

## State-of-the-Art Review

# Adult Neural Stem Cells and Repair of the Adult Central Nervous System

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### ABSTRACT

Neural stem cells are present not only in the developing nervous systems, but also in the adult central nervous system of mammals, including humans. The mature central nervous system has been traditionally regarded as an unfavorable environment for the regeneration of damaged axons of mature neurons and the generation of new neurons. In the adult central nervous system, however, newly generated neurons from adult neural stem cells in specific regions exhibit a striking ability to migrate, send out long axonal and dendritic projections, integrate into pre-existing neuronal circuits, and contribute to normal brain functions. Adult stem cells with potential neural capacity recently have been isolated from various neural and nonneural sources. Rapid advances in the stem cell biology have raised exciting possibilities of replacing damaged or lost neurons by activation of endogenous neural stem cells and/or transplantation of in vitro-expanded stem cells and/or their neuronal progeny. Before the full potential of adult stem cells can be realized for regenerative medicine, we need to identify the sources of stem cells, to understand mechanisms regulating their proliferation, fate specification, and, most importantly in the case of neuronal lineages, to characterize their functional properties. Equally important, we need to understand the neural development processes in the normal and diseased adult central nervous system environment, which is quite different from the embryonic central nervous system, where neural development has been traditionally investigated. Here we will review some recent progress of adult neural stem cell research that is applicable to developmental neurobiology and also has potential implications in clinical neuroscience.

### INTRODUCTION

**S**TEM CELLS ARE THE ESSENTIAL BUILDING BLOCKS of multicellular organisms (1–3). They have two defining properties—they can self-renew to produce more stem cells and they can differentiate to generate specialized cell types. Stem cells come in different varieties, depending on when and where they are produced during development and how versatile they are (1–3). Totipotent stem cells can give rise to a full organism once implanted in the uterus of a living animal. Both embryonic stem (ES) cells (produced in culture from the epiblast cells of a blastocyst) and embryonic germ (EG) cells (produced in cul-

ture from primordial germ cells of an early embryo) are pluripotent, capable of giving rise to every cell type of an organism, except the trophoblasts of the placenta (4). Somatic stem cells and germ-line stem cells are produced later in development from pluripotent stem cells. Hematopoietic stem cells are probably the best characterized somatic stem cells and are able to generate all the cell types of the blood and the immune system (1–3). Stem cells are also responsible for generation of other somatic systems during development, such as the central nervous system (CNS) (5–9). Neural stem cells (NSCs) in the central nervous system (CNS) have trilineage ability, capable of generating neurons, oligodendrocytes, and astrocytes (5–9).

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While diversification of cell types is largely completed at or soon after birth, many tissues undergo self-renewal in response to acute injury or normal usage throughout life (1–3). Therefore, some somatic stem cells are also maintained throughout the whole life span for the regeneration of specific tissues, such as the epidermis, hair, small intestine, and hematopoietic system (1–3).

The adult CNS was long thought to be largely post-mitotic with very limited ability to regenerate (10–14). Thus, it came as a surprise when the existence of NSCs in the adult CNS was discovered (6–9). Since the initial observation made over 40 years ago (15), neurogenesis, the generation of neurons, has been found in specific regions of the adult CNS of all mammals examined (6–9), including humans (16). The existence of NSCs in the adult CNS, the source of neurogenesis, was later suggested by the isolation of multipotent NSCs from the adult mice brain (17). Within the last decade, multipotent NSCs have been isolated from diverse regions of the adult CNS of both rodents and humans (17–22). These adult NSCs can be amplified *in vitro* through many passages without losing their multipotentiality, capable of giving rise to neurons, astrocytes, and oligodendrocytes both in culture and after transplantation to specific regions *in vivo* (7–9). In the last few years, the functionality of neurons derived from adult NSCs has been demonstrated both *in vitro* (23,24) and *in vivo* (25,26). Rapid advances in adult NSC biology have raised great expectations that these cells can be used as potential resources for neuronal replacement therapy after injury or degenerative neurological disease of the adult human CNS. In this review, we will focus on the recent progress in our understanding of how functional and integrated neurons are generated from adult NSCs in an apparently inhibitory adult CNS environment. The challenges in potential adult stem cell-based therapy for repairing the diseased adult CNS will also be discussed. For more details on CNS repair using various types of stem cells, several recent comprehensive reviews may be consulted (27–31).

### **MATURE CNS, AN UNFAVORABLE ENVIRONMENT FOR NEURONAL REGENERATION**

Damage of the CNS, caused by injury or degenerative neurological diseases, such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, leads to the severing of existing axonal connections and the death of neurons. Both intrinsic cell properties and extrinsic environmental factors contribute to the lack of regenerating capacity of the adult mammalian CNS (12–14,32–34).

First, unlike blood cells, which can be continuously replaced from hematopoietic stem cells, most of the lost

neurons are not replaced in the adult CNS (10,11). Active neurogenesis is found to be restricted to two specific regions in the adult CNS (6–9), namely the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ). Mostly glia cells, but not neurons, are generated in other regions of the intact CNS that are considered to be nonneurogenic (6–9). Cells with neurogenic potentials can, however, be derived from these nonneurogenic regions and exhibit multipotent stem cells properties in culture (6–9). Thus, NSCs in most regions of the mature CNS may have a latent neurogenic program that can be activated by the exposure to high concentrations of growth factors in culture (20). Alternatively, or in addition, the adult CNS environment may limit the neurogenic potentials of NSCs that have a broadly presence in the adult CNS.

Second, injured axons of mature neurons in the adult CNS do not spontaneously regenerate to reform connections (12–14). This is largely due to the presence of growth inhibitory factors in the adult CNS environment (12–14). Many inhibitory factors have been recently identified, including those associated with the CNS myelin (e.g., Nogo, myelin-associated glycoprotein, oligodendrocyte myelin glycoprotein) and the glia scar (e.g., chondroitin sulfate proteoglycan). Many developmental repulsive guidance cues (e.g., semaphorins, ephrins) have also been found to be present in the lesion area and may contribute to the lack of regeneration of axonal connections in the adult CNS (12–14). In addition to extrinsic environmental factors, changes in the intrinsic properties during neuronal maturation also contribute to the failure of axon regeneration (33,34). For example, neonatal rat retinal ganglion cells undergo a profound and apparently irreversible loss of intrinsic axon growth ability upon receiving signal(s) from amacrine cells (33). It has also been suggested that reduction of endogenous cAMP levels during neuronal maturation leads to the loss of regenerative capacity for many axons (34).

### **FUNCTIONAL NEUROGENESIS FROM ENDOGENOUS NEURAL STEM CELLS IN THE ADULT CNS**

If the mature CNS is such an inhibitory environment for neuronal regeneration, is it possible functionally to replace the lost neurons in the adult CNS? Recent landmark studies demonstrated that endogenous NSCs could generate new neurons not only in neurogenic regions in the adult CNS, but also in nonneurogenic regions under certain injury conditions (35,36). In addition, the adult CNS environment, otherwise inhibitory for regeneration of the existing mature neurons, is still permissive for the full spectrum of neuronal development of endogenous adult NSCs (25,26).

Traditionally, adult neurogenesis has been investigated with tracers that can be inherited by dividing cells and passed on to their progeny (7,37). The most commonly used tracer is bromodeoxyuridine (BrdU), a thymidine analog that incorporates into the DNA during the S phase of mitosis (37). In one study (35), Magavi and colleagues found that after the induced death of a specific subset of neocortical neurons followed by BrdU pulsing, a small number of neurons were labeled with BrdU with long-distance projections toward their targets in the neocortex. In another study (36), Nakatomi and colleagues induced ischemic brain injury to CA1 pyramidal neurons in the hippocampus followed by intraventricular infusion of two growth factors, epidermal growth factor (EGF) and basic fibroblast growth factor (FGF-2). They showed that neurons labeled with BrdU probably originated from endogenous NSCs located in the periventricular regions of the hippocampus and then migrated into the hippocampus to regenerate nearly half of the normal number of neurons in the CA1 region. In addition, significant functional recovery was observed (36). Assuming that no significant amounts of BrdU could be incorporated into nondividing cells (37), these studies suggested that endogenous NSCs could be induced to differentiate into neurons in situ outside neurogenic regions in the adult CNS.

Because demonstration of BrdU labeling requires fixation and immunostaining (37), the functionality of the neuronal progeny of adult NSCs cannot be examined. Due to these technical limitations and the fact that many neurons die soon after birth (38), it was not clear whether neurons generated from adult NSCs are actually functional and can be used by the CNS to replace diseased or damaged cells. The functional integration of electrically active new neurons in the adult CNS environment was only demonstrated recently (25,26). Using a strategy based on the finding that the integration of certain retroviruses and sustained expression of transgenes requires cell division (39), van Praag and colleagues were able to express green fluorescent protein (GFP) specifically in proliferating cells and their progeny by stereotaxic injection of engineered retroviruses into the dentate gyrus of the adult mice (25). This approach allows the GFP<sup>+</sup> neuronal progeny of dividing adult NSCs to be shown for their morphology and characterized for their physiological properties in acute brain slices with electrophysiological approaches. These studies revealed that newborn granule neurons send out long axonal projections and elaborate complex dendrite trees. More importantly, some of these newborn GFP<sup>+</sup> neurons can fire repetitive action potentials and receive functional synaptic inputs. Further comparative analysis showed that these newborn neurons exhibit functional properties very similar to those of mature granule neurons in the dentate gyrus (25). Using a similar retroviral strategy (26), Carleton and colleagues recently followed the developmental process

of newborn interneurons in the adult mice. These new neurons are first generated in the SVZ, and then migrate over a long distance through the rostra migratory stream (RMS) to the olfactory bulb. With a series of electrophysiological studies, they documented the functional maturation and integration of newborn interneurons in the olfactory bulb (26).

Taken together, these findings suggest that a neuronal developmental program could be recapitulated in both neurogenic regions and nonneurogenic regions of the mature CNS under certain conditions. In addition, newborn neurons from endogenous adult NSCs are capable of migrating, extending long axonal projections, and becoming functionally integrated into existing local circuits in the adult CNS environment.

### MECHANISMS REGULATING FUNCTIONAL NEUROGENESIS FROM ENDOGENOUS ADULT NEURAL STEM CELLS

During the last decade, we have witnessed tremendous progress regarding embryonic neural development that will clearly have a significant impact on our understanding of the repair mechanisms in the adult CNS (40). The significant difference between the embryonic and adult CNS environment suggests that there will be substantial differences in the mechanisms controlling the neural development in two different stages. Adult neurogenesis in SGZ and SVZ can therefore serve as model systems for investigation of the underlying mechanisms regulating the sequential steps of the neural development in the adult CNS environment. Elucidating the molecular mechanisms regulating neurogenesis from endogenous adult NSCs will also provide the framework for developing neuronal replacement therapy for CNS diseases with endogenous NSCs and/or transplantation of exogenous stem cells or their progeny. Here we will summarize our current understanding of functional neurogenesis from adult NSCs in the SGZ and SVZ.

#### *Proliferation of adult NSCs*

Proliferation of adult NSCs in the SVZ and SGZ is regulated by a variety of stimuli, including aging, stress, stroke, seizure, and physical activity (7–9,38). Neurotransmitters (e.g., serotonin, NMDA, nitric oxide) and steroid hormones are also known to regulate the proliferation of adult NSCs (7–9,38). In most cases, the identity of the proliferation signals received by adult NSCs remains unknown. Recent findings, however, had suggested that local vasculature (41) and astrocytes (42,43) might serve potential cellular sources for the signals. In culture, astrocytes from hippocampus or SVZ promote

proliferation of adult NSCs (42,43). In the adult hippocampus, hot spots of cell proliferation were found to be associated with vascular structures (41) and astrocytes (43). Furthermore, factors promoting endothelial cell proliferation also increase neurogenesis in the adult songbirds (44), suggesting an important relationship between these two processes.

While the mitogenic factors from vascular and astrocytic sources remain to be identified, several molecules have been shown to be effective in inducing proliferation of adult NSCs *in vivo* (7–9,45–48). Infusion of EGF, FGF-2, or transforming growth factor- $\alpha$  (TGF- $\alpha$ ) into the brain was found to promote proliferation of adult NSCs both under normal and injury conditions (7,36,45). EGF and FGF-2 have also been used, almost exclusively, as mitogens for NSCs derived from the adult CNS, either in adhesive or in neurospheres cultures (7–9). The mitogenic effects of FGF-2 for cultured NSCs at clonal densities appear to require a co-factor, recently identified as the glycosylated form of cystatin C (46). Combined delivery of FGF-2 and cystatin C to the dentate gyrus in adult mice stimulates proliferation of NSCs in the SGZ (46). Very recently, Sonic hedgehog (Shh) signaling was shown to regulate proliferation of adult NSCs (47,48). Loss of Shh signaling resulted in abnormalities in both the dentate gyrus and olfactory bulb (48). Pharmacological inhibition or stimulation of Shh signaling in the adult brain also leads to decreased or elevated NSC proliferation in the hippocampus and SVZ, respectively (47,48). *In vitro*, Shh is sufficient to maintain the proliferation of NSCs derived from adult rat hippocampus (47), while mice SVZ progenitors that lack *Smoothed*, a key downstream effector of Shh, formed significantly fewer neurospheres (48). Other factors that are not traditionally regarded as mitogens have also been implicated in regulating proliferation of adult NSCs (49). For example, Eph/ephrin signaling has been shown to be involved in axon guidance, neural crest cell migration, establishment of segmental boundaries, and formation of angiogenic capillary plexi (50). A 3-day infusion of the ectodomain of either EphB2 or ephrin-B2 into the lateral ventricle not only disrupted migration of neuroblasts, but also increased proliferation of NSCs in the SVZ of adult mice (49).

The proliferation signals for adult NSCs activate an array of interconnected cytoplasmic signal transduction pathways that eventually lead to the activation of gene transcription in the nucleus. While many of these pathways have been intensively investigated in other cell types, specific cytoplasmic pathways involved proliferation of adult NSCs remain to be identified.

#### *Fate specification of adult NSCs*

Several lines of evidence support the crucial roles of local environment in fate specification of adult NSCs

(7–9). Proliferating cells that generate only glia under normal conditions in broad regions of the adult CNS can generate neurons, either after isolation and culture *in vitro* (7–9), or after specific types of injuries *in vivo* (35,36). Clonally derived adult NSCs from nonneurogenic regions when transplanted back to the regions of their origin only give rise to glia (51,52). They do, however, differentiate into neurons when transplanted into the neurogenic SGZ (51,52). Recent *in vitro* studies have provided some insights into the cellular elements that may constitute a neurogenic niche in the neurogenic regions of the adult CNS. Cultured astrocytes derived from neurogenic hippocampus actively regulate neurogenesis by promoting proliferation and neuronal fate specification of adult NSCs derived from the adult hippocampus (43). Similarly, cultured astrocytes from SVZ also promote the production of neurons from SVZ stem cells (42). In contrast, astrocytes derived from adult spinal cord, a non-neurogenic region, do not promote neurogenesis (43). These studies raise the intriguing possibility that the ability to generate neurons from adult NSCs may be in part due to the regionally specified astrocytes. During early development, most of the astrocytes are generated after the majority of CNS neurons are born (9), thus are unlikely to instruct neuronal differentiation of fetal NSCs. These studies support the notion that there will be substantial differences between mechanisms regulating neurogenesis in the developing embryonic CNS and those in the adult CNS.

The molecular mechanisms underlying fate specification of adult NSCs are largely unknown (9). Members of the bone morphogenic protein (BMP) were shown to be able to instruct adult NSCs to adopt a glial fate (53). In the neurogenic SVZ, the BMP inhibitor *noggin*, released from the ependymal cells in the lateral wall, can block the gliogenic effects of BMP (53). The instructive factors for neuronal differentiation of adult NSCs, including those released from hippocampal or SVZ astrocytes, remain to be identified.

#### *Navigation of neuronal progeny of adult NSCs*

One of the striking features of adult neurogenesis is the capability of newborn neurons to have long-range migration and axonal outgrowth in a CNS environment otherwise inhibitory for mature neurons and axons. For neurogenesis in the olfactory bulb, neuroblasts generated from SVZ stem cells first migrate tangentially through tubular structures formed by special astrocytes, then radially in the olfactory bulb to their final position (6). This is quite different from what occurs during embryonic development where newborn neurons are generally guided by radial glia cells (9). Several developmental guidance cues and adhesion molecules have been implicated in directing neuronal migration of these new neurons (7), including members of the ephrin-B family, *Slit*, and poly-

sialated glycoprotein neural cell adhesion molecule (PSA-NCAM). For neurogenesis in the dentate gyrus, limited neuronal migration occurs (7). Instead, newborn neurons extend long axonal projections to the CA3 region and their dendritic arborizations to the outer molecular layer in opposite direction (25). It is possible that newly generated neurons do not express functional receptors for factors that inhibit axon regeneration in the adult CNS, and/or have different internal states (e.g., high levels of neuronal cAMP) that permit them to grow in an environment otherwise inhibitory for regenerating mature neurons. The active mechanisms responsible for such stereotypic guidance behaviors of newborn neurons are unknown. Interestingly, many axon/dendrite guidance cues involved in the guidance of developing granule neurons during fetal development, such as semaphorins, maintain their expression in adulthood, thus may contribute to the guidance of these newborn neurons (54).

#### *Maturation and integration by neuronal progeny of adult NSCs*

While the functional roles of adult neurogenesis remain elusive (7,38), recent studies provided convincing evidence that newly generated neurons are able to integrate into the existing neuronal circuits in the adult CNS (25,26). Functional studies of the maturation and integration process during adult neurogenesis have also revealed some unique features of neuronal development in the adult CNS that are different from that being observed during embryonic and neonatal stages (25,26).

In the case of neurogenesis in the dentate gyrus, about half of new newborn cells die within 2 weeks after birth (38). Four weeks after retroviral labeling, some of the remaining neurons become electrically active and start to receive synaptic inputs, as shown by electrophysiological recordings (35). Whether these newborn granule neurons also make functional synaptic connections with their target neurons, thus actively involved in the information flow, remains to be demonstrated. The complexity of their dendrites and density of the dendritic spine, the major sites for excitatory synapses, continue to increase for at least several months (25). Thus, the course of neuronal maturation for newborn granule neurons in the adult CNS appears to be much more protracted than those generated during embryonic stages.

In the case of neurogenesis in the olfactory bulb, electrophysiological recordings showed that tangentially migrating neurons express extrasynaptic GABA<sub>A</sub> and AMPA receptors, while NMDA receptors appear later in radially migrating neurons (26). The sequential expression of receptors for neurotransmitters is different from what occurs during embryonic neuronal development, where expression of NMDA receptors normally precedes AMPA receptors. These newborn olfactory neurons be-

come synaptically connected soon after migration has been completed (26). However, spiking activity does not occur until the neurons are almost fully mature. This is also different to developing embryonic neurons, which can fire action potentials and release neurotransmitters even before they are connected (55). One hypothesis for this unique neuronal maturation process is that the delayed maturation of excitability can prevent the newborn cells from disrupting the function of circuitry already in place in the adult.

The mechanisms regulating maturation and synapse formation by adult NSCs are largely unknown. In vitro studies with adult hippocampal NSCs suggest that local hippocampal astrocytes play essential roles in the maturation and synapse formation process (24). In the absence of astrocytes, the neuronal progeny of cultured adult NSCs remain immature, both morphologically and functionally. They display simple morphology, limited membrane excitability, inability to fire action potentials, and little functional synaptogenesis (24). In contrast, adult NSC derived neurons in the presence of astrocytes acquired physiological properties comparable to those of mature CNS neurons (24). Similar active roles of astrocytes in regulating synapse formation have been previously observed for neonatal neurons (56).

### **ADULT STEM CELL BASED THERAPY FOR CNS REPAIR**

The discovery of the existence of active functional neurogenesis in the adult CNS and rapid advances in stem cell technology have fueled our hope to cure currently intractable CNS diseases by replacing damaged or lost neurons. Many types of stem cells with neurogenic potential have been identified, including pluripotent ES and EG cells and multipotent fetal NSCs (4–9,17–22). Besides ethical concerns and political restrictions, immunocompatibility is another unique advantage of using adult stem cells over other cell sources. The realization that endogenous NSCs may be broadly present in the adult CNS and can be induced to generate new functionally integrated neurons in situ has raised exciting possibilities for self-repair of the damaged CNS by activation of endogenous NSCs, even without transplantation. Many strategies in using stem cells for neuronal replacement therapy in different CNS diseases have been proposed (27–31,57). Here we will focus our discussion on several key steps and challenges ahead for neuronal replacement therapy using adult stem cells.

#### *Source of adult stem cells*

Unlike hematopoietic stem cells, which can be directly isolated with cell surface markers, the precise identifica-

tion of NSCs occurs only retrospectively, and we are still in search of effective methods for prospective isolation of NSCs (7–9). Nonetheless, many types of adult stem cells with neural potentials have been derived (4–9,17–22,58–60). Some are derived from neural tissues, such as multipotent NSCs from the adult CNS, multipotent neural crest stem cells from gut and oligodendrocyte precursors or astrocytes that can be reprogrammed to generate neurons (58–60). Surprisingly, some appear to be derived from nonneural tissues (58–60), such as blood, bone marrow, or skin. In most cases, the neuronal identity is determined merely based on the expression of certain markers (e.g., Tuj1, NeuN), rather than their functional properties (58–60). Interpretation of some of the transplantation experiments are further complicated by the potential fusion events that have occurred between the transplanted cells and the host cells (58–60).

Because functionality is the foundation for the success of neuronal replacement therapy, it is essential to characterize the physiological properties of neurons derived from different types of adult stem cells. For adult multipotent NSCs derived from human white matter (22) and multipotent adult progenitor from bone marrow of mice (61), electrophysiological studies have shown that the neuronal progeny of these stem cells are electrically active, capable of firing action potentials. For adult NSCs derived from hippocampus, extensive functional analysis showed that these cells retain the ability to give rise to electrically active and functional neurons with all essential characteristic properties of mature CNS neurons, even after extensive propagation and amplification in cultures (24). Whether adult NSCs derived from nonneurogenic regions exhibit similar abilities remains to be investigated.

There is undeniable evidence that stem cells derived from very early embryos could be used to make numerous types of neural cell, including the principal projection neurons, most of which are born during embryogenesis (58). It remains to be seen whether any kind of adult stem cells has the potential to generate neuronal subtypes as diverse as ES cells. Studies with cultured adult NSCs derived from hippocampus did show some plasticity in generating neuronal subtypes (7,58,62). When transplanted to the RMS of adult rats, adult hippocampal NSCs can migrate to olfactory bulb and give rise to neurons expressing tyrosine hydroxylase, a phenotype never observed in hippocampus (62).

#### *Amplification of adult stem cells*

In addition to EGF and FGF-2, some other factors, such as Shh (47) and neuregulin (63), also appear to be sufficient in maintaining the self-renewal and multipotentiality of adult NSCs from rodents. Whether adult NSCs propagated in different mitogens will exhibit differential

responses to differentiation stimuli is unknown. It also remains to be investigated whether different mitogens will change the epigenetic properties of proliferating adult NSCs, resulting in a different capacity in generating neuronal subtypes. Because extensive amplification may be required to generate enough cells for transplantation, stem cells need to be routinely examined for potential abnormality, such as their karyotypes and growth factor dependency.

#### *Differentiation of stem cells into defined lineages*

Numerous attempts to transplant multipotent NSCs directly into the nonneurogenic regions of the adult CNS failed to generate significant numbers of new neurons (7); however, transplantation of neuronal lineage-restricted progenitors did generate neurons (64), suggesting that the neuronal fate specification is the limiting step. Therefore, understanding the neurogenic environment, by comparative studies with nonneurogenic conditions, will be essential for developing strategies to activate endogenous NSCs. It may be also beneficial to transplant neuroblasts or immature neurons instead of NSCs directly into the adult CNS. The molecular mechanisms regulating neuronal fate specification and neuronal subtype differentiation will be an area of intensive investigation in the immediate future. Understanding how these developmental processes occur during embryonic stages will clearly facilitate our efforts. Protocols have already been developed to differentiate ES cells effectively into different neuronal subtypes (65), including dopaminergic neurons, GABAergic neurons, and motor neurons. It is expected that similar strategies will also be developed to coax the adult stem cells (61). The functionality of neuronal progeny for stem cells, including membrane excitability and release of neurotransmitter, is an essential piece of the task, and should be rigorously examined.

#### *Survival and functional integration by newly generated neurons*

A significant percentage of neurons, either derived from endogenous NSCs (38) or from transplantation (66), died soon after in the adult CNS. Thus, strategies to support the survival of endogenous and/or transplanted cells are of apparent importance (66). Interestingly, enriched environment and learning appear to increase the survival of newborn neurons in the rodent hippocampus (38). Adult NSCs and their progeny can be genetically modified either *in situ* or *in vitro* to become the cellular sources for growth factors and neurotrophins that may promote or support the survival of themselves and the surrounding neurons.

To achieve functional integration by the newborn neurons from either endogenous NSCs or transplantation, we

will have to understand the mechanisms that control the neuronal migration, axon/dendrite guidance, and synapse formation in both normal and diseased adult CNS environment. Extensive characterizations are necessary for elucidating the expression of developmental guidance cues in normal and abnormal adult CNS. In addition, novel approaches will need to be developed to monitor the correct integration of newborn neurons.

### CONCLUDING REMARKS

The remarkable progress in isolation of adult stem cells with neural capacities and in understanding of basic mechanisms controlling functional neurogenesis in the adult CNS has reinvigorated our hope to repair the diseased CNS with adult stem cell-based neuronal replacement therapy. The possibility of activation of endogenous adult NSCs and/or autologous transplantation of progeny of adult stem cells expanded in vitro will circumvent the logistical, safety, and ethical issues associated with other types of stem cells. However, these enthusiasms have to be coupled with challenges before the dream can be realized. The first is to identify and prospectively isolate stem cells from adult sources. With the success in isolation of adult stem cells, it is of immediate concern to determine the functionality of the progeny of these adult stem cells. The essential factors within the local environment need to be identified and fine-tuned to allow survival, maturation, and targeting of newborn neurons. Finally, the integration of new neurons in the existing neuronal circuits needs to be monitored to avoid adverse effects. As Anderson has pointed out (8), there is a big difference between what stem cells normally do (the “actual”) and what they can do (the “possible”). From the perspective of cell therapy, we are actively pursuing the “possible.” The “actual,” however, can provide the blueprint for us to achieve the goal. Thus, both developmental neuroscientists, who are interested in the normal brain development, and clinical scientists, who are interested in using stem cells for therapy, have to be united to bring stem cells research and therapy to a new height, enabling the dream of CNS repair to come true.

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### REFERENCES

- Weissman IL. (2000). Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 100: 157–168.
- Fuchs E and JA Segre. (2000). Stem cells: a new lease on life. *Cell* 100:143–155.
- Weissman IL, DJ Anderson and F Gage. (2001). Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. *Annu Rev Cell Dev Biol* 17:387–403.
- Smith AG. (2001). Embryo-derived stem cells: of mice and men. *Annu Rev Cell Dev Biol* 17:435–462.
- McKay R. (1997). Stem cells in the central nervous system. *Science* 276:66–71.
- Alvarez-Buylla A and S Temple. (1998). Stem cells in the developing and adult nervous system. *J Neurobiol* 36: 105–110.
- Gage FH. (2000). Mammalian neural stem cells. *Science* 287:1433–1438.
- Anderson DJ. (2001). Stem cells and pattern formation in the nervous system: the possible versus the actual. *Neuron* 30:19–35.
- Temple S. (2001). The development of neural stem cells. *Nature* 414:112–117.
- Ramon y Cajal S. (1928). *Degeneration and Regeneration of the Nervous System*. Hafner, New York.
- Rakic P. (1985). Limits of neurogenesis in primates. *Science* 227:1054–1056.
- Schwab ME, JP Kapfhammer and CE Bandtlow. (1993). Inhibitors of neurite growth. *Annu Rev Neurosci*. 16:565–595.
- Fournier AE and SM Strittmatter. (2001). Repulsive factors and axon regeneration in the CNS. *Curr Opin Neurobiol* 11:89–94.
- Filbin MT. (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nature Rev Neurosci* 4:703–713.
- Altman J. (1962). Are neurons formed in the brains of adult mammals? *Science* 135:1127–1128.
- Eriksson PS, E Perfilieva, T Bjork-Eriksson, AM Alborn, C Nordborg, DA Peterson and FH Gage. (1998). Neurogenesis in the adult human hippocampus. *Nature Med* 4:1313–1317.
- Reynolds BA and S Weiss. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255:1707–1710.
- Gottlieb DI. (2002). Large-scale sources of neural stem cells. *Annu Rev Neurosci* 25:381–407.
- Weiss S, C Dunne, J Hewson, C Wohl, M Wheatley, AC Peterson and BA Reynolds. (1996). Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J Neurosci*. 16:7599–7609.
- Palmer TD, EA Markakis, AR Willhoite, F Safar and FH Gage. (1999). Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J Neurosci* 19:8487–8497.
- Arsenijevic Y, JG Villemure, JF Brunet, JJ Bloch, N Delgion, C Kostic, A Zurn and P Aebischer. (2001). Isolation of multipotent neural precursors residing in the cortex of the adult human brain. *Exp Neurol*. 170:48–62.

22. Nunes MC, NS Roy, HM Keyoung, RR Goodman, G Mc-Khann 2nd, L Jiang, J Kang, M Nedergaard and SA Goldman. (2003). Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nature Med* 9:439–447.
23. Toda H, J Takahashi, A Mizoguchi, K Koyano and N. Hashimoto. (2000). Neurons generated from adult rat hippocampal stem cells form functional glutamatergic and GABAergic synapses in vitro. *Exp Neurol* 165:66–76.
24. Song HJ, CF Stevens and FH Gage. (2002). Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nature Neurosci* 5:438–445.
25. van Praag H, AF Schinder, BR Christie, N Toni, TD Palmer and FH Gage. (2002). Functional neurogenesis in the adult hippocampus. *Nature* 415:1030–1034.
26. Carleton A, LT Petreanu, R Lansford, A Alvarez-Buylla and PM Lledo. (2003). Becoming a new neuron in the adult olfactory bulb. *Nature Neurosci* 6:507–518.
27. Bjorklund A and O Lindvall. (2000). Cell replacement therapies for central nervous system disorders. *Nature Neurosci* 3:537–544.
28. Zandstra PW and A Nagy. (2001). Stem cell bioengineering. *Annu Rev Biomed Eng* 3:275–305.
29. Rossi F and E Cattaneo. (2002). Opinion: neural stem cell therapy for neurological diseases: dreams and reality. *Nature Rev Neurosci* 3:401–409.
30. Tsai RY, R Kittappa and RD McKay. (2002). Plasticity, niches, and the use of stem cells. *Dev Cell* 2:707–712.
31. Ostenfeld T and CN Svendsen. (2003). Recent advances in stem cell neurobiology. *Adv Tech Stand Neurosurg* 28:83–89.
32. Kruger GM and SJ Morrison. (2002). Brain repair by endogenous progenitors. *Cell* 110:399–402.
33. Goldberg JL, MP Klassen, Y Hua and BA Barres. (2002). Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. *Science* 296:1860–1864.
34. Cai D, J Qiu, Z Cao, M McAtee, BS Bregman and MT Filbin. (2001). Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. *J Neurosci* 21:4731–4739.
35. Magavi SS, BR Leavitt and JD Macklis. (2000). Induction of neurogenesis in the neocortex of adult mice. *Nature* 405:951–955.
36. Nakatomi H, T Kuriu, S Okabe, S Yamamoto, O Hatano, N Kawahara, A Tamura, T Kirino and M Nakafuku. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* 110:429–441.
37. Rakic P. (2002). Neurogenesis in adult primate neocortex: an evaluation of the evidence. *Nature Rev Neurosci* 3:65–71.
38. Fuchs E and E Gould. (2000). Mini-review: in vivo neurogenesis in the adult brain: regulation and functional implications. *Eur J Neurosci* 12:2211–2214.
39. Lewis PF and M Emerman. (1994). Passage through mitosis is required for oncoretroviruses but not for the human immunodeficiency virus. *J Virol* 68:510–516.
40. Jessell TM and JR Sanes. (2000). Development. The decade of the developing brain. *Curr Opin Neurobiol* 10:599–611.
41. Palmer TD, AR Willhoite and FH Gage. (2000). Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425:479–494.
42. Lim DA and A Alvarez-Buylla. (1999). Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. *Proc Natl Acad Sci USA* 96:7526–7531.
43. Song H, CF Stevens and FH Gage. (2002). Astroglia induce neurogenesis from adult neural stem cells. *Nature* 417:39–44.
44. Louissaint A Jr, S Rao, C Leventhal and SA Goldman. (2002). Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron* 34:945–960.
45. Kuhn HG, J Winkler, G Kempermann, LJ Thal and FH Gage. (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J Neurosci* 17:5820–5829.
46. Taupin P, J Ray, WH Fischer, ST Suhr, K Hakansson, A Grubb and FH Gage. (2000). FGF-2-responsive neural stem cell proliferation requires CCg, a novel autocrine/paracrine cofactor. *Neuron* 28:385–397.
47. Lai K, BK Kaspar, FH Gage and DV Schaffer. (2003). Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. *Nature Neurosci* 6:21–27.
48. Machold R, S Hayashi, M Rutlin, MD Muzumdar, S Nery, JG Corbin, A Gritli-Linde, T Dellovade, JA Porter, LL Rubin, H Dudek, AP McMahon and G Fishell. (2003). Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron* 39:937–950.
49. Conover JC, F Doetsch, JM Garcia-Verdugo, NW Gale, GD Yancopoulos and A Alvarez-Buylla. (2000). Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. *Nature Neurosci* 3:1091–1097.
50. Kullander K and R Klein. (2002). Mechanisms and functions of Eph and ephrin signalling. *Nat Rev Mol Cell Biol* 3:475–486.
51. Shihabuddin LS, PJ Horner, J Ray and FH Gage. (2000). Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. *J Neurosci* 20:8727–8735.
52. Lie DC, G Dziejczapolski, AR Willhoite, BK Kaspar, CW Shults and FH Gage. (2002). The adult substantia nigra contains progenitor cells with neurogenic potential. *J Neurosci* 22:6639–6649.
53. Lim DA, AD Tramontin, JM Trevejo, DG Herrera, JM Garcia-Verdugo and A Alvarez-Buylla. (2000). Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28:713–726.
54. Skutella T and R Nitsch. (2001). New molecules for hippocampal development. *Trends Neurosci* 24:107–113.
55. Sun YA and MM Poo. (1987). Evoked release of acetylcholine from the growing embryonic neuron. *Proc Natl Acad Sci USA* 84:2540–2544.
56. Ullian EM, SK Sapperstein, KS Christopherson and BA Barres. (2001). Control of synapse number by glia. *Science* 291:657–661.
57. Lie DC, H-j Song, SA Colamarino, G-l Ming and FH Gage. (2004). Neurogenesis in the adult brain: new strategies for



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- central nervous system diseases. *Annu Rev Pharmacol Toxicol* 44:399–421.
58. Temple S. (2001). Stem cell plasticity—building the brain of our dreams. *Nature Rev Neurosci* 2:513–520.
59. Svendsen CN, A Bhattacharyya and YT Tai. (2001). Neurons from stem cells: preventing an identity crisis. *Nature Rev Neurosci*. 2:831–834.
60. Raff M. (2003). Adult Stem Cell Plasticity: Fact or Artifact. *Annu Rev Cell Dev Biol* 19:1–22.
61. Jiang Y, D Henderson, M Blackstad, A Chen, RF Miller and CM Verfaillie. (2003). Neuroectodermal differentiation from mouse multipotent adult progenitor cells. *Proc Natl Acad Sci USA* 100:11854–11860.
62. Suhonen JO, DA Peterson, J Ray and FH Gage. (1996). Differentiation of adult hippocampus-derived progenitors into olfactory neurons in vivo. *Nature* 383: 624–627.
63. Falk A and J Frisen. (2002). Amphiregulin is a mitogen for adult neural stem cells. *J Neurosci Res* 69:757–762.
64. Han SS, DY Kang, T Mujtaba, MS Rao and I Fischer. (2002). Grafted lineage-restricted precursors differentiate exclusively into neurons in the adult spinal cord. *Exp Neurol* 177:360–375.
65. Barberi T, P Klivenyi, NY Calingasan, H Lee, H Kawamata, K Loonam, AL Perrier, J Bruses, ME Rubio, N Topf, V Tabar, NL Harrison, MF Beal, MA Moore and L Studer (2003). Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nature Biotechnol* 21:1200–1207.
66. Brundin P, J Karlsson, M Emgard, GS Schierle, O Hansson, A Petersen and RF Castilho. (2000). Improving the survival of grafted dopaminergic neurons: a review over current approaches. *Cell Transplant* 9:179–195.

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