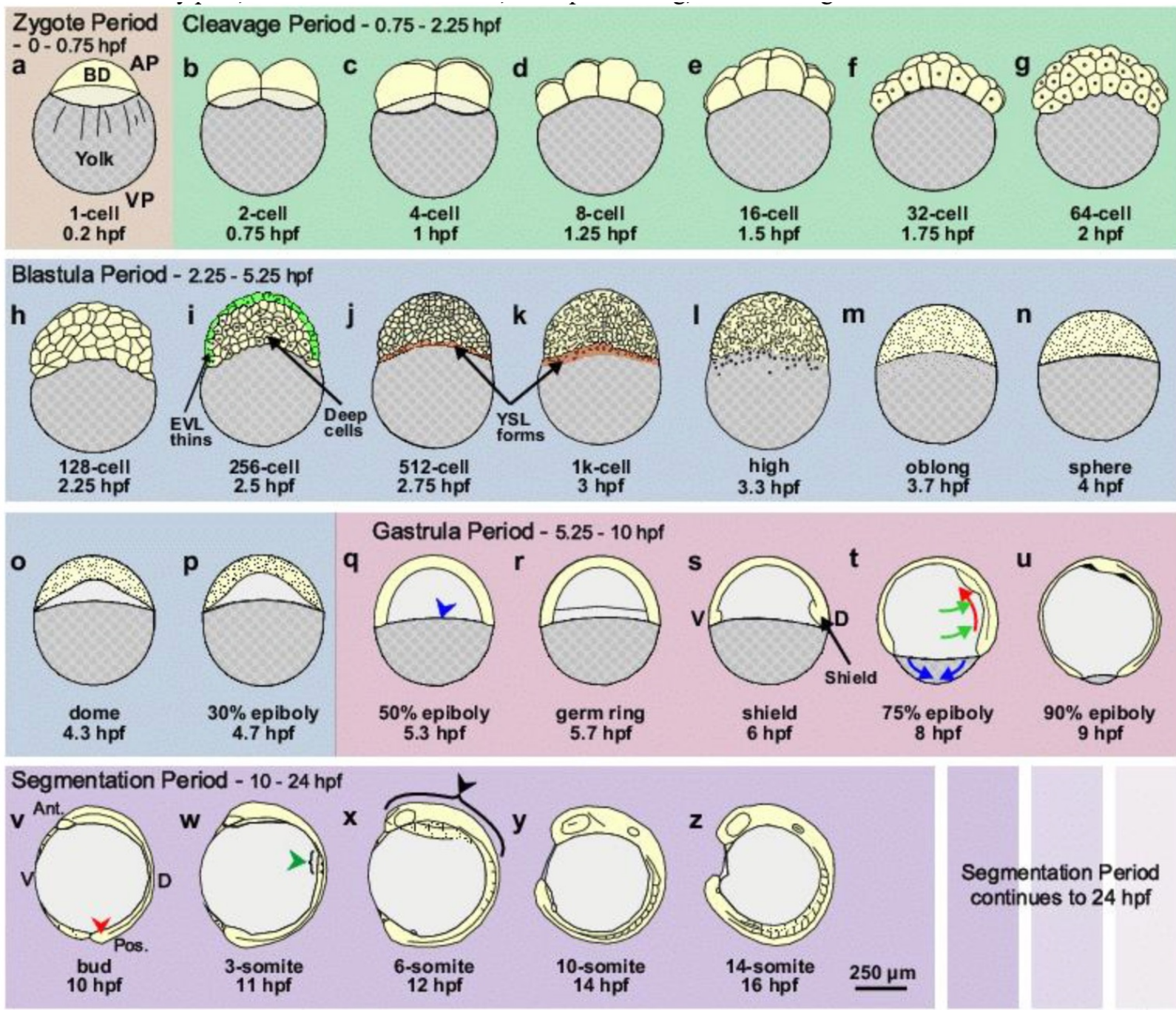

Zebrafish Gastrulation

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

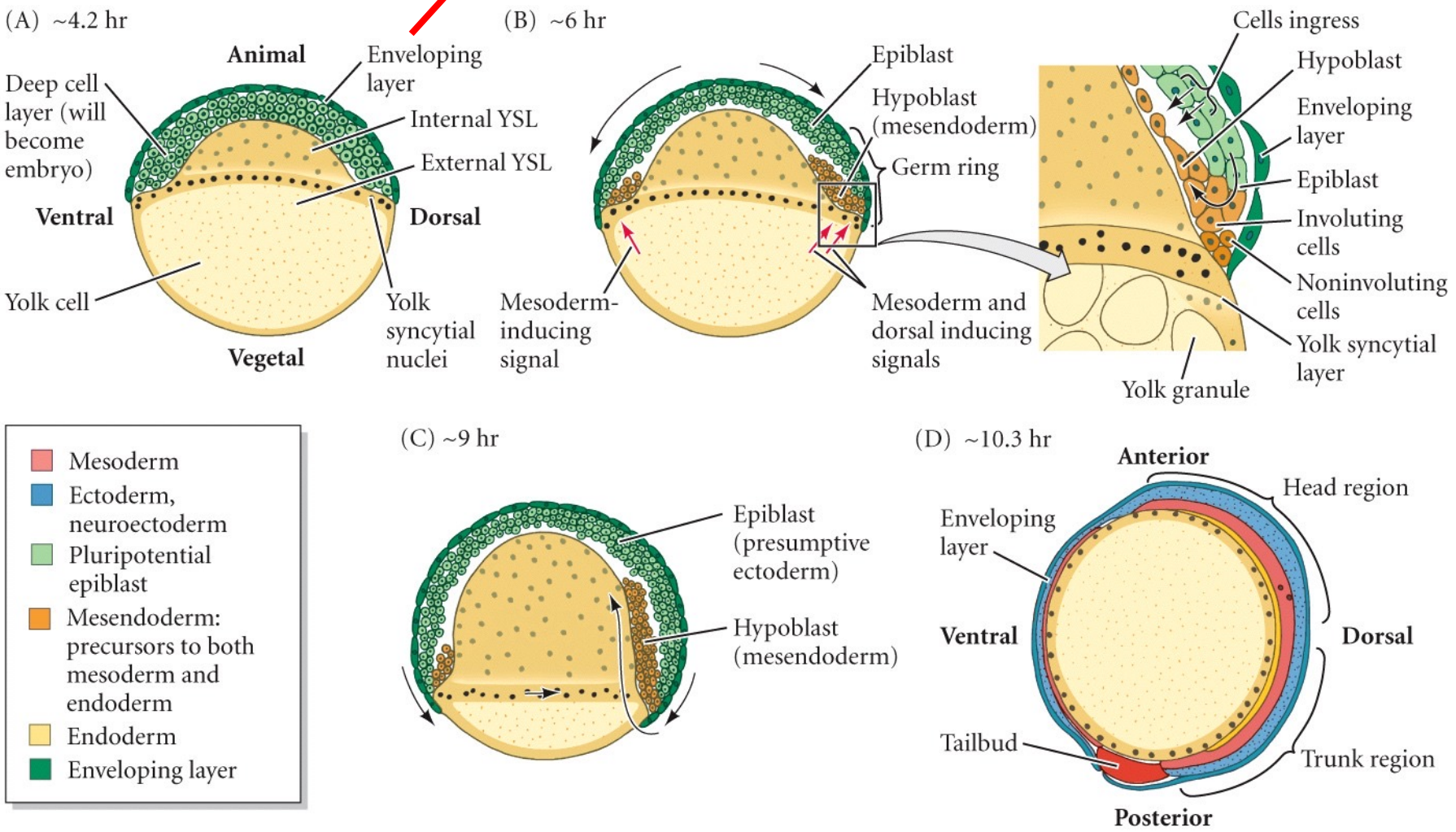

Zygote Period
- 0 - 0.75 hpf
a
BD
AP
Yolk
VP
1-cell
0.2 hpf
Cleavage Period - 0.75 - 2.25 hpf
b
2-cell
0.75 hpf
c
4-cell
1 hpf
d
8-cell
1.25 hpf
e
16-cell
1.5 hpf
f
32-cell
1.75 hpf
g
64-cell
2 hpf
Blastula Period - 2.25 - 5.25 hpf
h
128-cell
2.25 hpf
i
EVL
thins
Deep
cells
256-cell
2.5 hpf
j
512-cell
2.75 hpf
k
YSL
forms
1k-cell
3 hpf
l
high
3.3 hpf
m
oblong
3.7 hpf
n
sphere
4 hpf
Gastrula Period - 5.25 - 10 hpf
o
dome
4.3 hpf
p
30% epiboly
4.7 hpf
q
50% epiboly
5.3 hpf
r
germ ring
5.7 hpf
s
V
D
Shield
shield
6 hpf
t
75% epiboly
8 hpf
u
90% epiboly
9 hpf
Segmentation Period - 10 - 24 hpf
v
Ant.
V
D
Pos.
bud
10 hpf
w
3-somite
11 hpf
x
6-somite
12 hpf
y
10-somite
14 hpf
z
14-somite
16 hpf
250 µm
Segmentation Period
continues to 24 hpf

Gastrulação no peixe zebra

Uma camada protetora

Nota que a terminologia no peixe <u>difere</u> relativamente aos amniotas:

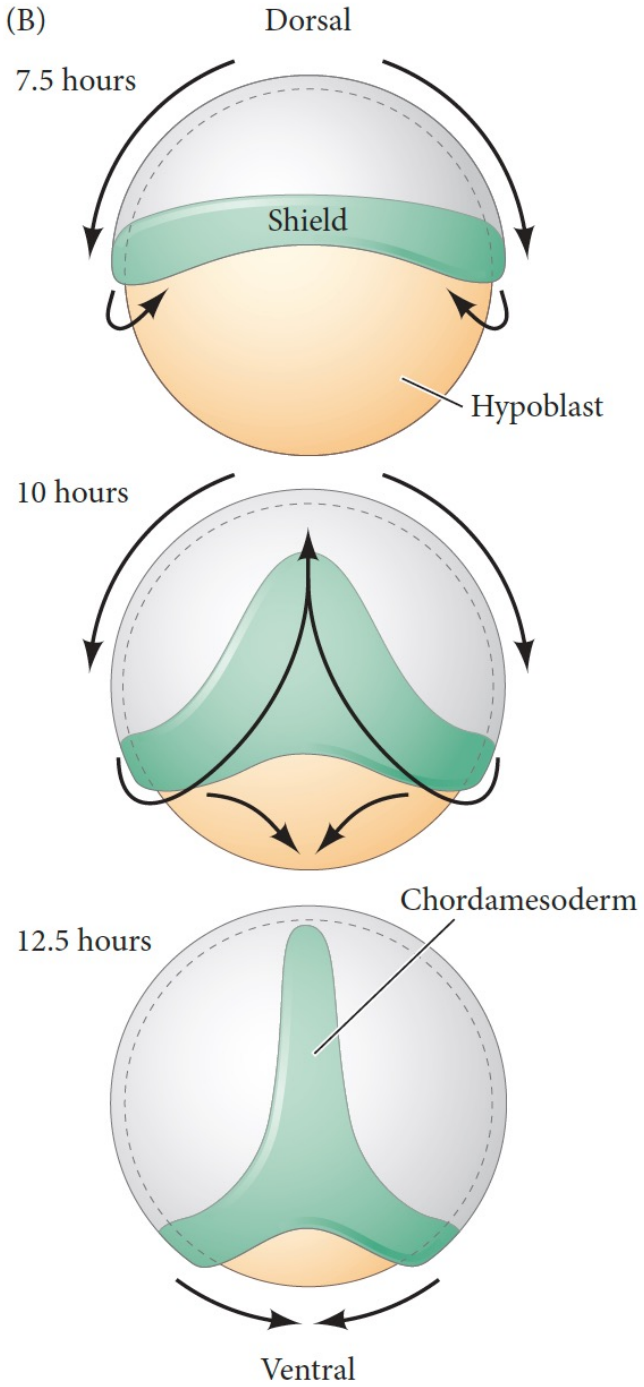
- Corion peixe=camada vitelina outros embriões;
- Deep cell layer peixe=epiblasto amniotas;
- Epiblasto peixe=ectoderme presuntiva amniotas;
- Hipoblasto peixe=mesoderme e endoderme presuntiva amniotas

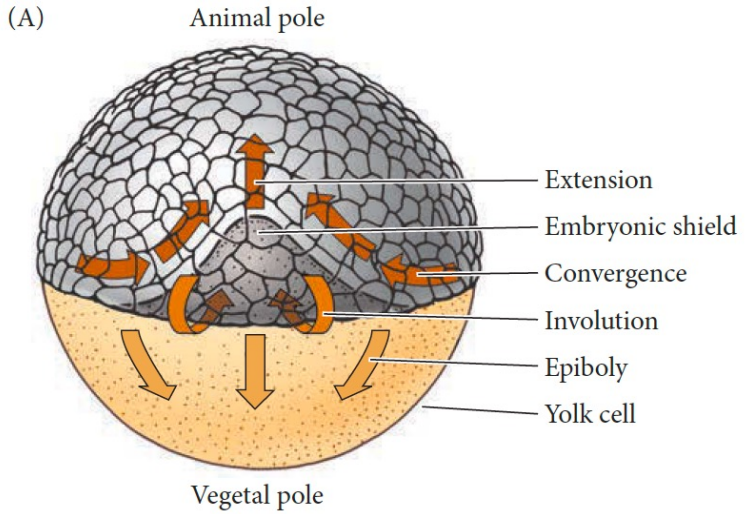


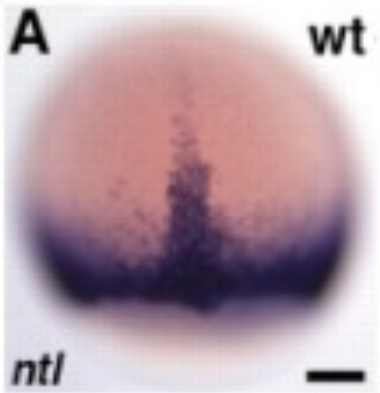
***DEVELOPMENTAL BIOLOGY 10e*, Figure 8.41**

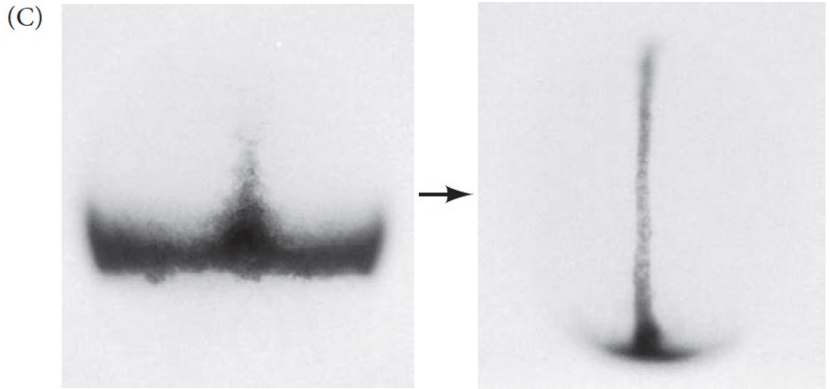
Vista lateral

Gastrulation in fish embryos









Vistas posteriores

O organizador do embrião de peixe foi descoberto com a experiência clássica de transplantação

the embryonic shield is functionally equivalent to the dorsal blastopore lip of amphibians,

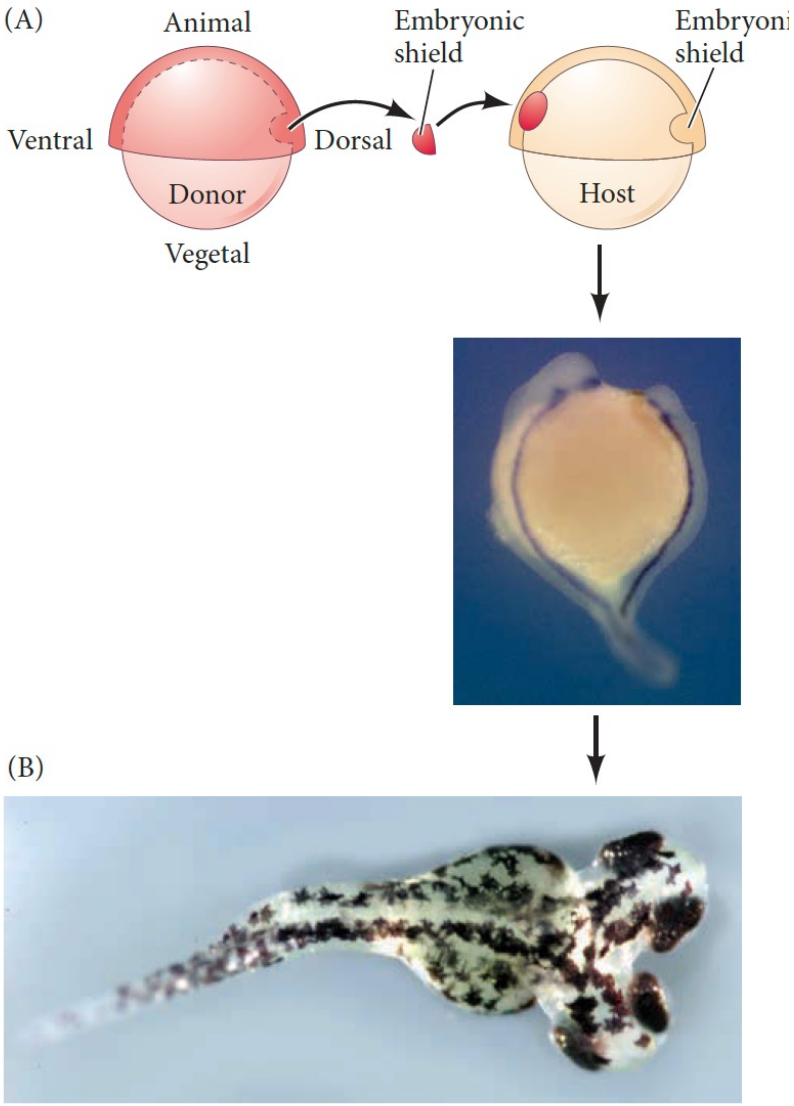
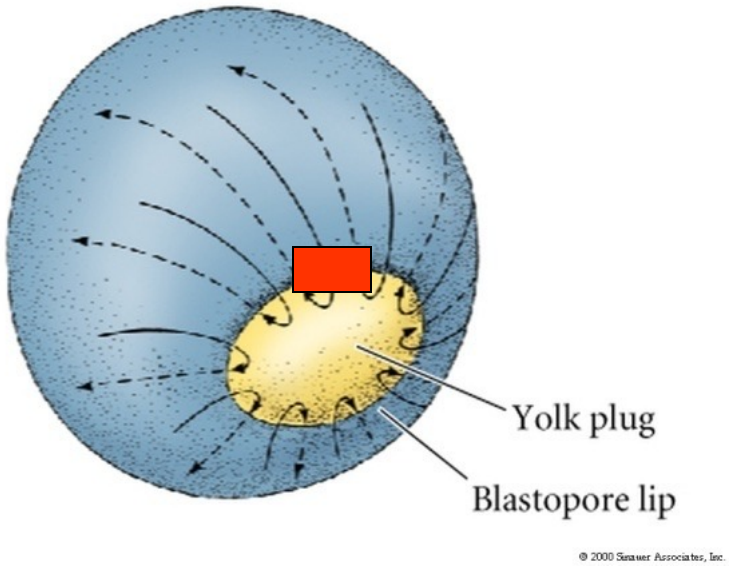
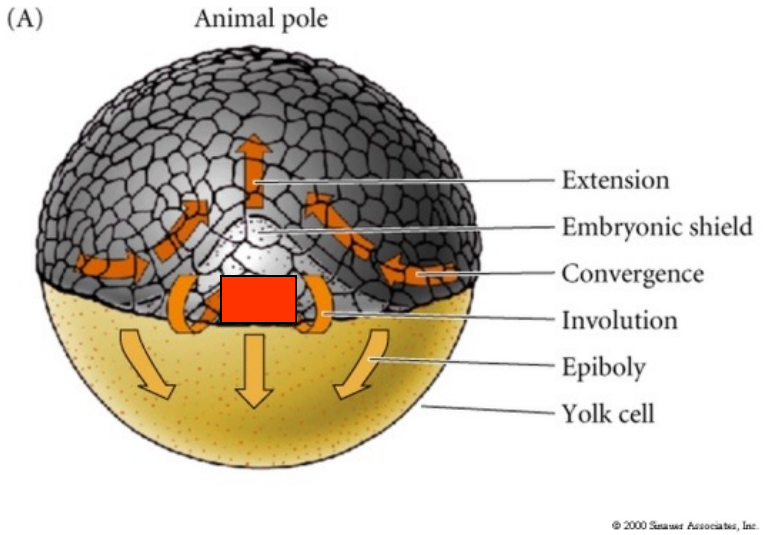
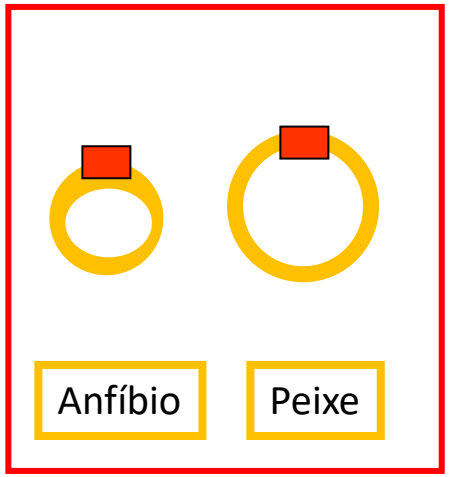


FIGURE 11.40 The embryonic shield

Os organizadores dos embriões de anfíbio e peixe







= organizador

= “blastóporo” lateral/ventral

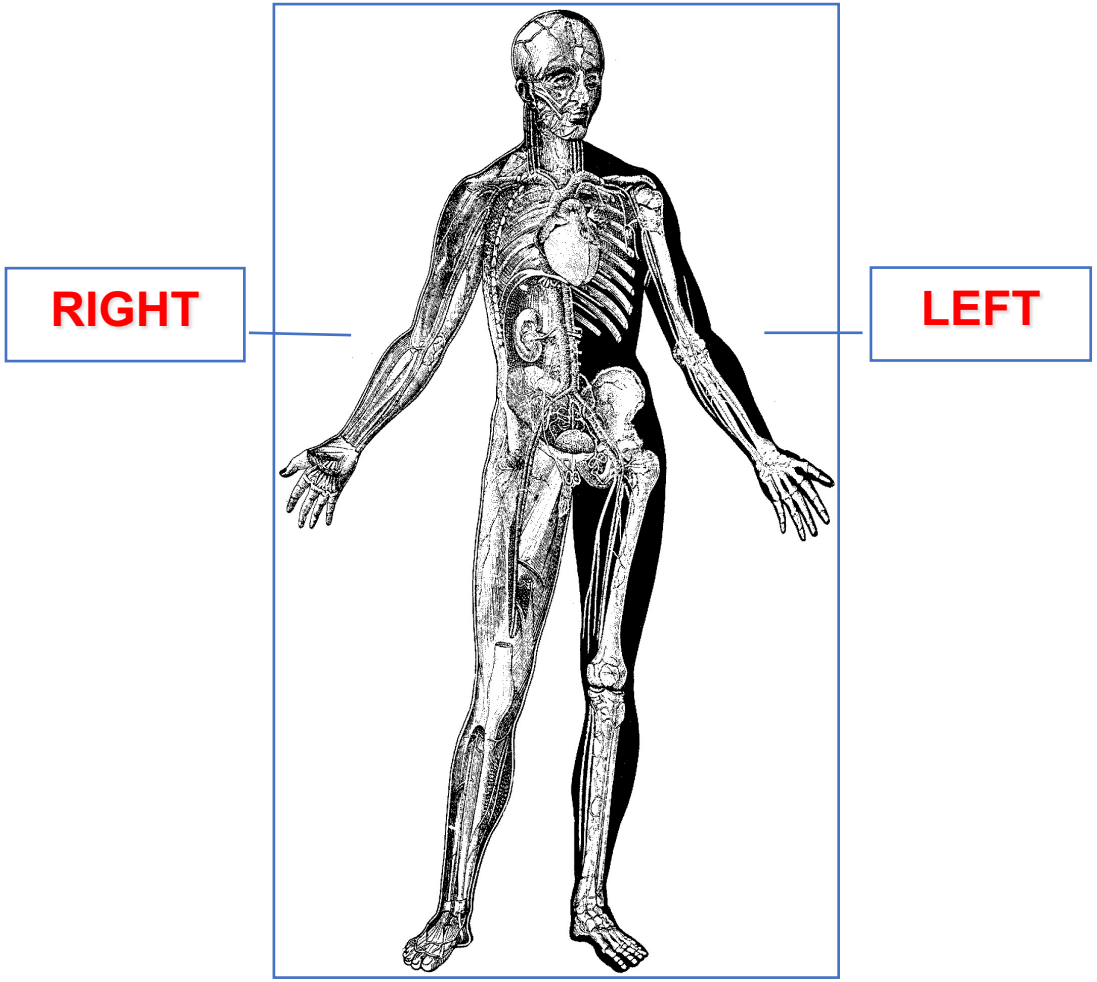
Left-Right Development

Susana S. Lopes

Department of Animal Biology
Faculty of Sciences
University of Lisbon

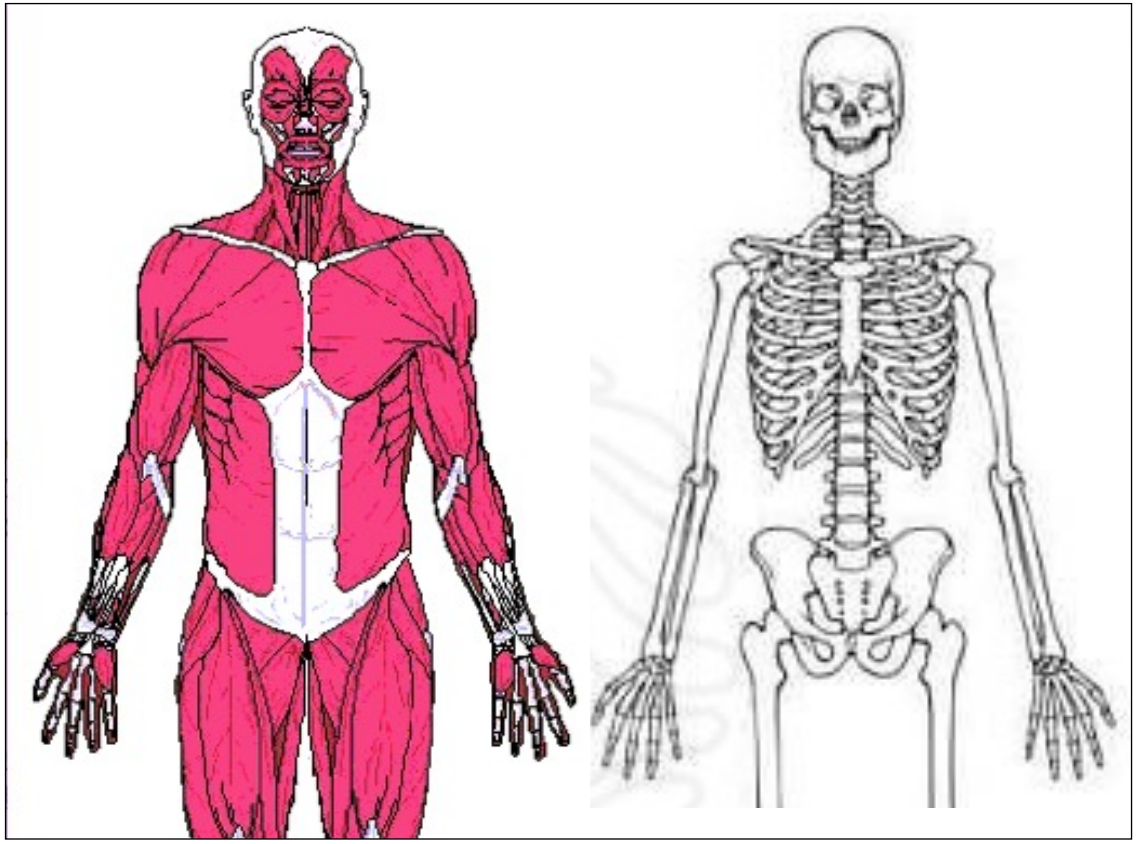
ssalopes@ciencias.ulisboa.pt

Body laterality

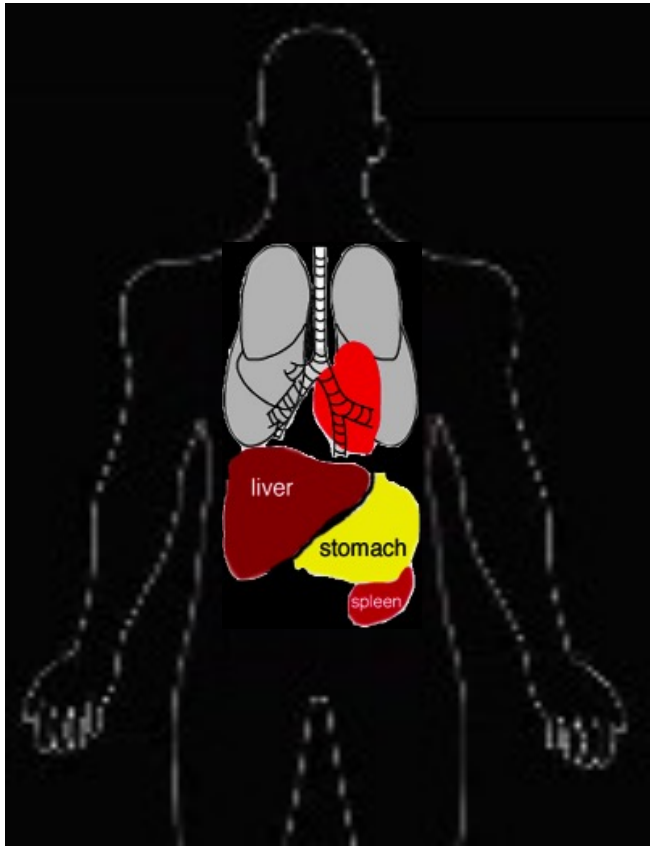


Symmetric outside asymmetric inside

Symmetric
skeleton and axial muscles

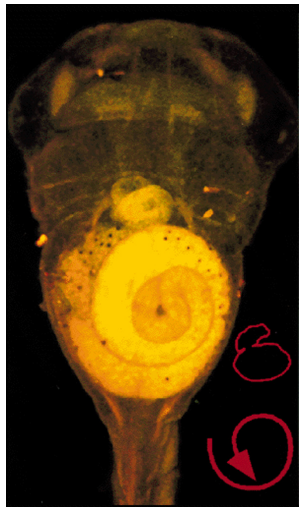


Asymmetric
distribution of the internal organs

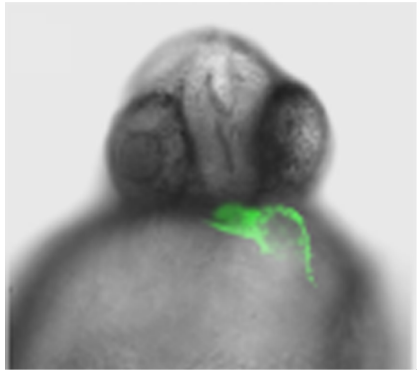


Left-Right Axis

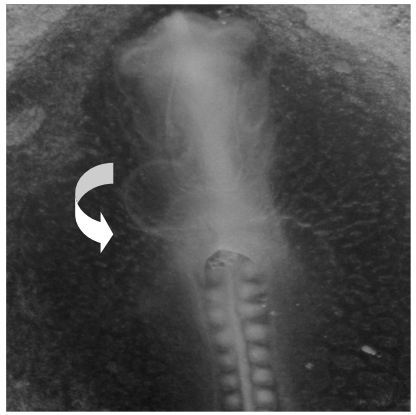
Asymmetric distribution of the internal organs



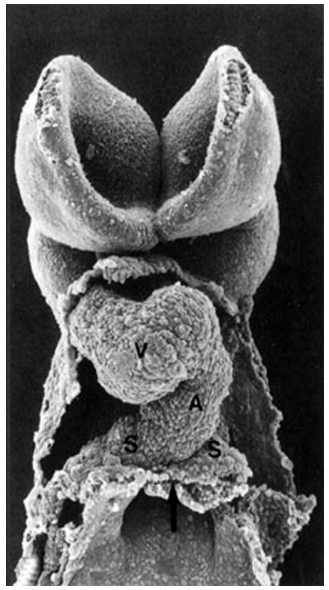
frog



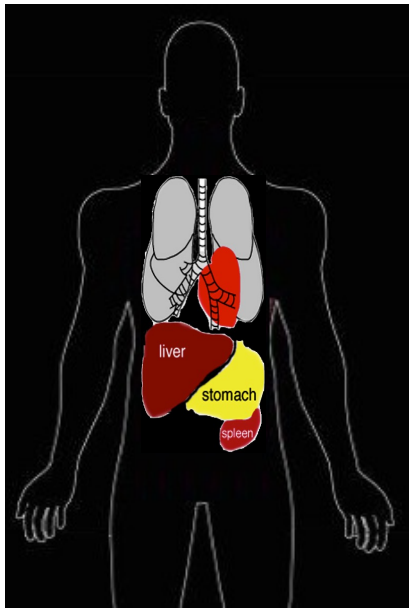
zebrafish



chicken

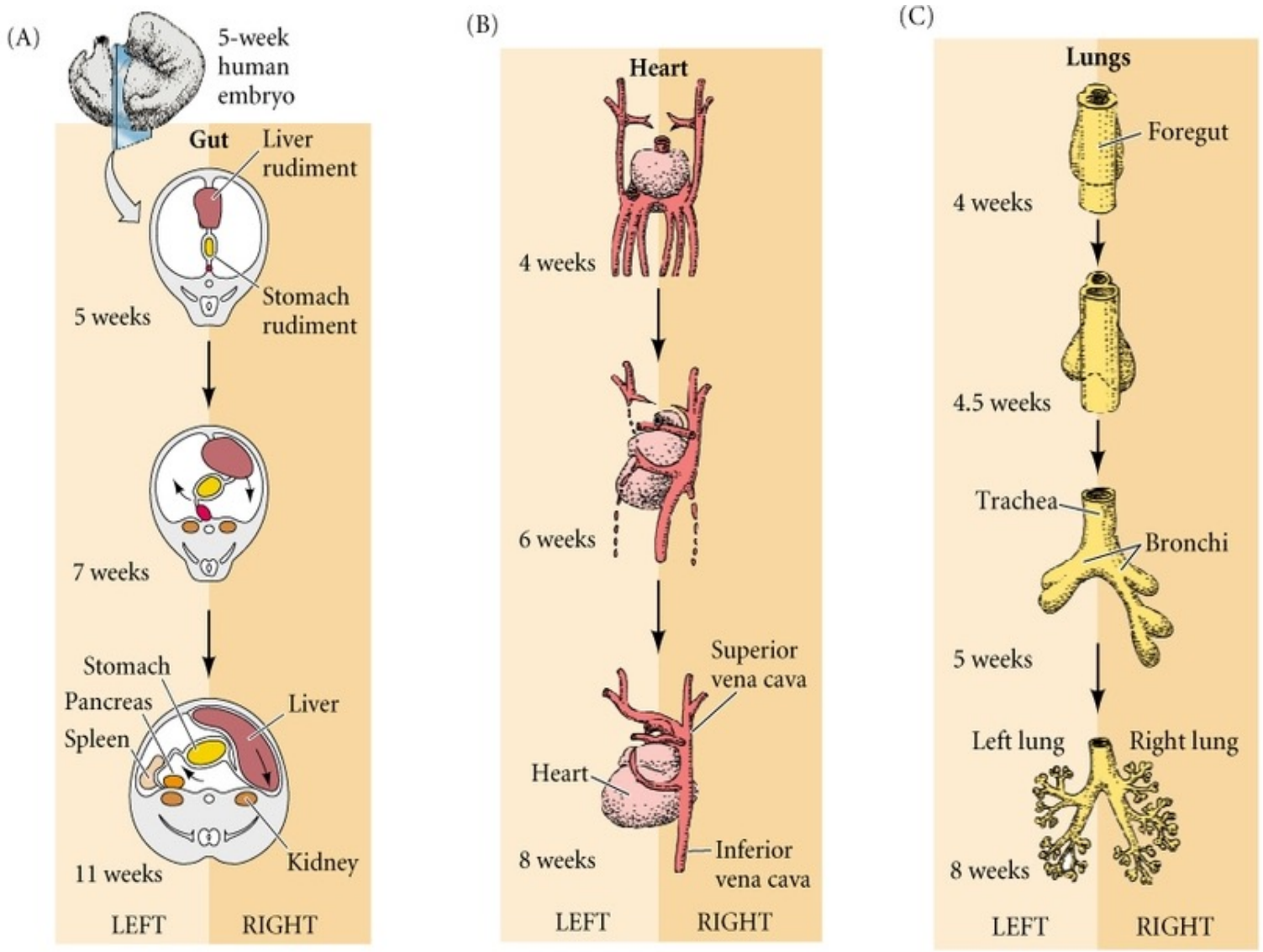


mouse



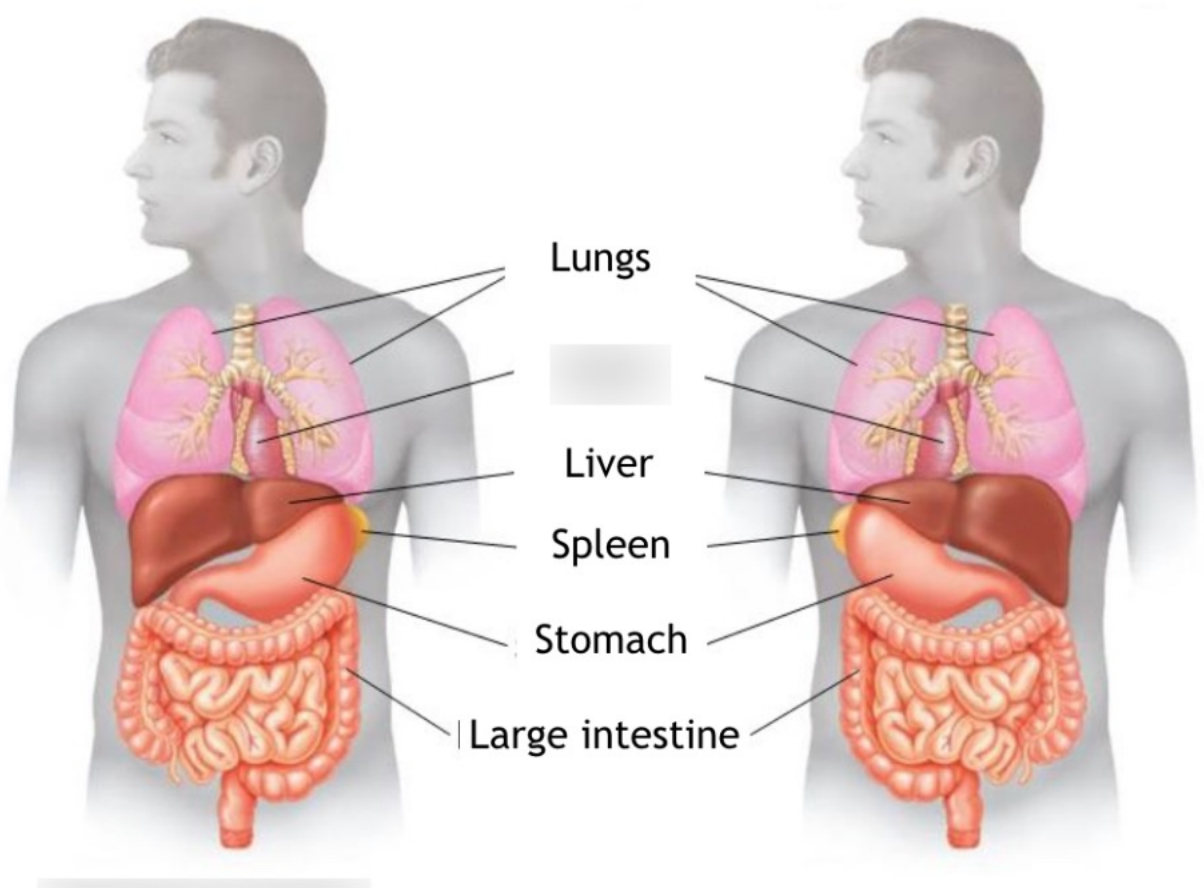
human

Asymmetry of organs

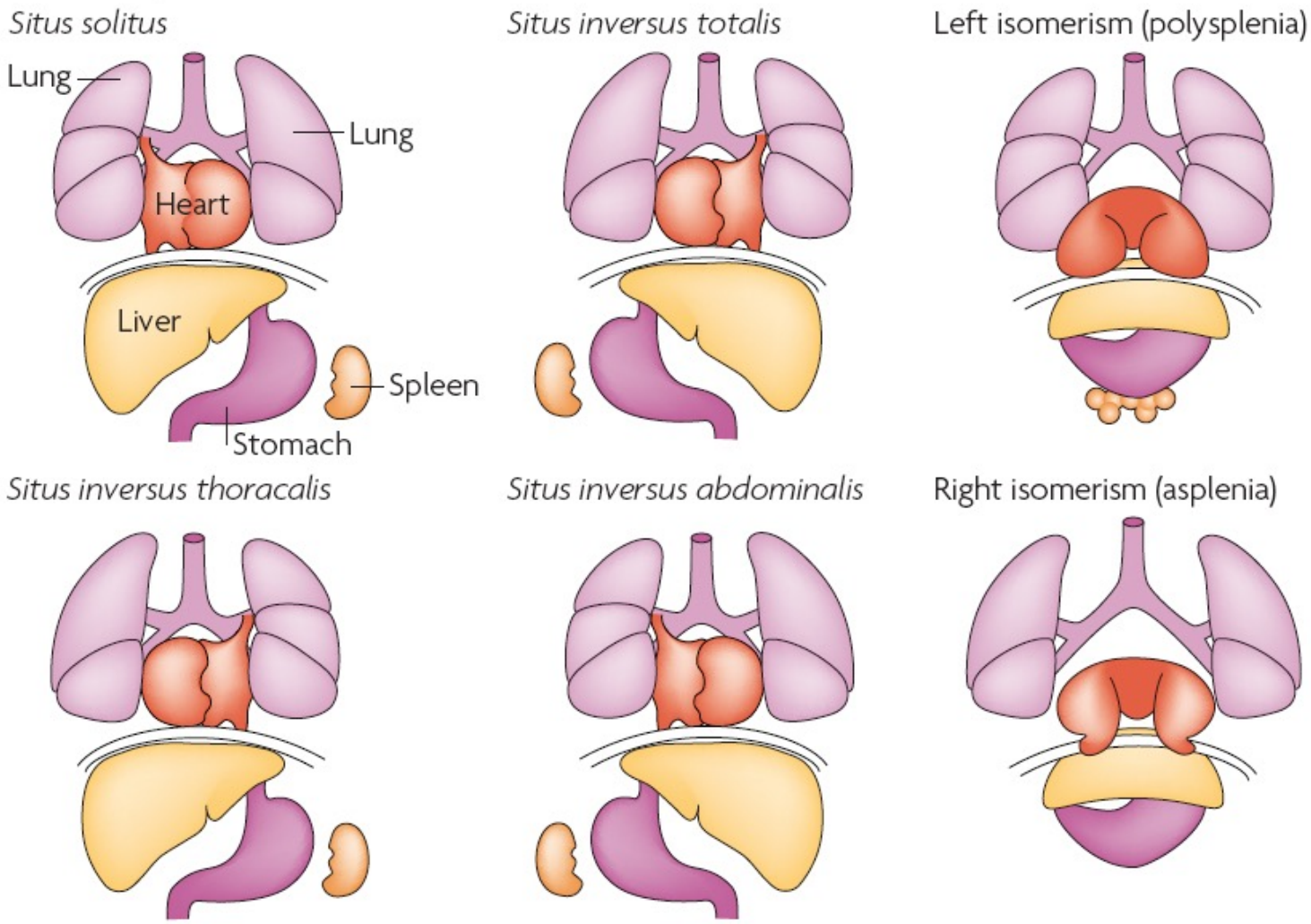


How is body asymmetry generated?

What is the difference?

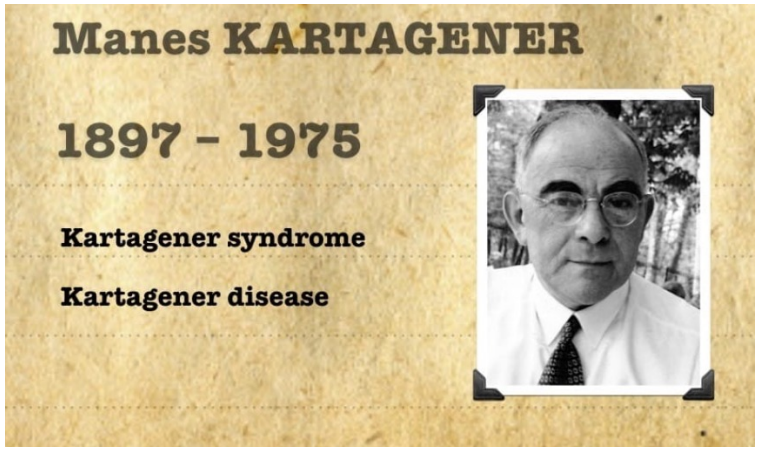


Laterality defects in humans



Fliegauf et al. Nature Rev. Mol.Cell Biol. (2007)

Human patients display a triad of symptoms – Kartagener syndrome



1 - Sinusitis

2 - Bronchiectasis

3 - *situs inversus*

Afzelius BA

Later Afzelius added infertility to the list…

What is the link?

Sinusitis	→	**Upper respiratory epithelium is ciliated**
Bronchiectasis	→	**Lower respiratory epithelium is ciliated**
Infertility	→	**Sperm cells have 1 flagellum**
situs inversus	→	**????**

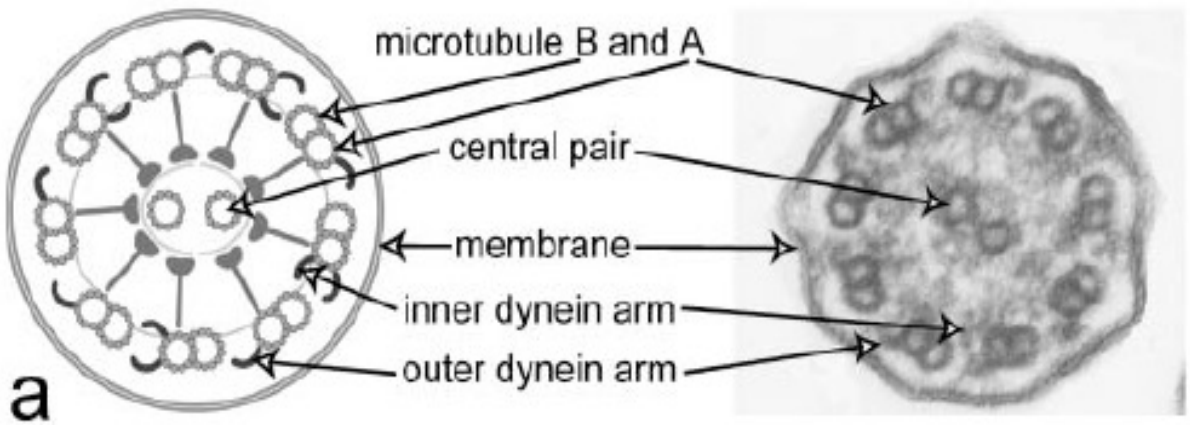
A Human Syndrome Caused by Immotile Cilia

Abstract. *Four subjects who produced immotile sperm were studied. In three of the subjects, who had frequent bronchitis and sinusitis, there was no mucociliary transport, as measured by tracheobronchial clearance. Electron microscopy indicated that cilia from cells of these patients lack dynein arms.*

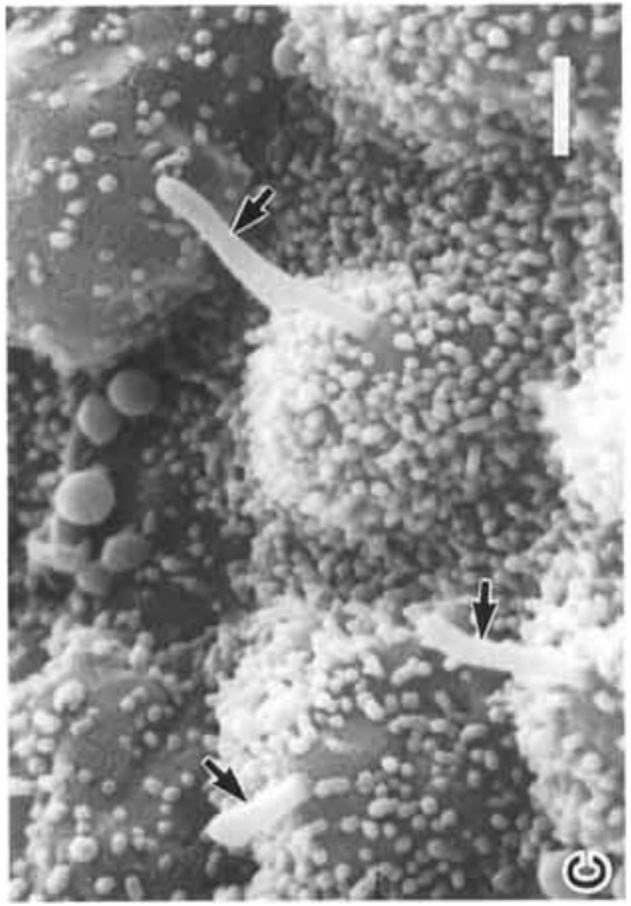
... Three of the subjects have situs inversus totalis ...

... Visceral asymmetry is determined through the movements of cilia of some embryonic epithelial tissues.

Afzelius, Science (1976)

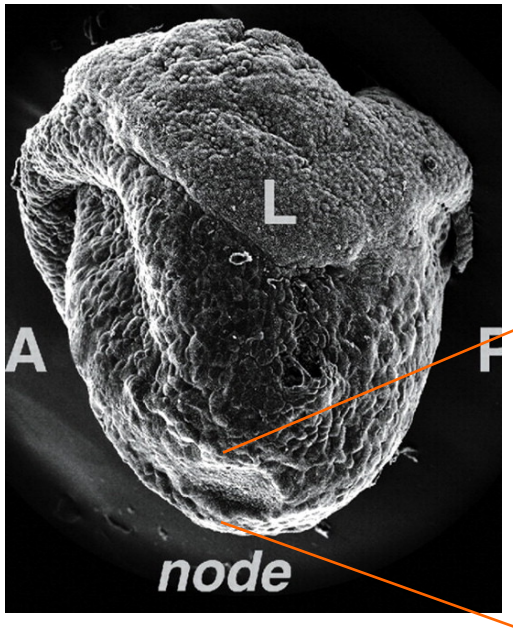


What is the embryonic epithelial tissue?

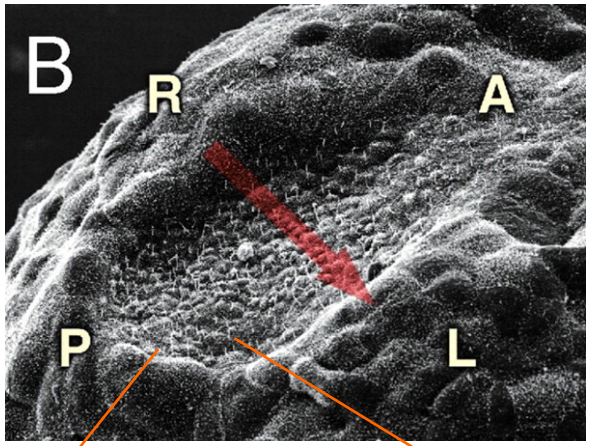


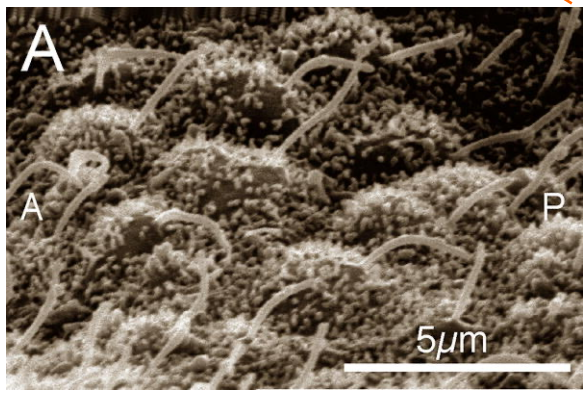
Sulik K, Dehart DB, Inagaki T, Carson JL, Vrablic T, Gesteland K, Schoenwolf GC. 1994. Morphogenesis of the murine node and notochordal plate. *Dev Dyn* 201: 260–278.

What is the embryonic epithelial tissue?



in the mouse embryonic node

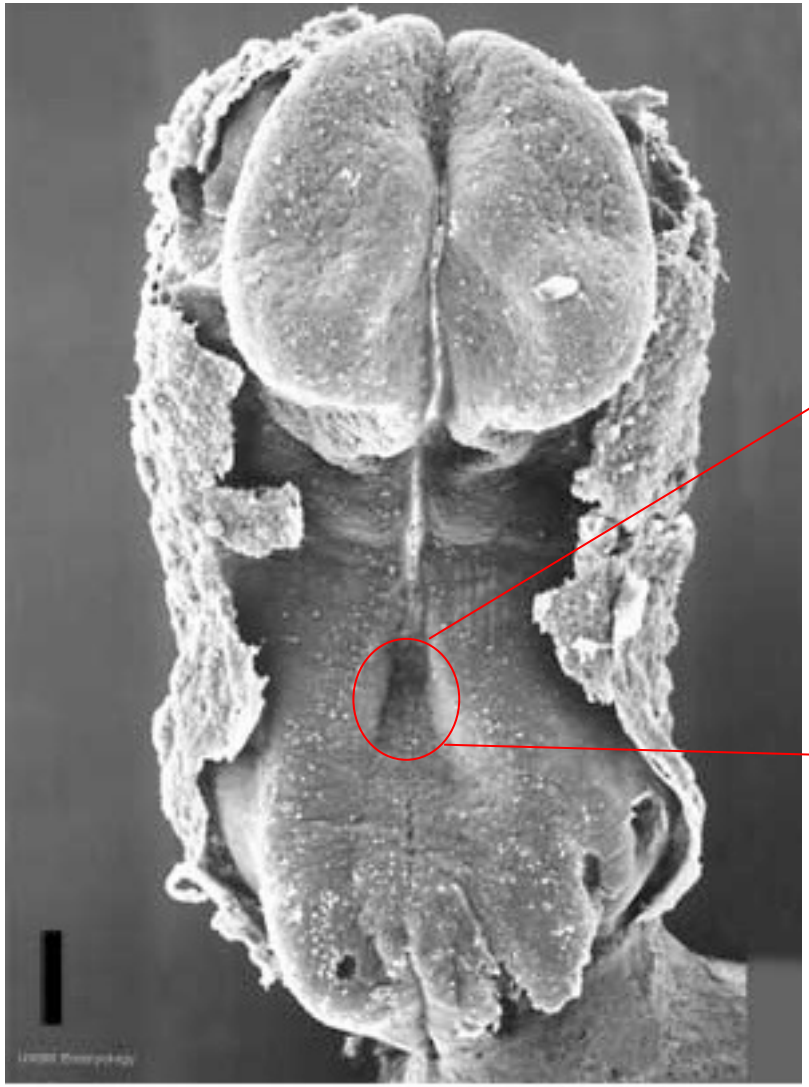


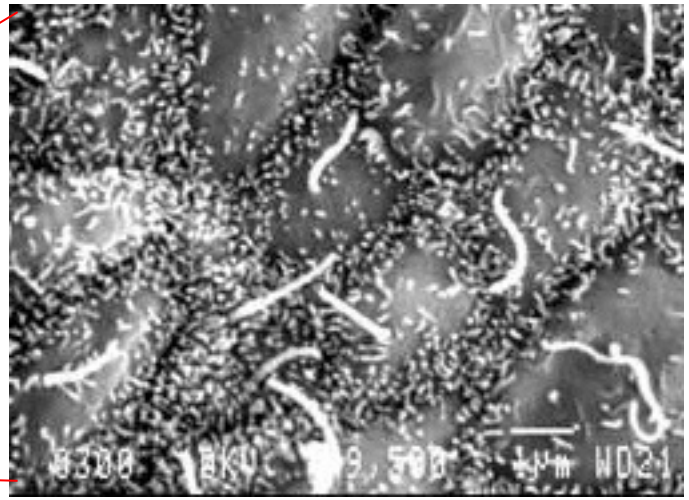


Cilia are on the surface of node cells

Shiratori and Hamada, Development. 2006

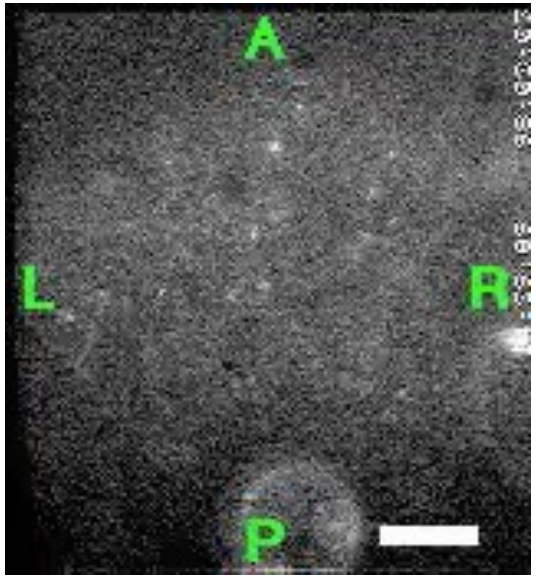
Human primitive node = L-R organizer





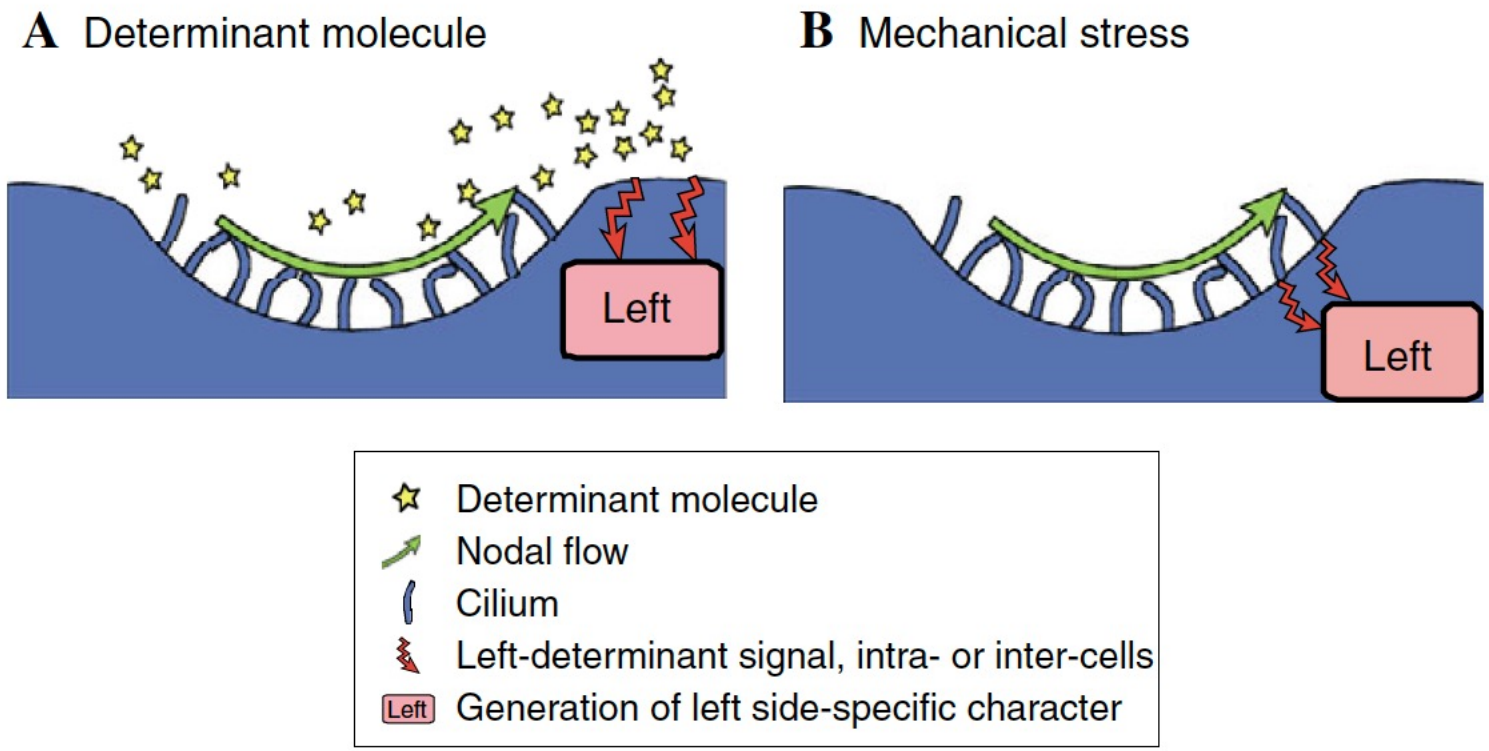
3 weeks

Node cilia generate a directional fluid flow towards the left



Nonaka et al. Cell (1998)

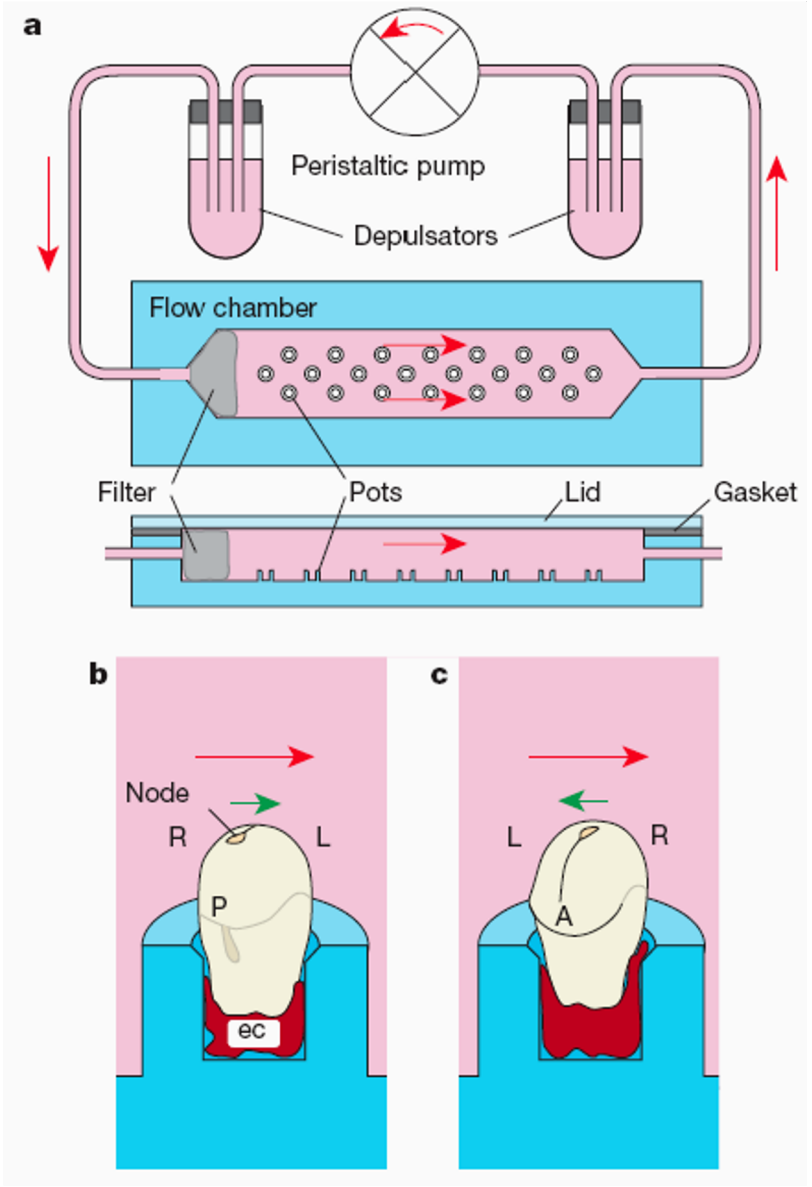
Mouse mutants with immotile, shorter or absent cilia have laterality defects



Nonaka et al. Cell (1998)

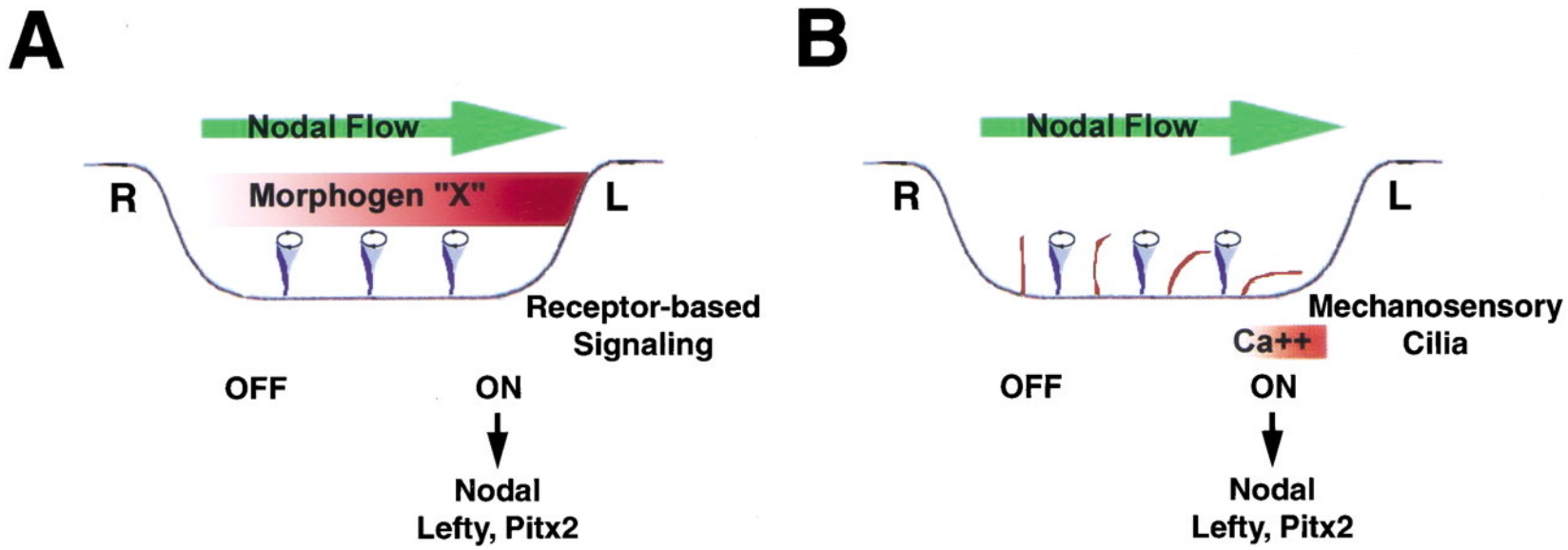
How could we test if the direction of fluid flow is important?

Testing the role of the nodal flow



(Nonaka *et al.* Nature 2002)

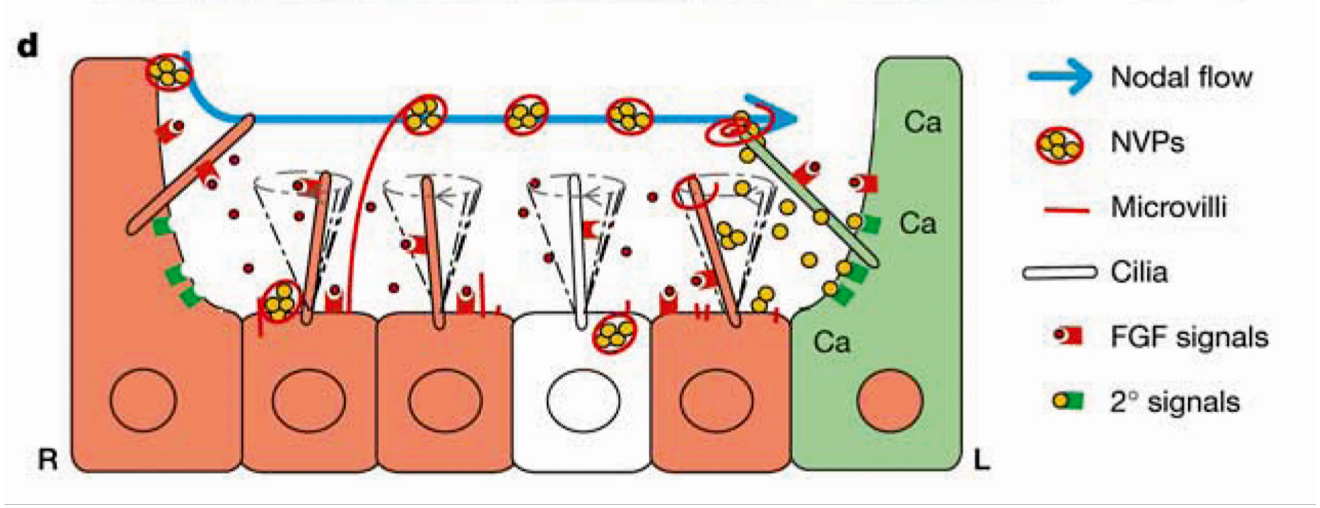
Alternative models of the proposed signaling events functioning downstream of nodal flow.



Clifford J. Tabin, and Kyle J. Vogan Genes Dev. 2003;17:1-6

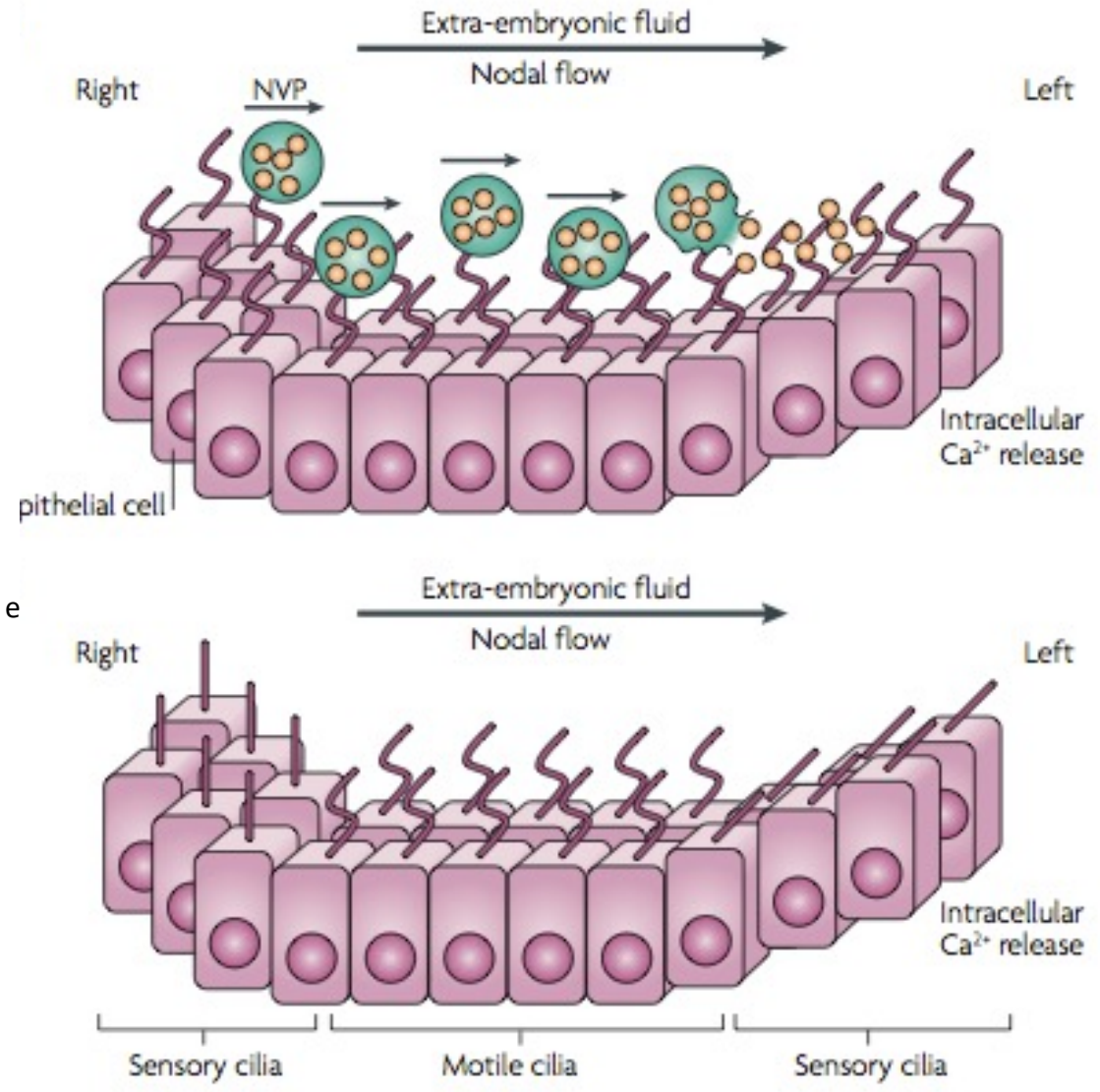
How does the Fluid Flow confer LR asymmetry?

“The updated morphogen model to NVP model”



Tanaka, Y., Okada, Y. and Hirokawa, N. (2005). FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. Nature 435, 172-177.

Are these two models compatible?



Fliegauf, M., Benzing, T. & Omran, H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* **8**, 880–893 (2007). https://doi.org/10.1038/nrm2278

Physicists asked: How can rotating cilia generate a laminar flow?

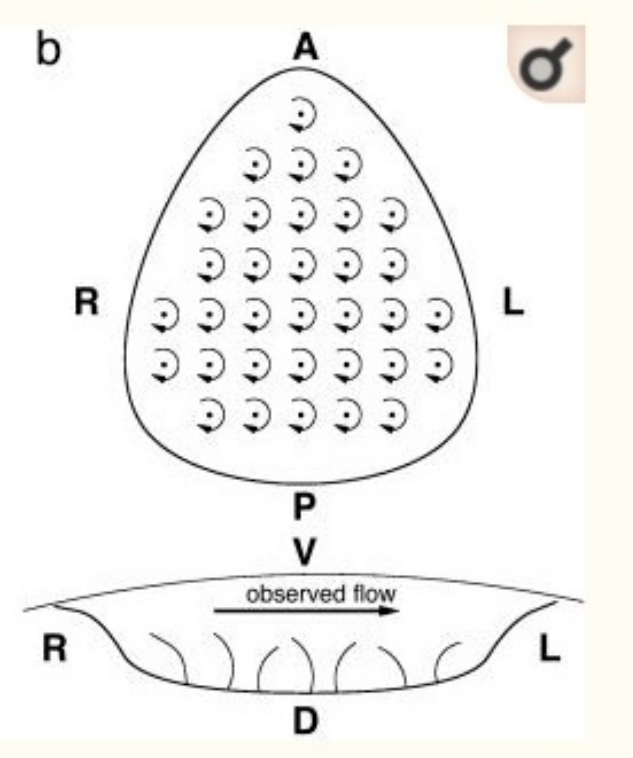
The relevance of numerical simulations

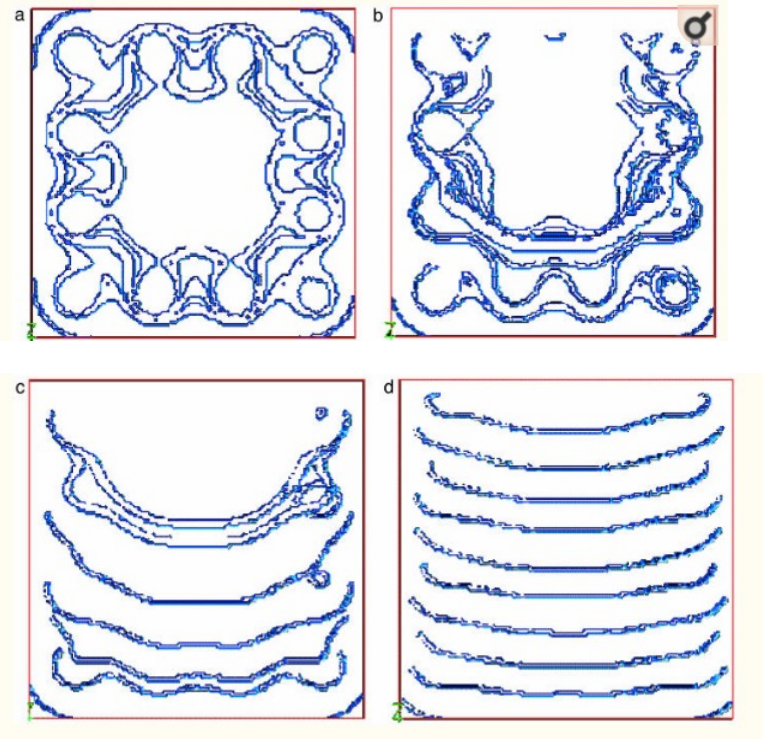
Proc Natl Acad Sci U S A. 2004 May 11; 101(19): 7234–7239.
Published online 2004 Apr 26. doi: 10.1073/pnas.0402001101
Developmental Biology, Physics

PMCID: PMC409902
PMID: 15118088

Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates

Julyan H. E. Cartwright,*† Oreste Piro,‡† and Idan Tuval‡†



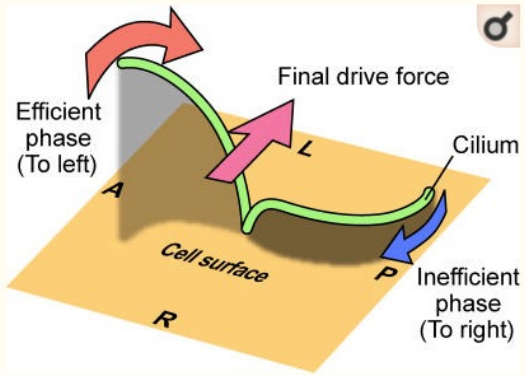


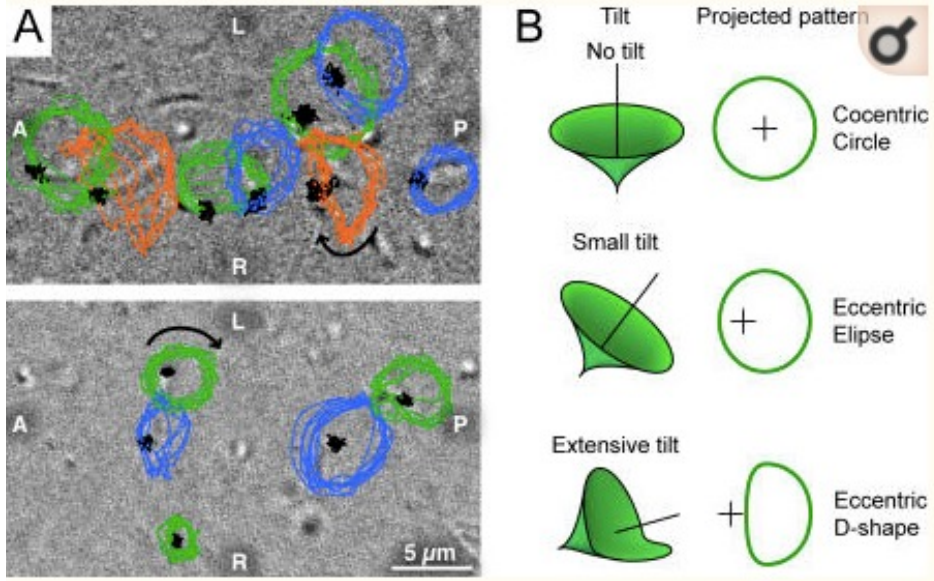
Biologists confirmed cilia were tilted

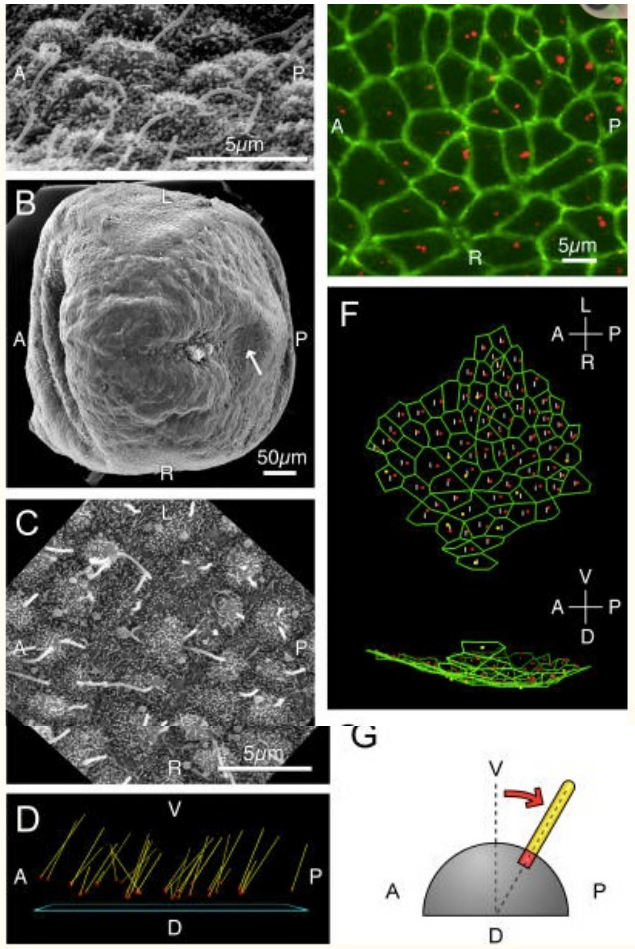
PLoS Biol. 2005 Aug; 3(8): e268.
Published online 2005 Jul 26. doi: 10.1371/journal.pbio.0030268
PMCID: PMC1180513
PMID: 16035921

De Novo Formation of Left–Right Asymmetry by Posterior Tilt of Nodal Cilia

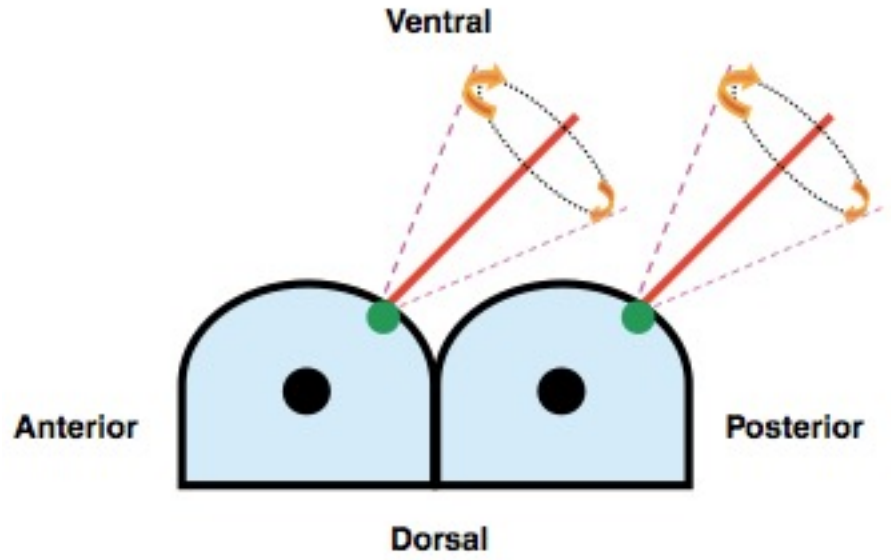
Shigenori Nonaka,[1,2] Satoko Yoshiba,[1,3] Daisuke Watanabe,[1,4] Shingo Ikeuchi,[1,3] Tomonobu Goto,[5] Wallace F Marshall,[2] and Hiroshi Hamada[1,3]

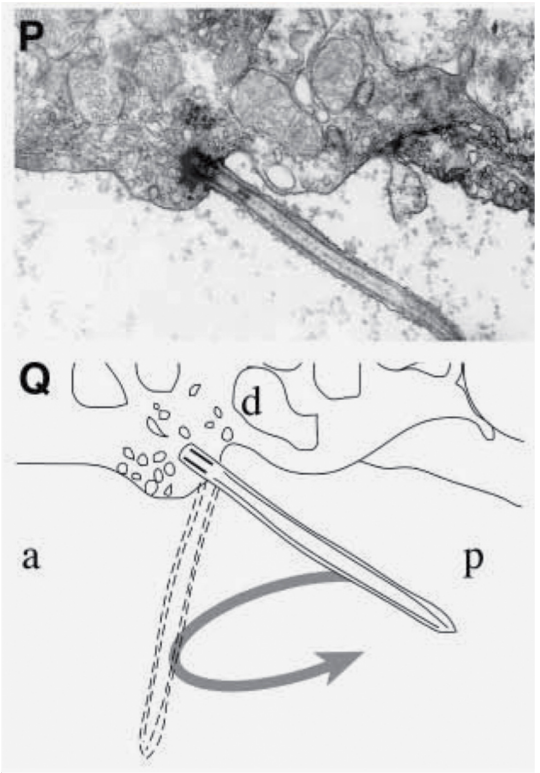






Zebrafish LRO cilia also have a posterior tilt





Kramer-Zucker *et al.*, ***Development*** (2005)

Polarity of the epithelial cells - planar cell polarity

Physicists asked: How can NVPs be thrown and explode at low Reynolds number ?

$$Re = \frac{\rho u L}{\mu}$$

Re = reynolds number

ρ = density of the fluid

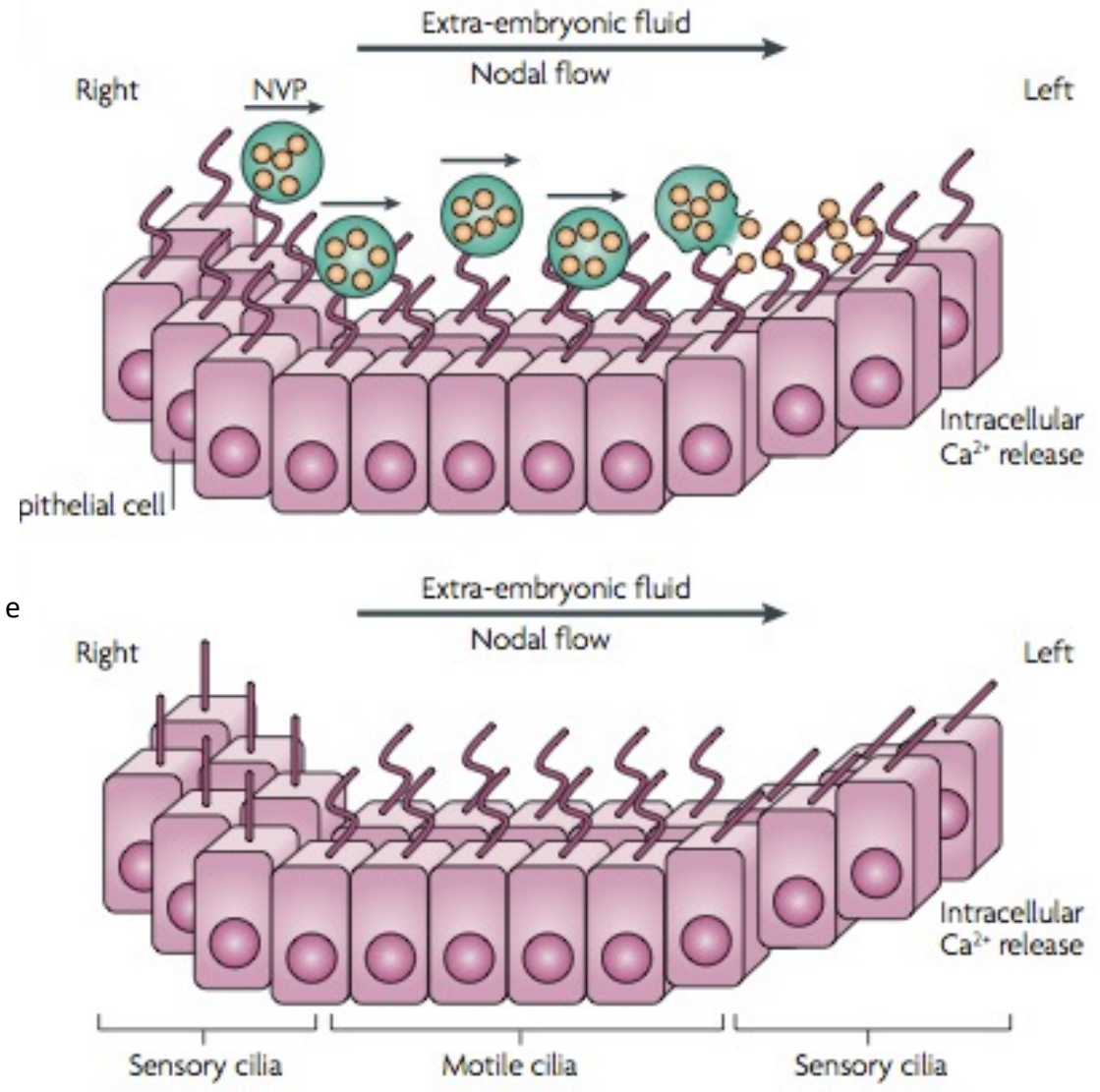
u = flow speed

L = characteristic linear dimension

μ = dynamic viscosity of the fluid

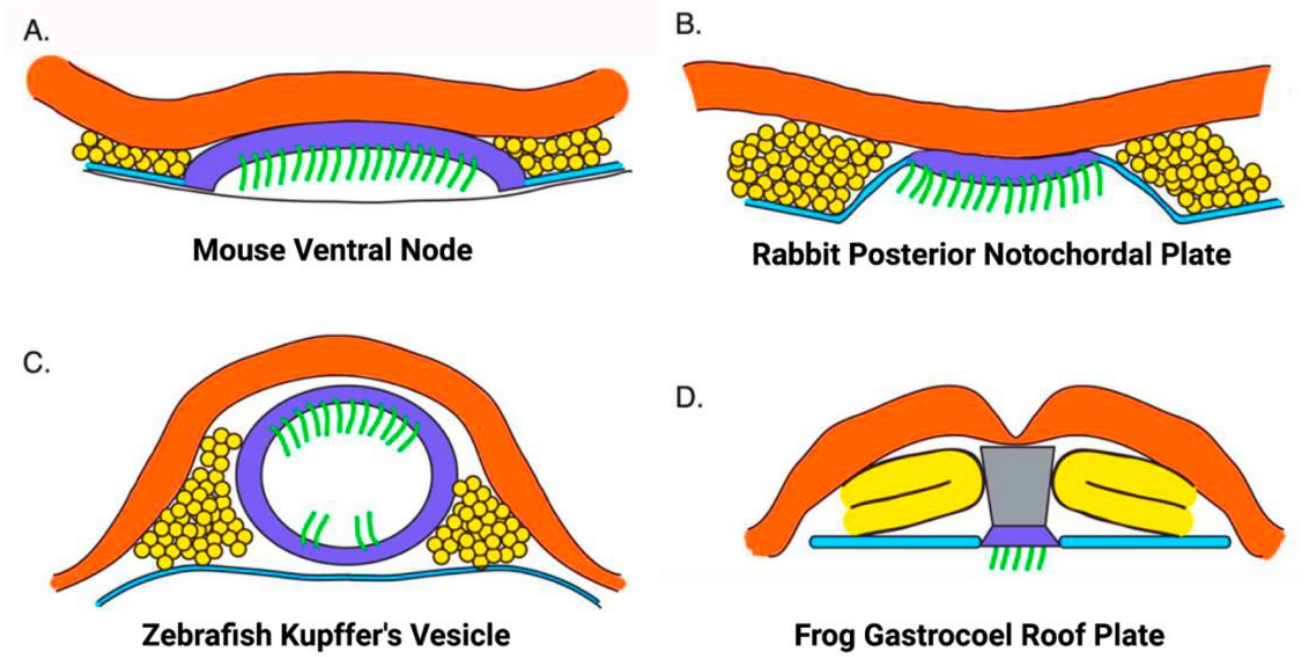
laminar flow occurs at low Reynolds numbers, where viscous forces are dominant, and is characterized by smooth, constant fluid motion.

Problem for the 1st model



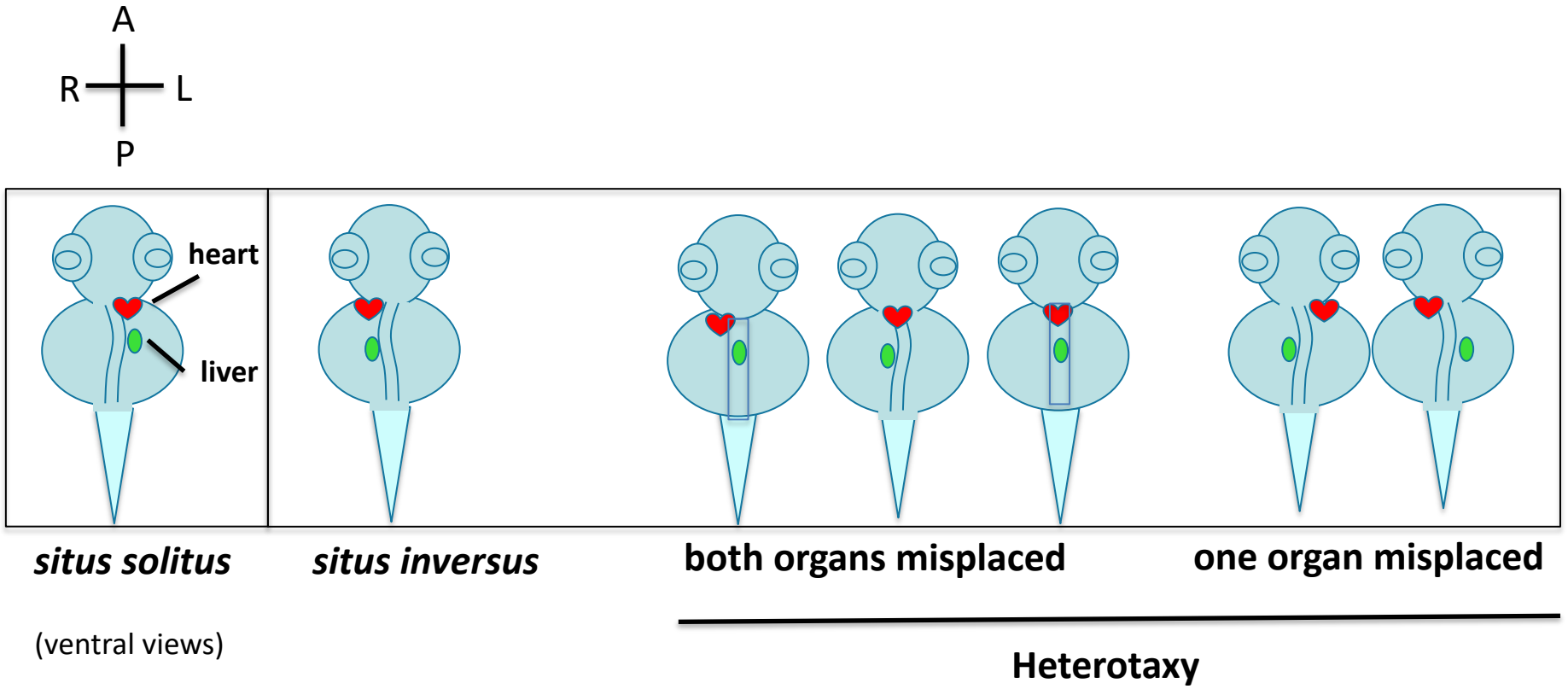
Fliegauf, M., Benzing, T. & Omran, H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* **8**, 880–893 (2007). https://doi.org/10.1038/nrm2278

Morphological diversity in left-right organizers amongst vertebrate species

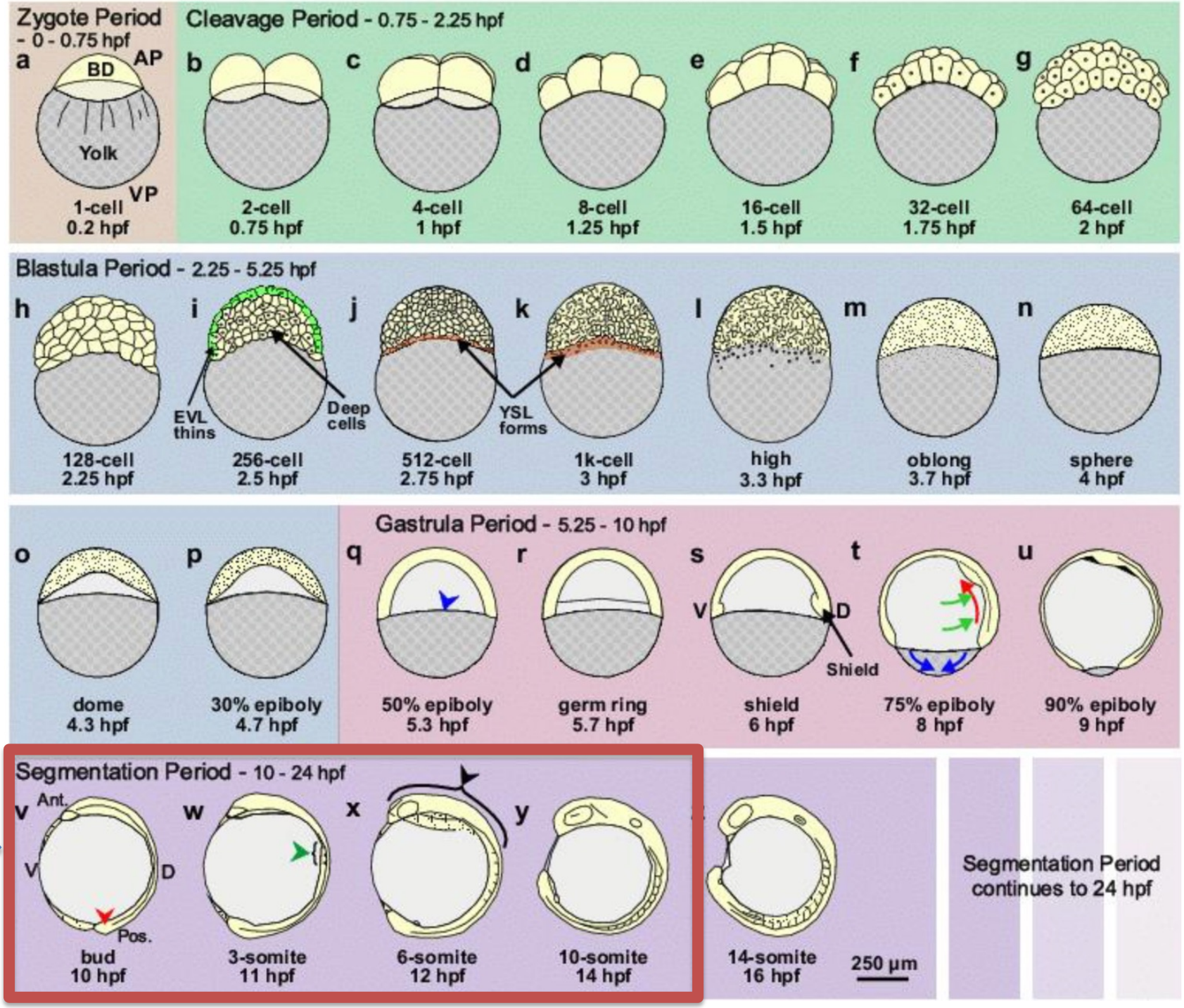


Transverse view, dorsal is up. Ectoderm is orange, paraxial mesoderm is yellow, endoderm/hypoblast is blue, left-right organizer (LRO) is purple, and cilia within the LRO are green. (A) The mouse ventral node and (B) the rabbit posterior notochordal plate are situated beneath the ectoderm and are laterally contiguous with the endoderm. Unlike in rabbit, the ventral pit of the mouse node is enclosed by Reichardt's membrane. (C) Zebrafish Kupffer's vesicle is an enclosed sphere with cilia concentrated on the dorsal anterior surface. (D) The Xenopus gastrocoel roof plate is contiguous with the lateral endoderm, and cilia point into the gastrocoel cavity. Panels are not size-matched. Figure and description adapted from (Lee & Anderson, 2008a).

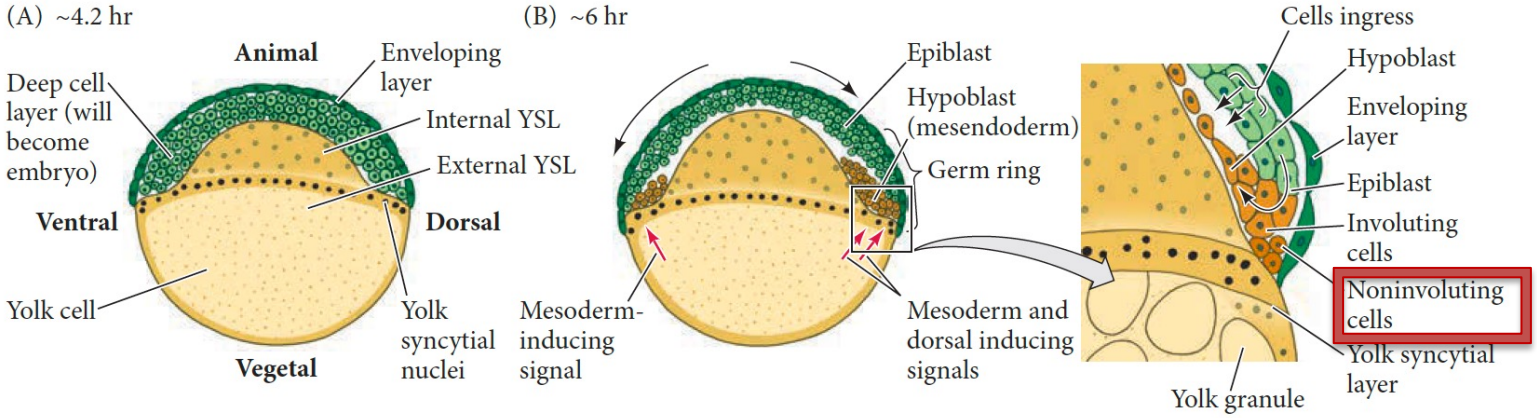
Organ laterality is also affected in zebrafish laterality mutants



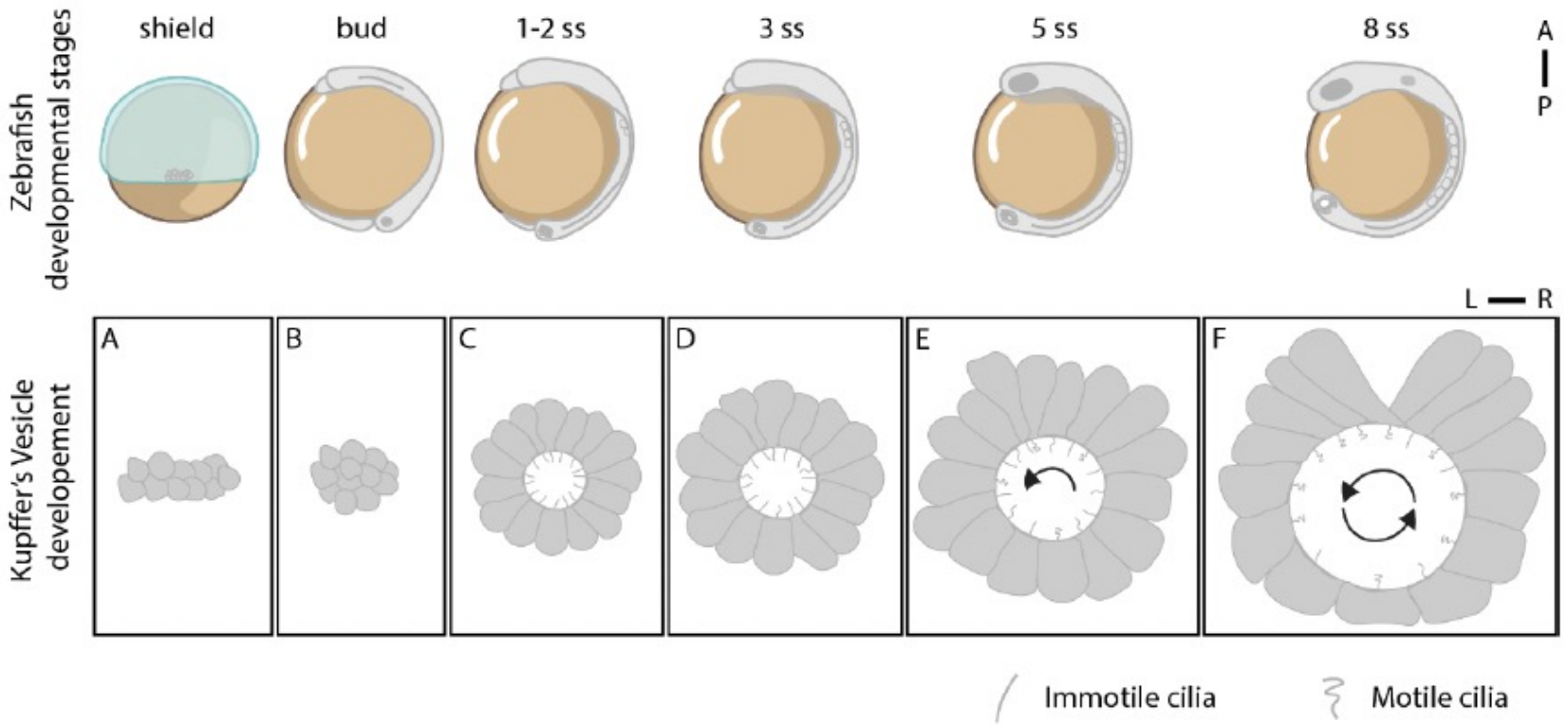
Zebrafish is ideal to study left-right development



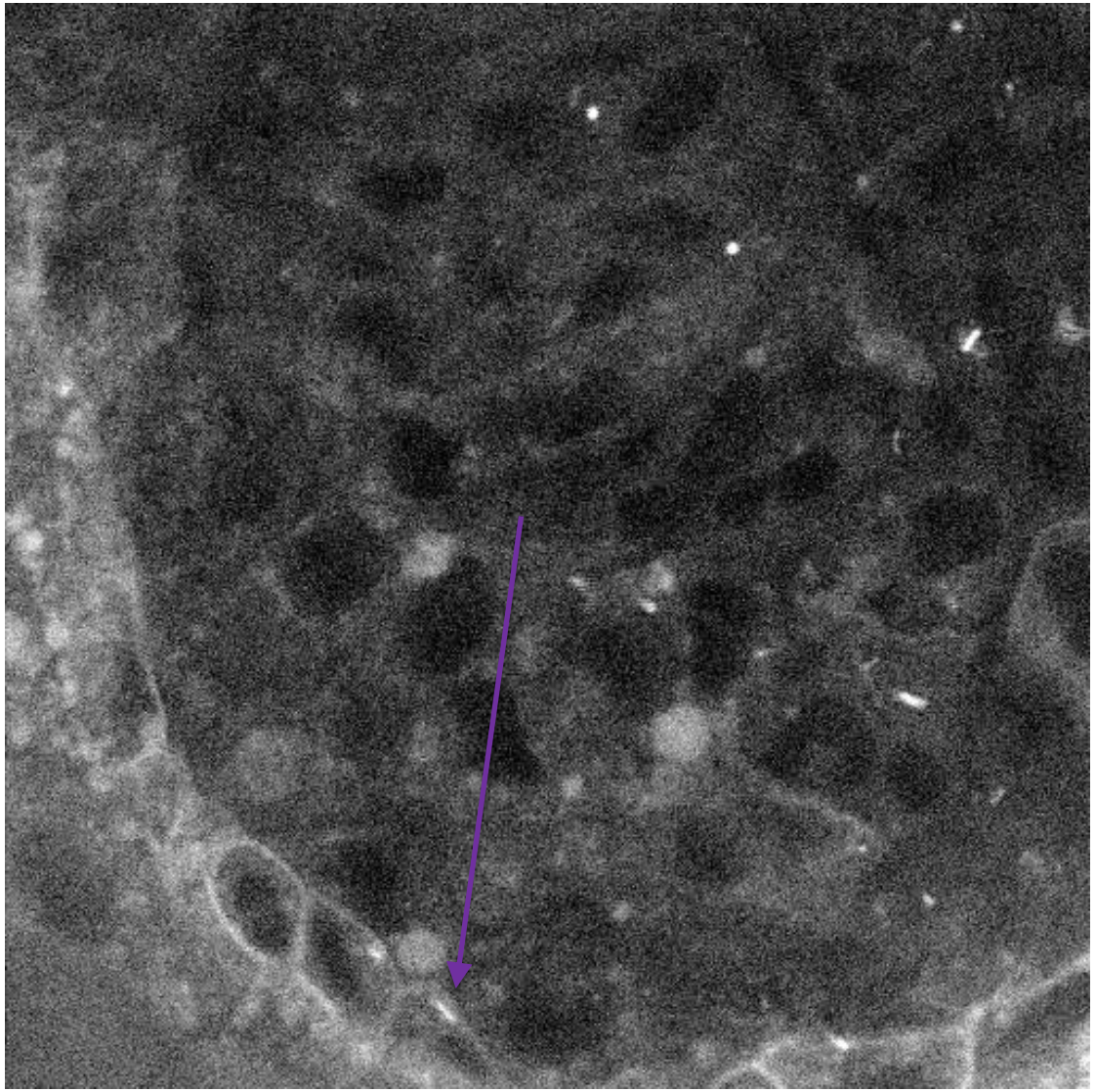
LRO development



Non-involuting cells
give rise to the Kupffer's vesicle
(the fish left-right organizer).



The zebrafish LR organizer has 2 types of cilia

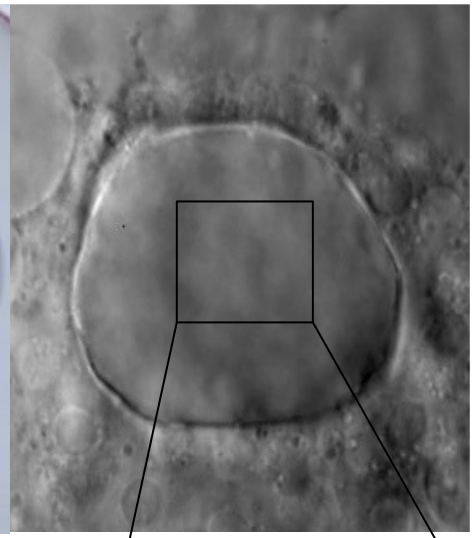


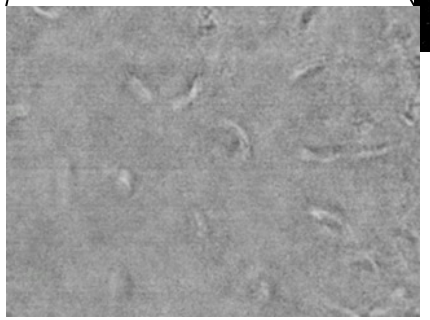
Standing Question!

Tavares et al. eLife (2017)

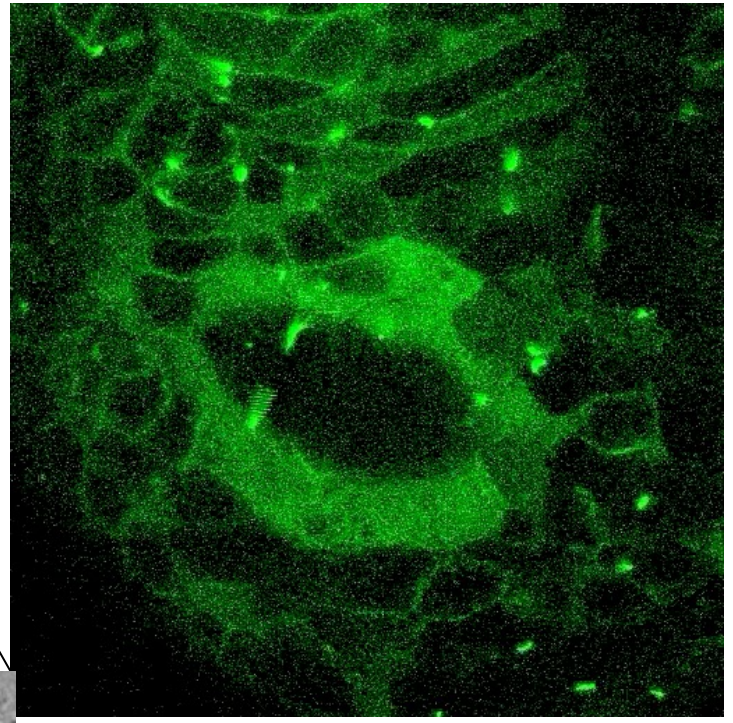
Tg(arl13b-GFP; *foxj1a*:GFP)

The zebrafish embryo is transparent allowing non-invasive live imaging of the left-right organizer





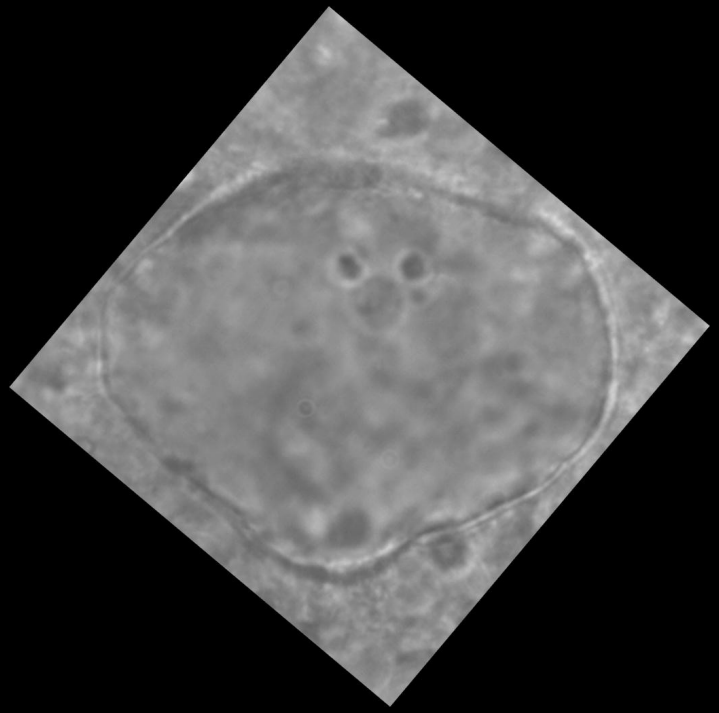
High speed video-microscopy
(2000 fps)



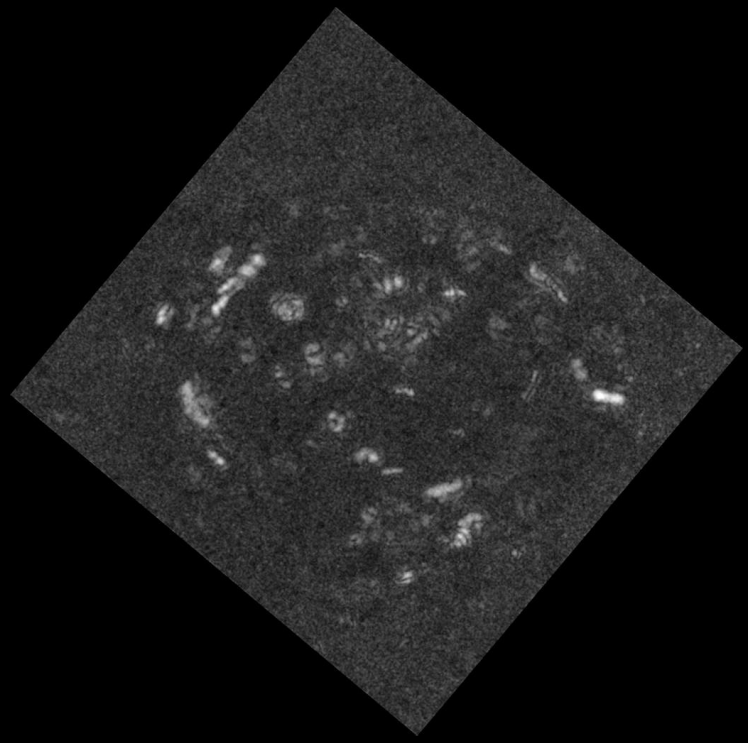
Confocal microscopy
Tg (foxj1a:GFP) + *arl13b* mRNA

Motile cilia also generate flow in the zebrafish LRO

Light microscopy

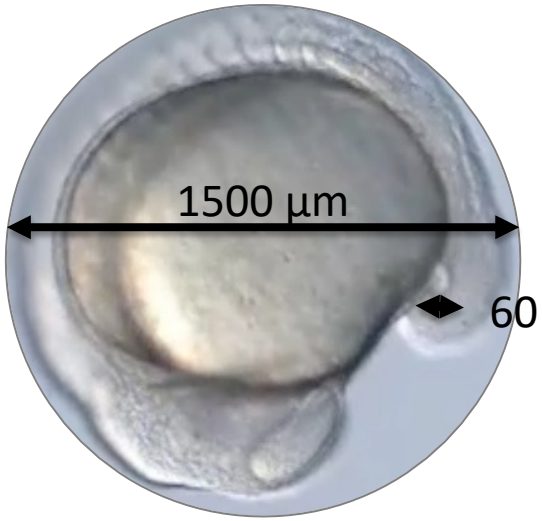


Native particles

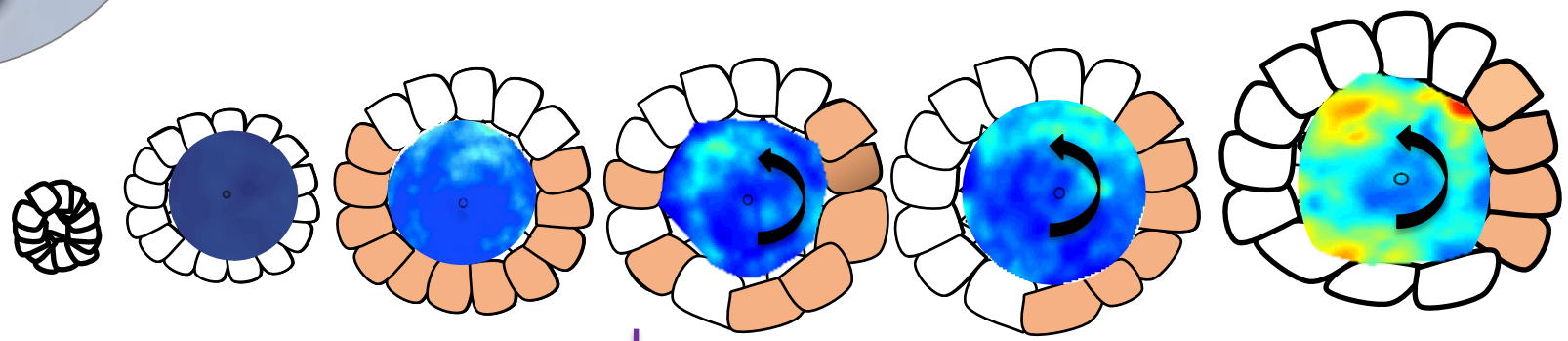


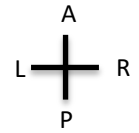
Native particle tracking

dand5 is the first asymmetric gene expressed in the left-right organizer

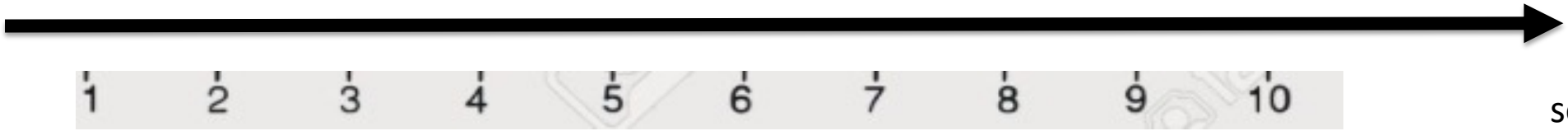


dand5



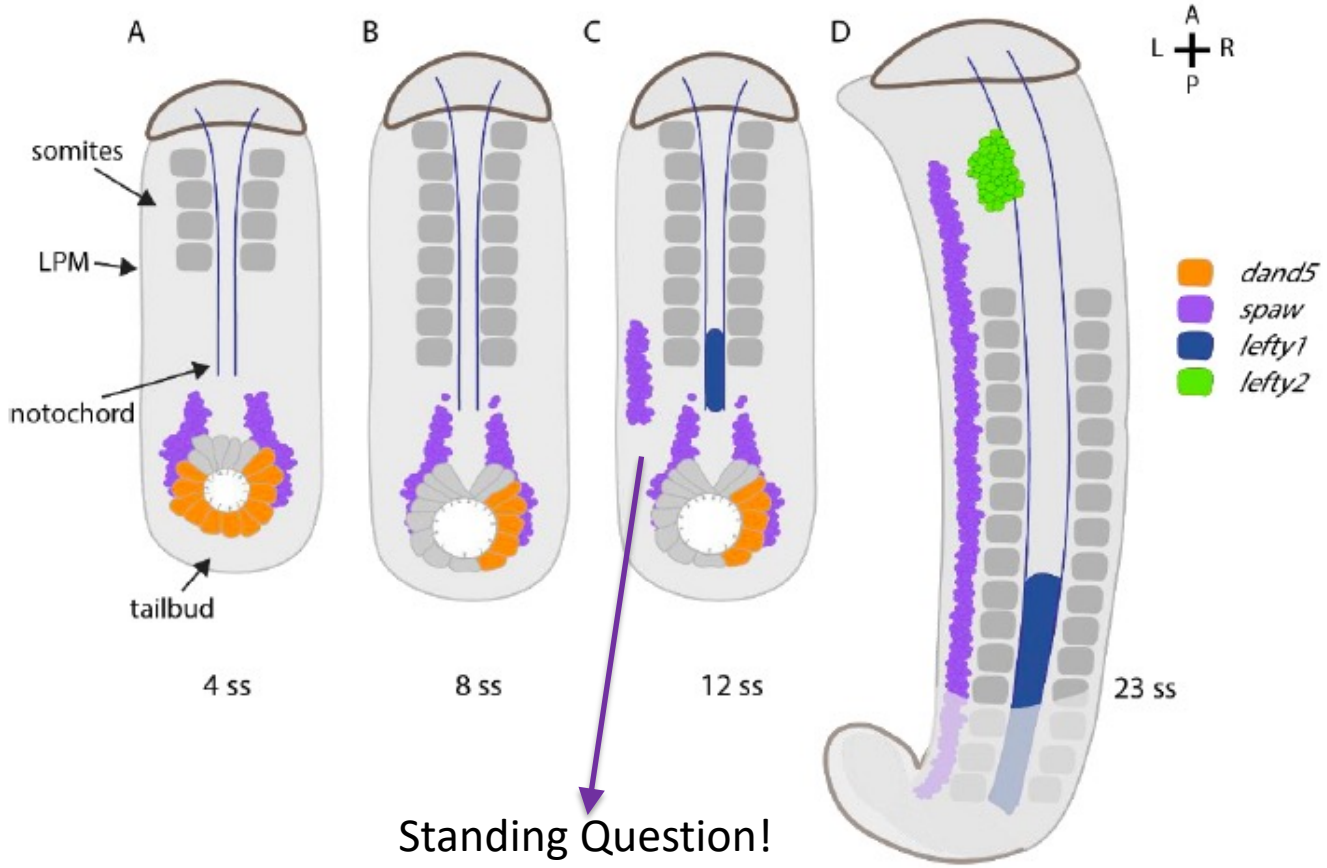


Standing Question!



somite stages

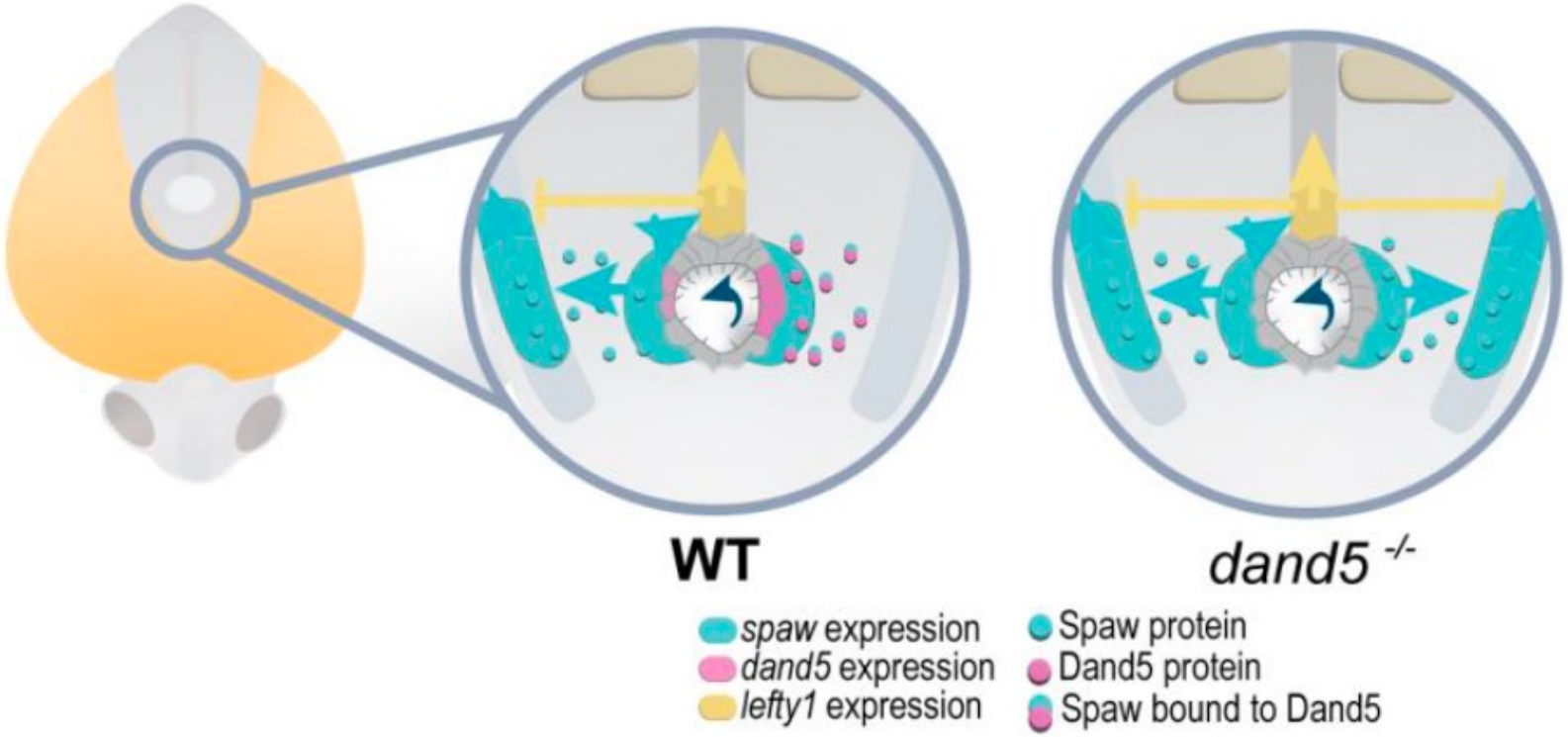
The conserved L-R Asymmetry cascade



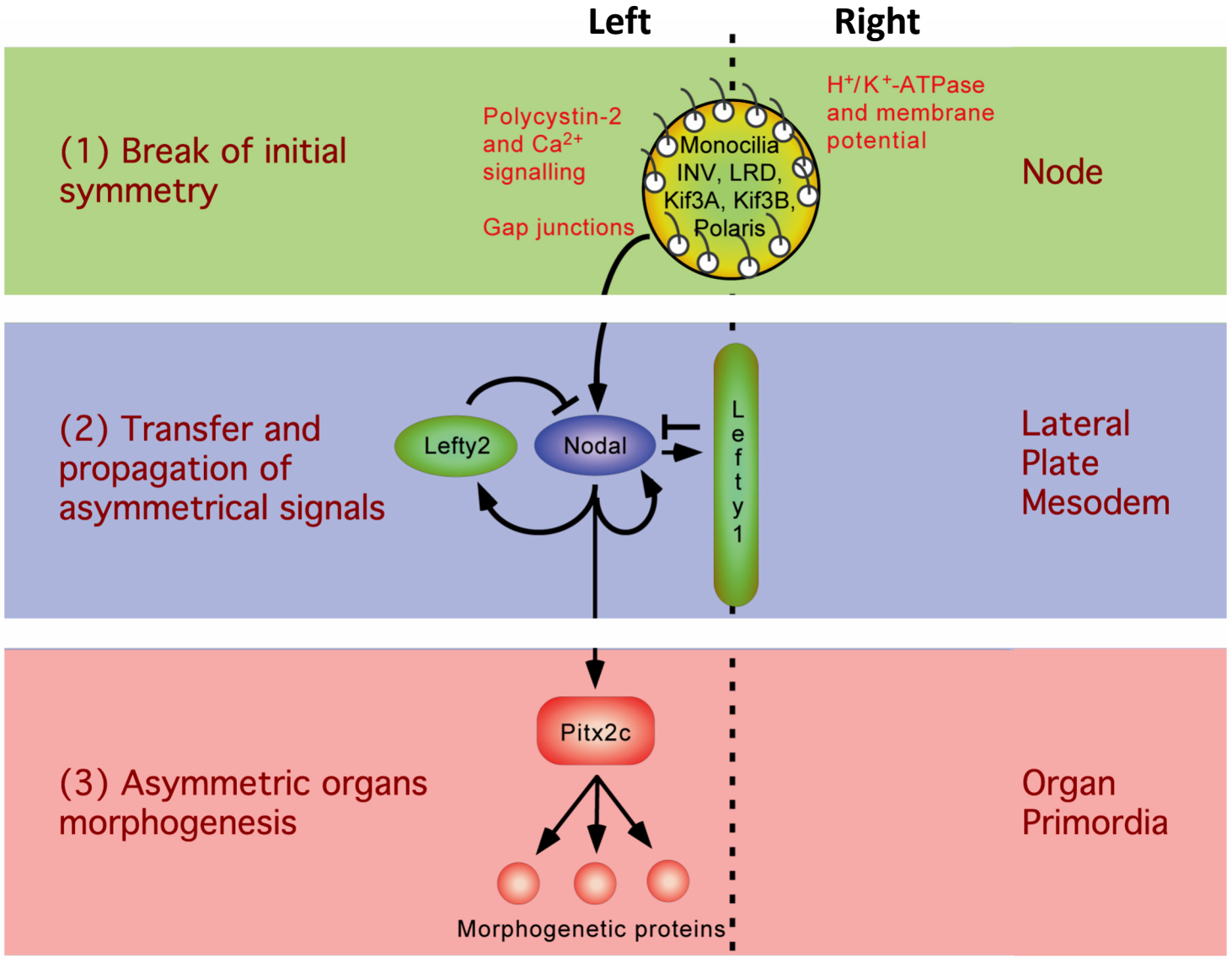
spaw=nodal

Nodal = secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression.
Lefty1 = secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Expressed in the notochord as mRNA.
Lefty2 = secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Expressed in the heart field as mRNA.
Dand5 = secreted protein antagonist of BMP and Nodal. Expressed in the Left-Right organizer as mRNA.

Dand5 inhibits Spaw on the right side of the LRO



The conserved L-R Asymmetry cascade



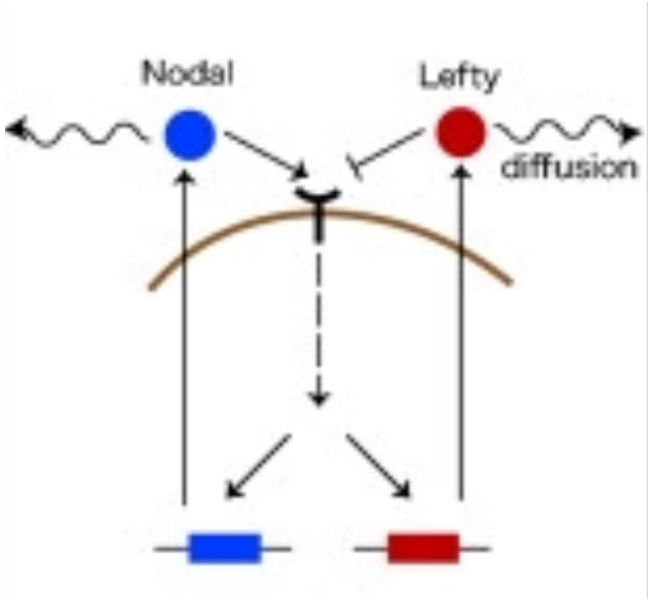
adapted from Mercola, **J. Cell Science** (2003)

The SELI system

Nodal and Lefty constitute a reaction-diffusion system, a theoretical model that involves two diffusible molecules, an activator and a feedback inhibitor (Turing, 1952).

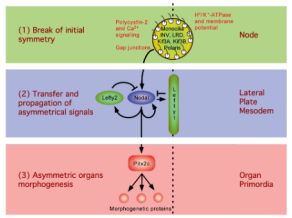
A reaction-diffusion system has been proposed to underlie pattern formation during development because a “self-enhancement and lateral-inhibition” nature of the model can produce “self-organizing patterns” (Meinhardt and Gierer, 2000, Meinhardt, 2001).

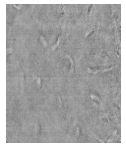
Importantly, this model has a potential to convert a small difference between two separated regions into a robust difference through local activation and long-range inhibition.



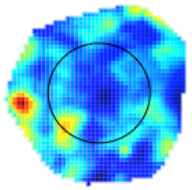
Nakamura et al. Generation of Robust Left-Right Asymmetry in the Mouse Embryo Requires a Self-Enhancement and Lateral-Inhibition System, Developmental Cell, 2006

The conserved L-R sequence of events

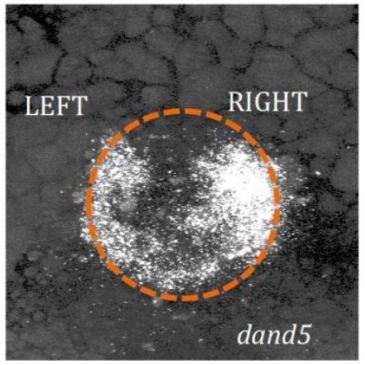




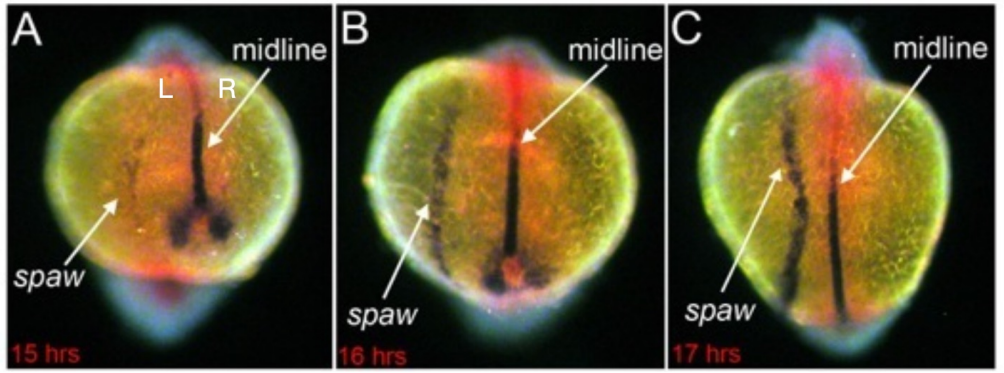
cilia

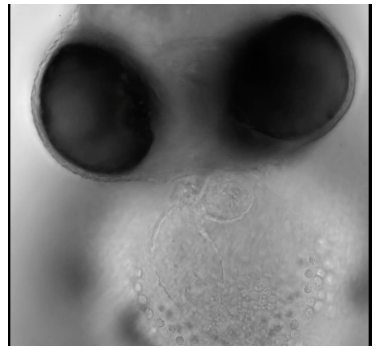


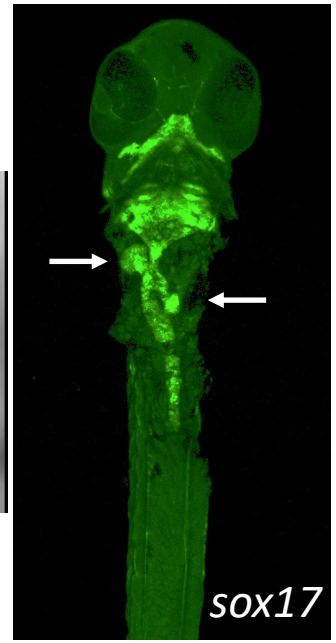
Asymmetric flow



Asymmetric *dand5* gene expression



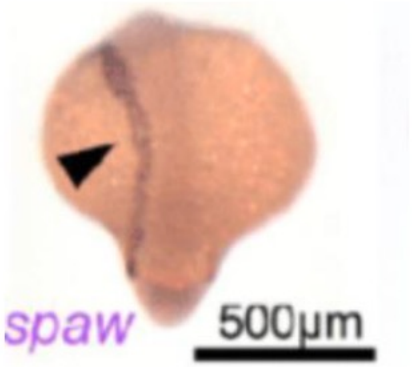


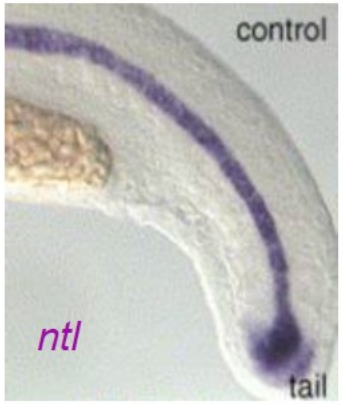


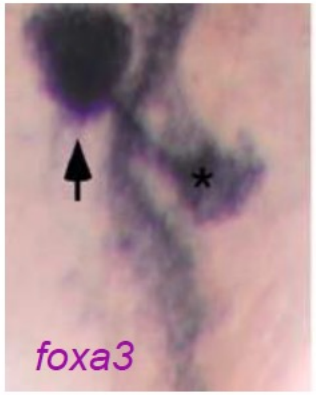
LR organizer (LRO) → Lateral Plate Mesoderm (LPM) → Heart, Liver and Pancreas

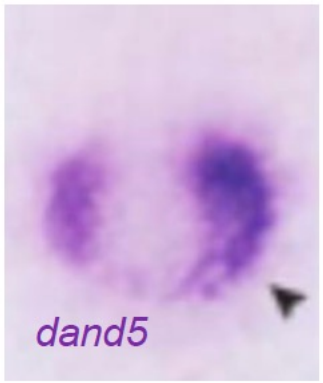
10 hrs 12 hrs 14 hrs 16 hrs 20 hrs 27 hrs 40 hrs

The conserved L-R sequence of events *by in situ* hybridization

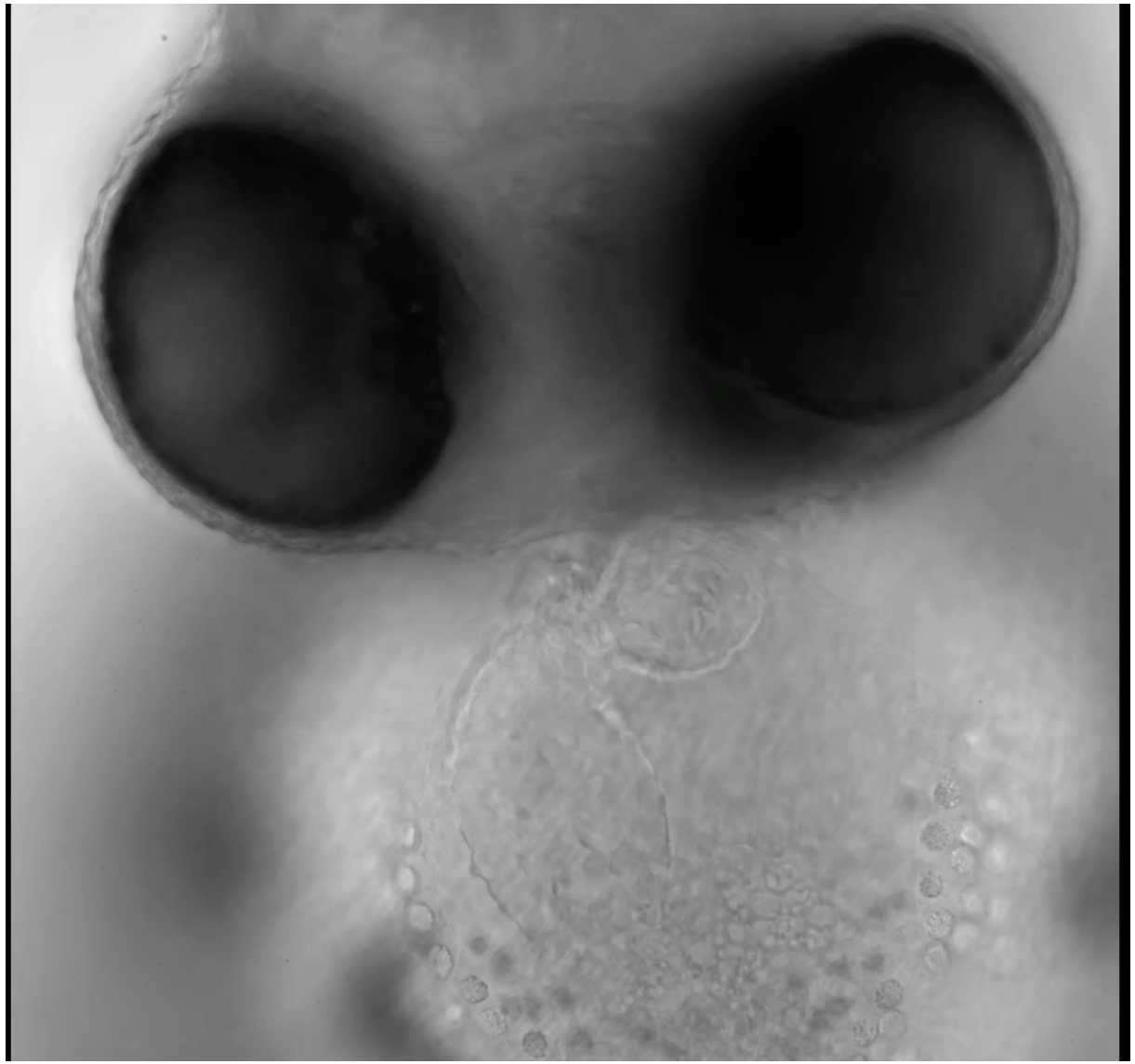








The heart looping



The gut asymmetric organs

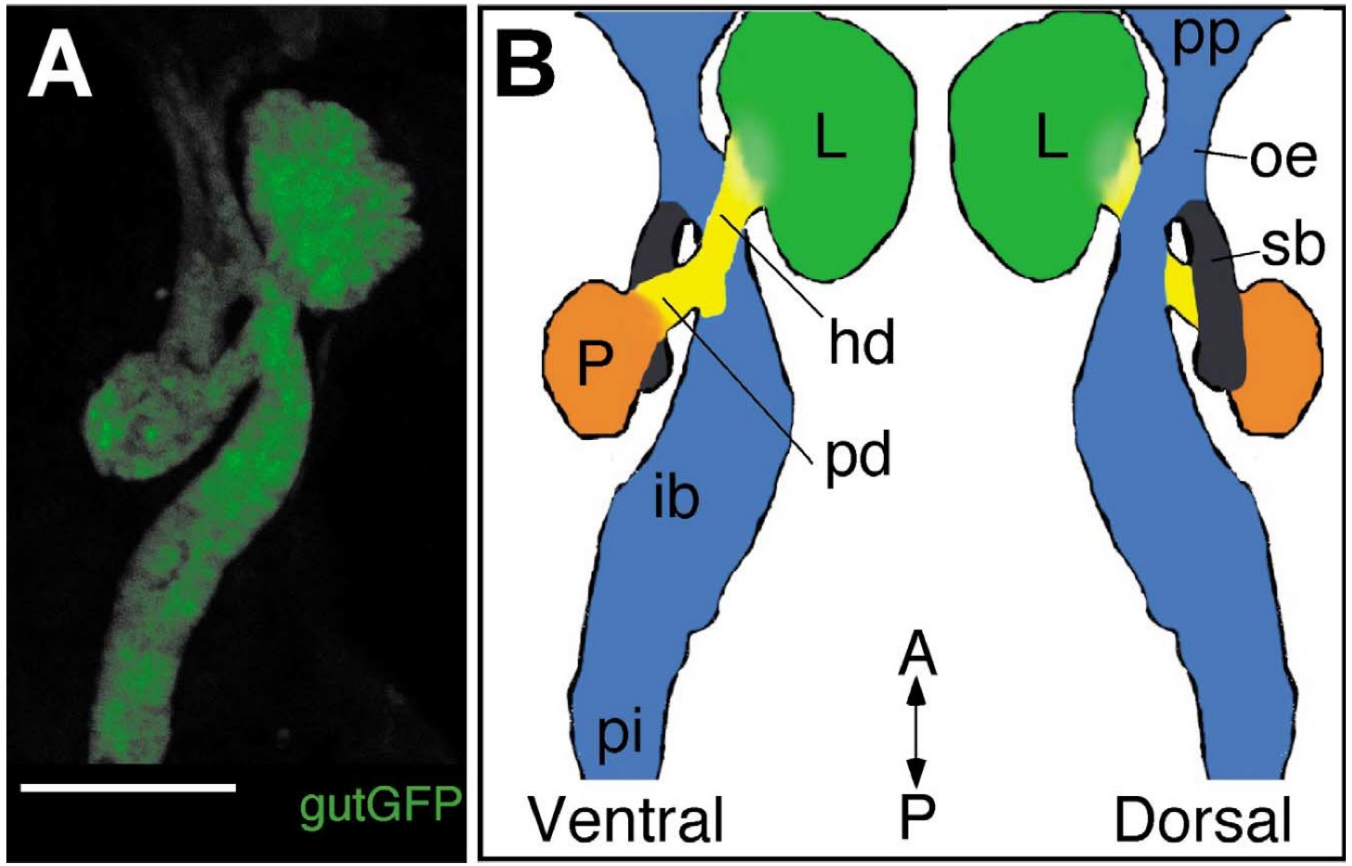


Fig. 1. The 52-hpf zebrafish digestive system as visualized in the stable transgenic gutGFP line. (A) Two-dimensional projection of a confocal stack, ventral view with anterior to the top. GFP expression occurs in all organs of the digestive system as well as the endodermal lining of the swim bladder. Scale bar, 100 μm. (B) Schematic drawings (ventral and dorsal views, anterior to the top) showing the identity and location of GFP-expressing organs at 52 hpf. L, liver; hd, hepatic duct; pd, pancreatic duct; P, pancreas; ib, intestinal bulb; pi, posterior intestine; pp, posterior region of the pharynx; oe, oesophagus; sb, swim bladder.