Preface

Neural differentiation is an early embryonic event that occurs soon after germ layer specification from the blastula. The early formed ectoderm undergoes further patterning to separate into two identifiable components, the presumptive neural ectoderm and the presumptive epidermis. Neural tissue segregates as a clearly demarcated epithelium termed the neuroepithelium (or neuroectoderm). This neuroepithelium generates the central nervous system (CNS), whereas cells at the margins of the neuroepithelium will generate the peripheral nervous system (PNS). A variety of evidence has been accumulated to show that the process of neural differentiation involves a sequential restriction in differentiation potential.

A fundamental breakthrough in our understanding of nervous system development was the identification of multipotent neural stem cells (neurospheres) about 10 years ago. Dr. Samuel Weiss and colleagues showed that EGF (epidermal growth factor)-dependent stem cells could be harvested from different brain regions at different developmental stages and that these could be maintained over multiple passages in vitro. The original finding that EGF-dependent neural stem cells exist has been replicated and extended by many investigators, and there has been a veritable explosion of research on stem cells, their role in normal development, and their potential therapeutic uses. Different classes of neural stem cells have been identified, new markers described, cell lines generated, and factors that regulate the differentiation process characterized. Other investigators have shown that these pluripotent stem cells likely generate CNS and PNS derivatives via the generation of intermediate lineage restricted precursors that differ from each other and from multipotent stem cells. The therapeutic implications of accessing a virtually unlimited population of homogenous progenitor cells to treat CNS disorders or for gene and drug discovery has not escaped investigators, and several companies have been formed to exploit stem cell technology and several research institutions have initiated transplant studies. This rapid transition from a basic discovery to clinical trials is both surprising and unprecedented.

In *Stem Cells and CNS Development,* I have invited some of the leading authorities in the field of neural stem cell biology to summarize their findings and describe how these results may lead to novel therapies. The first part of the book surveys the various kinds of stem cells, progenitor cells, and precursors that have been described, while the second half describes how these cells are beginning to be used for therapeutic purposes. It is my hope that this book will serve as a valuable compendium of practical information on the current state of the field for all those engaged in this research.

Mahendra Rao

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Multipotent Stem Cells in the Embryonic Nervous System

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Neural stem cells are defined by a number of properties, including their ability to proliferate, to maintain themselves (self-renew), to retain multilineage potential over time, and to generate large numbers of progeny, often through transient amplification of intermediate progenitor pools. Although self-renewal can occur through symmetric cell divisions that generate two identical daughter cells, asymmetric cell divisions that generate a renewable stem cell and a more lineage-restricted daughter cell are a hallmark of stem cells in many organ systems. Cells that do not self-renew but that nevertheless proliferate and have the capacity to generate multiple phenotypes are often referred to as multipotential progenitor cells, but they will be included in a broad definition of stem cells for the purposes of this review. Other stem cell-derived precursor populations that are able to proliferate but that have more restricted lineage potential (e.g., glial restricted or neuronal restricted cells) are discussed elsewhere (see Chapters 5 and 6; *1*).

At present there are no generally accepted markers that allow the unambiguous identification of stem cells in the developing nervous system in vivo. Expression of the intermediate filament proteins nestin and/or vimentin coupled with the lack of expression of markers of more differentiated progeny is frequently used to identify putative neural stem/progenitor cells in culture. Neural stem cells arise from generative zones, derived from the inner lining of the neural tube, that extend from periventricular regions of the telencephalon to the spinal cord within the mammalian central nervous system (CNS) *(2)*. These zones initially consist of pseudostratified ventral epithelium (ventricular zone [VZ]) that gives rise during late embryonic life to secondary subventricular zones (SVZs) that persist in an attenuated form

From: *Stem Cells and CNS Development*
Edited by: M. S. Rao © Humana Press Inc., Totowa, NJ

into the adult state. Neurons and radial glia are generated predominantly within the early embryonic VZ, whereas oligodendrocytes and astrocytes are largely generated during perinatal and early postnatal periods within regional cortical SVZs.

Patterns of labeling and growth of putative stem cells within the early embryonic cerebral cortical VZ suggest that the earliest cell divisions are symmetric, with the elaboration of equivalent daughter cells *(3,4)*. This process presumably allows exponential expansion of the resident progenitor population. Later, progenitor cell development and migration involve asymmetric cell divisions that cause the elaboration of apical and basal daughter cells, with neuronal differentiation of the basal cell and subsequent migration of the basal neuroblast to regions of the developing cortical plate. Following early neuroblast migration, an additional wave of symmetric cell divisions is essential for coordinating cortical laminar organization. Similarly, in slice cultures of developing ferret brain, early cell divisions are oriented primarily in a plane vertical to the ventricular surface and generate two apparently similar daughter cells. By contrast, later cell divisions occur predominantly in a horizontal plane and generate two different daughter cells by asymmetric cell division *(5)*. Interestingly VZ stem cells in low-density culture undergo stereotyped patterns of both symmetric and asymmetric cell divisions, suggesting that patterns of division are governed at least in part by cell-intrinsic programming *(6)*.

Although the mechanisms underlying asymmetric division of vertebrate stem cells remain unclear, important clues have emerged from studies of *Drosophila* development. A number of proteins have been identified that show a polarized distribution during asymmetric division of neural precursors, including Inscuteable, Miranda, Prospero, Staufen, Bazooka, and Numb proteins. Asymmetric localization of these proteins is microfilament dependent and coordinated with positioning of the mitotic spindle, leading to unequal distribution to daughter cells during cell division. Numb is required for the asymmetric cell division of at least some neural lineages *(7)*. Vertebrate homologs of Numb have been identified that are asymmetrically localized in cells in the developing mouse cortex and that appear to participate in the process of asymmetric cell division *(8)*. Interactions of Numb with Notch provide at least one way of integrating cell intrinsic and extrinsic mechanisms in determining the asymmetric fate of daughter cells *(9,10)*.

REGULATION OF STEM CELL PROLIFERATION AND SURVIVAL

Proliferation and survival of stem cells are regulated by a variety of factors including members of the fibroblast growth factor (FGF) and epider-

mal growth factor (EGF) families *(11–14)*. Studies of stem cells in culture have provided insight into some of the mechanisms governing progenitor cell proliferation and survival. Embryonic and postnatal stem cells do not survive well in culture in the absence of added growth factors, but they survive and proliferate when cultured in the presence of mitogens such as basic (b)FGF or EGF. In the absence of a culture substratum that promotes cell adherence, stem cells proliferate as clonal aggregates of cells ("neurospheres") ranging in size from a few cells to hundreds of cells. Stem cells that proliferate as neurospheres in the presence of these mitogens remain largely undifferentiated as judged by continued expression of nestin and vimentin and lack of expression of markers of more mature progeny, but they generate both neurons and glia when replated onto a culture substratum and upon withdrawal of the mitogen *(12,15,16)*. This facilitates uses of "neurosphere assays" in which the percent of neurospheres and the percent of cells within a neurosphere that commit to specific phenotypes are determined. By contrast, primary cultures of stem cells proliferate as a monolayer of cells when plated onto an adherent substratum in the presence of bFGF or EGF. Clonal analyses of low-density cultures have allowed examination of the developmental potential of single mitotic stem cells and the effects of defined epigenetic signals in altering cell fate *(17–19)*.

Stem cells exhibit differing requirements for EGF and bFGF during neural development. The preponderance of evidence suggests that the survival and proliferation of early embryonic progenitor species are regulated by bFGF *(11,14,20)*. Although early embryonic stem cells generated under the influence of bFGF are multipotential, they appear to be predisposed toward neuronal differentiation. Early embryonic VZ progenitor cells do not express the EGF receptor (EGFR), and the cells do not respond to ligand *(21,22)*. However, the receptor is progressively expressed during development by later SVZ progenitors and EGF and/or transforming growth factor-α (TGF-α) (which is another ligand for the EGFR) then regulate cellular proliferation and survival *(21–23a)*. Most evidence suggests that EGF-responsive progenitor cells are the predominant species present during the period of perinatal gliogenesis. The EGF-responsive cells appear to be derived from FGF-responsive stem cells, although they display different kinetics of proliferation *(20,24)*. EGF-responsive progenitor cells retain the capacity to generate all cellular phenotypes, but they appear to be predisposed to differentiate into glia.

There is substantial evidence that FGF and EGF receptor activation regulate stem cell proliferation and survival in vivo. Mice lacking functional bFGF have reduced tissue mass and reduced numbers of both neurons and glia in the cerebral cortex *(25,26)*, and injection of neonates with neutraliz-

ing antibodies to bFGF reduces DNA synthesis in several areas of brain *(27)*. Conversely, injection of bFGF into the cerebral ventricles of rat embryos increases the volume of cerebral cortex and the number of neurons generated *(26)*, and subcutaneous administration of bFGF to neonatal rats increases neuroblast proliferation in regions still undergoing neurogenesis *(28)*. Finally, ligands of the FGF family including bFGF are expressed contiguous to generative zones in the developing brain in vivo from early embryogenesis into adulthood *(29,30)*. Similarly, targeted deletion of the EGF receptor leads to defects in cortical neurogenesis *(23)*, and deletion of functional TGF-α (which activates EGF receptors) leads to diminished proliferation of precursors in the SVZ of mature animals. Additional evidence involving injection of EGF receptor ligands into brains of mature rats supports a role for these ligands in stem cell proliferation in adults (see Chapter 3). Finally, TGF-α is expressed in the developing brain in vivo from E13 into adulthood *(22)*.

A number of other factors have been implicated in the control of stem cell proliferation. *Sonic hedgehog* (Shh) is a member of the *hedgehog* (hh) multigene family that encodes signaling proteins involved in induction and patterning processes in vertebrate and invertebrate embryos (for review, see ref. *31*). However, in addition to its effects on axial patterning and cellular differentiation, Shh appears to regulate stem cell proliferation. Ectopic overexpression of Shh in the mouse dorsal neural tube increases rates of proliferation of embryonic spinal cord progenitor cells *(32)*. Although Shh increases proliferation of cultured neural stem cells, unlike bFGF or EGF it does not enhance cell survival *(33)*. The factor is a potent mitogen for cultured retinal progenitor cells, cerebellar granule cell precursors *(34)*, neuronal restricted precursors in the spinal cord *(35)*, and skeletal muscle cells, and overexpression of the Shh gene leads to basal cell carcinoma (for review, see ref. *31*). To activate target genes, the N-terminal signaling domain of Shh (Shh-N) binds to the Shh-binding protein, Patched (Ptc), which is complexed with Smoothened (Smo), to counteract the inhibition by Ptc of constitutive signaling activity mediated by Smo (for review, see ref. *36*). Disruption of the gene encoding Ptc leads to meduloblastoma and other primitive neuroectodermal tumors. These cumulative observations suggest that Shh is an important regulator of neural stem cell proliferation.

Stem cell proliferation may also be influenced by other types of regulatory signals including the wnt pathway, glutamate, γ-aminobutyric acid (GABA), biogenic amines, opioid peptides, and other peptides such as vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP). For example, injection of pregnant mice from E9 to

E11 with a VIP antagonist reduces bromodeoxyuridine (BrdU) labeling in germinal zones in the developing embryonic brains and reduces the subsequent size of the ventricular and intermediate zones *(37)*. Disruption of wnt and wnt-3a leads to deficits in expansion of dorsal neural prognitor cells *(38)*. However the precise role of these regulatory influences on stem cell proliferation in vivo are less well-characterized than the effects of bFGF, EGF, and Shh.

Stem cell proliferation also appears to be regulated by factors that actively promote exit of the cells from cell cycle. For example, treatment of cultured stem cells with members of the bone morphogenetic protein (BMP) family of factors promotes rapid exit of the cells from cell cycle, even in the presence of mitogens such as bFGF, EGF, or Shh *(33,39)*. Proliferation of cortical precursor cells cultured in the presence of bFGF is diminished by cotreatment with either neurotrophin 3 (NT3) *(40)* or GABA *(41)*. Glutamate treatment of embryonic cortical explants significantly reduces proliferation of putative progenitor cells *(42)*, and treatment with PACAP decreases proliferation of embryonic cortical precursors *(43)*. In most instances exit from cell cycle induced by these factors is associated with enhanced differentiation of the surviving cells.

Stem cell numbers appear to be regulated primarily by rates of proliferation that in turn reflect cell cycle duration, the length of time during which exponential expansion of cell numbers occurs, and the ratio of asymmetric to symmetric cell divisions *(4)*. However, there is now increasing recognition that stem cell numbers may also be influenced by apoptotic cell death within proliferative periventricular generative zones. Cell death is rare during early embryogenesis (E10), peaks during the E14–15 period, and begins to recede in late embryonic life *(44)* Targeted disruption of either caspase-9 or caspase-3 leads to decreased programmed cell death of cortical precursors, causing expansion and exencephaly of the forebrain as well as supernumerary neurons in the cerebral cortex *(45)*. By contrast, disruption of the c-Jun N-kinase signaling pathway leads to precocious degeneration of cerebral precursors *(45)*. Selective survival of different populations of progenitor cells may be important not only for regulation of cell numbers and specification of cellular phenotypes, but also for the morphogenesis of the brain. For example, the BMPs induce apoptosis of selected rhombencephalic neural crest-associated progenitor species, resulting in segmentation of the rhombencephalon *(46)*. Furthermore, the BMPs also induce apoptosis of VZ stem cells in culture *(19)* and of forebrain precursor cells in explants *(47)*. Stem cell numbers in different regions of the neuraxis thus appear to reflect regional rates of survival as well as proliferation.

DEVELOPMENTAL CHANGES IN STEM CELLS

Although stem cells retain the ability to generate neurons, oligodendroglia, and astrocytes throughout the embryonic and postnatal periods (and even in the adult — see Chapter 3), there are clearly developmental changes in both their bias toward differentiation into specific cell types and their responses to epigenetic signals. Early VZ stem cells in culture are predisposed to become neurons and to a lesser extent oligodendroglia, whereas SVZ stem cells are biased toward astrocytic differentiation. As noted previously, EGFRs are not expressed by VZ stem cells but are expressed by the cells in the SVZ. Although neural stem cells thus become progressively more biased toward a glial fate during development coincident with an increase in expression of EGFRs, the role of EGF signaling in this change is unclear. Retroviral introduction of extra EGFRs into VZ progenitor cells results in premature expression of traits characteristic of later SVZ progenitors including the bias toward astrocytic differentiation *(21)*. This suggests that developmental increases in levels of EGFRs expressed by progenitor cells may mediate changes in their responses to environmental signals and their tendency to differentiate into astrocytes. However, similar experiments involving introduction of extra copies of the EGFR into early embryonic retinal progenitor cells does not bias the cells toward a glial fate *(48)*. More importantly, pharmacologic blockade of EGFR signaling does not alter the developmentally increased bias of cultured progenitor cells to undergo astrocytic differentiation *(48a)*, suggesting that the competence to generate glia is temporally regulated by other mechanisms. There are also striking differences between VZ and SVZ stem cell responses to differentiating signals such as the BMPs *(49)* or leukemia inhibitory factor (LIF) *(50)*, so the same signals may induce different phenotypes at different developmental stages. Thus analysis of the factors regulating stem cell differentiation requires knowledge of the developmental stage and history of the cell.

MAINTENANCE OF STEM CELL FATE

The stem cell phenotype is maintained by both daughter cells during the period of symmetric cell divisions and rapid expansion of the stem cell pool in early embryos, and it is maintained by one daughter cell of each pair during later asymmetric cell divisions. Although the mechanisms underlying the maintenance of the stem cell phenotype in vertebrates are not yet well-described, it has become increasingly evident that there are active cell intrinsic as well as extrinsic mechanisms that inhibit lineage commitment by these cells. The most intensively studied example of such inhibitory signaling involves the Notch pathway. Notch and its ligands Delta and Serrate are integral membrane proteins that

generally transmit signals only between cells in direct contact. Overexpression of Delta1 (i.e., activation of Notch) suppresses neurogenesis, whereas overexpression of a dominant negative inhibitor of Delta1 leads to premature commitment of stem cells to the neuronal fate *(51)*. Activation of Notch also regulates transcriptional activity, including inhibition of production of Notch ligands by that cell. Through a process termed lateral inhibition, cells that produce ligand force neighboring cells to produce less ligand, thereby enabling the ligand-producing cells to increase production even further. The effect of such a feedback loop is to amplify small differences between neighboring cells and to drive them into different developmental pathways. Delta1 is expressed by a scattered subset of cells (nascent neurons; *52*) in the outer part of the VZ zone, whereas Notch1 is expressed throughout the VZ *(53)*. Delta production by daughter cells undergoing neuronal differentiation activates Notch in their dividing partners, thereby inhibiting neuronal differentiation and maintaining a cohort of stem cells so that neurogenesis can continue. Notch1 signaling also inhibits differentiation into alternative fates such as oligodendroglial differentiation *(54)*. More recent studies suggest that notch signaling promotes the generation of radial glia *(54a)*.

Stem cells express a number of other proteins whose function appears to be related to the maintenance of the undifferentiated state. For example, HES1 *(55,56)* was originally isolated as a mammalian homolog of hairy and Enhancer of Split, which negatively regulate neurogenesis in *Drosophila*. HES1 negatively regulates transcriptional activation mediated by basic helix-loop-helix (bHLH) genes and normally functions to repress the commitment of multipotent progenitor cells to the neuronal lineage, thereby maintaining their self-renewing state *(57)*. Overexpression of Hes1 prevents both migration of neural stem cells out of the VZ and expression of neuronal markers *(58)*, whereas HES1-deficient brains prematurely express neurofilaments *(59)*. These observations suggest that the gene is required for the negative regulation of neuronal differentiation.

Stem cell fate may also be maintained by the four members of the ID (*i*nhibitor of *D*NA binding and *i*nhibitor of cell *d*ifferentiation) family of proteins that resemble bHLH factors but that lack a basic region necessary for DNA binding. The ID proteins act as dominant negative inhibitors by preferentially dimerizing with a subset of bHLH factors to form inactive complexes, thereby decreasing bHLH-mediated transcriptional activity (for review, see ref. *60*). Members of the ID family are expressed throughout the nervous system during neurogenesis with localization of ID transcripts within putative neural stem cells *(61,62)*. Targeted disruption of both ID1 and ID3 in the same animals results in premature withdrawal of neuroblasts from cell cycle and expression of neuron-specific differentiation markers

(63). These observations suggest that expression of ID proteins is necessary to maintain stem cells in the undifferentiated, proliferative state.

TRANSCRIPTION FACTORS THAT PROMOTE LINEAGE COMMITMENT BY STEM CELLS

The observation that maintenance of the stem cell phenotype requires inhibition of bHLH factors by ID proteins and HES1 suggests that bHLH factors are involved in directing stem cell differentiation. There is, in fact, a large body of evidence that regulatory cascades of bHLH and other transcription factors play essential roles in mammalian neurogenesis. Detailed discussions of the relationship of neurogenesis to neural induction and of the genes involved in neurogenesis are beyond the scope of this chapter but are available in a number of recent reviews *(64,65)*.

Briefly, bHLH neurogenic factors are thought to regulate neuronal development positively at the level of both commitment and of postmitotic differentiation. Overexpression of bHLH genes such as *Mash1*, *neurogenin* (*Ngn*), or *neuroD* induces ectopic expression of neurons, whereas targeted deletion leads to deficits in the generation of neurons (for review, see refs. *66* and *67*). These factors act through stereotyped cascades; for example, *Ngn* expression precedes that of *neuroD*, and *Ngn* activates *neuroD* but not vice versa, suggesting that *Ngn* acts upstream of *neuroD* in neuron production.

The cascades may involve other types of transcription factors and homeobox genes. For example, the zinc finger transcription factor MyT1 is involved in neurogenesis, and blocking MyT1 function inhibits ectopic neurogenesis induced by *Ngn (68)*. This suggests that MyT1 activation is part of the cascade initiated by *Ngn*. Similarly, *Mash1* regulation of noradrenergic neuron differentiation depends in part on the homebox gene *Phox2a*, and targeted deletion of *Phox2a* abolishes the locus coeruleus, the major noradrenergic center in the brain *(69,70)*.

In summary, neuronal lineage commitment and progressive neuronal differentiation involve the coordinated interplay of positive and negative regulatory signals, including cascades of transcription factors that regulate lineage-specific gene expression. Furthermore, multiple signal cascades are involved in the generation of different populations of neurons, and activation of these cascades reflects the effects of both cell intrinsic and extrinsic factors that promote cell differentiation.

FACTORS REGULATING LINEAGE COMMITMENT BY STEM CELLS

Lineage commitment by stem cells results from the confluence of the effects of cell intrinsic and extrinsic signals, and there is substantial overlap

among the factors involved in proliferation and survival and those that regulate lineage commitment and cellular differentiation. For example, in addition to effects on survival and proliferation, bFGF and other members of the FGF family influence lineage commitment by embryonic neural stem cells. Withdrawal of bFGF from stem cells in vitro promotes generation of neurons and glia, suggesting that the factor represses intrinsic programs of stem cell differentiation. Furthermore, exposure of stem cells to bFGF alters their subsequent developmental bias. Treatment of cultured stem cells with bFGF promotes expression of the EGF receptor *(20,71)* and enhances expression of differentiated traits such as the catecholamine biosynthetic enzyme tyrosine hydroxylase *(72)*. Furthermore, the concentrations of bFGF to which embryonic stem cells are exposed in vitro influences cell fate; low concentrations of bFGF favor neuronal differentiation, whereas higher threshold concentrations favor oligodendroglial differentiation *(14)*.

This may reflect preferential activation of different subtypes of FGF receptors by different concentrations of the factor, a conclusion supported by observations of the differential effects of other FGF family members. For example, treatment of cultured stem cells with FGF-1 in the presence of heparan sulfate proteoglycan preferentially promotes neuronal differentiation, whereas bFGF (FGF-2) treatment of sister cultures preferentially promotes proliferation *(73)*. FGF-8 collaborates with Shh to induce dopaminergic neurons in the mid/hindbrain, whereas FGF-4 in association with Shh induces a serotonergic cell fate *(74)*.

Shh also appears to be involved in the induction of neuronal phenotypes in the brain (for review, see ref. *31*) and in the induction of oligodendrocyte lineage commitment in the spinal cord *(75,76)*. Shh treatment of cultured neural stem cells promotes the elaboration of both neuronal and oligodendroglial lineage species *(33)*, suggesting that its differentiating effects reflect direct actions on neural stem cells. Furthermore, neural stem cells express smoothened *(33)*, the signaling component of the Shh receptor, and constitutively active forms of smoothened reproduce inductive effects of Shh *(77)*, suggesting that Shh exerts its inductive effects directly on stem cells. However, the final phenotype of cells induced by Shh depends on other inductive signals and on other genes expressed by progenitor cell populations. For example, mutation of the homeobox gene *Nkx2.2* in progenitor cells alters the inductive effects of Shh in specifying the neuronal identity (motor neuron vs interneuron) of the progeny *(78)*. As noted above, interactions between the effects of Shh and other growth factors including wnt, FGF-4, and FGF-8, are critical for specifying alternate cellular phenotypes in the brain *(74)* and for patterning of the dorsal compartment of the somite. Interactions between Shh and members of the BMP gene family are

important for the specification of dorsal and intermediate dorsoventral cell types (for reviews, see *31* and *79*), and Shh inhibits BMP signaling, in part by inducing the endogenous BMP inhibitor noggin *(80)*. Shh and BMP signaling exert directly opposing effects on both proliferation and differentiation of cultured neural stem cells *(33)*.

Neuronal differentiation of stem cells is thus regulated by a diversity of factors including the Notch/delta pathway, NUMB family members *(10,81)*, FGF family members, Shh, BMP family members *(19,82,83)*, wnt family members *(84)*, retinoid-activated pathways *(85,86)*, and other signaling molecules (for review, see ref. *87*). The existence of so many pathways for neuronal lineage commitment and differentiation presumably reflects the diversity of neuronal phenotypes that must be generated. There is clearly diversity among stem/progenitor cell populations even at early developmental stages (for review, see refs. *11* and *88*), and there are developmental changes in stem cells that lead to markedly different cell fate decisions in response to the same factors at different developmental stages *(49)*. Commitment and differentiation of stem cells to specific neuronal lineages thus reflect complex patterns of events and parallel pathways for neurogenesis.

Just as there are multiple pathways of neuronal differentiation, there are several different pathways leading to astrocytic lineage commitment by neural stem cells. The peak period of gliogenesis occurs during late embryonic and early perinatal cerebral cortical development, and SVZ stem cells are biased toward astrocytic differentiation compared with VZ stem cells. Treatment with the BMPs promotes the elaboration of mature astrocytes from late embryonic SVZ-derived stem cells in culture as well as from early postnatal cerebral cortical multipotent and bipotent oligodendroglial-type 2 astroglial (O-2A) progenitor cells *(39,89)*. Ciliary neurotrophic factor (CNTF) and LIF also potentiate the generation of astrocytes from embryonic neural stem cells; genetic and developmental analyses confirm that a CNTF/LIF subgroup of factors that interacts with gp130/LIF-β receptors participates in astrogliogenesis *(90,91)*. However, BMP-2 treatment of progenitor cells cultured from animals that are deficient in the LIF-β receptor induces astrocytic lineage commitment, indicating that astrocytic differentiation does not require signaling through gp130/LIFRs *(91)*. CNTF and LIF signal through the JAK/STAT signaling pathway, whereas the BMPs signal through Smad-mediated pathways. Formation of a complex between STAT3 and Smad1, bridged by the transcriptional coactivator p300, may mediate cooperative effects of these two classes of factors on stem cell commitment to the astrocytic lineage *(92)*.

In view of the foregoing observations regarding multiple pathways of neuronal and astrocytic lineage commitment, it is not surprising that oligodendroglia (OLs) also appear to be generated from multiple lineages in response to a number of different epigenetic signals (for review, see ref. *93*). During embryonic development in the spinal cord, the expression of Jagged, a Notch ligand, coincides with the elaboration of foci of OL precursors from paramedian generative zones. Shh, a notochord-derived signal, supports the generation of mature OL lineage species from caudal regions of the neuraxis (spinal cord), but its role as an obligate developmental signal for more anterior regions of the central nervous system is unclear. Oligodendroglia are first generated in the embryonic spinal cord in response to signals derived from the floor plate and notocord. Treatment of spinal cord explants with Shh induces both OLs and neurons *(76)*, and antibodies that neutralize Shh prevent OL lineage commitment *(75)*. Shh treatment of cultured embryonic stem cells derived from neurospheres also induces both oligodendroglial and neuronal differentiation *(33)*, suggesting that OL lineage commitment reflects direct effects of Shh on stem cells. However, other factors produced by neurons influence this process, and accumulating evidence suggests that members of the neuregulin family may be involved *(94)*. Other factors are capable of promoting OL lineage commitment by cultured neural stem cells; for example, increased concentrations of bFGF or brief exposure to thyroid hormone foster OL differentiation *(13,14)*. The regulation of later stages of OL differentiation from glial restricted precursors is described elsewhere (Chapter 6).

PATTERNS OF STEM CELL DIFFERENTIATION IN VIVO

Tracking the fate of stem cell progeny in vivo became possible after development of techniques for labeling individual VZ cells with replication-defective retroviral vectors that label all daughter cells with an inheritable marker such as β-galactosidase. In early experiments, injection of retroviruses into E14 murine lateral ventricles gave rise to small, scattered clones that largely consisted of a single cell type, neuron, oligodendrocyte, or astrocyte. Mixed clones of neurons and glia were uncommon (<1% of clones), and most neuronal clones were of a single cell type, pyramidal or nonpyramidal *(95–98)*. This gave rise to a concept that the VZ consisted largely of different progenitor cell types committed to specific lineages. However, there was a surprisingly large degree of scattering of cells, suggesting that unexpectedly large amounts of cell movement in vivo might be blurring clonal boundaries. Walsh and Cepko *(99)* addressed this problem by using a library of heterogeneous retroviral vectors with numerous

genetic tags. Any daughter cells containing precisely the same mixture of tags were presumed to arise from the same progenitor, even if the progeny were scattered widely. Injection into E15–17 rat embryos generated some spread clones of clusters of different cell types and an equivalent number of smaller clones of a single cell type *(99,100)*. This is the pattern that would be expected if the retrovirus infected asymmetrically dividing cell pairs since one cell would display the multipotent stem cell phenotype, and the other would display the phenotype of a committed cell. In turn, this suggests that most cells generated by the VZ during this time period arise from multipotent progenitor cells undergoing active asymmetric division, a conclusion consistent with studies of stem cells in culture *(101,102)*. However, the precise proportions of stem cells, uncommitted progenitors, and committed progenitors at differing developmental stages and in different regions of the generative zones are unknown.

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