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Neural Stem Cell Regulation in Zebrafish

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Keywords

adult neurogenesis, zebrafish, radial glia, neural stem cell, Notch

Abstract

Neural stem cells (NSCs) are progenitor cell populations generating glial cells and neurons and endowed with long-lasting self-renewal and differentiation potential. While some neural progenitors (NPs) in the embryonic nervous system are also long-lived and match this definition, the term NSC classically refers to such progenitor types in the adult. With the discovery of extensive NSC populations in the adult brain of *Danio rerio* (zebrafish) and of their high neurogenic activity, including for neuronal regeneration, this model organism has become a powerful tool to characterize and mechanistically dissect NSC properties. On these bases, this article will consider NSCs in the adult zebrafish brain, with a focus on its most extensively characterized domain, the telencephalon (notably its dorsal part, the pallium). Whenever necessary, we will also refer to other brain subdivisions, embryonic processes, and the mouse adult brain, whether for comparative purposes or because more information is available in these other systems.

1. TYPES OF ZEBRAFISH ADULT NEURAL STEM CELLS AND THEIR ORIGIN

1.1. Neurogenesis Is a Continuous Process in Zebrafish

Adult neurogenesis relies on the proliferation and differentiation of neural stem cells (NSCs) that are capable of long-term self-renewal and, at minimum, bipotency (47). In zebrafish, neurogenesis is a widespread and continuous process that is initiated in the early embryo and persists throughout the lifespan (approximately 2 years, comparable to mice) (1, 53), continuing throughout juvenile stages and reaching a steady-state coincident with the onset of sexual maturity at ~3 months postfertilization (mpf) (74). Various proliferative zones have been described in the adult brain that contain progenitor populations that contribute to both constitutive neurogenesis and an increased capacity for regeneration in response to injury (1, 31, 46, 50, 53, 74) (**Figure 1**).

Adult NSCs originate from embryonic neural progenitors (NPs). During development, highly proliferative neuroepithelial cells (NECs) in the embryonic neural tube divide symmetrically, amplifying the progenitor pool before differentiating to radial glia (RG). RG in turn undergo asymmetric divisions that allow for self-renewal while also producing further committed NPs that give rise to neurons through terminal differentiation. NECs and RG that persist beyond these developmental stages and into adulthood serve as the adult NSCs, remaining proliferative, neurogenic, and self-renewing over time (32, 46, 48). Consistent with the embryonic origins of adult NSCs, adult neurogenesis largely resembles that in the embryo, as shared developmental programs are deployed in an adult context (77, 118, 126).

1.2. Neural Stem Cell Types Within the Adult Telencephalon

The adult telencephalon consists of two domains—the dorsal pallium and the ventral subpallium—both of which contain proliferative progenitors (1, 55). In the pallium, NSCs are RG that are largely quiescent and express astroglial and ependymal markers such as glial fibrillary acidic protein (Gfap), brain lipid-binding protein (Blbp), nestin, glutamine synthetase (Gs), s100 β , and Sox2 (24, 31, 35, 55, 81, 93) (**Figure 2**). These NSCs, along with further committed NPs, form a monolayer on the dorsal surface of the pallium. With lineage progression, NSCs downregulate the expression of astroglial and ependymal markers, generating NPs that undergo a limited number of divisions before delaminating from the ventricular surface, giving rise to Hu-positive neurons in the parenchyma below (1, 6, 35, 92).

By contrast, NSCs within the subpallium are highly proliferative NEC-like cells identified by the expression of the intermediate filament nestin but negative for Gfap expression (1, 90). Subpallial NECs give rise to tyrosine hydroxylase (TH)-positive neurons of the ventral telencephalon, as well as to migratory progenitors that transit tangentially alongside blood vessels through the rostral migratory stream to the olfactory bulb where they produce GABAergic and dopaminergic neurons (1, 55, 75).

1.3. Pallial Neurogenesis by Radial Glia Cells

The rest of this review focuses on studies conducted on the most extensively characterized domain within the adult zebrafish brain, the dorsal telencephalon (the pallium). Zebrafish pallial RG form neurogenic territories that are homologous to the mammalian subventricular/subependymal zone (SVZ/SEZ) of the lateral ventricle and subgranular zone (SGZ) in the dentate gyrus of the hippocampus (32, 101) and also include neocortical areas, which remain neurogenic over a lifetime (37, 79). Due to their location on the dorsal surface of the telencephalon, pallial RG are highly accessible for study both in situ using whole-mount tissues and in vivo by live imaging, during which NSCs can be monitored within their endogenous niche (6, 35, 36, 92, 126). Pallial NSC

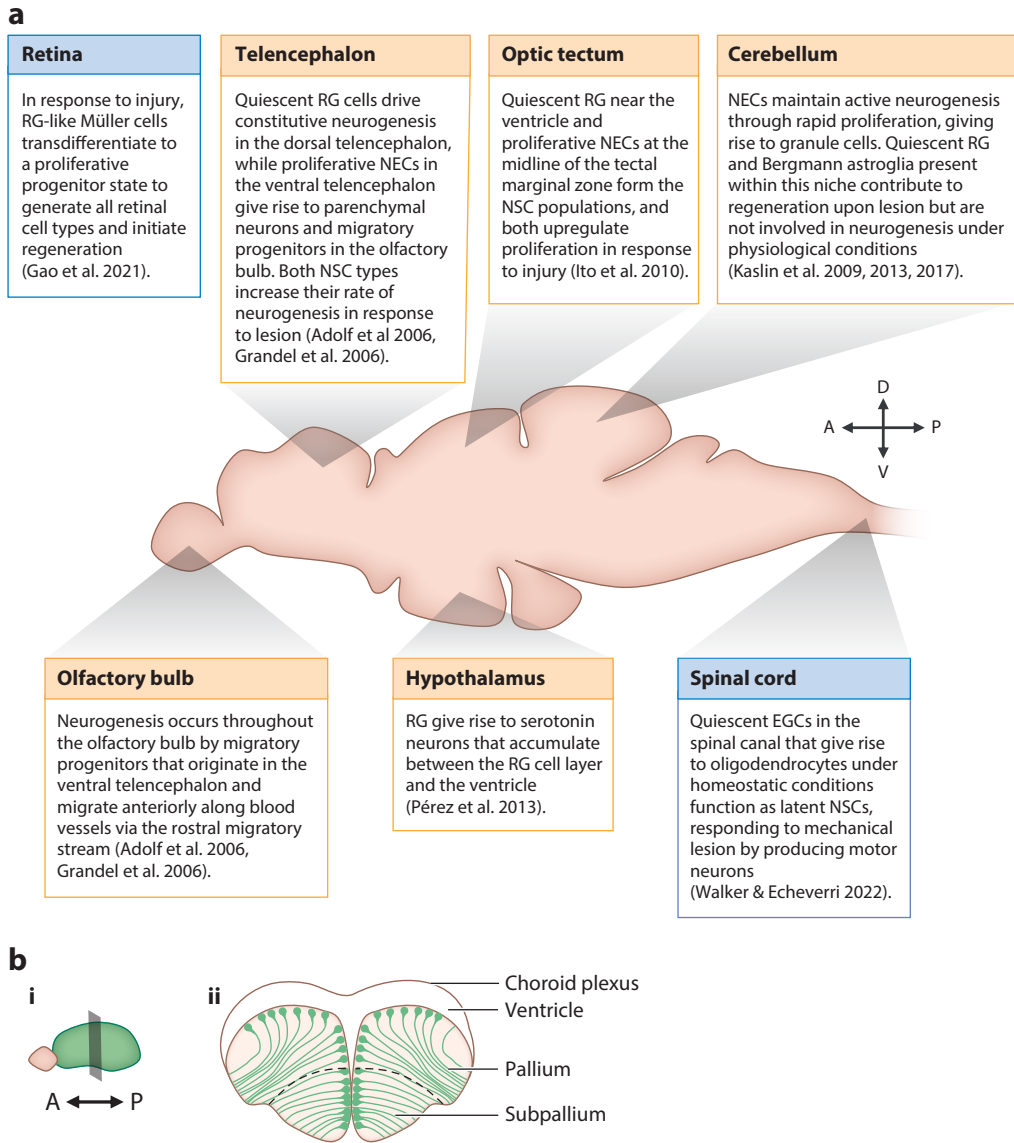


Figure 1

(a) Constitutive and regenerative neurogenic niches within the adult zebrafish. Several neurogenic niches among those identified in adult zebrafish are listed, along with the progenitor cell types found in each niche. Orange boxes indicate regions in which constitutive neurogenesis takes place under homeostatic conditions, while blue boxes highlight regions containing only latent progenitors known to reactivate in response to injury. Data from References 1, 50, 55, 65, 67–69, 111, and 131. (b) Schematic of a coronal section through the telencephalon (i) revealing two domains within this region (ii), the dorsal pallium and the ventral subpallium. The ventricle, covered by the choroid plexus, is also labeled. Abbreviations: A, anterior; D, dorsal; EGC, ependymal glial cell; NEC, neuroepithelial cell; NSC, neural stem cell; P, posterior; RG, radial glia; V, ventral.

dynamics, including division, lineage progression, and delamination, have been well studied using these approaches.

Adult RG maintain their apicobasal polarity, extending radial processes through the underlying parenchyma that terminate at blood vessels at the pial surface (81). The vast majority of pallial

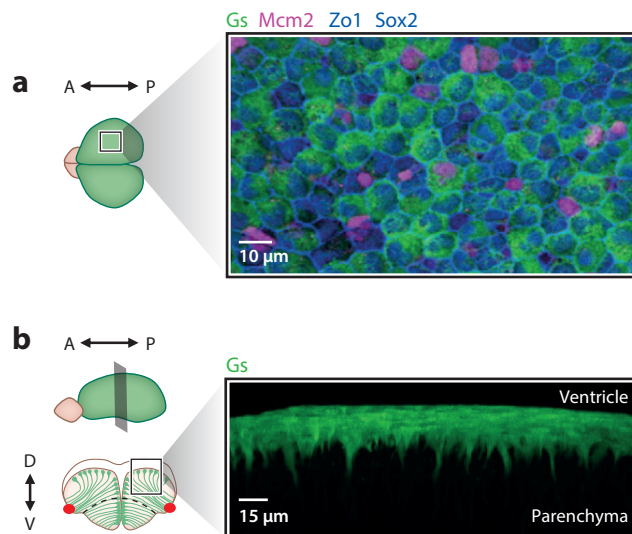


Figure 2

RG are the adult NSCs of the pallium and form a progenitor layer within the subventricular zone. (*a*) Dorsal view of RG in the pallium identified by Gs (green) and nuclear Sox2 (blue) expression. Apical cell contours are outlined by Zo1 (blue). The cell cycle marker Mcm2 (purple) marks dividing cells. (*b, left*) Schema of the adult telencephalon illustrating a cross section in which the RG and their basal processes are visible (green), which terminate at blood vessels at the pial surface (red dots). Dashed line depicts the boundary between the pallial (*top*) and subpallial (*bottom*) domains. (*Right*) A transverse section of the telencephalon by three-dimensional reconstruction following confocal microscopy highlighting the radial processes of adult NSCs labeled with Gs staining. Abbreviations: A, anterior; D, dorsal; Gs, glutamine synthetase; NSC, neural stem cell; P, posterior; RG, radial glia; V, ventral.

NSCs are maintained in quiescence, a reversible state of cell cycle arrest, with only 5% of RG estimated to be actively proliferating at any given time (4, 23, 93). While continuous proliferation by embryonic NPs is required to generate the central nervous system during development, repeated cycling by adult RG in mice is associated with NSC exhaustion and impaired neurogenesis (40, 64, 71, 83). For reasons that remain unclear, exhaustion occurs with continuous proliferation by NSCs, despite their capacity for asymmetric self-renewing divisions. Quiescence is therefore critical for maintaining the adult NSC pool over the lifespan and is initiated in zebrafish among larval RG between 7–10 days postfertilization (dpf) (4).

Within the dorsal ventricular progenitor layer, RG are interspersed among further committed Sox2⁺ nonglial NPs, akin to transit-amplifying progenitors (TAPs) in mammals (23, 35, 36, 49, 93, 117). As these cells progress along the neural lineage and transition to an NP state, they upregulate Notch ligands at the cell surface and induce Notch-mediated lateral inhibition in neighboring NSCs, thereby maintaining quiescence at the population level (23, 35, 36, 127). Eventually these NPs delaminate from the ventricular surface and migrate only 1–2 cell diameters from the proliferative ventricular zone into the underlying parenchyma, terminally differentiating to neurons and integrating into local circuits (6, 32, 35, 46, 117). Neurogenesis is a continuous process in the everted zebrafish pallium, and, in contrast to mouse, there is no radial migration of newborn neurons. Thus, the zebrafish pallium is composed of layers of neurons that are spatially ordered according to their sequence of birth, with recently born neurons closest to the NSC layer. Because the zebrafish pallium displays an everted morphology, early-born neurons are located in deeper pallial layers, while late-born neurons are located more superficially (32, 46, 117, 126).

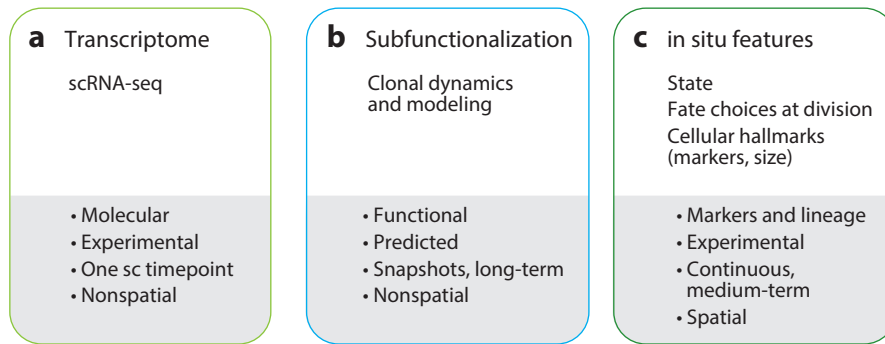


Figure 3

Single-cell heterogeneities among NSCs of the adult zebrafish pallium. Heterogeneities have been documented in (a) NSC transcriptomes, (b) fate, or (c) several features detectable by in situ analyses. These correspond to different modalities (bottom, gray background), which complicate their integration into a lineage. Abbreviations: NSC, neural stem cell; sc, single-cell; scRNA-seq, single-cell RNA sequencing.

1.4. Section 1 Take-Home Messages

Take-home messages for Section 1 include the following:

- Adult NSCs in the zebrafish pallium originate from embryonic progenitors that remain continuously neurogenic throughout the lifespan.
- Continuous neurogenesis in the adult zebrafish telencephalon provides a unique model for the study of lifelong progenitor maintenance.

2. NEURAL STEM CELL HETEROGENEITIES IN STEM CELL MAINTENANCE AND NEUROGENESIS

Recent studies reveal extensive heterogeneities between NSCs within neurogenic niches. These heterogeneities notably concern NSC morphologies, transcriptomes, capacities for self-renewal, frequency of activation, and quiescence depth, which is experimentally revealed by the propensity to reactivate upon blockade of quiescence-promoting pathways (Figure 3). A major and urgent goal today is to understand what these heterogeneities mean in terms of NSC trajectories: Do they reflect substates that individual NSCs transit through (i.e., changing over time in individual NSCs in the absence of cell division), cell hierarchies, parallel lineages, or a combination of these features?

2.1. Transcriptomic Heterogeneities

Single-cell RNA-sequencing (scRNA-seq) was used to characterize transcriptomic heterogeneities among adult pallial NSCs (25, 82, 98, 101). RG were identified postprofiling based on their expression of RG/astroglia marker genes such as *fatty acid binding protein 7* (*fabp7* or *blbp*) and *glutamine synthetase* (*glula* or *gs*) and progenitor marker genes such as *hairy-related 4* (*her4*). The studies profiling the highest numbers of RG (25, 98, 101) could distinguish quiescent from proliferating RG and several putative clusters among quiescent RG. The difficulty comes when trying to identify the biological meaning of the clusters, in particular because the RG population is maintained in homeostasis and with preserved neurogenic potential long-term in the adult zebrafish telencephalon, such that different ages cannot serve as longitudinal time points. While the first study (25) largely interpreted quiescent clusters as being regional (i.e., enriched in RG located in different pallial or subpallial ventricular domains), our recent work suggests that

clustering is additionally driven by properties such as RG position along the neurogenic lineage and/or quiescence duration or depth (101). Indeed, at least in the dorsal pallium, whole-mount in situ hybridization reveals a scattered expression of cluster gene markers within distinct pallial territories, in addition to broad expression domains or gradients. Further, some clusters differ in their transcriptomic proximity to RG-like cells (NSCs) versus astrocytes of the adult mouse SEZ and SGZ niches, suggesting different activation potential or frequency and/or capacity for neurogenesis. Direct cell tracing in situ is needed to resolve these questions and understand NSC trajectories within the transcriptomic space, including whether and which transitions between clusters do take place within the life of an NSC and which of them involve a division.

2.2. Heterogeneities in Fate

Insight into NSC heterogeneities can also be gained using long-term clonal tracing following Cre-lox-mediated genetic labeling and modeling clonal dynamics. In the adult zebrafish pallium, such analyses were conducted in the dorsal pallium with full (whole-mount) clonal characterization and very long chase times (>500 days) (126). This revealed the existence of two embedded dynamics, interpreted to reflect two hierarchically organized NSC pools: an upstream reservoir and a downstream operational pool. Reservoir NSCs divide asymmetrically to self-renew and concomitantly populate the operational pool, while operational NSCs are involved in neurogenesis. The modeling further predicts that operational NSCs stochastically choose between three division modes at any time (symmetric amplifying, symmetric differentiative, or asymmetric), although overall the dynamics of the pool is fated towards neurogenesis. Finally, NSCs are also predicted to differ in their quiescence duration, being on average much longer for reservoir than for operational NSCs. Together, this work predicted that the NSC population and adult neurogenesis can cohabit in equilibrium via an NSC hierarchy. Because this study did not use any subpopulation markers, however, it cannot be directly related to the scRNA-seq data, nor to a directly tractable behavior in situ.

2.3. Heterogeneities Revealed by Intravital Imaging

Finally, pallial NSCs also appear to differ in several cellular hallmarks that can be revealed in situ, such as the nuclear localization of microRNA-9 and argonaute-2 (70), lysosomal content (80), apical size and shape (92), and likely many other features. The spatial distribution of each of these parameters among NSCs harboring these different features are distributed in a scattered manner within the population. These parameters broadly correlate with the quiescent versus activated NSC states, but finer analyses reveal heterogeneities between quiescent NSCs themselves. As a first step to make sense of these heterogeneities in the context of neurogenesis, we recently used longitudinal intravital imaging with a focus on the molecular marker *deltaA*:GFP to assemble a predominant NSC lineage spanning months of an NSC life in situ (92). This revealed that *deltaA*-negative (*deltaA^{neg}*) NSCs are hierarchically upstream of *deltaA*-positive (*deltaA^{pos}*) NSCs, which they generate through a self-renewing and molecularly asymmetric division; *deltaA^{pos}* NSCs are then bound to neurogenesis, although this process can take weeks to months and several divisions. These behaviors correlate with additional features, *deltaA^{neg}* NSCs being generally of larger apical area and of longer quiescence than their *deltaA^{neg}* counterparts. These observations are very reminiscent of the hierarchy predicted when modeling clonal dynamics.

This new understanding of the NSC lineage, where in situ parameters and markers are embedded with quiescence duration, cell division, and fate, now offers an invaluable canvas on which to progressively position additional heterogeneous features to better understand the neurogenesis and NSC maintenance processes. These will urgently need to include, in addition to *deltaA* expression, the detailed heterogeneities of the scRNA-seq transcriptomic space. Together, this strongly

highlights the power of zebrafish to integrate multimodal information to unravel the regulation of adult neurogenesis in situ. Another major output will be to clarify the compared functional and lineage properties between adult NSCs in zebrafish and mouse. Along these lines, a striking observation is that the major cluster enriched in *delta*^{Δ^{neg}} NSCs in scRNA-seq is also transcriptomically closer to adult mouse astrocytes than it is to adult mouse RG-like NSCs (101). This could mean that the zebrafish adult pallial niche includes an upstream self-renewing NSC subpopulation that lost its neurogenic potential under physiological conditions in the adult mouse brain.

2.4. Section 2 Take-Home Messages

Take-home messages for Section 2 include the following:

- Heterogeneities between NSCs have been revealed at many levels, even within the same pallial niche.
- Intravital imaging offers a possibility to integrate the different heterogeneity levels.
- Many of these heterogeneities correspond to transient steps in the life of NSCs and are therefore dynamic.
- Many of these heterogeneities indicate a progressive and oriented neurogenesis process.
- Lineage progression correlates with decreased quiescence duration/depth and smaller apical size.

3. MOLECULAR CONTROL OF NEURAL STEM CELL PROPERTIES AT THE SINGLE-CELL LEVEL

The preservation of adult NSCs relies on multiple fate decisions along the neurogenic lineage, which includes the possibility of NSCs to enter a proliferative state as well as the choice of cell division modes. A combination of cell-autonomous mechanisms and extrinsic cues therefore orchestrates the regulation of adult NSC states and fates to ensure their long-term maintenance.

3.1. Regulation of Neural Stem Cell Quiescence in the Adult Zebrafish Brain

The vast majority of adult NSCs are found in quiescence and divide only rarely (79, 129). A variety of internal and external regulatory processes are involved in the control of NSC quiescence. Several reviews have summarized important findings on the topic (29, 79), and while we mention key factors at play in the adult zebrafish pallium, we highlight here the importance of Notch-mediated regulations (Figure 4).

3.1.1. Notch signaling is a master regulator of neural stem cell quiescence. The Notch signaling pathway is essential for both quiescent and proliferative states of NSCs (62, 64). It is classically initiated between neighboring cells that express transmembrane Notch receptors (Notch1–Notch3 in the zebrafish brain) and ligands (Delta and Jagged). Upon signaling activation, and following several cleavage steps, the Notch intracellular domain (NICD) transits to the nucleus, where it forms a transcriptional complex with Rbpj and triggers the transcription of effector genes, including members of the *Hes/her* (zebrafish ortholog) and *hey* families. *Hes/Her* factors repress the expression of proneural genes, such as *Achaete-scute complex-like 1 (Ascl1)*, thereby inhibiting neuronal differentiation and maintaining NP cells (63). *notch* receptor genes and *her* genes are highly expressed in NSCs and NPs of the adult zebrafish telencephalon (23, 24). In particular, both quiescent and activated NSCs express *notch3*, while *notch1b* expression is restricted to activated NSCs (23, 93). Multiple lines of evidence demonstrate that Notch signaling promotes adult NSC quiescence (79, 129). Pharmacological blockade of the Notch signaling pathway as well as selective abrogation of Notch3 by mutation or morpholino results in the activation of NSCs and

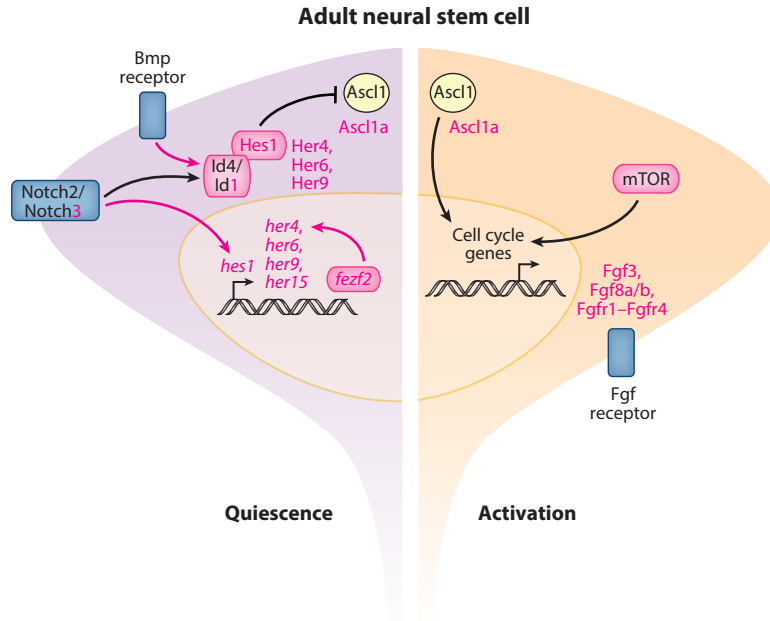


Figure 4

Factors regulating NSC quiescence and activation in the adult zebrafish pallium. The schema summarizes the main pathways controlling adult NSC quiescence (*left, purple*) and activation (*right, orange*) characterized in the zebrafish (*pink*) and mentioned in this review. Abbreviations: Ascl, achaete-scute like; BMP, bone morphogenetic protein; *fezf2*, *fez* family zinc finger 2; Fgf, fibroblast growth factor; Fgfr, fibroblast growth factor receptor; Her, hairy-related; Hes, hairy/enhancer of split; Id, inhibitor of DNA binding; mTOR, mammalian target of rapamycin; NSC, neural stem cell.

the expansion of the stem cell pool (4, 23, 72). By contrast, loss of Notch1 in the adult impairs NSC self-renewal and leads to their neuronal differentiation (4). Consequences of a long-term blockade of Notch3 on the NSC population has, however, not been investigated so far. Expression of *notch2* was not found in NSCs of the zebrafish pallium but is enriched in adult NSCs of the mouse brain, where it also promotes quiescence (41, 134).

3.1.2. Other intrinsic and extrinsic mechanisms regulating adult neural stem cell quiescence. In the adult zebrafish brain, several regulators of NSC quiescence have been unraveled in addition to Notch signaling. Intrinsic mechanisms include the expression of specific transcription factors, epigenetics, and metabolism, while interactions with soluble factors, the extracellular matrix, and other cells provide extrinsic signals from the niche. Among known modulators, the transcription factor Fez family zinc finger 2 (*Fezf2*) is highly expressed in quiescent NSCs and drives *her4.1* expression. Constitutive and conditional knockdowns demonstrated its requirement for maintaining adult NSC quiescence (11). Besides Notch signaling, bone morphogenetic protein (Bmp) also acts as a major quiescence-promoting pathway, and its role was mainly demonstrated through the modulation of its downstream target gene inhibitor of DNA-binding/differentiation (*id1*). *id1* is mostly expressed in quiescent NSCs, and its expression is upregulated in a subset of NSCs in the ventricular zone upon telencephalic injury, which prevents their depletion (28, 30, 116). Gain- and loss-of-function experiments argue for a role of *id1* in promoting NSC quiescence (132, 133). By contrast, fibroblast growth factor (Fgf) signaling exerts a proactivating function in NSCs. Expression of Fgf pathway members, in particular *fgf3*, *fgf8a/fgf8b*, and *fgfr1-fgfr4*, has been

detected in the adult telencephalon, albeit with heterogeneities between telencephalic domains (49, 128). Blocking Fgf signaling through the expression of a dominant negative form of Fgfr1 reduces NSC proliferation in the subpallium, while overexpression of Fgf8a results in increased NSC activation (49). Finally, studies in several stem cell systems have highlighted the importance of mammalian target of rapamycin (mTOR) signaling in the regulation of self-renewal, proliferation, and differentiation (96). In mice, quiescent NSCs maintain low mTOR complex 1 (mTORC1) activity by expressing Mfge8a (135). Increased mTOR signaling also inhibits autophagy in activated NSCs (21). Presently, little is known about the function of mTOR in the regulation of adult NSCs in zebrafish, and further studies will be required to reveal what the mTOR requirements are for the regulation of specific neural stem and progenitor substates.

3.1.3. Notch effectors dictate adult neural stem cell quiescence and proliferation. The dual role of Notch to promote both proliferation and quiescence is enabled by the levels and dynamic expression of its effector protein Hes1, mostly studied in mouse (89, 124). In mouse embryonic NPs, the activation or inhibition of the Notch signaling pathway determines whether a progenitor proliferates or differentiates, respectively. It has been shown that *Hes1* expression autonomously oscillates due to negative autoregulation (59, 84). These oscillations periodically repress the proneural gene *Ascl1*, thereby driving *Ascl1* oscillations and inducing NP proliferation. Conversely, Notch inhibition results in a downregulation of Hes1 expression, leading to sustained expression of *Ascl1* and the differentiation of NPs (61). In adult mouse NSCs, Hes1 expression dynamics also differentially controls NSC proliferation: While oscillatory Hes1 expression stimulates adult NSC proliferation, for NSCs in the embryo, high and sustained Hes1 expression induces quiescence (124). As a consequence of repression by oscillating Hes proteins downstream of Notch signaling, the levels of *Ascl1* transcript and protein also oscillate every 2–3 h in NPs. It has been demonstrated that forced oscillatory *Ascl1* expression is sufficient to promote NSC proliferation, whereas sustained *Ascl1* expression induces cell cycle exit and neuronal differentiation (61, 124). Corroborating these gain-of-function experiments, loss of *Ascl1* in adult mouse NSCs in vivo prevents proliferation and differentiation, demonstrating that *Ascl1* is required for the activation of quiescent NSCs (5). In addition, the return to quiescence of adult hippocampal NSCs after division is promoted by the proteasomal degradation of *Ascl1* protein by the ubiquitin ligase HECT, UBA, and WWE domain-containing 1 (Huwe1) (130).

The genes that control adult NSC state downstream of Hes1 and *Ascl1* remain to be determined. In mouse embryonic NSCs, transcriptional targets of Hes1 and *Ascl1* include cell cycle regulators (22, 89). Hes1 oscillations downregulate, while Hes1 stability increases, the expression of the cyclin-dependent kinase inhibitor p21, thereby respectively promoting or blocking proliferation (89). In addition, genes involved in cell cycle progression, such as *cdk1* and *e2f1*, can be upregulated by *Ascl1* (22). The mechanisms involved in this selection of proliferation-promoting target genes by *Ascl1* remain unclear. It has been hypothesized that this selection is permitted by the presence of active Notch signaling in proliferating progenitors in contrast to differentiating cells where the Notch pathway is inactive. Such mechanisms might also be involved in the regulation of adult NSCs and require further investigation.

In the adult zebrafish pallium, similar mechanisms might control NSC quiescence, with a potential interaction between Bmp and Notch signaling pathways. *Id* genes are expressed in the adult zebrafish brain, with *id1* found mostly in quiescent NSCs of the ventricular zone of the telencephalon (27). Bmp signaling, by inducing *Id1* expression in adult NSCs, promotes quiescence (128). *Id* proteins have also been shown to interact with Her proteins (Her4.1, Her4.5, Her6, and Her9) in vitro (110). In addition, Her4 is expressed in NSCs and interacts with Notch3 to drive quiescence (122). Studies have demonstrated that *Id1* might stabilize Hes1, leading to high

expression levels, thereby maintaining NSCs in quiescence in the mouse adult brain (125). Id4 downstream of Notch2 also promotes quiescence of mouse hippocampal NSCs (15, 125, 134). The functional cooperation of Id1 and Her has not yet been demonstrated in zebrafish.

3.1.4. The link between quiescence and stemness. Quiescence is not an indispensable property of adult stem cell systems, as observed in the skin and the gut where stem cell pools do not become depleted despite remaining continuously proliferative without quiescence under physiological conditions (3, 7, 12). In the brain, however, quiescence seems tightly linked to stemness, as regulatory mechanisms for these properties, such as the Notch signaling pathway, are shared. In addition, recent evidence suggests that NSC quiescence heterogeneities might reflect differences in stemness potential (T. Foley, unpublished data). Elucidating the molecular mechanisms underlying the relationship between quiescence and stemness (or proliferation and lineage progression) in NSCs, and how these mechanisms differ relative to other stem cell systems, will be fundamental to our understanding of NSC regulation.

3.2. Regulation of Adult Neural Stem Cell Lineage Progression in the Zebrafish Brain

Lineage progression among NSCs is regulated by a number of factors that act at both the single-cell and population levels. Through the integration of these signals, cells determine whether to remain self-renewing and long-lived within the progenitor pool or to progress toward terminal differentiation. This decision is often coupled with proliferation, yet the mechanisms underlying this relationship remain poorly described. In this section, recent work investigating both cell-autonomous regulatory mechanisms and cell–cell signaling pathways involved in coordinating lineage progression across the NSC population are described.

3.2.1. Neural stem cell division modes. Retroviral infection, genetic lineage tracing, and intravital live-cell imaging have demonstrated the potential of some pallial RG to undergo self-renewing divisions and to generate neurons at the single-cell level (6, 36, 92, 117, 126). In particular, these studies revealed the presence of a population of deeply quiescent NSCs that are capable of long-term self-renewal. These NSCs divide asymmetrically and give rise to a more active population of NSCs, the division mode of which is variable, including asymmetric and symmetric differentiative divisions (92, 126). In mice, most studies have explored NSC modes of division by addressing terminal fates, that is, the division outcomes in relation to progenitor versus neuronal daughter fates. Genetic lineage tracing and *ex vivo* time-lapse imaging of adult mice ventricular–subventricular zone (V-SVZ) NSCs suggest that asymmetric divisions at the individual NSC level are rare. Instead, NSCs choose stochastically between symmetric proliferative or differentiative divisions. While some symmetrically self-renewing NSCs can remain in the V-SVZ for months, the vast majority of NSCs undergo consuming symmetric divisions. Therefore, NSC maintenance was proposed to instead be regulated at the population level through population asymmetry. Whether it relies on the existence of different NSC subtypes or transient changes in NSC behavior remains to be addressed (8, 104). In the adult mouse hippocampus, intravital imaging of NSCs and their progeny indicates that the division modes of adult NSCs are similar to that of embryonic NSCs. Adult hippocampal NSCs switch division modes from initial symmetric self-renewing divisions to asymmetric and symmetric differentiative divisions (17, 112). Finally, direct conversion of NSCs into neurons was also reported to participate in neurogenesis in the zebrafish pallium (6, 35, 98, 126). scRNA-seq and RNA metabolic labeling analyses of the adult zebrafish telencephalon predict that direct NSC differentiation follows a separate trajectory and involves different adult NSC states compared to NSC proliferative differentiation (98), although this remains to be experimentally validated.

Even though we gained some insight into the division modes used by adult NSCs, little is known about the mechanisms at play and whether and how they differ from embryonic NSCs. Studies in the developing brain have highlighted the importance of regulation by signals from the stem cell niche and the asymmetric distribution of cell fate determinants. In particular, the Notch signaling pathway governs the specification, proliferation, and differentiation of embryonic NPs (18, 57, 119). In zebrafish embryos, Notch1 is required for the maintenance of proliferating NPs, which involves Notch-mediated lateral inhibition (4, 23, 127). Progenitors committed to differentiation express high levels of Notch ligands, delivering strong Notch signals to the surrounding progenitors. Active Notch signaling in these cells represses the expression of proneural genes and Notch ligands themselves, preventing neuronal differentiation. Mutual lateral inhibition between neighboring progenitors thus drives an intercellular feedback loop. When RG divide, Notch-mediated binary cell fate decisions allow sister cells to adopt different fates, a process referred to as directional intralinear Notch signaling (33). It remains poorly understood how the NSC division mode is regulated in the adult brain. Similar to that in embryonic NPs, the segregation of apicobasal domains, as well as segregation of signaling components, participates in the regulation of adult NSC fate behavior in mouse. Time-lapse imaging of NSCs in mouse SEZ slices showed that the inheritance of the basal process upon division represents a fate determinant (104). The asymmetric segregation of Delta1 has also been observed in SEZ NSCs *in vitro* and is associated with neuronal fate (71). Finally, the kinase Dyrk1A also biases daughter cell fate *in vitro* by stabilizing the epidermal growth factor receptor (EGFR) and Notch signaling (43). There is no functional information on the NSC division mode *in vivo* in the zebrafish adult brain so far. However, it is notable that the asymmetric divisions of *deltaA^{neg}* NSCs are a systematic behavior (92), suggesting that it may be controlled in a cell-autonomous manner.

3.2.2. Proneural factors in lineage progression. The transcription factor Ascl1 is a key regulator of neural fate decisions, including NSC proliferation and cell cycle exit, as well as neural specification in the embryonic and adult nervous system (13, 22, 56, 102). In mice, Ascl1 is expressed at low levels in some activated adult NSCs and at high levels in intermediate progenitor cells (5, 73, 109). Genetic lineage tracing revealed that many Ascl1:Cre-targeted progenitors are short-term self-renewing and biased toward neurogenic fate in the adult mouse hippocampus (73, 112). Inactivation of the Notch signaling pathway induces Ascl1 expression, resulting in NSC activation and a transient burst of neurogenesis before NSC depletion (5, 124). Conversely, the repression of Ascl1 maintains quiescent NSCs and inhibits neurogenesis (124). In addition to the transcriptional regulation of Ascl1, posttranslational modifications emerge as major regulators of its function. Ascl1 protein is regulated by cyclin-dependent multisite phosphorylation that restrains its ability to drive differentiation (2). The signaling cues driving Ascl1 phosphorylation status remain, however, to be characterized. In addition, Ascl1 has a short half-life and can be targeted for degradation by ubiquitin-mediated proteolysis in both embryonic and adult NSCs (53, 56, 58, 130). In zebrafish, *ascl1a* is expressed in some activated NSCs and progenitors (L. Bally-Cuif laboratory, unpublished data) and might play similar roles as in mice. However, the study of such mechanisms is limited by the availability of working Ascl1 antibodies in zebrafish as well as by our lack of understanding of the progressive neurogenic process. For instance, the identity and diversity of neurons in general (98, 101, 107) and newborn neurons in particular in the adult zebrafish telencephalon are just starting to be unraveled. Both GABAergic interneurons and aminergic neurons were previously described in the population of newborn neurons of the telencephalon (1, 55). More recently, the prospective isolation and scRNA-seq after short-term lineage tracing of RG progeny revealed the cellular identity of some adult newborn and mature neurons in the zebrafish telencephalon. Notably, RG from the *her4.1^{pos}* lineage constitutively generate glutamatergic neurons in the zebrafish pallium (82).

3.2.3. Signal integration by neural stem cells. NSCs receive and integrate multiple signals to determine the appropriate decision. How these signals interact and how signal dynamics influences NSC fate choices are poorly understood. In the future, exploring how these cues are integrated in time and space will be of major interest for the field.

3.3. Section 3 Take-Home Messages

Take-home messages for Section 3 include the following:

- By preventing premature differentiation and exit from quiescence, Notch signaling maintains the NSC population during adult neurogenesis; at least in mice, it interplays with proneural factors such as *Ascl1* in both processes.
- In adult NSCs, quiescence and stemness (or proliferation and lineage progression) are linked; this is observed in some, but not all, stem cell systems.
- Results from both mice and zebrafish highlight hierarchies among adult NSCs, with an upstream subpopulation of more quiescent and self-renewing NSCs and a downstream pool of more cycling NSCs and progenitors.
- Lineage progression involves NSC divisions of different modes (symmetric or asymmetric), the control of which in vivo remains poorly understood.

4. REGULATION OF NEURAL STEM CELL BEHAVIOR AT THE POPULATION LEVEL

NSCs of the adult dorsal pallium form a monolayered population of intermingled states and fates, which reflect asynchronous individual NSC trajectories. This population is maintained in a dynamic steady state (maintaining itself while producing neurons), although individual NSCs at any time can make different decisions. The fact that, in numbers, these fate decisions equilibrate NSC gains and losses (126) strongly suggests the existence of feedback mechanisms whereby one NSC state or decision influences others. In addition, the NSC population ensures the production of neurons in a spatially controlled manner along the mediolateral and anteroposterior pallial axes over time (32, 46). This is important for the regular growth of the pallium and likely also for its functionality: In the zebrafish pallium, the final position of a neuron directly depends on the position of its mother NSC (46) and therefore on the spatial distribution of neurogenic, and then terminally committed, NSCs within the population. Thus, it is also likely that spatial coordination operates at the population level to position individual NSC states relative to each other at any time.

These observations together lead us to propose that stemness, defined as long-term NSC self-renewal and neuronal generation for a functional pallium, is a population property controlled in both time and space via the spatiotemporal coordination of individual NSC trajectories generating an intrinsic niche (37). Thus, to understand stemness, we need to understand the population or emergent properties that control cell numbers (population size), cell potential (capacity to activate, maintenance of neurogenesis), and the spatial distribution of cell states.

4.1. Interactions Predicted by Molecules and Morphology

One striking feature of NSCs is their elaborate apicobasally polarized morphology (**Figure 5**). Radially, each NSC extends a primary cilium into the cerebrospinal fluid and a long basal process across the pallial parenchyma, contacting neurons and blood vessels. These connections presumably transmit systemic information to each NSC. In addition, along the ventricular plane, each NSC is tightly connected to its apical neighbors via direct cytoplasmic connections (inferred from the expression of connexins) and tight junctions and extends horizontal processes that run

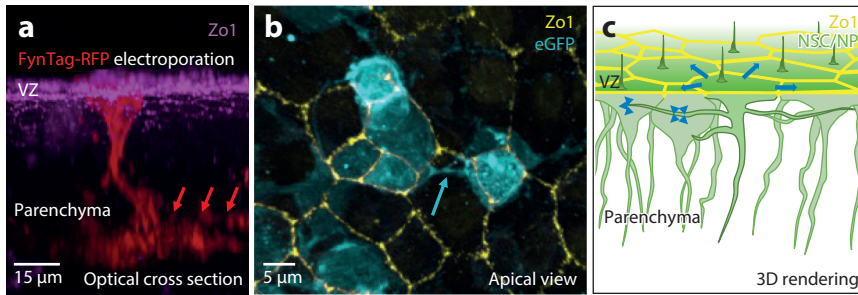


Figure 5

The tight NSC/NP arrangement at the ventricular zone, and horizontal cellular extensions, could facilitate cell–cell communication even at a distance. (a) Visualization of the lateral extensions of an individual radial glial cell (red arrows) inside the parenchyma. One RG of the adult pallium was electroporated in situ with a FynTag-RFP plasmid. Panel shows double IHC for the tight junction marker Zo1 (purple) and RFP. Panel provided by S. Ortica. (b) Visualization of the subapical processes (blue arrow) of *deltaA*-expressing NSCs/NPs, showing double IHC for Zo1 (yellow) and GFP (blue) in a Tg (*deltaA:egfp*) transgenic adult in a whole-mount pallium, apical view. (c) 3D rendering of a tilted whole-mount view illustrating possible signaling routes between progenitor cells (NSCs and NPs, green) in the adult pallium. Blue arrows represent apical interactions; blue arrowheads represent subapical interactions. Abbreviations: GFP, green fluorescent protein; IHC, immunohistochemistry; NP, neural progenitor; NSC, neural stem cell; RFP, red fluorescent protein; RG, radial glia; VZ, ventricular zone; Zo1, zona occludens-1; 3D, three-dimensional.

just below the apicobasal interface or at a few micrometers deep and can connect cells within up to a few cell diameters (92, 103). Expression of numerous exosomal components by NSCs have also been noted (103). Such connections, whether to immediate or more distant neighbors, could be important to ensure spatiotemporal coordination within the NSC population. Horizontal processes emanate from *deltaA*-expressing NSCs (92) (see **Figure 5**) and also express the phosphorylated insulin-like growth factor (Igf) receptor (Tyr1161 P-Igf1r) (103). In the future, it will be highly interesting to probe the existing scRNA-seq data sets for the differential expression, between NSC subtypes/substates, of receptor–ligand pairs or other families of factors that could convey horizontal information between NSCs.

4.2. Notch Signaling and Spatiotemporal Neural Stem Cell Coordination

The most studied pathway to date for its role in spatiotemporal coordination of NSC decisions in the adult zebrafish pallium is Notch signaling, specifically Notch3. As described in Section 3.1.1, blocking Notch3 in individual NSCs increases their likelihood to divide after 3 days (4). The Notch ligand DeltaA is expressed in a subpopulation of NSCs and in NPs and could serve as the signal for Notch activation (and the promotion of quiescence) in neighboring NSCs (23, 92). Although not experimentally tested to date, these interactions are expected to slow down the rate of NSC activation, thus establishing the rate of generation of neurogenic NSCs and neurons, that is, the overall size of the NSC population and of its output. *deltaA*^{pos} NSCs themselves are also sensitive to Notch signaling for their activation rate (92). Importantly, because Delta–Notch signaling operates between neighboring cells, it is also predicted to influence the spatial pattern of receiving versus sending cells, as amply demonstrated, for example, in the embryonic neural tube (16, 100). In the pallial germinal population, this should notably affect the distribution of quiescent NSCs (a majority of which are *deltaA*^{neg}) relative to *deltaA*^{pos} cells (which include most activated cells). Spatial statistics, modeling, and experimental blockade of Notch signaling were used to test this idea in situ, demonstrating that NSC activation events indeed tend to avoid the vicinity of activated NPs. Spatial modeling further predicts that, in the absence of these interactions, a drift will be observed

long-term (over 1 year) toward a clustered distribution of adult-born neurons (36). Together, these results indicate that Notch signaling, likely mediated by immediately adjacent *delta.A^{pos}* NPs, acts as both a state-determining and spatial-positioning signal to control the size and the physical distribution of the activated NSC subpopulation within the NSC pool. Several points remain to be addressed to achieve a dynamic and spatiotemporal understanding of Notch signaling in situ as a basis for NSC fate coordination. Among them, we note the possible effect of activated NSCs, the role of other Notch ligands expressed within the germinal zone (23), whether and how interactions (or information) propagate at large scale, and how different NSC activation frequencies are encoded in different pallial domains and how the system deals with spatial boundaries (35).

As discussed above, in addition to activation, NSCs encounter many other decision points along their trajectories, for example, in the choice of daughter cell states and fates and in their progression within the molecular space between divisions (92, 101, 126). The overall dynamic equilibrium of the NSC population suggests that all these decisions might involve spatiotemporal coordination, that is, population control of both their rate and spatial pattern. This remains an important field to establish, first through a precise quantitative and dynamic description of spatial patterns over time, and then through mechanistic analyses, to understand the interplay between population feedback and constrained cell-autonomous programs (internal clocks or determinants) in the generation of population stemness. Such analyses will be important beyond NSCs to understand the maintenance of stem cell pools, and important studies along similar conceptual lines of spatiotemporal regulation are being conducted, for example, in interfollicular epithelial stem cells of the skin (97, 99).

4.3. Section 4 Take-Home Messages

Take-home messages for Section 4 include the following:

- The control of NSC activation by Notch–Delta signaling ensures that activation events are widespread across the ventricular niche, in addition to controlling their rate.
- The balanced fates of individual NSCs suggest the existence of feedback interactions on NSC division modes.

5. THE SYSTEMIC AND ENVIRONMENTAL IMPACT ON NEURAL STEM CELL REGULATION TO INCREASE BRAIN PLASTICITY

Neurogenesis in the adult zebrafish is a constitutive and additive process sensitive to a wide variety of both systemic and environmental factors and much more than a passive means of accommodating increased body size over time. This is evidenced by stimulus-induced neurogenesis that fine-tunes sensory processing within brain territories responsible for specific input modalities, allowing for adaptation to a changing environment (85, 95). In addition to increased sensory input, NSC activation is also upregulated in response to lesion, bestowing the zebrafish with an enhanced capacity for neural repair (39, 76, 94). While we will elaborate on NSC dynamics in the contexts of stab-wound lesions and chronological aging below, additional systemic and environmental influences on NSC behavior are detailed in the sidebar titled Systemic and Environmental Influences on Adult Neurogenesis.

5.1. Increased Neurogenesis Facilitates Tissue Repair Upon Mechanical Lesion

Various methods for creating lesions have been used to study adult zebrafish NSCs activity in the context of regeneration, including chemical injury and mechanical lesions, among others (9, 76–78, 94, 121). The rapid and transient response to mechanical lesions by stab-wound injury to the

SYSTEMIC AND ENVIRONMENTAL INFLUENCES ON ADULT NEUROGENESIS

Hormonal Regulation

Both proliferation and differentiation by neural stem cells (NSCs) are sensitive to systemic hormone levels. Disordered insulin signaling and hyperglycemia negatively impact NSC proliferation and cognition, in part through perturbations to the blood–brain barrier and increased inflammation (20, 34, 51, 110). Similarly, proliferation is decreased among NSCs in the telencephalon, hypothalamus, cerebellum, and optic tectum of female zebrafish exposed to exogenous estrogen (91).

Sensory Stimuli

In adult zebrafish, neurogenesis increases with increased visual and olfactory input. Increased visual input results in increased neurogenesis within regions of the optic tectum, while increased olfactory stimulation increases neurogenesis within the olfactory bulb (85). This demonstrates that adult neurogenesis is adaptive, increasing in territories responsible for processing corresponding sensory modalities.

Social Interaction

Social interaction mediates the balance between proliferation by neural progenitors and the production of newborn neurons. Isolation during developmental and adult stages decreases proliferation by progenitors in the olfactory bulb and optic tectum while increasing neuron production, affecting the balance between progenitor amplification and lineage progression within the progenitor pool (85).

Spatial Learning

Both proliferation and the production of newborn neurons are increased in subregions of the adult telencephalon in response to spatial learning, allowing for positional information to be integrated with visual cues and thereby increasing cognitive ability (95).

Nutrition

High caloric intake is associated with premature aging accompanied by reduced proliferation by both neural stem and progenitor cells across all neurogenic niches (88, 123). Within the cerebellum specifically, a calorically rich diet is further predicted to impair terminal differentiation by neural progenitors (NPs). This, in combination with defective migration away from the proliferation zone, contributes to decreased neuronal production and impaired cognition over time (123).

telencephalon has been widely demonstrated, with proliferation and neurogenesis accelerated by ventricular RG to restore tissue architecture and neural connectivity (6, 79, 80, 89, 98, 117).

5.1.1. Unknown mechanisms link neural stem cell division mode to activation stimulus.

Under physiological conditions, most NSC divisions in the zebrafish are asymmetric, generating one NSC and one further committed progenitor (6, 92, 126). Following stab-wound injury, NSC divisions become largely symmetric and neurogenic, accelerating the production of newborn neurons to facilitate wound repair at the expense of the NSC pool (6). This is unlike mammalian systems where injury induces inflammation and the formation of a glial scar through the recruitment of reactive astrocytes, a process that is prohibitive to regeneration (19, 27, 45, 51, 120, 122). In contrast to mechanical lesion, 48 h γ -secretase inhibition by LY411575 to perturb downstream Notch signaling increases proliferation among pallial RG and biases these divisions toward symmetric

gliogenic fates (4). Examination of the molecular trajectories and extracellular signaling pathways that are instructive for cells to transition between division modes in the context of lesions, and how these trajectories compare to those observed under physiological conditions, would provide insight into different molecular pathways to activation and the regulation of cell fate choices.

5.1.2. Heterogeneity among neural stem cells may result in differential responses to lesion.

Following a lesion injury, heterogeneous cellular responses occur. Many NSCs enter the cell cycle, upregulating proliferation and neurogenesis at the population level. A subset of RG, however, remains quiescent and increases the expression of the transcription factor gene *id1* and the secreted signal gene *midkline a (mdka)* compared to control NSCs (87, 116, 132, 133). *id1* expression is promoted by Bmp signaling and, importantly, is needed to preserve long-term stemness. Indeed, consecutive lesions lead to NSC loss and impaired reparative neurogenesis in *id1* loss-of-function mutants (133). These results are important and deserve further thought. At the individual NSC level, NSC loss could result from a direct bias in fate (e.g., biased cell division mode) (6) or from exhaustion secondarily caused by excessive activation. Zhang et al. (133) found evidence in favor of the latter hypothesis, as NSC numbers in *id1* mutants only drop after a second lesion. At the population level, there are also several possible interpretations. The induction of differential responses to a lesion within the NSC population may result from the state each cell was in at the time of the lesion—with NSC asynchrony in lineage progression ensuring different responses—or from crosstalk balancing decisions between NSCs. Recent studies identified subpopulations of RG that appear to differ along a continuum of quiescence depth, with subpopulations highly responsive to activating cues thought of as shallow in their quiescence depth compared to others in deep states of quiescence that are more resistant to activation (80, 101). Among RG in deep quiescence, a subpopulation of progenitors involved in constitutive neurogenesis resembles mammalian astrocytes at the transcriptomic level (101). While astrocytes are absent from adult zebrafish, RG in the telencephalon are thought to perform many of the functions carried out by astrocytes in mammalian systems (66, 105), with these astrocyte-like RG perhaps being preferentially recruited to activation upon injury. Additionally, mapping the spatial distribution of responsive versus preserved NSCs to measure the degree of spatiotemporal coordination in the process will be important.

5.1.3. Lesion-induced neurogenesis can involve the recruitment of latent progenitors.

In territories other than the pallium, neurogenic activity by specialized RG-like cells, known as latent progenitors, in response to injury has also been observed. Latent progenitors are differentiated cell types that are not involved in neurogenesis under physiological conditions but are specifically responsive in the context of lesion. Through their activation, latent progenitors produce adult-born neurons through tissue-specific transformations that include dedifferentiation to a progenitor state, transdifferentiation to alternative lineages, or through reactivation from quiescence. In the retina, lesion injury results in dedifferentiation of RG-like Müller cells that change their morphology and gene expression profile as they become migratory and revert to a proliferative progenitor state from which all retinal cell types can be specified (38, 42, 44, 50, 54). In the spinal cord, highly quiescent ependymal glial cells (EGCs) that line the spinal cord canal form the latent progenitor compartment and reactivate in response to spinal cord injury. These cells are slow cycling and typically only give rise to oligodendrocytes under homeostatic conditions yet become highly proliferative upon injury to generate motor neurons that integrate into the spinal circuitry to facilitate regeneration (52, 60, 108, 113, 131). Through their dynamic response to injury, these and other latent progenitor cell types facilitate repair in regions that lack constitutive progenitors, thereby expanding the regenerative capacity of adult zebrafish.

5.2. Aging

As in mammals, NSC activity decreases in zebrafish with increased chronological age, impacting both constitutive and regenerative neurogenesis (14, 39). With increasing age, decreased proliferation by NSCs is associated with a decrease in the production of NPs and newborn neurons, slowing the rate of neurogenesis over time (39). In response to lesion, aged RG maintain their ability to increase proliferation at the population level but do so to a lesser extent than that observed in younger fish. Nevertheless, when activation among aged NSCs occurs, the same level of amplification along the lineage is observed, with similar proportions of progenitors being generated. These observations demonstrate that decreased neurogenesis with chronological aging results from a reduction in cell cycle frequency, rather than from the capacity for NSCs to differentiate to neuronal fates, and further highlight the close relationship between cell activation and lineage progression. In the zebrafish telencephalon and other stem cell systems, stem cell hierarchies have been identified wherein progenitors with relatively long quiescence durations and a high capacity for self-renewal supply cells to more proliferative and neurogenic pools downstream (17, 92, 126). Decreased neurogenesis among aging zebrafish may result from depletion of specific NSC subpopulations that are more proliferative than others, as has been shown in mice (17). It may be that more proliferative NSC subpopulations experience depletion over the lifespan, leaving only deeply quiescent NSCs over large timescales that divide too infrequently to sustain the rate of neurogenesis observed in younger counterparts.

The sidebar titled Unanswered Questions Related to Systemic and Environmental Impacts of Neural Stem Cell Behavior summarizes the numerous fundamental yet unanswered questions that the zebrafish model could help address relating to the response of NSCs to challenge and physiological status.

UNANSWERED QUESTIONS RELATED TO SYSTEMIC AND ENVIRONMENTAL IMPACTS OF NEURAL STEM CELL BEHAVIOR

Division Modes

The mechanisms linking proliferation to lineage progression have yet to be uncovered. Further, the molecular trajectories instructive for division mode upon activation in the context of lesion injury and pharmacological inhibition of proquiescence signaling pathways have yet to be identified and compared, both with each other and with those active under physiological conditions.

Activation by Astrocyte-Like Radial Glia Upon Lesion

It has yet to be determined whether the pallial radial glia (RG), similar to mammalian astrocytes, are specifically recruited to proliferate and differentiate in response to lesion injury, thereby participating in a regenerative program akin to reactive neurogenesis in mammals but without the formation of a glial scar.

Recruitment of Latent Progenitors Upon Lesion

A better understanding of the molecular responses by niche-specific latent progenitors at the single-cell level may uncover novel regulatory mechanisms involved in de- and transdifferentiation during tissue regeneration. By comparing these responses to the response by RG and neuroepithelial cells (NECs) during constitutive neurogenesis, molecular trajectories that are either shared or specific to each process could be identified.

(Continued)

(Continued)

Enhanced Quiescence Among RG Postlesion

Identification of molecular mechanisms by which stem cell exhaustion is prevented by an increase in quiescence depth among a subset of RG postlesion will contribute to our understanding of how the balance between tissue regeneration and NSC maintenance is regulated upon challenge.

Altered Neurogenic Output with Chronological Age

Whether decreased neurogenesis with chronological aging results from changes in the behavior of the entire NSC population or whether specific neurogenic subpopulations are more vulnerable to aging than others is not known.

5.3. Section 5 Take-Home Messages

Take-home messages for Section 5 include the following:

- The adult zebrafish possesses a dynamic pool of constitutive and latent NSCs capable of fine-tuning neurogenic activity in response to systemic and environmental cues.
- This neurogenesis-based plasticity enhances adaptability to a changing environment and increases the capacity for regeneration upon injury.

6. CONCLUSIONS

The zebrafish was selected as a promising model for the study of adult neurogenesis based on its numerous neurogenic niches, life-long neurogenesis, and strong neuronal regeneration capacity. The possibility of tracking NSCs at work in their niche using intravital imaging in the pallium, under fully noninvasive conditions, later proved invaluable for approaching issues such as spatiotemporal NSC coordination for the first time in an adult vertebrate brain. Recent work also demonstrates the additional benefit of intravital imaging to integrate multimodal information related to single-cell NSC heterogeneities. Our understanding of the adult pallial neurogenic lineage is now such that it can be put in parallel with lineages in mice, highlighting that the NSC subpopulation that self-renews long-term in zebrafish may have lost this capacity in mice to become astrocytes, which are not neurogenic under physiological conditions.

In spite of these advantages and key findings of the zebrafish model, our understanding of the molecular mechanisms of neurogenesis in the adult zebrafish brain remains partial and should be the focus of more work ahead. Along these lines, it may be interesting to work out more practical models for functional assays. In mice, remarkable advances were made in understanding NSC behaviors *in vitro*. The neurosphere assay has been instrumental to evaluate self-renewal and differentiation at the single-cell level (114, 115), while monolayer systems have helped characterize NSC patterns of division and lineage progression by live imaging (26, 106). Moreover, NSC heterogeneity is to some extent maintained *in vitro* (10). In spite of early reports of zebrafish neurospheres (86), modeling the specificity of adult zebrafish NSCs states *in vitro* such as quiescence, activation, proliferation, and commitment remains challenging and will require further efforts.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

T.F., M.T., and L.B.-C. jointly discussed the outline of the review, wrote the text, and prepared the figures.

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