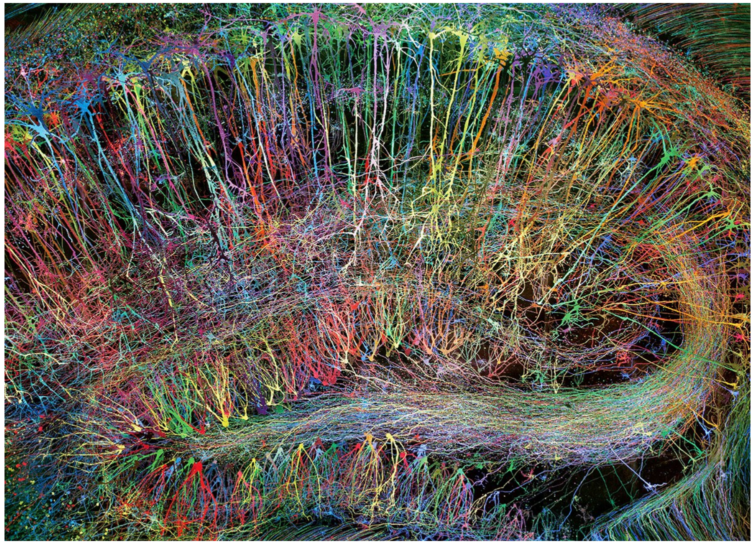
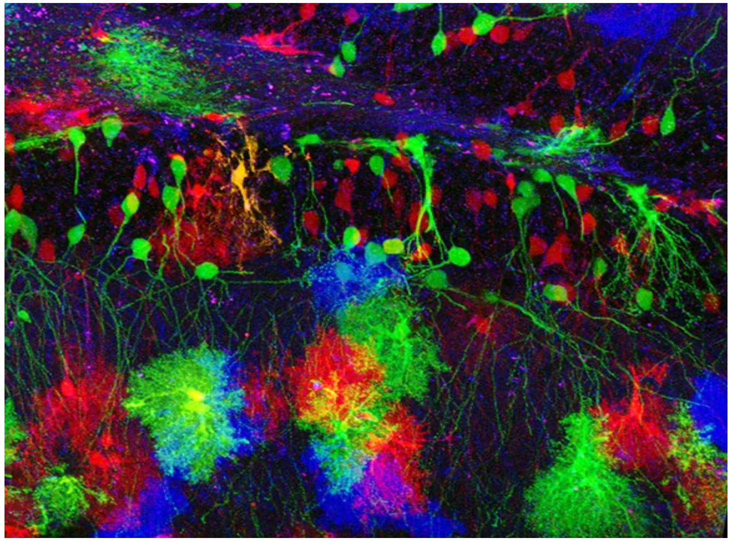
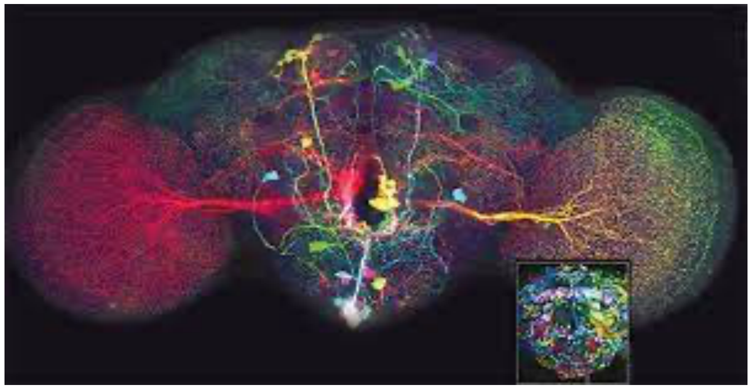
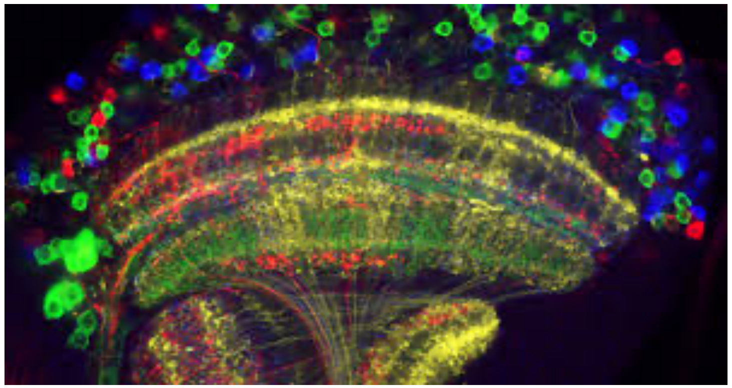
Cell Cycle and Cell Determination

Michel.gho@sorbonne-universite.fr

Cell Cycle and Cell Determination

Formation of organisms from a totipotential cell (the zygote), or the formation of body part from precursor cells, involves processes such as:

Cell proliferation
Cell determination
Cell differentiation
Cell shape changes
Cell migration

All take place over time.

How these processes are temporally and spatially regulated/coordinated?

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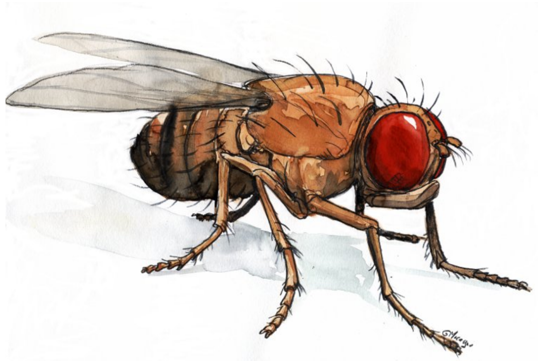
Cell Cycle and Cell Determination



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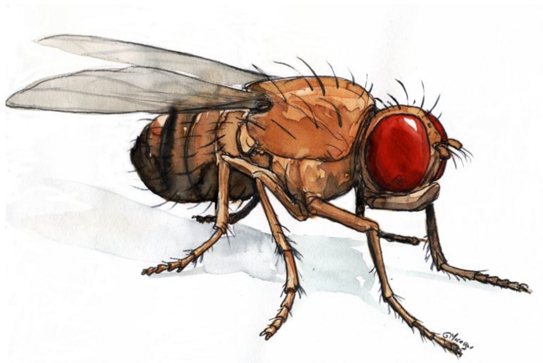
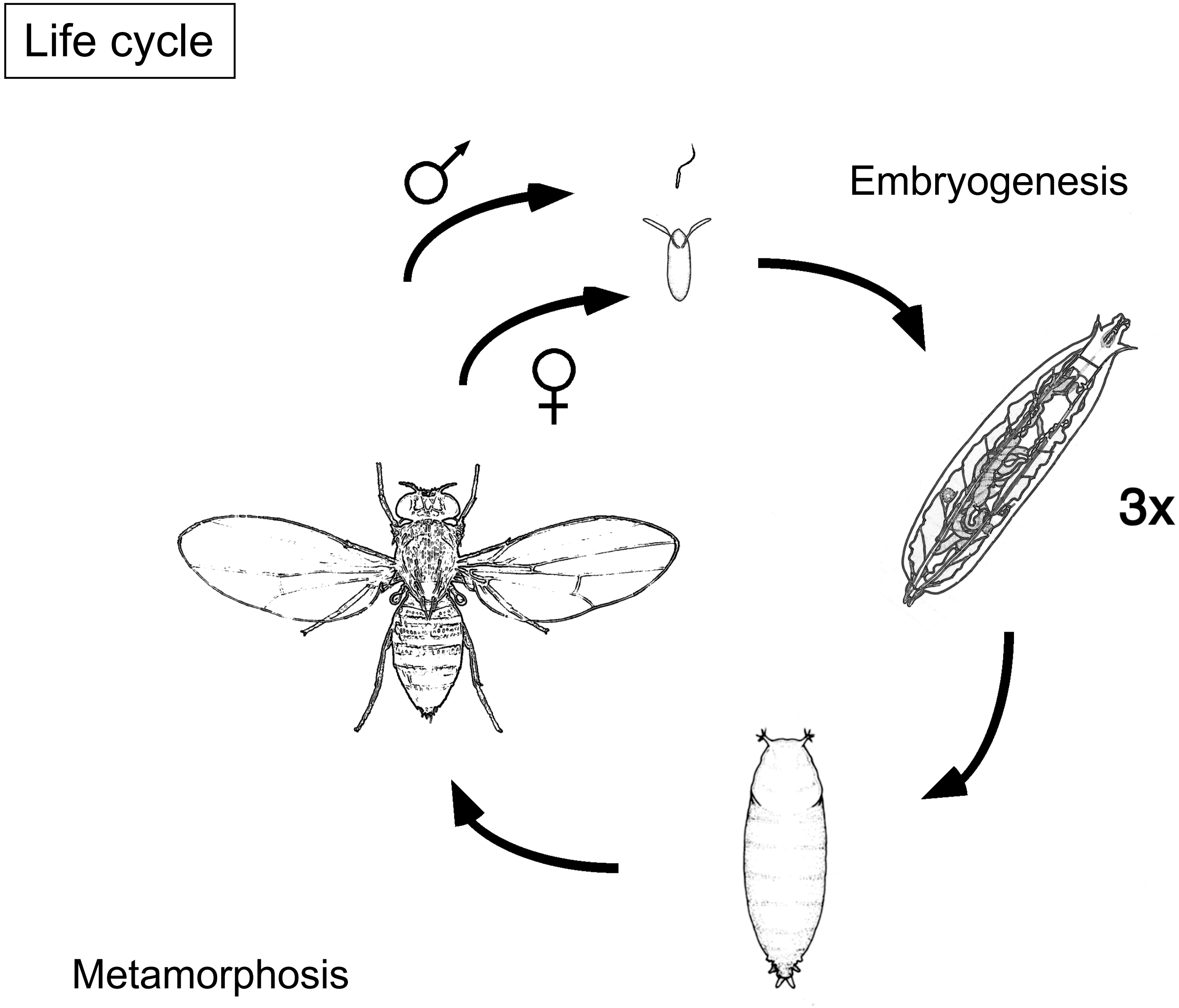
Neurogenesis in *Drosophila*



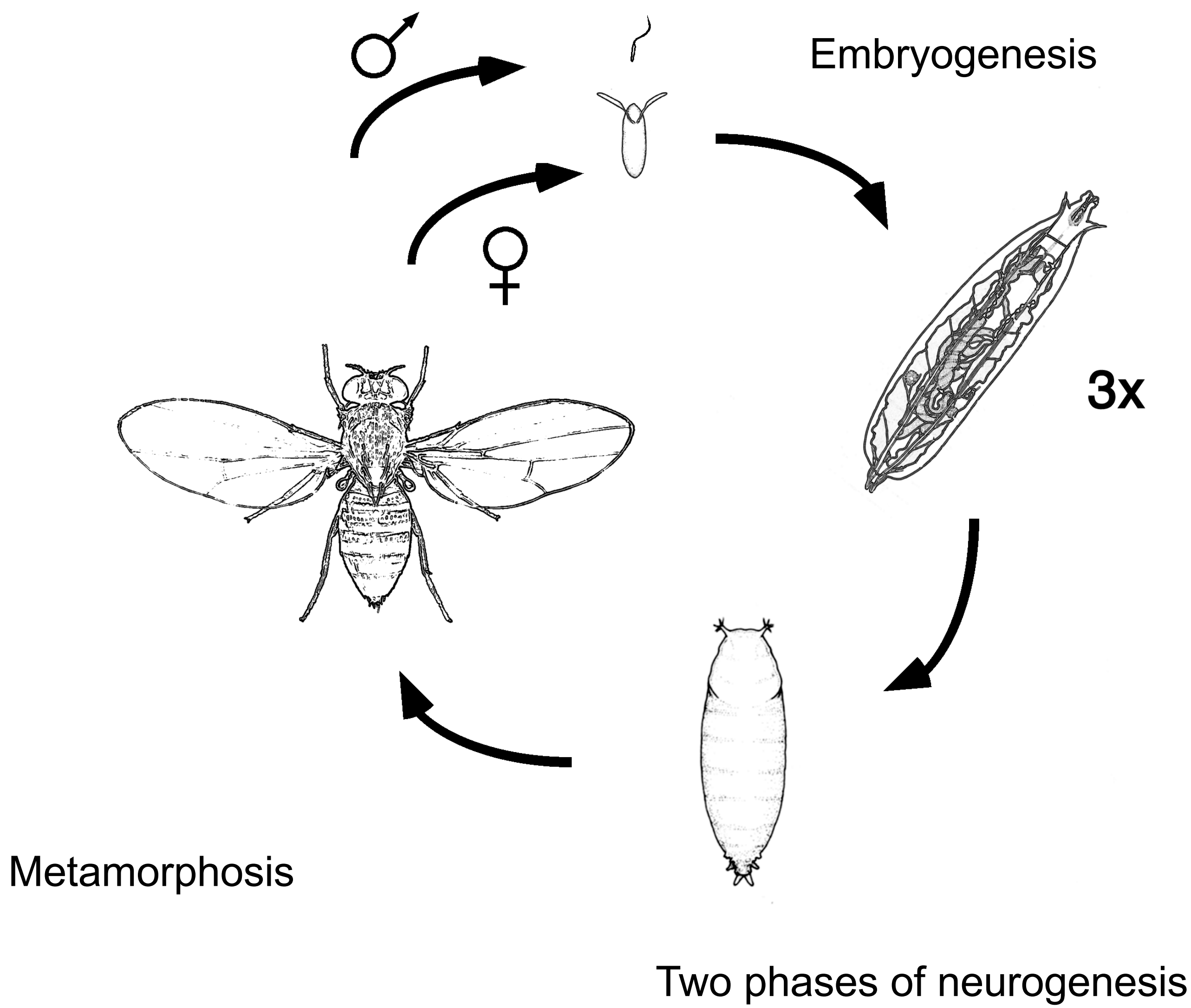
Cell cycle and neural cell determination in *Drosophila*

- Life cycle
- Neurogenesis
  - Mutual and lateral inhibition
  - Asymmetric cell division
- Origin of neural precursor cell diversity
  - Spatial clues
  - Temporal clues
- Time in development
  - Temporal patterning

Technical goals : Molecular and genetic tools commonly used in *Drosophila*

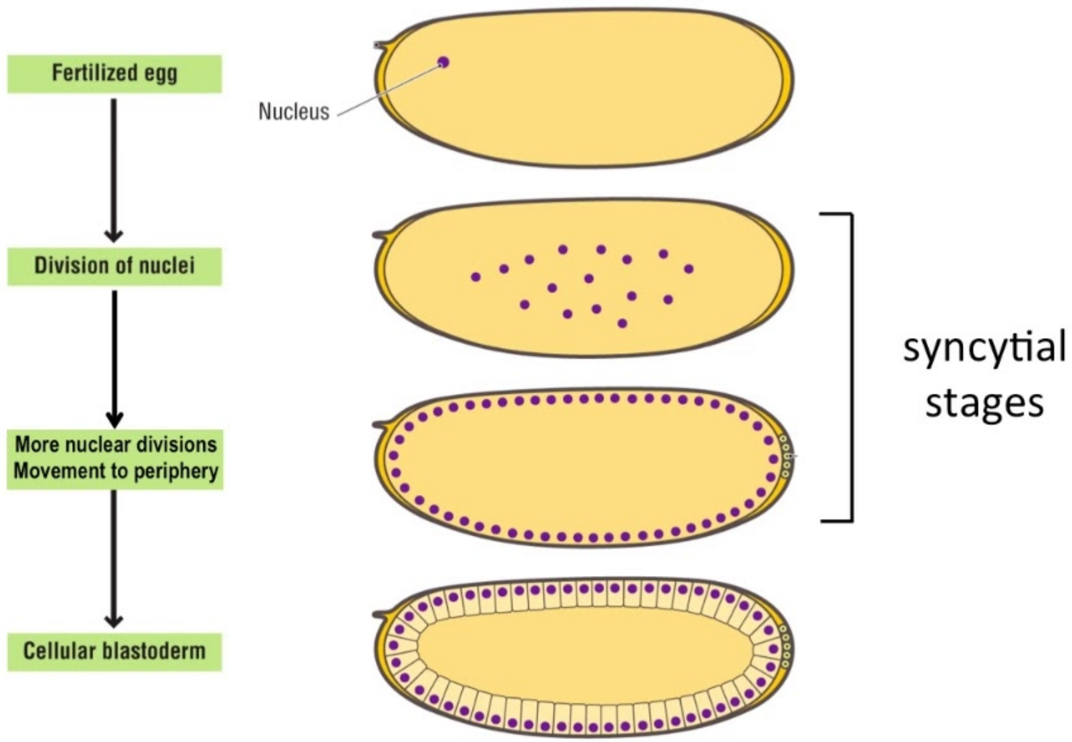

Life cycle
♂
♀
Embryogenesis
3x
Metamorphosis

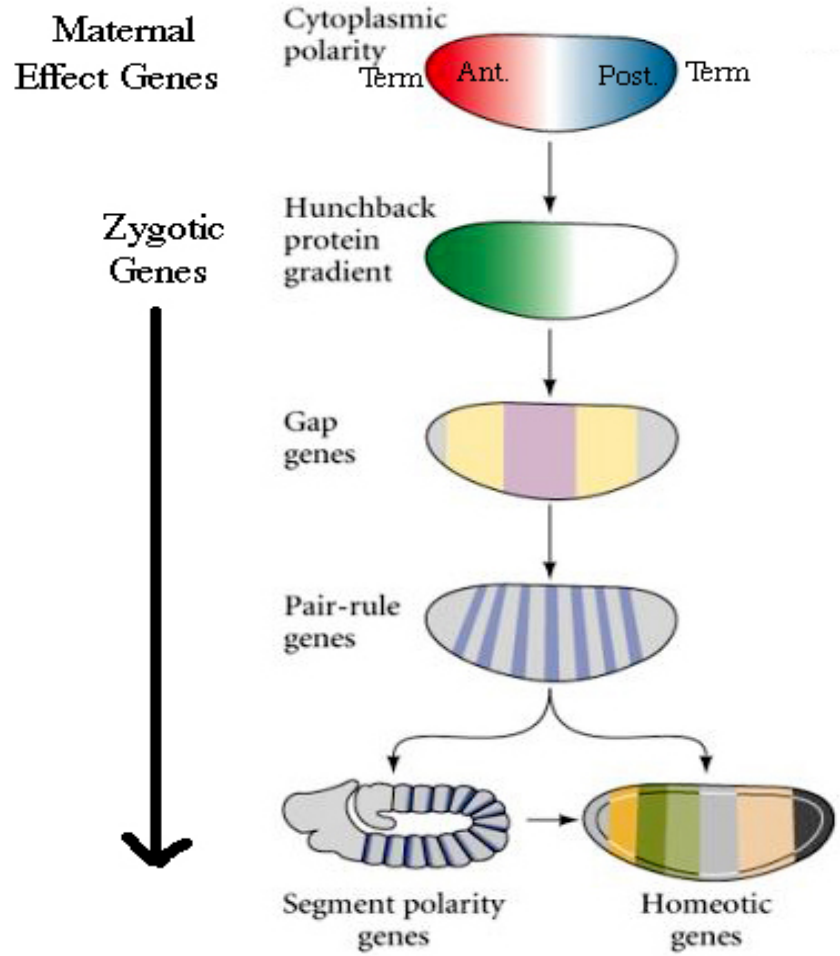
Life cycle

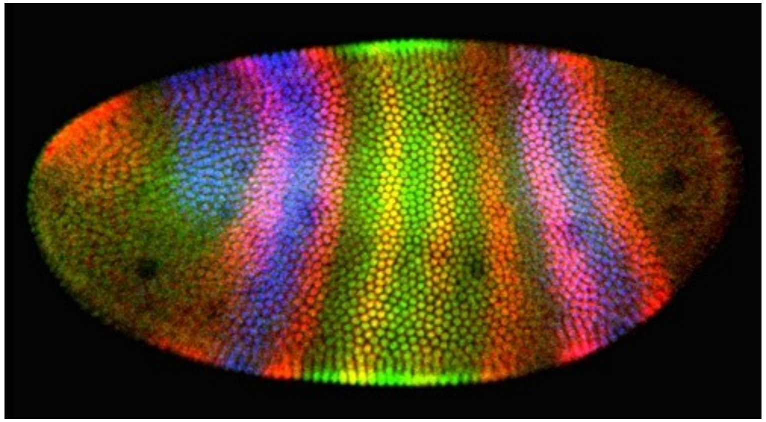


Embryogenesis
3x
Metamorphosis
Two phases of neurogenesis

Early stages of *Drosophila* development





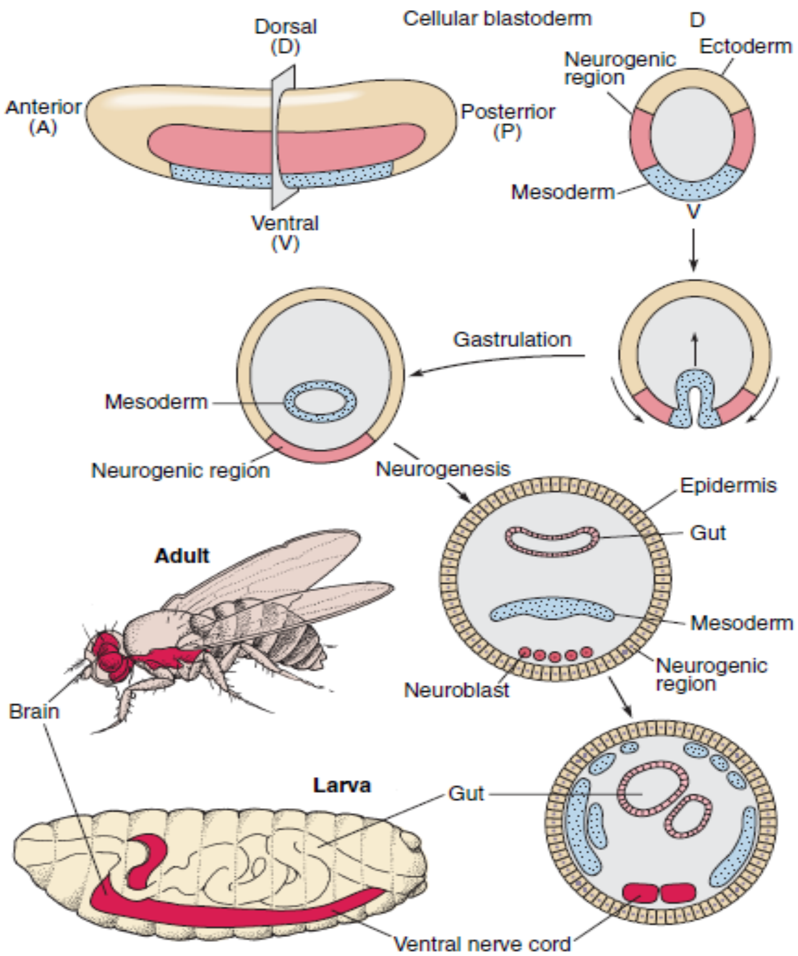


Hairy Krüppel Giant

Neurogenesis

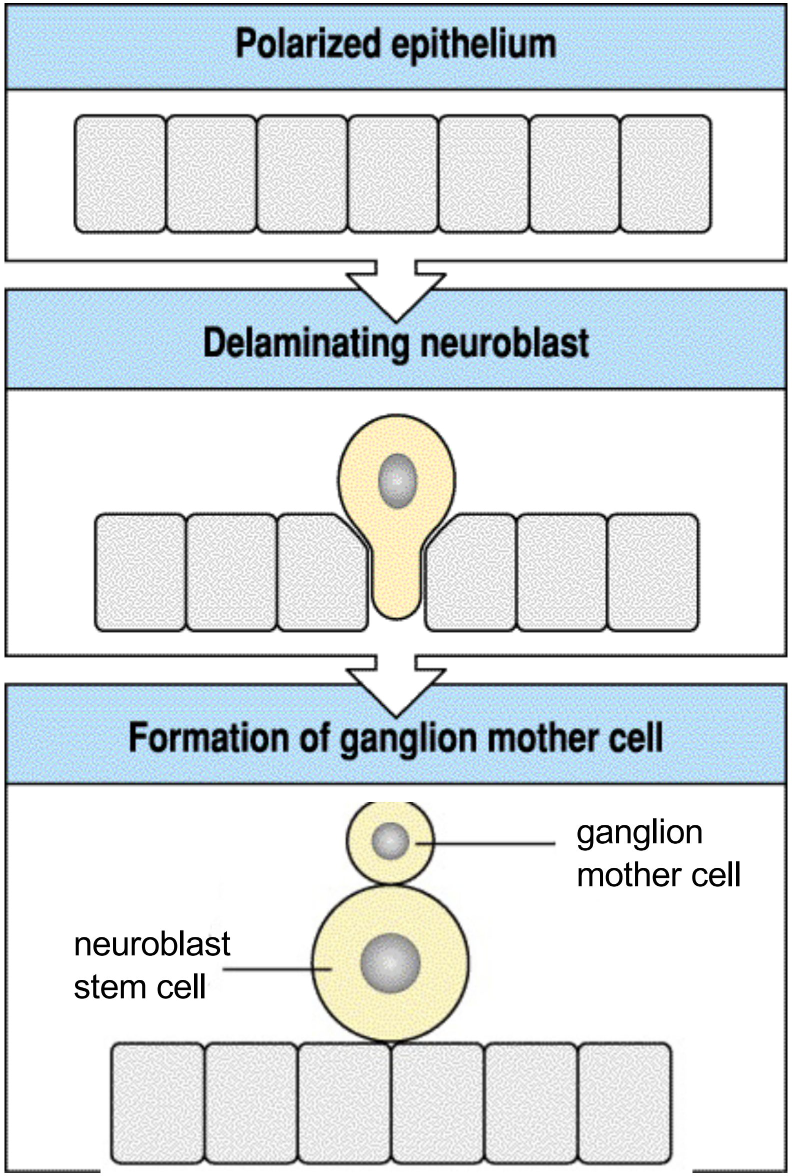
Neuroblast formation

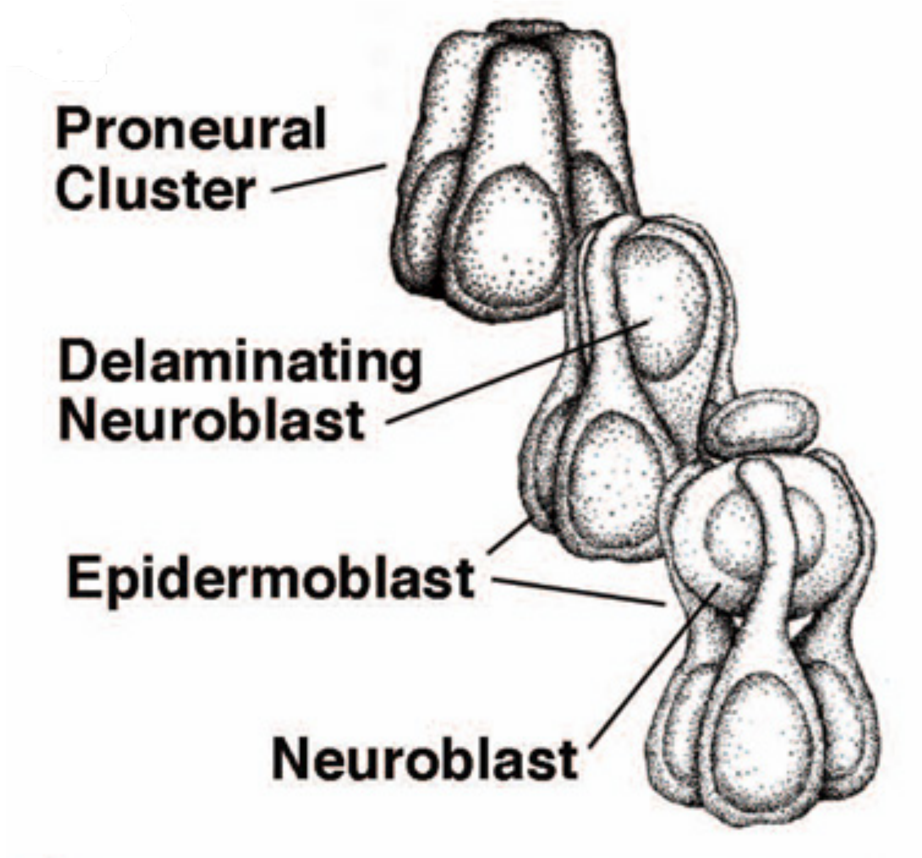
Neurons are generated from ventrolateral part of embryo (neuroectoderm)



- Cellularisation -> blastoderm
- Ventral furrow marks start of gastrulation
- Mesoderm invagination
- Ventral midline – future site of neurogenesis

Neuroblast formation

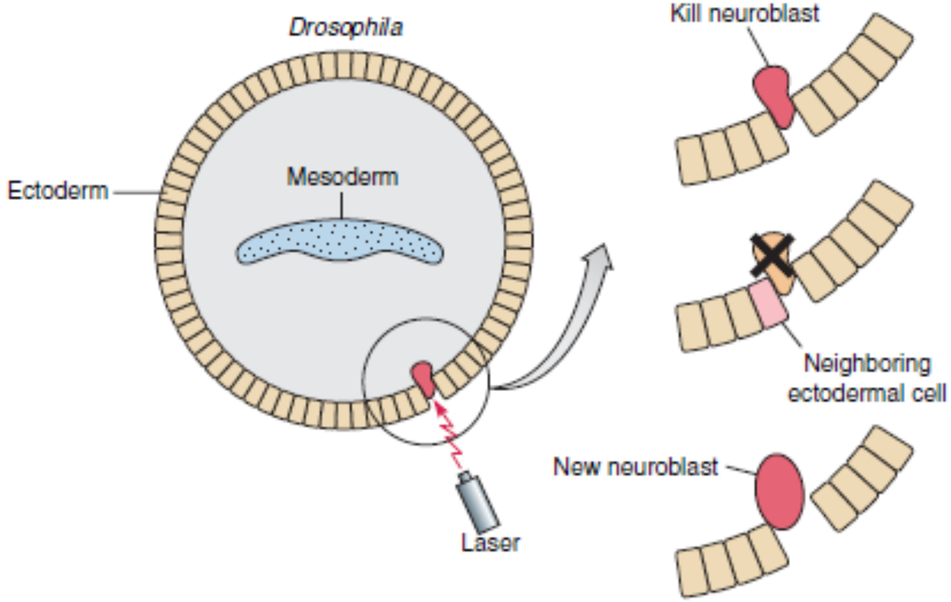




Neuroblast formation

Neuroblast selection is an active process

Neuroblasts inhibit neighboring cells to become a neuroblast



- The ablation of a delaminating neuroblast induces the redetermination of a neighbouring ectodermal cell to becomes a neuroblast

- Neuroblast inhibits neighbouring cells from becoming neuroblasts

Neuroblast formation

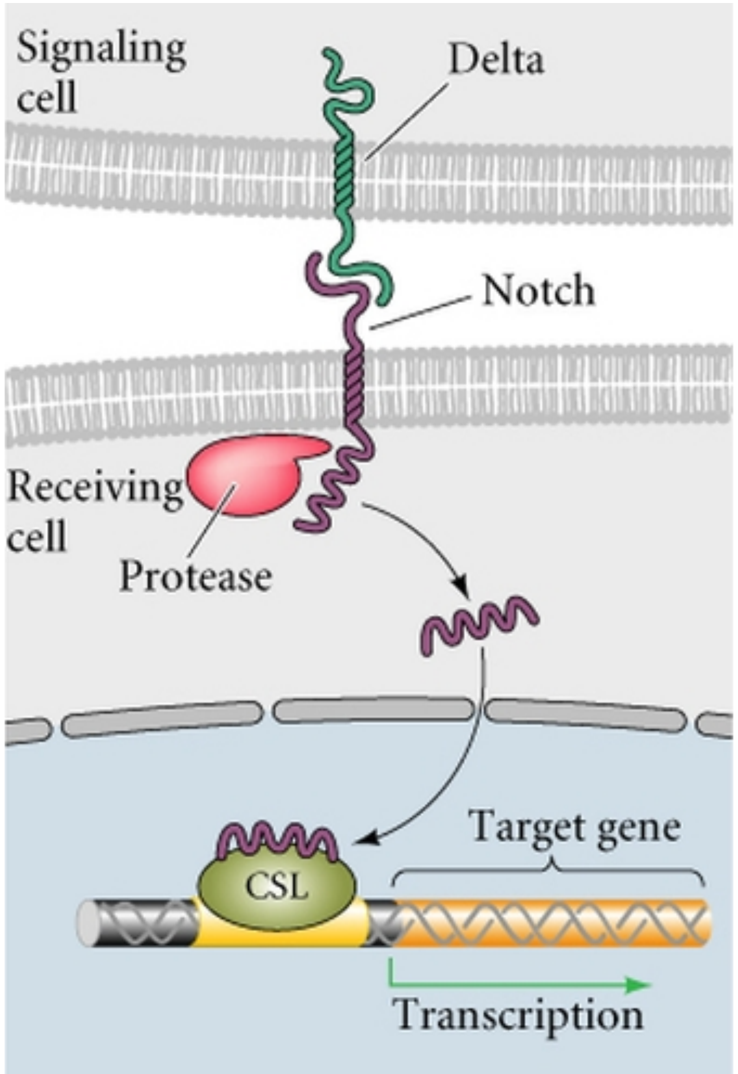
Neuroblast selection is an active process

Proneural Genes

- Achaete-Scute complex of genes (AS-C)
  - Achaete (ac), scute (sc), lethal of scute (lsc), asense (as)

Neurogenic Genes

- Notch-Delta pathway
  - Notch (N), Delta (Dl), Enhancer of split genes (E(spl)), Hairless (H), Suppressor of Hairless (Su(H)), brainiac (brn), mastermind (mam), neuralized (neu), kuzbanian (kuz), big brain (bib)…



Neuroblast selection is an active process

Proneural Genes

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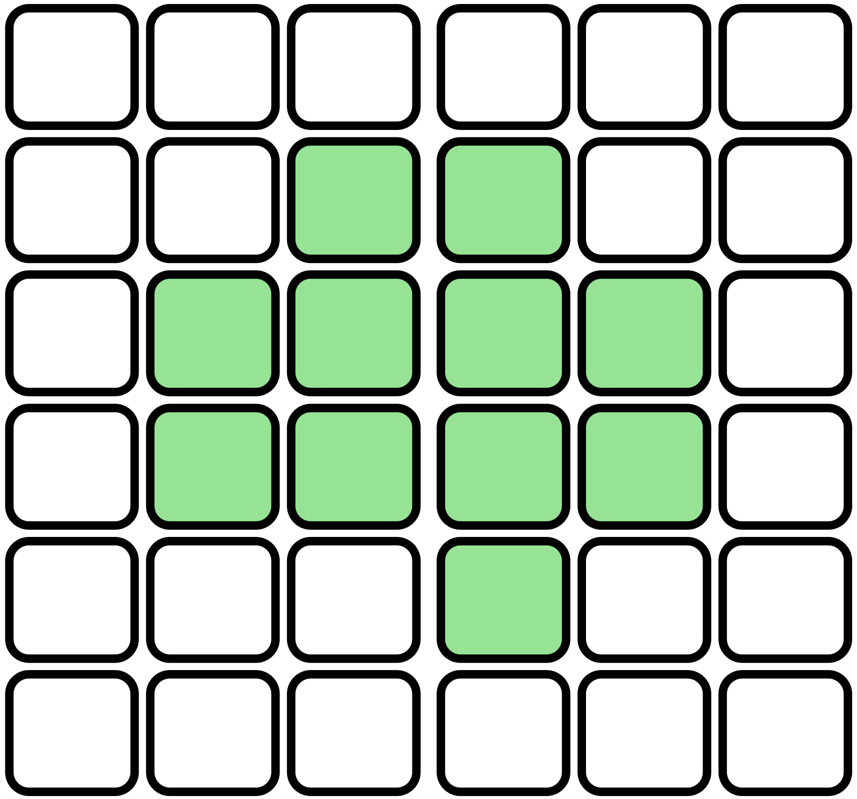
Neuroblast formation



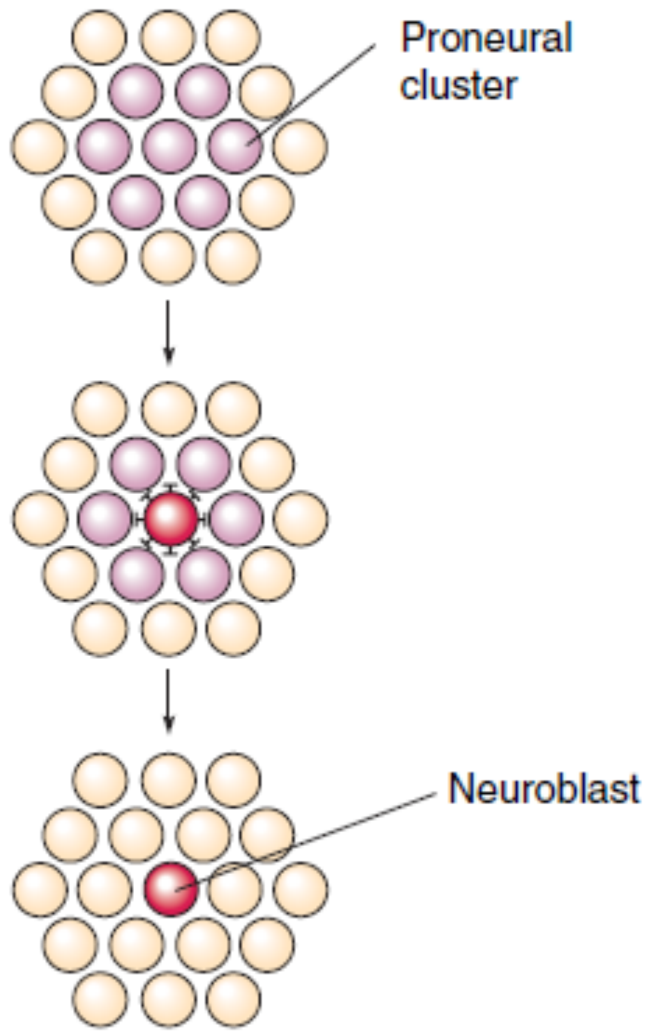
Equivalent cells

Cells expressing proneural genes become competent to acquire a neuroblast fate

Neuroblast selection is an active process

Example of an emergent property since it is not possible to know which cell will be neurogenic and which will be epithelial

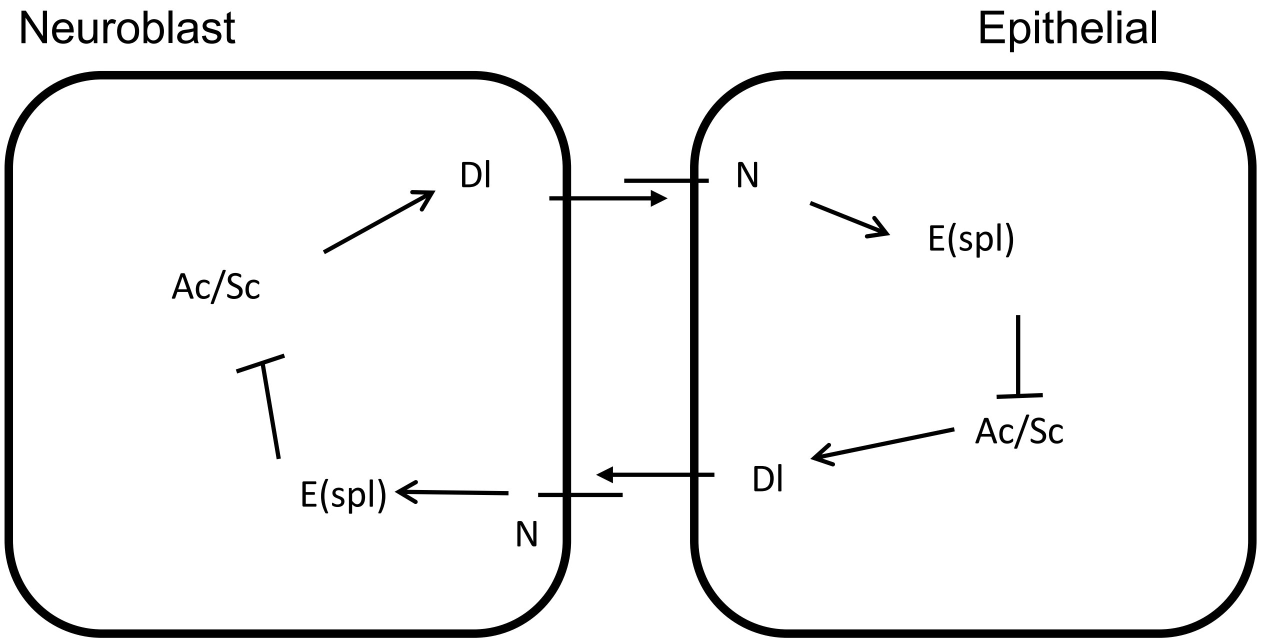
Neuroblast formation



Fortini ME Developmental Cell (2009)

Neuroblast formation

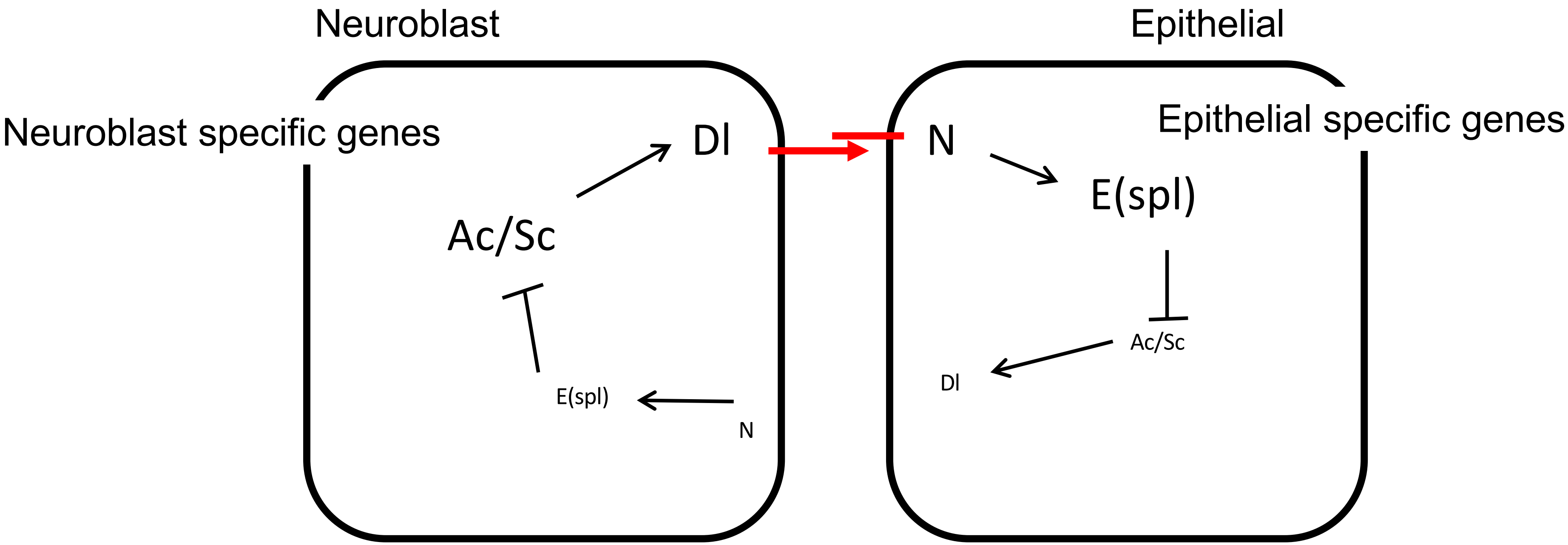
How does lateral inhibition take place?



Cells present similar levels of Notch activity
(Mutual inhibition)

Neuroblast formation

How does lateral inhibition take place?

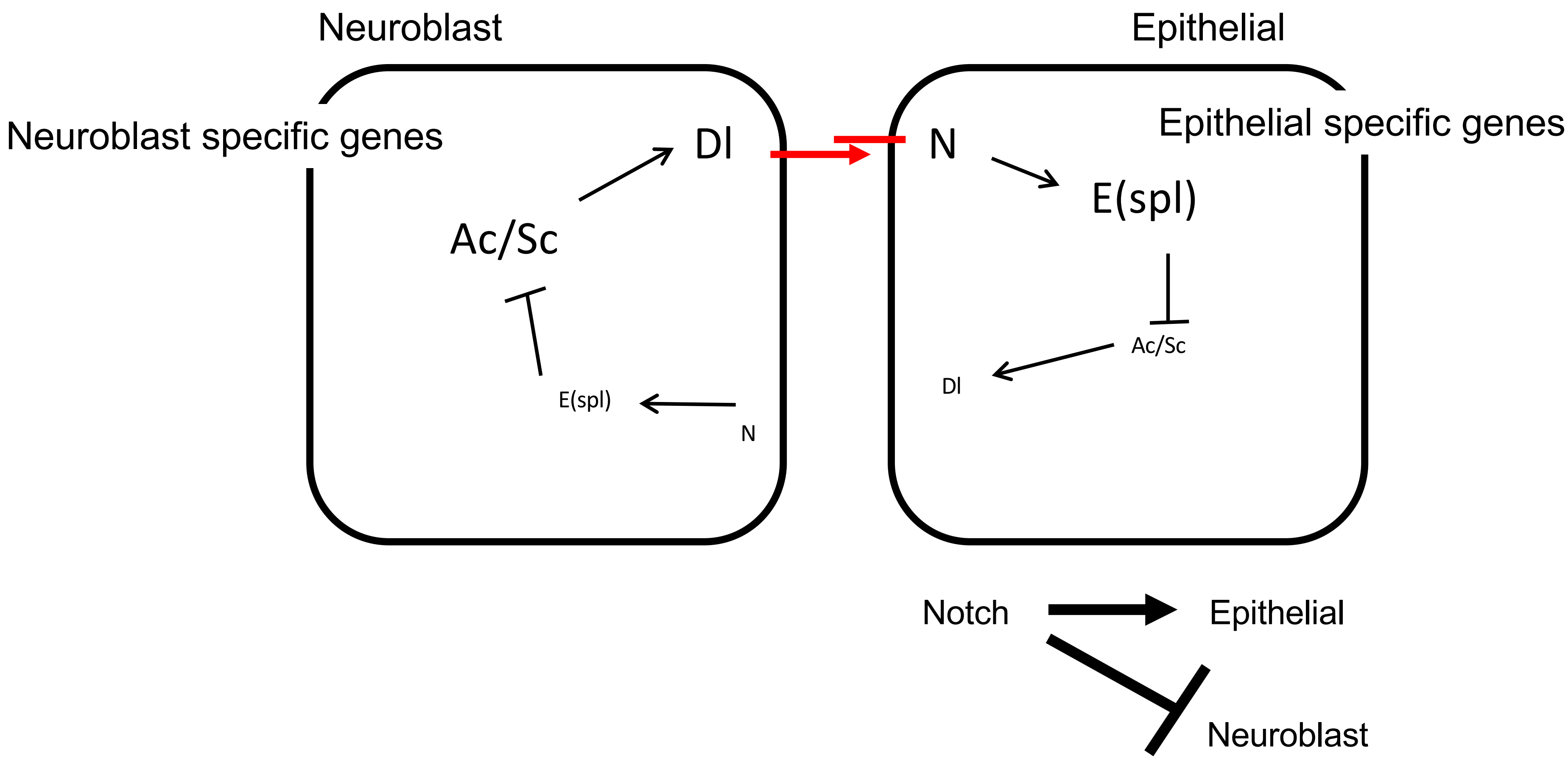


Due to a stochastic (random) events one cell expresses more Delta ligand than the other.

The net signal is unidirectional.
The activation of Notch receptor results in decreased Delta expression in cell that receive the signal.

Neuroblast formation

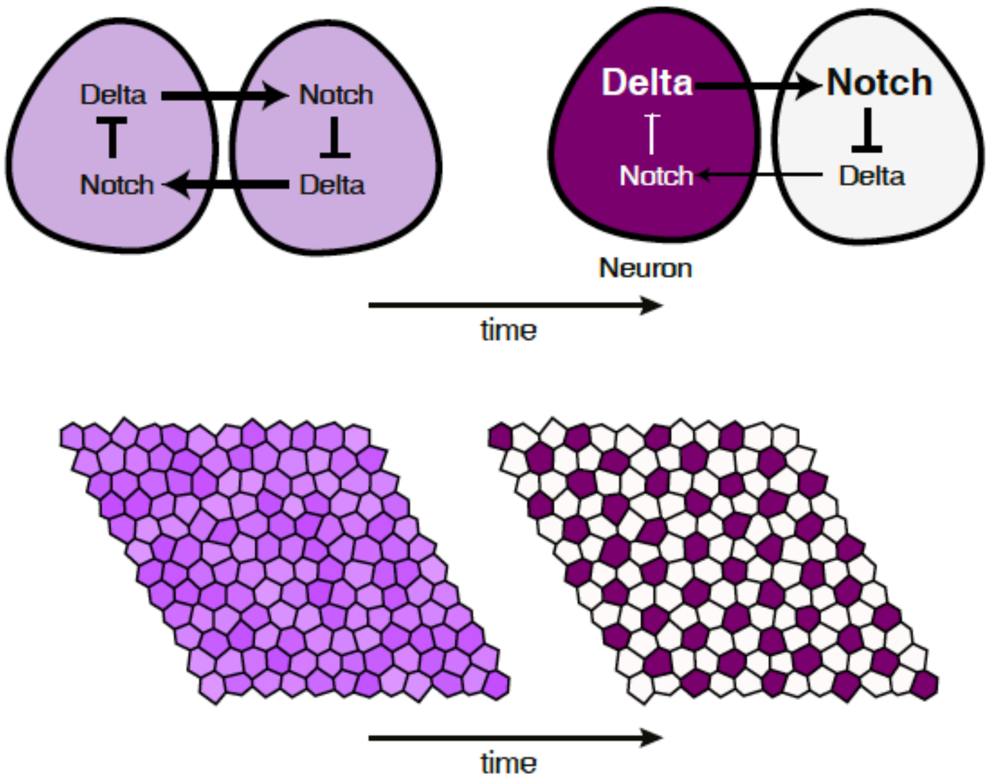
How does lateral inhibition take place?



When Notch or Delta are absent, the ectoderm cells become neurogenic.
The cell that becomes the neuroblast inhibits the surrounding cells from this fate and become epithelial cells instead (lateral inhibition).

Neuroblast formation

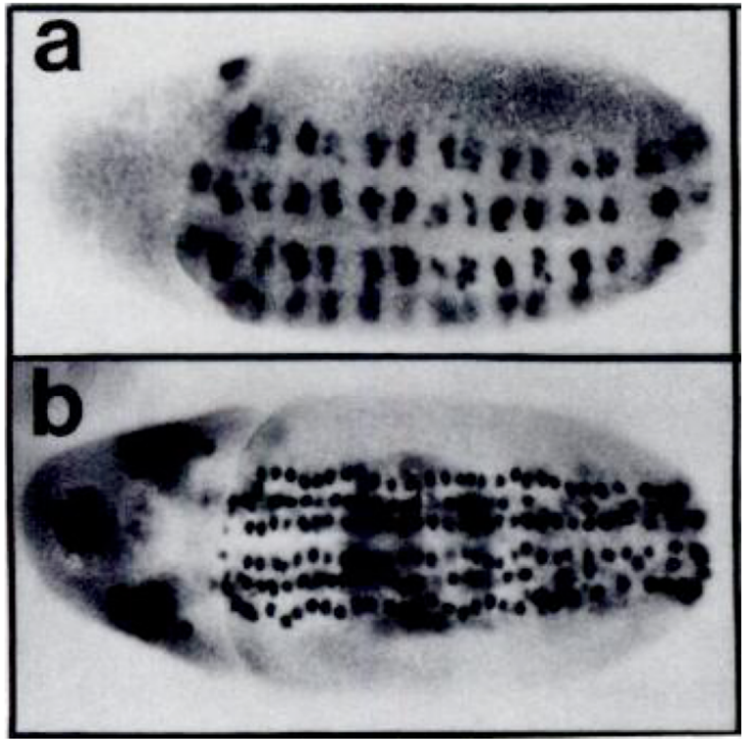
How does lateral inhibition take place?

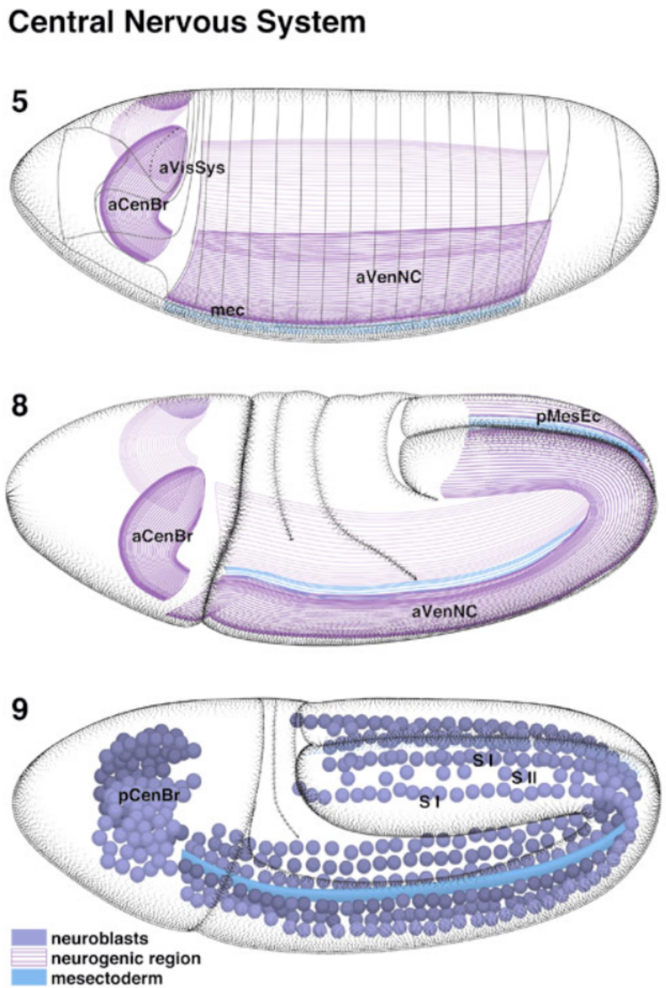


Neurogenic selection through lateral inhibition

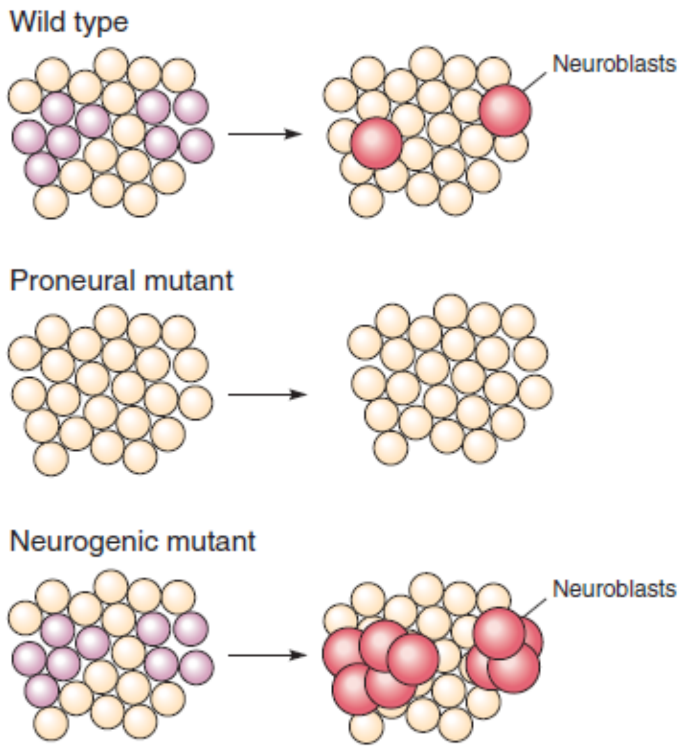
Neuroblast formation

Lateral inhibition during neurogenesis in *Drosophila*





achaete-scute complex genes and the Notch pathway are required for neuroblasts formation



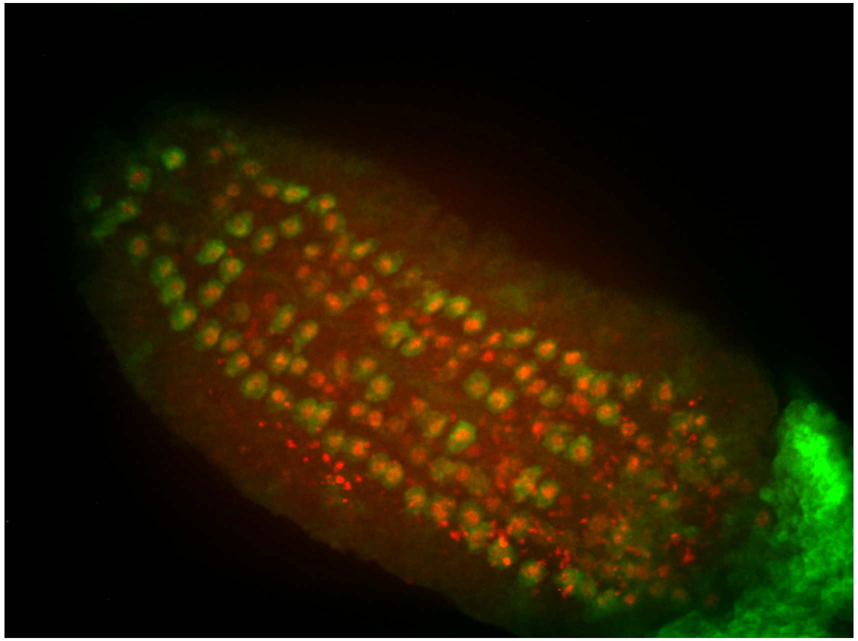
- Only one of proneural cells becomes a neuroblast in a normal conditions
- Flies mutant for proneural genes (achaete/scute) don't form neuroblasts
- Flies mutant for Notch or Delta – form ectopic neuroblasts

Genetic control of neuroblasts formation

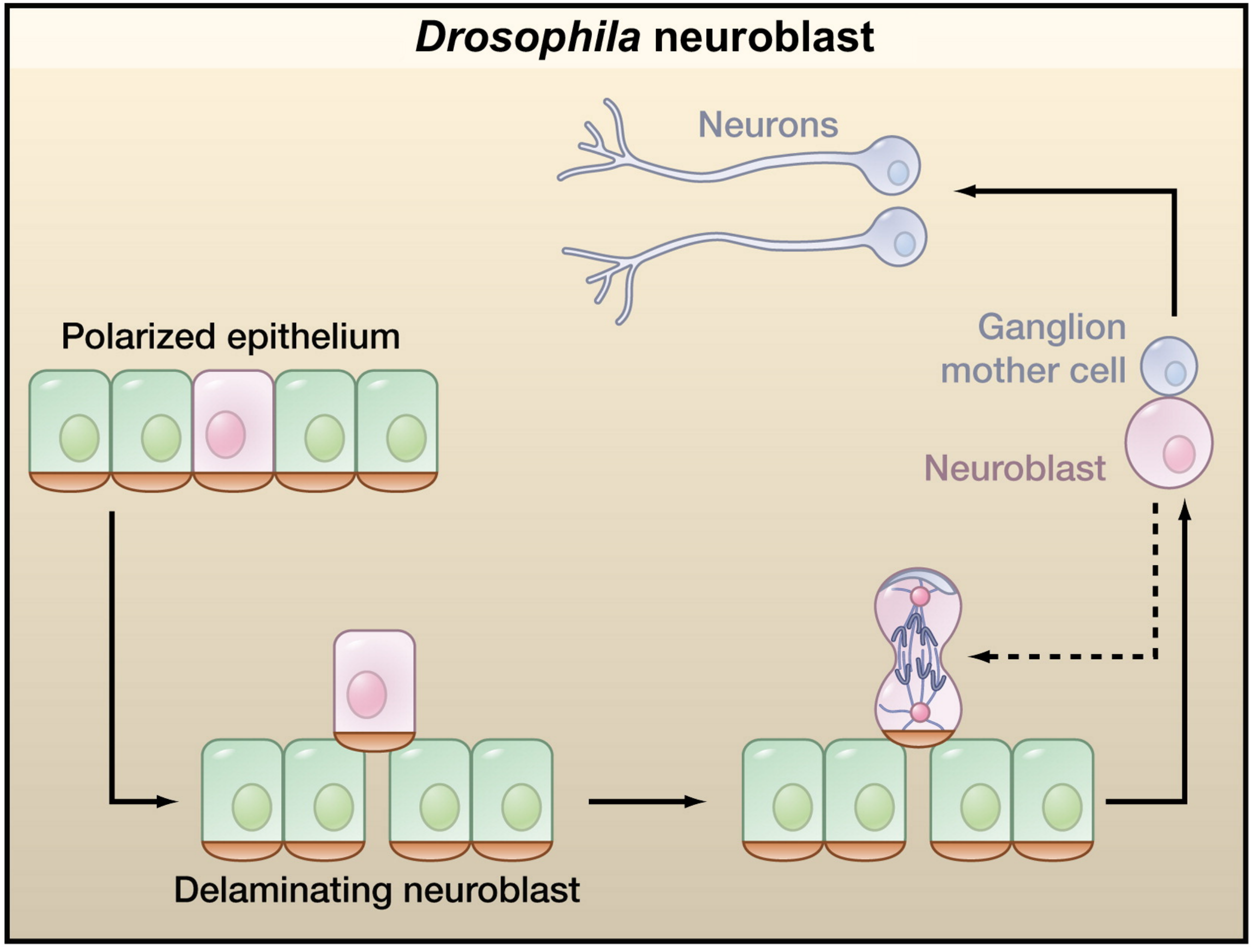
1) Specific genes expression pattern the neuroectoderm and pre-define territories with neural competence (pre-pattern genes)

2) In this territories, the expression of so-called proneural genes specified proneural fate in all cells in these clusters (proneural clusters). These genes belong to the Achaete-Scute complex (AS-C) and encode conserved basic-helix-loop-helix transcription factors

3) Single neuroblasts are specified within proneural clusters via the activation of so-called neurogenic genes –the Notch pathway (mutual followed by lateral inhibition)

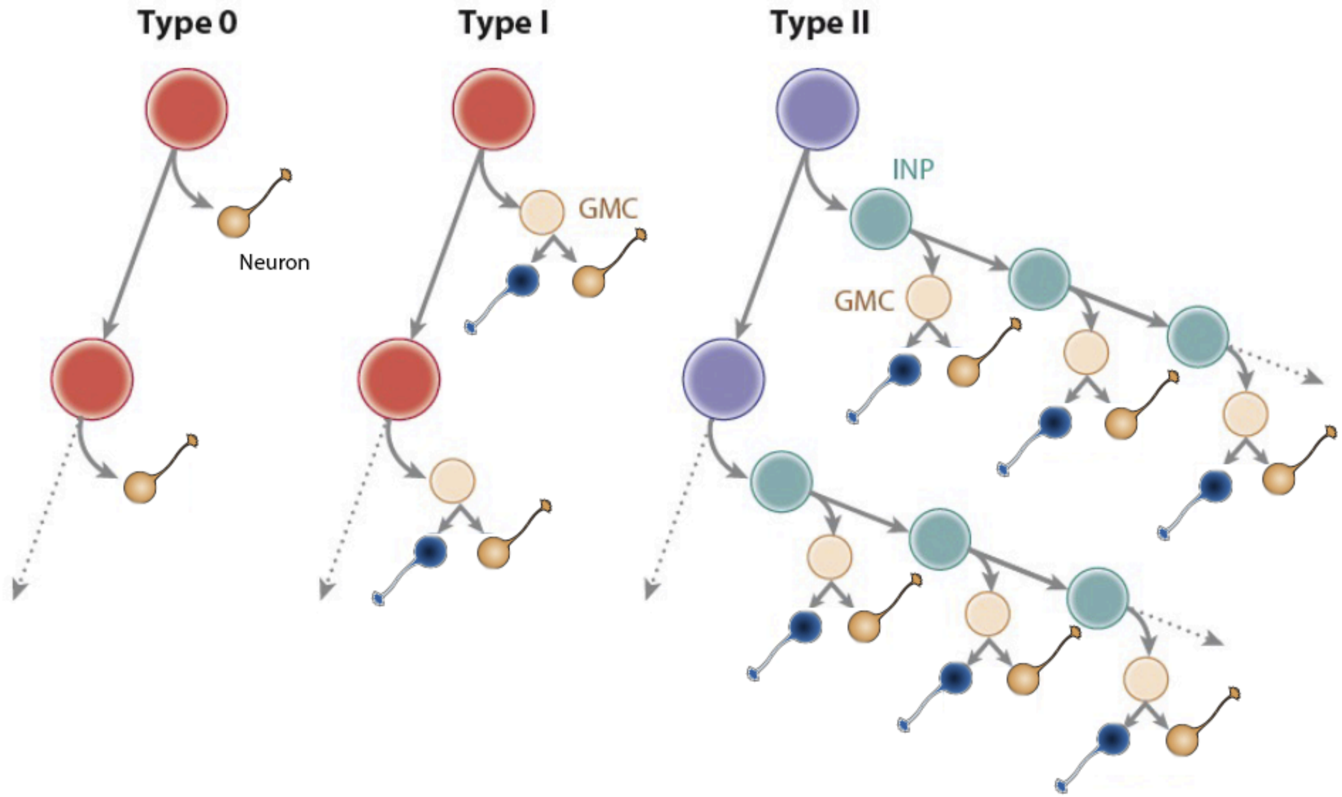


Neuroblasts undergo a self-renewing asymmetric division



(Simons & Clevers 2011)

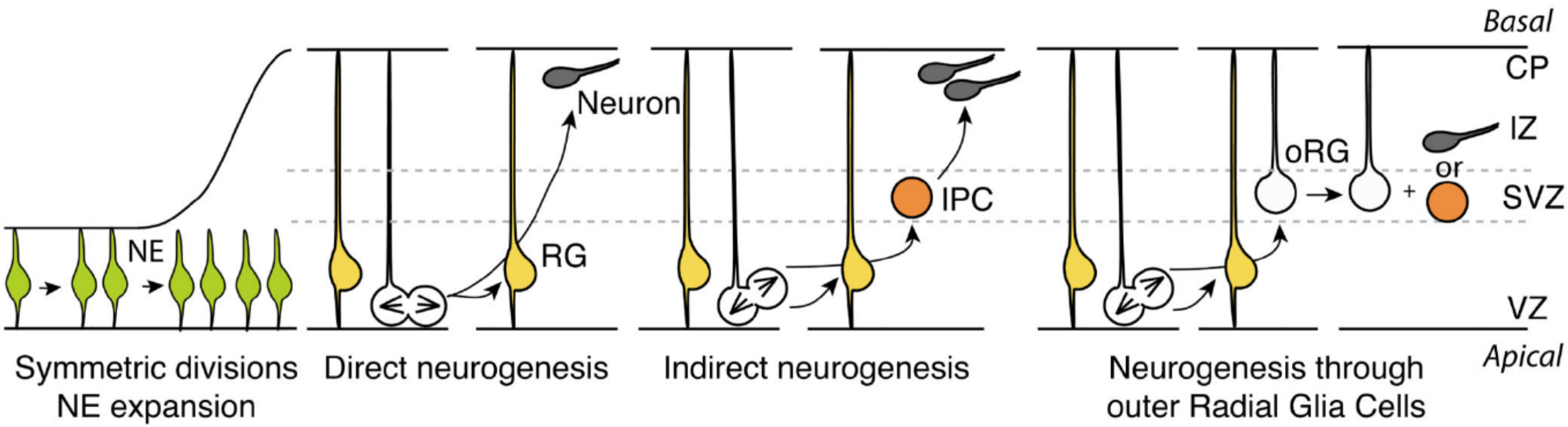
Neuroblast cell lineage



(Doe C, 2017)

Three modes of NB cell division. The largest cells are NBs. Abbreviations: GMC, ganglion mother cell; INP, intermediate neural progenitor; n, neurons [all sibling neurons are either Notch_{ON} (n∗) or Notch_{OFF} (n)].

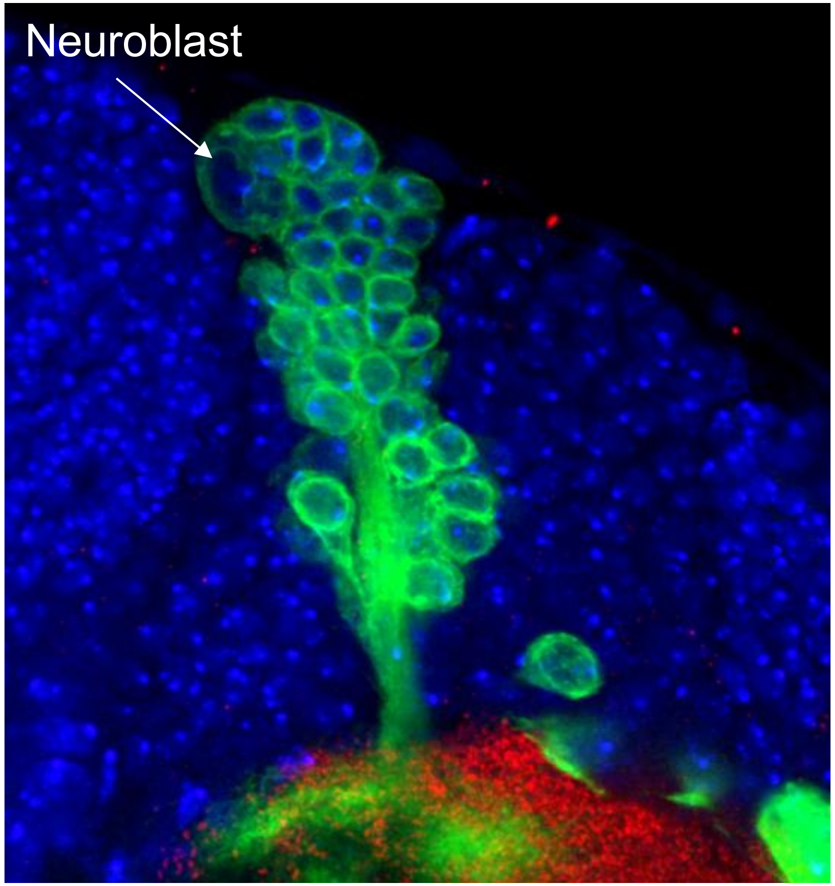
Neuroblast cell lineage



Homem et al (2015)

Development of the mouse neocortex.
NE neuroepithelial cells. RG radial glia cells. IPC Intermediate progenitor cell. oRG outer rafial glia.

Neuroblast cell lineage



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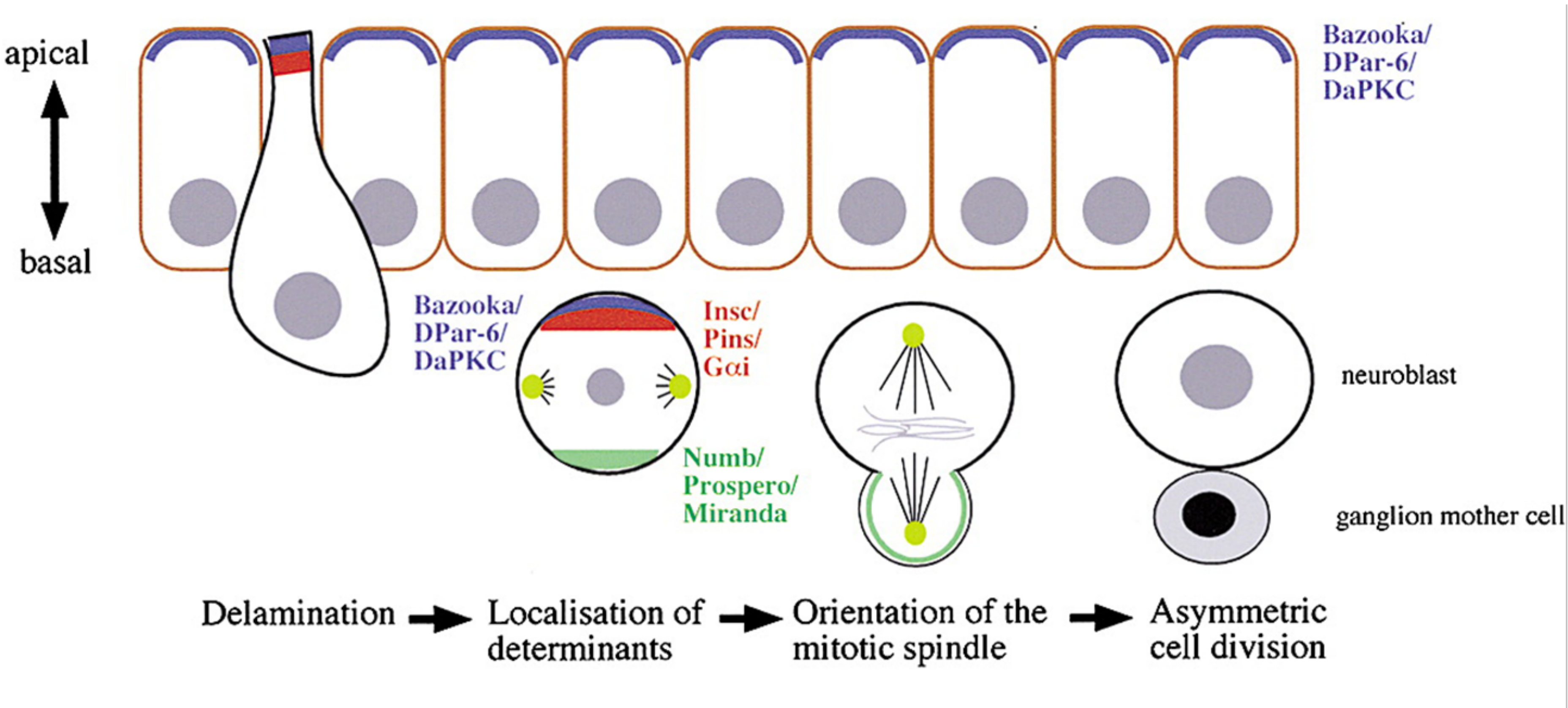
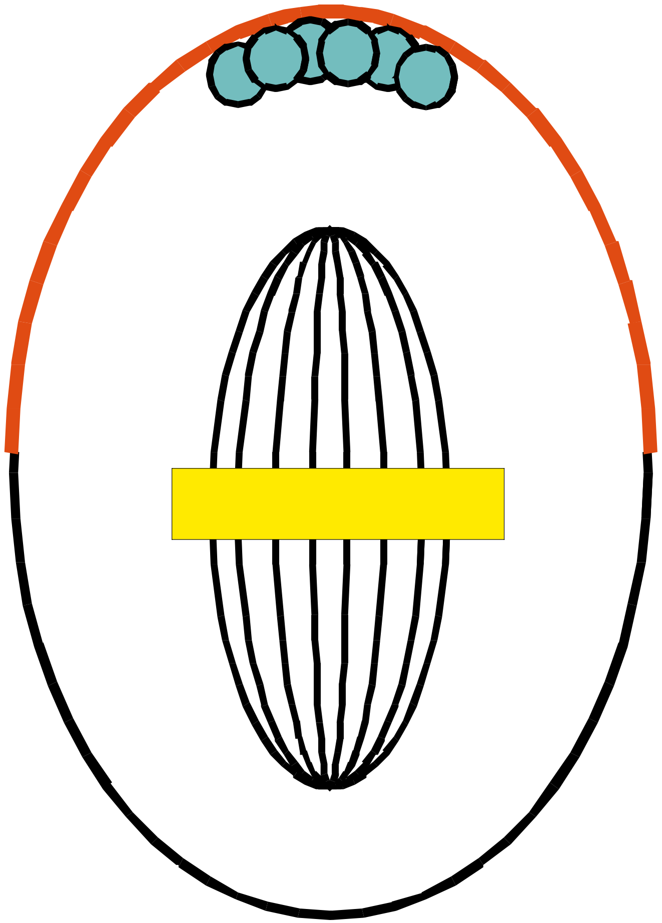


Neuroblasts divide asymmetrically

Asymmetric cell division

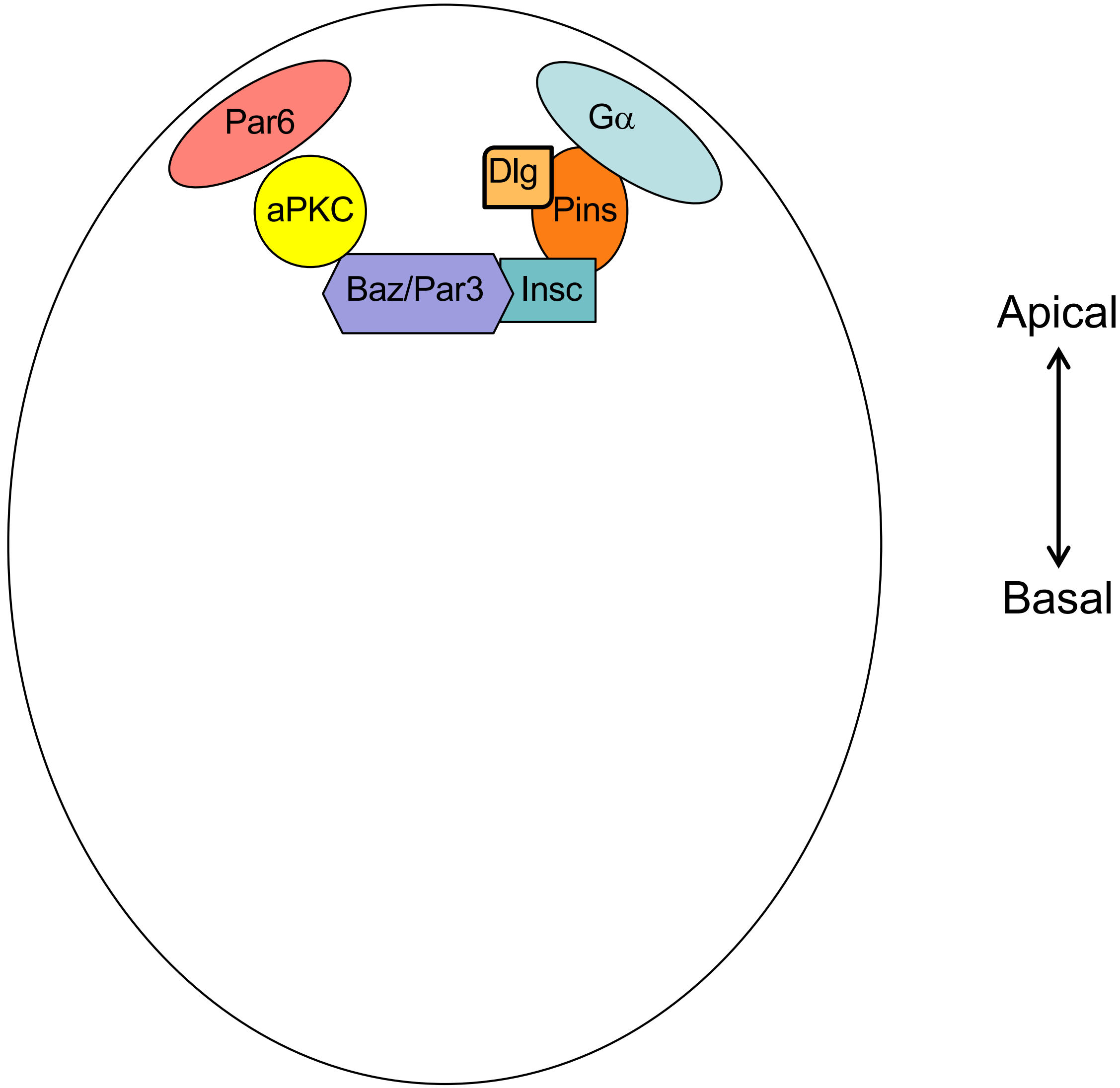
Requirements

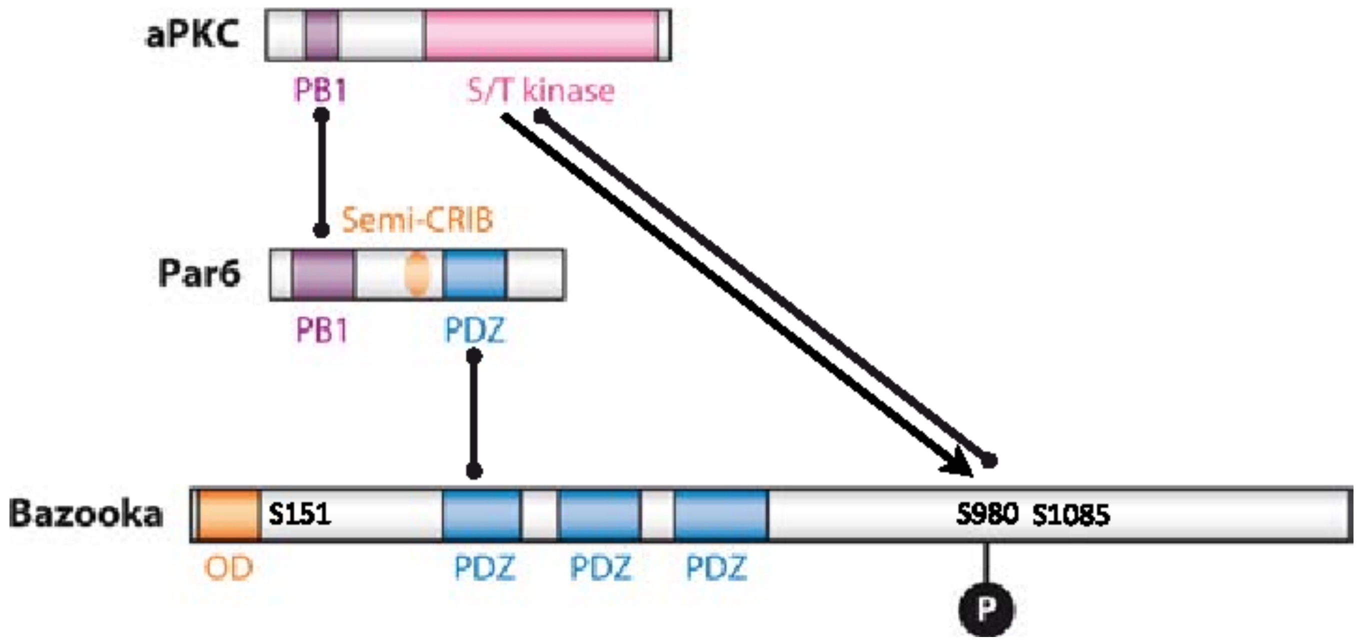


(1) Cell polarity
(2) Localisation of cell determinants
(3) Mitotic spindle orientation

Neuroblast asymmetric cell division

The apical complex

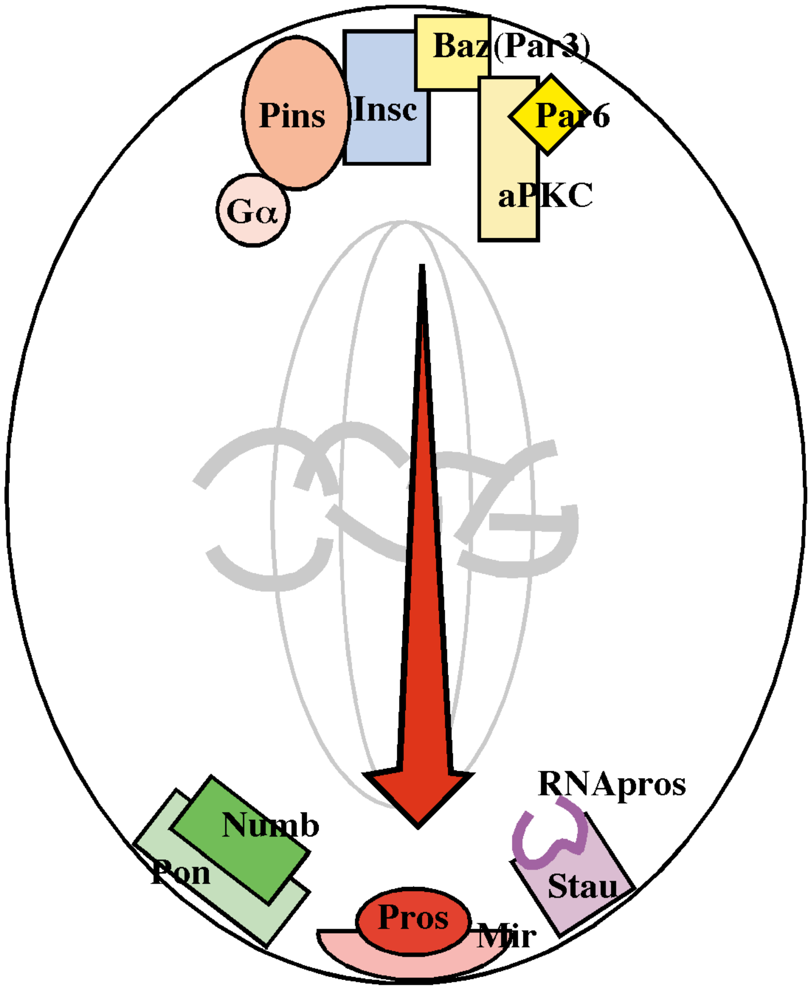




aPKC
PB1
S/T kinase
Semi-CRIB
Par6
PB1
PDZ
Bazooka
S151
S980
S1085
OD
PDZ
PDZ
PDZ
P

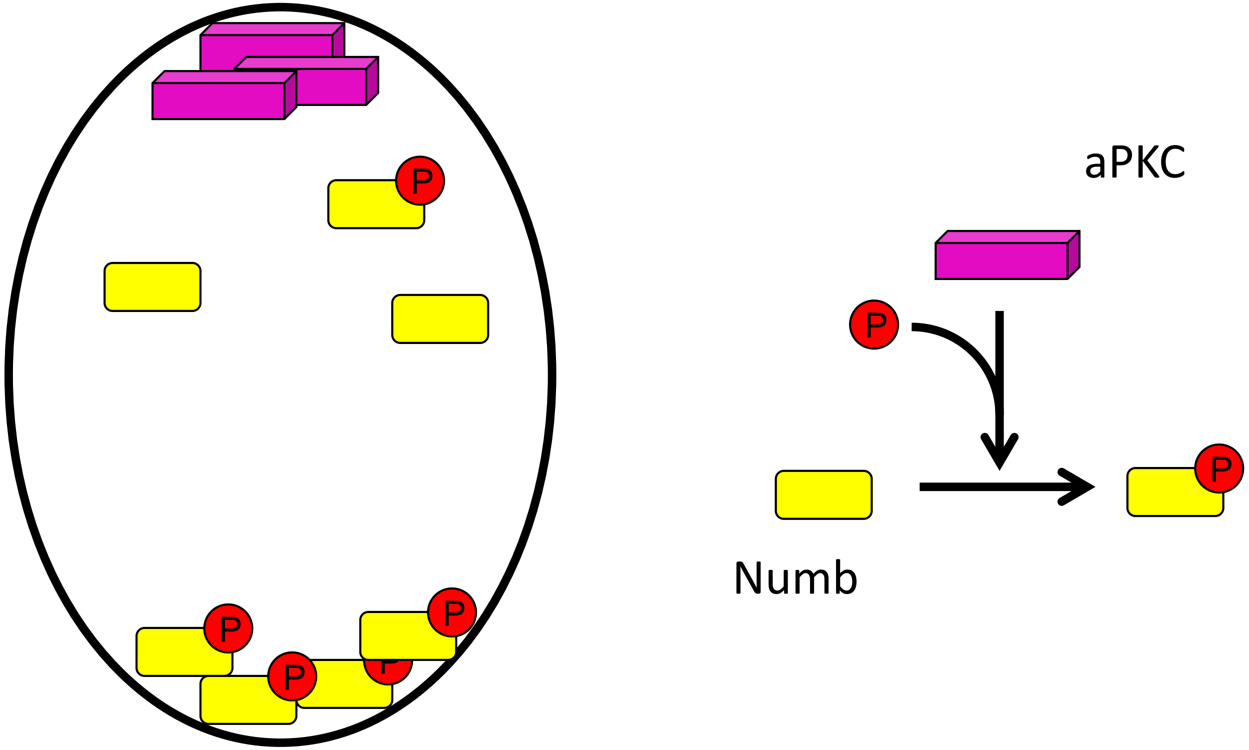
Neuroblast asymmetric cell division

Apical complex controls basal localisation of factors during neuroblast division



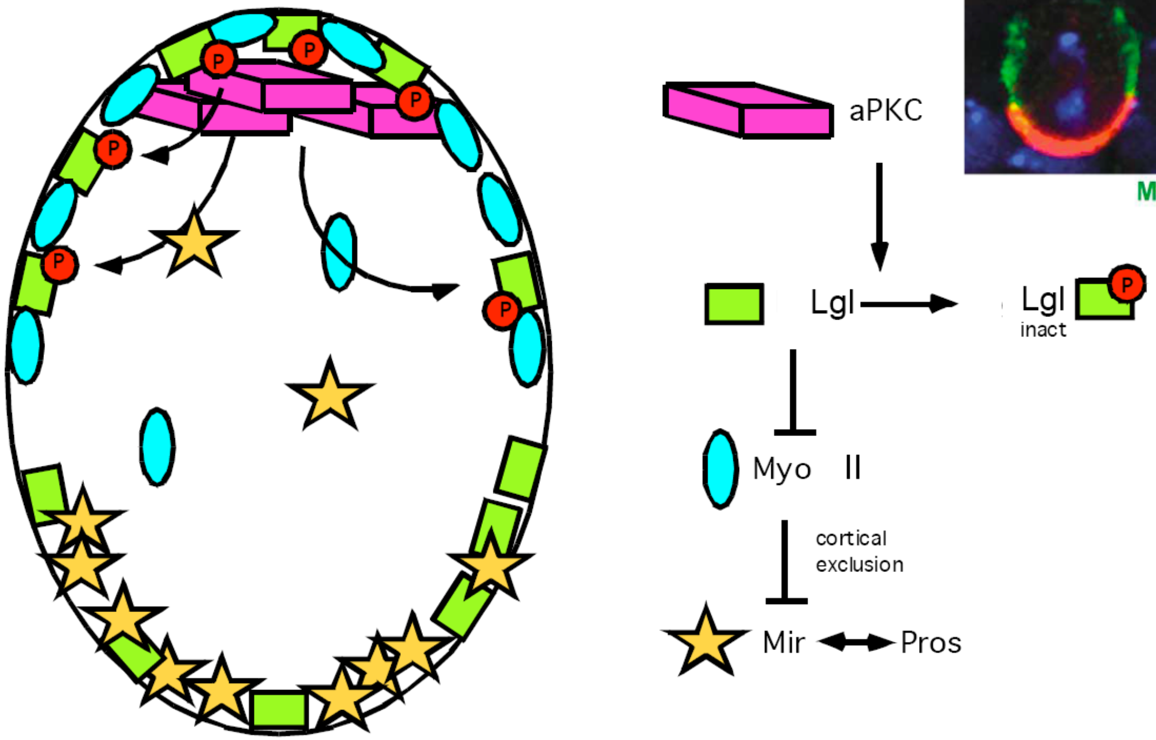
Neuroblast asymmetric cell division

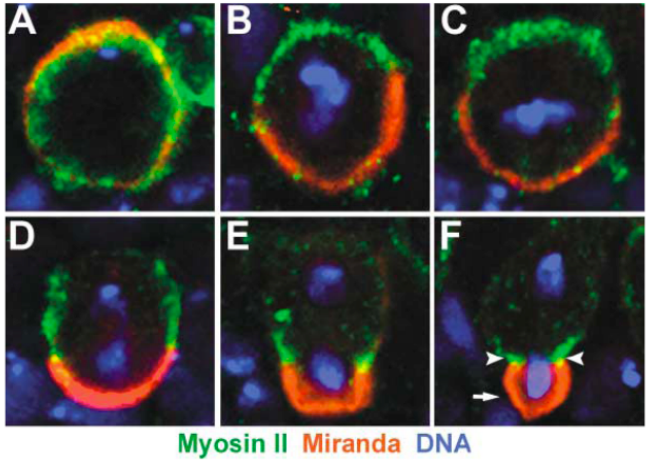
Numb



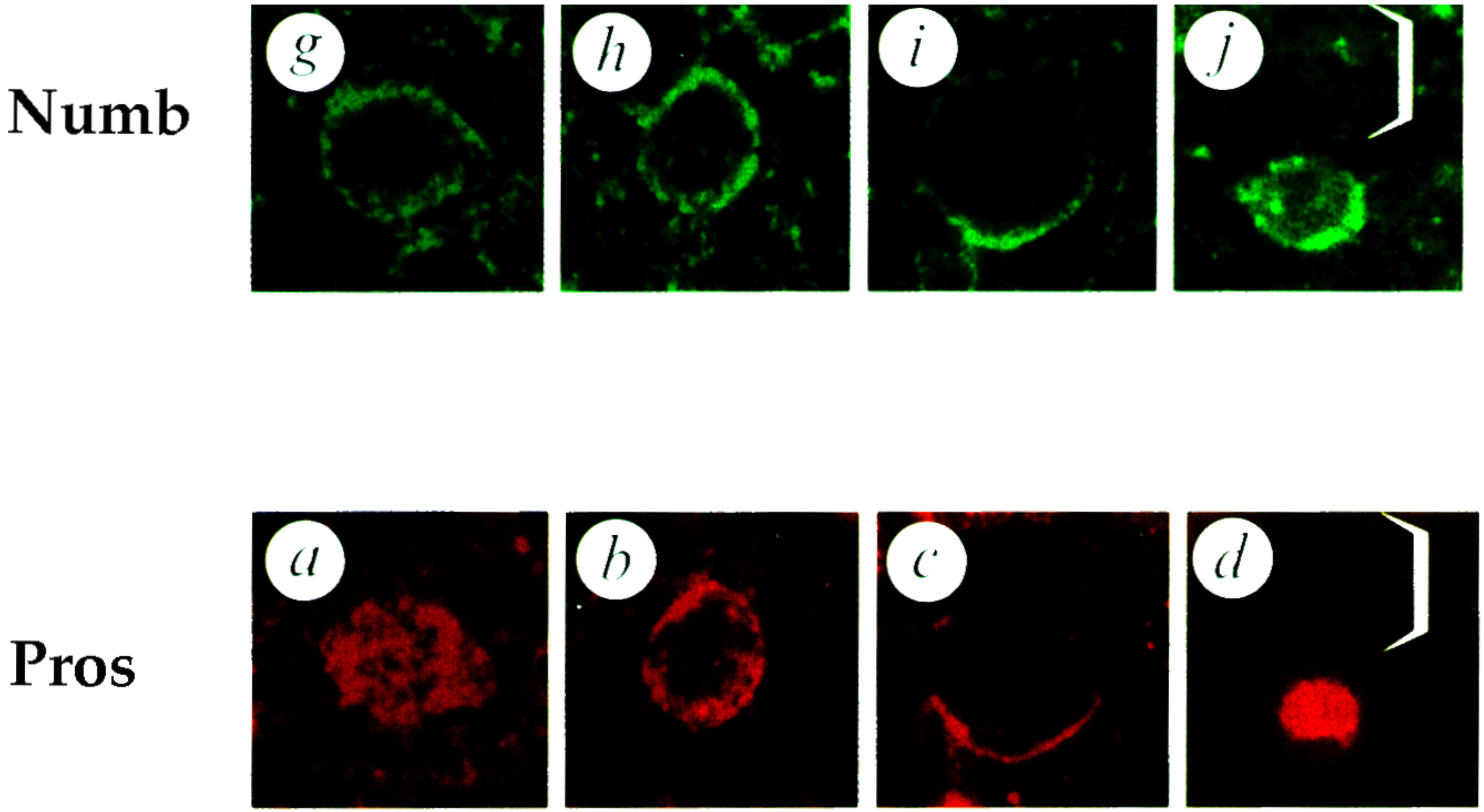
Neuroblast asymmetric cell division

Prospero



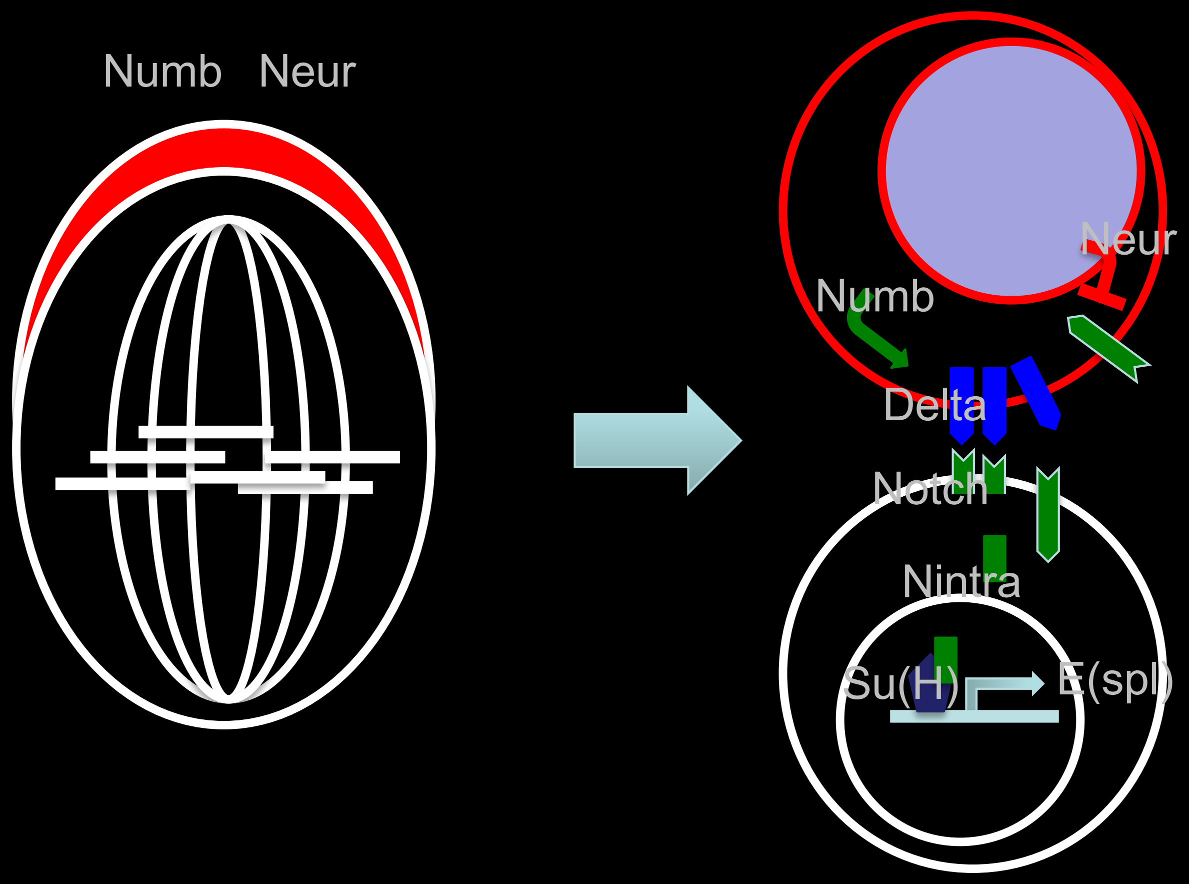


Neuroblast asymmetric cell division

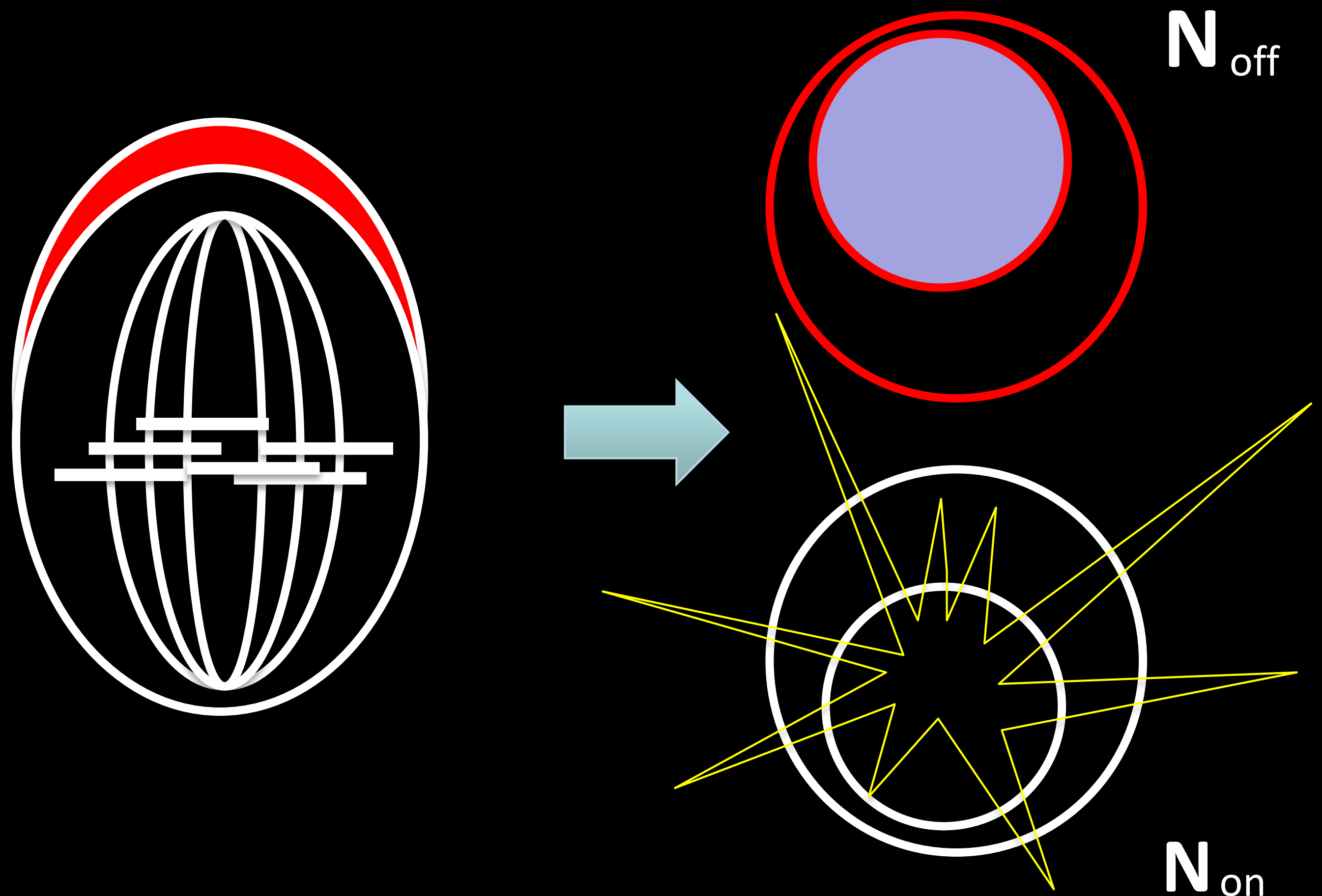


Knoblich (1995)

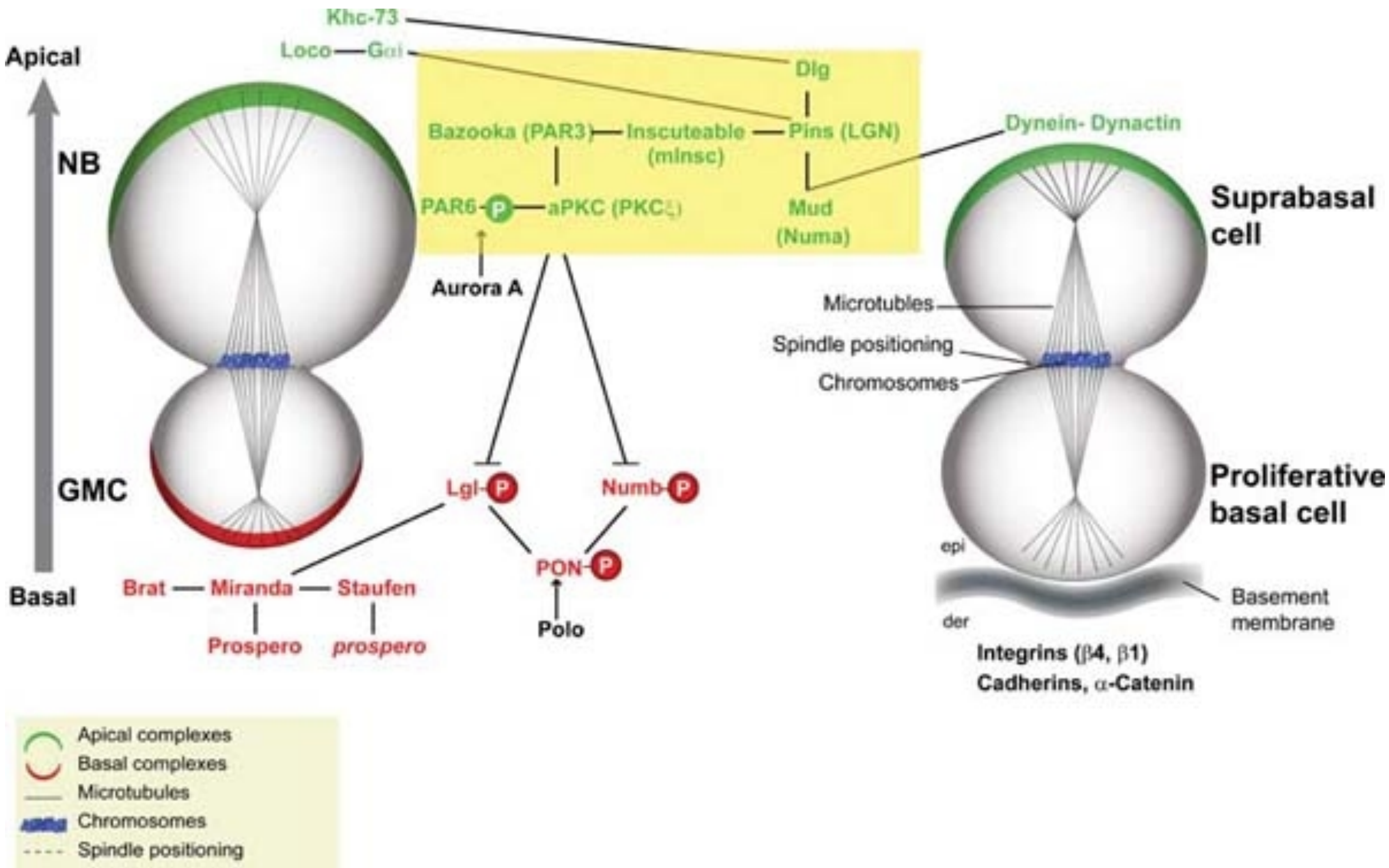
Binary cell decisions and asymmetric cell divisions



Binary cell decisions and asymmetric cell divisions

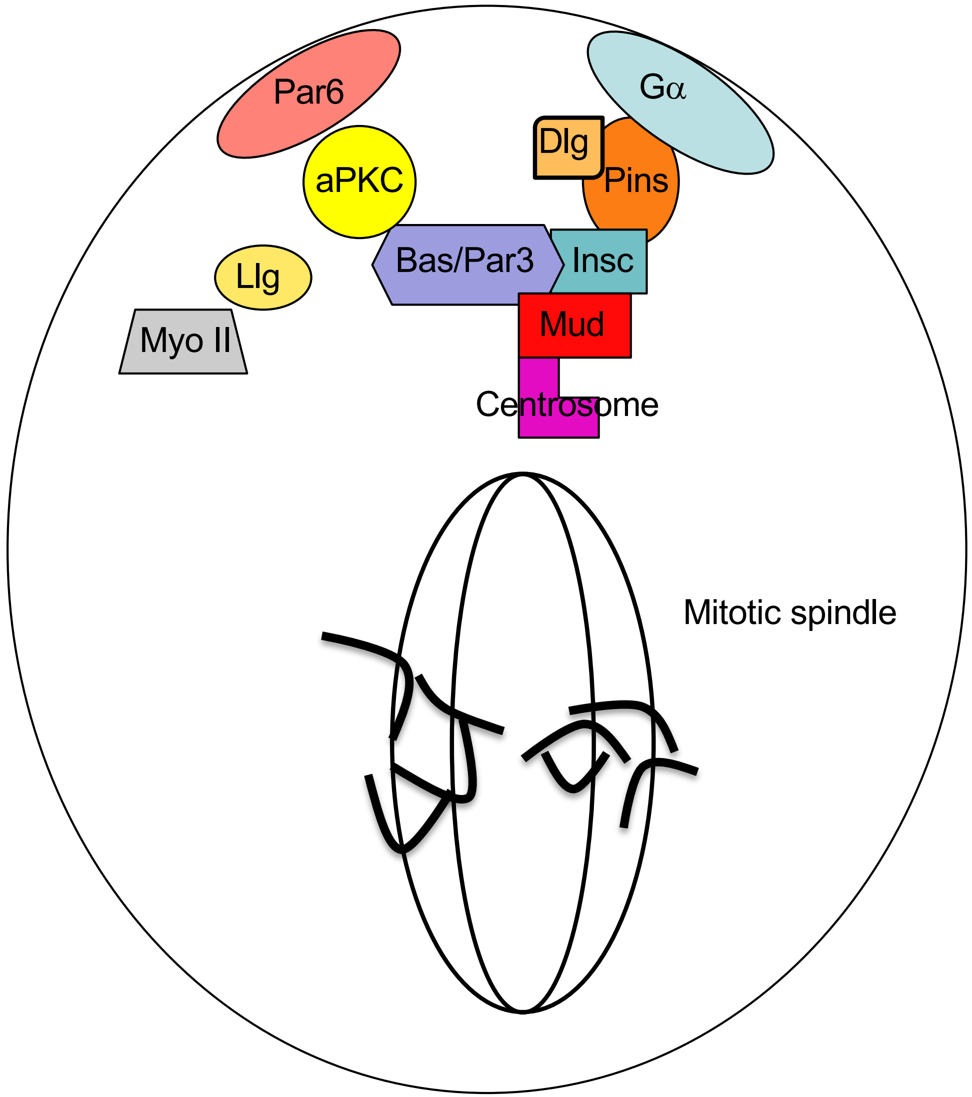


Neuroblast asymmetric cell division



Baker NE, Brown NL. All in the family: proneural bHLH genes and neuronal diversity. (2018) Development;145.

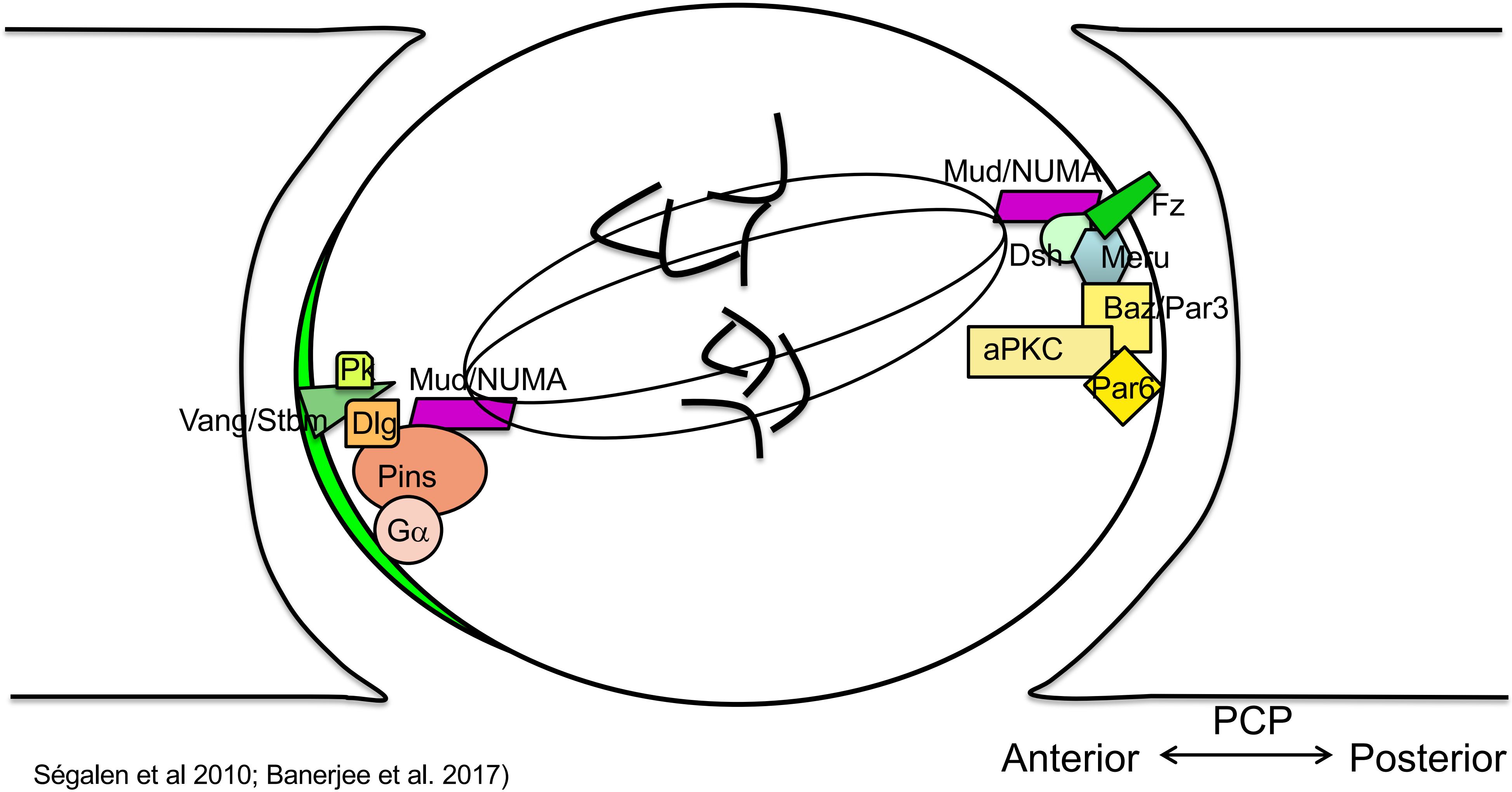
Neuroblast asymmetric cell division



SOP asymmetric cell division

Asymmetry is induced via the planar cell polarity (PCP) pathway

Posterior and anterior complexes control fat determinants segregation and spindle orientation



Ségalen et al 2010; Banerjee et al. 2017)

The Peripheral Nervous System

Sensory organs of the notum

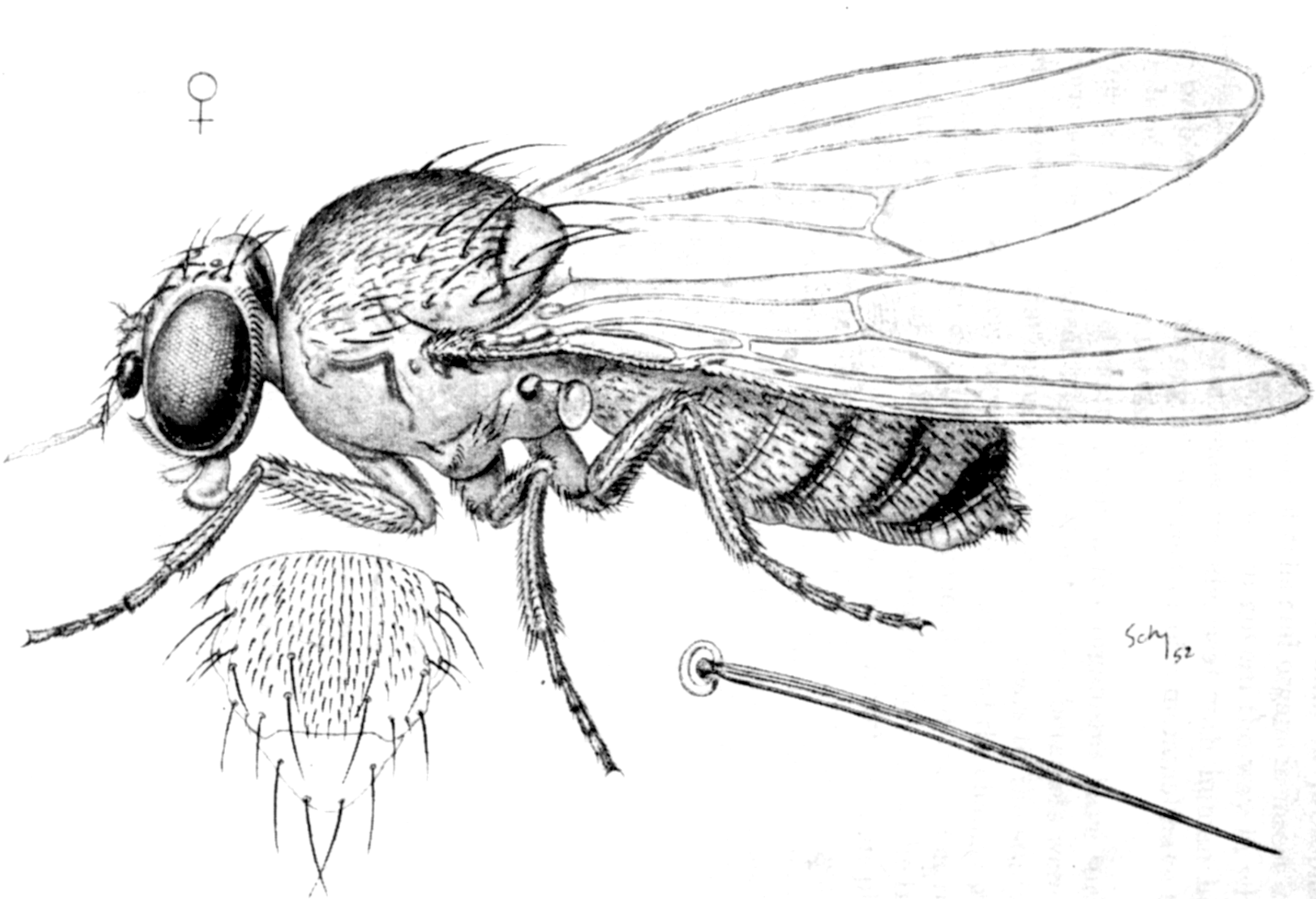
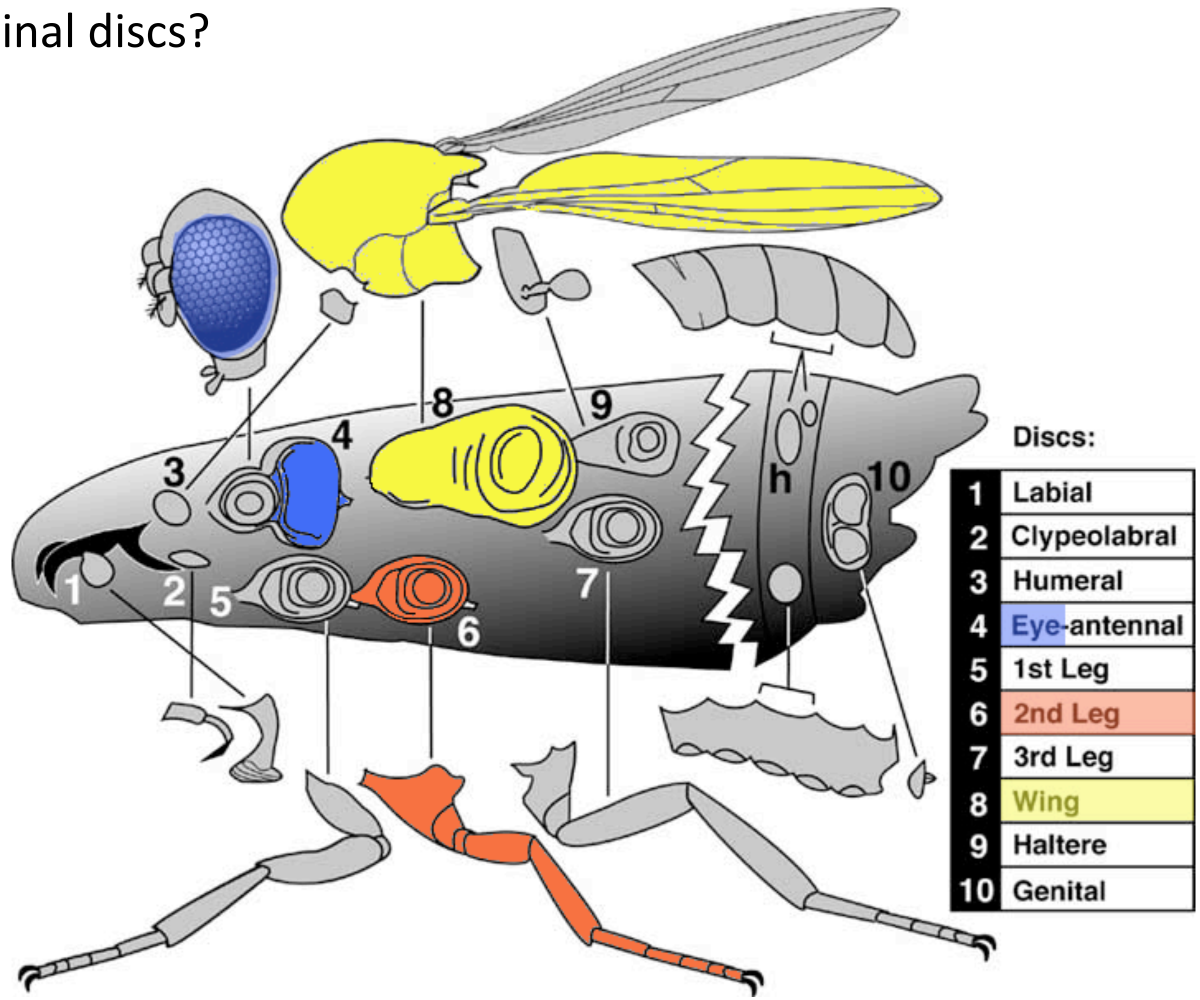


FIG. 1. *Drosophila melanogaster*. *Above*, side view, slightly oblique, of a female. *Below, left*, dorsal view of the thorax showing pattern of bristles and hairs. *Below, right*, a bristle in its socket.

Sensory organs of the notum

Selection of the SOP/pI cell

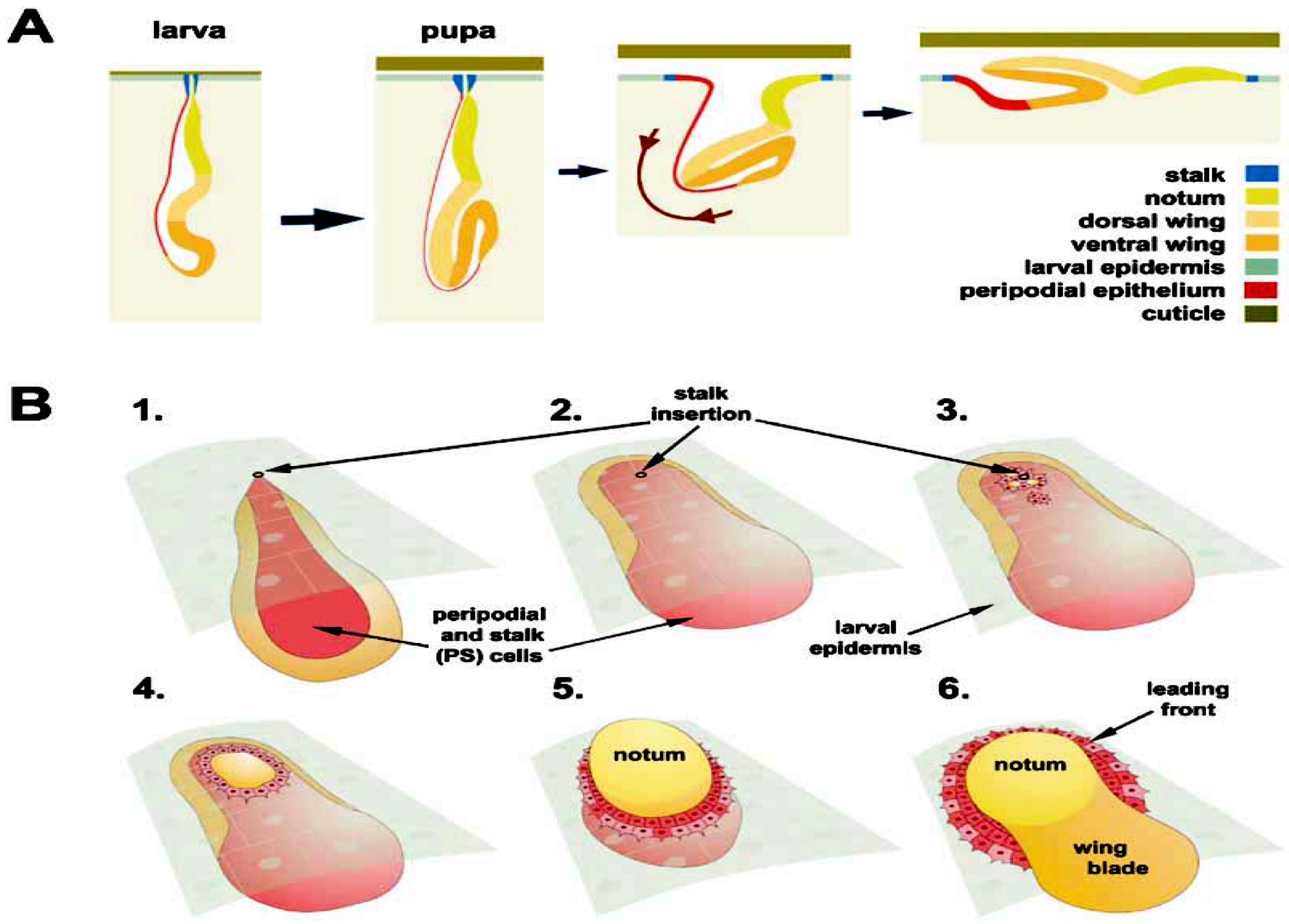
What are the imaginal discs?



Sensory organs of the notum

Selection of the SOP/pI cell

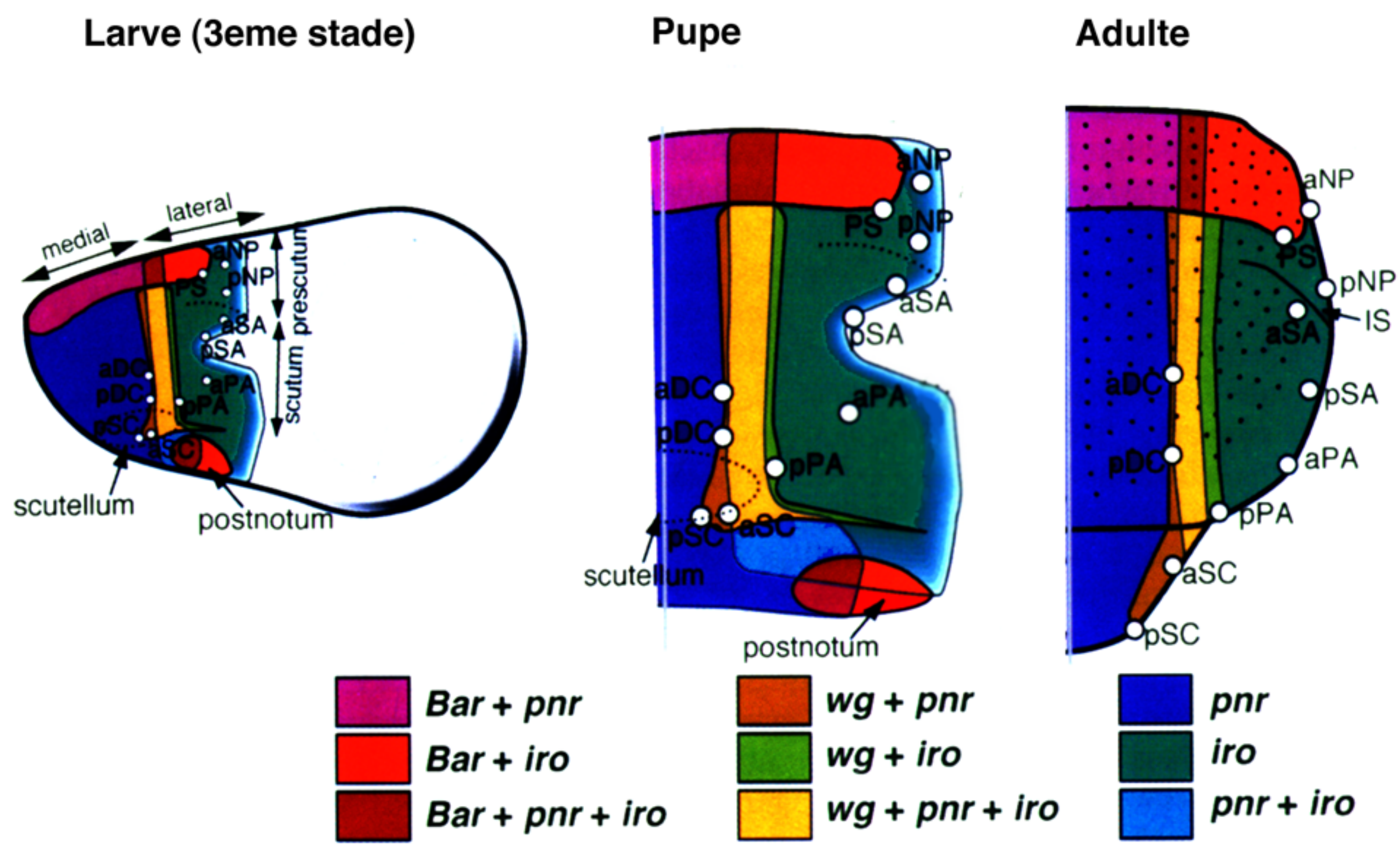
Wing discs eversion during metamorphosis



Sensory organs of the notum

Selection of the SOP/pI cell

Prepattern genes

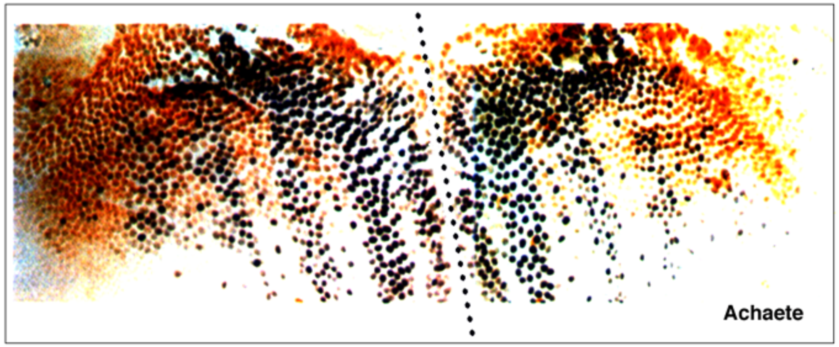


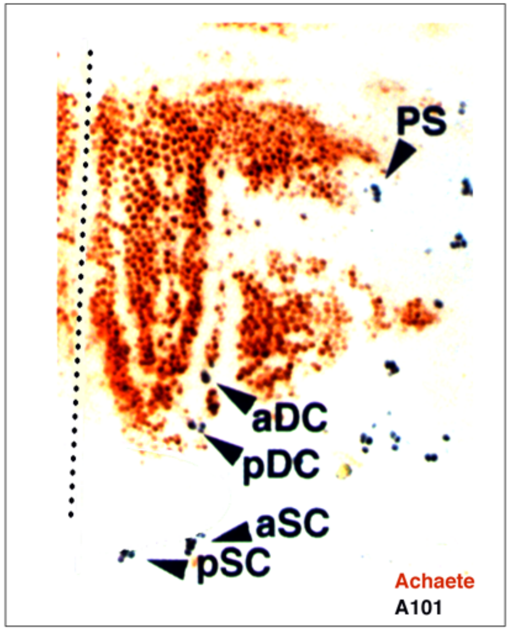
Sensory organs of the notum

Selection of the SOP/pI cell

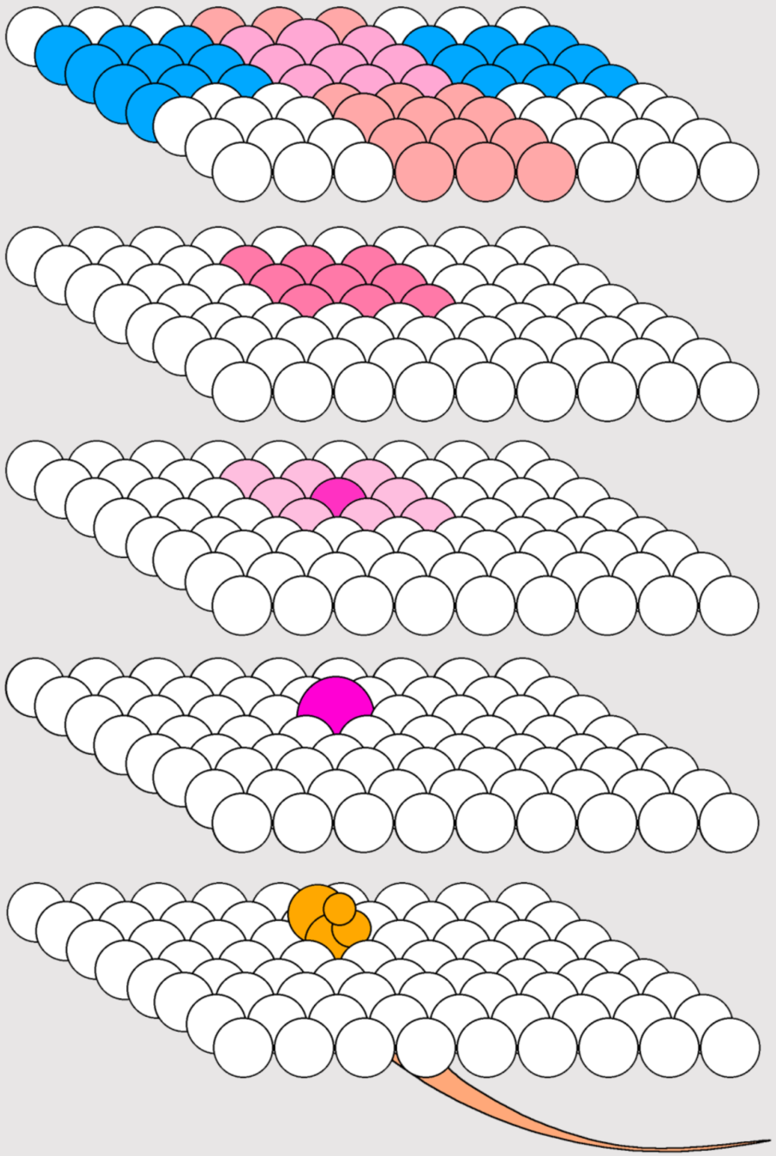
Proneural genes

Expression d'achaete dans le notum (8 hrs APF)





Sato et al., (1999) Development 126; 1457-1466



Pre-pattern

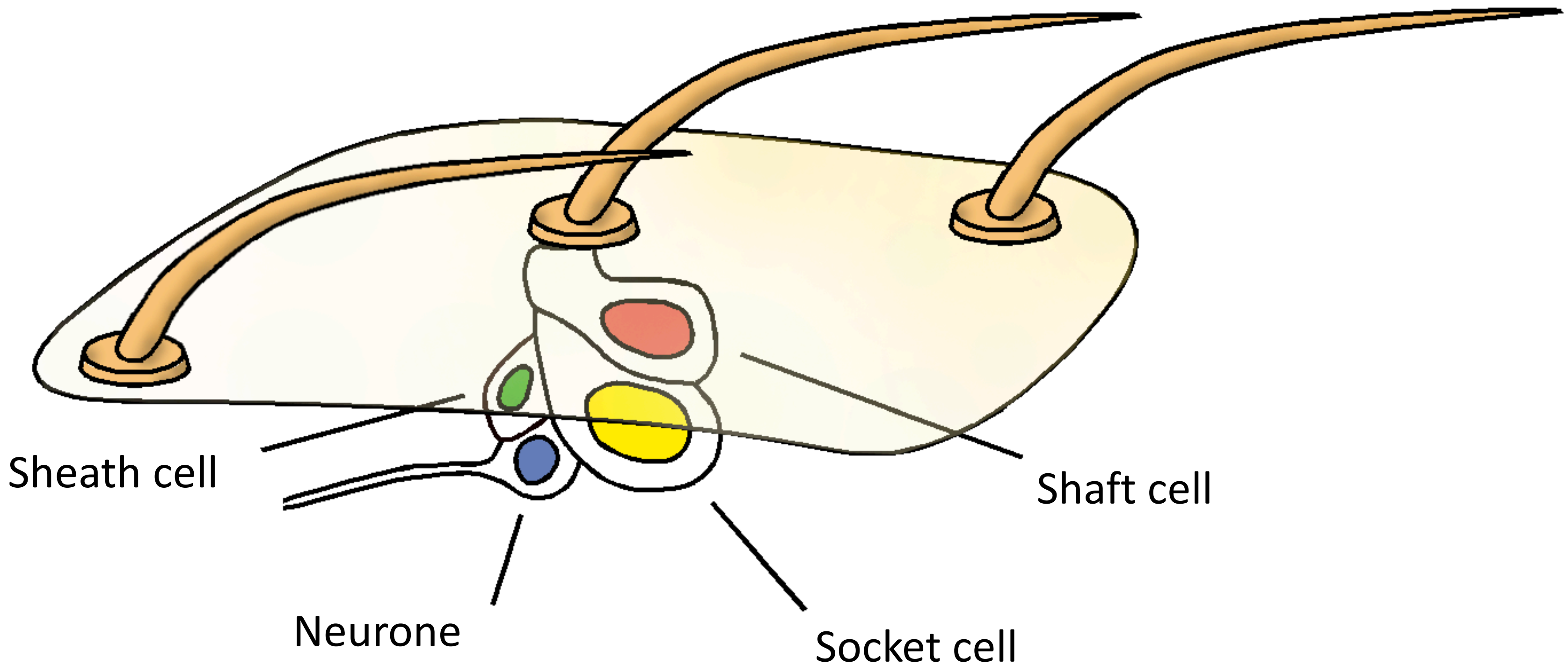
Formation d'un groupe proneural
gènes proneuraux

Sélection d'une SOP
gènes neurogéniques

Spécification de la SOP
gènes d'identité cellullaire

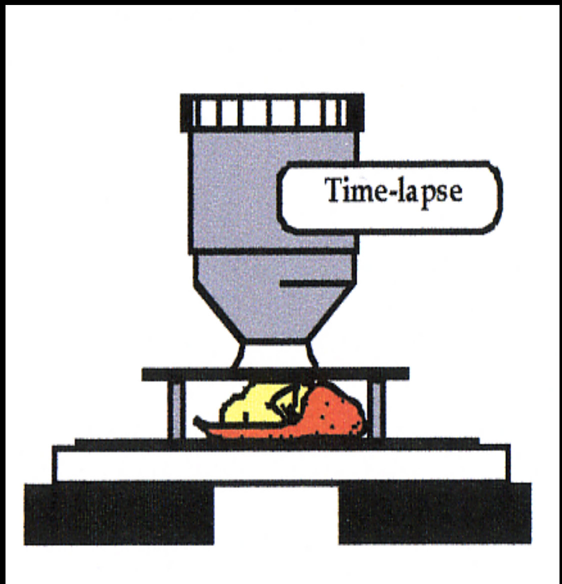
Division de la SOP et
différenciation de l'organe sensoriel
génes d'identité cellulaire

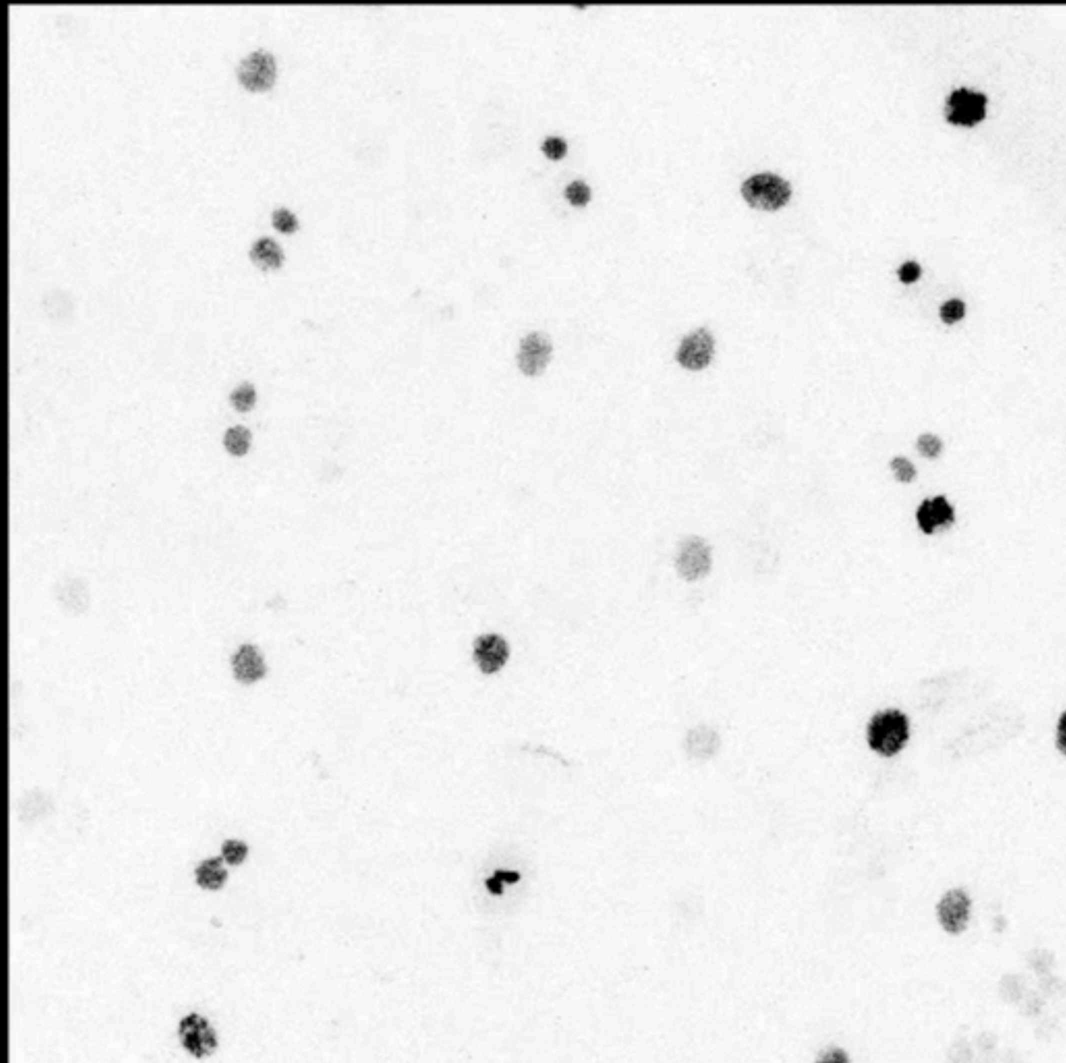
Sensory organs of the notum



Sensory organs of the notum

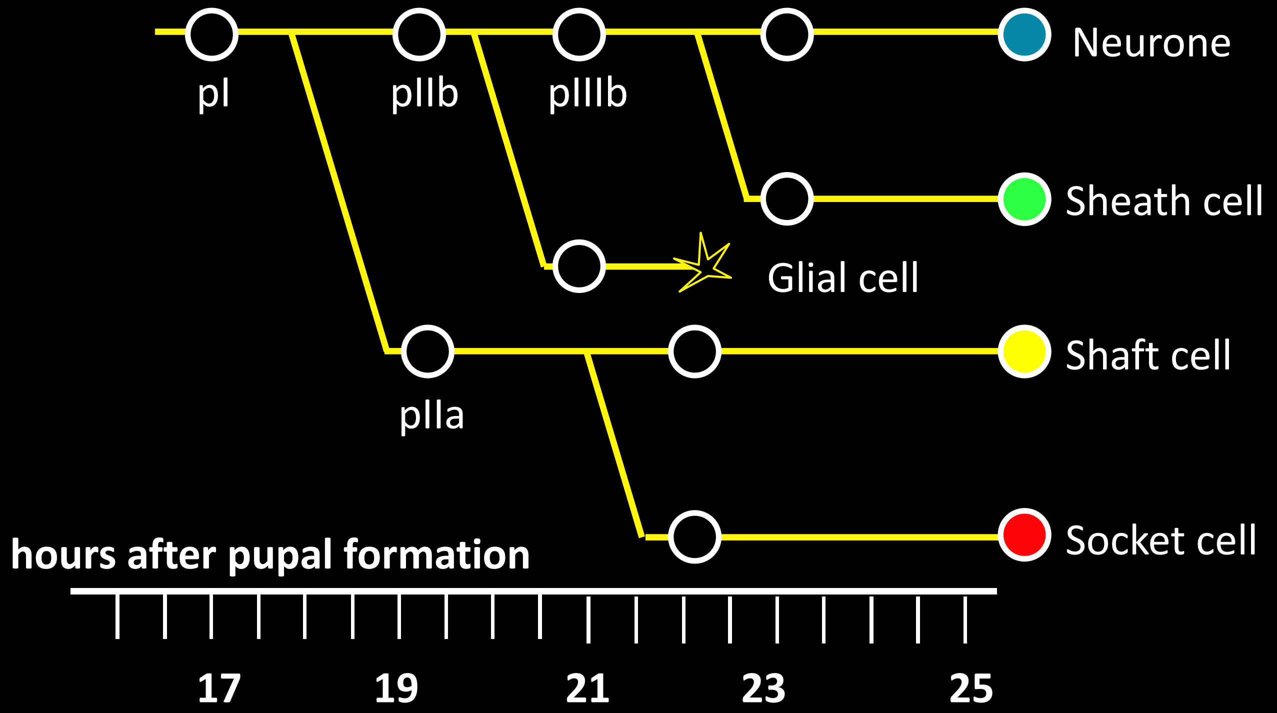
The bristle lineage





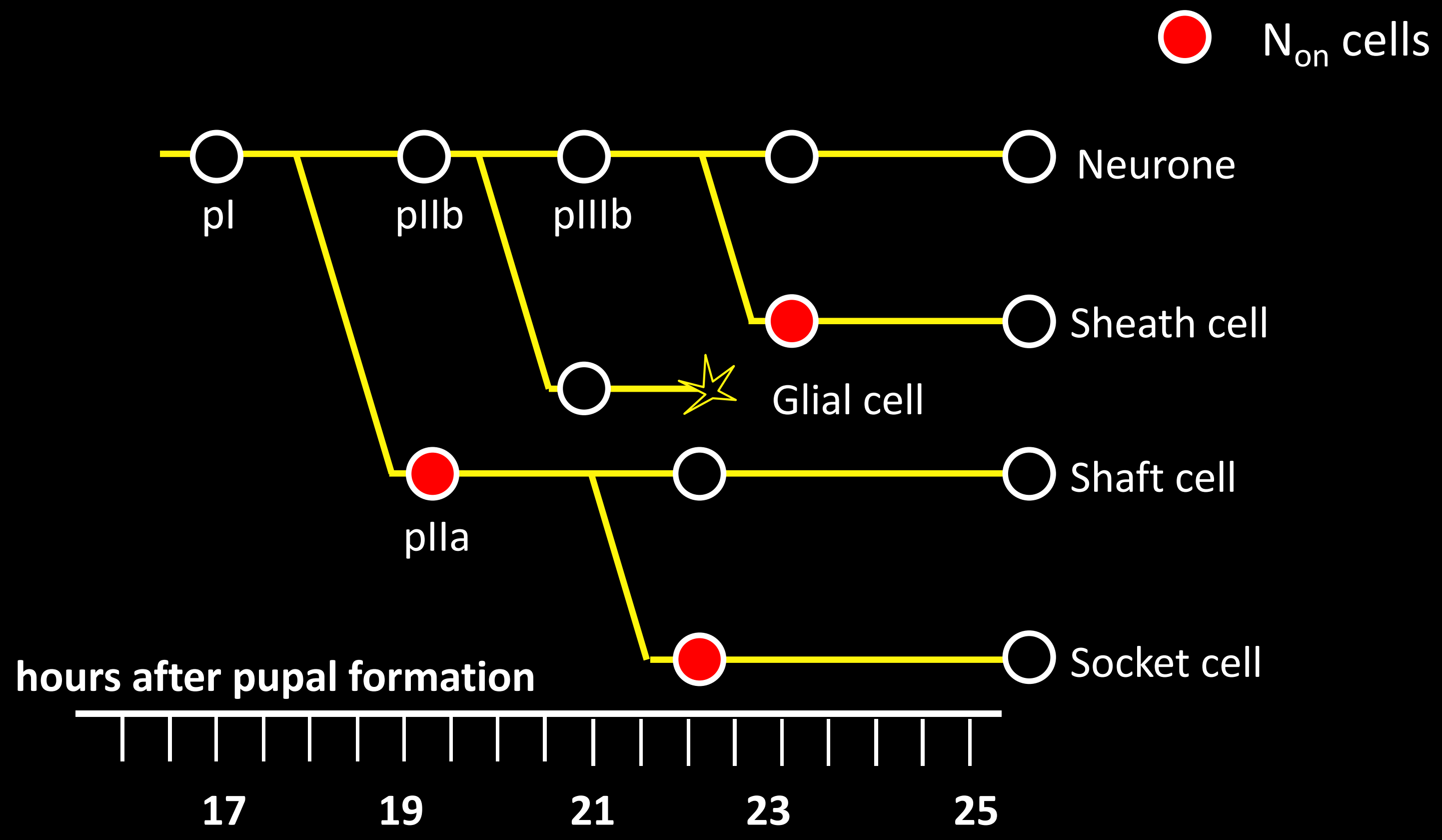
neu-Gal4; UAS-H2B::YFP

Drosophila mechanosensory organs



The bristle lineage (a model system to study cell diversification)

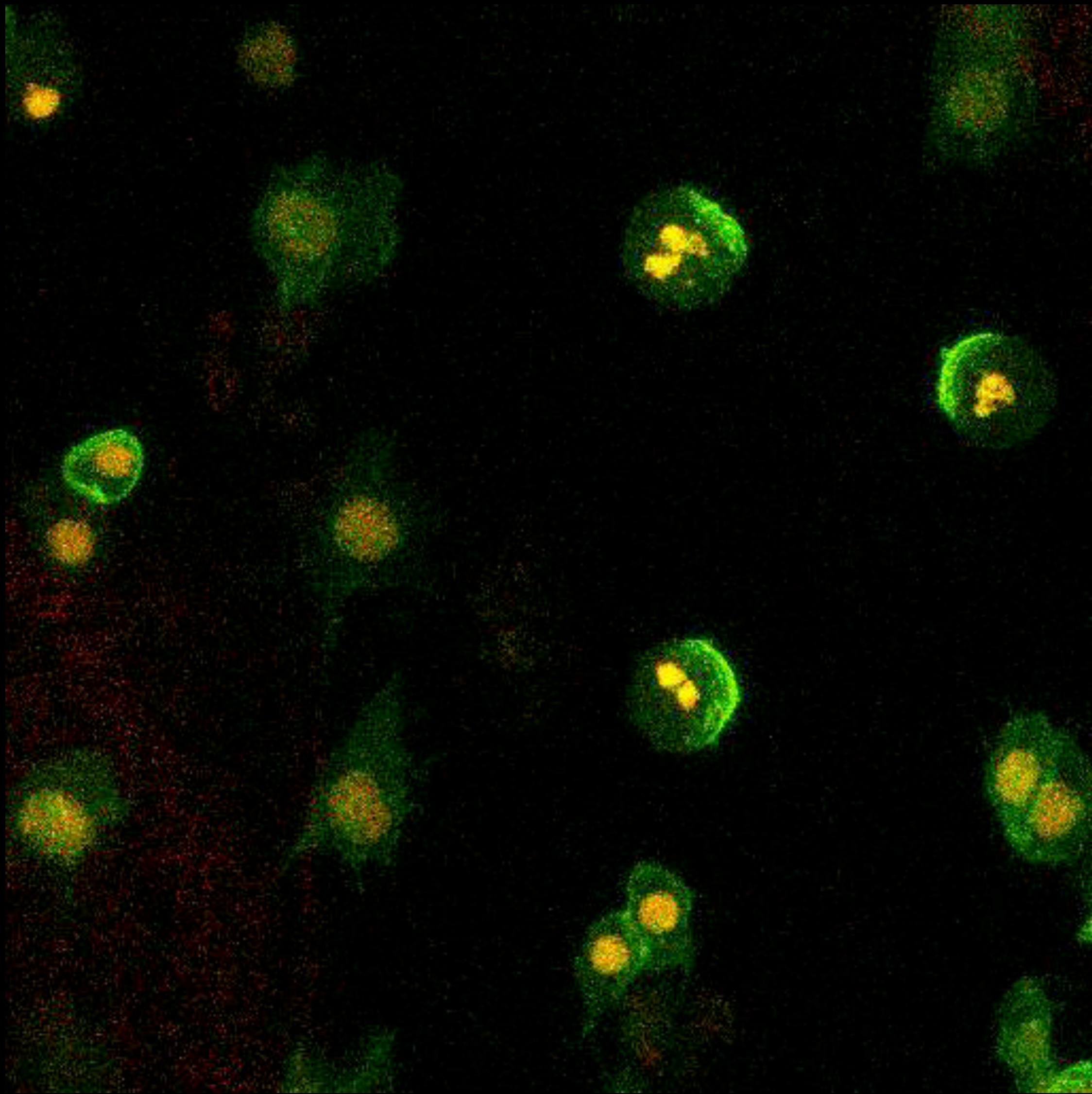
Drosophila mechanosensory organs



Binary cell fate decisions: The Notch pathway

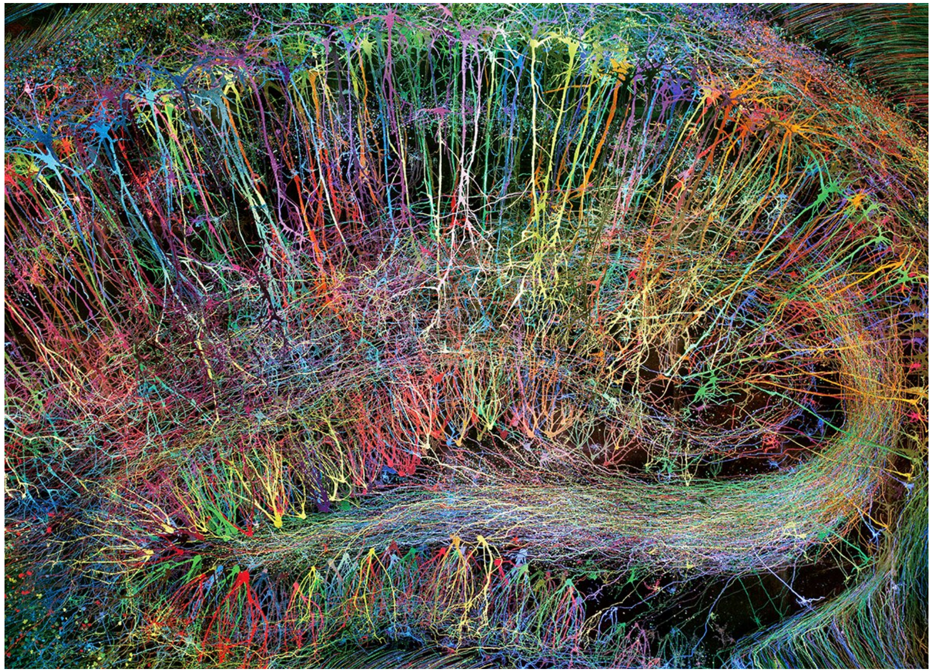
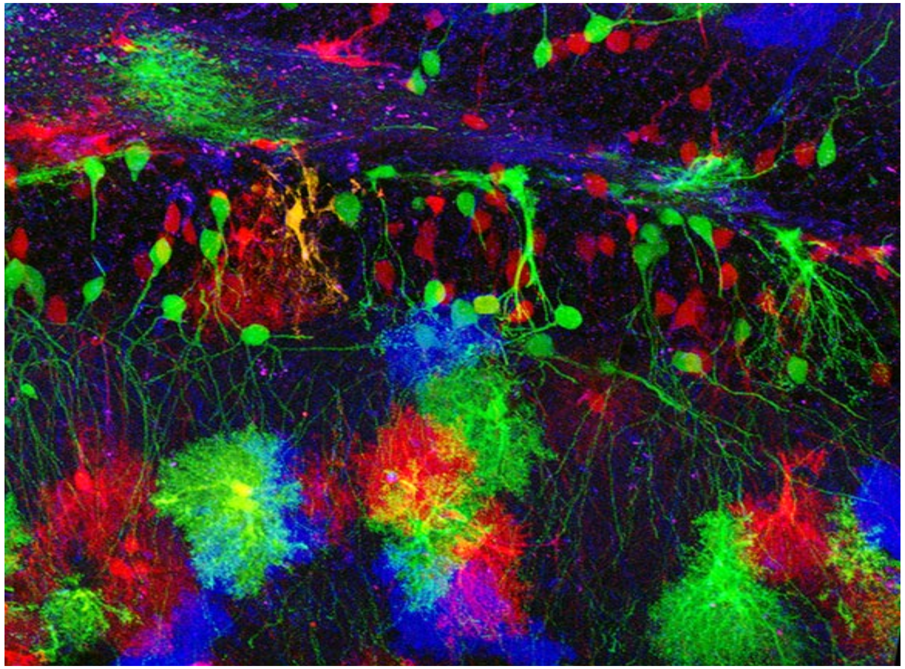
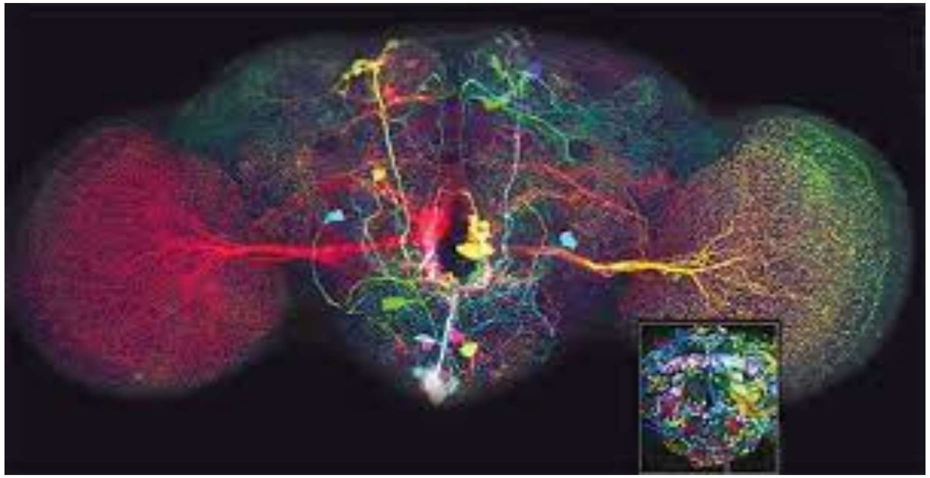
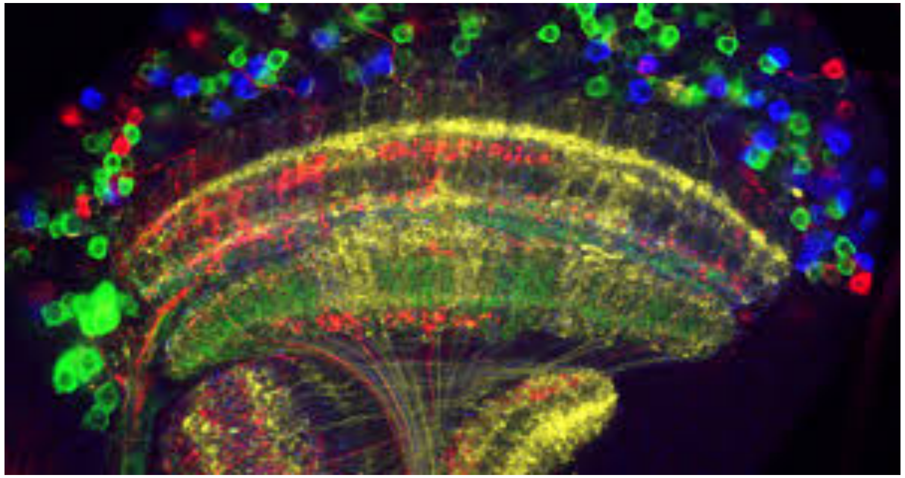
Sensory organs of the notum

Asymmetric cell divisions



HisH2B-RFP Pon-GFP

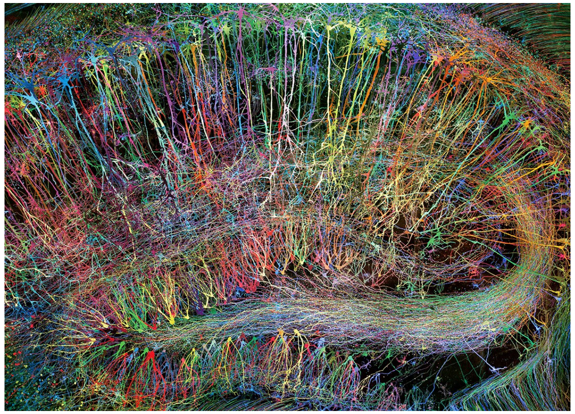
Origin of neuron diversity

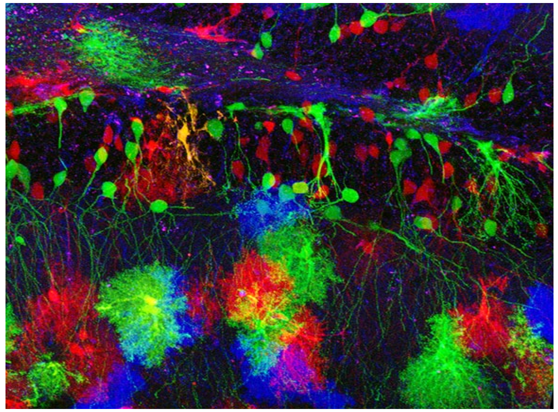





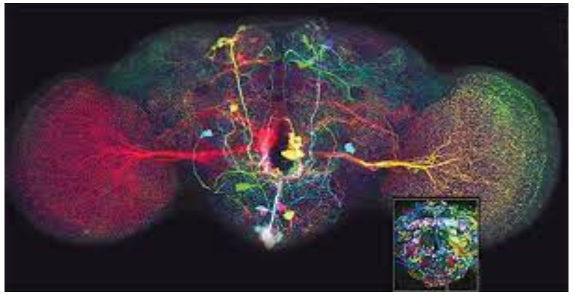
Origin of neuron diversity

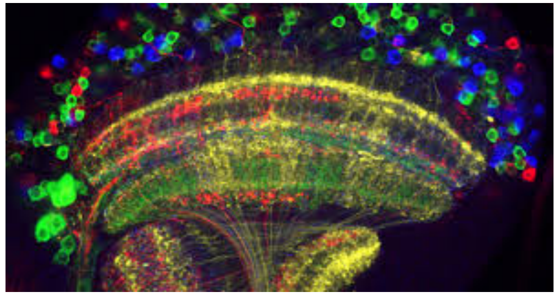
Spatial clues

Temporal clues

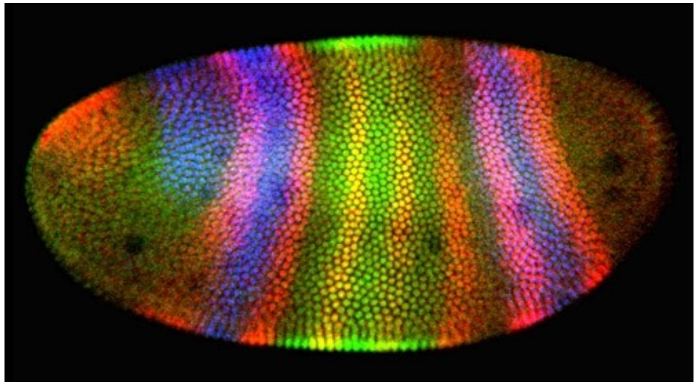




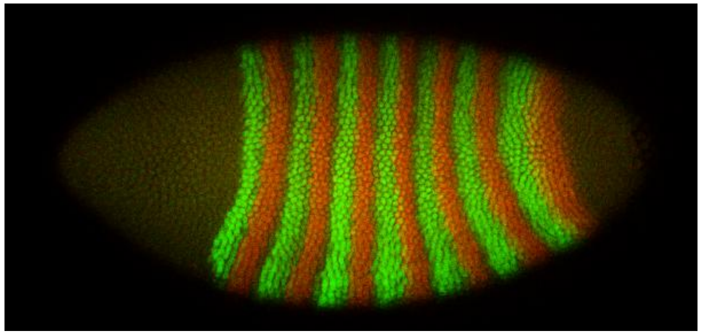




Spatial regulation of neuroblast identity

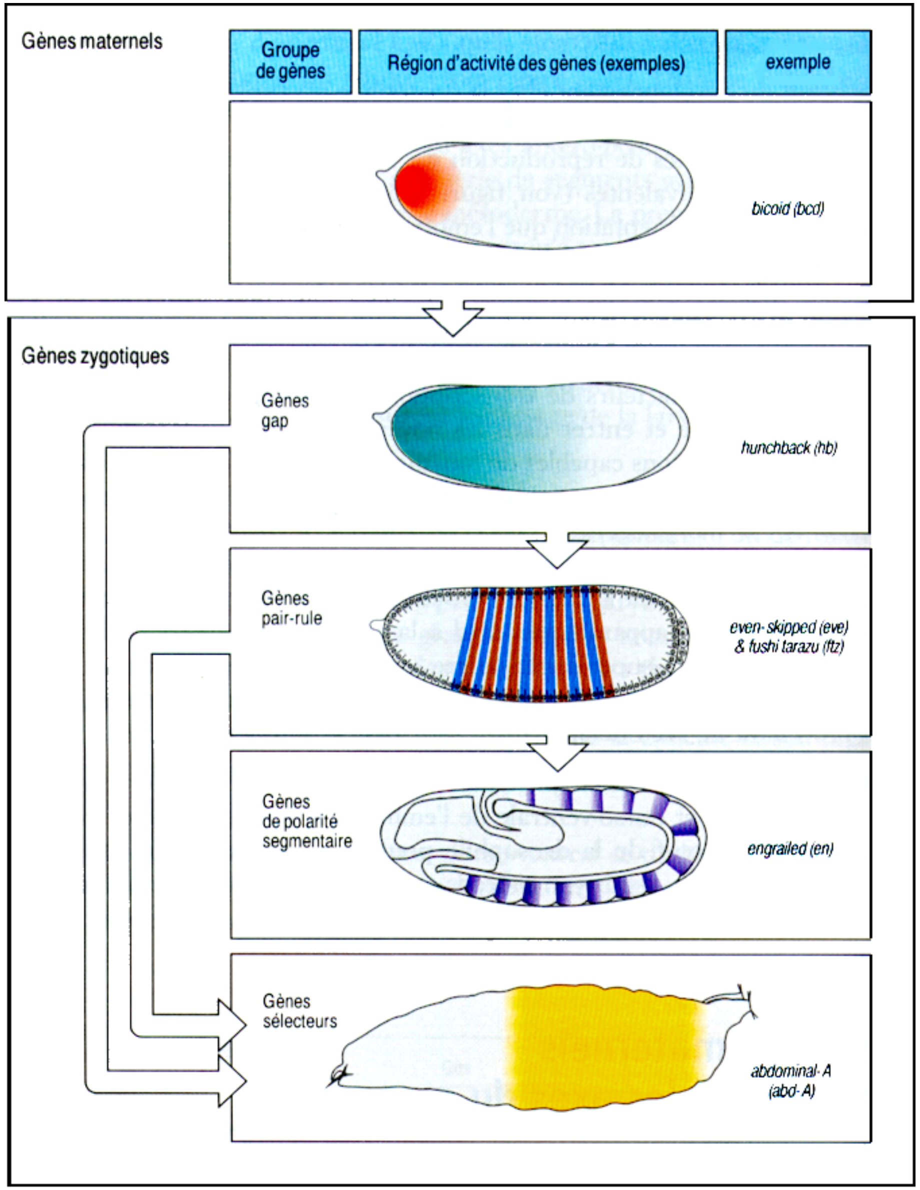


hairy krüppel giant



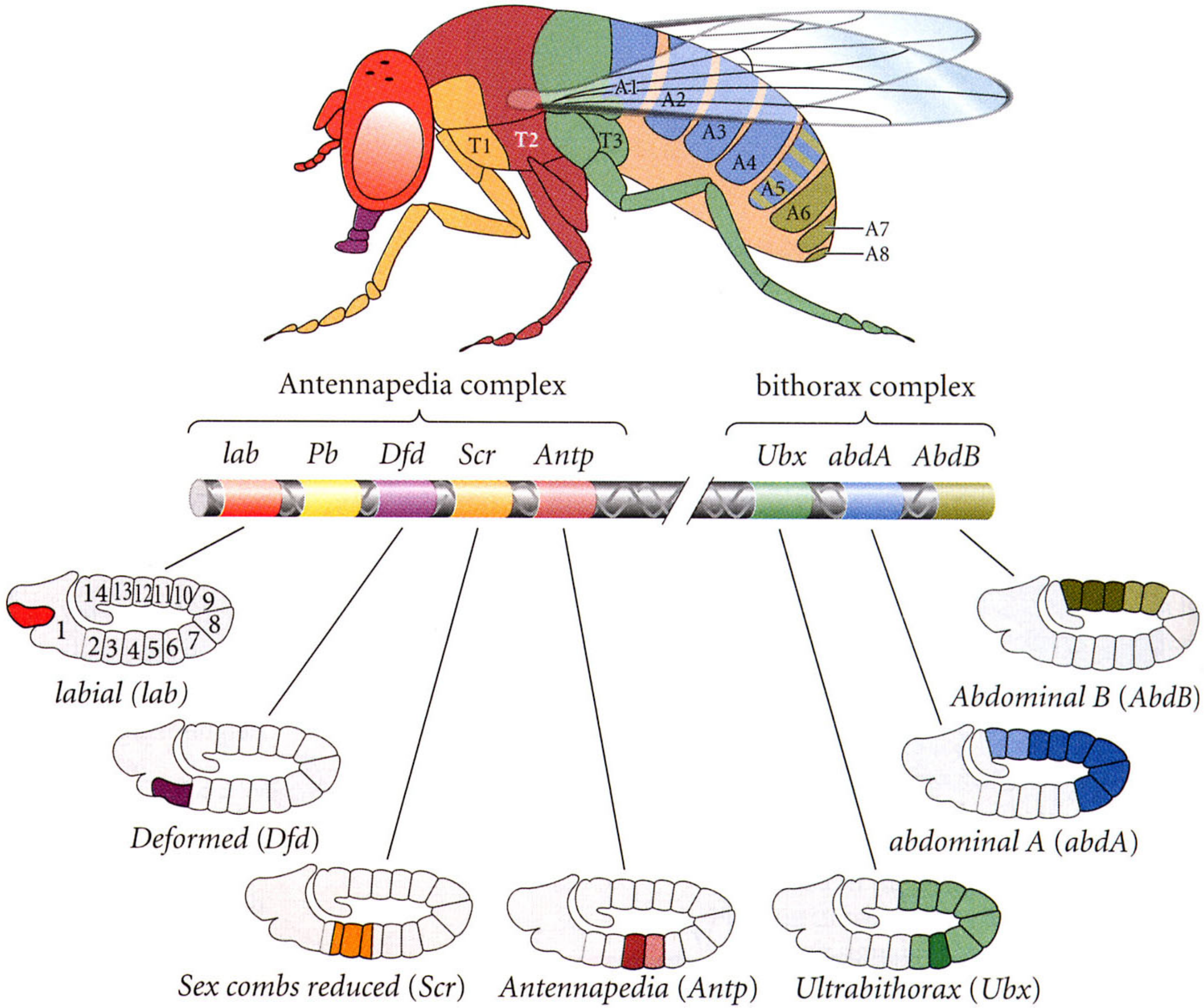
even-skipped fushi tarazu

Genes patterning following antero-posterior axis

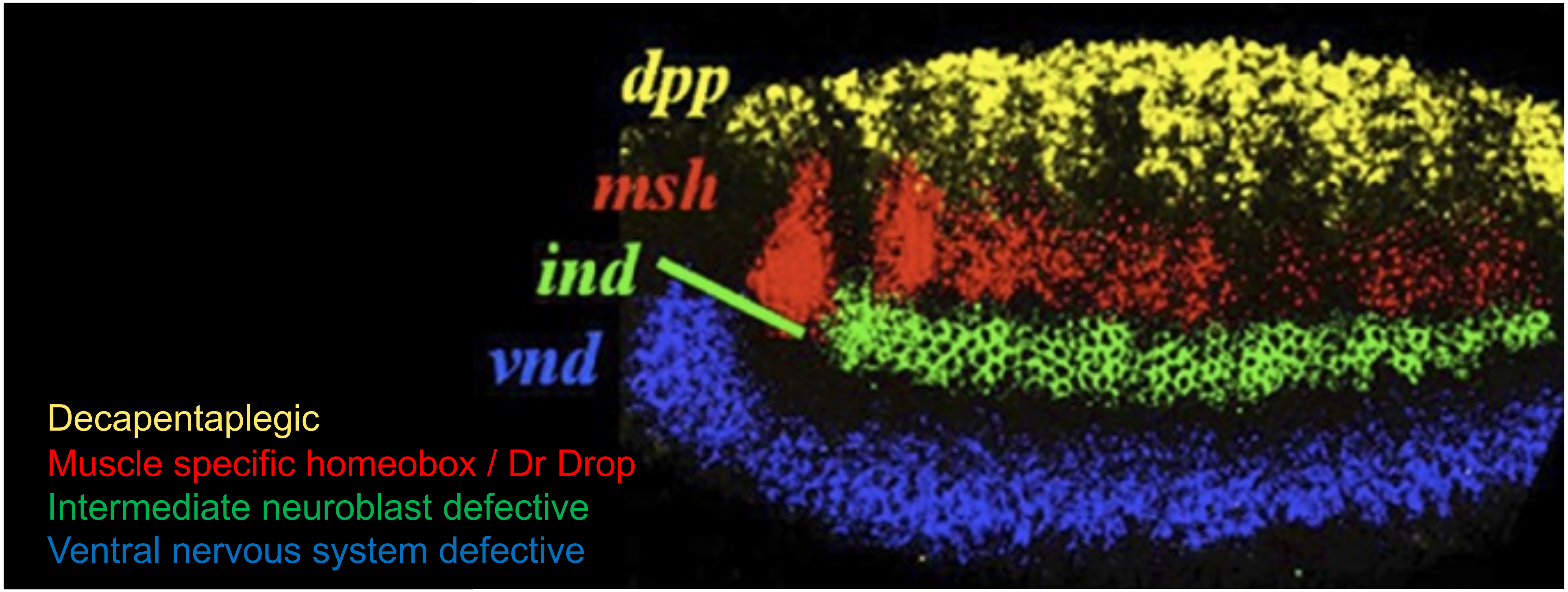


Spatial regulation of neuroblast identity

Hox genes



Spatial regulation of neuroblast identity

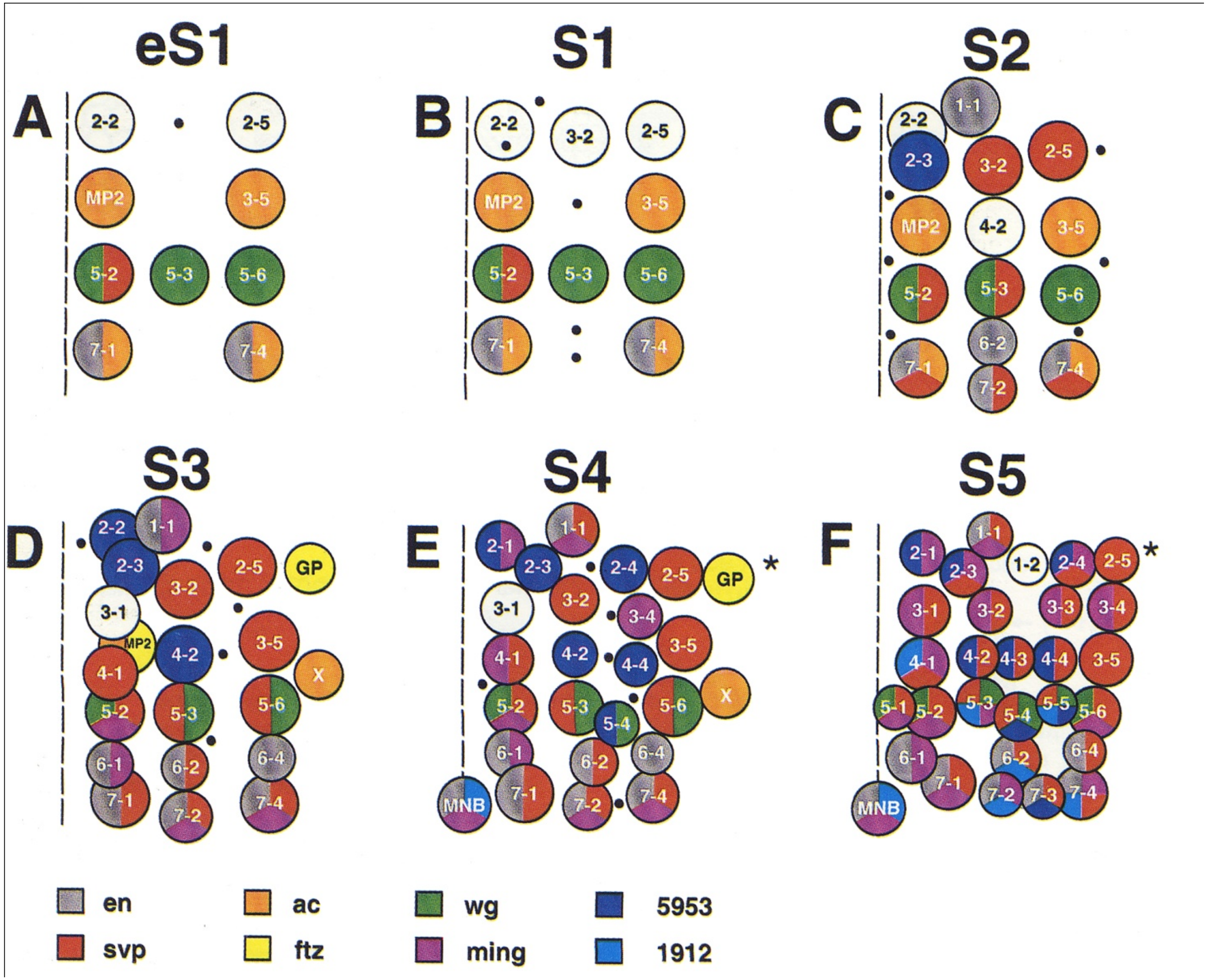


Mizutani et al. (2006)

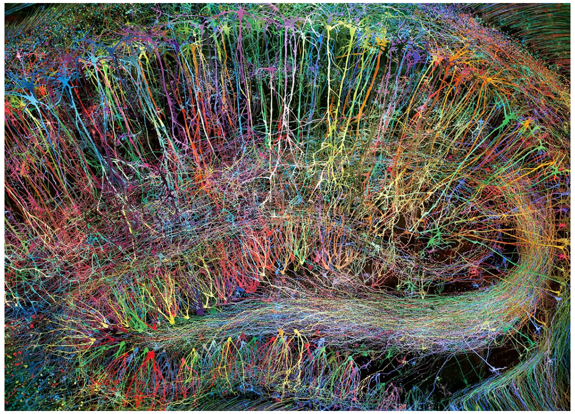
Genes patterning following medio-lateral axis

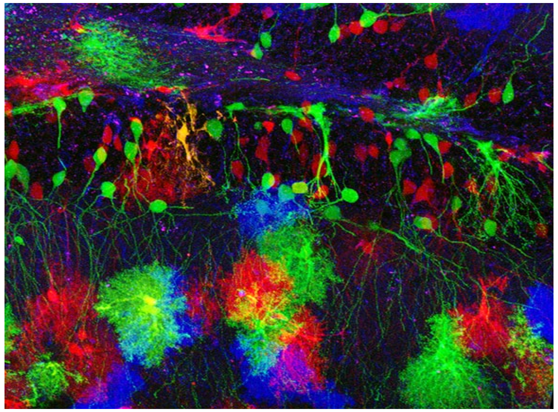
Spatial regulation of neuroblast identity

Neuroblasts in the embryonic ventral nerve cord



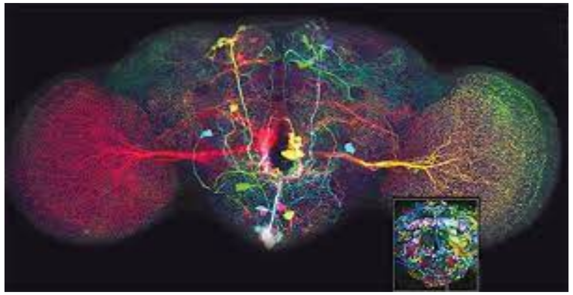
Origin of neuron diversity

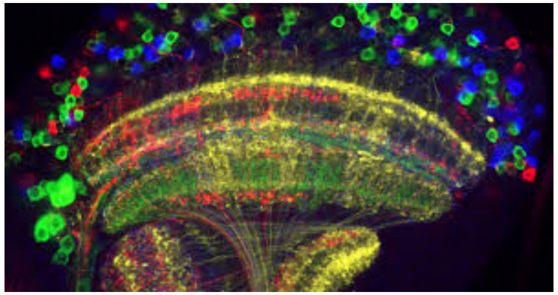




Spatial clues

Temporal clues





Temporal patterning in neurogenesis

A key challenge during neural development is to coordinate the birth and specification of diverse neuronal and glial cell types.

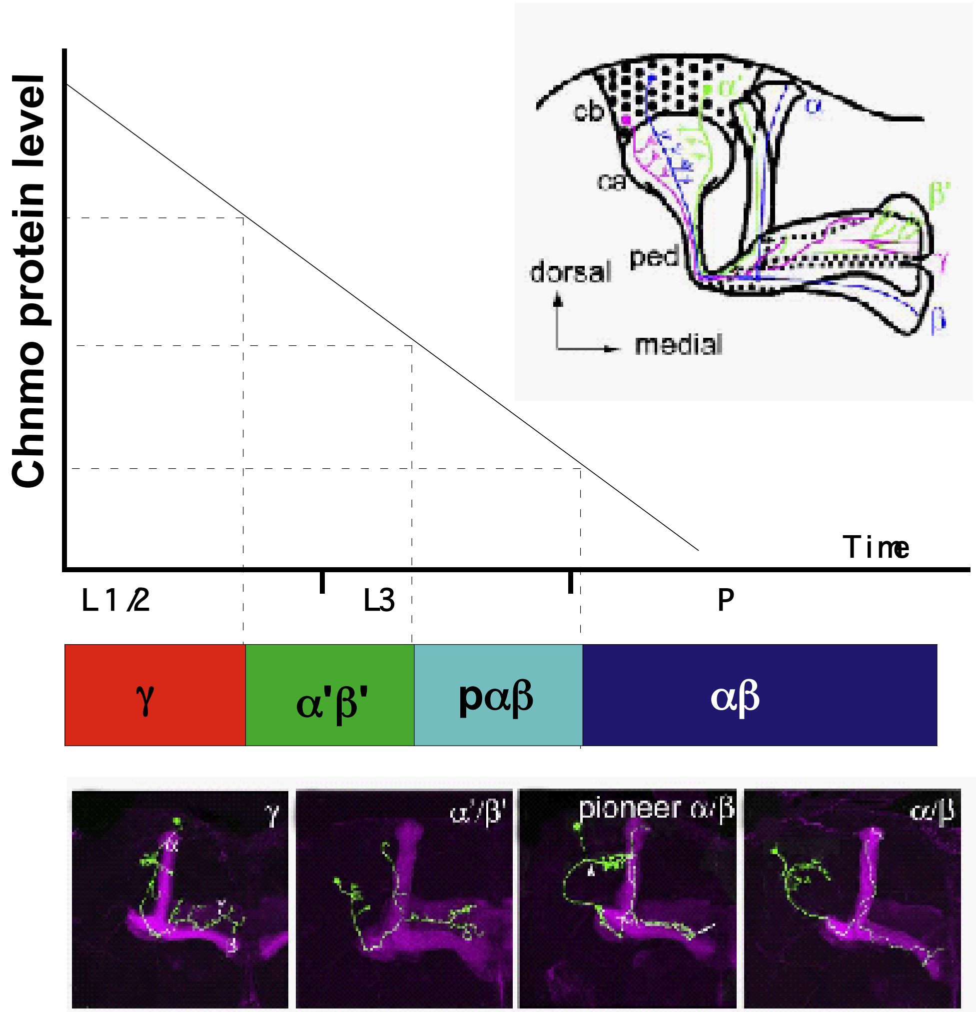
Gradients of the *Drosophila* Chinmo BTB-Zinc Finger Protein Govern Neuronal Temporal Identity

Sijun Zhu,[1,2,5] Suewei Lin,[3] Chih-Fei Kao,[3] Takeshi Awasaki,[3] Ann-Shyn Chiang,[4] and Tzumin Lee[1,3,*]

Cell 127, 409–422, October 20, 2006

Origin of neuroblast diversity

Temporal control of neuroblast identity



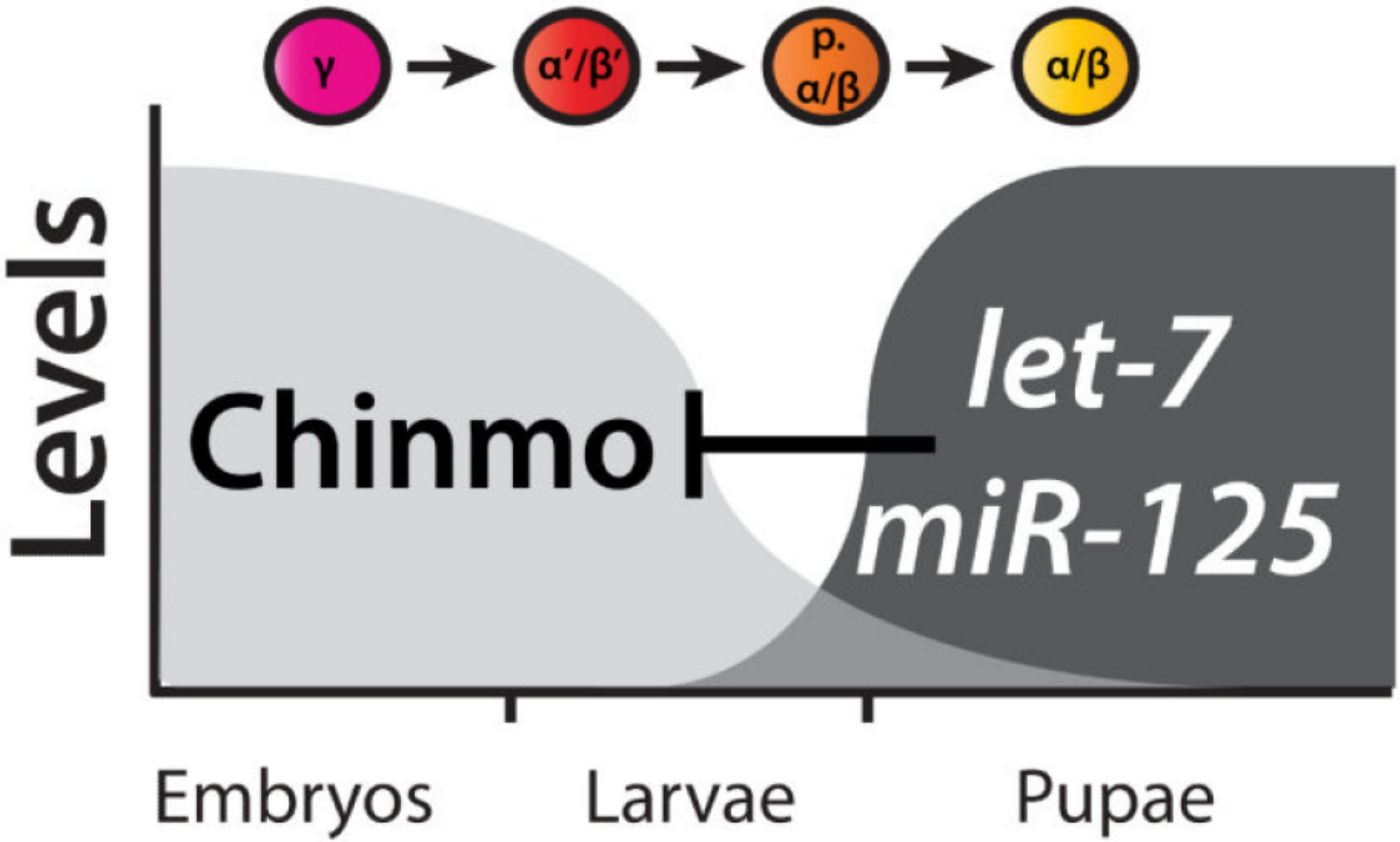
Zhu et al. (2006)

Temporal gradient of the Chinmo protein governs neuronal temporal identity

Origin of neuroblast diversity

Temporal control of neuroblast identity

Wu..Sokol (2012)



Temporal gradient of the Chinmo protein governs neuronal temporal identity

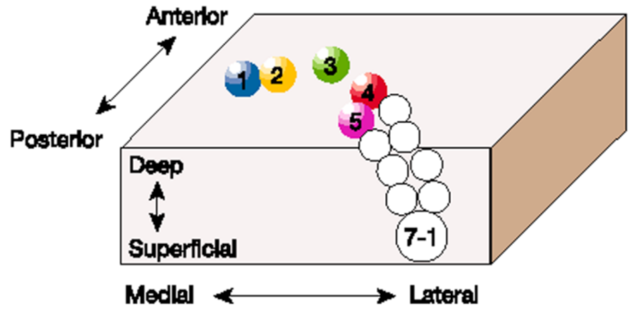
Cell, Vol. 106, 511–521, August 24, 2001, Copyright ©2001 by Cell Press

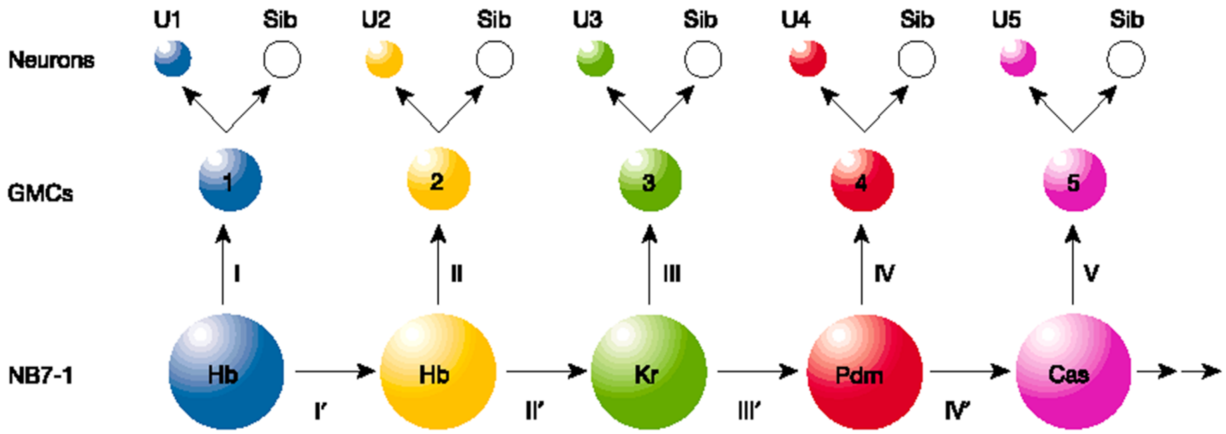
Drosophila Neuroblasts Sequentially Express Transcription Factors which Specify the Temporal Identity of Their Neuronal Progeny

Takako Isshiki, Bret Pearson, Scott Holbrook,
and Chris Q. Doe[1]

Origin of neuroblast diversity

Temporal control of neuroblast identity



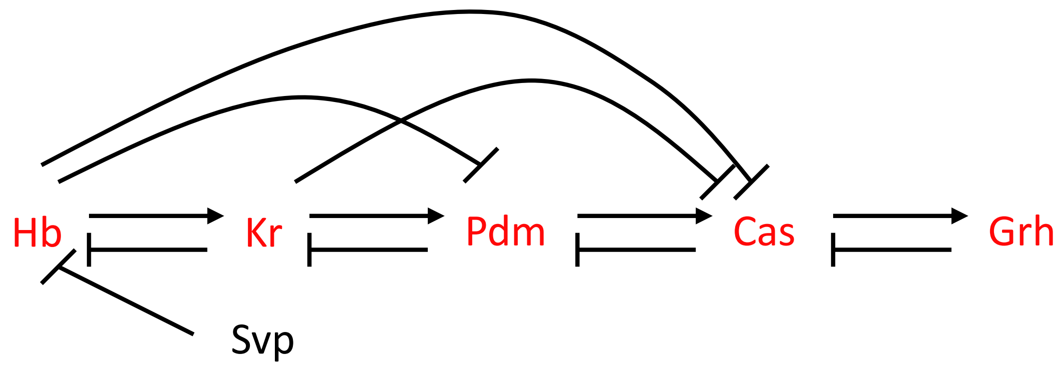


Doe (2003, 2005)

Multiple temporal identity transcription factors specify distict neuronal fates within a single early competence window

Origin of neuroblast diversity

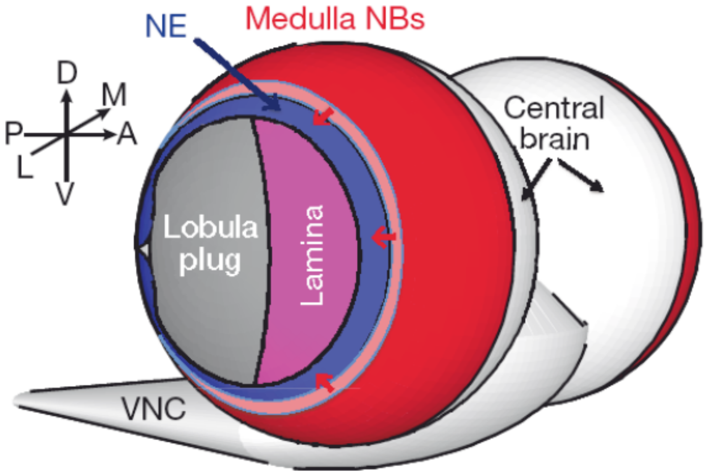
Temporal control of neuroblast identity

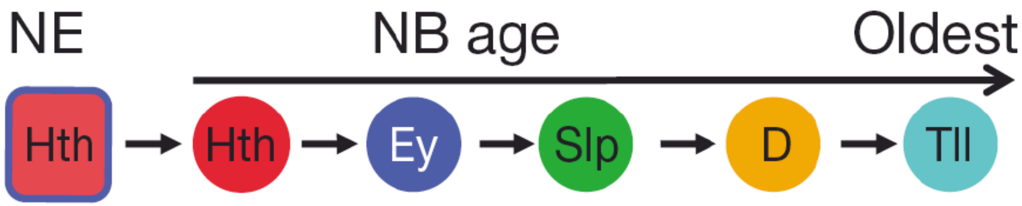


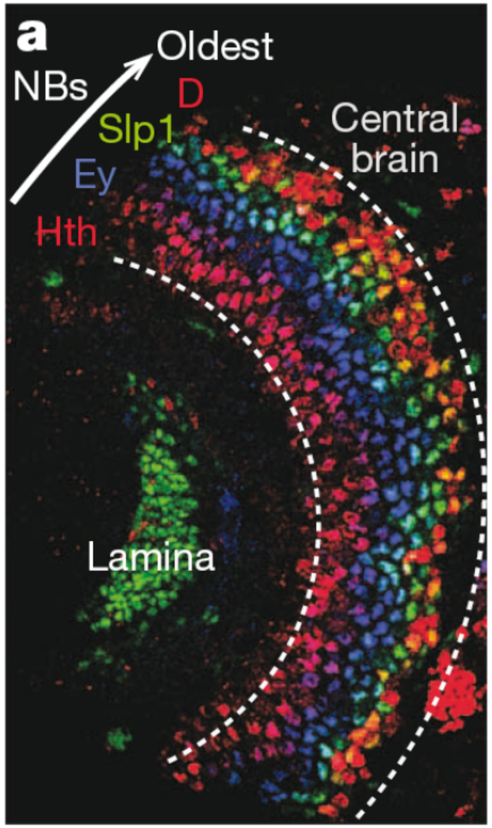
Transtions between TFS is controlled by feedforward and feedback repression cross-regulations

Adult Neurogenesis

Temporal patterning of Drosphila medulla neuroblasts







Li … Desplan (2013)

Origin of neuroblast diversity

Temporal control of neuroblast identity

VNC Nbs

Hunchback (Hb)
↓
Krüppel (Kr)
↓
Pdm (Pdm)
↓
Castor (Cas)
↓
Grainyhead (Grh)

Medulla Nbs

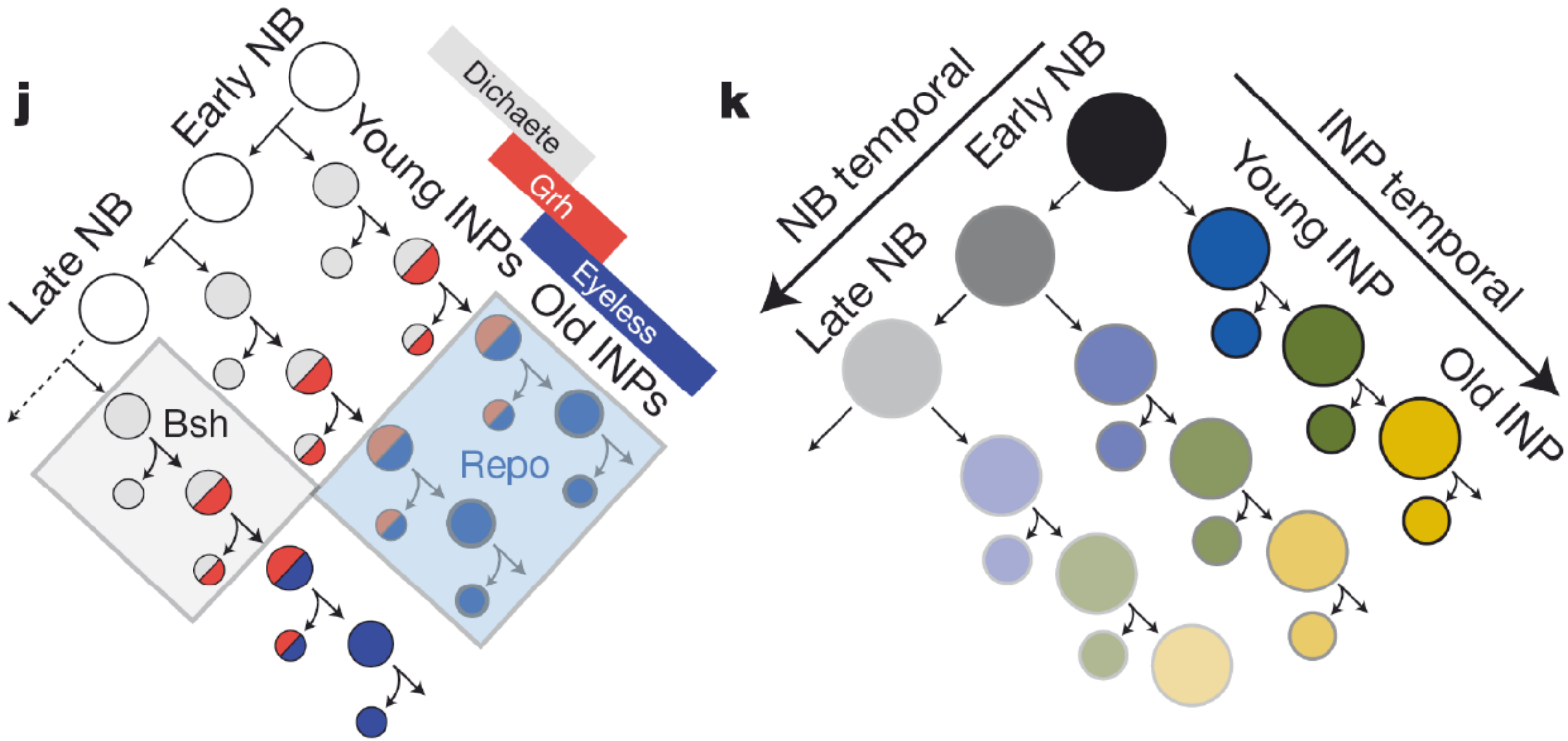
Homothorax (Hth)
↓
Klumpfuss (Klu)
↓
Eyeless (Ey)
↓
Sloppy-paired (Slp)
↓
Dichaete (D)
↓
Tailess (Tll)

INPs of type II Nbs

Dichaete (D)
↓
Grainyhead (Grh)
↓
Eyeless (Ey)

These TFs do not specify a certain neuron type, but control the birth-order-depedent neuronal identity

Adult Neurogenesis



Distinct neural progeny are born from early versus late type II neuroblast lineages neuroblast and Intermedial Neural Progenitors temporal patterning act together to generate neural diversity

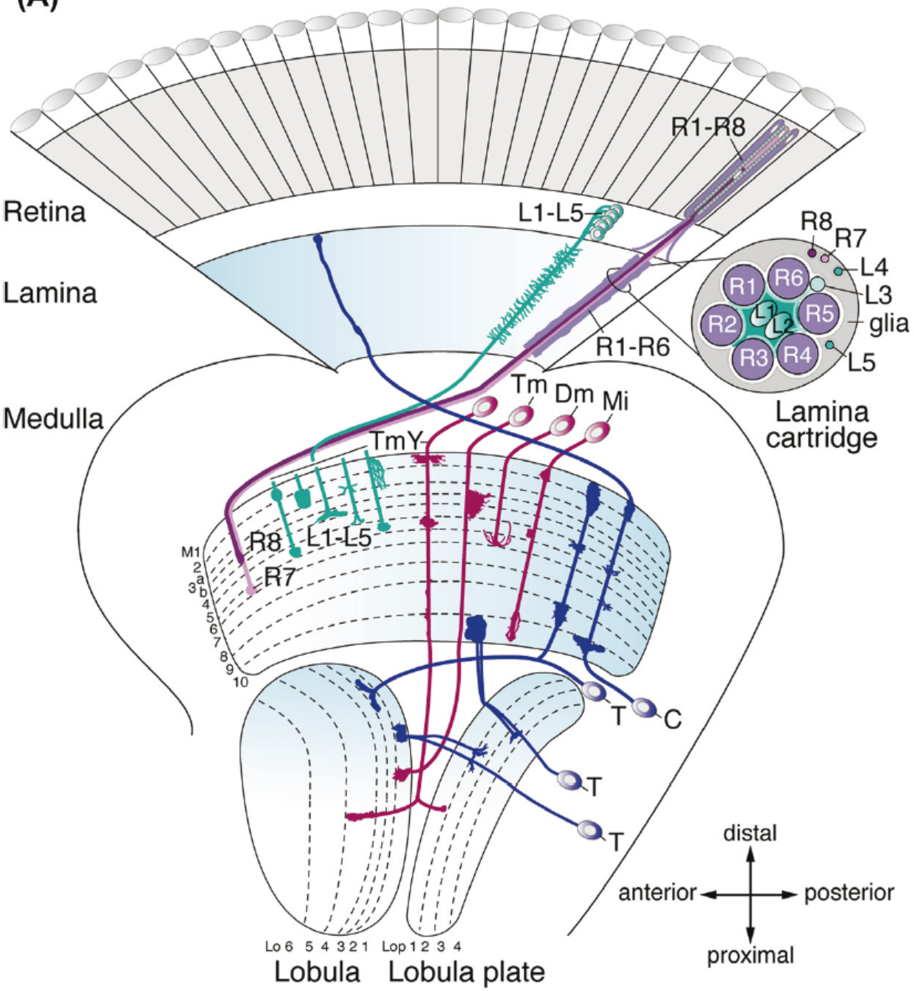
TF sequences on the two temporal axes act combinatorially to generate larger neural diversity

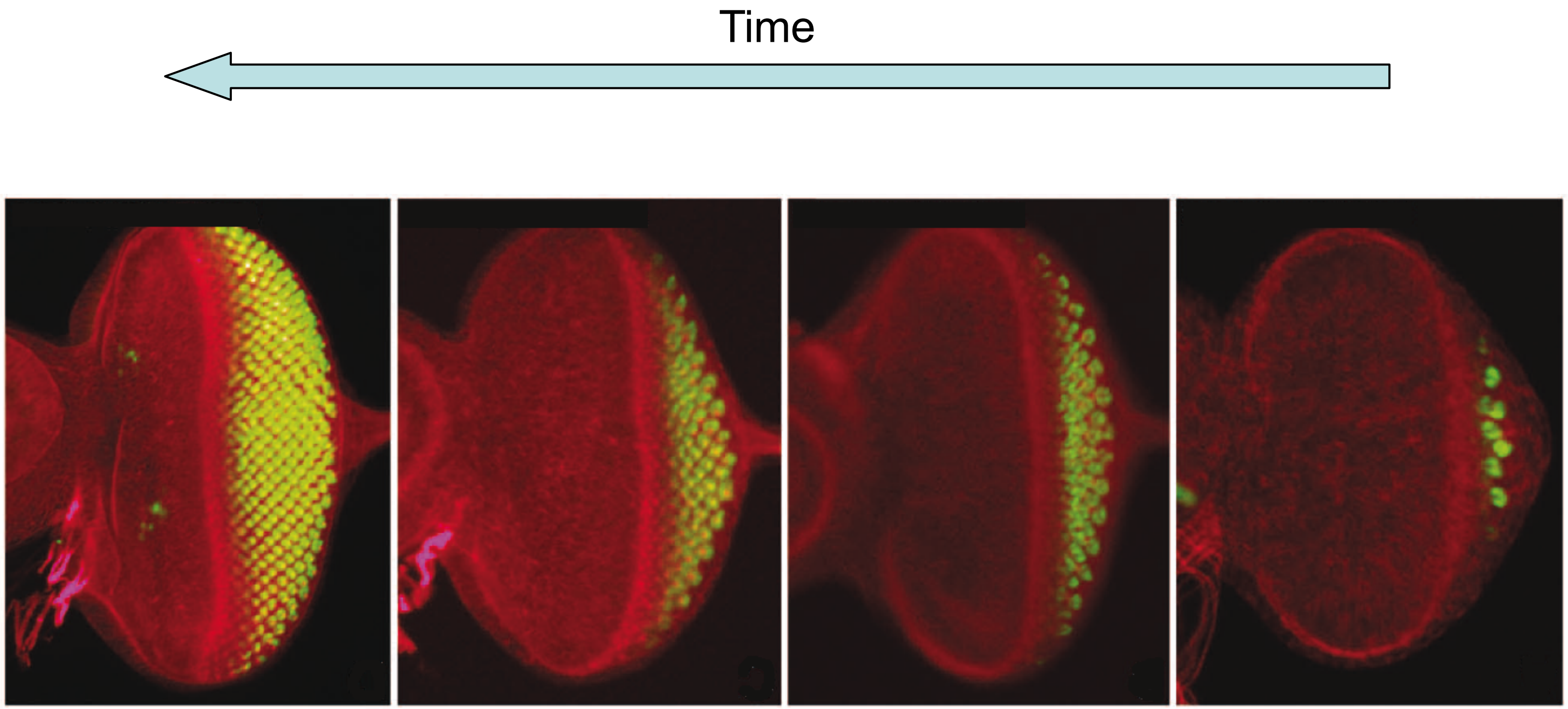
Glia relay differentiation cues to coordinate neuronal development in *Drosophila*

Vilaiwan M. Fernandes,*† Zhenqing Chen,†‡ Anthony M. Rossi, Jaqueline Zipfel,§ Claude Desplan

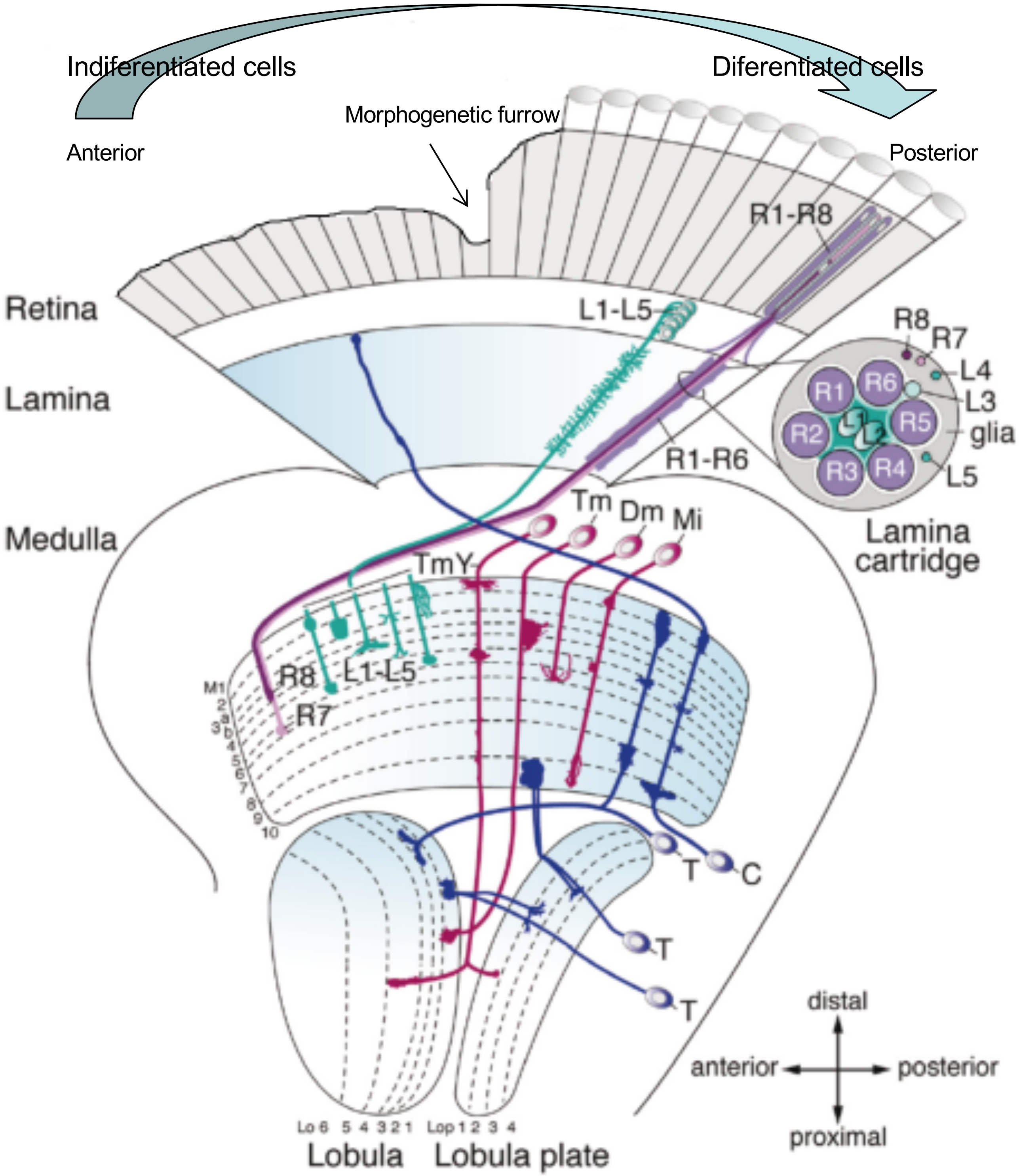
, *Science* **357**, 886–891 (2017) 1 September 2017

(A) Adult visual system

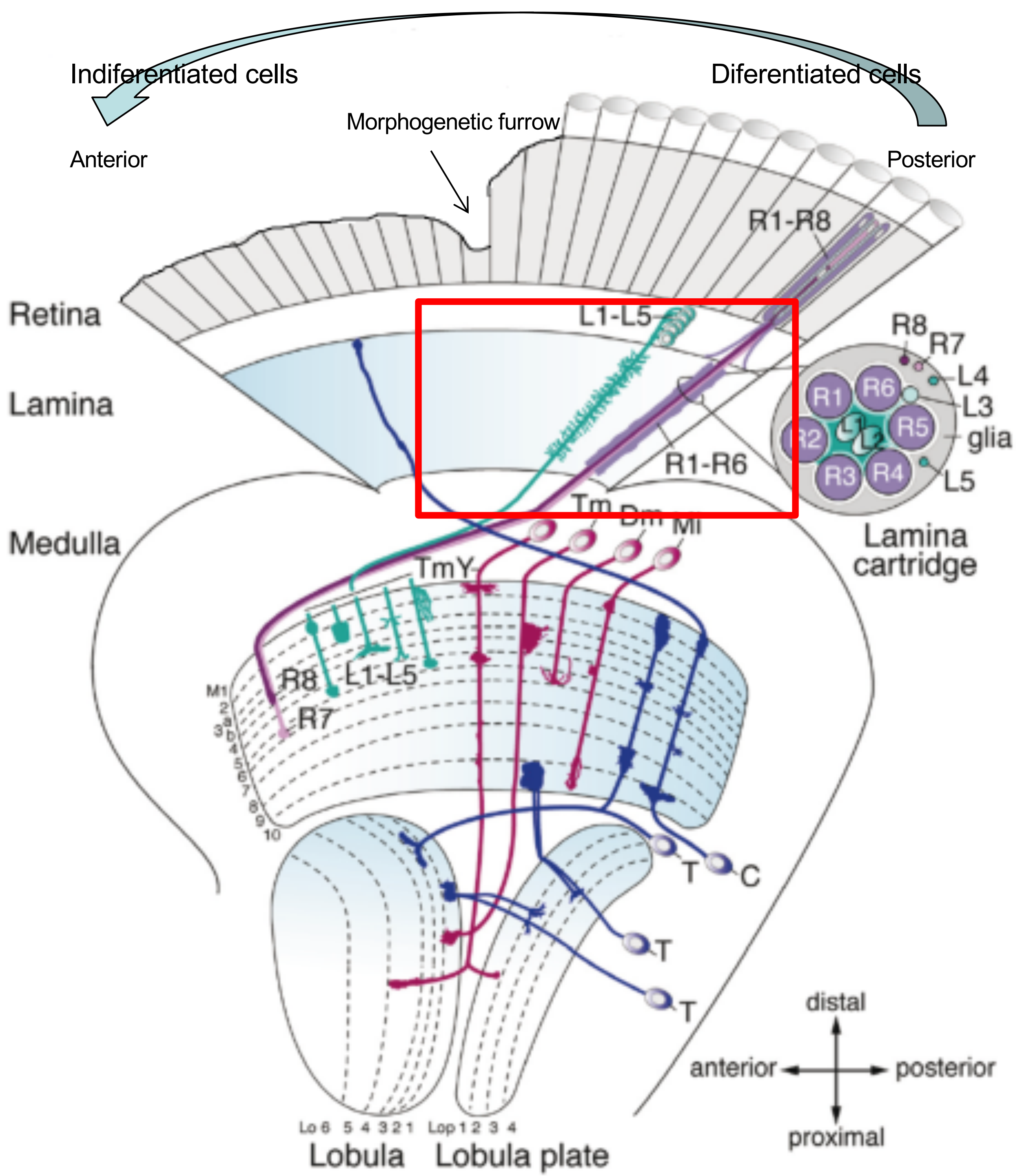




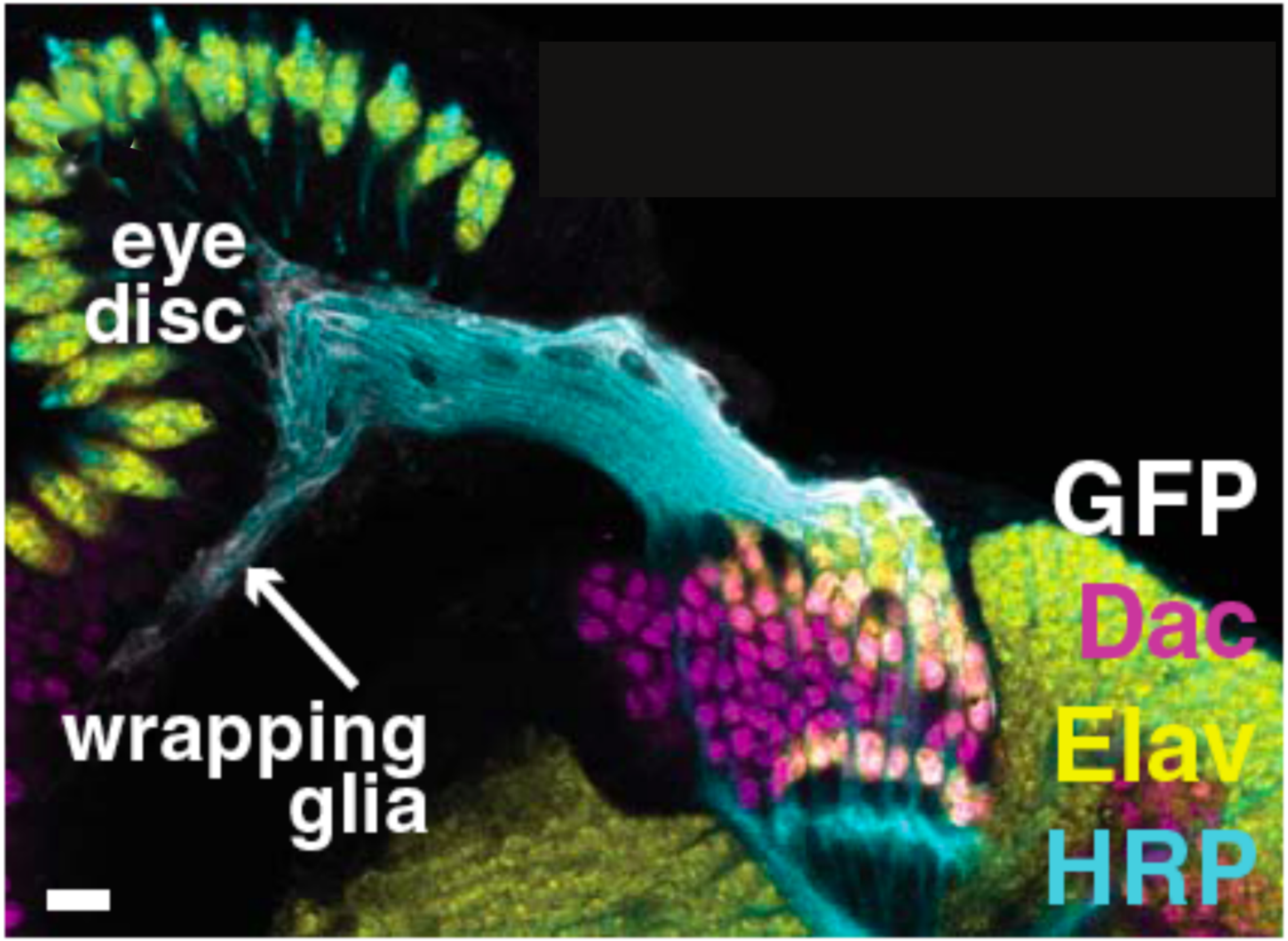
Progression of the morphogenetic furrow across the developing eye field of Drosophila

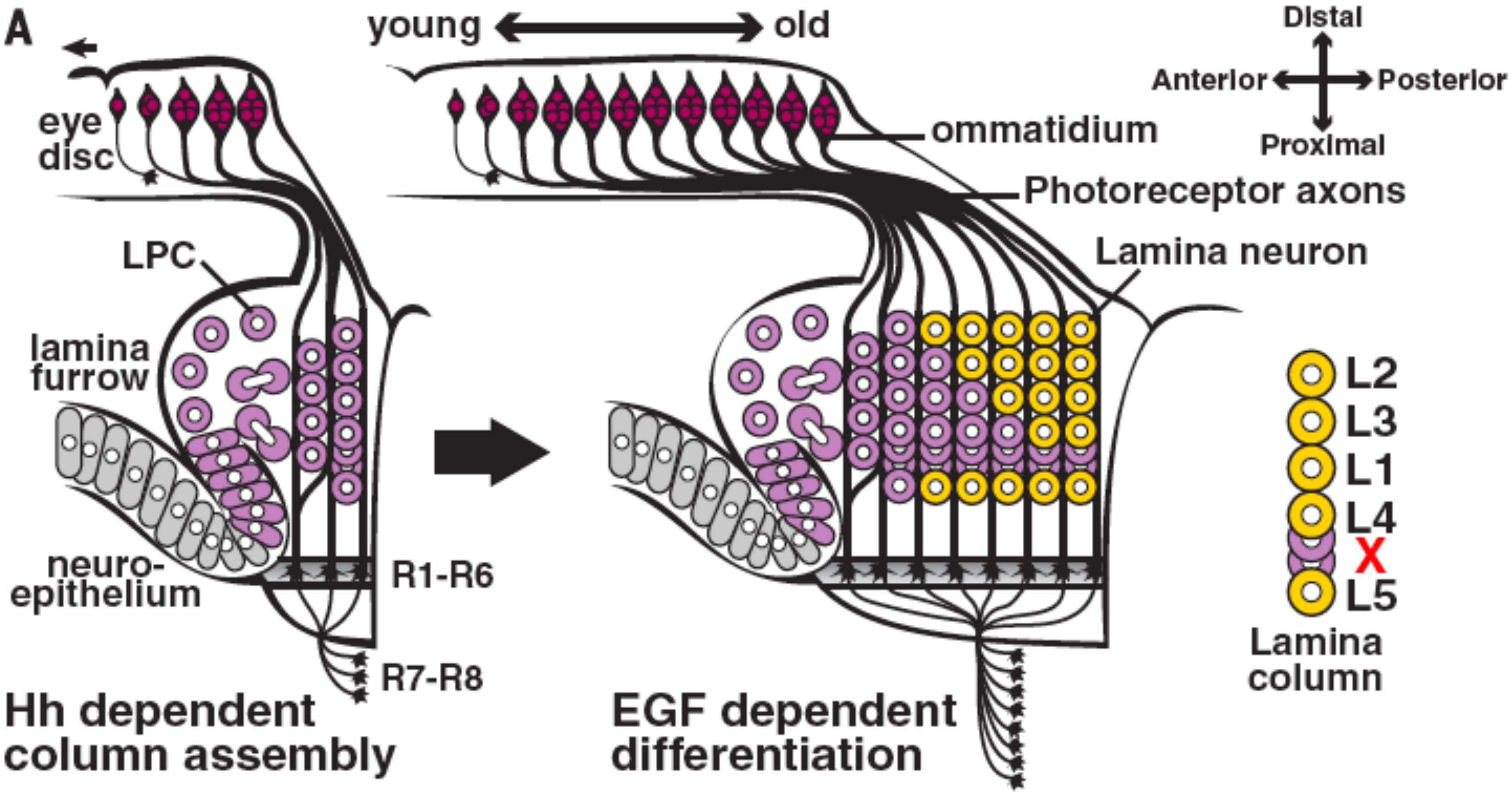


Indiferentiated cells
Diferentiated cells
Morphogenetic furrow
Anterior
Posterior
R1-R8
Retina
L1-L5
R8
R7
L4
L3
glia
L5
R1
R6
R5
R2
R3
R4
Lamina
R1-R6
Lamina cartridge
Medulla
Tm
Dm
Mi
TmY
R8
L1-L5
R7
T
C
T
T
distal
anterior
posterior
proximal
Lo 6 5 4 3 2 1
Lop 1 2 3 4
Lobula
Lobula plate



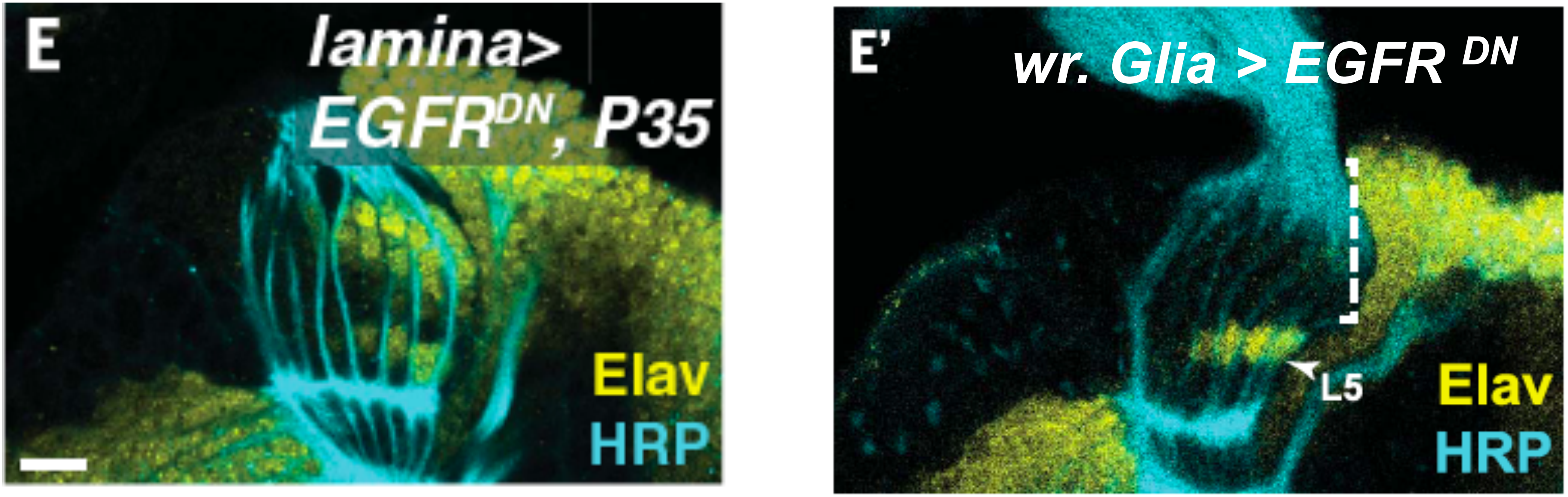
Indiferentiated cells
Diferentiated cells
Morphogenetic furrow
Anterior
Posterior
Retina
R1-R8
L1-L5
Lamina
R1-R6
R8
R7
L4
L3
glia
L5
R1
R6
R2
R5
R3
R4
L1
L2
Lamina
cartridge
Medulla
Tm
Dm
Mi
TmY
R8
L1-L5
R7
T
C
T
T
distal
anterior
posterior
proximal
Lo 6 5 4 3 2 1
Lop 1 2 3 4
Lobula
Lobula plate


eye disc
wrapping glia
GFP
Dac
Elav
HRP

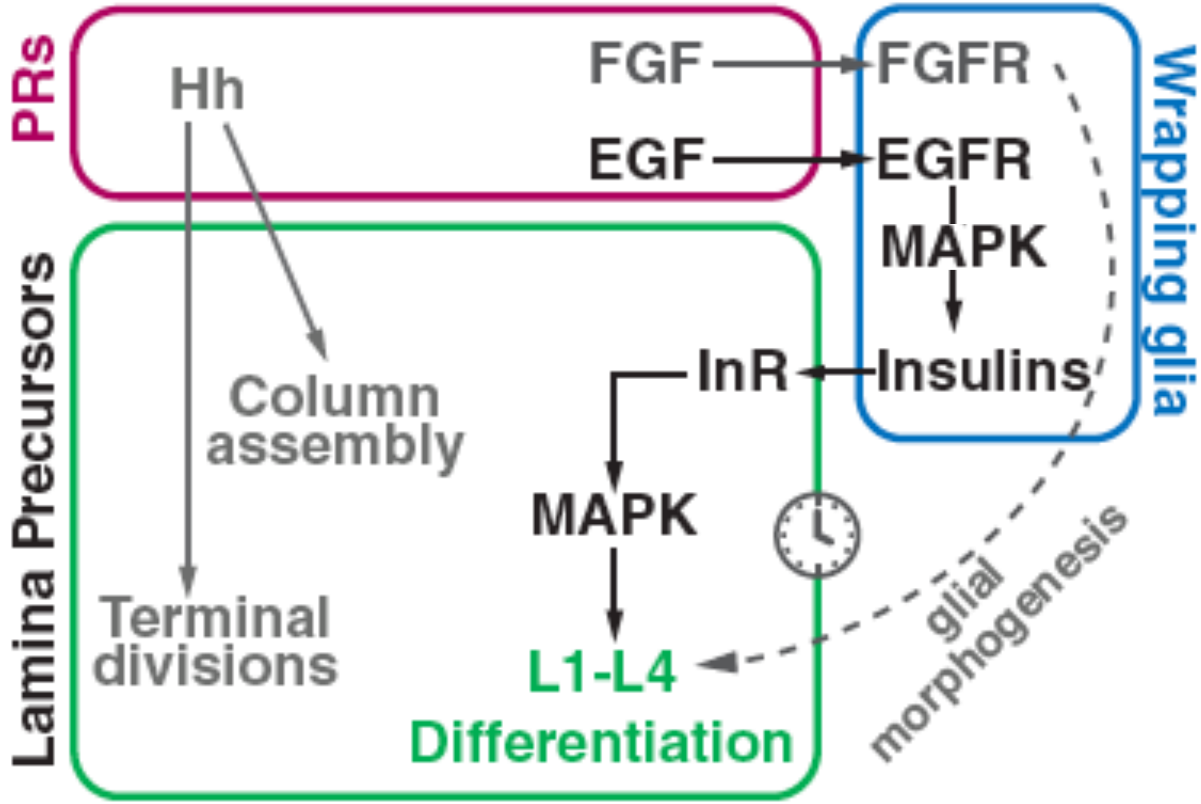


Signals from photoreceptors axons control lamina precursor cells cell divisions, column assembly and differentiation

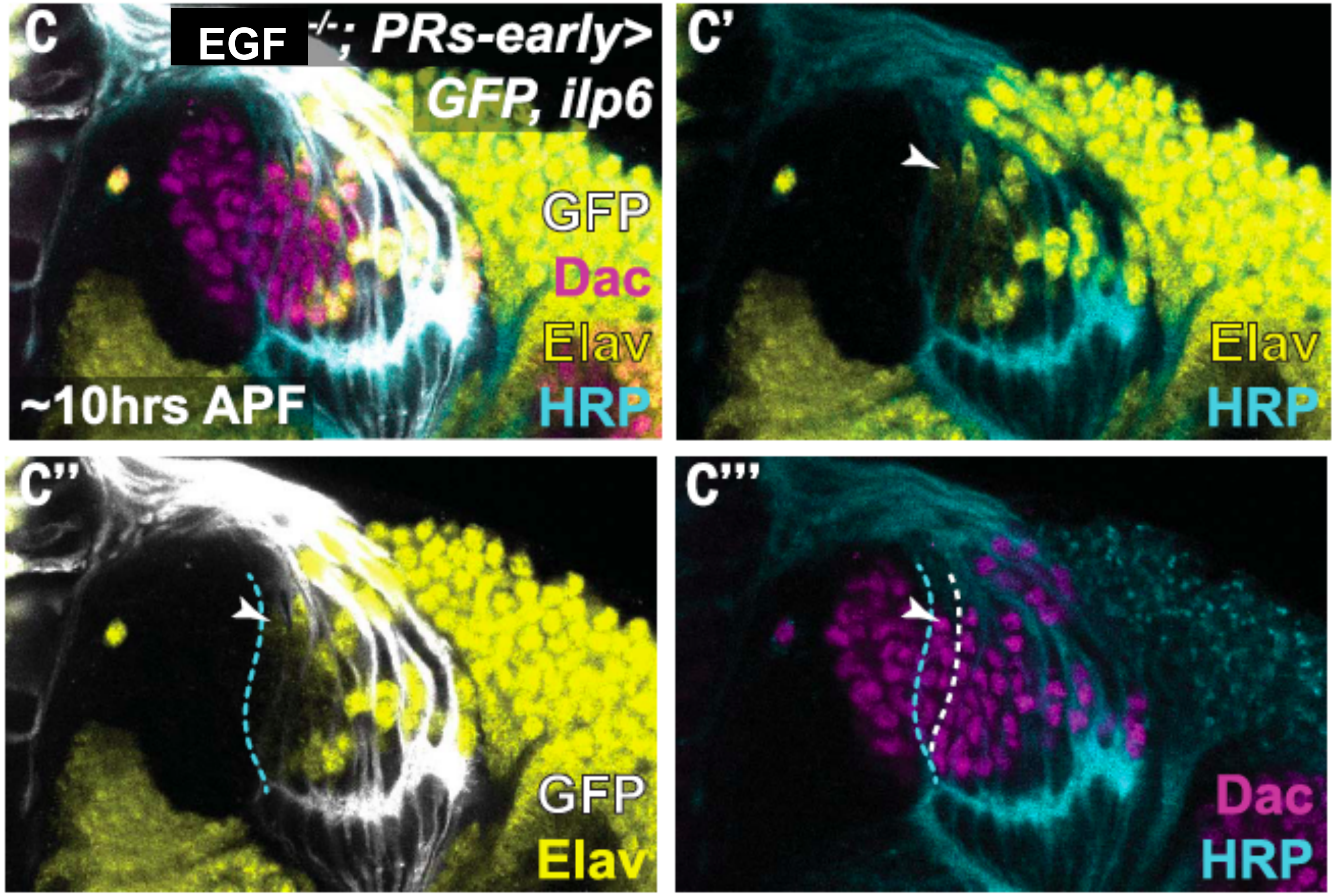
However, precursors differentiate only after column assembly is completed.



EGFR in wrapping glia, but not in lamina precursor cells, is required for lamina cells differentiation into neurons.



The connection between from photoreceptors to wrapping glia to lamina precursors induced a delay in the MAPK signalling that results in the assembly of lamina cells in columns followed by they differentiation



Activation of MPAK signalling in lamina precursor cells directly by photoreceptor axons disorganises column assembly.

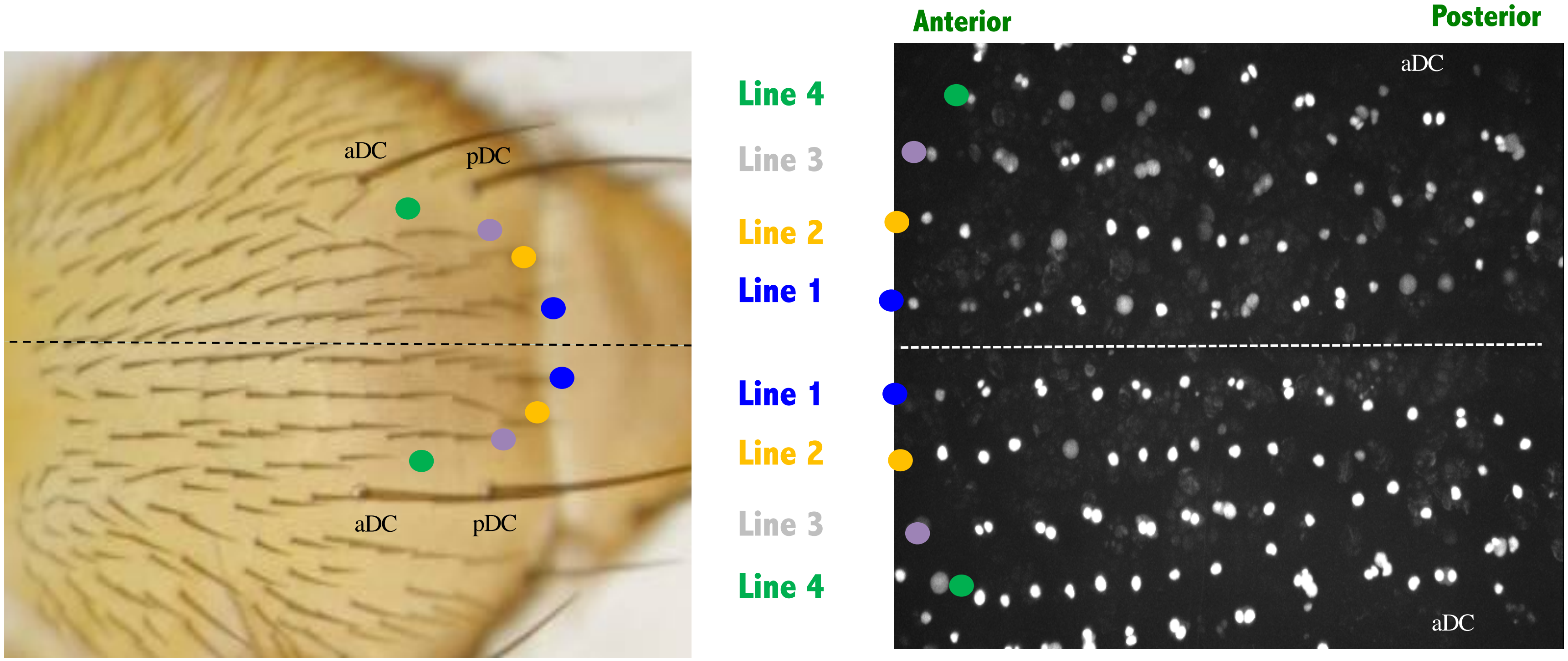
The connection between from photoreceptors to wrapping glia to lamina precursors induced a delay in the MAPK signalling that results in the assembly of lamina cells in columns followed by they differentiation

Elife. 2022 Mar 7;11:e75746. doi: 10.7554/eLife.75746.

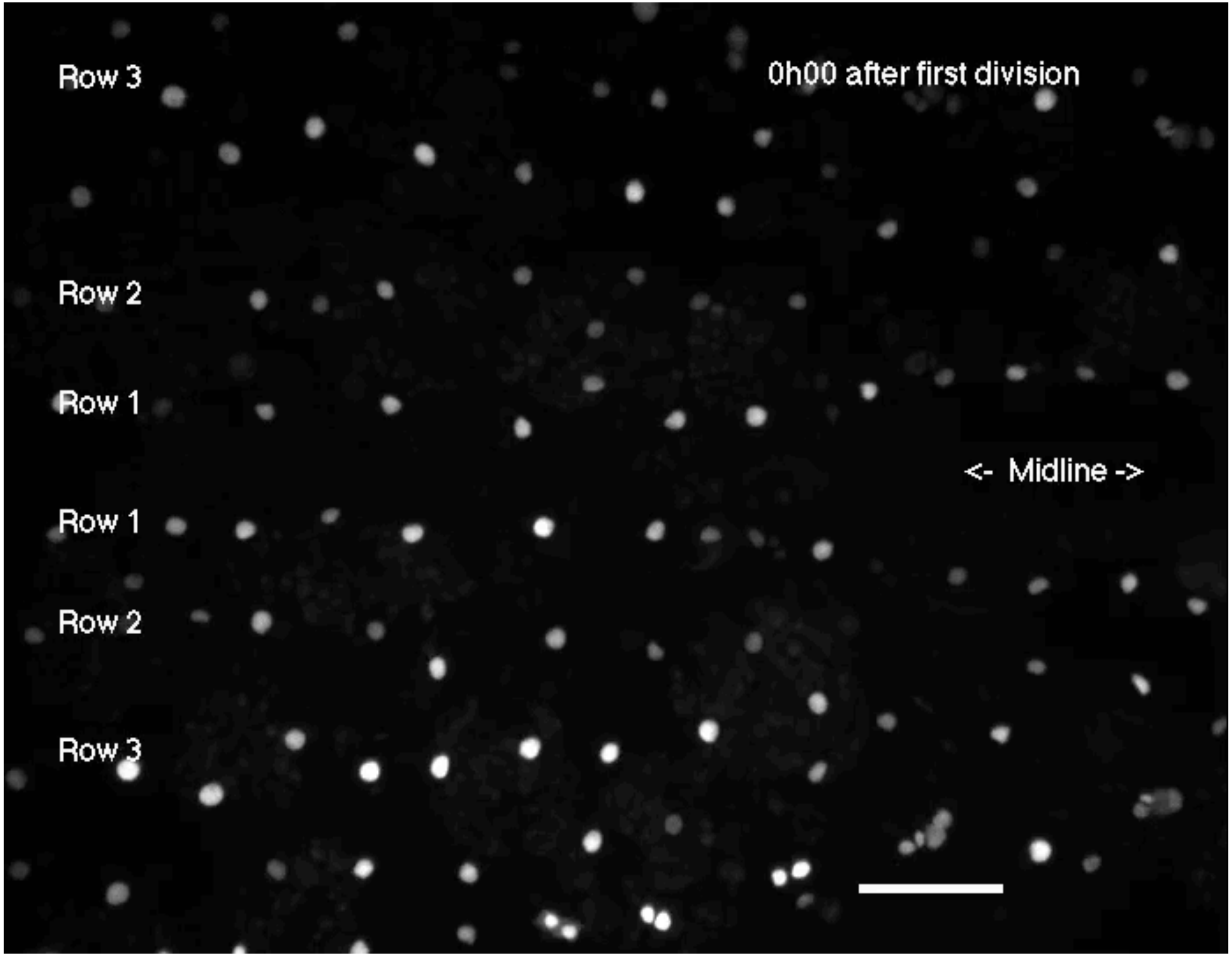
A neural progenitor mitotic wave is required for asynchronous axon outgrowth and morphology

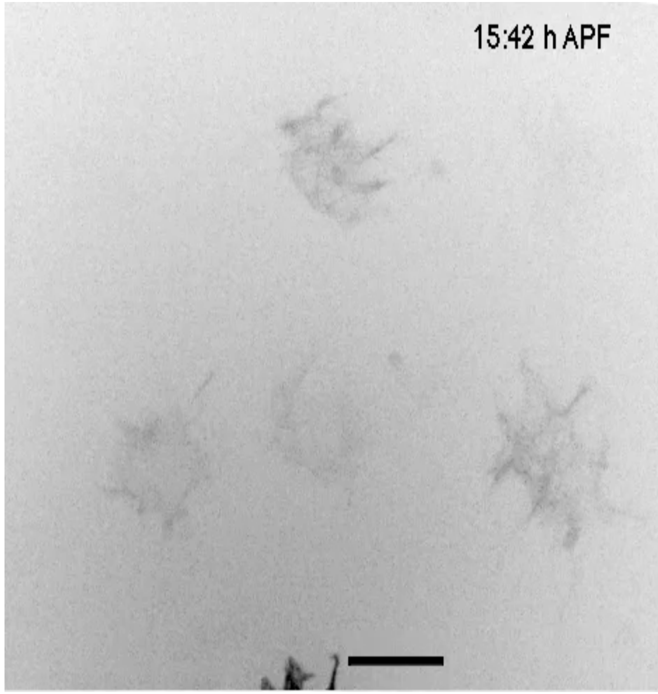
Jérôme Lacoste [1], Hédi Soula [2], Angélique Burg [1], Agnès Audibert [1], Pénélope Darnat [1], Michel Gho [#] [1], Sophie Louvet-Vallée [#] [1]

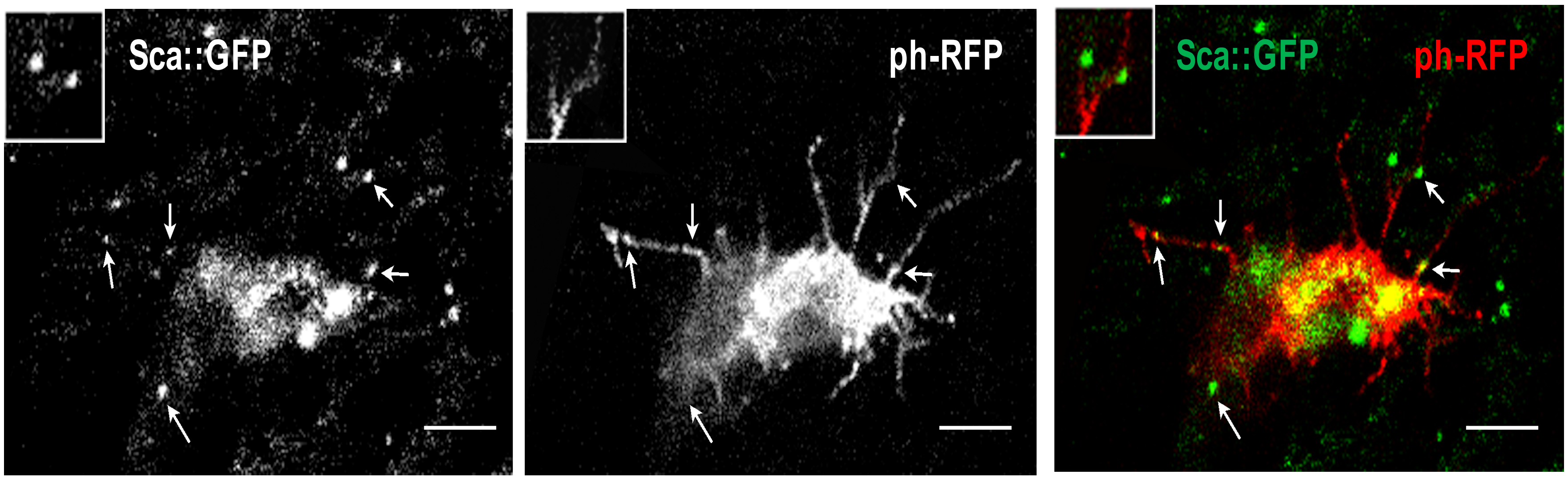
The Sensory Organ Precursor cells (SOP cells) producing the mechanosensory organs in *Drosophila* are among the few cases of normal G2-arrested somatic cells.

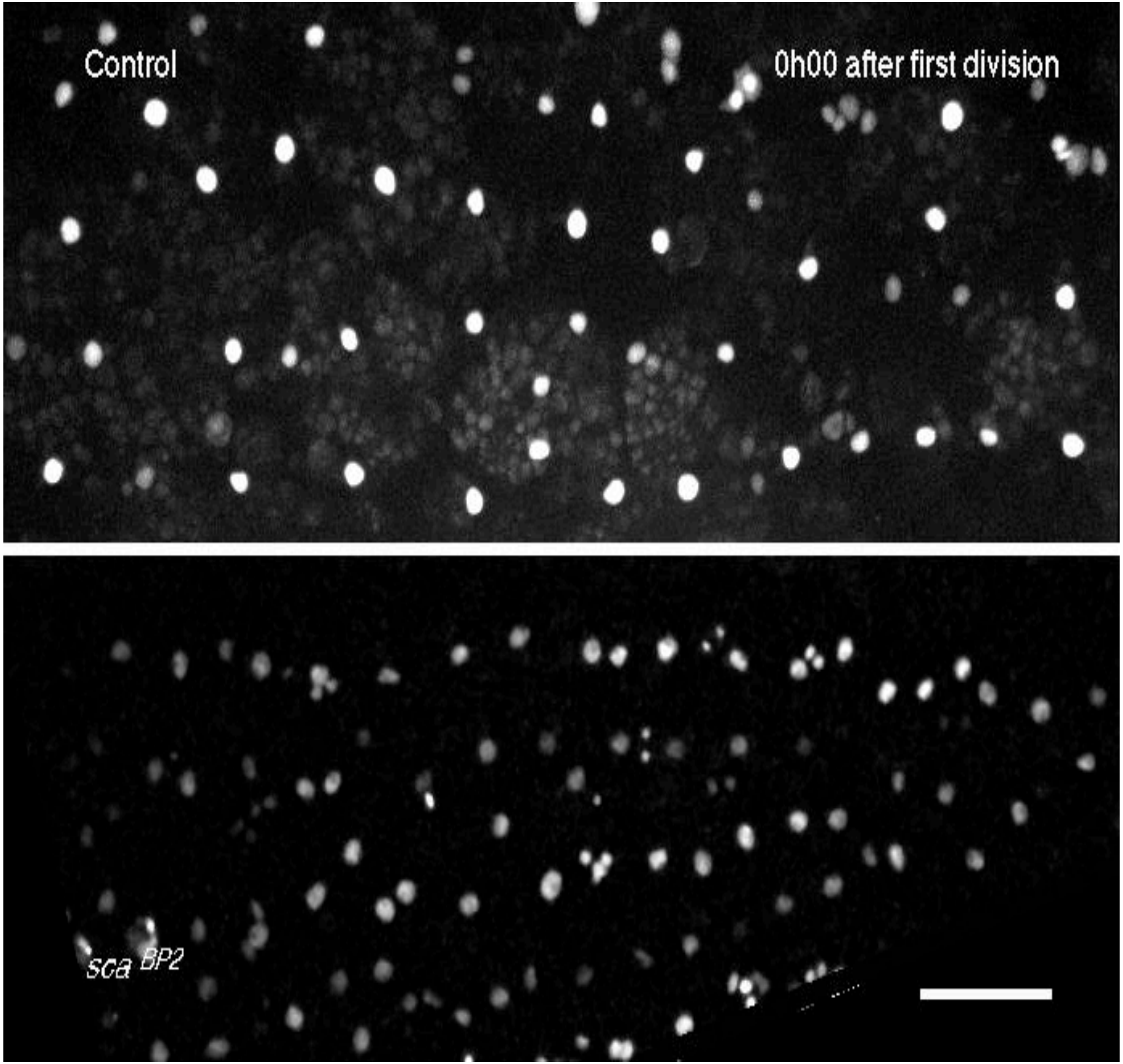


What is the dynamics of the resumption of the G2-arrest in these cells?


Row 3
0h00 after first division
Row 2
Row 1
<- Midline ->
Row 1
Row 2
Row 3


15:42 h APF

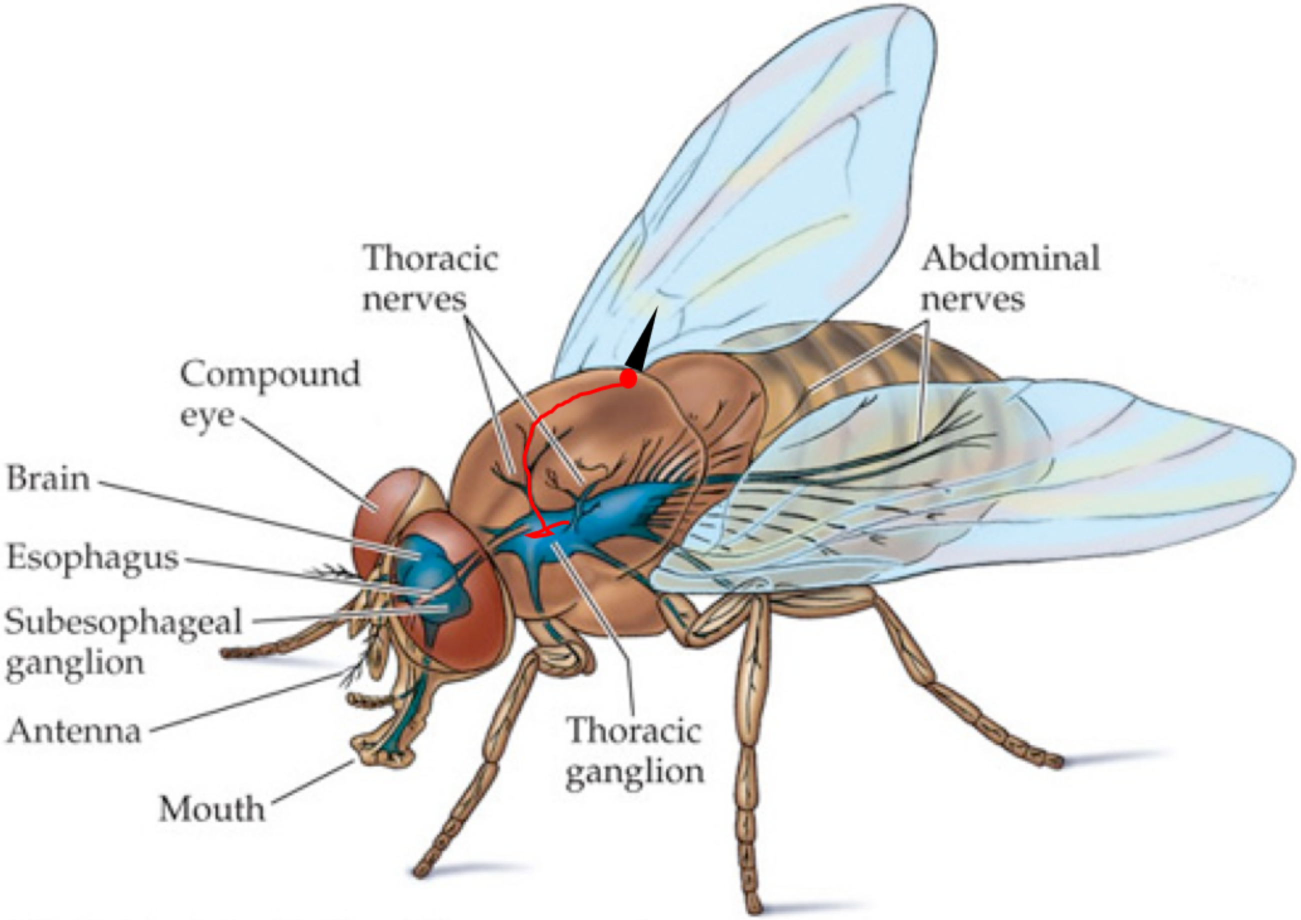

Sca::GFP
ph-RFP
Sca::GFP
ph-RFP



Control
0h00 after first division
sca BP2

The mitotic wave: physiological consequences

Morphometry of axonal projections in the thoracic ganglion

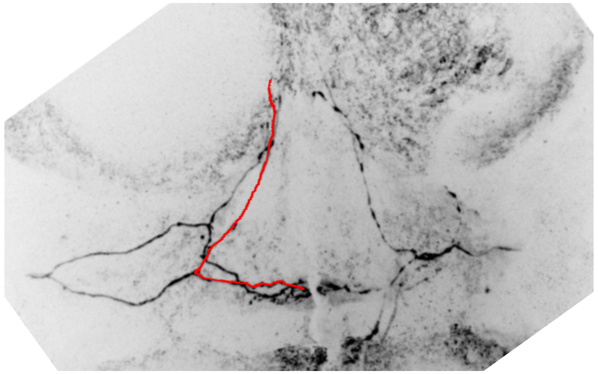


The mitotic wave: physiological consequences

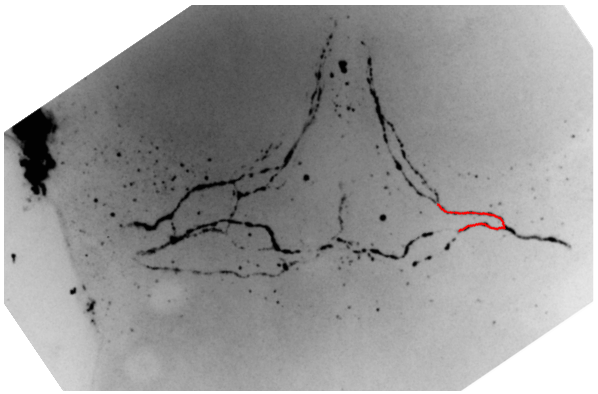
Morphometry of axonal projections in the thoracic ganglion

- **Pnr Gal4 > UAS-PH::GFP**

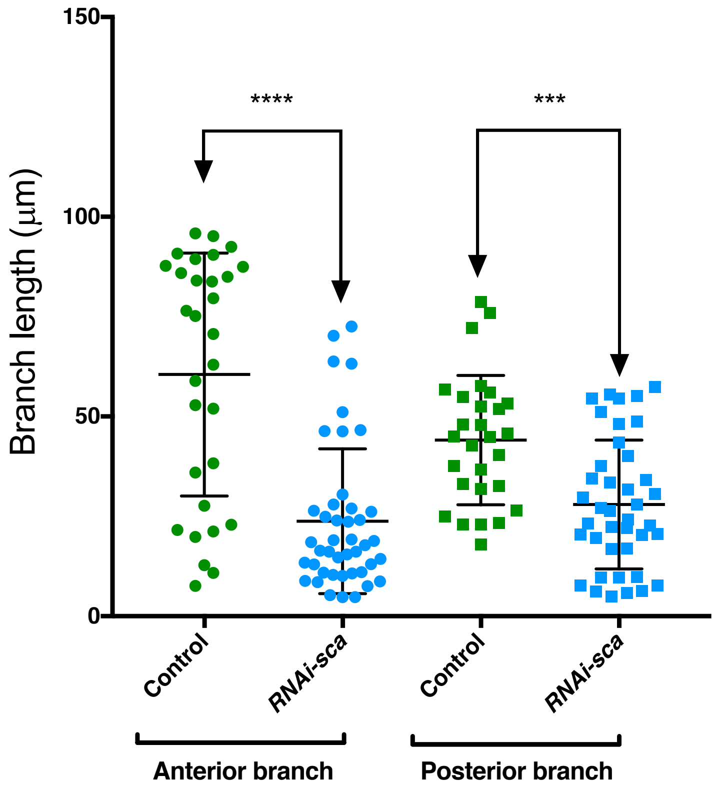
The mitotic wave: physiological consequences



Control



RNAi-sca



unpaired t test : *** p=0,0001 ; **** p<0,0001

When mitotic wave was specifically abolished, sensory organ axon terminals branched more than in control flies.

The cleaning reflex

The mitotic wave: physiological consequences

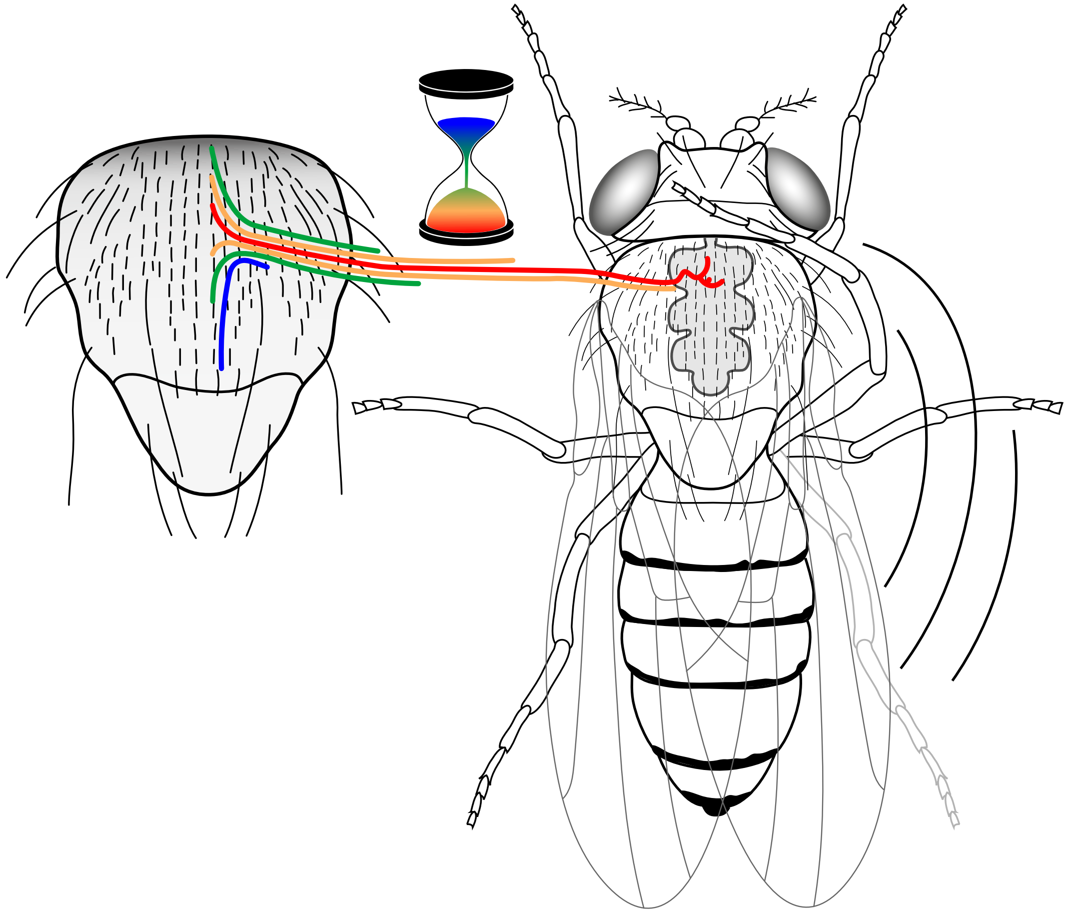
The cleaning reflex

Control

The mitotic wave: physiological consequences

The cleaning reflex

When mitotic wave
was abolished



The mitotic wave observed in precursor cells is related to progressive arrival of axon terminals into the central ganglion, and that this is required for a correct axon branching and appropriate behavior

In development, time is not only a dimension but also a parameter