

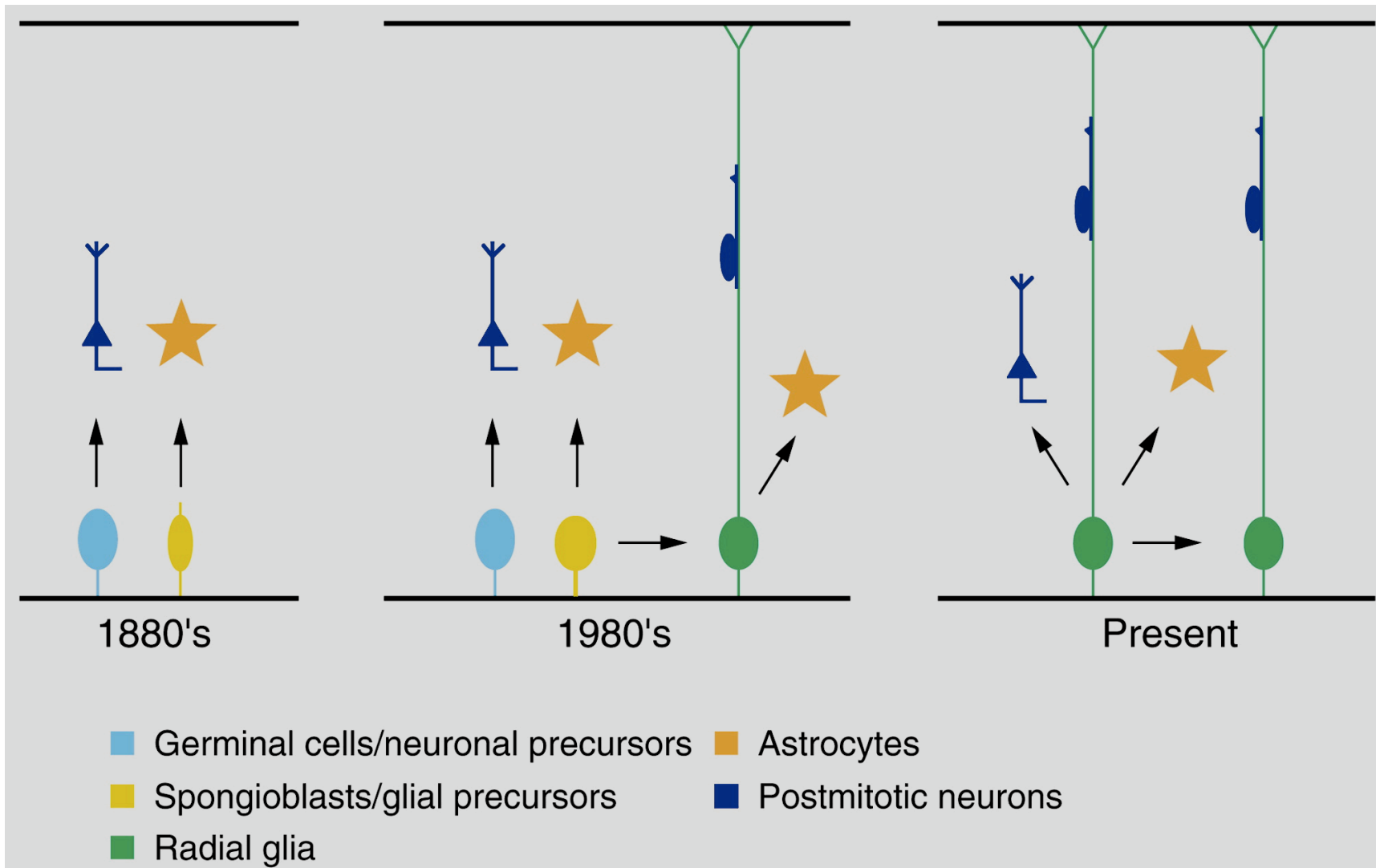
Neural stem cells

- Neurons in CNS
- Macroglial cells
 - Astrocytes
 - Oligodendrocytes

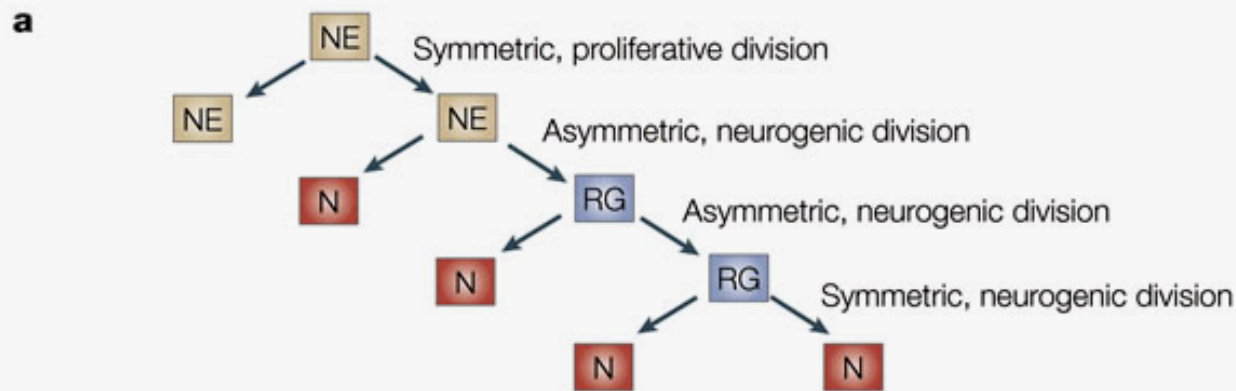
Neural Stem Cells

- Self-renewing (may be limited)
- Multipotent or unipotent
- Neuroepithelial cells can be considered as neural stem cells

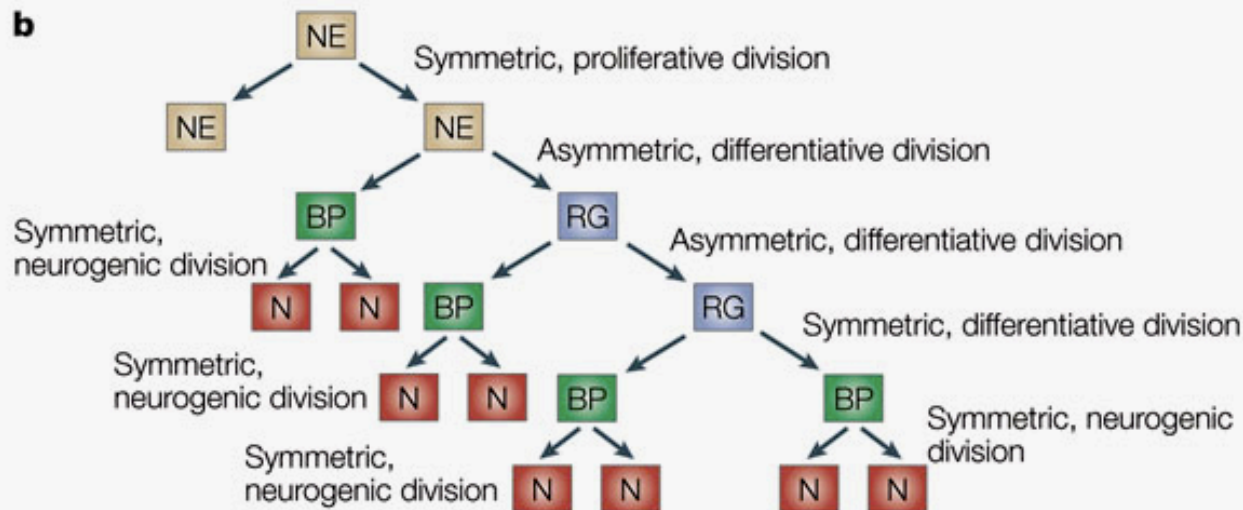
Radial glial cells



Lineage trees of neurogenesis



NE – neuronal epithelium



N – Neuron

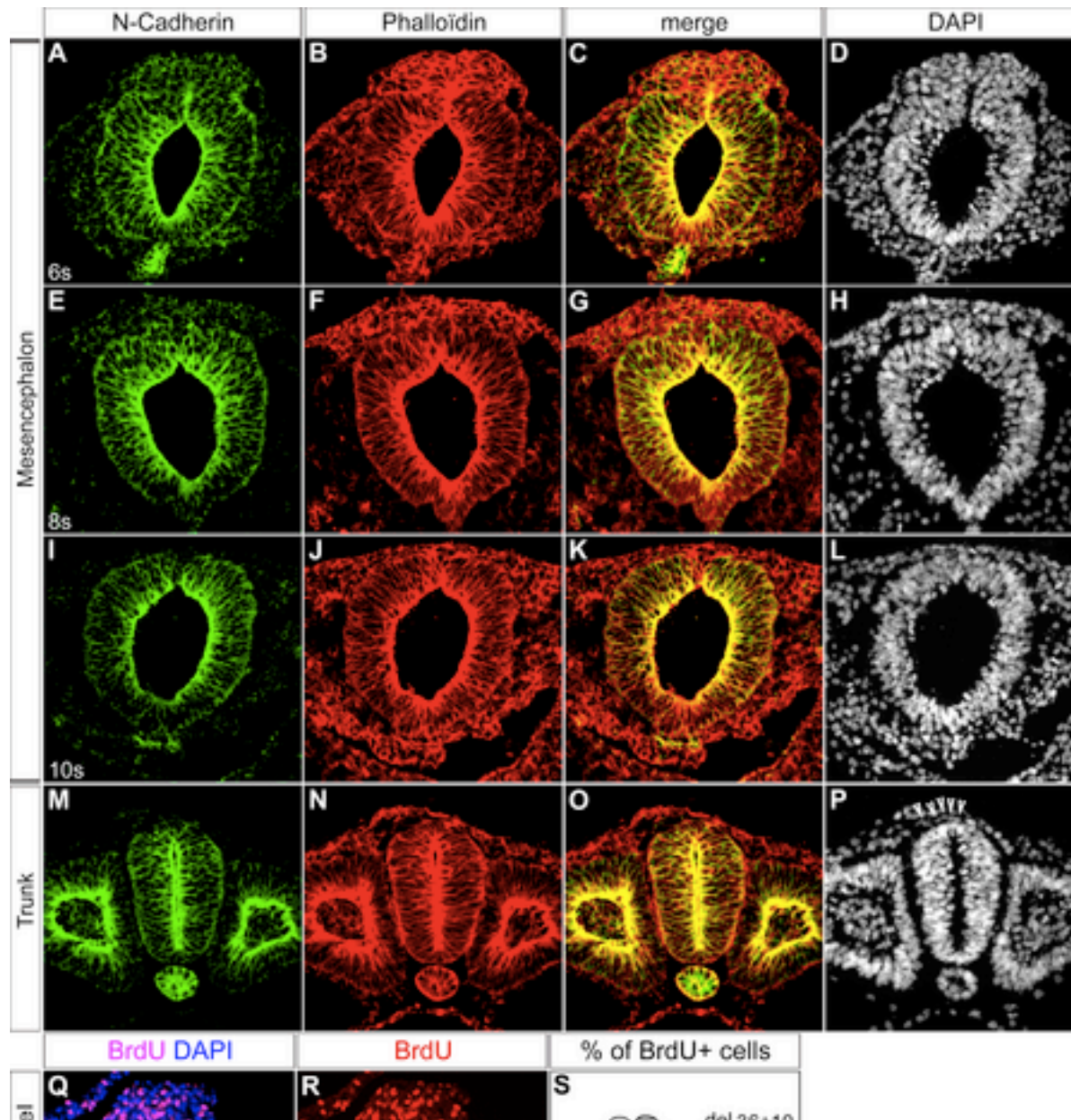
RG- radial glial

BP – basal progenitors

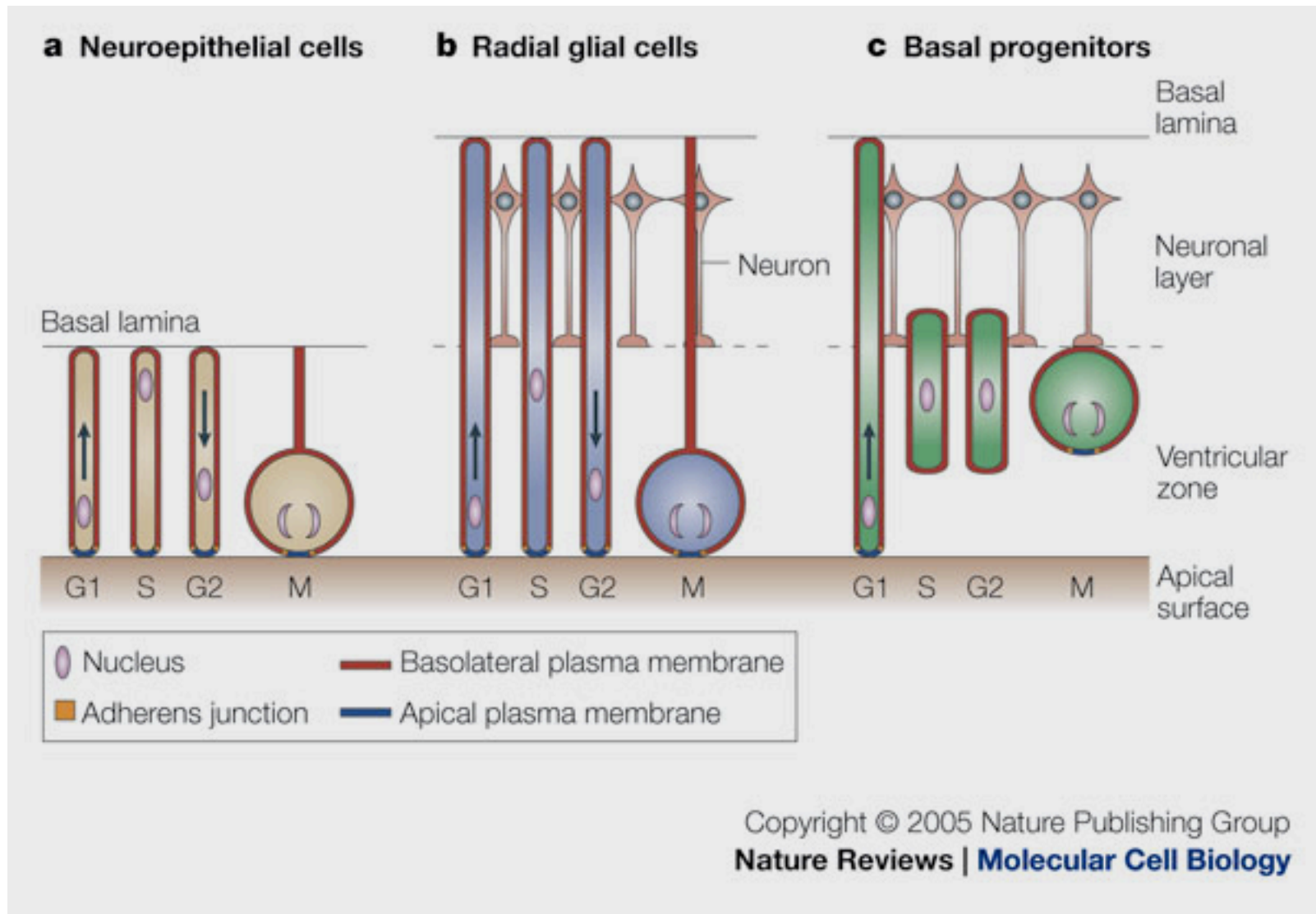
Neuroepithelium

- Formed of single layer of cells that appear stratified (pseudostratified) because of the nuclear positioning
- Nuclear positioning is due to interkinetic nuclear migration

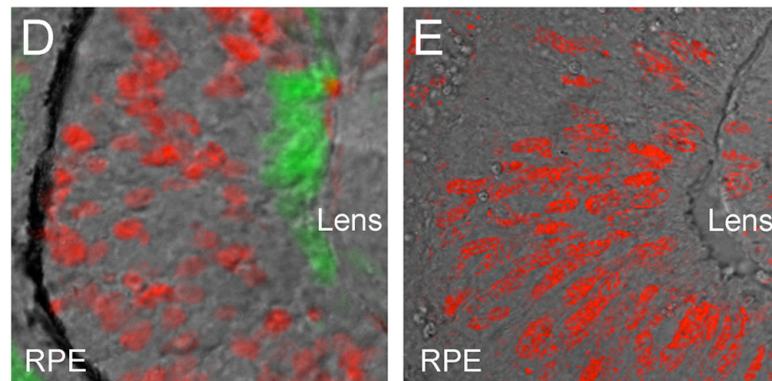
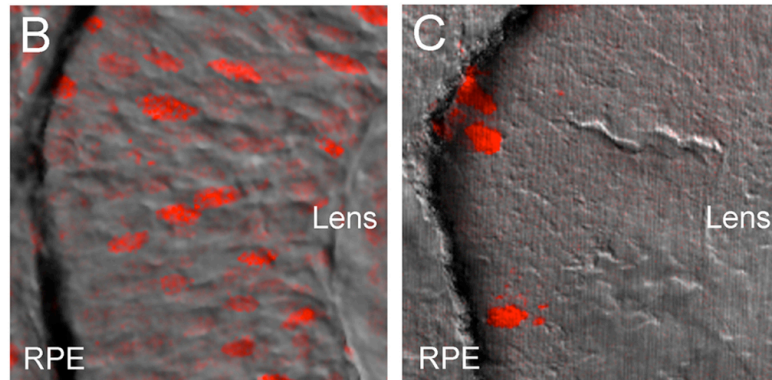
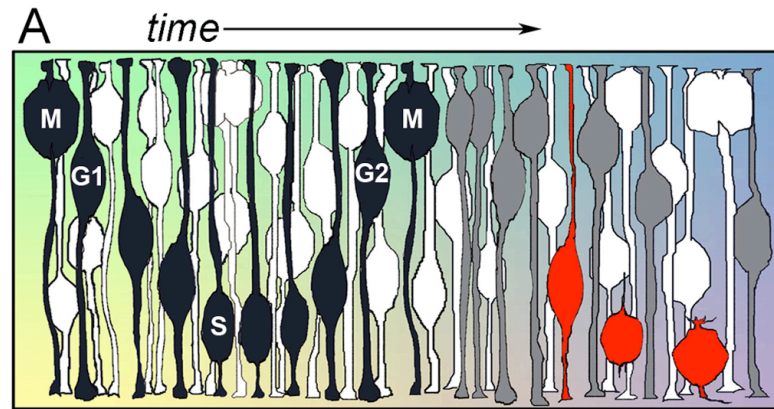
Neural epithelium



Polarize features of neuroepithelial and Radial glial cells

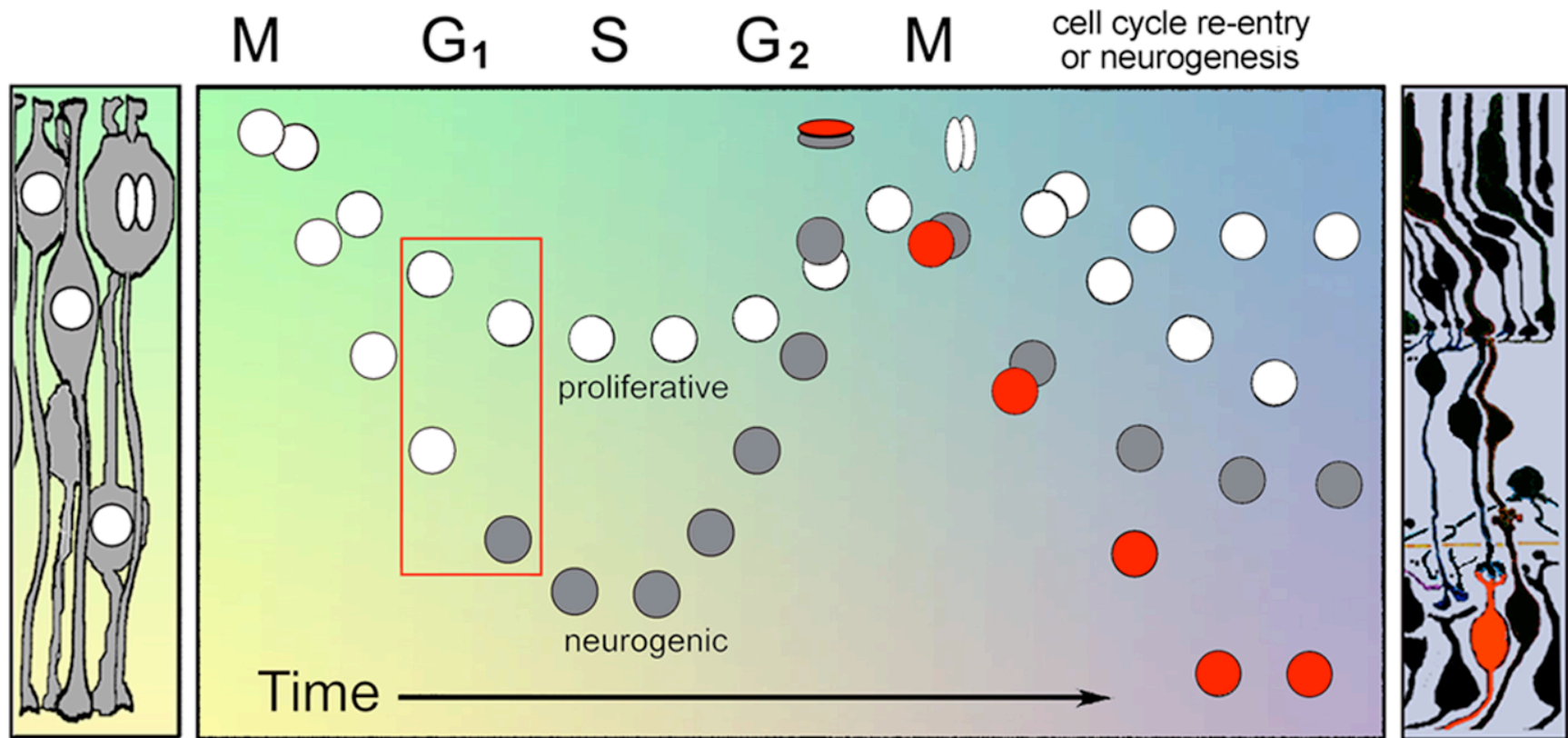


Interkinetic nuclear migration

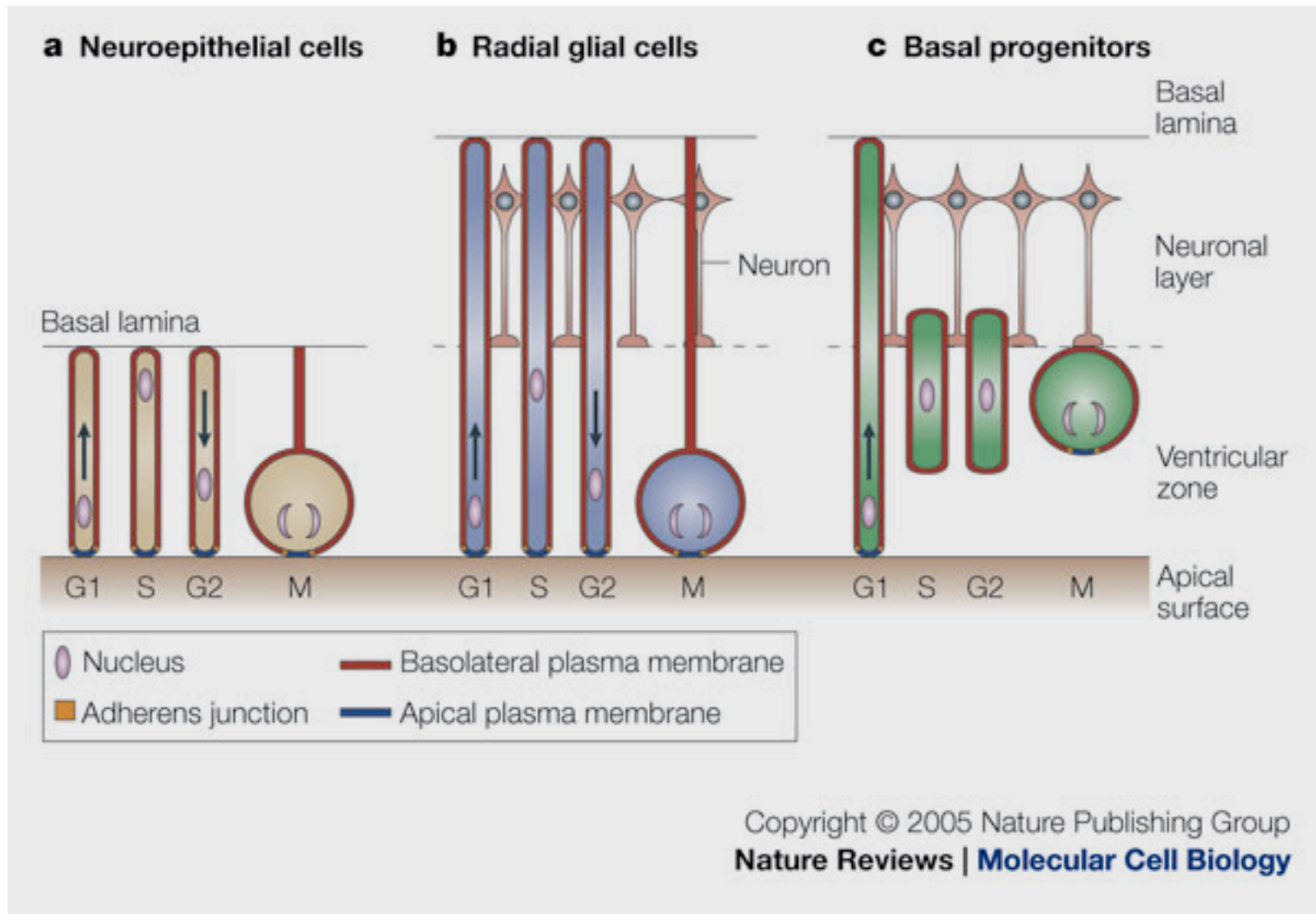


Retinal pigment
epithelium

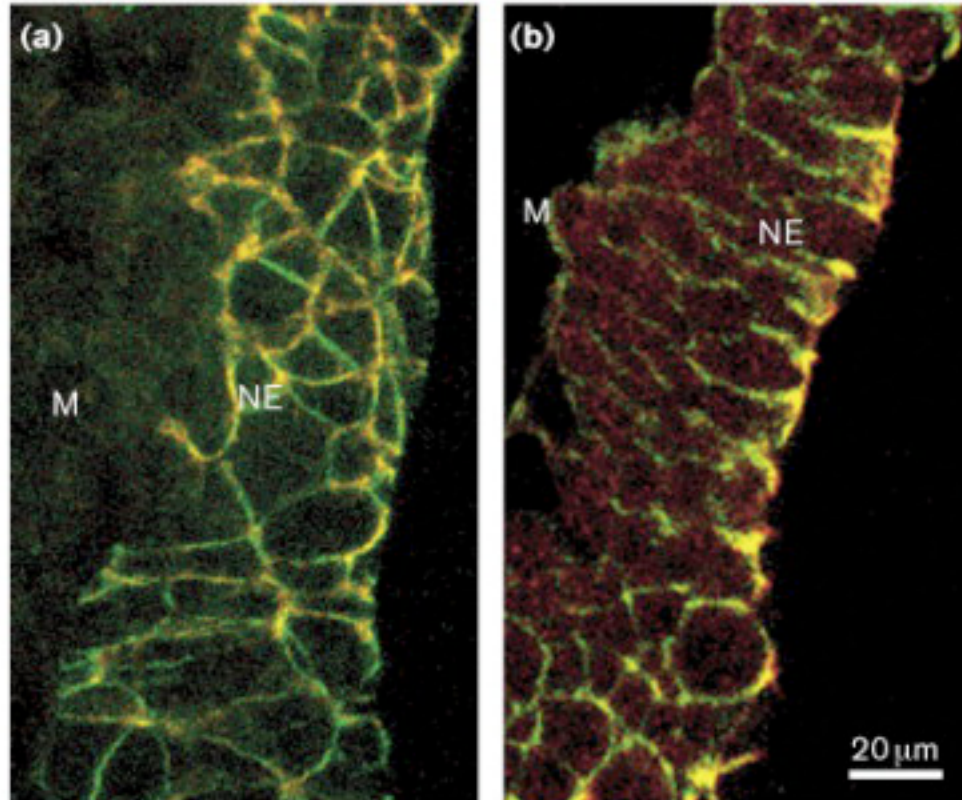
Interkinetic nuclear migration



Polarize features of neuroepithelial and Radial glial cells



Polar nature of neuralepithelial cells



Current Biology

Localization of e-cadherin in neuroectoderm

Neuroepithelium/Radial Glial Cells

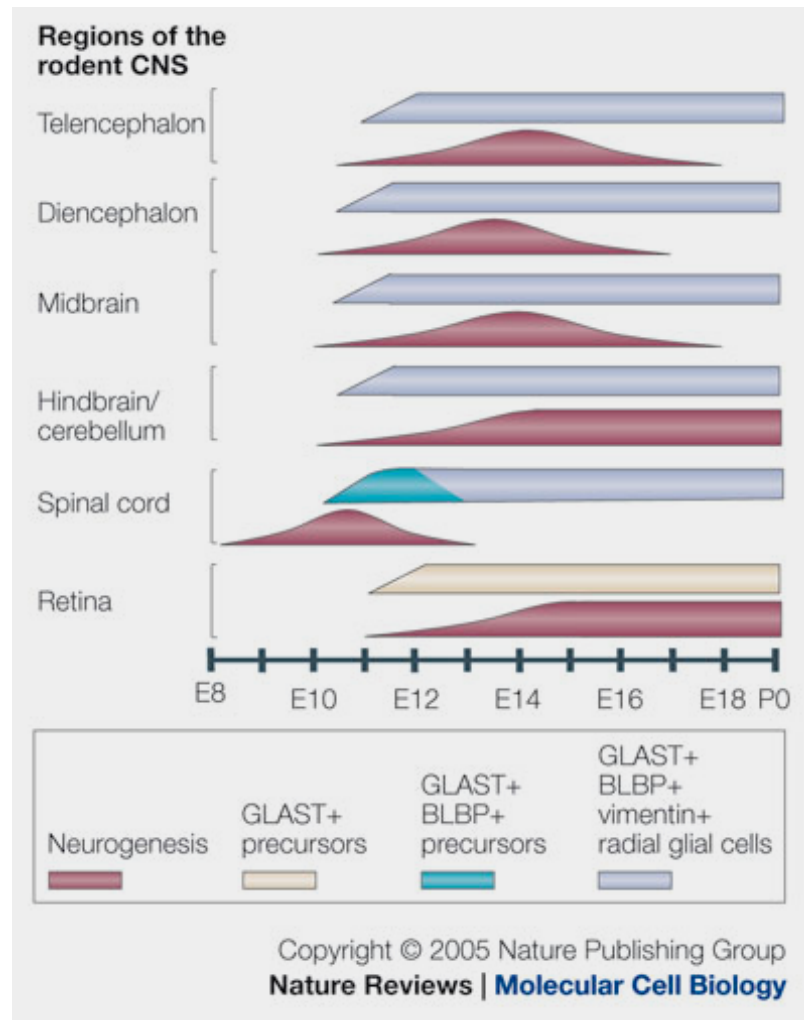
Table 1 | **Comparison of the properties of neuroepithelial and radial glial cells**

Property	Neuroepithelial cells	Radial glial cells
Interkinetic nuclear migration	Apical–basal	Apical–basal to the boundary of the ventricular or subventricular zone
Apical surface	Present	Present
Apical–basal polarity	Present	Present, but downregulated
Tight junctions	Present (early stages)	Absent
Adherens junctions	Present	Present
Basal lamina contact	Present	Present
Nestin expression	Present	Present
Astroglial markers	Absent	Present
<i>Tis21</i> expression*	Confined to the neurogenic subpopulation	Present in the neurogenic subpopulation
Neurogenesis	First phase	Subsequent phases

*The antiproliferative gene *Tis21* is a molecular marker that is selectively expressed in virtually all neuroepithelial cells that are about to undergo a neurogenic division, but not in proliferating neuroepithelial cells⁷⁴.

Nestin – intermediate filament – specific to neurons

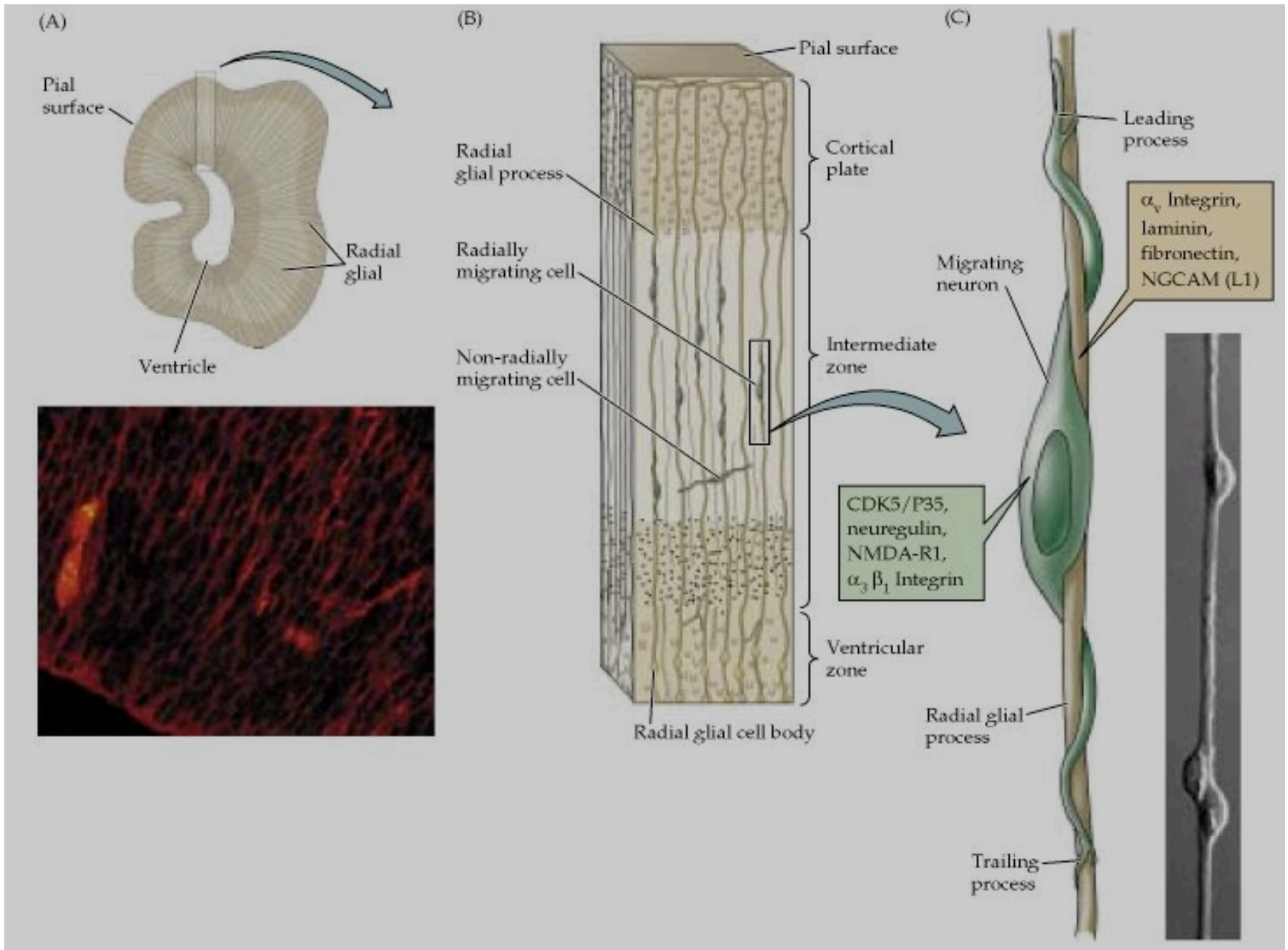
Radial glial cells in various regions rodent CNS



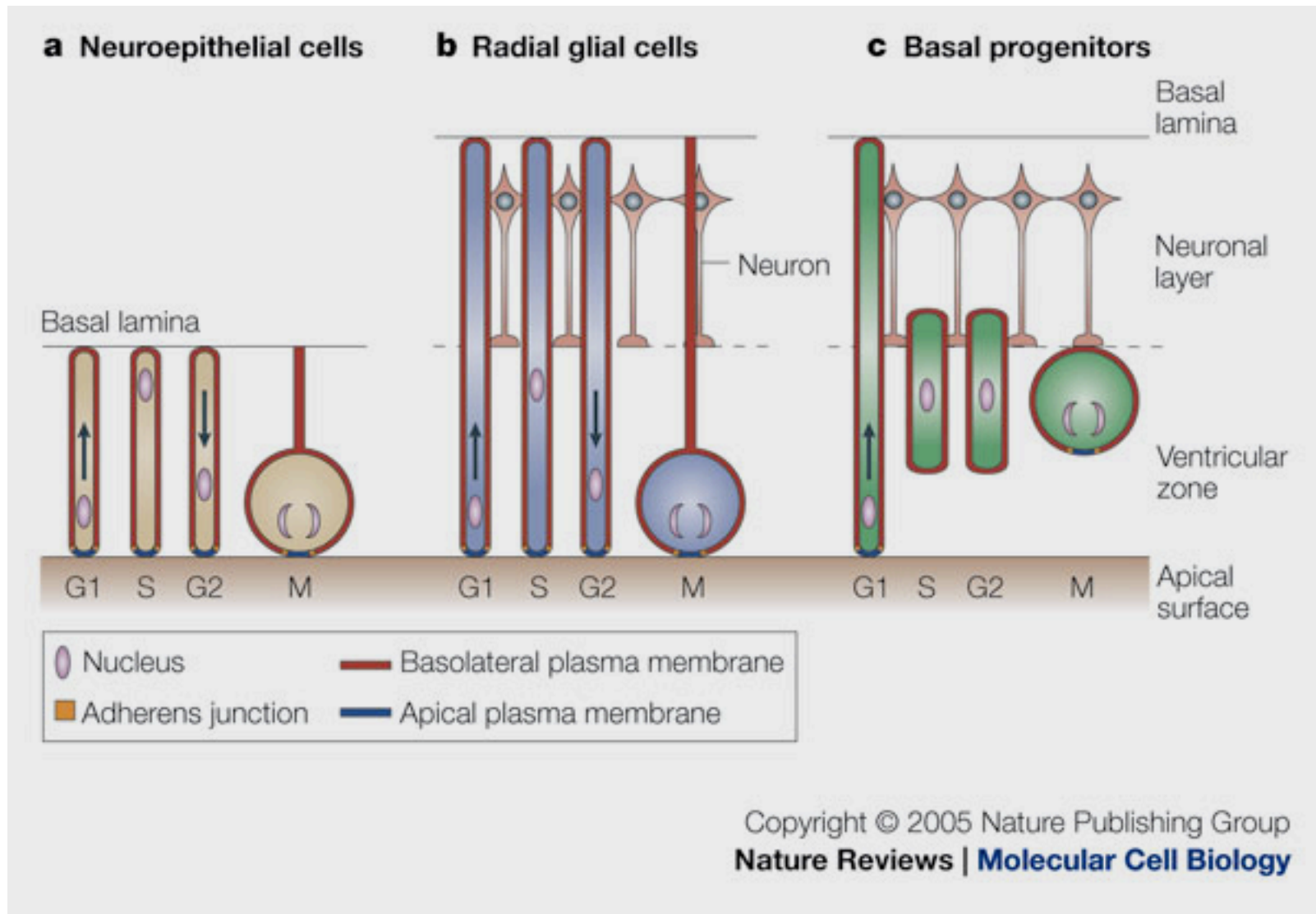
Astrocyte-specific
glutamate
transporter
(GLAST)

Brain-lipid-
binding protein
(BLBP)

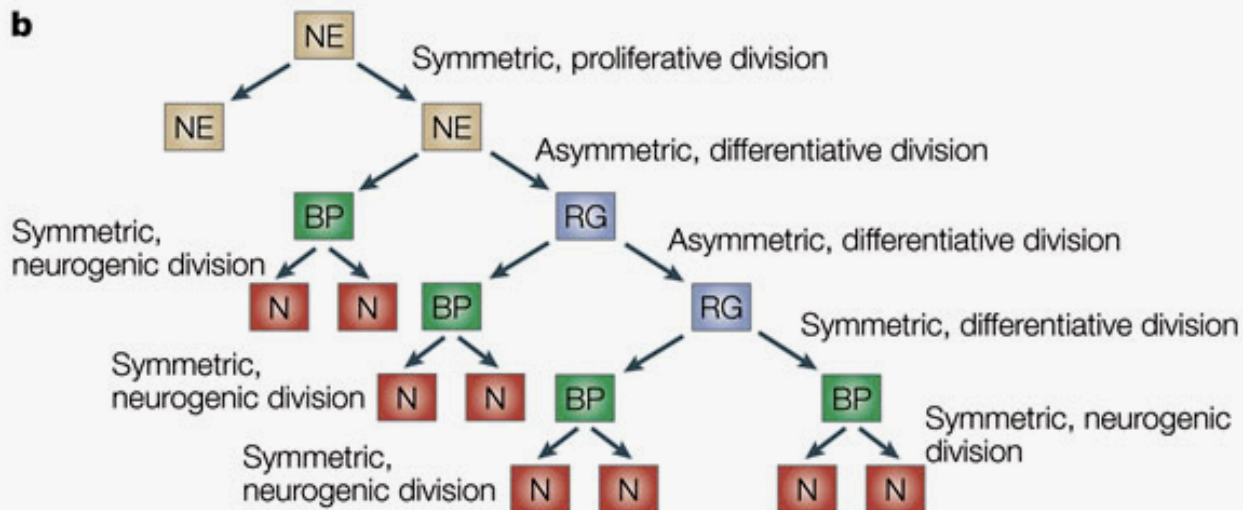
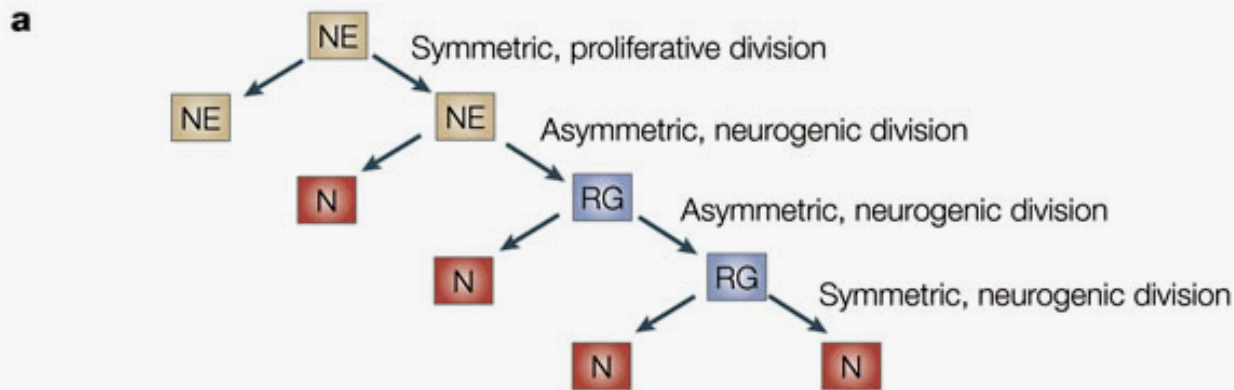
Vimentin – neuron
specific intermediate
filament



Basal progenitors



Basal progenitors



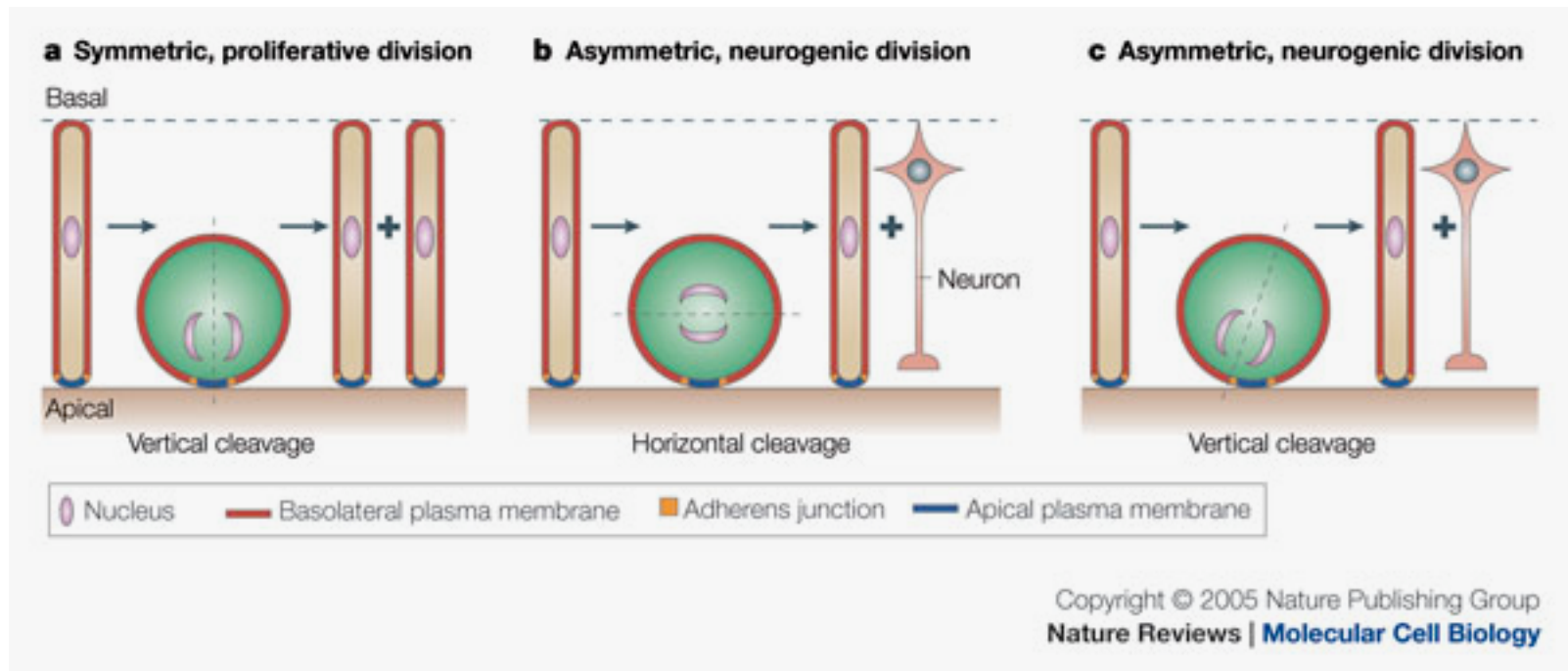
NE – neuronal epithelium

N – Neuron

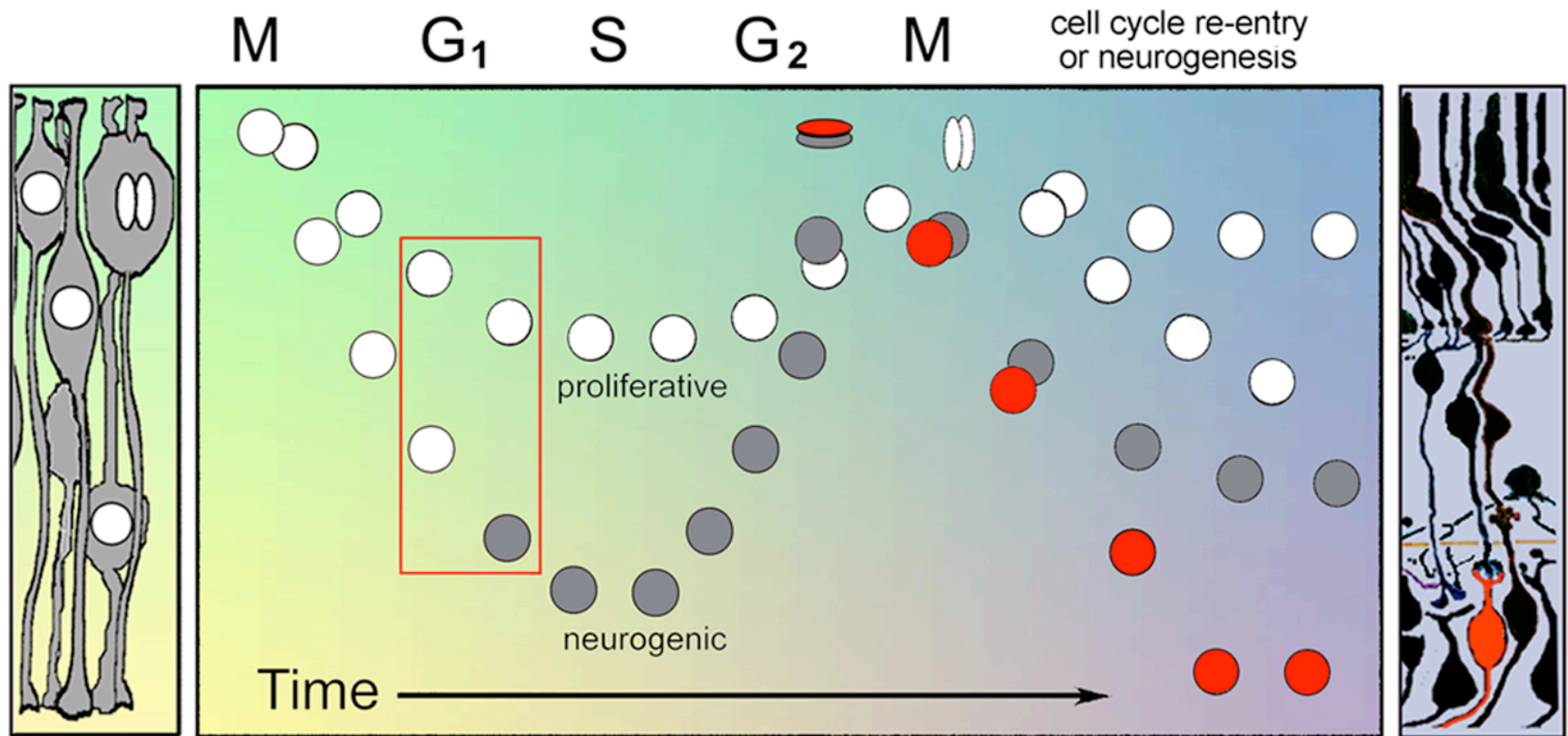
RG- radial glial

BP – basal progenitors

Cleavage plane orientation and cell polarity

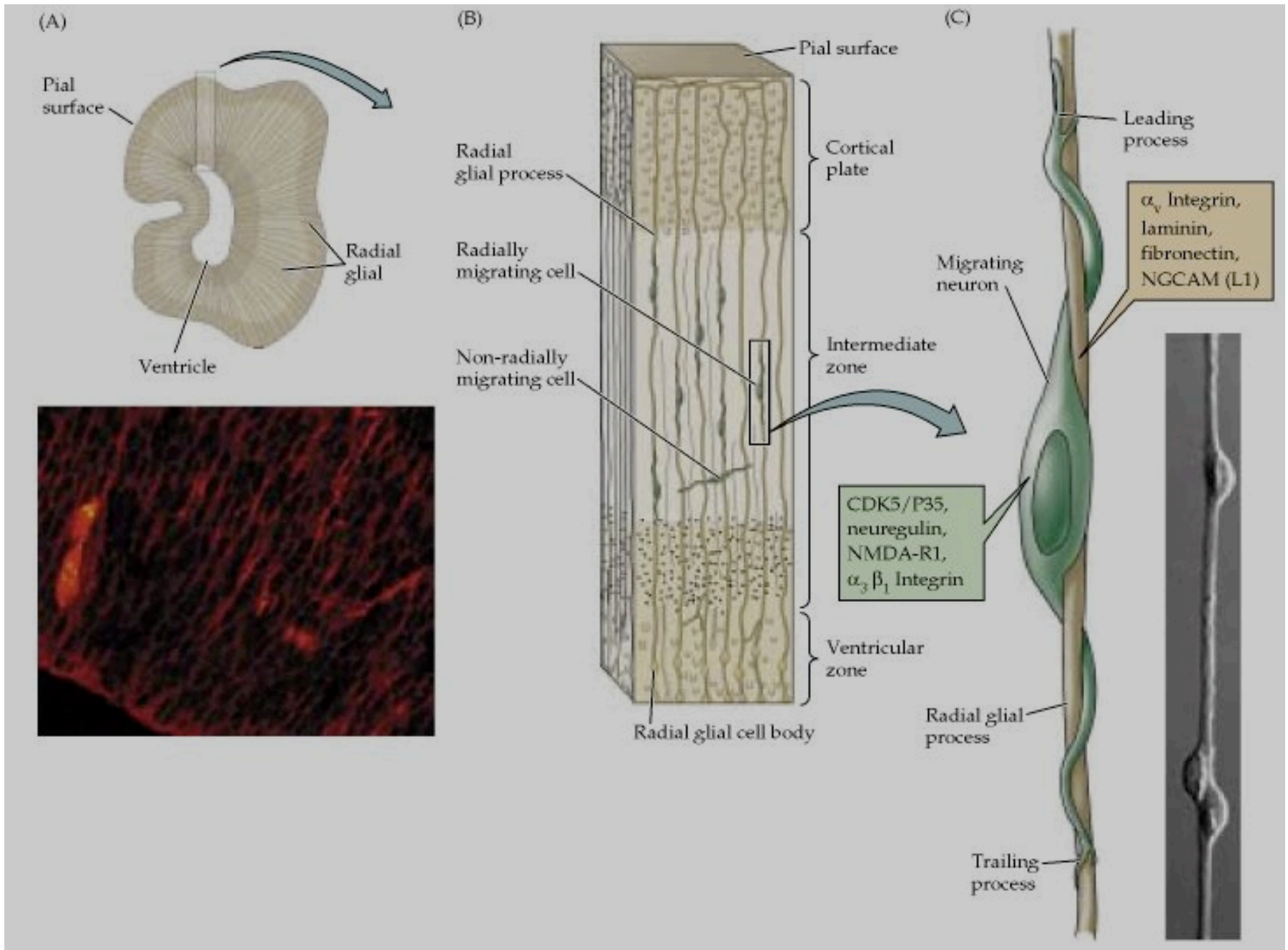


Interkinetic nuclear migration



Neurogenesis

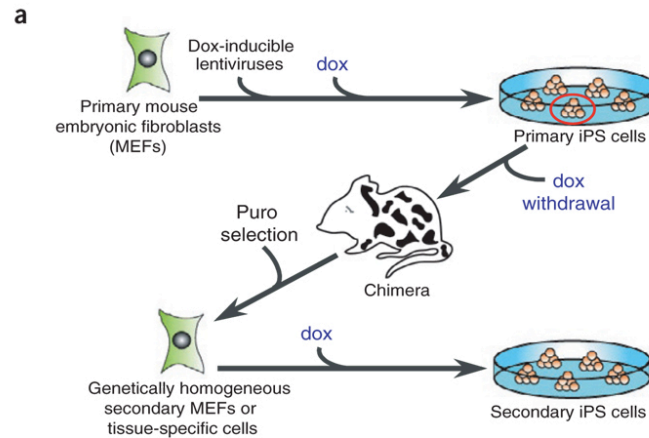
- The role of interkinetic migration is not well understood
- The plane of division and the duration of cell division during early stages of neurogenesis is controversial and not well understood
- A systematic analysis of gene regulation and gene products during early neurogenesis could resolve some of the issues



iPS

- **Takahashi, K. & Yamanaka** - *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676 (2006)*
- **Marius Wernig et al.** - *In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state Nature 448, 318-324 (19 July 2007)*
- **Genes needed for complete reprogramming - *Oct4, Sox2, Klf4 and c-Myc***

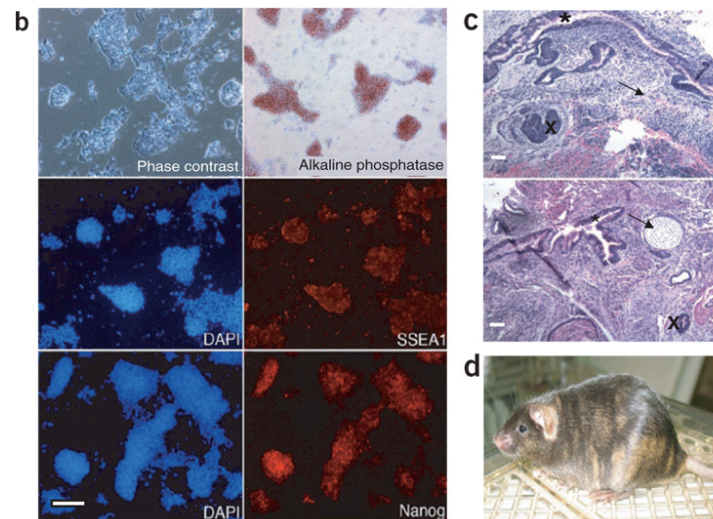
Drug inducible iPS



Background of MEFs used for primary infections:

Nanog-neo MEFs: ROSA26-rTA/pgk-puro
Collagen1A1-tetO-Oct4
Nanog-neo

Nanog-GFP MEFs: ROSA26-rTA/pgk-puro
Nanog-GFPiresPuro



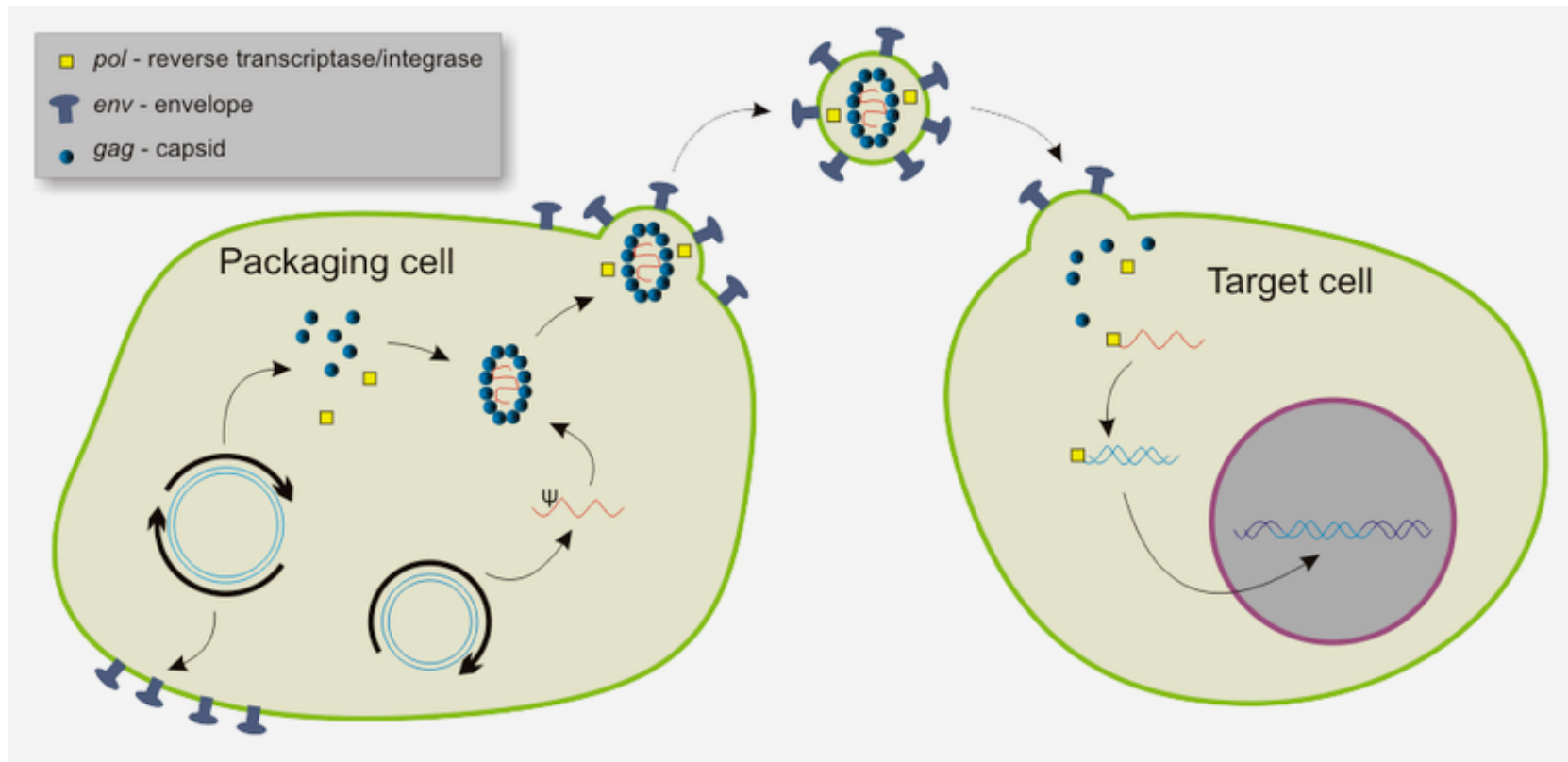
Transformation of differentiated cells to other cell fates

- Basic helix–loop–helix (bHLH) transcription factor MyoD (also called Myod1) can induce muscle-specific properties in fibroblasts but not hepatocytes
- Ectopic expression of interleukin (IL)-2 and granulocyte–macrophage colony-stimulating factor receptors can lead to myeloid conversion in committed lymphoid progenitor cells
- FGF – induction of neural cells

Direct conversion of fibroblasts to functional neurons by defined factors -Marius Wernig Lab

- Used TauEGFP knock-in mice
- Prepared MEF cell-lines without neuronal cell contamination (used immunofluorescence, PCR, FACS to verify)
- Rare *Tuj1* positive cells which were *TauEGFP* negative
- Used a lentiviral vector to introduce 19 TF

Lentiviral life cycle



Neuronal specific TF tested

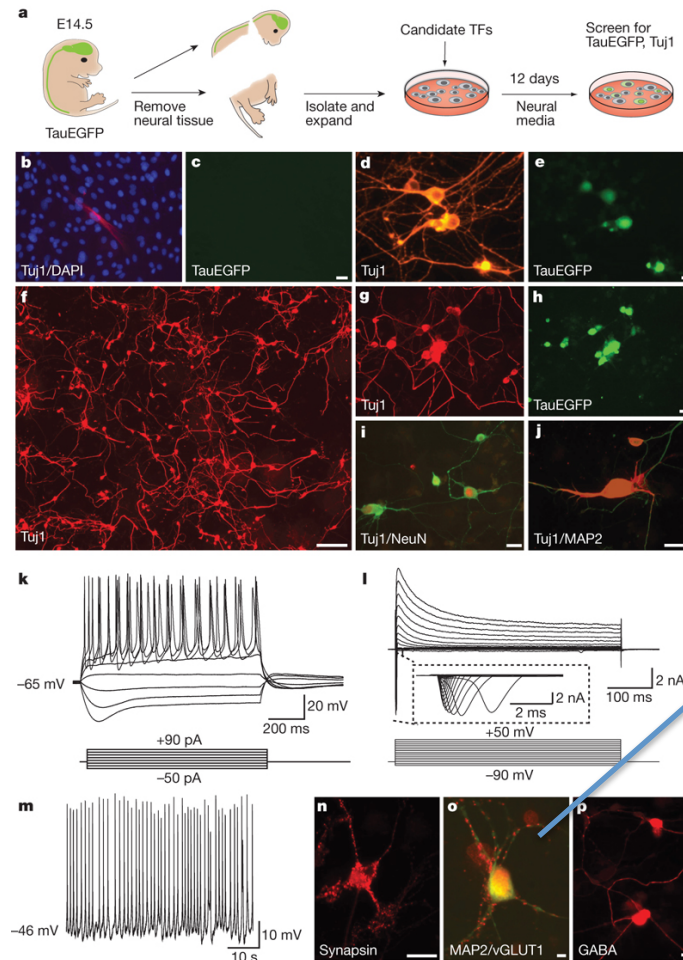
doi: 10.1038/nature08797

SUPPLEMENTARY INFORMATION

Supplemental Table 1: Transcription factors screened for neuron-inducing activity in MEFs

Gene Name	Gene Bank
Ascl1	NM_008553
Brn2	NM_008899
Brn4	NM_008901
c-myc	NM_010849
Dlx1	NM_010053
Hes5	NM_010419
Id1	NM_010495
Id4	NM_031166
Klf4	NM_010637
Lhx2	NM_010710
Mef2c	NM_025282
Myt1l	NM_001093775
NeuroD1	NM_010894
Nhlh1	NM_010916
Nr2f1	NM_010151
Olig2	NM_016967
Pax6	NM_013627
Sox2	NM_011443
Zic 1	NM_009573

A screen for neuronal-fate-inducing factors and characterization of MEF-derived iN cells.

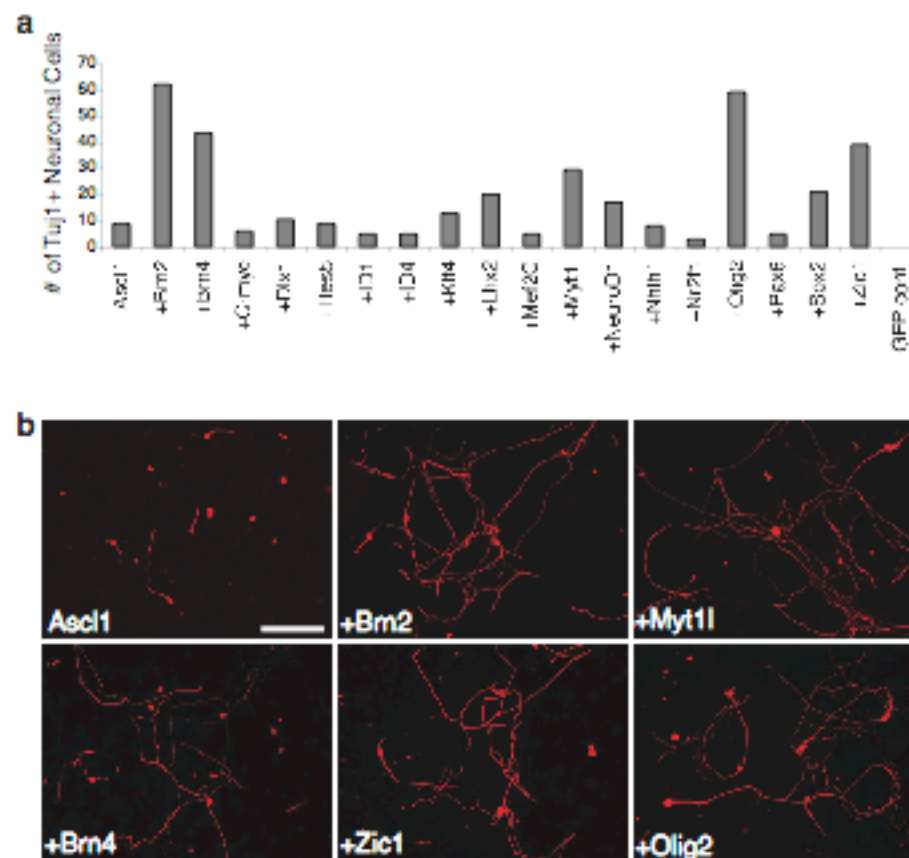


• vGLUT1-positive-
indicating the presence of
excitatory, glutamatergic
neurons

• GABA positive, the major
inhibitory
neurotransmitter in brain

T Vierbuchen *et al.* *Nature* **000**, 1-7 (2010) doi:10.1038/nature08797

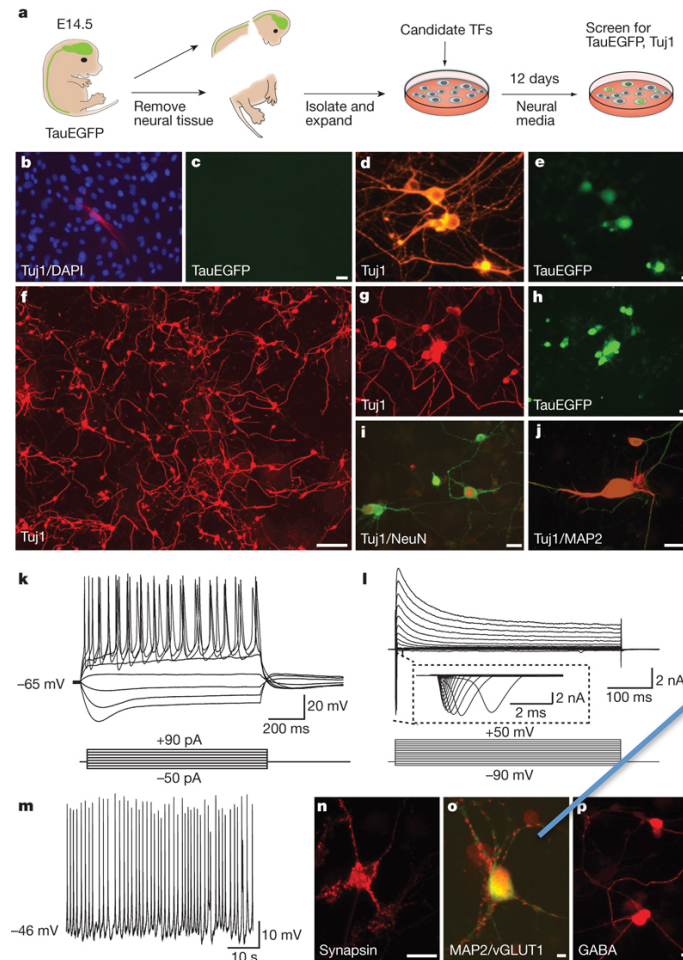
nature



Supplementary Figure 2: Screen for enhancers of Ascl1-induced conversion

a, The effect of 18 transcription factors in combination with Ascl1 on neuronal induction 13 days post infection. Shown are the average numbers of Tuj1-positive cells with a process three times longer than the cell body derived from two randomly selected, low magnification visual fields. **b**, Representative Tuj1-positive cells 13 days after infection with Ascl1 alone or in combination with the indicated genes. Note the increased complexity of the neurites in the Ascl1+Myt1 condition.

A screen for neuronal-fate-inducing factors and characterization of MEF-derived iN cells.



- vGLUT1-positive-
indicating the presence of
excitatory, glutamatergic
neurons

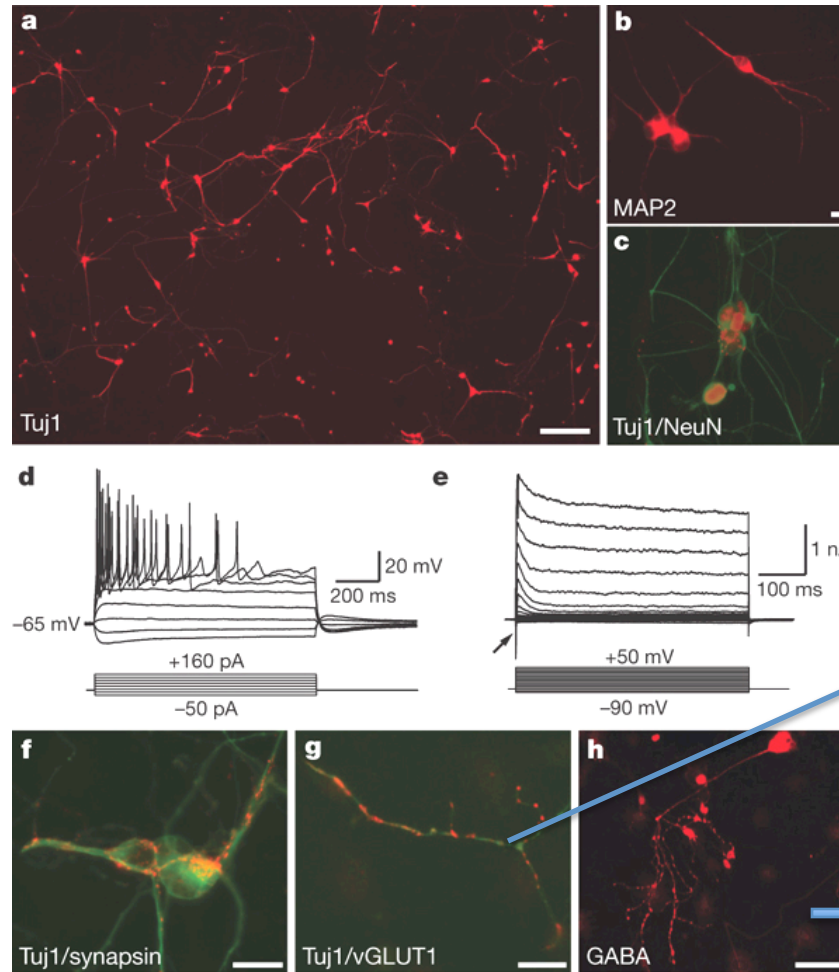
- GABA positive, the major
inhibitory
neurotransmitter in brain

T Vierbuchen *et al.* *Nature* **000**, 1-7 (2010) doi:10.1038/nature08797

nature

Efficient induction of neurons from perinatal tail-tip fibroblasts.

Tails of 3 day old mice



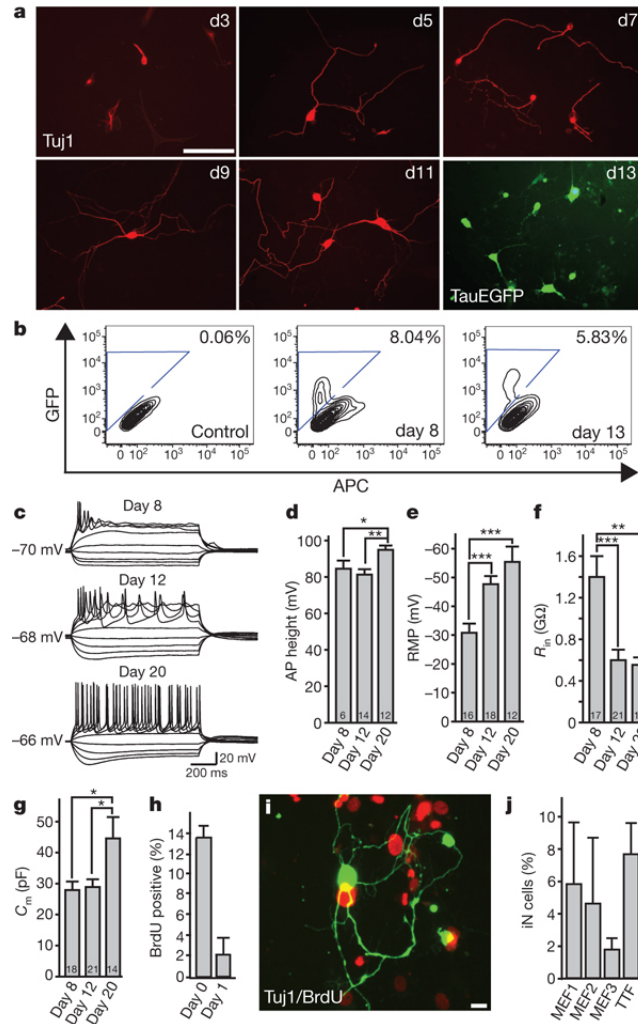
• vGLUT1-positive-
indicating the
presence of excitatory,
glutamatergic neurons

• GABA positive, the
major inhibitory
neurotransmitter in
brain

5F pool - *Brn2*, *Myt1l*, *Zic1*, *Olig2* and *Ascl1*

T Vierbuchen *et al.* *Nature* **000**, 1-7 (2010) doi:10.1038/nature08797

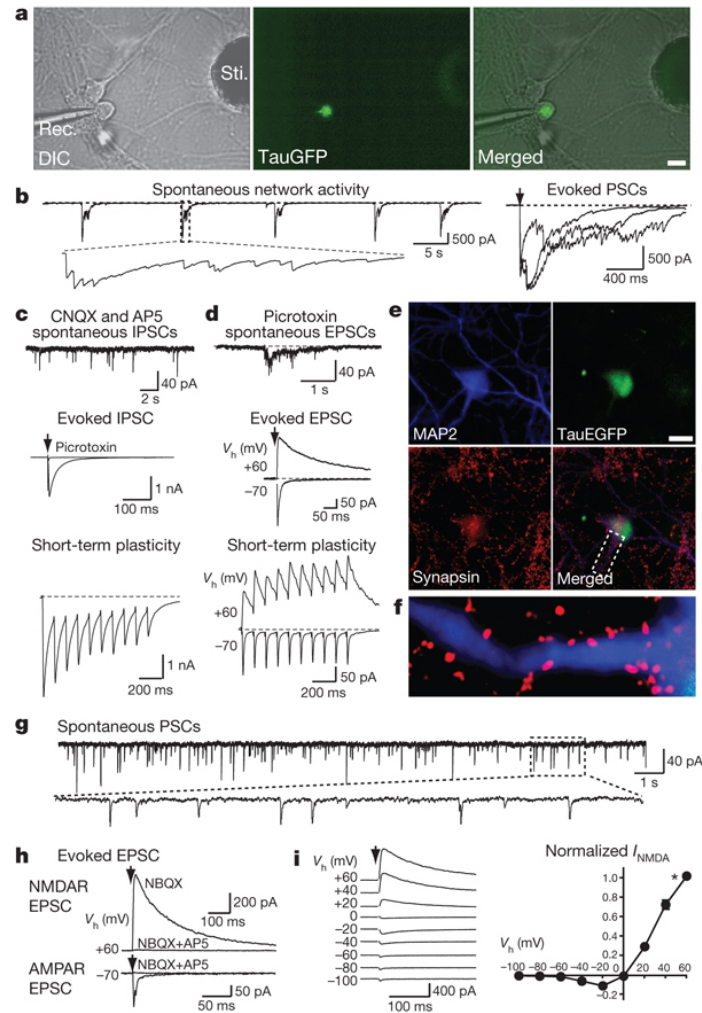
The 5F-pool-induced conversion is rapid and efficient.



T Vierbuchen *et al.* *Nature* **000**, 1-7 (2010) doi:10.1038/nature08797

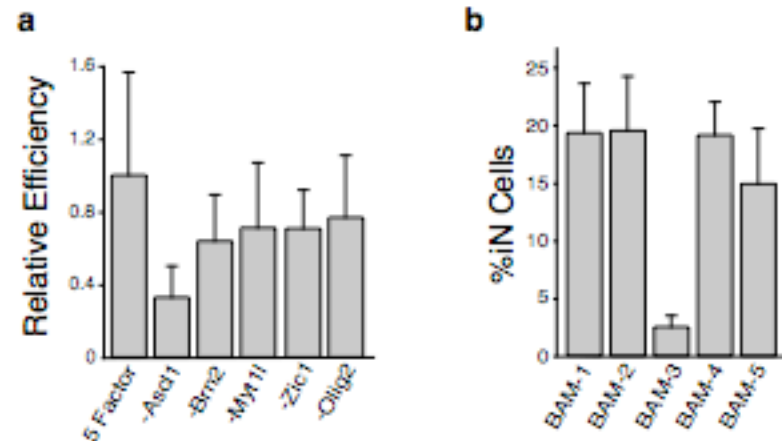
nature

MEF-derived iN cells show functional synaptic properties.



T Vierbuchen *et al.* *Nature* **000**, 1-7 (2010) doi:10.1038/nature08797

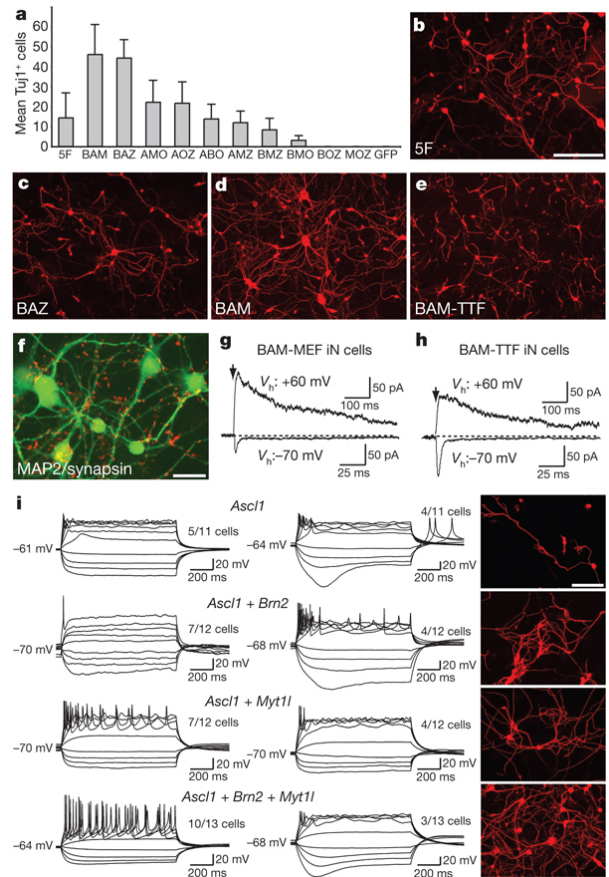
nature



Supplementary Figure 6: Additional neuronal induction efficiency estimates

a, Effect of removing single genes from the 5F pool. The average number of Tuj1-positive neuronal cells visible in a 20x field is normalized to the 5F condition (n=30 visual fields). **b**, Reproducibility of BAM-iN cell generation. Each bar represents an independent experiment. %iN cells is calculated from the number of plated cells (see methods). The low efficiency in BAM-3 is likely due to suboptimal lentiviral titer, however, the iN cells that are present in this condition still exhibit mature neuronal morphologies. Error bars = S.D.

Defining a minimal pool for efficient induction of functional iN cells.



BAM pool – *Ascl1*,
Brn2 and *Myt1*

BAZ pool - *Ascl1*,
Brn2 and *Zic1*