Monofocal origin of telencephalic oligodendrocytes in the anterior entopeduncular area of the chick embryo

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SUMMARY

Oligodendrocytes are the myelin-forming cells in the central nervous system. In the brain, oligodendrocyte precursors arise in multiple restricted foci, distributed along the caudorostral axis of the ventricular neuroepithelium. In chick embryonic hind-, mid- and caudal forebrain, oligodendrocytes have a basoventral origin, while in the rostral fore-brain oligodendrocytes emerge from alar territories (Perez Villegas, E. M., Olivier, C., Spassky, N., Poncet, C., Cochard, P., Zalc, B., Thomas, J. L. and Martinez, S. (1999) Dev. Biol. 216, 98-113). To investigate the respective territories colonized by oligodendrocyte progenitor cells that originate from either the basoventral or alar foci, we have created a series of quail-chick chimeras. Homotopic chimeras demonstrate clearly that, during embryonic development, oligodendrocyte progenitors that emerge from the alar anterior entopeduncular area migrate tangentially to invade the entire telencephalon, whereas those from the basal rhombomeric foci show a restricted rostrocaudal distribution and colonize only their rhombomere of origin. Heterotopic chimeras indicate that differences in the migratory properties of oligodendroglial cells do not depend on their basoventral or alar ventricular origin. Irrespective of their origin (basal or alar), oligodendrocytes migrate only short distances in the hindbrain and long distances in the prosencephalon. Furthermore, we provide evidence that, in the developing chick brain, all telencephalic oligodendrocytes originate from the anterior entopeduncular area and that the prominent role of anterior entopeduncular area in telencephalic oligodendrogenesis is conserved between birds and mammals.

Key words: Oligodendrocyte, Migration, Neural chimera, Proteolipid protein, Myelin, Chick, Quail

INTRODUCTION

Oligodendrocytes are the myelin-forming cells of the vertebrate central nervous system (CNS). In the adult, they are ubiquitously distributed throughout the white matter and to a lesser extent in the grey matter. During embryonic development, their precursors arise from discrete territories of the neuroepithelium (Small et al., 1987; Wharf et al., 1991), and segregate in the ventricular zone (Noll and Miller, 1993) where they can be identified by the expression of PDGFRα (Pringle and Richardson, 1993; Hall et al., 1996) or plp/dm-20 transcripts (Timsit et al., 1995; Spassky et al., 1998).

The origin of oligodendrocytes has mainly been studied in the spinal cord. In the lumbar and thoracic regions, from E12.5 in the mouse and E6.5 in the chick, oligodendrocyte precursors form two lateroventral columns of ventricular cells on either side of the ependyma (Pringle et Richardson, 1993; Ono et al., 1995). In the chick, pre-oligodendrocytes labeled by the O4 monoclonal antibody migrate radially, both lateroventrally and ventrodorsally, from these ventricular foci (Ono et al., 1995; Miller and Ono, 1998). In addition to radial migration, retroviral labeling experiments have demonstrated tangential migration of oligodendrocyte progenitors over a distance of more than 300 µm along the rostrocaudal axis of the spinal cord (Leber and Sanes, 1995; Leber et al., 1996). Furthermore, there is evidence for dorsoventral migration of putative dorsal oligodendrocyte progenitors (Cameron-Curry and Le Douarin, 1995). This pathway remains, however, subject to controversy, as illustrated by recent investigations (Pringle et al., 1998).

In the embryonic brain, oligodendroglial migrations have mainly been observed in the optic nerve (Noll and Miller, 1993; Cochard and Giess, 1995; Ono et al., 1997). The focal origin of a subpopulation of optic nerve oligodendrocytes has been localized in the ventricular neuroepithelium of the third ventricle, by fluorochrome labeling experiments performed in the chick embryo (Ono et al., 1997). However, the topological relationship between the ventricular foci of oligodendrocyte precursors and the different populations of brain...
oligodendrocytes has not yet been established, and the differential expansion of oligodendrocytes from basal and alar ventricular foci has not yet been investigated.

To address these issues, we have used avian neural chimeras (Le Douarin, 1969; Le Douarin, 1993; Alvarado-Mallart and Sotelo, 1984) to define a fate map of the ventricular foci of oligodendrogenesis in the prechordal and epichordal domains of the brain. Homotopic transplantations of either telencephalic or rhombencephalic plp/dm-20+ territories demonstrated that all oligodendrocytes in the telencephalon originated monofocally in the anterior entopeduncular area (AEP), while in the hindbrain, segmentally restricted populations of oligodendrocytes developed from metameric basal foci of plp/dm-20+ precursors. However, rhombencephalic oligodendrocytes adopted long-range migrations when developing ectopically in the telencephalon of heterotopic chimeras, while telencephalic oligodendrocytes showed restricted rostrocaudal distribution when transplanted into the hindbrain, suggesting the importance of local environmental cues on oligodendrocyte migration pathways.

MATERIALS AND METHODS

Microsurgery
White Leghorn chicken and Japanese quail eggs were incubated in a forced draft incubator at 38°C. Heterotopic transplantations in avians were performed on the second day of incubation. In all experiments, the quail embryo was the donor and the chick was the host. The grafts were performed at stage 2-7 somites (HH7-9) in the prosencephalon and at stage 10-16 somites (HH11-12) in the rhombencephalon (Hamburger and Hamilton, 1951; Zacchei, 1961). Pieces of neuroepithelium from the neural plate/neural tube were taken up unilaterally with sharp tungsten needles and inserted, either homotopically or heterotopically, into a site previously excised in the host. In the case of avian heterotopic chimeras, the basal plate of rhombencephalon (r3-r5) and ventral territories of telencephalon were exchanged unilaterally. Heterotopic homotopic mouse-chick chimeras were constructed by transplanting E9.5 plp-shble-lacZ transgenic mouse neuroepithelium (Spassky et al., 1998) into stage HH8-9 chick embryos. The rostral pole of mouse telencephalic vesicle containing the anlage of anterior subpallial areas, i.e. the entopeduncular area and part of the pallidum (Bulfone et al., 1993a; Rubenstein et al., 1998), was dissected in ice-cold PBS supplemented with 0.6% of glucose, then grafted into the chick neural plate as described above for the avian chimeras. The ventrodorsal and rostrocaudal polarity was maintained in all the grafts. After the operation, the chick eggs were sealed with tape and returned to the incubator until the time selected for fixation. Although many grafts integrated perfectly into the host, in a number of cases the donor tissue formed a small independent vesicle. These cases were not processed. Macroscopically normal chimeric embryos were sacrificed and fixed either in Clarke solution (acetic acid/100% ethanol, 1:3 V/V) for analysis on paraffin-embedded sections, or in PBS containing 4% paraformaldehyde for analysis on cryosections.

Tissue preparation and staining
Conditions for fixation and treatment of embryos for whole-mount in situ hybridization (ISH), or ISH and immunolabeling on paraffin, vibratome or cryosections, as well as procedures for indirect immunofluorescence were described in Spassky et al. (Spassky et al., 1998) and Perez-Villegas et al. (Perez-Villegas et al., 1999). For double immunolabeling on paraffin or cryostat sections, after incubation with the first antibody (Ab) (QCPN or AA3 Abs), the peroxidase substrate diaminobenzidine (DAB) was supplemented with 15 μg of nickel-ammonium sulfate, to obtain the black reaction product. Incubation with the second primary antibody (AA3 or QCPN monoclonal antibodies (mAbs), respectively) was followed by the classical secondary peroxidase-conjugated antibody and DAB reaction, resulting in a brown stain. To identify neurons, a similar procedure was used, combining QCPN mAb and either TuJ1 mAb or anti-CaBP Ab. Double-immunofluorescence staining was performed on vibratome sections that were examined and photographed by confocal microscopy (Omnichrome ion laser power supply on a Leica DRMB microscope). Heterospecific mouse/chick chimeras were fixed 10 days after surgery (HH 37) and processed for detection of β-galactosidase enzymatic activity on cryostat sections (40 μm; Spassky et al., 1998).

Antibodies
The O4 (IgM, Sommer and Schachner, 1981) and AA3 (IgG rat, Yamamura et al., 1991) mAbs were used as pan-specific oligodendrocyte markers. The QCPN mAb (IgG1, Hybridoma Bank, Iowa) distinguished quail cells from chick cells in quail-chick chimeras. TuJ1 mAb (IgG2A; Hybridoma Bank, Iowa) and anti-CaBP Ab (rabbit polyclonal; Swant Swiss Abs) were used as neuronal markers.

RESULTS

In the chick embryo, ventricular precursors expressing plp/dm-20 transcripts emerge from multiple segmental foci along the rostrocaudal axis, between E2.5 and E4.5 (Perez-Villegas et al., 1999). The subsequent distribution of plp/dm-20+ oligodendrocytes in the subventricular and mantle layers suggests that they expand differently in the rostral and caudal domains of the brain. In the alar foci of the prechordal brain, oligodendrocytes spread tangentially along the rostrocaudal axis, whereas those originating in the basal plate of the epichordal domain of the brain, between rhombomere 7 (r7) and prosomere 2 (p2), have a metamic pattern of distribution. We examined this differential expansion of brain oligodendrocytes from their foci of origin, in vivo, in quail-chick chimeras. As the patterns of plp/dm-20 expression in quail and chick embryonic brain are similar (data obtained by in situ hybridization on E2-E10 brain cryosections, not shown), plp/dm-20-expressing territories can be transplanted from quail to chick, and their progeny followed in the host by immunolabeling quail nuclei with the QCPN mAb.

In birds telencephalic oligodendrocytes derive from the AEP
At E5, the AEP is the most rostral domain where plp/dm-20 expression is observed in the ventricular layer of the prosencephalon (Perez-Villegas et al., 1999), and is superimposable to the telencephalic territory of expression of the transcription factor Gbx-2 (Bulfone et al., 1993b) (Fig. 1). The prospective AEP territory has been mapped to the more rostral domain of the cephalic neural plate at stage HH7-8 (Rubenstein et al., 1998). To investigate the contribution of AEP to the oligodendrocyte population in the telencephalon, a series of AEP chimeras was generated. The prospective AEP was excised from quail embryo before dorsal closure of the cephalic neural plate (stage HH7-8) and transplanted homotopically in a chick embryo at the same stage of development (Table 1). The chimeras were analyzed at E13-E14 (stage HH39-40), by double-labeling brain sections with
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QCPN mAb, which identify quail cells, and AA3 mAb, a pan-oligodendrocyte marker in birds (Perez-Villegas et al., 1999). The oligodendrocytes derived from the grafted territory were therefore defined as QCPN+ /AA3 + cells. To control the quality of the grafts, we compared the neural territory of QCPN + cells in AEP chimeras with the pattern of expression of \( Gbx2 \). In a first group of AEP chimeras (\( n = 2 \)) the QCPN + labeling in the ependymal cells lining the lumen of the third and telencephalic ventricles and derived structures was never observed out of the \( Gbx2 \) -expressing domain (compare Fig. 1A,B with Fig. 2B,E). The quail neuroepithelium grafted at stage HH7-8 thus corresponded to the prospective territory of AEP. Outside the ventricular neuroepithelium, the respective distributions of oligodendrocytes originating from either the host or the graft were carefully examined in diencephalic and telencephalic structures of these chimeras. A large number of AA3 + cells were observed, both in the vicinity of the AEP and in the telencephalon far from the grafted AEP. All these AA3 + cells were of quail origin (Fig. 2H). Caudal to the graft (in the stria medullaris), although the majority of the AA3 + oligodendrocytes were QCPN+, some were QCPN− (not shown). In a second group of AEP chimeras (\( n = 5 \)), the QCPN + cells in the ventricular layer were observed only in part of the AEP (Fig. 2A,D). In these quail smaller grafts of the AEP, only a subpopulation of the oligodendrocytes in the telencephalon were QCPN+, and the others were QCPN−, suggesting they originated from the host (Fig. 2G). In a third group of AEP chimeras (\( n = 3 \)), the graft was larger, i.e. included the whole prospective AEP territory plus a part of the adjacent pallidal and striatal domains. r3-7, rhombomeres 3-7; AEP, anterior entopeduncular area; POA, preoptic area; bp, basal plate; Rh, rhombencephalon.

Table 1. Homotopic and heterotopic quail-chick chimeras

<table>
<thead>
<tr>
<th>Type of graft</th>
<th>Territory of quail donor (stage of graft)</th>
<th>Territory of chick host (stage of graft)</th>
<th>Stage of fixation</th>
<th>Number of analyzed chimeras</th>
</tr>
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<tbody>
<tr>
<td>Homotopic AEP</td>
<td>AEP (HH7-8)</td>
<td>AEP (HH7-8)</td>
<td>E9.5-18.5</td>
<td>10</td>
</tr>
<tr>
<td>Homotopic Ctx</td>
<td>Cortex (HH 9-9+)</td>
<td>Cortex (HH 9-9+)</td>
<td>E13.5-17.5</td>
<td>5</td>
</tr>
<tr>
<td>Homotopic Rh</td>
<td>r3-r4 (HH10)</td>
<td>r3-r4 (HH10)</td>
<td>E9-14</td>
<td>6</td>
</tr>
<tr>
<td>Heterotopic Rh</td>
<td>AEP-POA (HH8-9+)</td>
<td>AEP-POA (HH8-9+)</td>
<td>E11.5-17.5</td>
<td>3</td>
</tr>
<tr>
<td>Homotopic AEP</td>
<td>AEP (HH 8-9+)</td>
<td>r3-r5 (HH10-12)</td>
<td>E13.5-16.5</td>
<td>6</td>
</tr>
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Homotopic grafts were performed within the prosencephalon (AEP and cortex) and within the rhombencephalon (r3-r4 and r6-r7). Heterotopic chimeras were produced by exchanging rhombencephalic and prosencephalic territories. Only the samples where the graft was perfectly integrated and developed normally in the host were processed. Among the homotopic AEP chimeras, two were restricted to the AEP, five included only part of the AEP and three included the whole AEP plus adjacent pallidal and striatal domains. r3-7, rhombomeres 3-7; AEP, anterior entopeduncular area; POA, preoptic area; bp, basal plate; Rh, rhombencephalon.

QCPN mAb, which identify quail cells, and AA3 mAb, a pan-oligodendrocyte marker in birds (Perez-Villegas et al., 1999). The oligodendrocytes derived from the grafted territory were therefore defined as QCPN+/AA3+ cells. To control the quality of the grafts, we compared the neural territory of QCPN+ cells in AEP chimeras with the pattern of expression of \( Gbx2 \). In a first group of AEP chimeras (\( n = 2 \)) the QCPN+ labeling in the ependymal cells lining the lumen of the third and telencephalic ventricles and derived structures was never observed out of the \( Gbx2 \)-expressing domain (compare Fig. 1A,B with Fig. 2B,E). The quail neuroepithelium grafted at stage HH7-8 thus corresponded to the prospective territory of AEP. Outside the ventricular neuroepithelium, the respective distributions of oligodendrocytes originating from either the host or the graft were carefully examined in diencephalic and telencephalic structures of these chimeras. A large number of AA3+ cells were observed, both in the vicinity of the AEP and in the telencephalon far from the grafted AEP. All these AA3+ cells were of quail origin (Fig. 2H). Caudal to the graft (in the stria medullaris), although the majority of the AA3+ oligodendrocytes were QCPN+, some were QCPN− (not shown). In a second group of AEP chimeras (\( n = 5 \)), the QCPN+ cells in the ventricular layer were observed only in part of the AEP (Fig. 2A,D). In these quail smaller grafts of the AEP, only a subpopulation of the oligodendrocytes in the telencephalon were QCPN+, and the others were QCPN−, suggesting they originated from the host (Fig. 2G). In a third group of AEP chimeras (\( n = 3 \)), the graft was larger, i.e. included the whole prospective AEP territory plus a part of the adjacent pallidum and striatum (Fig. 2C,F). All the AA3+ in the telencephalon were of quail origin (Fig. 2I). However, even though more cells from quail origin were present in the chimera, the distribution of AA3+ oligodendrocytes was similar than in the previous cases and no quail oligodendrocytes were observed ventral to the epithalamus, or in the caudal diencephalon. Altogether, these results strongly suggest that telencephalic oligodendrocytes originate from the AEP, and that structures adjacent to the AEP do not contribute to the oligodendroglial population.

To determine whether part of the oligodendroglial

Fig. 1. Gene expression domains of ventral telencephalon in the chick embryo at stage HH40 (E14). Sagittal section of telencephalon processed for double in situ hybridization to detect the transcripts of \( Gbx2 \) (in purple) and \( Otx2 \) (in red-orange). The domain of expression of \( Gbx2 \) is restricted to the structures derived from the AEP neuroepithelium (A). The patterns of expression of different genes in the subpallial structures of telencephalon are illustrated by distinct colours on a schematic sagital view on the midline (B). \( Gbx2 \) and \( shh \) (Shimamura and Rubenstein, 1997) are restricted to the AEP (red), \( Nkx-2 \) (Rubenstein et al., 1998) is detected in the AEP and the adjacent pallidum (orange), \( Dlx-2 \) (Bulfone et al., 1993a) extends in the AEP, pallidum and striatum (yellow). Ac, anterior commissure; AEP, entopeduncular area; chp, choroidal plexus; DP, dorsal pallium; DT, dorsal thalamus; EMT, eminentia thalami; LP, lateral pallium; MP, medial pallium; OB, olfactory bulb; oc, optic chiasm; PAL, pallidum; pc, pallial commissure; POA, preoptic area; SC, suprachiasmatic area; ST, striatum; VP, ventral pallium; VT, ventral thalamus.
population could nevertheless derive from the embryonic cortex, we generated avian pallial chimera. The prospective cortex was dissected unilaterally from quail embryos at stage HH9 and transplanted homotopically into chick embryos at the same stage of development (Fig. 3A; Table 1). This series of chimeras (n=5) was analyzed at E15.5-17.5 on vibratome sections double-labeled with QCPN and O4 mAbs. In the brain of these chimeras (Fig. 3B), all the cortical territories, the neostriatum and the olfactory bulb, as well as a dorsal portion of the striatum, derived from the graft (Fig. 3C,E,G). Although O4+ oligodendrocytes were detected in these structures, none was of quail origin (Fig. 3D,F,H). These data strongly suggest that the oligodendrocytes of the embryonic telencephalon originate solely from the AEP.

**Timecourse and migratory pathways followed by oligodendrocytes in the telencephalon**

The progression of oligodendrocytes from the AEP was followed by immunostaining with O4 mAb, which labeled pre-oligodendrocytes (Fig. 4). As previously described (Perez-Villegas et al., 1999), in the telencephalon, O4+ cells were first detected at E6 (HH 27-28) in close vicinity of the AEP. From this focus of emergence, O4+ cells spreaded in the telencephalon following three major streams of tangential migration: septal, lateral and rostral. Following the septal pathway, O4+ cells were first observed in the septum at E7 (HH 31-32), then in the hippocampus at E7-E8 and finally reached the hyperstriatum at E9 (HH 35-36). Along the lateral pathway, O4+ cells were detected in the longitudinal forebrain bundle at E7, then in the pallidum (pallistriatum primitivum) and the striatum and in the neostriatum at E8 (HH 34). In addition, O4+ cells also migrated ventrorostrally through the medial ganglionic eminence towards the subpial surface and reached the olfactory bulb at E9. Examination of homotopic AEP chimeras between E9 and E18.5 by double immunolabeling with QCPN and AA3 mAbs, allowed us to follow the progression of differentiated oligodendrocytes, and confirmed the observation performed with O4 mAb. At E9, AA3+ oligodendrocytes were observed in the vicinity of the graft, in the optic chiasm and the anterior commissure (Fig. 5A-C). In addition, a few AA3+/QCPN+ cells were observed in the septum and the pallidal and striatal territories (not shown). At E13, numerous AA3+ cells were observed at a distance of the grafted AEP along the lateral forebrain bundle, and in both the subpallial (pallidum-striatum complex) and pallial structures. Within the pallial subdivision, quail oligodendrocytes were localized in the caudal neo- and hyperstriatum, and notably in the dorsal part of hyperstriatum (Fig. 5D,E). Other longitudinal fascicules like the stria medullaris and the septomesencephalic tract, were also colonized by quail oligodendrocytes. Between E13 and E18.5, the AA3+/QCPN+ cells expanded rostrally in the marginal zone and within the lateral forebrain bundle and the stria medullaris. At E18.5, oligodendrogial cells, exclusively of quail origin, had invaded the entire telencephalon. They were abundant in the axonal tracts, but rare in the cortex. In the lateral forebrain bundle (Fig. 5F), the AA3+/QCPN+ AEP-derived oligodendrocytes represented 80±5% of QCPN+ cells, while in the hyperstriatum ventral, only 3±1% of QCPN+ cells
were AA3+. The majority of AA3+/QCPN+ AEP-derived cells observed in the latter cortical territory were immunolabeled with anti-CaBP Ab and thus most likely GABAergic interneurons (not shown). In the dorsal and rostral diencephalon, and specifically in the stria medullaris, most of the oligodendrocytes were also derived from the grafted AEP. However, no quail oligodendrocytes were detected ventral to the epithalamus or in the caudal diencephalon. This suggests that the stria medullaris is the exclusive pathway for the caudal migration of oligodendroglial cells derived from the AEP.

Extensive migration of AEP-derived oligodendrocytes in mouse-chick chimeras

To assess whether the ability of long-range migration of AEP-derived oligodendroglial cells is conserved in birds and mammals, heterospecific homotopic mouse-chick chimeras were created. For these experiments we took advantage of the plp-shble-lacZ transgenic mouse in which the regulatory elements of the oligodendrocyte-specific plp gene drive the expression of the E. coli lacZ reporter gene exclusively in oligodendroglial cells (Spassky et al., 1998). We performed homotopic grafts (n=6) of the AEP (including a part of the pallidum) from plp-shble-lacZ mouse at E9.5 into stage HH8 chick embryo (Fig. 6A). The presence of β-galactosidase-positive (β-gal+) cells in the chimeric brains was investigated at E11 (n=3) by staining with the X-gal substrate. Indicative of a mouse origin, β-gal+ oligodendrocytes were detected in the entopeduncular neuroepithelium. In addition, a massive population of β-gal+ cells was observed in the subventricular and mantle layers of the host telencephalon. Similar to the above reported observations in the avian AEP-chimeras, numerous mouse oligodendroglial cells had migrated out of the AEP explant to reach not only the pallidum (Fig. 6B,C) and striatum, but also the ventral and lateral pallium (Fig. 6B,D). In the control contralateral telencephalon of chimeras, as well as in the brain of normal chick embryos at the same stage of development, β-gal+ cells were never detected after the X-gal reaction.

Restricted migration of oligodendrocyte progenitors in the hindbrain

We next examined whether oligodendrocytes developed
Fig. 4. Migration pattern of AEP-derived cells from the oligodendrocyte lineage. The colonization of telencephalic structures by oligodendrocyte progenitors originating from the AEP, has been followed on chick forebrain coronal sections immunolabeled with O4 mAb between E6 and E9. (Top) A schematic 3D representation of the forebrain, showing the progression of O4+ cells, which are first detected in the vicinity of the AEP at E6. The table below indicates the time course of detection of O4 cells in each territory. From their site of emergence in the AEP, O4+ cells follow three major migratory pathways: a septal migration (red arrow) through the septum (Se), hippocampus (Hi) and hyperstriatum (H); a lateral migration (green arrow), through the longitudinal forebrain bundle (lfb), the pallidum (pallistriatum primitivum) (PAL), the striatum (ST), the neostriatum (N) and the hyperstriatum; a rostral (ventral) migration (yellow arrow) towards the olfactory bulb (OB).

Environmental control of oligodendrocyte migratory properties

The extensive tangential migration of oligodendrocyte progenitors originating in the rostro-alar territory of AEP contrasts with the segmentally restricted migration of those originating in the rhombencephalic basal plate. These strikingly different migratory properties, in the rostral versus the caudal brain, could either be controlled by intrinsic features of each population, or depend on environmental cues. We thus examined, in vivo, whether the migratory behavior of oligodendrocyte progenitors was conserved in an ectopic environment. Heterotopic chimeras were constructed at HH8-9 and the migration of QCPN+ oligodendrocytes analyzed between E11.5 and E17.5 (Table 1).

In one series of experiments (Rh→AEP chimeras), the AEP of HH8-9 chick host was unilaterally replaced by pieces of neuroepithelium corresponding to the basal plate of r3-r5
rhombomeres dissected from stage HH9-11 quail embryos \((n=3)\) (Fig. 8A,B). In these Rh→AEP chimeras, the basal rhombencephalic neuroepithelial cells generated oligodendrocyte progenitors (Fig. 8C) that migrated long distance from the graft. At E11.5, numerous AA3+/QCPN+ oligodendrocytes were identified in the hippocampus (103±10 cells/20,000 \(\mu m^2\)) (Fig. 8D) or in the septum (8±3 cells/20,000 \(\mu m^2\)), and most of oligodendrocytes observed in these territories originated from the graft (68% in the hippocampus and 90% in the septum). The AA3+/QCPN+ oligodendrocytes were also distributed within the fiber tracts of the prosencephalon: in the stria medullaris (9±1 cells/20,000 \(\mu m^2\)) or in the lateral forebrain bundle (31±4 cells/20,000 \(\mu m^2\)). At E11.5, in the dorsal telencephalon, i.e., neostriatum, hyperstriatum and cortex, very few quail oligodendrocytes were detected (<10 cells/140,000 \(\mu m^2\)). But at E17.5, O4+/QCPN+ oligodendrocytes were numerous in all telencephalic domains, and in the dorsal telencephalon they
showed the same pattern of distribution as in AEP-homotopic chimeras. In the RhAEP chimeras, in addition to quail oligodendrocytes, QCPN+/AA3− cells were also observed at a distance from the graft. These cells were identified as neurons by immunolabeling with TuJ1 mAb. These neurons were not CaBP+, thus probably not GABAergic, and have not been further characterized.

In corollary experiments (AEP→Rh chimeras), the AEP of HH8-9 quail were grafted into the ventral hindbrain of HH10-12 chick hosts (n=6). In the AEP→Rh chimeras, the migration of AEP-derived oligodendrocytes remained restricted to the rhombomeric domain of the graft (not shown), similar to the distribution of rhombencephalic-derived quail oligodendrocytes observed in the homotopic experiments above described (Fig. 7). Altogether, these data suggest that the differences in the migration pathways observed between oligodendrocytes originating from ventral or alar ventricular territories are most likely due to environmental cues.

DISCUSSION

Metameric development of oligodendrocytes in the hindbrain

We show here that independent populations of oligodendrocytes are compartmentalized by inter-rhombomeric boundaries within the caudal hindbrain (Fig. 9). The oligodendroglial cells distributed along the plurisegmental medial and lateral longitudinal fascicules maintain their segmental organization and myelinate axons in sectors determined by their metameric origin.

A similar metameric pattern of development has already been described for neurons of the reticular formation (Clarke et al., 1998) and rhombencephalic motoneurons (Lumsden and Keynes, 1989; Simon and Lumsden, 1993; Lumsden and Krumlauf, 1996). Fate maps have also revealed the metameric origin of adult sensory nuclei (Marin and Puelles, 1995). This compartmental restriction of cell development persists in the hindbrain during the period when the epithelium is predominantly germinative (Birgbauer and Fraser, 1994). Later in development, however, young neurons escape this rhombomeric restriction by tangential migration through the mantle layer, once they have acquired their positional specification (Wingate and Lumsden, 1996). In contrast, even at the latest stage examined (E14), oligodendrocytes remained confined within their rhombomere of origin.

Extensive production of telencephalic oligodendrocytes from the anterior entopeduncular area

The anterior entopeduncular area (AEP) is a discrete alar territory, which generates a cell population that generates the ventral pallidum, the medial septum and the dorsocaudal part of the preoptic area, i.e. the ventral structures of prosomeres P5 and P6 (Rubenstein et al., 1998). We show that, at the early stage of neural plate development, before the dorsal closure of the prosencephalic plate (stages HH7-8, i.e. 2 to 4 somites), the most rostral quarter of the cephalic neural plate is the prospective territory of AEP. In the AEP chimeras, the grafted neuroepithelium develops into a quail ventricular domain which coincides with the expression pattern of Gbx-2, specific of AEP in the telencephalon (Bulfone et al., 1993b). Our results are consistent with previously reported fate map of the prosencephalic neural plate (Rubenstein et al., 1998). We demonstrate that the AEP is the unique source of the large population of cortical and extracortical oligodendrocytes in the telencephalon of the avian embryo. In addition, we provide evidence that the oligodendrogenic potential of AEP is conserved in the mouse, by transplanting the AEP from plp-
Migration of embryonic brain oligodendrocytes

shble-lacZ transgenic mouse into a chick host. In mammals, other observations indicate that the AEP is a precocious and potent site of oligodendrogenesis in the prosencephalon. The AEP ventricular territory indeed expresses oligodendroglial markers at early stages of mouse brain development. The plp/dm-20 transcripts are detected from E9.5 (Timsit et al., 1995; Spassky et al., 1998) and the oligodendrocyte-specific basic-helix-loop-helix transcription factors Olig1 and Olig2 show, in the forebrain, a similar ventricular pattern of expression at E9-E10.5 (Lu et al., 2000). However, taking into account the much larger development of cortical structures in mammals, we cannot exclude the existence of additional sites of oligodendrogenesis in the mouse telencephalon.

The molecular mechanisms governing the considerable oligodendroglial potential of the AEP and the extensive expansion of its progeny, which are unique features in the telencephalon, are still unknown. However, the molecular inducer sonic hedgehog (Shh), already associated with the specification of oligodendrocytes in the spinal cord (Pringle et al., 1996; Poncet et al., 1996; Orentas et al., 1999), is very likely to be involved in these events. Expression of shh is restricted to the ventromedial domain of the telencephalon including the AEP (Shimamura and Rubenstein, 1997; Perez-Villegas et al., 1999) and treatment of E10.5 mouse AEP explants with anti-Shh mAb abolishes the genesis of oligodendrocytes (data not shown). The specific or collaborative effects of regulatory genes such as Dlx homeobox genes (Bulfone et al., 1993a; Bulfone et al., 1993b; Liu et al., 1997) and Nkx2.1 gene (Guazzi et al., 1990; Shimamura et al., 1995; Sussel et al., 1999), expressed in the pallidal domain of telencephalon, might also contribute to the control of oligodendrogenic activity in the AEP.

Extensive migration of AEP-derived cells

In the avian embryonic brain, the AEP-derived oligodendrocyte progenitors are widely distributed throughout subpallial and pallial domains, as well as the preoptic area. They migrate tangentially, dorsally and rostrally following the septum and the developing lateral forebrain bundle towards the dorsal telencephalon, and caudally through the stria medullaris.
towards the dorsal region of rostral diencephalon (Fig. 9). This observation corroborates previous descriptions of tangential migrations in the avian telencephalon obtained either with the neural chimera system (Balaban et al., 1988), or by labeling chick telencephalic progenitor cells at E2.5 with a library of retroviral vectors encoding alkaline phosphatase and a variety of molecular tags (Szele and Cepko, 1998). In addition, the heterospecific homotopic mouse-chick chimeras, which show important migrations of mouse AEP-derived oligodendrocytes into the subpallial and pallial structures of chick telencephalon, suggest a conservation between birds and mammals of the crucial role of the AEP in the generation of telencephalic oligodendrocytes. The molecular mechanisms controlling the migration of rostral oligodendrocytes might thus be similar, if not identical, in the chick and the mouse.

The present data converge with recent reports that cells from a germinal region adjacent to the AEP, the medial ganglionic eminence (MGE), in the mouse embryonic forebrain, are widely dispersed (Wichterle et al., 1999; Lavdas et al., 1999). In both embryonic brain slice cultures and in vivo grafts, MGE-derived cells spread tangentially through multiple regions of the embryonic brain. Sagittal representation of the brain at stage HH 27 (E5-6).

In the ventricular layer, the basoventral territories of emergence are colored in green, the alar domains in red. Arrows indicate the migratory pathways. Note that oligodendrocyte progenitors emerging from the basoventral foci, in the epicordal domain of the brain, migrate radially (black arrows), while those arising from the alar plate, in the precordal domain of the brain, follow extensive tangential migratory pathways (orange arrows). AEP, anterior entopeduncular area; ET, eminentia thalami; p, prosomere (p1 to p6); r, rhombomere (r1 to r7); sc, spinal cord; ZLI, zona limitans intrathalamica.
both embryonic and adult brain, whereas the migratory potential of progenitors derived from the cortex was strictly limited (Wichterle et al., 1999).

In addition, a robust neurogenesis has been detected in the AEP-MGE territory of AEP chimeras, which provides a large contribution to the population of GABAergic interneurons. In the mouse, a part of sibling cells of the MGE also corresponds to GABAergic neurons widely distributed in the forebrain (Wichterle et al., 1999; Lavdas et al., 1999; Sussel et al., 1999) and studies using highly unbalanced mouse stem cell chimeras have underlined that the predominant tangential migration of GABAergic neurons progenitors contrasted with the radial dispersion of neurons expressing glutamate (Tan et al., 1998). Therefore, in the forebrain of birds and mammals, the AEP-MGE germinative territory shows a dual potentiality to generate oligodendrocytes and GABAergic interneurons with common tangential and long-range migratory properties.

Migratory properties of oligodendrocytes depend on regional factors

The first demonstrations that specification of neural cell fate depends on environmental cues resulted from studies of transplanted cells in the quail-chick chimera system (LeDouarin, 1986; Martinez et al., 1991). Using a similar approach, we show that oligodendrocyte progenitors, transplanted between the rostral and caudal domains of embryonic brain, change their migratory properties. Rhombencephalic oligodendrocytes adopt long tangential migrations when transplanted into the ventral telencephalon, and telencephalic oligodendrocytes show limited tangential migrations when grafted into the hindbrain. Tangential migrations are therefore restricted by hindbrain cues, while they are promoted by the forebrain environment. The mechanisms underlying these regional properties controlling oligodendroglial migration are unknown. They could involve extracellular matrix molecules that may provide, depending on their loco-regional expression, preferred pathways for the migration of oligodendrocyte precursors (Kiernan and ffrench-Constant, 1993). Different combinations of neural cell adhesion molecules (Payne and Lemmon, 1993; Redies and Takeichi, 1993) and integrins (Milner and ffrench-Constant, 1994), might then characterize rostral versus caudal axonal tracts and also account for the different pattern of oligodendroglial migrations. Oligodendroglial migrations could also be controlled by regional heterogeneities within the radial glial scaffolding of the embryonic brain (Hatten, 1990). The elongated radial glial fibers, which guide the movement of neocortical neurons (Rakic, 1972; Bayer et al., 1991; McConnell, 1991), have been suggested to regulate spatio-temporally the migration patterns of glial progenitors in the rat subventricular zone (Levison et al., 1993). Although they are not strictly radially aligned in the telencephalon, especially in the mediodorsal and ventrolateral cortex (Misson et al., 1988; Edwards et al., 1990), their pattern of extension does not correspond to the pathways used by oligodendrocyte progenitors emerging from the AEP (Harfuss et al., 2001). It is thus unlikely that the long distance tangential-like migrations of oligodendrocytes in the developing telencephalon are driven by radial glial cells. In contrast to this gliophilic pathway, migration of oligodendrocyte progenitors can be guided by axonal signals. This is most probably the case for the optic nerve, a structure devoided of radial glia, and where oligodendrocyte progenitors are closely associated with retinal ganglion cell axons during their migration from the chiasmatic end towards the lamina cribosa (Ono et al., 1997). The same axonophilic cues may be involved for oligodendrocyte migration in the septal fimbria, the lateral forebrain bundle, or the stria medullaris, which are colonized from the AEP. Although the molecular codes involved in this axon signaling remain to be discovered, the ephrin and Eph receptors (O’Leary and Wilkinson, 1999) may be reasonable candidates. In the hindbrain, oligodendroglial movements are limited along the medial and lateral longitudinal fascicles. This limitation of tangential migration accurately follows the rhombomeric boundaries, suggesting that oligodendrocyte progenitors (whether from rhombencephalic or telencephalic origins) are unable to cross these boundaries. Finally, we cannot exclude the possibility that the tangential and radial glia-independent migration of oligodendroglial progenitors in the forebrain may also respond to repulsive or attractive mechanisms, similar to those described for the tangential migrations of neurons guided by Slit in the olfactory system (Wu et al., 1999) and neocortex (Hu, 1999; Zhu et al., 1999).

In conclusion, oligodendroglial development differs in the epichordal and prechordal domains of embryonic chick brain. Segmental oligodendrogenesis occurs in the more primitive epichordal domain, whereas oligodendroglia expand from a single focus, the AEP, into several prosomeres of the earlier evolving prechordal domain of the brain. In mammals, the AEP also plays a major role in telencephalic oligodendrogenesis, but further studies are needed to understand to what extent this ventricular territory might contribute to the subventricular zone (SVZ) of the lateral ventricles and to the final oligodendroglial populations of the cortex in mammals.

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