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Timing Is Everything: Making Neurons versus Glia in the Developing Cortex

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During development of the mammalian nervous system, neural stem cells generate neurons first and glia second, thereby allowing the initial establishment of neural circuitry, and subsequent matching of glial numbers and position to that circuitry. Here, we have reviewed work addressing the mechanisms underlying this timed cell genesis, with a particular focus on the developing cortex. These studies have defined an intriguing interplay between intrinsic epigenetic status, transcription factors, and environmental cues, all of which work together to establish this fascinating and complex biological timing mechanism.

During development of the vertebrate central nervous system, neurons are generated first, and glial cells second (Bayer and Altman, 1991), while in lower organisms such as flies, these two cell types appear coincidentally. This timed cell genesis in vertebrates makes good biological sense, since the bare bones of neuronal circuitry are initially established, and then the numbers and positions of glia are matched to that circuitry. However, while the underlying rationale is apparent, until recently the responsible mechanisms were less so. In this regard, studies addressing various aspects of nervous system development have now converged to provide us with insights into this issue. Here, we will review studies addressing this issue in the developing mammalian cortex. These studies have defined an intriguing interplay between intrinsic developmental programs and environmental cues, thereby providing us with a coherent overview of what has turned out to be a fascinating, novel, and perhaps not surprisingly, complex biological timer.

Much of the work examining the neurogenic-to-gliogenic switch has focused upon the developing mammalian neocortex for several major reasons. First, genesis of different cortical cell populations is temporally segregated; in rodents, neurons are generated from embryonic day 12 (E12) to E18, astrocytes appear at around E18, with their numbers peaking in the neonatal period, and differentiated oligodendrocytes are first seen postnatally (Figure 1) (Bayer and Altman, 1991). Even neurons of the different cortical layers are sequentially generated in an "inside-out" fashion, with the latest-born neurons being the most superficial. Second, this timed genesis also occurs in culture. Cultured primary E10–E12 cortical precursors (a term used here to encompass both cortical stem cells and their more biased progeny) generate only neurons for the first few days, followed by the sequential genesis of astrocytes and oligodendrocytes. Remarkably, Sally Temple and her colleagues demonstrated that this timed genesis of neurons versus glia and even early versus late-born neurons occurred within clones of single precursor cells (Qian et al., 2000; Shen et al., 2006). Third, retroviral lineage tracing studies demonstrated that, in vivo, single precursors contributed to both the neuronal and glial lineages, and that the repertoire of cell types generated by individual precursors changed over development (Walsh and Reid, 1995). For example, when the very early cortex was transduced (McCarthy et al., 2001), many precursors made only neurons, some made both neurons and glia, and some, surprisingly, made only glia, potentially because they waited until later time points to differentiate. Thus, multipotent cortical precursors change their behavior over time in vivo, generating first neurons and then glia, and the underlying timer mechanism(s) is maintained in clones of isolated precursor cells.

What then, have we learned about the neurogenic-to-gliogenic switch? Tremendous progress has been made in this area, largely by addressing the following specific questions. What limits precursors from making glia during the neurogenic period? What is the signal that directs precursors to start making glia at the expense of neurons, and does the same signal inhibit the genesis of neurons during the gliogenic period? Are precursor cells equally competent to generate neurons and glia at all developmental time points? If not, are the underlying intrinsic changes reversible or are they hardwired? Do perturbations in these mechanisms impact on developmental disorders or injury/degenerative responses in the mature nervous system? Recent studies addressing these questions support two major conclusions. First, precursor cells change in terms of their competence over time, being more biased to make neurons early, and glia late. Second, the extrinsic environment that a precursor finds itself in is a key determinant of its differentiation. For example, embryonic

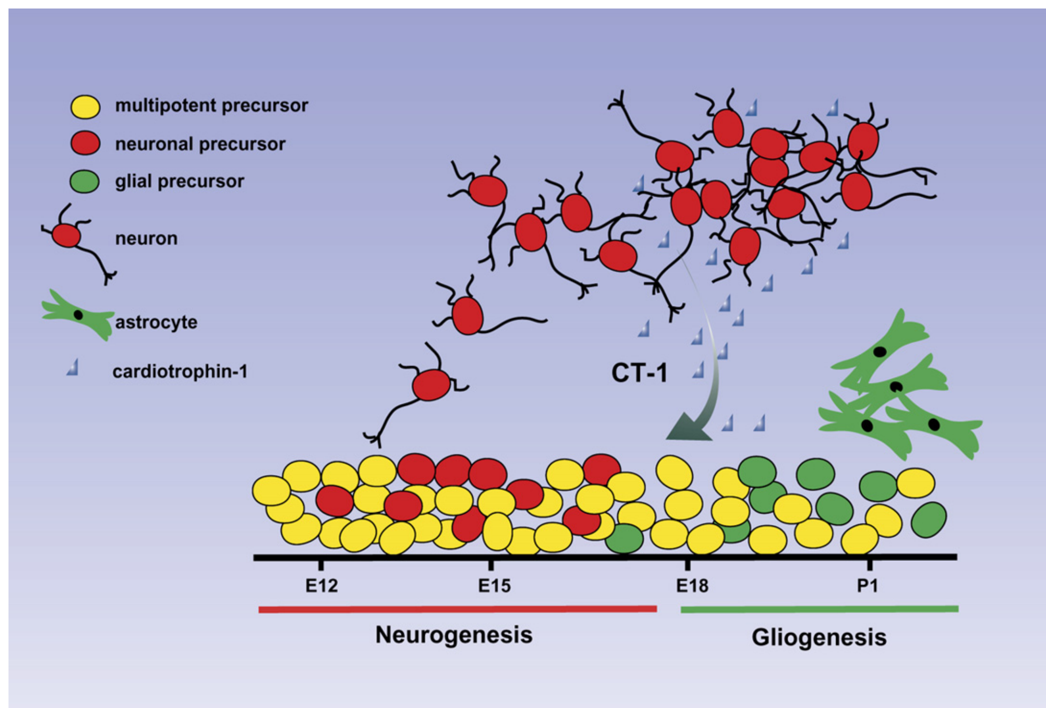

Figure 1. The Neurogenic-to-Gliogenic Switch in the Developing Neocortex
During development of the vertebrate central nervous system, neurons are generated first and glia second. Within the developing rodent neocortex, multipotent precursors generate neurons from approximately E12 to E18, and then the newly born cortical neurons secrete CT-1, which acts in concert with other gliogenic environmental cues such as BMP2 and the Notch ligands to induce the onset of astrocyte formation at approximately E18.

cortical precursors make neurons when cultured on embryonic cortical slices, but astrocytes when cultured on postnatal cortical slices (Morrow et al., 2001). Thus, the neurogenic-to-gliogenic switch depends upon the interplay between developmental biases and potent environmental signals, the nature of which will be described below.

The Trouble with Studying Neural Precursors…

Before reviewing the studies that have contributed to our understanding of the neurogenic-to-gliogenic switch, it is important to consider a number of experimental limitations. Astrocytes are the first glia to appear in the neocortex, and most studies have thus focused upon the switch from making neurons to astrocytes. However, many early studies utilized only GFAP, a late astrocyte protein, as a surrogate for astrogenesis due a paucity of appropriate markers. This approach has two caveats. First, GFAP expression does not distinguish astrogenesis from terminal astrocyte differentiation. Second, many embryonic and adult neural precursors express proteins previously thought to be astrocyte specific, including GFAP. For example, embryonic radial "glial" cells were recently shown to be neural precursors that generate both neurons and astrocytes (Gotz and Barde, 2005). These radial precursors express astrocyte markers such as BLBP, RC2, and outside of the cortex, GFAP. Thus, in response to these considerations, recent studies have examined additional astrocyte markers/genes and, in the large majority of cases, have confirmed the earlier conclusions.

A second experimental consideration is the lack of markers that distinguish neural stem cells from their more biased progeny. The neuroepithelium of the developing cortex contains multiple populations of precursors, including true multipotent stem cells that self-renew. Clonal analysis in culture also indicates the existence of unipotent and bipotent precursors, including a bipotent glial precursor. However, we are not yet able to prospectively isolate these different precursor populations or to definitively distinguish them in vivo, meaning that most studies manipulate multiple precursor types simultaneously. Complicating the analysis further are findings indicating that the progression from a multipotent precursor to a terminally differentiated cell type may not be a one-way street. For example, in the male germ cell lineage, a subset of transit-amplifying cells can move into vacated stem cell niches and start behaving like stem cells with regard to self-renewal (Nakagawa et al., 2007). Similarly, biased oligodendrocyte precursors will dedifferentiate to become multipotent neural stem cells in culture (Kondo and Raff, 2000). Thus, the precise point at which a given signal regulates the transition from a multipotent stem cell to a terminally differentiated neuron or glial cell can be difficult to ascertain. Nonetheless, the studies reviewed here have established a clear framework for understanding the neurogenic to gliogenic switch, and as we develop

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more and better markers/approaches, we will obtain an even more sophisticated and complete view of this complex transition.

A Central Role for a Neuron-Driven Cardiotrophin-1-gp130-JAK-STAT Pathway in the Initiation of Gliogenesis

The aforementioned study by Morrow et al. (2001) demonstrated that neurogenic cortical precursors became gliogenic if placed in a postnatal cortical environment. So what is the extrinsic cue that induces the gliogenic switch? The answer to this came from two coincident lines of study, both of which implicated cytokines of the IL-6 family. This particular subfamily of cytokines includes ciliary neurotrophic factor (CNTF), leukemia inhibitor factor (LIF), and cardiotrophin-1 (CT-1), all of which require and induce heterodimerization of two signal-transducing β subunits, the coreceptors LIFRβ and gp130. These receptors activate a number of signaling cascades, including the JAK-STAT pathway, where the JAKs are kinases that associate with and are activated by gp130, and the STATs are transcription factors that are phosphorylated and activated by the JAKs (reviewed in Ernst and Jenkins, 2004). In one set of studies, mice lacking either LIFRβ or gp130 were shown to have profound deficits in astrocyte formation (Ware et al., 1995; Koblar et al., 1998; Nakashima et al., 1999a). This phenotype was directly due to perturbations in astrogenesis, since (1) cultured *gp130*$^{-/-}$ or *lifrβ*$^{-/-}$ neural precursors were deficient in astrocyte formation (Koblar et al., 1998; Nakashima et al., 1999a), and (2) an acute knockdown of gp130 in cortical precursors caused a decrease in the number of precursors that generated early astrocytes in vitro, and a cell-autonomous decrease in astrocyte formation in vivo (Barnabé-Heider et al., 2005). The second group of studies demonstrated that CNTF and LIF were sufficient to induce astrogenesis (Johe et al., 1996; Bonni et al., 1997; Nakashima et al., 1999b), and that this action required LIFRβ and gp130 (Bonni et al., 1997; Nakashima et al., 1999a, 1999b). A subsequent study then demonstrated that ectopic expression of CNTF induced premature cortical astrocyte formation in vivo (Barnabé-Heider et al., 2005), indicating that at least a subset of cortical precursors were competent to generate astrocytes during the neurogenic period, and confirming that exposure to gliogenic cytokines could regulate the timing of astrogenesis.

These and subsequent studies then addressed the underlying mechanisms and demonstrated that cytokine-mediated gliogenesis involved activation of the JAK-STAT pathway. In particular, CNTF, LIF, CT-1, and other members of this family activated the JAKs and STAT1 and STAT3 in neural precursors, and the inhibition of STAT3 signaling abolished their ability to regulate astrocyte formation either in culture (Bonni et al., 1997; Rajan and McKay, 1998) or in vivo (Barnabé-Heider et al., 2005). Important insights into this activity came with the demonstration that the STATs caused direct transcriptional activation of two astrocytic genes, *gfap* and *s100β*, via STAT binding sites within their promoters (Bonni et al., 1997; Nakashima et al., 1999b; Namihira et al., 2004). This transactivation of glial genes required interactions between STATs and p300/CBP, two related coactivators that associate with a wide variety of transcription factors to promote transcription, in part by acetylating histones and inducing an active chromatin conformation (Kalkhoven, 2004). Moreover, overexpression of p300 was sufficient to enhance cytokine-mediated *gfap* expression, implying that coactivator levels might be limiting for gliogenesis (Nakashima et al., 1999b). Interestingly, the neurogenic bHLH ngn1 also bound to p300/CBP, and this interaction inhibited p300/CBP from associating with STAT3, thereby providing a way in which neurogenic bHLHs could directly suppress cytokine-mediated gliogenesis (discussed in more detail below) (Sun et al., 2001). Together, these studies provided compelling evidence that ligand binding to the LIFRβ and gp130 coreceptors was necessary for astrogenesis in the developing neocortex. In addition, since astrocytes are profoundly decreased throughout the brain and spinal cord of mice genetically deficient in these receptors (Ware et al., 1995; Nakashima et al., 1999a), then this suggests that they may well play a similar role throughout the developing CNS.

But what was the relevant LIFRβ/gp130 ligand? The first clue came from studies showing that the switch from making neurons to astrocytes in culture required signaling via gp130 and STAT3, implying that cortical precursors and/or their progeny make a gliogenic cytokine(s) (Nakashima et al., 1999a; Barnabé-Heider et al., 2005). The relevant cytokine was, however, unlikely to be CNTF or LIF (the two most widely used exogenous ligands), since neither is expressed until postnatal life (Stockli et al., 1991; Patterson and Fann, 1992), and mice lacking these genes demonstrated no (in the case of *cntf*) or very modest (in the case of *lif*) deficits in astrocyte number (Masu et al., 1993; Bugga et al., 1998). A recent study then demonstrated that CT-1 was a key gliogenic ligand. Specifically, Barnabé-Heider et al. (2005) showed that CT-1 was expressed in newly born cortical neurons, that ablation of this neuron-derived CT-1 completely blocked the neurogenic to gliogenic transition in cultured cortical precursors, and that *ct-1*$^{-/-}$ mice had 50%–70% deficits in the level of cortical astrogenesis. Thus, a major extrinsic mechanism for regulating the onset of gliogenesis apparently involves a feedback loop, wherein the first-born cell type, neurons, produces CT-1, which then instructs the parent cortical precursors to generate a second cell type, astrocytes (Figure 1).

BMPs and Notch Activation Instructively Promote Astrogenesis in Collaboration with the JAK-STAT Pathway

Two other important developmental signals, the BMPs and Notch, instructively promote astrogenesis, collaborating at least in part with the gliogenic JAK-STAT pathway. The relevant BMP family members are BMP2 and BMP4,

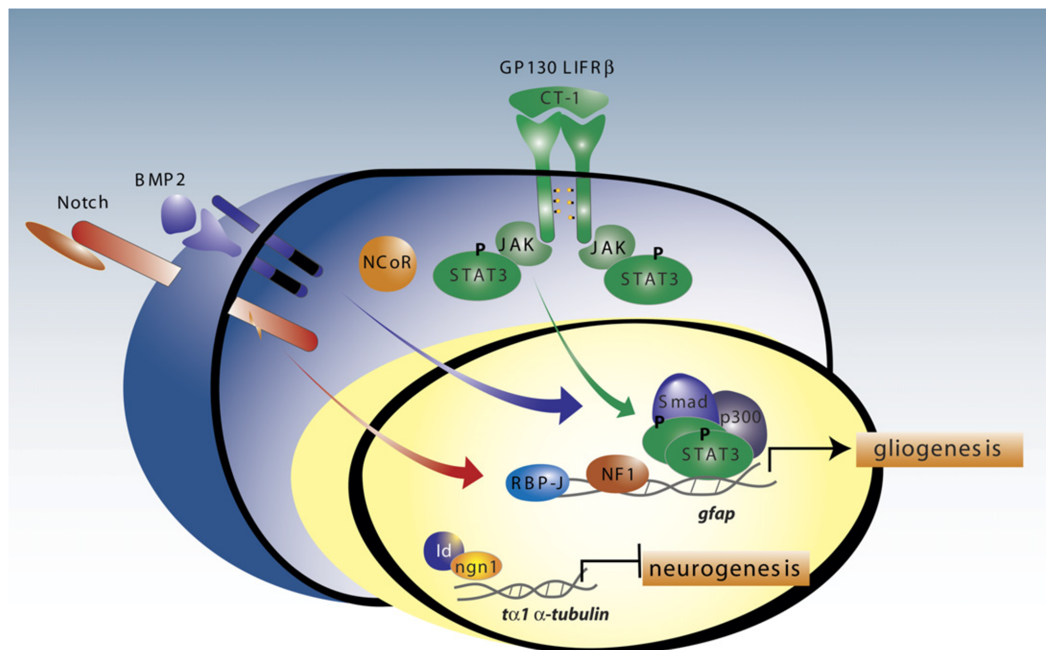

Figure 2. Multiple Environmental Cues Converge to Promote the Gliogenic Switch
At least three different environmental signals, CT-1, BMPs, and Notch ligands, converge to regulate the appropriate timing of gliogenesis. Foremost among these is the gliogenic cytokine CT-1, which binds to the gp130 and LIFRβ coreceptors, which then signal via the JAKs to phosphorylate and activate the STAT3 transcription factors. STAT3 then forms a complex with the Smads, which are downstream of activated BMP receptors, and the coactivator p300/CBP. This complex binds to the promoter of glial genes such as *gfap* to directly promote transcription. The gliogenic cytokine CT-1 also causes translocation of the repressor protein NCoR from the nucleus to the cytoplasm. At the same time, Notch activation causes its downstream effector, the RBP-Jκ transcription factor, to bind and transactivate the *gfap* promoter. Finally, a third key proastrocytic transcription factor, NF1, also binds to the *gfap* promoter. It is the coordinated actions of these different effector pathways that determine the timing and number of astrocytes that are ultimately formed.

which bind to their heterotrimeric serine/threonine kinase receptors to signal largely via activation of the downstream transcription factors, Smads 1, 5, and 8 (reviewed in Chen et al., 2004). These growth factors have multiple effects on neural cell genesis. For example, in cortical precursors BMP2 enhances neurogenesis during the neurogenic period (Li et al., 1998; Mabie et al., 1999) and instructively promotes astrocyte formation during the gliogenic period (Gross et al., 1996; Gomes et al., 2003). Insights into the underlying gliogenic mechanism came from a key study showing that, in precursors exposed to gliogenic cytokines and BMP2, Smad1 forms a transcriptional complex with activated STAT3 and p300/CBP (Nakashima et al., 1999b). Interestingly, binding of Smad1 to p300/CBP is independent of interactions between p300/CBP and the STATs or ngn1 (Sun et al., 2001), providing a potential molecular explanation for the dual actions of BMP2. In this model, during the gliogenic period, when ngn1 levels are low, exposure to BMP2 and gliogenic cytokines causes formation of a Smad:p300/CBP:STAT complex that transactivates gliogenic genes. Under these conditions, BMPs also cause expression of inhibitory HLHs such as Id1 (Nakashima et al., 2001) that can antagonize any neurogenic bHLHs expressed in the same precursors, thereby ensuring that precursors make glia and not neurons (Figure 2). In contrast, during the neurogenic period, precursors express high levels of bHLHs like ngn1, and BMP2 exposure then causes formation of a Smad:p300/CBP:ngn1 complex that inhibits gliogenesis by sequestering p300/CBP from the STATs, and that can potentially participate in transactivation of neuronal genes (Sun et al., 2001) (Figure 3). However, while this competitive model is attractive, we still don't know how important BMPs are for gliogenesis in vivo, since mice carrying knockouts of many of the pathway components are embryonic lethal (Chen et al., 2004), and conditional inactivation of *bmpr1a* and/or *bmpr1b* in the nervous system has only been reported to perturb neuronal development (Wine-Lee et al., 2004; Qin et al., 2006).

Like the BMPs, the Notch pathway has been the subject of intense investigation (reviewed in Kadesch, 2004; Louvi and Artavanis-Tsakonas, 2006). In very basic terms, Notch, upon binding to its ligands, is activated and cleaved, and the Notch intracellular domain (NICD) then translocates to the nucleus, where it interacts with RBP-Jκ (also termed CSL, CBF1, and suppressor of hairless) to form a transcriptionally active complex. RBP-Jκ directly regulates transcriptional events, including transcription of the prototypic Notch effectors, the *hes* inhibitory *bhlh*

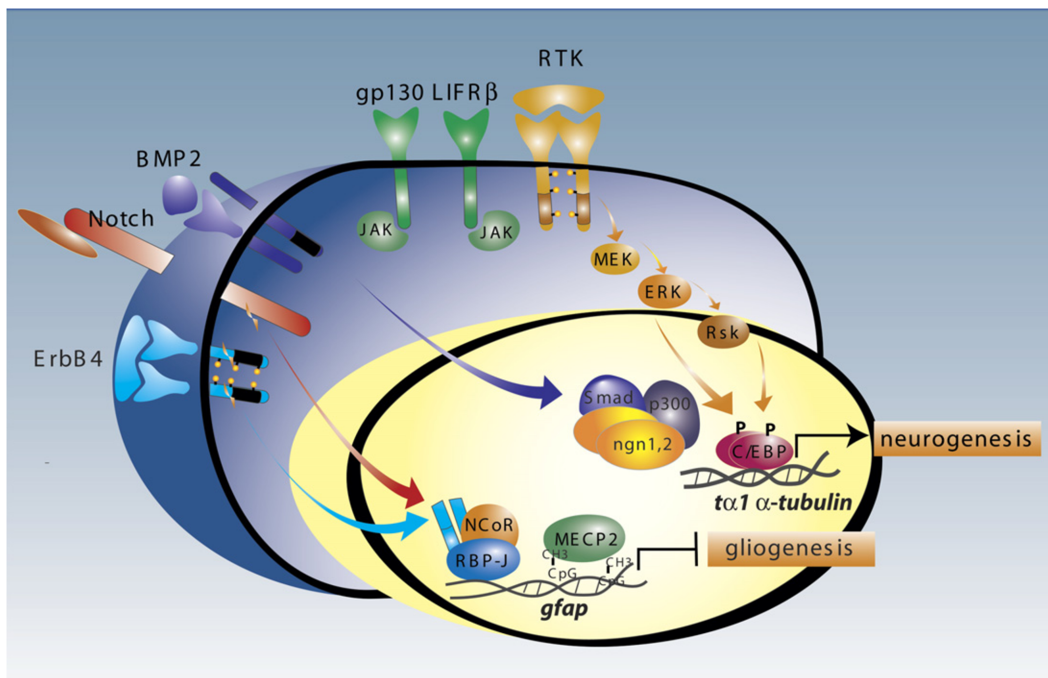

Figure 3. Intrinsic Mechanisms and Environmental Signals Work in Concert to Promote Neurogenesis and Repress Gliogenesis during the Neurogenic Period

Multiple mechanisms determine the timing and numbers of neurons that are generated during the neurogenic period. Foremost among these are the neurogenic bHLH proteins, which directly promote transcription of neuronal genes and at the same time inhibit gliogenesis by sequestering the p300/CBP cofactors. During the neurogenic period, BMPs like BMP2 also signal via their cognate receptors to promote neurogenesis, at least partially by interactions between the activated Smad transcription factors and p300/CBP. In addition, receptor tyrosine kinase receptors bind to growth factors such as the neurotrophins and PDGF and directly activate a MEK-ERK-Rsk pathway that induces phosphorylation of the C/EBP family of transcription factors. The C/EBPs then bind directly to the promoters of neuronal genes, such as that encoding Tα1 α-tubulin, and drive transcription of those genes. At the same time, gliogenesis is repressed by both intrinsic and extrinsic mechanisms during the neurogenic period. In particular, glial genes such as the *gfap* and *s100β* genes are methylated, and the MeCP2 protein binds to these methylated CpGs and promotes formation of an inactive chromatin conformation. A second repressive mechanism involves neuregulin binding to the ErbB4 receptor, which causes translocation of the NCoR repressor protein to the nucleus, where it complexes with the Notch effector RBP-Jκ and binds directly to the *gfap* promoter. Finally, pathways such as the MEK-ERK-C/EBP pathway that promote neurogenesis at the same time inhibit gliogenesis by as yet undefined mechanisms.

genes. With specific regard to mammalian neural precursors, activation of the Notch pathway has two distinct and temporally dissociable effects. During the neurogenic period, Notch signaling inhibits neurogenesis and promotes the maintenance of neural precursors such as radial glia (Gaiano et al., 2000). This important role is particularly evident in animals lacking components of the Notch signaling pathway, all of which demonstrate depletion of neural precursors and premature neurogenesis (reviewed in Yoon and Gaiano, 2005). Notch also instructively promotes gliogenesis, as first demonstrated in the peripheral nervous system and retina (Morrison et al., 2000; Furukawa et al., 2000). Similar findings were then reported for embryonic telencephalic precursors (Chambers et al., 2001; Grandbarbe et al., 2003) and adult hippocampal precursors (Tanigaki et al., 2001), suggesting that this is a general Notch function.

How does Notch promote gliogenesis? Evidence indicates that it may do so via both RBP-Jκ and the Hes bHLH transcription factors. With regard to RBP-Jκ, this transcription factor binds directly to the *gfap* promoter and promotes transcription, but only when the JAK-STAT pathway is coincidentally activated (Ge et al., 2002). When the JAK-STAT pathway is not activated, RBP-Jκ instead binds to a repressive cofactor protein, NCoR (Hermanson et al., 2002), which functions to repress gliogenic genes (described in detail below). As for the Hes proteins, Hes1 and the Hes-related proteins Hesr1 and Hesr2 all inhibit neurogenesis during the neurogenic period and, when ectopically expressed during the gliogenic period, promote astrogenesis (Sakamoto et al., 2003; Wu et al., 2003; Furukawa et al., 2000; Kageyama et al., 2005). The Hes proteins apparently mediate these effects by inhibiting neurogenic bHLHs (which promote neurogenesis and repress gliogenesis, as discussed in detail below), and by promoting activation of the JAK-STAT pathway (Kamakura et al., 2004). These various studies provide evidence that RBP-Jκ and Hes1 require coincident activation of the JAK-STAT pathway for their gliogenic actions, thereby suggesting that, like BMP2, environmental cytokines like CT-1 are necessary for Notch to become gliogenic. However, the in vivo importance of

Notch signaling relative to these other gliogenic pathways is still unclear, since mice genetically deficient in the Notch pathway exhibit early embryonic lethality, and a conditional knockout of the Notch receptors in the developing nervous system has not yet been reported.

Silence Is Golden: Gliogenesis Is Repressed during Neurogenesis by Intrinsic Mechanisms

While these studies demonstrate the importance of extrinsic positive signals for driving gliogenesis, they do not indicate how gliogenesis is silenced during the neurogenic period. This is a key issue, since at least two gliogenic cytokines, neuropoietin (Derouet et al., 2004) and cardiotrophin-like cytokine (Uemura et al., 2002), are expressed in the early embryonic cortex and yet there is no gliogenesis. However, insights into this issue derive from recent work showing that precursor cells change in their competence to respond to gliogenic cytokines over development. For example, a comparison of E10/11 and E14 cortical precursors showed that the younger precursors required prolonged culturing before they made astrocytes and responded poorly to cytokines with regard to JAK-STAT pathway activation and *gfap* transactivation (He et al., 2005). Thus, precursors change intrinsically over time, and these intrinsic changes are important for the neurogenic/gliogenic timing mechanism.

What are these intrinsic repressive mechanisms? One mechanism involves epigenetic silencing of genes that are necessary for astrocyte formation via DNA methylation and/or chromatin modifications. The first demonstration of this involved the finding that the STAT3 binding site in the *gfap* promoter was preferentially methylated in neurogenic versus gliogenic cortical precursors, and that this methylation inhibited STAT3 association and *gfap* transcription (Takizawa et al., 2001). The in vivo importance of this methylation was then demonstrated by analysis of a conditional knockout of the DNA methyltransferase 1 (*dnmt1*) gene in neural precursors (Fan et al., 2005). Brains of these mice displayed decreased numbers of neurons, precocious astrogenesis, and aberrant upregulation of the *gfap* and *s100*β genes, the latter of which is also methylated in early cortical precursors (Namihira et al., 2004). Interestingly, genes in the gp130-JAK-STAT pathway were also derepressed in *dnmt*$^{-/-}$ precursors, and STAT3 inhibition abolished the observed increase in gliogenesis (Fan et al., 2005). A subsequent study provided further support for the idea that methylation of genes in the gp130-JAK-STAT pathway regulated the gliogenic potential of cortical precursors and demonstrated that cytokines themselves derepressed the pathway, thereby defining a positive feedforward loop (He et al., 2005). Thus, in early neural precursors, DNA methylation represses genes encoding astrocyte-specific genes and the gp130-JAK-STAT pathway, and this repression is lifted as precursors develop.

A second mechanism for repressing gliogenesis during early neural development involves the neurogenic bHLHs. While many studies have defined a role for bHLHs like ngn1, ngn2, and Mash1 in neurogenesis and neuronal differentiation (reviewed in Bertrand et al., 2002), it has only recently been appreciated that they also regulate gliogenesis. This was first shown in studies ablating or overexpressing these bHLHs. Mice carrying mutations in *mash1* and *math3* (Tomita et al., 2000), or to a lesser extent, *mash1* and *ngn2* (Nieto et al., 2001) exhibited decreased neurogenesis, and enhanced and premature astrogenesis. Conversely, overexpression of neurogenic bHLHs either in vivo during the gliogenic period (Cai et al., 2000) or in cultured precursors exposed to CNTF (Sun et al., 2001) promoted neurogenesis at the expense of gliogenesis. Moreover, inhibition of neurogenic bHLHs by ectopic expression of their endogenous inhibitors, the HLHs Id1 and Id2, inhibited neurogenesis (Toma et al., 2000; Cai et al., 2000) and promoted gliogenesis (Cai et al., 2000). Thus, neurogenic bHLHs bias precursors to make neurons and at the same time inhibit cytokine-mediated astrocyte formation. Interestingly, one of the mechanisms underlying the repressive gliogenic effect involves a direct interaction between neurogenic bHLHs and the JAK-STAT pathway; Ngn1 binds to and sequesters CBP/p300, so that it is not available to bind to activated STAT3, thereby inhibiting gliogenic transcription (Sun et al., 2001). Such a mechanism would ensure that precursors biased to make neurons by high levels of neurogenic bHLHs would not be permitted to become astrocytes in response to gliogenic cytokines.

Silence Is Golden II: Environmental Signals Regulate Precursor Cell Competence

In addition to these intrinsic mechanisms, the gliogenic competence of neural precursors is regulated by environmental signals. One example of this involves receptor tyrosine kinase (RTK)-mediated activation of a SHP-2-MEK-ERK-Rsk pathway. This signaling cascade promotes neurogenesis (Ménard et al., 2002; Barnabé-Heider and Miller, 2003; Paquin et al., 2005; Liu et al., 2006; Gauthier et al., 2007), at least in part by enhancing phosphorylation of the C/EBP family of transcription factors, which are well known for regulating developmental cell genesis outside of the nervous system (Lane et al., 1999; Yamanaka et al., 1998). Phosphorylation of the C/EBPs then promotes neurogenesis via direct transactivation of neuronal genes such as *t*α*1* α*-tubulin* (Ménard et al., 2002) and *math2* (Uittenbogaard et al., 2007). At the same time, this pathway utilizes several distinct mechanisms to regulate the timing and extent of astrocyte formation. First, RTK-mediated activation of the protein tyrosine phosphatase SHP-2 both enhances activation of the MEK-ERK pathway and directly represses the gp130-JAK-STAT pathway, thereby promoting neurogenesis and ensuring that astrogenesis does not occur during the neurogenic period (Gauthier et al., 2007). Second, the C/EBPs, and their upstream activators MEK and ERK, potently repress astrocyte formation via an as-yet-undefined mechanism (Ménard et al., 2002; Barnabé-Heider and Miller, 2003; Paquin et al., 2005; Liu et al.,

2006). Interestingly, like ngn1, the C/EBPs bind to p300/CBP (Mink et al., 1997), suggesting that they too might inhibit gliogenesis by sequestering this essential cofactor from STAT. However, the C/EBPs both activate and repress transcription, suggesting that they might also act by directly repressing gliogenic genes. Thus, at least one growth factor-driven signaling pathway functions to inhibit gliogenesis during the neurogenic period.

In addition to the C/EBPs and bHLHs, which directly bias precursors to make neurons rather than glia, several transcription factors repress gliogenic genes as part of a mechanism to maintain precursor cells in an undifferentiated state. One of these is ATF5, which inhibits cortical precursors from differentiating into neurons or glia (Angelastro et al., 2003, 2005; Mason et al., 2005). Interestingly, at least some ATF5 family members can interact with C/EBPs and alter their DNA binding specificity (Shuman et al., 1997), suggesting that ATF5 might inhibit differentiation by binding to and regulating C/EBPs and/or other leucine zipper transcription factors that promote differentiation. A second growth factor-regulated signaling pathway that represses gliogenesis during the neurogenic period involves the growth factor neuregulin-1, and the protein N-CoR, a corepressor for multiple transcription factors that acts by forming a complex with histone deacetylases. The first indication that N-CoR was important for repressing gliogenesis came from a study showing that gliogenesis was prematurely and robustly activated in the *n-cor*$^{-/-}$ embryonic forebrain, and that cultured *n-cor*$^{-/-}$ cortical precursors did not self-renew, but instead differentiated into astrocytes (Hermanson et al., 2002). Overexpression of N-CoR could also inhibit cytokine-mediated gliogenesis, and this repression was dependent upon an interaction between N-CoR and the Notch effector RBP-Jκ, which together bound to the *gfap* promoter. Importantly, CNTF stimulation caused translocation of N-CoR to the cytoplasm, thereby providing a potential mechanism for derepressing glial genes in response to a gliogenic environment. More recently, Sardi et al. (2006) demonstrated that this repressive action of N-CoR was regulated by environmental signals; binding of neuregulin-1 to its ErbB4 receptor led to cleavage and release of the receptor intracellular domain, which formed a complex with the adaptor protein TAB2, and N-CoR. This complex translocated to the nucleus, where it associated with and repressed transcription of both the *gfap* and *s100β* promoters. Further evidence that neuregulin-1 suppresses astrogenesis came from studies showing that inhibition of the neuregulin-1 receptor ErbB2 in cortical radial glial precursors caused them to prematurely transform into astrocytes (Schmid et al., 2003). Together, these findings support a model where, during the neurogenic period, coincident ErbB2/ErbB4 and Notch activation cause formation of an ErbB4/TAB2/NCoR/RBP-Jκ complex that directly and potently represses gliogenic genes. When precursors are exposed to CT-1 during the gliogenic period, N-CoR translocates to the cytoplasm, STATs are activated, and RBP-Jκ is derepressed so that it can enhance STAT-mediated gliogenic gene expression (Figures 2 and 3).

While these studies defined environmental cues that repressed gliogenic competence during the neurogenic period, two other growth factors, EGF and FGF2, were shown to promote gliogenic competence. In this regard, increased expression of EGFR in neural precursors enhances astrogenesis, an effect that requires at least some JAK-STAT pathway activation (Burrows et al., 1997; Viti et al., 2003). Moreover, EGFR is expressed asymmetrically in neural precursors, segregating differentially to daughter cells with differing fates, with high-EGFR daughters coexpressing radial glia/astrocytic markers (Sun et al., 2005). However, it has been difficult to define the necessity for EGFR in regulating glial competence, since studies of *egfr*$^{-/-}$ mice indicate that this receptor also plays a key role in astrocyte differentiation, migration, and apoptosis (Sibilia et al., 1998; Kornblum et al., 1998; Wagner et al., 2006). Perhaps an acute knockdown of EGFR mRNA in neural precursors will resolve this issue. As for FGF2, it is a potent and important mitogen for neural precursors, and *fgf2*$^{-/-}$ mice have smaller brains and decreased numbers of both neurons and glia (Dono et al., 1998; Ortega et al., 1998; Vaccarino et al., 1999). In cultured precursors, FGF2 does not on its own induce astrocyte formation, but it does promote cytokine-mediated astrocyte formation, at least partially by promoting an active chromatin conformation around the *gfap* gene (Song and Ghosh, 2004). Interestingly, conditional ablation of the Brg1 ATP-dependent chromatin remodeling factor in neural precursors in vivo caused precocious neuronal differentiation and a failure of glial differentiation (Matsumoto et al., 2006), indicating that such environmentally regulated chromatin remodeling plays a key role in regulating all aspects of precursor cell fate determination during embryogenesis. Importantly, one of the major conclusions from this body of work is that, while multiple environmental cues regulate the neurogenic to gliogenic switch, many of them do so at least partially by converging to repress, derepress, or promote signaling via the gp130-JAK-STAT pathway.

A Transcription Factor Code for Astrocyte Formation?

The concept of a transcription factor code for cell genesis in the mammalian nervous system arose largely out of work demonstrating such a code in the spinal cord (Lee and Jessell, 1999), and studies demonstrating that combinations of positively acting bHLHs were essential for neurogenesis (Bertrand et al., 2002). Subsequent work demonstrating that oligodendrocyte development also required positively acting bHLHs, the oligs (Lu et al., 2002; Zhou and Anderson, 2002), supported this idea. However, until recently no specific proastrocytic transcription factor(s) had been identified, other than those that were downstream of gliogenic signaling pathways, such as the STATs and RBP-Jκ. This situation has changed in the past several years, with identification of

several proastrocytic transcription factors, the most important of which appears to be nuclear factor-1 (NFI). NFI-A, -B, -C, and -X are a transcription factor family that is expressed with overlapping but unique patterns of expression throughout the entire animal (reviewed in Gronostajski, 2000). While NFIs activate and repress multiple genes in many tissues, the first hint that they might be important for astrocyte development came from work showing that the *gfap* promoter contains a transcriptionally important NFI binding site (Krohn et al., 1999; Gopalan et al., 2006; Cebolla and Vallejo, 2006). At the same time, analysis of embryonic *nf1-a*$^{-/-}$ and *nf1-b*$^{-/-}$ brains demonstrated developmental disruption of the corpus callosum, a large reduction in embryonic midline glia, and 5- to 10-fold decreases in GFAP mRNA and protein (das Neves et al., 1999; Shu et al., 2003; Steele-Perkins et al., 2005). The precise role that NF1 plays during astrocyte development was then elucidated when it was found that NFIA/B is necessary and sufficient for the appropriate timing and levels of astrogenesis in the developing spinal cord (Deneen et al., 2006). Interestingly, this same study showed that NFIA is also necessary for oligodendrocyte fate specification, and for the repression of neurogenesis, the latter an effect that it mediates via the Notch effector Hes5. NFI may also inhibit neurogenesis directly, since it can bind to and repress transcription of at least one neuron-specific gene (Adams et al., 1995). Thus, NFI is expressed in neural precursors immediately prior to gliogenesis, at which point it may well collaborate with the CT-1-mediated JAK-STAT pathway to promote astrocyte formation, potentially by direct interactions between NFI and the STATs, as is seen in nonneural cells (Mukhopadhyay et al., 2001). Moreover, NFI, like the STATs, Ngn1, and the C/EBPs, binds to p300/CBP (Leahy et al., 1999), and thus CBP may provide a locus for coordination of all of these various gliogenic and neurogenic signals.

In addition to NFI, which appears to be required for astrogenesis throughout the developing CNS, two positively acting astrogenic bHLHs have more circumscribed functions. One of these, scl (for stem cell leukemia, also known as Tal1), is important for cell determination in the hematopoietic system and was recently shown to be essential for genesis of astrocytes within the p2 domain of the developing spinal cord (Muroyama et al., 2005). Scl was also required for the development of multiple populations of neurons (Bradley et al., 2006), indicating that it is not a proastrogenic bHLH per se, but rather appears to be a prodifferentiation factor for multiple neural cell types. A second positively acting bHLH, neurogenin3 (ngn3), and Sox9 have also both been reported to be important for gliogenesis in the developing spinal cord (Lee et al., 2003; Stolt et al., 2003). Interestingly, in both cases, animals lacking these transcription factors displayed deficits in both oligodendrocytes and in astrocytes, suggesting that ngn3 and Sox9 likely act to promote the genesis, maintenance, and/or differentiation of a bipotent glial precursor, rather than to specifically promote astrogenesis.

In this regard, one final issue that needs to be raised here concerns the transcriptional mechanisms that are involved in specifying astrocytes versus oligodendrocytes. As indicated in the introduction, the onset of gliogenesis in the cortex commences with the appearance of differentiated astrocytes, and this is followed by differentiated oligodendrocytes. However, until very recently it was thought that cortical oligodendrocytes did not develop from the same cortical precursors as did neurons and astrocytes, but that biased oligodendrocyte precursors were instead generated in the ventral forebrain, and these migrated into and populated the developing cortex. Recent work has challenged this idea and has instead defined a second wave of late-differentiating oligodendrocyte precursors that derive from multipotent cortical precursors within the dorsal cortex (Kessaris et al., 2006). Thus, a key question for the future is how bipotent gliogenic precursors choose to make astrocytes versus oligodendrocytes. Interestingly, a number of recent papers indicate that olig bHLHs, which are best known for their ability to promote oligodendrocyte formation (Ligon et al., 2006) while repressing astrogenesis (Zhou and Anderson, 2002), perform the latter function both by binding to p300/CBP and sequestering it from STAT3 (Fukuda et al., 2004), and by antagonizing the proastrocytic effects of NFI (Deneen et al., 2006). Moreover, evidence indicates that Hes1 both promotes astrogenesis via interactions with the JAK-STAT pathway (Kamakura et al., 2004) and inhibits oligodendrocyte formation by antagonizing the olig bHLHs (Wu et al., 2003). Thus, multiple mechanisms likely exist to regulate the choices made by gliogenic precursors, and it will be interesting to determine how these mechanisms ensure the correct timing and numbers of these two cell types.

What's Next?

Together, these studies have provided us with an integrated view of the neurogenic switch, with multiple extrinsic and intrinsic mechanisms acting in concert to repress gliogenesis during the neurogenic period, and then to induce gliogenesis when an appropriate number of neurons has been generated (Table 1). However, many questions remain. First, what keeps neurogenesis turned off during the gliogenic period? We know that gliogenic precursors can still make neurons under some conditions, since overexpression of neurogenic bHLHs is sufficient to promote neurogenesis in the postnatal CNS (Cai et al., 2000). We also know that gp130-JAK-STAT signaling (Bonni et al., 1997; Barnabé-Heider et al., 2005) and NFI (Deneen et al., 2006) both repress neurogenesis at the same time that they promote gliogenesis, but we don't have a detailed understanding of how this happens. Such information is important, not only with regard to development, but also with regard to the adult nervous system. For example, why do adult precursors from the SVZ and in the hippocampus make neurons, even though transplant studies have shown us that the adult CNS is a gliogenic environment? Would such information allow us to

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Table 1. Molecular Perturbations that Lead to Premature Astrocyte Formation In Vivo

Gene	Reference	Manipulation and Phenotype
Growth factors, growth factor receptors, and signaling pathways		
ErbB2	Schmid et al., 2003	Inhibition of ErbB2 in radial glia and astrocytes in transgenic mice by expression of a dominant-negative ErbB2 from the human *gfap* promoter led to appearance of astrocytes in the cortex by E16.
ErbB4	Sardi et al., 2006	Analysis of *erbB4*$^{-/-}$ mice in which the heart defect was rescued demonstrated robust astrocyte formation in the cortex by E17.5.
EGFR	Burrows et al., 1997	Overexpression of EGFR in E12 rat cortical explants led to premature appearance of S100β+, GFAP+ astrocytes 4 days later (equivalent to E16).
CNTF	Barnabé-Heider et al., 2005	Ectopic expression of CNTF in cortical precursors at E13.5 using in utero electroporation led to the appearance of GFAP+, S100β+ astrocytes in the cortex by E16
SHP-2	Gauthier et al., 2007	Genetic knockdown of SHP-2 in cortical precursors at E13/14 caused premature formation of GFAP+ astrocytes at E16/17.
Transcriptional regulators		
Dnmt1	Fan et al., 2005	Conditional ablation of *dnmt1* in neural precursors, achieved by crossing nestin:Cre mice with floxed *dnmt1* mice, led to premature appearance of GFAP+ cells in the spinal cord as early as E12, and in the cortex, by E18.
N-CoR	Hermanson et al., 2002	Analysis of *n-cor*$^{-/-}$ mice demonstrated robust premature astrogenesis by E15.5 in the developing neocortex of more than 70% of the knockout mice.
Neurogenic bHLHs	Tomita et al., 2000; Nieto et al., 2001	Analysis of *mash1*$^{-/-}$;*math3*$^{-/-}$ double mutant mice revealed premature astrocyte formation within both the midbrain and hindbrain by E15.5. Analysis of the cortex of *mash1*$^{-/-}$;*ngn2*$^{-/-}$ embryos demonstrated the presence of RC2+ cells with astrocyte morphology at E15.5 in all mice, and premature GFAP+ astrocytes at E18.5 in one of three embryos.
Olig bHLHs	Zhou and Anderson, 2002	Analysis of *olig1*$^{-/-}$;*olig2*$^{-/-}$ double mutant embryos revealed ectopic astrocytes within the embryonic spinal cord.
Id1	Cai et al., 2000	Overexpression of Id1 in cortical precursors at E12 using retroviruses in vivo led to premature genesis of cells with glial morphology by E16.
NFIA/B	Deneen et al., 2006	Overexpression of NFIA/B in embryonic chick spinal cord precursors caused premature induction of cells expressing both early and late astrocyte markers.
Scl	Muroyama et al., 2005	Overexpression of scl in embryonic chick spinal cord precursors caused appearance of ectopic early astrocytes.

manipulate the environment of the mature, injured CNS to promote neurogenesis rather than gliogenesis?

A second key question is how intrinsic changes in precursor cell responsiveness might be regulated by environmental cues. Several potential mechanisms are suggested by studies in other cell types. In epithelial cells, MEK can induce selective promoter methylation by dnmt3 (Pruitt et al., 2005) suggesting that one way that the MEK-ERK-C/EBP pathway might repress gliogenesis is by causing methylation of astrocyte genes. Moreover, STAT3 itself can directly form a complex with dnmt1 (Zhang et al., 2005), suggesting that the gliogenic JAK-STAT pathway may also directly regulate epigenetic silencing in the developing nervous system. Finally, the methyl-CpG-binding protein 2 (MeCP2), which binds to methylated CpG dinucleotides and modifies chromatin structure, was recently shown to be phosphorylated in response to Ca^{2+} influx in neurons (Zhou et al., 2006). Since MeCP2 is mutated in a variety of neurodevelopmental disorders, including Rett syndrome (Moretti and Zoghbi, 2006), and since it binds to the promoters of glial genes in early precursors (Namihira et al., 2004; Fan et al.,

2005), then this provides a mechanism whereby neurotransmitter or growth factor receptor activation could directly regulate chromatin structure. Thus, there are multiple ways that the environment could regulate the ''intrinsic'' changes seen in cortical precursors, and a key issue is what, if any, role these play in regulating the gliogenic timing mechanism.

A third and final question for the future is whether perturbations of the neurogenic-to-gliogenic timer play any role in neurodevelopmental disorders that result in cognitive dysfunction or mental retardation. One would imagine that alterations in the timing and/or numbers of different cells that were generated during embryogenesis would have a profound impact on the subsequent establishment and function of neural circuitry. Support for this idea comes from a recent study demonstrating that enhanced activation of SHP-2 in a mouse model of Noonan syndrome, a human genetic disorder where one-third to one-half of affected individuals have learning disabilities or mental retardation, caused perturbations in cortical cell fate genesis (Gauthier et al., 2007). Interestingly, the neurogenic SHP-2-Ras-MEK-ERK pathway is also involved in the related genetic disorders Costello syndrome and cardio-facial-cutaneous syndrome, both of which display mental retardation as part of their phenotype (reviewed in Bentires-Alj et al., 2006). Moreover, even heterozygosity for a loss-of-function allele of the coactivator CBP, which as discussed above is a key integrator of multiple neurogenic and gliogenic transcription factors, is sufficient to cause mental retardation in Rubinstein-Taybi syndrome (Josselyn, 2005). All of these genetic perturbations would be predicted, by the studies reviewed here, to alter cell fate genesis in the embryonic nervous system, and to thereby set the stage for a miswired and dysfunctional CNS. However, with the exception of the Noonan syndrome mouse, we still don't know whether such developmental dysgenesis actually occurs, and/or its relative importance for the development of human cognitive dysfunction. Addressing this and the other key issues raised here will provide us with insights into both normal and aberrant development and may also help us to recruit endogenous and exogenous neural stem cells to repair the injured or degenerating nervous system.

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