Neurogenesis and Neuronal Migration

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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>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Protein type</th>
<th>Chromosome^a</th>
<th>References</th>
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<td>ApoER2</td>
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<td>MARCKS</td>
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<td>Ncam1</td>
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<td>Pax2</td>
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<td>Sox6</td>
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<td>Sfn-1</td>
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<td>WldS</td>
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</table>

^aN.D. Not determined.

^bMice 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:

![Graph showing birthdating analysis for wild type and reeler mutant mice across different embryonic days and layers. The bars indicate the number of births for each day and layer.]
The *reeler* mutant mouse

- Birthdating analysis of *reeler* mutant:
- Timing of layer production is inverted
Anatomy of developing cortex in reeler
Molecular identification of Reelin

- Secreted protein
- Produced by Cajal-Retzius cells
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

decreased folding
reduced white matter
enlarged ventricles

cerebellum
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
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

- Truncation of cytoplasmic domain
  - No effect
- Truncation in extracellular domain
  - Dominant-negative
  - Still binds Sema3
  - Signaling fails
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

- Pluripotent 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 (∅)

![Genealogy Diagram](image)

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:

- **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 --

Current models for Radial Glial Cell asymmetric division

Current models for Radial Glial Cell asymmetric division

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