Radial Glia and Neural Progenitors in the Adult Zebrafish Central Nervous System

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The adult central nervous system (CNS) of the zebrafish, owing to its enrichment in constitutive neurogenic niches, is becoming an increasingly used model to address fundamental questions pertaining to adult neural stem cell (NSC) biology, adult neurogenesis and neuronal repair. Studies conducted in several CNS territories (notably the telencephalon, retina, midbrain, cerebellum and spinal cord) highlighted the presence, in these niches, of progenitor cells displaying NSC-like characters. While pointing to radial glial cells (RG) as major long-lasting, constitutively active and/or activatable progenitors in most domains, these studies also revealed a high heterogeneity in the progenitor subtypes used at the top of neurogenic hierarchies, including the persistence of neuroepithelial (NE) progenitors in some areas. Likewise, dissecting the molecular pathways underlying RG maintenance and recruitment under physiological conditions and upon repair in the zebrafish model revealed shared processes but also specific cascades triggering or sustaining reparative NSC recruitment. Together, the zebrafish adult brain reveals an extensive complexity of adult NSC niches, properties and control pathways, which extends existing understanding of adult NSC biology and gives access to novel mechanisms of efficient NSC maintenance and recruitment in an adult vertebrate brain.

Key words: radial glia, neuroepithelial progenitors, zebrafish, adult neurogenesis

Introduction

Aquatic vertebrates (fish, amphibians) have long been recognized for their sustained adult neurogenesis. Among them, the zebrafish is becoming increasingly popular to address issues of adult neural stem cell (NSC) biology and regeneration. In most subdivisions of the zebrafish adult central nervous system (notably the telencephalon, retina and spinal cord), radial glia (RG) were identified as a prominent progenitor cell type, whether under physiological or regenerative conditions. More recently, nonglial progenitors were also found to constitute persistent niches contributing to adult neurogenesis and, in some instance, to the generation of long-lasting, self-renewing RG pools. This highlights the complexity of adult neurogenesis and NSC processes in zebrafish, and the power of this model, unfairly qualified as “simple”, to gain insight into the biology of NSCs/adult progenitors. What is the exact hierarchical relationship of these different progenitor types? How do they compare with characterized mammalian NSCs? Do they truly exhibit characters of adult NSCs, in spite of a morphology resembling that of embryonic progenitors and the constant growth of zebrafish individuals through life? What can we learn from zebrafish adult neurogenesis that can be translated to other adult vertebrates and in particular to an adult mammal?

To bring insight into these questions, we will first summarize the characteristics and properties of mammalian RG, the “standard” in the field. We will then review the status of progenitor subtypes in the adult zebrafish, their physiological function under normal and regenerative conditions, and what is known of their underlying molecular and cellular control pathways.

The Standard: Radial Glia in Mouse

Radial glia (RG) arise in the developing mouse CNS around the onset of neurogenesis and are classically defined by the combination of several features: (i) the expression of glial markers typical of astroglial cells, (ii) an elongated (radial) morphology bridging the apical and basal surfaces of the...
neural tube, and (iii) the maintenance of an apico-basal polarity. These features are a RG signature together but not individually, as many astroglial markers are shared with astrocytes and/or ependymal cells, while the morphological and polarity criteria are also found in neuroepithelial (NE) cells, the first progenitors of the neural plate and tube from which RG emerge during development.

RG markers include expression of intermediate filaments such as Vimentin and glial fibrillary acidic protein (GFAP), expression of the glutamate transporters GLAST and GLT-1 and of glutamine synthase (GS), S100β, tenascin-C, metabolic enzymes such as the aldolase Aldhl1 and the brain lipid binding protein (BLBP), and the oestrogen biosynthesis enzyme aromatase B (Dimou and Götz, 2014; Götz, 2013). They also express various neurotransmitter receptors and voltage-dependent ion channels, and can release chemical messengers (Götz, 2013). These proteins attest of functional astroglial properties deeply involved in brain homeostasis, such as transmitter uptake and release, the buffering of extracellular K+ concentration, the provision of metabolic support to neurons, the generation of calcium waves, and oestrogen synthesis.

Apico-basal polarity is manifested by an apical membrane compartment limited by tight and adherens junctions, and organized in microdomains. The latter are enriched in proteins of the Par complex (Par3, Par6 and APKc), cdc42, cadherins and the glycoprotein Prominin-1. The apical domain also harbours a primary cilium protruding into the cerebrospinal fluid (Peyre and Morin, 2012). In contrast, the basal process is elongated and connected to the basement membrane of the pial surface of the neural tube. In addition, RG share with NE cells their expression of the intermediate filament Nestin and the transcription factor Sox2, directly or indirectly implicated in the neural progenitor properties of both NE and RG cells (Park et al., 2010). Finally, both cell types undergo interkinetic nuclear migration (INM), a process by which their nucleus translocates from apical to more basal positions between the S and M phases of the cell cycle.

RG are mostly transient in mammals: they act as progenitors, functional and structural components of the developing CNS, while they largely differentiate into astrocytes and ependymal cells at postnatal stages. However, glial cells with radial features can be found in the adult brain, some being located in specialized niches where they act as constitutive adult progenitor cells: the so-called “B” cells of the subependymal zone (SEZ) of the lateral ventricle, “type 1” cells of the subgranular zone (SGZ) of the dentate gyrus of the hippocampus, and, most likely, tanyocytes bordering the hypothalamic ventricle (Dimou and Götz, 2014; Morrens et al., 2012). Of these, “B” and “type 1” cells share all markers with RG, except GS and S100β. They extend a parenchymal process contacting the basement membrane surrounding blood vessels, and, in addition, B cells contact the brain ventricle through a specialized apical membrane domain comparable to embryonic RG. Figure 1 illustrates the shared and divergent properties of RG and related constituent cell types of the developing and mature CNS.

Radial Glia Localization and Physiological Properties in the Adult Zebrafish Central Nervous System

Progenitor cells with an overt radial morphology, apico-basal polarity and displaying INM can be identified around mid-embryogenesis in the developing zebrafish telencephalon (Dong et al., 2012), retina (Baye and Link, 2007; Das et al., 2003) and hindbrain (Alexandre et al., 2010; Coolen et al., 2012; Lyons et al., 2003), and are likely broadly distributed at this stage in the CNS. However, the expression of some “RG markers” is initiated as early as the neural plate stage (e.g., late epiboly for blbp (fabp7a), 1-somite stage for gfap (Thisse et al., 2001), making it perhaps more difficult than in rodents to precisely define a transition from NE to RG. Other markers are turned on during later embryogenesis and...
juvenile stages, to reach a mature RG state very similar to mammals (Fig. 1).

Contrasting with the situation in mammals, however, RG are widely maintained in the zebrafish adult CNS. At least in part, this is likely in functional relation with two specific features of the adult CNS in aquatic vertebrates: its maintenance of widespread niches of constitutive neurogenesis - where adult RG can, like in the embryo, serve as progenitor cells (see below)-, and its relatively small size, which may alleviate the need to “delocalize” astroglial cells into the parenchyma to ensure proper astroglial function (Chapouton et al., 2007). With the exception of the optic nerve (Koke et al., 2010), the zebrafish brain is devoid of bona fide astrocytes (Grupp et al., 2010), and only some ventricular domains (e.g., the diencephalon or the spinal cord) contain ependymal-like cells, characterized by the absence of expression of markers such as GFAP or Vimentin and the presence of multiple beating cilia extending in the cerebrospinal fluid (Kishimoto et al., 2011). Correlatively, in the adult zebrafish brain, RG express the water channel Aquaporin 4 (a property of multiple beating cilia extending in the cerebrospinal fluid (Kishimoto et al., 2011). Little is known of other functional properties of adult zebrafish RG (neuronal migration, synaptic plasticity, gli-neuron communication, brain homeostasis…) except for an extensive series of studies demonstrating their strong expression and activity of steroidogenic enzymes, suggesting their production of neurosteroids (Pellegrini et al., 2005). This property is shared with mammalian astrocytes and neurons. In addition, RG in the trout optic tectum exhibit voltage-gated Na+ inward currents and K+ outward currents (Rabe et al., 1999).

The following sections highlight the location and cellular characteristics of RG in the zebrafish adult brain.

**RG in the Adult Telencephalon**
The telencephalon of teleosts, like that of mammals, is composed of a dorsally located pallium and a ventrally located subpallium. Due to a peculiar morphogenesis and growth process during development, dorsal structures are displaced outward. This process, called “eversion”, (i) generates a pallium with inverted medio-lateral organization compared with other vertebrates and (ii) exposes the pallial ventricular zone (VZ) dorsally, covered by an extended choroid plexus (Dirian et al., 2014; Folgueira et al., 2012). Developmental, hodological, functional and gene expression information support a regional organization of the pallium in a lateral domain (Dl) containing hippocampal-like structures, a medial domain (Dm) containing amygdala-like nuclei (overlapping the pallial-subpallial junction) and a dorsal/central domain (Dd/Dc) corresponding to the neocortical area in mammals (Mueller and Wullimann, 2009). Homologies in the subpallium remain more uncertain (Ganz et al., 2012).

**Pallium.** Cells with RG characteristics are aligned along the pallial ventricle, covering the large majority of the VZ (Fig. 2A). RG cell bodies organize into a monolayer in contact with the CSF, while their long processes extend into the pallial parenchyma to reach the pial surface of the brain in contact with large blood vessels. Various RG morphologies have been described in different pallial areas; they differ in cell body shape and the length and degree of branching of the radial process (März et al., 2010a). Expression of markers, e.g., Nestin, GFAP, BLBP, Cyp19b (Aromatase B), S100B, Vimentin, GS and Fezf2 also shows some degree of heterogeneity (März et al., 2010a; Berberoglu et al., 2014). These heterogeneities remain to be fully characterized and their functional significance determined.

**Subpallium.** The VZ of the subpallium is bordered with a pseudo-stratified monolayer of radial, actively proliferating cells with ventricular (apical) and pial (basal) contacts. These cells display elongated nuclei, apicobasal polarity, express glial markers (e.g., GFAP, Vimentin, Cyp19b, BLBP, S100B) at very weak levels, and generally divide close to the ventricular surface while completing their S phase at a distance, suggesting that they may undergo INM (März et al., 2010a). A subpopulation of these cells expresses the transcription factor gene olig2 (März et al., 2010b), indicating some degree of heterogeneity within this population.

**RG in the Adult Retina**
Müller glia constitute the main glial population of the vertebrate retina and display a radial shape, with cell bodies located in the inner nuclear layer and extending processes throughout the retina’s radial depth, from the apical epithelial surface to the basal lamina. They express classical glial markers such as GFAP and GS, and also stain positive for carbonic anhydrase (Linser et al., 1985; Peterson et al., 2001). Following the constant growth of the retina driven from the ciliary marginal zone (CMZ) in teleosts, the least mature Müller glia are located in proximity to the CMZ; these cells also express BLBP (Raymond et al., 2006). It is to note that nonradial astrocytes from the optic nerve partly extend into the retina, along the inner limiting membrane; these are GFAP-negative but express Cytokeratin (Koke et al., 2010).

**RG in the Adult Diencephalon**
The diencephalon of the zebrafish includes the preoptic area, epithalamus, dorsal and ventral thalamus, posterior tuberculum, hypothalamus (consisting of dorsal, ventral, and caudal zones), syencephalon, and pretectum. RG cells expressing
GFAP, BLBP or AroB have been observed lining the ventricle of most subdivisions, although their density varies (Menuet et al., 2005). In the periventricular region of the hypothalamus (periventricular organ, PVO), a region particularly enriched in RG cells, the nuclei of BLBP- or AroB-positive RGs are located in a subventricular location and RG extend a short cytoplasmic
process toward the ventricle (Fig. 2B). In this territory, cerebrospinal fluid-contacting neurons intervene between the RG cell bodies and the ventricular surface (Pellegrini et al., 2007).

**RG in the Adult Mesencephalon**
The mesencephalon comprises the alar tectum opticum (TeO), bordered medially by the torus longitudinalis and laterally by the torus semicircularis, and the basal tegmentum. A prominent layer of RG cells has been described in the TeO, with their cell bodies aligned along the ventricle of the deepest tectal layer (periventricular gray zone) and extending a basal process reaching into upper TeO layers (Fig. 2C). The end feet of these RG cells form a glial limitans in the more superficial marginal zone, and also contact blood vessels (Corbo et al., 2012). This RG arrangement however excludes the marginal proliferation zone of the TeO, where no RG cells are present. Tectal RG cells express markers such as GFAP, BLBP, S100β, and AroB (Meneut et al., 2005; Ito et al., 2010). RG markers-expressing cells are also observed along the ventricle of the tegmentum and torus semicircularis, although their processes are shorter and/or not coursing radially (Meneut et al., 2005).

**RG in the Adult Rhombencephalon and Spinal Cord**

**Cerebellum.** Cell types in the adult zebrafish cerebellum have been extensively characterized, in link with the prominent neurogenesis occurring in this territory. GFAP, BLBP, S100β and Vimentin-positive RG cells can be found at the cerebellar midline in the molecular layer, close to the main neurogenic site generating granule neurons (Fig. 2D; Kaslin et al., 2009). Their processes extend into the molecular layer and are postulated to guide neuronal migration. An additional set of RG cells, so called Bergmann glia, can be observed sparsely distributed in the inner molecular layer throughout the two anterior cerebellar lobes (Fig. 2E). Compared with RG cells in other brain subdivisions, they harbour a process with multiple main branches.

**Medulla Oblongata and Spinal Cord.** GFAP-positive radial cells are present bordering the medulla and spinal cord ventricle in the adult zebrafish (Fig. 2F). Cells processes reach the spinal cord surface, and some have also been observed contacting small vessels (Kawai et al., 2001; Tomizawa et al., 2000). Some radial cells positive for BLBP expression also line the spinal ventricle. Heterogeneity within the spinal radial population is notably suggested by the nonubiquitous expression of molecular markers such as *olig2*, expressed in a discrete bilateral population of BLBP-positive but GFAP-negative radial cells in the basal plate (Park et al., 2007). In the juvenile, this population is distinct from another radial subpopulation expressing dbx1a (Briona and Dorsky, 2014). A population of radial cells of similar morphology and expressing Shh is also found lining the most ventral aspect of the central canal (Reimer et al., 2009).

**Zebrafish Adult Radial Glia as Neural Progenitors**
The discovery that RG cells are neural progenitors during development in the mouse (Anthony et al., 2004; Malatesta et al., 2000, 2003; Noctor et al., 2002), and that neural stem cell potential is maintained by glial cells in the adult rodent brain, strongly suggested that adult RG may be, at least in part, at the origin of the extensive neurogenesis observed in adult teleost fish. Recent work exploring this possibility in detail in the adult zebrafish confirmed this hypothesis but also revealed a more complex situation, where distinct RG pools may be recruited in constitutive versus reactive neurogenesis.

Active neurogenesis persists in most brain subdivisions of the zebrafish adult central nervous system, albeit at different rates, being notably low in the spinal cord. Overall, 16 constitutively neurogenic domains have been described (Grandel et al., 2006). RG or radial cells were directly implicated in constitutive neurogenesis in some of these domains: the pallium, the subpallium, and the hypothalamus. The pallial population can be further boosted in reaction to lesion. In addition, reactive neurogenesis recruits normally silent RG populations such as Müller glia in the retina, or ventricular ependymoglial cells in the spinal cord. The neuronal progenitor status of other RG or radial populations remains to be assessed, notably in the optic tectum.

Although this process was much less studied to date, glial cells are also generated lifelong in the adult zebrafish brain. The generation of new RG from RG progenitors was

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**FIGURE 2:** RG in the zebrafish adult central nervous system, revealed by *gfap:egfp* expression. Cross sections of the telencephalon (A), hypothalamus (B), optic tectum (C), cerebellum (D and E), and spinal cord (F) from *gfap:egfp* adult transgenic zebrafish (Bernardos and Raymond, 2006), immunostained for GFP (photographed under confocal microscopy). Arrows point to RG cell bodies (Bergman glia in E). The identification of GFAP-positive cells as RG originate from the following publications: Ito et al., 2010; Kaslin et al., 2009; Marz et al., 2010a; Meneut et al., 2005; Park et al., 2007. Abbreviations are from Wullimann et al., 1996: C: central canal, CCE: corpus cerebellaris, CCEr: commissura cerebelli, CTeC: commissura tecti, D: dorsal telencephalon (pallium), Dc: central subdivision of D, Dd: dorsal subdivision of D, Dm: medial subdivision of D, DL: lateral subdivision of D, DH: dorsal horn, DIV: diencephalic ventricle, EG: eminentia granularis, Hc: central zone of periventricular hypothalamus, Hd: dorsal zone of periventricular hypothalamus, LR: lateral recess of the hypothalamic ventricle, MA: Mauthner axon, MLF: medial longitudinal fascicle, PGZ: periventricular gray zone, Pit: pituitary, PPa: posterior preoptic area, RV: rhombencephalic ventricle, SRF: superior reticular formation, SY: sulcus ypsiloniformis, TeO: tectum opticum, TeV: tectal ventricle, TL: torus longitudinalis, TS: torus semicircularis, VH: ventral horn, VOT: ventrolateral optic tract, Vs: ventral subdivision of the subpallium.
formally documented in the pallium, and can be indirectly inferred from the growth of ventricular zones coupled with RG proliferation in other areas. The generation of oligodendrocytes is still prominent at adult stages, from source cells that seem to vary in nature between CNS territories (Chapouton et al., 2006).

These findings are detailed below.

**RG or Radial Progenitors in Constitutive Neurogenesis**

**Pallial RG are Life-Long Constitutive Neural Progenitors and a Main Amplification Step of the Neurogenic Lineage.**

**Neuronal generation.** BrdU labeling, and expression of positive proliferation markers such as Proliferating Cell Nuclear Antigen (PCNA) or Mini-Chromosome Maintenance proteins (e.g., MCM5) indicated that dividing cell types along the pallial ventricle include RG (Adolf et al., 2006a).

Following BrdU administration, neurons (expressing the proteins HuC/D) start appearing in the pallium parenchyma after a few days of chase, and their number increases with time until ca. 2–3 weeks (Adolf et al., 2006a). Lentivirus-mediated cell transduction likewise indicated that pallial RG generate neurons that reach a mature neuronal phenotype (with expression of synaptic proteins and the firing of axon potential) 4–8 weeks after birth (Rothenaigner et al., 2011). Cre-lox genetic tracing provided the direct demonstration of a RG origin for pallial neurons (Fig. 3A). Driver transgenic lines expressing an inducible form of the Cre recombinase under control of regulatory elements of the \( e(spl) \) her4 gene, selectively expressed in pallial RG, were crossed into reporter lines expressing a fluorescent protein upon recombination.

**FIGURE 3: Stem-like progenitor cells in the adult zebrafish central nervous system and their fate under normal and regenerative conditions.** RG (green) and NE (blue) progenitors are schematized on cross sections of the pallium (A), optic tectum (B), retina (C), cerebellum (D), and spinal cord (E). In the pallium, optic tectum and retina, NE stem-like progenitors are associated with a highly proliferating growth zone (pink). The activation status ("-", "+") and fate (arrows) of these progenitors under physiological conditions ("norm.") and under conditions of neuronal regeneration ("reg.") are also indicated on each section and in the tables (yellow, orange, pink, red: neurons of different identities, including rods (yellow ovals); gray: oligodendrocytes). (A) RG in the pallium generate RG and neurons. They likely can increase their repertoire of generated neuronal subtypes under regenerative conditions. NE progenitors in the lateral pallium generate RG NSCs. (B) RG in the optic tectum are endogenously mostly silent, but their fate, notably in freshly generated RG close to the growth zone, has not been directly assessed. NE progenitors at the posterior edge of the PGZ generate RG and neurons, most likely via a direct mode that does not involve RGs. Regenerative contexts have not been studied yet in the adult TeO. (C) Müller glia in the central retina are engaged in a slow rate generation of rods (yellow ovals), but can generate all neuronal subtypes upon repair activation. NE progenitors of the CMZ generate Müller glia and all other neuronal types; their recruitment and fate upon repair has not been analyzed in detail. (D) NE progenitors generate granule neurons, while medially located RG, and Bergman glia, are silent. The fate of these cells upon repair has not been analyzed. (E) RG lining the central canal are mostly silent and engaged in a slow rate generation of oligodendrocytes. Following spinal transection, RG activate and can generate motoneurons.
Activation of Cre at late juvenile stage (1.5 month) or at adult stage resulted in labeled neurons in the adult pallium (Kroehne et al., 2011).

We still know very little of the neuronal subtypes generated by RG in the pallium during juvenile and adult life. Parvalbumin-positive and calbindin-positive interneurons are present in high numbers, respectively concentrated in the central/lateral and medial pallium (Mueller et al., 2011; von Trotha et al., 2014). In addition, some GABAergic and glutamatergic neurons are scattered throughout the pallium (Mueller et al., 2011; von Trotha et al., 2014). The entire lateral pallium, including all its constituent neurons, is generated from juvenile stages onward; in contrast, neurons of the dorso-medial pallium are generated from both embryonic and postembryonic stages (Dirian et al., 2014). As a first step, a recent study demonstrated that neurons born in the ventral-most area of the medial pallium at around 1mpf were recruited in goal-directed behavior in adults (von Trotha et al., 2014). It remains a key point to analyze how the generation of distinct neuronal subtypes is orchestrated by juvenile or adult RG—at the single RG or the RG population level—and how they integrate into the distinct pallial areas and contribute to their function. Adult neurogenesis in the mammalian hippocampus is associated with a high rate of neuronal replacement (Spalding et al., 2013). It is possible that distinct integration processes are at stake in the zebrafish adult pallium, where very little cell death has been observed and adult neurogenesis is associated with neuronal addition and pallial growth.

**Division modes and rate.** During a cell cycle, PCNA and MCM5 concentrations increase during late G1 phase, peak in S, and decrease thereafter. The average proportion of PCNA- or MCM5-positive RG is of 8–10% in the medial and dorsal pallium of a 3–6-month-old (3 cm-long) adult zebrafish (Adolf et al., 2006a; März et al., 2010a). Measurements of cell cycle length based on the doubling time of activated RG indicate that the G1-S-G2-M cycle is accomplished in ca. 2 days (Alunni et al., 2013). Following cumulative labeling and several weeks of chase, the BrdU label is retained in 1–2% of RG, some of which also stain positive for PCNA or MCM5 (Ganz et al., 2010; März et al., 2010a). Clonal analyses 4 weeks after lentiviral transfection also indicate that most (75%) transduced RG have remained as single cells, while the remainder generated small clones of 2–4 RG cells (Rothenaigner et al., 2011). Together, these data attest of the overall slow and to some extent heterogeneous proliferation rate of adult pallial RG. Under physiological conditions, it remains unknown whether this heterogeneity reflects the existence of RG subtypes, a hierarchy in the RG recruitment cascade (as postulated for mammalian neural and other stem cells), or simply stochastic variations within a population of otherwise equivalent cells. Abrogation of Notch signaling, a major pathway gating RG proliferation in the adult zebrafish pallium, can bring all RG into cycle, indicating that all have the capacity to cycle (Alunni et al., 2013; Chapouton et al., 2010). Finally, pulses of different thymidine analogues separated by a few days demonstrate that a second cycle of the same RG virtually never happens during this time frame but rather that different RG activate over time (unpub.). These data together suggest that the RG population is largely composed of quiescent RG that divide asynchronously. Analysis of the fucci zebrafish lines, as well as centrosome labeling, further indicates that most nondividing RG are held in the G0 phase of the cell cycle (unpub).

Progenitor cells of the pallial germinal zone comprise, in addition to RG, nonglial proliferating cells, interpreted as intermediate neuronal progenitors (März et al., 2010a), and the current model postulates that (at least) some of these progenitors are generated from RG. Short-term tracing of pallial RG divisions following lentiviral transduction or BrdU incorporation indicated a predominance of symmetric gliogenic divisions (ie. divisions generating two RG) over asymmetric divisions (ie. divisions generating one RG and one intermediate progenitor; Fig. 4A; Rothenaigner et al., 2011). Whether all individual RG are capable of both division modes, and how the switch between these modes is controlled, remains unknown. The high frequency of symmetric divisions contrasts with the behavior of adult mouse NSCs (Costa et al., 2011; Suh et al., 2007). In an opposite manner, mouse intermediate progenitors, at least in the SEZ, are considered actively cycling to amplify neuronal production (Doetsch et al., 1999), while nonglial progenitors in the adult zebrafish pallium follow a low number of divisions before differentiation (Rothenaigner et al., 2011). Thus, the amplification steps that underlie pallial neurogenesis in mammals and zebrafish likely differ, and it was proposed that the amplification potential of zebrafish RG accounts for the persistence of a large population of active and recruitable NSCs in adults.

**Radial Progenitors of the Adult Subpallium Are Constitutive Neuronal Progenitors.**

**Neuronal generation.** BrdU administration to zebrafish adults results in intense labeling of subpallial progenitors. These can be chased to reveal the neogeneration of neurons in two areas, the olfactory bulb and subpallium proper, over a few weeks (Adolf et al., 2006a; Grandel et al., 2006). Many of the labeled progenitors express PSA-NCAM, suggesting that they are migrating (März et al., 2010a). The horizontal migration of neuronal progenitors along the subpallial ventricular zone into the olfactory bulb could also be followed over a few micrometers in time-lapse imaging on explants (Kishimoto et al., 2011). This and the contribution to OB
neurogenesis led to the conclusion that progenitors found in the subpallium include, likely in addition to the radial progenitors (see above), a population in part equivalent to the “rostral migratory stream” (RMS) of mammals (Adolf et al., 2006a). The “zebrafish RMS” however differs from the mammalian RMS by the absence of glial migration guides. The lineage relationship between subpallial radial progenitors, the migrating PSA-NCAM-positive progenitors, and OB or subpallial parenchymal neurons remains unclear and an important point to assess. Supporting the hypothesis that radial progenitors and PSA-NCAM-positive migrating progenitors are intermingled progenitors of at least partially distinct lineages, it is to note that no radial progenitors have been described intermingled with the population of PSA-NCAM-positive progenitors in posterior subpallial locations.

In the olfactory bulb, adult-born neurons include GABAergic interneurons in the internal layer and TH-positive and GABAergic interneurons in the glomero-laminar layer, comparable to the situation in mammals (Adolf et al., 2006b). Unlike in mammals however, adult-born neurons in the zebrafish subpallium also settle radially into the subpallial parenchyma proper. These include GABAergic and TH-positive neurons, and likely other neuronal subtypes, yet to be characterized (Adolf et al., 2006b). In addition, goal-directed behavior (von Trotha et al., 2014) and light avoidance behavior (Lau et al., 2011) involve subpallial neurons, but the birthdate of these neurons has not been determined.

Division modes and rate. BrdU incorporation and the proportion of ventricular progenitors expressing PCNA are much higher in the subpallium than in the pallium, indicating overall a faster cell cycle. Detailed analyses of progenitors properties in this region are however complicated by the intermingling of radial and migrating progenitors, which cannot be distinguished in BrdU tracing experiments. Overall, bona fide NSC properties have not been demonstrated in the subpallium, and the division mode(s) of progenitors remain to be characterized. The high proliferation rate of these progenitors makes it possible that they correspond to “transit amplifying”-like compartment. It also remains unclear whether slow cycling progenitors exist in (or feed into) this domain, where they are located, and what is their nature. Label retention assays indicate the presence of some slower-cycling progenitors in this location (Adolf et al., 2006a; Lam et al., 2009; Marz et al., 2010a), but whether this is a transient property of all resident progenitors or reflects a specific subpopulation is not known. Subpallial progenitors, for their very low expression of glial markers, have been considered by some authors as NE progenitors (Ganz et al., 2010). We find the distinction difficult to make at this point.

Radial Progenitors and Constitutive Neurogenesis in the Hypothalamus. BrdU incorporation and neuronal generation was observed in several diencephalic areas, including the preoptic area, the thalamic ventricle, and the hypothalamus. Adult-born TH-positive neurons were documented in all these domains, and serotonin (5HT)-positive neurons in the PVO in the hypothalamus (Grandel et al., 2006). However, the exact origin of these different neurons remains uncertain, as BrdU incorporating cells include RG but also nonradial progenitors in their vicinity (Pellegrini et al., 2007). One exception may be the PVO, where the large majority of proliferating cells is comprised within the RG population, leading to the conclusion that these RG are at least a source of the PVO 5HT neurons (Perez et al., 2013).
Müller Glia and Constitutive Neurogenesis in the Retina. Constitutive neurogenesis in the teleost retina originates from two sources, the ciliary marginal zone (CMZ, also called circumferential germinial zone, which persists until adulthood) and Müller glia (Fig. 3C). While the CMZ generates all retinal cell subtypes, Müller glia are specialized in the generation of a specific lineage, rod photoreceptors. Proliferating at low frequency, Müller glia generate a small population of amplifying “rod precursors” that migrate into the ONL and are the primary source of new rods in the central retina (Bernardos et al., 2007). In goldfish, it has been proposed that this constitutive low-rate generation of rods responds to the lowering in rods density that results from the retinal stretching accompanying eye growth (Raymond et al., 1988). It may also result from a low rate of rod death (Morris et al., 2008).

RG or Radial Progenitors in Constitutive Gliogenesis

Amplification of RG Populations During Adult Life. Brain growth is observed lifelong in zebrafish, accompanying body growth, and is paralleled with increased brain ventricular surfaces (unpub.). Ventricular zones generally display a continuous arrangement of RG, suggesting that most of these cells are generated during adulthood. This was directly demonstrated by tracing symmetric gliogenic divisions of RG in the adult pallium (Rothenaigner et al., 2011). The progenitor cells at the origin of adult-born RG in other brain territories remains uncertain.

RG at the Origin of Oligodendrocytes in the Spinal Cord. The proliferative capacity of parenchymal Olig2;Sox10-double positive cells in the telencephalon suggests that these cells, rather than ventricular RG, are at the origin of adult-born oligodendrocytes in this territory. The availability of Cre-expressing lines driven by RG-specific promoters (Boniface et al., 2009) will however make it possible to directly assess the contribution of RG to adult oligodendrogenesis in many brain areas.

In the spinal cord, RG cells expressing Olig2 can be found to divide, incorporating BrdU or expressing PCNA. Tracing using GFP stability of the olig2:gfp transgene further suggests that, under physiological conditions, these progenitors most likely do not generate neurons. The increased number of oligodendrocytes over time in the adult spinal cord, and their expression of a common set of markers with ventricular RG progenitors (Olig2, Sox10), suggest that the latter cells may be involved in oligodendrocyte generation (Fig. 3E; Park et al., 2007).

RG or Radial Progenitors in Reactive Neurogenesis

Reactive neurogenesis has been induced in the zebrafish adult central nervous system by various means: stab wounds or toxic compounds in the telencephalon, retina and cerebellum, phototoxical lesions in the retina, or trans-sections in the spinal cord. In striking contrast with the situation in mammals, these lesions in zebrafish are followed by an efficient regenerative process generally leading to full functional recovery within a few weeks. In all cases studied, RG are involved in the regenerative response, as attested by a boost in their proliferation. Hence, such paradigms are instrumental both in enhancing the activation potential of constitutively active RG (e.g., in the pallium) and in revealing the progenitor potential of RG in domains where they are normally largely silent, such as the retina and spinal cord. In many cases however, it remains to be precisely demonstrated where activated RG sit in the cellular hierarchy(ies) leading to neuronal repair, and whether they are also involved in replenishing glial pools. Finally, it will be important to determine to which extent the molecular cascades that trigger RG activation and the division mode they adopt in response to lesion recapitulate an endogenous activation process.

Radial Glia and Reactive Neurogenesis in the Pallium: Boosting Existing Neurogenesis. Stab wounds of various sizes have been inflicted to the telencephalon using sticks that were inserted into the brain either through the nostrils, perforating the subpallium and pallium longitudinally (Ayari et al., 2010; Baumgart et al., 2012; Kroehne et al., 2011), or radially or obliquely through the dorsal pallial surface, lesioning concomitantly the choroid plexus and germinal zone itself (Diotel et al., 2013; Kishimoto et al., 2012; Márz et al., 2011). RG activation (ie. cell cycle re-entry) is a shared response of all these paradigms (Kizil et al., 2012a; Schmidt et al., 2013) between approximately 2–3 days postlesion (dpl) and 7 dpl. In one important study, RG fate was genetically traced using her4:CreERT-mediated recombination, the expression of which is specific of quiescent RG prior to lesion. At 21 dpl, genetically labeled Hu-positive neurons were found at the site of lesion, unambiguously demonstrating that initially quiescent RG were recruited to generate neurons in the repair process (Fig. 3A; Kroehne et al., 2011). Different RG markers nonetheless highlight different proportions of RG re-entering division upon lesion (e.g., an important reactivation of her4-positive cells, some reactivation of S100β- or BLBP-positive cells, no reactivation of AroB-positive cells; Baumgart et al., 2012; Diotel et al., 2013; Márz et al., 2011; Kroehne et al., 2011), suggesting the existence of subpopulations of RG with different functions and/or sensitivity to the repair process. Further, some RG remaining silent were shown to upregulate a quiescence-promoting factor, Id1, suggesting an active refractory response to reactivation that may be used to preserve in part the NSC pool (Schmidt et al., 2014). In addition to RG, proliferation is
boosted in pallial nonglial “intermediate” (“transit amplifying”-like) progenitors upon lesion (Baumgart et al., 2012; Máz et al., 2011), suggesting that progenitor recruitment is possible directly at a level downstream of NSCs. Whether this differential recruitment is a general phenomenon or is dictated by the type or extent of the lesion, for example, remains to be assessed. Overall, which exact cellular hierarchies are involved in the neuronal regeneration process, and possibly also in the GZ replenishment process following its recruitment for repair, is unknown.

RG properties other than activation rate may be modified upon parenchymal lesion. Interestingly, in all paradigms studied, the precursors of regenerated neurons migrate to the lesioned area, which is located far deeper into the parenchyma than the area normally populated by adult-born neurons. In addition, the germinal zone areas activated during the repair process can be from a different neuroanatomical domain (this is obviously more evident in the case of small wounds, for example, where RG in the medial and dorsal pallium—Dm and Dd domains—are reactivated upon a lesion in the central pallium—Dc; Baumgart et al., 2012; Kishimoto et al., 2012). Although this has not been formally demonstrated, these observations suggest that the neuronal subtypes generated from stab wound-activated RG may be different from those they would generate under constitutive neurogenesis. A recent study on the telencephalic lesion model using quinolinic acid further supports this conclusion: lesion-activated RG, traced with her4:CreERT, could regenerate long-distance projection neurons located deep into the parenchyma at the site of lesion and extending axons contra-laterally through the anterior commissure (Skaggs et al., 2014). Thus, lesion-activated RG participated in re-establishing a circuitry normally not generated at this stage by RG NSCs during constitutive neurogenesis.

Radial Glia and Reactive Neurogenesis in the Retina: Activating Müller Glia. The best-studied repair process to date involving RG is certainly retinal regeneration. Over the years, several lesion paradigms have been developed (chemical, thermal, light, mechanical, cytotoxic, or genetic), which differ in the extent of the lesion generated and the cell types affected. Specifically, it was shown that most injury modes stimulate a proliferative response from Müller glial cells, and are followed by the regeneration of the lesioned neuronal subtypes (Fig. 3C; reviewed in Goldman, 2014; Gorsuch and Hyde, 2014; Lenkowski and Raymond, 2014). Müller glia (followed by the gfp:gfp or alpha1tubulin:CreERT transgenes or GS expression) were suspected to be at the origin of neuronal regeneration in a number of studies, since they generally constitute the main proliferating cell type of the regenerating retina (after a transient microglial reaction; Fausett and Goldman, 2006), and BrdU tracing indicates that proliferating progenitors then migrate and give rise to bipolar and amacrine cells in the INL and to cone photoreceptors in the ONL (Fausett and Goldman, 2006). Genetic tracing, conducted using a alpha1tubulin:CreERT2 transgene following a small mechanical lesion, confirms that reactivated Müller glial cells are recruited to give rise to cone photoreceptors, bipolar and amacrine cells, and Müller glia, under these conditions (Ramachandran et al., 2010). Thus, reactive Müller glia can bias their normal fate (rods) in response to lesion to regenerate the sensing neuronal subtypes. Not all Müller glia however reinitiate division, highlighting heterogeneity within this population, and single Müller glial cells (as opposed to the population, e.g., using sparse recombination) were not traced to assess multipotency.

Proliferation of Müller glia is (directly or indirectly) necessary for the regenerative response, since blocking proliferation with PCNA morpholinos results in Müller glia death and a failure to generate new neurons (Thummel et al., 2008). Like in embryonic NE cells and RG, INM accompanies the reactive proliferation of Müller glia (Nagashima, 2004). Although the exact regeneration cascade from Müller glia has not been traced in all cases, their proliferation response to regenerate rods following light-induced damage, and INL neurons following ouabain treatment, involves asymmetrical self-renewing divisions that, like constitutive rod neurogenesis, generate intermediate amplifying progenitors in the ONL (Nagashima, 2004). These observations argue that reactive Müller glia behave as bona fide, self-renewing NSCs. Interestingly, within this sequence of events, the extent of the lesion within the rod population, and the specificity of the lesion, may determine the step at which progenitors are recruited: large rod lesions and lesions affecting other retinal neuronal types recruit Müller glia cells, while small rod lesions primarily recruit ONL-located precursors (Montgomery et al., 2010). How this specificity is controlled, as well as which exact cellular hierarchies are involved in reparative neurogenesis, remain important questions that will benefit from generating targeted genetic lesions and tracing.

Radial Glia and Reactive Neurogenesis in the Optic Tectum. In terms of progenitor cells and growth, the organization of the teleost TeO is very similar to that of the retina, with the maintenance of a marginal nonglial proliferation zone responsible for life-long neuronal addition (see below), while tectal RG appear nonproliferating (Fig. 3B; Alunni et al., 2010; Iro et al., 2010). Thus, it is tempting to speculate that tectal RG could, like Müller glial cells, serve as an additional progenitor source activated for repair upon lesion (and perhaps for low-rate neuronal production under physiological conditions). To date, no studies directly address this question.
Radial Glia and Reactive Neurogenesis in the Spinal Cord. Lesions in the spinal cord are generally inflicted mechanically, by transection. Proliferation, starting 3 days after lesion and peaking at 2 weeks, is induced around the central canal in ependymoglial cells (Reimer et al., 2008). Using GFP stability as a tracer in *olig2:gfp* transgenics together with BrdU labeling, the generation of motorneurons (HB9- or Isl1/2-positive cells) from activated Olig2-positive progenitors could be documented (Fig. 3E; Reimer et al., 2008). Thus, the normal fate of these progenitors, i.e., oligodendrocytes generation, is modified toward producing motorneurons in response to lesion. More dorsally located ependymoglial cells, expressing the transcription factors Pax6 and Nkx6.1 and activated as well upon lesion, may be at the origin of regenerated V2 interneurons (Kuscha et al., 2012b). The fate of other activated ependymoglial cells, e.g., oligodendrocytes generation, is modified toward producing motorneurons in response to lesion. More dorsally located ependymoglial cells, expressing the transcription factors Pax6 and Nkx6.1 and activated as well upon lesion, may be at the origin of regenerated V2 interneurons (Kuscha et al., 2012b). The fate of other activated ependymoglial cells, e.g., those expressing *shh* ventrally or those located in the alar plate, has not been traced to date (Reimer et al., 2009). Other neuronal subtypes, including Pax2-positive interneurons and spinal serotonergic neurons, are regenerated as well (Kuscha et al., 2012a, 2012b). To date, the regenerating spinal cord is the only case in the zebrafish central nervous system where some hints on the factors modulating the neuronal identities produced have been proposed: the neurotransmitter dopamine, originating from descending projection neurons, biases toward motorneuron production at the expense of V2 interneurons (Reimer et al., 2013).

Single cell fate has not been determined within the spinal ventricular ependymoglial population upon lesion. The increased number of such cells in reaction to lesion indicates the occurrence of symmetric gliogenic divisions (Reimer et al., 2009). Which division type is involved in the generation of neurons, and whether division mode is controlled at the single cell or population level, is not known.

Activating Adult Radial Glia

Work conducted in mice in a number of organs, including the brain, suggests that the frequency of SC activation events conditions, in the long term, SC maintenance (for example, see Calzolari et al., 2015; de Haan et al., 1997; Encinas et al., 2011; Vaziri et al., 2013). This may be linked with some senescence or lack of self-renewal taking place over time. This makes it crucial to understand which molecular and cellular mechanisms gate SC activation from quiescence.

In most areas discussed above (pallium, hypothalamus, retina, spinal cord), RG progenitors involved in constitutive neurogenesis are characterized by a strong quiescence. Whether different RG subtypes exist with respect to this parameter remains unclear, but the presence of label-retaining cells in most of these domains was demonstrated: RG having incorporated and maintained BrdU can be found in division (ie. expressing proliferation markers such as PCNA or MCM proteins) after a few weeks of chase (März et al., 2010a). While this, per se, does not attest of NSC character, it shows that at least some RG divide slowly and recurrently. Zebrafish pallial RG also display a decreased activation frequency with aging, and can even get lost in the medial pallium in some individuals, with a fusion of the two pallial hemispheres (Edelmann et al., 2013). Perhaps in link with the regionalized occurrence of this phenomenon, the medial pallium belongs to the germinal zone domain where telencephalic RG cells are the earliest formed in embryos and maintained as neurogenic RG until adulthood (Dirian et al., 2014), and harbors the highest activation rate along the dorso-medial ventricular zone in young adults (Alunni et al., 2013). Together, these points suggest that adult zebrafish RG progenitors can help provide information on the control of the quiescence/activation balance of adult NSCs and its impact on NSC maintenance.

Significant contributions on this front were made recently, which both stress striking similarities in the control of adult NSC physiology between zebrafish and mammals and allowed moving beyond current understanding. Various signaling pathways, morphogens, neurotransmitter and environmental stimuli were identified to promote or restrict NSC activation. An important question remains to determine the extent to which these same pathways are involved in activating RG in regenerative contexts.

Notch Signaling as a Gatekeeper of Endogenous NSC Activation

RG in most subdivisions of the zebrafish adult central nervous system express high levels of *notch* receptor genes (Chapouton et al., 2010). Although, to our knowledge, it has proven very difficult to date to provide convincing immunohistochemical staining for Notch intracellular fragments in this system (but see Berberoglu et al., 2014), several Notch signaling target genes are also expressed in RG, among which the basic helix-loop-helix (bHLH) protein-encoding genes of the *hairless(spl)* family such as her4 and her15 (related to mammalian *Hes5*), and her6 and her9 (*Hes1*; Chapouton et al., 2010, 2011; Ganz et al., 2010), attesting of active Notch signaling in RG. In one recent report, the NICD fragment was also detected in the nuclei of RG (Berberoglu et al., 2014), at higher level in quiescent than in activated RG, in a manner reminiscent of *her4* (Berberoglu et al., 2014).

The function of Notch in RG was directly assessed in the pallium, retina, and spinal cord. Lowering Notch signaling systemically with gamma-secretase inhibitors leads to the progressive activation of all pallial RG, which turn on expression of PCNA and MCM proteins and complete a first division within 2 days and further divisions at this rate if the
Notch signaling was evidenced in the context of regeneration spinal cord, a comparable quiescence-promoting effect of knock-down of (Alunni et al., 2013). Accordingly, morpholino-mediated entry in some M

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Although some discrepancies exist, possibly due to the efficiency of following a first division (Alunni et al., 2013). Whether the latter observation reflects different RG subtypes, or is in link with the position of the cell within the quiescence phase, is not known. In line with the second interpretation, we demonstrated that RG found in S phase at a given time are very rarely found in S phase again a few days later, suggesting the existence of a refractory phase in RG activation following a first division (Alunni et al., 2013). Whether upstream and downstream regulators are shared remains to be verified.

The mechanisms leading to Notch activation in RG (and spatio-temporally controlled inhibition to control RG activation events) largely remain to be identified. In the pallium, the zinc-finger transcription factor Fezf2, expressed at high levels in quiescent RG, is necessary for the maintenance of quiescence and correlates with a high level of Notch signaling (Berberoglu et al., 2014), suggesting that it may act upstream of Notch (or indirectly affect Notch signaling via its regulation of RG state). The Notch ligand DeltaA is strongly expressed in activated RG and nonglial proliferating progenitors along the pallial VZ, suggesting that these cells may promote quiescence in neighboring RG, in a negative feedback-like mechanism limiting proliferation and recruitment within the pool of progenitor cells (Chapouton et al., 2010). Other ligands, such as Jagged, are also expressed in this location.

Other Pathways Controlling RG Quiescence/ Activation: Learning from Regeneration

Not surprisingly, most pathways promoting RG activation have been identified in the context of reparative neurogenesis. In the retina, Ascl1a and Stat3 are necessary for the proliferative response and neurogenesis upon mechanical lesion (Conner et al., 2014; Faussett et al., 2008). Ascl1a upregulates expression of Pax6 and Wnt signaling, and directly binds the promoters of the alpha1-tubulin and lin-28 genes, which are both activated during the repair process in reactive Müller glia (Faussett et al., 2008). The RNA-binding protein Lin-28, through repression of the miRNA let-7, in turn permits the lesion-dependent increased expression of hspd1, klf4, oct4 (pou5f3) and c-myc and induced expression of pax6, ascl1a and c-myc (Ramachandran et al., 2010a). Wnt signaling is also sufficient to elicit a similar gene expression response and
Müller glia activation (Ramachandran et al., 2011). Several pathways cross-talking and synergizing with Wnt/β-Catenin were recently identified, including Insulin, Insulin-like growth factor 1 (IGF-1), FGF2, Heparin-binding EGF-like growth factor (HB-EGF; Wan et al., 2014). Except for IGF-1 and FGF2, which are potent together but have little effect alone, activation of these pathways in isolation is sufficient to induce Müller glia activation and the partial reprogramming necessary to confer them, at the population level, a multipotent progenitor status capable of generating the different retinal neuronal subtypes. These pathways converge onto the activation of MAPK and PI3K, and, downstream, Stat3 and β-Catenin (Wan et al., 2014, reviewed in Goldman, 2014). In parallel, expression of the transcriptional co-repressors Tgif1 and Six3b, induced upon photoreceptor lesion and likely antagonizing the activity of Smad2/3, limit TGFβ signaling to permit the reactive proliferation of Müller glia and lower the gliosis response (Lenkowski et al., 2013; Lenkowski and Raymond, 2014). These factors may also promote activity of Ascl1a, Pax6 and Wnt (Lenkowski et al., 2013).

In the pallium, mechanical lesions induce activation of the transcription factor Gata3 in RG, which is necessary to promote RG activation. Expression of Gata3 in this context depends on Fgf signaling. Fgf also promotes progenitor cell proliferation under physiological conditions (Ganz et al., 2010) but, both in the naïve and lesioned contexts, whether this regulation directly takes place in RG is not clear. In contrast to the factors above, however, Gata3 alone is not sufficient to trigger the re-entry of RG into cycle, indicating that its activity depends on a regenerative context, or at least on a context prone to NSC activation (Kizil et al., 2012b).

Finally, in the spinal cord, one of the earliest factors over-expressed in ventricular ependymogial cells after injury is Sox2, and blocking Sox2 function lowers the proliferative response (Ogai et al., 2014). Sox2 may act redundantly with Sox11 (Guo et al., 2011). Activation of ependymogial cells along the central canal upon lesion also depends, at least in part, on Shh signaling, which is enhanced during repair in ventral ependymogial cells (Reimer et al., 2009). The upregulation of Shh itself is promoted by the neurotransmitter Dopamine, released from transected descending axons (Reimer et al., 2013).

Activation of these pathways/factors in RG could be triggered by cytokines produced during the inflammatory response, or by factors released by injured neurons. In the pallium, the leukotriene C4 (LTC4) inflammation pathway, mediated by the cysteinyl leukotriene receptor 1 (CysLT1) is activated in RG upon lesion, is necessary for RG reactive proliferation, and is by itself sufficient to induce Gata3 expression in RG and cell cycle re-entry (Kyrizis et al., 2012). TNFα released by dying neurons activates Müller glia in the lesioned retina (Nelson et al., 2013; Wán et al., 2014).

Importantly, while activation of several of these pathways/factors is sufficient to trigger RG activation in the uninjured germinial zones, they are not expressed at detectable levels under physiological conditions. This is the case for Wnt/Insulin/IGF-1/FGF2/HB-EGF components, as well as for Lin-28/Sox2/Nanog/c-Mycb in the retina, and for Gata3 in the pallium. Because activated RG are generally rare under physiological conditions, they may be difficult to sample in expression analyses. Thus, one may wonder whether activation of these pathways truly reflects the use of unique cascades during regenerative RG recruitment, or may be shared with the “normal” activated state of RG under physiological conditions. Addressing this question directly remains key for the future.

In link with this, how to interpret RG activation needs to be discussed. In the retina, Müller glia activation during regeneration is generally interpreted as a partial “dedifferentiation” process, permitting cells to re-enter the cell cycle and act as progenitor cells. Arguments in favor of this interpretation are the concomitant downregulation of glial marker genes such as GFAP, and the re-expression of pluripotency factors such as Lin-28, Sox2, Nanog, and c-Mycb. To which extent this corresponds to the “normal” activated state of Müller glia under physiological conditions is not clear. Further, the low level expression of another subset of pluripotency factors in the uninjured retina (Klf4, Oct4, and c-Mycb), and the hypomethylation status of several pluripotency genes in quiescent Müller glia (ascl1a, insm1a, hbegfa, lin28, sox2, oct4, nanog, c-mycb, and klf4), suggest that Müller glia constantly retain, albeit partially, some progenitor signature (Powell et al., 2013). RG activation in the pallium is a more frequent event and, to our knowledge, is not accompanied by downregulated expression of glial attributes. Pluripotency factors such as Sox2 for example are steadily expressed in both quiescent and activated RG. Thus, it is possible that the physiological differentiation status of pallial and retinal RG are different. Finally, it will be important to determine whether “dedifferentiation” and cell cycle re-entry can be uncoupled. In the retina for example, whether Notch blockade alone has the full potency to re-induce pluripotency markers remains to be shown.

Preserving NSCs, Limiting the Regenerative Response, and Limiting Gliosis in Response to Lesion

Among the factors discussed above, Notch3 signaling and Id1 appear upregulated upon lesion in the pallium, and antagonize RG proliferation in this context (de Oliveira-Carlos et al., 2013). Thus, a quiescence-promoting, negative response to lesion is induced, which involves an enhancement of the normal quiescence-maintenance cascade. This reaction could be
necessary to limit RG recruitment for repair and avoid exhaustion of the SC pool. Along similar lines, expression of the transcription factor Insm1a is initially induced by Ascl1a to potentiate its proliferation/dedifferentiation effects in reactive Müller glia, but displays a later expression phase a few days postlesion to limit the reactive zone. This is achieved by inhibiting expression of HB-EGFα, and induces the differentiation of newly formed neuronal precursors. Insm1a acts in the latter process, in part, by upregulating expression of p57kip (Ramachandran et al., 2012).

Other levels of control must also take place, for instance to adjust the identity of regenerated neurons to those needing replacement, or to prevent the overproduction of neurons in response to lesion. On the latter point, we note that this control can be bypassed in the case of extensive retinal lesions, where an overproduction of neurons can be observed, or when a reactive response is initiated by stimulating inflammation without injury (hence without neurons to be replaced) in the pallium (Kyritsis et al., 2012; Sherpa et al., 2014).

Finally, a striking difference between zebrafish and mammals is the lack of glial scaring in the former species, which likely plays an important role in the efficiency of regenerative neurogenesis. Like in mammals, an early upregulation in the expression of GFAP and BLBP by Müller glia follows lesion in the retina, but this response is transient. In the case of large wounds in the pallium, RG also upregulate their expression of intermediate filament proteins such as GFAP, Vimentin or Nestin and of the calcium-binding protein S100β, and exhibit hypertrophic processes. This resembles a reactive gliosis response, although the displacement of RG cells toward the lesion, and scar formation, appear minimal and transient (Baumgart et al., 2012; März et al., 2011). Given the deleterious effects of reactive gliosis on repair in mammals, it is of high interest to understand the bases for this attenuated glial response in zebrafish. In the retina, endogenous limitation of the TGFβ response plays a key role in this process (Lenkowski et al., 2013).

**Physiological Status and Environmental Stimuli**

Variations in the physiological status of the animal, outside of regenerative conditions, are important players controlling the activation state of germinal zones in the mammalian forebrain. These aspects remain understudied in the zebrafish. The lack of standardized animal maintenance conditions (including the lack of inbred lines) introduces a large degree of variability between animals that complicates the reading of subtle effects. Nevertheless, several neurotransmitters and hormones were recently shown to impact RG activation. In the PVO for example, serotonine (5HT) is necessary for the proliferation of radial progenitors (Perez et al., 2013), and the projection of 5HT processes toward pallial germinal zones suggests that it may be the case in this location as well (Lillesaar et al., 2009).

AromataseB (cyp19a1b), which converts androgens to estrogen, is strongly expressed in zebrafish RG (like in mammalian astrocytes under physiological conditions), and is itself expressed in response to oestrogens, indicating that RG both produce and receive this signal (Menuet et al., 2005; Mouriec et al., 2009). Blocking Aromatase activity in adult zebrafish led to a trend increase in the number of proliferating progenitors in several brain areas, notably the pallium, suggesting that oestrogens lower proliferation (Diotel et al., 2013). Whether they primarily target RG or nonglial proliferating progenitors remains however to be assessed. In line with a quiescence-promoting effect on RG, RG maintaining AroB expression after injury are not activated and presumably do not participate in the repair process (Diotel et al., 2013). The roles of other hormones in zebrafish have not been assessed yet.

Finally, recent works addressed the effect of behavioral challenges or sensory stimuli on zebrafish adult neurogenesis. Most interestingly, it was found that sensory stimulation (chemosensory or visual stimulations) selectively affected the neurogenic niches in brain areas involved in processing these stimulations, and this in different ways: for example, enriched odors increased the number of adult-born neurons in the OB and vagal lobe, while modifying the spectrum of light or brightness decreased the size of the progenitor niche in the TeO area (Lindsey et al., 2014). Changes in the social context, such as social isolation or social novelty, also affect (decrease) the number of proliferating progenitors in sensory niches and increase the number of newborn neurons, in a manner independent of the levels of cortisol, the steroid hormones released in response to stress (Lindsey and Tropet, 2014). The number and complexity of neurogenic niches in teleosts, and the complex repertoire of behaviors that a zebrafish can exhibit, suggest that much is to be learned from this model in terms of the environmental modulation of adult stem cell pools and neurogenesis.

**Neurogenesis Cascades From Adult RG**

Adult neurogenesis cascades have not been extensively studied using the zebrafish. The expression of proneural factors (Ascl1, Neurog1) in proliferating, non-RG progenitors, as well as the involvement of Notch1 in progenitor maintenance, suggests a recapitulation of embryonic cascades starting from the amplifying neurogenic progenitors step, after these are produced from activated RG (Alunni et al., 2013; Chaperon et al., 2010).

The factors driving RG toward neurogenic divisions and/or the generation of neuronal progenitors remain also understudied.
Non-RG Adult NSCs

RG are not however the only NSC type in the zebrafish adult brain. Indeed, an interesting finding of recent years is the fact that cells with neuroepithelial (NE) characteristics, rather than RG, are retained in some territories of the adult zebrafish brain and may serve as adult neural progenitors under physiological and/or regenerative conditions. In most instances, key questions remain to define the cellular hierarchies involving these NE progenitors during adult neurogenesis, and to determine whether they are also at the origin of the RG considered as NSCs. The long-lasting maintenance of these NE pools is also an issue given that, in several instances, they happen to be highly proliferative. Finally, whether such pools could have equivalent(s) in mammals remains a provocative but interesting issue.

Neuroepithelial Progenitors in the Retina and Optic Tectum

The first territory where a long-lasting neurogenic NE pool was described is certainly the adult zebrafish retina, where highly proliferating cells negative for mature glial markers (e.g., GFAP) but positive for progenitor and NSC markers (Nestin, BLBP, Sox2) are present at the CMZ, at the interface between the neural retina and the ciliary epithelium (Fig. 3C; Raymond et al., 2006). Tracing individual CMZ cells from the embryonic and juvenile Medaka demonstrates that the CMZ contains multipotent retinal stem cells that generate all the neuronal subtypes, as well as Müller glia, found in the adult eye (with the exception, under physiological conditions, of the central retina; Centanin et al., 2011, 2014). The persistence of such multipotent stem cells into adulthood is suggested by the fact that progeny clones from one embryonic or juvenile CMZ progenitor retain long-lasting contact with the CMZ. In agreement with such multilineage capacity, progenitors located at the adult CMZ tip maintain expression of early markers of retinal identity, such as *rx1*, *wnt2/Cdx10* or *pax6a*. Starting from this territory (peripheral CMZ), the CMZ is organized as concentric rings (middle and central CMZ) of progressively increasing commitment. Expression of the *hairy/spl* gene *her6* is restricted to the peripheral CMZ. Like NE progenitors, CMZ progenitors also strongly express Cadherin2 (N-Cad), contact both the apical surface and basal lamina of the neural retina. Pioneural genes such as *asc11a*, and *notch/delta* pathway genes, are most prominently expressed in the middle CMZ, and genes activated at differentiation stages, such as *neuroD*, in the central CMZ. In *Xenopus* at postembryonic stages, it was shown that the transition from tip CMZ stem cells to committed progenitors was controlled by the antagonistic actions of Wnt (active at the tip) and Shh (active further centrally) signaling (Borday et al., 2012).

Together, these results suggest that, while adult CMZ progenitors are at the origin of Müller glia, which can themselves act as neural progenitors, their primary contribution to adult neurogenesis does not involve a transition through the RG state. From the genes expression analyzed, retinal adult neurogenesis also largely recapitulates an embryonic neurogenesis sequence. Many questions remain open pertaining to the cellular properties of adult CMZ cells as potential NSCs. The first is to determine which cellular hierarchies, notably within the CMZ tip, sustain persistent neurogenesis in the adult: do quiescent CMZ tip cells exist that generate the bulk of the CMZ proliferating pool, and which relevant markers do these cells express? Are these cells inherited from selected embryonic long-lasting progenitors that maintain the same properties and role through life? Also, are they multipotent at the single cell level, as was shown in the embryo, or do they exhibit some degree of fate specialization? Finally, what are the relevant factors of their niche and, in that respect, is their localization “at the tip” a meaningful characteristic?

A seemingly similar organization is found at the posterior edge of the periventricular gray zone (PGZ) of the adult TeO, where a persistent growth zone has been described through life in goldfish, Medaka and zebrafish (Fig. 3B; Raymond and Easter, 1983). Most cells of the adult posterior tectal zone display a high proliferative activity, express progenitor markers (e.g., Sox2, Musashi, Bmi1), show polarized expression of apical markers (ZO-1, gamma-tubulin, APKe), but do not express glial markers, hence exhibiting characteristics of NE progenitors (Alunni et al., 2010). Their tracing with BrdU indicates that they contribute, as a population, to glutamatergic or GABAergic neurons in the PGZ layer, as well as to oligodendrocytes, and also generate the RG lining the ventral side of the PGZ. Successive pulses with different thymidine analogues, followed by a chase, also indicate that this zone, like the retina CMZ, is ordered by progressive stages of commitment: the most proliferating and least committed progenitors are found close to the “tip” (i.e. in the most posterior location) and commitment is increasing with cell’s “age” as one moves toward anterior. Interestingly, in its most posterior domain, the TeO growth zone ends in a narrow column of aligned cells with NE characteristics, bridging the TeO (along the torus longitudinalis), the tegmentum and the cerebellar valvula (Alunni et al., 2010; Chapouton et al., 2006; Ito et al., 2010; Lindsey et al., 2014). This population is possibly inherited from an equivalent cell pool described at embryonic and early postembryonic stages and referred to as the “posterior marginal layer” (PML; Recher et al., 2013). Both the embryonic and adult PMLs display a similar columnar arrangement and express the *hairy/spl* gene *her5* (Chapouton et al., 2006). Long-term BrdU tracing indicates that the tip of the TeO growth zone contains a
Neuroepithelial Progenitors in the Adult Pallium

Unexpectedly, NE progenitors were also recently discovered in the adult pallium (Dirian et al., 2014; Fig. 3A). As described above, the large majority of the adult pallial ventricular zone is occupied by RG that act as constitutive NSCs. However, the exact location and nature of the adult progenitor cells driving tectal (and segmental) growth remains uncertain. Whether these are a specific subpopulation of cells, or rather a temporary cell state, which are their specific markers, whether they exhibit NSC properties at the single cell level, and whether they are inherited from long-lasting progenitors maintaining such properties through life, remain open questions. The exact cellular hierarchies driving neurogenesis in this domain are also unclear. However, like for the retina CMZ, the fact that RG generated from the growth zone rapidly become quiescent makes it very likely that the nonglial progenitors of the growth zone do directly act as a major source of adult-born neurons, ie. without an obligatory transition step through a RG identity.

Neuroepithelial Progenitors in the Adult Cerebellum

Neurons are continuously added to the adult cerebellum, albeit at a decreased rate in adults, from a ventricular progenitor zone located dorso-medially along the cerebellar recess, a remnant of the IVth ventricle, in the corpus and valvula cerebelli (Kaslin et al., 2009). Progenitors in this cap-like structure do not express RG markers but are positive for the progenitor markers Nestin, Sox2, Meis, and Musashi, and exhibit apico-basal polarity, illustrated by the ventricular localization of ZO-1. They generate proliferating granule cell precursors that eventually differentiate into granule cell interneurons, the main neuronal subtype generated in the adult zebrafish cerebellum (Fig. 3D). These neurons settle individually into the cerebellar granule zone to complete the existing circuitry. This adult progenitor domain is inherited from embryonic cerebellar upper rhombic lip (URL) progenitors, the URL thus being involved in generating granule interneurons through life (Kizil et al., 2012a; Kaslin et al., 2013). BrdU pulse-chase assays demonstrate that the cap-like progenitor pool contains label-retaining cells, although, again, whether this identifies a specific subpool of progenitors remains unclear. In contrast, the embryonic and juvenile ventricular zone progenitors, involved in generating other cerebellar cell types, give rise to quiescent RG located below the cap structure in the adult cerebellum and that are mostly silent in adult. Thus, because of the dual origin of cerebellar neurons and the differential maintenance of the corresponding progenitors through life, neurogenesis from NE progenitors in the adult zebrafish cerebellum does not generate all neural subtypes of the adult structure (Kaslin et al., 2013). This contrasts with the situation in the retina, TeO and pallium. Furthermore, adult cerebellar neurogenesis does not involve a local growth zone. Whether adult NE progenitors of the cap-like structure would be capable of increasing their neurogenic repertoire under regenerative conditions remains to be determined.

Stem or Progenitor Cells?

The definition of a stem cell is based on functional criteria (self-renewal and multipotency, at the single cell level) that
are often difficult to rigorously assess experimentally, in particular through the life of an individual.

Of the zebrafish RG progenitors discussed above, the only one tested, and demonstrated, to get close to the rigorous definition of a NSC are RG of the pallium. Indeed, brainbow-mediated tracing from embryonic stages until adulthood revealed a large number of clones consisting of ventricular RG and parenchymal neurons produced at all stages of developmental juvenile and adult life (until the time of analysis; Dirian et al., 2014; Fig. 4C). The presence of several RG per clone, together with the known progenitor status of adult RG, demonstrates the occurrence of at least some (symmetric) self-renewing RG divisions from the embryonic progenitor that was uniquely labeled, and the additional presence of neurons attests of bipotency of this original progenitor. The individual properties of adult pallial RG over successive divisions during adulthood, however, have not been tested using such a clonal method yet, and the potential heterogeneity of the adult RG population in terms of the individual potency and division frequency of each cell is not known. Accordingly, it remains unclear whether the pallial germinial zone uses single cell- or population-based rules to maintain its homeostasis, and how this compares with the clonal rules used in other NSC systems (Calzolari et al., 2015; Klein and Simons, 2011; Simons and Clevers, 2011).

The NSC status of other RG in the zebrafish adult central nervous has been tested, at best, over a limited number of cell divisions. Self-renewing and multipotent cell divisions were observed in clonal analyses of Müller glia under regenerative conditions. This interesting study demonstrated the maintenance, in each clone, of one cell expressing RG markers following Müller glia reactivation (Nagashima, 2004). Whether the Müller glia generated through this reactive asymmetrical division is however capable of itself being reactivated and self-renewing if challenged in additional lesional events, hence whether it harbors bona fide NSC properties, remains an important point to assess.

The discovery of maintained NE progenitors in the adult brain, and, in several cases (TeO, retina, pallium), their generation of RG that could act as progenitors, poses the question of their NSC character and their position relative to RG in the NSC hierarchy. In the lateral pallium, which is generated from the NE progenitor pool, brainbow tracing revealed that individual embryonic NE progenitors generate clones containing ventricular RG and lateral pallial neurons, demonstrating multipotency. Whether each of these clones retains a/some adult NE progenitor(s), or whether new NE progenitors populate the late NE pool from another source to accommodate RG generation and neurogenesis in the lateral pallium, has not been rigorously assessed (Fig. 4B,C). As mentioned, a surprising feature of the NE pool is its overall high proliferating activity, a feature that cannot be immediately reconciled with the long-lasting maintenance of stem cells. One could imagine either the existence of a few steadily quiescent cells within this pool, of quiescence phases within the life of each cell of this pool, or of a regular reconstruction of the pool from another, quiescent source. On the latter point, it is difficult to imagine that the neighboring RG could play such a role, since the genetic tracing of pallial RG never gave rise to cells in the position of the NE pool. Thus, the upstream quiescent source would be located elsewhere. The same series of questions remains to be asked for all other adult NE pools described above.

What Did/Can Zebrafish Adult RG Teach Us of NSC Biology?

The shared molecular and cellular attributes of mammalian and zebrafish NSCs in the adult pallium was an initial justification for using the zebrafish model to gain relevant and evolutionary insight into adult NSC biology. Much beyond this, the maintenance of germinal niches containing long-lasting progenitors in many areas of the zebrafish adult central nervous system, the recently revealed huge diversity of adult NSCs in this model, the varieties of their potential and the diversity of their fates, make the zebrafish an invaluable source of information to appreciate the different strategies that can sustain adult neurogenesis and NSC maintenance in an adult vertebrate. One hope is to be able to discriminate the obligatory versus diverged components of the adult NSC state and fate. Another is to make use of the high neurogenic activity and reactivation potential of zebrafish germinal pools, both under physiological and regenerative conditions, to gain insight into the relevant regulatory cascades involved and to evaluate the strategies leading to efficient adult neurogenesis. Finally, the discovery of distinct NSC subtypes in the zebrafish pallium, the territory homologous to that hosting mammalian adult NSCs, raises the question of the evolutionary conservation of these different progenitor subtypes and cascades in this domain.

NSCs and Neurogenesis in the Adult Zebrafish Are Not a Mere Continuation of Embryogenesis and Teach Us About an Adult Process

Under proper nutritional conditions, the zebrafish grows lifelong. In addition, little cell death has been observed among adult-born neurons, suggesting that most of these do add to the existing circuits (Rothenaigner et al., 2011). This has been interpreted to mean that adult neurogenesis is primarily taking place to accommodate growth, in a manner more akin to embryonic neurogenesis than to the adult process of interest in mammals. Several strong arguments, pertaining to NSCs or to neurogenesis, stand however against this interpretation.
As discussed, zebrafish adult RG are largely quiescent. In the pallium, where RG are involved in constitutive neurogenesis, the average time between two divisions has been estimated to one month (Rothenaigner et al., 2011), which is of the range postulated in mouse for adult NSCs of the SEZ and SGZ. Adult pallial RG require a primary step of activation before contributing to the neurogenesis, and this step is gated by Notch3 signaling (Alunni et al., 2013). This is unlike embryonic neural progenitors, which do not enter quiescence, and do not express notch3. In the adult CNS, Notch3 maintains NSCs, but we postulate that this is rather indirect through limiting the number of NSC divisions and NSC exhaustion, given that Notch3 blockade primarily triggers NSC amplification. In contrast, the maintenance of embryonic neural progenitors requires Notch1 activity, thus resembles the process involved in both the adult zebrafish and mouse to maintain the progenitor status of transit amplifying progenitors (Ables et al., 2010; Ehms et al., 2010; Imayoshi et al., 2010). The role of Notch3 in gating NSC activation in the adult zebrafish pallium is also reminiscent of the molecular process controlling activation of satellite cells in the adult muscle, where Notch3 also limits satellite cell activation (Bjornson et al., 2012; Kitamoto and Hanaoka, 2010; Mourikis et al., 2012; Mourikis and Tadjbakhsh, 2014). Thus, the cell cycle characteristics of zebrafish adult pallial RG, and its molecular control, is similar to the processes controlling SC activation in mature organs such as the brain and muscle in the adult mouse.

A second important argument lies in the selectivity of adult neurogenesis in zebrafish. Unlike an embryonic process, which would continuously generate all neuronal subtypes, adult neurogenesis is limited to a subset of neurons in some of the neurogenic niches (such as the adult cerebellum, for example, where adult progenitors solely produce granule neurons under physiological conditions (Kaslin et al., 2009). In addition, recent studies were able to link the rate of adult neurogenesis with ongoing processing demand of the external environment. Hence, for example, new neurons are more actively produced in the visual processing centers upon challenge of visual stimulation. These observations together argue against neurogenesis being an additive process for the sole purpose of growth (Lindsey and Tropepe, 2014; Lindsey et al., 2014). The zebrafish is capable of complex behaviors, in particular regarding social behaviors. Thus, it will also help unravel new links between neurogenesis control and physiological status. In fact, juvenile/adult neurogenesis may also be particularly involved in processing reward (Webb et al., 2009).

**The NSC Niche**

The “niche”, the cellular or chemical microenvironment necessary to maintain stemness, is an important concept of the SC field, but is experimentally more difficult to define, as it is often not easy to disentangle between direct and indirect effects on SC fate. In the adult mammalian SEZ, the morphology of NSCs, contacting a tight arrangement of ependymal cells and the cerebro-spinal fluid at the apical side, as well as blood vessels and/or pericytes by their basal process, suggested that these components were involved in maintaining NSC stemness, hence were part of the niche (recently reviewed in Lim and Alvarez-Buylla, 2014; Silva-Vargas et al., 2013; Urban and Guillemot, 2014). Experimental manipulations confirmed this hypothesis. In the embryonic mouse cortex, a further signal driving RG maintenance was identified as Notch signaling emanating from neuronal precursors, in a negative feedback mechanism (Yoon et al., 2008). The data obtained so far in the adult zebrafish stress the importance of secreted signaling factors (for example, Fgf signaling in the subpallium or cerebellum; Kaslin et al., 2009; Ganz et al., 2010) in controlling the maintenance of RG or NE progenitors. The source of these factors however remains to be studied in detail; they could emanate from neighboring cells, the CSF or blood vessels, with which RG cells also establish tight contacts.

An interesting aspect of most ventricular surfaces in the zebrafish adult CNS, and in particular the telencephalon, is the quasi-absence of ependymal cells. Ependymal cells in the forebrain have only been described in the diencephalon (Lindsey et al., 2012), and the apical membranes of RG themselves constitute virtually all of the pallial and subpallial ventricular surface. This excludes the ependymal layer as a niche factor in these domains. In fact, analysis of the pallial RG stressed the importance of local interactions taking place within the progenitor population itself as major homeostasis factors. Activation of the major quiescence pathway identified to date, Notch signaling, is triggered by contact with cells expressing the Notch ligands Delta or Jagged. Expression analyses showed that these ligands (DeltaA, Jagged1b) are essentially expressed by activated RG and proliferating neuronal progenitors along the ventricular zone (Alunni et al., 2013; Chapouton et al., 2010; de Oliveira-Carlos et al., 2013). In addition, following a short pulse of Notch blockade, RG activation primarily takes place close to cells having recently divided (Chapouton et al., 2010). These observations suggest that dividing progenitors are major activators of Notch signaling in RG, contributing directly (Notch1) or indirectly (Notch3) to their maintenance and/or their silencing, in a feedback mechanism. Together, these results are pointing to the germinal pool of progenitors itself as a major, self-regulating niche, capable of adjusting the demand in NSC recruitment to ongoing neurogenesis.

In addition to feedback control from activated progenitors, analysis of the zebrafish adult pallial ventricular zone highlights a striking widespread pattern of activation events.
across the RG sheet (unpub.). Thus, some additional large scale regulation must take place, at any given time point and independently of proliferating neuronal progenitors (which are uniformly distributed just below the RG layer) to spread out RG activation events across the RG pool. Whether such a mechanism is also at play within the mouse SEZ and/or SGZ pools was not analyzed to date, in part due to the complex geometry of germinal zones, their relative paucity in NSCs and the presence of ependymal cells, in this model. Understanding the large-scale cues that control the spatio-temporal pattern of NSC activation, recruitment and properties will bring important insight on the spatio-temporal organization of the niche. Thus, for the first time, the zebrafish will provide invaluable access to the organization of NSC niches at a large scale, and to the homeostasis of NSC pools at the population level with spatial resolution.

Finally, the joint analysis of adult NE pools in the zebrafish adult brain points to common properties in their location: in most instances, these are positioned in marginal aspects of the neural plate/tube, at the junction between neurogenic and choroid plexus tissues. In the lateral pallium, the NE pool is juxtaposed to the tela choroidea bridging the first ventricle, this from the earliest developmental stages following formation of the neural rod until adulthood (Dirian et al., 2014). In the cerebellum, progenitors of the URL, at least at embryonic and juvenile stages, are bordered by the tela choroidea covering the fourth ventricle (Kaslin et al., 2009). At the posterior edge of the TeO, NE progenitors are associated/ partially overlapping with a small tela bridging the PGZ and the cerebellar valvula, possibly deriving from the anterior tip of the fourth ventricle tela (Chapouton et al., 2006). Finally, in the retina, the CMZ is located at the junction between the neural retinal tissue and the nonpigmented ciliary epithelium, a cellular monolayer with secretory properties (Kasper et al., 1987). In addition to the secretory activity of choroid plexuses, which may provide NE cells with immediate access to secreted factors, these junctional territories themselves are often also signaling centers. This is well known in the embryo, where the neural tube roof plate is bordered by Wnt1-expressing cells, for example, and where the juxtaposition of territories of different positional identities can be sufficient to trigger the generation of signaling centers. But signaling activity is also present next to zebrafish adult NE pools. Indeed, a sustained expression of the signaling factor Wnt3 is apparent at the lateral pallial edge (Dirian et al., 2014), or of Wnt factors at the CMZ tip (Gorsuch and Hyde, 2014; Lenkowski and Raymond, 2014). Expression of Wnt at the posterior edge of the TeO, to our knowledge, has not been analyzed in the adult, but is present in the embryo and may be maintained. Finally, there is an Fgf signaling source close to the cap-like progenitor pool in the cerebellum (Kaslin et al., 2009). Another important common molecular signature of these adult domains is the expression of “Notch-independent” e(spl) genes, such as her6 in the retinal CMZ (Raymond et al., 2006), her5 in the posterior TeO (Chapouton et al., 2006), and her9 in the NE pool of the adult pallium (Dirian et al., 2014). Similar expression is found in the corresponding NE pools at embryonic stages. Given that such junctional locations exist between neurogenic tissue and choroid plexuses in all vertebrates, it would be highly interesting to examine the status of neural cells in their proximity. The pallium is an especially promising territory, given that it harbours neurogenic domains in rodents. Most interestingly, a late source for SGZ NSCs was recently identified as a Gli1-positive territory at the edge of the tel- di-encephalon (Li et al., 2013), in a location that could be homologous to the zebrafish lateral pallial edge. Analyzing these source cells in more detail will reveal they have the characteristics of a long-lasting NE pool.

**NSC Heterogeneity**

An arising debate in the adult mammalian NSC field is the degree of heterogeneity of NSCs within germinal niches. In fact, genetic tracing using various “NSC promoters” (e.g., GFAP, BLBP, GLAST, Nestin...) to drive conditional Cre recombination revealed a variety of fates, suggesting that supposedly “generic” molecular markers in fact distinguish NSC subtypes or substrates (Giachino and Taylor, 2014). In addition to the basic difference between NE and RG pools highlighted above, zebrafish adult germinal zones also display a high degree of heterogeneity between RG located within an individual pool. In the pallium for example, some degree of heterogeneity was revealed when comparing the markers her4, GFAP, BLBP, AroB, S100b, and Nestin on individual sections. Our unpublished data further document large-scale heterogeneities between pallial areas, which can be more or less enriched in the proportion of RG expressing a given marker, associated with different properties (different birth dates for example, or different growth rates). Together, it is likely that the cellular compositions of zebrafish neurogenic RG niches are similar to that of the mammalian SEZ and SGZ in both heterogeneity and richness of cellular states (Lindsey et al., 2012; Marz et al., 2010a), and that the zebrafish will be an invaluable model to approach the meaning of these heterogeneities. Several key questions may be related to these heterogeneities in RG, including the degree of stemness, the degree of quiescence, or neurogenic competence. Importantly, the zebrafish adult pallial germinal zone encompasses the territories homologous to the mammalian SEZ and SGZ, which will permit direct comparative and cross-feeding studies between these species to understand the dynamics of NSC properties.
Concluding Remarks
In conclusion, the zebrafish adult CNS does not simply retain a high number of progenitor cells with activatable neurogenic potential. It offers a number of niches containing bona fide NSCs of adult characteristics, sharing cellular attributes and molecular recruitment pathways with mammalian adult NSCs, and organized in pools of rich complexity and precise spatial regionalization. Like in mammals, these pools respond differentially to environmental demand, notably producing neurons dedicated to distinct functions. Through these properties, this model will provide key insight into the molecular mechanisms controlling the adult NSC state(s) and fate(s), in their heterogeneity, their dynamic transitions, and their regulation at the population scale. The variety of locations (niches) and NSC subtypes (RG, NE progenitors) present in the zebrafish adult CNS will further expand our understanding of the possible strategies sustaining efficient NSC maintenance and neurogenesis. Finally, the efficiency of zebrafish neuronal repair pathways includes both the boosting of constitutively neurogenic NSCs, the partial dedifferentiation/reactivation of silent progenitors, and the induction of NSC protection mechanisms limiting reactive NSC recruitment, a diversity that will be instrumental to design the best strategies when trying to stimulate adult neurogenesis for repair in mammals.

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