Part 2

Development

Stem Cells: Neural Stem Cells in Cerebral Cortex Development

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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, namely, 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 *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 primates. Key differences exist, however, and they are pinpointed appropriately. We also highlight some areas of intense current research and mention ideas that may 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 that help to shape the architecture of the cortex. These features are the general cell structure and apical-basal polarity, interkinetic 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.

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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 either a limited or unlimited number of cell divisions, and (2) *multipotency*, the ability to give rise, directly or indirectly, to different types of differentiated cells, such as the different types of neurons and glial cells. In the developing cerebral cortex, diverse NSCs and progenitors derived from them may exist that generate all, some, or just one of these cell types. Present evidence suggests that all such subtypes of precursor cells exist, but their precise identification and characterization will require more 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. 6.1). There, an initial population of neuroepithelial cells 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 well-organized 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 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 extra-cellular signals from the notochord. This generates a tubular structure, called the neural tube, along the anteroposterior axis of the embryo. The complete 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

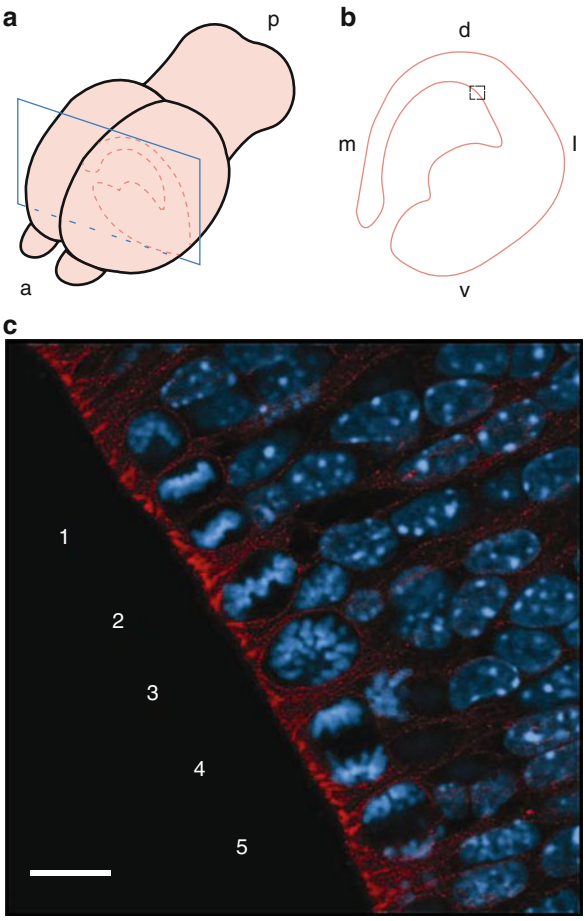

Fig. 6.1 The apical surface of the ventricular zone (VZ) of the dorsal telencephalon. (**a**) Schematic representation of an embryonic day 14.5 (E14.5, mid-neurogenesis) mouse brain with a coronal section (*blue rectangle*) through the medial part of the telencephalon; *a* anterior, *p* posterior. (**b**) Schematic representation of the 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. *Red*, immunofluorescence for cadherins, which are concentrated at the apical adherens junctions; *blue*, DAPI staining of DNA showing the nuclei and chromosomes. Note the mitotic cells and their chromosomes at the ventricular surface: *1*, anaphase; *2*, metaphase; *3*, prometaphase; *4*, anaphase; *5*, anaphase. Scale bar: 10 μm

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 whole 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. 6.2).

The caudal region of the neural tube gives rise to the spinal cord and the rostral region 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

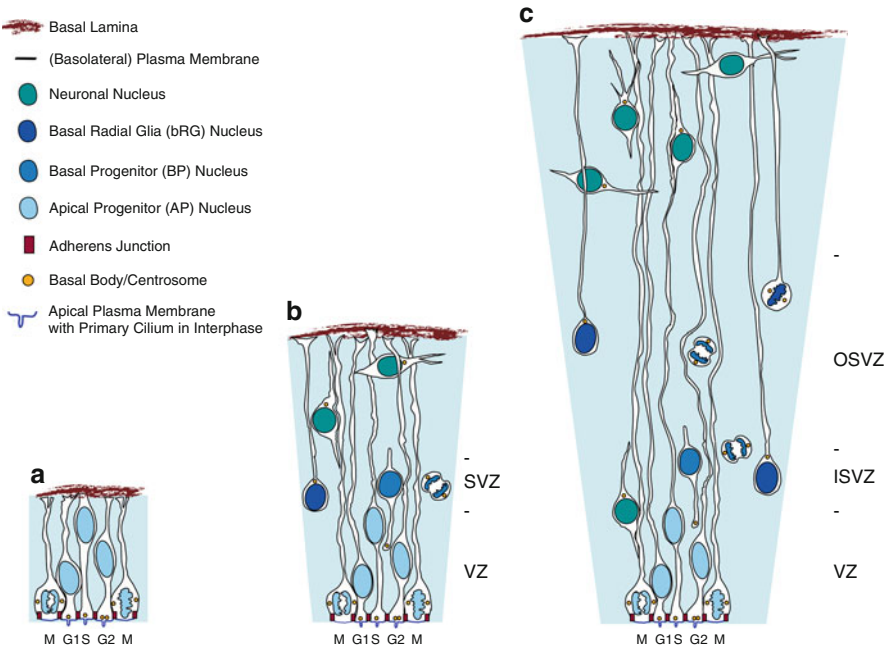

Fig. 6.2 General structure and major cell types of the developing cerebral neocortex. (**a**) Before neurogenesis, the AP population is composed of neuroepithelial cells that are attached to both the apical surface and the basal lamina of the neuroepithelium. APs are connected to each other via adherens junctions, collectively forming 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 at the apical surface, 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. Other progenitors derived from the APs accumulate in the SVZ. In rodents, most of these are BPs, which have lost both their apical and basal attachments. They typically do not self-renew and give rise to two neurons. Also present, but much less abundant, are bRG cells, 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 brains such as those of primates, the cortical wall expands further basally, for example, the SVZ, which can also be subdivided into an inner SVZ (ISVZ) and outer SVZ (OSVZ). bRG cells increase in relative abundance

through a five-vesicle stage in which further subdivisions appear. The hindbrain is divided into the metencephalon, which forms the pons and cerebellum, and the myelencephalon, from which the medulla arises. The midbrain, or mesencephalon, 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, hypothalamus, and retina. The telencephalon (Fig. 6.1) generates the basal ganglia, hippocampus, amygdala, olfactory bulbs, and 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 symmetry, is one of the basic mechanisms that allow NSCs to choose their developmental path (Fig. 6.3). *Symmetric cell divisions* generate two daughter cells with the same fate. These divisions can be further classified as *symmetric proliferative divisions*, which generate two daughter NSCs (Fig. 6.3a), and *symmetric neurogenic divisions*, where both daughter cells become postmitotic neurons (Fig. 6.3d, 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-like 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. 6.3b, c). During neurogenesis, cell divisions can also be asymmetric neurogenic, with one daughter becoming a neurogenic, non-stem-cell-like 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, and 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 neural 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 final symmetric neurogenic division (Fig. 6.3d).

Some of these division types were first deduced from cell-lineage-tracing experiments where cells were marked by retroviruses that specifically labelled 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 Cerebral 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, their cellular properties and their supracellular organization. We now describe the main types of NSCs and other progenitors in the dorsal telencephalic neuroepithelium (Fig. 6.1b). We then discuss each of their cell biological hallmarks and how they help shape the developing neocortex.

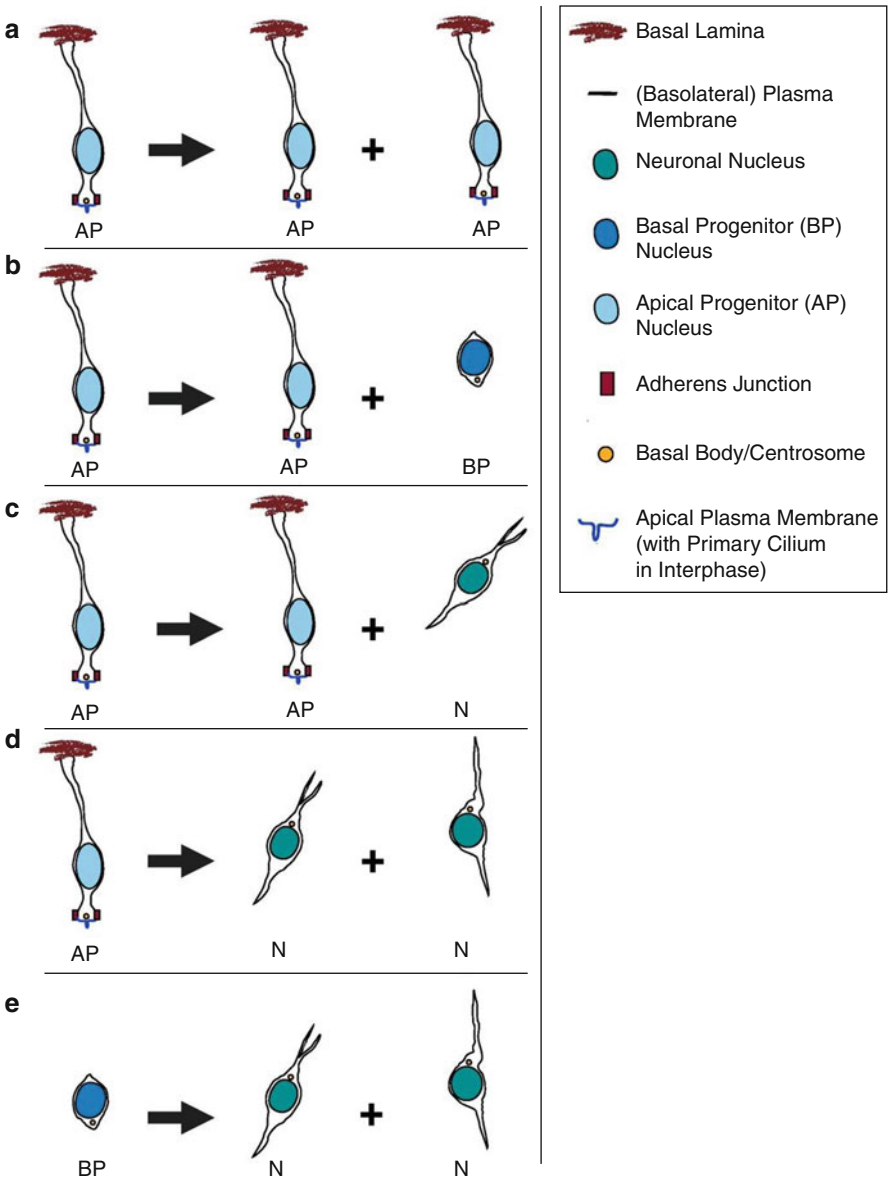

Fig. 6.3 The main types of NSC and progenitor cell divisions resulting in proliferation, self-renewal, and the production of differentiated progeny. (**a**) *Symmetric proliferative cell divisions* generate two daughter cells with the same fate as the mother cell. Thus, in the case of APs, they generate two additional APs. (**b**) *Asymmetric self-renewing divisions* (*type I*) generate one daughter cell with a similar fate as the mother AP and a second cell with a different fate. During mammalian neocortical neurogenesis, the majority of these divisions generate an AP and a non-stem-cell BP. (**c**) *Asymmetric self-renewing divisions* (*type II*) generate an AP and a neuron (N) directly. When neurogenesis advances, asymmetric neurogenic divisions (not shown) also occur,

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* (*NE*) cells and the *radial glial* (*RG*) cells. These two types of progenitors are closely related, as the entire NE cell population progressively turns into RG during early neurogenesis. NE cells maintain their general architecture but start to 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 attachments, and contacts are made with the endothelial cells of the developing 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 RG cells keep their nuclei in the apical-most layer of the neuroepithelium, contact the ventricle with their apical surface, and divide close to it. Therefore, we collectively refer to them as *apical progenitors* (*APs*, Fig. 6.2a–c).

Other types of progenitor cells exist in the developing CNS. *Basal progenitors* (*BPs*, called also intermediate progenitors) are generated from APs but lose both the apical and basal attachments (Fig. 6.2b, c). In contrast to APs, the ability to self-renew seems very limited for most BPs. Therefore, they are considered non-stem-cell-like progenitors. Another type of progenitor maintains only a basal contact and is thought to have a higher self-renewal potential. Since these progenitors are derived from, and retain properties of, RG, but their nucleus is located more basally, we refer to them as *basal radial glial cells* (*bRGs*. They have also been referred to as outer or intermediate RG cells) (Fig. 6.2c). In addition, the existence of *short neural precursors*, which may maintain only an apical contact, has been reported. It is possible that these general types of progenitors encompass or give rise to more subtypes of progenitors. A comprehensive characterization of all basally located progenitors 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 NE cells arranged side by side (Fig. 6.2a). The expansion of the early neuroepithelium is mostly lateral and occurs by symmetric proliferative divisions of the NE cells. 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

Fig. 6.3 (continued) with one daughter becoming a neuron and the other becoming a neurogenic, non-stem-cell progenitor, such as a BP. (**d**) *Symmetric neurogenic divisions* (*type I*) also occur in advanced stages of neurogenesis, with a non-stem-cell, terminal AP producing two daughter cells that become neurons. (**e**) *Symmetric neurogenic divisions* (*type II*) are the main division type in BPs, and produce two daughter neurons

possible because NEs 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, tube-like 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 polar extensions and contacts with the apical and basal sides have been widely implicated in AP fate and are discussed below. With the cells 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 NE cells, this early non-neurogenic neuroepithelium is referred to as being pseudostratified (Fig. 6.2a).

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 basally by radial expansion of the tissue. In this process, the neuroepithelium expands to form the developing cortical wall. The cell bodies of the NE cells now constitute the layer facing the ventricle, referred to as the ventricular zone (VZ) (Fig. 6.2a).

The RG cells that arise from the NE cells maintain the general polarized and elongated architecture of their progenitors, including the apical and basal processes and contacts (Fig. 6.2b). The elongation of the processes of these APs is part of the growth of the tissue, together with progenitor expansion and diversification, tissue vascularization and neuron production 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 by the progeny of single APs, which tend to migrate radially along the apical-basal axis, following AP basal processes. In this manner, bRGs, BPs, 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. 6.2b). The SVZ is mostly formed by the accumulation and divisions of delaminated BPs, although it also contains some AP nuclei and the AP basal processes. In organisms with longer neurogenesis periods and development of larger brains, such as primates, the early SVZ can be subdivided into an inner SVZ (ISVZ) and an outer SVZ (OSVZ) (Fig. 6.2c). BPs and bRGs accumulate in these subzones and are thought to strongly contribute to the increase in neurons and the expansion of the 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. 6.2b, c). At the end of development, there are six such layers in the mammalian cerebral cortex, which contain different types of neurons.

The Apical Domain of Neural Stem Cells

On the apical pole of APs, the end of the process forms an end foot called the apical domain. This domain, which accounts for only a minute fraction of the total cell plasma membrane, 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. 6.1c and 6.2). The apical domain is therefore the contact zone of the APs with the cerebrospinal fluid (CSF) that fills the lumen of the ventricle. This fluid has been shown to play important nutritional and signaling roles in neurogenesis.

The Apical Plasma Membrane

The fate of NE cells is thought to be influenced by extracellular signals, some of which are present in the ventricle. In this context, transmembrane proteins that are enriched in the apical membrane may take part in such signaling processes. An example of an apical membrane protein is prominin-1, which interacts with cholesterol, is present in the protrusions of the apical membrane and is considered a marker of somatic stem cells in general. Megalin, a lipoprotein receptor, is another example, and it may be involved in transducing signals of cholesterol-bearing morphogens such as Sonic hedgehog (Shh). SNARE (snap receptor) distribution may also be different in 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 aPKC, and adherens junction components.

The Adherens Junctions and Apical Cell Cortex

The adherens junctions and their components, such as cadherins (Fig. 6.1c) and catenins, have been broadly implicated in the polarity and fate of NE cells. These junctions help to keep the apical domains of APs together and maintain the integrity of the neuroepithelium (Figs. 6.1c and 6.2). 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.

The Primary Cilium and Centrosomes

The intracellular side of the apical domain is also the place where the primary cilium forms (Fig. 6.2). 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

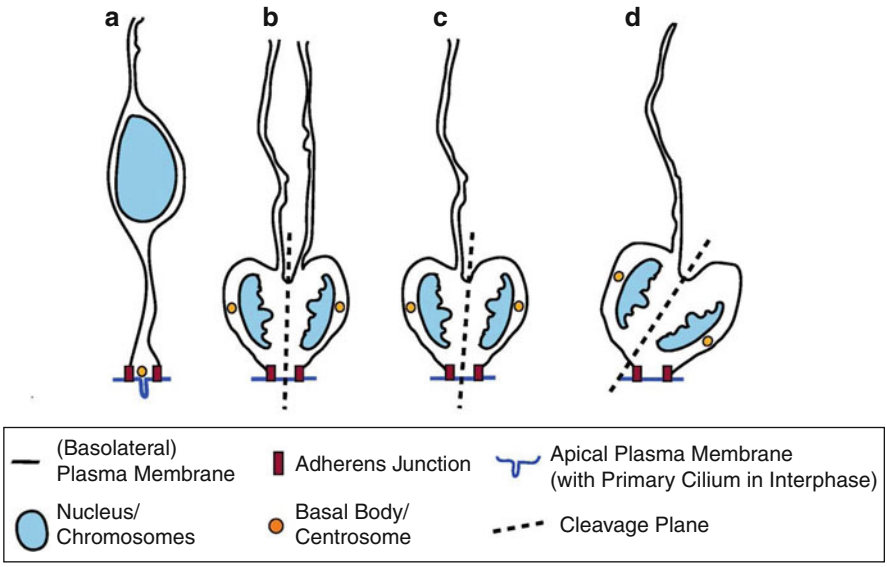

Fig. 6.4 The main cleavage modes during NSC divisions. (**a**) APs contact the ventricular surface via their apical domain, composed of adherens junctions surrounding the apical membrane. During interphase, APs carry a primary cilium in their apical domain that protrudes into the ventricle and is surrounded by CSF. During mitosis (**b**, **c**, **d**), the cilium has been disassembled to allow the centrosomes to become the poles of the mitotic spindle (not illustrated). 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, and both daughter cells remain therefore apically and basally attached after mitosis. (**c**) Asymmetric divisions with respect to the basal process can generate a symmetric distribution of the apical domain, with both daughter cells inheriting apical junctions and plasma membrane. (**d**) Asymmetric divisions with respect to the apical domain can also occur, with only one cell inheriting it and the other one losing the apical attachment. These divisions are typically produced by a tilting of the cleavage plane with respect to the orientation and position of the apical domain

nucleates the cilium shaft, called the axoneme. The growth of the axoneme then causes the apical plasma membrane to protrude into the ventricle, where it is surrounded by CSF. This makes the cilium behave like 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. 6.2 and 6.4). 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 NE cells is disassembled. Each pair of centrioles forms a centrosome, and both centrosomes can interact 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 asymmetric divisions. It has been proposed that the centrosome containing the mother centriole is typically inherited by the self-renewing daughter, whereas the centrosome containing the daughter centriole typically goes to the neurogenic daughter.

The Basal Attachment 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 to form a basal process that typically reaches the basal end of the neuroepithelium 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. 6.2). The functional significance of these branches is presently unknown. Interesting hypotheses include a more efficient communication with the basal and pial compartments via multiple contacts, and the branches serving as diversified tracks or cues for the radial migration of neurons.

Another feature often found in basal processes is the presence of discrete widenings in process diameter. These varicosities occur more frequently in mitotic than in interphase cells and may result from an irregular flow of cytoplasm toward the nucleus that differentially affects the diameter of the process. Most varicosities disappear during interphase, concomitantly with more cytoplasm flowing into the process. Similar to basal branching, a functional significance of these varicosities has not been established, yet they could constitute a specialized compartment to carry out distinct cellular functions, such as signal transduction, translation, or 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 constitutes a surface for basal attachment of the cells in the neuroepithelium (Fig. 6.2). The attachment 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. The basal lamina itself is covered by the inner surface of the pia mater, which is the innermost 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 basal attachment site. The meninges also have signaling functions, like that exerted by the neurogenesis-inducing retinoic acid.

Basally Located Progenitors

The SVZ has been shown to contain a diverse population of progenitors. BPs are abundant in the SVZ and have lost apical-basal polarity, attachments and typically

divide symmetrically with a mostly randomly oriented cleavage plane to give rise to two neurons (Fig. 6.2b, c). By contrast, bRGs in the SVZ are thought to have a higher self-renewal potential. The basal attachment that many of them retain could contribute to their self-renewal by gathering signals present in the surrounding tissue or coming from the more basal compartments. Since they have lost their apical attachment, bRGs are largely unipolar (Fig. 6.2b, c). While bRGs have been detected in the developing neocortex of all mammalian species studied so far, they have been found at a higher relative abundance in the SVZ of gyrencephalic species and a lissencephalic primate. Changes in the relative abundance of basally located progenitors may therefore strongly influence the development and size of the neocortex across species.

Interkinetic Nuclear Migration 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, the VZ (Fig. 6.2). 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. 6.1c). 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 during apical-to-basal migration, S phase in the basal portion of the VZ, and G2 during basal-to-apical migration (Fig. 6.2).

More specifically, before cortical neurogenesis, the interphase nuclei of APs move very close to the basal lamina. During neurogenesis, NE cells become RGs, which maintain the general cellular architecture and INM of NE cells. However, even though the basal processes of the RG elongate together with the thickening of the cortical wall, most nuclei remain inside the VZ (Fig. 6.2b, 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 and eventually reach the apical domain, where the next round of cell division takes place. Basally located progenitors, such as BPs and bRGs, do not undergo INM, presumably due to the lack of an apical attachment. However, when these progenitors arise in the VZ, their nucleus does move in a mostly basal direction, and they divide in the SVZ (Fig. 6.2b, c).

The Advantages of an Apical Mitosis

During the evolution of neural development, mechanisms that increase the efficiency of proliferation are likely to be favored. In this context, INM has been proposed to be a way to increase the efficiency of AP proliferation by facilitating the symmetric distribution of the epithelial structure to the daughter cells (see Fig. 6.4 and the “NSC Divisions and Process inheritance” section below).

The cell biological architecture of APs suggests that divisions at the apical surface are necessary for efficient proliferation. This is because the G2 nucleus of APs migrates apically and meets the centrosomes, which are located at the apical domain after the primary cilium has been disassembled. Then, a mitotic spindle forms, and APs can complete M phase. In order to maximize the number of apical mitoses in a rapidly expanding tissue, it appears necessary to have nuclei that have completed mitosis migrate away from the apical surface. This liberates space that can be occupied by the more rounded and wider mitotic cell bodies, which stretch even further parallel to the apical surface during anaphase (Fig. 6.1c). INM may therefore be a key cellular mechanism for the expansion of the AP pool. This could explain why it is advantageous for the nucleus to migrate apically to meet the centrosomes, instead of having the centrosomes migrate basally to reach the nucleus. Such a coordination of INM and M-phase onset could 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. However, the establishment of other proliferative zones in later stages of neurogenesis, such as the SVZ, brought about by the basal migration and division of BPs and bRGs, suggests that even strong AP proliferation near the apical surface of the VZ is not enough to sustain all cortical neurogenesis.

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.

In the case of microtubules, these are the dynein-dynactin motor complex and 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. How is the migration direction controlled? This could be achieved by cell-cycle-dependent switches that 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. 6.2a), 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 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 progression, 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-like BPs is also shorter than in proliferating APs. BPs, however, show a longer total cell cycle, which results mainly from a longer G1. The functional significance of this G1 increase during neurogenesis and in BPs 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* in general 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 surface. 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 is impaired by perturbing fundamental centrosome components, such as pericentrin or microcephaly-related proteins, cell divisions fail to take place normally and the NSC pool can become depleted, which results in reduced neurogenesis. Similar observations have been made when perturbing fundamental microtubule-associated proteins and molecular motors, 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 general requirements have 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 cleavage orientation. While cytokinesis onset and progression are governed by spindle orientation and cleavage furrow positioning and ingression, the completion of cytokinesis is achieved by the fusion of the plasma membranes at both ends of the cleavage furrow. 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, it often fuses with the more 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, neurogenic or gliogenic, division.

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 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. 6.1c and 6.4b, c). Deviations from this cleavage orientation may increase moderately in mid-neurogenesis (Fig. 6.4d), but they very 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 Attachment Inheritance

Both the apical and basal attachments have been implicated in the proliferative capacity of APs. Cells that maintain both of these attachments, such as APs, or at least one, such as bRGs, have in general a higher self-renewal potential than those that lose both, such as the neurogenic BPs. 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, but its bisection in such symmetric divisions is a more difficult task, given its increased length. However, at least some of the early APs do manage to bisect it before and during early neurogenesis, ensuring that both daughter cells immediately inherit a basal contact, without having to regrow one (Fig. 6.4b).

This immediate inheritance of the entire apical-basal architecture, including both attachments and the adherens junctions, is important to maintain the neuroepithelial structure and thus favor an efficient proliferation in the early stages of CNS development. It could also help to explain why the unidirectional cleavage furrow

ingresses exclusively in a basal-to-apical direction (Fig. 6.4), rather than the lateral direction seen in *Drosophila*. A bidirectional cleavage furrow along the highly elongated processes of such cells is also more difficult to achieve and coordinate. During more advanced stages of neurogenesis, the longer basal process is more unlikely to be bisected. Hence, if an AP were to undergo a symmetric proliferative division, one of the AP daughters would have to regrow a basal process for it to remain attached to the basal lamina. It has been proposed that the regrowth of the basal process 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. 6.4d). These 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 finite number of rounds.

A generalized effect of cleavage orientation and attachment inheritance across neurogenesis remains unclear. This is also because the results of experimentally perturbing cleavage orientation seem to heavily depend on which components have been perturbed. For example, the effects on neurogenesis of perturbing LGN are minor, whereas those of perturbing Lis1 or the Lfc-mediated cortical regulation of RhoA both heavily reduce neurogenesis. It is possible that the different genetic perturbations used in those studies also have effects other than just increased spindle orientation variability, which could explain the different outcomes. In addition, the differential activation of compensatory mechanisms could impact each particular case.

Together, these findings are consistent with the idea that the inheritance and maintenance of general epithelial features is important to keep the proliferation capability and at least to some extent also the self-renewal capacity. This is also supported by studies showing loss of proliferation and cortical tissue structure when cell polarity and junctions are acutely perturbed. Also, the apical contact remains important for those NSCs that persist throughout embryonic neurogenesis and remain in the SVZ of the adult brain. The specific contributions of each component and attachment, 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 lateral 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 and leads to the commitment of the progenitor cells, limiting the kinds of daughter cells they can generate. In the context of the developing cortex, this 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; and (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 neuroepithelium. 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 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 astroglial cell types.

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 beta-catenin stability, which is also 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 bFgf (basic FGF 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 (TF) 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 specifically 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, 2, and 3), as well as gliogenesis. Proneural TFs also determine neuronal subtype specification. For example, Ascl1 induces differentiation into cortical pyramidal neurons, while Ngn2 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 (Olig2, Nkx2.2, Ascl1) and astrocyte-specific (SCL/Tal1) 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 and epigenetic modulators.

MicroRNAs

MicroRNAs (miRNAs) are 20–25 nucleotide-long non-coding 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 such as Bmi-1, a member of polycomb repressor 1, and

High-mobility group AT-hook 2 (Hmga2) 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 remodelling, seem to play a similar role.

Radial Glial Cells Link Embryonic and Adult Neurogenesis

In general, adult NSCs are located in restricted regions of postnatal and adult brains and produce both glia and neurons. Adult CNS neurons in mammals arise mainly in the SVZ of the lateral ventricles and migrate to the olfactory bulb where they continually replace local interneurons. Neurogenesis also continues in the hippocampus, in the subgranular zone (SGZ) of the dentate gyrus.

Adult NSCs are related to embryonic NSCs. Just as the RG cells in the embryonic VZ, NSCs in the adult SVZ 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 compartments, 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 the RG cells, the apical end feet of these adult NSCs have specialized apical junctions and a primary cilium. They may therefore receive signals from the CSF in ways similar to the embryonic RG cells. The shared basic properties between adult NSCs in the SVZ and embryonic RG cells suggest that adult SVZ NSCs are modified RG cells that retain stem cell function throughout life.

The second area where adult neurogenesis occurs is in the SGZ of the dentate gyrus of the hippocampus. Adult SGZ NSCs also have some RG-like features, and since they originate from 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 transverse the granular cell layer, contacting the so-called molecular layer that overlays it, while 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 BPs), 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 for their functions in tissue

and in vivo. We have reviewed the current knowledge on the various 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 progenitor subtype. We also do not know what determines the size of the different progenitor populations, or how many rounds of division each progenitor 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 many neurodegenerative disorders and lesions of the nervous system.

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