

REVIEW ARTICLE

Regulation of active and quiescent somatic stem cells by Notch signaling

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Abstract

Somatic stem/progenitor cells actively proliferate and give rise to different types of mature cells (active state) in embryonic tissues while they are mostly dormant (quiescent state) in many adult tissues. Notch signaling is known to regulate both active and quiescent states of somatic stem cells, but how it regulates these different states is unknown. Recent studies revealed that the Notch effector *Hes1* is expressed differently during the active and quiescent states during neurogenesis and myogenesis: high in the quiescent state and oscillatory in the active state. When the *Hes1* expression level is high, both *Ascl1* and *MyoD* expression are continuously suppressed. By contrast, when *Hes1* expression oscillates, it periodically represses expression of the neurogenic factor *Ascl1* and the myogenic factor *MyoD*, thereby driving *Ascl1* and *MyoD* oscillations. High levels of *Hes1* and the resultant *Ascl1* suppression promote the quiescent state of neural stem cells, while *Hes1* oscillation-dependent *Ascl1* oscillations regulate their active state. Similarly, in satellite cells of muscles, known adult muscle stem cells, high levels of *Hes1* and the resultant *MyoD* suppression seem to promote their quiescent state, while *Hes1* oscillation-dependent *MyoD* oscillations activate their proliferation and differentiation. Therefore, the expression dynamics of *Hes1* is a key regulatory mechanism of generating and maintaining active/quiescent stem cell states.

KEYWORDSactive stem cell, *Hes1*, Notch signaling, oscillatory expression, quiescent stem cell

1 | INTRODUCTION

Somatic stem/progenitor cells have different characteristics in embryonic tissues than they do in adult tissues. In embryonic tissues, they actively divide and give rise to many mature cells, whereas in many adult tissues, they are mostly dormant/quiescent and only occasionally proliferate to give rise to some mature cells. The molecular nature of these differences between the embryonic active state

and the adult quiescent state of stem cells is unknown, but recent data suggest that Notch signaling plays an essential role in maintaining both active and quiescent stem cells (Imayoshi, Sakamoto, Yamaguchi, Mori, & Kageyama, 2010). Differentiating cells express Notch ligands such as Delta-like1 (*Dll1*), which activate Notch signaling in neighboring cells. Upon activation of Notch signaling, the transmembrane protein Notch is processed in the neighboring cells, releasing the Notch intracellular domain (NICD). NICD then transits

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to the nuclei, forms a complex with the Notch signaling mediator Rbpj, and induces transcriptional repressors such as Hes1 and Hes5 (Figure 1; Artavanis-Tsakonas, Rand, & Lake, 1999). Hes1 and Hes5 inhibit cell differentiation by antagonizing cell fate determination factors, such as the neurogenic factors Ascl1 and Neurogenin2 (Neurog2) (Figure 1; Kageyama, Ohtsuka, & Kobayashi, 2007). Thus, differentiating cells inhibit neighboring cells from differentiating by activating Notch signaling, a process called lateral inhibition. However, recent data suggest that Notch signaling molecules exhibit more dynamic expression patterns than previously thought and that such dynamic expression is important for morphogenesis (Kageyama, Ohtsuka, Shimojo, & Imayoshi, 2008). In this review, we discussed the functional significance and mechanism of Notch signaling in regulating active and quiescent stem cells, focusing mainly on neural stem cells.

2 | NEURAL STEM CELLS IN THE EMBRYONIC AND ADULT BRAIN

In the developing nervous system, neuroepithelial cells, the earliest form of neural stem/progenitor cells, actively proliferate (Figure 2a). Neuroepithelial cells are gradually elongated and transformed into radial glial cells that retain their cell bodies in the innermost neural tube layer, called the ventricular zone (VZ), and extend their processes, called radial fibers, to the external surface of the brain. Radial glial cells undergo asymmetric cell division, each dividing into two distinct cell types: one radial glial cell and one immature neuron or

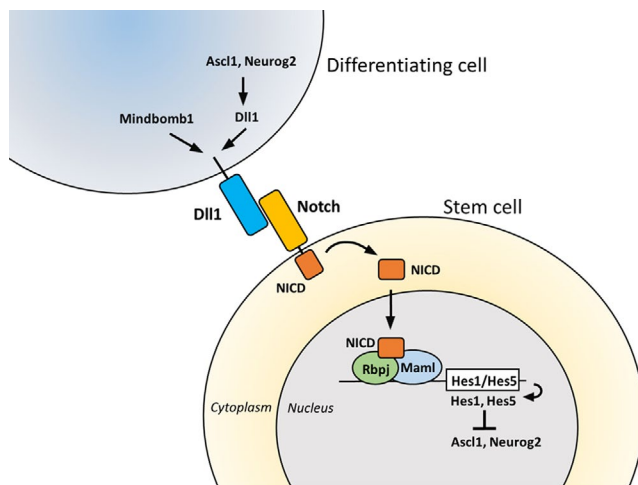


FIGURE 1 Lateral inhibition of differentiation via Notch signaling. A differentiating cell expresses Notch ligands such as Delta-like1 (DII1), which activate Notch signaling in a neighboring cell. The transmembrane protein Notch is then processed in the neighboring cell, releasing the Notch intracellular domain (NICD), which transits to the nucleus, forms a complex with the Notch signaling mediator Rbpj and the co-activator Mastermind-like (Maml), and induces transcriptional repressors such as Hes1 and Hes5. Hes1 and Hes5 inhibit cell differentiation and maintain a somatic stem cell by antagonizing cell fate determination factors such as the neurogenic factors Ascl1 and Neurog2

an intermediate progenitor (Figure 2a; Fujita, 2003; Götz & Huttner, 2005). Immature neurons migrate outside of the VZ into outer layers of the brain, where they become mature neurons, while intermediate progenitors migrate into the subventricular zone (SVZ), divide a few times, and give rise to more neurons (Figure 2a). In higher mammals such as primates, some radial glial cells detach from the inner (apical) surface, form a new outer SVZ (OSVZ) layer, and undergo asymmetric cell divisions, producing more neurons (Figure 2a). Neurons are formed in an inside-out pattern such that inner-layer neurons are generated first, followed by outer-layer neurons. Thus, cortical neuron subtypes are sequentially determined by birthdate through progressive lineage restriction of common radial glial cells. After producing neurons, radial glial cells finally differentiate into glial cells (oligodendrocytes and astrocytes), although some of them are maintained as neural stem cells in the postnatal and adult brain. It has been shown that some neural stem cells become slowly dividing or quiescent during embryonic stages and remain as adult neural stem cells (Fuentealba et al., 2015; Furutachi et al., 2015) while others become quiescent postnatally (Berg et al., 2019).

In the adult mouse brain, neural stem cells are present in two regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus (Figure 2b) and the SVZ of the lateral ventricles (Doetsch, 2003; Kriegstein & Alvarez-Buylla, 2009). These adult neural stem cells, which have a radial glial cell morphology, are mostly quiescent/dormant, and only occasionally become activated and divide to produce transit-amplifying cells (Figure 2b). Transit-amplifying cells divide a few times and soon differentiate into neurons that integrate into the preexisting neural circuits (Bonaguidi et al., 2011; Encinas et al., 2011; Imayoshi et al., 2008; Lagace et al., 2007; Pilz et al., 2018; Seri, García-Verdugo, McEwen, & Alvarez-Buylla, 2001). Thus, the characteristics of neural stem cells are totally different during the embryonic active and adult quiescent states.

3 | NOTCH SIGNALING IN ACTIVE AND QUIESCENT NEURAL STEM CELLS

Notch signaling plays an essential role in maintaining active neural stem cells in the developing mouse nervous system (Androutsellis-Theotokis et al., 2006; Chambers et al., 2001; Imayoshi et al., 2010; Mason et al., 2005; Mizutani, Yoon, Dang, Tokunaga, & Gaiano, 2007). Inactivation of the Notch signaling effector genes *Hes1*, *Hes3*, and *Hes5* upregulates the expression of proneural genes such as *Ascl1* and *Neurog2*, accelerates neurogenesis, and prematurely depletes neural stem cells in the developing nervous system except for the telencephalon (Hatakeyama et al., 2004; Imayoshi & Kageyama, 2014; Ishibashi et al., 1995; Ohtsuka et al., 1999). In the telencephalon, the *Hes1*-related gene *Hey1* is highly expressed, suggesting that it compensates for the *Hes* deficiency. The *Hes3;Hes5;Hey1*-null nervous system seems to develop normally, but additional deletion of *Hes1* upregulates the expression of *Ascl1* and *Neurog2*, accelerates neurogenesis, and prematurely depletes neural stem cells in the developing telencephalon (Sueda, Imayoshi, Harima, & Kageyama, 2019),

indicating that these four *Hes/Hey* genes cooperatively regulate telencephalic development. Similar defects in the developing nervous system were also observed in the absence of the Notch mediator Rbpj (Imayoshi et al., 2010). Thus, the Notch-Rbpj-Hes1/Hes3/Hes5/Hey1 pathway appears to play an essential role in maintaining active neural stem cells in the developing mouse nervous system.

Interestingly, Notch signaling is also important for maintaining quiescent neural stem cells in the adult brain (Ables et al., 2010; Ehm et al., 2010; Imayoshi et al., 2010; Nyfeler et al., 2005; Veeraraghavalu, Choi, Zhang, & Sisoda, 2010). Again, while adult neurogenesis is not significantly affected in the absence of *Hes3*, *Hes5*, and *Hey1*, additional deletion of *Hes1* upregulates the expression of *Ascl1*, accelerates neurogenesis, and prematurely depletes quiescent neural stem cells in the adult brain (Sueda et al., 2019). Furthermore, similar defects in the adult neurogenesis occur in the absence of *Rbpj* (Imayoshi et al., 2010), indicating that the Notch-Rbpj-Hes1/Hes3/Hes5/Hey1 pathway plays an essential role in maintaining quiescent neural stem cells in the adult brain.

Thus, Notch signaling regulates the maintenance of both embryonic active and adult quiescent neural stem cells. But, how does Notch signaling lead to active and quiescent states in the embryonic

and adult brains? Our recent data suggest that the dynamics of *Hes1* expression are involved in these different states.

4 | DYNAMIC EXPRESSION OF NOTCH SIGNALING GENES IN ACTIVE NEURAL STEM CELLS

While *Hes* genes are required to maintain embryonic neural stem cells, immunostaining and in situ hybridization analyses indicated that their expression levels are variable, exhibiting a granular, salt-and-pepper pattern in the VZ. Live-imaging analyses showed that in embryonic neural stem cells, *Hes1* expression oscillates with 2–3-hr periodicity regulated by negative feedback from *Hes1* protein (Figure 3). Notch signaling activates the expression of *Hes1*, whose protein product represses its own expression by directly binding to its promoter (Hirata et al., 2002; Shimojo, Ohtsuka, & Kageyama, 2008). When the *Hes1* promoter is repressed, both *Hes1* mRNA and *Hes1* protein disappear rapidly because they are extremely unstable, which cancels the negative feedback and initiates the next round of expression. Thus, *Hes1* expression oscillates autonomously

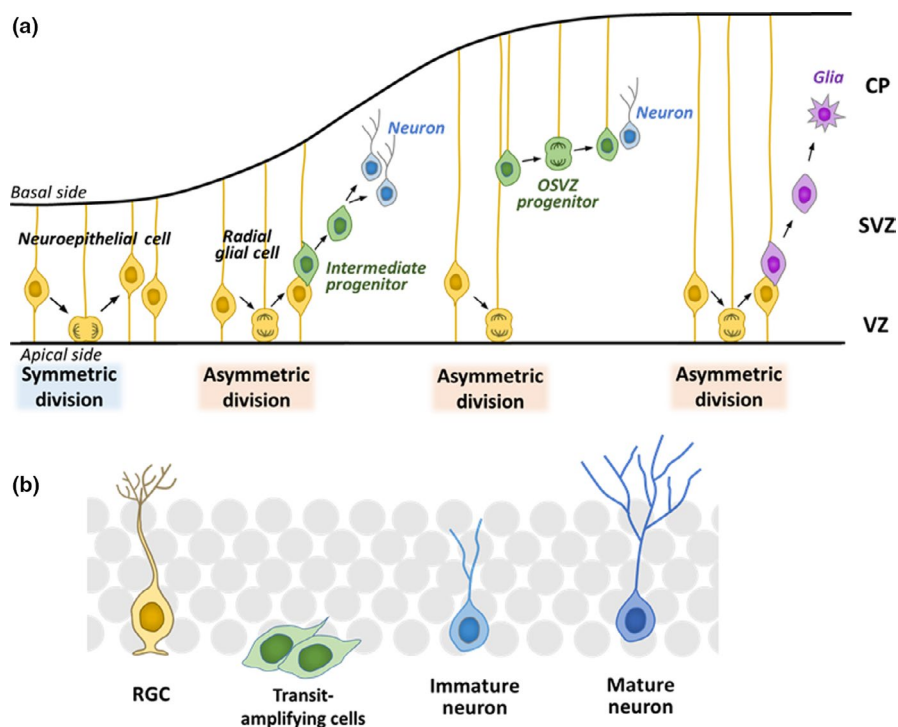


FIGURE 2 Neurogenesis in the embryonic and adult brain. (a) Differentiation of neural stem/progenitor cells in the embryonic cortex. Neuroepithelial cells initially undergo repeated self-renewal by symmetric division. As development proceeds, neuroepithelial cells elongate to become radial glial cells, which have cell bodies in the inner region (the ventricular zone, VZ) of the neural tube and long processes (radial fibers) that reach the outer surface. Radial glial cells give rise to neurons and/or intermediate progenitors. Each intermediate progenitor migrates into the subventricular zone (SVZ) and produces neurons. Neurons migrate outward to form the cortical plate (CP). Some radial glial cells detach from the inner (apical) surface, form a new layer called the outer SVZ (OSVZ), and undergo asymmetric cell divisions, producing more neurons. After producing neurons, radial glial cells differentiate into glia (oligodendrocytes and astrocytes). Both neuroepithelial cells and radial glial cells are considered embryonic neural stem/progenitor cells. (b) Neurogenesis in the subgranular zone (SGZ) of the hippocampal dentate gyrus of the adult brain. Neural stem cells with a radial glial cell (RGC) morphology are mostly quiescent/dormant and only occasionally divide to produce transit-amplifying cells, which divide a few times and differentiate into neurons

(Hirata et al., 2002), and a snapshot of Hes1 oscillations in embryonic neural stem cells exhibits a salt-and-pepper pattern in the VZ. In these cells, Hes1 oscillations periodically repress the expression of proneural genes and Dll1; therefore, these genes are also expressed in an oscillatory manner and exhibit salt-and-pepper patterns in the VZ (Figure 3; Shimojo et al., 2008).

It has been shown that the proneural gene *Ascl1* has dual, opposing functions: activating the proliferation of neural stem cells and inducing cell cycle exit and subsequent neuronal differentiation (Castro et al., 2011). In neural stem cells, where Notch signaling is active, Hes1 oscillations induce *Ascl1* oscillations, while in differentiating neurons, where Notch signaling is inactive, Hes1 expression disappears, enabling *Ascl1* to be expressed continuously. Hence, different expression dynamics may be involved in the dual opposing functions of *Ascl1*. To investigate this possibility, we used an optogenetic method that induces gene expression upon blue-light illumination (Figure 4a; Wang, Chen, & Yang, 2012). This analysis demonstrated that *Ascl1* activates proliferation of neural stem cells when its expression is oscillatory and induces cell cycle exit and neuronal differentiation when its expression is sustained (Figure 4b; Imayoshi et al., 2013). Thus, *Ascl1* oscillations, which normally depend on Hes1 oscillations, are important for proliferation of neural stem cells.

This oscillatory expression is also advantageous to keep a group of cells from differentiating. According to the "lateral inhibition" model of Notch signaling, neurons express Dll1 and activate Notch signaling in neighboring cells, which are inhibited from differentiating into neurons (Figure 1). Therefore, neurons are required to activate Notch signaling and maintain neural stem cells. However, the question arises as to how neural stem cells are maintained before neurons are born. It was found that at an early stage of development, neural stem cells express *Hes1*, proneural genes, and *Dll1* in an oscillatory manner (Shimojo et al., 2008). It is likely that neural stem cells mutually activate Notch signaling at an early stage of development, as follows

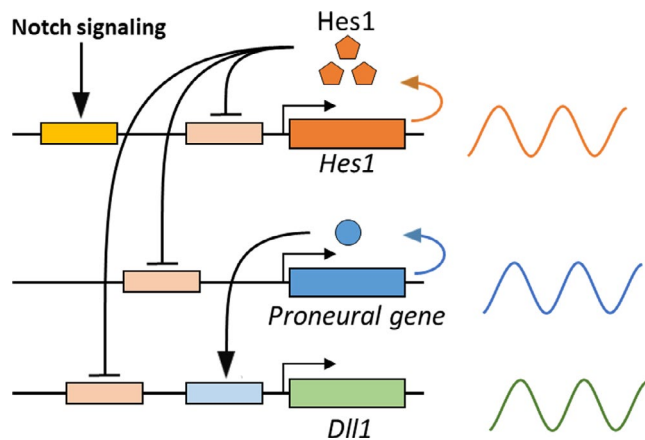


FIGURE 3 Dynamic expression of Notch signaling genes in active neural stem cells. Notch signaling activates the expression of *Hes1*, which oscillates with 2–3-hr periodicity regulated by negative feedback of *Hes1* protein. *Hes1* oscillations periodically repress the expression of proneural genes and *Dll1*. Therefore, these genes are also expressed in an oscillatory manner (shown on the right)

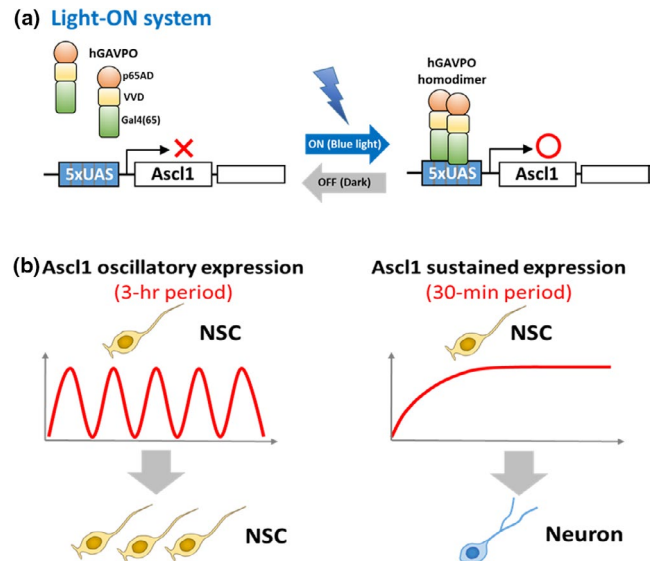


FIGURE 4 Optogenetic expression of the proneural gene *Ascl1*. (a) Optogenetic Light-ON system. Under dark conditions, hGAVPO is an inactive monomer. Upon blue-light illumination, hGAVPO forms a dimer, binds to the upstream activation sequence (UAS) promoter, and activates the expression of downstream genes. (b) The Light-ON system can differentially induce oscillatory and sustained *Ascl1* expression by changing blue light illumination patterns. *Ascl1* activates proliferation of neural stem cells (NSC) when its expression is oscillatory, and induces neuronal differentiation when its expression is sustained

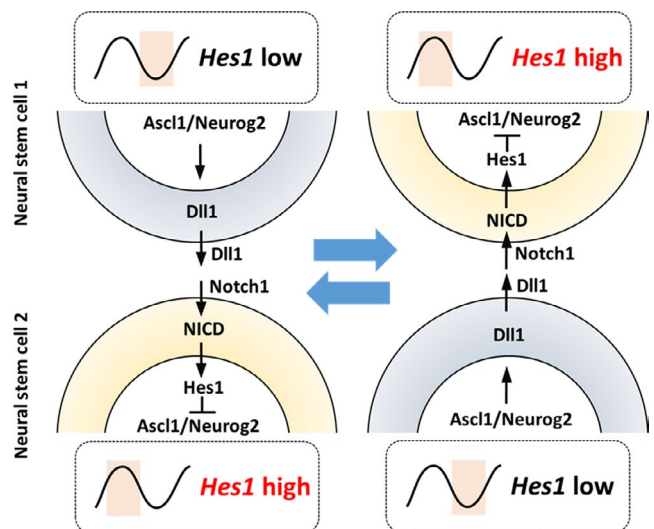


FIGURE 5 Mutual activation of Notch signaling by oscillations. When *Ascl1/Neurog2* are high in a neural stem cell (cell 1), they induce the expression of *Dll1*, which activates Notch signaling in a neighboring neural stem cell (cell 2). In this neighboring cell, activated Notch signaling induces the expression of *Hes1*, which represses *Ascl1/Neurog2* and *Dll1* expression. However, due to the 2–3-hr periodicity of oscillations, *Hes1* levels soon become lower, enabling *Ascl1/Neurog2* and *Dll1* to be expressed once again, which activates Notch signaling in the first cell (cell 1). In this way, neighboring cells (cell 1 and cell 2) can mutually activate Notch signaling via *Dll1* oscillations. NICD, Notch intracellular domain

(Figure 5): When *Ascl1*/*Neurog2* levels are high in one neural stem cell (cell 1), they induce the expression of *Dll1*, which activates Notch signaling in a neighboring neural stem cell (cell 2). In this neighboring cell, activated Notch signaling induces the expression of *Hes1*, which represses the expression of *Ascl1*/*Neurog2* and *Dll1*. However, due to the 2–3-hr periodicity of the oscillations, *Hes1* levels soon become lower, enabling the expression of *Ascl1*/*Neurog2* and *Dll1*, which activates Notch signaling in the first cell (cell 1). In this way, neighboring cells can mutually activate Notch signaling via *Dll1* oscillations (Shimojo et al., 2008). Mathematical modeling suggests that the timing of *Dll1* expression is very important for its oscillation and that accelerating or delaying the timing of *Dll1* expression dampens its oscillation. Indeed, when the timing of *Dll1* expression is accelerated or delayed by shortening or elongating the *Dll1* gene, respectively, *Hes1* oscillations are severely dampened (Shimojo et al., 2016). Under these conditions, the maintenance and proliferation of neural stem cells are impaired, leading to accelerated neurogenesis and microcephaly (Shimojo et al., 2016). Thus, oscillatory expression is necessary for the maintenance and proliferation of neural stem cells.

5 | DIFFERENT EXPRESSION DYNAMICS OF NOTCH SIGNALING GENES IN QUIESCENT NEURAL STEM CELLS

Immunohistological analysis indicates that not all cells exhibit the granular, “salt-and-pepper” staining patterns of *Hes1* in the

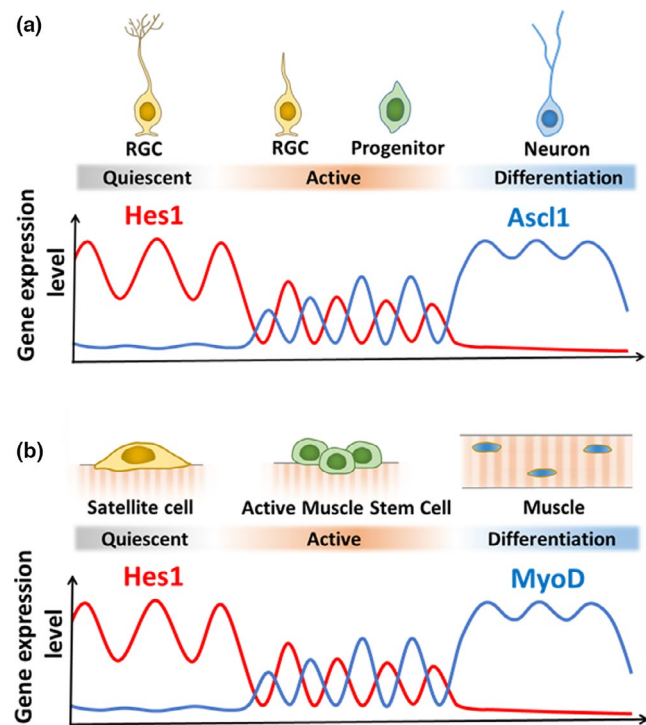


FIGURE 6 Gene expression dynamics during neurogenesis and myogenesis in adults. (a) Expression dynamics of *Hes1* and *Ascl1* during neurogenesis. (b) Expression dynamics of *Hes1* and *MyoD* during myogenesis

developing nervous system. *Hes1* levels are high and sustained, while proneural proteins are lacking in the boundary regions, such as the roof plate, floor plate, and isthmus (Baek, Hatakeyama, Sakamoto, Ohtsuka, & Kageyama, 2006). In these regions, cells are mostly quiescent, suggesting that patterns of gene expression may be involved in the quiescent versus active state. In agreement with this notion, it was previously shown that induction of high and sustained levels of *Hes1* inhibits cell proliferation and that induced inactivation of *Ascl1* expression impairs proliferation of neural stem cells (Baek et al., 2006; Castro et al., 2011). These results suggest that high and sustained *Hes1* and the resultant suppression of proneural genes may enhance the quiescence of neural stem cells.

How about neural stem cells in the adult brain? The process of activating quiescent neural stem cells in the adult brain has been intensively analyzed. Small fractions of neural stem cells are activated to produce transit-amplifying cells, which proliferate and soon generate neuroblasts (Figure 2b). Expression of the proneural gene *Ascl1* plays a critical role in the activation of quiescent neural stem cells and subsequent formation of neuroblasts in the mouse brain. *Ascl1* is expressed at low levels in some activated neural stem cells and at high levels in transit-amplifying cells (Andersen et al., 2014; Kim, Ables, Dickel, Eisch, & Johnson, 2011; Pastrana, Cheng, & Doetsch, 2009). Furthermore, live-imaging analysis showed that *Ascl1*-expressing neural stem cells exclusively generated neurons in the adult mouse hippocampus (Pilz et al., 2018). By contrast, in the absence of *Ascl1*, all neural stem cells remained quiescent, indicating that *Ascl1* is absolutely required to activate quiescent neural stem cells (Andersen et al., 2014). Recent live-imaging analysis of the adult mouse brain demonstrated that *Hes1* levels were oscillatory, although the peaks and troughs were higher than those in active neural stem cells, causing *Ascl1* expression to be continuously suppressed (Figure 6a; Sueda et al., 2019). Inactivation of the Notch pathway upregulates *Ascl1* expression, activates neural stem cells, and transiently enhances neurogenesis, but neural stem cells are soon depleted, ending neurogenesis prematurely (Ables et al., 2010; Andersen et al., 2014; Ehm et al., 2010; Imayoshi et al., 2010; Sueda et al., 2019). Conversely, activation of Notch signaling or induction of sustained *Hes1* expression represses *Ascl1* expression, inhibits neurogenesis, and maintains quiescent neural stem cells in the adult brain (Imayoshi et al., 2010; Mason et al., 2005; Mizutani et al., 2007; Sueda et al., 2019). These results indicate that high levels of *Hes1* and the resultant suppression of *Ascl1* promote quiescence in neural stem cells in the adult brain (Figure 6a). Indeed, induction of *Ascl1* oscillations efficiently activates neural stem cells to produce new neurons in the adult brain (Sueda et al., 2019).

The detailed mechanism by which high levels of *Hes1* are maintained in quiescent neural stem cells remains to be determined. It was shown that bone morphogenetic protein (BMP) signaling is important for maintaining quiescent neural stem cells in the adult brain (Mira et al., 2010). BMP signaling induces the expression of *Id1* (Miyazono & Miyazawa, 2002), and *Id1* is highly expressed by quiescent neural stem cells in the adult brain (Nam & Benezra, 2009). Interestingly, *Id1* inhibits *Hes1* activities by forming a complex. *Hes1*

oscillations are controlled by negative feedback (Hirata et al., 2002), but high levels of *Id1* interact with *Hes1* and inhibit its negative feedback, thereby upregulating *Hes1* expression (Bai et al., 2007; Boaretto, Iber, & Taylor, 2017). These results suggest that the BMP-*Id*-*Hes* axis may be the major pathway for upregulating *Hes1*, suppressing *Ascl1*, and maintaining quiescent neural stem cells in the adult brain.

6 | ROLES OF HES1 IN ACTIVE AND QUIESCENT MUSCLE STEM CELLS

Similar to neural stem cells, muscle stem cells have different characteristics in embryonic and adult tissues. For example, muscle stem cells derived from the dermomyotome of the somite actively proliferate and generate skeletal muscles in embryos. By contrast, satellite cells in the adult skeletal muscle (known muscle stem cells) are quiescent (Shi & Garry, 2006; Wang & Rudnicki, 2012). When muscles are injured, satellite cells are activated and contribute to muscle regeneration. During this process, the muscle determination gene *MyoD* plays an important role in muscle formation. It was reported that *MyoD* not only induces the expression of genes involved in muscle differentiation but also up-regulates the genes involved in cell proliferation (Cao et al., 2010). Indeed, *MyoD* directly activates the expression of *Id* genes (Cao et al., 2010), which inhibit muscle differentiation by antagonizing *MyoD* activity. Thus, *MyoD* also appears to have dual activities in muscle development, the initial maintenance of dividing muscle stem cells and the subsequent cell cycle exit and muscle differentiation, similar to that of *Ascl1* in neural development. However, it was not clear how *MyoD* controls these dual activities.

Recent studies showed that in satellite cells of adult muscles, *MyoD* expression is continuously repressed by the Notch-*Hes1* pathway (Lahmann et al., 2019; Mourikis et al., 2012). When satellite cells are activated, *Hes1* expression oscillates with 2–3-hr periodicity, and *Hes1* oscillations periodically repress *MyoD* expression, thereby driving *MyoD* oscillations (Lahmann et al., 2019). By contrast, when satellite cells begin muscle differentiation, *Hes1* expression disappears, while *MyoD* expression becomes sustained (Lahman et al., 2019). Furthermore, when *Hes1* is inactivated, *MyoD* expression becomes sustained immediately, and muscle differentiation proceeds without sufficient proliferation of satellite cells. All these data suggest that *Hes1* oscillations-dependent *MyoD* oscillations regulate proliferation of active muscle stem cells, whereas sustained *Hes1* and resultant suppression of *MyoD* enhance quiescence in muscle stem cells (Figure 6b).

7 | CONCLUSIONS

Notch signaling controls the production of active and quiescent somatic stem cells by oscillatory and sustained *Hes1* expression, respectively. Oscillating levels of *Hes1* drive the oscillatory expression

of cell fate determination factors such as *Ascl1* and *MyoD*, which activate proliferation, whereas high, sustained levels of *Hes1* suppress cell fate determination factors, leading to quiescence. The oscillatory expression of cell fate determination factors may keep somatic stem cells active, which then proliferate and give rise to mature cells. Thus, the induction of oscillations of cell fate determination factors may be useful to manipulate endogenous stem cells for tissue regeneration. For example, activation of endogenous neural stem cells by *Ascl1* oscillations can generate many new neurons, which may ameliorate neurodegenerative diseases such as Alzheimer disease and Huntington disease (Benraiss et al., 2013; Choi et al., 2018). Optogenetic and *Hes5*-promoter-induced oscillations of *Ascl1* can activate endogenous neural stem cells in the adult brain, but so far this procedure was not able to maintain active neural stem cells for more than a month (Sueda et al., 2019), probably because *Ascl1* expression tends to accumulate and become sustained after a few weeks, which may induce neuronal differentiation. Improvements of this kind of approach should lead to long-term activation of neural stem cells and may be useful for regenerative medicine in the future.

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AUTHOR CONTRIBUTION

RS made figures; RS and RK wrote the manuscript.

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