# Lecture 5. *Drosophila* embryonic and larval neurogenesis.

24.10.24

# *Drosophila* Embryonic CNS Development: Neurogenesis, Gliogenesis, Cell Fate, and Differentiation

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**ABSTRACT** The *Drosophila* embryonic central nervous system (CNS) is a complex organ consisting of ~15,000 neurons and glia that is generated in ~1 day of development. For the past 40 years, *Drosophila* developmental neuroscientists have described each step of CNS development in precise molecular genetic detail. This has led to an understanding of how an intricate nervous system emerges from a single cell. These studies have also provided important, new concepts in developmental biology, and provided an essential model for understanding similar processes in other organisms. In this article, the key genes that guide *Drosophila* CNS development and how they function is reviewed. Features of CNS development covered in this review are neurogenesis, gliogenesis, cell fate specification, and differentiation.

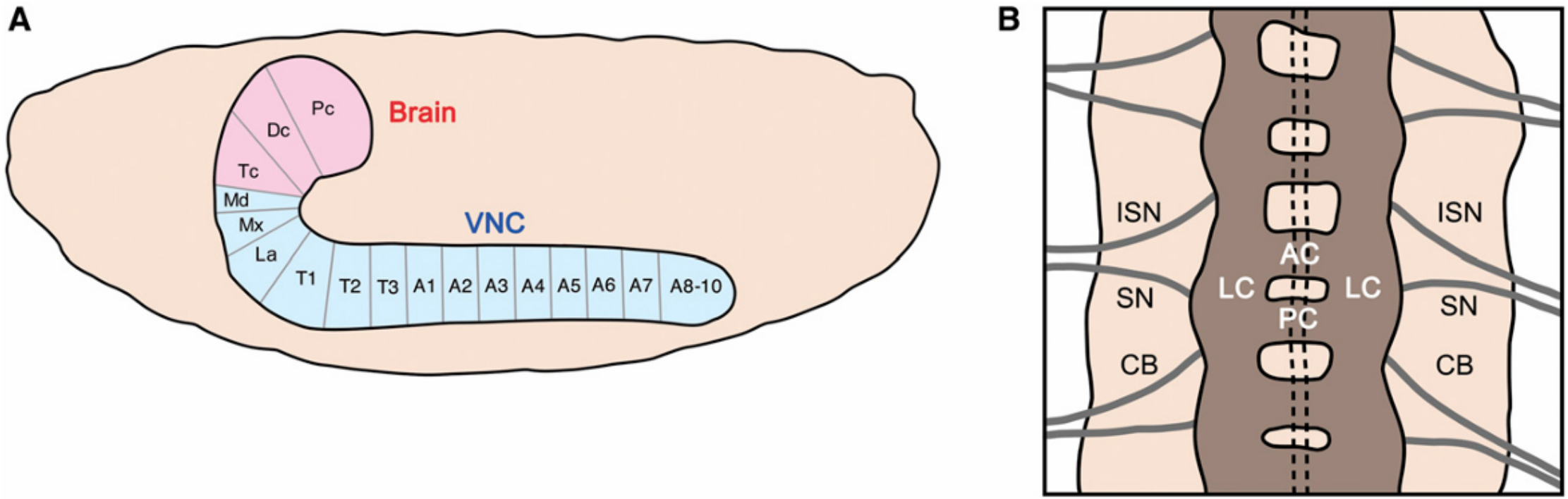



**Figure 1** Structure of the *Drosophila* embryonic CNS. (A) Schematic of a sagittal view of the CNS including brain (red) and ventral nerve cord (VNC; blue). Anterior is left and dorsal is top; neuromere names are listed in the text. (B) Horizontal (dorsal) view of three neuromeres of the VNC; anterior is top. The axon scaffold is shown in dark brown with the anterior commissure (AC), posterior commissure (PC), and lateral connectives (LC) indicated in one of the neuromeres. The cell bodies (CB) of the VNC are shown in tan; nerves shown include the intersegmental nerve (ISN) and segmental nerve (SN). The dotted lines represent the location of the CNS midline cells.

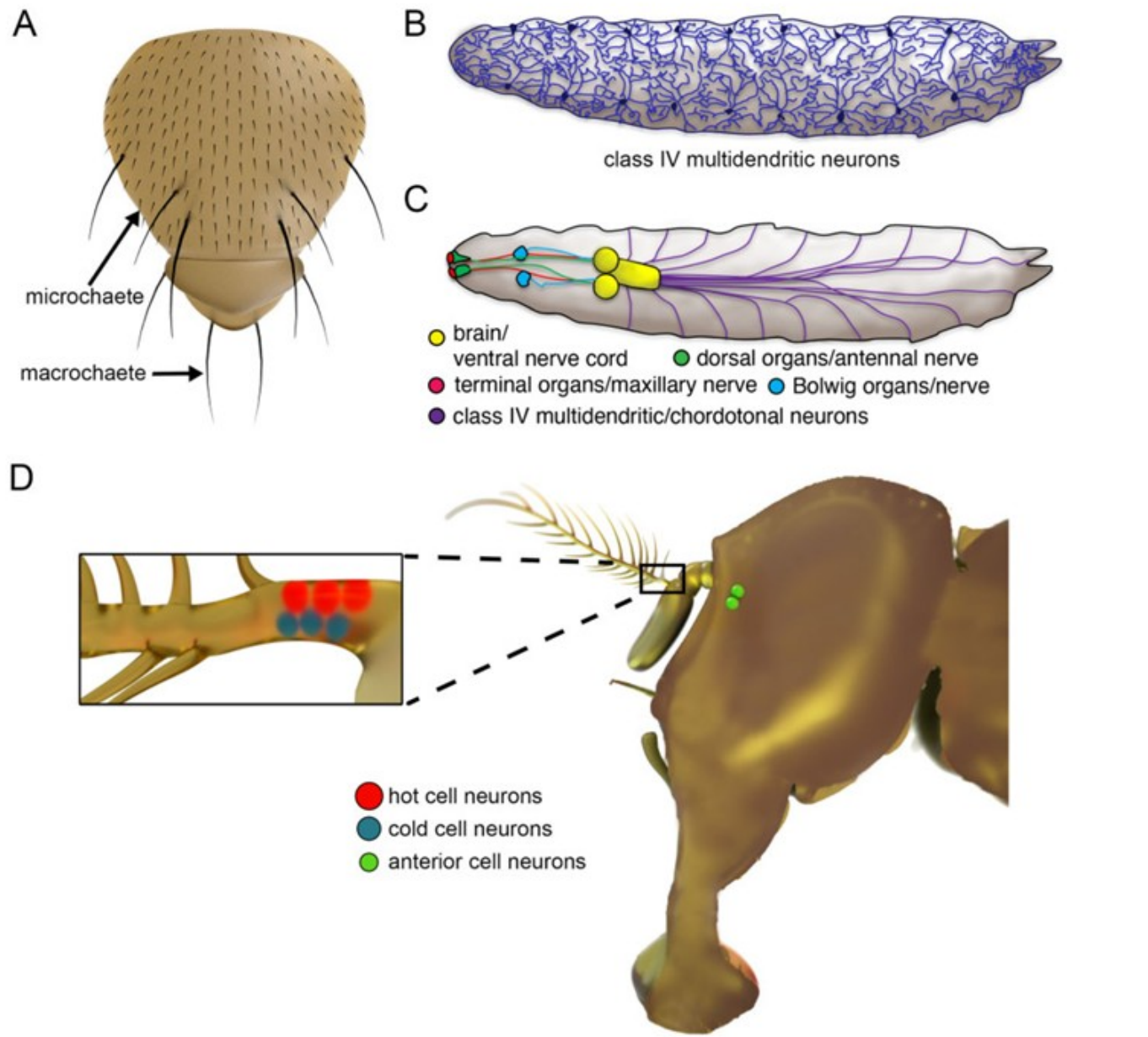



**Figure 7.** Mechanosensory sensilla on an adult thorax, thermosensory neurons in the arista, and sensory organs and neurons in larvae. (A) Distribution of microchaetae and macrochaetae on the adult thorax. (B) Arborization of class IV multidendritic neurons, which tile the body wall. (C) Sensory organs in larvae. (D) Side view of a head showing the positions of the anterior cell neurons in the brain (green) as well as the hot cell neurons (red cells) and cold cell neurons (blue) in the arista, a portion of which is magnified to the left.

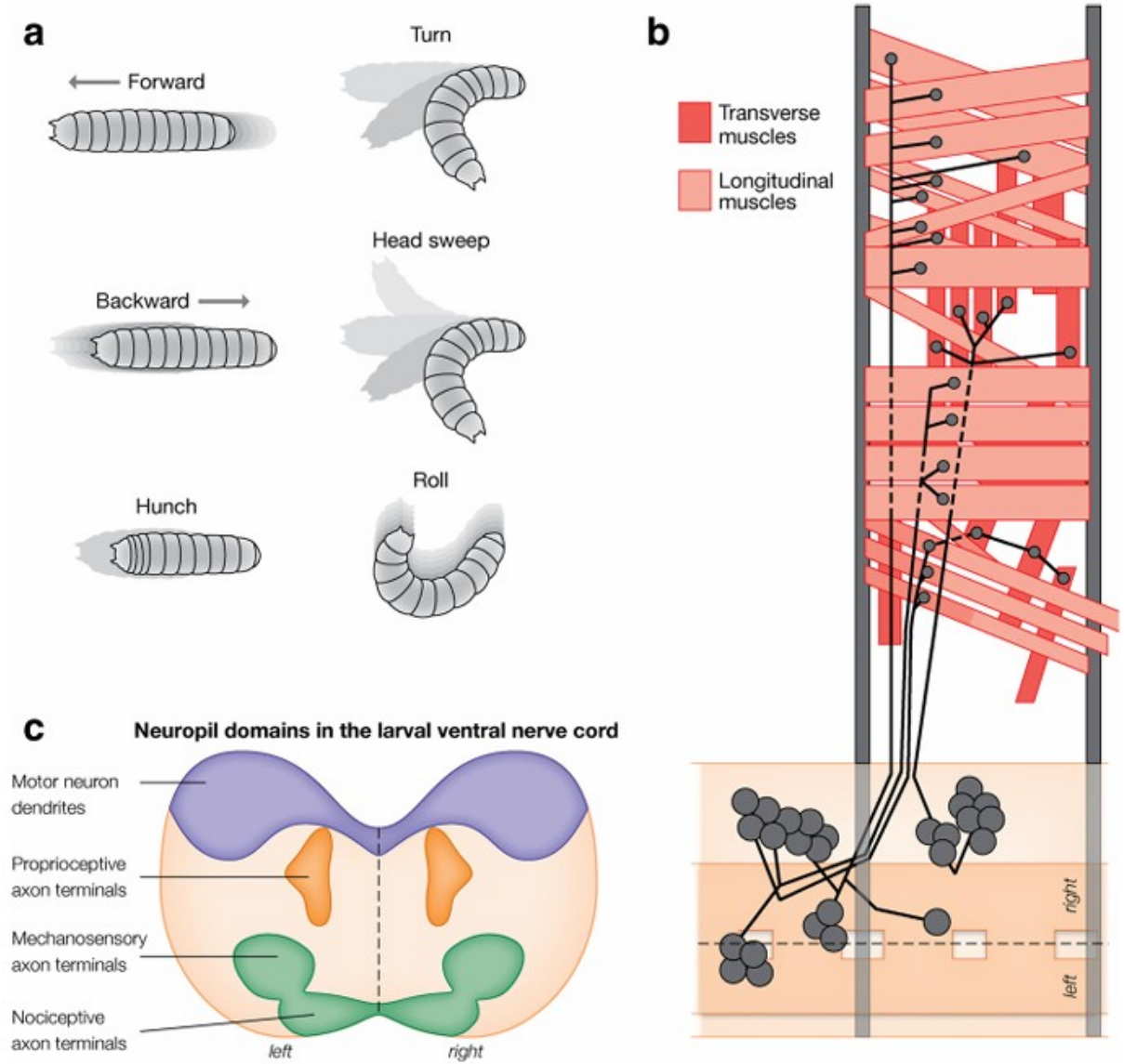



**Fig. 2** Muscles and motor neurons that drive various locomotor behaviors. **a** Larval locomotor behaviors. **b** Abdominal motor neurons and muscles in a single hemisegment. Only the type Ib motor neurons are shown (big bouton/single muscle target). Longitudinal muscles are light red, transverse muscles are darker red. Anterior to left; ventral midline, dashed line; dorsal midline at top of panel. **c** Cross-section schematic of abdominal neuropil; surrounding cell bodies are not shown. Motor dendrites target the dorsal (most internal) domain, sensory axons target ventral (most superficial) domains, with the exception of proprioceptive axons that target an intermediate domain. Ventral midline separating left/right sides, dashed line

Spatial transcription factors determining embryonic neuroblast identity

Dorso-ventral, segment polarity and homeotic gene control

DV first

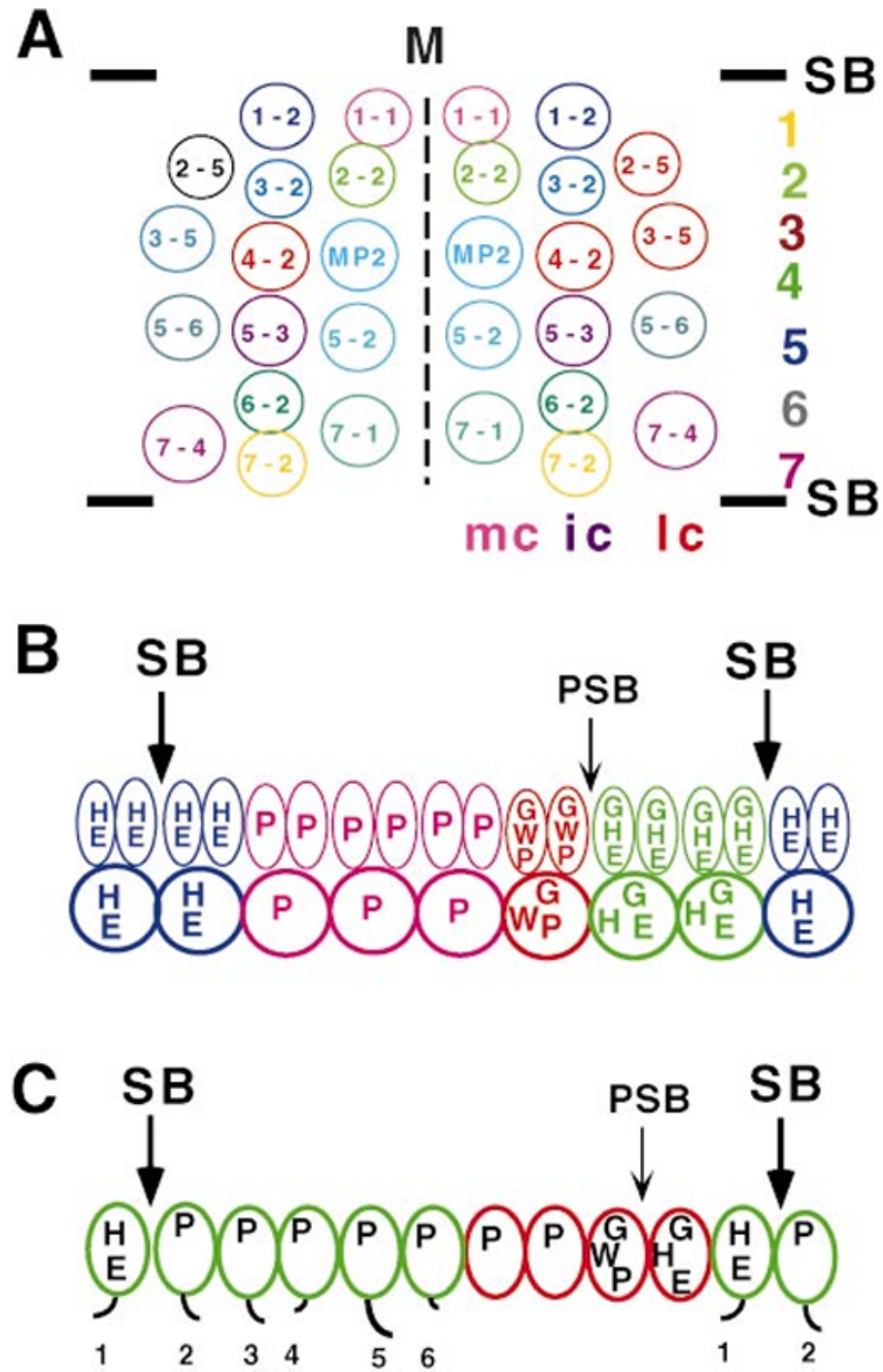



**Figure 1. A**: Neurogenesis in the ventral nerve cord of the Drosophila embryo. Schematic diagram of ventral view of a segment (neuromere) of an early stage 9 embryonic CNS (~ 4.5 hours of development at 22°C) is shown. The segmental boundary (SB) is shown by two small horizontal lines on each side. Each segment is divided into two hemisegments, separated by the midline (marked by the broken line, M). Thus, a total of 28 half-segments (or 28 hemineuromeres) are present in an embryo. In each hemisegment, ~ 30 neuroblasts are formed over a period of ~ 3 hours, however, in an early stage 9 embryo, only half of the total number of neuroblasts have delaminated. These neuroblasts are formed in rows (1–7, indicated by the numbers on the side) and columns (mc, medial column; ic, intermediate column; and lc, lateral column; please note that in an older stage embryo, one can discern as many as five columns) in a stereotypical manner on the ventral neurogenic region of the embryo. The numbers inside the neuroblasts represent their identity. Each of the neuroblasts is color-coded to indicate their distinct identity. The neuroblast numbering system in *Drosophila* is based on the system of neuroblast rows and columns that was adopted in the grasshopper and is in a medio-lateral order (smaller the number, more medial). Thus, NB4-2 for example, means a neuroblast formed in fourth row from the segmental border and second column from the midline. The Drosophila neuroblast map is less orthogonal compared to the grasshopper, but the numbering system is maintained to keep the grasshopper tradition. **B**: Expression of segment polarity genes in the neuroectoderm and neuroblasts. Saggital view is shown. About two rows of neuroectodermal cells correspond to one row of neuroblasts, thus, for example, *wg* is expressed in two rows of neuroectodermal cells whereas it is expressed only in one row of neuroblasts (see also Fig. 3 for expression of these genes in the neuroectoderm and neuroblasts). Note that expression of Gsb in the most posterior row of cells is restricted to only the cells closest to the midline; these cells give rise to NB7-1. **C**: Saggital view of the epidermal cells alternating the denticles

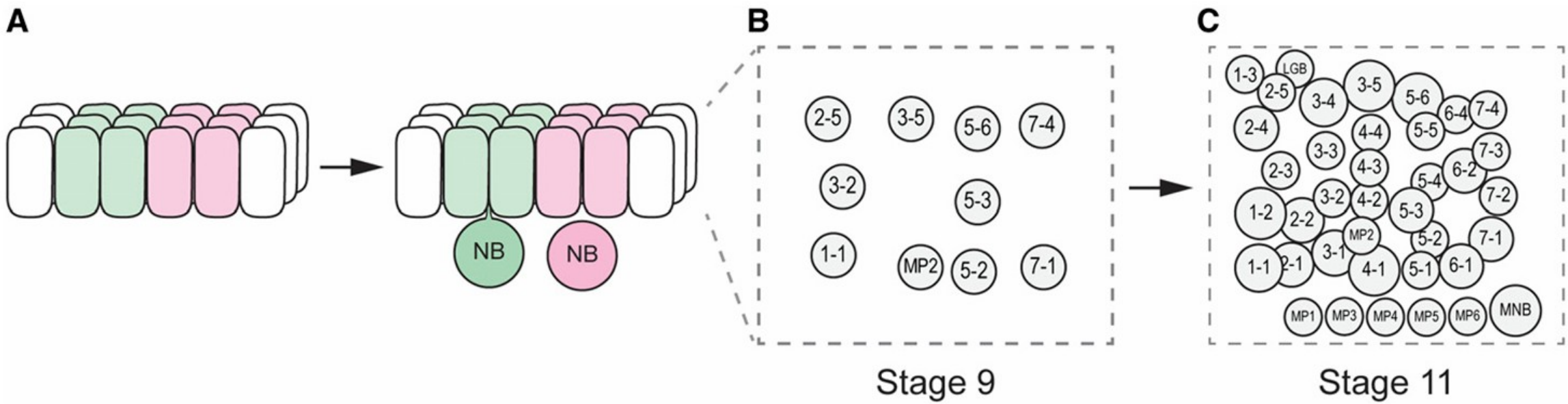


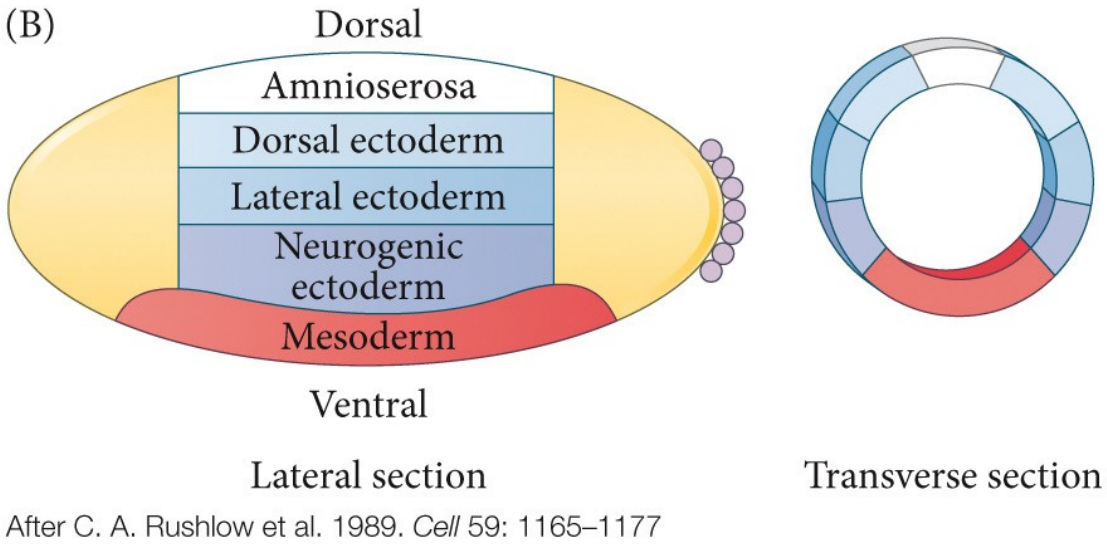

**Figure 2** Neuroblast formation. (A) Neuroblasts (NB) form and delaminate from the neuroectodermal layer. Proneural clusters of neuroectodermal cells give rise to a single NB. (B and C) Shown are hemi-neuromeres (anterior to the left; midline at bottom). At stage 9 (B) ~10 NBs have formed, and, by stage 11 (C), there are 32 NBs including MP2 and the longitudinal glioblast (LGB); midline precursors include the MNB and five MPs. Adapted by permission from Springer Nature: Nature Neuroscience Reviews (Kohwi and Doe 2013) copyright (2013), and by permission from the The Company of Biologists: Development (Urbach *et al.* 2016) copyright (2016).

# Figure 10.28 Specification of cell fate by the Dorsal protein

(A)

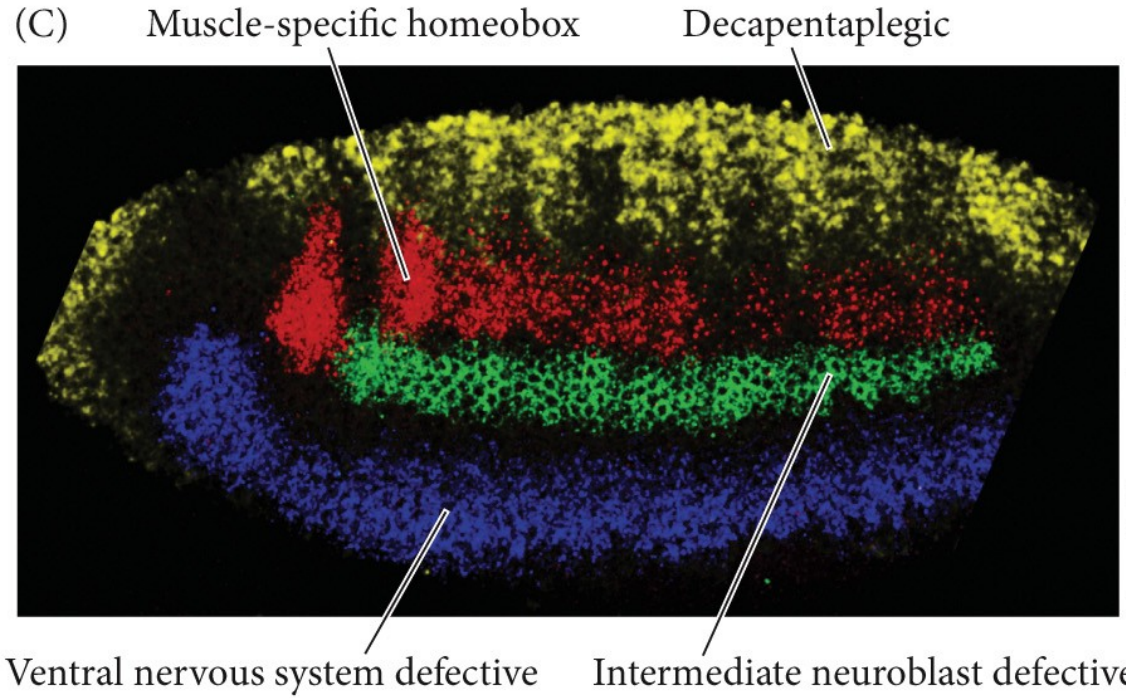
From S. Roth et al. 1989. *Cell* 59: 1189–1202, courtesy of the authors

(B)



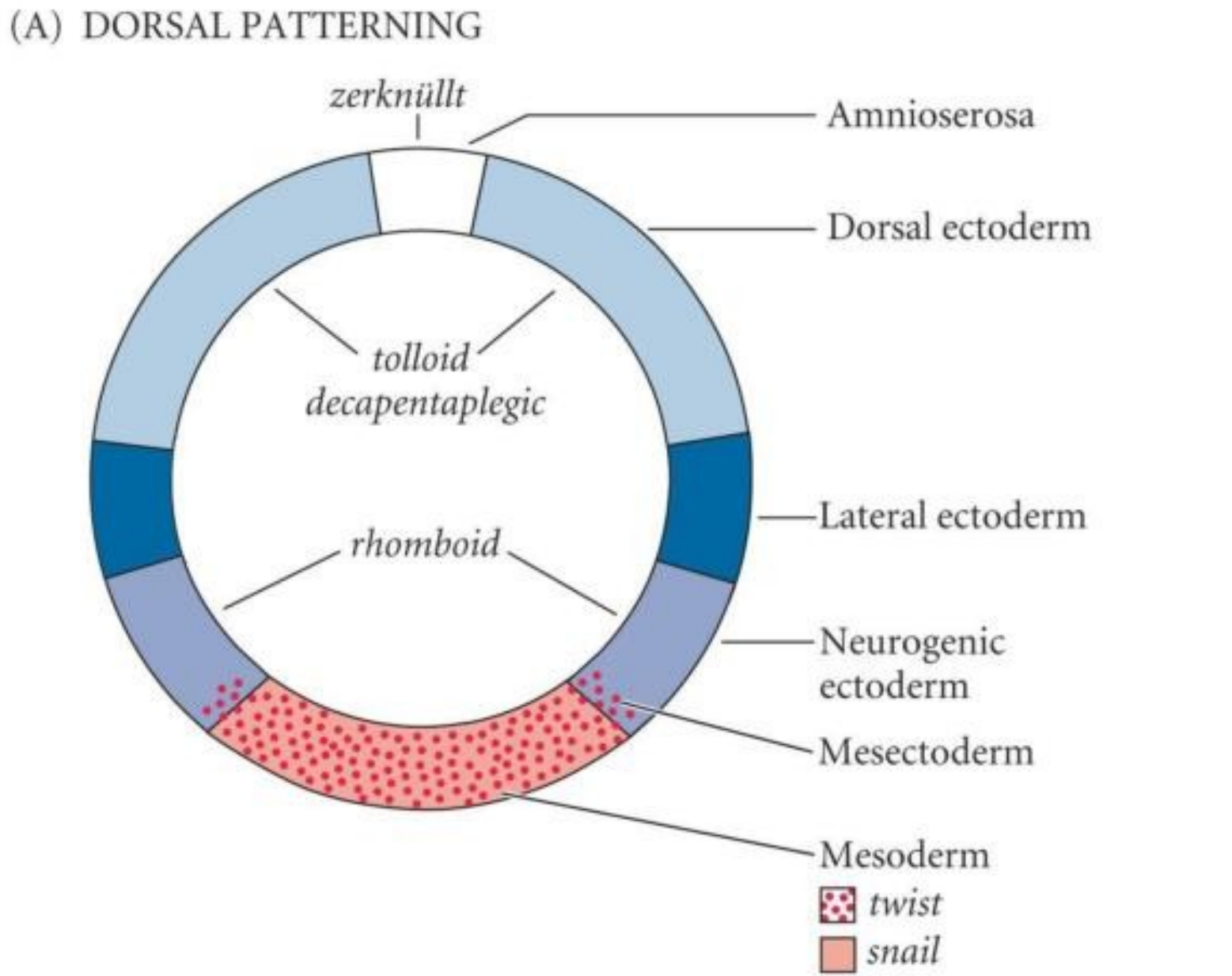

After C. A. Rushlow et al. 1989. *Cell* 59: 1165–1177

(C)



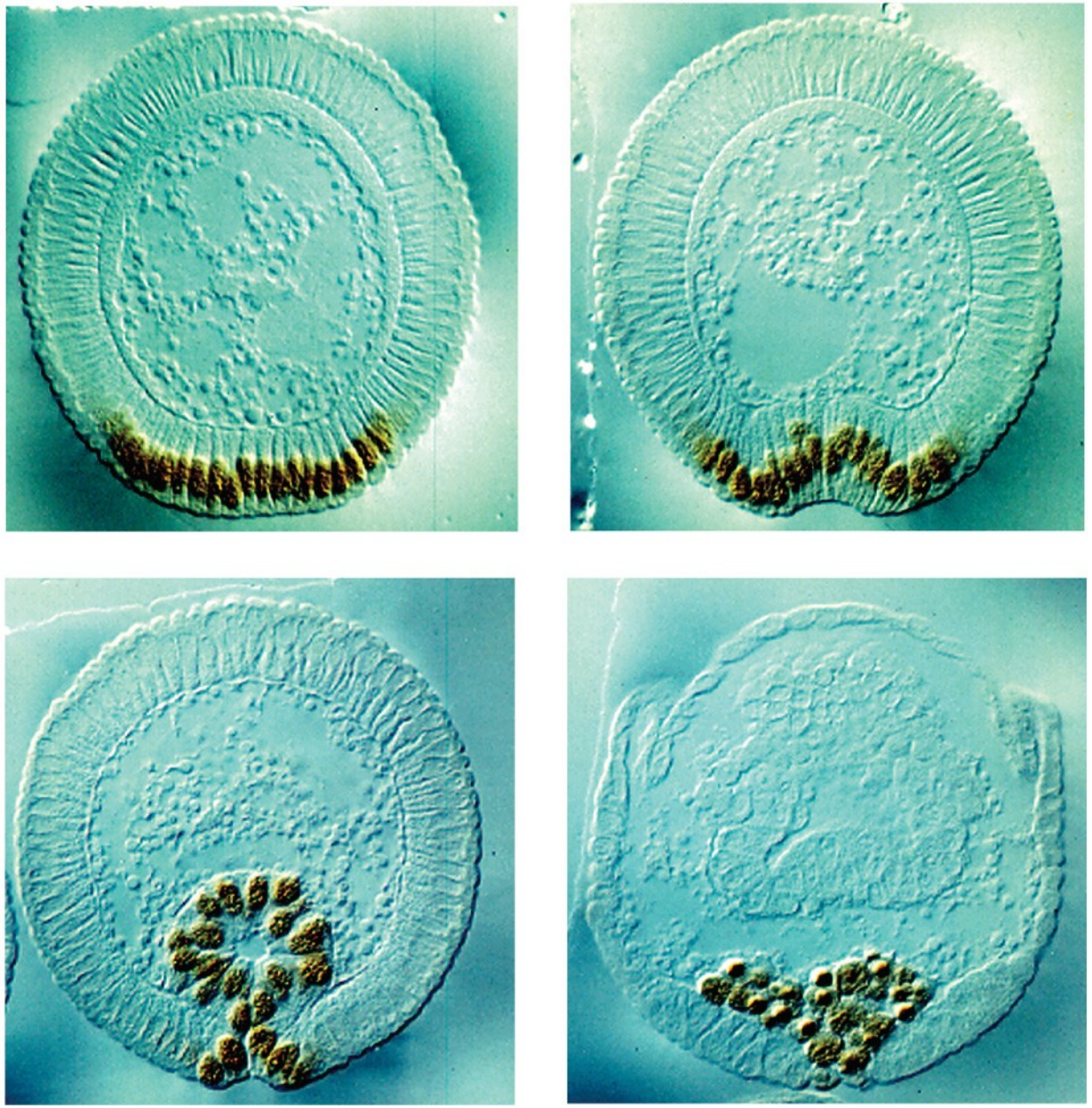

From D. Kosman et al. 2004. *Science* 305: 846, courtesy of D. Kosman and E. Bier

# Zygotically expressed genes




DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 9.41 (Part 1) Sinauer Associates, Inc.

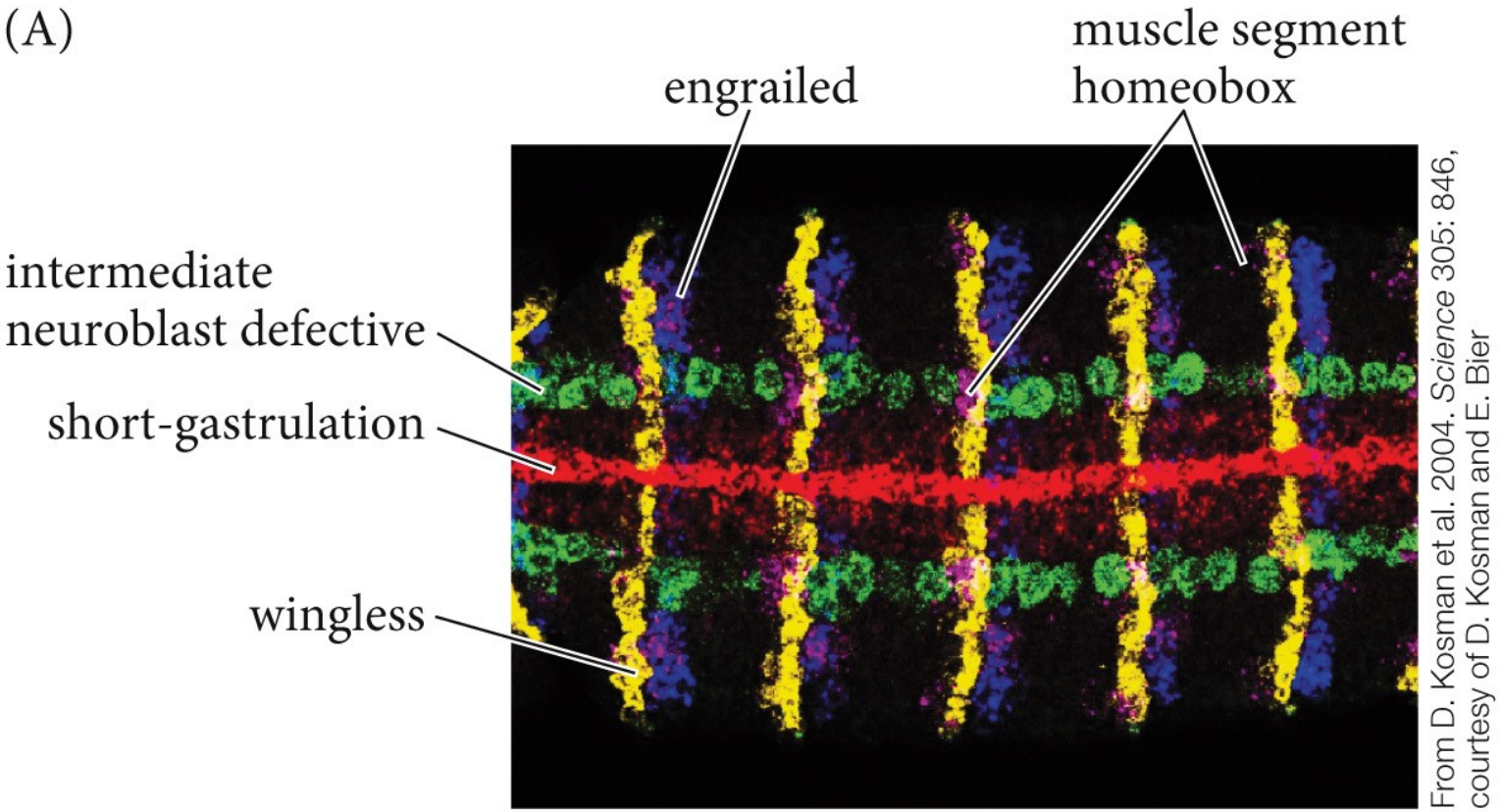
Figure 10.29 Gastrulation in *Drosophila*



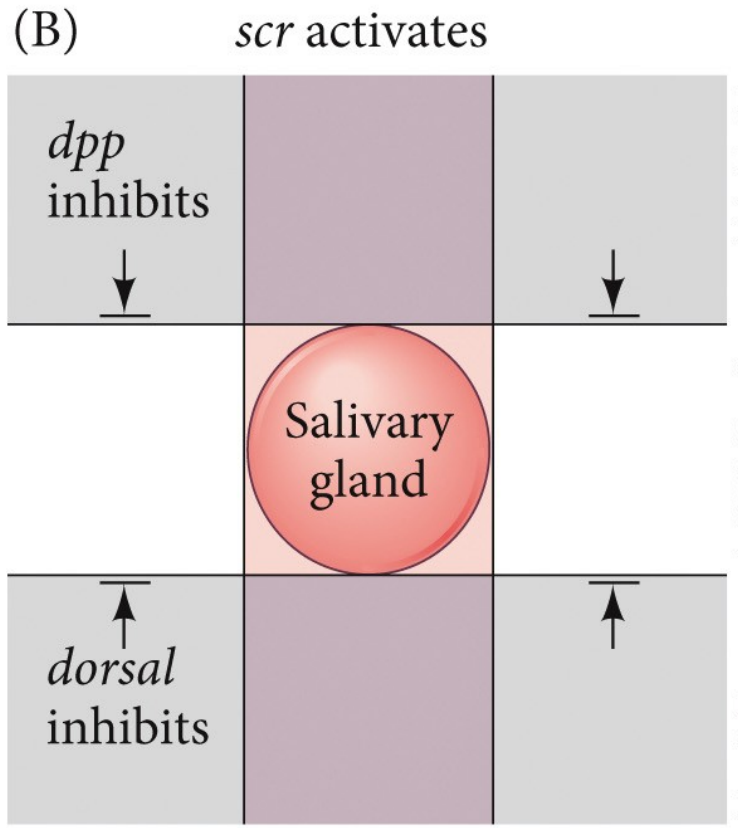
From M. Leptin. 1991. In *Gastrulation: Movements, Patterns, and Molecules*, R. Keller et al. (Eds.), pp. 199–212. Plenum: New York, courtesy of M. Leptin

*DEVELOPMENTAL BIOLOGY 13e*, Figure 10.29

Figure 10.30 Cartesian coordinate system mapped out by gene expression patterns




From D. Kosman et al. 2004. *Science* 305: 846, courtesy of D. Kosman and E. Bier




After S. Panzer et al. 1992. *Development* 114: 49–57

*DEVELOPMENTAL BIOLOGY 13e*, Figure 10.30

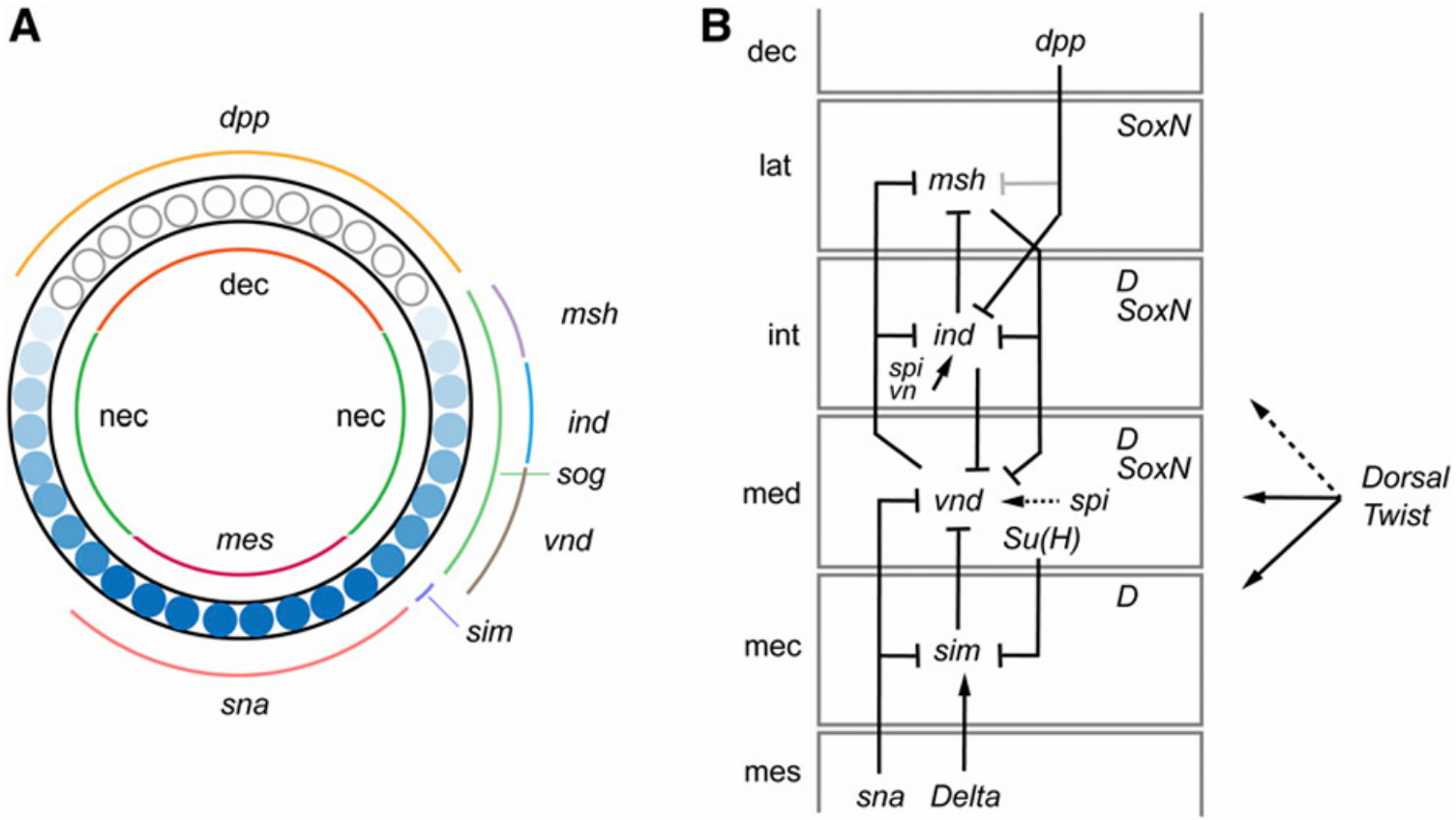



**Figure 3** Dorsal–ventral (D–V) patterning and neural identity genes. (A) Cross-section of a blastoderm embryo showing major cell types, gradient of Dorsal protein, and expression of D–V patterning genes (ventral is bottom). Inside shows the distribution of the three main cell types: mesoderm (mes), neuroectoderm (nec), and dorsal ectoderm (dec). The blue circles represents blastoderm nuclei and indicate the levels of Dorsal protein with dark shades equivalent to high levels of nuclear protein. The domains of expression of D–V patterning genes are shown on the outside. Adapted from Hong *et al.* 2008, copyright (2008) National Academy of Sciences. (B) Genetic interactions and expression patterns occurring in the different neuroectodermal domains that promote neural precursor identity. Neuroectodermal domains are lateral (lat), intermediate (int), and medial (med) neuroectoderm, and mesectoderm (mec). Also shown are dorsal ectoderm (dec) and mesoderm (mes). *dpp* is a stronger repressor of *ind* expression (dark) than *msh* expression (gray). Maintenance of *vnd* expression by *spi* signaling is indicated by a dashed arrow. Dorsal-Twist regulation: solid lines indicate regulation by both TFs and dotted line indicates regulation by only Dorsal. *Dichaete* (*D*) and *SoxN* are shown in their columns of expression.

# AP axis
# Segment polarity and homeotic genes

# Segment polarity genes in neuroblast formation and identity specification during *Drosophila* neurogenesis

**Krishna Moorthi Bhat**

**Summary**

The relatively simple central nervous system (CNS) of the *Drosophila* embryo provides a useful model system for investigating the mechanisms that generate and pattern complex nervous systems. Central to the generation of different types of neurons by precursor neuroblasts is the initial specification of neuroblast identity and the *Drosophila* segment polarity genes, genes that specify regions within a segment or repeating unit of the *Drosophila* embryo, have emerged recently as significant players in this process. During neurogenesis the segment polarity genes are expressed in the neuroectodermal cells from which neuroblasts delaminate and they continue to be expressed in neuroblasts and their progeny. Loss-of-function mutations in these genes lead to a failure in the formation of neuroblasts and/or specification of neuroblast identity. Results from several recent studies suggest that regulatory interactions between segment polarity genes during neurogenesis lead to an increase in the number of neuroblasts and specification of different identities to neuroblasts within a population of cells. *BioEssays* 21:472–485, 1999. 

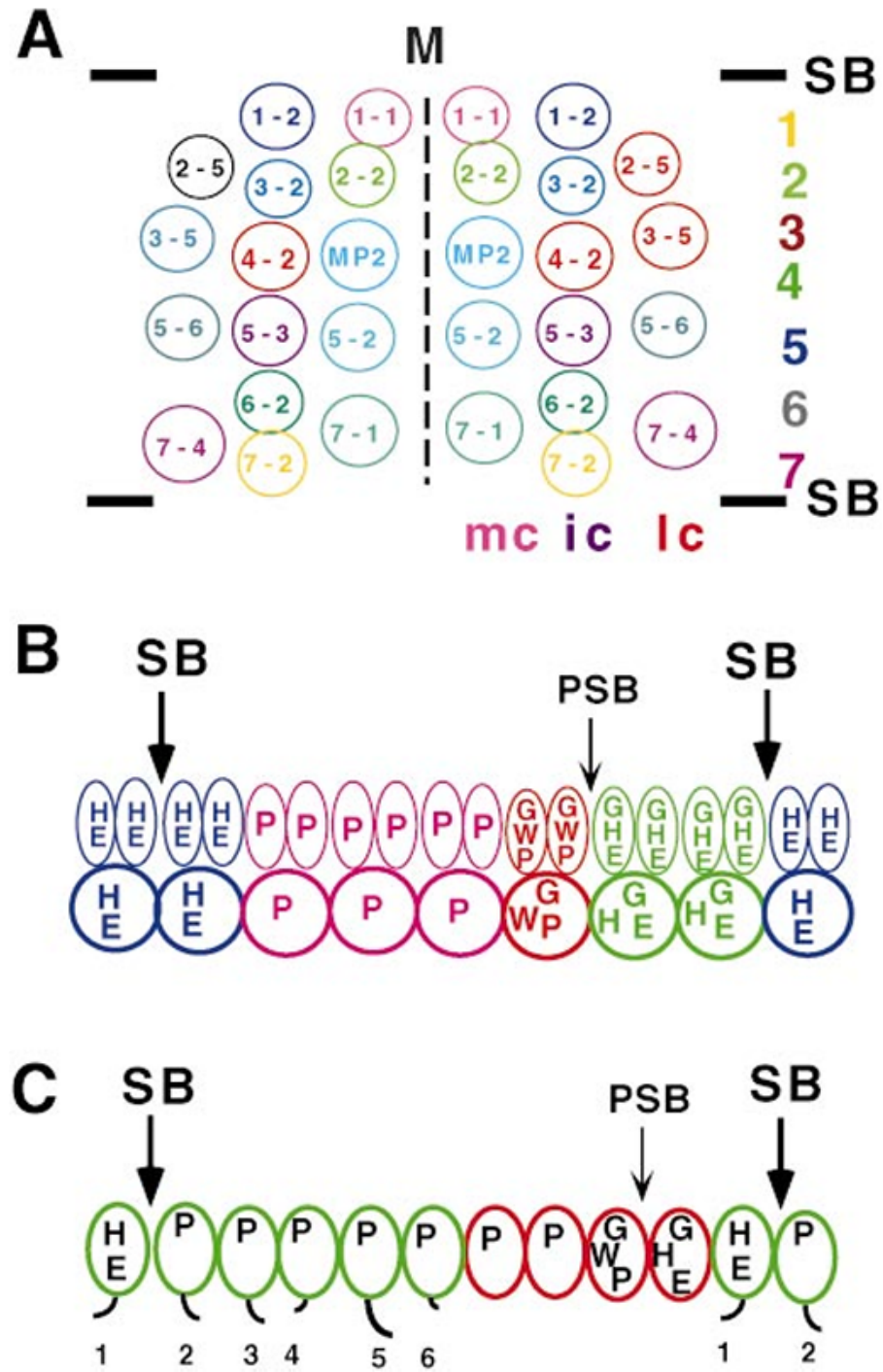



**Figure 1. A**: Neurogenesis in the ventral nerve cord of the Drosophila embryo. Schematic diagram of ventral view of a segment (neuromere) of an early stage 9 embryonic CNS (~ 4.5 hours of development at 22°C) is shown. The segmental boundary (SB) is shown by two small horizontal lines on each side. Each segment is divided into two hemisegments, separated by the midline (marked by the broken line, M). Thus, a total of 28 half-segments (or 28 hemineuromeres) are present in an embryo. In each hemisegment, ~ 30 neuroblasts are formed over a period of ~ 3 hours, however, in an early stage 9 embryo, only half of the total number of neuroblasts have delaminated. These neuroblasts are formed in rows (1–7, indicated by the numbers on the side) and columns (mc, medial column; ic, intermediate column; and lc, lateral column; please note that in an older stage embryo, one can discern as many as five columns) in a stereotypical manner on the ventral neurogenic region of the embryo. The numbers inside the neuroblasts represent their identity. Each of the neuroblasts is color-coded to indicate their distinct identity. The neuroblast numbering system in *Drosophila* is based on the system of neuroblast rows and columns that was adopted in the grasshopper and is in a medio-lateral order (smaller the number, more medial). Thus, NB4-2 for example, means a neuroblast formed in fourth row from the segmental border and second column from the midline. The Drosophila neuroblast map is less orthogonal compared to the grasshopper, but the numbering system is maintained to keep the grasshopper tradition. **B**: Expression of segment polarity genes in the neuroectoderm and neuroblasts. Saggital view is shown. About two rows of neuroectodermal cells correspond to one row of neuroblasts, thus, for example, *wg* is expressed in two rows of neuroectodermal cells whereas it is expressed only in one row of neuroblasts (see also Fig. 3 for expression of these genes in the neuroectoderm and neuroblasts). Note that expression of Gsb in the most posterior row of cells is restricted to only the cells closest to the midline; these cells give rise to NB7-1. **C**: Saggital view of the epidermal cells alternating the denticles

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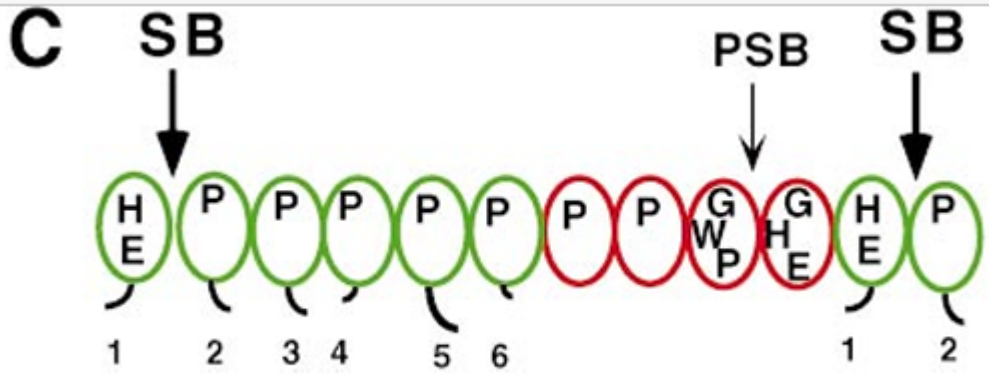


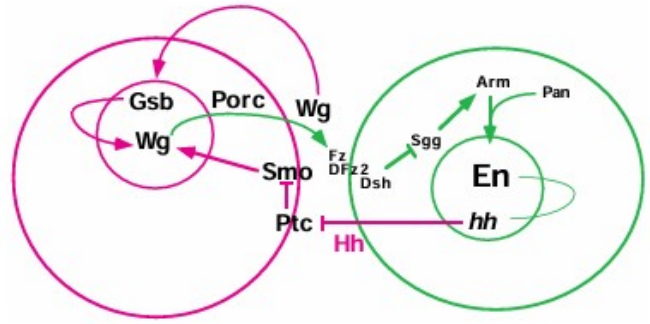



**Figure 2.** The Wg-signaling pathway and its interaction with other segment polarity genes during ectodermal patterning. The *wg* gene product is secreted out from the *wg*-expressing cells. This secretion is mediated by Porc. The Wg signal is then transduced across the membrane involving Frizzled proteins (such as Fz and DFz2) in the adjacent receiving cells. The cytoplasmic protein, Dsh, appears to be part of the Wg-reception complex and is involved in the transmission of the Wg signal to interior. How Dsh mediates this signal transduction has not been understood at the molecular level. This signal inactivates the Sgg/ZW3/GSK3 protein kinase, thus preventing Sgg from phosphorylating the Arm/β-Catenin protein. Although a hyper-phosphorylated Arm stays in the cytoplasm, a hypo-phosphorylated Arm (as a consequence of the Wg signal transduction) makes a complex with Pangolin/TCF-1/LEF-1 and translocates to the nucleus. In the epidermis, this pathway ultimately activates *en* (the ultimate targets of this pathway in the CNS is not known). One of the functions of En is to maintain *hh* expression. The secreted Hh interacts with its receptor Ptc, thus relieving the repression of Ptc on Smo. Smo ultimately activates downstream genes such as *wg* or *ptc*. Wg can also autoregulate via Gsb. The interaction between these genes changes depending upon the timing or particular stage of development.

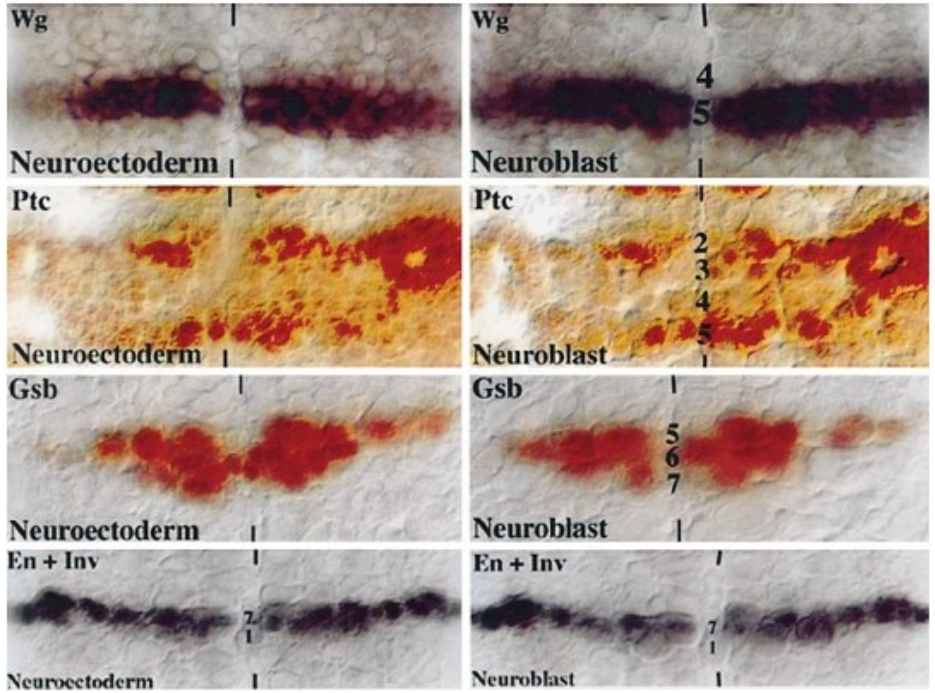



**Figure 3.** Neuroectodermal and neuroblast expression of segment polarity genes during early neurogenesis. The embryos are stained with various antibodies as indicated in different panels. The anterior end is up and the midline is marked by vertical lines. The embryos are ~ stage 10, approximately 5. 5 hours old. The numbers along the midline indicate rows of neuroblasts. The neuroectodermal staining is observed by a more ventral focusing on the surface of the embryo whereas neuroblast staining is observed at the layer beneath the surface neuroectodermal cells. Also, the neuroectodermal cells are smaller compared to neuroblasts.

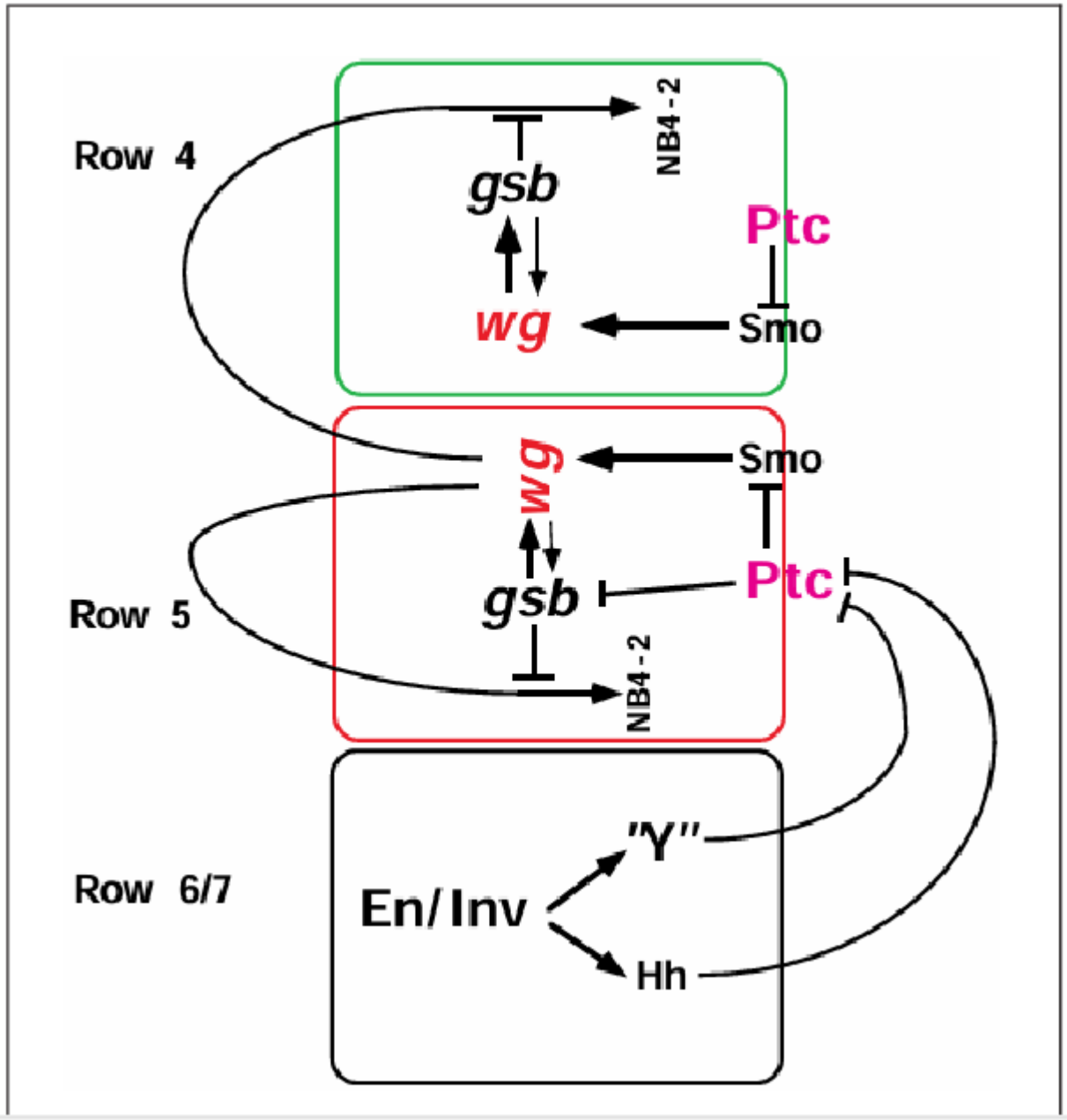



**Figure 4.** Interaction of signaling pathways mediated by *wg*, *ptc*, *hh*, *gsb*, and *en/inv* during neuroblast identity specification. In row 4 cells, Ptc represses expression of *gsb* by repressing *wg*, an activator of *gsb*. This allows the Wg signal from row 5 to specify NB4-2 identity to a row 4 cell. Ptc blocks the expression of *gsb* in row 4 cells by repressing *wg*, an activator of *gsb* in row 4. On the other hand, the activation of *wg* in row 4 in *ptc* mutants, is Smo-dependent. Since the level of Gsb in rows 4–6 and NB7-1 in row 7 in *ptc* mutants is higher than wild-type (Gsb is expressed only in rows 5, 6, and NB7-1 in wild-type, see Figs. 1 and 2), Ptc may also repress *gsb* expression either directly or via repression of an activator of *gsb*. Gsb also maintains the wild-type level and pattern of *wg* expression in rows 4 and 5 in *ptc* mutants and in row 5 in wild-type embryos. In row 5 cells, the negative regulatory circuit mediated by Ptc is relieved by En/Inv acting through an unidentified signaling molecule "Y". "Y" must be distinct from (but perhaps redundant to) Hh since unlike loss of *en/inv*, loss of Hh activity does not relieve the repression of Ptc on *gsb* or *wg*. The uncoupling of the *ptc-gsb* regulatory circuit in row 5 by *en/inv*-induced signal also allows Gsb to block an autocrine Wg signal from specifying NB4-2 identity to the NB5-3 precursor. With the postulated En/Inv→"Y" pathway, the En/Inv→Hh-signaling pathway is required for wild-type *wg* expression. The *hh* is activated in row 6/7 cells by *en/inv* and the Hh protein secreted from these cells interacts with Ptc allowing the activation of *wg* expression by Smo. Interaction of Hh (and/or "Y") with Ptc also relieves the repression of *wg* by Ptc. The interaction of these genes shown here appear to occur during neuroblast identity specification between late stage 8 and late stage 9 but not during neuroblast formation.

**TABLE 1.** The *Drosophila* Segment Polarity Genes and Their Requirements During Neurogenesis in the Embryonic CNS (see text for details).

| Genes | Mutant effect on neuroblast formation | Mutant effect on neuroblast identity specification | Comments | References |
|---|---|---|---|---|
| *wingless* | Affects NBs in rows 4, 5 and 6 | Affects NB4-2 identity (affect on other NBs ND) | Expressed in row 5<br>Required in rows 4, 5 and 6 | 17,21,31 |
| *hedgehog* | Affects NBs in rows 3 and 6 | ND | Expressed in rows 7, 1, 2 | 21,22,47 |
| *patched* | Affects NBs in rows 2–5 | Affects NB4-2 identity (affect on other NBs ND) | Expressed in rows 2–5<br>Represses expression of *wg* and *gsb* in row 4 | 20,21,24 |
| *gooseberry* | Affects NBs in row 5 (affect on row 6 and NB7-1 ND) | Transformation of NB5-3 to NB4-2 (affect on other NBs ND) | Expressed in rows 5, 6, NB7-1<br>Prevents Wg from specifying NB4-2 identity to NB5-3 | 18,19,20 |
| *engrailed, invected* | ND (+) | Transformation of NB5-3 to NB4-2 (affect on other NBs ND) | Expressed in rows 6, 7, NB1-2<br>Prevents Ptc from repressing *gsb* in row 5 | 21, 75 |
| *patched, gooseberry* | Affects NBs in rows 2–5 | Loss of NB4-2 identity is rescued (affect on other NBs ND)<br>The NB4-2 formation defect is not rescued | Loss of *gsb* allows Wg to specify NB4-2 identity.<br>NB5-3 is still transformed to NB4-2 | 20,21,24 |
| *patched, gooseberry, wingless* | Affects NBs in rows 2–6 | Loss of NB4-2 identity | The rescue of NB4-2 identity in *ptc, gsb* double mutants is Wg-dependent | 20, 21 |
| *engrailed, invected, gooseberry* | Affects NBs in row 5 (affect on other rows ND) | Transformation of NB5-3 to NB4-2<br>An additional NB is also transformed to NB4-2 | This triple mutant effect is Wg-dependent | 21 |

ND, not determined; *ptc, patched; wg, wingless; gsb, gooseberry.*

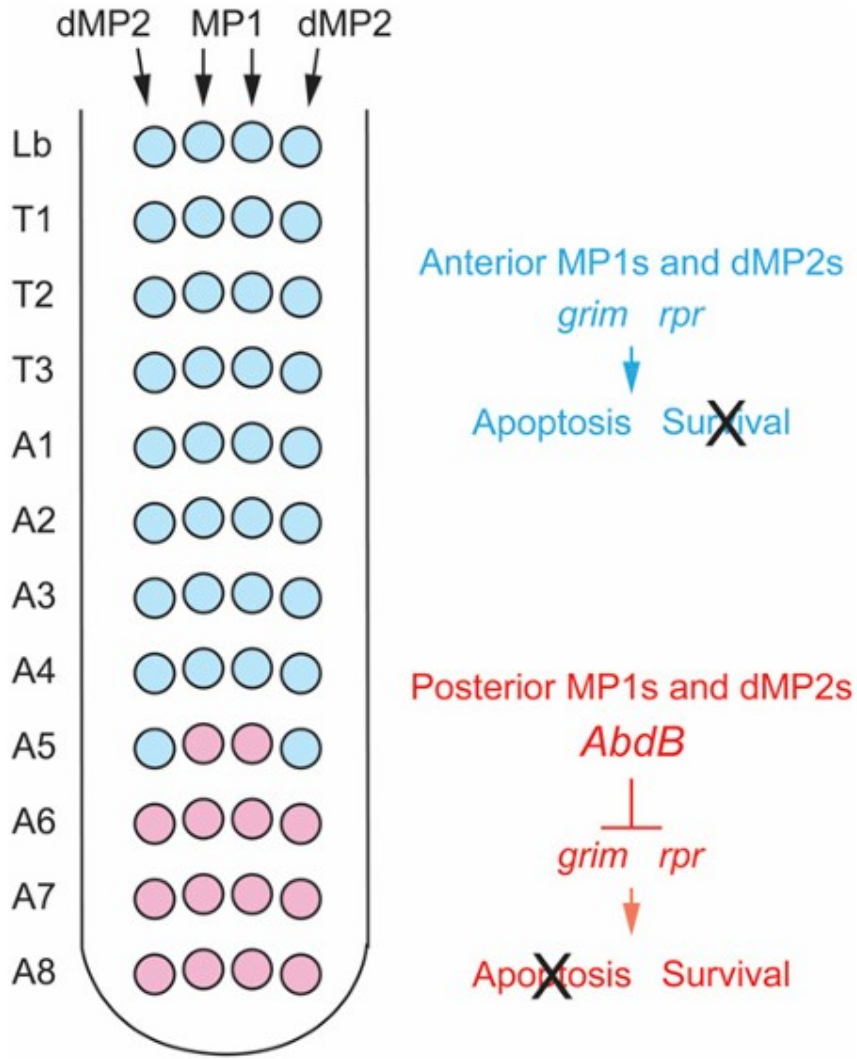


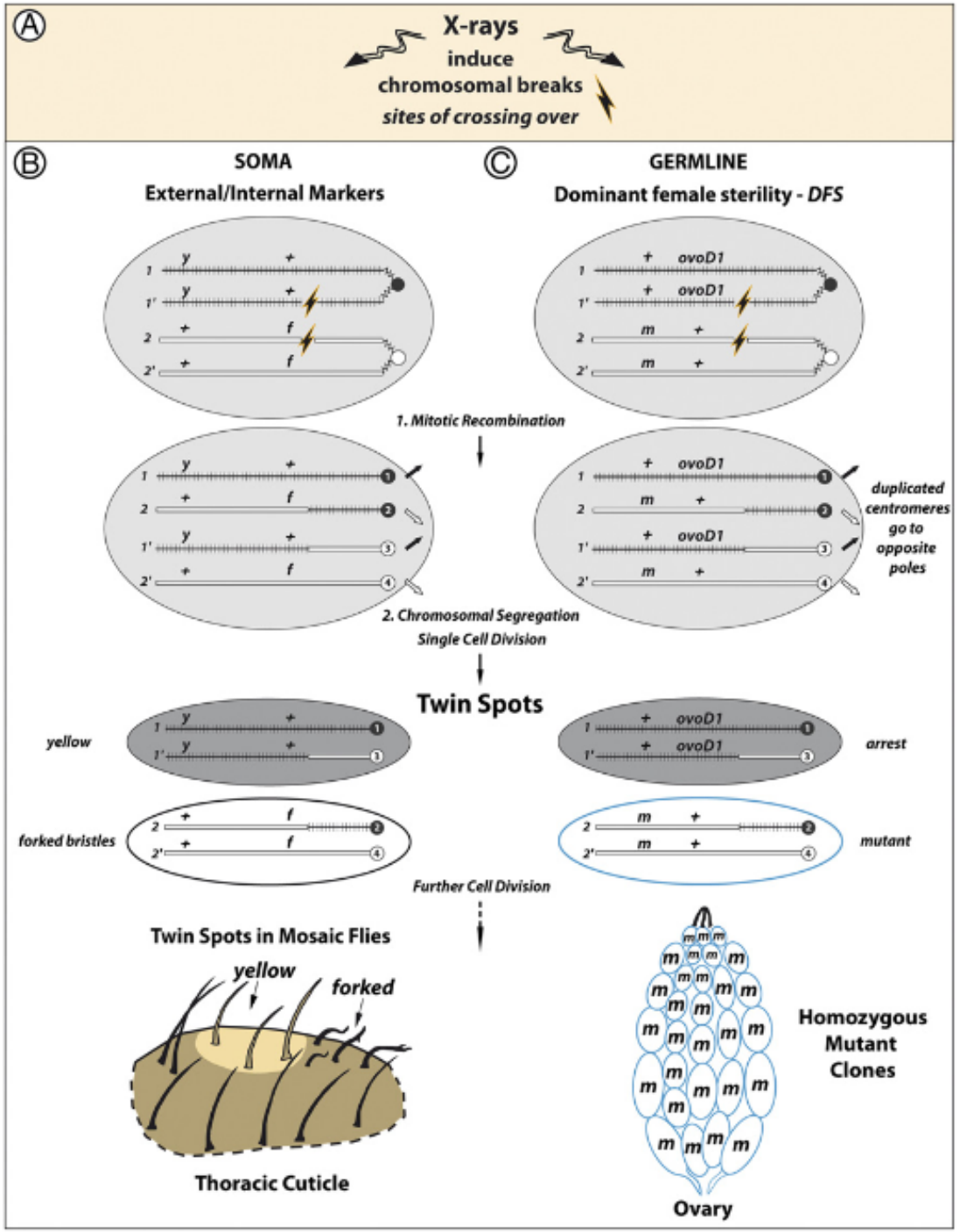

**Figure 15** *Hox* gene control of neuronal apoptosis. In anterior neuromeres, dMP2s undergo apoptosis (Lb → A5) and MP1s undergo apoptosis in Lb → A4. In posterior neuromeres, the *Abd-B Hox* gene inhibits apoptosis resulting in survival of dMP2s in A6 → A8 and dMP1s in A5 → A8. Adapted by permission from The Company of Biologists: Development (Miguel-Aliaga and Thor 2004) copyright (2004).

# Making germline clones efficiently with Flp/FRT to study neuroblast divisions

## Mitotic recombination

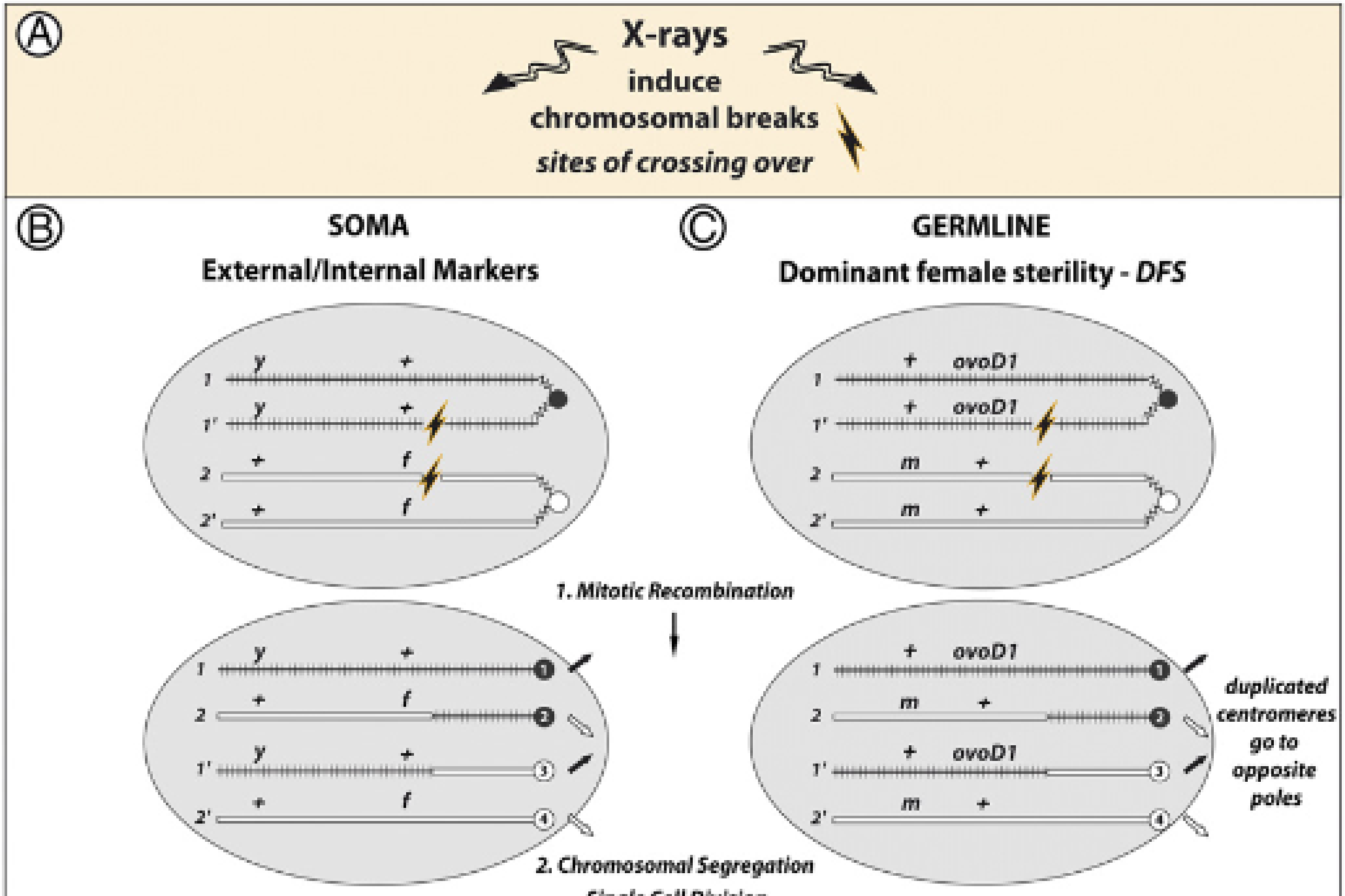
Somatic clones. Twin spots on fly cuticle show recessive mutant markers in side-by side clones arising from a single mitotic recombination event (Curt Stern, 1930).

Germline clones in ovaries using the dominant female sterile (DFS) mutant *OvoD*



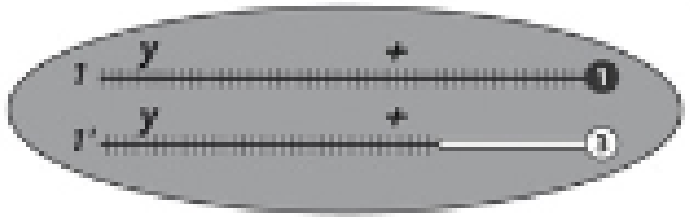
# MR = mitotic recombination to form clones of recombinant cells

**Fig. 1.** Mitotic recombination and generation of twin spots for clonal analyses. (*A*) X-ray irradiation causes chromosomal breaks and induces MR in the G2 phase of the cell cycle. (*B* and *C*) The underlying mechanisms of MR are the same in somatic (*B*) and germ-line (*C*) tissues, but the techniques for visualization of induced twin spots are different. MR causes an exchange of chromosomal arms distal to the site of crossing over. All genes downstream of the chromosomal breakpoint are homozygozed. In a heterozygote, the pattern of chromosomal segregation determines genotype. In G2-X segregation the recombined chromosomes migrate to different poles in a 1:3/2:4 configuration. Cytokinesis generates a mosaic fly with homozygous twin spots: one wild-type (+/+) and the other mutant (–/–); the rest of the cells are heterozygous (+/–). All twin spot genotypes shown in this and following figures arise from this type of segregation pattern. (*B*) In the soma, MR creates within the original heterozygous background multicellular homozygous clones of wild-type and mutant tissue of varying sizes, depending upon the number of further cell divisions. In early clonal analysis studies the homozygous mutant twin spot was identified by external phenotypes such as cuticle color or forked thoracic bristles; later, with the development of fluorescent protein markers, clones were identified within internal organs. (*C*) The DFS technique. Flies heterozygous and homozygous for *DFS* do not lay eggs; however, following MR in a heterozygote, double-positive germ cells will develop and produce normal eggs. If a mutation is positioned *in trans* to *DFS*, *m*/*m* homozygous clones will be generated in the germ line. *OvoD1* ovaries degenerate very early, and wild-type eggs are lost. Thus, the only eggs that develop, if *m* does not interfere with germ-line development, will be homozygous mutants.

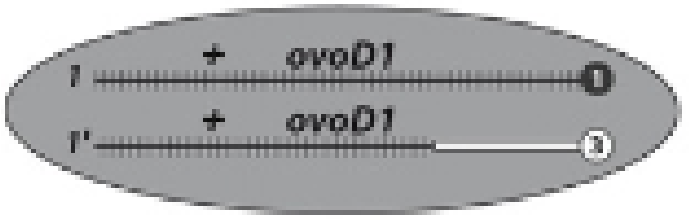

A
X-rays
induce
chromosomal breaks
sites of crossing over
B
SOMA
External/Internal Markers
C
GERMLINE
Dominant female sterility - DFS
y
+
f
ovoD1
m
1. Mitotic Recombination
2. Chromosomal Segregation
duplicated centromeres go to opposite poles

## Twin Spots

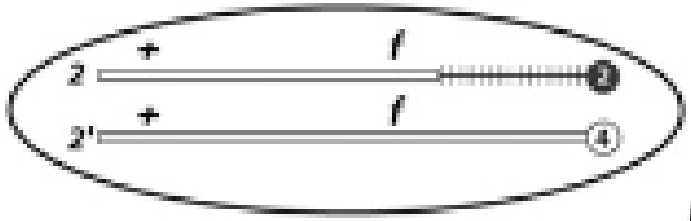
yellow



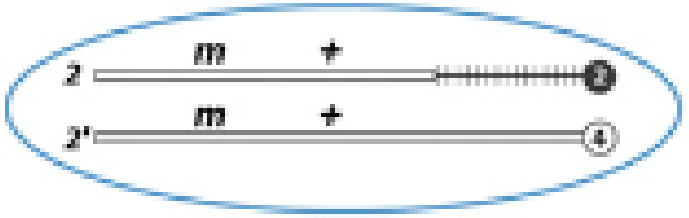

arrest




forked bristles

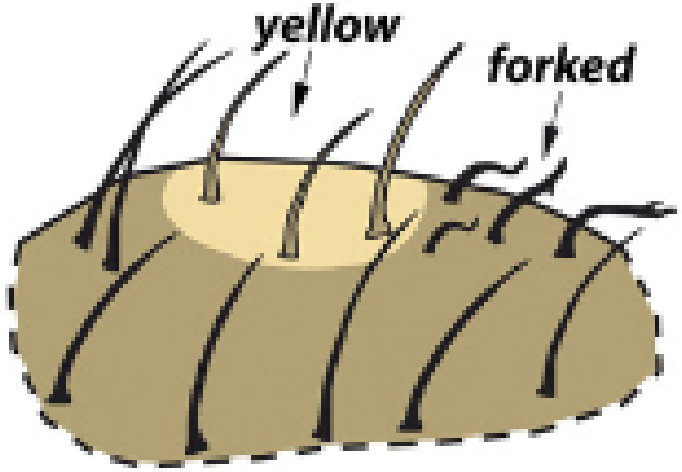



mutant

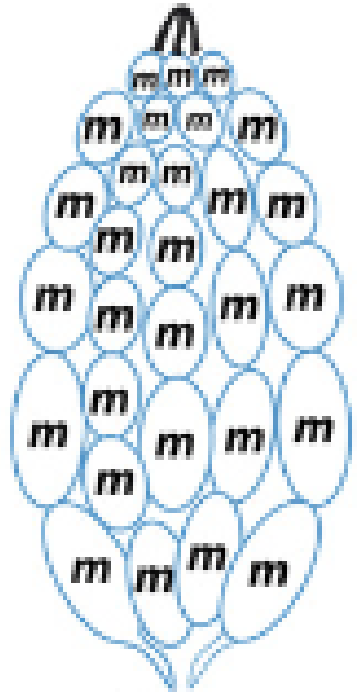



*Further Cell Division*

## Twin Spots in Mosaic Flies




Thoracic Cuticle

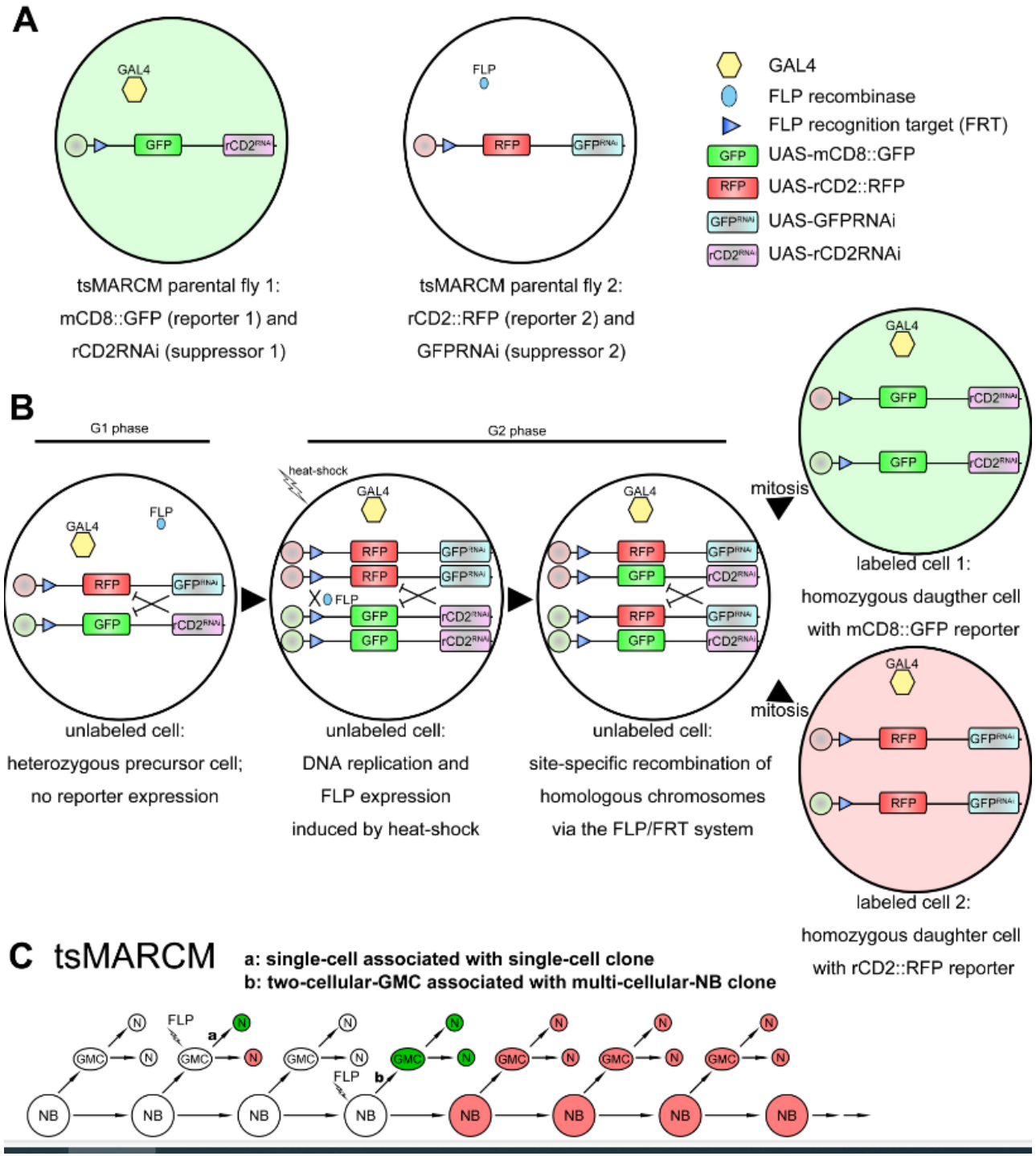



Ovary

Homozygous Mutant Clones

# Twin-spot MARCM

# Flp/FRT with RNAi as repressors



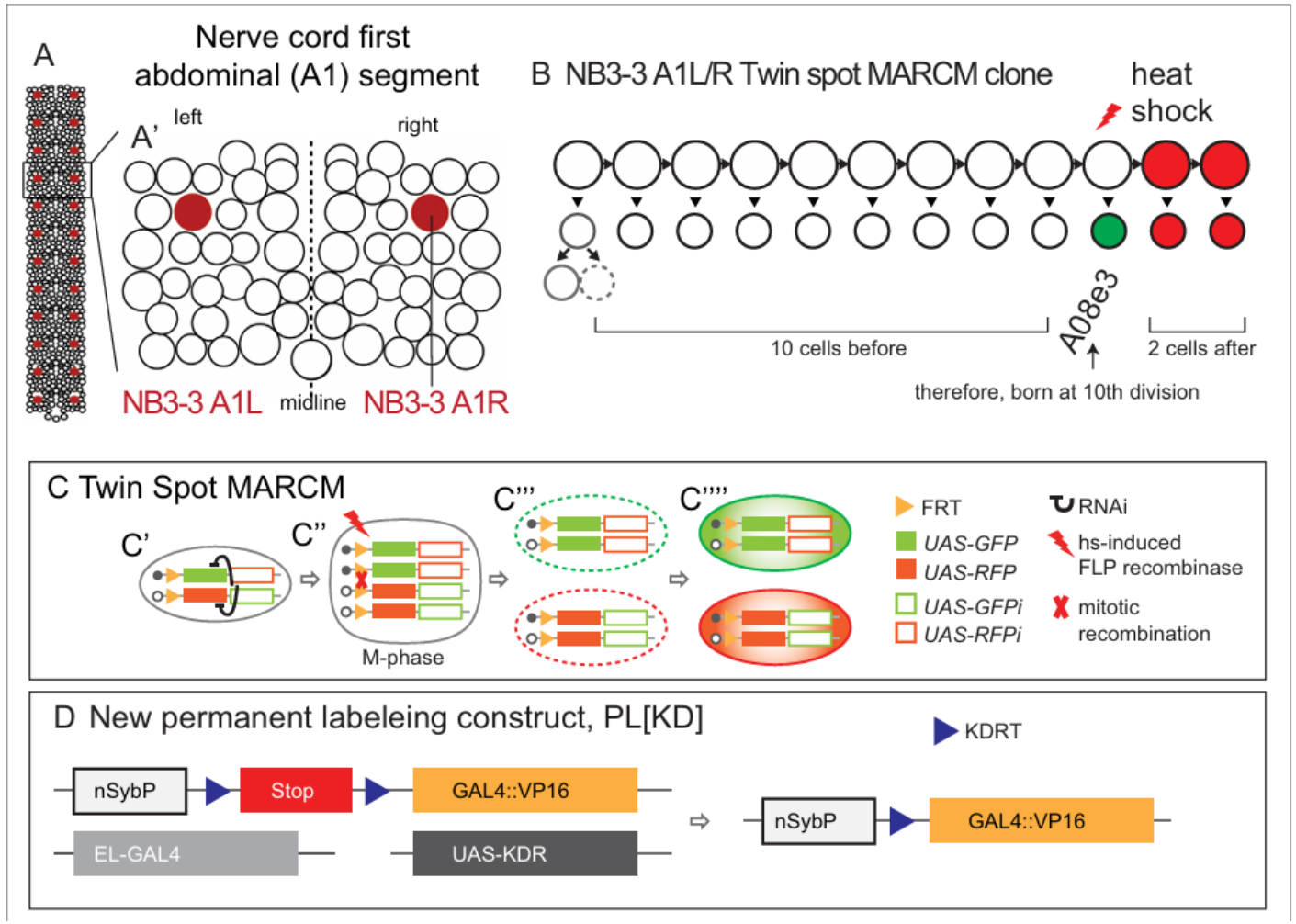
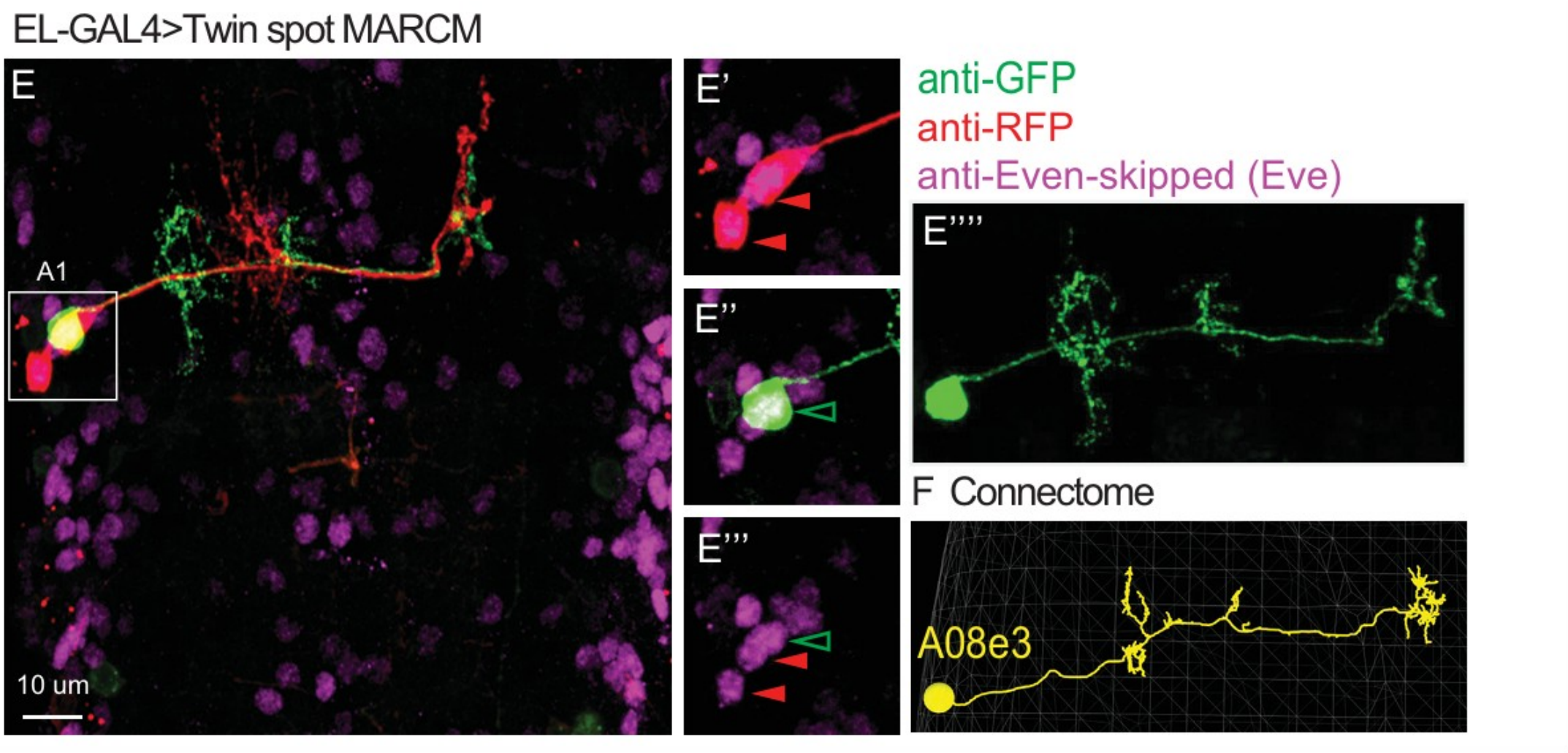



**Figure 1.** Twin-spot mosaic analysis with a repressible cell marker (ts-MARCM) determines the birth order and morphology of NB3-3A1L/R neurons. (**A, B**) Illustrations of *Drosophila* neuroblasts. (**A**) The nerve cord is left–right symmetrical and segmented. Each circle represents one neuroblast with NB3-3 in maroon. Segment A1 (boxed) is enlarged in (**A'**). It contains 30 types of neuroblasts. (**B**) NB3-3 lineage progression is shown with an example ts-MARCM clone overlaid. Each circle represents one cell and each arrow represents a cell division. First, NB3-3 divides to self-renew and generate a ganglion mother cell, which divides to generate a motor neuron (solid circle) and an undifferentiated cell (dashed circle). Then, NB3-3 directly generates

*Figure 1 continued on next page*




to the strong transcriptional activator VP16. (**E, F**) Image of a ts-MARCM clone and a corresponding neuron in the connectome. (**E**) Many segments of the nerve cord are shown in dorsal view with anterior up. The boxed region in segment A1 is enlarged at the right. In this ts-MARCM clone, two neurons are labeled in red and one in green (arrowheads), and all are Eve(+) ELs. The singly labeled EL is enlarged to highlight morphological detail. The corresponding neuron in the connectome is shown in (**F**). Specific genotype is listed in ***Supplementary file 4***.

The online version of this article includes the following figure supplement(s) for figure 1:

**Figure supplement 1.** NB3-3-GAL4 line.

*Figure 1 continued*

ELs. In ts-MARCM, a heat shock is provided (red lightning bolt) as NB3-3 divides. In this example, a singly labeled neuron is shown in green (A08e3), and two alternatively labeled neurons are shown in red. Because the total number of neurons in the lineage is known, counting labeled neurons allows inference of neuronal birth order. The identity of the singly labeled neuron is determined by matching the labeled neuron to the corresponding neuron in the connectome using morphological criteria (see 'Materials and methods'). (**C, D**) Illustration of ts-MARCM genetic constructs used in this study. Our updated version of ts-MARCM system has four components (**C, D**). (1) It uses a pair of genetically modified chromosomes. On one chromosome is an FRT recombinase site (yellow triangle) followed by a *UAS-GFP* (solid green box) and a *UAS-RFP-RNAi* (hollow red box) construct. On the other chromosome is an FRT site followed by a *UAS-RFP* (solid red box) and a *UAS-GFP-RNAi* (hollow green box) construct. When cells are heterozygous for these chromosomes, the GFP- and RFP- RNAi constructs ensure repression of GFP and RFP protein expression, respectively (black curves, **C'**). (2) It has a heat-shock-inducible FLP recombinase (red lightning bolt). By varying the heat shock protocol, we control both the timing and amount of FLP supplied. Heat shocks induce FRT-based chromosomal recombination in dividing cells (red X, M-phase cell, **C''**). A subset of recombination events produce a pair of post-mitotic progeny, one of which is homozygous for the *UAS-GFP, UAS-RFP-RNAi* construct, and the other homozygous for the *UAS-RFP, UAS-GFP-RNAi* construct. In these cells, RNAi is no longer able to repress GFP or RFP expression (**C'''**). (3) A cell-type-specific GAL4 line, (e.g., *EL-GAL4,* light gray box in **B**) is used to drive expression of *UAS-RFP* or *UAS-GFP* (**C''''**). (4) To get robust ts-MARCM labeling in early-stage larvae, it was often necessary to amplify GAL4 expression. To do so, we generated a new permanent labeling construct (**D**). Specifically, a neuron-specific nSyb promoter (white box) is upstream of a Stop (red box) flanked by KDRT (blue triangles) recombination sights. When the KDR recombinase (from UAS-KD, dark gay box) is supplied, the Stop is removed, and nSyb drives expression of a the new GAL4 (yellow box). This new GAL4 is the GAL4 DNA binding domain tethered to the strong transcriptional activator VP16. (**E, F**) Image of a ts-MARCM clone and a corresponding neuron in the connectome. (**E**) Many segments of the nerve cord are shown in dorsal view with anterior up. The boxed region in segment A1 is enlarged at the right. In this ts-MARCM clone, two neurons are labeled in red and one in green (arrowheads), and all are Eve(+) ELs. The singly labeled EL is enlarged to highlight morphological detail. The corresponding neuron in the connectome is shown in (**F**). Specific genotype is listed in ***Supplementary file 4***.

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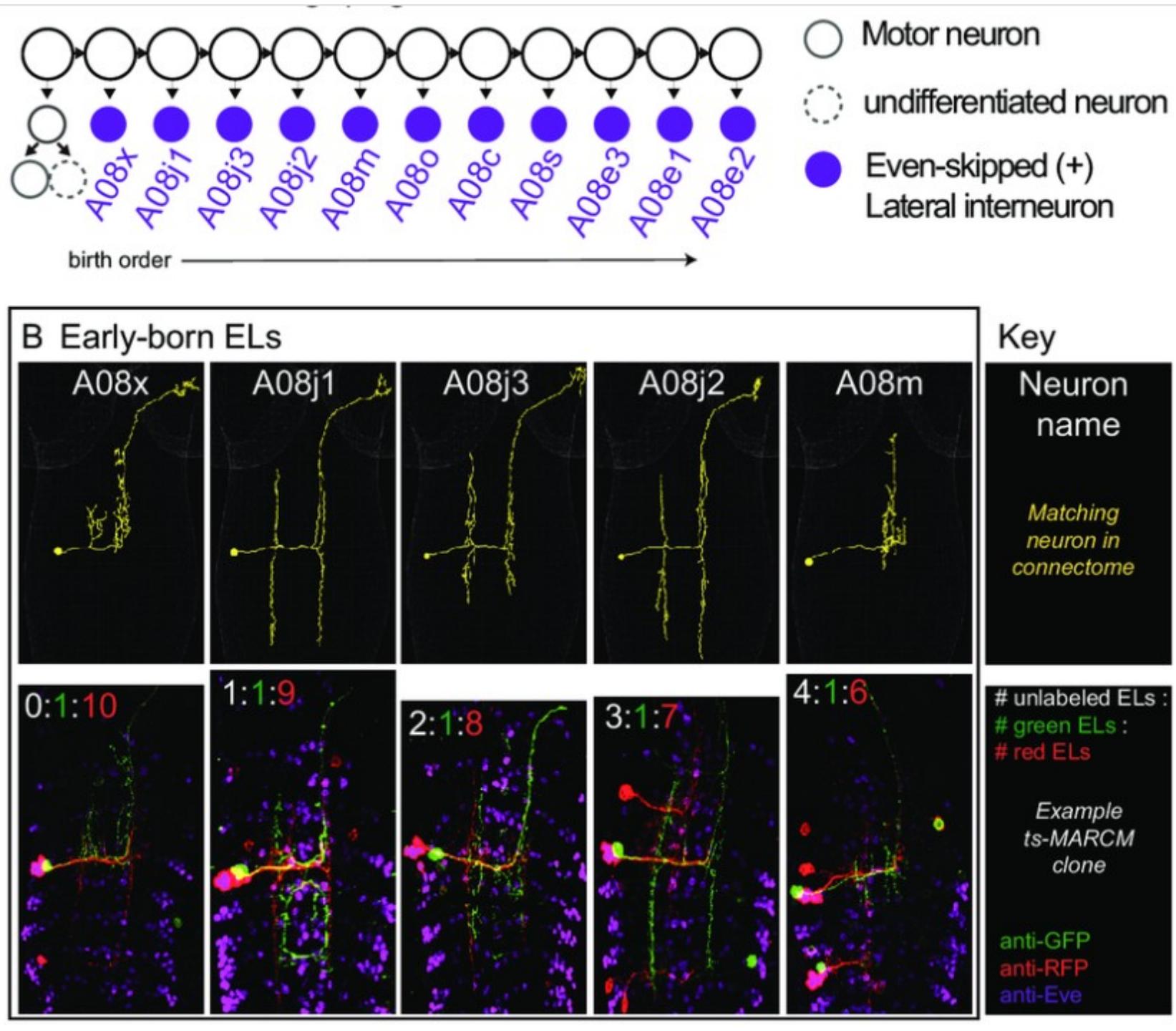
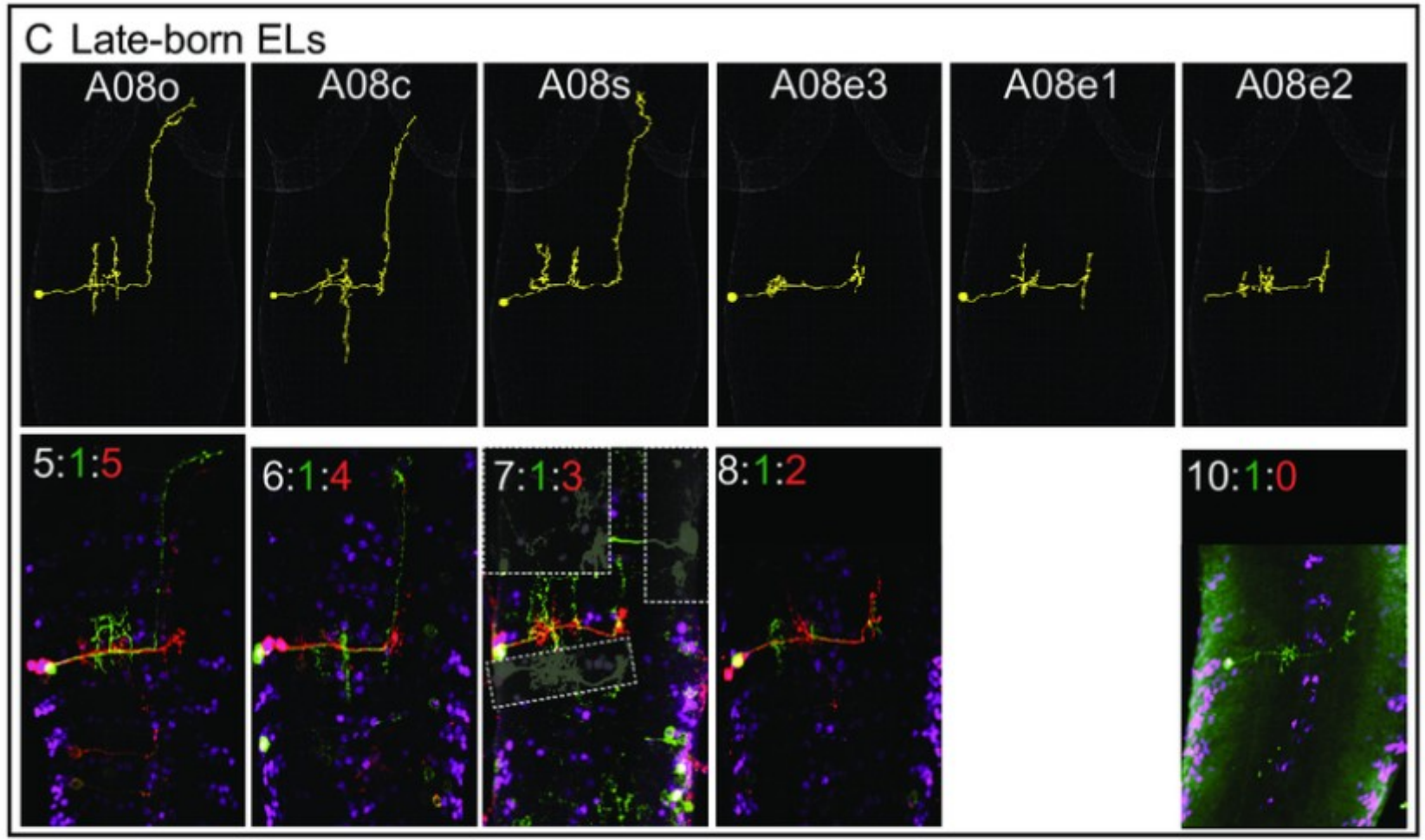
**Figure supplement 1.** NB3-3-GAL4 line.


Motor neuron
undifferentiated neuron
Even-skipped (+)
Lateral interneuron
A08x
A08j1
A08j3
A08j2
A08m
A08o
A08c
A08s
A08e3
A08e1
A08e2
birth order
B Early-born ELs
A08x
A08j1
A08j3
A08j2
A08m
0:1:10
1:1:9
2:1:8
3:1:7
4:1:6
Key
Neuron name
Matching neuron in connectome
# unlabeled ELs :
# green ELs :
# red ELs
Example ts-MARCM clone
anti-GFP
anti-RFP
anti-Eve




Twin-spot mosaic analysis with a repressible cell marker (ts-MARCM) provides birth order for all neurons in the NB3-3A1L/R lineage. (A) Schematic of NB3-3A1 lineage progression is shown with EL birth order. Each circle represents one cell, and each arrow represents a cell division. (B, C) Images of individually labeled ts-MARCM clones shown in birth order. Early-born ELs are show in the top box, and late-born ELs are shown in the bottom. An image key is shown to the right of the early-born ELs. Briefly, all images are shown in a dorsal view with anterior to the top. The neuron Figure 2 continued on next page

# Temporal transcription factors in successive asymmetric neuroblast divisions

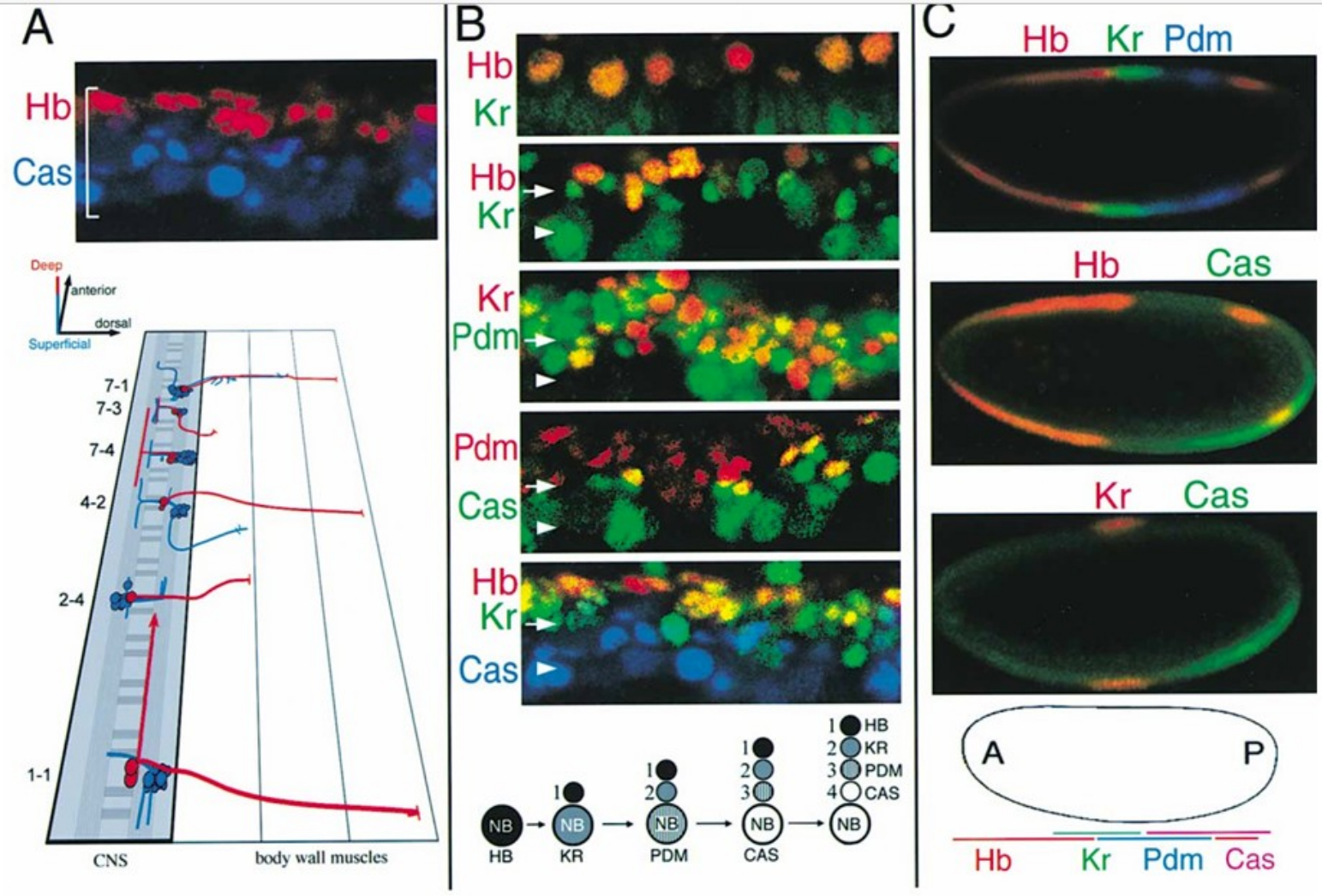



Figure 1. Relationship between Hunchback, Krüppel, Pdm, and Castor during Neurogenesis and Segmentation

(A) Hb$^+$ neurons occupy a deep layer and have long projections. Top: optical cross-section of a stage 12 CNS; deep layer at top; CNS thickness, bracket. Hb$^+$ neurons (red) occupy the deepest layer, and Cas$^+$ neuroblasts and GMCs (blue) are in a superficial layer. Bottom: schematic of six DiI neuroblast clones (Schmid et al., 1999). Early-born Hb$^+$ neurons (red) are in the deepest layer and have the longest axons of the clone; later-born neurons (blue) are superficial and have shorter axons.

(B) Hb, Kr, Pdm, and Cas are sequentially expressed in neuroblasts. Top: Optical cross-sections of embryos stained for the indicated proteins. Neuroblast layer, arrowhead; GMCs/neuron layer, arrow. Embryo stages from top to bottom: 9, 10, 11, 12, and late 12. Bottom: summary showing transient Hb, Kr, Pdm, and Cas expression in neuroblasts, followed by stable Hb, Kr, Pdm, and Cas expression in neuroblast progeny (gene expression overlaps not shown for clarity; see text for details).

(C) Hb, Kr, Pdm, and Cas are expressed in progressively more posterior domains in cellular blastoderm embryos. Anterior, left; ventral, down.

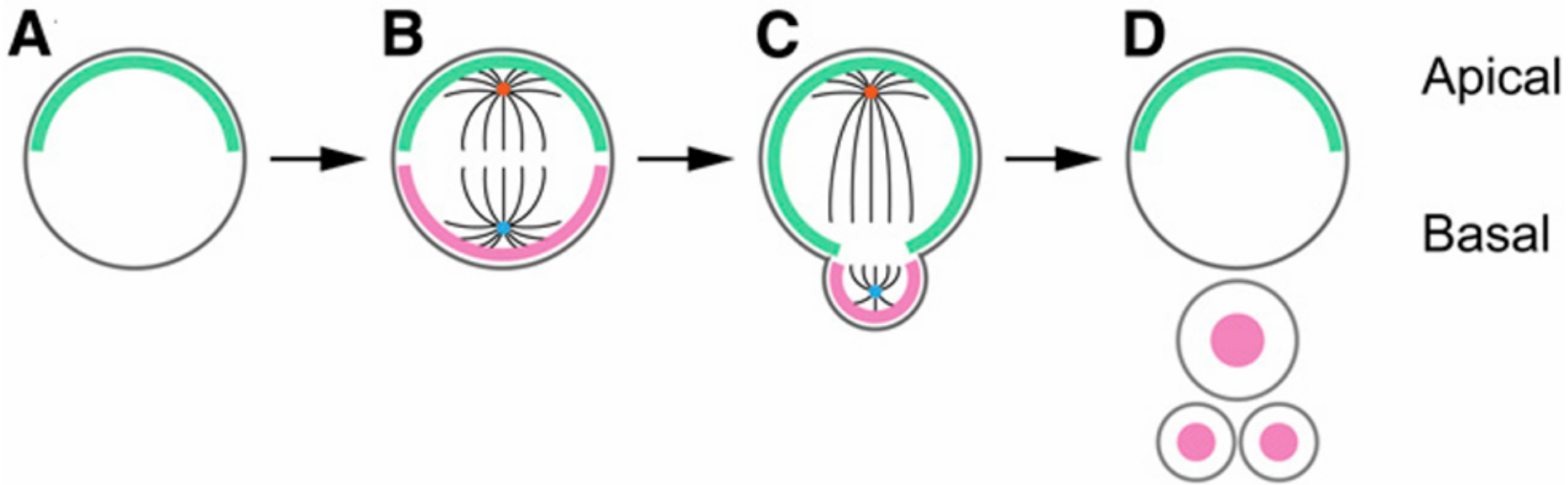
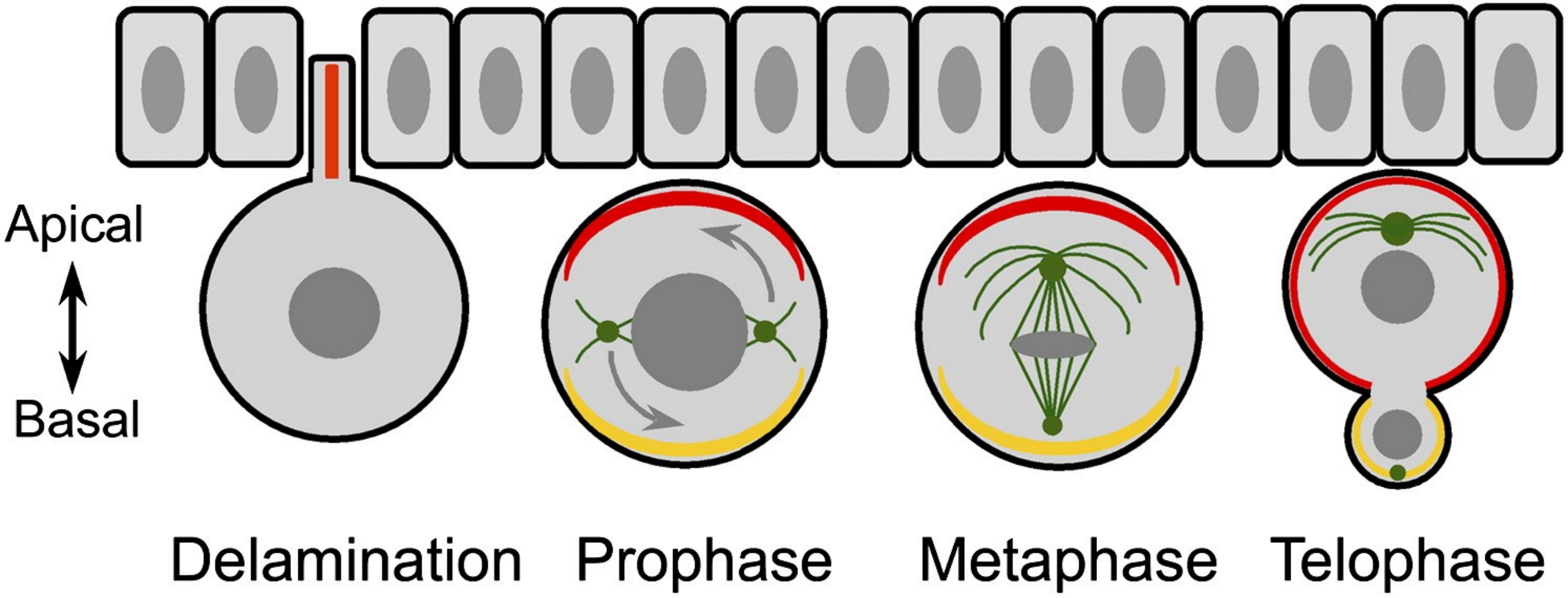



**Figure 6** Asymmetric NB division. (A) PAR complexes (green) form on the apical side of the NB cortex. (B) NB is polarized at metaphase with apical (green) and basal (red) complexes. Mother centrosome (older) is blue, and daughter centrosome (younger) is orange. (C) At telophase, the NB retains apical complexes, while the developing GMC has cortical basal factors. (D) After division, apical material again forms in the NB, whereas the basal factors enter the nucleus of GMCs and neurons. Adapted by permission from Springer Nature: Cell and Tissue Research (Kang and Reichert 2015) copyright (2014).


Apical
Basal
Delamination
Prophase
Metaphase
Telophase
Apical proteins (Par/Insc, Pins/Gαi/Loco)
Basal proteins (Numb/Pon, Pros/Mira)

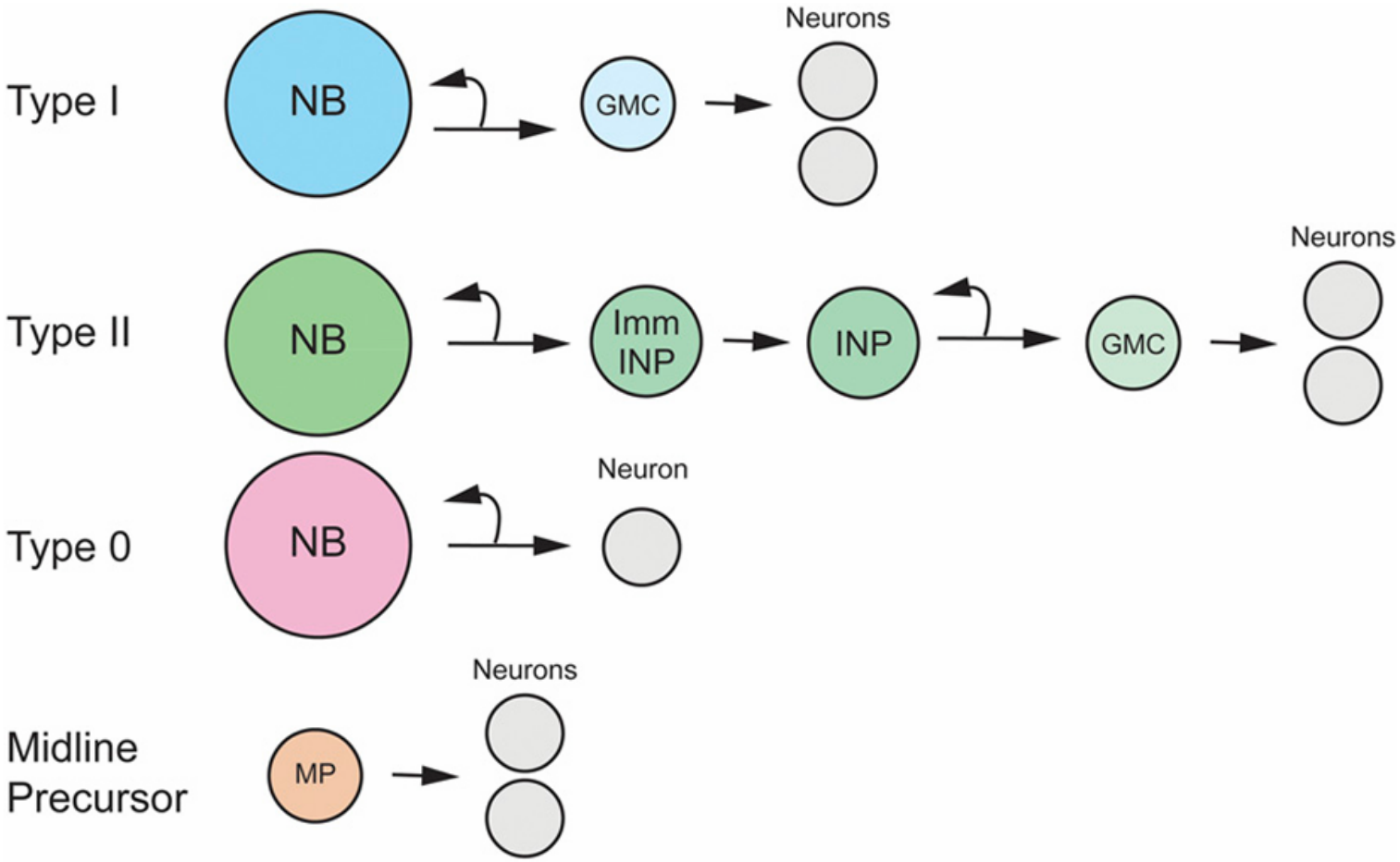



**Figure 5** Patterns of embryonic neural precursor divisions. Shown are Type I, Type II, Type 0 NB and Midline Precursor division modes. Type I and Type II division modes include GMCs, and Type II divisions include immature INP (Imm INP) and INP cell types.

ANNUAL REVIEWS

*Annual Review of Cell and Developmental Biology*

# Temporal Patterning in the *Drosophila* CNS

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Annu. Rev. Cell Dev. Biol. 2017. 33:219–40

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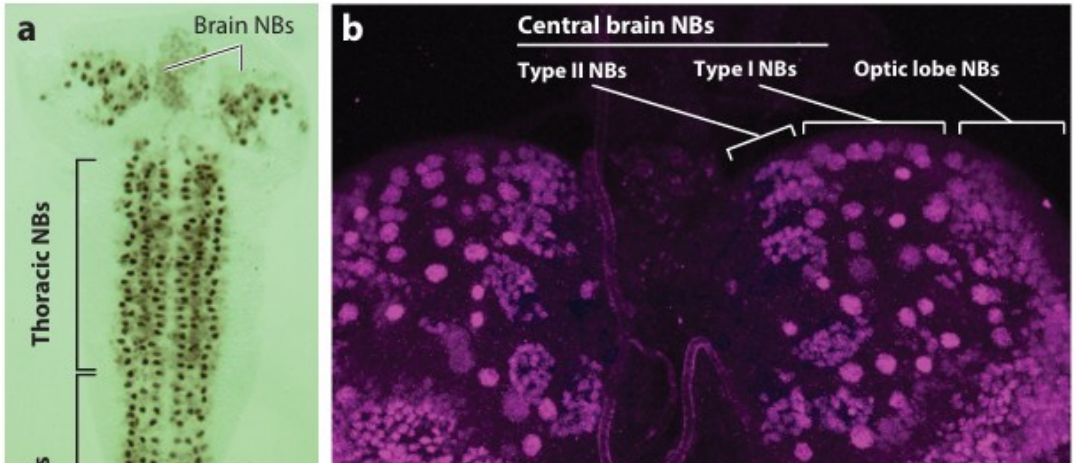
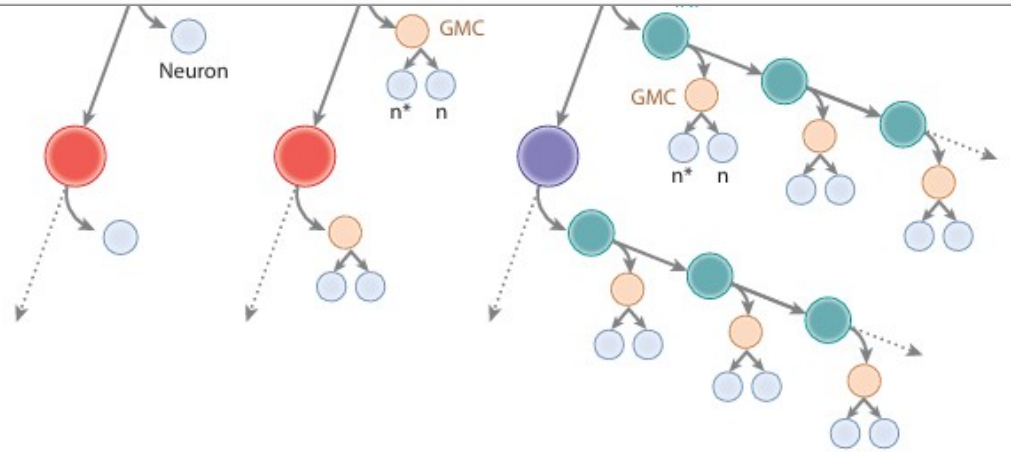
**Keywords**

temporal identity, neuroblast, neural stem cell, neural diversity, subtemporal




## Figure 1

(*a*) Stage 10 embryo flat mounted and stained for Snail protein to identify brain, thorax, and abdominal neuroblasts (NBs). (*b*) Third-instar larval brain and thoracic CNS stained for Deadpan to identify the indicated pools of NBs. (*c*) 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)].



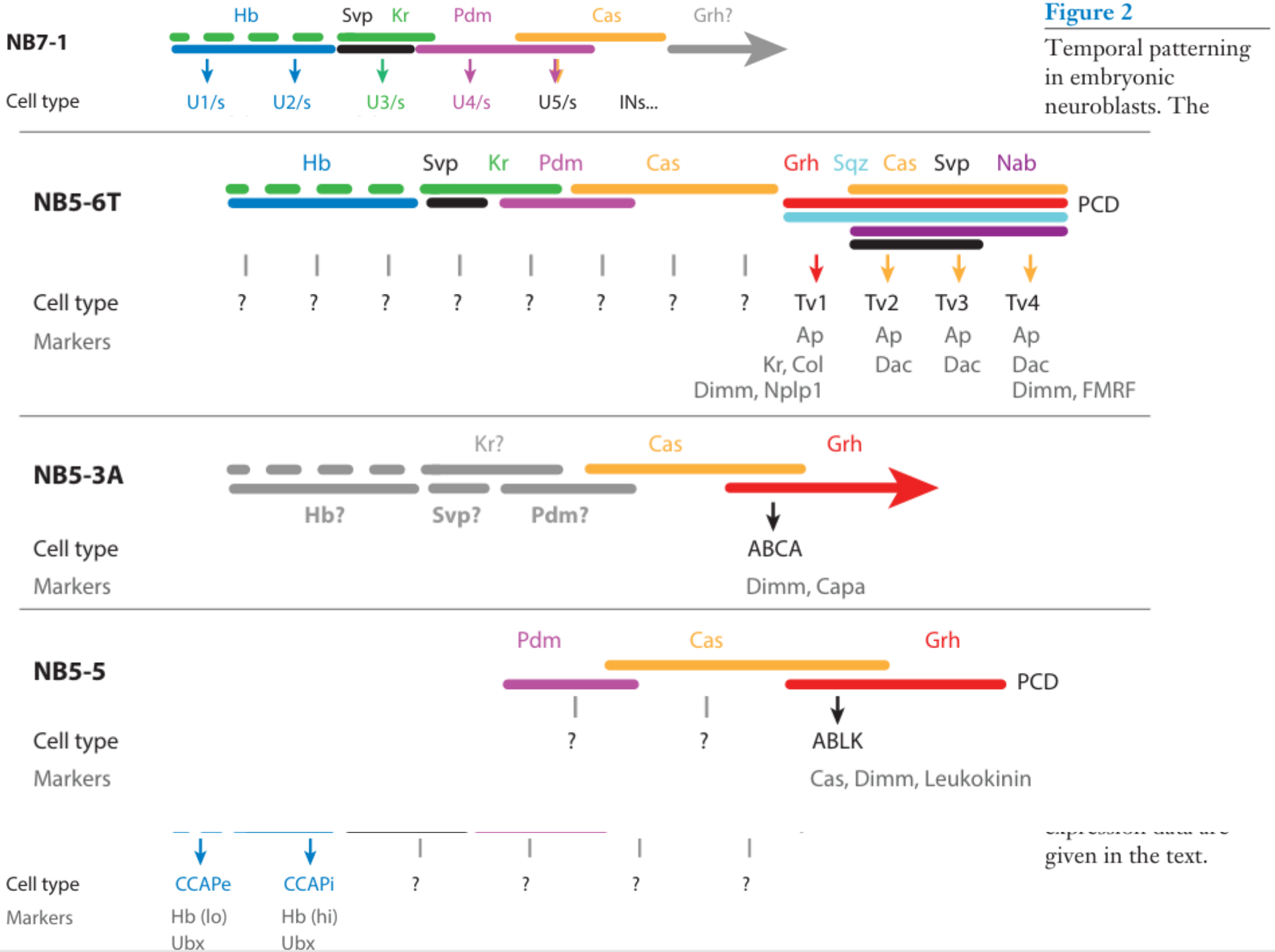
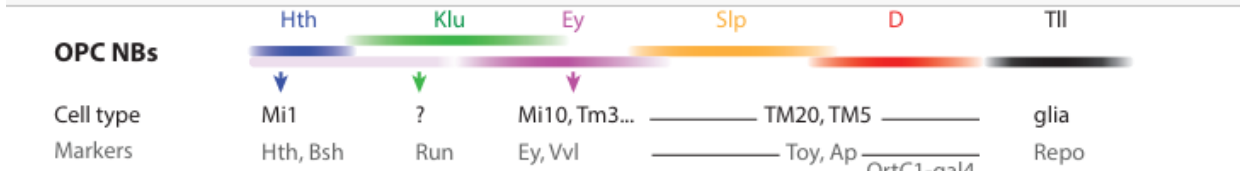



**Figure 2**

Temporal patterning in embryonic neuroblasts. The

given in the text.




## Figure 3

Temporal patterning in larval neuroblasts (NBs). Where known, hours after larval hatching are shown at the top. The lines at the top of each panel represent gene expression over time (early to late from *left* to *right*). The identity of each line is color coded with the gene name above [for example, in the mushroom body (MB) NB panel, Imp is shown in *dark green*, whereas Chinmo is shown in *light green*]. In the indicated NB population, line gradients reflect approximate expression gradients. Downward arrows indicate that the NB temporal factor is required to specify a particular neuronal identity, lack of an arrow indicates a correlation only between NB factor expression and a specific neuronal identity, a T bar indicates an inhibitory relationship, and curved upward arrows in the anterodorsal (AD) NB panel indicate the cell fate transformations occurring in the absence of Kr or Chinmo. In the AD NB panel, E denotes embryo, and L denotes larva. The "Cell type" row indicates the neuron(s) born during each expression window. The "Markers" row indicates the molecular markers that distinguish neurons within a lineage. The "Progeny" row in the thoracic NB panel indicates temporal expression in NB progeny. Abbreviations: MNs, motor neurons; OPC, outer proliferation center; tOPC, tip OPC.

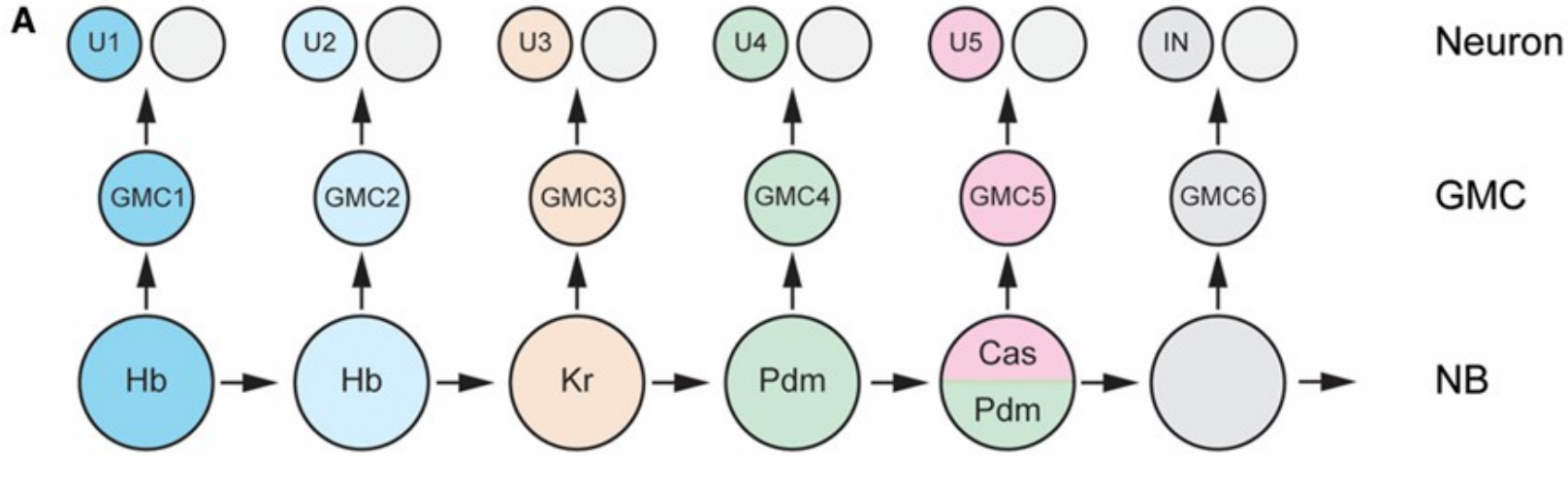
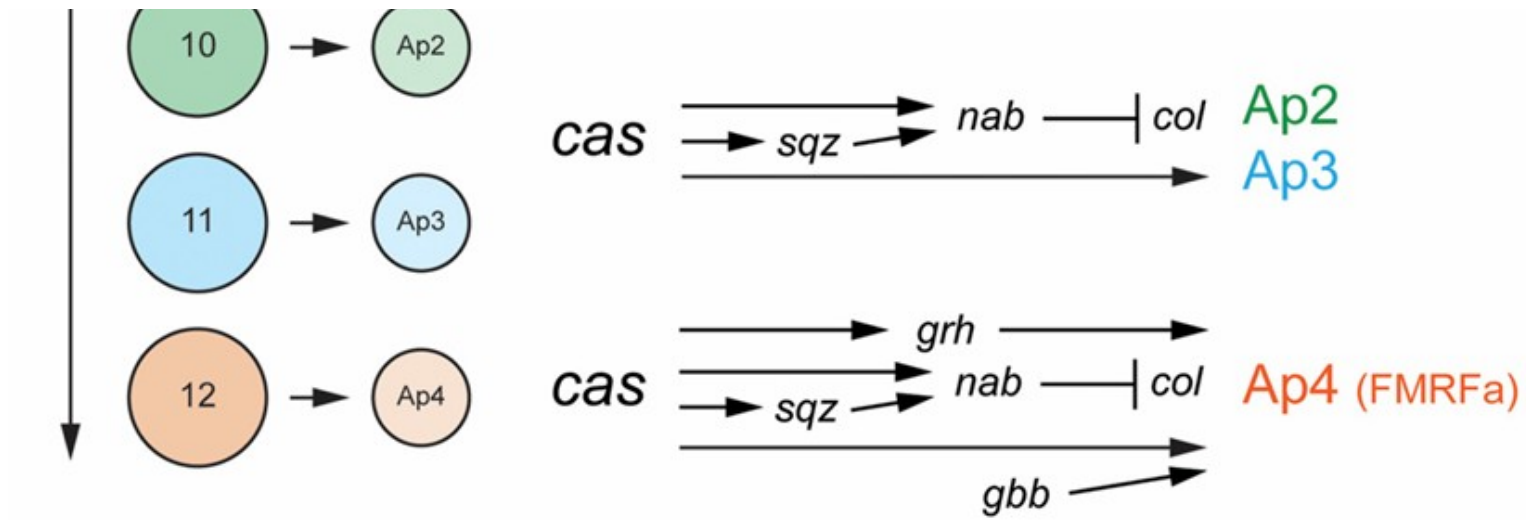



**Figure 7** Temporal transcription factor (TTF) and subtemporal transcription factor (STTF) gene cascades. (A) Progression of TTF in the NB 7-1 lineage. The U1–5 neurons are generated from GMCs 1–5. The corresponding NBs express Hb → Kr → Pdm → Cas and Pdm. Levels of Hb are higher in NB 1 (dark blue) in comparison to NB 2 (light blue). (B) In the NB 5–6T lineage, the AP1–4 neurons are generated from Type 0-dividing NBs that are present in a Cas temporal window. AP1 and AP4 are peptidergic (Nplp1 and FMRFa, respectively) and AP2/3 are not peptidergic. AP2-4 are distinct from AP1 due to the action of the Sqz and Nab STTFs that repress col. Adapted by permission from Elsevier: Cell (Baumgardt *et al*. 2009) copyright (2009).



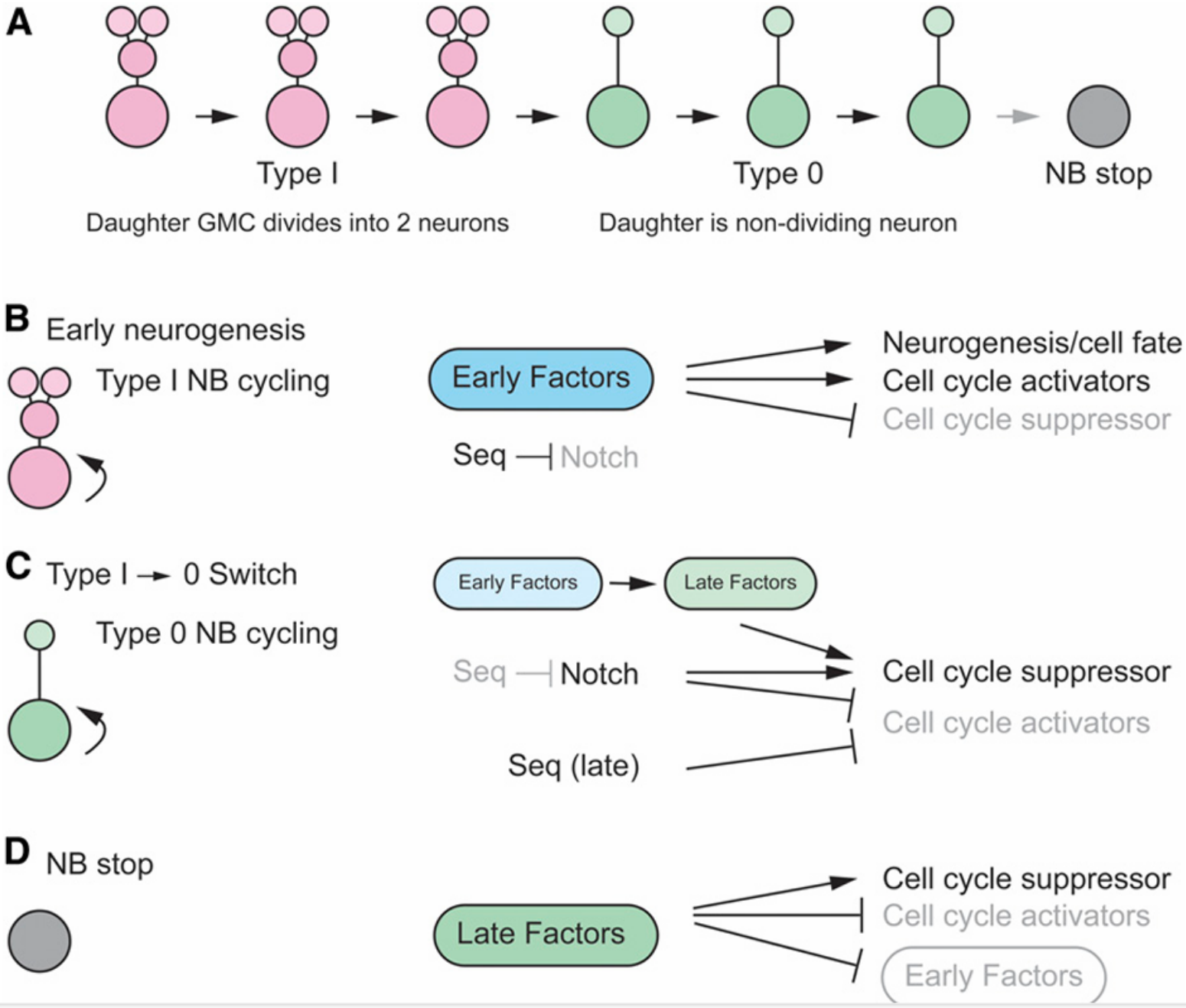



**Figure 8** Control of Type I cycling and the Type I→Type 0 switch. (A) In NB lineages, Type I cycling leads to a Type 0 division mode, followed by a stop in NB division. Type I GMC daughters have limited proliferative potential, dividing once, whereas type 0 NB daughters do not divide. (B) High levels of early factors (dark blue) promote neurogenesis (Type I NB cycling) by activating cell cycle factors. They also influence neural cell fate. Notch triggers the Type 0 switch but is suppressed by Seq during Type I cycling. High activity (black letters); low activity (gray letters). (C) As early factor levels decline (light blue), late factor levels rise (light green), and this promotes the Type I→0 switch in combination with Notch signaling and late-acting Seq: these genes activate expression of the *dap* cell cycle suppressor. Notch and late-acting Seq repress expression of cell cycle activators. (D) The stop in NB proliferation is accompanied by high levels of late factors (dark green) activating the Dap cell cycle suppressor and suppressing cell cycle activators, while also repressing early factor expression. Adapted by permission from Elsevier: Developmental Cell (Bahrampour *et al*. 2017) copyright (2017).

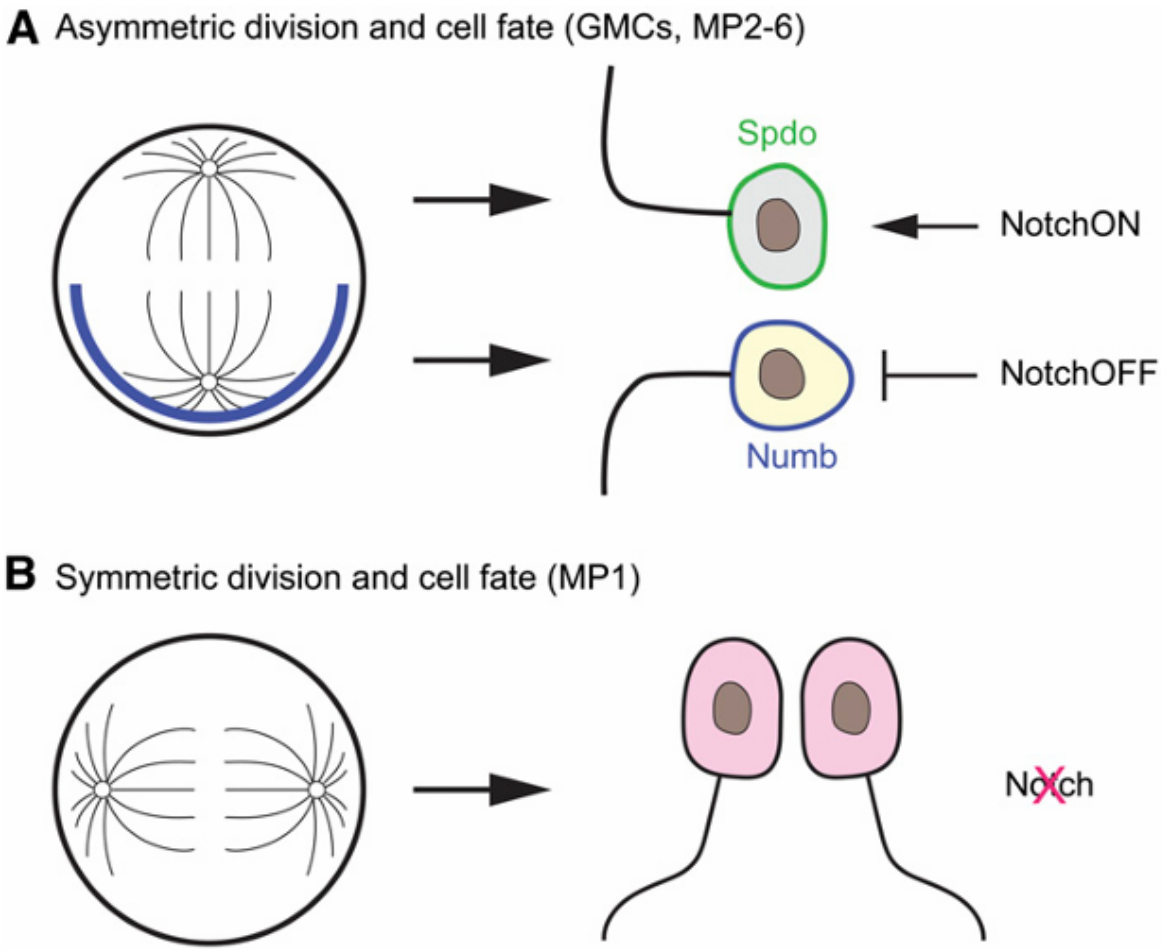
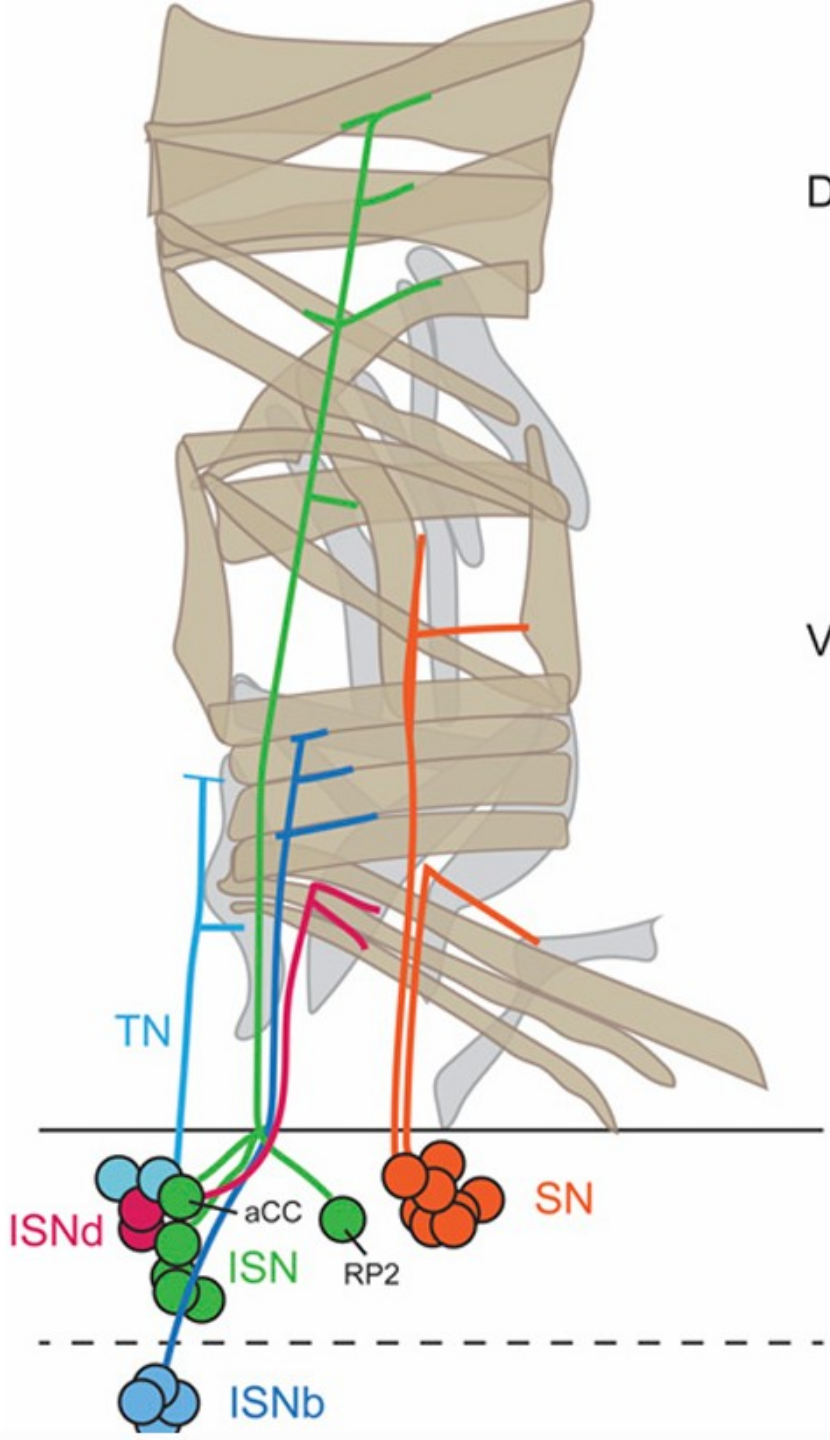



**Figure 9** Asymmetric divisions of GMCs and MPs. (A) During asymmetric division of GMCs and MP2-6, Numb accumulates at the basal side of the GMC/MP and is localized in one of the daughter neurons. Spdo accumulates at the membrane of the daughter neuron without Numb and facilitates Notch signaling (NotchON). The appearance of Numb in the other daughter neuron results in the inhibition of Notch signaling (NotchOFF). (B) The MP1 cell undergoes a symmetric division to form two identical MP1 neurons. Notch signaling is not utilized to generate MP1 neuronal fates.


TN
ISNd
aCC
ISN
RP2
SN
ISNb

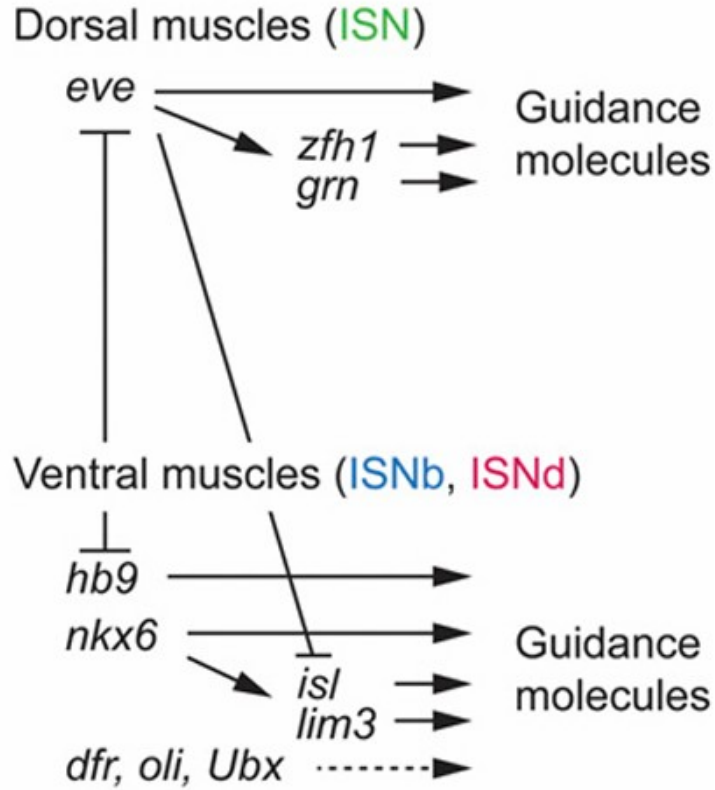

Dorsal muscles (ISN)
eve
zfh1
grn
Guidance molecules
Ventral muscles (ISNb, ISNd)
hb9
nkx6
isl
lim3
dfr, oli, Ubx
Guidance molecules

**Figure 10** Motoneuron cell fate and axon guidance. (A) Schematic showing somatic muscles present in a hemi-segment and representative motoneurons that contribute to the transverse nerve (TN), intersegmental nerves ISN, ISNb, and ISNd, and segmental nerve (SN). The ISN aCC and RP2 motoneurons are shown. (B) Shown are TFs that control motoneuron fate, differentiation, and the guidance of ISN, ISNb, and ISNd axons that project to dorsal muscles and ventral muscles. Adapted by permission from Elsevier: Seminars in Cell & Developmental Biology (Zarin and Labrador 2017) copyright (2017).

# Reading

- ***Drosophila*** Embryonic CNS Development: **Neurogenesis**, Gliogenesis, Cell Fate, and Differentiation
- ST **Crews** - Genetics, 2019 - academic.oup.com

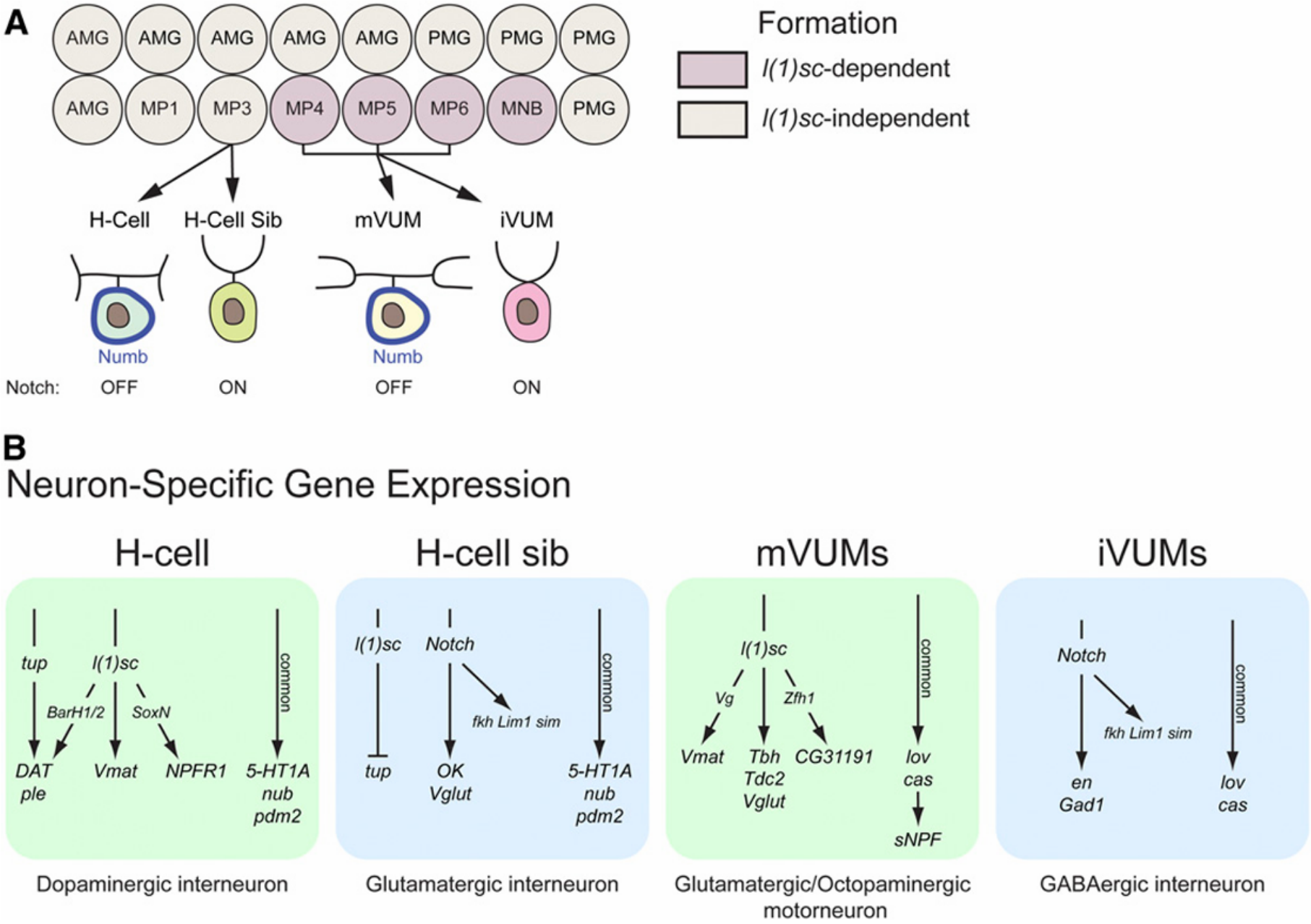



**Figure 11** Diverse neuronal types are generated from midline neural precursors. (A) H-Cell and H-cell sib derive from the asymmetric division of MP3, whose formation does not require the *l(1)sc* proneural gene. MP4–6 require *l(1)sc* for formation, and each gives rise to an mVUM and iVUM. (B) H-cell and mVUMs are NotchOFF neurons that utilize *l(1)sc* to control cell-type specific development. The regulation of differentiation genes requires multiple intermediate TFs. H-cell sib and iVUMs are NotchON neurons. Neural gene expression common to both progeny of an MP (H-cell/H-cell sib or mVUMs/iVUMs) employ regulatory pathways distinct from the *l(1)sc* and *Notch* pathways.

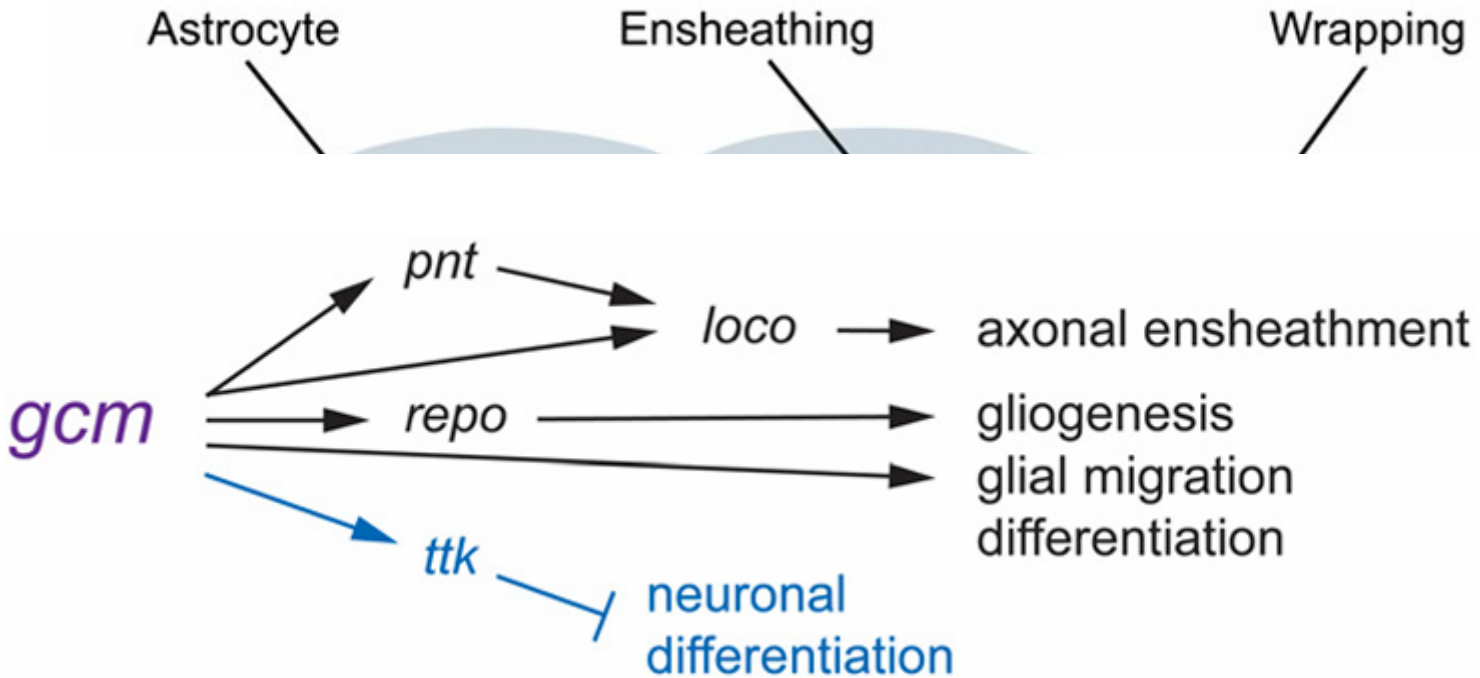



**Figure 13** GCM control of glial development. GCM controls glial formation and differentiation (black), and inhibits neuronal differentiation (blue). Modified by permission from John Wiley and Sons: Wiley Interdisciplinary Reviews: Developmental Biology (Altenhein *et al.* 2016) copyright (2015).

types of CNS glia are shown (purple). Modified by permission from Cold Spring Harbor Laboratory Press: Cold Spring Harbor Perspectives in Biology (Freeman 2015) copyright (2015).

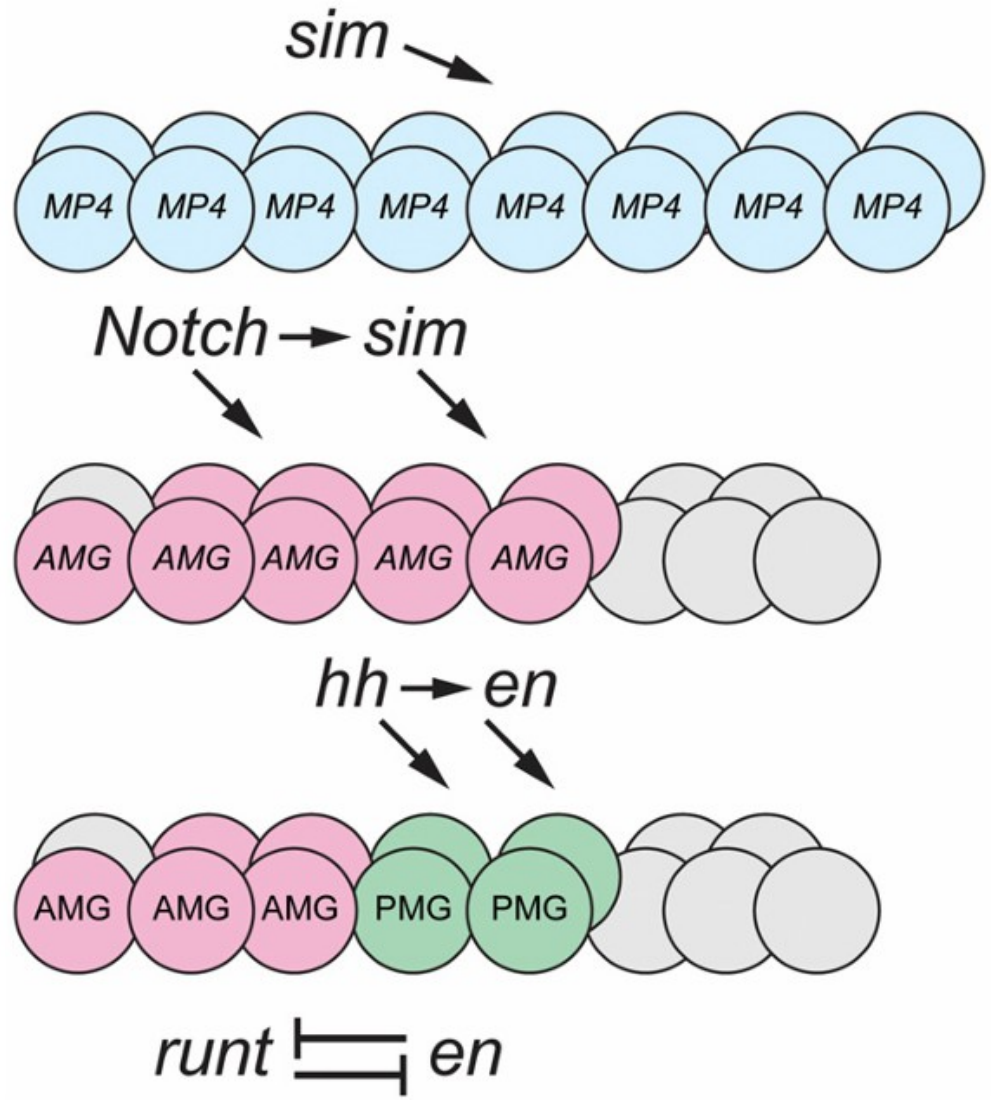



**Figure 14** Formation of midline glia. The *sim* gene is a master regulator of midline cell fate, and initially commits midline cells to an MP4 neural precursor fate. Notch signaling directs a group of midline cells to become AMG, in part by maintaining expression of *sim* in midline glia. Hh signaling directs formation of PMG, in part by activating expression of *en*. The Runt and En TFs cross-repress and lock in AMG and PMG fates.