Early events of neural development

Goals:
1) to discuss the origins of cells in the nervous system
2) to discuss how neural stem cells generate diverse cell types in the nervous system

The next four lectures will cover:
Induction (Jan 22)...emergence of the nervous system
Regionalization (Jan 24)...acquisition of positional information of neural cells
Discussion of a journal article (Jan 26)

Cell division and cell lineage (Jan 29)
Neuronal fate specification (Jan 31)
Discussion of a journal article (Feb 2)

We will deal with glia later in the course!

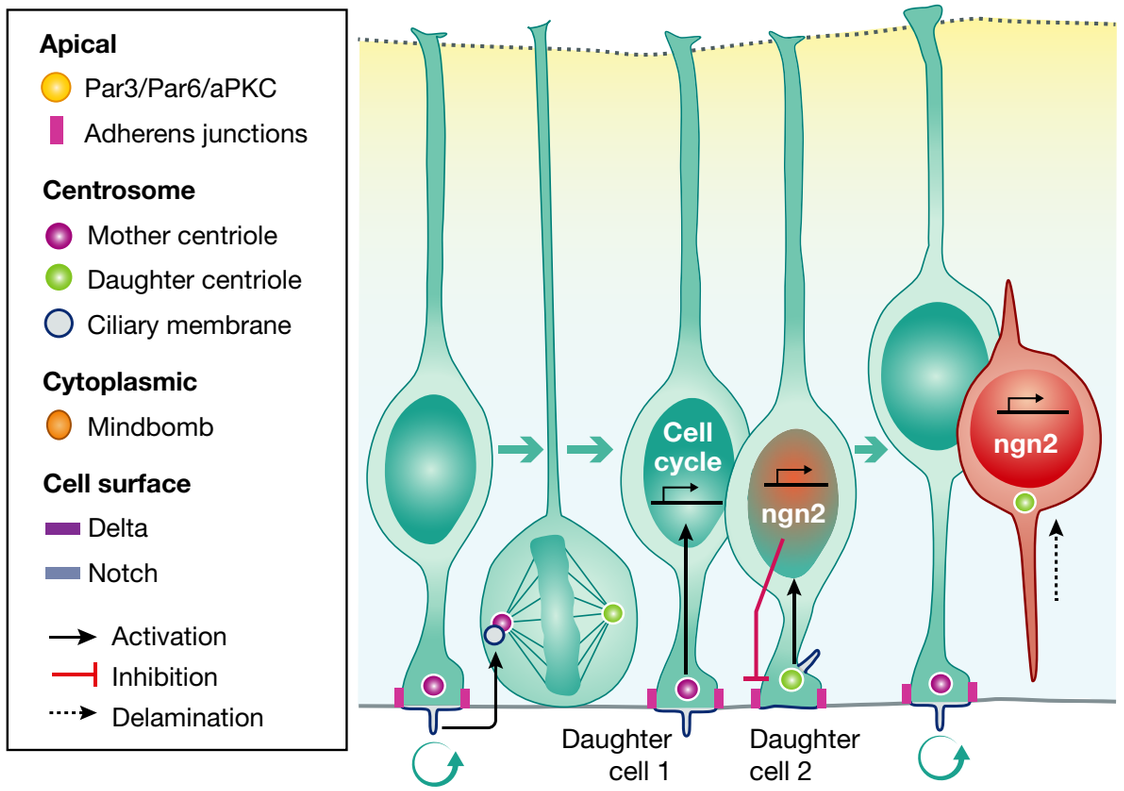
Outline of this lecture

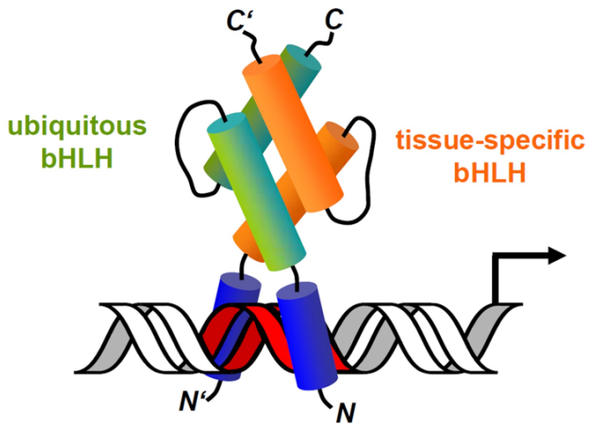
Control of daughter cell fates after cell division (RGC vs differentiating cell)
-Notch-Delta signaling
-proneural basic helix-loop-helix (bHLH) transcription factors

Identity of progenitor cells influence the type of neurons that the progenitor cell produces.
-regional identity (outcome of regionalization)
-temporal identity (RGCs changes as they undergo asymmetric divisions)

Neuronal fate can be controlled postmitotically.

Proneural transcription factors promote neuronal differentiation





bHLH-mediated DNA transcription

The differentiating daughter cell generated after an asymmetric division of a RGC expresses the proneural transcription factor Neurogenin 2 (Neurog2 or Ngn2).

Neurogenin 2 belongs to a family of transcription factors with a basic helix-loop-helix (bHLH) domain.

Neurogenin 2 promotes neuronal differentiation.

Neurogenin 2 promotes transcription of Tbr2, an IPC marker.

What determines whether the differentiating daughter cell becomes an IPC or a neuron is not well understood.

Notch signaling and proneural bHLH factors

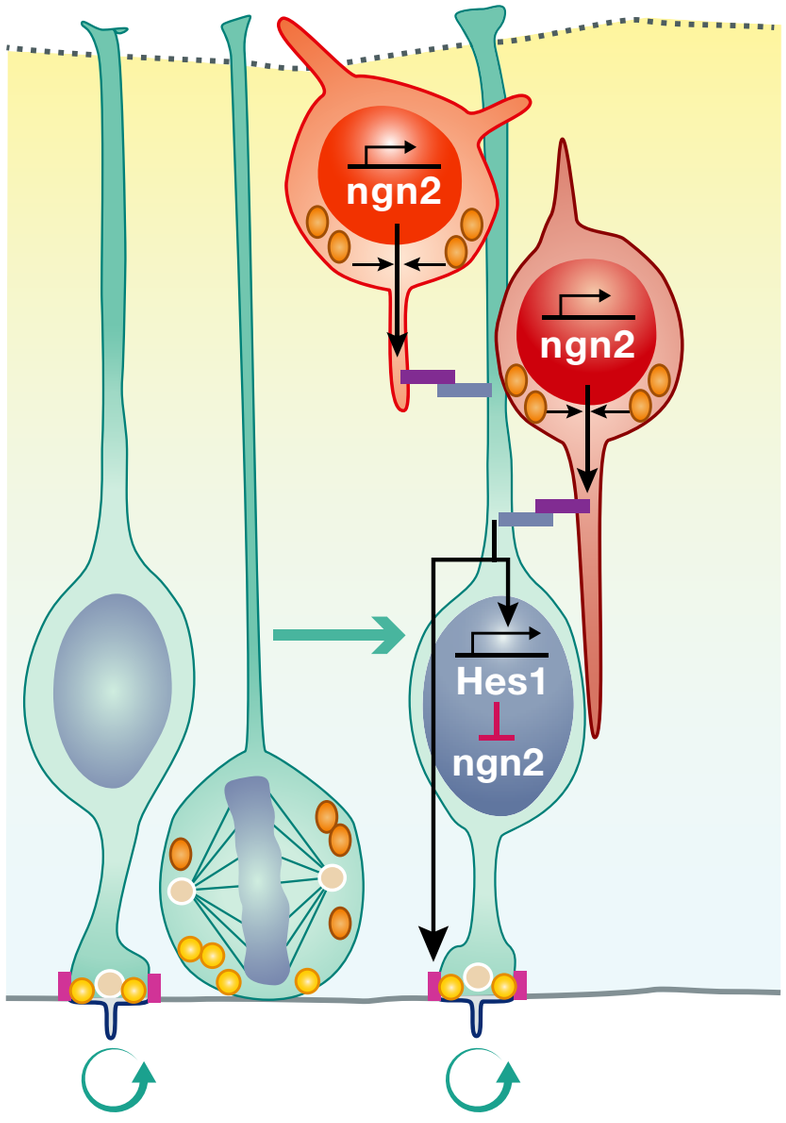
Apical
- Par3/Par6/aPKC
- Adherens junctions

Centrosome
- Mother centriole
- Daughter centriole
- Ciliary membrane

Cytoplasmic
- Mindbomb

Cell surface
- Delta
- Notch

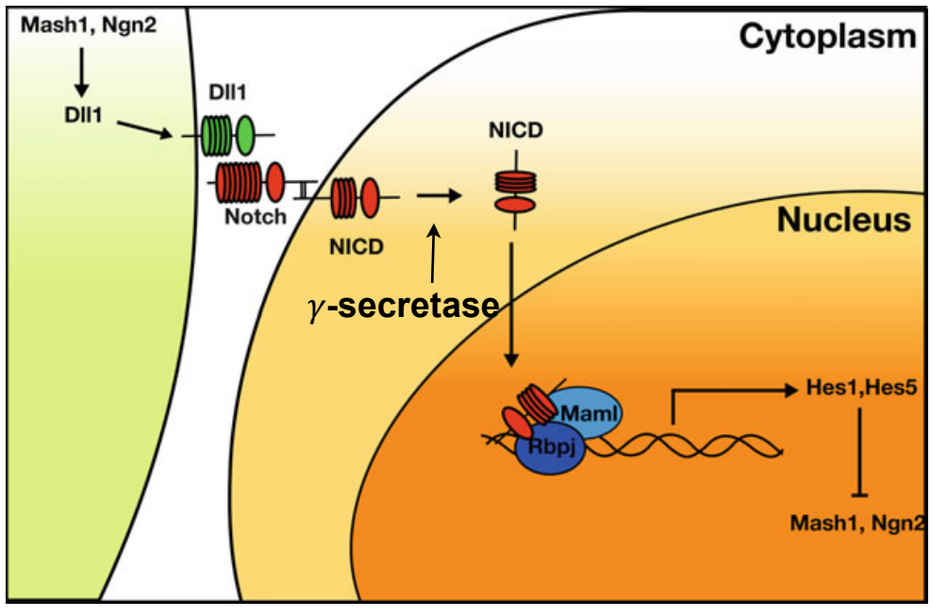
- Activation
- Inhibition
- Delamination



Ngn2 promotes transcription of Delta, a ligand for Notch, which is expressed in RGCs.

Notch signaling promotes transcription of Hes1, which inhibits Ngn2 functions and promote the RGC fate.

Notch signaling pathway



Shimojo et al. (2013)

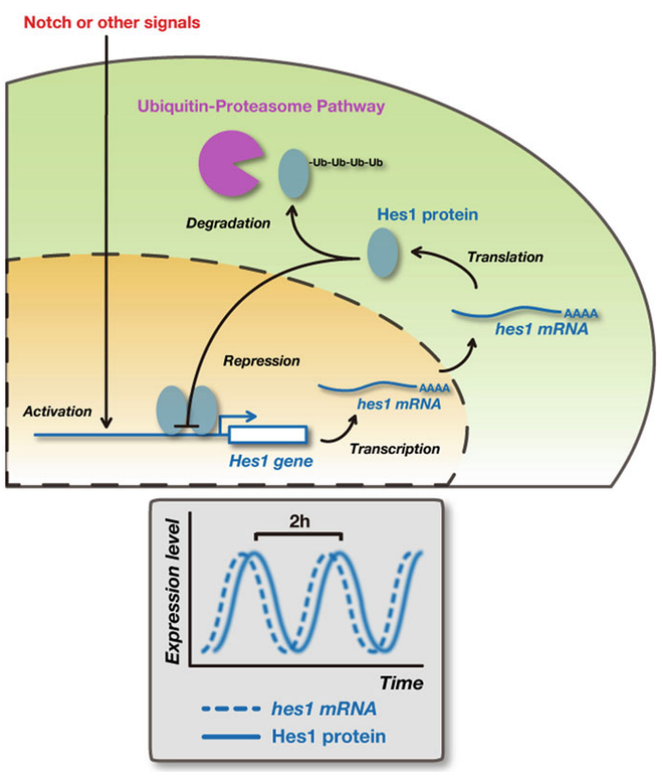
When Delta binds to Notch, γ-secretase cleaves Notch, resulting in the release of intracellular domain (Notch-ICD) into the cytoplasm and into the nucleus.

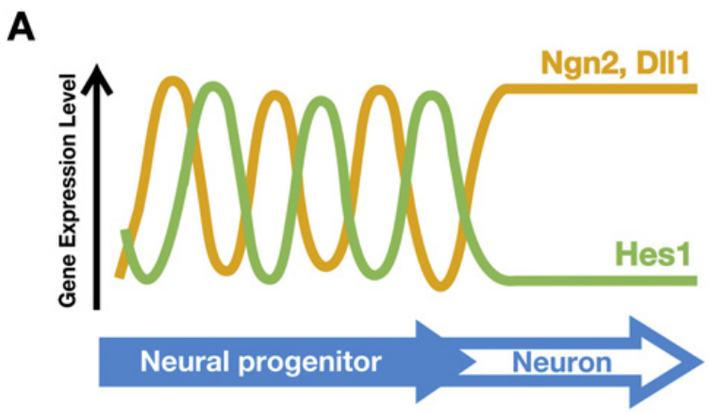
Together with Rbpj and MAML, Notch activates transcription of downstream genes such as Hes1 and Hes5.

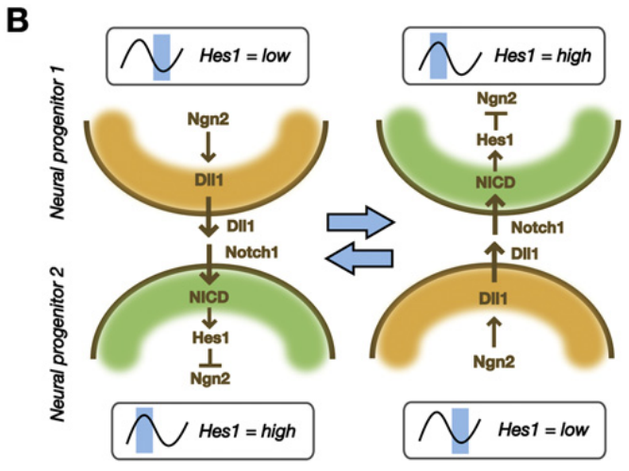
Hes inhibits proneural bHLH proteins.

Hes inhibits its own expression and oscillates rapidly in a reciprocal manner with Ngn2 in neural progenitor cells.

In differentiating cells, Hes expression disappears and Ngn2 expression becomes sustained.

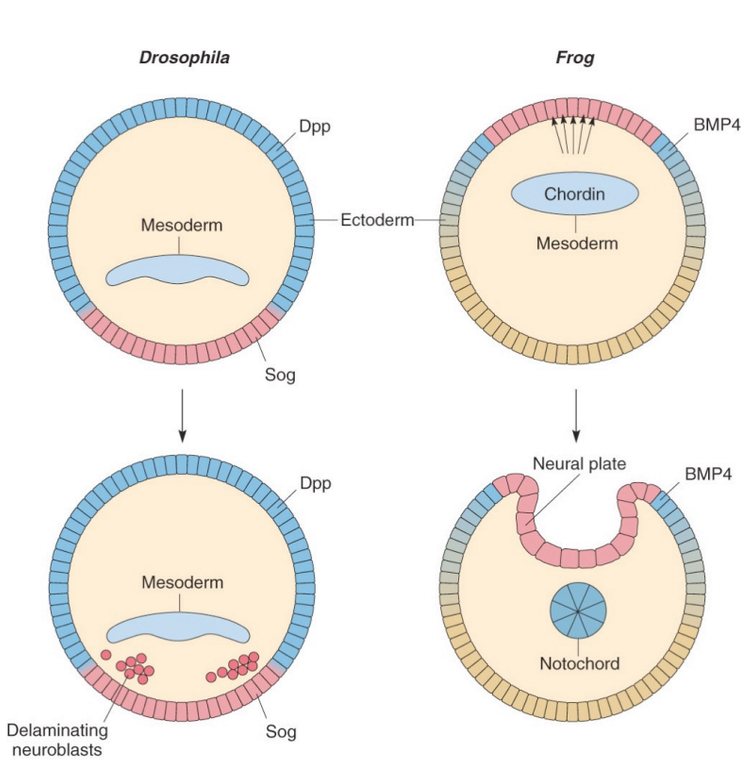


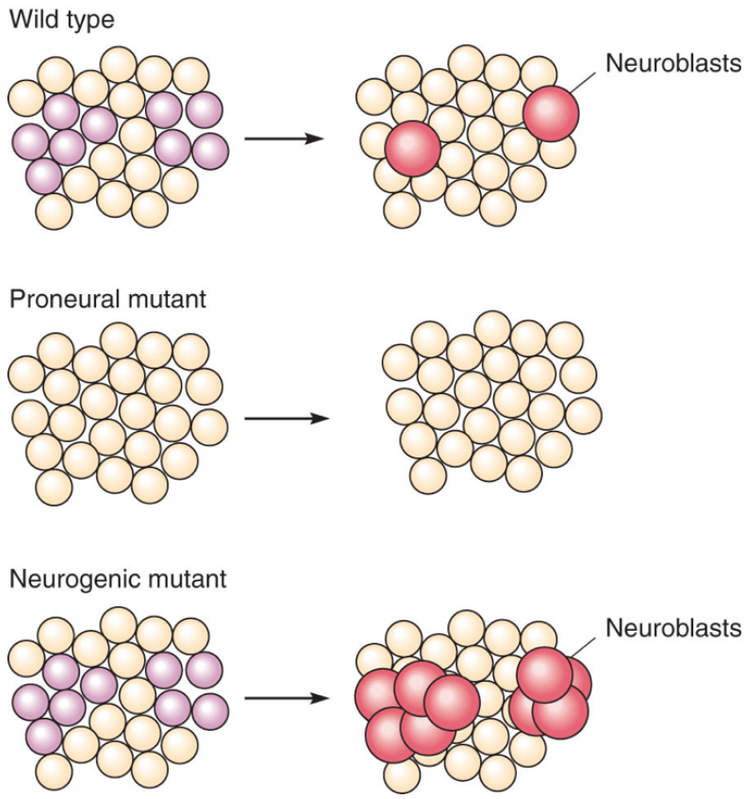




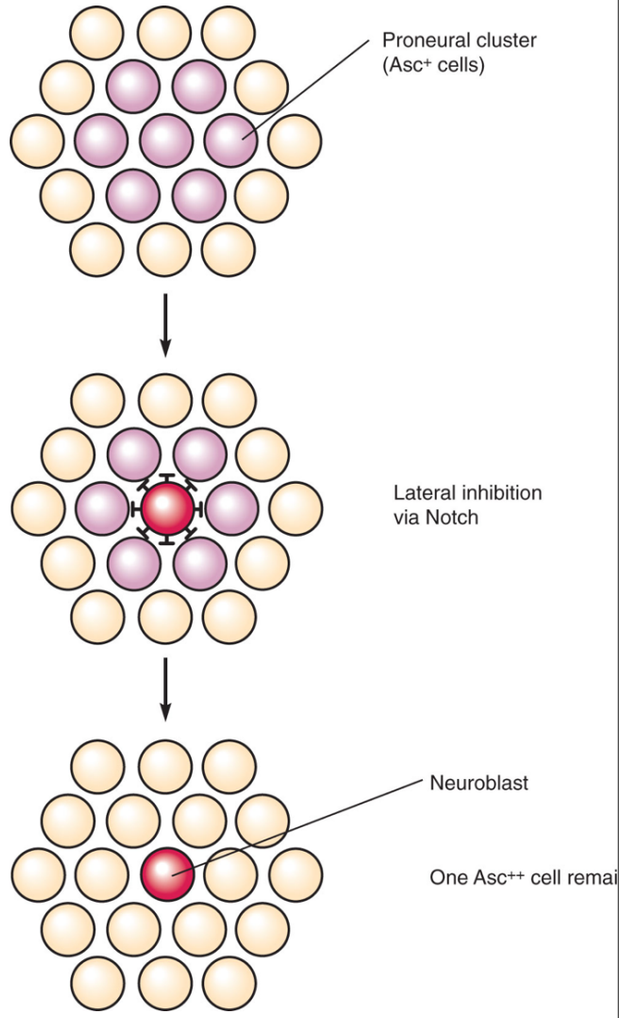
Shimojo et al. (2008)

Notch-delta signaling was discovered by Drosophila genetics





Sanes (2011)



“Proneural genes” encode basic-helix-loop-helix transcription factors.

“Neurogenic gene” encodes the transmembrane protein Notch.

Lateral inhibition selects a single cell in a proneural cluster to become a neuroblast.

Many intrinsic and extrinsic factors regulate daughter cell fate after asymmetric cell divisions

Input from environment
CSF, meninges

Neuronal feedback

Notch signaling **ON** → Proneural genes ↓

Shh signaling **ON** → Cell cycle ↑

Wnt signaling **ON** → Cell cycle ↑

Inheritance of cell fate determinants

Silencing of proneural genes
miR, epigenetic modification

Patterning → TF expression

Input from environment
CSF, meninges, blood vessels, afferents

Neuronal feedback

Notch signaling **OFF** → Proneural genes ↑

Shh signaling suppression → Differentiation

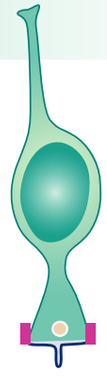
Wnt signaling **ON** → Differentiation

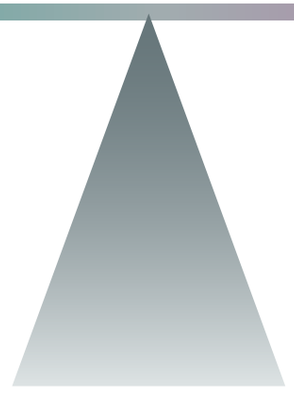
Inheritance of cell fate determinants

Silencing of NPC TF expression
miR, epigenetic modification

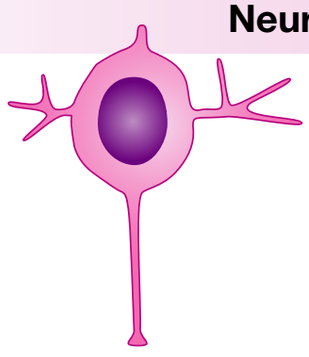
Sequential TF expression

Neural progenitor





Neuron



Paridaen and Huttner (2014)

Outline of this lecture

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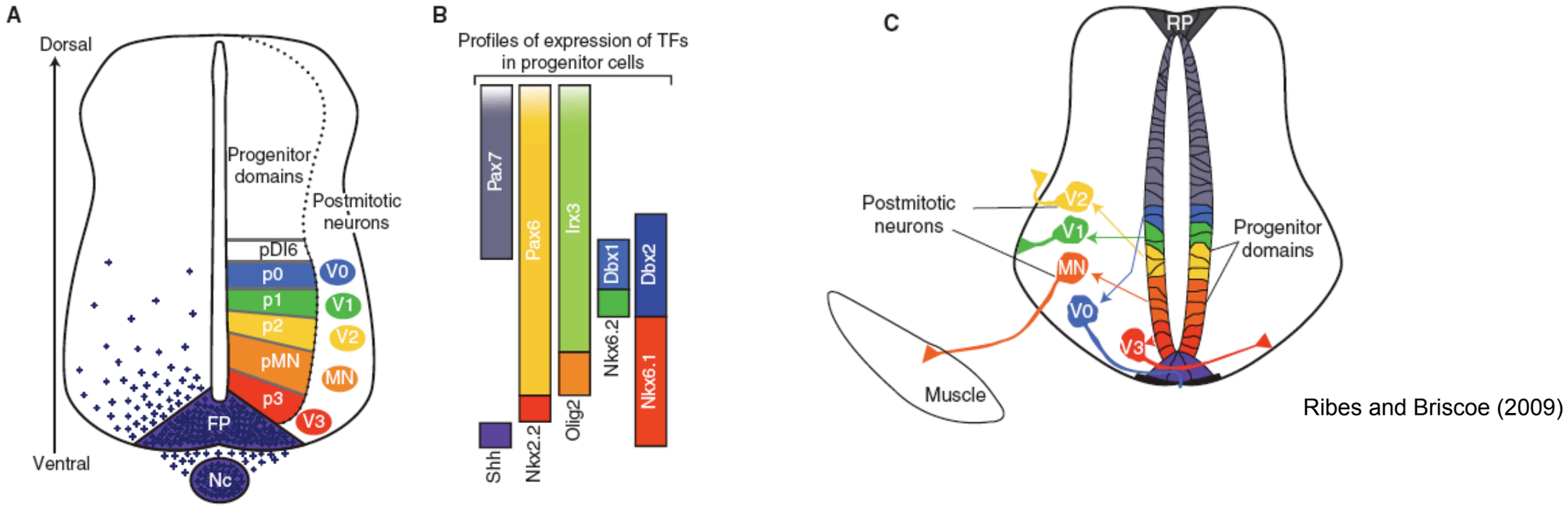
Identity of progenitor cells influence the type of neurons that the progenitor cell produces.
-regional identity (outcome of regionalization)
-temporal identity (RGCs changes as they undergo asymmetric divisions)

Neuronal fate can be controlled postmitotically.

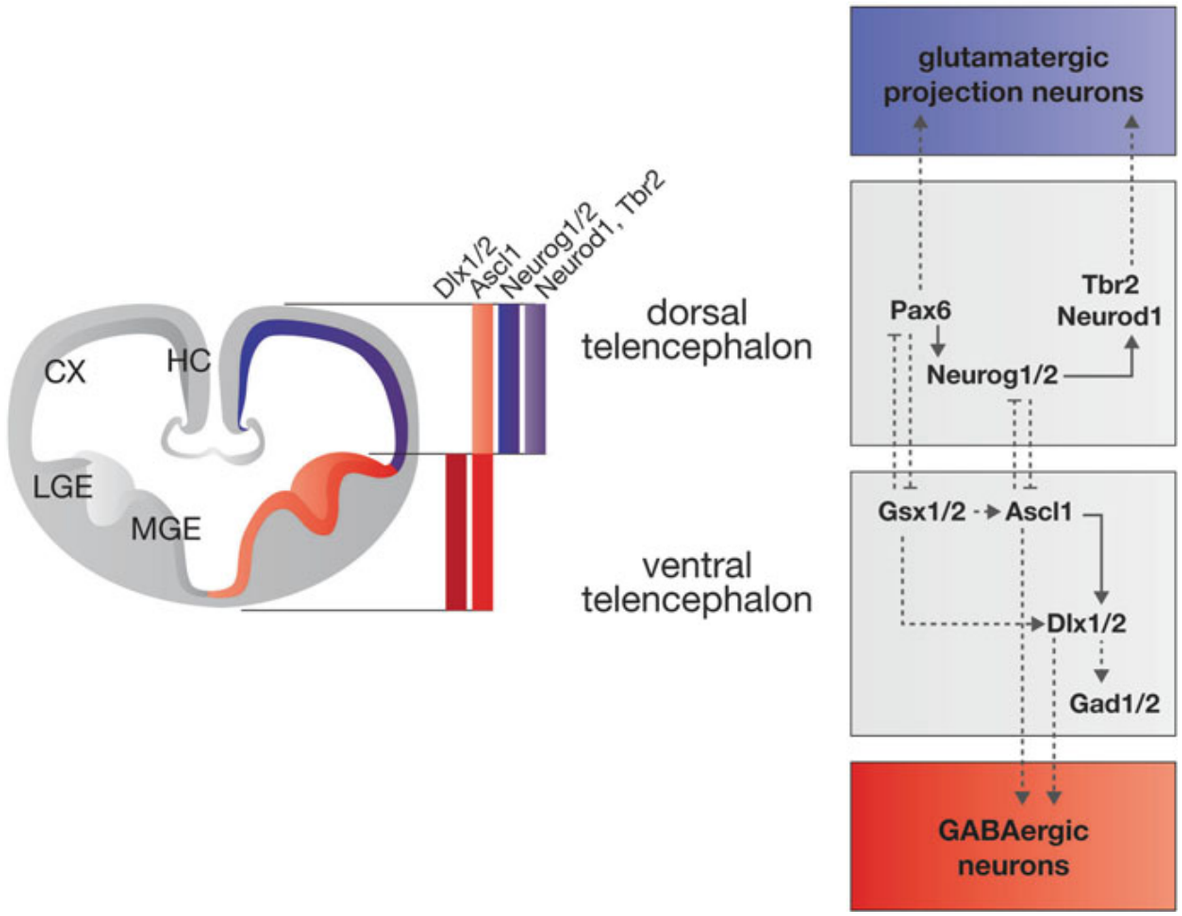
Spatial diversity of progenitor cells contributes to generating diversity of neuronal types



Ribes and Briscoe (2009)

Distinct subtypes of interneurons (V0-V3) and motor neurons (MN) are generated from each of the six progenitor domains in the ventral spinal cord.

Regionalization controls differential expression of proneural genes

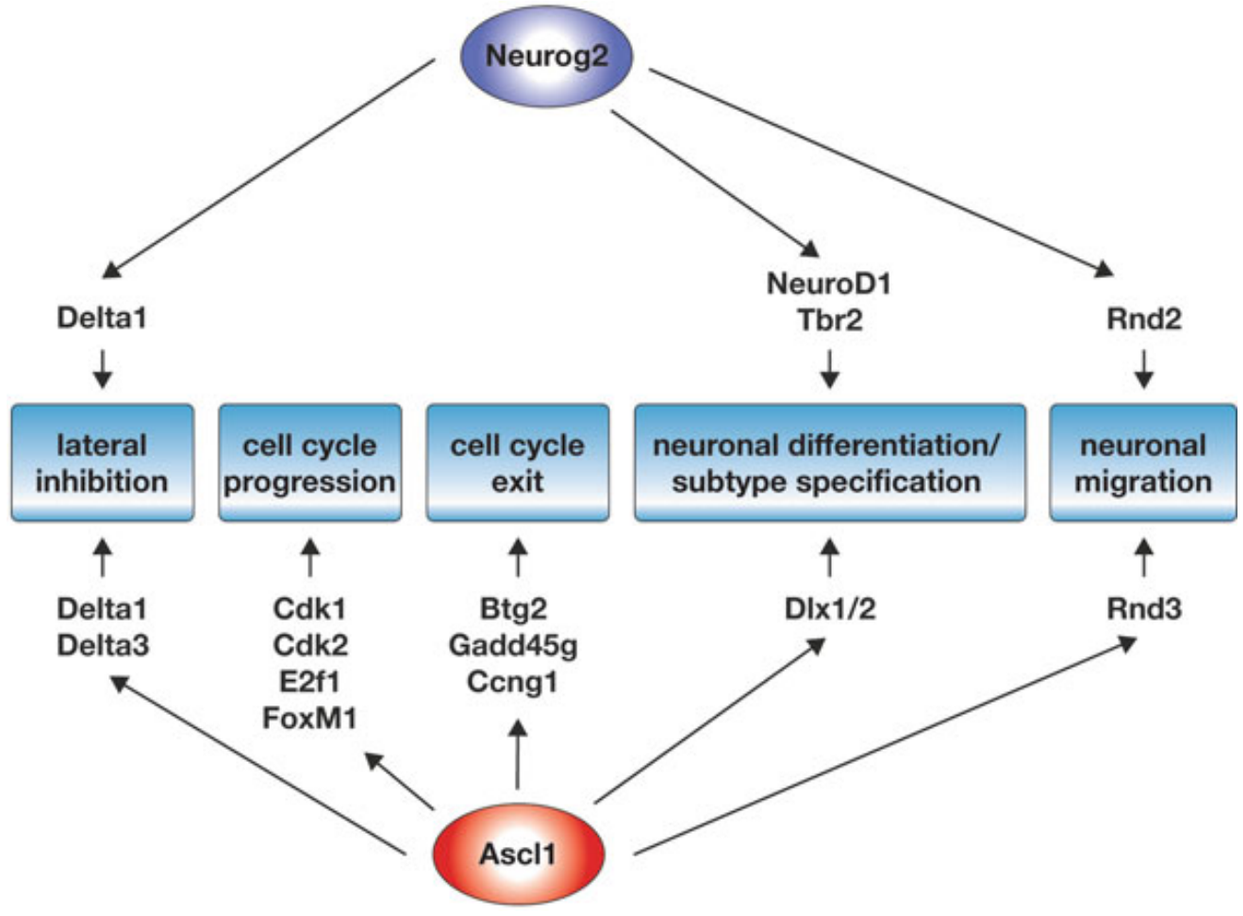


Heng and Guillemot (2013)

Expression of Neurogenins and Ascl1 are differentially regulated by regionalization mechanisms and are usually expressed in complementary manner in progenitor cells.

In telencephalon, Neurogenins are expressed in the dorsal part, which generates glutamatergic neurons. Ascl1 is expressed in ventral telencephalon, which generate mainly GABAergic inhibitory neurons.

Neurog2 and Ascl1 control the major steps of neurogenesis through direct regulation of multiple genes

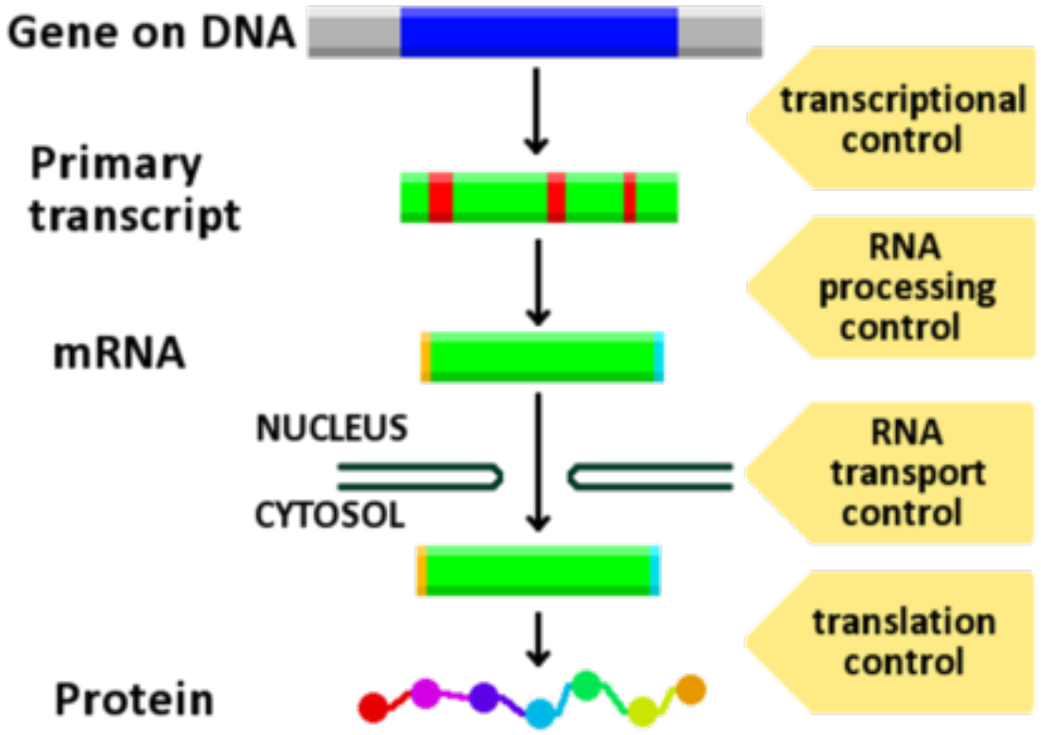


Heng and Guillemot (2013)

General functions of proneural genes: promote neuronal differentiation

Specific proneural genes (Neurogenin vs Ascl1) control specification of glutamatergic vs GABAegic neuronal fates

Patterns of gene expression are an intrinsic determinant of cell identity



Regulation of gene expression:

-epigenetic modification of chromatin (histone and DNA)

-transcription factors (DNA binding proteins that control initiation of transcription)

-micro RNAs and long-noncoding RNAs

-mRNA is also modified (“epitranscriptomics”)

-post-translational modification

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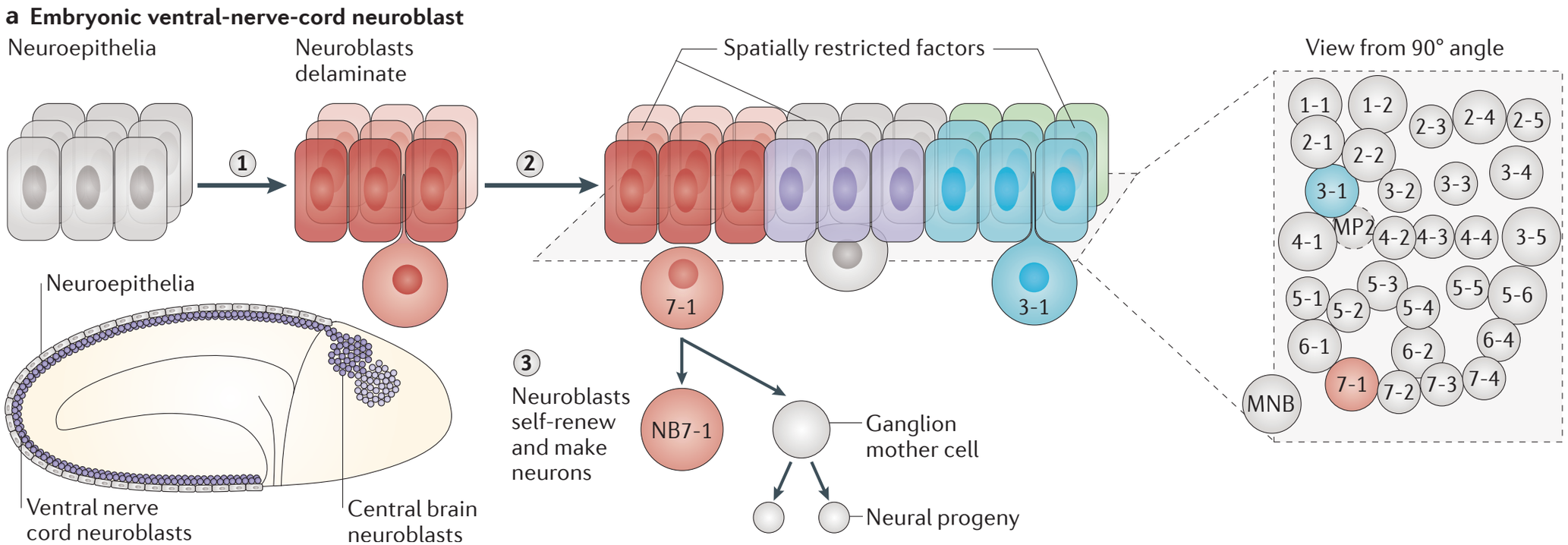
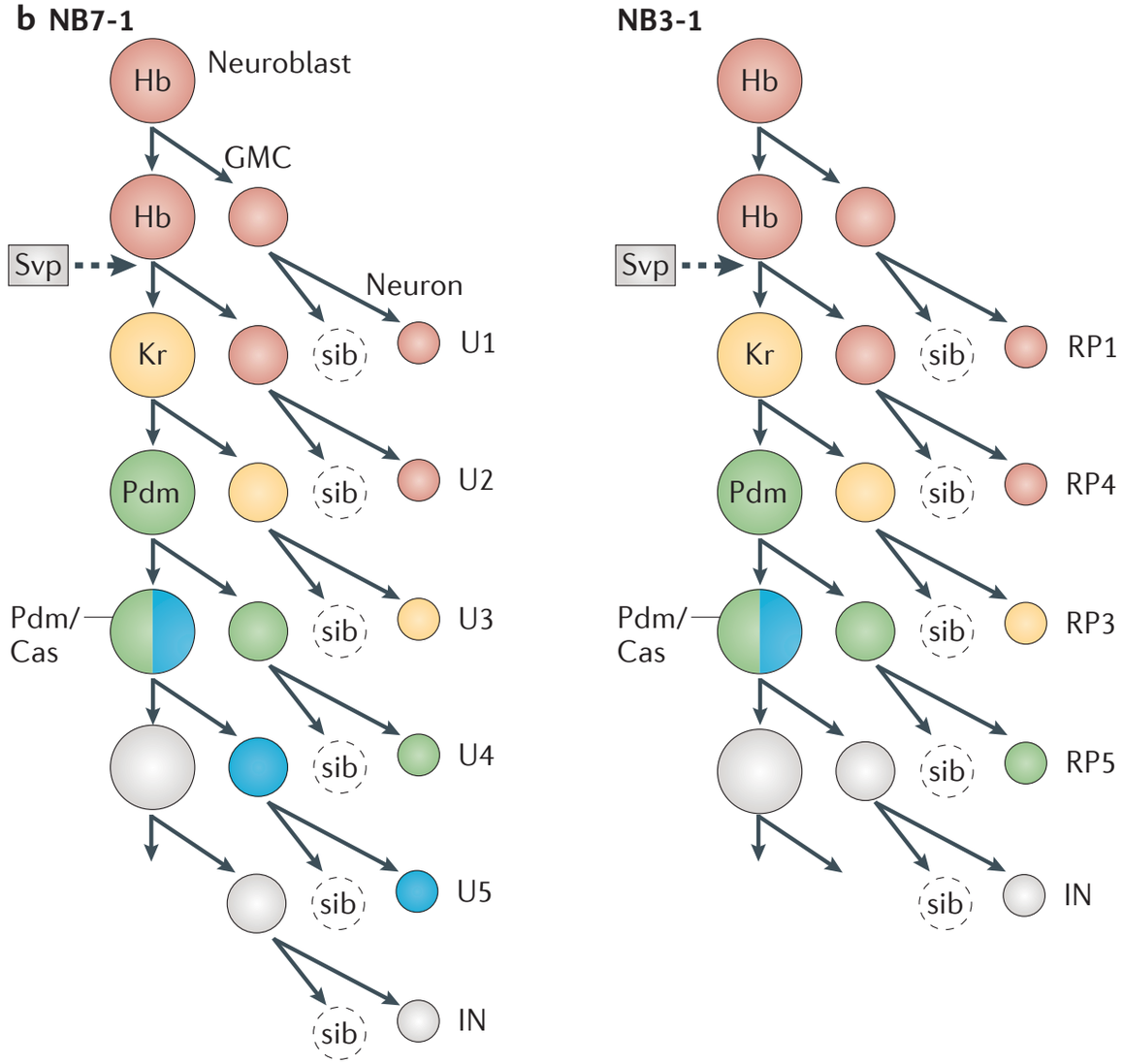
Neurogenesis in ventral nerve cord neuroblast lineages in the Drosophila embryo



-About 30 neural progenitor cells (neuroblasts) are arranged in a segmentally repeated pattern in the ventral CNS of Drosophila embryos.

-With each cell cycle (~1hr long), a small ganglion mother cell (GMC) buds off and divides once more to generate a pair of neurons or glia.

Neurogenesis in ventral nerve cord neuroblast lineages in the Drosophila embryo



-Each neuroblast is uniquely identified by the position and expression of specific molecular markers.

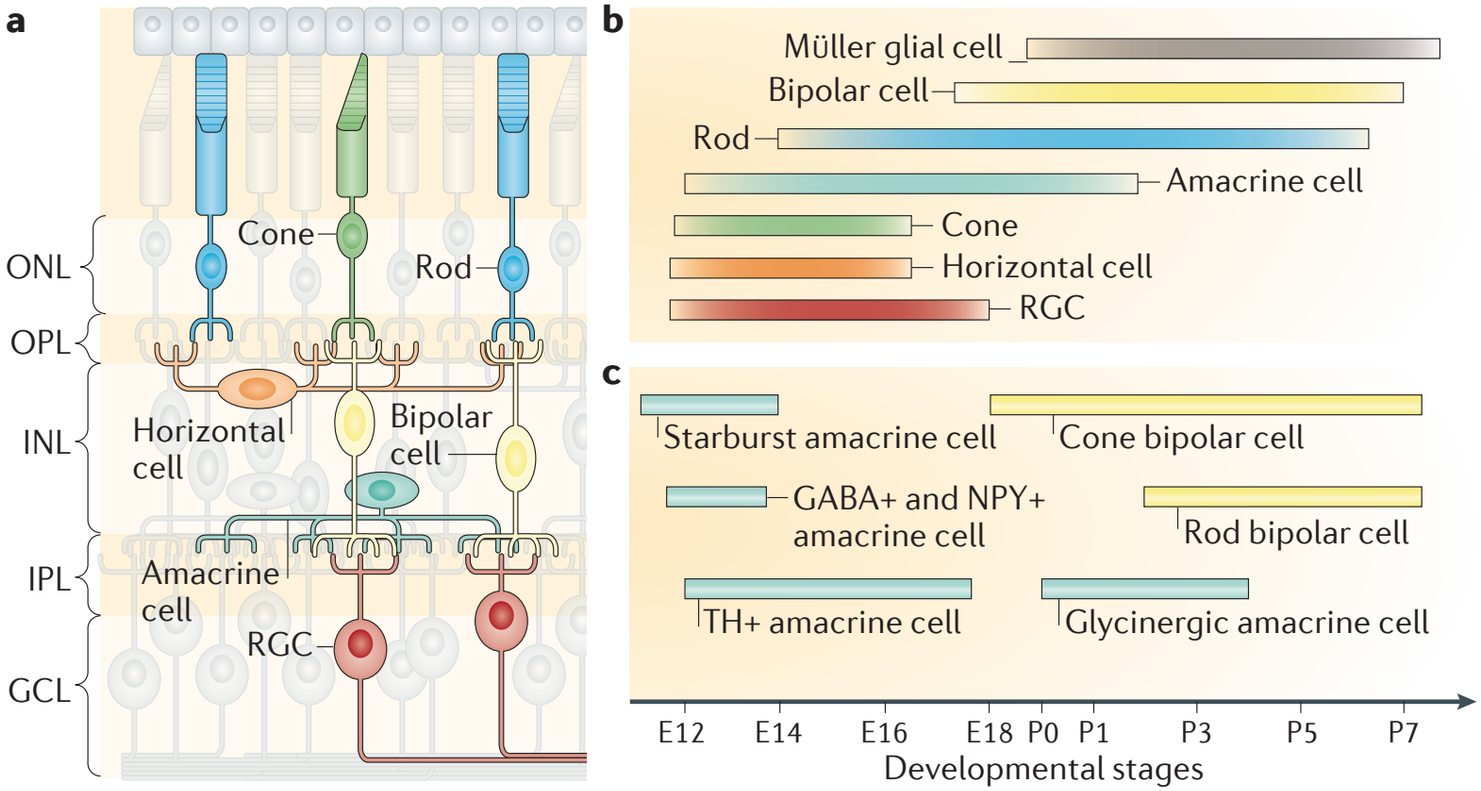
-A specific neuroblast gives rise to a reproducible set of neural progeny in the same birth order.

-Sequential expression of temporal identity factor: Hb→Kr→Pdm→Cas

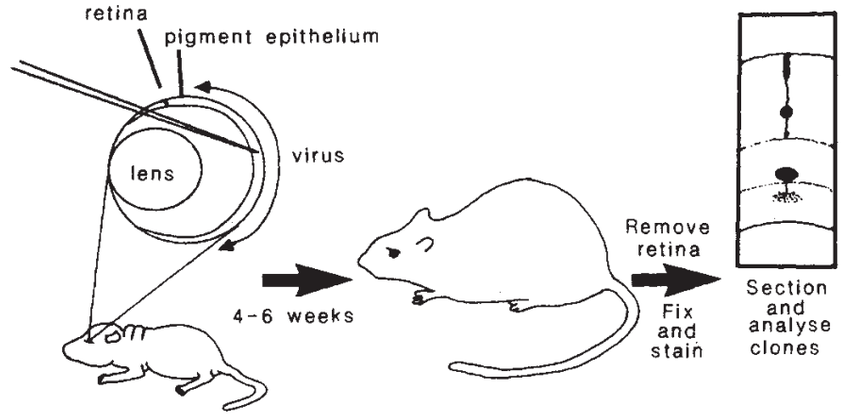
Hb (Hunchback; Ikaros family zinc finger transcription factor) is necessary and sufficient for early-born neural identity in multiple neuroblast lineages.

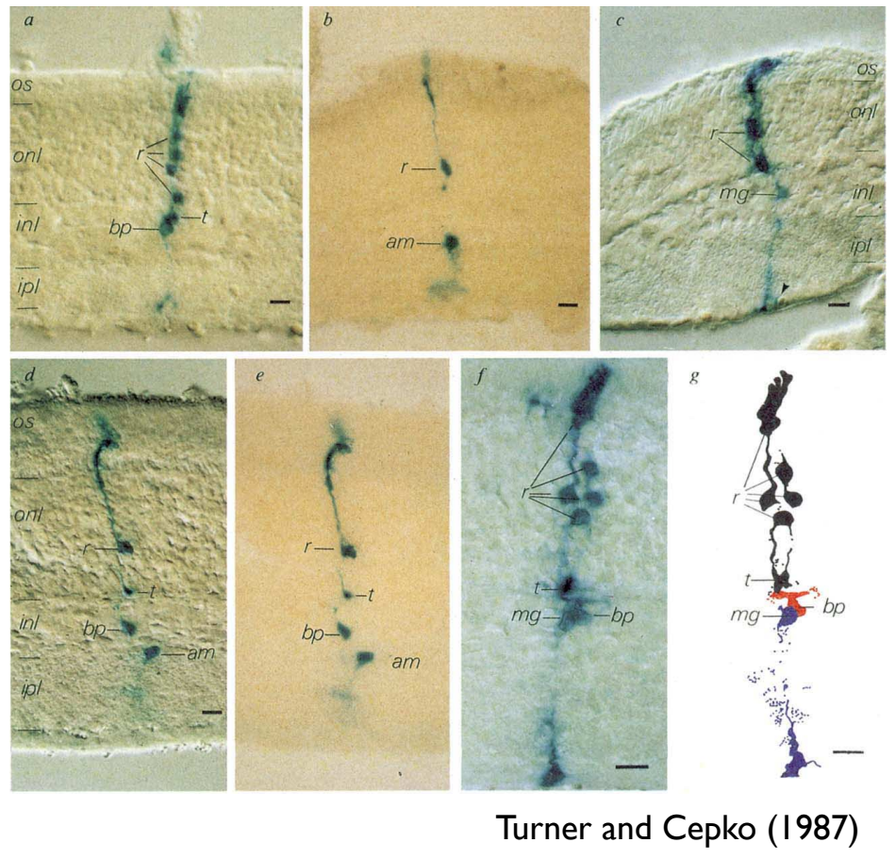
-Svp (Sevenup; COUP-family nuclear receptor) regulates the transition from Hb expression to Kr expression. The transition also requires cytokinesis.

Temporal fate specification in the mammalian retina



Cepko (2014)

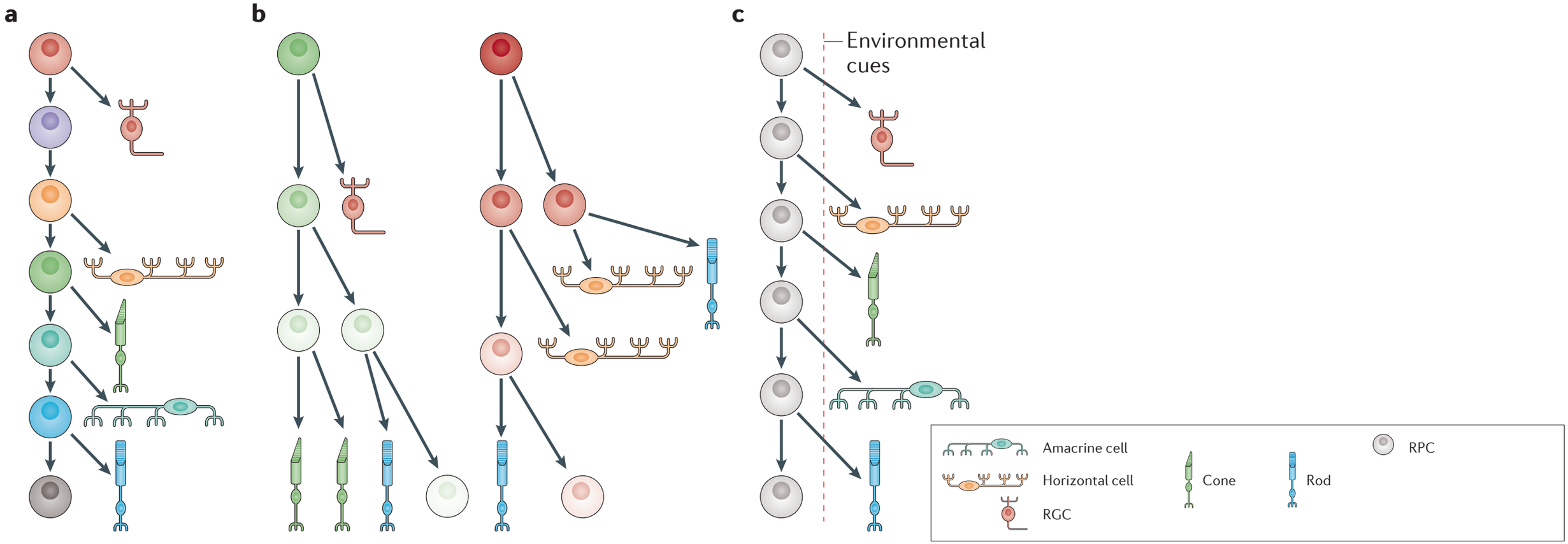




Turner and Cepko (1987)

-All the cells in the retina are derived from retinal progenitor cells (RPCs).
-*In vivo* lineage tracing (with retrovirus, etc.) shows that individual retinal progenitor cells are multi-potent.
-There is an evolutionarily conserved order of generation of neurons and glia.

Models of retinal cell fate determination



intrinsic, temporal order of competency states

intrinsic model with multiple, distinct types of RPCs that generate distinct sets of cell types

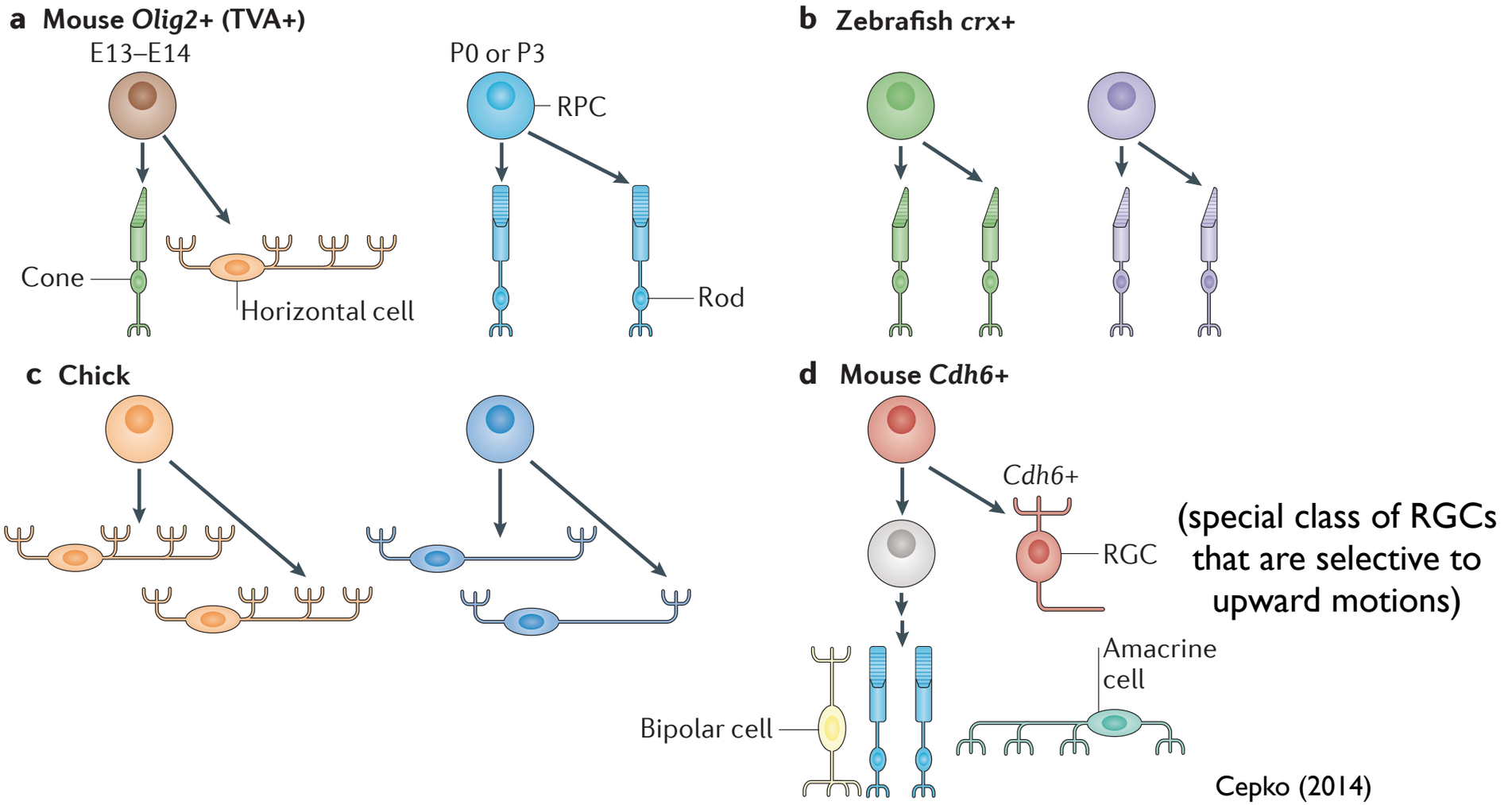
extrinsic model

Cepko (2014)

-Analysis of gene expression in single retinal progenitor cells show that they are extremely heterogeneous.

-Are differences in the expression of a particular mRNA correlated with the number or types of daughter cells produced by retinal progenitor cells?

Biases in the types of neurons produced by specific RPCs

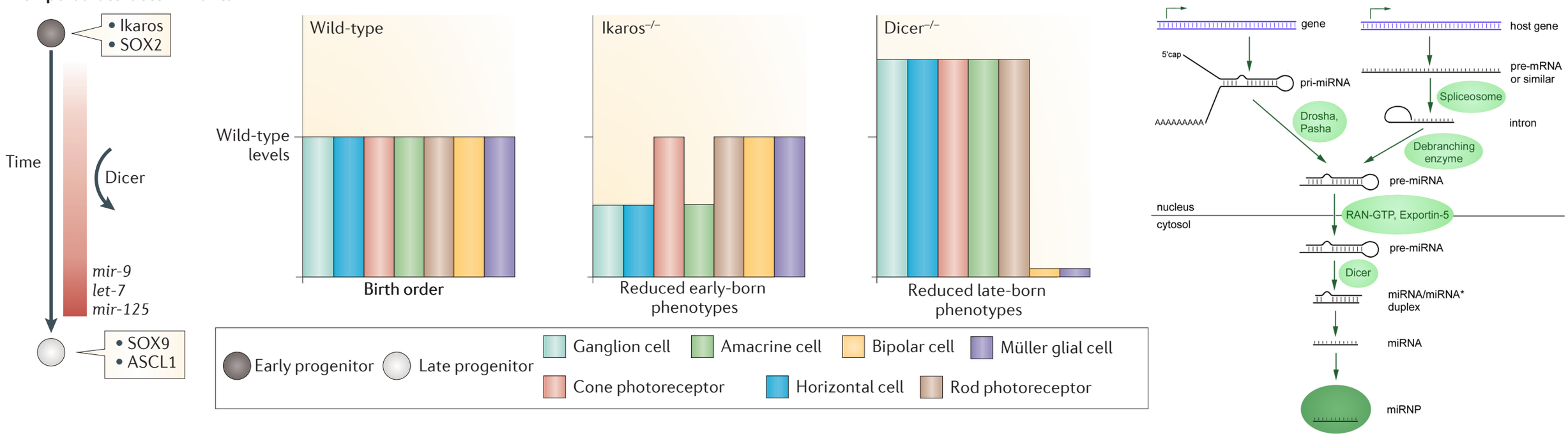


-Olig2-expressing RPCs divide only once to produce two neurons, even early in development.

-These cells seem to represent a subpopulation of terminally dividing cells.

-Cdh6-expressing RPCs generate larger clones, indicating the existence of bias in non-terminal division.

Temporal fate specification in the mammalian retina



Kohwi and Doe (2013)

-Ikaros is expressed in early progenitor cells and is necessary and sufficient for specification of early-born neuronal types (Eliott et al., 2008).

-Retina-specific deletion of *Dicer*, which is required for production of microRNA, results in the increased number of early-born cell types and the reduction of late-born cell types. Suggested target miRNAs include miR-9, let-7 and miR-125 (their over-expression accelerates early-to-late fate switch).

-Counting the number of cell cycles does not seem to regulate temporal progression of the cell fate (mice with too few or too many cell cycles still produce early- and late- cell types in the normal ratio).

Extrinsic cues affect temporal progression of retinal cell fates

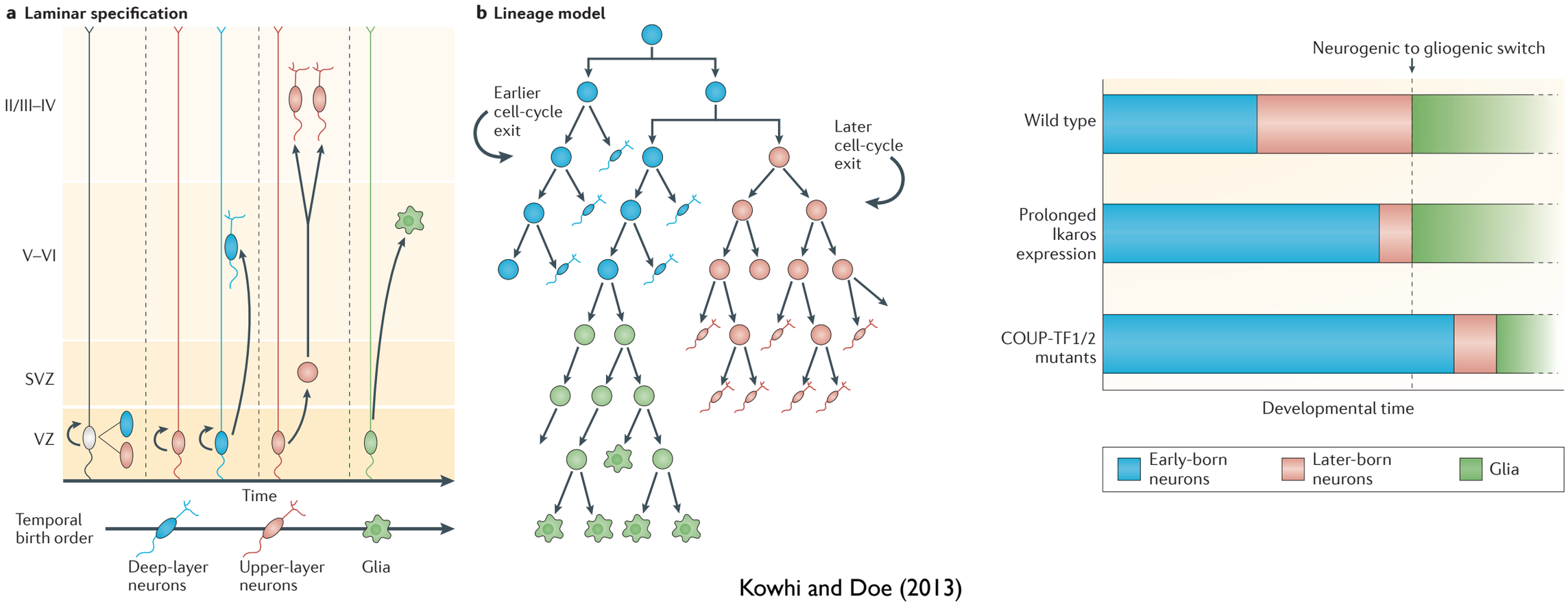
Depletion of early-born cell types (retinal ganglion cells (RGCs), amacrine cells) does not impair the production of late-born cells.....evidence against the feed-forward regulations)

Feedback inhibition does exist:

-Existing RGCs limit the production of additional RGCs through soluble factors (Shh, Gdf11).

-Existing amacrine cells limit the production of additional amacrine cells.

Temporal fate specification in the mammalian neocortex



Kowhi and Doe (2013)

-inside-out pattern of neurogenesis followed by gliogenesis
-Early progenitor cells express Ikaros.
-Prolonged Ikaros expression or deletion of COUP-TF genes delays transition.

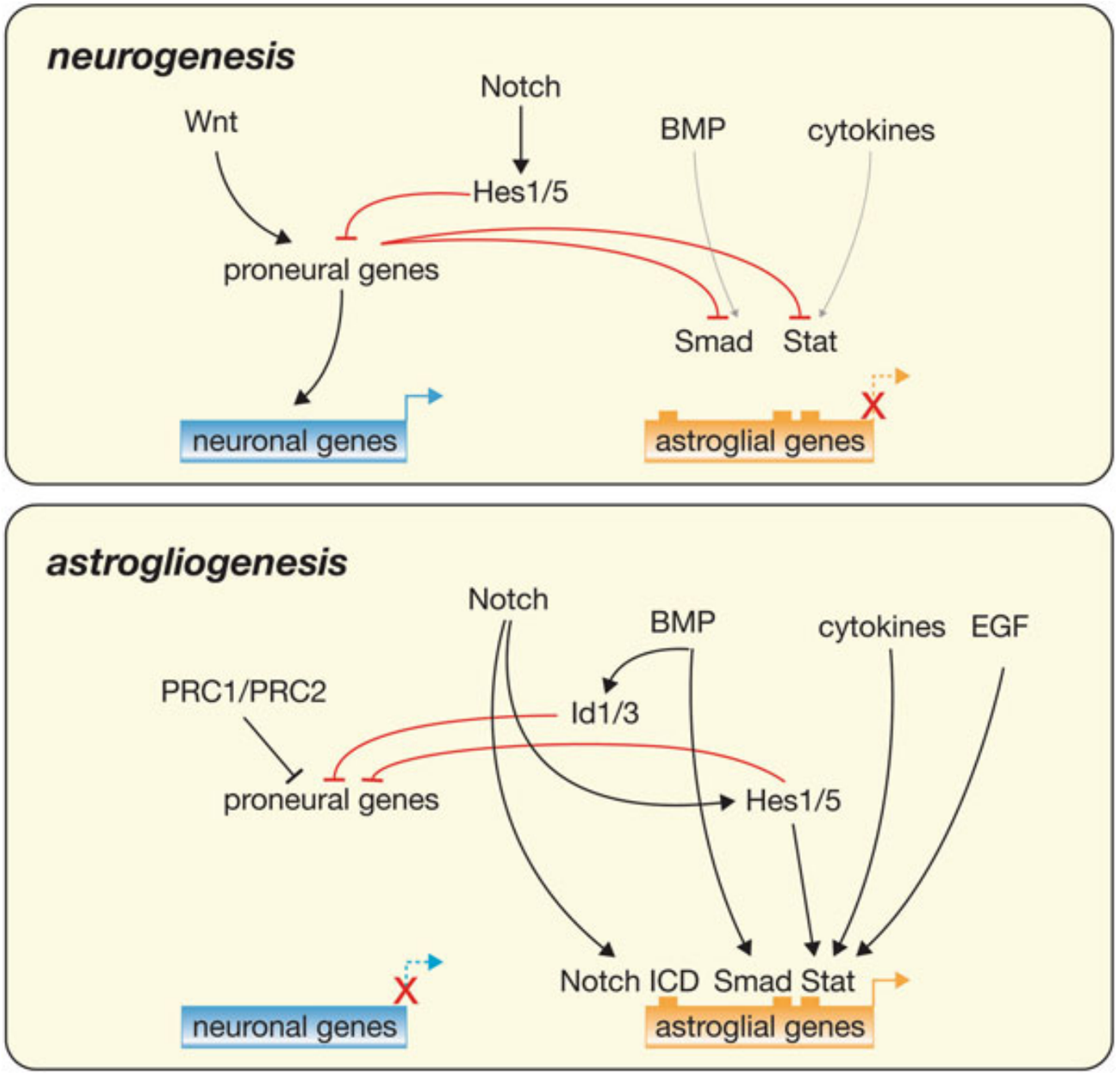
Switch from neurogenesis to gliogenesis

Intrinsic factors:
-"proneural" basic bHLH transcription factors promote neurogenesis and inhibit gliogenesis

-Gliogenic factor Sox9 is required for the timely neuron-to-glia switch.

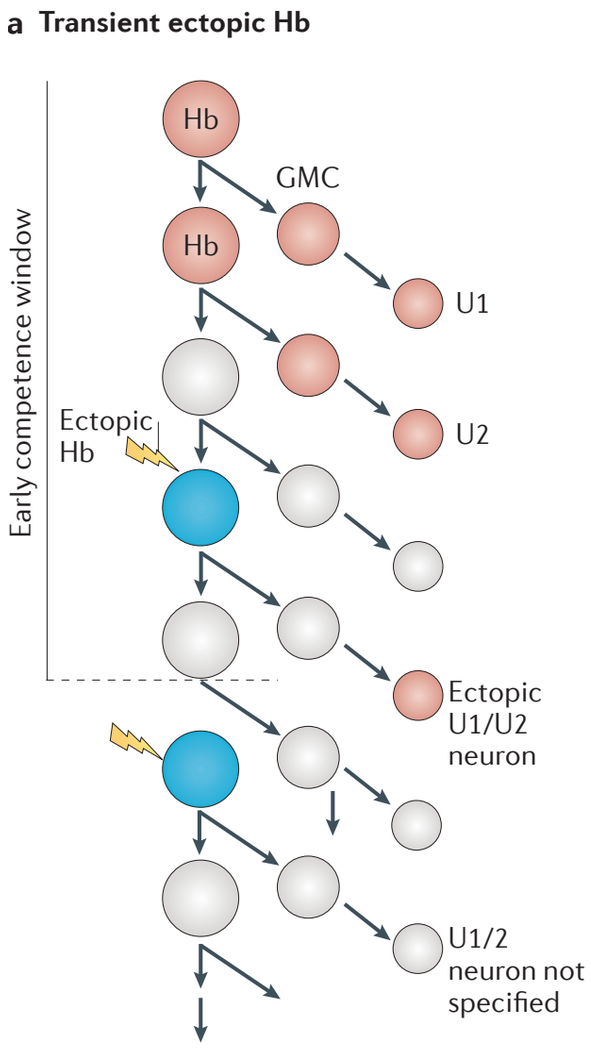
Extrinsic factors:
-cytokines activate *Gfap* transcription and promote gliogenesis
  ciliary neurotrophic factor (CNTF)
  leukemia inhibitory factor (LIF)
  cardiotropin I (CT1)...produced by newborn cortical neurons
-other factors that promote gliogenesis
  BMP
  Notch ligands

Mammalian proneural genes regulate the neuronal vs glial fate decisions

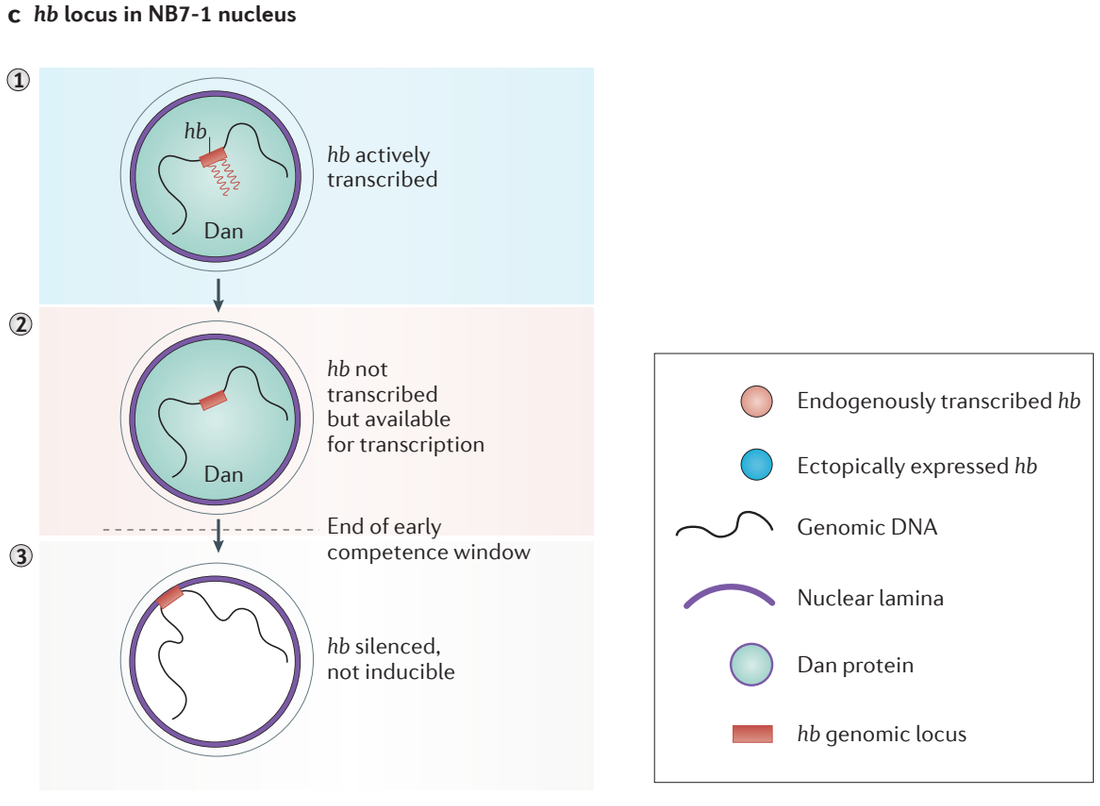


Heng and Guillemot (2013)

Changes in progenitor competence

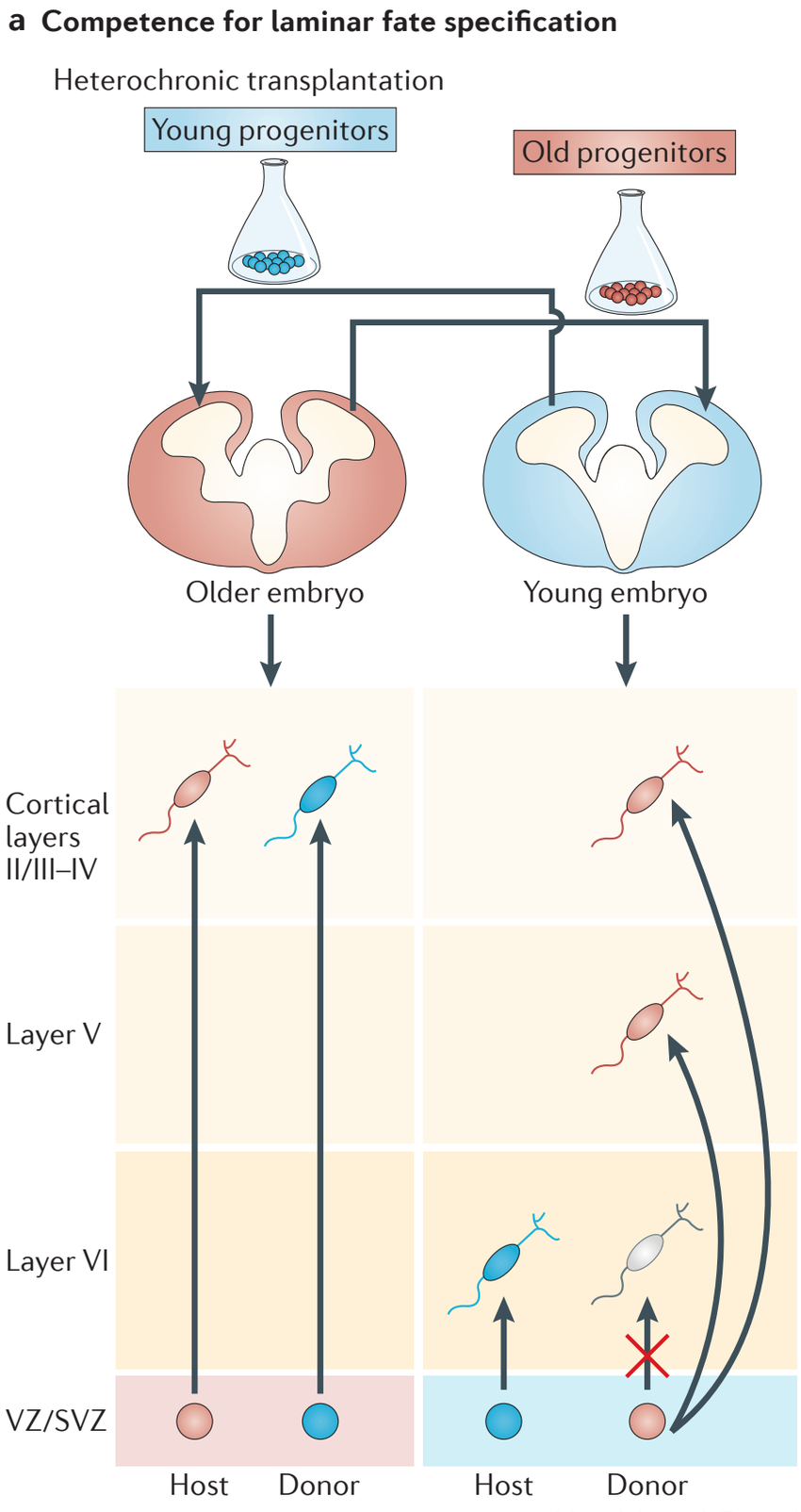


-Pulsed ectopic *Hb* expression can produce early-born neurons only up to the 5th division.
-Permanent silencing of *Hb* gene locus occurs at the end of the "competence window".
-*Hb* genomic locus is associated with the nuclear lamina, a gene silencing hub.
-Genetic disruption of the nuclear lamina reduce *Hb* gene-lamina association and delays the closure of the competence window.



Kowhi and Doe (2013)

Changes in progenitor competence



Kowhi and Doe (2013)

-Heterochronic transplantation of ferret cortical progenitor cells shows that progenitor cells lose competence to specify early-born, deep-layer neurons (McConnell, 1988; Desai and McConnell, 2000).

-Young progenitors (producing layer 6) are able to follow the host program (producing layers 2-3) only when the progenitors were transplanted prior to undergoing S-phase of the cell cycle.

-Very old progenitors (producing layers 2/3) are NOT able to follow the host program (producing layer 6) even if they undergo one or more cell divisions in the young environment.

-When old progenitor (producing layer 4) are transplanted to young (producing layer 6) donor, they do not produce layer 6 neurons but they produce layer 5 neurons.

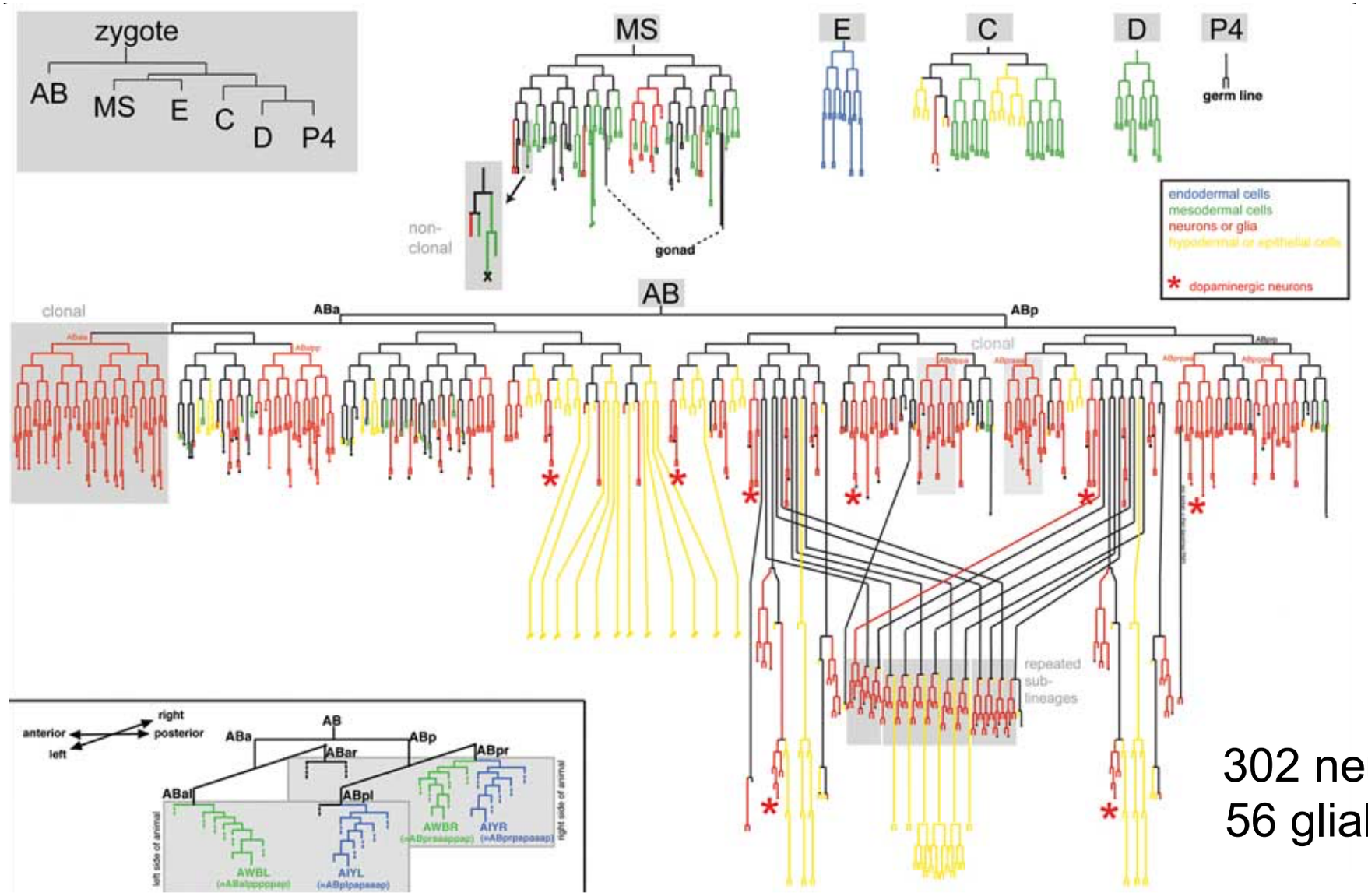
-This suggests that competence to specify temporal identity persists for a short amount of time.

Methods for analyzing cell lineage

Vertebrate models are behind C. elegans and Drosophila, in which cell lineages have been described.

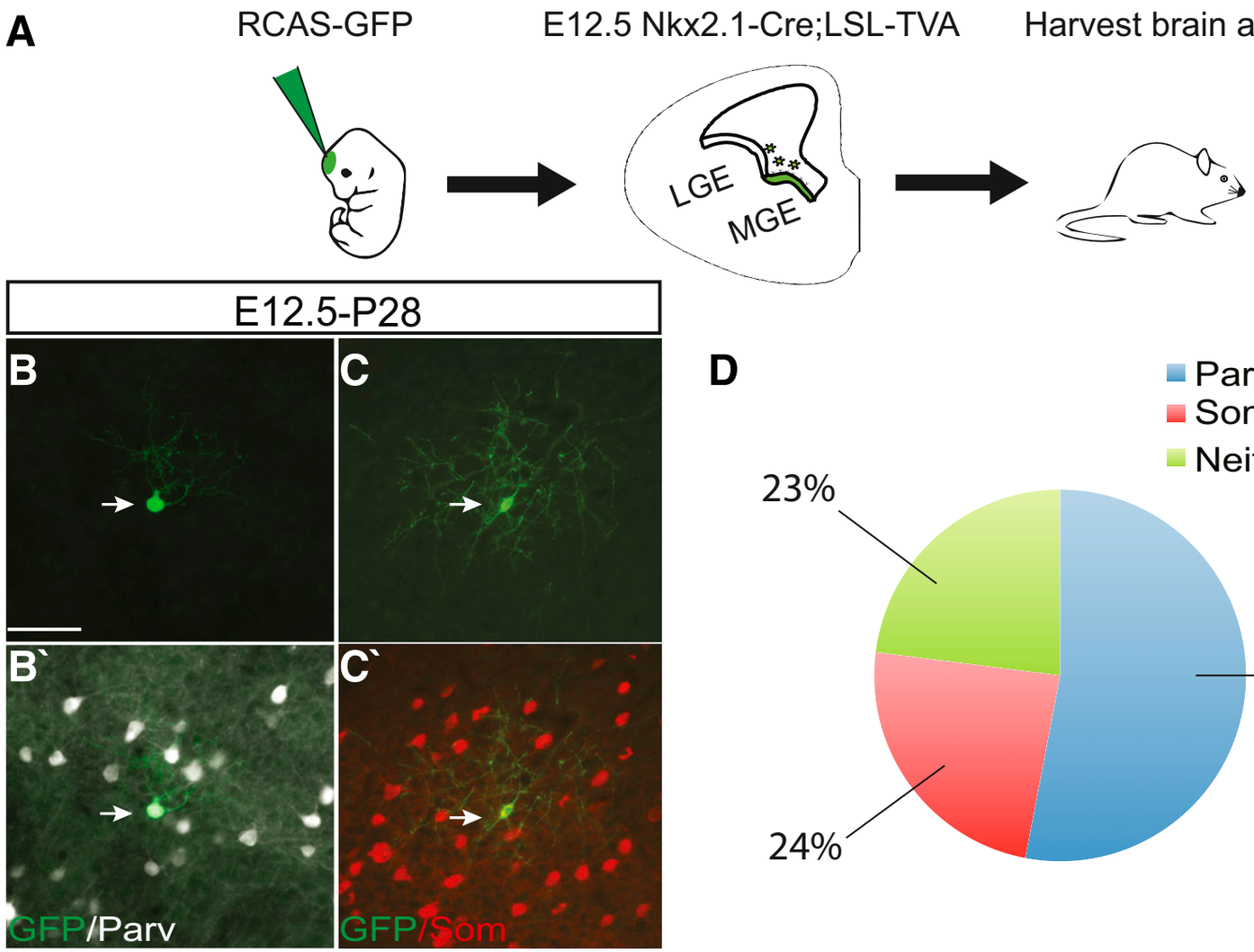
It is important to develop a clonal analysis of cell lineage in order to better understand mechanisms of cell divisions and cell fate in vertebrate nervous systems.

neural cell lineages in C. elegans



302 neurons
56 glial cells

Retrovirus vectors for lineage tracing



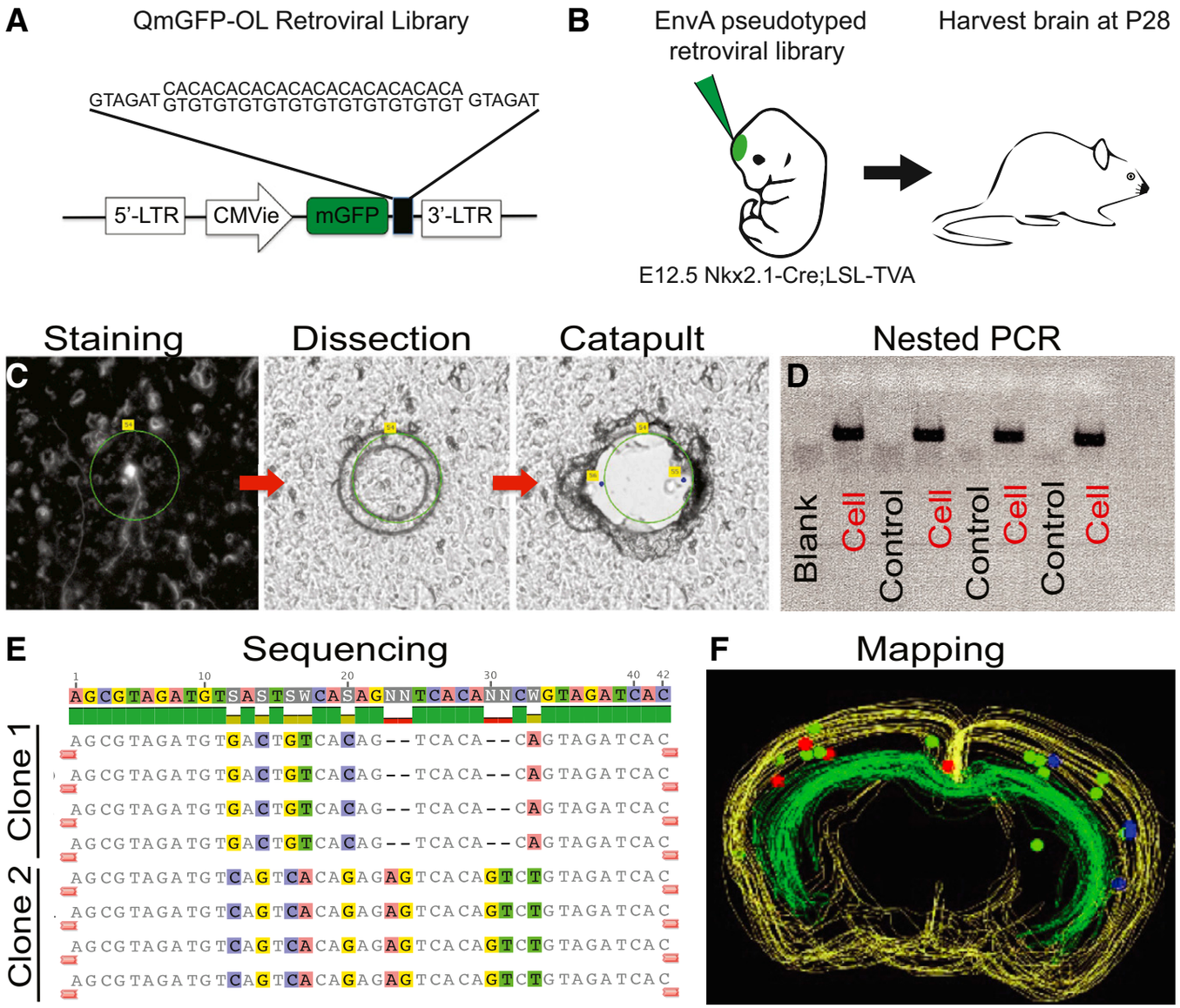
Harwell et al., 2015

Key to provide cell-type specificity
-retrovial vector (RCAS) combined with trangene-mediated supply of the receptor TVA in a specific type of neural progenitor cells expressing Nkx2.1.

-Virus can enter the mammalian cells that express TVA but cannot be released from the infected cells.

Key to provide sparse labeling
-use a low virus titer

Increasing the fidelity of single cell labeling

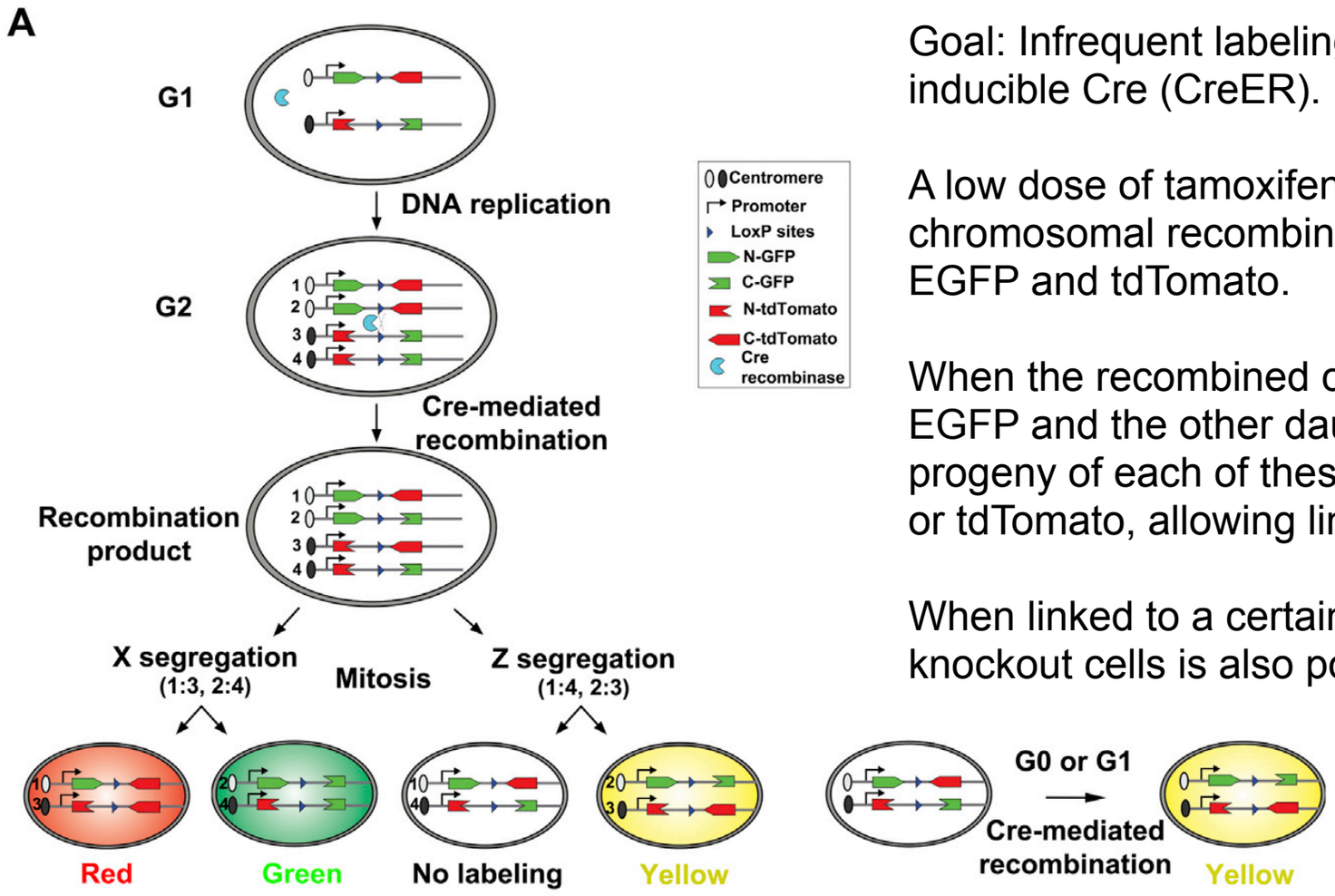


Harwell et al., 2015

Use of barcode retrovirus library
-different barcode (24bp) almost certainly guarantees a unique clone.

A limitation of the retroviral tracing
-very robust silencing underestimates lineage capabilities of stem cells

Genetic lineage tracing with MADM (mosaic analysis of dual markers)



Goal: Infrequent labeling of neural progenitor cells that express an inducible Cre (CreER).

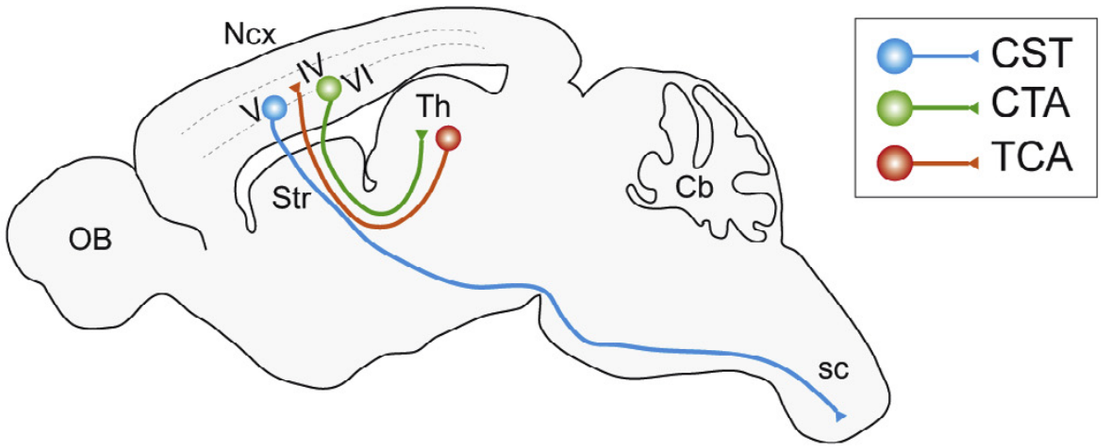
A low dose of tamoxifen allows an extremely low efficiency of inter-chromosomal recombination that results in a full reading frame of EGFP and tdTomato.

When the recombined cell divides, one daughter cells expresses EGFP and the other daughter cell expresses tdTomato. All the progeny of each of these daughter cells continue to express EGFP or tdTomato, allowing lineage tracing.

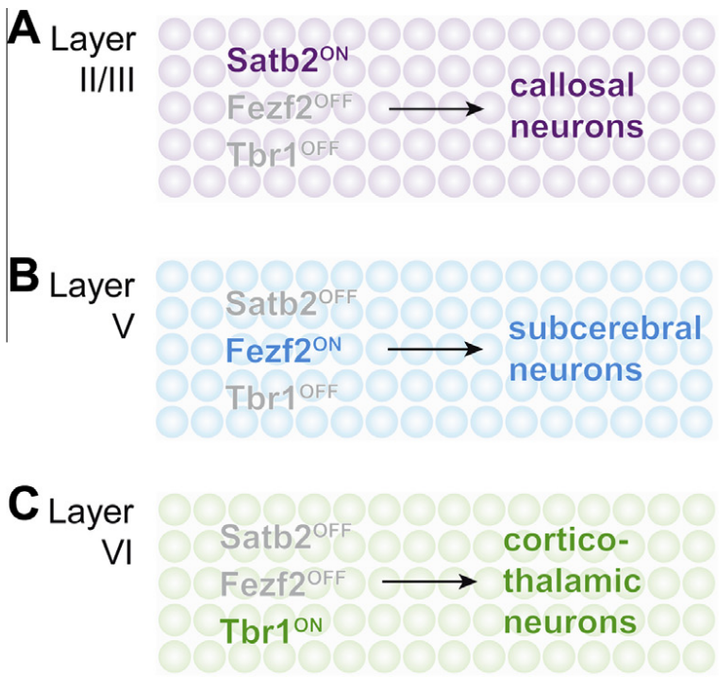
When linked to a certain gene mutation, in vivo mosaic analysis of knockout cells is also possible.

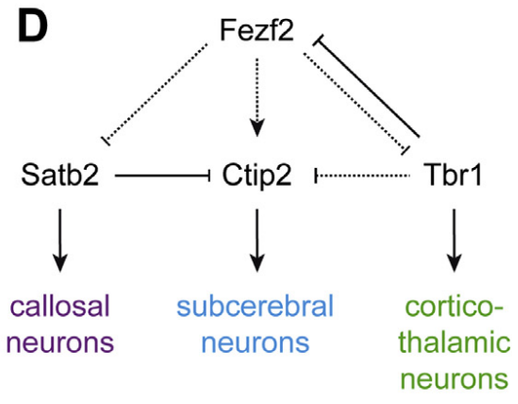
Gao et al., 2014

Regulation of postmitotic cell fate by transcription factors



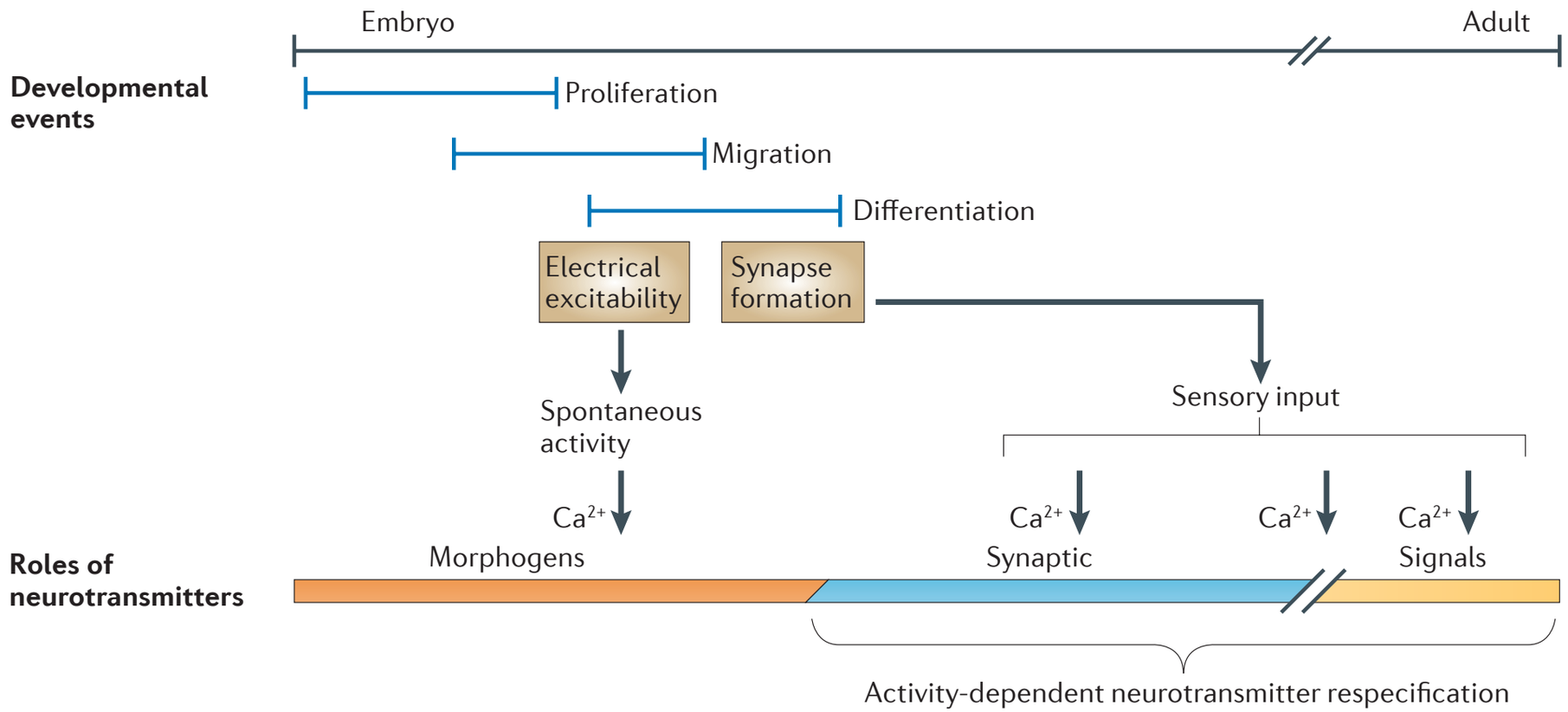
-Ectopic expression of Fezf2 in upper cortical neurons can reprogram them to adopt the fate of deep layer neurons (marker expression and subcortical projection). But the switch of fate occurs only for a brief time window after the final mitosis.





Lopez-Bendito (2013)

Regulation of postmitotic cell fate by neuronal activity



Spitzer (2012)

Neurons elicit spontaneous activity (Ca spikes) and transmitter release before synapse formation.

Summary

Control of daughter cell fates after cell division (RGC vs differentiating cell)
-The daughter cell receiving Notch signaling stays as an RGC by activating the bHLH protein Hes1 and Hes5.
-The daughter cell expressing proneural bHLH transcription factors (e.g. Neurogenins or Ascl1) and Delta (=Notch ligand) undergoes differentiation. Proneural bHLH factors promote neuronal differentiation and Delta keeps the neighboring progenitor cell from differentiating.

Identity of progenitor cells influence the type of neurons that the progenitor cell produces.
-regionalization establishes positional identity of neural progenitor cells.
-Ngn2 and Ascl1 are differentially regulated in developing brain by regionalization, and promote differentiation of glutamatergic and GABAergic neurons, respectively.
-RGCs changes their identity and fate potential as they undergo asymmetric divisions.

Neuronal fate can be controlled postmitotically.
-Many transcription factors are known to regulate genes that are specific to certain neuronal types.
-Neuronal activity also regulates gene expression, thus neuronal identity.