Neural stem cells (NSCs) are primary progenitors that give rise to neurons and glia in the embryonic, neonatal and adult brain. In recent years, we have learned three important things about these cells. First, NSCs correspond to cells previously thought to be committed glial cells. Second, embryonic and adult NSCs are lineally related: they transform from neuroepithelial cells into radial glia, then into cells with astroglial characteristics. Third, NSCs divide asymmetrically and often amplify the number of progeny they generate via symmetrically dividing intermediate progenitors. These advances challenge our traditional perceptions of glia and stem cells, and provide the foundation for understanding the molecular basis of mammalian NSC behavior.

Introduction

The central nervous system (CNS) develops from a small number of highly plastic cells that proliferate, acquire regional identities and produce different cell types. These cells have been defined as neural stem cells (NSCs) on the basis of their potential to generate multiple cell types (e.g. neurons and glia) and their ability to self-renew in vitro. However, this definition does not necessarily reflect the behavior of these cells in vivo. In the brain, it appears that NSCs are specified in space and time (see section below on ‘The developing NSC’), becoming spatially heterogeneous and generating a progressively restricted repertoire of cell types. It is also not clear how strictly NSCs self-renew in vivo, since their potential progressively changes and they generate different progeny over the course of development. In this review we will define a NSC, or primary progenitor, as a neural cell that maintains the potential to generate multiple cell types over long periods of time. NSCs from the same region that maintain these characteristics but change in appearance and/or potential over time are said to belong to the same lineage.

The lineage of mammalian NSCs lining the lateral ventricular wall has now been identified: neuroepithelial cells probably transform into radial glia, which transform into astrocyte-like adult stem cells. This integrated view of NSCs is a dramatic departure from the view that early-embryonic, late-embryonic and adult NSCs are distinct, unrelated populations. There are many other basic properties of stem cells that we are just beginning to understand. NSCs often produce intermediate progenitors that exist transiently and amplify the number of daughter cells produced by a NSC division. We have also learned that in the adult brain, oligodendrocytes are produced not just by restricted progenitors but also by astrocytes residing in a stem cell niche. It has been speculated that these astrocytes or their progeny play a role in brain tumor formation. In this review, we will discuss some of these recent advances and important questions that remain in the field. Particular attention will be given to the murine brain, where much of the work was done.

Primary progenitors

The CNS begins as a sheet of cells made up of primary progenitors known as neuroepithelial cells. The edges of this sheet fold together to form the neural tube, the fluid-filled center of which later becomes the ventricular system and spinal canal. Neuroepithelial cells are radially elongated and contact both the apical (ventricular) and basal (pial) surfaces (Figure 1a). They divide at the ventricular surface, forming a ventricular zone, but pull their nucleus toward the pial surface during interphase. Initially, neuroepithelial cells divide symmetrically to increase the pool of stem cells but later divide asymmetrically, generating a stem cell that remains in the ventricular zone and a daughter cell that migrates radially outward [1*]. The accumulation of daughter cells thickens the developing brain and radially stretches neuroepithelial cells.

By the onset of neurogenesis, neuroepithelial cells were thought be replaced by a different NSC: the radial glial cell [2*]. Like neuroepithelial cells, radial glial cells divide in the ventricular zone and maintain contact with the pial surface via a radially projecting basal process (Figure 1b). Since they share many characteristics (reviewed in [3,4]), it is likely that neuroepithelial cells transform directly into radial glial cells, although this has not been shown experimentally. Though it is...
Neural stem cells (NSCs) and their progeny in the developing forebrain. The NSCs (shown in blue) of the lateral ventricular wall change their shape and produce different progeny as the brain develops. They begin as neuroepithelial cells and transform into radial glial cells, which mature into astrocyte-like cells. NSCs maintain contact with the ventricle, into which they project a primary cilium. The potential of an individual stem cell in vivo is not known and the progeny shown in this schematic are produced by the NSC population. Stem cells produce progeny either directly or via an intermediate progenitor (shown in green), which has been either included or omitted for clarity. Different types of progeny may be produced by different intermediate progenitors, although just one is shown here. (a) At early developmental stages the CNS is a tubular structure. It is made up of neuroepithelial cells, which divide symmetrically at the ventricular surface to expand the stem cell pool. At this time, some early-born neurons such as Cajal-Retzius cells are produced. (b) Neuroepithelial cells probably differentiate into embryonic radial glial cells, which divide to generate striatal neurons and oligodendrocytes either directly or via an intermediate progenitor in the subventricular zone (SVZ). The radial processes of radial glial cells support the migration of neuroblasts (shown in red). (c) Radial glial cells persist in the neonatal brain, where they generate oligodendrocytes, olfactory bulb interneurons, and ependymal cells. They also generate astrocytes, some of which remain stem cells in the adult. (d) In the adult brain, neurogenic astrocytes often retain a radial process and contact both the ventricle and the basal lamina of blood vessels. They generate oligodendrocytes and olfactory bulb interneurons. Stri, striatum; VZ, ventricular zone.
expression of glial fibrillary acidic protein (GFAP) [9,10,11]. Neurogenic astrocytes are principally found in two regions: the subventricular zone of the lateral wall of the lateral ventricle (Figure 1d) and the subgranular zone of the dentate gyrus of the hippocampus. The astrocytic nature of NSCs has been confirmed in work in which GFAP+ cells were conditionally ablated in the adult mouse brain, resulting in an almost complete loss of neurogenesis [10,11]. By contrast, there is no support for the claim that the ependymal cells lining the lateral ventricle are stem cells. Ependymal cells are post-mitotic and are not generated in the adult [12] even if stimulated to proliferate with exogenous growth factors [13].

Since radial glial cells and subventricular zone astrocytes share many properties, it has been hypothesized that they belong to the same lineage [14]. In particular, radial glial cells of the neonatal lateral ventricular wall (Figure 1c) occupy the same region as the astrocytic stem cells of the adult subventricular zone (Figure 1d). This hypothesis was confirmed in a recent study in which radial glial cells were specifically labeled using a Cre-lox-based strategy [15]. This work showed that labeled radial glial cells generate neurons, oligodendrocytes, ependymal cells and parenchymal astrocytes, as well as subventricular zone astrocytes that act as NSCs both in vivo and in vitro. Radial glial cells may directly transform into subventricular zone astrocytes, since some labeled subventricular zone cells retain a radial process and express GFAP [15]. In summary, the current evidence suggests that NSCs gradually transform from neuroepithelial cells to radial glial cells to astrocyte-like cells (Figure 1).

The recognition that cells of this neurogenic lineage have glial characteristics has changed our perception of glia [16–18]. However, we still do not know the fundamental characteristics that distinguish neurogenic astrocytes from the vast population of non-neurogenic astrocytes elsewhere in the brain. It may be possible to identify and purify these two populations using a combination of astrocytic markers and markers that are enriched in adult stem cells. Comparing the gene expression profiles of these purified populations may shed light on the molecular basis of stem cell behavior.

**Intermediate progenitors**

Primary NSCs persist in the ventricular zone of the developing brain through asymmetric self-renewing divisions. Intermediate progenitors, on the other hand, divide symmetrically in a region just above the ventricular zone known as the subventricular zone [19,20]. This division in the subventricular zone amplifies the number of cells produced by a given NSC division and may be an important determinant of brain size, since species with larger brains have a larger pool of intermediate progenitors [21]. We have learned much about intermediate progenitors from time-lapse imaging studies, which reveal the dynamic appearance and behavior of NSCs and their progeny.

Haubensak and colleagues used time-lapse imaging to follow cells expressing GFP under the Tis21 promoter, which is specifically active in neurogenic cells. Labeled cells were present not only in the ventricular zone but also in the subventricular zone or basal ventricular zone from the beginning of neurogenesis. They found that primary NSCs divide asymmetrically in the ventricular zone while intermediate progenitors divide symmetrically in the subventricular zone [19]. This conclusion is supported by an earlier study by Noctor and colleagues, who followed cells in the developing cortex expressing high levels of cytoplasmic GFP. This allowed detailed morphological analysis of both parent and daughter cells, and revealed a curious phenomenon. When intermediate progenitors reach the subventricular zone, they send out numerous processes before they divide symmetrically, as if sensing local factors [19].

Environmental factors do indeed influence adult NSCs and intermediate progenitors. In the subventricular zone, these cells are also known as type B and type C cells, respectively. Among the many factors thought to regulate neural progenitors (reviewed in [22–24]) are, unexpectedly, neurotransmitters. Synaptically released dopamine stimulates C cell proliferation via the D2 receptor [25]. On the other hand, B cells are inhibited by γ-aminobutyric acid (GABA) non-synaptically released from newly born neurons in the subventricular zone [26].

While it is unclear whether C cells are specified by environmental cues or by their parent NSC, it is clear that they are a heterogeneous population. Whereas most, if not all, adult C cells express the transcription factor Mash1 [27] and many C cells produce neuroblasts, some express Olig2 and appear to produce oligodendrocytes [28,29] (Figure 1d). Olig2 is a basic helix–loop–helix transcription factor required for oligodendrocyte and motor neuron production. Recently, it has been shown to induce the production of oligodendrocytes [28,30] and perhaps astrocytes [30] in the postnatal subventricular zone. Olig2+ C cells may be derived from a subset of subventricular zone astrocytes that express PDGFα, a receptor for platelet-derived growth factor (PDGF) [31]. PDGF stimulates the proliferation of the widely dispersed but glial-restricted oligodendrocyte precursor cells (OPCs) [32].

Interestingly, PDGFα+ cells in the subventricular zone hyperproliferate in vivo in response to exogenous PDGF and form tumor-like masses of intermediate progenitor-like cells, many of which are Olig2+ [31,33]. Many human brain tumors express Olig2 [34] and have overactive PDGF signaling [35]. These findings are of particular interest since it has been postulated that brain...
tumors are propagated by a cancerous ‘tumor stem cell’ [36]. The precursor to the tumor stem cell has not been identified, but it may be an NSC or intermediate progenitor. NSCs that have acquired tumor suppressor gene mutations are poised to hyperproliferate in response to a second insult [37,38] and brain tumors are frequently found near neurogenic niches [39]. Understanding the role of neural progenitors in tumor formation may lead to more effective treatment for brain tumors and uncover the mechanisms that regulate stem cells in the normal and diseased brain.

The developing neural stem cell
Mammalian NSCs produce different cell types at different points in development. The degree to which older stem cells retain the ability to generate earlier-born cell types remains a fundamental question since certain NSCs might be used to replace brain cells lost in trauma or disease. To answer this question, we must first understand how NSCs change over time and discover the mechanisms underlying these changes.

Over the course of development, NSCs change their morphology and produce different progeny, but also change their gene expression profiles [40]. Though some genes are common to both young and old NSCs, others are expressed only at certain time points. Temporally specific genes may coordinate an intrinsic developmental program, since progenitors grown in culture produce certain progeny only when grown with specific progenitors in vivo [41**]. This program seems to run forwards, but not backwards. After several days in vitro, cortical progenitors are unable to produce earlier-born cell types even when co-cultured with younger progenitors [41**], suggesting that NSCs cannot be respecified by environmental cues. This phenomenon has been described in fruit flies [42] and in the vertebrate retina and CNS [43*] where NSCs become unresponsive to the environmental cues that specified them earlier.

Such commitment, or resistance to respecification, can be tested by challenging NSCs with heterochronic or heterotopic transplantation. Recent work shows that postnatal cerebellar progenitors only give rise to postnatally born cell types when transplanted into the embryonic brain, whereas transplanted embryonic progenitors give rise to all appropriate cell types [44]. This finding holds true even when the progenitors are grown in vitro before transplantation [45]. These studies confirm the classic work in ferrets showing that cortical progenitors are increasingly committed over time [46].

The mechanisms of mammalian NSC commitment are largely unknown, but may include DNA and histone modification and/or changes in transcription factor expression and chromatin structure (reviewed in [47,23]). Our molecular understanding of invertebrate stem cell specification and commitment is based on our identification of their stem cell lineage. Now that the mammalian NSC lineage is known, perhaps similar advances can be made in vertebrate stem cell biology.

To understand how the potential of lineally related stem cells changes over time, one could label individual NSCs at different developmental stages and trace their progeny in vivo. Alternatively, NSCs could be labeled in the early embryo, isolated after defined time points, and either transplanted or grown in culture. At the same time points, the gene expression profiles of these NSCs could be determined and correlated with their potential. Such approaches would advance our understanding of the molecular basis of NSC specification and commitment. This knowledge is essential to effectively design and use NSC-based therapies.

Conclusions and perspectives
Though recently we have greatly advanced our understanding of mammalian stem cells, there are still many unanswered questions. What is the in vivo potential of a NSC, and how does it differ from what we see in vitro? What distinguishes a neurogenic astrocyte from an astrocyte elsewhere in the brain? What factors permit a cell to continue acting as a stem cell? What factors specify NSCs, and what mechanisms are responsible for their commitment? Is their commitment reversible? Can NSCs be induced to generate a different repertoire of cell types? To answer these questions, it is important to have a solid grasp of NSC identity and behavior. The identification of the neural stem cell lineage and the intermediate progenitors they produce is an important step in this direction.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


   This paper, along with [19**,20], demonstrates that intermediate progenitors generate neurons via symmetric divisions in the ventricular zone from early developmental stages.


   Contrary to reference [3], this paper suggests that radial glial cells are the principal stem cell of the embryonic mouse brain.

Neural stem cells in mammalian development

Merkle and Alvarez-Buylla


This work, along with reference [10], demonstrates that GFAP+ cells are the principal stem cells of the adult forebrain, since neurogenesis is effectively eliminated when GFAP+ cells are conditionally ablated.


This paper demonstrates that radial glia of the neonatal mouse striatum generate the neurogenic stem cells of the adult mouse subventricular zone. Both of these cell types generate neurons in vivo and act as stem cells in vitro and thus are linearly related NSCs.


This work clearly illustrates not only the different division modes of primary and intermediate progenitors, but also the complex morphological changes they and their progeny undergo. Unexpectedly, intermediate progenitors pause in the subventricular zone and extend numerous processes. Neuroblasts do the same when they reach the SVZ, after which they sometimes migrate back down toward the ventricle before changing course again and continuing up to the cortical plate. The role of this behavior is unknown.


This paper demonstrates that the proneural basic helix-loop-helix transcription factor Mash1 is expressed in the adult mouse brain and specifies neurons and oligodendrocytes. Mash1 expression is largely eliminated after treatment with the antimitotic drug Ara-C but reappears with the same timecourse as the rapidly proliferating intermediate progenitor (type C) cell, suggesting that these are the primary Mash1 expressing cell type in the adult subventricular zone.


This work describes a subset of astrocytic subventricular zone stem cells that produce neurons and oligodendrocytes and express the PDGF receptor alpha. In response to exogenous PDGF, these NSCs proliferate rapidly and produce brain tumor-like growths. The production of neurons is diminished in response to PDGF, suggesting that PDGF drives multipotent adult NSCs to produce oligodendrocytes rather than neurons.


6 Cell differentiation


Zhu et al. show that mice lacking two functional tumor suppressor genes form glioblastoma-like tumors. These tumors seem to initiate in or near the adult subventricular zone. This work suggests that the source of brain tumors might be neural stem cells or their progeny.


This paper convincingly shows that cortical progenitors have an intrinsic developmental program which progresses in vitro as well as in vivo. Cells isolated from older animals and cultured with younger cells were unable to adopt the greater plasticity of their younger neighbors, suggesting that the restriction in stem cell potential is irreversible, at least by factors present in the embryonic brain. Knocking down expression of the transcription factor Foxg1 was able to induce mid-gestation progenitors to produce some earlier-born cell types, but the potential of older ones remained unaffected.


In this detailed review, Pearson and Doe describe and compare the temporal specification of NSCs in different regions of the mammalian and invertebrate nervous systems. Many mechanisms appear to be conserved, including the stereotyped production of different progeny at different time points and the progressive loss of progenitor potential.


