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A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus

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Continuously generated new neurons promote circuitry plasticity within specialized regions and contribute to specific functions of the adult mammalian brain. A number of recent studies have investigated the cellular origin of adult neurogenesis in the hippocampus, yielding divergent models of neural stem cell behavior. An essential question remains whether these models are overlapping or fundamentally discrete. We review evidence that primary neural precursors in the adult hippocampus exhibit significant heterogeneity in their properties of self-renewal, multi-lineage differentiation and regulation, representing a range from unipotential committed precursors to *bona fide* self-renewing multipotent neural stem cells. We further present a testable unifying hypothesis of adult neural stem cell behavior *in vivo* to outline a common framework for future studies of molecular and cellular mechanisms regulating adult neural stem cells and how these cells may contribute to hippocampal function and repair.

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Introduction

The adult mammalian brain is a plastic structure, capable of dynamic cellular and molecular remodeling in response to an individual's interactions with the outside world. One dramatic example of structural plasticity in the mature brain is the birth and addition of newborn neurons to the existing circuitry, a process named adult neurogenesis [1]. Under physiological conditions, new neurons arise from neural precursors within two specialized micro-environments, the subventricular zone along the lateral ventricle and the subgranular zone (SGZ) in the dentate gyrus, leading to modifications of the olfactory bulb and hippocampal circuitry, respectively [2]. Adult hippocampal neurogenesis draws much interest because newborn hippocampal neurons have been suggested to adapt the brain to temporal events in external space, including spatial learning and retention, pattern discrimination and clearance of memory traces [3,4]. An emerging concept is that newborn neurons at specific stages of maturation are preferentially recruited in the circuitry owing to their high excitation/inhibition balance and enhanced synaptic plasticity [5–7]. In addition, adult hippocampal neurogenesis is involved in response to antidepressants [8], stress [9], and brain injuries and may play a role in the pathophysiology of mental disorders [2,10,11]. A basic understanding of precursor properties and their niche interactions will illuminate how neural precursors sense and respond to changes in the external environment to promote tissue homeostasis or repair.

Adult neurogenesis is thought to arise from precursors with properties of neural stem cells (NSCs) [12]. Technical advances have made it possible to dissect basic cellular processes of neural precursor behavior *in vivo* and their contribution to the adult neurogenesis process (Table 1). Several recent studies have examined properties of primary neural precursors in the adult mouse hippocampus, including *in vivo* clonal analysis of activation, self-renewal and fate choice decisions from a prospectively identified precursor [13•,14••,15]. These approaches allow investigation of intrinsic and extrinsic mechanisms contributing to hippocampal neurogenesis and homeostasis. For any lineage-tracing studies, careful identification of the precursor source is critical for interpretation. For example, three recent studies performed lineage-tracing to investigate NSC properties in the adult mouse hippocampus, but arrived at different conclusions [14••,16••,17•]. Two studies interpreted that NSCs self-renew [14••,17•], while a third did not observe precursor maintenance [16••]. NSC potential also differs among studies, including the generation of astroglia and additional NSCs. Though conclusions drawn are seemingly contradictory, they may result partly from labeling different precursor populations using divergent approaches. In this review, we summarize *in vivo* evidence suggesting the existence of neural precursor heterogeneity in the adult rodent hippocampus. We also

Table 1

Fate-mapping approaches for studying adult hippocampal neural stem cells. Five different approaches to perform fate-mapping of neural precursors are illustrated. In cell culture, isolated precursors are grown *in vitro* as a monolayer or non-adherent 'neurospheres'. For slice culture, the hippocampus is sectioned to partially maintain local tissue architecture *ex vivo*. For *in vivo* studies, analysis can be based upon the incorporation of nucleotide analogs during DNA replication in the S-phase of cell cycle, the expression of transgenes from retroviruses requiring integration of the retroviral genome during the M phase, or transgenic mice expressing reporters under specific promoters. Some advantages and disadvantages of these five approaches are listed. Additionally, next-generation tools are either under development or recently become available

	Cell culture	Slice culture	Thymidine analogs	Retrovirus	Transgenic animals
Species applicability	Broad	Broad	Broad, limited in human	Broad, limited in human	Mice
Preparation	*In vitro*	*Ex vivo*	- *In vivo* - *Ex vivo* - *In vitro*	- *In vivo* - *Ex vivo* - *In vitro*	- *In vivo* - *Ex vivo* - *In vitro*
Cell population	Good	Good	Limited	Limited	Good
Single cell analysis	Good	Good – video microscopy	Limited	Limited	Good
Cell targeting	Quiescent/mitotic	Quiescent/mitotic	Mitotic	Mitotic	Quiescent/mitotic
Visualization	Direct/processing	Direct/processing	Processing	Direct	Direct/processing
Morphology	Whole cell	Whole cell	Nuclear	Whole cell	Whole cell
Birth dating	Good	Good	Good	Good	Poor
Temporal resolution	High	High	Moderate	Moderate	Low
Injury	High	Moderate-high	No	Moderate	No
Concerns and other limitations	- Niche removal - Reprogramming - Cell of origin?	- Tissue damage - Tissue survival	- Rarely targets quiescent stem cells - DNA repair	- Rarely targets quiescent stem cells - Invasive	- Specificity - Labor intensive
Next-generation approaches	- No growth factors - Video analysis	*In vivo* video microscopy	Dual/triple labeling	Split-Cre specific lentivirus	- Multicolor reporters - Birth-dating

discuss characteristics of neural precursors as defined by their identity, potential and regulation to outline a testable unifying hypothesis of NSC behavior in the adult hippocampus.

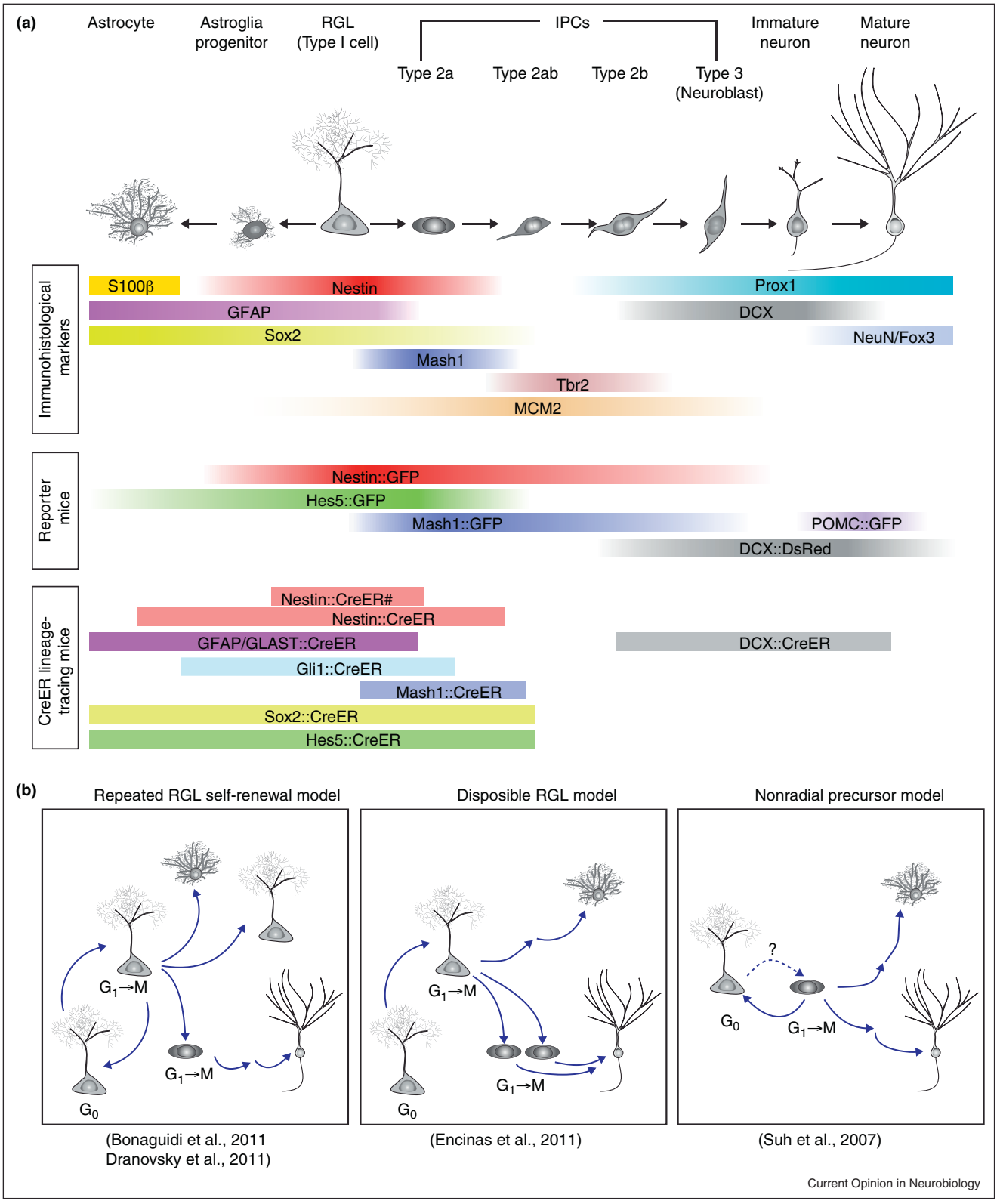
Adult neural stem cells: identity and potential

First established by culturing isolated cells, NSCs are defined by their potential to both self-renew and generate neurons and glia from a single cell [12,18]. Emphasis on *in vivo* NSC properties became more prevalent, because reprogramming studies have raised the question whether cultured lineage-restricted neural progenitors acquire potential not evident *in vivo* [19–21]. Unlike in *C. elegans* and *Drosophila* where certain somatic stem cells can be identified by location, *in vivo* lineage-tracing studies in mammalian systems utilize retrospective analysis, which requires knowledge of the identity of originally labeled cells to perform fate-mapping. Given the presence of multiple precursor subtypes in the adult hippocampus, a thorough understanding of the prospective identity is needed to elucidate to what degree primary precursors self-renew and generate multiple progeny.

Radial glia-like precursors

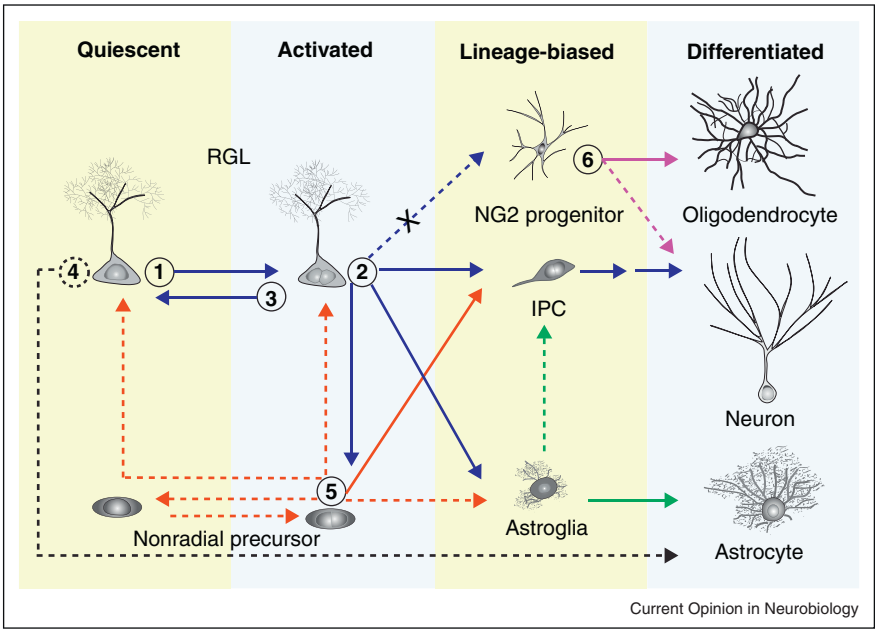
In the prevailing model of adult hippocampal neurogenesis, quiescent radial glia-like cells (RGL, or Type-1 cells) generate proliferative precursors known as intermediate progenitors (IPCs, or Type-2 cells), which give rise to neuroblasts (Type 3 cells) and then immature neurons (Figure 1a). RGLs express nestin, GFAP and Sox2, and possess a defining radial branch extending through the granule cell layer. Evidence supporting RGLs as NSCs comes from anti-mitotic treatment recovery [22], genetic ablation [23], and transgenic fate-mapping [17•,24,25]. *In vivo* clonal analysis further revealed that a single RGL undergoes several rounds of self-renewal and differentiation to produce both neurons and astrocytes over a long duration, demonstrating characteristic stem cell properties by individual RGLs [14••]. Alternative RGL properties have also been postulated (Figure 1b). Encinas *et al.* proposed that RGLs do not possess long-term maintenance; instead, RGLs repeatedly enter cell cycle once activated and generate only neurons before terminally differentiating into astrocytes [16••]. While this model is seemingly at odds with conclusions drawn by Bonaguidi *et al.* [14••], interpretations may differ partly owing to the selection of different RGL subpopulations. Using the same *Nestin-CreER-Z/EG* mouse line for fate-mapping [26], Encinas *et al.* targeted proliferative RGLs with BrdU and performed population analysis, whereas Bonaguidi *et al.* sparsely labeled predominantly quiescent RGLs and performed clonal analysis. Therefore, technical differences may account for preferential labeling of divergent RGLs. The same may be true for another recent study by Dranovsky *et al.* concluding that RGLs expand, instead of decline, over time using a different *Nestin-CreER* mouse line for population analysis [17•]. Notably, clonal analysis of quiescent RGLs also revealed substantial NSC heterogeneity, including unipotent clones and clones depleted of

Figure 1



Models for neural stem cell (NSC) behavior in the adult hippocampus. **(a)** Neural lineages according to the radial glia-like cell (RGL) model. RGLs, or Type 1 cells, are quiescent precursors that generate new neurons and astrocytes through intermediate progenitor cell (IPC) and astroglia progenitor phases, respectively. IPCs proliferate while progressively differentiating through multiple stages into neurons, while astroglia progenitors possess

Figure 2



A unified NSC model in the adult hippocampus under basal conditions. Multiple precursor subtypes co-exist in the adult dentate gyrus to coordinate tissue homeostasis under basal conditions. Quiescent RGLs undergo three known decisions: (1) Activation; (2) Cell fate choice; and (3) Maintenance/self-renewal. Once exited from quiescence, RGLs can generate new RGLs, nonradial precursors, IPCs and astroglia, but not the oligodendrocyte lineage (2). Afterwards, RGLs can remain in a proliferative state, or return to quiescence (3). While not yet directly observed, a RGL may differentiate into an astrocyte without proliferation (4). Yet to be demonstrated directly, nonradial precursors may generate RGLs and astroglia in addition to the known neuronal generation, while maintaining the precursor state (5). Likewise, NG2 progenitors may choose among proliferation, oligodendrocyte generation and precursor maintenance (6). Importantly, heterogeneity of decisions exists within a single precursor type and ranges from unipotent non-self-renewing progenitors to multipotent self-renewing stem cells. Arrows indicate direct cell generation. Dotted arrows represent potential choices that need further experimental evidence. Double arrows represent multistep cell generation. Arrows with an 'X' represent choices not experimentally observed.

RGLs [14••]. These findings demonstrate that RGL subpopulations with apparently similar morphological and molecular identity exhibit varying levels of self-renewal and differentiation capacity and probably represent a range from unipotential committed precursors to *bona fide* self-renewing multipotent NSCs. Whether heterogeneity reflects differences in intrinsic properties or extrinsic regulation remains a fundamental question in stem cell biology. Future studies are needed to address mechanisms underlying RGL heterogeneity and potential hierarchy.

Nonradial neural precursors

Nonradial precursors generate new neurons in the adult SGZ and have also been proposed to act as primary precursors (Figure 1b) [13•]. Nonradial precursors lack any radial process and some contain parallel extensions to the dentate granule cell layer. These precursors express Sox2, but not GFAP, and are labeled in *Hes5:gfp* reporter mice [27••]. They are more mitotic than RGLs, but most are not always in cell cycle [13•,27••]. The identity of nonradial precursors is not clearly delineated from early IPCs (Type-2a cells), which share similar morphological and molecular characteristics (Figure 1a) [27••,28,29]. The potential of nonradial precursors and their lineage relationship to other precursors also remain unclear. *In vivo* clonal analysis has demonstrated that RGLs can give rise to nonradial Sox2$^+$ cells (Figure 2) [14••]. Retrovirus-mediated lineage-tracing of individual Sox2$^+$ cells in the adult SGZ showed that most labeled clones exhibited

comparatively small proliferative capacity. Stage-specific markers are shown for each cell type. Short-term fate-mapping from various cells of origin can be achieved using fluorescent reporter mice under the regulatory control of stage-specific promoters. Long-term indelible lineage-tracing can be performed from various precursors using inducible Cre-LoxP technology in combination with 'floxed-on' reporters. The exact cell type to be labeled is controlled by the combination of the CreER driver line, specific reporter line and dose of tamoxifen (Nestin::CreER#: induction with a low dose of tamoxifen). References for each reporter and lineage-tracing mouse line are listed in the Appendix A: Supplementary Data online. **(b)** Three proposed NSC models. In the 'Repeated RGL self-renewal' model, RGLs can cycle between quiescent and mitotic states. Once activated, RGLs can divide symmetrically to generate additional RGLs, or asymmetrically to produce neuronal and astroglial lineages. In the 'Disposable RGL' model, once activated, RGLs repeatedly divide to generate only the neuronal lineage without returning to quiescence and then terminally differentiate into astrocytes. In the 'Nonradial precursor' model, proliferative cells lacking a radial process generate neurons, astrocytes, and even RGLs. Arrows indicate direct cell generation. Dotted arrows represent unknown choices. Double arrows represent multistep cell generation.

limited self-renewal and unipotent differentiation, while no clones displayed both self-renewal and multipotentiality [13•]. Interestingly, one among 300 examined clones contained a neuron and an astrocyte and another contained an RGL and a neuron, suggesting that nonradial precursors may act as NSCs and can generate RGLs (Figures 1 and 2). These events occurred at extremely low frequency and whether RGLs were initially labeled with retrovirus was not reported [13•].On the contrary, retroviruses preferentially target mitotic cells and label one of the two progeny [30], raising the possibility that the originating NSC was not labeled. Therefore, the nonradial precursor identity, potential and lineage relationship with other NSCs remain elusive and require future studies using alternative single-cell fate-mapping approaches.

Other neural precursors

There are additional proliferating cell populations in the adult hippocampus, which may act as NSCs under certain conditions. IPCs are the most proliferative cell type in the adult dentate gyrus and locate exclusively to the SGZ region (Figure 1a). These cells possess small tangential processes, express Tbr2 and preferentially incorporate BrdU [31]. Derived from both radial and nonradial precursors (Figure 2) [14••,16••,27••,28], these cells are considered secondary transient amplifying precursors because they soon express DCX and Prox1, markers of committed immature neurons (Figure 1a) [31], and the population is quickly replenished following anti-mitotic treatments [22]. IPCs re-enter cell cycle on average 2.5 times [16••]. It remains unclear whether they can also produce astroglia or maintain as precursors over a long duration.

$NG2^+$ oligodendrocyte precursors constitute the major proliferative population in the adult dentate non-neurogeneic areas and are also found in the SGZ [16••]. NG2 cells possess long wispy processes, express the characteristic proteoglycan NG2 as well as PDGFRalpha, Sox10 and Olig2 [32]. So far, NG2 cells and oligodendrocytes have not been observed arising from RGLs in the adult SGZ (Figure 2) [14••], suggesting that they may represent a discrete precursor population with different embryonic origins. Despite substantial debate over NG2 cell potential [33,34], the emerging consensus is that they produce astroglia and oligodendrocytes during development, but are restricted to oligodendrocyte generation in the adult nervous system (Figure 2) [32]. Whether NG2 cells exhibit expanded potential under injury or other conditions remains an interesting question.

Astroglia are a potential third and understudied precursor population. In the adult SGZ, these cells exhibit horizontal or bushy morphology and express GFAP, S100β and Aldh1l1 [35]. Under basal conditions, astroglia are largely not considered as a neuronal precursor cell type because they lack nestin expression and are incredibly quiescent [14••,16••,36]. However, rare astroglia can be labeled with cell cycle markers and share many immunohistological similarities with RGLs including the neuronal determinant Mash1 [35,37]. Interestingly, new astroglia are generated from RGLs in the adult SGZ before they migrate to the hilus or molecular layer [14••]. Whether these astroglia can act as transient precursors before leaving the neurogenic niche or even remain as progenitors for an extended period remains to be determined (Figure 2). It will be also interesting to examine whether hippocampal reactive astroglia exhibit greater potential under pathological conditions.

Adult neural stem cells: regulation at multiple decision points

The adult neurogenesis process is remarkably responsive to external alteration under physiological and pathological conditions [2]. The cellular targets of environmental effects are becoming increasingly known and most commonly influence later stages of neurogenesis [2]. An emerging concept is that early precursors exhibit differential responsiveness to specific external stimuli [27••]. Primary precursors and their progeny reside in a niche surrounded by neuronal circuitry with glia and endothelial cells [2]. Signaling from the niche is proposed to control many aspects of stem cell behavior, including their mitotic state, cell fate specification and precursor maintenance. Therefore, understanding how various niche components, signaling pathways and environmental stimuli differentially regulate NSC behavior will reveal how NSCs contribute to the homeostasis and repair. Recently, it has become clear that NSCs face multiple decision points during adult hippocampal neurogenesis (Figure 2).

Activation of quiescent adult neural precursors

RGLs and nonradial cells exist primarily in a quiescent state in the adult hippocampus, but can shuttle between quiescent and activated states, or exist as a relatively stable mitotic population [14••,16••,27••]. It remains unclear what mechanisms distinguish between return to quiescence and retention in mitosis, which may represent another precursor decision point. Rather, mechanisms regulating the activation of quiescent precursors are comparatively well described owing to their ease of analysis using cell cycle indicators in combination with cell type-specific markers [2]. A common theme amongst the known mechanisms is the active suppression of proliferation. For example, conditional disruption of BMP, Notch/RBP-J or REST signaling in RGLs results in rapid activation of NSCs accompanied by a transient increase in IPC numbers and production of new neurons in the adult hippocampus [38–40]. The function of quiescence may serve as a general protective mechanism that counteracts stem cell exhaustion under basal

conditions to retain the capacity to be mobilized in meeting local physiological and pathological tissue demands over the life-time [41]. Intriguingly, environmental alterations have been recently identified to differentially promote RGL and nonradial precursor proliferation. For example, RGLs, but not nonradial cells, are activated in response to exercise, whereas both cell populations proliferate upon seizure-inducing stimuli [27••]. It will be interesting to investigate how niche cells relay various environmental cues and molecular signaling mechanisms that regulate precursor exit from and return to quiescence.

Fate specification of adult neural precursors

New neurons, astroglia and oligodendrocytes are generated in the adult hippocampal region. Under physiological conditions single RGLs have been shown to generate additional RGLs, nonradial cells, neuron-generating IPCs and astroglia, but not oligodendrocytes (Figure 2), indicating that cell fate specification occurs within RGLs [14••]. However, mechanisms regulating NSC fate specification *in vivo* are not well understood, probably reflecting the choice of technical approaches (Table 1). BrdU and retrovirus lineage-tracing preferentially label proliferative IPCs, which may be limited in their fate potential, thus under represent gliogenesis levels in the SGZ [14••,36]. Additionally, transgenic approaches at the population level, which do label RGLs, routinely assess cell fate choice by analyzing the number of primary precursors and final amount or ratio of neurons and astrocytes [17•]. This input/output relationship approach is complicated by the pleotropic nature of many molecules known to regulate various stages of neurogenesis, including the proliferation and death of intermediate cell types [2]. While tedious, single-cell fate mapping can potentially resolve *in vivo* NSC decisions from additional downstream effects. For instance, *PTEN* deletion within individual RGLs promotes symmetric RGL divisions at the expense of both neuronal and astroglial cell fate choices [14••]. Interestingly, the decrease in IPC generation from RGLs is negated by an increase in neuroblast survival, which increases the final production of newborn neurons.

Fate specification of NSCs may also be subject to dynamic regulation under diverse physiological, environmental or pathological conditions to reflect brain adaptation over time. For example, social isolation stress promotes RGL expansion, which may prepare the brain for increased neurogenic potential when more favorable conditions return [17•]. The signals and molecular mechanisms dictating fates of the NSC lineage remain to be determined. As several molecular mechanisms have been described to affect NSC fate specification *in vitro*, they may serve as appealing candidates for investigation *in vivo* [12,18]. Of particular interest is to address how niche components couple neuronal circuitry activity to direct regulation of NSCs fate decisions under both normal physiological and pathological conditions.

Maintenance of adult neural precursors

The balance of NSC maintenance and neurogenesis is essential to ensure continuous generation of new hippocampal neurons throughout life without depleting the NSC pool. For example, the long-term consequence of excessive activation is subsequent depletion of NSC compartment and impaired maintenance of NSCs, which ultimately leads to the loss of regenerative capacity of the NSC population and subsequent neuronal production in the adult hippocampus [38–40]. Similarly in the neurogenic subventricular zone, loss of *FoxOs* or *Dlk1* leads to initial increase of NSC activation during early postnatal stages, followed by NSC depletion and defects in adult neurogenesis [42,43]. Direct evidence is still lacking with regard to whether failure of NSC maintenance is owing to increased astrocytic differentiation of radial NSCs, lack of activation of quiescent NSCs, or cell death of their downstream neuronal progeny. Although the source of most niche signals remains to be fully characterized, it becomes increasingly clear that these signals play an important role in fine-tuning the number of quiescent NSCs and the amount of neurogenesis in the adult brain possibly through adaptive and feedback mechanisms. Ultimately, the total NSC pool reflects a summation of NSC decisions over time: maintenance through quiescence or asymmetric self-renewal, reduction through terminal differentiation, and expansion through symmetric self-renewal (Figure 2). Future studies are needed to identify intrinsic and extrinsic mechanisms underlying each decision.

Adult neural stem cells: heterogeneity as a unifying principle

Stem cell heterogeneity is an emerging principle in many adult somatic tissues, which can display differences among stem cell identity, potential and regulation [44,45]. First, stem cells with apparently similar identity can display different potential and regulation. For example, long-term hematopoietic stem cells (LT-HSC) with similar molecular profiles exist as either relatively mitotic or extremely quiescent subpopulations and differentially contribute to tissue maintenance and injury responses, respectively [46]. Meanwhile, LT-HSC can possess fate bias, preferentially generating the myeloid or lymphoid lineages despite undergoing extensive self-renewal [47]. Second, stem cells with different identities may contribute to separate functions within the same tissue. In the intestine, $Lgr5^{+}$ highly mitotic intestine stem cells (ISC) constantly remodel the host tissue [48], while quiescent $Bmi^{+}Lgr5^{-}$ ISCs act as a reserve pool that regenerate lost or damaged $Lgr5^{+}$ ISCs [49]. In the adult olfactory neuroepithelium, globose basal cells and horizontal basal cell serve as house-keeping and reserved NSCs, respectively [50]. These findings from different

somatic systems suggest that multiple stem cells co-exist within a tissue to satisfy particular local demands.

The dentate gyrus in the adult brain also contains multiple precursor populations of various identities, including RGLs, nonradial precursors, IPCs, NG2 cells and astroglia. While still unproven, these multiple cell types may represent the co-existence of multiple NSC populations in the adult brain to serve different tissue demands [27••] (Figure 2). In addition, subpopulations within each cell type may possess a range of potential and undergo differential regulation as evident using single-cell lineage studies within the RGL pool [14••]. RGL subtypes may vary from unipotential 'disposable' stem cells [16••] to self-renewing and multi-lineage-generating NSCs [17•,25]. In many somatic tissues, the most quiescent precursor acts as a stem cell reservoir, responds to injury and can replace other stem cell subtypes. In the adult SGZ, approximately 10% of RGL clones remain as single cells after one year of tracing [14••]. Could these cells be long-term injury-responsive NSCs? Are there other precursor populations containing NSC properties? Are NSCs intrinsically diverse, or are they equipotent but respond selectively to different environmental cues? Ultimately, the concept of precursor heterogeneity within a given region may be a unifying hypothesis on adult NSC properties in the mammalian brain. This principle provides a platform for determining NSC capacity for brain alteration, exploring underlying mechanisms, and understanding how they may act as a compensatory mechanism to promote tissue homeostasis or repair.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.conb.2012.03.013.

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