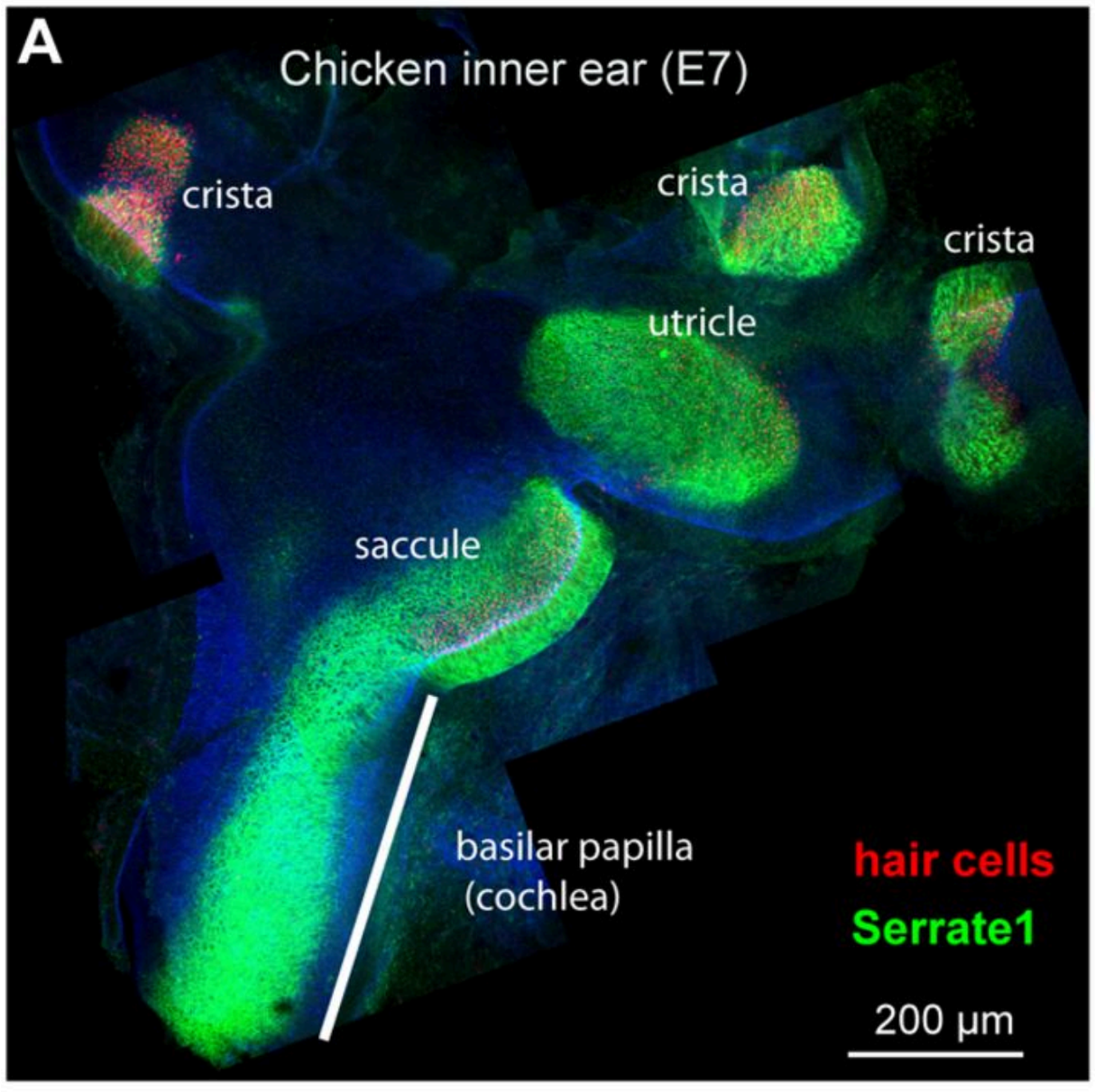
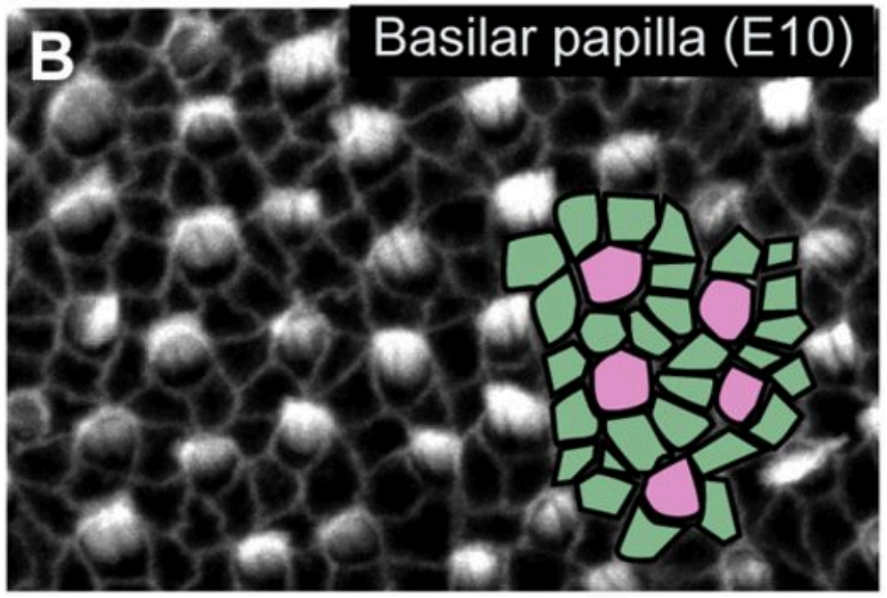
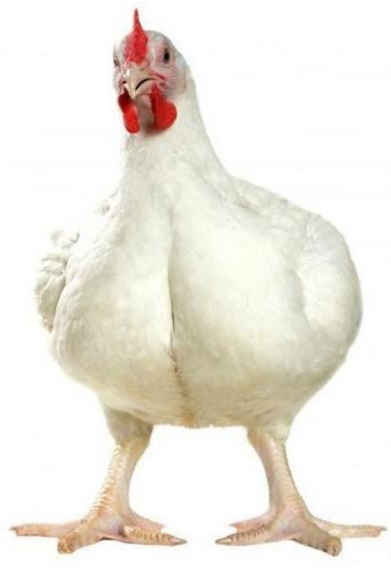
Big questions

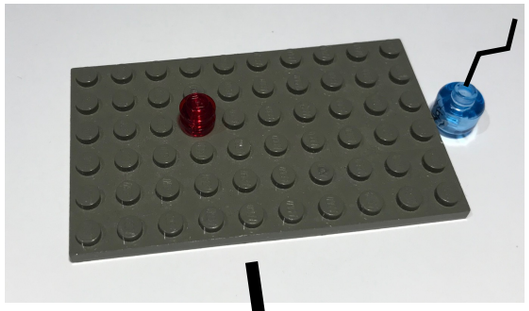




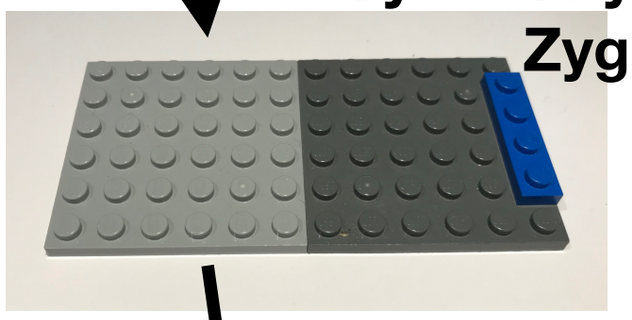


Chrysostomou et al,. 2012

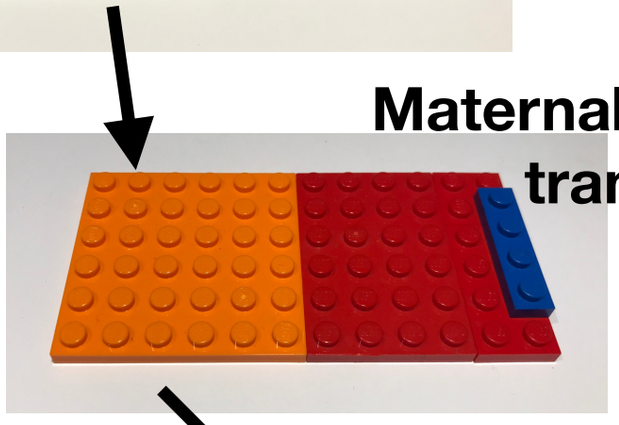
LEGO embryo - where are we? Fine-grained patterns



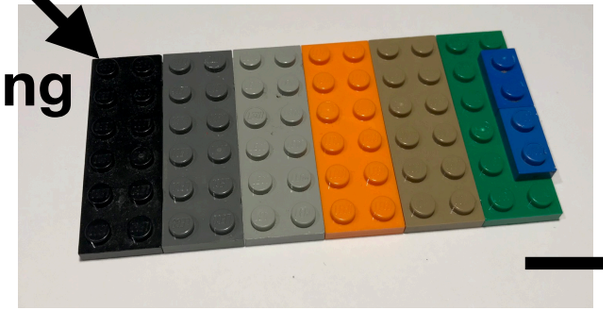
Fertilisation



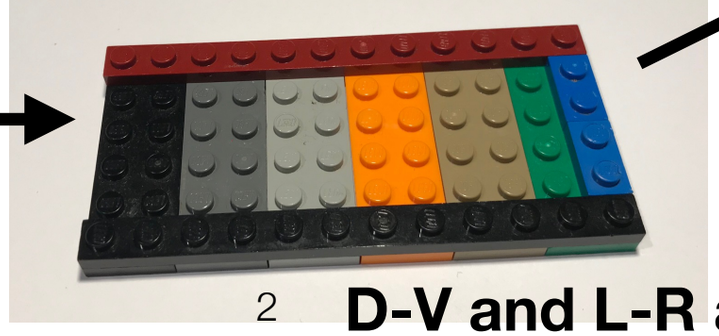
Symmetry breaking
Zygote



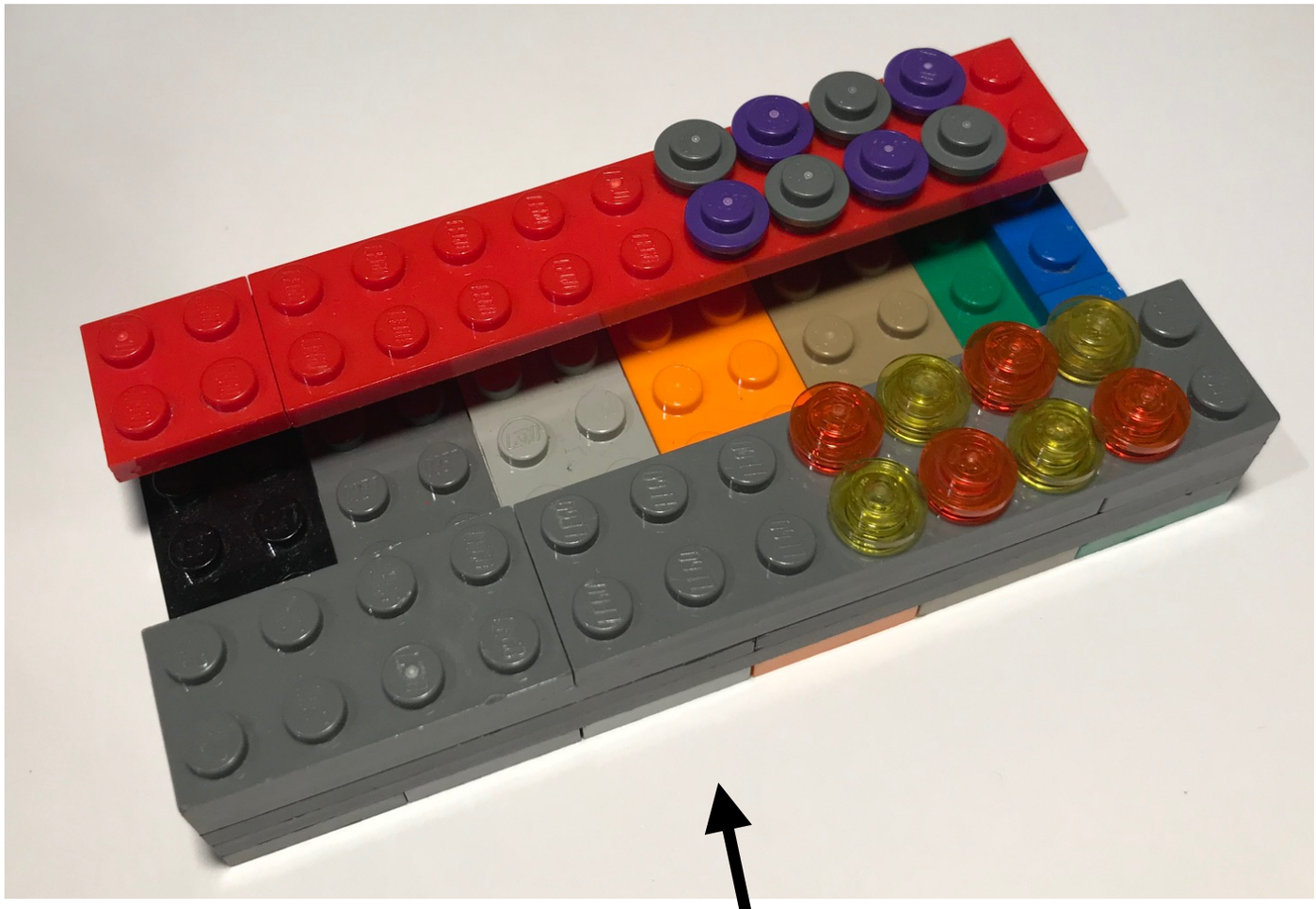
Maternal-to-Zygotic
transition



Early patterning
A-P axis



D-V and L-R axes

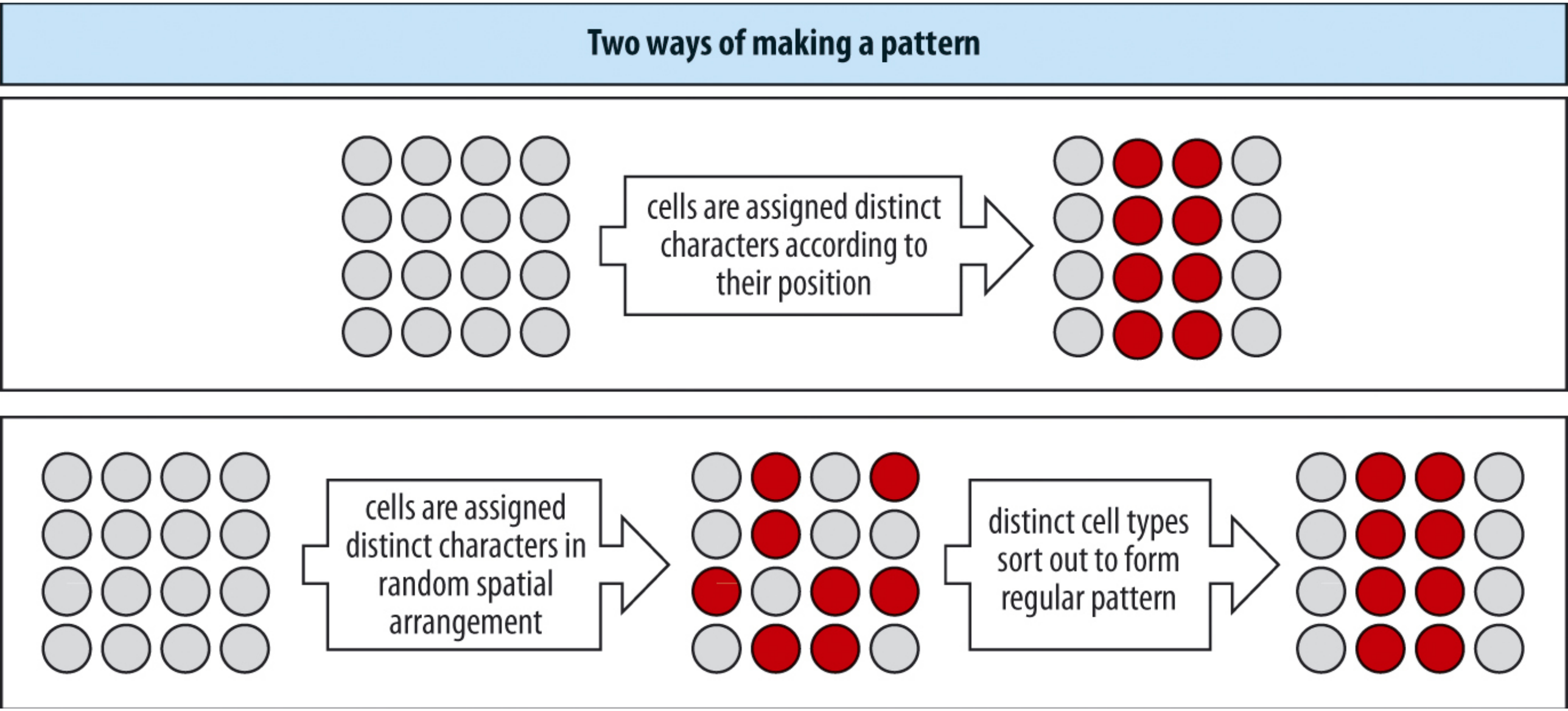


Morphogenesis:
Gastrulation and folding

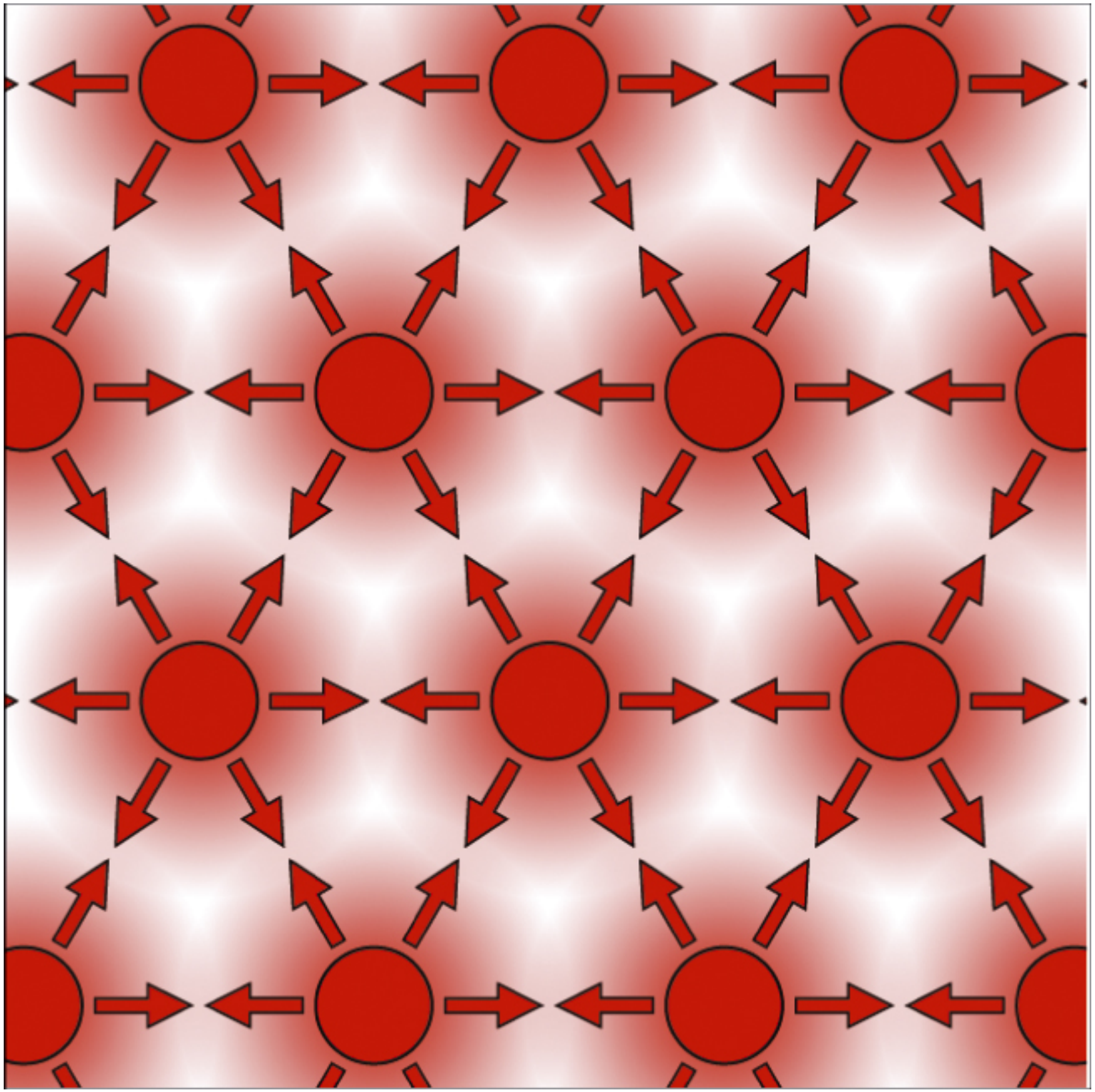
Today's menu

- **Lateral inhibition**
- Paracrine and Juxtacrine signalling
  - Receptor Tyrosine Kinase pathway
  - Delta Notch pathway
- Equivalence groups
- Worm vulva
- **Neurogenesis in insects and vertebrates**
- Pulling forces in Delta-Notch signalling

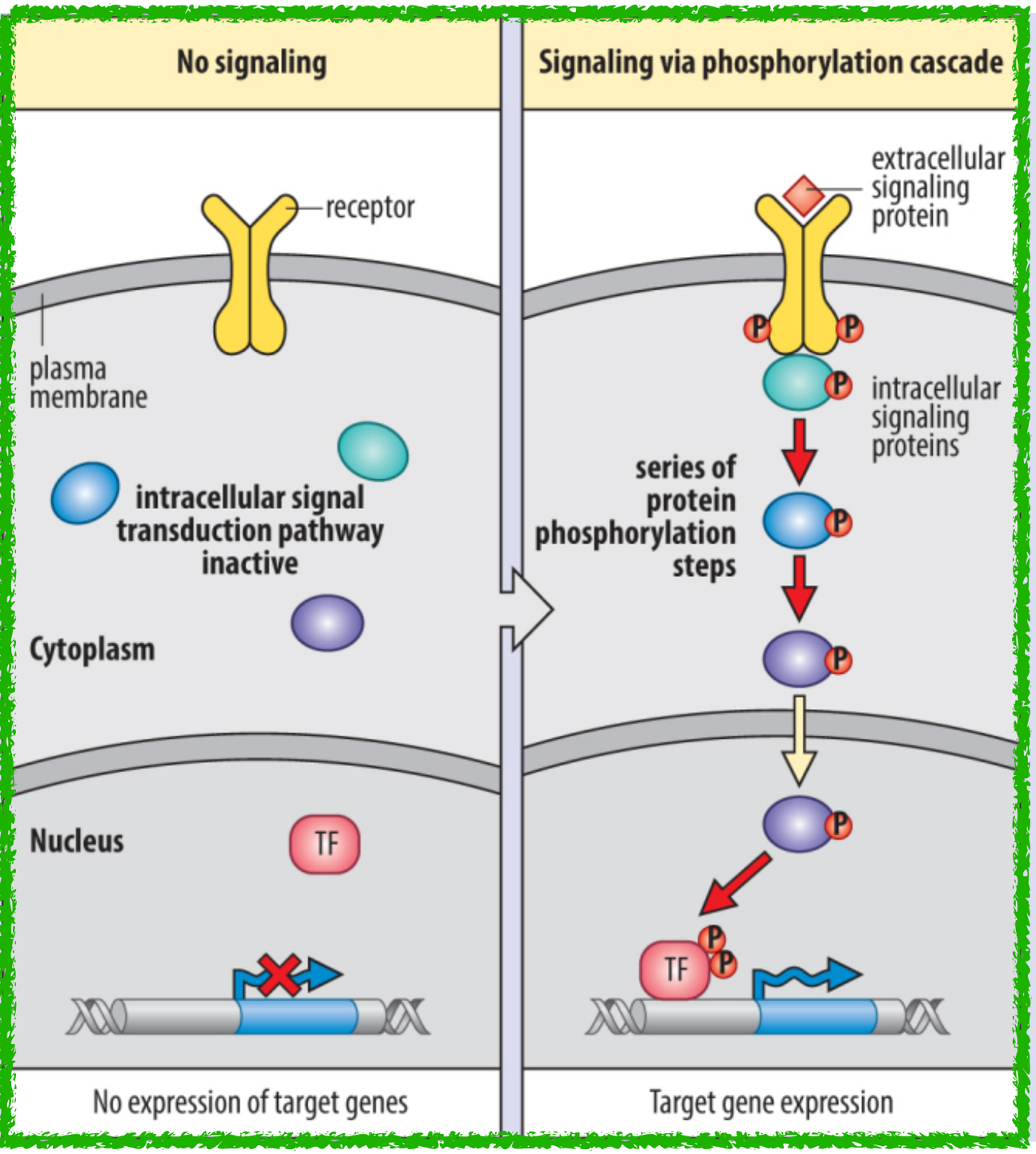
Position first, or position second?



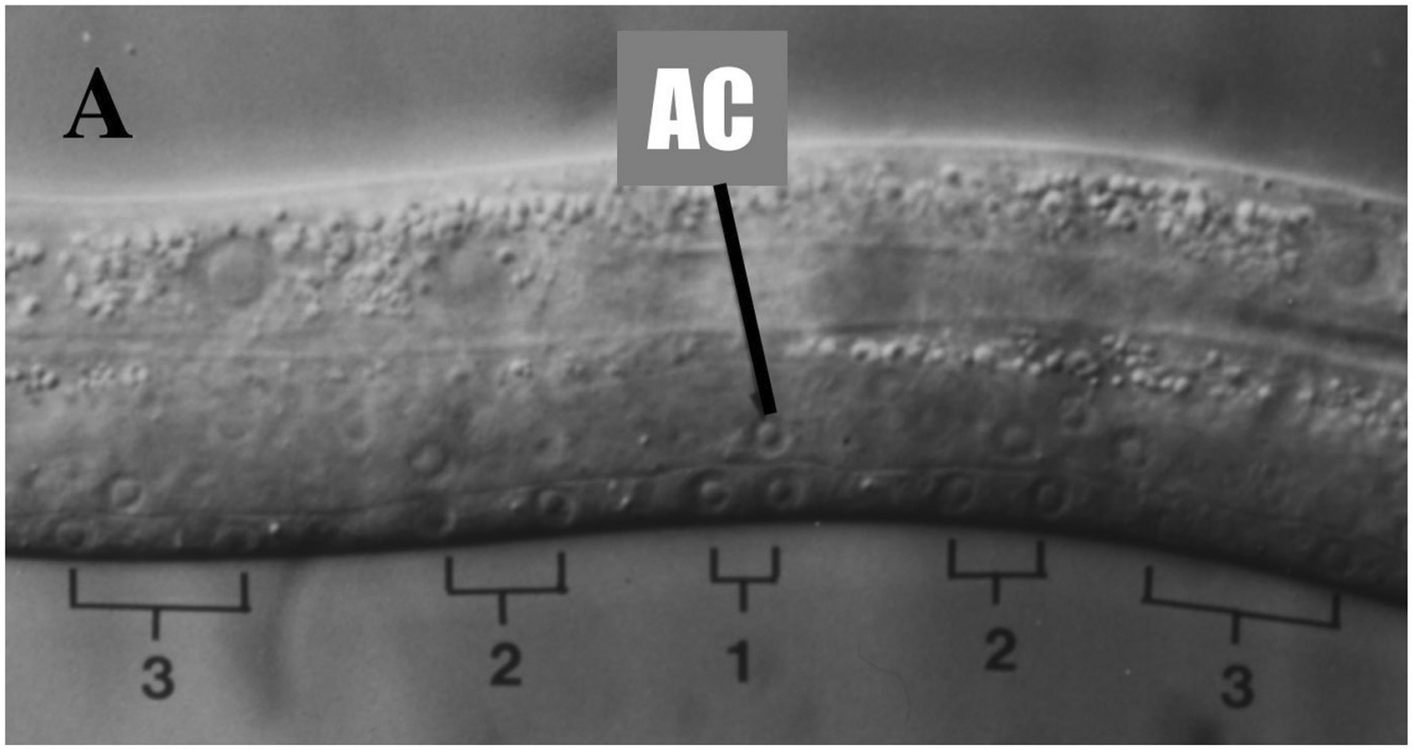
Third option: Lateral inhibition - positional “negotiation”



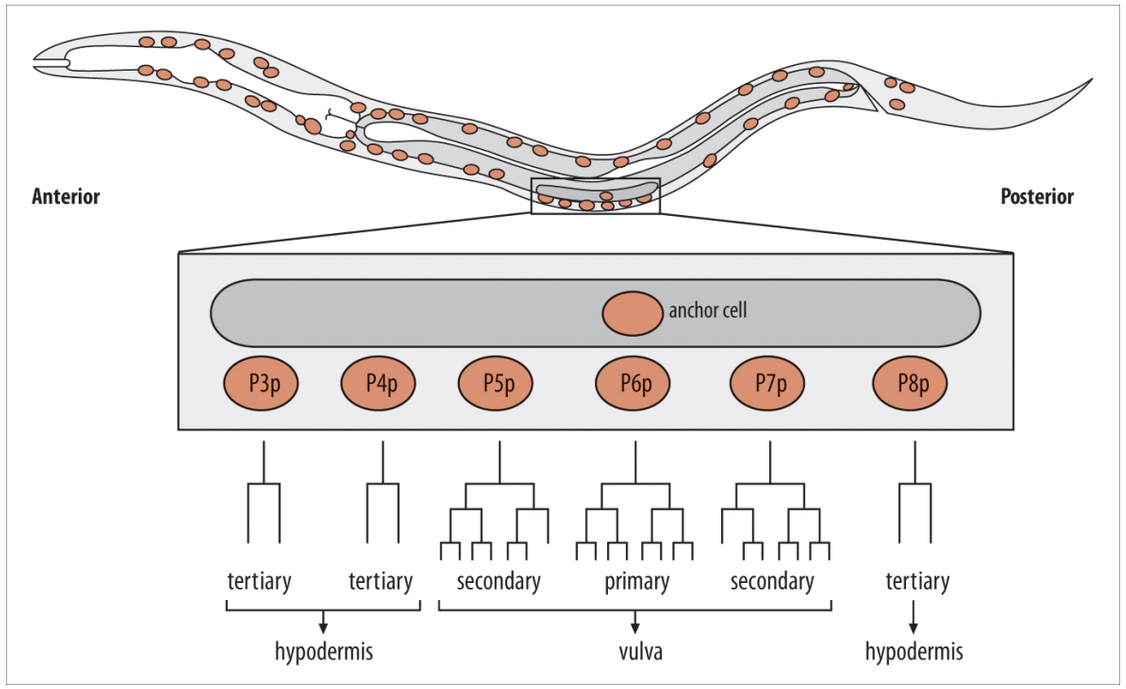
Signal transduction and intracellular pathways

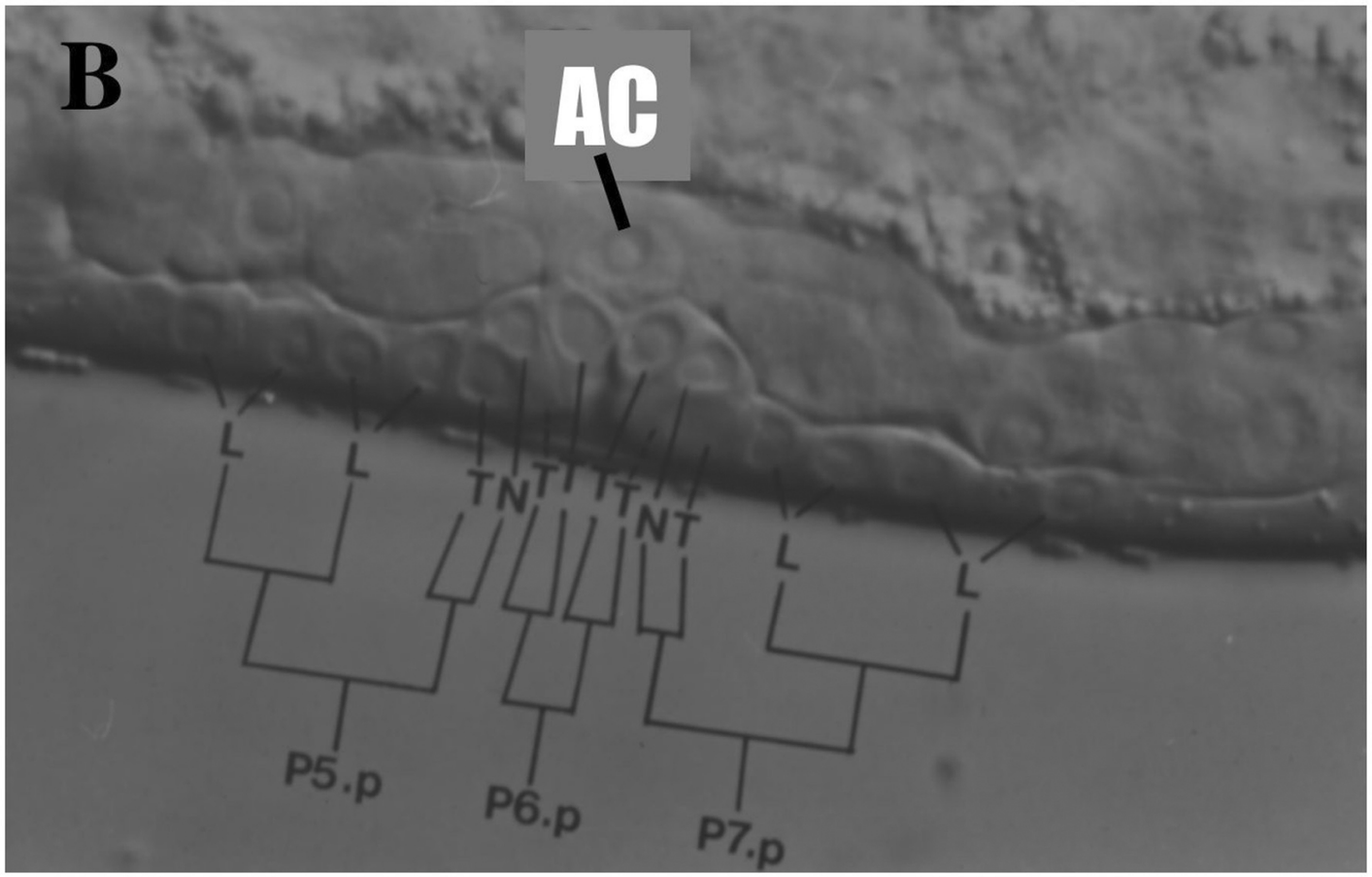


Development of the vulva in *C. elegans*



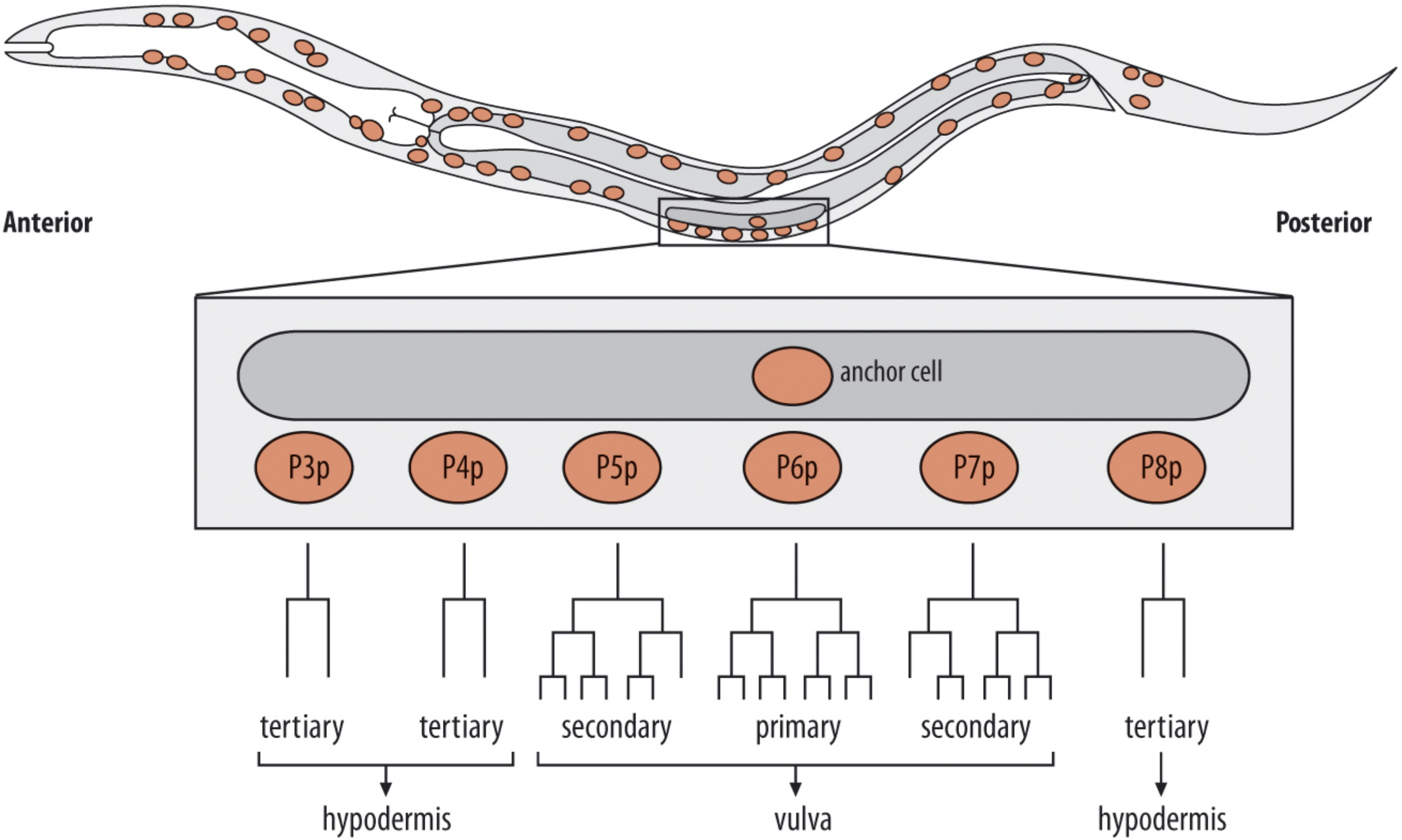
Early L3



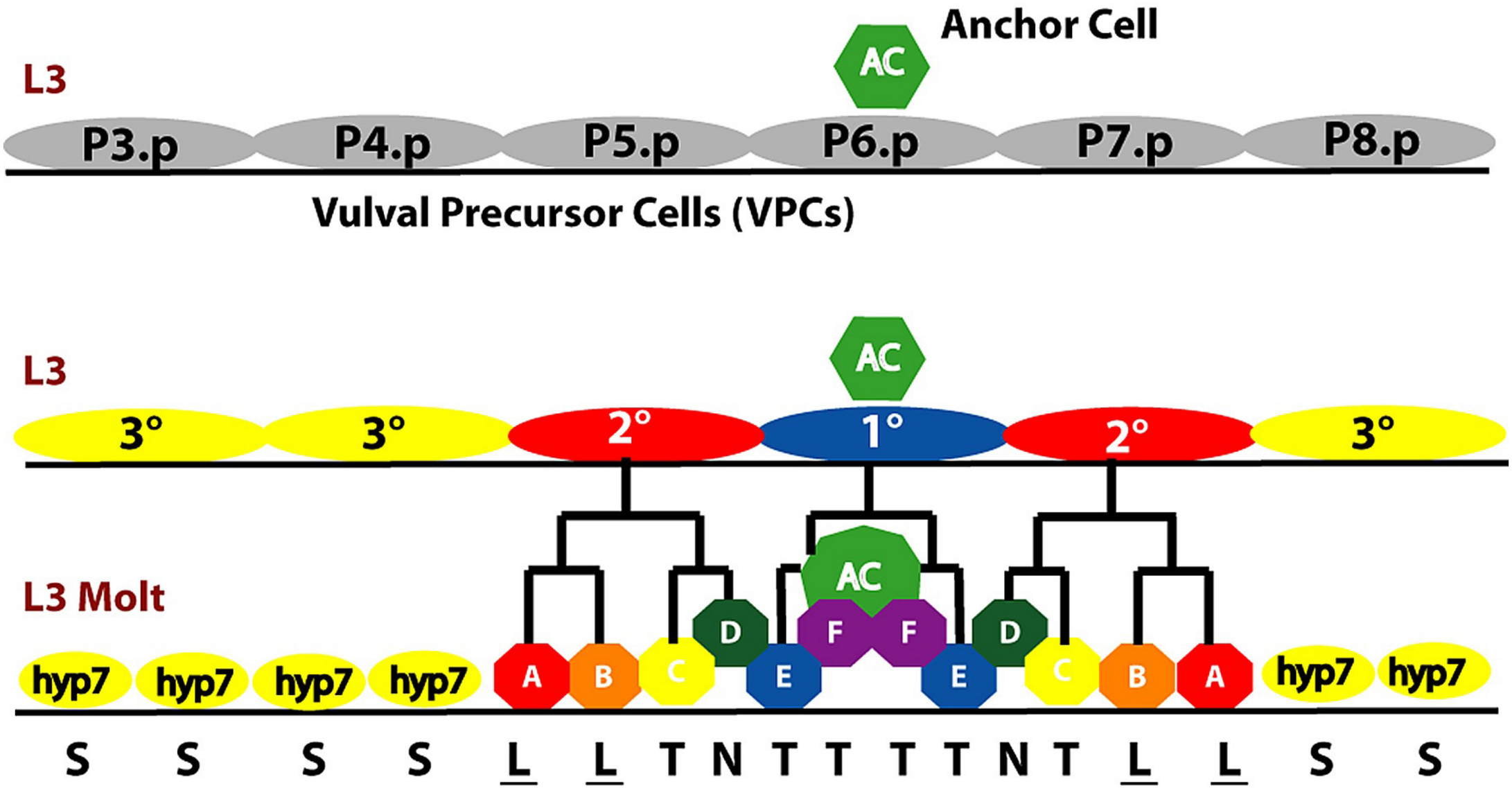


Late L3

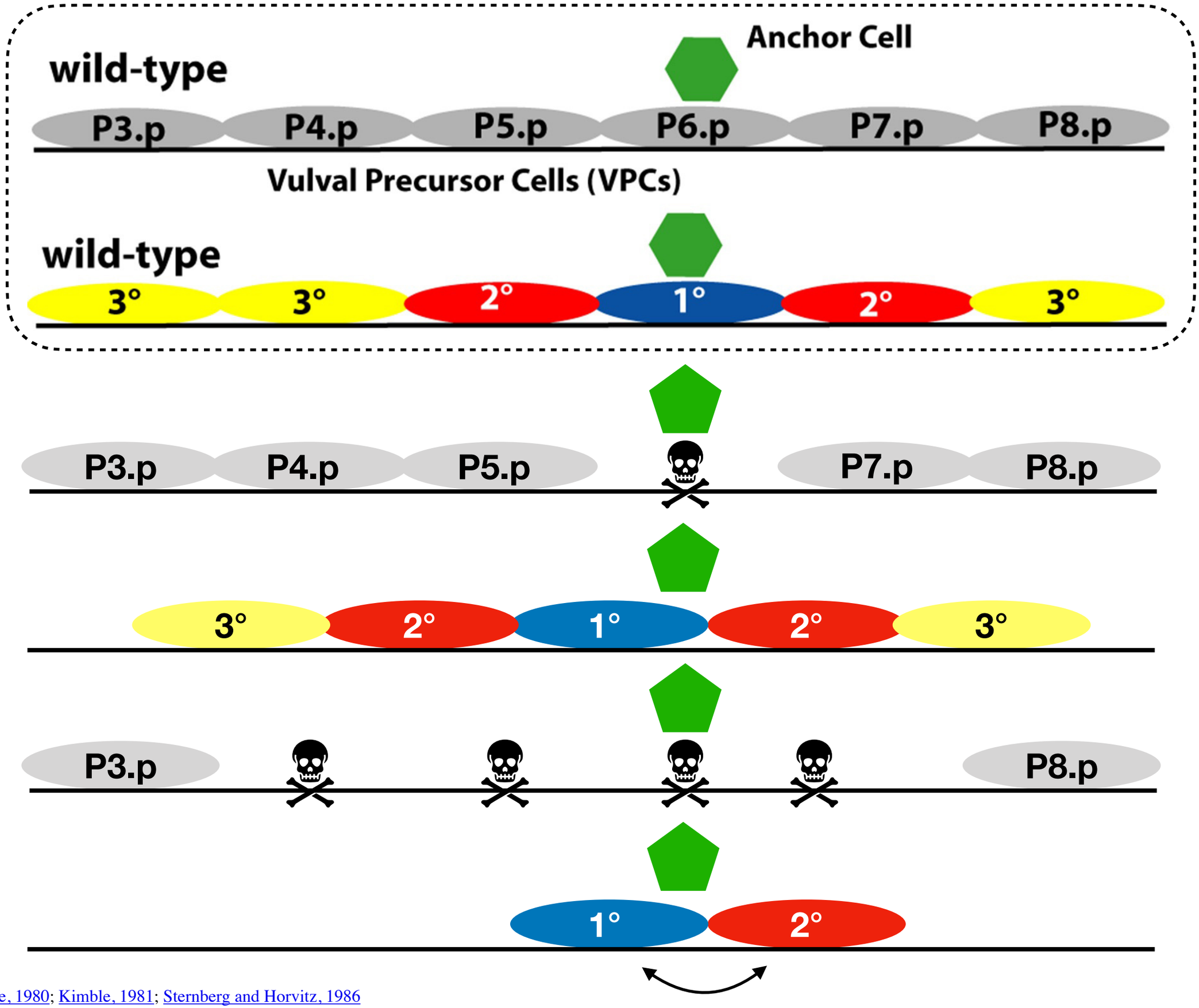
Development of the vulva in *C. elegans*



Development of the vulva in *C. elegans*

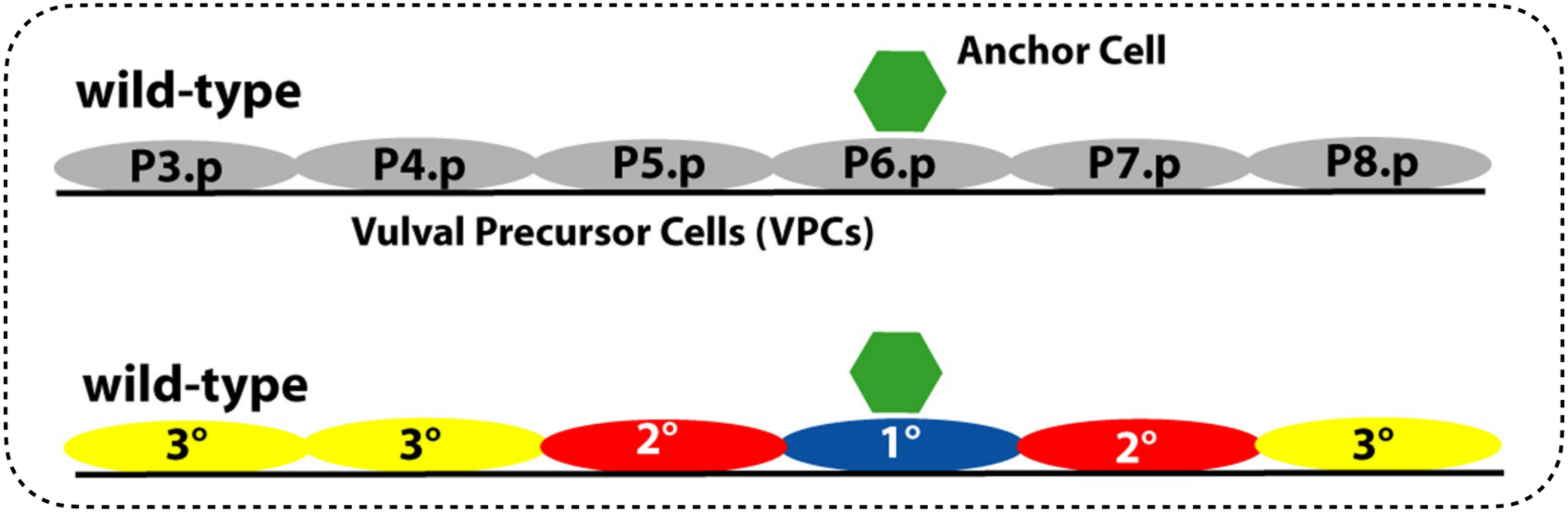


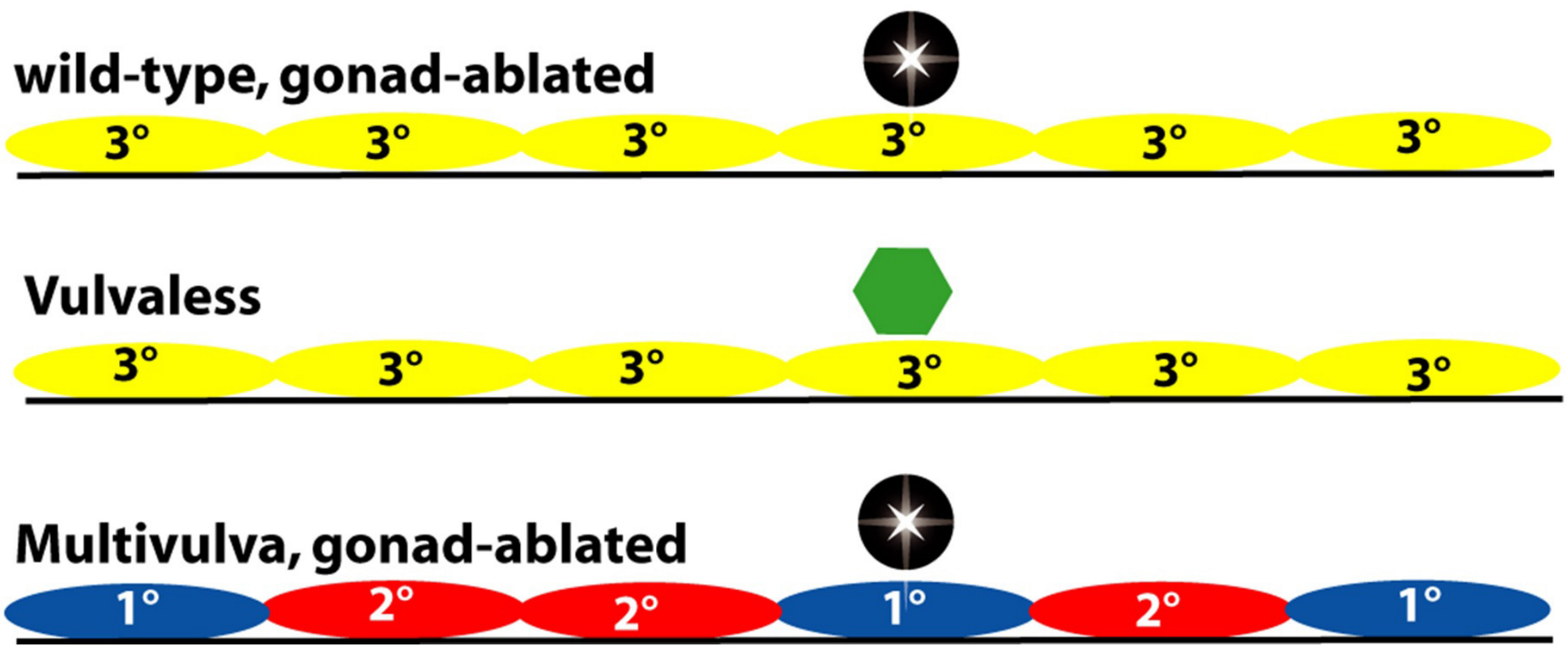
Equivalence group - ablation of Pn.p cells



Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1986

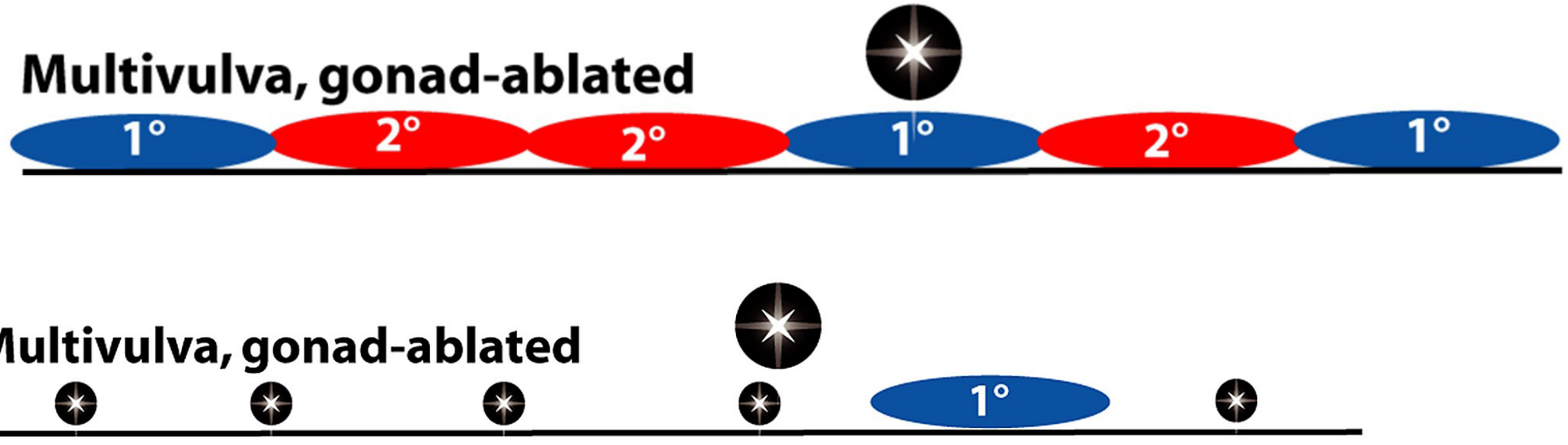
Equivalence group - anchor cell and mutant classes



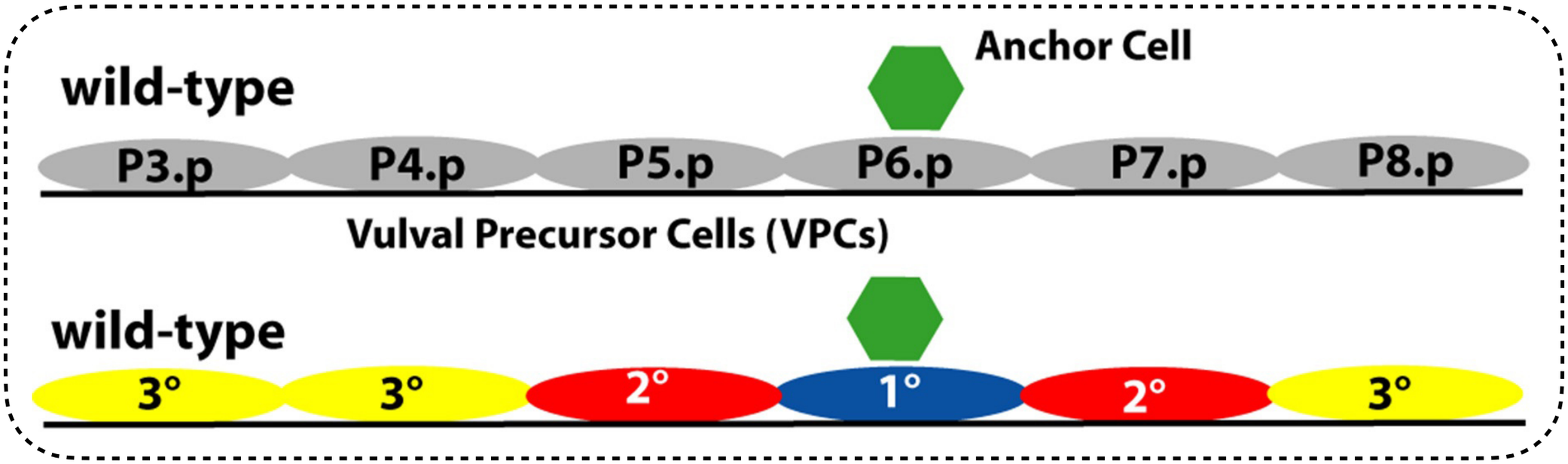


lin-15 reveals a global inhibitory signal

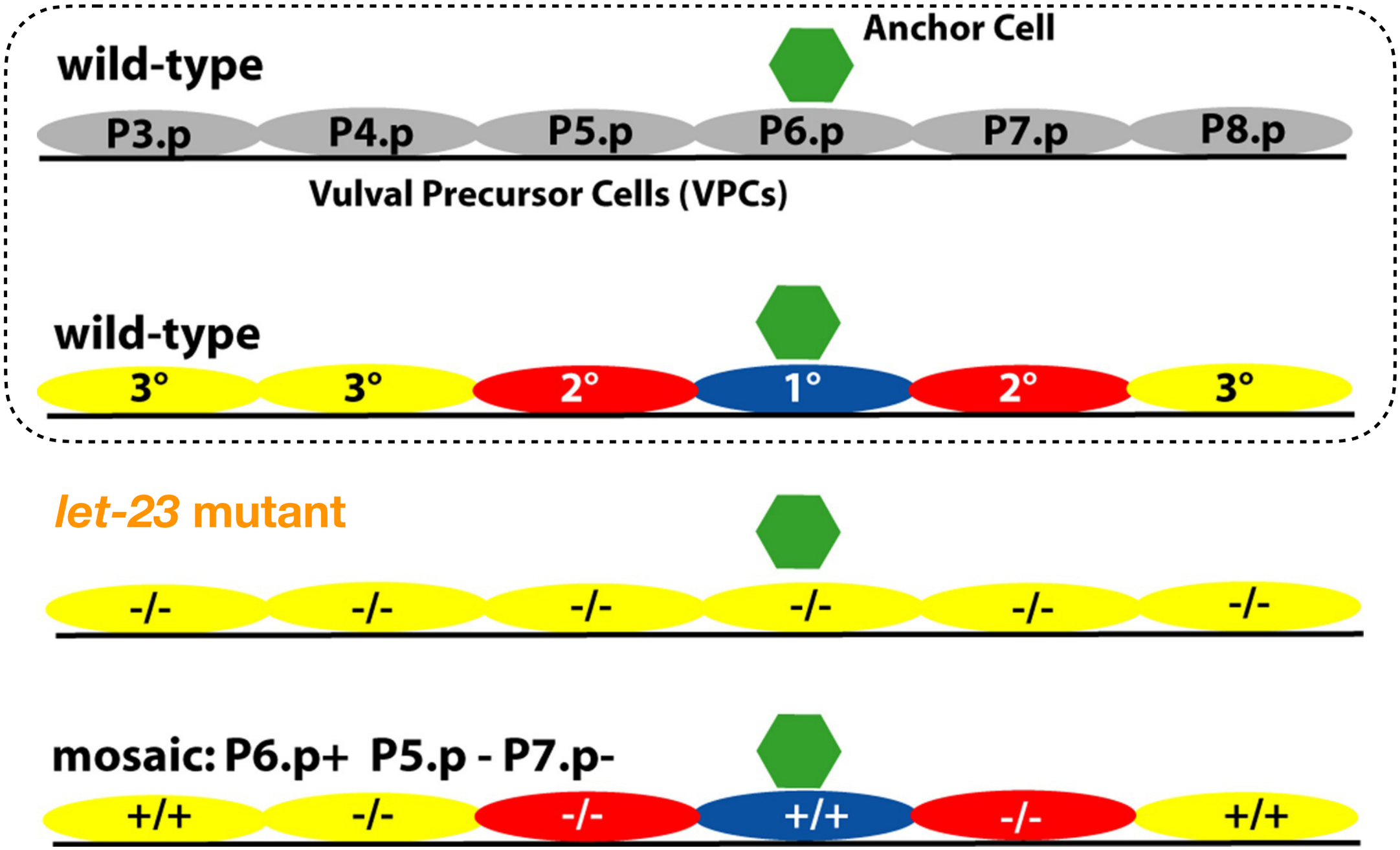
Mutant
lin-15



lin-3 and induction



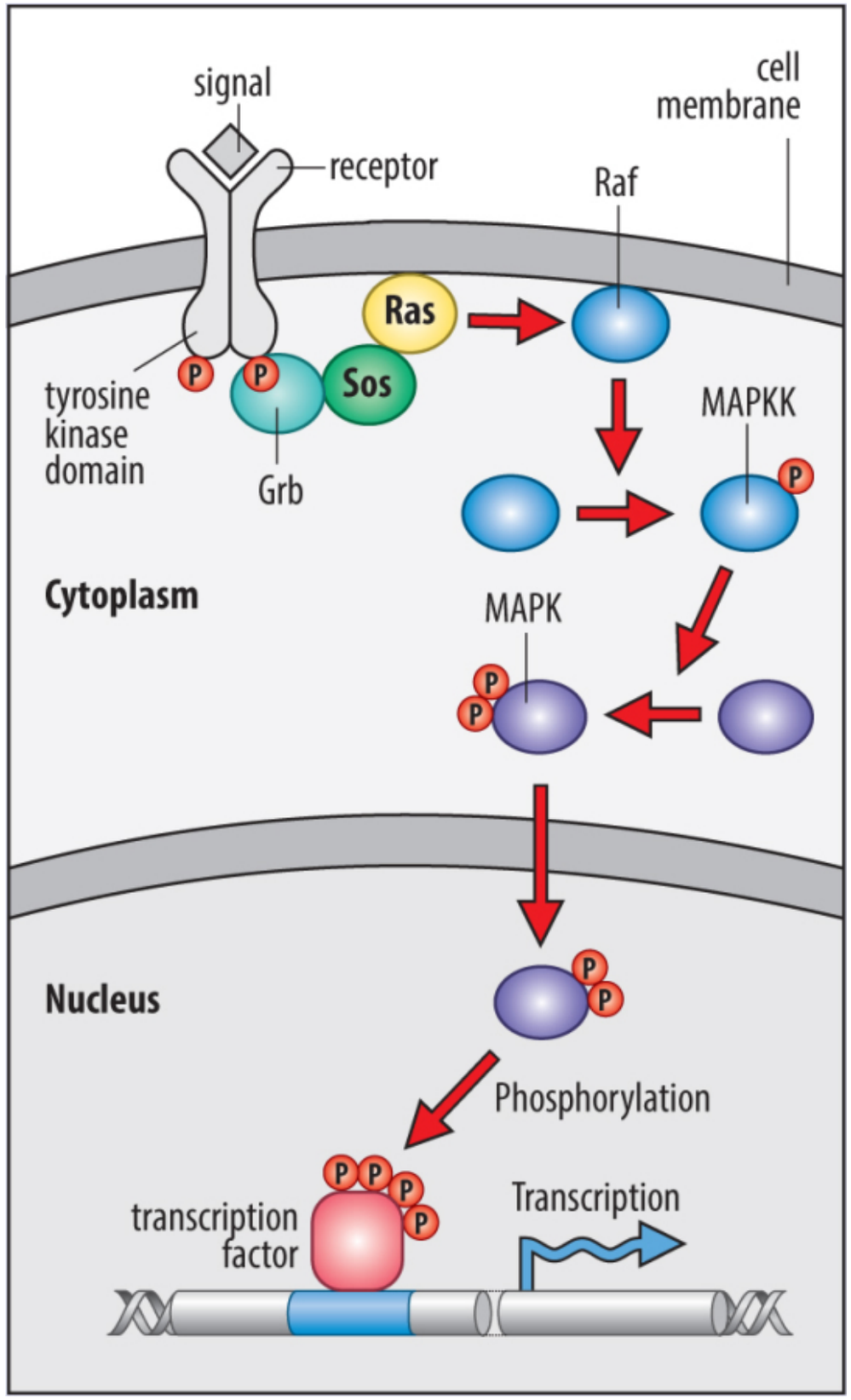
Let-23 and induction



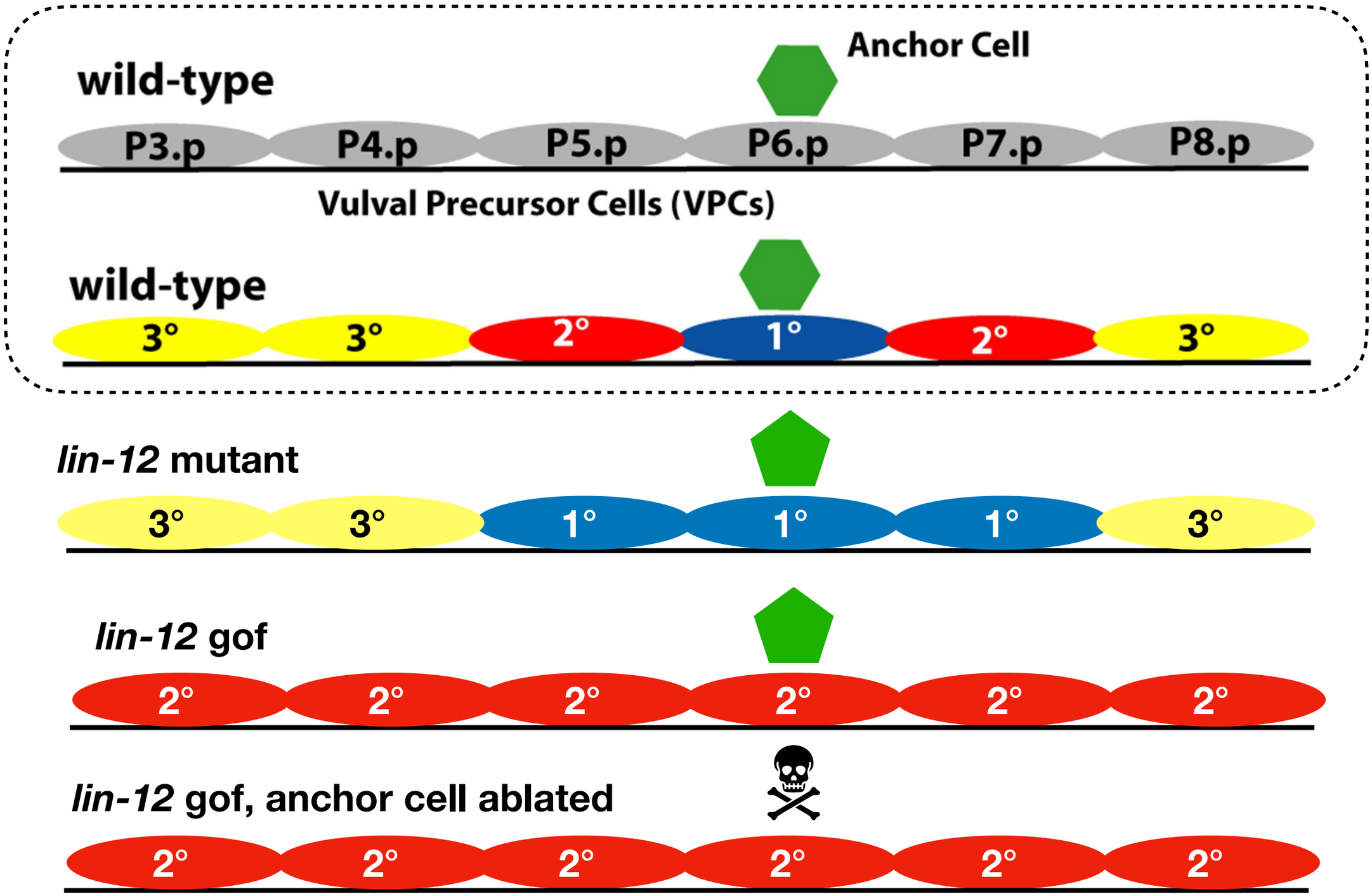
Individual puzzle

The Receptor Tyrosine Kinase signalling pathway

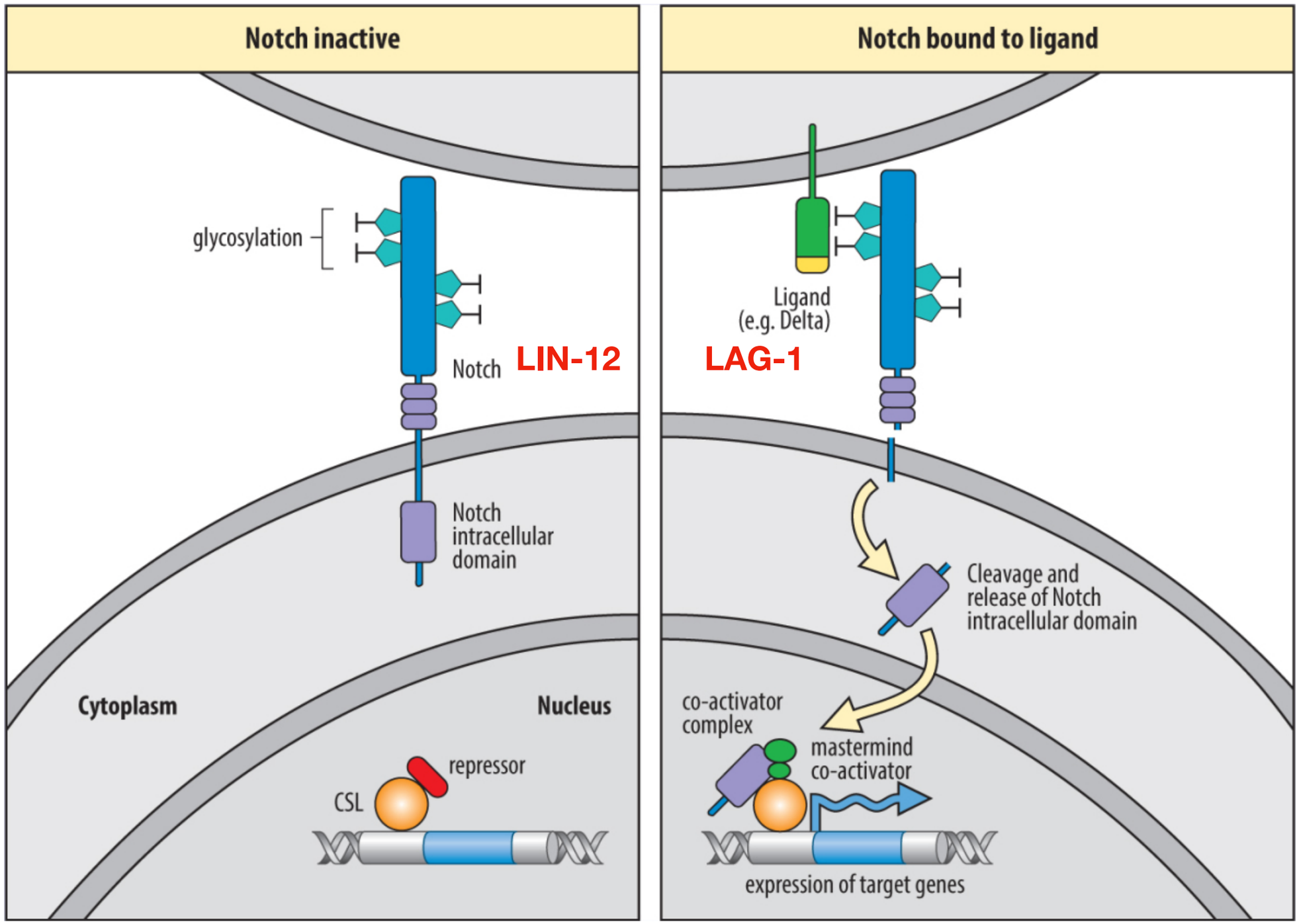
Also: EGF, FGF, NGF, c-Kit…



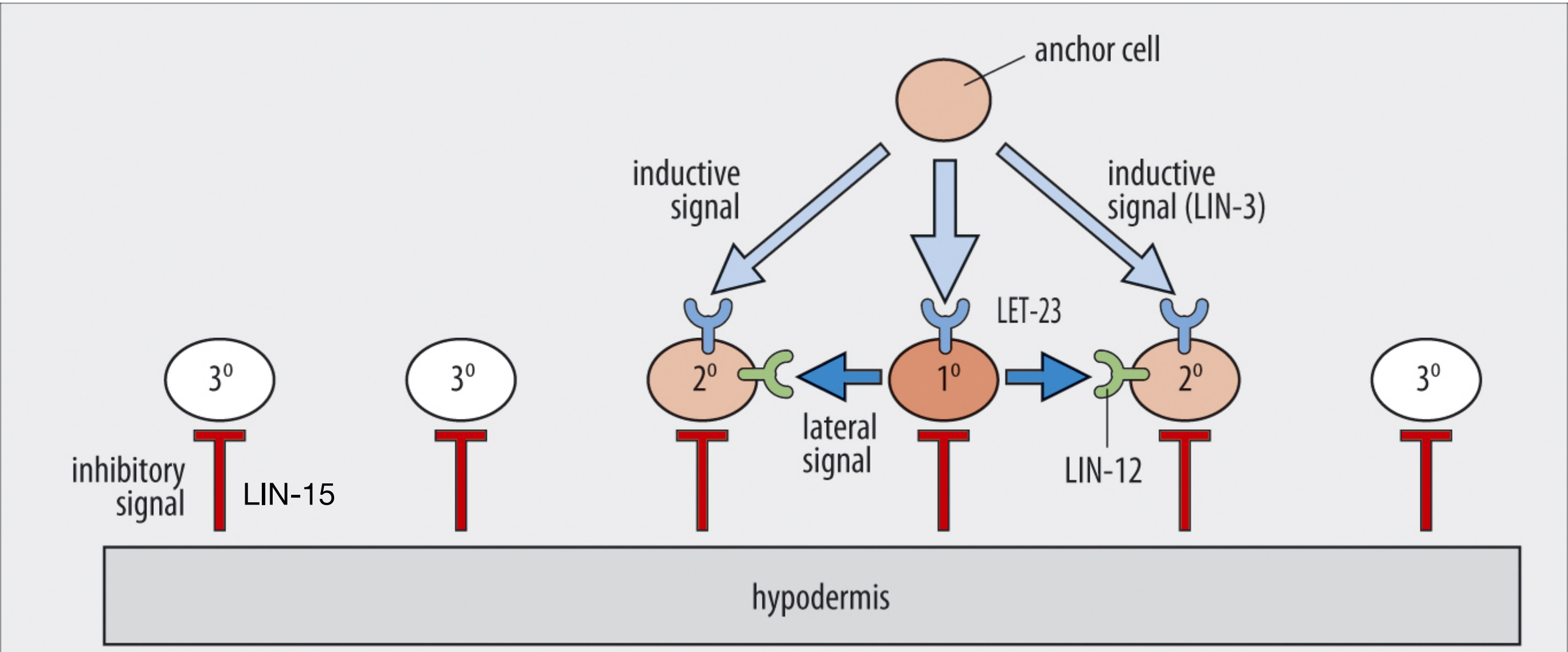
lin-12 in the Pn.p cells



The Notch signalling pathway



Inhibition, induction, and lateral inhibition



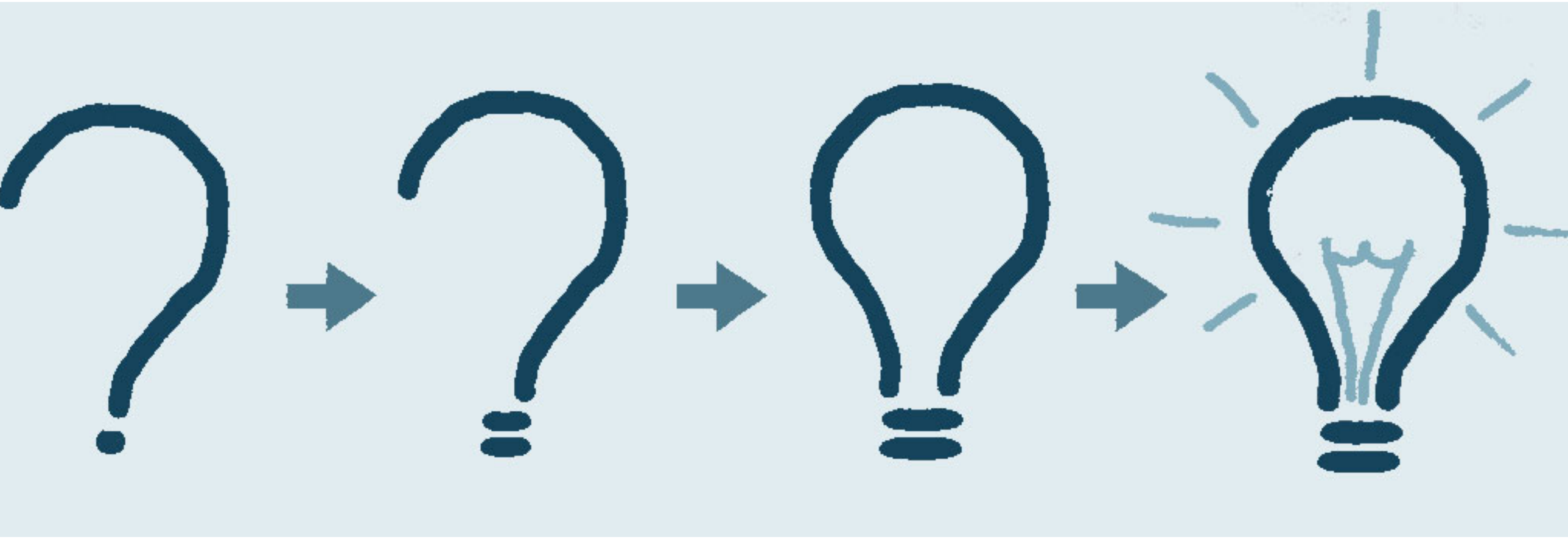
Summary

- **Pattern with a single cell resolution**
- Short range induction - paracrine
- Cell-cell inhibition - juxtacrine

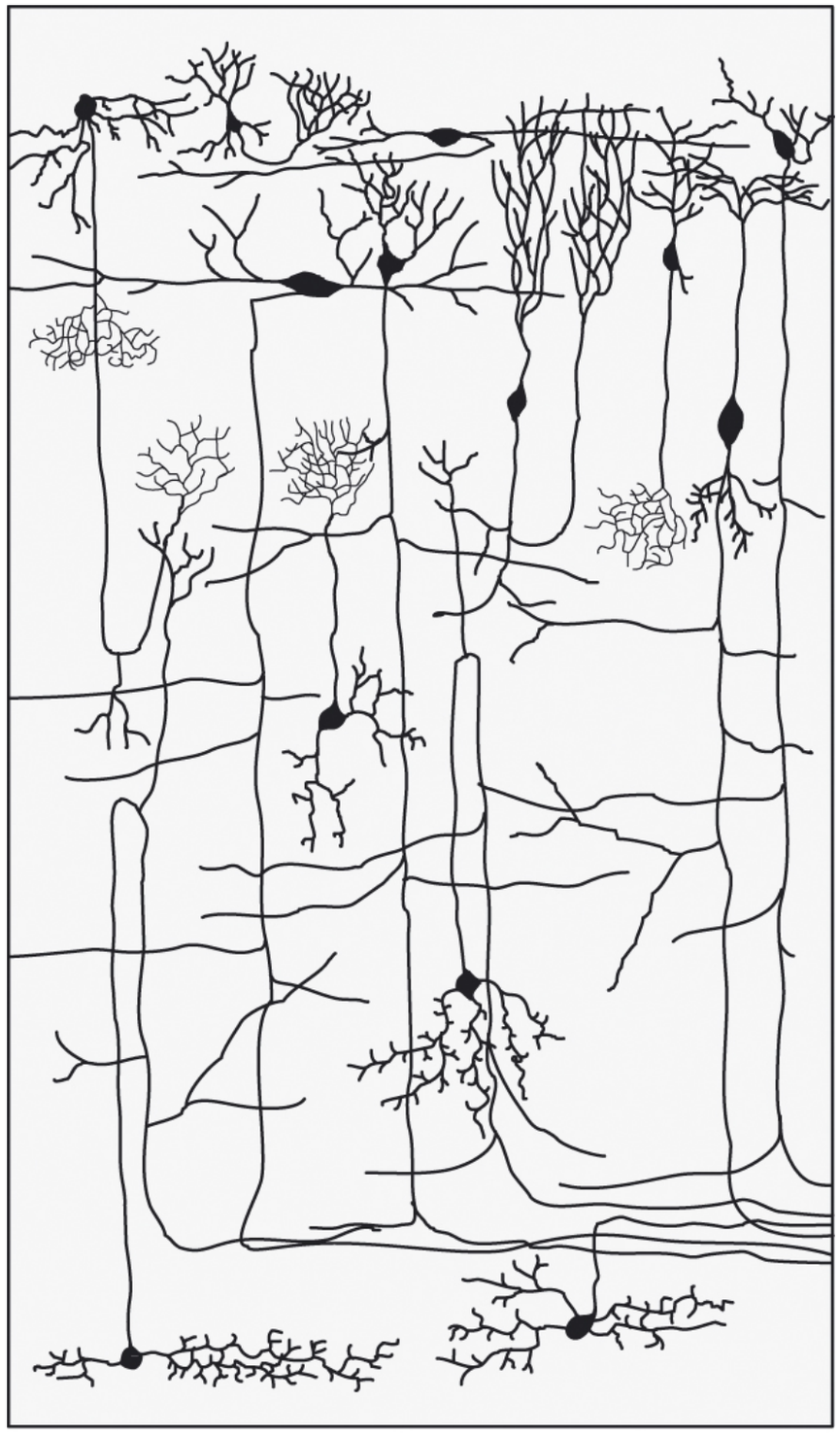
- Pattern set by interplay between global inhibition, local induction and lateral inhibition

- Equivalence group (commitment group) ablation and genetics

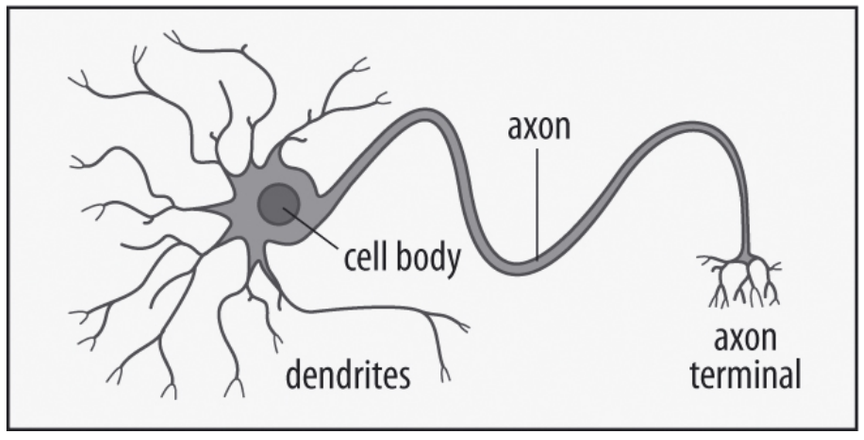
Questions?



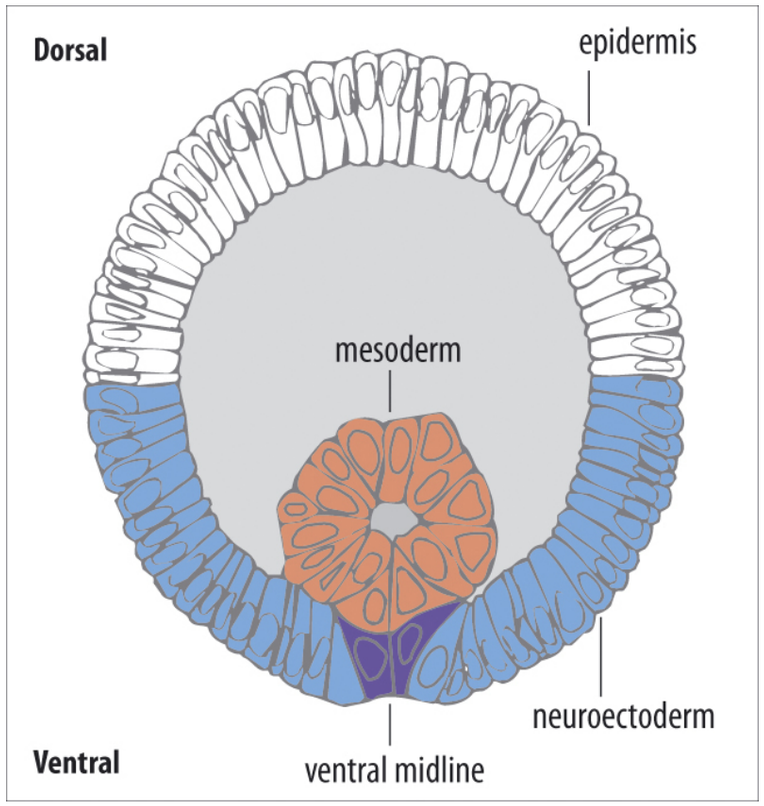
Take a break

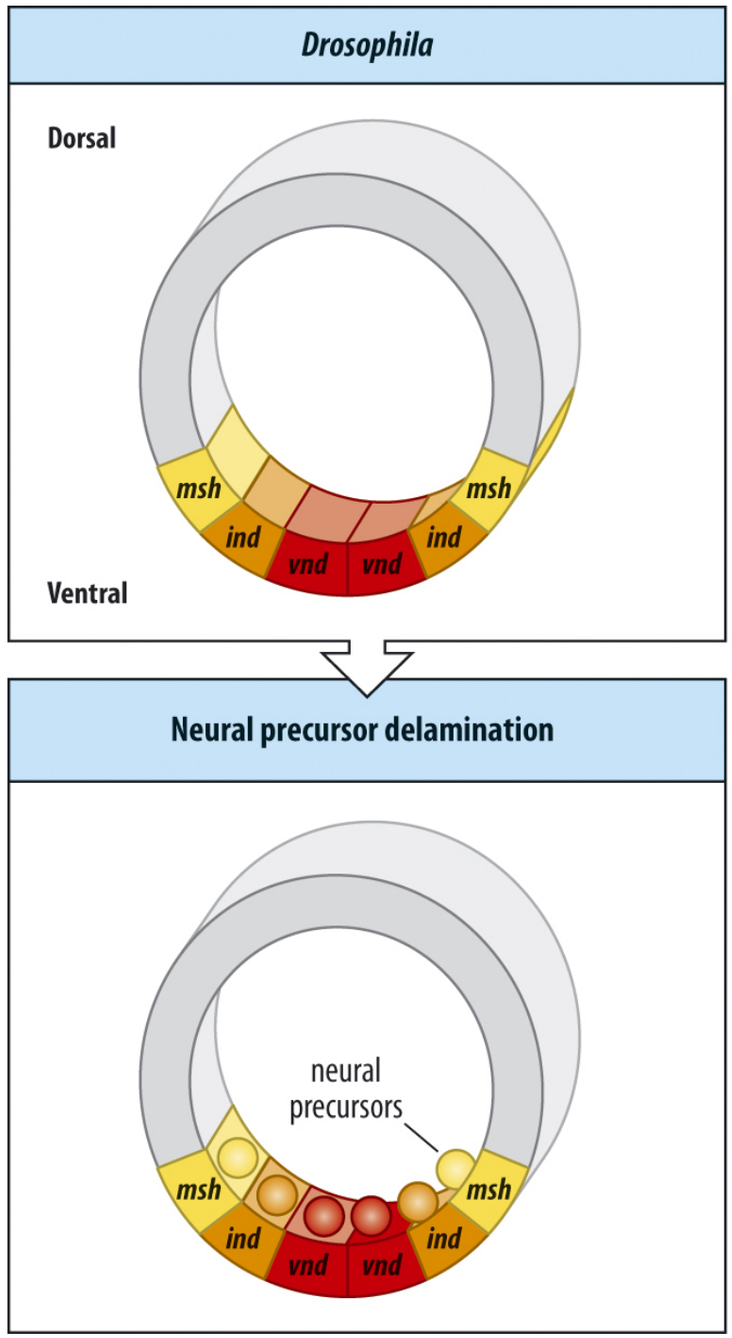


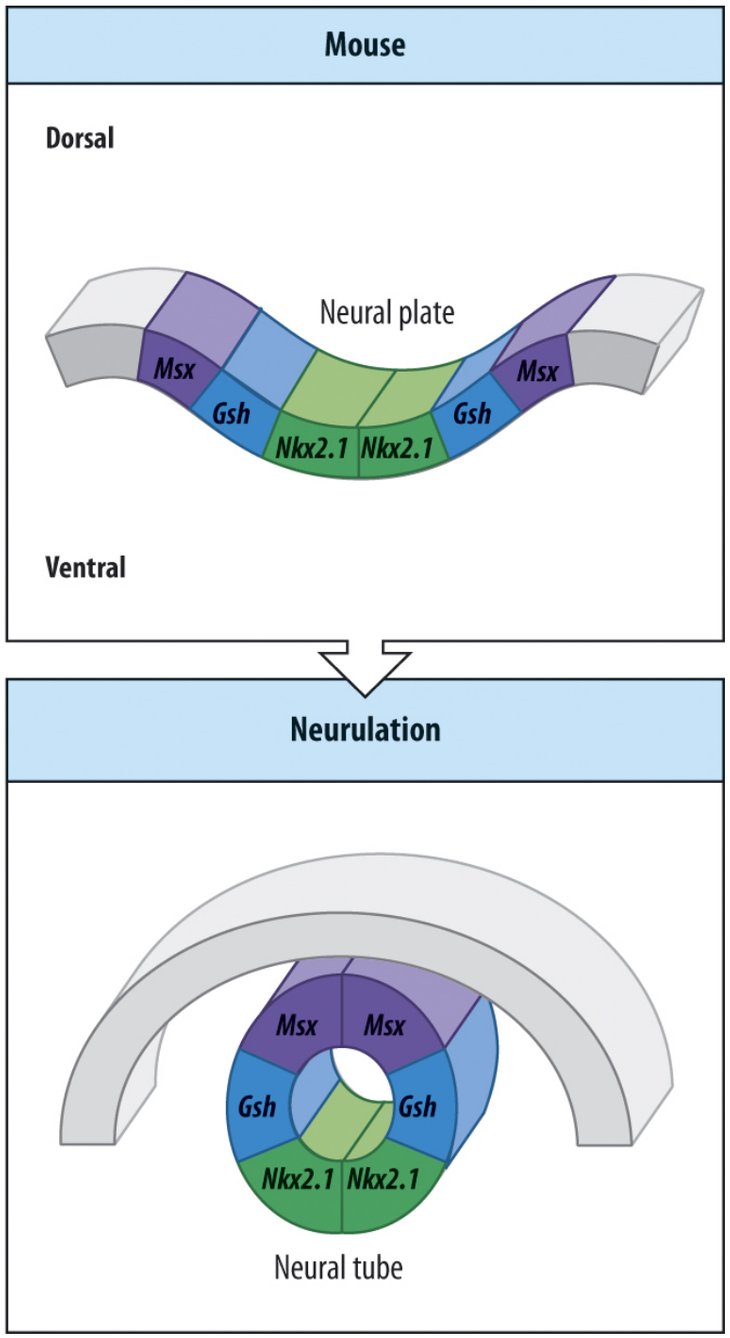
Neurogenesis
Neuroblasts → neurons



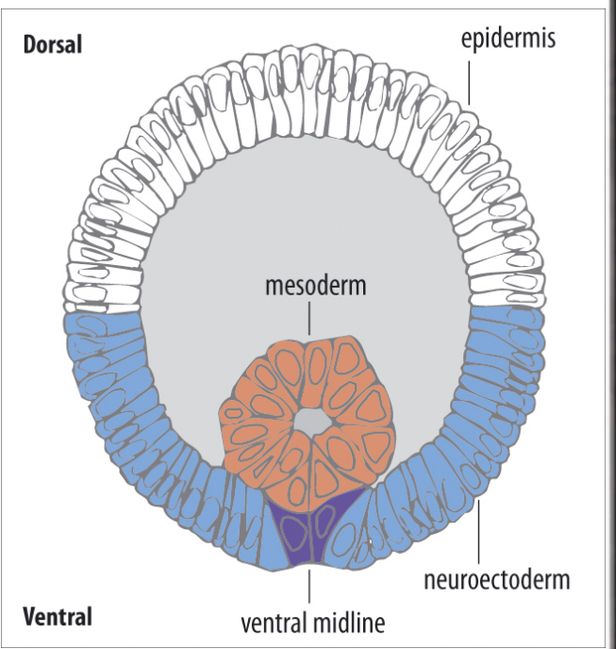
Neuroectoderm makes neural precursors (neuroblasts, 2D epithelial sheet)



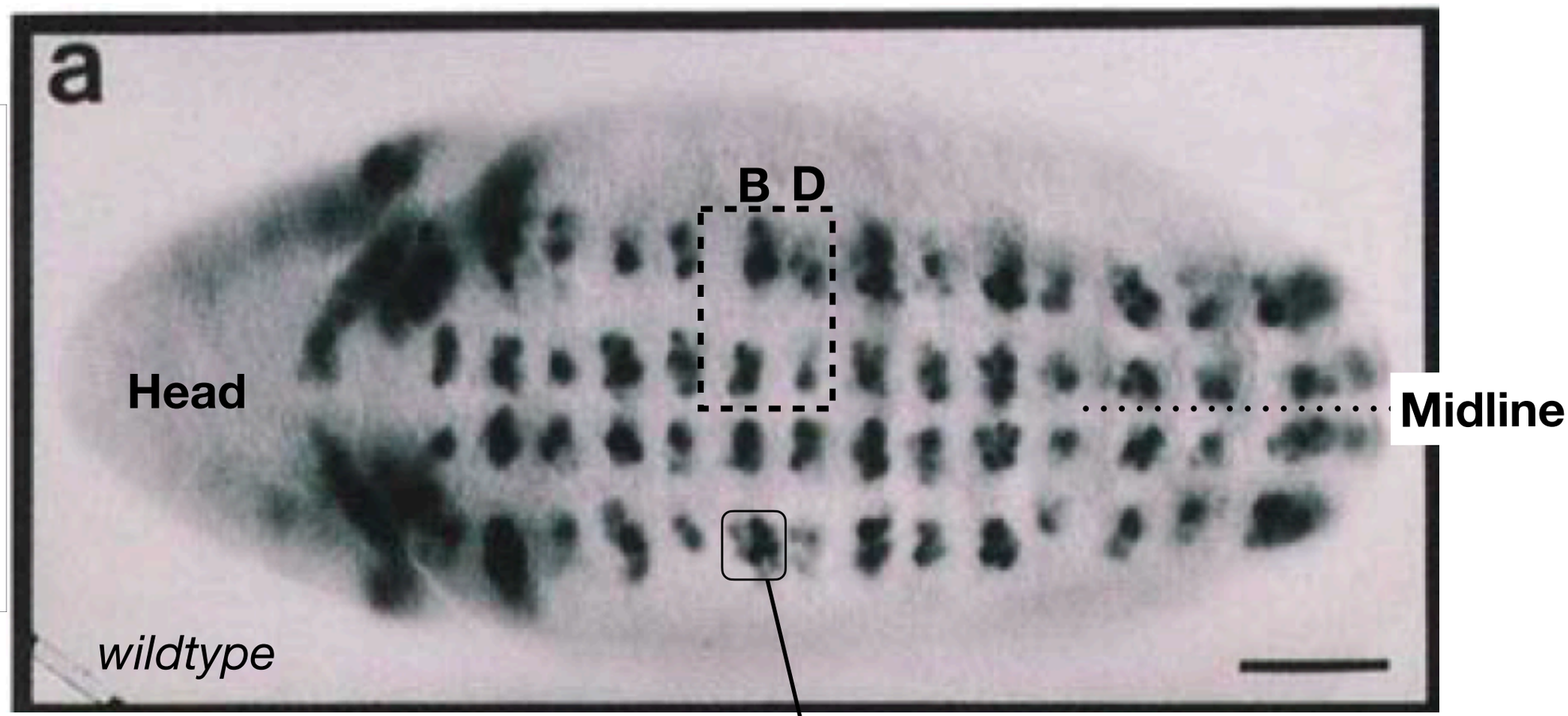




Proneural clusters in every segment



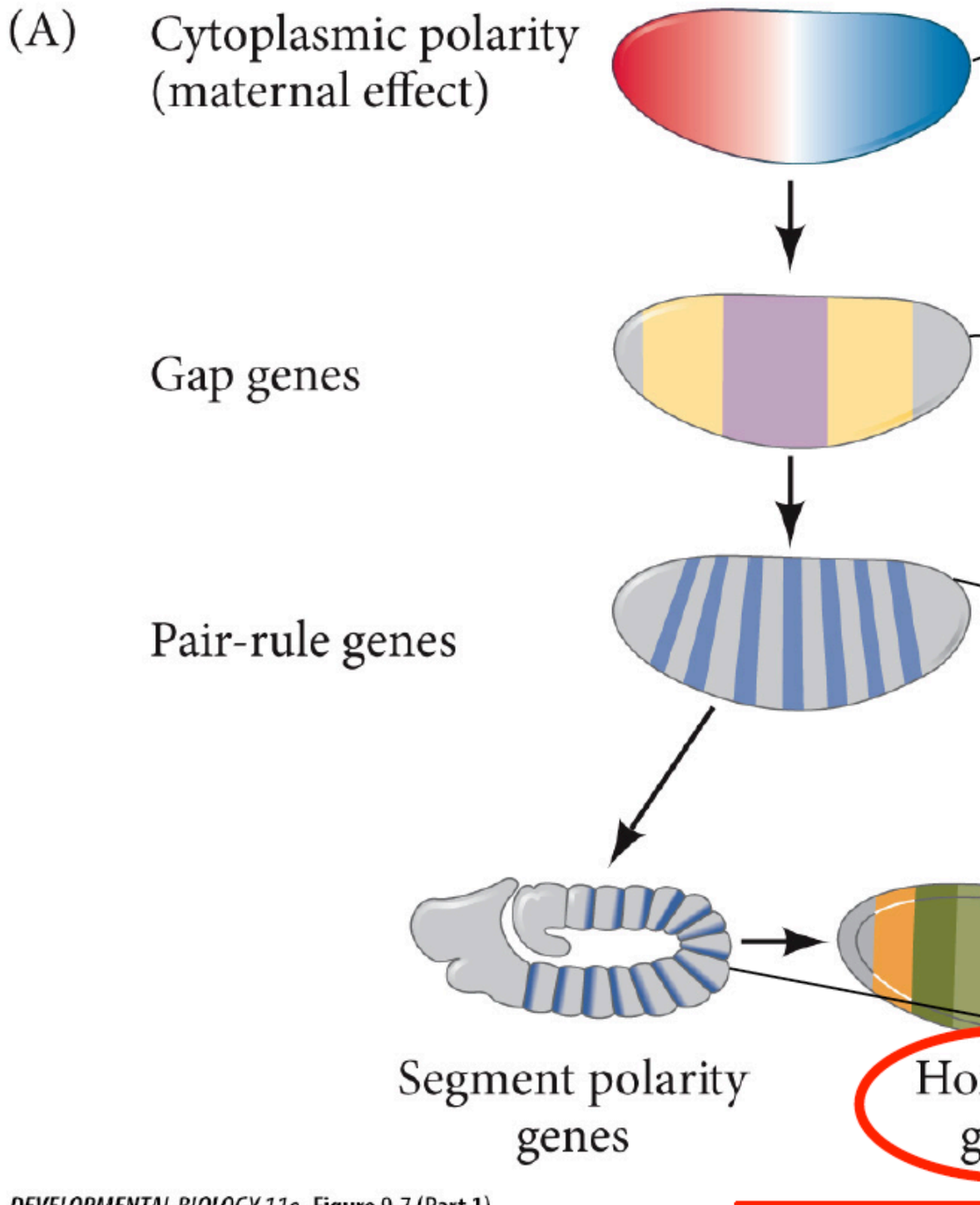
***achaete* gene expression**



5-7 cells in each proneural cluster

Skeath et al., 1992

And so on and so forth



DEVELOPMENTAL BIOLOGY 11e, Figure 9.7 (Part 1)
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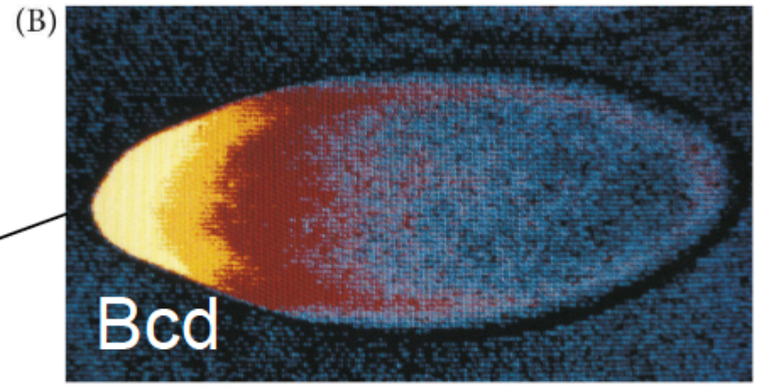

DEVELOPMENTAL BIOLOGY 11e, Figure 9.7 (Part 2)
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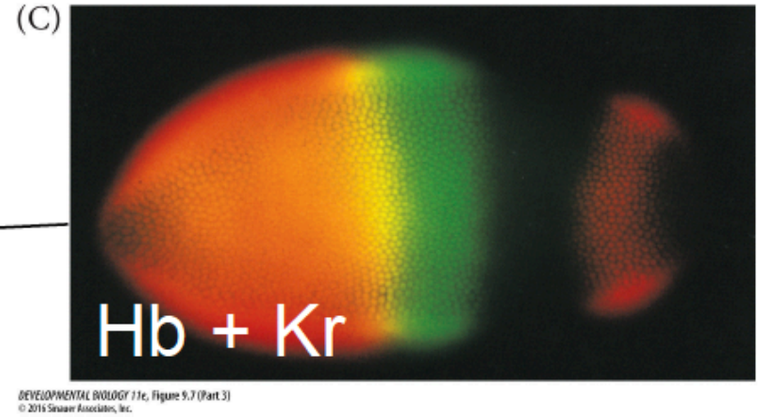

DEVELOPMENTAL BIOLOGY 11e, Figure 9.7 (Part 3)
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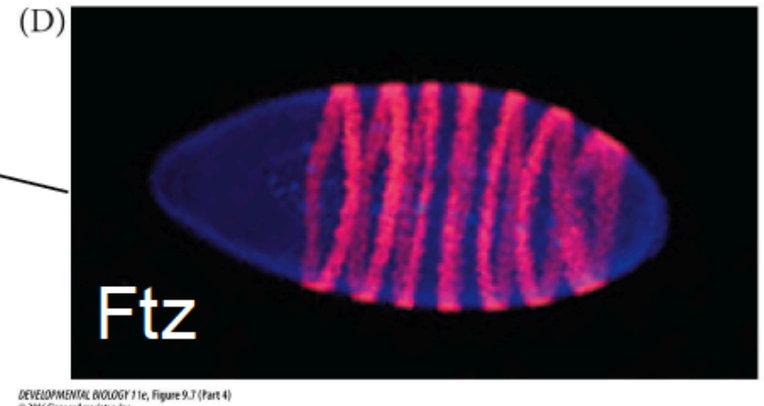

DEVELOPMENTAL BIOLOGY 11e, Figure 9.7 (Part 4)
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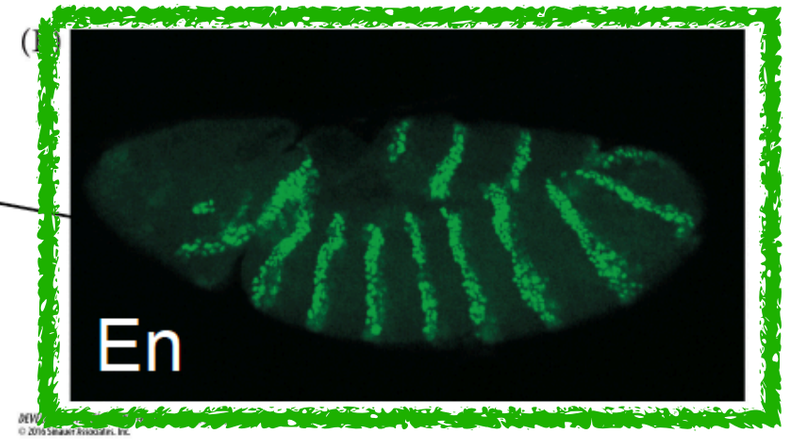
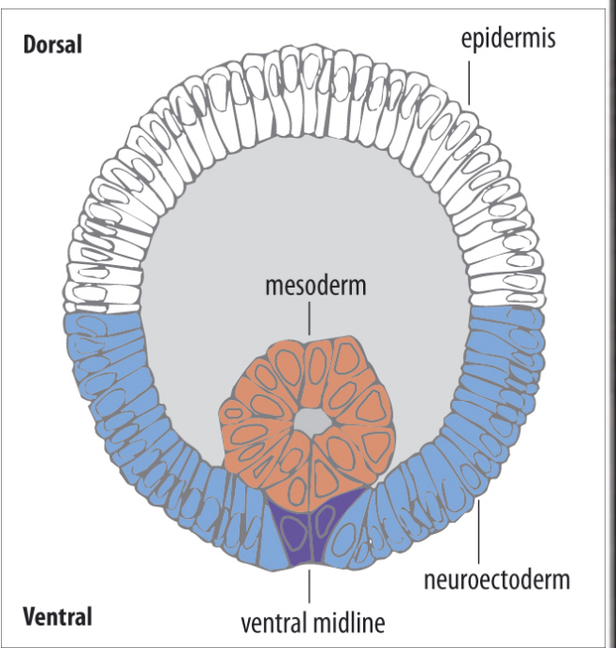
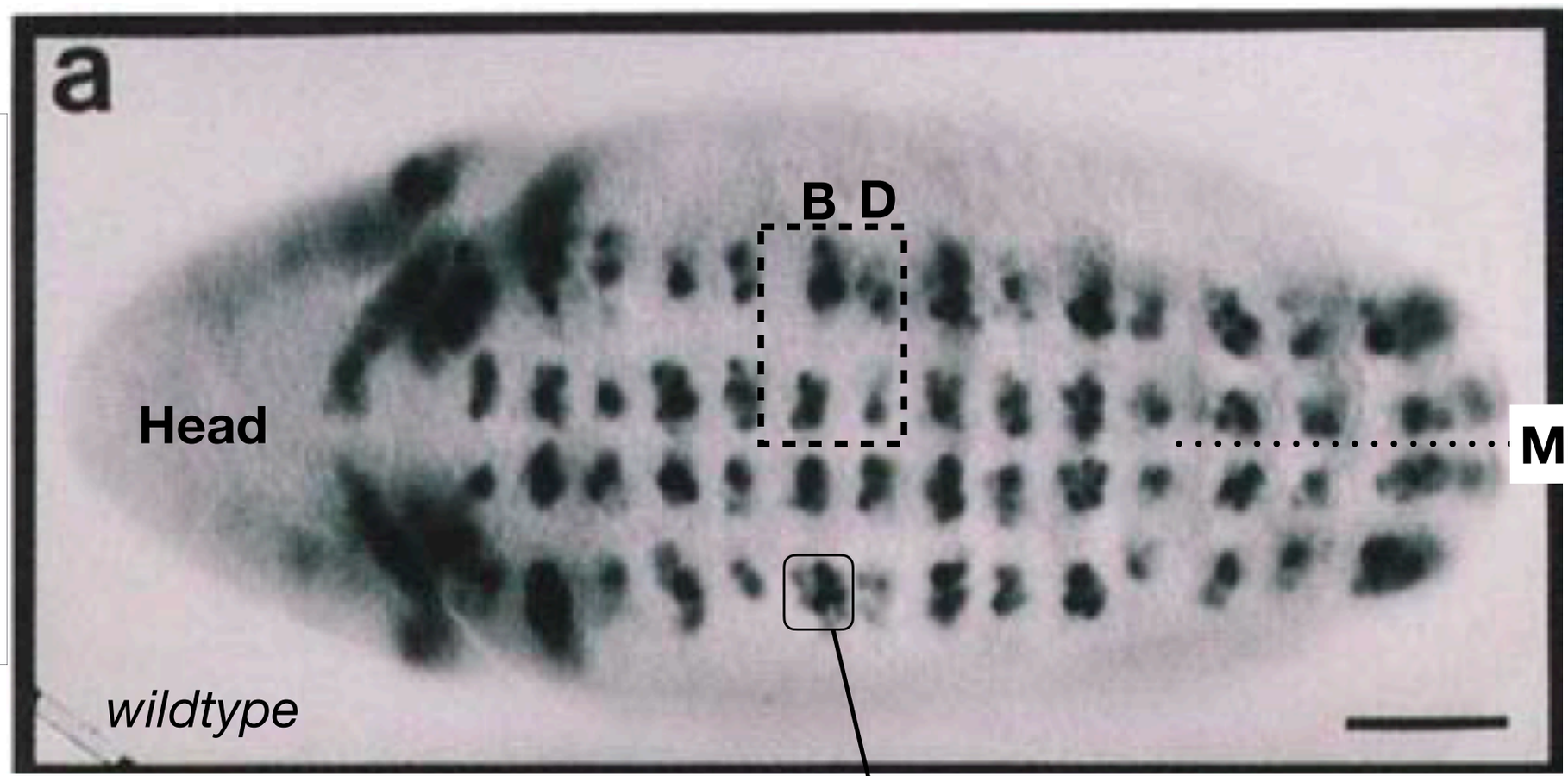


Will be back in Week **13**

Proneural clusters in every segment



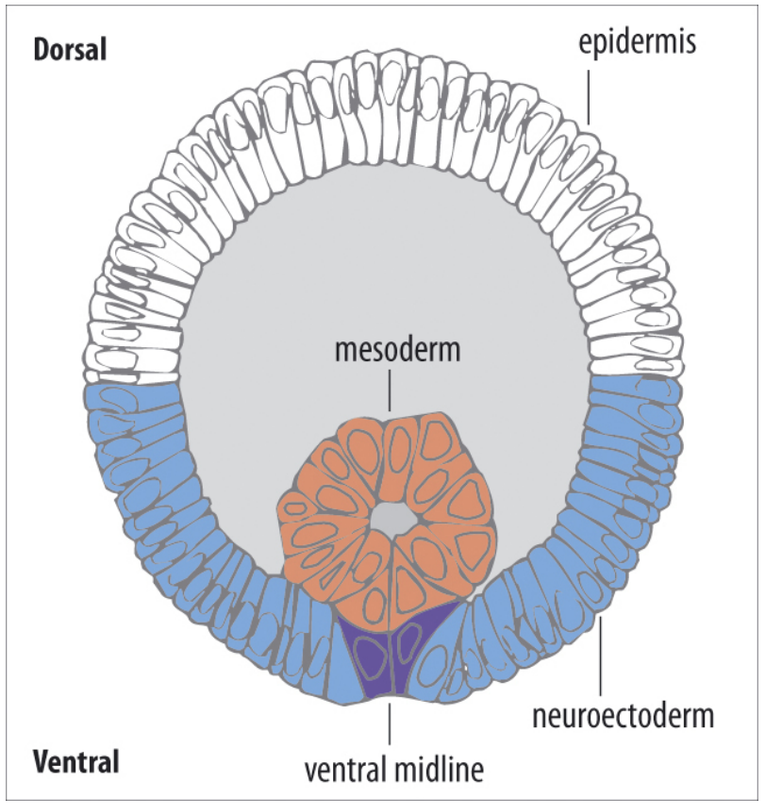
***achaete* gene expression**

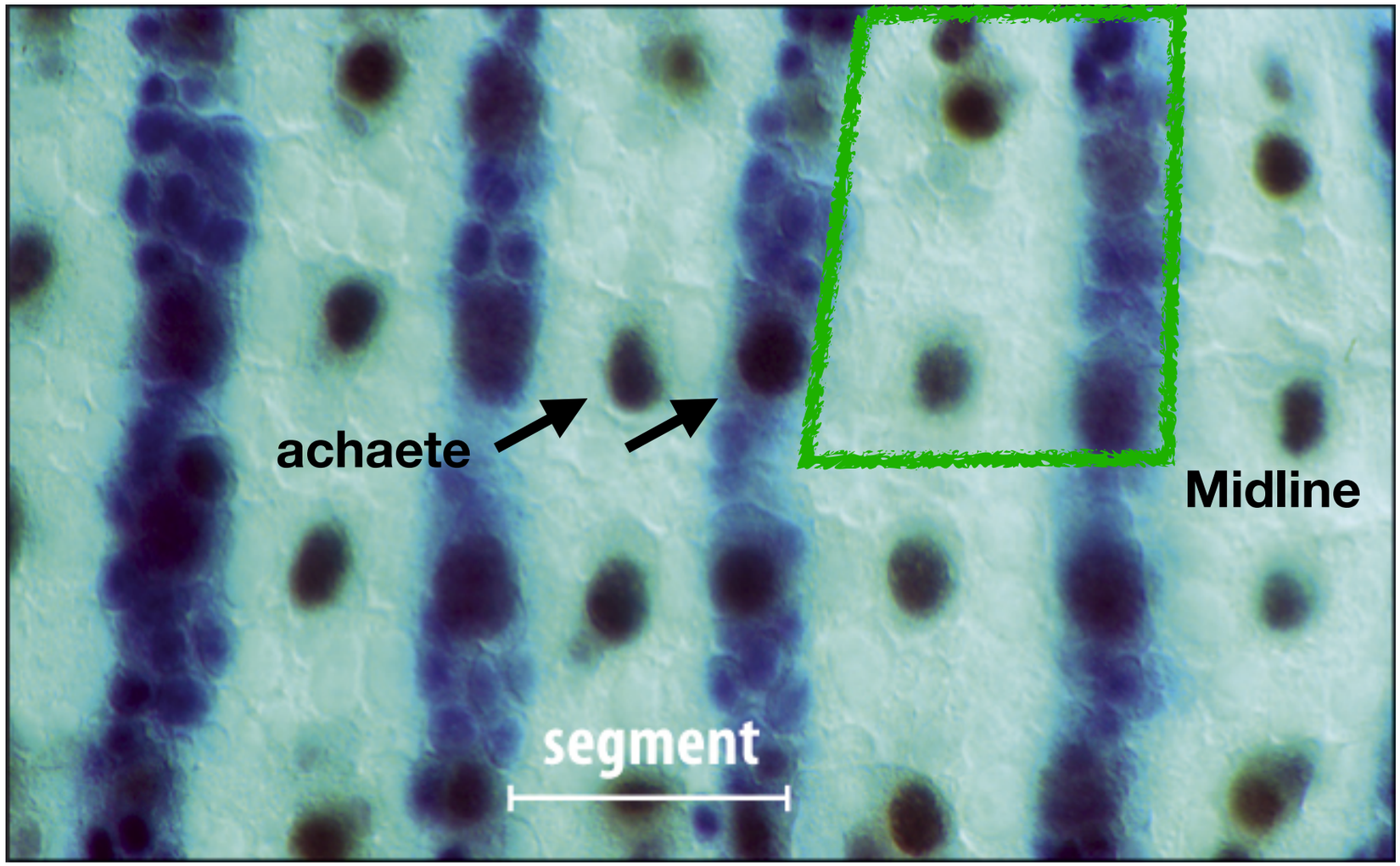


5-7 cells in each proneural cluster

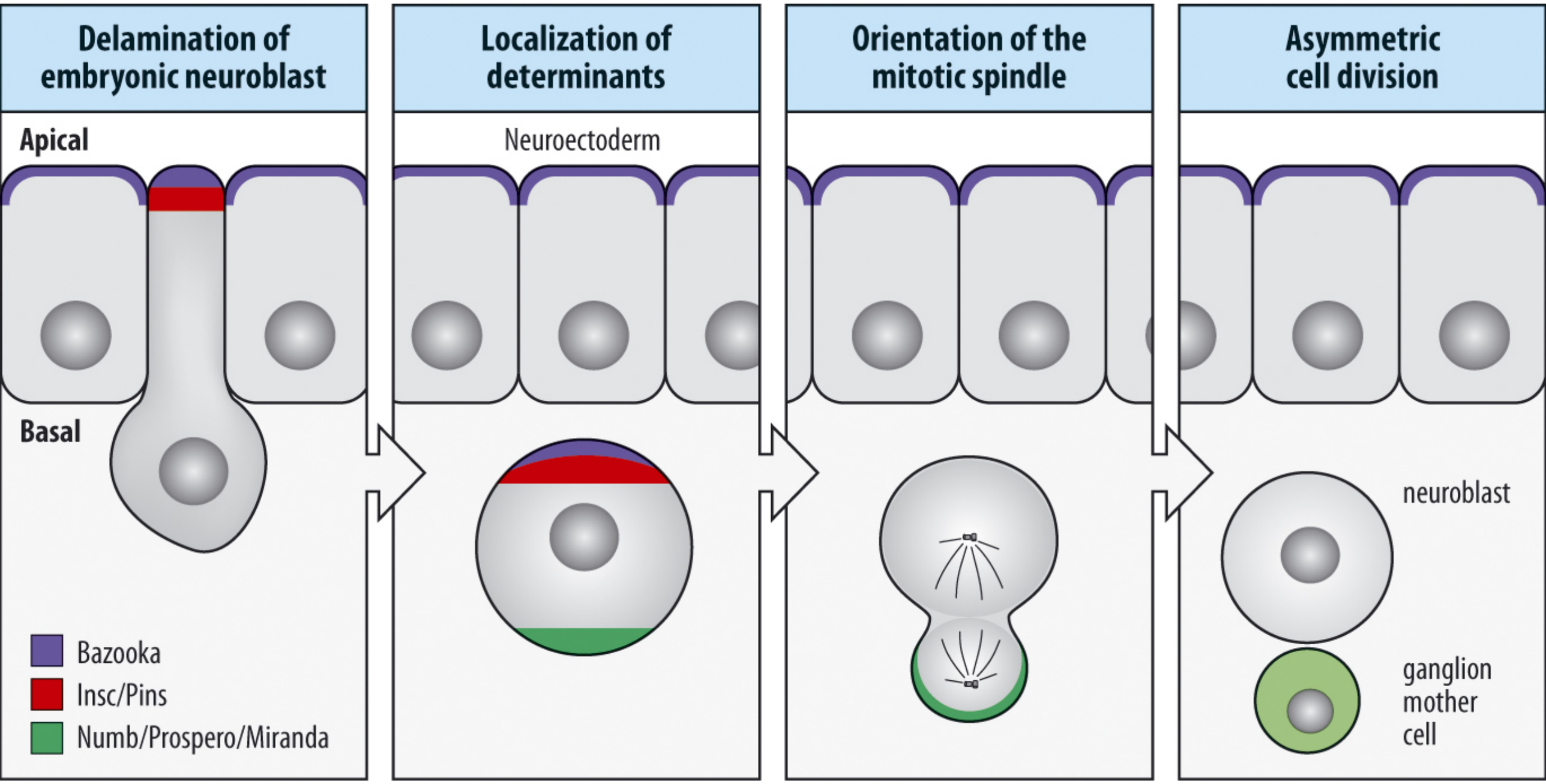
Skeath et al., 1992

Proneural clusters resolve into single neuroblasts



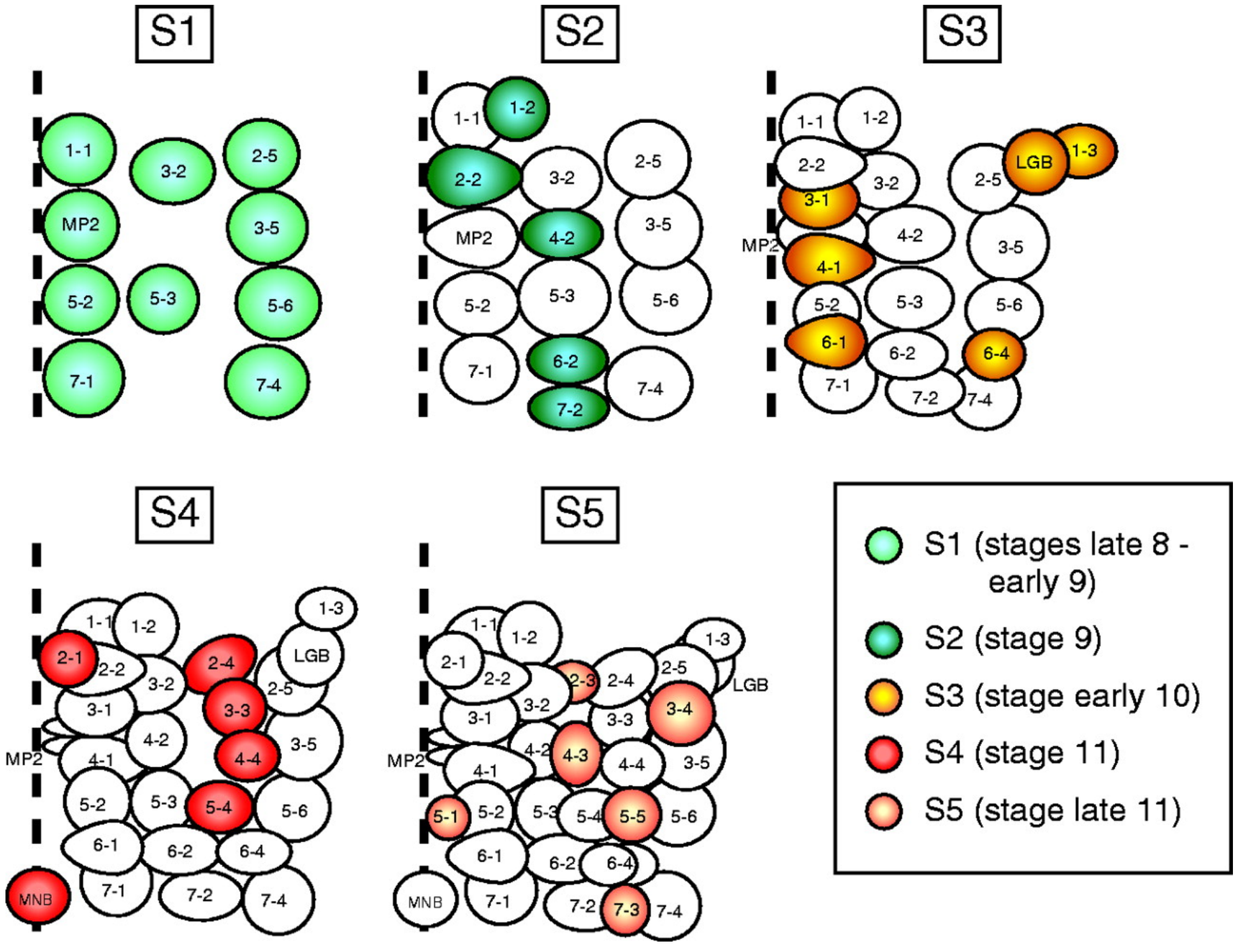


Delamination of the neuroblast



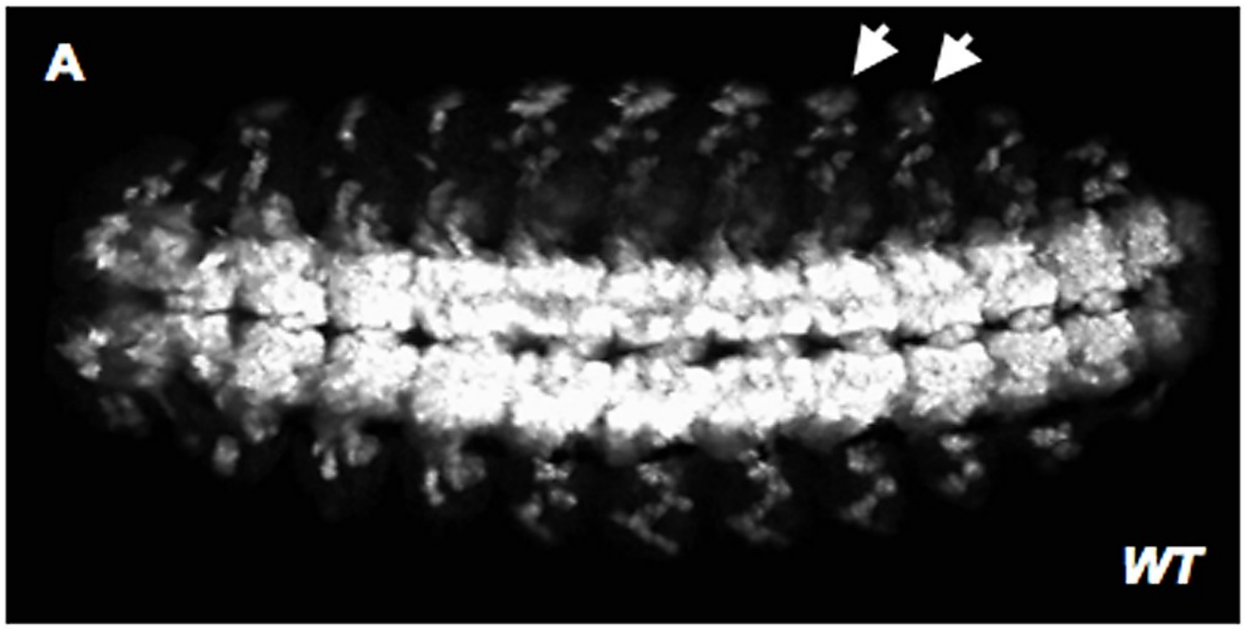
Five waves of neuroblast specification

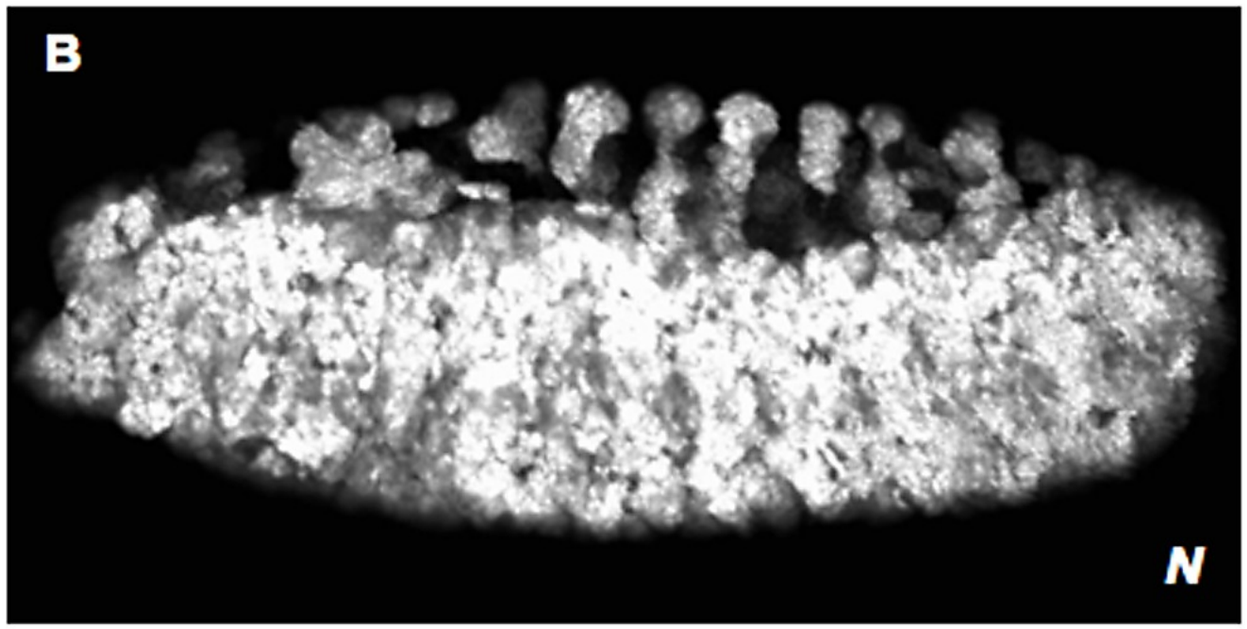
30 cells per hemisegment



Berger et al., 2001

Neurogenic mutants make too many neurons
Little ectoderm left behind

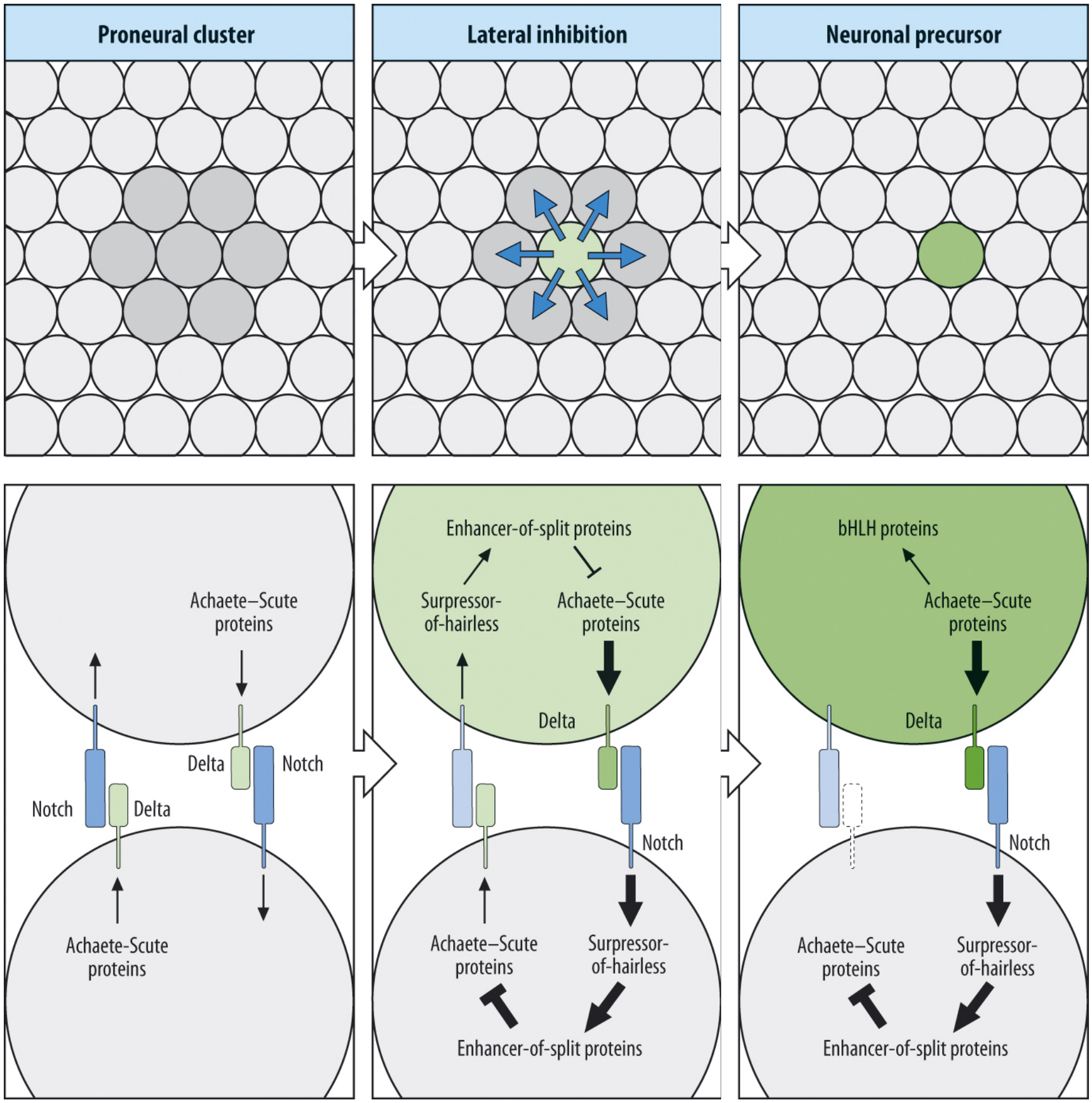




***Notch* mutant**

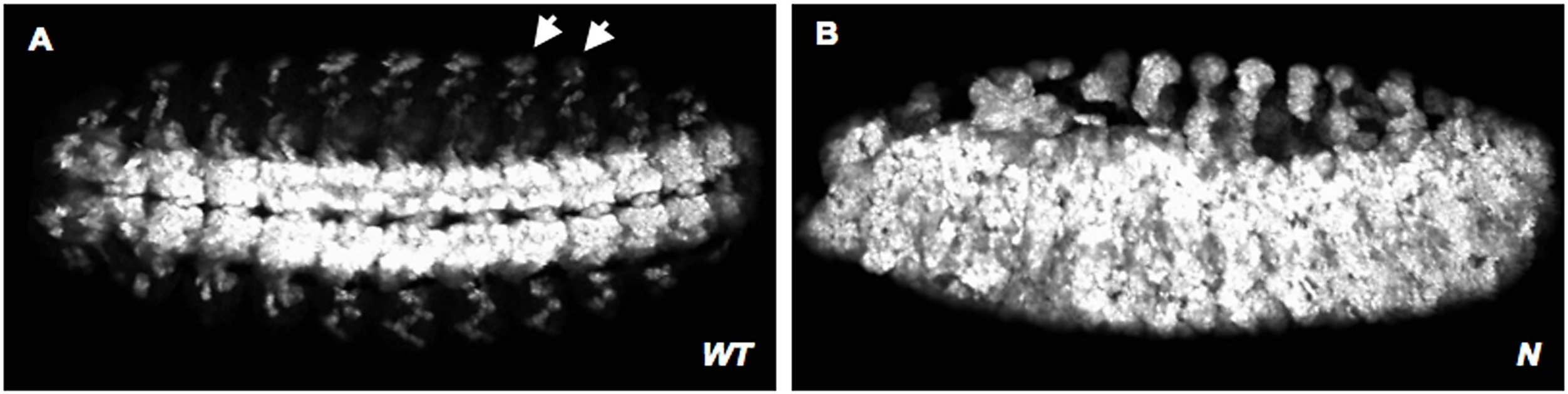
Lake et al., 2009

Notch signalling and lateral inhibition in 2D



Group puzzle

Neurogenic mutants make too many neurons
Little ectoderm left behind

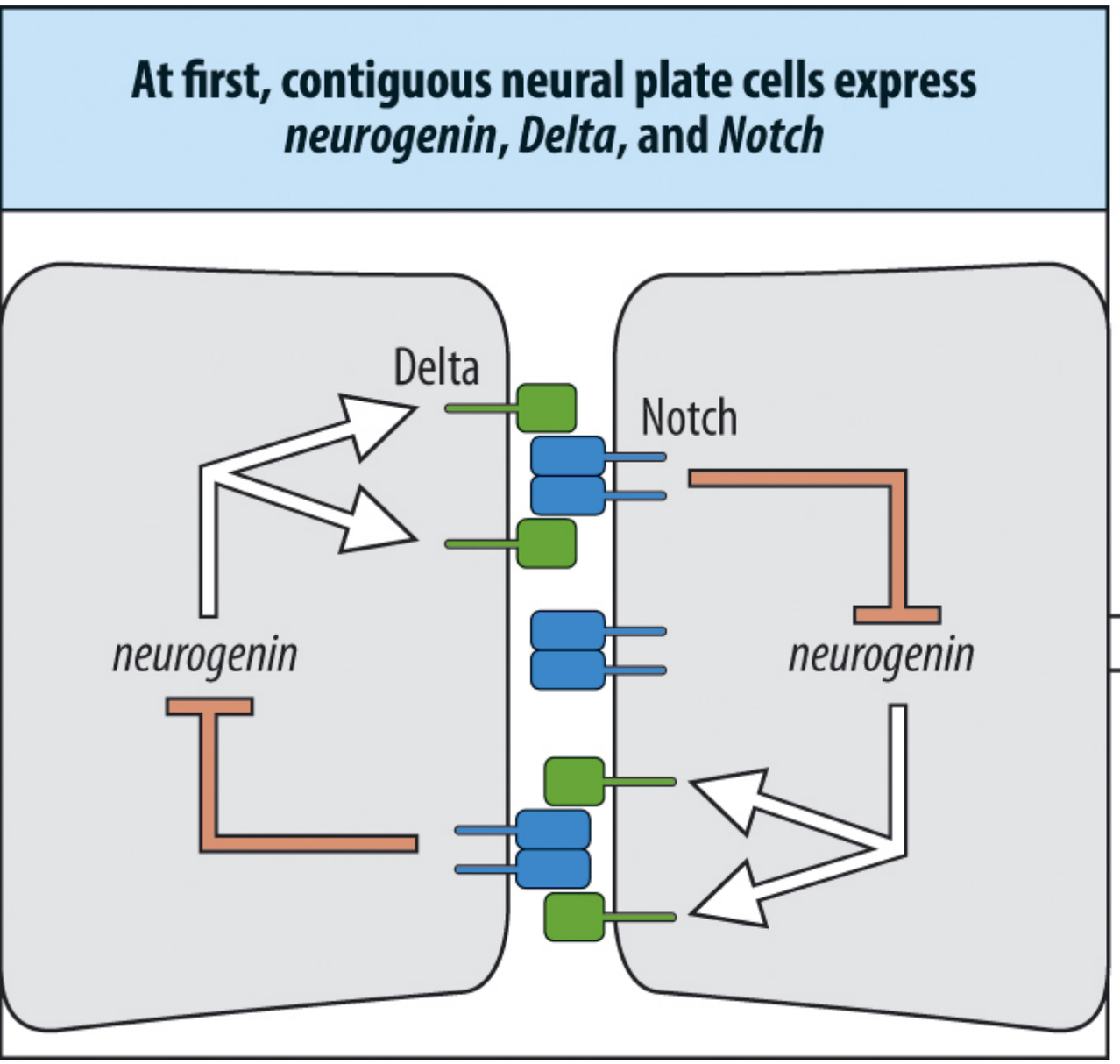


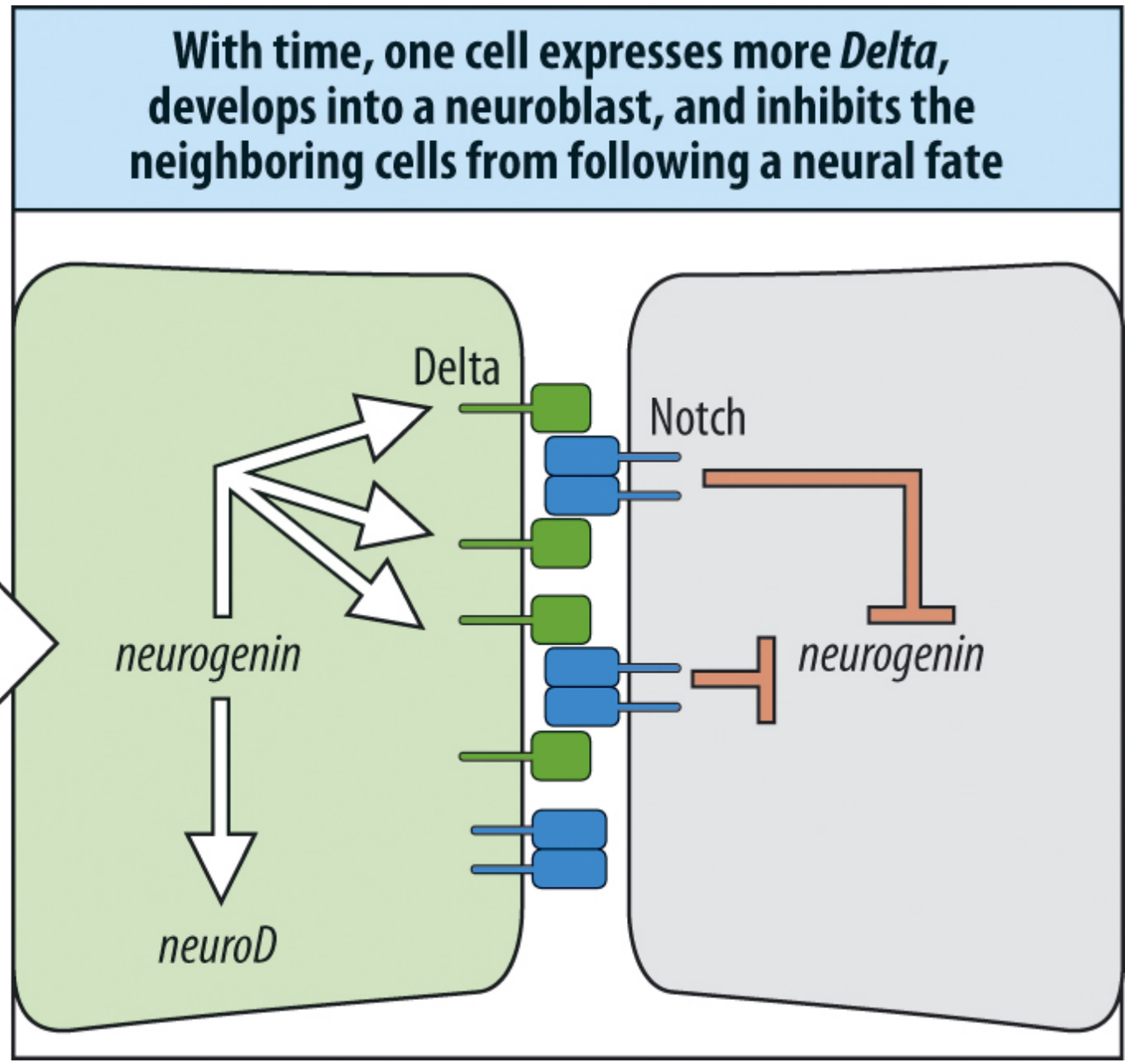
***Notch* mutant**

In which cell type is Notch required?
The delaminating neuroblast, the cells that remain in the neuroectoderm, both?
How would you test this?

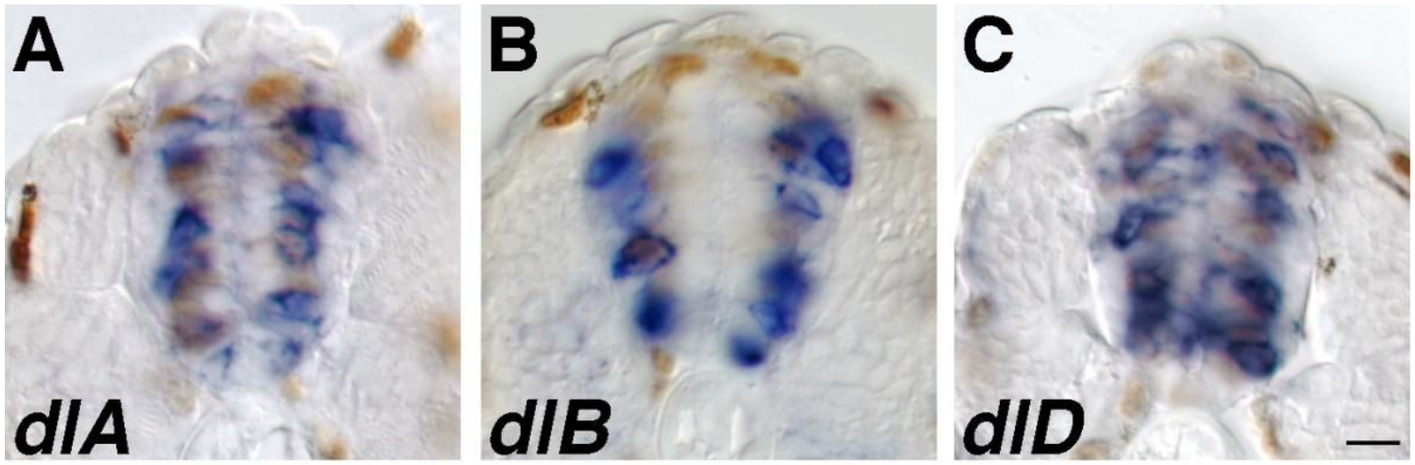
Lake et al., 2009

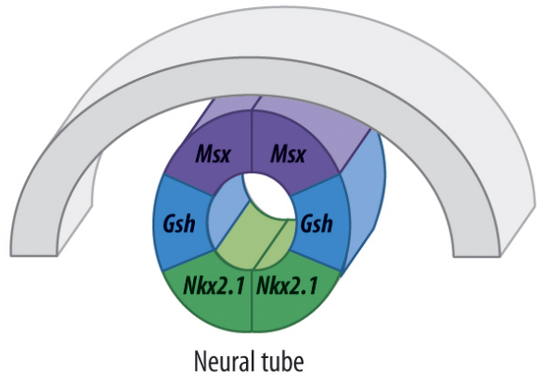
Neuronal precursor specification in the vertebrate CNS

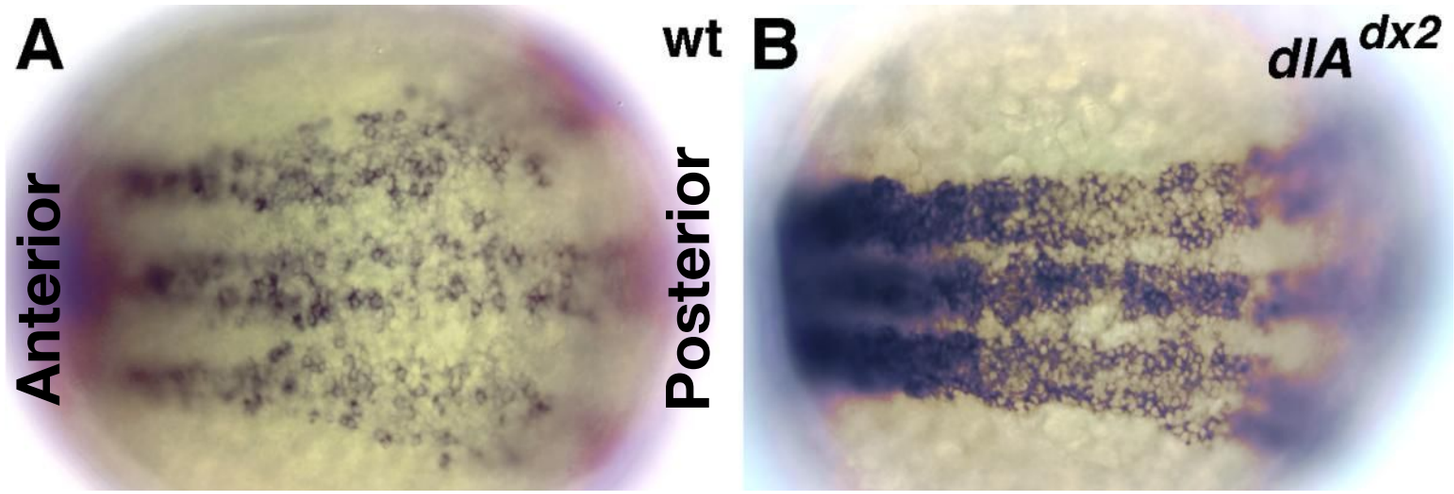


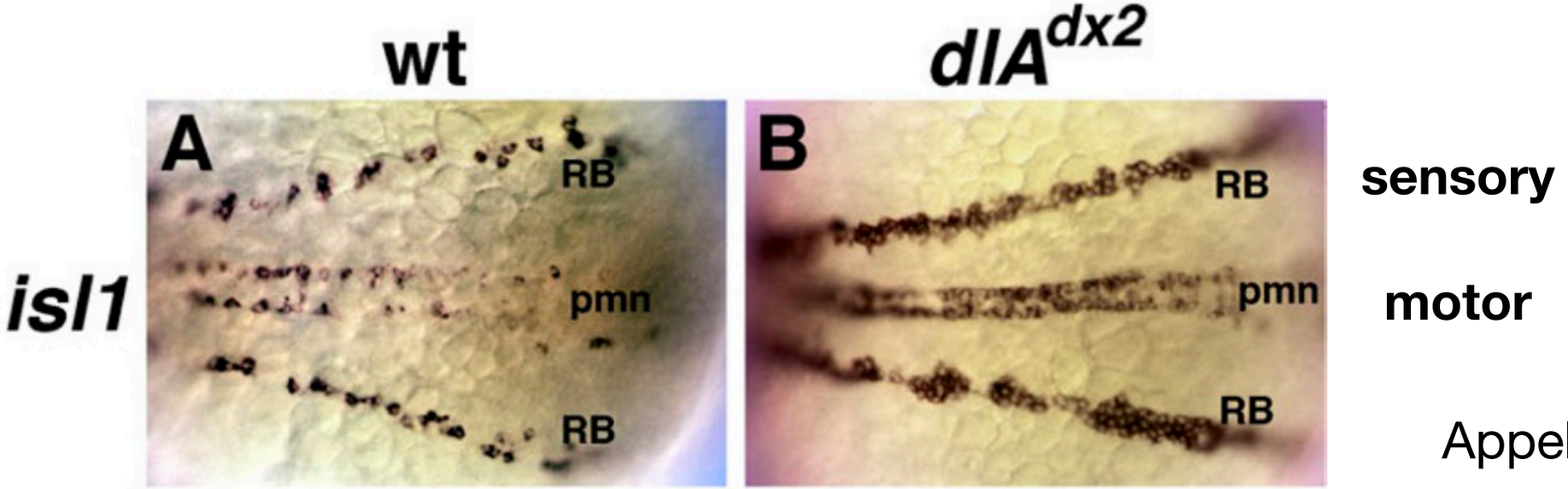


Delta & *Notch* mutants cause too many neurons *neurogenic*





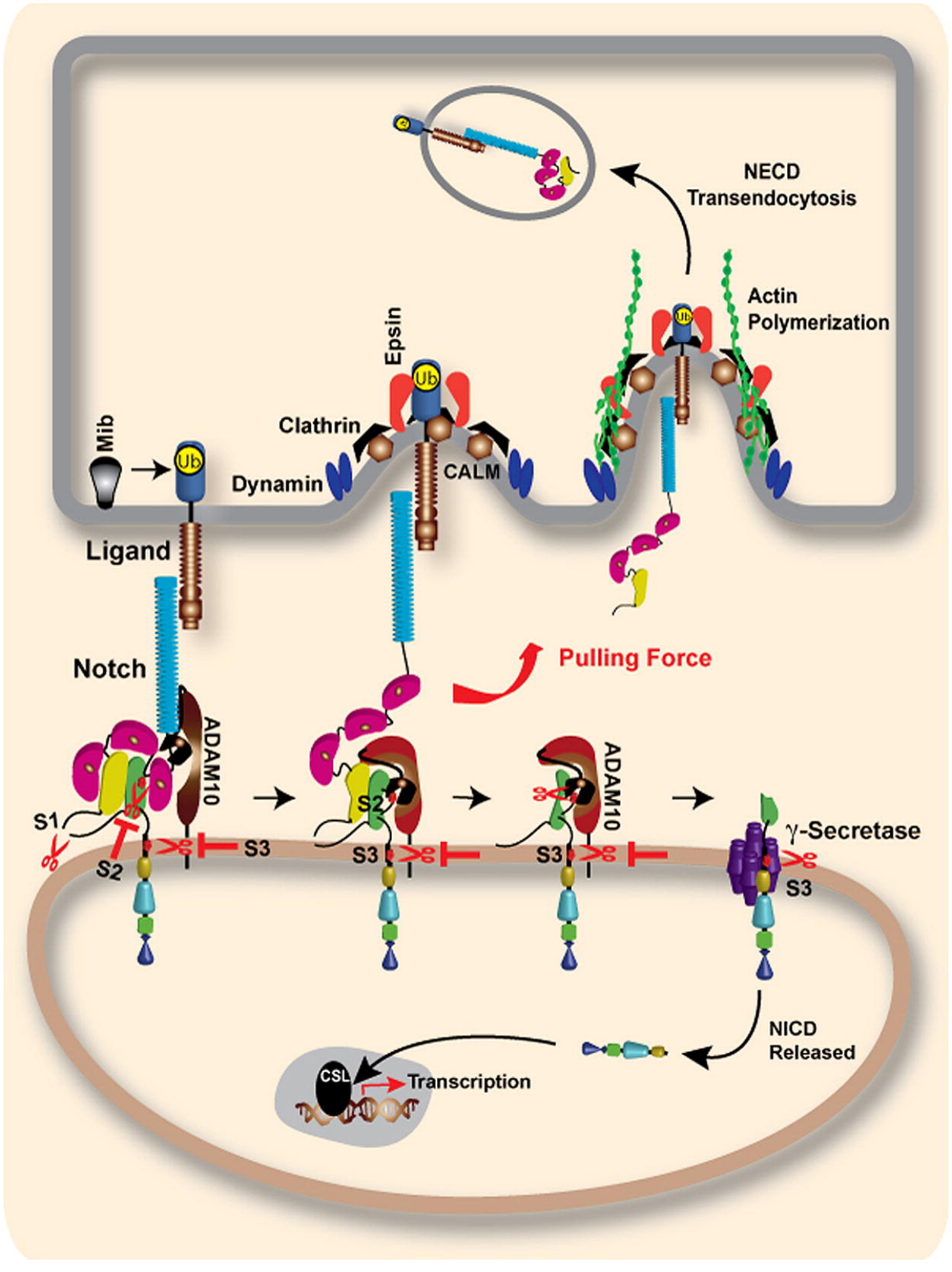




Appel et al., 2001


KEEP
CALM
AND USE
THE
FORCE

Notch singling requires three proteolytic cleavages



S1 in Golgi

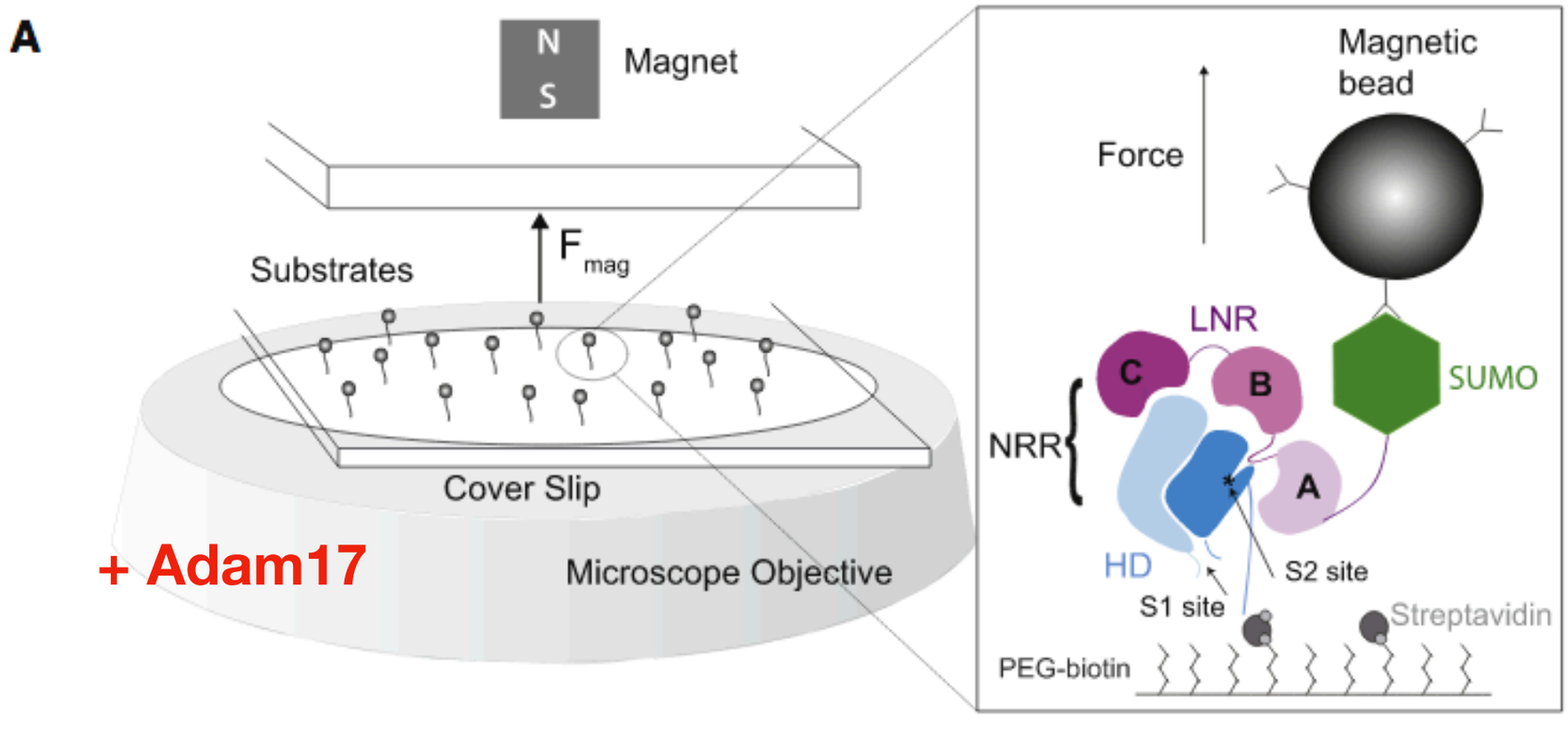
S2 on surface after Delta binding (ADAM)

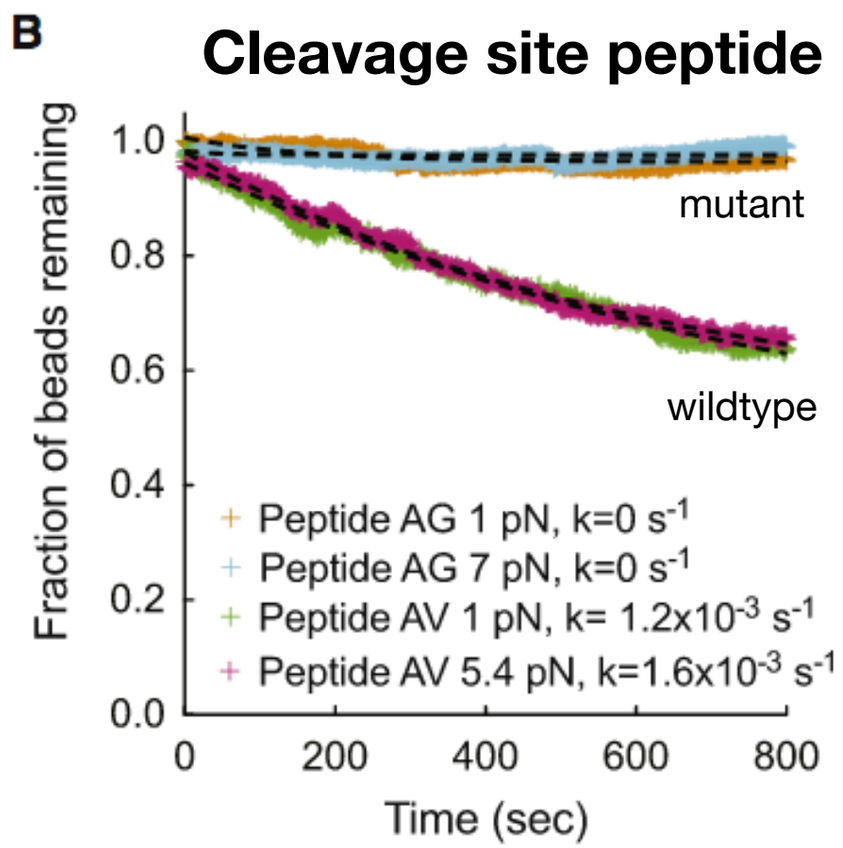
S3 in membrane after Delta internalisation (γ-secretase)

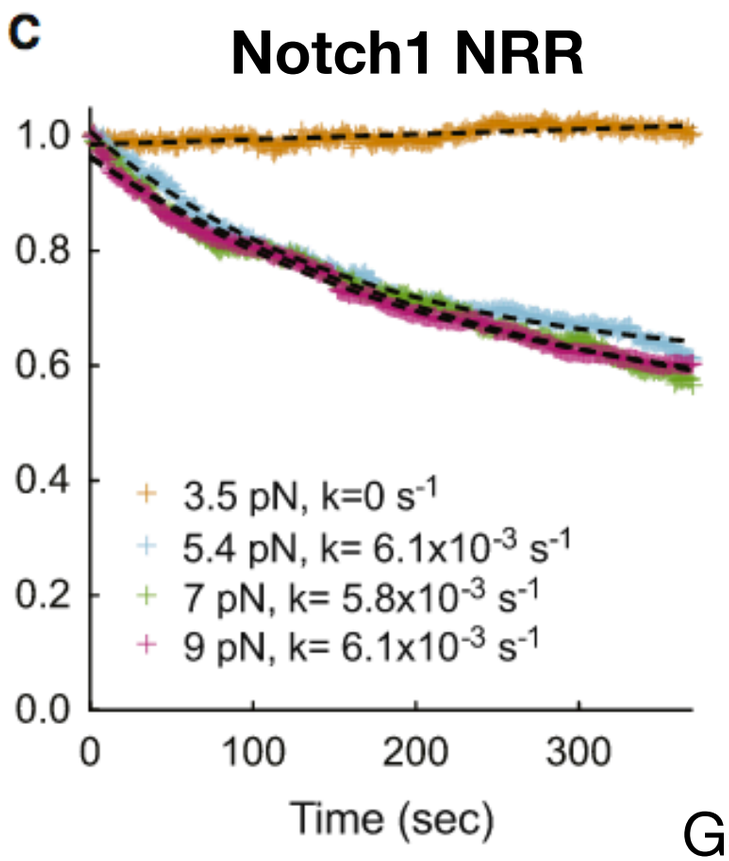
Dynamin, epsin and clathrin mutants block Delta-Notch signalling

Meloty-Capella et al., 2012

Notch cleavage at ~ 5 pN pulling force

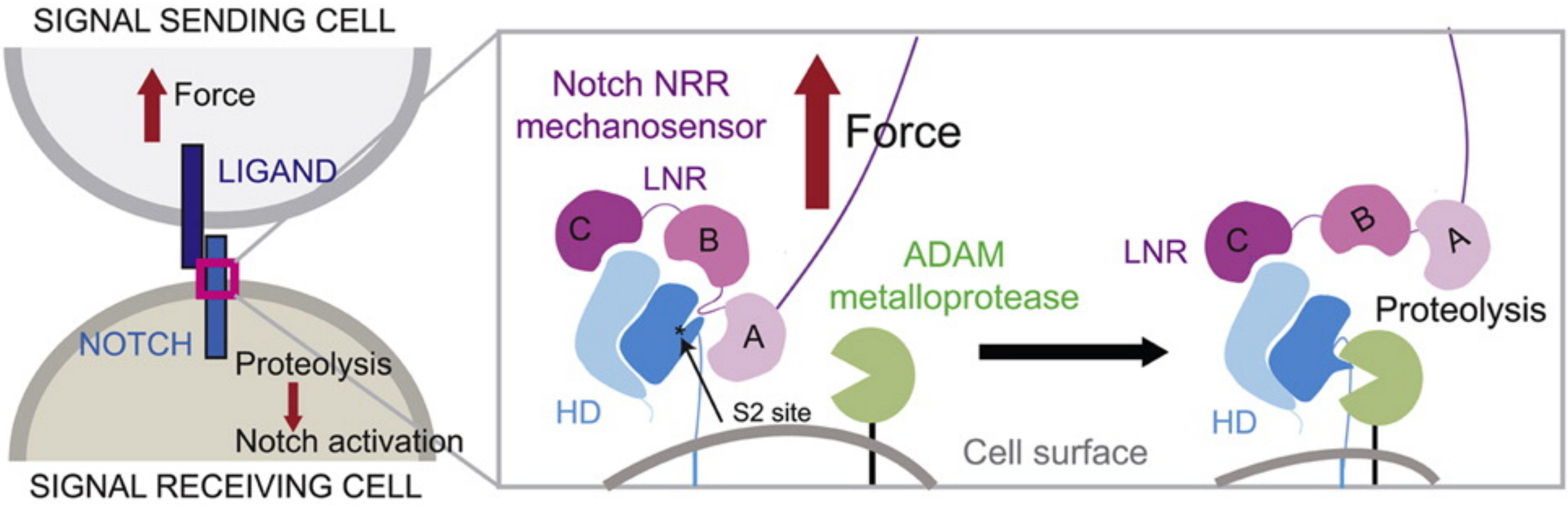






Gordon et al., 2015

Model for force-dependent Notch signalling



Is the Notch pathway a mechanosensor?
When is a chemical signal a mechanical signal?

Summary

- **Selection of a single cell from a 2D sheet**
- Delta-Notch signalling in equivalence group
- Force dependent Notch cleavage
- Pattern set by interplay between induction and lateral inhibition
- Patterning, selection, delamination, asymmetric cell division
- Positional negotiation

Questions?