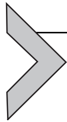


CHAPTER TWO

Ontogeny of adult neural stem cells in the mammalian brain

Allison M. Bond[a], **Guo-li Ming**[a,b,c,d], **and Hongjun Song**[a,b,c,e,*]

[a]Department of Neuroscience and Mahoney Institute for Neurosciences, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
[b]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
[c]Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, PA, United States
[d]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
[e]The Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
[*]Corresponding author: e-mail address: shongjun@pennmedicine.upenn.edu

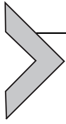
Contents

1. Introduction 68
2. Embryonic origins of adult neural stem cells 69
  2.1 Origins of subgranular zone adult neural stem cells 71
  2.2 Origins of ventricular-subventricular zone adult neural stem cells 72
3. Transformation of neural stem cell properties from development to adulthood 75
4. Establishment of adult neurogenic niches 80
5. Mechanisms regulating establishment of the adult neural stem cell pool 84
6. Concluding remarks and future perspectives 88
Acknowledgments 91
References 91

Abstract

Neural stem cells (NSCs) persist into adulthood in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and in the ventricular-subventricular zone (V-SVZ) of the lateral ventricles, where they generate new neurons and glia cells that contribute to neural plasticity. A better understanding of the developmental process that enables NSCs to persist beyond development will provide insight into factors that determine the size and properties of the adult NSC pool and thus the capacity for life-long neurogenesis in the adult mammalian brain. We review current knowledge regarding the developmental origins of adult NSCs and the developmental process by which embryonic NSCs transition into their adult form. We also discuss potential mechanisms that might regulate proper establishment of the adult NSC pool, and propose future directions of research that will be key to unraveling how NSCs transform to establish the adult NSC pool in the mammalian brain.

Current Topics in Developmental Biology, Volume 142
ISSN 0070-2153
https://doi.org/10.1016/bs.ctdb.2020.11.002
Copyright © 2021 Elsevier Inc.
All rights reserved.



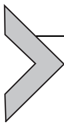
1. Introduction

Neural stem cells (NSCs) are resident stem cells of the nervous system and are responsible for populating the brain with neurons and glia during development. Though their numbers decline as development is completed, some NSCs are retained where they continue to generate neurons and glia throughout adulthood. The role of NSCs in the adult brain is very different than during development—NSCs generate progeny during development to establish neural circuits, whereas NSCs generate progeny in adulthood to modify circuits through neural plasticity. In particular, neurogenesis, or the generation of new neurons from NSCs, provides a source of new neurons which can be added to neural circuits to induce plasticity (Ma, Bonaguidi, Ming, & Song, 2009; Ming & Song, 2011). Active neurogenesis does not occur in all regions of the adult mammalian brain under physiological conditions, but instead is limited to two discrete regions: NSCs in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) region generate dentate granule neurons (DGNs) and astrocytes, and NSCs in the ventricular-subventricular zone (V-SVZ) of the lateral ventricles generate olfactory bulb interneurons and oligodendrocytes (Bond, Ming, & Song, 2015; Ming & Song, 2005). A fundamental question in NSC biology is how NSCs are maintained in the SGZ and V-SVZ beyond development. To get at this question, we need to understand the developmental ontogeny of adult NSCs.

The earliest neural precursors in the embryonic brain are neuroepithelial cells, which divide symmetrically to expand the precursor pool. Neuroepithelial cells transform into radial glia (RG) by acquiring features associated with glial cells, such as astroglial marker expression and initiation of specialized contact with endothelial cells in the developing vasculature (Kriegstein & Alvarez-Buylla, 2009). RG first divide asymmetrically to generate neurons and neuronal progenitors, called intermediate progenitor cells (IPCs), and later divide to generate glia and glial progenitors (Kriegstein & Alvarez-Buylla, 2009; Namba & Huttner, 2017). While the majority of embryonic RG are not maintained beyond development, a small proportion of RG transform into adult NSCs and are maintained throughout life. More specifically, adult NSCs in the SGZ and V-SVZ are called RGLs (radial glia-like NSCs) (Bonaguidi et al., 2011) and B cells (Doetsch, Garcia-Verdugo, & Alvarez-Buylla, 1997), respectively. Though the behavior and function of adult NSCs has been extensively studied, the developmental ontogeny of adult NSCs, particularly the transformation of RG into

adult NSCs, has only recently become a focus of adult NSC research (Berg, Bond, Ming, & Song, 2018; Obernier & Alvarez-Buylla, 2019). One defining feature of adult NSCs is their quiescent state, a reversible state in which a cell has exited cell cycle but retains the capacity to divide (Urban, Blomfield, & Guillemot, 2019). The transition from a proliferative state to quiescence is thought to be a critical step in the transformation from a developmental RG state to an adult NSC state and has been used as a developmental milestone marking the transition to an adult NSC phenotype.

In this chapter, we present an overview of our current understanding of the ontogeny of adult NSCs in the mammalian brain, focusing on studies in rodent models. We start by discussing embryonic origins of adult NSCs, including the developmental timing of RG to adult NSC transformation, the lineage relationship between RG of the embryonic brain and adult NSCs, and differences in the embryonic origins of adult NSCs in the SGZ and V-SVZ. Next, we review how intrinsic cellular and molecular properties of NSCs change as they transform from RG into adult NSCs, and the developmental establishment of adult NSC niches, which are specialized microenvironments that support NSC maintenance and continuous neurogenesis. Finally, we summarize potential cellular and molecular mechanisms that regulate transformation of RG to adult NSCs. We conclude with remaining unanswered questions and future directions. We hope that by taking a developmental perspective to understand how adult NSCs emerge from development, we will gain insight into mechanisms that maintain long-term NSC potential, which can be applied to enhance plasticity and regenerative capacity of the adult mammalian brain.



2. Embryonic origins of adult neural stem cells

Adult NSCs in the SGZ and V-SVZ have different embryonic origins and arise through distinct developmental processes (Fig. 1). The major differences between origins of adult SGZ and V-SVZ NSCs are (1) the timing of entry into quiescence and (2) the continuity of progeny fate. Within each of the two adult NSC populations, there also appears to be some variety in their embryonic origin, which has been linked to adult NSC heterogeneity. Thus, understanding developmental origins of adult NSCs may provide insight into adult NSC function. In this section, we discuss the developmental timing of adult NSC emergence and lineage relationships between adult NSCs, embryonic RG and differentiated cell types in the brain.

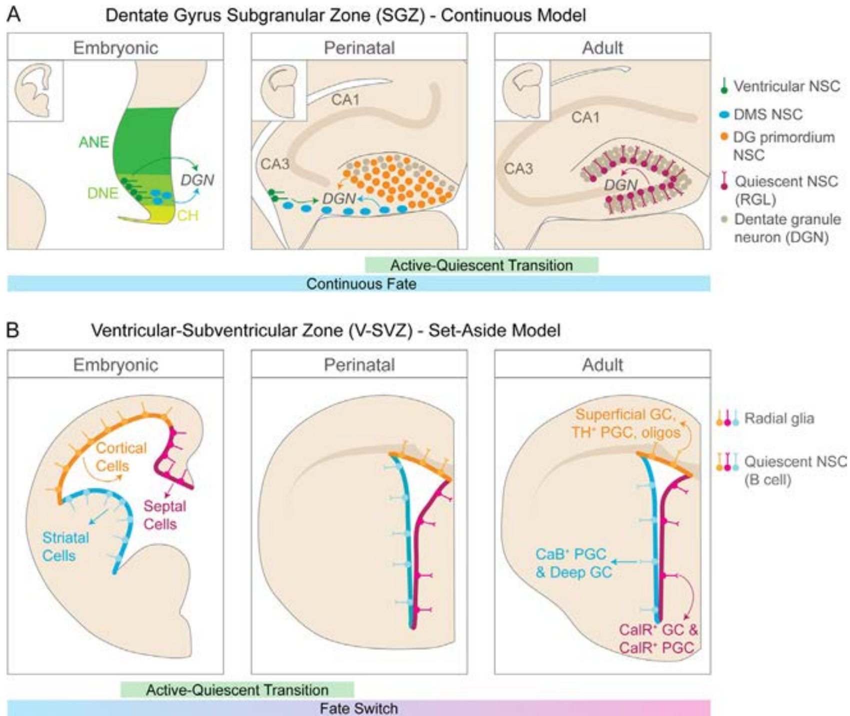


Fig. 1 Two models for the origin of adult neural stem cells in the subgranular zone and the ventricular-subventricular zone. (A) Radial glia (RG) in the embryonic dentate neuroepithelium (DNE) divide and begin to detach from the ventricle to enter the dentate migratory stream (DMS). Dentate gyrus (DG) neural stem cells (NSCs) from the DMS continuously arrive in the dentate primordium perinatally, where they divide and contribute to peak DG cytogenesis. During early postnatal development, DG NSCs transition to a quiescent state in the SGZ until reactivated in adulthood. At each developmental stage, DG NSCs give rise only to dentate granule neurons (DGNs) and astrocytes, but not CA1 or CA3 neurons, which originate from the ammonic neuroepithelium (ANE). CH, cortical hem. (B) RG in the embryonic ventricular zone generate cells for different regions of the brain depending on their location along the ventricle—RG in the medial pallium, dorsal pallium, and subpallium give rise to cells in the septum, cortex, and striatum, respectively. During mid-embryonic development, a subpopulation of RG transition to a quiescent state and remain quiescent until reactivated in adulthood. In adulthood, quiescent NSCs (B cells) give rise to different types of olfactory bulb interneurons and oligodendrocytes depending on their location along the ventricle, which corresponds to their embryonic origin—dorsal B cells give rise to superficial granule cells (GCs), tyrosine hydroxylase (TH)-expressing periglomerular cells (PGCs) and oligodendrocytes, medial B cells give rise to calretinin (CalR)-expressing GCs and PGCs, and ventral B cells give rise to calbindin (CaB)-expressing PGCs and deep GCs. Therefore, NSCs in the V-SVZ lineage undergo a fate switch between embryonic development and adulthood.

2.1 Origins of subgranular zone adult neural stem cells

The emergence of adult RGLs in the SGZ is inextricably linked to DG development. To understand origins of adult RGLs, we must first understand DG development (Fig. 1A). The earliest DG precursors in mice emerge around embryonic day 10.5 (E10.5) in the dentate neuroepithelium (DNE). The DNE is a small region of the medial pallium flanked dorsally by the ammonic neuroepithelium (ANE), which gives rise to CA regions of the hippocampus, and ventrally by the cortical hem, a source of Wingless-related (Wnt) and bone morphogenetic protein (BMP) signaling that patterns the hippocampus and neocortex (Altman & Bayer, 1990; Bayer & Altman, 1974; Caronia-Brown, Yoshida, Gulden, Assimacopoulos, & Grove, 2014). RG in the ANE and the DNE maintain distinct progeny fate specification, only generating neurons for their respective regions of the hippocampus throughout development (Berg et al., 2019). During early embryonic brain development, precursors in the DNE divide to expand the precursor population and then begin to migrate medially to forge the dentate migratory stream (DMS) around E15.5. Unlike most RG which remain at the ventricular surface, embryonic RG in the DG detach from the ventricle and migrate in the DMS to the primitive DG where the majority of cytogenesis occurs during perinatal development (Bayer & Altman, 1974; Berg et al., 2019). The proliferative capacity of the DG NSC population changes drastically during early postnatal development (Fig. 1A). Co-localization of the proliferative marker Mcm2 with NSC markers Hopx and Nestin revealed that DG NSCs are highly proliferative through postnatal day 3 (P3), and become less proliferative starting at P7, with a rapid decline in NSC proliferation between P3 and P14 (Berg et al., 2019). Additionally, retroviral labeling in the neonatal DG showed that dividing precursors give rise to adult RGLs, in addition to DG neurons and astrocytes (Namba et al., 2005). Thus, the first postnatal week is a time of peak cytogenesis in the DG and a turning point in the proliferative capacity of DG NSCs. Multiple studies have used thymidine analog label-retaining experiments to show that most DG NSCs enter long-term quiescence during the first postnatal week, with a peak at P3 (Berg et al., 2019; Noguchi, Castillo, Nakashima, & Pleasure, 2019; Ortega-Martínez & Trejo, 2015). Though the majority of developmental cytogenesis occurs during the first postnatal week, morphogenesis and organization of DG sub-structures, such as the hilus, granule cell layer, molecular layer and SGZ, is not complete until after P14 (Bayer & Altman, 1974; Nicola, Fabel, & Kempermann, 2015).

Separate lineage-tracing studies have identified two potentially different embryonic sources of adult SGZ RGLs. The first study used a *Gli1-CreER*^{T2}

mouse line to fate-map perinatal Hedgehog (Hh)-responsive precursors located in the ventricular zone near the ventral hippocampus at E17.5. These Hh-responsive precursors migrate dorsally over the course of neonatal development to populate the entire septo-temporal axis of the DG with long-lived adult RGLs (Li, Fang, Fernandez, & Pleasure, 2013). The second study used the *Hopx-CreER*^{T2} mouse line to clonally lineage trace embryonic precursors located in the DNE at E11.5 (Berg et al., 2019). Hopx-expressing embryonic precursors divide, migrate along the DMS and arrive in the primitive DG by E18.5. Postnatally (at P8 and P30), each Hopx^{+} precursor-derived clone contained RGLs, in addition to DGNs and astrocytes, and cells within a single clone could spread along up to ~900 μm of the septotemporal axis, which is ~30% of the entire adult DG (Berg et al., 2019). Throughout embryonic and early postnatal development, RG in the dentate ventricular zone constantly divide and migrate along the DMS to the primitive DG (Nelson et al., 2020). The above two studies identify embryonic RG populations that migrate away from the dentate VZ at different times: the Hopx^{+} precursors are located at the ventricle in the dentate neuroepithelium at E11.5 but have largely re-located in the primitive DG by late embryonic development, while the Hh-responsive precursors are located at the ventricle during late embryonic development, specifically in the ventral hippocampus. It remains unclear whether adult RGLs generated by these two different populations of embryonic NSCs are functionally different. These studies highlight temporal and spatial differences in embryonic NSCs within the dentate germinal zone, which may instruct adult RGL heterogeneity. For example, the dorsal-ventral location along the ventricle or timing of migration away from the ventricle could define different subpopulations of adult RGLs. Future studies will need to examine how differences in developmental origin may impact adult RGL function, potentially generating heterogeneity within the RGL population in the adult hippocampus (Bonaguidi et al., 2016; Gebara et al., 2016).

2.2 Origins of ventricular-subventricular zone adult neural stem cells

Quiescent adult V-SVZ B cells are generated through a distinct process that occurs well before the cessation of development (Fig. 1B). Two separate studies used different label-retaining assays (BrdU or H2B-GFP) to determine that the majority of adult V-SVZ B cells enter a slow-cycling, quiescent state between E13.5 and E15.5 in mice and remain largely quiescent until at least young adulthood (P21–P28) (Fuentealba et al., 2015;

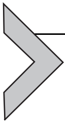
Furutachi et al., 2015). Ependymal cells, which line the walls of brain ventricles in the adult and play a critical role in cerebral spinal fluid (CSF) flow through coordinated beating of their cilia, are also generated between E13.5 and E15.5 from embryonic RG (Del Bigio, 2010; Spassky et al., 2005). Two recent studies using multiple methods of clonal lineage tracing revealed that ependymal cells and B cells share a common embryonic precursor (Ortiz-Álvarez et al., 2019; Redmond et al., 2019). A subpopulation of RG divides between E13.5 and E15.5 to give rise to differentiated ependymal cells and quiescent B cells; most B cells are generated through E-B divisions and most ependymal cells are generated through E-E divisions, though a small number of B–B cell divisions also occur (Ortiz-Álvarez et al., 2019). Ependymal cells and B cells then undergo a lengthy maturation process from E17.5 to P21, during which coordinated expansion of the ependymal cell apical domain and reduction of the B cell apical domain ultimately results in the formation of the characteristic pinwheel ultrastructure in the adult V-SVZ (Mirzadeh, Merkle, Soriano-Navarro, Garcia-Verdugo, & Alvarez-Buylla, 2008; Redmond et al., 2019). Thus, B cells originate from a distinct subpopulation of RG that become quiescent during embryogenesis and can generate ependymal cells, a cell type that is fundamental to the physical structure of the adult V-SVZ niche.

Adult B cell heterogeneity is well-defined and has been directly linked to B cell location and embryonic origin (Fig. 1B). As a population, adult B cells can generate many different subtypes of olfactory bulb interneurons, as well as oligodendrocytes, but the fate of individual B cells is restricted to certain cell types. First, neurons and oligodendrocytes are generated from different B cell populations. Live imaging of single adult V-SVZ NSCs and their progeny in vitro revealed that individual B cells generate either neurons or oligodendrocytes, but never both (Ortega et al., 2013). In addition, most B cell-derived oligodendrogenesis occurs in the dorsal V-SVZ, with only scarce oligodendrogenesis occurring in the ventral V-SVZ, suggesting that B cell fate is spatially organized (Ortega et al., 2013). Similarly, the regional location along the ventricle dictates which interneuron subtype is generated by neurogenic B cells (Merkle et al., 2014; Merkle, Mirzadeh, & Alvarez-Buylla, 2007; Ortega et al., 2013; Ventura & Goldman, 2007). For example, B cells located in the dorsal V-SVZ generate mostly superficial granule cells (GCs) and dopaminergic (TH^+) periglomerular cells (PGCs), and a small number of glutamatergic juxtaglomerular neurons, B cells in the ventral V-SVZ produce deep GCs and calbindin ($CalB^+$) PGCs, and B cells in the medial V-SVZ generate calretinin ($CalR^+$) GCs and $CalR^+$ PGCs

(Brill et al., 2009; Delgado & Lim, 2017; Lledo, Merkle, & Alvarez-Buylla, 2008). The positional identity and subsequent fate potential appear to be an intrinsic property of the B cells because region-specific V-SVZ NSC cultures can maintain their positional identity after multiple serial passages in vitro and also when heterotopically grafted (Delgado, Lu, & Lim, 2016; Merkle et al., 2007). Though it was initially thought that the adult V-SVZ arose from the lateral ganglionic eminence (LGE) region of the embryonic brain, it is now clear that other regions of the developing telencephalon contribute to the adult V-SVZ as well (Fig. 1B). Clonal lineage tracing using genetically barcoded retrovirus showed that adult B cells share a common embryonic precursor with cells from the cortex, striatum, or septum, but lose this relationship around E15.5 when RG generate quiescent B cells (Fuentealba et al., 2015). The number of clones analyzed in this study was low and future independent approaches will be needed to validate these results. Regionalization of fate specification in the embryonic brain occurs when morphogen gradients induce discrete territories of transcription factor expression within the VZ (Alvarez-Buylla, Kohwi, Nguyen, & Merkle, 2008). Some of these regions are retained in the adult V-SVZ, suggesting that adult B cells maintain a memory of their embryonic positional identity through consistent transcription factor expression, which results in NSC fate potential heterogeneity in the adult brain (Brill et al., 2008; Fuentealba et al., 2015; Merkle et al., 2014; Young, Fogarty, Kessaris, & Richardson, 2007). Recent work showed that sonic hedgehog (Shh) signaling induces a ventral identity in embryonic NSCs, which is then maintained by mixed-lineage leukemia 1 (Mll1)-dependent epigenetic mechanisms throughout development and into adulthood (Delgado et al., 2020). Thus, adult V-SVZ B cell fate is predetermined by regional patterning of embryonic precursors and is directly linked to their embryonic origin.

In summary, developmental origins of adult NSCs in the SGZ and V-SVZ are distinct. Precursors to SGZ RGLs generate DGNs and glia throughout DG development and become quiescent and transform into RGLs during the first postnatal week (Fig. 1A). RGLs continue to generate DGNs when re-activated during adulthood. SGZ RGLs are therefore generated through a process that is integrated into DG development, and DG NSCs maintain continuous fate specification from development through adulthood. In contrast, V-SVZ B cells are generated through a separate process in which a subpopulation of RG are set aside in a quiescent state embryonically, while most RG continue proliferating and contributing to brain development (Fig. 1B). Precursors to V-SVZ B cells first generate neurons

and glia for the cortex, striatum or septum before entering quiescence embryonically. Then quiescent V-SVZ B cells generate olfactory bulb interneurons and oligodendrocytes when they are reactivated in adulthood, exhibiting a different fate than their embryonic counterparts. Adult NSC heterogeneity has been directly linked to the embryonic origin of NSCs in the V-SVZ, but a potential link between origin and heterogeneity in the SGZ has not been explored. Further investigation into the developmental origin(s) of adult SGZ NSCs may reveal new insights into NSC heterogeneity and the relationship between origin and function.



3. Transformation of neural stem cell properties from development to adulthood

Transformation of developmental RG into adult NSCs is a multi-step process that begins with entry into a quiescent state. Quiescence is a common characteristic of many adult somatic stem cell populations and is critical to their maintenance and function (Urban et al., 2019; van Velthoven & Rando, 2019). In addition to entrance into quiescence, there are many other cellular properties, such as morphology, progeny fate and cell division mode, that change as NSCs transition from a developmental to adult state. We discuss how properties of NSCs in the SGZ and V-SVZ change during the transition from development to adulthood and associated molecular changes.

Morphological features, such as cell polarity, influence NSC function. Embryonic RG in the developing brain have an apical endfoot contacting the ventricle and extend a basal process that contacts the pial surface (Kriegstein & Alvarez-Buylla, 2009; Taverna, Gotz, & Huttner, 2014). The apical endfoot plays a role in signaling from the CSF, intercellular communication, and cell–cell adhesion, while the basal endfoot is critical for conveying extracellular matrix signals from the basal lamina (Taverna et al., 2014). Adult NSCs in the V-SVZ maintain contact with the ventricle through their apical processes, which cluster together in the center of ependymal cell pinwheel structures where they can access the CSF (Mirzadeh et al., 2008). However, DG NSCs lose ventricular contact early on during development when they enter the DMS (starting at ~E15.5 in mice), well before they transition to quiescence and an adult RGL state (Fig. 2) (Berg et al., 2019). The mechanisms that direct DG NSCs to detach from the ventricle and enter the DMS remain largely unexplored, but the process is reminiscent of outer radial glia (oRG) observed in higher order mammals, such as in the human cortex (Florio & Huttner, 2014; Molnár et al., 2019). Recent work in the mouse

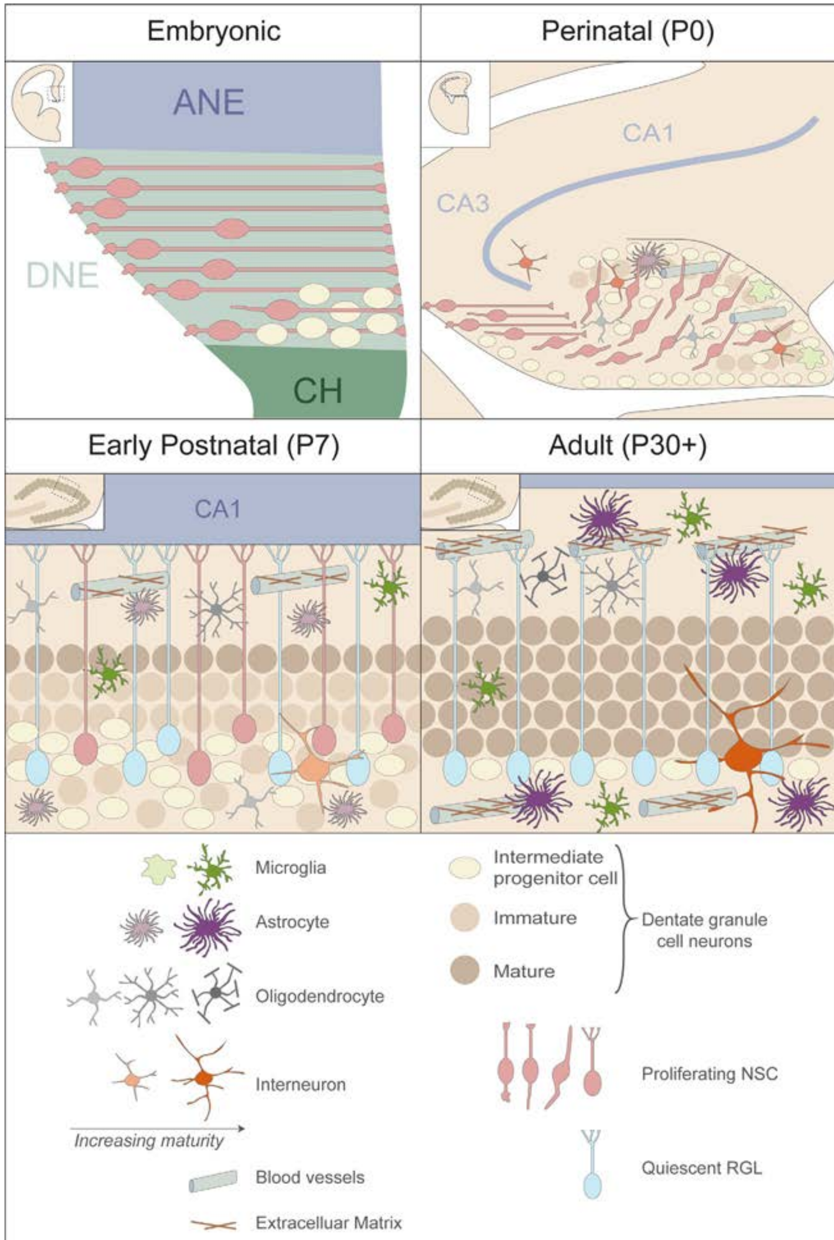


Fig. 2 Establishment of the adult neural stem cell pool and mature neurogenic niche in the dentate gyrus of the hippocampus. Embryonic dentate gyrus (DG) radial glia (RG) in the dentate neuroepithelium (DNE) divide to expand the RG population and generate intermediate progenitor cells, which serve as scaffolding for migrating RG that have

suggests that DG NSCs follow pioneering Tbr2$^+$ IPCs through the DMS and into the primitive DG and that this interaction is mediated by Notch signaling (Nelson et al., 2020). The characteristic radial morphology of adult RGLs and cell body localization in the SGZ begins to emerge between P3 and P7 and is fully present by P14, correlating with the transition into quiescence (Fig. 2) (Berg et al., 2019; Brunne et al., 2010; Nicola et al., 2015; Seki et al., 2014). In addition, while the basal processes of developmental RG reach the pial surface, the basal processes of adult NSCs in both the SGZ and V-SVZ are more likely to contact the vasculature, where they receive important contact-mediated signaling (Mirzadeh et al., 2008; Palmer, Willhoite, & Gage, 2000; Tavazoie et al., 2008).

NSC fate is guided by temporal and spatial patterning during development, creating neuronal subtype diversity in the brain. The V-SVZ B cell lineage follows this developmental principle and undergoes a fate switch from development to adulthood, initially generating neurons for the septum, cortex or striatum prior to entering quiescence and then generating olfactory bulb interneurons when adult NSCs are reactivated (Fig. 1B) (Fuentealba et al., 2015). Though olfactory bulb interneurons begin to be generated from RG around E14.5, the majority of olfactory bulb interneurons are generated perinatally (Batista-Brito, Close, Machold, & Fishell, 2008; Bayer, 1983). However, embryonic precursors to adult B cells enter quiescence between E13.5 and E15.5, suggesting that developmentally-born and adult-born olfactory bulb interneurons are generated by distinct NSC populations. The mechanism that induces a change in fate potential in the V-SVZ B cell lineage remains unknown. A recent study showed that Shh triggers the fate switch of cortical RG from generating glutamatergic pyramidal neurons to generating astrocytes, oligodendrocytes and GABAergic olfactory bulb interneurons during late embryonic development (Zhang et al., 2020). Shh ligand is

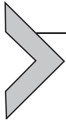
detached from the ventricle and entered the dentate migratory stream (DMS). The dentate primordium emerges during perinatal development, a time of peak cytogenesis and cell migration. NSCs continuously migrate into the dentate primordium from the DMS, peak neurogenesis occurs, astrocytes are generated, angiogenesis occurs, and microglia, oligodendrocyte precursor cells and immature interneurons migrate into the DG. The gross morphology of the DG begins to take shape during early postnatal development, as the DG NSCs transition to a quiescent state and transform into radial glia-like NSCs (RGLs). Cytogenesis declines and many cell types, including neurons and glia, begin a maturation process that extends across the first 3–4 postnatal weeks. The DG gains its adult form around P30 when most RGLs are quiescent and located in the SGZ, and other cell types have reached full maturation.

secreted from ventral sources during embryonic development, but during perinatal development migrating cortical interneurons and cells in the choroid plexus provide a dorsal source of Shh, which induces the fate switch of cortical RG (Winkler et al., 2018). Thus, it is possible that B cell olfactory bulb interneuron fate is acquired in a similar manner during late embryonic development and after B cells have entered quiescence. This is in stark contrast to DG NSCs, which exhibit continuity of fate specification, participating in developmental DGN generation embryonically before entering quiescence and continuing to generate DGNs throughout adulthood when reactivated (Fig. 1A) (Berg et al., 2019). It remains unknown whether the DGN fate is permanently induced in DG RG by morphogens during early embryonic development or if it is continuously reinforced by environmental cues throughout development. In addition, DGNs born at different times during development/adulthood have distinct morphological features, physiology, and rates of survival, suggesting that DG NSCs may generate different subtypes of DGNs at different times (Ciric, Cahill, & Snyder, 2019; Cole et al., 2020; Kerloch, Clavreul, Goron, Abrous, & Pacary, 2019; Mathews et al., 2010; Save, Baude, & Cossart, 2019; Snyder, 2019).

In addition to fate potential, other functional properties change in NSCs across time, including self-renewal capacity and cell division mode. Analysis of in vitro neurosphere cultures of DG progenitors suggested that P7 progenitors are intrinsically more proliferative and more capable of forming neurospheres than P28 progenitors (Gilley, Yang, & Kernie, 2011). Time-lapse imaging of glial fibrillary acid protein (GFAP)-expressing DG NSCs between P4 and P6 revealed largely symmetric cell division to generate either 2 NSCs or 2 IPCs (Namba et al., 2011), while clonal lineage-tracing and live in vivo imaging have revealed largely asymmetric cell division of adult RGLs (Bonaguidi et al., 2011; Pilz et al., 2018). In contrast, the cell division of adult B cell precursors is largely asymmetric to generate IPCs and then one ependymal cell and one quiescent B cell (Kriegstein & Alvarez-Buylla, 2009; Ortiz-Álvarez et al., 2019; Redmond et al., 2019), but adult V-SVZ B cells divide symmetrically, with mostly consuming divisions and only a small proportion of self-renewing divisions (Obernier et al., 2018). There are undoubtedly additional NSC property differences between developmental and adult NSCs, and understanding how these properties change over time will provide insight into mechanisms driving the transition from developmental to adult NSCs.

Many molecular changes accompany cellular property changes during the transition from developmental RGs into adult NSCs. For example, astroglial marker gene expression increases with the transformation into

adult NSCs. DG NSCs do not express the astroglial marker brain lipid binding protein (Blbp) until around P3, when it is expressed very weakly, and then expression of Blbp in NSCs rapidly increases around P7 as NSCs extend radial processes and become localized to the SGZ (Matsue et al., 2018; Nicola et al., 2015). V-SVZ NSCs concomitantly downregulate expression of RC2 (Nestin), an RG marker, and increase GFAP expression around P6, the time when ependymal cells and B cells mature (Merkle, Tramontin, García-Verdugo, & Alvarez-Buylla, 2004). Other studies have used more global methods to investigate developmental changes in NSC gene expression. An early study used microarrays to compare the molecular profiles of NSCs from P7 to P28 DG in vivo and found differential expression of genes involved in cell adhesion, lipid metabolism, glutamate transport and gap junctions (Gilley et al., 2011). More recently, RNA-sequencing (RNA-seq) studies of NSCs across developmental time points have identified a distinct molecular shift in NSCs that corresponds to the timing of entry into quiescence (Berg et al., 2019; Hochgerner, Zeisel, Lönnerberg, & Linnarsson, 2018; Yuzwa et al., 2017). For example, single-cell RNA-seq of the DG across a spectrum of ages identified a molecular shift in NSCs during early postnatal development (Hochgerner et al., 2018), and single-cell RNA-seq of cortical NSCs across embryonic development identified a molecular shift in NSCs between E11.5 and E13.5 (Yuzwa et al., 2017). Further bulk RNA-seq characterization of the molecular evolution of Hopx-expressing DG NSCs at embryonic, postnatal and adult stages suggests that changes in cell signaling and metabolism correspond to the transition from developmental NSCs to adult RGLs (Berg et al., 2019). RNA-sequencing studies have also shown that developmental RG share a core molecular identity with their adult counterparts in both the SGZ and V-SVZ, which is conserved across all stages of development and represents a defined NSC signature, including expression of genes involved in NSC maintenance and cell cycle, as well as less well-characterized genes within the neural precursor context (Berg et al., 2019; Yuzwa et al., 2017). Though molecular changes likely underlie shifts in NSC properties as developmental RG transform into adult NSCs, few studies have linked changes in the molecular landscape to changes in NSC behavior across development. Future studies will need to determine causal links between molecular mechanisms discovered by single-cell sequencing studies and NSC behavior in vivo to identify mechanisms that instruct the transition from developmental RG to adult NSC. More in depth studies that break down the transformation of developmental RG into adult NSCs into simpler sequential steps will be required to understand the mechanisms that regulate this complex process (Shin et al., 2015).



4. Establishment of adult neurogenic niches

The adult neurogenic niches are composed of both neural and non-neural cell types. These cells transduce local and long-range signals that support NSC function and modulate NSC behavior, such as the balance between quiescence and activation. Each cell type has its own developmental timeline of generation, migration and maturation, such that the adult neurogenic niche is gradually assembled over the course of development (Bjornsson, Apostolopoulou, Tian, & Temple, 2015). Here we review how the SGZ (Fig. 2) and V-SVZ (Fig. 3) neurogenic niches evolve during development and how the timing of niche development might affect NSC function and behavior.

Multiple non-neural cell types, such as choroid plexus, vascular cells and microglia, contribute to the adult neurogenic niches. The choroid plexus is a secretory tissue located in each ventricle. The choroid plexus secretes growth factors and morphogens into the CSF, which regulate the growth, proliferation and maintenance of embryonic RG and adult V-SVZ B cells that have direct contact with the CSF (Lehtinen et al., 2013). The cocktail of secreted factors changes across development and age, resulting in dynamic regulation of NSC behavior (Lehtinen et al., 2011; Silva-Vargas, Maldonado-Soto, Mizrak, Codega, & Doetsch, 2016). For example, the concentration of CSF-borne insulin-like growth factor 1 (Igf1) peaks during late embryonic development and promotes progenitor proliferation (Lehtinen et al., 2011). In addition to secreted factors, the CSF supplied by the choroid plexus provides mechanical pressure which is required for normal brain enlargement and development (Desmond & Jacobson, 1977). Vascular cells, including endothelial cells, pericytes and vascular smooth muscle cells, play a multifaceted role in the adult NSC niches, providing oxygen through blood flow and providing secreted and contact-derived neurogenic signals (Palmer et al., 2000; Shen et al., 2004). The composition of blood-borne circulatory factors changes across development due to the developmental timeline of source cell types, and the effects of blood-borne factors on the niche are mediated by the level of vascular infiltration. Angiogenesis in the brain begins around E9.5–E10, and peak endothelial cell proliferation occurs at the end of the first postnatal week (Marin-Padilla, 1985; Robertson, Du Bois, Bowman, & Goldstein, 1985). Throughout development, the neural progenitor population promotes angiogenesis and blood vessel maturation, while the vasculature promotes NSC self-renewal, proliferation and neurogenesis, creating a symbiotic relationship

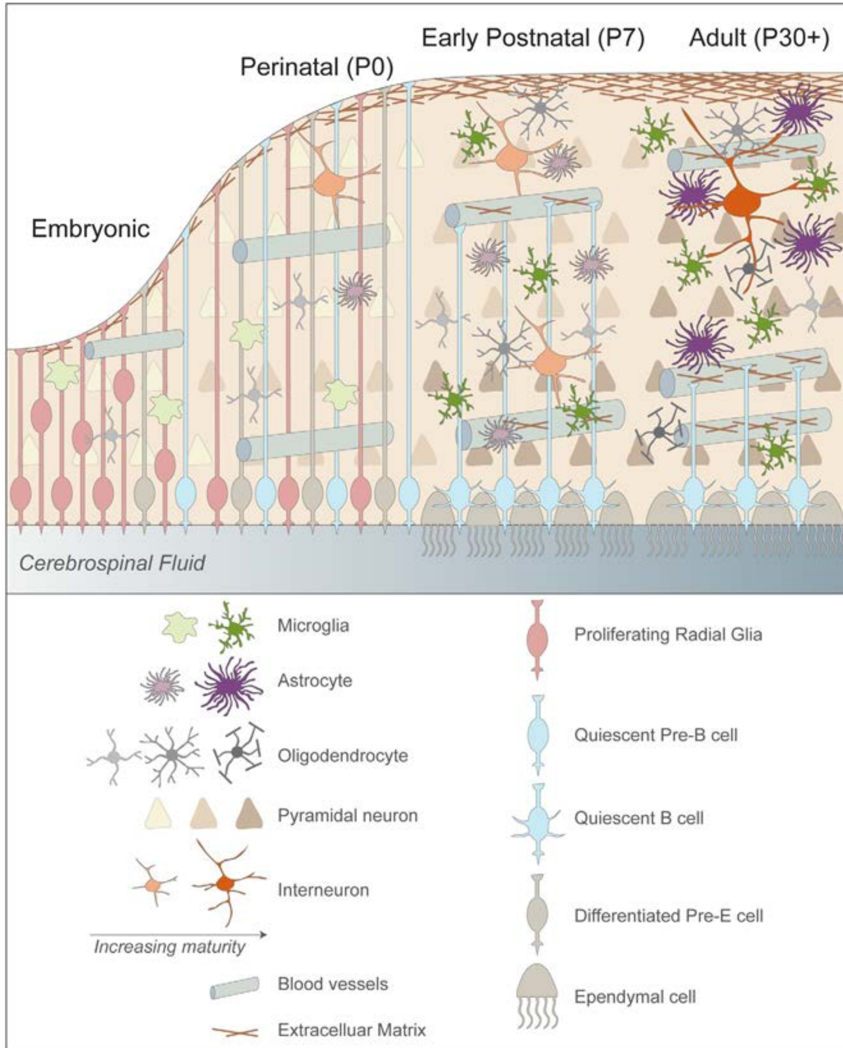


Fig. 3 Establishment of the adult neural stem cell pool and mature neurogenic niche in the ventricular-subventricular zone. Embryonic radial glia (RG) divide to expand the RG population and then to generate neurons. During mid-embryonic development, some RG divide to generate immature ependymal cells and quiescent RG. Infiltration of oligodendrocyte precursor cells and microglia and angiogenesis begin in the embryonic ventricular zone and continue throughout perinatal development. In addition, astrocyte generation and interneuron migration occur perinatally, as embryonically born neurons begin to mature. During early postnatal development, ependymal cells become multiciliated and become distinguishable from quiescent B cells, gliogenesis declines, and cellular maturation of many cell types continues. The V-SVZ gains its adult form around P30 when ependymal cells have expanded to their full size, niche cell types have completed their maturation and quiescent B cells can become reactivated to generate olfactory bulb interneurons. In addition to these stepwise changes, there are gradual changes in the composition of the cerebral spinal fluid and the accumulation of extracellular matrix over the course of development.

which lasts into adulthood (Gerhardt et al., 2004; Ma & Huang, 2015; Palmer et al., 2000). Microglia are resident macrophages which mediate immune responses in the central nervous system (Tay, Savage, Hui, Bisht, & Tremblay, 2017). Microglia begin colonizing the brain around E9.5 and undergo rapid proliferation to expand between E10.5 and P0 (Kierdorf et al., 2013). The microglial population continues to increase during the first 2 postnatal weeks, decreases by 50% from week 3 to 6, and then stabilizes to adult levels (Nikodemova et al., 2015). Microglia in the developing brain have an amoeboid morphology and play a role in supporting neurogenesis and clearing apoptotic cells, while microglia in the adult brain have a ramified morphology and play a larger role in synaptic regulation, but continue to clear apoptotic cells in neurogenic zones (Sierra et al., 2010; Tay et al., 2017). Thus, the role of non-neural cell types in regulating NSCs changes across development as these cell types are generated, migrate and mature in the NSC environment.

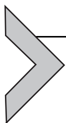
Neuronal activity contributes to the adult neurogenic niche and affects NSC behavior directly through neurotransmitter signaling and indirectly through activity-induced release of neurogenic factors from neurons (Berg, Belnoue, Song, & Simon, 2013; Jang & Song, 2008; Song, Sun, Olsen, Ming, & Song, 2015; Vicidomini, Guo, & Sahay, 2020). Adult NSCs express receptors for many neurotransmitters, including both glutamate (excitatory) and GABA (inhibitory) (Berg et al., 2013; Shin et al., 2015). Activation of $GABA_A$ receptors maintains quiescence in both SGZ RGLs and V-SVZ B cells (Giachino et al., 2014; Liu, Wang, Haydar, & Bordey, 2005; Song et al., 2012). Both adult neurogenic niches are innervated by neural inputs from local and long-range projections. For example, local parvalbumin interneurons directly regulate DG RGLs by tonic GABA activation (Song et al., 2012), and hypothalamic proopiomelanocortin neurons send projections that innervate the anterior ventral V-SVZ and promote proliferation of B cells (Paul, Chaker, & Doetsch, 2017). DGN activity induces expression of pro-neurogenic factors like brain-derived neurotrophic factor (BDNF) and fibroblast growth factor (FGF) and downregulates expression of anti-neurogenic factors like secreted frizzled-related protein 3 (sFRP3), a Wnt inhibitor (Jang et al., 2013; Ma et al., 2009; Sun et al., 2015). Neuronal regulation of adult NSCs has primarily been studied in the adult when neurons are fully mature, so it remains unknown when adult-like neuronal regulation of NSCs is established during development. Most neurogenesis occurs embryonically, as does axonal targeting, but synapse development occurs during the first 3 postnatal weeks (Reemst, Noctor, Lucassen, & Hol, 2016). Specifically, DG neurogenesis is a protracted process that peaks even later during

perinatal development (Bayer & Altman, 1974). Thus, neuronal regulation of NSCs is likely dynamic until neuronal maturation is complete during the postnatal period.

Glia also play a critical role in regulating adult NSCs through paracrine signaling (Falk & Götz, 2017; Ma, Ming, & Song, 2005; Song, Stevens, & Gage, 2002). Astrocytes provide many different secreted and contact-derived signals that regulate adult NSCs in the SGZ and V-SVZ. For example, astrocyte derived interleukins 1β and 6 (IL-1β and IL-6) and ephrin-B signaling promote neuronal differentiation in the SGZ (Ashton et al., 2012; Barkho et al., 2006), and astrocyte derived ligands of the BMP, Wnt and Notch signaling pathways regulate self-renewal and neuronal differentiation in the V-SVZ (Ferrón et al., 2011; Moreno-Estellés et al., 2012; Peretto et al., 2004). Astrogliogenesis in the brain begins in late embryonic stages (E16–E18) and occurs throughout the first 2 weeks of postnatal life (Bandeira, Lent, & Herculano-Houzel, 2009). Once born, astrocytes gradually acquire mature astrocyte functionality over the first postnatal month (Akdemir, Huang, & Deneen, 2020; Bushong, Martone, & Ellisman, 2004). Oligodendrocytes are the myelinating cells of the CNS that form a myelin sheath to facilitate rapid transmission of axon potentials and provide metabolic support to the axons. Demyelination through administration of cuprizone and rapamycin inhibits adult SGZ RGL proliferation and reduces the dendritic spine densities of adult-born DGNs through unknown molecular mechanisms (Zhang et al., 2020). To date, the role of the oligodendrocyte lineage in adult neurogenic niches appears to be indirect through modulation of neuronal circuitry, but it is also possible that the oligodendrocyte lineage provides direct paracrine signals that contribute to the niche microenvironment through yet to be discovered mechanisms. Oligodendrocytes are generated from oligodendrocyte precursor cells (OPCs), which are generated in three waves in the developing brain: (1) OPCs emerge from the MGE around E12.5, (2) soon after the first wave, OPCs are generated from the LGE, and (3) soon after birth, OPCs are generated from the cortical VZ (Bergles & Richardson, 2015; Kessaris et al., 2006). After birth, MGE-derived OPCs are eliminated and the majority of oligodendrocytes in the adult brain are those derived from the cortical VZ (third wave), with a small contribution from LGE-derived oligodendrocytes (Tripathi et al., 2011). Oligodendrogenesis and myelination are protracted and arise from cumulative processes that occur throughout postnatal development and in adulthood (Monje, 2018). Ependymal cells are specialized glia that have epithelial characteristics and line the ventricles of the brain. Ependymal cells contribute to structural integrity of ventricles and play a functional role in the

V-SVZ niche as the source of signaling factors and by beating their cilia to propel CSF. For example, Noggin, an inhibitor of BMP signaling, exerts a pro-neurogenic effect on B cells, blocking astrocyte differentiation (Lim et al., 2000), and pigment epithelium-derived factor (PEDF) promotes B cell self-renewal (Ramirez-Castillejo et al., 2006). Ependymal cells are generated from RG between E14 and E16 (Spassky et al., 2005). Although ependymal cells are generated embryonically, multiciliated ependymal cells are not detectable until birth and are not widespread throughout the lateral ventricles until the first postnatal week (Spassky et al., 2005). Mature ependymal cell characteristics, such as Foxj1 expression, loss of Vcam1 expression, expansion of the apical domain, and pinwheel ultrastructural formation, emerge between late embryonic development and the first postnatal week, suggesting that ependymal cell differentiation largely occurs during postnatal development (Redmond et al., 2019).

Many niche cell types are generated postnatally, and even those that are generated embryonically must undergo an extended maturation period, which frequently lasts weeks into postnatal development (Figs. 2 and 3). Thus, SGZ and V-SVZ neurogenic niches reach their adult form long after NSCs have entered quiescence. This is especially true for V-SVZ NSCs, which become quiescent embryonically. The offset timing of NSC quiescence and niche establishment prompts multiple questions. How is NSC quiescence maintained before the adult niche has matured? How does niche maturation affect quiescent NSC function over early postnatal development? Do quiescent NSCs go through their own maturation process once they exit cell cycle and is this maturation process affected by the developing niche environment? To address these questions, future research must focus on the interaction between niche maturation and the progression of NSC properties during late embryonic and early postnatal development.



5. Mechanisms regulating establishment of the adult neural stem cell pool

Proper establishment of the adult NSC pool requires expansion of the precursor RG population, transition to adult NSC properties and maintenance of self-renewal capacity. Most studies have investigated how manipulations during embryonic development affect the number, proliferation and morphology of adult NSCs. However, few studies have focused on more specific manipulations during the period of adult NSC transformation in the SGZ (P3–P7) and the V-SVZ (E13.5–E15.5). We summarize broad

cellular mechanisms that are likely critical for proper establishment of the adult neural stem cell pool, and give examples of molecular mechanisms that impact the development of adult NSCs. Both intrinsic and extrinsic molecular mechanisms regulate the establishment of adult NSC pools, but are often distinct for SGZ and V-SVZ NSCs.

The size of a stem cell population is regulated by its cell division dynamics. During cortical development, a period of symmetric self-renewing cell division first expands the RG population and is followed by a period of asymmetric cell division when neurogenesis begins. Altering the cell division mode or the overall rate of proliferation has long-term effects on the output of the NSC population. For example, if the cell division mode is switched to asymmetric division or differentiation prematurely, then the NSC pool will be smaller and fewer neurons will be generated. The number of adult NSCs in the SGZ and the V-SVZ decreases with age, suggesting that once the NSC pool transitions to its adult form, the NSC population loses the innate ability to expand (Encinas et al., 2011; Obernier et al., 2018). Therefore, the size of the adult NSC pool is largely dependent on the cell division dynamics of the precursor pool prior to entering quiescence. Molecular and environmental manipulations have begun to reveal mechanisms that regulate developmental NSC cell division and subsequently impact the establishment of the adult NSC pools. Shh is a morphogen critical for dorso-ventral patterning in the developing brain. Decreased Shh signaling in embryonic NSCs, through deletion of pathway components Sufu or Smoothened, impairs NSC proliferation in the developing DG, resulting in a smaller pool of quiescent adult RGLs (Han et al., 2008; Li et al., 2013; Noguchi et al., 2019). In contrast, reduced Shh signaling in embryonic cortical RG, as a result of Sufu deletion, induces expansion of the dorsal V-SVZ in the adult brain due to increased number and proliferation of B cells (Gomez et al., 2019). Surprisingly, despite increased B cell proliferation, Sufu deletion reduces the number of transit amplifying progenitor cells, resulting in lower levels of adult neurogenesis. Thus, Shh signaling is critical to the establishment of appropriate levels of adult neurogenesis in both the SGZ and V-SVZ, but affects the behavior of the two NSC pools differently. Erk1 and Erk2 are key players in the MAP kinase signaling cascade, which transduces signals from cell surface receptors to effect changes in transcription. Double knockout of Erk1 and Erk2 severely impairs DG morphogenesis due to reduced number and proliferation of RG in the DG ventricular zone at E16.5, resulting in reduced embryonic neurogenesis and near complete absence of postnatal NSCs or neurogenesis (Vithayathil, Pucilowska,

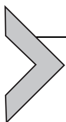
Goodnough, Atit, & Landreth, 2015). Alterations to both the maternal and early postnatal environment can impact adult NSC establishment. Modeling maternal diabetes by maternal leptin receptor haploinsufficiency ($Lepr^{db/+}$) or by maternal exposure to the circulating metabolite methylglyoxal, which is increased during maternal diabetes, results in offspring with depleted adult NSC pools in both the SGZ and V-SVZ (Yang et al., 2016). The reduced population of adult V-SVZ B cells is a result of a premature switch to neurogenesis in embryonic precursors, which stunts the expansion of the precursor pool. However, other interventions can increase the size of the adult NSC pool. Maternal infection during pregnancy results in an immediate inflammatory response, which can be modeled by maternal injection of IL-6. A single injection of IL-6 at E13.5 increases the size and proliferation of the adult V-SVZ NSC pool due to increased proliferation and self-renewal of embryonic precursors (Gallagher et al., 2013). It is unclear whether more embryonic RG transform into adult B cells or whether increased proliferation of adult B cells expands the adult NSC population. In contrast, IL-6 injection at E13.5 did not elicit a phenotype in the adult SGZ NSC pool, suggesting that some mechanisms controlling the establishment of the two adult NSC pools are time and/or niche specific.

The establishment of the V-SVZ B cell population is linked to the establishment of the ependymal cell population. The Geminin superfamily, composed of Geminin, GemC1 and Mcidas, control DNA replication and multiciliogenesis in cells throughout the body by antagonistically regulating centriole amplification—Geminin inhibits centriole amplification, while GemC1 and Mcidas promote centriole amplification (Balestrini, Cosentino, Errico, Garner, & Costanzo, 2010; McGarry & Kirschner, 1998; Pefani et al., 2011). The balance of Geminin superfamily members expressed in embryonic RG regulates the choice between ependymal vs B cell fate and can have long-term effects on the adult V-SVZ NSC pool. Overexpression of Mcidas or GemC1 in embryonic RG induces premature differentiation into ependymal cells at the expense of B cell generation (Kyrousi et al., 2015; Ortiz-Álvarez et al., 2019). In contrast, GemC1 deficiency in embryonic RG prevents the appearance of multiciliated ependymal cells during postnatal development and promotes B cell generation, increasing the number of B cells in the adult V-SVZ and the level of neurogenesis (Kyrousi et al., 2015; Lalioti et al., 2019). Similarly, overexpression of Geminin biases embryonic RG toward the B cell fate, increasing the number of RG that symmetrically divide to give rise to two B cells immediately preceding quiescence, a cell division mode which rarely occurs under endogenous conditions

(Lalioti et al., 2019; Ortiz-Álvarez et al., 2019). Multiple upstream signaling pathways impact the Geminin/GemC1/Mcidas regulation of fate choice between ependymal cells and B cells (Kyrousi, Lygerou, & Taraviras, 2017). For example, Gli3 knockout results in a mixed B cell/ependymal cell phenotype, suggesting that Shh signaling regulates proper establishment of the ependymal cell and B cell populations in the V-SVZ (Wang, Kane, Lee, & Ahn, 2014). In addition, Notch signaling prevents GemC1 and Mcidas from promoting the ependymal cell fate program in embryonic RGs (Kyrousi et al., 2015). Thus, the establishment of the adult V-SVZ NSC population is directly influenced by ependymal cell generation and is regulated by the Geminin superfamily members.

Developmental NSCs begin their transformation into adult NSCs by transitioning into a quiescent state. Although mechanisms regulating adult NSC re-entry into cell cycle from quiescence have been studied (Shin et al., 2015), mechanisms regulating developmental NSC exit from cell cycle into a quiescent state remain relatively unexplored. Adult NSCs maintain their quiescence largely due to extrinsic niche mechanisms, and disruption of these niche mechanisms often results in precocious entry into cell cycle and rapid depletion of the NSC pool (Urban et al., 2019). Because the adult niches are not fully formed or mature until after NSCs enter quiescence, it is possible that intrinsic mechanisms trigger quiescence entry during development. For example, cell cycle regulators are key regulators of adult NSC establishment. Cyclin-dependent kinase (CDK) inhibitors restrain cell cycle progression, often functioning as tumor suppressors. The CDK inhibitor p57 is required for the emergence of the quiescent B cell population in the V-SVZ. Knockout of p57 embryonically increases the proliferation of embryonic NSCs but reduces the size of the adult B cell population, resulting in lower levels of adult V-SVZ neurogenesis (Furutachi et al., 2015). In contrast, germline knockout of p21 or p27 results in increased RGL and progenitor cell proliferation in the adult SGZ, resulting in increased levels of neurogenesis (Andreu et al., 2015; Pechnick, Zonis, Wawrowsky, Pourmorady, & Chesnokova, 2008; Qiu et al., 2009). The cellular mechanism that results in increased RGL proliferation in the absence of p21 and p27 remains unclear because all three studies used germline knockout and only analyzed the adult brain. It is possible that p21 and p27 restrain the proliferation of both developmental and adult NSCs in the DG, but future studies will need to use temporally-restricted deletion of p21 and p27 to better define their role in NSC quiescence. The nuclear factor one (NFI) family of transcription factors, composed of NFIA, NFIB

and NFIX, have been implicated in activating differentiation genes and repressing self-renewal genes in the developing hippocampus. Mice lacking any one of the NFI transcription factors exhibit hippocampal malformations (Barry et al., 2008; Heng et al., 2014; Piper et al., 2010). Adult NSCs in the DG of Nfix null mice are more proliferative and have impaired radial morphology and localization to the SGZ (Heng et al., 2014; Martynoga et al., 2013). The NFI transcription factors bind to enhancer regions specific to the quiescent adult NSC state, and NFIX is required and sufficient to induce aspects of quiescence in adult NSC cultures (Martynoga et al., 2013). Thus, NFIX regulates DG NSCs across development and may play a role in the establishment of the adult RGL pool, potentially through regulating proliferation/quiescence. However, temporally-restricted inactivation of NFIX has not been explored, and NFIX may have distinct effects during different stages of DG development. DNA methyltransferase 1 (DNMT1) is an enzyme responsible for maintenance of DNA methylation during DNA replication and is highly expressed in proliferating precursors in the embryonic DG. Tamoxifen-inducible Cre-mediated deletion of Dnmt1 in embryonic NSCs using *Nestin-CreER*^{T2} transgenic mice results in a dramatically smaller DG, which becomes apparent during early postnatal development, suggesting that the DNMT1 may play a role in DG development during the critical period of NSC transformation (Noguchi et al., 2016). Conditional knockout of Dnmt1 reduced the proliferation of postnatal NSCs, resulting in fewer RGLs and impaired neurogenesis in the adult (Noguchi et al., 2016). Dnmt1 knockdown in neonatal DG-derived NSC cultures recapitulated the decreased NSC proliferation observed in vivo and linked the proliferative defect to increased levels of p21 and p57 in the absence of DNMT1 (Noguchi et al., 2016). To date, most studies have used germline knockouts or conditional embryonic deletion of genes of interest, so it is unclear whether the mechanisms described above regulate proliferating embryonic precursors, transitioning NSCs, or both. Future studies will be needed to understand how molecular factors affect NSCs at different stages of development and how manipulating these factors during development ultimately affects the adult NSC pools.



6. Concluding remarks and future perspectives

Though most adult neurogenesis research in mammals has focused on mechanisms that regulate NSC behavior in the adult brain, recent research has begun to investigate the developmental establishment of adult NSCs and

their niches. The field of adult NSC ontogeny is in its infancy, but has the potential to greatly impact our current concept of adult neurogenesis and NSC maintenance. The vast majority of embryonic NSCs do not survive beyond development, which suggests that the subpopulation of developmental NSCs that are maintained into adulthood possess a distinct capacity to be very long-lived. Understanding why some precursors transition into an adult NSC state while others do not will give us insight into mechanisms of NSC maintenance in the mammalian nervous system.

Multiple principles have already emerged from the burgeoning field of adult NSC ontogenesis. First, the adult NSC niche is established well after NSCs enter a quiescent state. Decades of work have shown that adult NSCs are extremely responsive to the surrounding niche environment, which provides both proliferation- and quiescence-inducing signals. Pro-quiescence signals dominate in the adult niches, resulting in a largely quiescent adult NSC population. Thus, one possibility was that the emergence of the adult NSC niche during development might trigger NSCs to enter quiescence, transitioning them to an adult NSC state. However, recent work in the SGZ and the V-SVZ indicates that NSCs in both compartments enter quiescence long before the adult niche is fully formed. The adult NSC niches are composed of many different cell types, including neurons, glia, and other non-neural cell types, which each have their own developmental timelines. Though most neurogenesis throughout the brain occurs embryonically, neuronal maturation and synapse development occurs largely postnatally. Gliogenesis occurs postnatally, followed by a maturation period that lasts well into the first month of life. Angiogenesis and microglial establishment continue to be dynamic throughout the first month of life. Collectively, this suggests that the adult NSC niches are not assembled in their adult form until around 1 month of age in mice, weeks after the restricted periods of development when NSCs enter long-term quiescence (E13.5–E15.5 in the V-SVZ and P3–P7 in the SGZ). Thus, intrinsic mechanisms and/or transient developmental niche mechanisms likely orchestrate the NSC transition to quiescence. However, the precise cellular and molecular mechanisms that trigger NSC entry into quiescence remain unknown and will be a major focus of future studies. It will also be interesting to explore how niche regulation of quiescent NSCs changes as niche maturation occurs across early postnatal development. NSCs in both the SGZ and V-SVZ go through an extended maturation period after they have entered quiescence, which is potentially impacted by the gradual formation of the adult neurogenic niche. Studying adult NSC establishment in parallel with adult niche formation will

likely reveal synergistic interactions that link NSC function to its changing environment and may give us insight into how early life experience can have a long-term impact on adult neurogenesis. A second principle that has emerged from the study of adult NSC ontogenesis is that the adult SGZ and V-SVZ NSC populations are established through distinct developmental processes. Two separate models have been proposed—the "Continuous Model" for SGZ NSCs (Berg et al., 2019) and the "Set-Aside Model" for V-SVZ NSCs (Fuentealba et al., 2015; Furutachi et al., 2015)—to reflect major differences between SGZ and V-SVZ NSC establishment, including the timing of quiescence and whether NSC fate changes after entering quiescence (Fig. 1). In addition, a handful of studies have suggested that different molecular mechanisms regulate the establishment of SGZ and V-SVZ NSCs, showing that some mechanisms regulate the establishment of one adult NSC population but not the other, whereas other mechanisms regulate two populations in different ways. Differences in properties of adult NSCs in the SGZ and V-SVZ may be reflective of their distinct embryonic origins and trajectories across development. Gaining a deeper understanding of SGZ and V-SVZ adult NSC ontogeny as separate developmental phenomena may also provide insight into why adult neurogenesis in the DG appears to be more conserved in humans than adult olfactory bulb neurogenesis (Kempermann et al., 2018).

We are just beginning to unravel adult NSC ontogenesis and many unanswered questions remain. What triggers NSC entry into quiescence? Does an intrinsic clock initiate quiescence within a subpopulation of NSCs at a developmentally-specified time or do transient signals from the developing environment cause NSCs to enter quiescence? How is the size of the quiescent NSC pool determined? Can the establishment of the quiescent NSC pool be influenced by environmental factors and/or experience? Future studies should aim to better define and identify developmental precursors to adult NSCs. The cellular properties and molecular profiles of precursors to adult NSCs are not well-defined and it is unclear whether precursors to adult NSCs possess an innate identity that makes them very long-lived or whether long-term maintenance is an acquired state that occurs stochastically during transient stages of development. Though we have begun to identify precursors to adult RGLs in the SGZ, the identity of precursors to adult B cells remains unknown and may turn out to be heterogeneous. Defining precursor identity will be critical to developing targeted methods to specifically manipulate developmental precursors to adult NSCs. In addition, future studies should use what is known about the timeline of adult NSC ontogenesis to design temporally-specific experiments to interrogate

mechanisms that regulate distinct developmental events, such as NSC pool expansion, generation of differentiated cell types, entry into quiescence, and quiescent NSC maturation. For example, recent work has identified the timing of NSC entry into long-term quiescence in both the SGZ and V-SVZ, but it remains unknown when the size of either adult NSC pool is established. A cyclical process of defining endogenous developmental processes, then interrogating their molecular mechanisms using cell type-specific and temporally-specific methodology, will drive forward adult NSC ontogenesis research.

Adult neurogenesis confers a unique layer of plasticity to specific regions of the mammalian brain by generating new neurons that integrate and modify the existing neural circuitry. Neurogenesis in the adult mammalian brain is wholly dependent on the long-term maintenance of a subset of embryonic NSCs, but we are just beginning to understand the developmental processes that promote NSC maintenance beyond development. Research investigating adult NSC ontogenesis will undoubtedly expand our understanding of NSC maintenance and will provide much needed insight into how developmental disorders and interventions can have long-lasting impacts on the adult NSC pool and subsequent neural plasticity in the adult mammalian brain.

Acknowledgments

We thank D.A. Berg for comments and members of the Ming and Song laboratories for discussion. This work was supported by grants from the National Institutes of Health (R35NS116843 to H.S. and R35NS097370 to G-l.M.).

References

Akdemir, E. S., Huang, A. Y., & Deneen, B. (2020). Astrocytogenesis: Where, when, and how. *F1000Res*, *9*.

Altman, J., & Bayer, S. A. (1990). Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. *The Journal of Comparative Neurology*, *301*, 325–342.

Alvarez-Buylla, A., Kohwi, M., Nguyen, T. M., & Merkle, F. T. (2008). The heterogeneity of adult neural stem cells and the emerging complexity of their niche. *Cold Spring Harbor Symposia on Quantitative Biology*, *73*, 357–365.

Andreu, Z., Khan, M. A., González-Gómez, P., Negueruela, S., Hortigüela, R., San Emeterio, J., et al. (2015). The cyclin-dependent kinase inhibitor p27 kip1 regulates radial stem cell quiescence and neurogenesis in the adult hippocampus. *Stem Cells*, *33*, 219–229.

Ashton, R. S., Conway, A., Pangarkar, C., Bergen, J., Lim, K. I., Shah, P., et al. (2012). Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. *Nature Neuroscience*, *15*, 1399–1406.

Balestrini, A., Cosentino, C., Errico, A., Garner, E., & Costanzo, V. (2010). GEMC1 is a TopBP1-interacting protein required for chromosomal DNA replication. *Nature Cell Biology*, *12*, 484–491.

Bandeira, F., Lent, R., & Herculano-Houzel, S. (2009). Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 14108–14113.

Barkho, B. Z., Song, H., Aimone, J. B., Smrt, R. D., Kuwabara, T., Nakashima, K., et al. (2006). Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. *Stem Cells and Development*, *15*, 407–421.

Barry, G., Piper, M., Lindwall, C., Moldrich, R., Mason, S., Little, E., et al. (2008). Specific glial populations regulate hippocampal morphogenesis. *The Journal of Neuroscience*, *28*, 12328–12340.

Batista-Brito, R., Close, J., Machold, R., & Fishell, G. (2008). The distinct temporal origins of olfactory bulb interneuron subtypes. *The Journal of Neuroscience*, *28*, 3966–3975.

Bayer, S. A. (1983). 3H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Experimental Brain Research*, *50*, 329–340.

Bayer, S. A., & Altman, J. (1974). Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. *The Journal of Comparative Neurology*, *158*, 55–79.

Berg, D. A., Belnoue, L., Song, H., & Simon, A. (2013). Neurotransmitter-mediated control of neurogenesis in the adult vertebrate brain. *Development*, *140*, 2548–2561.

Berg, D. A., Bond, A. M., Ming, G. L., & Song, H. (2018). Radial glial cells in the adult dentate gyrus: What are they and where do they come from? *F1000Res*, *7*, 277.

Berg, D. A., Su, Y., Jimenez-Cyrus, D., Patel, A., Huang, N., Morizet, D., et al. (2019). A common embryonic origin of stem cells drives developmental and adult neurogenesis. *Cell*, *177*. 654–668 e615.

Bergles, D. E., & Richardson, W. D. (2015). Oligodendrocyte development and plasticity. *Cold Spring Harbor Perspectives in Biology*, *8*, a020453.

Bjornsson, C. S., Apostolopoulou, M., Tian, Y., & Temple, S. (2015). It takes a village: Constructing the neurogenic niche. *Developmental Cell*, *32*, 435–446.

Bonaguidi, M. A., Stadel, R. P., Berg, D. A., Sun, J., Ming, G. L., & Song, H. (2016). Diversity of neural precursors in the adult mammalian brain. *Cold Spring Harbor Perspectives in Biology*, *8*, a018838.

Bonaguidi, M. A., Wheeler, M. A., Shapiro, J. S., Stadel, R. P., Sun, G. J., Ming, G. L., et al. (2011). In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. *Cell*, *145*, 1142–1155.

Bond, A. M., Ming, G. L., & Song, H. (2015). Adult mammalian neural stem cells and neurogenesis: Five decades later. *Cell Stem Cell*, *17*, 385–395.

Brill, M. S., Ninkovic, J., Winpenny, E., Hodge, R. D., Ozen, I., Yang, R., et al. (2009). Adult generation of glutamatergic olfactory bulb interneurons. *Nature Neuroscience*, *12*, 1524–1533.

Brill, M. S., Snapyan, M., Wohlfrom, H., Ninkovic, J., Jawerka, M., Mastick, G. S., et al. (2008). A dlx2- and pax6-dependent transcriptional code for periglomerular neuron specification in the adult olfactory bulb. *The Journal of Neuroscience*, *28*, 6439–6452.

Brunne, B., Zhao, S., Derouiche, A., Herz, J., May, P., Frotscher, M., et al. (2010). Origin, maturation, and astroglial transformation of secondary radial glial cells in the developing dentate gyrus. *Glia*, *58*, 1553–1569.

Bushong, E. A., Martone, M. E., & Ellisman, M. H. (2004). Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *International Journal of Developmental Neuroscience*, *22*, 73–86.

Caronia-Brown, G., Yoshida, M., Gulden, F., Assimacopoulos, S., & Grove, E. A. (2014). The cortical hem regulates the size and patterning of neocortex. *Development*, *141*, 2855–2865.

Ciric, T., Cahill, S. P., & Snyder, J. S. (2019). Dentate gyrus neurons that are born at the peak of development, but not before or after, die in adulthood. *Brain and Behavior: A Cognitive Neuroscience Perspective*, *9*, e01435.

Cole, J. D., Espinueva, D., Seib, D. R., Ash, A. M., Cooke, M. B., Cahill, S. P., et al. (2020). Adult-born hippocampal neurons undergo extended development and are morphologically distinct from neonatally-born neurons prolonged development of adult-born neurons. *The Journal of Neuroscience*, *40*, 5740–5756.

Del Bigio, M. R. (2010). Ependymal cells: Biology and pathology. *Acta Neuropathologica*, *119*, 55–73.

Delgado, R. N., & Lim, D. A. (2017). Maintenance of positional identity of neural progenitors in the embryonic and postnatal telencephalon. *Frontiers in Molecular Neuroscience*, *10*, 373.

Delgado, R. N., Lu, C., & Lim, D. A. (2016). Maintenance of neural stem cell regional identity in culture. *Neurogenesis (Austin)*, *3*, e1187321.

Delgado, R. N., Mansky, B., Ahanger, S. H., Lu, C., Andersen, R. E., Dou, Y., et al. (2020). Maintenance of neural stem cell positional identity by mixed-lineage leukemia 1. *Science*, *368*, 48–53.

Desmond, M. E., & Jacobson, A. G. (1977). Embryonic brain enlargement requires cerebrospinal fluid pressure. *Developmental Biology*, *57*, 188–198.

Doetsch, F., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (1997). Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *The Journal of Neuroscience*, *17*, 5046–5061.

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J. H., Tordo, J., Peterson, D. A., et al. (2011). Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. *Cell Stem Cell*, *8*, 566–579.

Falk, S., & Götz, M. (2017). Glial control of neurogenesis. *Current Opinion in Neurobiology*, *47*, 188–195.

Ferrón, S. R., Charalambous, M., Radford, E., McEwen, K., Wildner, H., Hind, E., et al. (2011). Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature*, *475*, 381–385.

Florio, M., & Huttner, W. B. (2014). Neural progenitors, neurogenesis and the evolution of the neocortex. *Development*, *141*, 2182–2194.

Fuentealba, L. C., Rompani, S. B., Parraguez, J. I., Obernier, K., Romero, R., Cepko, C. L., et al. (2015). Embryonic origin of postnatal neural stem cells. *Cell*, *161*, 1644–1655.

Furutachi, S., Miya, H., Watanabe, T., Kawai, H., Yamasaki, N., Harada, Y., et al. (2015). Slowly dividing neural progenitors are an embryonic origin of adult neural stem cells. *Nature Neuroscience*, *18*, 657–665.

Gallagher, D., Norman, A. A., Woodard, C. L., Yang, G., Gauthier-Fisher, A., Fujitani, M., et al. (2013). Transient maternal IL-6 mediates long-lasting changes in neural stem cell pools by deregulating an endogenous self-renewal pathway. *Cell Stem Cell*, *13*, 564–576.

Gebara, E., Bonaguidi, M. A., Beckervordersandforth, R., Sultan, S., Udry, F., Gijs, P. J., et al. (2016). Heterogeneity of radial glia-like cells in the adult hippocampus. *Stem Cells*, *34*, 997–1010.

Gerhardt, H., Ruhrberg, C., Abramsson, A., Fujisawa, H., Shima, D., & Betsholtz, C. (2004). Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. *Developmental Dynamics*, *231*, 503–509.

Giachino, C., Barz, M., Tchorz, J. S., Tome, M., Gassmann, M., Bischofberger, J., et al. (2014). GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. *Development*, *141*, 83–90.

Gilley, J. A., Yang, C. P., & Kernie, S. G. (2011). Developmental profiling of postnatal dentate gyrus progenitors provides evidence for dynamic cell-autonomous regulation. *Hippocampus*, *21*, 33–47.

Gomez, H. G., Noguchi, H., Castillo, J. G., Aguilar, D., Pleasure, S. J., & Yabut, O. R. (2019). Suppressor of fused regulates the proliferation of postnatal neural stem and precursor cells via a Gli3-dependent mechanism. *Biology Open*, *8*, bio039248.

Han, Y. G., Spassky, N., Romaguera-Ros, M., Garcia-Verdugo, J. M., Aguilar, A., Schneider-Maunoury, S., et al. (2008). Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nature Neuroscience*, *11*, 277–284.

Heng, Y. H., McLeay, R. C., Harvey, T. J., Smith, A. G., Barry, G., Cato, K., et al. (2014). NFIX regulates neural progenitor cell differentiation during hippocampal morphogenesis. *Cerebral Cortex*, *24*, 261–279.

Hochgerner, H., Zeisel, A., Lönnerberg, P., & Linnarsson, S. (2018). Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nature Neuroscience*, *21*, 290–299.

Jang, M. H., Bonaguidi, M. A., Kitabatake, Y., Sun, J., Song, J., Kang, E., et al. (2013). Secreted frizzled-related protein 3 regulates activity-dependent adult hippocampal neurogenesis. *Cell Stem Cell*, *12*, 215–223.

Jang, M. H., Song, H., & Ming, G.-L. (2008). Regulation of adult neurogenesis by neurotransmitters. In F. H. Gage, G. Kempermann, & H. Song (Eds.), *Adult neurogenesis* CSHL Press.

Kempermann, G., Gage, F. H., Aigner, L., Song, H., Curtis, M. A., Thuret, S., et al. (2018). Human adult neurogenesis: Evidence and remaining questions. *Cell Stem Cell*, *23*, 25–30.

Kerloch, T., Clavreul, S., Goron, A., Abrous, D. N., & Pacary, E. (2019). Dentate granule neurons generated during perinatal life display distinct morphological features compared with later-born neurons in the mouse hippocampus. *Cerebral Cortex*, *29*, 3527–3539.

Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., & Richardson, W. D. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nature Neuroscience*, *9*, 173–179.

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience*, *16*, 273–280.

Kriegstein, A., & Alvarez-Buylla, A. (2009). The glial nature of embryonic and adult neural stem cells. *Annual Review of Neuroscience*, *32*, 149–184.

Kyrousi, C., Arbi, M., Pilz, G. A., Pefani, D. E., Lalioti, M. E., Ninkovic, J., et al. (2015). Mcidas and GemC1 are key regulators for the generation of multiciliated ependymal cells in the adult neurogenic niche. *Development*, *142*, 3661–3674.

Kyrousi, C., Lygerou, Z., & Taraviras, S. (2017). How a radial glial cell decides to become a multiciliated ependymal cell. *Glia*, *65*, 1032–1042.

Lalioti, M. E., Kaplani, K., Lokka, G., Georgomanolis, T., Kyrousi, C., Dong, W., et al. (2019). GemC1 is a critical switch for neural stem cell generation in the postnatal brain. *Glia*, *67*, 2360–2373.

Lehtinen, M. K., Bjornsson, C. S., Dymecki, S. M., Gilbertson, R. J., Holtzman, D. M., & Monuki, E. S. (2013). The choroid plexus and cerebrospinal fluid: Emerging roles in development, disease, and therapy. *The Journal of Neuroscience*, *33*, 17553–17559.

Lehtinen, M. K., Zappaterra, M. W., Chen, X., Yang, Y. J., Hill, A. D., Lun, M., et al. (2011). The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron*, *69*, 893–905.

Li, G., Fang, L., Fernandez, G., & Pleasure, S. J. (2013). The ventral hippocampus is the embryonic origin for adult neural stem cells in the dentate gyrus. *Neuron*, *78*, 658–672.

Lim, D. A., Tramontin, A. D., Trevejo, J. M., Herrera, D. G., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (2000). Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron*, *28*, 713–726.

Liu, X., Wang, Q., Haydar, T. F., & Bordey, A. (2005). Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nature Neuroscience*, *8*, 1179–1187.

Lledo, P. M., Merkle, F. T., & Alvarez-Buylla, A. (2008). Origin and function of olfactory bulb interneuron diversity. *Trends in Neurosciences*, *31*, 392–400.
Ma, D. K., Bonaguidi, M. A., Ming, G. L., & Song, H. (2009). Adult neural stem cells in the mammalian central nervous system. *Cell Research*, *19*, 672–682.
Ma, S., & Huang, Z. (2015). Neural regulation of CNS angiogenesis during development. *Frontiers in Biology (Beijing)*, *10*, 61–73.
Ma, D. K., Jang, M. H., Guo, J. U., Kitabatake, Y., Chang, M. L., Pow-Anpongkul, N., et al. (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science*, *323*, 1074–1077.
Ma, D. K., Ming, G. L., & Song, H. (2005). Glial influences on neural stem cell development: Cellular niches for adult neurogenesis. *Current Opinion in Neurobiology*, *15*, 514–520.
Marin-Padilla, M. (1985). Early vascularization of the embryonic cerebral cortex: Golgi and electron microscopic studies. *The Journal of Comparative Neurology*, *241*, 237–249.
Martynoga, B., Mateo, J. L., Zhou, B., Andersen, J., Achimastou, A., Urbán, N., et al. (2013). Epigenomic enhancer annotation reveals a key role for NFIX in neural stem cell quiescence. *Genes & Development*, *27*, 1769–1786.
Mathews, E. A., Morgenstern, N. A., Piatti, V. C., Zhao, C., Jessberger, S., Schinder, A. F., et al. (2010). A distinctive layering pattern of mouse dentate granule cells is generated by developmental and adult neurogenesis. *The Journal of Comparative Neurology*, *518*, 4479–4490.
Matsue, K., Minakawa, S., Kashiwagi, T., Toda, K., Sato, T., Shioda, S., et al. (2018). Dentate granule progenitor cell properties are rapidly altered soon after birth. *Brain Structure & Function*, *223*, 357–369.
McGarry, T. J., & Kirschner, M. W. (1998). Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell*, *93*, 1043–1053.
Merkle, F. T., Fuentealba, L. C., Sanders, T. A., Magno, L., Kessaris, N., & Alvarez-Buylla, A. (2014). Adult neural stem cells in distinct microdomains generate previously unknown interneuron types. *Nature Neuroscience*, *17*, 207–214.
Merkle, F. T., Mirzadeh, Z., & Alvarez-Buylla, A. (2007). Mosaic organization of neural stem cells in the adult brain. *Science*, *317*, 381–384.
Merkle, F. T., Tramontin, A. D., García-Verdugo, J. M., & Alvarez-Buylla, A. (2004). Radial glia give rise to adult neural stem cells in the subventricular zone. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 17528–17532.
Ming, G. L., & Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. *Annual Review of Neuroscience*, *28*, 223–250.
Ming, G. L., & Song, H. (2011). Adult neurogenesis in the mammalian brain: Significant answers and significant questions. *Neuron*, *70*, 687–702.
Mirzadeh, Z., Merkle, F. T., Soriano-Navarro, M., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (2008). Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell*, *3*, 265–278.
Molnár, Z., Clowry, G. J., Šestan, N., Alzu'bi, A., Bakken, T., Hevner, R. F., et al. (2019). New insights into the development of the human cerebral cortex. *Journal of Anatomy*, *235*, 432–451.
Monje, M. (2018). Myelin plasticity and nervous system function. *Annual Review of Neuroscience*, *41*, 61–76.
Moreno-Estellés, M., González-Gómez, P., Hortigüela, R., Díaz-Moreno, M., San Emeterio, J., Carvalho, A. L., et al. (2012). Symmetric expansion of neural stem cells from the adult olfactory bulb is driven by astrocytes via WNT7A. *Stem Cells*, *30*, 2796–2809.
Namba, T., & Huttner, W. B. (2017). Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex. *Wiley Interdisciplinary Reviews: Developmental Biology*, *6*.

Namba, T., Mochizuki, H., Onodera, M., Mizuno, Y., Namiki, H., & Seki, T. (2005). The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus. *The European Journal of Neuroscience*, *22*, 1928–1941.

Namba, T., Mochizuki, H., Suzuki, R., Onodera, M., Yamaguchi, M., Namiki, H., et al. (2011). Time-lapse imaging reveals symmetric neurogenic cell division of GFAP-expressing progenitors for expansion of postnatal dentate granule neurons. *PLoS One*, *6*, e25303.

Nelson, B. R., Hodge, R. D., Daza, R. A., Tripathi, P. P., Arnold, S. J., Millen, K. J., et al. (2020). Intermediate progenitors support migration of neural stem cells into dentate gyrus outer neurogenic niches. *eLife*, *9*.

Nicola, Z., Fabel, K., & Kempermann, G. (2015). Development of the adult neurogenic niche in the hippocampus of mice. *Frontiers in Neuroanatomy*, *9*, 53.

Nikodemova, M., Kimyon, R. S., De, I., Small, A. L., Collier, L. S., & Watters, J. J. (2015). Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. *Journal of Neuroimmunology*, *278*, 280–288.

Noguchi, H., Castillo, J. G., Nakashima, K., & Pleasure, S. J. (2019). Suppressor of fused controls perinatal expansion and quiescence of future dentate adult neural stem cells. *Elife*, *8*.

Noguchi, H., Murao, N., Kimura, A., Matsuda, T., Namihira, M., & Nakashima, K. (2016). DNA methyltransferase 1 is indispensable for development of the hippocampal dentate gyrus. *The Journal of Neuroscience*, *36*, 6050–6068.

Obernier, K., & Alvarez-Buylla, A. (2019). Neural stem cells: Origin, heterogeneity and regulation in the adult mammalian brain. *Development*, *146*.

Obernier, K., Cebrian-Silla, A., Thomson, M., Parraguez, J. I., Anderson, R., Guinto, C., et al. (2018). Adult neurogenesis is sustained by symmetric self-renewal and differentiation. *Cell Stem Cell*, *22*. 221-234.e228.

Ortega, F., Gascon, S., Masserdotti, G., Deshpande, A., Simon, C., Fischer, J., et al. (2013). Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. *Nature Cell Biology*, *15*, 602–613.

Ortega-Martínez, S., & Trejo, J. L. (2015). The postnatal origin of adult neural stem cells and the effects of glucocorticoids on their genesis. *Behavioural Brain Research*, *279*, 166–176.

Ortiz-Álvarez, G., Daclin, M., Shihavuddin, A., Lansade, P., Fortoul, A., Faucourt, M., et al. (2019). Adult neural stem cells and multiciliated ependymal cells share a common lineage regulated by the geminin family members. *Neuron*, *102*. 159–172.e157.

Palmer, T. D., Willhoite, A. R., & Gage, F. H. (2000). Vascular niche for adult hippocampal neurogenesis. *The Journal of Comparative Neurology*, *425*, 479–494.

Paul, A., Chaker, Z., & Doetsch, F. (2017). Hypothalamic regulation of regionally distinct adult neural stem cells and neurogenesis. *Science*, *356*, 1383–1386.

Pechnick, R. N., Zonis, S., Wawrowsky, K., Pourmorady, J., & Chesnokova, V. (2008). p21Cip1 restricts neuronal proliferation in the subgranular zone of the dentate gyrus of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 1358–1363.

Pefani, D. E., Dimaki, M., Spella, M., Karantzelis, N., Mitsiki, E., Kyrousi, C., et al. (2011). Idas, a novel phylogenetically conserved geminin-related protein, binds to geminin and is required for cell cycle progression. *The Journal of Biological Chemistry*, *286*, 23234–23246.

Peretto, P., Dati, C., De Marchis, S., Kim, H. H., Ukhanova, M., Fasolo, A., et al. (2004). Expression of the secreted factors noggin and bone morphogenetic proteins in the subependymal layer and olfactory bulb of the adult mouse brain. *Neuroscience*, *128*, 685–696.

Pilz, G. A., Bottes, S., Betizeau, M., Jorg, D. J., Carta, S., Simons, B. D., et al. (2018). Live imaging of neurogenesis in the adult mouse hippocampus. *Science*, *359*, 658–662.

Piper, M., Barry, G., Hawkins, J., Mason, S., Lindwall, C., Little, E., et al. (2010). NFIA controls telencephalic progenitor cell differentiation through repression of the Notch effector Hes1. *The Journal of Neuroscience*, *30*, 9127–9139.

Qiu, J., Takagi, Y., Harada, J., Topalkara, K., Wang, Y., Sims, J. R., et al. (2009). p27Kip1 constrains proliferation of neural progenitor cells in adult brain under homeostatic and ischemic conditions. *Stem Cells*, *27*, 920–927.

Ramirez-Castillejo, C., Sanchez-Sanchez, F., Andreu-Agullo, C., Ferron, S. R., Aroca-Aguilar, J. D., Sanchez, P., et al. (2006). Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nature Neuroscience*, *9*, 331–339.

Redmond, S. A., Figueres-Oñate, M., Obernier, K., Nascimento, M. A., Parraguez, J. I., López-Mascaraque, L., et al. (2019). Development of ependymal and postnatal neural stem cells and their origin from a common embryonic progenitor. *Cell Reports*, *27*. 429-441.e423.

Reemst, K., Noctor, S. C., Lucassen, P. J., & Hol, E. M. (2016). The indispensable roles of microglia and astrocytes during brain development. *Frontiers in Human Neuroscience*, *10*, 566.

Robertson, P. L., Du Bois, M., Bowman, P. D., & Goldstein, G. W. (1985). Angiogenesis in developing rat brain: An in vivo and in vitro study. *Brain Research*, *355*, 219–223.

Save, L., Baude, A., & Cossart, R. (2019). Temporal embryonic origin critically determines cellular physiology in the dentate gyrus. *Cerebral Cortex*, *29*, 2639–2652.

Seki, T., Sato, T., Toda, K., Osumi, N., Imura, T., & Shioda, S. (2014). Distinctive population of Gfap-expressing neural progenitors arising around the dentate notch migrate and form the granule cell layer in the developing hippocampus. *The Journal of Comparative Neurology*, *522*, 261–283.

Shen, Q., Goderie, S. K., Jin, L., Karanth, N., Sun, Y., Abramova, N., et al. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science*, *304*, 1338–1340.

Shin, J., Berg, D. A., Zhu, Y., Shin, J. Y., Song, J., Bonaguidi, M. A., et al. (2015). Single-cell RNA-Seq with waterfall reveals molecular cascades underlying adult neurogenesis. *Cell Stem Cell*, *17*, 360–372.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*, *7*, 483–495.

Silva-Vargas, V., Maldonado-Soto, A. R., Mizrak, D., Codega, P., & Doetsch, F. (2016). Age-dependent niche signals from the choroid plexus regulate adult neural stem cells. *Cell Stem Cell*, *19*, 643–652.

Snyder, J. S. (2019). Recalibrating the relevance of adult neurogenesis. *Trends in Neurosciences*, *42*, 164–178.

Song, H., Stevens, C. F., & Gage, F. H. (2002). Astroglia induce neurogenesis from adult neural stem cells. *Nature*, *417*, 39–44.

Song, J., Sun, J., Olsen, R. H. J., Ming, G.-I., & Song, H. (2015). Neuronal circuitry mechanisms regulating adult mammalian neurogenesis. In *Cold spring harbor perspective in biology* (in press).

Song, J., Zhong, C., Bonaguidi, M. A., Sun, G. J., Hsu, D., Gu, Y., et al. (2012). Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature*, *489*, 150–154.

Spassky, N., Merkle, F. T., Flames, N., Tramontin, A. D., Garcia-Verdugo, J. M., & Alvarez-Buylla, A. (2005). Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. *The Journal of Neuroscience*, *25*, 10–18.

Sun, J., Bonaguidi, M. A., Jun, H., Guo, J. U., Sun, G. J., Will, B., et al. (2015). A septo-temporal molecular gradient of sfrp3 in the dentate gyrus differentially regulates quiescent adult hippocampal neural stem cell activation. *Molecular Brain*, *8*, 52.

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., et al. (2008). A specialized vascular niche for adult neural stem cells. *Cell Stem Cell*, *3*, 279–288.

Taverna, E., Gotz, M., & Huttner, W. B. (2014). The cell biology of neurogenesis: Toward an understanding of the development and evolution of the neocortex. *Annual Review of Cell and Developmental Biology*, *30*, 465–502.

Tay, T. L., Savage, J. C., Hui, C. W., Bisht, K., & Tremblay, M. E. (2017). Microglia across the lifespan: From origin to function in brain development, plasticity and cognition. *The Journal of Physiology*, *595*, 1929–1945.

Tripathi, R. B., Clarke, L. E., Burzomato, V., Kessaris, N., Anderson, P. N., Attwell, D., et al. (2011). Dorsally and ventrally derived oligodendrocytes have similar electrical properties but myelinate preferred tracts. *The Journal of Neuroscience*, *31*, 6809–6819.

Urban, N., Blomfield, I. M., & Guillemot, F. (2019). Quiescence of adult mammalian neural stem cells: A highly regulated rest. *Neuron*, *104*, 834–848.

van Velthoven, C. T. J., & Rando, T. A. (2019). Stem cell quiescence: Dynamism, restraint, and cellular idling. *Cell Stem Cell*, *24*, 213–225.

Ventura, R. E., & Goldman, J. E. (2007). Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. *The Journal of Neuroscience*, *27*, 4297–4302.

Vicidomini, C., Guo, N., & Sahay, A. (2020). Communication, cross talk, and signal integration in the adult hippocampal neurogenic niche. *Neuron*, *105*, 220–235.

Vithayathil, J., Pucilowska, J., Goodnough, L. H., Atit, R. P., & Landreth, G. E. (2015). Dentate gyrus development requires ERK activity to maintain progenitor population and MAPK pathway feedback regulation. *The Journal of Neuroscience*, *35*, 6836–6848.

Wang, H., Kane, A. W., Lee, C., & Ahn, S. (2014). Gli3 repressor controls cell fates and cell adhesion for proper establishment of neurogenic niche. *Cell Reports*, *8*, 1093–1104.

Winkler, C. C., Yabut, O. R., Fregoso, S. P., Gomez, H. G., Dwyer, B. E., Pleasure, S. J., et al. (2018). The dorsal wave of neocortical oligodendrogenesis begins embryonically and requires multiple sources of sonic hedgehog. *The Journal of Neuroscience*, *38*, 5237–5250.

Yang, G., Cancino, G. I., Zahr, S. K., Guskjolen, A., Voronova, A., Gallagher, D., et al. (2016). A Glo1-methylglyoxal pathway that is perturbed in maternal diabetes regulates embryonic and adult neural stem cell pools in murine offspring. *Cell Reports*, *17*, 1022–1036.

Young, K. M., Fogarty, M., Kessaris, N., & Richardson, W. D. (2007). Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. *The Journal of Neuroscience*, *27*, 8286–8296.

Yuzwa, S. A., Borrett, M. J., Innes, B. T., Voronova, A., Ketela, T., Kaplan, D. R., et al. (2017). Developmental emergence of adult neural stem cells as revealed by single-cell transcriptional profiling. *Cell Reports*, *21*, 3970–3986.

Zhang, H., Kim, Y., Ro, E. J., Ho, C., Lee, D., Trapp, B. D., et al. (2020). Hippocampal neurogenesis and neural circuit formation in a cuprizone-induced multiple sclerosis mouse model. *The Journal of Neuroscience*, *40*, 447–458.

Zhang, Y., Liu, G., Guo, T., Liang, X. G., Du, H., Yang, L., et al. (2020). Cortical neural stem cell lineage progression is regulated by extrinsic signaling molecule sonic hedgehog. *Cell Reports*, *30*. 4490-4504.e4494.