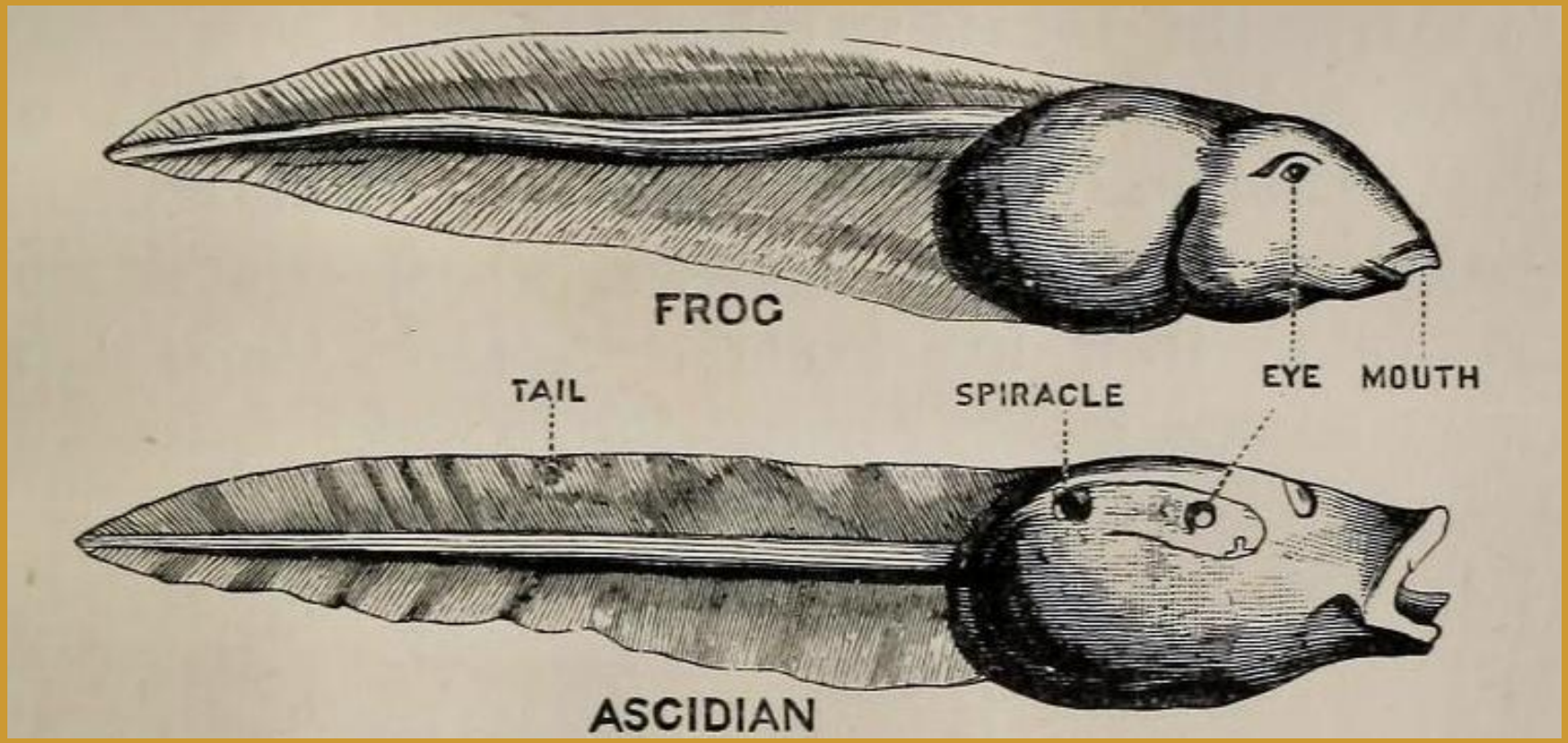
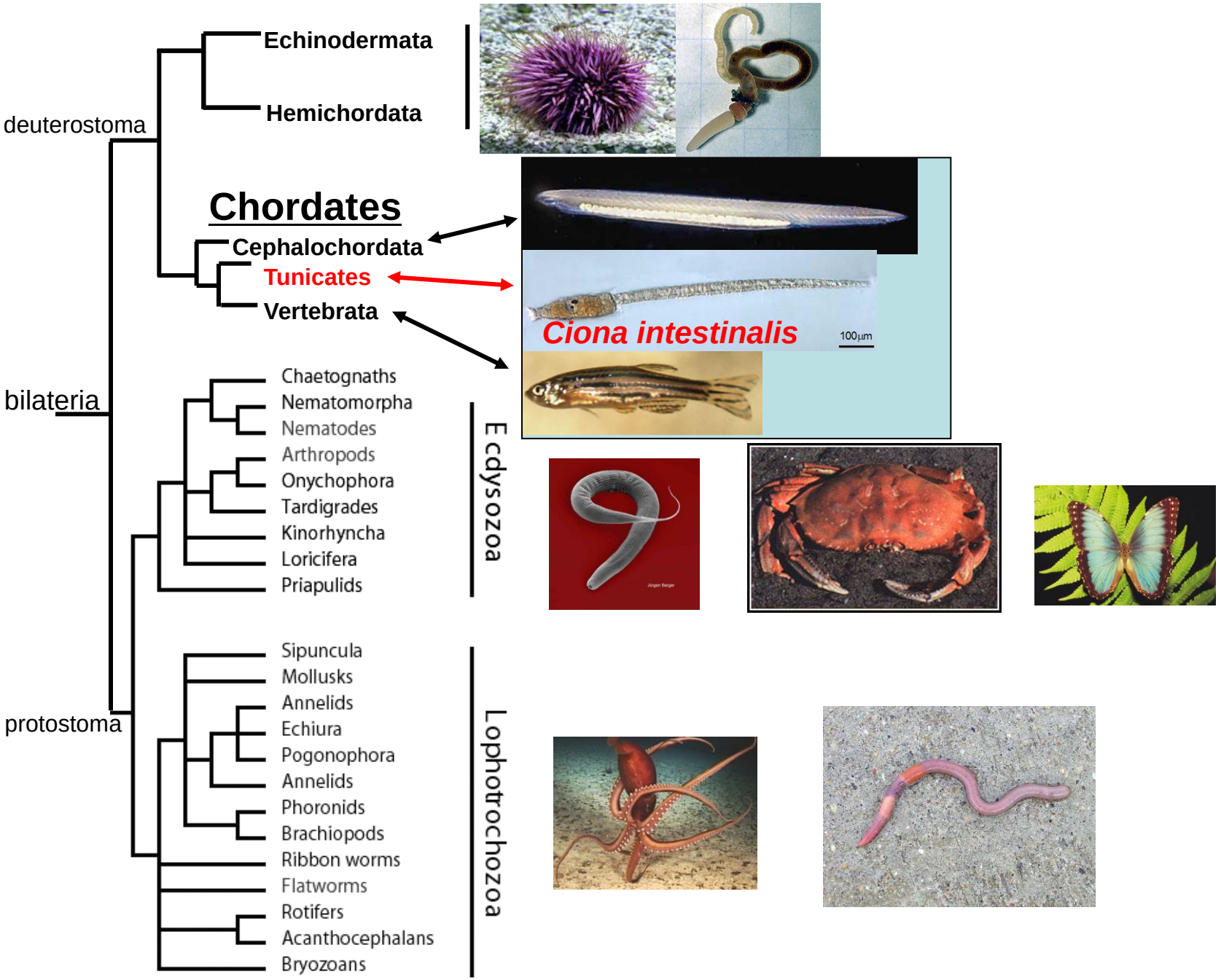
Morphogenetic analysis in a simple chordate



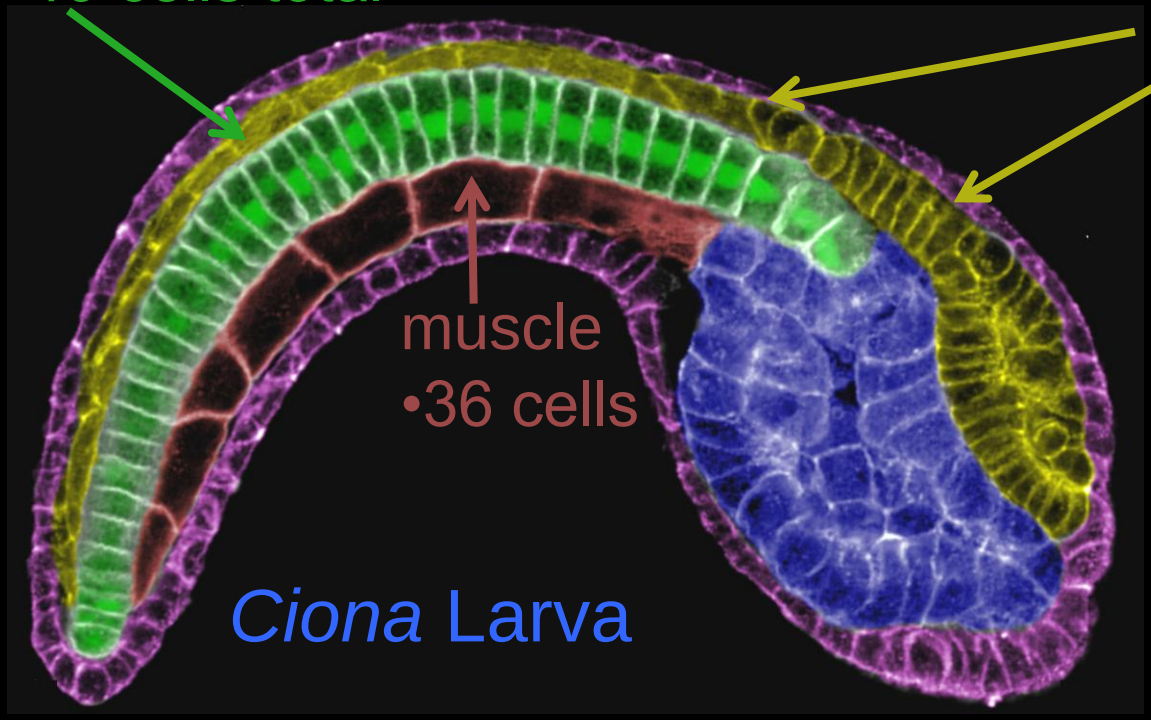
A Guide to the shell and starfish galleries of the British Museum, 1901


Echinodermata
Hemichordata
deuterostoma
Chordates
Cephalochordata
Tunicates
Vertebrata
Ciona intestinalis
100µm
bilateria
Chaetognaths
Nematomorpha
Nematodes
Arthropods
Onychophora
Tardigrades
Kinorhyncha
Loricifera
Priapulids
Ecdysozoa
protostoma
Sipuncula
Mollusks
Annelids
Echiura
Pogonophora
Annelids
Phoronids
Brachiopods
Ribbon worms
Flatworms
Rotifers
Acanthocephalans
Bryozoans
Lophotrochozoa

ascidians have a chordate body plan with simplified embryology and genomics

notochord
•40 cells total

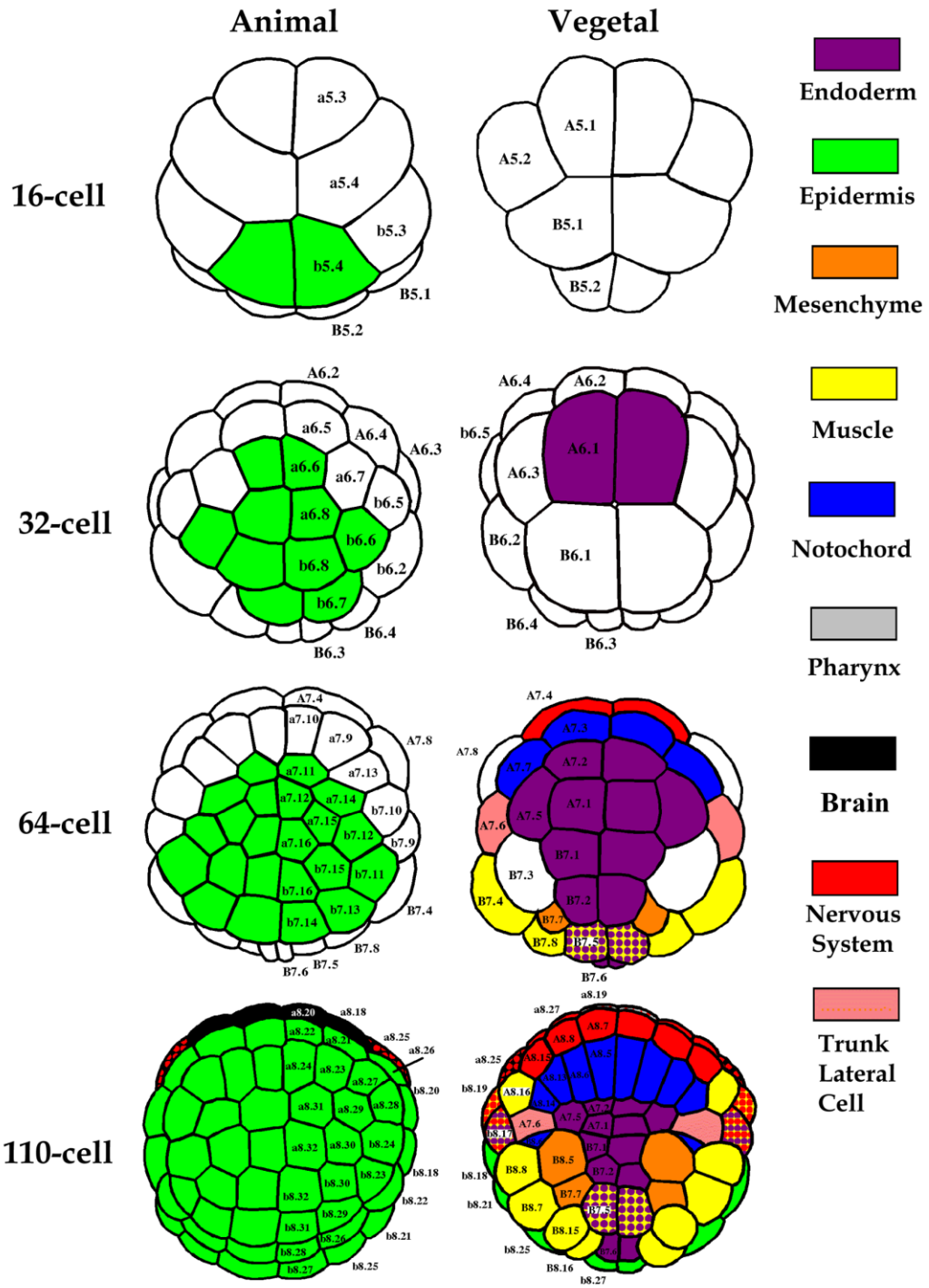
central nervous system

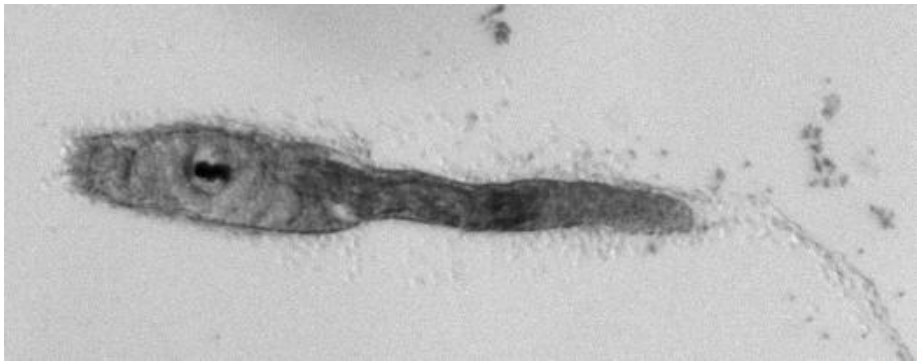


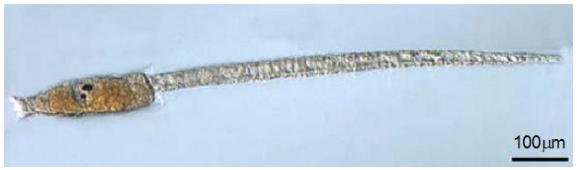
- ≈ 130 neural cells
- ≈ 230 glial cells
- central photo- and gravity-receptors
- 10 motor neurons

- genome: ≈5% the size of vertebrates and ≈½ the number of genes

and ascidians develop according to a fixed and well-characterized cell lineage……

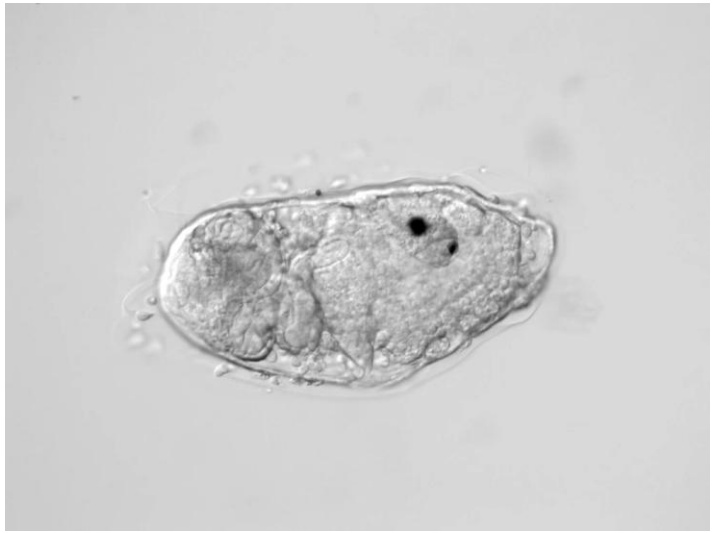


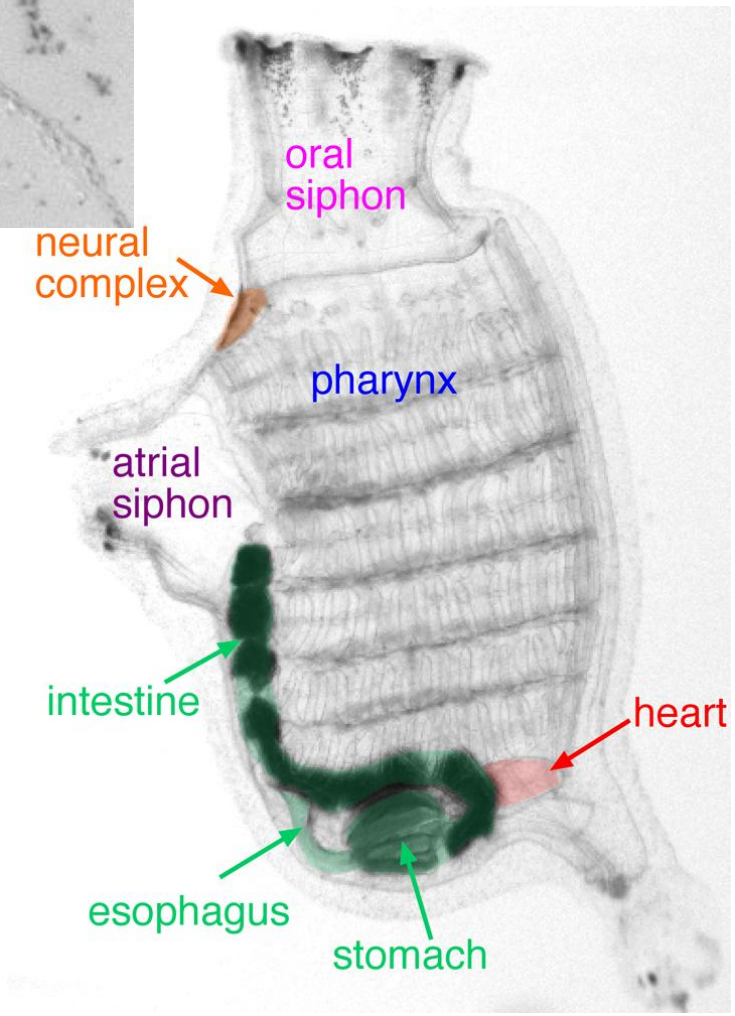




≈1 mm

metamorphosis





Forward Genetics in *Ciona*

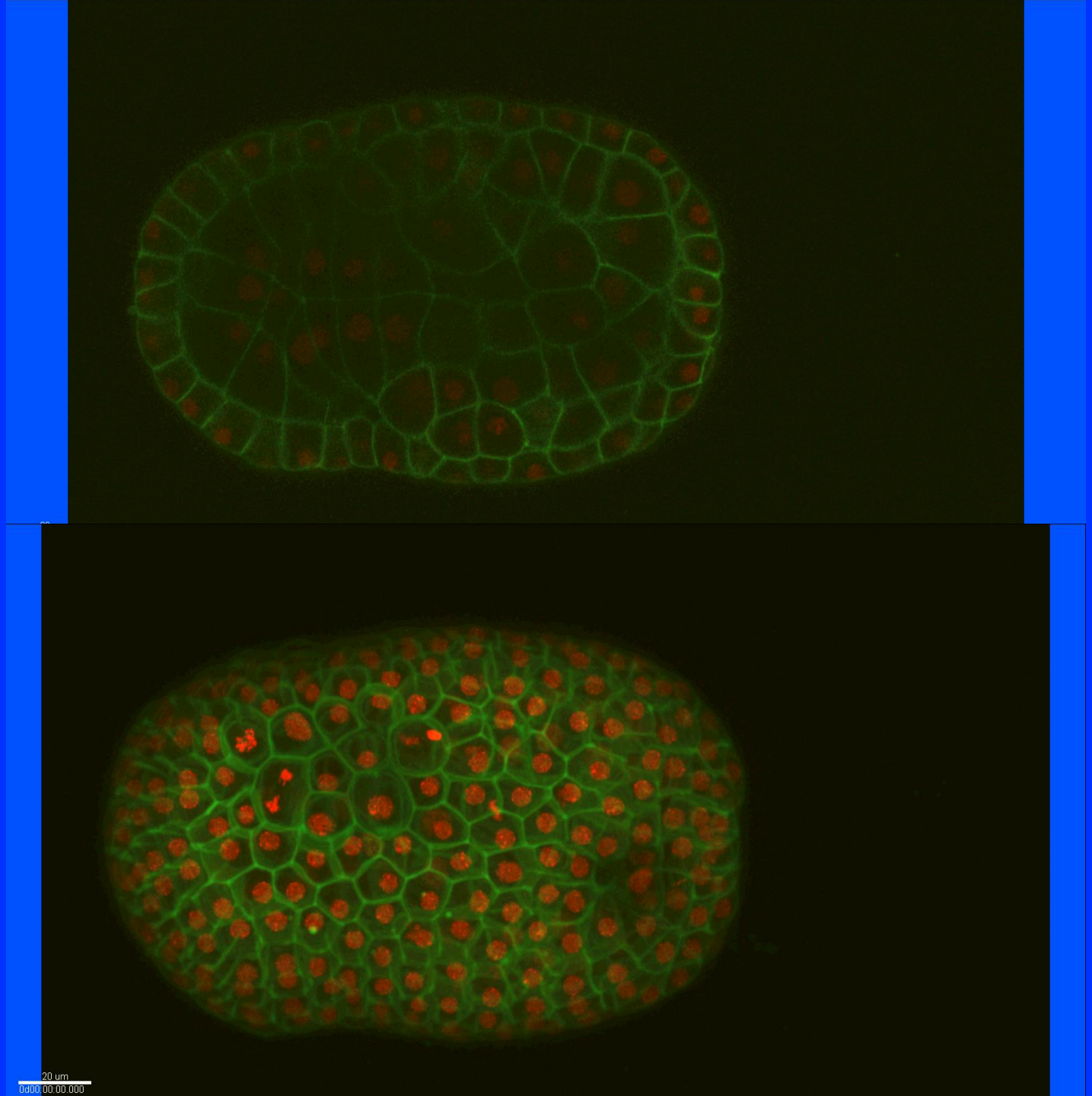
- adults are self-fertilizing hermaphrodites
- mutant lines isolated by screening wild population
- generation time is about 4-6 months
- mutations mapped by deep sequencing

ascidian embryos are ideal for live imaging…

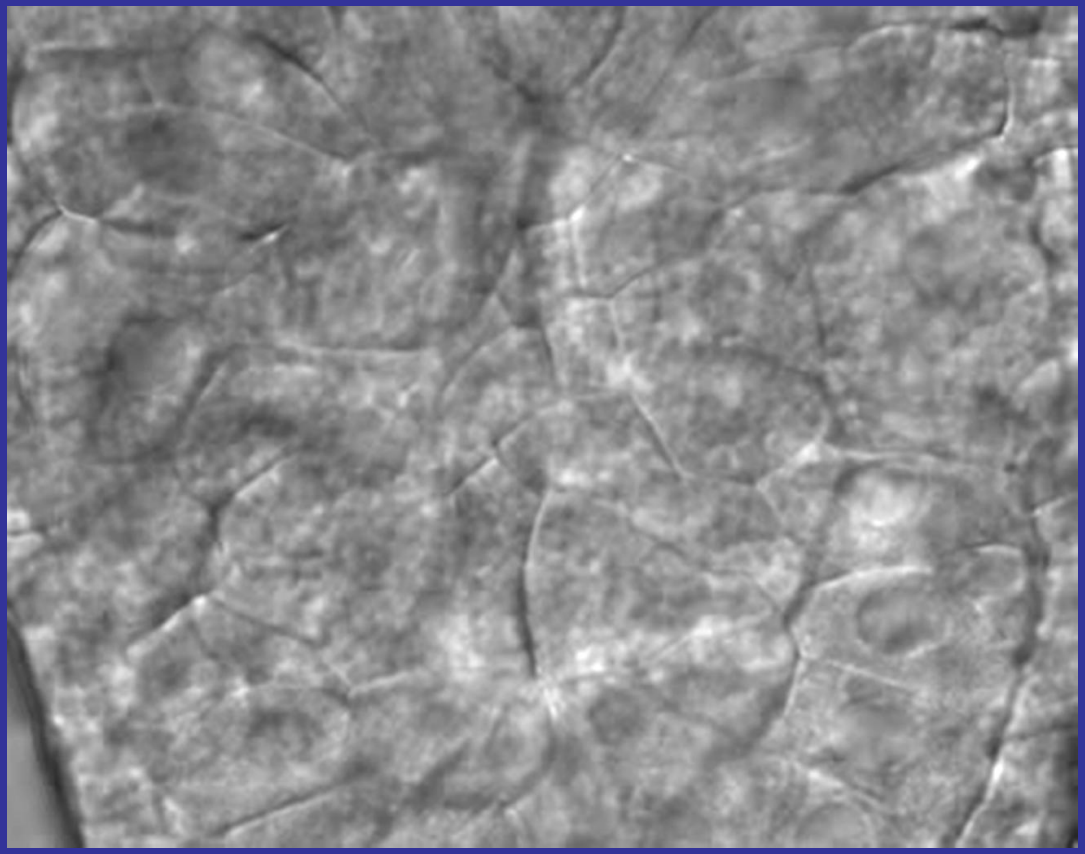
Xenopus larva
>50,000 cells

Ciona larva
~2,600 cells

- basic chordate body plan
- more than an order of magnitude fewer cells
- certain species are extraordinarily transparent


20 um
0d00:00:00.000

Notochord Morphogenesis Project



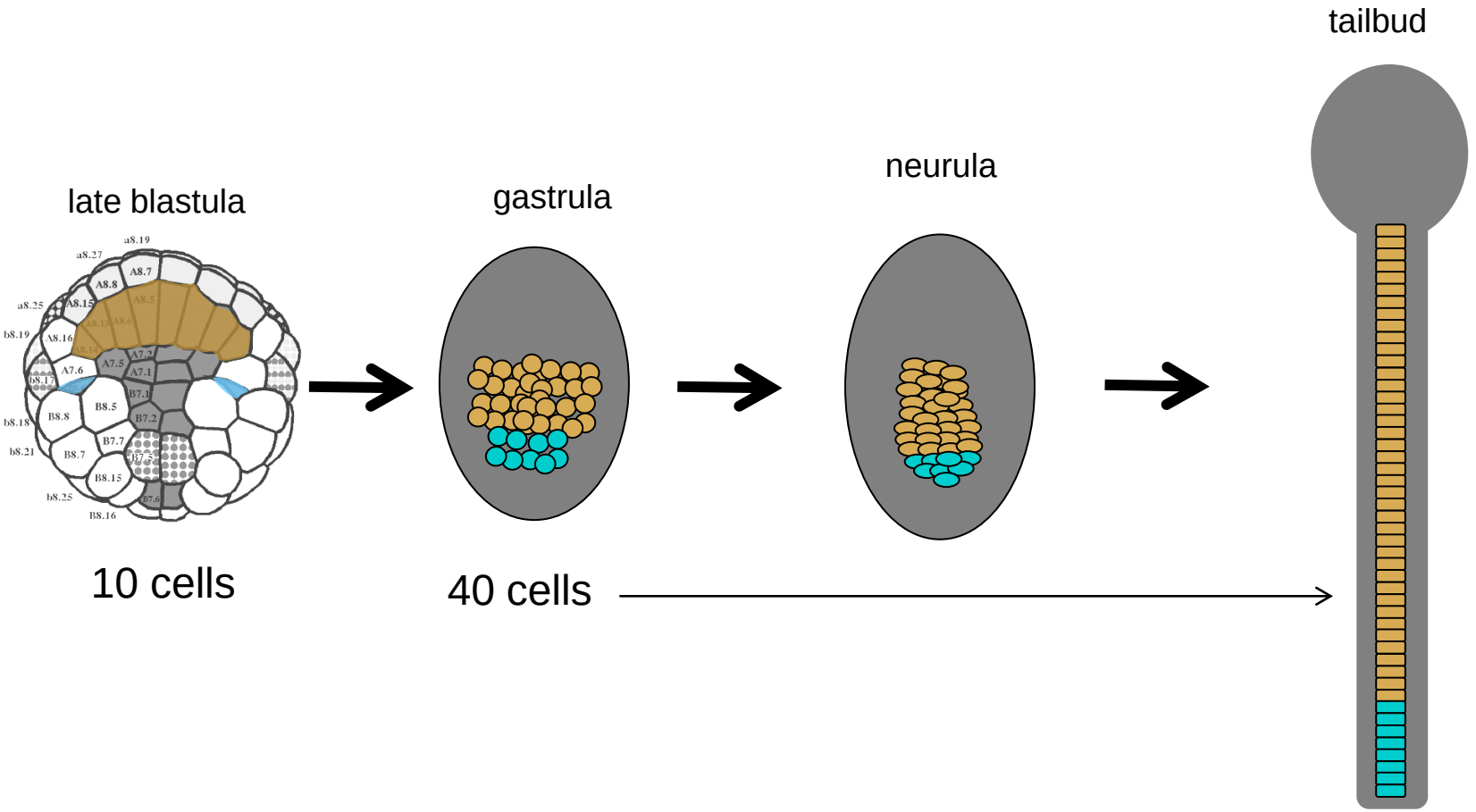
why the notochord?

- essential organ for the development of all chordates
- earliest organ to form in development
- serves as a model for organogenesis and coordinated cell behavior

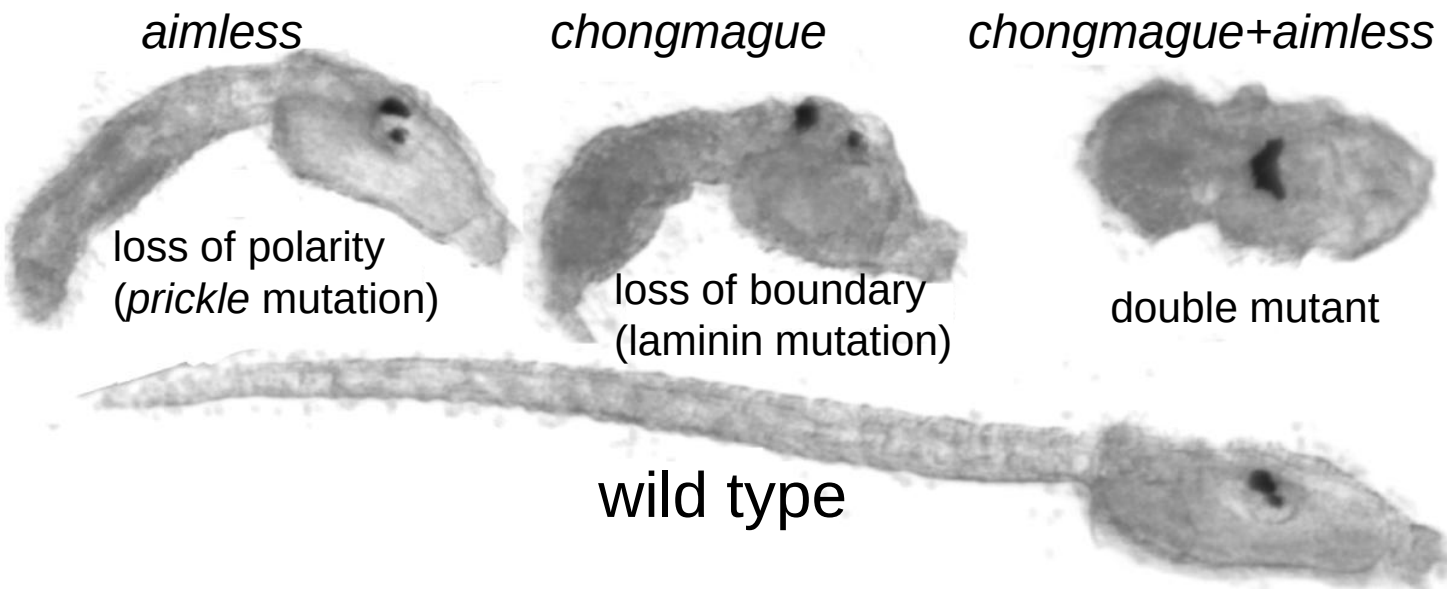
former:
Di Jiang - postdoc
Michael Veeman -postdoc
Wendy Reeves -postdoc
Benoit Maury -postdoc

current:
Matthew Kourakis
Erin Newman-Smith
Wang Hao

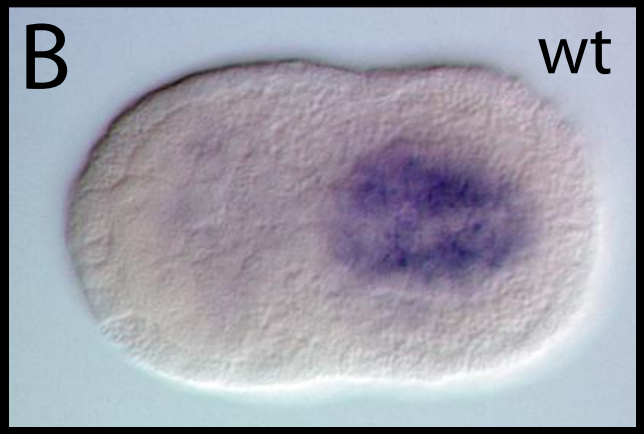
Ciona notochord morphogenesis proceeds with 40 cells



spontaneous mutants disrupting notochord development…

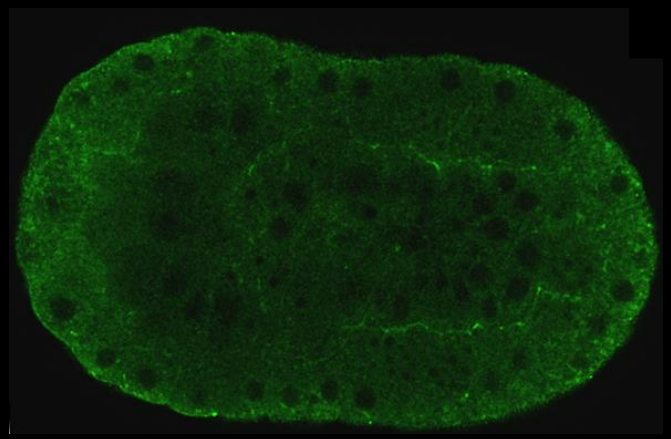


in situ

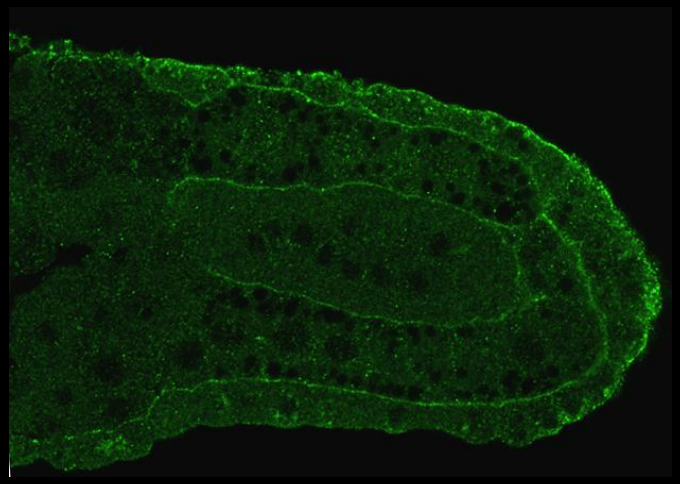


chongmague- a null mutation in α-laminin3,4,5

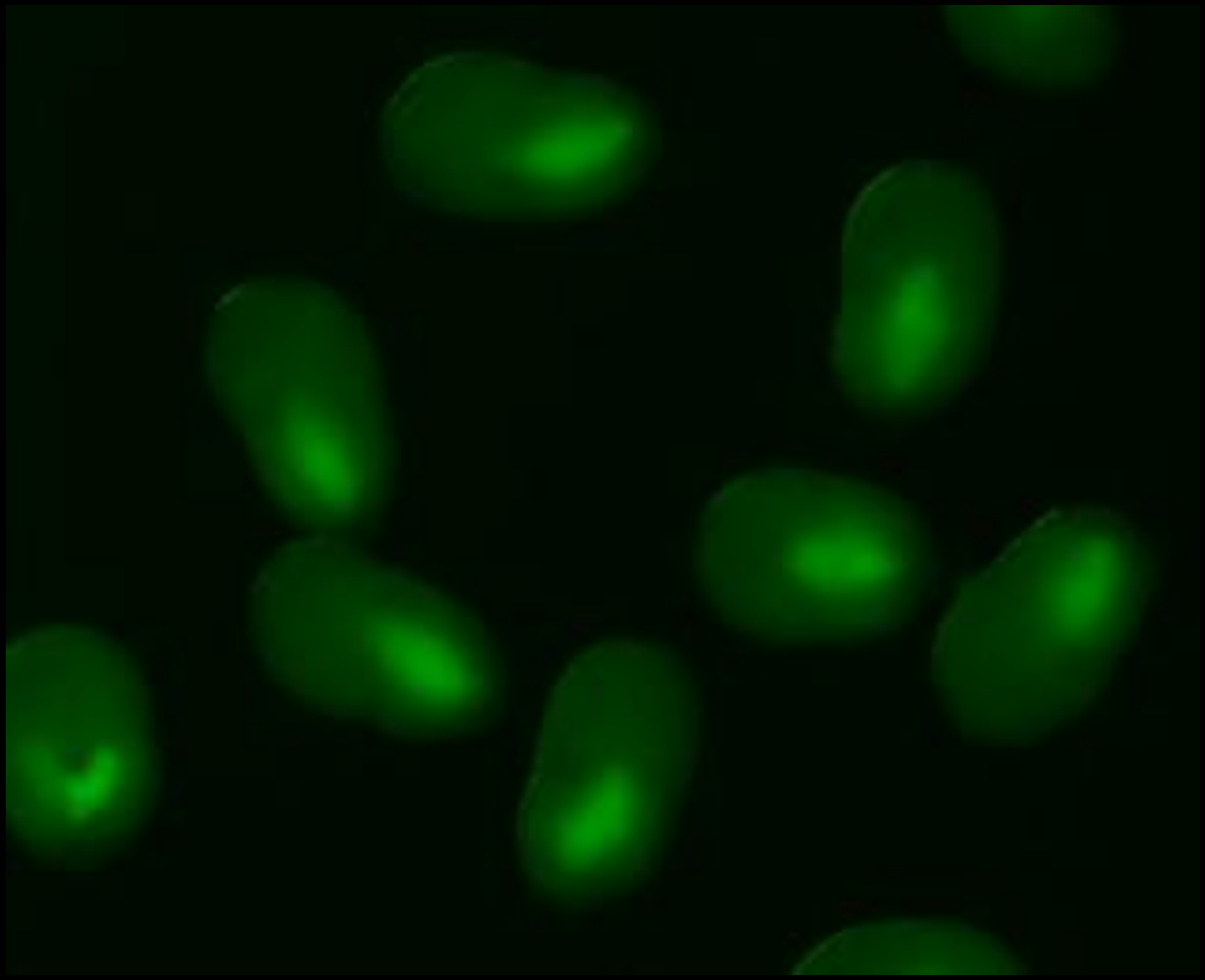
antibody (in wild type)

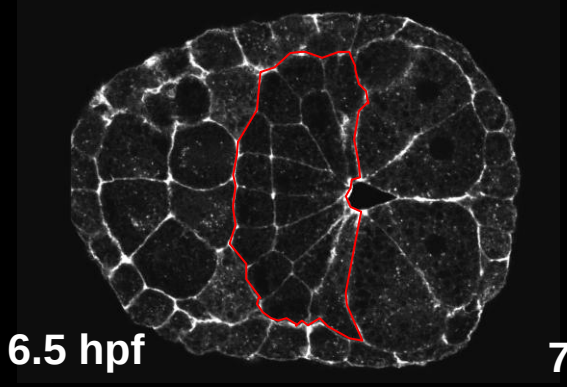


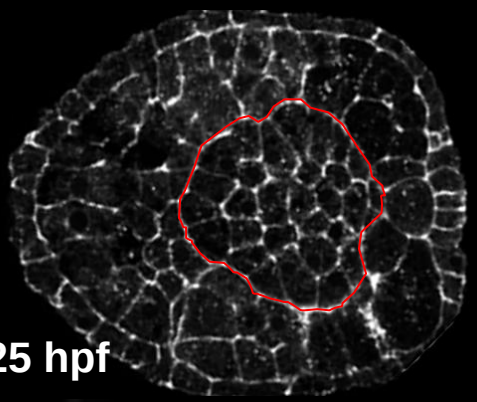
neurula

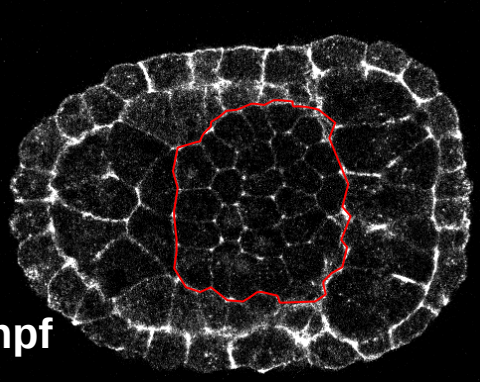


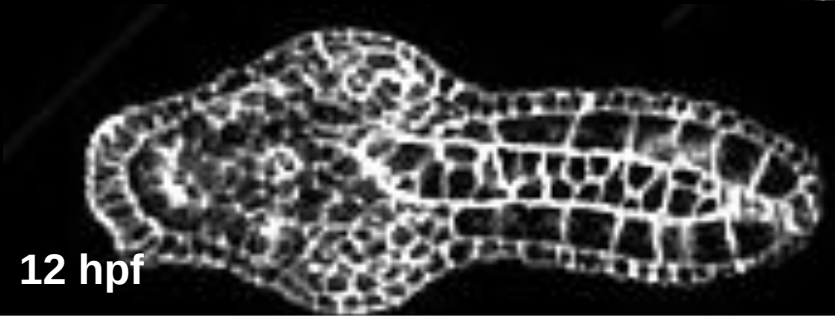
tailbud



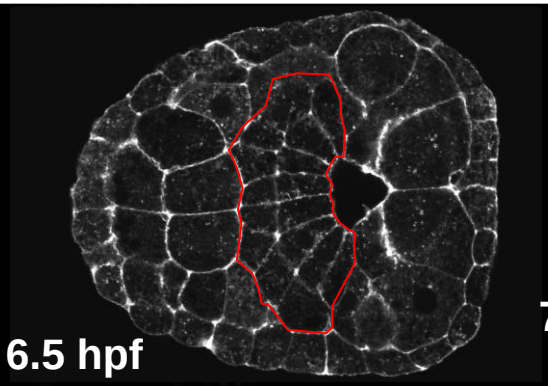


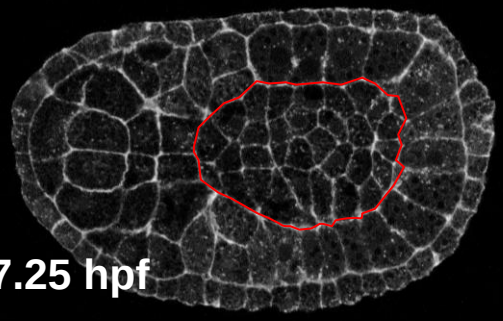


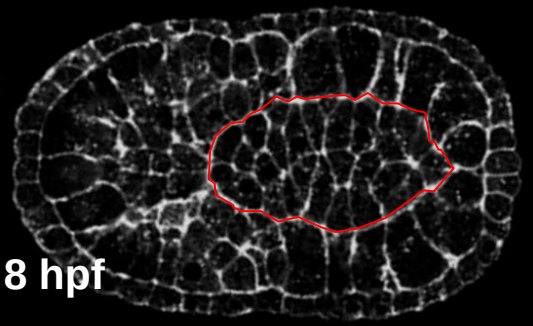


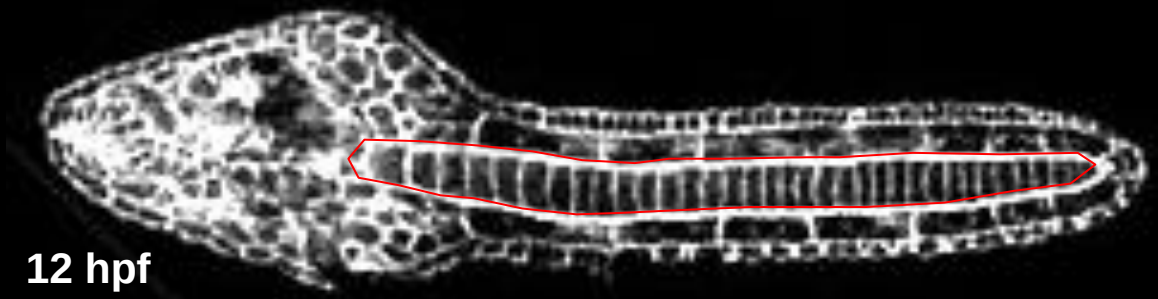


•loss of gene product *prickle.*
Notochord cells remain motile, but are not polarized.



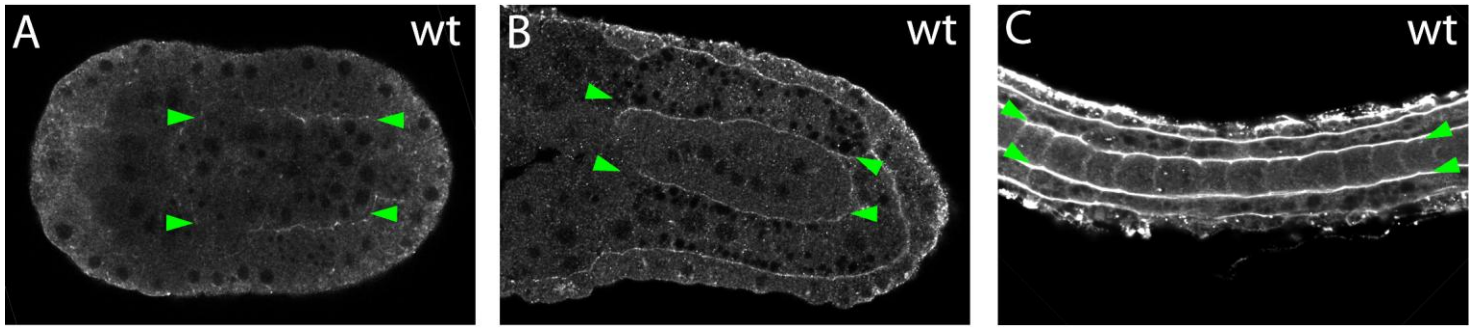




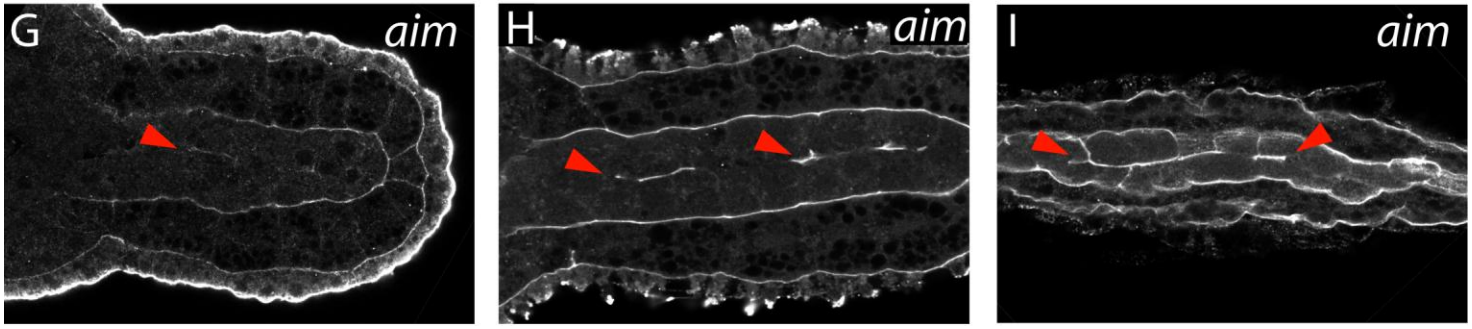


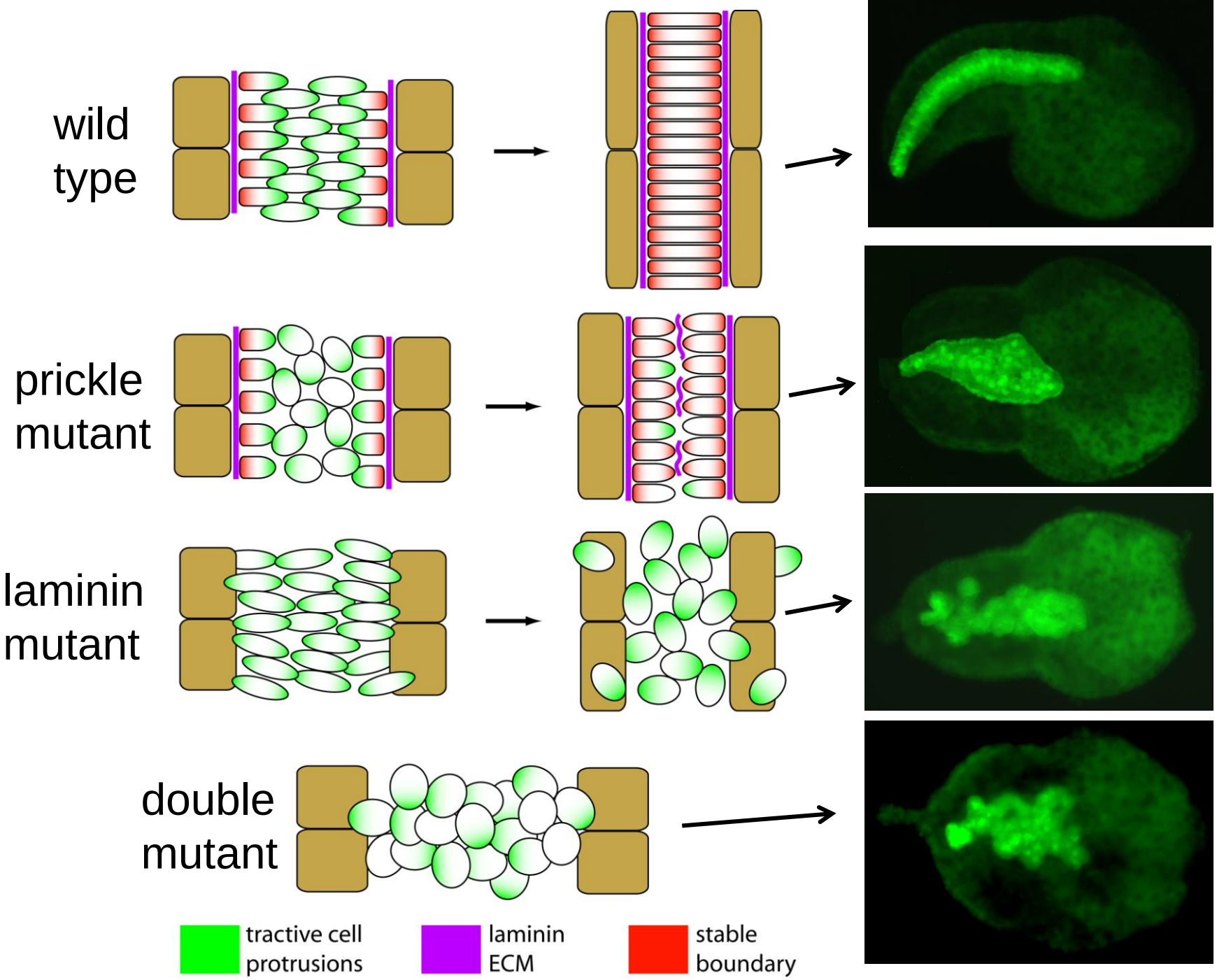
normal embryo

α-laminin3,4,5 antibody

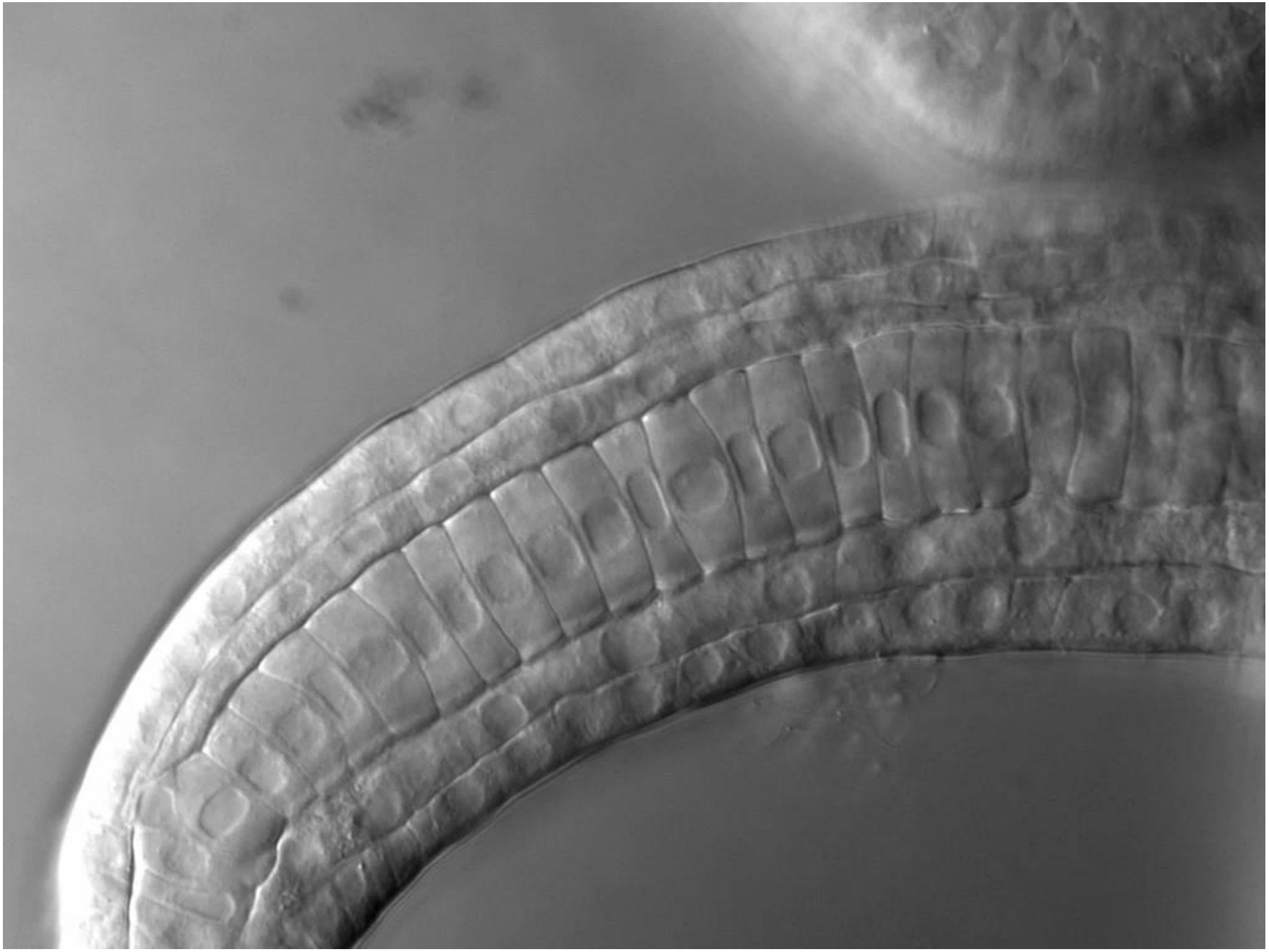


in *pk* mutant background, laminin localization become unpolarized:

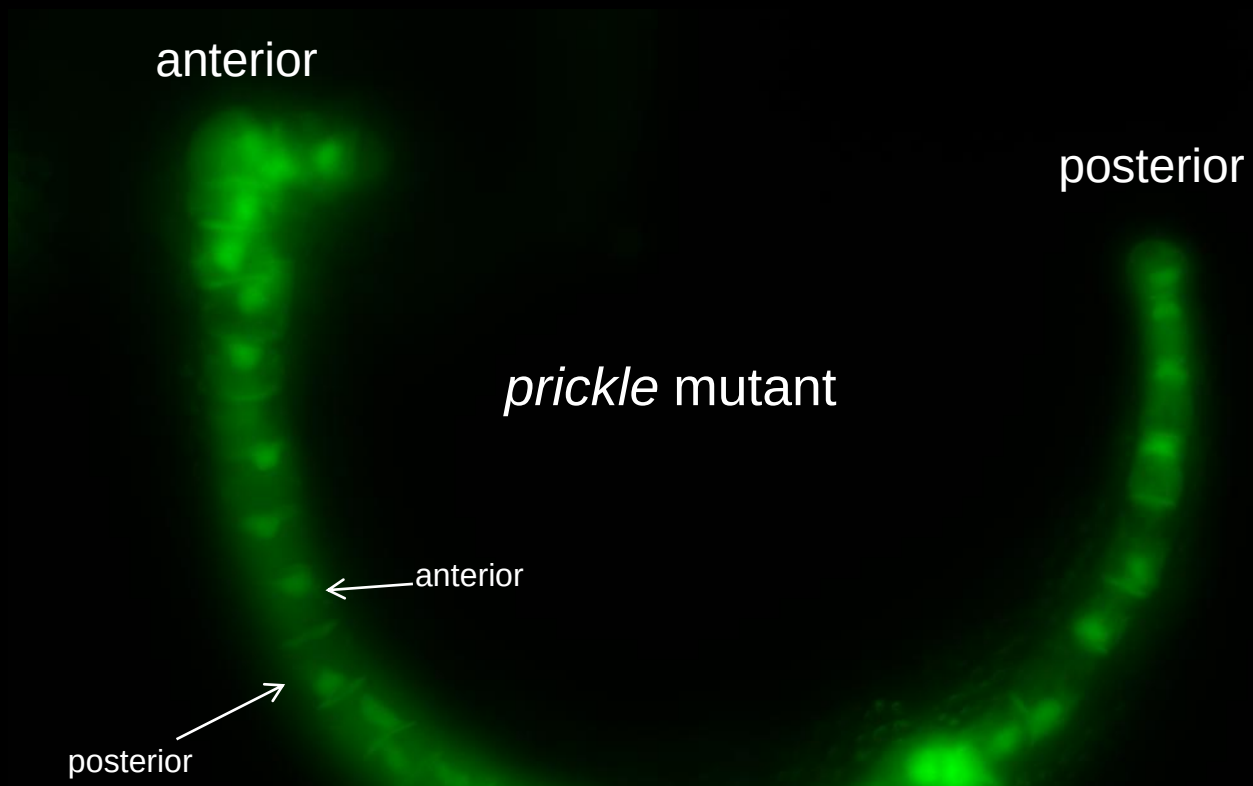



wild type
prickle mutant
laminin mutant
double mutant
tractive cell protrusions
laminin ECM
stable boundary

after intercalation the cells are polarized in the anterior/posterior axis



in *pk*-null background polarity is randomized

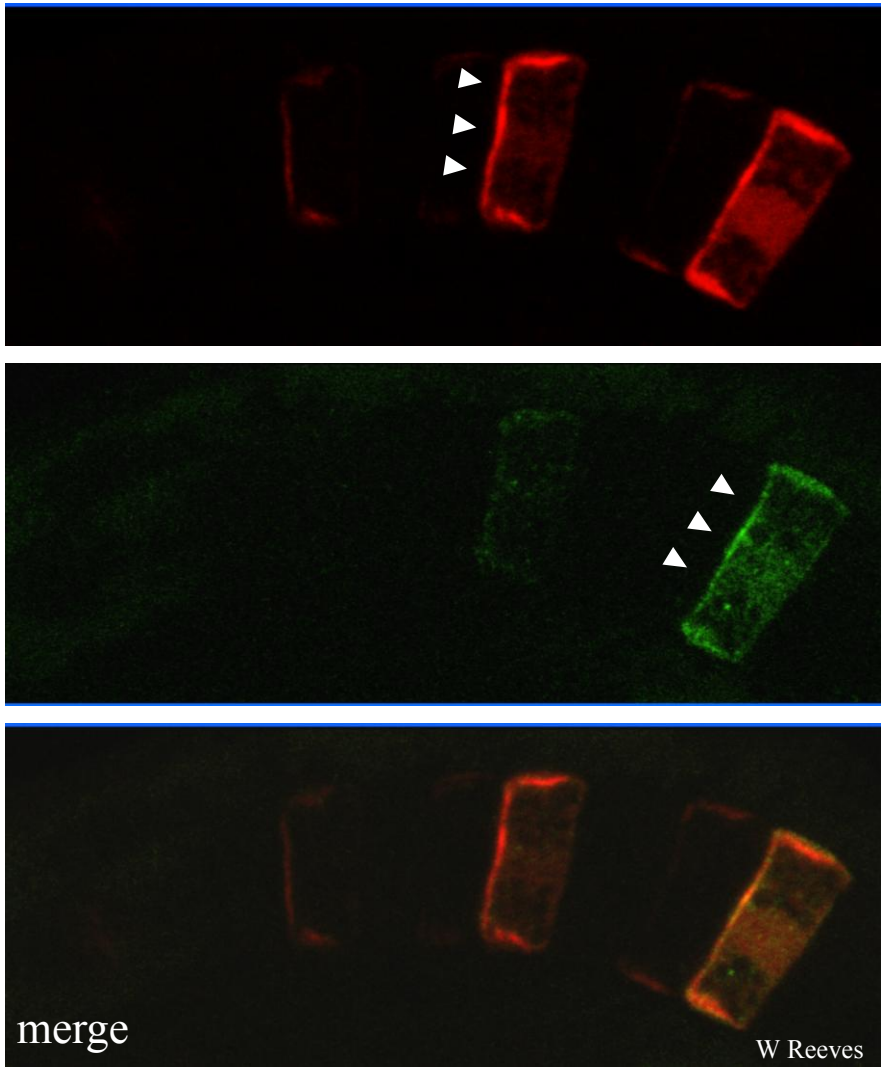


pk and *m-rlc*

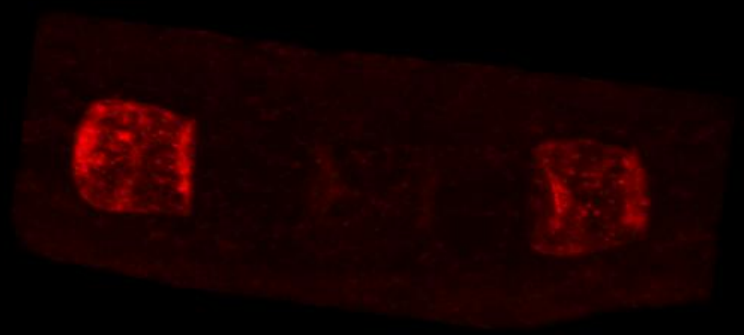
- earliest known markers of A-P polarity

asymmetric anterior expression begins simultaneously *after* completion of intercalation

myosin-rlc
prickle

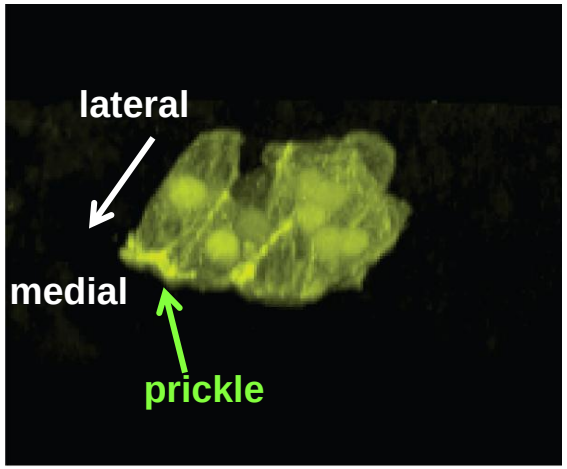


mid tail II



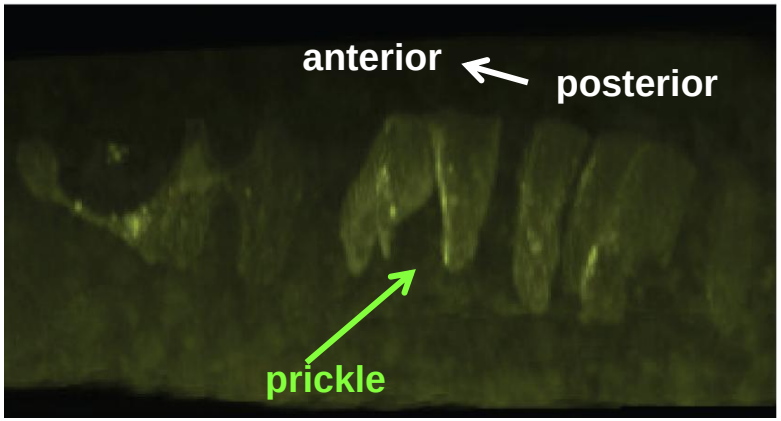
red = strabismus

yellow = prickle



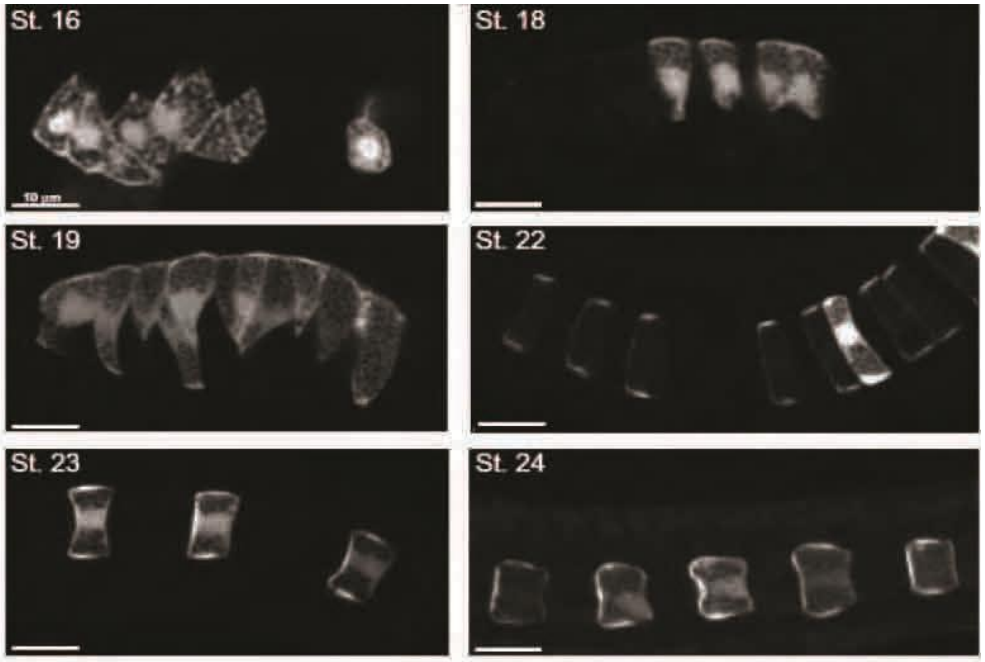
mid-intercalation

prickle relocalizes as polarity changes

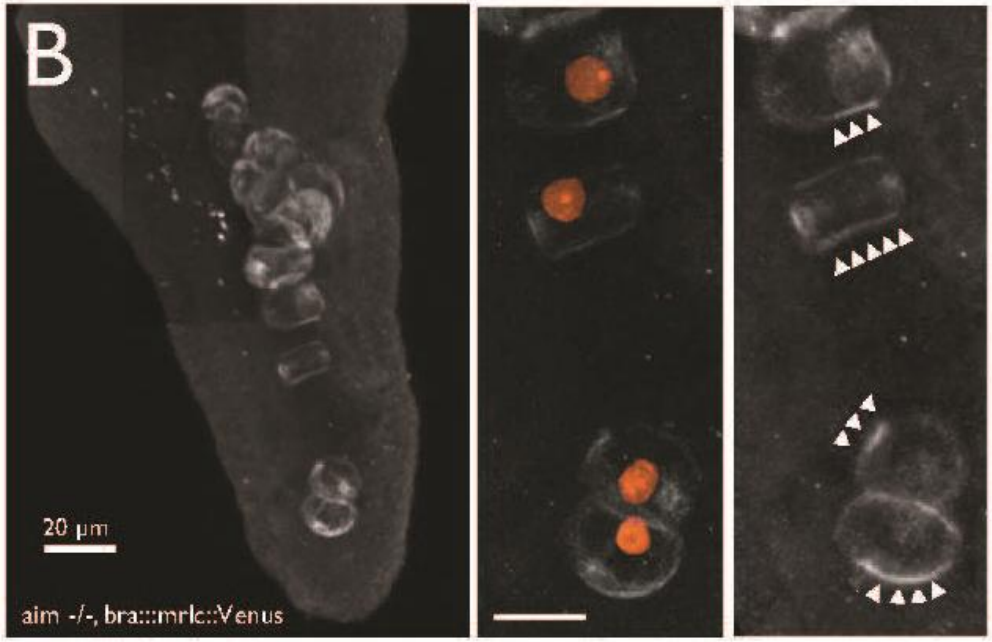


late-intercalation

full-intercalation

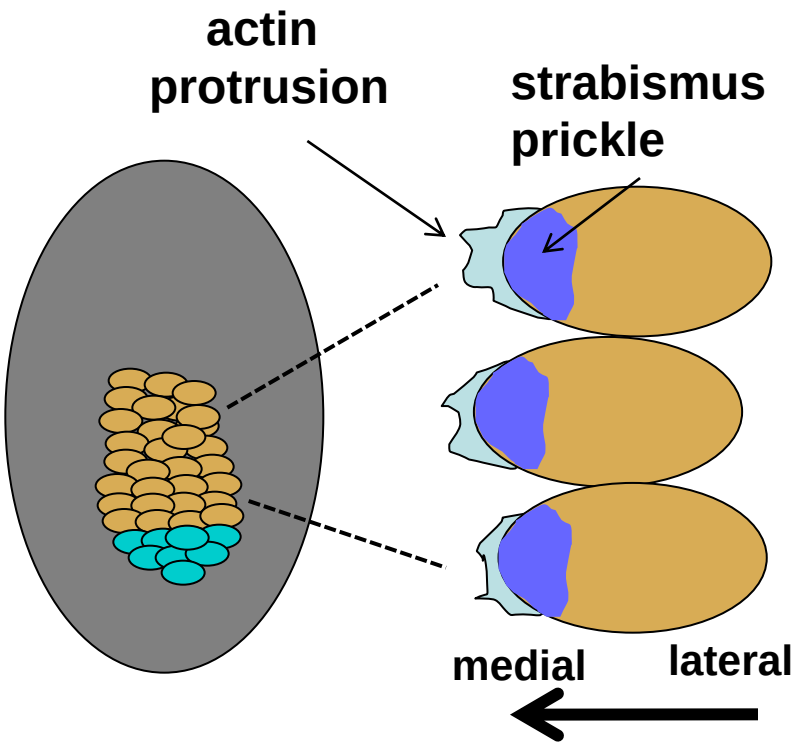


mrlc polarity onset coincides with the completion of intercalation



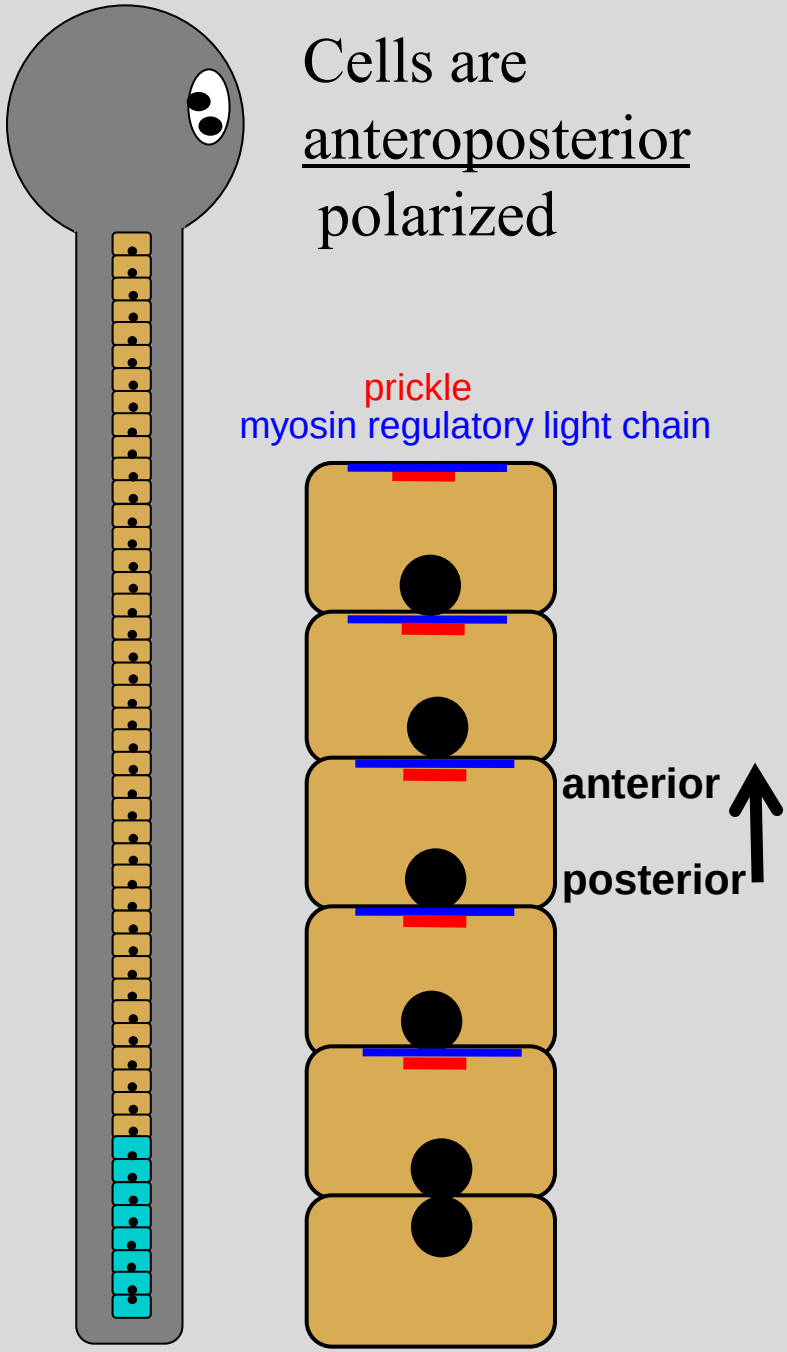
in a pk-null background ***mrlc*** polarity is lost

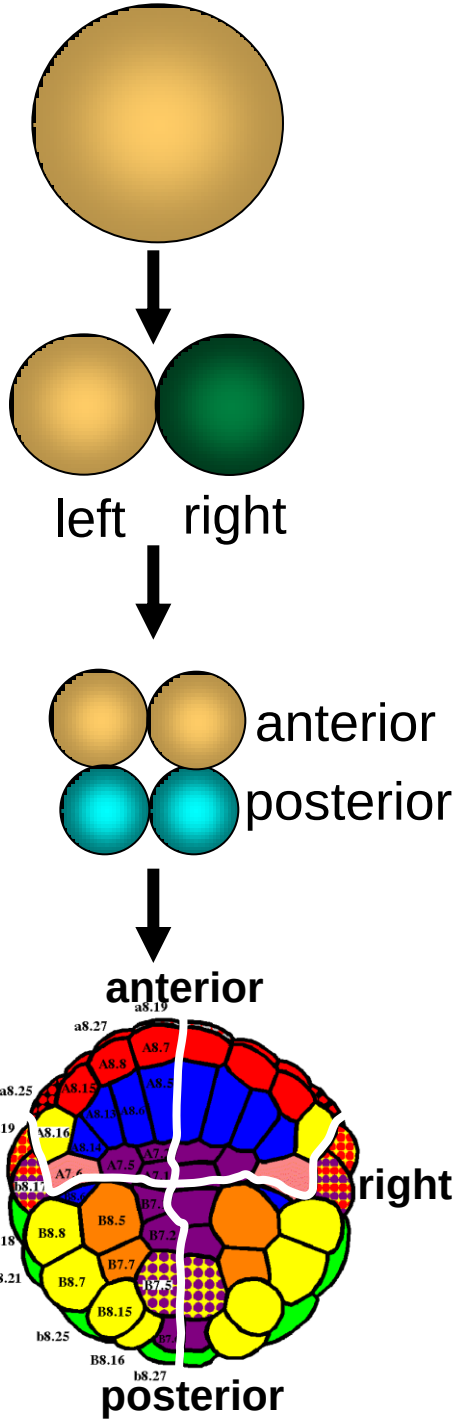
Cells are mediolateral polarized

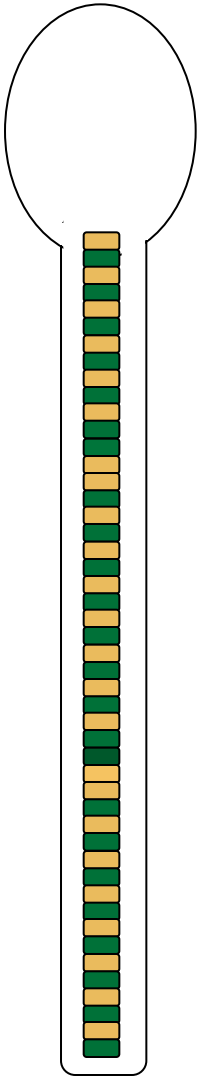


≈4 hours

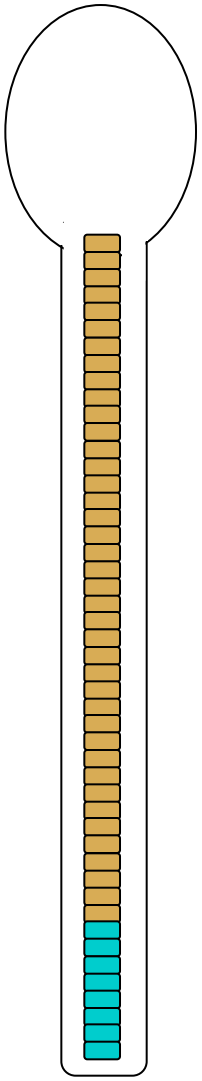
Cells are anteroposterior polarized





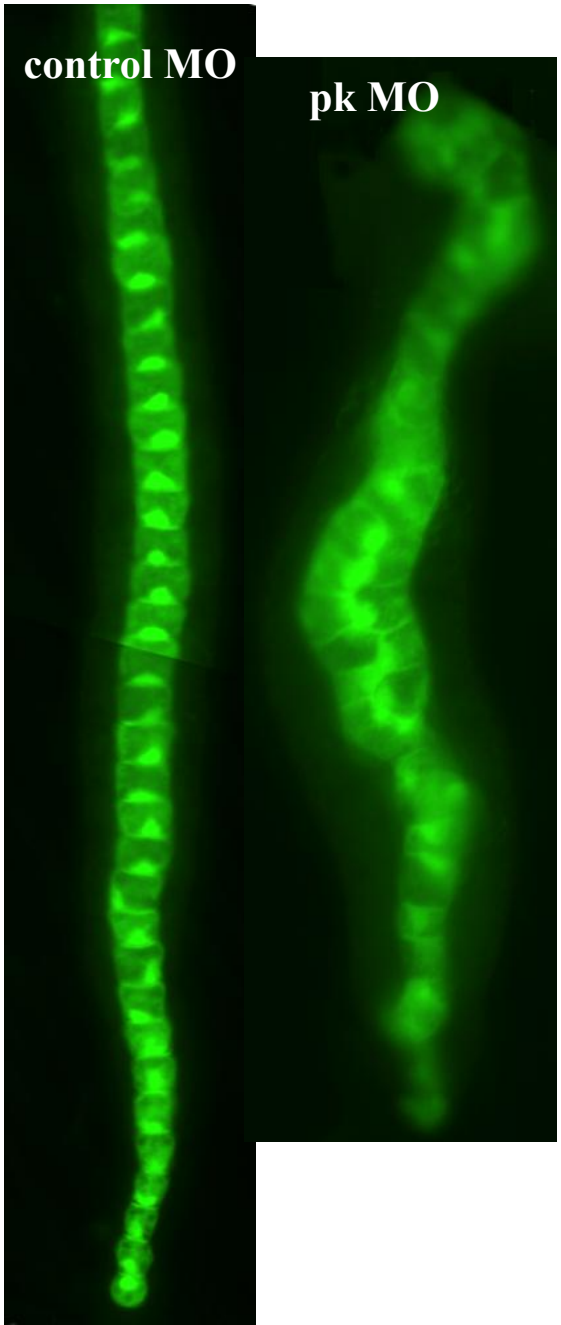


left/right
notochord
fates

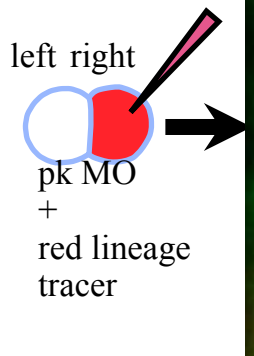


anterior/posterior
notochord
fates

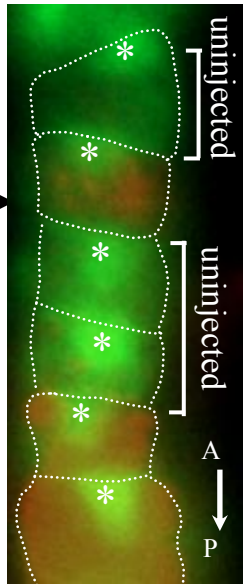
Polarity-defective mosaic, pk-MO injection



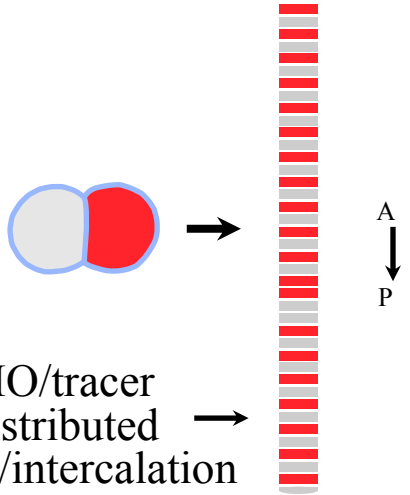
morpholino
pk MO



left or right injected

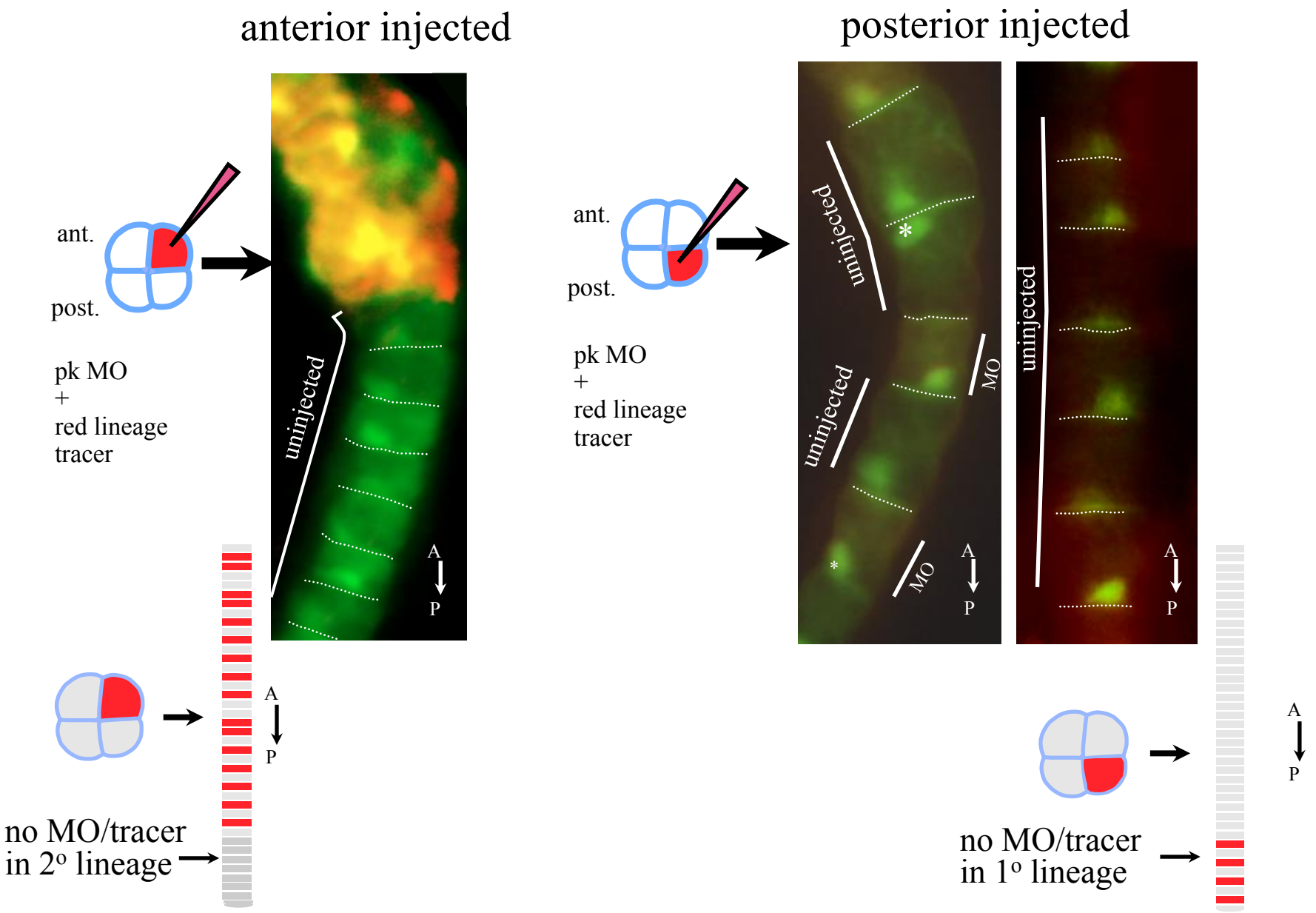


cell non-autonomous



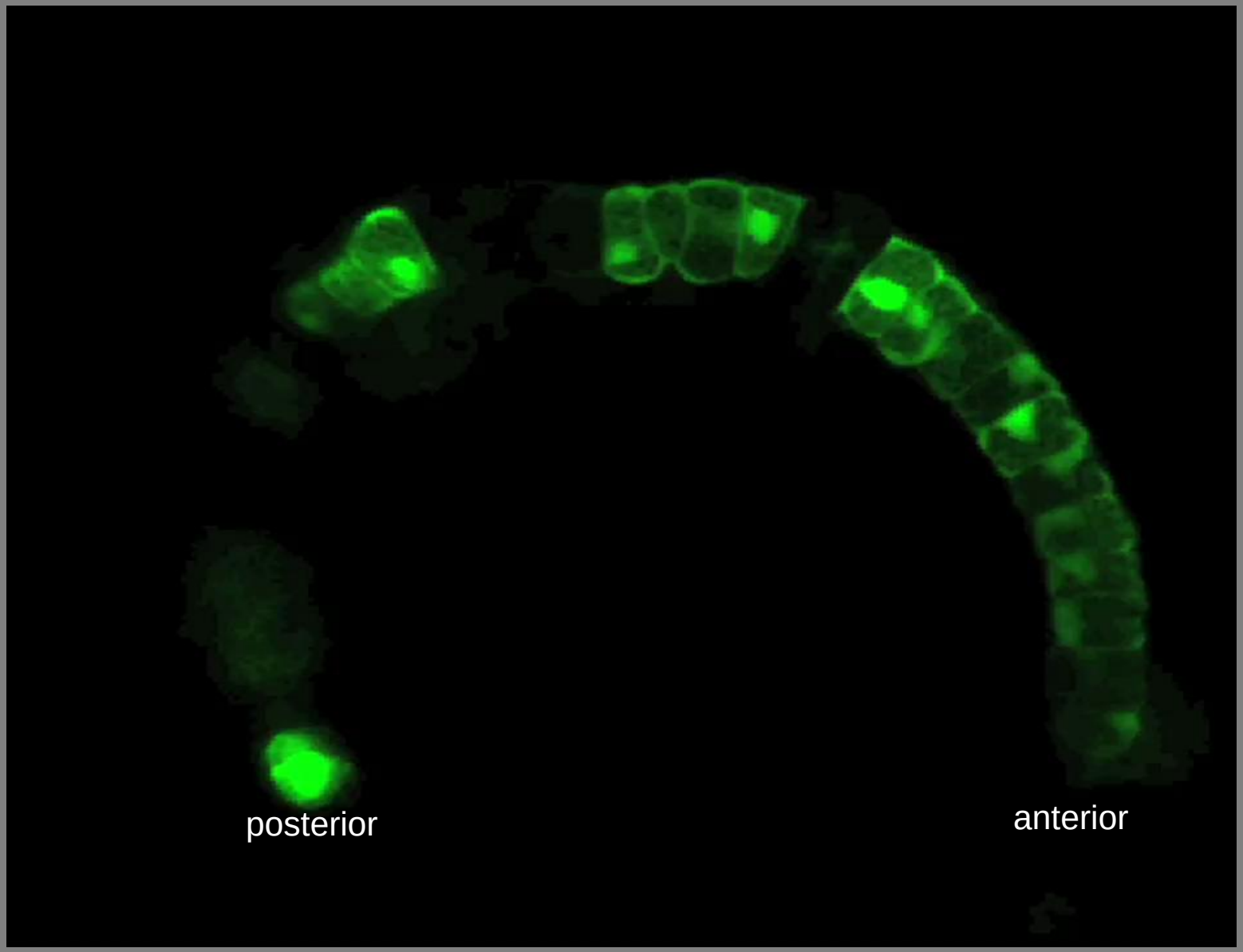
MO/tracer
distributed
w/intercalation

pk-MO, cell non-autonomous, but only local



laser ablation of a single cell……..

(time = 60min)

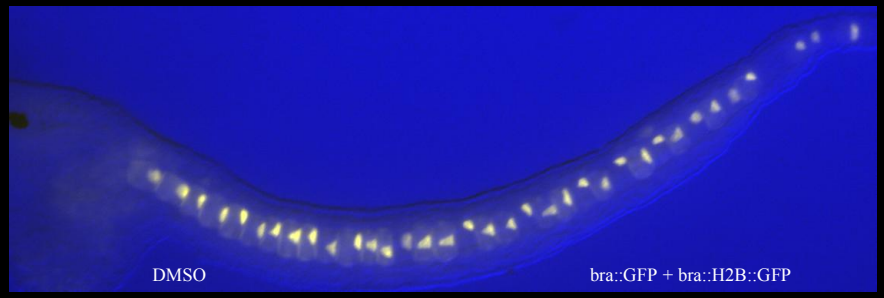


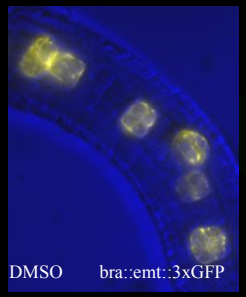
- core PCP pathway coordinates polarity between neighboring cells.

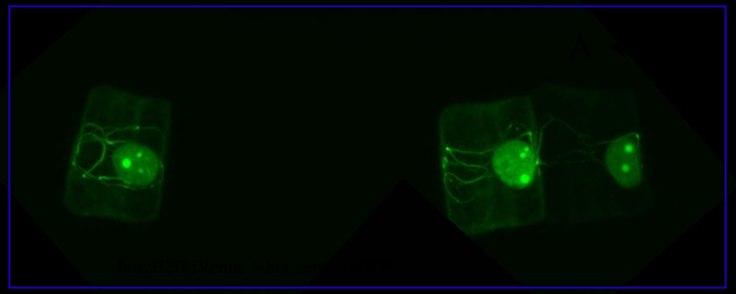
- cell to cell coordination does not set global polarity

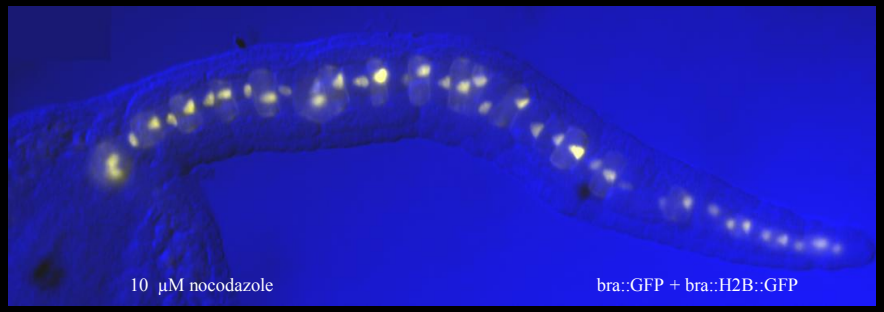
Does nuclear polarity require microtubule network?

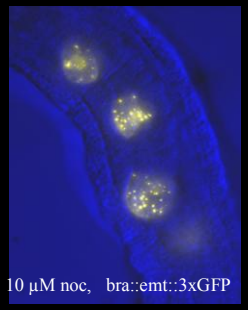
- *Polarity unchanged after mt inhibitor.*
  nocodazole - blocks mt polymerization
  add after intercalation until otolith formation

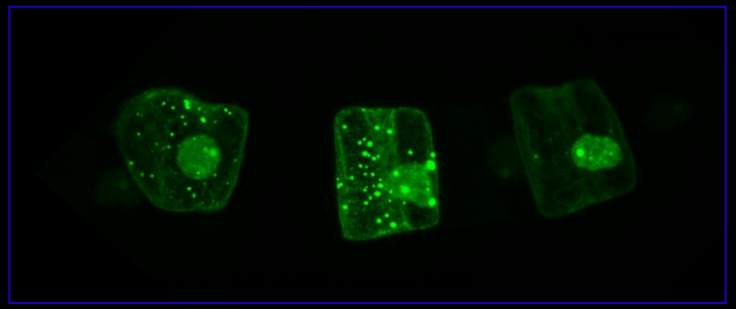










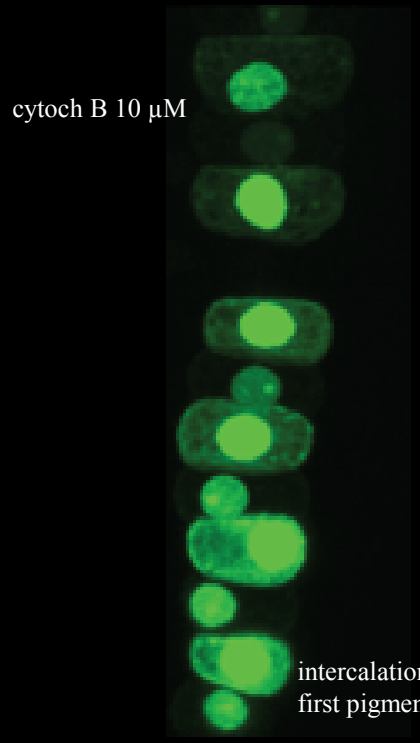


Does nuclear polarity require intact actin network?

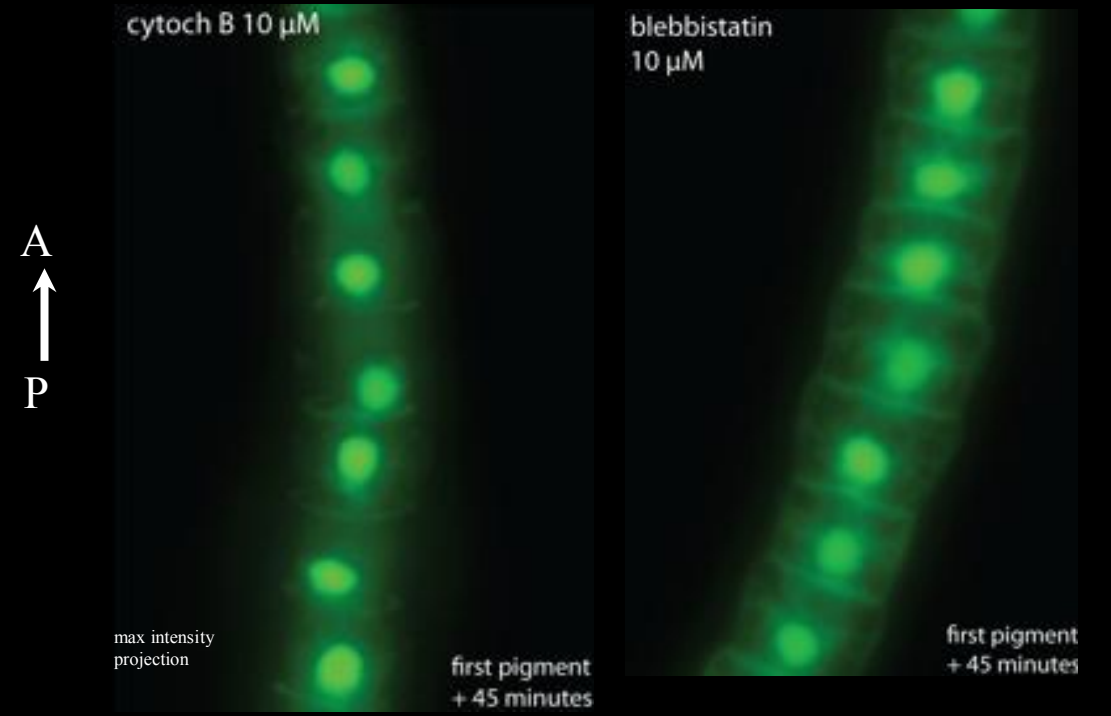
cytochalasin B (f-actin) or blebbistatin (myosin head)

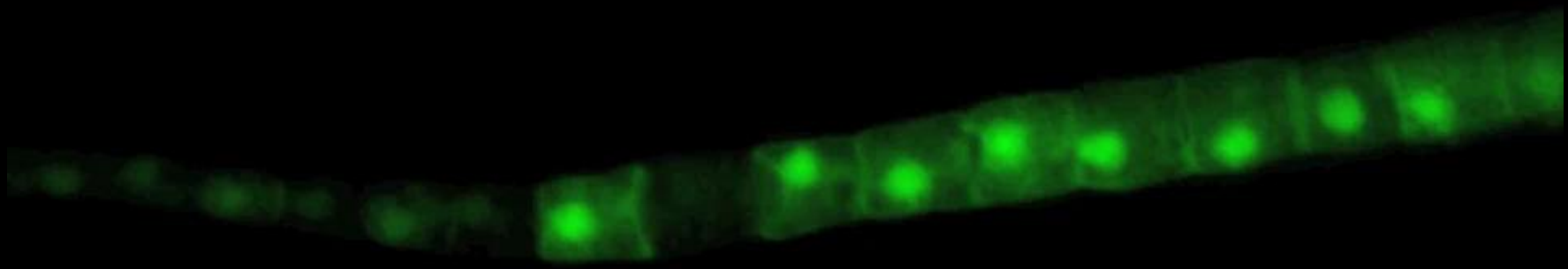
- *Required for initiation and maintenance*

Drug added *after* intercalation



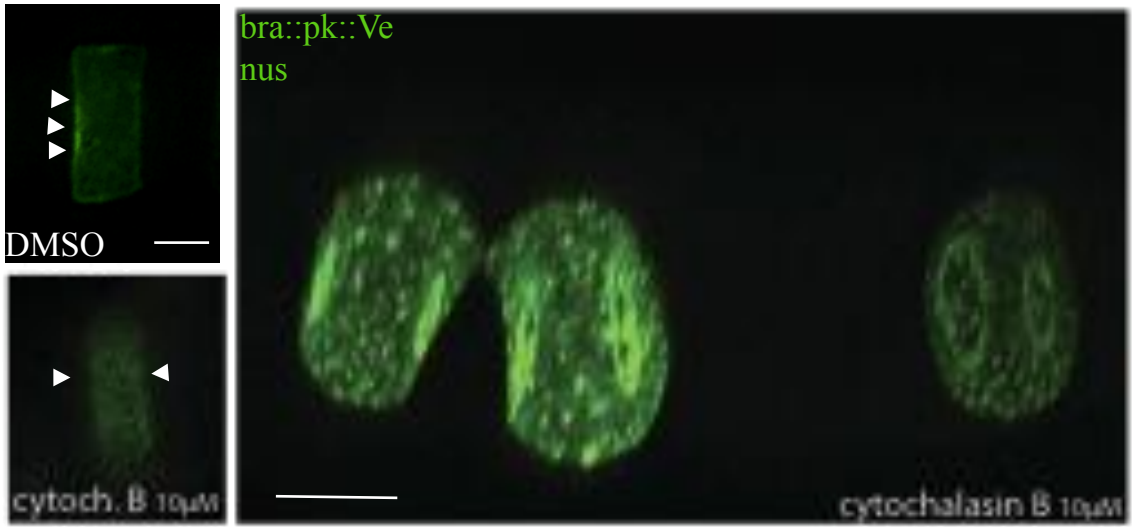
Drug added *after* nuclei polarize



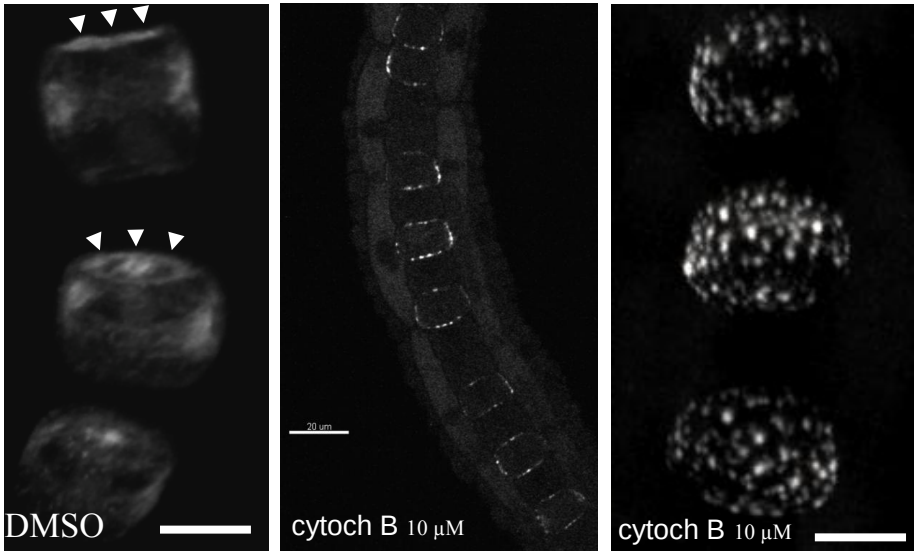


Polarized protein localization is disrupted after cytochalasin treatment

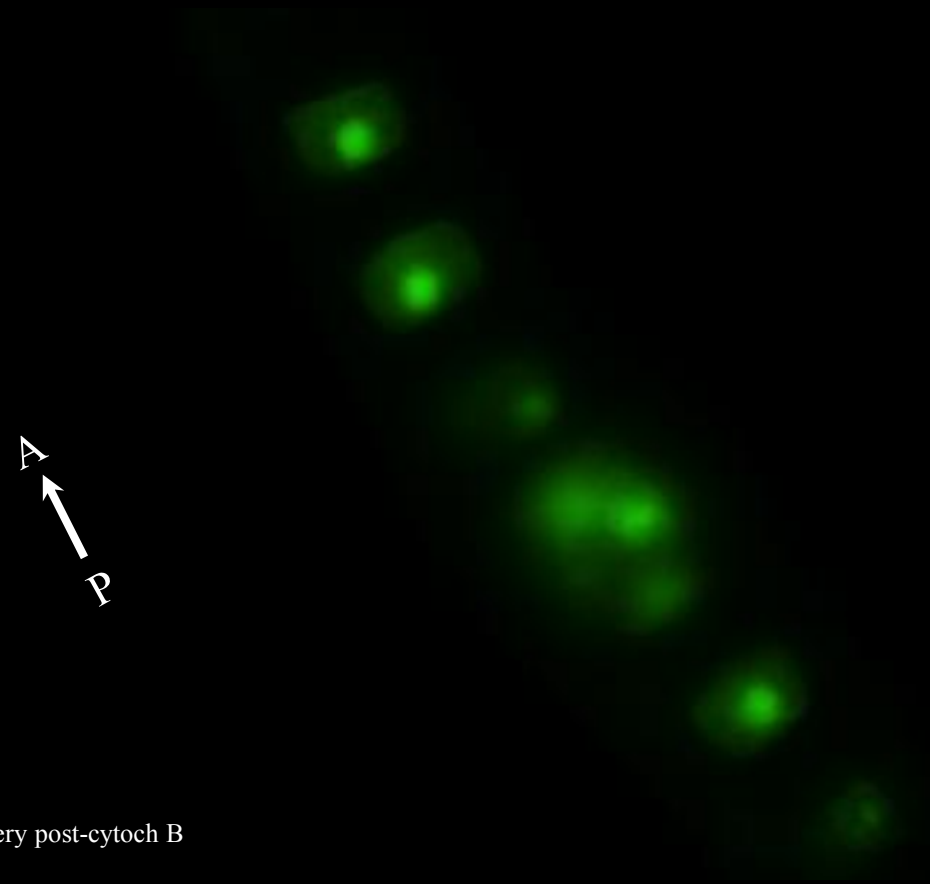
prickle



myosin-rlc

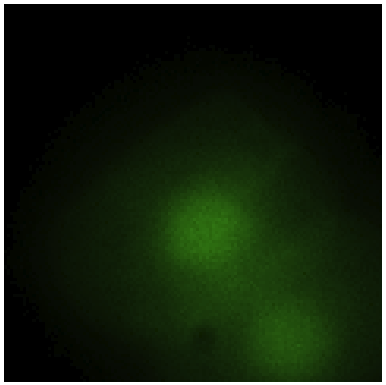


Nuclei re-polarize after removal from cytochalasin B

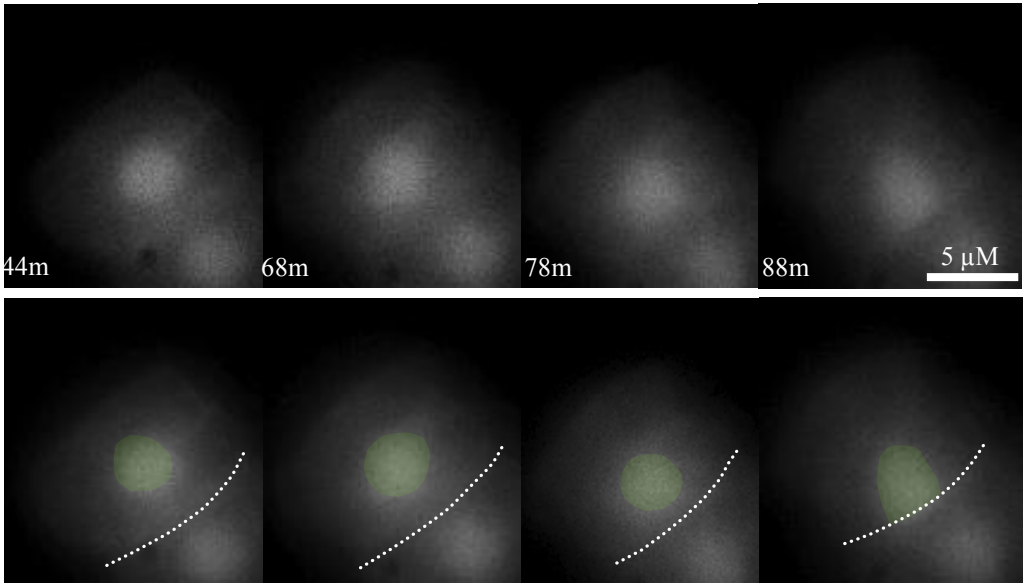


1hr recovery post-cytoch B

Nuclei re-polarize after removal from cytochalasin B



44-88m after wash from cytoch B

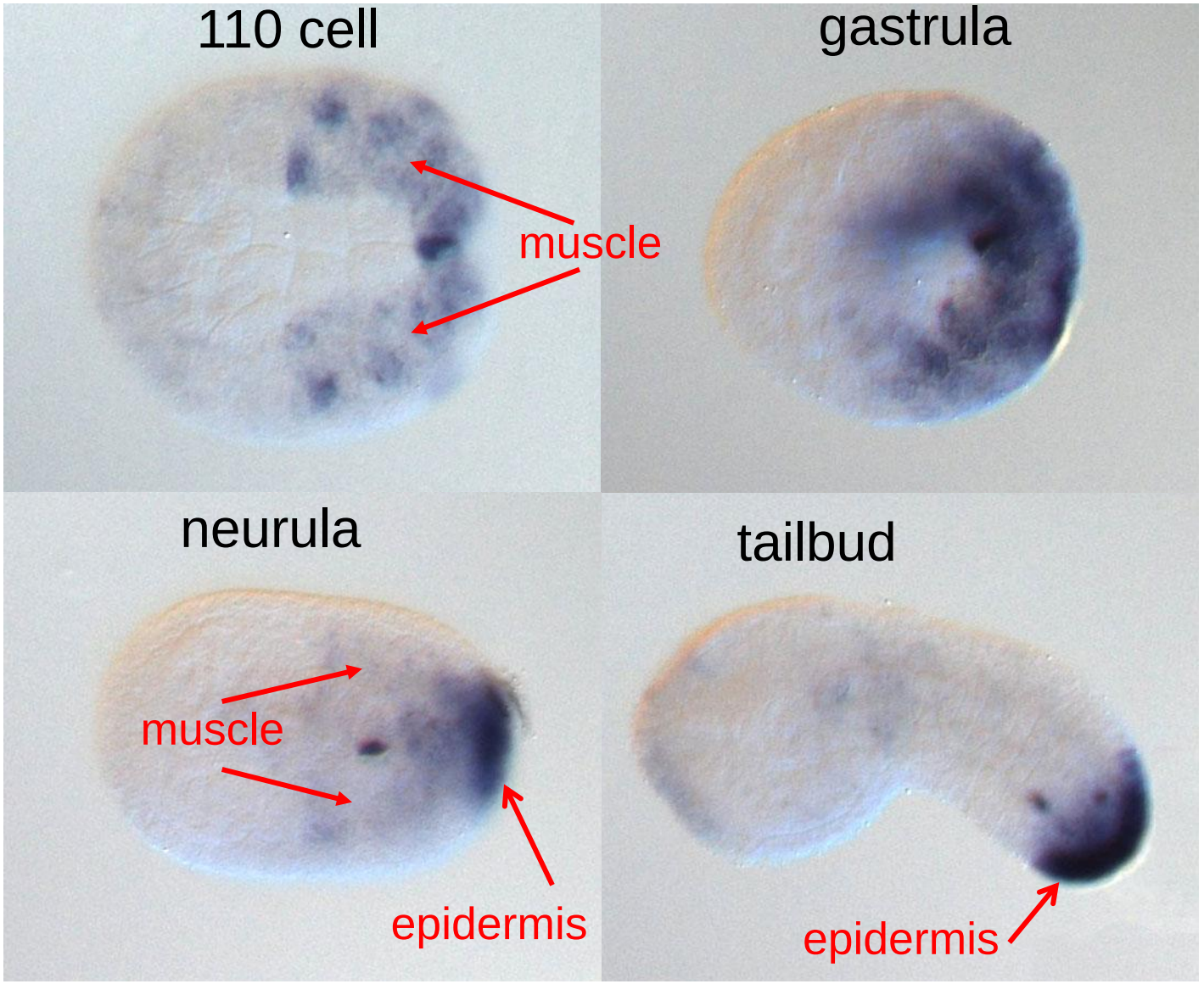


→ *Re-polarization <u>always to posterior</u>*

- polarity information still present
  even after cytoskeleton depolymerized

•**wnt5**

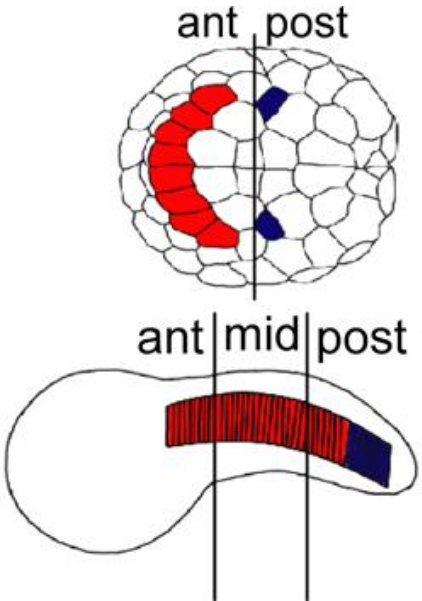
•could this be the global polarity signal?

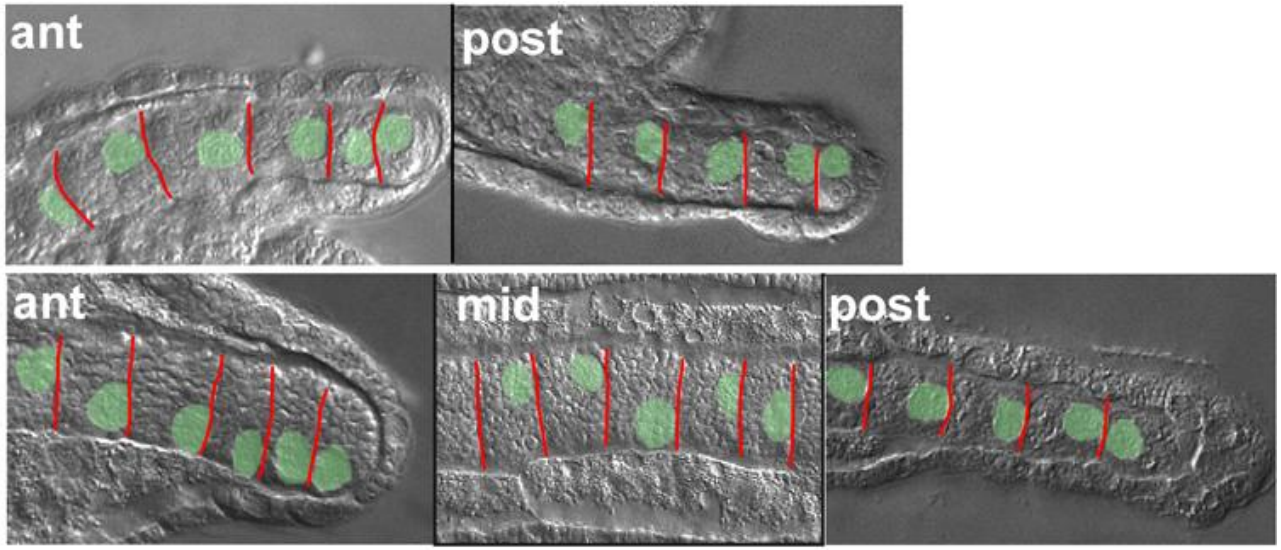


loss of **early** muscle wnt5 disrupts intercalation

wnt5 morpholino (1-cell stage injection)

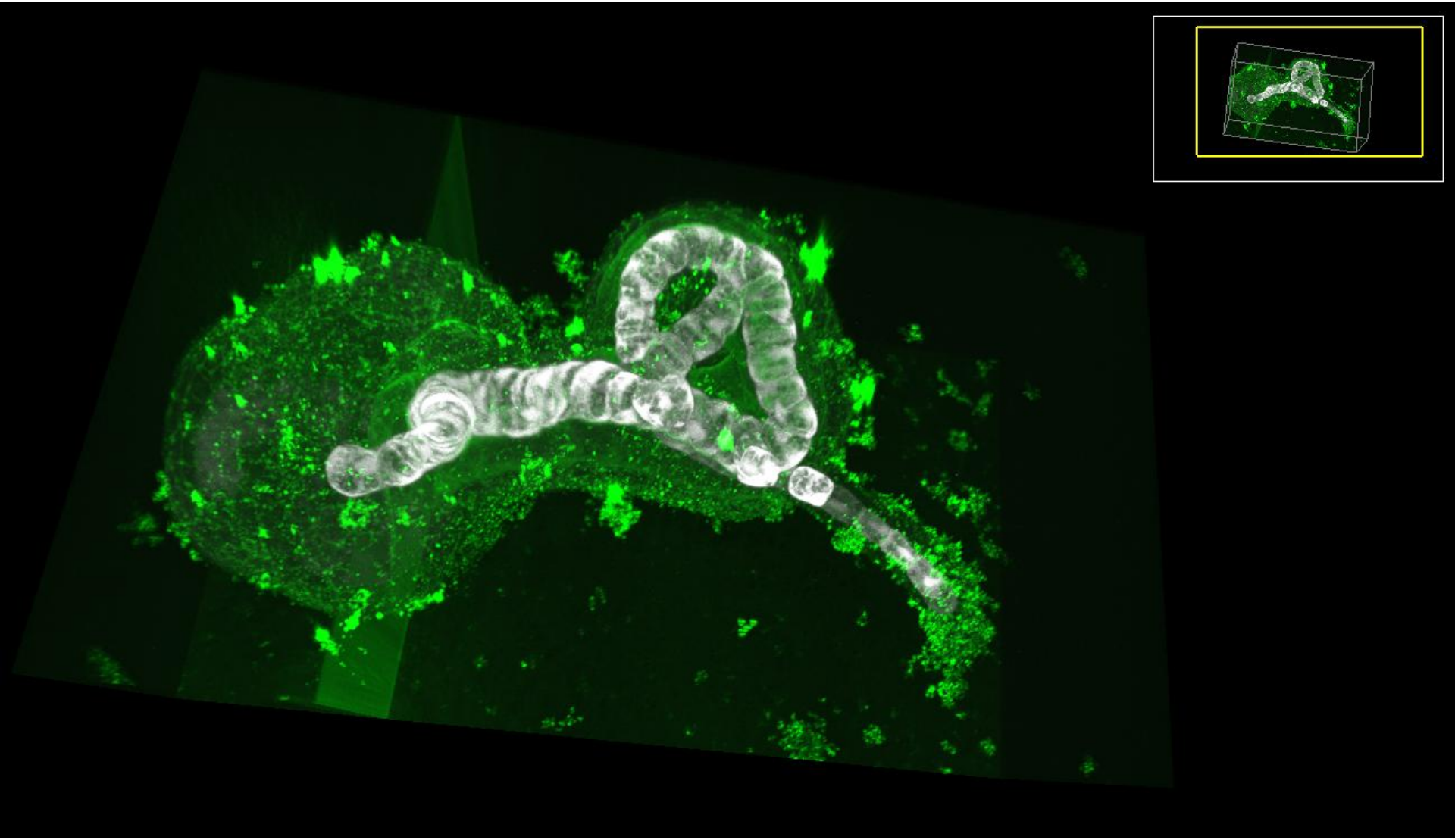
loss of **late** epidermal wnt5 does *not* disrupt A/P polarity



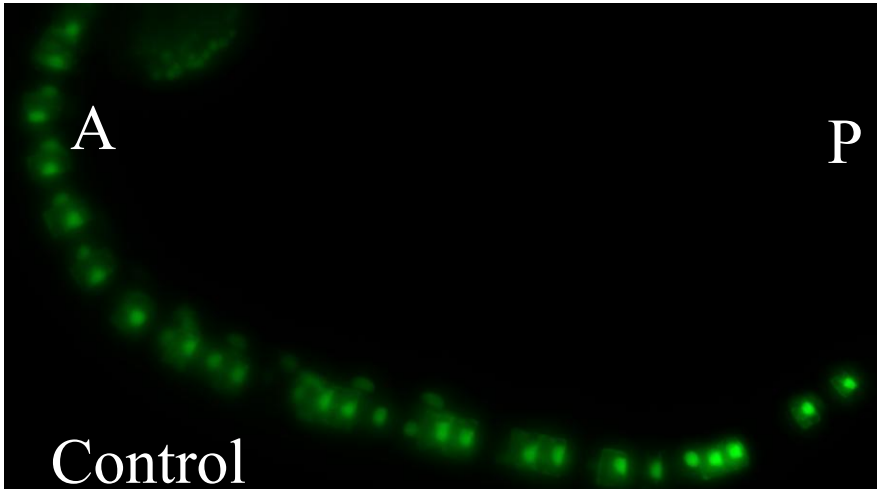


MO knockdown of the non-classical cadherin ***dachsous*** causes two distinct phenotypes

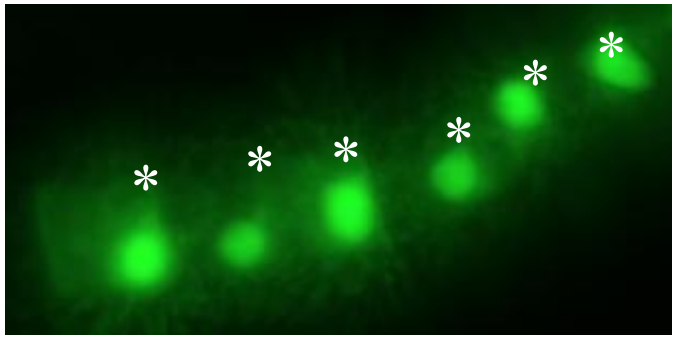
phenotype 1: splitting of the notochord

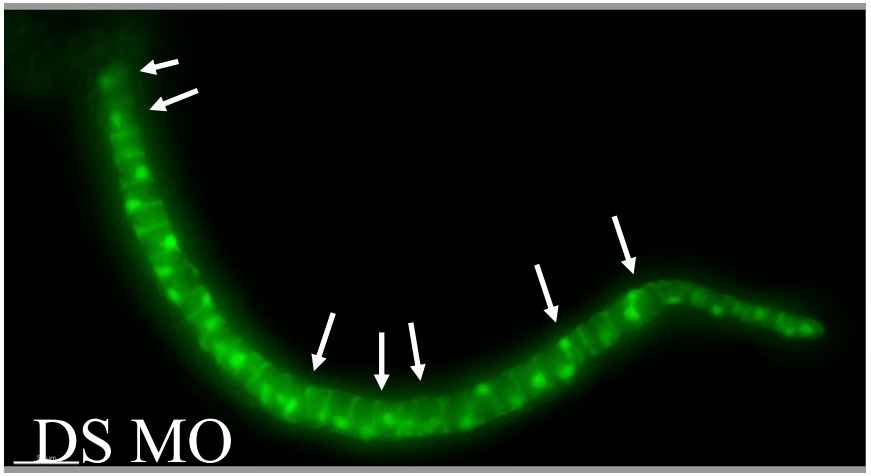


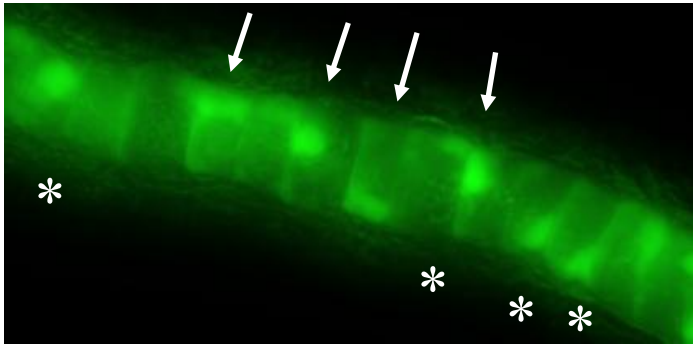
dachsous knockdown also causes A/P polarity defects



green--BraP::GFP

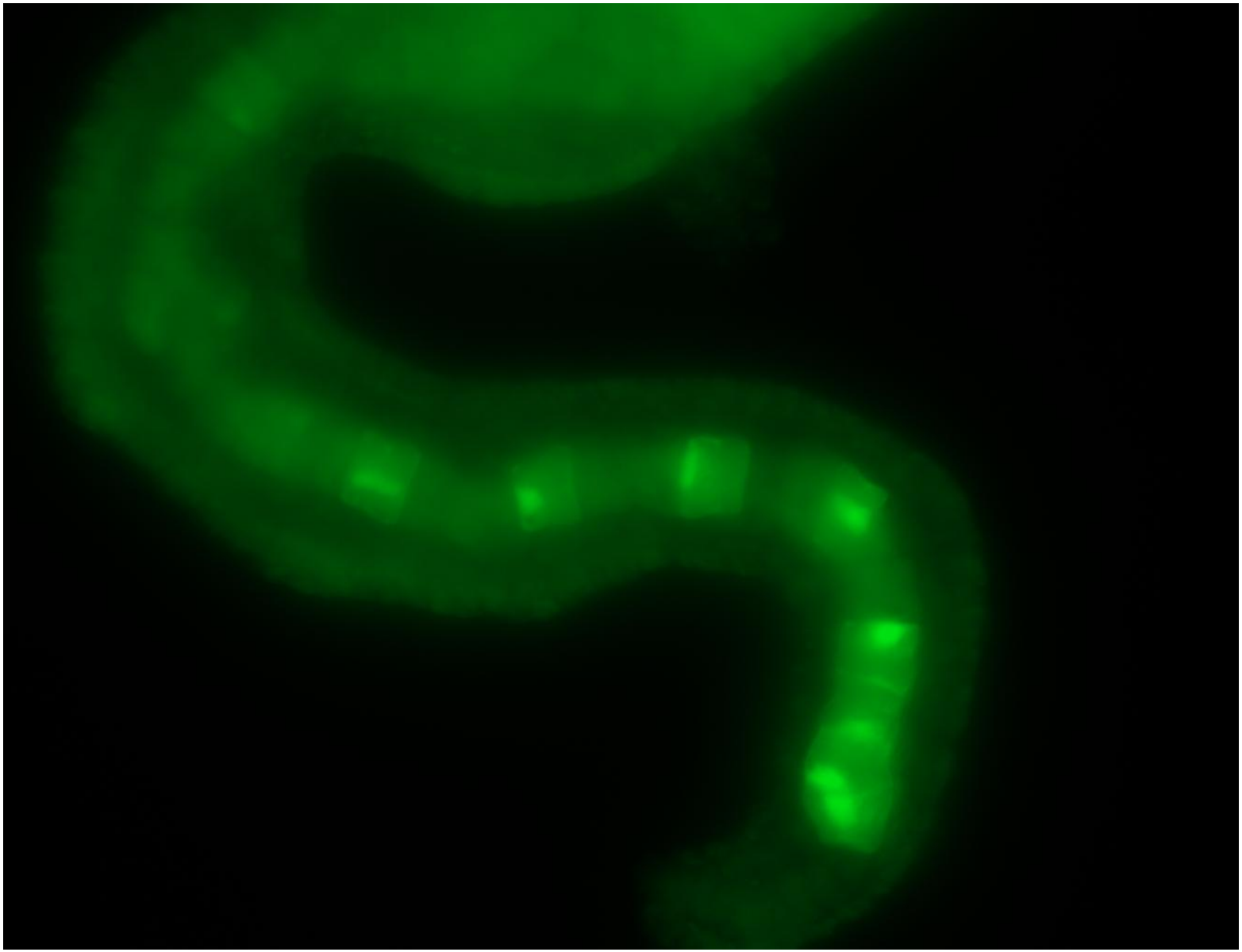






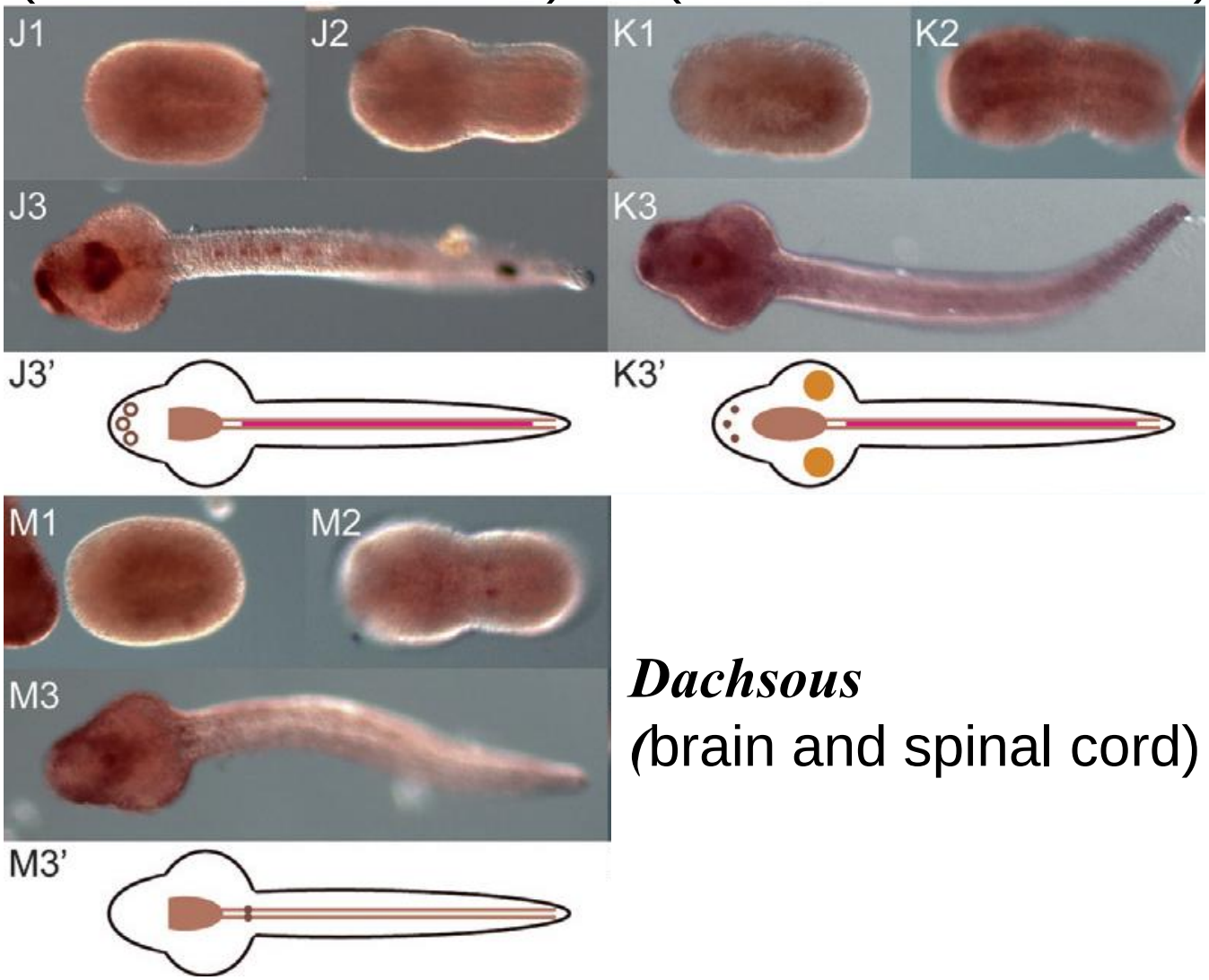
* **Normally positioned nuclei**

→ **Mis-localized nuclei**



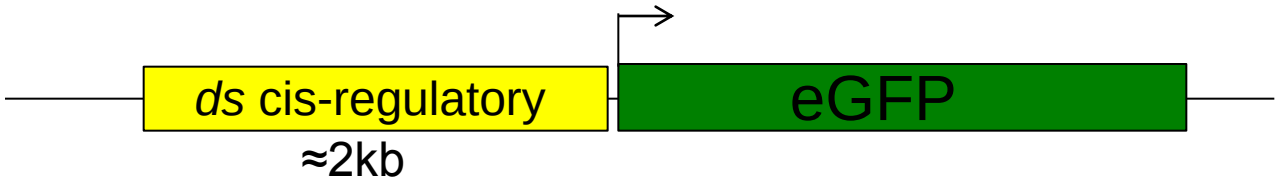
Fat4
(brain and notochord)

Fat1,2,3
(brain and notochord)

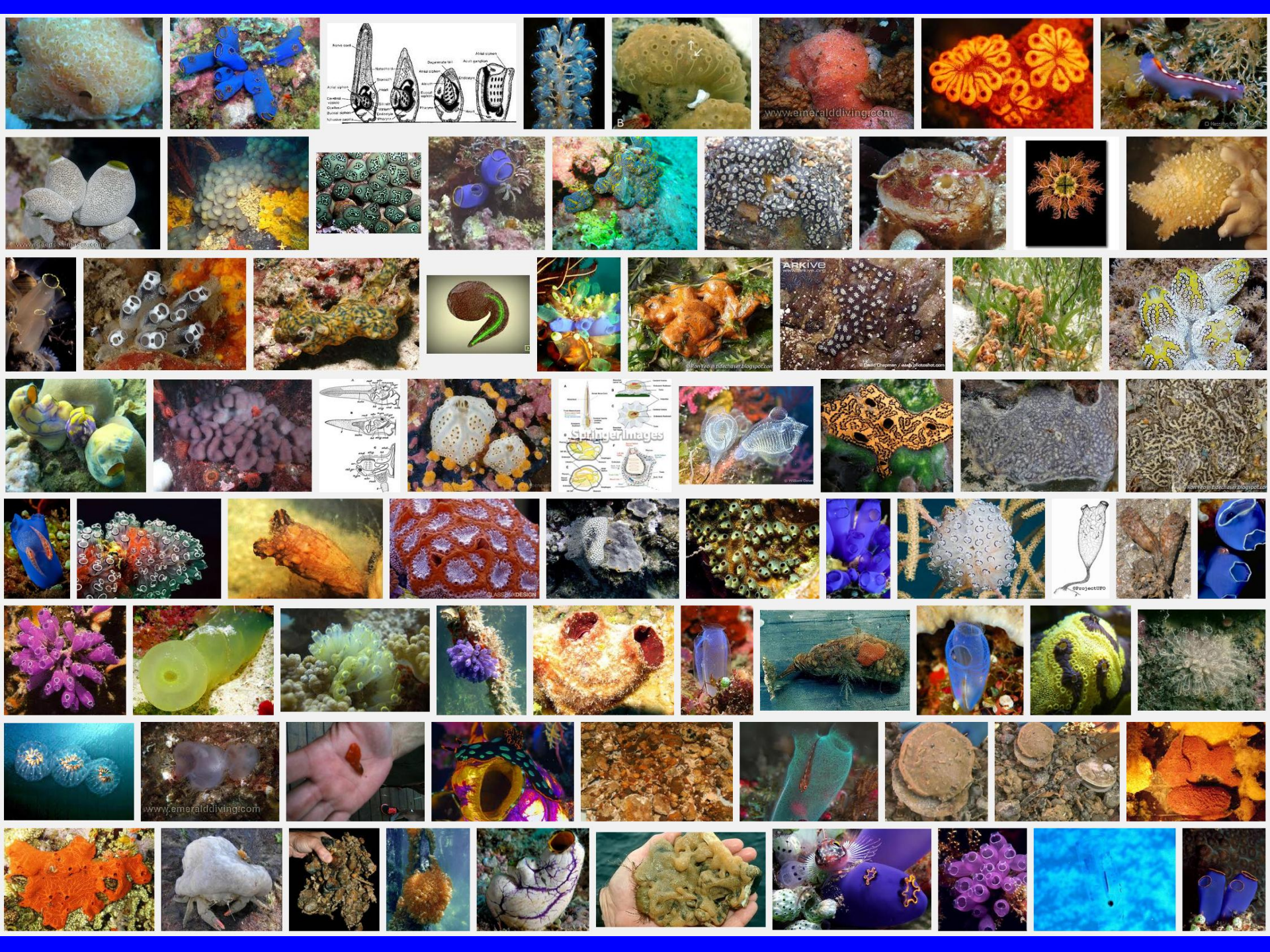


Dachsous
*(*brain and spinal cord)

T. Noda, N. Satoh / Gene Expression Patterns 8 (2008) 349–356


ds cis-regulatory
eGFP
≈2kb

- global polarity signal does not require intact actin cytoskeleton
- ***wnt5*** is essential for intercalation, but probably does not give positional information
- ***ds/fat*** system appear to be essential for proper A/P polarity, but not for intercalation
- preliminary results suggest the ***fat4*** and ***fat1,2,3***, are in the notochord, while ***ds*** is in the overlying spinal cord.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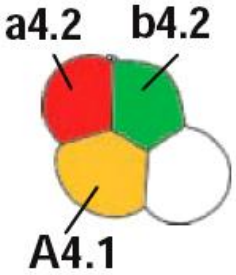
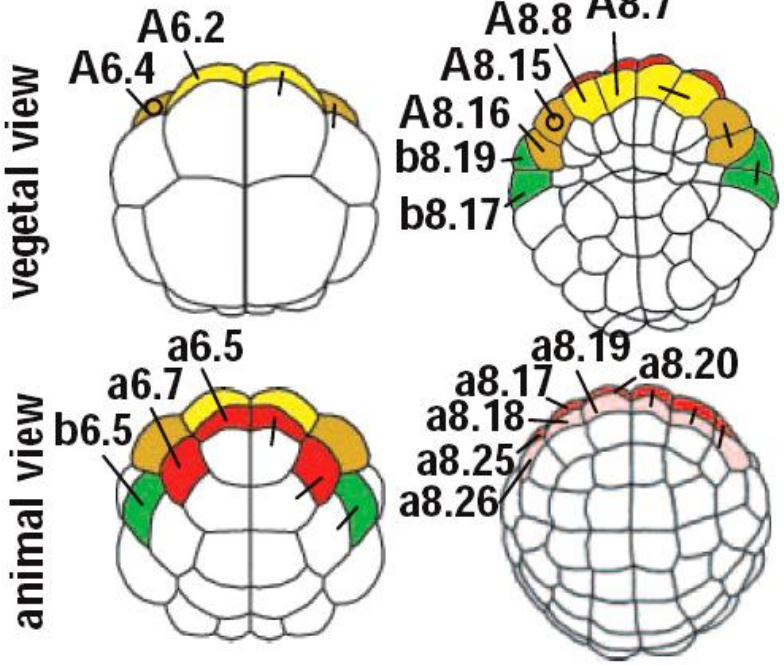
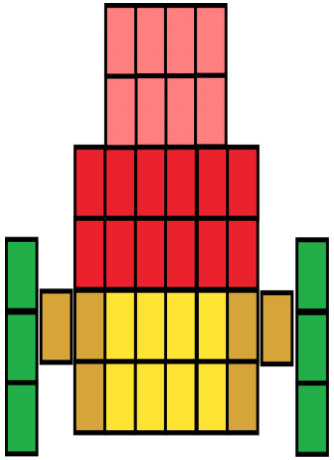
www.arkive.org
#ProjectUFO

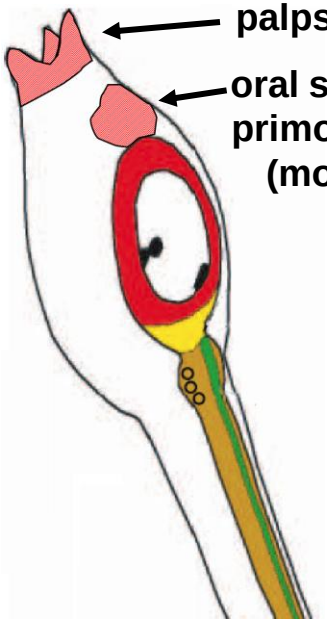
Ciona central nervous system

8-cell stage **32-cell stage** **110-cell stage** **neurula stage** **larval stage**









modified from:
Hudson and Yasuo (2005) *Development*

ectoderm lineage
foxA-a

epidermis

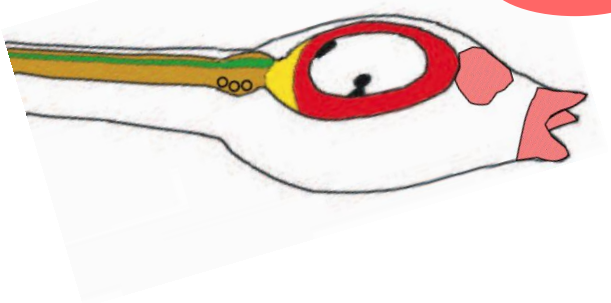
A-lineage
FGF9/16/20

induction

a-lineage
dmrt1
otx

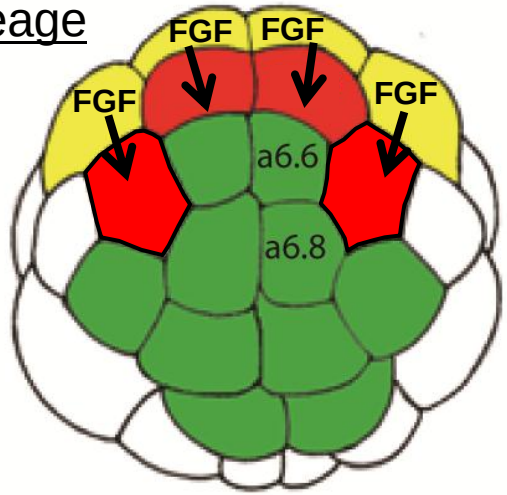
arrestin
six3/6

isl

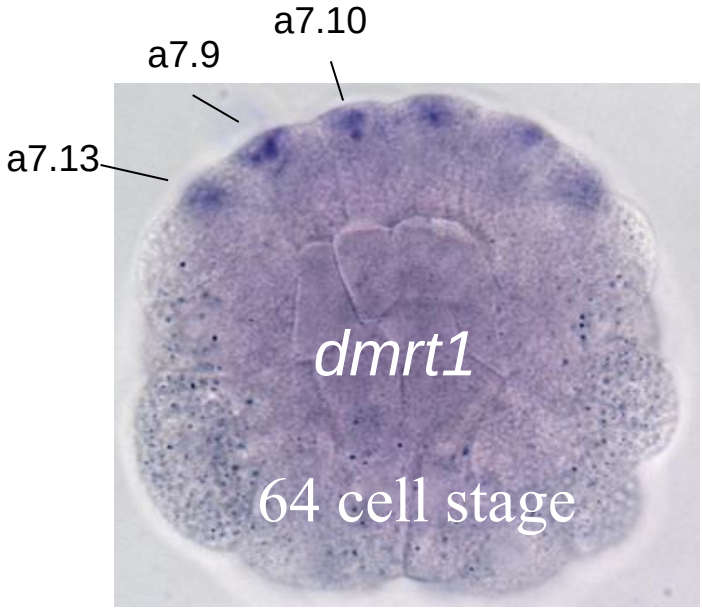


A-lineage

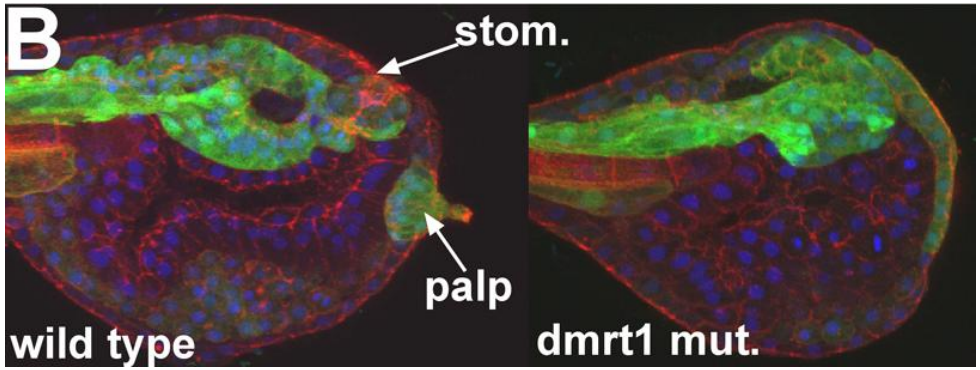
a-lineage



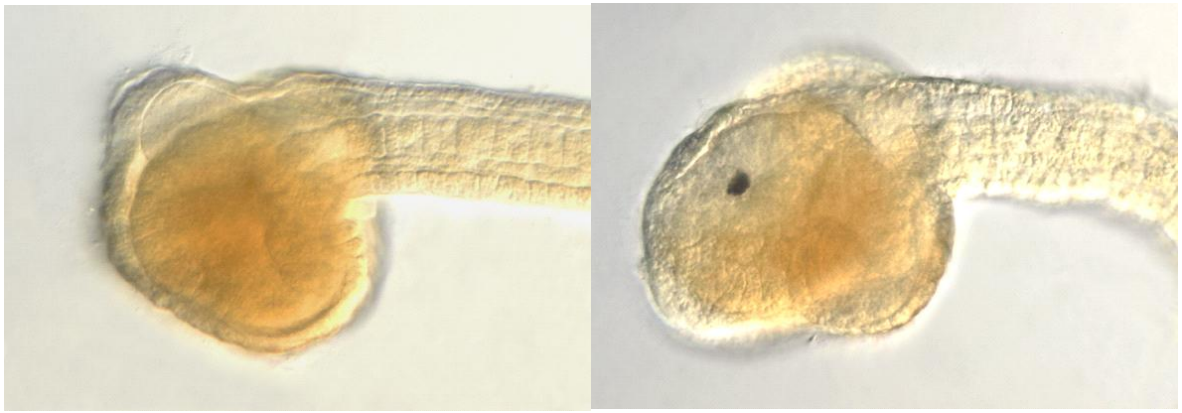
32 cell stage



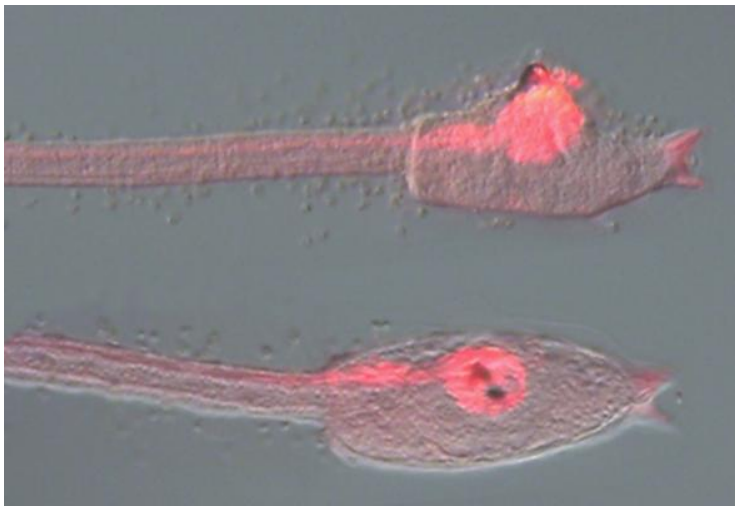
mutations disrupting “small a” lineage

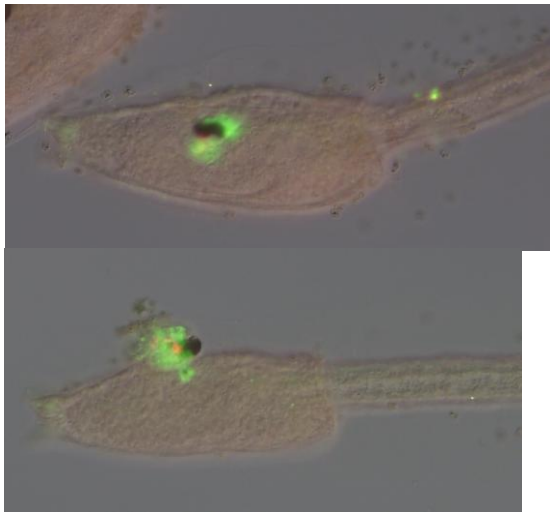


VAGABOND-null deletion in transcription factor *dmrt1*. Loss of anterior brain, mouth (stom.) and palps.



FRIMOUSSE- brain, mouth, palps absent. Failure of anterior neurulation. Posterior CNS-no obvious problems

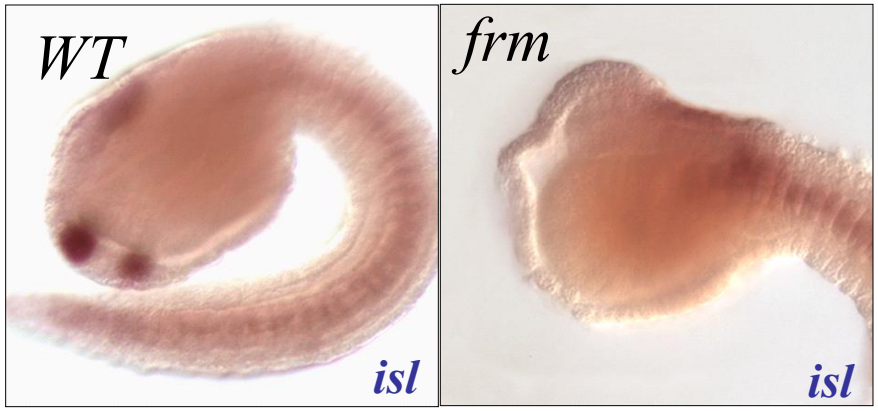




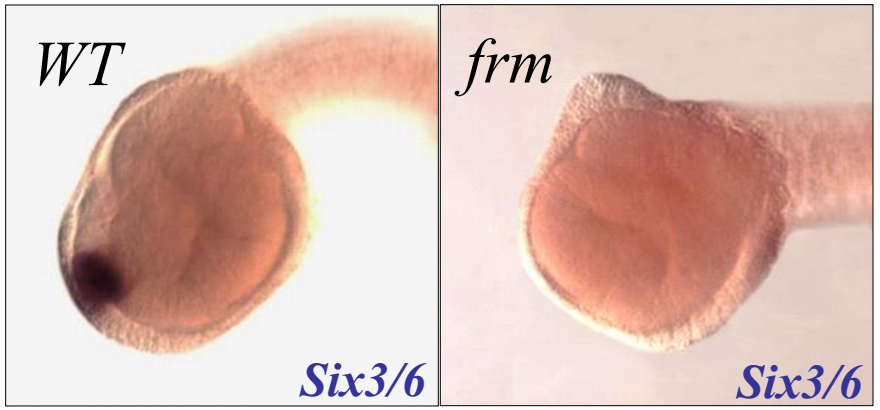
BUGEYE- Normal CNS differentiation. Failure to close anterior neural tube during neurulation.

In *frimousse* embryos markers for the palps, mouth and anterior brain are lost (a-lineage)

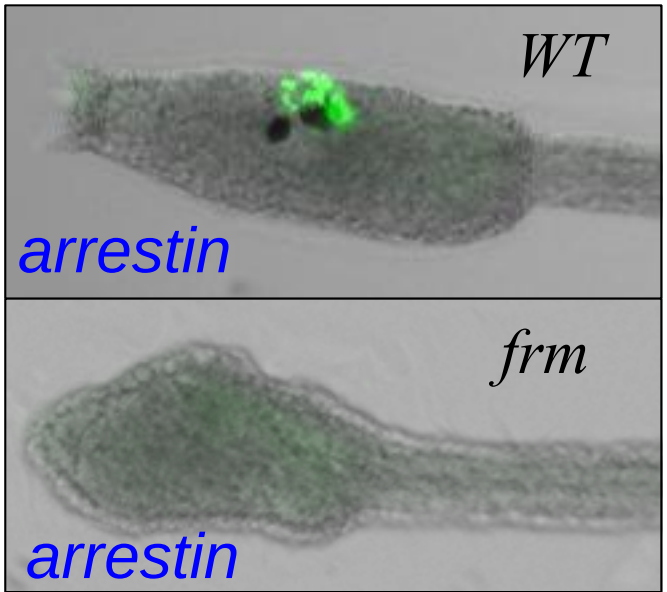
palp



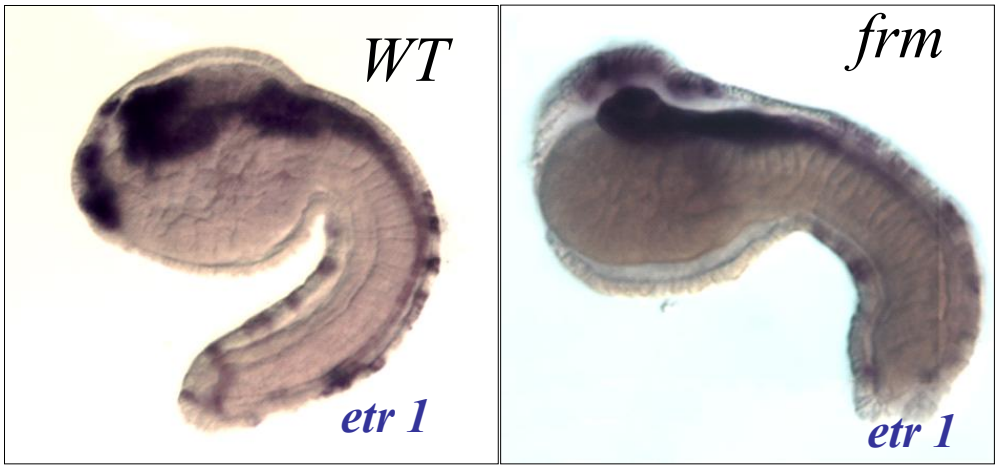
mouth



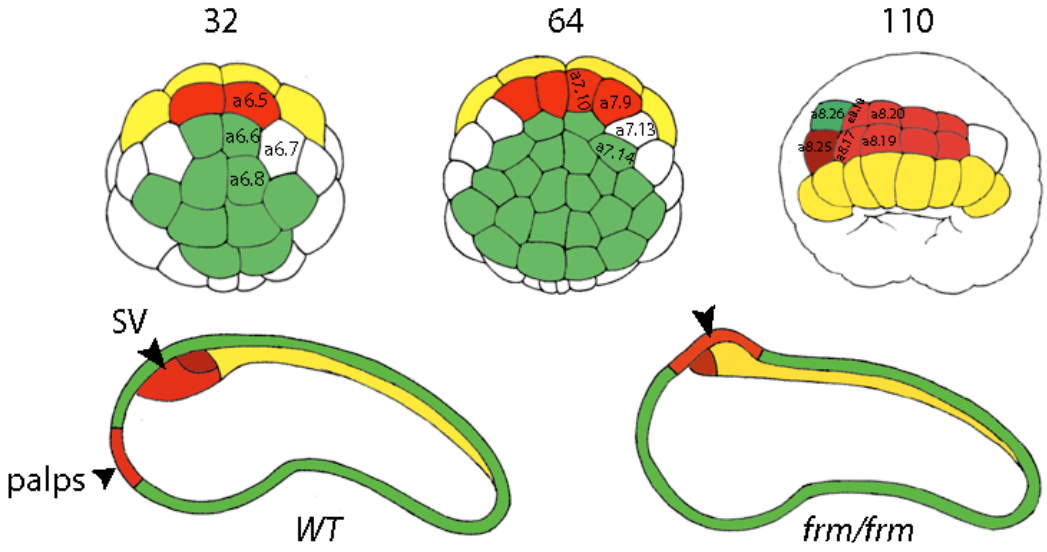
anterior brain

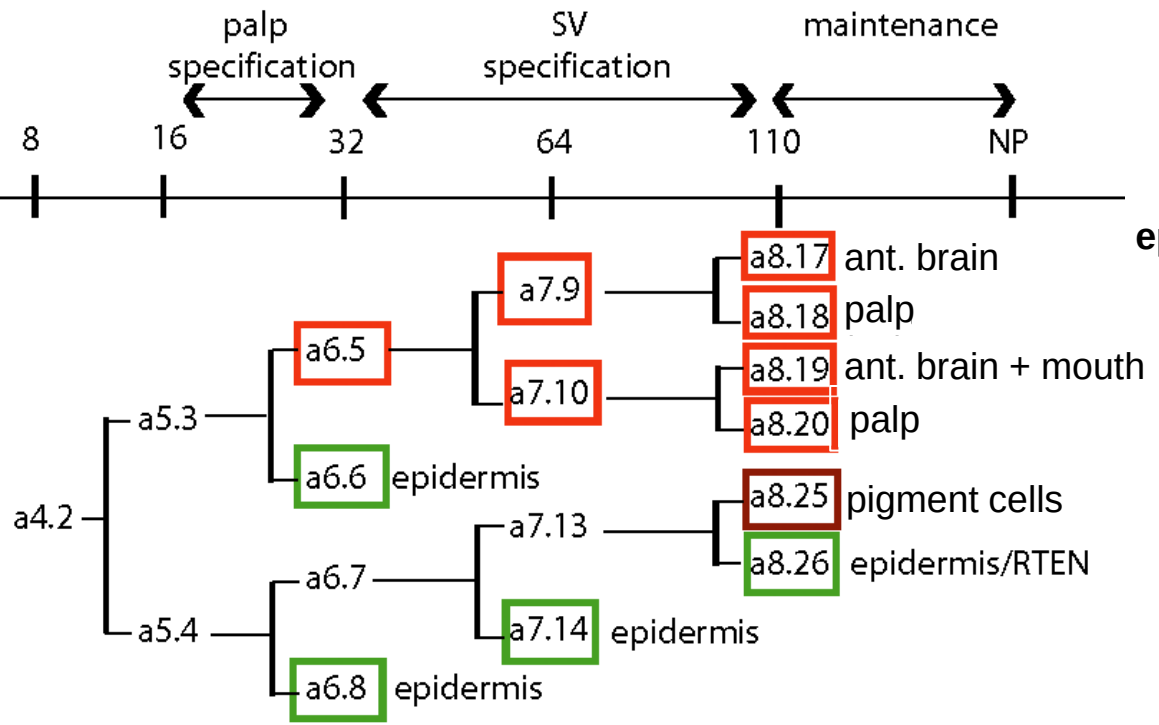


pan-neural

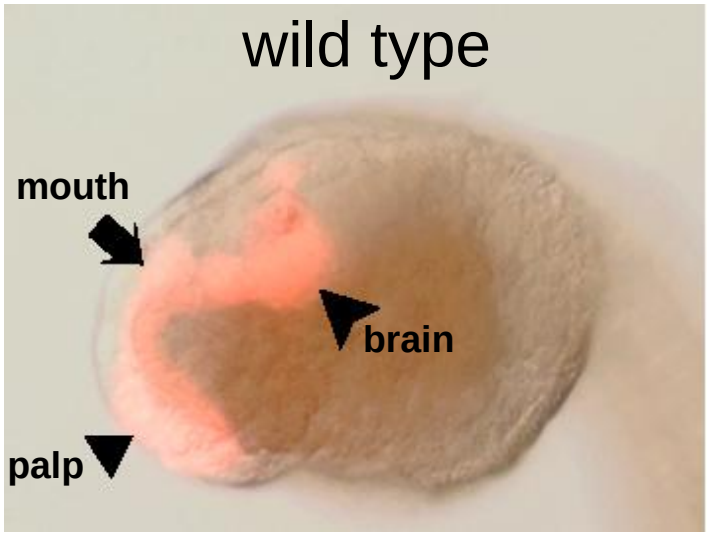


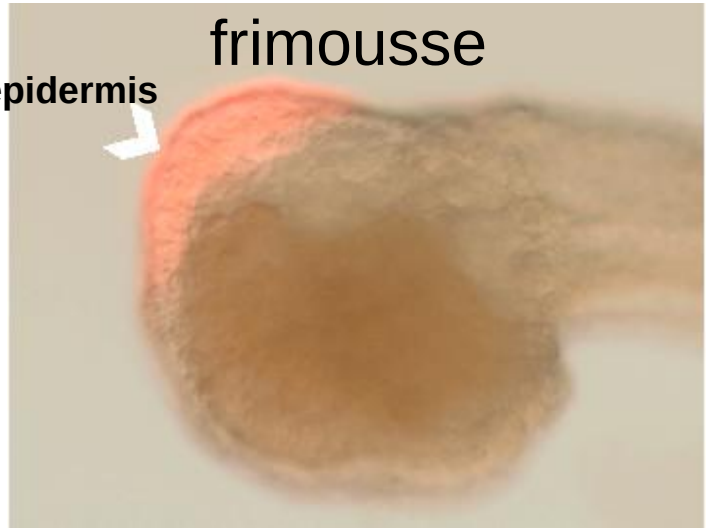
frimousse has a cell-fate transformation





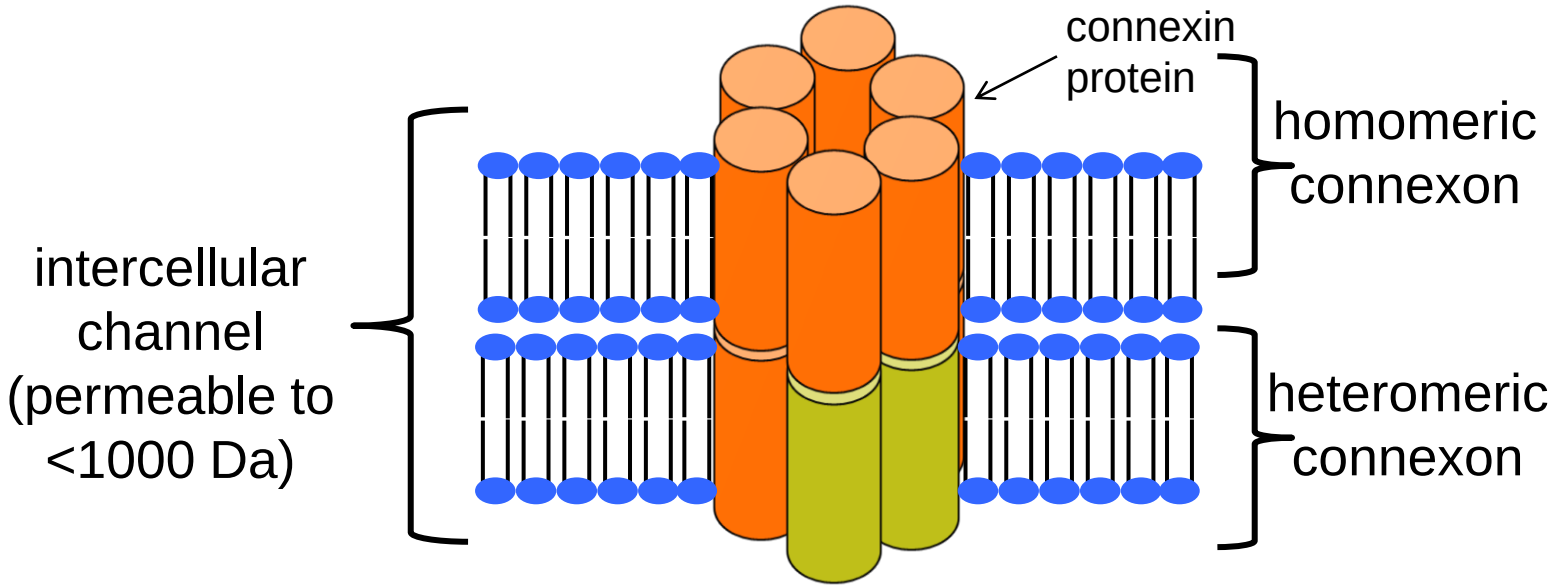
a6.5 derivatives



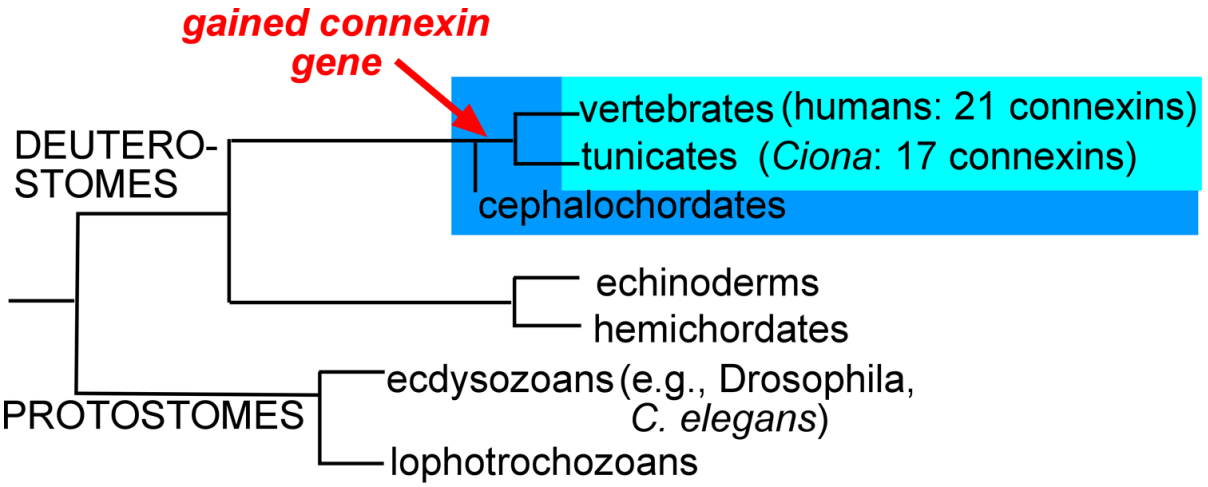


frimousse mutation maps to a **connexin** gene (cnx11)

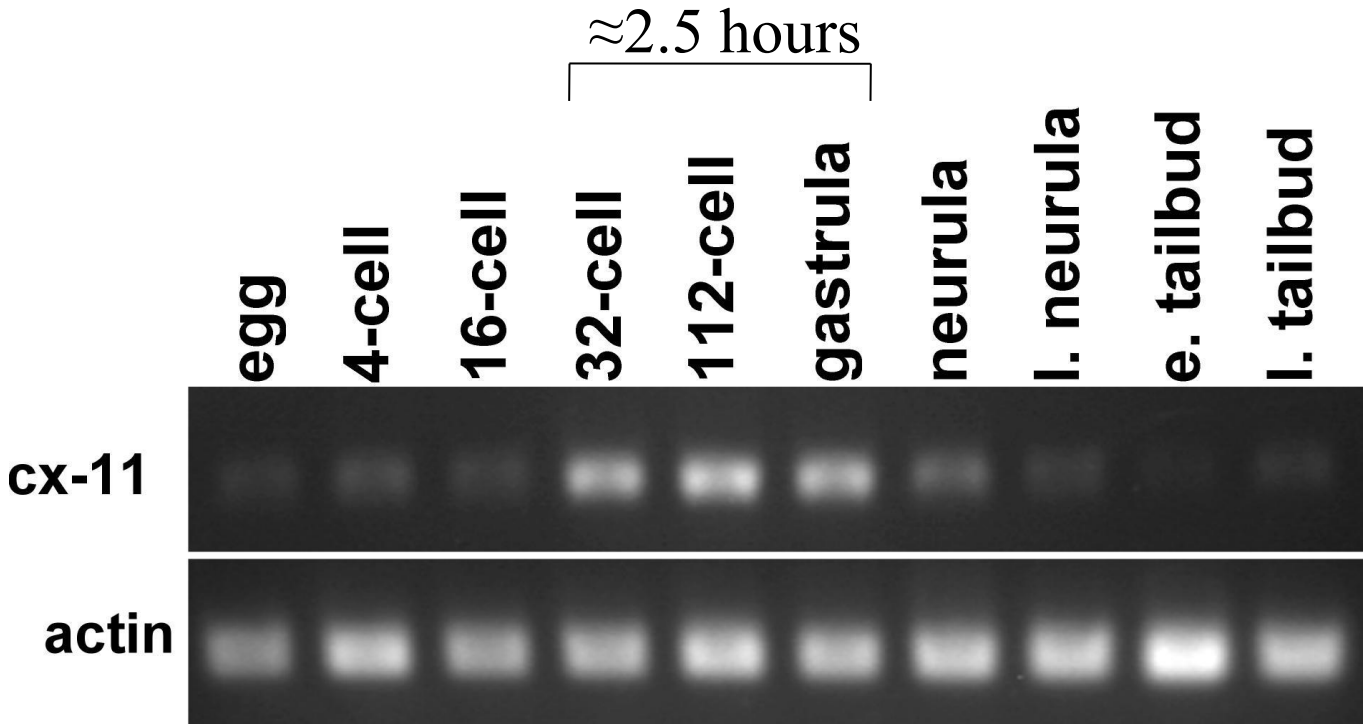
- connexin proteins make gap junctions:



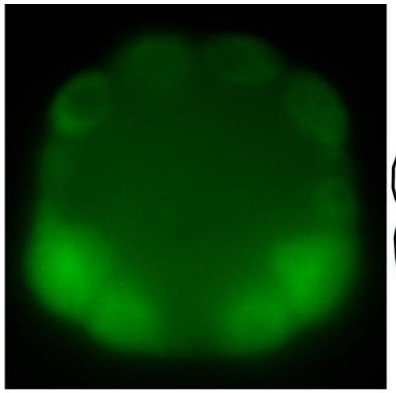
- connexin genes are only found in vertebrates and tunicates:

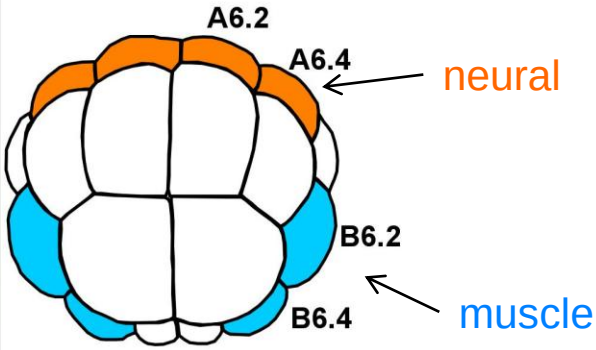


•causitive gene of *frm* mutation (*connexin11*) is up-regulated during neural induction

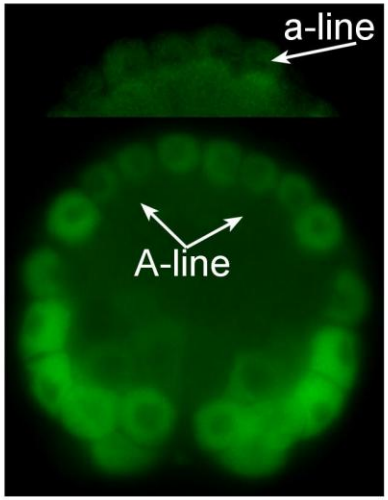


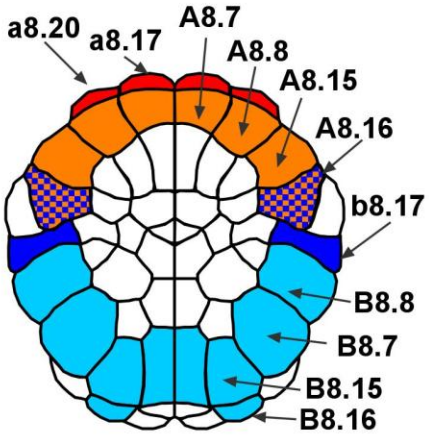
32-cell



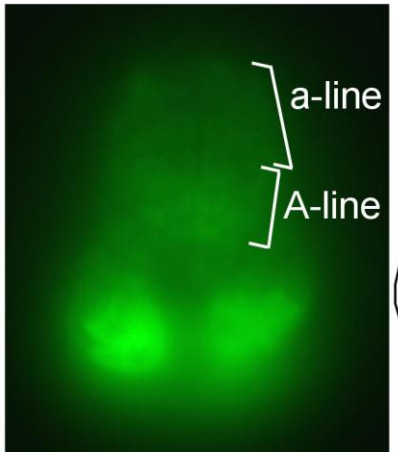


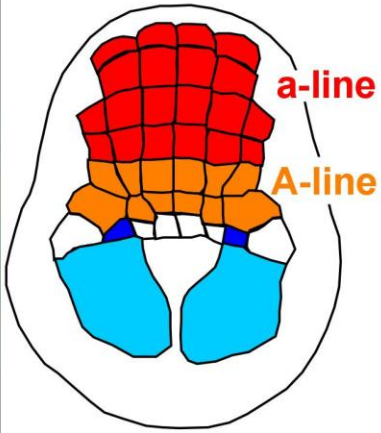
gastrula



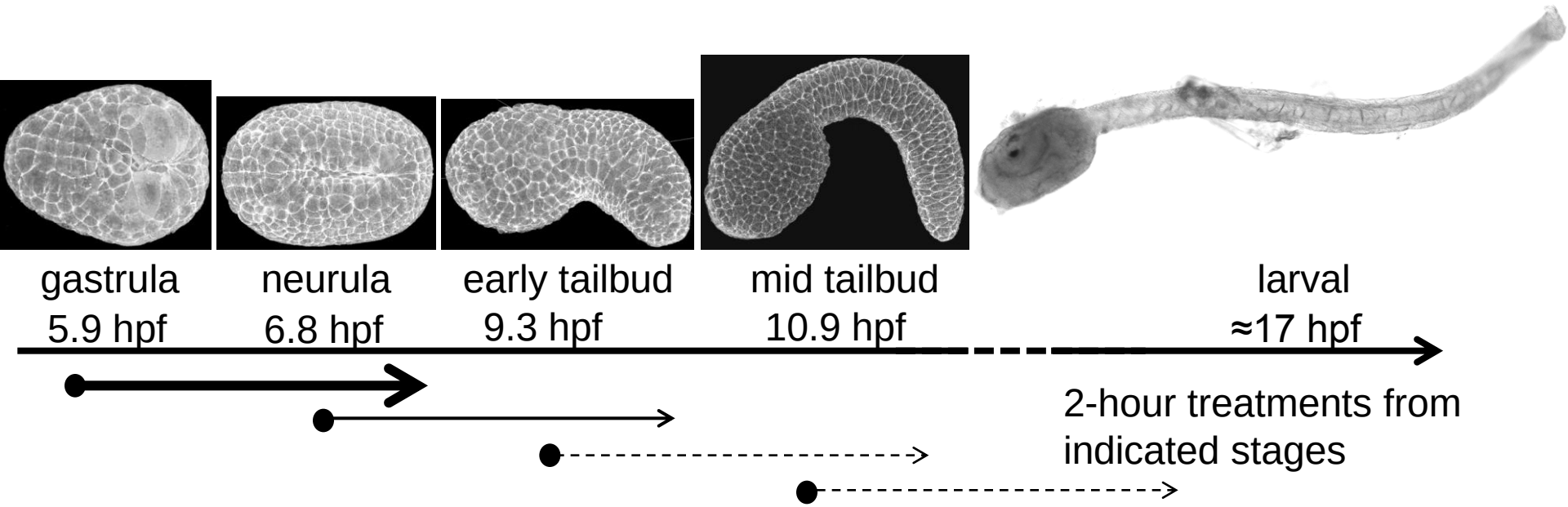


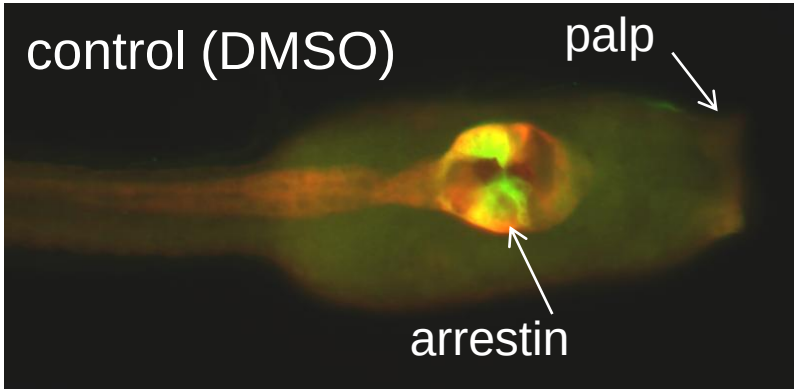
neurula

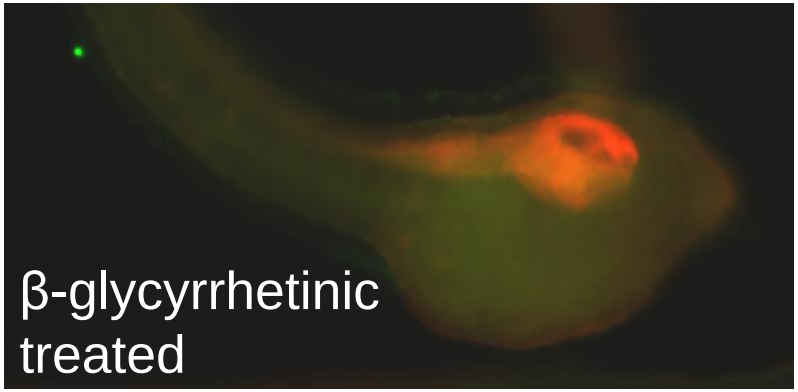




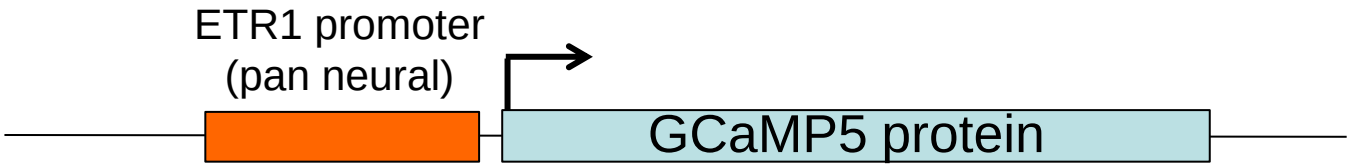
•**gap junction inhibitor β-glycyrrhetinic acid phenocopies the *frimousse* mutation**



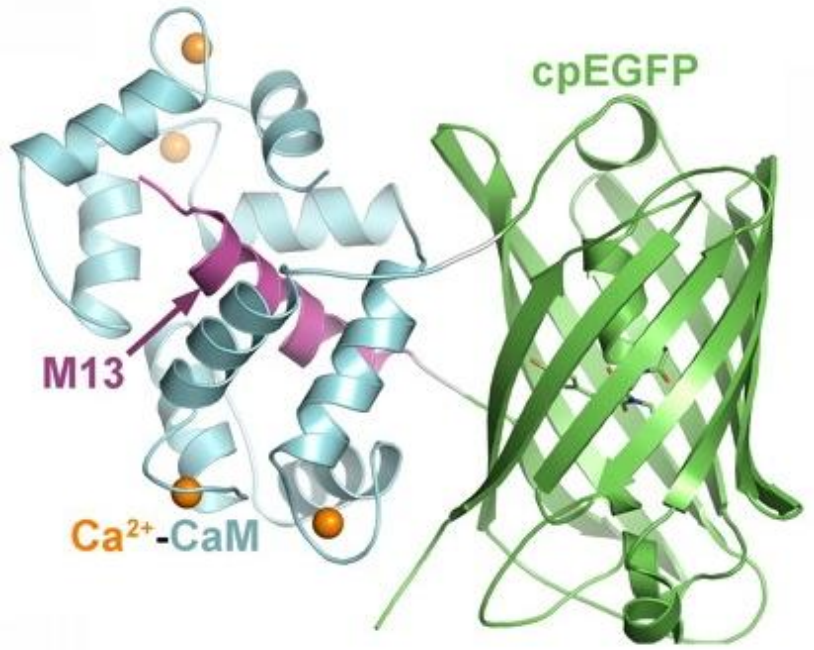




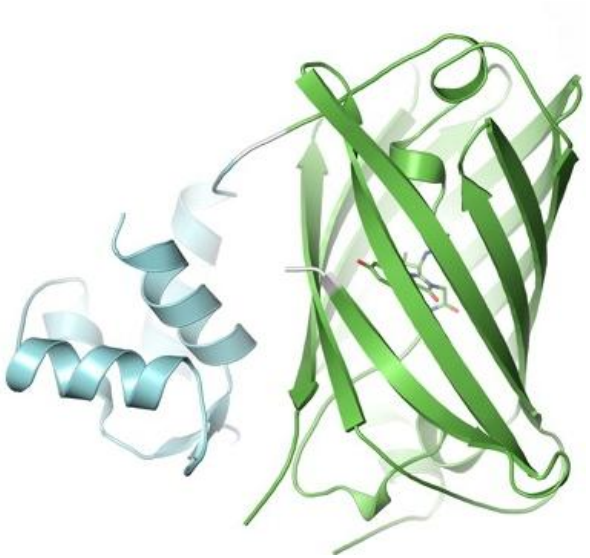
What are the gap junctions doing?



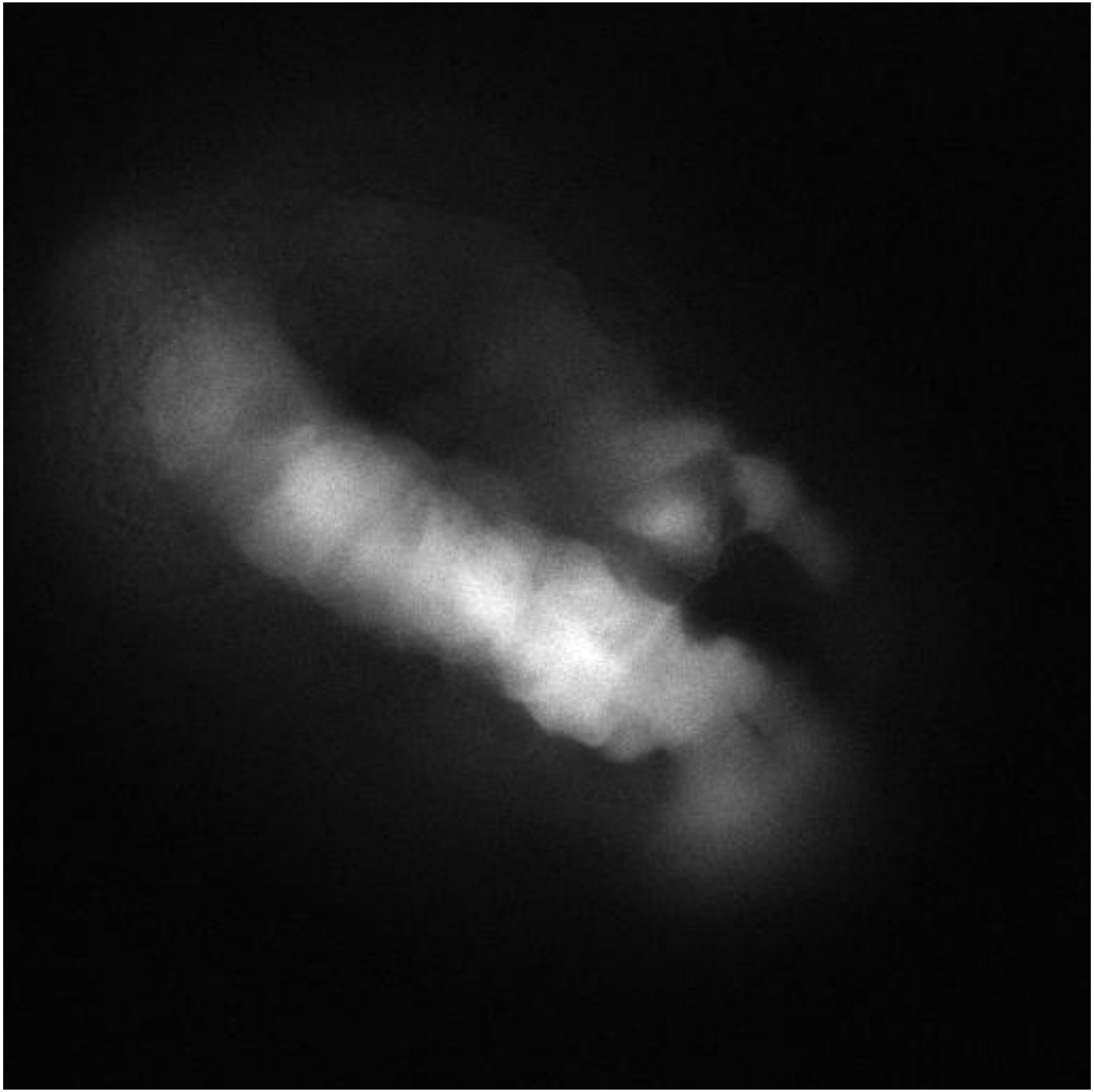
transgenic construct



GCaMP5 + Ca^{2+}
High Fluorescence



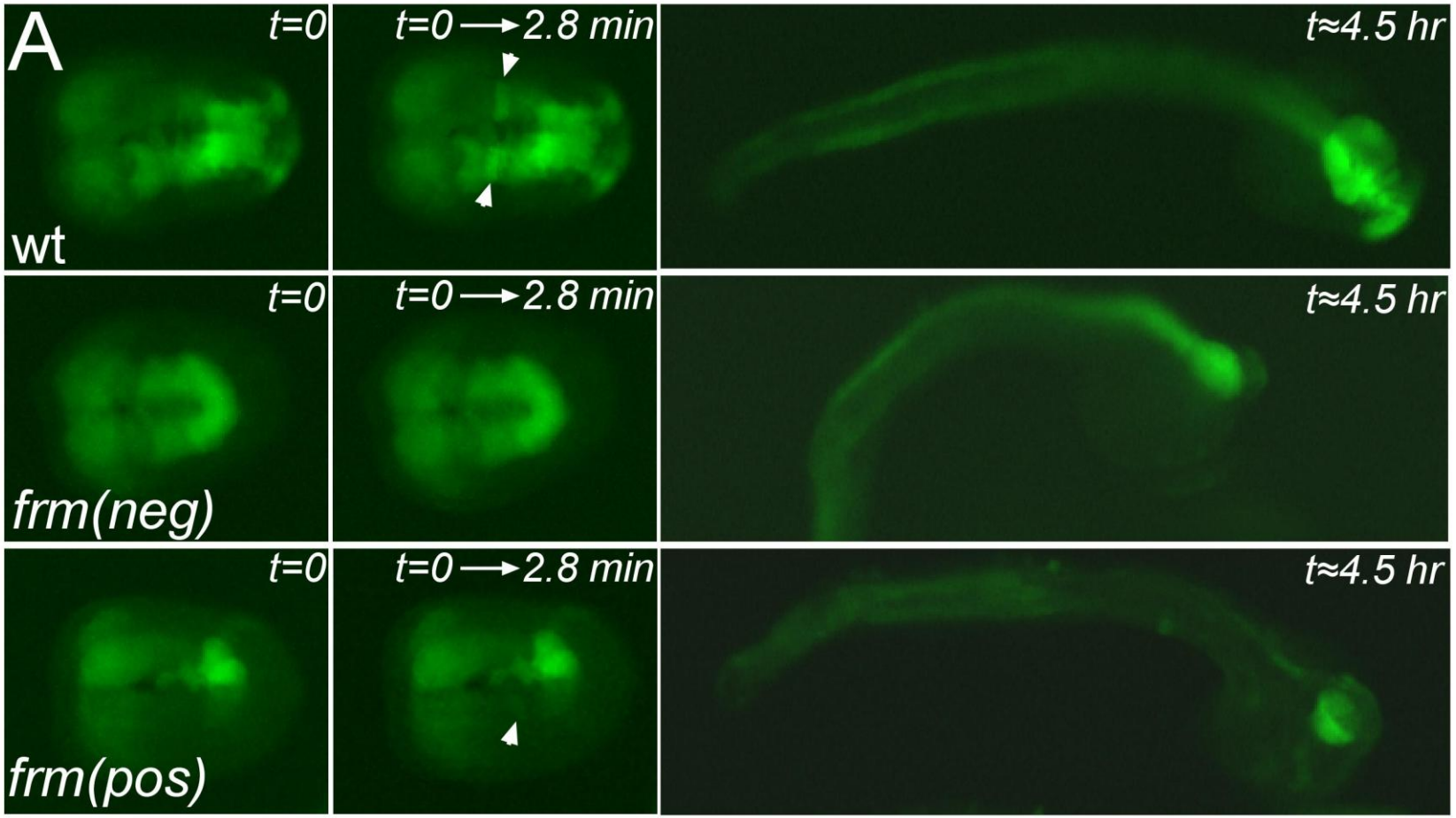
GCaMP5 w/o Ca^{2+}
Low Fluorescence

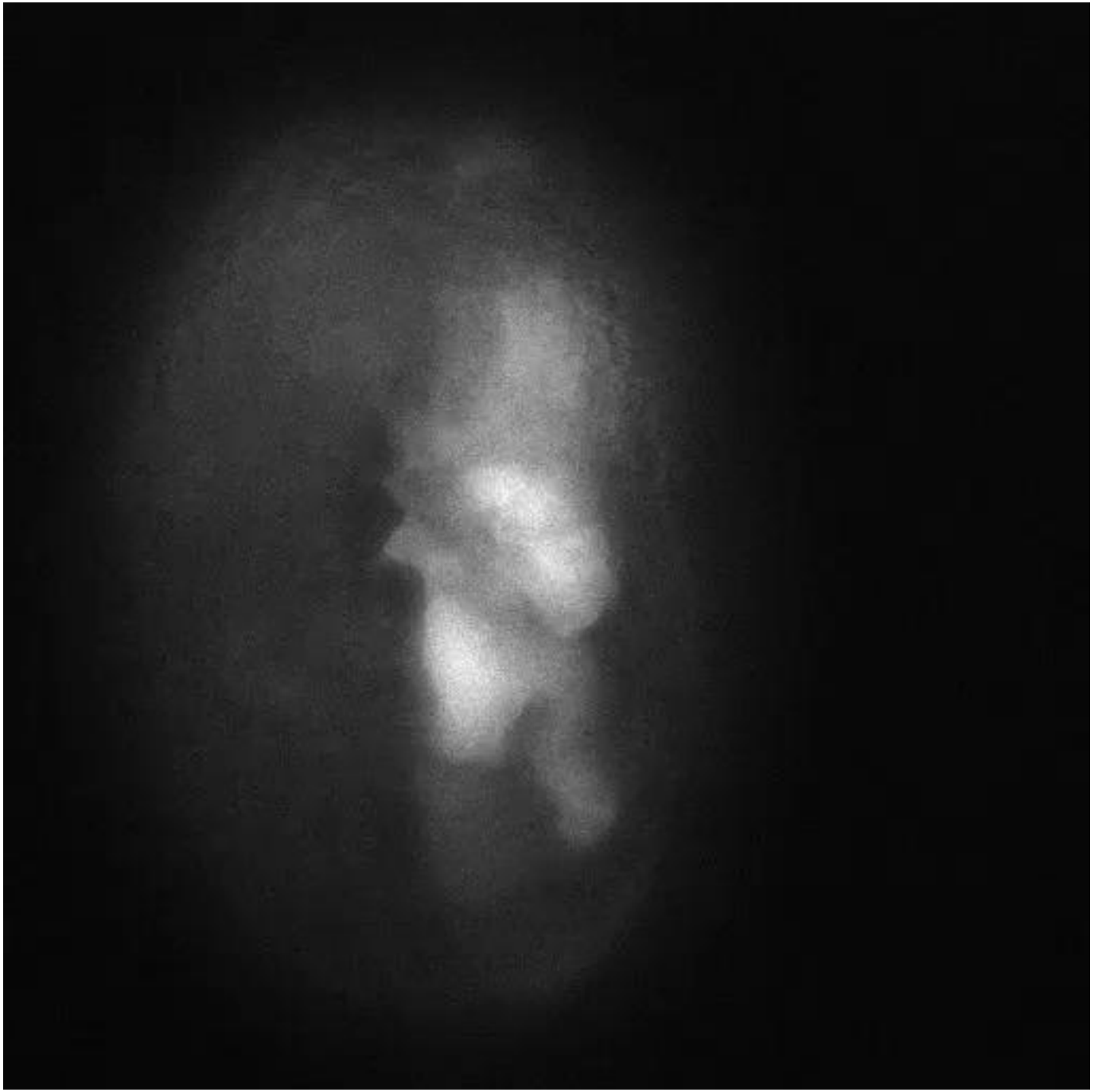


Ca^{2+} transients

5-10 second duration

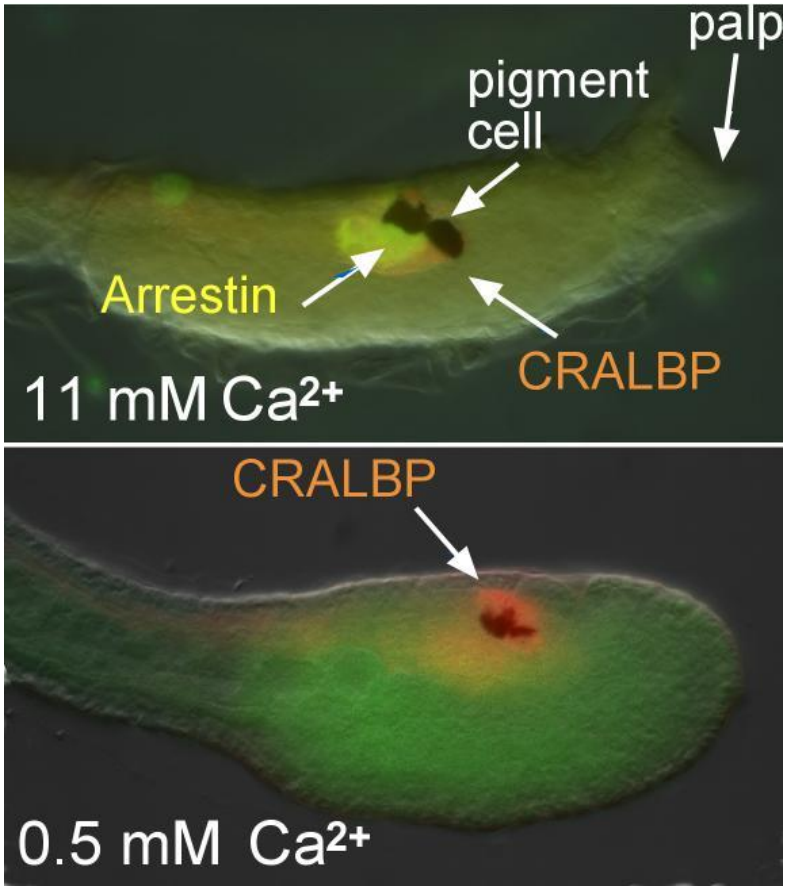
•Ca^{2+} transients are eliminated in *frimousse* mutation (essentially)

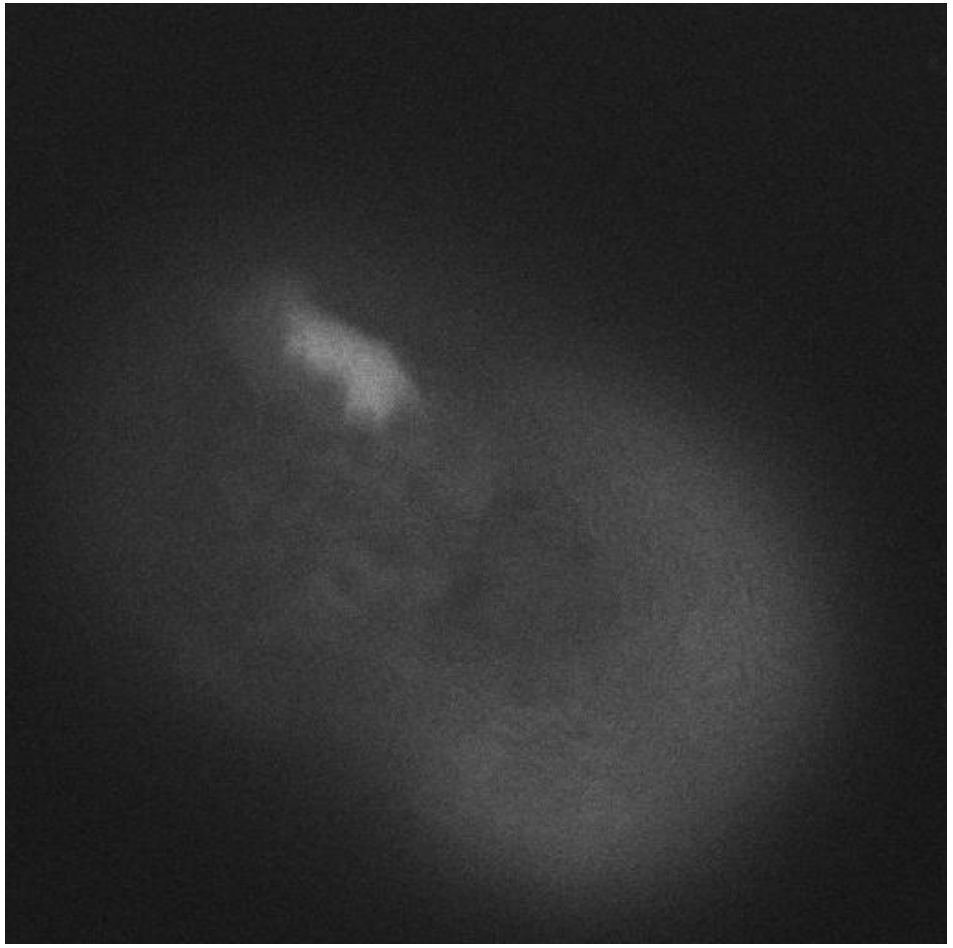


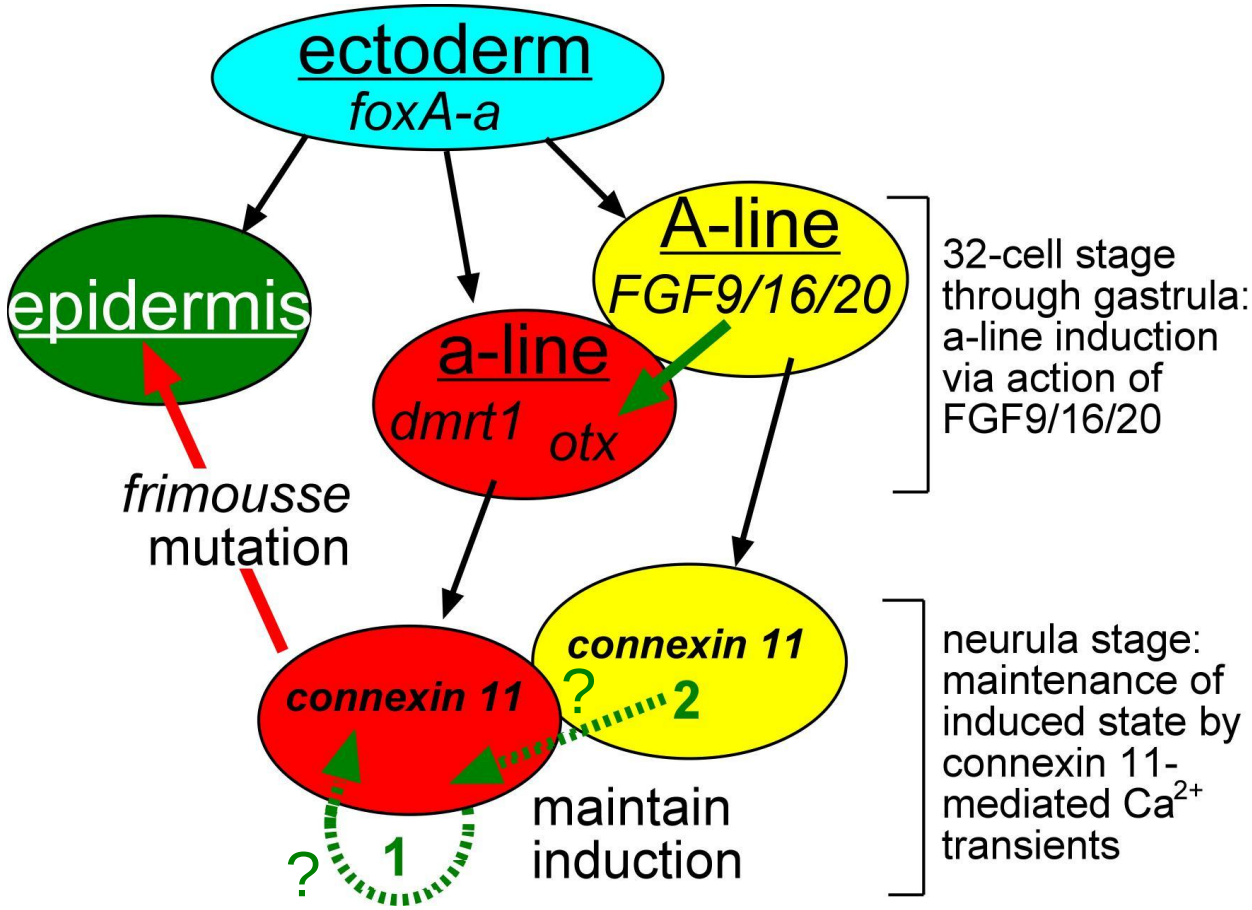


Ca^{2+} depletion (0.5 mM versus 11 mM) at the critical stage:

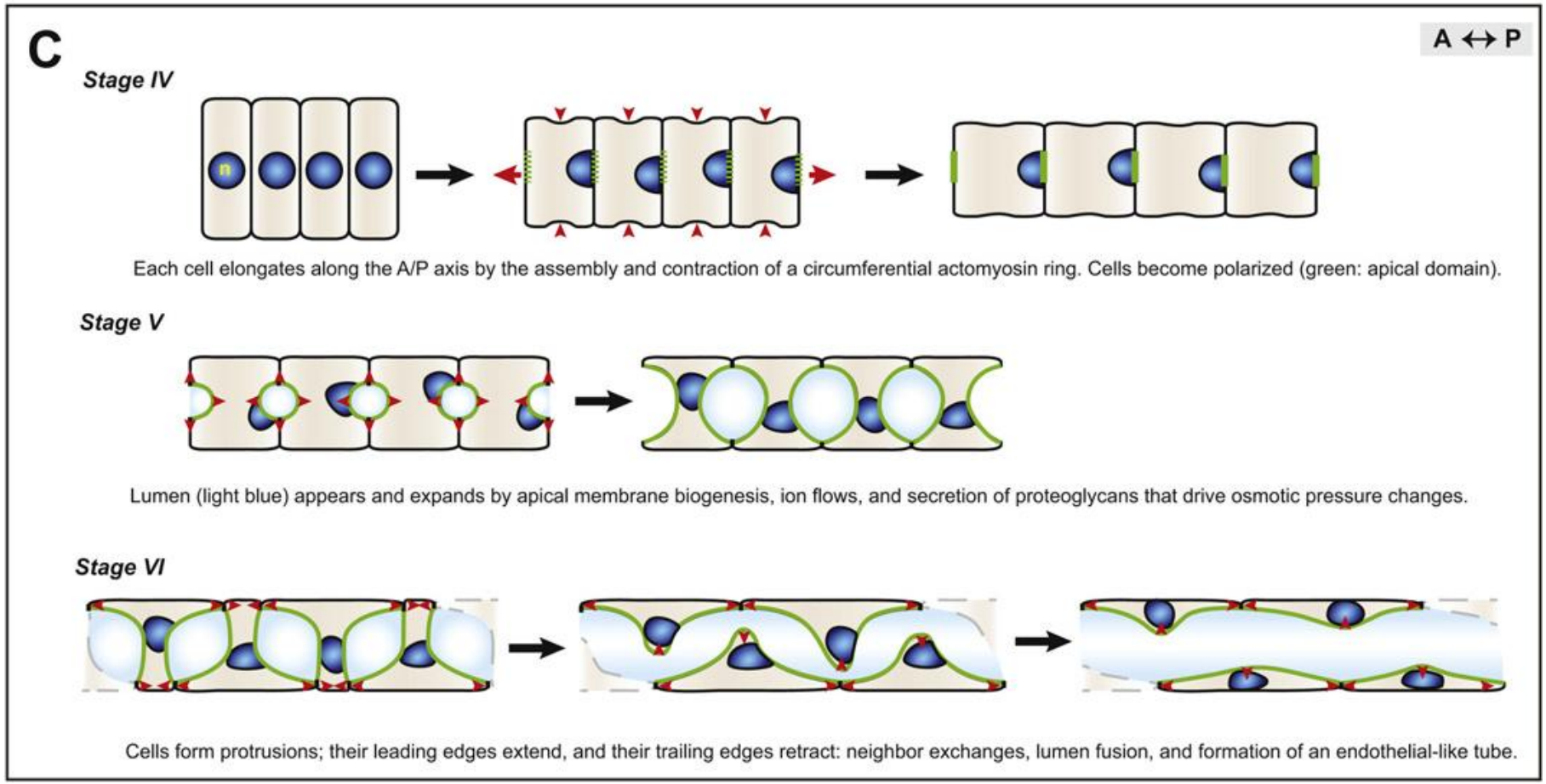
1. phenocopies mutation *and* 2. eliminates Ca^{2+} transients







- gap junction communication within the neural plate is required to maintain neural induction
- Ca^{2+} transients appear to play a role
- how and why?...stay tunned



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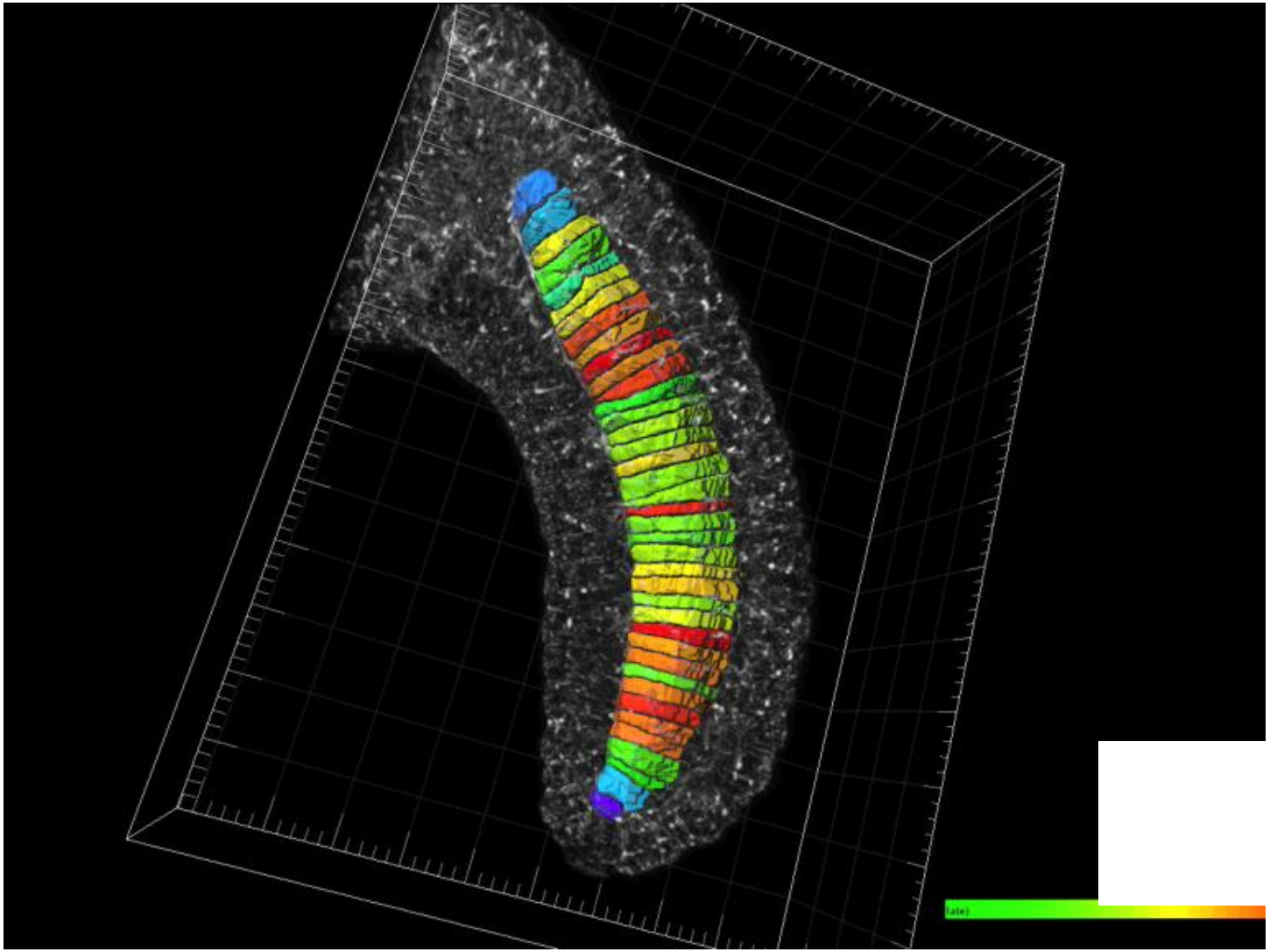
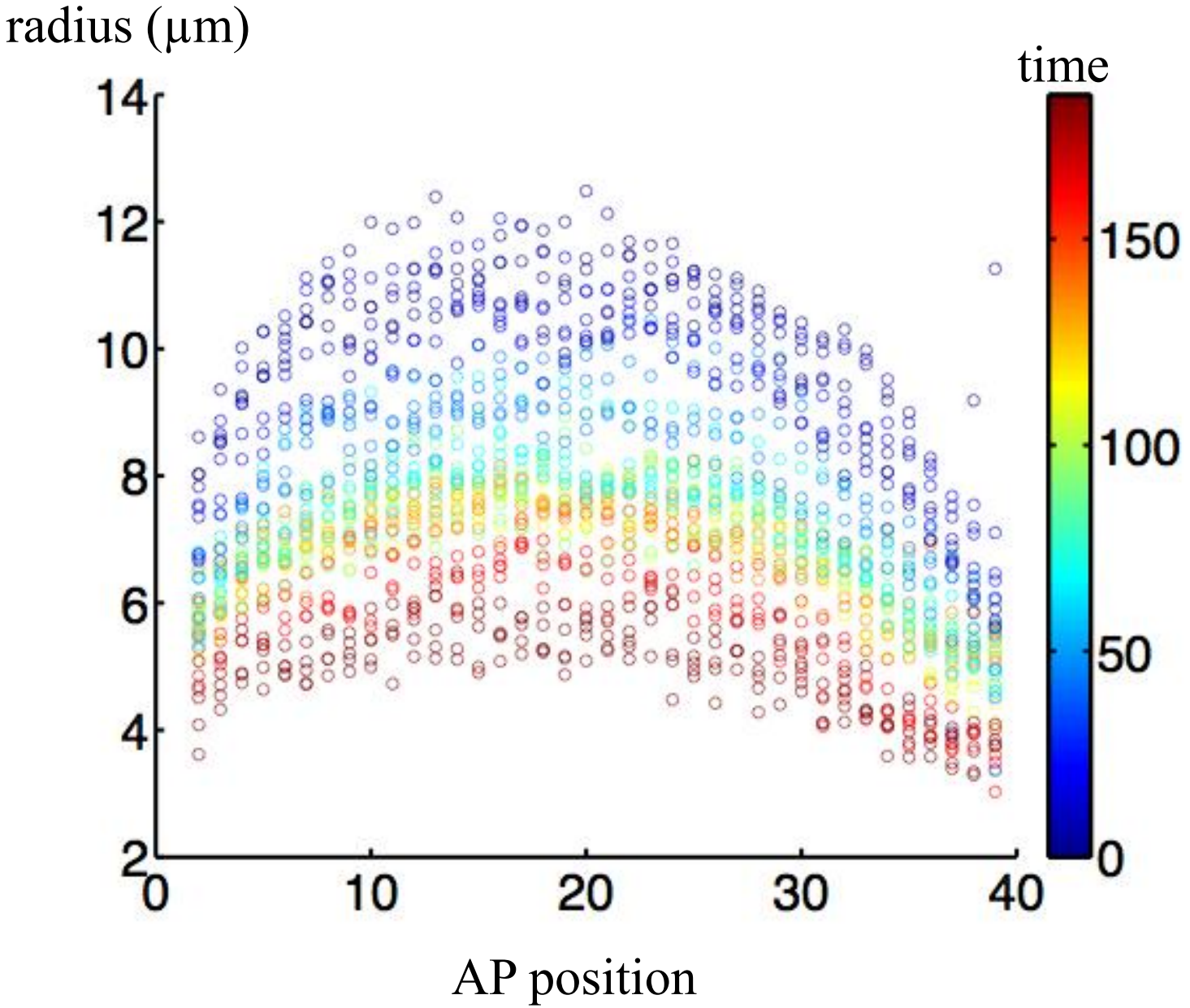
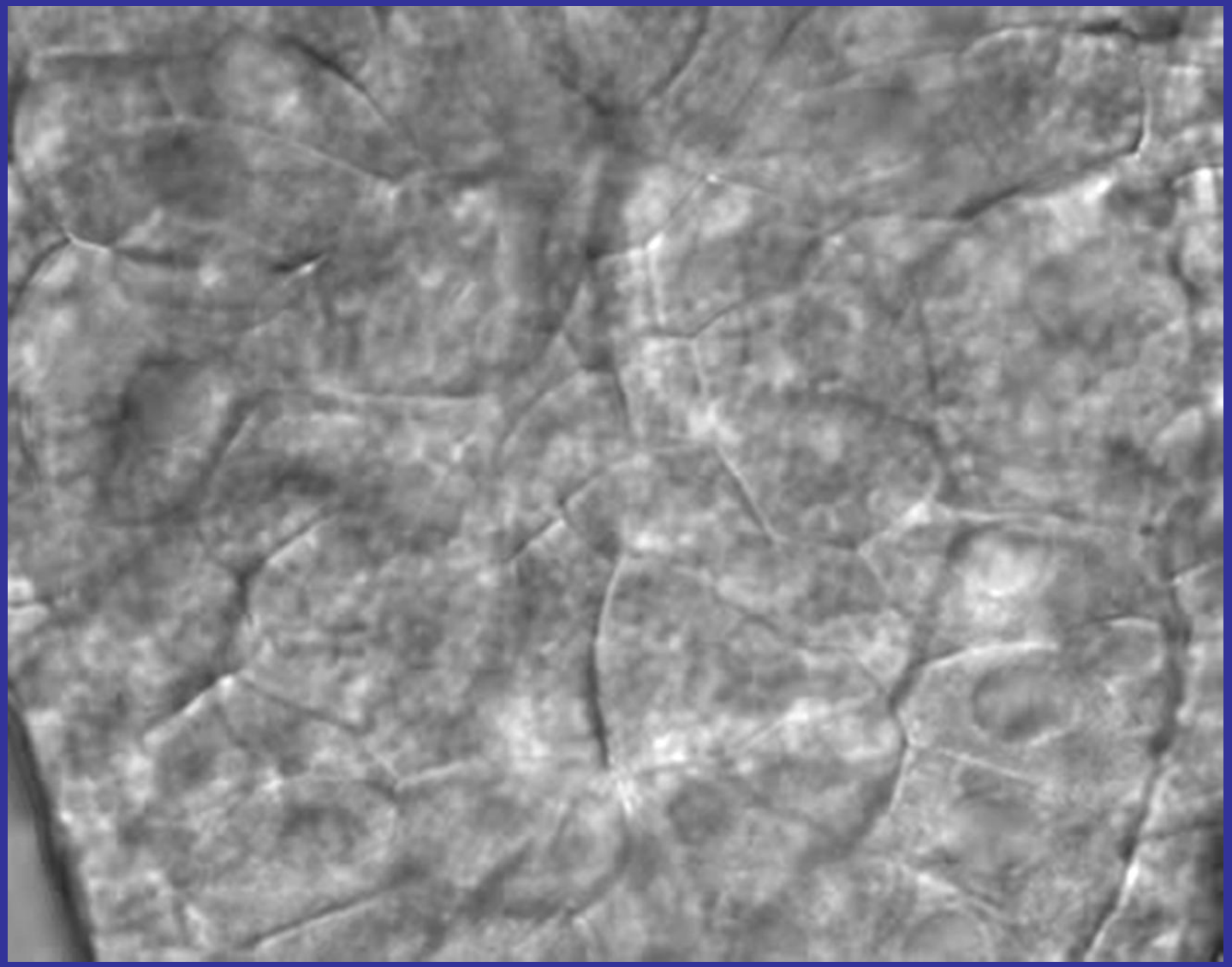
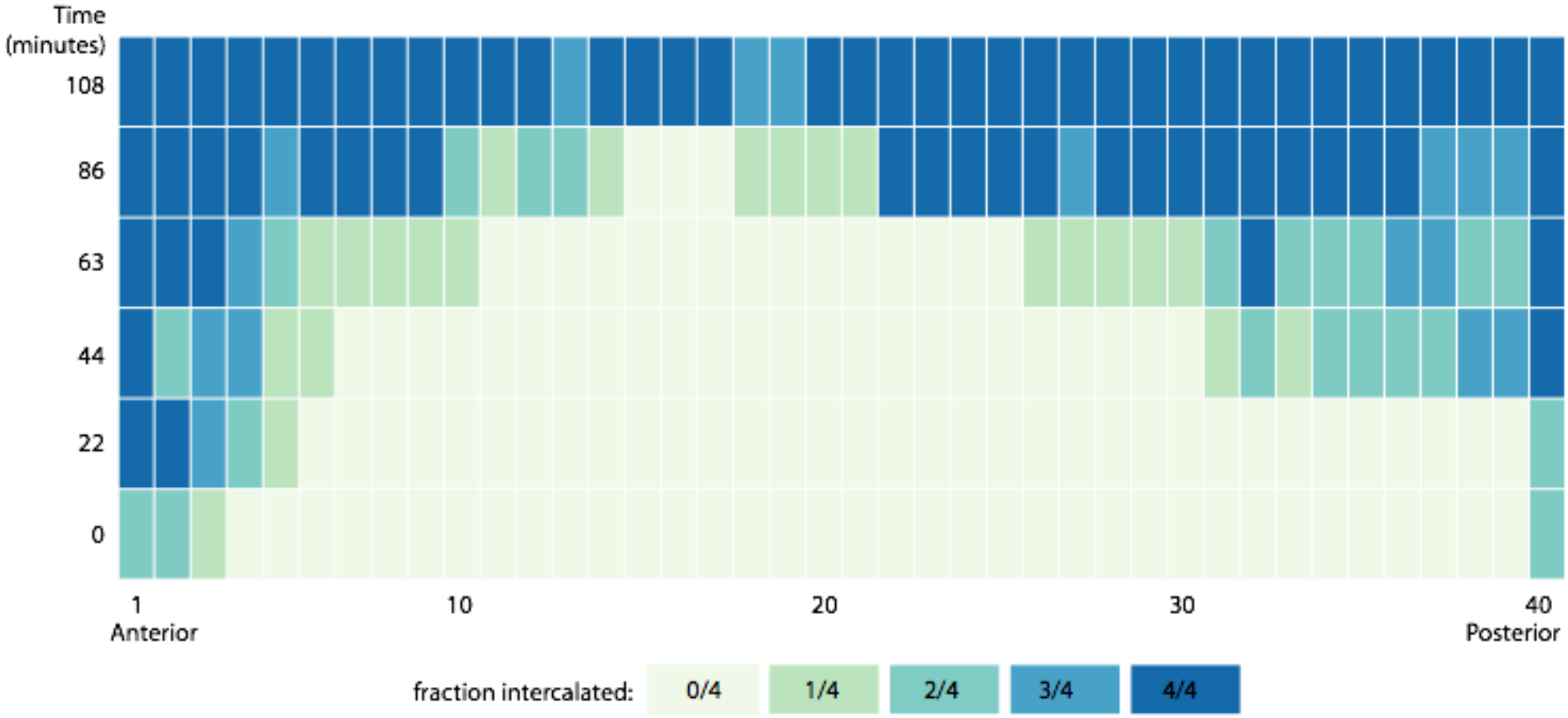
What is the basis of the tapered shape of the notochord?

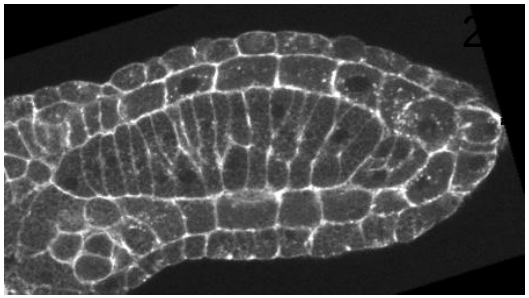


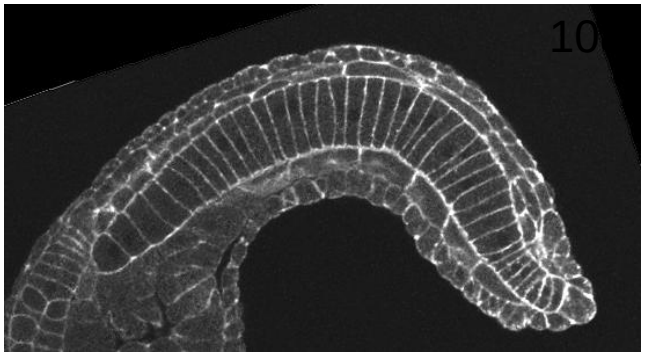

radius (μm)
time
14
12
10
8
6
4
2
0
10
20
30
40
150
100
50
0
AP position

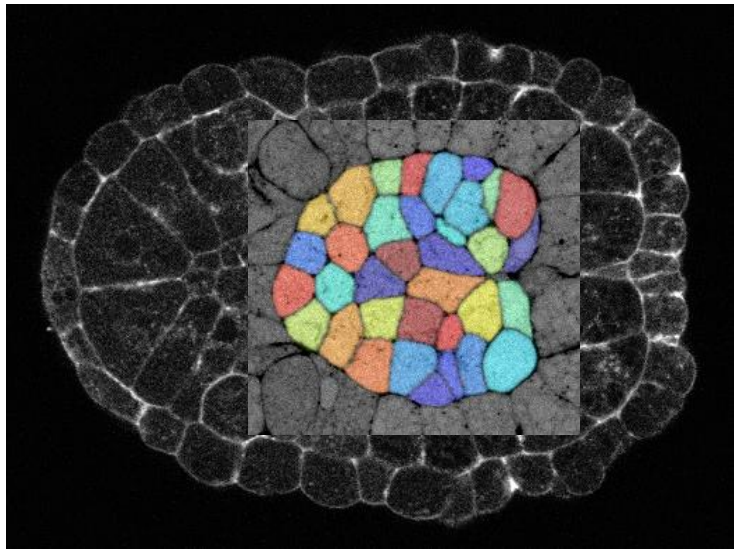


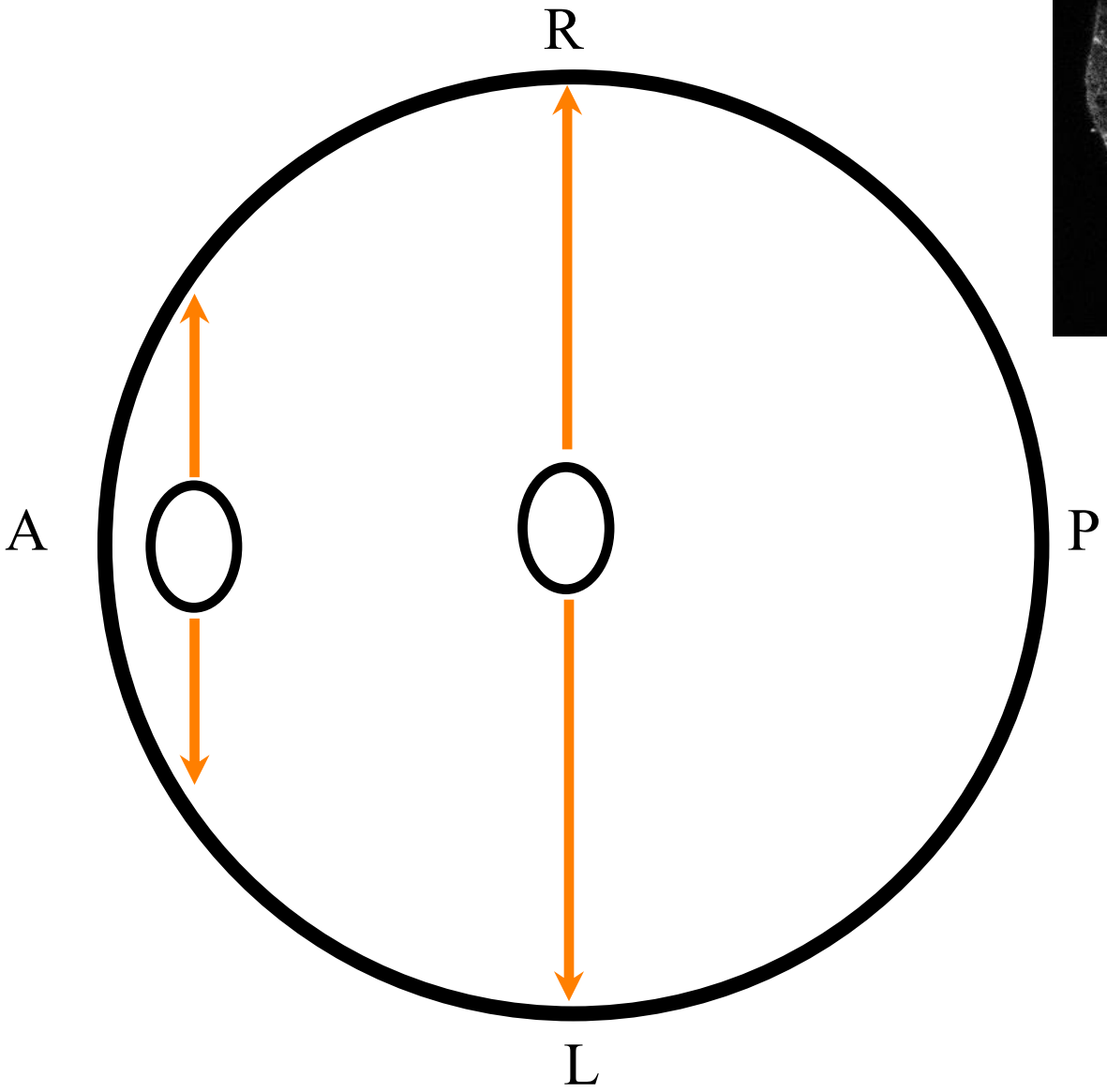
cells at the ends have a head-start in elongation

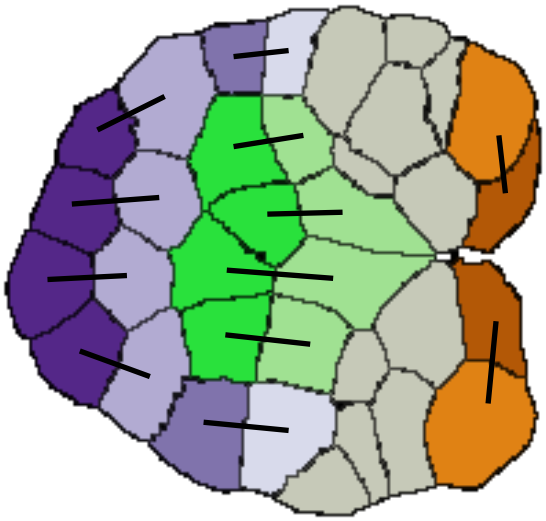




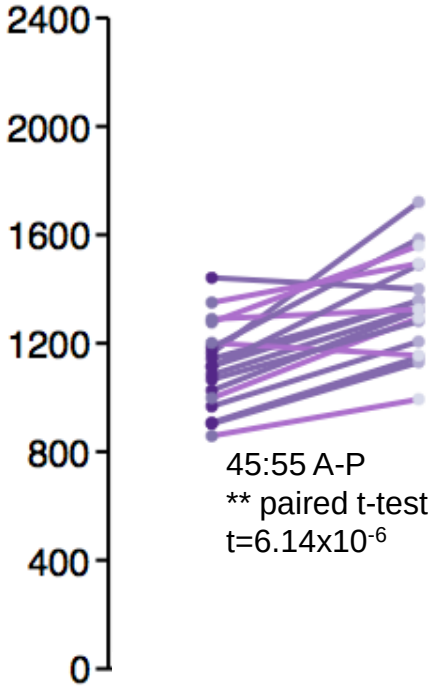





R
A
P
L



Notochord Cell Volume (μm^3)

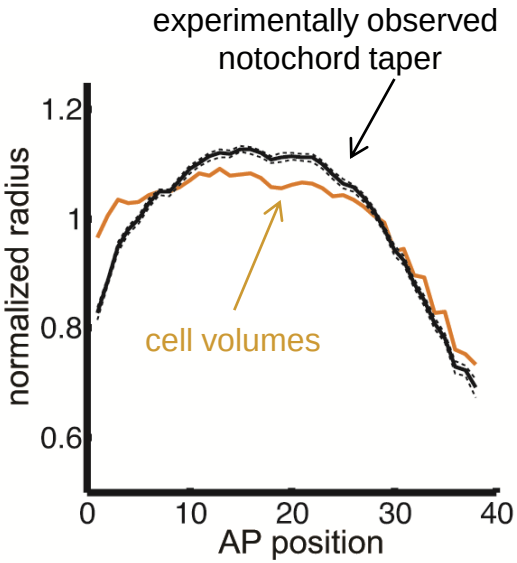


45:55 A-P
** paired t-test
$t=6.14x10^{-6}$

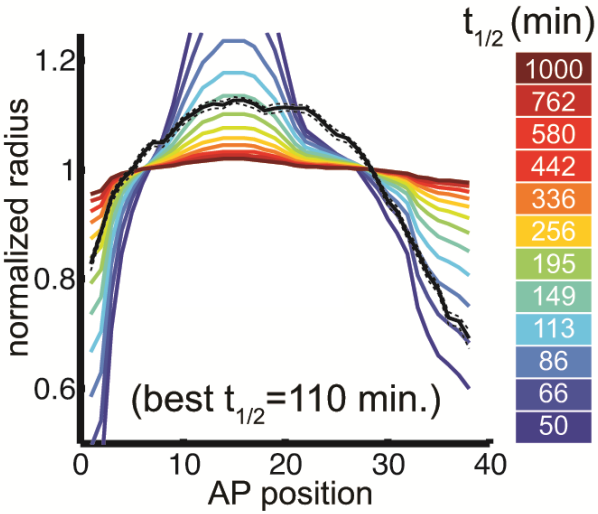
51:49 A-P

65:35 L-M
** paired t-test
t=0.008

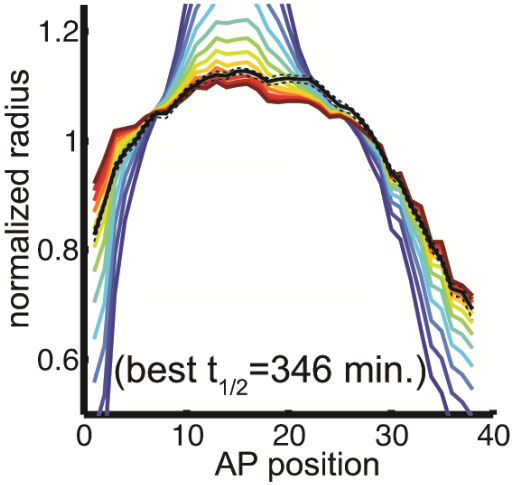
modeling notochord taper



cell volume
only-model



head start
only-model



head start
and volume
-model