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The Hes gene family: repressors and oscillators that orchestrate embryogenesis

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Embryogenesis involves orchestrated processes of cell proliferation and differentiation. The mammalian Hes basic helix-loop-helix repressor genes play central roles in these processes by maintaining progenitor cells in an undifferentiated state and by regulating binary cell fate decisions. Hes genes also display an oscillatory expression pattern and control the timing of biological events, such as somite segmentation. Many aspects of Hes expression are regulated by Notch signaling, which mediates cell-cell communication. This primer describes these pleiotropic roles of Hes genes in some developmental processes and aims to clarify the basic mechanism of how gene networks operate in vertebrate embryogenesis.

Introduction

Embryogenesis depends on the timely proliferation of progenitor cells and their subsequent differentiation into multiple cell types. Regulation of the timing of cell differentiation and cell fate choice are key issues for making organs of the right size, shape and cell composition. In many organs, cell proliferation and differentiation are antagonistically regulated by multiple basic helix-loop-helix (bHLH) activator and repressor genes. In particular, the Hes bHLH repressor genes play an essential role in the development of many organs by maintaining progenitor cells and by regulating binary cell fate decisions. For example, in the developing nervous system of mouse embryos, progenitor cells proliferate and sequentially give rise to different types of cells by changing their differentiation competency. Without Hes genes such as *Hes1*, however, progenitor cells prematurely differentiate into certain types of neurons only, and are depleted before they have proliferated sufficiently and generated all neuronal and glial cell types. This results in small and deformed brain structures. Hes genes also function as biological clocks, measuring time in developmental events, such as somite segmentation. In these processes, Hes genes function as effectors of Notch signaling, which coordinates cellular events via cell-cell interactions.

In this primer, we describe the key features of Hes factors and detail their roles in some representative processes of embryogenesis: namely, in the development of the nervous and digestive systems, two well-characterized processes, where *Hes1* (and *Hes3* and *Hes5* in the nervous system) regulates cell proliferation and differentiation, and in the process of somite segmentation, where *Hes7* functions as a biological clock. We will mainly focus on the roles of the mouse Hes genes, but also compare them with the zebrafish Hes genes (called the her family of genes) (Table 1).

Hes factors: structures and functions

Hes genes are mammalian homologs of the *Drosophila* genes *hairy* and *Enhancer of split* [*E*(*spl*)]. There are seven members in the Hes family (*Hes1-7*), although *Hes4* is absent in the mouse genome (Table 1). Hes genes encode nuclear proteins that repress transcription both actively and passively. In this section, we describe the structural and transcriptional features of Hes factors.

Conserved functional domains of Hes factors

Three conserved domains confer transcriptional functions on all Hes factors: the bHLH, Orange and WRPW domains (Fig. 1A). The bHLH domain consists of two regions: the basic region for DNA binding and the helix-loop-helix region for dimerization. Unlike most other bHLH factors, Hes factors have a proline residue in the middle of the basic region. This proline is proposed to confer to Hes factors unique DNA-binding activity. Most bHLH factors bind to a consensus sequence called the E box (CANNTG) that is present in the promoter region of their target genes. This sequence is further classified into two groups: class A and class B. However, Hes factors bind with highest affinity to different target sequences than the other bHLH factors. These sequences are called the class C site CACG(C/A)G or the N box CACNAG (Akazawa et al., 1992; Sasai et al., 1992; Ohsako et al., 1994). The Orange domain has two amphipathic helices and regulates the selection of bHLH heterodimer partners (Dawson et al., 1995; Taelman et al., 2004). The C-terminal WRPW domain, which consists of the tetrapeptide Trp-Arg-Pro-Trp, represses transcription. This sequence also acts as a polyubiquitylation signal (Kang et al., 2005): Hes factors are polyubiquitylated and degraded by the proteasome, and thus they have very short half-lives (~20 minutes) (Hirata et al., 2002). These domains endow Hes factors with unique features as repressors and oscillators.

Hes factors as repressors

Hes factors repress transcription by at least two different mechanisms: active and passive repression (Fig. 1B,C) (Sasai et al., 1992). Active repression depends on the WRPW domain, which interacts with the co-repressors encoded by the Transducin-like E(spl) (TLE) genes/*Groucho-related gene* (*Grg*), homologs of *Drosophila groucho* (Paroush et al., 1994; Fisher et al., 1996; Grbavec and Stifani, 1996). It is known that chromatin is activated by histone acetyltransferase and inactivated by histone deacetylase, and it has been shown that *Drosophila* Groucho inhibits transcription by recruiting histone deacetylase. Thus, it is likely that the Hes–Groucho-homolog complex represses transcription by inactivating chromatin (active repression, Fig. 1B). Hes factors not only form homodimers, but they also form heterodimers with other bHLH repressors, such as Hey1 (Hes-related with YRPW motif1) and Hey2. Heterodimers of Hes1 and Hey1 or Hey2 bind to the class C site at their targets with a higher affinity and repress transcription more efficiently than homodimers do (Fig. 1B) (Iso et al., 2001). Hes factors also form heterodimers with bHLH activators that bind to the E box. However, these heterodimers cannot bind to DNA (Fig. 1C). Thus, Hes factors display a dominant-negative effect on E box-binding bHLH activators (passive repression).

Direct targets for Hes1 include the bHLH activators Mash1 (also known as Ascl1 – Mouse Genome Informatics) and E47 (also known as Tcfe2a – Mouse Genome Informatics), which are the

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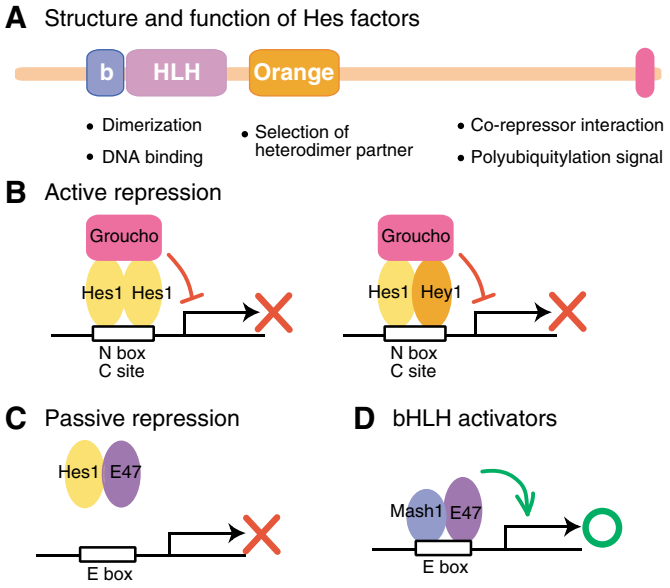


Fig. 1. Structure and function of Hes factors. (**A**) The conserved domains of Hes factors. The basic (blue), HLH (mauve), Orange (orange) and WRPW (pink) domains and their functions are indicated. (**B**) Active repression: Hes factors bind to the N box or class C site by forming homodimers (left panel) or heterodimers with Hey (right panel) and actively repress transcription by interacting with co-repressors, such as Groucho homologs. (**C**) Passive repression: Hes factors form non-DNA-binding heterodimers with bHLH activators such as E47 and inhibit transcriptional activation. (**D**) Activation: bHLH activators such as Mash1 and E47 form heterodimers that bind to the E box and activate transcription.

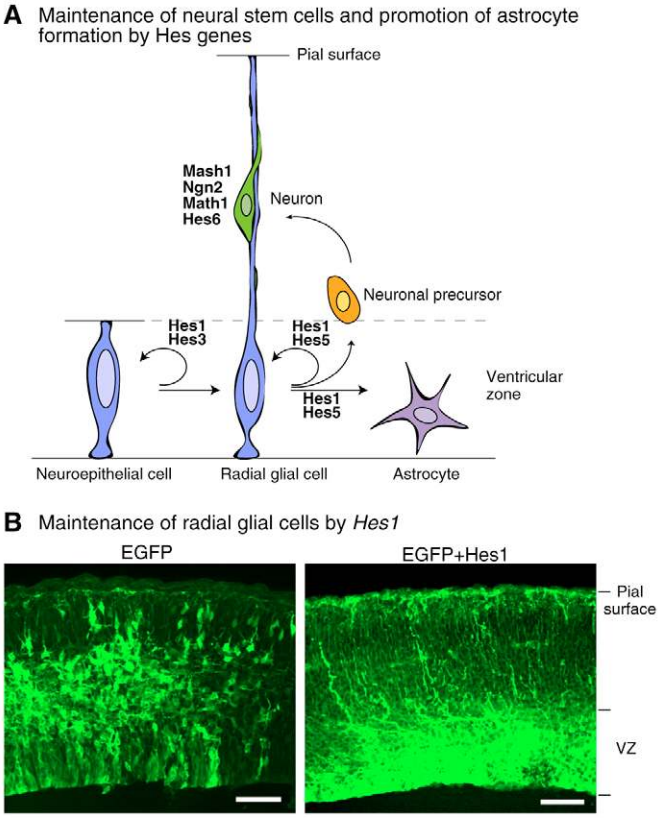


Fig. 2. Maintenance of neural stem cells and promotion of astrocyte formation by Hes genes. (**A**) Regulation of cell differentiation by bHLH genes. Neuroepithelial cells initially form the neural plate. These cells gradually develop into radial glial cells, which have a cell body in the ventricular zone (VZ) and a radial fiber reaching the pial surface. Radial glial cells give rise to neurons by asymmetric cell divisions. After the production of distinct types of neurons, radial glial cells finally differentiate into astrocytes. Hes genes maintain neuroepithelial cells and radial glial cells during early development, and promote astrocyte formation during late development. Proneural genes such as *Mash1*, *Ngn2* and *Math1* promote neurogenesis. Unlike other Hes genes, *Hes6* also promotes neurogenesis. The broken line indicates the border of the VZ. (**B**) Transfection experiments in mouse embryonic brains. Many of the cells transfected with a control vector that only drives enhanced green fluorescent protein (EGFP) expression in radial glial cells have migrated out of the VZ (at the bottom of the image) and differentiated into neurons, whereas cells transfected with a vector that directs the co-expression of *Hes1* and *EGFP*, remain in the VZ and display radial glial cell morphology [from Ohtsuka et al. (Ohtsuka et al., 2001)]. Scale bars: 100 μm.

mammalian homologs of the proteins encoded by the *Drosophila* proneural genes *achaete-scute complex* (also known as *Cyp4g1* – FlyBase) and *daughterless*, respectively. Mash1 can form a heterodimer with E47 and activate neuronal-specific gene expression by binding to the E box (Fig. 1D) (Johnson et al., 1992), whereas Hes1 inhibits Mash1 and E47 activities by forming non-DNA-binding heterodimers with them (passive repression, Fig. 1C). Furthermore, Hes1 represses *Mash1* expression by directly binding to the class C site in the *Mash1* promoter (active repression; Fig. 1B) (Chen et al., 1997). Similarly, in the developing pancreas, Hes1 actively and passively inhibits other bHLH activators, such as *pancreas specific transcription factor 1a* (*Ptf1a*) and *neurogenin 3* (*Ngn3*, also known as *Neurog3*), which specify exocrine and endocrine cell fates, respectively (Lee et al., 2001; Fukuda et al., 2006), as discussed in more detail below.

Hes genes regulate the maintenance of stem cells and progenitors

Hes genes regulate the maintenance of stem cells and progenitors and the normal timing of cell differentiation by antagonizing the effects of bHLH activators. Here, we describe roles for Hes genes in the nervous and digestive systems.

Hes genes regulate the maintenance of stem cells in neural development

During neural development, neuroepithelial cells form the neural plate and proliferate by symmetric cell division (Fig. 2A) (Fishell and Kriegstein, 2003; Götz and Huttner, 2005). As development proceeds, neuroepithelial cells become radial glial cells, which have a cell body in the ventricular zone and a radial fiber reaching the pial surface (Fig. 2A). Both neuroepithelial and radial glial cells are considered to be embryonic neural stem cells. Radial glial cells undergo asymmetric cell divisions, giving rise to a further radial glial cell and a neuron or neuronal precursor (Fishell and Kriegstein, 2003; Götz and Huttner, 2005). Radial glial cells can change their competency and can generate distinct types of neurons over time. In the mouse cortex, different neurons migrate along radial fibers to settle in different layers: early-born neurons in deeper layers and later-born neurons in more-superficial layers. After the production of neurons, radial glial cells can differentiate into oligodendrocytes and finally into astrocytes (Fig. 2A) (Fujita, 2003). *Hes1* and *Hes3*

Table 1. Hes and related genes in mouse and zebrafish

	Expression	Function	Reference
Mouse			
Hes1	CNS, PNS	Repression of neurogenesis (both Notch-dependent and -independent)	(Ishibashi et al., 1995 ; Ohtsuka et al., 1999)
	PSM (cyclic)	Not known (Notch-dependent)	(Jouve et al., 2000)
Hes2	CNS, PNS	Not known	(Nishimura et al., 1998)
Hes3	CNS, PNS	Repression of neurogenesis (Notch-independent)	(Hirata et al., 2001; Hatakeyama et al., 2004)
Hes4	Exists in human but not in mouse		
Hes5	CNS, PNS	Repression of neurogenesis (Notch-dependent)	(Ohtsuka et al., 1999)
	PSM (cyclic)	Not known	(Dunwoodie et al., 2002)
Hes6	CNS, PNS	Promotion of neurogenesis (antagonizes Hes1)	(Bae et al., 2000)
Hes7	PSM (cyclic)	Somite segmentation (Notch-dependent)	(Bessho et al., 2001)
Zebrafish			
her1	PSM (cyclic)	Somite segmentation (Notch-dependent)	(Müller et al., 1996; Holley et al., 2002)
her3	CNS, MHB, Inter-proneuronal domains	Repression of neurogenesis (Notch-independent)	(Hans et al., 2004; Bae et al., 2005)
her4.1	CNS, Proneuronal domains	Repression of neurogenesis (Notch-dependent)	(Takke et al., 1999)
	PSM (non-cyclic)	Somite segmentation (Notch-dependent)	(Pasini et al., 2004)
her5	MHB	Repression of neurogenesis (Notch-independent)	(Geling et al., 2003; Geling et al., 2004)
her6	CNS	Repression of neurogenesis (Notch-dependent)	(Pasini et al., 2001; Cunliffe, 2004)
	PSM (non-cyclic)	Somite segmentation (Notch-dependent)	(Pasini et al., 2004)
her7	PSM (cyclic)	Somite segmentation (Notch-dependent)	(Oates and Ho, 2002)
her9	CNS, Inter-proneuronal domains	Repression of neurogenesis (Notch-independent)	(Leve et al., 2001; Bae et al., 2005)
her11	MHB		(Sieger et al., 2004)
	PSM (cyclic)	Somite segmentation (Notch-dependent)	
her12	CNS, Proneuronal domains		(Sieger et al., 2004)
	PSM		
her13.2	PSM (non-cyclic)	Somite segmentation (FGF-dependent)	(Kawamura et al., 2005)
her15	CNS		(Sieger et al., 2004)
	PSM		

CNS, central nervous system; PNS, peripheral nervous system; PSM, presomitic mesoderm; MHB, midbrain-hindbrain boundary.

are widely expressed by neuroepithelial cells, whereas *Hes1* and *Hes5* are mainly expressed by radial glial cells (Fig. 2A), although there is some redundancy between the Hes genes (Hatakeyama et al., 2004).

Misexpression of *Hes1* or *Hes5* in the developing mouse brain at embryonic day (E) 13.5 inhibits neuronal differentiation and maintains a radial glial cell identity (Fig. 2B) (Ohtsuka et al., 2001). In the absence of *Hes1* and *Hes5*, radial glial cells are not maintained and prematurely differentiate into neurons. Despite the correct formation of neuroepithelial cells in *Hes1; Hes3; Hes5* triple-knockout mice, these cells prematurely differentiate into early-born types of neurons and become depleted without giving rise to the later-born cell types (Hatakeyama et al., 2004). Thus, Hes genes maintain neural stem cells until later stages of mouse development, ensuring that sufficient numbers of cells with a full cell-type diversity are generated. In these Hes-mutant mice, proneural bHLH genes, such as *Mash1* and *Ngn2* (also known as *Neurog2*), are highly upregulated, which accounts for the premature neurogenesis (Hatakeyama et al., 2004). The intracellular domain of Notch (NICD), which is constitutively active, also promotes the maintenance of radial glial cells by inhibiting neurogenesis (Gaiano et al., 2000). However, in the absence of *Hes1* and *Hes5*, the NICD cannot inhibit neurogenesis, indicating that *Hes1* and *Hes5* are essential effectors of Notch signaling in the nervous system (Box 1 and Table 1) (Ohtsuka et al., 1999).

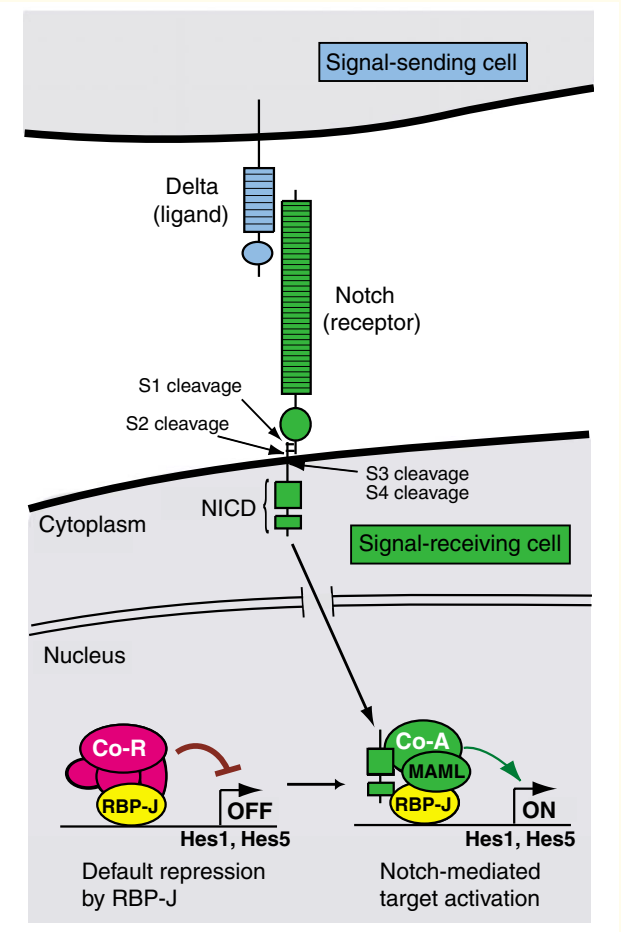
Strikingly, in *Hes1; Hes5* double-knockout mice, the optic vesicles, which are normally formed on the right and left sides of the diencephalon, are absent (Hatakeyama et al., 2004). In the presumptive eye regions, non-retinal neurons prematurely differentiate as early as E9.5, although no neurogenesis normally occurs in the wild-type optic vesicles at this stage (Hatakeyama et al., 2004). Thus, retinal stem cells/progenitors are neither maintained nor properly specified in the absence of *Hes1* and *Hes5*.

The other Hes genes (*Hes2* and *Hes6*) are also expressed in the developing nervous system, but the expression and function of *Hes6* is unique among the Hes genes. Unlike *Hes1*, *Hes3* and *Hes5*, *Hes6* is expressed by differentiating neurons. Furthermore, Hes6 inhibits Hes1 by forming a non-functional heterodimer, which supports Mash1 activity, and thereby promotes neuronal differentiation (Fig. 2A and Table 1) (Bae et al., 2000).

Hes genes regulate boundary formation

The developing nervous system is partitioned into many compartments by boundaries, such as the zona limitans intrathalamica (Zli) and the isthmus (Fig. 3A). The Zli is the boundary between the thalamus and the prethalamus, whereas the isthmus is the boundary between the midbrain and the hindbrain. Boundaries are formed by specialized neuroepithelial or radial glial cells, which have unique features, including slow proliferation, delayed or no neurogenesis and organizer activities that regulate specificity of neighboring compartments. For example, the Zli and the isthmus function as organizing centers by secreting sonic hedgehog and Fgf8, respectively, and by regulating the regional specification of neighboring compartments (Kiecker and Lumsden, 2005) (Fig. 3A). Cells migrate within each compartment but do not usually cross boundaries; thus, each compartment forms a unit that consists of a distinct set of cell types (Kiecker and Lumsden, 2005). Although *Hes1* is expressed in both compartments and boundaries, the mode of expression is different in the two structures (Baek et al., 2006). In compartments, Hes1 levels are variable: high levels occur

Box 1. Notch signaling



Notch family members are type I transmembrane receptors for the DSL (Delta, Serrate, Lag-2) family of type I transmembrane ligands (Artavanis-Tsakonas et al., 1999; Selkoe and Kopan, 2003). In mammals, there are four Notch receptors (Notch1-4) and five ligands [Jagged1, Jagged2, Delta-like 1 (Dll1), Dll3 and Dll4]. The newly synthesized Notch molecule is processed by a furin-like convertase at the S1 site into two fragments that remain associated to form the functional heterodimeric receptor. Notch signaling is triggered when the ligands (DSL family) expressed on the surface of neighboring cells interact with Notch receptors. Upon ligand binding, Notch receptors undergo successive proteolytic cleavage. The first cleavage at the S2 site by an extracellular protease of the ADAM (a disintegrin and metalloprotease) family, TNF-α converting enzyme (TACE), generates an active membrane-tethered form of Notch. The truncated product is further processed at two endomembrane sites, S3 and S4, by the γ-secretase activity of Presenilins 1 and 2, which release the intracellular domain of Notch (NICD) from the plasma membrane. The NICD translocates to the nucleus and associates with the DNA-binding transcription factor RBP-J (also known as Rbpsuh) in mice, Su(H) in *Drosophila*, and LAG-1 in *C. elegans*. As a result, RBP-J is converted from a transcriptional repressor to an activator. In this process, NICD, RBP-J and Mastermind-like proteins (MAML family, MAML1-3) assemble on target DNA and form a RBP-J–NICD–MAML ternary complex. This transcriptional activation complex is formed through displacement of the co-repressor complex and recruitment of the co-activators. Thus, Notch signaling activates the transcription of target genes, such as *Hes1* and *Hes5* (Jarriault et al., 1995). Hes factors then subsequently repress the transcription of proneural genes such as *Mash1*.

in some cells, whereas, in others, levels are lower (Fig. 3Ba) (Baek et al., 2006). Hes1 levels could be oscillating in these cells (see below). By contrast, Hes1 is persistently expressed at high levels by many boundary cells (Fig. 3Bb) (Baek et al., 2006). There is an inverse correlation between Hes1 and Mash1 levels: cells that express high levels of Hes1 express low levels of Mash1 and vice versa (Fig. 3B). Within boundaries, persistent and high levels of *Hes1* expression constitutively repress the expression of proneural genes, such as *Mash1*, thereby inhibiting neurogenesis (Fig. 3Bb) (Baek et al., 2006). In the absence of Hes genes, proneural genes are ectopically expressed in boundaries, leading to ectopic neurogenesis and the loss of organizer activity (Hirata et al., 2001; Baek et al., 2006). In zebrafish, *her3* and *her5* have similar activities, inhibiting neurogenesis and contributing to the formation of the midbrain-hindbrain boundary (Table 1). It has been reported that the expression of *her3* and *her5* does not depend on Notch signaling (Table 1), suggesting that Hes expression in boundaries of the mouse nervous system could also be independent of this pathway.

It has been shown that Hes1 regulates cell cycle progression. During the G1 phase, cyclin-dependent kinase (CDK) promotes cell cycle progression by forming complexes with cyclins, whereas the CDK inhibitors p21 and p27 antagonize this process. Low levels of Hes1 promote cell proliferation by downregulating p21 and p27 (Murata et al., 2005). However, persistent and high levels of *Hes1* expression have been shown to inhibit the cell cycle, probably because Hes1 also represses the expression of some cell cycle regulators such as E2F-1, which promotes the G1-S phase transition (Castella et al., 2000; Ström et al., 2000; Hartman et al., 2004; Baek et al., 2006). Thus, within boundaries, persistent and high levels of *Hes1* expression may contribute to slowing cell proliferation as well as to the inhibition of differentiation, raising the possibility that persistent versus variable *Hes1* expression differentially regulates boundary versus compartment characteristics (Fig. 3B).

Hes1 regulates the maintenance of stem cells and progenitors in digestive organs

The dorsal and ventral pancreatic buds, which fuse to form the pancreas, grow from the endodermal epithelium of the foregut (Murtaugh, 2007). The pancreatic epithelium gives rise to both exocrine and endocrine cells: exocrine progenitors become acinar cells, which secrete digestive enzymes, whereas endocrine cells emigrate from the epithelium to form islets (Fig. 4A). The liver and biliary systems also originate from the endodermal epithelium of the foregut. Thus, the gut, pancreas, liver and biliary systems share the same origin.

In the developing pancreas, the bHLH gene *Ptf1a* promotes exocrine cell differentiation, whereas the bHLH gene *Ngn3* promotes the differentiation of all four endocrine cell types [α (glucagon-producing), β (insulin-producing), δ (somatostatin-producing) and PP (pancreatic polypeptide-producing) cells] (Fig. 4A) (Krapp et al., 1998; Gradwohl et al., 2000; Kawaguchi et al., 2002). Inactivation of *Hes1* in mice leads to the upregulation of *Ngn3*, an acceleration of post-mitotic endocrine cell differentiation and severe pancreatic hypoplasia (Jensen et al., 2000). Similar defects are observed following inactivation of the Notch ligand delta-like 1 (Dll1) or the Notch effector Recombination signal sequence-binding protein (RBP-J) (Box 1), or by the overexpression of *Ngn3* (Apelqvist et al., 1999), suggesting that the Dll1–Notch–RBP-J–Hes1 pathway inhibits premature endocrine differentiation. Hes1 also represses *Ptf1a* expression by directly binding to the promoter of this gene. Similarly, NICD inhibits acinar

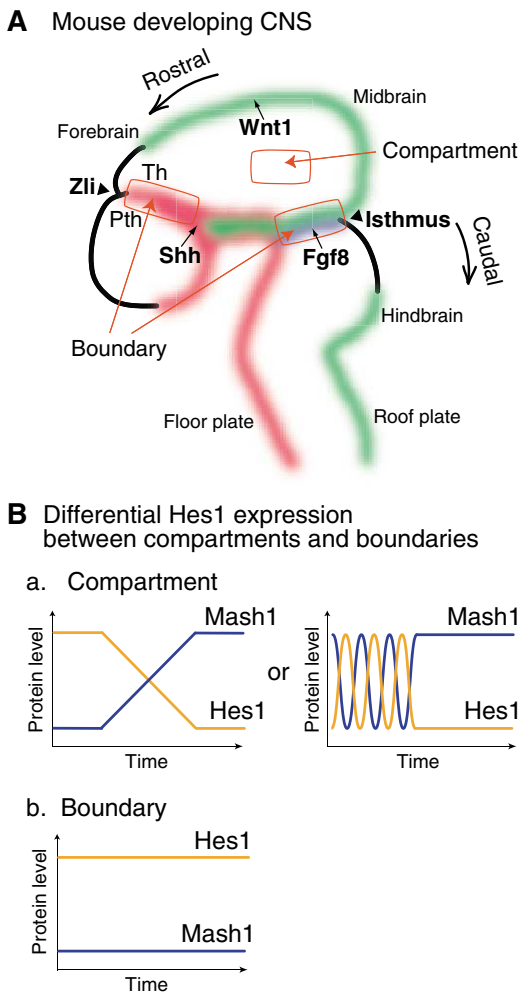


Fig. 3. Differential *Hes1* expression between compartments and boundaries. (**A**) Lateral view of the developing central nervous system (CNS) in the mouse at E10.5. The CNS is partitioned into many compartments by boundaries, such as the zona limitans intrathalamica (Zli) and the isthmus. These boundaries and the roof and floor plates function as organizing centers by expressing *Shh* (pink), *Wnt1* (green) or *Fgf8* (purple). Cells in these compartments undergo active proliferation and neurogenesis, whereas those in boundaries undergo slower proliferation and no neurogenesis. Pth, prethalamus; Th, thalamus. (**B**) The *Hes1* expression mode is different between compartments and boundaries: (**Ba**) variable expression (could be oscillatory) in compartments and (**Bb**) persistently high expression in boundaries. In compartments, when Hes1 levels are low, Mash1 levels are high, and vice versa. These cells finally lose *Hes1* expression and differentiate into neurons. By contrast, in boundaries, *Hes1* is persistently expressed at high levels, and neurogenesis is inhibited. This difference in the *Hes1* expression modes may confer compartment versus boundary characteristics.

cell differentiation by antagonizing Ptf1a function (Hald et al., 2003; Murtaugh et al., 2003; Esni et al., 2004; Fujikura et al., 2006). Thus, Notch-Hes1 signaling promotes the maintenance of pancreatic stem cells/progenitors by antagonizing *Ptf1a* and *Ngn3* (Fig. 4A). Interestingly, in *Hes1*-null mice, *Ptf1a* and *Ngn3* are ectopically expressed in the common bile duct, stomach and duodenum, leading to the formation of an ectopic pancreas (Sumazaki et al., 2004; Fukuda et al., 2006).

In embryonic and adult intestinal epithelium, stem cells give rise to four principal cell types: enterocyte (absorptive), goblet (mucous-secreting), enteroendocrine (hormone-secreting) and Paneth (antimicrobial peptide-secreting) cells (Fig. 4B). In the adult intestine, stem cells are located near the bottom of crypts (see Fig. 4B): the cells moving upwards towards the top of the crypt and towards the villi differentiate into goblet cells, enteroendocrine cells and enterocytes, whereas those moving downwards towards the bottom of the crypt differentiate into Paneth cells (Fig. 4B) (Crosnier et al., 2006). The bHLH activator gene *Math1*, a mammalian homolog of the *Drosophila* proneural gene *atonal*, promotes the development of goblet, enteroendocrine and Paneth cells, whereas the activation of Notch-Hes1 signaling represses *Math1*, and thereby inhibits the generation of *Math1*-dependent cells (Jensen et al., 2000; Yang et al., 2001; Suzuki et al., 2005; van Es et al., 2005; Fre et al., 2005; Stanger et al., 2005). The inactivation of Notch signaling in the adult intestine causes the loss of *Hes1* expression and the concomitant upregulation of *Math1*, leading to the differentiation of virtually all crypt cells into postmitotic goblet cells (van Es et al., 2005). Thus, the Notch-Hes1 pathway regulates the maintenance of stem cells/progenitors by repressing *Math1*.

Loss of the tumor suppressor gene *adenomatosis polyposis coli* (*Apc*) is associated with the development of intestinal adenomas (Kinzler et al., 1991). Interestingly, these adenoma cells express *Hes1* at high levels, suggesting that the Notch-Hes1 pathway contributes to this transformation of progenitor cells to adenomas. Strikingly, the treatment of mice with a γ-secretase inhibitor, which inhibits Notch signaling by preventing the release of the NICD, leads to the upregulation of *Math1* and the subsequent transformation of adenomas into collections of goblet cells (van Es et al., 2005). Thus, the Notch-Hes1 pathway also plays an important role in intestinal adenoma development, and γ-secretase inhibitors may be useful for treating these tumors (van Es et al., 2005).

Hes genes regulate binary cell fate decisions

In addition to maintaining stem cells, Hes genes regulate binary cell fate decisions. For example, they regulate the determination of an astrocyte versus a neuronal fate, thereby generating cell type diversity (Kageyama et al., 2005).

Hes1 and *Hes5* regulate astrocyte versus neuron fate specification

During late development, Hes genes promote astrocyte formation in the developing nervous system. It has been shown that proneural bHLH activators such as neurogenin 1 (Ngn1, also known as Neurog1 – Mouse Genome Informatics) have dual activities: they not only activate neuronal-specific gene expression, but they also inhibit astrocyte-specific gene expression by sequestering co-activators of these genes, such as p300 (Sun et al., 2001). Furthermore, mice lacking proneural genes display a blockade of neurogenesis and show accelerated formation of astrocytes at E15.5 (Tomita et al., 2000; Nieto et al., 2001). Thus, one of the mechanisms of Hes-induced astrocyte versus neuron fate specification is through the inhibition of proneural bHLH activators. Hes1 can also induce astrocyte development through another pathway. Lif (leukemia inhibitory factor) and Bmp2 (bone morphogenetic protein 2) synergistically induce astrocyte formation (Nakashima et al., 1999; Kamakura et al., 2004). Lif signaling activates the Janus kinase Jak2, which then activates the downstream transcription factor Stat3 by phosphorylation. It has been shown that Hes1 promotes Jak2-mediated phosphorylation of Stat3 by forming a complex with Jak2 and Stat3. This then induces the differentiation

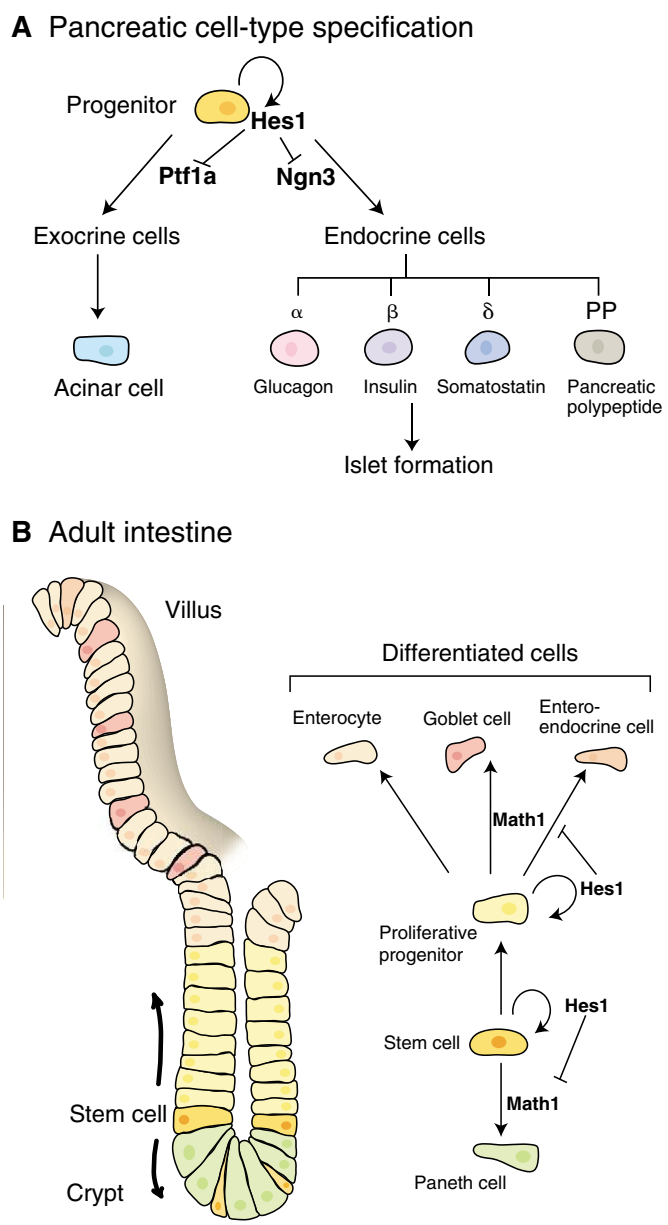


Fig. 4. Regulation of pancreatic and intestinal cell differentiation by *Hes1*. (**A**) In the developing pancreas, the epithelium gives rise to both exocrine and endocrine cells; exocrine cells form acini, whereas endocrine cells emigrate from the epithelium and form islets. The bHLH gene *Ptf1a* regulates exocrine cell differentiation, whereas *Ngn3* promotes the differentiation of all four types of pancreatic endocrine cells [α (glucagon), β (insulin), δ (somatostatin) and PP (pancreatic polypeptide) cells]. Hes1 regulates the maintenance of progenitors by inhibiting the expression of *Ptf1a* and *Ngn3*. (**B**) In the adult small intestine, stem cells are found near the bottom of the crypt. The cells moving upwards towards the crypt top and towards the villi differentiate into goblet cells, enteroendocrine cells and enterocytes, whereas those moving downwards towards the crypt bottom differentiate into Paneth cells. *Hes1* regulates stem cell maintenance and enterocyte versus non-enterocyte fate choice by repressing *Math1*, which promotes the development of goblet, enteroendocrine and Paneth cells.

of astrocytes (Kamakura et al., 2004). It remains to be determined, however, why Hes genes cannot induce astrocyte formation at early stages of development. One possibility is that the epigenetic status of astrocyte-specific genes is different between the early and late neural stem cells; in early stem cells, transcription from astrocyte-specific promoters is repressed by hypermethylation, whereas these promoters are hypomethylated in later stem cells (Takizawa et al., 2001). Thus, the intrinsic properties of neural stem cells change over time, correlating with their differential response to Hes genes.

Hes1 regulates binary cell fate decisions in digestive organs

In the developing intestine of *Hes1*-knockout mice, the bHLH activator *Math1* is upregulated, leading to an increase in goblet, enteroendocrine and Paneth cells (Fig. 4B) (Jensen et al., 2000; Suzuki et al., 2005; van Es et al., 2005). Similarly, in zebrafish, goblet and enteroendocrine cells are formed at the expense of enterocytes by the inactivation of Notch signaling (Crosnier et al., 2005). Thus, it is likely that Notch-Hes1 signaling promotes the enterocyte versus non-enterocyte specification, although the downregulation of Notch signaling is required for the maturation of enterocytes.

Liver progenitors give rise to two types of cells: hepatocytes and biliary epithelial cells. In the absence of *Hes1*, hepatocytes form normally, whereas intrahepatic bile ducts are completely absent (Kodama et al., 2004). This phenotype is similar to that of Alagille syndrome, which is associated with mutations in the human Notch ligand *jagged 1* (Oda et al., 1997; Li et al., 1997). Thus, it is likely that the specification of a biliary fate versus a hepatocytic one is brought about by Jagged-mediated Hes activity.

Hes genes are molecular oscillators

In mouse embryos, a bilateral pair of somites is formed every 2 hours, indicating that an innate timing mechanism regulates this process (Dubrulle and Pourquié, 2004). It has been shown that the expression of *Hes1* and *Hes7* oscillates with a periodicity of 2 hours, and may function as a biological clock; *Hes1* seems to regulate the timing of biological events in many cell types, such as fibroblasts, whereas *Hes7* functions as the segmentation clock.

Hes1 is a cellular oscillator

Expression of *Hes1* can be induced following serum stimulation or Notch activation in many cell types, such as fibroblasts, myoblasts and neuroblasts; however, strikingly, the induced expression is oscillatory (Hirata et al., 2002). Hes1 oscillation is cell-autonomous and depends on negative autoregulation (Fig. 5A). After induction, Hes1 protein represses the expression of its own gene by directly binding to its promoter. This repression is short-lived due to the short half-life of the mRNA and protein. In this way, Hes1 autonomously initiates oscillatory expression with a periodicity of approximately 2 hours, suggesting that Hes1 acts as a biological clock (Fig. 5A). This oscillation is transient and dampened after three to six cycles (6-12 hours). However, this observation is not due to dampened oscillation within individual cells, in which *Hes1* expression is still cyclical even after nearly 2 days, as revealed by real-time imaging analysis (Masamizu et al., 2006). Rather, the dampening arises because the oscillation period varies from cycle to cycle within a cell, and, therefore, oscillations among cells easily fall out of synchrony. This loss of synchrony explains why, after several cycles, the oscillatory expression of *Hes1* is not detected by northern or western blot analysis. Similarly, even in stationary cultured cells where Hes1 levels seem to be constant by northern and western blot analyses, *Hes1* expression is found to be dynamically changing at the single-cell level (Masamizu et al., 2006). Thus, at any given time,

Hes1 expression levels are variable within and between cells, which may enable a cell to mediate a different response to the same stimulus. Hes1 oscillation may regulate the timing of cellular events, such as the cell cycle, but its precise roles are unknown.

Hes7 is an essential component of the segmentation clock

Somites, the segmental units that later give rise to the vertebrae, ribs, skeletal muscles and dermis, are formed by the segmentation of the anterior region of the presomitic mesoderm (PSM; Fig. 5B) (Dubrulle and Pourquié, 2004). This event is repeated every 2 hours in mouse embryos. It has been suggested that this periodic event is controlled by a biological clock, called the segmentation clock. It was first reported that the expression of *chairy1*, a chick homolog of mouse *Hes1*, is periodically propagated in a wave-like fashion initiating at the posterior end and moving towards the anterior region of the PSM (propagation is classified into three phases; Fig. 5Bb) (Palmeirim et al., 1997). Each wave leads to the generation of a pair of somites. In the mouse PSM, *Hes1*, *Hes5* and *Hes7* are expressed in a similar fashion to each other (Fig. 5B), but *Hes7* is the most important for somite segmentation. This wave-like expression of *Hes7* is elicited by its oscillatory expression in each PSM cell (Fig. 5Bc). In the absence of *Hes7*, somites fuse, which results in fused vertebrae and ribs (Bessho et al., 2001). Interestingly, a lack of oscillation due to persistent *Hes7* expression also leads to somite fusion (Hirata et al., 2004). Thus, oscillatory expression is very important for periodic somite segmentation. In zebrafish, *her1* and *her7* have oscillatory expressions and regulate somite segmentation (Table 1).

Hes7 oscillation, like that of Hes1, is regulated by negative feedback (Fig. 5A) (Bessho et al., 2003). One of its downstream target genes is the glycosyltransferase *lunatic fringe* (*Lfng*), which regulates Notch activity by glycosylation (Moloney et al., 2000; Brückner et al., 2000). Hes7 protein represses transcription from the *Lfng* promoter as well as from its own, and thus *Lfng* expression oscillates in phase with *Hes7* expression (Bessho et al., 2003). In the absence of *Hes7*, *Lfng* is constitutively expressed in the PSM (Bessho et al., 2001). *Lfng* oscillation is also crucial for segmentation, as both the loss of and persistent expression of *Lfng* has been shown to lead to severe somite fusion (Evrard et al., 1998; Zhang and Gridley, 1998; Serth et al., 2003). Lfng periodically inhibits Notch signaling and thereby generates oscillations in Notch activity (Dale et al., 2003; Morimoto et al., 2005; Huppert et al., 2005), which may in turn influence Hes7 oscillation. It is thought that the combined effects of these coupled negative-feedback loops on *Hes7* and *Lfng* expression are important for sustained and synchronized oscillations and for the correct timing of the biological clock.

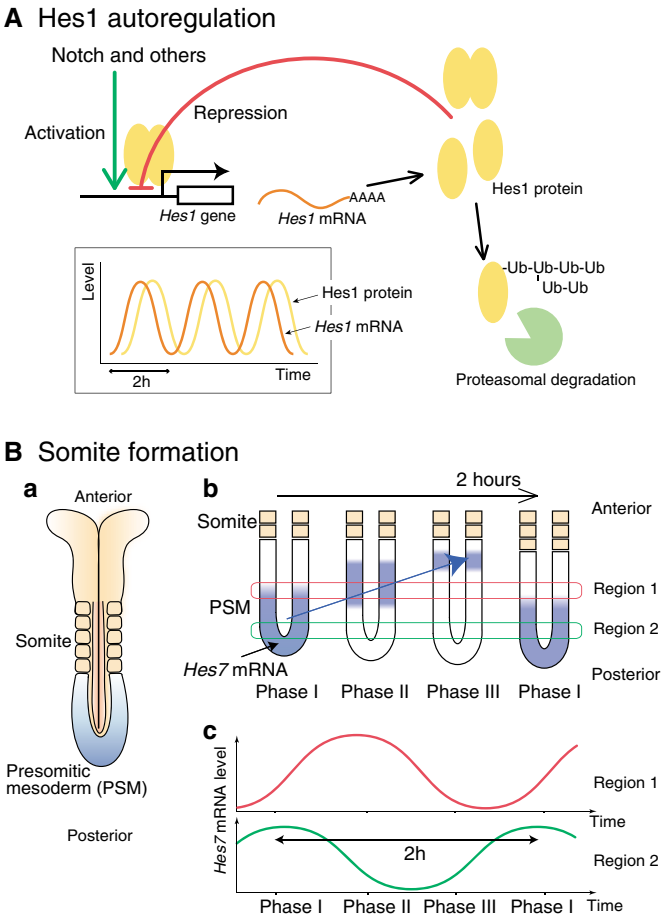


Fig. 5. Hes genes are molecular oscillators. (**A**) Oscillatory expression of *Hes1* is regulated by negative feedback. Promoter activation (green), as induced by Notch, for example, induces the production of Hes1 protein, which represses expression of its own gene (red). Then, both *Hes1* mRNA and protein disappear rapidly because they have very short half-lives, allowing the next round of expression. In this way, *Hes1* expression autonomously oscillates. (**B**) Hes7 oscillation in somite segmentation. (**Ba**) Ventral view of a mouse embryo at the five-somite stage. Somites form periodically by segmentation of the anterior region of the presomitic mesoderm (PSM, shown in blue). (**Bb**) *Hes7* expression is periodically propagated, like a wave, from the posterior end to the anterior region of the PSM (shown by blue arrow, classified into three phases), and each wave leads to the generation of a pair of somites (buff). (**Bc**) This dynamic change is elicited by oscillatory expression in each PSM cell with a slight delay from the posterior to the anterior direction.

Conclusion

It has been shown that Hes genes not only promote the maintenance of stem cells/progenitors, but they also regulate the binary cell fate decisions in many organs, only some of which are described here. Hes genes also regulate the timing of several developmental events, such as somite segmentation. Although *Hes1* expression oscillates in many cell types, the significance of this oscillation in non-PSM cells remains to be determined. In mouse neural stem cells, *Hes1* expression is variable, suggesting that it oscillates in these cells. Real-time visualization of *Hes1* expression supports this suggestion (R.K. and T.O., unpublished data). However, the significance of Hes1 oscillations to stem cell proliferation and differentiation is unclear. Although *Hes1* is required for the maintenance of stem cells, persistent and high levels of *Hes1* expression inhibit both cell proliferation and differentiation (Baek et al., 2006). Thus, oscillatory expression may be important for stem cell maintenance. Furthermore, recent studies have revealed that, other than the circadian clock genes, Hes genes are not the only oscillators (Nelson et al., 2004; Lahav et al., 2004). This raises the possibility that more genes may be identified that display oscillatory expression, and that these oscillations could be a required general feature of many cellular events. Our understanding of how gene networks operate in cell proliferation and differentiation during development is continually improving. It is now apparent that Hes genes play an indispensable role in different developmental contexts, as well as in the crucial maintenance of the biological clock.

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