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REVIEW

A star is born: new insights into the mechanism of astrogenesis

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Abstract Astrocytes emerge as crucial cells for proper neuronal functioning in the developing and adult brain. Neurons and astrocytes are sequentially generated from the same pool of neural stem cells (NSCs). Tight regulation of the neuron-to-astrocyte switch is critical for (1) the generation of a balanced number of astrocytes and neurons and (2) neuronal circuit formation, since newborn astrocytes regulate synapse formation. This review focuses on signaling pathways that instruct astrogenesis, incorporating recently discovered intrinsic and extrinsic regulators. The canonical pathway of astrocytic gene expression, JAK/STAT signaling, is inhibited during neurogenesis to prevent premature astrocyte differentiation. At the onset of astrogenesis, Notch signaling induces epigenetic remodeling of astrocytic genes like glial fibrillary acidic protein to change NSC competence. In turn, astrogenesis is initiated by signals received from newborn neurons. We highlight how key molecular pathways like JAK/STAT and Notch are integrated in a complex network of environmental signals and epigenetic and transcriptional regulators to determine NSC differentiation. It is essential to understand NSC differentiation in respect to future NSC-based therapies for brain diseases, as transplanted NSCs preferentially become astrocytes. As emphasized in this review, many clues in this respect can be learned from development.

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Introduction

Astrocytes emerge as crucial cells for proper neuronal functioning in the developing and adult brain. Astrocytes are important regulators of neuronal function, including synaptic transmission and information processing by neural circuits. Moreover, astrocytes maintain central nervous system homeostasis by regulation of ion, pH, and neurotransmitter metabolism as well as blood flow [1]. During development, neurons and astrocytes are generated from neural stem cells (NSCs), the so-called radial glia. Radial glial cells are multipotent and represent the majority of progenitor cells in the developing brain. Early in development, radial glia produce intermediate progenitor cells which, in turn, produce neurons. At the onset of astrogenesis, radial glia directly differentiate into astrocytes or generate intermediate progenitors. Newborn astrocytes are crucial for neuronal development since they regulate the formation of developing synapses [2] and regulate synaptic connectivity [3]. A tight regulation of the switch from a neuronal to an astrocytic NSC potential is critical for normal brain formation. During the neurogenic phase, proneural basic helix-loop-helix (bHLH) transcription factors, such as Neurogenin 1 (Ngn1), drive neurogenesis and simultaneously prevent the generation of astrocytes. Astrogenesis is mainly repressed by inactivation of the JAK/STAT pathway, the key signaling pathway promoting astrocyte

differentiation. Notch activity, which closely interacts with the JAK/STAT pathway, is critical to drive NSC differentiation into an astrocytic fate by demethylation of astrocytic genes. Upon the release of astrocytic repressors, gliogenic cytokines secreted by newborn neurons activate the JAK receptor. STAT3 is phosphorylated and, in turn, is activated. Active STAT3 binds together with the co-activator complex p300/CBP to the promoter of astrocytic signature genes, such as glial fibrillary acidic protein (GFAP). Activation of astrocytic gene expression initiates astrocyte differentiation [4]. This review discusses the current concept of the developmental neuron-to-astrocyte fate switch. Specifically, it highlights recent findings that demonstrate a complex interplay of different molecular pathways controlling the onset of astrogenesis. The majority of the reviewed literature focuses on the development of the cortex if not stated otherwise in the text. Unraveling the regulatory pathways that control astrocyte differentiation is of crucial importance in developing novel approaches to selectively differentiate NSCs into astrocytes or in preventing astrocyte differentiation in the diseased brain. As emphasized in this review, many clues in this respect can be learned from development.

We will discuss the mechanisms that inhibit astrogenesis ("Brakes of astrogenesis"), how the brakes of astrogenesis are released ("Releasing the brakes of astrogenesis: changes in NSC competence"), and the initiation of astrocytic differentiation by Notch signaling ("Notch signaling, a master regulator of astrogenesis") and newborn neurons ("Newborn neurons trigger the initiation of astrogenesis").

Brakes of astrogenesis

Inhibition of JAK/STAT signaling prevents premature astrogenesis

Inhibition of astrogenesis during the neurogenic phase is essential for the generation of a sufficient amount of neurons necessary to build all neuronal layers and connections in the brain. To this end, silencing of the JAK/STAT pathway, the canonical pathway regulating astrocytic gene expression, is critical, since gliogenic signals are already present early in the embryonic brain [5, 6]. Upon activation of JAK receptors, phosphorylation and acetylation activates STAT3, which then dimerizes and translocates to the nucleus to activate gene transcription. STAT3 activity is of crucial importance for astrogenesis to occur [5]. Therefore, current research focuses on the mechanism of how JAK/STAT signaling is inhibited at the level of STAT3.

During neurogenesis, sequestration of the p300/CBP co-activator complex from STAT3 results in inhibition of STAT3-dependent transcription in NSCs. p300/CBP has an intrinsic acetyltransferase activity. Dissociation of p300/CBP might reduce acetylation at the STAT3 binding site of the GFAP promoter [7]. The pro-neuronal factor Ngn1 binds the p300/CBP complex, preventing its interaction with STAT3 [8]. Hence, Ngn1 suppresses astrogenesis by inhibition of the JAK/STAT pathway [9], and in parallel promotes neuronal differentiation by activating the expression of neuronal genes [8]. Other proneural bHLH transcription factors such as Ngn2, NeuroD1, and Mash1 are essential for neuronal differentiation as well. Loss of each of these bHLH factors induces astrocyte differentiation [10, 11]. Therefore, hypothetically, Ngn2, NeuroD1, and Mash1 could also inhibit astrocyte differentiation by sequestering p300/CBF.

In addition, JAK/STAT signaling is inhibited by methylation of STAT3 binding sites in the promoters of astrocytic genes. Methylation results in condensation of the chromatin, which hinders binding of the transcription factor STAT3 at the promoter of the astrocytic genes GFAP and S100B [12]. DNA methyltransferase I (DNMT1) is a potential candidate for methylation of astrocytic genes during neurogenesis. Indeed, conditional deletion of DNMT1 in NSCs induces the switch from neurogenesis to astrogenesis [13]. DNA methylation at the STAT3 binding site of the promoter is likely to recruit methyl CpG binding proteins such as methyl CpG binding protein 2 (MeCP2), which has been shown to bind to promoters of glial genes in early precursors [13, 14]. Some first evidence from human embryonic carcinoma cells indicates that MeCP2 forms a repressor complex together with the SIN3 transcription regulator homolog A (Sin3A) at the methylated STAT3 binding site of the GFAP promoter [7]. MeCP2-Sin3A is a known repressor complex which condenses the chromatin and thereby represses gene expression at numerous gene promoters [15].

Interestingly, upon JAK/STAT activation in non-neuronal cells, SIN3A interacts with STAT3 to promote de-acetylation of the STAT3 protein. De-acetylation of STAT3 is associated with low STAT3 activity [16]. Similarly, Sin3A could be involved in the inhibition of glial genes in two ways: by epigenetic silencing of STAT3 binding sites in the GFAP promoter and by suppression of basal STAT3 activity. Further experimental proof for SIN3A-dependent silencing of STAT3 action in NSCs is necessary to define the role of the repressor complex of SIN3A and MeCP2 in suppressing JAK/STAT signaling.

The importance of silencing STAT3 activity to inhibit glial gene expression is emphasized by the result of overexpression of a repressor of STAT3 expression. Such a repressor is a suppressor of cytokine signaling 3 (Socs3), which inhibits astrogliogenesis and increases neurogenesis [17]. Intriguingly, STAT3 silencing might actively promote neurogenesis through induction of the proneuronal transcription

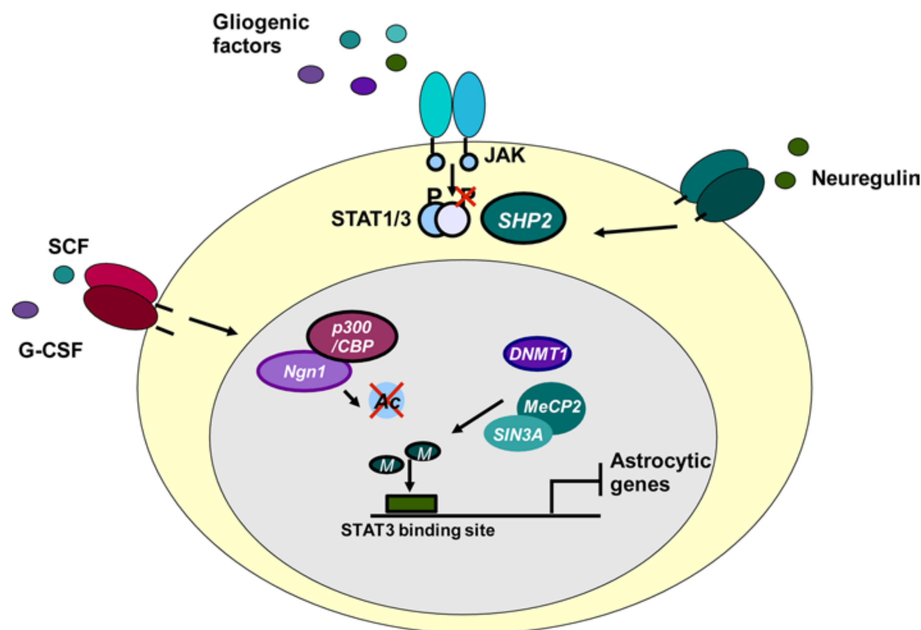

Fig. 1 Silencing of astrocytic JAK/STAT signaling during neurogenesis. Inhibition of astrogenesis early in development is critical for the generation of a sufficient amount of neurons. Multiple mechanisms repress the JAK/STAT pathway, the canonical pathway of astrocytic gene expression since activation of astrocytic gene promoters by the STAT3 transcription factor is crucial for astrocyte differentiation. The phosphatase SHP2, which is regulated by neuregulin, de-phosphorylates, and in turn inhibits STAT3. Moreover, sequestering of the co-activator complex p300/CBP by the neurogenic bHLH factor Ngn1 blocks acetylation of STAT3 binding sites (Ac). The hematopoietic growth factors SCF and G-CSF were shown to induce Ngn1 expression. Finally, methylation (M) of STAT3 binding sites silences astrocytic gene promoters. The DNA methyltransferase DNMT1 and the repressor complex of MeCP2-Sin3A induce methylation and in turn condensation of the chromatin

factors Math1, Ngn3, and NeuroD [18]. Consistently, conditional deletion of STAT3 stimulates differentiation of NSCs into neurons and prevents astrogenesis [18, 19].

Taken together, the JAK/STAT pathway is a key regulator of astrogenesis. During neurogenesis, several mechanisms inhibit STAT3 activity and in turn astrocytic JAK/STAT signaling to prevent astrogenesis. Firstly, the co-activator complex p300/CBP is sequestered by the proneural transcription factor Ngn1, leading to prevention of STAT3-dependent transcription and reduced acetylation at the STAT3 binding site of the GFAP promoter. Secondly, methylation of STAT3 binding sites in astrocytic gene promoters condenses the chromatin and inhibits binding of STAT3. Finally, STAT3 may be inactivated by de-acetylation of the STAT3 protein which may, in addition to preventing astrogenesis, induce neurogenesis by induction of proneural gene expression (see Fig. 1).

What triggers inhibition of the JAK/STAT pathway?

Extrinsic cues that can induce inhibition of JAK/STAT activity are neurotrophins. Neurotrophins, such as brain-derived neurotrophic factor (BDNF), activate the neurogenic SHP2-Ras-MEK-ERK pathway to promote neurogenesis [20]. SHP2, a phosphatase modulating the MEK-ERK pathway [21], is an important regulator of the neuron-to-astrocyte switch as well. Knockdown of SHPs in NSCs in vivo stimulated premature astroglial differentiation during the late neurogenic period [22]. SHP2 possesses a dual role during early development. SHP2 expression triggers pro-neural MEK-ERK signaling, and in addition, inhibits the astrocytic JAK/STAT pathway. In non-neural cells, it was shown that SHP2 dephosphorylates STAT3 to inhibit JAK/STAT activity [23]. Consistently in NSCs, absence of SHP2 increases phosphoSTAT3 levels [22]. Although neurotrophins inhibit JAK/STAT signaling during early development by dephosphorylating STAT3, the SHP2-Ras-MEK-ERK pathway may repress STAT3 activity in additional ways. MEK was shown to induce selective promoter methylation by DNA methyltransferase 3 in non-neural cells [24]. Thus, SHP2-Ras-MEK-ERK activity could inhibit STAT3-dependent transcription by methylation of astrocyte genes as well. To date, no specific activator of the SHP2-Ras-MEK-ERK pathway is identified which induces the inhibition of astrogenesis. BDNF is a potential candidate since it is (1) expressed before the astrocytic phase [25] and (2) an important regulator of NSC differentiation during early development [26].

Another inhibitor of JAK/STAT activity is Reelin signaling. Reelin signals through the cytoplasmic adapter

protein Disabled 1 (Dab1). Reelin-Dab1 activity is crucial for diverse events during development, such as neuronal migration along the radial glial fiber, dendrite formation, synaptic plasticity, and radial glial morphology [27]. Dab1 suppresses astroglial differentiation through induction of the proneural bHLH factor NeuroD [28]. NeuroD, in concert with other proneuronal bHLH factors, is an inhibitor of the JAK/STAT pathway [9]. Upon deletion of Dab1 in NSCs, NeuroD expression decreases, which in turn enhances STAT phosphorylation [28]. The effect of Reelin itself is still under debate. In isolated NSCs in culture, Reelin has no effect on astrogenesis, but loss of Reelin in vivo indeed results in an increased number of astrocytes in the mouse hippocampus [29].

There are also indications that hematopoietic growth factors inhibit astrogenesis. Stem cell factor (SCF) and granulocyte colony stimulating factor (G-CSF) block astrogenesis during early development. The receptors for SCF and G-CSF are expressed in the embryonic ventricular zone and in primary NSCs in vitro. In culture, SCF and G-CSF increase the pro-neural bHLH gene Ngn1 [30]. Ngn1 inhibits the JAK/STAT pathway by sequestering the co-activator complex p300/CBP [8] and hence, suppresses astrogenesis (see Fig. 1). Erythropoietin, another hematopoietic growth factor, promotes neuronal differentiation via Ngn1 expression in adult NSCs [31]. So far, the role of erythropoietin in astrogenesis has not been addressed. High expression levels of the erythropoietin receptor in neurogenic regions during development indicate that it has a role in embryonic NSC regulation [32].

JAK/STAT independent brakes of astrogenesis

Inhibitors of astrogenesis have been identified which act independently of JAK/STAT silencing. ErbB4-NCoR signaling is crucial to prevent premature astrogenesis in vivo. ErbB4 is a tyrosine kinase receptor of the EGF receptor family, which forms a repressor complex together with NcoR. Upon activation of the receptor, the intracellular domain of the ErbB4 receptor translocates together with NcoR into the nucleus to inactivate GFAP expression [33]. Neuregulin secretion by neurons activates ErbB4-NCoR signaling. Hence, newborn neurons might contribute to the inhibition of astrogenesis. At the onset of astrogenesis, it is thought that downregulation of ErbB4 releases the brake and allows for the generation of astrocytes [34]. How silencing of ErbB4 is regulated is still unclear. Neuregulins have diverse functions in the embryonic brain such as regulating neuronal migration, axonal guidance, and synapse formation [35]. Thus, it needs to be determined how these different biological effects of neuregulin signaling are regulated at distinct stages of development. The pathways identified to prevent astrogenesis are depicted in Fig. 1 and Table 1.

Releasing the brakes of astrogenesis: changes in NSC competence

Epigenetic remodeling of the GFAP promoter

At the onset of astrogenesis, NSC competence changes from a neurogenic to an astrocytic potential, which is marked by the activation of astrocytic signature genes such as GFAP. The chromatin state of the GFAP promoter changes significantly in order to allow active gene transcription. DNA methylation, as well as histone methylation and acetylation, play a crucial role in the epigenetic regulation of GFAP transcription. For the majority of genes, hallmarks of active gene transcription are high levels of trimethylation of histone3-lysine 4 (H3K4), whereas a repressed chromatin state is marked by DNA and H3K9 and H3K27 methylation. In addition, H3K9 and H3K14 acetylation are associated with an open chromatin state [36]. Several factors were identified, which induce opening of the chromatin at the GFAP promoter (see Fig. 2).

The JAK/STAT pathway component p300/CBP has an intrinsic acetyltransferase activity. It is suggested that binding of p300/CBP triggers its own activity and induces H3K9 and H3K14 acetylation at the STAT3 binding site of the GFAP promoter [7]. The availability of p300/CBP is regulated by Ngn1. Before the onset of astrogenesis, p300/CBP is sequestered by Ngn1 to inhibit binding of the complex to the GFAP promoter (see Fig. 1). During astrogenesis, Polycomb group (PcG) proteins silence Ngn1 expression [37]. PcG proteins are an important epigenetic regulation system in the cell [38].

PcG proteins induce silencing of the Ngn1 promoter by reducing histone acetylation and increasing H3K27 methylation [37]. Ngn1 downregulation releases p300/CBP. In turn, p300/CBP forms a co-activator complex with STAT3 at the GFAP promoter to activate its expression. Consistently, loss of PcG proteins prolongs Ngn1 expression, represses astrocytic gene expression, and in turn delays the astrocytic phase of NSCs in vivo [37]. Acetylation via p300/CBP was shown in non-neuronal cells to facilitate STAT3 dimerization and STAT3-dependent gene transcription [39]. The exact mechanism how p300/CBP activates GFAP expression is still elusive.

In addition to acetylation, the methylation status of the GFAP promoter is also crucial for astrocyte differentiation [12]. DNA methylation condenses the chromatin and hinders binding of transcriptional activators such as STAT3. The astrocytic transcription factor nuclear factor 1 A (NF1A) plays a central role in the demethylation of the GFAP promoter. NF1A expression is upregulated during astrogenesis [40]. Binding of NF1A to the GFAP promoter [41] induces the dissociation of the methyltransferase DNMT1 from the GFAP promoter. Since no enzyme has been identified which

Table 1 Regulators of astrogenesis

Gene (References)	Manipulation and effect
Suppressors of astrogenesis	
SCF, G-CSF [30]	Treatment of rat NSCs (isolated at E18) promotes neuronal differentiation and inhibits astroglial differentiation (GFAP-positive cells)
Neurogenic bHLH Ngn1 [8]	Overexpression of Ngn1 in rat NSCs (isolated at E14) using retrovirus or adenovirus promotes neurogenesis and decreases GFAP expression
DNMT1 [13]	Conditional deletion of DNMT1 in NSCs (using Nestin-Cre) results in premature GFAP-positive cells in the cortex by E18
ErbB4-NCoR [33]	Depletion of ErbB4 results in premature GFAP-negative, S100B-positive cells in the cortex by E17.5
Fbxw7 [68]	Conditional deletion of Fbxw7 in NSCs (using Nestin-Cre) results in an increased number of GFAP-positive cells in primary cerebral cultures isolated at P0.5
Lhx2 [61]	Conditional deletion of Lhx2 using in utero electroporation at E15 to express Cre recombinase in the hippocampus results in aberrant GFAP-positive cells 7 days later
	Overexpression of Lhx2 (using in utero electroporation at E15 or E17) reduced the GFAP-positive cells 7 days later
Emx [62]	Overexpression of Emx in NSCs (isolated at E11.5) promotes neurogenesis and reduces the number of S100B-positive cells
Socs3 [17]	Overexpression of Socs3 using adenovirus in rat NSCs (isolated day 17–18) reduces the amount of GFAP-positive cells
SHP2 [22]	shRNA-mediated knockdown of SHP2 in the cortex at E13/14 induces premature generation of GFAP-positive astrocytes at E16/17
Activators of astrogenesis	
LIF [9, 71]	Treatment of primary mouse NSCs (isolated at E11) promotes astrocyte differentiation as measured by GFAP and S100B-positive cells [9]
	Depletion of LIF results in no changes of astrocyte numbers (measured by GFAP) at P3 [71]
CNTF [71, 74]	Treatment of primary mouse NSCs (isolated at E14 or E17) with CNTF increases the number of GFAP-positive cells [74]
	Overexpression of CNTF (using in utero electroporation at E13.5 or E15) results in ectopic S100B and GFAP-positive cells 3 days later [71]
CT-1 [71]	NSCs isolated at E13 from CT-1 knockout mice display decreased GFAP expression after 6 days in culture
BMPs [77, 79]	Treatment of primary mouse NSCs (isolated at E18.5) with BMP4 promotes GFAP expression and BMP4 overexpressing animals display increased numbers of GFAP-positive cells [77]. Exposure of murine NSCs (isolated at E17) to BMP 2, 4, 5, or 6 increases GFAP expression and enhances morphological complexity [79]
Tgf-β1 [86]	Treatment of primary mouse NSCs (isolated at E14) with Tgf-β1 enhances the number of GFAP-positive cells
RA [53]	Treatment of primary mouse NSCs (isolated at E14.5) with RA together with LIF enhances GFAP expression
Notch [42]	Overexpression of NICD using a retrovirus in mouse NSCs (isolated at E11.5) increases the amount of GFAP-positive cells
STAT3 [18, 19]	Conditional depletion of STAT3 using in vitro expression of Cre recombinase in mouse NSC (isolated at E14–15) promotes neurogenesis and reduces GFAP expression [19]
	Expression of a dominant negative form of STAT3 in rat NSCs (isolated at day 17–18) reduces GFAP expression 3 days later [18]
NF1A [42–44]	Overexpression of NF1A using a retrovirus in mouse NSCs (isolated at E11.5) increases the amount of GFAP-positive cells [42]
	Depletion of NF1A severely reduces GFAP expression in midline glia at E17 [44] and the cortex and hippocampus of 3-month-old mice [43]
Oasis [70]	Depletion of OASIS results in reduced GFAP and S100B-positive cells in the cortex of at E16.5

actively demethylates the promoter, passive loss of methylation via release of DNMT1 was suggested as the main mechanism for demethylation of the GFAP promoter [42]. In NF1A knockout mice, methylation is preserved and GFAP expression is severely reduced in cortical [43, 44] and midline glia populations [45]. NF1B knockout mice display similar defects in GFAP expression, suggesting that NF1B might also be involved in demethylation of GFAP [44].

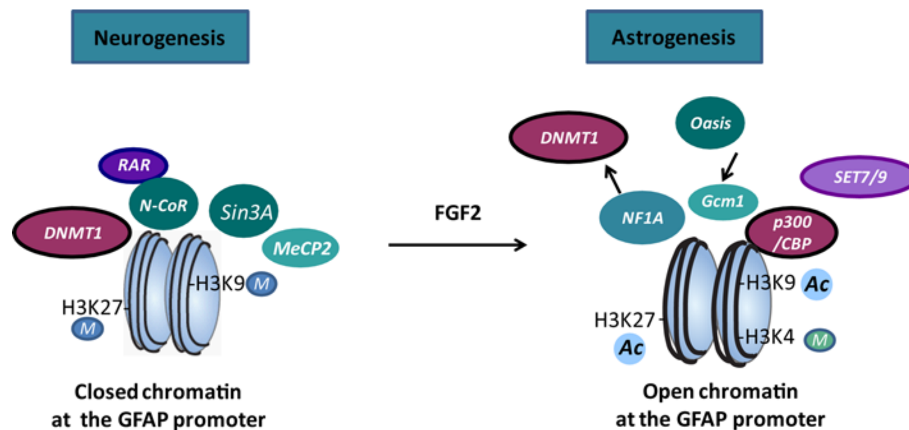

Fig. 2 Epigenetic remodeling of the GFAP promoter. During the neurogenic phase, the astrocytic gene GFAP is epigenetically silenced by methylation (M) induced by the DNA methyltransferase I (DNMT1). The repressor complex of MeCP2-Sin3A as well as of the RA receptor (RAR)N-CoR might be involved in condensation of the chromatin. At the onset of astrogenesis, the methylation status of the GFAP promoter significantly changes. Binding of the Notch downstream target NF1A induces the release of DNMT1. An additional trigger for demethylation is OASIS-Gcm1 signaling. Histone acetylation by p300/CBP potentiates opening of the chromatin. FGF2 signaling acts as external stimulus to remodel the methylation pattern of histones possibly via the methyltransferases SET7/9

What triggers chromatin remodeling at the GFAP promoter?

A key signal for opening the chromatin state at the GFAP promoter is fibroblast growth factor 2 (FGF2). Song and Ghosh demonstrated that FGF2 suppresses H3K9 methylation at the STAT3 binding site and promotes H3K4 hypermethylation. In cultured cortical NSCs, FGF2-mediated chromatin remodeling itself does not initiate astrogenesis but requires additional astrocytic stimuli, such as the gliogenic cytokine ciliary neurotrophic factor (CNTF), to induce GFAP expression [46]. In vivo, lack of FGF2 reduces GFAP expression in the grey matter, and specifically in this region, H3K4 methylation at the STAT3 binding site of the GFAP promoter [47]. The regulatory pathways downstream of FGF2 signaling are still under investigation. FGF2 signaling might target methyltransferases to regulate K9 versus K4 methylation [46]. A possible candidate is the methyltransferase SET7/9. First, SET7/9 is shown to regulate methylation of H3K4 [48] and second, overexpression of SET7/9 potentiates astrogenesis induced by FGF2 [46]. Additional enzymes regulating epigenetic changes downstream of FGF2 signaling are still elusive. Taken together, FGF2 sets an example of how extrinsic factors of the neurogenic niche control the developmental competence of NSCs via the methylation status of the GFAP promoter.

An additional extrinsic stimulus that controls the epigenetic state of the GFAP promoter is retinoic acid (RA). Reporter mice for RA signaling demonstrate activity early in the developing brain [49–51]. In the absence of RA, the RA receptor (RAR) forms a repressor complex with N-CoR to induce deacetylation of gene promoters by recruitment of histone deacetylases (HDACs). Induction of RA signaling during astrogenesis might induce the replacement of HDACs with histone acetylases at the GFAP promoter [52]. Indeed, RA promotes acetylation of H3 around STAT3 responsive elements of the GFAP promoter in vitro. In turn, relaxation of the chromatin might facilitate STAT3 binding and initiate GFAP transcription. Consistent with this hypothesis, RA potentiates the differentiation of NSCs into astrocytes [53].

An additional step in the epigenetic remodeling of the GFAP promoter could be the release of the repressor complex SIN3A-MeCP2. First evidence indicates that SIN3A-MeCP2 condenses the chromatin at the GFAP promoter during neurogenesis (see Fig. 1). This complex is released from the GFAP promoter at the onset of astrogenesis [7]. Dissociation of SIN3A-MeCP2 might potentiate the relaxation of the chromatin to enhance binding of the astrocytic transcription factor STAT3. The identity of histone modifying enzymes that are regulated upon the release of SIN3A-MeCP2 is still elusive. The regulators identified to remodel the GFAP promoter are depicted in Fig. 2.

The complex epigenetic regulation of GFAP transcription demonstrates that the activity of histone-modifying enzymes is tightly regulated during the course of development. Histone-modifying enzymes are widely expressed in different cell types and regulate the transcription of hundreds of genes simultaneously. Nevertheless, modification of single histone marks requires local activity of enzymes. Moreover, the epigenetic state needs to be regulated differentially at distinct developmental stages. Future research is necessary to understand the synchronized action of multiple histone-modifying enzymes at different genomic loci under a tight temporal

control. This goal demands isolation of distinct cell populations at different developmental stages to be able to analyze the epigenetic status of these cells. Hence, improvement of isolation techniques using cell type-specific surface markers is of crucial importance [54]. Although GFAP is an important astrocyte signature gene, the focus of the current literature solely on changes in the GFAP promoter introduces a bias in the analysis of regulatory mechanisms controlling astrogenesis. Investigation of additional promoters, such as S100B or GLT1, is necessary to complete our insight into the initiation of astrocytic gene expression.

In summary, recent studies identified a distinct histone code at active GFAP promoters during astrogenesis. Increased acetylation and decreased methylation triggers the relaxation of the chromatin around STAT3 binding sites of the GFAP promoter. In turn, binding of the transcriptional activator STAT3 initiates GFAP transcription. Extrinsic signals like FGF2 and RA orchestrate epigenetic changes to elicit astrocytic gene expression at the onset of astrogenesis.

Notch signaling, a master regulator of astrogenesis

Notch signaling is a key regulator of the NSC fate during development. The role of Notch activity is studied extensively using knockout mice, overexpression studies, or pharmacological inactivation of the pathway. The canonical signaling pathway in mammals is activated by the Notch ligands Jagged and Delta-like proteins. Upon ligand binding, Notch is cleaved by the γ-secretase complex containing presenilin1, releasing the notch intracellular domain (NICD). NICD translocates into the nucleus, where it regulates transcription of Notch target genes via DNA binding proteins such as the c-promoter binding factor 1 (CSL) [55]. Notch signaling fulfils different roles during the course of development. During the neurogenic phase, Notch is critical for the maintenance of the NSC pool via repression of neurogenesis. The Notch transcriptional downstream targets hairy enhancer of split (Hes), Hes1 and Hes5, are crucial inhibitors of neuronal differentiation [56]. When development proceeds, newborn neurons are thought to stimulate Notch signaling in NSCs by expression of Notch ligands such as Jagged 1 and Delta-like 1 [42]. At this stage, Notch activation is necessary and sufficient to induce astrocyte differentiation. Expression of NICD in mouse embryonic NSCs results in demethylation of the GFAP promoter and, in turn, activation of GFAP expression. Notch signaling induces the epigenetic remodeling of the GFAP promoter through its downstream target NF1A [42]. As mentioned previously, NF1A is a crucial transcription factor that induces the release of DNMT1 from the GFAP promoter, thereby allowing for astrocytic gene expression (see Fig. 2). So far, it is not known whether NF1A simultaneously activates additional astrocytic gene promoters or if it exerts its effect exclusively on GFAP. Moreover, how the Notch response switches from being self-renewing to astrogenic during the course of development is to date still elusive.

Notch signaling is a major point of convergence between different regulators of the neuron-to-astrocyte switch. JAK/STAT and Notch signaling act synergistically to promote astrogenesis (see Fig. 3). Their interaction is mainly

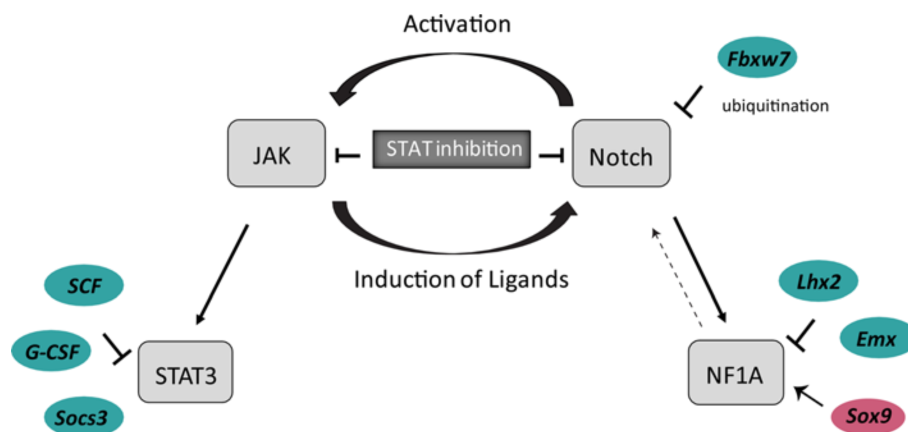

Fig. 3 Synergistic effect of JAK/STAT and Notch signaling at the onset of astrogenesis. A positive feedback loop of the key astrocytic signaling pathways JAK/STAT and Notch stimulates astrogenesis. STAT3 induces Notch ligands which, in turn, activate Notch signaling. Notch activity induces phosphorylation of STAT3 to activate JAK/STAT signaling. Inhibitors of the JAK/STAT pathway, like Socs3 and the growth factors SCF and G-CSF also repress Notch signaling. Notch activity is regulated by (1) Fbxw, which marks the Notch1 receptor for degradation by the proteasome, (2) its downstream target NF1A, which itself inhibits Notch signaling. Multiple transcription factors (Lhx2, Sox9, Emx) control NF1A expression and in turn the onset of astrogenesis. Inhibitory factors are labeled in *green* and activating factors in *red*

established by STAT3. STAT3 induces the Notch ligand DLL1, which in turn activates Notch signaling in adjacent cells. So far, it is still elusive whether neurons or astrocytes are the predominant cell type responsible for the non-cell-autonomous interplay of JAK/STAT and Notch signaling [57]. In agreement with this finding, an inhibitor of STAT3 transcription, Socs3, suppresses Hes5 expression [17]. Moreover, dominant-negative STAT3 blocks astrocyte differentiation induced by the Notch pathway via reduction of Notch1, Hes1, and Hes5 expression [18, 58]. Finally, the growth factors SCF and G-CSF, which repress JAK/STAT activity (see Fig. 1), decrease expression of Notch 1 and Hes1 [30]. Hes1 itself induces phosphorylation of STAT3 to activate JAK/STAT signaling. To this end, Hes1 directly binds STAT3 in the nucleus, facilitating its interaction with the kinase JAK2. Recruitment of JAK2 induces phosphorylation and, in turn, activation of STAT3 [58]. Hence, JAK/STAT and Notch signaling establish a positive feedback loop to initiate astroglial differentiation (see Fig. 3).

Since Notch signaling is a very potent stimulus for astrocyte differentiation, its activity needs to be tightly regulated. The Notch target NF1A plays a central role in controlling Notch activity. NF1A itself limits Notch activity by repressing Hes1 [59]. This negative feedback loop restrains Notch signaling and prevents excessive generation of astrocytes. Recently, the lim-homeodomain transcription factor Lhx2 was found to inhibit Notch signaling through NF1A. Lhx2 is a known transcriptional regulator of cerebral cortical fate and neuronal differentiation [60]. In the hippocampus, Lhx2 serves as a brake, which prevents astrogenesis until neurogenesis is complete [61]. Subramanian and colleagues suggest a model in which Lhx2 inhibits Notch-mediated astrogenesis through its downstream target NF1A, since (1) down-regulation of Lhx2 produces astrocytes only if NF1A is functional, and (2) Lhx2 expression blocks astrogenesis induced by NF1A overexpression [61]. Another inhibitor of NF1A that might contribute to the regulation of astrogenesis is the empty spiracles homolog 2 (Emx2). Emx2 is a patterning factor involved in central nervous system morphogenesis and promotes neurogenesis at the expense of astrogenesis [62]. Microarray analysis of isolated NSCs lacking Emx2 showed upregulation of NF1A [63]. Hence, Emx2 may repress NFIA to avoid premature astrogenesis.

A positive regulator of NF1A expression is the Notch downstream target Sox9, an HMG box transcription factor. Sox9 is expressed early during development and ablation of Sox9 in nestin-positive NSCs in vivo prolongs neurogenesis and delays astrogenesis [64]. Recently, Kang and colleagues demonstrated that the regulation of astrogenesis by Sox9 is NF1A-dependent. Sox9 associates with NF1A, which induces the expression of NF1A itself as well as other target genes involved in astrogenesis [65]. Hence, Sox9 regulates the timing of NF1A expression. Upon NF1A expression, astrogenesis is initiated through epigenetic remodeling of the GFAP promoter allowing for JAK/STAT-mediated gene transcription. Taken together, the Notch downstream target NF1A is a key regulator of astrocyte differentiation. Inhibition of NF1A function prolongs neurogenesis whereas its activation induces the onset of astrogenesis.

In addition, Notch activity is controlled by degradation of the Notch 1 receptor. The F-box- and WD repeat domain-containing protein 7 (Fbxw7) is a ubiquitin ligase, which ubiquitinates Notch1, targeting it for proteasomal degradation [66]. Hence, loss of Fbxw7 in nestin-positive NSCs leads to the accumulation of the Notch1 receptor. Hoeck and colleagues demonstrated that increased Notch1 levels after Fbxw7 deletion at early development, such as E16.5, blocks NSC differentiation and decreases neurogenesis [67]. This finding emphasizes the prominent role of Notch signaling in maintaining self-renewal of NSCs and inhibiting neurogenesis early in development. Matsumoto and colleagues analyzed the generation of astrocytes in Fbxw7 knockout mice. At birth, high levels of Notch1 in the absence of Fbxw7 enhance astrogenesis [68]. Hence, regulation of Notch activity by Fbxw7 controls neurogenesis, as well as astrogenesis depending upon the developmental stage of NSCs.

In summary, Notch signaling is crucial for the initiation of astrogenesis (1) by inducing epigenetic remodeling of the GFAP promoter via its downstream target NF1A and (2) by potentiating JAK/STAT signaling through activation of STAT3. Notch activity needs to be tightly regulated to prevent premature astrogenesis. An important brake is the transcription factor Lhx2, which inhibits expression of the downstream target NF1A. Moreover, the ubiquitin ligase Fbxw7 degrades the Notch1 receptor limiting an activation of the pathway. The regulators identified to control Notch activity are depicted in Fig. 3. A future challenge is the dissection of Notch function in astrogenesis independently of its role in NSC maintenance in early development. Conditional knockout of Notch components specifically during the astrogenic phase could provide insights into Notch-mediated astrogenesis [67].

Oasis-Gcm1 signaling

Recently, an additional pathway was identified that changes NSC competence at the onset of astrogenesis. Saito and colleagues demonstrated that Oasis-Gcm1 signaling evokes demethylation of the GFAP promoter. Oasis is a CREB/ATF transcription factor activated by an unfolded protein response (UPR) in NSCs. The UPR comprises a network of signaling pathways that control the maintenance of the endoplasmic reticulum (ER) function to fold

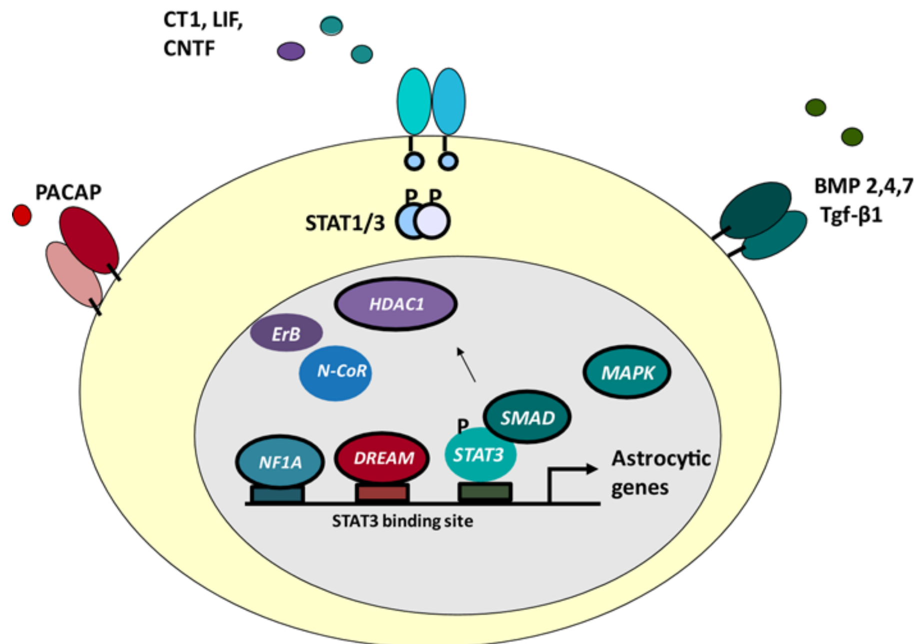

Fig. 4 An integrated network of astrocytic stimuli converges to initiate astrogenesis. Gliogenic cytokines such as CT-1, LIF, and CNTF activate JAK/STAT signaling, the canonical pathway for astrocytic gene expression. In addition, gliogenic cytokines may release repressor complexes such as ErbB-NCoR and HDAC1 from astrocytic gene promoters. The effect of the cytokines is potentiated by the growth factors BMPs and TGF-β1. They signal via SMAD proteins that form a STAT3-SMAD co-activating complex on the STAT3 binding site of the GFAP promoter. Finally, the calcium-regulated PACAP-DREAM pathway acts synergistically with the JAK/STAT pathway, and depends on the transcription factor NF1A, a downstream factor of Notch signaling

proteins. The UPR balances the rate of incoming proteins with the folding capacity of the ER. Disruption of this balance leads to ER stress [69]. Saito and colleagues state a model in which mild ER stress in NSCs activates Oasis. Disruption of this regulation by ablation of Oasis in mice reduced the number astrocytes and delayed the onset of astrogenesis while increasing neuronal precursors [70]. The downstream target of Oasis, glial cell missing (Gcm1), partially restored astrogenesis through demethylation of the GFAP promoter. Thus, this study provides a first link between ER function and astrocyte differentiation. It is important to define the role of the newly discovered Oasis-Gmc1 pathway in relation to Notch activation in order to understand how the two pathways converge to control astrocytic gene expression.

Newborn neurons trigger the initiation of astrogenesis

Gliogenic signals initiate differentiation of NSCs into astrocytes (see Fig. 4; Table 1). It is hypothesized that newly generated neurons secrete cytokines to stimulate astrocyte differentiation. The gliogenic cytokine cardiotrophin-1 (CT-1) is of crucial importance, since loss of CT-1 results in massive defects in astrogenesis [71]. In contrast, depletion of leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) has only a small impact of astrogenesis in vivo [72], since endogenous expression is initiated after birth [73]. Nevertheless, LIF and CNTF stimulate JAK/STAT signaling significantly in vitro [9, 71, 74]. CNTF and LIF activate the JAK receptor on NSCs leading to STAT1/3 phosphorylation [75]. In turn, STAT-dependent gene transcription is activated. Consistently, CNTF-induced astrogenesis can be blocked by inhibitors of the JAK/STAT pathway [17].

In addition to activating JAK/STAT signaling, gliogenic cytokines may release repressor complexes from astrocytic gene promoters. The presence of LIF decreases the association of the NCoR and HDAC1 with the GFAP promoter [9]. Consistently, induction of another repressor complex ErbB4-NCoR antagonizes cytokine-induced increase in GFAP expression [33]. Neuregulin was shown to promote ErBb4 expression, thus the amount of neuregulin in the neurogenic niche might control cytokine-induced astrogenesis.

Gliogenic cytokines act synergistically with bone morphogenetic proteins (BMPs) to promote astrogenesis (see Fig. 4). BMP signaling alone is not efficient in inducing astrocyte differentiation [9, 76]. Nevertheless, BMP signaling has a synergistic effect on cytokine-mediated

upregulation of GFAP. BMPs mainly signal through SMAD1. BMP potentiates JAK/STAT signaling through the formation of a STAT-SMAD1 co-activating complex on the STAT3 binding site of the GFAP promoter [76]. The complex is initiated by p300, which bridges the formation of a complex between STAT3 and SMAD1.

The astrocytic JAK/STAT signaling seems to determine the astrocytic effect of BMPs. As shown for BMP4, the effect of BMP signaling depends on the developmental context. BMP4 participates in astrocyte differentiation in late development [77, 78]. In contrast, during early development, BMP4 induces neuronal differentiation [79, 80]. It is hypothesized that the effect of BMP might depend on the balance between neuronal and astrocytic differentiation pathways [81]. Thus, high JAK/STAT activity at the onset of astrogenesis may instruct BMP signaling to promote astrogenesis.

In addition, BMP signaling is integrated in the positive feedback loop of JAK/STAT and Notch signaling, which enhances astrogenesis (see Fig. 4). BMP2 induces expression of the Notch downstream target Hes5, which inhibits the proneural bHLH Ngn1 [80]. In turn, repression of the pro-neural gene Ngn1 releases coactivators of STAT3 to activate astrocytic gene expression (see "Brakes of astrogenesis"). In this way, extrinsic BMP signaling couples induction of astrogenesis with inhibition of neurogenesis. So far, BMP2 and BMP4 were considered to be the main BMP family members promoting astrogenesis. Recently, BMP7 was identified as a crucial regulator of astrocyte differentiation since intraventricular injection of BMP7 in embryonic mice initiates premature astrogenesis [82]. Interestingly, BMP induces GFAP expression in astrocytes that are morphologically distinct from LIF-induced astrocytes [77]. Hence, different astrogenic stimuli might induce particular astrocyte subpopulations. Investigation of astrocyte markers such as S100B or GLT1 in addition to GFAP is critical for a better characterization of distinct astrocytic subpopulations.

Tgf-β1, an additional member of the Tgf superfamily, might be an important activator of astrogenesis as well. Tgf-β1 receptors are expressed in early development [83] and cortical neurons secrete Tgf-β1 in vitro [84]. Moreover, Tgf-β1 activates SMAD2/3 signaling, which interacts with the JAK/STAT pathway to promote GFAP expression in cortical NSCs in culture [85]. Thus, similar to BMPs, Tgf-β1 could promote the cooperation between SMAD proteins and STAT3 to enhance JAK/STAT-mediated astrogenesis. In addition, Tgf-β1 was demonstrated to induce non-canonical MAPK/PI3 K signaling to control the differentiation of NSCs into astrocytes [86].

A mediator of Tgf-β1 signaling pathway is TAB 2 [87]. Interestingly, a key astrocytic repressor complex ErbB4-NCoR (discussed in "Brakes of astrogenesis") forms a complex with TAB 2 at the promoter of astrocytic genes during neurogenesis [33]. Stipursky and colleagues proposed a model in which activation of Tgf-β1 might sequester TAB 2 from the inhibitor complex. The dissociation of the repressor complex could promote transcription of glial genes and in turn astrogenesis [85]. The cross-talk between neuregulin inducing the ErbB4-NCoR complex and Tgf-β1 signaling limiting the action of the ErbB4-NCoR complex may fine-tune the switch from the neurogenic to the gliogenic phase.

In addition to JAK/STAT and Notch signaling, calcium signaling stimulates astrogenesis. The cAMP-dependent pituitary adenylate cyclase-activating polypeptide (PACAP) signaling to the downstream regulatory element antagonist modulator (DREAM) plays an important role in this regulation [88]. Vallejo suggested a model in which the PACAP activates intracellular cAMP production and in turn entry of calcium in NSCs. Calcium activates the transcription factor DREAM, which binds to responsive elements in the GFAP promoter. Hence, GFAP expression is stimulated. Consistently, loss of DREAM delays astrogenesis in vivo. Synergistic effects between the PACAP-DREAM pathway and the JAK/STAT pathway were observed, which depend on the transcription factor NF1A, a downstream factor of Notch signaling. Hence, calcium signaling and growth factor-regulated pathways cooperate to activate astrogenesis.

Taken together, astrocytic stimuli from newborn neurons are the key trigger for astrogenesis. The most potent signals are gliogenic cytokines, which activate JAK/STAT signaling, the canonical pathway for gliogenic gene expression. Growth factors of the TGF superfamily like BMPs and Tgf-β1 act synergistically with gliogenic cytokines to activate the JAK/STAT pathway. In addition, calcium signaling cooperates with growth factors to induce the generation of astrocytes. Hence in the presence of astrocytic stimuli, an integrated network of different signaling pathways converges to initiate astrogenesis (see Fig. 4; Table 1).

Maintenance of astrocytic identity

Once new astrocytes are generated in the developing brain, they need to maintain their glial identity. To this end, repression of neuronal genes is of crucial importance. A key player in silencing neuronal genes in astrocytes might be the repressor element 1-silencing transcription factor (REST). Neuronal gene silencing by REST is studied in several non-neural tissues [89, 90]. Recent findings demonstrate a role for REST in silencing of proneuronal genes also during the astrogenic phase. Microarray analysis of mouse NSCs at the onset of astrogenesis revealed that REST, together with the cofactor CoREST, targets genes involved in key developmental pathways of astrocyte specification including Notch, JAK/STAT, FGF, EGF, and BMP

signaling [91]. It was demonstrated for BMP signaling that the astrocytic stimulus BMP2, itself, induces the transcription of REST [92]. In this way, initiation of astrogenesis might be coupled to suppression of neuronal genes.

REST inhibits gene expression via epigenetic silencing of neuronal gene promoters. To this end, chromatin repressors such as SIN3A associate with REST [93]. Intriguingly, SIN3A was recently suggested to repress astrocytic gene expression during the neurogenic phase (see Fig. 2). Dissociation of the repressor from astrocytic promoters upon initiation of astrogenesis might allow its binding to neurogenic promoters. Hence, initiation of astrogenesis might promote suppression of neuronal genes in astrocytes.

In differentiated astrocytes, REST occupies neuronal gene promoters. Depletion of REST induces expression of neuronal genes like β-III-tubulin, suggesting an inhibition of neural gene expression [92]. Nevertheless, expression of neuronal markers like doublecortin (Dcx) is observed in human cortical astrocytes in the adult brain [94]. Moreover, simultaneous expression of GFAP and β-III-tubulin suggests an intermediate cell state, the so-called "asterons" [95]. Interestingly, loss of SHP2, which is part of the proneuronal SHP2/MAP/ERK pathway, triggers asterons [22]. Whether SHP2 is involved in the regulation of REST function is currently unclear.

Taken together, REST activity is critical for silencing neuronal genes in differentiated astrocytes. Gliogenic stimuli might induce REST expression coupling the onset of astrogenesis to suppression of neuronal gene expression. Thus, the astrocyte fate is maintained.

Outlook

Here we review the increasing molecular and genetic evidence demonstrating a key role for the JAK/STAT as well as the Notch signaling pathway in promoting astrocyte differentiation. Activation of these pathways is tightly regulated by a complex network of intrinsic and extrinsic factors in order to ensure a correct timing of astrogenesis. Despite the increasing insight into astrocyte specification, some major questions still remain unanswered. For example, to date, it is unclear which factors trigger silencing of neurogenic transcription factors by PcG proteins to initiate astrogenesis. An additional limitation of the current literature is the fact that multiple studies rely solely on GFAP as a marker for astrocytes. It is important to realize that not all astrocytes express GFAP [96], thus focusing solely on the regulation of GFAP excludes the investigation of additional astrocyte populations. Moreover, focusing on GFAP will bias the analysis of regulatory mechanisms of astrocyte differentiation, since alternate astrocyte markers like S100B or GLT-1 are regulated differentially upon astrocytic stimuli. For example loss of FGF2 in vivo, a potential epigenetic regulator of astrocyte differentiation, only reduces expression of GFAP but not of other astrocyte markers [47]. The functional role of GFAP in astrocytes is still elusive. So far, it is unclear whether GFAP expression initiates astrocyte specification or, alternatively, is crucial for terminal differentiation. Therefore, the analysis of markers besides GFAP is critical to study the process of astrogenesis. Comparison of astrocytic gene profiles at different stages of development could help to find markers for distinct stages of differentiation. Finally, GFAP is not exclusively expressed in astrocytes but is also present in NSCs in the developing and adult brain, typifying them as neurogenic astrocytes [97]. This observation complicates the interpretation of the regulation of astrogenesis solely based on GFAP expression. To date, the different roles of GFAP in NSCs versus astrocytes are still elusive.

Gene modulation specifically during the astrocytic phase is essential in understanding astrocyte differentiation. For example, modification of Notch signaling was performed during the neurogenic phase and secondary effects were studied on astrogenesis [42]. Manipulation during the gliogenic phase could help in specifying Notch function for astrocyte differentiation. Moreover, an important question is whether proliferation and differentiation are coupled similarly during astrogenesis as in the neurogenic phase. The "cell cycle length hypothesis" suggests that inhibition of the cell cycle favors neurogenesis and prevents proliferation of NSCs [98]. The same concept might hold true for NSCs during the astrogenic phase. Overexpression of cell cycle inhibitor p57kip2 triggers the switch from proliferation to neuronal differentiation at E14.5. At later stages of development, p57kip2 promotes premature astrocyte differentiation [99]. Intriguingly, several other studies also showed increased astrogenesis upon reduction of NSC proliferation [100, 101]. Nevertheless, definite proof is missing that cell cycle inhibition induces NSC differentiation also during astrogenesis, as it does in neurogenesis.

The current concept of astrogenesis focuses on a temporal regulation of the neuron-to-astrocyte switch: radial glial cells generate neurons first, and only later in development change their competence to become astrogenic. However, it is nowadays recognized that different progenitor cell types act as NSCs in the developing brain. Although radial glial cells produce the majority of astrocytes, basal progenitors also give rise to astrocytes in ventral areas of the brain [102]. Moreover, radial glial cells were shown to differentiate into intermediate progenitors to expand in number before producing astrocytes. These different progenitors are present in distinct areas of the brain [2] suggesting that, in addition to a temporal regulation, astrogenesis is spatially regulated. Future research is necessary to define how regional production of astrocytes by different progenitor

cells is regulated and whether all progenitors switch from a neuron to astrocyte competence in a temporal manner.

Astrocytes themselves might contribute to an expansion of the glia population. Ge and colleagues suggest a model in which local proliferation of differentiated astrocytes is a major source of astrogenesis in the postnatal brain [103]. Hence, various cell types need to be considered as progenitors of astrocytes.

Studying human development is challenging and therefore many pathways of NSC differentiation described in the rodent brain are not yet confirmed in human cells. Recently, important regulators of rodent astrogenesis were tested for their potential to induce astrocyte differentiation of NSCs derived from human iPS cells or ESCs. To this end, iPS cells or ESCs were sequentially specified into embryonic bodies and NSC forming rosettes. Intriguingly, neuronal formation from NSCs derived from iPS cells and ESCs proceeds the generation of astrocytes, as observed for differentiation of endogenous NSCs during development [104–106]. In addition, key mechanisms of mouse astrogenesis also emerge as important regulators for astrocyte differentiation from human iPS cells or ESCs as demonstrated for the Notch pathway. Astrocytes derived from iPS cells express high levels of the Notch downstream target NF1A [107]. In mouse NSCs, NF1A expression and activation of the Notch pathway instructs astrogenesis by activation of the GFAP promoter (see "Notch signaling, a master regulator of astrogenesis"). Whether NF1A is crucial for demethylation of the GFAP promoter in human NSCs as in rodent NSCs is not clear. The onset of NF1A expression proceeds the one of GFAP, which would allow for a regulation of GFAP expression, and in turn, instruction of astrogenesis by NF1A [105].

Moreover, similar to mouse NSCs, blocking of the initiation of another Notch downstream target Sox9 during astrocyte differentiation impairs the Notch-induced astrogenesis in ESCs [104]. However, the effect of a lack of Sox9 on neurogenesis is different in an in vivo situation. Genetic ablation of Sox9 in vivo prolongs neurogenesis in mice, whereas in cultured human NSCs derived from human ESCs, Sox9 knockdown decreased the number of neurons and increased marker expression for NSCs [104]. In conclusion, astrocyte differentiation from iPS cell and ESC-derived NSCs follows the main principles of endogenous NSC differentiation. However, due to the lack of a complex network of intrinsic and extrinsic factors present in the neurogenic niche in vivo, the effect of specific astrogenic stimuli can differ in iPS cell- and ESC-derived NSC cultures. A similar conclusion can be drawn from studies testing gliogenic cytokines in human NSCs cultures. The cytokines LIF and CNTF increased GFAP expression in NSCs derived from ESCs similar to mouse NSCs [102, 103] and iPS cells [102]. However, in contrast to an important role of CT-1 in instruction of astrogenesis in the mouse brain, CT-1 treatment of iPS cells and ESCs did not result in an increase of GFAP expression [106].

Hence, in order to tailor protocols to differentiate astrocytes from of iPS cells and ESCs, careful validation of known astrogenic stimuli is necessary. Identification of factors instructing differentiation of human NSCs is of special importance for transplantation of NSCs. NSC transplantation is currently investigated as a treatment for different brain diseases. Interestingly, an inflammatory environment in the diseased brain might trigger astrocyte differentiation of transplanted NSCs. Activated microglia were shown to secrete LIF and other activators of JAK/STAT signaling, which induce astrocyte differentiation [108, 109]. Moreover, reactive astrocytes have the potential to promote astrogenesis in vitro, most likely via release of CNTF and LIF [110]. Future research will clarify whether astrocytic stimuli in the microenvironment of the diseased brain can determine the cell fate of grafted NSCs. Astrocyte differentiation might be beneficial for the recovery of brain function since newborn astrocytes can provide trophic support for damaged neurons. Alternatively, depending on the context of a specific disease, suppression of astrogenesis might favor neuronal differentiation and stimulate replacement of damaged neurons. Future research will determine whether astrocyte differentiation is "friend or foe" in the diseased brain, an outcome which will be dependent on the specific pathology of a disease.

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