Special Focus Review

Dynamic signaling for neural stem cell fate determination

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Central nervous system (CNS) development starts from neural stem cells (NSCs) which ultimately give rise to the three major cell types (neurons, oligodendrocytes and astrocytes) of the CNS. NSCs are specified in space- and time-related fashions, becoming spatially heterogeneous and generating a progressively restricted repertoire of cell types. Mammalian NSCs produce different cell types at different time points during development under the influence of multiple signaling pathways. These pathways act in a dynamic web mode to determine the fate of NSCs via modulating the expression and activity of distinct set of transcription factors which in turn trigger the transcription of neural fate-associated genes. This review thus introduces the major signal pathways, transcription factors and their cross-talks and coordinative interactions in NSC fate determination.

Introduction

The development of CNS begins as a sheet of cells made up of primary progenitors known as neuroepithelial cells. By the onset of CNS formation, neuroepithelial cells were thought to be replaced by different neural stem cells (NSCs).1 The current evidence suggests that NSCs presence in both embryo and adult. It gradually transform from neuroepithelial cells to radial glial cells and then to astrocyte-like cells.2-4

Mammalian NSCs produce different cell types at different points in development. During this course, NSCs change their morphology and produce different progeny, but also change their gene expression profiles. Different models have been proposed for the genesis of cells within CNS (Fig. 1). First model was built by in vitro work, which showed that NSCs could generate lineage-restricted progenitors, neural restricted progenitors and glial restricted progenitors, respectively.5 This model proposes a homogeneous NSC population within the CNS first generates lineage-restricted progenitors, NRP s and GRPs, which then generate neuronal and glial cell types, respectively. Model 2 has proposed that neurons and glia cell arise from bipotential progenitors in spatially distinct domains, which generate combinations of one neuronal and one glial cell type, rather than from lineage-restricted progenitors.6 Model 3 suggests that NSCs in the motor neuron progenitor (pMN) domain could sequentially generate separate olig-positive motor neurons, oligodendrocyte and probably olig-negative astrocyte precursors.7 Temporally specific genes may coordinate an intrinsic developmental program, since progenitors grown in culture are found to produce certain progeny at given times, much like what progenitors do in vivo.1

The mechanisms of mammalian NSC commitment are largely unknown, which may include extrinsic and intrinsic mechanisms, such as the changes of signal environment, transcription factor expression patterns and epigenetic modifications.8 This review will focus on the signaling pathways and transcriptional factors which are thought to be involved in NSC fate determination. The composition and features of major signaling pathways for cell fate determination in CNS, the signals and transcription factors which initiate and promote neurogenesis, astrogenesis and oligogenesis and the mechanisms that control the timing of neural specification will be discussed sequentially.

Major Signaling Pathways Involved in CNS Development

Despite the difference in themes, each of the development stages involves induction, proliferation, locomotion, cell type or subtype determination and, meanwhile, prevention of inappropriate cell formation. To reach this goal, a precise mechanism is established, by which different signaling pathways play their roles exactly and in coordinative manners according to the time, space and intensity. So far, a panel of pathways has been proved to be involved in CNS development in stage-related patterns (Fig. 2). The panorama of each of the signaling pathways becomes increasingly clear in terms of extracellular stimulators, the components of cytoplasmic signaling cascade, the molecular targets and the consequences on gene transcription.

Wnt pathway. In canonical Wnt pathway, coordinate interactions of Wnt proteins with other relevant components, β-catenin, adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β), Axin and Conuctin, regulate cell proliferation and differentiation in the processes of embryogenesis.9 Given lack of Wnt expression, the excess β-catenin in cytoplasm is phosphorylated by functional interactions of GSK-3β, Axin, Conuctin and APC, and subjected to degradation by the ubiquitin proteasome system. Activation of Wnt signaling pathway leads to inhibition of GSK-3β activity, resulting in accumulation of cytoplasmic β-catenin that then enter to nucleus. Wnt signaling can be accomplished when β-catenin molecules translocalize into the nucleus and complex with T-cell...
Figure 1. Three proposed models for the genesis of CNS cell components. (A) Model 1: NRP/GRP model; (B) Model 2: MNOP model; (C) Model 3: Sequential model. NRPs, neuronal restricted progenitors; GRPs, glial restricted progenitors; MNOP, motoneuron and oligodendrocyte common progenitor; NA, neuron and astrocyte common progenitor; OPC, oligodendrocyte precursor; pA, astrocyte precursor; pM, motoneuron precursor.

Figure 2. Signaling pathways involved in CNS development. The blue arrows indicate activations, the red lines represent repression.
factors (TCFs) and/or lymphocyte enhance factor (LEFs) to activate transcription of multiple target genes.\textsuperscript{10}

Wnt signaling is implicated in the control of cell growth and differentiation during CNS development.\textsuperscript{11} Many studies show that this signaling induces neuronal and astroglial differentiation but suppresses oligodendroglial differentiation.\textsuperscript{11-13} Wnt signaling promotes proliferation of early NPCs, while plays positive roles in neurogenic phase,\textsuperscript{14} presumably due to the difference of epigenetic statuses in the early and late NPCs.

**Notch pathway.** The Notch proteins are 300-kDa single-pass transmembrane receptor, which function in CNS development by guiding cell fate determination.\textsuperscript{15} So far, four Notch receptors (Notch1-Notch4) and five structurally similar Notch ligands, Delta 1, Delta 3, Delta 4, Jagged 1 and Jagged 2, have been identified in mammals. The extracellular domain of the two single-pass transmembrane ligands, delta and serrate (jagged), interacts with the extracellular domain of Notch receptor on the adjacent cell. This ligand binding triggers a series of proteolytic events that results in the cleavage of Notch to generate Notch intracellular domain (NICD). Since NICD contains nuclear import signals, it has the chance to translocate into the nucleus\textsuperscript{16} and then associates with the constitutive DNA binding protein CSL (CBF1, Suppressor of hairless, Lag-1). By this way, the CSL complex is converted from a transcriptional repressor to a transcriptional activator.\textsuperscript{17} In the nervous system, Notch signals activate expressions of HES (hairy and enhance of split paralogues) family genes such as Hes1 and Hes5 as well as the HES-related genes Hesr1 and Hesr2 which belong to basic helix-loop-helix (bHLH) transcriptional repressor.\textsuperscript{18} Recently, the gene encoding brain lipid-binding protein (BLBP) was identified as a Notch target.\textsuperscript{19} This gene has RBp\textsubscript{bHLH} (recombining binding protein suppressor of hairless) binding sites which respond to activated Notch. Additionally, both N-Myc and c-Myc are targeted by Notch, which play manifold roles in regulating cell maintenance, renewal and apoptosis. These evidences reflect the widely ranged importance of Notch signaling in the developmental processes such as stem cell self-renewal, cell fate determination and instruction of differentiation and apoptosis.\textsuperscript{15} During mammalian CNS development, Notch signaling plays active roles in maintaining neuronal progenitor renewal, inhibiting neuronal commitment, promoting astrocyte-oriented glial fates, preventing oligodendrocyte formation as well as brain morphogenesis and neuronal migration.\textsuperscript{20}

**BMP pathway.** BMPs (Bone Morphogenetic Proteins) are a large subclass (more than 20 members) of the transforming growth factor-beta (TGF\textbeta) super family, which are expressed in many tissues under physiologic conditions, and are regulated through reversible interactions with extracellular antagonists, including noggin, chordin, follistatin and DAN.\textsuperscript{21} BMPs are classified into several subgroups such as BMP2 and BMP4 on the basis of sequence similarities and homology.\textsuperscript{22} The action of BMP is mediated by heterotetrameric serine/threonine kinase receptors. There are two specific BMP receptor subunits, BMPRI (BMP Receptor Type-I) and BMPRII, which are required for high-affinity binding and signaling.\textsuperscript{23} The binding of BMP to its receptor complex results in BMPRI activation and then SMAD1, SMAD5 and SMAD8 phosphorylations.\textsuperscript{24} The phosphorylated BMP-specific SMADs form a complex with the co-SMAD or SMAD4 and then translocate into the nucleus to activate transcription of specific genes by binding with the GCCG

or CAGA motif in the promoter regions of many BMP-responsive genes.\textsuperscript{25} They are also recruited to the promoters of BMP-responsive genes by high affinity cofactors such as OAZ, which binds to the promoter of STAT3, leading to the transactivation of the glial fibrillary acidic protein gene, a marker for astroglial differentiation.\textsuperscript{23,24,26} BMPs are involved in many developmental processes, including cell proliferation, differentiation, apoptosis and intercellular interactions during morphogenesis in a gene dosage dependent manner. In the ectoderm, BMPs activate two biochemical pathways, one mediated by SMADs\textsuperscript{27} and the other mediated by the p38/MAPK (mitogen-activated protein kinase) pathway downstream of TAK1 (TGFB activated kinase-1).\textsuperscript{28}

In addition to promoting bone formation, BMPs, especially BMP2 and BMP4, exhibit diverse activities in brain development.\textsuperscript{29} They act at different stages of neural development and in different regions of the CNS to regulate proliferation and determine the cell fate and the manner of differentiation. BMP signaling also inhibits the transformation from primitive ectoderm into neural ectoderm, and once the neural tissue is established, BMP signaling has a positive influence in the regulation of dorsal neural cell type formation.\textsuperscript{30} Moreover, BMP signaling cross-talks with other key developmental pathways such as Wnt and Sonic Hedgehog (Shh) to coordinate cell proliferation and patterning, allowing the formation of the appropriate numbers and types of differentiated neurons.\textsuperscript{31} BMPs induce astrocyte specification rather than proliferation of pre-existing astrocyte precursors and inhibit oligodendrocyte specification.\textsuperscript{32}

**STAT3 pathway.** Signal transducer and activators of transcription 3 (STAT3) is an important family member of cytoplasmic proteins with SH2 (Src Homology-2) domains which act as signal messengers and transcription factors. STAT3 is activated via the tyrosine phosphorylation cascade after ligand binding with the cytokine receptor-kinase complex and growth factor-receptor complex such as IL-6 (Interleukin-6), OSM (Oncostatin-M), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF).\textsuperscript{33} The phosphorylated STAT3 proteins dimerize and move into the nucleus, recruiting p300 and binding to specific sequences in target gene promoter. STAT3-mediated signaling is one of the main mechanisms for promoting astrocyte differentiation by inhibiting neuronal differentiation in the embryonic cortex.\textsuperscript{34} It has been known that STAT3 can promote NSC proliferation, while in the presence of activated BMP and Notch signaling, it induces astrogensis.\textsuperscript{35,36} Because of the above molecular effects in different stages of embryonic CNS development, any alteration of this signaling may lead to the developmental defects\textsuperscript{37} or primitive neuroectodermal tumors such as medulloblastoma due to mal-differentiation.\textsuperscript{38}

**Shh pathway.** Sonic hedgehog (Shh) is a member of the hedgehog family. Shh was initially identified as a morphogen that is directly responsible for dorso-ventral patterning of the CNS.\textsuperscript{39} As a secreted protein, Shh undergoes autocatalytic processing and modification for gaining activity prior to secretion. The Shh precursor protein is cleaved to yield a ~19 kDa N-terminal domain (signaling domain; N-Shh) and a ~25 kDa C-terminal domain (catalytic domain). Cholesterol modification is important for secretion and long-range activity of the Shh protein.\textsuperscript{40} The canonical Hedgehog signaling is mediated by two transmembrane proteins, a twelve-pass membrane protein binds Hedgehog ligand Patched (Ptc) and a seven-pass membrane protein as a signal transducer Smoothened (Smo).\textsuperscript{41}
When Shh is absent, Ptc acts as a transcriptional repressor and constitutively interacts with and inhibits Smo activity. When Shh binds Ptc, the inhibitory effects of Ptc’s on Smo are released, leading to the nuclear translocation of Ci/Gli protein as a transcriptional activator. Gli is the key transcription factor for Shh signaling. There are 3 Gli genes with distinct transcriptional functions in vertebrates. The downstream genes of Shh signaling include proliferation-related genes N-Myc, cyclin D1, E2f1 and E2f2 and those involved in de novo vascularization during embryonic development such as all three VEGF-1 (vascular endothelial growth factor-1) isoforms and angiopoietins-1 and -2.42,43

Shh is involved in the determination of cell fate and embryonic patterning during early vertebrate development in time and space-associated patterns. Shh is able to promote proliferation of both neural and non-neural tissues. It confers on neural stem cells proliferating competence both temporally and spatially by regulating the expression of N-Myc, cyclin D1, E2f1 and E2f2.42,43 In addition to the roles in proliferation and differentiation, Shh signaling seems to control cell death,44 because some of its downstream genes such as N-Myc and cyclin D1 are deeply involved in the regulation of cell survival. Shh signaling has been implicated in the specification and subsequent development of oligodendrocytes. A possible molecular link between Shh signaling and oligodendrocyte specification was revealed with cloning a subclass of neural basic helix-loop-helix (bHLH) transcription factors, Olig1 and Olig2.44 Again, alterations of Shh signaling are closely associated with neurocortical tumors formation.45

**PDGF/EGF pathway.** FGF/PDGF/EGF belongs to receptor tyrosine kinase superfAMILY, which exerts their stimulatory effects by binding to both of isoforms of these tyrosine kinase receptors. Ligand binding induces receptor dimerization and autophosphorylation, allowing binding and activation of cytoplasmic SH2-domain (Src homology) containing signaling molecules.46 Activation of their intrinsic tyrosine-specific protein kinases results in increased turnover of phosphatidylinositol and the sequential release of inositol triphosphates. These events coincide with the activation of other tyrosine kinase pathways, because the most important downstream protein tyrosine kinases (Tyk) activated in this cascade belong to the Janus kinase (JAK) that leads to activation of JAK-STAT signaling and initiation of astrogenesis. Additionally, FGF/PDGF/EGF plays its roles together with other signaling pathways. For example, it functions coordinatively with Notch and beta1-integrin Pathways in neural stem cells.47 In neural precursors, the PDGF/EGF-activated JAK-STAT pathway involves two tyrosine kinases, JAK-1 and Tyk-2, resulting in nuclear translocation of two latent cytoplasmic transcription factors, STAT-1 and STAT-2. Both PDGF and EGF promote neuronal differentiation or oligodendrocyte in committed NSCs, while promote proliferation during the early phase of NSC development.48

**Regulatory Factors in Neuronal Fate Determination**

**Main signaling pathways for neuronal fate determination.** Wnt signaling. Wnt ligands and receptors are expressed and play their roles in both the expansion and neurogenic phases.39 It seems that β-catenin, a central player of Wnt signaling, exerts effects on proliferation and differentiation or both, depending on the context of other signaling cascades.12 The effects of Wnt signaling in cultured neural progenitor cells are modified from pro-differentiation to maintenance of the proliferating state by FGF2.12,49,50 Ectopic expression of stabilized β-catenin in early NPCs (E8) results in over proliferation of NPCs and an enlarged neocortex.49,51 Wnt exhibits its neurogenic effects on late NPCs (E13.5), because it causes cell cycle arrest and neuronal differentiation in developing neocortex.49,52,53 The neurogenic effect of Wnt signaling is powerful in terms of induction of neuronal differentiation because nuclear accumulation of β-catenin alone is sufficient to induce neuronal lineage commitment. Canonical Wnt pathway increases the expression of neurogenin1 (Ngn1) and Ngn2, a sort of proneural basic helix-loop-helix (bHLH) proteins, through direct activation of their promoters by the β-catenin/TCF complex.50,52 The critical role of β-catenin in Ngn1 and Ngn2 expression was also evidenced by conditional knockout of β-catenin gene in the cerebral cortex and neural crest stem cells.49,53,54 All these results indicate the instructive role of Wnt pathway in neurogenesis. Interestingly, the Wnt/β-catenin pathway promotes proliferation via upregulation of cyclin D1, cyclin D2, c-Myc expression in other systems, while it appears to promote neuronal differentiation via upregulating Ngn1 and Ngn2 expression in the neocortex.49,52

**Notch signaling**. NSCs may be manipulated and enriched via regulating Notch activation. Ectopic expression of the Notch target Hes1 in the developing forebrain of mouse embryos inhibited neuronal differentiation, keeping the HES1-positive cells at the embryonic ventricular or the adult ependymal zone.20 Altered expression of other Notch targets such as HES5, HESR1 and HESR2 may result in similar consequences. Therefore, Notch maintains pluripotent neural stem cell (NSC) renewal by inhibition of their differentiation until the correct differentiation cue is available.

For a normal CNS development, it is necessary to keep neurons in an appropriate proportion and to initiate gliogenesis in time. Therefore, the expression of proneural genes should be restrained as neurons have reached to suitable number at individual positions. A mechanism called 'lateral inhibition' is thus developed, which is defined as the commitment to a neural fate by one cell had the consequence of inhibiting its neighbors to follow the same fate.55 Notch ligand in the progenitor cells activates the Notch signaling cascade in neighboring cells, resulting in the expression of Hes/Her/Esr genes which, in turn, directly downregulate proneural gene expression. Through this approach, the expression of proneural genes is restricted to single cells which enter a neural-differentiation pathway.55 During vertebrate neurogenesis, proneural genes such as Mash1 and Ngn1 trigger transcriptional activation of the Notch receptor ligand Delta. Therefore, the commitment to a neural fate by one cell had the consequence of inhibiting its neighbors to follow the same fate. The embryos lacking Notch function may exhibit neurogenic phenotype because the disruption of Notch-dependent cellular communications causes all of the neighboring cells to develop into neuronal progenitors. When the mouse embryo harbors the mutant forms of those Notch effectors, virtually all the neural stem cells differentiate prematurely into neurons, resulting in severe disorganized neural tube and absence of normal brain structures, suggesting the significance of Notch signaling in controlling appropriate cellular composition of CNS by preventing surplus neuron formation and promoting glial progenitor-to-astrocyte transition.20

It has been found that Notch 1 and Notch 2 present in the nuclei of almost all cortical post-mitotic neurons and that many terminally
differentiated neurons expressed the compositional elements of Notch pathway. These evidences suggest that Notch signaling is paved in mature neurons but unable to reverse the differentiation process. In other words, Notch and its signaling components may be regarded as one of the terminal differentiation symbols of neurons. So far, the biological significance of those Notch proteins in mature neurons remains to be figured out.

**BMP signaling.** BMPs promote proliferation of early NPCs and to induce expression of BMP receptor-IB through BMP receptor-IA. When the amount of BMP receptor-IB reaches to a threshold level, BMPs induce mitotic arrest and neuronal differentiation through BMP receptor-IB. This evidence suggests that BMPs function differently as a mitogen at the early stage and as a differentiation factor at the later stage of CNS formation.

**Transcription factors in neuronal fate determination.** Proneural genes encode bHLH family transcription factors and play an important role in neurogenesis. Mutation analysis in the mouse showed clear proneural activity for Mash1, Ngn1 Ngn2, and possibly Mash1 and Mash5, whereas other neural bHLH genes are involved in specifying neuronal fates or in neuronal differentiation instead of proneural role. In the formation of spinal cord, Mash1, Ngn1 and Ngn2 are expressed in most progenitors, except in two domains which are located at the ventral and dorsal ends of the neural tube where Ngn3 and Math1 are expressed, respectively. Although the role of proneural genes has not yet been systematically examined in most regions of the brain, Mash1, Ngn1 and Ngn2 are co-expressed in the dorsal telencephalon, and account for the generation of all progenitors of the cerebral cortex. In contrast, Mash1 is the only known proneural genes to be expressed in the ventral telencephalon, and some progenitors persist and differentiate normally although a large fraction of progenitors is missing in this region in Mash1 mutants, indicating the presence of other genes with proneural activity.

Many of neuronal-differentiation genes are structurally related to proneural genes, suggesting that distinct bHLH genes acting in cascades underlie the sequential steps of cell determination and differentiation. In vertebrates, bHLH genes of the NeuroD family have the characteristics of differentiation genes as well. NeuroD is required for the proliferation, differentiation and survival of granule cells in the cerebellum and hippocampus. The genes of NeuroD family act downstream of vertebrate proneural genes in a manner very similar to as e and cato in Drosophila neurogenesis. Activation of NeuroD by Ngn5 is likely direct, because of the activation of ase by ac and sc. Nieto M, et al. showed that vertebrate proneural genes promoted neuronal fates and inhibited glial fates simultaneously, indicating their important role in the transition from neurogenesis to gliogenesis.

**Transcription factor and signaling interplays in neuronal fate determination.** The balance of proliferation and differentiation of NSCs is mainly regulated by the opposing activities of repressor bHLH and activator bHLH as well as Sox1-3 and Sox21 which are largely determined by the activities of Notch and Wnt signalings. Notch signals triggers the expression of Hes family genes such as Hes1 and Hes5 that in turn inhibit Sox21 expression via down-regulating the expression of proneural genes. Therefore, attenuation of Notch signaling is a critical event for neurogenic commitment of NSCs. Unlike Notch, Wnt signaling is regarded as one of the few signaling pathways that have been shown to promote the neuronal fate in an instructive manner. It induces neuronal differentiation through promoting proneural Ngn1 and Ngn2 expression and suppressing Hes1 and Hes5 expression in late embryonic and postnatal development stages (Fig. 3).

**Regulatory Factors in Astroglial Fate Determination.**

**Main signaling pathways for astroglial fate determination.** Signaling pathways involved in the induction of astrocyte specification are thought to be induced via cytokines such as CNTF, LIF and IL-6 as well as the signaling mediated by BMP and Notch proteins.

**STAT3 signaling.** The typical effect of STAT3 signaling is to promote GFAP expression, a specific biomarker of glial cells. As a transcription factor, STAT3 induces GFAP expression via binding with STAT-responsive element in GFAP promoter region when activated by CNTF and LIF. The capacity of STAT3 in triggering GFAP expression largely depends upon the stage where neural progenitor cells are in. When LIF and CNTF mediated phosphorylation occurs to STAT3, the phosphorylated STAT3 molecules translocalize into nucleus and trigger GFAP expression in relatively late-(E14.5) but not early-stage (E11.5) NPCs. Similar phenomena could be observed in Notch-induced GFAP expression. Instead of GFAP induction, Notch and LIF/CNTF-STAT3 promote self-renewal of early NPCs. Interestingly, CNTF induces almost the same levels of STAT3 phosphorylation in early and late NPCs, but GFAP is induced only in the late NPCs, suggesting that the same stimuli result in different biological outcomes in early and later NPCs. Among the genes activated by STAT1/3 in late embryonic NEPs, there are several composition elements of the Jak-Stat pathway itself, including gp130, Jak1, Stat1 and Stat3. Thus, STAT signaling triggers an auto-regulatory loop that reinforces itself and presumably tends to consolidate and stabilize the astrocyte phenotype.

**BMP signaling.** Bone morphogenetic proteins have been implicated in the development of gliocytes. It has been proved that BMPs are involved in astrocytic differentiation of neural precursor cells. In the nuclei, BMP-downstream transcription factors, i.e., Smad proteins, induce expression of astrocyte-specific genes in cooperation with another cytokine signaling. The typical example is that BMP signaling mutant mice exhibit glial cell maturation defects, indicating that BMP signaling is the critical element for astrocyte generation and maturation.

**Notch signaling.** Notch directly promotes the differentiation of many glial subtypes except oligodendrocytes in an instructive manner. Notch1 could be detected in the nuclei of radial glial cells but not in neuronal precursor cells positive in neuronal basic helix-loop-helix proteins or in the differentiating neurons of the embryonic forebrain. Furthermore, activated Notch signaling in retinal progenitors blocks rat neuronal differentiation and promotes the expression of Müller glia markers. In postnatal mouse retinae, Ectopic expression of Hes5 promotes gliogenesis at the expense of neurogenesis, conversely, erasing Hes5 expression results in significant reduction of Müller glia. These data thus suggest that Notch activation is essential for astrogenesis by inhibiting the tendency of neuronal formation. Notch appears to promote the astrocytic phenotype through its downstream effectors Hes1 and Hes5 which are known to promote astrocytic differentiation via suppressing the function of proneural bHLHs.
**Wnt signaling.** In addition to neuronal induction, Wnt signaling is able to promote astroglial differentiation and suppresses oligodendroglial differentiation in a phase-dependent fashion through indirectly regulating gliogenesis by inducing BMPs in neuronal cells.\(^{12,75}\) Wnt-induced BMPs are then secreted to the extracellular space to promote astroglial differentiation and inhibit oligodendroglial differentiation of surrounding cells. Oligodendroglial differentiation can only proceed when attenuation of Wnt and BMP signaling occurs. In the aspects of astrogenic promotion and oligogenic suppression, the functional similarities remain not only between Wnt and BMP but also between them two and Notch signaling.\(^{75}\) Therefore, a coordinative functioning of Wnt, BMP and Notch pathways is essential in determining the courses of neurogenesis and gliogenesis. In another word, Wnt signaling never plays its roles independently but through interplays with other signaling elements which provide positional information and induce stepwise cell fate specification.

**Transcription factors in astroglial fate determination.** Proneural bHLH transcription factors such as Ngn1 and Mash1 repress gliogenesis by inhibiting the activity of the Jak-STAT pathway.\(^{76}\) They achieve this goal through two approaches: (1) competing with STAT1/3 for binding to p300 and CBP which are present in limiting quantities in the cells and (2) directly inhibiting STAT activation. Sun and his colleagues\(^{77}\) proposed a sequestration model, where Ngn1 inhibits glial differentiation by sequestering activator complexes away from glial-specific genes and by directly inhibiting STAT3 activation when the cortical precursor cells are neurogenic. These evidences largely explain how the switch of neurogenesis is turned to gliogenesis during development. As gestation proceeds, Ngn1 expression is downregulated and STAT3 can form a complex with Smad1, bridged by p300, to effectively induce astrogenesis. The factor(s) leading to Ngn downregulation remains to be determined. It has been shown that growth hormone (GH) acts through the Jak/STAT pathway to activate the inhibitory STAT5, resulting in the downregulation of Ngn1 expression in cultured neurospheres.\(^{78}\) GH and its receptor are expressed in the rodent brain as early as embryonic day 10 and reach the peak just before birth. It is therefore conceivable that GH might play a role in the neuron-astrocyte switch. However, this could not easily explain the fact that neuronal-glielial switching occurs approximately on schedule in primary NEPs in the absence of added GH. An alternative option is that robust Jak/STAT signaling by itself might inhibit Ngn expression.\(^{79}\)

**Figure 3.** The involvement of Notch and Wnt signaling pathways and their relevant transcription factors in neurogenesis. The blue arrows indicate activating effects, and the red lines represent repressive effects. Notch is a negative signal while Wnt is a positive signal in neurogenesis through the mediation by bHLH and SOX transcription factors.
actions or crosstalks of STAT signaling with the ones mediated by BMP and Notch proteins.35,36,69,81 STAT3 might co-operate with BMP2 as well as Notch family members to induce glial fibrillary acidic protein (GFAP) expression. LIF and BMP2 were found to act in synergy on primary fetal neural progenitor cells to induce astrocyte formation by p300 bridged STAT3-Smad1 complex.82 LIF can also consequently activate BMP and Smad1 by triggering STAT3 activation.35 Although STAT3 signaling suppresses the expression of proneural bHLHs to inhibit neurogenesis by promote Notch signaling,83 it can be activated reversely by Hes proteins that directly recruit the upstream kinase JAK2.69 Additionally, Notch-Hes signaling is found to induce astrocytic differentiation through activation of STAT3 by directly recruiting the upstream kinase JAK2.69 In addition to the interaction with STAT3 signaling, Notch signaling also promotes astrogliogenesis via direct CSL-mediated GFAP activation.84 Furthermore, recruitment of p300 to the Notch intracellular domain (NIC)-containing complex can be facilitated by activated Smad1, indicating the contribution of BMP2-mediated enhancement of Notch-induced Hes-5 expression.85 Consequently, a functional coordination of Notch, BMP and STAT3 signaling is important in the accomplishment of astrogenesis by maintaining appropriate intensity of STAT3 signaling. It should be pointed out that, a positive autoregulatory loop of STAT signaling is formed during the onset of astrogliogenesis, by which STAT1/3 directly induces the expression of various components of the Jak-STAT pathway, including gp130, Jak1, STAT1 and STAT3.86 By this way, STAT signaling is further strengthened, leading to upregulation of its downstream genes and consolidated astrocyte phenotype.

Regulatory Factors in Oligodendroglial Fate Determination

Main signaling pathways for oligodendroglial fate determination. The origin of oligodendroglia has been debated for years. One of the well accepted notions is that oligodendroglia from both dorsal and ventral sources become active at different times during development. Oligodendrocyte precursors first arise in a restricted ventral part of the embryonic spinal cord and migrate laterally and dorsally from there; later on, secondary sources develop in the dorsal cord.87 Apparently, oligodendrogenesis can be induced by several signaling pathways depending on different origins.

Shh pathway. Shh signaling has been implicated in the specification and subsequent development of oligodendrocytes. Several studies have shown that Shh promotes the generation of pMN-derived OLPs (progenitor of motor neurons derived oligodendrocyte progenitor) in the spinal cord.88 In the absence of Shh signaling, ventrally derived OLPs fail to form in the spinal cord or forebrain, while transplating Shh-expressing tissue adjacent to the dorsal neural tube in vivo or adding Shh to spinal cord explants in vitro induces the formation of ectopic OLPs, highlighting the importance of spatially restricted Shh signalling in directing OLP generation.89 Several studies demonstrated that sonic hedgehog (Shh) signaling was specifically required during oligodendrocyte development. For instance, Shh promotes NSCs to transform to oligodendrocyte progenitor/OLP89,90 and induces pMN-derived OLPs (progenitor of motor neurons derived oligodendrocyte progenitor) in primary cultures of dissociated forebrain NPCs.91,92

Shh-independent pathway. Although the activated Shh signaling is important for oligogenesis, a Shh-independent pathway to oligodendrocyte specification is evidenced because NSCs from Shh or Smo null mice can still generate OLPs in culture.93 It was demonstrated that FGF2,93,94 also participate in oligodendrocyte specification according to different origin of oligodendroglia.92,95 NSCs from the cortex and the dorsal spinal cord can generate OLPs when they are exposed to FGF2 and cycloapamine, a potent inhibitor of Shh signaling. It is therefore possible that FGF might be responsible for oligodendrocyte induction in the dorsal spinal cord and/or forebrain, which are unlikely to be within the range of Shh from the floor plate (spinal cord) or MGE (forebrain).

In addition to Shh and FGF2, insulin-like growth factor I (IGF-I) has also been implicated to influence oligodendrocyte specification, because multipotent adult neural progenitors can be induced to generate oligodendrocytes in vitro after treating IGF-I, IGF-II or insulin.96 The oligodendrogenic activity of IGF-I is thought to be mediated at least in part by inhibition of BMP signaling.96 Notch signaling is essential at the early stages of specification of the oligodendroglial lineage, because constitutive activation of Notch signaling results in excess OLPs at the expense of motor neurons.97 On the other hand, persistent Notch signaling can inhibit in vitro oligodendrocyte maturation, and loss of Notch signaling leads to premature differentiation of OLPs to oligodendrocytes in vivo.98 These data thus suggest that Notch signaling might regulate both the specification of OLPs and oligodendrocytes maturation.

Opposite effects of BMPs on oligodendrogenesis. BMP signaling is another element that influences oligodendrocyte fate determination but in an opposite way of Shh signaling. The members of the BMP family have been proposed to act as negative regulators of oligodendrocyte specification because BMPs induce expression of Id proteins that interact with OLIG proteins to inhibit OLP differentiation and maturation.58 In vitro study revealed that when forebrain or spinal cord neural stem cells were incubated with BMPs, they failed to develop oligodendrocytes, irrespective to the presence of activated Shh or FGF2 signaling.99,100 Apparently, dynamic transition of signaling patterns with imbalanced but nonrandom signaling pressure is essential for NSC fate determination.

Transcription factors in oligodendroglial fate determination. bHLH genes. A possible molecular link between Shh signaling and oligodendrocyte specification was revealed by cloning a subclass of neural basic helix-loop-helix (bHLH) transcription factors, Olig1 and Olig2.91 Induced expression of Olig2 is sufficient for oligodendrocyte specification but not for motor neuron specification and astrocyte repression. On the other hand, when Olig1 and Olig2 are doubly knockout, the mice have no oligodendrocytes at E18.5, indicating that these two Shh-target genes are both necessary for oligodendrocyte formation.101 It has been found that ES cell-derived Olig2+ cells can give rise to both motor neurons and oligodendrocytes, depending on the time/phase at which differentiation is initiated. It should be noted that Shh conducts its functions such asmotor neuron specification much earlier than the time of oligodendrocyte specification. Therefore, in order to keep CNS development in a steady pace, other intrinsic and extrinsic factors may exist at that time to inhibit the oligogenic effects of Shh.

Olig1 and Olig2 were originally identified as two bHLH transcription factors which mark the oligodendrocyte lineage from very early stages.101 In the spinal cord, these two transcripts can be...
detected from embryonic day 9 onwards and known to be induced by Shh signaling. The transcripts are initially observed in the third ventral of the spinal cord but soon become restricted to the pMN domain. Olig2 expression persists in pMN throughout motor neurons development; it is rapidly extinguished in post-mitotic MNs but remains in OLPs. Expression of Olig1 is more dynamic at early times prior to the appearance of OLPs. Both Olig1 and Olig2 genes are expressed throughout the oligodendrocyte lineage including the mature oligodendrocytes. Functional studies on mice have demonstrated the significance of Olig2 in either motor neuron or oligodendrocyte specification. In a mouse embryonic stem (ES) cell line, Olig2 requires the cooperation of retinoic acid (RA) and Shh for motor neuron specification. Olig2 bypasses the need of Shh in oligodendrocyte specification but can not bypass the CNTF-STAT signaling to repress astrocyte differentiation. Unlike Olig2 that seems essential for oligodendrocyte development, initial evidence indicates a redundant, non-essential function for Olig1. However, more recent work with the Olig1 mutant mice questions the previous data and suggests that Olig1 is, in fact, a central regulator of oligodendrocyte maturation and myelination.

Another bHLH gene that has recently been found to play a role in oligodendrocyte development is the proneural gene Mash1. In the early telencephalon, Mash1 is co-expressed with Olig2 at the time of oligodendrocyte specification in the medial ganglionic eminence (MGE)/anterior entopeduncular area (AEP) and its expression is briefly maintained in initial populations of migrating OLPs. Although the data indicate both Mash1-dependent and Mash1-independent OLPs in the telencephalon, the precise function of Mash1 in the oligodendrocyte lineage remains to be further investigated.

Nkx 6.1, Nkx 6.2, and Nkx 2. Nkx 6.1 and Nkx 6.2 are two homeodomain transcription factors which are induced by Shh in the ventral spinal cord. Within the pMN domain, Nkx6.1 and Nkx6.2 appear to regulate the expression of Olig2 in a dose-dependent manner. In Nkx6.1/Nkx6.2 mutants, all the motor neurons and oligodendrocytes generated from the pMN domain are eliminated, demonstrating an essential role for these two genes in oligodendrocyte specification from this region;

REGULATING THE TRANSITION FROM NSC TO PROLIFERATION.

As referred above, the transition of proliferation to differentiation of NSCs is mainly regulated by the opposing activities of repressor bHLH and activator bHLH as well as Sox1-3 and Sox21 which are largely determined by the activities of Notch and Wnt signalings. Among these signalings, the Wnt/β-catenin pathway is noticeable because it could promote proliferation via upregulation of cyclin D1, cyclin D2, c-Myc and c-jun expression in other systems, while it appears to promote neuronal differentiation via upregulating Ngn1 and Ngn2 expression in the neocortex.

Wnt signaling together with its nuclear effector, β-catenin and T-cell factor (TCF) DNA-binding proteins, form a regulatory system, by which β-catenin/TCF complexes distinguish precisely between genes to be activated and genes to remain silent in a given cell system and/or at a given time. It has been known that activated Wnt signaling is a major initiation and promotion factor of neurogenesis because of its ability to selectively triggering a panel of neuronal-associated gene expression in NSC differentiation. It therefore suggests that the transition of NSC to neuron is determined to a certain signaling such as Wnt by selectively triggering a panel of neural fate-determination genes instead of genes directed proliferation. The possibility of the dynamic features of the gene responses to Wnt signaling may be the influence of epigenetic factors including DNA methylation and chromatin re-modeling.

It has been found that only Wnt-responsive promoters are bound by specific subsets of TCFs, whereas nonresponsive Wnt target promoters remain unoccupied. It is also found that Wnt-responsive, TCF-bound states correlate with DNA hypomethylation, histone H3 hyperacetylation, and H3K4 trimethylation, whereas inactive, nonresponsive promoter chromatin shows DNA hypermethylation, is devoid of active histone marks, and additionally can show repressive H3K27 trimethylation. These data suggest that epigenetic modifications of promoter chromatin and differential promoter occupancy by functionally distinct TCF proteins jointly determine susceptibility to Wnt signaling. This result begins to unveil why Wnt signaling play different roles in neural stem cell.
self-renewal and neurogenesis which exist a long time.122

Controlling stepwise transition from neurogenesis to gliogenesis. JAK-STAT pathway is a main pathway controlling the onset of astrocyte formation. The neurobiological functions of STAT signaling are phase dependent. CNTF can induce almost the same levels of STAT3 phosphorylation in early and late NPCs, but GFAP is induced only in the later cells,123 indicating that the capacity of STAT3 in triggering GFAP expression largely depends upon the developmental stage of neural progenitor cells.9 This phenomenon again strongly suggests that the same stimuli may result in different biological outcomes during stepwise CNS development. It is therefore possible that different sets of intrinsic factors may present in early and late NPCs, which determine the ways to respond external stimuli such as STAT3 and Wnt signaling via modulating the status of gene accessibility.14,49,124 In this context, the influence of epigenetic factors in NSC fate determination should be taken into account.

STAT3 promotes astrogenesis by promoting GFAP expression. A CpG motif within the STAT-binding element has been found in the promoter region of GFAP gene. It is methylated in early NPCs, but becomes demethylated and accessible to STAT3 later during development or culture.123 Fan GP, et al. indicated that DNA methylation was a key mechanism in inhibiting the expression of various components of the astrogliogenic JAK-STAT pathway,125 suggesting a causal relationship between DNMT1 (DNA methyltransferase 1) expression and the control of the onset of astrogliogenesis in NPCs. It should be addressed that DNMT1 molecule has also been shown to directly inhibit gene transcription with its N-terminal transcription repression domain, which interacts with other transcription repressor components, including histone deacetylases and histone lysine-methyltransferases for inactive chromatin remodeling.126 DNA methylation not only inhibits astroglial marker genes but also the genes which are essential for JAK-STAT signaling. Thus, demethylation of these two groups of genes and subsequent elevation of STAT activity are key mechanisms which control the timing and magnitude of astroglial differentiation. JAK-STAT activation in neural precursor cells is under the tight control of several independent mechanisms.125 Additionally, the transcriptional activity of GFAP promoter can also be regulated via modification of histone H3.127

This stage-specific DNA methylation and chromatin modification of the important DNA elements might be part of a general mechanism that accounts for some of the intrinsic differences among different stages of NPCs. Though GFAP stands as a specific biomarker for glial cells, it is merely the Type III of intermediary filament proteins and by no means a crucial protein for astrocytic differentiation. Therefore, more important astrocytic determinant genes may be epigenetically regulated during stepwise brain development, leading to differential cellular and molecular responses to the same signaling.

Concluding Remarks

Neural cell fate determination is complicated, which requires diverse signal pathways, transcription factors and epigenetic machineries to participate in. Multiple extracellular signals and corresponding intrinsic transcriptional factors may exert different effects in time- and stage-dependent fashions. Wnt and Notch pathways are important in the specification of neural fates; the former is thought to be a positive regulator, while the later plays the opposite roles. The mediators connecting Wnt or Notch signaling with the fates of neural stem cells are proneural genes which encode bHLH family transcription factors, such as Ngn1, Ngn2 and Mash1. The BMPs and STAT signaling pathways can act independently of one another but also synergize in some circumstances to specify astrocytes. A coordinative function of Wnt, BMP, Notch and STAT signaling is essential in determining the courses of neurogenesis and gliogenesis. Shh signaling has been implicated in the specification and subsequent development of oligodendrocytes. FGF2, insulin-like growth factor I (IGF-I) has also been suggested to influence oligodendrocyte specification according to different origin of oligodendroglia. Transcriptional factors play important roles as mediators between different signaling pathways and neural cell fate determination. Given the availability of the above extracellular and interior molecular elements, the responsiveness of NSCs to these signals is the key point for determining the direction of NSC differentiation, which is largely controlled by epigenetic mechanisms such as DNA methylation, histone modifications and non-coding RNA. As a matter of fact, the involvement of epigenetic mechanisms in embryonic CNS development has been increasingly documented, especially in the recent years. The manners of molecular response to neural-associated signaling are largely regulated by the epigenetic factors which determine the accessibility of the corresponding genes. And the enzymes which regulate epigenetic program often interact with transcription factors to form a panel of complexes to control global gene expression pattern. By this way, local and overall chromatin identities are created, which suit for distinct fate determination of NSCs. The continuous progress in epigenetics of developmental neuroscience may help us to answer how cell fate is precisely determined, remembered and inherited through disclosing the manners of local and global epigenetic programming and re-editing during the transitions of NSC differentiation.

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