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. Cells increase in number and diversity from \( \sim 10^3 \) cells to \( \sim 10^{11} \) 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

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

**difference between regions (linked to regionalization)**

**difference between species**

Cell division in the nervous system is most studied in mammalian neocortex (mouse and human).
How are cell divisions regulated?

**cell cycle behavior**

1. length
2. exit

**mode of division**
1. symmetric
2. asymmetric

**cleavage plane of division influences division mode**
- vertical
- horizontal
- oblique

expand cell populations
generate diverse cell types

What are the intrinsic and extrinsic regulators of these processes?
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).

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

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[Diagram showing cell cycle with $[^3]$H-thymidine incorporation 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

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).

Paridaen and Huttner (2014)
Neuroepithelial cells transition into radial glial cells

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.

Judith TML Paridaen & Wieland B Huttner

Paridaen and Huttner (2014)
-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).
Radial glia are neural stem cells

two criteria for a stem cell:
self-renewal
production of multiple cell types

- 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.

![Diagram of radial glial (RG) cell division and migration](image)
Radial glia and astrocytes

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 (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)
"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

<table>
<thead>
<tr>
<th>Cell-cycle parameters (h)</th>
<th>APs (Pax6+/Tbr2−)</th>
<th>Tis21-GFP−</th>
<th>Tis21-GFP+</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{c} )</td>
<td>14.1</td>
<td>14.1</td>
<td>14.0</td>
</tr>
<tr>
<td>( T_{s} )</td>
<td>5.0</td>
<td>8.3</td>
<td>1.8</td>
</tr>
<tr>
<td>( T_{c} )</td>
<td>19.1</td>
<td>22.4</td>
<td>15.8</td>
</tr>
<tr>
<td>( T_{g2} )</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>( T_{M} )</td>
<td>0.9</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>( T_{G1} )</td>
<td>11.6</td>
<td>11.4</td>
<td>11.7</td>
</tr>
</tbody>
</table>

| BPs (Tbr2+/Tbr1−)       | 23.3              | 23.0       | 23.4       |
| \( T_{c} \)             | 3.2               | 6.4        | 2.8        |
| \( T_{s} \)             | 26.5              | 29.4       | 26.2       |
| \( T_{c} \)             | 1.6               | 1.6        | 1.6        |
| \( T_{g2} \)            | 0.5               | 0.5        | 0.5        |
| \( T_{M} \)             | 21.2              | 20.9       | 21.3       |

| Tis21-GFP− NPCs         | 8.0               | 23.3       | 1.6        |
| Tis21-GFP+ NPCs         | 2.4               | 21.7       | 1.6        |

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

Arai et al. (2011)

- 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. (2001)
Inside-out pattern of neurogenesis in the neocortex

$^{3}$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

In the vertebrate developing brain, early RGC divisions feature cleavage planes perpendicular to the ventricular surface (vertical cleavage, Fig 2B, C left). The spindle orientation of symmetric RGC divisions is tightly regulated by mechanisms involving the centrosomes, astral microtubule positioning, and interaction with proteins present at the cell cortex [27]. The mitotic spindle is anchored to the cell cortex by astral microtubules via dynein and the LGN/Ga2i/NuMa complex. Localization of the LGN complex components to the lateral membrane of NECs/RGCs is essential for maintaining early symmetric RGC divisions in vertebrate neurogenesis (Fig 2C, left) [28–30]. In addition, Lis1, a gene that causes lissencephaly (“smooth” brain) in humans when mutated, mediates capture of the astral microtubules by the cell cortex through interaction with dynein and Ndel1 [31]. Perturbation of the Lis1/Ndel1 complex severely disrupts the expansion of the NEC/RGC pool by inducing random cleavage planes [31–33].

In asymmetric divisions in Drosophila, Insc induces horizontal cleavage planes through recruitment of the LGN complex to the apical domain by interaction of Insc with polarity proteins (Fig 2C, right). However, horizontal cleavages are less common in vertebrate developing brains. For example, in the mammalian neocortex, oblique and horizontal cleavage planes appear only in later developmental stages (Fig 2C, middle) [34,35]. These cleavages generate basal progenitors such as IPs and bRG that are proposed to be important during evolutionary cortical expansion [36,37]. Disruption of mInsc at later stages of neurogenesis interferes with the spindle orientation of these asymmetric divisions [35], suggesting that release of the tight regulation of spindle orientation is important for inducing basal progenitors.

Indeed, mutations in genes regulating spindle orientation cause brain disorders such as lissencephaly and microcephaly in humans [38]. Interestingly, most known microcephaly genes encode centrosomal proteins, which often have a role in regulating spindle orientation, such as Aspm, Cdk5rap2, and MCPH1 [38–40]. Centrosome overduplication in mouse RGCs leads to multipolar mitotic spindles, eventually causing microcephaly due to RGC apoptosis and subsequent reduction in NPCs [41]. In general, besides regulating spindle orientation, the function of microcephaly genes is related to control of centriole duplication, centrosome maturation, and/or entry into mitosis. However, it is still unclear how disruption of symmetric proliferative NEC – RGC transition Asymmetric neurogenic Additional NPC types (e.g., mammalian neocortex)
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.

- 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.

Early nerve cord “ground state”: Hox-free in NBs

Thoracic lineage

Anterior abdominal lineage

Posterior abdominal lineage

Late nerve cord: Hox gradients in NBs → cell cycle

Thoracic lineage

Anterior abdominal lineage

Posterior abdominal lineage

NB proliferation

type I→0 daughter proliferation switch

Lineage size

Modenero Cobeta et al. (2017)
Regional differences in neurogenesis

2. Neurogenic gradients within each brain region

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

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.
3. Evolution
difference in neocortical organization between human and mouse
Dehay and Kennedy (2007)

Figure 1 | Differences in the anatomical organization of the rodent and primate embryonic cortex.
These schematics are transects through the presumptive area 17 of the embryonic cortex in mouse (a) and monkey (b) at comparable developmental stages. The depth of each layer is drawn to a common scale. Gestation period is 19 days in the mouse and 165 days in the monkey. Cortical neurogenesis lasts 8 days in the mouse (embryonic day 11 (E11) to E19) and spans 60 days in the monkey visual cortex (area 17). In primates, an early appearing (at approximately E55) outer fibre layer (OFL) forms a major landmark during development. The ventricular zone (VZ) declines progressively after E65. By contrast the subventricular zone (SVZ) increases progressively in depth and by E72 is divided into an inner subventricular zone (ISVZ) and outer subventricular zone (OSVZ) by an intruding inner fibre layer (IFL). The OSVZ exhibits a number of unique features. It is histologically similar to the VZ and also has a compact radial organization. This alone distinguishes the primate OSVZ from the loosely organized SVZ of rodents and primates. Contrary to what is observed in the rodent, where the VZ is the main germinal compartment throughout corticogenesis, the primate VZ declines rapidly during corticogenesis and is paralleled by the early appearance of the SVZ followed by the OSVZ. The monkey cortical plate (CP) appears as early as E46. The sub-plate (SP) is evident after E55. The marginal zone (MZ) is minimal before E65. FL, fibre layer; IP, inner plexiform layer; PP, pre-plate. Reproduced with permission from REF. 34 © (2002) Oxford Journals.

basics of cortical histogenesis
-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.
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

Mode of division
- 1. symmetric
- 2. asymmetric

What are the intrinsic and extrinsic regulators of these processes?

Cleavage plane of division influences division mode
- vertical
- horizontal
- oblique

Expand cell populations
Generate diverse cell types
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

<table>
<thead>
<tr>
<th>CYCLIN–CDK COMPLEX</th>
<th>VERTEBRATES CYCLIN</th>
<th>CDK PARTNER</th>
<th>BUDDING YEAST CYCLIN</th>
<th>CDK PARTNER</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁-Cdk</td>
<td>cyclin D*</td>
<td>Cdk4, Cdk6</td>
<td>Cln3</td>
<td>Cdk1**</td>
</tr>
<tr>
<td>G₁/S-Cdk</td>
<td>cyclin E</td>
<td>Cdk2</td>
<td>Cln1, 2</td>
<td>Cdk1</td>
</tr>
<tr>
<td>S-Cdk</td>
<td>cyclin A</td>
<td>Cdk2, Cdk1**</td>
<td>Clb5, 6</td>
<td>Cdk1</td>
</tr>
<tr>
<td>M-Cdk</td>
<td>cyclin B</td>
<td>Cdk1</td>
<td>Clb1, 2, 3, 4</td>
<td>Cdk1</td>
</tr>
</tbody>
</table>

* 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

Regulation of G1/S transition

- 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})

- 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.
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.
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 p21cip1, p27kip1 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

SHH signaling requires the primary cilium

Paridaen and Huttner (2014)
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.