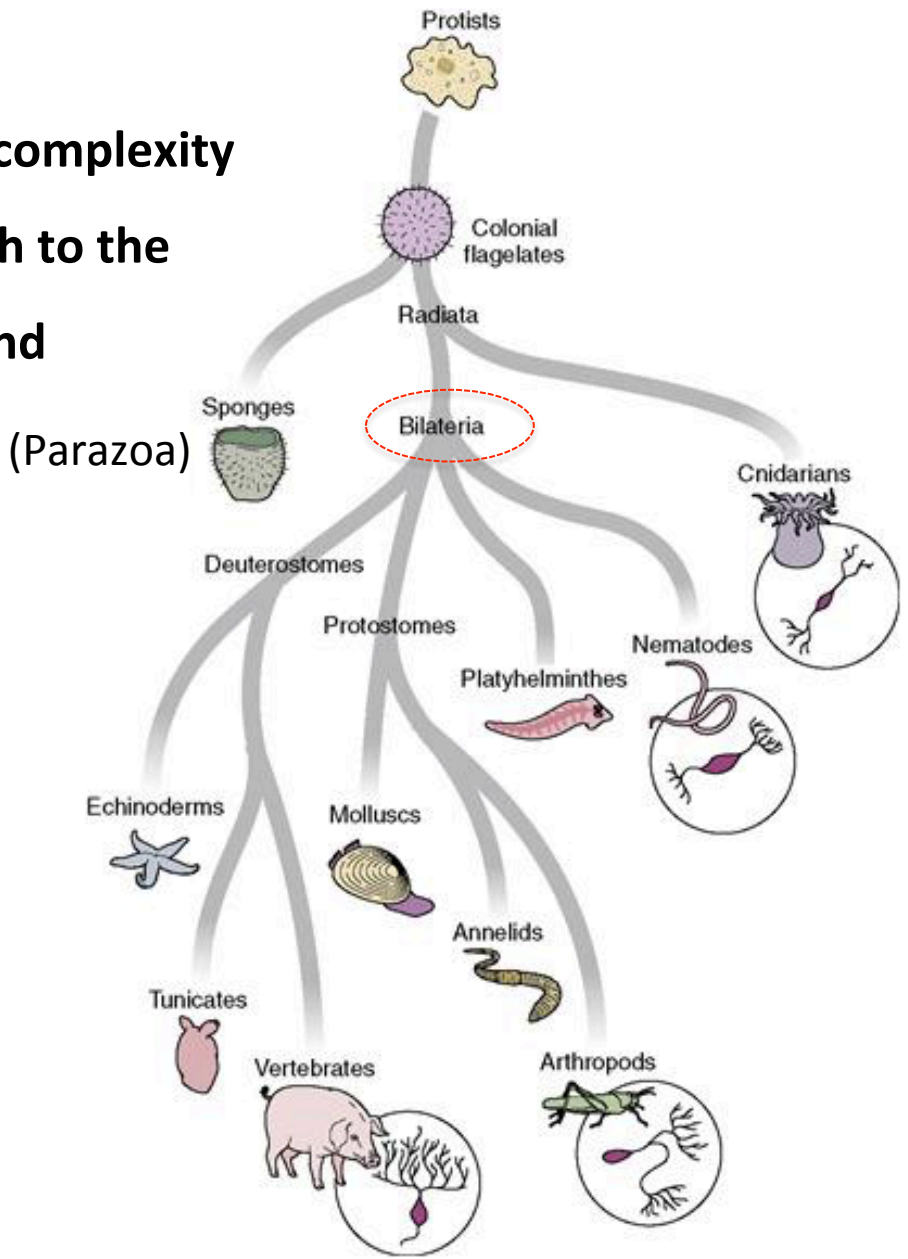
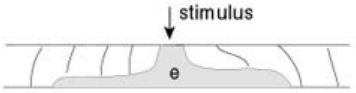
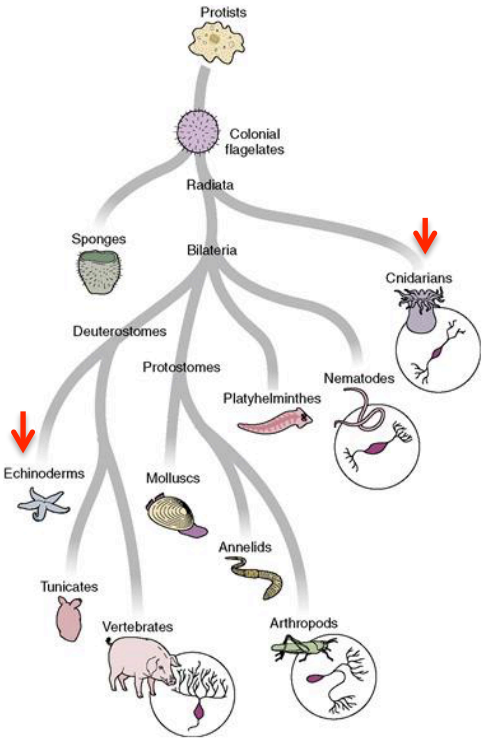
Derivation of the Neural Tissue

Metazoan nervous systems range in complexity from the simple nerve net of jellyfish to the complex nervous system of insects and vertebrates

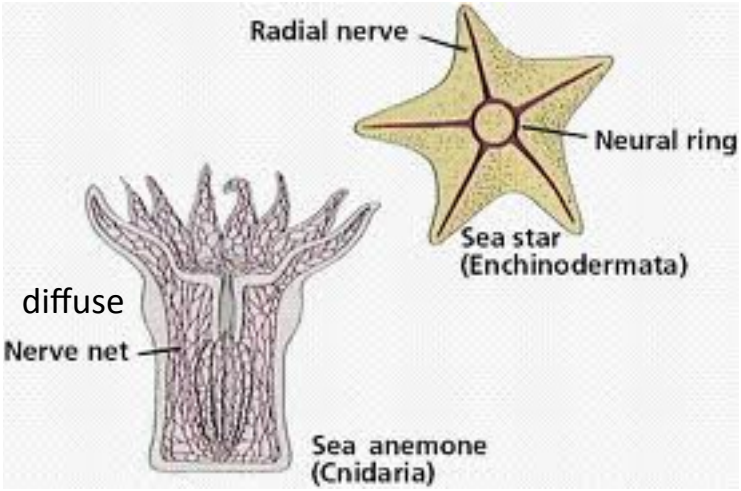


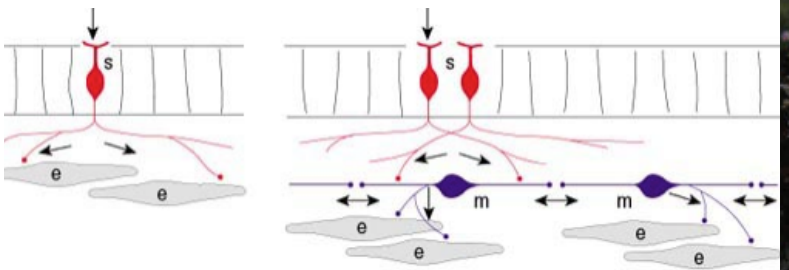
No nervous system

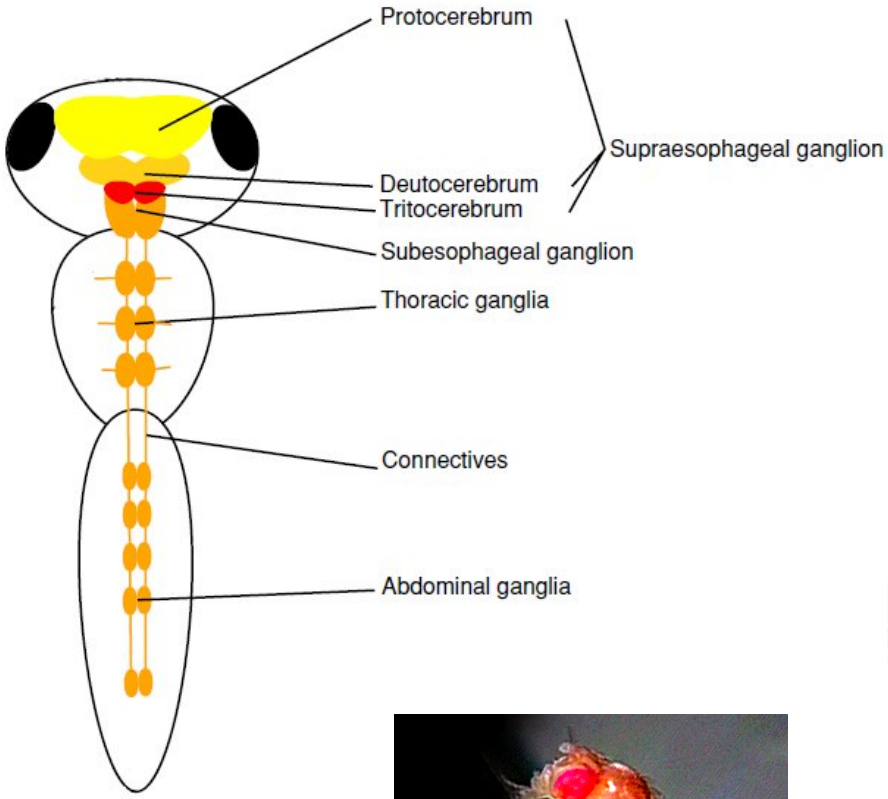




Nerve net - no ganglia - no cephalization

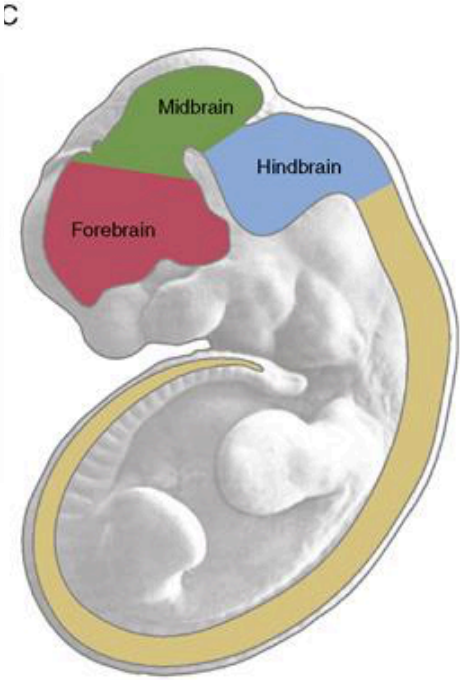






Complex nervous system
Ganglia
Cephalization

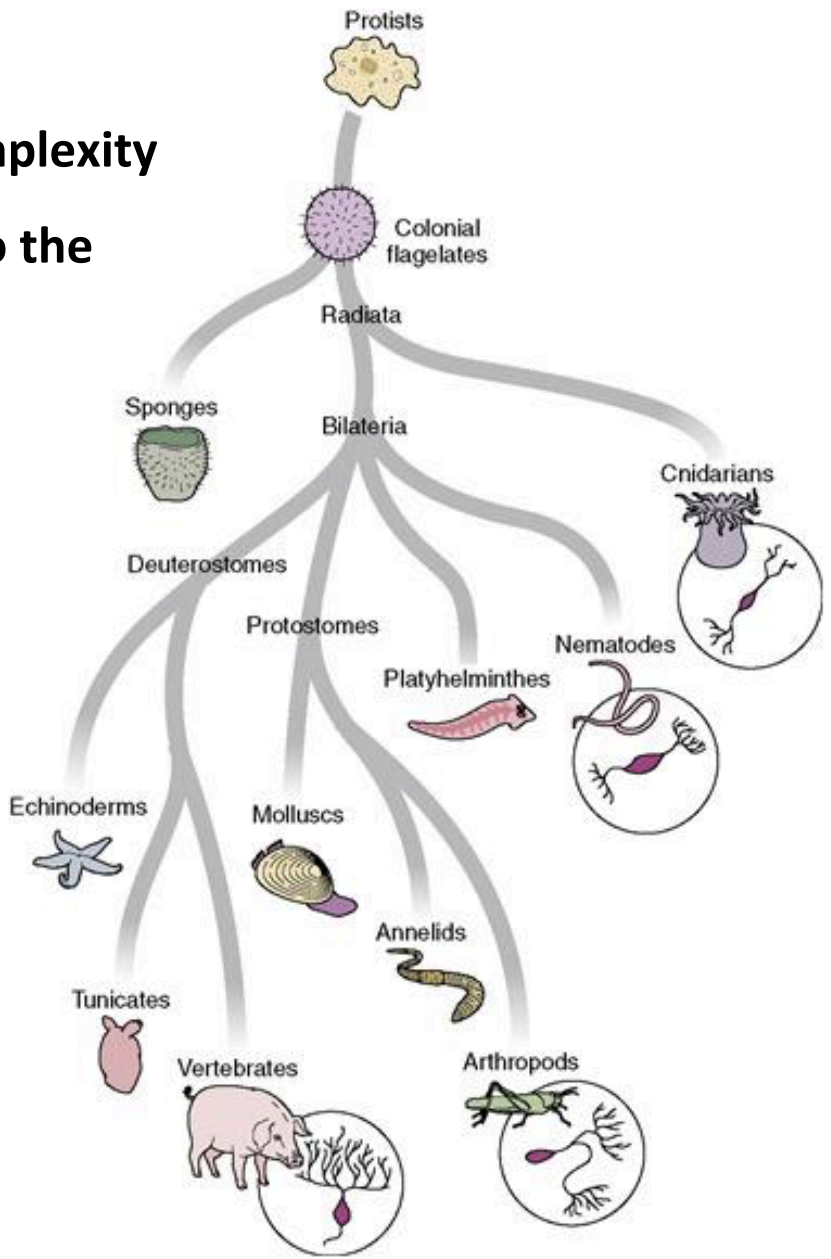
Arthropods



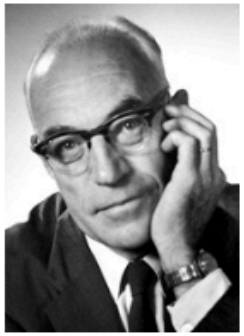
Vertebrates

Metazoan nervous systems range in complexity from the simple nerve net of jellyfish to the complex nervous system of insects and vertebrates

Neurons from different species share many common features

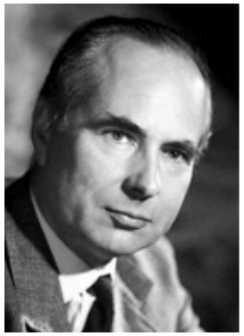


The Nobel Prize in Physiology or Medicine 1963



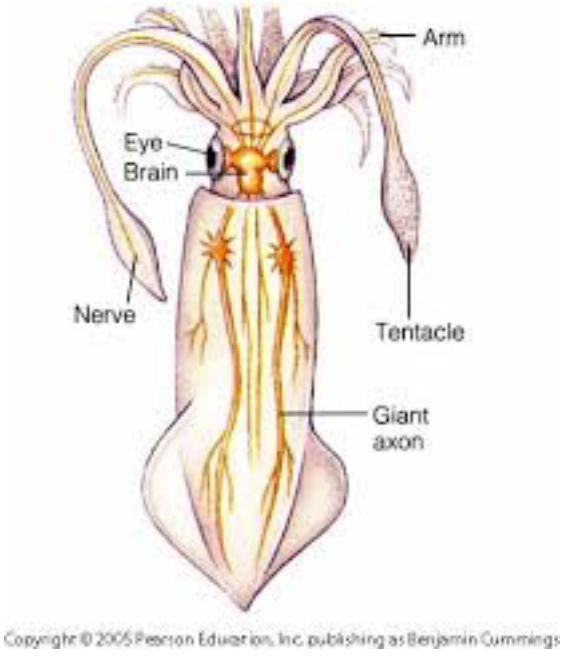
Sir John Carew Eccles
Prize share: 1/3

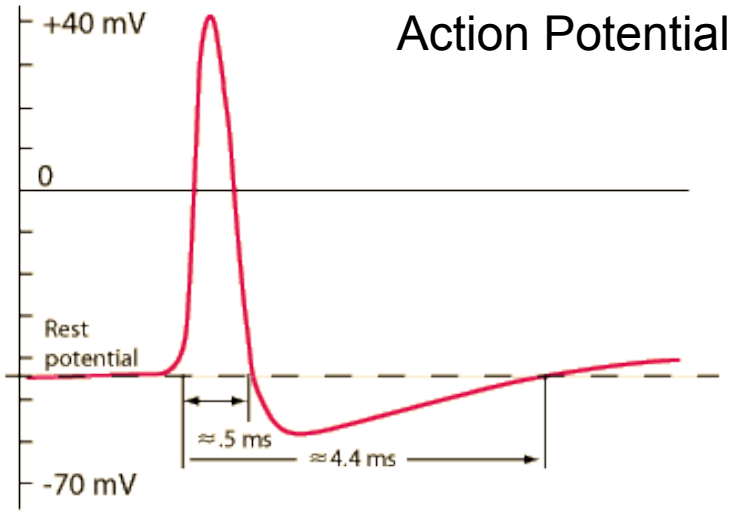
Alan Lloyd Hodgkin
Prize share: 1/3



Andrew Fielding Huxley
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1963 was awarded jointly to Sir John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley *"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane".*





Alan Hodgkin & Andrew Huxley

Brain function & behaviour

Learning & memory

"learning produces changes in behavior, not by altering basic circuitry, but by adjusting the strength of particular connections between nerve cells"

→ defined sets of genes and proteins that stabilize **synaptic connections** and trigger growth of new ones

Nobel Prize

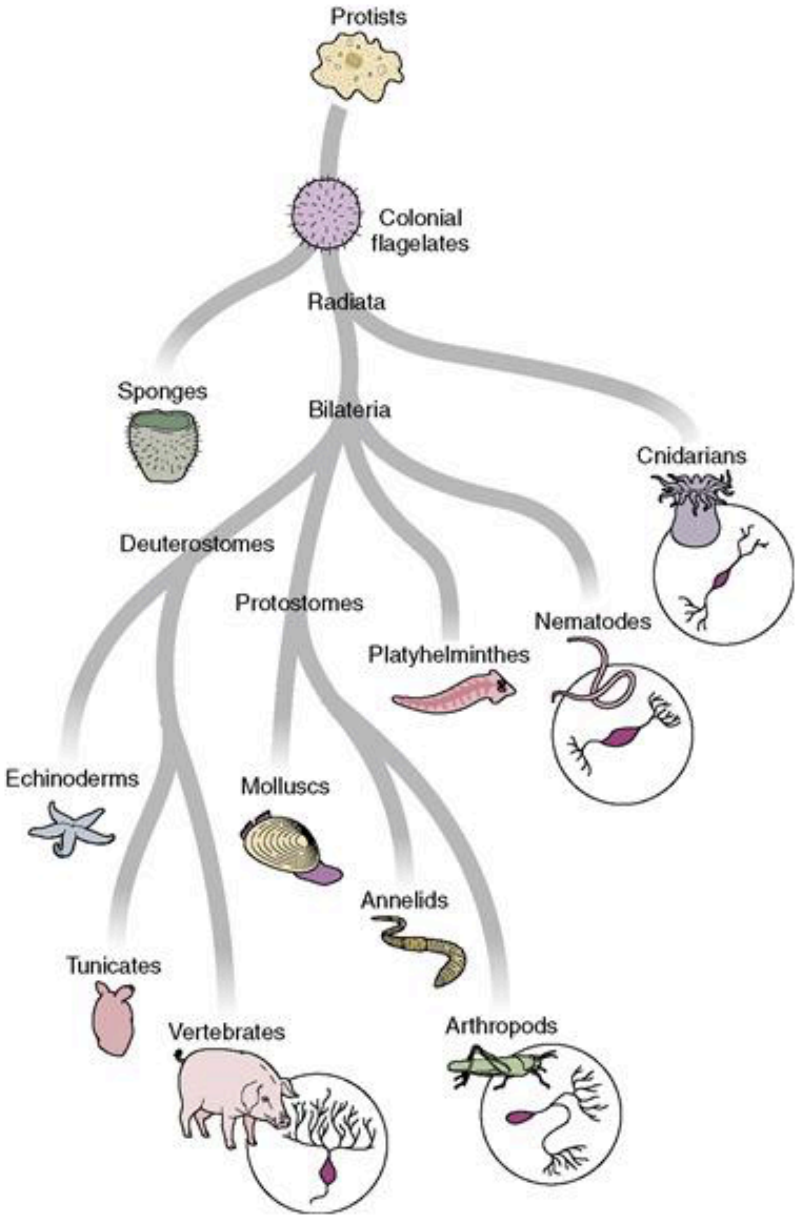
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2000/kandel-lecture.html

Despite the great diversity of animal forms

underlying principles of development

have been maintained throughout evolution

The study of neural development in diverse species is critical to the understanding of the development of anyone species



Model Organisms

Each organism has is own peculiar characteristic but due to the common descent of all living organisms and the conservation of metabolic patwhays and genetic material during evolution many aspects of biology (development) are similar in most organisms

- ✓ easy to maintain and breed in a laboratory setting
- ✓ short life cycle
- ✓ large number of offspring
- ✓ embryos easy to obtain
- ✓ particular experimental advantages

General principles can be derived …
but
care must be taken when extrapolating from one organism to another!!!

The development of the nervous system:

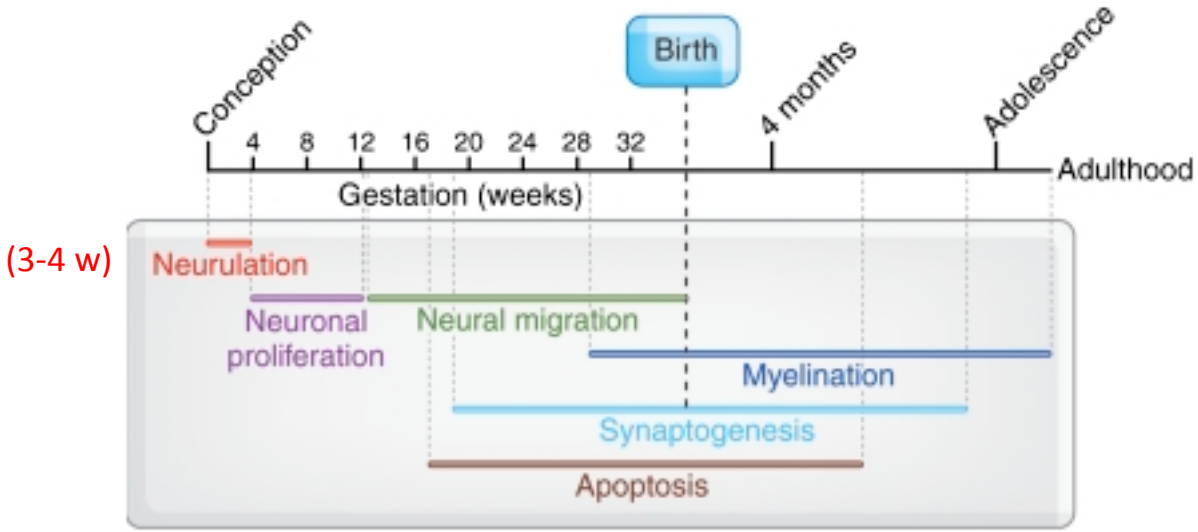
- Starts once the 3 primary germ layers are established
- Involves the segregation of neural cells from other cell types
- Involves the generation of neural precursor cells - mitotically active

→ Depending on the organism, it can occur in different way and at different time points

But the cellular/molecular mechanisms involved are highly conserved throughout evolution

→ The Neural system is one of the earliest systems to begin and the last to be completed after birth

Human

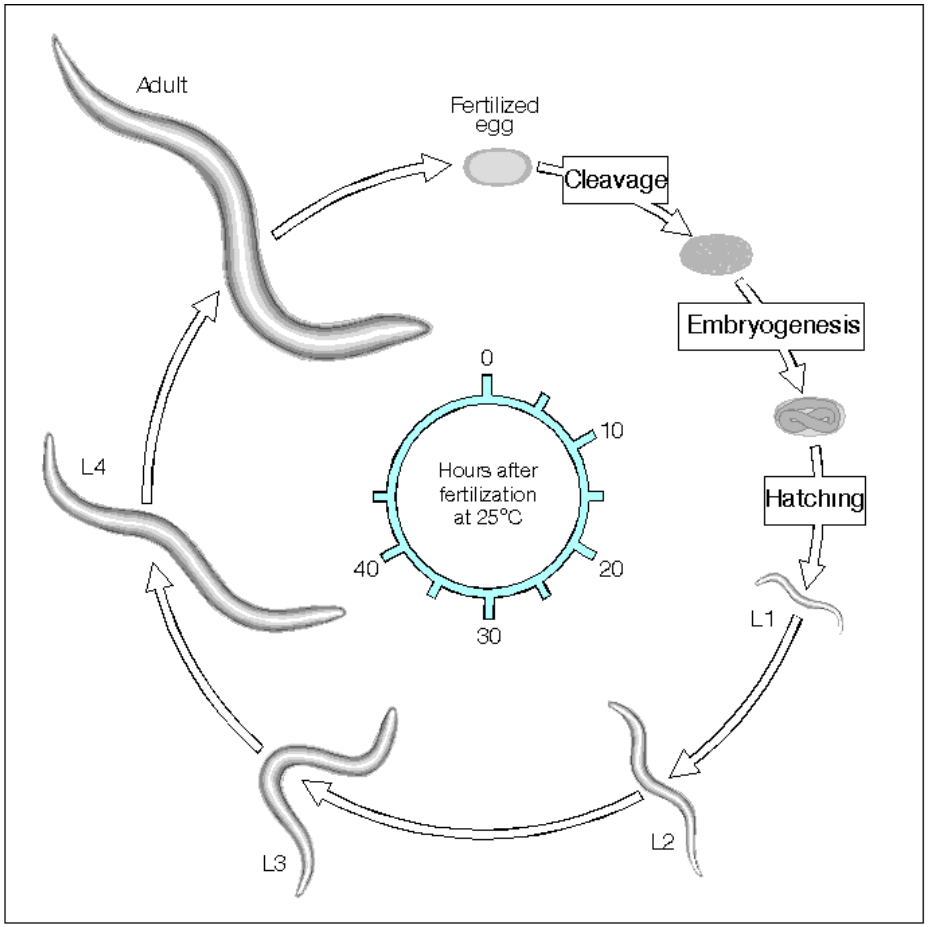


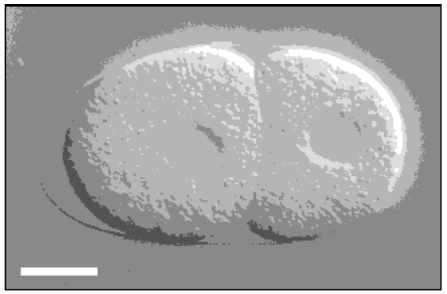
1. The neural tissue derives from the ectoderm

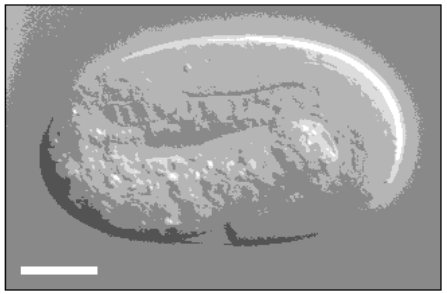
Two examples in invertebrates:

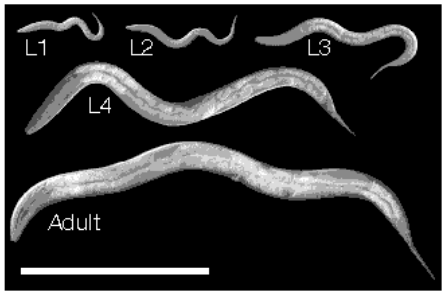
- *C. elegans*
- *Drosophila*

C. elegans









Short life cycle: from egg to egg takes about 3 days

Its life span is around 2 to 3 weeks
Simple structure
Transparent

Watch video

C. elegans

- Nervous system (*the most complex system in C.elegans*)

302* neurons (118 morphologically distinct neuron classes!!)
56 glial cells

Neurons are organized in several ganglia in the head and tail and into a spinal cord-like ventral nerve cord

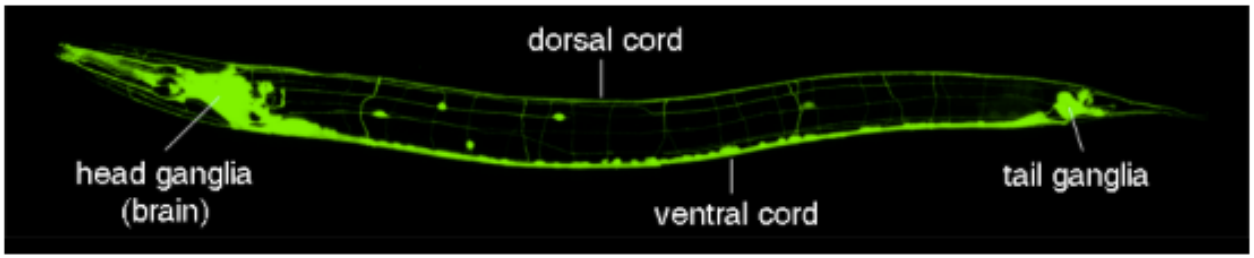
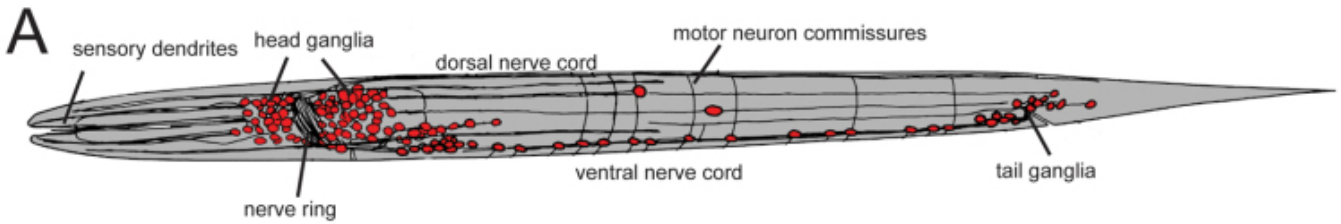


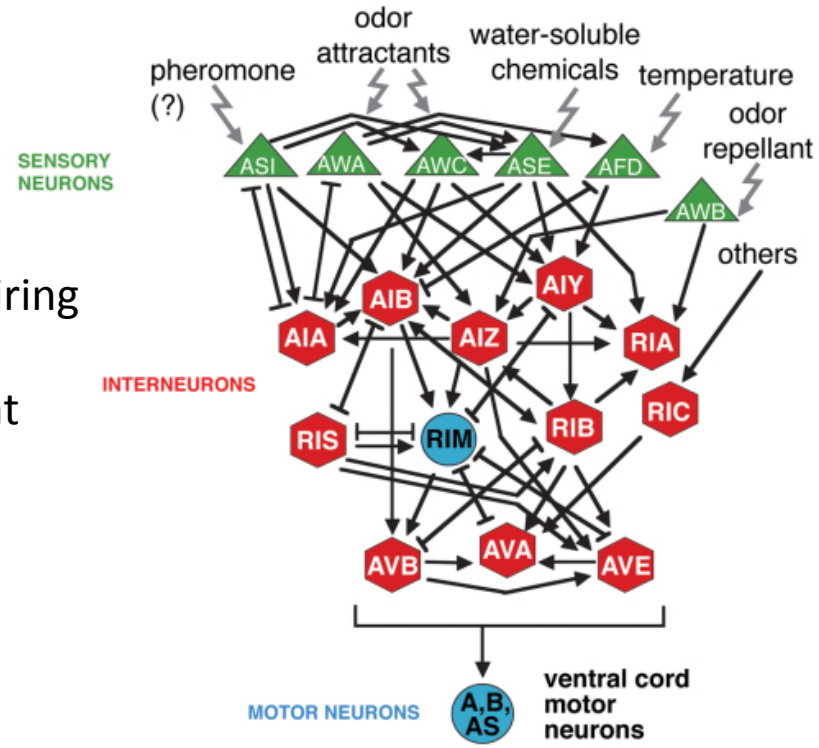
Figure 1: *C. elegans* nervous system: all neurons labelled with a fluorescent marker (GFP)



1° larval stage =222 neurons

*hermaphrodite
(383 in males)

B



complex patterns of wiring

(integration of different sensory modality)

→ Chemical synapses

⊣ Electrical synapses

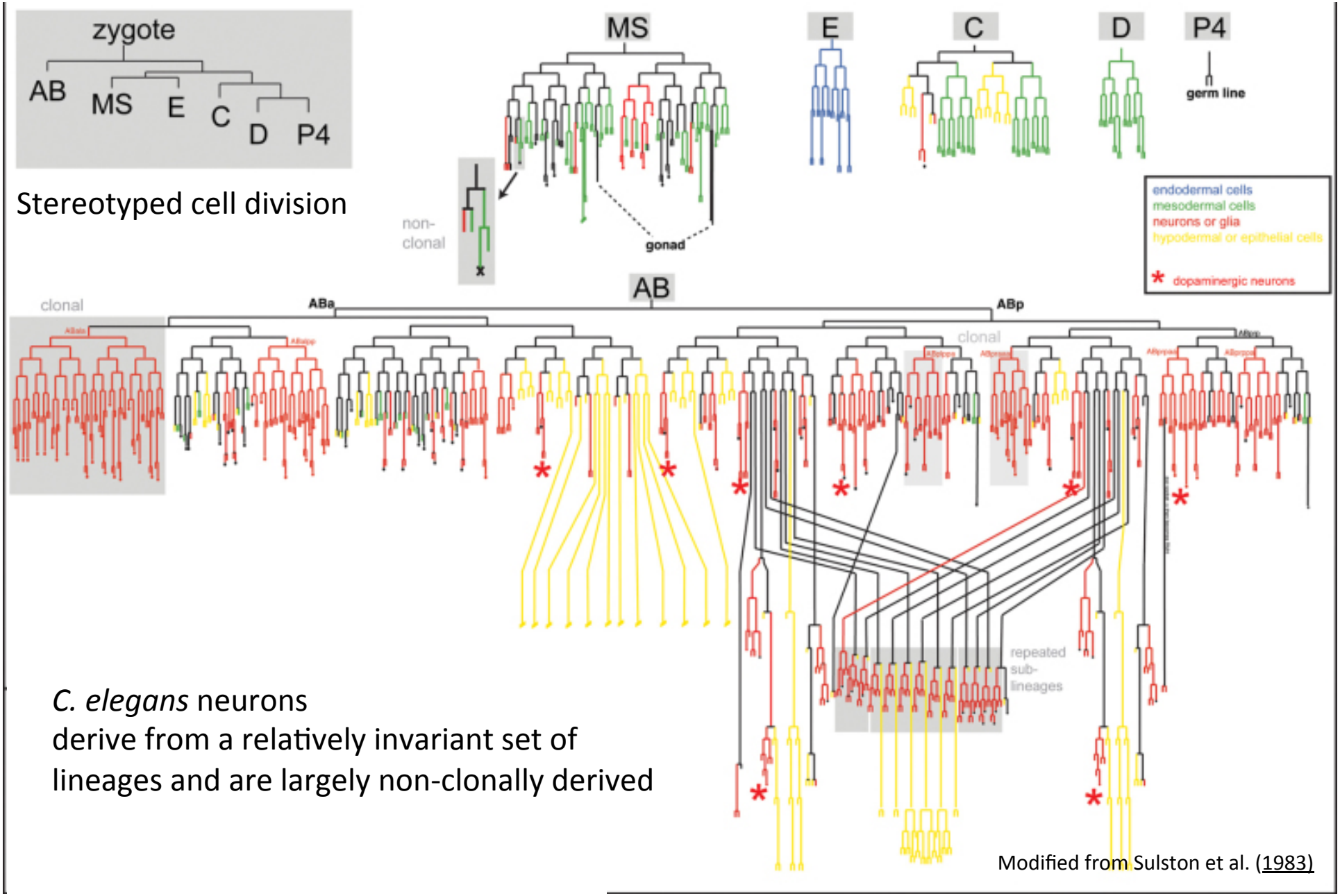
Combinatorial gene expression pattern → identifiers of neuronal terminal fate

		AIY	CAN	ADL	RID	AIZ	RME	AIA	SIA	ASE
Transcription factor	*ceh-10*	1	1	0	1	0	1	0	0	0
	ttx-3	1	0	1	0	0	0	1	0	0
	ceh-23	1	1	1	0	0	0	0	0	0
Terminal gene battery	*sra-11*	1	0	0	0	0	0	1	0	0
	kal-1	1	1	0	1	1	0	0	0	0
	hen-1	1	0	0	0	0	0	0	0	1
	unc-17	1	0	0	0	0	0	0	1	0
	ser-2	1	1	0	1	1	1	0	1	0

1 = gene expressed

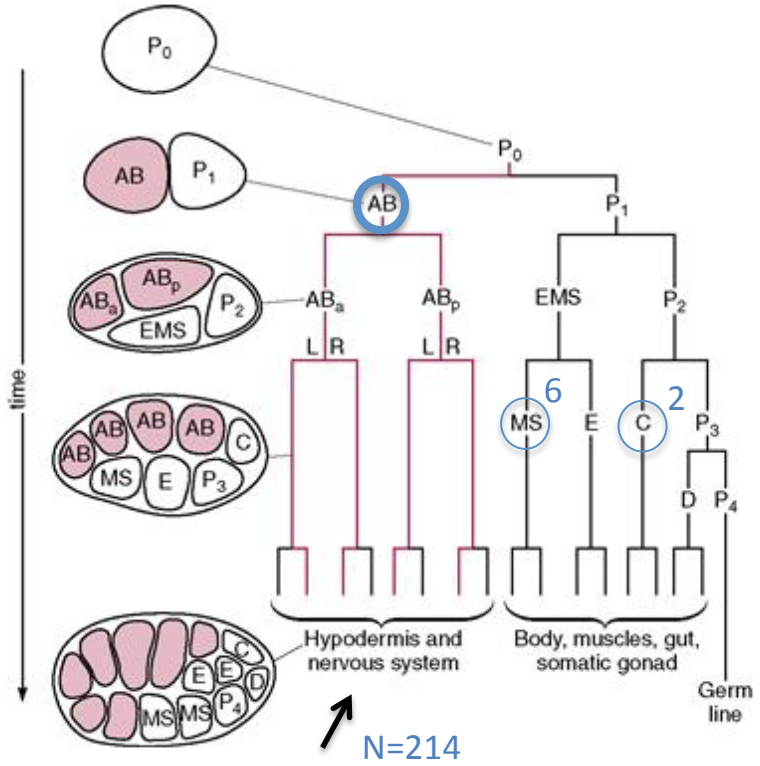
0 = gene not expressed

The complete lineage of the *C. elegans* nervous system



Shared lineage of hypodermal and neural cell fate

(most of the neurons derive from the AB lineage)



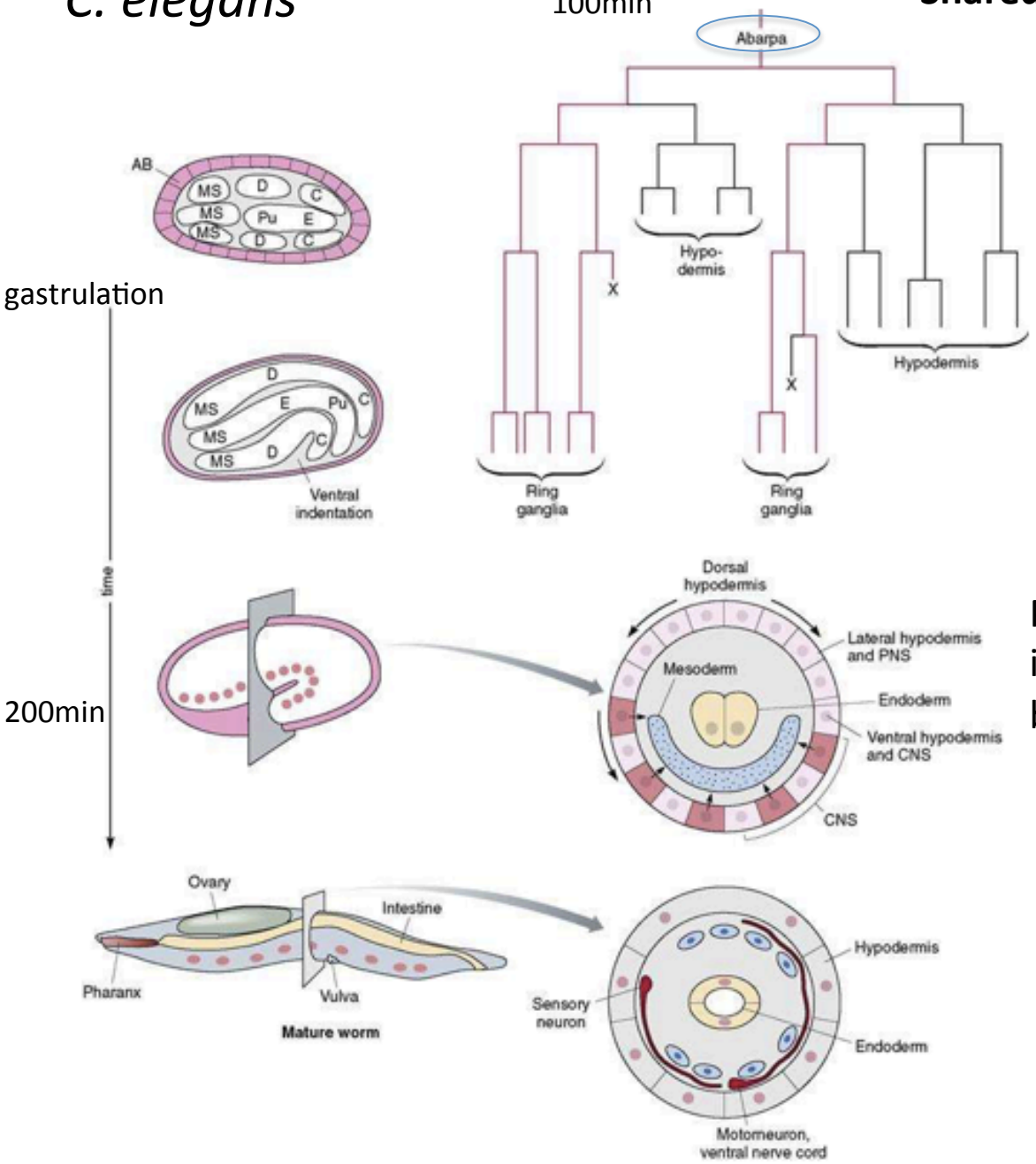
L1=222 neurons

N=214

(hypodermis→epidermis)

C. elegans

Shared lineage of hypodermis and neurons

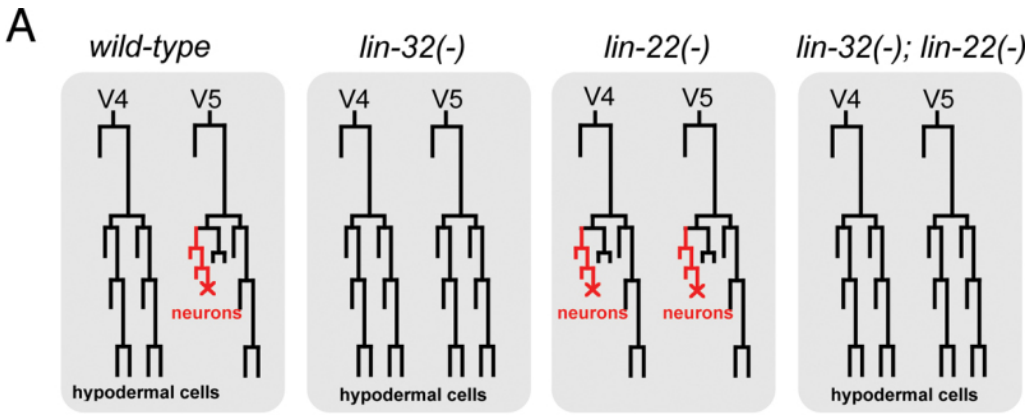


Abarpa progeny:

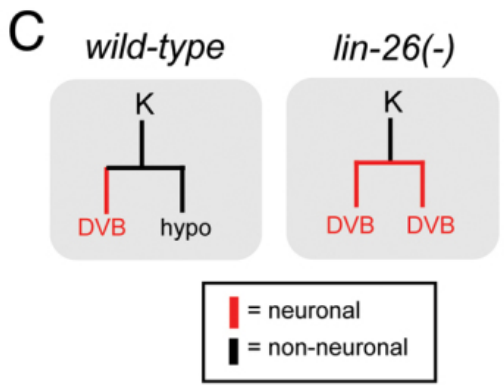
- 9 neurons
- 10 hypodermal cells

Progeny of Abarpa cells that move inside from the ventrolateral surface become nervous system

Neuronal vs. non-neuronal lineage transformations: genes controlling lineage decisions



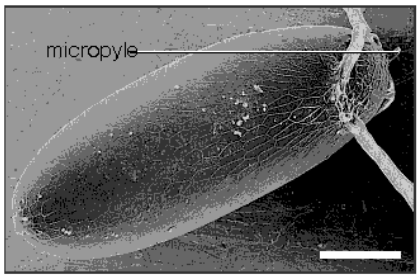
Cells derived from the postembryonic V ectoblasts lose their neuronal fate in lin-32 mutants transforming in hypodermal cells – or transform into neuronal fates in lin-22 or lin-26 mutants

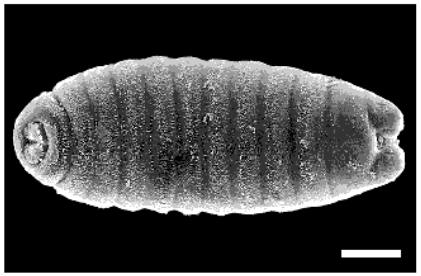


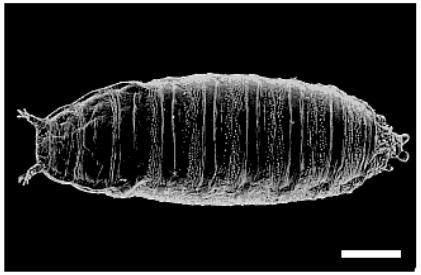
neuronal fate may be the "default" specification program in many lineages that is modified through the action of specific gene products

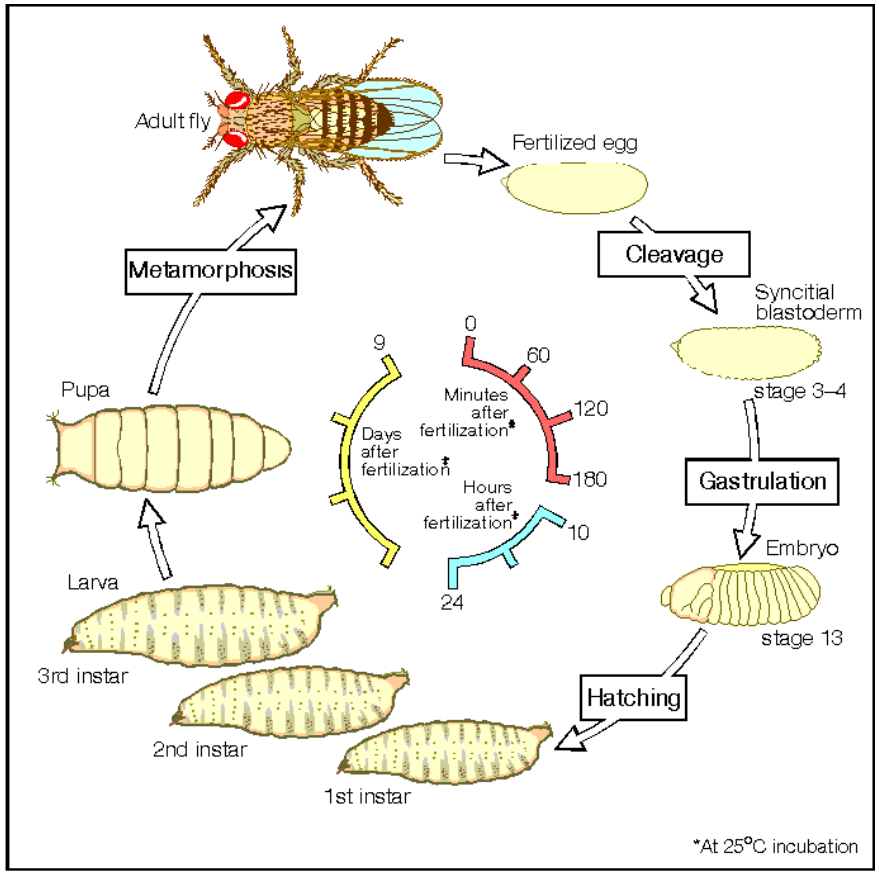
Lin-32* has a proneural function
Lin-22/Lin-26* have an anti-neural function

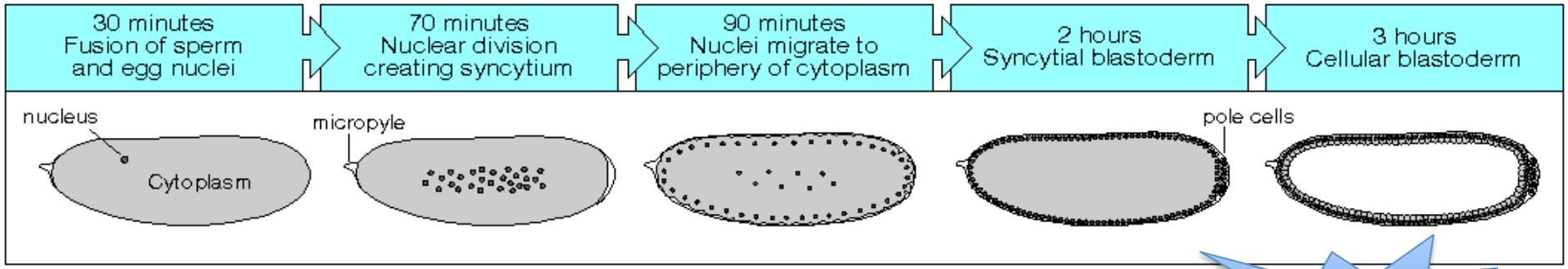
*TFs







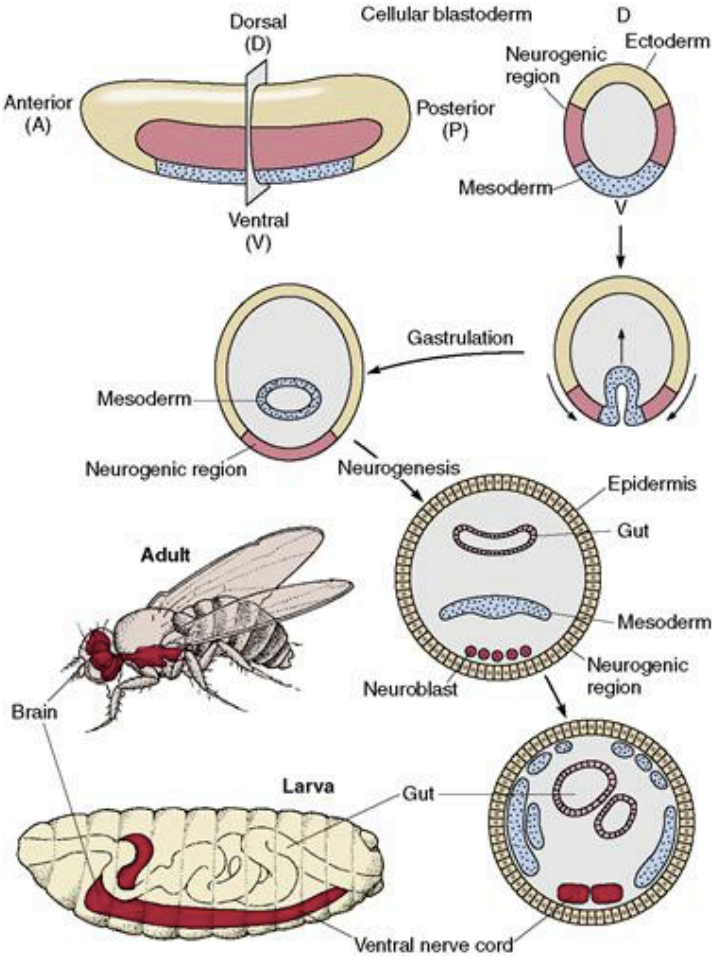




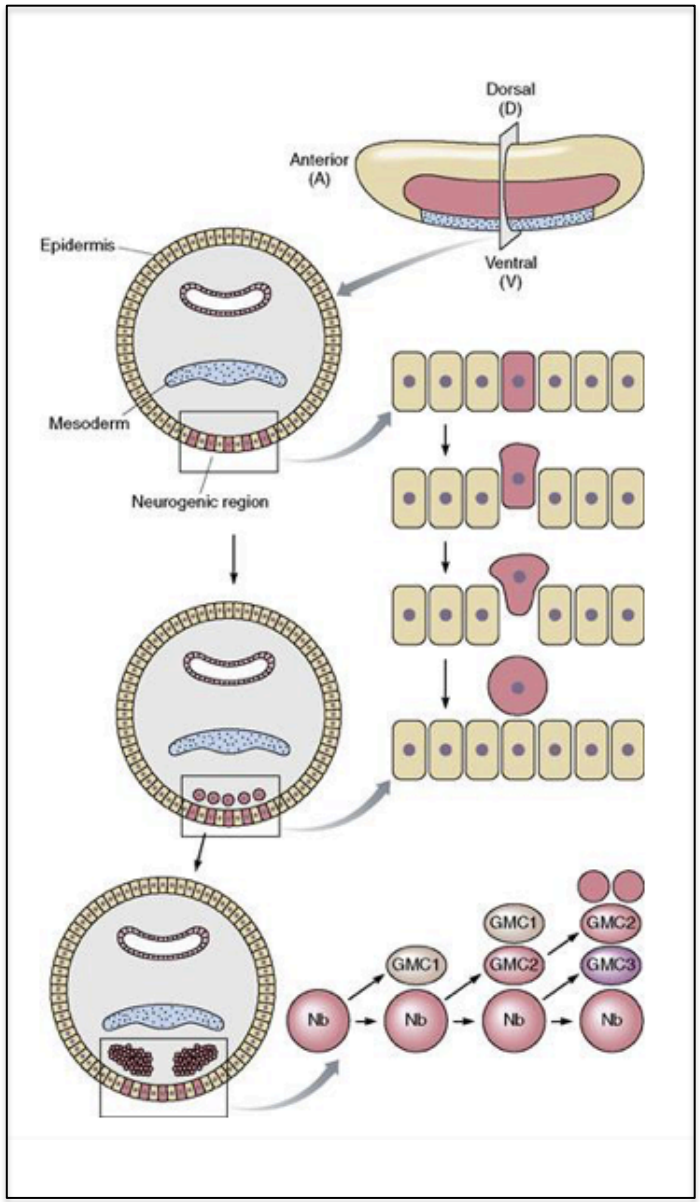
Short life cycle – 12 days

Watch video

Drosophila



- Most of the nervous system in Drosophila derives from the ventro-lateral part of the cellular blastoderm
- Following gastrulation, the neurogenic region (ectoderm) is at the ventral midline→ it will give rise to ventral nerve cord (CNS)
- The procephalic neurogenic region will give rise to the cerebral ganglia



Single neuroblasts separate from the ectoderm by **delamination** in several waves and move into the interior of the embryo to form neural precursor cells called neuroblasts (Nb)

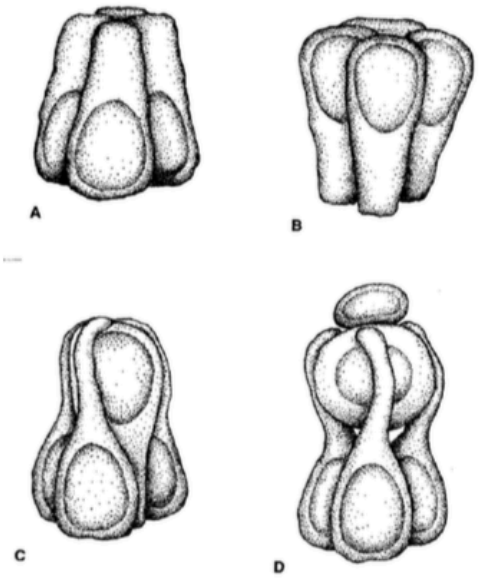
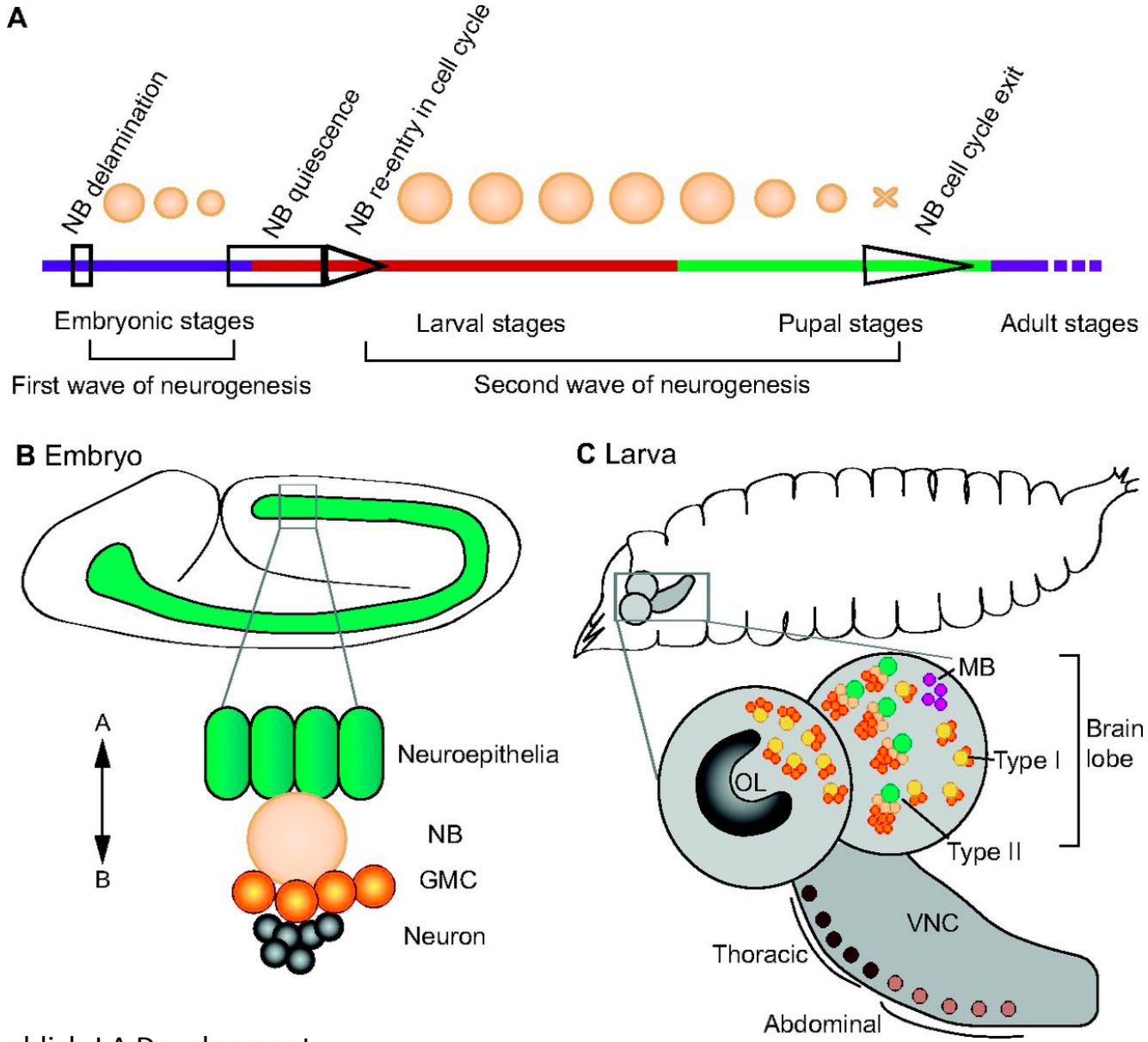


Fig. 1. Morphological differentiation of neuroblasts. The development of NB 4-2 is drawn based on camera-lucida tracings of embryos viewed with Nomarski optics. (A) Ectodermal cells at the 4-2 position have a uniform columnar morphology, with the nuclei located at the ventral surface of the cells. (B) The nuclei of one to four cells at the 4-2 position move towards the dorsal cell surface. (C) One cell begins to delaminate into the embryo; it shifts both cytoplasm and the nucleus dorsally relative to adjacent cells. (D) Delamination is complete and the new NB 4-2 divides asymmetrically, budding off a smaller ganglion mother cell (top cell) at its dorsal surface. Subsequently, the adjacent ectodermal cells withdraw their dorsal processes and regain a columnar morphology.

Doe, Development 1992

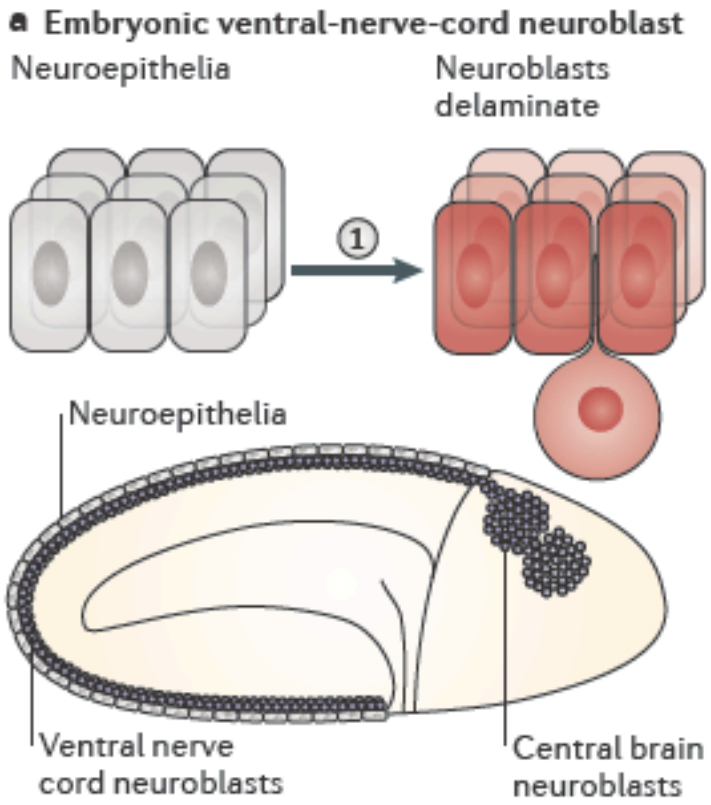
Once inside the embryo the Nb undergo a <u>stereotyped pattern</u> of asymmetric divisions giving rise to ganglion mother cells (GMCs) that in turn originate neurons or glia

Neurogenesis in Drosophila



Homem C C F , and Knoblich J A Development 2012;139:4297-4310

Interactions among the ectodermal cells in controlling neuroblast segregation



Proneural clusters→ lineage segregation

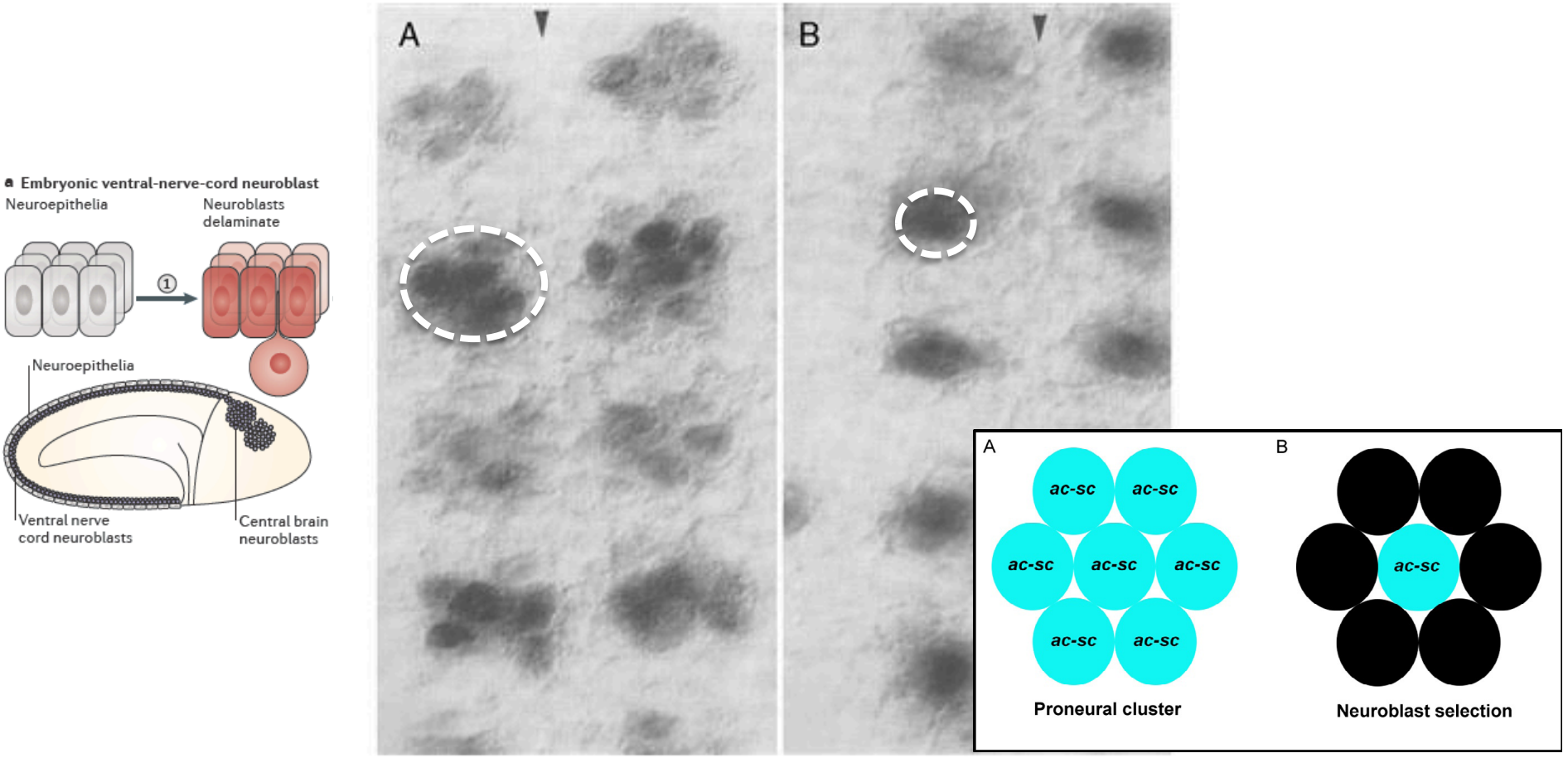
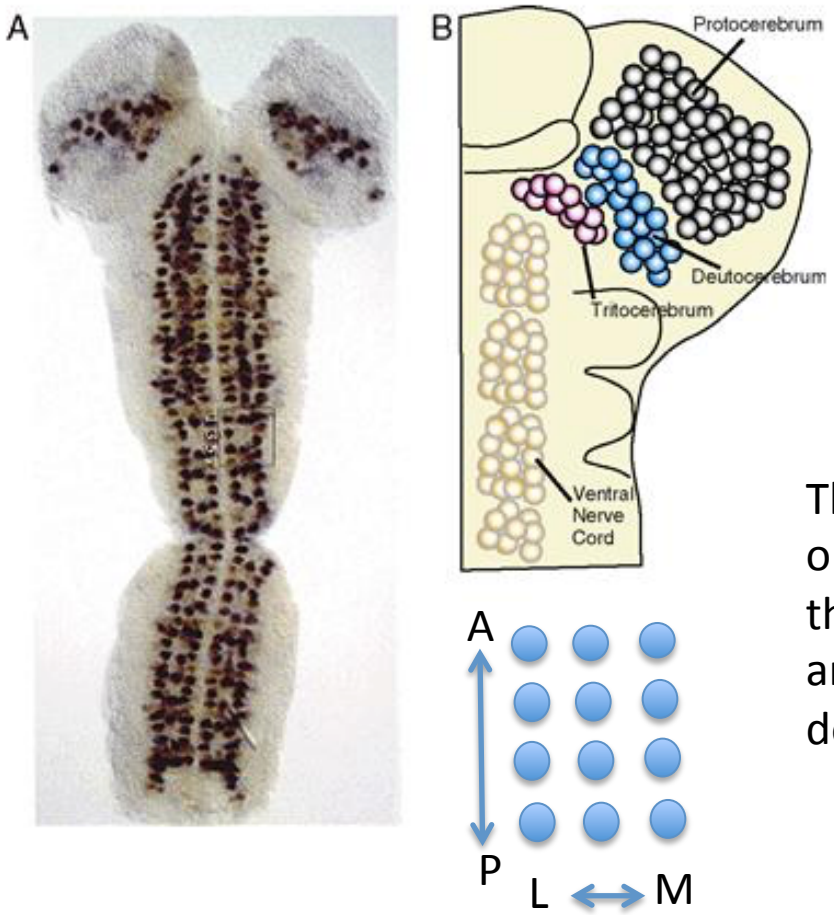


Fig. 1.23 Neuroblast segregation in the *Drosophila* neurogenic region proceeds in a highly patterned array. A. In this embryo stained with an antibody against *achaete-scute (as-c)* protein, clusters of proneural cells in the ectoderm express the gene prior to delamination. B. A single neuroblast develops from each cluster and continues to express the gene. The other proneural cells downregulate the *as-c* gene.(From Doe, 1992)



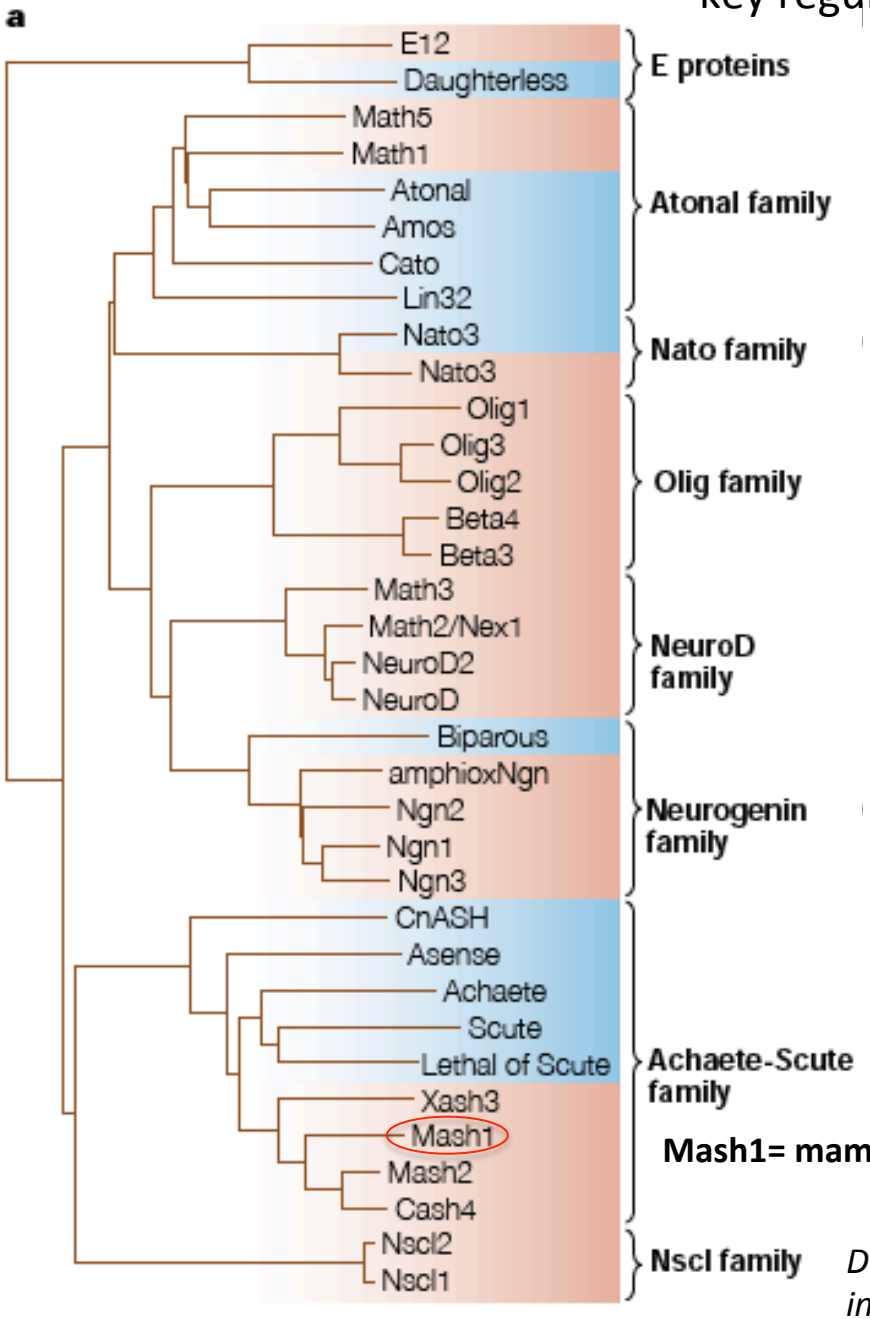
Ventral view of a Drosophila embryo

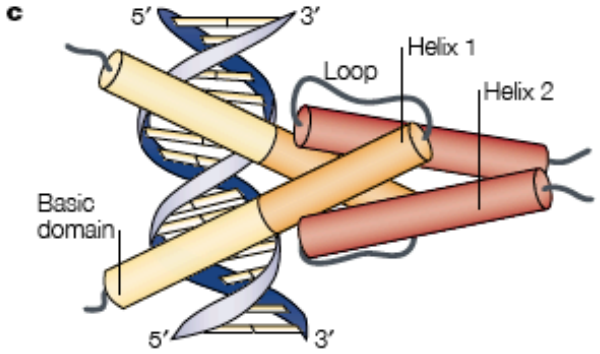
Ventral nerve cord stereotyped pattern

The early neuroblasts form an orthogonal grid of 4 rows along the anterior-posterior (AP) axis and 3 columns along the dorsoventral (DV) axis

Proneural genes

key regulators of neurogenesis





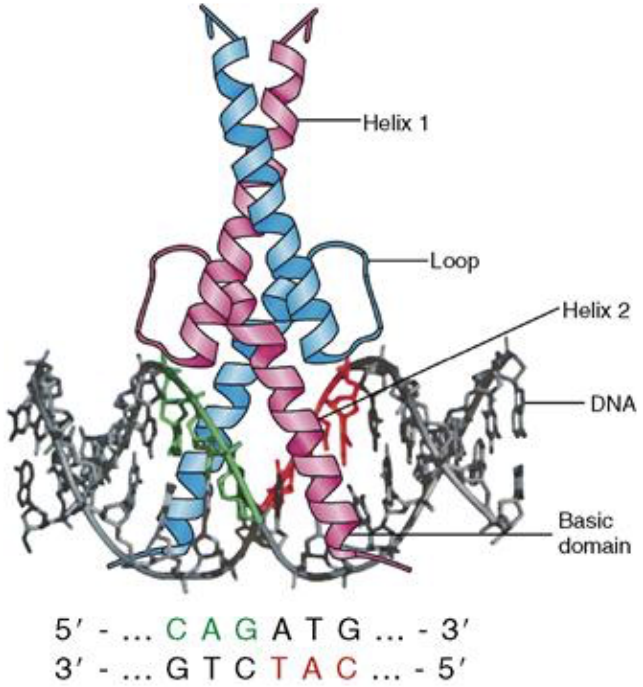
Proneural proteins bind E-box (CANNTG) DNA sequences as heterodimeric complexes formed with ubiquitously expressed bHLH proteins (E proteins)

Activate trascription of neuronal genes

Mash1= mammalian achaete scute homolog-1 (ascl1)

Dendrogram of the sequence of the basic helix-loop-helix domain of invertebrate (blue) and vertebrate (red)

Proneural genes
key regulators of neurogenesis



Multifaceted role in neural development:

- generic neural fate
- Specification of subtype identities

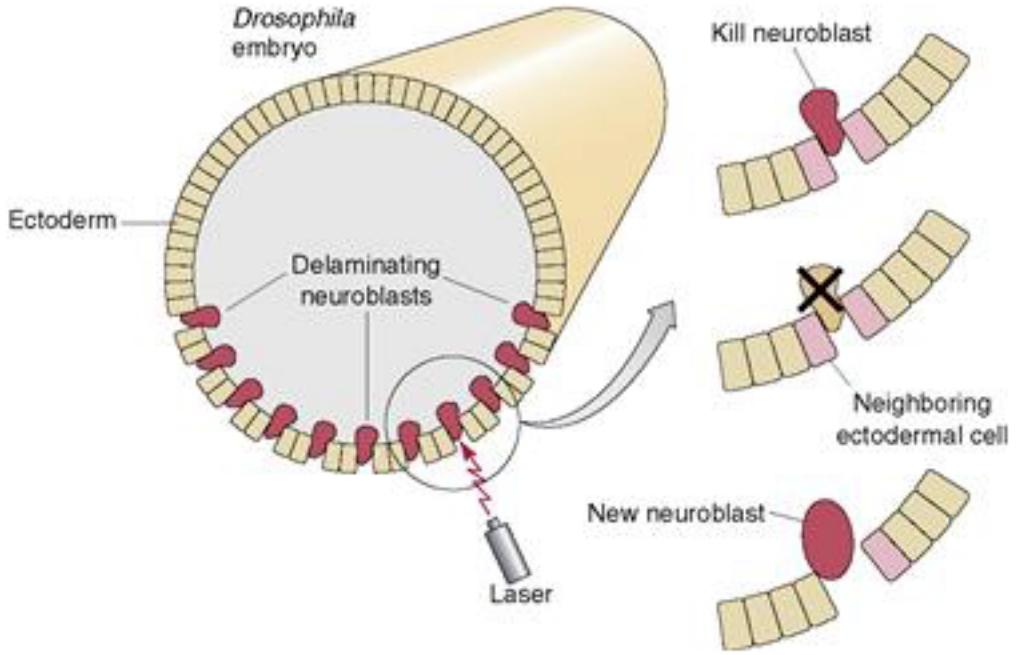


Fig. 1.26 Ablation of the delaminating neuroblast with a laser microbeam directed to the ventral neurogenic region of the fly embryo causes a neighboring ectodermal cell to take its place.

Expression of **achaete-scute** genes

determination of precursor cells towards neural fate (proneural)

Proneural genes inhibit their own expression in adjacent cells, preventing these cells from becoming neuroblasts:

How?

by **a molecular regulatory loop between neighbouring cells**

Notch pathway

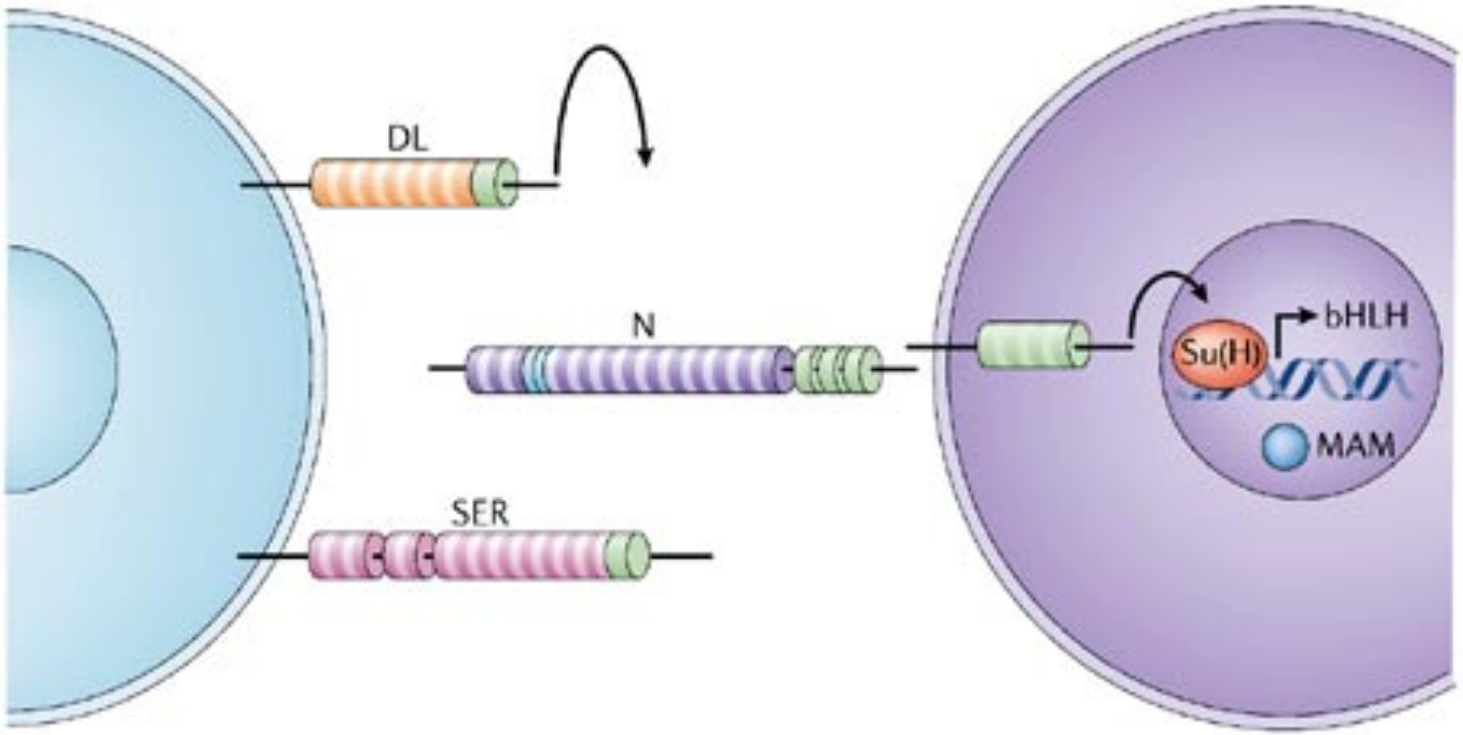
The developmental logic of **Notch**

Notch signaling couples cell fate acquisition by an individual cell to the cell fate choices made by its “next door neighbours”

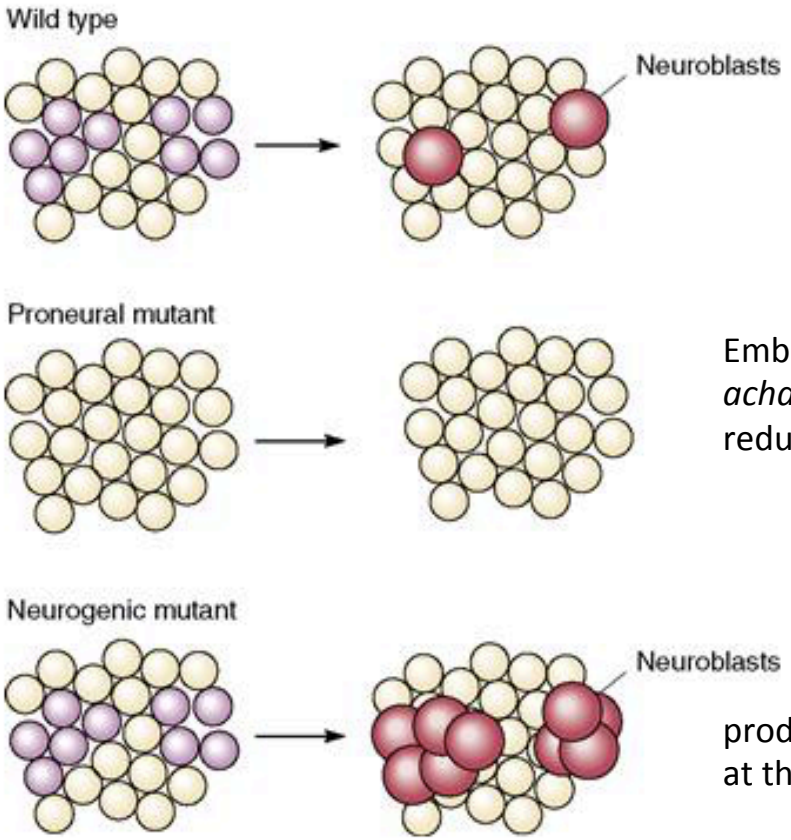
Cell-cell interaction: a membrane-bound receptor (Notch) on one cell interacts with a membrane bound ligand (e.g. Delta) on another cell

→ Lineage segregation

The core of Notch pathway



Drosophila



Embryos lacking the proneural genes *achaete/scute* or *lethal of scute* have a reduced number of neuroblasts

Notch/Delta mutants

produce a remarkable excess of neurons at the expense of epidermis

Fig. 1.24 Neurogenic genes and proneural genes were first identified in the *Drosophila* due to their effects on neural development. In the wild-type embryo (top), only one neuroblast (red) delaminates from a given proneural cluster in the ectoderm. However, in flies mutant for proneural genes (middle), like *achaete scute*, no neuroblasts form. By contrast, in flies mutant for neurogenic genes (bottom), like *notch* and *delta*, many neuroblasts delaminate at the positions where only a single neuroblast develops in the wild-type animal. Thus, too many neurons delaminate—hence the name "neurogenic."

Neurogenic mutant

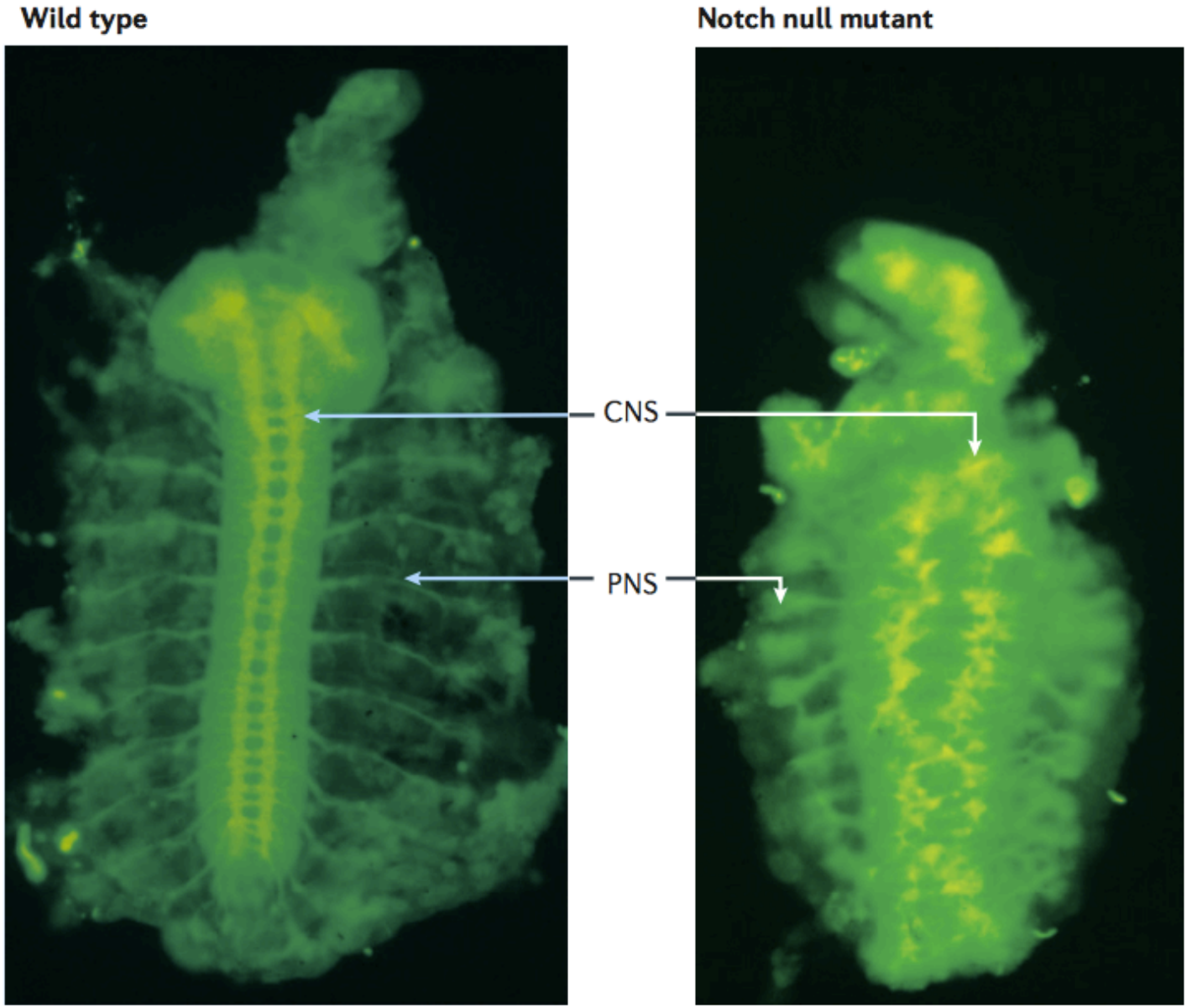
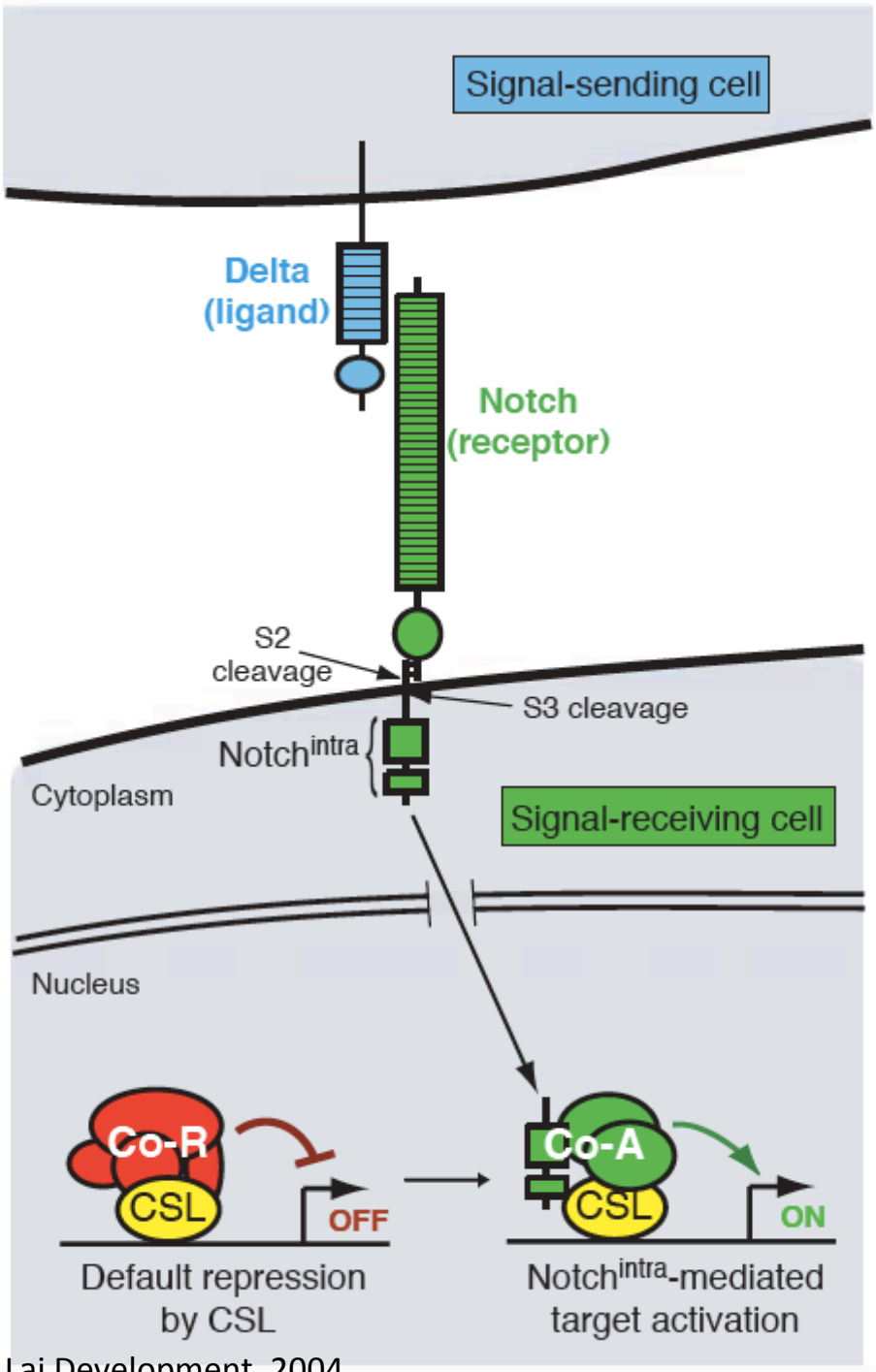


Figure 1 | ***Drosophila melanogaster* embryos stained with an antibody against horseradish peroxidase that recognizes neural tissue.** Wild-type and Notch null mutant *D. melanogaster* embryos, showing the hypertrophy of both the CNS and PNS that occurs in the absence of Notch. Image reproduced, with permission, from REF. 148 © (1989) Rockefeller University Press.

Box 1. Neurogenic genes

The field of Notch signaling originated with the study of 'neurogenic' fly embryos, which exhibit excessive neuronal differentiation. The term 'neurogenic' has persisted over the decades: partly out of deference to history; and partly out of the efficacy of the neurogenic phenotype in continuing to identify new genes that are functionally connected to Notch signaling, even to this day. However, the term 'neurogenic' has also been the source of some continuing confusion, as it might reasonably be assumed to refer to a gene that promotes neurogenesis and/or functions exclusively during neurogenesis. Therefore, it is important to understand that: (1) 'neurogenic' describes a loss-of-function condition (thus, 'neurogenic' genes actually serve to repress neurogenesis); and (2) 'neurogenic' genes do not function exclusively during neurogenesis (rather, they usually operate throughout development).



Lai Development, 2004

Basic operation of the Notch pathway

The key players are:

- Delta-type ligand,
- the receptor Notch,
- the CSL TF

Activation of Notch by its ligand triggers two proteolytic cleavages of Notch.

→ S3 cleavage (by a protease gamma-secretase) releases the Notch intracellular domain (Notch-ICD) which translocates to the nucleus and activates CSL.

→ In the absence of nuclear Notch-ICD, CSL associates with a co-repressor complex (Co-R), which actively represses the transcription of Notch target genes.

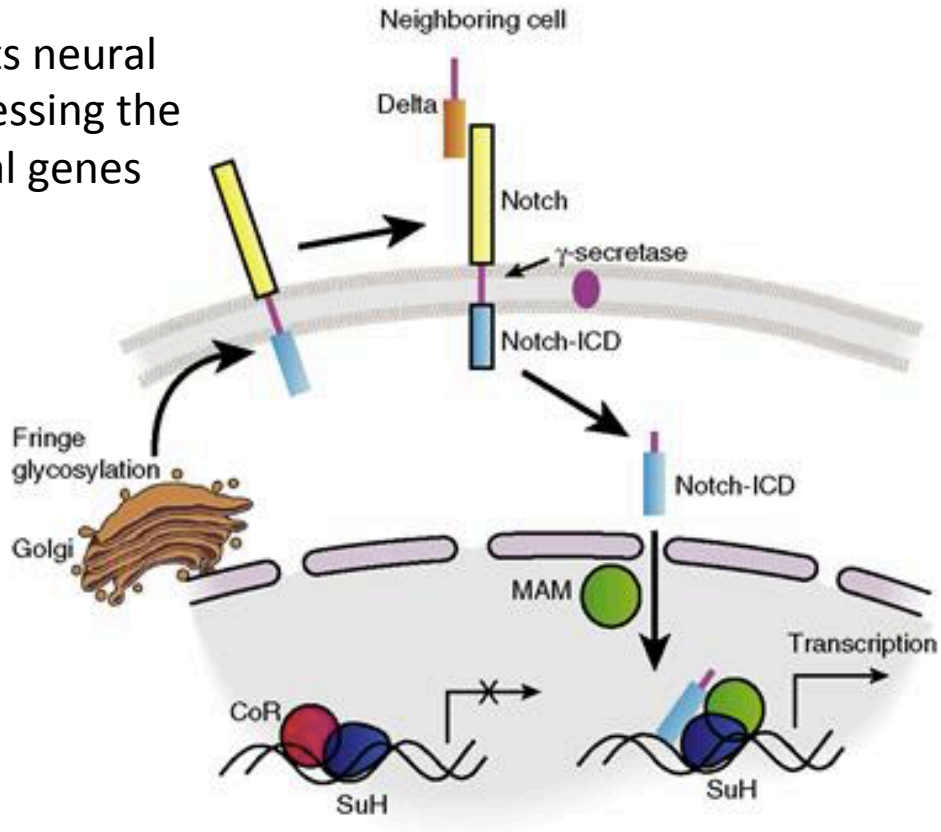
→ The CSL co-repressor complex is displaced by a co-activator complex containing Notch ICD.

CSL=CBF1/Su(H)/LAG1

Table 1. Names of core components of Notch signaling (ligand, receptor and transcription factor) in different species

Core component	*C. elegans*	*D. melanogaster*	Mammals
Ligand	LAG-2 APX-1 ARG-2 F16B12.2	Delta Serrate	Delta-like1 (DLL1) Delta-like2 (DLL2) Delta-like3 (DLL3) Jagged 1 (JAG1) Jagged 2 (JAG2)
Receptor (Notch)	LIN-12 GLP-1	Notch	Notch1 Notch2 Notch3 Notch4
Transcription factor (CSL)	LAG-1	Suppressor of Hairless [Su(H)]	CBF1/RBPJκ RBPL

Notch signaling restricts neural differentiation by repressing the expression of proneural genes



Drosophila

Espl –I proneural genes (bHLH→N-boxes)

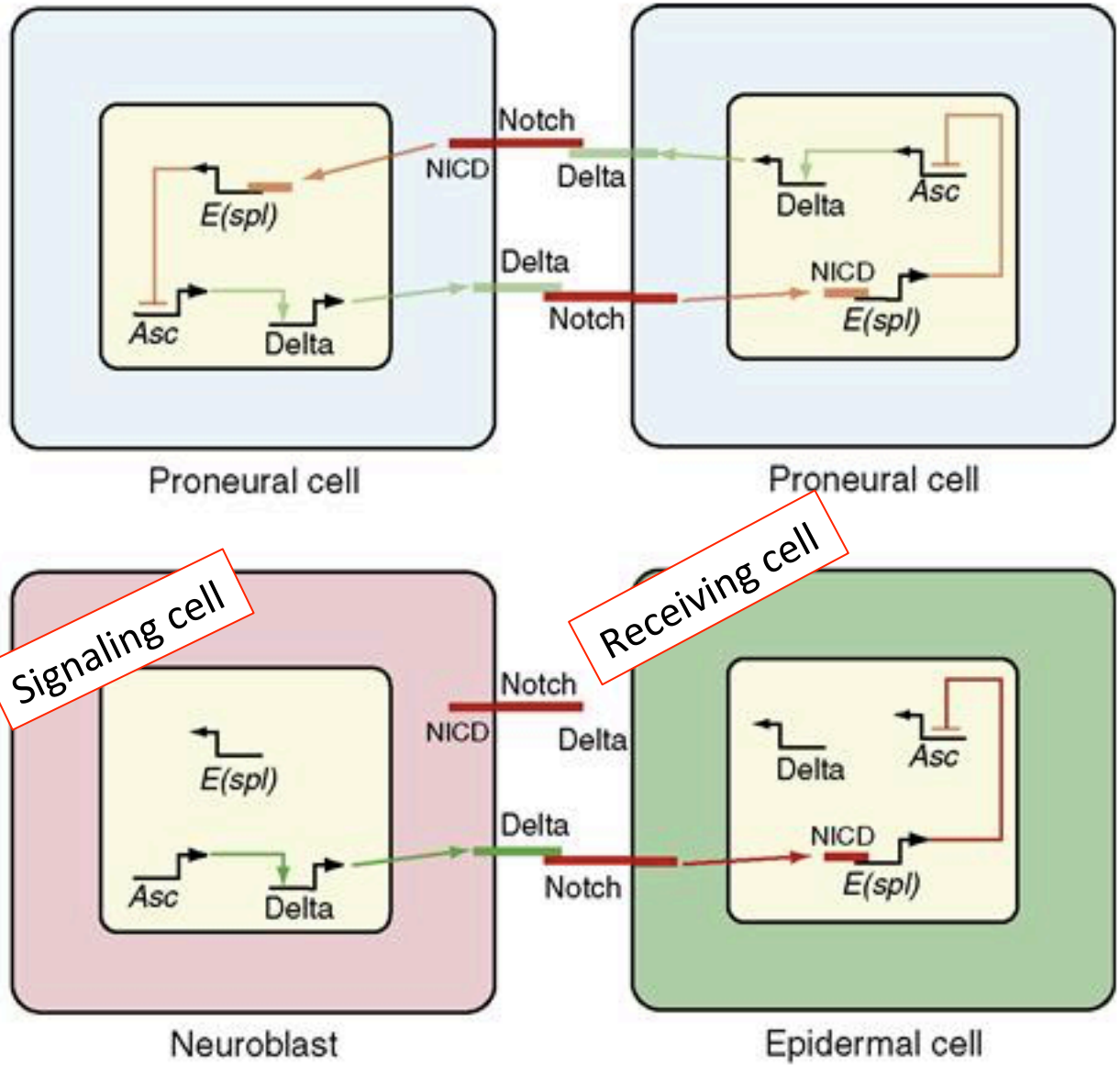
Fig. 1.27 The binding of Delta to Notch leads to a proteolytic cleavage of the molecule by a protease called gamma-secretase. This releases the intracellular part of the Notch molecule (called the Notch-ICD, for intracellular domain). The Notch-ICD interacts with another molecule, Suppressor of Hairless (SuH), and together they form a transcription activation complex to turn on the expression of downstream target genes, specifically *Enhancer of Split*. The E(spl) proteins are repressors of *Asc* gene transcription, and so they block further neural differentiation and reduce the levels of Delta expression.

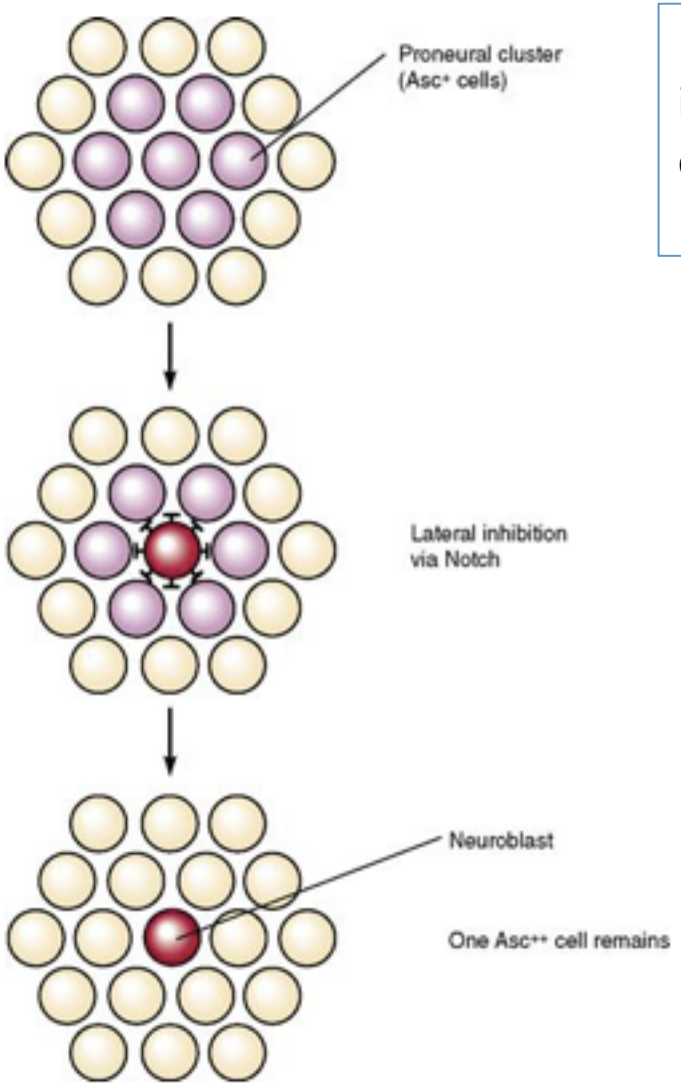
MAM = mastermind - function as coactivator of Notch signalling

Lateral inhibition and Notch/Delta pathway "the *Drosophila* neuralepidermal choice"

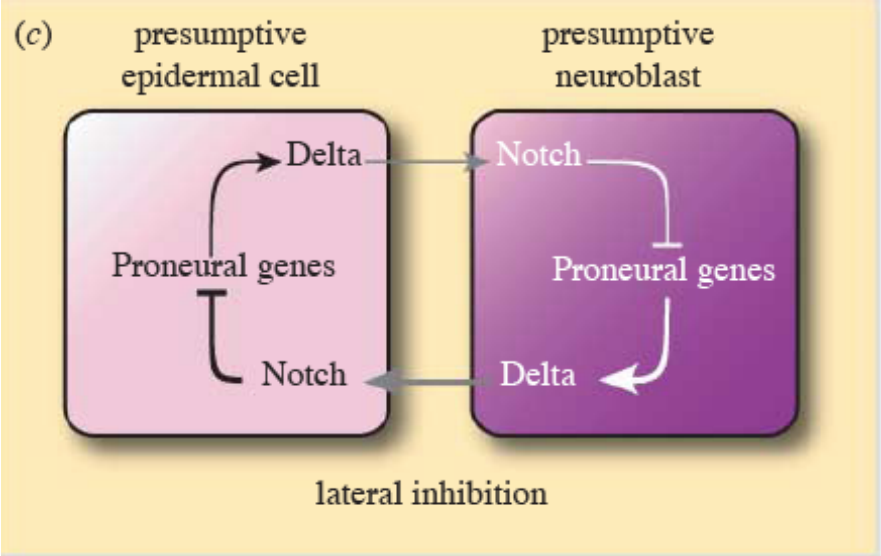
2 equivalent cells
(expression receptor and ligand)

non equivalent



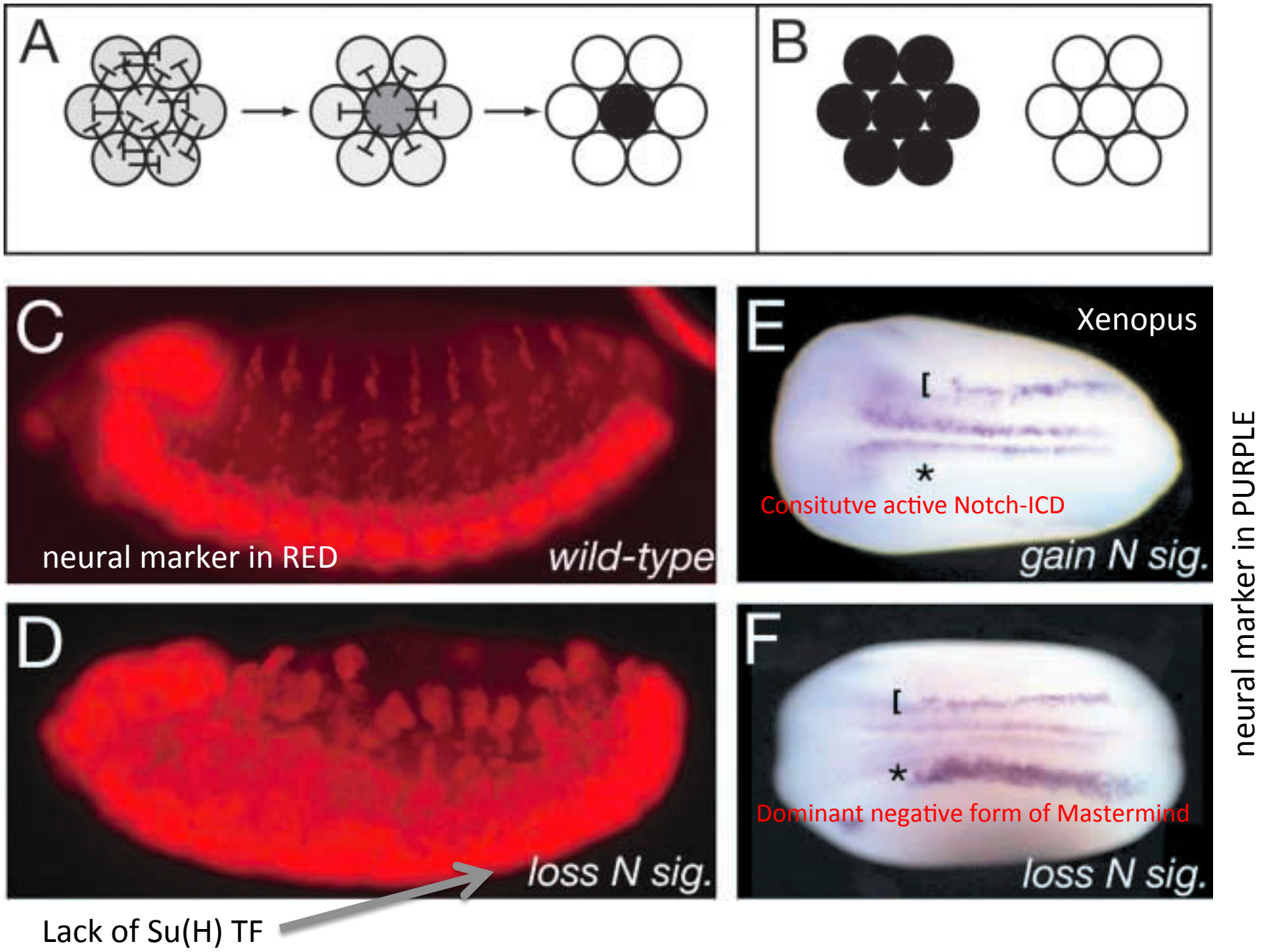


Key to the process of **lateral inhibition** in *Drosophila* is the direct and dose-dependent transcriptional activation of the Notch receptor ligand Delta by proneural genes



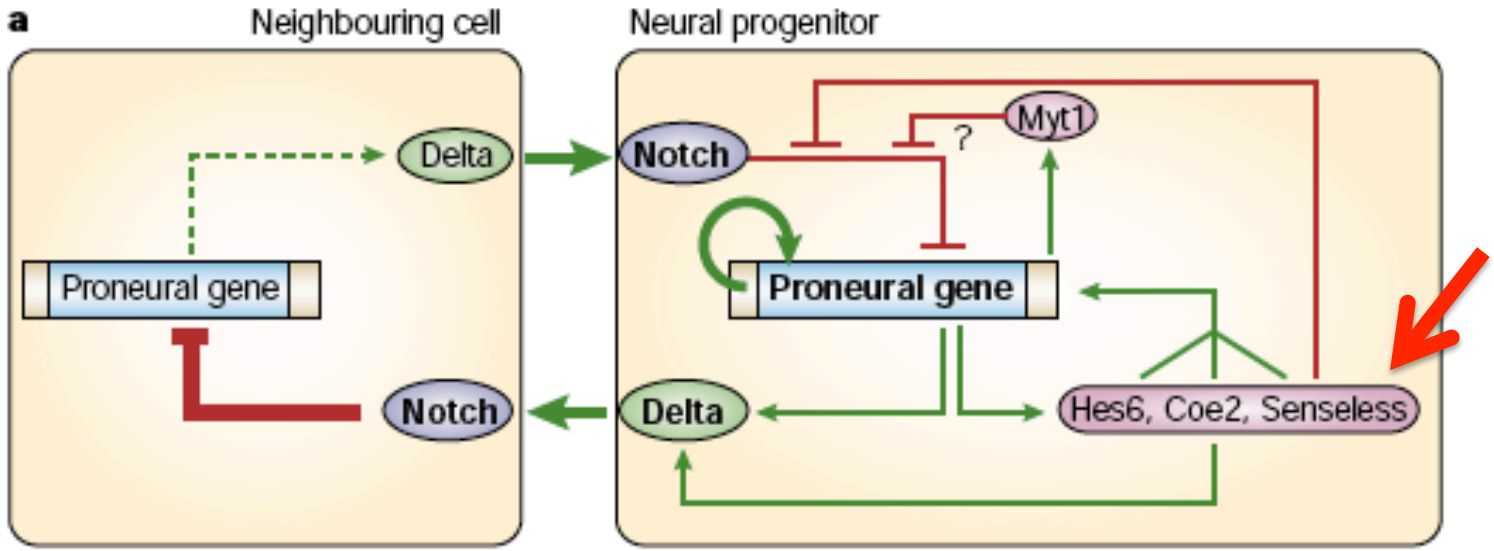
Low Notch activity→ neural fate
High Notch activity→ epidermal fate

→ *The cell that initially has higher levels of proneural genes or Delta expression (or lower levels of Notch expression) will become a neuroblast*

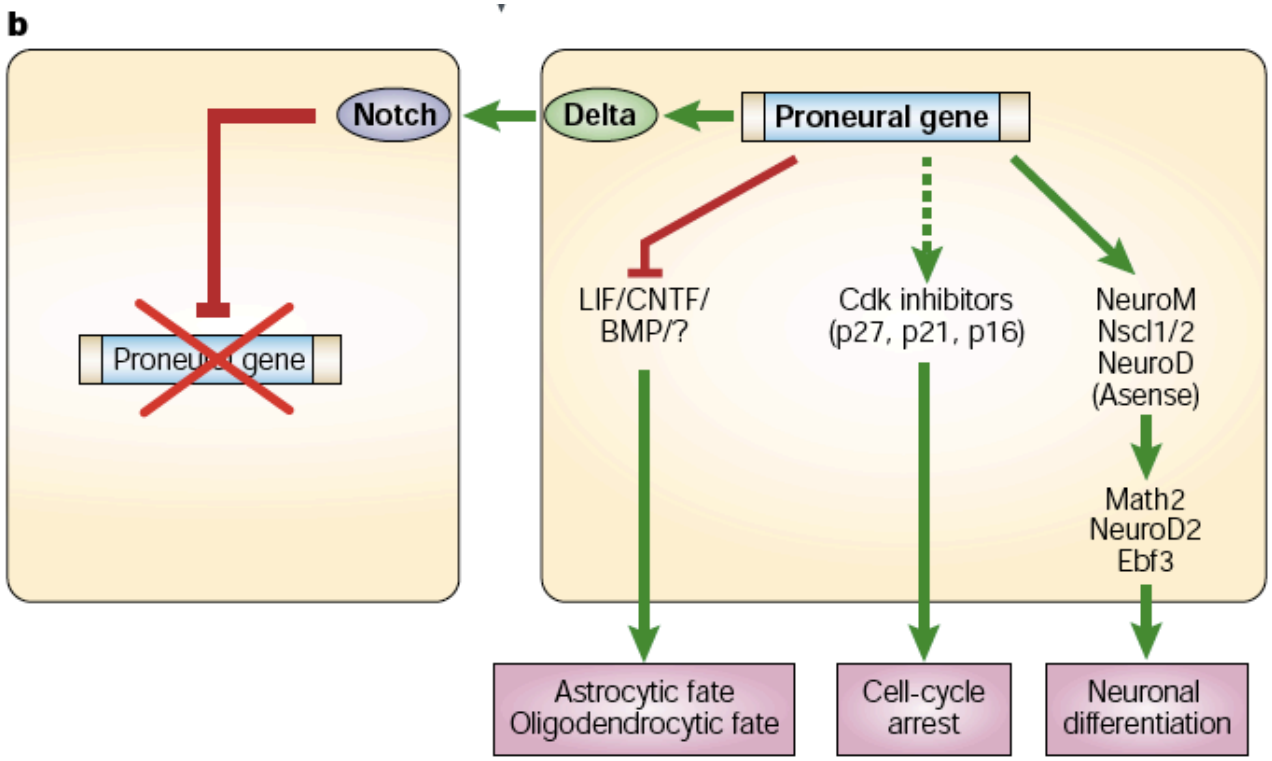

A
B
C
neural marker in RED
wild-type
D
loss N sig.
Lack of Su(H) TF
E
Xenopus
Consitutve active Notch-ICD
gain N sig.
F
Dominant negative form of Mastermind
loss N sig.
neural marker in PURPLE

Positive feedback loops maintain high proneural gene levels

Notch signalling is involved in the initial regulation of proneural gene expression but **other positive-feedback mechanisms** are required to increase and/or maintain the levels of proneural gene expression in the selected neural progenitors



Functional hierarchy of proneural bHLH genes
(vertebrates and invertebrates)



downstream regulatory genes implement neuronal differentiation programs

→ distinct bHLH genes act in cascade underling the sequential steps of cell determination and differentiation