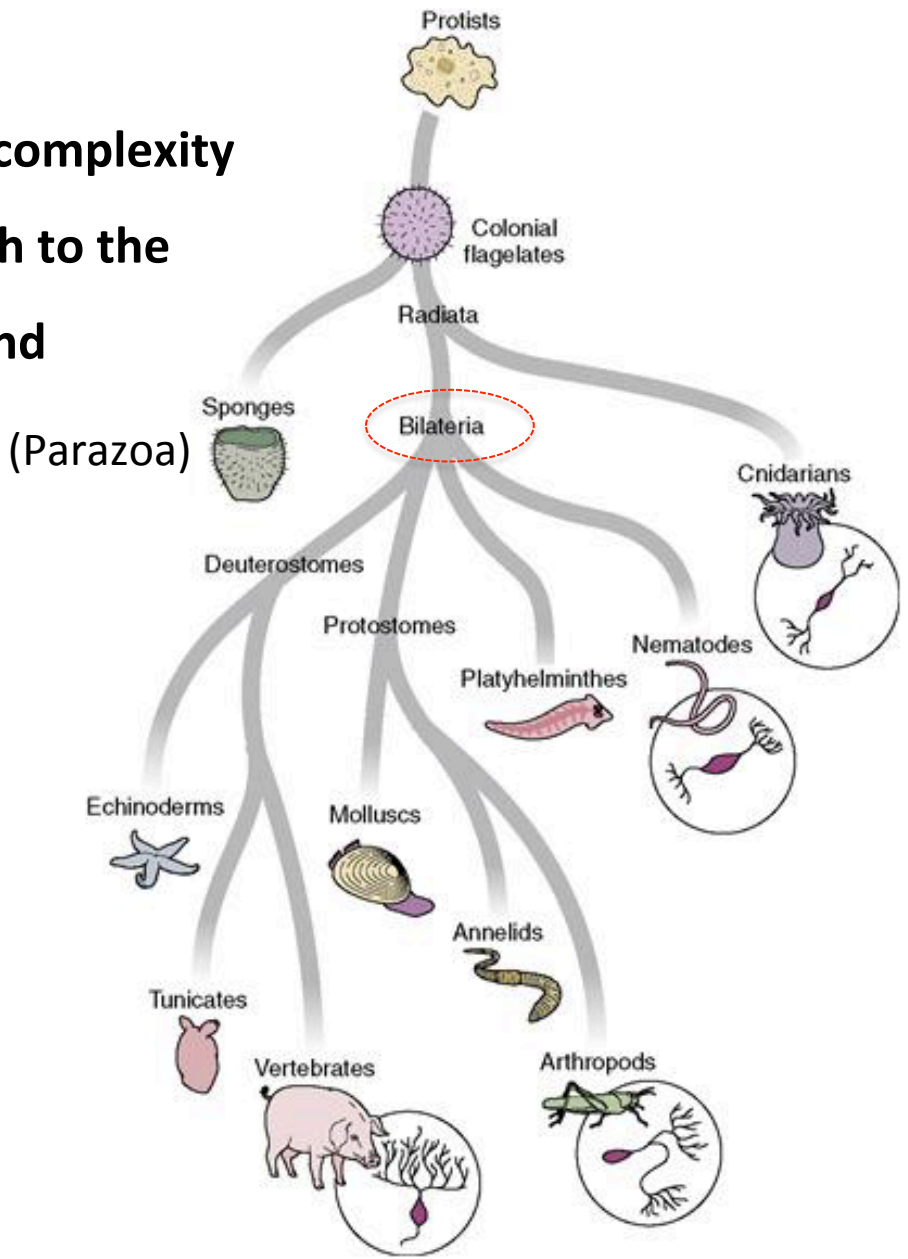
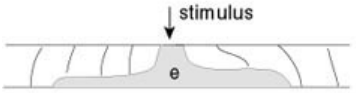
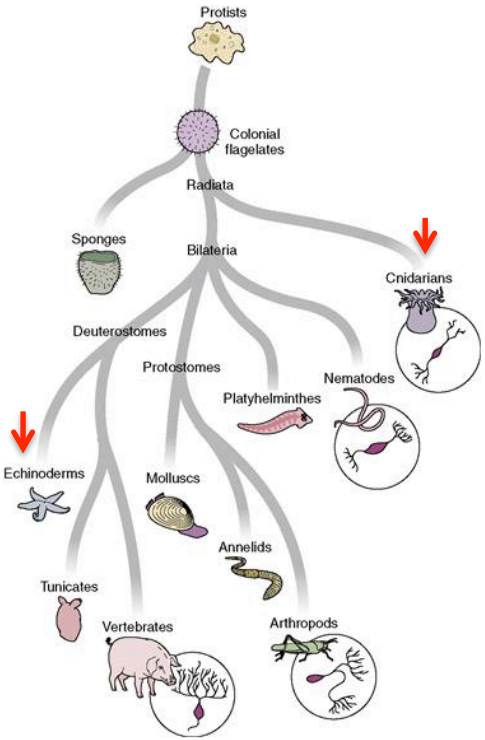
Derivation of the Neural Tissue

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

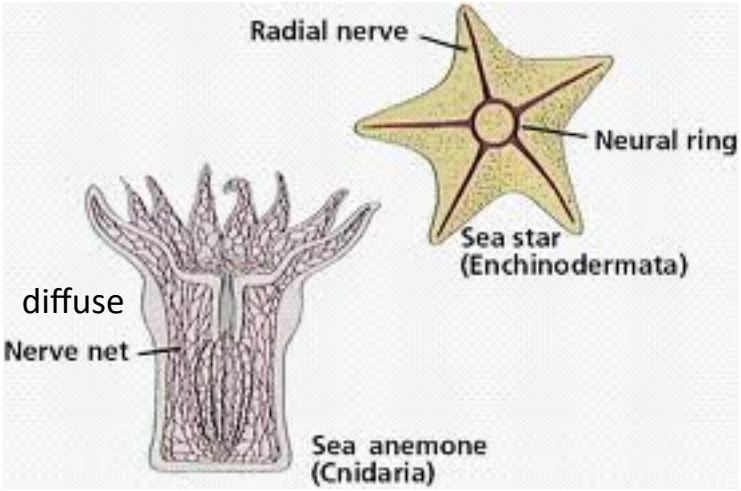


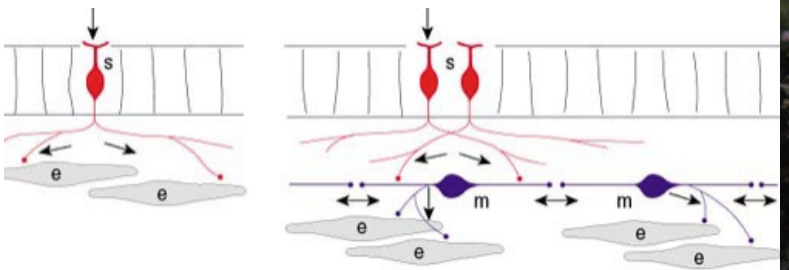
No nervous system

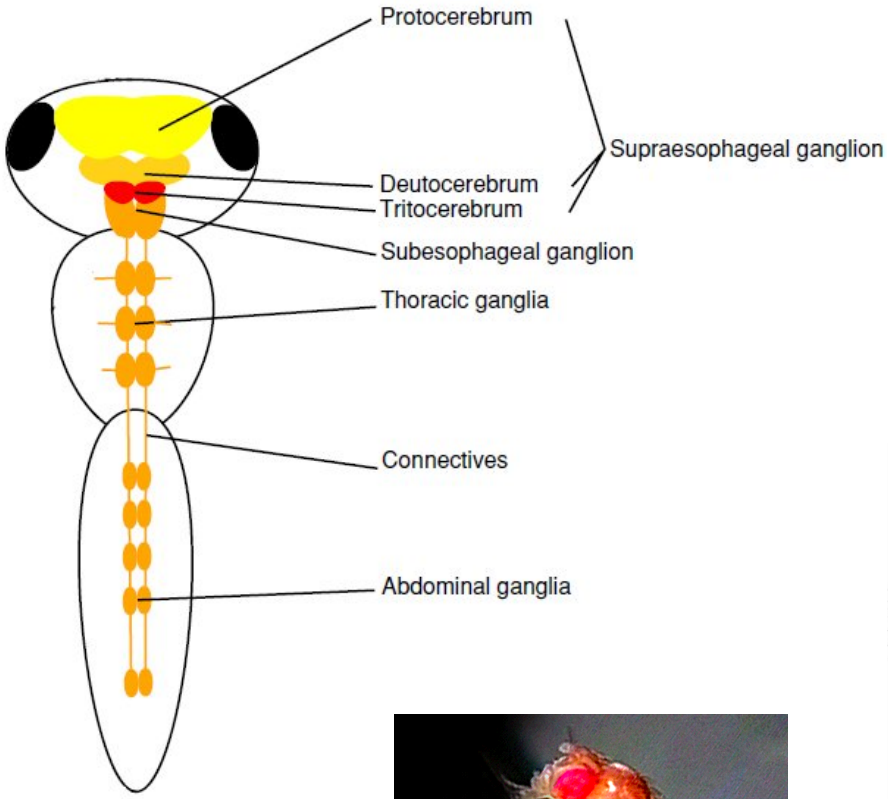




Nerve net - no ganglia - no cephalization

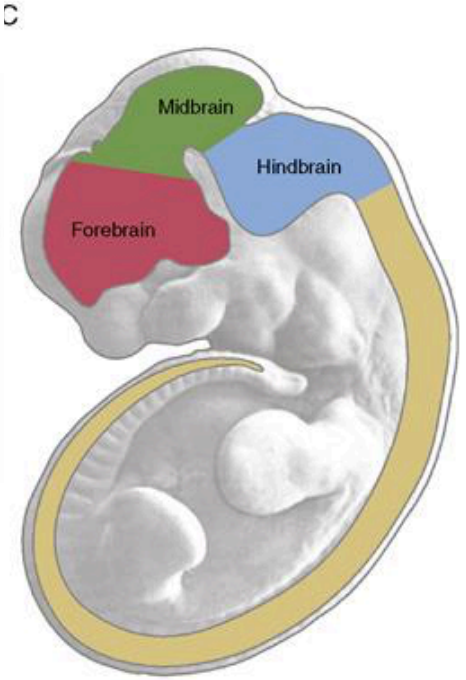






Complex nervous system
Ganglia
Cephalization

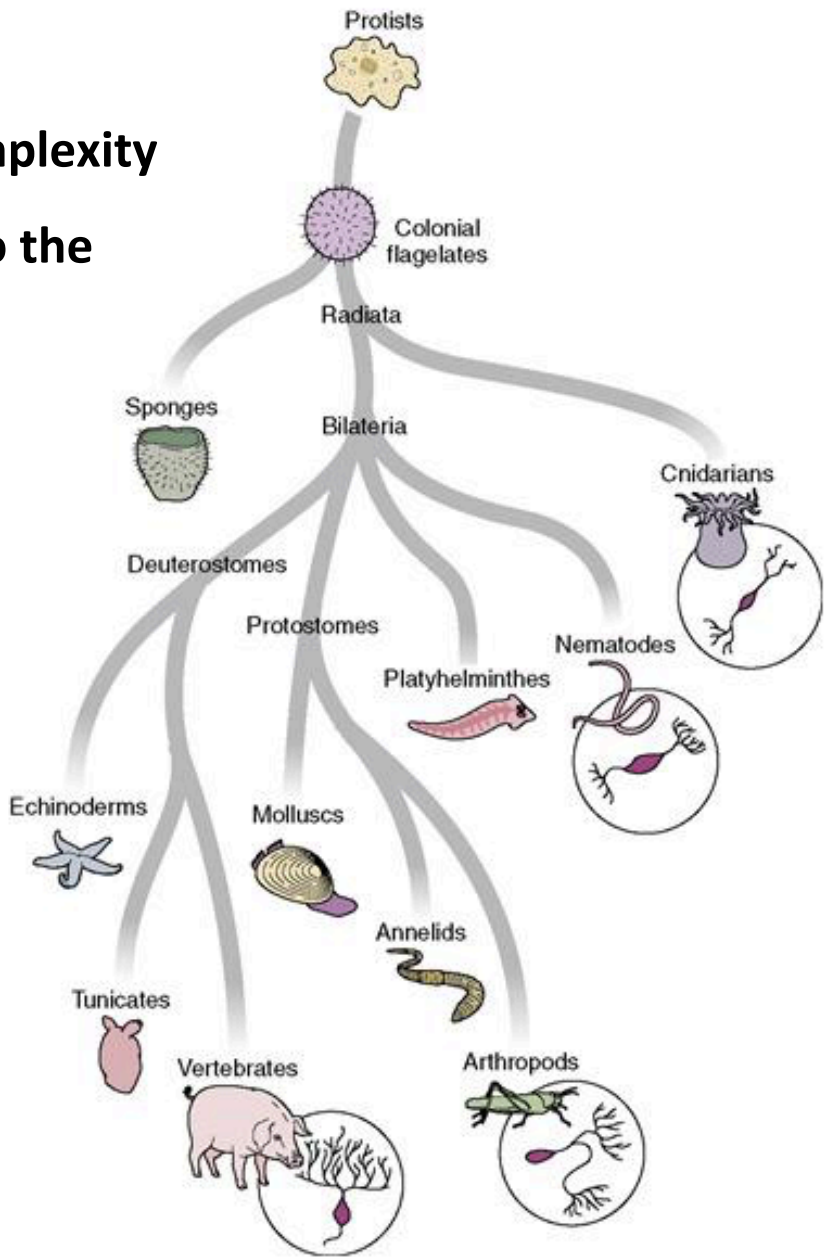
Arthropods



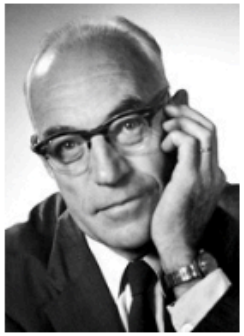
Vertebrates

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**

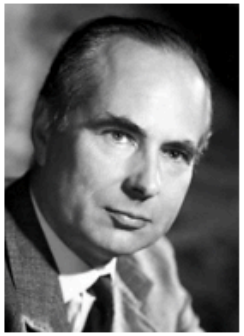


The Nobel Prize in Physiology or Medicine 1963



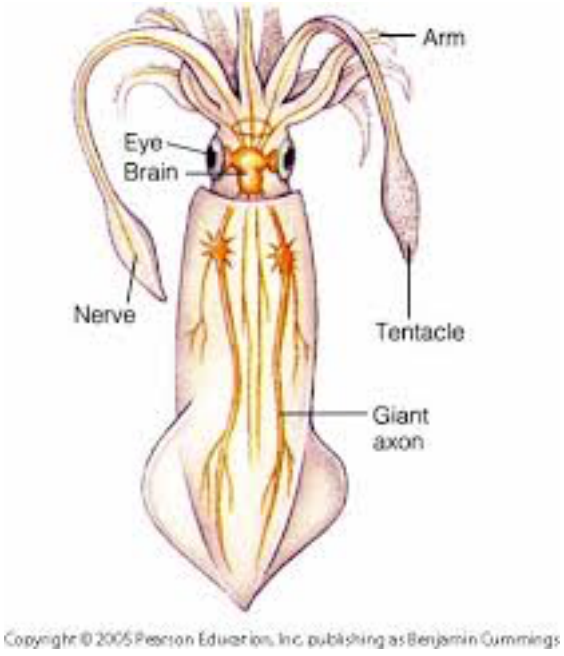
Sir John Carew Eccles
Prize share: 1/3

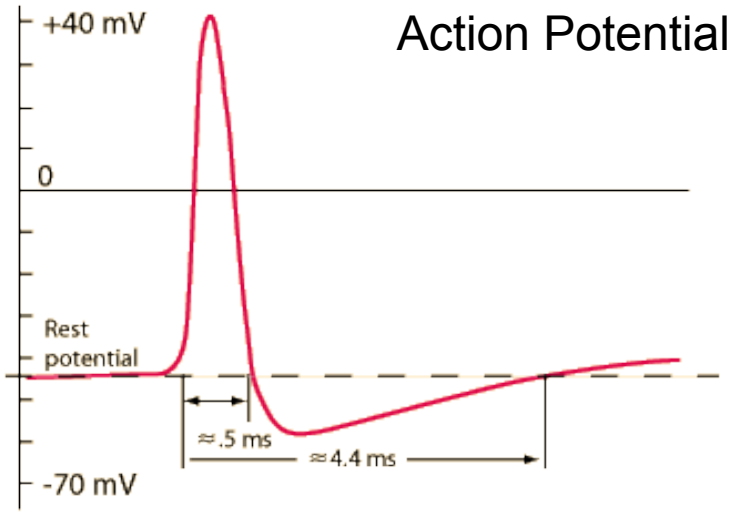
Alan Lloyd Hodgkin
Prize share: 1/3



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"

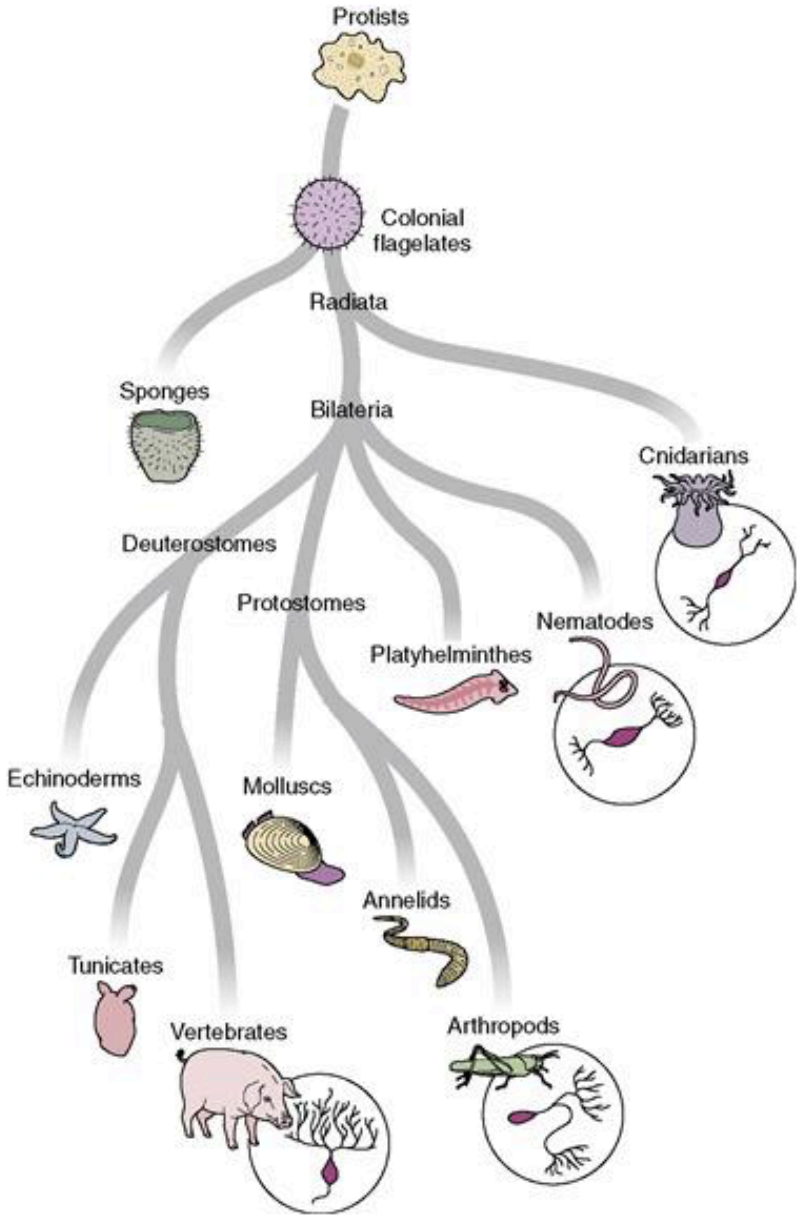
→ defined sets of genes and proteins that stabilize **synaptic connections** and trigger growth of new ones

Nobel Prize

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

The **developmental programs** that govern body plan and organization of the neural system are conserved throughout phylogeny

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



Model organisms

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:

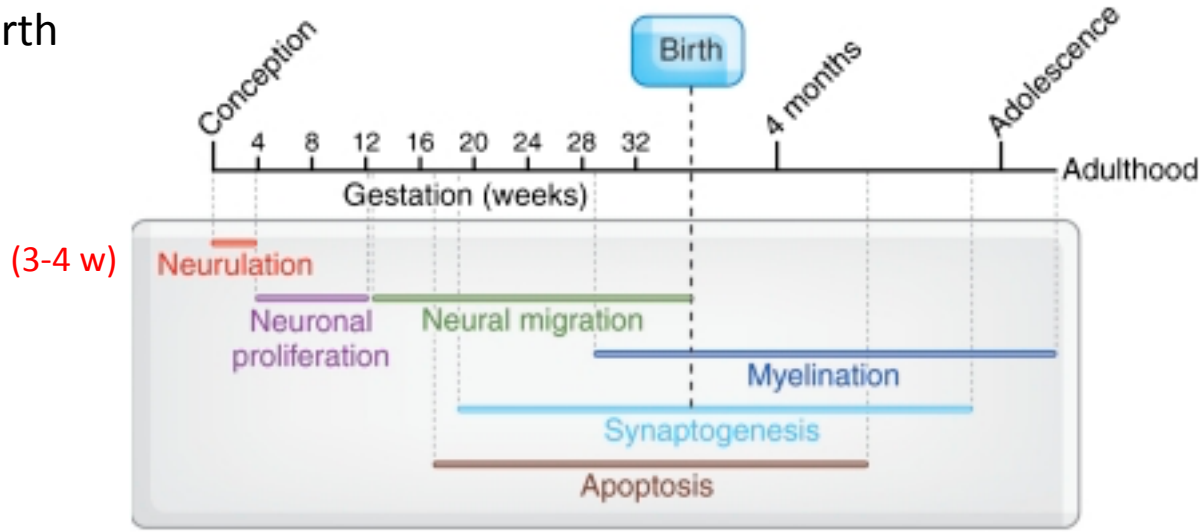
- Starts once the 3 primary germ layers are established
- Involves the **segregation of neural cells from other cell types**
- Involves the generation of neural precursor cells - mitotically active

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

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



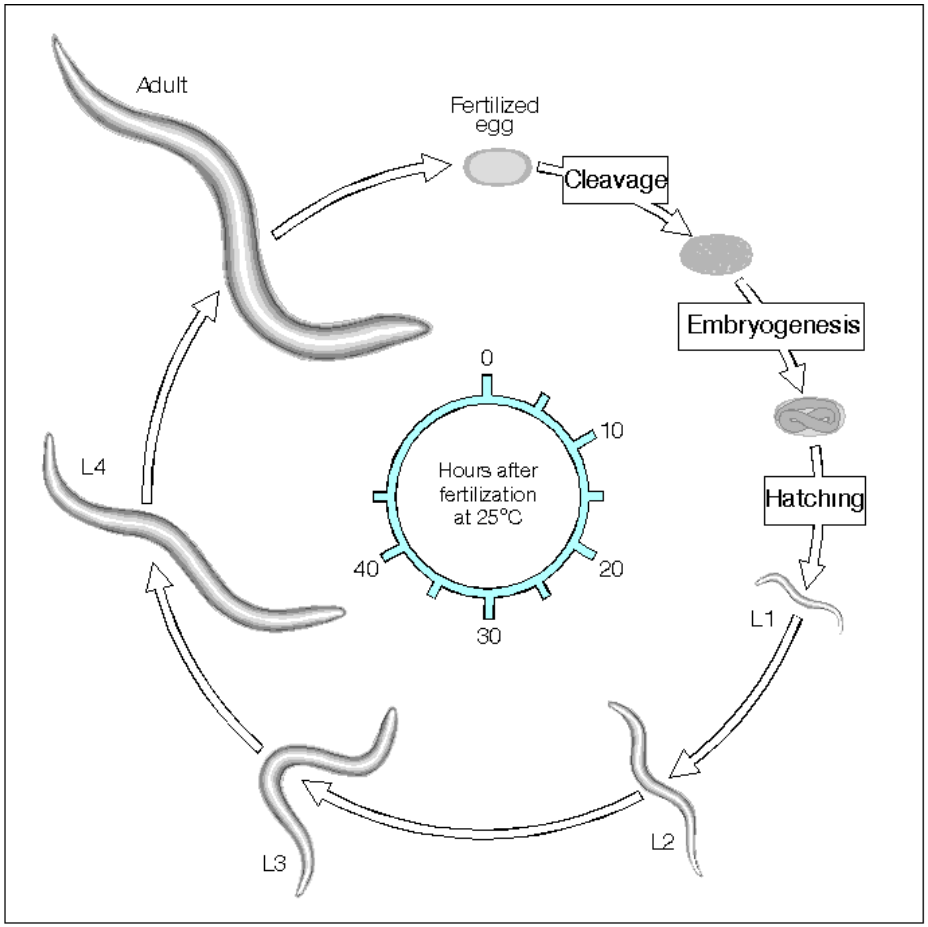
1. The neural tissue derives from the ectoderm*

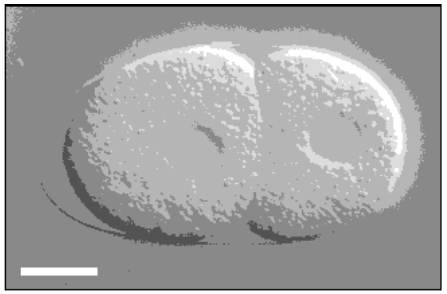
Two examples in invertebrates:

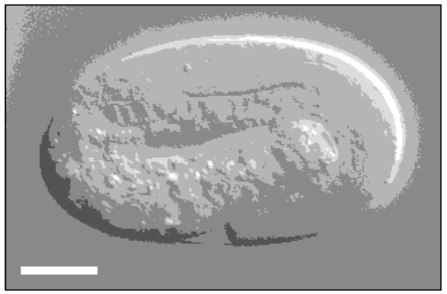
- *C. elegans*
- *Drosophila*

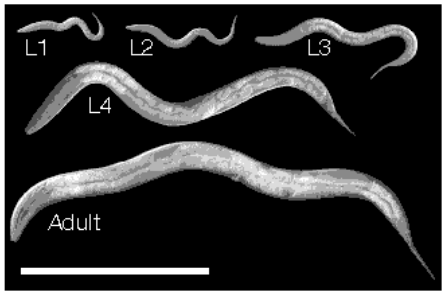
**in c. elegans a few neurons/glia cells derive from the mesoderm*

Caenorhabditis elegans (*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 videos

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

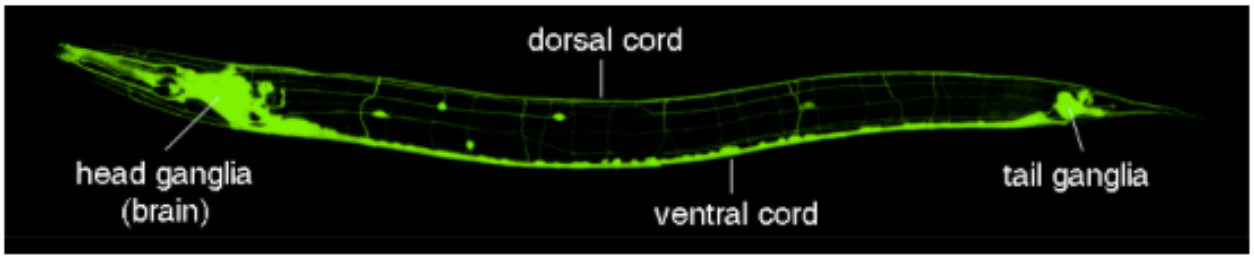
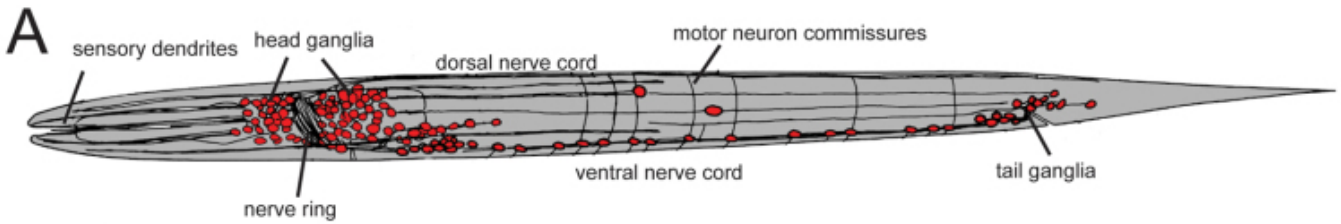


Figure 1: *C. elegans* nervous system: all neurons labelled with a fluorescent marker (GFP)

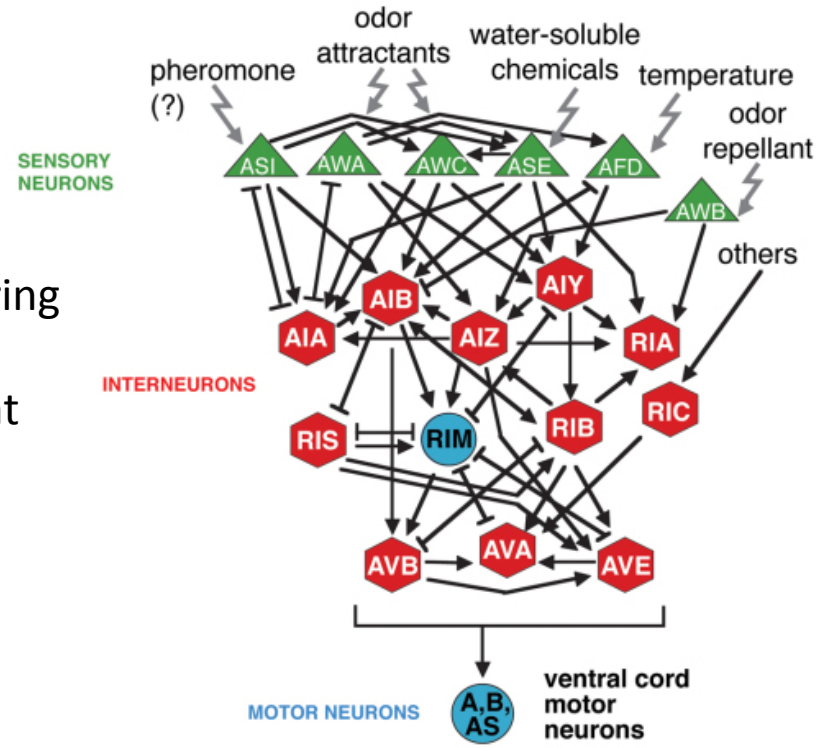
The nervous system is identical between individuals



1° larval stage =222 neurons

*hermaphrodite (383 in males)

B



complex pattern of wiring

(integration of different sensory modality)

→ Chemical synapses

⊣ Electrical synapses

Combinatorial gene expression pattern → identifiers of neuronal terminal fate

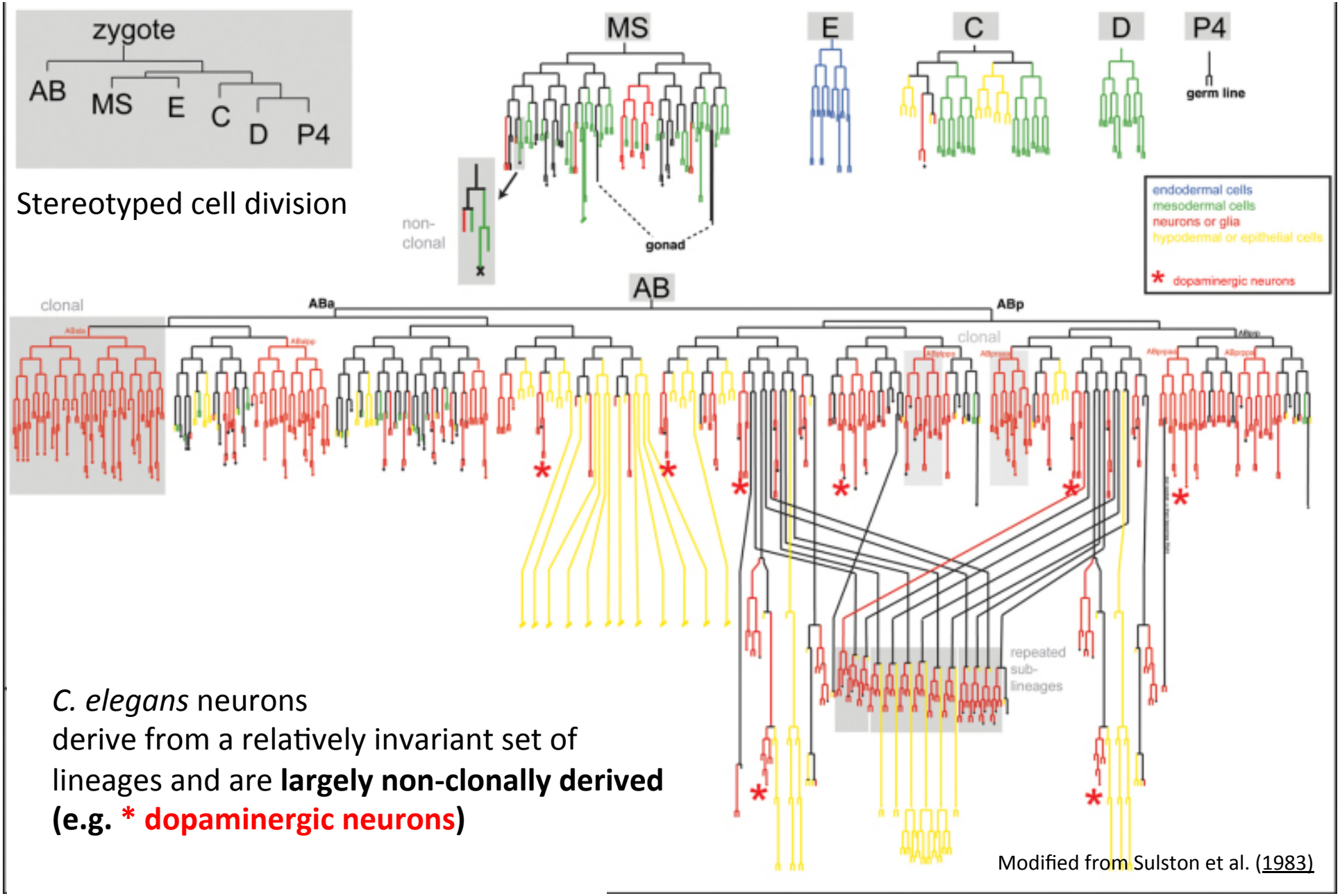
		AIY	CAN	ADL	RID	AIZ	RME	AIA	SIA	ASE
Transcription factor	*ceh-10*	1	1	0	1	0	1	0	0	0
	ttx-3	1	0	1	0	0	0	1	0	0
	ceh-23	1	1	1	0	0	0	0	0	0
Terminal gene battery	*sra-11*	1	0	0	0	0	0	1	0	0
	kal-1	1	1	0	1	1	0	0	0	0
	hen-1	1	0	0	0	0	0	0	0	1
	unc-17	1	0	0	0	0	0	0	1	0
	ser-2	1	1	0	1	1	1	0	1	0

1 = gene expressed
0 = gene not expressed

*Individual neuron types are defined not by the expression of unique terminal differentiation genes, but by the expression of **a unique combination** of more broadly expressed genes*

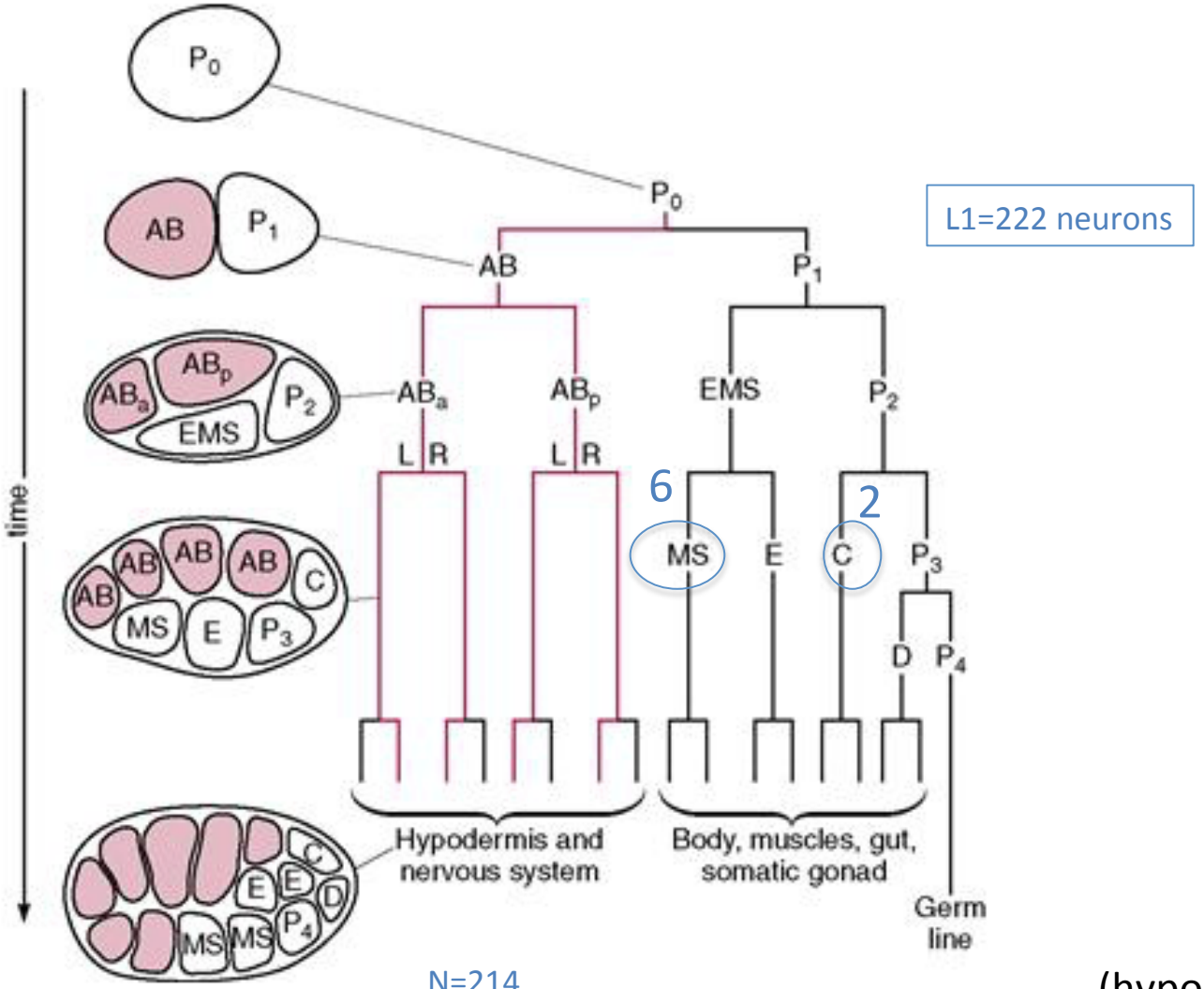
…differential levels of gene expression may also be instructive

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)



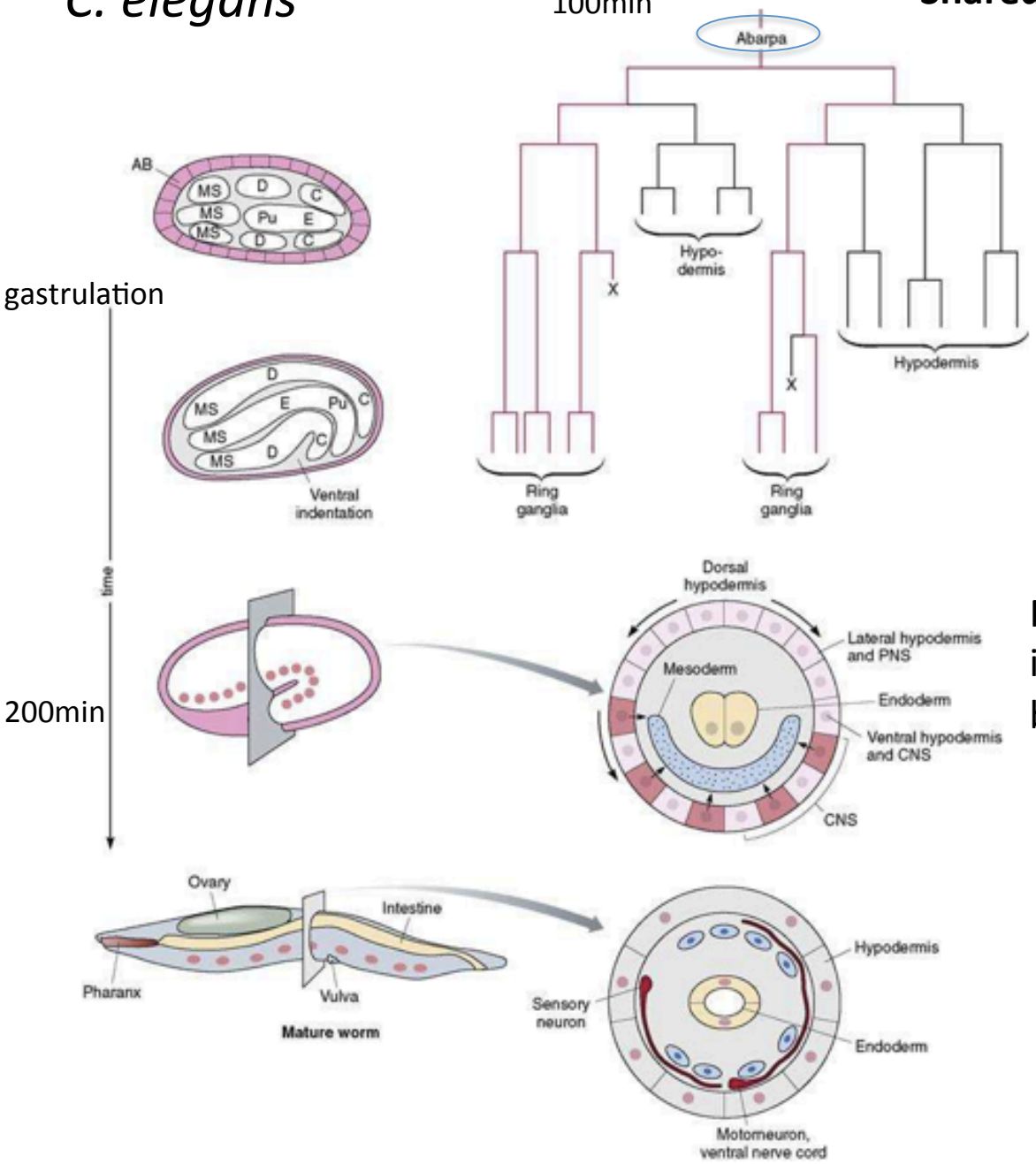
L1=222 neurons

N=214

(hypodermis→epidermis)

C. elegans

Shared lineage of hypodermis and neurons

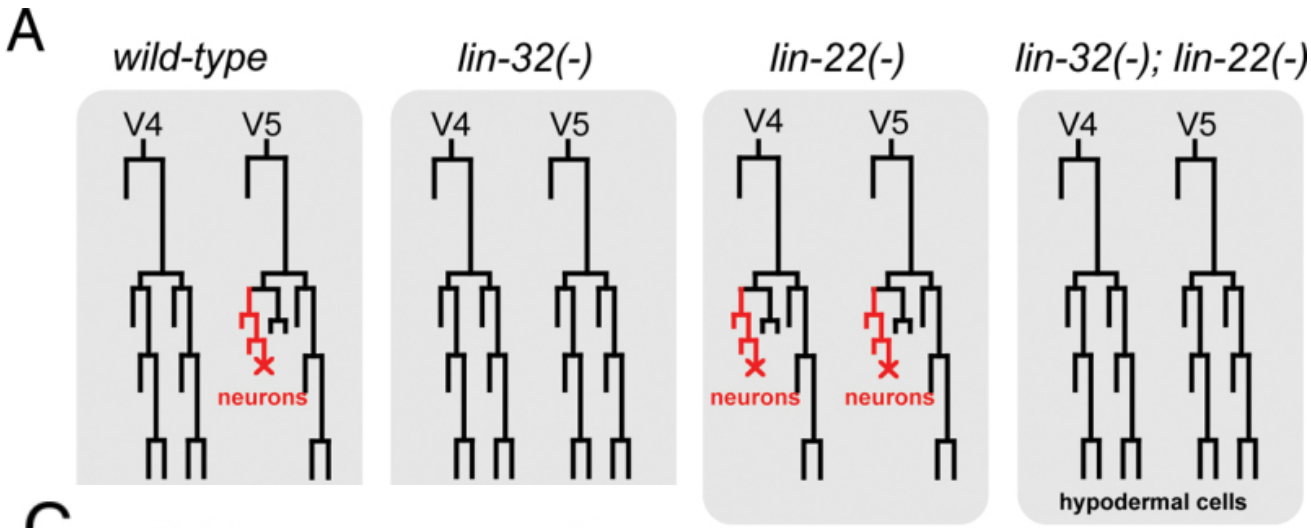


Abarpa progeny:

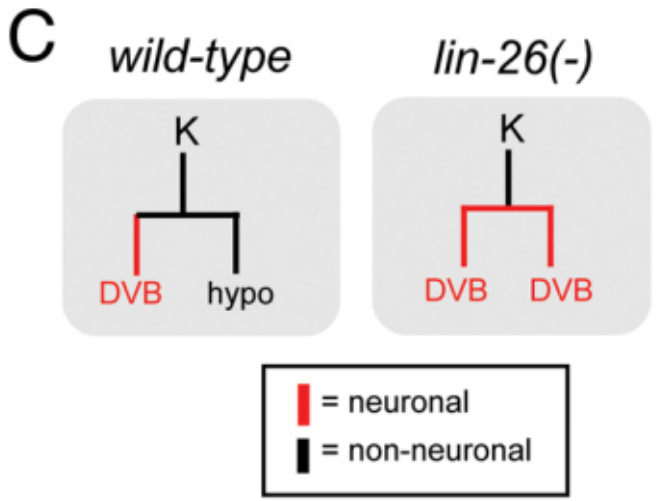
- 9 neurons
- 10 hypodermal cells

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

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



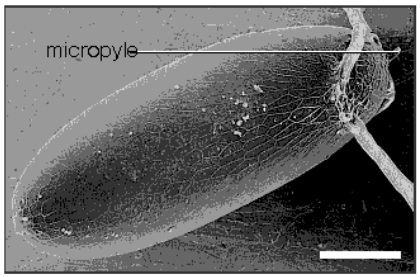
Cells derived from the post-embryonic V ectoblasts lose their neuronal fate in lin-32 mutants transforming in hypodermal cells – or transform into neuronal fates in lin-22 or lin-26 mutants

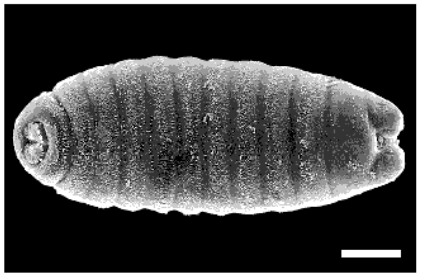


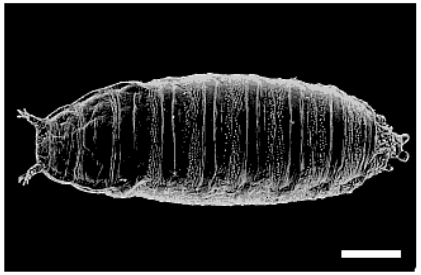
neuronal fate may be the "default" specification program in many lineages that is modified through the action of specific gene products

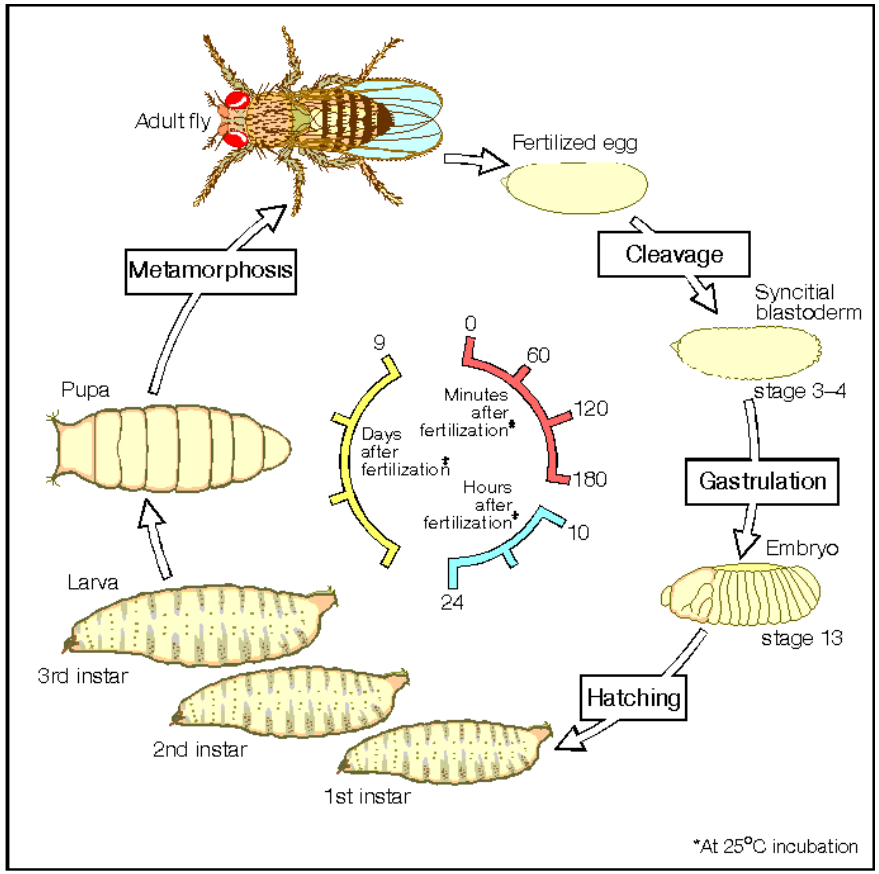
Lin-32* has a proneural function
Lin-22/Lin-26* have an anti-neural function

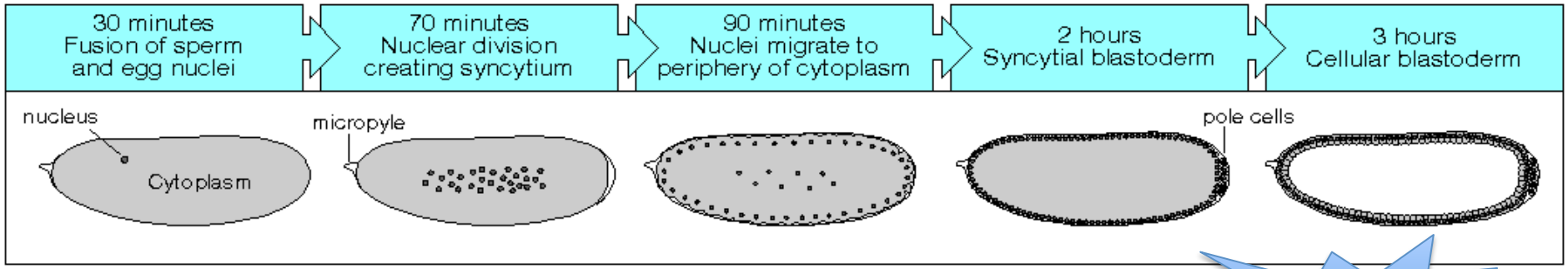
*TFs







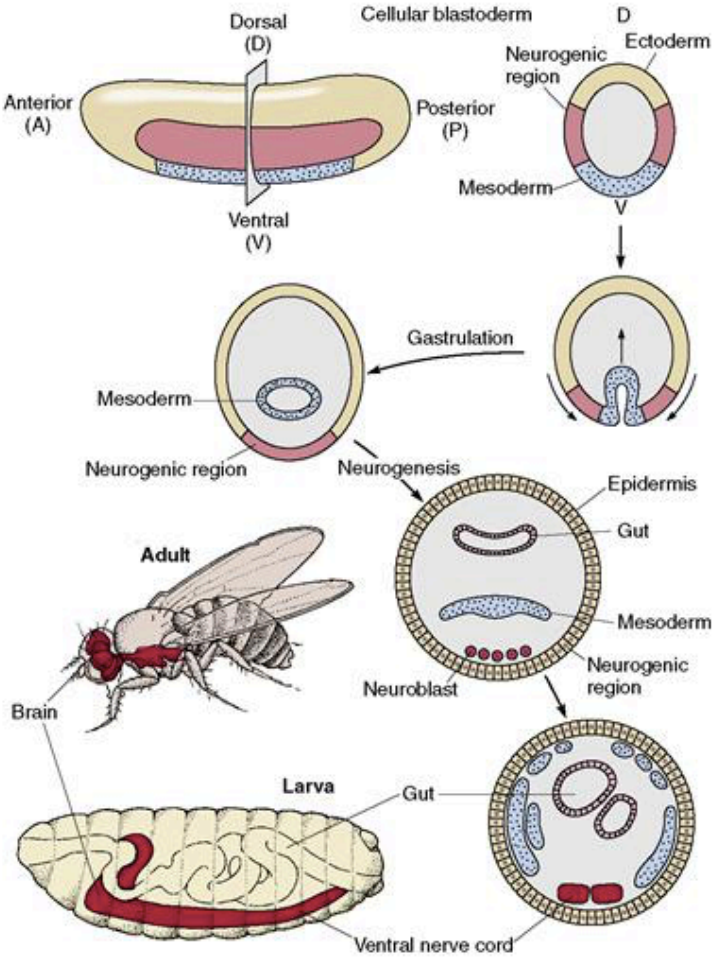




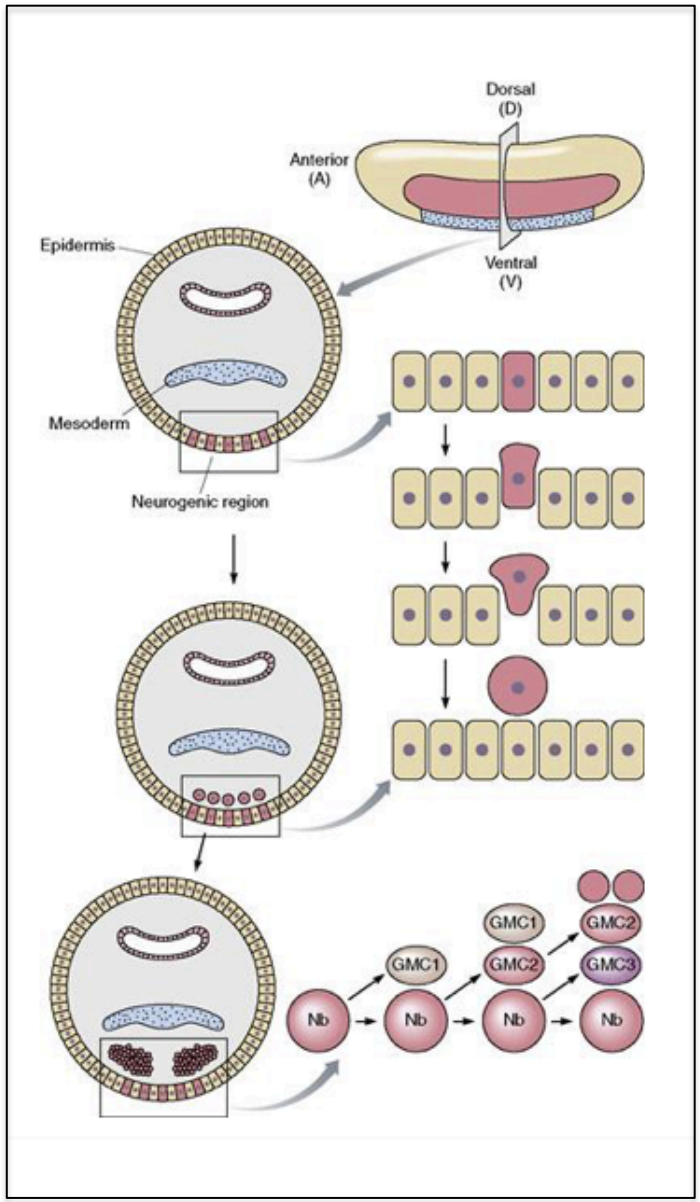
Short life cycle – 12 days

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 the ventral nerve cord (CNS)
- More anteriorly, the procephalic neurogenic region gives rise to the cerebral ganglia



Single cells 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)**

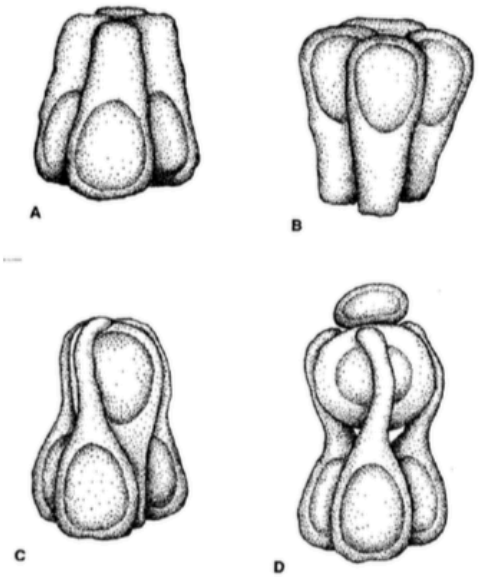
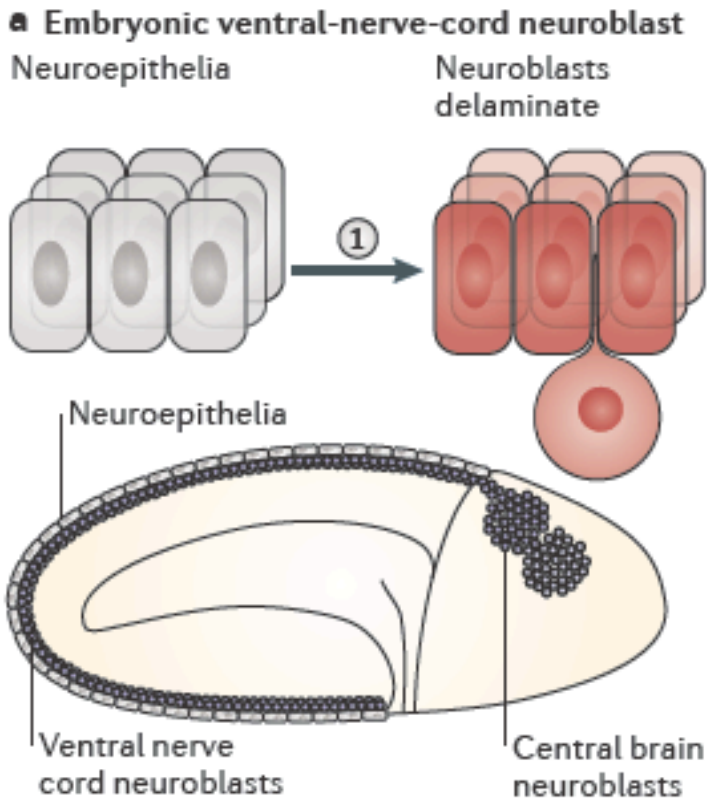


Fig. 1. Morphological differentiation of neuroblasts. The development of NB 4-2 is drawn based on camera-lucida tracings of embryos viewed with Nomarski optics. (A) Ectodermal cells at the 4-2 position have a uniform columnar morphology, with the nuclei located at the ventral surface of the cells. (B) 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.

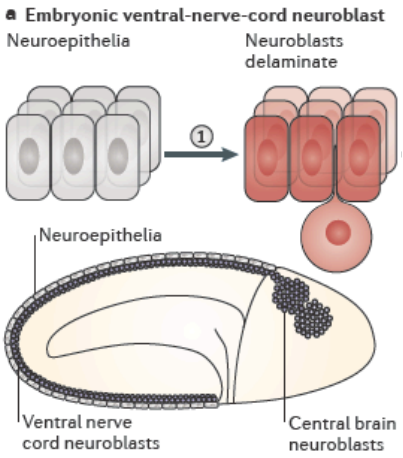
Doe, Development 1992

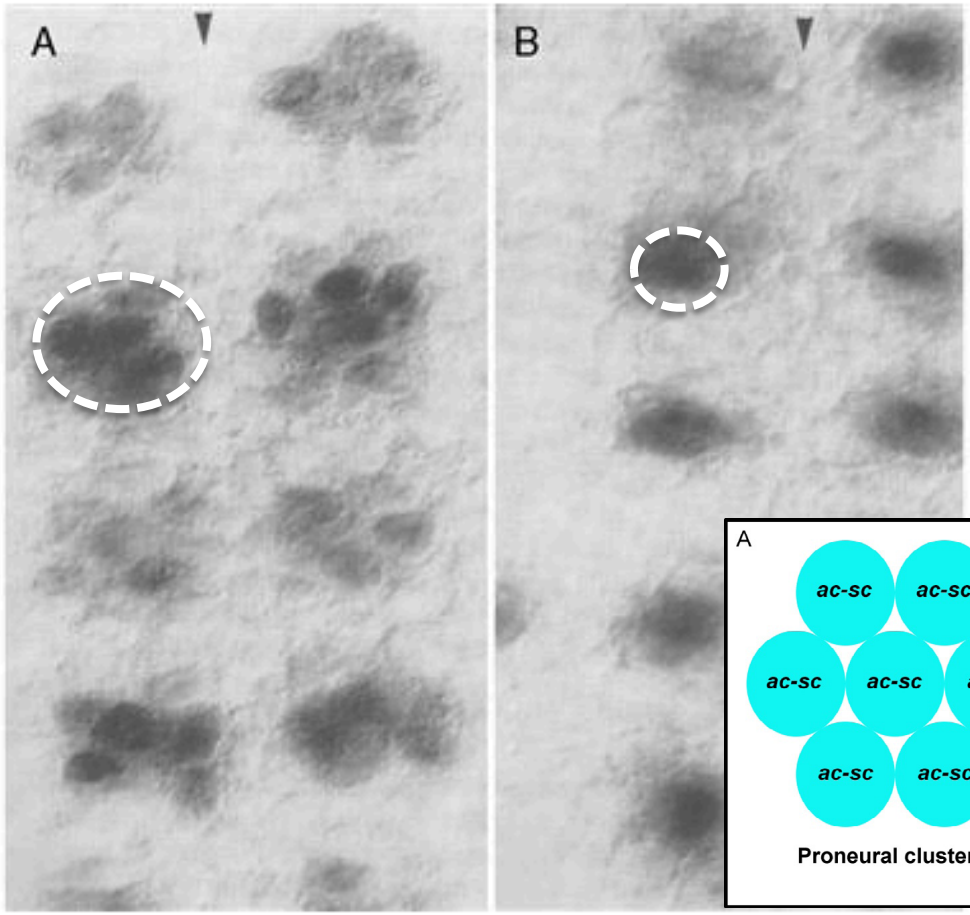
Once inside the embryo the Nb undergo a **stereotyped pattern** of **asymmetric divisions** giving rise to ganglion mother cells (GMCs) that in turn originate neurons or glia

Interactions among the ectodermal cells in controlling neuroblast segregation



Proneural clusters→ lineage segregation





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

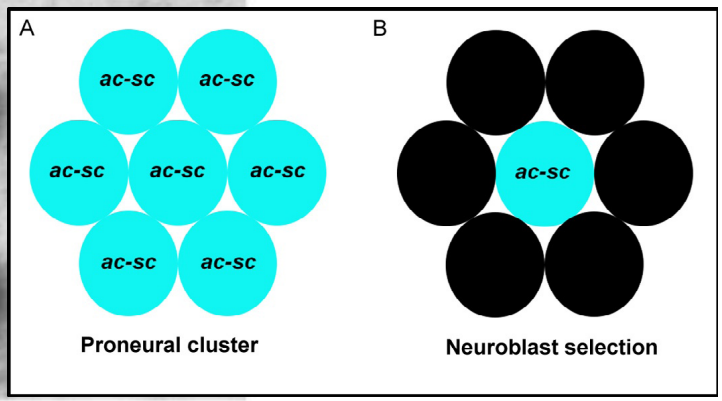
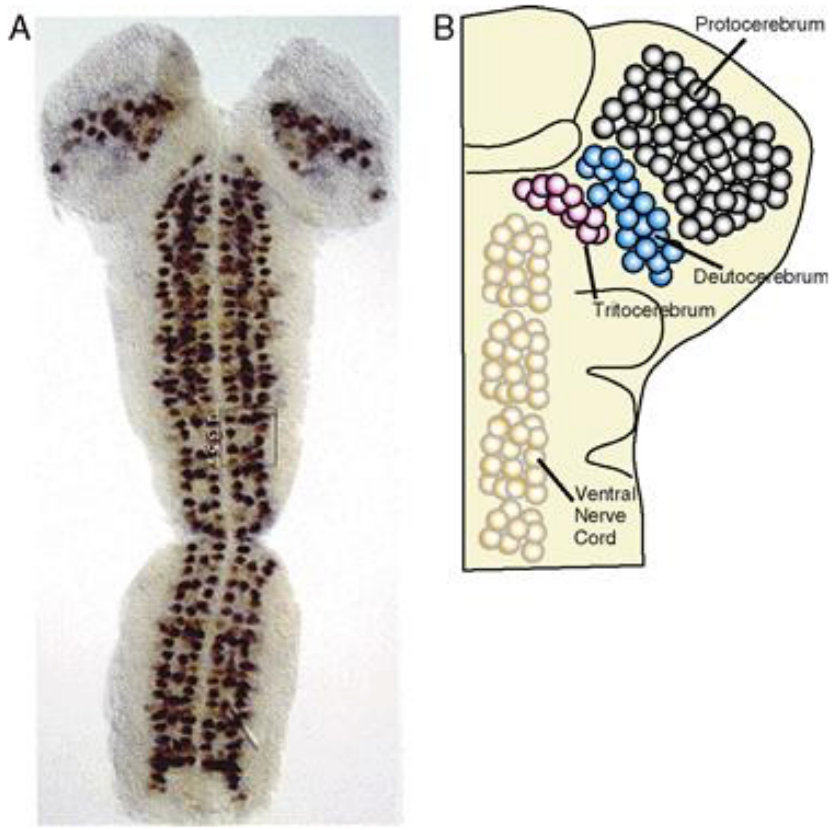


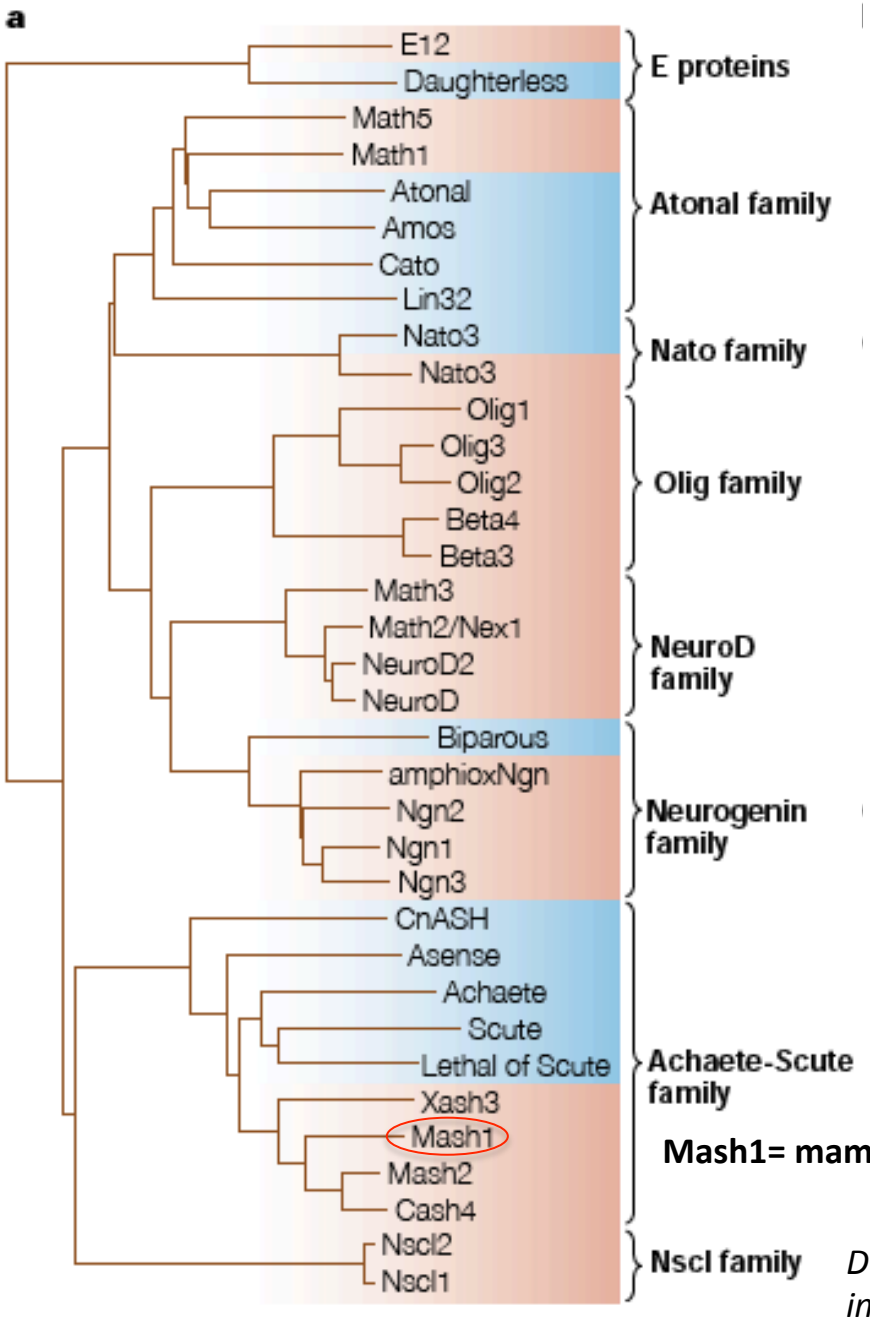
Fig. 1.23 Neuroblast segregation in the *Drosophila* neurogenic region proceeds in a highly patterned array. A. In this embryo stained with an antibody against *achaete-scute (as-c)* protein, clusters of proneural cells in the ectoderm express the gene prior to delamination. B. A single neuroblast develops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

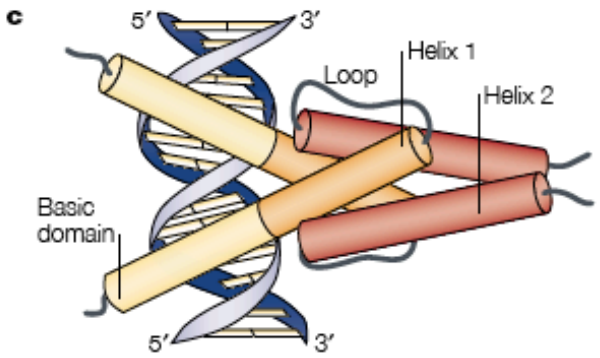


stereotyped pattern

Ventral view of a Drosophila embryo

Proneural genes
key regulators of neurogenesis





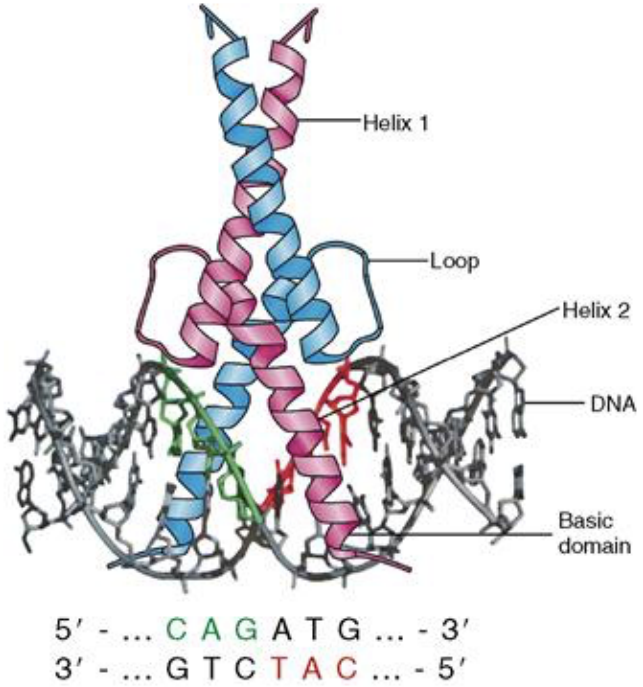
Proneural proteins bind E-box (CANNTG) DNA sequences as heterodimeric complexes formed with ubiquitously expressed bHLH proteins (E proteins)

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

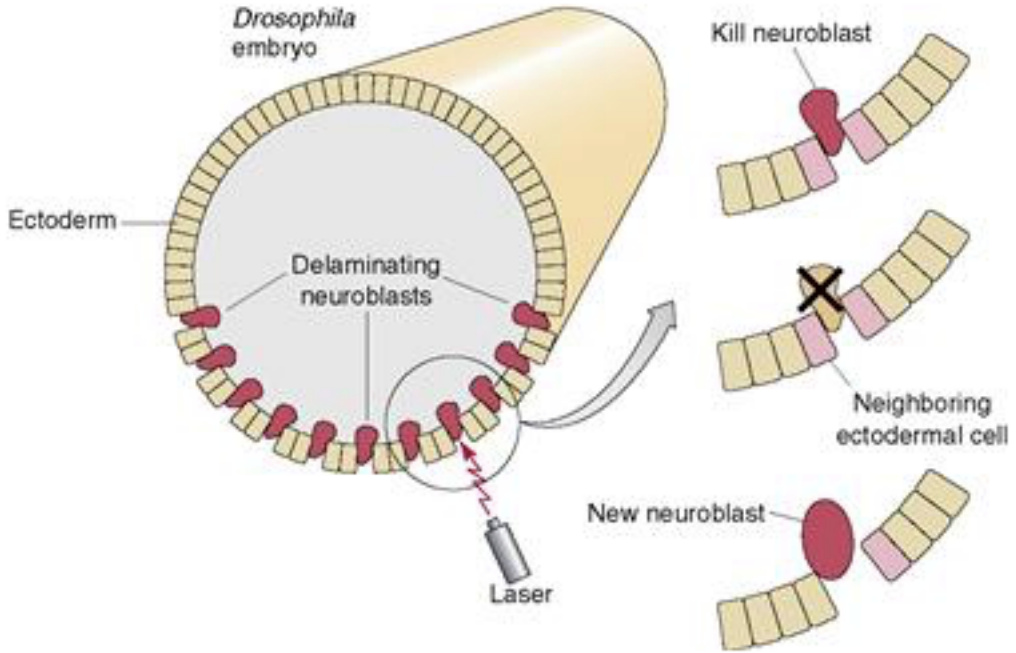


Fig. 1.26 Ablation of the delaminating neuroblast with a laser microbeam directed to the ventral neurogenic region of the fly embryo causes a neighboring ectodermal cell to take its place.

Neuroblasts are specified by cell interactions

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"

Local cell-cell interaction:

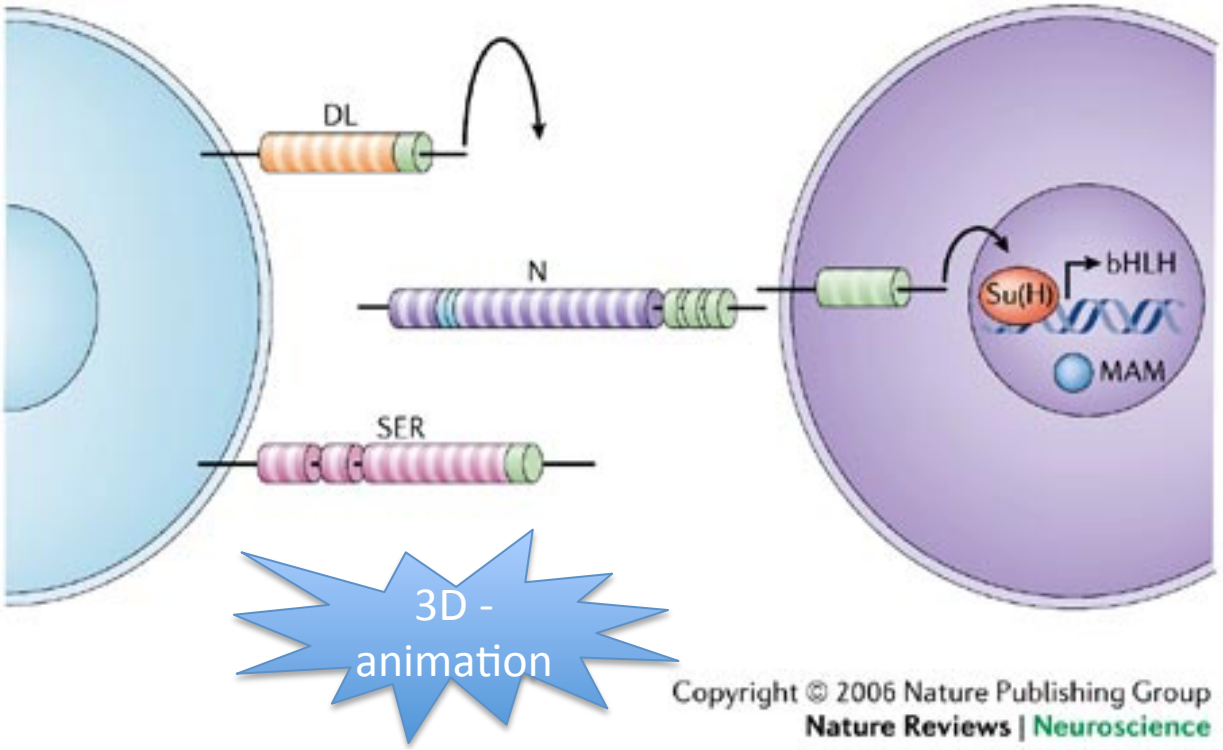
a membrane-bound receptor

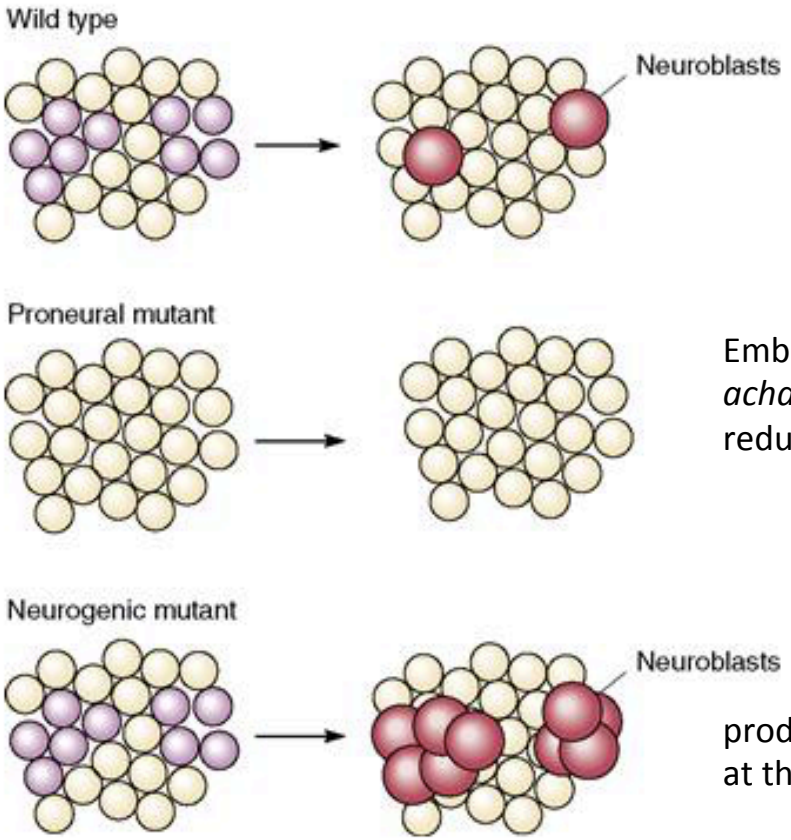
(Notch) on one cell **interacts** with

a membrane bound ligand (e.g.

Delta) on another cell

Lineage segregation





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

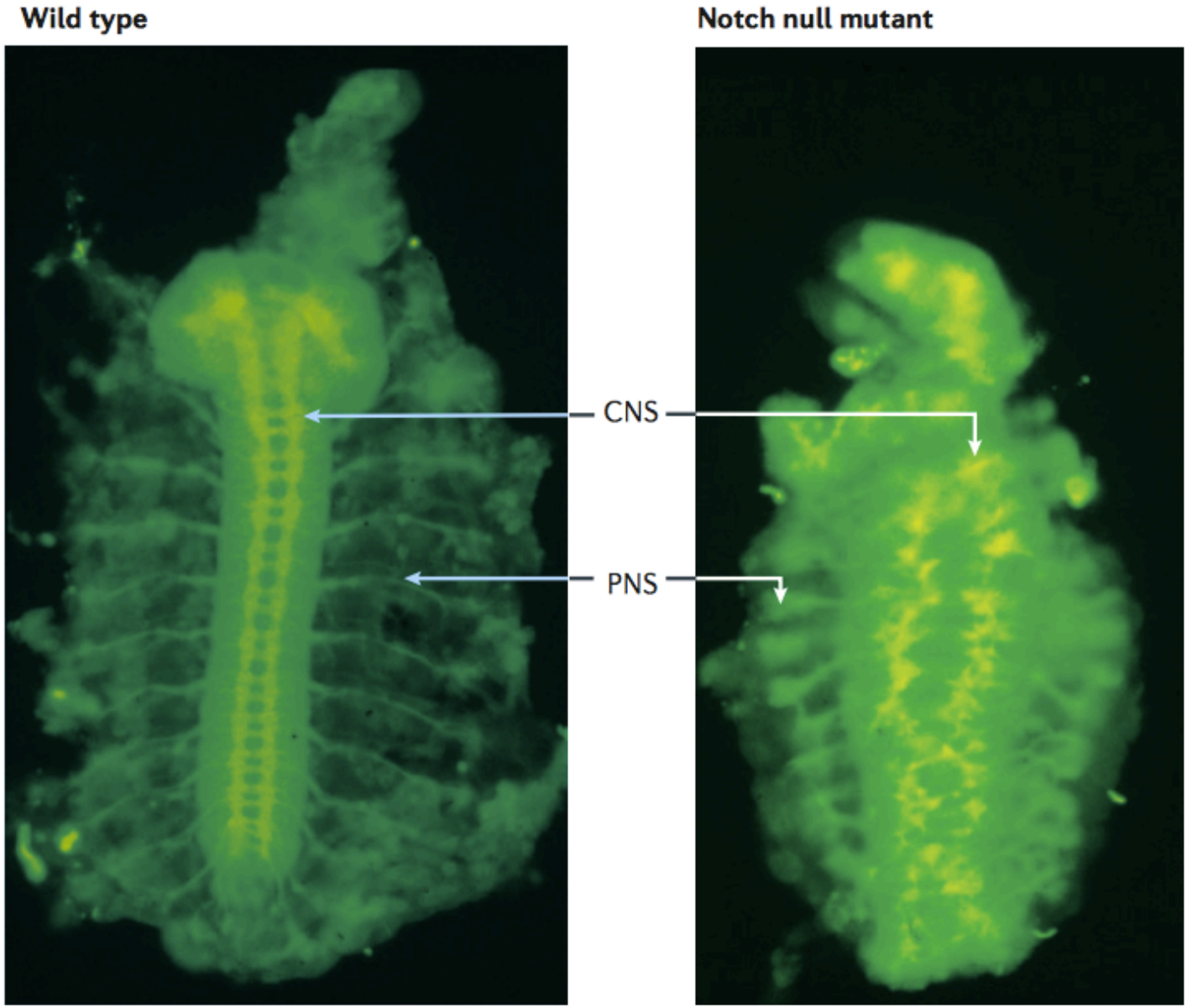
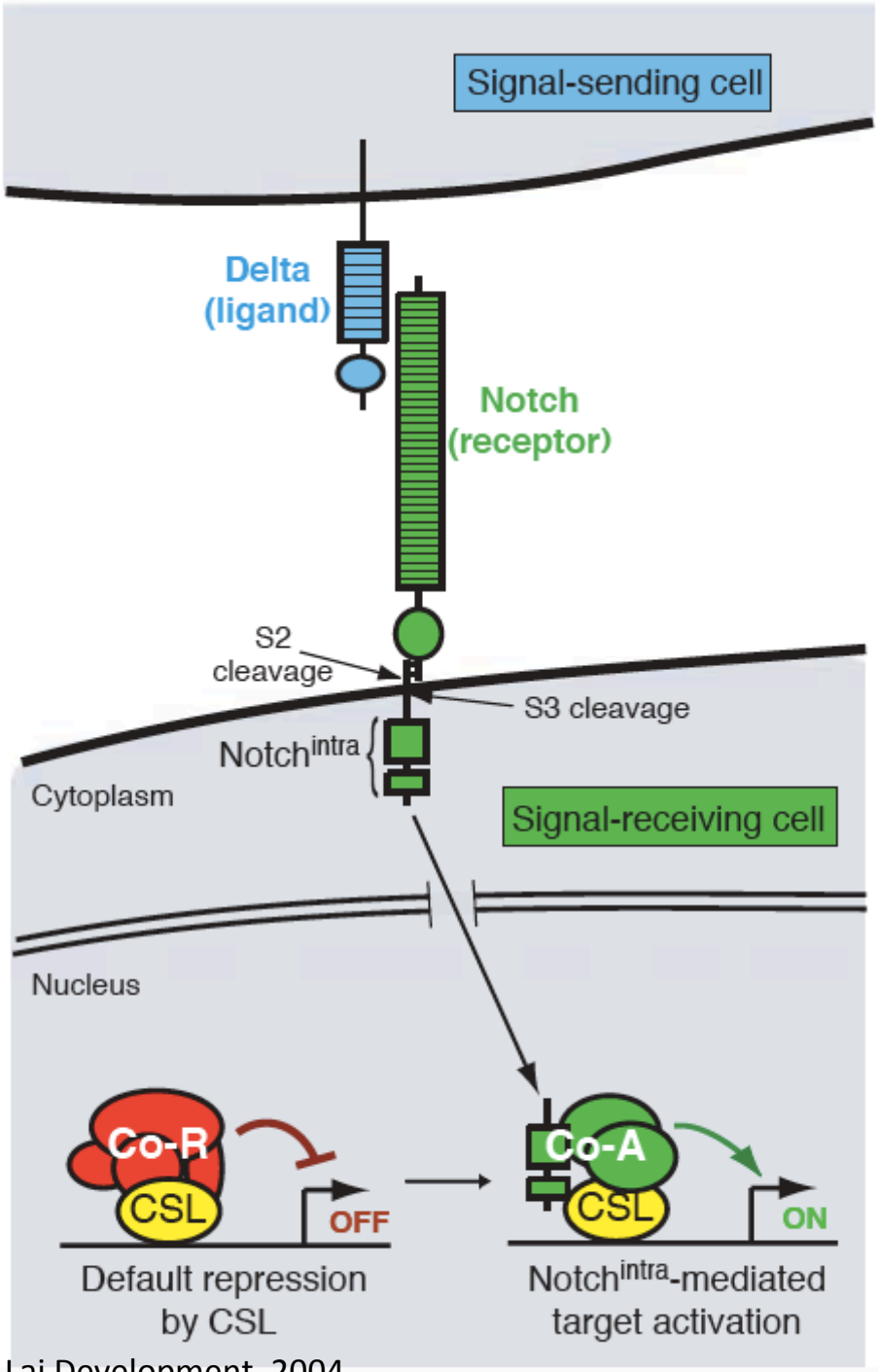


Figure 1 | ***Drosophila melanogaster* embryos stained with an antibody against horseradish peroxidase that recognizes neural tissue.** Wild-type and Notch null mutant *D. melanogaster* embryos, showing the hypertrophy of both the CNS and PNS that occurs in the absence of Notch. Image reproduced, with 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.

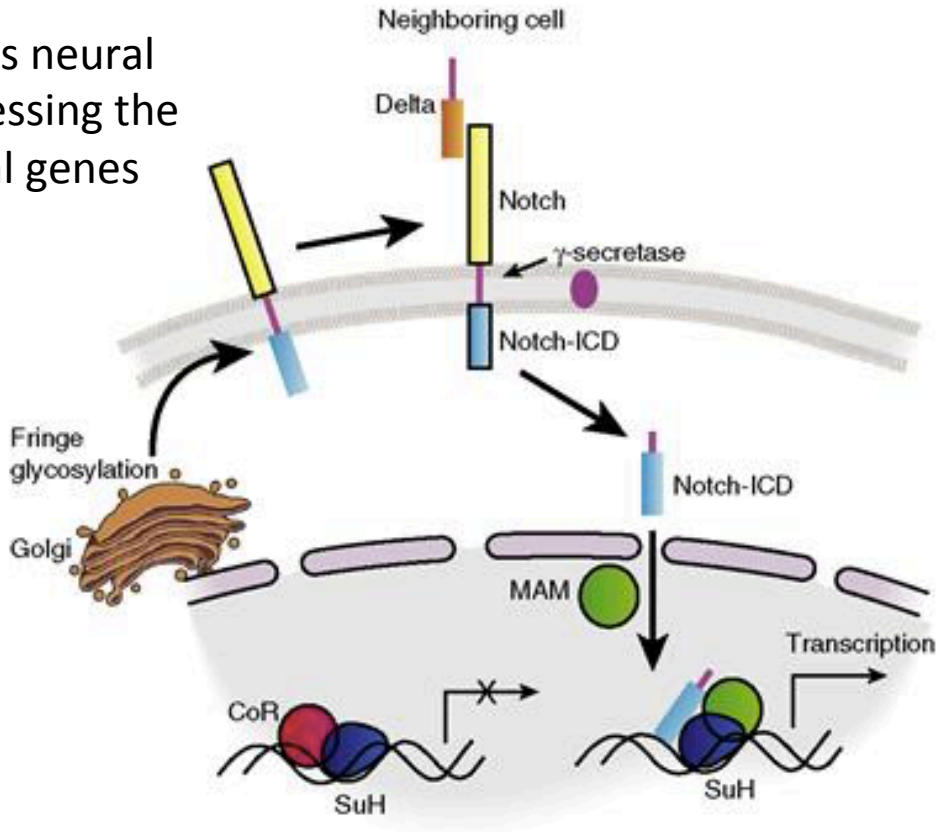
→ The CSL co-repressor complex is displaced by a co-activator complex containing Notch ICD.

CSL (CBF1, Suppressor of Hairless, Lag-1)

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



Espl complex
–l proneural genes

Drosophila

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.

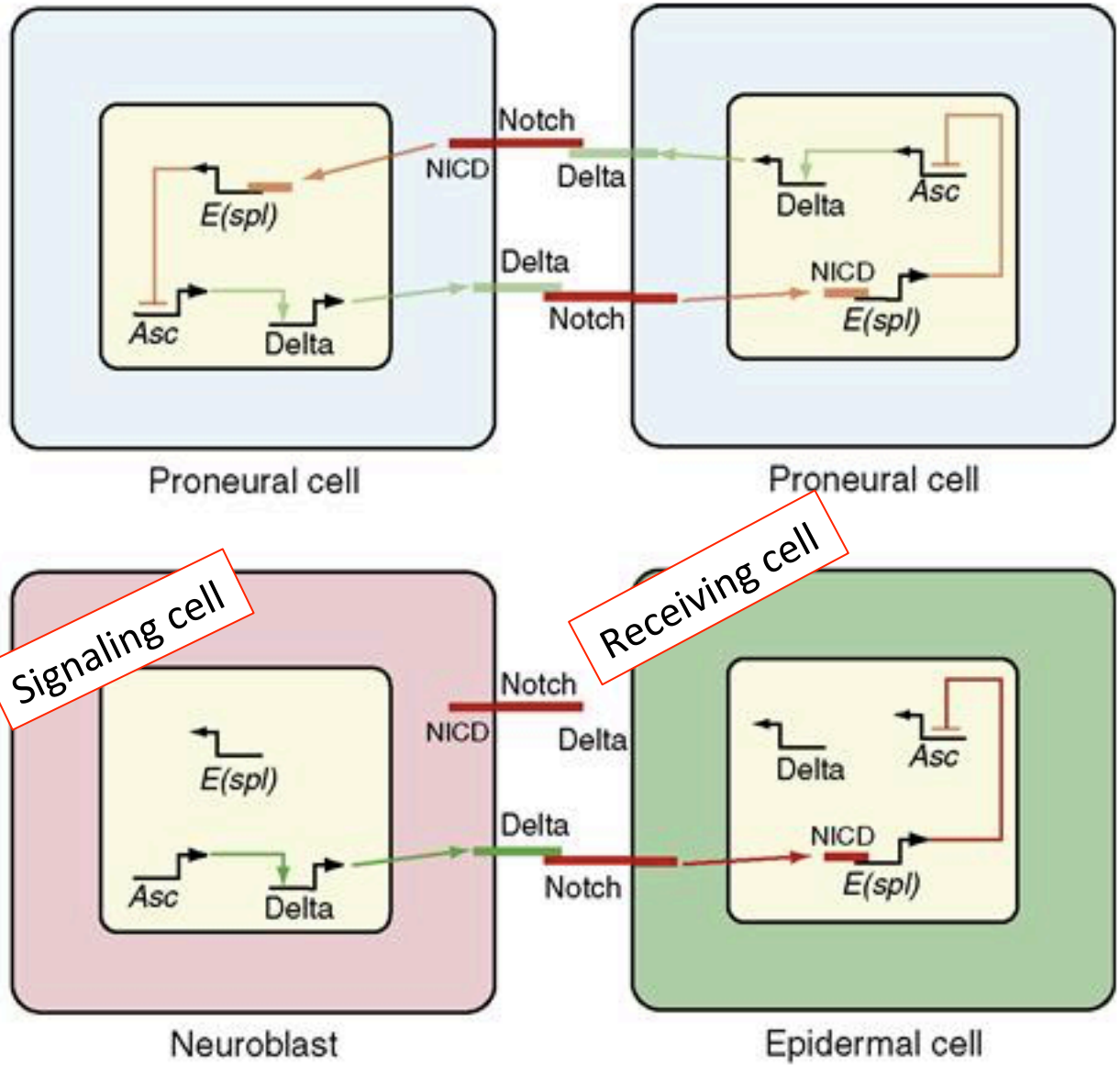
MAM = mastermind - function as coactivator of Notch signalling

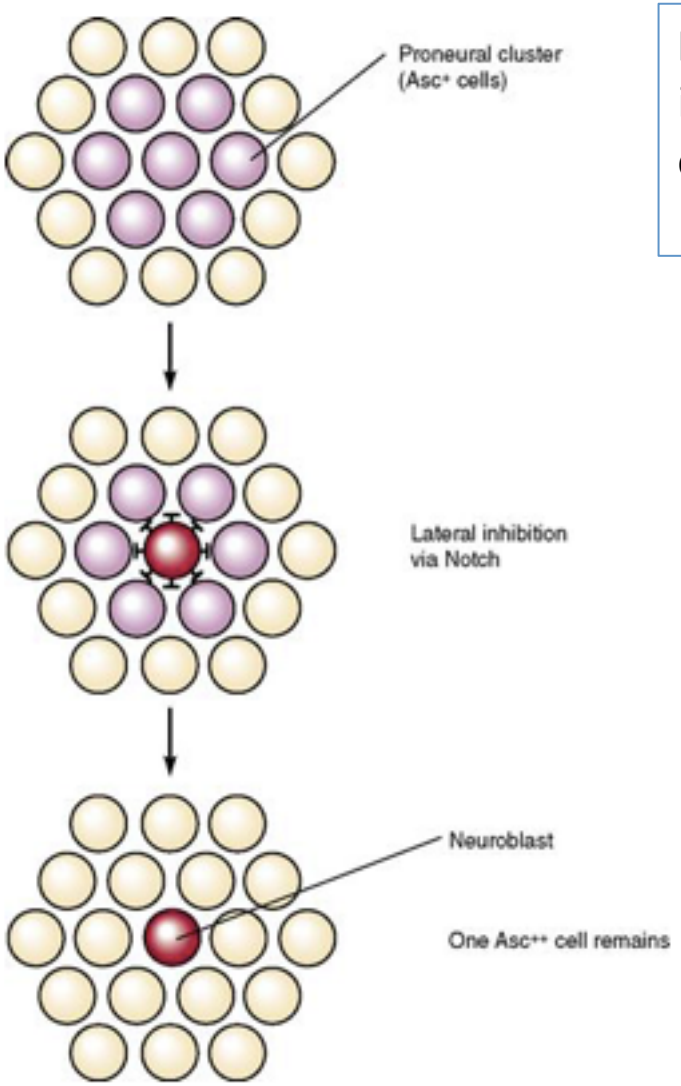
Lateral inhibition and Notch/Delta pathway

"the *Drosophila* neuralepidermal choice"

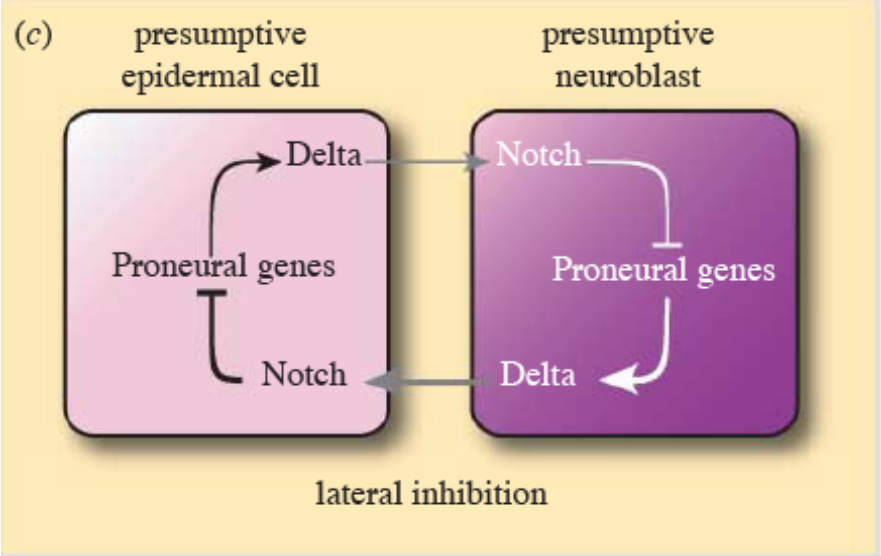
2 equivalent cells
(expression receptor and ligand)

non equivalent





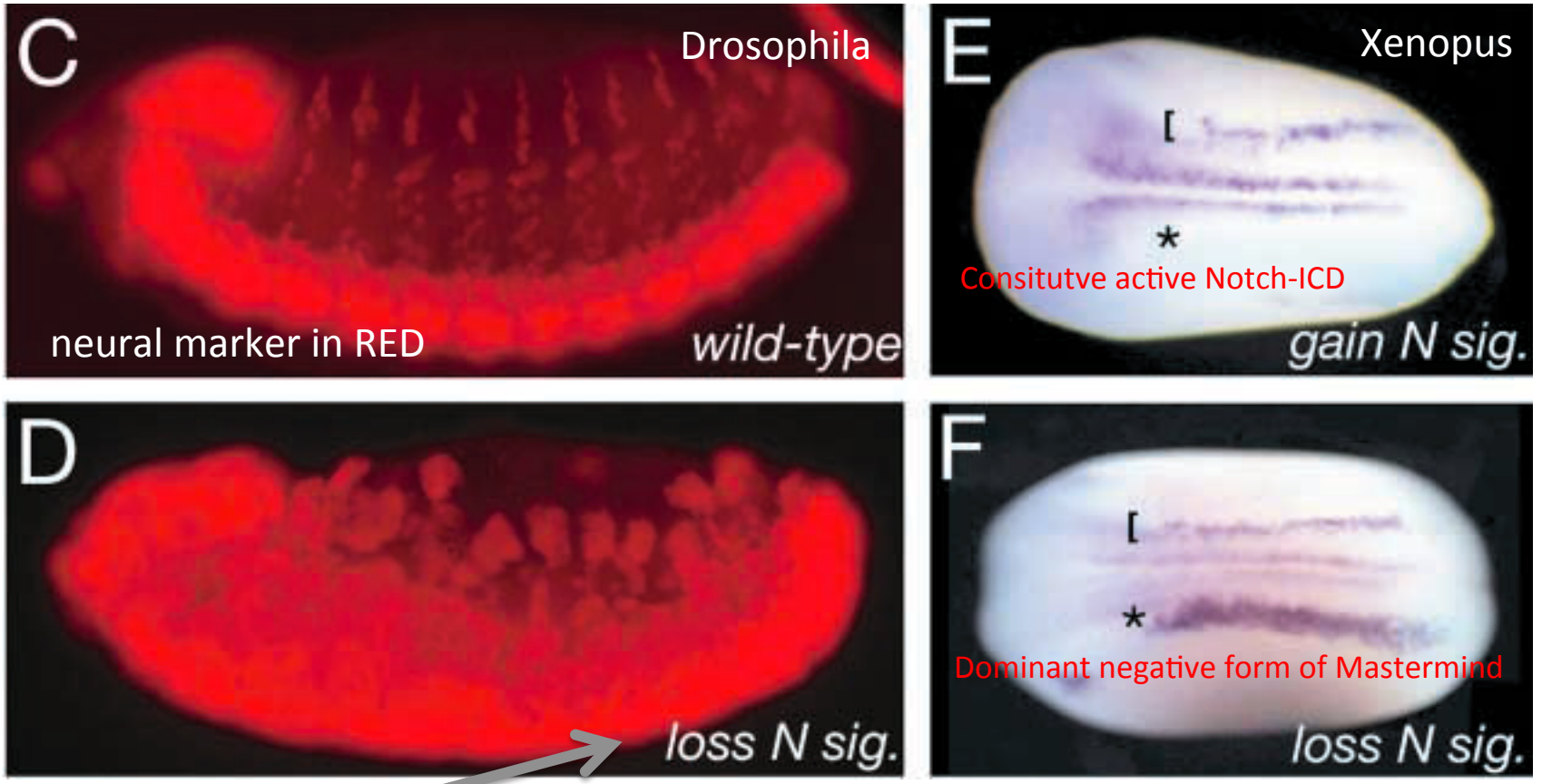
Key to the process of **lateral inhibition** in *Drosophila* is the direct and dose-dependent transcriptional **activation of the Notch receptor ligand Delta** by proneural genes

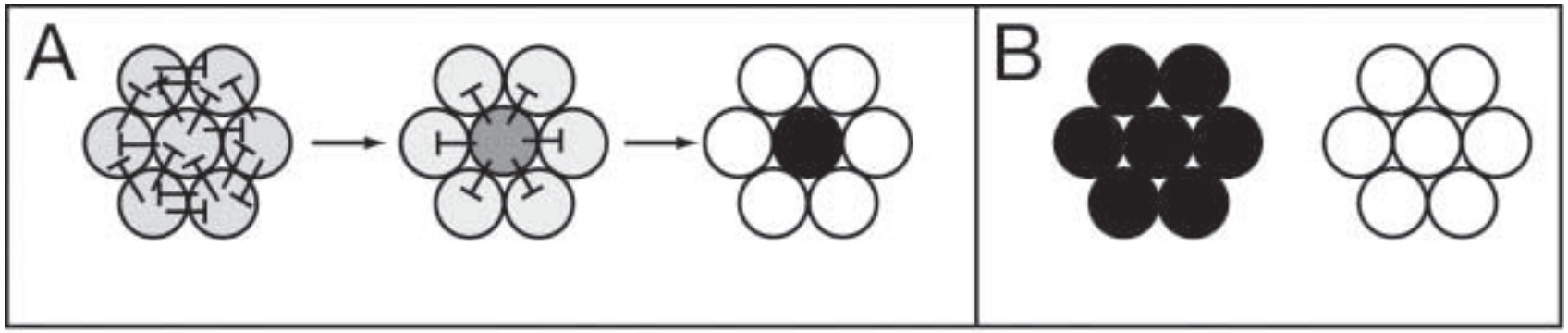


Low Notch activity→ neural fate
High Notch activity→ epidermal fate

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

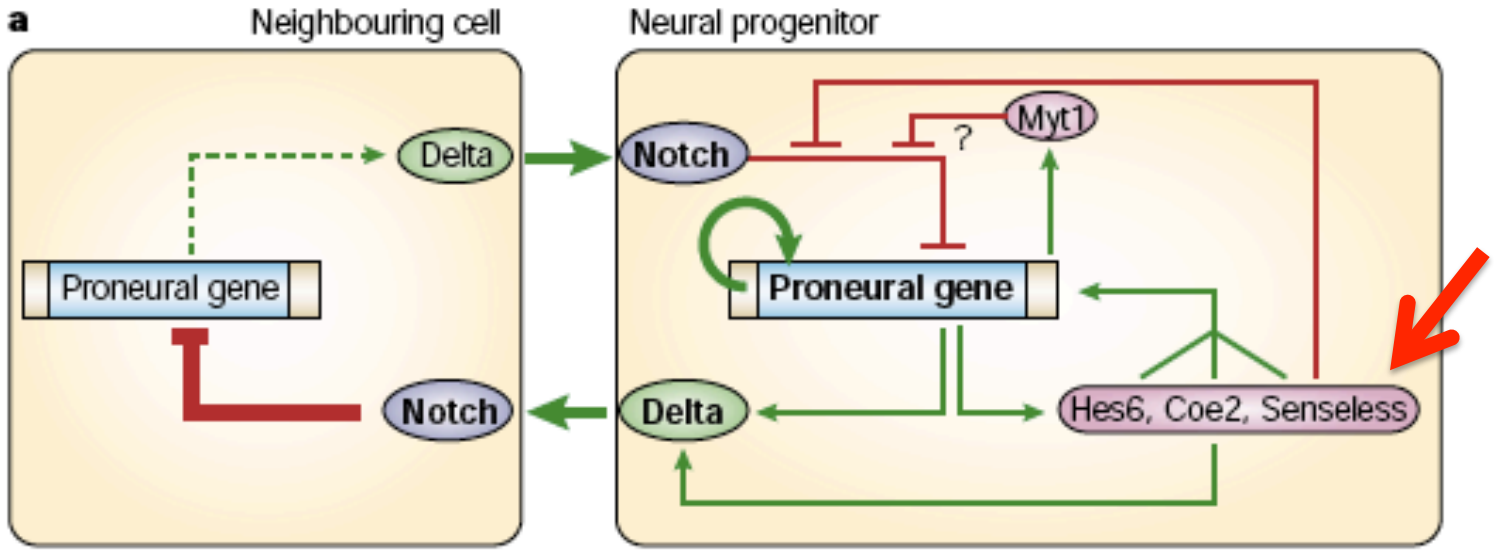
Notch signaling inhibits neurogenesis



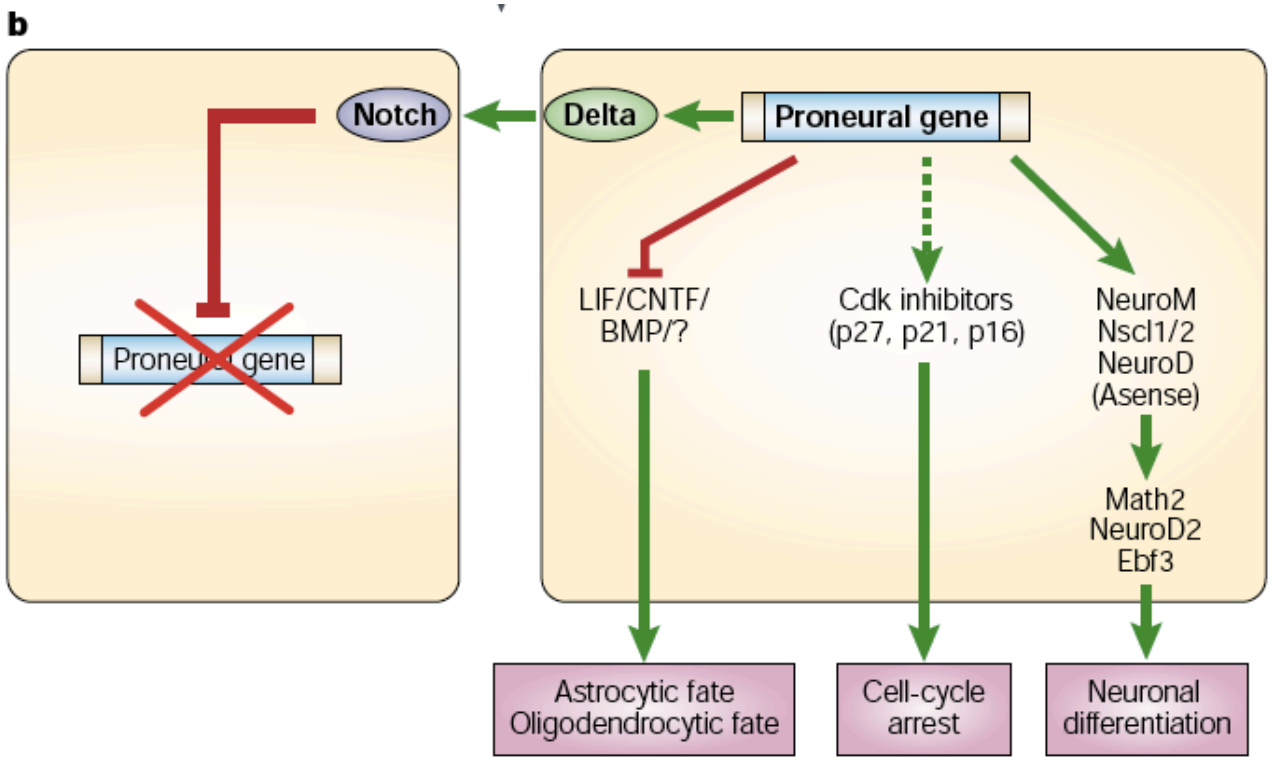

A
B

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



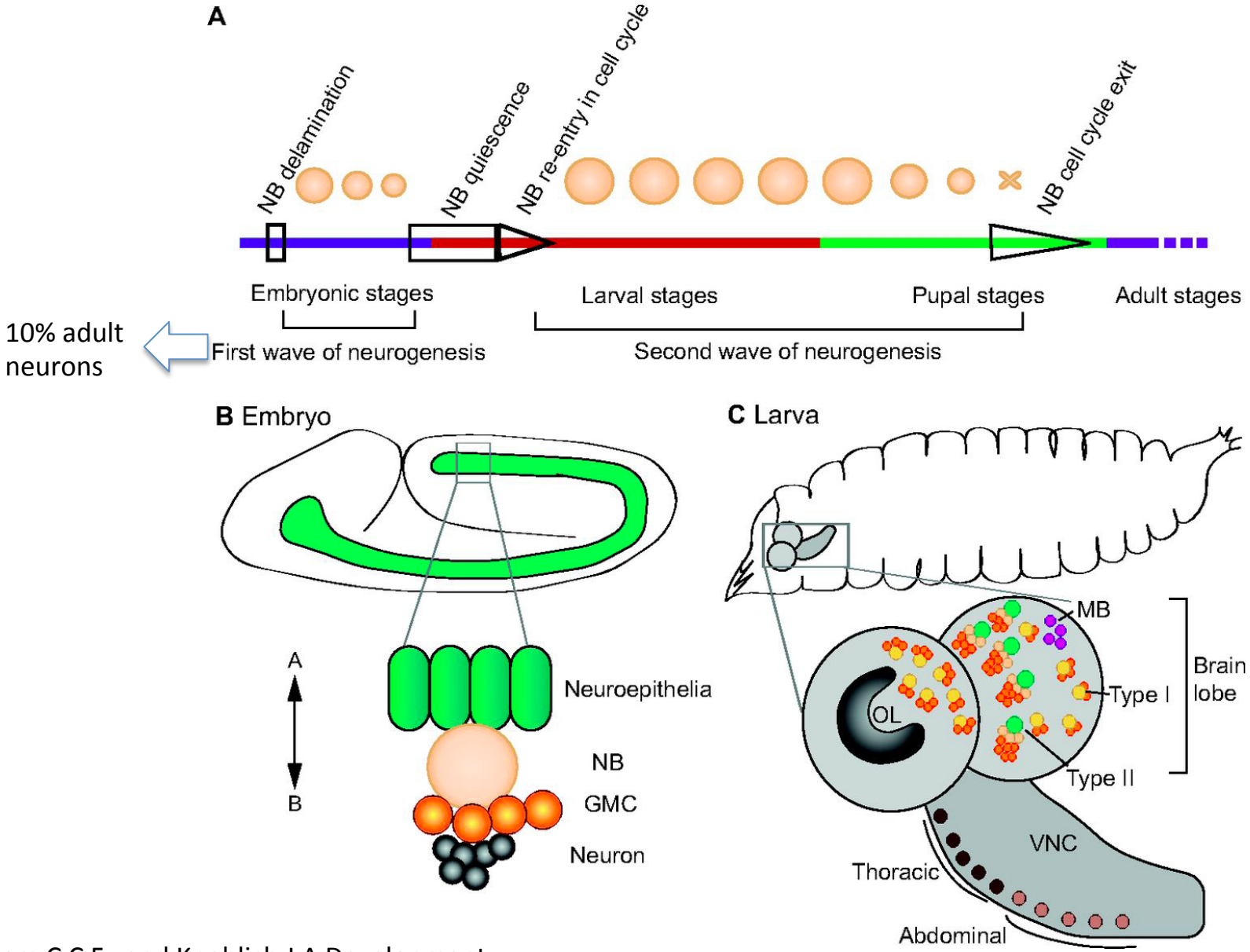
Functional hierarchy of proneural bHLH genes
(vertebrates and invertebrates)



downstream regulatory genes implement neuronal differentiation programs

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

Neurogenesis in Drosophila



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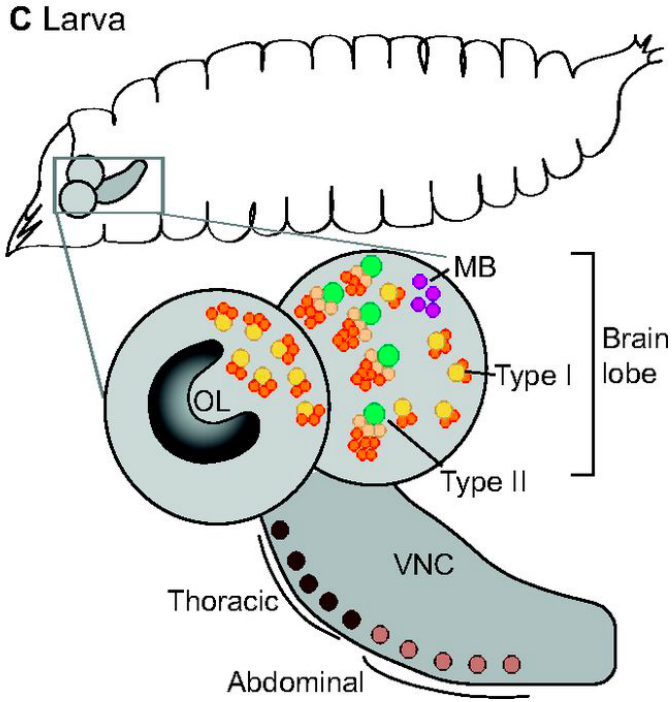
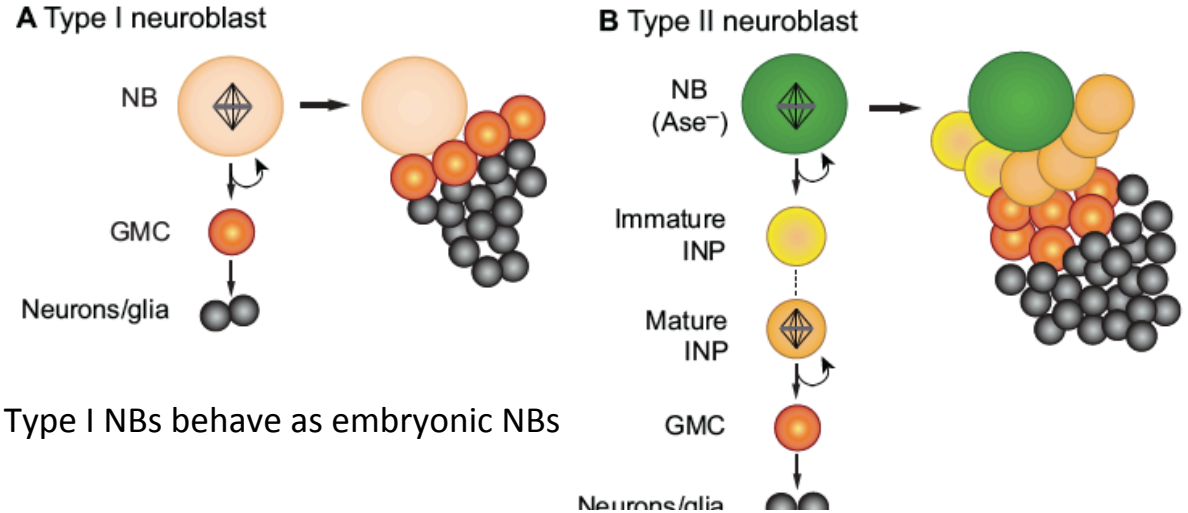
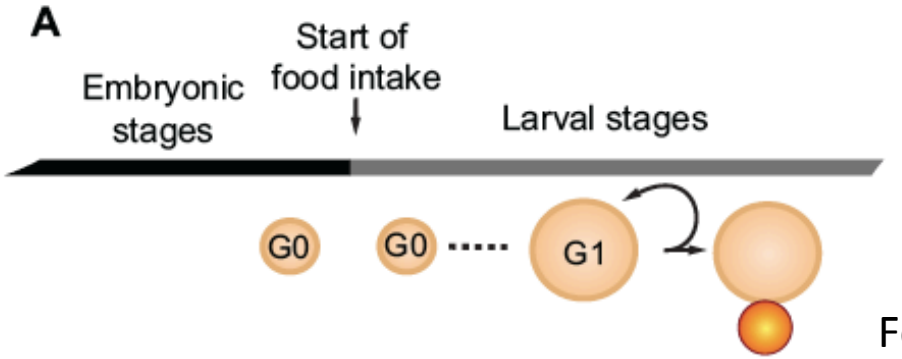


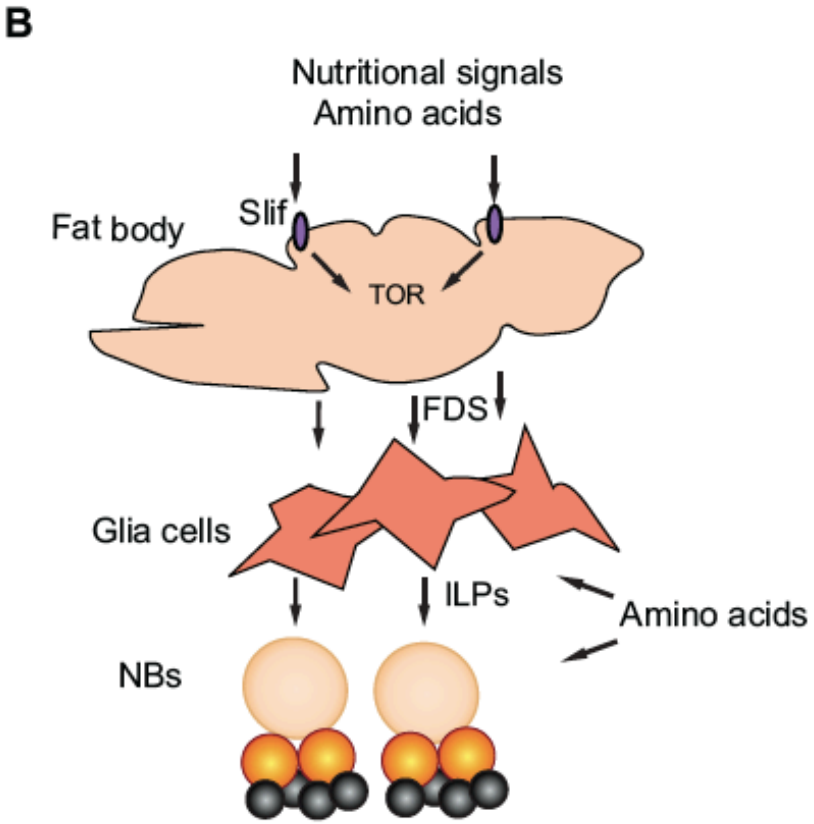


Type I NBs behave as embryonic NBs

Fig. 2. Type I and type II neuroblasts and their mode of asymmetric cell division. (**A**) Type I neuroblasts (NBs) divide asymmetrically to self-renew and generate a ganglion mother cell (GMC, orange). GMCs divide once to generate neurons or glia (gray). (**B**) Type II NBs divide asymmetrically to self-renew and generate an immature intermediate precursor (INP; yellow). After a period of maturation, INPs start dividing asymmetrically to self-renew and to generate a GMC. The GMCs divide once into two differentiating neurons or glial cells (gray). Through INPs, type II lineages give rise to more neurons than do type I NBs.

In young larval stages nutritional signals control NB growth and cell division





Nutrients provide the building blocks for macromolecular biosynthesis that drives cell growth and proliferation

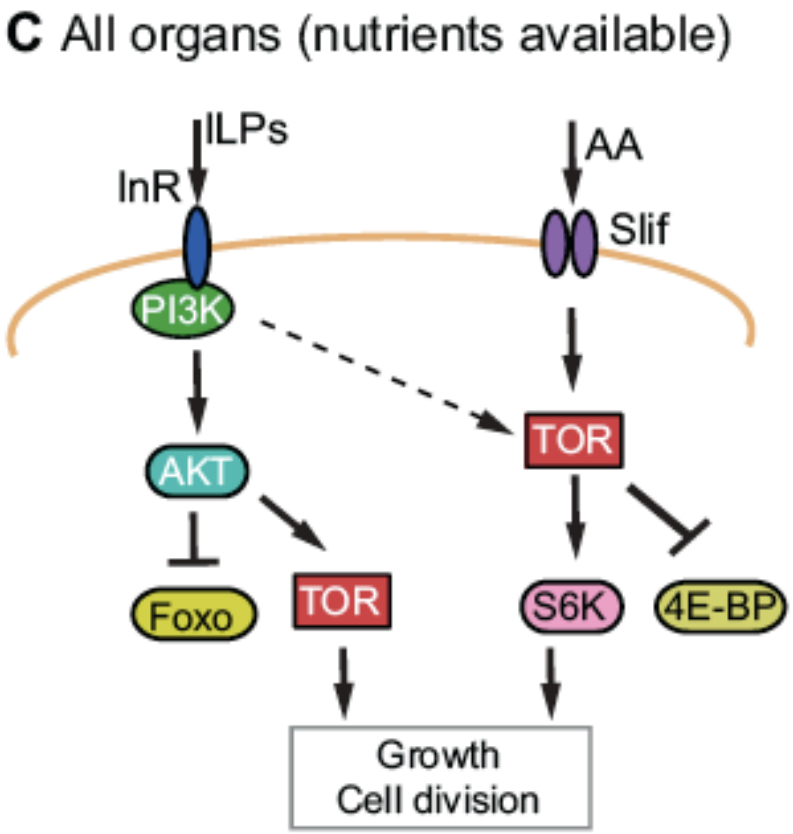
Food intake in larval stages induces NB* growth, transition from G0 to G1 (i.e. exit from quiescence), followed by cell division

Through the transporter Slimfast (Slif), amino acids are detected by the fat body where the target of rapamycin (**TOR**) pathway is activated.

TOR activation leads to production and secretion of a fat body-derived signal (**FDS**) **which activates the insulin pathway in glial cells**, which in turn release **insulin-like peptide 6** (ILP), inducing NB growth and division.

*all exept MB NBs

In young larval stages nutritional signals control NB growth and cell division

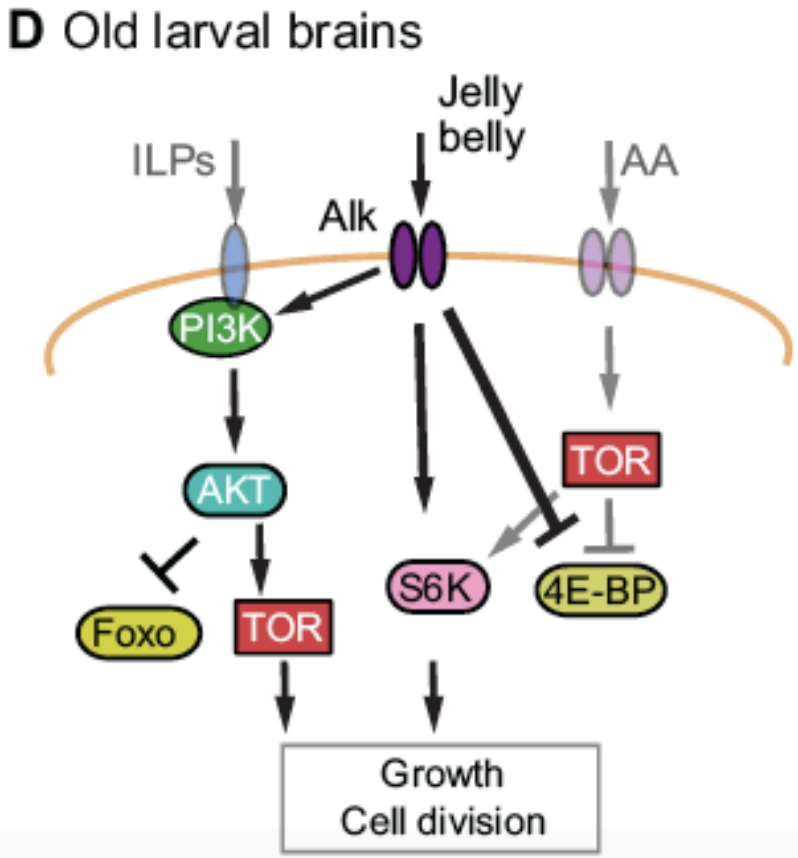


Signaling pathways activated downstream of insulin and amino acids.

ILPs bind the insulin receptor (InR, blue) and activate the PI3K/AKT pathway, which inhibits the growth inhibitor Foxo and activates TOR, leading to cell growth and division.

Circulating amino acids are detected by Slif, which also activates the TOR pathway. TOR activates S6K and inhibits 4E-BP, thus promoting protein translation, biosynthesis and ultimately cell growth and division.

→ **In late larval stages NBs are no more controlled by nutritional states**



Jelly belly (expressed by glial cells)

Alk= anaplastic lymphoma kinase

eLIFE elifesciences.org

RESEARCH ARTICLE

Eyeless uncouples mushroom body neuroblast proliferation from dietary amino acids in *Drosophila*

Conor W Sipe, Sarah E Siegrist*

Department of Biology, University of Virginia, Charlottesville, United States

Link to the article:
https://elifesciences.org/articles/26343

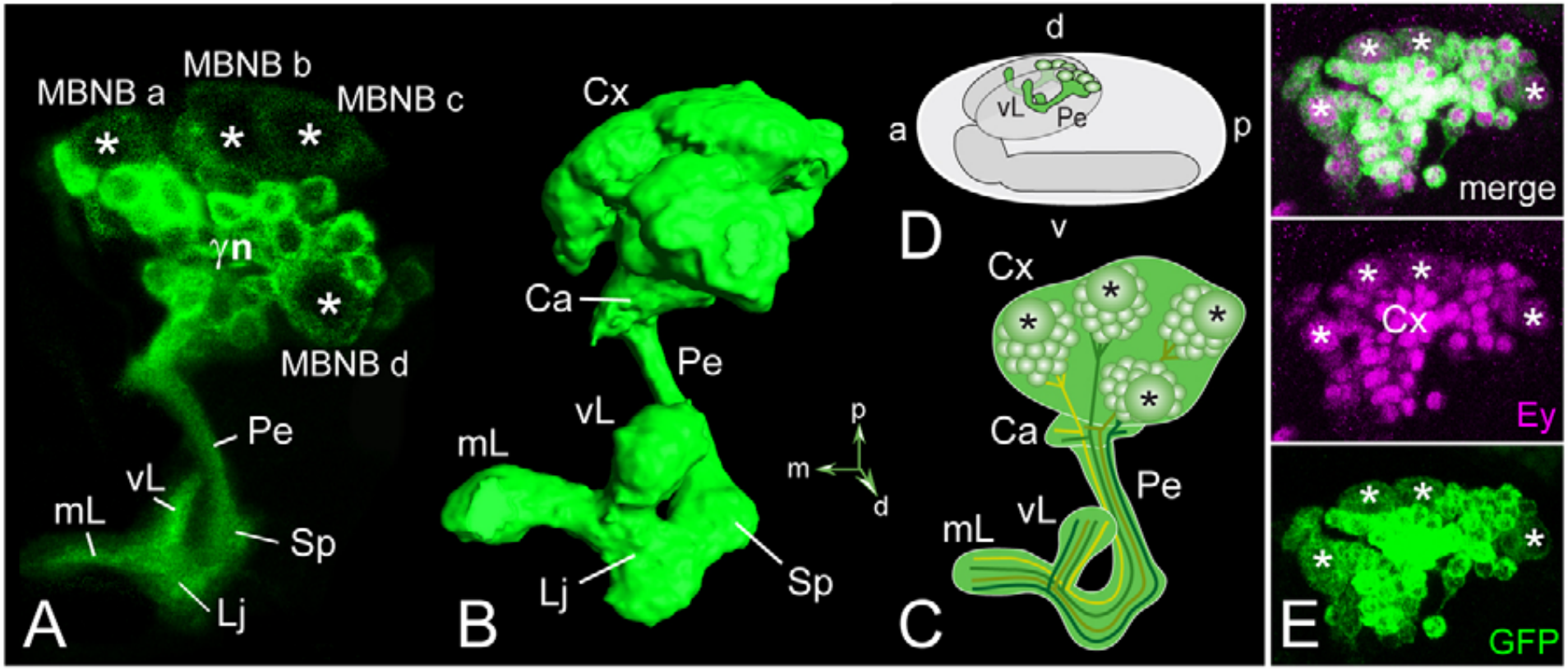


Fig. 1. Organization of the mushroom body in the *Drosophila* late embryo/early larva.

(A-C) Shown is the mushroom body (MB) in the right brain hemisphere in dorsal view.

(**A**) Composite confocal image of OK107(*ey*)>CD8::GFP visualizes the morphology of the whole MB. CD8::GFP is detected in γ-neurons (γn) and the four mushroom body neuroblasts (MBNBs; asterisks in A,C,E) in the late st17 embryo.

(**B**) Threedimensional reconstruction of the MB in the early L1 based on OK107(*ey*)>CD8::GFP expression.

(**C**) Scheme illustrates the four clonal subunits of the larval MB. γ-neurons form axonal tracts running through the peduncle (Pe) into the medial (mL) and vertical (vL) lobe and dendritic branches in the calyx (Ca).

(**D**) MBs in a lateral view within the CNS of the late embryo. (**E**) Co-expression of Ey and OK107(*ey*)>CD8::GFP in all cell bodies of the late st17 MB cortex (Cx). a, anterior; p, posterior, d, dorsal; v, ventral; m, medial; Lj, lobe junction; Sp, spur.

Kunz et al., Development 2012

Discussion in small groups (15'-20') followed by discussion in the class

General questions:

- Is the **title** appropriate, reflecting the content and conclusions of the article?
- Hypothesis: are the **abstract and introduction** accurate, informative, regarding the
- objectives/rational & indicate the significance of the work?
- Are the **results** clearly and succinctly presented, convincing and focused on objectives?
- Are the **main conclusions** consistent with the results?
- What do you think is the **main limit** of the study?
- Do you have **comments or criticisms** ?

Specific questions:

In the Introduction the authors state that *"While amino acids are required to reactivate quiescent NBs, it is unclear whether further dietary amino acid intake is required"* to your knowledge are there known regulators that can control NBs cell division independently from the nutritional status?

In the Results and discussion the authors write "*This reduction was not due to a change in NB number, suggesting that NBs require dietary amino acids to maintain proliferation*" can you comment on this sentence? What is its meaning?

Each group focussed on 1 figure of the paper addressing the following points:

1) What kind of experiments are shown?
2) Does the figure clearly show the data and support the conclusions reached by the authors? Is the legend explicative?
3) Do you have comments or criticism?