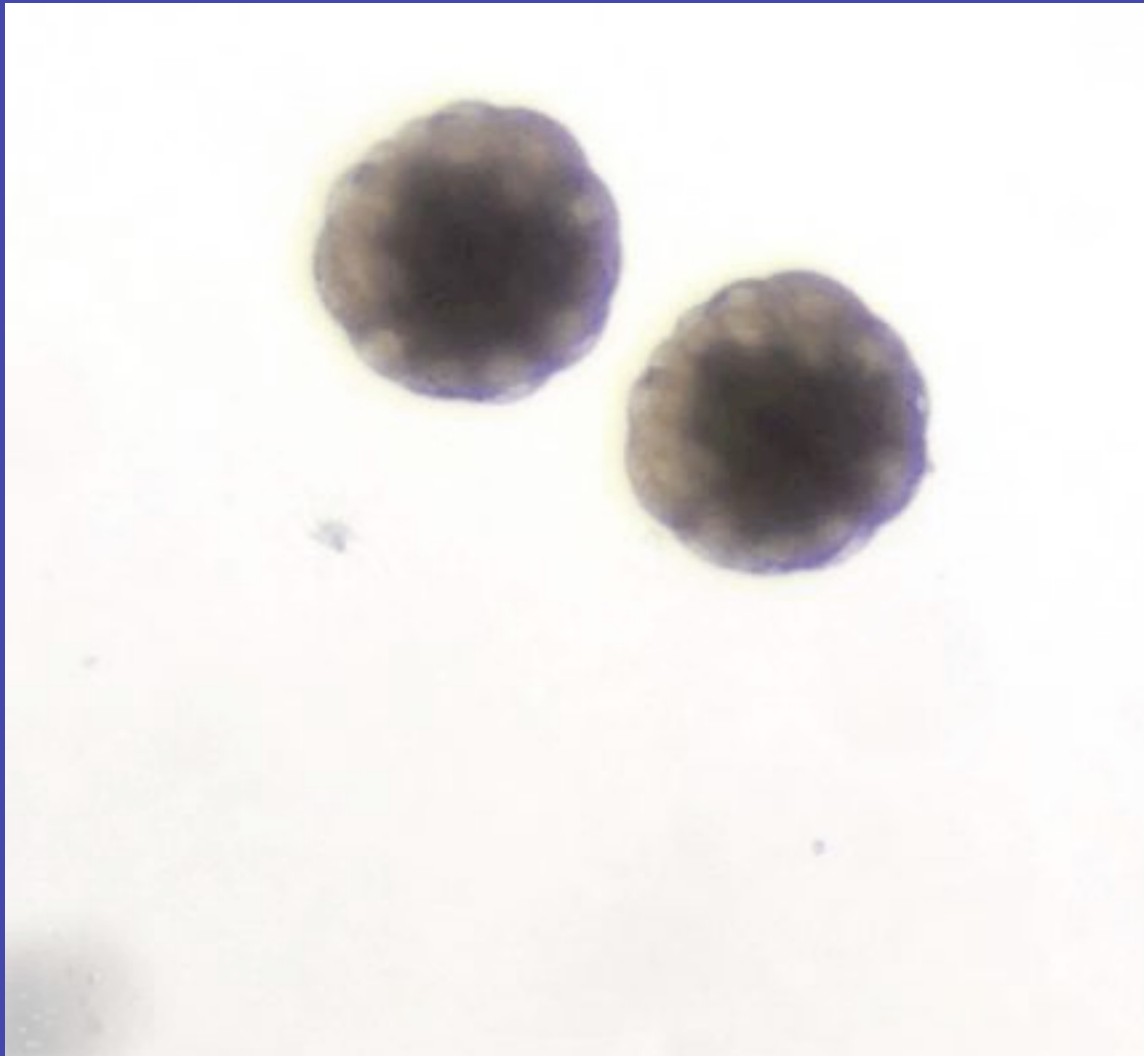


in toto dynamic imaging and modeling of chordate morphogenesis

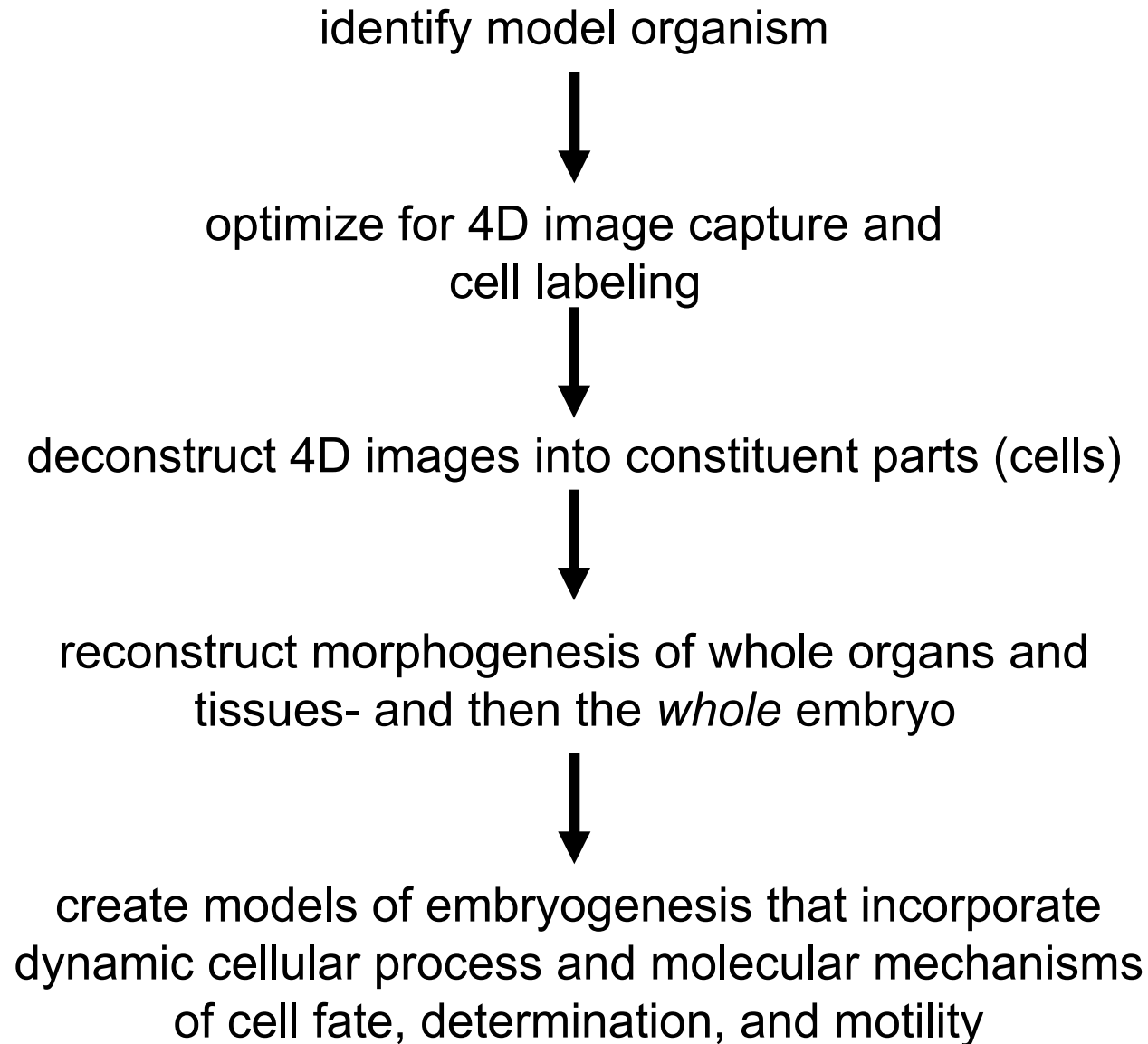


- morphogenesis driven by changes in cell shape, adhesion, contractility...

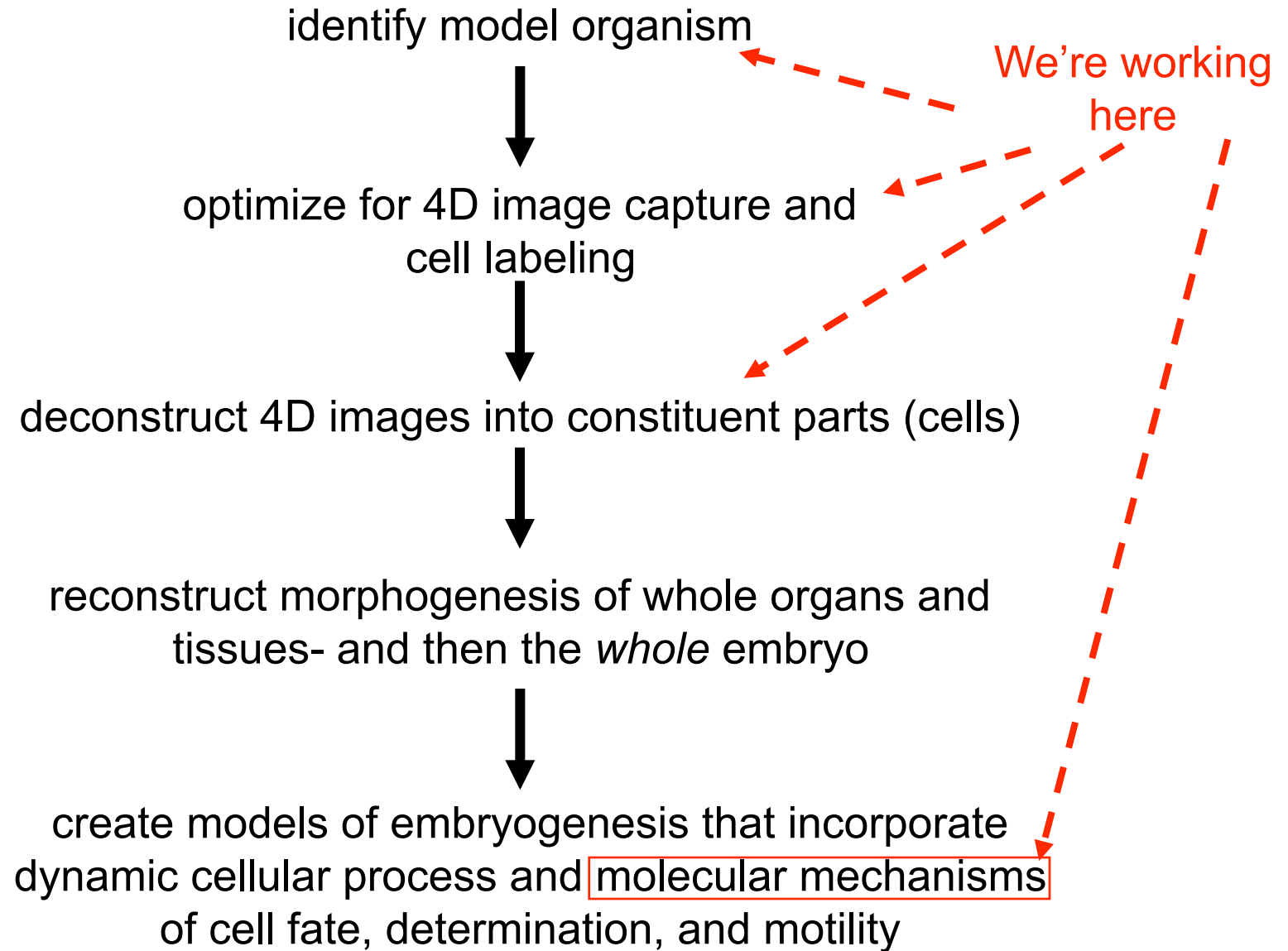
- our goal: a comprehensive understanding of processes driving morphogenesis

Development of ascidian from 64 cells to swimming larvae.
Elapsed time \approx 17 hours (embryo is not “growing” during morphogenesis)

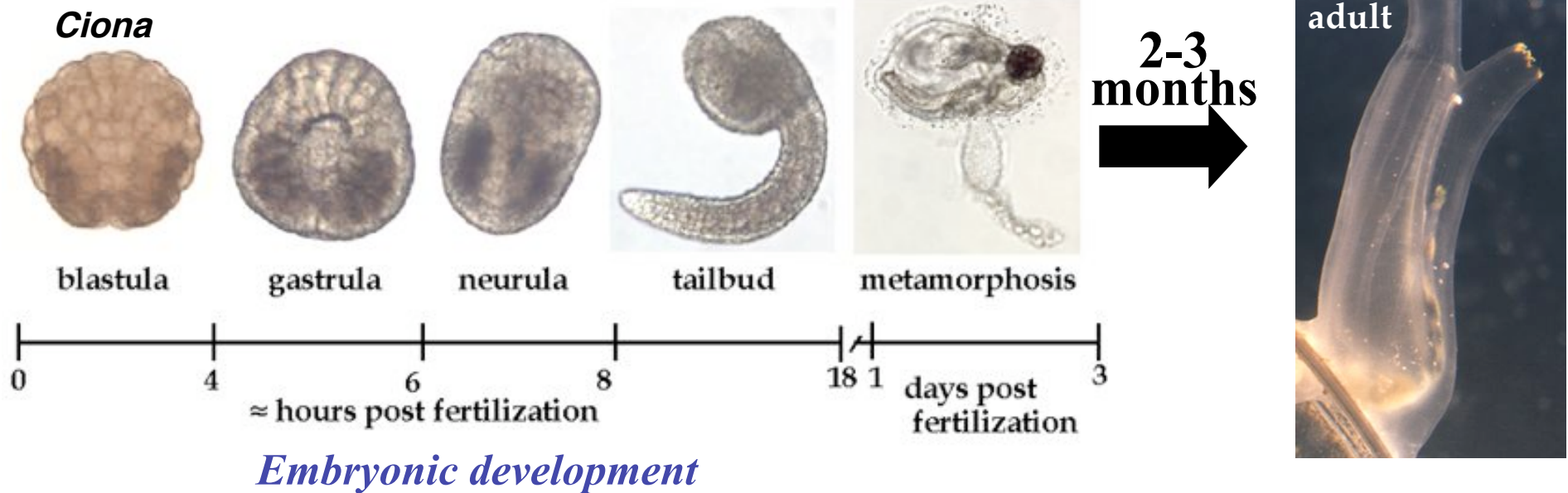
dynamic *in toto* embryogenesis modeling: the challenges



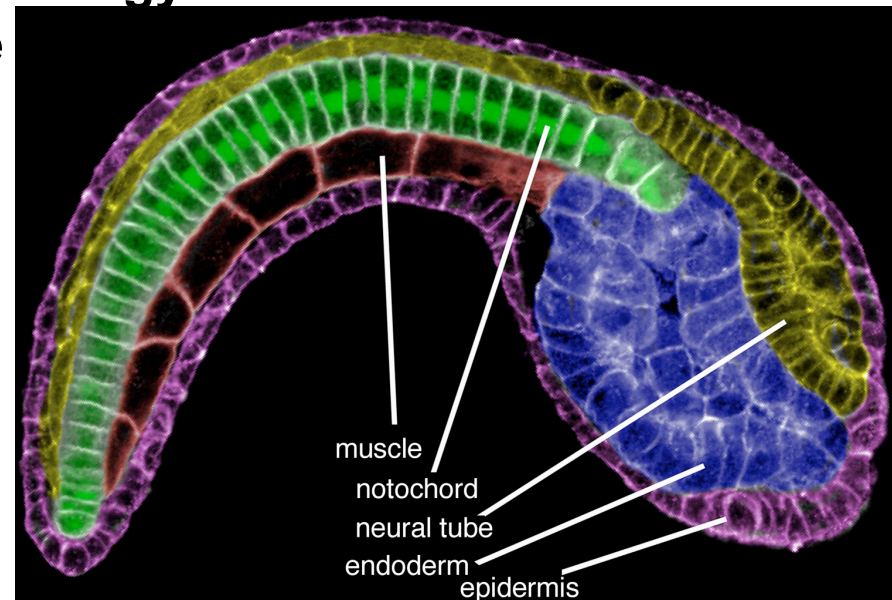
dynamic *in toto* embryogenesis modeling: the challenges



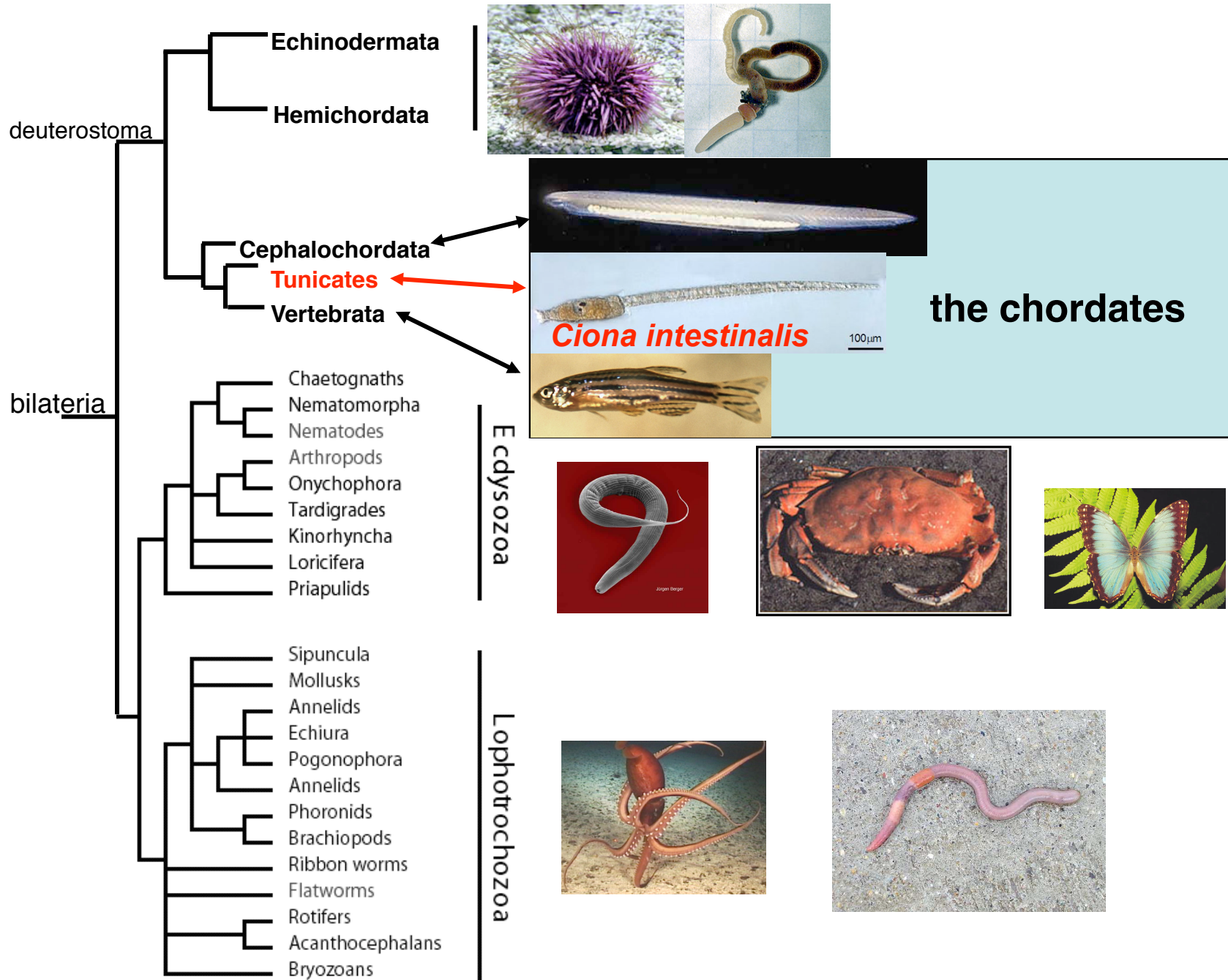
why morphogenesis of ascidians?



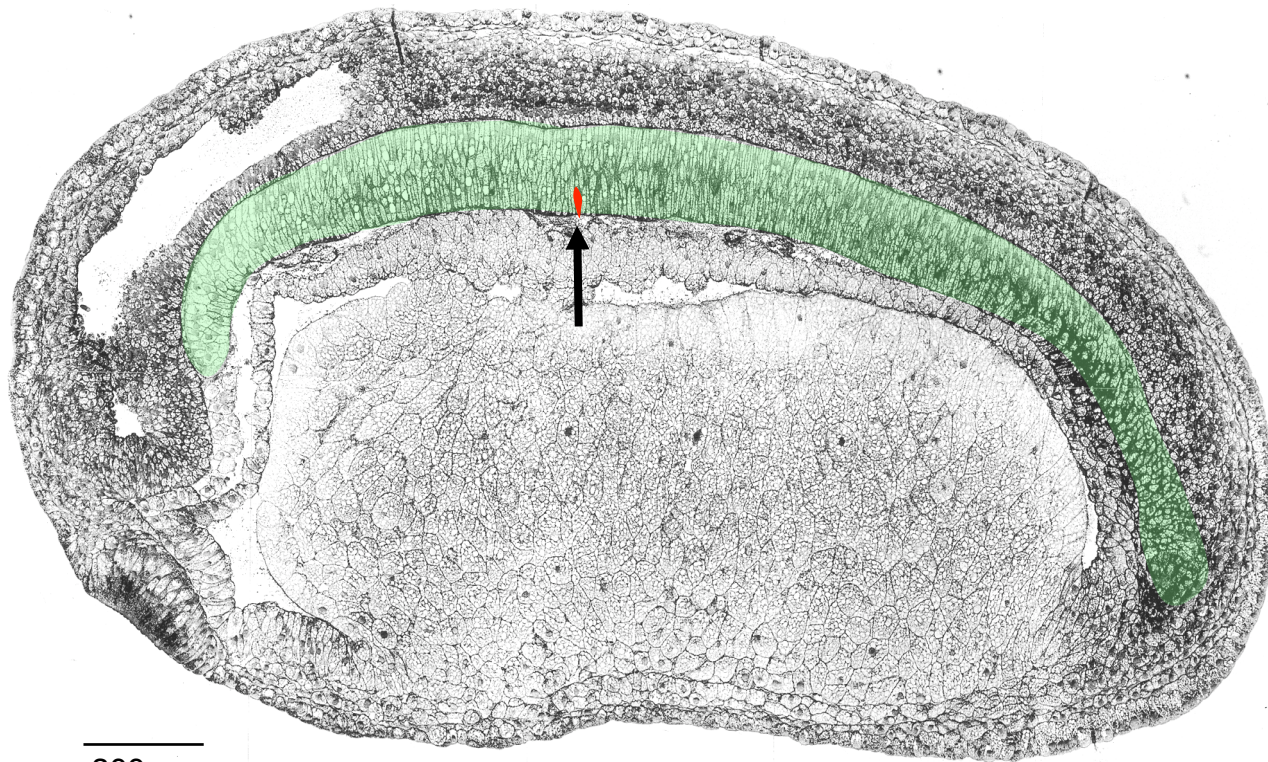
- 1) conserved embryology and physiology with vertebrates
- 2) embryos and larvae very simple
- 3) invariant cell lineage
- 4) small and sequenced genome
- 5) lower genetic redundancy
- 6) produces lots of progeny
- 7) stable transgenesis
- 8) self-fertilizing hermaphrodites
- 9) forward genetics



ascidians are our closest invertebrate relatives!

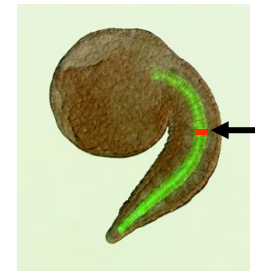


amphibian and ascidian embryos at equivalent stages of development (tailbud) - shown to scale



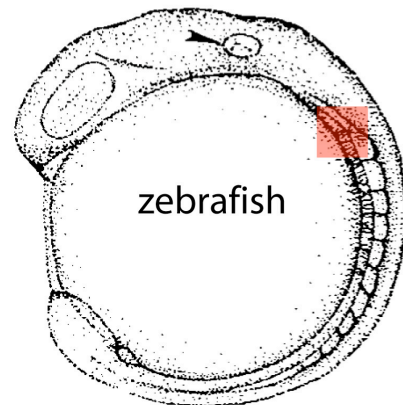
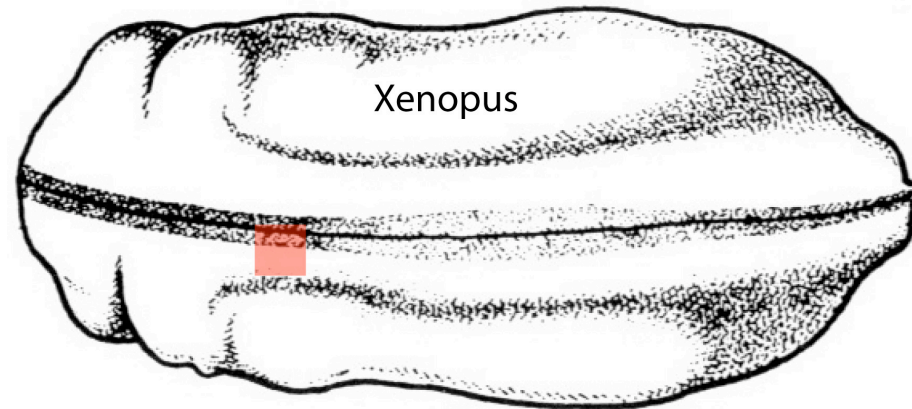
300 μm

Xenopus (amphibian)

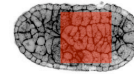


Ciona (ascidian)

the challenge of capturing live embryos at high resolution



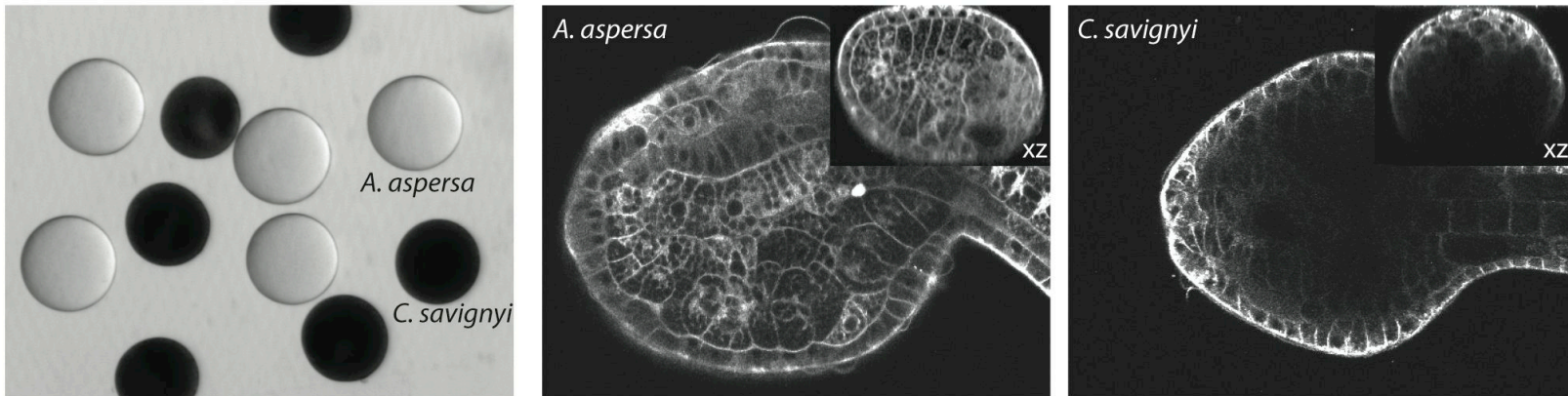
ascidian



*Comparable microscopic images of ascidian, amphibian and fish (~130x130microns).
Complete coverage of embryo requires tiling such images*

what is the ideal ascidian for this project?

Ascidiella versus *Ciona*



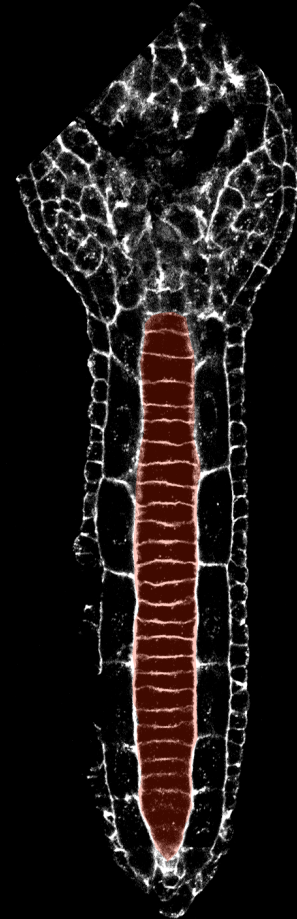
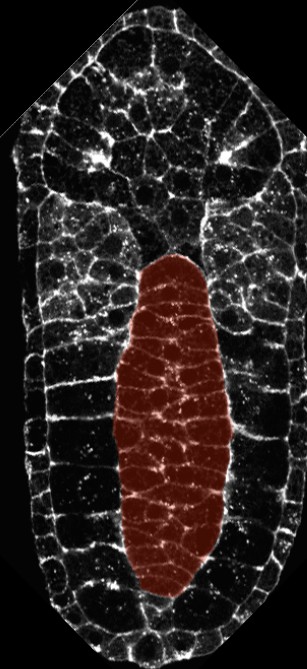
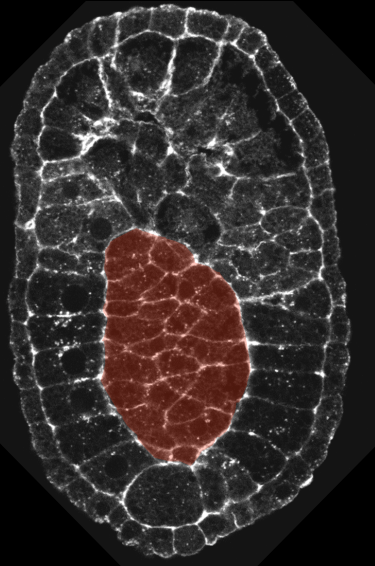
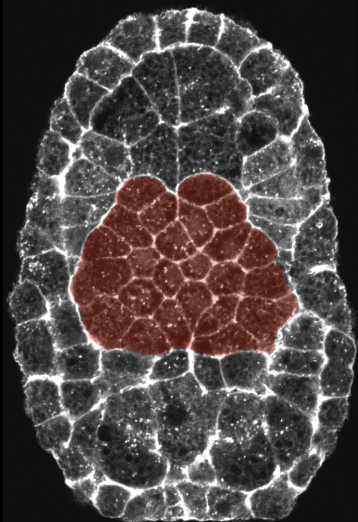
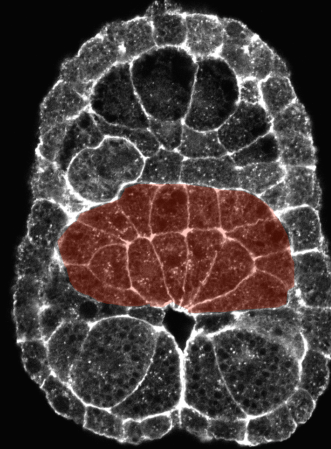
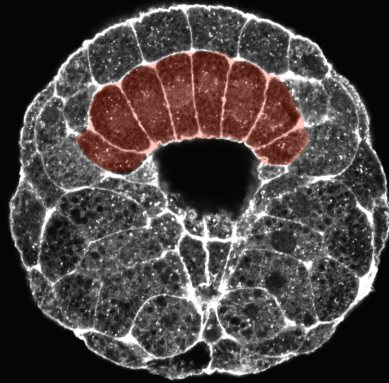
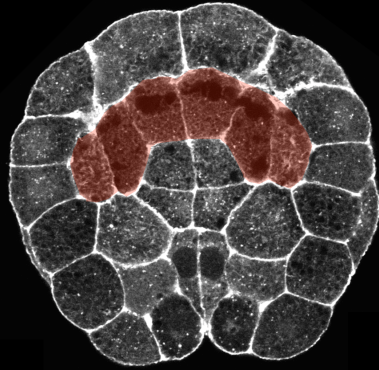
- *Ascidiella* much better optics + harder to work with
- *Ciona* much more widely studied + easy to work with

...where to start?????

notochord:

- first fully-formed organ to develop
- essential for morphogenesis of all chordates
- good molecular models of morphogenesis
- in ascidian, morphogenesis takes place with no cell division.
- in ascidian, small number (40) of fairly regularly-shaped cells

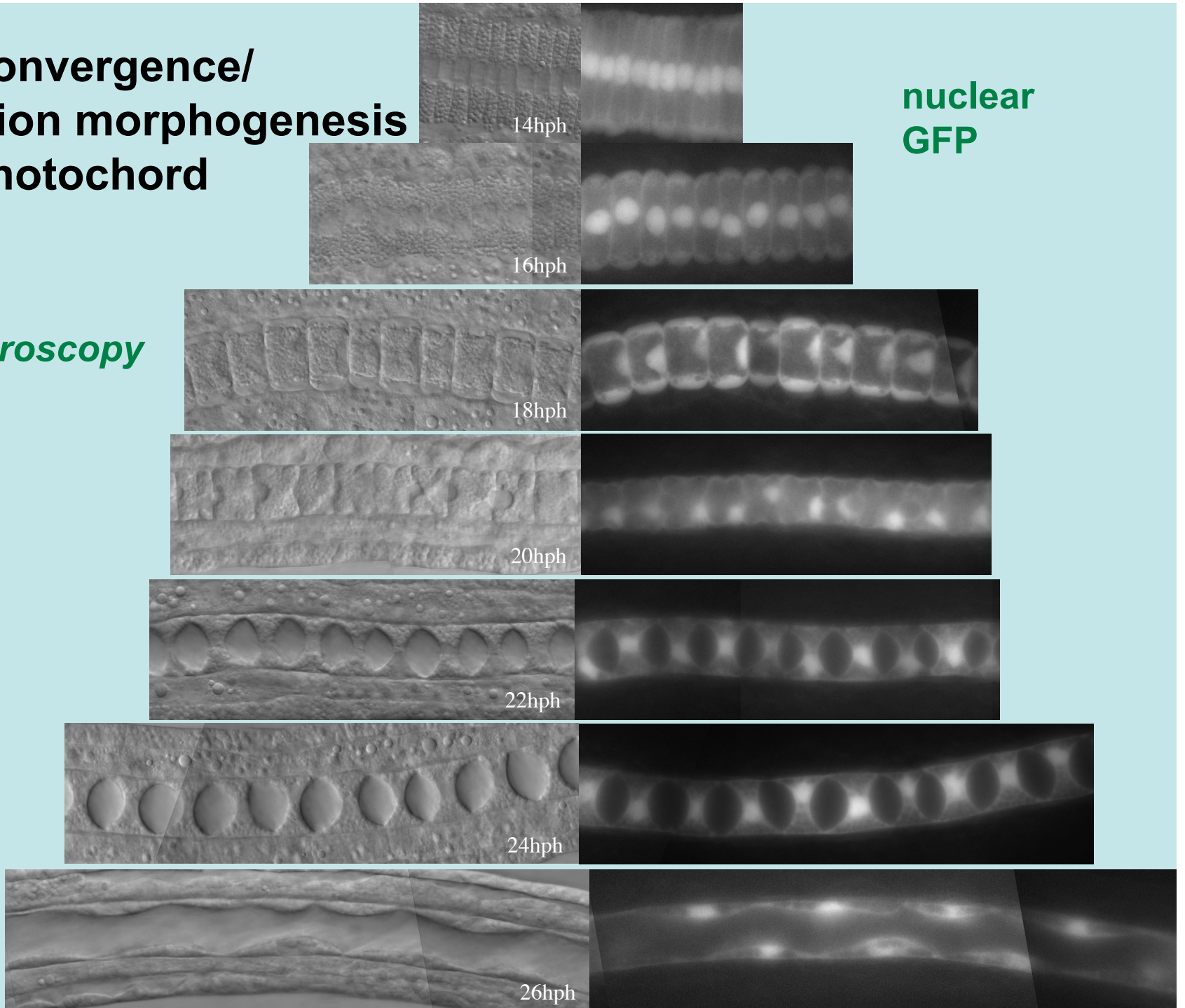
ascidian notochord morphogenesis



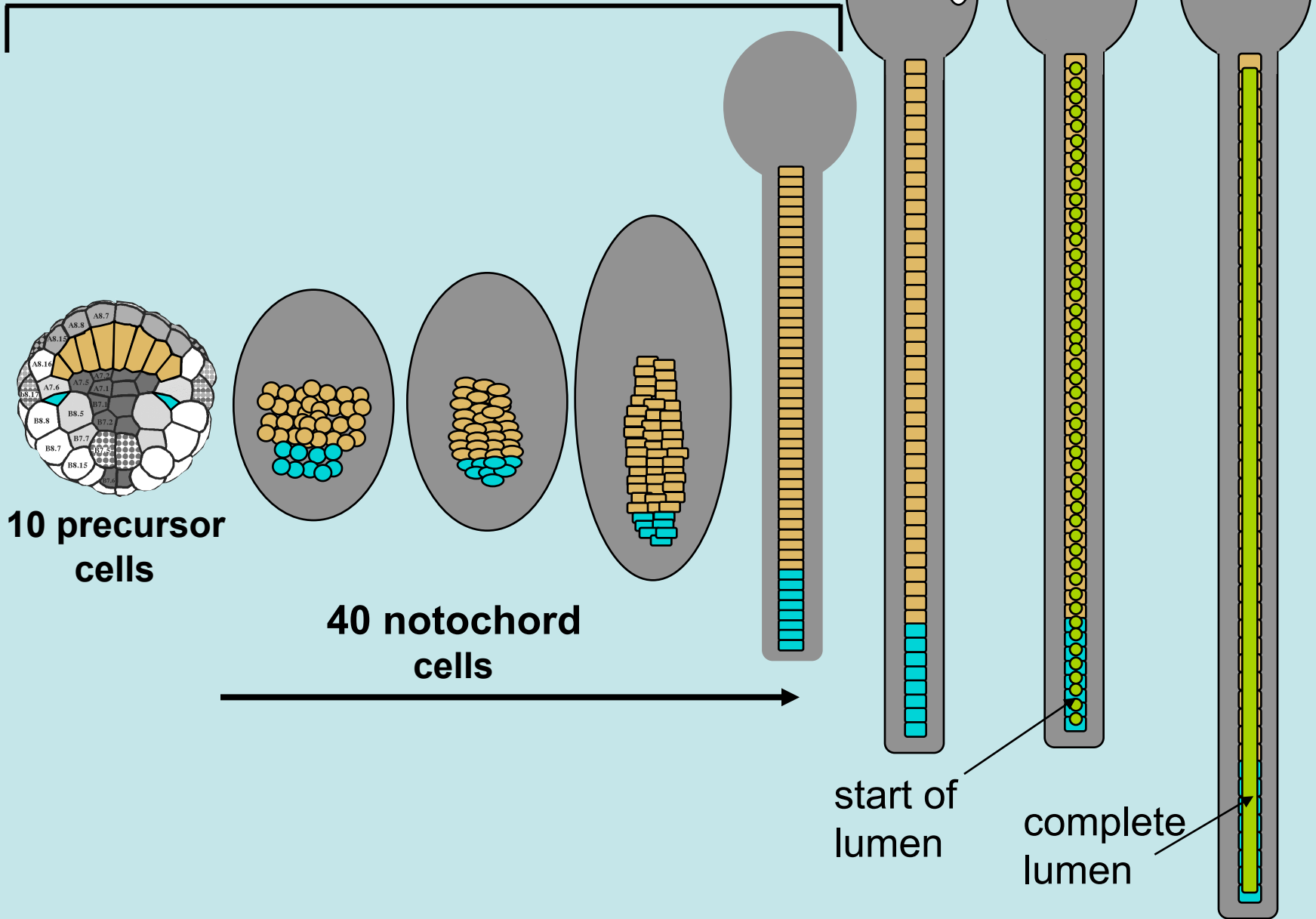
Post-convergence/ extension morphogenesis of the notochord

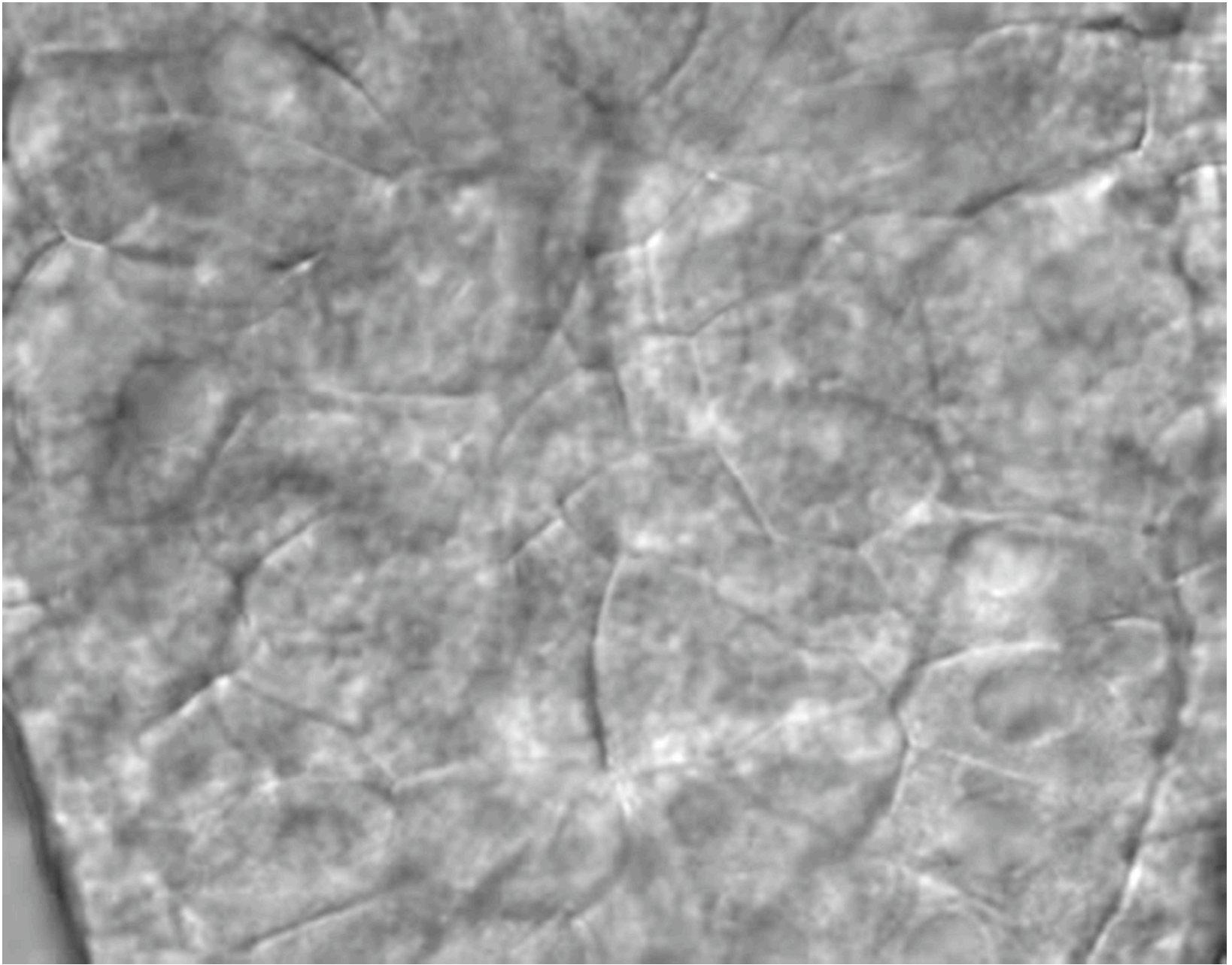
DIC
microscopy

nuclear
GFP

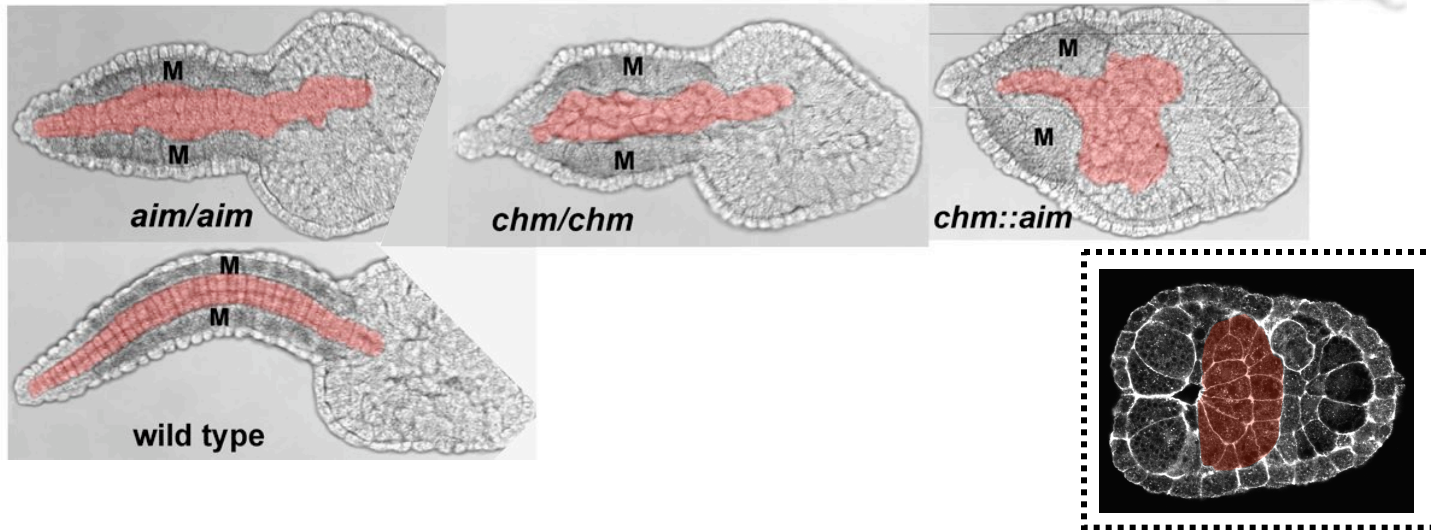
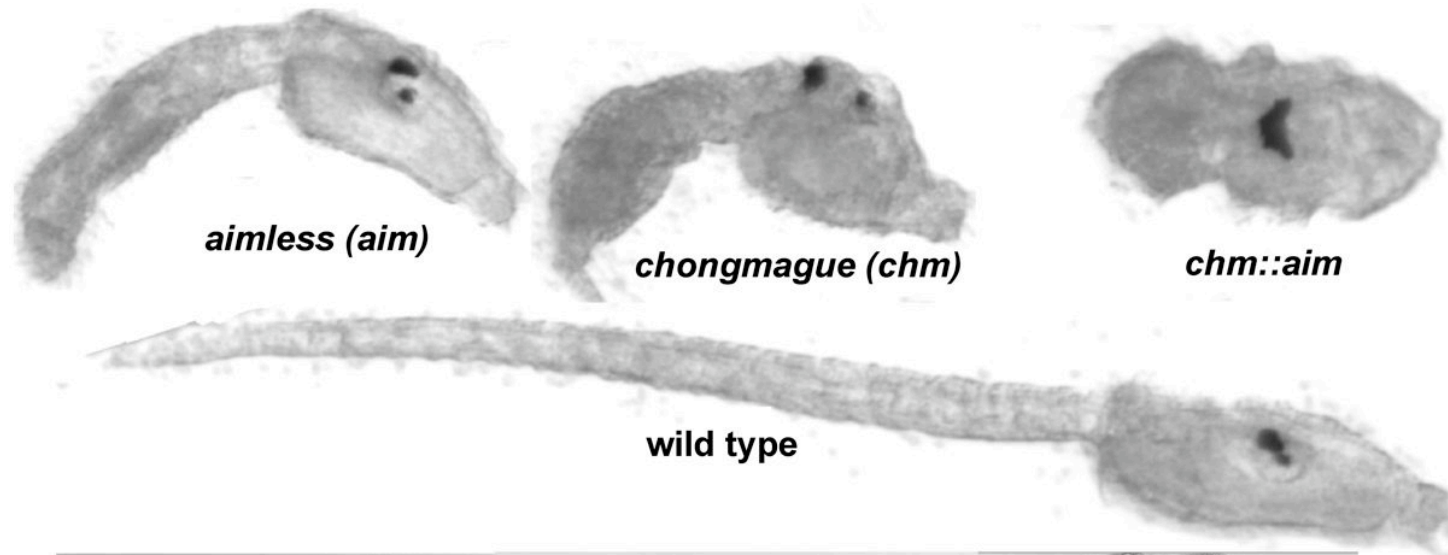


what are the morphogenic mechanisms that generates single-cell column?



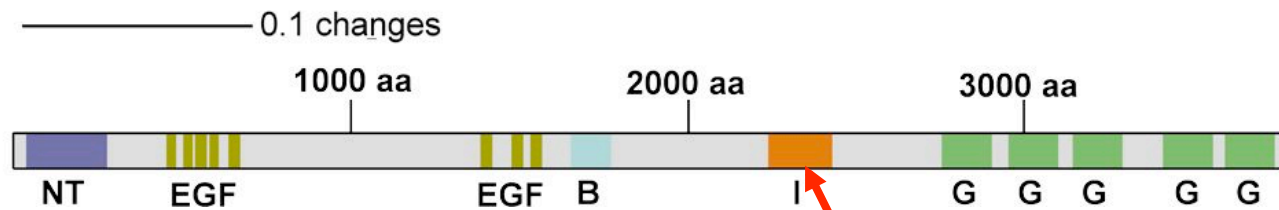
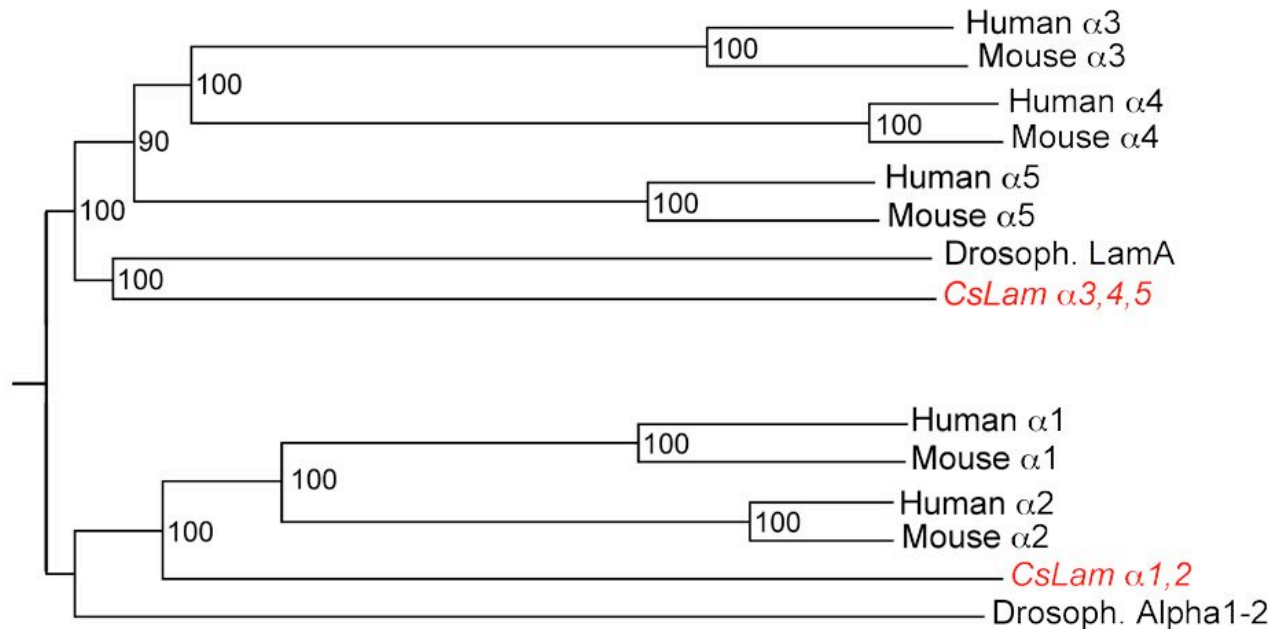
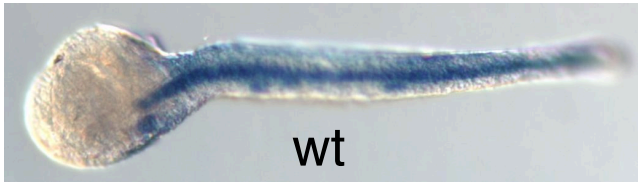


recessive mutations disrupting convergent extension



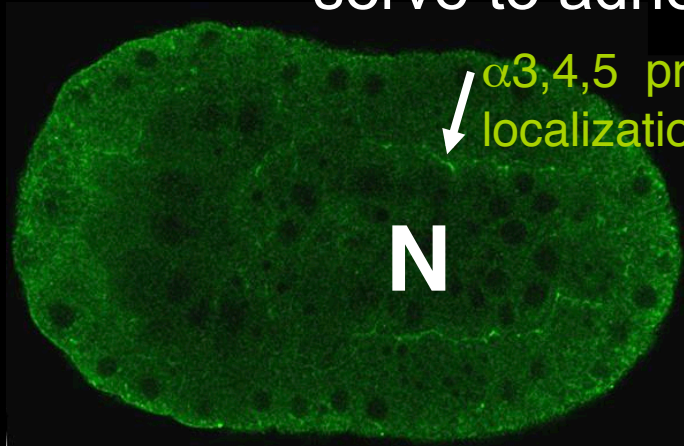
- *chongmague* has a null mutation disrupting notochord boundary
- *aimless* has probable null mutation in PCP pathway gene
- double mutant has complete failure of convergent extension

chm maps to a laminin (α -3,4,5 like)

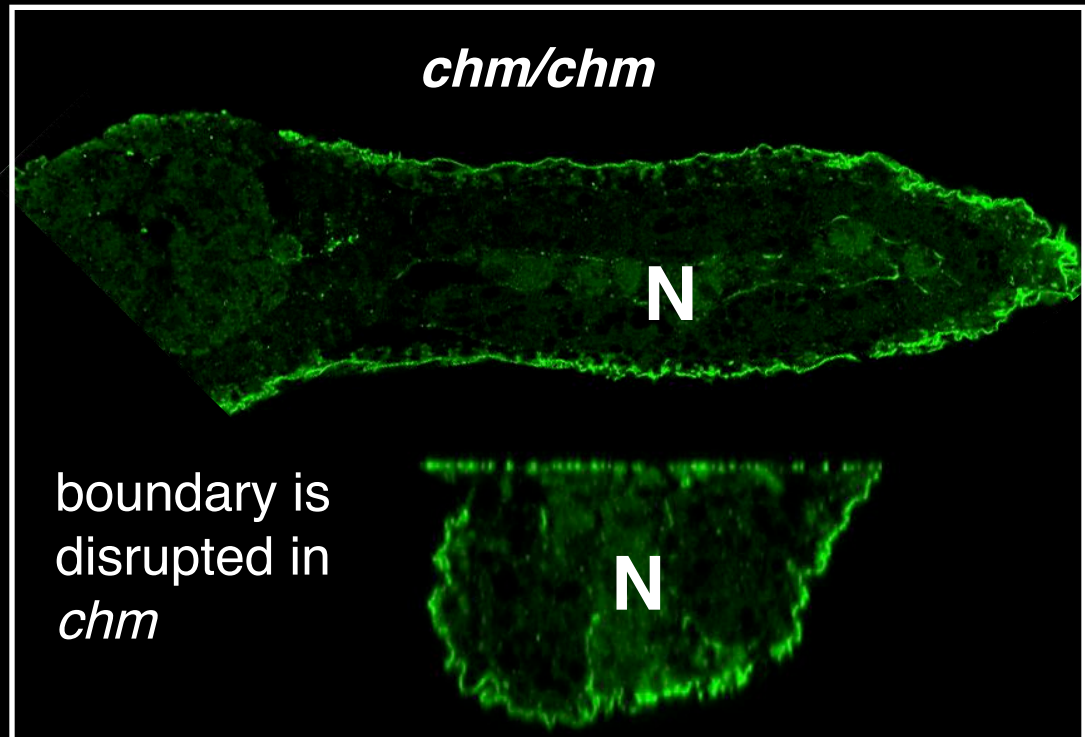
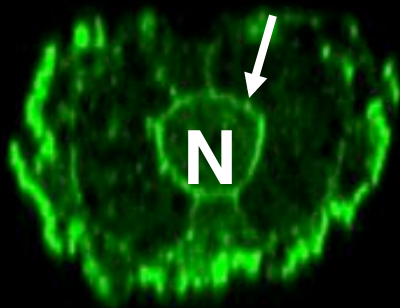
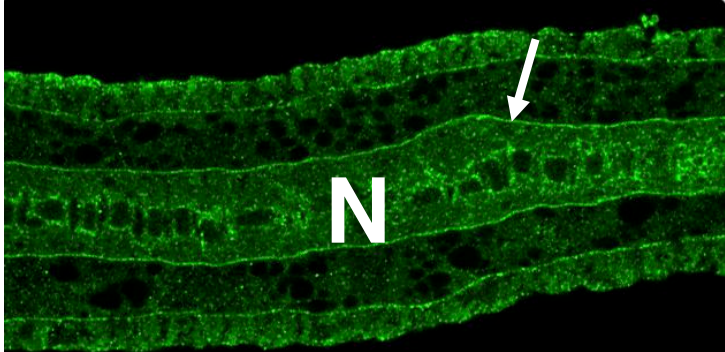
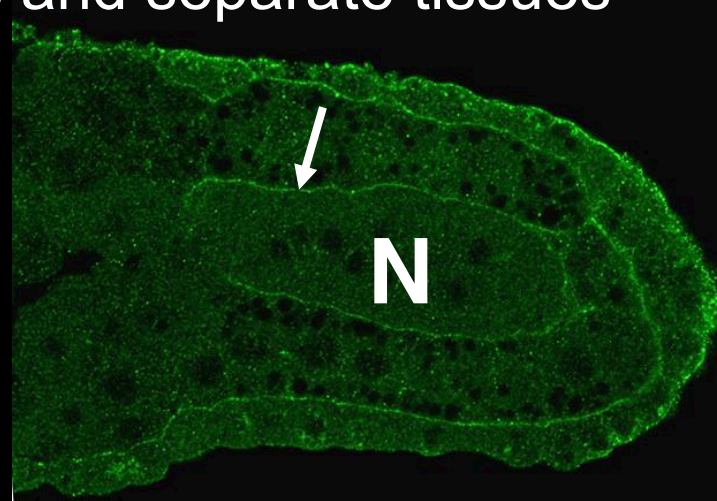


frame shift

laminins are components of **basement membranes**, which serve to adhere cells and separate tissues



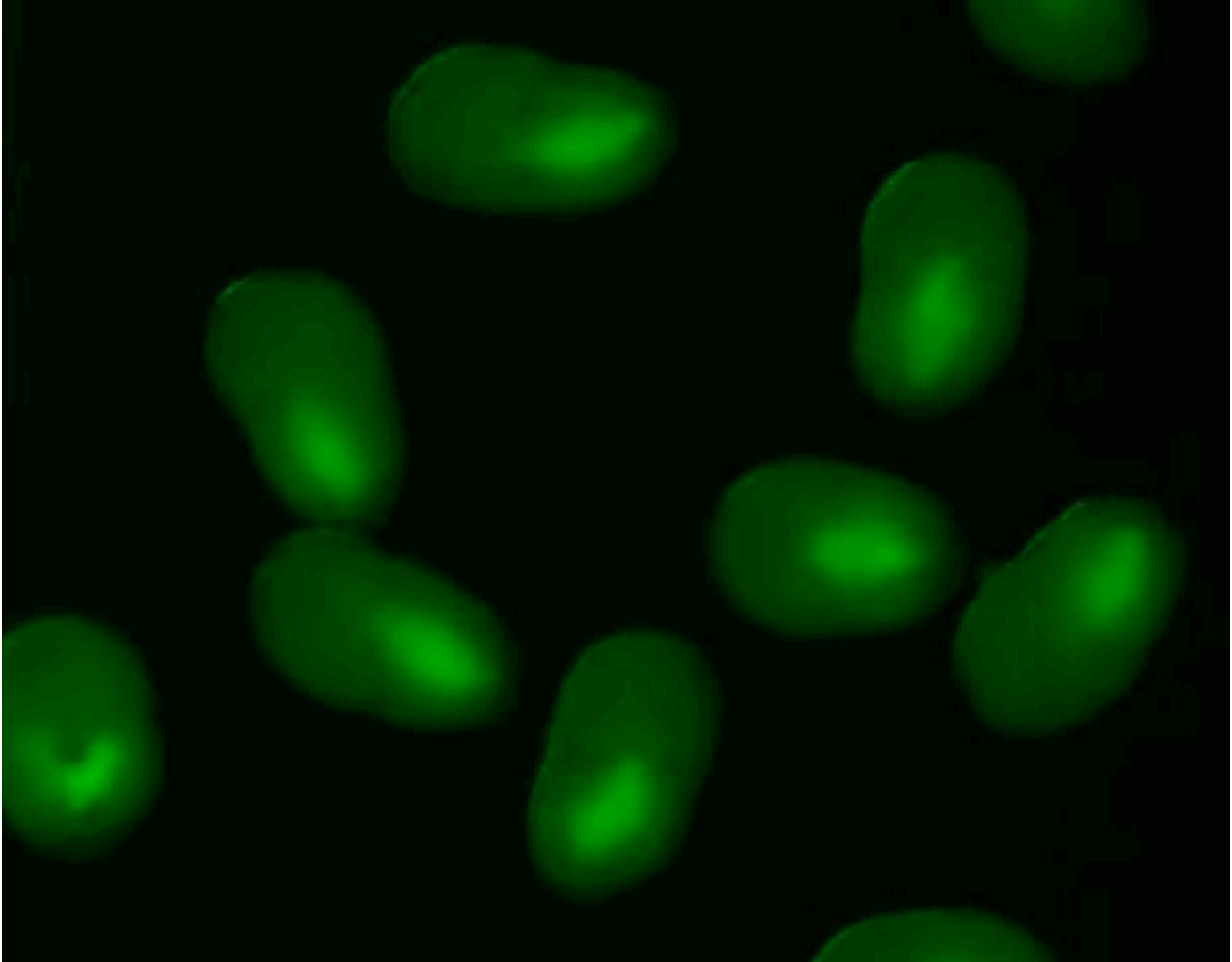
α 3,4,5 protein localization

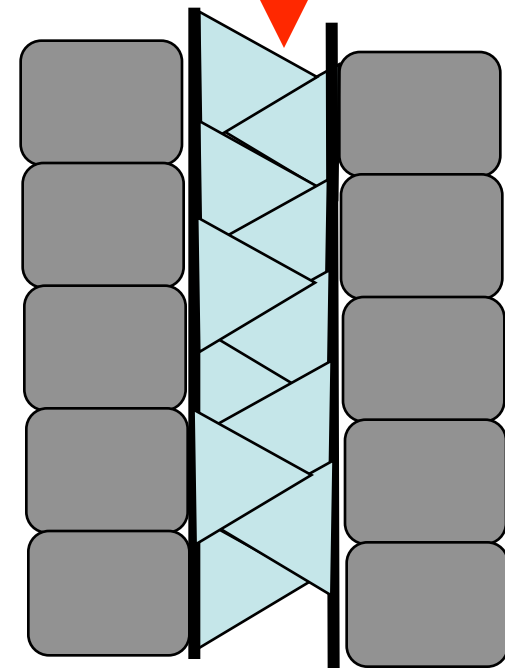
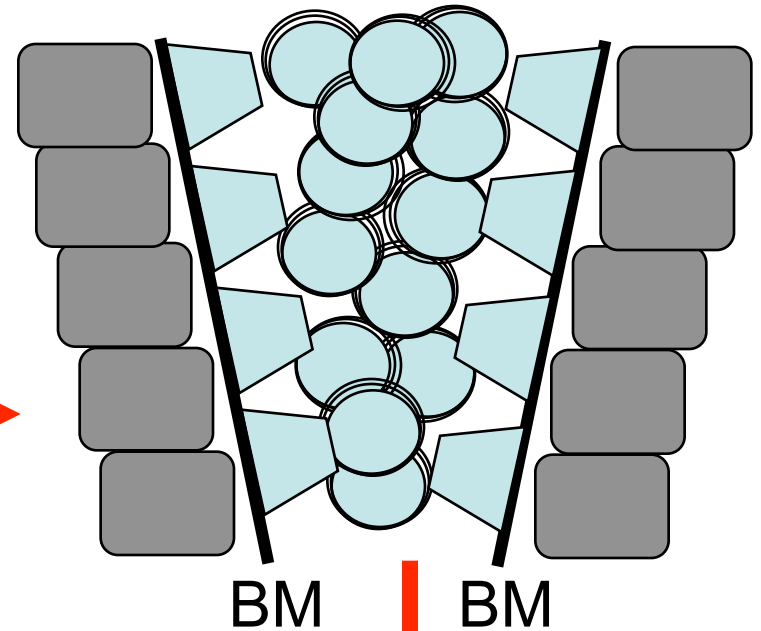
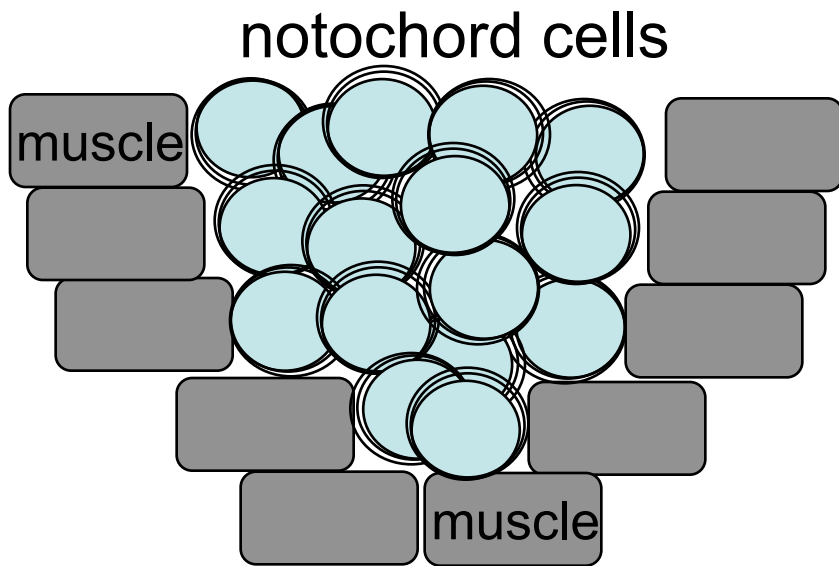


chm/chm

boundary is disrupted in *chm*

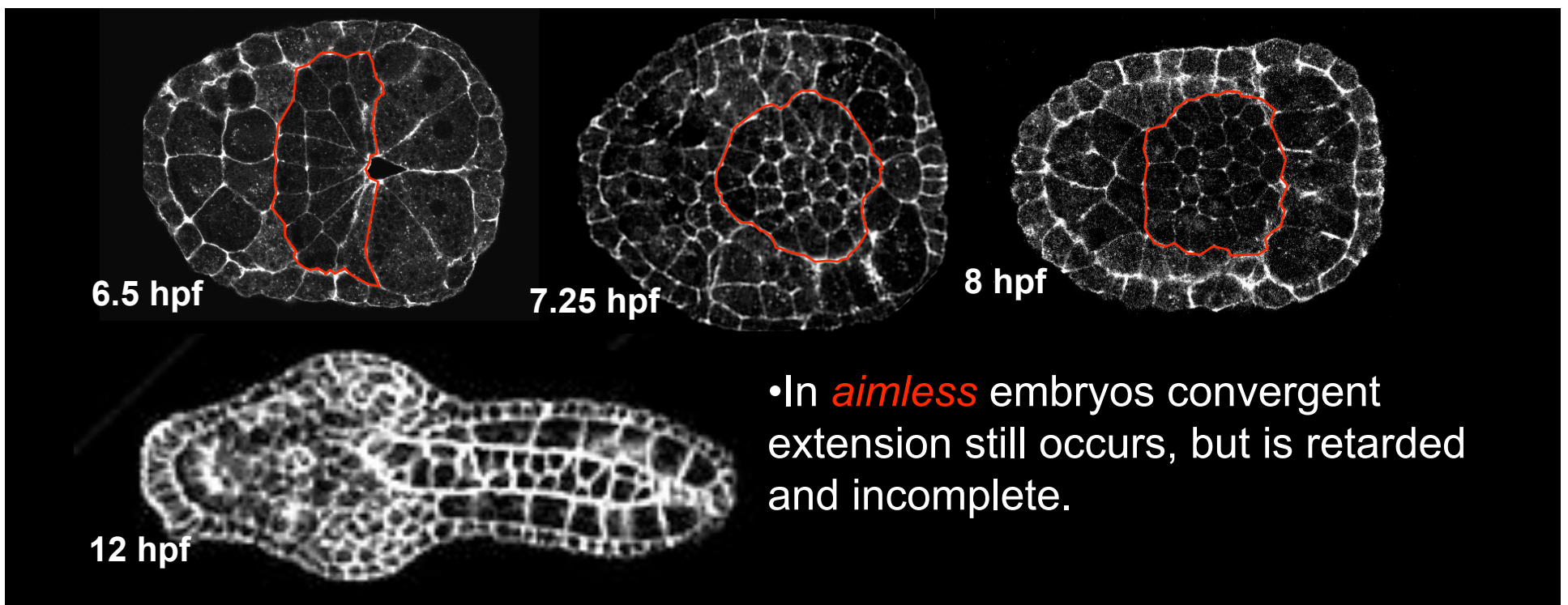
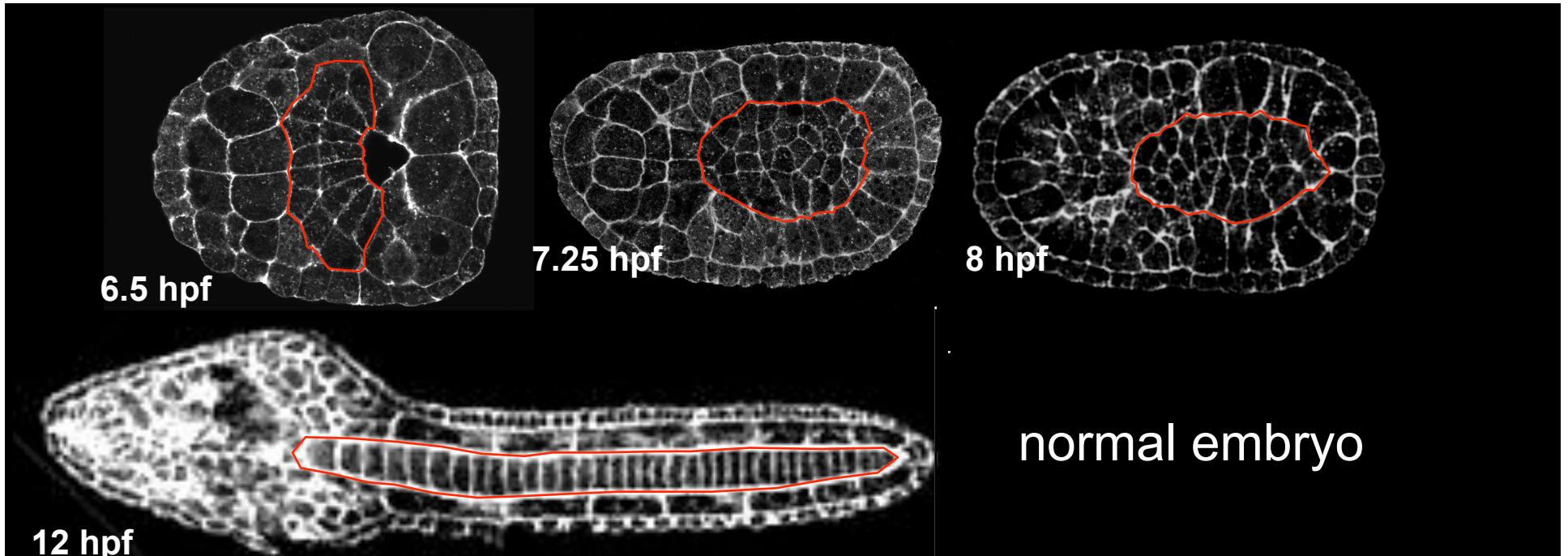
chm phenotype get progressively worse:



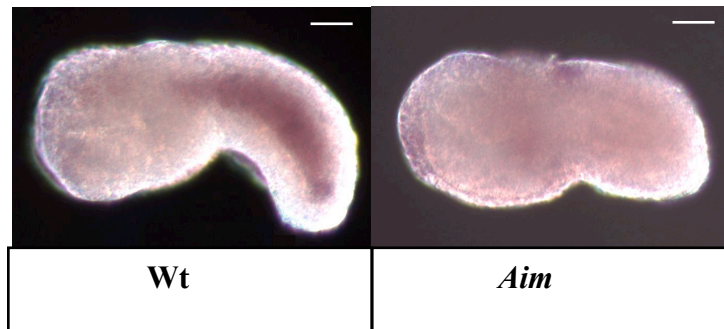
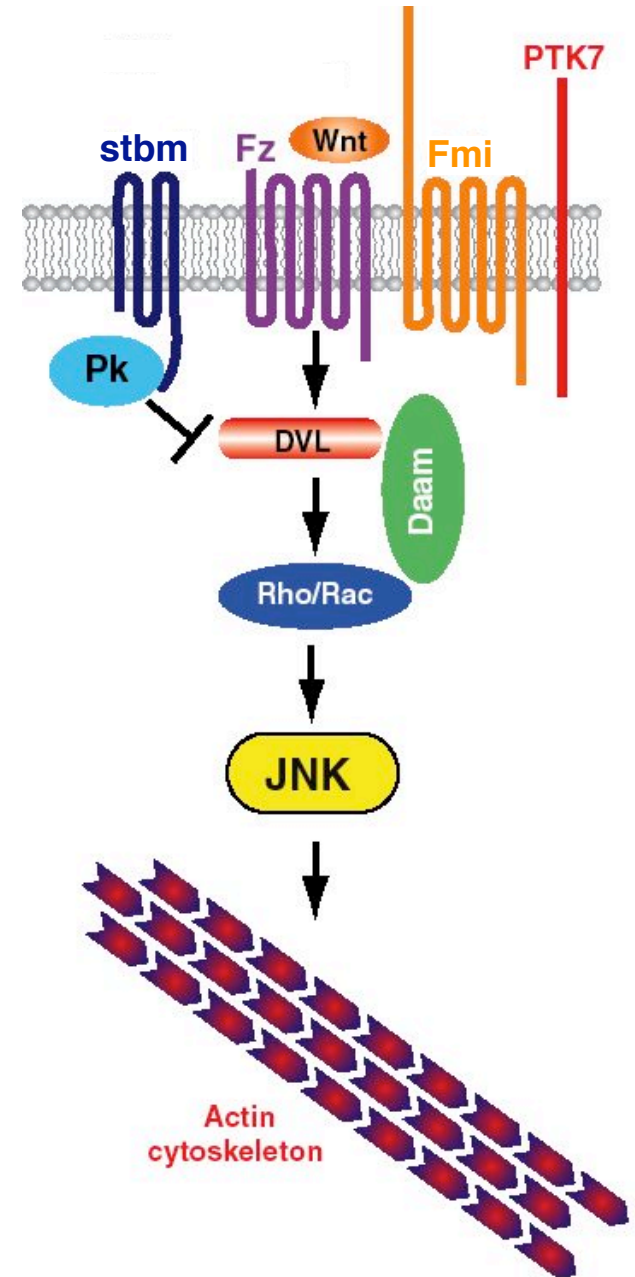
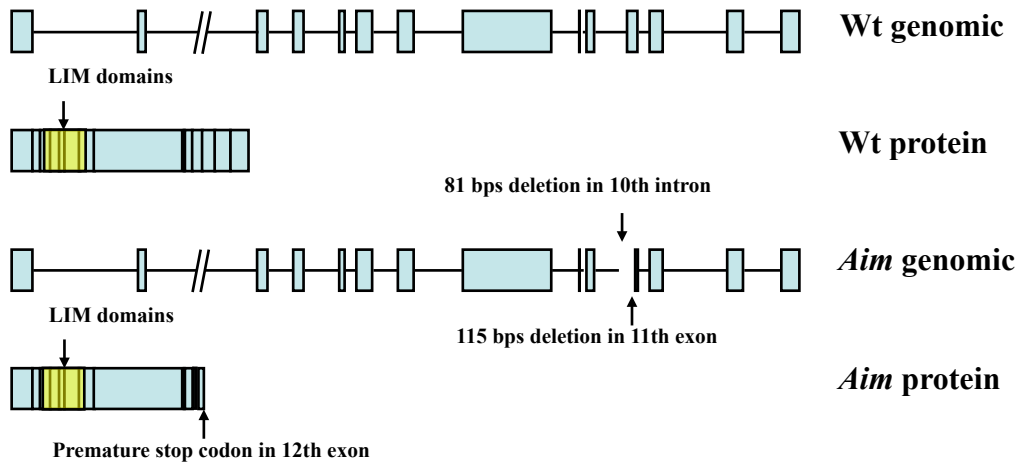


the model: boundary capture

- notochord cells exhibit random motility early in development.
- contact with the forming peri-notochord basement membrane captures them.
- the result is a convergence of the notochord cells even in the absence of polarized cell motility

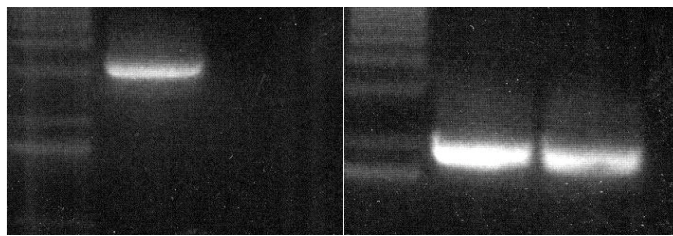


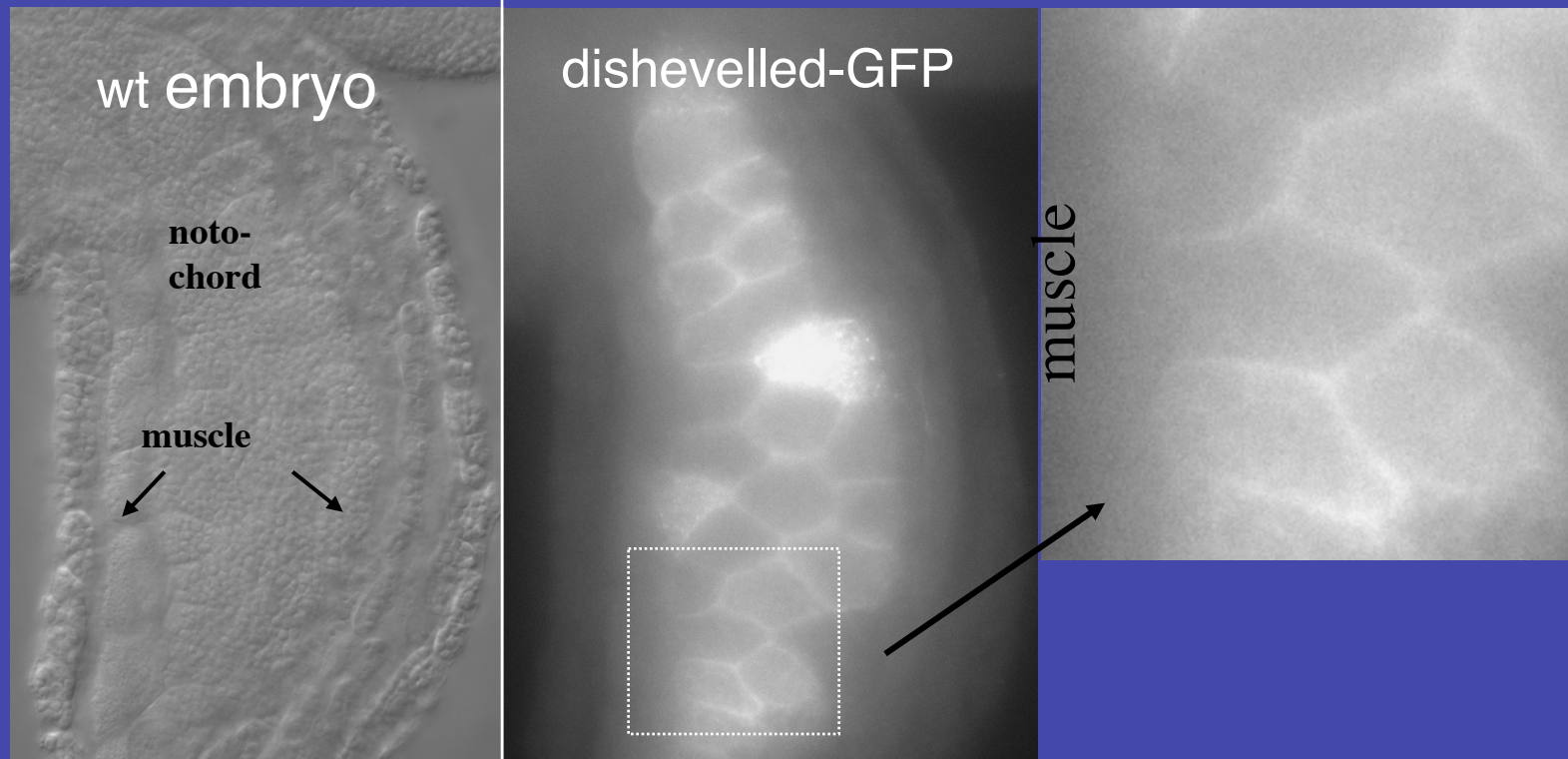
aimless line has deletion in the PCP-gene *prickle* (*pk*)



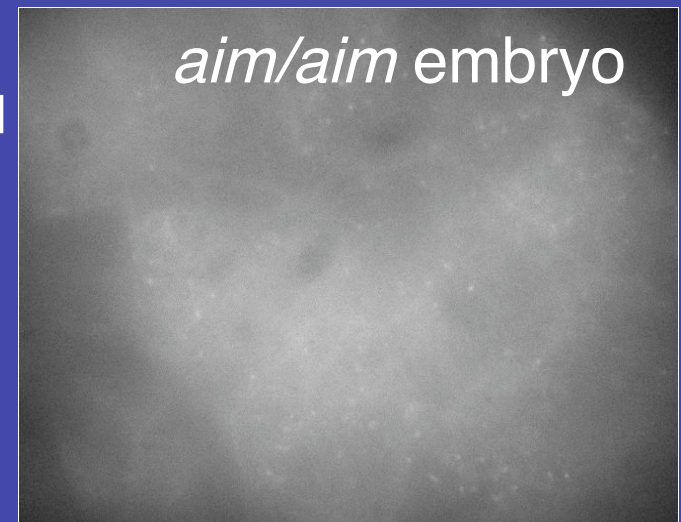
pk

dsh





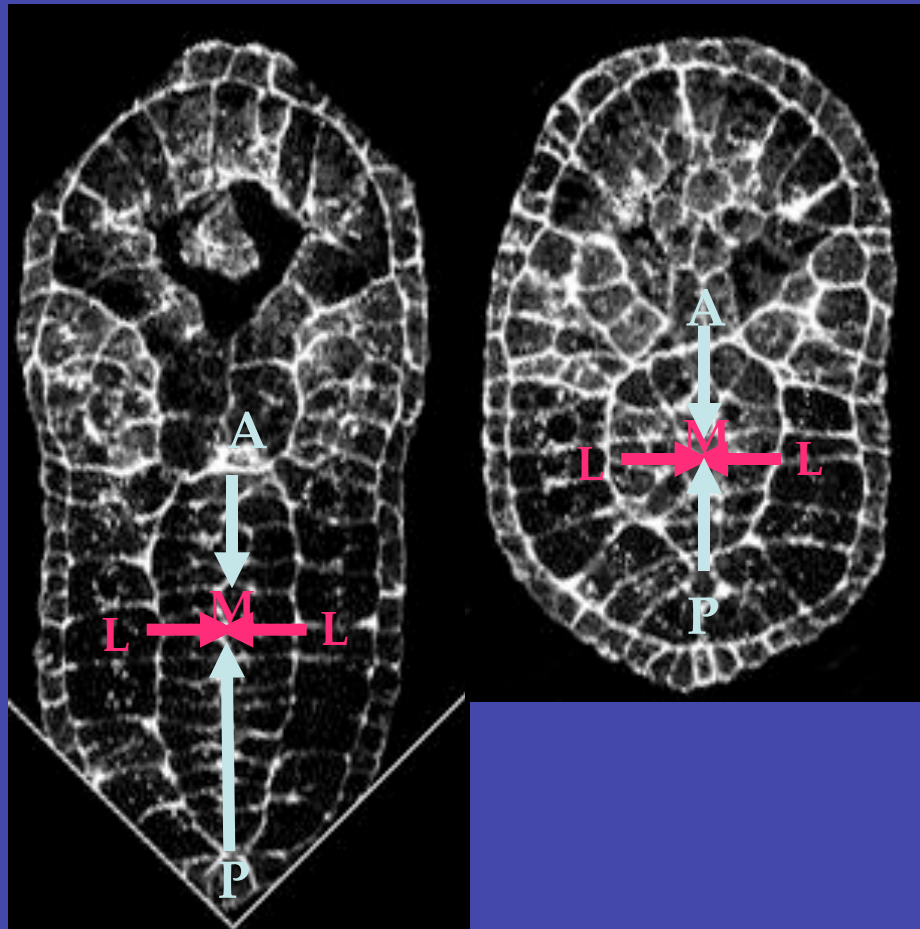
- in wt embryos *dishevelled* is membrane bound and polarized away from muscle boundary
- in *aim/aim* embryos both polarization and membrane localization of *dishevelled* is lost



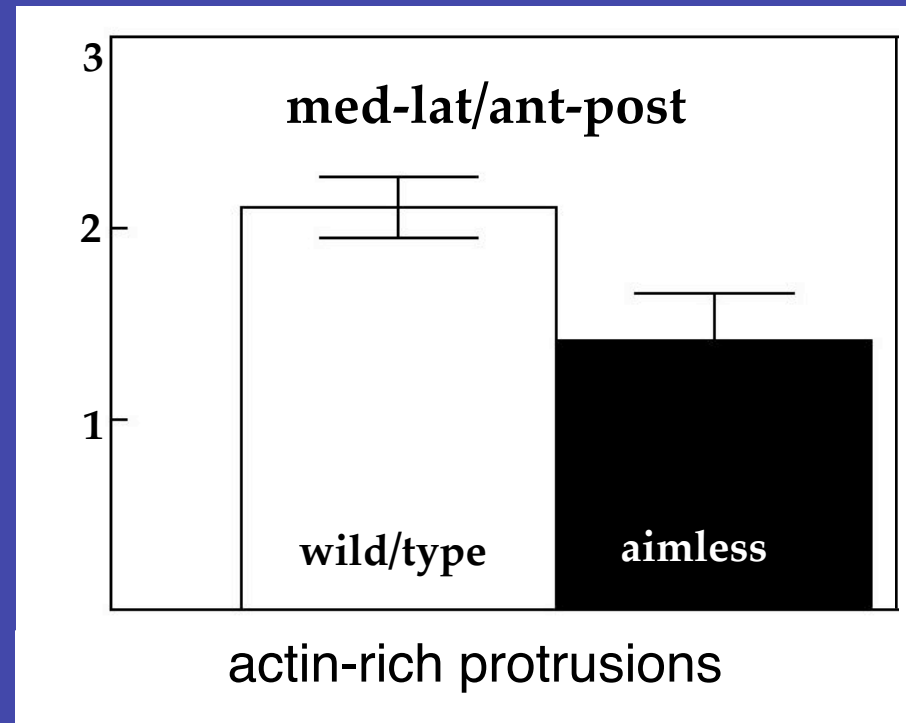
loss of notochord cell polarity in aim/aim embryos has multiple consequences

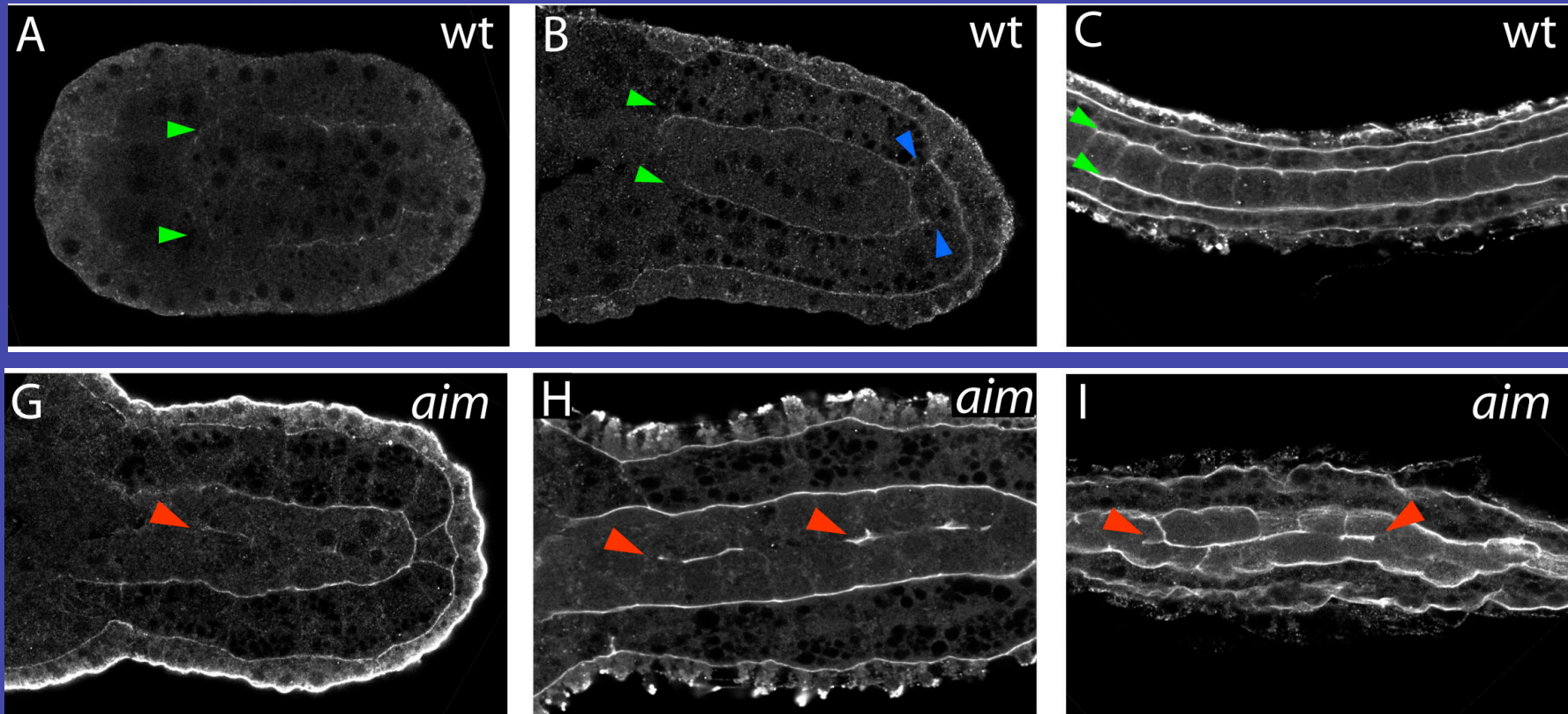
wild type

aimless



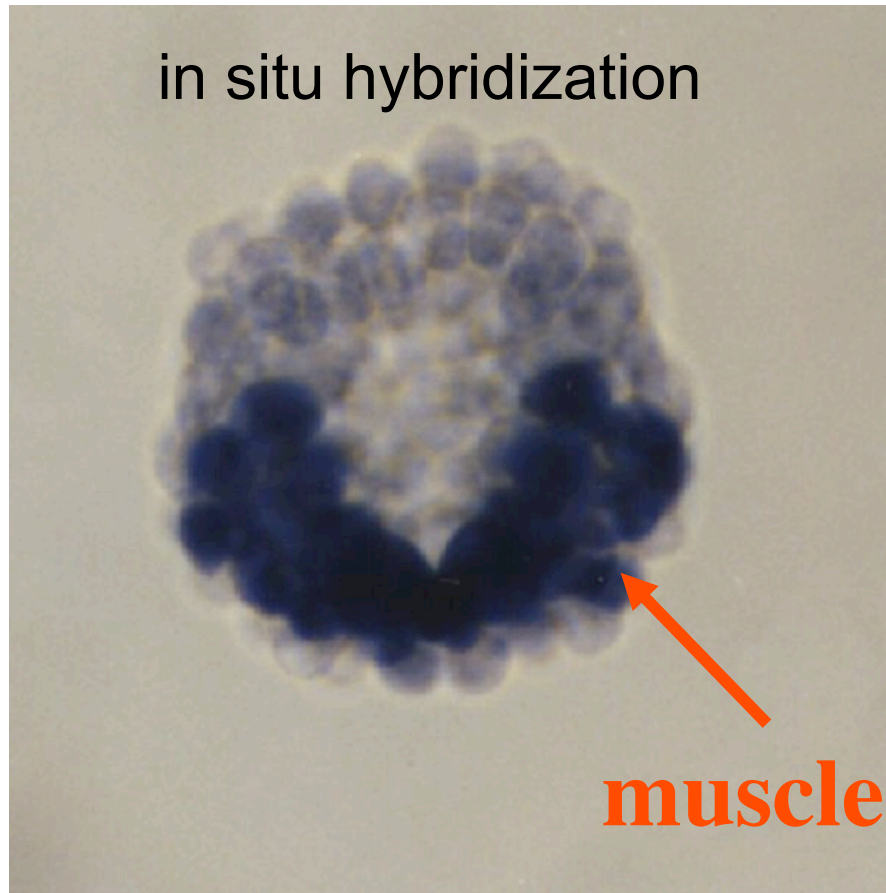
- loss of mediolateral-biased motility



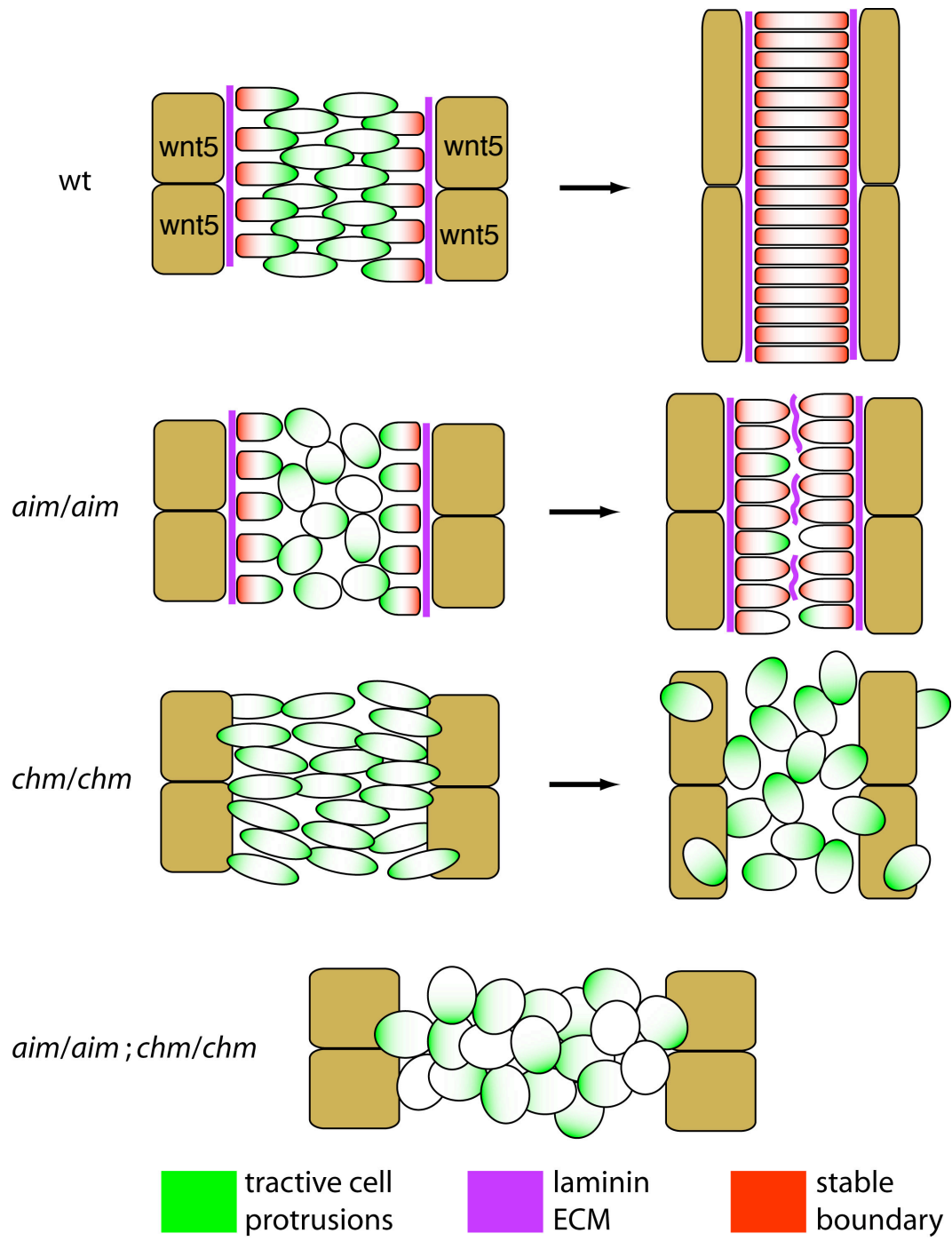


- loss of *pk* function also leads to a progressive disruption of the boundary
- probably prevents complete intercalation of notochord cells (rather than loss of polarized motility)

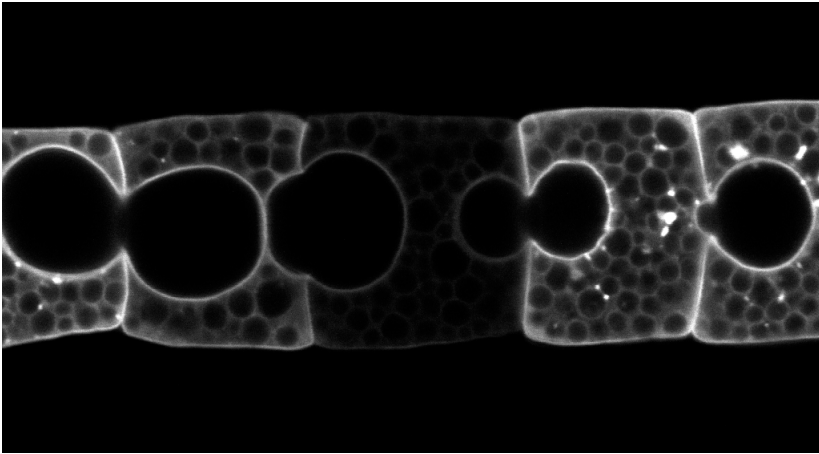
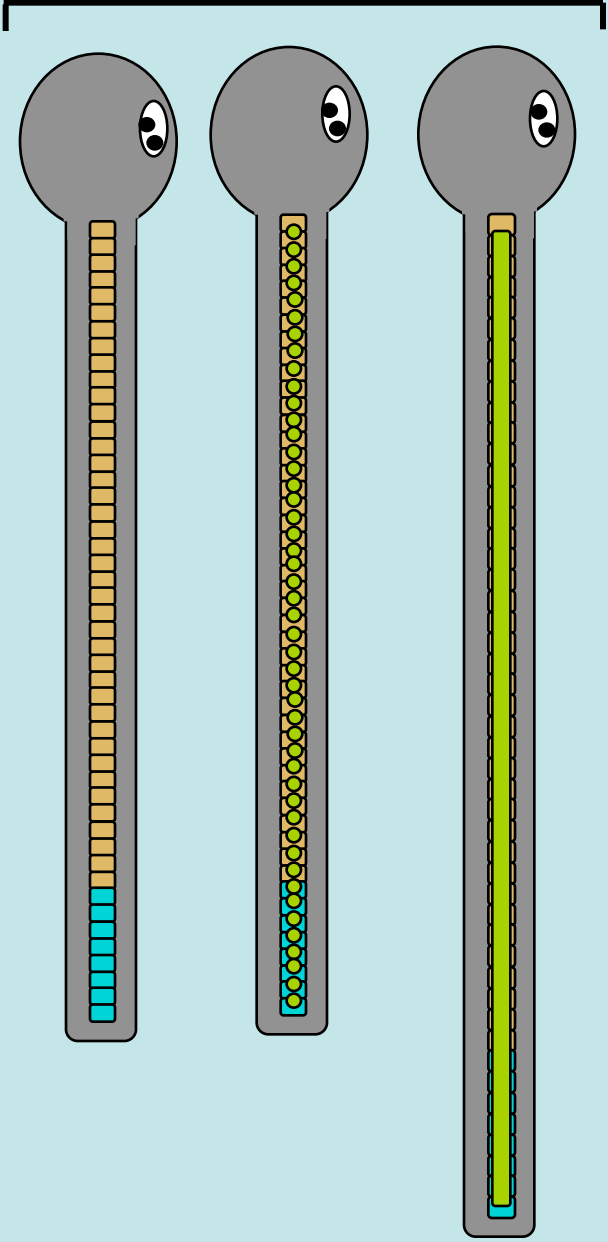
Ciona wnt5



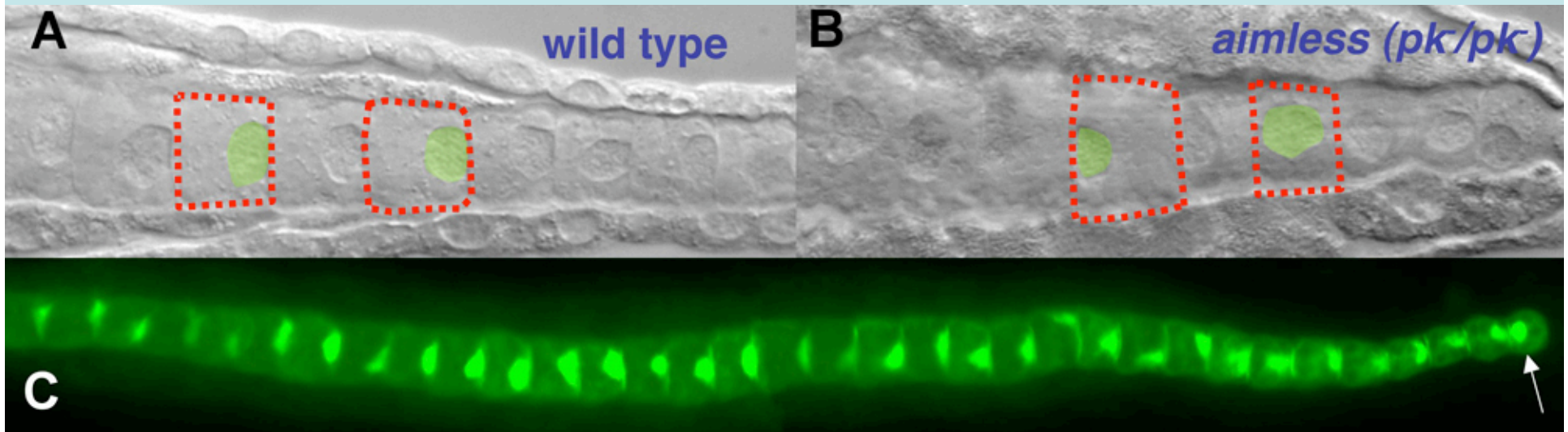
the muscle-derived *wnt5* is a strong candidate
for the directional cue



what are the morphogenic mechanisms that generates the lumen?

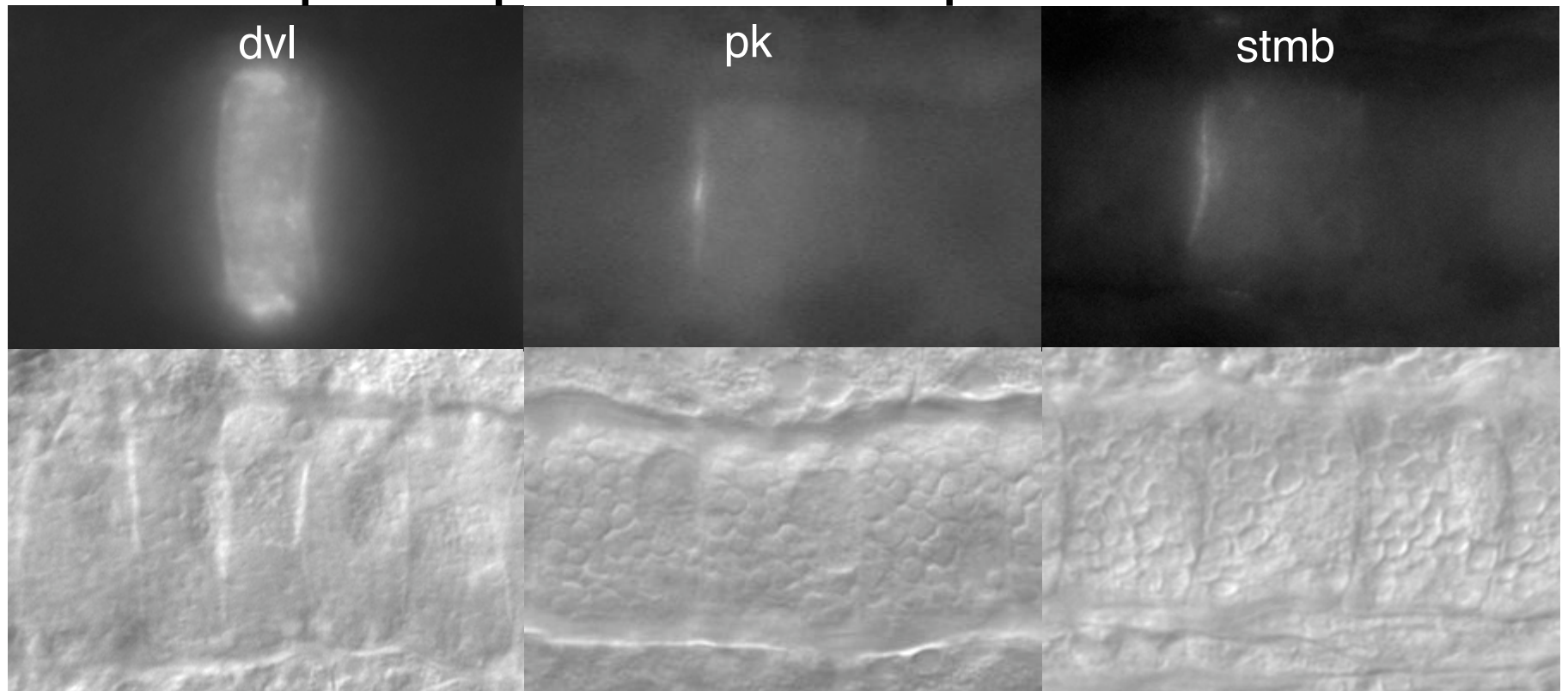


the notochord cells are polarized in the anterior/posterior axis

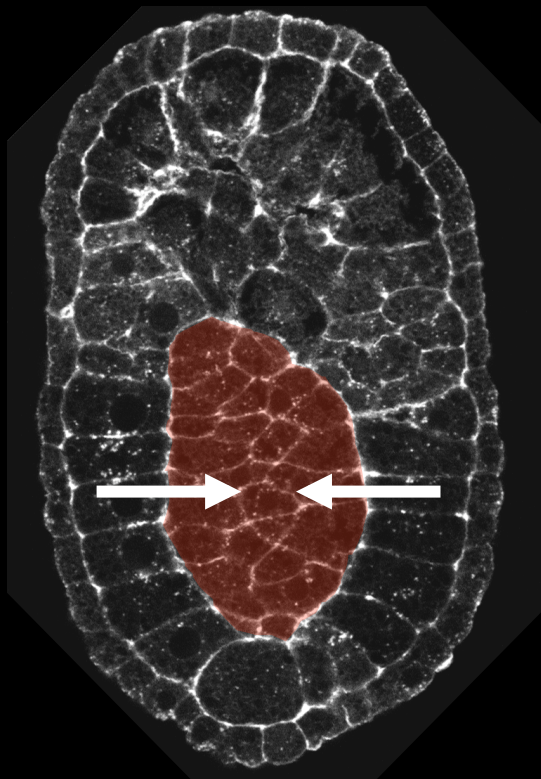


1. nuclei are invariably found at posterior edge of each cell, except for the most posterior cell
2. this polarity is only seen in notochord cells
3. polarity is not evident until after cells have intercalated into in single-celled column
3. mutation of the gene *prickle* (*pk*) disrupts this polarity

PCP proteins polarized in anterior/posterior axis

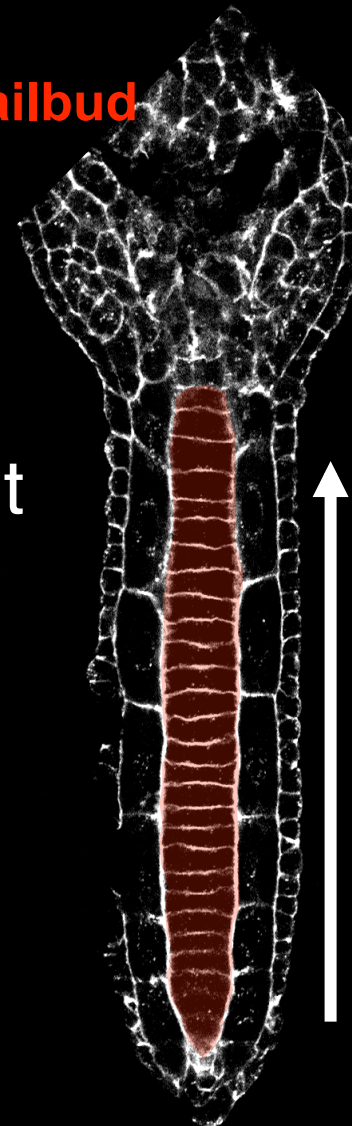


neurula



medio-lateral

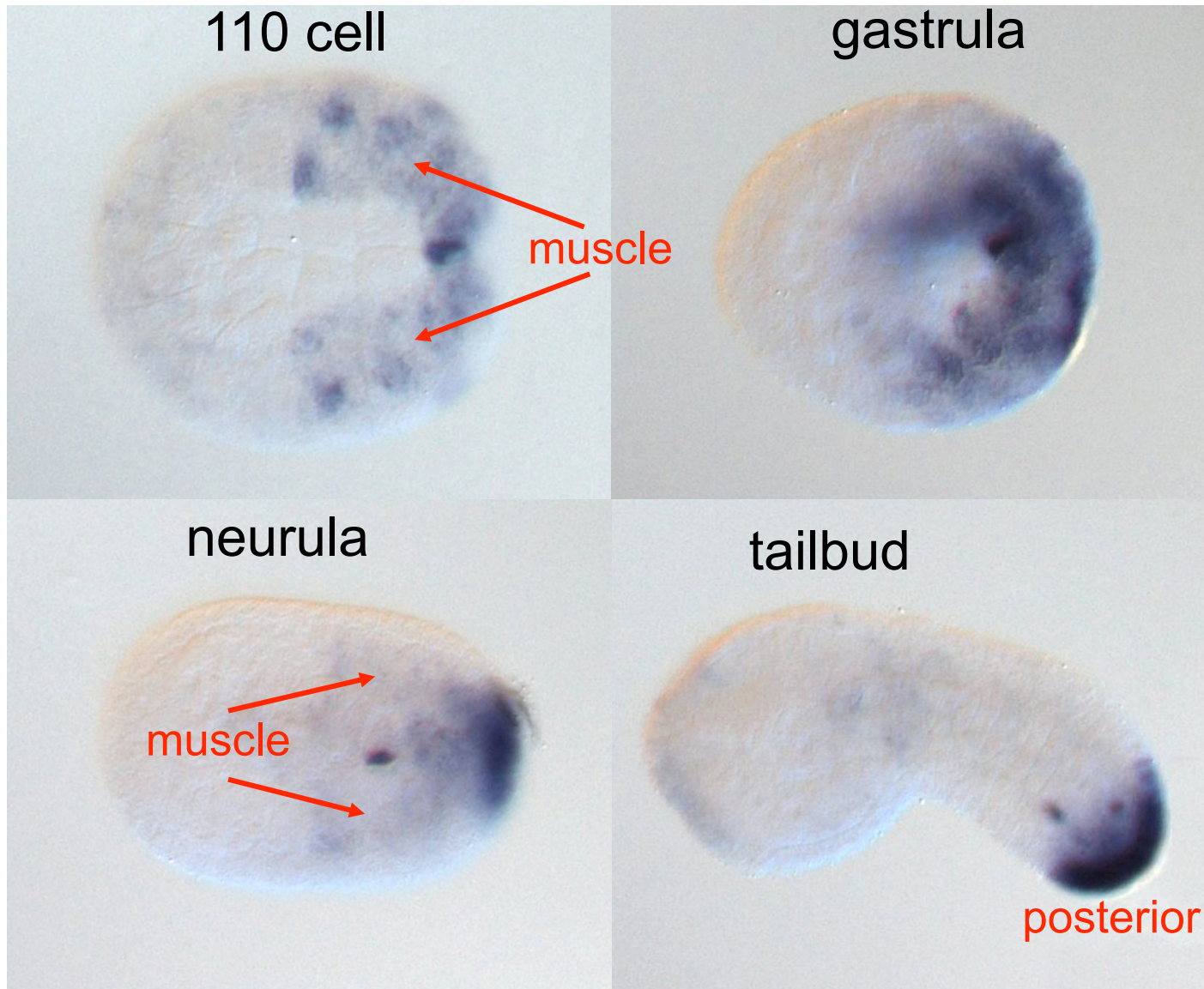
tailbud



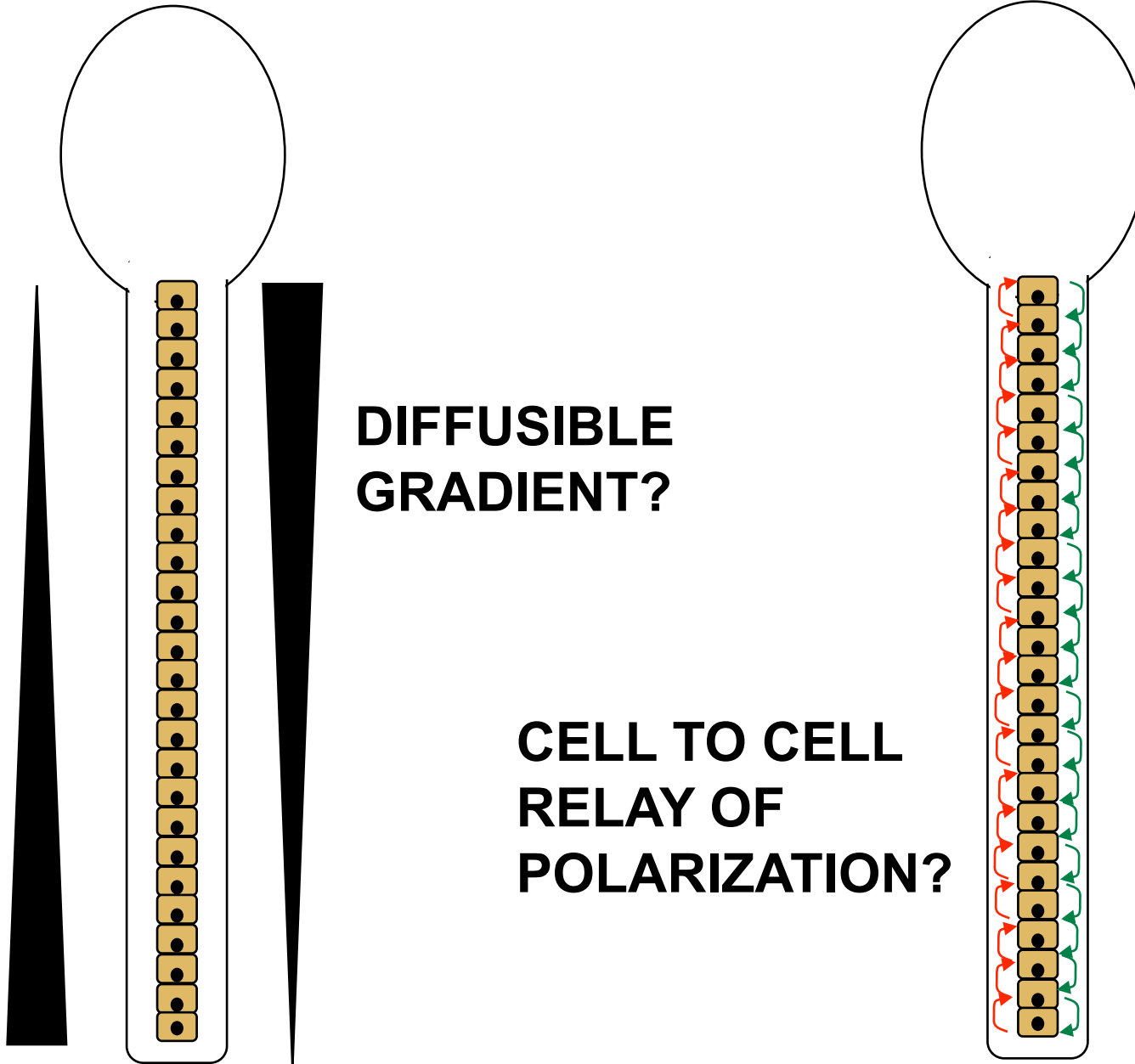
anterior-posterior

PCP-dependent
notochord
polarity shift

wnt 5

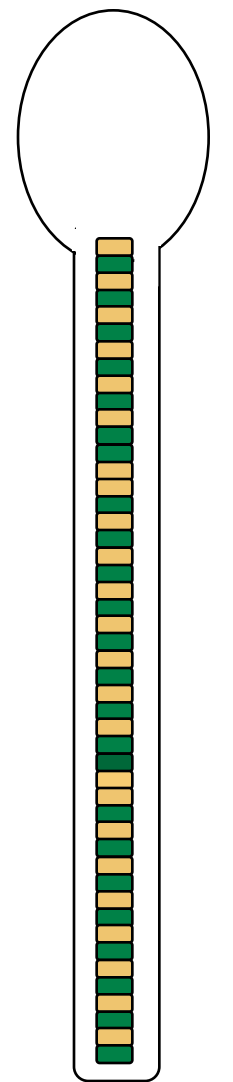
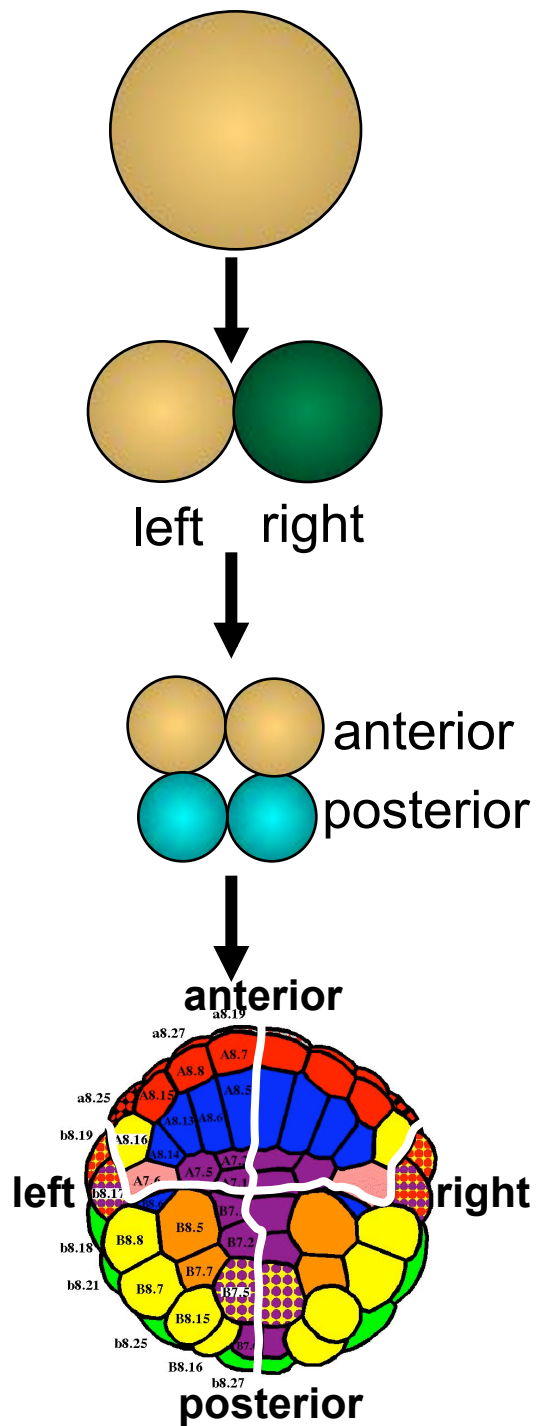


what is mechanism for generating AP polarity?

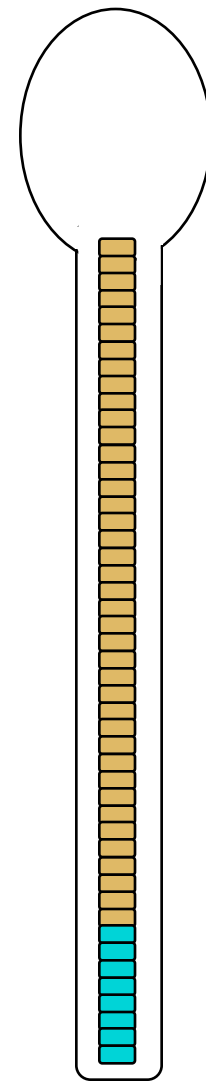


**DIFFUSIBLE
GRADIENT?**

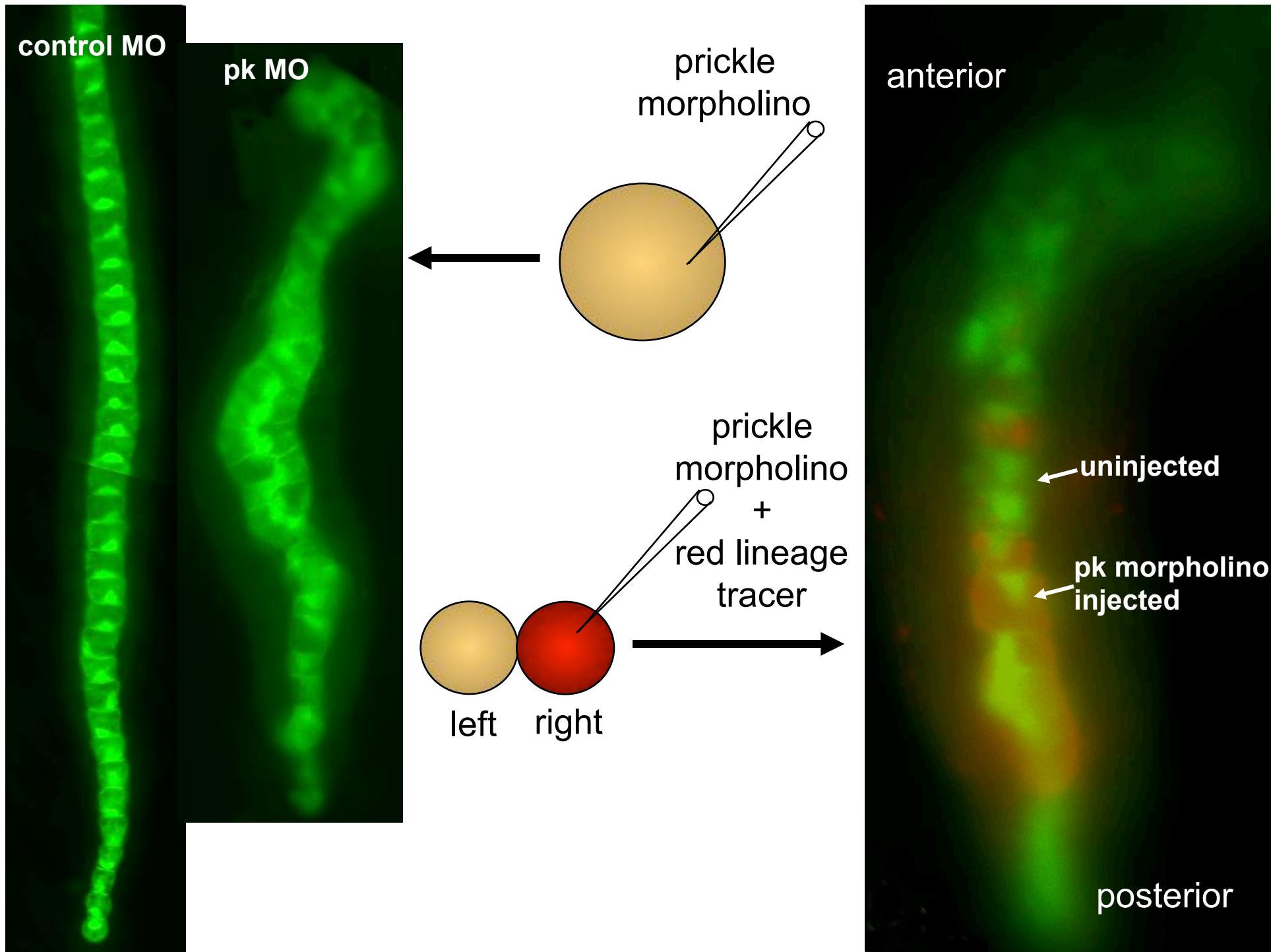
**CELL TO CELL
RELAY OF
POLARIZATION?**

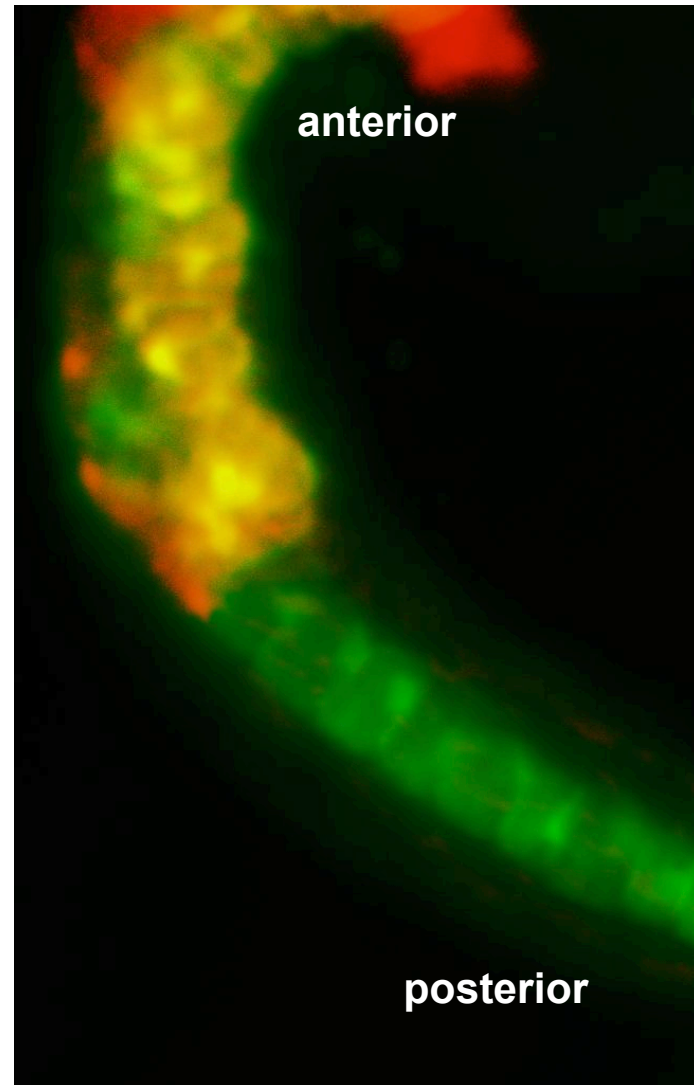
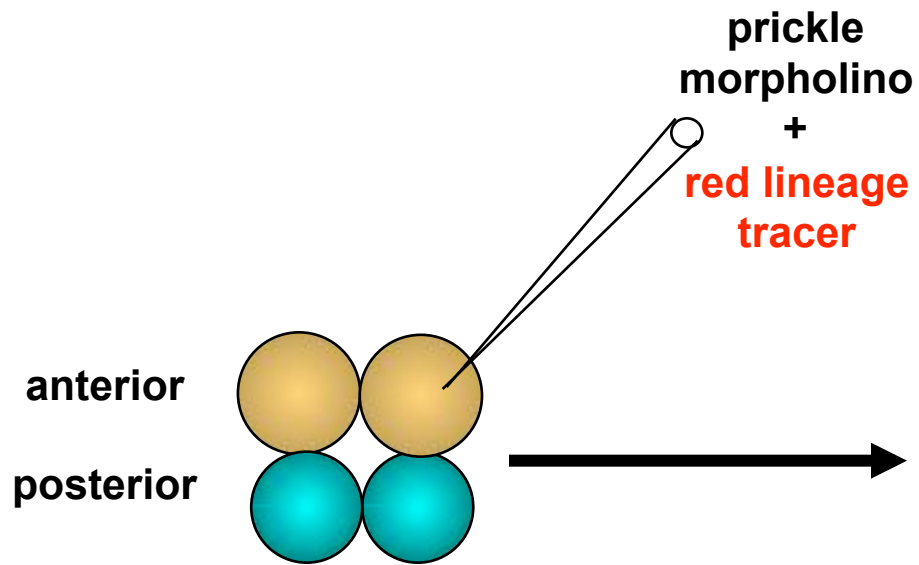


left/right notochord fates



anterior/posterior notochord fates

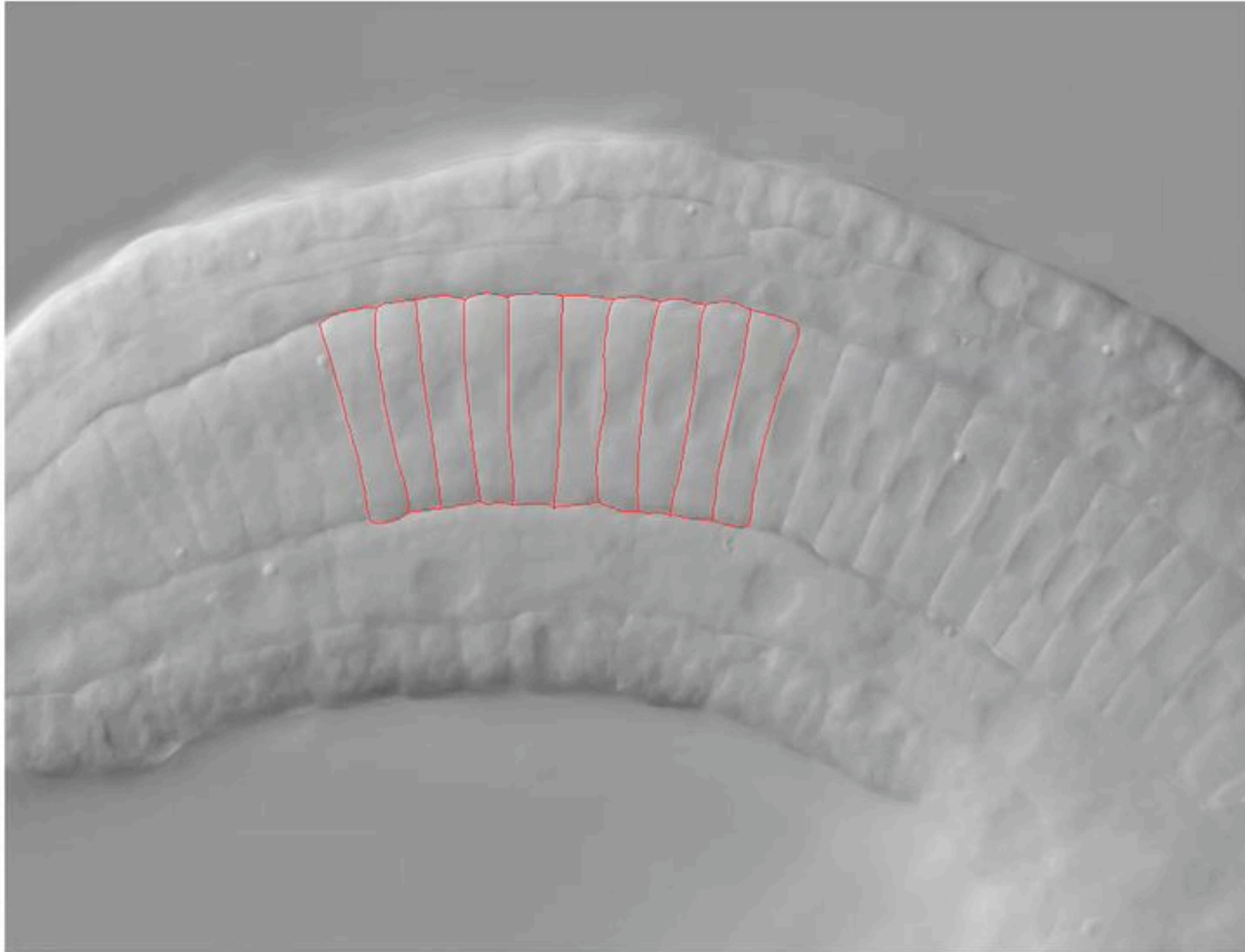




- genetic studies have provided insights into mechanisms driving notochord morphogenesis
- models based on our genetic studies predict certain types of cellular behavior. For example, cells should be quiescent at lateral edges after intercalation.



Final result, iter = 50



automated 2D + t segmentation of notochord cells from DIC images

Segmentation of notochord cells in fixed, stained embryos

mip



slice



0

20

45

67.5

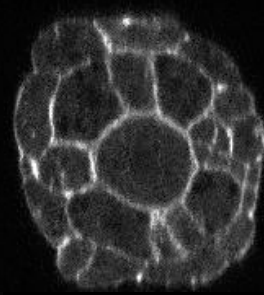
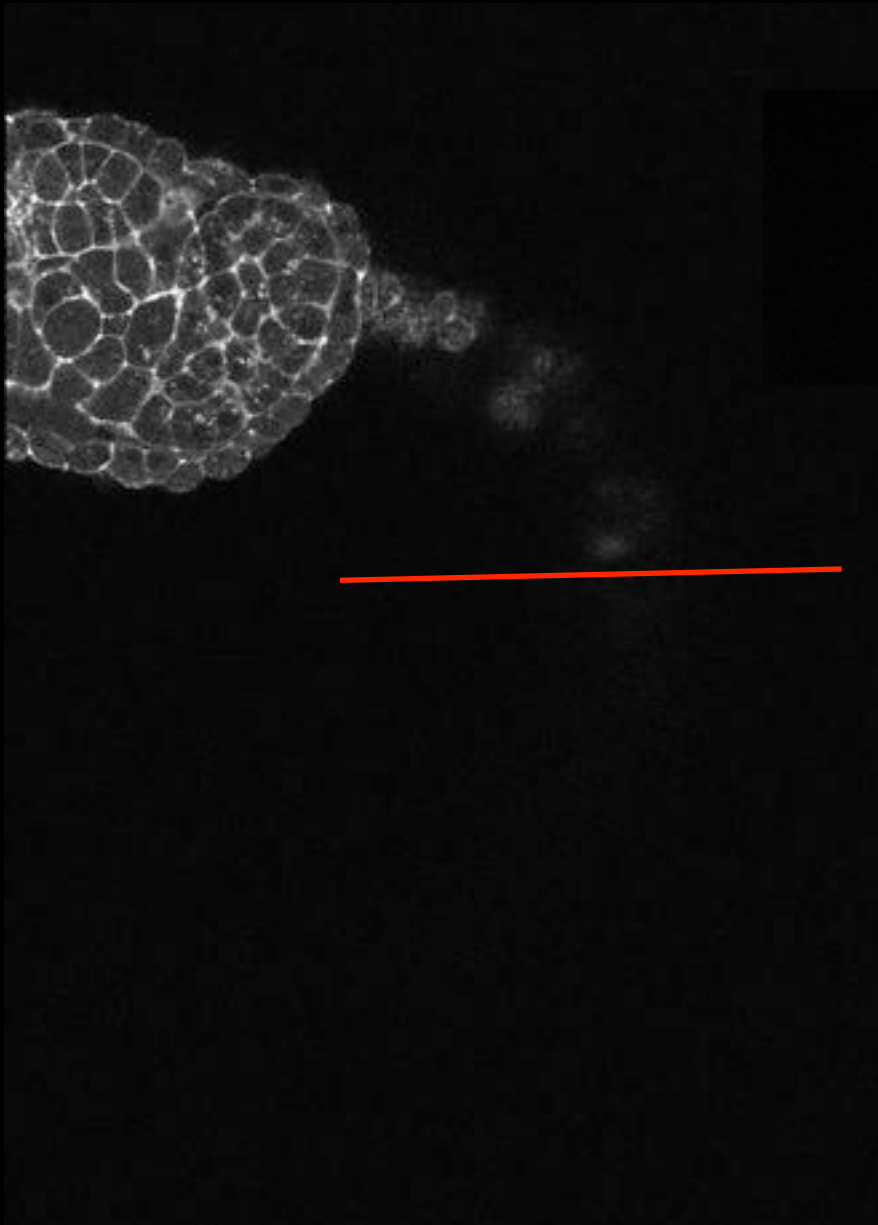
90

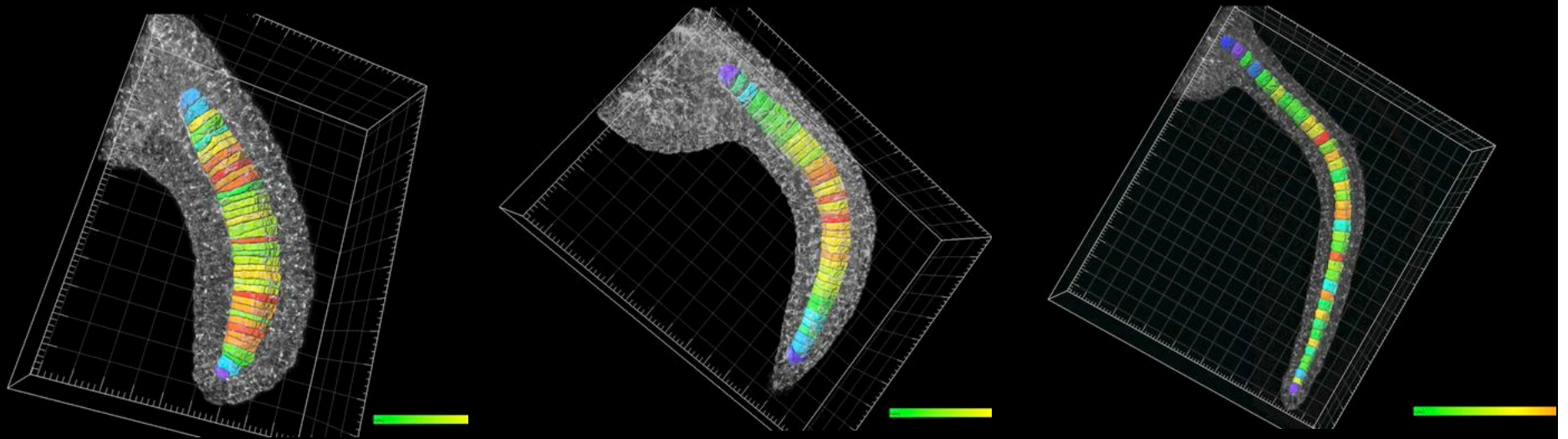
112.5

136

160

minutes



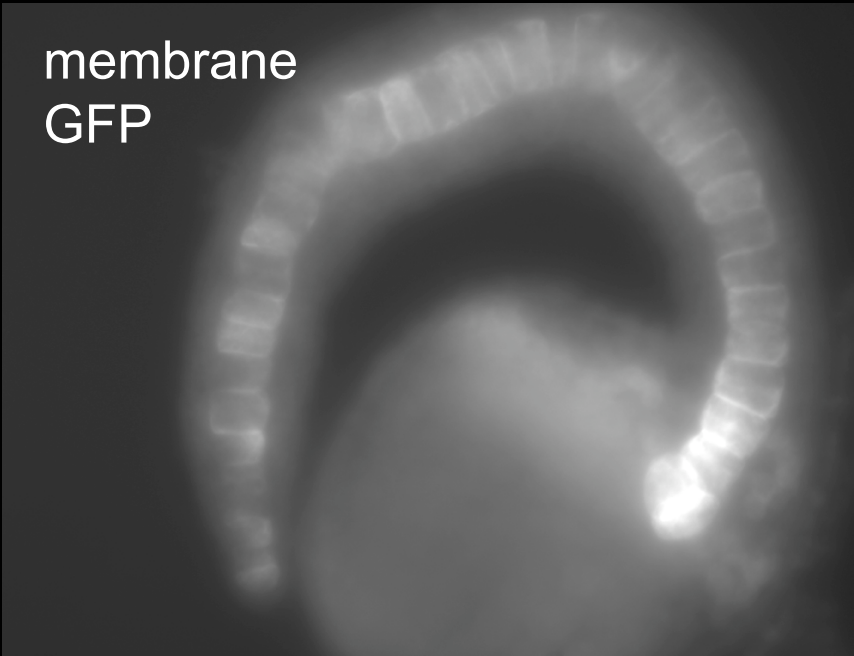


- images were segmented in 3D by watershed transformation
- segmentation works well for fixed, stained, cleared embryos.
- long term goal is segmentation of all stages of notochord development in **live** embryos

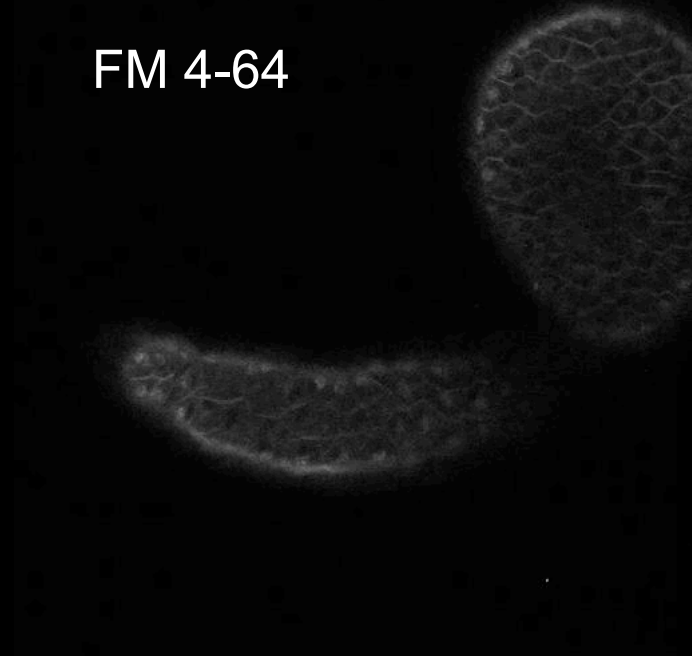
- with the goal is image capture and segmentation in live embryos:

- two approaches we are exploring

membrane
GFP



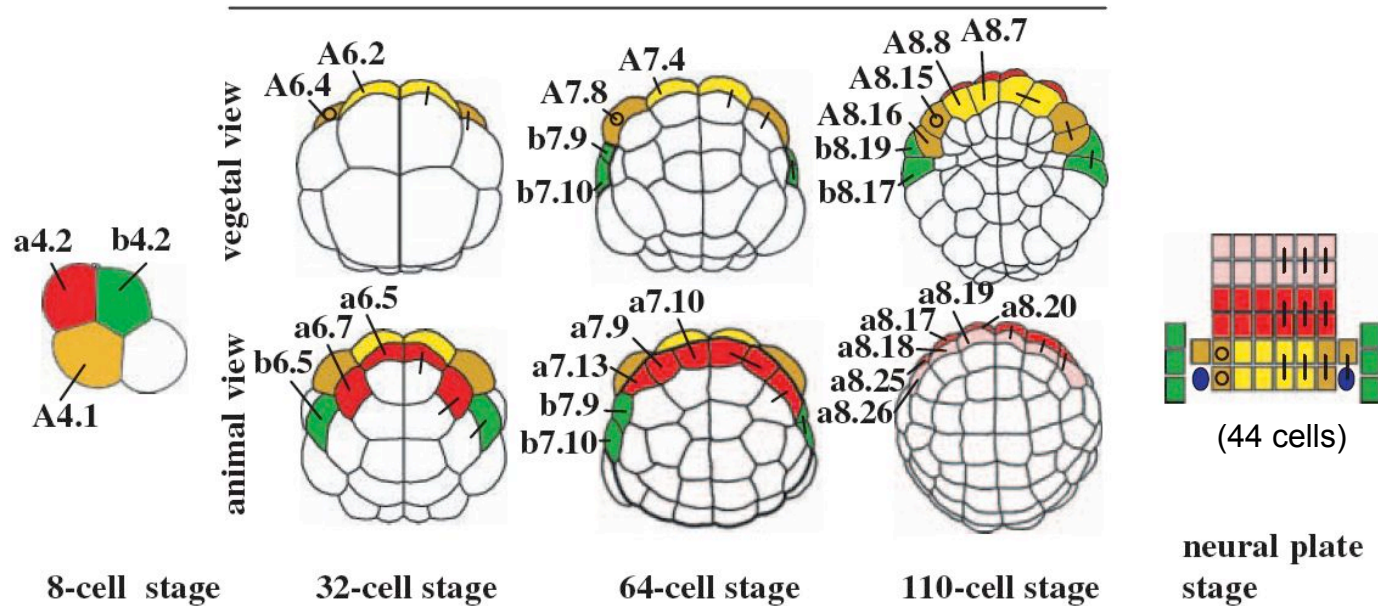
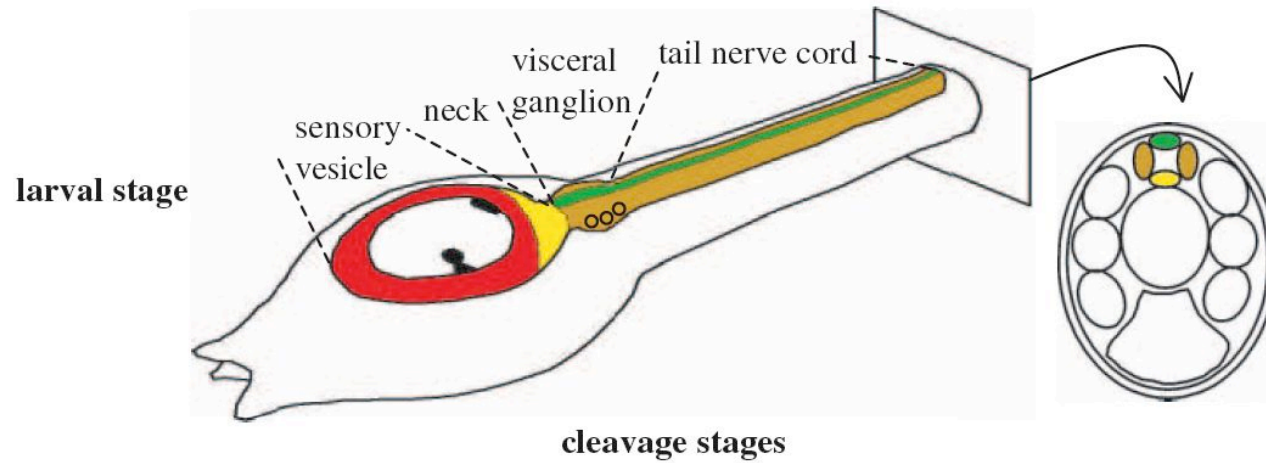
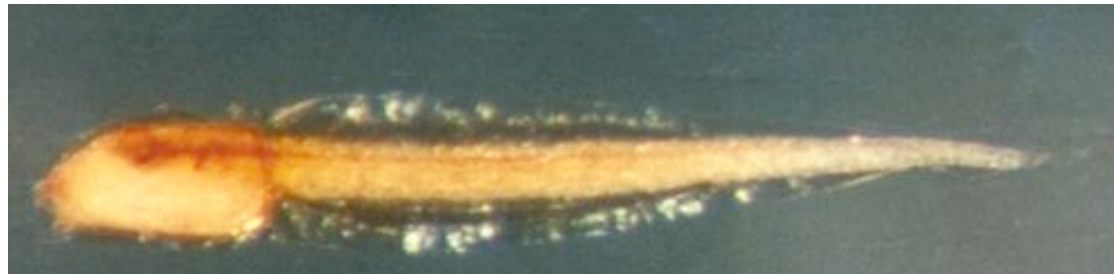
FM 4-64



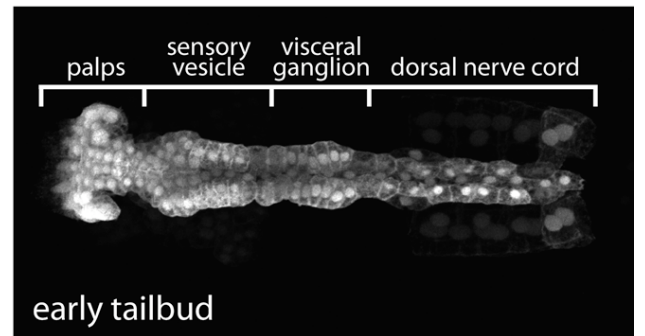
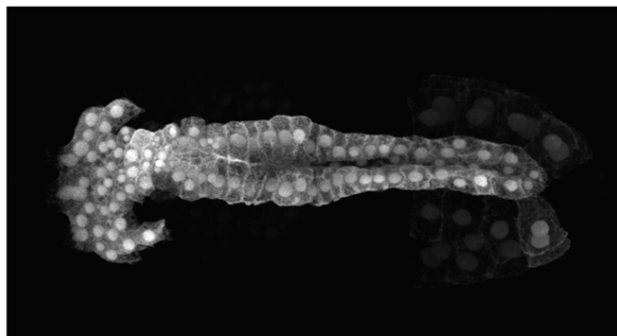
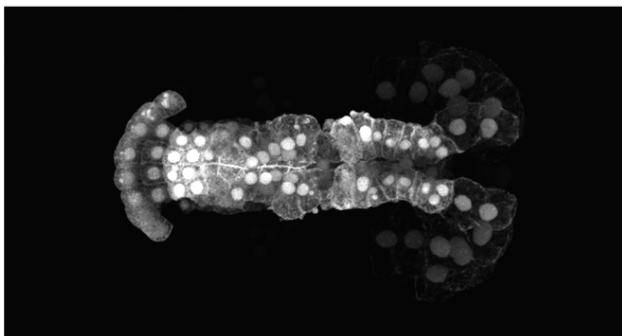
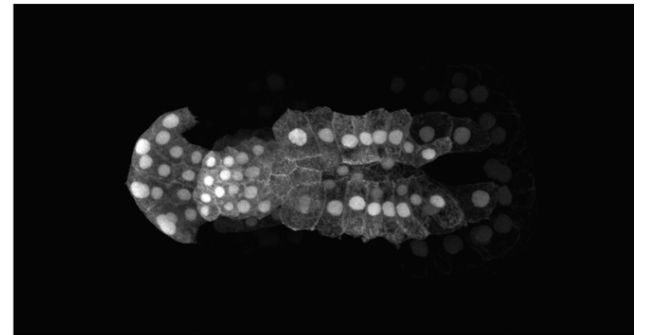
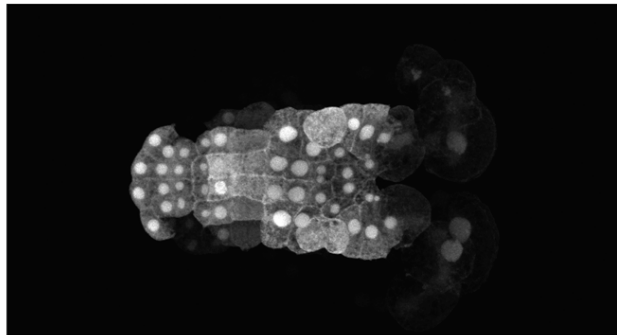
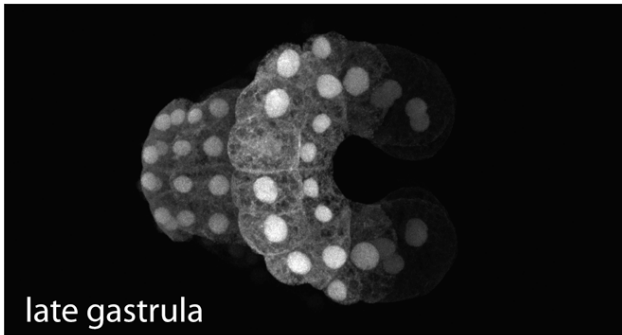
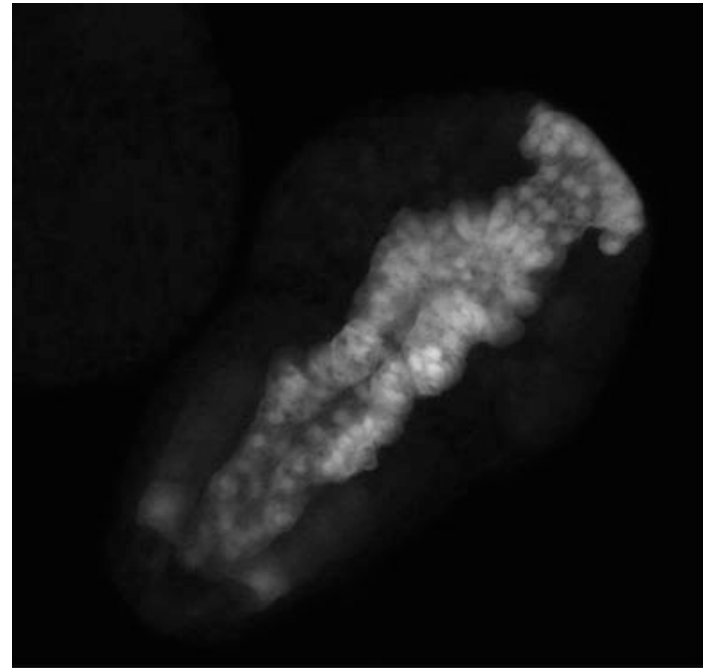
Ascidian Central Nervous System

free swimming larva

< 130 neural cells
 ≈ 230 glial cells

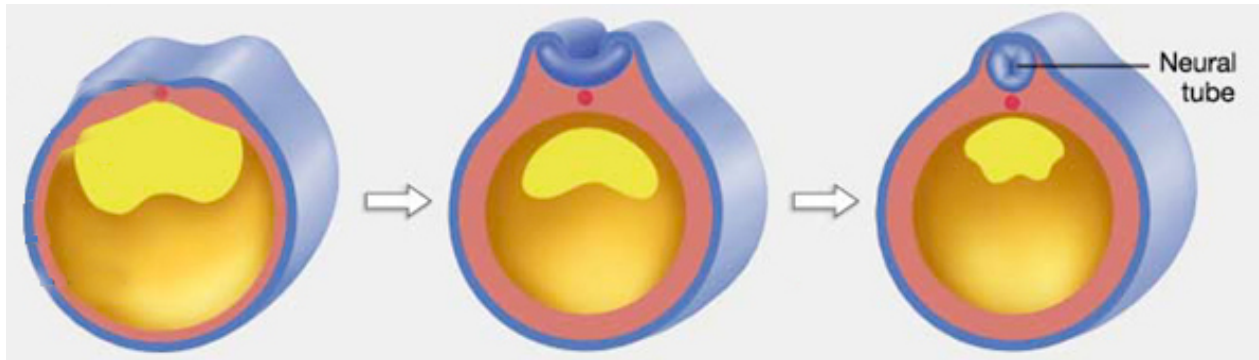


transgenic ascidian line gives us
cellular resolution of neurogenesis
in live embryos



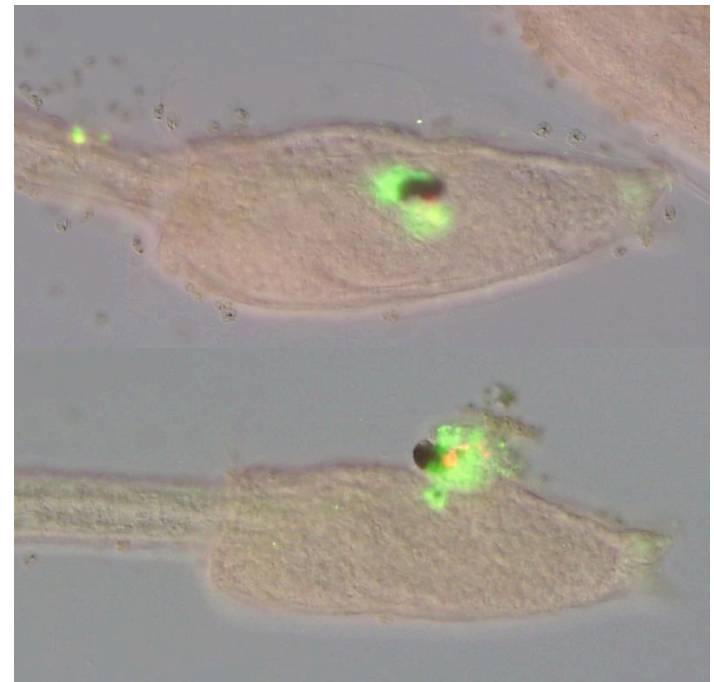
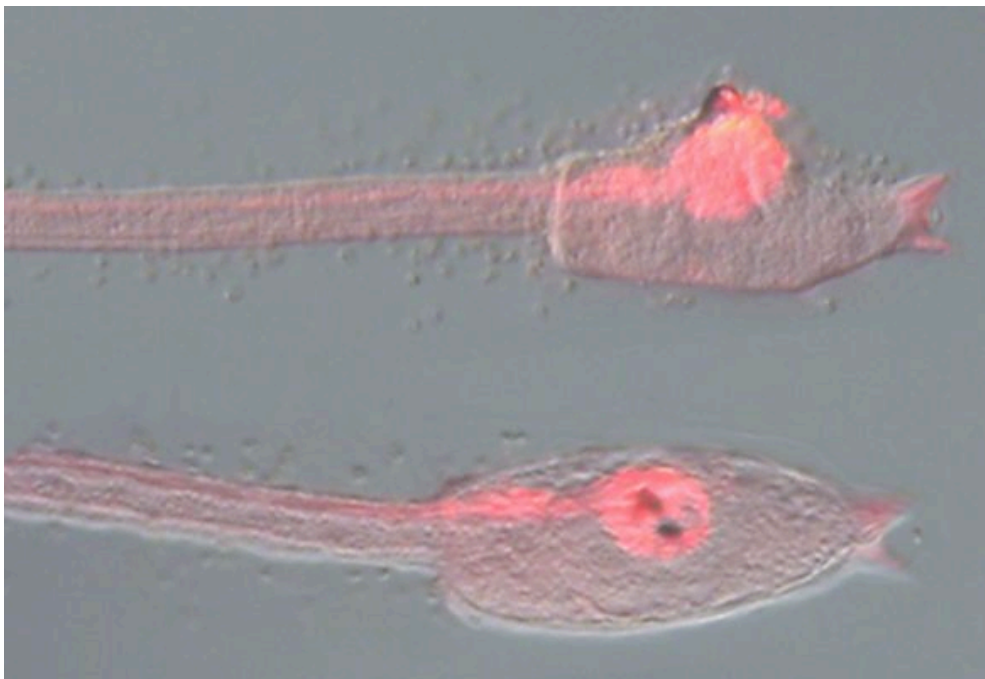
neurulation in the frog *Xenopus*

neurulation



exencephaly in the mouse

exencephaly mutant in *Ciona* (bugeye)

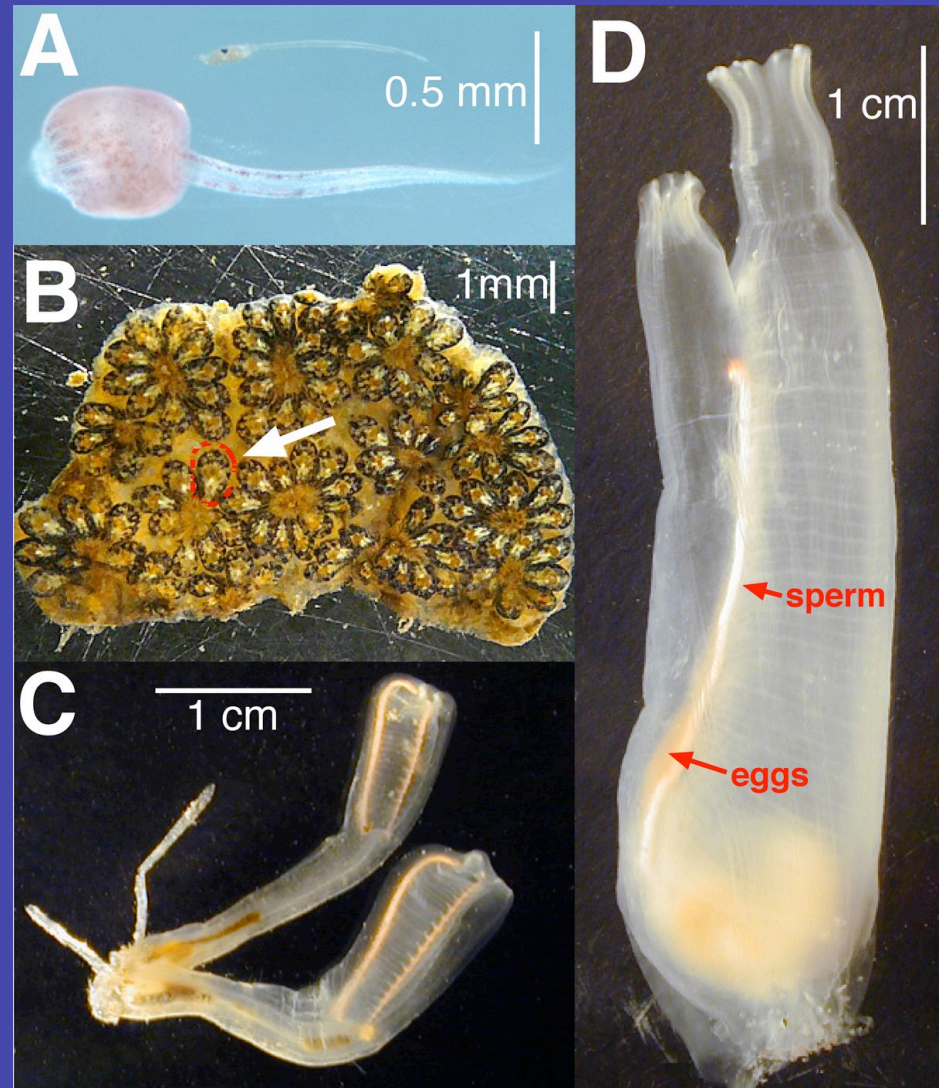


Ascidian Project:

Matt Kourakis
Shota Chiba
Michael Veeman
Erin Mulholland
Erin Newman-Smith
Danny El-Nachef

collaborators:

B. Manjunath
Boguslaw Obara



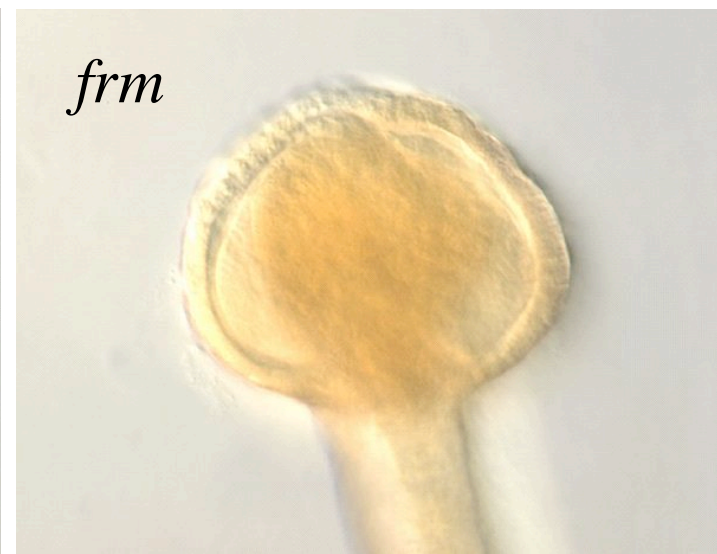
Funding:

Thanks NIH

Santa Barbara Ascidian Stock Center
<http://www.ascidiancenter.ucsb.edu/>

phenotype: **frimousse**

- anterior brain (sensory vesicle) is absent
- palps (adhesive gland) absent
- mouth (stomodeum) absent



Expression of markers of the palps, RTEN, stomodeum and anterior sensory vesicle is abolished

WT

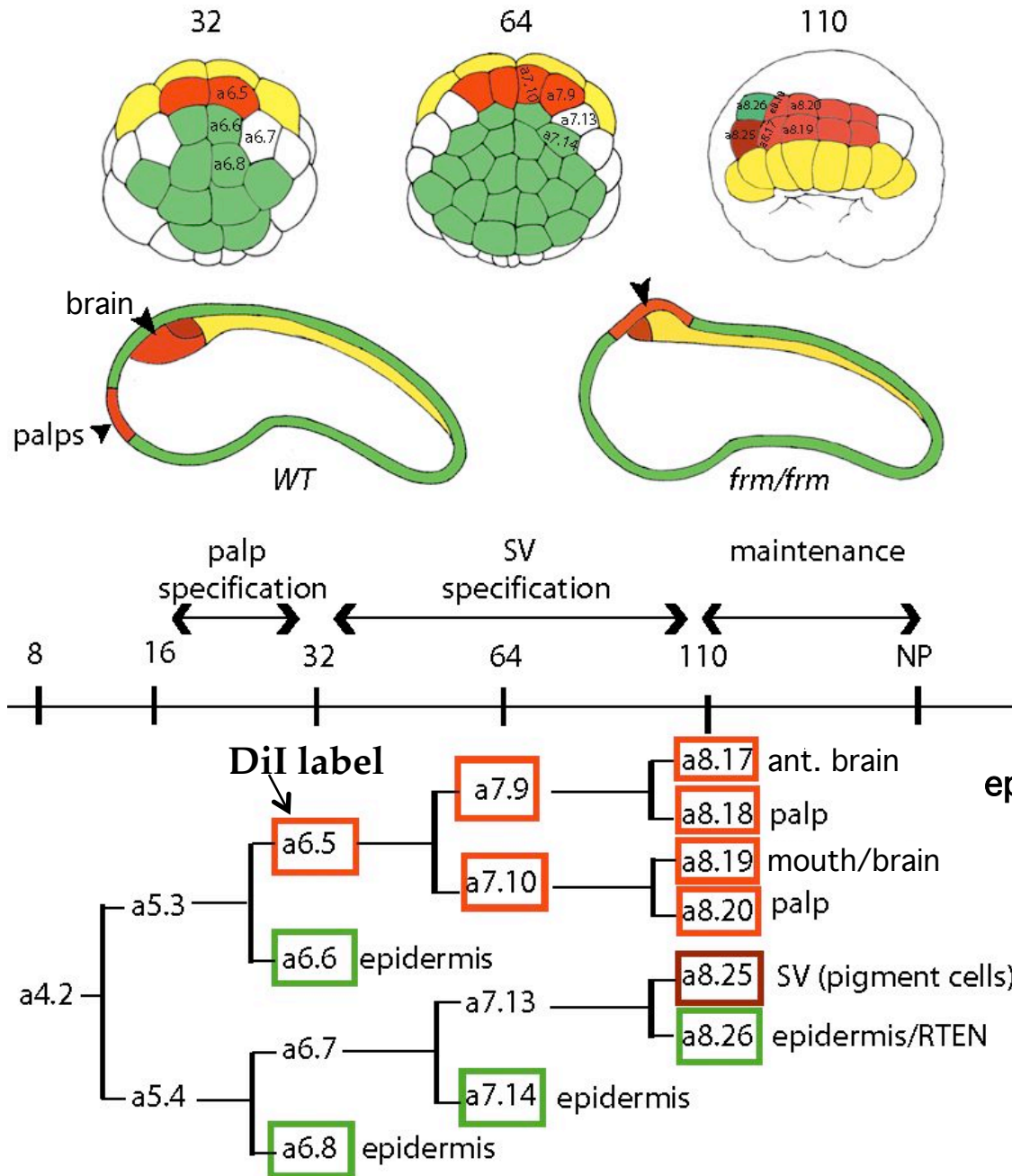
frm

WT

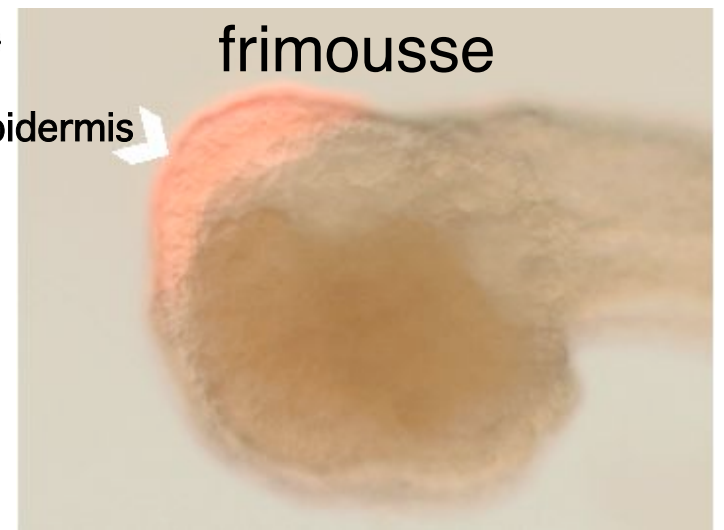
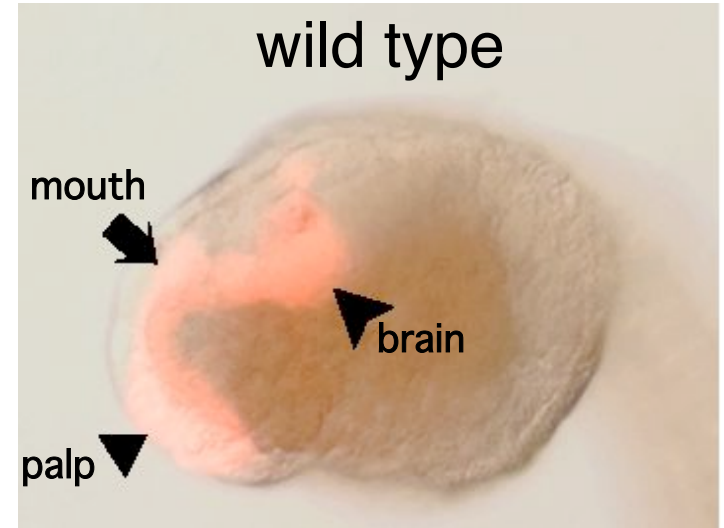
frm



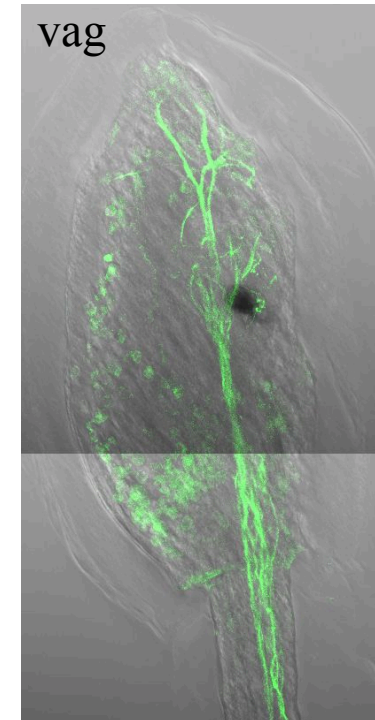
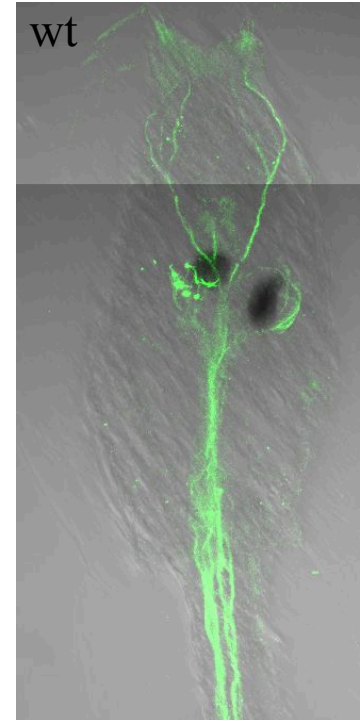
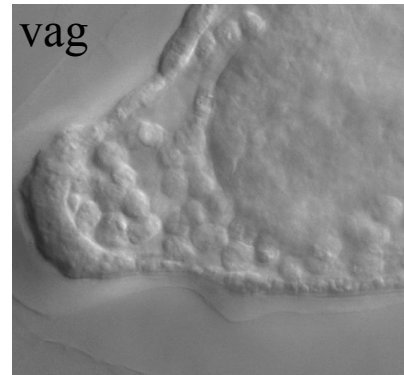
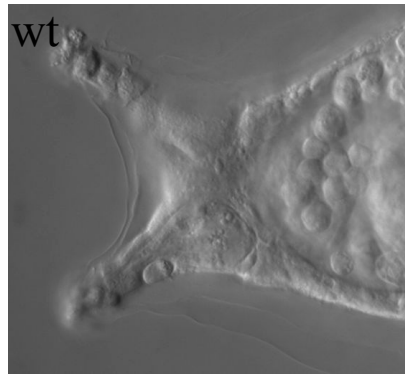
frm has cell fate transformation



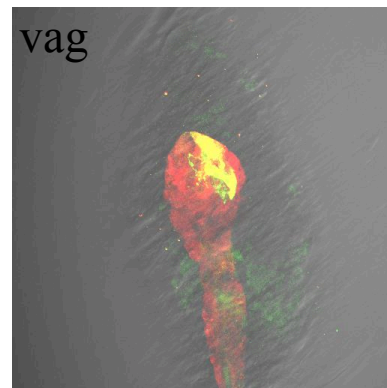
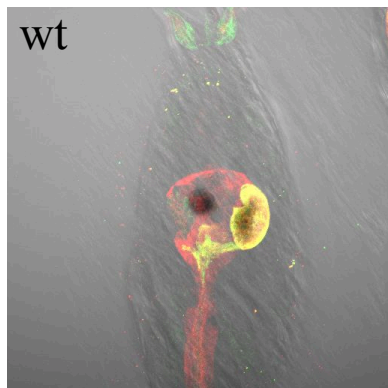
a6.5 derivatives



Vagabond



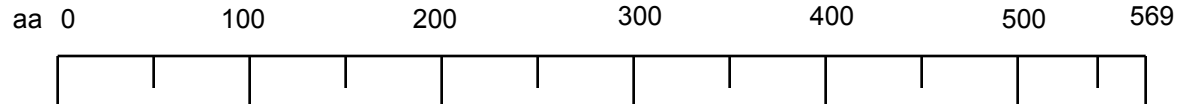
Synaptotagamin



Arrestin/CRALBP

- ENU induced mutation
- Defects apparent in:
 - adhesive appendages (palps)
 - pigmented sensory organs
 - palp sensory neurons
 - larval metamorphosis
- Homozygous lethal

Ciona Dmrt-1



wt

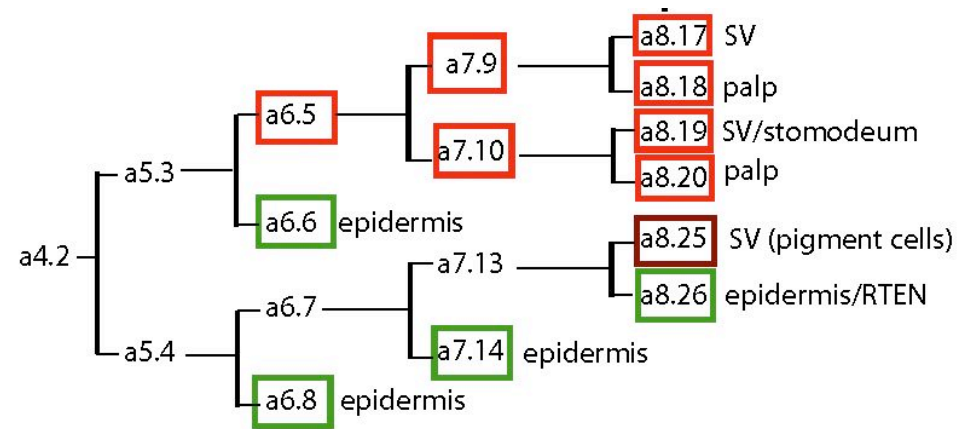
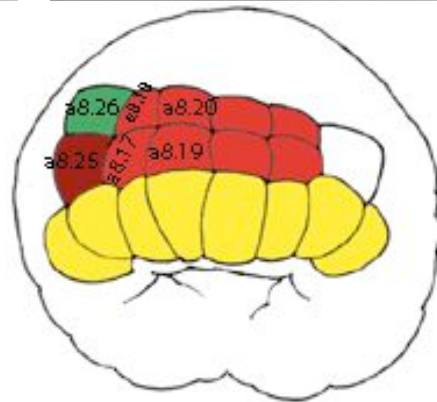
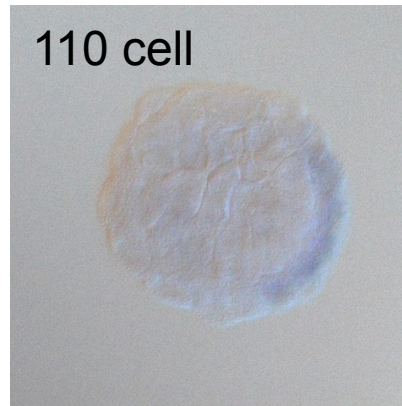
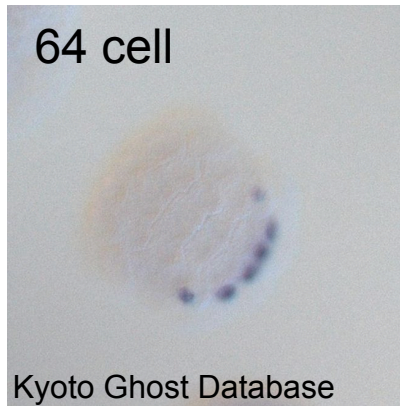
TGG

vag



TAG

DM Domain: Zinc finger DNA binding domain
DMA Domain: Unknown function



UC Santa Barbara

An aerial photograph of the UC Santa Barbara campus and surrounding area. The image shows the blue Pacific Ocean on the left, the green hills of the Santa Barbara mountains in the background, and the campus buildings and green spaces in the foreground. The sky is clear and blue.

- what are tunicates?
- tunicate biology
- tunicate genetics