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Gene regulation in adult neural stem cells. Current challenges and possible applications☆

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ABSTRACT

Adult neural stem and progenitor cells (NSPCs) offer a unique opportunity for neural regeneration and niche modification in physiopathological conditions, harnessing the capability to modify from neuronal circuits to glial scar. Findings exposing the vast plasticity and potential of NSPCs have accumulated over the past years and we currently know that adult NSPCs can naturally give rise not only to neurons but also to astrocytes and reactive astrocytes, and eventually to oligodendrocytes through genetic manipulation. We can consider NSPCs as endogenous flexible tools to fight against neurodegenerative and neurological disorders and aging. In addition, NSPCs can be considered as active agents contributing to chronic brain alterations and as relevant cell populations to be preserved, so that their main function, neurogenesis, is not lost in damage or disease. Altogether we believe that learning to manipulate NSPC is essential to prevent, ameliorate or restore some of the cognitive deficits associated with brain disease and injury, and therefore should be considered as target for future therapeutic strategies. The first step to accomplish this goal is to target them specifically, by unveiling and understanding their unique markers and signaling pathways.

Contents

1. Introduction

Several areas of the adult brain contain resident neural stem cells (NSCs) that maintain the capacity to generate different cell types, including copies of themselves. Lineage tracing studies have shown that under

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physiological conditions, the progeny of adult NSC is composed largely by new neurons and by a minority of other cell types, including astrocytes and oligodendrocytes. These cell types are not always generated directly from the division of NSCs and may originate from precursors with more restricted potential through multiple stages of amplification. These transit amplifying cells are termed intermediate progenitor cells (IPCs) and can generate neurons, oligodendrocytes or astrocytes [1].

The generative capacity of adult NSCs is most evident in the subgranular zone (SGZ) of the hippocampus [2] and the subventricular zone (SVZ) lining the lateral ventricles [3], and adult neurogenesis has been intensively studied in these areas. In humans, neurogenesis in the SGZ might be more relevant than in the SVZ, as judged by its persistence through aging in the hippocampus [4] versus its early decline in the lateral ventricle-olfactory bulb axis [5]. Importantly, alterations in the generative capacity of adult NSC in the hippocampus have been linked to various diseases such as epilepsy, age-related cognitive decline, depression, Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and others [6]. These observations have led to the idea that endogenous NSCs could be genetically or pharmacologically manipulated to promote brain repair [7]. However, such therapeutic strategies require a detailed understanding of the developmental programs that characterize adult hippocampal NSCs activation and differentiation. Adding to the complexity of this task, recent observations have revealed an unexpected diversity in NSC in the adult mammalian brain [8,9]. Together, these observations suggest that identifying gene expression profiles that characterize particular cell states and understanding how these profiles are regulated to permit programed cell state transitions is of crucial importance to advance into the therapeutic targeting of adult NSCs. In the next sections we will review the literature linking gene expression profiles to cell states identified in adult NSCs and will highlight current challenges associated with the desired specific manipulation of these cell states and their possible applications for targeting adult NSCs.

2. Cell states and cell identity

The term “cell state” is generally used in the literature in a loose way, commonly indicating a certain functional condition in which a cell is at a

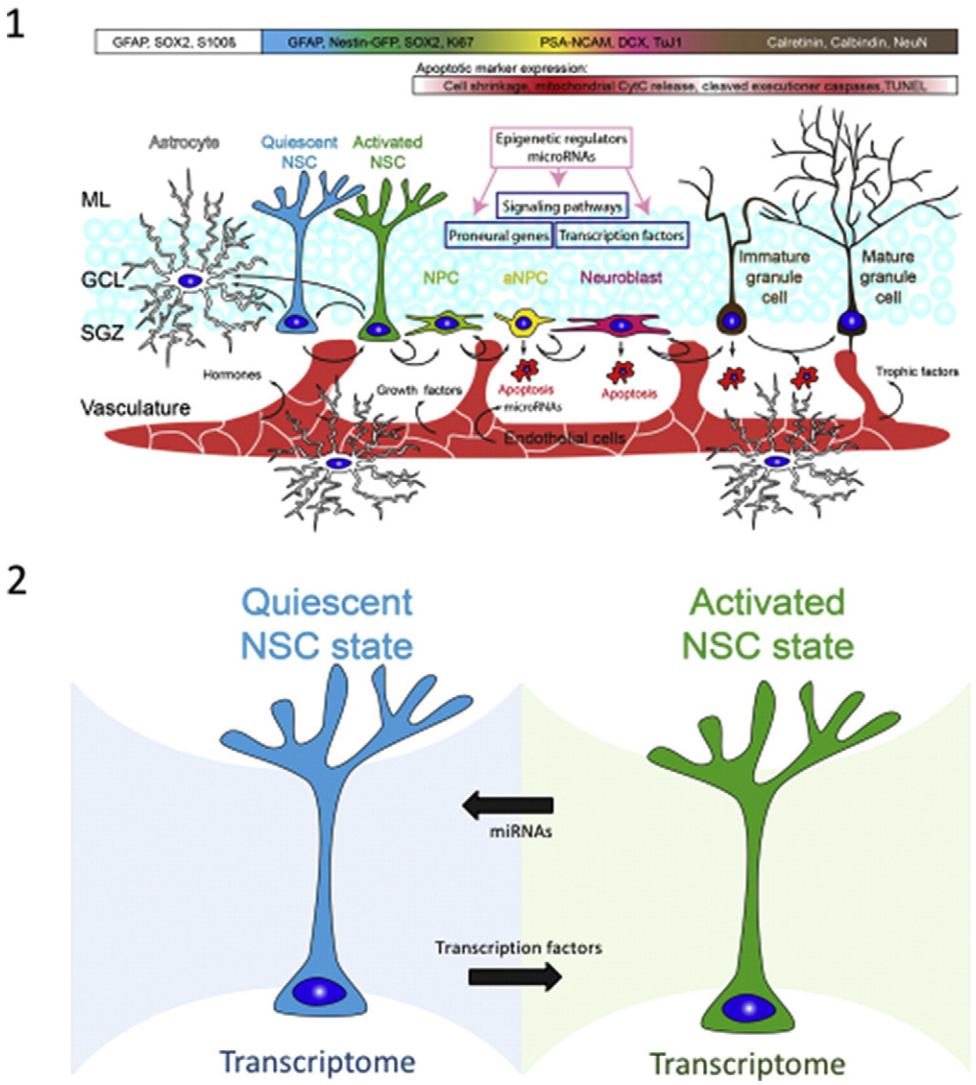

Fig. 1. 1) Overview of the different stages of neurogenesis in the adult hippocampus. Different cell types are identified by a combination of specific markers and morphological features. The complexity of this cellular microenvironment, consisting of multiple cell types in close association with the vasculature, allows for both local and distant regulation mediated by a combination of cell-intrinsic and -extrinsic factors including transcription factors and epigenetic mechanisms, including regulation by several miRNAs. 2) Cell state transitions and identity in NSCs. Focusing on the transition between quiescent NSC and activated NSC and IPCs, cell states characterized by expression profiles (transcriptome) have been identified (Section 3). These profiles define gene expression boundaries within which cells maintain a particular identity. These boundaries may be established by a coordinated regulation of gene expression mediated by transcription factors and miRNAs, which may reduce variability and confer robustness to genetic developmental programs. The figure has been adapted from [23].

given moment in time. In stricter terms, a cell state could be defined as the complete list of constituent molecules within a cell, including its transcripts, proteins, lipids, metabolites, and other molecules, present at a given moment and under defined conditions [10]. In this respect, "omics" profiles and in particular transcript, protein and microRNA (miRNA) profiles, have emerged as invaluable experimental tools to characterize and understand cellular states [11]. In stem cells, states are highly dynamic and state transitions can be induced by numerous cell-intrinsic and environmental factors [12] and the dynamic action of transcription factors is crucial for the regulation of some of these cell state transitions [13]. However, gene expression profiles that represent multiple cell states may all relate to a single cellular identity. In this scenario, cells must retain their phenotypical identities under environmental pressure and endogenous transcriptional noise that can result in significant variations in molecular repertoires. Therefore, gene expression profiles that determine cell function should be contained within certain boundaries outside which there is an inadequate amount of gene expression to achieve function [10] (Fig. 1).

Importantly, cell sates may also be defined in negative or subtractive terms, considering the part of the genome that is not expressed or is actively silenced at a given time under defined conditions [14]. Indeed, lineage-specific transcription factors may function in NSC not only by activating gene expression, but also through the spatially controlled repression of transcriptional repressors, promoting a de-repression strategy that results in neuronal fate specification [15]. Similarly, well-characterized epigenetic mechanisms repress the expression of non-lineage specific genes. In this respect, gene transcription is not only conditioned by the availability of transcription factors, but also by chromatin context that may favor or disfavor transcription factor binding to DNA, and may also maintain transcriptional states through the cell cycle. In particular, the study of epigenetic mechanisms that regulate NSC identity has provided valuable information to understand how the structural adaptation of chromosomal regions may register, signal or perpetuate gene expression profiles associated with particular cell states [16–18]. Many epigenetic modifications associated with active and inactive chromatin states have been identified in adult NSCs and it escapes the objective of this review to summarize all of them, therefore we direct the reader to some excellent and recent reviews on the topic [19,20]. Within the many epigenetic mechanisms that play a role in the regulation of adult NSC, in the next sections we will refer specifically to post-transcriptional regulation by miRNAs, which influences multiple aspects of adult neurogenesis, including stem cell maintenance and proliferation, fate specification, neuronal differentiation and ultimately functional integration by controlling synaptogenesis and impacting on diseases associated with adult neurogenesis (reviewed in [21–23]).

3. Mechanisms of regulation of gene expression and NSC identity

A commonly used operational definition of stem cell is an undifferentiated, potentially immortal cell, with the capacity of self-renewal and ability to differentiate into multiple cell types [24]. Therefore, as stem cells depart from their undifferentiated state they lose multipotency and commit to a particular cell lineage. As mentioned before, adult NSC can give rise to different types of neural cell lineages: neurons, astrocytes, and oligodendrocytes. Adult NSCs exist in particular microenvironments or "niches", which are composed of various cell types, in addition to the extracellular matrix, including ependymal cells, astro- and micro-glia cells, endothelial cells, immature progeny of adult NSC (IPCs, neuroblasts, immature neurons) and mature neurons, which together may provide extrinsic signals that influence cell-intrinsic transcriptional programs that control NSC self-renewal and differentiation [25].

The characterization of gene expression profiles that define a particular cell state and may determine cell identity in adult NSC is technically challenging because it requires the purification of specific cell types from a complex mixture of cells present in brain tissue. To tackle this problem, different experimental strategies have been used successfully but most of them rely on the identification of cell type-specific marker(s) with specific antibodies, followed by fluorescence-activated cell sorting to purify the positive population [26–29]. These studies have provided invaluable information about gene expression profiles characteristic to discrete cell populations of adult NSC and their immature neuronal progeny. However, they all share common experimental limitations emerging from the need to rely on antibody specificity, the artificial expression of fluorescent proteins under cell type-specific promoters and the loss of morphological and spatial information associated with tissue disruption before cell sorting. Much less information is available about gene expression profiles that may characterize the glial progeny of NSC, probably because under physiological conditions these represent a minority population. However, under the pathological conditions induced by strong epileptic seizures, adult NSC in the dentate gyrus generates an increased proportion of reactive astrocytes. Based on this observation, specific gene expression profiles induced by seizures in the DG have been identified, however, they lack cell-level resolution [30–32].

More recently, single-cell RNA-seq techniques have been applied to the characterization of gene expression profiles in adult NSC and their early progeny, resulting in the prospective identification of sequential stem cell states [33,34]. Although this approach may suffer from similar limitations, i.e. the need to rely on antibody specificity, expression of fluorescent proteins under cell type-specific promoters and the loose of morphological and spatial information associated with tissue disruption, single-cell RNA-seq generates gene expression profiles with single cell resolution and may thereby reveal cell states or cell types that cannot be faithfully recognized using purified discrete cell populations. Conversely, due to endogenous transcriptional noise, single-cell RNA-seq could overestimate cellular heterogeneity, and the incorporation of technical variability into the procedure is an obstacle that still needs to be overcome [35]. Shin and colleagues [33] used single cell RNA-seq in combination with an advanced bioinformatics approach to reconstruct adult NSC dynamics in NSC reporter mice in which fluorescent protein expression is driven by the nestin promoter [36–38]. Using this approach they identified gene expression profiles that suggest a set of sequential molecular transitions that characterize the cell states leading form a quiescent NSC (qNSC) to an activated NSC (aNSC) and early IPCs, indicative of developmental steps that give rise to new neurons in the dentate gyrus. In agreement with previous, but less detailed studies, Shin and colleagues found evidence for molecular pathways that led to NSC activation, composed in many cases by transcription factors that were previously linked to NSC and adult neurogenesis (reviewed in [39]; Table 1). Furthermore, Shin and colleagues identified groups of transcription factors that were previously linked to embryonic but not yet to adult neurogenesis regulation, and transcription factors with uncharacterized roles in neurogenesis. Following a similar approach, Llorens-Bobadilla and colleagues [34] isolated NSC from the mouse SVZ based on the expression of the markers GLAST and Prominin1 and performed single-cell RNA-seq. They identified a dormant NSC subpopulation that enters a primed-quiescent state upon brain injury. This primed cell state is characterized by downregulation of glycolytic metabolism, Notch, and BMP signaling and a concomitant upregulation of protein synthesis, long non-coding RNAs expressed in a cell-type- and state-specific manner and lineage-specific transcription factors. The primed state was associated with the expression of immediate early genes Egr1, Fos, SoxC factors (Sox4 and Sox11), and Ascl1, among others [34]. Considered together, these observations suggest that dynamic transcription factor expression profiles characterize the early steps of adult NSC activation and neurogenesis.

3.1. Transcription factors and intrinsic molecular cascades

The observations discussed in the previous section suggest that cell state changes involved in the transition from qNSCs to aNSCs involve a

Table 1
Transcriptions factors, their expression patterns and functions in the regulation of the hippocampal neurogenic lineage.

Transcription factor name (gene symbol)	Expression	Function	Reference
Notch1/RBPJk	NSCs	Regulation of Notch signaling; regulation of cell quiescence and proliferation.	Breunig et al. [160] Ables et al. [161] Ehm et al. [162] Lugert et al. [65]
Smad1/2	NSCs	Regulation of proliferation.	Mira et al. [126] Kandasamy et al. [163]
TLX	NSCs	Regulation of proliferation competence.	Shi et al. [132] Niu et al. [134] Zhang et al. [164]
ApoE4	NSCs	Regulation of BMP signaling; induction of glial differentiation at the expense of neurogenesis.	Theendekara et al. [165] Li et al. [166]
Sox2	NSCs, IPCs	Regulation of NSC multipotency and proliferation capacity.	Steiner et al. [167] Kuwabara et al. [168]
REST	NSCs, IPCs	Repression of neuron-specific genes; regulation of proliferation and self-renewal	Gao et al. [169]
NR3C1	NSCs, IPCs	Regulation of cell differentiation	Garcia et al. [170] Fitzsimons et al. [94]
TCF/LEF	NSCs, IPCs	Regulation of Wnt signaling; fate determination; regulation of proliferation	Song et al. [171] Lie et al. [172] Jang et al. [173]
Sox21	NSC, IPCs	Regulation of neuronal lineage commitment	Matsuda et al. [174]
Hmga1-rs1; Hmgb2/3; Smarcc1/Baf155; Smarce1/Baf57; Nfia, Nfix, Mxd3, Zeb2; Zeb3/Zfp; Hopx	NSC, IPCs	Not yet characterized	Shin et al. [33]
Mash1/Ascl1	IPCs	Regulation of cell quiescence, proliferation, specification and differentiation	Bertrand et al. [175] Castro et al. [176] Andersen et al. [69]
Foxg1	IPCs	Regulation of cell proliferation and survival	Shen et al. [177]
Tbr2	IPCs	Regulation of proliferation and neuronal lineage commitment	Hodge et al. [178] Hodge et al. [179]
Tis21	IPCs	Control of cell cycle progression; regulation of neuronal lineage commitment	Farioli-Vecchioli et al. [180] Farioli-Vecchioli et al. [181]
Prox1	IPCs	Regulation of cell survival	Lavado et al. [182] Karalay et al. [183]
NeuroD1	IPCs	Regulation of cell differentiation	Hsieh et al. [184]
Sox4/11	IPCs	Regulation of neuronal lineage commitment	Mu et al. [185] Wang et al. [186]

drift from a cell state dominated by the integration of extrinsic signals in qNSCs towards cellular states dictated by more deterministic cell-intrinsic programs involving dynamic changes in transcription factor expression [33]. This hypothesis is well in line with the idea that adult NSC activation and commitment of their progeny to specific cellular identities is controlled by environmental signals that are subsequently integrated into precise patterns of gene expression [40,41]. In particular, in NSCs, dynamic changes in the activity of several transcription factor activities have been characterized and they are important for accurate control of gene expression during the transition through different cell states associated with proliferation and differentiation [42–45] and recently reviewed in [46]. An extensive list of transcription factors with proven or putative role in the regulation of adult NSC has been identified (summarized in Table 1 and recently extensively reviewed in [39]). Based on this evidence, transcription factors are attractive targets for the in vivo manipulation of adult neurogenesis [47].

3.2. Epigenetic regulation and integration of extrinsic signals

Epigenetic mechanisms are important for the maintenance of cell-type-specific gene expression profiles and they coordinate cell-extrinsic environmental signals and cell-intrinsic genetic programs. In agreement with this concept and with gene expression profiles recently identified in qNSC and aNSC, discussed in previous sections, experimental observations suggest that epigenetic mechanisms play important roles in fine-tuning adult neurogenesis. Rather than reviewing the existing information again, we refer the reader to extensive previous reviews [20,41] and focus our discussion on postranscriptional regulation of gene expression by miRNAs.

Multiple non-coding RNAs, such as miRNAs, provide an extra level of epigenetic control in NSCs [23,48–50]. MiRNAs are short RNA molecules composed of approximately 22 nucleotides that are natural effectors of RNA interference [51]. They are transcribed as endogenous hairpin-shaped transcripts called pri-miRNAs by RNA polymerase II or III. Pri-miRNAs are then cleaved into pre-miRNAs in the nucleus by the Drosha/DGCR8 complex. After been exported to the cytoplasm by exportin 5, the pre-miRNA is cleaved, this time by the Dicer/TRBP complex, to form mature functional miRNAs, which ultimately recognize specific mRNAs and target them for translational repression and/or cleavage [52–54]. Interestingly, multiple miRNAs have been associated with the regulation of specific steps during neurogenesis progression (summarized in Table 2 and recently reviewed in [22]).

MiRNA profiles have demonstrated to be accurate markers of cell identity, allowing the distinction of different cell phenotypes and miRNAs may function to regulate the transition between cell states [10,55]. In agreement with this idea, miRNAs seem to regulate the "canalization" of genetic programs, i.e. the stabilization of cellular phenotype to reduce variability, and thereby confer robustness, during genetic developmental programs [56,57]. These functions, which may be exerted in many cases by the coordinated actions of more than one individual miRNA, have been demonstrated in NSCs during embryonic development, where for example, miR-9∗ and miR-124 bind to the 3′ untranslated region of BAF53a mRNA, repressing protein expression and thereby controlling a dynamic shift in subunit composition of the SWI/SNF complex, involved in regulating NSC multipotency in complex interactions with transcription factors and epigenetic mechanisms [58]. Thus, miRNAs may exert a coordinated distributed control over functionally related mRNAs, converging on the regulation of common pathways or biological functions in NSCs [32,59]. In summary, changes in cell identity may be closely regulated by transcription factors and miRNAs, which are frequently engaged in many regulatory feedback loops [10,23,46]. Based on the evidence discussed in this section,

Table 2
miRNAs that regulate neurogenesis, their targets, origin of identification and functions, adapted and extended from [22].

miRNA name	Target	Expression	Function	References
miR-184	Numbl	Mouse NSCs	Inhibits differentiation, promotes proliferation, maintenance of the neurogenic stem cell pool	Liu et al. [97]
miR-34a	Numbl	Mouse NPCs from embryonic cortex	Inhibits differentiation, promotes proliferation, maintenance of the neurogenic stem cell pool	Fineberg et al. [187]
	BCL2	Mouse cortex/SH-SY5Y cell line	Promotes apoptosis	Wang et al. [188]
	Cdk4	Primary keratinocytes	Inhibits cell cycle progression	Antonini et al. [189]
	Cyclin D2	Primary keratinocytes	Inhibits cell cycle progression	Antonini et al. [189]
	Synaptotagmin1	Mouse ES cells	Inhibits synaptic development	Agostini et al. [190]
	Syntaxin-1A	Mouse ES cells		Agostini et al. [190]
miR-106b/miR-25 cluster	IGF-signaling	Mouse primary NSC culture	Promotes NSPC proliferation, neuronal differentiation	Brett et al. [191]
miR-124	Sox9	Mouse NSCs	Promotes neuronal differentiation	Stolt et al. [192]
	STAT3	Mouse ESCs	Promotes neuronal differentiation	Krichevsky et al. [193]
	BCL2L13	Mouse DG	Inhibits apoptosis	Schouten et al. [32]
	Lhx2	Mouse hippocampus	Promotes neurite outgrowth	Sanuki et al. [194]
	Rap2a	Mouse NSCs	Promotes dendritic branching	Xue et al. [195]
miR-137	BCL2L13	Mouse DG	Inhibits apoptosis	Schouten et al. [32]
	Mib-1	Mouse hippocampus & mouse primary neuronal culture	Inhibits dendritic growth and spine formation	Smrt et al. [96]
	LSD1	Embryonic NSCs	Promotes proliferation	Sun et al. [196]
	Ezh2	Adult NSCs	Inhibits differentiation	Szulwach et al. [197]
miR-9	TLX	Mouse hippocampus	Promotes differentiation, inhibits differentiation	Zhao et al. [198]
	REST	NT2 cell line	Promotes neuronal differentiation	Packer et al. [199]
	Rap2a	Mouse NCSs	Promotes dendritic branching	Xue et al. [195]
	Stathmin	Human embryonic NPCs	Inhibits migration	Delaloy et al. [200]
Let-7b	TLX	Mouse NSCs & embryonic mouse brain	Inhibits proliferation, promotes differentiation	Zhao et al. [201]
	Cyclin D1	Embryonic mouse brain	Inhibits cell cycle progression	Zhao et al. [201]
miR-125b	Nestin	Rat NSPCs	Promotes neuronal differentiation	Cui et al. [202]
miR-145	Sox2	Mouse NSC	Promotes neuronal differentiation	Morgado et al. [203]
miR-26b	Ctdsp2	Zebrafish & P19 cells	Promotes neuronal differentiation	Dill et al. [204]
miR-19	Rapgef2	Mouse hippocampus & mouse NPCs	Stimulates migration	Han et al. [205]
miR-379/miR-410 cluster	N-Cadherin	Mouse embryonic cortex	Induces migration	Rago et al. [206]
miR-134	Limk1	Rat hippocampus	Inhibits spinogenesis	Schratt et al. [207]
	Dcx	Primary NPCs, primary neurons, mouse embryonic cortex	Inhibits NPC migration	Gaughwin et al. [208]
miR-138	APT1	Mouse hippocampus	Inhibits spinogenesis	Siegel et al. [209]
miR-17/miR-92 cluster	PTEN	Rat primary cortical neurons	Induces axonal outgrowth	Zhang et al. [210]
miR-132	P250GAP	Mouse hippocampus	Promotes spinogenesis	Impey et al. [211]
miR-124, -135, -139, -218, -370, -382, -411, -708, -134, -127, -376	Act synergistically on a group of common targets	Hippocampal NSCs	Neuronal fate determination	Pons-Espinal et al. [124]

miRNAs constitute another group of attractive targets for the in vivo manipulation of adult hippocampal neurogenesis.

The spatial folding of chromatin is another critical factor in the regulation of gene expression and spatial organization of chromatin, which may correlate with epigenetic features such as histone marks associated with active transcription during cellular differentiation [60,61]. To date, little is known about the role of spatial chromatin organization in adult NSC. Pioneering work from Beagan and colleagues has revealed a role for the architectural protein CTCF, which plays critical role in connecting higher-order chromatin folding, during early neural lineage commitment of NPCs isolated from neonatal mouse brains [62]. They demonstrate that CTCF interacts with the zinc-finger protein YY1 to coordinate three-dimensional chromatin organization during the transition from naïve/primed pluripotency to multipotent NPC. Interestingly, CTCF is required for neural development and accurate expression of clustered protocadherin genes in adult cortical and hippocampal neurons, indicating that three-dimensional (3D) chromatin organization plays a relevant role in controlling gene expression in these brain areas [63].

4. NSC quiescence and activation. Challenges and opportunities

The earliest point of regulation in the neurogenic cascade is the recruitment of NSCs to get activated and enter the cell cycle, and thus changes in this first step will be potentially translated to the next steps of the neurogenic cascade (Fig. 2). Therefore, learning to control the mechanisms governing this point of regulation offers the possibility of manipulating the levels of neurogenesis in a significant manner. There are, however, some aspects of NSC biology that should be further considered. Earlier reports had proposed that, although the rate of neurogenesis drastically decreases with age, the size of a radial-like astrocyte population in the dentate gyrus remains constant over time [64], and using an activated Notch reporter transgenic line (Hes5::GFP), that at least a subpopulation of NSCs remains present and responsive to external stimuli in the aged hippocampus [65]. Although still controversial or subject of debate, mounting evidence strongly suggests that the NSC populations of the hippocampus deplete overtime [37,66], and that this depletion is linked to its activation [37,67,68] and thus, blocking NSC activation may preserve its decline [67,69]. Therefore, it can be argued that increasing NSC activation might have the adverse effect of accelerating the depletion of the NCS population in the long term. Because of this reason, maintaining a balance between NSC quiescence and activation that provides sufficient neurogenesis to satisfy environmental demands and at the same time preserves a reserve of NSC throughout life remains a central challenge for the therapeutic use of adult NSCs.

The balance of quiescence and activation is a dynamic process and although it was generally assumed in the field that neuronal activity was a main regulator of NSC activity and neurogenesis, it has not been

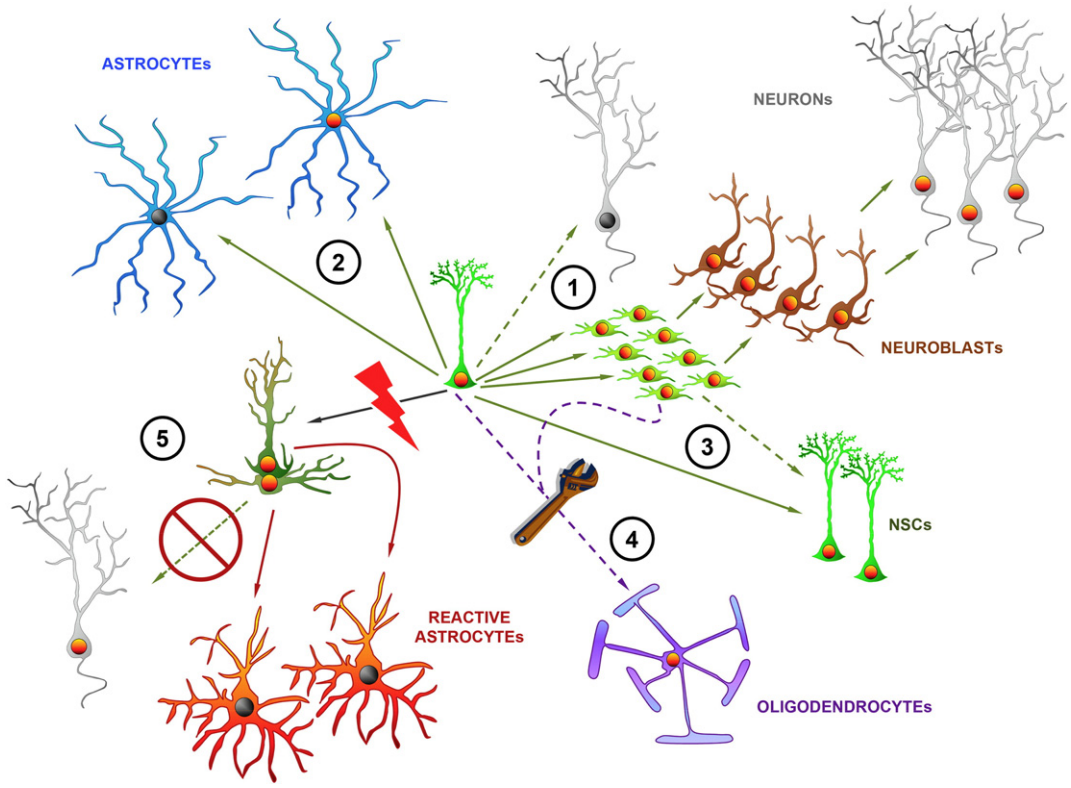

Fig. 2. Differentiation potential of hippocampal NSCs. 1). NSCs of the adult hippocampus were first demonstrated to generate neurons after entering cell division by GFAP-based retroviral labeling [157]. More recently, lineage tracing combined with clonal analysis suggests that NSCs can differentiate directly into neurons. 2). Lineage tracing combined with BrdU administration demonstrated that activated NSCs differentiated into astrocytes after finishing their round of neurogenic divisions [37]. Their astrocytic differentiation can also take place in absence of cell division as shown by clonal analysis-based lineage tracing [105]. 3). This type of analysis showed that in normal conditions, although with low probability, NSCs can divide symmetrically and generate copies of themselves [105]. 4). Through genetic modification using overexpression of ASCL1, also known as Mash1 [158] or deletion of Drosha [159] NSCs can generate oligodendrocytes. 5). Finally, after seizures NSCs become reactive NSCs, abandoning their neurogenic potential and ultimately differentiating into reactive astrocytes [30].

until recent times that a solid link has been established. NSCs respond directly to gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter released by interneurons. Song et al. [67] showed in 2012 that adult radial NSCs in the hippocampus express $GABA_A$ receptors ($GABA_AR$) and respond to GABA and its $GABA_AR$ agonists muscimol, whereas the $GABA_AR$ antagonist blocks the response. Further, diazepam enhanced the response to GABA by NSC, demonstrating the role of the γ2 subunit. Administration of diazepam robustly decreased the rate of NSC activation, as measured by EdU incorporation and MCM2 expression. In turn, nestin-based conditional deletion of the $GABA_A$ γ2 subunit induces a higher rate of NSC activation. Clonal analysis revealed that in addition, symmetric division and astrogliogenic asymmetric division of NSCs were increased in the conditional KO. Importantly, depletion of NSCs was also observed [67]. Later, it was shown that hippocampal NSCs express $GABA_B$ receptors and when $GABA_{B1}$ is conditionally removed in GFAP expressing cells a higher number of radial NSCs enters cell division, as measured by incorporation of BrdU [70]. Administration of the $GABA_BR$ agonist baclofen increased quiescence of NSCs in the Hes5::GFP reporter transgenic mouse, and although administration of the $GABA_BR$ antagonist CGP54626A resulted in an increase in the density of Hes5::GFP cells positive for the cell cycle marker PCNA, the total number of Hes5::GFP cells was not increased, suggesting that symmetric, self-renewing division was not promoted. This result is interesting because it suggests that GABAergic control of the NSC quiescence/activation balance can be more finely tuned than initially expected. In any case, modifying GABA levels or using agonists to preserve the population of NSCs may not be an appealing strategy, due to the wide effect that misbalancing GABA input in the brain may have.

Cyclins are a family of proteins that control cell cycle progression by activating downstream protein kinases called cyclin-dependent kinases (Cdks). Several members of the cyclin/Cdk family have been manipulated to successfully expand the NSPC pool. In embryonic stages, shortening the G1 phase in NSCs by overexpressing cyclin-dependent kinase 4 (Cdk4) and cyclin D1(CD1) triggers an expansion of NSCs by favoring symmetric division in detriment of neurogenic asymmetric division [71]. When CD1 and Cdk4 were overexpressed in the hippocampus of adult mice, in an inducible and transitory manner, an increase in the size of the NSC pool was found, with a concomitant reduction in neurogenesis [72]. This result may be due to the fact that only those NSCs that have already been activated accelerate their cell cycle and switch to a symmetric mode of division, but the quiescent NSCs are neither recruited nor activated. In a second phase, once that the overexpression of Cdk4 and CD1 has stopped, an increase in neurogenesis was observed. Therefore, this manipulation represents the best candidate so far to induce and expansion or "repopulation" of the NSC pool that translates later in a sustained enhancement of neurogenesis. Thus, CD1 and Cdk4 overexpression, and the identification of the underlying intracellular effects it brings about, presents one of the best opportunity to overcome the challenge imposed by the need to maintain a sustainable balance between NSC quiescence and activation.

5. Cell type specific targets for molecular intervention

One of the main challenges for NSPC manipulation is the relative lack of knowledge regarding the expression of particular genes expressed exclusively by the different cell types involved in the neurogenic cascade, that could be used to express reporter genes or drive the conditional knock in/out of other genes for functional assays. The challenge is easier to solve in the later postmitotic stages of cell maturation when newborn cells have already acquired neuronal fate commitment.

Markers such as doublecortin (DCX) [73], polysialic acid-neural cell adhesion molecule (PSA-NCAM) [74], pro-opiomelanocortin (POMC) [75] and glutamate decarboxylase 67 (GAD67) [76], can be used for unique and unambiguous detection and tagging of neuroblasts and immature neurons. On the other hand, NSCs and IPCs share common markers and with radial glia and astrocytes, and often secondary methods are required to selectively identify, visualize and manipulate these cells. One of the most widespread used markers is nestin, a marker of neuroepithelial stem cells [77] that has been used to generate several constitutive [38,78] and inducible [79–81] transgenic mouse lines, in which fluorescent protein expression driven by the nestin promoter is used in combination with cell morphology and other cell markers such as GFAP, to study NSPCs in the SVZ and the dentate gyrus. The main caveat of these systems is that nestin is expressed not only by NSCs and IPCs, but also at lower levels by oligodendrocyte progenitor cells (OPCs), pericytes [37] and reactive astrocytes [30]. Still, nestin has been more extensively used than GFAP, GLAST (expressed in astrocytes) or Sox2 and brain lipid binding protein (BLBP) (expressed in NSCs, IPCs and astrocytes). Other options that have been used for visualization, fate mapping and genetic manipulation are based on Sonic hedgehog (Shh) signaling. For instance, the glioma associated oncogene Gli1 (GLI1 Kruppel family member) labels NSPCs in the SVZ and the hippocampus [37,82] and the Notch target gene *Hes5* labels several types of NSCs and IPCs in the adult brain [65]. A recent report used the *Prss56* gene, expressed in radial glia during development, to visualize NSCs in the adult brain [83]. However, not only (a subpopulation of) NSCs were labeled in the postnatal and adult SVZ and the dentate gyrus, also astrocytes and neurons were found. A more promising marker recently reported to label NSCs with high specificity in the adult neurogenic niches is the lysophosphatidic receptor 1 (LPAR1) [84], which shares with nestin the capability of labeling the vast majority of hippocampal NSCs, as defined by co-expression of GFAP and the morphological features unique to them. Two considerations must be taken into account when using these tools to visualize and fate-map neurogenesis, and especially when using them to induce genetic alteration in cells of interest. One is that more than one single cell population is being targeted; and the second, that within the different populations targeted, the expression of the reporter gene or the genetic manipulation could take place in particular subpopulations of each cell type.

6. Genetic strategies to target quiescent and activated hippocampal NSCs specifically

The detailed study of adult hippocampal NSCs, including genetic profiling of individual cells, has been made possible by the existence of a group of techniques that have allowed the distinction and manipulation of individual NSC from other cells present in their surrounding microenvironment. Beyond their initial identification by the incorporation of synthetic nucleoside analogues during cell division, such as BrdU [85], other techniques such as retrovirus-mediated gene transfer have relied on the same concept, this is, the specific labeling of proliferating cells and their progeny in vivo [86,87]. More recently, genetic strategies based on the expression of constitutive or inducible reporters expressed under the control of cell type-specific promoters and the application of site-specific recombinases have allowed the visualization, tracking and alteration of specific neurogenic cell populations in the adult hippocampus [88,89]. However, many of these approaches have failed to label non-proliferating qNSC, which remain an elusive target due to their low abundance and lack of proliferation [28]. In some cases, lentiviruses have been successfully used for long-term gene transfer into adult NSCs and their neuronal progeny [90]. However, without the use of cell type-specific promoters, lentiviral vectors lack cell type specificity as they

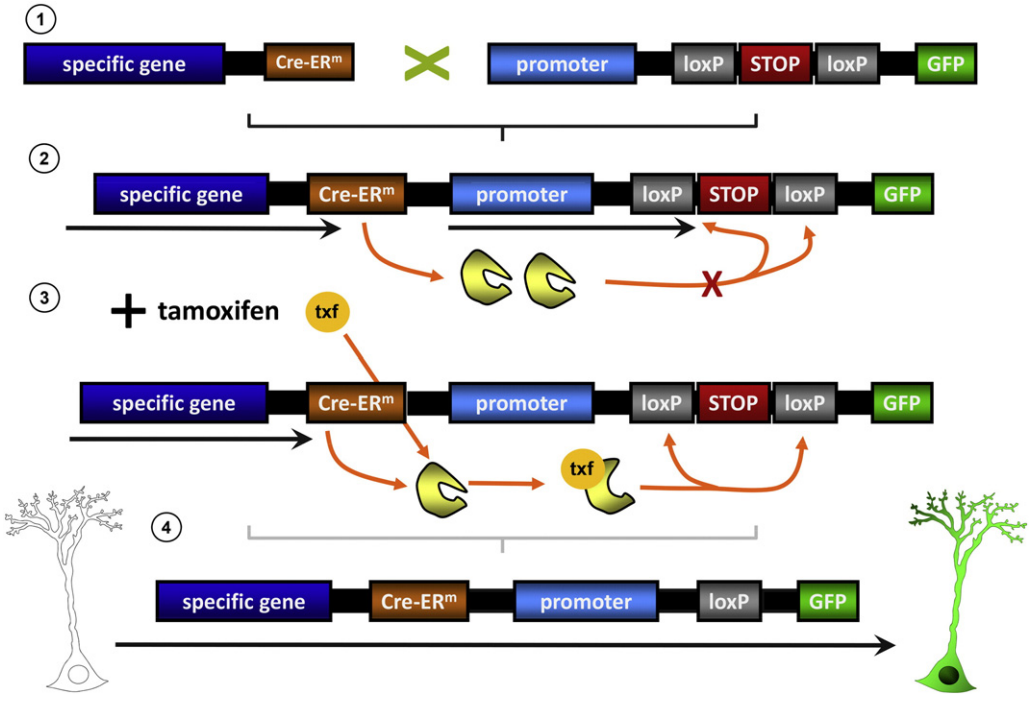

Fig. 3. Inducible expression of fluorescent proteins for lineage tracing. 1) Two lines of transgenic mice are crossed to generate double transgenic mice in which the cell type of interest, and its progeny, can be traced. In the first line the expression of a mutated version of the Cre recombinase is driven by the expression of a cell type-specific gene of interest. In the second line, the expression of a fluorescent protein (for instance GFP) is driven by a ubiquitous promoter (such as β-actin or Rosa 26) is often combined with an enhancer (for instance the cytomegalovirus, CMV, promoter). The expression of the fluorescent protein is prevented by the presence of a "stop" sequence that is flanked by "lox" sequences that the Cre recombinase can recognize. 2) In common inducible systems, the Cre recombinase is fused to a recombinant estrogen binding/nuclear localization site derived from the estrogen receptor. In this way, target "lox" sequences are not recombined unless the fusion protein is translocated to the nucleus upon binding to the artificial estrogen Tamoxifen. Endogenous estrogens do not bind to the mutated version of Cre recombinase. 3) When tamoxifen is administered to the double transgenic mice, the Cre recombinase can enter the nucleus and recombine the "lox" sequences, thus removing the "stop" sequence. Thus, GFP expression starts in those cells expressing the cell type-specific gene of interest, and will continue due to the ubiquitous promoter in those cells, as well as in their progeny.

label mainly neural progenitors and immature cells and only a small percentage of the labeled cells in the SGZ may be considered NSCs [91, 92]. Both lentiviral and retroviral vectors have been used to deliver RNA interference effectors in vivo to NSCs and IPCs and synthetic RNA interference effectors may also be injected directly into the DG [32, 93–97]. The introduction of cell type specific promoters controlling gene expression in the lentiviral vector provides a further level of selectivity and, as with retroviral vectors, a tighter cell type-specificity can be achieved by combining lentiviruses with the Cre-lox system in transgenic animals [87,98]. Diverse inducible forms of the Cre recombinase expressed under NSC- or IPC-specific promoters, such as nestin, GFAP or Ascl1 have been extensively used to study adult neurogenesis in the hippocampus and we will discuss these in the next section.

6.1. Cre/loxP-based genetic strategies

Since its development, the nestin-CreERT2/R26R-yellow fluorescent protein (YFP) model has been extensively used to investigate adult neurogenesis and recent studies have demonstrated that the commonly described short tamoxifen treatment used to activate the CreERT2 system in nestin-CreERT2/R26R-YFP mice does not have a measurable impact on adult neurogenesis or behavior, supporting its broad applicability [99]. In view of the extensive use of this model, the Cre-loxP system deserves a short discussion, particularly focused on its current advantages and limitations regarding its use to study neurogenesis. The Cre-loxP site-specific recombination system was first derived from coliphage P1 and since then extensively characterized [100,101]. In this system, a single protein with DNA recombinase activity, Cre, is both necessary and sufficient to induce recombination between two specific 34 bp-long sequences termed loxP sites, and this dual component system has been adapted to function in a mammalian cell [102,103]. Importantly, using cell type-specific promoters and combining these with inducible Cre mutants (sensitive to tamoxifen or doxycycline), the Cre/loxP system can be expressed in a time- and location- specific fashion (Fig. 3) [104]. Such inducible Cre/loxP systems have been readily adapted to the study of adult neurogenesis by using presumable NSC- and IPC-specific promotors [36,105,106].

One of the first Cre/loxP-based systems developed to study neurogenesis was the nestin-Cre mice, which presents recombination in neuroectodermal progenitor cell types [107]. However, some nestin-Cre lines displayed impairments in the acquisition of both contextual- and cued-conditioned fear, hypopituitarism and other defects that may be due to the insertion of a human growth hormone (hGH) mini-gene downstream of the Cre recombinase [108–110]. To overcome these limitations, inducible expression systems have been developed such as the previously mentioned nestin-CreERT2/R26R-YFP mice, which can be used to label, track, and phenotype stem cells and their progeny in the adult SVZ and SGZ after induction of recombination with the estrogen ligand tamoxifen (TAM) [80,81]. Similarly, other inducible systems have been developed using alternative NSC/IPC promoters, such as GFAP, Ascl1, GLAST, Sox-1 and others [111–114]. However, these CRE-driver lines express CRE in not completely overlapping NSPC populations, making difficult the comparison of results obtained with them [33,115,116]. The Cre-Flex system presents a more recent strategy that allows more flexibility and has been used in complex long-term fate mapping with adult NSCs [117]. This system consists in the combination of transgenic mouse lines with lentiviral vectors for inducible lineage tracing. In the lentiviral vector, GFP, or any other fluorescent protein, is expressed under an ubiquitous promoter. The GFP sequence is flanked by wild type loxP and mutated loxm2 sequences and the firefly luciferase (Fluc) can be added for in vivo non-invasive detection (Fig. 4). However, as mentioned before, the expression of NSC/IPC markers is rather widespread in the adult brain, where for example nestin and prominin1 are also expressed in ependymal cells lining the ventricle [28,118]. To overcome problems associated with using a single marker, different combinations of markers

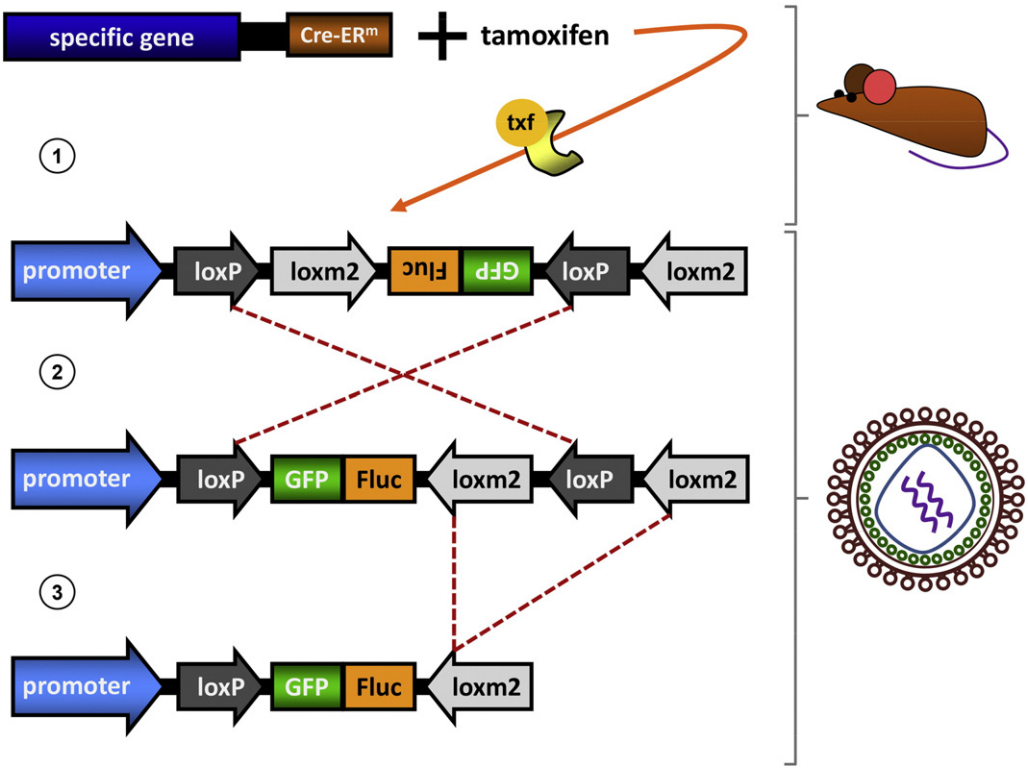

Fig. 4. Cre-Flex (Cre-mediated flip-excision) inducible expression of fluorescent proteins for lineage tracing. 1) Tamoxifen is administered to the transgenic mice of interest injected with the lentiviral vector. 2) The mutated Cre recombinase, bound to tamoxifen will recombine the equivalent sequences with opposite directions and the DNA sequence with the GFP will be flipped (Flip step). 3) The equivalent sequences with equal orientation will be excised (excision step), preventing further recombination. Alternatively, the Flip step can take place at the loxm2 sequences and the excision step at the loxP sequences, but the end product will be the same (Adapted from [117]).

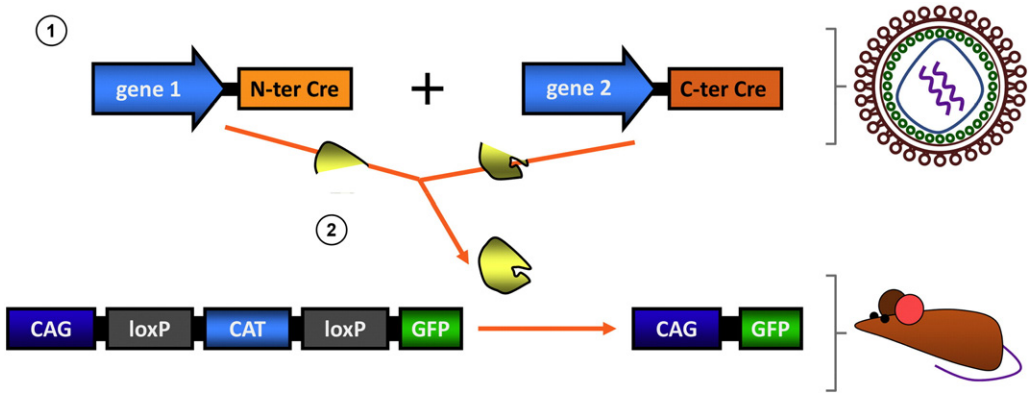

Fig. 5. Split-Cre system for lineage tracing. 1) To further increase the specificity of the labeling and tracing, two different lentiviral vectors with two different specific promoters, each one coding for one half of the Cre recombinase (for instance Prominin 1 and GFAP) can be injected in transgenic mice in which a fluorescent protein (such as GFP) is expressed under a strong promoter (for instance the cytomegalovirus-IE promoter, CAG). 2) In normal conditions GFP expression is prevented by a loxP-flanked "stop" sequence, such as a fragment of the chloramphenicol acetyltransferase, CAT. Those cells expressing both genes of interest will generate the whole, active, Cre-recombinase, which will remove the stop sequence and thereby promote GFP expression.

have been successfully used to detect and isolate NSC [28,119,120]. Following a similar reasoning, an inactive "split-Cre" strategy has been designed, in which the Cre recombinase is split into two complementary fragments that regain Cre activity when overlapping co-expression is controlled by two different promoters (Fig. 5) [121]. Because of its prospective general applicability, this system will be discussed in detail in the next section.

6.2. Split Cre-mediated gene regulation in quiescent and activated hippocampal NSC. A novel approach to gain targeting specificity

Genetic approaches based on the Cre/loxP system aim to provide experimental access to individual cell types within complex neural systems. However, the use of a single promoter is often insufficient to genetically define a distinct neural cell type and induce Cre/LoxP-mediated DNA-recombination specifically there [122]. This problem has been approached by constructing fusion proteins composed of a protein-protein interaction domain and either N- or C-terminal Cre fragments to generate a split Cre system (Fig. 4) [121]. Elegantly, each split-Cre protein alone was unable to induce DNA recombination, while both fragments readily assembled into a functional recombinase when co-expressed in the same cell in vivo in transgenic mice, or using viral vectors. Importantly, Hirrlinger and colleagues [121] demonstrated that in-vivo reconstituted Cre activity persistently labeled cells defined by transient, but coincident expression of both split-Cre fragments, thereby permitting the study of transient cell populations, such as those observed during adult neurogenesis. However, a functional Cre complementation may be determined by several experimental parameters such as temporal expression relations within the split-Cre fragments, the specificity of transgenic or viral expression, the efficiency of complementation and the specificity of the reporter gene used to detect recombination. For a detailed discussion of these parameters, we refer the reader to the original work from Hirrlinger and colleagues [121]. The same authors have adapted the split-Cre system for the temporal control of Cre-mediated DNA recombination by fusing split-Cre proteins with the tamoxifen inducible ERT2 domain [123].

Irrespectively of the possible limitations mentioned in the previous paragraph, the split-Cre system has been successfully used to study adult NSC [28,120,124]. In all three cases, the split-Cre fragments controlled by the hGFAP or the prominin1 promoter were delivered using two lentiviral vectors to adult NSC defined by the combinatorial expression of GFAP and prominin1. In the first example, split-Cre fate mapping was used to identify GFAP/prominin1-expressing cells as adult NSCs in vivo, distinguishing them from niche astrocytes and ependymal cells and allowing the bona-fide identification of gene expression profiles that characterize adult NSCs [28]. In the second example, Beckervordersandforth and colleagues [120], used the split-Cre system to specifically target adult NSCs in the dentate gyrus, demonstrating that Prominin1 labels GFAP+ radial glia-like NSCs cells and the long-term neurogenic lineage of Prominin1 +/hGFAP+ NSCs in the dentate gyrus. Finally, in the most recent example of the use of the split-Cre system to study adult NSCs, Pons-Espinal and colleagues demonstrated that split-Cre lentivirus-mediated ablation of the miRNA processing enzyme Dicer in vivo specifically impairs neurogenesis, but not astrogliogenesis in the dentate gyrus of Dicer^{flox}/Td-Tomato^{flox} mice. These three examples clearly demonstrate how the versatility of the split-Cre system can be used to target adult NSCs, providing an invaluable tool to gain targeting specificity in Cre-mediated DNA recombination.

7. Gene targeting in quiescent and activated hippocampal NSC

7.1. Possible opportunities for therapeutic applications

The adult NSC niches are controlled, in a similar fashion to their developmental counterparts, by Notch, Sonic hedgehog, bone morphogenetic proteins (BMPs), Wnt and other signaling pathways that eventually result in a transcription factor-dependent activation of specific genes [125]. As discussed before in Section 4, it is essential to consider that manipulation leading to increased neurogenesis may in turn deplete neurogenesis irreversibly through functional exhaustion or terminal differentiation of NSCs. The BMPs signaling cascade is one of the main regulators of quiescence/activation of NSCs. BMP receptor 1 (BMPr1) signaling is active in quiescent NSCs and antagonizing it, for instance through intra-hippocampal infusion of Noggin, leads to exit from quiescence in NSCs and increased neurogenesis. In the long term, however, the NSC and progenitor pools become depleted. Conversely, addition of BMPs to NSCs induces quiescence [126]. Wnt signaling is controlled by agonists and antagonists such as the Dickkopf (Dkk) family of secreted glycoproteins that exert their function by preventing the binding of Wnt ligand to LRP5/LRP6. Knock down of Dkk1 in nestin-expressing cells (using the Nestin-Cre-ER^{T2} system) triggers neurogenesis by increasing cell proliferation and/or survival of NSPCs, which translates into improved scores in anxiety/depression-like behavior and memory behavioral tests [127]. The long-term effect on the NSPC pool and neurogenesis was not assessed in this study, however, AHN seems to be decreased in the aging brain microenvironment by the action of a Wnt-mediated signaling cascade, providing a suitable mechanism for the gradual loss of neurogenesis associated with aging [128]. Regarding Notch signaling, its implication in regulating the

activity of NSPCs and neurogenesis in the adult SVZ was unveiled under normal physiological circumstances, with changes being observed in aging and ischemia [129]. Further detailed analysis showed that Jagged1, a member of the Serrate/Jagged family, considered to be canonical Notch ligands [130], is a key regulator of NSC self-renewal and differentiation in the adult SVZ. Partial Jagged 1 deletion in combination with partial Notch1 deletion leads to a loss of mitosis in the SVZ and the rostral migratory stream (RMS). Although the long-term effect on the NSC pool size and neurogenesis was not assessed, the in vitro data pointed towards an impairment of NSC self-renewal. The in vivo outcome cannot be easily inferred as, in agreement to what occurs in the dentate gyrus, self-renewal in vivo is limited in the SVZ, and does not counteract the depletion of NSCs [131].

Several other intrinsic factors such as Mash1, Mll1 and PTEN, have been demonstrated to participate in the maintenance of quiescence in hippocampal NSCs and might be used to increase the NSC population and neurogenesis in the long term. A pathway that has attracted major interest is that controlled by the nuclear TLX receptor (Table 1). Earlier results suggested that its expression is required for maintenance of proliferation and that its elimination leads to loss of NSPCs mitotic potential [132]. In addition, ablation of TLX expression leads to accelerated depletion of NSCs in both the SGZ and the SVZ [132,133] due to direct astroglial conversion, or at least to the induction of a quiescent cell state mediated by the p53 and p21/Cdkn1a pathways [134]. Knocking out of the tumor repressor PTEN in conditional Nestin-Cre-ERT2 transgenic mice, targeting preferentially NSCs by using a low single dose of tamoxifen, induces exit from quiescence and promotes symmetric division of NSCs as analyzed by clonal analysis. However, the rate of depletion through differentiation was again higher than the symmetric division rate and the authors reported a net loss of NSC clones over a one-month period. In addition, reduction of PTEN promoted astrocytic differentiation.

One manipulation that has been reported to increased NSC activation and lead to a larger population of NSCs and augmented neurogenesis in the aging brain is the administration of taurine [135]. This finding is intriguing, given the fact that taurine is a free amino acid found in many diets and can be readily purchased over the counter. A strategy that might be considered to preserve neurogenesis in situations in which it can become depleted through exhaustion, such as neuronal hyperexcitation [30] is to force NSC quiescence. Ascl1 (achaete-scute homolog 1), also known as Mash1, is a transcription factor expressed in NSPCs in the adult neurogenic niches (Table 1). Its conditional knockdown (based on Glast expression) in adult hippocampal NSCs prevents their activation, which in turn translates into a reduction in age-related depletion of the NSCs population [69]. When considering this manipulation, it must be noted that the effect is assumingly irreversible, and therefore the preservation of the NSCs population takes place at the expense of impairing neurogenesis in a chronic manner, and therefore on/off systems should be develop to control quiescence/activation at will. Finally, mixed-lineage leukaemia 1(Mll1, KMT2A) acts in postnatal NSCs by directly targeting distal-less homeobox 2 (Dlx2), whose activation is required for the generation of neurons from NSCs in the SVZ. Interestingly, Mll1 knockout in GFAP-expressing cells impaired only neurogenesis while gliogenesis was spared and stayed similar to control levels [136]. Together these results highlight the complexity behind the regulation of NSPCs function, and specially that the interplay between quiescence/activation, self-renewal and differentiation can have very different outcomes, and no assumption can be made when manipulating particular genes in NSPCs.

7.2. Novel approaches for NSC tracing and manipulation

In recent years, new strategies directed to enhance the regenerative potential of NSCs have been developed. Among them, the use of magnetic nanoparticles (MNP) is within the most advanced. MNPs can be used to both track cells in a non-invasive manner using them as contrast for magnetic resonance imaging, and for gene delivery. NSPCs have been successfully labeled with supramagnetic iron oxide (SPIO) particles employing direct magneto-electroporation [137], viral vectors such as the hemagglutinating virus of Japan envelopes (HVJ-Es) [138] or antibodies [139]. Magneto-electroporation with the FDA-approved SPIO compound Feridex has been most frequently used to label NSCs in transplantation studies with reportedly no negative effects on cell proliferation, migration or differentiation. Using this technique, Feridex-labeled human NSCs could be traced in mice up to 18 weeks after transplantation [140]. Importantly, gene expression profiling revealed that overall NSC gene expression remained largely unaltered following SIPO labeling [141], which again indicates largely a lack of negative effects on NSCs.

MNPs can be used also for genetic engineering aimed to increase their therapeutic potential. Transfection of NSC cultures with DNA-coated MNPs using magnetic fields (magnetofection) offers the advantage of greater biosafety and being less time and cost-consuming than the more widespread used viral vector-based transduction [142]. Magnetofection is an emerging field in which basic concepts are still been investigated, such as the benefit of the use of oscillating versus static fields [143], the type of SPIO particles to use [144] and the optimization of protocols for 3D cultures, which offer greater post-transplantation graft survival than more transfection-friendly monolayer cultures [145].

In parallel to magnetofection, the development of “soft” substrates and 3D matrices that resemble more closely the natural structural properties of NSC niches, may allow NSC to grow and develop in ways that resemble more accurately the situation in-vivo. In fact, both fields are closely related, as MNP uptake and transfection efficiency are highly dependent on NSC's biophysical properties and can be improved by using “biomimetic” or “neuromimetic” materials that better resemble the neural tissue in terms of elasticity, porosity, and surface properties [146].

Indeed, the mechano-elastic properties of biocompatible materials can be used to modify NSC's biological properties, inducing for instance neurogenic differentiation of human mesenchymal stem cells seeded on nanogratings of polydimethylsiloxane, which increased focal adhesion signaling pathways due to a higher number of periodic surface points of contact with the cells [147]. The discovery and characterization of the carbon structure graphene [148] has opened new possibilities for the development of biocompatible materials in which nanostructure and electric fields could be controlled to manipulate cell properties. Graphene foam can be generated with a 3D structure that increases NSCs proliferation over the 2D counterpart. At the same time, graphene is highly conductive and offers a large interface that allows charge transport. This may be an interesting feature in view of recent finding suggesting that electrical stimulation could guide NSC to damaged sites in the brain [149]. Thus, NSCs could be electrically stimulated via the same 3D structure in which their growth is promoted [150,151]. Besides electrical stimulation, NSCs growing in graphene nanostructures have also been shown to become more neurogenic, at the expense of their gliogenic fate, after direct photo-stimulation [152] and after near infra-red laser stimulation [153]. Interestingly, graphene nanoparticle structures also enhance neuronal differentiation and axonal growth of cultured human NSCs [154]. Furthermore, by increasing the amount of graphene oxide coating of a carbon nanofiber structure, olygodendrocytic differentiation can be potentiated over neurogenic differentiation [155].

The final purpose of these NSCs manipulations is their implementation in vivo, with the goal of regenerating neurons or neural tissue lost to injury or disease. Even though NSC transplantation into adult brain can be achieved nowadays, the main problem of this potential therapeutic approach is that the host tissue seems to be just not permissive to the appropriate long-term integration of the new neurons. Manipulating of the host tissue in order to make it more permissive or “development-like” is still inaccessible and therefore other strategies

potentiating the growth, survival and differentiation of the transplanted NSCs must be pursued. In this respect, the use of different forms, combinations and concentrations of graphene to boost the biomedical use of NSCs in regenerative medicine is just starting to deliver results [156]. The cellular and molecular mechanisms have not yet been explored and the in-vivo validation is still missing, but it is a very promising field for brain regenerative therapies.

8. Future perspectives

NSPCs in the adult brain, although constrained by a less plastic environment and localization restricted to discrete niches, are more multifunctional and flexible than previously thought. Thus, a next logical step is to make full use of the therapeutic opportunities that they provide, as NSPCs could be considered target for strategies to generate novel, or improve already existing, clinical approaches. For this at least three challenging objectives should be achieved: 1) Protect and preserve them in those conditions leading to their exhaustion such as neuroinflammation or neuronal hyperexcitation; 2) Increase or redirect their neurogenic output to restore the neuronal loss that characterizes neurological and neurodegenerative disorders such as stroke, traumatic brain injury, Alzheimer's disease, aging; 3) Modify their differentiation to generate glial cells that in turn could improve the injured or diseased niche in situations such as multiple sclerosis and hippocampal sclerosis.

We do not know yet if these manipulations would actually translate into better behavioral and cognitive outcomes in animal models and finally humans, but they are worth being explored. The first steps towards these goals are the clean, specific targeting and manipulation of particular NSPC types, a task which main difficult still dwells in the overall absence of unique and exclusive markers.

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