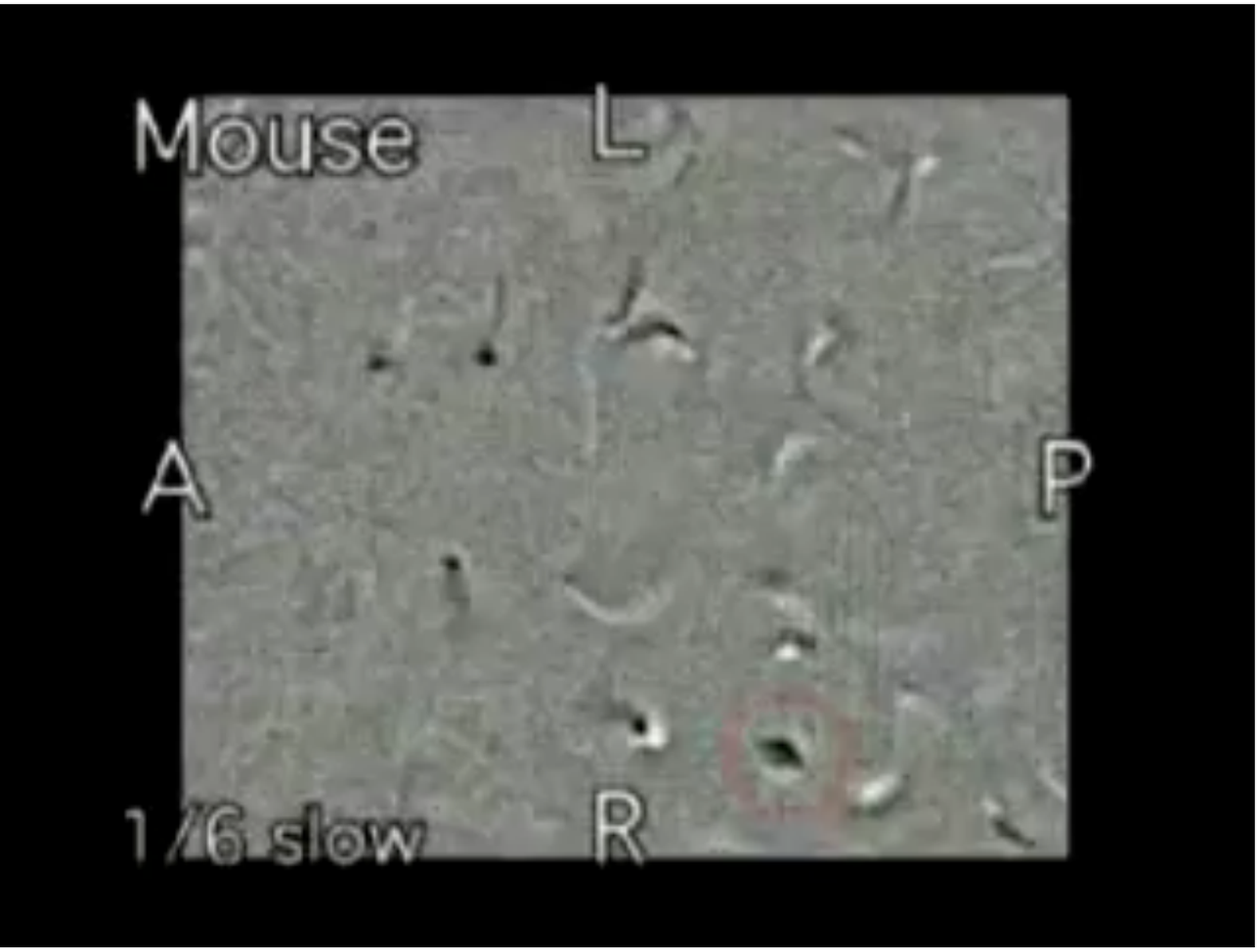
9+2 motile cilia

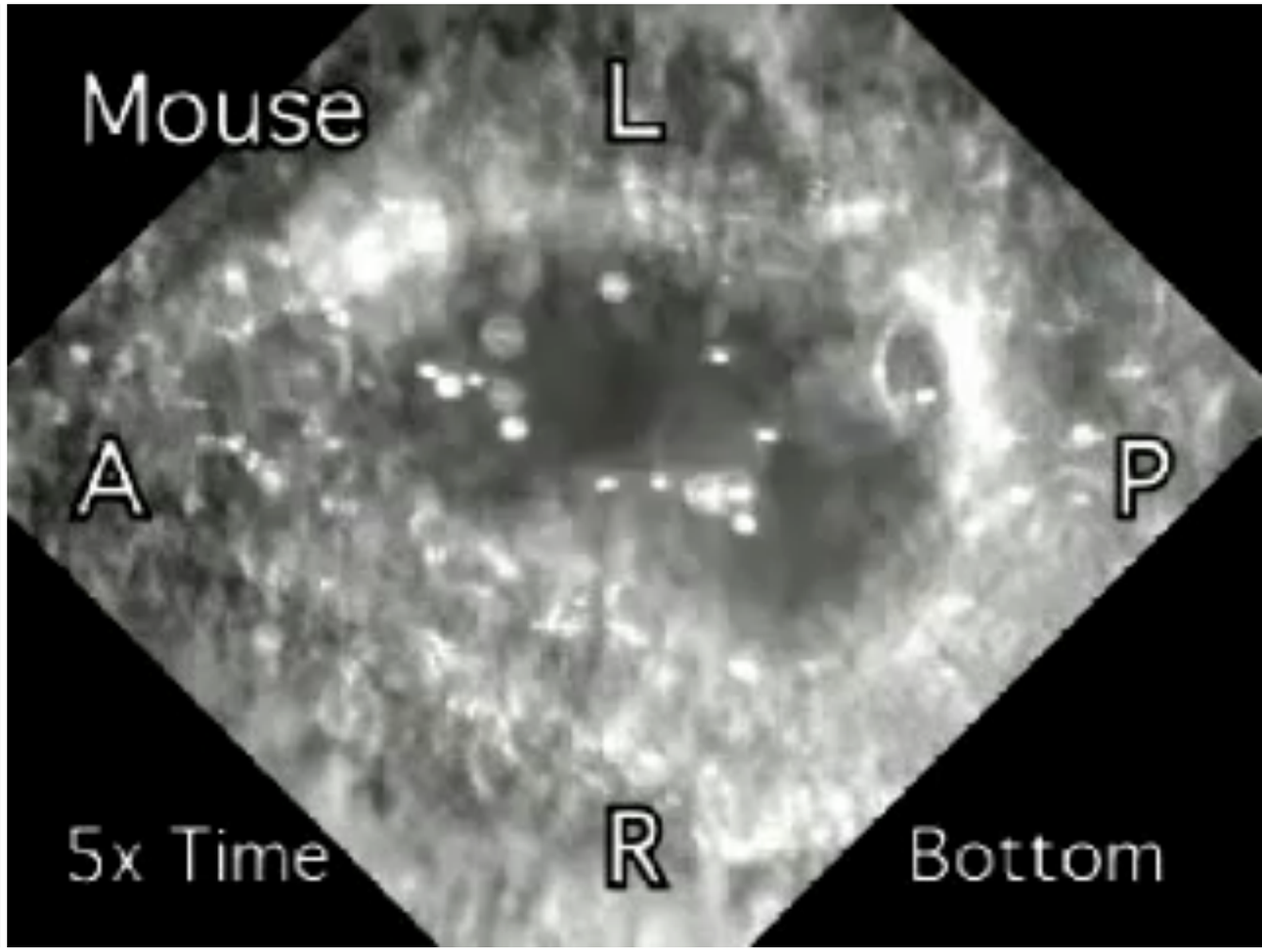
9+0 motile cilia

tilt?

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

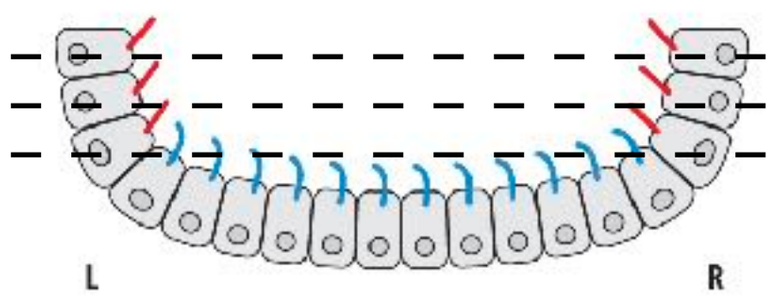
Cilia-induced fluid flow across node





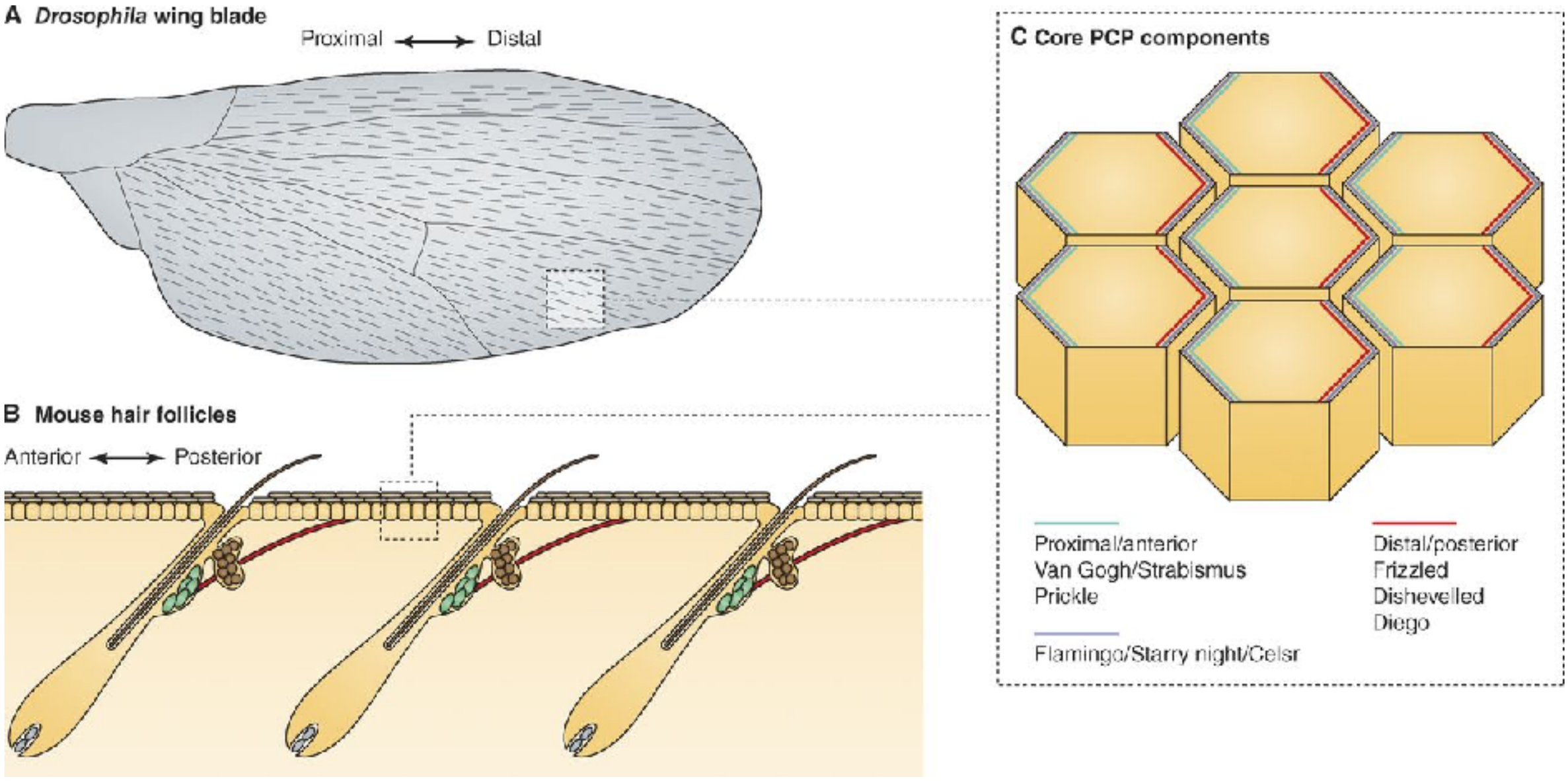
Cilia rotate clockwise,
Elevated power stroke,
Low recovery stroke,
Point posteriorly

Beads in flow
Fast and slow



Okada et al, 2005

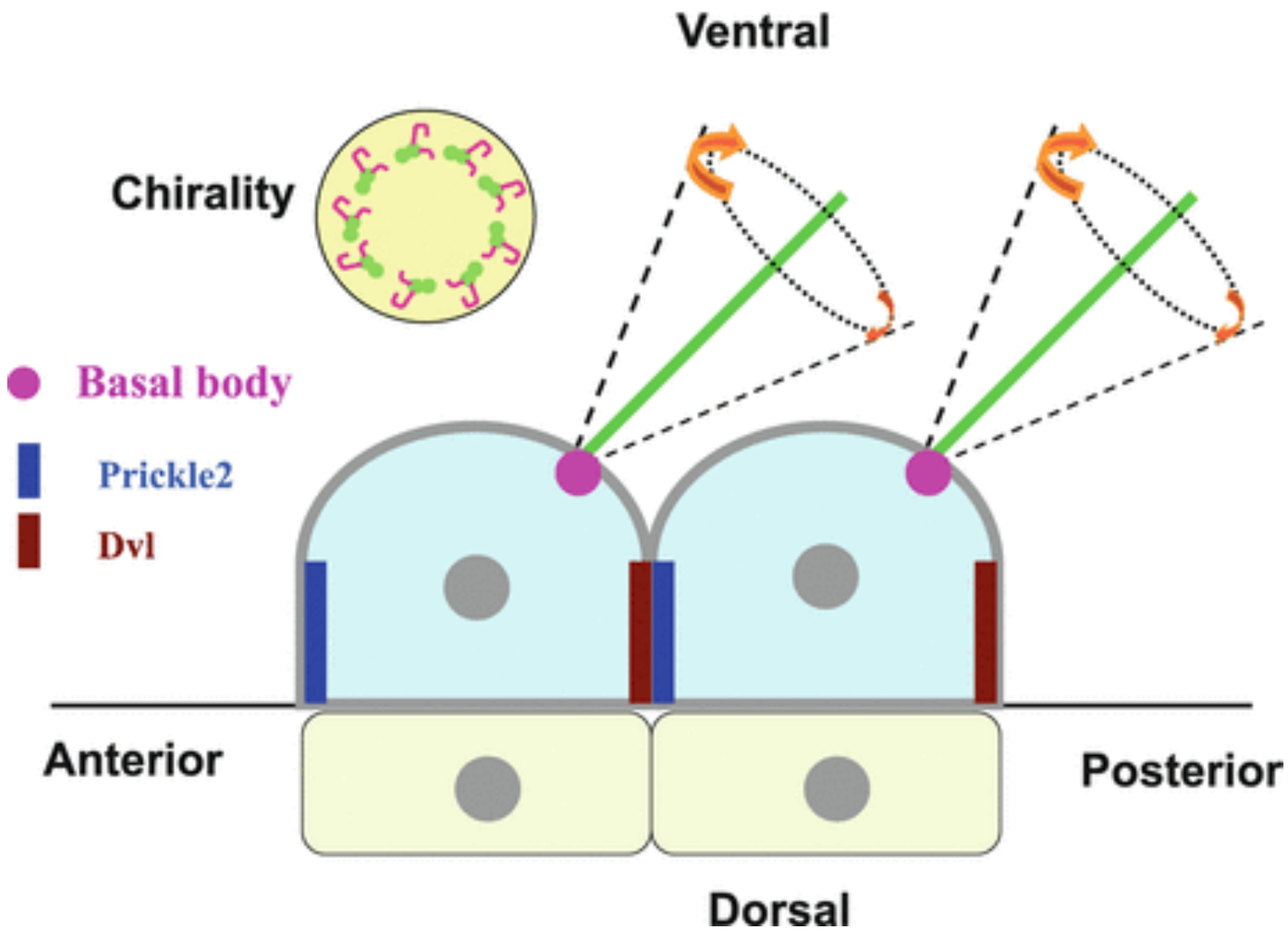
Planar polarity in a 2D epithelial sheet



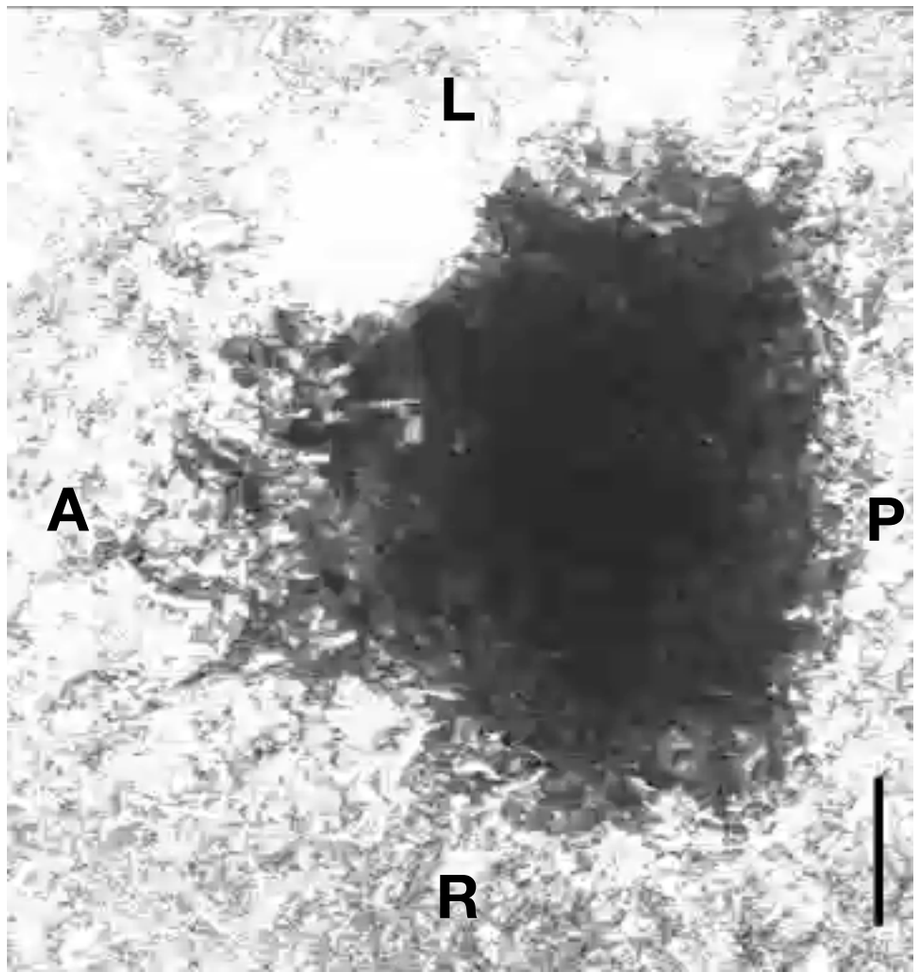
+ many other examples…

Devenport, 2014

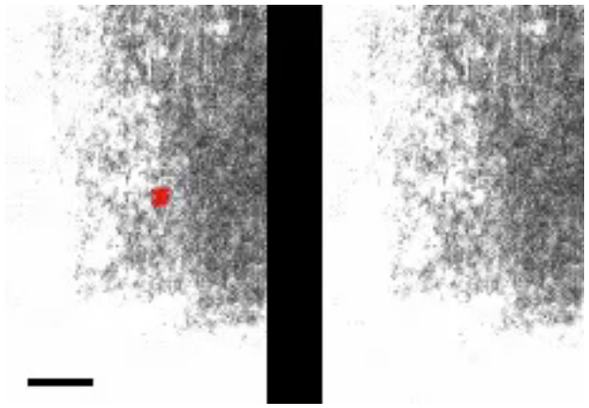
The node epithelium is polarised early along the A-P axis



Membrane particles move across node



Dil labelled membrane

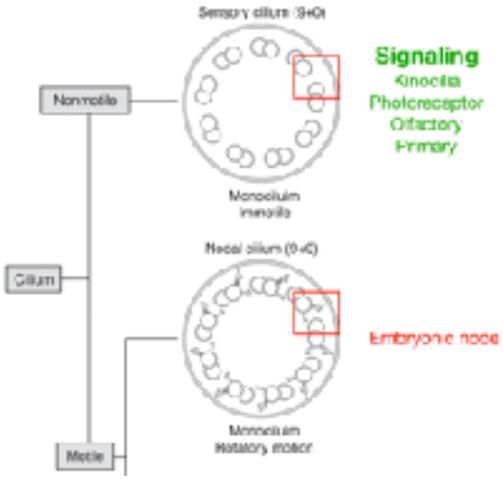


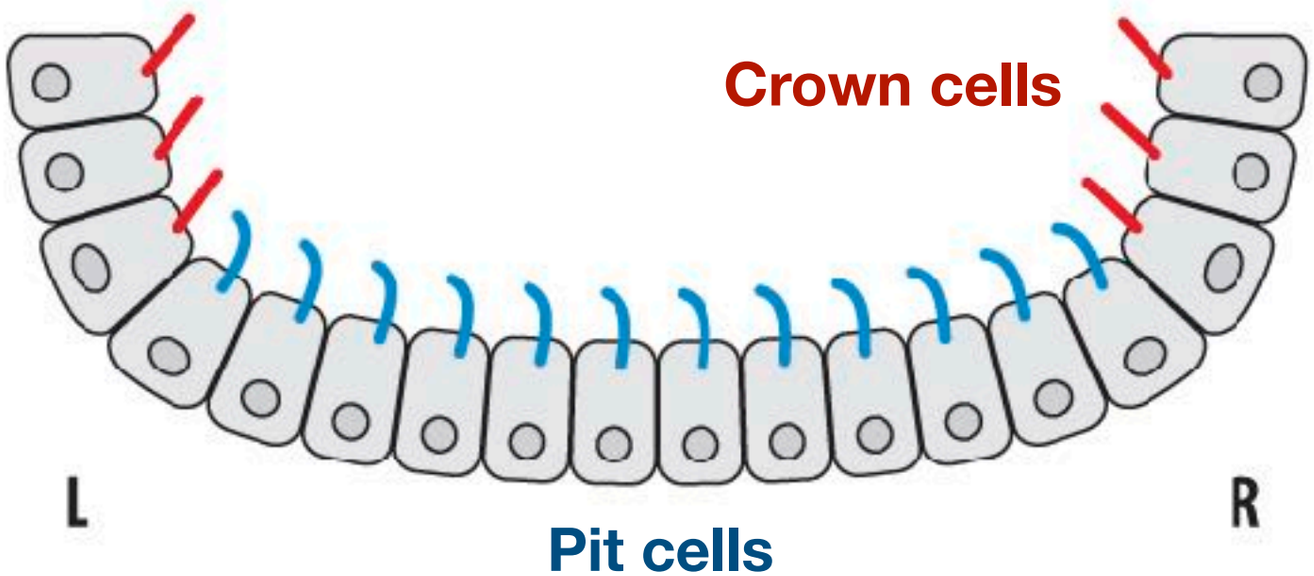
Release from cilium?

Disruption on wall?

Tanaka et al, 2005

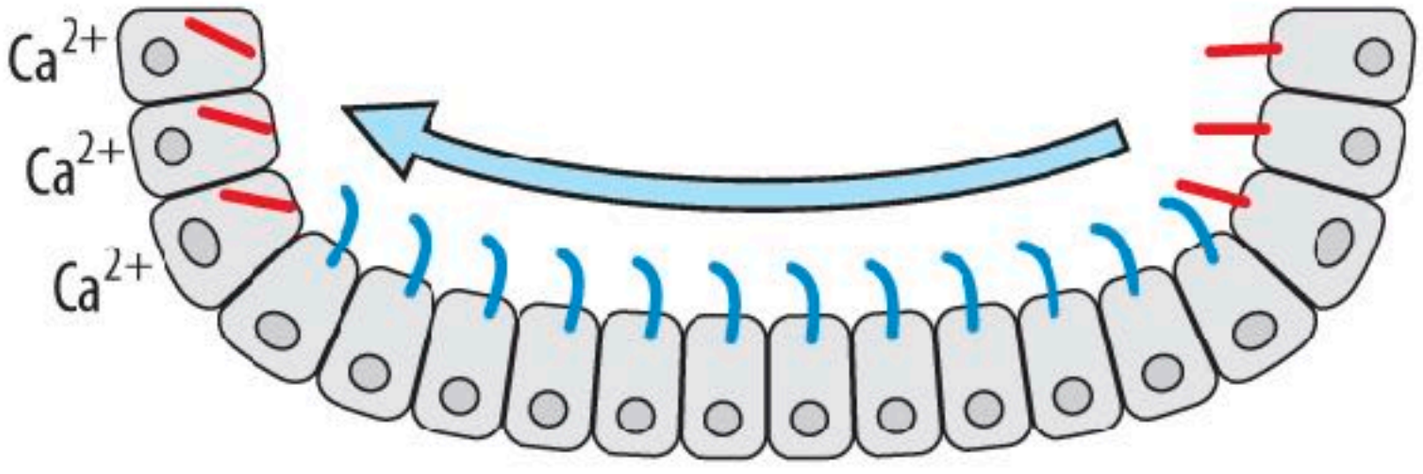
Two types of cilia inhabit the node





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



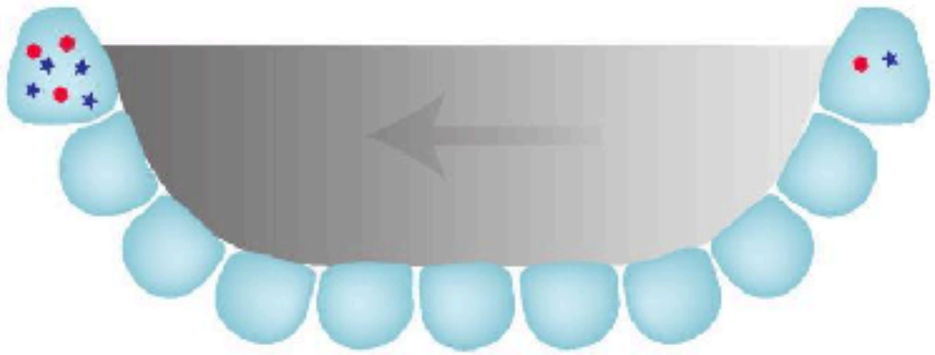
Nodal
Lefty

Yoshiba et al. 2012

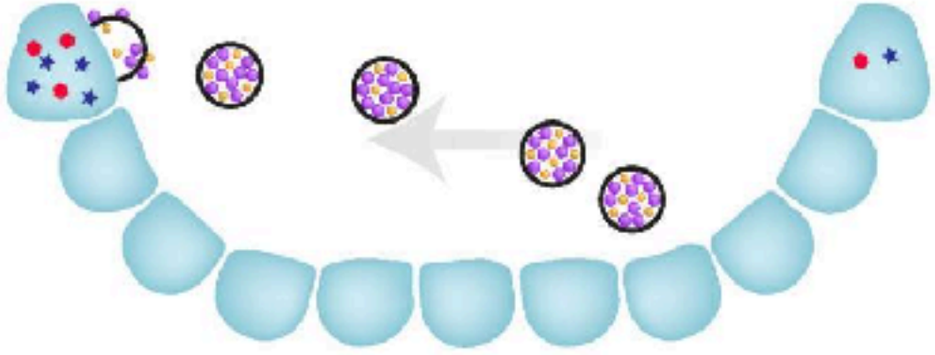
Group puzzle

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

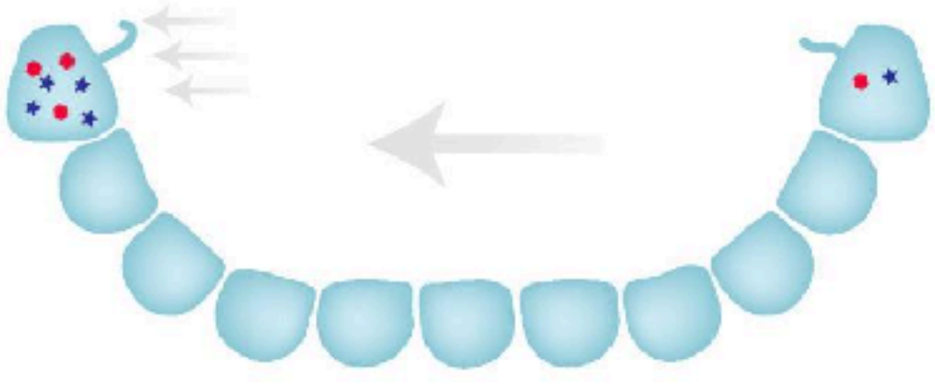
Morphogen gradient



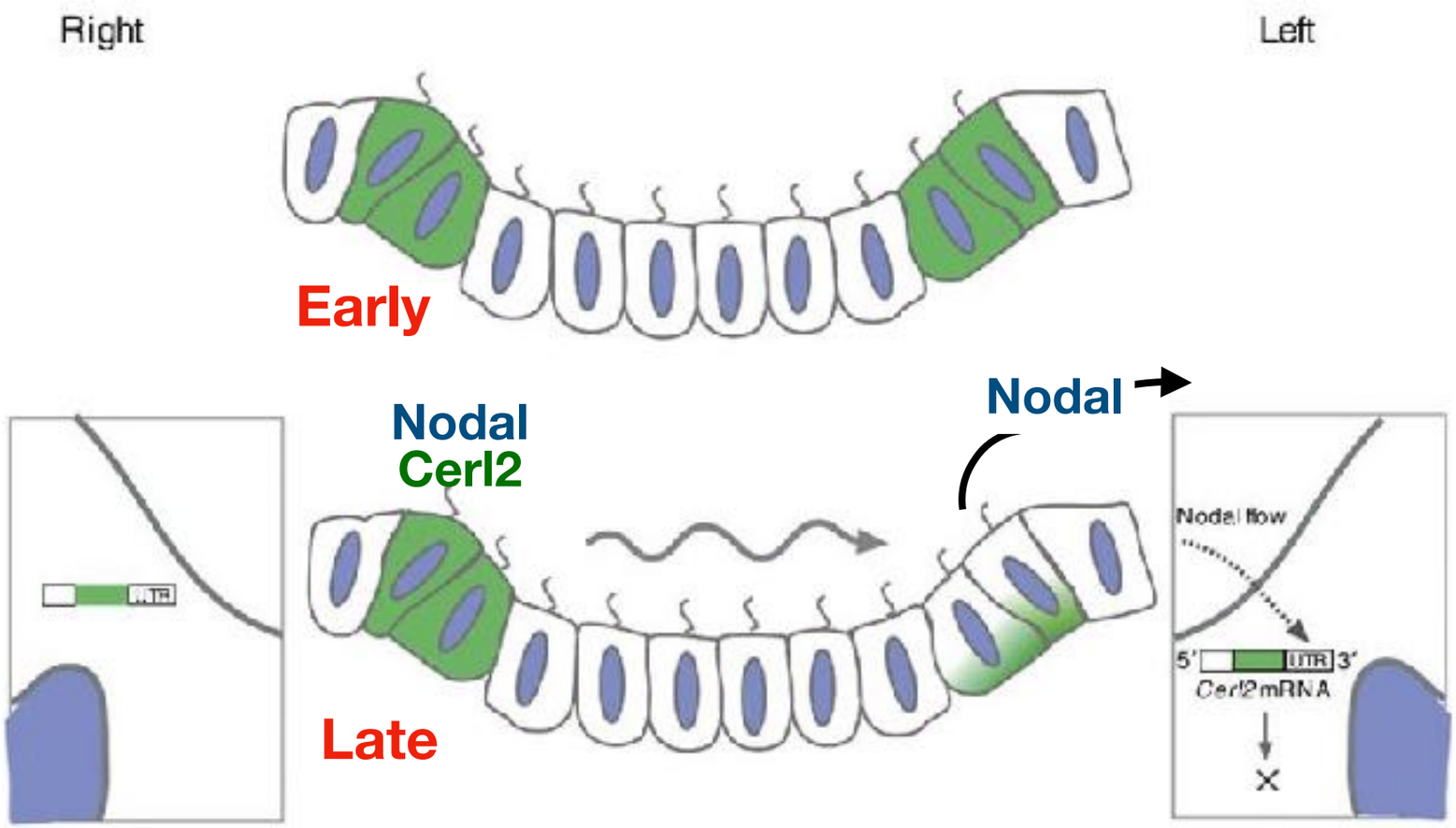
Nodal vesicular parcel (NVP)



Mechanosensation



Signal processing to release asymmetric Nodal



Cerl2 / Dand5

Earliest asymmetric mRNA

symmetric in *iv* mice

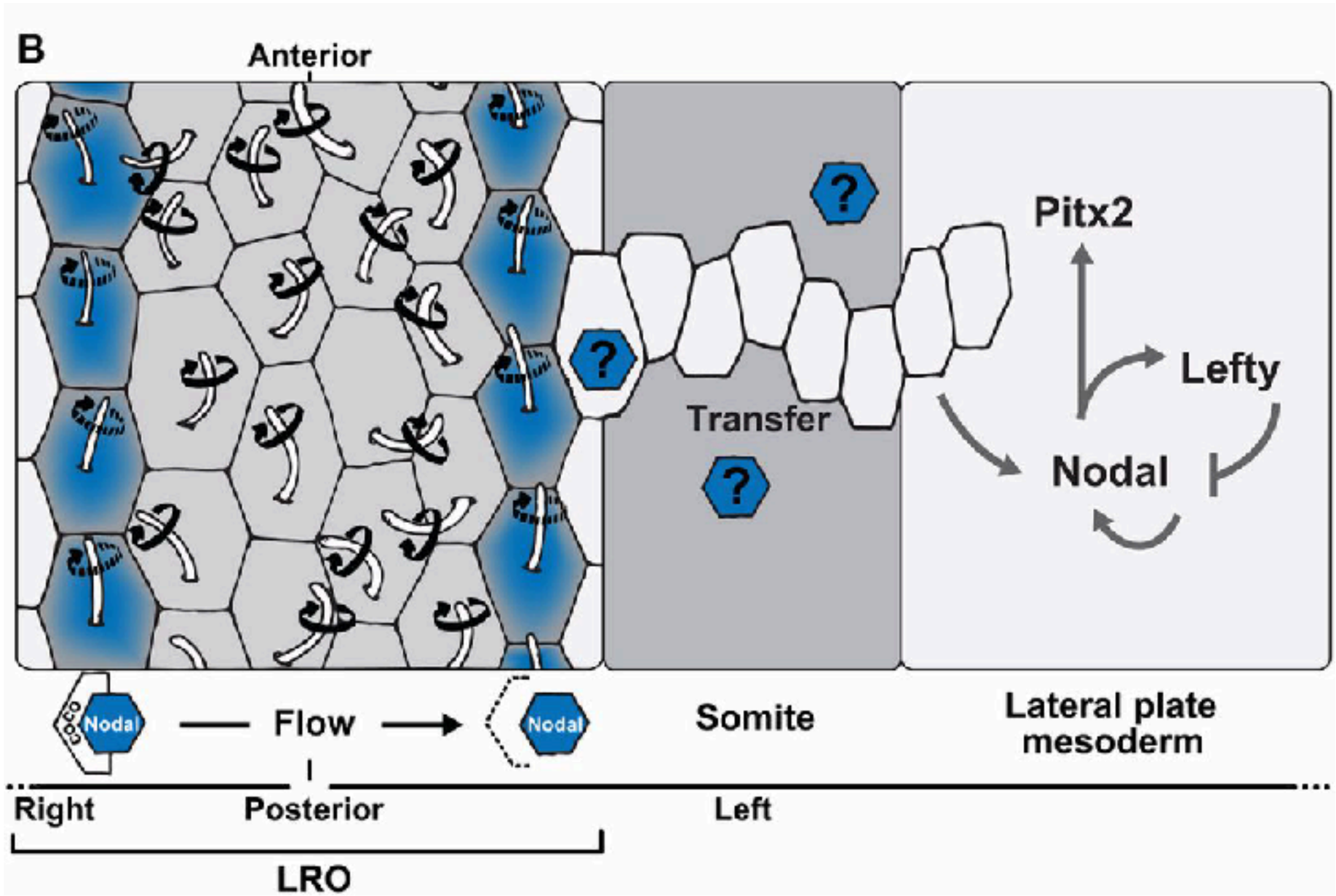
Nodal inhibitor(!)

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?

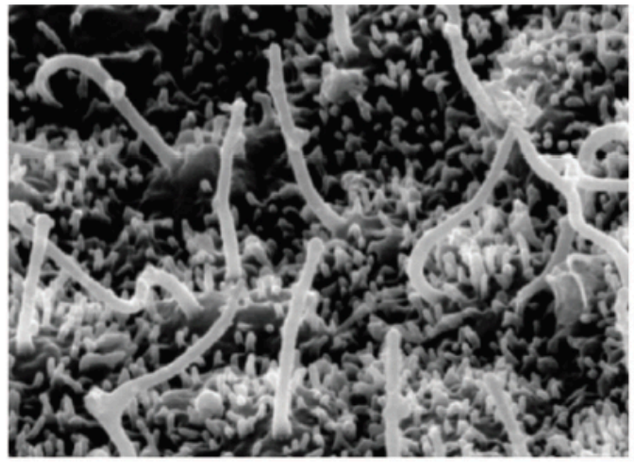
How is a small Nodal signal amplified along the LPM?

Summary

Symmetry breaking

Patterning

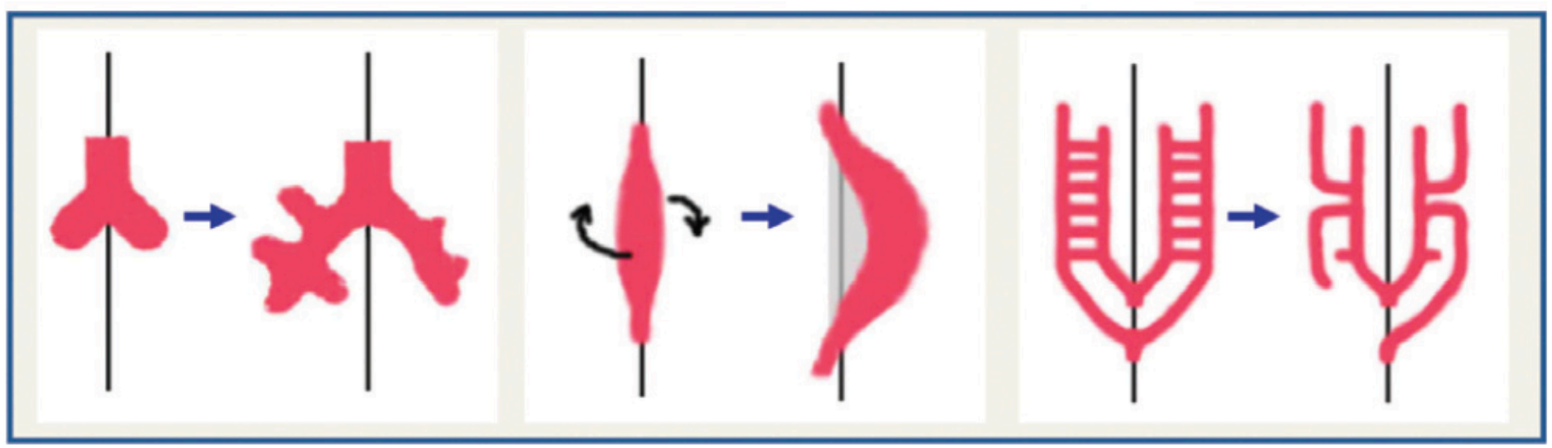
Organogenesis



Node cilia

R L

Nodal
Lefty



Pitx2

Questions?