Specification of CNS glia from neural stem cells in the embryonic neuroepithelium

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All the neurons and glial cells of the central nervous system are generated from the neuroepithelial cells in the walls of the embryonic neural tube, the 'embryonic neural stem cells'. The stem cells seem to be equivalent to the so-called 'radial glial cells', which for many years had been regarded as a specialized type of glial cell. These radial cells generate different classes of neurons in a position-dependent manner. They then switch to producing glial cells (oligodendrocytes and astrocytes). It is not known what drives the neuron–glial switch, although downregulation of pro-neural basic helix–loop–helix transcription factors is one important step. This drives the stem cells from a neurogenic towards a gliogenic mode. The stem cells then choose between developing as oligodendrocytes or astrocytes, of which there might be intrinsically different subclasses. This review focuses on the different extracellular signals and intracellular responses that influence glial generation and the choice between oligodendrocyte and astrocyte fates.

Keywords: CNS; oligodendrocyte; astrocyte; development

1. INTRODUCTION

The nervous system is composed of neurons and glial cells. Glial cells in the central nervous system (CNS) include both the 'macroglia', which are derived from the neural tube, and the 'microglia', which are derived from haemopoietic precursors. Microglia are the resident macrophages of the CNS and play a key role in immune surveillance and defence. This review is concerned with the two major classes of macroglia: astrocytes and oligodendrocytes. These cells were first visualized over a century ago (Andriezen 1893) and were given names that described their morphology in Golgi tissue preparations: astrocytes ('star-like') and oligodendrocytes ('sparsely branching'). The term 'glial' originally referred to the sticky nature of the substance we now know as white matter, the myelinated axon tracts. This seeded the idea that glial cells, which are abundant in white matter, have a purely passive support role—the 'glue' holding the neurons together (for a historical review see Pasik & Pasik 2004). Today, it is recognized that glial cells play active roles in nervous system function, even forming synaptic contacts with neurons and contributing in some way to information storage or processing. Oligodendrocytes have a well-defined function—formation of the insulating myelin sheaths around CNS axons—but they and their precursors also express voltage- and ligand-gated ion channels and have synaptic input from neurons. Astrocytes have many described functions: (i) they provide structural and trophic support for neurons, (ii) they interact intimately with blood vessels and induce formation of the blood–brain barrier, and (iii) they regulate CNS synaptogenesis and synaptic transmission. Despite the functional versatility of glial cells and the fact that they significantly outnumber neurons in most parts of the CNS, we know relatively little about their intrinsic diversity and how this arises during development.

The multitude of neuronal and glial cell types of the mature CNS are generated from an initially homogeneous pool of naive neural precursors (NPCs) in the embryonic neural tube, the so-called neuroepithelial precursor cells (NEPs). The NEPs first generate neurons, followed by glial cells. At early stages, NEPs are multipotent with the capacity to generate all types of CNS neurons and glia. With time, their potential is restricted by exposure to surrounding environmental signals that transmit positional information about both dorsal–ventral and anterior–posterior axes. These positional signals include morphogens such as Sonic hedgehog (Shh) and members of the bone morphogenetic protein (BMP) family. How this positional information is interpreted by precursor cells and how it leads them to adopt specific cell fates is one of the major questions in developmental biology today.

This review documents some of what we presently know about glial cell specification, where they are specified, and the key extracellular signals and the intracellular signal transduction pathways that are involved.

2. OLIGODENDROCYTES
(a) *The origins of oligodendrocytes*

Unlike neurons, which become post-mitotic soon after they are specified, oligodendrocytes are initially specified as proliferative, migratory precursor cells. These were first identified in cultures of rat optic nerve

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cells (Raff *et al.* 1983), at which time they were named oligodendrocyte-type-2 astrocyte progenitor cells (O-2A progenitors) to denote the fact that they can differentiate into either oligodendrocytes or astrocytes (type-2 subtype), depending on the culture medium. However, it has so far not proved possible to identify cells with the antigenic phenotype of type-2 astrocytes *in vivo*, so the precursor cells are now more usually referred to simply as oligodendrocyte precursor or progenitor cells (OLPs or OPCs (OLP used herein)). These go through several identifiable maturation stages characterized by distinct morphological and antigenic changes, before finally associating with axons and elaborating myelin. Since mature myelinating oligodendrocytes are ubiquitous throughout the mature CNS, they were originally presumed to be derived more-or-less equally from all parts of the embryonic germinal zones—the so-called ventricular zone (VZ) that surrounds the ventricles of the brain and the central canal of the spinal cord. However, with the discovery of early markers of the oligodendrocyte lineage, such as the platelet-derived growth factor receptor-alpha (Pdgfr-α; Pringle & Richardson 1993), the myelin proteolipid protein isoform DM20 (Timsit *et al.* 1995) and the O4 antigen (Noll & Miller 1993), came the realization that OLPs might be generated from defined, spatially localized subsets of NEPs.

In the spinal cord, the first OLPs are generated from ventral NEPs (Warf *et al.* 1991; Noll & Miller 1993; Pringle & Richardson 1993; Yu *et al.* 1994; Timsit *et al.* 1995) from a specific domain of the VZ called pMN that also generates motor neurons (MNs; Richardson *et al.* 1997; Sun *et al.* 1998; Lu *et al.* 2002; Takebayashi *et al.* 2002; Zhou & Anderson 2002). The proliferating OLPs migrate away and rapidly populate the entire spinal cord before differentiating into myelin-forming oligodendrocytes. Recent studies using mouse mutants that lack this ventral source of OLPs showed that dorsal NEPs also generate some OLPs (Cai *et al.* 2005; Vallstedt *et al.* 2005). This was confirmed by Cre-*lox* fate mapping in wild-type mice (Fogarty *et al.* 2005). The dorsal half of the spinal cord neuroepithelium contributes approximately 20% of all oligodendrocytes in the cervical spinal cord and up to 50% of the oligodendrocytes in the dorsal funiculus (dorsal axon tracts; Fogarty (2005); for a review see Richardson *et al.* (2006); figure 1). In the mouse, the dorsally derived OLPs start to appear around embryonic day 15, approximately 2 days later than their ventrally derived counterparts. When they first appear, they have a distinctive radial morphology and co-label with the radial glial marker RC2 (Fogarty *et al.* 2005), verifying previous suggestions that oligodendrocytes as well as astrocytes might form by direct transformation of radial glia (Choi *et al.* 1983; Hirano & Goldman 1988; Voigt 1989; Culican *et al.* 1990; Barry & McDermott 2005).

A similar theme is observed at more anterior levels of the neuraxis. In the forebrain, the first Pdgfr-α-positive OLPs appear in the ventral neuroepithelium (anterior entopeduncular area, AEP; medial ganglionic eminence, MGE), then spread throughout the forebrain by migration and proliferation (Spassky *et al.* 1998; Olivier *et al.* 2001; Spassky *et al.* 2001; Tekki-Kessaris *et al.* 2001). Subsequent waves of OLP generation emanate
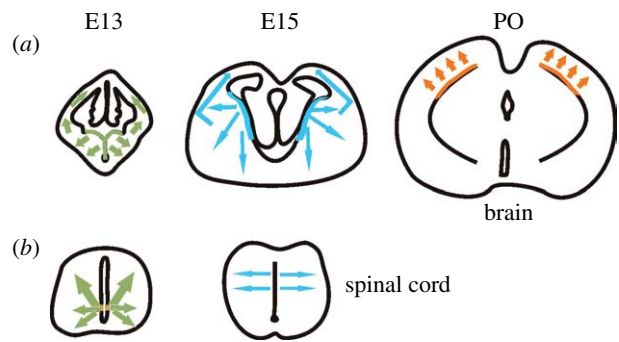

Figure 1. Developmental sites of origin of OLPs. The diagrams depict the sites of origin and migration pathways of OLPs during embryonic and perinatal development. (*a*) Drawings of coronal sections through the developing telencephalon at the indicated ages. Colour marks the sites of origin of OLPs in the VZ and arrows show their presumed migration pathways. (*b*) Transverse sections of the cervical spinal cord showing the early ventral and later dorsal sources along with their dispersal routes. Note: The brain sections are drawn to scale relative to each other, as are the spinal cord sections, but the spinal cord is drawn disproportionately large compared with the brain. The ventral sources of OLPs are induced by Shh from the floor plate of the spinal cord or the VZ of the ventral forebrain. The dorsal sources (including the cerebral cortex of the brain) are thought to be Shh independent, possibly relying on fibroblast growth factor. Dorsal OLP generation might also be helped by BMP downregulation in dorsal structures during later embryonic development. See text for a more complete description. E13, embryonic day 13; E15, embryonic day 15; PO, postnatal day 0.

from more dorsal regions (the lateral and the caudal ganglionic eminences (LGE/CGE)) and finally from within the cortex (Kessaris *et al.* 2006). Thus, there is a progressive wave of OLP production from ventral to dorsal regions (figure 1). The very first OLPs generated in the ventral forebrain (MGE/AEP) almost disappear during post-natal life and are replaced by other populations including the LGE/CGE-derived population. This might reflect some selective advantage of more recently generated OLPs that leads to competitive elimination of ventrally derived cells. Alternatively, continual turnover (loss and replacement) of oligodendrocytes during adult life might lead to gradual loss of the MGE/AEP-derived population because stem cells in the adult subventricular zone (SVZ)—the ultimate source of new oligodendrocytes in the adult (Levison & Goldman 1993, 1997; Luskin & McDermott 1994)—are derived from the embryonic LGE/CGE and cortex with little contribution from the MGE/AEP (Young *et al.* submitted).

(b) *Oligodendrocyte specification: cell-extrinsic signals*

(i) *Induction of oligodendrogenesis*

In the spinal cord, Shh is secreted from the notochord and subsequently from the floor plate at the ventral midline of the neural tube. Diffusion of Shh from these regions generates a morphogenetic gradient and, together with complementary signals from the roof plate, initiates a programme of NEP cell fate specification. This involves activation or repression of a set of homeobox, paired-box (Pax) and basic

helix–loop–helix (bHLH) transcription factor genes, which have been classified as either class I or class II depending on whether they are activated (class II) or repressed (class I) by Shh (Briscoe *et al.* 2000). The combinatorial expression of these genes defines progenitor domains along the dorsal–ventral axis and influences the neuronal subtypes they generate. Shh is thus essential for the generation of different classes of ventral spinal cord neurons. Several studies have shown that Shh also promotes the generation of pMN-derived OLPs in the spinal cord (Poncet *et al.* 1996; Pringle *et al.* 1996; Orentas *et al.* 1999) and is a potent inducer of OLP formation in primary cultures of dissociated forebrain NEPs (Tekki-Kessaris *et al.* 2001; Kessaris *et al.* 2004). In the absence of Shh signalling, ventrally derived OLPs fail to form in the spinal cord or forebrain (Orentas *et al.* 1999; Nery *et al.* 2001; Tekki-Kessaris *et al.* 2001). Furthermore, transplanting Shh-expressing tissue adjacent to the dorsal neural tube *in vivo* (Orentas & Miller 1996; Poncet *et al.* 1996; Pringle *et al.* 1996), or adding Shh to spinal cord explants *in vitro* (Agius *et al.* 2004), induces the formation of ectopic OLPs, highlighting the importance of spatially restricted Shh signalling in directing OLP generation.

The mechanism by which Shh acts in vertebrates is still unclear (Ingham & McMahon 2001). Shh binds to its transmembrane receptor Patched (Ptc), causing disinhibition of its co-receptor Smoothened (Smo), a seven-pass transmembrane G-protein-coupled receptor (GPCR). In *Drosophila*, this eventually promotes nuclear translocation of the full-length form of the transcription factor Cubitus interruptus (Ci) which acts as a transcriptional activator. In the absence of Shh, Ci is proteolytically cleaved to a repressor form of the protein. The mammalian homologues of Ci are the zinc finger proteins of the Gli family (Gli1–Gli3). Owing to the existence of the three closely related members, it has been difficult to decipher their individual contributions to Shh signalling (Jacob & Briscoe 2003). Mouse Gli1 is not proteolytically cleaved and acts as a transcriptional activator with redundant functions during development. Although Gli2 possesses both activator and repressor activities, it is the activator properties that are required for ventral neural tube patterning. Gli3, on the other hand, is thought to act as an inhibitor of Shh signalling. Indeed, several aspects of Shh signalling are restored in mouse mutants lacking both Shh and Gli3 (Litingtung & Chiang 2000), indicative of Gli3 having a repressor function. The current model for Shh patterning in the neural tube is that Shh signalling leads to the formation of a ventral-to-dorsal (high–low) gradient of Gli activities with activator functions predominating ventrally and repressor functions dorsally (Jacob & Briscoe 2003). The requirements for Gli activity within the pMN domain are still unclear, as all three mouse Gli mutants lack defects in MN differentiation. However, mice lacking Gli2 function exhibit delayed and reduced oligodendrogenesis from the pMN domain (Qi *et al.* 2003). The proliferation of OLPs is unaffected in the Gli2 null mice and normal oligodendrocyte numbers are restored before birth, indicating a specific function for Gli2 at the early stages of oligodendrocyte generation (Qi *et al.* 2003).

NEPs from Shh or Smo null mice, the latter lacking all hedgehog (Hh) signalling, can still generate OLPs in culture (Chandran *et al.* 2003; Cai *et al.* 2005). This clearly demonstrates the existence of a Shh-independent pathway to oligodendrocyte specification. *In vitro* evidence suggests that FGF2 might be an alternative inducer of OLPs (Chandran *et al.* 2003; Gabay *et al.* 2003; Kessaris *et al.* 2004). NEPs from the cortex and the dorsal spinal cord can generate OLPs if cultured in the presence of fibroblast growth factor-2 (FGF2) and cyclopamine, a potent inhibitor of Hh signalling (Chandran *et al.* 2003; Gabay *et al.* 2003; Kessaris *et al.* 2004). It is therefore possible that FGF might be responsible for inducing oligodendrocytes in the dorsal spinal cord and/or forebrain, which are unlikely to be within range of Shh from the floor plate (spinal cord) or MGE (forebrain). However, conditional deletion of FGFR1 in the cortical neuroepithelium did not affect the capacity of these cells to generate oligodendrocytes *in vivo* (our own unpublished observations). Whether this indicates that FGF is not involved in oligodendrocyte generation from this region or else reflects redundancy among members of the FGF receptor family remains to be determined.

In addition to Shh and FGF2, insulin-like growth factor I (IGF-I) has also been suggested to influence oligodendrocyte specification (Hsieh *et al.* 2004). Previous evidence had shown that multipotent adult neural progenitors can be induced to generate oligodendrocytes *in vitro* when cultured in the presence of IGF-I, IGF-II or insulin. This effect is independent of the activity of IGFs on oligodendrocyte maturation or survival. The oligodendrogenic activity of IGF-I is thought to be mediated at least in part by inhibition of BMP signalling (see §2*b*(ii)). Whether insulin/IGF plays an important role in the oligodendrocyte specification *in vivo* is not yet known, although several studies have demonstrated the importance of this family of growth factors in oligodendrocyte maturation (Carson *et al.* 1993; Beck *et al.* 1995; Ye *et al.* 1995, 2002)

(ii) *Inhibition of oligodendrogenesis*
BMPs are members of an extended family of transforming growth factor-beta (TGF-β)-related proteins that also includes activins, dorsalin-1 (DSL1), growth/differentiation factors (GDFs) and glial cell-derived neurotrophic factor (GDNF). Several of these proteins (BMPs, GDFs and DSL1) are expressed in the dorsal part of the neural tube including the roof plate, where they are required for specification of subclasses of dorsal spinal interneurons (Liem *et al.* 1997; Lee *et al.* 1998). Members of the BMP family have been proposed to act as negative regulators of oligodendrocyte specification. Forebrain or spinal cord neural stem cells cultured in the presence of BMPs fail to develop oligodendrocytes, even in the presence of Shh or FGF2 (Gross *et al.* 1996; Mekki-Dauriac *et al.* 2002; Yung *et al.* 2002; Vallstedt *et al.* 2005). This raises the question of whether BMPs also regulate oligodendrocyte development *in vivo*. Ectopic expression of BMPs in chick ventral spinal cord inhibits OLP development (Mekki-Dauriac *et al.* 2002);

conversely, inhibition of BMPs with the BMP antagonist Noggin causes an expansion of the oligodendrogenic domain. The same effect is also caused by surgical removal of the roof plate (Mekki-Dauriac *et al.* 2002). In all these experiments, effects on OLP and astrocyte production are reciprocal (see §3). Collectively, the data suggest that endogenous BMP signals spatially restrict neurogenesis and oligodendrogenesis to defined neuroepithelial regions (Hall & Miller 2004).

There is evidence that, in addition, BMPs also inhibit differentiation of OLPs by inducing expression of the *inhibitors of differentiation* (*Id*) genes (Samanta & Kessler 2004). The encoded Id proteins are bHLH proteins related to Olig1/2 (see §2*c*) but lacking a DNA-binding domain; they act as dominant-negative inhibitors of Olig1/2 and other bHLH proteins by binding to them and preventing their interaction with target genes. BMP induces cultured mouse NPCs to express all four Id proteins (Id1–4), but it is mainly Id4 (and to a lesser extent Id2) that inhibits OLP differentiation and expression of maturation markers of the oligodendrocyte lineage such as MBP and CNPase. This inhibition effect is mediated by binding and sequestering not only the Olig proteins, but also their required bHLH cofactors E12 and E47 (Samanta & Kessler 2004). In these experiments the Olig/Id complexes were retained in the cytoplasm and excluded from the cell nucleus.

Members of the Wnt family of proteins are also secreted from the roof plate and might influence gliogenesis in the dorsal neural tube. Unlike BMPs, Wnts are not involved in patterning the dorsoventral axis. They are known to be mitogenic for NPCs (Megason & McMahon 2002), although Wnt-3A was found not to promote proliferation of OLPs *in vitro* (Shimizu *et al.* 2005). Recent spinal cord explant studies indicate that Wnts do not influence OLP specification *per se*, but regulate the differentiation of OLPs to more mature oligodendrocytes (Shimizu *et al.* 2005).

(iii) *Delta–Notch signalling in oligodendrocyte development*
Activation of the Notch receptor through its ligands Delta and Serrate is one of the key mechanisms controlling cell fate decisions in the embryo. In the CNS, neurons are generated before glia and active Notch signalling is essential to maintain a population of proliferative NEPs into the gliogenic period. Accordingly, zebrafish embryos that have deficient Notch signalling have excess neurons in the spinal cord at the expense of OLPs (Appel *et al.* 2001; Park & Appel 2003). Conversely, expression of a constitutively active form of the Notch receptor resulted in excess OLPs at the expense of MNs (Park & Appel 2003). This led to the suggestion that Notch signalling is essential at the early stages of specification of the oligodendrocyte lineage. Notch plays another role later in the oligodendrocyte lineage; cell culture studies showed that constitutive activation of Notch signalling can inhibit oligodendrocyte maturation *in vitro* (Wang *et al.* 1998). Subsequently, it was found that loss of Notch signalling leads to premature differentiation of OLPs to oligodendrocytes *in vivo* (Genoud *et al.* 2002; Givorgi *et al.* 2002), while expression of an active form of the Notch receptor in transgenic zebrafish embryos prevented

OLP differentiation (Park & Appel 2003). Collectively, these data indicate that Notch signalling might regulate both the specification of OLPs and their subsequent developmental maturation to oligodendrocytes. Remyelination studies in rodents, however, have shown that the expression of Notch1 in adult OLPs and its ligand Jagged in surrounding cells has no effect on the rate of remyelination, suggesting that distinct mechanisms of OLP maturation may operate during development and in the adult (Stidworthy *et al.* 2004).

(c) *Oligodendrocyte specification: cell-intrinsic determinants*
(i) *bHLH genes*
Olig1 and Olig2 were originally identified as two bHLH transcription factors that mark the oligodendrocyte lineage from the very early stages (Lu *et al.* 2000; Takebayashi *et al.* 2000; Zhou *et al.* 2000). In the spinal cord, *Olig1* and *Olig2* transcripts can be detected from embryonic day 9 onwards. Their expression is induced by Shh signalling. Transcripts are initially observed in the ventral third of the spinal cord but soon become restricted to the pMN domain. *Olig2* expression persists in pMN throughout MN development; it is rapidly extinguished in post-mitotic MNs but remains on in OLPs. Expression of *Olig1* is more dynamic at early times prior to the appearance of OLPs. Both the *Olig* genes are expressed throughout the oligodendrocyte lineage including in mature oligodendrocytes.

Functional studies in mice have demonstrated the significance of Olig2 in both MN and oligodendrocyte specification (Lu *et al.* 2002; Takebayashi *et al.* 2002; Zhou & Anderson 2002). In the absence of functional Olig2 protein, all spinal MNs and oligodendrocytes fail to develop. Embryos lacking Olig2 die at birth or shortly after due to the absence of motor function. At more anterior levels of the neuraxis, small pockets of oligodendrocytes develop, especially within the fore- and hindbrain, suggesting redundancy between Olig1 and Olig2 (Lu *et al.* 2002). Embryos lacking the function of both the *Olig* genes show complete absence of all oligodendrocytes throughout the CNS (Zhou & Anderson 2002).

Olig2 is thought to have multiple roles in the precursors of MNs. Initially, Olig2 promotes pMN identity by repression of alternative fates (Mizuguchi *et al.* 2001; Novitch *et al.* 2001). For example, Olig2 represses expression of Irx3, a transcription factor that promotes V2 interneuron identity in the ventral spinal cord. Ventral expansion of the p2 domain and the V2 interneuron population can thus be observed in embryos lacking Olig2 function. Olig2 also acts to maintain the MN precursor pool by repressing the expression of MN differentiation factors within the neuroepithelium. By binding directly to E-box elements within the gene encoding MN determinant HB9, Olig2 can repress its expression and inhibit premature differentiation of MNs (Lee *et al.* 2005). In addition, Olig2 is thought to promote the expression of Neurogenin-2 (Ngn2), a pro-neural gene that acts to promote neurogenesis (Lee *et al.* 2005). Olig2 and Ngn2 compete for binding to E-box elements as well as to other bHLH partners, creating a negative feedback loop that controls the differentiation of MNs and the

maintenance of the pMN pool for subsequent generation of oligodendrocytes (Lee *et al.* 2005). Although Olig2 is expressed in MN precursors, its expression is downregulated in differentiating MNs. Unlike proneural proteins and other bHLH transcription factors, which mostly act as transcriptional activators, Olig2 appears to function as a transcriptional repressor (Bertrand *et al.* 2002). Direct targets of Olig2 presumably include MN determinants (Lee *et al.* 2005) and astrocyte specification factors (see §3), although such targets are as yet poorly characterized.

Unlike Olig2, which seems to have essential roles in oligodendrocyte development, initial evidence indicated a redundant, non-essential function for Olig1. Mice lacking Olig1 showed a slight delay in oligodendrocyte maturation but were otherwise normal. By contrast, Olig2 mutant mice lacked all oligodendrocytes and MNs in the spinal cord (Lu *et al.* 2002), although oligodendrogenesis in the fore- and hindbrain was apparently normal. However, more recent work with the Olig1 mutant mice questions the previous data and suggests that Olig1 is in fact a central regulator of oligodendrocyte maturation and myelinogenesis (Xin *et al.* 2005). These authors found that, in the absence of Olig1, the mice developed severe neurological deficits and died within the third post-natal week. While oligodendrocyte progenitors developed normally, maturation was severely affected and markers of myelinating oligodendrocytes were absent. Although the reason for the discrepancies in different loss-of-function studies is not clear, all data support a functional role for Olig1 in oligodendrocyte maturation. This is also consistent with the recent striking finding that remyelination does not occur in *Olig1* null adult mice with experimentally induced focal demyelination (Arnett *et al.* 2004).

Another bHLH gene that has recently been found to play a role in oligodendrocyte development is the proneural gene Mash1 (Parras *et al.* 2004). In the early telencephalon, Mash1 is co-expressed with Olig2 at the time of oligodendrocyte specification in the MGE/AEP and its expression is briefly maintained in initial populations of migrating OLPs (Parras *et al.* 2005). At later stages, Mash1-negative OLPs populate the telencephalon. In newborn mice, expression of Mash1 can be observed in a large fraction of OLPs, and Mash1-deficient pups have severely reduced numbers of oligodendrocytes in the olfactory bulb (Parras *et al.* 2004). Although the data so far indicate that there are both Mash1-dependent and Mash1-independent OLPs in the telencephalon, the precise function of Mash1 in the oligodendrocyte lineage is still unknown.

(ii) *Homeobox genes*
Nkx6.1 and Nkx6.2 (also known as Gtx1) are two homeodomain transcription factors that are expressed in the ventral spinal cord under the inductive influence of Shh. Expression is restricted to the ventral neuroepithelium where they are thought to have several patterning roles. Within the pMN domains, Nkx6.1 and Nkx6.2 appear to regulate the expression of Olig2 in a dose-dependent manner (Vallstedt *et al.* 2001, 2005). Loss-of-function studies in mouse have suggested a redundant or non-essential function for Nkx6.2 in the spinal cord, whereas, in the absence of

Nkx6.1, expression of Olig2 is delayed and greatly diminished and the generation of MNs (and more dorsally located interneurons) is severely impaired (Sander *et al.* 2000; Liu *et al.* 2003). In compound Nkx6.1/Nkx6.2 mutants, all the MNs and oligodendrocytes generated from the pMN domain are eliminated demonstrating an essential role for these two genes in oligodendrogenesis from this region (Cai *et al.* 2005; Vallstedt *et al.* 2005). Interestingly, oligodendrocyte development from dorsal precursors is unaffected, perhaps indicating a significant difference in oligodendrocyte specification from dorsal and ventral precursors (Cai *et al.* 2005; Vallstedt *et al.* 2005). Whether this difference simply reflects alternative patterning requirements within different neuroepithelial regions or a specific function for Nkx6 proteins in ventral oligodendrogenesis is not known. In addition to its patterning role, Nkx6.2 is expressed in myelinating oligodendrocytes, where it appears to be involved in regulating axon–glial interactions at myelin paranodes (Southwood *et al.* 2004).

Another homeobox transcription factor that is involved in oligodendrogenesis in the neural tube is Nkx2.2. The expression of Nkx2.2 is induced by Shh at the time of neural plate induction and is restricted to a small region of the VZ adjacent to the floor plate (the p3 domain). Until the end of neurogenesis, the expression domains of Olig2 and Nkx2.2 are non-overlapping. This mutually exclusive relationship is established by cross-repressive interactions between the two genes (Mizuguchi *et al.* 2001; Novitch *et al.* 2001; Sun *et al.* 2001, 2003; Zhou *et al.* 2001). With the onset of oligodendrogenesis, expression of Nkx2.2 expands dorsally into the pMN domain, suggesting a switch from a cross-repressive to a cooperative relationship between Nkx2.2 and Olig2. However, despite this suggestive expression pattern, it is now clear that Nkx2.2 does not play an important role in oligodendrocyte specification *per se*, although it is important during the maturation of oligodendrocytes from OLPs (Qi *et al.* 2001; for further discussion see Richardson *et al.* 2006).

(iii) *Sox genes*
The Sox family of transcription factors has a prominent role in oligodendrocyte development. There are at least 20 mammalian Sox proteins, three of which have so far been implicated in oligodendrocyte generation. Sox9, Sox8 and Sox10 are expressed at different stages of oligodendrocyte development. Sox9 is expressed in the entire neuroepithelium of the spinal cord at embryonic day 10.5 (Stolt *et al.* 2003). At later stages, it is expressed in proliferating oligodendrocyte progenitors as well as in astrocytes, where its expression persists throughout life (Stolt *et al.* 2003). The expression of Sox8 slightly precedes that of Sox10, and both are restricted to the oligodendrocyte lineage from the early stages of their development until maturation (Stolt *et al.* 2002, 2004, 2005). Mice lacking Sox9 have reduced numbers of oligodendrocytes (and astrocytes) indicating a requirement for this factor in glial specification (Stolt *et al.* 2003). Compound Sox8/Sox9 deficient mice exhibit complete loss of oligodendrocytes suggesting a redundancy between the two

genes (Stolt *et al.* 2005). The loss of Sox10, on the other hand, results in normal numbers of oligodendrocyte progenitors but a failure to mature, indicating that Sox10 is required for terminal differentiation (Stolt *et al.* 2002).

(d) *Oligodendrocyte diversity?*

Oligodendrocyte heterogeneity has been reported on the basis of morphology and the observation that mature cells myelinate either a single large diameter axon or several small axons (Bunge 1968; Bjartmar *et al.* 1994; Butt *et al.* 1997). It is not clear whether this variation reflects intrinsic differences among oligodendrocyte subtypes or phenotypic plasticity. Heterogeneity has also been proposed on the basis of expression of markers such as Pdgfra or PLP/DM20 (Le Bras *et al.* 2005). The possibility that there might be different functional subclasses of oligodendrocyte becomes more intriguing owing to the discovery of dorsally derived as well as ventrally derived OLPs and the likelihood that these are specified by different signals (Chandran *et al.* 2003; Kessaris *et al.* 2004). Therefore, a major question in the field is whether different signalling pathways generate different oligodendrocyte subtypes. An attempt was made to address this question by ablating oligodendrocyte subpopulations in the forebrain, based on their region of origin in the VZ (Kessaris *et al.* 2006). This did not reveal any obvious phenotypic consequences that could point to different functional subclasses of oligodendrocytes. This tentatively suggests that the described morphological and/or molecular differences among oligodendrocytes might reflect phenotypic plasticity (Kessaris *et al.* 2006). Nevertheless, this question is still far from settled.

3. ASTROCYTES

(a) *Astrocytes: one cell type or several?*

Astrocytes were originally described more than 100 years ago as star-like cells in Golgi tissue preparations. Two morphological variants were later described: 'fibrous' astrocytes in white matter tracts and 'protoplasmic' astrocytes in grey matter. It is not clear whether the different morphology of fibrous versus protoplasmic astrocytes simply reflects their different physical environments—fibrous astrocytes in white matter being constrained to align with axons, for example—or whether they are intrinsically different cells that are specified independently, follow different developmental pathways and perform different functions in the mature CNS. Since morphology is an unreliable way of classifying cells, astrocytes later came to be defined by their high content of cytoplasmic intermediate filaments (Vaughn & Peters 1967; Choi & Lapham 1978), a major component of which is the glial fibrillary acidic protein (GFAP). GFAP immunoreactivity subsequently became their defining mark (Bignami & Dahl 1974, 1977; Antanitus *et al.* 1975). However, defining a cell population on the basis of one protein is unsatisfactory as it could mislead us into lumping different types of cells together as one. Moreover, there are cells in grey matter with a protoplasmic astrocyte-like morphology that do not

normally express GFAP, but can upregulate the protein after local CNS injury or disease (Gabbott & Stewart 1987; Stichel *et al.* 1991; Walz & Lang 1998; Scotti 2003). These cells have been called 'GFAP-negative protoplasmic astrocytes' (Walz 2000). To better characterize astrocyte-like glial cells, two approaches have been used: (i) there has been a search for alternative molecular markers that can either unify or subdivide the astrocyte population and (ii) there have been efforts to define glia on the basis of their electrophysiological properties *in vitro* and *in situ*.

Molecular markers that are useful for defining astrocyte-like glia are still few in number. They include the calcium-binding protein S100β (Ludwin *et al.* 1976), the enzyme glutamine synthetase (GS; Norenberg & Martinez-Hernandez 1979), the glutamate transporter GLAST (Schmitt *et al.* 1997), fibroblast growth factor receptor-3 (Fgfr3; Pringle *et al.* 2003), the water channel protein aquaporin-4 (Aqp4; Vitellaro-Zuccarello *et al.* 2005) and others. Not all these are completely specific (e.g. Cammer 1990; Hachem *et al.* 2005) and it is still far from clear whether they can define astrocytes as a single population of cells or several subgroups. However, there are indications that astrocytes might be heterogeneous. For example, in the post-natal rat spinal cord, there are regionally restricted populations of GFAP-positive white matter astrocytes that either do or do not co-express GLAST (Barry & McDermott 2005).

On top of this, there are regionally specialized types of GFAP-positive glial cells such as Bergmann glia in the cerebellum and Műller glia in the retina. Moreover, it has recently become clear that in some animal species, GFAP is also expressed on radial glial cells in the VZ of the embryonic neural tube and in 'subependymal astrocytes' in the adult (Dahl *et al.* 1981; Levitt *et al.* 1981; Walz & Lang 1998; Malatesta *et al.* 2000; Andrae *et al.* 2001; Barry & McDermott 2005). Radial glia and sub-ependymal astrocytes are now thought to correspond to multipotent NPC (stem) cells in the embryo and adult, respectively; they can generate neurons and oligodendrocytes as well as differentiated astrocytes *in vivo* (Doetsch *et al.* 1999; Hartfuss *et al.* 2001; Götz *et al.* 2002; Kriegstein & Gotz 2003; Anthony *et al.* 2004; Merkle *et al.* 2004). Whether all these cell types should be regarded as specialized subtypes of astrocytes based purely on their expression of GFAP is debatable.

Ultimately what is needed are functional definitions of astrocytes, but we are still some way from that. For example, electrophysiological studies of astrocytes are confusing and inconclusive. Early work on adult optic nerve astrocytes led to the conclusion that all astrocytes are electrically 'passive', lacking voltage-gated currents and displaying a linear voltage–current relationship (Kuffler *et al.* 1966). Subsequent studies on astrocytes from younger animals revealed more complex electrophysiology, including inward and outward K^+ currents and an inward Na^+ current (for review see Barres 1991). This has led to the notion that there are two astrocyte subtypes: passive and complex. However, different laboratories disagree as to the relative frequencies of the passive and complex forms, whether and how their ratio changes with CNS maturation,
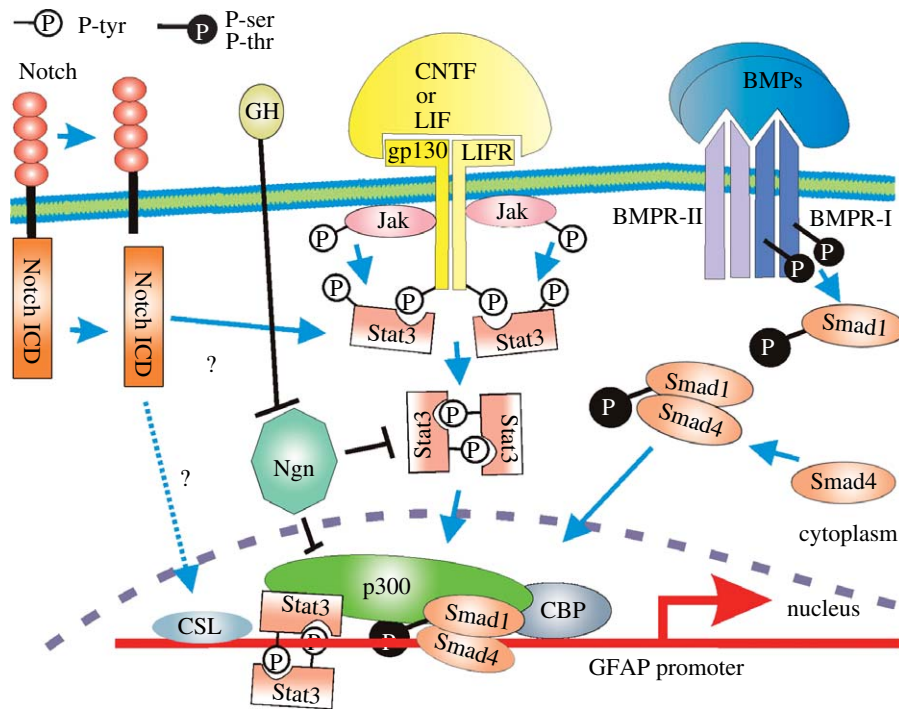
Figure 2. Signalling pathways involved in the induction of astrocyte specification/differentiation. Astrocyte differentiation is thought to be induced via cytokine and/or BMP signalling pathways. Cytokines (LIF or CNTF) induce dimerization of the LIF receptor (LIFR) with co-receptor gp130, leading to phosphorylation and activation of Janus kinases (Jaks). Receptor dimerization also creates docking sites for the transcription factor Stat3, which is tyrosine phosphorylated by Jaks. The phosphorylated Stats dimerize and move into the nucleus, recruiting p300 and binding to specific sequences in the GFAP promoter. BMP dimers induce the tetramerization of the BMPR-I and BMPR-II receptors, which are serine–threonine kinases. BMPR-II then phosphorylates and activates BMPR-I, which phosphorylates Smad1. Phosphorylated Smad1 associates with Smad4 (unknown stoichiometry) and the complex moves into the nucleus, recruiting p300 and CBP, which probably bind directly to the GFAP promoter and initiate transcription. Smads and Stats bind opposite ends of p300, which presumably allows synergistic integration of LIF/CNTF and BMP signalling pathways. These signalling pathways can be inhibited by neurogenins, which are thought to act by blocking Stat dimerization and also by sequestering p300. The activity of neurogenin can be inhibited, in an undefined manner, by growth hormone. Notch signalling (via the cleaved/released intracellular domain) might also interact with the Stat pathway. See text for a fuller description.

whether the two phenotypes can be assigned to GFAP-positive and GFAP-negative molecular sub-groups and so on. Some of the controversies might be attributable to different methodologies, regional vari-ation, the criteria according to which cells were selected for recording (e.g. on the basis of morphology) and the bias that this might introduce (Steinhauser *et al.* 1992; Matthias *et al.* 2003; for review see Walz 2000). It is also possible that the passive and complex subtypes can interconvert rapidly and this might be influenced by the way cells or tissue slices are treated prior to and during recording. An additional complication results from the fact that astrocytes are extensively coupled via gap junctions *in vivo*, which tends to linearize current–voltage relationships (passive phenotype). A recent study concluded that the complex pattern is found only during early post-natal development and that most or all astrocytes in the mature hippocampus are of the passive variety (Zhou *et al.* 2006). This suggests that complex astrocytes might be glial precursors, and passive astrocytes their differentiated progeny.

It should be clear from the above discussion that, when seeking to define the mechanisms of astrocyte specification and subsequent development, we are on uncertain ground. As long as we remain unsure whether astrocytes comprise one group of pheno-typically plastic cells or several intrinsically distinct

subgroups, it will be difficult to know whether different laboratories are studying the same or different developmental pathways. In practice, most workers rely on induction of GFAP expression *in vivo* or in neural cell cultures as a surrogate for astrocyte specification. This raises additional questions about whether we are all studying normal developmental mechanisms (astrogenesis) or the acute activation of GFAP expression that accompanies CNS injury or disease (astrocytosis).

(b) The embryonic origins of astrocytes
Astrocytes, like oligodendrocytes and neurons, are ultimately formed from the NEPs that line the lumen of the embryonic neural tube. However, there is a persuasive body of evidence that astrocytes—at least fibrous astrocytes—are formed indirectly from NEPs via radial glial cells. The evidence supporting this idea was largely circumstantial. First, immunohisto-chemical studies have identified cells with antigenic and morphological phenotypes intermediate between radial glia and astrocytes (Choi *et al.* 1983; Hirano & Goldman 1988; Voigt 1989; Barry & McDermott 2005). Second, radial glial cells begin to disappear during development at about the same time as astro-cytes appear (Choi & Lapham 1978; Schmechel & Rakic 1979; Misson *et al.* 1988) and the cells

interpreted as intermediate forms are also observed during this period. Recently, more direct evidence has come from genetic fate mapping experiments. When introduced into transgenic mice, a short promoter fragment from the human GFAP gene drives reporter gene expression not only in differentiated astrocytes, but also earlier in radial glia. When these radial glia were purified from transgenic brains and cultured *in vitro*, they gave rise to astrocytes as well as neurons, providing the first indication that radial glia can be pluripotent NPCs (Malatesta *et al.* 2000). It has now been confirmed that radial glia are also pluripotent precursors *in vivo* (Noctor *et al.* 2002; Malatesta *et al.* 2003; Anthony *et al.* 2004; Fogarty *et al.* 2005).

It is not known whether all astrocytes are generated directly from radial glia. It is also not known whether all radial glia generate astrocytes, or whether they generate different subtypes of astrocytes from different parts of the VZ. In the embryonic spinal cord, oligodendrocytes and astrocytes are generated preferentially from different parts of the VZ, with most oligodendrocytes being derived from the pMN domain (Sun *et al.* 1998; Lu *et al.* 2002; Takebayashi *et al.* 2002; Zhou & Anderson 2002). Other progenitor domains generate predominantly astrocytes (Pringle *et al.* 1998; Lu *et al.* 2002; Pringle *et al.* 2003; Fogarty *et al.* 2005). By Cre-*lox* fate mapping in mice, we showed that the *Dbx1*-expressing domain (p1, p0, dP6 and dP5—four progenitor domains centred on the dorsoventral midline) generates significant numbers of protoplasmic astrocytes, the majority of which lie in grey matter and do not express detectable GFAP immuno-reactivity. The *Dbx1* domain also generates fibrous astrocytes in the white matter and a small number of oligodendrocytes, in addition to neurons (Fogarty *et al.* 2005). Furthermore, the *Nkx2.2*-expressing domain (p3, the ventral-most domain abutting the floor plate) generates protoplasmic and fibrous astrocytes in addition to neurons and small numbers of oligodendrocytes (R. Taveira-Marques, N. Kessaris & W. D. Richardson 2006, unpublished observations). The protoplasmic astrocytes that are generated from *Dbx1* and *Nkx2.2* domains are morphologically similar, but occupy different territories in the adult spinal cord. It remains to be determined whether they are functionally or physiologically distinguishable.

(c) *Astrocyte specification*

As discussed previously, oligodendrocyte specification in the ventral spinal cord and telencephalon relies on the induction of *Olig* gene expression by Shh. A hedgehog-independent, FGF-dependent mechanism of *Olig* gene induction and oligodendrocyte specification has also been identified. What are the extracellular signals and cell-intrinsic factors that specify astrocytes? Two principal signalling systems have been identified: (i) the BMPs and (ii) cytokines such as ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF) and interleukin-6 (IL-6). These two signalling pathways can act independently of one another but also synergize in some circumstances.

(i) *Mutually antagonistic effects of BMPs and Shh on astrocyte and oligodendrocyte specification*

Several BMPs (BMP2, 4, 5 and 7) have been shown to induce multipotent NPCs from the perinatal mouse forebrain SVZ to differentiate as GFAP-positive astrocytes in culture (Gross *et al.* 1996; Mehler *et al.* 2000). In these experiments, GFAP upregulation was accompanied by rapid and irreversible changes in cell shape and size as well as inhibition of cell division, suggesting that BMPs induced astrocyte specification rather than proliferation of pre-existing astrocyte precursors. Concomitantly, oligodendrocyte production was strongly inhibited (Gross *et al.* 1996).

The inducing activity of Shh on oligodendrocytes has been clearly demonstrated and the inhibitory activity of BMPs on oligodendrocyte specification has been confirmed both in explant cultures of embryonic chick spinal cords (Wada *et al.* 2000; Mekki-Dauriac *et al.* 2002) and *in vivo* (Mekki-Dauriac *et al.* 2002; see §2). In complementary experiments, Agius *et al.* (2004) showed that Shh not only induces OLP production, but also simultaneously inhibits astrocyte generation: when Shh is added to cultured 'open book' chick spinal cord explants, Olig2 expression and OLP production expand dorsally from pMN into p2 and beyond, and astrocyte production is shut down, judging by the loss of Fgfr3 and GLAST expression. Blocking Shh signalling with cyclopamine had the opposite effect: Olig2 expression was obliterated and astrocyte production expanded ventrally from p2 through pMN to the floor plate. These experiments clearly demonstrate that BMPs and Shh are mutually antagonistic as far as gliogenesis is concerned, and suggest that the normal pattern of astrocyte and OLP production along the dorsal–ventral axis is controlled by a fine balance between Shh, BMPs and BMP inhibitors from both dorsal and ventral sources (Mekki-Dauriac *et al.* 2002; Vallstedt *et al.* 2005). It has also been suggested that the later generation of OLPs from precursors in the dorsal spinal cord might be triggered by temporal downregulation of dorsally derived BMPs (Vallstedt *et al.* 2005). This interpretation implies that precursor cells at all dorsal–ventral levels might be inherently capable of generating either astrocytes or OLPs (or both) once neuron production is over, but can be biased towards one or the other cell type by locally prevailing signals. This would be compatible with a lineage model whereby all NEPs generate neurons followed by glial-restricted precursors (GRPs or glioblasts; Rao *et al.* 1998; Noble *et al.* 2004), which could then respond to positional signals to generate predominantly astrocytes or oligodendrocytes.

(ii) *The Jak–Stat pathway*

When OLPs, derived from perinatal rat optic nerve or other parts of the CNS, are cultured in defined medium, they differentiate into oligodendrocytes. In contrast, in the presence of 10% foetal calf serum (FCS), they start to express GFAP and adopt a process-bearing morphology; these cells were called 'type-2 astrocytes' to distinguish them from flat, fibroblast-like 'type-1 astrocytes' that are abundant in cultures established from many parts of the CNS

(Raff *et al*. 1983). The type-2 astrocyte-inducing activity in FCS can be mimicked by the cytokine CNTF, in combination with extracellular matrix (ECM). CNTF by itself induces reversible GFAP expression, ECM being required for full morphological transformation and stable GFAP expression (Lillien *et al*. 1990). Since type-2 astrocytes have not yet been clearly identified *in vivo* (Fulton *et al*. 1992), it is not clear whether OLPs are exposed to CNTF during normal development. However, it has subsequently been shown that CNTF and the related cytokines IL-6 and LIF can also induce NEPs, growing as neurospheres or dissociated cell cultures, to express GFAP (Johe *et al*. 1996; Bonni *et al*. 1997; Rajan & McKay 1998). The response of primary NEPs to cytokines depends on their developmental stage, mirroring the normal developmental progression of NEPs from neurogenesis to gliogenesis *in vivo*. Thus, cells taken from the neurogenic embryonic day 11 mouse cerebral cortex do not activate GFAP during short-term (2-day) culture with cytokines. However, embryonic day 11 cortical NEPs acquire the ability to express GFAP in response to cytokines after 5 days in culture (the equivalent of embryonic day 16; He *et al*. 2005).

CNTF, IL-6 and LIF all bind to cell-surface receptors that share the same co-receptor gp130. Following ligand binding and dimerization, gp130 triggers activation of cytoplasmic tyrosine kinases of the Janus kinase (Jak) family. These in turn phosphorylate and activate Stat family transcription factors, Stat1 and Stat3 (for reviews see Darnell 1997; Levy & Darnell 2002). The activated Stat proteins dimerize, translocate to the nucleus and regulate transcription by binding to specific sites in the DNA. In addition, Stats can bind cofactors of the p300/CREB-binding protein (CBP) group (figure 2). These large (approx. 2500 residues) proteins bridge between the Stats (or other transcription factors) and RNA polymerase II, forming a complex that increases transcription, possibly by loosening chromatin structure in the vicinity of the gene promoter (Darnell 1997). This might occur because p300 has intrinsic histone acetylase activity and also can bind other acetylases.

He *et al*. (2005) showed that the GFAP-inducing activity of LIF in NEP cultures was dependant on Stat1/3. The GFAP 1.9 kb promoter region contains a canonical-binding site for Stats and binding of phosphorylated Stat1 or Stat3 to this element was demonstrated in electrophoretic mobility shift assays (Bonni *et al*. 1997; He *et al*. 2005). A GFAP promoter-driven reporter gene was not LIF responsive in early (neurogenic) cortical progenitors, but became responsive to LIF after several days in culture, just like the endogenous GFAP gene. Stat1/3 activity was enriched in the micro-dissected cortical VZ/SVZ of late (P0), compared to early (embryonic day 12), embryos, supporting the idea that late activation of the Jak–Stat pathway contributes to gliogenesis *in vivo*. This is also supported by the fact that mice deficient in components of cytokine signalling (gp130, LIF and LIF receptor) have perturbed astrocyte development (Bugga *et al*. 1998; Koblar *et al*. 1998; Nakashima *et al*. 1999*a*). As endothelial cells are a demonstrated

source of LIF in the CNS and can induce astrocyte differentiation in cultured rat optic nerve precursors (Mi *et al*. 2001), it is possible that blood vessels are also important for triggering astrocyte development.

Among the genes activated by Stat1/3 in late embryonic NEPs were several components of the Jak–Stat pathway itself, including gp130, Jak1, Stat1 and Stat3. Thus, Stat signalling triggers an autoregulatory loop that reinforces itself and presumably tends to consolidate and stabilize the astrocyte phenotype. This, taken together with previous work, suggests a model for how the neuron–astrocyte fate switch might be controlled. At early times, neurogenic bHLH transcription factors such as Ngn1 and Mash1 repress gliogenesis (Nieto *et al*. 2001; Sun *et al*. 2001) by inhibiting the activity of the Jak–Stat pathway. They achieve this by competing with Stat1/3 for binding to p300 and CBP that are present in limiting quantities in the cells and also by directly inhibiting Stat activation (Sun *et al*. 2001). In the absence of a robust positive feedback loop, the Jak–Stat pathway remains at a low basal level, insufficient for activating astrocyte-specific gene expression. As expression of Ngn1/2 diminishes during later embryonic development, repression of the Stat1/3 pathway is relieved and Stat-mediated transcription of GFAP and other astrocyte genes is activated. It remains to be determined what leads to Ngn downregulation. It has been shown that growth hormone (GH), which acts through the Jak–Stat pathway to activate the inhibitory Stat5, can downregulate Ngn1 expression in cultured neurospheres. GH and its receptor are expressed in the rodent brain from as early as embryonic day 10, peaking just before birth (Hojvat *et al*. 1982; Turnley *et al*. 2002); so it is conceivable that GH might play a role in the neuron–astrocyte switch (figure 2). However, this would not easily explain the fact that neuron–glial switching occurs approximately on schedule in primary NEPs in the absence of added GH (see above). Another alternative is that robust Jak–Stat signalling might itself inhibit Ngn expression.

(iii) *The relationship between BMP and cytokine signalling*
How can we reconcile the observations that both BMPs and LIF-like cytokines can induce astrocyte specification? Perhaps these two pathways induce different subtypes of astrocytes, or perhaps they operate independently of one another in different parts of the CNS, or perhaps they act together to reinforce one another.

The latter idea is supported by the recent demonstration that sub-effective doses of BMPs and cytokines can synergize with one another for astrocyte induction (Nakashima *et al*. 1999*b*). Once again, p300/CBP is central to the story. BMPs act through a heterotetrameric complex of type I and type II serine–threonine kinase cell-surface receptor subunits. Following BMP binding, the receptor phosphorylates and activates Smad proteins, which are transcriptional regulators. Nakashima *et al*. (1999*b*) showed that the GFAP promoter region contains separate binding sites for Stat3 and Smad1 and postulated that a direct or indirect physical interaction between the two factors might underlie their synergism. Knowing that Smads can associate with p300/CBP family members (e.g. Janknecht *et al*. 1998), they investigated the idea that

p300 bridges Stat3 and Smad1. They discovered that indeed both factors bind non-competitively to p300, Stat3 mainly to the amino-terminal half and Smad1 to the carboxy-terminal region. This provides a simple and elegant explanation of how BMPs and cytokines might collaborate to induce astrocyte generation (figure 2).

(iv) *The Notch signalling pathway*

As mentioned previously, Notch signalling is integral to appropriate MN and oligodendrocyte generation. It has also been implicated in astrocyte induction (Tanigaki *et al.* 2001; Ge *et al.* 2002; Grandbarbe *et al.* 2003). In the canonical Notch signalling pathway, binding of a Notch ligand (e.g. Delta) to Notch at the cell surface leads to proteolytic cleavage and nuclear translocation of the released Notch intracellular domain, where it associates with the CSL DNA-binding protein and its partner SKIP. This converts CSL–SKIP from a transcriptional repressor to an activator of target genes, including the *hes* genes. A CSL-independent pathway also exists (for review see Louvi & Artavanis-Tsakonas 2006). A constitutively active Notch intracellular domain, transduced into cultured adult rat hippocampal progenitors, induced astrocyte production (GFAP expression) in a majority of the expressing cells (Tanigaki *et al.* 2001). In these experiments, the Notch-activated pathway was presumed to be independent of Jak–Stat signalling, because mutation of the Stat-binding element within the GFAP promoter did not impair its ability to be activated by Notch. However, it was later shown that in NEPs from mouse or rat embryonic cortex, a dominant-negative form of Stat3 blocked the ability of Notch to activate GFAP, implying that Notch signalling does interact with the Jak–Stat pathway (Ge *et al.* 2002). The form of the interaction remains unclear because the CSL-binding site in the GFAP promoter proved not to be necessary for Notch-mediated activation. Either a CSL-independent mechanism is responsible or a CSL-dependent mechanism might act indirectly on the GFAP promoter. In any case, Notch signalling seems not to be absolutely required for astrocyte development because astrocytes can still be generated by neural stem cells derived from the CSL null mutant embryo; however, their appearance is delayed relative to wild-type cells (Ge *et al.* 2002).

4. CONCLUSIONS

There has been a great deal of progress recently in identifying the molecular mechanisms of both oligodendrocyte and astrocyte specification. We know the major signalling pathways that specify the cells from their precursors: (i) Shh and possibly FGF for oligodendrocytes and (ii) BMPs and cytokines for astrocytes. We also have made significant headway in identifying the intracellular signal transduction pathways and transcription factors that elicit the earliest steps in astrocyte or oligodendrocyte differentiation. Inevitably, many questions remain. High on the list is the question of glial diversity: are there different 'shades' of oligodendrocytes and astrocytes and, if so, are these determined at the moment of specification, by subtle variations in the signalling environment of particular groups of precursor cells in the VZ, or later, by signals from the neurons and other glia with which they intermingle after leaving their birthplace? These are basic questions of anatomy and physiology that still have to be addressed. The discovery of oligodendrocyte lineage-specific transcription factors (Olig and Sox) has been a major boost not only for the insight they have given into the internal mechanism of cell specification, but also because they provide reliable histological markers for tracking the cells during development and regeneration, both *in vitro* and *in vivo*. Something similar for astrocytes is desperately needed and surely cannot be very far away. The discovery of the central importance of the Stat3 transcription factor for astrocyte gene transcription is exciting and will accelerate our understanding of general mechanisms of gene regulation and cell fate selection, revolving around the important role of the p300/CBP adaptor proteins in coordinating and integrating different signalling pathways including, and perhaps especially, epigenetic pathways.

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