REVIEW

doi:10.1038/nature09611

Developmental genetics of vertebrate glial–cell specification

David H. Rowitch[1,2,3] & Arnold R. Kriegstein[4]

Oligodendrocytes and astrocytes are macroglial cells of the vertebrate central nervous system. These cells have diverse roles in the maintenance of neurological function. In the embryo, the genetic mechanisms that underlie the specification of macroglial precursors *in vivo* appear strikingly similar to those that regulate the development of the diverse neuron types. The switch from producing neuronal to glial subtype-specific precursors can be modelled as an interplay between region-restricted components and temporal regulators that determine neurogenic or gliogenic phases of development, contributing to glial diversity. Gaining insight into the developmental genetics of macroglia has great potential to improve our understanding of a variety of neurological disorders in humans.

Glia make up 10–20% of the cells in the *Drosophila* nervous system and at least 50% of the cells in the human brain. These findings indicate that glial-cell function is crucial for the increase in complexity of neurological function that has emerged during evolution. The principal types of macroglial cell — astrocytes and oligodendrocytes — are derived from the neuroepithelium and are found throughout the mature central nervous system (CNS). By contrast, the other group of glia — microglia — are mesodermal (more specifically, haematopoietic) in origin.

Astrocytes provide structural support, regulate water balance and ion distribution, and maintain the blood–brain barrier. They also participate in cell–cell signalling by regulating calcium flux, releasing D-serine, producing neuropeptides and modulating synaptic transmission. In the CNS, myelin provides insulation for neuronal axons and allows saltatory conduction through the formation of nodes of Ranvier. Some invertebrates have ensheathing glia that produce components of myelin[1] and even cytoplasmic extrusions and node-of-Ranvier-like structures[2], but oligodendrocytes capable of forming compact myelin are present in all jawed vertebrates. Moreover, oligodendrocyte precursor cells (OPCs) in the mammalian brain form synapses with neurons, suggesting an even greater degree of complexity in the interactions between neurons and oligodendroglia[3].

The diverse macroglial-cell types can be distinguished both by their morphological characteristics and by their expression of various markers (Fig. 1). Mature oligodendrocytes of the white matter express a variety of myelin markers, including myelin basic protein, proteolipid protein 1 (PLP1) and adenomatous polyposis coli (APC) protein. OPCs, by contrast, express platelet-derived growth-factor receptor-α, the transcription factor SOX10 and the proteoglycan NG2 (also known as CSPG4). These cells maintain proliferative and migratory competence during development and in the adult, and are early responders to injury (Fig. 1). Mature astrocytes can be divided into two categories: fibrous astrocytes and protoplasmic astrocytes. Fibrous astrocytes populate the white matter and typically have a 'star-like' appearance with dense glial filaments that can be stained with the intermediate filament marker glial fibrillary acidic protein (GFAP). Protoplasmic astrocytes are found in the grey matter, have more irregular, 'bushy', processes than do fibrous astrocytes and typically have few glial filaments. These cells come into contact with and ensheath synapses by extending thousands of thin processes, some of which also contact blood vessels.

This review describes recent advances in understanding the developmental genetics that underlies macroglial cell-type specification in vertebrates. In the past decade, it has become clear that the way in which glia derived from the neuroepithelium are specified follows similar rules to the specification of the various neuron types. There is now enough evidence to make the case that glial-cell specification in the embryo is regulated according to a 'segmental template', with the developmental programs for oligodendrocytes and astrocytes being partly independent.

Overview of stem-cell precursors for macroglia

All neurons and macroglia in the developing CNS are derived from neuroepithelial cells that line the cerebral ventricles and spinal canal. At about embryonic day (E) 9–10 in mice, progressive waves of neurogenesis begin at caudal regions of the spinal cord and proceed rostrally, as well as along ventral–dorsal and lateral–medial gradients in the brain. Radial glial cells are the primary progenitor cells at embryonic stages of neurogenesis[4–6], and like the neuroepithelial cells from which they are derived, they line the forebrain ventricles and spinal canal, maintain apical–basal polarity, and undergo interkinetic nuclear migration in association with cell-cycle progression.

Radial glia differentiate into neurons and macroglia, as has been shown by tracking cells using Cre–*loxP* fate mapping with Cre expressed under the control of the brain lipid-binding protein (*Blbp*) gene promoter[7]. Time-lapse imaging of radial glia shows that they often undergo asymmetrical self-renewal division to produce neurons or intermediate progenitor cells [8–11]. Oligodendrocyte precursors and ependymal cells also derive from radial glial cells, but whether intermediate progenitors are involved is uncertain. Intermediate progenitors are the main proliferative cells of the subventricular zones of the embryonic telencephalon[8,10] and seem to be restricted to producing neurons or glia (Fig. 1).

The *in vivo* potential of neuroepithelial cells and radial glia becomes regionally restricted through the action of organizing signals such as sonic hedgehog (SHH), fibroblast growth factors (FGFs), WNTs and bone morphogenetic proteins (BMPs), all of which provide positional information through morphogen gradients in the dorsal–ventral, anterior–posterior and medial–lateral axes (discussed below in 'Embryonic pattern formation and gliogenesis') .

[1]Howard Hughes Medical Institute, [2]Department of Pediatrics, [3]Department of Neurological Surgery, [4]Department of Neurology, University of California, San Francisco, and the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, 513 Parnassus Avenue, San Francisco, California 94143-0525, USA.

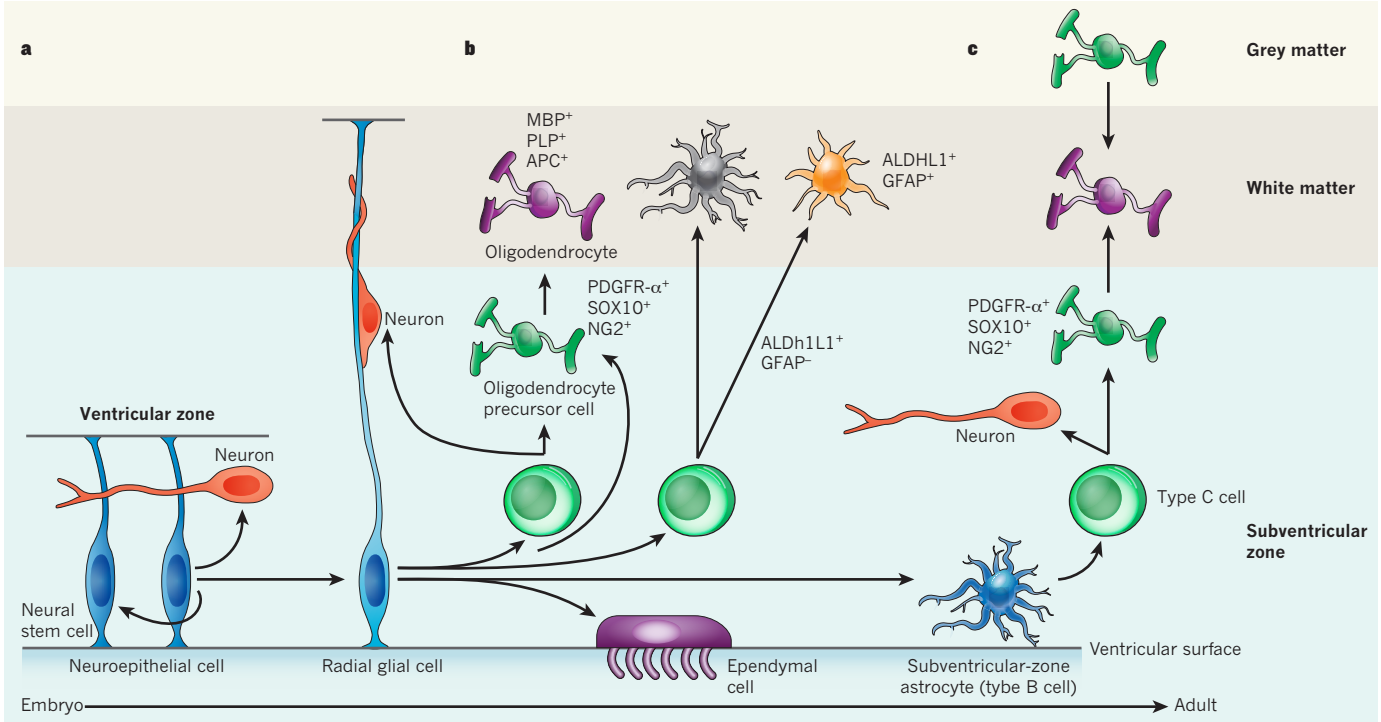

Figure 1 | Patterns of gliogenesis in embryonic and adult progenitor zones. The progression from the embryo to the adult is shown from left to right (**a** to **c**). Black arrows indicate self-renewal or differentiation from one cell type to another. Markers of macroglia and their precursors are listed. **a**, Self-renewing neuroepithelial cells line the ventricles throughout the neuraxis at the stages of neural tube closure. These cells may generate some neurons. Neuroepithelial cells are transformed into radial glial cells as neurogenesis begins. **b**, Radial glia produce intermediate progenitor cells and oligodendrocyte precursor cells (OPCs), which in turn produce neurons and oligodendrocytes, respectively. Radial glia can also become astrocytes, as well as producing intermediate progenitors that expand in number before producing astrocytes. Protoplasmic astrocytes and fibrous astrocytes might arise from common or independent progenitors. Radial glia also produce ependymal cells. **c**, In adults, oligodendrocytes are produced by two independent pathways: type B cells in the cortical subventricular zone produce transit-amplifying cells (known as type C cells), which in turn produce OPCs as well as neurons. The OPCs subsequently generate oligodendrocytes, and OPCs that are already resident in the grey matter also produce oligodendrocytes. ALDH1L1, aldehyde dehydrogenase 1 family, member L1; APC, adenomatous polyposis coli; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; PDGFR-α, platelet-derived growth-factor receptor-α; PLP, proteolipid protein 1. All green cells are intermediate progenitors, with type C cells being a subset of these, and all blue cells are neural stem cells (even though each blue cell is a different type).

Embryonic pattern formation and gliogenesis

A fundamental question in developmental neurobiology is how a relatively simple and undifferentiated neuroepithelium in the embryo can give rise to the remarkable cellular diversity and specialization of the mature CNS. It is now clear that both spatial and temporal mechanisms operate to generate diverse neuron and glial-cell types. Patterning along the neuraxis leads to segmentation of the neuroepithelium into progenitor domains (denoted p0, p1 p2, p3 and pMN) for distinct neuron types. On the basis of recent studies, this model can be extended to macroglia, but important questions remain about the role of the ultimate environment to further shape key characteristics of macroglia. In this section, we summarize how domains for neuronal and glial progenitors are established in the embryo.

Embryonic spinal cord

Organizing signals are required for pattern formation in the embryonic neural tube. SHH is a secreted protein that is essential for the activity of organizing structures of the ventral midline, such as the notochord and floor plate. Full-length SHH undergoes autoproteolysis and lipid modifications, which are crucial for its long-range signalling properties in the neural tube. Ventral SHH-mediated signalling is antagonized by patched, GLI3, HIP1 and dorsal BMP- and WNT-mediated signalling[12,13]. Together, these molecules establish a gradient of morphogenic activity that confers positional identity in the dorsal–ventral axis, resulting in generation of neural diversity.

An important function of SHH-mediated signalling is to regulate the expression of transcription factors that demarcate unique progenitor regions in the ventral neural tube[12] (Fig. 2a). The subsequent sharpening and maintenance of domain boundaries depends on cross-regulatory interactions: for example, repression of expression of the homeobox protein IRX3 by oligodendrocyte transcription factor 2 (OLIG2), and NKX2.2–PAX6 reciprocal repression (Fig. 2b). Combinatorial interactions within a given progenitor domain regulate its identity and, consequently, neuron type specification and further diversification[12].

SHH-mediated signalling is both necessary and sufficient for oligodendrocyte production in the spinal cord, another parallel with the development of motor neurons[14]. Both the concentration and timing of SHH exposure is important for establishing cell fate[15]. Indeed, Orentas *et al.*[14] found that SHH activity is required up until the time of OPC specification, at stage 24 in the chick (about E12.5 in mice). By contrast, later stages of OPC maturation are SHH independent[14,16], which is consistent with the normal sequence of OPC migration away from the source of SHH in the ventral midline (Fig. 3).

Functional analyses of transcription-factor-encoding genes have provided insight into the distinct gliogenic domains for oligodendrocytes and astrocytes in the ventral neural tube. *Olig1/2*null mutant mice show a failure in the development of motor neurons and OPCs in the spinal cord, as well as of all oligodendrocytes in the brain[17,18]. Data indicating that OLIG proteins function as transcriptional repressors show that the transcriptional targets of OLIG proteins might themselves be antagonists of motor neuron or oligodendrocyte development, such that OLIG proteins promote a neuron or glial-cell fate by repressing the repressors of that fate[19]. This mechanistic parallel between motor neuron and OPC

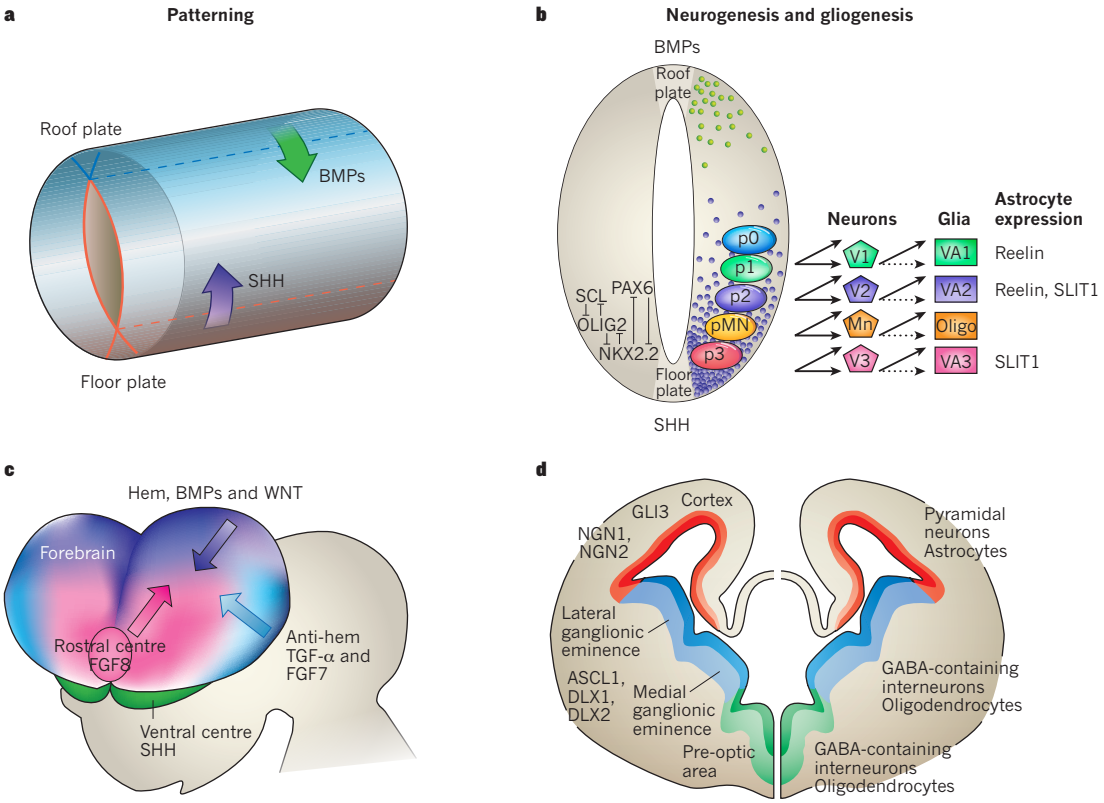

Figure 2 | Patterning of the neural tube generates unique domains for neuronal and glial progenitors. **a**, The primitive neuroepithelium of the neural tube is patterned by organizing signals. These signals emanate from the ventral floor plate (such as SHH, purple) and roof plate (BMPs and WNTs, green). **b**, A cross-sectional view of the neural tube is shown. Progenitors of motor neurons and interneurons are formed within distinct regionally restricted domains of the ventral neural tube: the p0, p1, p2 and p3 domains for interneuron subtypes, and the pMN domain for motor neurons. Dorsal domains are also similarly parcelled (not shown). Signalling mediated by SHH (gradient denoted by purple circles) regulates the expression of transcription factors (for example, NKX2.2, OLIG2, PAX6 and SCL) in the ventral neural tube. The interactions of these factors sharpen and maintain the domain boundaries. Embryonic OPCs are derived mainly from the pMN domain. OPCs are recognized by expression of PDGFR-α, SOX10 and NG2. Three astrocyte subtypes have been identified: VA1 astrocytes (which express PAX6 and reelin, derived from p1) are the most dorsal; VA3 astrocytes (which express NKX6.1 and SLIT1, derived from p3) are the most ventral; and VA2 astrocytes (which express PAX6, NKX6.1, reelin and SLIT1, derived from p2) are located in an intermediate white-matter domain. **c**, Organizing centres of the forebrain are shown. These include the cortical hem (purple), which is a dorsal source of BMPs and WNTs; a ventral centre (green), which is a source of SHH; and rostral (pink) and anti-hem (blue) regions, which are sources of growth factors such as FGF8, and transforming growth factor-α (TGF-α) and FGF7, respectively. **d**, A coronal view of the embryonic (~E14.5) forebrain showing its division into dorsal and ventral regions that are specialized for producing different neuron and glial-cell types. The dorsal region includes the cortex, a source of pyramidal neurons and astrocytes. The ventral region includes the lateral and medial ganglionic eminences and the pre-optic area, which are sources of GABA (γ-aminobutyric acid)-containing interneurons and oligodendrocytes. The green, blue and red shaded areas represent the pre-optic area, medial/lateral ganglionic eminences and neocortex, respectively (see Fig. 3). Transcription factors that are associated with dorsal (NGN1, NGN2, GLI3) and ventral (ASCL1, DLX1, DLX2) patterning and cell fate specification are indicated. NGN, neurogenin.

development, however, does not indicate that these cells originate from a common bipotent progenitor. Indeed, several lines of evidence indicate that the pMN domain (where motor neurons form) comprises independently segregating neuroblasts and glioblasts that are sequentially specified (Box 1).

Astrocyte development was increased in *Olig1/2* mutants, as indicated by more cells expressing GFAP. Such *Olig1/2* double mutants and *Olig2* single mutants lack the pMN domain and, instead, develop a ventrally expanded p2 domain and have more interneurons of the V2 class and more astrocytes. In other words, astrocytes develop despite a genetic deletion that eliminates the ultimate precursor population for OPCs, a finding that is incompatible with the bipotent glial-cell-restricted precursor model of development (Box 1).

Given these findings, ventral neural-tube astrocytes and oligodendrocytes seem to develop along mutually exclusive paths, but what would be the underlying molecular mechanisms of this? One possibility is that OLIG2 represses astrocyte fate by promoting an alternative fate. Alternatively, OLIG2 might repress a 'pro-astrocytic' transcriptional program in an adjacent progenitor domain. In support of the latter model, a basic helix–loop–helix (bHLH) code operates in the neural tube, and this is required for astrocyte differentiation within the p2 domain[20]. Whereas deletion of *Olig2* expands the p2 domain ventrally, generating astrocytes instead of oligodendrocytes[18], deletion of p2-associated *Scl* (also known as *Tal1*) resulted in dorsalized OLIG2 expression, increasing oligodendrocyte production at the expense of astrocytes. Data showing that cross-repressive interactions between the bHLH transcription factors SCL and OLIG2 are required to maintain astrocyte generation suggested a model whereby astrogenesis might generally take place in restricted regions of the neural tube.

This idea of a 'segmental model' for glial-cell type specification is further supported by work by Hochstim *et al.*[21], who showed that combinatorial expression of PAX6 and NKX6.1 specified three molecularly distinct subtypes of ventral astrocytes (termed VA1, VA2 and VA3), which were identified on the basis of their expression of *Slit1* and *Reelin*, which encode axon and neuronal migration factors. These astrocyte subtypes show dorsal–ventral positional identity in white matter, which mirrors the arrangement of progenitors in the p1, p2 and p3 domains. NKX2.2 seems to be required for SLIT1 expression in ventral astrocyte precursors,

BOX 1

Investigating the potential of glial precursors

Here we consider proposed models for glial-cell lineage progression, caveats to these models and new directions that might better explain the nature of glial precursor cells *in vivo*.

Are there bipotent precursors for glial-cell types *in vivo*?

The developmental potential of the neural progenitor cells that give rise to astrocytes and oligodendrocytes remains the subject of extensive debate[84]. Seminal studies by Raff and colleagues identified two types of astrocyte precursor *in vitro* (type 1 and type 2 astrocytes) and indicated that type 2 astrocytes and oligodendrocytes developed from a common 'O-2A' precursor[85]. Subsequently, others reported a glial-restricted precursor (GRP) cell, which was found to be competent to generate all three glial-cell types on the basis of *in vitro* analysis of cultured cells and their potential after transplantation[86,87].

In vivo studies, however, do not strongly support the model of normal development through such a bipotent oligodendrocyte–astrocyte (O-A) GRP cell. Subjecting glial precursors *in vivo* (reviewed in ref. 88) to retroviral fate mapping (an approach that targets proliferating cells) indicates that mixed astrocyte and oligodendrocyte clones were rarely or never observed in embryos. This finding is in keeping with data from *Olig2*-knockout animals and from animals in which the pMN domain has been ablated, both of which completely lack embryonic OPCs but have unaffected astrocyte production[89]. This result indicates that OPCs and astrocytes develop in different regions of the neural tube by independent mechanisms (see Fig. 2b). By contrast, the Goldman[90] and Parnavelas[91] groups have shown that bipotent O-A clones could be labelled with retrovirus in the neonatal forebrain. However, such cells represent only 10–15% of glia-generating precursors. Together, these data indicate that the bipotent O-A GRP is only a minor pathway in gliogenesis, at a transient neonatal stage of brain development. A common bipotent O-A precursor might have a larger role in the setting of injury[92,93].

Another alternative model has also been put forward. The lack of motor neurons and OPCs in *Olig2*-null animals might be explained by the existence of a restricted bipotent motor-neuron–OPC precursor cell. However, several findings indicate, instead, that the pMN domain comprises independently segregating neuroblasts and glioblasts rather than a bipotent precursor[27]. Collectively, the studies above argue against glial-cell development through restricted bipotent O-A or neuron–oligodendrocyte precursors.

Restriction of neuron versus glial precursor potential

In vertebrate systems, many lines of evidence are consistent with a model of general restriction of CNS precursor cells to the production of neurons at early stages, followed by a later phase of glial-cell production (see the section 'The switch from neuron to glial-cell production'). In this way, a precursor at the phase of glial-cell production might be called 'glial-restricted' in the sense that it cannot normally produce neurons. The actions of signalling pathways (for example, Delta-like–NOTCH signalling) and the 'pro-glial-cell' transcription factors (for example, SOX9 and nuclear factor I (NFI) proteins) are generally required in all dorsal–ventral domains of the neural tube for the transition to glial-cell production. Such general mechanisms must work together with region-specific factors to establish different glial-cell types (oligodendrocytes or astrocytes) in particular domains (Fig. 2). Finally, in addition to such 'active' programs of glial-cell type specification, the default mechanism by which radial glia become astrocytes (Fig. 1) is predicted to contribute astrocytes to all domains.

Phases of neuron and glial-cell production from progenitors might alternate. In invertebrates, the production of neuroblasts and glioblasts from a common progenitor can switch back and forth[34]. Type B cells of the adult rodent subventricular zone can give rise to neurons and/or oligodendrocytes[94] at different times in response to environmental cues[95]; however, further work is needed to establish whether this takes place through a common bipotent precursor or reflects segregation of independent neuron- and glial-cell-dedicated type B cells.

In summary, published studies indicate that the classic bipotent glial-restricted precursor defined in *in vitro* studies is unlikely to be the ancestor of most glia *in vivo*. A challenge for developmental genetics is to identify the precise mechanisms that account for astrocyte type specification and lineage progression.

New genetic tools for investigating the astrocyte lineage

Newer fate-mapping approaches might help to address further the issues of glial-cell lineage progression. These approaches include constitutive and inducible recombinase technologies (Flp and Φ31)[96], in combination with specialized reporter systems such as mosaic analysis with double markers (MADM)[97] and Brainbow[98]. These technologies allow for more precise targeting of progenitor populations, for example using two types of recombinase under the control of different yet overlapping enhancers, or using reporters that can be activated in a very few cells, allowing clonal origins to be assessed *in vivo*. However, a major technical hurdle is identifying regulatory sequences that will drive expression of the recombinase specifically in astrocytes and their intermediate progenitor cells. Although human *GFAP*-regulatory sequences have been used extensively in transgenic mice, GFAP is expressed by a heterogeneous group of cells in the CNS. Therefore, careful attention must be paid to the precise expression characteristics of available transgenic mice that use the *GFAP* enhancer in such systems. Other markers of astrocytes and/or their precursors have been reported, including FGF receptor 3 (ref. 99), GLAST (also known as SLC1A3)[100], fatty-acid-binding protein 7 (ref. 101), brain lipid-binding protein (BLBP)[7], SOX9 (ref. 102) and NFIA and NFIB[36]. However, some of these markers (for example, BLBP) are also expressed during neurogenic stages and therefore do not exclusively mark ventricular-zone cells committed to the astrocyte lineage. Other markers are also expressed in non-astrocyte lineages (for example, FGFR3 in oligodendrocytes). Recently, gene-expression profiling of purified astrocyte populations[103,104] has uncovered several potential new markers, such as the gene encoding the folate metabolic enzyme ALDH1L1 (refs 103, 105). Clearly, a priority for the field is identification of markers that are specific for key stages of astrocyte development throughout the CNS, as well as possibly heterogeneous astrocyte precursors and mature astrocytes.

independent of its function to repress PAX6 from the p3 domain[22].

In summary, it is clear that the diverse astrocytes and oligodendrocytes of the ventral neural tube are generated according to the domain organization of the neural. It remains to be seen whether the functional heterogeneity of astrocytes is similarly regulated and whether this 'segmental model' applies to gliogenesis in the dorsal regions of the spinal cord and in the rostral CNS.

Neuroepithelium of the forebrain

As indicated in Fig. 2d, forebrain excitatory pyramidal cells arise in the dorsal telencephalon, whereas inhibitory interneurons arise in the ventral telencephalon. Moreover, different classes of interneuron are derived from specific progenitor domains within the ventral telencephalon. How is such diversity achieved? Similar to the spinal cord, patterning centres located at key points along the neuraxis induce the

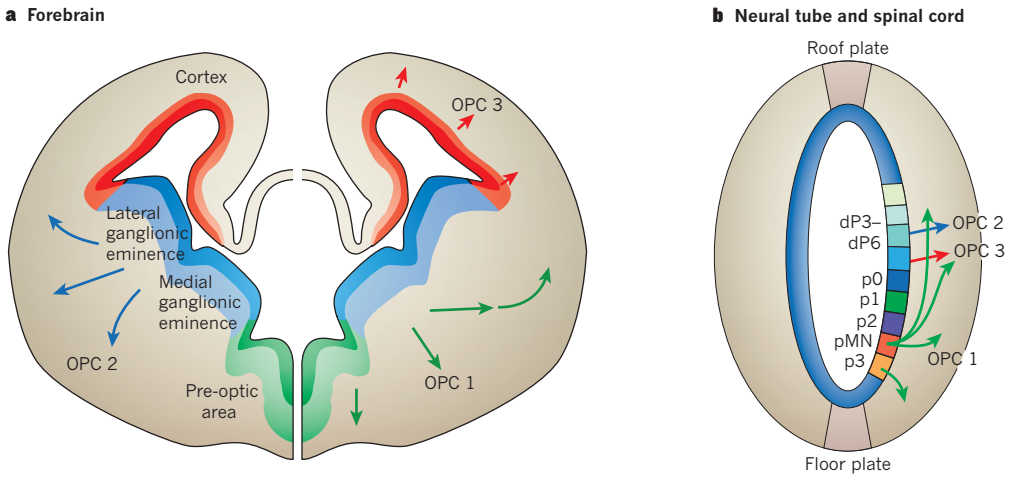

Figure 3 | Multiple waves of oligodendrocyte production in the mammalian CNS. **a**, Three sequential waves of OPCs (OPC 1, OPC 2 and OPC 3) are generated from different regions of the forebrain ventricular zone: OPC 1 (green arrows) arises from *Nkx2.1*-expressing precursors in the medial ganglionic eminence, starting at E12.5; OPC 2 (blue arrows) arises from precursors expressing the homeobox gene *Gsx2* in the lateral and medial ganglionic eminences, starting at E15.5; and OPC 3 (red arrows) arises from precursors expressing the homeobox gene *Emx1* in the cortex, starting at birth. Work from the Richardson lab[70] shows that the OPC 1 population is replaced by later waves[70]. **b**, Two distinct waves of OPCs emanate from the ventral region and the dorsal region of the spinal cord in the embryo and fetus, respectively. A third wave begins after birth. In the first wave (OPC 1, green arrows), ventral OPCs arise from *Olig2*-expressing progenitor cells in the pMN domain at E12.5 and subsequently migrate to populate the entire neural tube. The development of these cells depends on SHH-mediated signalling and is inhibited by dorsally derived BMPs and WNT proteins. In the second wave (OPC 2, blue arrows), dorsal OPCs develop from *Olig2*-expressing cells of the dP3, dP4, dP5 and dP6 domains during the fetal phase (at day E15.5) in an SHH-independent manner. The origins of the third wave (OPC 3, red arrows), which occurs after birth, remain unclear. These OPCs might arise from progenitor cells, which remain around the central canal, or from proliferative *Ng2*-expressing precursor cells throughout the parenchyma. OPCs from ventral and dorsal regions are intermixed in the spinal cord at birth, with a heavy predominance of pMN-derived cells. The relative contributions at adult stages are unclear.

graded expression of transcription factors along the rostral–caudal and dorsal–ventral axes, resulting in the parcellation of the neuroepithelium into progenitor domains that lead to spatially distinct origins for most types of neuron[23].

Like in the spinal cord, patterning molecules establish spatial domains of proneural and homeodomain protein expression within forebrain progenitor cells. Proneural proteins are bHLH transcription factors — for example, ASCL1 (also known as MASH1), neurogenin 1 (NGN1), NGN2 and NGN3, and ATOH1 (also known as MATH1) — that initiate neurogenesis (reviewed in ref. 24). The commitment of progenitors to a neuronal fate involves not only the promotion of neurogenesis but also the coordinate inhibition of self-renewal and the suppression of gliogenic programs[25,26].

The switch from neuron to glial-cell production

Gliogenesis generally follows neurogenesis in the developing mammalian CNS, with the same progenitor domains switching developmental programs from neuron production mainly to oligodendrocyte or astrocyte production. In the ventral spinal cord, for example, there is a developmental switch from the production of neurons to oligodendrocytes within the same progenitor domain. (It should be kept in mind that radial glia in all domains of the CNS are thought to transform into astrocytes, which would yield a small proportion of astrocytes and ependymal cells, even in domains that primarily produce oligodendrocytes, such as the pMN domain[27].)

The spinal-cord pMN domain

The pMN domain of the ventral spinal cord, with its well-defined outputs of motor neurons and oligodendrocytes, is a suitable model for studying how the generation of successive waves of neuron and glial-cell progeny from a discrete precursor population is regulated. The actions of SHH and OLIGs are consistently required throughout the processes of pattern formation and motor neuron and oligodendrocyte specification, but several lines of evidence indicate that the 'tone' of both SHH-mediated signalling and the levels of OLIG2 proteins are crucial for maintaining cell fate and the decision to produce differentiated first-wave motor neurons or, later, glia[28]. However, as described below, many other factors must be taken into account.

Proneural factors are repressors of gliogenesis. NGN2 is expressed in a subset of OLIG2-expressing cells in the pMN domain at the time of motor neuron production, and this pattern is conserved across species[18,19,29]. OPC production is preceded by downregulation of *Ngn2* (ref. 28), leading to the proposal that *Ngn2* downregulation is a determinant of the neuron–glial-cell switch. NGN activity and the neuron–glial-cell switch are also modulated in the pMN domain by factors involved in pattern formation. In contrast to early embryonic expression of NGN2 in the ventral neural tube, ASCL1 is expressed from E16 into the gliogenic phases, where it is required for oligodendrocyte development[30].

NOTCH proteins are also involved in gliogenesis. These proteins are single-pass, heterodimeric transmembrane receptors that bind to their transmembrane ligands (for example, the Delta-like proteins) at the surface of adjacent cells. Ligand binding results in cleavage of the intracellular domain of the receptor, which then translocates to the nucleus and recruits a complex that includes the transcriptional effector RBP-J. Forced expression of activated NOTCH1 promotes the formation of radial glia in the brain[31]. Analyses in zebrafish[29] and mice[32] show that oligodendrocytes fail to form in embryos that lack NOTCH signalling. Progenitors in the pMN domain contribute solely to motor neuron production in NOTCH mutants, whereas forced expression of activated NOTCH1 blocks neurogenesis and results in excess OPCs[33]. These studies indicate that the general role of NOTCH signalling is permissive rather than instructive for glial-cell fate acquisition.

'Pro-glial-cell' transcription factors are also required for the neuron–glial-cell switch. The studies discussed in the previous section are consistent with a broad range of data that illustrate the importance of the proneural protein–NOTCH program in regulating the decision to produce neurons or glia in the brain. However, additional 'pro-glial-cell' transcription programs are required.

Analysis of *Sox9* loss-of-function mutations in mice provided evidence that a gliogenic stage-specific transcriptional program is needed

for the generation of oligodendrocytes and astrocytes in the vertebrate neural tube[34]. *Sox9* mutants showed defects in the specification of oligodendrocytes in the pMN domain, as well as in the production of an apparent deficit of astrocytes in the p2 domain. Such animals also had more motor neurons and V2 interneurons, indicating that SOX9 is a general molecular component of the neuron–glial-cell switch in the developing spinal cord. Recent studies indicate that SOX9 also has a role in hindbrain cell-fate choice[35].

The initiation of gliogenesis in the embryonic spinal cord, and the differentiation of astrocytes later in gliogenesis, is controlled by the nuclear factor I (NFI) genes, which encode a family of transcription factors that bind CAATT boxes. Gain-of-function manipulations in the embryonic chick spinal cord indicate that NFIA expression is sufficient for gliogenesis, whereas loss of NFIA expression led to a loss of glial progenitors and a concomitant increase in neurogenesis that resulted from loss of NOTCH activity[36]. NFI proteins seems to have an instructive role in collaborating with NOTCH to promote gliogenesis. Furthermore, knockout of *Nfia* or *Nfib* results in decreased GFAP expression[37,38], in keeping with studies indicating that NFI genes directly regulate expression of this gene [39,40].

In summary, studies of the pMN domain indicate that the neuron–glial-cell switch is regulated in a complex manner that requires the following: ongoing (perhaps modulated) activity of SHH and OLIG2, downregulation of *Ngn2* expression, Delta-like–NOTCH signalling to preserve progenitors for producing second-wave domain-specific progeny, and activation of a pro-gliogenic phase-specific transcriptional program involving SOX9 and NFI transcription factors.

The neuron to glial–cell switch in the forebrain

Progenitor domains generate different types of neuron in a temporally specific manner. For example, in the dorsal pallium, laminar-specific neuron subtypes that are thought to arise from common progenitor cells are sequentially produced (deep-layer neurons first and upper-layer neurons later). This developmental program is largely cell-intrinsic, because cultured neural progenitors derived from the embryonic CNS, as well as embryonic-stem-cell-derived neural progenitors, generate neuron subtypes in a precise temporal and laminar order[41–43] but can also be affected by signals from the local environment[44]. In the anterior entopeduncular area, the homeobox proteins DLX1 and DLX2 regulate interneuron versus oligodendrocyte cell fate[45].

Early in forebrain development, the promoters of *GFAP* and *S100B* are methylated, and astrocyte development is repressed. At these early stages, neuroepithelial cells are insensitive to cytokines[46,47]. However, radial glia become competent to respond through demethylation of the promoters of astrocytic genes, possibly through epigenetic regulation[48]. When multipotent progenitor cells become competent to generate astrocytes, they are held in check by extrinsic signals that repress gliogenesis. For example, stimulation with FGF2 prompts the nuclear receptor co-repressor (NCOR) to act directly to the proximal *Gfap* promoter, thereby repressing transcription[49]. Neurogenesis is also promoted by neuregulin 1, which activates a presenilin-dependent nuclear signalling pathway involving ERBB4, a member of the epidermal growth-factor receptor family. This pathway antagonizes the actions of astrogenesis-promoting signals such as ciliary neurotrophic factor (CNTF)[50] through a mechanism that involves NCOR[49]. Interestingly, a developmental reduction in ERBB2 expression occurs at the end of neurogenesis when radial glia are transformed into astrocytes[51], and introduction of ERBB2 into adult astrocytes can restore their neurogenic potential[52]. Recent evidence highlights the role of epigenetic events in neuron–glial-cell fate decisions by inducing gene silencing through methylation and deacetylation. For example, the Polycomb group complex epigenetically suppresses the genes encoding proneural bHLH factors, promoting the neuron–astrocyte fate switch in neural precursor cells[53].

NOTCH signalling has been implicated in the switch to astrogenesis[51]. Neuron-committed intermediate progenitors and young neurons express the NOTCH ligands jagged 1 and Delta-like 1 (refs 54–56), which activate NOTCH signalling in radial glia, promoting astrogenesis. NOTCH promotes astrogenesis, in part, through HES proteins, which inhibit neurogenic bHLH factors, but also through promoting cytokine-mediated activation of the JAK–STAT pathway, leading to demethylation and upregulation of astrocyte-specific genes[57]. Thus, by increasing NOTCH activation, newly formed neurons may prime multi-potent progenitors to respond to gliogenic cytokines[54].

Gliogenesis might also be regulated by another neuronal feedback mechanism: cytokines secreted by neurons have been proposed to promote gliogenesis[58]. Neurons secrete gliogenic cytokines — particularly members of the interleukin 6 (IL-6) family, including leukaemia inhibitory factor (LIF), CNTF and cardiotrophin 1 (CT1; also known as CTF1) — which bind to a receptor complex that contains the α-subunit of the LIF receptor (LIFR) and gp130, activating the gp130–JAK–STAT pathway in cortical precursor cells and promoting gliogenesis[58–60]. Thus, mice lacking LIFR or gp130 have deficits in astrogenesis[61–63], and knockdown of expression of gp130 in cortical precursors decreases astrogenesis *in vitro* and *in vivo*[58]. During development of the cortex, CT1, in particular, has been shown to promote the neuron–glial-cell switch in multipotent precursor cells *in vivo*, as well as *in vitro*[58], supporting the concept that neuronal feedback may help to regulate the developmental switch to gliogenesis.

BMPs have a dual role in this switch, depending on the levels of certain growth factors that are present. BMPs promote neurogenesis during the neurogenic period and astrogenesis during the gliogenic period[64]. Exposure to BMP2 together with gliogenic cytokines promotes the formation of a SMAD–p300–CREB-binding-protein–STAT complex that transactivates astrocyte-associated genes[63]. At the same time, BMPs also exert a coordinate antagonism of proneural bHLH factors, suppressing neurogenesis[65] and perhaps gliogenesis. BMPs suppress oligodendrocyte development and promote astrocyte development. In addition, the proneural bHLH factor ASCL1 has a role in supporting oligodendrocyte development in the telencephalon in collaboration with OLIG2 (ref. 24) and an ongoing function in neuron and oligodendrocyte production in postnatal brain[66]

In contrast to the roles of OLIG2 in repressing astrogenesis in the embryonic spinal cord (described above), OLIG2 has also been proposed to support the determination of astrocyte cell fate in the forebrain. At day 7 after birth, in the subventricular zone of the mouse brain, OLIG2 was observed in gliogenic progenitors that produce both astrocytes and oligodendrocytes, and forced expression studies indicated that OLIG2 repressed differentiation into neurons and promoted glial-cell fates[67]. However, conditional ablation of *Olig2* in astrocytes expressing GFAP, and their precursors, reduced the proliferation of reactive astrocytes, suggesting that the functions of OLIG2 in astrocytes might relate to roles in cell cycle progression.

Temporally distinct waves of gliogenesis

So far we have focused on the initial phases of glial-cell specification. Now we turn to the later stages of gliogenesis, which differ in many respects, at least for oligodendrocytes.

Oligodendrocytes are produced in several distinct spatiotemporal waves in the spinal cord and brain

Although OPCs are derived from the ventral pMN domain in the embryonic spinal cord, there are additional sources of OPCs that emerge during fetal development in dorsal CNS[16,68]. Such fetal OPCs had a molecular phenotype distinct from their embryonic counterparts, with transient expression of the dorsal progenitor markers PAX7 and GSX1 and GSX2, but no functional differences between these cells have been demonstrated. Using a fate-mapping approach, Fogarty and colleagues[68] provided an additional line of evidence that OPCs develop from the ventricular-zone progenitors of the dorsal spinal cord. Together, these studies suggest that the contribution to the early postnatal spinal cord of ventral OPCs to dorsal OPCs is about 4:1. However, it remains to be determined whether the relative contributions of dorsal OPCs increase

with age, which would further clarify the functional importance of these cells.

In the ventral domains of both the spinal cord and the forebrain, SHH is required for OPC production[14,69]. Additional OPCs arise in an SHH-independent manner in the dorsal domains of the forebrain at later stages of development[69]. The precise origins of forebrain OPCs have been explored using Cre–*loxP* fate mapping in transgenic mice[70]. Beginning at about E11.5, OPCs were found in the medial ganglionic eminence and the anterior entopeduncular area. By E14.5, they were found throughout the telencephalon, including the cortex. Over time, intermediate and dorsal OPCs were produced and were observed to replace or dilute out early-wave OPCs. Indeed, the early-appearing OPCs derived from the medial ganglionic eminence and anterior entopeduncular area had mostly disappeared at 10 days after birth. Thus, three waves of OPCs arise to populate the forebrain, and these follow a ventral–dorsal temporal progression (Fig. 3).

The authors of this study[70] also used a Cre–*loxP* strategy to ablate early-wave OPCs specifically, by eliminating cells expressing a *Sox10* transgene through expression of a diphtheria-toxin-encoding transgene. Although there were no significant neurological consequences, further studies are needed to understand the precise developmental, phenotypic and functional differences between embryonic and fetal OPCs. These findings could have important implications in the setting of CNS injury. For example, distinct populations of OPCs might preferentially contribute to remyelination and/or be better targets for therapeutic manipulation.

Spatial and temporal patterns of astrogenesis

Thymidine-labelling experiments that detect proliferating cells indicated that, whereas cortical neurogenesis begins at about E12, oligodendrogenesis begins around the time of birth. However, in these studies, the timing of glial-cell lineage specification was probably misjudged, because cells were labelled after exit from the cell cycle. A pool of committed precursors that proliferate early and differentiate later would therefore escape labelling. Indeed, as discussed above, specification of forebrain oligodendrocytes takes place in the embryo. Astrogenesis in the embryo is linked to the terminal phases of radial glial-cell function (Fig. 1). The precise timing remains unclear, however, because of a lack of definitive markers for astrocyte precursors (as distinct from radial glia) that could be used to monitor their emergence and proliferation at early developmental stages. In contrast to OPCs, which proliferate in the mammalian brain throughout life, mature astrocytes are generally thought to be quiescent but proliferate after injury[71]. However, further work is needed to determine the time frame of astrocyte precursor proliferation.

After astrocyte specification has occurred, astrocyte precursors migrate to their final positions, where they begin the process of terminal differentiation. Understanding the nature and extent of astrocyte precursor migration is essential for defining the relationship of mature astrocytes, if any, to their position of origin in the neural tube.

It is controversial whether astrocytes of the embryonic CNS migrate radially, tangentially or both. Most studies have relied on transplantation of cultured astrocytes or cortical explants into perinatal animals *in vivo* or in cortical slice cultures *in vitro*. These studies generally agree that astrocytes first migrate tangentially along white-matter tracts and then delaminate and move in a radial direction in the grey matter[72,73]. Some regions of the brain seem to exert a strong chemoattractive effect on astrocytes; for example, the substantia nigra attracts all astrocytes that have been transplanted in the vicinity of the midbrain[74]. However, a caveat of these studies is that *ex vivo* culture and transplantation conditions might select for an astrocyte subtype that is not representative of the type present in the embryo. In other studies, sparse retroviral labelling of proliferating multipotent precursors indicates that most clonally related cells (including astrocytes) are found in radial columns, as well as tangentially along fibre tracts[75]. It is possible that the modes of astrocyte migration vary in different regions of the nervous system. Comprehensive *in vivo* analysis of astrocyte migration — using improved markers that have become available, imaging tools (for example, time-lapse microscopy) and sophisticated fate-mapping techniques (Box 1) — will be useful for answering these questions.

Future directions and challenges

Astrocytes are a heterogeneous group of cells, both functionally and morphologically. In recent years, researchers have made impressive progress in understanding which cell-intrinsic factors regulate OPC maturation. This has, however, highlighted a large gap in the knowledge of many fundamental aspects of astrocyte developmental biology.

Analysis of vertebrate embryos has improved the understanding of the mechanisms that underlie the specification of macroglia. As a departure from the classical models of glial-cell development through a bipotent glial-cell-restricted precursor, recent studies indicate that oligodendrocyte and astrocyte precursors *in vivo* develop separately and in mutually exclusive domains of the ventral neural tube. Broader implications of this segmental model of gliogenesis, however, remain to be defined. For example, could the region-restricted production of astrocytes help to establish the molecular and functional diversity of this population? In the case of the adult subventricular zone, one study indicates that the dorsal–ventral patterning of type B cells is reflected in permanent restrictions on their potential to produce different types of interneuron[76].

To answer such questions, regulatory factors that are specific for astrocytes and their precursors will probably need to be identified, and new genetic tools for exploring the complex functions of glia will be needed (Box 1). In addition, the role of invertebrate model systems in uncovering new pathways of gliogenesis cannot be underestimated. Indeed, researchers are making rapid progress using such approaches, which form an exciting area of research[77].

It is clear that macroglia are crucial for maintaining neurological function, as has been shown in studies of human diseases. In individuals with Pelizaeus–Merzbacher disease, a congenital leukodystrophy, mutation of *PLP1* renders OPCs defective and incapable of myelin production. An understanding of OPC development has contributed to the rationale for cell-based therapies for this disorder[78]. Oligodendrocytes are also targets in individuals with the autoimmune disease multiple sclerosis and in newborns with injuries to the white matter that are associated with cerebral palsy. Mutation of *GFAP* in humans is the aetiological factor in the congenital disorder Alexander disease, which typically has an adult onset. The list of astrocyte-based diseases is expanding to include amyotrophic lateral sclerosis[79], epilepsy[80] and Parkinson's disease[81]. The availability of robust and specific markers for the developmental progression of the glial-cell lineage will facilitate the assessment of the contributions of glia and their precursors to a range of human neurological disorders. For example, in multiple sclerosis and periventricular leukomalacia, markers (such as OLIG2) identified in developmental studies have been examined in neuropathological studies, showing that OPCs are blocked in their differentiation and fail to carry out normal repair functions after white-matter injury[82,83]. These findings suggest that regulation — rather than replacement — of endogenous progenitors is a promising therapeutic approach. Further progress in studying the developmental genetics of macroglia will undoubtedly improve our understanding of a range of human neurological disorders. ■

1. Zalc, B. & Colman, D. R. Origins of vertebrate success. *Science* **288,** 271–272 (2000).
2. Roots, B. I. The phylogeny of invertebrates and the evolution of myelin. *Neuron Glia Biol.* **4,** 101–109 (2008).
3. Lin, S. C. & Bergles, D. E. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. *Nature Neurosci.* **7,** 24–32 (2004).
   This paper shows that OPCs of the hippocampus receive direct input from interneurons, suggesting that GABA (γ-aminobutyric acid)-induced chloride flux influences oligodendrocyte development and the efficacy of glutamate-induced signalling in OPCs.
4. Malatesta, P., Hartfuss, E. & Gotz, M. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage.

Development **127,** 5253–5263 (2000).
This paper highlights the neurogenic potential of radial glia. Using fluorescence-activated cell sorting, radial glia isolated from the embryonic neocortex were shown to generate neurons, as well as astrocytes.
5. Miyata, T., Kawaguchi, A., Okano, H. & Ogawa, M. Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* **31,** 727–741 (2001).
6. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. & Kriegstein, A. R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409,** 714–720 (2001).
This study used retroviral labelling *in utero* and showed that neurons were directly generated through the self-renewal division of radial glia.
7. Anthony, T. E., Klein, C., Fishell, G. & Heintz, N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* **41,** 881–890 (2004).
8. Haubensak, W., Attardo, A., Denk, W. & Huttner, W. B. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc. Natl Acad. Sci. USA* **101,** 3196–3201 (2004).
9. Miyata, T. *et al.* Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development* **131,** 3133–3145 (2004).
10. Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. & Kriegstein, A. R. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neurosci.* **7,** 136–144 (2004).
11. Noctor, S. C., Martinez-Cerdeno, V. & Kriegstein, A. R. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *J. Comp. Neurol.* **508,** 28–44 (2008).
12. Briscoe, J. & Novitch, B. G. Regulatory pathways linking progenitor patterning, cell fates and neurogenesis in the ventral neural tube. *Phil. Trans. R. Soc. B* **363,** 57–70 (2008).
13. Ulloa, F. & Marti, E. Wnt won the war: antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube. *Dev. Dyn.* **239,** 69–76 (2010).
14. Orentas, D. M., Hayes, J. E., Dyer, K. L. & Miller, R. H. Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. *Development* **126,** 2419–2429 (1999).
15. Dessaud, E. *et al.* Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism. *Nature* **450,** 717–720 (2007).
16. Vallstedt, A., Klos, J. M. & Ericson, J. Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. *Neuron* **45,** 55–67 (2005).
17. Lu, Q. R. *et al.* Common developmental requirement for *Olig* function indicates a motor neuron/oligodendrocyte connection. *Cell* **109,** 75–86 (2002).
18. Zhou, Q. & Anderson, D. J. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell* **109,** 61–73 (2002).
19. Novitch, B. G., Chen, A. I. & Jessell, T. M. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. *Neuron* **31,** 773–789 (2001).
20. Muroyama, Y., Fujiwara, Y., Orkin, S. H. & Rowitch, D. H. Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. *Nature* **438,** 360–363 (2005).
This paper was the first to suggest that astrocyte development is regulated by region-restricted mechanisms.
21. Hochstim, C., Deneen, B., Lukaszewicz, A., Zhou, Q. & Anderson, D. J. Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. *Cell* **133,** 510–522 (2008).
This paper shows that a homeodomain code in the ventral neural tube regulates the diversity of astrocytes. It provides the strongest evidence so far for the 'segmental model' of astrocyte development.
22. Genethliou, N. *et al.* Spatially distinct functions of PAX6 and NKX2.2 during gliogenesis in the ventral spinal cord. *Biochem. Biophys. Res. Commun.* **382,** 69–73 (2009).
23. Hoch, R. V., Rubenstein, J. L. & Pleasure, S. Genes and signaling events that establish regional patterning of the mammalian forebrain. *Semin. Cell Dev. Biol.* **20,** 378–386 (2009).
24. Parras, C. M. *et al.* The proneural gene *Mash1* specifies an early population of telencephalic oligodendrocytes. *J. Neurosci.* **27,** 4233–4242 (2007).
25. Bylund, M., Andersson, E., Novitch, B. G. & Muhr, J. Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nature Neurosci.* **6,** 1162–1168 (2003).
26. Sandberg, M., Kallstrom, M. & Muhr, J. Sox21 promotes the progression of vertebrate neurogenesis. *Nature Neurosci.* **8,** 995–1001 (2005).
27. Masahira, N. *et al.* Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. *Dev. Biol.* **293,** 358–369 (2006).
28. Lee, S. K., Lee, B., Ruiz, E. C. & Pfaff, S. L. Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. *Genes Dev.* **19,** 282–294 (2005).
29. Park, H. C. & Appel, B. Delta–Notch signaling regulates oligodendrocyte specification. *Development* **130**, 3747–3755 (2003).
30. Battiste, J. *et al.* Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. *Development* **134,** 285–293 (2007).
31. Gaiano, N., Nye, J. S. & Fishell, G. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* **26,** 395–404 (2000).
32. Yang, X. *et al.* Notch1 signaling influences v2 interneuron and motor neuron development in the spinal cord. *Dev. Neurosci.* **28,** 102–117 (2006).
33. Itoh, M. *et al.* Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. *Dev. Cell* **4,** 67–82 (2003).
34. Karcavich, R. & Doe, C. Q. *Drosophila* neuroblast 7-3 cell lineage: a model system for studying programmed cell death, Notch/Numb signaling, and sequential specification of ganglion mother cell identity. *J. Comp. Neurol.* **481,** 240–251 (2005).
35. Esain, V., Postlethwait, J. H., Charnay, P. & Ghislain, J. FGF-receptor signalling controls neural cell diversity in the zebrafish hindbrain by regulating *olig2* and *sox9*. *Development* **137,** 33–42 (2010).
36. Deneen, B. *et al.* The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. *Neuron* **52,** 953–968 (2006).
In this paper, the 'pro-glial-cell' transcription factor NFIA is described and shown to promote gliogenesis, inhibit neurogenesis in ventricular-zone spinal-cord progenitors and, at later stages, promote differentiation into astrocytes.
37. das Neves, L. *et al.* Disruption of the murine nuclear factor I-A gene (*Nfia*) results in perinatal lethality, hydrocephalus, and agenesis of the corpus callosum. *Proc. Natl Acad. Sci. USA* **96,** 11946–11951 (1999).
38. Steele-Perkins, G. *et al.* The transcription factor gene *Nfib* is essential for both lung maturation and brain development. *Mol. Cell. Biol.* **25,** 685–698 (2005).
39. Cebolla, B. & Vallejo, M. Nuclear factor-I regulates glial fibrillary acidic protein gene expression in astrocytes differentiated from cortical precursor cells. *J. Neurochem.* **97,** 1057–1070 (2006).
40. Wilczynska, K. M. *et al.* Nuclear factor I isoforms regulate gene expression during the differentiation of human neural progenitors to astrocytes. *Stem Cells* **27,** 1173–1181 (2009).
41. Gaspard, N. *et al.* An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* **455,** 351–357 (2008).
42. Shen, Q. *et al.* The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nature Neurosci.* **9,** 743–751 (2006).
43. Eiraku, M. *et al.* Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* **3,** 519–532 (2008).
44. Desai, A. R. & McConnell, S. K. Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development* **127,** 2863–2872 (2000).
45. Petryniak, M. A., Potter, G. B., Rowitch, D. H. & Rubenstein, J. L. *Dlx1* and *Dlx2* control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron* **55,** 417–433 (2007).
This paper shows that DLX1 and DLX2 repress oligodendrocyte production in an area of the forebrain and suggests that DLX1 and DLX2 have non-cell-autonomous effects or epigenetic effects in the oligodendrocyte lineage.
46. Temple, S. The development of neural stem cells. *Nature* **414,** 112–117 (2001).
47. Molne, M. *et al.* Early cortical precursors do not undergo LIF-mediated astrocytic differentiation. *J. Neurosci. Res.* **59,** 301–311 (2000).
48. Takizawa, T. *et al.* DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev. Cell* **1,** 749–758 (2001).
49. Hermanson, O., Jepsen, K. & Rosenfeld, M. G. N-CoR controls differentiation of neural stem cells into astrocytes. *Nature* **419,** 934–939 (2002).
50. Sardi, S. P., Murtie, J., Koirala, S., Patten, B. A. & Corfas, G. Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. *Cell* **127,** 185–197 (2006).
51. Fox, I. J. & Kornblum, H. I. Developmental profile of ErbB receptors in murine central nervous system: implications for functional interactions. *J. Neurosci. Res.* **79,** 584–597 (2005).
52. Ghashghaei, H. T. *et al.* Reinduction of ErbB2 in astrocytes promotes radial glial progenitor identity in adult cerebral cortex. *Genes Dev.* **21,** 3258–3271 (2007).
53. Hirabayashi, Y. *et al.* Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron* **63,** 600–613 (2009).
54. Namihira, M. *et al.* Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. *Dev. Cell* **16,** 245–255 (2009).
55. Campos, L. S., Duarte, A. J., Branco, T. & Henrique, D. mDll1 and mDll3 expression in the developing mouse brain: role in the establishment of the early cortex. *J. Neurosci. Res.* **64,** 590–598 (2001).
56. Yoon, K. J. *et al.* Mind bomb 1-expressing intermediate progenitors generate Notch signaling to maintain radial glial cells. *Neuron* **58,** 519–531 (2008).
57. Kamakura, S. *et al.* Hes binding to STAT3 mediates crosstalk between Notch and JAK–STAT signalling. *Nature Cell Biol.* **6,** 547–554 (2004).
58. Barnabe-Heider, F. *et al.* Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. *Neuron* **48,** 253–265 (2005).
This paper provides evidence that embryonic cortical neurons regulate astrocyte development by secreting the neurotrophic cytokine CT1, which activates the gp130–JAK–STAT signalling pathway.
59. Bonni, A. *et al.* Regulation of gliogenesis in the central nervous system by the JAK–STAT signaling pathway. *Science* **278,** 477–483 (1997).
60. Ochiai, W., Yanagisawa, M., Takizawa, T., Nakashima, K. & Taga, T. Astrocyte differentiation of fetal neuroepithelial cells involving cardiotrophin-1-induced activation of STAT3. *Cytokine* **14,** 264–271 (2001).
61. Ware, C. B. *et al.* Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* **121,** 1283–1299 (1995).
62. Koblar, S. A. *et al.* Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor. *Proc. Natl Acad. Sci. USA* **95,** 3178–3181 (1998).
63. Nakashima, K. *et al.* Synergistic signaling in fetal brain by STAT3–Smad1 complex bridged by p300. *Science* **284,** 479–482 (1999).
64. Mabie, P. C., Mehler, M. F. & Kessler, J. A. Multiple roles of bone morphogenetic

protein signaling in the regulation of cortical cell number and phenotype. *J. Neurosci.* **19,** 7077–7088 (1999).
65. Nakashima, K. *et al.* BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. *Proc. Natl Acad. Sci. USA* **98,** 5868–5873 (2001).
66. Parras, C. M. *et al.* Mash1 specifies neurons and oligodendrocytes in the postnatal brain. *EMBO J.* **23,** 4495–4505 (2004).
67. Marshall, C. A., Novitch, B. G. & Goldman, J. E. Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. *J. Neurosci.* **25,** 7289–7298 (2005).
68. Fogarty, M., Richardson, W. D. & Kessaris, N. A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. *Development* **132,** 1951–1959 (2005).
69. Nery, S., Wichterle, H. & Fishell, G. Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. *Development* **128,** 527–540 (2001).
70. Kessaris, N. *et al.* Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nature Neurosci.* **9,** 173–179 (2006).
This study used Cre–*loxP* fate-mapping technology and showed that oligodendrocytes develop in at least three distinct waves from the time of embryogenesis through the postnatal stages.
71. Buffo, A. *et al.* Origin and progeny of reactive gliosis: a source of multipotent cells in the injured brain. *Proc. Natl Acad. Sci. USA* **105,** 3581–3586 (2008).
72. Hatton, J. D., Nguyen, M. H. & U, H. S. Differential migration of astrocytes grafted into the developing rat brain. *Glia* **9,** 113–119 (1993).
73. Jacobsen, C. T. & Miller, R. H. Control of astrocyte migration in the developing cerebral cortex. *Dev. Neurosci.* **25,** 207–216 (2003).
74. Zhou, H. F. & Lund, R. D. Migration of astrocytes transplanted to the midbrain of neonatal rats. *J. Comp. Neurol.* **317,** 145–155 (1992).
75. Gray, G. E. & Sanes, J. R. Migratory paths and phenotypic choices of clonally related cells in the avian optic tectum. *Neuron* **6,** 211–225 (1991).
76. Merkle, F. T., Mirzadeh, Z. & Alvarez-Buylla, A. Mosaic organization of neural stem cells in the adult brain. *Science* **317,** 381–384 (2007).
This paper shows that in the adult the subventricular zone contains regions that produce different types of interneuron, which contribute to the rostral migratory stream and the olfactory bulb.
77. Freeman, M. Specification and morphogenesis of astrocytes. *Science* (in the press).
78. Goldman, S. A., Schanz, S. & Windrem, M. S. Stem cell-based strategies for treating pediatric disorders of myelin. *Hum. Mol. Genet.* **17,** R76–R83 (2008).
79. Nagai, M. *et al.* Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nature Neurosci.* **10,** 615–622 (2007).
80. Nicoll, J. A. & Weller, R. O. A new role for astrocytes: β-amyloid homeostasis and degradation. *Trends Mol. Med.* **9,** 281–282 (2003).
81. Saijo, K. *et al.* A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* **137,** 47–59 (2009).
82. Chang, A., Tourtellotte, W. W., Rudick, R. & Trapp, B. D. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *N. Engl. J. Med.* **346,** 165–173 (2002).
83. Billiards, S. S. *et al.* Myelin abnormalities without oligodendrocyte loss in periventricular leukomalacia. *Brain Pathol.* **18,** 153–163 (2008).
84. Richardson, W. D., Kessaris, N. & Pringle, N. Oligodendrocyte wars. *Nature Rev. Neurosci.* **7,** 11–18 (2006).
85. Raff, M. C., Miller, R. H. & Noble, M. A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* **303,** 390–396 (1983).
86. Herrera, J. *et al.* Embryonic-derived glial-restricted precursor cells (GRP cells) can differentiate into astrocytes and oligodendrocytes *in vivo*. *Exp. Neurol.* **171,** 11–21 (2001).
87. Rao, M. S., Noble, M. & Mayer-Proschel, M. A tripotential glial precursor cell is present in the developing spinal cord. *Proc. Natl Acad. Sci. USA* **95,** 3996–4001 (1998).
88. Costa, M. R., Bucholz, O., Schroeder, T. & Gotz, M. Late origin of glia-restricted progenitors in the developing mouse cerebral cortex. *Cereb. Cortex* **19** (suppl. 1), i135–i143 (2009).
89. Wu, S., Wu, Y. & Capecchi, M. R. Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors *in vivo*. *Development* **133,** 581–590 (2006).
90. Levison, S. W. & Goldman, J. E. Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* **10,** 201–212 (1993).
91. Parnavelas, J. G. Glial cell lineages in the rat cerebral cortex. *Exp. Neurol.* **156,** 418–429 (1999).
92. Zhao, J. W., Raha-Chowdhury, R., Fawcett, J. W. & Watts, C. Astrocytes and oligodendrocytes can be generated from NG2$^+$ progenitors after acute brain injury: intracellular localization of oligodendrocyte transcription factor 2 is associated with their fate choice. *Eur. J. Neurosci.* **29,** 1853–1869 (2009).
93. Tatsumi, K. *et al.* Genetic fate mapping of Olig2 progenitors in the injured adult cerebral cortex reveals preferential differentiation into astrocytes. *J. Neurosci. Res.* **86,** 3494–3502 (2008).
94. Menn, B. *et al.* Origin of oligodendrocytes in the subventricular zone of the adult brain. *J. Neurosci.* **26,** 7907–7918 (2006).
95. Jablonska, B. *et al.* Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. *Nature Neurosci.* **13,** 541–550 (2010).
96. Raymond, C. S. & Soriano, P. High-efficiency FLP and ΦC31 site-specific recombination in mammalian cells. *PLoS ONE* **2,** e162 (2007).
97. Zong, H., Espinosa, J. S., Su, H. H., Muzumdar, M. D. & Luo, L. Mosaic analysis with double markers in mice. *Cell* **121,** 479–492 (2005).
98. Livet, J. *et al.* Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450,** 56–62 (2007).
99. Pringle, N. P. *et al. Fgfr3* expression by astrocytes and their precursors: evidence that astrocytes and oligodendrocytes originate in distinct neuroepithelial domains. *Development* **130,** 93–102 (2003).
100. Shibata, T. *et al.* Glutamate transporter GLAST is expressed in the radial glia–astrocyte lineage of developing mouse spinal cord. *J. Neurosci.* **17,** 9212–9219 (1997).
101. Owada, Y., Yoshimoto, T. & Kondo, H. Spatio-temporally differential expression of genes for three members of fatty acid binding proteins in developing and mature rat brains. *J. Chem. Neuroanat.* **12,** 113–122 (1996).
102. Stolt, C. C. *et al.* The Sox9 transcription factor determines glial fate choice in the developing spinal cord. *Genes Dev.* **17,** 1677–1689 (2003).
103. Cahoy, J. D. *et al.* A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* **28,** 264–278 (2008).
104. Fu, H. *et al.* A genome-wide screen for spatially restricted expression patterns identifies transcription factors that regulate glial development. *J. Neurosci.* **29,** 11399–11408 (2009).
105. Anthony, T. E. & Heintz, N. The folate metabolic enzyme ALDH1L1 is restricted to the midline of the early CNS, suggesting a role in human neural tube defects. *J. Comp. Neurol.* **500,** 368–383 (2007).

Acknowledgements We apologize to those whose work we could not cite owing to space limitations. We thank A. Molofsky for comments and M. Jenner for formatting the manuscript. Work in our laboratories is supported by grants from the National Institutes of Health (A.R.K. and D.H.R.) and the California Institute for Regenerative Medicine (A.R.K.). D.H.R. is a Howard Hughes Medical Institute investigator.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence should be addressed to D.H.R. (rowitchd@peds.ucsf.edu) and A.R.K. (kriegsteina@stemcell.ucsf.edu).