Feature Review

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Oscillatory control of bHLH factors in neural progenitors

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The mammalian brain consists of a complex ensemble of neurons and glia. Their production during development and remodeling is tightly controlled by various regulatory mechanisms in neural progenitor cells (NPCs). Among such regulations, basic helix-loop-helix (bHLH) factors have key functions in the self-renewal, multipotency, and fate determination of NPCs. Here, we highlight the importance of the expression dynamics of bHLH factors in these processes. The oscillatory expression of multiple bHLH factors is correlated with the multipotent and self-renewable state, whereas sustained expression of a selected bHLH factor regulates fate determination. We also discuss potential mechanisms by which a single bHLH factor can exhibit versatile functions in NPC regulation as well as the hierarchical structure of the bHLH factor oscillatory network.

bHLH factors in neural development

The majority of neurons constituting the mammalian brain develop from NPCs (see Glossary) during embryonic periods (Box 1) [1–5]. After neuronal production, generation of glial cells from NPCs follows and continues in the postnatal brain. Neurons are also continuously produced from NPCs in two restricted regions of the adult brain, the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus; these neurons play important roles in learning and memory by remodeling the preexistent neural circuit [6–8]. It is therefore essential to maintain NPCs throughout life. NPCs are undifferentiated multipotent cells responsible for the production of three cell types, neurons, oligodendrocytes, and astrocytes. Intense studies have unveiled the molecular mechanisms governing the multipotency, self-renewal, and fate determination of NPCs.

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It has been shown that bHLH transcription factors play critical roles in the coordinated production of optimized numbers of neurons and glial cells in the appropriate brain regions at the proper times [5,9–11]. For example, proneural bHLH factors, such as Neurog1 and Neurog2 (Neurog1/2) and achaete-scute complex homolog 1 (Ascl1; formerly Mash1), regulate neuronal fate specification. Olig1 and Olig2 (Olig1/2) bHLH factors are critically important for the entirety of the generation and maturation of oligodendrocytes. Hairy and enhancer of split (Hes) bHLH factors are responsible for the maintenance of undifferentiated NPCs throughout development and promote the generation of astrocytes. Thus, bHLH factors are involved in all major processes of neural development. Recent studies revealed that bHLH factors are expressed in a dynamic manner during proliferation and differentiation of NPCs [5,12,13]. In this review, we discuss the functional significance of these expression dynamics as well as the hierarchical structure of the bHLH factor network in NPCs.

Versatile functions of bHLH factors in cell proliferation and differentiation

NPCs are maintained from the embryonic to the adult brain. This maintenance is very important for the sequential production of neurons and glia in the developing brain

Glossary

Basic helix-loop-helix (bHLH): a motif for DNA binding and dimer formation. A bHLH factor forms a homo- or hetero-dimer through the helix-loop-helix domain and binds to the target sequence through the basic region. Proneural bHLH factors such as Neurog2 and Ascl1 form hetero-dimers with another bHLH factor E47, bind to the E box (CANNTG), and activate target gene expression. By contrast, the bHLH factors Hes1 and Hes5 form homo-dimers, bind to the N box (CACNAG) and C site (CACG(C/A)G), and repress target gene expression.
Intermediate progenitor: a cell type derived from NPCs. These cells are present in the subventricular zone, adjacent to the ventricular zone, where the cell bodies of NPCs reside. Intermediate progenitors divide once or twice before differentiating into postmitotic neurons.
Neural progenitor cell (NPC): multipotent cells that give rise to neurons, oligodendrocytes, and astrocytes. NPCs are present as neuroepithelial cells and radial glial cells in the embryonic brain and GFAP-expressing astrocyte-like cells in the adult brain. NPCs can directly give rise to neurons, but in most cases, they first generate intermediate progenitors in the embryonic brain and transit-amplifying cells in the adult brain. Both intermediate progenitors and transit-amplifying cells further divide and differentiate into postmitotic neurons.

Box 1. NPCs

Neuroepithelial cells, the first form of embryonic NPCs, proliferate extensively by repeated symmetric cell division, whereby each neuroepithelial cell divides into two neuroepithelial cells [4]. As the wall of the neural tube becomes thicker, neuroepithelial cells gradually elongate, becoming radial glial cells, which have cell bodies in the innermost region (the ventricular zone) and radial fibers reaching the outer surface. Radial glial cells are the second form of embryonic NPCs. Radial glial cells undergo asymmetric cell division, whereby each radial glial cell divides into two distinct cell types, a new radial glial cell and an immature neuron or an intermediate progenitor [78–80]. In the developing neocortex, radial glial cells sequentially give rise to different types of neurons, initially deep layer neurons and then superficial layer neurons, by repeated asymmetric cell divisions [81]. Later, radial glial cells give rise to oligodendrocytes, ependymal cells, and astrocytes.

Neurogenesis continues in two regions of the adult brain, the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus. Each region contains NPCs that are similar to astrocytes [82]. Adult NPCs express the astrocyte marker GFAP, are mostly quiescent, and occasionally divide to give rise to transit-amplifying cells, which further divide several times to produce more neurons [54].

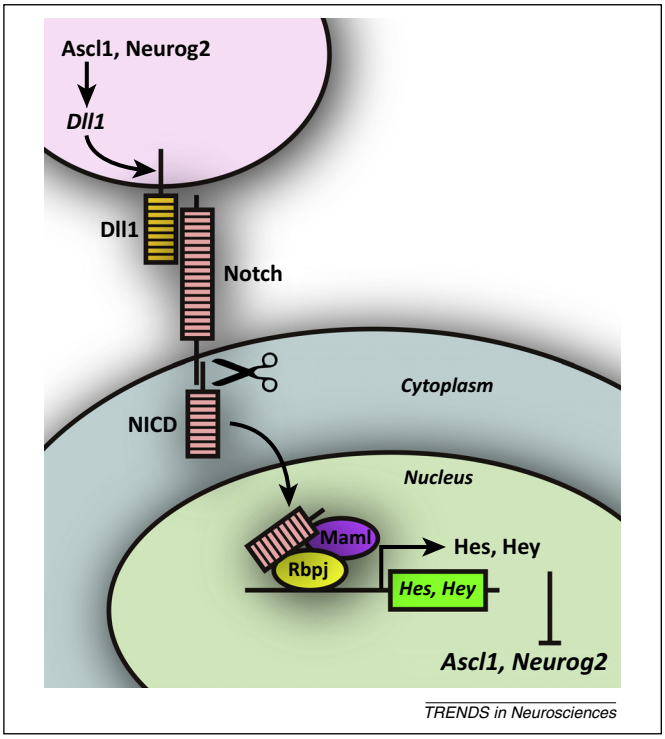

Figure 1. Notch signaling. Notch signaling is activated through cell–cell interaction, between ligand (Dll1)-expressing and receptor (Notch)-expressing cells. Proneural factors (Ascl1, Neurog2) induce the expression of Notch ligand, which activate Notch signaling in neighboring cells. Upon activation of Notch signaling, Notch protein is subjected to proteolysis, releasing its intracellular domain (NICD, Notch intracellular domain), which translocates to the nucleus. Nuclear NICD forms a complex with the DNA-binding protein Rbpj and the coactivator Maml and induces the expression of downstream genes such as *Hes* and *Hey*. Hes and Hey repress the expression of target genes including proneural genes such as *Ascl1* and *Neurog2*.

and for remodeling neural circuits in the adult brain. The coordinated contribution of various cell signaling pathways and gene products is indispensable for the long-term maintenance of NPCs. Above all, Hes transcriptional repressors play a central role in NPC maintenance and self-renewal under the control of Notch signaling [14–16].

Notch signaling is activated through cell–cell interaction, between ligand [Deltalike1 (Dll1) and Jagged1 (Jag1)]-expressing and receptor (Notch)-expressing cells (Figure 1) [17]. Upon activation of Notch signaling through this interaction, Notch receptors are subjected to intramembrane proteolysis, releasing their intracellular domain (NICD, Notch intracellular domain), which is then translocated to the nucleus. Nuclear NICD forms a complex with the DNA-binding protein Rbpj (recombination signal binding protein for immunoglobulin kappa J region) and the coactivator mastermind-like protein (Maml) and induces the expression of downstream genes, such as *Hes* and *Hes*-related *Hey* genes. Hes/Hey factors critically contribute to the maintenance of NPCs by repressing the expression of proneural factors, such as Neurog1/2 and Ascl1. In the absence of Notch signaling components, for instance in *Hes*-mutant mice, the expression of proneural factors is upregulated, leading to accelerated neurogenesis and premature exhaustion of NPCs [18,19]. Notch signaling is thought to work as a kind of feedback signal from newly born immature neurons to NPCs within the germinal zone. However, NPCs themselves also express Notch ligand proteins, and these cells as well as immature neurons and intermediate progenitors contribute to the activation of Notch receptors in NPCs [20–22]. In addition to the Notch pathway, inhibitor of DNA-binding (Id) factors, epigenetic factors, and other signaling pathways, including Wnt, fibroblast growth factor (FGF), epidermal growth factor (EGF), bone morphogenetic protein (BMP), and Sonic hedgehog (Shh) pathways, work in cooperation with the Notch–Hes pathway to achieve long-term maintenance of NPCs [5,6,23].

Proneural bHLH factors are transcriptional activators that induce neuronal production [10,24]. Their downstream target genes regulate a wide array of neuronal fate determination and differentiation processes, such as cell cycle exit, migration, neurite formation, and neurotransmitter release [25]. Proneural bHLH factors also induce the expression of the Notch ligand Dll1, which activates Notch signaling in neighboring cells, thereby maintaining NPCs [26]. In addition to the generic program of neuronal fate determination and differentiation, proneural bHLH factors have neuronal subtype-specific functions [10,24]. For example, Neurog1/2 and Ascl1 regulate specification of glutamatergic and GABAergic neurons, respectively [27,28]. High levels of Neurog1/2 or Ascl1 expression in NPCs can rapidly induce neuronal differentiation. However, as we discuss later, proneural bHLH factors also seem to be involved in NPC proliferation. For instance, it was shown that NPCs proliferate slowly in the absence of Ascl1, and that Ascl1 directly upregulates the expression of genes involved in cell cycle progression, in addition to inducing cell cycle exit and subsequent neuronal differentiation [13,29,30]. Thus, proneural bHLH factors have opposing functions, promoting NPC proliferation versus neuronal differentiation, although the exact mechanism remains to be analyzed.

The formation of major glial cells in the brain, oligodendrocytes and astrocytes, is also regulated by bHLH factors [11,31,32]. Olig1/2 bHLH factors control transcriptional regulation during oligodendrocyte fate specification and maturation. In the absence of Olig2 or Olig1/2, oligodendrocytes are completely missing in the brain [33–35]. It has