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# Astrocyte Development

Steven W. Levison, Jean de Vellis, and James E. Goldman

## INTRODUCTION

Astrocytes were first identified at the end of the 19th century as star-shaped glia within the CNS. Over the last century we have come to appreciate the myriad functions that they perform to maintain homeostasis in the brain (Andriezen, 1893; Lenhossék, 1895; Cajal, 1909). Great strides also have been made in understanding their origins from the neural ectoderm and the events that shape their development. We will begin by first reviewing the functions and types of astrocytes in the mature brain and then proceed to describe when they arise, where their precursors reside, how they migrate, and which intrinsic and extrinsic factors shape their development.

## FUNCTIONS AND TYPES OF ASTROCYTES IN THE CNS

In this chapter, we will define an astrocyte as a mature cell that possesses specific attributes and performs specific functions in the mature CNS. The functions of astrocytes include regulating extracellular ion concentrations, detoxifying xenobiotics, modifying synaptic efficacy, inactivating neurotransmitters, inducing and maintaining the blood–brain barrier and glial limitans, and providing nutrients and trophic support for neurons and oligodendrocytes. In the adult CNS, astrocytes are positioned rather uniformly across the parenchyma of the CNS where they associate with blood vessels, the pial surface, neurons, oligodendrocytes, and other astrocytes (Fig. 1).

Astrocytes are not randomly dispersed across the CNS, but rather form a "matrix" in which their cell bodies are evenly separated in space by a distance of approximately 50 μm (Chan-Ling and Stone, 1991; Levison and Goldman, 1993; Tout *et al*., 1993). It has been proposed that astrocytes contact each other during development and establish individual domains (Bushong *et al*., 2002). Astrocytes that contact each other form gap junctions, which allow them to communicate. These gap junctions are formed by connexins which allow calcium and other small molecules to flow between the cells. As a consequence of these contacts, the astrocytes form a syncytium that enables intracellular signals or ions to flow across rather large regions of the CNS. In addition to contacting each other, astrocytes create functional connections with the endothelial cells that line the blood vessels of the brain. Indeed, the blood vessel end foot specialization is one of the distinguishing characteristics of a mature astrocyte. The basal lamina secreted by the endothelial cells, as well as the basal lamina formed by the fibroblasts located at the pial surfaces of the brain are covered by end feet processes of astrocytes (Peters *et al*., 1976). Astrocytes also invest neuronal cell bodies, their dendrites, and synapses (Kosaka and Hama, 1986; Hama *et al*., 1993; Ventura and Harris, 1999). At these contacts, there are bidirectional signals that regulate synaptic efficacy and enhance the metabolism of the astrocytes (Haydon, 2001). The formation of these various types of cell contacts must be accounted for in a developmental model for astrocyte genesis.

### Differences in Astrocyte Types in Different Regions

#### Types of Astrocytes *In Vivo*

The broad category of cells referred to as astrocytes is traditionally subdivided into the subtypes of fibrous or protoplasmic on the basis of morphological criteria as well as by their localization to white matter or gray matter, respectively (Figs. 2 and 3). In the cerebellum, the Bergmann glia represent an additional astrocyte form. In brain regions other than forebrain, cerebellum, and spinal cord there are a number of other specialized CNS cells that share some characteristics with astrocytes. Included are pituicytes, tanycytes, ependymal cells, and Mueller

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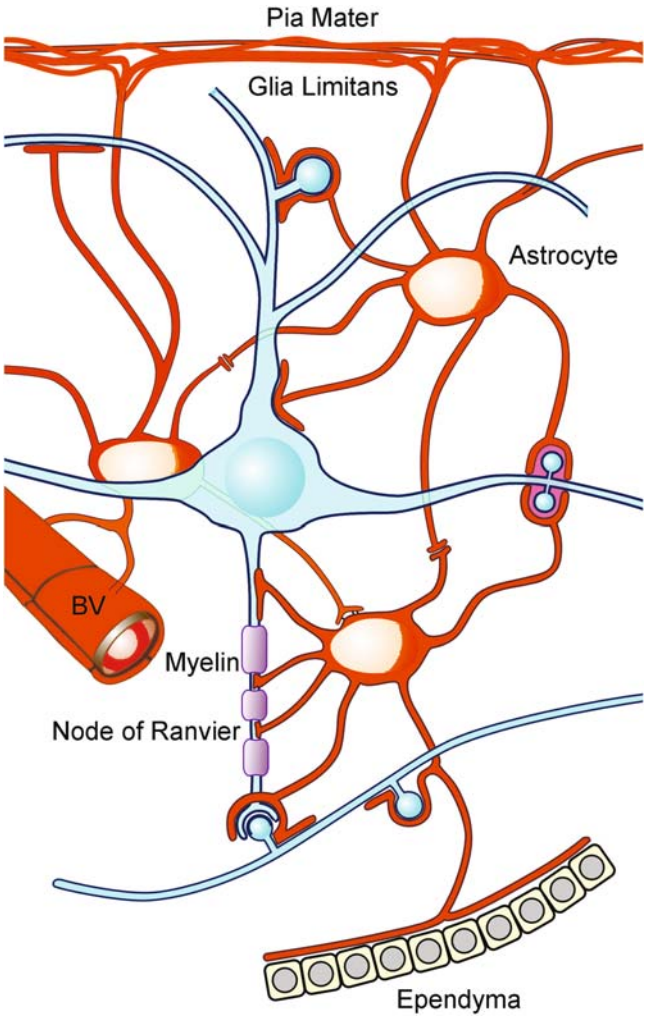


**FIGURE 1.** Astrocytes. In the adult CNS, astrocytes interact with multiple cell types. As depicted in this schematic, they form end feet on capillaries that induce the blood–brain barrier properties of the cerebral microvasculature. They interact with the cells along the pial surface, as well as at the ventricular lumen, and their processes interdigitate among the neurons, synapses, nodes of Ranvier, and oligodendrocytes. Additionally, they form gap junctions with other astrocytes.

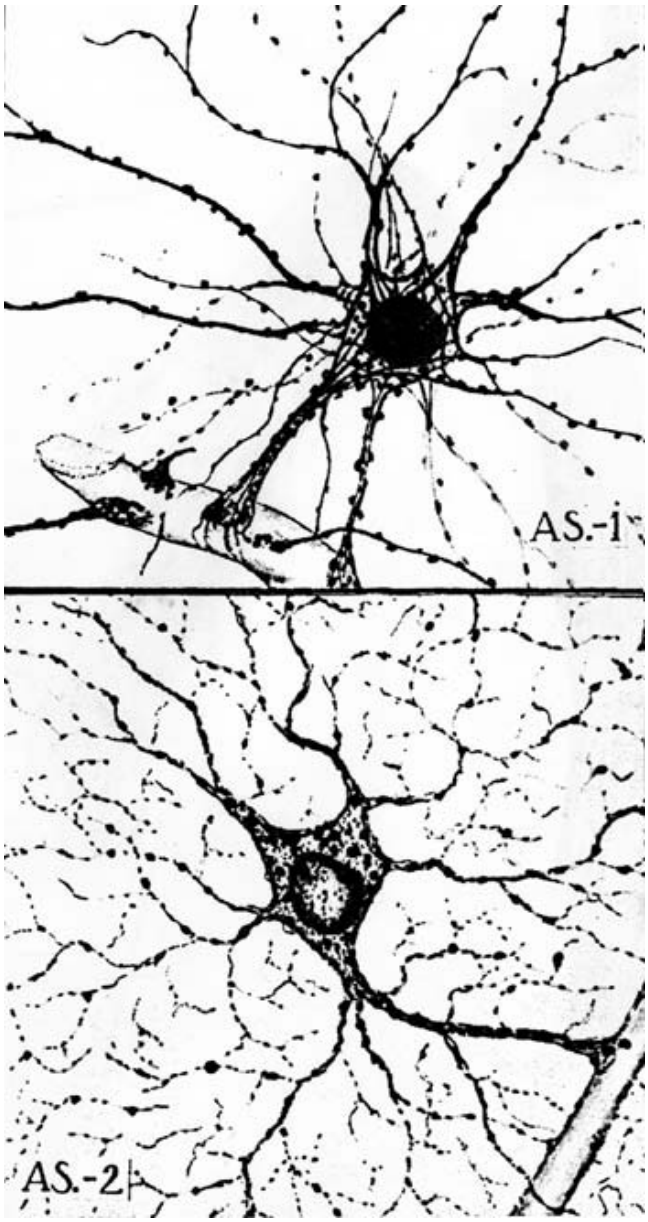


**FIGURE 2.** Types of astrocytes. Classical studies of astrocytes used metal impregnation techniques to reveal their structure. Histologists divided astrocytes into two main categories on the basis of their morphology. Reproduced here are two camera lucida drawings made by Wilder Penfield of a protoplasmic astrocyte and of a fibrous astrocyte (Penfield, 1932). Reproduced with permission.

glia. These glial cells will not be discussed at greater length; however, articles describing their development and functions can be found in the *Neuroglia* textbook (Kettenmann and Ransom, 1995).

Fibrous astrocytes have a predominantly star-like morphology, with many cylindrical processes radiating symmetrically from the cell soma. These processes extend for long distances, branch infrequently, and contain abundant intermediate filaments. They frequently form end feet on capillaries and at the nodes of Ranvier. Fibrous astrocytes have oval nuclei containing evenly dispersed chromatin. In electron microscopic preparations, their cytoplasm is lightly tinted, with scattered glycogen granules and a relatively low density of organelles.

Protoplasmic astrocytes have a more complex morphology than fibrous astrocytes. Their processes are highly branched and form membranous sheets that enfold neuronal processes and cell bodies; they also form end feet on capillaries and at the pial surface. Protoplasmic astrocytes have spherical to oval nuclei, with slightly clumped chromatin. At the electron microscopic level their cytoplasm is lightly tinted; it contains glycogen and some microtubules. Compared to fibrous astrocytes, protoplasmic astrocytes have fewer intermediate filaments and a greater density of organelles. Whether the more complex morphology of protoplasmic astrocytes is an intrinsic property of the cell or

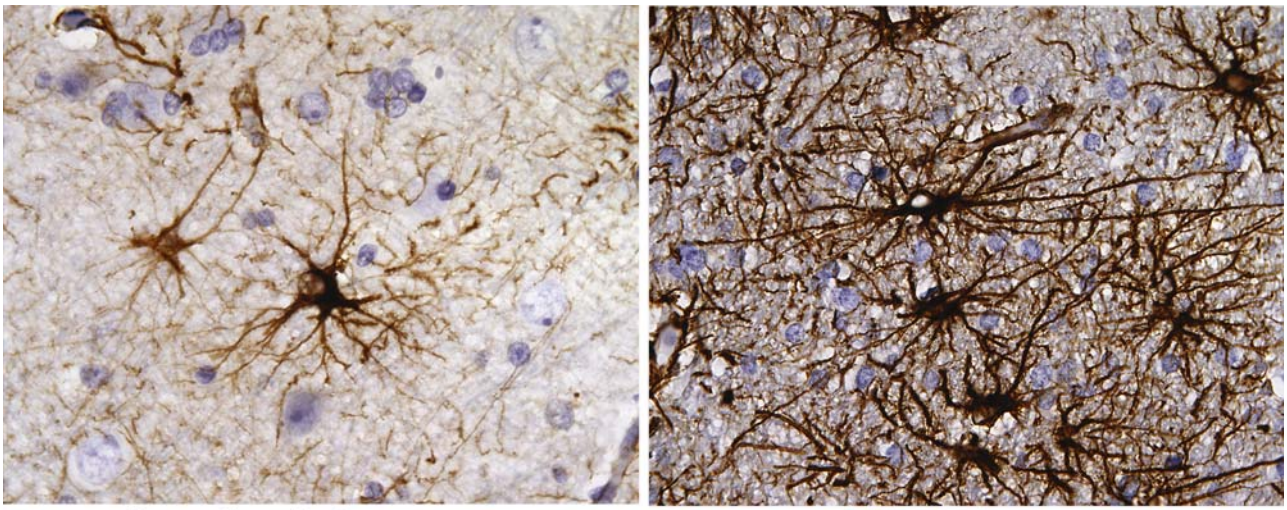


**FIGURE 3.** Human astrocytes stained for GFAP with a hematoxylin counterstain. Modern studies of astrocytes use immunohistochemical techniques to reveal their structure. Depicted here are protoplasmic astrocytes from the gray matter and fibrous astrocytes from the white matter stained for GFAP (brown) with a light hematoxylin counterstain (blue). Provided by S. W. Levison.

whether this form adopted as a consequence of the constraints of gray matter neuropil is not known. Since astrocytes can assume stellate shapes *in vitro* (see below), the process-bearing morphology of astrocytes is likely to reflect intrinsic biochemical and cytoskeletal properties.

Another astrocyte subtype known as the Bergmann glia, or Golgi epithelial cell, resides in the cerebellar cortex. The cell bodies of the Bergmann glia are present in the Purkinje cell layer, and these cells extend several long processes through the molecular layer, ending at the glial limitans of the pial surface and large blood vessels. Their processes ensheath Purkinje neurons and they send horizontal, lamellate expansions as they ascend through the molecular layer. These cells have a pale, bean-shaped nucleus that is usually oriented perpendicular to the pial surface. Their cytoplasm is typically pale, containing intermediate filaments, randomly oriented microtubules, glycogen, and scattered ribosomes.

The separation of astrocytes into the subcategories "fibrous" and "protoplasmic" has merit, but it is simplistic. For instance, Retzius (1894), drew images of at least six different types of astrocytes on the basis of Golgi metal impregnation staining, and Ramon-Molinar (1958) described cells stained with del Rio Hortega's modification of the Golgi method that displayed the star-like morphology of astrocytes, but had fewer processes, some of which were arranged in parallel, resembling those of oligodendrocytes. Additionally, there are astrocytes in white matter with a more protoplasmic topology, and astrocytes with mixed fibrous and protoplasmic features. Furthermore, classical histologists have described cells intermediate in form between oligodendrocytes and astrocytes that they have referred to as "transitional" neuroglia (Penfield, 1924; Wendell-Smith *et al*., 1966). The terms "fibrous" and "protoplasmic" continue to be used because the morphological distinctions are generally sound, and ultrastructural observations tend to confirm the separation of astrocytes into these two groups. However, the extent to which these differences are intrinsic, lineage-dependent properties, or are conferred upon the cells by the environments in which they reside is unknown. Whether "fibrous" and "protoplasmic" astrocytes are functionally distinct and whether they arise from the same progenitors are also unresolved issues.

## Astrocyte Development Is Not Uniform Across Different Regions of the CNS

Studies carried out in a variety of regions in the mammalian CNS have revealed that glial development does not follow one pattern. There are notable anatomic differences among regions of the developing CNS and consequently there are multiple mechanisms of astrogliogenesis. For example, in most regions of the CNS, astrocytes arise from precursors that are direct descendents of the primitive neuroepithelium (the ventricular zone, VZ), such as in the spinal cord. Cells in the VZ can produce astrocytes directly. However, in other regions of the CNS where there is a subventricular zone (SVZ), some astrocytes arise from the precursors of the SVZ. In regions of the CNS where there is no SVZ, such as the optic nerve, there is strong evidence that the astrocytes are generated in a single wave, consistent with there being a single astrocyte lineage in these regions (Skoff, 1990). However, in regions of the CNS where there are multiple germinal zones, there may be two waves of astrogliogenesis. Thus, a conclusion that may be reached is there are more types of astrocytes in the forebrain (where there is an SVZ) than in the optic nerve. Below we will review in more depth the development of astrocytes within the optic nerve, cerebellum, spinal cord, and forebrain.

## Molecular Markers for Astrocytes and Their Developmental Expression

In order to trace the development of an astrocyte from a precursor, one must have specific criteria that can be reliably used to classify different mature cell types. A number of immunological markers have been used over the years to identify specific cell types. Astrocyte intermediate filaments are composed of glial fibrillary acidic protein (GFAP), a protein enriched in astrocytes in the CNS, and vimentin, a less cell-specific filament protein (Bignami *et al*., 1972; Antanitus *et al*., 1975; Dahl *et al*., 1981a). Thus, a positive immunohistochemical reaction for GFAP has often been used as a major criterion for identifying astrocytes. However, GFAP expression cannot be used as the sole criterion for identifying an astrocyte. For example, in early astrocyte development, vimentin can be the major or only intermediate filament expressed (Schnitzer *et al*., 1981). Furthermore, some gray matter glia with the morphology and ultrastructural characteristics of astrocytes lack intermediate filaments (Herndon, 1964; Palay and Chan-Palay, 1974). Such astrocytes would be GFAP negative. Consistent with this observation, studies on GFAP protein levels and *in situ* hybridization for GFAP transcripts indicate that GFAP is expressed at lower levels in gray matter than in white matter (Kitamura *et al*., 1987). Further restraint is recommended in using GFAP as the gold standard for astrocyte identification since some cells that may wander into the brain, such as a subset of lymphocytes, can express GFAP, and GFAP is also expressed by other cells outside of the CNS, such as myoepithelial cells, osteocytes, and chondrocytes (Neubauer *et al*., 1996; Hainfellner *et al*., 2001). Consequently, molecular markers other than intermediate filaments have been used to define astrocytes. For example, the enzyme glutamine synthetase (GS) is enriched in astrocytes, and fibrous and protoplasmic astrocytes are equally labeled by antibodies to GS (Norenberg and Martinez-Hernandez, 1979). The calcium-binding protein S-100β, and more recently the glutathione-*S*-transferase mu, are also useful markers for astrocytes (Boyes *et al*., 1986; Cammer *et al*., 1989).

Few antigenic markers are absolutely specific. Some gray matter oligodendrocytes and astrocytes, in fact, share a number of markers, consistent with the view that these cell populations might be closely related. For instance, the "oligodendrocytic" markers carbonic anhydrase II (CA) and another glutathione-*S*-transferase form, the π subunit, are expressed at low levels in some gray matter astrocytes in rodent CNS (Cammer and Tansey, 1988; Cammer *et al*., 1989). Furthermore, the "astrocytic" marker GS has been demonstrated in some gray matter oligodendrocytes (D'Amelio *et al*., 1990; Tansey *et al*., 1991). Heterogeneous expression of these enzymes also illustrates the heterogeneity of astrocytes (also see below).

The macroglial cells of the CNS include many distinct types. Among these are several types of astrocytes, as well as progenitor cells. Due to this complexity, a full characterization of any given glial population should be based on a constellation of the attributes described above, including ultrastructure, the presence or absence of "astrocytic" markers (such as GFAP, S-100β, or GS) and the presence or absence of "oligodendrocytic" markers (such as 2′,3′ cyclic nucleotide 3′ phosphohydrolase [CNP] or myelin basic protein), and the absence of markers that are expressed by precursors. Examples of precursor markers could include nestin, vimentin, polysialic acid neural cell adhesion molecule (PSA-NCAM) and the NG2 proteoglycan.

# A SUBSET OF ASTROCYTES ARE DIRECT DESCENDANTS OF THE VENTRICULAR ZONE

## Radial Glia Arise from the VZ

Using metal impregnation staining, Lenhossék (1895) was the first to document the genesis of astrocytes. Using the Golgi method, which stains only a fraction of the cells in nervous tissues, cells were revealed that spanned the entire width of the developing neural tube, from ventricle to pial surface (Fig. 4).

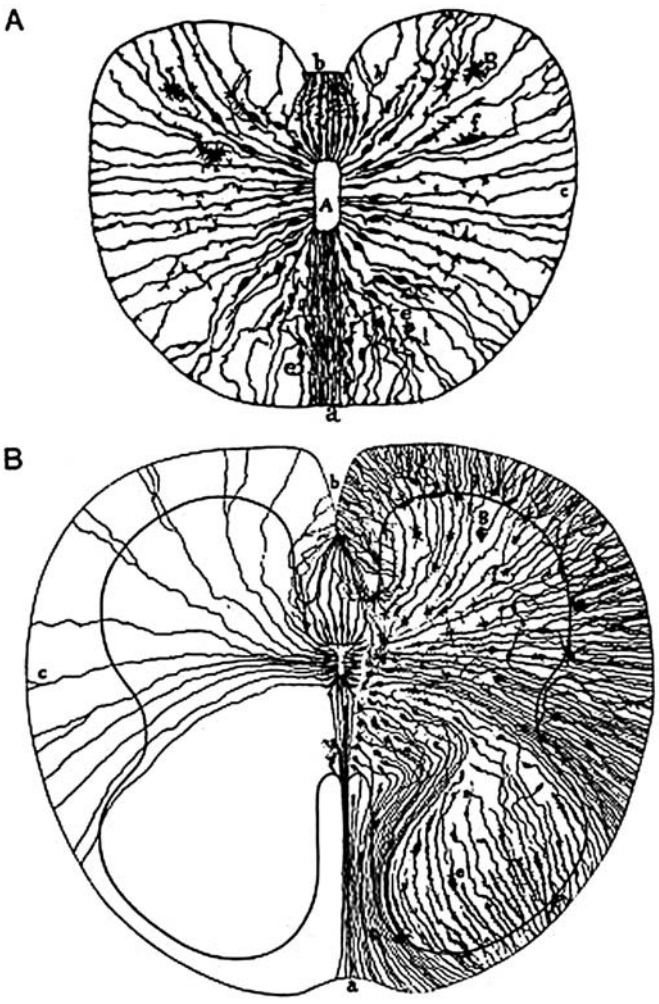


**FIGURE 4.** Radial glial cells in the spinal cord of the 9-day chick embryo (A) and 14 cm human embryonic spinal cord (B). These drawings depict golgi metal impregnation stains of radial glia, migrating precursors as well as radial glial transforming into astrocytes. (A) from Cajal (1894); (B) from Lenhossek (1893). Reproduced with permission.

Lenhossék provided a series of images that documented the transition of cells from these radial forms to cells that had the morphologies of mature astrocytes. The most primitive forms of these cells were classified using a number of terms and eventually given the name of radial glia by Rakic (1995). These radial cells had clearly evolved from the neuroepithelial cells of the VZ. Like earlier neuroepithelial cells these radial cells have processes that span the distance between the ventricular and pial surface, where they form pial end feet. As the CNS develops and more cells are added, the processes of the neuroepithelial cells elongate, but their cell bodies remain confined to the VZ. While these cells are referred to as radial glia, their processes may not, in fact, be strictly radial. Depending upon their location in the CNS, their processes may emerge and radiate perpendicular to the pial surface or they may curve substantially. As illustrated in Fig. 5, those radial glia that are located in the developing dorsomedial cerebral cortex extend processes that are perpendicular to the pial surface. Radial glial cells whose cell bodies reside in the lateral aspects of the VZ extend curvilinear process that first extend ventrolaterally, but then turn at right angles toward the pial surface. Based on both light and electron microscopic images of the developing brain, as well as time lapse video microscopy of brain slices or of immature brain cells *in vitro*, it is clear that the radial glia act as substrates along which cortical neurons migrate during early forebrain development (Rakic, 1971, 1972; Gasser and Hatten, 1990). Migrating neurons adhere closely to the radial glia and the migrating neurons extend a leading process that wraps around the radial glial cell, forming a temporary adhesion to the radial glial cell which facilitates its movement (see Chapter 8).

## The Radial Glial Cell Identity Is Actively Maintained by Neuron–Glial Cell Interactions

Radial glial cells receive signals from migrating neuroblasts that stabilize their status as radial glial cells. This type of signaling was first demonstrated using an *in vitro* paradigm where it was shown that immature granule neurons could induce immature cerebellar glia to adopt a radial glial morphology. To date, two ligand receptor signaling pairs have been discovered that likely mediate this effect; these are Delta/Notch and Neuregulin/erbB receptors (Fig. 6). Notch is a transmembrane receptor that is present on cells in the VZ and Notch is present on

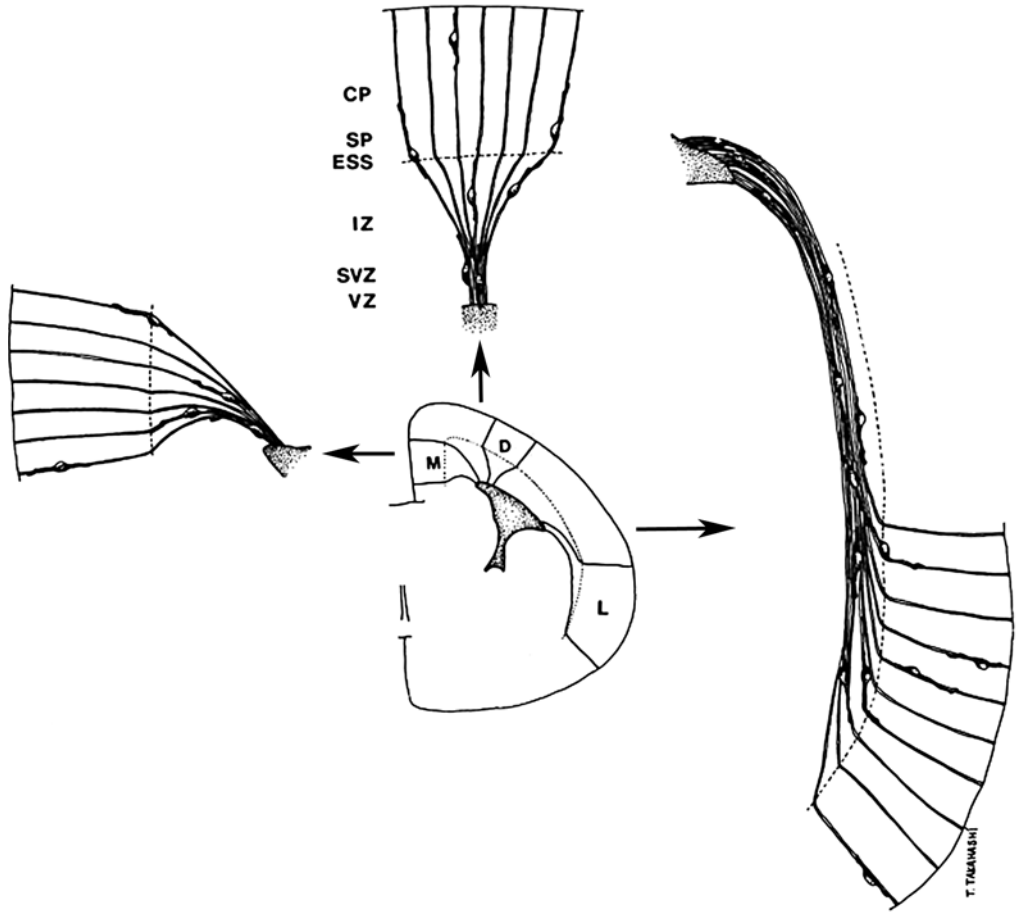


**FIGURE 5.** Radial glia. Schematic representation of patterns of radial glial fiber alignment and neuronal migration in medial (M), dorsal (D), and lateral (L) hemispheric regions. CP indicates cortical plate and SP indicates subplate. The somata of the radial glia are not depicted. From Misson *et al*. (1991), with permission.

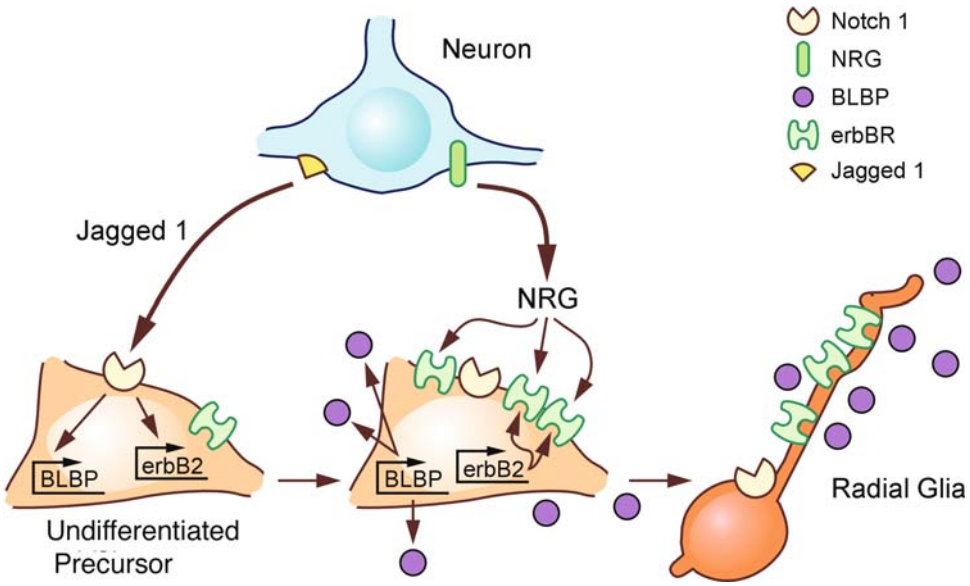


**FIGURE 6.** Neuronal induction of radial glia by sequential signaling through Notch and Erbb pathways. Initial contact by a Jagged1-expressing neuron activates Notch expression in the undifferentiated precursor. Notch signaling then induces expression of brain lipid-binding protein (BLBP) and erbB2 in the glial precursor. The increase in erbB receptor expression enhances the precursor's responsiveness to neuron-derived neuregulin (NRG), which subsequently induces the cell to adopt a radial morphology and to support neuronal migration.

radial glial cells. The ligands for Notch also are transmembrane proteins, and one of the ligands, Delta-1 is highly expressed by immature neurons. As the receptor is present on the radial glia, and the ligands are present on immature neurons, these molecules are appropriately situated to coordinate signals between migrating neurons and the radial glial cells they require to translocate to their appropriate destination. The introduction of an activated Notch receptor using replication deficient retroviruses increases the relative numbers of radial glia (Gaiano *et al*., 2000). As activated Notch receptors increase the numbers of radial glia in the developing brain, this receptor system can provide a signal that maintains the numbers of radial glia needed during CNS development. However, as reviewed below, Notch signaling also can induce the radial glia to differentiate into astrocytes, which demonstrates that the context in which a precursor receives a signal is essential to how it interprets and responds to that signal. This example also demonstrates that no single ligand is responsible for the specification of an astrocyte from a precursor.

An additional ligand–receptor signaling system that has been shown to play a role in maintaining radial glial cell numbers is the neuregulin/erbB signaling pair. Like Notch, erbB receptors are transmembrane signaling receptors that are highly expressed by radial glial cells in the developing brain; however, erbBs activate disparate second messengers than Notch, and thus these receptors are not likely redundant. In the developing cerebellum, Bergmann glia cells express erbB4 whereas in the neocortex the radial glia express erbB2. Migrating neurons in both regions express the ligand for these receptors, neuregulin (Anton *et al*., 1997; Rio *et al*., 1997). Radial glial cells fail to develop normally in erbB2 genetically deficient mice and when antibodies against neuregulin or against erbBs are introduced into radial glial/immature neuron cell cultures, the length of the radial glial cells decreases and these antibodies perturb the migration of the immature neurons on the radial glia. Furthermore, adding neuregulin to these same cultures enhances the length of the radial glial fiber and increases the rate of neuronal migration. As the levels of neuregulin in the CNS decrease concurrent with neuronal maturation, which precedes the maturation of the radial glia (reviewed below), neuregulin/erbB likely play an important role in radial glial cell maintenance and function.

## Radial Glia Express Markers of Immature Astrocytes

Radial glia express a number of molecules that are later shared by immature astrocytes. It is in part through the transient coexpression of these immature and mature cell markers that lineage relationships between radial glia and astrocytes have been deduced. The markers expressed by radial glia include (1) the intermediate filament proteins nestin and vimentin, and in primates, GFAP (Levitt *et al*., 1981; Dahl *et al*., 1981b; Benjelloun-Touimi *et al*., 1985; Hockfield and McKay, 1985; Levine and Goldman, 1988; Tohyama *et al*., 1992), (2) intermediate filament associated protein (IFAP) 300 (Yang *et al*., 1993), (3) brain lipid-binding protein (BLBP) (Hartfuss *et al*., 2001), (4) glutamate transporters, in particular GLAST (Shibata *et al*., 1997), (5) the aldolase isoform, zebrin II (Staugaitis *et al*., 2001; Marshall and Goldman, 2002), (6) the RNA-binding protein Musashi-1 (Sakakibara *et al*., 1996), and (7) the radial cell markers RC1 and RC2 (Culican *et al*., 1990; Edwards *et al*., 1990).

## Radial Glia Transform into Astrocytes at the End of Neurogenesis

The majority of radial glia undergo a transformation during the perinatal period to become astrocytes. As discussed earlier, Lenhossék used Golgi impregnations to reveal "intermediate" or "transitional" forms between radial glia and astrocytes in the early postnatal rodent brain and late gestational primate brain (Cajal, 1909; Choi and Lapham, 1978; Schmechel and Rakic, 1979b; Misson *et al*., 1988, 1991) (Fig. 7). Similar studies have been performed using antibodies to radial glial enriched molecules. These "intermediates" possess long processes that are frequently radially orientated, but they gradually become more complex, as the cells become astrocytic. The accumulation of GFAP in cells with transitional forms was used as supportive evidence for this developmental transition. While these observations do not directly prove a transformation, they are consistent with it. More direct proof that radial glial differentiate into astrocytes comes from two types of experiments. First, radial glia were labeled *in situ* in the neonatal ferret brain by placing crystals of the fluorescent dye, DiI, onto the pial surface and then the development of the labeled cells was followed as they gradually transformed into astrocytes (Voigt, 1989). Second, the transformation of radial glial cells into astrocytes was followed using

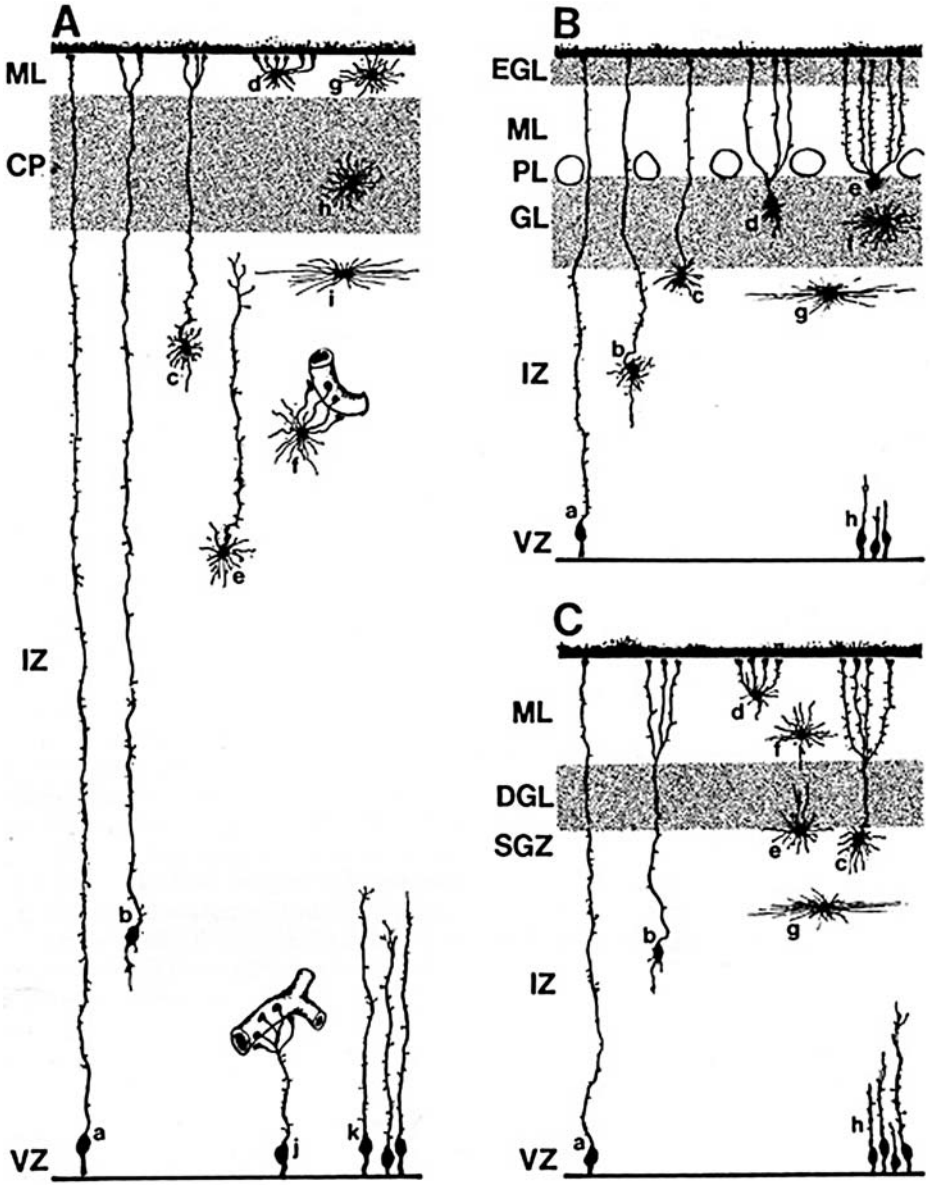


**FIGURE 7.** Radial glia are astrocyte precursors. After facilitating neuronal migration, radial glia differentiate into astrocytes. Depicted are radial glia from the cerebral cortex (A), cerebellar cortex (B), and dentate gyrus of the hippocampus (C). CP, cortical plate; EGL, external granule layer; GL, granular layer; DGL, dentate gyrus; IZ, intermediate zone; ML, molecular layer; PL, Purkinje cell layer; SGZ, subgranular zone; VZ, ventricular zone. From Cameron and Rakic, (1991), with permission.

replication deficient retrovirus infections. This type of analysis uses a retroviral vector to transfer a reporter gene, such as the gene encoding *Escherichia coli* beta-galactosidase, into the genome of dividing cells. Progeny of the infected cell will continue to express the transferred gene (Cepko, 1988; Sanes, 1989). In the case of beta-galactosidase, therefore, the descendants of an infected cell can be detected using either a histochemical stain for beta-galactosidase or by immunofluorescence with specific antibodies. When rat or chick embryos were injected with retroviruses and sacrificed at short intervals, the initial infected cells frequently had the morphologies of radial glial and they expressed markers of radial glia such as RC2 or vimentin. When infected animals were examined at later intervals after infection, many of the cells expressing β-galactosidase had the morphologies of astrocytes (Galileo *et al*., 1990; Gray and Sanes, 1992). Thus, these linage-tracing experiments also established a precursor product relationship between radial glia and astrocytes. A third piece of evidence has been gleaned from cell culture studies where immature astrocytes can be transformed back to radial cells by the addition of neurons (which have neuregulin on their surface) or by the addition of neuregulin.

## Radial Glial Are Bipotential Progenitors for Neurons and Astrocytes

Fate mapping studies using retroviruses have argued for a common progenitor for astrocytes and neurons in the retina (Turner and Cepko, 1987), optic tectum (Galileo *et al*., 1990; Gray *et al*., 1990; Gray and Sanes, 1992), cerebral cortex, and striatum (Halliday and Cepko, 1992; Walsh and Cepko, 1993). Studies that were performed when the retroviral lineage tracing technique was first applied to study CNS precursor differentiation reported that radial glia of the spinal cord and tectum could generate neurons and astrocytes, and the suggestion was made that the radial glia were self-renewing stem cells (Gray and Sanes, 1991) (Fig. 8). This latter hypothesis has been supported by more recent lineage studies and from time-lapse video microscopy to show that embryonic radial glial can divide asymmetrically to generate immature neurons, which then use the parent radial glial cell to ascend into the developing cortical plate (Malatesta *et al*., 2000; Noctor *et al*., 2001; Campbell and Gotz, 2002). When these radial guides are no longer required for neural precursor migration, most of the radial glia differentiate into astrocytes.

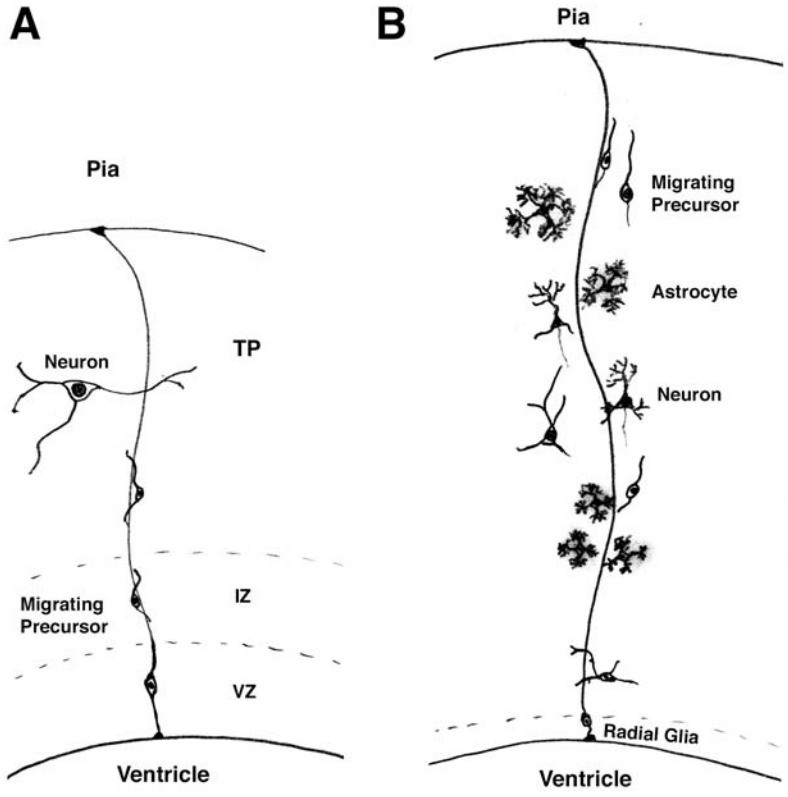


**FIGURE 8.** Radial glia are bipotential stem cells. Replication deficient retroviruses were injected into the tecta of early chick embryos to label dividing cells in the ventricular zone (VZ) and then examined over time to establish which cells were descended from the infected cells. (A) As depicted here, when analyzed within a few days of infection, clones of cells derived from the infected cells contained radial glia, cells migrating through intermediate zone (IZ) toward the tectal plate (TP) and the occasional neuron. (B) When analyzed more than a week after infection, the clones still contained one radial glial cell, but they also contained several types of neurons as well as astrocytes. Adapted from Gray and Sanes (1992).

## Some Radial Glia Persist into Adulthood in Rodents and Human

Some glial cells with radial morphologies persist into adulthood. These include the Bergmann glia of the cerebellar cortex, radial glia in the hippocampus, and radially oriented glia found in the brainstem and spinal cord (King, 1966; Flament-Durand and Brion, 1985; Mori *et al*., 1990; Reichenbach, 1990). Bergmann glia maintain their connections to the pial surface, while many of the radial glia in the brainstem and spinal cord maintain connections to the ventricular surface. These latter cells have been classically termed "tanycytes." Some of these radial cells guide migrating neurons during their development from the germinal zones to their final laminar locations. For example, cerebellar granule cells migrate from the external to the internal granule cell layer along Bergmann glial processes. Other radial glia in the adult brain function as conduits for ions or small proteins from the cerebral spinal fluid into the brain (Mori *et al*., 1990). Recent studies have suggested that some of the residual radial glia, especially those in the hippocampus and related cells in the SVZ, may have stem cell properties. That is, they retain the developmental plasticity of radial glia of the embryonic CNS.

In an experiment to determine whether those cells in the SVZ that retained the features of radial glia are indeed neural stem cells, cytosine arabinoside (Ara-C), a potent antimitotic drug, was infused onto the surface of the brain of adult CD-1 mice for six days. This treatment effectively eliminates all mitotically active cells. However, within 10 days of the ceasing the Ara-C treatment, the SVZ fully regenerates, indicating that neural stem cells are resistant to Ara-C. Those cells that were resistant to Ara-C showed the ultrastructural and antigenic features of radial glia and they were the first cell type to divide after halting the Ara-C treatment (Doetsch *et al*., 1999). Given their location and antigenic features, these stem cells are likely derivatives of the immature neuroepithelial cells of the VZ, although this lineage relationship has not been proven. Similarly, an immature astrocytic population that resides in the subgranular zone of the hippocampus generates neuroblasts that migrate into the dentate gyrus, where they differentiate into granule neurons (reviewed in Fabel *et al*., 2003). These data indicate that the neural stem cells that persist in the mature brain retain properties of radial glia. It would be, however, a mistake to conclude that all of the immature astrocytes throughout the CNS can function as neural stem cells as those cells that leave their niche near the ventricles lose their stem cell properties. One question that remains unresolved is whether these persistent radial glia are bipotential precursors that produce neurons and astrocytes like their predecessors during development, or whether they are multipotent neural stem cells that are capable of generating all three major CNS cell types.

# OTHER ASTROCYTES ARE DIRECTLY DESCENDED FROM THE SUBVENTRICULAR ZONE

The SVZs of the forebrain and cerebellum become prominent in late gestation and are highly productive. Indeed, during late gestation and during the first few weeks of postnatal life, the SVZs are the major sources of new astrocytes and oligodendrocytes. The SVZs contain large numbers of highly proliferative cells that are easily labeled by markers of DNA synthesis. Indeed, classic studies of forebrain gliogenesis inferred the migration of SVZ cells into white matter and gray matter and their differentiation into astrocytes and oligodendrocytes from thymidine pulse-labeling experiments (Altman, 1963; Paterson *et al*., 1973). However, the conclusions reached from such DNA-labeling experiments were somewhat ambiguous, since dividing progenitors outside of the SVZ were also labeled as a consequence of systemically administered the $^{3}$H-thymidine. Furthermore, interpretations of these experiments were confounded by the dilution of the label to undetectable levels with continued divisions of progenitor cells.

## Lineage Tracing Experiments Demonstrate That Postnatal SVZ Cells Give Rise to Gray Matter, Protoplasmic Astrocytes

To more accurately trace the lineage of SVZ cells, dividing cells within the SVZ have been labeled with replication-deficient retroviruses (Fig. 9). When retroviruses are injected into the dorsolateral SVZ of the forebrain of two-day old rats, 95% of the labeled cells (when examined one day later) reside within the SVZ and they have immature morphologies (Levison and Goldman, 1993). The labeled cells typically have an ovoid or spindle shape, and the majority possess a single process that is oriented toward

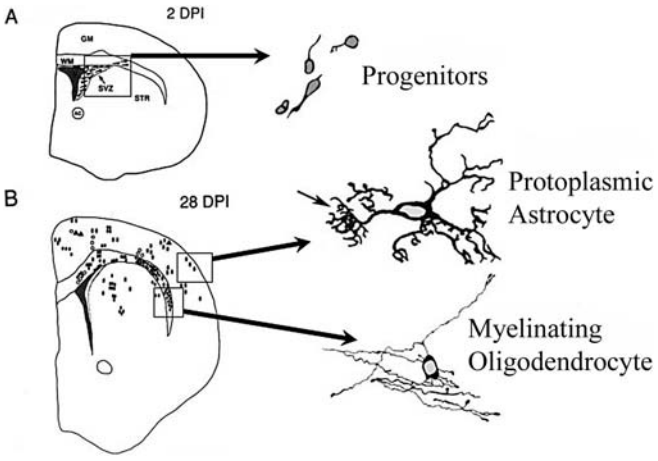


**FIGURE 9.** Schematic of postnatal SVZ descendants. Replication deficient retroviruses were injected into the SVZ of postnatal day two rats and the types of cells labeled were examined either 2 days post infection (DPI) or 28 DPI. The location of the labeled cells is indicated as well as the types of cells. At 2 DPI (A), the majority of labeled cells had simple unipolar morphologies and most of the labeled cells remained within the SVZ. At 28 DPI, labeled cells could be found dispersed throughout the forebrain, and these labeled cells could be classified as protoplasmic astrocytes (asterisks in B), myelinating oligodendrocytes (dark circles in B), or nonmyelinating oligodendrocytes (open circles in B). Camera lucida drawings of a typical protoplasmic astrocyte with an end foot on a blood vessel (arrow) and of a myelinating oligodendrocyte are provided. Adapted from Levison and Goldman (1993) and Levison *et al*. (1993), with permission.

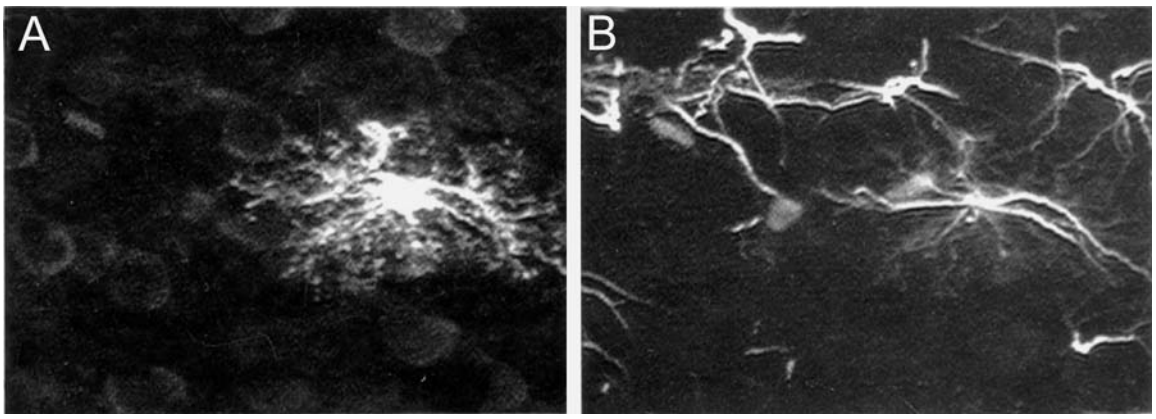


**FIGURE 10.** Protoplasmic astrocytes are descendants of the newborn SVZ. Panel A depicts immunofluorescence for the enzyme β-galactosidase encoded by the retroviral vector that had infected the precursor of this cell while it resided in the SVZ. The same cell is depicted in panel B stained for GFAP. From Levison and Goldman (1993), with permission.

the adjacent white matter. None of the labeled cells have the morphology of radial glia. Over time the labeled cells migrate out of the SVZ, generally moving laterally to colonize the adjacent striatum, or they migrate dorsally and laterally to reside in white matter and neocortical gray matter (Levison and Goldman, 1993; Levison *et al*., 1993; Luskin and McDermott, 1994). SVZ cell migration also has been studied using time-lapse video microscopy in brain slices. These studies have demonstrated that migrating SVZ cells can use several means to migrate including radial glial cells and white matter tracts as well as glial tunnels (Suzuki and Goldman, 2003). Astrocytes generated from SVZ cells distribute throughout all cortical layers, up to the pial surface, and to the striatum. However, SVZ cells that migrate into the white matter preferentially differentiate into oligodendrocytes and not astrocytes (Levison and Goldman, 1993) (Fig. 9).

## Contact with Blood Vessels Induces SVZ Precursors to Become Astrocytes

An interaction with blood vessels or the pial surface appears to be one of the first signs of astrocyte differentiation (Zerlin *et al*., 1995; Zerlin and Goldman, 1997). After leaving the SVZ, many of the migrating SVZ cells still retain their simple, largely unipolar morphology. After contacting a blood vessel with its leading process, the SVZ cell begins to ensheath the blood vessel and it will form the classic end foot of an astrocyte (Zerlin *et al*., 1995). During this ensheathment process, the cell slowly extends multiple fine processes into the surrounding parenchyma. The cell also acquires nestin and vimentin immunoreactivity—intermediate filaments characteristic of immature astrocytes that form the cytoskeleton of the cell (Zerlin *et al*., 1995). Approximately half of the morphologically characterized astrocytes also express detectable levels of GFAP (Fig. 10). While this observation seems contradictory, it is, in fact not surprising, given that protoplasmic astrocytes have few intermediate filaments. Although half of the cells characterized as protoplasmic astrocytes do not express GFAP, they uniformly express S-100β (Levison *et al*., 1999). Interestingly, protoplasmic astrocyte differentiation is predominantly observed within the gray matter, with 65% of those cells from the P2 rat SVZ differentiating into these cells. Many of these newly generated astrocytes in the gray matter reside in tightly knit clusters, consistent with the view that astrocyte precursors continue to divide after they reach their final destination. By contrast, only 8.5% of the progeny of the P2 SVZ cells migrating into the white matter display astrocytic features, indicating that white matter tracts in the postnatal brain may not be permissive for astrocyte differentiation from SVZ cells.

This early interaction with blood vessels thus constitutes an early stage in astrocyte development. In fact, contact with endothelial cells induces astrocyte differentiation in astrocyte progenitors cultured from optic nerve (Mi *et al*., 2001). That is, endothelial cells induce the expression of the astrocyte markers, GFAP and S-100β, in immature cells. This induction can be neutralized with antibodies to leukemia inhibitory factor (LIF), a growth factor expressed by endothelia (Mi *et al*., 2001) (see below for discussion of molecular signals for astrocyte differentiation).

## The Precursors in the SVZ That Generate Astrocytes Are Molecularly Distinct from VZ Derived Radial Glia

In contrast to the markers expressed by radial glia, precursors in the SVZ express a nonoverlapping set of molecular markers, which strongly suggests that the precursors in the SVZ that generate astrocytes are distinct from radial glia, which also give rise to astrocytes. As reviewed above, radial glia express a number of markers that include the intermediate filaments nestin, vimentin, and sometimes GFAP, IFAP-300, BLBP, GLAST, zebrin II, Musashi-1, and the radial cell markers RC1 and RC2. By contrast, the progenitors in the SVZ that give rise to glia do not express the majority of these markers. By contrast, a number of markers expressed by those SVZ cells that will go on to produce astrocytes are not expressed by radial glia. For example,

when SVZ cells are dissociated and stained *in vitro* approximately two-thirds of the cells that would have gone on to generate astrocytes and oligodendrocytes label for PSA-NCAM and they express the ganglioside $GD_3$ (Levison and Goldman, 1997). These cell surface molecules are neither expressed by radial glia, nor are they expressed by type 1 astrocytes, which are lineally related to radial glia (Culican *et al*., 1990). An additional molecule that is differentially expressed by SVZ cells and radial glia is the transcription factor Dlx2. Dlx2 is expressed by SVZ cells of the medial ganglionic eminence and it is not expressed by cells in the neocortical VZ (Anderson, 1997). Fate mapping studies performed using a Dlx2/tauLacZ knock-in mouse have shown that Dlx-2 expressing SVZ cells migrate into the striatum, white matter, and cerebral cortex where a subset differentiates into astrocytes (Marshall and Goldman, 2002).

## SVZ Cells That Generate Astrocytes Can Also Produce Neurons or Oligodendrocytes

A small proportion of clones generated by single CNS cells from the postnatal SVZ contain astrocytes, neurons, and oligodendrocytes. On the basis of multiple lines of evidence we have argued that the retroviruses preferentially label progenitors, rather than stem cells (Levison and Goldman, 1997). Therefore, these experiments indicate that progenitors in the postnatal SVZ have the potential to give rise to both glia and neurons (Young and Levison, 1996; Levison and Goldman, 1997). Interpreting retroviral lineage studies *in vivo* can be difficult, depending on how one defines a "clone" of cells. Many studies have found that retroviral-labeled cells tend to congregate in homogenous clusters, with a small proportion of heterogenous clusters (astrocytes and oligodendrocytes) (Luskin *et al*., 1988; Price and Thurlow, 1988; Grove *et al*., 1993; Levison and Goldman, 1993; Luskin and McDermott, 1994; Parnavelas, 1999). Forebrain gliogenesis has been reexamined using a retroviral "library" (Walsh and Cepko, 1992), so that the proximity of cells to one another becomes irrelevant in judging clonality and two related cells that happen to be separated in space by some distance can be found to come from the same retrovirally infected progenitor. Most clones are indeed homogeneous, but about 15–20% are composed of both astrocytes and oligodendrocytes, sometimes appearing in the same cluster of glia (Zerlin and Goldman, submitted). Thus, not all SVZ cells are irrevocably committed to an astrocytic or oligodendrocytic fate before they emigrate from the SVZ. In fact, some do not make a final fate decision until they have stopped migrating, at which time they continue to divide to generate heterogeneous or homogenous clusters. An unresolved question is whether this difference in differentiation potential is not only a result of the actions of extrinsic inducers of astrocyte differentiation, but whether there also are intrinsic differences in the types of progenitors that exit the SVZ. While the majority of SVZ cells are molecularly distinct from VZ cells, a subset of SVZ cells share properties with VZ derived radial glia. The expression of the markers zebrin II and GLAST by a subset of SVZ cells may indicate that these cells are of direct VZ origin, whereas the PSA-NCAM population may be a separate precursor population. Thus, for the PSA-$NCAM^+$ cell population, their differentiation may be regulated by instructions that they receive once they cease migrating, whereas the zebrin $II^+$ population may be committed to an astrocyte fate prior to leaving the SVZ. Alternatively, it may turn out that the astrocyte fate decision is probabilistic, rather than strictly determined at one specific place and time.

## Astrocyte Progenitors Emigrate from the SVZ along Radial Glial Guides

As progenitors emigrate from the SVZ, they usually leave in a radial orientation (Kakita and Goldman, 1999; Suzuki and Goldman, 2003). This suggests that they use radial glia to guide their exit from the SVZ. Indeed, examining progenitors as they leave the SVZ and as they migrate radially into white matter and cortex show that many contact radial glial processes (Suzuki and Goldman, 2003), which the CNS retains into early postnatal life. Glioblasts emigrating from the SVZ migrate radially, but once in the white matter they can move parallel to axons or radially into the cortex, where they can either continue or turn to migrate tangentially (Suzuki and Goldman, 2003). Furthermore, some SVZ cells migrate laterally along the white matter and then turn radially to enter the cortex, a pattern reminiscent of neuronal migration along radial glia (Bayer *et al*., 1991; Misson *et al*., 1991). (Fig. 11). Glioblasts migrate in a saltatory fashion at an average velocity of about 90 m per hr, but maximal speeds of up to 250 m per hr have been documented. By P14, however, the radial glia have transformed into astrocytes, and studies on the development of P14 SVZ cells have shown that progenitors at this stage either migrate into the subcortical white matter or the striatum, but they are unable to migrate into the neocortex. Thus, without radial glia, the SVZ cells are restricted from colonizing dorsal forebrain structures (Levison *et al*., 1993).

# DO DIFFERENT PATHWAYS OF ASTROCYTE DEVELOPMENT IN THE MAMMALIAN FOREBRAIN GIVE RISE TO DIFFERENT TYPES OF ASTROCYTES?

The studies summarized above indicate that there are two (at least) separate pathways through which the CNS provides astrocytes from immature cells: directly from radial glia and from SVZ cells. Is there any reason to think that these pathways generate different astrocyte types? Studies of glial development with retroviruses suggest that SVZ cells that migrate into gray matter preferentially generate astrocytes, whereas if they migrate into white matter they largely differentiate into oligodendrocytes (Levison and Goldman, 1993). This result suggests that many white matter astrocytes, at least in rodents, are not generated by SVZ cells, but from an earlier precursor pool, namely, the radial glia. Astrocytes constitute a heterogeneous group of cells, which vary in shape and molecular constituents, including growth factor expression, ion channels, levels of GFAP, and types of

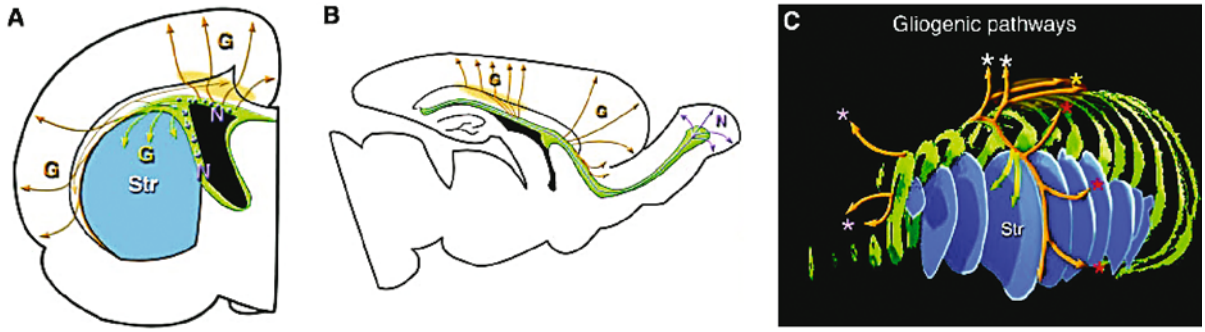


**FIGURE 11.** Models of migratory pathways of postnatal SVZ cells. (A) Coronal plane. Migratory pathways of SVZ cells into the white matter, cortex, and the striatum are shown by orange and yellow arrows, respectively. $SVZ_{DL}$ neuronal progenitor migration is depicted by purple dots and by short purple arrows. A region where cell accumulate between the corpus callosum and the subcortical white matter is indicated by light orange shades. (B) Sagittal plane. Representative migratory patterns of glial progenitors out of the anterior and posterior SVZ are shown by orange arrows. Migration of neuronal progenitors is shown by light purple arrows. (C) Reconstruction of the SVZ (green), the striatum (blue), and corresponding migration routes of glial progenitors. Migration toward the dorsal cortex (white asterisks), lateral cortex (red asterisks), and frontal cortex (pink asterisks) is shown. Yellow arrows indicate migration into the striatum. The orange ellipsoid shadow shows the cell accumulation layer between the corpus callosum and the subcortical white matter. Reproduced with permission from Suzuki and Goldman, (2003).

neurotransmitter transporters. How much of this heterogeneity is determined by lineage (radial glia vs SVZ), and how much is determined by local environmental factors (white matter vs gray matter) is not yet known.

Regardless of the source of a given astrocyte, the CNS must have a mechanism to generate large numbers of astrocytes in the perinatal period. It is in this time of rapid brain growth that the pial surface greatly enlarges, especially in larger mammals with gyriform brains, the vascular tree grows tremendously, and much of synaptogenesis peaks. As noted above, there seem to be constraints on astrocyte size; thus, as the brain enlarges there may be a requirement for more glia. It is likely that the need for additional astrocytes is satisfied by both the division of radial glia, the migration of astrocyte progenitors from the SVZ, and by the continued division of these progenitors within the parenchyma (Schmechel and Rakic, 1979a; Luskin and McDermott, 1994; Zerlin *et al*., 1995; Zhang and Goldman, 1996; Levison *et al*., 1999).

## ASTROCYTE DEVELOPMENT IN THE CEREBELLUM

The cerebellum contains fibrous astrocytes in white matter, velate astrocytes in the internal granule cell layer, and Bergmann glia, whose cell bodies lie within the Purkinje cell layer (Palay and Chan-Palay, 1974). Bergmann glia send long complex processes dorsally that branch extensively as they project toward the pial surface. These processes resemble those of radial glia and guide immature granule cells as they migrate from the external to the internal granule cell layer (Grosche *et al*., 1999). Early investigations using $^{3}$H-thymidine uptake and morphological analyses suggested that Bergmann glia arise from other Bergmann glia or from some sort of immature cells, of unknown nature (Basco *et al*., 1977; Moskovkin *et al*., 1978; Choi and Lapham, 1980). Fate mapping studies using recombinant retroviruses show that progenitors that originate in the VZ at the base of the cerebellum migrate from the base of the cerebellum through the white matter to reach the cortex to give rise to Bergmann glia and velate and white matter astrocytes (along with oligodendrocytes) (Miyake *et al*., 1995; Zhang and Goldman, 1996; Milosevic and Goldman, 2002). At least some of the migratory progenitors in the white matter begin to express astrocyte characteristics, such as GLAST, during migration (Milosevic and Goldman, 2002). Since the viral-labeled astrocytes occur in clusters, either their immediate precursors or the immature astrocytes themselves continue to proliferate after they migrate from the VZ. The details of Bergmann glial growth can in general be inferred from images of Golgi or retroviral-labeled cells or immunocytochemical staining in the postnatal Purkinje cell layer (Choi and Lapham, 1980; Zhang and Goldman, 1996; Yamada *et al*., 2000). The cell bodies arrest their transit from the VZ at the Purkinje cell layer and from there extend processes toward the pial surface. Over time, the numbers and complexity of their processes increase in dynamic synchrony with Purkinje cell dendritic growth and parallel fiber–Purkinje cell synapse formation. Interactions with neurons likely influence astrocyte morphology. Astrocytes derived from neonatal cerebellum assume Bergmann glial-like shapes, with elongated processes, when they are cocultured with migrating neurons, whereas they remain polygonal in the absence of neurons or in the presence of nonmigratory neurons (Hatten, 1985).

## ASTROCYTE DEVELOPMENT IN THE SPINAL CORD

A number of immunocytochemical studies suggest that radial glia in the spinal cord, as elsewhere, generate astrocytes (Choi, 1981; Hirano and Goldman, 1988), although as noted above, it is difficult to infer lineage pathways from a chronological series of static images. Recent studies have linked the development of motor neurons and interneurons of the spinal cord to

oligodendrocyte development, arguing that these three cell types arise from a single, ventral lineage, the specificity of which changes over time in a way correlated with changes in the expression of bHLH transcription factors that regulate patterning and cell fate (for review see Rowitch *et al*., 2002). Astrocytes, however, are not included in this lineage. Using fibroblast growth factor (FGF) receptor type 3 as a marker for astrocytes and their precursors in the developing cord, Pringle *et al*. (2003) have localized astrocyte precursors to both dorsal and ventral VZ, with the exception of the (ventral) pMN domain, which gives rise to oligodendrocytes and motor neurons. This is consistent with the earlier observation that astrocytes are generated from both dorsal and ventral parts of the neuroepithelium, while oligodendrocytes arise only from ventral parts (Pringle *et al*., 1998). Thus, the patterning of gliogenesis in the cord as reflected in the domains of the early neuroepithelium is different for astrocytes and oligodendrocytes and it is possible that cells in the pMN domain suppress the ability to generate astrocytes. In fact, in mice that are null for the bHLH factors Olig1 and Olig2, the pMN domain is converted to an adjacent homeodomain in the VZ, and that part of the VZ now generates interneurons followed by astrocytes (Takebayashi *et al*., 2002; Zhou and Anderson, 2002).

## MAINTENANCE OF ASTROCYTES IN THE ADULT BRAIN

### Astrocytes Are Maintained by Endogenous Precursors

Astrocytes continue to be generated in the adult CNS, albeit at an apparently very low rate. Studies that have counted astrocyte numbers or determined how many immature cells label in the adult brain with $^3$H-thymidine conclude that immature cells persist within the parenchyma of the adult brain, but that there is little or no net accumulation of astrocytes with age (Altman, 1963; Hommes and Leblond, 1967; Korr *et al*., 1973; Ling and Leblond, 1973; Vaughan and Peters, 1974; Kaplan and Hinds, 1980; Paterson, 1983; Reyners *et al*., 1986; McCarthy and Leblond, 1988). For instance, in a study by McCarthy and LeBlond (1988), the authors administered $^3$H-thymidine to nine-month-old "aged" male mice and then analyzed the astrocytes and oligodendrocytes of the corpus callosum. Some of the mice were given $^3$H-thymidine as a 2 hr pulse to determine which cells were dividing, while in other mice $^3$H-thymidine was infused for 30 days and the animals were sacrificed 60 or 180 days later. The $^3$H-thymidine pulse paradigm revealed that there are cells resident within the white matter that label after an acute pulse of $^3$H-thymidine. These cells have the morphological features of immature cells. After a 30-day infusion, approximately 12% of the astrocytes and 1% of the oligodendrocytes were labeled, indicating a net daily addition of 0.4% astrocytes and 0.04% oligodendrocytes per day. However, when the 30-day infusion was followed by 60 and 180 days without $^3$H-thymidine, the labeled astrocytes decrease to 5% and 0% over time, respectively, whereas the number of labeled oligodendrocytes did not change. These data indicate that there are resident immature cells in the corpus callosum that continue to divide across the life span to give rise to new astrocytes and oligodendrocytes. The production of new astrocytes appears to occur concurrent with cell turnover, so that the astrocyte population remains stable. By contrast, oligodendrocytes slowly accumulate in the adult brain, as confirmed by other studies (Ling and Leblond, 1973; Peters *et al*., 1991; Levison *et al*., 1999).

Several lines of evidence support the concept that the cells that are dividing in the mature brain are immature astrocytes rather than mature cells or bipotential glial precursors. For instance, when bulk fractionation techniques are used to isolate cells from the adult brain, a population of GD3 ganglioside$^+$ cells can be harvested from the white matter (a marker of immaturity). These GD3$^+$ cells from the adult brain are GFAP$^-$, divide in culture, and over time acquire mature astrocytic properties (such as GFAP), but not oligodendrocyte properties (Norton and Farooq, 1989). Using a retroviral strategy to label proliferating cells within the adult white matter, cells can be isolated that are able to generate oligodendrocytes in culture as well as a subset of cells that can generate astrocytes (Gensert and Goldman, 2001). The astrocyte "progenitor" subpopulation expresses the intermediate filament, vimentin, a cytoskeletal protein observed early in astrocyte differentiation.

### Astrocyte Precursors Are Different from NG2 Cells

In addition to the vimentin$^+$ proliferating glial cells that are distributed throughout the adult CNS, there is another cell population that expresses the NG2 proteoglycan. As these cells are quite abundant and cycle slowly, the question has been raised as to whether these cells generate astrocytes in the adult brain. One reason that this question has been posed is that a cell that also expresses this antigen *in vitro* is the oligodendrocyte-type 2 astrocyte (O-2A) progenitor, which, as reviewed below, can generate oligodendrocytes or type 2 astrocytes. Despite this apparent overlap in the expression of NG2, the bulk of evidence suggests that adult NG2$^+$ cells do not produce astrocytes in the normal mature CNS. It is now well established that NG2$^+$ cells *in vivo* do not express GFAP under normal or reactive conditions (Levine and Card, 1987; Levine *et al*., 1993; Levine, 1994; Nishiyama *et al*., 1996; Bu *et al*., 2001). Since GFAP is not detectable in many protoplasmic astrocytes, the lack of GFAP immunoreactivity in NG2$^+$ cells itself does not rule out the possibility that NG2$^+$ cells might represent protoplasmic astrocytes as suggested by one study (Levine and Card, 1987). However, several observations indicate that this is not the case. Double immunolabeling studies using other antigens known to be expressed by protoplasmic astrocytes, such as the calcium-binding protein S-100β and GS, have shown that NG2 cells do not express these astrocyte markers (Nishiyama *et al*., 1996; Reynolds and Hardy, 1997). Furthermore, NG2 cells also lack several other characteristics of astrocytes. They are not coupled by gap junctions, they do not generate calcium waves, and they lack glutamate transporters (for review see Lin and Bergles, 2002).

$NG2^+$ cells are morphologically distinct from astrocytes. In a recent study, where fluorescent dyes were injected into hippocampal astrocytes, cells with bushy, highly arborized processes were labeled of which only one tenth are labeled for GFAP (Bushong *et al*., 2002). Morphologically, these dye-filled cells showed striking similarity to classic images of protoplasmic astrocytes. By contrast, dye-filled cells that expressed NG2 had simpler morphologies with multiple thin processes emanating from the cytoplasm (Bergles *et al*., 2000), similar to the pattern of cell surface NG2 immunolabeling. Protoplasmic astrocytes have small cell bodies, similar to $NG2^+$ cells, but astrocytes have a greater number of tertiary branches, and their primary branches are wider near the soma and taper distally. The extensive arborization of the processes and the presence of spines give them a bushy, fuzzy appearance. These cells, typically, have relatively weak strands of GFAP immunoreactivity along a subregion of their proximal processes. Interestingly, the territories of the CNS parenchyma occupied by each cell type do not overlap (for review see Nishiyama *et al*., 2002).

## CELL CULTURE STUDIES REVEAL MULTIPLE LINEAGES OF ASTROCYTES

### Multipotent Neural Stem Cells Generate Astrocytes by Generating More Restricted Glial Precursors

The unambiguous demonstration of multipotent stem cells *in vitro* and *in vivo* suggests that differentiated astrocytes must be derived from an initially multipotent stem cell population. How these multipotent cells become restricted and specified toward neurons and glia is a focus of intense investigation. As reviewed in Chapter 4, the earliest multipotential precursors eventually become more restricted, so that over the course of development there emerge bipotent and eventually unipotent neural precursors. At the present time it is not clear how many intermediate precursors eventually form.

The first evidence for the existence of multiple glial precursors emerged from studies on the optic nerve. Martin Raff and his colleagues published studies in 1983 characterizing two types of astrocytes in optic nerve cultures. Designated type 1 and type 2 astrocytes, these cells were delineated morphologically, antigenically, and by their responses to soluble growth factors (Raff *et al*., 1983a) (Fig. 12). More recently, additional astrocyte types have been identified in forebrain and spinal cord cultures and these will be reviewed below.

Many studies on cell lineage have relied upon antibodies that react with either specific glial lineages or specific stages during the differentiation of cells within a lineage. Antibodies that have proven useful in studies of glial lineage *in vitro* include the antiganglioside antibodies A2B5, R24, and LB1 (the latter two react with GD3 ganglioside; Raff *et al*., 1983a; Goldman *et al*., 1986; Levi *et al*., 1986), the rat neural antigen-2 (Ran-2) (Bartlett *et al*., 1980), antibodies to fibronectin (Gallo *et al*., 1987), anti-chondroitin sulfate or anti-NG2 antibodies (Gallo *et al*., 1987; Levine and Stallcup, 1987), antibodies to the cell adhesion molecule J1 (ffrench-Constant *et al*., 1986), and antibodies to GAP-43 (Deloulme *et al*., 1990). All of these markers stain type 2 astrocytes and their progenitor, the O-2A progenitor, but they do not react with type 1 astrocytes, with the exception of Ran-2 and fibronectin, which stain subsets of type 1 and not type 2 astrocytes. Using markers alone is less than straightforward. For instance, it appears that type 2 astrocytes lose immunoreactivity for A2B5, LB1, and R24 with time in culture (Aloisi *et al*., 1988; Lillien *et al*., 1990; Levison and McCarthy, 1991) and they can become immunoreactive for Ran-2 after weeks in culture (Lillien and Raff, 1990). Furthermore, A2B5, LB1, and R24 are not specific for type 2 astrocytes, since some astrocytes that are clonally distinct from type 2 astrocytes express these markers (Vaysse and Goldman, 1990; Miller and Szigeti, 1991; Vaysse and Goldman, 1992).

### The Lineage of the Type 1 Astrocyte

Type 1 astrocytes were originally defined as flat, polygonal cells that expressed GFAP but did not bind the monoclonal antibody, A2B5 (Raff *et al*., 1983a). Optic nerve type 1 astrocytes can be distinguished from type 2 astrocytes by their

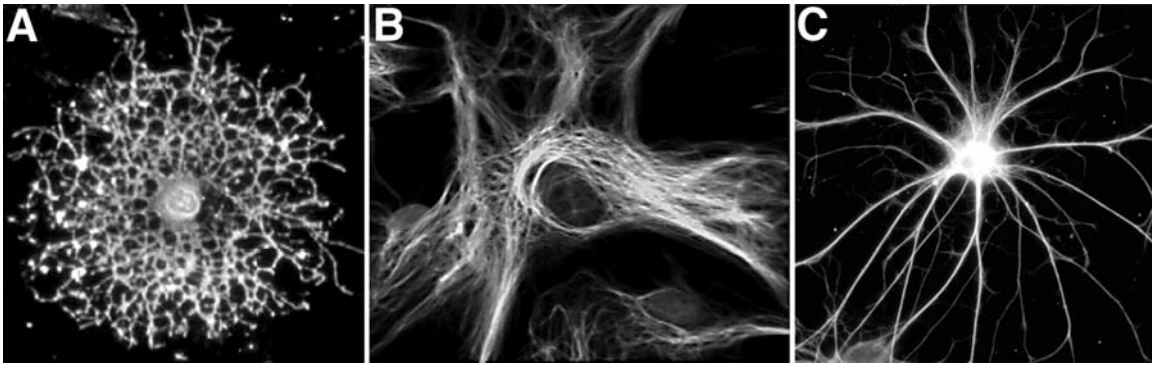


**FIGURE 12.** Three main types of macroglial cells in the CNS. Immunofluorescence micrographs of cells in culture. (A) Oligodendrocyte labeled on its surface with the $O_4$ monoclonal antibody. (B) Type 1 astrocyte labeled with antiserum against GFAP. (C) Type 2 astrocyte labeled with antiserum against GFAP. Photos provided by William Tyler and Phillip Albrecht.

immunoreactivity with the antibody Ran-2, by their absence of immunoreactivity with the other antibodies listed above, and by their separation from the oligodendrocyte lineage (Raff *et al*., 1984). Unlike the O-2A lineage cells, type 1 astrocytes proliferate in response to epidermal growth factor (EGF) (Raff *et al*., 1983a). Type 1 astrocytes develop early during gliogenesis. $GFAP^+/A2B5^-$ astrocytes first appear in cell suspensions of developing rat optic nerve on embryonic day 16 (E16) (Miller *et al*., 1985). Studies in forebrain cultures also support the early generation of astrocytes with a type 1 morphology and antigenic phenotype. For example, they are clonally distinct from the other glial lineages by E16 in rat forebrain cultures (Vaysse and Goldman, 1992). Culican *et al*. (1990) studied cultures from embryonic mouse forebrain and described cells with a radial glial-like morphology that bound the RC1 antibody, a monoclonal antibody that labels radial glia *in vivo* (Edwards *et al*., 1990). While initially $GFAP^-$, these cells became $RC1^+/GFAP^+$ with time, and eventually $RC1^-/GFAP^+$, a developmental and antigenic sequence that suggests type 1 astrocytes are generated *in vitro* from radial glia.

Applying the glial nomenclature derived from studies on optic nerve glia to other CNS regions can be problematic, since morphology and antigen expression can vary. For instance, studies of spinal cord astrocytes demonstrate that there is a greater variety of astrocyte types in the spinal cord than in optic nerve, and furthermore, that $A2B5^+$ cells from the spinal cord give rise to "pancake"-shaped spinal cord astrocytes that are distinct from type 1 astrocytes (Miller and Szigeti, 1991; Fok-Seang and Miller, 1992). While clonally related cells tended to be morphologically similar, some are morphologically heterogeneous. Furthermore, the expression of A2B5 and Ran-2 varies even among clonally related cells. These and other observations illustrate astrocyte heterogeneity in different CNS regions and argue that antigen expression can be regulated by both lineage-dependent and lineage-independent factors.

## Type 2 Astrocytes and the O-2A Lineage

Type 2 astrocytes were originally defined in optic nerve cultures (Raff *et al*., 1983b), but type 2 astrocytes have been obtained from cultures of cerebellum (Levi *et al*., 1986; Levine and Stallcup, 1987) and cerebral cortex (Goldman *et al*., 1986; Behar *et al*., 1988; Ingraham and McCarthy, 1989). As indicated above, a panel of additional cell markers is available that distinguish type 2 from type 1 astrocytes. In suspensions of developing brain, cells with the antigenic characteristics of type 2 astrocytes appear postnatally and derive from a bipotential O-2A progenitor (also referred to as an oligodendrocyte precursor cell or OPC) (Miller *et al*., 1985; Williams *et al*., 1985). O-2A progenitors differentiate into oligodendrocytes in a chemically defined medium, but into type 2 astrocytes in medium supplemented with fetal bovine serum (FBS) (Raff *et al*., 1983b). Studies have characterized the molecules that induce type 2 astrocyte differentiation. Lillien *et al*. (1988) demonstrated that ciliary neurotrophic factor (CNTF) causes a transient commitment of the O-2A progenitor toward a type 2 astrocyte fate, but that the presence of an extracellular matrix-associated molecule derived from endothelial cells or fibroblasts is required for this phenotype to be expressed stably (Lillien *et al*., 1990). Another stimulus that was partially characterized is the astrocyte-inducing molecule (AIM) that was isolated from the fetuin fraction of fetal bovine serum. Based on its biochemical properties, AIM may well turn out to be a member of the Galectins, since it has been recently demonstrated that Galectin-1, which is a fetuin-binding protein, can induce astrocyte differentiation from precursors (Sasaki *et al*., 2003).

Direct evidence that the O-2A lineage is distinct from the type 1 astrocyte lineage was provided by an experiment where A2B5 and complement were combined to lyse the O-2A progenitor and its progeny. While the type 1 lineage was unaffected, the descendants of the O-2A progenitor failed to develop (Raff *et al*., 1983b). Conversely, O-2A progenitors purified using fluorescence activated cell sorting (Williams *et al*., 1985; Behar *et al*., 1988), or grown as single cell microcultures (Temple and Raff, 1986) gave rise to oligodendrocytes or type 2 astrocytes, but not type 1 astrocytes. Furthermore, a retroviral analysis found that type 1 astrocytes are clonally distinct from oligodendrocytes in cultures from forebrain and spinal cord (Vaysse and Goldman, 1990). Whether type 2 astrocytes have a correlate *in vivo* has not yet been determined.

## Other Astrocyte Types

Another astrocyte type has been identified *in vitro* (Vaysse and Goldman, 1992). In cultures of striatum, spinal cord, and cerebellum, these cells are very large, flat, and extend many fine cytoplasmic processes. They express both GFAP and GD3 ganglioside and remain $GD3^+$ for at least eight weeks (the longest timepoint examined). Many, but not all of these cells, also stain with A2B5, but none express O4 or galactocerebroside (oligodendrocyte lineage markers). While these astrocytes antigenically resemble type 2 astrocytes, they are clonally distinct from type 1 astrocytes and from the O-2A lineage in the neonatal CNS. These astrocytes comprise a small percentage of the total cells and proliferate little, since the average clonal size is small. Whether these astrocytes have a correlate *in vivo* also has not yet been determined.

## Heterogeneity within Astrocyte Lineages *In Vitro*

Subclasses of astrocytes with a type 1 phenotype have been revealed by analyses of cytoskeletal proteins, neuropeptide content, neuroligand receptors, secreted peptides, surface glycoproteins, release of prostaglandins, and by their influence on neuronal arborization patterns (for review, see Wilkin *et al*., 1990). While many of these differences emerged by comparing cultures from different brain regions, subtypes have also been distinguished from the same brain region (McCarthy and Salm, 1991; Miller and Szigeti, 1991). Type 2 astrocytes also appear to be heterogeneous as revealed by receptor expression and class II MHC inducibility (Calder *et al*., 1988; Sasaki *et al*., 1989; Dave *et al*., 1991; Inagaki *et al*., 1991).

## RECENT STUDIES PROVIDE EVIDENCE FOR THE SEQUENTIAL SPECIFICATION OF PRECURSORS FROM NEURAL STEM CELLS TO GLIAL-RESTRICTED PRECURSORS TO ASTROCYTE PRECURSOR CELLS

### Glial-Restricted Precursors (GRPs) Are Cells That Can Differentiate into Type 1 Astrocytes, Oligodendrocytes, and Type 2 Astrocytes

*In vitro* experiments performed by several laboratories have identified a precursor that does not generate neurons, but which does produce type 1 astrocytes, oligodendrocytes, and under appropriate conditions, type 2 astrocytes. These precursors have been designated GRPs. Rao and colleagues have established that there are cells present in the developing spinal cord at E12 that are A2B5 and nestin immunoreactive (Rao and Mayer-Proschel, 1997; Rao *et al*., 1998; Gregori *et al*., 2002; Power *et al*., 2002). Spinal cord GRPs lack PDGFR-alpha immunoreactivity and synthesize detectable levels of PLP/DM-20. Furthermore, they do not stain for ganglioside GD3 or for PSA-NCAM. Since GRPs are the earliest identifiable glial precursor and they generate two kinds of astrocytes *in vitro*, they are clearly at an earlier stage of restriction than type 1 astrocyte precursors and O-2A progenitors. This sequence of appearance of progressively more restricted precursors suggests, though does not prove, that a lineage relationship exists between them. A hypothetical relationship is schematized in Fig. 13, which is supported by *in vitro* studies.

Work performed by Rao and colleagues supports the model depicted where there is a gradual restriction in the developmental potential of neural precursors from a multipotential neuroepithelial precursor (NEP) to a cell-type specific neural progenitor (Mayer-Proschel *et al*., 1997; Rao and Mayer-Proschel, 1997; Rao *et al*., 1998). At least three intermediate precursors have been shown to arise from spinal cord neural stem cells. When $A2B5^+$/$PSA\text{-}NCAM^-$ precursors are generated from spinal cord NEPs and grown in serum-containing medium, they generate A2B5-negative, flat astrocytes. When these same precursors are stimulated with CNTF and FGF-2, they generate oligodendrocytes, but not neurons. The transition from an NEP to a GRP, and the subsequent production of more restricted glial cell types provides evidence for the transformation of multipotential precursors into more restricted glial precursors.

Analogous experiments conducted on precursors from the forebrain SVZ show that there are GRPs within the SVZ that are descended from multipotential neural stem cells. Clonal analyses have shown that precursors in the newborn rat SVZ can generate type 1 and type 2 astrocytes as well as oligodendrocytes (Levison *et al*., 1993, 2003). In particular, when SVZ cells cultured under conditions that are permissive for neuronal differentiation, some SVZ derived progenitors generate astrocytes and oligodendrocytes, but they do not produce neurons. Thus, these cells can reasonably be called GRPs (Levison and Goldman, 1997). However, the markers expressed by GRPs from the SVZ appear

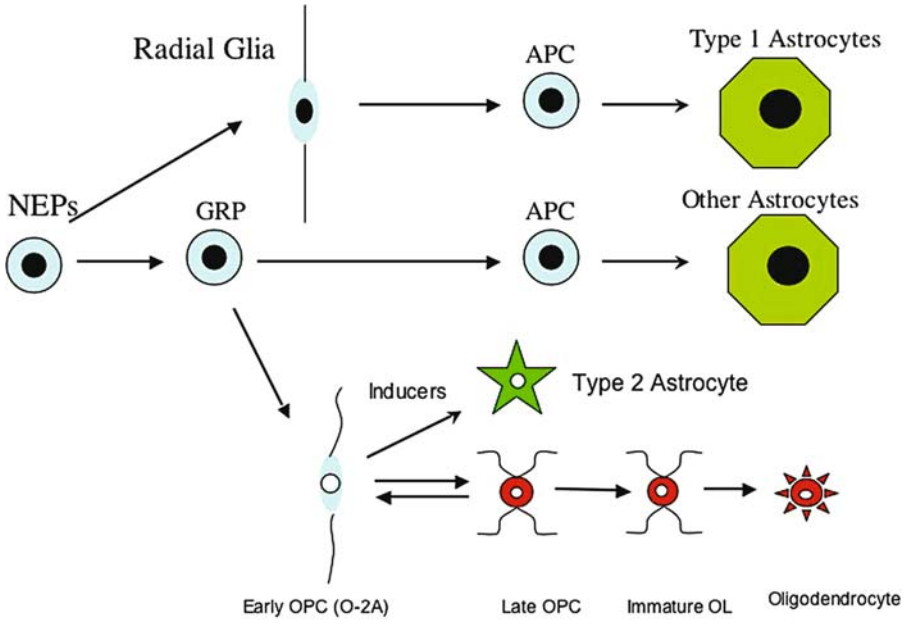


**FIGURE 13.** Model of astrocyte lineages. Depicted are several developmental pathways resulting in the production of a heterogeneous population of astrocyte types from neural epithelial precursors (NEPs). Depicted is the radial glia lineage which produces type 1 astrocytes through an intermediate astrocyte precursor cell (APC). Also depicted are the glial-restricted precursors (GRPs) such as those within the SVZ that produce both APCs as well as early oligodendrocytes progenitor cells (OPCs). These OPCs, *in vitro*, can be induced to produce type 2 astrocytes. Not depicted are other APCs, such as those in the optic nerve that are direct descendants of the NEPs without a radial glial intermediate.

to be different from the markers expressed by spinal cord GRPs in that SVZ GRPs express PSA-NCAM and ganglioside GD3 whereas these cell surface markers are not present on spinal cord GRPs (Levison *et al*., 1993; Avellana-Adalid *et al*., 1996; Ben-Hur *et al*., 1998; Zhang *et al*., 1999). Whether the properties and functional attributes of the astrocytes generated by spinal cord GRPs are different from the properties and functional attributes of the astrocytes generated by forebrain GRPs remains to be discerned.

## Several Astrocyte-Restricted Precursors Have Been Isolated

There is clear evidence from *in vivo* studies that radial glia generate a subset of astrocytes, and these *in vivo* studies are supported by *in vitro* studies. For instance, in the study reported by Culican *et al*. (1990) the authors used the monoclonal antibody RC1, which recognizes an epitope present on radial glial, to follow the development of RC1-labeled cells *in vitro*. They observed that the cells from the E13 mouse brain that labeled with RC1 resembled radial glial cells *in vivo*. These cells possessed long, thin unbranched processes. After 3–4 days *in vitro* in the absence of neurons, these cells retained their RC1 epitope, acquired GFAP, and exhibited a polygonal shape reminiscent of type 1 astrocytes. In the presence of neurons, the $RC1^+$ cells acquired GFAP, but they possessed a more complex morphology, reminiscent of the stellate-shape typical of astrocytes *in vivo*. Unfortunately, these authors did not more fully characterize the antigenic phenotype of this astrocyte population, therefore, it is not entirely clear which type(s) of astrocytes were produced.

Other astrocyte-restricted precursors have been purified from the optic nerve using immunopanning. Mi *et al*. (2001) purified a population of cells from the E17 optic nerve that are $Ran\text{-}2^+/A2B5^+/Pax\text{-}2^+/Vimentin^+$ and they are $S\text{-}100^-$ and $GFAP^-$. Although $A2B5^+$, apparently, these cells express low levels of A2B5 when compared to O-2A progenitors. These astrocyte precursor cells (APCs) are clearly different from immature astrocytes and from O-2A progenitors. When maintained in a serum-containing medium, the APCs do not differentiate, but die, whereas immature astrocytes will differentiate and will readily divide. Moreover, when maintained in a culture medium that is permissive for oligodendrocyte differentiation, these APCs do not generate oligodendrocytes. Finally, when stimulated with either CNTF or LIF, APCs differentiate into $A2B5^-/GFAP^+$ polygonal astrocytes and not into type 2 astrocytes. Thus, on the basis of these studies, the authors conclude that these cells represent an astrocyte intermediate between the multipotential neural stem cell and a type 1 astrocyte. Unfortunately, these authors did not use markers of radial glia to determine whether these APCs might be similar to radial glia. However, these authors report that neither Pax-2 nor Ran-2 are expressed by forebrain APCs, suggesting that these optic nerve APCs are distinct from APCs in other regions of the CNS. Whether these different groups have identified slightly different precursors or whether the same precursor has been isolated multiple times remains to be determined.

# MULTIPLE SIGNALS REGULATE ASTROCYTE SPECIFICATION

As alluded to earlier in this chapter, there are several sets of ligands and receptors that promote astrocyte differentiation: (1) the alpha helical family of cytokines and their receptors, (2) transforming growth factor beta (TGFβ) family members, particularly the bone morphogenetic proteins (BMPs) and BMP receptors, (3) Delta and Jagged ligands and Notch receptors, (4) FGFs and their receptors, (5) EGF family member ligands and the erbB family of receptors, and (6) Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and the PAC1 receptor.

## Members of the Alpha Helical Family of Cytokines Induce Astrocyte Specification through the LIF Receptor Beta and Activation of STATs

Hughes *et al*. (1988) initially found that CNTF would induce astrocyte differentiation in O-2A progenitors isolated from the postnatal optic nerve. Other members of the alpha helical cytokine family include leukemia inducing factor (LIF), interleukin-11, cardiotropin 1, and oncostatin M. The receptors for the alpha helical cytokines are expressed by cells in the VZ as well as by cells in the SVZ and CNTF has been shown to induce astrocytes from both cell populations (Johe *et al*., 1996; Bonni *et al*., 1997; Park *et al*., 1999). However, CNTF deficient mice do not have a defect in astrocyte production, indicating that CNTF is not essential for astroglial differentiation (DeChiara *et al*., 1995; Martin *et al*., 2003). Whereas CTNF is dispensable for astrocyte differentiation, the LIF receptor may be important since LIF receptor deficient mice have reduced numbers of $GFAP^+$ cells at E19 (Koblar *et al*., 1998).

Upon binding of alpha helical cytokines to their receptors, the janus kinases (JAKs) associated with those receptors become activated, whereupon they phosphorylate downstream signaling molecules such as the protranscription factors STAT3 and STAT1. Phosphorylating these protranscription factors enhances their ability to dimerize whereupon they form complexes with CBP/p300 (Bonni *et al*., 1997; Kahn *et al*., 1997) (Fig. 14). This transcriptional complex can then move into the nucleus where it can activate or repress genes that promote astrocyte differentiation as well as genes that are characteristic of astrocytes such as GFAP. Additionally, these cytokines will activate protein kinase B/AKT that will phosphorylate a transcriptional repressor known as N-CoR to keep that factor in the cytoplasm. When N-CoR is not phosphorylated it translocates into the nucleus, where it represses astrocyte differentiation. Indeed, astrocyte differentiation occurs prematurely in mice that lack N-CoR (Hermanson *et al*., 2002).

## Members of the TGF-β Family of Cytokines Induce Astrocyte Specification

During embryogenesis BMP signaling is essential for inducing mesoderm from ectoderm as well as for dorsal ventral

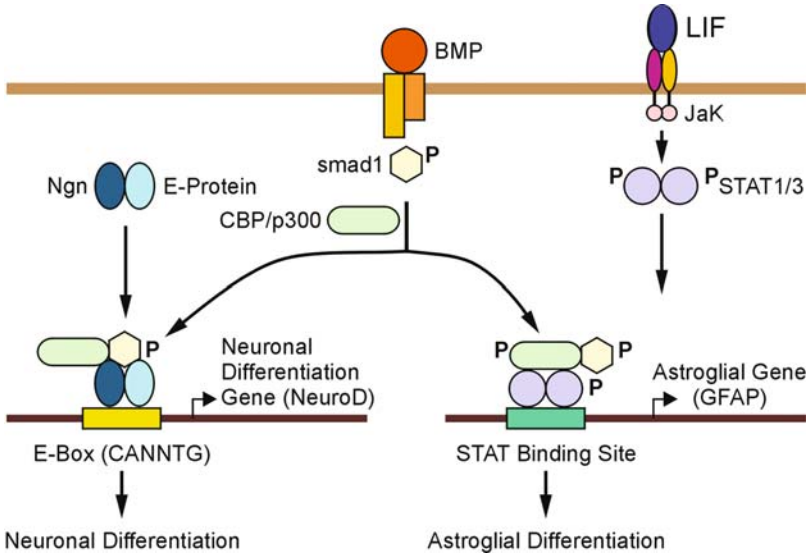


**FIGURE 14.** Model for developmental switch from neurogenesis to gliogenesis. The presence of neurogenin-1 in early VZ precursors inhibits glial differentiation by sequestering CBP–Smad1 away from glial-specific genes. When levels of neurogenin-1 decrease, CBP/p300 and Smad1, separately or together, are recruited to glial-specific genes (such as GFAP) by activated STAT1/ STAT3. Thus, neurogenin not only directly activates neuronal differentiation genes; it also inhibits glial gene expression.

patterning of the neural tube (Mehler, 1997). But later in development BMP homodimers and heterodimers potently induce astrocyte differentiation (D'Alessandro *et al*., 1994; Gross *et al*., 1996; Mabie *et al*., 1997). The BMP receptors are expressed at high levels in the VZs and SVZs from as early as E12, and BMP-4 also is expressed in these regions (Gross *et al*., 1996). *In vitro* studies have demonstrated that BMP ligands induce the differentiation of cells with the phenotypes of type 1 astrocytes or type 2 astrocytes depending upon which precursors are stimulated with ligand (Mabie *et al*., 1997; Zhang *et al*., 1998; Mehler *et al*., 2000). BMPs also inhibit precursor proliferation (even in the presence of mitogens like EGF), and they also increase the maturation of astrocytes (D'Alessandro and Wang, 1994; D'Alessandro *et al*., 1994). Comparative studies on the BMP ligands have shown that heterodimers comprised of BMP-2 and BMP-6, or BMP-2 and BMP-7, are potent at pico molar concentrations and that such heterodimers are more than three times more potent than homodimers of either ligand. Furthermore, they are much more potent than the related family member TGFβ1 which had been previously been implicated in astrocyte differentiation (Sakai *et al*., 1990; Sakai and Barnes, 1991; D'Alessandro *et al*., 1994; Gross *et al*., 1996).

BMPs signal through a heterodimeric receptor composed of type 1 and type 2 subunits, which are serine/threonine kinases. The BMP bind to the type 2 receptor which then associates with the type 1 receptor resulting in the phosphorylation of the type 1 subunit. This activates the receptor leading to the phosphorylation of the protranscription factor Smad-1. The phosphorylated Smad-1 can then dimerize with another Smad, such as Smad-4, to produce a transcriptionally active complex that can induce or repress target genes. Several of the genes regulated by BMP signaling are *Id1* and *Id3* which promote astrocytic differentiation and negatively regulate neuronal differentiation (Nakashima *et al*., 2001). Another means by which BMP signaling inhibits neuronal differentiation is by sequestering CBP/p300, thus preventing neuronal specification (Fig. 14). Supporting these models, BMPs increase the percentage of astrocytes from neural stem cells while decreasing the production of neurons (as well as oligodendrocytes) without concurrent cell death, consistent with the concept that BMPs promote the specification of astrocyte-restricted precursors (Gross *et al*., 1996; Nakashima *et al*., 2001; Sun *et al*., 2001).

## Fibroblast Growth Factor-8b Promotes Astrocyte Differentiation

There are at least 21 FGFs, and these signaling molecules have long been known to affect astrocyte development. For instance, FGF-2 is a potent mitogen for type 1 astrocytes and their precursors and FGFs will increase GFAP and GS levels in cultured astrocytes (Morrison *et al*., 1985; Perraud *et al*., 1988). The FGFs exert their effects by stimulating one of four transmembrane tyrosine kinase FGF receptors and three of these receptors (FGFRs 1–3) are expressed by neural precursors in the VZ and SVZ (Bansal *et al*., 2003). While the majority of studies have focused on FGF-2, a screen of nine FGF ligands (FGF-1, 4, 6, 7, 8a, 8b, 8c, 9, and 10) on embryonic rat neocortical precursors found that FGF-8b potently promoted the differentiation of a subpopulation of neocortical precursors toward astrocytes (Hajihosseini and Dickson, 1999). The other FGF8 ligands did not have this effect at the concentrations tested. As the precursors

expressed FGFRs 1–3, it is not presently clear which FGFR is mediating this inductive effect. FGFR3 does not appear to be essential since FGFR-3 null mice have more astrocytes than their wild-type counterparts (Oh *et al*., 2003). FGF-2 can have a similar effect to FGF8b, but at concentrations 10 times higher than are required for FGF8b (Qian *et al*., 1997).

## Signaling through the EGF Receptor Induces Astrocyte Specification

As discussed earlier, the ligand neuregulin, which binds to the erbB receptors, is produced and secreted by migrating neurons to prevent radial glia from differentiating into astrocytes (Anton *et al*., 1997; Rio *et al*., 1997). When the levels of neuregulin decrease, as they do during neuronal maturation, the radial glia become receptive to other astrocyte differentiating signals. As neural precursors become competent to generate astrocytes the levels of another receptor, the EGF receptor, increase, as does the level of one of its ligands, TGFα. In elegant experiments where the levels of the EGF receptor are experimentally increased, precursors that would not normally generate astrocytes do so precociously (Burrows *et al*., 1997). This occurs because raising the levels of EGF receptor confers competence to these early progenitors to respond to LIF (Viti *et al*., 2003). Indeed studies on early rat or mouse neural precursors or on precursors genetically deficient in EGF receptor show that LIF is incapable of inducing GFAP expression in cells lacking EGF receptors (Molne *et al*., 2000; Viti *et al*., 2003). In addition to providing competence to early progenitors to generate astrocytes, signaling through the EGF receptor has long been known to increase the proliferation of immature astrocytes (Leutz and Schachner, 1981). Thus, signaling through the EGF receptor coordinates several aspects of astrocytes development.

## PACAP, Increases cAMP to Induce Astrocyte Differentiation

The neuropeptide PACAP and one of its receptors, PAC1, are expressed highly in the VZ during late gestation and the PAC1 receptor is expressed by E17 neocortical precursors *in vitro*. As this receptor is known to increase cAMP within cells, and as it had been shown previously that elevating cytosolic cAMP increases the expression of GFAP by immature astrocytes (Shafit-Zagardo *et al*., 1988; Masood *et al*., 1993; McManus *et al*., 1999), Vallejo and Vallejo (2002) asked whether PACAP might induce astrocytic differentiation from fetal precursors. When they stimulated E17 forebrain precursors with PACAP, they observed increased levels of cAMP within 15 min, and the elevated levels of cAMP lead to phosphorylation of the transcription factor CREB. When examined 2 days later, PACAP exposed cells, or cells treated with a cAMP analog assumed a stellate shape, they had elevated levels of GFAP and they had decreased levels of nestin (McManus *et al*., 1999). Prolonged treatment with PACAP was not necessary as a 30-min exposure was sufficient to induce GFAP expression and stellation. Finally, inhibiting the increase in cAMP is sufficient to inhibit the increased GFAP expression induced by PACAP. Thus, elevating cAMP by PACAP will induce astrocytic specification from fetal precursors (Fig. 15).

## Notch Activation Can Promote Astrocyte Specification

The transmembrane signaling receptor Notch functions in a context dependent manner to regulate multiple aspects of neural development. The family of Notch transmembrane receptors control cell fate decisions by interaction with Notch ligands expressed on the surface of adjacent cells. As discussed earlier,

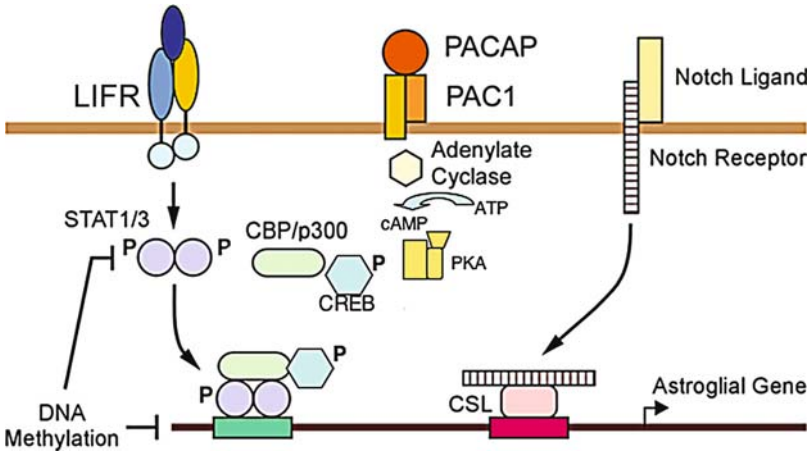


**FIGURE 15.** Signals regulating astrocyte specification. The LIF receptor (LIFR) activates the JAKs, and STATs, which can then combine with CBP/p300 to form a transcriptional regulator. Methylation of specific promotors will inhibit this complex from acting. The PAC1 receptor for PACAP increases levels of cAMP within the cell, which activates protein kinase A (PKA) to phosphorylate CREB, another transcription factor. Finally, cleavage of Notch receptors subsequent to binding by a Notch ligand releases the intracellular domain, which can combine with CSL to directly regulate genes involved in astrocyte specification.

Notch signaling promotes radial glial cell formation, and other studies have demonstrated that Notch inhibits differentiation at later stages in neural lineages as well. However, several recent studies show that Notch can instructively promote astrocytic differentiation. Studies by Tanigaki *et al*. (2001) and Ge *et al*. (2002) using either hippocampal-derived multipotent or E11 neocortical precursors, respectively, showed that introducing the signaling component of either the Notch1 or Notch3 receptors induces the expression of GFAP, increases the size of the cells and stimulates process formation. Moreover, activated Notch appears to act instructively as it reduces the number of neuronal and oligodendroglial cells while increasing the percentage of astrocytes. This effect of Notch on astroglial differentiation is not likely indirect, since the intracellular signaling domain of Notch forms a transcriptional complex with CSL and SKIP that binds to specific elements of the GFAP promotor to initiate transcription of GFAP. Notch signaling also induces the downstream target transcriptional regulator, Hes-1 (but not Hes-5). While Notch can clearly regulate GFAP expression, Hes-1 likely mediates some of Notch's effects on astrocyte differentiation. In experiments where the Hes transcription factors are overexpressed in glial-restricted precursors, overexpressing Hes-1, but not Hes-5, promotes astrocytic differentiation (as indicated by increased GFAP and CD44 expression) at the expense of oligodendrocyte differentiation (Wu *et al*., 2003). Importantly, this effect of Hes-1 is stage-specific because Hes-1 does not promote the astrocyte fate when overexpressed in neuroepithelial cells. Altogether, these experiments demonstrate that Notch can directly induce astroglial gene expression by forming a transcriptional complex with CSL and SKIP, and that this transcriptional complex also induces downstream signaling molecules like Hes-1 that also regulate astrocyte differentiation.

## An Interplay of Multiple Pathways Contributes to Astrocyte Genesis

The competence of neural precursors to respond to extracellular signals is certainly one mechanism that regulates the onset of astroglial differentiation. One intrinsic feature that may determine whether a precursor will generate neurons or glia is the balance between "neurogenic" and "gliogenic" transcription factors. For instance, early neuroectodermal precursors express higher levels of Neurogenin 1, which correlates with the preference for these cells to differentiate into neurons rather than glia (Fig. 14). Overexpressing Neurogenin 1 in embryonic neuroepithelial cells not only promotes neurogenesis, but also decreases the ability of these cells to respond to astrocyte inducing signals, such as LIF (Sun *et al*., 2001). Sun *et al*. (2001) demonstrated that neurogenin 1 binds to the same CBP/p300, complex as the STATs. Furthermore, the Neurogenin-1-binding domain overlaps with the STAT-binding domain on CBP/p300; thus, Neurogenin 1 and STAT cannot physically bind to CBP/p300 simultaneously. Consequently, the relative levels of neurogenin 1 and STAT3 may in part determine whether an immature cell becomes a neuron or an astrocyte. Furthermore, Neurogenin 1 inhibits STAT phosphorylation. Thus, competition between Ngn1 and STAT for these transcriptional coactivators as well as negative regulation of STAT phosphorylation provides a viable mechanism for determining a neocortical precursor's fate. However, merely overexpressing Neurogenins or Mash 1 by retroviral infection does not alter dramatically the numbers of neurons vs astrocytes that develop, suggesting that it is not just the levels of the transcription factor that determines cell fate *in vivo*. Similarly, knocking out both Neurogenin 2 and Mash 1 does not produce a dramatic decrease in neurons and increase astrocytes, although the cortices of these mice displayed marked disorganization of laminar patterning (Nieto *et al*., 2001).

DNA and histone methylation also regulate the intrinsic capacity of neural precursors to differentiate into astrocytes. A CpG dinucleotide within the STAT3-binding element of the GFAP promotor is highly methylated in early neuroepithelial cells, and the methylation of this site prevents STAT3 from binding. Consequently, the STATs cannot act as transcriptional activators of GFAP. This site is demethylated during CNS development, coincident with transcriptional activation by STATs and commensurate with astroglial differentiation (Takizawa *et al*., 2001). Furthermore, growth factors that have been shown to increase the competence of early precursors to generate astrocytes increase the methylation of Histone H3 at specific lysines which results in changes in chromatin conformation, again enabling specific genes involved in astroglial differentiation to be expressed (Song and Ghosh, 2004).

How might other extrinsic signaling molecules regulate astrocyte development *in vivo*? As discussed above, most of the soluble factors that can instructively drive astrocyte development are present in the developing CNS and some are present quite early. For instance, BMP-4 is present as early as E14, which is when neurons are produced, yet BMP-4 does not induce neuronal generation from early precursors. One reason is that the BMP antagonist, Noggin, is expressed in the developing cortex (Li and LoTurco, 2000) and in adult rodents, Noggin is found in ependymal cells (Lim *et al*., 2000). There it may function to counteract BMP-induced astrocytic development. LIF, which can induce astrocytes, also is present in the VZ quite early, and indeed, signaling through the LIF receptor is required to maintain the complement of neural stem cells. However, as reviewed above, in the absence of EGF receptor signaling, alpha helical cytokines cannot induce astrocyte differentiation. CNTF/LIF may be insufficient to induce astrocytes from SVZ cells later in development as factors present in the extracellular matrix may be required (Lillien *et al*., 1990). As discussed above, immature astrocytes derived from the SVZ interact with basal laminae at blood vessels and at the pial surface, and blood vessel interactions appear to be an early step in astrocyte differentiation (Zerlin and Goldman, 1997; Mi *et al*., 2001). Altogether, these examples demonstrate that astrocyte differentiation is coordinately regulated by the intrinsic properties of neural precursors as well as by the simultaneous signaling from multiple extrinsic signaling molecules.

## CONCLUSION

We began this chapter by reviewing the types of astrocytes that populate the mature brain and then proceeded to discuss where and how astrocytes form. While there remain gaps in our knowledge, it is clear that there are multiple sources of astrocytes. In the forebrain, both the VZ and the SVZ produce astrocytes. The radial glia, which are direct descendants of the neuroepithelium, are one source of astrocytes. SVZ cells, which emerge later in development, are a second source, and they produce a subset of gray matter astrocytes. In the cerebellum, astrogliogenesis may proceed in a fashion similar to that established for the forebrain, but astrocyte generation in the spinal cord is different. Great strides continue to be made in defining the precursor product relationships between different types of phenotypically defined glial precursors and the cells that they produce. Moreover, elegant *in vitro* analyses are beginning to unravel the relative roles of the intrinsic competences of precursors at defined stages of development to respond to specific extrinsic signaling molecules. Multiple extrinsic signals have been identified that coordinate astrocyte differentiation. These include the alpha helical cytokines, BMPs, Notch ligands, FGF8b, EGF ligands, and PACAP, and as more is learned about the transcriptional regulators that they use, it may turn out that the internal signals used to establish an astrocytic fate are less complicated than the multiple signals that impinge upon their precursors. Clearly much has been learned over the last century when astrocytes were first discerned as a recognizable cell type, yet there are still many basic issues that remain to be addressed. We hope that this chapter has provided a conceptual framework onto which you, the reader, may incorporate the forthcoming answers.

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