Neural Stem Cells in Cerebral Cortex Development

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Abstract

Neural stem cells (NSCs) in the developing neuroepithelium, as well as the progenitors they generate, eventually give rise to all the neurons of the mammalian central nervous system (CNS). In addition, they generate other essential neural cells, mainly the two types of macroglial cells in the CNS, astrocytes and oligodendrocytes. This chapter focuses on the cellular and molecular aspects of neural stem cell biology during CNS embryonic neurogenesis, the process through which these initially multipotent cells undergo fate restriction steps that will eventually lead to all the neuronal subtypes. We describe neurogenesis mainly in the developing cerebral neocortex, although the principles highlighted here describe also many aspects of the development of other CNS regions. We take the rodent brain as the base model system, because many principal hallmarks of brain development are evolutionarily conserved between rodents and other mammals, including hominids. Key differences exist, however, and they are described where appropriate. We also highlight some areas of intense current research and mention ideas that could contribute to our understanding of CNS development and function.

Keywords

Apicobasal polarity · Apical progenitors · Apical radial glia · Asymmetric cell division · Basal progenitors · Basal radial glia · Brain development · Brain evolution · Cell cycle regulation · Cell division · Cell lineage · Cerebral cortex · Cleavage furrow ingression · Differentiation · Embryonic development · Interkinetic nuclear migration · Neocortex · Neural development · Neural plate · Neural progenitors · Neural stem cells · Neural tube · Neuroectoderm · Neuroepithelium · Neuroepithelial cells · Neurogenesis · Outer radial glia · Primary cilium · Proliferation · Radial glia · Self-renewal · Spindle orientation · Stem cells · Telencephalon · Transcription factors.

Abbreviations

APs	apical progenitors
aRG	apical radial glia
bIPs	basal intermediate progenitors
BPs	basal progenitors
bRG	basal radial glia
CNS	central nervous system
CSF	cerebrospinal fluid
FGFs	fibroblast growth factors
INM	interkinetic nuclear migration
ISVZ and OSVZ	inner and outer SVZ
NECs	neuroepithelial cells
NSCs	neural stem cells
Shh	Sonic hedgehog
SVZ	subventricular zone
TFs	transcription factors
VZ	ventricular zone

Introduction

Neural stem cells (NSCs) in the developing neuroepithelium, as well as the progenitors they generate, eventually give rise to all the neurons of the mammalian central nervous system (CNS). In addition, they generate other essential neural cells, mainly the two types of macroglial cells in the CNS, astrocytes and oligodendrocytes. This chapter focuses on the cellular and molecular aspects of neural stem cell biology during CNS embryonic neurogenesis, the process through which these initially multipotent cells undergo fate restriction steps that will eventually lead to all the neuronal subtypes. We describe neurogenesis mainly in the developing cerebral neocortex, although the principles highlighted here describe also many aspects of the development of other CNS regions. We take the rodent brain as the base model system, because many principal hallmarks of brain development are evolutionarily conserved between rodents and other mammals, including hominids. Key differences exist, however, and they are described where appropriate. We also highlight some areas of intense current research and mention ideas that could contribute to our understanding of CNS development and function.

After defining and introducing general features of NSCs, we trace the developmental origin of NSCs, from the establishment of the neuroectoderm to the formation of the different brain segments, such as the forebrain and the telencephalon. We then explore cellular and molecular aspects that impact the ability of NSCs to multiply and differentiate during the proliferation and neurogenic periods and which contribute to shaping the architecture of the cortex. These features are the general cell structure and apicobasal polarity, nuclear migration and cell cycle

control, cleavage plane orientation, signaling, and gene expression. Finally, we introduce the developmental origin of adult NSCs and the features they share with their embryonic progenitors. Adult neurogenesis is covered in detail in a following chapter.

Definition and General Features of Neural Stem Cells

In this chapter, the term "neural stem cell" refers to precursor cells that are present at different developmental stages and fulfill two criteria:

1. ***Proliferation*** (i.e., stem cell duplication) ***or self-renewal*** (i.e., maintenance of one daughter cell as a stem cell), for a high or unlimited number of cell divisions.
2. ***Multipotency***, the ability to give rise, directly or indirectly, to various types of differentiated neural cells, such as the different types of neurons and glial cells.

Nevertheless, in the developing cerebral cortex, NSCs and other progenitor cells derived from them exist as part of mixed and dynamic populations. Each subpopulation may then have variable and changing degrees of proliferation, self-renewal, and multipotency and may thus generate all, some, or just one type of differentiated cells. Present evidence suggests that most, if not all, such subtypes of precursor cells may exist. Their comprehensive identification and precise characterization is ongoing and will require extensive research.

Neural Stem Cells Generate All Neural Cells in a Temporally Controlled Manner

The first NSCs appear very early in embryonic development, with the formation of the neuroectoderm and the neural plate. The differentiation potential of NSCs then progressively narrows as development proceeds, limiting the kinds of cells that they can originate. A good example is found in the telencephalon (Fig. 1). There, an initial population of multipotent neuroepithelial cells (NECs) proliferates before neurogenesis and then, when neurogenesis begins, gives rise to different progenitor cell types that in turn originate all the differentiated cells that will form the adult cerebral cortex. It has also been suggested that levels of fate restriction may already exist in some progenitors before neurogenesis. Most of neurogenesis occurs during embryonic development, and only small populations of NSCs remain in specific niche locations of the adult body.

The regulation of the duration of the embryonic neurogenesis period is emerging as a key mechanism for neocortex evolution and may be sufficient to determine the final neocortex size in different mammals. For example, similar proliferation and neurogenesis rates, but maintained during longer neurogenic periods, can explain the larger neocortex sizes in great apes, including humans, compared to those of monkeys. The maintenance of these rates for different durations could be achieved

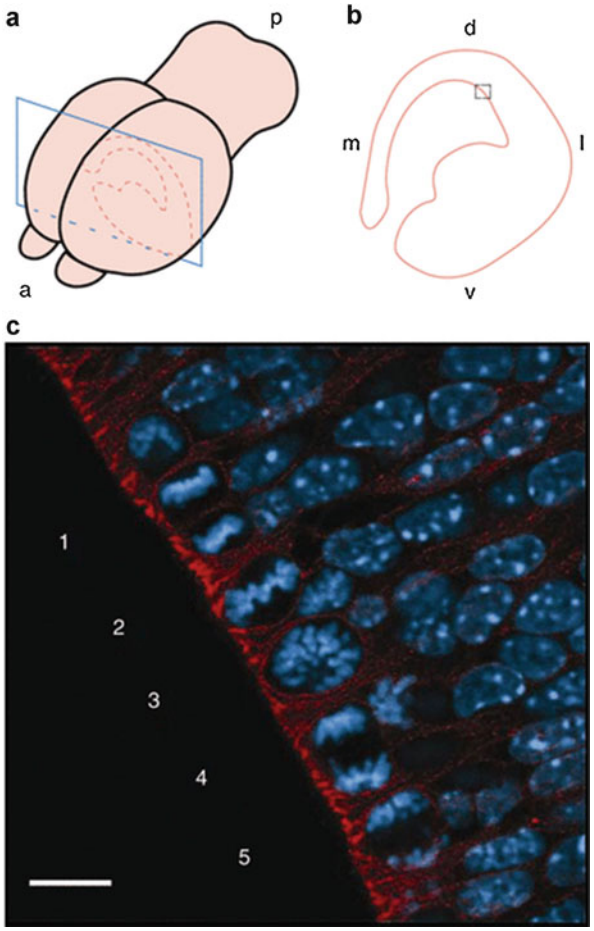

Fig. 1 The apical region of the ventricular zone (VZ) in the dorsal telencephalon. (**a**) Schematic representation of an embryonic day 14.5 (E14.5, mid-neurogenesis) mouse brain showing a coronal section (*blue rectangle*) through the medial part of the telencephalon; *a* anterior, *p* posterior. (**b**) Schematic representation of the tissue section indicated by the *pink dashed line* in **a**; *m* medial, *l* lateral, *d* dorsal, *v* ventral. The *black square* indicates the apical region of the VZ shown in **c**. (**c**) Fluorescence image of the apical region of the VZ, where apical progenitors (APs) divide. *Red*, immunohistofluorescence for cadherins, which are concentrated at the adherens junctions of apical domains; *blue*, DAPI staining of DNA showing the chromatin in the nuclei and the mitotic chromosomes of mostly apical radial glia (aRG), the most common type of AP at this developmental stage. Note the mitotic cells and their chromosomes next to the ventricular surface in the following phases: 1, anaphase; 2, metaphase; 3, prometaphase; 4, anaphase; 5, anaphase. Scale bar: 10 μm

by species differences in cell and molecular characteristics of neocortical NSCs and progenitors that are discussed in the following sections, for example, epithelial features and gene regulatory and metabolic mechanisms required to maintain proliferation.

The diverse cell types that form the mature cerebral cortex are produced following a specific order: neurogenesis begins first and is later followed by gliogenesis. The neurons in the neocortex are contained in six distinct neuronal layers, which are generated in an inside-first, outside-last order. The first generated neurons establish the preplate, followed by cortical plate neurons that arise in a layer-specific temporal order: early-born neurons form the deep layers, while late-born ones migrate past them to establish the superficial or upper layers.

Embryonic Origin and Early Development of the Nervous System

All cells in the vertebrate nervous system are derived from the ectoderm, the outermost of the three cell layers that compose the developing embryo after gastrulation. A portion of the ectoderm differentiates into the neuroectoderm and thickens to form the so-called neural plate, a defined patch of columnar epithelium where neuroepithelial cells begin the neural differentiation program. The neural plate undergoes a folding process called neurulation, in response to extracellular signals from the notochord. This generates a tubular structure, called the neural tube, along the anteroposterior axis of the embryo. The entire CNS arises from the neural tube. During neurulation, the neural plate narrows and folds on itself, forming a longitudinal inward groove, while its edges, called neural folds, elevate and fuse to create the neural tube. An independent progenitor cell population, the neural crest cells, derives from the neural folds and becomes interspersed in the surrounding tissue. They are the progenitors of the peripheral nervous system. The entire neural tube is composed of neuroepithelial tissue lining a fluid-filled inner space called the central cavity. Such a spatial disposition has important implications for tissue architecture, signaling, and polarity, which are discussed below (Fig. 2).

The caudal region of the neural tube gives rise to the spinal cord, and the rostral region gives rise to the brain. Varying rates of proliferation along this axis allow for the differential lateral expansion of certain regions. Early in development, the rostral portion of the tube divides into three brain vesicles: the hindbrain or rhombencephalon, the midbrain or mesencephalon, and the forebrain or prosencephalon. The complexity of the embryonic brain increases as development proceeds, going through a five-vesicle stage in which further subdivisions appear. The hindbrain is divided into the metencephalon, which forms the pons and the cerebellum, and the myelencephalon, from which the medulla arises. The midbrain remains as one vesicle but later gives rise to the tectum and the cerebral peduncles. The forebrain is subdivided into the diencephalon and the telencephalon. The diencephalon gives rise to the thalamus, the hypothalamus, and the retina. The telencephalon (Fig. 1) generates the basal ganglia, the hippocampus, the amygdala, the olfactory bulbs, and the cerebral cortex. The dorsolateral part of the telencephalon gives rise to the neocortex, which is exclusively found in

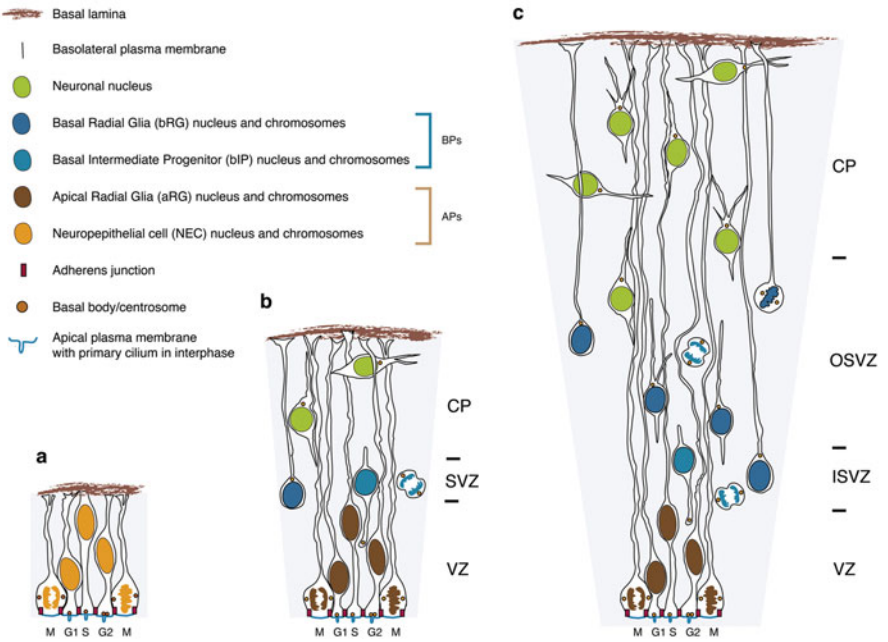

Fig. 2 General structure and major cell types of the developing neocortex. (**a**) Before neurogenesis, the AP population is composed of neuroepithelial cells (NECs) that are attached to the apical surface and contact the basal lamina of the neuroepithelium. APs are connected to each other via the adherens junctions that surround each apical plasma membrane. This forms the apical domains that collectively constitute the ventricular surface. In interphase, APs also have a primary cilium in the apical domain, which is disassembled for mitosis. During interkinetic nuclear migration (INM), AP cell divisions occur apically, after which the nuclei migrate basally during G1, undergo S phase near the basal lamina, and migrate apically during G2 to divide again. (**b**) During neurogenesis, the NECs turn into apical radial glia (aRG) while the tissue expands basally. The nuclei of APs continue to perform INM but are mostly restricted to the VZ. Basal progenitors (BPs), derived from the APs, accumulate in the SVZ. In rodents, most of these are basal intermediate progenitors (bIPs), which have lost both their apical and basal contacts. They typically do not self-renew and undergo a terminal division to give rise to two neurons. Also present, but much less abundant, are basal radial glia (bRG), which have more self-renewal capacity. Neurons produced by all these progenitors migrate basally to the cortical plate (CP). Note that the other layers basal to the SVZ, including the six neuronal layers characteristic of the mammalian cerebral cortex, are not depicted in detail. (**c**) In larger brains, such as those of primates, the cortical wall expands further basally and in a more conical pattern (shaded area). The SVZ can also be subdivided into an inner SVZ (ISVZ) and outer SVZ (OSVZ), which contain more BPs than in rodents

mammals and is the last main type of cerebral tissue that appeared during mammalian evolution. The neocortex is centrally involved in higher cognitive abilities, such as abstract thought and language in humans, and is the main focus of the next sections.

The Cell Biology of Neural Stem and Progenitor Cells

Cell Division Modes Impact Proliferation vs. Neurogenesis

From the early development of the cerebral cortex, the mode of cell division, and specifically its degree of symmetry, is one of the basic mechanisms that can determine the subsequent developmental paths of the NSC progeny (Fig. 3).

Symmetric cell divisions generate two daughter cells with a similar fate. These divisions can be further classified as ***symmetric proliferative***, which generate two daughter NSCs (Fig. 3a), and ***symmetric neurogenic***, where both daughter cells become neurons. Newborn neurons will not divide further and are characterized as postmitotic (Fig. 3d, e). In the case of symmetric neurogenic divisions, the mother progenitor cell cannot be classified a posteriori as a stem cell anymore, since no self-renewal occurred, and is classified as a terminal or non-stem cell progenitor.

Asymmetric divisions generate two daughter cells with a different fate. ***Asymmetric self-renewing*** divisions generate one daughter cell with a similar stem cell fate as the mother cell and the second cell with a different fate. The non-stem cell daughter may be either a non-stem cell progenitor or a neuron (Fig. 3b, c). During neurogenesis, cell divisions can also be ***asymmetric neurogenic***, with one daughter becoming a neurogenic, non-stem cell progenitor and the other becoming a neuron. In these divisions, again no self-renewal occurs.

Cell Division Modes Change During Development

During the early stages of development, prior to but also during early neurogenesis, NSCs mostly undergo symmetric proliferative divisions. During mid- and later stages of neurogenesis, NSCs progressively undergo more asymmetric self-renewing divisions. Asymmetric neurogenic divisions also become more abundant. The non-stem cell progenitors derived from them typically undergo symmetric neurogenic divisions. Finally, during the last stage of neurogenesis, most of the remaining neuronal progenitors have lost their stem cell-like properties and eventually produce two neurons, for example, by undergoing a terminal, or consumptive, symmetric neurogenic division (Fig. 3d). Some of these division types were first deduced from lineage-tracing experiments, where cells were marked by retroviruses that specifically labeled dividing cells with a cellular tag. With the development of better microscopy and tissue culturing techniques, these observations were confirmed and expanded by time-lapse observations of living organotypic brain slices, where cells were followed using green fluorescent protein and other markers.

Main Characteristics of Neural Stem Cells in the Developing Neocortex

During early development, NSCs reside in the neuroepithelium, a highly specialized epithelium that lines the lumen of the lateral ventricle. The structure and properties of the neuroepithelium, from which the developing cortical wall and eventually the

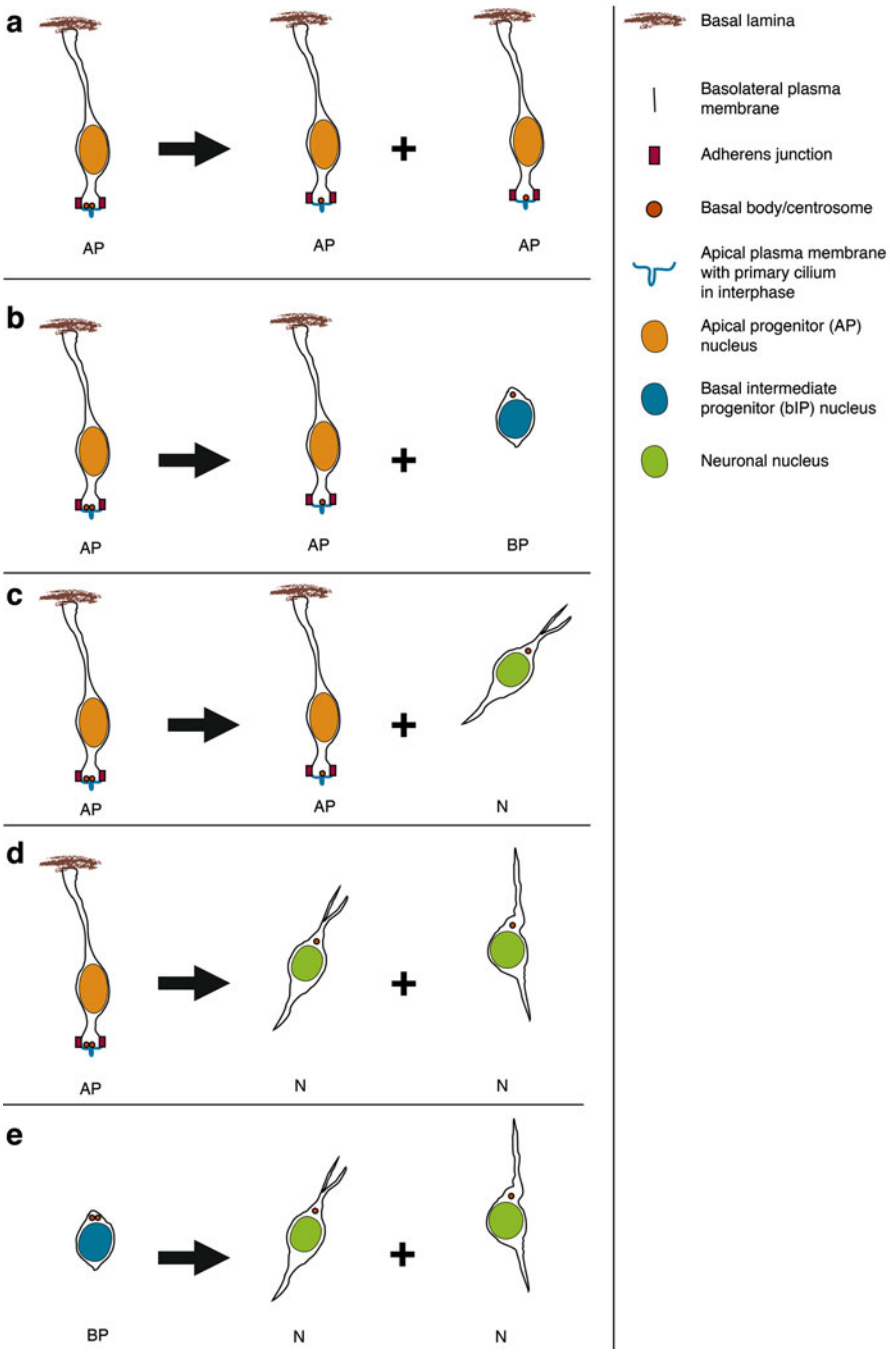

Fig. 3 Main types of neural stem cell (NSC) and progenitor cell divisions resulting in proliferation, self-renewal, and the production of differentiated progeny. (**a**) Symmetric proliferative divisions generate two daughter stem cells with a similar fate as the mother cell. Thus, in the case of apical

mature cerebral cortex arise, are largely determined by the neural stem and progenitor cells it contains, by their cellular and molecular properties and their supracellular organization. We now describe the main types of NSCs and other progenitors in the dorsolateral telencephalon (Fig. 1b). We then discuss each of their main cell biological hallmarks and how they help shape the developing neocortex.

The Main Types of Neocortical Stem and Progenitor Cells

There are two primary types of neural progenitor cells that have stem cell-like properties: the **neuroepithelial cells (NECs)** and the **radial glial (RG) cells**. These two types of progenitors are closely related in that the entire NEC population progressively turns into **apical RG (aRG)** during early neurogenesis. Throughout this transition, NECs maintain most of their general architecture but progressively express astroglial proteins, such as the astrocyte-specific glutamate transporter (GLAST), the brain lipid-binding protein (BLBP), and the intermediate filament glial fibrillary acidic protein (GFAP). Also, glycogen storage granules appear in the basal process, and contacts are made with the endothelial cells of the nascent vasculature. These contacts are similar to those made later by differentiated glia, such as astrocytes. As will be discussed in the following section, both NECs and the aRG keep their nuclei in the apical-most layer of the developing neocortex, they directly contact the ventricle with an apical attachment, and their mitosis occurs very close or directly at this apical domain. Therefore, they are collectively referred to as **apical progenitors (APs**, Fig. 2a–c). A population of recently described non-stem cell APs, called apical intermediate progenitors, have only an apical attachment and undergo one terminal division.

In addition to APs, another main category of progenitor cells exists in the developing CNS, called **basal progenitors (BPs)**. BPs originate from APs and delaminate from the apical surface. They do so either acutely, during an asymmetric division, when the cleavage furrow bypasses the AP apical domain and one of the daughter cells therefore loses the apical attachment (see “Neural Stem Cell

Fig. 3 (continued) progenitors (APs), they typically generate two additional APs. (**b**) Asymmetric self-renewing divisions (type I) generate one daughter cell with a similar stem cell fate as the progenitor mother, e.g., an AP or a basal radial glia (bRG, not shown), and a second cell with a different progenitor fate. During rodent neocortical neurogenesis, the majority of these divisions generate an AP and a non-stem cell basal intermediate progenitor (bIP). In primates and other large-brain mammals, these divisions can also generate a self-renewing bRG and a bIP. (**c**) Asymmetric self-renewing divisions (type II) generate an AP, or bRG, and a neuron (N) directly. For (c) and (d), note that direct neuron generation from APs is considered rare. When neurogenesis advances, asymmetric neurogenic divisions (not shown) also occur, with one daughter becoming a neurogenic non-stem cell progenitor, such as a bIP, and the other becoming a neuron. (**d**) Symmetric neurogenic divisions (terminal type I) may also occur in advanced stages of neurogenesis, with a non-stem cell AP or bRG producing two daughter cells that become neurons. (**e**) Symmetric neurogenic divisions (terminal type II) are the main division type of bIPs and produce two neurons

Divisions and Contact Inheritance" below), or independently of division, by a progressive downregulation of the apical attachment components, like adherens junctions. Delamination is therefore a major step of apical-basal polarity loss for BPs and is thought to contribute to a reduction in stem cell properties. The main types of BPs are **basal radial glia (bRG**, also called outer radial glia) and **basal intermediate progenitors (bIPs)**. Despite losing their apical attachment and dividing basally, most bRG maintain a level of polarized apicobasal structure during mitosis that can include an apically directed process (that however does not reach the apical surface) and/or a basal process that often contacts the basal lamina. bRG in primates and other relatively large-brained mammals, but less so in rodents, can self-renew and/or have proliferative capacity (Fig. 2c). bIPs lose both the apical and basal processes and have therefore no clear apicobasal polarity (Fig. 2b, c). In rodents, bIPs have typically lost the ability to proliferate or self-renew and are considered non-stem cell progenitors that undergo one terminal division to produce two neurons. In primates and other large-brained mammals, some bIPs can exhibit proliferative or self-renewal potential. Both bIPs and bRG have been identified in the developing neocortex of all mammalian species studied to date, and bRG show a higher abundance in species with relatively large and gyrencephalic brains, such as primates.

Changes in the relative and total abundance of the different APs and BPs can influence the development and size of the neocortex across species and are likely to have played crucial roles in the evolution of mammalian brain structure and function. It is also likely that these main types of progenitors encompass or give rise to more subtypes. A comprehensive characterization of all cortical stem and progenitor cells remains the focus of intense research.

The Epithelial Nature of the Primary Neural Stem Cells

Apical Progenitors Are Elongated and Highly Polarized

Before neurogenesis starts, the neuroepithelium is formed by a single layer of NECs arranged side by side (Fig. 2a). The expansion of the early neuroepithelium is mostly lateral and occurs by symmetric proliferative divisions of the NECs. These cells typically reach both the apical and the basal sides of the neuroepithelium, with the nuclei located along the apicobasal axis. This is possible because NECs are highly elongated and polarized along this axis and continue to elongate during development to keep their apical and basal contacts as the tissue grows radially. Their cell body is usually widest where the nucleus is located, with a diameter of around 5–10 μm.

The long, tube-like extensions that reach the apical and basal sides, called processes, are usually thinner than the nucleus, at around 1 μm or less. This is in contrast to the length of these processes, which can grow hundreds of μm during development. Each nucleus has therefore the appearance of a "bead on a string." These polarized extensions and contacts with the apical and basal sides have been

widely implicated in AP fate and function and are discussed below. With the cells tightly arranged side by side, the distribution of the nuclei in different positions along the apical-basal axis may give, at first sight, the impression of the tissue being stratified, i.e., composed of different layers. However, since there is actually only one layer of NECs, this early non-neurogenic neuroepithelium is referred to as being pseudostratified (Fig. 2a).

Different Zones Arise from the Neuroepithelium During Neurogenesis

When the neural tube has closed and neurogenesis starts, a true stratification begins, and additional zones grow on the basal side while the tissue expands radially. In this period, the neuroepithelium expands to form the developing cortical wall. The apical processes and cell bodies of the NECs now constitute the layer facing the ventricle, referred to as the **ventricular zone (VZ)** (Fig. 2a).

The RG cells that arise from the NECs downregulate some epithelial features, like tight junctions, but maintain the general polarized and elongated architecture of their progenitors, including the apical and basal processes and contacts (Fig. 2b). The further elongation of these processes constitutes part of the growth of the tissue, together with the expansion and diversification of the progenitor populations, tissue vascularization, and neuron production, growth, and migration from neighboring regions. On a cellular level, radial tissue expansion is thought to mainly follow the "radial unit hypothesis." Radial units are composed of the progeny of single APs, which tend to migrate radially along the apical-basal axis, following the basal processes of adjacent APs. In this manner, bRG, bIPs, neurons, and other cells derived from an AP accumulate in the newly forming basal zones. This expansion follows a conical pattern of growth, with the tip of the cone located apically and the cone base broadening basally, which is also thought to contribute to the formation of gyri and sulci in gyrencephalic species.

The next area that arises basally to the VZ is the **subventricular zone (SVZ)** (Fig. 2b). The SVZ is mostly formed by the accumulation and divisions of delaminated BPs, but it is also supported by the growing basal processes of APs. In many organisms with longer neurogenesis periods and development of larger brains, such as primates, the growing SVZ becomes subdivided into an **inner SVZ (ISVZ) and an outer SVZ (OSVZ)** (Fig. 2c) that are separated by an inner fiber layer composed of processes from the neurons migrating tangentially from ventral parts of the cortex. BPs accumulate in these subzones and underlie the increase in the number of neurons that sustains the strong expansion of the neocortex in for example hominids. The neurons being born from these progenitors migrate further radially and accumulate beyond an outer fiber layer, at the basal-most side of the developing cortical wall and form the neuron-containing layers of the cerebral cortex (Fig. 2b, c). At the end of development, six such layers are established in the cerebral cortex, each of which contains different populations of neurons.

The Apical Components of Neural Stem Cells

On the apical pole of APs, the end of the process directly contacts the ventricle by forming a stable apical attachment with neighboring APs. This end foot is called the apical domain. It accounts for only a minute fraction of the total cell membrane and is composed of a core of apical plasma membrane delimited by a ring of adherens junctions. Apical domains are flanked on all sides by the apical domains of other APs, and they are linked to each other via the adherens junctions. Collectively, the joint apical domains form the ventricular surface that faces the lumen (Figs. 1c and 2). The apical domain is therefore the contact zone of the APs with the cerebrospinal fluid (CSF) that fills the ventricle. This fluid has been shown to play important nutritional and signaling roles in neurogenesis.

The Apical Plasma Membrane

The fate of APs is thought to be influenced by extracellular signals, some of which are present in the ventricle. Transmembrane proteins that are enriched in the apical membrane may thus take part in such signaling processes. An example is prominin-1, which interacts with cholesterol, is present in the protrusions of the apical membrane, and is considered a general marker of somatic stem cells. Megalin, a lipoprotein receptor, is another example. It may be involved in transducing signals of cholesterol-bearing morphogens, such as Sonic hedgehog (Shh). Snap receptor (SNARE) distribution may also be different between the apical and basolateral plasma membranes, and the SNARE vesicle fusion machinery has been implicated in AP fate determination through the localization of apical cortex proteins, such as atypical protein kinase C (aPKC), and adherens junctions components.

The Adherens Junctions and Apical Cell Cortex

The adherens junctions and their components, such as cadherins (Fig. 1c) and catenins, have been broadly implicated in the polarity and fate of APs. These junctions help to keep the apical domains of APs together and maintain the integrity of the neuroepithelium (Figs. 1c and 2). Cadherins, which are transmembrane proteins, interact with the cadherins of neighboring APs and establish a junctional contact between them. This contact is supported by other proteins, such as catenins, that are located on the intracellular side and link the cytoplasmic domains of cadherins to the F-actin cytoskeleton. Interestingly, beta-catenin is also involved in the Wnt signaling pathway and promotes the proliferation of APs. Other proteins linked with apical-basal polarity and fate determination in APs are also associated with adherens junctions and the cell cortex of the apical domain. One example is the Par complex, containing Par3, Par6, and aPKC, which plays important roles in maintaining cell polarity and proliferation by favoring Notch signaling. In addition to adherens junctions, gap junctions

also participate in cellular communication via the alignment of connexin hemichannels between neighboring cells. Small molecules and ions, such as calcium, are shared through these channels to regulate nuclear and cell movements.

The Primary Cilium and Centrosomes

The intracellular side of the apical domain is also the place where the primary cilium forms (Fig. 2). This leaves the cilium in an optimal location to receive signals coming from the CSF. The root of the cilium is the basal body, which nucleates the cilium shaft, called the axoneme. The growth of the microtubules in the axoneme pushes the apical plasma membrane that surrounds the cilium to protrude into the ventricle, where it becomes essentially immersed in CSF. This positions the cilium as an "antenna" for signals present in the CSF. Several signaling pathways, including Shh and Wnt, have been linked to primary cilium function. Shh signaling participates, via the cilium, in progenitor expansion in the adult dentate gyrus of the hippocampus, and a similar function during development is plausible.

The only phase of the cell cycle when cells do not have a cilium is during M phase (Figs. 2 and 4). This is because the cilium and the mitotic spindle share a basic component: the centrioles. During interphase, the centriole that constitutes the basal body duplicates, as does its associated daughter centriole. Then, at the transition between G2 and M phase, the primary cilium of APs is disassembled. The centrosomes, each of which contains a pair of centrioles, can then interact with each other to form a bipolar mitotic spindle that congresses and then segregates the chromosomes. Interestingly, the inheritance of either the mother or daughter centriole-containing centrosome may influence progenitor cell fate during asymmetric cell divisions. The centrosome containing the mother centriole seems to be typically inherited by the more self-renewing daughter, whereas the centrosome containing the daughter centriole preferentially goes to the more neurogenic daughter.

The Basolateral and Basal Components of Neural Stem Cells

The Basolateral Membrane and Radial Processes

The plasma membrane that is basal to the adherens junction belt, which delimits the small apical domain of APs, is called the basolateral membrane. This membrane always surrounds the nucleus and extends beyond it. The segment of AP basolateral membrane that stays within the VZ forms the **apical process**. Therefore, the apical process is unique in that it contains both an apical segment, the apical domain, and a basolateral segment, the rest of the apical process. Recent work has shown that, unlike in all other known cells, the AP Golgi apparatus is not pericentrosomal during interphase. Instead, it is mostly distributed along the apical process. This could be important for the biogenesis of the basolateral membrane of the apical process and for the polarized structure of APs. The segment of basolateral membrane that goes

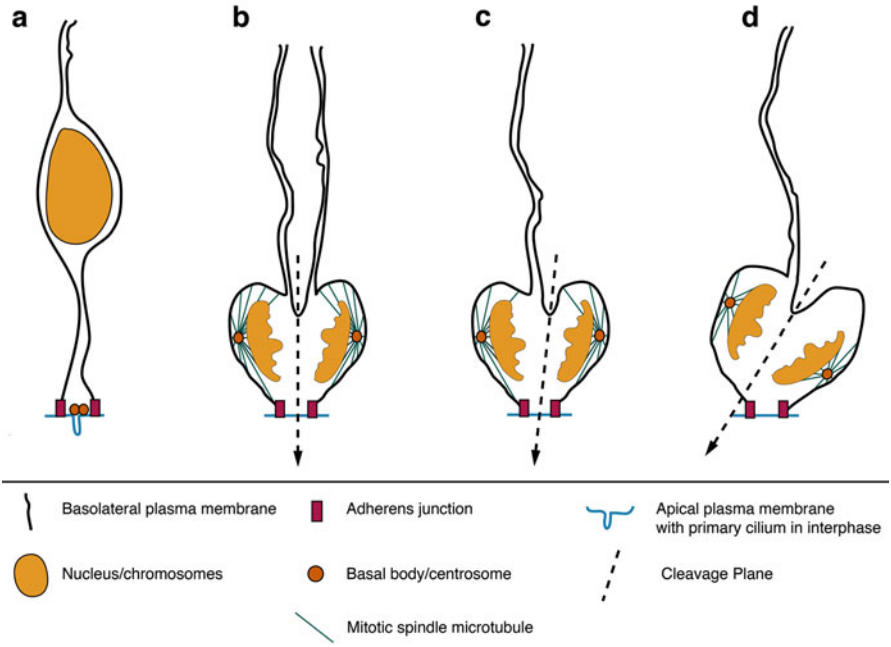

Fig. 4 Major cleavage modes during the divisions of neuroepithelial cells (NECs), the primary neural stem cells (NSCs) of the mammalian brain. These modes generally also apply to apical radial glia (aRG), the other main type of apical progenitor (APs), which derive from NECs, although (b) may be specific to some NECs. (**a**) APs contact the ventricle via their apical domain, composed of adherens junctions surrounding the apical membrane. During interphase, APs carry a primary cilium in their apical domain, nucleated by the basal body, which protrudes into the ventricle, where it is surrounded by CSF. During mitosis (**b**, **c**, **d**), the primary cilium has been disassembled to allow the centrosomes to become the poles of the mitotic spindle. During AP cell divisions, the cleavage furrow typically ingresses in a basal-to-apical "vertical" direction. (**b**) Before and during early neurogenesis, NECs can undergo symmetric divisions that bisect both the basal process and the apical domain. Both daughter cells therefore immediately inherit an apical and a basal contact. (**c**) In APs where the basal process is not bisected, asymmetric divisions that distribute it to only one daughter cell can nevertheless distribute the apical domain symmetrically, and both daughter cells therefore inherit apical plasma membrane and junctions. (**d**) Highly asymmetric divisions, with respect to both the basal contact and the apical domain can occur with a range of orientations, from slightly oblique to fully horizontal (not shown). Only one of the daughter cells will keep the contact to the ventricle after mitosis (some of these cells may regrow a basal process and maintain an AP identity, while others may delaminate later and become a BP). The other daughter cell, which is acutely delaminated by the cleavage furrow, can immediately become a BP if the apical contact is not re-established. These divisions usually result from strong tilting of the spindle leading to the tilted ingression of the cleavage furrow, which can be due to fewer astral microtubules anchoring the spindle to the cell cortex

beyond the VZ forms a **basal process** that often reaches the basal end of the developing cortical wall and contacts the basal lamina.

The morphological variability of the apical and basal processes has been linked to fate determination in APs but also in BPs that have delaminated from the apical

surface. Progenitors with both apical and basal processes, such as APs, typically show a high proliferation or self-renewing potential. Interestingly, this also tends to hold true for those delaminated BPs that maintain both processes. Furthermore, among those BPs with only a basal process, those with a process that is longer and/or branched out also show more proliferation or self-renewing potential. Consistent with this idea, mammals with relatively large brains, such as primates, have higher proportions of BPs with more complex process morphologies, especially of the basal process. The importance of longer and/or branched basal processes is that they can offer an increased access to nutrients, oxygen, and diverse signals in the basal compartments, e.g., proliferation signals from extracellular matrix components around blood vessels, in the SVZ, and in the layers basal to it.

In contrast to the apical process, which only exhibits one contact to the ventricle, the basal process can often branch out right before reaching its basal end, thereby contacting the basal lamina in several places (Fig. 2). The functional significance of these basal end foot branches remains under investigation. Interesting possibilities include a broader, more efficient and perhaps differentiated communication of progenitors with different segments of the basal and pial compartments. It could also be a way to further compartmentalize local organelles and activities, such as translation of basally transported mRNAs. Branches may also serve as diversified tracks or cues for the final steps of radial migration and positioning of neurons.

The apical process can also have small lateral protrusions, called lamellate expansions. These may further increase the exchange of nutrients and signals with their surroundings. Interestingly, lamellate expansions have also been found in the basal processes of bRG from animals with a large cortex, such as primates, but not in those of rodents, suggesting they may play a role in the bRG self-renewal and/or proliferation required for a large cortex. Another morphological feature often found in basal processes is the presence of discrete broadenings in the diameter of the process. These so-called varicosities are more frequently seen in mitotic cells and may be caused by an irregular flow of cytoplasm toward the nucleus. This may be influenced by uneven distributions of organelles, cell membrane components, and other molecules along the process. Most varicosities disappear during interphase, concomitant with more cytoplasm flowing into the process. A functional significance for these varicosities has not been established. They may constitute specialized compartments for cellular functions relevant to cell polarity, such as signal transduction, translation, intracellular trafficking, and storage of basal components.

The Basal Lamina and Pial Surface

The basal lamina is composed of a network of extracellular matrix proteins, such as collagen, laminin, and fibronectin. These are secreted by cells on the outer surface of the brain, such as astrocytes and endothelial and pial cells. It covers the neocortex and constitutes a surface where cells can establish basal contacts (Fig. 2). The contact of the basal process to the basal lamina through membrane receptors, such as integrins, has been shown to participate in the maintenance of the AP population. Similarly, the basal

contact that many bRG retain can contribute to their self-renewal by gathering signals present in the surrounding tissue or coming from the more basal tissues. The basal lamina itself is covered by the inner surface of the pia mater, the innermost layer of the meninges. The meninges are a complex network of three layers composed of fibers, blood vessels, and cells that covers and protects the outer surface of the brain and can also serve as a site for basal contacts. The meninges also have signaling functions, for example, those exerted by the neurogenesis-inducing retinoic acid (Fig. 2b, c).

Nuclear Movements and Cell Cycle Progression

The nuclei of APs typically migrate basally and apically during interphase. This process is called interkinetic (or intermitotic) nuclear migration (INM), and it explains the pseudostratification of the early neuroepithelium and, later, of the VZ (Fig. 2). At the time of mitosis, the nucleus has usually migrated to the apical-most side of the cell, so the majority of AP divisions occur with the spindle and the chromosomes located next to the apical domain that lines the ventricle (Fig. 1c). At the end of mitosis and during G1, the daughter nuclei migrate basally again. In short, cell cycle progression in APs is thus linked to INM in the following general way: **M phase** occurs at the apical surface, **G1** is during apical-to-basal migration, **S phase** is in the basal portion of the VZ, and **G2** is during basal-to-apical migration (Fig. 2).

More specifically, before cortical neurogenesis, the G1 nuclei of NECs migrate basally and can end up very close to the basal lamina. During early neurogenesis, NECs become aRG but retain their characteristic architecture and INM. A notable difference, however, is that even though the basal processes of aRG elongate together with the radial thickening of the cortical wall, their nuclei remain inside the VZ and therefore within the apical process (Fig. 2b, c). APs undergo S phase to replicate their chromosomes with their nuclei in a distinctly basal position. When S phase is completed and cells enter G2, the nuclei migrate apically to reach the apical domain, where the next round of cell division takes place. BPs, on the other hand, do not undergo INM, due to the lack of an apical attachment and, consequently, of full apical-basal polarity. Despite not undergoing INM, the nuclei of BPs do migrate but in a mostly basal direction. In this way, most BPs divide in the SVZ, the zone they effectively help to create with their presence (Fig. 2b, c). In some BPs, notably bRG, there is an acceleration of this movement during mitotic onset known as mitotic somal translocation. While a function for this accelerated part of the basal migration remains under investigation, it could help speed up the growth of the basal zones.

The Advantages of an Apical Mitosis for APs

The neocortex is a rapidly expanding tissue with a complex organization that combines pseudostratification and stratification during neurogenesis. During the evolution of its development, mechanisms that increased the efficiency and accuracy of proliferation are likely to have been favored. INM could be one of those mechanisms. The

cell biology of APs suggests that cell divisions close to the apical domain are necessary for efficient proliferation, while other cell cycle stages are best moved basally. This is because the G2 nucleus of APs migrates apically and meets the centrosomes, which remain near the apical domain even after the primary cilium has been disassembled. A mitotic spindle then forms, and APs can divide apically (Fig. 2). This facilitates the symmetric distribution of the apical domain to both daughter cells (see Fig. 4 and the "Neural Stem Cell Divisions and Contact Inheritance" section below).

In order to maximize the number of apical divisions while maintaining tissue organization, cells that have completed mitosis move their nuclei away from the apical surface. This liberates space that can be occupied by the incoming mitotic cell bodies, which are more rounded and wider than in interphase, and which stretch even further parallel to the apical surface during anaphase (Fig. 1c). INM can therefore act as a key cellular mechanism for the expansion of the AP pool, while avoiding overcrowding of interphase nuclei near the ventricle. In this way, it is advantageous for the nucleus to migrate apically to meet the centrosomes, instead of having the centrosomes migrate basally to reach the nucleus. A tight coordination of INM and M phase onset can ensure that the G2-M nucleus arriving at the apical side readily meets the newly assembled centrosomes and quickly proceeds with mitosis. Also in this context, signals that are apically enriched and favor proliferation by acting directly on the cell body, for example, via the primary cilium, could also make it worthwhile for the nucleus to migrate apically and favor keeping this cilium assembled for as long as possible before mitosis. Such a "nuclear residence hypothesis" is also supported by studies in fish. Notch signaling in the fish retina, which prevents progenitor differentiation, is especially active on the apical side of the neuroepithelium and acts during the basal-to-apical phase of INM.

The Advantages of a Basal Mitosis for BPs

The establishment of other proliferative zones in later stages of neurogenesis, such as the SVZ, constitutes strong evidence that even maximized AP proliferation near the apical surface of the VZ is not sufficient to sustain all cortical neurogenesis. Consistent with this idea, BPs have been found in all mammalian species studied to date. The basal migration and division of BPs is likely to play an analogous "decongestion" role in the radial growth of the neocortex to that played by INM for APs in the VZ: dividing BPs spread by migrating basally through the tissue to help reduce crowding while the tissue expands basally. In a similar but hypothetical growth situation with no BPs, all intermediate progenitors and neurons would have to be born near the ventricle, and each newborn neuron would have to migrate all the way to the neuronal layers, likely a slower and less efficient process than the establishment of basal germinal zones. From an evolutionary perspective, species with a relatively large cortex tend to have larger pools of bRG, the BPs that are typically most capable of self-renewal and/or proliferation. Therefore, to favor cerebral cortex expansion, not only the purely neurogenic divisions are shifted basally but also many of the proliferating and self-renewing divisions.

Mechanisms of Interkinetic Nuclear Migration

Microtubule and actin-based movements have been implicated in both the apical-to-basal and basal-to-apical parts of INM. The relative importance of each seems to vary between taxa and between tissues and remains the focus of intensive research. Both mechanisms involve motor proteins that are typically associated with each of those cytoskeletal structures.

Microtubule motor proteins that have been implicated in INM are the dynein-dynactin motor complex and the kinesins. For either motor system, it is thought that the nucleus moves as a huge cargo along microtubule tracks, with the force being provided by motor complexes that link the nuclear envelope to the microtubules. Consistent with the fact that interphase microtubules have their plus end-oriented toward the basal side, plus end-directed kinesins have been implicated in the apical-to-basal migration during G1. Conversely, the minus-end-directed motor dynein has been implicated in basal-to-apical migration during G2. It is possible that the direction of migration may be controlled by cell cycle-dependent switches. These may activate the kinesin-dependent movement during G1 and the dynein-dependent movement during G2. Both mechanisms would then be either turned off or balanced during S phase.

The actin cytoskeleton has also been implicated in both INM directions, and motor complexes involving myosin play major roles. As opposed to microtubules, the actin cytoskeleton does not provide continuous tracks for directional movement of the nucleus. Instead, it is thought that actomyosin contractility could be orchestrated to produce a polarized constriction of the cell cortex along the apical-basal axis. If the constriction happens at the apical side, the nucleus would be pushed basally and vice versa. Such an apical-basal alternation of the direction of the "push by squeezing" could also be coordinated via signals that are specific to the phase of the cell cycle.

Interdependence of Interkinetic Nuclear Migration and Cell Cycle Progression

Even though INM and cell cycle progression accompany each other tightly in APs (Fig. 2a), cell cycle progression in the mammalian neuroepithelium does not necessarily require INM. This was established by showing normal cell cycle progression kinetics upon inhibition of the actin-myosin motor complex during the apical-to-basal part of INM. Interestingly, the converse dependence of INM on cell cycle progression seems to be stronger. This was concluded when drug treatments that arrested cells in S phase also stopped INM. Moreover, when S phase progression was not arrested, but only delayed, a corresponding slowdown effect was observed in INM kinetics, with cells migrating more slowly. Together, these findings place cell cycle progression, the fundamental guideline of general cell proliferation, as the more dominant event in the coordination between the two. However, this interdependence also appears to vary among different regions of the CNS and different taxa, and more research is needed to clarify them.

Cell Cycle Progression and Stem Cell Fate

As in all cells, proteins called cyclins control cell cycle progression in NSCs. To function, cyclins must associate with partner cyclin-dependent kinases (CDKs). Specific cyclin-CDK complexes phosphorylate downstream effectors, regulating the progression of the cell in and out of each cell cycle phase. During neurogenesis, the increase in neuron production is accompanied by a general lengthening of the cell cycle. This is mostly due to a lengthening of the G1 phase. Interestingly, experimental manipulations of the cell cycle, and G1 in particular, have confirmed that a shorter cell cycle is generally linked to proliferation and self-renewal, while a longer cell cycle is generally linked to neurogenesis.

In mid-stages of neurogenesis, however, the total length of the cell cycle is longer in proliferating APs as compared to neurogenic APs. This difference results from proliferating APs having a longer S phase. This suggests that DNA replication is happening more slowly, and perhaps more carefully, to minimize the risk of mutations arising and expanding in the stem cell population. Consistent with this, S phase in the neurogenic, non-stem cell bIPs is also shorter than in proliferating APs. bIPs, however, show a longer total cell cycle, which results mainly from a longer G1. The functional significance of this G1 increase in bIPs remains under investigation but could mean that a longer phase of protein synthesis during G1, or a longer time of exposure to extracellular signals, may be required for the specification of their fate.

Neural Stem Cell Divisions in Proliferation Versus Neurogenesis

Cell Division Machinery and Progression

The cell division machinery common to most somatic cells is also implicated in the NSC division modes that are relevant for cell fate specification. The mitotic spindle and its main components have all been shown to play basic roles in the regulation of symmetric and asymmetric divisions. During **prophase** in APs, the centrioles liberated from the disassembling primary cilium can then assemble the centrosomes and relocate toward the center of the cell soma. During **prometaphase**, the centrosomes nucleate and organize mitotic microtubules to build a bipolar spindle. It is positioned in an equatorial plane of the cell soma by astral microtubules-cell cortex interactions and congresses the chromosomes into a metaphase plate via kinetochore (k-fibers) and other microtubules. During **metaphase**, when all chromosomes are tightly congressed into a plate in a central plane of the cell soma (Figs. 1c and 2), the orientation of the spindle is also established by astral microtubules-cell cortex interactions and can show dynamic variability. The orientation during **anaphase** onset will typically remain as the cleavage orientation. In proliferating APs, this orientation is usually parallel to that of the local apical surface of the tissue, resulting in a cleavage plane perpendicular to it and along the apicobasal axis of the tissue (Figs. 1c and 4). This favors symmetric divisions. As neurogenesis advances, the spindle can experience more orientation variability, which tends to favor an increase

in asymmetric divisions. BPs share most of these fundamental division characteristics, yet they tend to have a more variable spindle and cleavage orientation, due to their reduced (bRG) or missing (bIP) apicobasal polarity. During **telophase**, when chromosomes decondense, and beyond, daughter nuclei and/or cells are usually oriented to continue their radial migration, either as part of INM for APs or basally for BPs.

When bipolar spindle assembly or stability is impaired, for example, by perturbing fundamental centrosome proteins, cell division fails to take place normally, and the NSC pool can become depleted. This results in reduced neurogenesis. Examples of these proteins include pericentrin and those directly involved in causing autosomal recessive primary microcephaly, such as ASPM or CDK5RAP2. The NSC pool is also affected when fundamental microtubule-associated proteins and molecular motors are perturbed, such as cytoplasmic dynein and its binding partners.

Cell Cortex and Cytokinesis

The integrity of the cell cortex and its interplay with microtubules are necessary for normal mitosis and cytokinesis. The beginning of cleavage furrow ingression depends on actomyosin interactions with scaffold and activator proteins, such as anillin and RhoA. Likewise, the orientation of the cleavage plane depends on spindle orientation and interactions of the dynamic actomyosin cortex with populations of astral and mid-zone microtubules. These fundamental requirements have also been shown to influence AP division symmetry. In addition, the perturbation of other proteins that link mitotic microtubules with the cell cortex, such as LGN, NuMA, and their binding partners, also significantly affects spindle and cleavage orientation. Then, cytokinesis onset and progression are governed by spindle orientation and cleavage furrow positioning and ingression. Afterward, the completion of cytokinesis is achieved by the fusion of the plasma membranes at both ends of the cleavage plane. In a symmetric division, the basolateral cleavage furrow membrane that ingresses in the basal-to-apical direction fuses with the apical membrane, establishing a heterophilic membrane fusion. In an asymmetric division, however, the cleavage furrow membrane often fuses with similar basolateral membrane, establishing a more homophilic membrane fusion. It is possible that these membrane differences play a role in the establishment of symmetric versus asymmetric divisions, and they could result from unequal distributions of lipids and membrane proteins, such as SNAREs.

The Distribution of Polarized Components During Neural Stem Cell Division

The high apical-basal polarity of APs has motivated researchers to investigate the role of the symmetric versus asymmetric distribution of polarized cellular components in the determination of NSC fate. As with many other aspects of mammalian

development, those investigations have been inspired by pioneering studies in the fruit fly *Drosophila melanogaster*. *Drosophila* also has polarized NSCs called neuroblasts, whose polarity is mainly due to the asymmetric localization of proteins along the apical-basal axis. For example, the PAR/aPKC complex is enriched, as in mammals, on the apical cell cortex. This apical enrichment is in turn necessary for the differential mitotic localization of cell fate determinants, such as the neurogenic Prospero and Numb at the basolateral side. During a neurogenic mitosis, these components are distributed asymmetrically by a cleavage plane that bisects the neuroblast perpendicularly to its apical-basal axis. This effectively creates one apical daughter that continues to have the neuroblast stem cell-like identity and one basal daughter, called a ganglion mother cell, which undergoes a further division, either neurogenic or gliogenic.

Some of those general features are evolutionarily conserved in mammalian neurogenesis, where the inheritance of polarized components is also ultimately determined by the orientation of the cleavage furrow and cytokinesis. Nevertheless, key differences set it apart from the canonical *Drosophila* system. In mammals, the orientation of the AP mitotic spindle in metaphase remains largely parallel to the plane of the apical surface. This implies that the plane of cleavage furrow ingression is typically oriented perpendicular to the apical surface and along the apical-basal axis (Figs. 1c and 4b, c). Deviations from this cleavage orientation can nevertheless increase during neurogenesis (Fig. 4d) and may sometimes reach the 90° rotation observed in *Drosophila*.

In addition, *Drosophila* neuroblasts typically lose their apical contact early on and do not exhibit the long basal processes and contact with the basal lamina that persist through several generations of mammalian APs. These and other structural differences may be at the heart of the regulatory differences between these systems and could also help explain the bigger size and higher complexity observed in the mammalian brain.

Neural Stem Cell Divisions and Contact Inheritance

Both the apical and basal contact have been implicated in the proliferative capacity of APs. Cells that maintain both of these contacts, such as most APs, or at least one contact, such as many bRG, generally have a higher proliferation and/or self-renewal potential compared to cells with neither contact, such as neurogenic bIPs. When mammalian APs undergo highly symmetric proliferative divisions, they are typically cleaved along the apical-basal axis of the cell, and the apical domain is bisected. Therefore, both daughter cells inherit an apical domain. In some cases, proliferative APs that have momentarily lost the apical contact during a division that bypassed it can re-establish it soon afterward. The basal process is kept during mitosis, and its bisection is a more difficult task, given its long and narrow nature. However, some APs can bisect it. These are mostly NECs that divide before and during early neurogenesis, ensuring that both daughter cells immediately inherit a basal contact (Fig. 4b).

This immediate inheritance of the entire apical-basal architecture, including both contacts and the adherens junctions, is important to maintain the neuroepithelial structure and thus favor an efficient and orderly proliferation in the early stages of CNS development. It could also help explain why, unlike in most other cell types, the AP cleavage furrow ingresses unidirectionally and exclusively in a "vertical," basal-to-apical direction (Fig. 4), rather than the "lateral" direction seen in *Drosophila*. A bidirectional cleavage furrow along the highly elongated apicobasal processes of such cells would be more difficult to coordinate and achieve, given the presence of the apical domain. During advanced stages of neurogenesis, the increasingly longer basal process is unlikely to be bisected, but a process regrowth to regain basal lamina attachment is possible. It has been shown that such a regrowth, in APs left with only an apical attachment after mitosis, involves Notch signaling.

The increase in cleavage plane variability in mid- and late stages of neurogenesis increases the number of divisions that do not bisect, but rather bypass, the apical domain (Fig. 4d). These highly asymmetric divisions typically leave one cell that inherits only the apical contact and is more likely to delaminate and differentiate and another cell that inherits the basal contact, which is more likely to self-renew, albeit probably for a limited number of rounds.

Spindle Structure and Orientation in NSC Fate Determination

A generalized causal effect of spindle and cleavage orientation in NSC fate determination has long been suspected but difficult to establish. The results of experimentally perturbing spindle orientation seem to heavily depend on which spindle or spindle-related factors are targeted. For example, higher cleavage plane variability by perturbing LGN in mice increases the number of bRG-like cells, but this shows little effect on rodent neurogenesis. In mammals with larger brains, a higher cleavage plane variability has also been associated with an increased production of bRG and also with neocortex expansion. On the other hand, while perturbing either Lis1 or the Lfc-mediated cell cortical regulation of RhoA is also associated with higher spindle variability, this results in heavily reduced neurogenesis. Perturbation of centrosome-associated proteins involved in microcephaly also leads to spindle misorientation and neurogenesis reduction, likely due to premature depletion of the AP pool. This suggests that the different gene and protein perturbations used in those studies not only change spindle orientation but might also have additional effects that may explain the different outcomes. To overcome these limitations, recent efforts modified spindle orientation by targeting astral microtubules, which are fundamental components of spindle structure rather than associated factors. Reducing the number of specific astral microtubules associated to the apical and basal poles of the cell soma is sufficient to change NSC fate and increase neurogenesis. These astral microtubules likely regulate spindle and cleavage orientation variability by acting as dynamic "anchors" between the spindle and the cell cortex.

Taken together, these findings are consistent with the notion that epithelial features are generally important to favor the proliferation and self-renewal capacity

of NSCs. This is also supported by studies showing loss of proliferation and cortical tissue architecture when cell polarity and junctions are acutely perturbed. In addition, the importance of the apical attachment for long-term stemness is underscored by its persistence in those NSCs that remain throughout embryonic neurogenesis and can later be found in the SVZ of the adult brain as adult neural stem cells. The specific contributions of each cellular and tissue component, and their interplays, remain under intense scrutiny.

Molecular Mechanisms of Neural Stem Cell Maintenance and Differentiation

The Fate of NSCs Is Influenced by a Diversity of Molecular Factors

The cell types derived from NSC divisions throughout development depend greatly on when and where they are generated. This is because each distinct location of the developing nervous system is under the influence of different extracellular signals. These signals are active in defined patterns and gradients along the anteroposterior, dorsoventral, and mediolateral axes. The complex and dynamic interactions among them establish different spatial domains, which confer a specific positional identity to the progenitor cells present in them. This positional information interacts with the intrinsic factors in each cell, leading to the commitment of the progenitor cells and thus limiting the kinds of daughter cells they can generate. In the context of the developing cortex, this process leads to the subdivision of the cortical area into what is known as a "protomap," in which the lineages of neurons that will be formed are to some extent already pre-established.

Signaling in Neural Stem Cells

Apart from the most thoroughly studied signaling systems, each of which is briefly discussed below, many other factors influence the fate of NSCs. Some notable examples are:

1. Neurotrophins, growth factors that influence cell survival and proliferation, mostly in the peripheral nervous system.
2. Reelin, an extracellular matrix glycoprotein that regulates progenitor cell differentiation and neuronal migration.
3. Retinoic acid, a metabolite of vitamin A that contributes to anteroposterior patterning and promotes neurogenesis.
4. The cerebrospinal fluid, which has a complex and dynamic signaling protein composition.
5. The extracellular matrix composition, which has effects on cell proliferation and survival through cell-matrix contacts via adhesion molecules.

6. The vascular environment, both through the signaling molecules transported in the blood and the particular niche established by the basal lamina surrounding the endothelial cells.
7. The input from other cells within the nervous system, such as the thalamocortical afferents in the cerebral cortex.
8. Factors delivered from the mother during pregnancy that affect the neurogenic output of the NSCs in the late stages of neurogenesis by regulating, e.g., the length of the neurogenic period.

Shh Acts Mainly in Patterning

During neural tube development, a gradient of the morphogen Shh triggers the downstream events that establish dorsoventral identity of the NSCs. Shh signaling is active in the ventral side of the developing neural tube with a ventral^{high}-to-dorsal^{low} expression. This ventral^{high} Shh suppresses dorsalizing transcription factors (TFs) like Gli3 and Paired box 6 (Pax6). The dorsally active expression of Gli3 suppresses Shh signaling in the dorsal domain, and Pax6 acts as a patterning TF, suppressing the expression of ventralizing TFs like Ascl1and Gsh2 to then establish the dorsal identity. Although Shh is a key regulator in establishing dorsoventral identity of the NSCs, Shh-independent pathways also act during development to pattern the telencephalon.

At later stages of cortical development (E14.5 in mouse), Shh signaling is also important for the cell cycle kinetics of NSCs and the migration of neurons. The role of Shh in NSCs is particularly important, as Shh signaling is essential for high proliferative capacity of both APs and BPs in gyrencephalic species like human.

Wnt/Beta-Catenin Signaling Has a Dorsalizing Effect

Within the dorsal domain of the developing telencephalon, the signaling from the roof plate and cortical hem specifies the dorsomedial and dorsolateral fate. One of the key signaling pathways active in this region is Wnt signaling. The main source of the Wnt signaling is the cortical hem, which is a transient structure appearing at the dorsal midline. The secreted Wnt ligands are expressed along the dorsal midline along the anteroposterior axis. They also present a specific radial distribution throughout the cortical wall, being expressed mainly in the apical side of the VZ and in the cortical plate. Wnt ligands act mainly by binding to the Frizzled family of receptors and promoting the stability of beta-catenin, a component of the adherens junction complex. This stabilization allows beta-catenin to reach the nucleus, where it associates with TCF/LEF transcription factors and promotes the expression of specific proliferation genes, such as myc or cyclin D.

In early development (E8.5–E9.5 in mouse), Wnt signaling in the dorsal domain is required for the expression of dorsally expressed genes (like Emx1/2 and Ngn2) and to suppress the expression of ventrally restricted genes (like Gsh2, Mash1, Dlx2/5). However, it is important to note that this dorsalizing effect of Wnt signaling is only restricted to early stages, before the start of neurogenesis. Also, in these early NSCs, Wnt signaling is essential for their symmetric proliferative divisions and therefore NSC expansion. At later stages (after the onset of neurogenesis), Wnt signaling is not

required to maintain a dorsal vs. ventral fate, but it regulates AP-to-BP transition and the neuronal differentiation of BPs. Due to its multiple roles, Wnt signaling is considered to have pleiotropic effects on cortical development, depending on the stage of development and the region where it is active. Also, emerging evidence shows that a huge diversity in Wnt receptors might be responsible for the different downstream events that are activated by Wnt signaling.

Notch Inhibits Neurogenesis

The Notch signaling pathway comprises a family of transmembrane receptors, called Notch receptors, which bind to specific DSL (Delta/Serrate/LAG-2) ligands in the membrane of neighboring cells. Notch activity is distributed in gradients along the apicobasal axis of the developing cortical wall. Its activation is highly dynamic in individual cells, varying with cell cycle progression and therefore with INM. Notch signaling activates Hes and Hey genes, inhibitory TFs that repress proneural genes, thus inhibiting neurogenesis and maintaining progenitor cell character. In newborn neurons, proneural gene expression induces Notch activation in the neighboring cells, repressing neuronal differentiation in them. This process is termed lateral inhibition and helps the maintenance of the progenitor cell pool even in the presence of differentiation-inducing signals. Notch signaling has a context-dependent effect on the fate specification of progenitor cells (favoring the maintenance of APs rather than their differentiation into BPs) and neurons. It also participates in neuronal maturation and later in development promotes gliogenesis, primarily the generation of astroglia. Recent studies have established a role of Notch signaling in brain evolution by showing that NOTCH2NL, a human-specific paralog of NOTCH, works via the Notch ligand DLL1 and increases Notch signaling in NSCs. This increased Notch activity is thought to be essential for the higher proliferative capacity of the human NSCs as compared to the mouse NSCs.

BMPs Have Multiple Effects in Neurogenesis

Bone morphogenetic proteins (BMPs) are mostly expressed in the dorsal part of the neural tube. The downstream targets of the BMP pathway include cell cycle regulators (such as cyclin D1 and CDK4), the inhibitor of differentiation (Id) family of genes, repressors of proneural genes, and Wnt ligands. As most of the signaling molecules implicated in neural development, BMPs have pleiotropic effects. The main role of BMPs is in patterning, but depending on the receptor type that is activated, BMPs can also induce either progenitor cell proliferation or neurogenesis. These effects are mostly achieved by a mitogenic effect, meaning that they promote cell division in the target cells, but this depends on the specific BMP, the cell environment, and the interplay with other intrinsic and extrinsic factors.

FGFs Can Inhibit or Promote Neurogenesis

Many of the fibroblast growth factors (FGFs) are mitogenic and promote the self-renewal of NSCs and their maintenance in an undifferentiated state. This is the case with basic FGF (bFGF) or Fgf2, which is expressed in the developing VZ and SVZ. However, certain members of the family, like Fgf8 or Fgf4, promote differentiation

and cell cycle exit and, hence, neurogenesis. Fgf8 also has a prominent role in the initial patterning of the CNS, as it is secreted from signal organizing centers such as the isthmus.

Intrinsic Mechanisms of Neural Stem Cell Maintenance

Numerous Transcription Factors and Species-Specific Transcripts Contribute to NSC Maintenance and Fate Determination

Once the dorsoventral and mediolateral domains are determined, the local environment initiates the expression of various TFs within each domain. The functions of these TFs include, in sequential order, patterning of the neural tube, NSC commitment, and neuronal fate specification and differentiation. The core cell-intrinsic TFs that are activated under the influence of different local environment vary in different cortical regions, thus giving rise to a wide diversity of neurons that are generated from these NSCs. For example, the events leading to the expression of the TF Ngn2 in the dorsal domain induce differentiation into cortical pyramidal neurons, whereas the events leading to the expression of the TF Ascl1 in the ventral domain drive differentiation of GABAergic inhibitory neurons.

The TFs active in NSCs giving rise to pyramidal neurons are very well characterized and include those important for the generation and proliferation of both APs and BPs and their neuronal differentiation. As mentioned above, at the onset of neocortical neurogenesis, a high Notch signaling in the APs leads to the expression of pro-proliferative TFs like Hes1/Hes5/Hey1. These direct downstream targets of Notch signaling promote symmetric proliferative divisions of the NSC by repressing proneural TFs like Ngn1/Ngn2. Interestingly, both these pro-proliferative and proneural TFs exhibit an oscillatory expression, and when the expression of pro-proliferative TFs is decreased, this leads to the stable expression of proneural TFs, which then promote differentiation of NSCs to become committed to neuron production. These proneural TFs, in addition to activating the program for the differentiation to a neuron, have also been implicated in the AP-to-BP transition and their migration to the SVZ. For example, Ngn2 promotes the expression of T-box brain protein 2 (Tbr2) and the SNAG family TFs Insm1, Scratch 1 and Scratch 2, where Tbr2 and Insm1 have been implicated in the generation of the bRG and Scratch 1 and Scratch 2 have been implicated in their migration.

In addition to Notch signaling, other local cues (e.g., Wnt, FGF, and BMP signaling) trigger the expression of additional TFs that play an essential role in the maintenance of both APs and the BPs. For example, the TFs SMAD1/5, two canonical effectors of BMP signaling, promote AP self-renewal and prevent premature neurogenesis via posttranscriptional regulation of the TF YAP, a core component of the Hippo signaling pathway that is essential for NSC proliferation. Additionally, at least in vitro, FGF2 determines the pro-proliferative vs. proneural effects of the Wnt signaling via ß-catenin. These examples present a picture where the local cues regulating the regional fate specification of the NSCs (dorsal

vs. ventral and lateral vs. medial) additionally regulate the proliferation vs. differentiation decision of the NSCs in that region. A cross talk between these local signals fine-tunes the balance between proliferation and differentiation of the NSCs.

Recent advances in transcriptomic studies of NSCs have shown that many BPs in gyrencephalic species exhibit a transcription profile similar to APs, which is likely the reason for their high proliferative capacity. Additionally, many species-specific transcripts have also been identified and shown to regulate the proliferative capacity of the NSCs. A prominent example is *ARHGAP11B*, which encodes a truncated Rho GTPase-activating-protein and is the first human-specific gene shown to regulate BP expansion. Moreover, *TBC1D3* is a hominin-specific gene encoding a protein of the RABGAP family, which has been shown to be important for bRG generation and their high proliferative capacity. Finally, TMEM14B is a primate-specific, bRG-enriched transmembrane protein implicated in BP expansion.

Neuronal differentiation into specific pyramidal neuron subtypes also requires the action of specific TFs. Whereas TFs like Fezf2, Id4, and Ngn1 are expressed in the NSCs giving rise to deep-layer neurons, TFs like Cux1/2 and Brn1/2 are enriched in the NSCs giving rise to upper-layer neurons. A similar orchestration is true for gliogenesis, which is controlled by both oligodendrocyte-specific and astrocyte-specific TFs. Many complex interactions are established between the different transcription factors, and their activity can also have pleiotropic effects depending on their localization during development. This core regulatory network acts through different cell-intrinsic mechanisms, such as the regulation of microRNAs (miRNAs) and epigenetic modulators.

MicroRNAs

MicroRNAs (miRNAs) are 20- to 25-nucleotide-long noncoding RNAs that regulate the stability and translation of target messenger RNAs, adding a level of complexity to the fine-tuning of cell functions. In the last decade, several miRNAs have been identified that display dynamic spatiotemporal patterns of expression during brain development, and many have been shown to regulate specification, proliferation, and differentiation of NSCs. miRNAs have been shown to execute their functions by regulating multiple signaling pathways/events in the NSCs. For example, miR-124 regulates Notch signaling and alternate splicing, and miR-9 regulates Wnt signaling. Mounting evidence has revealed an active cross talk between miRNAs and epigenetic changes in NSCs. For example, miR-137 expression is epigenetically regulated by MeCP2, a member of the DNA methyl-CpG-binding protein family, and miR-137 in turn targets Ezh2, a histone methyltransferase and member of the PcG protein family. In addition, both miR-9 and miR-124 have been shown to repress the expression of the Baf53a subunit of the BAF chromatin-remodeling complex in NSCs, just before they exit mitosis. This allows the replacement of this subunit with the Baf53b subunit, which is a subunit in the BAF complex with postmitotic functions. Lastly, a strong positive correlation between the cognitive abilities of a given species and the number of miRNAs expressed in its brain has led to the

hypothesis that miRNAs might also play a role in regulating the neurogenic pathways essential for the development of higher cognitive abilities.

Epigenetic and Epitranscriptomic Regulators

The core transcriptional network controls the epigenetic regulation of many genes. For example, histone modifiers regulate both the repression of differentiation genes and the activation of multipotency-related ones by controlling the acetylation (e.g., by the NuRD complex) and methylation (e.g., by the PcG complex) of the histones in the nucleosomes that organize their DNA. Modifications at specific DNA sequences (e.g., by DNA methytransferases) influence DNA-protein interactions and thus regulate the transcriptional output. Temporal changes in the patterning of these acetylations and methylations allow the expression of specific sets of genes required for the transition of NSCs from progenitor fate to neuronal differentiation and for the acquisition of a specific neuronal subtype identity. Other chromatin modifiers, e.g., the BAF complex, promote the rearrangement of certain chromatin regions in order to allow access of further transcriptional regulators. This can impact the expression of effectors of various signaling pathways involved in NSC proliferation and neuronal differentiation.

In addition to modifications at the DNA level, studies of the modification at the RNA level, termed epitranscriptomics, have recently emerged as a new field. These modifications have been shown to have critical roles in influencing the fate of NSCs. Different types of such modifications, like pseudouridine (Ψ), 5-methylcytidine (m^5C), and N6-methyladenosine (m^6A), have been identified on mRNAs and have been shown to influence the dynamics of translation or decay of the targeted RNA, thereby making them particularly important for the regulation of genes that require transient expression, such as the proneural genes.

Radial Glial Cells Link Embryonic and Adult Neurogenesis

Adult NSCs are located in restricted regions of postnatal and adult brains and produce both glia and neurons. Most adult-born CNS neurons in mammals are interneurons destined to the olfactory bulb. They arise mainly in the SVZ of the lateral ventricles and migrate toward the olfactory bulb through a route known as the rostral migratory stream. Neurogenesis also continues in the hippocampus, in the subgranular zone (SGZ) of the dentate gyrus.

Adult NSCs Come from Embryonic NSCs

Just as the RG cells in the embryonic VZ, NSCs in the adult SVZ express glial markers and maintain many epithelial characteristics, such as processes that allow them to contact both the surface of the ventricle on the apical side and

the basal lamina of blood vessels. Therefore, these cells probably receive messages from both the apical and basal zones, which may regulate their stem cell properties. However, unlike the embryonic RG cells, adult NSCs do not extend a long process that contacts the pial surface of the brain. Interestingly, the adult NSCs in the SVZ of the lateral ventricle do contact the ventricle directly, with their apical process intercalating between ependymal cells. Similar to those present in RG cells, the apical end feet of these adult NSCs have specialized apical junctions and a primary cilium. They may therefore communicate with each other and receive signals from the CSF in ways similar to embryonic RG cells. These shared basic properties between the adult NSCs in the SVZ and the embryonic RG suggest that adult SVZ NSCs are modified RG that retain stem cell function throughout life. In fact, tracing experiments have shown that a subpopulation of slow-cycling and long-lived embryonic aRG, which is specified during early stages of embryonic neurogenesis, gives rise to adult NSC.

The second area where adult neurogenesis occurs is the SGZ of the dentate gyrus of the hippocampus. Adult SGZ NSCs also have some RG-like features, and since they originate in the embryonic telencephalon, they may also be derived from embryonic RG cells. However, their cellular architecture is different, and they do not contact any ventricular surface. Instead, their basal processes cross the granular cell layer and contact the so-called molecular layer that overlays it. Their cell bodies remain in the SGZ proper, between the granule cells and the hilus. The division pattern of SGZ progenitors is reminiscent of the neurogenic scheme in the telencephalon: RG-like progenitors divide, giving rise to rounded progenitor cells lacking processes (similar in morphology and marker expression to telencephalic bIPs), which eventually generate neurons.

Perspectives in Embryonic Neural Stem Cell Research

In this chapter, we have described NSCs of the cerebral cortex in their developmental context, focusing on the aspects that are central to their functions in tissue and in vivo. We have taken the neocortex as our main area of interest and have reviewed the current knowledge on the main types of NSCs, their specific cell biological features, and their ability to proliferate, self-renew, and generate differentiated progeny. Many key questions remain incompletely answered, however, such as which specific genes, which functional gene networks, and, ultimately, which cellular and molecular pathways are responsible for the specification of each NSC and progenitor subtype. Despite ongoing contributions, we also still lack a full understanding of how the size of the different NSC and progenitor populations is determined or how many rounds of division each of those subpopulations will undergo.

On the other hand, an increasing stream of new tools continues to increase our understanding of NSC identity and function, especially across hominid species. Notably, and despite their limitations to accurately represent native tissue, brain organoids are helping uncover characteristics that are specific to species for which

native tissue is difficult or impossible to obtain, such as humans and other great apes. In addition, we are seeing ever more precise characterizations of subpopulations and single cells that are supported by "omics" methods (e.g., genomics, transcriptomics, and proteomics.) Obtaining answers to fundamental questions about the nature of NSCs will be critical for understanding the evolution, development, and normal functioning of the brain. This knowledge is also a prerequisite for finding sensible ways of applying NSC biology to the development of therapies for the different neurodevelopmental, neuropsychiatric, and neurodegenerative disorders, as well as for more acute lesions of the nervous system that may benefit from regeneration approaches.

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