19 Chen HL, Pistollato F, Hoeppner DJ et al. Oxygen tension regulates survival and fate of mouse central nervous system precursors at multiple levels. Stem Cells 2007:25:2291–2301.
20 Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: A critical component of the stem cell niche. Cell Stem Cell 2010:7:150–161.
21 Wright WE, Shay JW. Inexpensive low-oxygen incubators. Nat Protoc 2006;1:2088–2090.
22 Moeller BJ, Dreher MR, Rabbani ZN et al. Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. Cancer Cell 2005;8:99–110.
23 Zhou Z, Hong EJ, Cohen S et al. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 2006;52:255–269.
24 Lois-Caballe C, Baltimore D, Qin X-F. Method for expression of small antiviral RNA molecules within a cell. US Patent0 059 944 Al. 2003.
25 Mutoh T, Miyata T, Kashiwagi S et al. Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede. Exp Neurol 2006;200:430–437.
26 Papkovskii DB, Yaropolov AI, Savitskii AP et al. Fibre-optic oxygen sensor based on phosphorescence quenching. Biomed Sci 1991;2: 536–539.
27 Hatada I, Namihira M, Morita S et al. Astrocyte-specific genes are generally demethylated in neural precursor cells prior to astrocytic differentiation. PLoS One 2008;3:e3189.
28 Dunwoodie SL. The role of hypoxia in development of the Mammalian embryo. Dev Cell 2009;17:755–773.
29 Arteel GE, Thurman RG, Raleigh JA. Reductive metabolism of the hypoxia marker pimonidazole is regulated by oxygen tension independent of the pyridine nucleotide redox state. Eur J Biochem 1998; 253:743–750.
30 Raleigh JA, La Dine JK, Cline JM et al. An enzyme-linked immunosorbent assay for hypoxia marker binding in tumours. Br J Cancer 1994;69:66–71.
31 Iyer NV, Kotch LE, Agani F et al. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 1998;12:149–162.
32 Jain S, Maltepe E, Lu MM et al. Expression of ARNT, ARNT2, HIF1 Alpha, HIF2 alpha and Ah receptor Mrnas in the developing mouse. Mech Dev 1998;73:117–123.
33 Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. Mol Cell 2008:30:393–402.
34 Palmer LA, Semenza GL, Stoler MH et al. Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. Am J Physiol 1998;274:L212–L219.
35 Roitbak T, Surviladze Z, Cunningham LA. Continuous expression of HIF-1alpha in neural stem/progenitor cells. Cell Mol Neurobiol 2011; 31:119–133.
36 Hirsila M, Koivunen P, Gunzler V et al. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J Biol Chem 2003;278:30772–30780.
37 Martinez R, Esteller M. The DNA methylome of glioblastoma multiforme. Neurobiol Dis 2011;39:40–46.
38 Quinn GE, Dobson V. Outcome of prematurity and retinopathy of prematurity. Curr Opin Ophthalmol 1996;7:51–56.
39 Blackburn S. Environmental impact of the NICU on developmental outcomes. J Pediatr Nurs 1998;13:279–289.
40 Deulofeut R, Critz A, Adams-Chapman I et al. Avoiding hyperoxia in infants < or = 1250 g is associated with improved short- and long-term outcomes. J Perinatol 2006;26:700–705.

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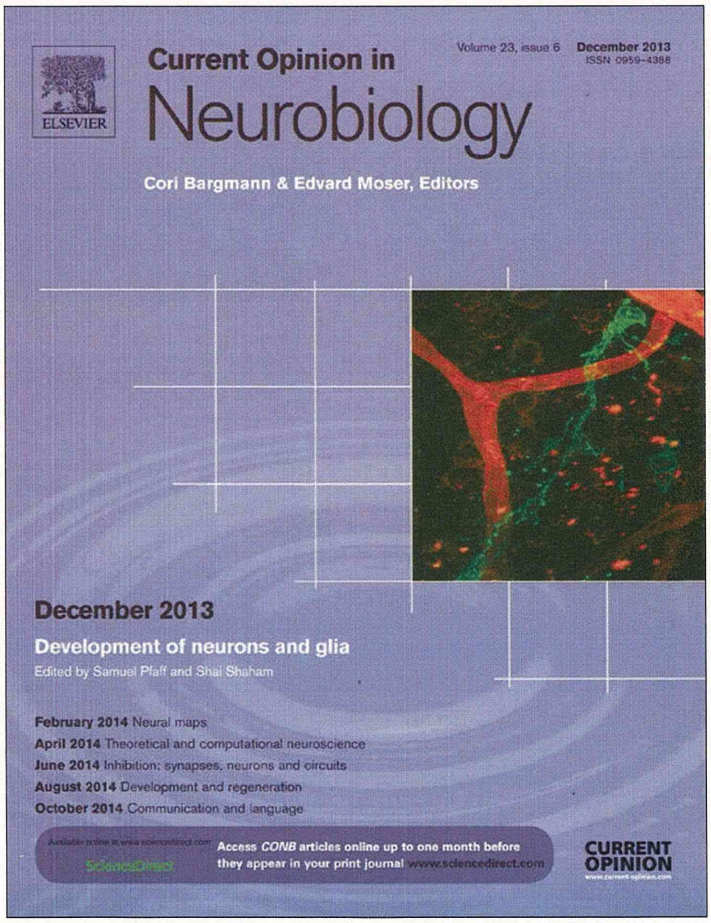

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Current Opinion in Neurobiology

Mechanisms of astrocytogenesis in the mammalian brain

Masakazu Namihira[1] and Kinichi Nakashima[2]

In the mammalian central nervous system, astrocytes are the most abundant cell type and play crucial roles in brain development and function. Astrocytes are known to be produced from multipotent neural stem cells (NSCs) at the late gestational stage during brain development, and accumulating evidence indicates that this stage-dependent generation of astrocytes from NSCs is achieved by systematic cooperation between environmental cues and cell-intrinsic programs. Exemplifying the former is cytokine signaling through the gp130-Janus kinase/signal transducer and activator of transcription 3 pathway, and exemplifying the latter is epigenetic modification of astrocyte-specific genes. Here, we introduce recent advances in our understanding of the mechanisms that coordinate astrocytogenesis from NSCs by modulating signaling pathways and epigenetic programs, with a particular focus on the developing mammalian forebrain.

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Introduction

Nearly 50% of the cells in the adult human brain are glial cells, among which astrocytes are the most abundant cell type and are ubiquitous throughout the brain and spinal cord [1]. Astrocytes play a variety of crucial roles in brain development and function, such as structural support, maintenance of water balance and ion distribution, and construction of the blood–brain barrier to control the passage of substances from the blood into the brain. During mammalian development, astrocytes are generated from neural stem cells (NSCs) located in the ventricular zone and subventricular zone in the gliogenic phase of late gestation [2]. NSCs, known as neuroepithelial cells in early-gestational to mid-gestational stages, divide both symmetrically, to increase their own numbers, and asymmetrically, to generate early neurons (Figure 1). As development proceeds, the morphology of NSCs changes: the cells display extended radial processes to the pial surface from their somata, which reside in the ventricular zone [3], and become known as radial glial cells (Figure 1). These NSCs generate more neurons but now also have the potential to differentiate into astrocytes. At the late embryonic and early postnatal stages, most NSCs begin to detach from the apical side and migrate into the cortex, where they differentiate into astrocytes [4,5] (Figure 1), although a recent report demonstrates that the majority of astrocytes in the postnatal cortex arise from the local proliferation, during the early postnatal period, of cells that have already differentiated [6•].

In this review, we focus on recent molecular insights into astrocytogenesis from NSCs in the developing mouse brain. It is well established that there are two important components in astrocytogenesis during mammalian brain development. One is the epigenetic derepression of astrocytic gene transcription, a prerequisite for the progressive acquisition of astrocyte differentiation potential by NSCs; the other is the activation of cytokine signaling through the gp130-Janus kinase/signal transducer and activator of transcription 3 (JAK–STAT3) pathway to induce astrocytic gene transcription. Accumulating evidence indicates that various factors contribute to astrocyte production from NSCs by regulating these two processes. In this review, we survey reports investigating astrocytogenesis from NSCs in the mammalian central nervous system, with a special emphasis on the developing forebrain.

Regulators modulating the signaling pathway of astrocyte-inducing cytokines

The JAK–STAT3 pathway is activated by members of the interleukin (IL)-6 family of cytokines, including leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and cardiotrophin-1 (CT-1), through homodimerization or heterodimerization of the common signal transducer gp130 either with itself or with another receptor component such as LIFRβ [7]. Activated STAT3 then induces astrocyte differentiation by activating astrocytic genes such as glial fibrillary acidic protein (*gfap*) [8,9] (Figure 2). In a convergent pathway, the action of another group of cytokines, the bone morphogenetic proteins (BMPs), is mediated by heterotetrameric serine/threonine kinase receptors and their downstream transcription factors Smad1, 5, or 8. After being phosphorylated, these

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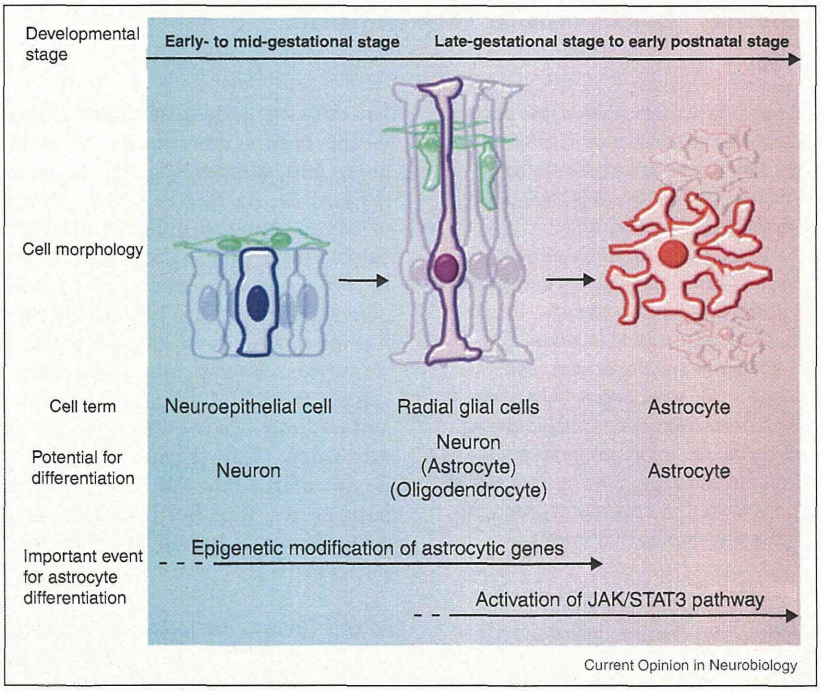
Figure 1



Temporal development of neural stem cells (NSCs), exemplified by the ventricular zone of the cerebral cortex. In early-gestational to mid-gestational stages (left), NSCs, referred to as neuroepithelial cells (blue), divide symmetrically and asymmetrically to increase their own numbers and to generate early neurons (green). As development proceeds (middle), the morphology of NSCs changes to have and the cells acquire radial processes extending to the pial surface from their somata, which reside in proximity to the ventricular boundaries; the cells are now referred to as radial glial cells (violet). These NSCs also generate many neurons (green) but have the additional potential to differentiate into astrocytes and oligodendrocytes. At the late-gestational and early postnatal stages (right), most NSCs begin to detach from the apical side and migrate into the cortex, and differentiate into astrocytes (red). In this scheme, there are two important events for astrocytogenesis. The first is epigenetic derepression of astrocytic gene transcription for the acquisition of astrocyte differentiation potential by NSCs, which is sustained until late embryonic stages; the second is activation of cytokine signaling through the JAK/STAT3 pathway for the induction of astrocytic gene transcription.

transcription factors form a complex with Smad4 which is translocated into the nucleus to activate the transcription of specific genes. Smads are known to participate in the induction of astrocytic gene expression by forming a complex with STAT3 that is bridged by the transcriptional coactivators p300/CBP [10].

Deletions of the genes encoding LIF [11], LIFRβ [12], gp130 [13] or STAT3 [14] all result in impaired astrocyte differentiation *in vivo*, indicating that the JAK–STAT3 pathway is essential for astrocytogenesis in the developing brain. It has also been reported that CT-1 secreted from neurons is important for astrocyte differentiation from NSCs in embryonic stages [15]. Furthermore, the proneuronal transcription factor Neurogenin 1 (Neurog1) may suppress astrocyte differentiation from NSCs by sequestering the p300/CBP-Smads complex away from STAT3, leading to the suppression of STAT3 target genes [16,17] (Figure 2). Neurog1 is expressed during the neurogenic period, but not the astrocytogenic period, of neocortical development. Its expression is precisely controlled by polycomb group proteins through chromatin modification [18], thus contributing to the mechanisms for fate-switching of NSCs from neurogenic to astrocytogenic as development proceeds.

Recently, the RAF/MEK/ERK pathway, which is among the best-characterized signaling cascades in mediating the effects of extracellular factors, has been shown to participate in astrocytogenesis from NSCs by modulating the JAK–STAT3 pathway. Li et al. [19•] reported that mutation of the core RAF/MEK/ERK pathway components MEK1 (MAP2K1) and MEK2 (MAP2K2) in NSCs caused a severe reduction in the number of astrocyte precursor cells expressing the aldehyde dehydrogenase 1 family member L1 [20] in late-gestational forebrain. They also found that the JAK/STAT3 pathway in NSCs derived from *Mek1/2* null mutant embryos was attenuated due to a marked reduction in the expression of gp130, which is critical for activation of the pathway by IL-6

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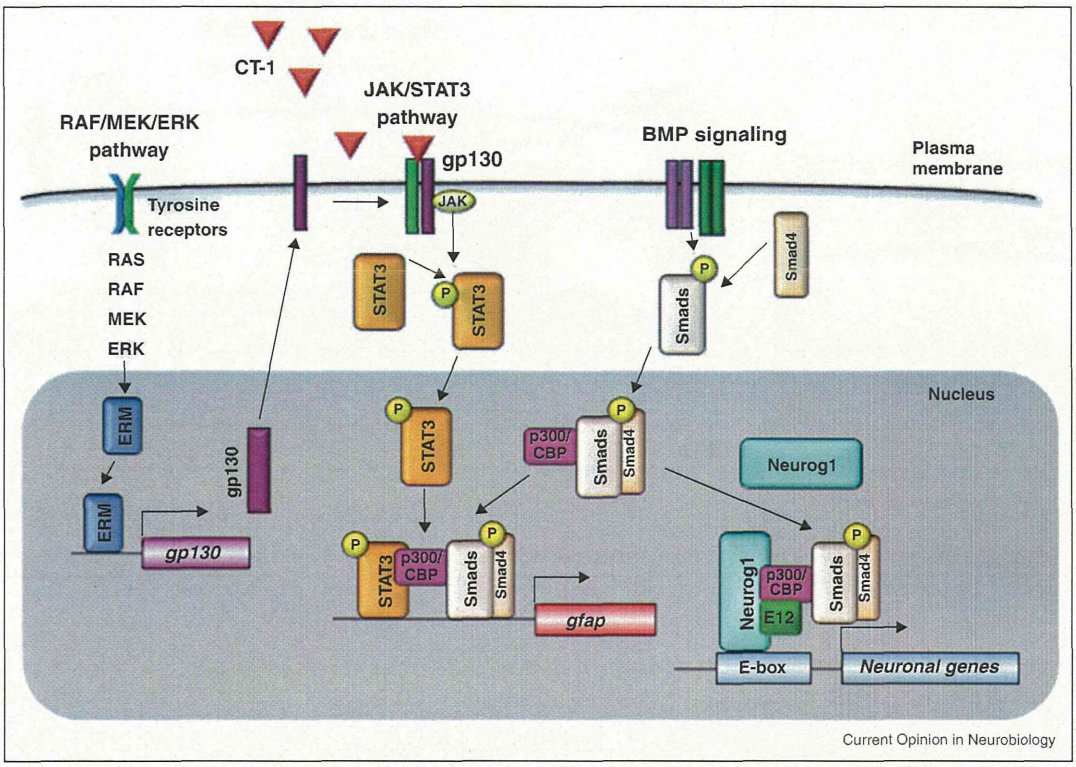
Figure 2



Regulators modulating the signaling pathway of astrocyte-inducing cytokines. Synergistic integration of the JAK/STAT3 pathway and BMP/Smads signaling is achieved by the formation of a complex involving their respective downstream transcription factors, STAT3 and Smads, together with p300/CBP. Neuron-secreted CT-1 is important for activation of the JAK/STAT3 pathway in NSCs during embryonic stages. Neurog1 sequesters the p300/CBP-Smads complex away from STAT3, leading to the suppression of STAT3 target genes. The RAF/MEK/ERK pathway also plays an important role in astrocytogenesis from NSCs by regulating gp130 expression.

family cytokines. In addition, Li et al. identified the Ets transcription factor family member Etv5/Erm as a downstream target of MEKs in NSCs [19•]. Overexpression of Etv5/Erm could rescue the impairment of cytokine-induced differentiation into astrocytes in MEK null mutant NSCs, suggesting that Etv5/Erm regulates gp130 expression in NSCs. Collectively, these observations indicate that the RAF/MEK/ERK pathway plays an important role in astrocytogenesis from NSCs through the regulation gp130 expression (Figure 2).

Epigenetic modifiers of astrocytic genes in NSCs

Although it is clear that activation of the JAK–STAT3 pathway by IL-6 family cytokines induces astrocyte differentiation from NSCs, as described above, early-gestational and mid-gestational-stage NSCs are insensitive to these cytokines in terms of astrocyte differentiation; in other words, astrocyte differentiation is inhibited in NSCs during these developmental stages [21]. This inhibition is known to be achieved by a particular epigenetic modification, DNA methylation. Since the promoters of astrocytic genes such as *gfap* are highly methylated in NSCs at early-gestational and mid-gestational stages, STAT3 binding to these promoters is impeded (Figure 3). As gestation proceeds, astrocytic gene promoters are demethylated and the NSCs then become competent to express these genes in response to astrocyte-inducing cytokines, suggesting that DNA methylation plays an important role in defining the timing of astrocytogenesis from NSCs during development [21,22]. We have demonstrated previously that Notch signaling and its downstream target nuclear factor IA (NFIA) play a crucial role in the demethylation of astrocytic gene promoters [23] (Figure 3). We confirmed that Notch ligands are indeed expressed in committed neuronal precursor cells and young neurons, and that these ligands activate Notch signaling in the residual NSCs. Moreover, forced expression of Notch intracellular domain (NICD), which can mimic Notch signal activation in mid-gestational NSCs, upregulated NFIA expression. This in turn accelerated the demethylation of astrocytic

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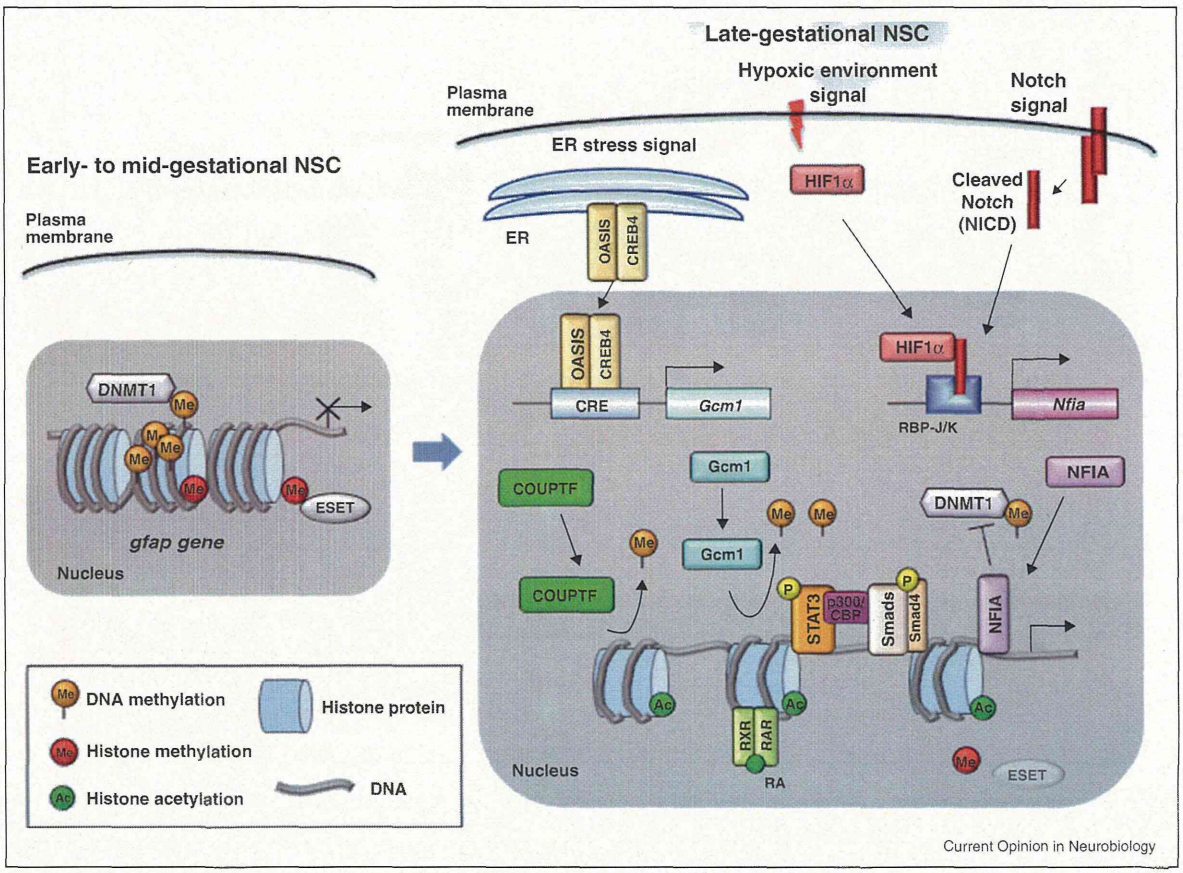
Figure 3



Epigenetic modifiers of astrocytic genes in NSCs. (Left) The *gfap* gene promoter in NSCs is highly methylated at early-gestational and mid-gestational stages, and STAT3 binding to the promoter is thus impeded. ESET also associates with the *gfap* promoter, inducing hypermethylation of H3K9 to repress *gfap* transcription. (Right) When Notch is activated, its intracellular domain NICD is cleaved and then translocated into the nucleus, where it forms a complex with RBP-J/k. This complex activates transcription of *Nfia*, and NFIA in turn accelerates demethylation of the *gfap* promoter by preventing the association of DNMT1. Hypoxia activates HIF1α, which associates with NICD to enhance the transcriptional activity of NICD. COUP-TFI and II also contribute to the demethylation of the *gfap* promoter. The ER stress-transducing protein OASIS induces expression of the transcription factor Gcm1, which may cause active demethylation of *gfap*. Histone H3 acetylation by RA, via its cognate nuclear receptors RAR/RXR, enables STAT3 to gain more efficient access to the *gfap* promoter. ESET is downregulated as gestation proceeds.

gene promoters by preventing their association with DNA methyltransferase 1 (DNMT1), which is essential for the maintenance of methylation patterns on the genome during cell division, and thus allowed precocious astrocyte differentiation of NSCs in response to LIF stimulation [23]. Recently, we have shown that the oxygen sensor hypoxia-inducible factor 1α (HIF1α) is important for astrocytic gene demethylation in developing NSCs by enhancing the activity of the Notch signaling pathway [24•] (Figure 3). It is generally known that embryonic tissues including the brain are hypoxic, and we further showed that astrocyte differentiation of NSCs was impaired when the embryos were incubated in a hyperoxic environment. The oxygen level surrounding NSCs is thus apparently critical for the appropriate scheduling of astrocytogenesis through its capacity to affect epigenetic modification.

The murine homologs of chicken ovalbumin upstream promoter transcription factors I and II (COUP-TFI/II) also contribute to the demethylation of the *gfap* promoter during development. Using a mouse embryonic stem cell (mESC)-derived NSC culture that recapitulates mouse central nervous system development observed *in vivo* [25,26], Naka et al. reported that in *Coup-tfI/II* double-knockdown NSCs, the hypermethylated status of the *gfap* promoter was maintained, and the switch from neurogenesis to astrocytogenesis was thereby inhibited [26].

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Furthermore, knockdown of *Coup-tfI/II* in the developing mouse forebrain also resulted in impaired astrocyte differentiation of NSCs [26]. Although the mechanism remains unknown, these results indicate that COUP-TFI and II are important factors for *gfap* promoter demethylation (Figure 3).

Recently, the unfolded protein response (UPR), which is triggered by unfolded protein accumulation-induced endoplasmic reticulum (ER) stress, has been implicated in astrocyte differentiation during brain development. The ER stress transducer protein Old Astrocyte Specifically Induced Substance (OASIS) plays a key role in astrocytogenesis from NSCs by regulating *gfap* gene promoter methylation [27•] (Figure 3). OASIS belongs to the CREB/ATF family of transcription factors and modulates cell-specific or tissue-specific unfolded protein response signaling [28]. *Oasis* knockout in NSCs resulted in inhibited astrocyte differentiation, attributable to diminished DNA demethylation of the *gfap* promoter. Furthermore, expression of the transcription factor Gcm1, a mammalian homolog of Drosophila GCM [29], which is essential for glial differentiation in Drosophila, was upregulated by OASIS, and Gcm1 overexpression accelerated demethylation of the *gfap* promoter [27•] (Figure 3). Hitoshi et al. showed recently that GCM1 and GCM2 are involved in the demethylation of *Hes5* promoter DNA during the production of early-gestational NSCs from primitive neuroepithelium in mouse embryos [30••]. This report interestingly suggests that, unlike the case of NFIA on the *gfap* promoter, demethylation of the *Hes5* promoter by GCM1 and GCM2 is an active demethylation, which is independent of DNA replication. Thus, the active demethylation of astrocytic genes by GCM1 may also contribute to the acquisition of astrocytogenetic potential by NSCs during development.

Chromatin modification is also important for the regulation of astrocytogenesis from NSCs during development. The best-characterized chromatin modifications are acetylation and methylation of lysine (K) residues of the core histones, H3 and H4. An increase in histone acetylation by histone acetyltransferases causes remodeling of chromatin from a tightly to a loosely packed configuration (euchromatin), which leads to transcriptional activation. Our group suggested that histone H3 acetylation by retinoic acid (RA) via its cognate nuclear receptors, RAR/RXR, on the *gfap* promoter facilitates LIF-induced astrocyte differentiation of NSCs by allowing STAT3 to gain more efficient access to the promoter [31] (Figure 3). In contrast to acetylation, methylation of histones can result in either activation or repression of gene transcription, depending on which residue is methylated [32]. For instance, H3K4 methylation marks transcriptionally active chromatin, whereas methylated H3K9 and H3K27 mark transcriptionally inactive chromatin. The H3K9 methyltransferase ESET (also called Setdb1 or KMT1E) is known to repress gene expression in euchromatin by interacting with the co-repressor KAP1 (Trim28) [33]. Tan et al. [34•] showed that ESET is highly expressed at early stages of mouse brain development, but is downregulated as gestation proceeds. They further suggested that inactivation of *ESET* impaired neurogenesis at early embryonic stages, but accelerated astrocyte production *in vivo*. Tan et al. also noted that ESET binds to the *gfap* promoter to induce H3K9 methylation in wild-type NSCs, whereas in *ESET* mutant NSCs H3K9 is hypomethylated, suggesting that H3K9 methylation is important for the appropriate timing of astrocytogenesis in the embryonic mouse brain [34•] (Figure 3).

Closing remarks

Space constraints prevent us from introducing many other transcriptional factors that participate, directly or indirectly, in astrocyte differentiation of NSCs, notably Olig2 [35], serum response factor [36], RP58 [37••], and LIM-homeodomain transcription factor 2 (Lhx2) [38••]. The findings described here and in previous reports reveal clearly that an exquisite interplay among a great variety of factors — including transcriptional factors, epigenetic modifiers, and the environment surrounding the cells — systematically coordinates the generation of astrocytes from NSCs during brain development. Given the astrocyte's roles in diverse brain functions, the impairment of astrocyte development may contribute to neurological disorders at postnatal and adult stages, such as brain tumors, epilepsy and psychiatric diseases. For example, Alexander disease, which is caused by a mutation in GFAP, is characterized by macrocephaly, abnormal white matter, and developmental delay [39]. It has been reported that GFAP expression is increased in some Alexander disease brains, and that overexpression of wild-type GFAP in mice resulted in the formation of Rosenthal fibers, pathological marks of Alexander disease, that are indistinguishable from those found in afflicted patients [40]. In addition, gene expression profiling of mouse brains has revealed the misexpression of many genes involved in glutathione metabolism, peroxide detoxification and iron homeostasis in neurons [41]. Thus, further progress in the investigation of astrocyte development will contribute greatly to our understanding of human neurological disorders.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S: **Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain**. *J Comp Neurol* 2009, **513**:532-541.

2. Marin-Padilla M: **Prenatal development of fibrous (white matter), protoplasmic (gray matter), and layer I astrocytes in the human cerebral cortex: a Golgi study**. *J Comp Neurol* 1995, **357**:554-572.

3. Tramontin AD, Garcia-Verdugo JM, Lim DA, Alvarez-Buylla A: **Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment**. *Cereb Cortex* 2003, **13**:580-587.

4. Kriegstein A, Alvarez-Buylla A: **The glial nature of embryonic and adult neural stem cells**. *Annu Rev Neurosci* 2009, **32**:149-184.

5. Juliandi B, Abematsu M, Nakashima K: **Epigenetic regulation in neural stem cell differentiation**. *Dev Growth Differ* 2010, **52**:493-504.

6. • Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY: **Local generation of glia is a major astrocyte source in postnatal cortex**. *Nature* 2012, **484**:376-380.

This paper shows that the majority of astrocytes in the postnatal cortex are produced by local proliferation of differentiated cells during the early postnatal period.

7. Ernst M, Jenkins BJ: **Acquiring signalling specificity from the cytokine receptor gp130**. *Trends Genet* 2004, **20**:23-32.

8. Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD, Greenberg ME: **Regulation of gliogenesis in the central nervous system by the JAK–STAT signaling pathway**. *Science* 1997, **278**:477-483.

9. Rajan P, McKay RD: **Multiple routes to astrocytic differentiation in the CNS**. *J Neurosci* 1998, **18**:3620-3629.

10. Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T: **Synergistic signaling in fetal brain by STAT3–Smad1 complex bridged by p300**. *Science* 1999, **284**:479-482.

11. Bugga L, Gadient RA, Kwan K, Stewart CL, Patterson PH: **Analysis of neuronal and glial phenotypes in brains of mice deficient in leukemia inhibitory factor**. *J Neurobiol* 1998, **36**:509-524.

12. Koblar SA, Turnley AM, Classon BJ, Reid KL, Ware CB, Cheema SS, Murphy M, Bartlett PF: **Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor**. *Proc Natl Acad Sci U S A* 1998, **95**:3178-3181.

13. Nakashima K, Wiese S, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Yoshida K, Kishimoto T, Sendtner M, Taga T: **Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation**. *J Neurosci* 1999, **19**:5429-5434.

14. He F, Ge W, Martinowich K, Becker-Catania S, Coskun V, Zhu W, Wu H, Castro D, Guillemot F, Fan G *et al.*: **A positive autoregulatory loop of Jak–STAT signaling controls the onset of astrogliogenesis**. *Nat Neurosci* 2005, **8**:616-625.

15. Barnabe-Heider F, Wasylnka JA, Fernandes KJ, Porsche C, Sendtner M, Kaplan DR, Miller FD: **Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1**. *Neuron* 2005, **48**:253-265.

16. Sun Y, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X, Fan G, Greenberg ME: **Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms**. *Cell* 2001, **104**:365-376.

17. Guillemot F: **Spatial and temporal specification of neural fates by transcription factor codes**. *Development* 2007, **134**:3771-3780.

18. Hirabayashi Y, Suzki N, Tsuboi M, Endo TA, Toyoda T, Shinga J, Koseki H, Vidal M, Gotoh Y: **Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition**. *Neuron* 2009, **63**:600-613.

19. • Li X, Newbern JM, Wu Y, Morgan-Smith M, Zhong J, Charron J, Snider WD: **MEK is a key regulator of gliogenesis in the developing brain**. *Neuron* 2012, **75**:1035-1050.

This study shows that RAF/MEK/ERK pathway plays an important role in astrocytogenesis from NSCs through its regulation of the JAK/STAT3 pathway.

20. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA *et al.*: **A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function**. *J Neurosci* 2008, **28**:264-278.

21. Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, Fujita N, Nakao M, Taga T: **DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain**. *Dev Cell* 2001, **1**:749-758.

22. Namihira M, Nakashima K, Taga T: **Developmental stage dependent regulation of DNA methylation and chromatin modification in a immature astrocyte specific gene promoter**. *FEBS Lett* 2004, **572**:184-188.

23. Namihira M, Kohyama J, Semi K, Sanosaka T, Deneen B, Taga T, Nakashima K: **Committed neuronal precursors confer astrocytic potential on residual neural precursor cells**. *Dev Cell* 2009, **16**:245-255.

24. • Mutoh T, Sanosaka T, Ito K, Nakashima K: **Oxygen levels epigenetically regulate fate switching of neural precursor cells via hypoxia-inducible factor 1alpha-notch signal interaction in the developing brain**. *Stem Cells* 2012, **30**:561-569.

This report indicates that in the environment surrounding NSCs, a low oxygen level is important for astrocytic gene demethylation in developing NSCs by enhancing the activity of the Notch signaling pathway.

25. Okada Y, Matsumoto A, Shimazaki T, Enoki R, Koizumi A, Ishii S, Itoyama Y, Sobue G, Okano H: **Spatiotemporal recapitulation of central nervous system development by murine embryonic stem cell-derived neural stem/progenitor cells**. *Stem Cells* 2008, **26**:3086-3098.

26. Naka H, Nakamura S, Shimazaki T, Okano H: **Requirement for COUP-TFI and II in the temporal specification of neural stem cells in CNS development**. *Nat Neurosci* 2008, **11**:1014-1023.

27. • Saito A, Kanemoto S, Kawasaki N, Asada R, Iwamoto H, Oki M, Miyagi H, Izumi S, Sanosaka T, Nakashima K, Imaizumi K: **Unfolded protein response, activated by OASIS family transcription factors, promotes astrocyte differentiation**. *Nat Commun* 2012, **3**:967.

The authors show that the ER stress transducer protein OASIS plays a key role in astrocytogenesis of NSCs via demethylation of the gfap gene promoter, and that Gcm1, the downstream target of OASIS, may achieve this demethylation.

28. Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, Iseki K, Wanaka A, Imaizumi K: **OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes**. *Nat Cell Biol* 2005, **7**:186-194.

29. Kim J, Jones BW, Zock C, Chen Z, Wang H, Goodman CS, Anderson DJ: **Isolation and characterization of mammalian homologs of the *Drosophila* gene glial cells missing**. *Proc Natl Acad Sci U S A* 1998, **95**:12364-12369.

30. •• Hitoshi S, Ishino Y, Kumar A, Jasmine S, Tanaka KF, Kondo T, Kato S, Hosoya T, Hotta Y, Ikenaka K: **Mammalian Gcm genes induce Hes5 expression by active DNA demethylation and induce neural stem cells**. *Nat Neurosci* 2011, **14**:957-964.

This report shows that Gcm1 and Gcm2 are involved in demethylation of the Hes5 promoter during the production of early-gestational NSCs from primitive neuroepithelium in mouse embryos, and that demethylation of Hes5 promoter by GCM1 and GCM2 is the active demethylation independently of DNA replication.

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31. Asano H, Aonuma M, Sanosaka T, Kohyama J, Namihira M, Nakashima K: **Astrocyte differentiation of neural precursor cells is enhanced by retinoic acid through a change in epigenetic modification**. *Stem Cells* 2009, **27**:2744-2752.

32. Yoo CB, Jones PA: **Epigenetic therapy of cancer: past, present and future**. *Nat Rev Drug Discov* 2006, **5**:37-50.

33. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd: **SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins**. *Genes Dev* 2002, **16**:919-932.

34. • Tan SL, Nishi M, Ohtsuka T, Matsui T, Takemoto K, Kamio-Miura A, Aburatani H, Shinkai Y, Kageyama R: **Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development**. *Development* 2012, **139**:3806-3816.

This study indicates that H3K9 methylation by ESET is important for the appropriate timing of astrocytogenesis in the embryonic mouse brain.

35. Cai J, Chen Y, Cai WH, Hurlock EC, Wu H, Kernie SG, Parada LF, Lu QR: **A crucial role for Olig2 in white matter astrocyte development**. *Development* 2007, **134**:1887-1899.

36. Lu PP, Ramanan N: **A critical cell-intrinsic role for serum response factor in glial specification in the CNS**. *J Neurosci* 2012, **32**:8012-8023.

37. •• Hirai S, Miwa A, Ohtaka-Maruyama C, Kasai M, Okabe S, Hata Y, Okado H: **RP58 controls neuron and astrocyte differentiation by downregulating the expression of Id1–4 genes in the developing cortex**. *EMBO J* 2012, **31**:1190-1202.

This report indicates that the transcription factor RP58 downregulates all four Id genes in the developing cerebral cortex. RP58 KO mice display enhanced astrocytogenesis, and the phenotypes of their NSCs were mimicked by the overexpression of all Id genes in wild-type cortical progenitors. These observations implicate RP58 as a novel key regulator that controls the differentiation of NSCs and restricts astrocytogenesis by repressing Id genes during corticogenesis.

38. •• Subramanian L, Sarkar A, Shetty AS, Muralidharan B, Padmanabhan H, Piper M, Monuki ES, Bach I, Gronostajski RM, Richards LJ *et al.*: **Transcription factor Lhx2 is necessary and sufficient to suppress astrogliogenesis and promote neurogenesis in the developing hippocampus**. *Proc Natl Acad Sci U S A* 2011, **108**:E265-E274.

Subramanian et al. show that Lhx2 KO in the embryonic hippocampus by in utero electroporation, and in organotypic slice culture, caused the premature production of astrocytes at stages when neurons are normally generated. Furthermore, Lhx2 overexpression was able to override and suppress the activation of the gfap promoter by NFIA. However, this activity of Lhx2 is spatially restricted to the hippocampus: loss of Lhx2 function in the neocortex did not result in premature astrogliogenesis. This study therefore suggests that Lhx2 plays an important role in the neuron–glia cell fate decision in the hippocampus.

39. Quinlan RA, Brenner M, Goldman JE, Messing A: **GFAP and its role in Alexander disease**. *Exp Cell Res* 2007, **313**:2077-2087.

40. Messing A, Head MW, Galles K, Galbreath EJ, Goldman JE, Brenner M: **Fatal encephalopathy with astrocyte inclusions in GFAP transgenic mice**. *Am J Pathol* 1998, **152**:391-398.

41. Hagemann TL, Gaeta SA, Smith MA, Johnson DA, Johnson JA, Messing A: **Gene expression analysis in mice with elevated glial fibrillary acidic protein and Rosenthal fibers reveals a stress response followed by glial activation and neuronal dysfunction**. *Hum Mol Genet* 2005, **14**:2443-2458.

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Bidirectional promoters are the major source of gene activation-associated non-coding RNAs in mammals

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Abstract

Background: The majority of non-coding RNAs (ncRNAs) involved in mRNA metabolism in mammals have been believed to downregulate the corresponding mRNA expression level in a pre- or post-transcriptional manner by forming short or long ncRNA-mRNA duplex structures. Information on non-duplex-forming long ncRNAs is now also rapidly accumulating. To examine the directional properties of transcription at the whole-genome level, we performed directional RNA-seq analysis of mouse and chimpanzee tissue samples.

Results: We found that there is only about 1% of the genome where both the top and bottom strands are utilized for transcription, suggesting that RNA-RNA duplexes are not abundantly formed. Focusing on transcription start sites (TSSs) of protein-coding genes revealed that a significant fraction of them contain switching-points that separate antisense- and sense-biased transcription, suggesting that head-to-head transcription is more prevalent than previously thought. More than 90% of head-to-head type promoters contain CpG islands. Moreover, CCG and CGG repeats are significantly enriched in the upstream regions and downstream regions, respectively, of TSSs located in head-to-head type promoters. Genes with tissue-specific promoter-associated ncRNAs (pancRNAs) show a positive correlation between the expression of their pancRNA and mRNA, which is in accord with the proposed role of pancRNA in facultative gene activation, whereas genes with constitutive expression generally lack pancRNAs.

Conclusions: We propose that single-stranded ncRNA resulting from head-to-head transcription at GC-rich sequences regulates tissue-specific gene expression.

Keywords: Bidirectional promoter, Non-coding RNA, CpG island, Directional RNA-Seq, Gene activation

Background

Protein-coding regions account for only about 1.5% of the human genome [1], but the FANTOM Consortium and the ENCODE Project Consortium revealed that more than 62% of the genomic DNA acts as a template for transcription [2,3], indicating that there are a large number of non-coding RNAs (ncRNAs) in living cells. Recently, many functional ncRNAs have been identified. It is well known that small RNAs, such as miRNAs and piRNAs, act in post-transcriptional regulation by forming RNA-RNA duplexes [4,5]. In addition to these RNAs, many kinds of long ncRNAs have been shown to function in post-transcriptional regulation, such as RNA editing, splicing and translation, by forming RNA-RNA duplexes [6-13]. Indeed, 4,520 sense-antisense transcript (SAT) pairs in mice have the potential to form RNA-RNA duplexes [14]. RNA-RNA duplexes also play a role in transcriptional gene silencing through DNA methylation and histone modifications [15-18]. Thus, it is clear that the formation of RNA-RNA duplexes is important for the mRNA silencing triggered by ncRNA.

However, several studies have reported that some long ncRNAs cause transcriptional activation of genes without

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