Roades of Hes bHLH Factors in Neural Development

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Abstract

Hes genes, mammalian homologues of Drosophila hairy and Enhancer of split genes, encode basic helix-loop-helix (bHLH) transcriptional repressors. There are seven members in the Hes family, among which Hes1, Hes3, and Hes5 are expressed by embryonic neural stem cells. Mutations in these Hes genes lead to up-regulation of proneural bHLH gene expression and concomitantly premature neuronal differentiation. As a result, neural stem cells are prematurely depleted without proliferating sufficiently, and without generating later born cell types such as astrocytes and ependymal cells. In addition, premature depletion of neural stem cells leads to the disruption of brain structures, because these cells constitute a framework by forming the inner and outer barriers. Thus, Hes genes regulate the generation of cells not only in the correct number but also in their full diversity and in an organized manner by maintaining neural stem cells. At later stages, Hes genes promote gliogenesis. In contrast, proneural bHLH genes induce Hes6, which antagonizes Hes1 activity and promotes neuronal specification. This antagonistic regulation between Hes1/3/5 and proneural bHLH genes is important for the normal timing of neural stem cell differentiation. Hes genes are also required for maintenance of the isthmic organizer, which specifies the midbrain and hindbrain by secreting morphogens. Thus, Hes genes regulate formation of complex brain structures with appropriate size, shape, cytoarchitecture and specification by controlling neural stem cells and the organizing center.

1.1 Introduction

The neural plate consists of neuroepithelial cells, and these cells divide symmetrically to produce more neuroepithelial cells (Fig. 1.1). This cell type is the earliest form of embryonic neural stem cells (Alvarez-Buylla et al., 2001; Fujita, 2003). After neural tube formation, neuroepithelial cells become radial glial cells, which have a cell body in the ventricular zone and long radial fibers extending from the internal
surface to the pial (outer) surface of the neural tube (Fig. 1.1). This cell type was long thought of as a specialized glial cell that guides neuronal migration along the radial fibers, but it has been recently shown that radial glia are embryonic neural stem cells. Radial glial cells divide asymmetrically, forming one radial glial cell and one neuron (or a neuronal precursor) from each division. Neurons migrate along the radial fibers to the outer layers. Radial glial cells are later differentiated into ependymal cells, which form the internal lining of the neural tube (Fig. 1.1) (Spassky et al., 2005). After production of neurons, radial glial cells give rise to oligodendrocytes and finally to astrocytes (Fig. 1.1). At around the time of birth, the radial glial cells disappear, but recent studies have shown that some astrocytes or astrocyte-like cells are neural stem cells, which remain in the adult brain. Thus, neural stem cells change their characteristics in both morphology and competency during development (Alvarez-Buylla et al., 2001; Fujita, 2003). Because it takes a certain period of time for neural stem cells to change their characteristics, maintenance of these cells until later stages is required for generation of cells not only in the correct number but also in their full diversity.

Another important aspect of neural development is that the nervous system is partitioned into several compartments such as the midbrain and hindbrain. These compartments are divided by specialized boundary cells, which secrete morphogens and specify the adjacent compartments, thus acting as the organizing center. For example, the isthmus, the boundary demarcating the midbrain and hindbrain, secretes Wnt1 and Fgf8 and thereby regulates midbrain and hindbrain development (the isthmic organizer, see Fig. 1.11) (Lumsden and Krumlauf, 1996; Joyner et al., 2000; Mason et al., 2000; Wurst and Bally-Cuif, 2001). Premature loss of the isthmus leads to mis-specification of the midbrain and hindbrain neurons. Thus, maintenance of the boundary cells is very important for development of region-specific neurons.

Recent studies have shown that Hes genes, which encode basic helix-loop-helix (bHLH) transcriptional repressors, play a critical role in maintenance of both neural stem cells and boundary cells in the developing nervous system. In the absence of Hes genes, neural stem cells and boundary cells are prematurely lost, leading to severe impairment of neural development. In this chapter, we describe the structures, expression, regulation and functions of Hes factors in neural development.

1.2 Structure and Transcriptional Activities of Hes Factors

Hes genes are mammalian homologues of Drosophila hairy and Enhancer of split [E(spl)] genes, which negatively regulate neural development (Akazawa et al., 1992; Sasai et al., 1992; Feder et al., 1993). There are seven members in the Hes family (Fig. 1.2B), among which Hes1 and Hes4 are more similar to hairy in structure while the other members are more similar to E(spl). There are also several Hes-related bHLH genes such as Hesr/Hey/HRT/Herp/CHF/Gridlock (Iso et al., 2001) and Heslike (Miyoshi et al., 2004), which form distinct subfamilies (Fig. 1.2B,C). Among the Hes
family members, *Hes1*, *Hes3* and *Hes5* are expressed by neural stem cells in mouse developing nervous system. Each Hes factor has the following three conserved domains: the bHLH domain; the Orange domain (the helix 3–helix 4 domain); and the WRPW (Trp-Arg-Pro-Trp) domain, which are essential for transcriptional activities (Fig. 1.2A).

The bHLH domain involves dimer formation and DNA binding. bHLH factors form homodimers and heterodimers through the HLH domain while binding to DNA targets via their basic regions. Strikingly, a proline residue is conserved in the middle of the basic region of all Hes factors as well as of *Drosophila Hairy* and E(spl) proteins (Fig. 1.2A,B, asterisk). It has been suggested that this proline residue may be involved in the specificity of the target DNA sequences, although the exact significance of this conservation remains to be determined. Hes1 binds to the N box (CACNAG) and the class C site (CACGCG) with a higher affinity than to the E box (CANNTG) (Sasai et al., 1992; Chen et al., 1997), unlike most other bHLH factors.

**Fig. 1.1** Change of morphology and competency of neural stem cells during development. Neuroepithelial cells divide symmetrically to increase the cell number. After neural tube formation, neuroepithelial cells become radial glial cells, which have a cell body in the ventricular zone and long processes extending from the internal to the outer surface. Radial glial cells divide asymmetrically to produce one radial glial cell and one neuron (or neuronal precursor) from each division. Neurons migrate along the radial fibers to the outer layers. After neurogenesis, radial glial cells give rise to oligodendrocytes, ependymal cells and finally astrocytes.
Fig. 1.2  Features of Hes bHLH factors. (A) Three conserved domains of Hes factors, the bHLH, Orange and WRPW domains. (B) Sequence alignment of the bHLH domain of Hes and related factors. Proline is conserved in the middle of the basic region of Hes factors (asterisk). (C) Phylogenetic tree of Hes and related factors. (This figure also appears with the color plates.)
which bind to the E box with a higher affinity. Hes1 can bind to these sites not only as a homodimer (Fig. 1.3A) but also as a heterodimer with Hes-related bHLH factors such as Hesr (Iso et al., 2001). In contrast, when Hes1 forms a heterodimer with other bHLH factors such as Mash1 and E47, these heterodimers do not bind to DNA (non-functional heterodimers) (Fig. 1.3B) (Sasai et al., 1992). The HLH factor Id, which lacks the basic region (Benezra et al., 1990), also forms a heterodimer with Hes1, but this heterodimer does not bind to DNA either (Jogi et al., 2002).

The Orange domain, located just downstream of the bHLH domain, is suggested to consist of two amphipathic helices (see Fig. 1.2A). This domain is shown to confer specificity for bHLH factor interactions (Dawson et al., 1995; Taelman et al., 2004). For example, the Hes-related bHLH factor Hairy interacts with the bHLH factor Scute efficiently, while another Hes-related bHLH factor, E(spl)m8, does not, and this difference in the interaction specificity maps to the Orange domain (Dawson et al., 1995). This domain is also known to mediate transcriptional repression (Castella et al., 2000), although a corepressor interacting with this domain is not known.

The WRPW domain is located at or near the carboxyl terminus (see Fig. 1.2A). This domain acts as a repression domain and interacts with the corepressor TLE/Grg, a homologue of *Drosophila* Groucho (Paroush et al., 1994; Fisher et al., 1996; Grbavec and Stifani, 1996). It is suggested that Groucho mediates long-range transcriptional repression that can function over distances of several kilobases in *Drosophila* embryos (Zhang and Levine, 1999). Groucho modifies the chromatin structure by recruiting the histone deacetylase Rpd3 and thereby actively represses transcription (called “active repression”; Fig. 1.3A) (Chen et al., 1999). In addition to Groucho-mediated “active repression”, Hes1 represses transcription by forming nonfunctional heterodimers with bHLH activators such as Mash1 and E47, as described.
cause it is very unstable (see above), allowing the next round of Hes1 promoter activation. As a result, Hes1 autonomously exhibits oscillatory expression with a periodicity of 2 h (Fig. 1.5), indicating that Hes1 functions as a biological clock with a 2–h cycle (Hirata et al., 2002). This oscillatory expression of Hes1 is widely observed in many cell types, including neural progenitors. In the presomitic mesoderm, Hes7 expression oscillates with a 2–h periodicity and regulates somite segmentation, which occurs every 2 h in mouse embryos. In the absence of Hes7, somites are severely fused, indicating that Hes7 is an essential component of the somite segmentation clock (Bessho et al., 2001, 2003; Hirata et al., 2004). The significance of the Hes1 clock in neural development, however, remains to be determined.

Fig. 1.5 Oscillatory expression of Hes1 by a negative feedback loop. Hes1 seems to act as a 2–h cycle biological clock in many systems. Activation of Hes1 promoter leads to synthesis of Hes1 mRNA and Hes1 protein, which in turn represses its own transcription by binding to the Hes1 promoter (negative feedback loop). Because Hes1 protein is polyubiquitinated and degraded by the proteasome system, Hes1 protein disappears rapidly when the transcription is repressed. Disappearance of Hes1 protein allows the next round of the Hes1 promoter activation. As a result, Hes1 autonomously exhibits oscillatory expression with a periodicity of 2 h.

1.4 Expression of Hes Genes in the Developing Nervous System

At the initial neuroepithelial cell stage, Hes1 and Hes3 are widely expressed by neural stem cells (Allen and Lobe, 1999; Hatakeyama et al., 2004). However, Hes3 expression is gradually down-regulated in the ventral part of the neural tube (Fig. 1.6) and later disappears from most regions at the radial glial stage except for the isthmus, the boundary between the midbrain and hindbrain (Allen and Lobe, 1999; Hirata et al., 2001). As Hes3 expression is down-regulated, Hes5 expression is up-regulated (Fig.
1.6). Up-regulation of Hes5 expression coincides with that of Notch and Delta expression, suggesting that Hes5 expression is controlled by Notch signaling while initial Hes1 and Hes3 expression is not. Hes1 expression is maintained even after Hes3 expression is repressed, and it is likely that Hes1 expression at later stages may depend on Notch signaling.

During the early stages of radial glial cells, Hes1 and Hes5 expression is mostly complementary to each other (Fig. 1.6) (Hatakeyama et al., 2004). For example, Hes5 is strongly expressed in the midbrain and hindbrain but not in the isthmus, while Hes1 is expressed in the isthmus (Fig. 1.6; see also Fig. 1.11). In the optic vesicle at early stages, Hes1 but not Hes5 is expressed. Interestingly, in the absence of either Hes1 or Hes5, expression of the remaining Hes genes is up-regulated in many regions. For example, Hes5 expression occurs ectopically in both the isthmus and the optic vesicles of Hes1–null embryos. Similarly, Hes1 and Hes5 expression domains are expanded in the spinal cord of Hes5–null and Hes1–null embryos, respectively. These results suggest that Hes1 and Hes5 may functionally compensate for each other. At later stages, the apparent complementary expression patterns of Hes1 and Hes5 are lost, and these genes seem to be coexpressed by many neural stem cells.

1.5 Maintenance of Neural Stem Cells by Hes Genes

Roles for Hes genes in neural stem cells have been investigated by gain-of-function and loss-of-function experiments. Mis-expression of Hes1, Hes3 or Hes5 in the embryonic brain inhibits neuronal differentiation and maintains radial glial cells (Ishibashi et al., 1994; Hirata et al., 2000; Ohtsuka et al., 2001). Hes1 is known to
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repress expression of the proneural gene Mash1 by binding to the class C site of the Mash1 promoter (Chen et al., 1997). Conversely, in the absence of Hes1 and Hes5, many radial glial cells are not maintained and prematurely differentiate into neurons (Ishibashi et al., 1995; Tomita et al., 1996; Ohtsuka et al., 1999; Cau et al., 2000; Nakamura et al., 2000; Hatakeyama et al., 2004). In Hes1:Hes5 double-mutant embryos, expression of the proneural bHLH genes Mash1 and Math3 is highly up-regulated, which may lead to premature neuronal differentiation (Hatakeyama et al., 2004). Furthermore, Hes1(−/−):Hes5(−/−) neurospheres do not expand properly even in the presence of bFGF and EGF, in contrast to the wild-type neurospheres, which proliferate extensively (Ohtsuka et al., 2001). Thus, Hes1 and Hes5 regulate maintenance of neural stem cells by preventing premature onset of the proneural bHLH gene expression in the embryonic brain.

In Hes1:Hes5 double-mutant embryos, some radial glial cells are still maintained, suggesting that Hes3 may compensate for Hes1 and Hes5 deficiency. Agreeing with this notion, in the absence of Hes1, Hes3 and Hes5, even neuroepithelial cells are prematurely differentiated into neurons, in contrast to the wild-type neuroepithelial cells, which are never differentiated into neurons (Hatakeyama et al., 2004). Furthermore, virtually all radial glial cells are prematurely differentiated into neurons and become depleted without generating the later-born cell types (later born neurons, oligodendrocytes, astrocytes and ependymal cells) (Fig. 1.7) (Hatakeyama et al., 2004). Thus, Hes1, Hes3 and Hes5 are essential to generate cells in correct numbers and in their full diversity by maintaining neural stem cells until later stages.

Fig. 1.7 Premature neuronal differentiation in Hes1:Hes3:Hes5 triple knock-out mice. The horizontal sections of the neural tube of mouse embryos at day 10. In the wild type, cell bodies of radial glia (Ki67+) are located in the ventricular zone while neurons (TuJ1+) reside in the outer layers. In the absence of Hes1, Hes3 and Hes5, neuronal differentiation is severely accelerated. As a result, virtually all cells become neurons and neural stem cells are depleted. Adopted from Hatakeyama et al. (2004). (This figure also appears with the color plates.)

Strikingly, even in Hes1:Hes3:Hes5 triple-mutant mice, the neuroepithelial cells are initially formed, indicating that formation of neural stem cells is independent of Hes gene activities (Fig. 1.8). However, in the absence of Hes genes, neuroepithelial cells and radial glial cells are prematurely differentiated, indicating that their mainte-
1.5 Maintenance of Neural Stem Cells by Hes Genes

Maintenance depends on Hes gene activities (Hatakeyama et al., 2004). Neural stem cells thus change their characteristics over time as follows: Hes-independent neuroepithelial cells, Hes-dependent neuroepithelial cells, and Hes-dependent radial glial cells (Fig. 1.8). Based on their expression patterns, Hes1 and Hes3 are important for the maintenance of neuroepithelial cells, while Hes1 and Hes5 are required for most radial glial cells.

![Fig. 1.8](image)

**Fig. 1.8** Change of characteristics of neural stem cells. Neuroepithelial cells are formed independently of Hes genes, but their maintenance critically depends on Hes genes and not on Delta/Notch. Radial glial cells depend on Delta, Notch and Hes activities. Thus, neural stem cells change their characteristics as follows: Hes-independent neuroepithelial cells, Hes-dependent neuroepithelial cells, and Hes-dependent radial glial cells.

It has been shown that there are at least two types of neural stem cells depending on the developmental stage, namely primitive and definitive (Hitoshi et al., 2004). Definitive neural stem cells are derived from later stages and depend on Notch signaling, while primitive neural stem cells are from earlier stages and do not depend on Notch signaling: rather, they depend on LIF signaling (Hitoshi et al., 2004). Because initial Hes1 and Hes3 expression in neuroepithelial cells is not controlled by Notch signaling, this expression could be controlled by LIF signaling or by a related signaling pathway. Primitive neural stem cells thus could be Hes-dependent or Hes-independent neuroepithelial cells, while definitive neural stem cells could be Hes-dependent radial glial cells.

Hes-related bHLH genes, Hesr1 and Hesr2, are also expressed by neural stem cells in the embryonic brain, and mis-expression of Hesr1 or Hesr2 promotes maintenance of neural stem cells (Sakamoto et al., 2003). Hesr expression is also controlled by Notch signaling, and Hesr and Hes proteins form heterodimers and act as repressors (Iso et al., 2001). Thus, it is possible that Hesr and Hes cooperatively regulate maintenance of neural stem cells.
Cytokine signaling is known to regulate neural stem cells. In response to the activation of cytokine receptors, JAK2 phosphorylates tyrosine residues of STAT3, and this phosphorylated STAT3 can promote maintenance of neural stem cells. Interestingly, JAK2–STAT3 signaling depends on Notch signaling (Kamakura et al., 2004). The Notch effectors Hes1 and Hes5 physically interact with both JAK2 and STAT3, and this complex facilitates the phosphorylation and activation of STAT3 by JAK2 (Kamakura et al., 2004), thus highlighting the significance of the cross-talk between the Notch-Hes and JAK-STAT pathways in neural stem cells.

Neural stem cells are essential for neural development because they give rise to all cell types, but the analysis of Hes-mutant mice has revealed another important function of this cell type. Both neuroepithelial cells and radial glial cells have epithelial features: they carry the tight junction and adherens junction at the apical side and form the basal lamina at the basal side (Fig. 1.9). These apical and basal structures constitute the inner and outer barriers of the neural tube, respectively. In the absence of Hes genes, both the apical and basal structures are disrupted due to the premature loss of neural stem cells, leading to spilling of neurons into the lumen as well as into the surrounding tissues (Fig. 1.9) (Hatakeyama et al., 2004). Therefore, neural stem cells are essential for the structural integrity of the nervous system. In wild-type embryos, by the time neural stem cells disappear, the ependymal cells are differentiated at the apical side and form the apical junctional complex, while astrocytes are differentiated and contribute to the basal lamina formation at the basal side. Hes genes are required to maintain neural stem cells until formation of ependymal cells and astrocytes and are thus essential for their structural integrity.

**Fig. 1.9** Epithelial features of radial glia. Radial glial cells carry the tight junction and adherens junction at the apical side and form the basal lamina at the basal side. These apical and basal structures constitute the inner and outer barriers of the neural tube, respectively. In the absence of Hes genes, both the apical and basal structures are disrupted due to premature loss of neural stem cells, leading to spilling of neurons into the lumen as well as into the surrounding tissues.
Promotion of Gliogenesis by Hes Genes

Proneural bHLH genes such as *Mash1* override the inhibitory activities of *Hes* genes and promote neuronal differentiation. This process involves another member of the *Hes* family, *Hes6*. *Hes6* can form a heterodimer with *Hes1*, but this complex does not bind to DNA (Bae et al., 2000). Furthermore, *Hes6* was shown to inhibit the interaction between *Hes1* and Groucho/TLE/Grg and induce degradation of *Hes1* protein (Gratton et al., 2003). As a result, *Mash1* is relieved from *Hes*-induced inhibition. Thus, *Hes6* inhibits *Hes1* but supports *Mash1* and promotes neuronal differentiation in the developing brain and retina (Fig. 1.10) (Bae et al., 2000; Koyano-Nakagawa et al., 2000; Gratton et al., 2003). *Hes6* expression is induced by proneural bHLH genes such as *Neurogenin* (Fig. 1.10) (Koyano-Nakagawa et al., 2000). Thus, the proneural bHLH genes inhibit *Hes1/3/5* genes by inducing *Hes6*, while *Hes1/3/5* genes inhibit the proneural bHLH genes, indicating that these bHLH genes regulate each other in a mutually antagonistic manner.

1.6 Promotion of Gliogenesis by Hes Genes

At later stages, when gliogenesis occurs, *Hes1* and *Hes5* are transiently expressed by astrocytes in the developing brain (Nakashima et al., 2001; Wu et al., 2003) and by Müller glial cells in the developing retina (Hojo et al., 2000; Furukawa et al., 2000). Mis-expression of *Hes1* and *Hes5* at later stages promotes generation of astrocytes in the brain and Müller glial cells in the retina (Fig. 1.10) (Hojo et al., 2000; Furukawa et al., 2000; Ohtsuka et al., 2001; Takatsuka et al., 2004). Conversely, in the absence of *Hes1* or *Hes5*, production of Müller glial cells is decreased (Hojo et al., 2000; Furukawa et al., 2000; Takatsuka et al., 2004). Thus, *Hes1* and *Hes5* are involved in gliogenesis at later stages, indicating that *Hes* genes exhibit different activities depending on their developmental stage: maintenance of neural stem cells at early stages and promotion of gliogenesis at later stages. It remains to be determined whether *Hes1* and *Hes5* instruct neural stem cells to adopt a glial fate at later stages, or whether *Hes1* and *Hes5* just maintain neural stem cells until the gliogenic phase. Interestingly, it has been shown that the proneural bHLH gene *Neurogenin1* (*Ngn1*) has two activities: promotion of neurogenesis and inhibition of gliogenesis (Sun et al., 2001). *Ngn1* sequesters the CBP-Smad1 transcriptional complex away from the glial-specific promoters and recruits the complex to the neuronal-specific promoters, thereby promoting neurogenesis while inhibiting alternative fates. Conversely, inactivation of the proneural genes *Mash1*, *Ngn2* and *Math3* blocks neurogenesis while enhancing gliogenesis (Tomita et al., 2000; Nieto et al., 2001). Thus, suppression of the proneural genes could be one of the major mechanisms for *Hes1*– and *Hes5*–induced gliogenesis.
Fig. 1.10  The bHLH gene network in neural development. *Hes1*, *Hes3* and *Hes5* repress proneural bHLH gene expression and maintain neural stem cells. In contrast, proneural bHLH genes induce *Hes6*, which inhibits *Hes1* and promotes neuronal differentiation. *Hes1/Hes5*–expressing cells finally become glial cells.

In the postnatal retina, *Hesr2*, but not *Hesr1* or *Hesr3*, is specifically expressed by Müller glial cells (Satow et al., 2001). Furthermore, mis-expression of *Hesr2* promotes generation of Müller glial cells (Satow et al., 2001). It is thus possible that *Hesr2* regulates gliogenesis by forming a heterodimer with *Hes1* or *Hes5*.

1.7 Maintenance of the Isthmic Organizer by *Hes* Genes

As development proceeds, the nervous system is partitioned into several compartments, which are demarcated by boundary cells. These cells secrete morphogens and specify the adjacent compartments, thus acting as organizing centers. One such example is the isthmus, the boundary between the midbrain and hindbrain (Lumsden and Krumlauf, 1996; Joyner et al., 2000; Mason et al., 2000; Wurst and Bally-Cuif, 2001). The isthmic cells secrete Wnt1 and Fgf8 and regulate development of the midbrain and hindbrain (Fig. 1.11). The isthmic cells express *Hes1* and *Hes3* (Fig. 1.11A) and do not give rise to any neurons. In the absence of *Hes1* and *Hes3*, however, the isthmic cells prematurely lose Wnt1 and Fgf8 expression (Fig. 1.11B) and are ectopically differentiated into neurons (Hirata et al., 2001). As a result, the midbrain and hindbrain neurons are not properly specified. For example, oculomotor and trochlear nuclei and dopaminergic neurons of the midbrain and locus ceruleus neurons of the hindbrain are missing in *Hes1*:*Hes3* double-mutant embryos (Hirata et al., 2001). Thus, *Hes1* and *Hes3* are essential for maintenance of the
Isthmic organizer and development of the midbrain and hindbrain. Similar functions have been reported for Hes-related bHLH genes Her5 and Her11/Him in zebrafish isthmus (Ninkovic et al., 2005).

![Isthmic organizer and development](image)

**Fig. 1.11** Roles of Hes1 and Hes3 in the isthmic organizer. (A) Expression patterns of Hes, Wnt1 and Fgf8 genes. Hes1 and Hes3 are expressed in the isthmic organizer, which secretes Wnt1 and Fgf8 and specifies the midbrain and hindbrain. (B) Wnt1 and Fgf8 expression in the isthmic organizer. In the absence of Hes1 and Hes3, the isthmic cells prematurely lose Wnt1 and Fgf8 expression and differentiate into neurons. (This figure also appears with the color plates.)

### 1.8 Perspective

It has by now become clear that the bHLH genes Hes1, Hes3 and Hes5 regulate the maintenance of virtually all neural stem cells. However, many questions are still unaddressed, regarding Hes functions in neural stem cells. First, neural stem cells have two important activities – self-renewal and differentiation – but it is not known how coordinately neural stem cells undertake these two different activities. The persistent expression of Hes1 induces self-renewal but inhibits differentiation, while loss of Hes1 induces differentiation but inhibits self-renewal. Thus, both persistent expression and loss of expression of Hes1 impairs neural stem cell activities, suggesting that dynamic changes of Hes1 expression seem to be important for neural stem cells. Because Hes1 expression oscillates with a 2–h periodicity, this oscillatory expression might help perform the self-renewal and differentiation activities coordinately. For example, at the cell cycle checkpoint for self-renewal versus
Differentiation, the cells at high levels in Hes1 oscillation could adopt self-renewal, while those at low levels could adopt differentiation. Clearly, further studies are required to substantiate this model.

Second, while Hes1, Hes3 and Hes5 functionally compensate for each other, it remains to be determined whether these three Hes genes have the same activities in neural stem cells. During early stages, Hes1 and Hes3 are expressed, but at later stages, Hes3 is down-regulated while Hes5 is up-regulated. It is not known why Hes3 and Hes5 expression is switched during development. Expression of Hes1 and Hes3 at early stages is independent of Notch signaling while that of Hes5 (and probably Hes1 also) is dependent on Notch signaling at later stages. Furthermore, Hes1/Hes5–expressing radial glial cells give rise to neurons while Hes1/Hes3–expressing neuroepithelial cells do not, raising the possibility that Hes1/Hes5 at later stages allow neurogenesis while Hes1/Hes3 at early stages do not. Thus, Hes genes could have different activities.

During embryogenesis, Hes-expressing cells remain as neural stem cells but finally become astrocytes in the brain and Müller glia in the retina, indicating that Hes genes exhibit different activities depending on developmental stages. These different activities could be due to other factors that are coexpressed with Hes, but those factors specific for neural stem cells and glial cells remain to be analyzed.

Another important issue is that although Hes genes are essential for the maintenance of neural stem cells, they are not required for the initial formation of neural stem cells. The initial formation of neural stem cells might be regulated by other Hes-related bHLH genes or by totally different factors. Further analysis of Hes and related bHLH genes is definitely required to understand the molecular dynamics of the regulation of neural stem cells.

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**Abbreviations**

- bHLH: basic helix-loop-helix
- CRE: cAMP-responsive element
- ICD: intracellular domain
- PKC: protein kinase C


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Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y. 2004. Hes binding to STAT3 mediates crosstalk be-
Taelman V, Van Wayerbergh R, Solter M, Pichon B, Pieler T, Christophe D, Belle-


