Development 117, 525-533 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage

Nigel P. Pringle and William D. Richardson

Department of Biology (Medawar Building), University College London, Gower Street, London WC1E 6BT, UK

SUMMARY

During rat embryogenesis, PDGF alpha receptor (PDGF-αR) mRNA is expressed in the ventral half of the spinal cord in two longitudinal columns, one each side of the central canal. Initially, these columns are only two cells wide but the cells subsequently appear to proliferate and disseminate throughout the spinal cord. Our previous studies of PDGF-αR expression in the developing CNS suggested that PDGF-αR may be a useful marker of the oligodendrocyte lineage in situ. The data presented here complement those studies and lead us to propose that the earliest oligodendrocyte precursors in the spinal cord originate in a very restricted region of the ventricular zone during a brief window of time around embryonic day 14 (E14). In the embryonic brain, migrating PDGF-αR$^+$ cells appear to originate in a localized germinal zone in the ventral diencephalon (beneath the foramen of Monro). Our data demonstrate that gene expression and cell fate can be regulated with exquisite spatial resolution along the dorsoventral axis of the mammalian neural tube.

Key words: PDGF receptors, rat CNS, glial cells, development, in situ hybridization

INTRODUCTION

One of the major challenges in developmental neurobiology is to determine how the enormous variety of neuronal and glial cell types in the mature central nervous system (CNS) is generated from the undifferentiated neuroepithelial cells of the embryonic neural tube. We are approaching this problem by focussing on the development of oligodendrocytes, the myelinating glia of the CNS. During development, oligodendrocytes arise from glial progenitor cells known as O-2A progenitors (see Richardson et al., 1990 for review). There is evidence that O-2A progenitors are migratory cells in vivo, invading developing white matter tracts from nearby germinal zones (Altman, 1966; Paterson et al., 1973; Small et al., 1987; Reynolds and Wilkin, 1988; LeVine and Goldman, 1988a,b), but their sites of origin are, in general, unknown. It is possible that the ultimate origin of the oligodendrocyte lineage, in common with most neuronal cell lineages, is in the germinal zones around the ventricles of the brain and spinal cord (ventricular and subventricular zones; Altman, 1966; Paterson et al., 1973; LeVine and Goldman, 1988a,b). However, it also has been suggested that oligodendrocytes in the spinal cord develop from de-differentiated radial glia (Choi et al., 1983; Choi and Kim, 1985; Hirano and Goldman, 1988; Aloisi et al., 1992). It has not been possible to resolve this or other related issues because of the lack of specific molecular markers that can be used to identify oligodendrocyte lineage cells in sections of CNS tissue. We recently presented evidence that, during late neurogenesis in the rat, expression of the alpha receptor for platelet-derived growth factor (PDGF-αR) might be restricted to cells of the oligodendrocyte lineage (Pringle et al., 1992). This raised the possibility that reagents directed against PDGF-αR might be useful for studying the earlier development of the oligodendrocyte lineage in situ. This paper describes in situ hybridization experiments to visualize PDGF-αR$^+$ cells in the embryonic spinal cord and brain. These experiments revealed a transient singularity of PDGF-αR expression in the basal ventricular zone of the embryonic spinal cord. The location of these early PDGF-αR$^+$ cells and their subsequent behaviour corresponds closely with what is known about the development of oligodendrocyte precursors in the embryonic spinal cord (Warf et al., 1991). We also present evidence suggesting that PDGF-αR$^+$ cells in the forebrain and midbrain originate in a restricted region of the ventricular zone of the third ventricle (beneath the foramen of Monro), in the ventral half of the developing diencephalon. Our data provide a striking illustration of the high degree of spatial resolution with which gene expression and cell fate can be regulated along the dorsoventral axis of the neural tube.

MATERIALS AND METHODS

Preparation of tissue sections

Sprague-Dawley rat embryos aged E12, E13, E14, E15, E16 and E18 were used in this study. To time the pregnancies, female rats were caged with males overnight and then removed: this was taken

as day zero of the pregnancy. Embryos of approximately the right ages were checked according to the morphological criteria of Long and Burlingame (1938). Two or three animals were examined at each age. In some instances (E12, E14, E16 and E18) animals from separate litters were used; there were no obvious differences. Tissues were fixed for 24 hours in ice cold 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) and cryoprotected by immersion in 0.5 M sucrose in PBS for 24 hours at 4°C. Tissues were immersed in OCT embedding compound (BDH) and frozen in aluminium foil boats placed onto dry ice. Tissue was kept at −70°C until required. Frozen sections were cut (15 μm nominal thickness) on a cryostat and were collected on 3-aminopropyltriethoxysilane (APES)-coated glass microscope slides. Sections were air dried for approx. 2 hours at 20˚C and postfixed in 4% paraformaldehyde in PBS for 15 minutes at room temperature. After two 5-minute washes in PBS, the sections were dehydrated in increasing concentrations of ethanol (30, 60, 80, 95 and 100% for 1 minute each) and stored at −70˚C.

Animals were sectioned in three different planes (coronal, horizontal and sagittal). At E12 and E13, serial coronal and horizontal sections were taken through the animal at approximately 100 μm intervals. For E14, E15 and E16 animals, coronal sections were taken at approximately 100 μm intervals and horizontal sections at approximately 200 μm intervals. At E18 serial coronal sections were taken every 300 μm and horizontal sections every 400 μm.

In situ hybridization

Our in situ hybridization procedure, based on that of Lawrence and Singer (1985), has been described previously (Pringle et al., 1989), except that we omitted the preincubation in non-radioactive α-thio UTP, and we included an extra wash in 4× SSC for 1 hour at room temperature immediately following the hybridization step. This latter modification seemed to be important for eliminating occasional high backgrounds. Our ^{35}S-labelled RNA probe was generated as described previously by in vitro transcription from an approx. 1.5 kb *Sac*I-*Pvu*II fragment encompassing most of the extracellular domain of the rat PDGF-αR, cloned into pGEM1. For autoradiography, the slides were coated with Ilford K5 nuclear emulsion, exposed for 1-2 weeks in the dark at 4˚C, and developed in Kodak D-19. Some sections were lightly counterstained with Hematoxylin (Gills No.3, Sigma).

Computer imaging

Corresponding bright- and dark-field micrographs were converted to digital images using a video camera and frame grabber connected to a Macintosh computer. The monochrome images were assigned false colours and superimposed using Adobe Photoshop software, and the composite images photographed directly from the computer monitor.

RESULTS

To map the distribution of PDGF-αR mRNA in the developing rat CNS, we performed in situ hybridization experiments with a ^{35}S-RNA probe corresponding to part of the extracellular domain of the rat PDGF-αR. This probe specifically recognizes PDGF-αR mRNA and does not cross-hybridize with PDGF-βR mRNA (Pringle et al., 1992), which is also known to be present in the developing rat CNS (Sasahara et al., 1991; Smits et al., 1991). Having previously investigated PDGF-αR expression in the CNS of rats aged E16 and above, in this study we concentrated on animals at earlier stages of development. Cryostat sections (15 μm nominal thickness) of embryonic rats (E12, E12.5, E13, E14, E15, E16 and E18) were hybridized in situ with the PDGF-αR probe, as described in Materials and methods. Subsequently, sections were autoradiographed, lightly stained with hematoxylin and examined in the light microscope under bright- and dark-field illumination. Control experiments with ^{35}S-RNA probes homologous to PDGF-αR mRNA ('sense' probe) gave no autoradiographic signal above background (Pringle et al., 1992).

PDGF-αR expression in the alar ventricular zone of the developing spinal cord

Development of the spinal cord proceeds with a systematic temporal lag in both the rostral-to-caudal and ventral-to-dorsal directions (Nornes and Das, 1974; Altman and Bayer, 1984). Therefore, by taking a series of sections from the lumbar (caudal) level to the cervical (rostral) level, approximately one-and-a-half days of development can be studied in one animal. The ventricular zone of the spinal cord is subdivided at its dorsoventral midline (sulcus limitans) into the so-called alar (dorsal) and basal (ventral) plates. At E12, the earliest age that we examined, we could just detect low levels of PDGF-αR expression in the alar plate in the more anterior parts of the spinal cord and in the brainstem. PDGF-αR expression subsequently increased in intensity and spread into the more posterior regions of the spinal cord by E13. Fig. 1 shows a transverse (coronal) section through an E13 spinal cord (lumbar level) and brainstem, photographed under bright- and dark-field illumination. At this age, PDGF-αR expression at the lumbar level of the spinal cord is restricted to a narrow band in the dorsal half of the alar ventricular zone (Fig. 1A,C), but at the cervical region of the cord, and in the brainstem, PDGF-αR expression extends throughout most of the alar ventricular zone (Fig. 1B,D). PDGF-αR expression in the alar plate diminishes after E13 and by E15 disappears completely (Fig. 4). This transient PDGF-αR signal in the alar plate is presumably derived from developing interneurons, raising the question of whether these neurons can respond to PDGF during development and, if so, how (see Discussion).

PDGF-αR expression in the ventral half of the developing spinal cord

In addition to the rather diffuse PDGF-αR hybridization signal in the alar plate of the brainstem at E13, we observed a few very intensely labelled cells in the basal ventricular zone (arrow in Fig. 1D). Because of the possible link between these PDGF-αR$^+$ cells and oligodendrocyte lineage cells, which are also thought to arise in the ventral half of the spinal cord (Warf et al., 1991), we decided to look more carefully at the development of these ventrally located cells in the spinal cord.

We cut transverse serial sections through most of the length of the spinal cord of an E14 rat embryo, and subjected them to the in situ hybridization procedure. At the lumbar level of the cord, there were two tiny foci of PDGF-αR$^+$ cells in the basal ventricular zone, one on either side of the central canal (Fig. 2A). Examination of these foci at higher power revealed that they consisted of just two adjacent cells at the ventricular surface; these cells had no features that distinguished them from neighbouring cells that

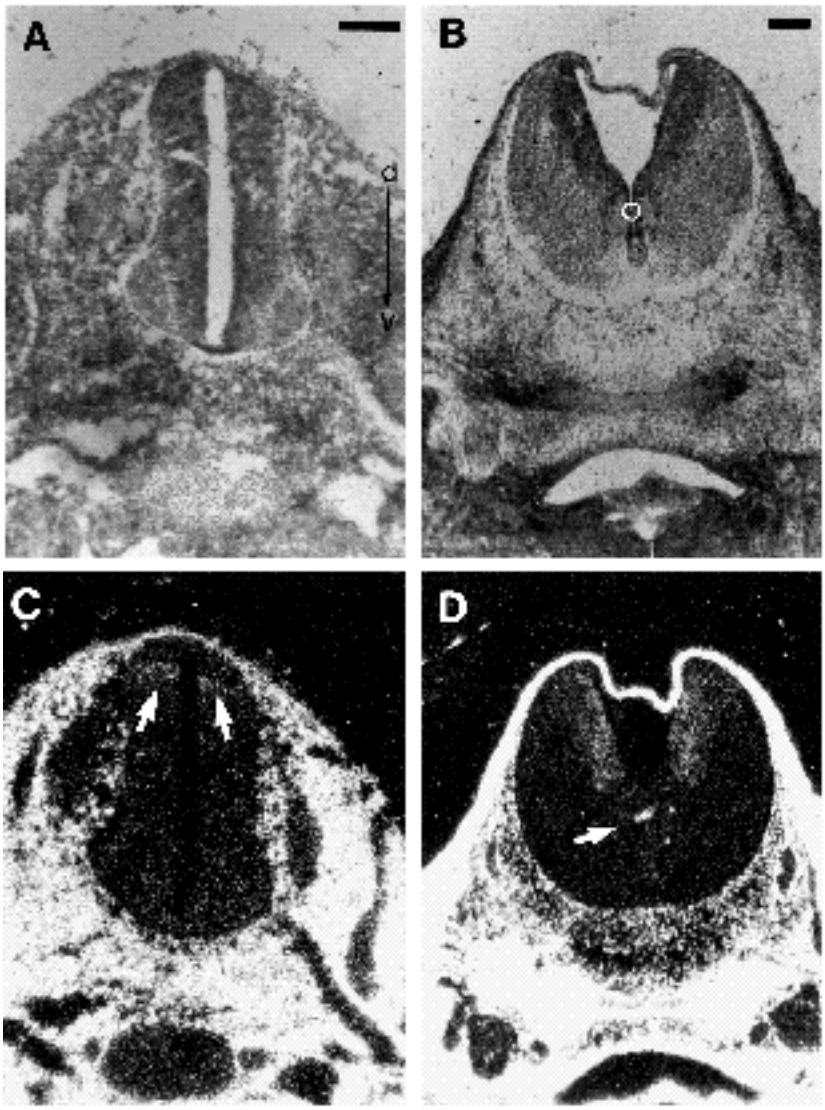

Fig. 1. PDGF-αR expression in the E13 rat spinal cord and brainstem. Transverse sections through the lumbar region of an E13 rat spinal cord (A,C) and brainstem (B,D) were subjected to in situ hybridization with a ^{35}S-RNA probe to PDGF-αR, followed by autoradiography. (A,C and B,D) Corresponding bright- and dark-field images. In the lumbar spinal cord at E13, the PDGF-αR signal is restricted to a narrow stripe in the dorsal aspect of the alar plate (arrows in C). In the hindbrain (about a day-and-a-half more advanced in development) PDGF-αR expression extends throughout the alar plate; in addition, there are a few intensely labelled cells in the basal plate (arrow in D, circle in B). Non-CNS tissues are intensely labelled. Scale bars (A and B), 250 μm.

did not express PDGF-αR (data not shown). The PDGF-αR$^+$ cells were present in the basal plate all along the length of the spinal cord (Fig. 2A-C), but at more rostral levels the number of PDGF-αR$^+$ cells in the transverse plane was greater, and individual PDGF-αR$^+$ cells were present at a distance from the ventricular zone as if they had detached themselves from the central cluster and migrated into the gray matter (Fig. 2C). The columnar organization of the PDGF-αR$^+$ cells in the E14 spinal cord was more obvious in a longitudinal plane of section (Fig. 3). Two days later, at E16, the cluster of PDGF-αR$^+$ cells had gone from the ventricular zone (Fig. 4). Instead, individual PDGF-αR$^+$ cells were scattered throughout the gray and white matter, though they were located predominantly in the ventral half of the cord at this age. After a further two days, at E18, the ventral-to-dorsal gradient of PDGF-αR$^+$ cells had almost, but not quite, disappeared (Fig. 5). This temporal sequence of events suggests that PDGF-αR$^+$ cells arise at a defined point of the ventricular zone at E14 and subsequently migrate away from there and proliferate to populate the rest of the spinal cord.

We also performed in situ hybridization on sagittal sections of E14 and E16 rat embryos (Fig. 6A and B, respectively). From such sections it is clear that PDGF-αR is expressed strongly in many tissues outside the CNS, as described previously (Orr-Urtreger et al., 1992; Morrison-Graham et al., 1992; Schatteman et al., 1992; Pringle et al., 1992). The thin columns of PDGF-αR expression in the ventral half of the E14 spinal cord are apparent where the plane of section is appropriate; it appears to extend from the spinal cord into the developing hindbrain and even some way into the midbrain (arrowheads in Fig. 6A). At E16, there are many more PDGF-αR$^+$ cells in the brain and spinal cord; in the spinal cord these appear to be distributed in both a ventral-to-dorsal and an anterior-to-posterior gradient. The last vestiges of the column of PDGF-αR$^+$ cells in the basal ventricular zone can be observed at the lumbar level of the E16 spinal cord (Fig. 6B).

PDGF-αR expression in the embryonic brain

Since the spinal cord and brain both develop from the embryonic neural tube, we thought it possible that there

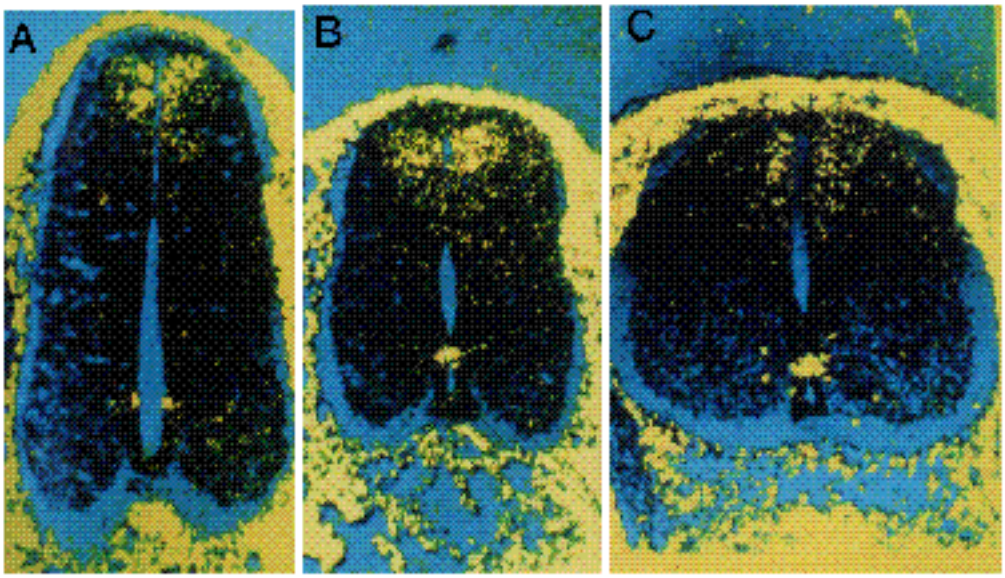

Fig. 2. PDGF-αR expression in the E14 rat spinal cord. Transverse sections through an E14 rat spinal cord were subjected to in situ hybridization and autoradiography to visualize PDGF-αR mRNA. Corresponding bright-field and dark-field images were assigned false colours and superimposed electronically as described in Materials and methods. The bright-field image is blue and the dark-field image yellow. The figure illustrates the lumbar (A), thoracic (B) and cervical (C) levels of the cord (dorsal side uppermost). The approximate levels of these sections are indicated by black arrowheads in Fig. 6A. PDGF-αR mRNA is expressed strongly in non-CNS tissues outside of the spinal cord, by presumptive neuronal precursors in the dorsal region of the spinal cord and, most strikingly, in a small focus of cells (oligodendrocyte precursors?) located in the ventral half of the cord.

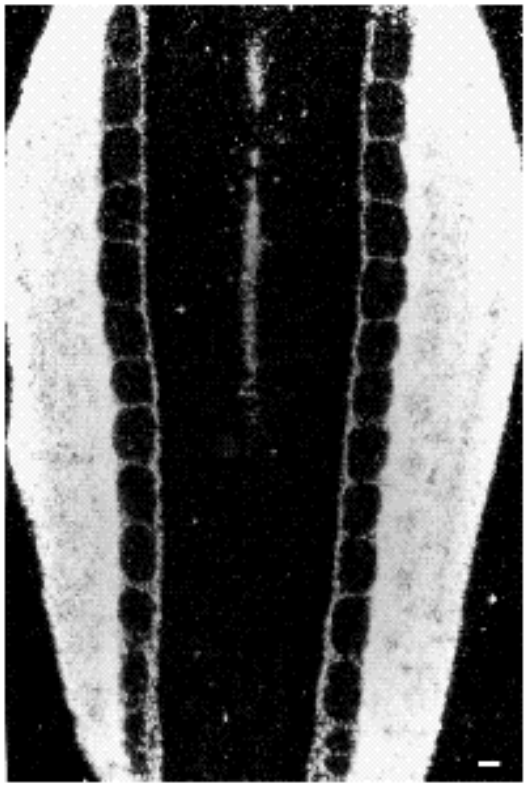

Fig. 3. PDGF-αR expression in the E14 rat spinal cord. A longitudinal section through an E14 spinal cord was subjected to in situ hybridization, autoradiography and dark-field micrography. Part of the column of PDGF-αR^+ cells at the midline of the basal plate is visible, where the plane of section is appropriate. Scale bar (lower right), 100 μm.

might be germinal centers for PDGF-αR^+ cells in the brain that are topologically equivalent to those in the spinal cord. To explore this possibility we cut sections of embryonic rat brain and subjected them to the in situ hybridization procedure with our PDGF-αR probe. Fig. 7 shows coronal sections through different parts of the diencephalon of an E13 rat brain photographed under dark-field illumination. There are two separate regions of PDGF-αR expression in the ventricular and subventricular zones, one in the dorsal half and one in the ventral half of the diencephalon. In the more anterior section only the dorsal region of expression is visible (arrow in A); this presumably corresponds to the anterior tip of the wedge-shaped zone of PDGF-αR expression in the posterior part of the diencephalon, visible in sagittal sections of E13 (not shown) or E14 (Fig. 6A) rats. The existence of a dorsal and a ventral region of PDGF-αR expression in the developing diencephalon suggested that, by analogy with the spinal cord, the ventral zone of PDGF-αR^+ might be the source of the many individual PDGF-αR^+ cells that later appear throughout the forebrain and midbrain (Pringle et al., 1992; Fig. 6B). We therefore examined the ventral zone of PDGF-αR expression more carefully in series of coronal sections of E14 and E15 rat heads. Three sections through the forebrain of an E15 rat are shown in Fig. 8. At this age there are very few, if any, PDGF-αR^+ cells in the most anterior part of the forebrain (Fig. 8A). More posteriorly (Fig. 8B), individual PDGF-αR^+ cells are visible throughout the developing thalamus and hypothalamus; these cells are mainly located outside the subventricular zones of the lateral ventricles, and are absent from the most dorsal extremities (developing cerebral cortex). Further posteriorly (Fig. 8C), a region of intense PDGF-αR expression is encountered in the ven-

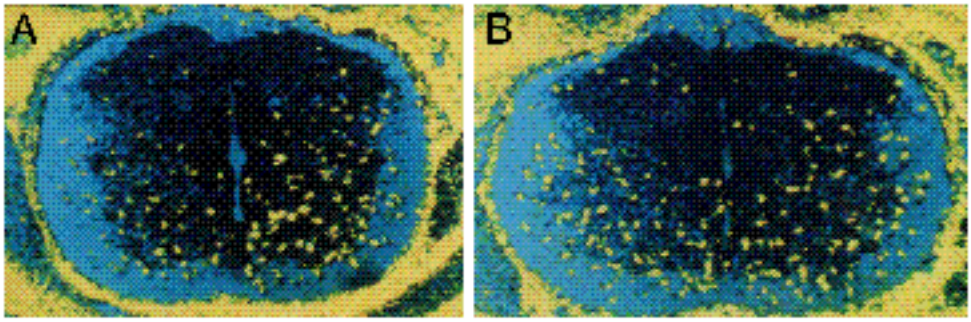

Fig. 4. PDGF-αR expression in the E16 rat spinal cord. Transverse sections through an E16 spinal cord were subjected to in situ hybridization and autoradiography; bright- and dark-field images are displayed as outlined in the legend to Fig. 2. Sections from the thoracic (A) and cervical levels (B) are shown. The approximate levels of these sections are indicated by black arrowheads in Fig. 6B. PDGF-αR cells are no longer present at the ventricular surface; instead, many individual cells are distributed throughout the gray and white matter, mainly in the ventral half of the cord.

tricular and subventricular zones of the third ventricle, particularly beneath the ducts that connect the third and the lateral ventricles (foramen of Monro). Outside this germinal center, in the ventral half of the forebrain, there are many individual PDGF-αR^+ cells, which we presume originated in the ventricular zone just described. These individual PDGF-αR^+ cells later propagate in the anterior and dorsal directions to populate the entire cerebral cortex by the day of birth (Pringle et al., 1992).

DISCUSSION

A focus of PDGF-αR expression in the ventricular zone of the embryonic spinal cord: the origin of the oligodendrocyte lineage?

The most salient finding of the present work is the discovery of a transient longitudinal column of PDGF-αR expression in the basal ventricular zone of the embryonic spinal cord. In cross-section, this column appears as an island of PDGF-αR mRNA in the midline of the spinal cord or, where the central canal is dilated, as two islands flanking the midline. Because of the systematic temporal lag in development along the length of the spinal cord, we have been able to observe these foci of PDGF-αR^+ cells over about one-and-a-half days of development in one animal. In the lumbar region of the cord at E14, the region of PDGF-αR expression is tiny, comprising only two cells in the cross-sectional (coronal) plane. It is not possible to distinguish these PDGF-αR^+ cells from their PDGF-αR^- neighbours by morphology using the light microscope. At more rostral levels (later developmental time) the island of PDGF-αR expression enlarges, presumably as a result of cell proliferation, and individual PDGF-αR^+ cells appear at a distance from the midline, apparently having detached themselves from the central focus of cells and migrated into

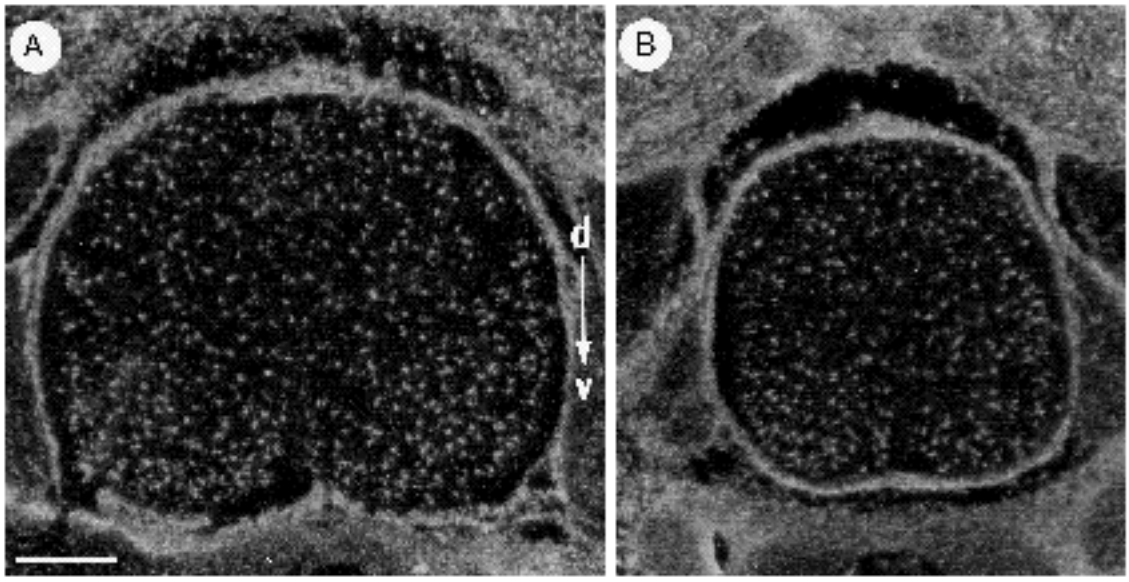

Fig. 5. PDGF-αR expression in the E18 rat spinal cord. Transverse sections through the thoracic region of an E18 spinal cord (A) and brainstem (B) were treated as outlined in the legend to Fig. 1. PDGF-αR^+ cells are distributed throughout the cross-section of the cord, including the most dorsal region. The ventral-dorsal gradient of PDGF-αR^+ cells has almost, but not quite, disappeared. Scale bar (in A), 250 μm.

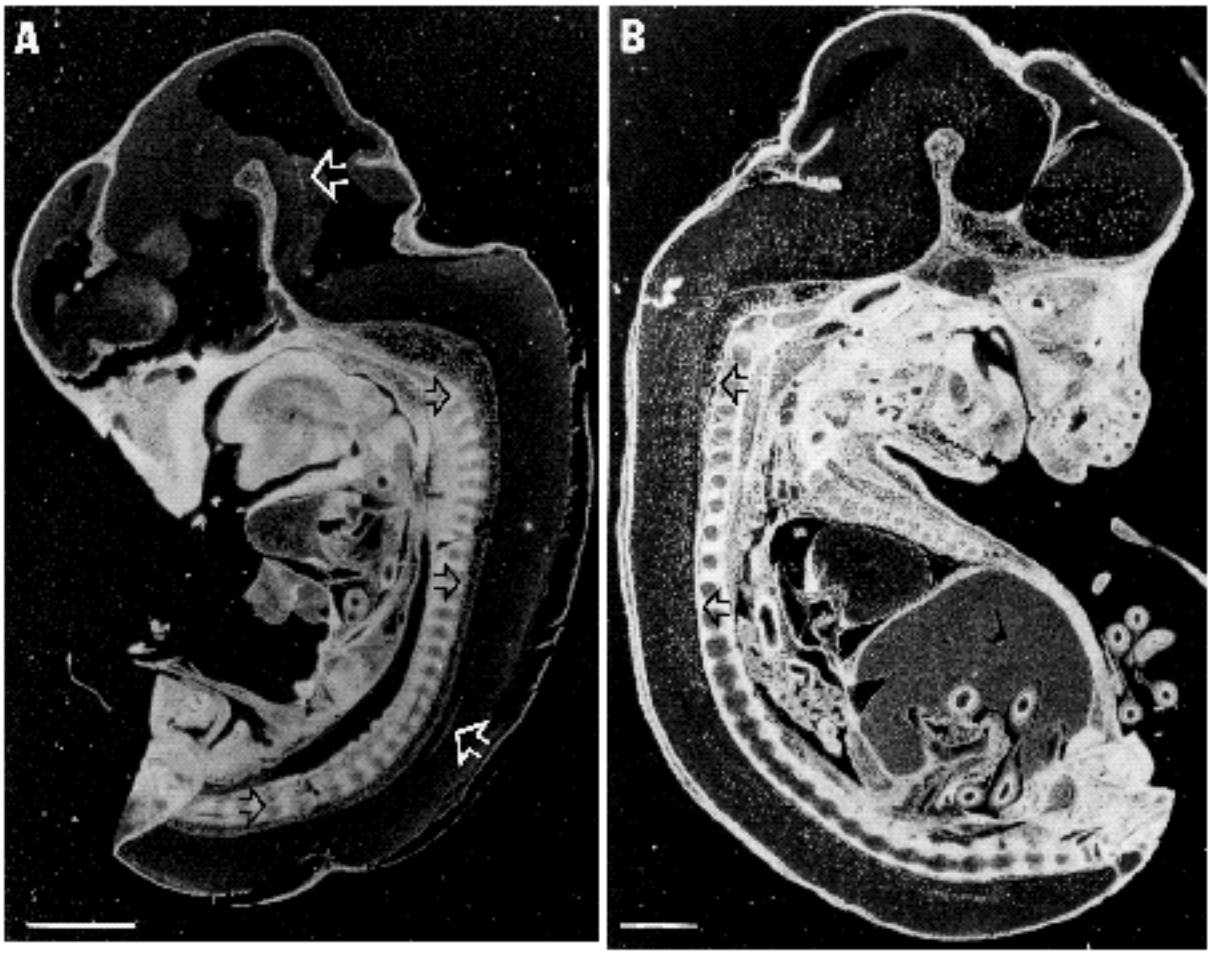

Fig. 6. PDGF-αR expression in the CNS of E14 and E16 rat embryos. Sagittal sections of E14 (A) and E16 (B) rat embryos were treated as outlined in the legend to Fig. 1. Large white arrowheads in A indicate the columns of PDGF-αR^+ cells in the basal ventricular zone of the spinal cord and hindbrain, where the plane of section happens to be suitable. Localized PDGF-αR expression is also apparent in the diencephalon at E14 (A), and in many mesodermal and neural crest-derived tissues (A and B). Black arrowheads indicate the approximate levels of the transverse sections illustrated in Figs 2 and 4. Scale bars, 100 μm.

the gray matter. Two days later, at E16, the cluster of PDGF-αR^+ cells has disappeared from the ventricular zone; instead, there are many PDGF-αR^+ cells throughout the gray and white matter in the ventral half of the spinal cord, although there are still relatively few in the dorsal half. By E18, PDGF-αR^+ cells are distributed more-or-less uniformly throughout the cross-section of the cord. This temporal sequence of events strongly suggests that PDGF-αR^+ cells originate at a defined point in the ventricular zone and subsequently migrate away from there into the surrounding tissue. The fact that PDGF-αR^+ cells are present in the ventricular layer for only a day or two suggests that the founding PDGF-αR^+ cells and/or their progeny move away from the ventricular zone soon after they are formed and that most proliferation of these cells takes place outside the ventricular layer.

It is likely that the PDGF-αR^+ cells originating in the basal ventricular zone represent glial cells, not neurons. Cells in the basal plate are known to give rise to motor neurons, which come to reside in the ventral horns of the spinal cord, but motor neuron production ceases by E13, about one day before we first detect PDGF-αR expression in the basal ventricular zone (Nornes and Das, 1974; Altman and Bayer, 1984). Indeed, most mitotic activity in the basal plate has ceased, and the ventricular zone has largely regressed, before PDGF-αR^+ cells first appear in the basal plate at E14. Thus, the appearance of PDGF-αR^+ cells in that region of the spinal cord is a relatively late neurogenic event, suggesting that these cells might be glial rather than neuronal in nature. This conclusion is supported by the observation that most proliferation of PDGF-αR^+ cells seems to take place outside the ventricular germinal zones whereas the precursors of neurons in the spinal cord and brain undergo their final divisions within the ventricular zone before migrating outwards into the developing gray matter as postmitotic neuronal progenitors. Moreover, after E14 the PDGF-αR hybridization signal is associated with cells possessing small, round nuclei that stain intensely with haematoxylin, characteristics that are generally regarded as hallmarks of glial cells, whereas cells that are clearly neuronal, based on their location and their large nuclei (e.g. motor neurons), are negative for PDGF-αR. At least some of the

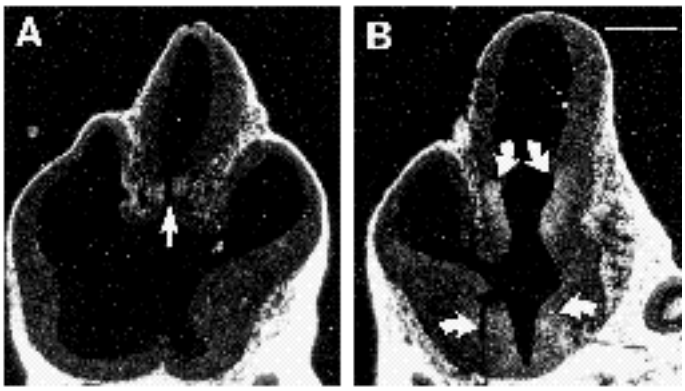

Fig. 7. PDGF-αR expression in the E13 rat diencephalon. Approximately coronal sections through an E13 rat head, at the level of the diencephalon, were treated as outlined in the legend to Fig. 1. The section in A is about 200 μm anterior to that in B. Arrows indicate areas of PDGF-αR expression in the ventricular and subventricular zones. Scale bar (in B), 500 μm.

PDGF-αR$^+$ cells must be glial cells, since they are present in the white matter of the spinal cord which is devoid of neuronal cell bodies. Blood vessels are readily apparent in these spinal cord sections, often being marked by rows of cells with elongated nuclei. These cells, which presumably represent microvascular endothelial cells and/or circulating blood cells, are always negative for PDGF-αR.

We believe that the PDGF-αR$^+$ cells arising in the basal ventricular zone include the earliest precursors of the oligodendrocyte lineage. First, it is known that O-2A progenitor cells, the immediate precursors of oligodendrocytes, express PDGF-αR in vitro and in vivo (Hart et al., 1989; McKinnon et al., 1990); we have recently presented circumstantial evidence from in situ hybridization that, during later neurogenesis (after E16), PDGF-αR expression might be restricted to the O-2A lineage (Pringle et al., 1992). Second, the time and site of origin of PDGF-αR$^+$ cells in the ventral half of the spinal cord is entirely consistent with what is known about the appearance of oligodendrocyte precursors in the cord. Warf et al. (1991) divided the spinal cord of embryonic rats into longitudinal segments which they further dissected into ventral and dorsal halves. They dissociated the cells and looked for the presence of oligodendrocytes, either immediately following dissociation or after maintaining the cells in culture for some time. At E14 they found no oligodendrocytes anywhere in the spinal cord, but oligodendrocytes developed in cultures of cells derived from all rostrocaudal levels, implying that oligodendrocyte precursors, but not mature oligodendrocytes, were present all along the spinal cord at E14. However, only the ventral half of the E14 spinal cord had the capacity to give rise to oligodendrocytes in culture. During later development (after E16), the dorsal half of the spinal cord gradually acquired the capacity to generate oligodendrocytes in vitro, although the numbers of oligodendrocytes forming in cultures of dorsal cells were significantly less than in cultures of ventral cells until E18. These data implied that oligodendrocyte precursors first appear at E14 in the ventral half of the spinal cord and later spread to the dorsal half. Thus, there are striking similarities between the results of Warf et al. (1991) and our own data on PDGF-αR expression, strongly suggesting that at least some of the PDGF-αR$^+$ cells that we observe in the basal plate correspond to precursors of oligodendrocytes. It is possible that the PDGF-αR$^+$ cells at the ventricular surface have already been committed to the exclusive production of oligodendrocytes, or they might be pluripotent precursor cells with the capacity to give rise to other types of glial cells or neurons in addition to oligodendrocytes. For example, Miller and Szigeti (1991) found evidence for several distinct types of astrocytes in the developing rat spinal cord, although it is not yet known whether any of these express PDGF-αR.

PDGF-αR expression by presumptive neuronal precursors in the spinal cord

At the cervical level of the spinal cord, PDGF-αR is expressed transiently between E12.5 and E15 near the dorsal tip of the central canal (dorsal aspect of the alar ven-

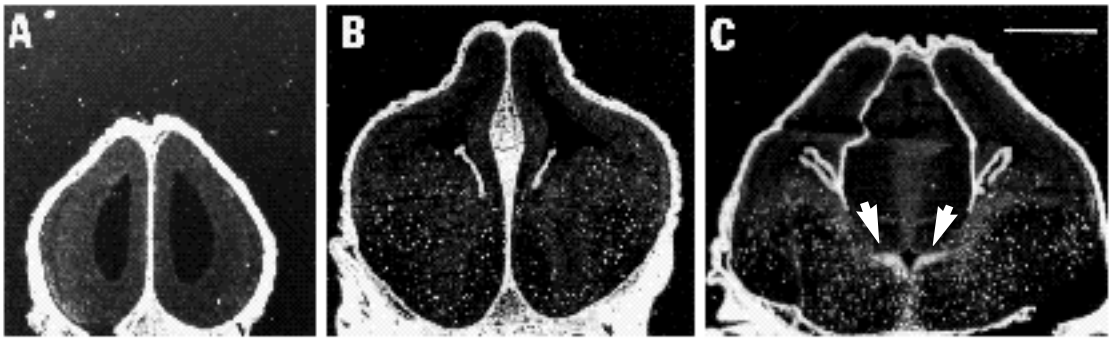

Fig. 8. PDGF-αR expression in the developing forebrain of an E15 rat embryo. Coronal sections taken at different levels of the forebrain were treated as outlined in the legend to Fig. 1. The sections are roughly equally spaced. No PDGF-αR$^+$ cells are visible in the most anterior parts of the forebrain (A). Individual PDGF-αR$^+$ cells are distributed throughout the developing thalamus and hypothalamus (B), but are not present in the developing cerebral cortex. Intense PDGF-αR signal is present in a restricted region of the ventricular zone beneath the foramen of Monro (C); this might be the source of the individual cells that are scattered throughout the ventral region of the diencephalon at this age, and that expand into all regions of the forebrain and midbrain by the day of birth (E21/P0). Scale bar (in C), 1 mm.

tricular zone). This region of the ventricular zone gives rise to interneurons of the substantia gelatinosa, the last major population of neurons to form in the spinal cord (Nornes and Das, 1974; Altman and Bayer, 1984). The first interneurons become post-mitotic at the cervical level of the cord around E14, and the peak of interneuron production occurs around E15 (Nornes and Das, 1974; Altman and Bayer, 1984). Therefore, PDGF-αR expression in the alar ventricular zone appears to precede interneuron differentiation. PDGF A-chain is expressed by many cells in the developing spinal cord from E14 to adulthood (Yeh et al., 1991; H. Mudhar and W.D.R., unpublished data). It is possible, therefore, that PDGF might act as a mitogen or survival factor for interneuron precursors in the later stages of their development. It will be interesting to discover if other populations of spinal cord neurons that are born earlier than interneurons (e.g. motor neurons) also express PDGF-αR before their final mitotic division.

Evidence for a localized source of migrating PDGF-αR^+ cells in the brain

In a previous study (Pringle et al., 1992), we visualized PDGF-αR^+ cells in the rat CNS at ages from E16 to adult. At E16 there were few PDGF-αR^+ cells in the anterior forebrain, and these were located outside and inferior to the subventricular zones of the lateral ventricles, leading us to suggest that PDGF-αR was not expressed in these cells until shortly before, or after, they had started migrating away from the germinal zones towards their final destinations. In the present study, we looked earlier and in greater detail at the genesis of PDGF-αR^+ cells in the developing brain. In the diencephalon at E12-E14 there are two separate domains of PDGF-αR expression. Both are in the ventricular and subventricular zones, one located in the dorsal half of the diencephalon and the other in the ventral half. As in the spinal cord, the dorsal region of expression is transient; it appears around E12-E13 and is gone by E15. We presume that these PDGF-αR^+ cells are developing neurons. The ventral region of PDGF-αR expression first appears at E13. The hybridization signal in this region intensifies during the next two days, and individual cells appear at a distance from the ventricular surface, apparently radiating out into the developing thalamus and hypothalamus and, later (E18-P0), into more dorsal brain regions including the cerebral cortex (see Figs 4 and 5 of Pringle et al., 1992). At E15, and possibly earlier, the ventricular PDGF-αR signal is localized, extending no more than 1 mm in the anterior-posterior direction and mainly restricted to the conjunction of the lateral ventricles and the third ventricle (foramen of Monro). If, as we suppose, these PDGF-αR^+ cells include representatives of the oligodendrocyte lineage, our data raise the possibility that oligodendrocytes in the forebrain and midbrain might all develop from migrating precursor cells that originate in a specialized region of the ventricular zone located in the developing diencephalon. The reason we failed to observe PDGF-αR^+ cells in the germinal zones of the forebrain in our previous study (Pringle et al., 1992) was because PDGF-αR expression in the ventricular and subventricular zones is both transient and localized.

It has been suggested (Choi et al., 1983; Choi and Kim, 1985; Hirano and Goldman, 1988; Aloisi et al., 1992) that astrocytes and oligodendrocytes arise, not from ventricular cells, but by transdifferentiation of radial glial cells, a transient population of cells in the embryonic CNS that are thought to provide structural support and guidance for migrating neuronal progenitors. Our own studies of PDGF-αR expression tend not to favor this model, but lend support to the alternative notion that the oligodendrocyte lineage originates in the ventricular zones of the spinal cord and brain.

Control of gene expression in the dorsoventral axis of the neural tube

The spatially restricted patterns of PDGF-αR expression in the alar and basal ventricular zones of the developing spinal cord are presumably established by regulatory gene products, such as the Hox and Pax transcription factors, that are themselves distributed non-uniformly along the dorsoventral axis (Graham et al., 1991; Rangini et al., 1991; Gruss and Walther, 1992). The initial focus of PDGF-αR^+ cells in the basal region of the spinal cord is much smaller than any of the domains of gene expression defined by these transcription factors or, for that matter, any other gene investigated to date. How can PDGF-αR gene expression be regulated with such high spatial resolution along the dorsoventral axis? Perhaps, by analogy to the anterior-posterior axis of *Drosophila* (Ingham, 1988), several regulatory factors with distinct but overlapping patterns of expression can combine to subdivide the dorsoventral axis of the mammalian neural tube with greater precision than could any one factor acting alone.

We thank K.-H. Lee and R. Reid for sending us the rat PDGF-αR probe. This work was supported by grants from the Medical Research Council and the Multiple Sclerosis Society. We thank all our colleagues in the Medawar Building for encouragement, advice, and/or useful comments on the manuscript, and J. Parnavelas and M. Fitzgerald for sharing their enthusiasm and knowledge of neuroanatomy. We also thank Colin Hopkins and Mark Shipman for help with computer imaging.

REFERENCES

Aloisi, F., Giampaolo, A., Russo, G., Peschle, C. and Levi, G. (1992). Developmental appearance, antigenic profile, and proliferation of glial cells of the human embryonic spinal cord: an immunocytochemical study using dissociated cultured cells. *Glia* **5**, 171-181.

Altman, J. (1966). Proliferation and migration of undifferentiated precursor cells in the rat during postnatal gliogenesis. *Exptl. Neurol.* **16**, 263-278.

Altman, J. and Bayer, S. A. (1984). The development of the rat spinal cord. *Adv. Anat. Embryol. Cell Biol.* **85**, 1-166.

Choi, B. H., Kim, R. C. and Lapham, L. W. (1983). Do radial glia give rise to both astroglial and oligodendroglial cells? *Dev. Brain. Res.* **8**, 119-130.

Choi, B. H. and Kim, R. C. (1985). Expression of glial fibrillary acidic protein by immature oligodendroglia and its implications. *J. Neuroimmunol.* **8**, 215-235.

Graham, A., Maden, M. and Krumlauf, R. (1991). The murine *Hox-2* genes display dynamic dorsoventral patterns of expression during central nervous system development. *Development* **112**, 255-264.

Gruss, P. and Walther, C. (1992). Pax in development. *Cell* **69**, 719-722.

Hart, I. K., Richardson, W. D., Heldin, C.-H., Westermark, B. and Raff, M. C. (1989). PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. *Development* **105**, 595-603.

Hirano, M. and Goldman, J. E. (1988). Gliogenesis in the rat spinal cord:

Evidence for origin of astrocytes and oligodendrocytes from radial precursors. *J. Neursci. Res.* **21**, 155-167.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in Drosophila. *Nature* **335**, 25-34.

Lawrence, J. B. and Singer, R. H. (1985). Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. *Nucl. Acids Res.* **13**, 1777-1799.

LeVine, S. M. and Goldman, J. E. (1988a). Spatial and temporal patterns of oligodendrocyte differentiation in rat cerebrum and cerebellum. *J. Comp. Neurol.* **277**, 441-455.

LeVine, S. M. and Goldman, J. E. (1988b). Embryonic divergence of oligodendrocyte and astrocyte lineages in developing rat cerebrum. *J. Neurosci.* **8**, 3992-4006.

Long, J. A. and Burlingame, P. L. (1938). The development of the external form of the rat with observations on the origin of the extraembryonic coelom and foetal membranes. *University of California publications in Zoology* **43** 143-184.

McKinnon, R. D., Matsui, T., Dubois-Dalcq, M. and Aaronson, S. A. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5**, 603-614.

Miller, R. H. and Szigeti, V. (1991). Clonal analysis of astrocyte diversity in neonatal rat spinal cord cultures. *Development* **113**, 353-362.

Morrison-Graham, K., Schatteman, G. C., Bork, T., Bowen-Pope, D. F. and Weston, J. A. (1992). A PDGF receptor mutation in the mouse (*Patch*) perturbs the development of a non-neuronal subset of neural crest-derived cells. *Development* **115**, 133-142.

Nornes, H. O. and Das, G. D. (1974). Temporal pattern of neurogenesis in spinal cord of rat. I. An autoradiographic study. Time and sites of origin and migration and settling patterns of neuroblasts. *Brain Res.* **73**, 121-138.

Orr-Urtreger, A., Bedford, M. T., Do, M.-S., Eisenbach, L. and Lonai, P. (1992). Developmental expression of the α receptor for platelet-derived growth factor, which is deleted in the embryonic lethal *Patch* mutation. *Development* **115**, 289-303.

Paterson, J. A., Privat, A., Ling, E. A. and Leblond, C. P. (1973). Investigation of glial cells in semithin sections III. Transformation of subependymal cells into glial cells as shown by radioautography after ^{3}H-thymidine injection into the lateral ventricle of the brain of young rats. *J. Comp. Neurol.* **149**, 83-102.

Pringle, N., Collarini, E. J., Mosley, M. J., Heldin, C.-H., Westermark, B. and Richardson, W. D. (1989). PDGF A chain homodimers drive proliferation of bipotential (O-2A) glial progenitor cells in the developing rat optic nerve. *EMBO J.* **8**, 1049-1056.

Pringle, N. P., Mudhar, H. S., Collarini, E. J. and Richardson, W. D. (1992). PDGF receptors in the CNS: during late neurogenesis, expression of PDGF alpha receptors appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* **115**, 535-551.

Rangini, Z., Ben-Yehuda, A., Shapira, E., Gruenbaum, Y. and Fainsod, A. (1991). *CHox E*, a chicken homeogene of the *H2.0* type exhibits dorso-ventral restriction in the proliferating region of the spinal cord. *Mech. Dev.* **35**, 13-24.

Reynolds, R. and Wilkin, G. P. (1988). Development of macroglial cells in rat cerebellum. II. An in situ immunohistochemical study of oligodendroglial lineage from precursor to mature myelinating cell. *Development* **102**, 409-425.

Richardson, W. D., Raff, M. and Noble, M. (1990). The oligodendrocyte-type-2 astrocyte lineage. *Semin. Neurosci.* **2**, 445-454.

Sasahara, M., Fries, J. W. U., Raines, E. W., Gown, A. M., Westrum, L. E., Frosch, M. P., Bonthron, D. T., Ross, R. and Collins, T. (1991). PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* **64**, 217-227.

Schatteman, G. C., Morrison-Graham, K., Weston, J. A. and Bowen-Pope, D. F. (1992). PDGF receptor alpha-subunit expression during mouse development: pattern of expression and consequences of gene disruption. *Development* **115**, 123-131.

Small, R. K., Riddle, P. and Noble, M. (1987). Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature* **328**, 155-157.

Smits, A., Kato, M., Westermark, B., Nistér, M., Heldin, C.-H. and Funa, K. (1991). Neurotrophic activity of PDGF: rat neuronal cells possess functional PDGF β-receptors and respond to PDGF. *Proc. Natn. Acad. Sci. USA* **88**, 8159-8163.

Warf, B. C., Fok-Seang, J. and Miller, R. H. (1991). Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. *J. Neurosci.* **11**, 2477-2488.

Yeh, H.-J., Ruit, K. G., Wang, Y.-X., Parks, W. C., Snider, W. D. and Deuel, T. F. (1991). PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell* **64**, 209-216.

(Accepted 26 October 1992)