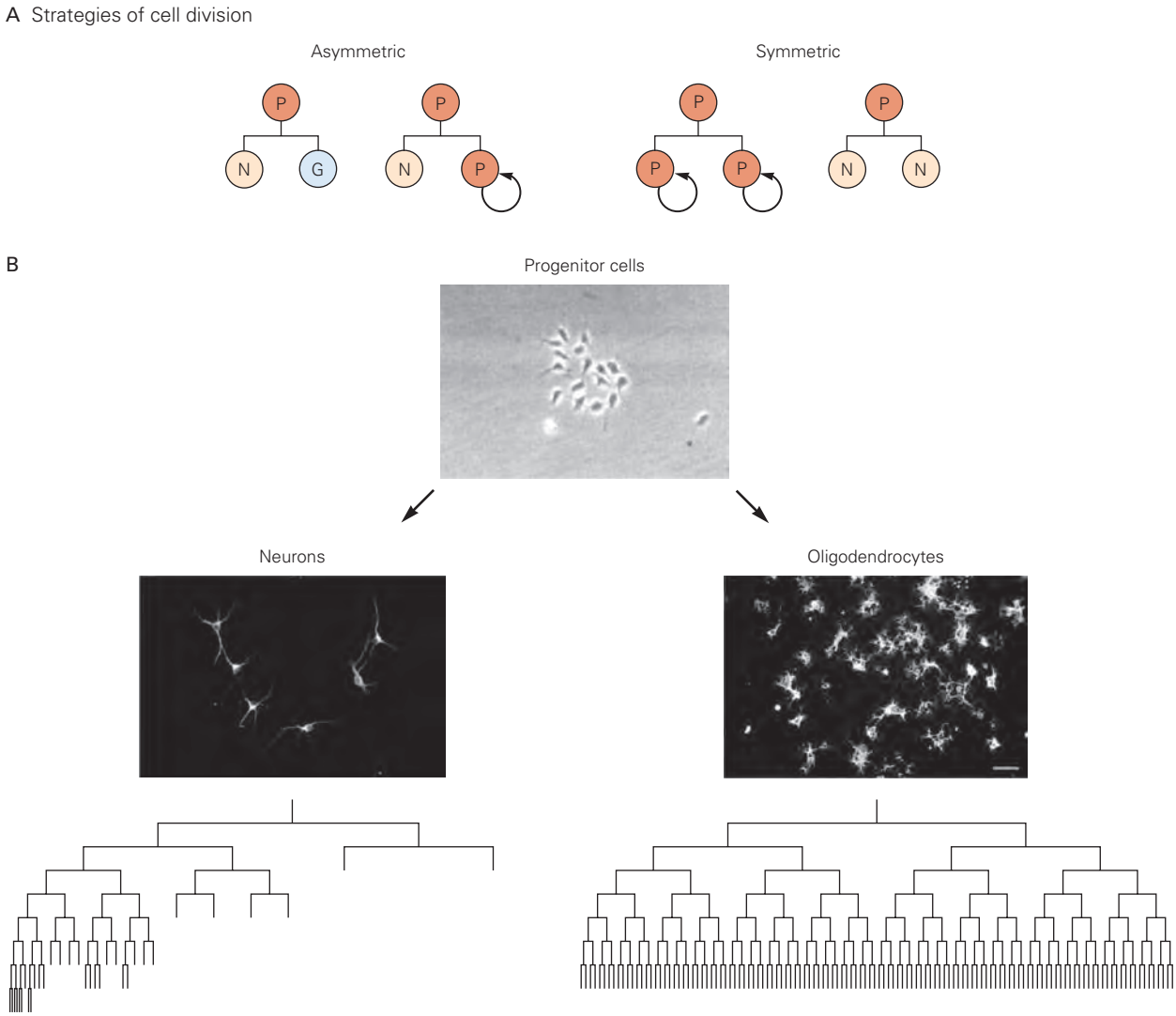


Figure 46–1 Neural progenitor cells have different modes of division.

A. Asymmetric and symmetric modes of cell division. A progenitor cell (P) can undergo asymmetric division to generate a neuron (N) and a glial cell (G), or a neuron and another progenitor. This mode of division contributes to the generation of neurons at early stages of development and of glial cells at later stages, typical of many regions of the central nervous system. Progenitor cells can also undergo symmetric division to generate two additional progenitor cells or two postmitotic neurons.

B. Time-lapse cinematography captures the divisions and differentiation of isolated cortical progenitor cells in the rodent. Lineage diagrams illustrate cells that undergo predominantly asymmetric division, giving rise to neurons, or symmetric division, giving rise to oligodendrocytes. (Adapted, with permission, from Qian et al. 1998. Permission conveyed through Copyright Clearance Center, Inc.)

transmembrane ligand Delta and its receptor Notch. This signaling system was revealed in genetic studies in *Drosophila*. Neurons emerge from within a larger cluster of ectodermal cells, called a *proneural region*, all of which have the potential to generate neurons. Yet within the proneural region, only certain cells form neurons; the others become epidermal support cells.

Delta and Notch are initially expressed at similar levels by all proneural cells (Figure 46–3A). With time, however, Notch activity is enhanced in one cell and suppressed in its neighbor. The cell in which Notch activity is highest loses the potential to form a neuron and acquires an alternative fate. The binding of Delta to Notch results in proteolytic cleavage of the Notch cytoplasmic domain, which then enters the nucleus.

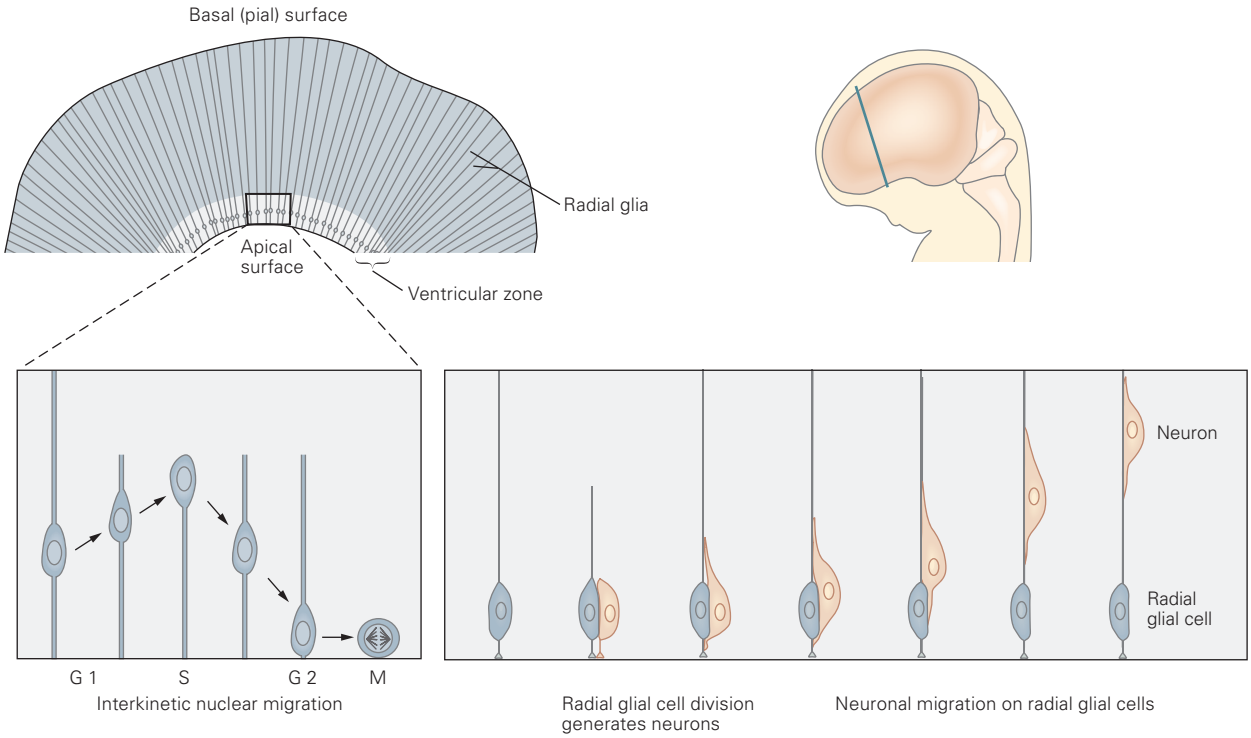


Figure 46–2 **Radial glial cells serve as precursors to neurons in the central nervous system and also provide a scaffold for radial neuronal migration.** The nuclei of progenitor cells in the ventricular zone of the developing cerebral cortex migrate along the apical-basal axis as they progress through the cell cycle. *Left*: During the G1 phase, the nuclei rise from the inner (apical) surface of the ventricular zone. During the S phase, they reside in the outer (basal) third of the ventricular zone. During the G2 phase, they migrate apically, and mitosis (**M**) occurs when the nuclei reach the ventricular surface. *Right*: During cell division, radial glial cells give rise to postmitotic neurons that migrate away from the ventricular zone using radial glial cells as a guide.

There, it functions as a transcription factor, regulating the activity of a cascade of other transcription factors of the basic helix-loop-helix (bHLH) family. The bHLH transcription factors suppress the ability of the cell to become a neuron and reduce the level of expression of the ligand Delta (Figure 46–3B,C).

The initial difference in Notch levels between cells may be small and in some cases stochastic (random). Through this feedback pathway, however, these initial minor differences are amplified to generate all-or-none differences in the status of Notch activation and, consequently, the fates of the two cells. This basic logic of Delta-Notch and bHLH signaling has been conserved in vertebrate and invertebrate neural tissues.

How does Notch signaling regulate neuronal and glial production in mammals? At early stages in the development of the mammalian cortex, Notch signaling promotes the generation of radial glial cells by activating members of the Hes family of bHLH transcriptional repressors. Two of these proteins, Hes1 and Hes5, appear to maintain radial glial cell character by activating the expression of an ErbB class tyrosine kinase receptor for neuregulin, a secreted signal that promotes radial glial cell identity. The Notch ligand Delta1 as well as neuregulin are expressed by newly generated cortical neurons; thus, the radial glial cells depend on feedback signals from their neuronal progeny for continued production.

At later stages of cortical development, Notch signaling continues to activate Hes proteins, but a change in the intracellular response pathway results in astrocyte differentiation. At this stage, the Hes proteins work by activating a transcription factor, STAT3, which recruits the serine-threonine kinase JAK2, a potent inducer of astrocyte differentiation. STAT3 also activates expression of astrocyte-specific genes such as the glial-fibrillary acidic protein (GFAP).

The generation of oligodendrocytes, the second major class of glial cells in the central nervous system, follows many of the principles that control neuron

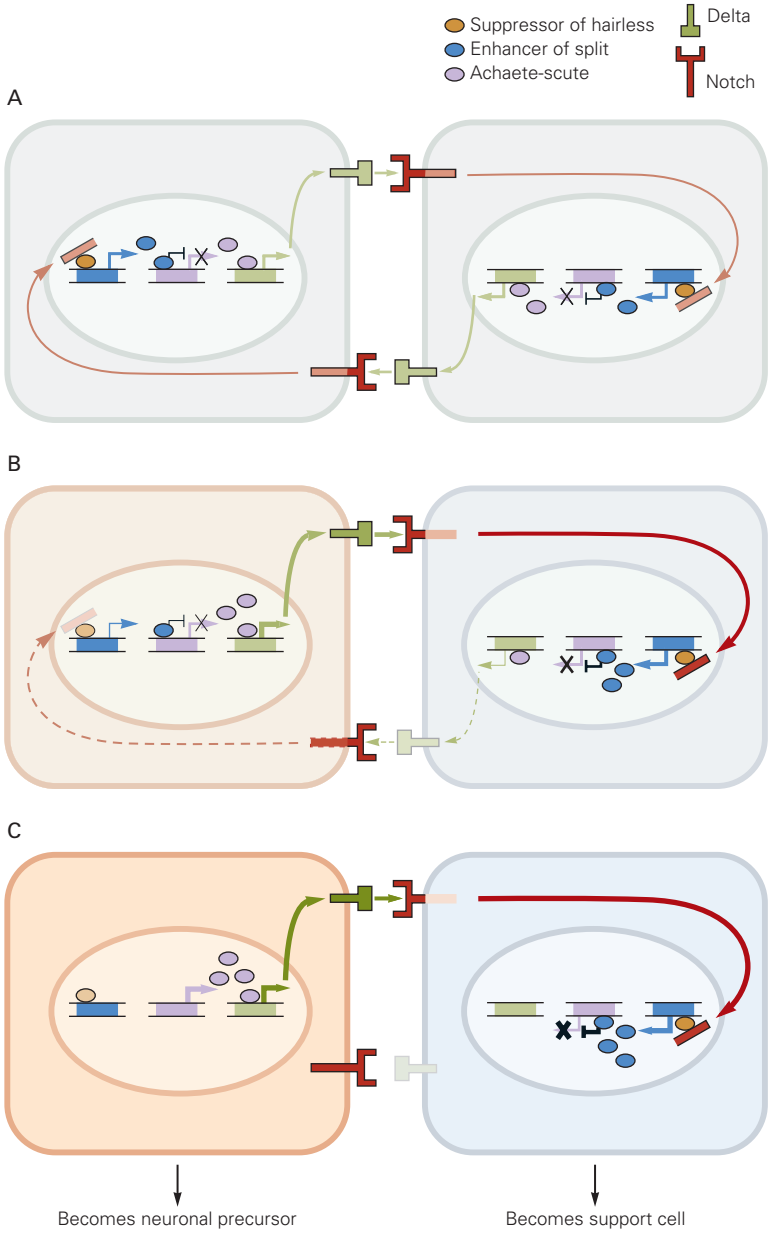


Figure 46–3 Delta binds the receptor Notch and determines neuronal fate.

A. At the onset of the interaction between two cells, Delta engages the receptor Notch. Delta and Notch are expressed at similar levels in each cell, and thus their initial signaling strength is equal.

B. A small imbalance in the strength of Delta-Notch signaling breaks the symmetry of the interaction. In this example, the left cell provides a slightly greater Delta signal, thus activating Notch signaling in the right cell to a greater extent. On binding by Delta, the cytoplasmic domain of Notch is cleaved to form a proteolytic fragment called Notch-Intra, which enters the nucleus of the cell and initiates a basic helix-loop-helix (bHLH) transcriptional cascade that regulates the level of Delta expression. Notch-Intra forms a transcriptional complex with a bHLH protein, suppressor of hairless, which binds to and activates the gene encoding a second bHLH protein, enhancer of split. Once activated, enhancer of split binds to and represses expression of the gene encoding a third bHLH protein, achaete-scute. Achaete-scute activity promotes expression of Delta. Thus, by repressing achaete-scute, enhancer of split decreases transcriptional activation of the Delta gene and production of Delta protein. This diminishes the ability of the cell on the right to activate Notch signaling in the left cell.

C. Once the level of Notch signaling in the left cell has been reduced, suppressor of hairless no longer activates enhancer of split, and the level of expression of achaete-scute increases, resulting in enhanced expression of Delta and further activation of Notch signaling in the right cell. In this way, a small initial imbalance in Delta-Notch signaling is rapidly amplified into a marked asymmetry in the level of Notch activation in the two cells. In the mammalian central nervous system, cells with high levels of Notch activation are diverted from neuronal fates, whereas cells with low levels of Notch activation become neurons.

and astrocyte production (Figure 46–4). Notch signaling regulates the expression of two bHLH transcription factors, Olig1 and Olig2, which have essential roles in the production of embryonic and postnatal oligodendrocytes.

Additional mechanisms exist to ensure that the effects of Notch signals are avoided in cells destined to become neurons. One involves a cytoplasmic protein called Numb. The key role of Numb in neurogenesis was first shown in *Drosophila*, where it determines the neuronal fate of daughter cells of asymmetrically dividing progenitors. In the mammalian cortex, Numb is preferentially localized in neuronal daughters and antagonizes Notch signaling. Loss of Numb activity causes progenitor cells to proliferate extensively. The inhibition of Notch signaling results in the expression of several proneural bHLH transcription factors, notably Mash1, neurogenin-1, and neurogenin-2. Neurogenins promote neuronal production by activating downstream

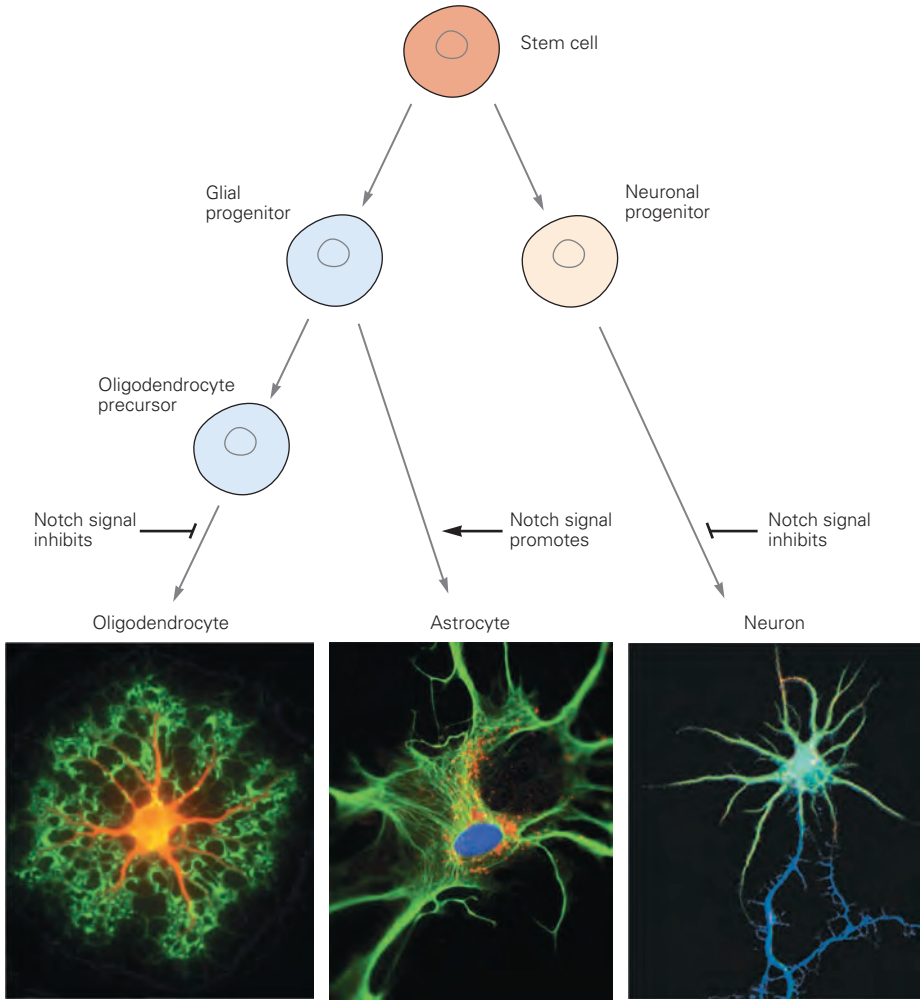


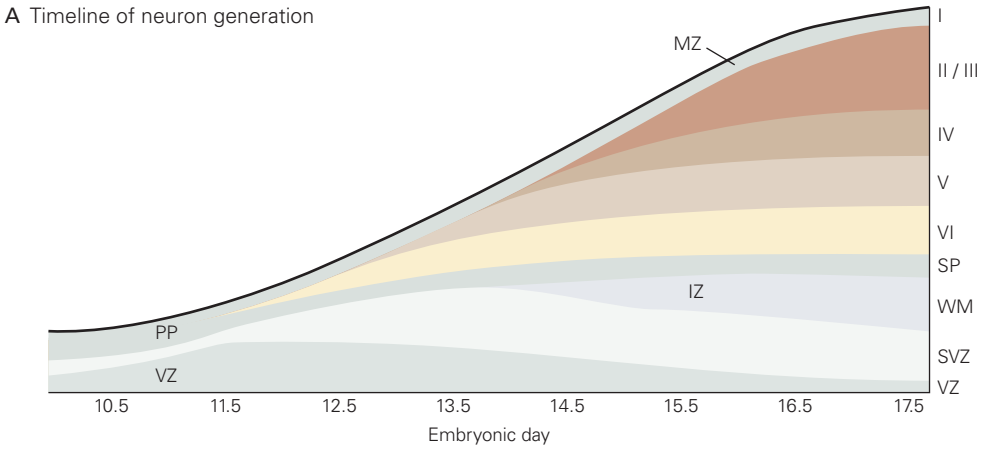
Figure 46–4 Notch signaling regulates the fate of cells in the developing cerebral cortex. Notch signaling has several roles in cell differentiation in the developing cerebral cortex. Activation of Notch signaling in glial progenitor cells results in differentiation of the cells as astrocytes and inhibits differentiation as oligodendrocytes (left pathway). Notch signaling also inhibits progenitor cells from differentiating into neurons (right pathway). (Photo of oligodendrocyte reproduced, with permission, from David H. Rowitch; photo of astrocyte reproduced, with permission, from SAASTA on behalf of photographers Edward Nyatia and Dirk Michael Lang; photo of neuron reproduced, with permission, from Masatoshi Takeichi.)

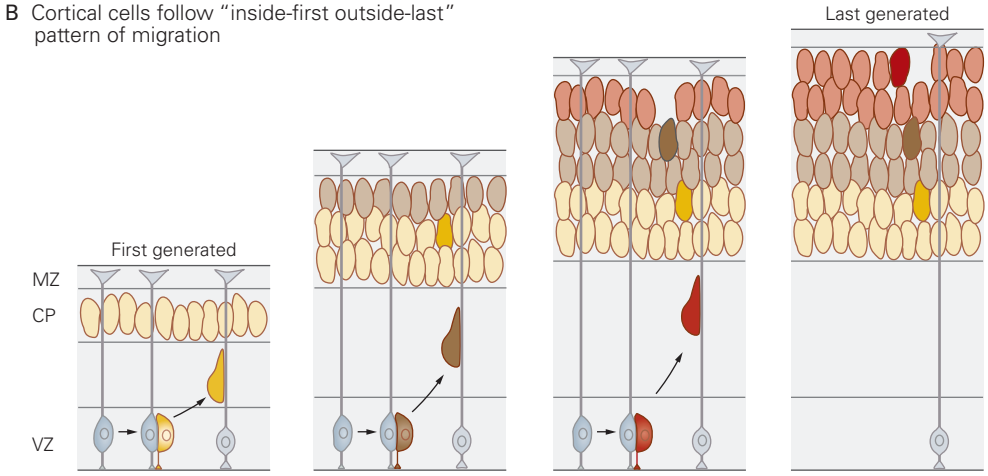
bHLH proteins such as neuroD, and they block the formation of astrocytes by inhibiting JAK and STAT signaling.

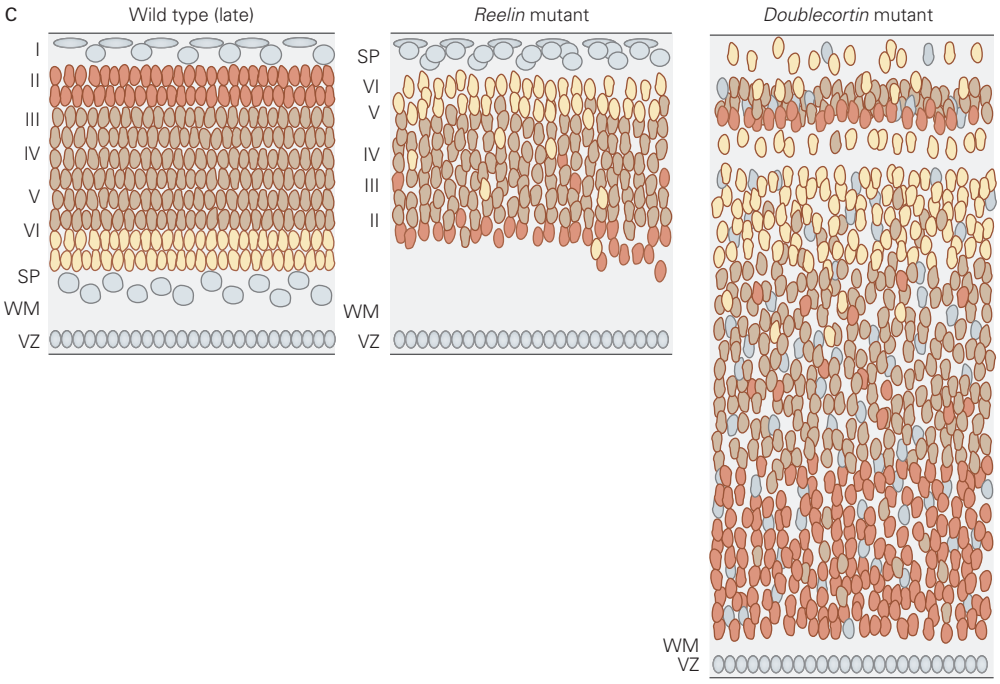
Although Delta-Notch signaling and bHLH transcription factor activators lie at the heart of the decision to produce neurons or glial cells, several additional transcriptional pathways augment this core molecular program. One important transcription factor, REST/NRSF, represses the expression of neuronal genes in neural progenitors and glial cells. REST/NRSF is rapidly degraded as neurons differentiate, permitting the expression of neurogenic bHLH factors and other neuronal genes. Homeodomain transcription factors of the SoxB class also play an important role in maintaining neural progenitors by blocking neurogenic bHLH protein activity. The differentiation of neurons therefore requires the avoidance of REST/NRSF and SoxB protein activity.

The Layers of the Cerebral Cortex Are Established by Sequential Addition of Newborn Neurons

The ventricular zone in the most anterior portion of the mammalian neural tube gives rise to the cerebral cortex in a series of steps. Cells from the ventricular zone, which is on the apical edge of the neuroepithelium, initially migrate basally to form a subventricular zone, which houses a set of progenitor cells with a more restricted set of fates. Next to form is an intermediate zone, through which newly formed neurons migrate, and a preplate, which houses the earliest-born neurons. Additional neurons migrate to form a layer called the cortical plate, which lies within the preplate. The cortical plate thereby divides the preplate into an apical subplate and a basal marginal zone (Figure 46–5A).


A Timeline of neuron generation
MZ
I
II / III
IV
V
VI
SP
IZ
WM
PP
SVZ
VZ
VZ
10.5
11.5
12.5
13.5
14.5
15.5
16.5
17.5
Embryonic day


B Cortical cells follow "inside-first outside-last" pattern of migration
Last generated
First generated
MZ
CP
VZ


C
Wild type (late)
Reelin mutant
Doublecortin mutant
I
II
III
IV
V
VI
SP
WM
VZ
SP
VI
V
IV
III
II
WM
VZ
WM
VZ

Once within the cortical plate, neurons become organized into well-defined layers. The layer in which a neuron settles is correlated precisely with the neuron's *birthday*, a term that refers to the time at which a dividing precursor cell undergoes its final round of cell division and gives rise to a postmitotic neuron. Cells that migrate from the ventricular and subventricular zones and leave the cell cycle at early stages give rise to neurons that settle in the deepest layers of the cortex. Cells that exit the cell cycle at progressively later stages migrate over longer distances and pass earlier-born neurons before settling in more superficial layers of the cortex. Thus, the layering of neurons in the cerebral cortex follows an inside-first, outside-last rule (Figure 46–5B).

Neurons Migrate Long Distances From Their Site of Origin to Their Final Position

The migration of neurons from the cortical ventricular zone to the cortical plate follows a process termed *radial migration.* In this mode, the neurons move along the long unbranched processes of radial glial cells to reach their destinations. In contrast, interneurons enter the cortex from subcortical sites by a process called *tangential migration*. We discuss these modes in turn and then describe a third migratory strategy, *free migration*, which predominates in the peripheral nervous system.

Excitatory Cortical Neurons Migrate Radially Along Glial Guides

Classical anatomical studies of cortical development in the 1970s provided evidence that neurons generated in the ventricular zone migrate to their settling position along a pathway of radial glial fibers. Radial glial cells serve as the primary scaffold for radial neuronal migration. Their cell bodies are located close to the ventricular surface and give rise to elongated fibers that span the width of the developing cerebral wall. Each radial glial cell has one basal end-foot at the apical surface of the ventricular zone and processes that terminate in multiple end-feet at the pial surface (Figure 46–6). Radial glial scaffolds are especially important in the development of the primate cortex, where neurons are required to migrate over long distances as the cortex expands. A single radial glial cell scaffold can support the migration of up to 30 generations of cortical neurons before eventually differentiating into an astrocyte.

What forces and molecules power neuronal migration on radial glial cells? After a neuron leaves the cell cycle, its leading process wraps around the shaft of the radial glial cell and its nucleus translocates within the cytoplasm of the leading process. Although the leading process of the migrating neuron extends slowly and steadily, the nucleus moves in an intermittent, stepwise manner because of complex rearrangements of the cytoskeleton. A microtubular lattice forms a cage around the nucleus; movement of the nucleus depends on a centrosome-like structure, termed a *basal body*, from which a system of microtubules projects into the leading process, providing tracks along which the nucleus moves (Figure 46–7A).

Neuronal migration along radial glia also involves adhesive interactions between cells. Adhesive receptors such as integrins promote neuronal extension on radial glial cells. The migration of neurons along glial fibers is nevertheless different from the extension of axons driven by growth cones (Chapter 47). In neuronal migration, the leading process is devoid of the structured actin filaments that typify growth cones and more closely resembles an extending dendrite, an inference made first by Santiago Ramón y Cajal.

Disruption in the migratory and settling programs of cortical neurons underlies much human cortical pathology (Figure 46–5C). For example, in lissencephaly

Figure 46–5 (Opposite) **The migration of neurons within the embryonic cerebral cortex leads to layered cortical organization.** (Adapted from Olsen and Walsh 2002.)

A. This temporal sequence of neurogenesis is for the mouse cerebral cortex. Neurons begin to accumulate in the cortical plate during the last 5 days of embryonic development. Within the cortical plate, neurons populate the deep layers before settling in the superficial layers. (Abbreviations: **IZ**, intermediate zone; **MZ**, marginal zone; **PP**, preplate; **SP**, subplate; **SVZ**, subventricular zone; **VZ**, ventricular zone; **WM**, white matter.)

B. During normal cortical development, neurons use radial glial cells as migratory scaffolds as they enter the cortical plate. As they approach the pial surface, neurons stop migrating and detach from radial glial cells. This orderly inside-out pattern of neuronal migration results in the formation of six neuronal layers in the mature cerebral cortex, arranged between the white matter and subplate. (Abbreviation: **CP**, cortical plate.)

C. In the mouse mutant *reeler,* which lacks functional reelin protein, the layering of neurons in the cortical plate is severely disrupted and partially inverted. In addition, the entire cortical plate develops beneath the subplate. In *doublecortin* mutants, the cortex is thickened, neurons lose their characteristic layered identity, and some layers contain fewer neurons. A similar disruption is observed in *Lis1* mutants, which underlies certain forms of human lissencephaly.

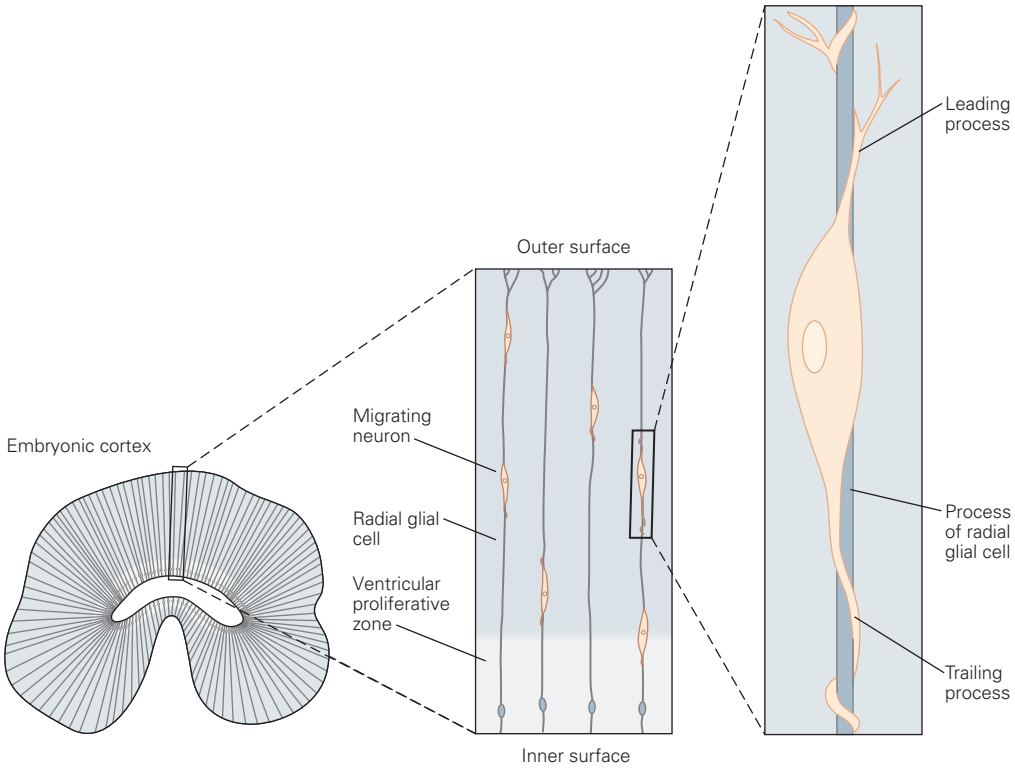


Figure 46–6 Neurons migrate along radial glial cells. After their generation from radial glial cells, newly generated neurons in the embryonic cerebral cortex extend a leading process that wraps around the shaft of the radial glial cell, thus using the radial glial cells as scaffolds during their migration from the ventricular zone to the pial surface of cortex.

(Greek, smooth brain, referring to the characteristic smoothing of the cortical surface in patients with the disorder), neurons leave the ventricular zone but fail to complete their migration into the cortical plate. As a result, the mature cortex is typically reduced from six to four neuronal layers, and the arrangement of neurons in each remaining layer is disordered. Occasionally, lissencephaly is accompanied by the presence of an additional group of neurons in the subcortical white matter. Patients with lissencephalies from mutations in the *Lis1* and *doublecortin* genes often suffer severe intellectual disability and intractable epilepsy. The Lis1 and doublecortin proteins have been localized to microtubules, suggesting that they are involved in microtubule-dependent nuclear movement, although their precise functions in neuronal migration remain unclear.

Mutations that disrupt the reelin signaling pathway disrupt the final stage of neuronal migration through the cortical subplate. The reelin protein is secreted from the Cajal-Retzius cells, a class of neurons found in the preplate and marginal zone. Signals from these cells are crucial for the migration of cortical neurons. In mice lacking functional reelin, neurons fail to detach from their radial glial scaffolds and pile up underneath the cortical plate, disobeying the inside-out migratory rule. As a consequence, the normal layering of cell types is partially inverted and the marginal zone is lost. Reelin acts through cell-surface receptors that include the ApoE receptor 2 and the very-low-density lipoprotein receptor. The binding of reelin to these receptors activates an intracellular protein, Dab1, which transduces reelin signals. Not surprisingly, the loss of proteins that transduce reelin signals produces similar migratory phenotypes.

Cortical Interneurons Arise Subcortically and Migrate Tangentially to Cortex

Progenitor cells in the cortical ventricular zone were initially believed to give rise to all cortical neurons. However, as better molecular labels for distinct neuronal types became available, it was found that interneurons arise in the ventricular zone of subcortical structures. Most of them originate in regions of the ventral telencephalon called the ganglionic eminences (Figure 46–8).

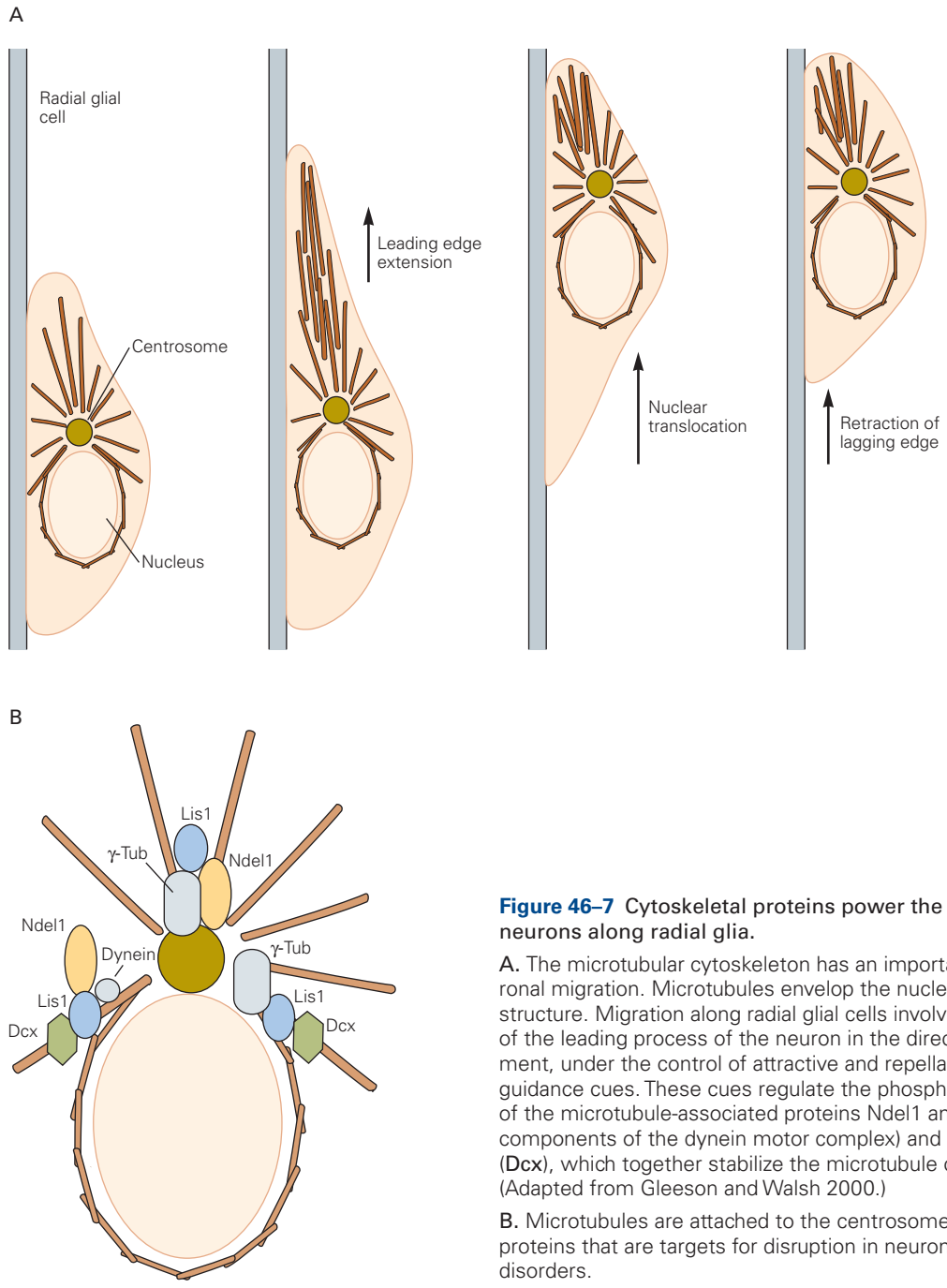


Figure 46–7 Cytoskeletal proteins power the migration of neurons along radial glia.

A. The microtubular cytoskeleton has an important role in neuronal migration. Microtubules envelop the nucleus in a cage-like structure. Migration along radial glial cells involves elongation of the leading process of the neuron in the direction of movement, under the control of attractive and repellant extracellular guidance cues. These cues regulate the phosphorylation status of the microtubule-associated proteins Ndel1 and Lis1 (two components of the dynein motor complex) and of doublecortin (**Dcx**), which together stabilize the microtubule cytoskeleton. (Adapted from Gleeson and Walsh 2000.)

B. Microtubules are attached to the centrosome by a series of proteins that are targets for disruption in neuronal migration disorders.

The medial and central eminences generate most cortical interneurons, which migrate dorsally from their sites of origin to enter the cortex. Some enter through the intermediate zone, while others enter through the marginal zone (Figure 46–5A). Once they reach particular anterior-posterior and mediolateral positions, they switch to a radial mode of migration to travel the final distance to appropriate layers. Distinct populations of neurons generated in the ganglionic eminences migrate at different times and through different routes, contributing to the diversity of the interneuronal population. Precise relationships between time and place of origin, migratory route, and ultimate fate remain to be determined.

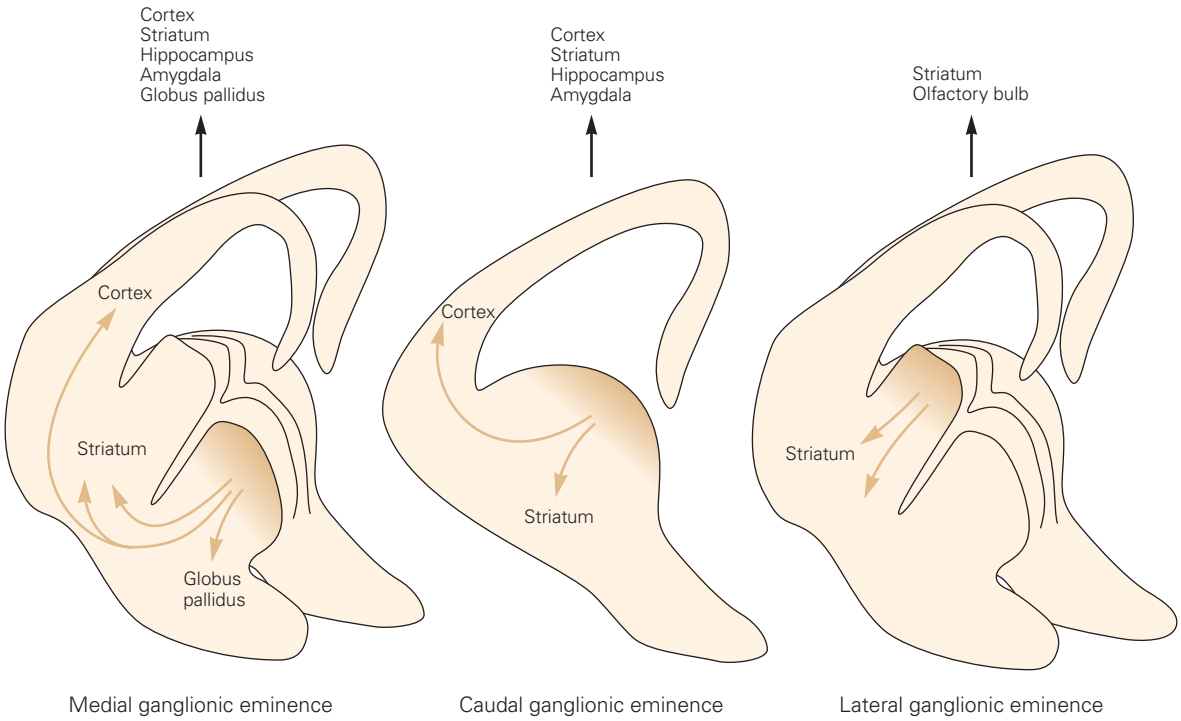


Figure 46–8 Forebrain interneurons are generated in the ventral telencephalon and migrate tangentially to the cerebral cortex. Neurons generated in the ganglionic eminences migrate to and settle in many regions of the forebrain, where they differentiate into interneurons. Cortical interneurons arise from medial and caudal ganglionic eminences. Other cells generated in these regions migrate in other directions, populating the hippocampus, striatum, globus pallidus, and amygdala with interneurons. The lateral ganglionic eminence generates cells that migrate to the striatum and the olfactory bulb. Cells migrating to the bulb use neighboring migrating cells as substrates for migration, a process called chain migration. (Adapted, with permission, from Bandler, Mayer, and Fishell 2017. Copyright © 2017 Elsevier Ltd.)

Nonetheless, it is now clear that cortical neurons originate from two sources: excitatory neurons from the cortical ventricular zone and interneurons from the ganglionic eminences.

Interneurons in other forebrain structures also arise from the ganglionic eminences, as well as a few other subcortical sites such as the preoptic area. Cells migrating caudally from the medial and caudal eminences populate the hippocampus, while cells migrating ventrolaterally from these regions populate the basal ganglia. In contrast, neurons generated in the lateral ganglionic eminence migrate rostrally and contribute the periglomerular and granule interneurons of the olfactory bulb. In this rostral migratory stream, neurons use neighboring neurons as substrates for migration (chain migration). In the adult brain, neurons that follow the rostral migratory stream originate instead in the subventricular zone of the striatum.

Transcription factors control the character of ganglionic eminence neurons. The homeodomain proteins Dlx1 and Dlx2 are expressed by cells in the ganglionic eminences. In mice lacking Dlx1 and Dlx2 activity, the resultant perturbation of neuronal migration leads to a profound reduction in the number of GABAergic interneurons in the cortex. Other transcription factors are responsible for differences among ganglionic eminences. For example, Nkx2.1 is selectively expressed by cells in the medial ganglionic eminence. In its absence, interneurons generated in this region take on characteristics of those normally generated in the lateral and caudal ganglionic eminences. Yet other transcription factors specify the distinct characteristics of subpopulations of neurons within each ganglionic eminence.

One of the main features that these transcription factors specify is the migratory path that the newborn interneurons take. A host of soluble and cell surface factors produced by cells in and near the ganglionic eminences provide repulsive cues that lead to expulsion of cells from the ventricular zone, so-called motogenic (movement-promoting) cues that speed their migration and attractive cues that direct them to their targets. These factors include slits, semaphorins, and ephrins, all of which we will encounter in Chapter 47 as molecules that guide axons to their targets.