Neurogenesis and Neuronal Migration

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March 1, 2004

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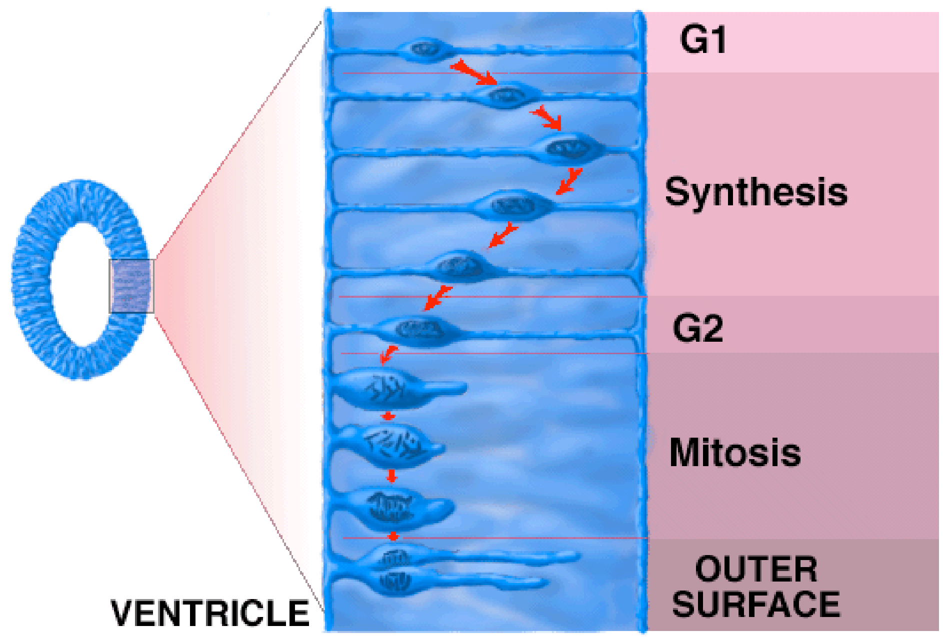
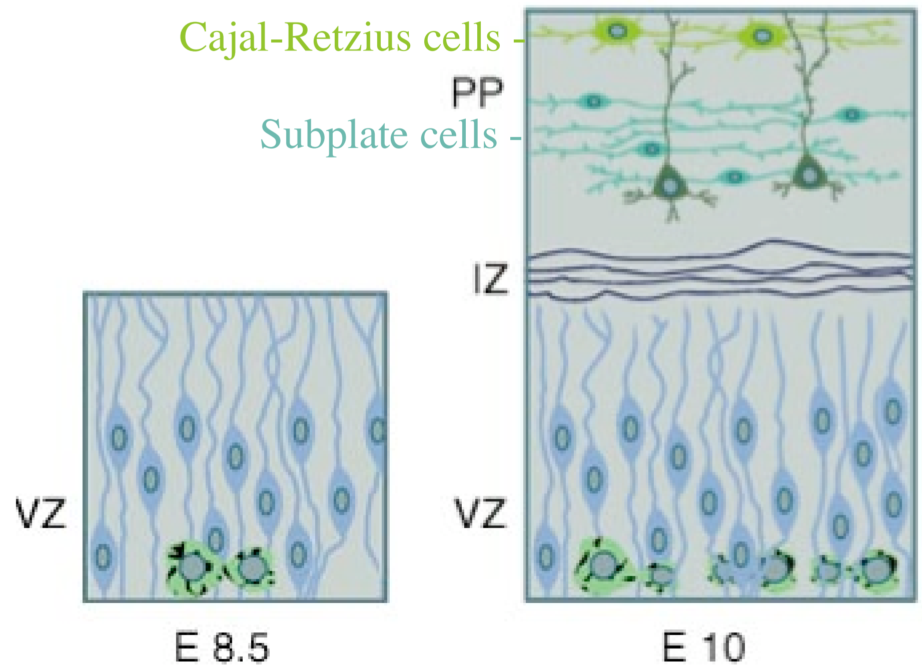
Development of cortical layers

- Cortex starts out as monolayer epithelium
- Nuclei/cells move up and down according to their cell cycle phase



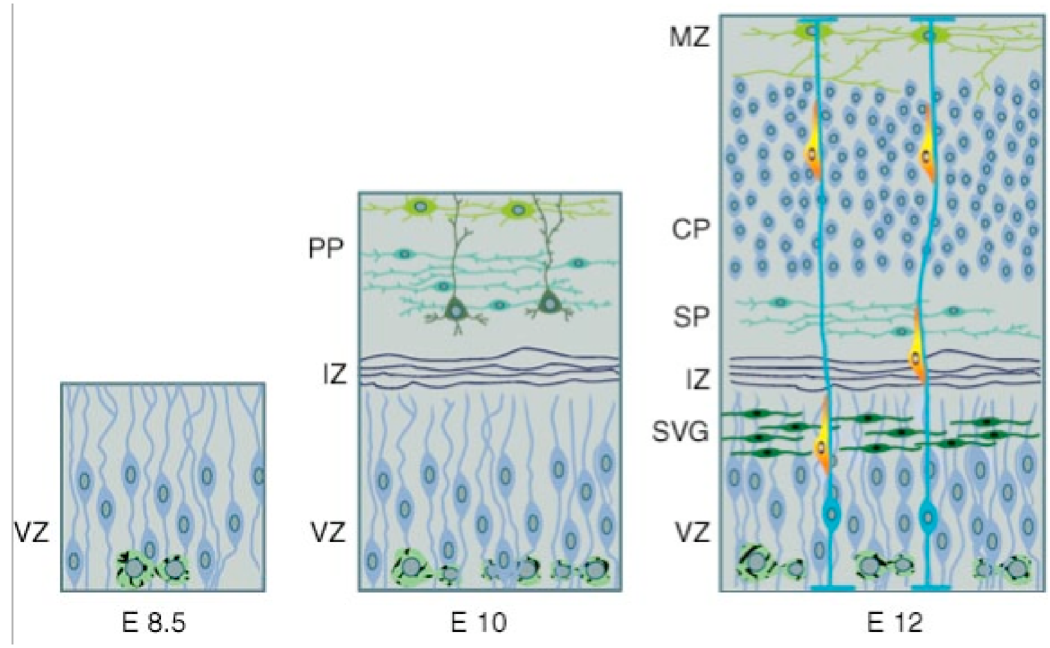
Development of cortical layers

- **Neurogenesis initiates:**
  - Some cells begin to leave cell cycle -- rise
    - Form preplate
      - Cajal-Retzius cells
      - Subplate cells
  - Many cells continue to divide
    - Ventricular zone (VZ)
  - Axons enter cortex: Intermediate Zone (IZ)
    (bidirectional cortex/thalamus connections)



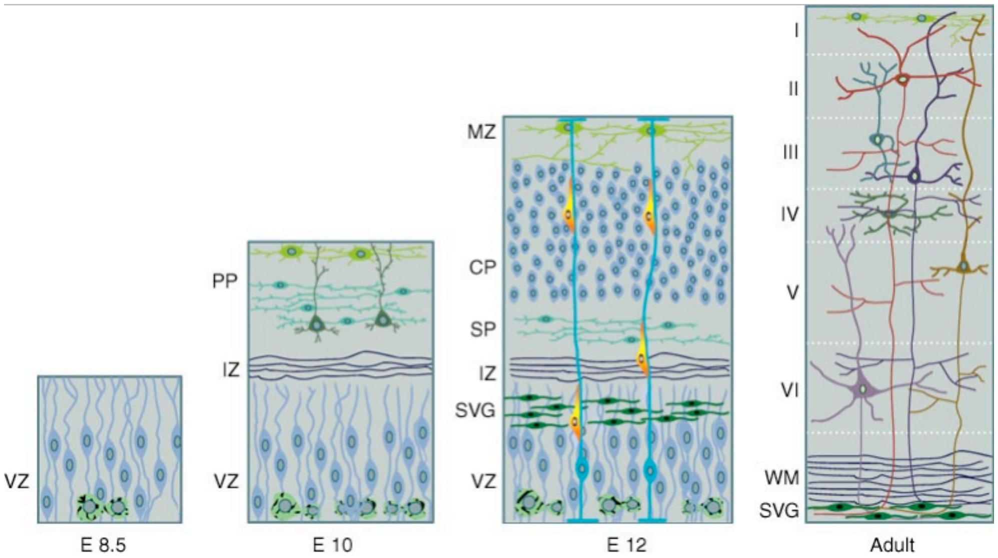
Development of cortical layers

- Newly generated neurons migrate through subplate
- Stop beneath Cajal-Retzius cells
- Form cortical plate



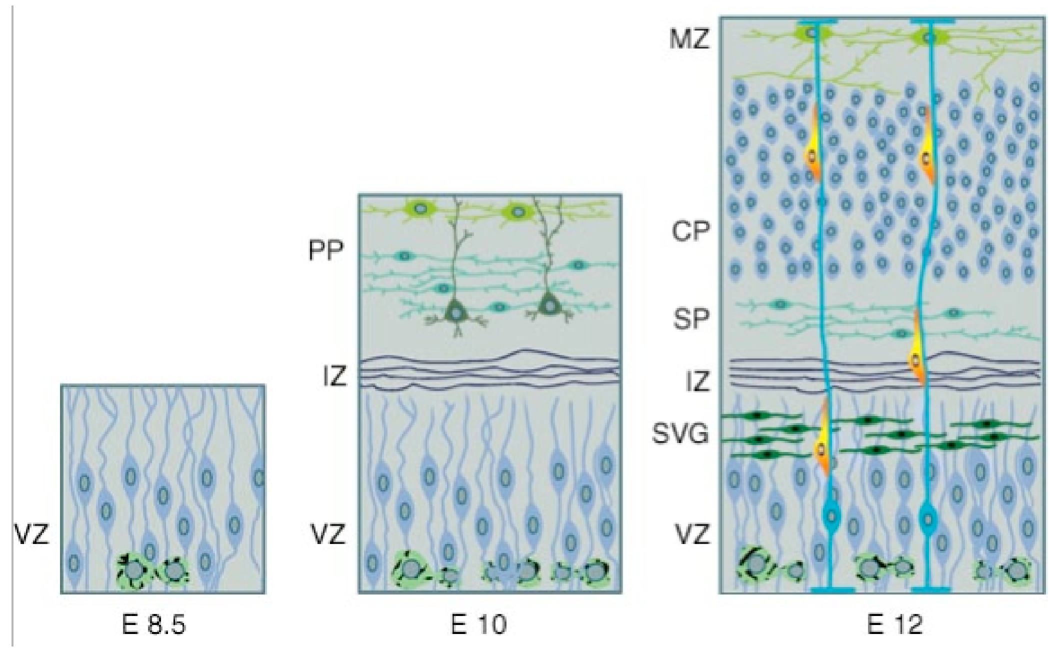
Development of cortical layers

- **Cortical Plate differentiates to form cortical layers**



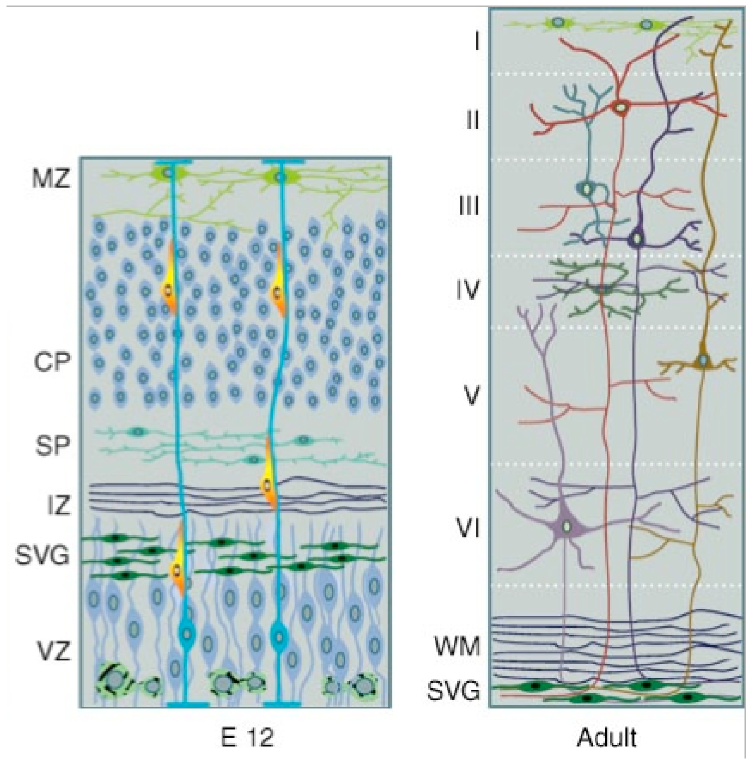
Development of cortical layers

- **As cortical plate forms**
- **Subpopulation of proliferating cells forms above VZ :**
  - **Subventricular Zone (SVG)**



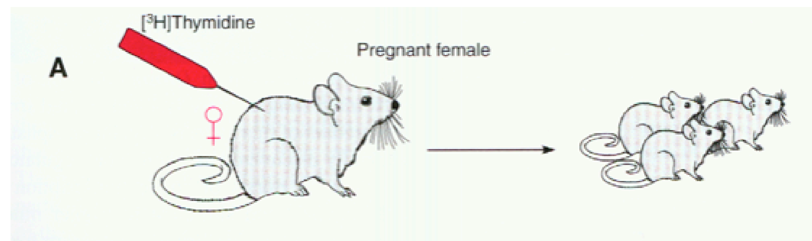
Subventricular Zone

- **Secondary zone of neurogenesis**
- **Proliferate through post-natal period**
  - **Generate multiple cell types:**
    - **Glia**
    - **Neurons**
    - **Include cells that migrate to olfactory bulb**



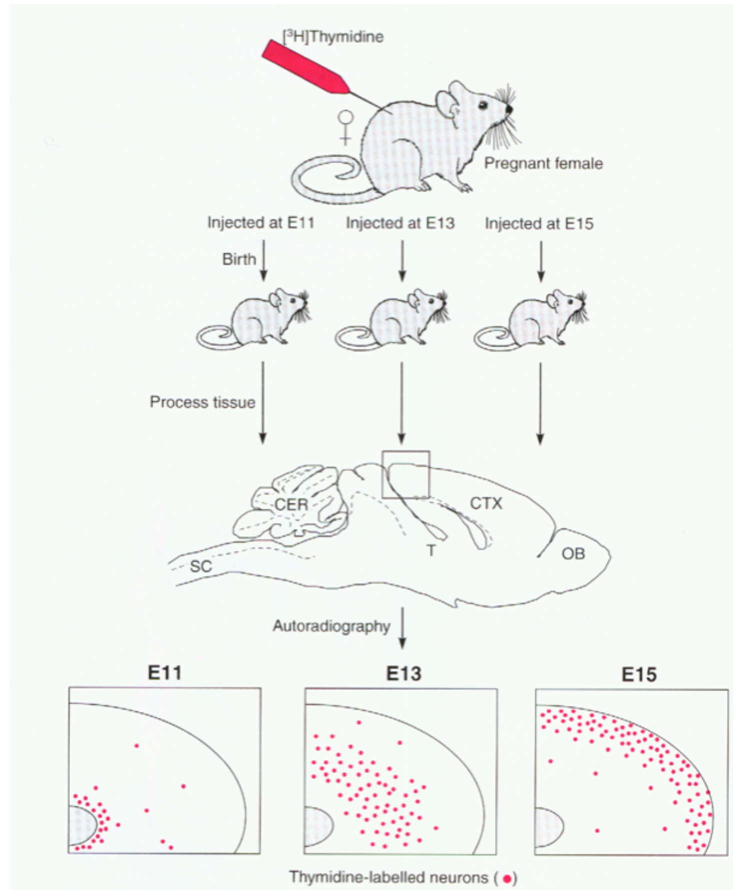
Are cortical layers generated in any temporal sequence?

- **Birthdating analysis**
- **Inject mother with tritiated thymidine**



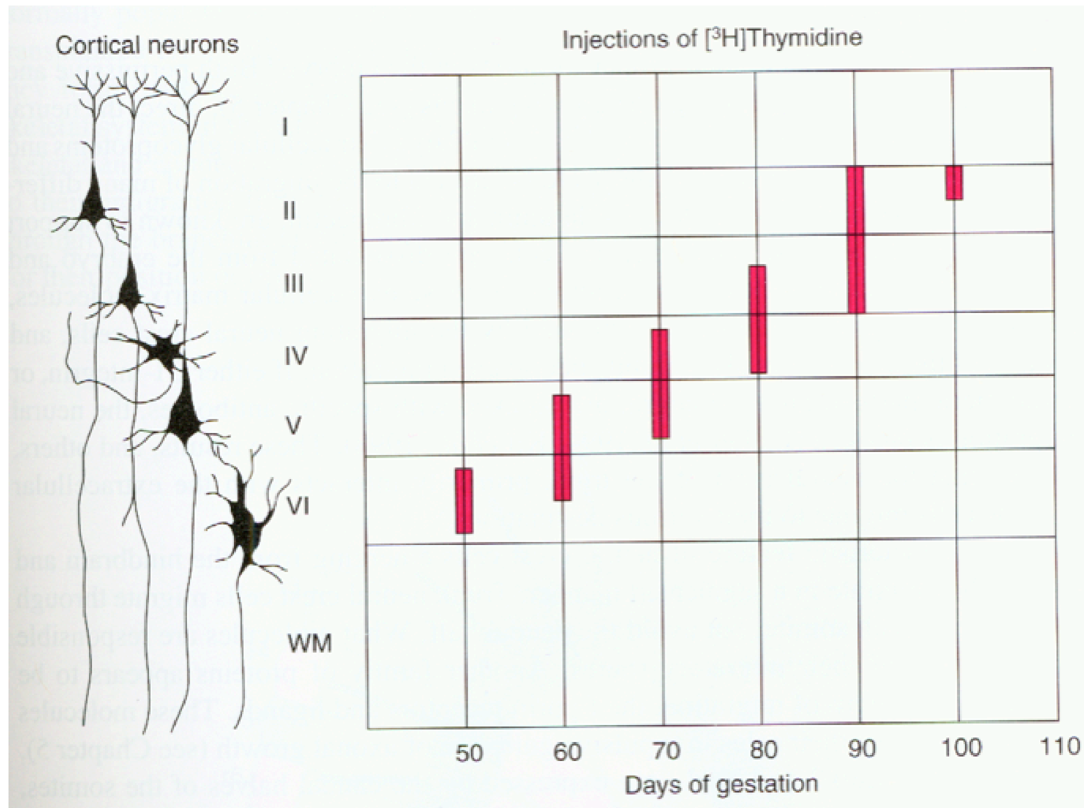
Birthdating of cortical layers in rodents

- Inject at multiple time points
- Detect using autoradigraphy
- Answer: Cortical layers generated "inside-out"



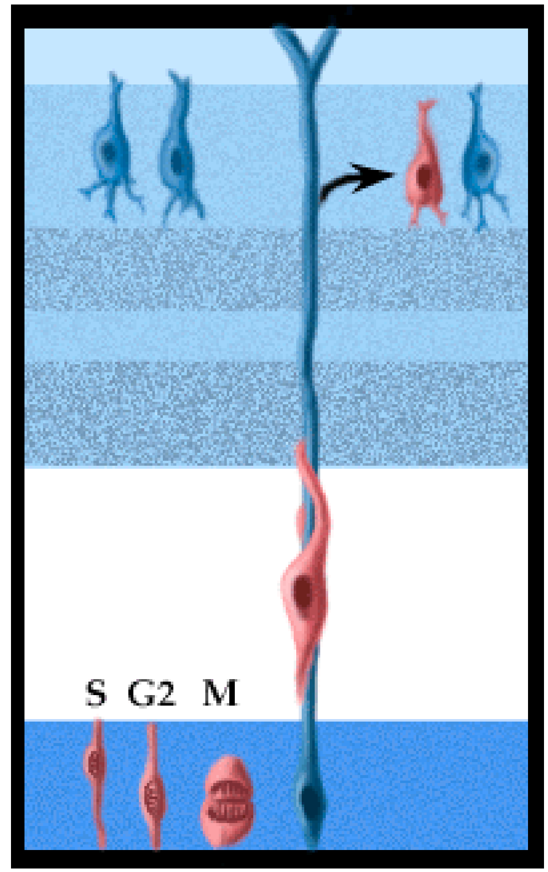
Birthdating in monkey cerebral cortex

Primates:
Cortical layers also generated "inside-out"



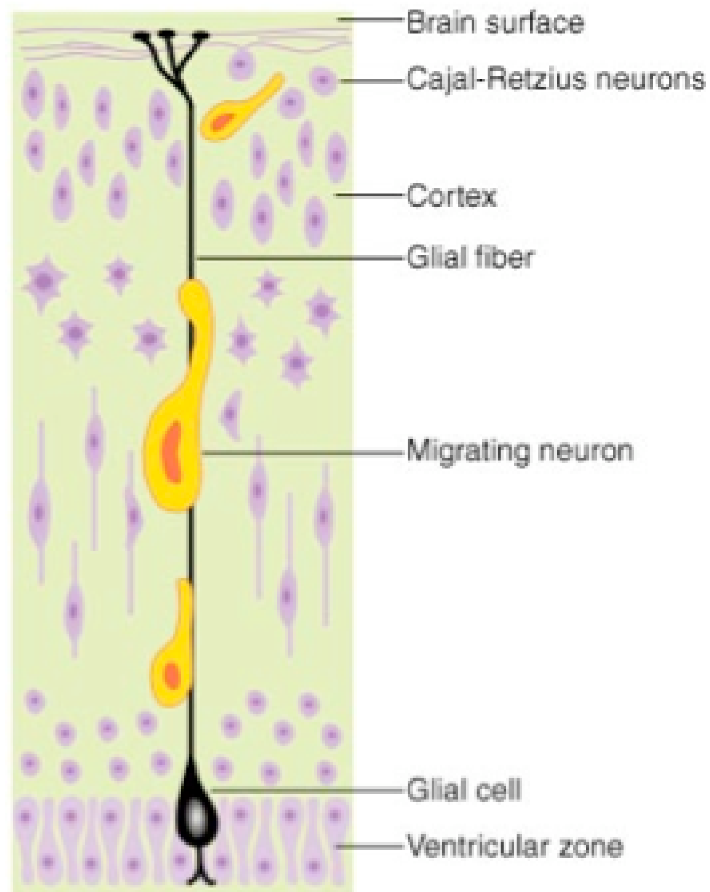
Radial migration

- **Post-mitotic neurons migrate away from ventricular zone toward brain surface**

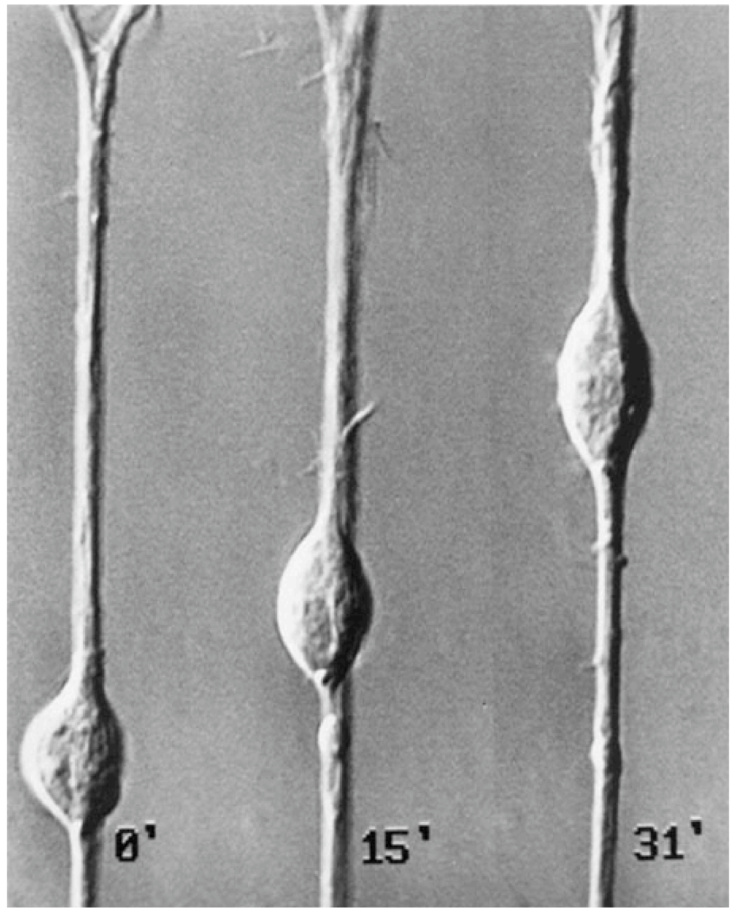


Pattern of migration

- **Newly generated cells migrate beyond earlier cells**

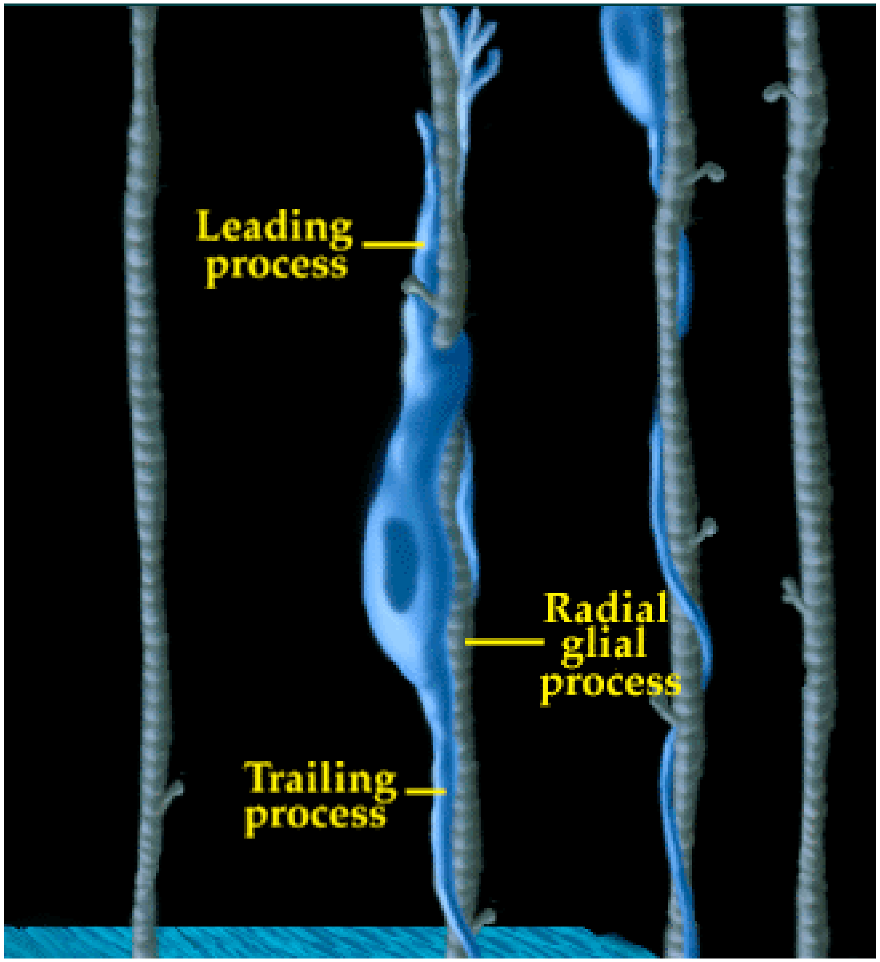


Neuron growing along glial cell in culture

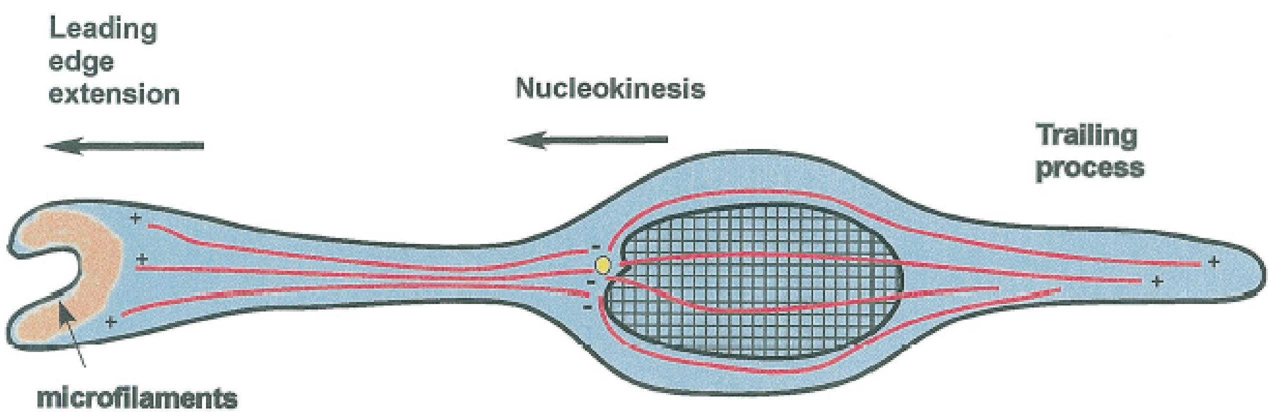


Migration along radial glial cells

- Radial glial cells span the developing cortex
- Neurons appear to migrate in close contact along them



Neuronal migration



How is neuronal migration regulated?

- **Molecular pathways controlling neuronal migration identified through human and mouse mutants:**

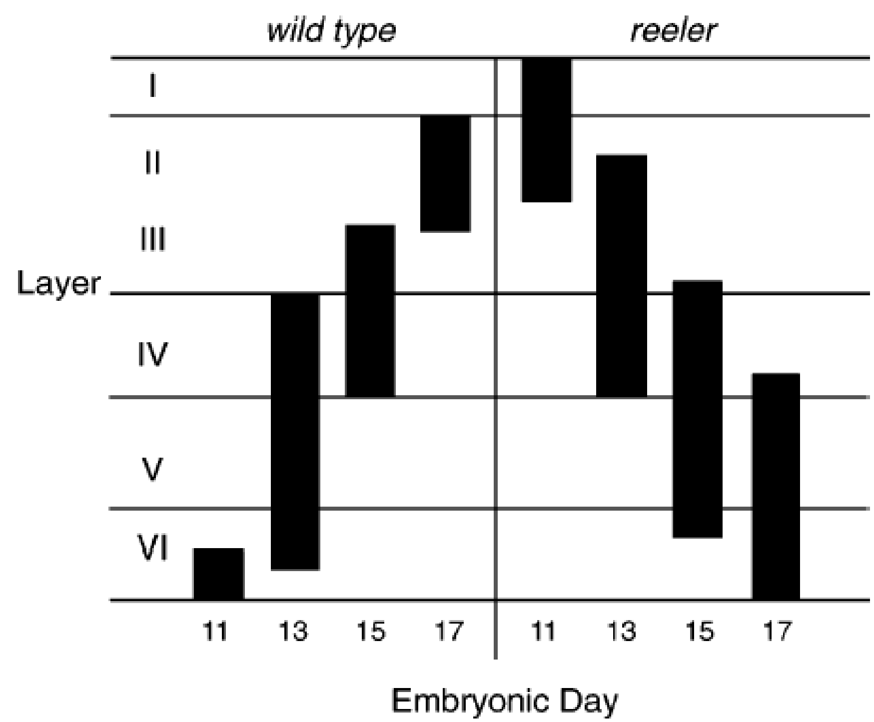
Table 1. *Mouse and human genes associated with migration in the CNS*

			Chromosome[a]		
Gene	Name	Protein type	mouse	human	References
ApoER2	*apolipoprotein E receptor 2*	transmembrane	N.D.	1p34	Trommsdorff et al. (1999)
Astn	*astrotactin*	secreted glycoprotein	N.D.	1q25.2	Hatten (1999)
Cdk5	*cyclin-dependent kinase 5*	cyclin-dependent kinase	5	7q36	Ohshima et al. (1996)
Cdk5r	*p35*	Cdk5 regulatory subunit	11	N.D.	Chae et al. (1997)
Cxcr4	*chemokine receptor 4*	receptor	1	2	Zou et al. (1998)
Dab-1	*disabled 1*	signal transduction	4	1q	Howell et al. (1997b); Sheldon et al. (1997)
Dlx-1,2	*distal-less homeobox 1,2*	transcription factor	2	2q32	Anderson et al. (1997)
Dcx	*doublecortin*	microtubule associated protein	X	Xq22–23	des Portes et al. (1998); Gleeson et al. (1998)
Erbb4[b]	*avian erythroblastosis oncogene B4*	tyrosine kinase receptor	N.D.	2q34	Rio et al. (1997)
Fgf-2	*fibroblast growth factor 2*	growth factor	3	4q25–27	Dono et al. (1998)
Flna	*filamin α*	actin-binding protein	X	Xq28	Fox et al. (1998)
FCMD	*fukutin*	secreted glycoprotein	N.D.	9q31	Kobayashi et al. (1998)
itga3	*integrin α 3*	integrin receptor subunit	11	N.D.	Anton et al. (1999)
Itga6	*integrin α 6*	integrin receptor subunit	2	2	Georges-Labouesse et al. (1998)
KAL1	*Kallmann's syndrome*	novel protein	N.D.	Xp22.3	Franco et al. (1991)
Lama2	*laminin α 2*	extracellular matrix	10	6q22–23	Helbling-Leclerc et al. (1995)
Pafah1b1	*lissencephaly-1*	subunit of platelet activating factor acetylhydrolase	11	17p13.3	Reiner et al. (1993)
MARCKS	*myristoylated alanine-rich protein kinase C substrate*	neural substrate for PKC	10	6q22	Blackshear et al. (1997)
Ncam	*neural cell adhesion* molecule 180	adhesion molecule	9	11q22.2	Tomasiewicz et al. (1993)
Ntn-1	*netrin-1*	extracellular ligand	11	17q12	Bloch-Gallego et al. (1999)
Ntf-4/5†	*neurophin 4/5*	neurotrophin	7	19	Brunstrom et al. (1997)
Hg 1[b]	*neuregulin*	growth factor	N.D.	8	Anton et al. (1997)
Pax6	*paired box gene 6*	transcription factor	2	11p13	Caric et al. (1997)
Pex2	*peroxisome assembly factor 1*	peroxisome membrane protein	N.D.	8	Faust and Hatten (1997)
Pex5	*peroxisome receptor 1*	peroxisomal import receptor	N.D.	12p13	Baes et al. (1997)
Ptn	*pleiotrophin*	ligand for receptor-like protein tyrosine phosphatase	6	7q33	Maeda and Noda (1998)
Reln	*reelin*	extracellular protein	5	7q22	D'Arcangelo et al. (1995)
Slit-1	*Slit Drosophila homolog 1*	secreted	N.D.	10q23.3	Wu et al. (1999)
Unc5h3	*UNC-5 C. elegans* homolog 3	netrin receptor	3	N.D.	Ackerman et al. (1997); Leonardo et al. (1997)
Vldlr	*very low density lipoprotein receptor*	transmembrane	19	9p24	Trommsdorff et al. (1999)

[a](N.D.) Not determined.
[b]Mice deficient in these genes die at E10.5; therefore, their role in neuronal migration in vivo is not known.

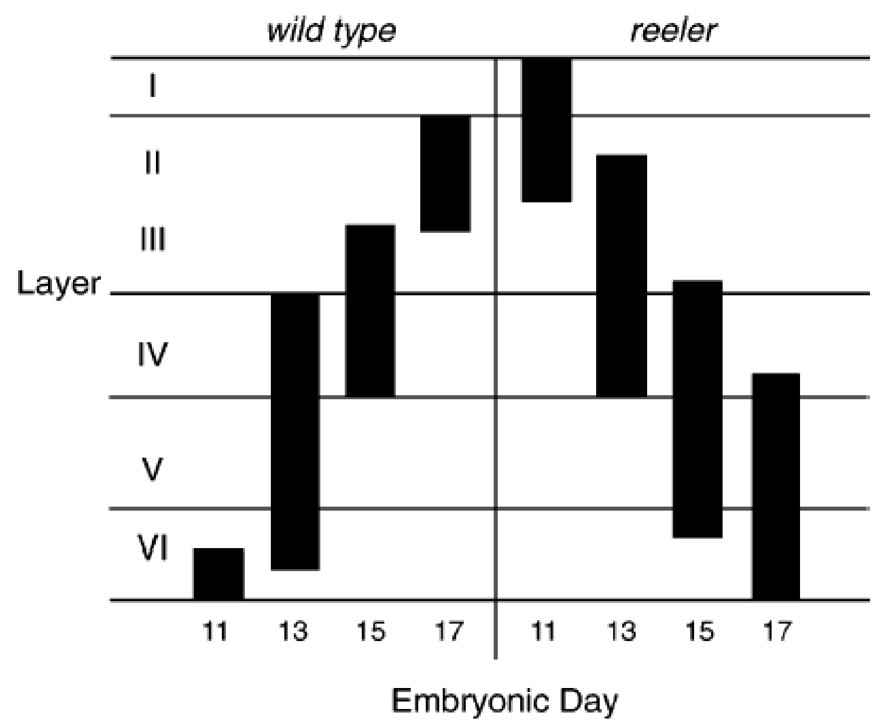
The *reeler* mutant mouse

- **Birthdating analysis of reeler mutant:**

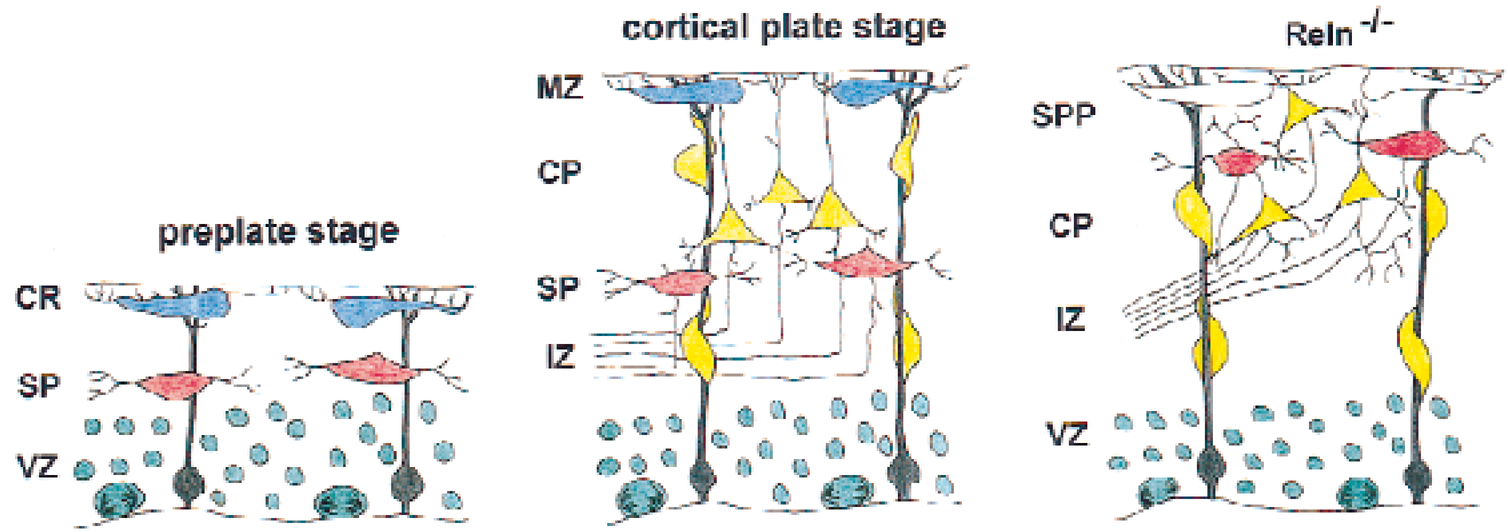


The *reeler* mutant mouse

- Birthdating analysis of reeler mutant:
- Timing of layer production is inverted

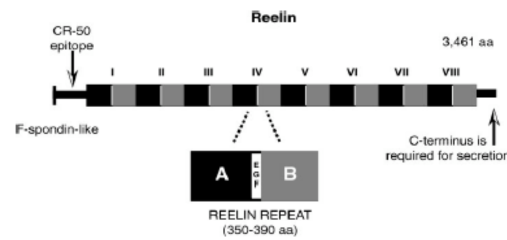


Anatomy of developing cortex in *reeler*

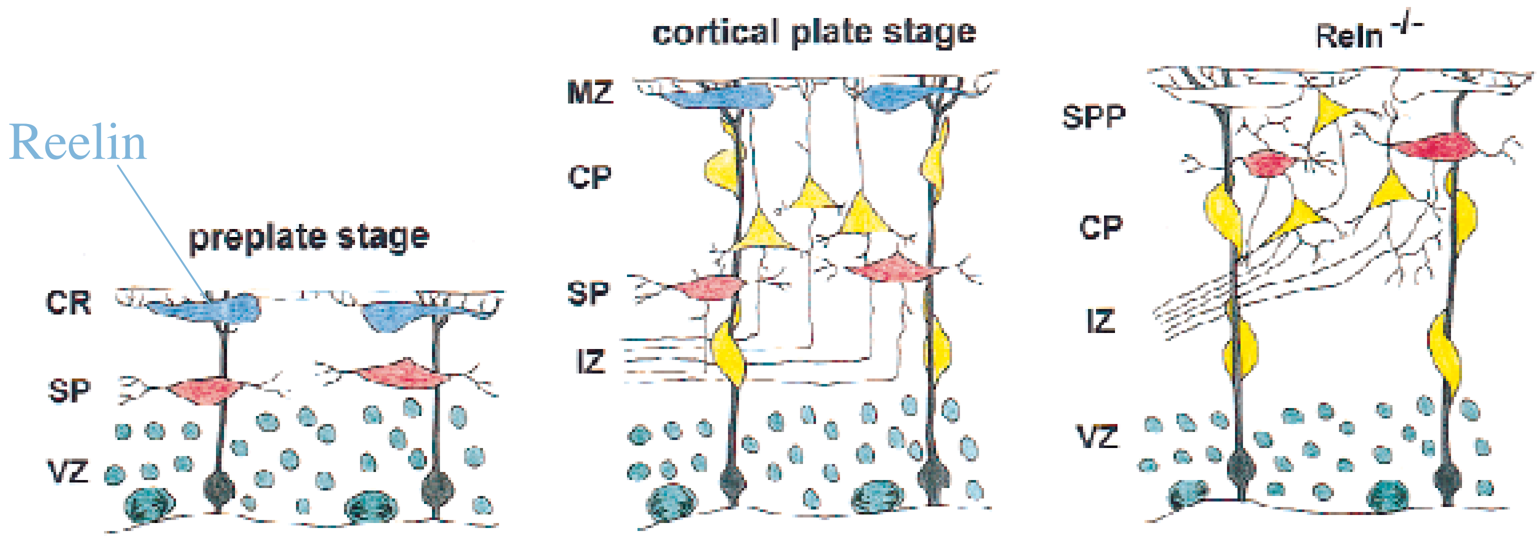


wild type reeler

Molecular identification of Reelin



- Secreted protein
- Produced by Cajal-Retzius cells

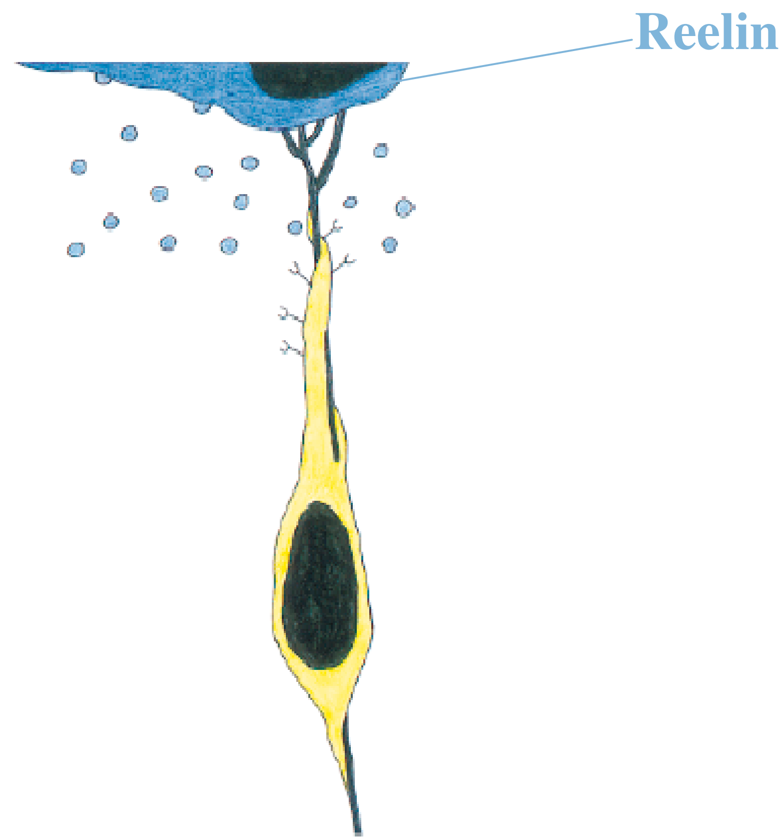


wild type

reeler

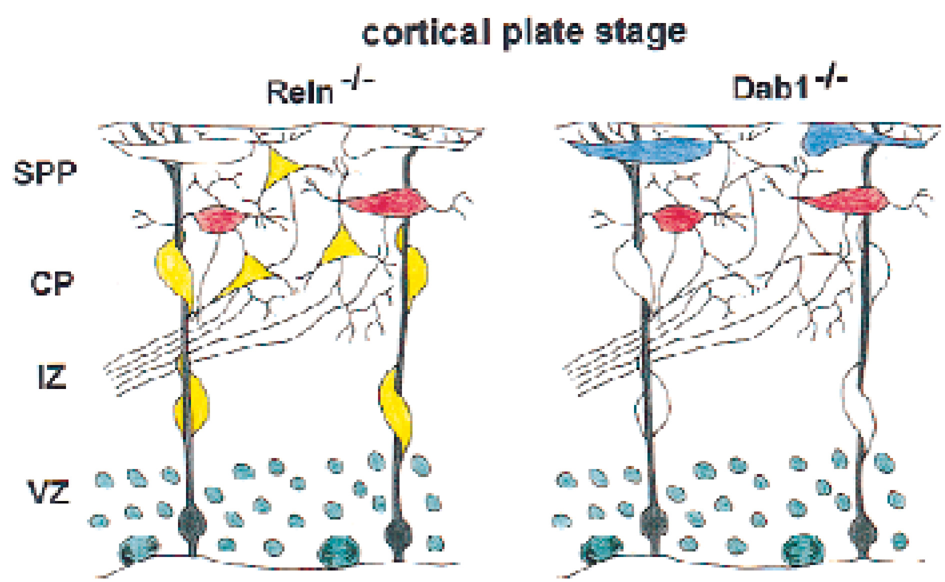
Model for Reelin function

- **Signal promoting migration along and/or detachment from radial glial cell**



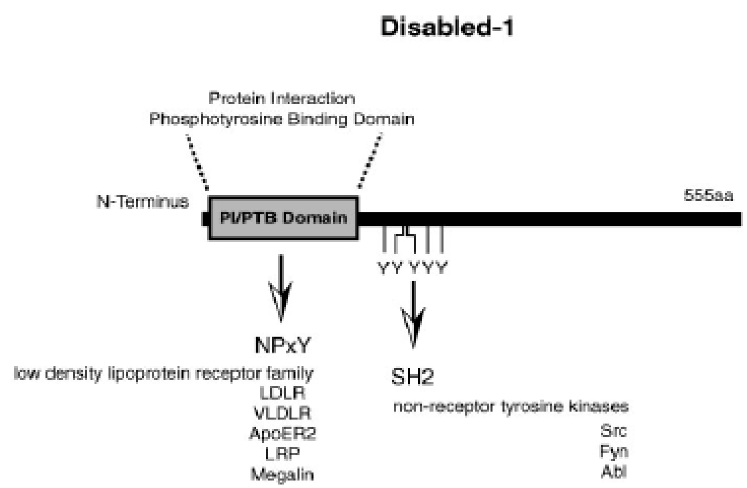
The Reelin pathway

- **Other mutant mice found with same phenotype: eg., *Dab1***



Dab1

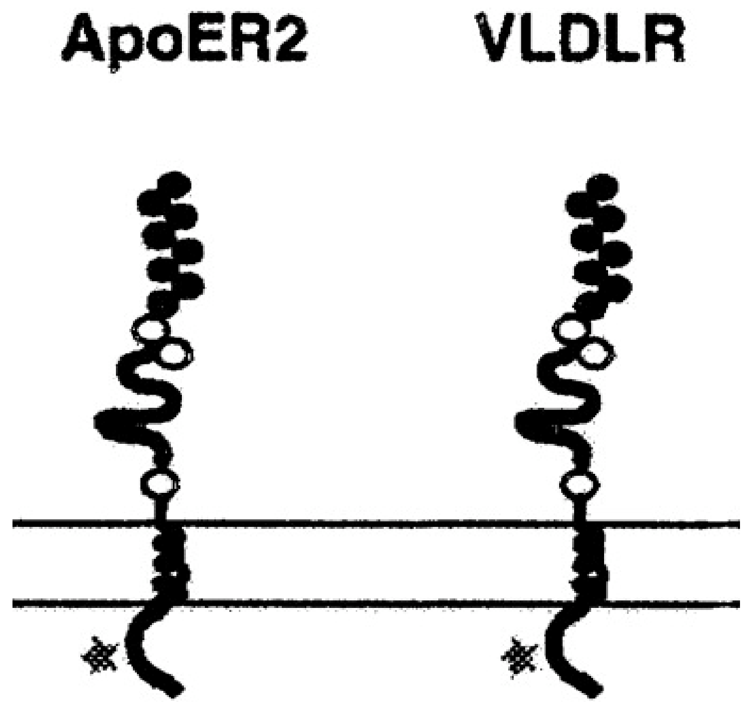
- **Cytoplasmic adaptor protein**
- **Binds to receptors**
- **Binds to cytoplasmic protein kinases**



Receptors for Reelin

- **Animals double mutant for ApoER2/VLDLR resemble *reeler***
- **Well-known lipoprotein receptors**
- **Expressed in migrating neurons**
- **Bind Reelin**

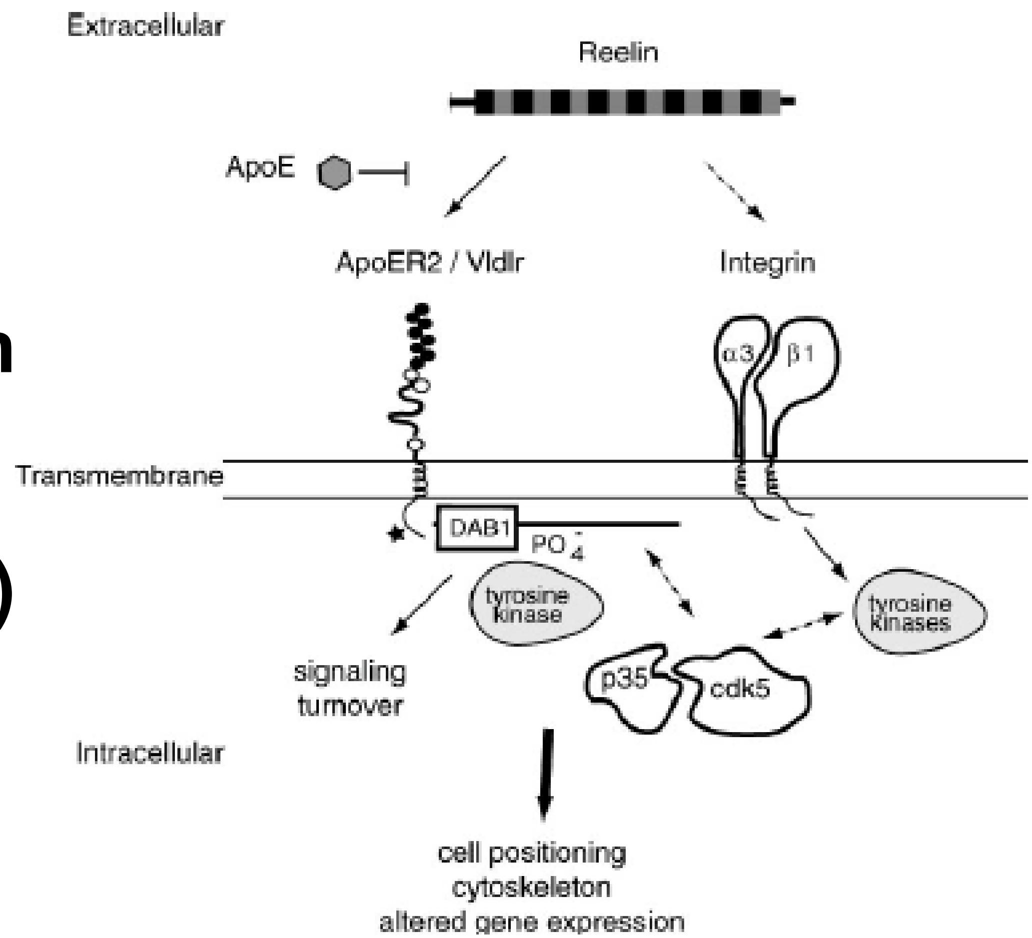
Reelin also binds integrins -- co-receptor?



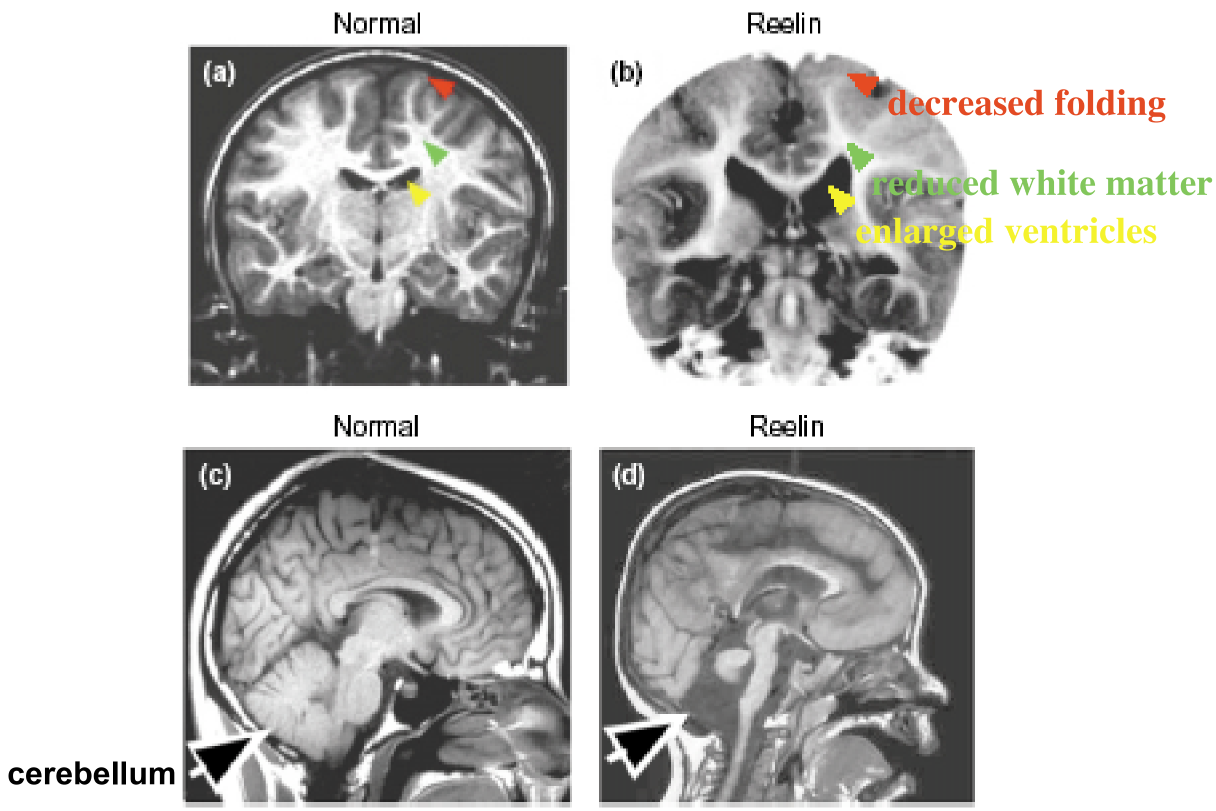
Reelin signaling pathway

ApoER2/VLDLR bind Dab1!

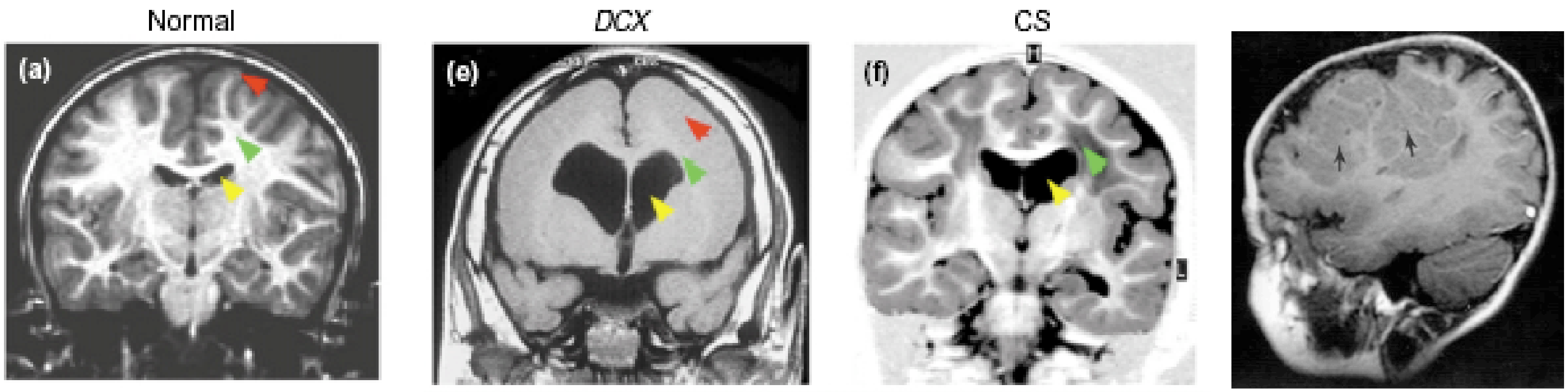
-- in addition: mutants in P35 and cdk5 (which function together as kinase complex) have similar phenotypes to reeler



Disorders of neuronal migration in human disease



Regulators of migration found as human disease genes

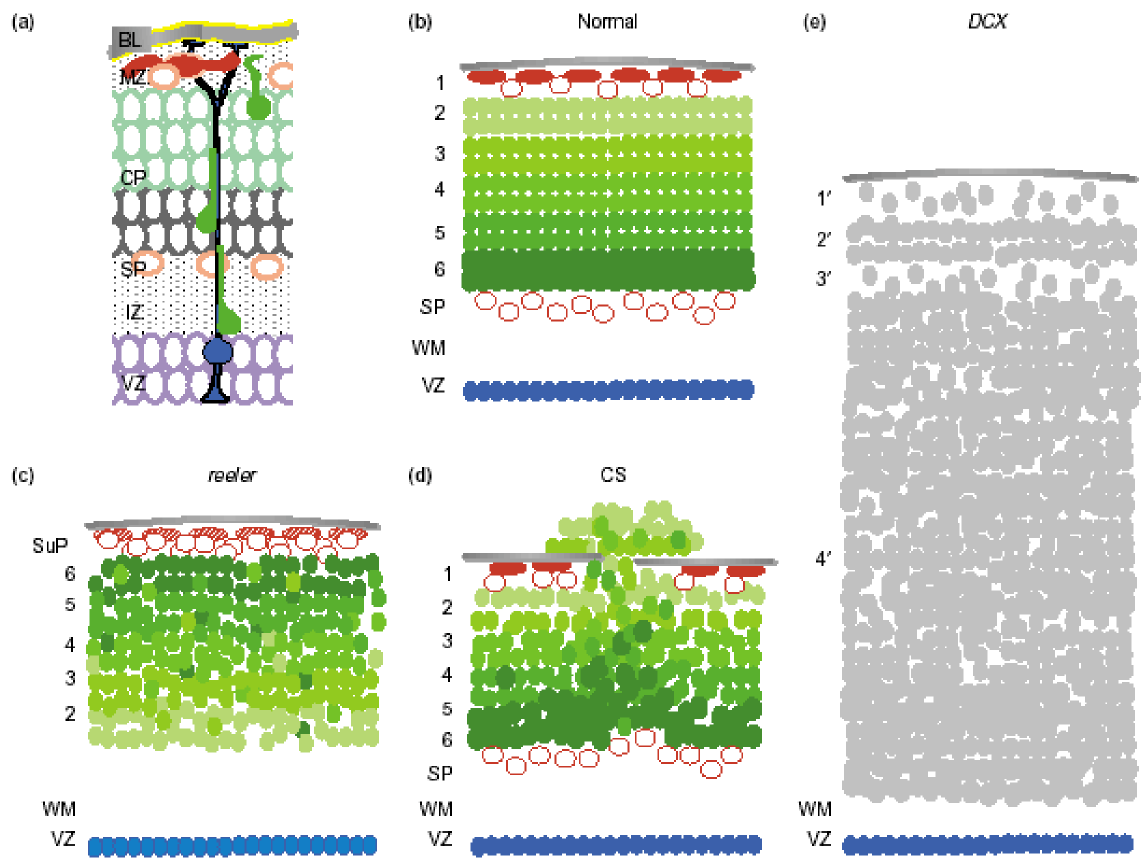


Lissencephaly (smooth brain)

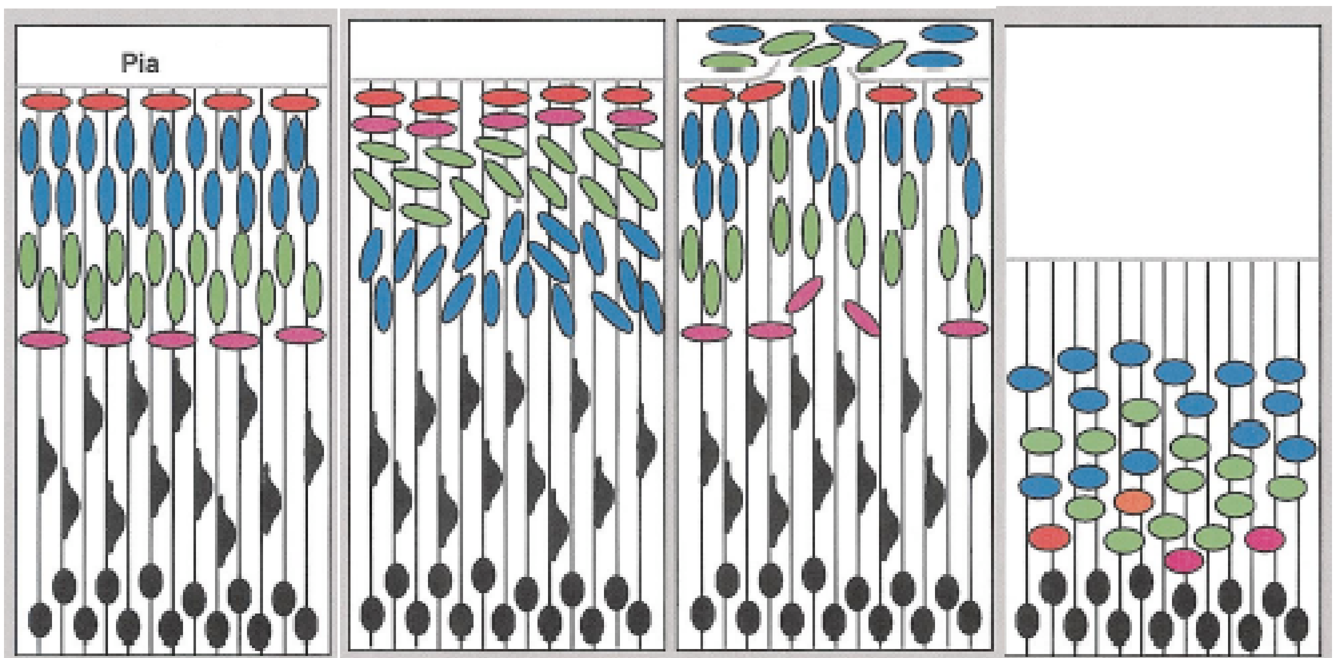
Cobblestone cortex

Cortical heterotopia [sideview]

Cortical layering in patients



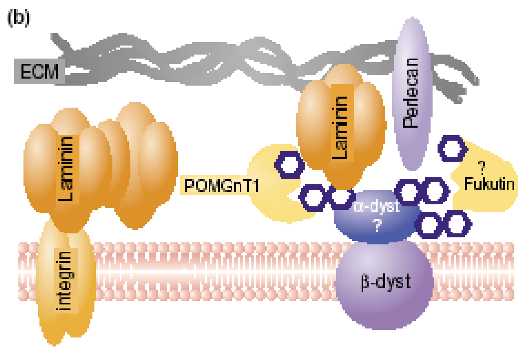
Schematic of how layering defects may be generated



Normal Classical Lissencephaly Cobblestone cortex Cortical Heterotopia

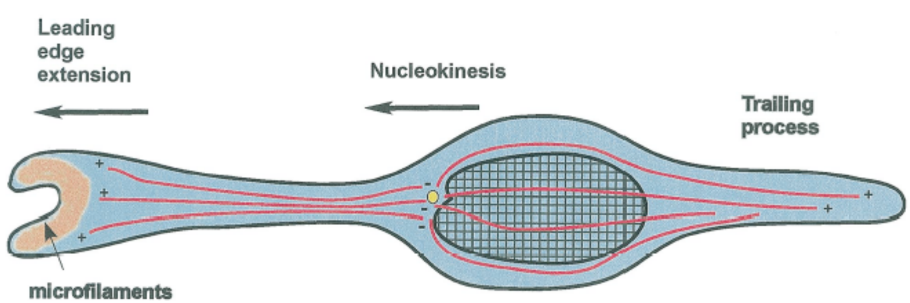
Cobblestone cortex

- Abnormal basal lamina/extracellular matrix
  - Fukutin: glycoprotein/glycolipid modifying enzyme
  - Muscle-eye-brain (MEB) disease protein: protein glycosylating enzyme
  - May disrupt basal lamina surrounding brain



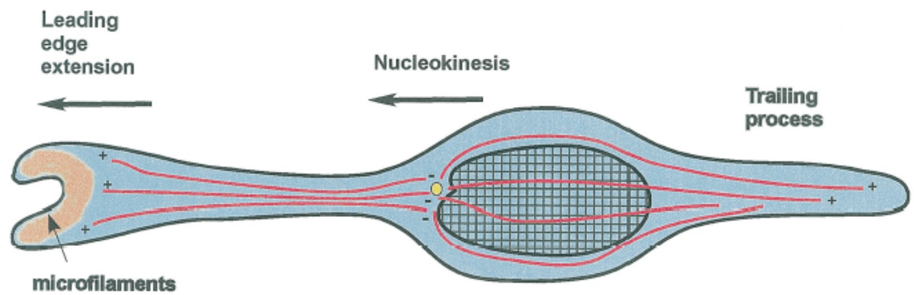
Lissencephaly genes: microtubule regulators involved in nuclear migration

- **Genes that interact with microtubules:**
  - **Lis1 (homolog of NudF -- required for nuclear migration in *Asperigillus nidulans*)**
    - **Interacts with microtubule organizer (centrosome)**
    - **Interacts with Dynein (microtubule motor protein) -- multiple roles including nuclear movement**
  - **DCX (microtubule binding protein)**



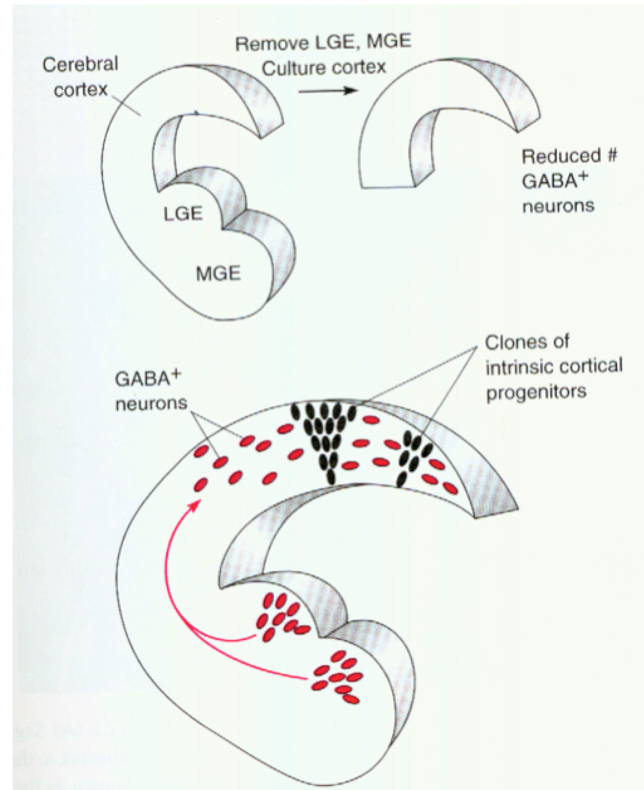
X-linked periventricular heterotopia

- Mutant in Filamin
  - Actin-associated protein
  - Associates with multiple regulators of actin cytoskeleton
- Both actin and microtubule cytoskeletons important in migration



Tangential migration in cortex

- Embryological and labelling experiments demonstrated that not all cortical cells arise from radial migration
- Lose GABA-ergic interneurons in mutant mice with disrupted LGE and MGE development
- GABA-ergic interneurons migrate in from region of basal telencephalon (medial ganlionic eminence, MGE)

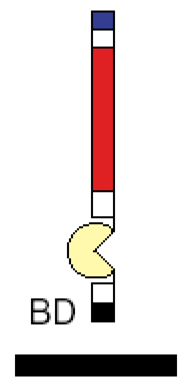


Molecular mechanisms of tangential migration

- Differs from radial migration:
  - Does not require reelin, dab or cdk5

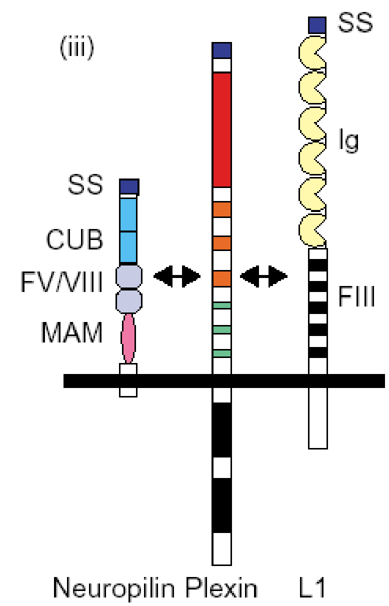
Regulators of tangential migration

- **Semaphorins: family of guidance cues: attract and repel cells and processes**
  - **Sema 3: secreted signal**



Regulators of tangential migration

- Semaphorin 3 receptors:
  - Neuropilin (ligand-binding subunit)
  - Plexin (trans-MB signal transducer)
  - L1 (modulator)

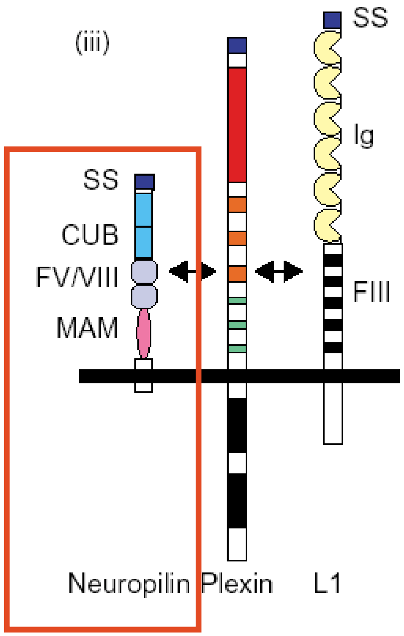


Semaphorin signaling in tangential migration

- Neuropilin (receptor) expressed on migrating cells
- Semaphorin 3 expressed on pathway
- Examined effect of disrupting Neuropilin signaling via:
  - Nrp2 knock-out mouse
  - Nrp1 dominant-negative
    - How to make a dominant-negative receptor?

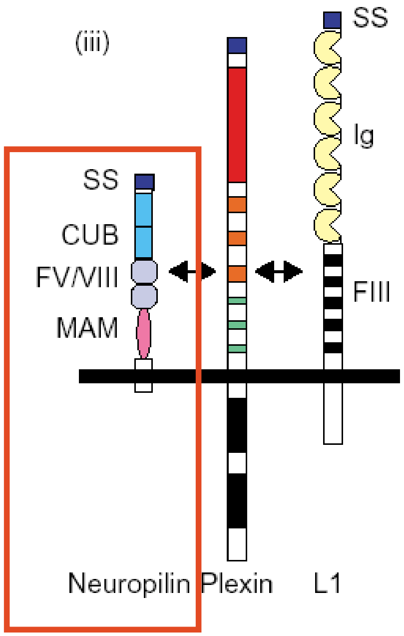
Dominant-negative neuropilin

- Truncation of cytoplasmic domain
  - No effect



Neuropilin dominant-negative receptor

- Neuropilin functions:
  - Bind Sema 3
  - Initiate signal transduction



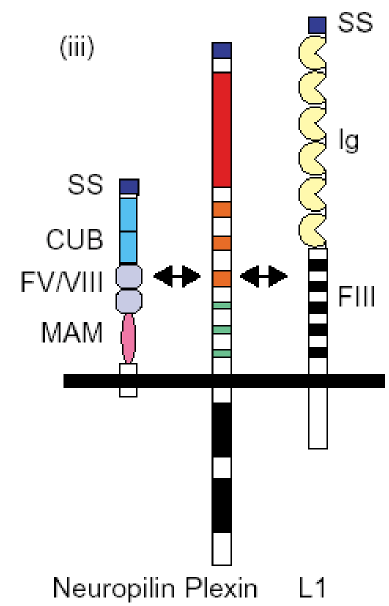
Dominant-negative neuropilin

- Truncation of cytoplasmic domain
  - No effect
- Truncation in extracellular domain

  -Dominant-negative

  -Still binds Sema3

  -Signaling fails



Dominant-negative neuropilin

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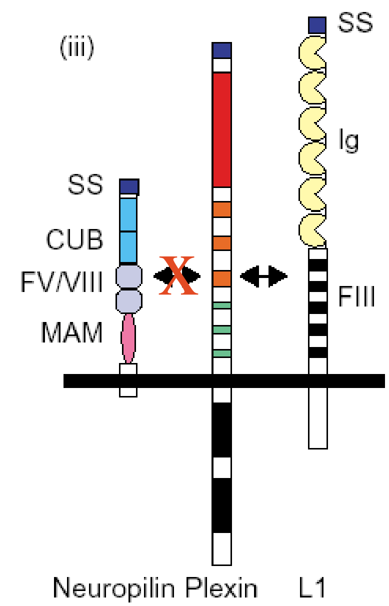
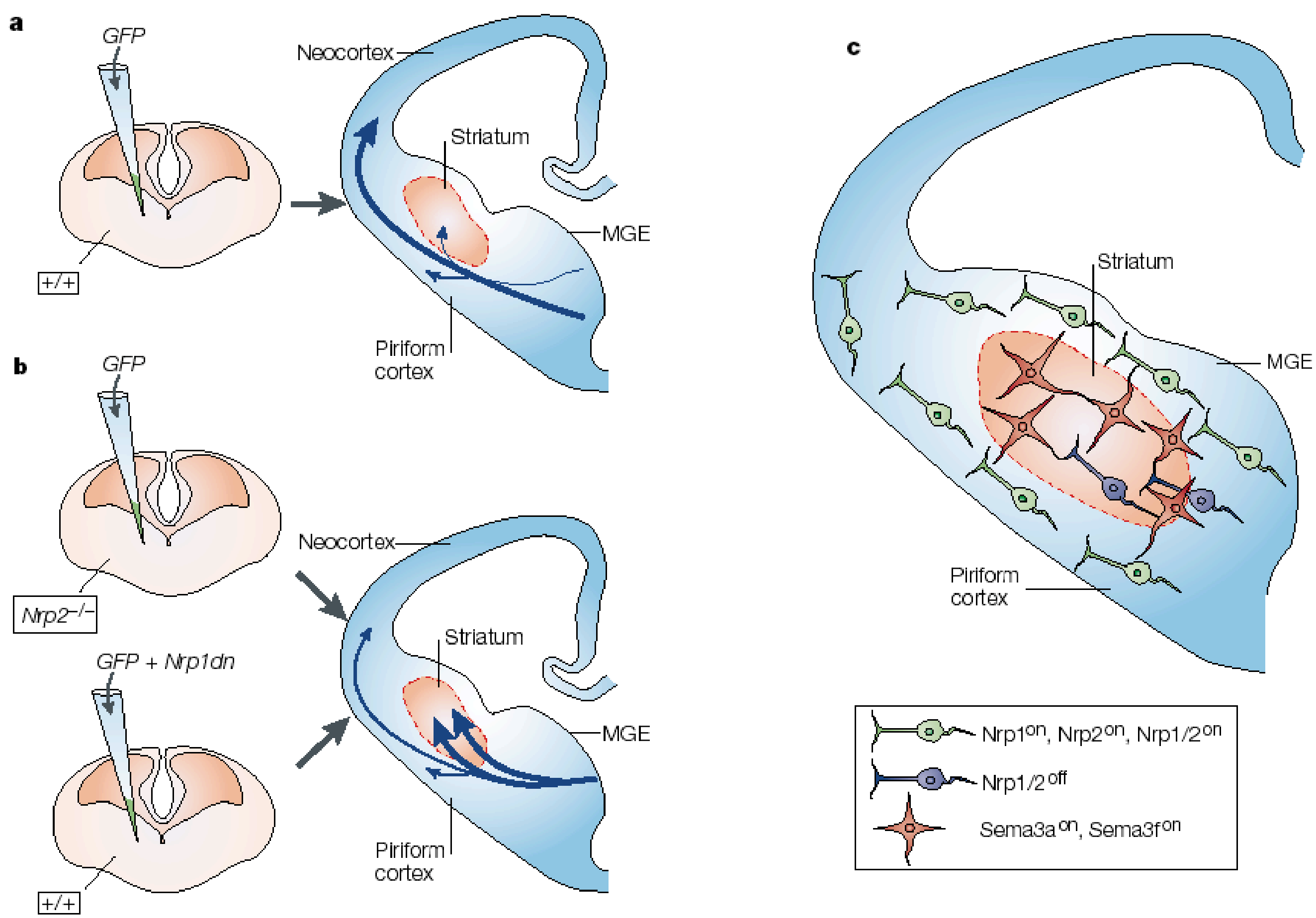
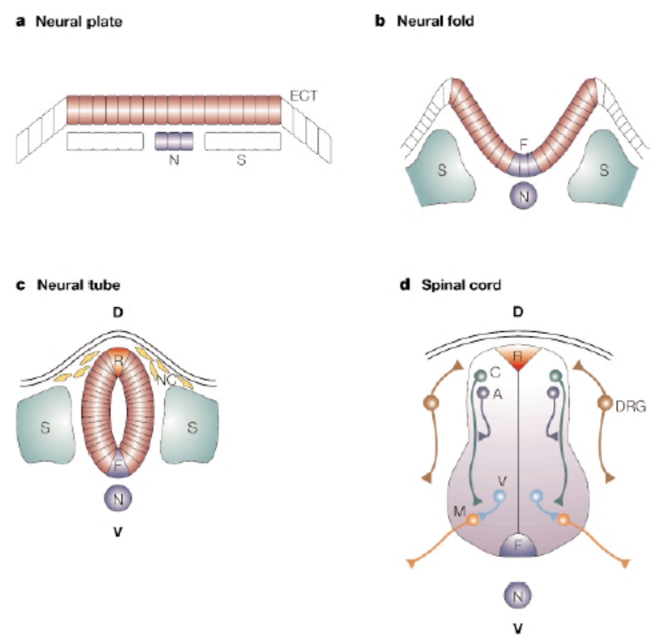


Neuropilin signaling regulates tangential migration

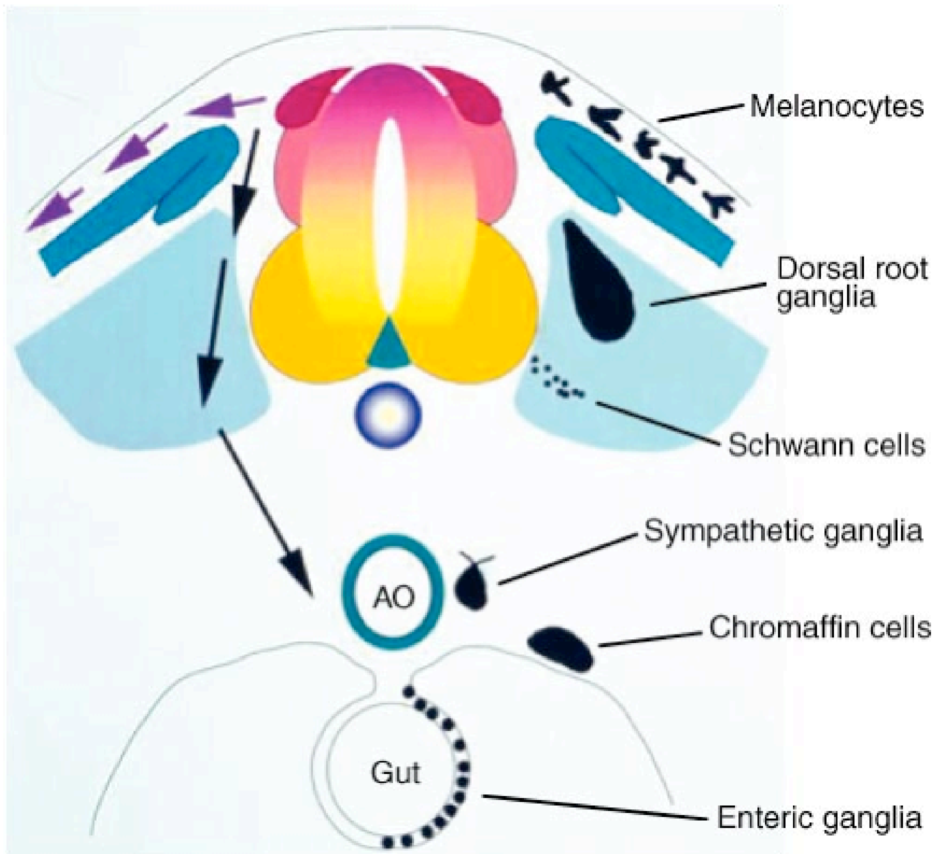


Dorsal/Ventral Axis patterning

- Structures along DV axis of Neural Tube
  - Roof plate (R)
  - Floor plate (F)
  - Notochord (N)
  - Neural crest (NC)
  - Paraxial mesoderm/somites (S)

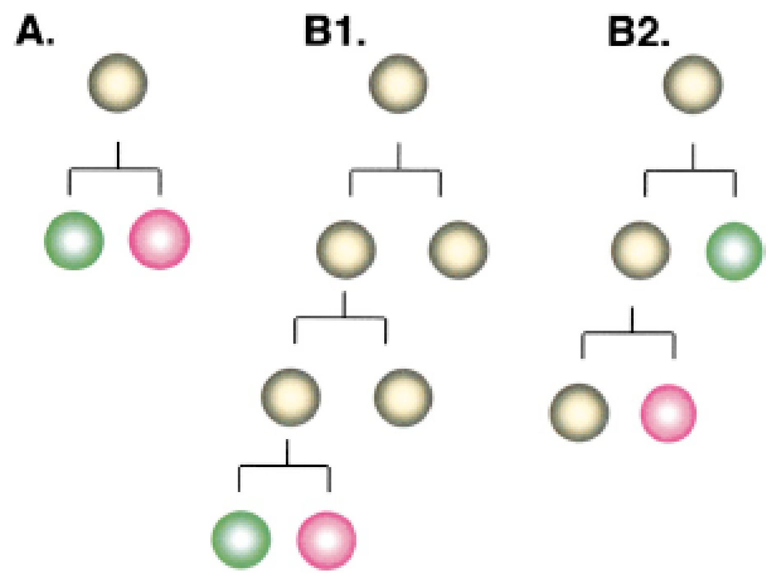


Neural Crest Cells



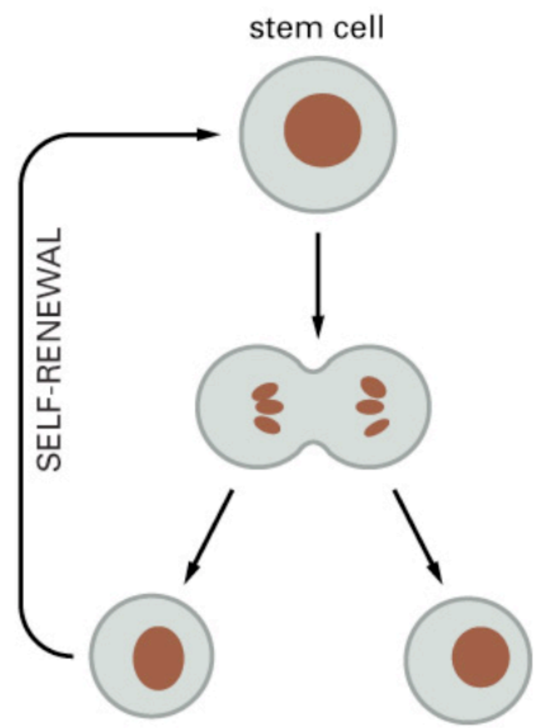
Generation of appropriate numbers of cells

- A) Non-self-renewing progenitor: generates two differentiating cells
- B) Self-renewing: generates at least one cell same as parent



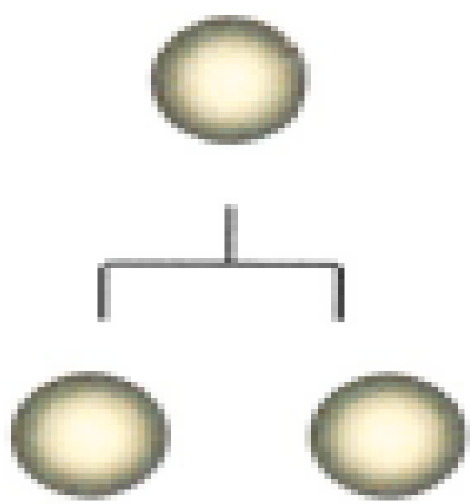
Nervous system progenitors

- Nervous systems undergo enormous expansion in cell number during development
- Relies on cells that can self-renew: stem cells

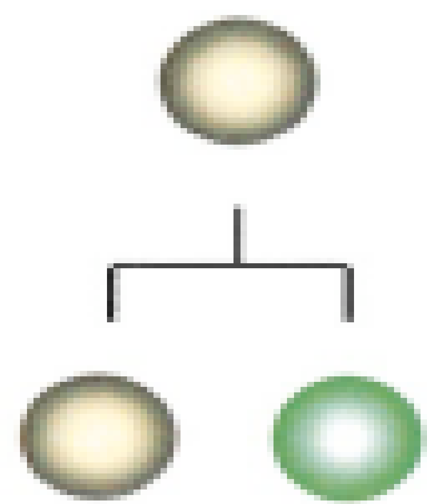


Stem cell divisions

- Symmetric division:
  - Generates two stem cells



- Asymmetric division:
  - Regenerates stem cell and produces a novel cell



Stem cells in the hematopoietic system

- Plutipotent stem cells can generate stem cells with progressively restricted potential fates
- Restriction can proceed in more than one step as generate increasingly committed progenitors

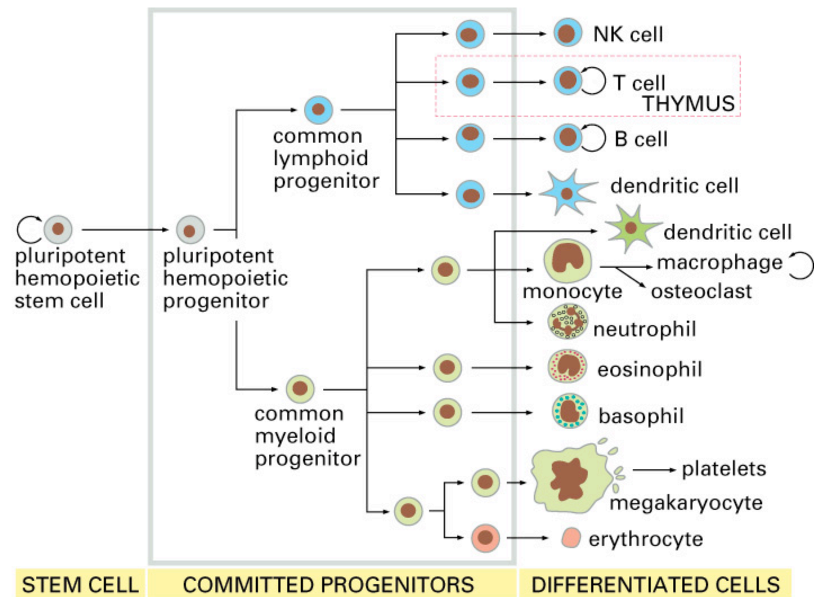


Figure 22–35. Molecular Biology of the Cell, 4th Edition.

Neural stem cells

- Key properties:
- Multipotent -- generate multiple different types of progeny
- Self-renewing

Sample genealogy of cortical neuronal stem cell

- **Self-renewing**
- **Undergo symmetric (diamond, circle) and asymmetric (*) divisions**
- **Multipotent: generates neurons (N) and glia (_)**

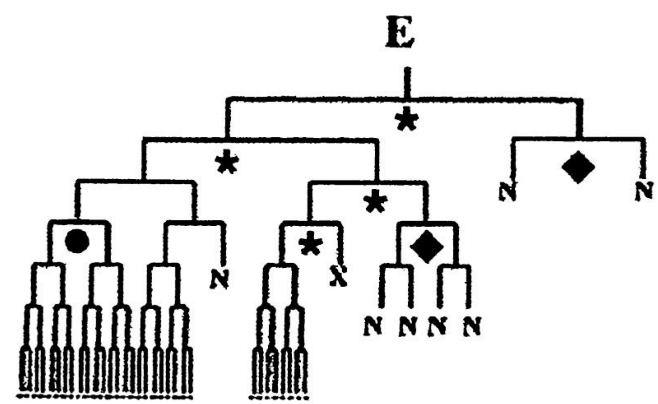


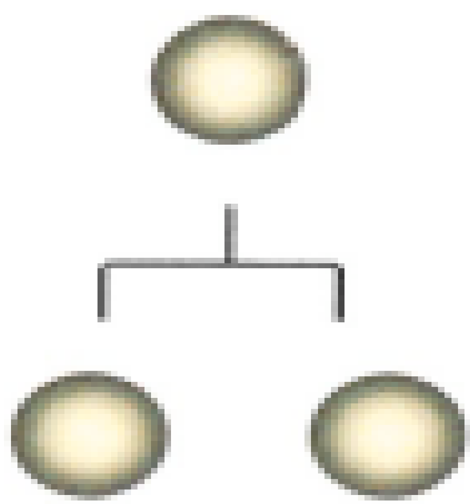
Figure 2. Cortical NSC Lineages In Vitro

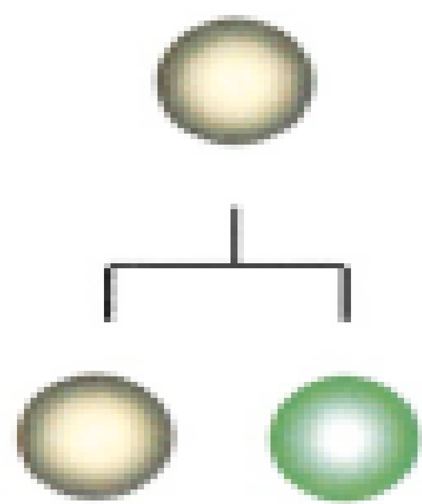
Actual genealogies of individual founder cells (E, F, G, and H) reconstructed from time lapse video recordings of cortical NSCs grown in defined medium in the absence of other cell types (Qian et al., 2000). Note that the sequential generation of neurons (N) and then glia (–) observed in vivo is reproduced in vitro. Asterisks (E) indicate examples of asymmetric divisions; closed circle indicates symmetric division producing only nonneuronal cells; closed diamonds indicate symmetric divisions producing only neurons. "X" indicates dead cell. Reproduced with permission from Qian et al. (2000).

Stem cell divisions

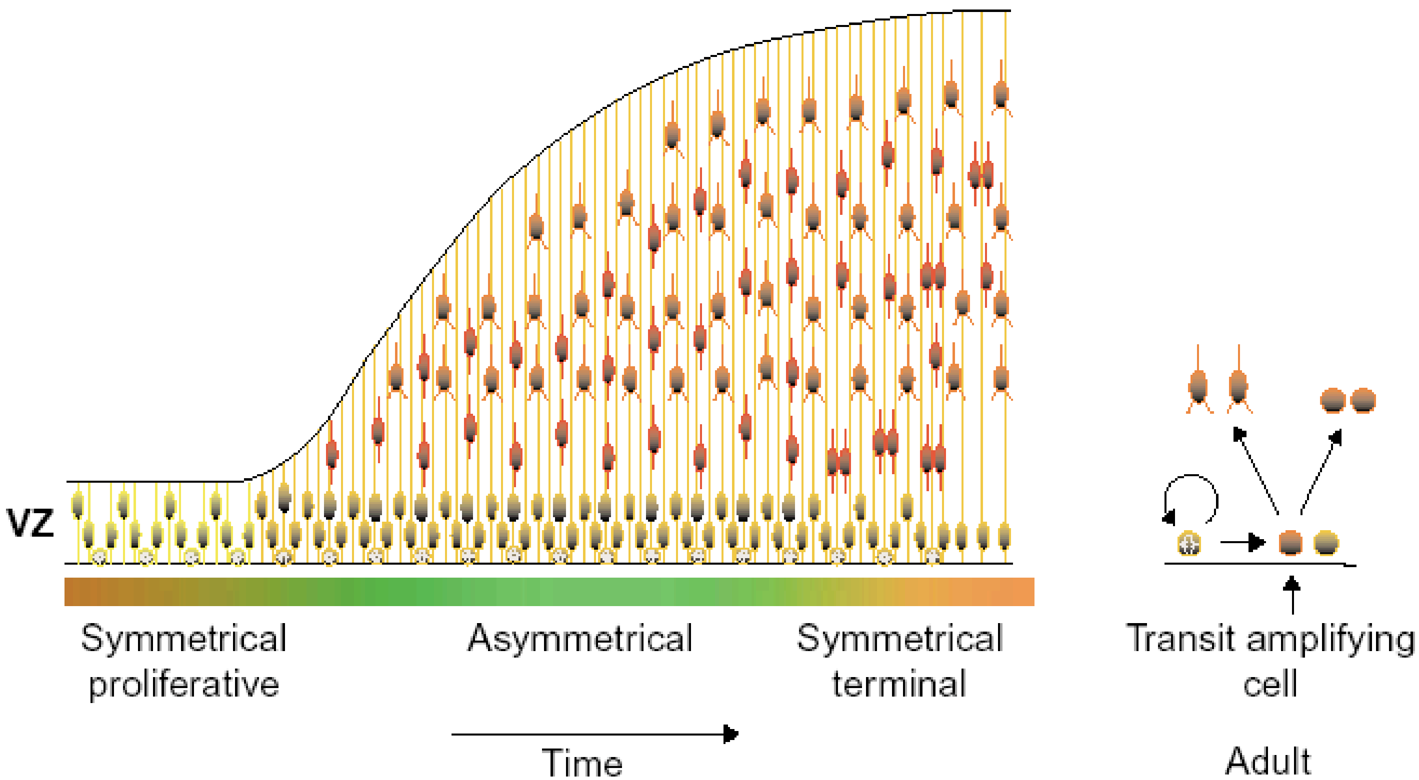
- **Symmetric division:**
  - Generates two stem cells

- **Asymmetric division:**
  - Regenerates stem cell and produces a novel cell

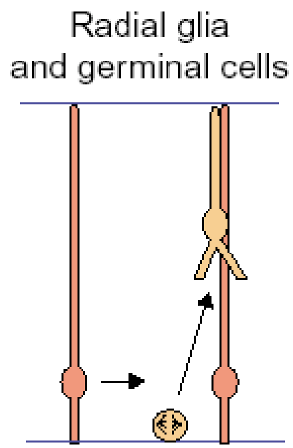




Shifts in fraction of pattern of stem cell division with time



Radial glial cells:



(Rakic, 1970s)

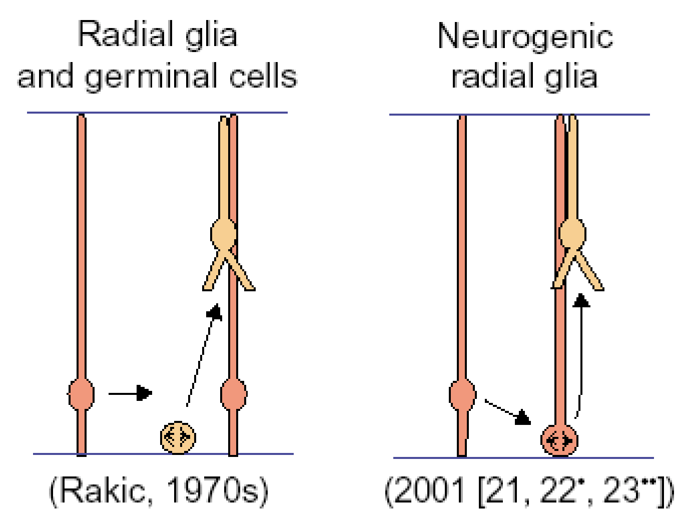
- **Classic view:**
- **Radial glial cells act as substrates for neural migration**
- **A distinct population of cells generates neurons**

Radial glial cells: (c. 2001)

- Radial glial cells are mitotically active
- What do they produce?
  - Infect radial glia with GFP retrovirus
  - Identify single, labelled radial glia cells at 24h
  - Wait 2 more days (forms a clone of cells)

Radial glial cells: more than just substrate for migration

- What do labelled radial glial cells produce?
  - See labelled:
    - mitotically active radial glia -- divide in VZ
    - post-mitotic neurons
  - Post-mitotic neurons migrate along clonally related radial glial cells --



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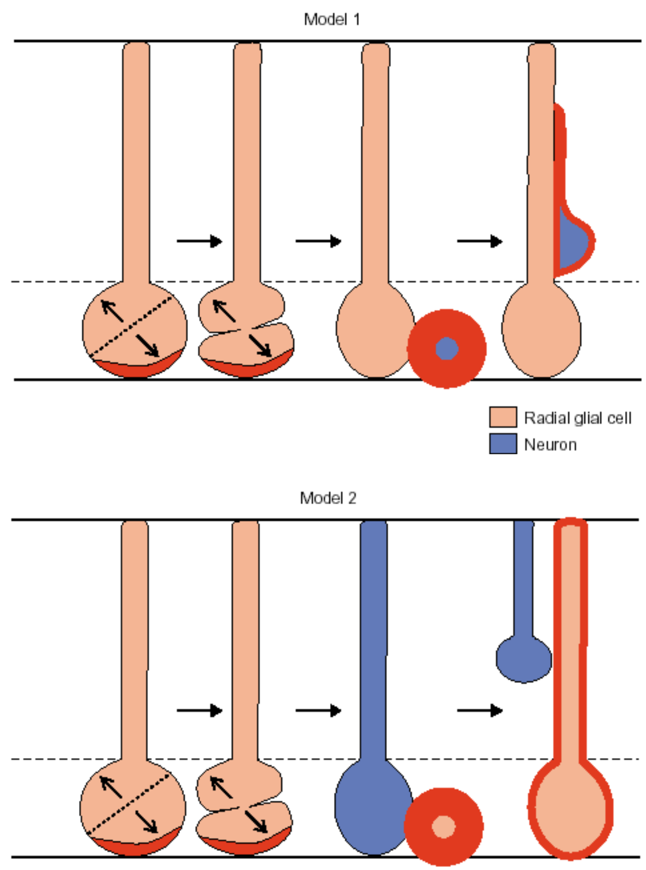
Current models for Radial Glial Cell asymmetric division



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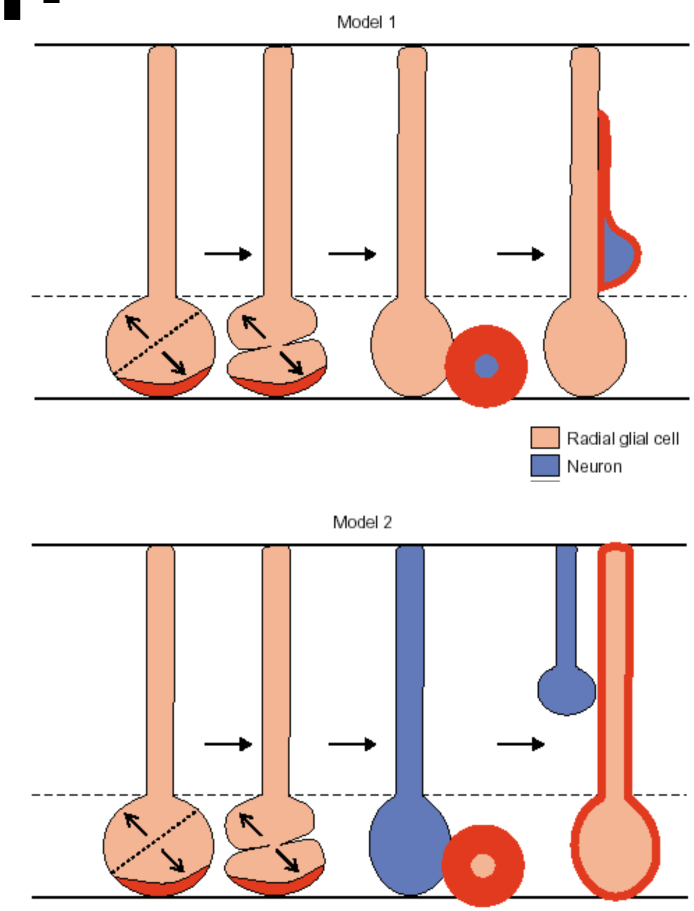
Current models for Radial Glial Cell asymmetric division

- Current evidence suggests that both “translocation” and “migration” are used



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