# Early events of neural development

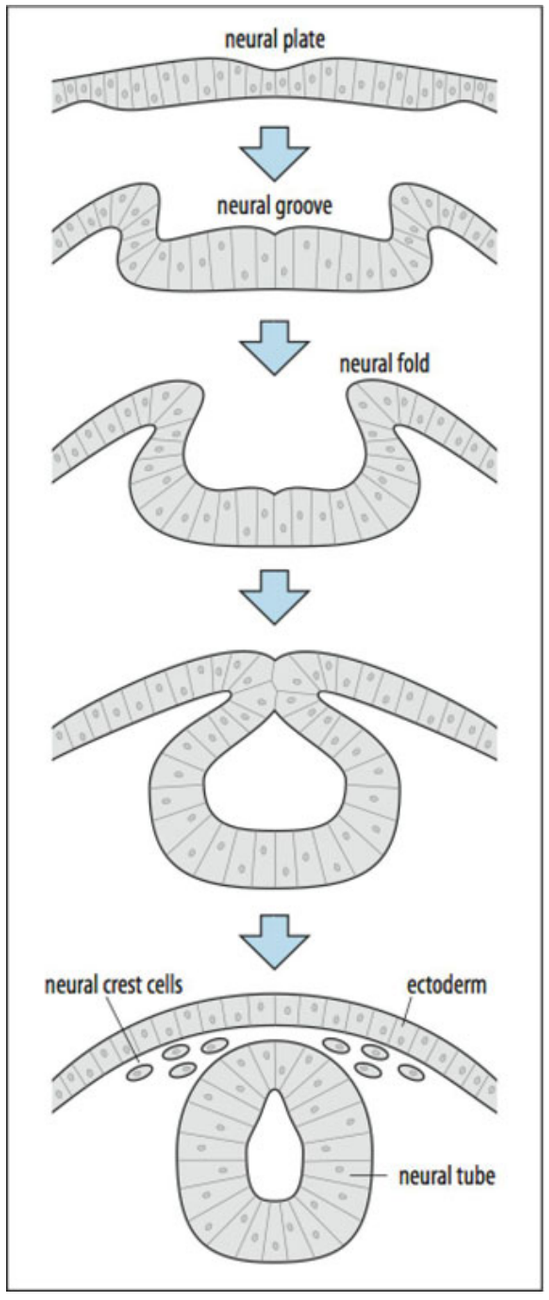
Goals:
1) to discuss the origins of cells in the nervous system
2) to discuss how neural stem cells generate diverse cell types in the nervous system

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)

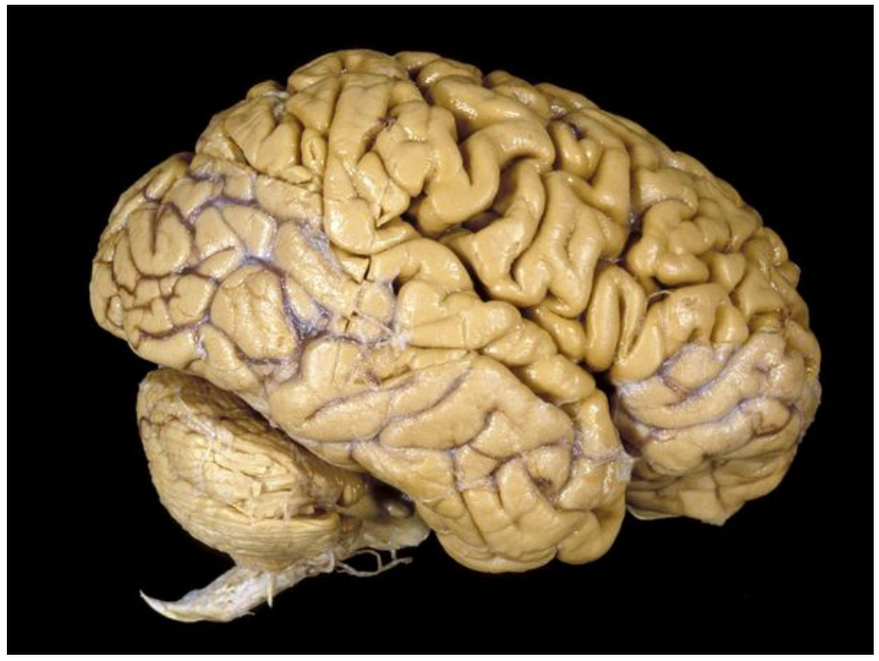
We will deal with glia later in the course!

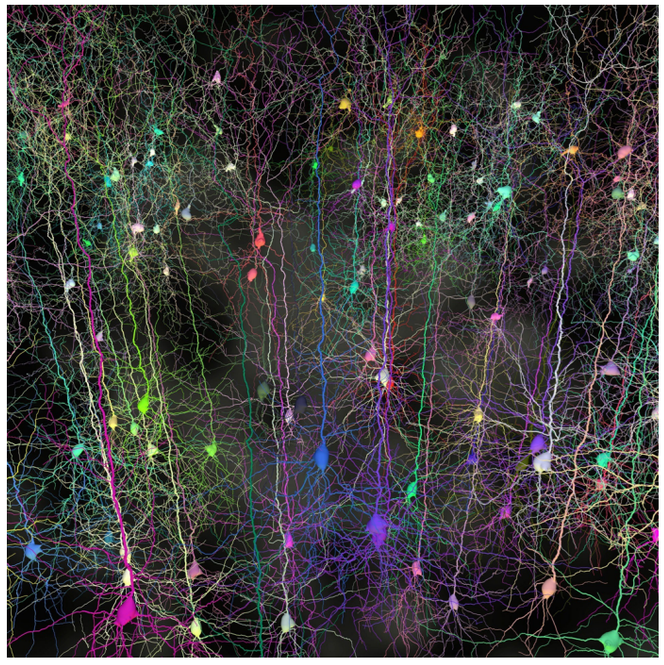
# The nervous system undergoes a huge increase in cell number during development




~$10^3$ cells

~$10^{11}$ cells





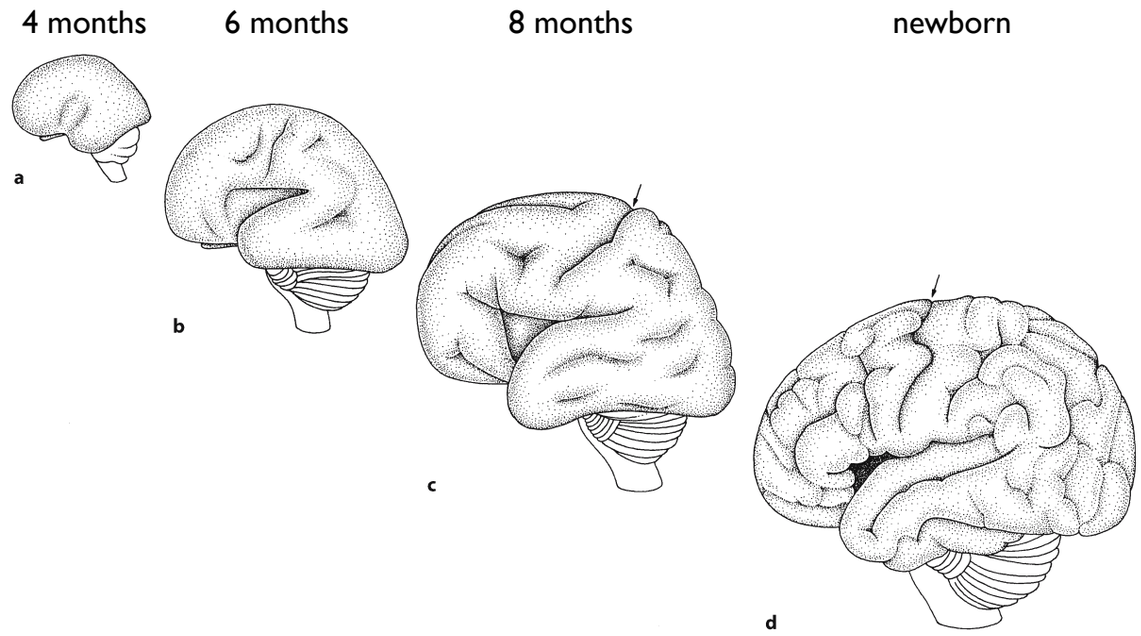
Cells increase in
-number $10^8 \approx 2^{27}$
-diversity

**Final outcome of regulated cell divisions**
=generation of an optimal number of each cell type in each brain region (essential for normal brain functions)

# Cell division is highly regulated during neural development

<u>temporal regulation</u>

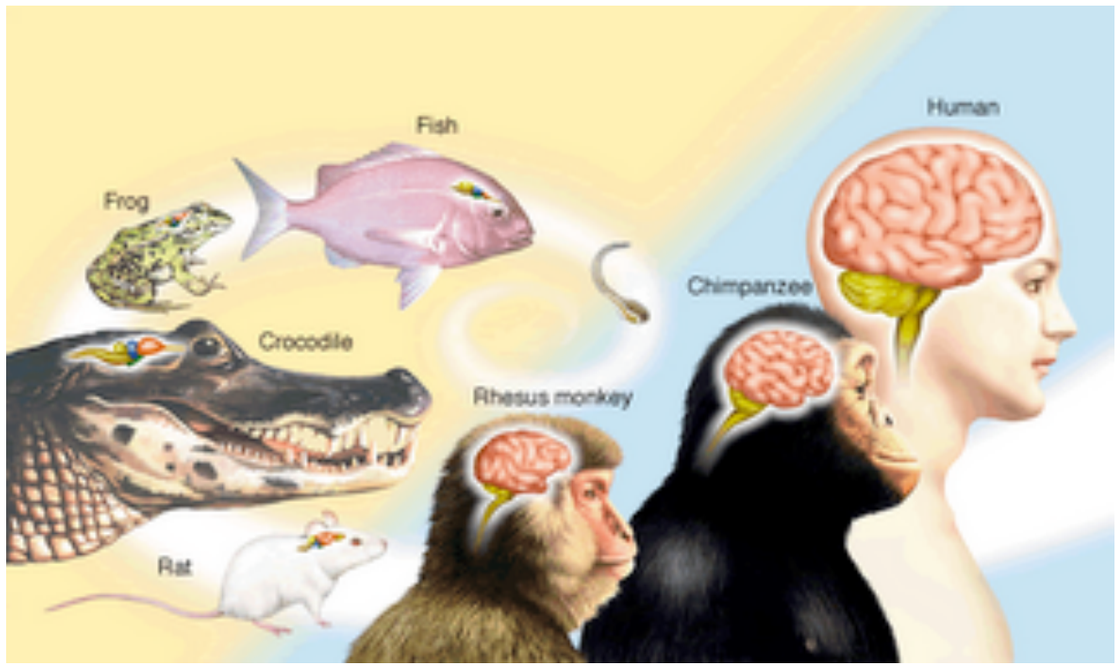
-initial exponential growth
-emergence of more differentiated progenitor cell types
-onset of neurogenesis
-cell division eventually terminated




<u>difference between regions (linked to regionalization)</u>

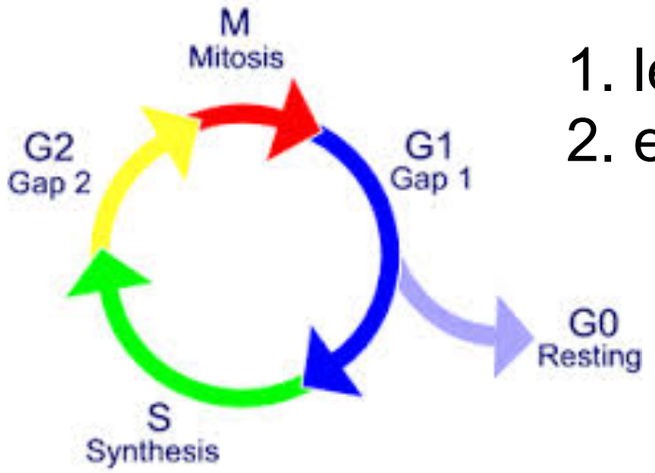
<u>difference between species</u>

Cell division in the nervous system is most studied in mammalian neocortex (mouse and human).



# How are cell divisions regulated?

<u>cell cycle behavior</u>

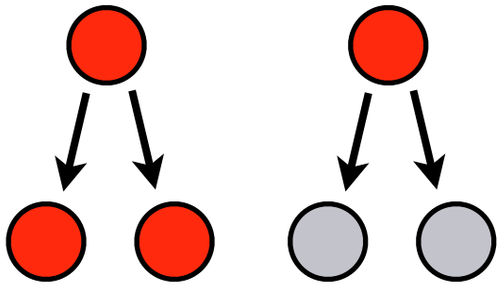



1. length
2. exit

**What are the intrinsic and extrinsic regulators of these processes?**

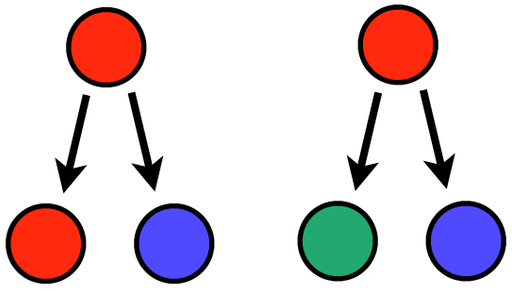
<u>mode of division</u>

1. symmetric



expand cell populations

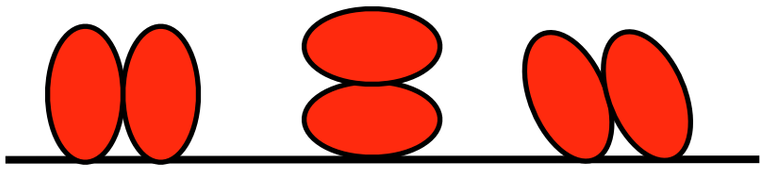
2. asymmetric



generate diverse cell types

cleavage plane of division influences division mode

vertical horizontal oblique



# Regulators of cell division

**Extrinsic regulators**
-secreted growth factors (EGFs, FGFs, IGFs, WNTs, Shh, etc.)
-small molecules (glutamate, GABA, serotonin, etc.)
-direct cell-cell interactions (cadherins, Delta-Notch, etc.)
-extracellular matrix (collagen, laminin, etc.)

**Sources of extrinsic regulators**
-progenitor cells
-neurons (feedback regulation)
-cerebrospinal fluid (CSF)
-other types of cells (e.g., microglia, blood vessels, meninges)

**Intrinsic regulators**
-transcription factors
-cell cycle regulators
-cell polarity regulators

# Cell division changes over time, space and evolution during neural development

1. Time

-initial exponential growth
    symmetric division by neuroepithelial cells (NECs)

-onset of neurogenesis
    asymmetric division by apical radial glial cells (aRGCs)

-emergence of more differentiated progenitor cell types
    basal intermediate progenitor cells (bIPCs), basal radial glial cells (bRGCs)
    This occurs in mammalian neocortex but other brain regions may not undergo this step.

-division is eventually terminated

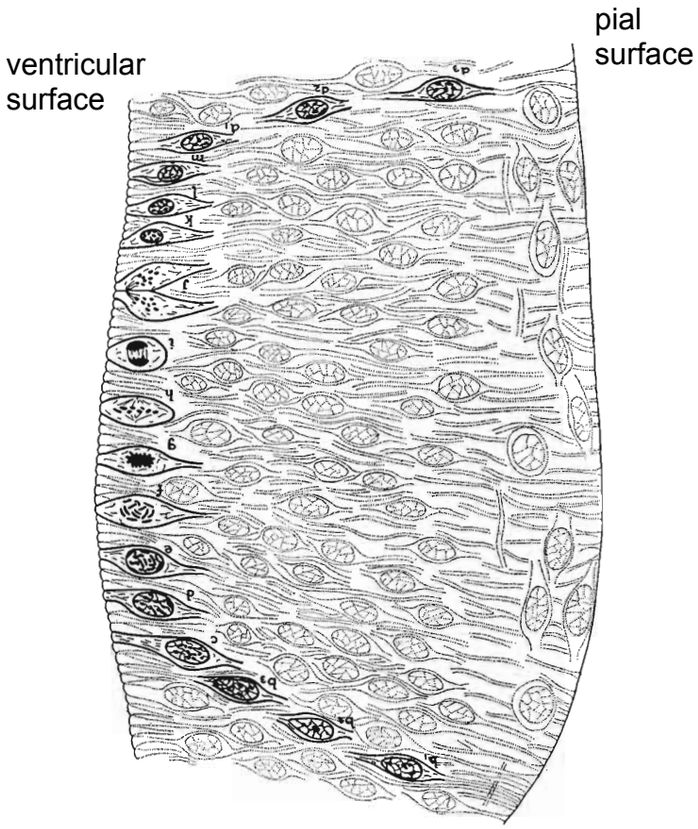
# Progenitors at the earliest stage: neuroepithelial cells (NEs)

## Sauer (1935):

-Early CNS is composed of “pseudostratified” neuroepithelium.

-“Germinal cells” are anchored by thin cytoplasmic processes to the inner and outer surfaces of the neuroepithelium.

-Nuclei of the neuroepithelial cells may undergo a to-and-fro movement during the cell cycle (**interkinetic nuclear migration**).

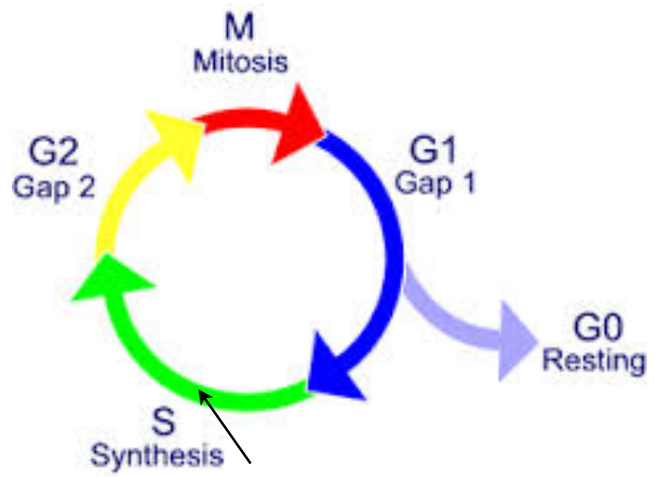



Sauer (1935)

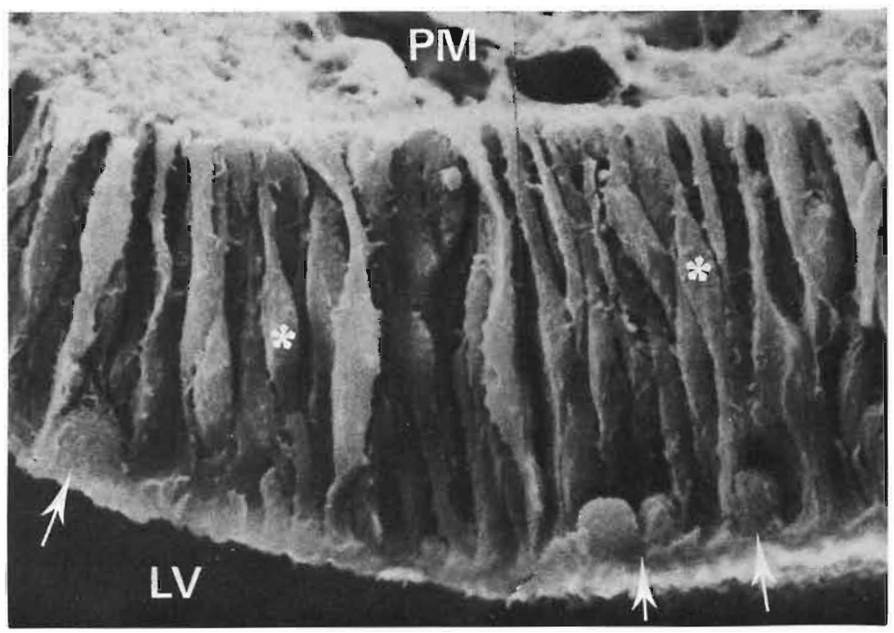
## Sidman et al. (1959):

-performed [$^3$H]-thymidine autoradiography and verified interkinetic nuclear migration

1-2 hours after injection of [$^3$H]-thymidine:
labeled cells near the pial surface
several hours later:
labeled cells near the lumen of the mitotic zone

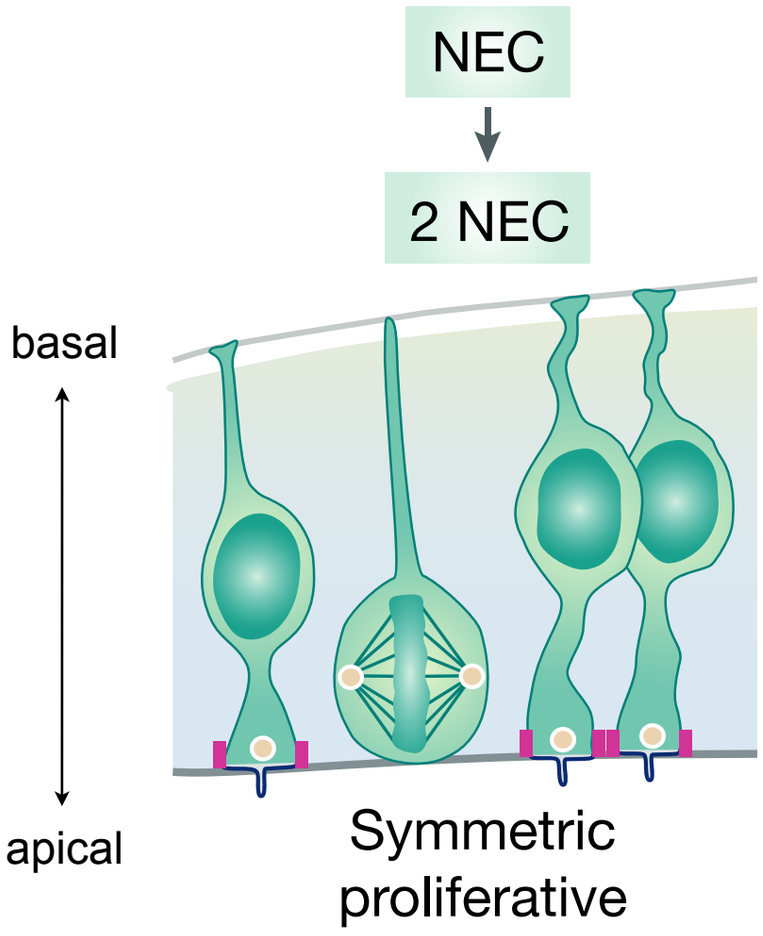



[$^3$H]-thymidine incorporated into DNA in S-phase




scanning electron micrograph of E12 rat telencephalon (Seymour and Berry, 1975)

# Neuroepithelial cells have a apical-basal polarity and undergo symmetric divisions




Paridaen and Huttner (2014)

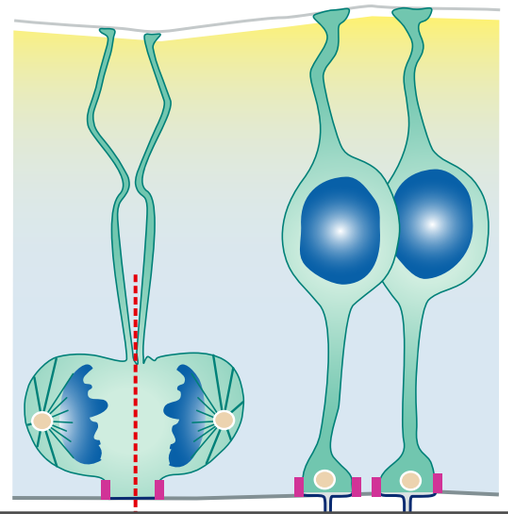
Neuroepithelial cells (NECs) are highly polarized.

Apical membrane is exposed to the ventricle has a single primary cilium, which detect signals in the CSF.

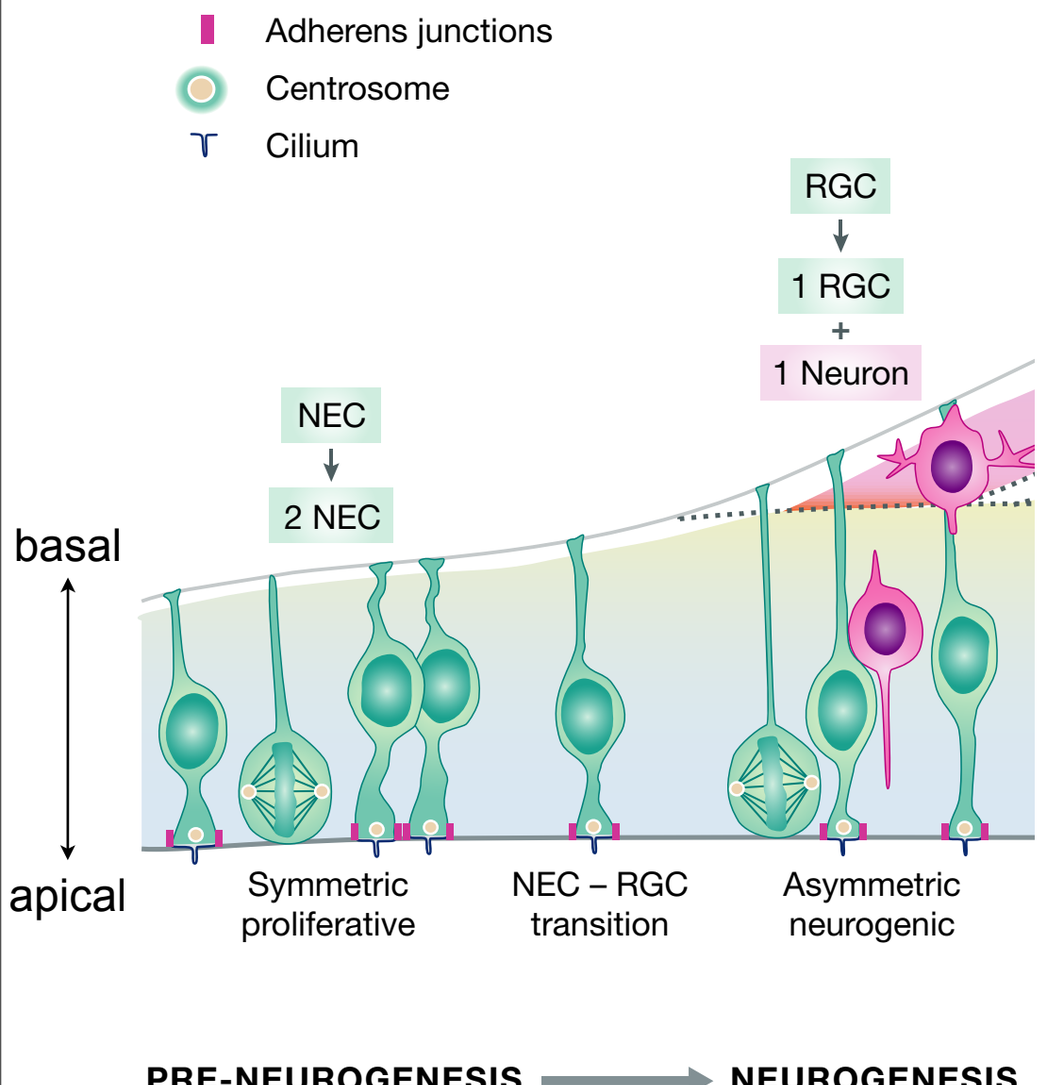
Apical membrane of the neighboring NECs are attached to each other by cell adhesion via adherens junctions and tight junctions.

Basal membrane is attached to the basal lamina immediately under the pia.

NECs divide symmetrically. The cleavage plane is perpendicular to the ventricular surface (vertical cleavage).



# Neuroepithelial cells transition into radial glial cells



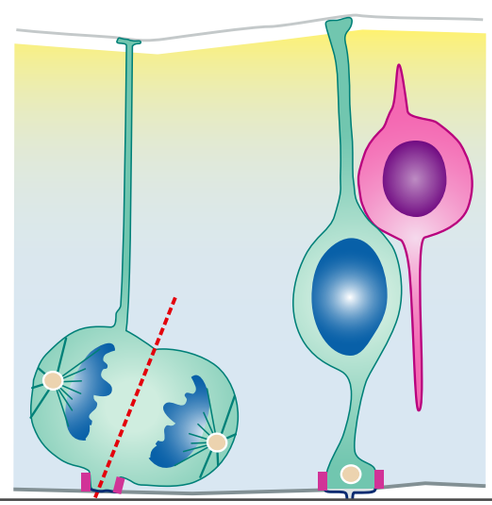

Paridaen and Huttner (2014)

Radial glial cells (RGCs) appear as neurogenesis starts

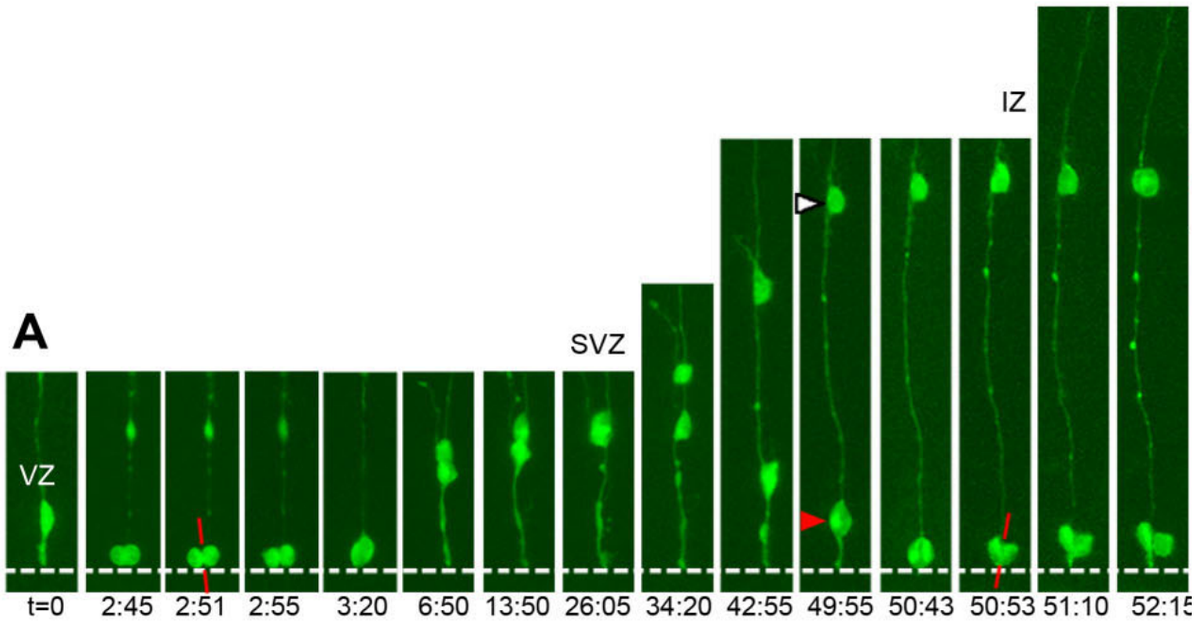
RGCs and NECs share:
-apical-basal polarity
-interkinetic nuclear migration
-adherens junctions
-markers (Nestin, Sox2, etc.)

RGCs are different from NECs:
-express astroglial markers (GLAST, BLBP)
-have lost tight junctions.

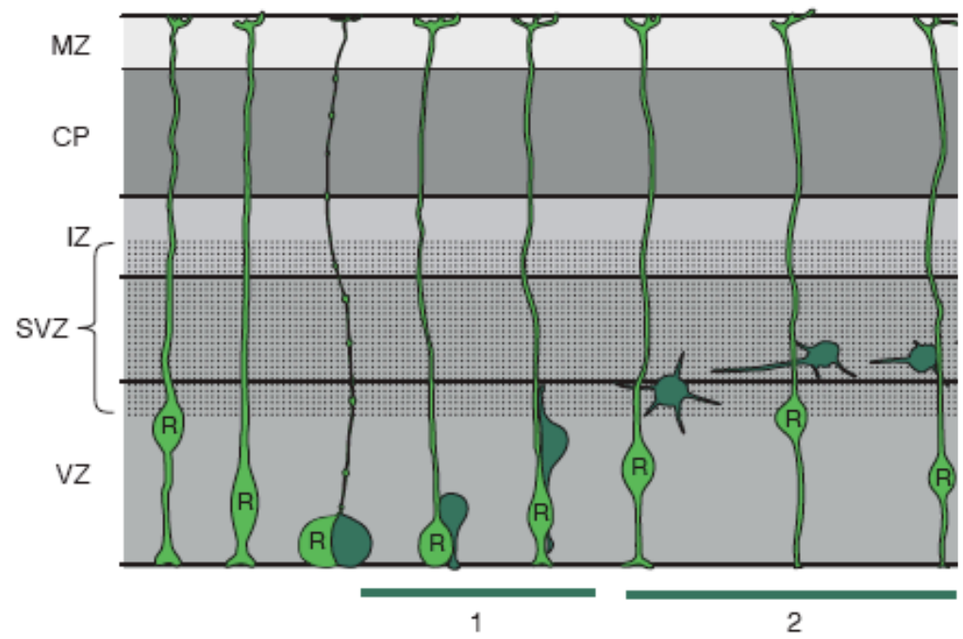
**RGCs divide asymmetrically.** The cleavage plane is slightly oblique to the ventricular surface.



# Time-lapse analysis of radial glia divisions




Noctor et al. (2008)




Kriegstein and Noctor (2004)

-EGFP-expressing retrovirus was infected in rat cortex. EGFP continues to be expressed in all progeny after the infected cell divides.

-EGFP$^+$ cells in cultured slices were analyzed with time-lapse microscopy over ~3 days.

-RGCs divided (t=2:51) at the apical surface.
-One daughter cell migrated towards the pial surface.
-The other daughter cell underwent interkinetic nuclear migration and divided again (t=49:55) at the apical surface.

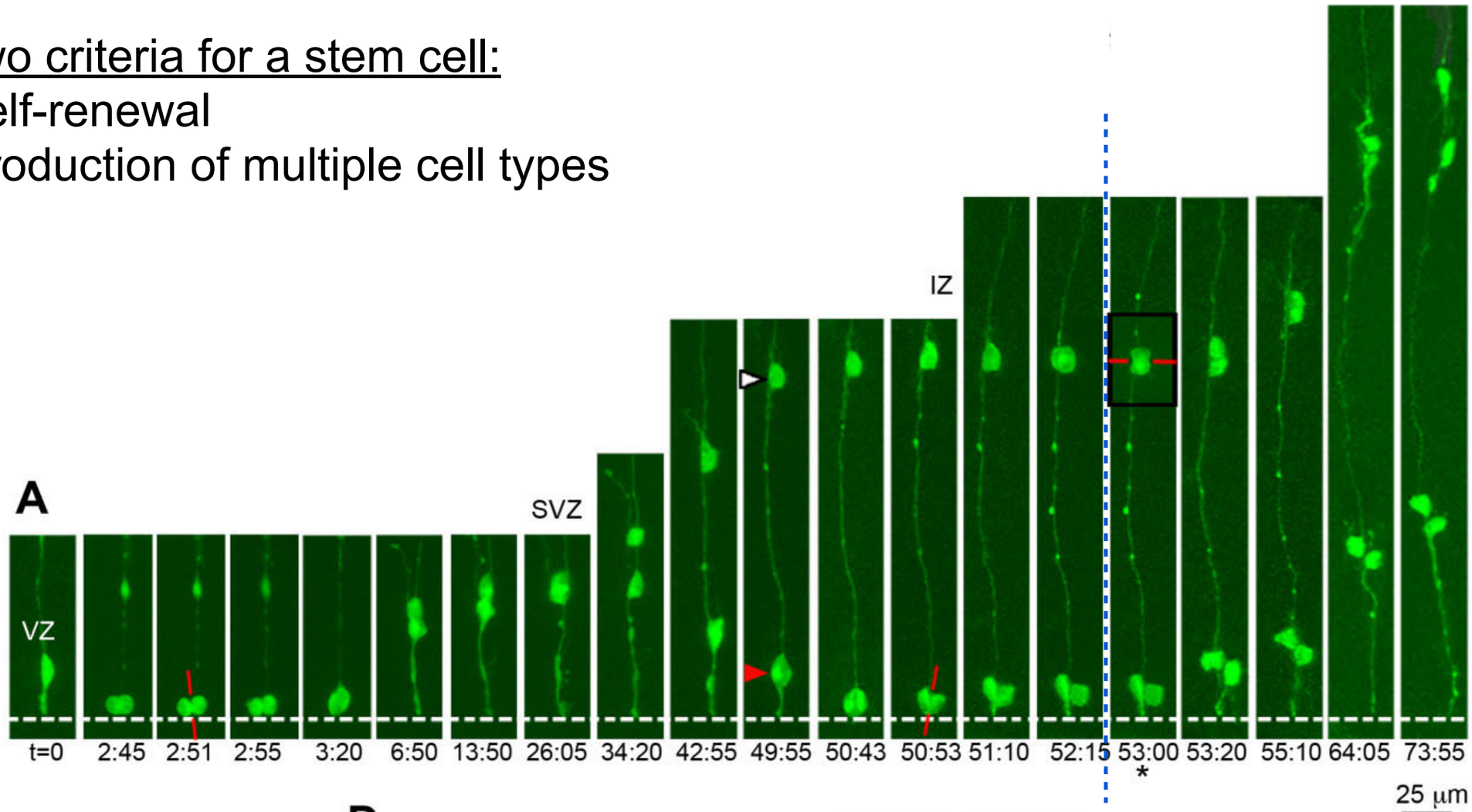
The range of positions that RGCs take defines the ventricular zone (VZ).

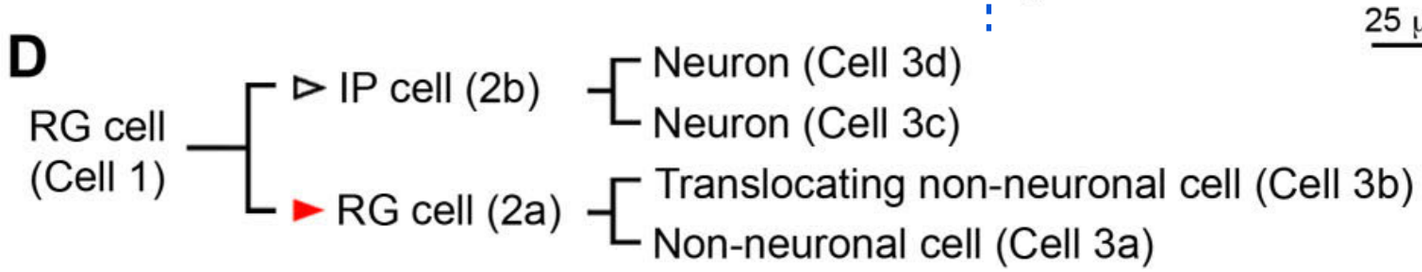
R: radial glial cell

VZ: ventricular zone
SVZ: subventricular zone
IZ: intermediate zone
CP: cortical plate
MZ: marginal zone

# Radial glia are neural stem cells

<u>two criteria for a stem cell:</u>
self-renewal
production of multiple cell types

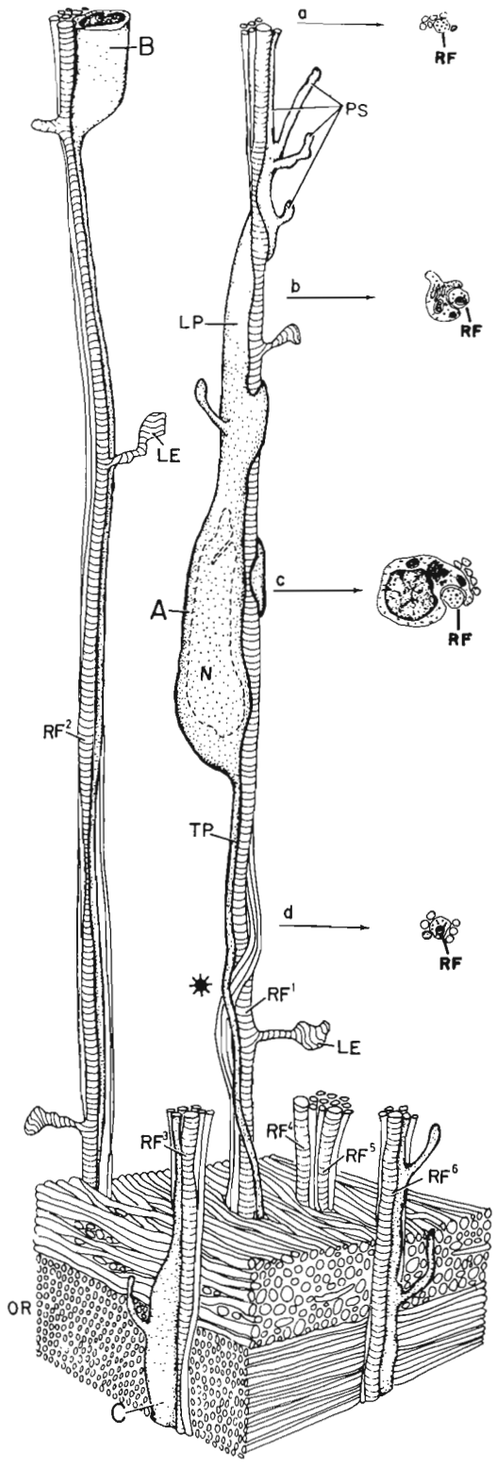



Noctor et al. (2008)

-Both daughter cells divided again!
-RGC division produced one RGC and a differentiating cell (intermediate progenitor cells, IPC), which divides again horizontally to produce neurons.
-RGCs eventually undergoes a “self-consuming” division.

# Radial glia and astrocytes




Rakic (1972)

Radial glial cells were initially considered as specialized glial cells with a unique developmental role in guiding neuronal migration (Rakic 1972, 1988).

Common features between radial glia and astrocytes:

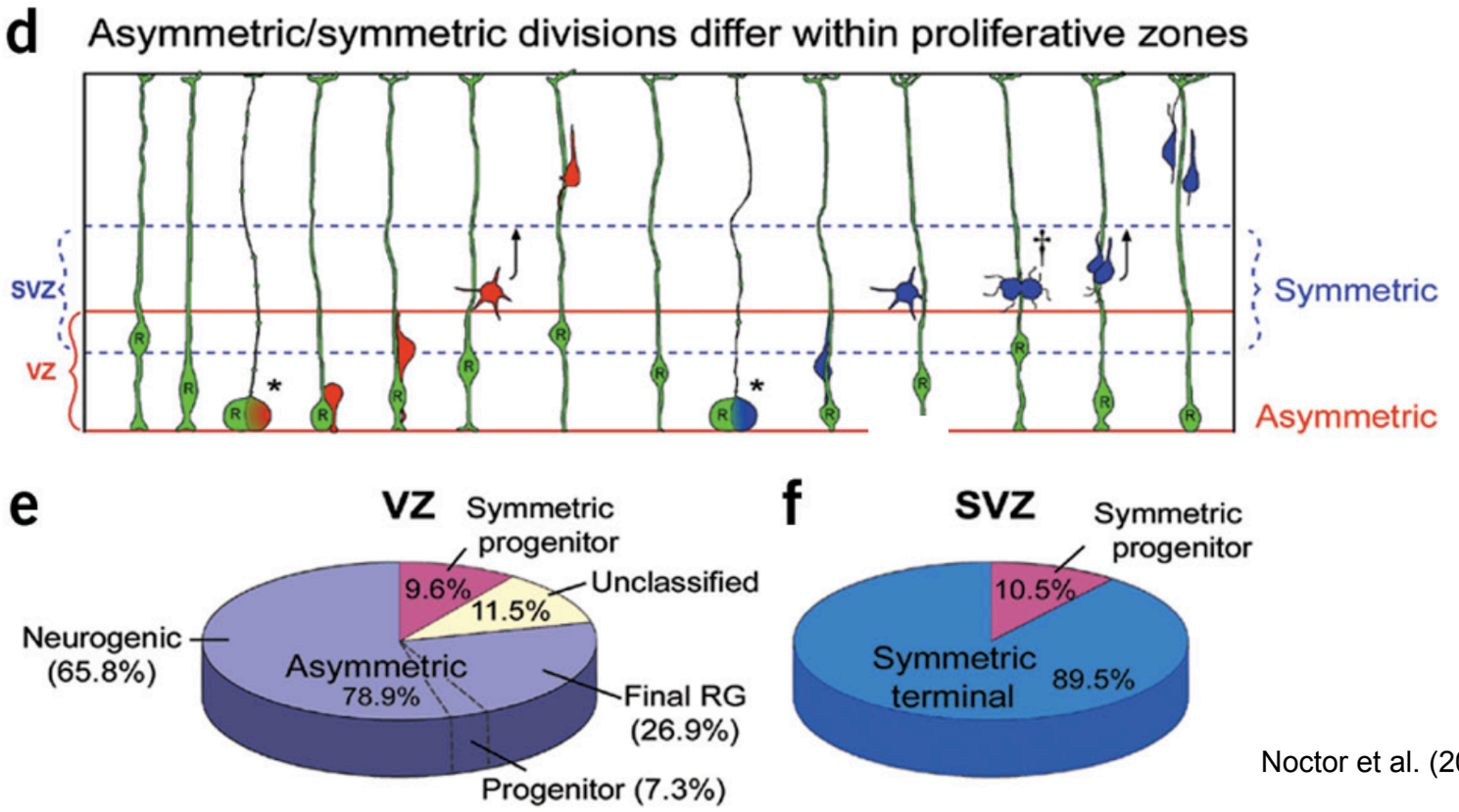
- marker expression
- contacts with blood vessels
- contain glycogen storage granules
- coupled together by gap junctions
- sustain intracellular Ca waves

Radial glia turns into astrocytes.

Time-lapse analysis of embryonic cortical slices discovered that radial glia divide and produce neurons.

Similar to radial glia in developing brains, neural stem cells in the adult brain also express astrocyte markers (e.g. GFAP) and share some of the above properties with astrocytes.

# Radial glia generate neurons directly or indirectly

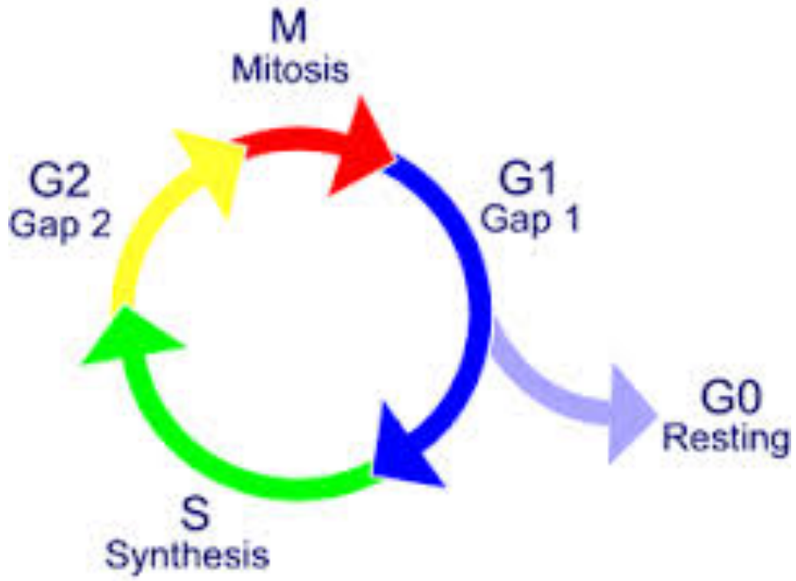



Noctor et al. (2004)

Radial glia (RGCs) divide at the apical (ventricular) surface and produce:
-one RGC and one neuron (red)...direct neurogenesis
or
-one RGC and one intermediate progenitor cell (IPC, blue). The IPC divides symmetrically in the subventricular zone (SVZ) once or twice to generate 2 or 4 neurons...indirect neurogenesis

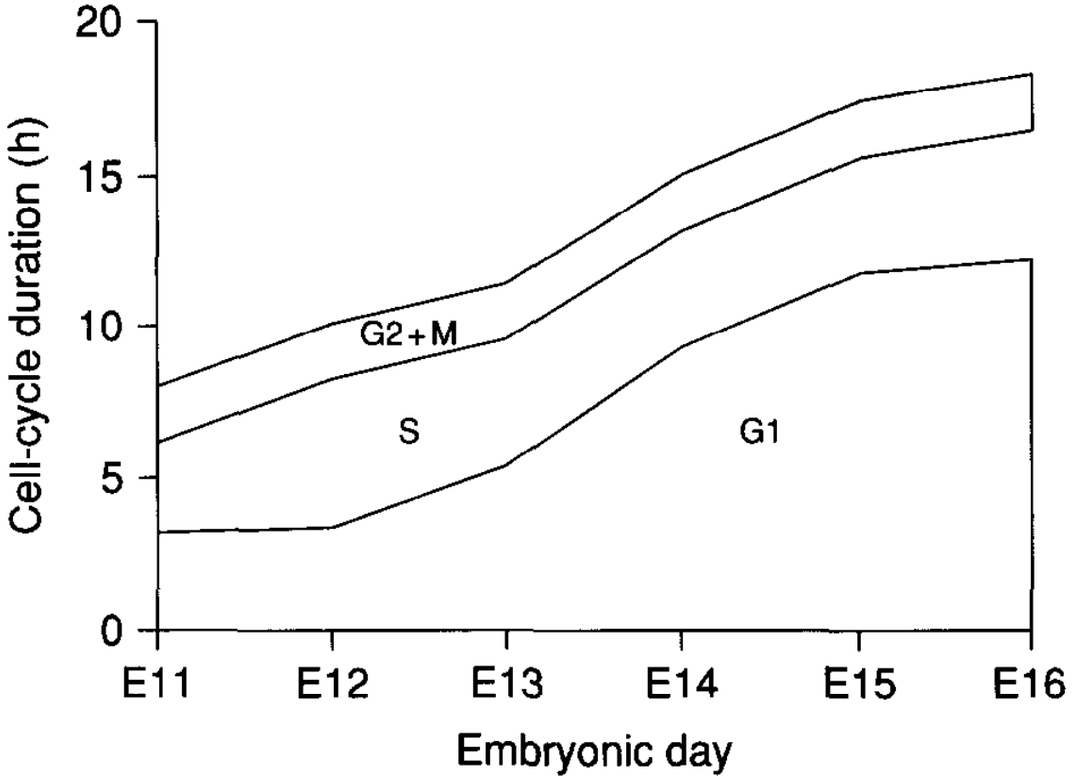
RGCs sometimes produce an RGC and a basal radial glia (bRG), which divide like an RGC or an NEC but are outside of the VZ and lack an apical process.

# Regulation of the cell cycle



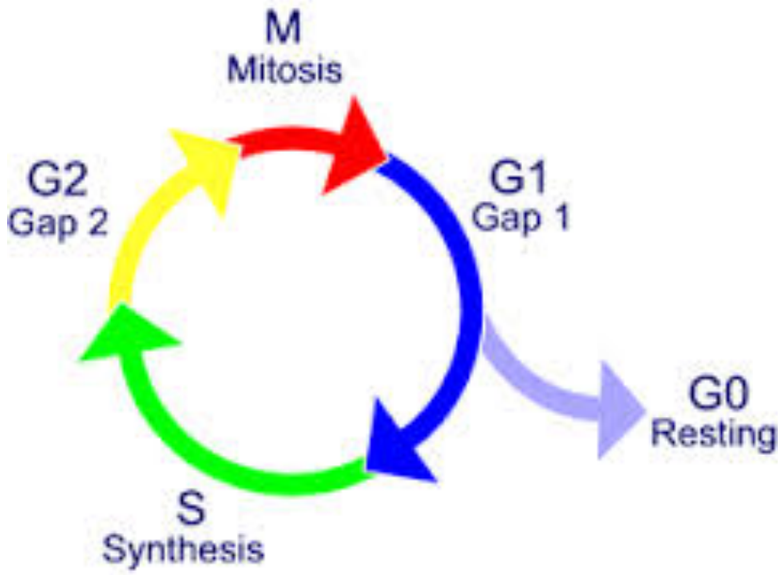

-In embryonic mouse neocortex, cell cycle time lengthens as development proceeds.

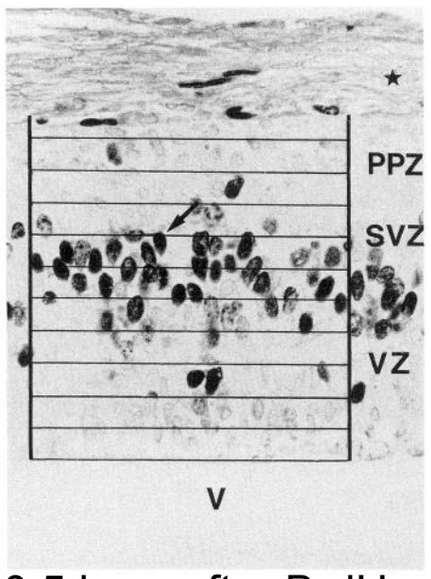
-Lengthening of cell cycle time is mostly due to the lengthening of the G1 phase.



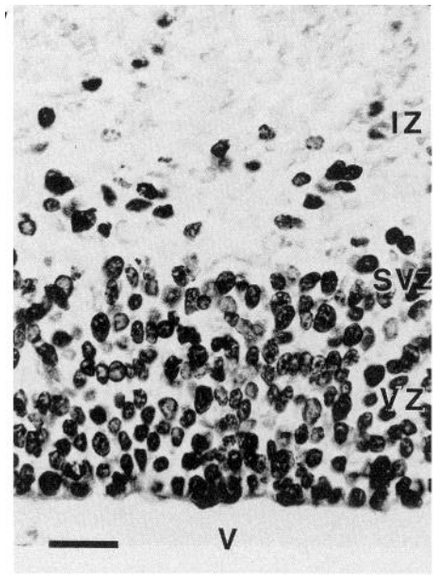

Caviness et al. (1995)

# How can we measure cell cycle parameters?




0.5 hour after BrdU




14 hour after BrdU

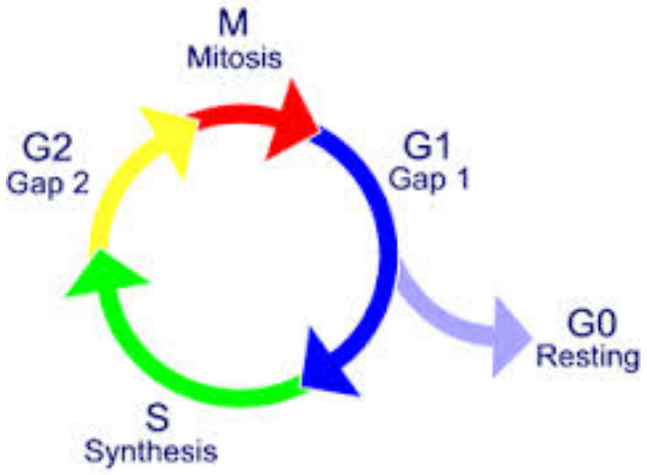
”cumulative S phase labeling” with thymidine analogs (bromodeoxyuridine (BrdU), ethynyldeoxyuridine (EdU) etc.) allows determination of S phase length and total cell cycle length.

Mitotic figures in nuclear staining (e.g., DAPI) or the use of M-phase markers allows detection of cells in mitosis, thus he M phase length. Phosphorylated histone H3 (PH3) also marks cells in M-phase.

After a single labeling of a thymidine analog, G2 phase can be measured by the time it takes for labeled cells to to enter the M phase.

Labeling different progenitor populations by specific markers will allow to determine cell cycle parameters separately for RGCs and IPCs.

# RGCs and IPCs appear to show different cell cycle parameters




| | Cell-cycle phases (h) | | | | | |
|---|---|---|---|---|---|---|
| | $T_C$-$T_S$ | $T_S$ | $T_C$ | $T_{G2}$ | $T_M$ | $T_{G1}$ |
| APs (Pax6+/Tbr2−) | 14.1 | 5.0 | 19.1 | 1.6 | 0.9 | 11.6 |
| *Tis21*-GFP− | 14.1 | 8.3 | 22.4 | 1.6 | 1.1 | 11.4 |
| *Tis21*-GFP+ | 14.0 | 1.8 | 15.8 | 1.6 | 0.7 | 11.7 |
| BPs (Tbr2+/Tbr1−) | 23.3 | 3.2 | 26.5 | 1.6 | 0.5 | 21.2 |
| *Tis21*-GFP− | 23.0 | 6.4 | 29.4 | 1.6 | 0.5 | 20.9 |
| *Tis21*-GFP+ | 23.4 | 2.8 | 26.2 | 1.6 | 0.5 | 21.3 |
| *Tis21*-GFP− NPCs | | 8.0 | 23.3 | 1.6 | 1.0 | 12.7 |
| *Tis21*-GFP+ NPCs | | 2.4 | 21.7 | 1.6 | 0.6 | 17.1 |

Arai et al. (2011)

APs (apical progenitors)...radial glia (PAX6+)
BPs (basal progenitors)...intermediate progenitors (TBR2+)

-Total cell cycle time is shorter for RGCs than IPCs.
-G1 phase is twice as long for IPCs than for RGCs.
-G2 phase is similar between IPCs and RGCs.
-S phase is shorter for IPCs.

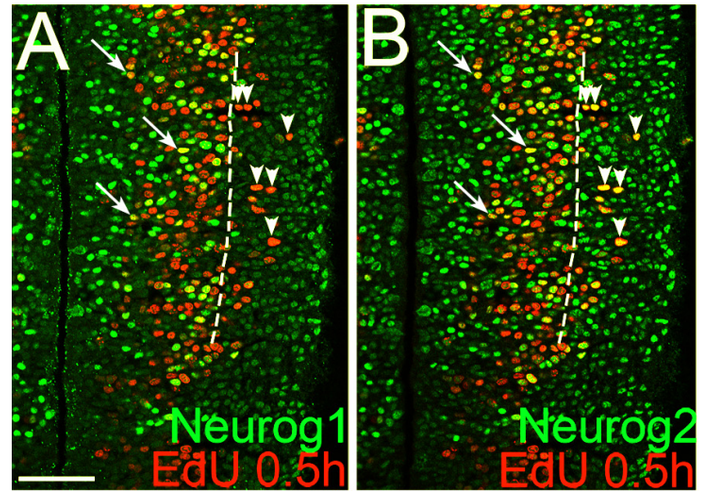
# Uses of S-phase labeling for “time stamping”

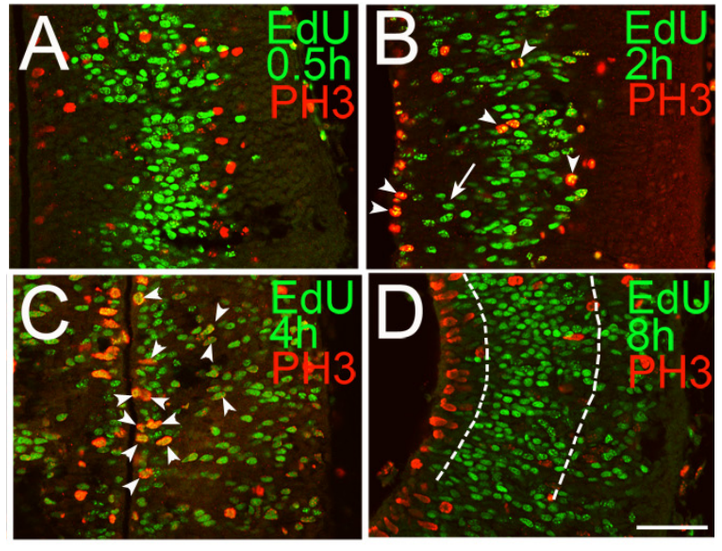
Short survival time (e.g., 30min) allows detection of cells in S-phase.

Increased post-injection survival time results in labeled cells at progressively later phases of the cell cycle.

Label will be diluted in cells that continue to divide.

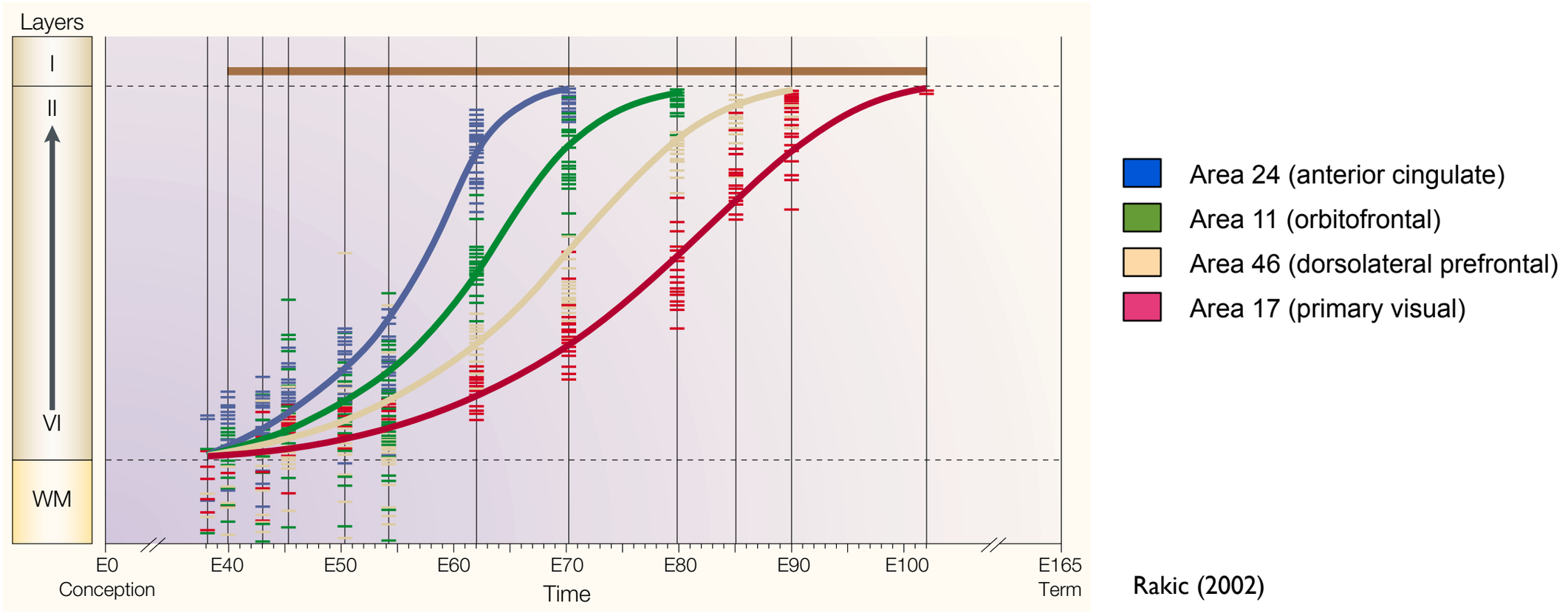
Labeled cells that become postmitotic following division, retain the label throughout life (birth-dating).




Wang, Bluske et al. (20011)

# Inside-out pattern of neurogenesis in the neocortex




Rakic (2002)

$^{3}$H-thymidine was injected on a selected embryonic day and the monkey was killed postnatally.

Deep layer (VI)  neurons become postmitotic first. Upper layer (II) neurons are the latest to be born.

Generally, neurogenesis occurs earlier in more anterior (rostral) and more lateral regions of the neocortex (neurogenic gradient).

# Summary 1-changing cell division over time

**Cells change their morphology, gene expression and division mode during neurogenesis.**

Before neurogenesis starts, neuroepithelial cells (NECs) increase the pool size of by dividing symmetrically.

Once neurogenesis starts, apical radial glial cells (aRGCs) divide asymmetrically and produce neurons either directly or indirectly via more differentiated progenitor cells including:

-basal intermediate progenitor cells (bIPCs): divide symmetrically
-basal radial glia (bRGs or bRGCs): various division mode, high proliferative capacity, abundant in primate neocortex

NECs and aRGCs share many features (morphology, gene expression, etc.) and are considered as neural stem cells.

Over time, cell division slows down, and neurogenesis stops and is taken over by gliogenesis. In a few brain regions, RGCs turn into adult neural stem cells and continues to produce into adulthood.

# Overview of neurogenesis in embryonic vertebrate CNS

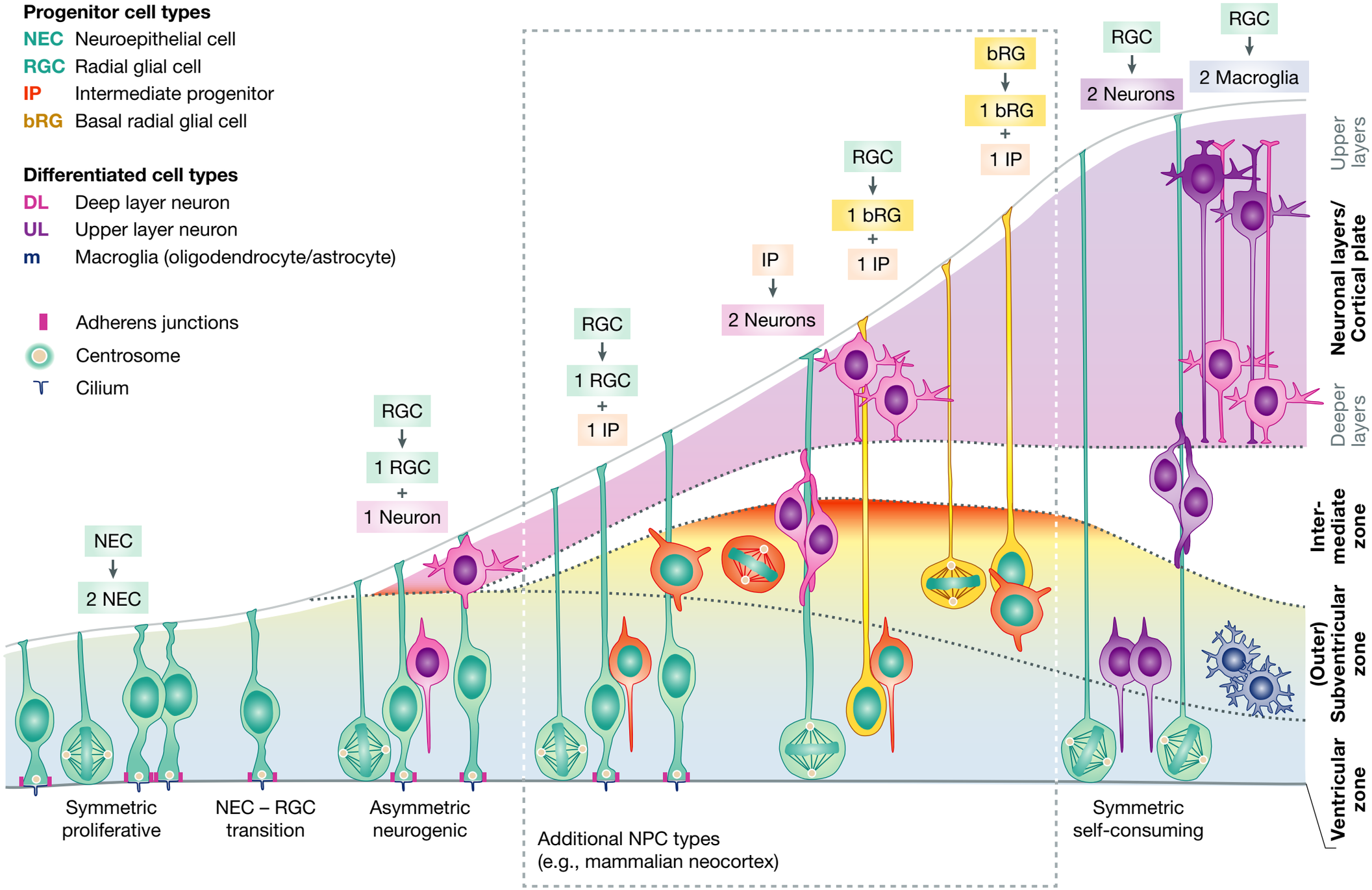
**Progenitor cell types**

**NEC** Neuroepithelial cell
**RGC** Radial glial cell
**IP** Intermediate progenitor
**bRG** Basal radial glial cell

**Differentiated cell types**

**DL** Deep layer neuron
**UL** Upper layer neuron
**m** Macroglia (oligodendrocyte/astrocyte)

Adherens junctions
Centrosome
Cilium




Paridaen and Huttner (2014)

# Cell division changes over time, space and evolution during neural development

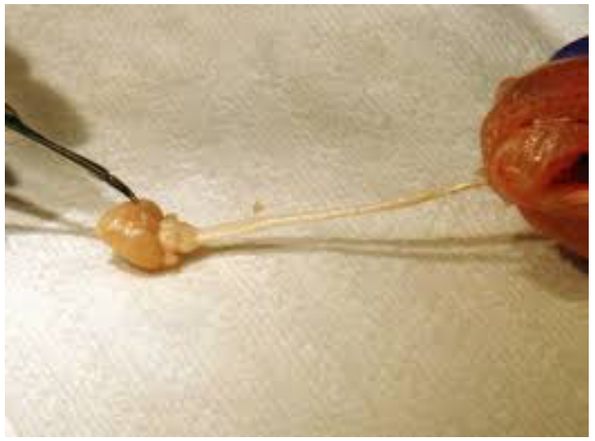
2. Region
anterior vs posterior size difference
neurogenic gradients
areal difference in the neocortex

# Regional differences in neurogenesis

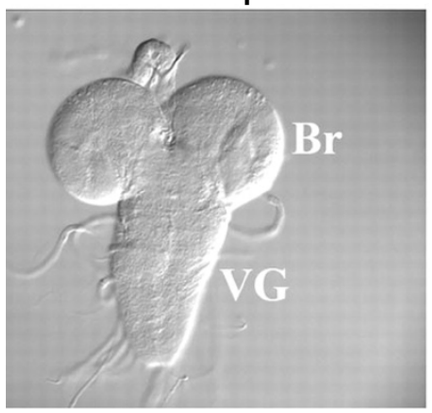
1. Rostral neural tissue becomes bigger (with more cells) than caudal neural tissue.

human

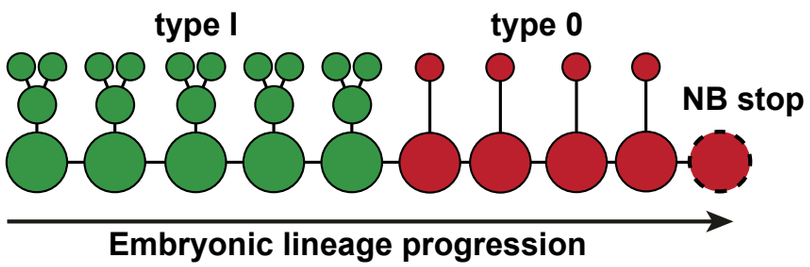
mouse



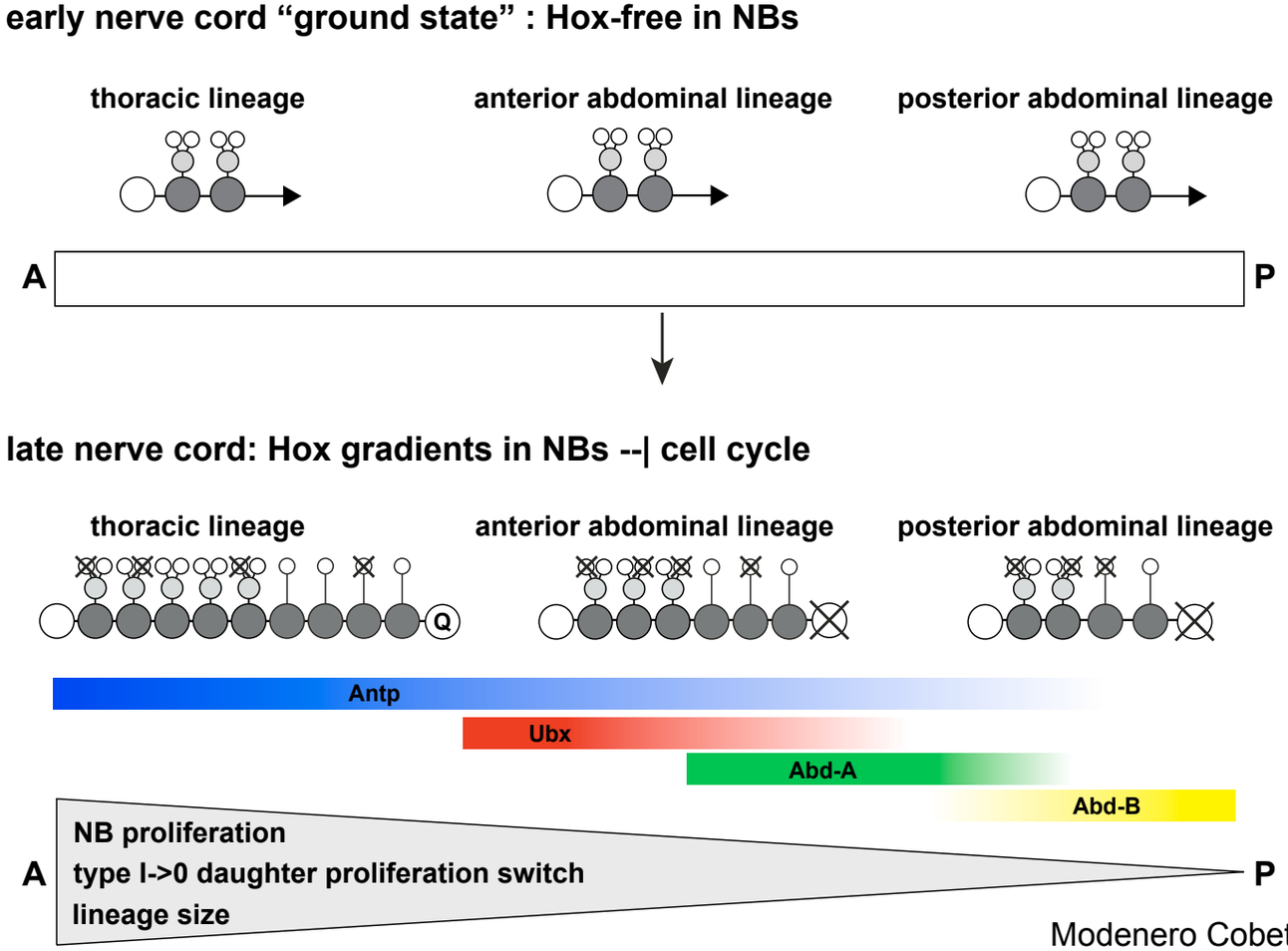
Drosophila




-In mice, indirect neurogenesis by IPCs is more abundant in rostral CNS than hindbrain and spinal cord.
-In Drosophila, switch from indirect to direct neurogenesis occurs early in caudal CNS due to overlapping expression of Hox genes.



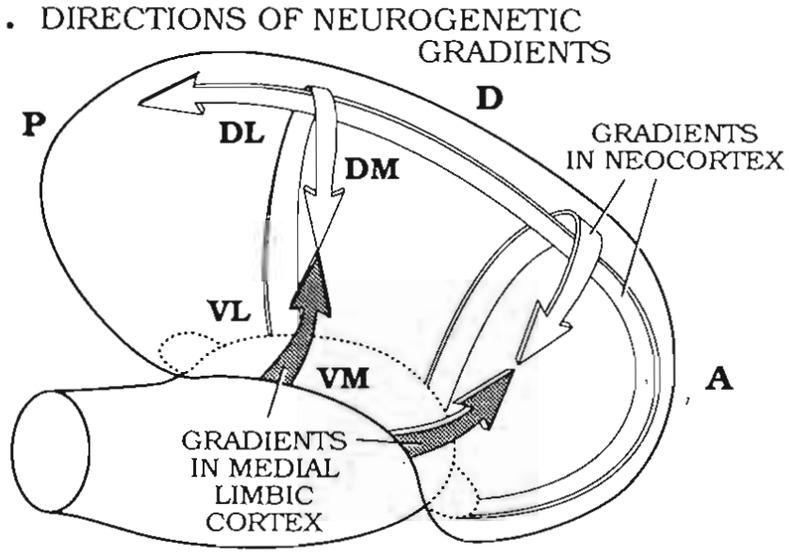

Bahrampour et al. (2017)




Modenero Cobeta et al. (2017)

# Regional differences in neurogenesis

## 2. Neurogenic gradients within each brain region



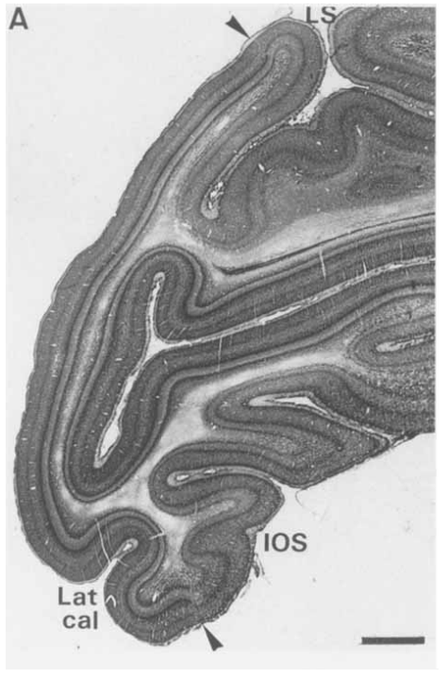

Bayer and Altman (1991)

The onset and progression of neurogenesis are not uniform across the cortex.
-anterior early, posterior late
-lateral early, medial late

In the spinal cord, motor neurons are first generated ventrally. Neurons in the dorsal spinal cord are generated later.

Neurogenic gradients are likely to be linked to mechanisms of regionalization.

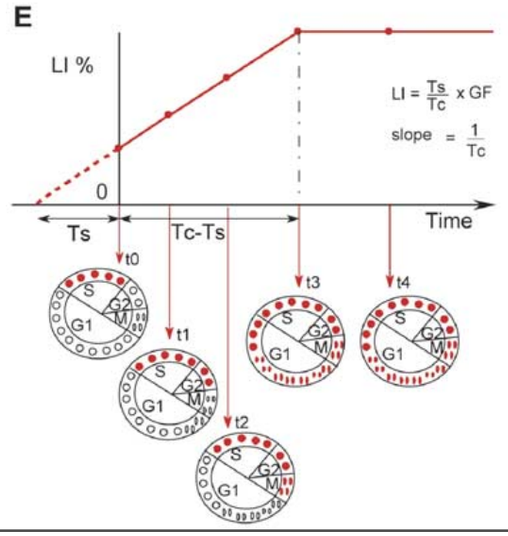
## 3. Difference between two adjacent cortical areas

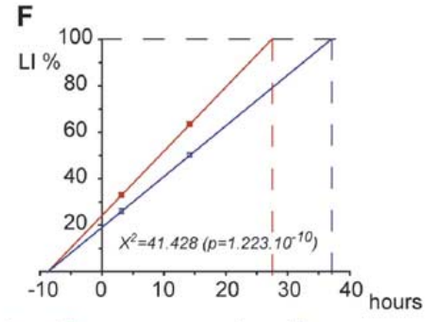



Dehay and Kennedy (1996)

In upper layers of primate visual cortex, area 17 (primary visual area) has a much higher neuronal density than the adjacent area 18 (secondary visual area).

The cell cycle length and S phase length are shorter in area 17 than in area 18 when upper layer neurons are being generated.




Area 17
Tc = 36 h
Tg1+Tg2+Tm = 27 h
Ts = 9 h

Area 18
Tc = 46 h
Tg1+Tg2+Tm =37 h
Ts = 9 h

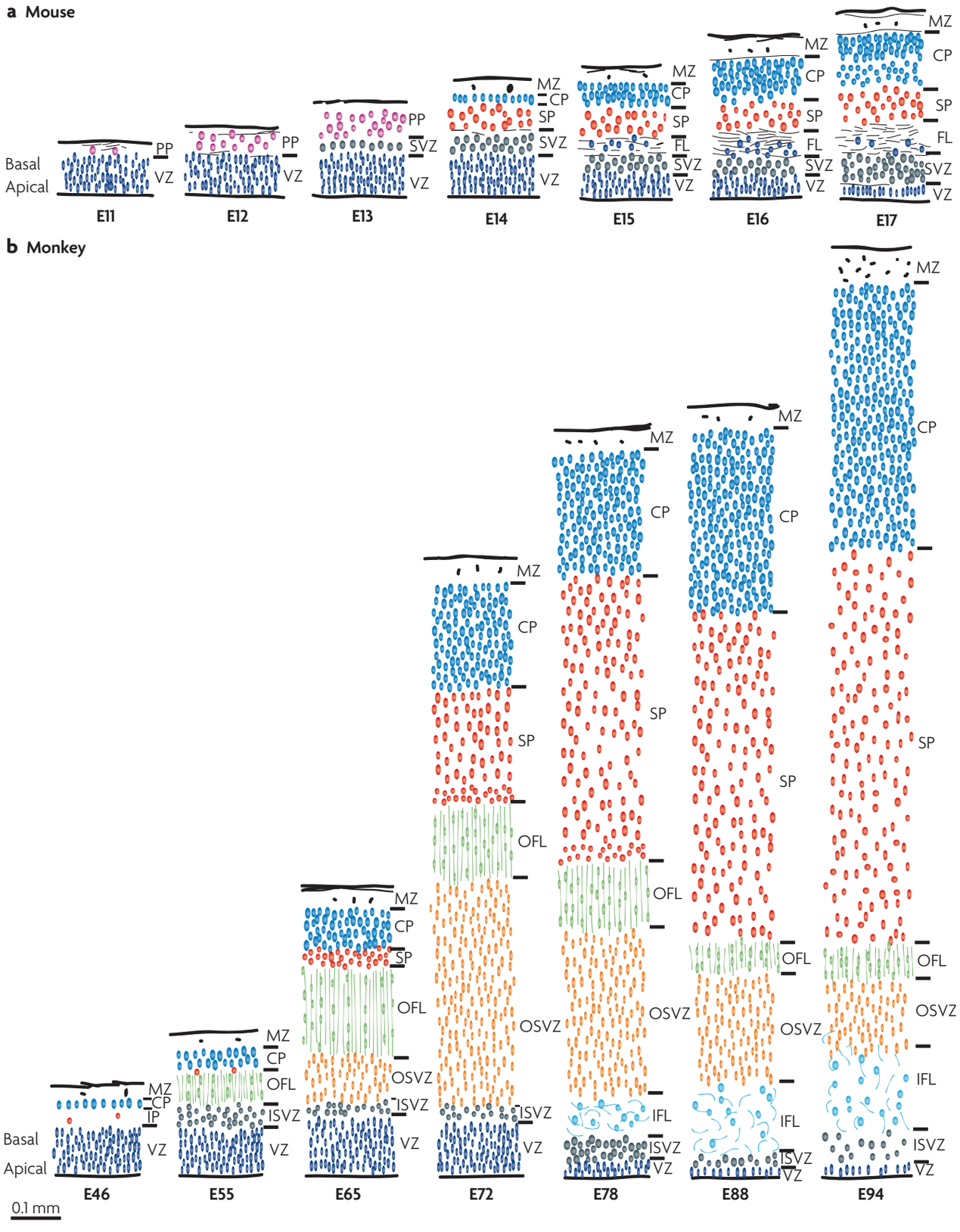
Lukaszewicz et al. (2005)

# Description of cell division during neural development across time, space and evolution

3. Evolution

difference in neocortical organization between human and mouse

# Difference between mouse and human cortex




Dehay and Kennedy (2007)

<u>basics of cortical histogenesis</u>

-PP (preplate) is the layer of earliest-born postmitotic neurons. Many of these cells come from outside of the neocortex.

-Neurons generated from neocortical progenitor cells migrate radially and form the cortical plate (CP).

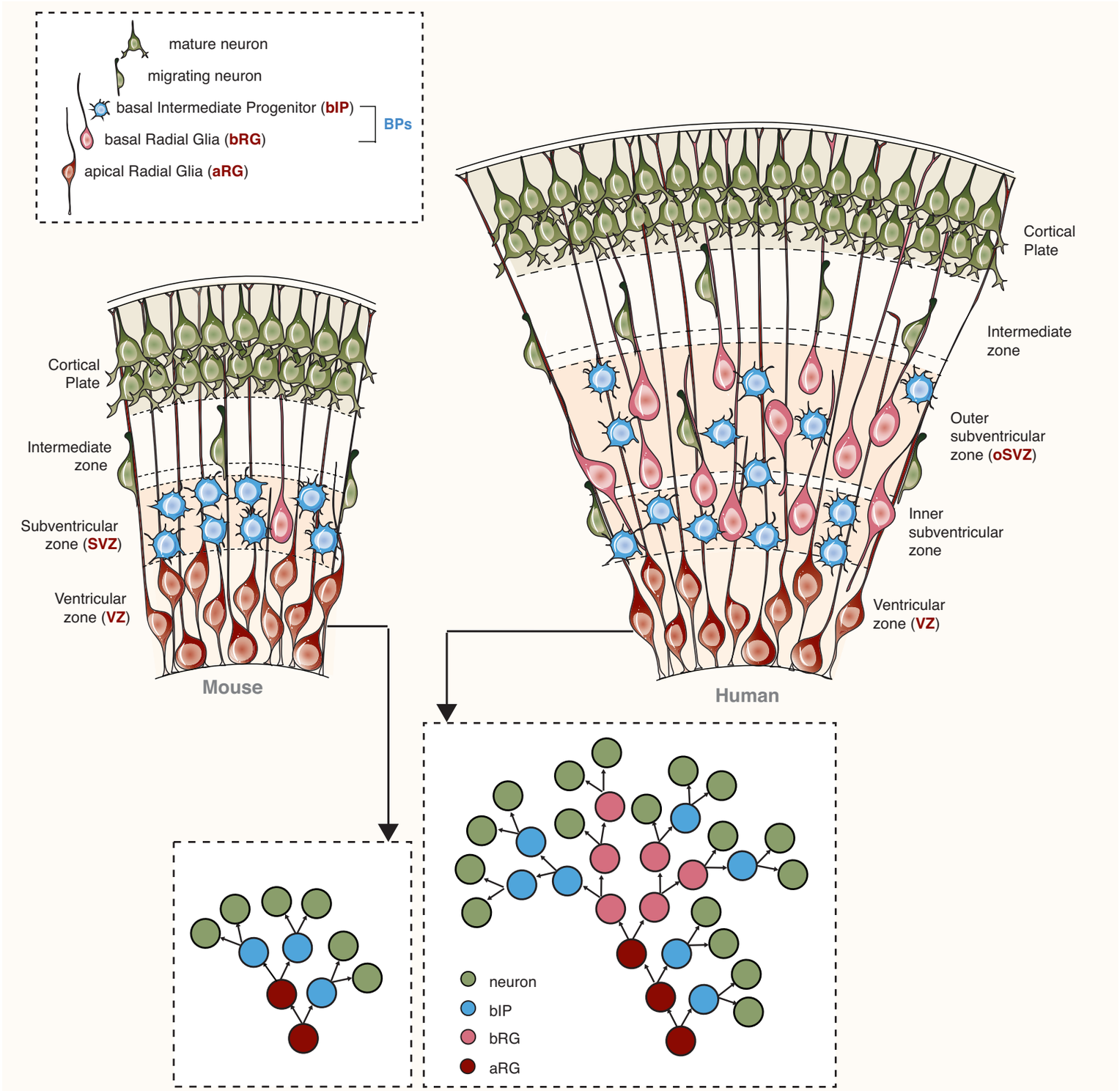
-CP “splits” PP, which becomes marginal zone (MZ) and subplate (SP).

-In mice, axons to and from cortical neurons form a fiber layer (“FL”) between SP and SVZ.

-In primates, there is an additional thick layer of progenitor cells called outer subventricular zone (OSVZ).

Basal radial glia (bRGs) are much more abundant in primate cortex and are located mainly in OSVZ.

# Basal radial glia have a high proliferative capacity




bRGs have a high proliferative capacity possibly due to their attachment to the basal lamina via basal membrane.

Abundance of bRGs may explain the immense number of neurons in primate cortex.

Florio, Borrell, Huttner (2017)

# Summary 2

Progression of cell division is not uniform across the brain.

There is a global regional difference in size and temporal progression of neurogenesis.
-This is likely to be tightly linked to brain regionalization

There is also a difference in cell division pattern between two adjacent cortical areas that have different neuronal density.

Rodent and primate brains are hugely different in size. Abundance of basal radial glia, which have a contact with the basal lamina and have high proliferative capacity, may contribute to this difference.

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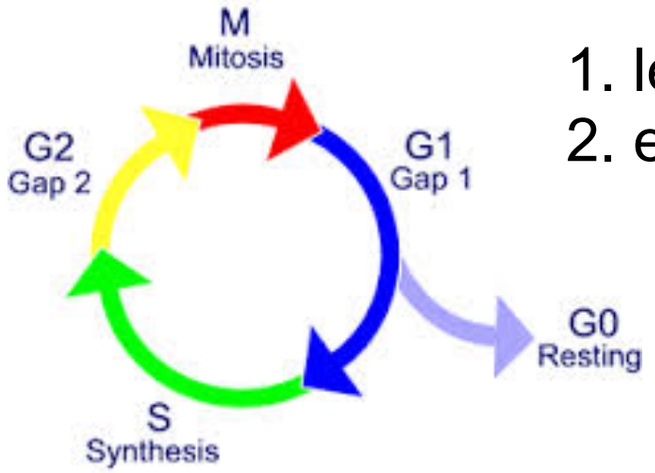
# Control of cell division

Control of cell cycle progression
Control of division mode (symmetric vs asymmetric)

# Control of cell division

cell cycle behavior

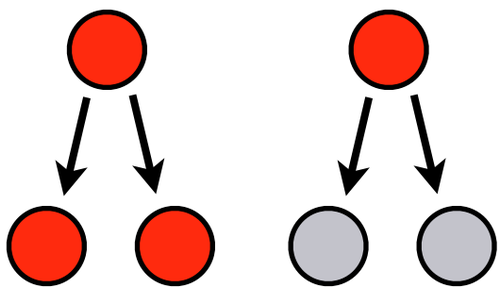



1. length
2. exit

**What are the intrinsic and extrinsic regulators of these processes?**

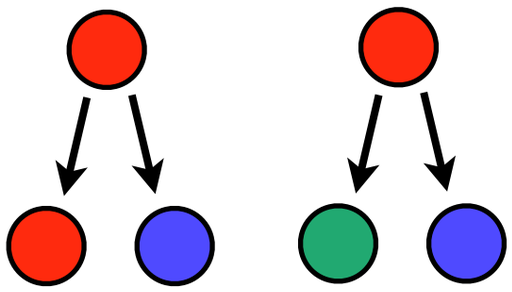
mode of division

1. symmetric



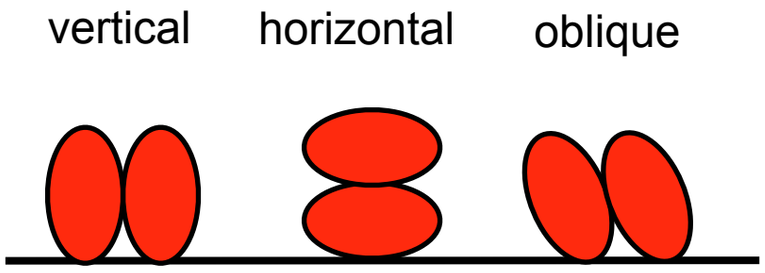
expand cell populations

2. asymmetric

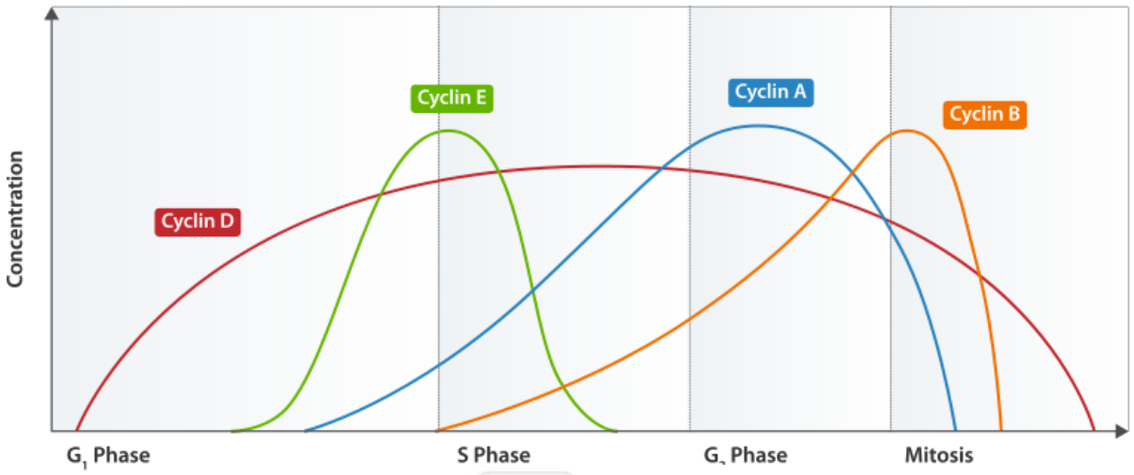


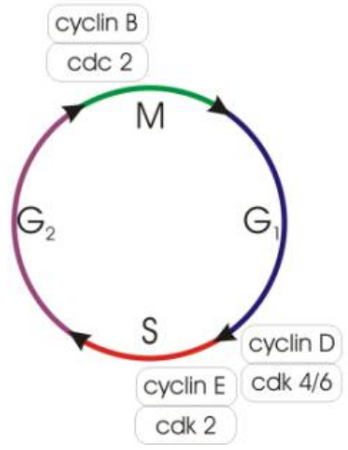
generate diverse cell types

cleavage plane of division influences division mode



# Regulation of cell cycle progression




-Cyclically activated cyclin-dependent protein kinases (Cdks) control the cell cycle.

-Protein levels of cyclins oscillate during the cell cycle, while concentrations of Cdks do not change.

-The G1 cyclins (cyclin D) help govern the activities of the G1/S cyclins, which control the progression into S phase.

**Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast**

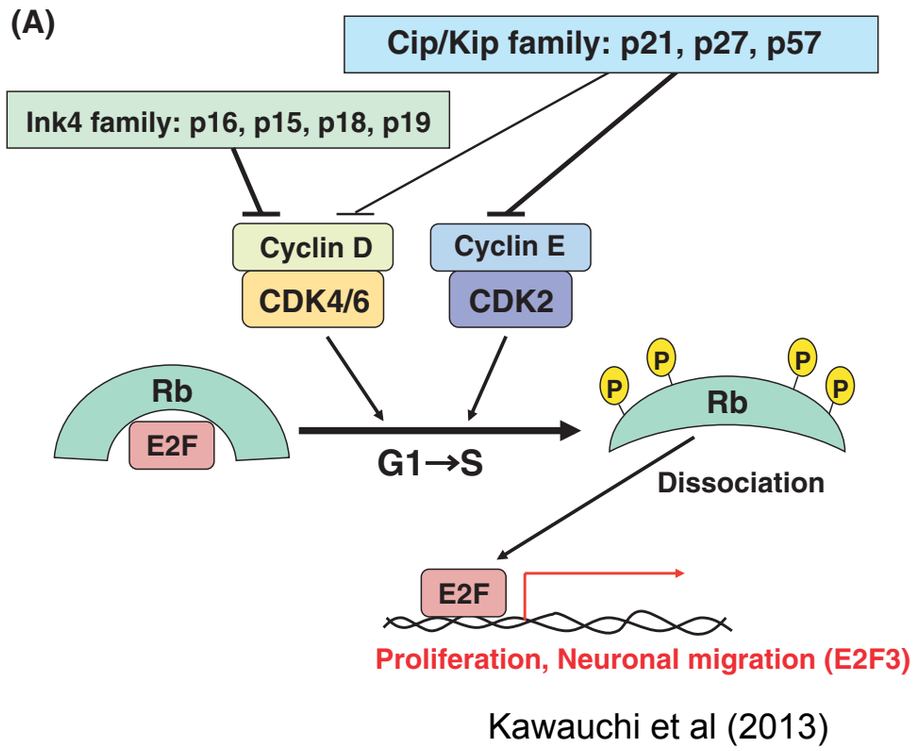
| CYCLIN–CDK COMPLEX | VERTEBRATES | | BUDDING YEAST | |
|---|---|---|---|---|
| | CYCLIN | CDK PARTNER | CYCLIN | CDK PARTNER |
| $G_1$-Cdk | cyclin D* | Cdk4, Cdk6 | Cln3 | Cdk1** |
| $G_1$/S-Cdk | cyclin E | Cdk2 | Cln1, 2 | Cdk1 |
| S-Cdk | cyclin A | Cdk2, Cdk1** | Clb5, 6 | Cdk1 |
| M-Cdk | cyclin B | Cdk1 | Clb1, 2, 3, 4 | Cdk1 |

*** There are three D cyclins in mammals (cyclins D1, D2, and D3).**
**** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.**

Table 17-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Alberts et al (2008)

# Regulation of G1/S transition




Kawauchi et al (2013)

-Cyclin D-Cdk4/6 and Cyclin E-Cdk2 activation phosphorylates Rb protein.

-Phosphorylation of Rb protein dissociates E2F family transcription factors. Both E2F1 and E2F3 promote G1-S transition.

-Rb (retinoblastoma) is a tumor suppressor. Children who inherit a mutant copy of the *Rb* gene develop retinoblastoma when the second allele of this gene is mutated in a progenitor cell in the retina. In the absence of Rb protein, E2F is free to activate the genes that cause uncontrollable progression of the cell cycle.

-Activities of Cyclin-Cdk complexes are suppressed by CDK inhibitor proteins.
CDK inhibitor families:
Kip family ($p21^{cip1}$, $p27^{kip1}$, $p57^{kip2}$)
Ink4 family ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, $p19^{Ink4d}$)

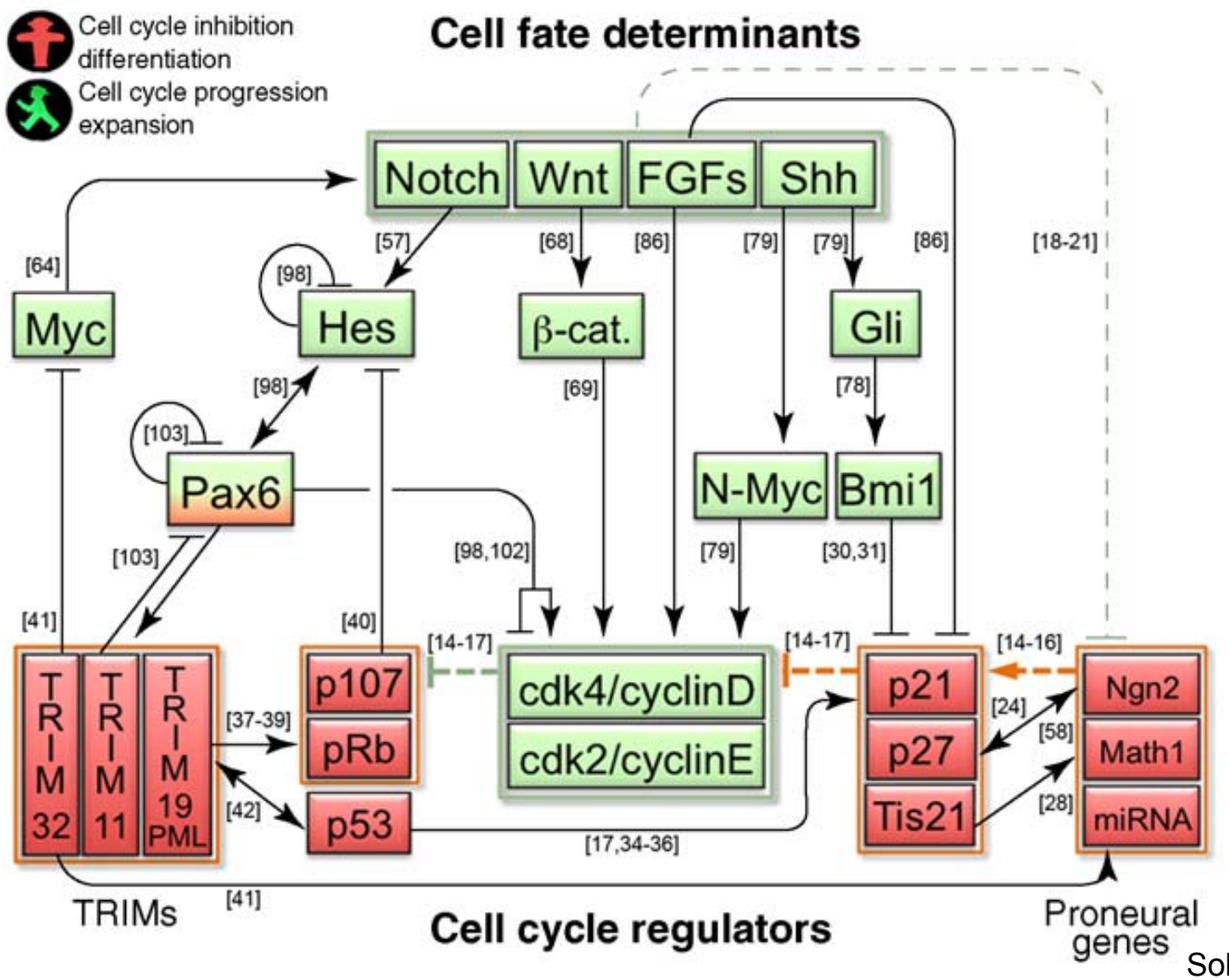
# Cell cycle length (in particular the length of G1 phase) is a fate determinant

-Lengthening G1 phase in progenitors by pharmacological inhibition of cyclin E-Cdk2 or RNAi-mediated silencing of cyclin D1-Cdk4 enhanced neurogenic divisions.

-G1 shortening by over-expression of cyclin D1, cyclin E1 or Cdk4-cyclin D1 increases proliferation and delays differentiation.

-Interkinetic nuclear migration (INM) depends on cell cycle progression. G1 phase arrest by over-expressing $p18^{Ink4c}$ led to an accumulation of nuclei at basal position of VZ of mouse cortex.

# Regulation of G1/S transition



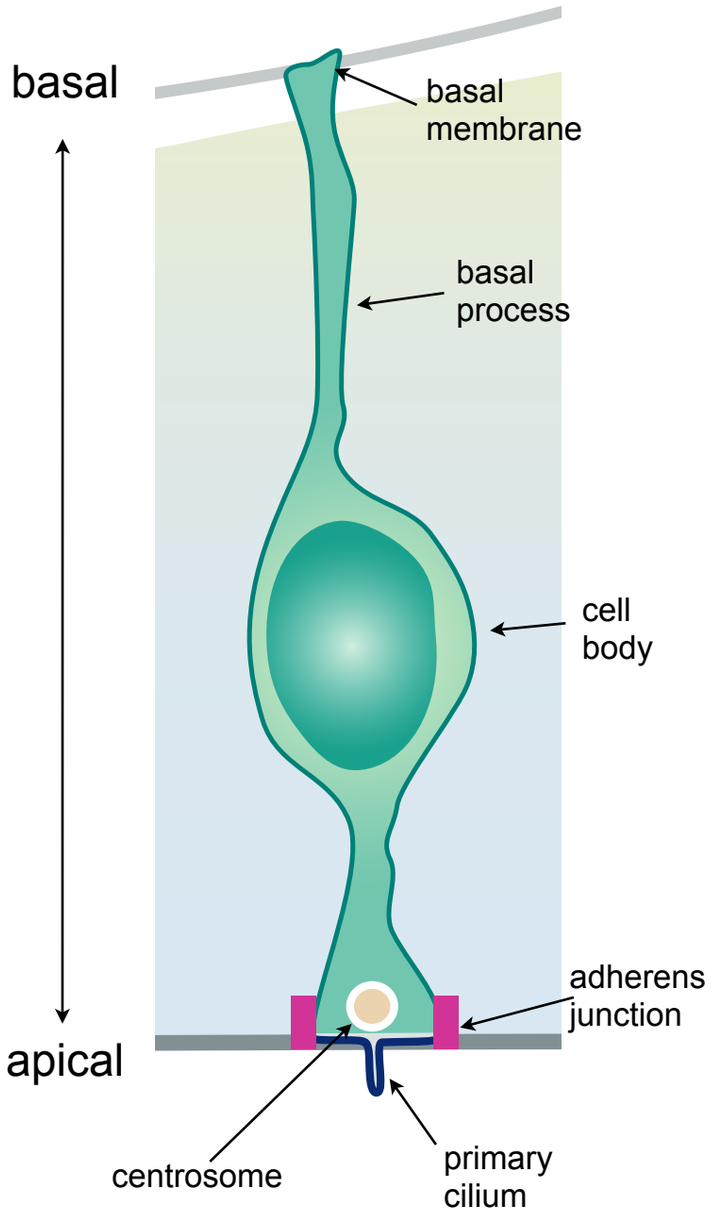

Solomoni and Calegari (2010)

-A number of signaling pathways affect different molecules that control G1-S transition.

-Wnt/β-catenin and FGF pathways upregulate cyclinD1 expression and shorten the cell cycle in neural progenitors. FGF10 has no effect on cell cycle length but is important for the transition from NECs to apical RGCs.

-Other growth factors have different targets. Shh/Gli pathway inhibits $p21^{cip1}$, $p27^{kip1}$ and Tis21 and indirectly activates G1-S transition.

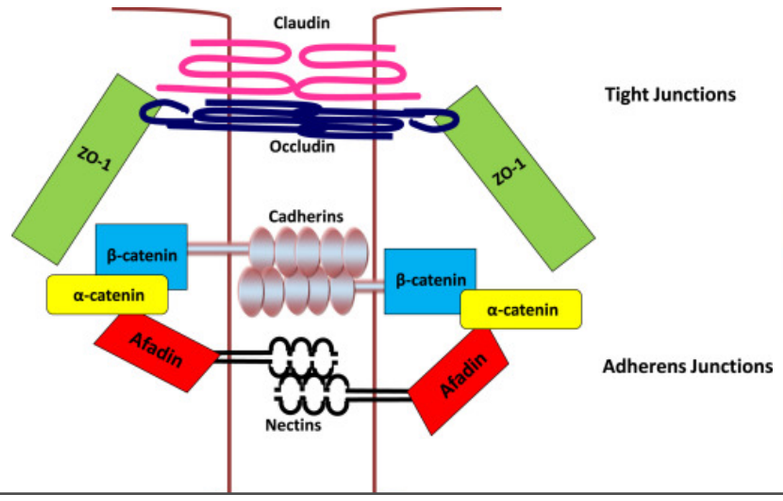
# Neuroepithelial cells and apical radial glia have an apical-basal polarity and specialized structures

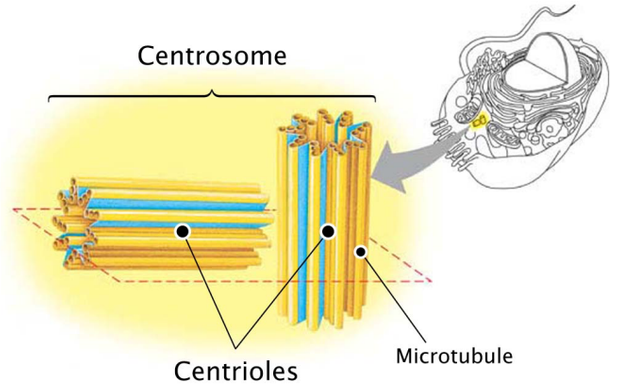


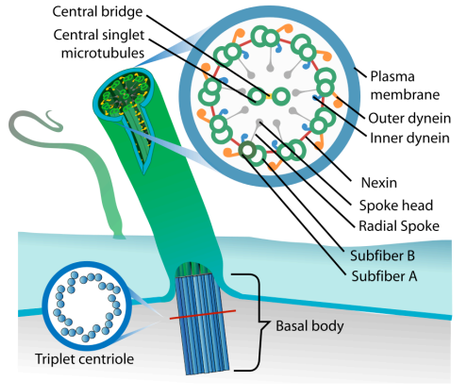

Basal structures:
Basal membrane is attached to the basal lamina.
-expresses integrin molecules, which may be important for maintenance of proliferative capacity

Apical structures:
-Adherens junctions consist of cadherins and catenins, attached to the actin cytoskeleton...allows cohesion of neighboring neuroepithelial cells

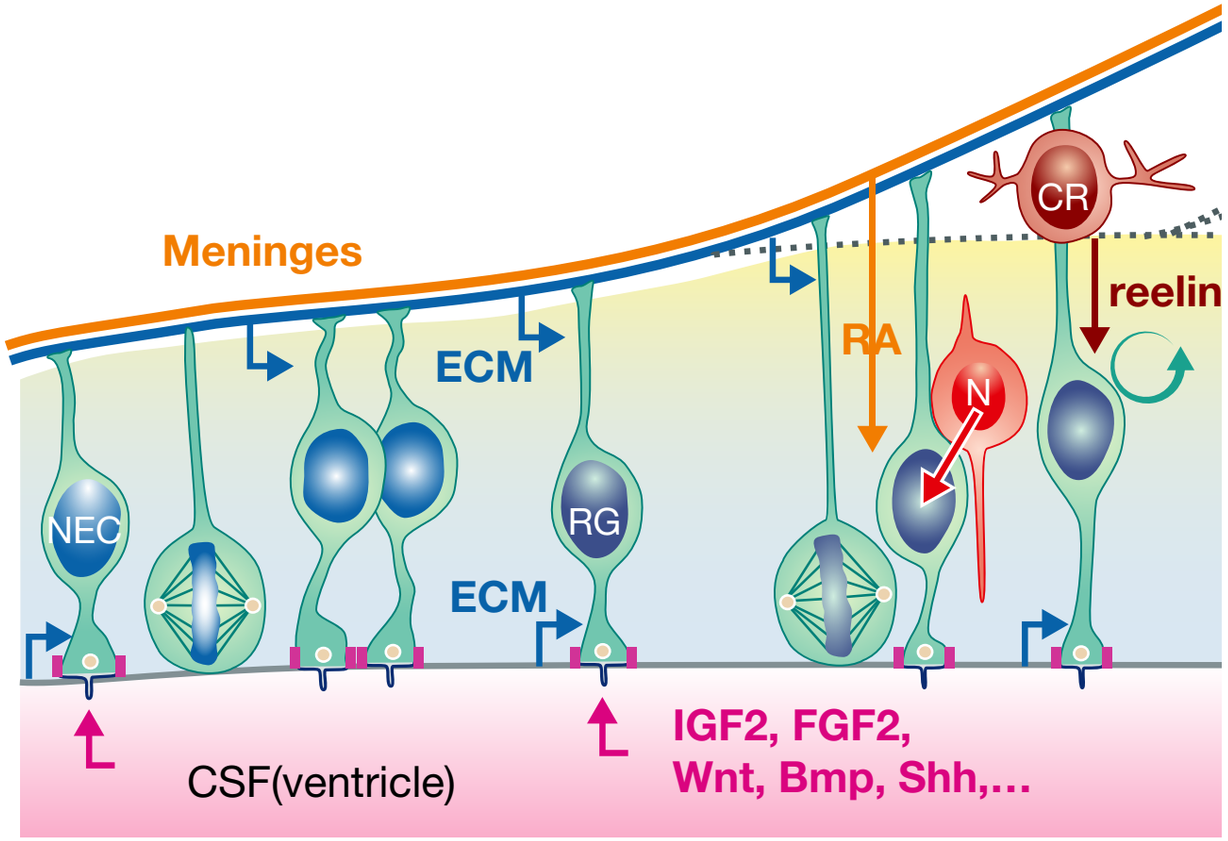
-Centrosome is docked to the apical membrane and forms the base for the primary cilium.
The primary cilium is an antenna for various growth factors contained in CSF (e.g. IGF-I, SHH).



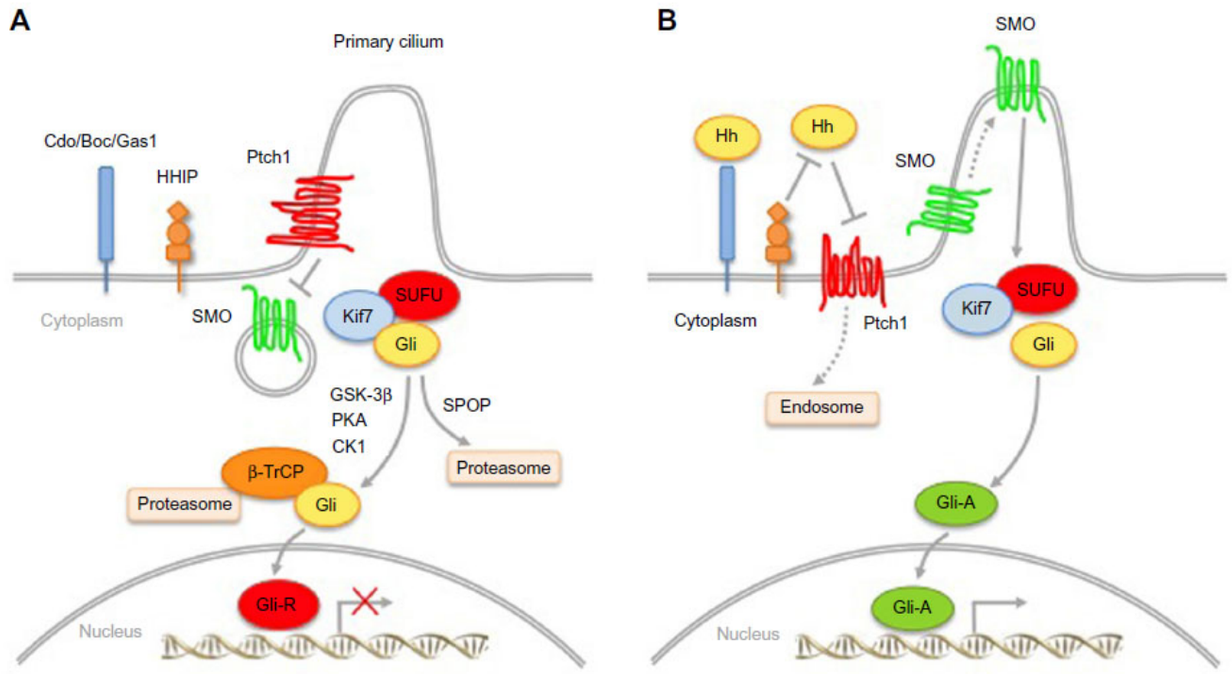
# Regulators of cell divisions come from various sources




**PRE-NEUROGENESIS** → **NEUROGENESIS**

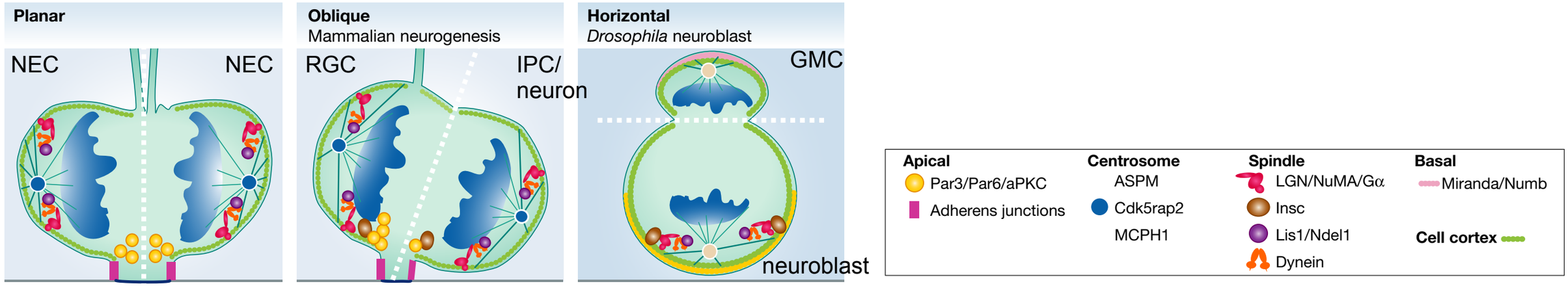
Paridaen and Huttner (2014)

SHH signaling requires the primary cilium



# Regulation of division pattern

1. What determines spindle orientation?
2. What determines daughter cell fates in asymmetric divisions?




In Drosophila neuroblasts, horizontal cleavage results in asymmetric distribution of fate determinants, leading to different fates of the two daughter cells.

In mammalian cortex, localizing the LGN complex to the lateral membrane is essential for a planar (vertical) division.

**Inheritance of basal process**

-A planar division results in an inheritance of basal and apical structures by both daughter cells. Basal process is either split or one of the daughter cells regrows it after the division.

-After an oblique division of an RGC, the daughter cell that inherits the basal process stays as an RGC and the other daughter cell that loses the basal process becomes an IPC or a neuron.

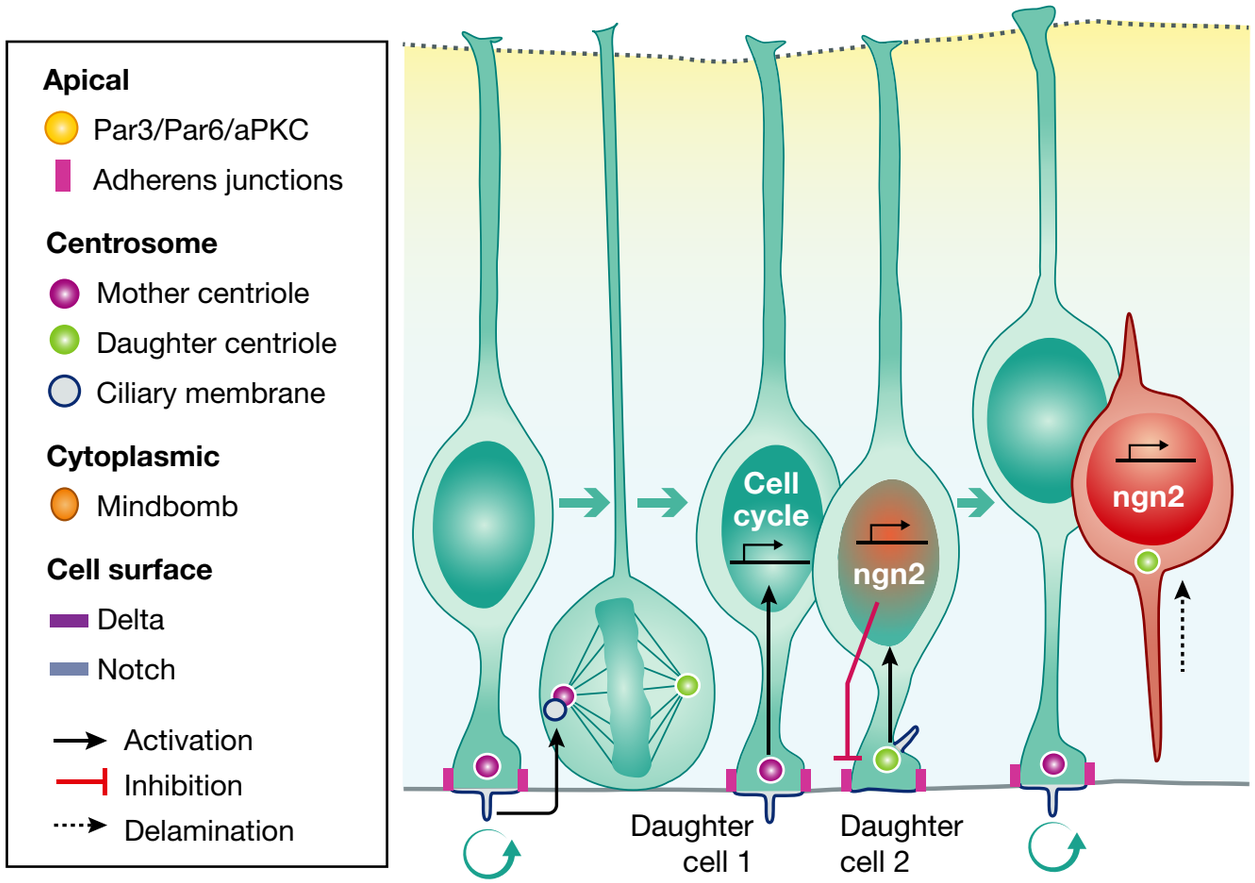
# Regulation of division pattern

**centrosome asymmetry, ciliogenesis and daughter cell fate**

Centrosome is composed of two orthogonally arranged centrioles (mother and daughter centrioles).

In interphase cells, the mother centriole forms the basal body at the base of the primary cilia. In oblique division of RGC, the daughter cell that inherits the other centriole maintains the cilial membrane and reforms the primary cilium into the ventricle. This cell stays as an RGC.

The other daughter cell that inherits the daughter centriole grows a primary cilium at the basolateral membrane, thereby not receiving growth factor signal from the CSF and differentiate.




Many genes encoding centrosomal proteins are mutated in patients with primary microcephalies, a group of diseases resulting in a dramatic decrease in brain size at birth.

# Summary 3-regulators of cell division

Cell division is controlled at cell cycle progression (length, exit) and division mode (symmetric vs asymmetric).

Cyclins control cell cycle progression by activating CDKs, which control transition of different phases of the cell cycle by phosphorylating their targets.

For G1-S transition, CDKs phosphorylate Rb, which allows E2F transcription factors to promote transition to the S phase.

Many growth factors regulate the cell cycle in developing brain. They come from different sources and utilize various specialized structures of NECs and RGCs like basal and apical membranes (and primary cilium).

Spindle orientation determines symmetry vs asymmetry of the NEC/RGC division. Asymmetric division results in asymmetric distribution of both basal and apical components as well as many molecules.

Failed regulation of cell division may results in abnormal cell numbers in the brain.