Cell Division & Cell Linage I

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Course News

Coffee Hour Dr. Nakagawa & Dr. McLoon
Tuesday, Sept 25
10:00-11:00am
Surdyk's Café in Northrop Auditorium

Stop by for a minute or an hour!

Cell division in the developing nervous system is a highly regulated process.

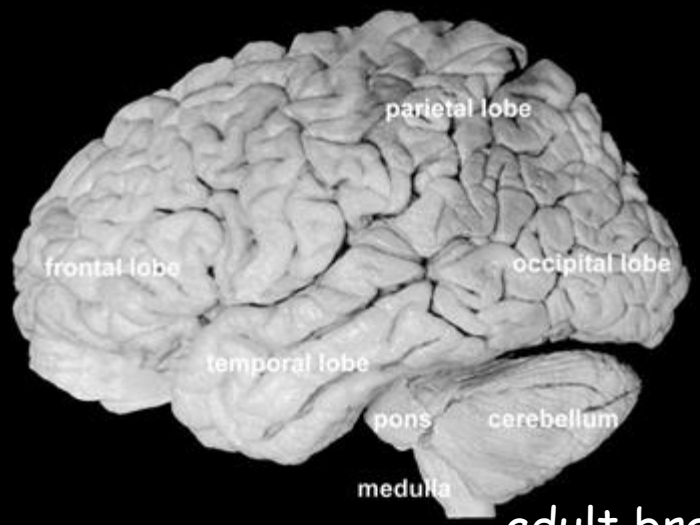
Unicellular organisms have a strong selective pressure to grow and divide as rapidly as possible. Their rate of division is usually controlled by the availability of nutrients and temperature.

Cells of multicellular organisms gave up the potential for unlimited division in order to maintain an optimal number of each cell type for the overall good of the organism. The division of a cell is determined by the genetic program for each specific cell type and is regulated by cell extrinsic factors.

Cell division in the developing nervous system is a highly regulated process.

neural plate
~2,000 cells

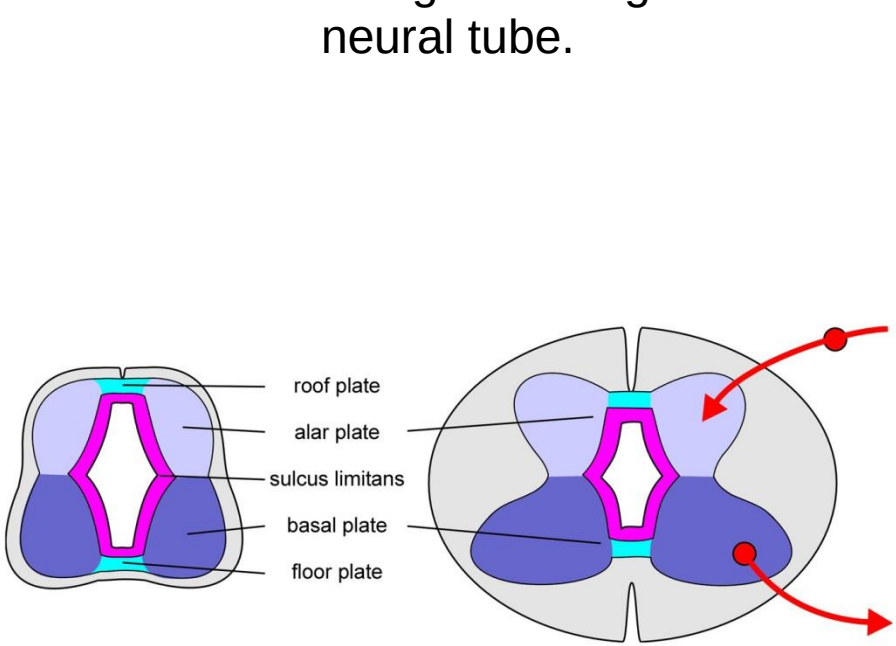
- Significant cell division is required to generate the nervous system.
- Cell division must terminate when the system reaches the adult size.

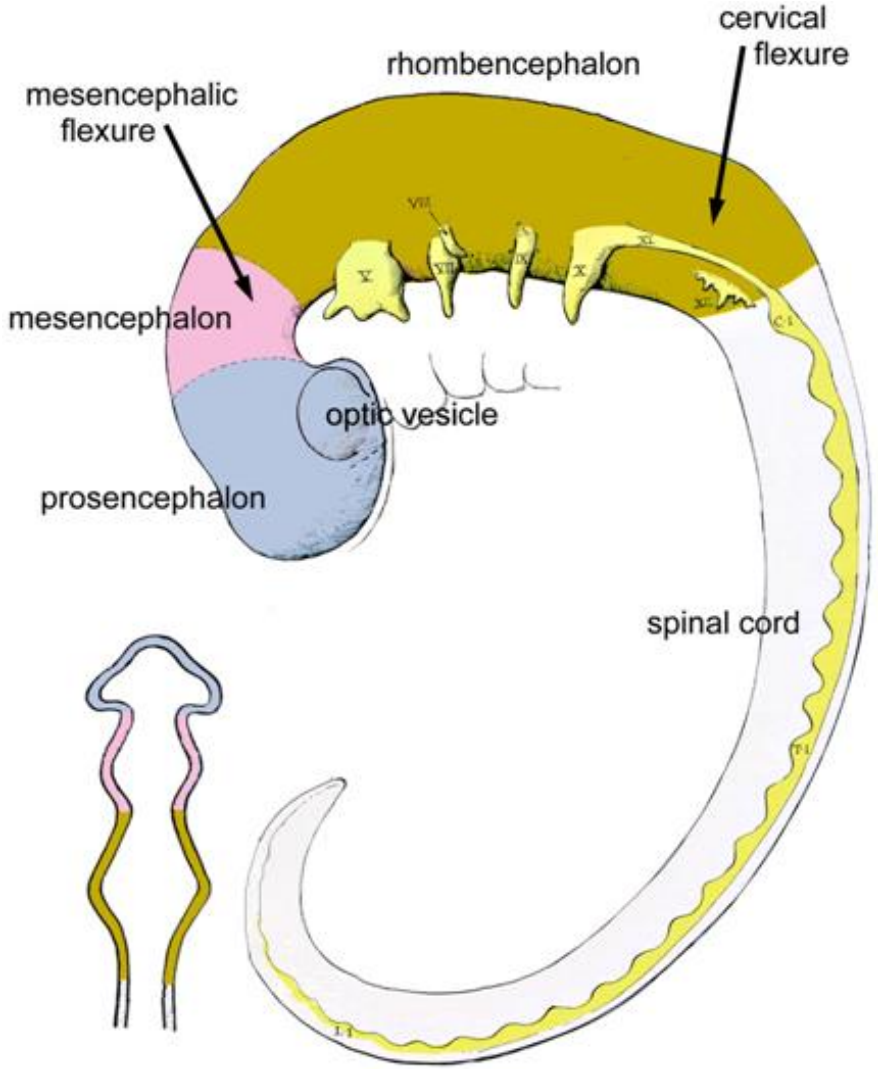


adult brain
~100,000,000,000 cells

Cell division in the developing nervous system is a highly regulated process.

- The amount and rate of cell division are not uniform along the length or around the circumference of the neural tube.





Names for Dividing Cells in the Developing Nervous System

progenitor cells

neural epithelial cells

radial glial cells

neuroblasts

stem cells / neural stem cells

ventricular zone cells

apical progenitor cells

basal progenitor cells

intermediate progenitor cells

subventricular zone cells

outer subventricular zone cells

outer radial glia cells

Names for Dividing Cells in the Developing Nervous System

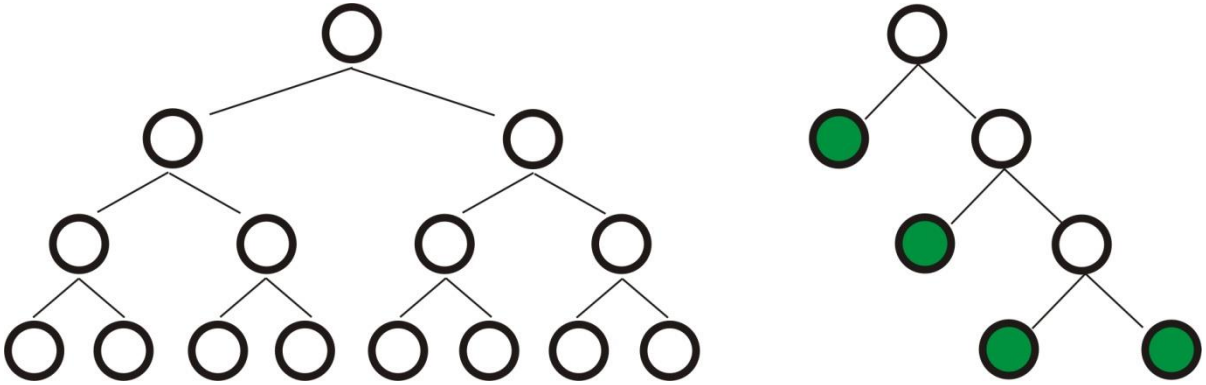
- Early progenitor cells of the neural tube are called neuroepithelial cells. These cells span the thickness of the neural tube.
- In brain regions that grow the largest (e.g. cortex and cerebellum), the neuroepithelial cells later in development can express markers of astrocytes such as GFAP, and then are called radial glia.
- The distinction between a neuroepithelial cell and radial glia is not well defined.

Names for Dividing Cells in the Developing Nervous System

- Early in neural tube development, all cell division is in the ventricular zone or layer. These progenitor cells can be called ventricular zone cells.
- Later, some cell division can take place outside the ventricular zone. These cells can have different names depending on where they divide (e.g. subventricular zone cells, outer radial glia).

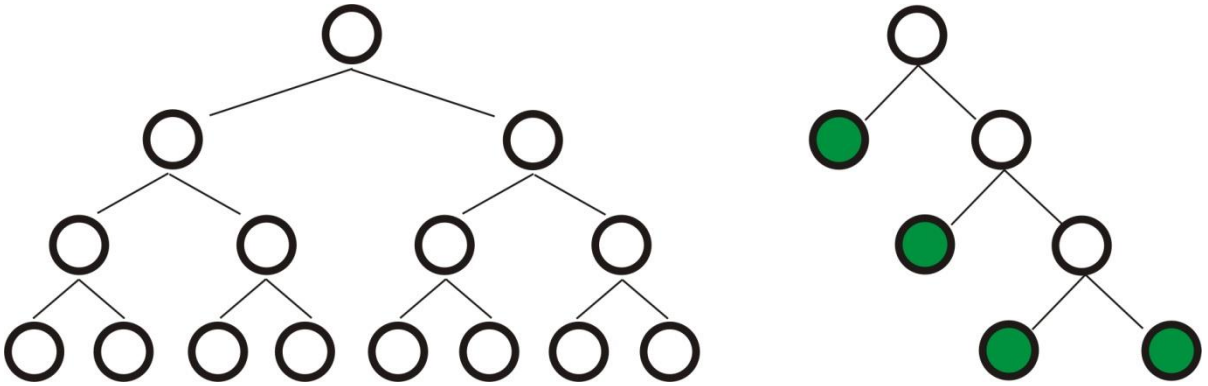
Modes of Cell Division

- The developing nervous system has three modes of cell division:
    - symmetric dividing (preneurogenic)
    - asymmetric (neurogenic)
    - symmetric differentiating (neurogenic)

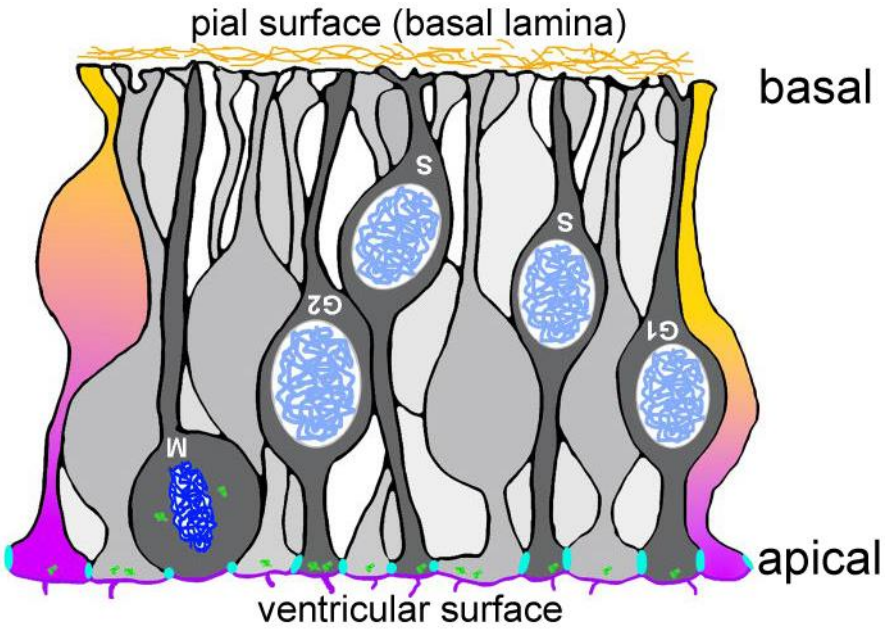


Modes of Cell Division

- The length of time the progenitor cells spend in the symmetric dividing (preneurogenic) mode of division is a major determinant of the final size of a tissue.
- Neurogenic divisions are required to generate a functional nervous system.

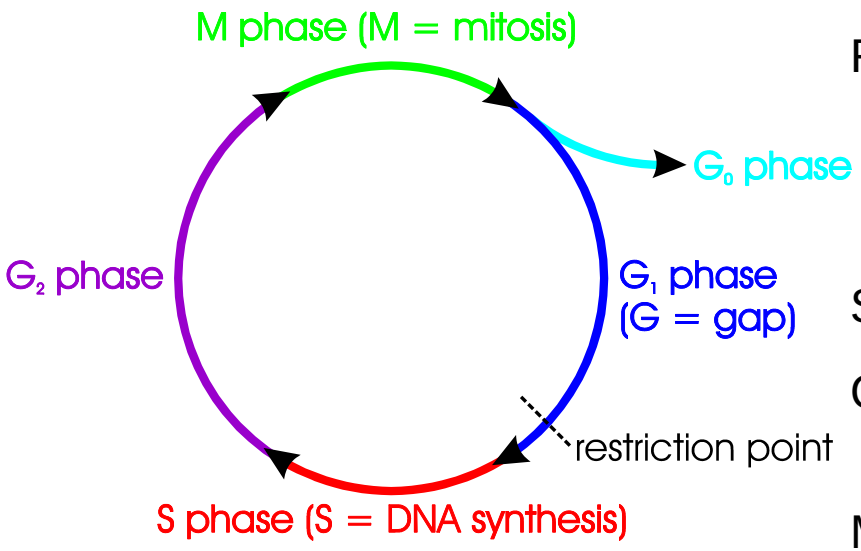


Neuroepithelial Cells



- Bipolar cells with apical and basal ends
- Neighboring progenitor cells are linked at the apical ends by adherens junctions.
- Apical end has a cilium projecting into the ventricle, which is lost during M-phase.
- Basal end is in contact with the basal lamina.
- Many regulatory proteins are asymmetrically distributed in the apical-basal axis.

Phases of the Cell Cycle



G_1 period during which proteins that initiate or block division are expressed

Restriction point - a condition during in which a cell is destined to progress through mitosis regardless of any changes in the environment of the cell

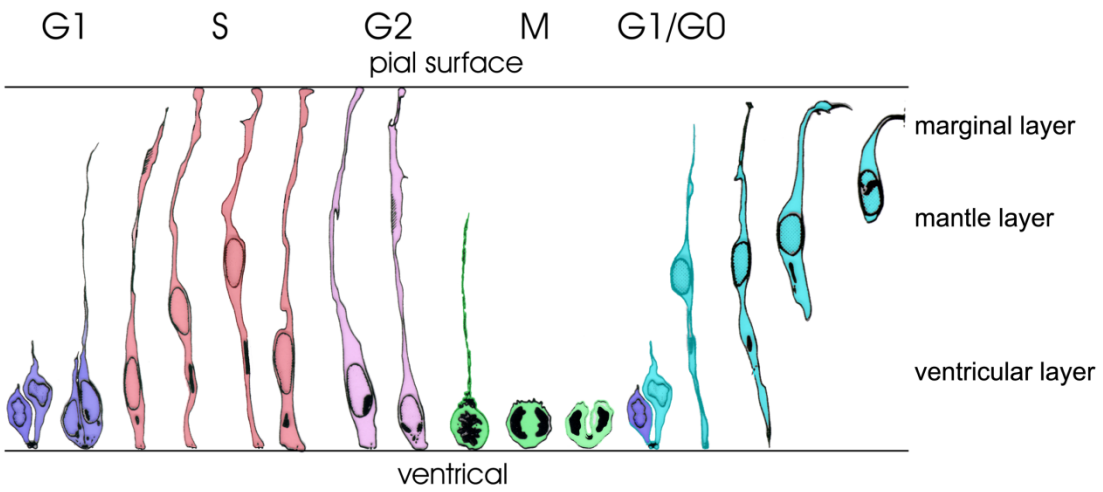
S period during which DNA is replicated

G_2 period during which proteins needed for mitosis are expressed

M period during which cell divides into two; steps are: prophase, metaphase, anaphase, telophase and cytokinesis

G_0 permanent arrest in G_1; period during which neurons differentiate and function

Interkenetic Nuclear Migration during the Cell Cycle in the Early Neural Tube



G1 nucleus translocates from the ventricular (apical) surface towards the pial (basal) surface

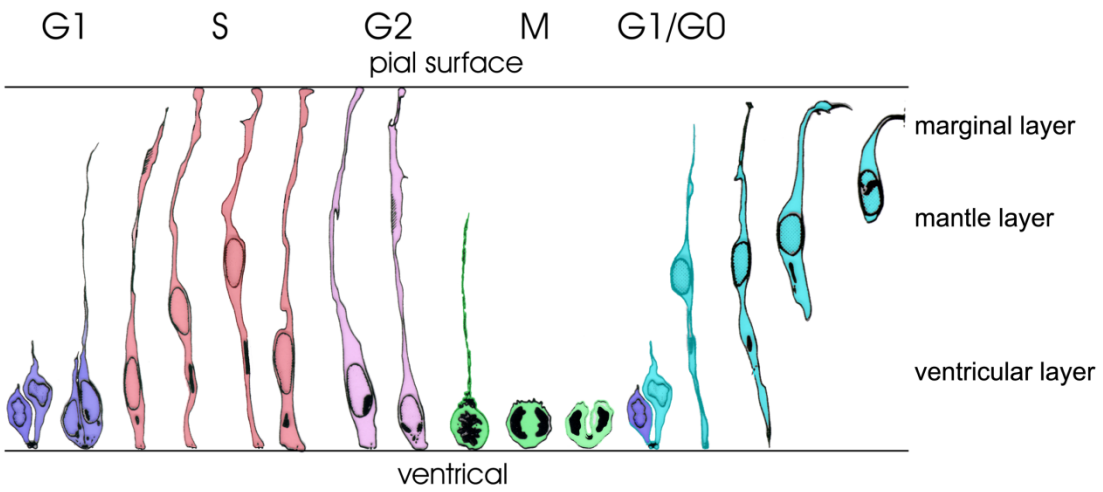
S DNA replicates

G2 nucleus translocates towards the ventricular (apical) surface

M cell divides at the ventricular (apical) surface

G0 cell loses attachment to the ventricular surface and migrates towards the pial surface to differentiate

Layers of the Early Neural Tube



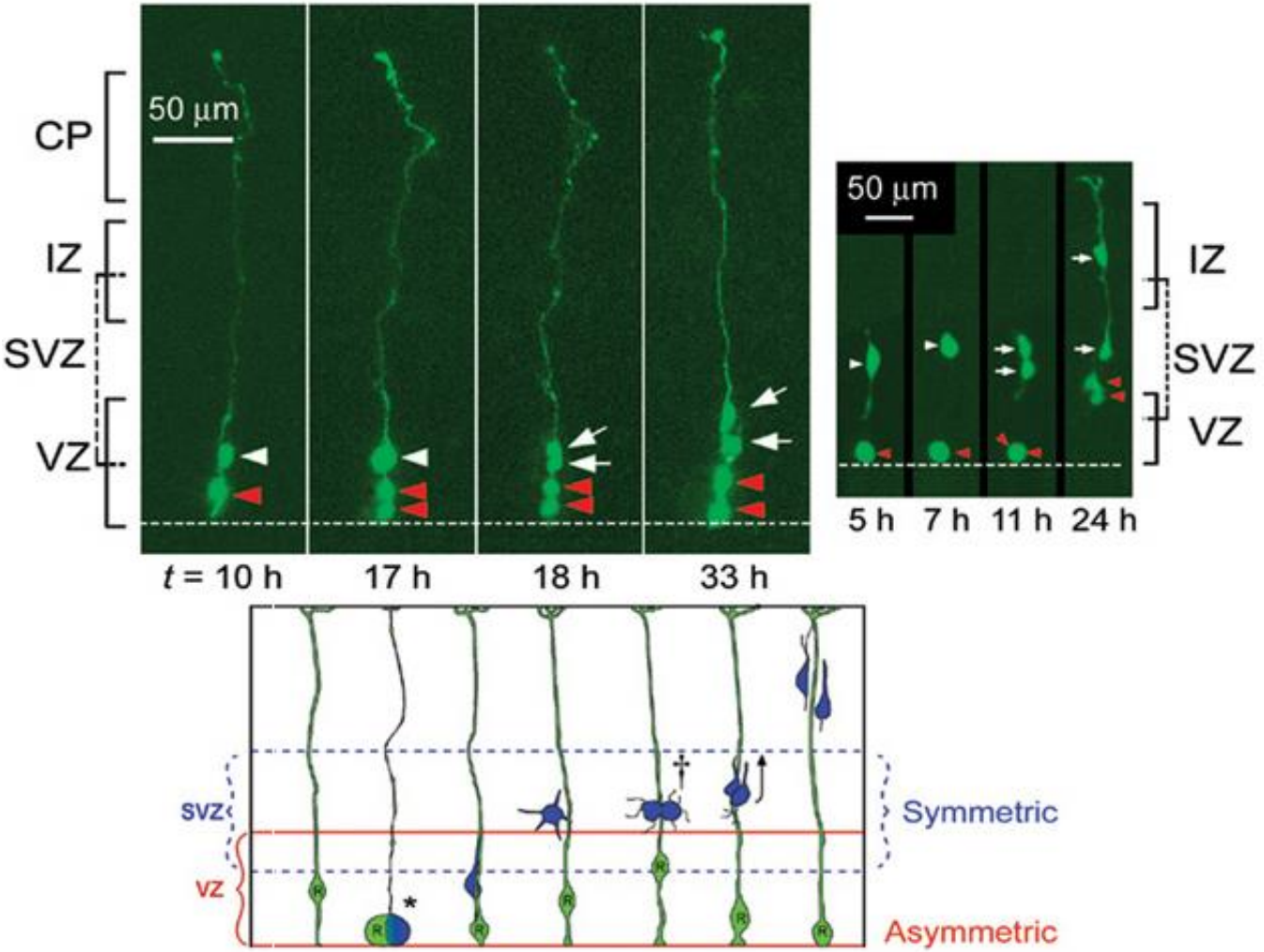
ventricular layer – dividing cells

mantle layer – postmitotic, differentiating cells

marginal layer – axons below pial surface

In some regions, progenitor cells migrate away from the ventricular zone and establish a secondary site of cell division.

e.g. subventricular zone of developing cortex



In some regions, progenitor cells migrate away from the ventricular zone and establish a secondary site of cell division.

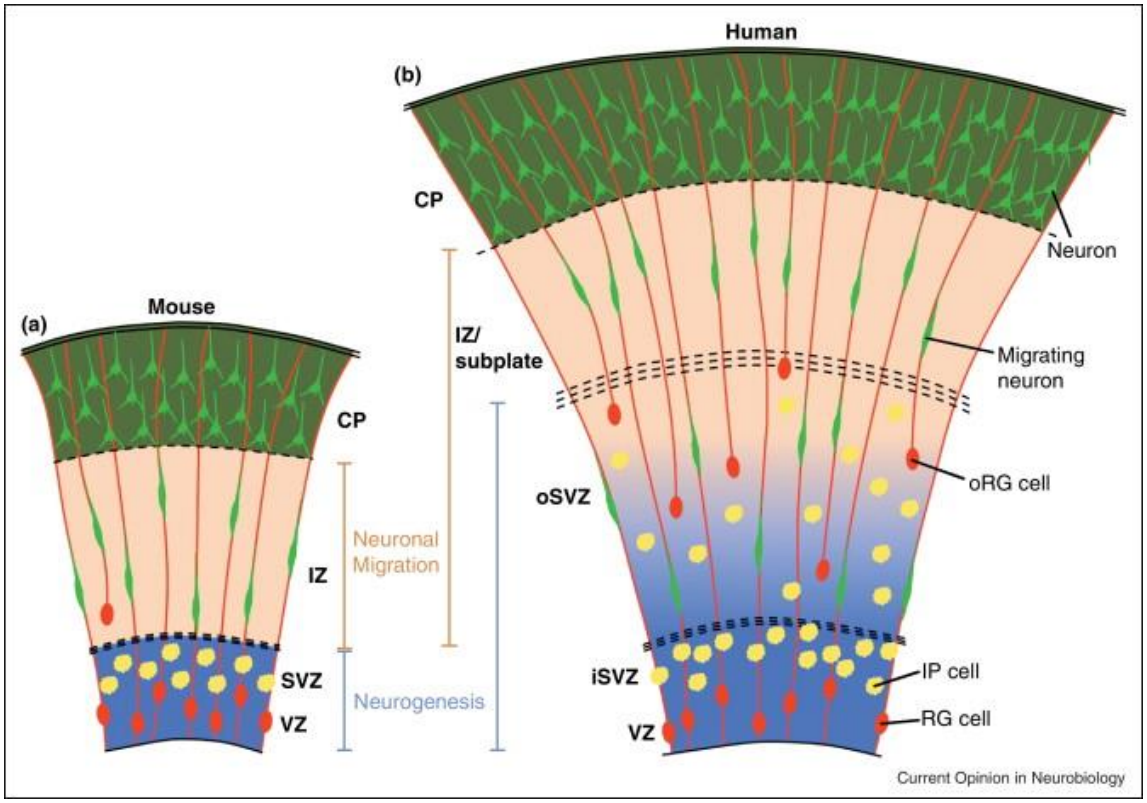
- Cells that divide in these secondary (i.e. non-ventricular) sites have been called:
    - intermediate progenitor cells
    - basal progenitor cells
    - subventricular zone cells
    - transit amplifying cells

In some regions, progenitor cells migrate away from the ventricular zone and establish a secondary site of cell division.

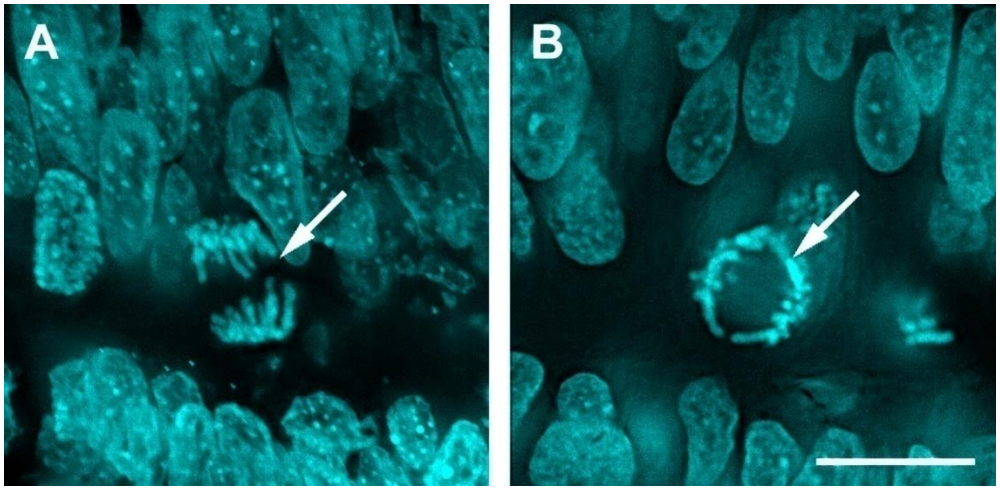
- Intermediate progenitor cells:
  - are not polarized.
  - lack apical or basal processes.
  - divide symmetrically.
  - typically undergo a limited number of divisions.

Outer Subventricular Zone Progenitor Cells (Outer Radial Glia)

- reside in cortex above the subventricular layer
- have a basal process extending to the basal lamina
- lack an apical process
- abundant in primate cortex; rare in rodent cortex

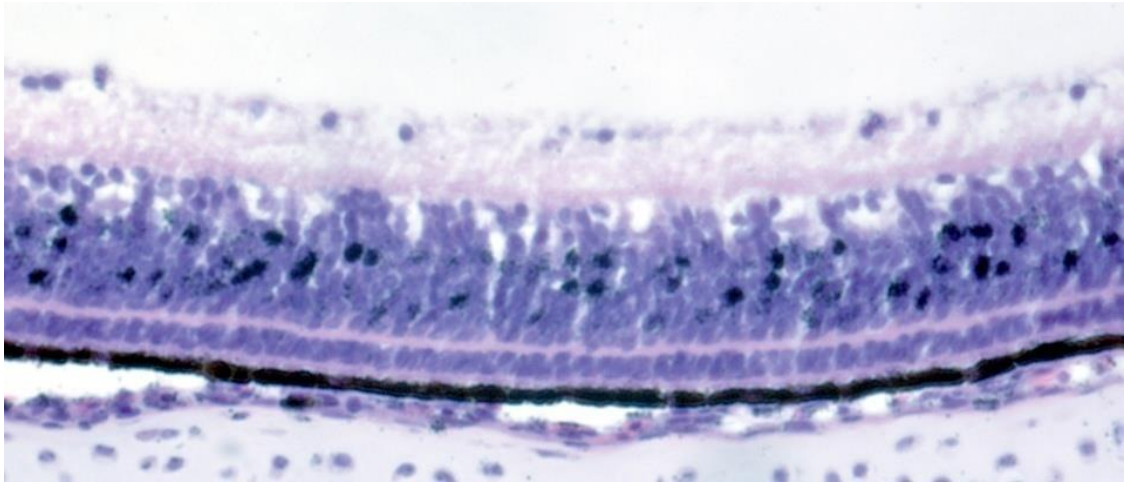


Methods Used to Study Cell Division Experimentally



Nuclear stains show M-phase cells.

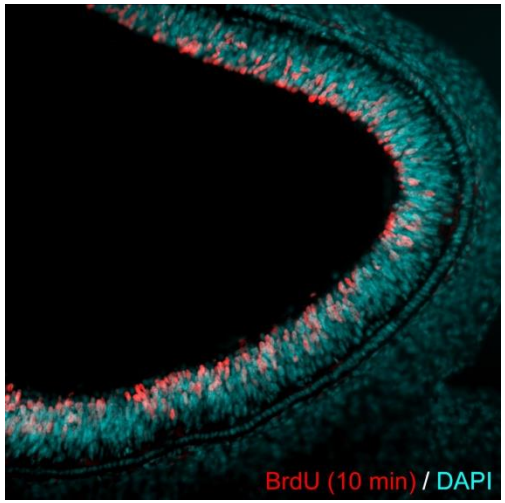
Methods Used to Study Cell Division Experimentally

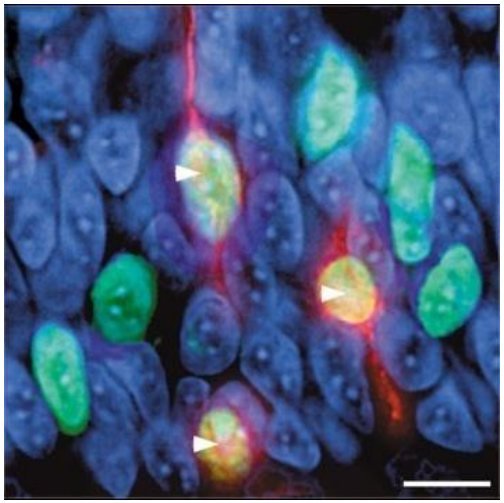


S-phase labeling:

- ^{3}H-thymidine labeling coupled with autoradiography

Methods Used to Study Cell Division Experimentally





S-phase labeling:

- bromodeoxyuridine (BrdU) labeling coupled with immunohistochemistry

Methods Used to Study Cell Division Experimentally

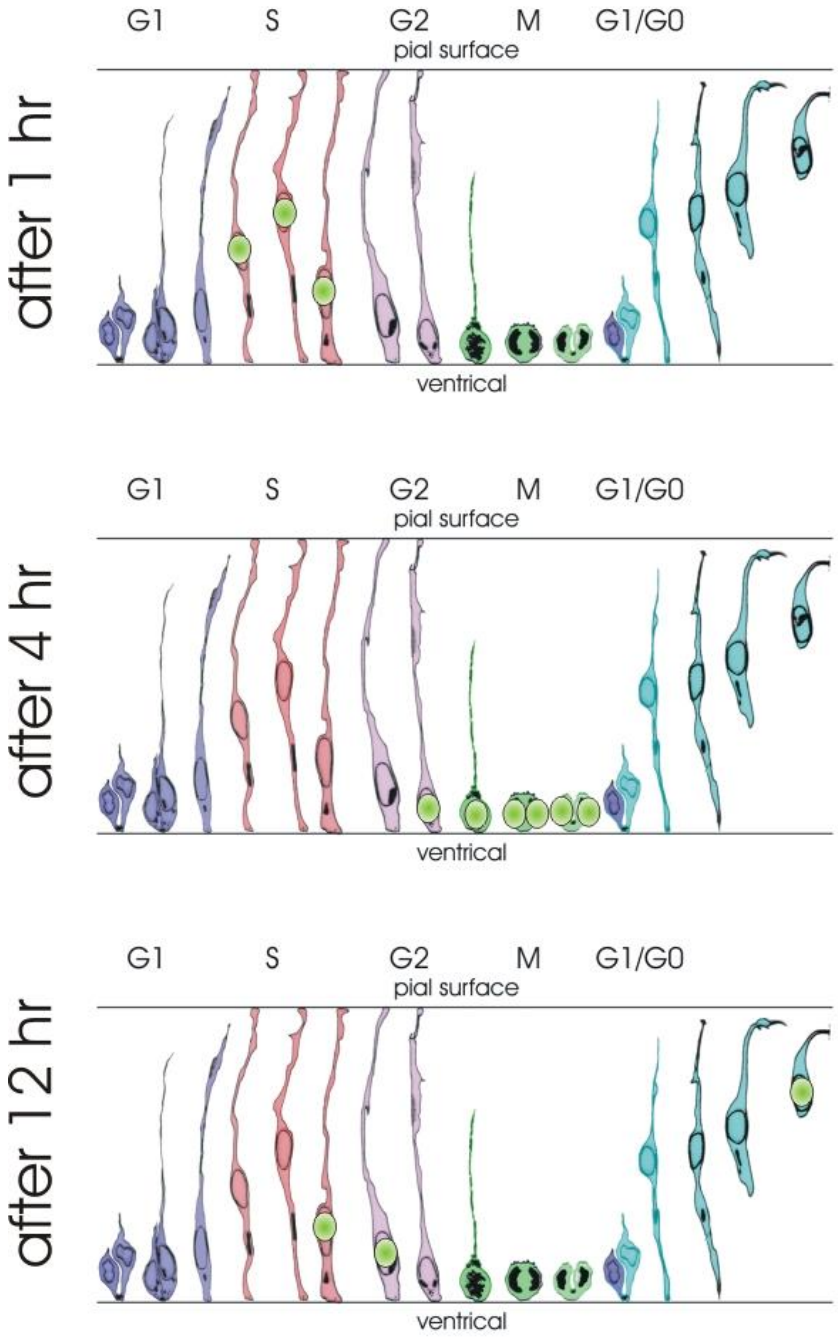
S-phase labeling:

- in mammals, label is available for 1-4 hrs following the injection

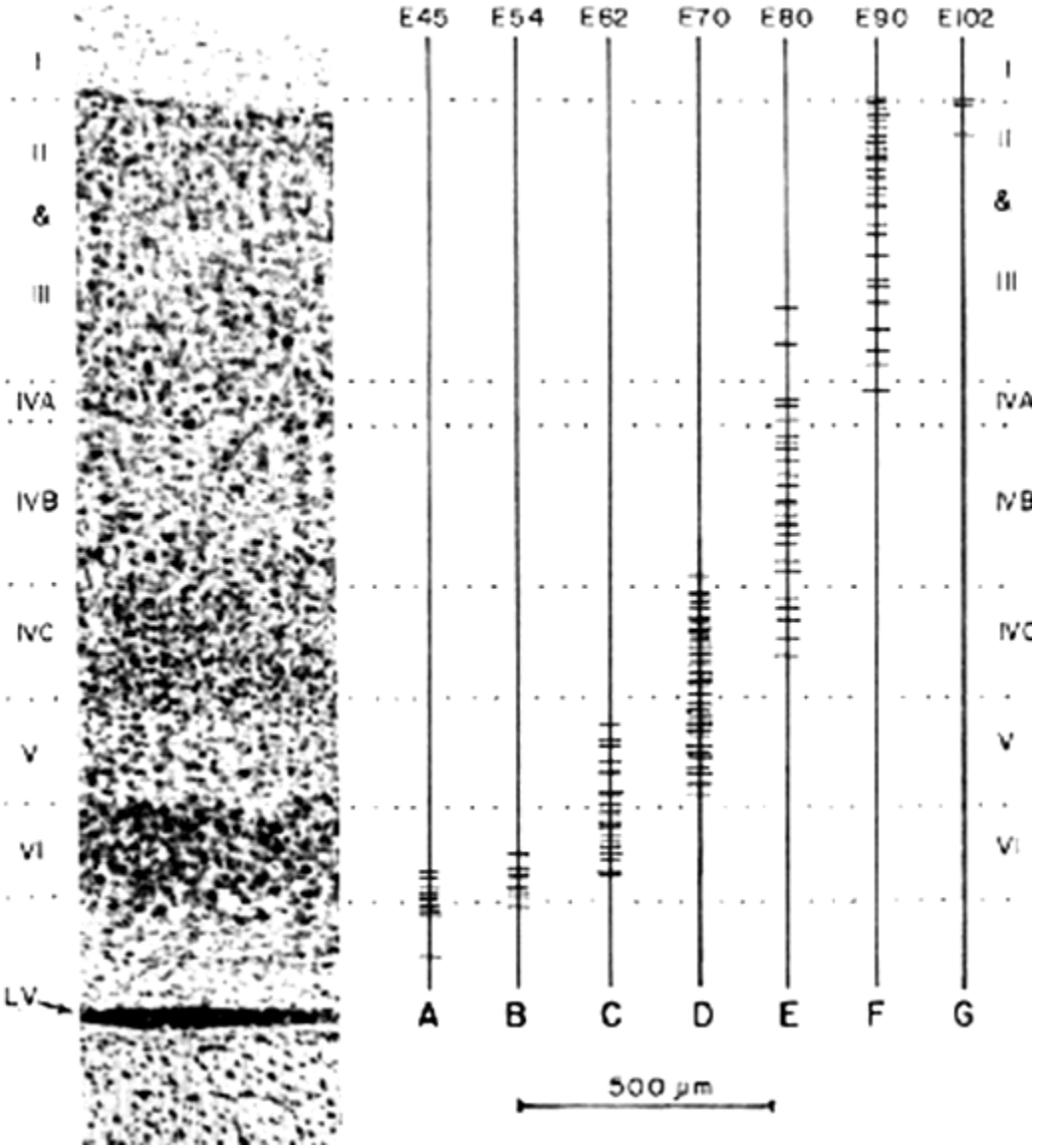
- increased post-injection survival time results in labeled cells at progressively later phases of the cell cycle

- labeled cells that differentiate following division, retain the label throughout life

- label will be diluted in cells that continue to divide


after 1 hr
G1
S
G2
M
G1/G0
pial surface
ventrical
after 4 hr
G1
S
G2
M
G1/G0
pial surface
ventrical
after 12 hr
G1
S
G2
M
G1/G0
pial surface
ventrical

Cell Birth Dates in Cerebral Cortex



[Rakic (1974) Science 183]

Methods Used to Study Cell Division Experimentally

Immunohistochemistry or in situ hybridization to detect expression of certain proteins:

Cell cycle markers-
- Proliferating Cell Nuclear Antigen – G1/S phase
- Phosphohistone H3 – G2/M phase
- Ki67 – all phases

Neural progenitor cell markers-
- Nestin
- Sox2
- Pax6

Differentiating cell markers-
- Proneural bHLH transcription factors
  - (Ngn, Ngn2, Ash1, etc.)
- p27/kip1
- HuC/D

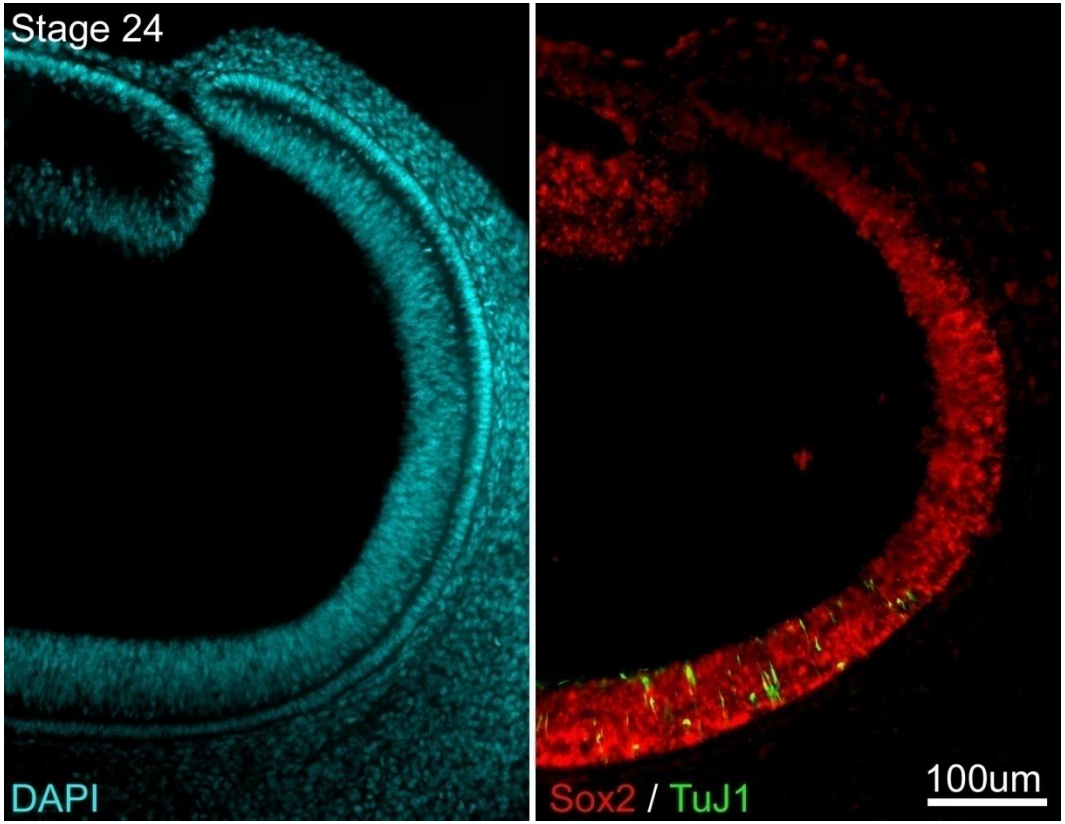
Methods Used to Study Cell Division Experimentally

Immunohistochemistry or in situ hybridization to detect expression of certain proteins:

Differentiated neuron-
- B3-tubulin (neuronal tubulin, TuJ1)
- Islet1
- Neurofilament (x3)

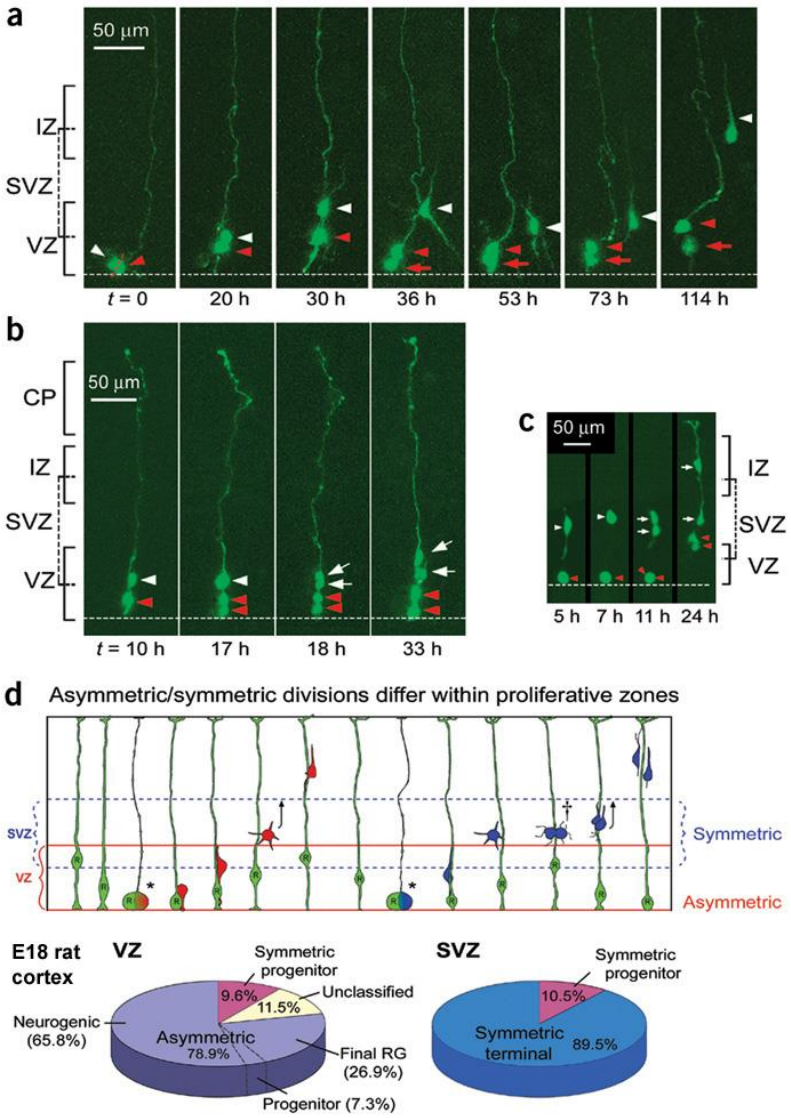
Differentiated astrocyte-
- Glial fibrillary acidic protein (GFAP)
- Glutamine synthetase

Sox2 = neural progenitor cell
TuJ1 (neuronal tubulin) = differentiating neuron



Methods Used to Study Cell Division Experimentally

Live imaging of fluorescently labeled cells



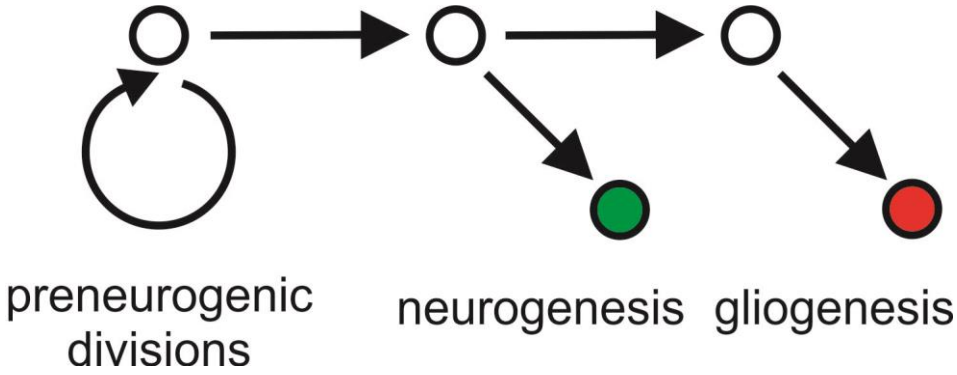
[Noctor et al. (2004) Nature Neurosci.]

Live imaging revealed the architecture of cell division.

- Ventricular layer cells retain attachment to the pial (basal) surface during division.
- One cell inherits the basal process and typically will remain in the ventricular layer.
- The cell that did not inherit the process can grow a new process and continue dividing, can migrate and differentiate or can migrate and divide again.

Generalizations on the Pattern of Differentiation in the CNS

- neurons before glia
- large, projection neurons before small interneurons



Generalizations on the Pattern of Differentiation in the CNS

- basal plate (motor) before alar plate (sensory)
- ontogeny parallels phylogeny

Cell Cycle Length

- varies; 12 hrs is typical
- increases during development
- difference between slow and fast dividing cells is typically the time spent in G_1
- length of S, G_2 and M phases are nearly constant for all cells of an organism

Table 2.1. Cell Cycle of Neurepithelial Germinal Cells

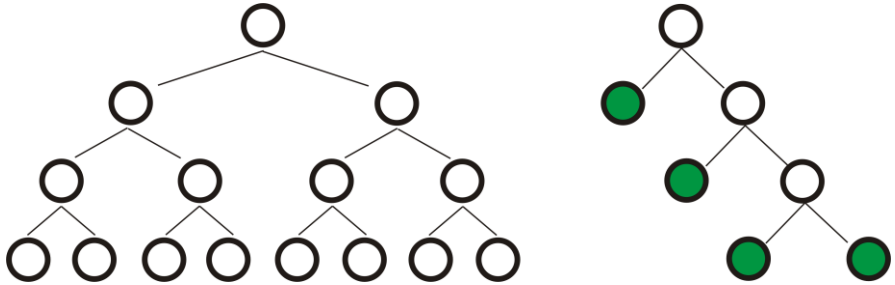
Tissue	Species	Age	Cycle time	S	G_2	M	G_1	Reference
Neural tube	Chick	E1	5	—	—	—	—	Fujita (1962)
	Mouse	E10	8.5	4.6	0.6	1.3	2	Kauffman (1968)
	Mouse	E11	10.5	5.4	1.2	1.3	2.7	Same
	Mouse	E11	11	5.5	1	1	3.5	Atlas and Bond (1965)
Telencephalon	Mouse	E10	7	5.1	1	0.8	0.1	Hoshino *et al.* (1973)
	Mouse	E13	15.5	6.9	1	0.8	6.8	Same
	Mouse	E17	26	10.4	1	0.8	13.8	Same
Cerebral cortex	Mouse	E15	11	7.5	2	2	—	Langman and Welch (1967)
	Rat	E12	11	6–8	2	—	3.7	Waechter and Jaensch (1972)
	Rat	E18	19	6–8	2	—	11.2	Same
Cerebral subventricular zone	Rat	E18	19	6–7	—	—	10	Same
	Rat	P1	18.3	10	3.7	—	3.1	Same
	Rat	P6	17.2	10.8	2.5	—	2.5	Same
	Rat	P21	20.1	12.4	2.1	—	5.2	Lewis and Lai (1974)
Hippocampal dentate gyrus granule cells	Rat	P1–12	15.1–17.7	10.1–11.7	2.5–3.3	—	1.1–2.4	Lewis (1978)
Neural retina	Chick	E6	10	—	—	—	—	Fujita (1962)
	Mouse	P2	28	12.5	1.5	0.8	13	Denham[a] (1967)
Optic tectum	Chick	E3	8	4	1.5	0.3	2.2	Wilson (1973, 1974)
	Chick	E4	9	5	1.5	0.4	2.1	Same
	Chick	E5	13	4	1.5	0.8	6.7	Same
	Chick	E6	15	5	1.5	1.4	7.1	Same
	Mouse	E10	8.5	5	1	1	1.5	Wilson (1974)
	Mouse	E11	11	6	0.9	1.2	2.9	Same
Rhombic lip	Rat	E14	12	—	—	—	—	Ellenberger *et al.* (1969)
Cerebellum external granule cells	Mouse	P1–10	21.5	7	2	0.6	11.9	Fujita *et al.* (1966)
	Mouse	P7–14	24	—	5	—	—	Miale and Sidman (1961)
	Mouse	P2–10	18	8.3	2	—	7.8	Mares *et al.* (1970)

[a] Time in hours.

Symmetry of Cell Division

- early divisions are symmetric with both daughter cells dividing again

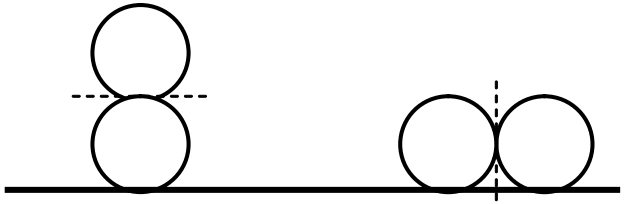
- later divisions generate one or two cells that differentiate



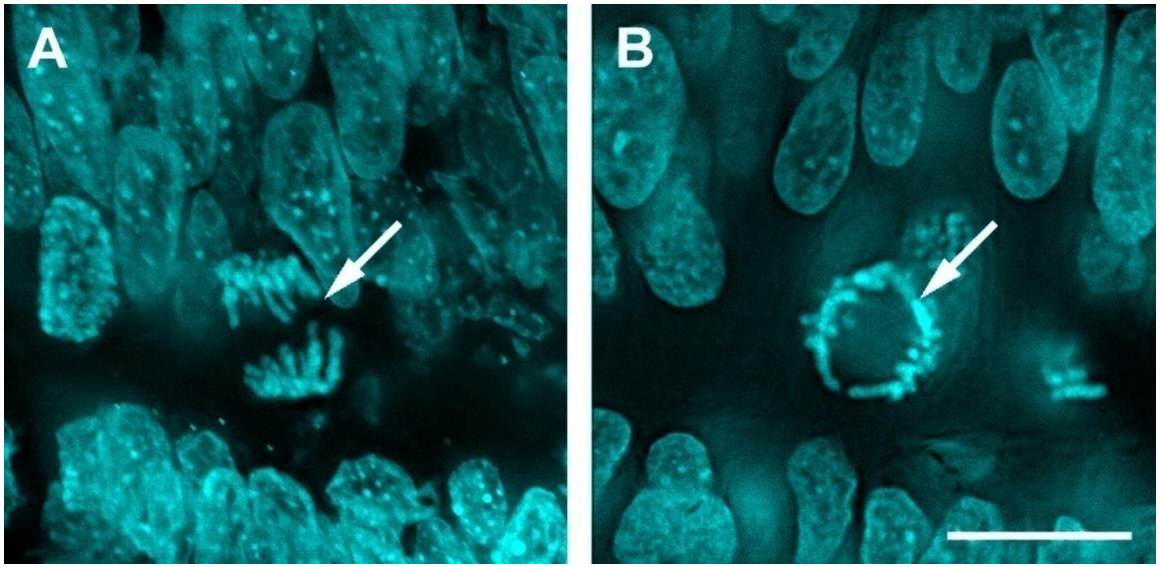
- Emx2, a transcription factor, is expressed by the preneurogenic progenitor cells in the cerebral cortex; misexpression of Emx2 in cortical cells delays differentiation and promotes division; knockout of Emx2 has the opposite effect and results in a smaller cortex.

Symmetry of Cell Division

- the plane of cell division could be linked to the mode of division:



[ventricle]



Symmetry of Cell Division

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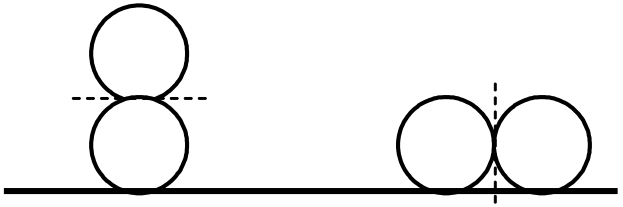


[ventricle]

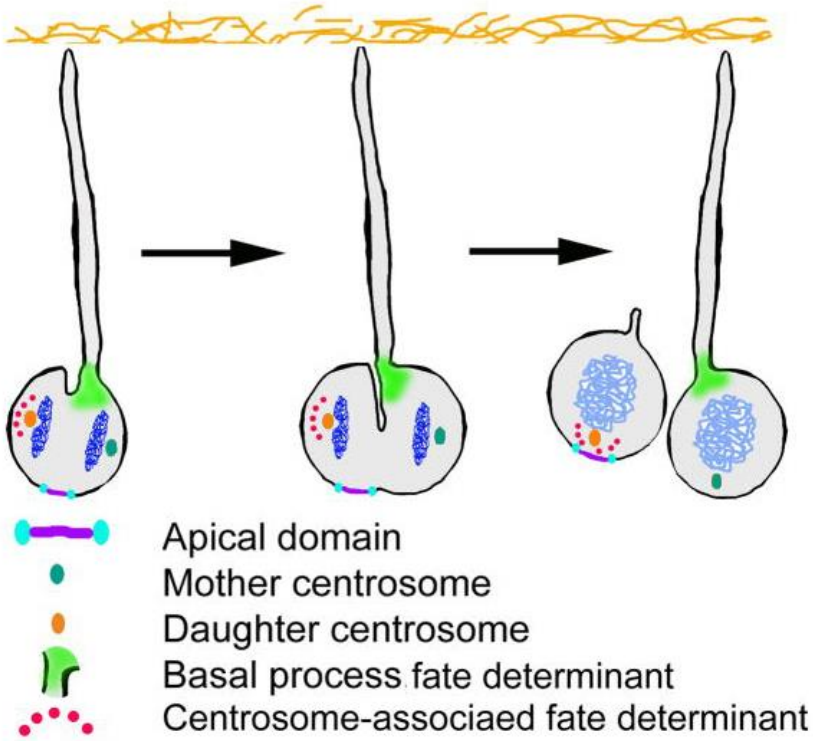
  - divisions with a cleavage plane parallel to the ventricular surface proposed to be asymmetric with one cell differentiating and the other cell dividing

  - divisions with a cleavage plane perpendicular to the ventricular surface proposed to be symmetric with both daughter cells dividing again

Symmetry of Cell Division

- however, many studies found that virtually all divisions have a plane perpendicular or nearly perpendicular to the ventricular surface

Symmetry of Cell Division



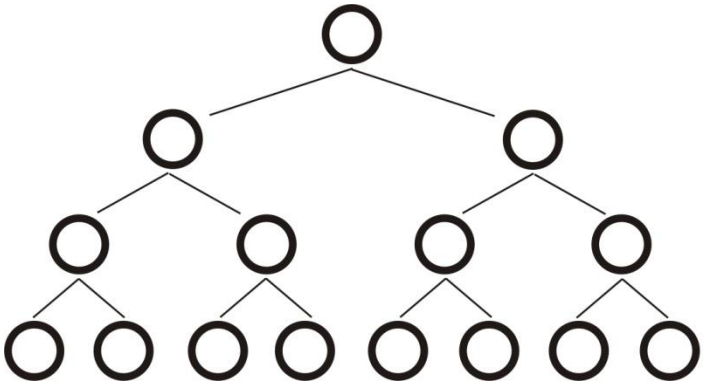
- The relative division of cell components between the two daughter cells appears to better predict the outcome of a division:
  - The cell that continues to divide will inherit the basal process.
  - The cell that differentiates will inherit the newly synthesized centrosome.
  - Prominin-1 and Par3/6 (cell membrane receptor-like proteins) are present in the daughter cell that divides and absent from the one that differentiates. The Par complex is important for the asymmetric distribution of various cellular elements.

Initiation of Differentiation

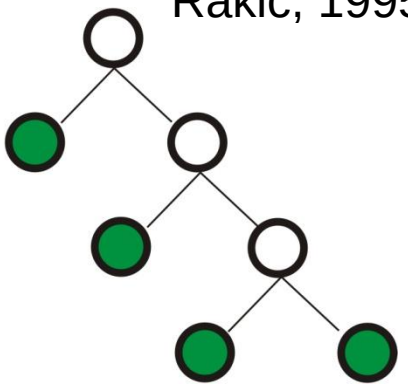
- The most significant factor in determining the final number of cells in a region of the adult nervous system is the time spent in the preneurogenic mode of division.

e.g. The conversion from preneurogenic to neurogenic division in human cortex is two days later than in monkey, which results in a considerably larger cortex.

Rakic, 1995



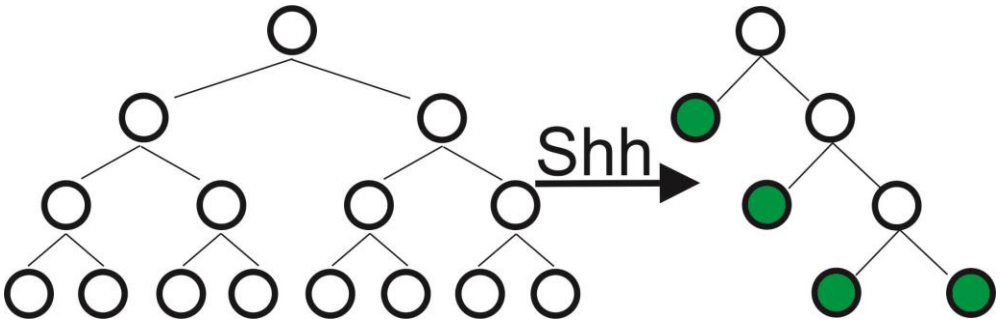
preneurogenic

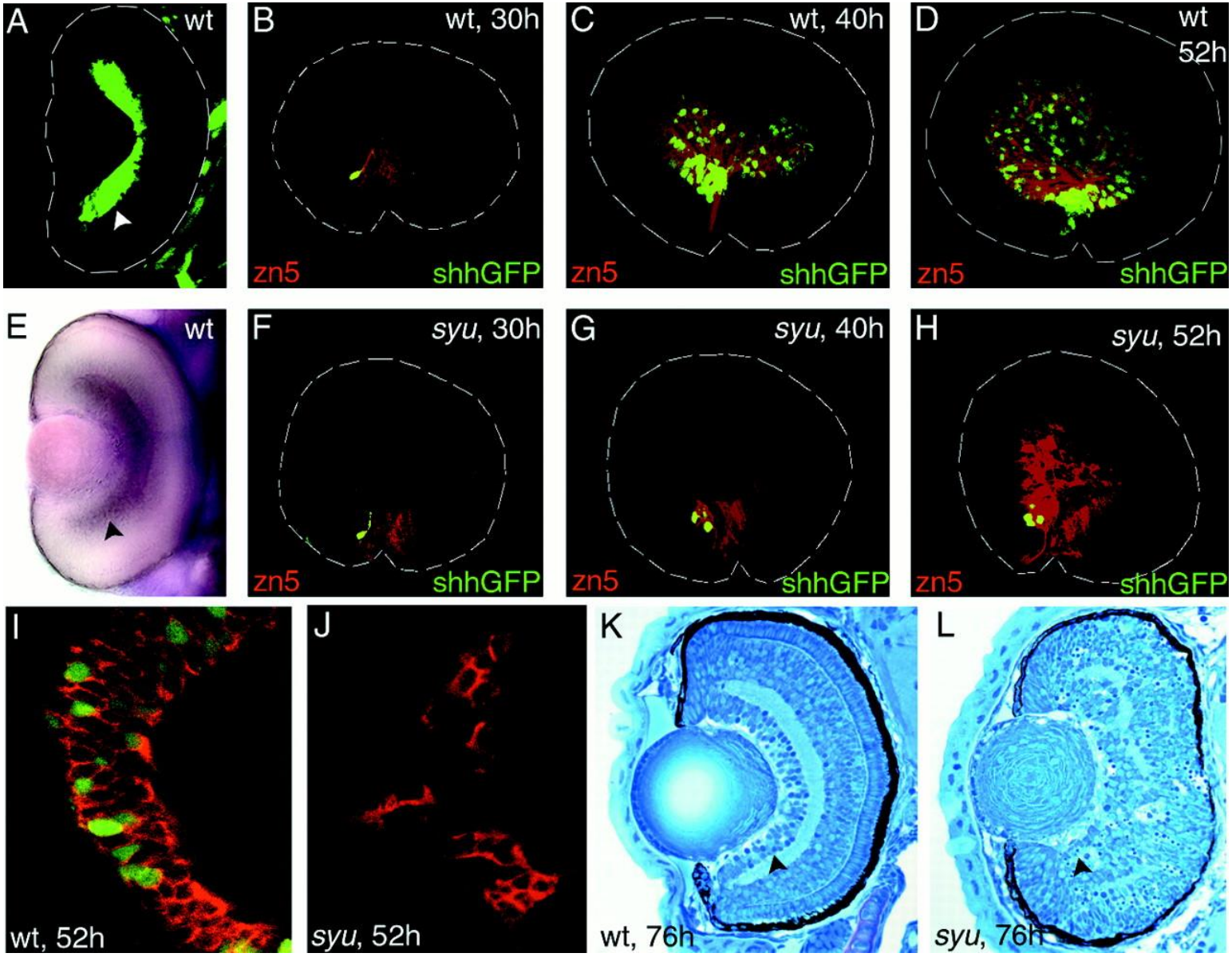


neurogenic

Initiation of Differentiation

- A study of developing zebrafish retina suggested that a self-propagating wave of sonic hedgehog (Shh) expression drives the onset of differentiation.





[Neumann & Nuesslein-Volhard (2000) Science 289:2137]

Initiation of Differentiation

nature
cell biology

Shh-mediated centrosomal recruitment of PKA promotes symmetric proliferative neuroepithelial cell division

Murielle Saade[1], Elena Gonzalez-Gobartt[1], Rene Escalona[1,2], Susana Usieto[1] and Elisa Martí[1,3]

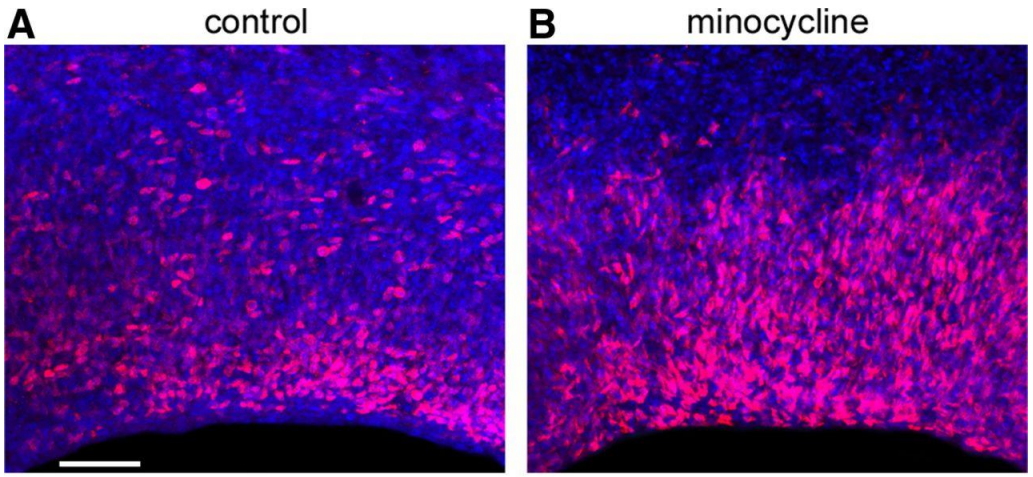
Tight control of the balance between self-expanding symmetric and self-renewing asymmetric neural progenitor divisions is crucial to regulate the number of cells in the developing central nervous system. We recently demonstrated that Sonic hedgehog (Shh) signalling is required for the expansion of motor neuron progenitors by maintaining symmetric divisions. Here we show that activation of Shh/Gli signalling in dividing neuroepithelial cells controls the symmetric recruitment of PKA to the centrosomes that nucleate the mitotic spindle, maintaining symmetric proliferative divisions. Notably, Shh signalling upregulates the expression of pericentrin, which is required to dock PKA to the centrosomes, which in turn exerts a positive feedback onto Shh signalling. Thus, by controlling centrosomal protein assembly, we propose that Shh signalling overcomes the intrinsic asymmetry at the centrosome during neuroepithelial cell division, thereby promoting self-expanding symmetric divisions and the expansion of the progenitor pool.

Initiation of Differentiation

- Studies in developing mouse cortex suggest that FGF10 or retinoic acid may drive the onset of differentiation.

Microglia regulate cell division.

- Microglia are present in the germinal layers of the developing cortex.
- Microglia phagocytize progenitor cells.
- Elimination of microglia with a drug resulted in more progenitor cells and a larger cortex.



In vitro organotypic slices

Cunningham et al. (2013) J. Neurosci. 33:4216