

Review

The significance of gene expression dynamics in neural stem cell regulation

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(Edited by Shigekazu NAGATA, M.J.A.)

Abstract: Neural stem cells (NSCs) actively proliferate and generate neurons and glial cells (active state) in the embryonic brain, whereas they are mostly dormant (quiescent state) in the adult brain. The expression dynamics of *Hes1* are different between active and quiescent NSCs. In active NSCs, *Hes1* expression oscillates and periodically represses the expression of proneural genes such as *Ascl1*, thereby driving their oscillations. By contrast, in quiescent NSCs, *Hes1* oscillations maintain expression at higher levels even at trough phases (thus continuous), thereby continuously suppressing proneural gene expression. High levels of *Hes1* expression and the resultant suppression of *Ascl1* promote the quiescent state of NSCs, whereas oscillatory *Hes1* expression and the resultant oscillatory *Ascl1* expression regulate their active state. Furthermore, in other developmental contexts, high, continuous *Hes1* expression induces astrocyte differentiation or the formation of boundaries, which function as signaling centers. Thus, the expression dynamics of *Hes1* are a key regulatory mechanism generating and maintaining various cell types in the nervous system.

Keywords: active state, bHLH factor, boundary cell, neural stem cell, oscillation, quiescent state

1. Introduction

Many forms of biological activity are continuous, and their amplitude and duration represent important information for cellular events. For

example, *Shh* signaling specifies progenitor cell identity, such as V3 interneuron or motor neuron progenitors in the spinal cord, in amplitude- and duration-dependent manners: higher amplitude or longer duration of *Shh* signaling induces V3 interneurons, whereas lower amplitude or shorter duration of *Shh* induces motor neurons.¹⁾ However, recent studies revealed that many other forms of biological activity are pulsatile or oscillatory, and that not only their amplitude and duration but also their frequency and phase convey essential information for cellular events.^{2)–4)} In some cases, the same factors can cause different outcomes depending on whether they exhibit pulsatile or continuous activity. For example, luteinizing hormone-releasing hormone (LHRH) is secreted in a pulsatile manner and activates estrogen and testosterone secretion.⁵⁾ However, when LHRH is administered continuously, its receptor is rapidly desensitized, losing the response to LHRH and suppressing estrogen and testosterone secretion.⁵⁾ Thus, pulsatile LHRH functions as an activator, but continuous LHRH functions as an inhibitor for estrogen and testosterone formation.

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Abbreviations: bHLH: basic helix-loop-helix; CP: cortical plate; Dll1: Delta-like 1; INP: intermediate progenitor; LHRH: luteinizing hormone-releasing hormone; NICD: Notch intracellular domain; NSC: neural stem cell; SVZ: subventricular zone; VZ: ventricular zone.

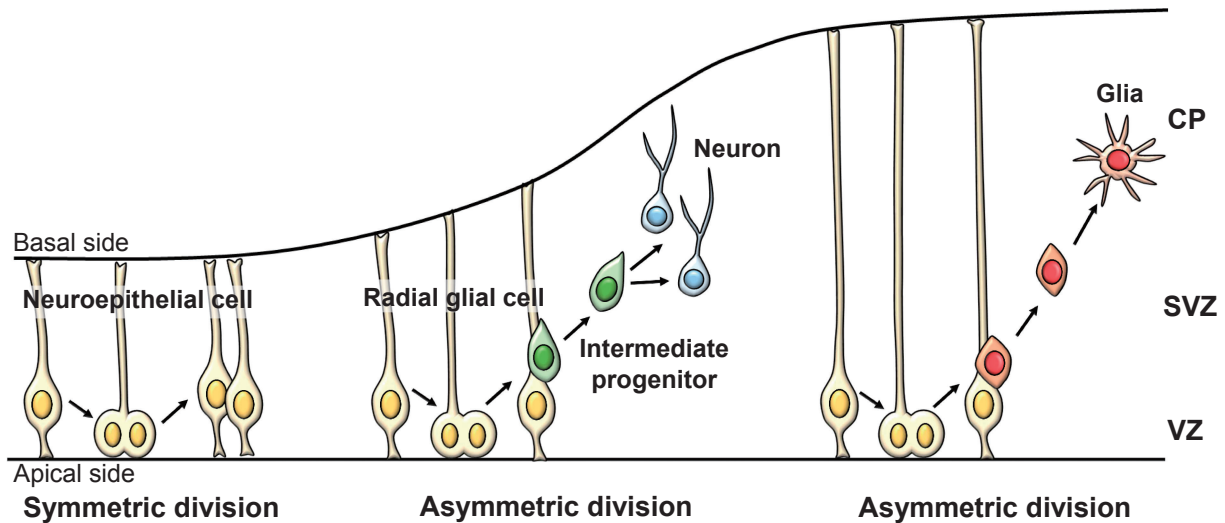


Fig. 1. Differentiation of NSCs in the embryonic cortex. Neuroepithelial cells repeatedly undergo self-renewal by symmetric division. As development proceeds, neuroepithelial cells elongate to become radial glial cells, which have cell bodies in the inner region (ventricular zone, VZ) of the neural tube and long processes (radial fibers) that reach the outer surface (Basal side). Radial glial cells give rise to intermediate progenitors or neurons. Each intermediate progenitor migrates into the subventricular zone (SVZ) and produces neurons. Neurons further migrate into the basal side and form the cortical plate (CP). After producing neurons, radial glial cells differentiate into glia (oligodendrocytes and astrocytes). Neuroepithelial cells and radial glial cells are both considered embryonic NSCs.

Another example is the somite segmentation clock gene *Hes7*. Somites are metamereric structures, which later give rise to the vertebrae, ribs, skeletal muscles, and subcutaneous tissues. Somites repeatedly form by segmentation of the anterior parts of the presomitic mesoderm, which is located in the caudal part of an embryo. *Hes7* expression oscillates in a synchronous manner in the presomitic mesoderm, and each cycle of *Hes7* oscillation leads to the segmentation of a pair of somites.^{6)–8)} When *Hes7* expression becomes sustained, all somites and their derivatives are severely fused.⁹⁾ Thus, oscillatory *Hes7* expression leads to periodic somite segmentation, but sustained *Hes7* expression results in severe somite fusion. Accumulating evidence suggests that oscillatory or sustained gene expression dynamics exhibit different activities in various biological events.

In this review, we discuss the significance and mechanisms of gene expression dynamics in tissue stem cells, particularly focusing on neural stem cells (NSCs). A *Hes7*-related gene, *Hes1*, causes various outcomes in NSCs, depending on its oscillatory or sustained expression.

2. NSC regulation by bHLH factors

In the developing nervous system, neuroepithelial cells, which constitute the wall of the neural tube,

proliferate actively (Fig. 1). As development proceeds, neuroepithelial cells elongate gradually and become 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 neural tube (Fig. 1). Neuroepithelial cells undergo symmetric cell division, in which each neuroepithelial cell produces two neuroepithelial cells, whereas radial glial cells undergo asymmetric cell division, in which each radial glial cell produces two distinct cell types, *i.e.*, one radial glial cell and one intermediate progenitor (INP) (Fig. 1).^{10),11)} INPs migrate outside of the VZ into the outer layer called the subventricular zone (SVZ), where they further divide a few more times and differentiate into mature neurons. After producing neurons, radial glial cells finally differentiate into glial cells (oligodendrocytes and astrocytes) (Fig. 1), although some of them are maintained as NSCs in the postnatal and adult brain. Neuroepithelial cells and radial glial cells are collectively called embryonic NSCs.

The maintenance of NSCs and their differentiation into neurons are antagonistically controlled by basic helix-loop-helix (bHLH) transcriptional activators and repressors.^{12)–14)} bHLH transcriptional activators include proneural factors such as *Ascl1* and *Neurog2*, and bHLH transcriptional repressors

include Notch signaling effectors such as Hes1 and Hes5. *Ascl1* and *Neurog2* up-regulate genes involved in neuronal differentiation, inducing the formation of INPs and neurons. By contrast, Hes1 and Hes5 repress *Ascl1* and *Neurog2* expression and thereby inhibit neuronal differentiation, leading to the maintenance of NSCs. The inactivation of *Hes1* and *Hes5* up-regulates *Ascl1* and *Neurog2* expression, accelerates neurogenesis, and prematurely depletes NSCs in the developing nervous system.^{15),16)} Thus, antagonistic regulation between bHLH transcriptional activators and repressors is essential for normal neural development. Of note, *Ascl1* and *Neurog2* up-regulate ligands of Notch signaling such as Delta-like 1 (*Dll1*), which activate Notch signaling in neighboring cells (Fig. 2A). Upon activation of Notch signaling, Notch intracellular domain (NICD), an active form of Notch signaling, is formed and induces Hes1 and Hes5 expression, thereby inhibiting neuronal differentiation (Fig. 2A). These results indicate that *Ascl1*- or *Neurog2*-expressing differentiating INPs and neurons activate Notch signaling in neighboring cells, which are inhibited from undergoing neuronal differentiation, a process called lateral inhibition.^{17),18)} Thus, Notch signaling is essential for the maintenance of NSCs in the developing nervous system.

3. NSC regulation by oscillatory gene expression

The above observations indicate that INPs and neurons play an important role in maintaining NSCs by *Dll1* expression; however, this raises the question of how NSCs are maintained before INPs and neurons are generated. Accumulating evidence suggests that Notch signaling regulation is not as simple as a one-way pathway from differentiating INPs and neurons to neighboring NSCs. *Ascl1*, *Neurog2*, and *Dll1* expression is not unique to differentiating INPs and neurons, but occurs in NSCs in a salt-and-pepper pattern (*i.e.*, a mixture of positive and negative cells) in the early stages of neural development, particularly before INPs and neurons are generated. These findings indicate that in addition to Hes1 and Hes5, *Ascl1*, *Neurog2*, and *Dll1* are expressed by subsets of NSCs, raising the possibility that NSCs mutually activate Notch signaling. Time-lapse imaging analysis revealed that Hes1 and Hes5 expression oscillates with a 2–3-h periodicity in NSCs.^{19),20)} Hes1 and Hes5 can repress their own expression by binding directly to their own promoters (negative feedback), leading to their down-regulation (Fig. 3).²¹⁾ However, when these factors disappear, negative feedback is can-

celled, allowing the next round of expression. Thus, Hes1 and Hes5 expression oscillates autonomously by negative feedback in NSCs (Fig. 3).²¹⁾ Hes1 and Hes5 oscillations then periodically repress *Ascl1*, *Neurog2*, and *Dll1* expression, which also oscillates in NSCs (Fig. 3). On the basis of this observation, the current view of Notch signaling during the early stages of development is as follows (Fig. 2B).^{18),19)} When Hes1 and Hes5 expression is low in certain cells, *Ascl1* and *Neurog2* expression increases, inducing *Dll1* expression, and high *Dll1* levels activate Notch signaling in neighboring cells, which express high levels of Hes1 and Hes5. However, because of oscillatory expression, Hes1 and Hes5 expression is suppressed 1–1.5 h later in the latter cells, resulting in high *Ascl1*, *Neurog2*, and *Dll1* expression, which activates Notch signaling in the former cells (Fig. 2B). Thus, according to these oscillations, NSCs can mutually activate Notch signaling, inhibiting neuronal differentiation of each other and maintaining a group of cells in an undifferentiated state. This two-way regulation is important in the early stages of development before INPs and neurons are generated. As development proceeds, this two-way regulation disappears, and *Dll1* signals are produced only by differentiating INPs and neurons.

The oscillations observed in the developing nervous system are anti-phase or out-of-phase between neighboring NSCs, unlike the in-phase oscillations of Hes7 in the presomitic mesoderm.^{22),23)} However, the significance of anti-phase or out-of-phase oscillations is not known. NSCs are required to produce diverse cell types during neural development, and anti-phase or out-of-phase oscillations may be useful for the generation of the diverse responses of NSCs. For example, Hes1-low cells can respond to certain signals, but Hes1-high cells cannot. Further analysis is required to test this hypothesis.

Oscillatory expression itself seems to be very important for the activity of NSCs. Hes1 expression oscillates in proliferating NSCs, but is higher even during the trough phase (thus continuous) in differentiating astrocytes (Fig. 4).²⁰⁾ Similarly, the proneural gene *Ascl1* and the oligodendrocyte determination gene *Olig2* are expressed in an oscillatory manner depending on Hes1 oscillations in proliferating NSCs, but become sustained and high in differentiating neurons and oligodendrocytes, respectively (Fig. 4).²⁰⁾ *Ascl1*, Hes1, and *Olig2* play important roles in the proliferation of NSCs, in addition to their respective neurogenic, astrogenic, and oligodendrogenic functions.^{20),24),25)} Thus, each factor has oppos-

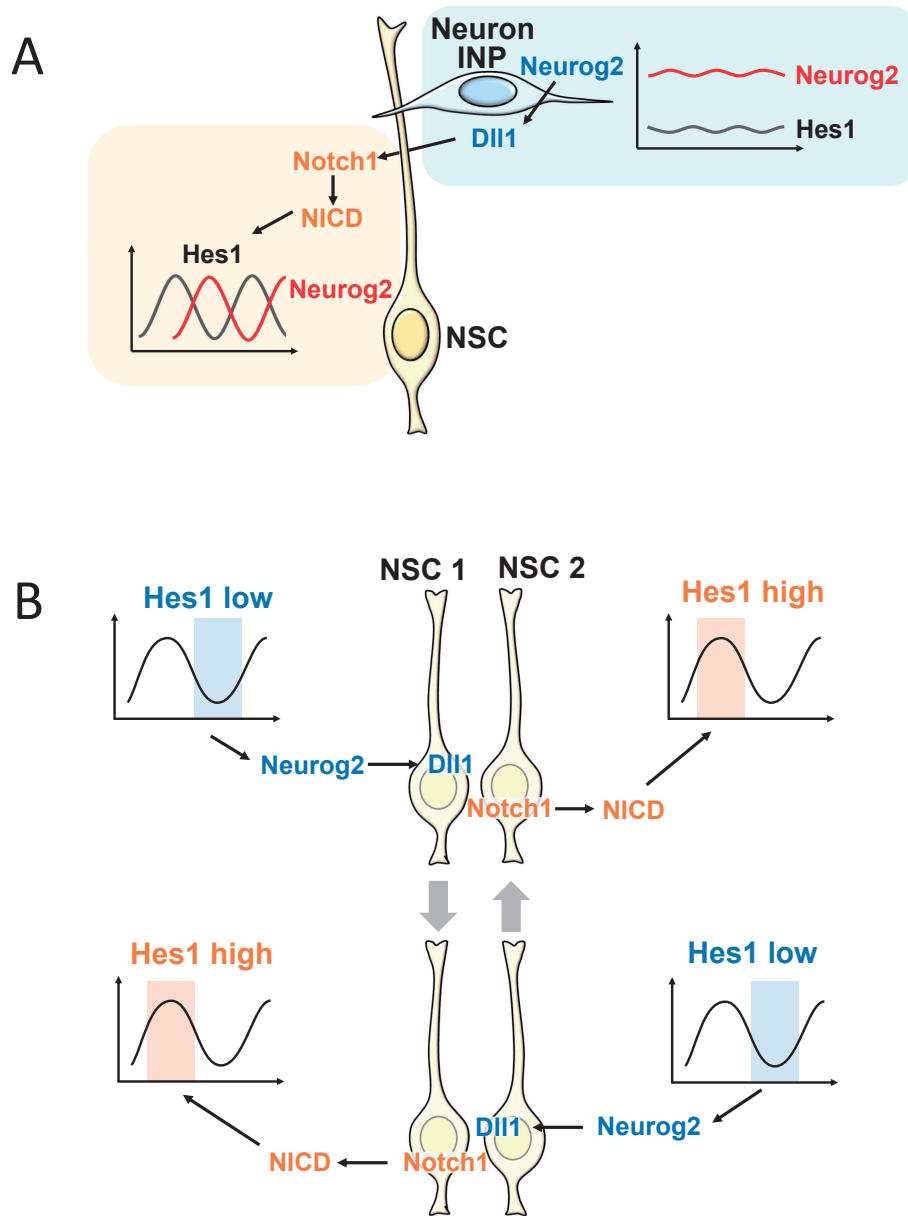


Fig. 2. Dynamic control of Notch signaling. (A) In INPs and neurons, proneural factors induce Dll1 expression, which activates Notch signaling in neighboring cells. The activation of Notch signaling releases the Notch intracellular domain (NICD), which induces Hes1 and Hes5 expression. This process is called lateral inhibition. In NSCs, Hes1 and Hes5 expression autonomously oscillates, driving the oscillatory expression of proneural genes such as Neurog2. (B) Before INPs and neurons are generated, the oscillatory expression of Dll1 enables the mutual activation of Notch signaling between neighboring NSCs, suggesting that oscillatory expression is beneficial for the maintenance of a group of undifferentiated cells.

ing functions, *i.e.*, maintaining NSCs and promoting the differentiation of specific cell types, but it was unknown how these factors regulate their opposing functions. The observation that these factors exhibit different expression dynamics (oscillatory or sustained) between NSCs and differentiating cells suggested a hypothesis that such different expression

dynamics are responsible for the regulation of their opposing functions. Indeed, optogenetic analysis, which can induce pulsatile or sustained expression in cultured NSCs by changing light illumination patterns, showed that *Ascl1* induces neuronal differentiation when its expression is sustained, but activates NSC proliferation when its expression is

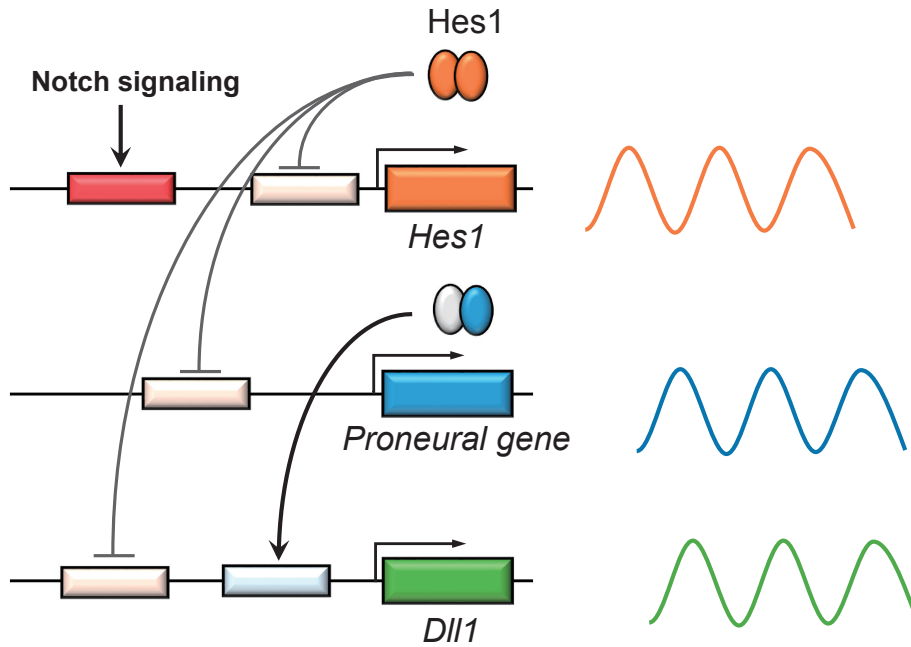


Fig. 3. Dynamic gene expression in active NSCs. Notch signaling activates Hes1 expression, which represses its own expression. From this negative feedback, Hes1 expression oscillates autonomously with a 2–3-h periodicity. Hes1 oscillations periodically repress the expression of proneural genes and Dll1. As a result, these genes are also expressed in an oscillatory manner. Adapted from Ref. 51.

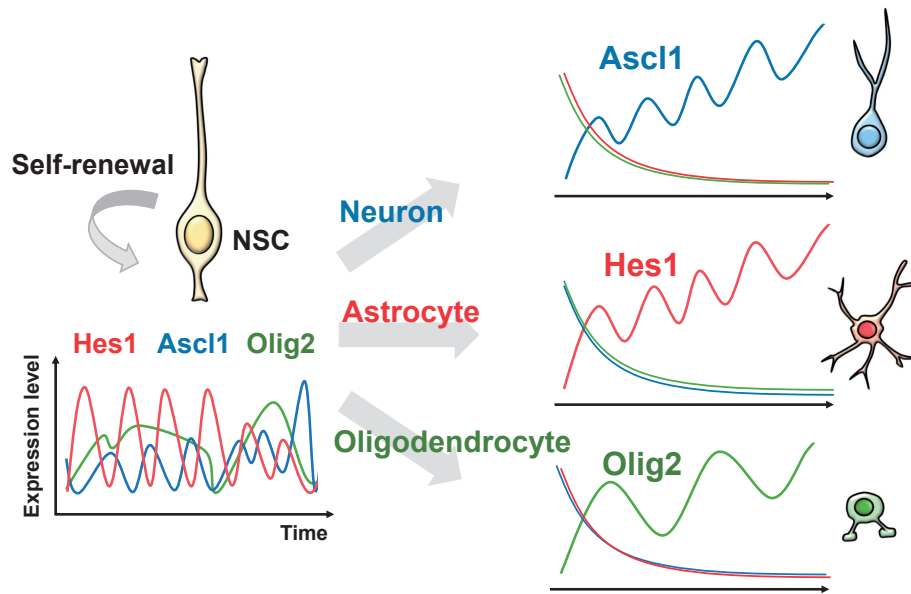


Fig. 4. Expression dynamics of bHLH factors in multipotency and cell fate choice. The proneural gene *Ascl1*, the astrogenic gene *Hes1*, and the oligodendrogenic gene *Olig2* are expressed in an oscillatory manner in proliferating NSCs, but become sustained and high in differentiating neurons, astrocytes, and oligodendrocytes, respectively. Adapted from Ref. 14.

oscillatory.²⁰) These observations suggest that cell fate determination factors such as *Ascl1*, *Hes1*, and *Olig2* exhibit opposing functions depending on their expression dynamics.

4. *In vivo* significance of oscillatory expression in NSCs

The significance of oscillatory expression in

neural development *in vivo* has been analyzed in a number of studies. According to mathematical modeling, the time delay required for Dll1-Notch signaling transmission between cells is important for oscillatory dynamics.²²⁾ With appropriate time delays, neighboring cells exhibit in-phase oscillations, similar to *Hes7* oscillations in the presomitic mesoderm, or out-of-phase oscillations, as observed in NSCs. However, when the time delays are shortened or elongated, both in-phase and out-of-phase oscillations are dampened, and in the most extreme cases, expression becomes steady, a condition known as amplitude/oscillation death.²⁶⁾ To dampen the oscillations, the time delay required for Dll1-Notch signaling transmission between cells was changed by shortening the *Dll1* gene by deleting all introns (type-1 mutant) or elongating it by inserting an extra sequence (type-2 mutant).²²⁾ The shortened and elongated *Dll1* genes exhibit accelerated and delayed expression, respectively. As a result, the time delay in Dll1-Notch signaling transmission is shortened and elongated in type-1 and type-2 Dll1 mutants, respectively. Notably, both types of Dll1 mutant mice exhibit dampened *Hes1* oscillations in NSCs and dampened *Hes7* oscillations in the presomitic mesoderm.²²⁾ Dampened *Hes1* oscillations inhibit the proliferation of NSCs, accelerating neuronal differentiation, while dampened *Hes7* oscillations lead to severe fusion of somites and their derivatives, such as vertebrae and ribs.²²⁾ Another approach was to shorten the time delay required for negative feedback. According to mathematical modeling, oscillatory expression critically depends on the proper time delay in negative feedback, *i.e.*, accelerated negative feedback dampens oscillations, leading to steady expression.⁹⁾ Intronic delay, which includes the transcription of intronic sequences and the removal of introns by splicing, constitutes a major part of the time delay required for negative feedback. Deletion of all introns from the *Hes7* locus accelerates negative feedback, leading to steady *Hes7* expression and severe somite fusion.⁹⁾ Similarly, all introns were removed from the *Hes1* locus (*Hes1*-intron(-)) to dampen *Hes1* oscillations (Fig. 5A).²⁷⁾ In such mutant mice, *Hes1* oscillations in NSCs are dampened (Fig. 5B), and NSC proliferation decreases slightly.²⁷⁾ The phenotype is rather mild, probably because *Hes1* oscillations were not abolished completely, *i.e.*, *Hes1* expression still oscillated in the mutant, although the amplitude was smaller. Another reason is that the functions of *Hes1* can be compensated for by *Hes1*-related genes, such as *Hes3*

and *Hes5*. Indeed, although *Hes3*-null;*Hes5*-null mice are almost normal, the introduction of the *Hes1*-intron(-) mutation significantly inhibits NSC proliferation and accelerates neurogenesis, leading to microcephaly (Fig. 5C).²⁷⁾ These studies clearly indicated that oscillatory expression in NSCs is important for normal brain morphogenesis *in vivo*.

Another example for the significance of expression dynamics is observed in boundary regions such as the isthmus, which separates the midbrain and the hindbrain, and the roof plate and floor plate, which separate the right and left halves of the neural tube.²⁸⁾⁻³¹⁾ In these regions, cells neither proliferate nor produce neurons, unlike proliferating NSCs. In addition to separating regions, boundary cells function as signaling centers to specify their neighboring regions, *e.g.*, the isthmus secretes Fgf8, whereas the roof plate and floor plate secrete Wnt and Shh, respectively.²⁸⁾⁻³¹⁾ Notably, boundary cells express *Hes1* at high levels in a sustained manner and do not express proneural genes, suggesting that sustained *Hes1* expression contributes to the non-proliferative and non-neurogenic properties of boundary cells.³²⁾ The inactivation of *Hes1* and *Hes1*-related genes leads to the ectopic expression of proneural genes and down-regulation of signaling molecules in the boundary regions, whereas the induction of sustained *Hes1* expression represses proneural gene expression and inhibits NSC proliferation.³²⁾ Thus, high and sustained *Hes1* expression and the resultant suppression of proneural genes are important for the non-proliferative and non-neurogenic properties of boundary cells. However, it remains to be determined how sustained *Hes1* expression differentially controls astrocyte differentiation and boundary cell formation.

5. Transition from oscillatory to sustained gene expression

When NSCs start neuronal differentiation, the expression of *Hes1* changes from oscillatory to repressed, whereas *Ascl1* expression changes from oscillatory to sustained.^{19),20)} How the transition from oscillatory to repressed *Hes1* expression is controlled remains to be analyzed. Because *Hes1* expression is regulated by Notch signaling, one possibility is that Notch signaling becomes inactive when neuronal differentiation starts. Treatment with the γ -secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, which inhibits Notch signaling, down-regulates *Hes1* expression and up-regulates *Ascl1* expression in NSCs, leading to neuronal differentiation.²⁰⁾ The Notch ligand Dll1 is

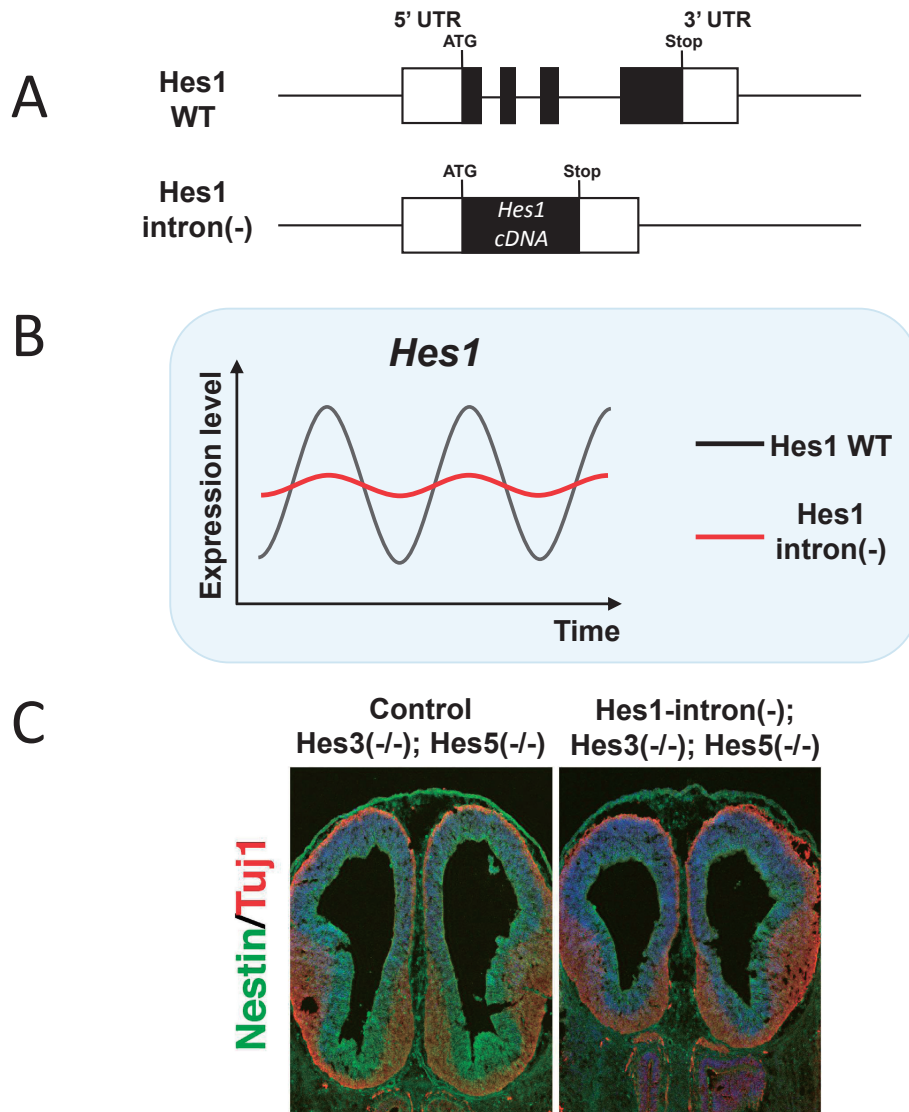


Fig. 5. Reduced NSC proliferation by dampened Hes1 oscillations. (A) Schematic structures of the wild-type and *Hes1-intron(-)* *Hes1* locus. (B) Hes1 expression dynamics in NSCs. Hes1 oscillations are dampened in *Hes1-intron(-)*-mutant NSCs. (C) The telencephalon of a control *Hes3(-/-);Hes5(-/-)* mouse and a *Hes1-intron(-);Hes3(-/-);Hes5(-/-)* mouse. Immunohistochemical analysis shows Nestin⁺ NSCs and Tuj1⁺ neurons. Adapted from Ref. 27.

mainly expressed by INPs in the SVZ when neurogenesis occurs. During this stage, NSCs undergo asymmetric cell division at the apical surface (the innermost region of the neural tube), and each cycle of cell division produces one NSC and one INP (Fig. 1). NSCs retain radial fibers, which can receive Dll1 signals from INPs in the SVZ (Fig. 2A), whereas newly formed INPs, which are present near the apical surface and separate from the SVZ, do not carry radial fibers and, therefore, do not receive Dll1 signals from INPs in the SVZ. Indeed, Notch signaling is

active in NSCs but inactive in INPs.³³⁾ These observations suggest that asymmetric cell division automatically generates a pair of cells, one with active Notch signaling and the other with inactive Notch signaling.

Time-lapse imaging analysis of Hes1 expression suggested that the situation is not that simple. At several hours before asymmetric cell division begins, Hes1 expression disappears, whereas proneural gene expression is up-regulated in a sustained manner, raising the possibility that even when Notch signaling

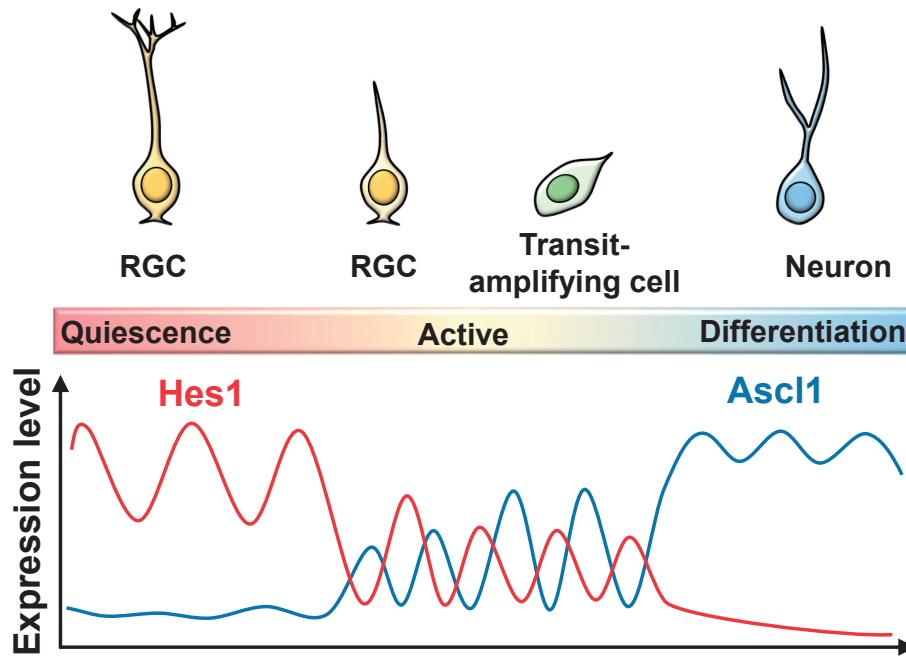


Fig. 6. Expression dynamics of *Hes1* and *Ascl1* in quiescent and active NSCs, transit-amplifying cells, and differentiating neurons. RGC, radial glia-like NSC. Adapted from Ref. 38.

is still active, *Hes1* expression is repressed before asymmetric cell division.²⁰⁾ One possible hypothesis is that a repressor of *Hes1* gradually accumulates in NSCs before asymmetric cell division. *Hes1* oscillations drive proneural gene oscillations in NSCs. Many downstream genes of proneural genes are expressed only after neuronal differentiation starts, and are not expressed in NSCs. However, some downstream genes such as *Dll1* are expressed under the control of proneural genes in NSCs. *Dll1* expression oscillates in NSCs because *Dll1* protein is unstable,^{19);22)} but if protein products are stable, proneural gene oscillations should lead to the gradual accumulation of such proteins. It is possible that such proteins may repress *Hes1* expression when their levels reach a certain threshold. Further analyses are required to test this hypothesis.

6. High and sustained *Hes1* expression in quiescent NSCs in the adult brain

In the adult mouse brain, NSCs are present in two regions, the subgranular zone of the hippocampal dentate gyrus and the SVZ of the lateral ventricles.^{34)–36)} These adult NSCs, which have a radial glial cell morphology, are mostly quiescent/dormant, and only occasionally become activated and divide to produce transit-amplifying cells (Fig. 6). Transit-

amplifying cells divide a few times and soon differentiate into neurons that integrate into the preexisting neural circuits. Thus, the characteristics of NSCs are totally different between the embryonic active and adult quiescent states.

Notch signaling plays an essential role in maintaining active NSCs in the developing nervous system. The inactivation of the Notch signaling effector genes *Hes1* and *Hes1*-related genes up-regulates the expression of proneural genes such as *Ascl1* and *Neurog2*, accelerates neurogenesis, and prematurely depletes NSCs from the developing nervous system.¹⁶⁾ Similarly, the inactivation of the Notch mediator *Rbpj* causes the same defects.³⁷⁾ Thus, the Notch-*Rbpj*-*Hes1* pathway appears to play an essential role in maintaining active NSCs. Of note, Notch signaling is also important for maintaining quiescent NSCs in the adult brain. The inactivation of *Hes1* and *Hes1*-related genes up-regulates *Ascl1* expression, accelerates neurogenesis, and prematurely depletes quiescent NSCs from the adult brain.³⁸⁾ Furthermore, similar defects in adult neurogenesis occur in the absence of *Rbpj*,³⁷⁾ indicating that the Notch-*Rbpj*-*Hes1* pathway plays an essential role in maintaining quiescent NSCs in the adult brain. Thus, Notch signaling regulates the maintenance of embryonic active and adult quiescent NSCs.

The next question is how Notch signaling leads to the active and quiescent states in embryonic and adult brains. Our recent data suggest that the dynamics of *Hes1* expression are involved in these different states. The proneural gene *Ascl1* plays a critical role in the activation of quiescent NSCs and subsequent formation of neuroblasts in the adult brain.³⁹⁾ *Ascl1* is expressed at low levels in some activated NSCs and at high levels in transit-amplifying cells.^{39)–41)} Furthermore, live-imaging analysis showed that *Ascl1*-expressing NSCs exclusively generate neurons in the adult mouse hippocampus.⁴²⁾ By contrast, in the absence of *Ascl1*, all NSCs remain quiescent, indicating that *Ascl1* is absolutely required for the activation of quiescent NSCs.³⁹⁾ Live-imaging analysis of the adult mouse brain demonstrated that *Hes1* expression is oscillatory in quiescent NSCs, although the peaks and troughs are higher than those in active NSCs, causing *Ascl1* expression to be suppressed continuously.³⁸⁾ The inactivation of the Notch-Rbpj-*Hes1* pathway up-regulates *Ascl1* expression, activates NSCs, and transiently enhances neurogenesis, but NSCs are soon depleted, ending neurogenesis prematurely.^{37),38)} Conversely, the induction of sustained *Hes1* expression represses *Ascl1* expression, inhibits neurogenesis, and maintains quiescent NSCs in the adult brain.³⁸⁾ These results indicate that high levels of *Hes1* and the resultant suppression of *Ascl1* promote quiescence in NSCs in the adult brain. Indeed, the induction of *Ascl1* oscillations efficiently activates NSCs to produce new neurons in the adult brain.³⁸⁾

The mechanism by which high levels of *Hes1* are maintained in quiescent NSCs remains to be determined. It was shown that bone morphogenetic protein signaling is important for maintaining quiescent NSCs in the adult brain.⁴³⁾ Bone morphogenetic protein signaling induces the expression of *Id1*, and *Id1* is highly expressed by quiescent NSCs in the adult brain.⁴⁴⁾ Notably, *Id1* interacts with *Hes1* and inhibits *Hes1* negative feedback, thereby up-regulating *Hes1* expression, although *Id1* cannot inhibit *Hes1* from repressing proneural gene expression.⁴⁵⁾ It was also reported that *Notch2* induces *Id4* expression in quiescent NSCs.⁴⁶⁾ These findings suggest that *Id1* and *Id4* may be responsible for the high levels of *Hes1* expression, thereby suppressing *Ascl1* and maintaining quiescent NSCs in the adult brain.^{46),47)}

Why *Hes1* oscillations promote cell proliferation and why sustained *Hes1* expression leads to quiescence still remain to be analyzed. Sustained *Hes1*

expression inhibits the proliferation of not only NSCs but also other cell types, such as muscle and hematopoietic stem cells, suggesting that this inhibitory activity of *Hes1* on proliferation is rather universal in many cell types.⁴⁸⁾ *Hes1* is also highly expressed by human fibroblasts when they enter quiescence due to serum deprivation or contact inhibition.⁴⁹⁾ These quiescent fibroblasts lose their ability to reenter the cell cycle and become senescent when *Hes1* is knocked down, whereas high, sustained *Hes1* expression is sufficient to prevent these cells from becoming senescent.⁴⁹⁾ By contrast, *Hes1* expression oscillates in proliferating muscle progenitors and pancreatic progenitors.^{48),50)} Thus, high levels of *Hes1* expression are a general feature of quiescence, whereas *Hes1* oscillations are a general feature of an active state.⁵¹⁾ One hypothesis is that the expression of genes involved in cell cycle progression is well maintained when *Hes1* expression is oscillatory but is totally suppressed when *Hes1* expression is high and sustained. Further analyses are required to characterize the relationship between *Hes1* oscillations and cell cycle progression.

7. Conclusions

Hes1 oscillations drive the oscillatory expression of the proneural gene *Ascl1*, leading to an active state, whereas high and sustained *Hes1* expression suppresses *Ascl1* expression, leading to quiescence. In other developmental contexts, high and sustained *Hes1* expression is associated with boundary cell formation and astrocyte differentiation. Therefore, the dynamics of *Hes1* expression are a key regulatory mechanism for generating various types of cells in the nervous system. Further understanding of the significance of gene expression dynamics will be useful for the manipulation of NSCs and the development of regenerative medicine in the future.

Acknowledgements

This work was supported by Core Research for Evolutional Science and Technology (CREST) (JPMJCR12W2, R.K.), Grant-in-Aid for Scientific Research on Innovative Areas (16H06480, R.K.) from Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan, AMED-CREST (19gm1110002h0003, R.K.) from Japan Agency for Medical Research and Development, and Scientific Research (C) (18K06254 to H.S.) and Research Fellowship for Young Scientists (17J02922 to S.O.) from Japan Society for the Promotion of Science.

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(Received June 9, 2020; accepted July 30, 2020)

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Profile

Shohei Ochi was born in Mie prefecture in 1989. He earned his B.S. degree in Pharmaceutical Science from Tokyo University of Science in 2013. He received his M.S. degree in Medical Science in 2015 and Ph.D. degree in Medicine in 2020 from Kyoto University where he was provided JSPS Research Fellowship for Young Scientists in 2018. He moved to Sendai in 2020 and worked as research assistant for the Neuro Global International Joint Graduate Program in the Department of Developmental Neuroscience, United Centers for Advanced Research and Translational Medicine (ART), at Tohoku University Graduate School of Medicine where he is investigating the molecular mechanism of sexual differentiation in the embryonic murine cortex.



Profile

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Profile

Hiromi Shimojo was born in Fukushima Prefecture in 1979 and graduated and received her D.V.M. from Nippon Veterinary and Life Science University in 2004. She received her Ph.D. in Medicine from Kyoto University in 2008. Thereafter, she began her postdoctoral training at the Institute for Virus Research in Kyoto University. From 2013, she worked at Institute for Integrated Cell-Material Sciences in Kyoto University as a program-specific research center assistant professor. From 2017, she worked at the Institute for Frontier Life and Medical Sciences in Kyoto University as a program-specific assistant professor. From 2020, She moved to the Graduate School of Frontier Biosciences in Osaka University and is currently working as an assistant professor. Her research interests are on the significance of various dynamics in gene expression and signal transmission during mammalian development.

