Developmental control of cortico-cerebral astrogenesis

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ABSTRACT A remarkable body of research over the last 15 years has been aimed at disentangling the cellular and molecular mechanisms which regulate murine cortico-cerebral astrogenesis. This research effort has allowed the reconstruction of the actual sizing of this process, as well as a better definition of its temporal, spatial and clonal articulation. Moreover, these investigations have shed substantial light on the cardinal molecular mechanisms governing the transition from pallial neuronogenesis to astrogenesis, as well as subsequent progress of the latter. It has turned out that proper temporal articulation of astrogenesis relies on a plethora of tightly interlaced mechanisms, which synergistically dampen astrogenesis prior to birth and promote it during peri- and postnatal life. The aim of this review is to provide a comprehensive and organic synthesis of these mechanisms, as well as a critical evaluation of their specific relevance to proper articulation of cerebral cortex astrogenesis in time and space.

KEY WORDS: cerebral cortex, astrocyte, development, molecular mechanism, chromatin

Introduction

Albeit less numerous than reported in old literature (Hilgetag and Barbas, 2009), astrocytes form a remarkable fraction of the cortico-cerebral neural complement. Non-neuronal cells numbers are an approximately linear function of brain mass, which conversely correlates with neuronal numbers according to a power law (the scale factor, depending on the CNS district and the genus the animal belongs to, equals 1.7 and 1.1 circa, in rodents and primates cerebral cortex, respectively) (Herculano-Houzel, 2012). Thus, non-neuronal cells are 47% and 79% of total cortico-cerebral cells, in mice and humans, respectively (Azevedo et al., 2009; Herculano-Houzel et al., 2011), resulting less abundant in grey compared to white matter (in humans 58% vs 94%, respectively (Azevedo et al., 2009)). Astrocytes - as documented in the rodent neocortex (Irriintchev, 2004) - amount to slightly less than half of these non-neuronal cells.

Astrocytes play a large variety of roles, in cortico-cerebral development, physiology and pathology (Wang and Bordey, 2008). They shape the morphology of neuronal dendrites (Ballas et al., 2009; Jacobs et al., 2010) and assist migration of some neuronal progenitors (Kaneko et al., 2010). They contribute to genesis and function of the blood-brain barrier (Tao-Cheng et al., 1987; Abbott et al., 2006; Alvarez et al., 2011; Bozoyan et al., 2012), provide structural and metabolic support to neurons (Allen and Barres, 2009; Bélanger et al., 2011; Prebil et al., 2011), and modulate synaptic transmission and information processing (Nedergaard et al., 2003; Eroglu and Barres, 2010; Sasaki et al., 2011; Min and Nevian, 2012). Finally, they react to pathological conditions, by upregulating specific gene products (intermediate filament glial librillary acidic protein (Gfap) and inhibitory extracellular matrix (ECM) proteins among them), and demarcate the damaged site from surrounding tissue with an ECM-rich scar, poorly permissive for axonal regeneration (Silver and Miller, 2004; Schachtrup et al., 2011).

Spatio-temporal and clonal architecture of the cortico-cerebral astrogenic compartment

Like elsewhere in CNS, even within the developing cerebral cortex astrocytes are mainly generated after neurons and before oligodendrocytes. Classical birthdating experiments showed that mouse astrogenesis initiates in the last third of the prenatal neuronogenic window, at around embryonic day 15 (E15). Astrocyte birthrates arise abruptly after neuronogenesis completion, peaking around postnatal day 3 (P3) (Ge et al., 2012). Then, they smoothly decrease. Consistently, Gfap* astroglial elements, just detectable around E16 and still rare at birth (<2.0%), double every 3-4 days in the first postnatal week (Qian et al., 2000), and reach their ab...
solute plateau around P21 (Bandeira et al., 2009).

Regional articulation of cortico-cerebral astrogenic matrices is relatively simple, as compared to neuronogenic and oligodendro-genic ones. Cortical neurons are generated in as many as seven tangentially distinct structures: pallium, generating the majority of glutamatergic neurons; cortical hem, cortical antihem and sep-tum, generating pioneer glutamatergic neurons of Cajal-Retzius; medi-al-, lateral- and caudal ganglionic eminences (MGE, LGE and CGE), generating murine gabaergic interneurons (Guillomet, 2005). Cortico-cerebral oligodendrocytes are also born in distinct birthplaces: MGE, LGE, pallium and, apparently, thalamus (Kessaris et al., 2006). Conversely, all cortico-cerebral astrocytes exclusively derive from pallial precursors, as robustly demonstrated by cre/loxP-mediated labelling, driven by the Emx1 promoter (Gorski et al., 2002; Tsai et al., 2012). Moreover, clonally-related astrocytes share their specific areal location. They may be found clustered along abventricular processes of remnants of radial glia from which they presumably originated (Tsai et al., 2012) or adjacent to apical (i.e. abventricular) dendrites of isoclonal projection neurons (Magavi et al., 2012). That was demonstrated by injecting little amounts of cre-encoding adenoviruses into P1 Z/EG reporter mice and immunoprofiling them at P4-P28 (Tsai et al., 2012), or examining cortical development in transgenic mice in which a random and sparse subset of neural progenitors undergoes CRE/lox recombination, so permanently labeling their progeny (Magavi et al., 2012).

Concerning clonal topology of the astrogenic compartment, it was addressed in vivo, at a variety of developmental stages, by labelling periventricular neural precursors with diluted tracer-viruses (retroviruses or retroviral libraries, both selectively infecting intermitotic elements) and immunotyping the resulting clones. Depending on cases, clones were singled out on the basis of geometrical vicinity among their components (case tracer-retroviruses) or based on sharing of the same molecular tag, regardless of distance (that is the case of libraries). Precursors with mixed neuronogenic and gliogenic potencies resulted to be frequent in the early preneuro-nogenic forebrain (McCarthy et al., 2001), more rare (circa 10%) in the rat E15 cortex (Walsh and Cepko, 1992, 1993; Reid et al., 1995). Glial clones containing both astrocytes and oligodendrocytes were found upon retroviral infection of the neonatal rat SVZ (at frequency of 15%) (Levison and Goldman, 1993), but even in mouse E9.5 forebrains (albeit at frequency of only 5%) (McCarthy et al., 2001). Finally, purely neuronal, astrocytic or oligodendrocytic clones were detected at high frequency when retroviruses were injected into the rat E15-16 cortex (Grove et al., 1993; Luskin et al., 1993) or the neonatal cortex (Luskin and McDermott, 1994). These data are consistent with a model characterized by a progressive restriction of histogenetic potencies displayed by proliferating precursors, from mixed neuronal-glial, through glial, to purely astrogenic or oligogenic. However, such progression seems to be not tightly synchronized among clones. In particular, the appearance of committed elements with restricted potencies may predate quite a lot the birth of their ultimate postmitotic progenies. Conversely, some precursors may retain their tripotency until relatively late developmental stages.

Concerning immunological identification of distinct astrogenic precursors, specific molecular markers are available, largely used in developmental studies. The A2B5+PSANCAM- antigenic profile has been specifically associated to bipotent glial progenitors, both rhombo-spinal and telencephalic, endowed with mixed astrogenic and oligogenic properties (Rao et al., 1998; Han et al., 2004; Strathmann et al., 2007). NG2+ cells may act as progenitors of oligodendrocytes and paleocortical grey matter astrocytes (Zhu et al., 2007). Cluster of differentiation 44 antigen (CD44) was described in unipotent astrogenic progenitors (Liu et al., 2004). Aldolase C (AldoC; Bachoo et al., 2004) and aldehyde dehydrogenase 1 family, member L1 (Aldh1L1; Cahoy et al., 2008) label all astrocytes, Gfap and S100b are preferentially associated to protoplasmatic and fibrous astrocytes of grey and white matter, respectively (see ref (Magavi et al., 2012)).

Concerning radial location of the different types of astrogenic precursors, the scenario is quite complex. About 13 year ago, Goetz and coll. unexpectedly found that radial glial cells, previously

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Fig. 1. Transmodulators interacting with the Gfap promoter. Shown are presumptive Gfap promoter configurations in OFF and ON states. Approximate positions of cis-active modules within the murine Gfap promoter refer to the transcriptional start site. They have been compiled on the basis of pooled mouse and rat data cited in the text, taking into account mouse/rat sequence homologies, as detected by Blat software at http:// genome.ucsc.edu. Note that physical interaction between pSmad2,4 and the Tgfβ1-RE has not yet been experimentally proven. Moreover, the quaternary complex sitting on the silent promoter around -180 was simply inferred on the basis of NCoR affinity for both RBP.Jk and ErbB4-ICD, the latter mediated by a Tab2 bridge (see Text).
considered as part of the glial lineage and commonly known as guiding cables for migrating neurons, also are neuronal precursors. Moreover, they showed that, only upon neurogenesis completion, radial glia shifts towards generation of astrocytes (Malatesta et al., 2000, 2003). These new concepts took quite a long time to be fully metabolized by the scientific community (Malatesta and Götz, 2013). Nowadays they have been widely accepted and early multipotent elements generating both neurons and glia are commonly supposed to coincide with a subset of prenatal radial glia (Kriegstein and Alvarez-Buylla, 2009). Moreover, it has been shown that, after completion of prenatal neurogenesis, many radial glial cells lose their contract with the ventricular cavity and get straightly transformed in astrocytes (Noctor et al., 2004). In addition, radial glia is further believed to generate subventricular precursors (Rakic, 1998) committed to gliogenesis, bipotent or unipotent (Marshall et al., 2003), which populate the perinatal and neonatal subventricular zone and supply cerebral cortex with a substantial part of its astrocytic and oligodendrocytic complements (Zerlin et al., 1995; Kakita and Goldman, 1999; Kakita et al., 2003; Kriegstein and Alvarez-Buylla, 2009). More recently, however, it has been shown that neocortical astrocytes are actually born in different places, depending on their ultimate radial location. When EGFP-encoding plasmids were transfected into SVZ/radial glial cells of P0-P2 mice, by intraventricular DNA injection and electroporation, 16-20 days later, 75% and 22% of labelled astrocytes were found in SVZ/white matter and layers V/V, respectively, and only 3% in layers IV-I. This suggests that, at P0-P2, progenitors of deeper and more superficial astrocytes are near and far from the ventricle, respectively. On the other hand, injection of high titer EGFP-encoding gammaretroviruses into the cortex of P0-P2 mice was followed, 7-10 days later, by high frequency labelling of astrocytes (almost 50%), regardless of their radial location. This means that, albeit far from the ventricle, the almost totality of layer IV-I astrocytes is generated by local proliferating progenitors (Ge et al., 2012). Actually, it was found that they derive from precursors which, still intermitotic, had left periventricular layers and undergone an almost complete astroglial differentiation (astrocyte-like dividing cells, astrolke-D cells). These cells constitute a substantial (and progressively decreasing) fraction of cortico-cerebral astrocytes (from 19% at P3, to 0.3% at P50). They give rise to repeated, symmetric divisions and supply superficial layers of grey matter with the almost totality of their astrocytic complement (Ge et al., 2012; Magavi et al., 2012).

**Intrinsic and extrinsic mechanisms controlling astrocyte fate choice**

Two seminal studies published about 12 year ago formally demonstrated that the switch from neurogenesis to astrogenesis taking place in the rodent cerebral cortex around birth is the result of two key concurrent factors: the advancement of an intrinsic “developmental clock” hardwired in neural multipotent precursors (as proposed by Martin Raff and coll. in mid 80’s, for optic nerve), and the activity of paracrine regulatory signals, impinging on these precursors from their surroundings.

The first study, performed by the Temple group (Qian et al., 2000), showed that isolated E10-E11 mouse cortico-cerebral stem cells, grown in Fgf2-containing basal medium, give rise to large clonal trees including both neurons and astrocytes, the latter ones generated 2-5 generations past the former ones. That means that early cortical stem cells, even in the absence of extrinsic cues, are intrinsically programmed to switch from early neuronogenesis to late astrogliogenesis, according to a biologically plausible timetable.

The second study, performed by the Ghosh group (Morrow et al., 2001), conversely showed that proliferating neural precursors obtained by dissociation of mouse E15 cortico-cerebral tissue, give preferentially rise to neurons or astrocytes, depending on the substrate on which they are cultivated, rat E18 and P15 cortical slices, respectively. That was not due to differential survival or expansion of the committed subclones originating from these elements. Rather it reflected a differential commitment of these proliferating precursors to distinct histogenetic pathways. This commitment was induced by the surrounding micro-environment and diffusible substances were apparently responsible for it. That was suggested by the replication of these results in the presence of semipermeable barrier, interposed between the inducer tissue and the induced precursors. However, when P5 mouse precursors were assayed by this test, these cells always differentiatied as astrocytes, regardless of the developmental age of the substrate supporting them. This means that, albeit the behaviour of cortical precursors is influenced by the surrounding environment, however their plasticity is limited and their histogenetic properties change irreversibly as development proceeds.

Molecular mechanisms by which intrinsic and extrinsic control of astrogenesis are implemented have been subject of an articulated dissection, still in progress. This dissection has been largely focused on factors and constraints regulating transcription of select genes, such as Glial and S100β, chosen for historical and objective reasons as models of astroglia-specific transcription. Consistently with the classical findings of Temple and Ghosh, it emerged that astrocyte-specific gene expression is regulated by specific extracellular ligands secreted by surrounding cells, acting on multipotent neural precursors and modulating their histogenetic properties. Information carried by these ligands is generally conveyed to the nucleus of neural precursors via dedicated receptors and transducers. This ultimately results in differential nuclear availability of transcription factors, which, interacting with chromatin of astroglial genes, may modulate its epigenetic state and regulate its current transcription rate. Collectively, expression levels of these receptors and transducers as well as the epigenetic state of this chromatin dictate precursors ability to respond to astrogenic stimuli, namely the other key determinant of astrogenesis progression.

**Transactive pathways modulating astrogenesis**

It has been shown that at least 5 main trans-active pathways regulate transcription from astrocytic promoters (Glial and S100β the best characterized ones): (1) cardiotophin 1 (Ct1)/janus kinase 2 (Jak2)/Signal transducer and activator of transcription 1 and 3 (Stat1 and Stat3), (2) Delta/Notch, (3) Neuregulin 1 (Nrg1)/v-erb-a Erythroblastic leukemia viral oncogene homolog B4 (ErbB4), (4) Transforming growth factor β1 (Tgfβ1)/Tgfβ-receptors I and II (TgfRI and TgfRⅡ)/Small mothers against decapentaplegic homologs 2 and 4 (Smad2 and Smad4), and (5) Pituitary adenylate cyclase-activating polypeptide (Pacap)/Pituitary adenylate cyclase (Pac)/Downstream regulatory element antagonist modulator (Dream) (Fig. 1). These pathways apparently impact astrocytic promoters in direct ways.
Some of them are further provided of “auxiliary branches”, finely tuning their activity. The majority of them are functionally interconnected, according to an intricate topology.

Cardiotrophin-1-type ligands released by neurons and astrocytes, signalling via the Jak2/Stat3 axis, are the main promoters of astrogial commitment and astrocytic differentiation (He et al., 2005). Nuclear translocation of Notch-IntraCytoplasmic Domain (Notch-ICD), induced by Delta-type signals (from the neuronal lineage), promotes astrogial specification as well (Ge et al., 2002). Nuclear translocation of ErbB4-IntraCytoplasmic Domain (ErbB4-ICD), induced by Nrg1 (from the neuronal lineage), conversely antagonizes it (Sardi et al., 2006). Finally, further positive inputs to astrogensis promotion come from firing of the pSmad2,3 pathway, triggered by Tgfβ1s (from both neuronal and astrocytic lineages) (Stipursky and Gomes, 2007), as well as from Pac/Dream signalling, stimulated by Pacap available within the perinatal periventricular zone (Cebolla et al., 2008). An overview of these pathways follows.

**The Ct1/Jak2/Stat3 cardinal pro-astrogenetic pathway**

A number of IL6-related ligands, often referred to as “astrogenic cytokines” and including Cardiotrophin 1 (Ct1), Leukemia Inhibiting Factor (Lif), Ciliary Neurotrophic Factor (Cntf), Neuroepoietin (Np) and Cardiotrophin-like Cytokine (Clc), are able to strongly promote expression of astroglial genes, by acting on cortico-cerebral NSCs and astrogenic progenitors which express the corresponding receptor complex (Bonni et al., 1997; Rajan and McKay, 1998; Nakashima et al., 1999a; Ochiai et al., 2001; Uemura et al., 2002; Derouet et al., 2004; Barnabé-Heider et al., 2005). These astrogenic cytokines bind to their α-coreceptors (including the plasmamembrane-bound ciliary neurotrophic factor receptor α, Cntfrα, and its secreted variant sCntfrα) and trigger the heterodimerization of the two β-subunits of their main receptor, glycoprotein 130 (gp130) and Lif-receptor β (LifR β)”. Such β-subunits contain one, plasmamembrane-proximal suppressor of high-copy PP1 protein 2 (Shp2)-binding site (YxxV) as well as several, membrane-distal Signal transducer and activator of transcription 1 and 3 (Stat1/3)-binding sites (YxxL). Ligand-induced heterodimerization of β-subunits is followed by their multiple Y-phosphorylation as well as by further Y-phosphorylation of the gp130/LifRβ-associated signalling mediators Stat1,3 and Shp2 (reviewed in ref (Ernst and Jenkins, 2004)). All these Y-phosphorylations are catalyzed by Janus tyrosine kinases 1 and 2 (Jak1,2), which are constitutively associated to gp130/LifRβ and are phosphorylated upon gp130/LifRb stimulation. Y-phosphorylations are followed by the detachment of pStat1,3 and pShp2 from cytokine receptors (reviewed in ref (Ernst and Jenkins, 2004)). pStat1 and pStat3 homo- and heterodimers translocate into the nucleus, where they interact with chromatin (Ernst and Jenkins, 2004) and transactivate astrocyte-specific genes, such as Gfap, S100β, aquaporin, etc (He et al., 2005). pShp2 conversely stimulates the mitogen-activated Erk kinase (Mek)/extracellular signal-regulated kinase (Erk) and the Akt cascades (Ernst and Jenkins, 2004), not directly implicated in transactivation of astroglial genes (Barnabé-Heider et al., 2005), and is involved in self-inhibition of the main astrogenic axis (Lehmann et al., 2003).

The primary role of the Ct1/Jak2/Stat3 axis in astrogensis promotion has been thoroughly documented. mRNAs of Cntfrα (Derouet et al., 2004), gp130, LifRβ, Jak1, Stat1 and Stat3 (He et al., 2005) were found in the pallial neuroepithelium as early as at E11-12, albeit at low level. Moreover, all these genes resulted to be subsequently upregulated, peaking around birth and afterwards (Derouet et al., 2004; He et al., 2005). Knock-out of gp130 almost abolished Gfap expression in Lif- or Cntf-treated E14.5 telencephalic precursors, as well as in the E18.5 brain (except fimbria and hippocampus) (Nakashima et al., 1999b). A similar in vivo suppression of Gfap expression was elicited by inactivation of LifRβ (Koblar et al., 1998). Moreover, pharmacological inhibition of Jak by AG490 (but not inhibition of Mek by PD98059) reduced the gliogenic effect elicited by CNTF (Barnabé-Heider et al., 2005). Finally, as for Stat3, its positive implication in stimulation of astroglial genes was proven by a variety of approaches. It was observed upon transduction of rodent telencephalic precursors with artificial alleles of Stat3, encoding for a gain-of-function version of this protein (Stat3c, constitutively dimerizing, however still requiring Lif stimulation for its transactivating properties), or encoding for dominant-negative versions of it (Stat3f, unable to get Y-phosphorylated, and Stat3d, unable to bind to DNA) (He et al., 2005). Moreover, astrogenic properties of pStat3 were evident upon cre/loxP-mediated ablation of Stat3 in murine E14.5 cortico-cerebral NSCs (Cao et al., 2010), as well as after knock-down of Stat3 mediated by RNAi (Aberg et al., 2001; Barnabé-Heider et al., 2005; He et al., 2005). Interestingly, reduced astrogenesis caused by Stat3-LOF manipulations was often associated to an excess of neuronal differentiation (Gu et al., 2005; Cao et al., 2010).

Concerning real ligand(s) triggering the astrogenic cascade in the developing cerebral cortex, the situation is as follows. Cntf (Derouet et al., 2004) and Lif (Barnabé-Heider et al., 2005) are not expressed in the embryonic CNS. Np-mRNA is detectable throughout murine embryonic neuroepithelia since E11-12, disappearing around birth (Derouet et al., 2004). Clc- and C1-mRNAs, quite scarce at E12-14, are readily detectable by E17.5 (Uemura et al., 2002; Barnabé-Heider et al., 2005). Moreover, Ct1 is specifically expressed by cortical neurons and the medium conditioned by these neurons can promote Gfap upregulation. Remarkably, this upregulation can be prevented by the addition of a neutralizing antibody against Ct1 as well as by genetic ablation of Ct1. No prevention effects are conversely obtained by an anti-Lif neutralizing antibody or via Lif knockout. Consistently, the Gfap+ astroglial complement, as evaluated at P3, is reduced by 50-75% in Ct1−/− mutants and almost unaffected in Lif−/− mutants (Barnabé-Heider et al., 2005) (except a 30% decrease in the dentate area (Koblar et al., 1998)). All that suggests that perinatal cortical astrogenesis is mainly triggered by Ct1 released by previously born neurons, possibly with the help of Lif in some regions. Later, astrogenesis might be further promoted by Cntf released by astrocytes (Lillien et al., 1988), so self-sustaining its advancement.

**Auto-regulation of the Ct1/Jak2/Stat3 axis**

The Ct1/Jak2/Stat3 axis is provided of positive (Fig. 2A) and negative (Fig. 2B) regulatory loops, which finely tune its capability to sense Ct1 and Ct1-like signals and transduce them to the nucleus. These loops are crucial to proper timing of astrogenesis and appropriate regulation of the astrogenic-to-neuronogenic balance. They may be classified as follows:

**Modulating gp130, Jak1, Stat1 and Stat3 expression levels**

gp130, Jak1, Stat1 and Stat3 expression levels are very low in E11 cortico-cerebral precursors, they arise more and more during neurogenesis progression and finally peak around birth. This
upregulation is promoted by the astrogenic cytokines, through the C1/Jak2/Stat3 axis. This was proven, by transducing cortico-cerebral precursors with mutated alleles of Stat3, encoding for gain-of-function or dominant-negative versions of it (He et al., 2005). Remarkably, such upregulation is direct. In fact, evolutionarily conserved, pStat-binding sites are in the promoters of all four genes and - as proven by ChiP - they are specifically enriched in pStat3 upon Lif stimulation (He et al., 2005).

Modulating Jak2 phosphorylation

Two molecular devices, triggered by C1 stimulation and encoded by suppressor of cytokine signalling gene 3 (Socs3) and Shp2, provide a negative feedback, limiting levels of phospho-Jak2 upon cytokine stimulation and so concurring to proper balance between astrogenesis and neuronogenesis.

Socs3, upregulated by Jak/Stat signalling, binds to a single, membrane-proximal Y residue of LifRb or gp130 (Y757 of mouse gp 130), upon phosphorylation of this residue which follows cytokine-dependent stimulation of the receptor complex (Schmitz et al., 2000). In this way, Socs3 recruits the proteasome to the ligand-occupied receptor complex and triggers degradation of its components as well as inhibition of Jak2 phosphorylation (Krebs and Hilton, 2001). Relevance of Socs3 to proper tuning of astrogenic rates was demonstrated by adenoviral transduction of Socs3 to rat E15-17 striatal precursors, followed by exposition of these cells to Lif or Lif/Fgf2 (Cao et al., 2006). It was found that Socs3 downregulates the astroglial output elicited by cytokine stimulation, in as little as 1 day. This was associated to early upregulation of NSC markers and delayed increase of the percentage of microtubule associated protein 2-expressing (Map2+ elements originating from the culture. That reasonably reflected an inhibition of glial commitment of NSCs, which, even in the presence of gliogenic citokines, retain their identity and are consequently available for alternative histogenetic pathways (Cao et al., 2006).

The phosphorylation-dependent Shp2 tyrosine phosphatase also binds to the LifRb/gp130 phospho-tyrosine residues which interact with Socs3, upon cytokine stimulation (Schmitz et al., 2000). Following that, Shp2 dephosphorylates the adjacent Jak2, so attenuating Jak/Stat signalling (Lehmann et al., 2003). In humans, mutations leading to constitutive activation of SHP2 cause the Noonan Syndrome (NS), which includes learning disabilities and mental retardation. In the mouse, knockdown of Shp2 in cultured cortical precursors or in the developing embryonic cortex inhibits neuronogenesis, anticipates astrogenesis and enlarges the astrocytic complement. Conversely, expression of an constitutively active Shp2 mutant causes an opposite phenotype, like it also happens in a mouse model of human NS. Thus, in normal cortogenesis, Shp2 channels early neural precursors to make neurons and not astrocytes, so contributing to postposition of astrogenesis to late gestational and early postnatal ages (Gauthier et al., 2007).

Hetero-regulation of the C1/Jak2/Stat3 axis, by orthogonal modulatory plugins

Several orthogonal regulatory branches converge onto the C1/Jak2/Stat3 axis, finely tuning its firing rates (Fig. 3). Based on position of their entry points on this axis, these branches may be classified as follows.

Regulating levels of the C1-receptor (gp130)

Two heterologous players regulate gp130 expression levels, the proneural machinery and the Mek/Erk signalling machinery (Fig. 3A). As shown by both gain- and loss-of-function experiments, Neu-

![Fig. 2. Autologous regulatory loops modulating the astrogenic cytokine signalling axis. Positive (A) and negative (B) self-regulating loops shaping the temporal firing profile of the main astrogenic signalling axis. (A) pStat1,3-responsive elements located within the promoters of gp130, Jak1, Stat1 and Stat3 mediate the progressive surge of the corresponding mRNAs and protein products, upon activation of this axis by C1-type ligands. (B) pStat1,3 also directly promote transcription of Socs3, whose protein product binds the most plasma membrane-proximal tyrosine residue of gp130/LifRb, phosphorylated upon C1 signalling. From this position, Socs3 recruits the proteasome to the gp130/LifRb-bound phosphorylated Jak, so paving the way to its degradation. (B) Finally, upon C1 signalling, the phosphorylated, most plasma membrane-proximal tyrosine residue of gp130/LifRb gets bound by the Shp phosphatase, which dephosphorylates the gp130/LifRb-bound phospho-Jak and so prevents further phospho-Jak-dependent, Stat1,3 activation.](image-url)
Neurogenin1 (Neurog1) and Neurogenin2 (Neurog2)” do repress gp130 expression (He et al., 2005). These two factors peak during the neurogenic period and so contribute to postponing astrogenesis to perinatal and postnatal phases.

Concerning the Mek/Erk axis, it has been recently shown that co-ablation of Mek1 and Mek2 in radial glial cells (RGCs), by Nestin-Cre, hGFAP-Cre or electroporated pCMV-Cre, inhibits the switch of RGCs from neurogenesis to gliogenesis and prevents appearance of astrocyte- and oligodendrocyte-restricted progenitors. Consequently, surviving Mek1/2-deleted mice exhibit cortices almost devoid of astrocytes and oligodendroglia and undergo extensive neurodegeneration. A similar but less drastic phenotype follows co-ablation of Erk1 and 2. Conversely, electroporation of a constitutively active form of Mek1 (caMek1) leads to precocious activation of astrogenesis (Li et al., 2012). Remarkably, the effects of the Mek/Erk machinery on astrogenesis are largely mediated by the Ets transcription family member Etv5/Erm, which is detectable in the VZ at E14.5-E18.5 - is necessary and sufficient for activation and progression of astrogenesis (Li et al., 2012). Puzzlingly, however, extracellular signals triggering astrogenic firing of the rat sarcoma (Ras)/rapidly accelerated fibrosarcoma (Raf)/Mek/Erk axis have been poorly defined. For example, Fgf2, an established stimulator of this axis (Dorey and Amaya, 2010), promotes astroglial differentiation in the absence of any gp130 upregulation (Song and Ghosh, 2004), namely a major effect of Mek/Erk firing in this context. This suggests that Fgf2 impact on astrogenesis may be mediated by alternative pathways (Dorey and Amaya, 2010). On the other hand, Mek/Erk stimulation of astrogenesis is consistent with the capability of Nf1 to limit gliogenesis (Dasgupta and Gutmann, 2005; Hegedus et al., 2007; Wang et al., 2012). In fact the product of Nf1, Neurofibromin1, is a Ras-GTPase that converts the GTP-bound active form of Ras to the inactive, GDP-bound form (Schefzik et al., 1997), so that Nf1 inactivation leads to hyperactivation of the Ras/Raf/Mek/Erk pathway. Nf1 was inactivated constitutively (Dasgupta and Gutmann, 2005), limited to E12.5 radial glia and its progenies (by Brain lipid binding protein gene-driven cre (Blbp-cre)^-cre and Glap-cre deleters) (Hegedus et al., 2007; Wang et al., 2012), as well as in postnatal SVZ B cells and their progenies (by a Nestin-creET deleter and tamoxifen administration at P26-30) (Wang et al., 2012). In all cases a dramatic increase of gliogenesis was reported. In constitutive and early embryonic Nf1-knock-out models that was associated to a remarkable enlargement of the subventricular zone and hyperthrophy of the corpus callosum (Dasgupta and Gutmann, 2005; Hegedus et al., 2007). In postnatal knock-out models, NSC-restricted Nf1 deletion resulted in increased gliogenesis at the expense of neurogenesis (Wang et al., 2012). Remarkably, exaggerated glial commitment triggered by Nf1 ablation could be phenocopied by constitutive overactivation of the Ras pathway (Hegedus et al., 2007), and rescued by small inhibitors of Mek/Erk signalling (Wang et al., 2012).

The key mechanism mediating astrogenic properties of the Ras/Raf/Mek/Erk axis is Mek1,2-dependent upregulation of gp130, encoding for one of the two subunits of the cardiotrophin 1-receptor. This upregulation, in fact, considerably sensitizes neural precursors to Ct1-family astrogenic cytokines. However, additional mechanisms concur to astrogenic properties of the Ras/Raf/Mek/Erk axis. First, Mek1,2 contribute to glial commitment triggered by Nf1 ablation could be phenocopied by constitutive overactivation of the Ras pathway (Hegedus et al., 2007), and rescued by small inhibitors of Mek/Erk signalling (Wang et al., 2012).

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to its active pStat3 form (see below). Second, firing of this axis stimulates proliferation and selective expansion of astrogial lineage elements. This happens upon defective N1f-dependent Ras inhibition in embryonic NSCs, resulting in increased self-renewal of these elements and more frequent late-born Olig2⁺ glial progenitors originating from them (Hegedus et al., 2007). In a similar way, sustained Mek1,2 signalling forces astrocytes to keep proliferating for longer times after their generation, so further amplifying the glial output of the system in vivo (Li et al., 2012).

Regulating Jak1 levels

During the early neurogenic period, Jak1 protein levels are kept low by proneural genes (Fig. 3A), which contributes to delayed activation of astrogenesis (He et al., 2005).

Modulating Stat1 and Stat3 levels

Two main players modulate expression levels of Stat proteins, namely the proneural machinery and the Egf transduction machinery (Fig. 3A).

Downregulation of Stat1 and Stat3 by Neurog1 and Neurog2 was demonstrated by both gain- and loss-of-function approaches (He et al., 2005). It is a key mechanism preventing precocious activation of astrogenesis.

Conversely, signalling through EgfR (aka ErbB1) promotes cortico-cerebral astrogenesis. EgfR is expressed at low level in the ventricular zone of the neuronogenic pallium and, at higher and higher levels in basal proliferative layers of the developing cortex (Caric et al., 2001). Already at the end of 90's, it was reported that mice lacking EgfR suffer of delayed astrocyte development (Komblum et al., 1998; Sibilia et al., 1998). In 1997 and 2003, two seminal papers from the Lilien group, clarified cellular and molecular modalities of EgfR-dependent regulation of astrogenesis (Burrows et al., 1997; Viti et al., 2003). In the former study, the authors found that retrovirus-mediated overexpression of EgfR, in the E15 rat brain or in E12/E15 organotypic cultures of rat cortex, upregulates their astrocytic output, whereas delivery of the two main EgfR ligands, Egf and Tgfa, to EgfR-wt brains of the same developmental ages does not alter astroglial rates. These data suggest that, at both E12 and E15, EgfR levels, rather than ligand levels, limit firing of the Egf transduction pathway and, therefore, astrogenesis rates. Only starting from E18, endogenous EgfRs levels would become sufficiently high to be saturating for astrocytes generation. Further dissection of this phenomenon by clonal analysis showed that the increased astrogenic output elicited by EgfR/Egf manipulations actually has a dual origin. It reflects an enhanced astroglial commitment of multipotent precursors and it is amplified by more pronounced proliferation of committed elements. Remarkably, whereas combined EgfR/Egf manipulation was required to increase astroglial commitment at E12 and E15, simple delivery of exogenous ligand was sufficient for that at E18. This suggests that minimal firing of the Egf transduction pathway necessary for astroglial commitment may be higher than that required for promotion of proliferation (Burrows et al., 1997). Consistently with results of EgfR-GOF studies, cortical explants from E17 EgfR⁺ mutant mice did not react to exogenous Lif administration. They differed from wt controls, which conversely responded with a quite enhanced astrogenic output (Viti et al., 2003).

Concerning molecular mechanisms underlying EgfR promotion of astrogenesis, Egf signalling upregulates astrogenesis rates, mainly by facilitating transmission of the C11 signal through the Jak2/Stat3 axis via EgfR-dependent upregulation of Stat3 expression. In fact, retroviral transduction of EgfR into murine, wild type cortico-cerebral precursors of different developmental ages considerably increases the frequency of Stat3⁺ cells (ten-fold at E11, two-fold at E16), whereas EgfR knock-out in E16 precursors grown in vitro for 4 days reduces this frequency by about 5/6-fold (Viti et al., 2003). On the other hand, following retroviral transduction of Stat3 into E11.5 precursors and in the only presence of endogenous astrogentic cytokines, frequencies of pStat3⁺ elements and S100β⁺ derivatives reach plateau values (not further upregulatable by exogenous Lif stimulation), which may be elicited in Stat3-wt cells of similar age by simple administration of exogenous Lif. In other words, the upregulation of Stat3 triggered by EgfR sensitizes embryonic neural precursors to the astrogentic cytokines, sufficiently to achieve a pronounced astrogenic response, even in front of low levels of these ligands (Viti et al., 2003).

However, pro-astrocytogenic activities of EgfR signalling cannot be fully accounted for in the light of Stat3 upregulation. In fact, experimental overexpression of EgfR in early cortical precursors (E11-13) upregulates frequencies of both S100β⁺ and Gfap⁺ astrocytes, whereas straight Stat3 overexpression in the same cells increases S100β⁺ cells, but not Gfap⁺ ones. That suggests that an Egf-dependent mechanism different from Stat3 upregulation may be selectively required to activate Gfap. This mechanism cannot be Stat3 phosphorylation, as Lif stimulation elicits similar levels of pStat3 in both EgfR-GOF and Stat3-GOF early cortical precursors (E11-13). It could be something else, working in parallel to pStat3 or downstream of it (Viti et al., 2003). On the other side, the expansion of the Gfap⁻ compartment triggered by EgfR stimulation might partially have nothing to do with Gfap activation, being alternatively due to increased proliferation of astroglially-committed progenitors (Gadient et al., 1998).

Concerning the ligand triggering the EgfR-mediated astrogenic response in vivo, it is possible that this function is early shared by Egf and transforming growth factor α (Tgfα) and subsequently played by Tgfα only. In fact, both ligands are expressed in the neuronogenic cortical primordium (the former throughout it, the latter confined to its lateral border) (Assimacopoulos et al., 2003), but only Tgfα (and no Egf at all) is detectable in postnatal periventricular precursors (Romero-Grimaldi et al., 2011). Consistently with this prediction, mice expressing reduced Tgfα levels have a decreased number of astrocytes (Weickert and Blum, 1995). Remarkably, the activity of A disintegrin and metalloproteidase domain (Adam) “sheddases” is required to make Egf/Tgfα ligands available in the extracellular space (Blobel, 2005), so enabling astrogenesis. Both Adam10 and Adam17 are co-expressed in the VZ of the mid-neuronogenic cortical primordium (Diez-Roux et al., 2011) as well as in periventricular precursors of postnatal cortex (Romero-Grimaldi et al., 2011). However only Adam17 seems implicated in astrogenesis promotion. In fact, Adam17 knock-down impairs astrogenesis and its expression levels go up in damaged brain regions undergoing astrogliosis. That, conversely, does not apply to Adam10 (Romero-Grimaldi et al., 2011).

Modulating Stat3 phosphorylation

Mechanisms mediating astrogenic properties of Notch include promotion by the Delta/Notch/Hes axis of signalling through the Jak/Stat pathway. That is achieved by the Notch-effectors hairy
enhancer of split homologs 1 and 5 (Hes1 and Hes5), which act as bridges between Jak2 and Stat3 and facilitate phosphorylation of the latter by the former (Fig. 3B) (Kamakura et al., 2004). As a consequence of that, overactivation of the Notch signalling in murine E12 telencephalic precursors, followed by their in vitro expansion for 9 days, leads to a considerable enhancement of their astroglial differentiation. Consistently, such enhancement is fully suppressed, when a dominant-negative form of Stat3 is introduced in these cells (Kamakura et al., 2004).

Among mechanisms by which proneural genes inhibit astrogensis, there is Neurog1 inhibition of Lif/Ct1-induced Stat1,3 phosphorylation (Fig. 3B). Molecular details of this inhibition are poorly known. It has been suggested that such inhibition contributes to diversify the responses exhibited by both E13.5 and E17.5 murine cortical precursors to Lif/Ct1. Both precursor types undergo phosphorylation, only the latter phosphorylates Stat3 (Sun et al., 2001). As a consequence of that, E13.5 are only neurogenic, E17.5 are mainly astrogenic. Consistently, the cortex of mutants double knock-out for Neurog2 and Mouse achaete scute homolog 1 (Mash1) has increased numbers of gial progenitors and activates astrogensis earlier (Nieto et al., 2001).

Finally, a reciprocal regulatory loop, including Neural myelocytomatosis prooncogene (N-myc) and INK4a p19 protein/alternate reading frame of the INK4a/ARF locus (p19(Arf)), regulates Stat3 phosphorylation, thus channelling Jak/Stat signalling machinery to distinct OFF and ON states (Fig. 3B). N-Myc and p19(Arf) are expressed by NSCs prevalently during the embryonic and the perinatal/postnatal phases of cortico-cerebral histogenesis, respectively. The former promotes NSC self-renewal and neurogenesis, the latter sustains astrogenesis. Both are induced by Fgf2 and Egf, along a fast and a slow kinetics, respectively. The latter is further promoted by CNTF-dependent Stat3; N-Myc and p19(Arf) reciprocally inhibit their expression. N-Myc counteracts CNTF-dependent phosphorylation of Stat3 at Y705, p19(Arf) facilitates this phosphorylation, via p53. In this way, the N-Myc/p19(Arf) loop concurs to neatly define the functional state of the Ct1/Jak2/Stat3 axis, OFF or ON, during the embryonic and the post-natal life, respectively (Nagao et al., 2008).

**Acting downstream of or in parallel with pStat3**

It was shown that in vitro cultured telencephalic precursors from E14.5 mouse embryos activate Gfap in as little as 2 days under combined Lif/Bmp2 stimulation, taking at least 2 days more in the presence of either cytokine (Nakashima et al., 1999c). Remarkably, such capability of Lif and bone morphogenetic factor 2 (Bmp2) to synergically promote astrogenesis was confirmed in E16.5 cortico-cerebral precursors kept under thyroid hormones (Adachi et al., 2005). Looking for molecular mechanisms underlying this phenomenon, it was found that no direct interaction occurs between the two key transducers of Lif and Bmp signalling, Stat3 and Smad1, respectively. Conversely, they are bridged by the transcriptional coactivator p300/CBP, interacting with Stat3 at its amino terminus (even in the absence of Lif/Ct1 stimulation) and with Smad1 at its carboxyl terminus (upon Bmp stimulation). This leads to the formation of a pStat3-p300/CPB-pSmad1 ternary complex, which is recruited to the Gfap promoter and mediates cooperative promotion of its transcription by Lif and Bmp2 (Fig. 3C). More generally, it accelerates induction of astrocytes from neural progenitors and amplifies the astrogenic output (Nakashima et al., 1999a). Intriguingly, addition of Bmp alone to gp130/- neural cultures, insensitive to Lif, elicits a moderate but reproducible activation of the Gfap promoter, suggesting that pSmads might also directly transactivate such promoter, independently of pStat3 (Nakashima et al., 1999c).

As reported above, the proneural factor Neurog1 inhibits astrogenesis by counteracting Stat3 phosphorylation. However, it also inhibits pStat3 activity, by competing with pStat3 for binding to p300/CPB-pSmad1 (Fig. 3C) and so diverting such heterodimer to neuron-specific promoters (Sun et al., 2001). Actually, it was shown that in cultures of rat E18 cortico-cerebral precursors, Ngn1 downregulates Gfap promoter-driven transcription, even upon administration of Cntf or Bmp. On the other hand, overexpression of either p300/CPB or Smad1 signficantly rescues glial differentiation from Ngn1 suppression. Moreover, both Cntf/p300 and Smad1 potentiate the transcription of a Ngn1-responsive promoter (Sun et al., 2001). Interestingly, in extracts from rat E14 cerebral cortex, p300/CPB is associated with both Neurog1 and Smad1, but not with Stat3 (which - nevertheless - is expressed at high levels in these extracts). In contrast, in extracts of P3 cortico-cerebral SVZ, rich of astroglial precursors and not expressing Neurog1 anymore, Stat3 is associated to p300/CPB (Sun et al., 2001). The most parsimonious explanation of these data is that the p300/CPB-pSmad1 heterodimer may potentiate both neuron- and astrocyte-specific transcriptions, by complexing Neurog1 and pStat3, respectively. However, p300/CPB-pSmad1 is available only to a limited extent, so that a competition takes place between its interactors for binding to it. This competition is intrinsically biased in favor of Neurog1. Therefore, until proneural genes are abundantly expressed, it is won by their products, so resulting in competitive silencing of astrogial transcriptions. Only after nonogenogenesis completion, proneural factors disappear, so allowing astrocyte-specific transcriptions to rise (Sun et al., 2001). This capability of Ngn2 to antagonize astrogial transcriptions might be the main mechanism underlying exaggerated generation of astrocytes, which has been recently found in cultures of pallial precursors knock-out for Pax6 (Sakayori et al., 2012), namely a direct transactivator of Neurog1 and Neurog2 promoters (Scardigli, 2003; Blader, 2004).

An additional transcription factor set limiting pStat3 activity includes the leucin-zipper family transcription factors CCAAT/enhancer binding protein (C/EBP)-α, -β, and -δ. These are expressed by E12-18 cortico-cerebral precursors, are activated by Mek/Erk-dependent phosphorylation, and are necessary and sufficient to promote nonogenogenesis. That was shown in vitro, transfecting E12 precursors with plasmids encoding for two distinct, dominant-negative variants of C/EBP-β (counteracting all C/EBP paralogs) or a constitutively active, phosphomimetic variant of it, and scoring their progenies 3-4 days later. This was confirmed in vivo, electroporating E15 brains with these constructs and analyzing them 3 days later (Ménard et al., 2002; Paquin et al., 2005). Remarkably, in addition to impairment of nonogenogenesis, functional knock-down of C/EBPs also promotes Cntf-dependent astroglial differentiation, suggesting that C/EBPs may normally contribute to prevent precocious activation of this process. However mechanisms mediating these phenomena are still matter of debate. The increased astroglial output obtained upon C/EBPs knock-down might simply reflect the higher fraction of nonogenenic precursors which retain the stem state upon this manipulation, so resulting available to subsequent glial differentiation (Ménard et al., 2002; Paquin et al., 2005). However, overexpression of a phospho-dead variant of C/EBP-β,
while repressing neuronogenesis, did not affect astrogenesis at all (Paquin et al., 2005), suggesting that mechanisms mediating C/EBP-dependent regulation of astrogenesis should at least partially differ from those impacting neuronogenesis. It was shown that C/EBP-β is able to bind CBP/p300 (Mink et al., 1997). As such, similar to Neurogins and Olig2 (Sun et al., 2001; Fukuda et al., 2004), it might sequester the p300/CBP-<br>NSmad1 heterodimer (Fig. 3C), making it not available to bind pStat3 and so preventing transcription of glial genes.

Finally, it has been shown that the basic, helix-loop-helix (bHLH) factor Olig2, implicated in cortico-cerebral gliogenesis, may also counteract the astrogligenic activity of pStat3, in a Neurog1-like way (Fig. 3C) (Fukuda et al., 2004). Olig2 is widely expressed in the GABA-ergic neuronal lineage as well as by oligodendroglial cells at different stages of their maturation (Cai et al., 2007). Within the astrocytic lineage, it gives rise to a transient perinatal activation wave (Cai et al., 2007; Ono et al., 2008), subsequently declines and fully disappears by P21 (Cai et al., 2007). Such Olig2 downregulation in differentiating astrocytes is fully replicated in vitro, in 4 days-old cultures of murine E14.5 telencephalic precursors, kept under Lif (Fukuda et al., 2004). Remarkably, Olig2, if overexpressed in perinatal cortical precursors, competes with pStat3 for binding to the p300/CBP-<br>NSmad1 dimer, so antagonizing astrocytic differentiation (Fukuda et al., 2004). This mechanism might be crucial to restrict full activation of the Gfap promoter to mature astrocytes and avoid any leakage of it in the oligodendroglial lineage. Moreover, it could help explaining some traits of the complex phenotype displayed by Olig2−/− mutants, such as reversion of Gfap and S100β relative expression levels in layers II-IV of neocortex (Cai et al., 2007).

The anti-astrogenic Nrg1/ErbB4 pathway

A major role in setting up the proper onset of astrogenesis is played by the ligand Neuregulin1 (Nrg1), expressed by neurons and neural precursors, and its ErbB4 receptor, transducing its signal within cortico-cerebral precursors. This was demonstrated by Sardi et al. (Sardi et al., 2006), co-manipulating the Nrg1/ErbB4 and the Ct1/Jak2/Stat3 pathways in telencephalic precursors and assaying consequences of that on the activation of astroglial genes. Transduction of the Nrg1 signal along the “canonical” pathway starts with Nrg1 binding to ErbB receptors, inducing their hetero- or homodimerization. That results into activation of their intracellular tyrosine kinase activity and generation of docking sites for adaptor proteins, in turn activating the Raf/Mek/Erk and the phosphoinositide-3-kinase (PI3K) machineries (Mei and Xiong, 2008). Remarkably, this canonical pathway is not sufficient to control the phosphoinositide-3-kinase (PI3K) machineries (Mei and Xiong, 2008), it might sequester the p300/CBP-pSmad1 heterodimer (Fig. 3C), making it not available to bind pStat3 and so preventing transcription of glial genes.

The pro-astrogenic Delta/Notch pathway

It has been shown that artificial overactivation of Notch signalling in mouse E11.5 or rat E13 cortico-cerebral precursors leads - 2-3 weeks later - to increased astrocytic outputs. Consistently, an opposite phenotype occurs upon blockade of Notch signalling by the γ-secretase inhibitor N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyI ester (DAPT; Ge et al., 2002). As reported above, Notch signalling might sustain the astrogenic program thanks to the capability of Hes1 and Hes5 to physically connect Hes1 and Hes5 to the RBPJk binding site of the Gfap promoter in correspondence of its RBPJk binding

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NCoR into the nucleus, where each component of it can be found associated to the chromatin of Gfap and S100β (Sardi et al., 2006). Interestingly, the ErbB-4-ICD capability to drive cytoplasm-to-nucleus NCoR translocation is dominant over the opposite ability of the firing Jak/Stat axis to extrude this cofactor from nucleus. Actually, binding sites of the ternary ErbB4-ICD/Tab2/NCoR complex within Gfap and S100β promoters were not mapped. It was proposed that this complex would be further connected to recombination signal binding protein for for immunoglobulin kappa J region (RBPKj), for which NCoR has a very high affinity, and, in such a way, it might bind to the Gfap promoter in correspondence of its RBPKj binding site (Miller and Gauthier, 2007). Remarkably, ErbB4 cleavage, its Tab2-mediated interaction with NCoR, and nuclear translocation of the ErbB4-ICD/Tab2/NCoR complex, all do require binding of Nrg1 to the jα isoform of ErbB4. The last two steps further rely on the first. Interestingly, when the “non canonical” ErbB4 pathway is overactivated in rat E14.5 telencephalic precursors in vitro, this suppresses the increase of Gfap and S100β-promoter-driven transcription, otherwise observable after subsequent Cntf stimulation. Conversely, no effect is exerted by ErbB4 pathway overactivation on basal Gfap- and S100β- transcription levels detectable in the absence of Cntf (Sardi et al., 2006). Consistent data were obtained after experimental manipulation of the non canonical Nrg1/ErbB4 axis in vivo. Gfap expression levels were dramatically upregulated in the E17.5 cerebral cortex of mutant mice with CNS-restricted inactivation of ErbB4. Electroporation of human jM-<br>ErbB4 into the brain of these mice, at E13.5, specifically rescued this phenotype.

In synthesis, Nrg1/ErbB4 seems to mainly act as a brake, filtering the astrogenic outcome of the cardinal Ct1/Jak2/Stat3 pathway and dictating the temporal frame of its emergence. In particular, strong Nrg1 signalling in early (<E14.5) murine cortico-cerebral precursors would prevent premature activation of astrogenesis, despite of early firing of the Ct1/Jak2/Stat3 axis. Then, subsequent dampening of Nrg1 signalling, due to late ErbB4 downregulation (Kornblum et al., 2000; Fox and Kornblum, 2005), would lead to progressive derepression of astrogenesis, eventually becoming the prevalent histogenetic process, at E18.5 and later.
precursors, astrogenesis initiates normally about 7 days later. This suggests that Notch signalling is not sufficient to trigger this process and additional conditions have to be fulfilled in order to unveil its pro-gliogenic power. Intriguingly, even artificial overstimulation of the Delta/Notch axis, while enhancing astrogenesis, does not anticipate it. Moreover, the astrogenic activity of Notch signalling requires Jak/Stat signalling, but not vice versa (Ge et al., 2002). Key of these phenomena might be the transcriptional corepressors NCOR and temporal regulation of its subcellular distribution pattern. In fact, this molecule is able to bind RBPJk (Kao et al., 1998) and is prevalently located within the nucleus of young neural precursors (Sardi et al., 2006). Therefore, specifically within these precursors, it might convert RBPJk into a trans-repressor, silencing astroglial genes. Then, when firing of the Jak2/Stat3 axis translocates NCOR to the cytoplasm (Sardi et al., 2006), RBPJk, freed from NCOR, would become prevalently complexed by Notch-IntraCytoplasmic-Domain (NICD) (Miller and Gauthier, 2007). As such, after E14.5, it might promote more and more transcription of astroglial genes. Consistently with this model, 70% of NCOR-embryos show prominent cortico-cerebral overexpression of Gfap as early as E14.5, a time at which wild type cortices barely express this marker (Hermanson et al., 2002).

**The pro-astrogenic Tgfβ1/Tgfβ1RI,II pathway**

It was originally reported that Tgfβ1 secreted by neurons and astrocytes induces the activation of the Gfap promoter in cultured astrocytes, so stimulating their maturation. In this respect, E15 neurons, expressing higher levels of Tgfβ1, resulted to be more efficient inducers than E18.5 neurons and late embryonic astrocytes were more responsive to Tgfβ1 than late post-natal ones. Moreover, this Tgfβ1-dependent upregulation of Gfap was peculiar to cerebral cortex. Midbrain and cerebellar astroglia, while able to respond to Tgfβ1, did not display any increase of Gfap levels (De Sampaio e Gomes, 2007). However, two key aspects of progliogenic Tgfβ1-signalling transduction, Smad2,3 and 4, are already expressed by the mouse E14.5 nestin-positive astroglial precursors, by Pacap and Cntf, elicits an additive incremental effect on frequency of Gfap+ cells. Pertinently, cortical precursors from Dream−/− mice fail to express Gfap in response to Pacap, whereas Dream+ neural precursors, treated by Bdnf, generate about 2-fold more neurons. That suggests that the Pacap pathway contributes to the astrogenic-vs-neuronogenic histogenetic choice. Consistently, the neonatal cortex of Dream−/− mice exhibits a reduced content of astrocytes. However this is a transient phenomenon, as Gfap+ cells of these mutants, about 66% of controls at P1, bounce to 120% in as little as one week (Cebolla et al., 2008). Interestingly, the Pacap pathway seems to act independently of Jak2/Stat3. In fact, Dream knock-out does not affect the responsiveness of cortico-cerebral precursors to Lif/Ctnf (Cebolla et al., 2008). Moreover, combined suboptimal stimulation of cortical precursors, by Pacap and Ctnf, elicits an additive incremental effect on frequency of Gfap+ cells (Cebolla and Vallejo, 2006). However, more subtle parameters of astroglial differentiation, such as the Gfap content of treated cultures and the morphological complexity of astrocytes, display a more than additive upregulation by Pacap and Ctnf, so implying some cryptic functional interaction among the corresponding transcription pathways (Cebolla and Vallejo, 2006).

**Accessibility of chromatin to transcription**

It has been shown that, in addition to trans-active machineries impinging on astroglial promoters, proper activation and adequate progression of cortico-cerebral astrogenesis requires an appropriate temporal regulation of chromatin accessibility to transcription.
Large-scale changes of chromatin structure

A first correlation has been described to occur between large scale chromatin configuration and the developmental choice taken by NSCs towards neuronal or astrocytic lineages. It turns out that the open configuration displayed by chromatin in young NSCs preferentially leads to self-renewal and neuronal differentiation, whereas its subsequent, progressive compaction biases stem cells to make astrocytes (Kishi et al., 2012). It is not clear how generalized changes of chromatin accessibility impact the histogenetic choice of NSCs. It is possible that such changes primarily reduce transcription rates of proneural genes and, because of the major trans-inhibitory role that proneural proteins exert on astrocyte-specific transcriptions (He et al., 2005), indirectly depress astrogenesis. At least two gene sets have been implicated in modulating this epigenetic change.

High mobility group A (HmgA) proteins, 1 and 2, expressed at high level during early neuronogenesis, effectively compete with histone H1 for binding internucleosomal DNA linkers. In this way they keep chromatin accessible and promote the turnover of factors interacting with it, which is associated to prevalent neuronal differentiation. By the end of embryonic life, Hmga levels are reduced, which is possibly experimental to upregulation of astrogenesis rates taking place at that time (Kishi et al., 2012). Consistently with this model, experimental manipulations of Hmga1/2 expression levels, both in vitro and in vivo, affect neurono-to-astrogenic ratios, indicating that these genes are both necessary and sufficient to orientate NSCs to neuronogenesis and inhibit gliogenesis (Kishi et al., 2012).

N-myc, expressed at high level during early neuronogenesis as well, conversely upregulates the histone acetyl-transferase gene GCNs5. In this way, it promotes the acetylation of histones H3 and H4 as well as a more open chromatin configuration (Knoepfler et al., 2006). That may contribute to the capability of N-myc to sustain neuronogenesis. It may help N-myc to counteract expression and activity of p19(Arf), which is strongly expressed in late NSCs and channel them to astroglial differentiation (Nagao et al., 2008).

Epigenetic changes at proneural genes

A neat trans-regulatory effect of the epigenetic state of neuronal genes on astrogenesis rates has been rigorously documented in the case of the proneural gene Ngn1. It has been found that the repressive mark trimethyl-histone 3 lysine 27 (H3K27me3) becomes more and more frequent near the Ngn1-TSS, in mouse E11.5 cerebral precursors kept in vitro for 3, 6 and 9 days. That is consistent with the progressive downregulation of Ngn1 occurring in vivo, around the end of the neuronal phase, and it is instrumental to late activation of astrogenesis (Hirabayashi et al., 2009). Actually, H3K27 trimethylation at the Ngn1-TSS depends on one of the two H3K27-methyltransferases included in the Polycomb Repressive Complex 2 (PRC2), enhancer of zeste homolog 2 (Ezh2). If Ezh2 is conditionally knocked-out at E13.5-14.5, then H3K27me3 levels at the Ngn1-TSS persist low and no downregulation of Ngn1 can be found around E17.5. Remarkably, this conditional knock-out is sufficient to shift the response of cortico-cerebral precursors to prodifferentiating agents in vitro, from as in neuronogenesis. In vivo, it dramatically reduces the frequency of S100β+ cells, as evaluated in P1.5 cortices (Hirabayashi et al., 2009). Interestingly, a depression of astrogenesis may be also achieved by knocking down embryonic ectoderm development (Eed) (which encodes for another subunit of PRC2) or inactivating ring finger protein 1 gene (Ring1b) (which encodes for a subunit of the Polycomb Repressive Complex 1 (PRC1), a functional partner of PRC2) (Hirabayashi et al., 2009). Remarkably, the timing of these genomic manipulations is crucial to disclose the antigliogenic activity of PRC2, reasonably because of the involvement of this complex in additional decisional processes which precede the neurono-to-astrogenic switch. In fact, if Ezh2 is disrupted prior to neuronogenesis, then neuronogenesis rates are exaggerated, at expenses of precursors self-renewal, and the activation of astrogenesis is paradoxically anticipated (Pereira et al., 2010).

Epigenetic changes at astroglial genes

In addition to large scale chromatin dynamics and proper epigenetic tuning of proneural genes, it has been proven that a major pre-condition for the switch from neuronogenesis to astrogenesis is the progressive “opening” of astrocytic chromatin. This is a complex process which takes place while neuronogenesis is on, from E11.5 to E14.5 and beyond, and essentially consists in the acquisition by the astroglial genes of the capability to get bound by their specific transactivators (Hatada et al., 2008).

This capability depends first on the methylation state of DNA. Murine E11.5 cortico-cerebral precursors express the full set of molecular transducers of LIF-like signals (LIF-receptor subunits, Jak and Stats among these). Exposed to LIF, they upregulate pStat3 to some extent. However, as it also happens in neurons and other non-neural cells, this does not result in Gfap transcription, because in these cells pStat3 cannot interact with its binding site, 1.5kb upstream of the Gfap-TSS (Takizawa et al., 2001). In normal conditions, in fact, such interaction starts to be allowed only from E14.5, just upon natural CpG demethylation at this site. Remarkably, if DNA methylation is inhibited by 5-azacytidine (5azaCdR) administration, then Gfap is activated already in E11.5 precursors, by only 4 days (Takizawa et al., 2001). An anticipation of astrogenesis also occurs upon knock-out of the “maintenance” DNA methyl transferase gene, Dnmt1. In this case, E11.5 precursors, exposed to LIF, massively activate Gfap and S100β in as little as 2 days in vitro, whereas their wild type counterpart would take 7 more days for that (Fan et al., 2005). More recently, demethylation of astroglial genes just prior to glial differentiation of NSCs has been described in a variety of other cases (AldoC, ATP-sensitive inward rectifier potassium channel 10 gene (Kcnj10), serpin peptidase inhibitor b8 gene (Serpinb8) and SRY-box containing gene 8 (Sox8) among them). Nowadays it is considered as a pervasive phenomenon, crucial to timely activation of the astrogenic program (Hatada et al., 2008).

However, the preparation of astrocytic genes to transcription is

### Table 1

<table>
<thead>
<tr>
<th>Effectors</th>
<th>Effects on the Gfap promoter</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Ni1α</td>
<td>5meC↓, H3K9-m2↓, H3K4-m2↑</td>
<td>(Namihira et al., 2009)</td>
</tr>
<tr>
<td>Fgf2*</td>
<td>5meC↓, H3K9-m2↓, H3K4-m2↑</td>
<td>(Song and Ghoosh, 2004)</td>
</tr>
<tr>
<td>Coup/d1.2</td>
<td>5meC↓, H3K9-m2↓, H3K4-m2↑, H3-ac↑</td>
<td>(Naka et al., 2008)</td>
</tr>
<tr>
<td>RA</td>
<td>H3-ac↑</td>
<td>(Asano et al., 2009)</td>
</tr>
<tr>
<td>Eset</td>
<td>H3K9-m3↑, H3K9-ac↓</td>
<td>(Tan et al., 2012)</td>
</tr>
<tr>
<td>Brg1</td>
<td>chrom remodeling</td>
<td>(Matsumoto et al., 2006)</td>
</tr>
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* requires subsequent Ctnf stimulation to be effective
not limited to proper regulation of their DNA methylation levels. It encompasses also a fine modulation of the covalent modification profile of histones associated to them, as well a conformational rearrangement of chromatin (Matsumoto et al., 2006). The former includes the replacement of the dimethyl-histone 3 lysine 9 mark (H3K9me2) by the dimethyl-histone 3 lysine 4 mark (H3K4me2), a phenomenon originally documented at the pStat3-BS of Gfap, within rat E15.5 cortico-cerebral precursors kept 2 days in vitro under Fgf2. Remarkably, this switch is associated to H3 hyperacetylation and was proven to be sufficient and necessary to make Gfap suitable to transcription (Song and Ghosh, 2004).

Complex molecular mechanisms dictate the epigenetic state of astroglial chromatin. At the moment, at least six main players have been shown to master this control: Notch (via Nuclear Factor Ia, NF1a), Fgf2, Coupft1&2, retinoic acid, RA (via RA-Receptors α, β and X, RARα, RARβ and RXR), the histone 3 lysine 9-methyltransferase (H3K9-MT) ERG-associated protein with SET domain (ESET) and X, RARb receptor, leading to replacement of NCoR-HDAC by p300/CBP. As a consequence of that, the Notch effector RBPJκ binds to the NF1a promoter, about 2.0 kb upstream of its TSS, and stimulates its transcription (Namihira et al., 2009). In turn, NF1a binds to the Gfap promoter (at least four NF1a binding sites were found within the Gfap promoter (Cebolla and Vallejo, 2006; Piper et al., 2010)). This is necessary and sufficient to detach Dnmt1 from this region and get it demethylated, so paving the way to the astrogenic program (Namihira et al., 2009). That was assessed, by overexpressing NF1a (or a dominant negative version of it) in E11.5 cortico-cerebral precursors, and assaying their behaviour in vitro, after 4 days of Lif stimulation. Thus, NF1a mediates the pro-astrogenic function of the Delta/Notch axis, being transcribed in response to Notch signalling and possibly competing with Dnmt1 for binding to the Gfap promoter (Namihira et al., 2009). Remarkably, overstimulation of the Delta/Notch/NF1a axis is not sufficient per se to enhance astrogenesis. The pro-astrogenic activity of this pathway only emerges, provided that the system is co-stimulated by overexpressed Lif.

Fig. 4. Epigenetic regulation of the Gfap promoter by NF1a and RA. (OFF state). In the absence of NF1a, Dnmt1 C5-methylates the surroundings of the pStat1,3-binding site, at -1.5kb, making such site not suitable to interact with its cognate transactivator. Meanwhile, in the absence of RA, the RARα/RXR receptor sits on the RARE at -2.5kb and, via an NCoR bridge, recruits histone deacetylases (HDACs). These ones deacetylate the Gfap promoter, around both -2.5kb and -1.5kb, so contributing to shut down transcription. (ON state). NF1a binds to the Gfap promoter, possibly at as many as four distinct locations, and inhibits Dnmt1 interaction with it, so allowing progressive lose of C5-methyl marks. Limited to the archicortex, Lhx2 counteracts this NF1a-dependent inhibition of Dnmt1, so antagonizing astrogenesis arousal. On the other side, RA changes the conformational state of its RARE-bound RARα/RXR receptor, leading to replacement of NCoR-HDAC by p300/CBP. As a consequence of that, the Gfap promoter is richly acetylated and so made prone to transcription.
by Lif (Namihira et al., 2009) and/or Pacap (Cebolla and Vallejo, 2006). Consistently, if the NF1a-binding site -79/-57 bps upstream of the Gfap-TSS is disrupted, then the responsibility of the Gfap promoter to both Ctnf and Pacap is suppressed and even basal transcription of this gene collapses (Cebolla and Vallejo, 2006). It is worth mentioning that this mechanism seems to be not limited to Gfap. In fact, NF1a-BSs can be found in the promoters of other astroglial genes (including S100β, aquaporin4, and clusterin). NF1a binds to these sites upon activation of the Notch pathway, and NF1a overexpression is sufficient to induce demethylation of the corresponding promoters (Namihira et al., 2009). More recently, it has been shown that electroporation of the LIM homeobox 2 TF gene Lhx2 into the E15/17 ventricular zone reduces astrogenesis rates by almost 2/3-fold and prevents exaggerated astrogenesis induced by NF1a overexpression. On the other side, disruption of Lhx2 activity, by E15.5 electroporation of its dominant-negative cofactor gene ClimDD, magnifies astrogenesis at expenses of neuronogenesis, only provided that NF1a is not ablated. In a few words, Lhx2 seems to inhibit transcription of astroglial genes, by preventing the activity of NF1a on their promoters (Subramanian et al., 2011). However, these phenomena were documented exclusively in the archicortical anlage, where the late gestational drop of Lhx2 expression levels may contribute to perinatal upregulation of astrogenesis. No gliogenic anomaly was conversely found upon manipulation of Lhx2 activity in neocortical precursors (Subramanian et al., 2011). Finally, in addition to promoting primary opening of Gfap chromatin, NF1a might further sustain transcription of this chromatin at more advanced steps of astroglial differentiation, synergizing with its later activated paralogs, NF1b, NF1c and NF1x (Wilczynska et al, 2009).

As for Fgf2, it was reported that the exposure of E18 rat corticocerebral precursors to this GF for as little as 6 hours makes them responsive to a subsequent 24 hours Cnft stimulation. This results into a remarkable upregulation of Gfap, which, conversely, does not respond to the Cnft treatment alone. Consistently, these precursors, exposed to combined Fgf2 and Cnft for 6 days, give rise to 3-fold less neuronal clones and 2-fold more astrocytic clones. Concerning mechanisms of action of Fgf2, this factor does not influence expression or phosphorylation of Stat3. As proven by ChIP, it conversely triggers the replacement of H3K9me2 with H3K4me2 at the pStat3-BS of the Gfap promoter. However how does it happen, it is not known (Song and Ghosh, 2004). Moreover, Fgf2, stimulating proliferation, contributes to the “dilution” of the 5methylcytosines originally clustered at the Gfap and S100β promoters, which occurs upon the Notch-induced detachment of Dnmt1 from these promoters (Namihira et al., 2009). In these ways, Fgf2 allows pStat3 to bind to the astrocytic promoters and stimulate transcription (Song and Ghosh, 2004; Namihira et al., 2009).

Among agents promoting the opening of astrocyte genes chromatin, there are also the two TF genes Coup-tf1 and 2. Coup-tfs are widely expressed in the telencephalic ventricular zone, where they display a transient peak around E12.5. Their involvement in astrogenesis was recognized by an elegant shRNA screening, run in embryoid body- (EB)-derived neurospheres. It was found that shRNA-mediated silencing of Coup-tfs juvenilizes these cultures, increasing the neuronal output obtainable from tertiary neurospheres under Lif/Bmp stimulation, and reducing the astrocytic one. Consistently, lentivirus-mediated repression of Coup-tfs in cortico-cerebral precursors, from E12.5 onward, resulted - at P20 - in doubling of their neuronal progenies as well as in a dramatic shrinkage of their astrocytic output. Moreover, when Coup-tfs knock-down was anticipated to E10.5, then the switch from deep layer- to superficial layer-neurons production was defective too. This suggests that beyond astrogenesis activation, these two factors may play a more general role as promoters of pan-histogenetic progression. Interestingly, however, neither Coup-tf1 nor Coup-tf2 are sufficient to anticipate the onset of astrogenesis, so pointing toward a permissive rather than instructive role. Remarkably, the two Coup-tfs exert only a moderate influence on the expression levels of key trans-active factors modulating astrogenesis. Their impact on gliogenesis is probably mediated by the epigenetic changes they evoke on the chromatin of astrocytic genes. That has been shown at the level of the pStat3-BS the Gfap promoter. Here, their knock-down upregulates H3K9me2 levels, at expenses of H3K4me2 and acetylated-histone 3 (H3ac), and leads to increased CpG methylation. However these effects are not direct. In fact, no straight interaction of Coup-tfs with the Gfap promoter was detected and both Coup-tfs were shown to play their pro-gliogenic role as transcriptional repressors (Naka et al., 2008).

Retinoic acid (RA), administered to murine E14.5 telencephalic precursors, synergizes with Lif in inducing the activation of the Gfap gene (within as little as 2 days). In the absence of RA, the RARα/β/RXR complex sits on a retinoic acid responsive element (RARE) located at circa 2.5kb upstream of the Gfap transcriptional start site, possibly recruiting specific corepressors (NCoR and/or silencing mediator for retinoid or thyroid-hormone receptors (SMRT)) to the chromatin. This makes histone hypacetylated, both near the RARE and around the major STAT3 BS, at -1.5kb, and inhibits transcription. Upon binding of RA to RARα, the RARα/β/ RXR complex releases the corepressor(s) and recruits the histone acetyl transferase (HAT) E1A binding protein p300/CRB binding protein (p300/CRBP), so increasing histone acetylation at both -2.5kb and -1.5kb. This makes the pStat3-BS suitable to be bound by pStat3 and leads to Lif-dependent transcriptional activation (Asano et al., 2009).

ESET mRNA, encoding for the histone methyl transferase KMT1E, is expressed by the neuronogenic cortico-cerebral ventricular zone at progressively decreasing levels, almost disappearing prior to birth. Its experimental inactivation derepresses transcription of non-neural genes and endogenous retroviral (ERV) sequences and distorts temporal articulation of the cortical histogenetic schedule. ESET ablation anticipates both upper layer neurons generation and astrogenesis onset. Moreover, it hastens the subsequent increase of astrocytic rates. Conversely, in utero electroporation of ESET antagonizes astrogenesis, although not fully suppressing it. KMT1E binds to the Gfap promoter, it is necessary for its H3K9 trimethylation and prevents its H3K9 acetylation. As such, it contributes to the complex machinery which controls timed opening of astroglial genes chromatin and allows timely activation of astroglial programs (Tan et al., 2012). Based on the detection of a number of Hes1-binding sites in its promoter, it has been suggested that the ESET transcription unit might “sense” cell-cycle-locked Hes1 pulsation, integrate digital information encapsulated within such pulsation and convert such information into a cell-cycle number-dependent analog output, in charge of modulating the rates of the histogenetic processes (Tan et al., 2012). Finally, it is worth mentioning that a supportive role for astrogenesis is played by Brg1, encoding for one of the two catalytic
ATPase subunits of the SWI/SNF chromatin remodeling complex (Matsumoto et al., 2006). Brg1 is expressed by cortical neurons and is activated in the cortical ventricular zone after E13.5. Its early ablation impairs self-renewal of neural precursors and results into a depleted astrogenic pool, as proven by the dramatic downregulation of CD44, S100β and Gfap. Interestingly, if Brg1 ablation is postponed (by infecting E14.5 Brg1fl/fl cortico-cerebral precursors with adenoviral cre-expressors, in vitro), then the proliferating pool is spared, however its astrogenial differentiation is still very poor, suggesting a specific involvement of this gene in activation of the astrogenic program. Molecular details of this involvement are presently unknown (Matsumoto et al., 2006).

Regulation of astrocyte committed progenitor proliferation

Our knowledge of such regulation is still very poor and only a few genes have been implicated in it. Fgf2 and Egf stimulate proliferation of neonatal rod astroblasts (Mayer et al., 2009; Riboni et al., 2001). Fibroblast growth factor 9 (Fgf9) promotes a substantial expansion of the perinatal astrogenic proliferating pool (Seuntjens et al., 2009) and delays terminal differentiation of mature astrocytes (Lum et al., 2009). Smad interacting protein 1 (Sip1) TF limits astroblasts proliferation, possibly by inhibiting Fgf9 expression (Seuntjens et al., 2009). A reduction of late astrocyte progenitors, compared to early, NSC-born ones, occurs upon overexpression of the TFs Foxg1 and Empty spiracle homolog 2 (Emx2) in NSCs, suggesting that astrocyte progenitors might undergo precocious terminal differentiation (Branacchio et al., 2010).

Finally, it has been shown that cortical ablation of the TF Olig2 leads to a dramatic upregulation of Gfap in the neocortical postnatal grey matter, pointing to a possible prolonged persistence of astrocytes in a proliferating state. However, kinetic analysis of Olig2-LOF mutants ruled out this hypothesis, suggesting that this phenotype might rather reflect defective Gfap silencing in S100β+ cells of grey matter (Cai et al., 2007). Conversely Olig2, upregulated in reactive adult astrocytes, is strictly necessary to sustain their proliferation and the formation of the glial scar (Chen et al., 2008).

Concluding remarks

In synthesis, several interfaced molecular machineries regulate proper progression of cortico-cerebral astrogenesis.

Astrogenesis timing is firstly determined by temporally regulated accessibility of astrocytic promoters to transactive complexes stimulating their transcription. Undoubtedly, this is the main factor preventing precocious astrocyte generation within the early neurogenic pallium. Distinct machineries, acting on both DNA and histones, contribute to such regulation. Three factors promote DNA demethylation at astrocytic promoters: the RBP.Jk target Nf1α removes the methylating Dnmt1 enzyme from these promoters; Fgf2 likely dilutes previously acquired 5-methyl marks; Couptf1,2 act according to still unknown mechanisms. As for histones, three factors promote a transcription-prone profile: Fgf2 and Couptf1,2 induce the replacement of H3K9-me2 by H3K4-me2; RA, secreted by telencephalic mengines, evokes H3 hyperacetylation. Conversely, progressive downregulation of the H3K9 trimethylating enzyme ESET possibly contributes to sharp astrogenesis activation in neural precursors which have completed neurogenesis.

Moreover, temporal tuning of astrogenic rates is further refined by regulated firing of a specific transactive pathways impinging on astrogial promoters.

Astocyte-specific transcriptions are mainly promoted by Ct1-family cytokines, via the intracellular Jak/Stat transducing axis. In this respect, both ligands availability and neural precursors responsivity to them are crucial to proper temporal progression of astrogenesis. Astrocyte-specific transcriptions are triggered by neuron-secreted Ct1, accumulating by the end of neuronogenesis, and they are later potentiated by Cntf, released by newborn astrocytes. Besides, sensitivity of neural precursor to these cytokines, very low around the onset of neuronogenesis, gets higher and higher, as neurogenesis proceeds. This is due to progressive autoactivation of the Jak/Stat transduction axis, via pStat3-dependent transcription of gp130, Jak1, Stat1 and Stat3, as well as to the effects of additional regulatory branches feeding this axis. These are: Mek/Erk and Egf/Tgfα signalling (arising during prenatal life), which upregulate gp130 and Stat3, respectively; Hes1.5 (expressed by apical precursors in response to neuronal-progenitor Delta signalling), which ease pJak2-dependent Stat3 phosphorylation; and p19 (arising in the late pallium upon decrease of its early repressor N-myc), which also facilitates pJak2-dependent Stat3 phosphorylation.

On the other side, two additional transactive agents, expressed at higher level during early neuronogenesis and fading out around its completion, Neurogenins (Ngns) and ErbB4, antagonize astrogene- sis, preventing its large scale activation around mid-neuronogenesis. Ngns inhibit transcription of four key effectors of the Ctx1 transduction axis (gp130, Jak1, Stat1 and Stat3), counteract pJak2-dependent Stat3 phosphorylation and further compete with pStat1,3 for limited amounts of the p300/ CBP-pSmad2,4 complex, available upon Bmp signalling. The intracytoplasmic domain (ICD) of ErbB4, detaching from cell plasma membrane upon Nrg1 stimulation, promotes the translocation of the transcriptional corepressor NCoR into the nucleus, where it contributes to inhibit Gfap transcription, being likely conveyed to its promoter by an RBP-Jk bridge.

Finally, relatively little is known about mechanisms which, once macroglial differentiation has been initiated, modulate proliferation and differentiation rates of astocyte-committed progenitors, so dictating the final astroglial output of the system. A few transcription factors (Sip1, Emx2 and Foxg1) as well a few secreted ligands (Fgf9 and Tgfα) have been implicated in this issue. However, molecular details of their action as well as their reciprocal epistatic relationships were poorly clarified. They will be likely subjects of future investigations.

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