

Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later

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Adult somatic stem cells in various organs maintain homeostatic tissue regeneration and enhance plasticity. Since its initial discovery five decades ago, investigations of adult neurogenesis and neural stem cells have led to an established and expanding field that has significantly influenced many facets of neuroscience, developmental biology, and regenerative medicine. Here we review recent progress and focus on questions related to adult mammalian neural stem cells that also apply to other somatic stem cells. We further discuss emerging topics that are guiding the field toward better understanding adult neural stem cells and ultimately applying these principles to improve human health.

Introduction

It is now established that neural progenitor/stem cells (NSCs) reside in the adult mammalian brain and contribute to brain plasticity throughout life (Kempermann and Gage, 1999). Joseph Altman first suggested that neurogenesis, or the generation of new neurons, occurs beyond development in the adult mammalian brain (Altman and Das, 1965). His findings sparked optimism that this endogenous process can be harnessed to repair the injured or diseased brain. Significant progress in the investigation of this phenomenon has since led to remarkable knowledge about adult NSCs and neurogenesis (Ming and Song, 2011). For example, pioneering in vitro analysis demonstrated self-renewal and multipotency of NSCs derived from the adult mammalian brain (Reynolds and Weiss, 1992). In vivo studies using nucleotide analog labeling, retroviral lineage-tracing, and genetic fate-mapping later revealed NSC population dynamics, differentiation capacities, regulatory mechanisms, and heterogeneity. Single-cell genetic lineage-tracing has illustrated the existence of endogenous adult mammalian NSCs with hallmark stem cell properties (Bonaguidi et al., 2011).

This Review focuses on topics of adult NSCs that can be applied more broadly to somatic stem cells in many other tissues, such as bone marrow, blood, endothelium, skin, fat, gastrointestinal tract, liver, lung, endocrine organs, and skeletal muscle (Li and Clevers, 2010). Adult somatic stem cells share fundamental properties, including self-renewal, relative quiescence, differentiation capacity, and residence within a specific environment or niche (Figure 1A). We focus on recent progress delineating the composition of specialized neurogenic niches, signaling mechanisms, and potential functions of NSCs in the adult mammalian brain. We also explore emerging topics in the adult somatic stem cell field, such as single-cell analysis, human stem cells, and reprogramming. We hope to provide an integrated view of adult NSCs and prompt new questions and theories about regulation and potential function of adult somatic stem cells.

NSCs in the Adult Mammalian Brain

There are two major neurogenic niches in the adult mammalian brain where endogenous NSCs reside, the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) within the dentate gyrus of the hippocampus (Figure 1B). The SVZ is located along the ependymal cell layer, which separates the ventricular space from the SVZ (Figure 2A). Adult SVZ NSCs (also named type B cells) extend a basal process to terminate on blood vessels and extend an apical process with a primary cilium that pokes through the ependymal cell layer to contact the cerebrospinal fluid (CSF) in the ventricle (Mirzadeh et al., 2008). Type B NSCs give rise to transient amplifying progenitors (C cells) (Doetsch et al., 1999), which divide a few times before becoming neuroblasts (A cells). Neuroblasts then form a chain and migrate into the olfactory bulb where they migrate radially and differentiate into different subtypes of interneurons. Radial glia-like NSCs (named RGLs or type 1 cells) in the SGZ, at the border between the inner granule cell layer and hilus, give rise to intermediate progenitor cells (IPCs) (Seri et al., 2001), which exhibit limited rounds of proliferation before generating neuroblasts (Berg et al., 2015) (Figure 2B). Neuroblasts migrate tangentially along the SGZ and develop into immature neurons, which migrate radially into the granule cell layer to differentiate into dentate granule neurons (Sun et al., 2015).

Two fundamental properties of stem cells are their abilities to self-renew and give rise to differentiated progeny (Figure 1A). It had long been postulated that adult neurogenesis originates from tri-potent NSCs with the capacity to generate neurons, astrocytes, and oligodendrocytes. The existence of self-renewing, multipotent adult NSCs was originally suggested by the long-term expansion and differentiation of neurospheres (non-adherent, spherical cultures of clonally derived precursors), or monolayer cultures into three neural lineages (Palmer et al., 1997; Reynolds and Weiss, 1992). However, recent genetic fate-mapping and clonal lineage-tracing of NSCs in the adult hippocampus in vivo has found the generation of neurons and

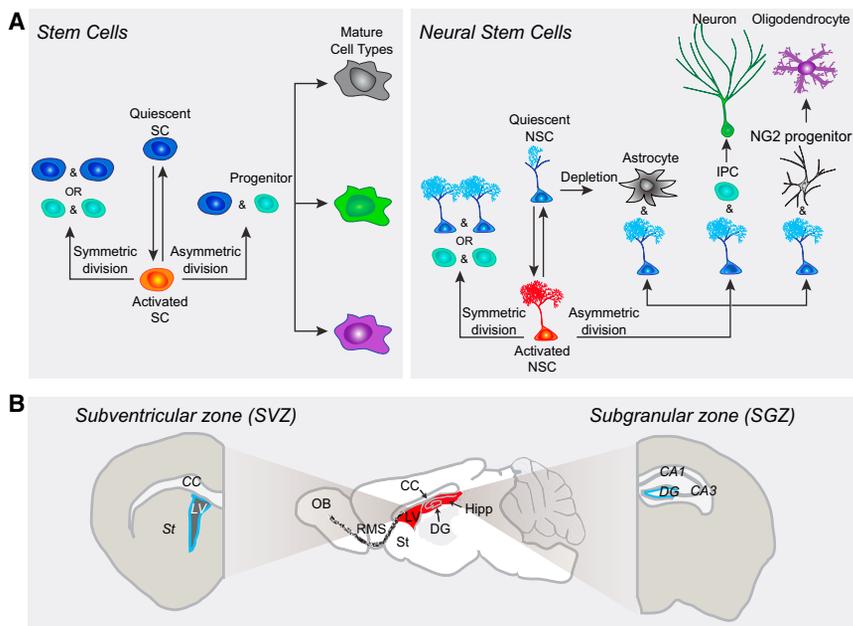


Figure 1. Behavior of Neural Stem Cells within Adult Niches

(A) A schematic diagram illustrating potential behavior of an adult stem cell and, more specifically, of an adult neural stem cell (NSC) over its life cycle. Adult NSCs can transition between quiescent and active states by exiting and entering the cell cycle, respectively. Once activated, NSCs choose between different modes of division. Asymmetric division is self-renewing and yields an NSC and a progenitor, while symmetric division yields either two NSCs (self-renewing) or two progenitors (not self-renewing). Progenitors may be fate restricted, meaning that they can only differentiate into a particular cell type, or they may be multipotent and must make a fate choice before differentiation. It is also possible that NSCs may directly differentiate into mature glial cell types. Though this diagram depicts a wide range of NSC activities, specific NSC populations may exhibit a predisposition for certain activities, such as asymmetric division or quiescence.

(B) A sagittal view of the adult rodent brain, focusing on two major niches where adult NSCs reside: the subventricular zone (SVZ) and the subgranular zone (SGZ). The SVZ is located along the lateral ventricle in the forebrain, while the SGZ is located in the hippocampus along

the dentate granule cell layer where it abuts the hilus. CC, corpus callosum; DG, dentate gyrus; Hipp, hippocampus; LV, lateral ventricle; NSC, neural stem cell; OB, olfactory bulb; RMS, rostral migratory stream; SC, stem cell; St, striatum.

astrocytes, but not oligodendrocytes (Bonaguidi et al., 2011). In the adult SVZ, population fate-mapping studies have shown the generation of both neurons and oligodendrocytes. However, recent *in vivo* clonal analysis has found only neuronal lineages from individual NSCs (Calzolari et al., 2015), whereas *in vitro* time-lapse analysis revealed the generation of either neurons or oligodendrocytes from acutely isolated individual precursor cells, but never both (Ortega et al., 2013). Therefore, whether endogenous NSCs with an intrinsic tri-lineage potential exist in the adult mammalian brain remains a fundamental question. In one model, NSCs are gradually specified during development and become restricted in the lineage potential in adult (Kriegstein and Alvarez-Buylla, 2009). Alternatively, adult NSCs could be intrinsically tri-potent, but with some latent lineage potentials constitutively suppressed by the niche. Whether endogenous adult NSCs exhibit long-term self-renewal is also under debate. In the adult SGZ, one population-level study suggested that NSC activation leads to multiple rounds of proliferation, which is coupled with their depletion via differentiation into astrocytes without their ever returning to quiescence (Encinas et al., 2011). Clonal analysis, on the other hand, showed cycles of activation, a return to quiescence, and re-activation of individual NSCs, with moderate depletion via astrocytic transformation (Bonaguidi et al., 2011). In the adult SVZ, clonal analysis revealed rapid progeny expansion and then depletion of NSCs (Calzolari et al., 2015). Different genetic labeling approaches could target different populations of NSCs and/or different states of the same NSC population, thereby producing seemingly disparate results. Given that the most primitive NSCs are rare, it will be critical to develop specific targeting approaches to examine their self-renewal capacity and lineage potential at the clonal level in the future.

Studies using an anti-mitotic drug to eliminate dividing precursors showed that adult NSCs are largely quiescent *in vivo*

(Doetsch et al., 1999). Quiescence is thought to allow adult stem cells to withstand metabolic stress and to preserve genome integrity over a lifetime. The quiescent state has long been viewed as dormant and passive. Emerging evidence suggests just the opposite. Recent single-cell transcriptome analysis of quiescent adult SGZ NSCs showed active expression of various receptors for niche signals and downstream signaling components, the majority of which are downregulated once NSCs become activated (Shin et al., 2015). Functionally, decreasing GABA (Song et al., 2012) or Wnt inhibitor expression (Jang et al., 2013) leads to quiescent NSC activation. The concept that quiescence is an actively maintained state is gaining broad empirical support in multiple somatic stem cell systems (Cheung and Rando, 2013).

Another emerging theme in stem cell biology is heterogeneity. In most adult somatic stem cell systems, quiescent and active stem cell populations co-exist (Li and Clevers, 2010). The most obvious example of heterogeneity among adult NSCs is the difference in their progeny between two niches: SVZ NSCs generate olfactory bulb interneurons and corpus callosum oligodendrocytes, whereas SGZ NSCs generate dentate granule neurons and astrocytes. Notably, NSCs derived from both niches generate all three neural lineages once propagated in culture with high concentrations of growth factors (Palmer et al., 1997; Reynolds and Weiss, 1992); therefore, the *in vivo* niche may limit adult NSC potential. Within the adult SVZ, progeny subtypes are further specified according to where the mother NSC is located along the ventricle. For example, ventral NSCs produce deep granule cells and calbindin-expressing periglomerular cells, whereas dorsal NSCs produce superficial granule cells and tyrosine-hydroxylase-expressing periglomerular cells (Merkle et al., 2007). This form of positional heterogeneity could be due to regional differences in the niche and/or intrinsic differences in

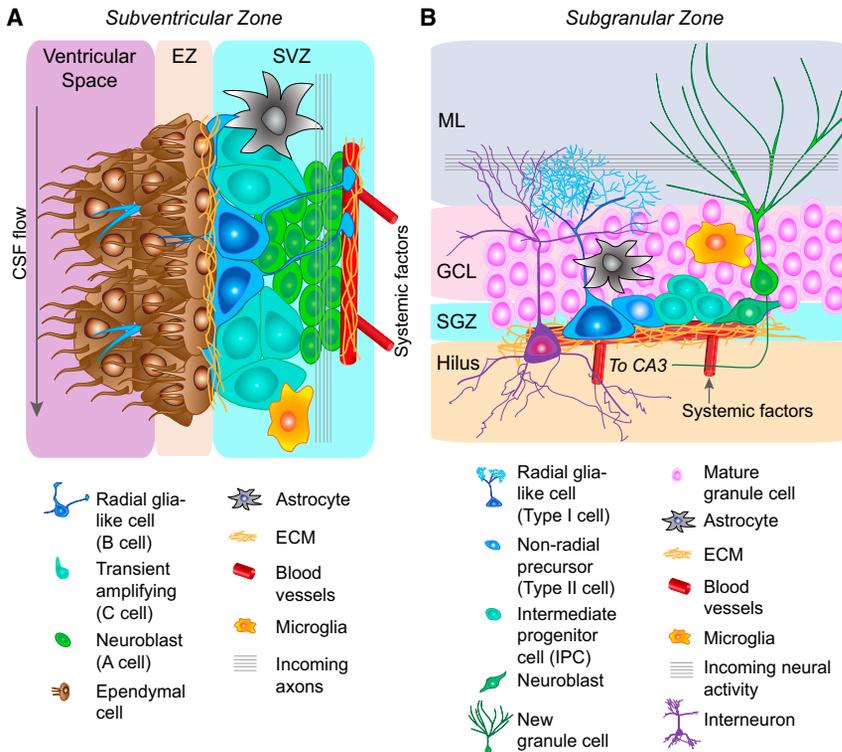


Figure 2. Adult NSC Niches

(A) A schematic diagram depicting cellular and molecular components of the SVZ niche. Ependymal cells are organized into rosette-shaped structures which line the lateral ventricle and border the SVZ. Radial glia-like neural stem cells (B cells) reside along the ependymal zone in the SVZ and extend a radial process to contact blood vessels. They also extend a single cilium through the ependymal rosettes to contact the cerebrospinal fluid in the ventricular space. Radial glia-like neural stem cells (NSCs) generate transit-amplifying cells (C cells), which generate neuroblasts (A cells). Neuroblasts migrate down the rostral migratory stream to the olfactory bulb where they differentiate into olfactory bulb neurons. In addition to the aforementioned cell types, astrocytes and microglia contribute to the cellular architecture of the niche. Molecular niche signals, including morphogens (e.g., BMPs, SHH, Wnts, and Notch), growth factors (e.g., VEGF, IGF, and EGF), neurotrophins (e.g., BDNF, NT-3, and NGF), cytokines (e.g., interleukins), neurotransmitters (GABA, 5-HT, Ach, and dopamine), extracellular matrix (e.g., laminins and proteoglycans), cell-cell signaling molecules (e.g., Ephrins and Connexins), and systemic factors (e.g., CCL11 and GDF11), contribute to both adult NSC niches.

(B) A schematic diagram depicting cellular and molecular components of the SGZ niche. Radial glia-like NSCs (type 1 cells) reside in the SGZ and extend a radial process through the granule cell layer of the dentate gyrus into the molecular layer. Radial glia-like NSCs generate intermediate progenitor cells (IPCs), which generate neuroblasts,

and these progenitor cells are closely associated with the vasculature. Neuroblasts differentiate into dentate granule cells, which migrate into the granule cell layer of the dentate gyrus. In addition to the aforementioned cell types, astrocytes, microglia, and interneurons contribute to the cellular architecture of the niche. ECM, extracellular matrix; EZ, ependymal zone; GCL, granule cell layer; ML, molecular layer; SGZ, subgranular zone; SVZ, subventricular zone.

NSCs. In the adult dentate gyrus, multiple inducible reporter lines, such as *GLAST-CreER^{T2}* and *Nestin-CreER^{T2}*, label adult NSC populations that react to environmental stimuli differently (DeCarolis et al., 2013). It should be noted that phenotypic heterogeneity could reflect distinct populations or different states of the same population of stem cells, which cannot be resolved via snap-shot or short-term lineage-tracing analyses. This represents a technical challenge for the whole somatic stem cell field, which may require a combination of single-cell long-term lineage-tracing, single-cell transcriptome analysis, and modeling to resolve.

A related question is how is adult NSC heterogeneity established during development, or more generally, what are the embryonic origins of adult NSCs? Adult SGZ NSCs appear to originate from the ventral hippocampus during late gestation and then re-locate to the dorsal hippocampus (Li et al., 2013). Two recent studies suggested that adult SVZ NSCs are regionally specified at an early embryonic stage and then remain largely quiescent until re-activation postnatally (Fuentelba et al., 2015; Furutachi et al., 2015). In contrast to our substantial knowledge about the origins of other somatic stem cells (Barker et al., 2010), very little is known about the identity, location, and properties of precursors that give rise to adult NSCs in the SGZ and SVZ.

The Adult NSC Niche

Somatic stem cells reside in niches with specific molecular and cellular characteristics. Adult NSC niches include ependymal

cells, vascular cells, and immature and mature lineages of NSCs (Ma et al., 2005). Unique to the adult SVZ, ependymal cells line the ventricular surface, and their apical surface is covered in cilia that beat the CSF, circulating it through the ventricle (Figure 2A). Ependymal cells are organized into pinwheel formations, and at the center of each pinwheel, NSCs extend a single apical cilium to directly contact the CSF (Mirzadeh et al., 2008) (Figure 2A). Ependymal cells secrete signaling factors, such as noggin, an inhibitor of BMP signaling, which allows activation of NSCs. Compared to non-neurogenic regions, the vasculature is highly organized with unique architecture throughout both adult SGZ (Sun et al., 2015) and SVZ (Shen et al., 2008), and adult NSCs extend processes that are closely associated with blood vessels (Figure 2). Both endothelial cells themselves and factors transported through the blood provide signals that impact NSCs. For example, endothelial cells secrete vascular endothelial growth factor (VEGF), which promotes NSC self-renewal, and neurotrophin-3 (NT-3), which promotes quiescence and long-term maintenance (Delgado et al., 2014). In addition, the blood-brain barrier is leaky near clusters of proliferating NSCs, which would allow these cells to more easily access factors in the blood (Tavazoie et al., 2008). Under homeostatic conditions, microglia phagocytose IPCs that have undergone apoptosis (Siererra et al., 2010), while activated microglia reduce neurogenesis (Ekdahl et al., 2003). It has become clear for multiple somatic stem cell systems that stem cells themselves, and their immature and mature progeny, are an integrated part of the niche. Adult NSCs release factors that contribute to autocrine and paracrine

signaling (Alfonso et al., 2012). Immediate progeny of NSCs provide diffusible or contact-mediated signals, such as the neurotransmitter GABA (Liu et al., 2005) and Notch ligand Delta-like 1 (Dll1) (Kawaguchi et al., 2013), to regulate NSC quiescence as a part of the feedback mechanism for homeostasis. Astrocytes secrete cytokines, such as IL-1 β and IL-6, which promote neuronal differentiation of NSCs (Barkho et al., 2006). Finally, adult NSCs extend long radial processes that potentially integrate signals along their length. In the SGZ, radial processes extend through the entire granule cell layer up into the molecular layer of the dentate gyrus where they branch out extensively, forming a tuft of processes. This structure may allow NSCs to sample and integrate neuronal circuitry activity. Indeed, adult NSCs are regulated by neurotransmitters released by mature neurons (Berg et al., 2013). Together, these results demonstrate that multiple cell types contribute to the signaling milieu of the niche, which represents a general theme for various adult somatic stem cells.

The extracellular matrix (ECM) is a network of molecules that provides a structural framework for surrounding cells and is involved in signaling. In adult neurogenic niches, the ECM contains molecules such as laminins, proteoglycans, and tenascin C (Kazanis and French-Constant, 2011). Fractones, the first ECM structures described in the adult niches, contact precursors and trap growth factors (Kerever et al., 2007). NSCs are embedded in the ECM and express cell adhesion molecules, which allow them to interact with the ECM. For example, vascular cell adhesion molecule-1 (VCAM-1) is highly expressed at the apical endfeet of SVZ NSCs and is necessary for the structural organization of the ependymal cell pinwheel architecture (Kokovay et al., 2012). In addition to its role in maintaining niche structures, the ECM can activate signaling cascades. Integrin-linked kinase (ILK) interacts with multiple proteins, including integrins; loss of ILK in adult NSCs results in increased cell proliferation (Porcheri et al., 2014).

Morphogens, such as BMPs, Notch, Wnts, and sonic hedgehog (SHH), are critical for tissue patterning and specification during embryonic development, and they continue to regulate adult NSCs (Faigle and Song, 2013). For example, BMP signaling negatively regulates neurogenesis by promoting SVZ NSC astroglial fate commitment and SGZ NSC quiescence (Lim et al., 2000; Mira et al., 2010). Notch signaling induces proliferation and maintenance of NSCs in both adult niches, whereas loss of signaling forces NSCs to exit the cell cycle and transition to a progenitor stage (Breunig et al., 2007). Wnt signaling is important for NSC maintenance, and Wnt inhibitors Dickkopf-1 (Dkk1) and secreted frizzled-related protein 3 (sFRP3) promote NSC quiescence (Jang et al., 2013; Seib et al., 2013). SHH signaling is required for SVZ NSC maintenance, but over-activation promotes symmetric division, which initially leads to NSC expansion but ultimately depletion (Feret et al., 2014). Growth factors and neurotrophins also play an important role in regulating late progenitors, but their impact on NSCs is less well described (Faigle and Song, 2013). Together, these studies highlight the importance of tight regulation of morphogens and growth factors within the niche; a disturbance in either direction can negatively impact the NSC population.

Though apparently lacking synapses, NSCs express receptors and respond to a variety of neurotransmitters (Berg et al.,

2013). GABA, the major inhibitory neurotransmitter of the brain, promotes NSC quiescence by blocking cell-cycle progression (Fernando et al., 2011). In the SVZ neuroblast-released GABA acts as a negative feedback mechanism to limit NSC division (Liu et al., 2005). SGZ NSCs can sense parvalbumin interneuron-released GABA; reducing GABA signaling results in NSC activation and symmetrical division (Song et al., 2012). The dopamine receptor antagonist haloperidol increases SVZ NSC division, suggesting that dopamine also promotes quiescence (Kippin et al., 2005). In contrast, serotonin and acetylcholine activate SVZ NSCs (Paez-Gonzalez et al., 2014; Tong et al., 2014).

Adult NSCs also communicate with other cells via gap junctions and direct cell-cell interactions. The majority of NSCs are coupled to each other by gap junctions formed by connexin 43 (Cx43) (Kunze et al., 2009). Loss of Cx30 and Cx43 in SGZ NSCs severely diminishes NSC numbers. NSCs also communicate via direct cell-cell interactions with endothelial cells of the vasculature and astrocytes. In the SGZ, astrocytic expression of ephrinB2 promotes neuronal differentiation of NSCs via EphB4 receptor-mediated pro-neuronal transcription factor upregulation (Ashton et al., 2012). In the SVZ, endothelial cell expression of ephrinB2 and Jagged1 promotes NSC quiescence by suppressing cell-cycle entry and inhibiting differentiation, and loss of either gene results in NSC activation and depletion (Ottone et al., 2014). These studies illustrate that the same signal could have different effects in the two niches.

Beyond the local niche, adult NSCs can also sense systemic changes via circulating factors. SVZ NSCs have direct access to CSF, which represents a complex reservoir of diverse signals. While its role remains to be characterized for adult NSCs, CSF in the embryonic brain promotes cortical neural progenitor proliferation via IGF-2 (Lehtinen et al., 2011). Heterochronic parabiont studies have shown that elevated circulating factors in the blood of aged mice, such as cytokines CCL11 (Villeda et al., 2011) and β 2-microglobulin (Smith et al., 2015), inhibit SGZ cell proliferation and neurogenesis, whereas factors in blood of young animals, such as GDF11, induce vascular remodeling and lead to increased SVZ NSC number and neurogenesis (Katsimpardi et al., 2014).

In summary, NSCs are dynamically regulated by a large number of synergistic and antagonistic factors in each neurogenic niche and beyond. Several key questions remain to be addressed. How does each individual NSC compute a constant barrage of niche signals and make the ultimate decision to stay in quiescence or enter the cell cycle and choose a particular fate? How does an animal's experience translate into changes in niche signaling that impact NSC behavior? Answers to these questions may reveal general principles for various adult somatic stem cells.

Mechanisms Regulating Adult NSCs

Transduction of extracellular niche signals triggers a signaling cascade that activates intracellular regulatory mechanisms, including transcription factors, epigenetics, and metabolism (Figure 3A). Although some of these regulators affect gene expression or cell function directly, there is a lot of crosstalk among regulators to form a complex web of interactions.

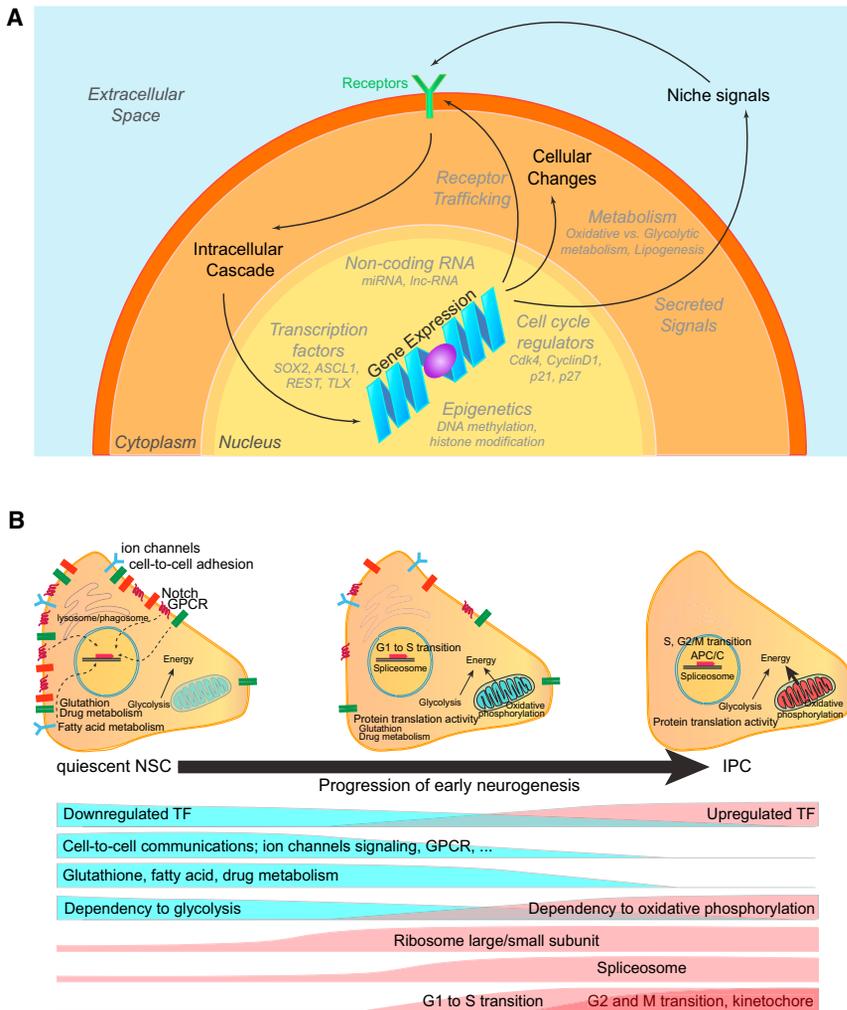


Figure 3. Adult NSC Regulation

(A) A schematic diagram depicting extrinsic and intrinsic mechanisms that regulate adult NSCs. Extrinsic niche signals activate receptors, which trigger intracellular cascades that induce changes in gene expression. In addition, intrinsic transcriptional programs can direct gene expression in NSCs. Regardless of how it occurs, modulation of gene expression results in cellular changes, which affect NSC behavior. Not surprisingly, intrinsic and extrinsic mechanisms that regulate NSCs are interconnected and feed back on one another.

(B) Schematic summary of molecular signatures of quiescent NSCs and molecular cascades underlying their activation and neurogenesis in the adult NSCs revealed by single-cell RNA-seq and Waterfall analyses (adapted from Shin et al., 2015). Shown on the top is an illustration of molecular signatures of adult qNSCs and their immediate progeny. Shown at the bottom are functional categories of genes that show a clear shift during adult qNSC activation and generation of IPCs. qNSCs exhibit intracellular and intercellular signaling to actively sense the local niche, rely mostly on glycolysis for energy, and have highly active fatty acid, glutathione, and drug metabolism. Upon activation, NSCs increase translational capacity and enter the cell cycle with G1 to S transition. Oxidative phosphorylation starts to be active and stem cell-specific properties are downregulated. IPCs maintain active cell-cycle genes, ribosomal activity, and fully active oxidative phosphorylation for energy generation.

Regulation of gene expression by transcription factors represents one fundamental mechanism that regulates adult NSCs (Hsieh, 2012). For example, the transcription factor SRY (sex determining region Y)-box 2 (Sox2) is highly expressed by adult NSCs in both niches, and its deletion results in NSC depletion. Sox2 expression requires active Notch signaling, and SOX2 has many targets, including Shh and Tlx. Another important transcription factor, achaete-scute homolog 1 (Ascl1), is expressed in a small subset of adult NSCs at a given time and activates NSCs by targeting cell-cycle regulators (Andersen et al., 2014). The nuclear receptor TLX is critical for NSC proliferation and maintenance, and repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST/NRSF) blocks pro-neuronal genes to prevent precocious neuronal differentiation while maintaining the NSC pool in the SGZ (Hsieh, 2012). In the SVZ, there are a host of transcription factors that specify the diverse populations of olfactory bulb neurons (Sequerra, 2014). Classically, individual transcription factors have been investigated for their role in regulating adult NSCs, but the recent availability of single-cell RNA-seq datasets for adult NSCs in both SVZ (Llorens-Bobadilla et al., 2015) and SGZ (Shin et al., 2015) allows, for the first time, a means to understand the logic of combinatorial tran-

scription factor regulation of NSC behavior at the whole-genome level. Epigenetic modifications, which are potentially heritable changes in gene expression that are not encoded in the DNA sequence, play important roles in adult neurogenesis (Ma et al., 2010). A common form of chromatin modification is DNA methylation, which normally suppresses gene expression. The DNA methyltransferase DNMT3a methylates intergenic regions of some neurogenic genes and, unlike most DNMTs, promotes their expression in adult SVZ NSCs (Wu et al., 2010). Active DNA demethylation, mediated by the TET-GADD45 pathway (Guo et al., 2011), regulates SGZ NSC proliferation both cell autonomously (Zhang et al., 2013) and non-cell autonomously via modulation of growth factor expression in mature granule neurons (Ma et al., 2009). Methyl-CpG binding proteins bind to methylated DNA to regulate gene expression and neurogenesis (Ma et al., 2010). For example, loss of Methyl-CpG binding protein 1 increases NSC proliferation (Liu et al., 2010). Methyl-CpG binding protein 2 balances NSC proliferation and differentiation by regulating specific miRNAs, including repression of miR-137 (Szulwach et al., 2010). Histone modifications also contribute to the epigenetic regulation of adult neurogenesis. For example, Bmi-1, a member of the polycomb group of chromatin remodeling factors, is critical for SVZ NSC self-renewal (Molofsky et al., 2003). Enhancer of zeste homolog2 (Ezh2) is a subunit of the polycomb repressive complex 2 and the methyltransferase of histone H3K27. Ezh2 is expressed in actively dividing NSCs in the SGZ and is thought to promote NSC proliferation

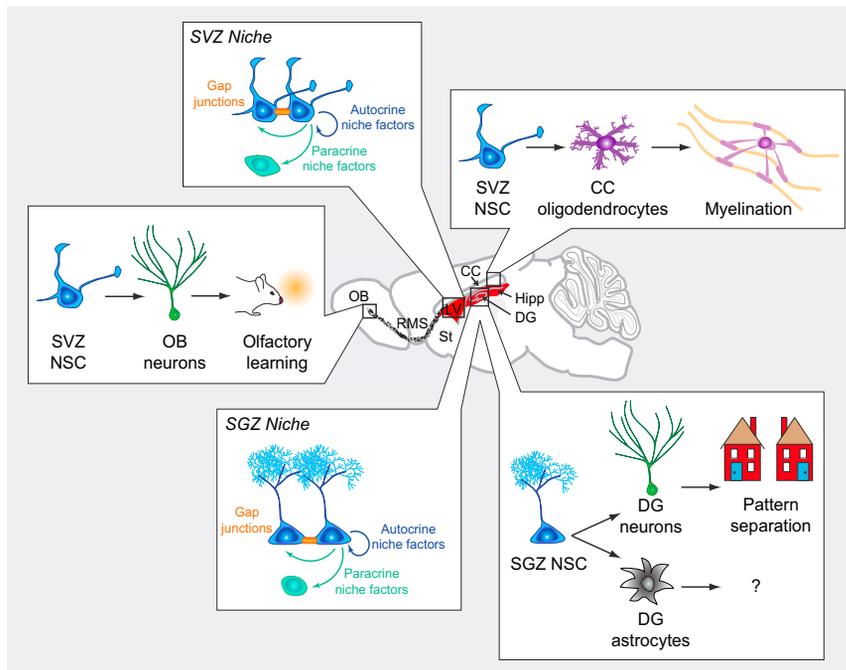


Figure 4. Impact of NSCs in the Adult Mammalian Brain

Adult NSCs directly impact the surrounding niche and indirectly impact neural circuitry through their progeny. NSCs in the SVZ and SGZ release autocrine and paracrine niche factors that contribute to the signaling milieu. In addition, NSCs form gap junctions to directly communicate with other NSCs. SVZ NSCs generate OB neurons and CC oligodendrocytes. OB neurons contribute to olfactory learning, while CC oligodendrocytes myelinate CC axons. SGZ NSCs generate DG neurons and astrocytes. DG neurons are important for pattern separation functions, while the functions of astrocytes remain to be explored. Thus adult NSCs impact not only the surrounding niche, but also the circuitry of multiple brain regions and ultimately behavior.

CC, corpus callosum; DG, dentate gyrus; Hipp, hippocampus; LV, lateral ventricle; NSC, neural stem cell; OB, olfactory bulb; RMS, rostral migratory stream; SGZ, subgranular zone; St, striatum; SVZ, subventricular zone.

by suppressing Pten expression and activating Akt-mTOR (Zhang et al., 2014). Histone deacetylase 3 regulates NSC proliferation via stabilization of cyclin-dependent kinase 1 (Jiang and Hsieh, 2014).

Epigenetic mechanisms often target other intracellular regulators, such as non-coding RNAs, creating a network of crosstalk interactions. Non-coding RNAs, including miRNAs and long non-coding RNAs (lnc-RNAs), play a regulatory role in adult neurogenesis (Li and Jin, 2010). miRNAs are small non-coding RNAs that regulate gene expression by binding to untranslated regions of target messenger RNAs, thus inhibiting protein synthesis. miR-124 promotes lineage progression in the SVZ by repressing SOX9 (Cheng et al., 2009), whereas miR-137 inhibits differentiation and promotes NSC proliferation through modulation of Ezh2 (Szulwach et al., 2010). lnc-RNAs are non-coding RNAs greater than 200 nucleotides that can regulate gene transcription, post-transcriptional mRNA processing, and epigenetic modifications. Two lnc-RNAs, Six3os and Dlx1as, promote neuronal differentiation and repress astrocytic differentiation of NSCs, whereas another lnc-RNA, Pnky, inhibits NSC differentiation (Ramos et al., 2013, 2015).

Recent studies have revealed changes in energetic demands during different stem cell behaviors (Jessberger, 2015). SVZ NSCs are more resistant to hypoxia and have a lower requirement for oxidative metabolism but are more dependent on glycolytic metabolism than neurons (Candelario et al., 2013). Spot14, a gene previously implicated in lipid metabolism, reduces lipogenesis and negatively regulates SGZ NSC proliferation (Knobloch et al., 2013). Recent single-cell RNA-seq analysis of adult NSC dynamics showed that activation of quiescent NSCs is accompanied by downregulation of glycolytic metabolism and upregulation of mitochondrial oxidation in both SGZ and SVZ (Figure 3B) (Llorens-Bobadilla et al., 2015; Shin et al., 2015). Emerging evidence supports a model that stem cells predomi-

nantly utilize glycolytic metabolism, which is thought to be important for maintaining self-renewal and lineage potency; upon lineage differentiation, cells switch to oxidative metabolism, which is needed to support the growing energetic demands of specialized progeny.

One hallmark of adult neurogenesis is its dynamic regulation by various physiological, pathological, and pharmacological stimuli, such as exercise, antidepressants, aging, epilepsy, and stroke (Ming and Song, 2011). The specific target or targets and underlying signaling mechanisms for many stimuli remain to be identified. It is also not always clear whether a particular signaling mechanism directly affects quiescent NSCs or their immediate progeny or whether subtypes of NSCs are differentially regulated. New tools that precisely target specific NSC sub-populations or different states of the same NSCs are needed to address these questions.

Potential Functions of Adult NSCs

Adult somatic stem cells normally play a homeostatic role in maintaining tissue organization. When adult NSCs were initially discovered, it was assumed that their function was to provide a regenerative source for new neurons, and there were hopes that these NSCs might be involved in functional brain repair after injury. Instead, cumulative evidence suggests that the primary function of endogenous adult NSCs is to confer an additional layer of plasticity to the brain via both direct and indirect mechanisms (Christian et al., 2014).

In both neurogenic niches, adult NSCs primarily generate new neurons, which are hyper-excitable and exhibit a lower threshold for long-term potentiation induction during a critical period when compared to mature neurons (Ge et al., 2008). These properties of developing neurons could allow them to differentially contribute to information coding compared to mature neurons in the same circuit (Figure 4). In addition, adult-born neurons promote plasticity of the existing circuitry by making new synaptic contacts with mature neurons (Ming and Song, 2011). Behavioral analyses suggest that adult neurogenesis is important for certain

types of hippocampus- or olfactory-bulb-dependent learning and memory (Christian et al., 2014; Deng et al., 2010; Sahay et al., 2011). Specifically, new dentate granule cells have been implicated in long-term spatial memory and pattern separation, a function that permits discrimination between two similar inputs, whereas new olfactory bulb interneurons are important for short-term olfactory memory and flexible olfactory associative learning. Excitement in this field continues with suggestions of additional functions of adult neurogenesis, such as buffering stress response (Snyder et al., 2011) and regulating “forgetting” or memory consolidation (Akers et al., 2014).

Though adult NSCs primarily generate new neurons, they also produce a small but significant proportion of glia. SGZ NSCs generate astrocytes, but the function of newborn glia cells remains largely unknown (Figure 4). Astrocytes are connected by gap junctions and form networks that can modulate the surrounding neural circuitry through multiple avenues. For example, astrocytes release factors, such as glutamate, adenosine triphosphate (ATP), and D-serine, collectively termed gliotransmitters, to modulate neuronal excitability, synaptic activity, and plasticity (Araque et al., 2014). Gliogenesis also occurs in the SVZ with predominantly oligodendrocytes. Oligodendrocyte precursors migrate to the corpus callosum to myelinate axons (Figure 4) (Xing et al., 2014). After demyelinating injuries, significantly more oligodendrocytes contribute to remyelination in the corpus callosum, suggesting that these precursors could potentially be a target for enhancing myelin repair. Though the proportion of gliogenic progeny may be modest, this is an understudied area and further studies are needed to determine the contribution of adult gliogenesis to brain function in order to fully understand functions of adult NSCs.

Adult NSCs themselves may also contribute to the niche through autocrine and paracrine signaling (Figure 4). Because it is challenging to determine the origin of niche signals, only a few studies have been able to present direct evidence that adult NSCs release factors to impact the niche. For example, upon increases in intracellular calcium, NSCs release vasodilating factors that activate pericytes on the vasculature to increase blood flow (Lacar et al., 2012). Adult SVZ NSCs and their immediate progeny secrete diazepam binding inhibitor, which antagonizes GABA signaling and promotes proliferation of neuroblasts (Alfonso et al., 2012). In addition to regulating the vasculature and progenitors, NSCs might also directly influence each other. For example, NSCs in the adult SGZ express both the VEGF receptor 3 and its ligand VEGF-C; VEGF receptor stimulation promotes NSC activation (Han et al., 2015). In addition, NSCs are connected to each other by gap junctions, potentially coordinating their actions (Kunze et al., 2009).

The role of adult NSCs in brain function is now expanding beyond that of being a mere source of neuronal progeny. Future studies will be needed to explore whether signaling or transcriptional modulation of adult NSCs in vivo can further expand their lineage potential and function to include therapeutic neural repair.

Emerging Topics in the Adult Stem Cell Field

Adult somatic stem cells are difficult to study because they are rare and heterogeneous and exhibit dynamic states. There is a need for technologies with precision to better probe adult stem

cells so that we can take advantage of their endogenous regenerative capacity for the benefit of human health. Classic studies of adult NSCs mostly relied on population-level analyses, which may mask properties of distinct groups of stem cells. Recent studies have begun using single-cell analysis to distinguish among different stem cell populations, including NSCs. For example, clonal genetic lineage-tracing has started to reveal properties of individual NSCs and their heterogeneity (Bonaguidi et al., 2011; Calzolari et al., 2015). It is expected that clonal analysis with genetic manipulations using different drivers to target sub-populations will lead to novel insights into adult NSC biology. Given recent advances in miniaturized microscope technology, it will also become possible to directly image the behavior of individual adult NSCs in vivo to explore how experience impacts NSC activation and division directly, as was achieved in adult fish (Barbosa et al., 2015) and for other adult somatic stem cells (Ritsma et al., 2014; Rempel et al., 2012). Another major technical advance is single-cell omics analyses (Shin et al., 2014), including transcriptomes via RNA-seq; epigenomes via methyl-seq, HiC, and ACTA-seq; and proteomics via mass cytometry. For example, single-cell RNA-seq analyses of adult NSCs in both SVZ (Llorens-Bobadilla et al., 2015; Luo et al., 2015) and SGZ (Shin et al., 2015) have started to provide a holistic picture of molecular signatures of quiescent adult NSCs and molecular dynamics of NSC activation and neurogenesis, including a switch in transcription factor expression and energy sources, changes in niche signaling capacity, and priming of protein translation machinery (Figure 3B). Instead of snapshot analyses of discrete time points, future studies will be greatly facilitated by novel bioinformatic tools, such as Monocle (Trapnell et al., 2014), SCUBA (Marco et al., 2014), and Waterfall (Shin et al., 2015), and novel sequencing technologies, such as FISSEQ (Lee et al., 2014), to reconstruct temporal, spatial, and lineage information to get a complete picture of the continuous and dynamic stem cell behavior.

Adult neurogenesis is conserved in mammals. Previous studies of postmortem tissues pulsed with BrdU have demonstrated the presence of adult neurogenesis in humans (Eriksson et al., 1998), and an innovative approach using ^{14}C from nuclear bomb testing has started to reveal quantitatively the extent of human neurogenesis over a lifetime. Understanding human adult NSCs and neurogenesis is not only important for therapeutic applications but also for basic knowledge of NSC biology given differences observed between humans and rodents. For example, the ^{14}C -based birth-dating strategy suggested that the age-dependent decline in hippocampal neurogenesis is much less dramatic in humans as compared to rodents (Spalding et al., 2013). In addition, NSCs residing in the adult human SVZ may generate neurons that migrate to the striatum, rather than into the olfactory bulb as in rodents (Bergmann et al., 2012; Ernst et al., 2014; Sanai et al., 2011; Wang et al., 2011). The major barrier to studying adult human stem cells is the inaccessibility of living tissue. Most studies have relied upon histological analysis using postmortem samples, but we are limited to a few markers, such as NESTIN and DCX (Knoth et al., 2010). Recent single-cell RNA-seq analysis may identify new markers for adult NSCs to facilitate the effort. In addition, spontaneous somatic nuclear and mitochondrial mutations have been used as clonal marks to track cell lineage in human brains (Evrony et al., 2015) and

intestinal stem cells (Baker et al., 2014). After further development, these approaches may enable clonal analysis of endogenous adult NSCs in humans via massive single-cell sequencing. Non-invasive imaging methods, such as magnetic resonance imaging and positron emission tomography, also have the potential to revolutionize adult NSC studies in humans, but they remain to be validated.

A series of discoveries on cellular reprogramming by Gurdon and Jaenisch, culminated by Yamanaka's demonstration of reprogramming somatic cells into pluripotent stem cells by defined transcription factors, has broken the conceptual barrier that development is a largely one-way process. Studies have since shown that mammalian cells can be reprogrammed in vivo, either by molecular or environmental factors, to exhibit expanded potentials that are not seen under physiological conditions (Arlotta and Berninger, 2014). For example, viral-mediated expression of exogenous Sox2 in resident astrocytes in the adult mouse reprograms them into proliferating neuroblasts in striatum and spinal cord, regions outside canonical neurogenic niches (Niu et al., 2013; Su et al., 2014). In the absence of exogenous reprogramming factors, striatal astrocytes turn on nestin expression and generate neurons in both an adult mouse stroke model (Magnusson et al., 2014) and an excitotoxic model of Huntington's disease (Nato et al., 2015) via downregulation of Notch signaling. Neuroblasts in the adult SVZ are converted to oligodendrocytes in an injury model of corpus callosum demyelination via upregulation of chordin (Jablonska et al., 2010). Though trans-differentiation could be a powerful method to generate new cells in regions that lack a stem cell niche, many unknowns remain. The molecular mechanisms, especially epigenetic mechanisms, underlying induced potential remain to be determined. One interesting mechanistic question is whether such plasticity in vivo involves a return to the NSC state, even transiently, followed by differentiation (Bar-Nur et al., 2015). Newly generated cells need to develop outside a niche in an environment that may lack appropriate signals to direct cellular identity and integration. Thus, the clinical relevance of in vivo reprogramming will hinge upon demonstration of proper integration, functional recovery, and minimal side effects.

Concluding Remarks

Over the past five decades, adult neurogenesis and NSCs have evolved from a topic of interest to a few optimistic scientists to an established field that has made tremendous progress to significantly impact our perspective of brain plasticity. Initial work focused on characterizing the process of adult neurogenesis, followed by efforts to identify underlying cellular and molecular mechanisms and its functional significance. Current work continues to examine and manipulate NSC regulatory mechanisms in an effort to take advantage of the regenerative capacity of adult NSCs. Though our understanding of adult NSCs has come a long way, there are still significant challenges for future research.

First, adult NSCs are a very heterogeneous population of cells, making it difficult to distinguish between different temporal states or distinct populations in vivo. Future studies will benefit from identification of sub-population- or state-specific markers, as well as from single-cell analysis to dissect properties of various NSC sub-populations and dynamic states. Second,

despite substantial advances in understanding numerous signaling pathways, little is known about how NSCs integrate many diverse signals to ultimately make choices, such as quiescence versus activation, symmetric versus asymmetric cell division, and maintenance versus fate commitment. Future research will need to focus on interactions between signaling pathways to identify signaling hubs and the hierarchy that coordinates the barrage of incoming signals. Third, human neurogenesis research has recently gained traction, and we have learned that despite sharing similar neurogenic niches, there are many differences between rodent and human adult neurogenesis. Significant advancements in the adult NSC field will require more human studies, as well as the development of new methods to probe NSCs in vivo. Non-invasive monitoring will be critical not only for human studies, but also for real-time and longitudinal studies to understand the life-long impact of neurogenesis. Finally, while the prospect of using adult NSCs therapeutically as a regenerative source for neural repair is very exciting, a major issue that must be addressed is the functional consequence of new neuron generation, particularly outside of the traditional neurogenic niches via reprogramming. Vigorous examination of neuronal integration and the impact of new neurons on the surrounding neuronal circuitry and behavior will be imperative for this line of research to be clinically relevant.

Adult NSCs and neurogenesis confer a unique mode of plasticity to the mature mammalian brain. One overarching goal of adult neurogenesis research is to manipulate NSCs to improve brain health. The field has spent the past five decades accumulating knowledge regarding neurogenesis and is starting to apply that knowledge in conjunction with new technologies to work toward this ultimate goal.

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