<table>
<thead>
<tr>
<th>Title</th>
<th>Dynamic regulation of Notch signaling in neural progenitor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Kageyama, Ryoichiro; Ohtsuka, Toshiyuki; Shimojo, Hiromi; Imayoshi, Itaru</td>
</tr>
<tr>
<td>Citation</td>
<td>Current Opinion in Cell Biology (2009), 21(6): 733-740</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2009-12</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/2433/89648">http://hdl.handle.net/2433/89648</a></td>
</tr>
<tr>
<td>Rights</td>
<td>© 2009 Elsevier Ltd. All rights reserved. Please cite only the published version. この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。</td>
</tr>
<tr>
<td>Type</td>
<td>Journal Article</td>
</tr>
<tr>
<td>Textversion</td>
<td>author</td>
</tr>
</tbody>
</table>

Kyoto University
Dynamic regulation of Notch signaling in neural progenitor cells

Ryoichiro Kageyama*, Toshiyuki Ohtsuka, Hiromi Shimojo, and Itaru Imayoshi

Institute for Virus Research, Kyoto University, Shogoin-Kawahara, Sakyo-ku, Kyoto 606-8507, Japan; Japan Science and Technology Agency, CREST, Shogoin-Kawahara, Sakyo-ku, Kyoto 606-8507, Japan

*Corresponding author: Ryoichiro Kageyama
Institute for Virus Research
Kyoto University
Shogoin-Kawahara, Sakyo-ku
Kyoto 606-8507
Japan
Tel: 81-75-751-4011
Fax: 81-75-751-4807
E-mail: rkageyam@virus.kyoto-u.ac.jp
Summary
In the developing nervous system, differentiating neurons express Delta and activate Notch signaling in their neighboring cells. As a result of Notch activation, neuronal differentiation is inhibited in neighboring cells and they remain neural progenitor cells. Thus, differentiation of neurons and maintenance of neural progenitor cells are well balanced due to Notch signaling. Recent studies revealed that Notch signaling is under the control of more complex and dynamic regulation than previously thought, such as cell cycle-dependent activation and oscillating gene expression. We discuss here recent advances in understanding how Notch signaling is regulated in the developing nervous system and what outcome each type of regulation of Notch signaling leads to. We highlight the role of Notch signaling in proliferation and differentiation of neural progenitor cells.

Introduction
During cortical development, neural progenitor cells (neuroepithelial cells/radial glial cells) initially undergo symmetric cell division: each neural progenitor cell divides into two neural progenitor cells (Fig. 1) [1-5]. By repeating symmetric cell division, neural progenitor cells proliferate extensively. Then, these cells undergo asymmetric cell division: each neural progenitor cell divides into two distinct cell types, one neural progenitor cell and one immature neuron or a basal progenitor cell (Fig. 1). Immature neurons migrate outside of the ventricular zone into the cortical plate, where these cells become mature neurons, whereas basal progenitor cells migrate into the subventricular zone, proliferate further and give rise to more neurons. By repeating asymmetric cell division, neural progenitor cells sequentially give rise to distinct types of neurons. Neural progenitor cells also undergo symmetric neurogenic division, by which each cell divides into two neurons. After production of neurons, neural progenitor cells finally differentiate into glial cells (Fig. 1). Thus, neural progenitor cells gradually change their competency in proliferation and differentiation during neural development. If these cells are prematurely depleted, not only is the number of cells reduced but also later-born cell types are lacking, indicating that maintenance of neural progenitor cells until the final stage in development is essential for achieving production of both a proper number of cells and a full diversity of cell types [6]. It has been shown that Notch signaling plays an important role in maintenance and differentiation of neural progenitor cells. The core
pathway of this signaling has been recently reviewed in detail [7,8]. Here we review the recent progress on different aspects of Notch signaling.

**Basic pathway of Notch signaling**
In the developing nervous system, proneural genes such as the basic helix-loop-helix (bHLH) transcriptional activators *Mash1* and *Neurogenin2* (*Ngn2*) induce the neuronal differentiation program [9,10]. These genes also activate the expression of ligands for the Notch receptor such as the transmembrane protein Delta1, which activate Notch protein of neighboring cells (Fig. 2). The ubiquitin ligase Mind bomb is essential for Delta-induced activation of Notch signaling [11,12]. Upon activation of Notch, the intracellular domain (NICD) is released from the transmembrane portion and transferred to the nucleus, where NICD forms a complex with the DNA-binding protein RBPj (Fig. 2) [13]. The NICD-RBPj complex is a transcriptional activator and induces expression of bHLH transcriptional repressors such as *Hes1* and *Hes5*. Hes1 and Hes5 then repress expression of proneural genes and Delta1, thereby leading to inhibition of neuronal differentiation and maintenance of neural progenitor cells (Fig. 2) [14]. Thus, a differentiating neuron prevents neighboring neural progenitor cells from differentiating, thereby promoting asymmetric division into one neural progenitor cell and one differentiating daughter neuron. This pathway involves many factors such as ligands (Delta), receptors (Notch), mediators (RBPj) and effectors (Hes), and it has been shown that the activity of Notch signaling is regulated or modulated at the levels of these factors.

**Biased distribution of Delta and Notch**
The nuclei of neural progenitor cells are known to move between the apical and basal ends of the ventricular zone depending on the phase of the cell cycle, and this process is termed elevator movement or interkinetic nuclear migration [3]. Nuclei are located at the apical side (the ventricular surface) during M phase and at the basal side of the ventricular zone during S phase (Fig. 3). Nuclei move from the apical to the basal side during G1 phase and in the opposite direction during G2 phase (Fig. 3). It has been suggested that this nuclear movement is involved in regulation of the rate of neurogenesis via Notch signaling [15]. Indeed, it was recently shown that Notch signaling activity changes according to the position of nuclei due to differential apical-basal distribution of Delta and Notch.
In the developing retina of zebrafish, Notch is expressed at higher levels at the apical side and forms the apical-to-basal gradient, whereas Delta is expressed in a gradient with the opposite orientation (Fig. 3) [16••]. The precise mechanism of how these gradients are formed is not known, but the stability of the gene products regulated according to the cell cycle seems to contribute to the gradient formation [17]. Interestingly, Notch signaling is activated as the nuclei move to the apical side whereas it is down-regulated as the nuclei move to the basal side [16••]. It is likely that cells whose nuclei are located in the basal region (Delta-high) send Delta signals to neighboring cells whose nuclei are located in the apical region (Notch-high), but it remains to be determined where in the developing retina Delta-Notch interaction occurs (apical, basal, or everywhere). These results suggest that the nuclear movement is important for activation of Notch signaling and maintenance of neural progenitor cells. In agreement with this notion, mok-mutant neural progenitor cells, whose nuclei are preferentially located in the basal side due to inactivation of the motor protein Dynactin1, receive less Notch signaling activity and prematurely differentiate into neurons [16••]. Since overexpression of NICD rescues this mutation, it is likely that the proper activation of Notch signaling depends on nuclear movement. In the developing dorsal telencephalon of mice, however, NICD and Hes1 are expressed more frequently in the basal side of the ventricular zone, suggesting that Notch signaling is active in the basal region, unlike in the zebrafish retina [18]. Whether nuclear movement is involved in activation of Notch signaling in regions other than the retina is currently unknown.

**Regulation of ligand expression**

In addition to Delta, Jagged1 and Jagged2 function as Notch ligands, and these ligands seem to be differentially used during development. In the developing cochlea of the inner ear, the sensory epithelial domain is initially specified, and then hair cells (sensing the sound) and support cells differentiate from the sensory epithelial domain. It has been shown that both the initial phase (the sensory epithelial specification) and the subsequent phase (hair cell versus support cell differentiation) are regulated by Notch signaling [19,20•,21•,22•]. Blockade of Notch signaling during the initial phase inhibits the specification of the sensory epithelial domain, leading to loss of both hair cells and support cells. In contrast, blockade of Notch signaling during the later phase leads to overproduction of hair cells at the expense of support cells. Thus,
inhibition of Notch signaling leads to different outcomes depending on developmental stages. It has been shown that Jagged1 is expressed in the presumptive sensory domain and regulates the formation of the sensory epithelium, while Delta1 and Jagged2 are expressed by hair cells and inhibit neighboring support cells from becoming hair cells [19]. Thus, it is likely that different Notch ligands have different activities in the developing cochlear. Although the precise mechanism of how different ligands make different outcomes remains to be determined, it seems that Jagged1 induces expression of Hey1/Hesr1 and Hey2/Hesr2, Hes-related bHLH transcriptional repressors, whereas Delta1 and Jagged2 induce expression of Hes1 and Hes5 [22•]. Further analysis is required to determine whether Hes1/5 and Hey1/2 have distinct activities in the developing cochlear.

**Regulation of RBPj activity**

RBPj is not always a mediator of Notch signaling, but its activity seems to be regulated, leading to a different outcome of Notch signaling. Whereas Notch signaling is required for maintenance of both neural progenitor cells and basal progenitor cells, the latter cells express less Hes1 and Hes5 than the former, suggesting that RBPj is not active in the latter [23••,24••]. Furthermore, knock-down of RBPj converts neural progenitor cells into basal progenitor cells [23••]. These results suggest that RBPj-dependent Notch signaling regulates neural progenitor cells, whereas RBPj-independent Notch signaling regulates basal progenitor cells. However, the expression level of RBPj is not different between these two types of cells, and thus RBPj may be posttranslationally inactivated in basal progenitor cells. Activation of RBPj however does not convert basal progenitor cells into neural progenitor cells, suggesting that the conversion is unidirectional, proceeding only from neural progenitor cells to basal progenitor cells.

In addition to inactivation of RBPj, up-regulation of Tbr2 is required for basal progenitor cell formation [25,26]. Tbr2 expression is induced by Ngn2, and it was shown that one of the daughter cells initiates expression of Ngn2 and Tbr2 about 2 hours and 4 hours, respectively, after asymmetric cell division in the mouse dorsal telencephalon [27•]. This Ngn2'Tbr2' daughter cell becomes a basal progenitor cell, while the other daughter cell is negative for Tbr2 (Ngn2 is either positive or negative, see below) and remains a neural progenitor cell. It is likely that Tbr2 expression is inhibited by RBPj-dependent Notch signaling in neural progenitor cells but not by
RBPj-independent Notch signaling in basal progenitor cells, but the precise mechanism of RBPj-independent Notch signaling remains to be determined.

**Hes1-driven oscillations in Notch signaling**

The Notch signaling pathway described above (differentiating neurons activate Notch signaling of neighboring cells) raises a question as to how neural progenitor cells are maintained during early stages before neurons are generated. It has been shown that proneural genes and Delta1 are expressed in a salt-and-pepper pattern before neurons are born [28-36]. In the mouse dorsal telencephalon, neuronal formation starts around E11, but the proneural gene Ngn2 and Delta1 as well as Notch are expressed by neural progenitor cells as early as E8.5, indicating that Notch signaling is active before neuronal formation. This result raises another question, why neurons are not born until E11 although Ngn2 is expressed as early as E8.5. Real-time imaging analysis [37] revealed that Ngn2 expression is oscillating in neural progenitor cells but sustained in differentiating neurons [38••]. The oscillating Ngn2 expression in neural progenitor cells is driven by Hes1 oscillations. Notch signaling induces Hes1 expression, but Hes1 represses its own expression by directly binding to the N box sequences in its own promoter. This negative feedback leads to disappearance of both Hes1 mRNA and Hes1 protein because they are extremely unstable, but the disappearance of Hes1 protein allows the next round of expression [39]. In this way, Hes1 expression oscillates with a period of about 2-3 hours in neural progenitor cells (Fig. 4A) [38••]. Oscillating Hes1 expression periodically represses Ngn2 expression, leading to oscillating Ngn2 expression (Fig. 4A). Strikingly, Delta1 expression also oscillates in neural progenitor cells under the control of periodic activation by Ngn2 and periodic repression by Hes1 (Fig. 4A) [38••]. Delta1 oscillations then seem to reciprocally activate Notch signaling each other and maintain a group of cells as neural progenitor cells (Fig. 4A).

Shutdown of Notch signaling represses Hes1 expression persistently and leads to sustained up-regulation of Ngn2 [38••]. Apparently, sustained expression of Ngn2 is required for neuronal differentiation, probably because many downstream genes required for neuronal differentiation respond rather slowly to Ngn2 [40,41]. When Ngn2 expression oscillates, only subsets of downstream genes such as Delta1 seem to be selectively expressed. Thus, Ngn2 oscillations may be advantageous for maintenance of neural progenitor cells by inducing Delta1 oscillations without
inducing neuronal differentiation. These results suggest that Ngn2 can induce two totally opposite outcomes depending on its expression mode: oscillating expression leads to maintenance of neural progenitor cells whereas sustained expression induces neuronal differentiation (Fig. 4B).

**Non-oscillatory Hes1 expression in dormant cells**

Not all cells express Hes1 in an oscillatory manner: cells in the roof plate, floor plate and boundary regions such as the isthmus seem to express Hes1 continuously [42]. These cells proliferate very slowly or do not proliferate at all. In addition, they usually do not give rise to any neurons because sustained Hes1 expression constitutively represses proneural gene expression. Furthermore, introduction of non-oscillatory Hes1 expression into neural progenitor cells inhibits their proliferation and neuronal differentiation [42]. However, these cells can initiate neuronal differentiation when Hes genes are inactivated [42], suggesting that cells with non-oscillatory Hes1 expression are rather dormant with regard to proliferation and differentiation activities but have potential to resume such activities when Hes1 expression become non-sustained. Thus, these cells have not irreversibly become post-mitotic cells but maintain the proliferation and differentiation potential. This feature is similar to the one observed in fibroblasts, where sustained Hes1 overexpression leads to reversible quiescence (can resume cell proliferation) but not to irreversible senescence (cannot resume cell proliferation) [43•]. Neural stem cells in the adult brain are also slowly dividing or mostly dormant, although continuous neurogenesis from these cells is essential for maintenance of structures and functions of the adult brain [44]. Hes1 seems to be highly expressed by adult neural stem cells [45], and it is possible that the dormancy of adult neural stem cells is regulated by sustained Hes1 expression.

The mechanism of how oscillatory versus non-oscillatory Hes1 expression is regulated remains to be determined, but it has been suggested that Jak-Stat signaling is involved in this regulation. In fibroblasts, Jak2 activates Stat3 by phosphorylation, and phosphorylated Stat3 (pStat3) induces Socs3 expression, which in turn inhibits Jak2 [46]. Because of this delayed negative feedback, pStat3 and Socs3 levels oscillate. In fibroblasts, blockade of this pathway by a Jak inhibitor leads to non-oscillatory Hes1 expression by stabilizing the Hes1 protein [46]. Similarly, treatment with a Jak inhibitor inhibits Hes1 oscillations in neural
progenitor cells, suggesting that Jak-Stat signaling is involved in regulation of oscillating Hes1 expression [38••]. Another possible mechanism is Id-mediated regulation of Hes1 [47••]. Id proteins, HLH factors without a basic region, form heterodimers with Hes1 through the HLH domains and inhibit Hes1 from binding to the N box in the Hes1 promoter but not from binding to the class C site in proneural gene promoters. As a result, Id factors prevent Hes1 from negative autoregulation and sustain Hes1 expression but allow Hes1 to repress proneural gene expression [47••].

Conclusions
Notch signaling is regulated at multiple steps. First, in the developing retina, neural progenitor cells with nuclei located at the basal side send Delta signals, while ones with nuclei located at the apical side receive Notch activation. Thus, the interkinetic nuclear migration is important for the proper Notch signaling activity. Second, different Notch ligands have different activities, and therefore the selection of appropriate Notch ligands is important for proper neural development. Third, Notch signaling is regulated at the level of RBPj. Notch signaling maintains both neural progenitor cells and basal progenitor cells, but RBPj-dependent pathway regulates the former, whereas RBPj-independent pathway regulates the latter. Inactivation of RBPj converts neural progenitor cells into basal progenitor cells. Fourth, expression of the effector Hes1 is dynamic in neural progenitor cells. Hes1 oscillations drive cyclic expression of Delta1, which in turn activates Notch signaling, thereby keeping a group of cells undifferentiated. Last, non-oscillatory Hes1 expression makes neural progenitor cells dormant with regard to proliferation and differentiation, and Jak-Stat and Id seem to be involved in the regulation of oscillating and non-oscillating Hes1 expression. It is now clear that Notch pathway is under more complex and dynamic regulation than previously thought. How these multiple regulatory mechanisms for Notch signaling are coordinated in the developing nervous system remains to be determined.

References


This paper demonstrated that Mind bomb 1, which modulates Notch ligand endocytosis in a ubiquitin-dependent manner, is expressed by intermediate progenitors (or basal progenitors) and newborn neurons in the developing mouse cortex. By making conditional knock-out mice, the authors revealed that this gene is required for Notch signaling by Delta-expressing cells.


This study demonstrated that interkinetic nuclear migration (INM) controls the Notch signaling activity during cell cycle in the retinal progenitor cells of zebrafish. Notch signaling is more activated on the apical side than on the basal side of the retina. Retinal progenitor cells having a mutation on Dynactin-1, which encodes an important regulator protein of INM, quickly passed through apical region, and thus are less exposed to Notch signaling and exit the cell cycle prematurely.


20•. Daudet N, Ariza-McNaughton L, Lewis J: Notch signalling is needed to maintain, but not to initiate, the formation of prosensory patches in the chick inner ear. *Development* 2007, **134**:2369-2378.

See 22.


See 22.


These studies showed that treatment with Notch inhibitors in the developing cochlear during an early phase leads to loss of the sensory epithelium (and loss of hair cells), whereas the same treatment during a later phase leads to overproduction of hair cells, indicating that Notch signaling has different activities at different stages. The Hes-related genes *Hey1/Hesr1* and *Hey2/Hesr2* are expressed during the sensory epithelium specification, but *Hes1* and *Hes5* are mainly expressed during hair cell versus support cell differentiation.


The authors showed that neural stem cells (NSCs) and intermediate neural progenitors (INPs) coexist in the developing telencephalic ventricular zone (VZ), and
that these cells can be distinguished by RBPj/CBF1 activity. RBPj-dependent Notch signaling promotes the NSC characters in the VZ, whereas RBPj-independent Notch signaling promotes the INP characters in the VZ and SVZ. This paper described that the neocortical VZ cells are heterogeneous and that Notch signaling differentially regulates NSCs and INPs.


This study demonstrated that neural (apical) progenitor cells, young basal progenitor cells in the ventricular zone and basal progenitor cells in the subventricular zone could be distinguished by gene expression profiles. This study also suggested that young basal progenitor cells are a major source of the Delta signaling. Notch signaling is necessary for preventing neural progenitor cells from prematurely differentiating into young basal progenitor cells. It was also shown that neural progenitor cells display significant variations in the expression level of the downstream component of the Notch signaling (Hes1, Hes5, Ngn2, Ascl1, Dll1 etc.), suggesting that neural progenitor cells are heterogeneous in the developing neocortex.


In this paper, using clonal DiI-labeling and time-lapse observation methods, authors carefully analyzed timeline of Ngn2 and it’s downstream target, Tbr2 in neural progenitors just after finishing their apical cell-divisions. Ngn2 expression is initiated asymmetrically in one of the daughter cells followed by Tbr2 expression. It was also shown that Notch signaling activity is weaker in apically located neural progenitors than in basally located progenitors, but Notch activation during the initial phase of life in apical surface-born daughter cells prevents the premature initiation of the Ngn2-Tbr2 cascade.


30. Hatakeyama J, Kageyama R: *Notch1 expression is spatiotemporally correlated with neurogenesis and negatively regulated by Notch1-independent Hes genes in the developing nervous system*. Cereb Cortex 2006, 16 Suppl. 1:i132-137.


34. Sommer L, Ma Q, Anderson DJ: *neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination
genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. 
\textit{Mol Cell Neurosci} 1996, 8:221-241.


Real-time imaging revealed that \textit{Hes1} expression oscillates with a period of about 2-3 hours in neural progenitor cells in the developing mouse neocortex. \textit{Hes1} oscillations drive \textit{Ngn2} and \textit{Delta1} oscillations, which in turn mutually activate Notch signaling between these cells. Inhibition of Notch signaling leads to repression of \textit{Hes1} and sustained up-regulation of \textit{Ngn2}, resulting in neuronal differentiation.


42. Baek JH, Hatakeyama J, Sakamoto S, Ohtsuka T, Kageyama R: Persistent and high levels of Hes1 expression regulate boundary formation in the developing central nervous system. *Development* 2006, **133**:2467-2476.

43. Sang L, Coller HA, Roberts JM: Control of the reversibility of cellular quiescence by the transcriptional repressor HES1. *Science* 2008, **321**:1095-1100. This paper demonstrated that Hes1 is required for the reversibility of cellular quiescence in fibroblasts and tumor cells. Increases Hes1 expression prevent both premature senescence and inappropriate differentiation of quiescent cells.


47. Bai G, Sheng N, xie Z, Bian W, Yokota Y, Benezra R, Kageyama R, Guillemot F, Jing N: Id sustains Hes1 expression to inhibit precocious neurogenesis by releasing negative autoregulation of Hes1. *Dev Cell* 2007, **13**:283-297. This study revealed the mechanism of how Id factors regulate maintenance of neural progenitor cells. Id proteins inhibit Hes1 from negative autoregulation by physical
interaction with Hes1, thereby sustaining Hes1 expression. This physical interaction
does not interfere with Hes1 ability to repress proneural gene expression. Thus, Id
proteins maintain neural progenitor cells by sustaining Hes1 expression.

Figure legends
Figure 1. Proliferation and differentiation of neural progenitor cells.
Neural progenitor cells (NPC) initially undergo symmetric cell division and
proliferate extensively. Then, these cells give rise to neurons (N) or basal progenitor
cells (BP) by asymmetric cell division. Neurons and basal progenitor cells migrate
into the cortical plate (CP) and the subventricular zone (SVZ), respectively. Basal
progenitor cells further divide in the SVZ and produce more neurons. Neural
progenitor cells undergo asymmetric cell division in the ventricular zone (VZ),
giving rise to many different types of neurons. After production of neurons, neural
progenitor cells finally differentiate into glial cells.

Figure 2. The core pathway of Notch signaling.
Proneural genes such as Mash1 and Ngn2 activate the neuronal differentiation
program and induce the expression of Delta, which in turn activates Notch in
neighboring cells. Upon activation of Notch, the intracellular domain (NICD) is
released from the transmembrane portion and transferred to the nucleus, where NICD
forms a complex with the DNA-binding protein RBPj. The NICD-RBPj complex
induces expression of transcriptional repressor genes such as Hes1 and Hes5. Hes1
and Hes5 then repress expression of proneural genes and Delta, thereby leading to
maintenance of neural progenitor cells.

Figure 3. Elevator movement/interkinetic nuclear migration.
Nuclei of neural progenitor cells are located at the apical side (the ventricular
surface) during M phase and at the basal side of the ventricular zone during S phase.
Nuclei move from the apical to the basal side during G1 phase and in the opposite
direction during G2 phase. In the developing retina of zebrafish, Notch is expressed
at higher levels at the apical side and forms the apical-to-basal gradient, whereas
Delta is expressed in a gradient with the opposite orientation. Due to this biased
distribution of Delta and Notch, the nuclear movement is important for activation of
Notch signaling and maintenance of neural progenitor cells.

Figure 4. Oscillations in Notch signaling.
(A) Hes1 expression oscillates with a period of about 2-3 hours in neural progenitor cells, and Hes1 oscillations drive Ngn2 and Delta1 oscillations by periodic repression. Delta1 oscillations then seem to activate Notch signaling and keep a group of cells as neural progenitor cells. (B) Hes1 oscillations drive Ngn2 oscillations in neural progenitor cells (NPC), whereas loss of Hes1 expression continuously up-regulates Ngn2 expression in neurons. Sustained Ngn2 expression induces neuronal differentiation, whereas Ngn2 oscillations lead to maintenance of neural progenitor cells.
Figure 1

Symmetric division phase

Asymmetric division (neurogenic) phase

Gliogenic phase

NPC

N

BP

glia

CP

SVZ

VZ
Figure 3: Neural progenitor cell cycle progression.