Neural Stem Cells in Cerebral Cortex Development

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Abstract

Neural stem cells (NSCs) in the developing neuroepithelium give rise, directly or indirectly, 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

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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 main model system, as many principal hallmarks of brain development are evolutionarily conserved between rodents and other mammals, including hominids. Key differences exist, however, and they are pinpointed 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

Apical-basal polarity • Apical process • Apical progenitors • Apical radial glia • Asymmetric cell division • Basal process • Basal progenitors • Basal radial glia • Bone morphogenetic protein • Brain development • Cell cycle regulation • Cell division • Cell lineage • Cleavage furrow ingression • Tissue differentiation • Embryonic development • Fibroblast growth factor • Interkinetic nuclear migration • Neural development • Neural plate • Neural stem cells • Neural tube • Neuroepithelium • Neuroepithelial cells • Neurogenesis • Notch • Primary cilium • Proliferation • Radial glial cells • Self-renewal • Sonic hedgehog • Spindle orientation • Stem cells • Telencephalon • Transcription factors • Wnt

Introduction

Neural stem cells (NSCs) in the developing neuroepithelium give rise, directly or indirectly, 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 main model system, as many principal hallmarks of brain development are evolutionarily conserved between rodents and other mammals, including hominids. Key differences exist, however, and they are pinpointed 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 introducing general features of NSCs, we trace the developmental origin of NSCs, from the establishment of the neuroectoderm until 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 proliferate and generate neurons, and which help to shape the architecture of the cortex. These features are the general cell structure and apical-basal 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 another chapter.

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) ***self-renewal***, for a high or unlimited number of cell divisions, and (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 populations. Each subpopulation may have variable degrees of self-renewal and multipotency and may thus generate all, some, or just one type of differentiated cells. Present evidence suggests that all such subtypes of precursor cells 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 neuroepithelial cells (NECs) gives rise to different progenitor cell types that in turn originate all the differentiated cells that will form the adult cerebral cortex. Most of neurogenesis occurs during embryonic development, and only small populations of NSCs remain in specific locations, or niches, of the adult body.

The diverse cell types that form the mature cerebral cortex are produced following a specific order: first neurons, then glia. 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, which 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 layers.

Embryonic Origin and 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

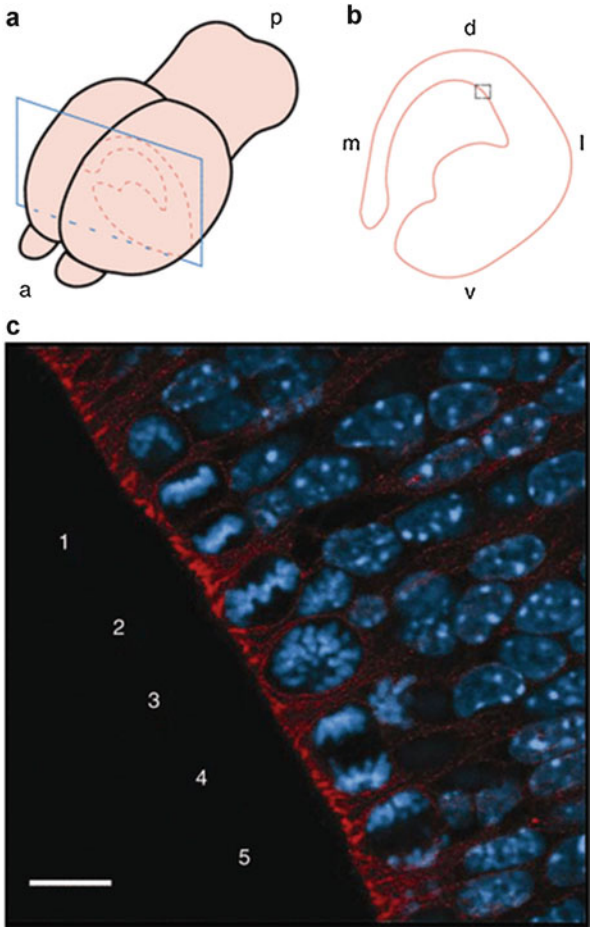

Fig. 1 The apical region of the ventricular zone (VZ) in the dorsal telencephalon. (**a**) Schematic representation of an embryonic day 14.5 (E14.5, mid-neurogenesis) mouse brain showing a coronal section (*blue rectangle*) through the medial part of the telencephalon; *a* anterior, *p* posterior. (**b**) 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*, immunofluorescence 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: 1, anaphase; 2, metaphase; 3, prometaphase; 4, anaphase; 5, anaphase. Scale bar: 10 μm

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, which is the focus of the next sections.

The Cell Biology of Neural Stem and Progenitor Cells

Cell Division Modes

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). Highly symmetric cell divisions generate two daughter cells with essentially the same fate. These divisions can be further classified as symmetric proliferative divisions, which generate two daughter NSCs (Fig. 3a), and symmetric neurogenic divisions, where both daughter cells become postmitotic neurons (Fig. 3d, e). In the latter case, the mother progenitor cell cannot be classified as a stem cell anymore since no self-renewal has occurred. We refer to these as terminal or non-stem cell progenitors.

Asymmetric self-renewing divisions, on the other hand, generate one daughter cell with a similar stem cell fate as the mother cell and a second cell with a different fate. The non-stem cell daughter may be either a non-stem cell-like 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.

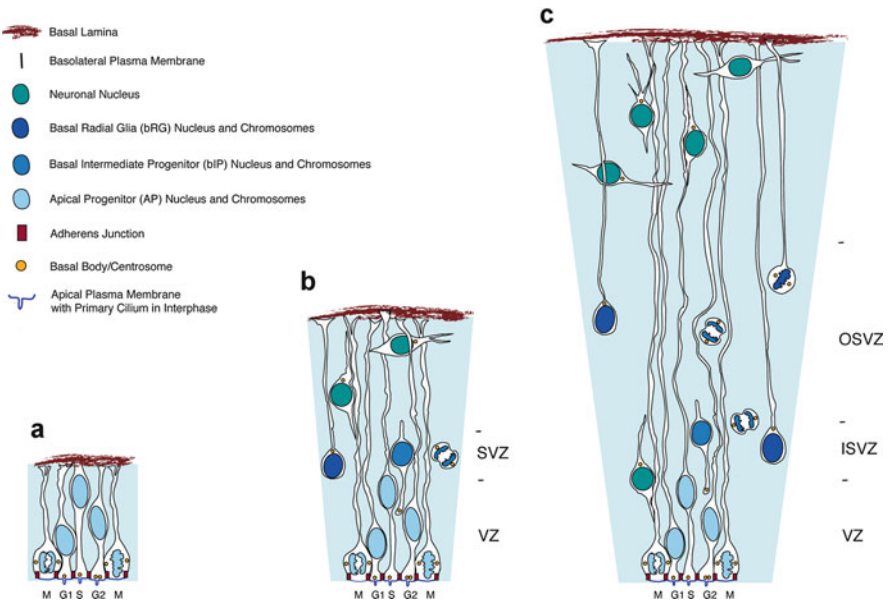

Fig. 2 General structure and major cell types of the developing neocortex. (**a**) Before neurogenesis, the AP population is composed of neuroepithelial cells (NECs) that are attached to the apical surface and contact the basal lamina of the neuroepithelium. APs are connected to each other via the adherens 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 tissue expands basally and 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. 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 the SVZ can also be subdivided into an inner SVZ (ISVZ) and outer SVZ (OSVZ), which contain more BPs than in rodents

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

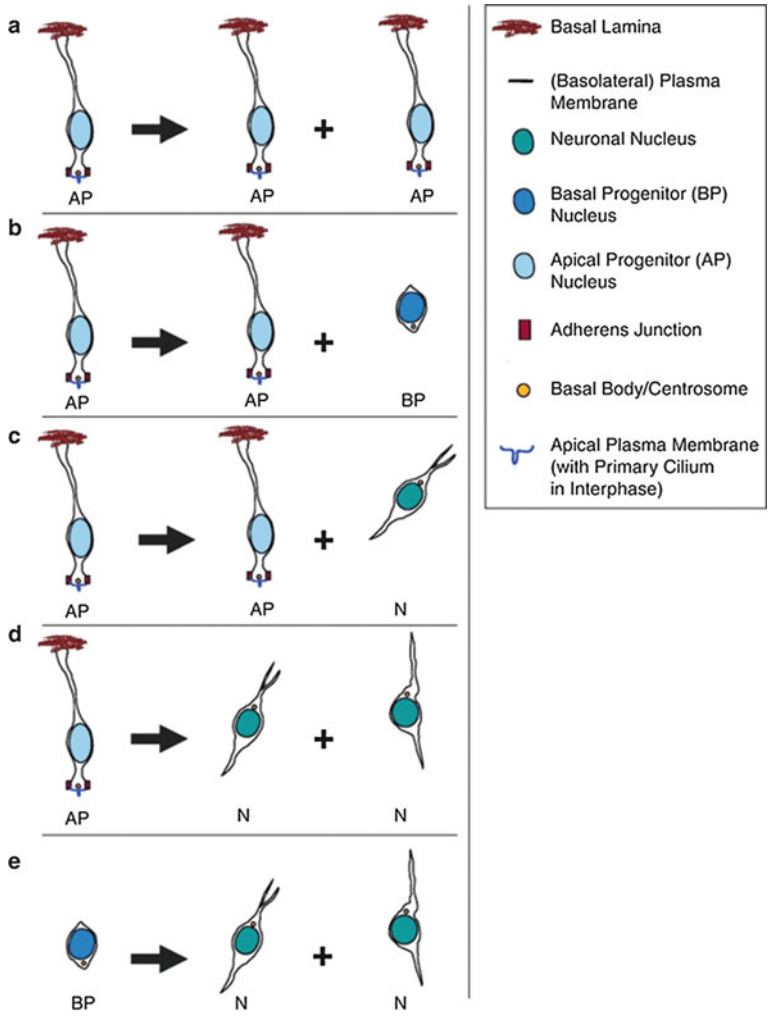

Fig. 3 The main types of neural stem cell (NSC) and progenitor cell divisions resulting in proliferation, self-renewal, and the production of differentiated progeny. (**a**) Symmetric proliferative divisions generate two daughter stem cells with the same fate as the mother cell. Thus, in the case of apical progenitors (APs), they generate two additional APs. (**b**) Asymmetric self-renewing divisions (type I) generate one daughter cell with a similar fate as the progenitor mother, for example, 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 self-renewing bRG. (**c**) Asymmetric self-renewing divisions (type II) generate an AP, or bRG, and a neuron (N) directly. 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 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

eventually produce two neurons, for example, by undergoing a terminal 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 (GFP) and other markers.

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 cerebral cortex arises, are largely determined by the neural stem and progenitor cells it contains, by their cellular properties and their supracellular organization. We now describe the main types of NSCs and other progenitors in the dorsal telencephalic neuroepithelium (Fig. 1b). We then discuss each of their 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: the entire NEC population progressively turns into RG during early neurogenesis. Throughout this transition, NECs maintain most of their general architecture but progressively express glial 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 by differentiated glia, such as astrocytes. As will be discussed in the following section, both NE and the apical RG (aRG) cells keep their nuclei in the apical-most layer of the developing neocortex, they contact the ventricle with an apical attachment integrated into the adherens junctions belt, and their mitosis occurs close to 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, but lose the canonical apical-basal polarity and are thought to have comparably reduced stem cell properties. The main types of BPs are basal radial glia (bRG) and basal intermediate progenitors (bIPs). bRG in primates, but not in rodents, can self-renew and can have some proliferative capacity (Fig. 2c). Despite losing their apical attachment and dividing basally, most bRG have a polarized structure during mitosis that can include an unattached apical process and also a basal process that often

contacts the basal lamina. bRG (also called outer or intermediate RG) have been detected in the developing neocortex of all mammalian species studied, but they show a higher abundance in species with relatively large and gyrencephalic brains, such as primates. Changes in the relative abundance of these and other BPs may therefore influence the development and size of the neocortex across species. bIPs are also generated from APs, but typically lose both the apical and basal processes and are therefore not polarized (Fig. 2b, c). bIPs have lost the ability to self-renew and are considered non-stem cell progenitors that undergo one terminal division to produce two neurons.

It is likely that these main types of progenitors encompass or give rise to more subtypes. For example, BPs similar to bIPs but able to proliferate have been termed transit amplifying progenitors. In addition, progenitors that can self-renew and divide in the basal part of the ventricular zone (VZ) while maintaining an apical contact have been termed subapical progenitors. A comprehensive characterization of all cortical stem and progenitor cells is the focus of intense current research.

The Epithelial Nature of 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 apical-basal 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. Their cell body is widest where the nucleus is located, with a diameter of around 5–10 μm.

The long, tubelike extensions that reach the apical and basal sides, called processes, are much thinner than the nucleus (less than 1 μm). 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 are discussed below. With the cells being side by side, the arrangement of the nuclei in different positions along the apical-basal axis gives, at first sight, the impression of the tissue being stratified or composed of different layers. However, since there is only one layer of NECs, this early non-neurogenic neuroepithelium is referred to as being pseudostratified (Fig. 2a).

Different Zones Arise in 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 process, the neuroepithelium expands to form the developing cortical wall. The 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 maintain the general polarized and elongated architecture of their progenitors, including the apical and basal processes and contacts (Fig. 2b). The 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 basally forming zones. This expansion follows a conical pattern of growth, with the tip of the cone located apically and the cone base broadening basally.

The next area that arises basally from 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 crossed by the basal processes of APs. In organisms with longer neurogenesis periods and development of larger brains, such as primates, the growing SVZ can become subdivided into an inner SVZ (ISVZ) and an outer SVZ (OSVZ) (Fig. 2c). BPs accumulate in these subzones and are thought to strongly contribute to the increase in the number of neurons that underlies the expansion of the hominid neocortex. The neurons being born from these progenitors accumulate at the basal-most side of the tissue 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 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 junction 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 axoneme then causes the apical plasma membrane that surrounds the cilium to protrude into the ventricle, where it becomes 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 during anaphase. Interestingly, the inheritance of either the mother or daughter centriole-containing centrosome may influence progenitor cell fate during

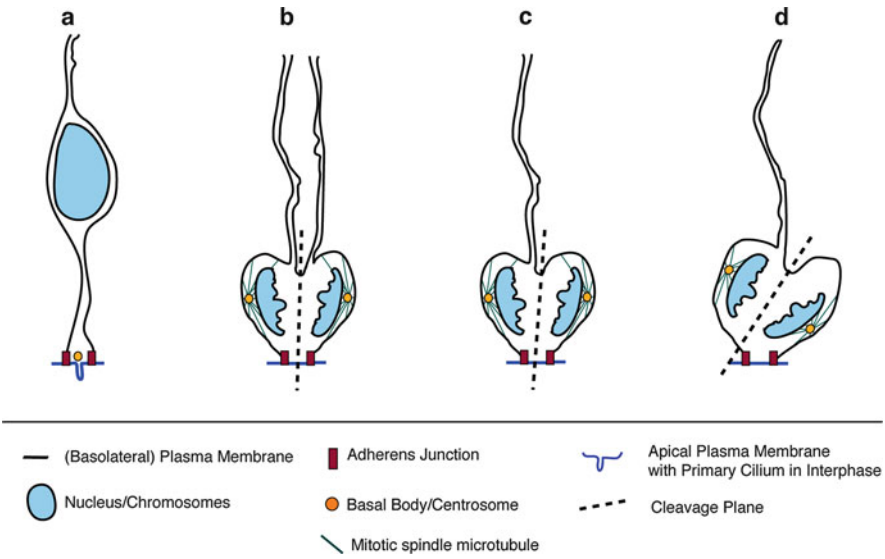

Fig. 4 Major cleavage modes during the divisions of neuroepithelial cells (NECs), the primary neural stem cells (NSCs) of the mammalian brain. These modes also apply to other apical progenitors (APs), such as apical radial glia (aRG), which derive from NECs. (**a**) APs contact the ventricle via their apical domain, composed of adherens junctions surrounding the apical membrane. During interphase, APs carry a primary cilium in their apical domain that protrudes into the ventricle and is surrounded by CSF. During mitosis (**b**, **c**, **d**), the 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 direction. (**b**) Before and during early neurogenesis, APs can undergo symmetric divisions that bisect both the basal process and the apical domain. Both daughter cells therefore inherit an apical and a basal contact. (**c**) Asymmetric divisions that distribute the basal contact 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 also occur. Only one of the daughter cells will contact the ventricle and the other one will become delaminated. These divisions can result from tilting of the spindle and the subsequent cleavage furrow, which can be due to fewer astral microtubules anchoring the spindle to the cell cortex

asymmetric cell divisions. Recent studies show that the centrosome containing the mother centriole is typically inherited by the more self-renewing daughter, whereas the centrosome containing the daughter centriole preferentially goes to the more neurogenic daughter.

The Basal Components of Neural Stem Cells

The Basolateral Membrane and Basal Process

The plasma membrane beyond the adherens junctions ring that delimits the small apical domain 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. Recent work has shown that, unlike in all other known cells, the AP Golgi apparatus is not pericentrosomal during interphase, as in all other cells described to date. Instead, it is mostly distributed along the apical process, which may be important for the polarized structure of APs. The segment of basolateral membrane that goes beyond the VZ forms a basal process that typically reaches the basal end of the developing cortical wall and contacts the basal lamina. Contrary to the apical process, the basal process often branches out shortly before reaching the basal end, thereby contacting the basal lamina in several places (Fig. 2). The functional significance of these branches is presently unknown. Interesting hypotheses include a broader and more efficient communication of progenitors with the basal and pial compartments and the branches serving as diversified tracks or cues for the radial migration and positioning of neurons.

Another 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 depend on an uneven distribution of organelles, cell membrane components, and other molecules along the process. Most varicosities disappear during interphase, concomitant with more cytoplasm flowing into the process. Similar to basal branching, a functional significance of 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, 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 inner-most layer of the meninges. These are a complex network 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 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 general cellular architecture and INM. A notable difference, however, is that even though the basal processes of aRG elongate together with the thickening of the cortical wall, their nuclei remain inside the VZ, in the apical process (Fig. 2b, c). It is with the nuclei in a distinctly basal position that APs undergo S phase to replicate their chromosomes. 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 divide in the SVZ, the zone they effectively help to create with their presence (Fig. 2b, c). In some BPs, the most accelerated part of this movement is at the beginning of mitosis and is known as mitotic somal translocation.

The Advantages of an Apical Mitosis

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, yet 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 also maintaining tissue organization, nuclei that have completed mitosis then migrate away from the apical surface. This liberates space that can be occupied by the following 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. It is thus 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 could also make it worthwhile for the nucleus to migrate apically. Such a "nuclear residence hypothesis" is 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. Nevertheless, the establishment of other proliferative zones in later stages of neurogenesis, such as the SVZ, suggests that even strong AP proliferation near the apical surface of the VZ is not sufficient to sustain all cortical neurogenesis. The basal migration and division of BPs is likely to play an analogous role to INM in the growth of the neocortex. Dividing BPs can spread in this way through the tissue to reduce crowding and facilitate the basal expansion of the tissue.

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.

The 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. The migration direction could be controlled by cell cycle-dependent switches, which 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 move basally and vice versa. Such an apical-basal alternation of the direction of the "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 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 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, cell cycle progression in NSCs is controlled by proteins called cyclins. 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 a slower, more careful DNA replication 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

The Cell Division Machinery

The cell division machinery common to most somatic cells is also implicated in NSC division modes. The mitotic spindle and each of its main components have all been shown to play basic roles in the regulation of symmetric and asymmetric divisions. Robust regulation of spindle positioning requires first the formation and maintenance of a bipolar spindle by a pair of centrosomes, which nucleate and organize mitotic microtubules, including kinetochore fibers and astral microtubules. During prometaphase, the centrosomes relocate to the equatorial plane of the AP cell soma at the apical domain. The centrosomes can then form a stable bipolar spindle, with the spindle axis typically oriented parallel to the plane of the apical surface during metaphase. When bipolar spindle assembly or stability is impaired, 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 and its binding partners, also significantly affects spindle and cleavage orientation. Cytokinesis onset and progression are governed by spindle orientation and cleavage furrow positioning and ingression. However, 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 ingressing 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 perpendicular 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 may increase moderately during neurogenesis (Fig. 4d), but they rarely 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 two structural differences may be at the heart of the regulatory differences between these systems and could 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 are 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 self-renewal potential compared to cells with neither contact, such as the neurogenic bIPs. When mammalian APs undergo symmetric proliferative divisions, they are cleaved along the apical-basal axis of the cell, and the apical domain is bisected. Therefore, both daughter cells inherit an apical domain. The basal process is kept during mitosis, and its bisection is a more difficult task, given its long and narrow nature. However, many APs manage to bisect it 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 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 may be more difficult to achieve and coordinate. During more 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 proposed that such a regrowth in APs with only an apical attachment involves Notch signaling.

The moderate 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 been difficult to establish. The results of experimentally perturbing spindle orientation seem to heavily depend on which cellular factors are targeted. For example, higher cleavage plane variability by perturbing LGN in mice increased the number of bRG, but this showed little effect on neurogenesis. On the other hand, perturbing either Lis1 or the Lfc-mediated cortical regulation of RhoA heavily reduced neurogenesis. This suggests that the different gene and protein perturbations used in those studies may have additional effects to the changes in spindle orientation, which may explain the different outcomes. In addition, the differential activation of compensatory mechanisms may impact each particular case. 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 was 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 idea that epithelial features are 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 is underscored by its persistence in those NSCs that remain throughout embryonic neurogenesis and are found in the SVZ of the adult brain. Nevertheless, the specific contributions of each cell 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 a "protomap," in which the lineages of neurons that will be formed are already preestablished.

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

Notch Inhibits Neurogenesis

The Notch signaling pathway comprises a family of transmembrane receptors, called Notch receptors, that 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 transcription factors (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.

Shh Acts Mainly in Patterning

The expression of the morphogen Shh is localized in ventral structures of the developing nervous system. The main effect of the Shh signaling cascade is the activation of the Gli transcription factor family. In the absence of Shh signaling, certain Gli proteins act as transcriptional repressors. When Shh signaling is active,

they function as transcriptional activators, promoting the expression of proliferation genes such as myc and cyclin D and thus maintaining progenitor cell identity. They also have important effects on dorsoventral patterning.

Wnt/Beta-Catenin Signaling Inhibits Neurogenesis and Has a Dorsalizing Effect

The secreted Wnt ligands are expressed along the dorsal midline along the anteroposterior axis of the neural tube, as well as in the cortical hem. 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. Wnt signaling has pleiotropic effects. This means that the same molecule can have different effects, depending on the context and mechanism of action. In this case, the developmental stage and location play a key role in the modulation of Wnt signaling. During neurogenesis, Wnt mainly promotes AP proliferation, maintaining them in an undifferentiated state, but it also plays roles in patterning of the neural tube and in neuron maturation.

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 Contribute to Fate Determination

Nervous system development is controlled by transcription factors (TFs) that belong to two main classes, containing either a homeodomain or a basic helix-loop-helix (bHLH) domain. Their functions include, in a sequential order, patterning of the neural tube, progenitor cell commitment, and neuronal fate specification and differentiation.

Patterning TFs, such as the homeodomain TF paired box 6 (Pax6), establish progenitor cell domains (e.g., Pax6 is expressed in the APs of the dorsal telencephalon) and contribute to the selection of the cell types those progenitors give rise to. Proneural TFs, like achaete-scute homolog 1 (Ascl1), neurogenins 1–3, or atonal homolog 1 (Atoh1 or Math1), mostly belong to the bHLH type. They inhibit self-renewal and multipotency-promoting genes, such as the SoxB1 family (Sox1, Sox2, and Sox3), as well as gliogenesis. Proneural TFs also contribute to neuronal subtype specification. For example, Ngn2 induces differentiation into cortical pyramidal neurons, while Ascl1 drives differentiation of GABAergic inhibitory neurons. These intrinsic effects are combined with those of the specific inductive signals present in the environment of the progenitor cells when they undergo their final division. Proneural TFs are expressed transiently around this time, promoting cell cycle exit and the start of neuronal differentiation.

Neuronal differentiation also requires the action of specific TFs such as NeuroM or NeuroD. The same 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 20–25-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. Certain miRNAs act specifically in neural tissue. For example, mir9 promotes neural progenitor proliferation, inhibiting differentiation and migration. At later stages it participates in neuronal differentiation, whereas in adult tissue it contributes to maintain the balance of progenitor cell differentiation and proliferation.

Epigenetic 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 and methylation of the histones in the nucleosomes that organize their DNA. Other chromatin modifiers promote the rearrangement of certain chromatin regions in order to allow the access of further transcriptional regulators. In NSCs, histone

deacetylases (HDACs) cause local chromatin condensation, repressing the transcription of neuronal differentiation genes. The degrees of histone and DNA methylation, as well as chromatin remodeling, seem to play a similar role.

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. Furthermore, 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 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 unanswered, 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. We also do not know what determines the size of the different NSC and progenitor populations, or how many rounds of division each of those populations can undergo. Obtaining answers to these fundamental questions 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 against the different neurodevelopmental and neurodegenerative disorders and lesions of the nervous system.

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Further Reading

Götz M, Huttner WB (2005) The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6:777–788

Guillemot F (2007a) Cell fate specification in the mammalian telencephalon. Prog Neurobiol 83:37–52

Guillemot F (2007b) Spatial and temporal specification of neural fates by transcription factor codes. Development 134:3771–3780 (Freely available online)

Hansen DV, Rubenstein JLR, Kriegstein AR (2011) Deriving excitatory neurons of the neocortex from pluripotent stem cells. Neuron 70:645–660

Hébert JM, Fishell G (2008) The genetics of early telencephalon patterning: some assembly required. Nat Rev Neurosci 9:678–685 (Freely available online)

Homem CC, Repic M, Knoblich JA (2015) Proliferation control in neural stem and progenitor cells. Nat Rev Neurosci 16(11):647–659

Kandel ER, Schwartz JH, Jessell TM (eds) (2000) Principles of neural science, 4th edn. McGraw-Hill, New York

Kriegstein AR, Alvarez-Buylla A (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149–184 (Freely available online)

Lui JH, Hansen DV, Kriegstein AR (2011) Development and evolution of the human neocortex. Cell 146:18–36

Miyata T, Okamoto M, Shinoda T, Kawaguchi A (2015) Interkinetic nuclear migration generates and opposes ventricular-zone crowding: insight into tissue mechanics (Freely available online). Front Cell Neurosci 8:473

Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD (2007) Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci 8:427–437

Mora-Bermúdez F, Huttner WB (2015) Novel insights into mammalian embryonic neural stem cell division: focus on microtubules. Mol Biol Cell 26(24):4302–4306 (Freely available online)

Stiles J, Jernigan TL (2010) The Basics of brain development. Neuropsychol Rev 20:327–348 (Freely available online)

Sur M, Rubenstein JLR (2005) Patterning and plasticity of the cerebral cortex. Science 310:805–810

Taverna E, Huttner WB (2010) Neural progenitor nuclei IN motion. Neuron 67:906–914

Taverna E, Götz M, Huttner WB (2014) The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. Annu Rev Cell Dev Biol 30:465–502