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Oligodendrocyte precursor (O-2A progenitor cell) migration; a model system for the study of cell migration in the developing central nervous system

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SUMMARY

Cell migration plays an important role in the development of complex multicellular organisms. The molecular mechanisms that regulate this migration are therefore of great interest. Unfortunately, however, analysis of cell migration in vertebrates is hampered by the inaccessability of the cells and the difficulty of manipulating their environment within the embryo. This review focusses on one particular migratory cell population, the oligodendrocyte precursor cell or O-2A progenitor cell, that gives rise to the myelin-forming oligodendrocytes within the CNS. These cells migrate extensively during normal development. They can be purified and grown in large numbers in cell culture, so allowing the use of reductionist approaches using cell and molecular biology techniques. Moreover, cultured cells will migrate within the CNS following transplantation. As a result, the migration of these cells in vivo can be analysed following manipulation in vitro. Taken together, we believe that the different properties of these cells makes them excellent candidates for studies addressing the control of cell migration in the developing nervous system.

Key words: cell migration, oligodendrocyte, O-2A progenitor cell, tenascin, extracellular matrix, cell adhesion, lamina cribrosa

INTRODUCTION

Cell migration is an essential component of neural development. The central nervous system (CNS) develops from a two-dimensional sheet of cells into a complex three-dimensional structure. Throughout development much of the cell proliferation and the birth of new cell types is restricted to localised areas near the centre of the developing brain, the ventricular and subventricular zones. Neurones and glial cells form in these zones then migrate to their final destination. The best known example of this is the extensive radial migration of neurones along radial glia (Rakic, 1972). More recently, however, it has become clear that glial cells as well as neurones migrate during neural development. In particular, a number of studies have shown that the oligodendrocytes that form myelin in the mature CNS develop from precursor cells that have migrated long distances prior to differentiation.

The purpose of this short review is to summarise the evidence for this migration and emphasise the value of this particular system as a model for the study of migration during neural development.

EVIDENCE FOR MIGRATION OF OLIGODENDROGLIAL LINEAGE CELLS DURING DEVELOPMENT

The first evidence for migration came from studies on the origin of oligodendrocytes using morphological techniques. These studies defined immature cells with the characteristics of oligodendroglia in the developing CNS, and taken in conjunction with autoradiographic experiments, suggested that these putative precursor cells arise from proliferating cells present within the subventricular zone (SVZ; Paterson et al., 1973). This restricted origin of oligodendrocyte precursors in the SVZ and the subsequent widespread distribution of oligodendrocytes implies the migration of oligodendrocytes or their precursors during development. More recent work has provided direct evidence of this migration using two different approaches - transplantation and in situ labelling.

In a series of elegant studies over the last decade, Gumpel and colleagues have used grafts of CNS tissue into *shiverer* mice brains to analyse oligodendrocyte precursor cell migration (Gumpel et al., 1989). In this system, the formation of myelin by transplanted cells can be analysed

by immunostaining for myelin basic protein (MBP), which is lacking in the *shiverer* host as a result of a deletion within the MBP gene. These experiments show the formation of myelin patches at a considerable distance from the original transplant, showing that oligodendroglial cells or their precursors must have migrated out of the transplant (Lachapelle et al., 1984). A similar conclusion as to the migratory potential of oligodendroglial cells within transplants was reached by Duncan et al. (1988) in studies on the adult myelin-deficient (md) rat. Three weeks after transplantation of CNS cell suspensions, patches of normal myelin were seen to extend as far as 6 mm from the site of implantation. In a study designed to analyse the developmental migration in more detail, Gansmuller et al. (1991) showed migration following transplantation into newborn *shiverer* hosts directly by labelling the transplant with the Hoechst 33342 dye. Hoechst-labelled cells were seen as far as 3 mm from the transplant after 18-20 days. It is likely that this represents an under-estimate of the degree of migration as patches of MBP+ myelin were seen at much greater distances; however none of these more distant MBP+ cells were labelled with Hoechst dye, presumably reflecting repeated cell division and dilution of the dye prior to differentation of the oligodendrocytes.

It could be argued that the *shiverer* brain, lacking normal myelin, represents an abnormal environment for oligodendrocyte precursors and that such transplantation experiments do not establish that migration occurs during normal development. However, two sets of labelling studies show migration in the normal CNS. Levison and Goldman (1993) injected replication-deficient retroviruses expressing the β-galactosidase gene into the SVZ of neonatal rats. The retrovirus incorporates into dividing cells within the SVZ but is then unable to replicate independently and so transfect neighbouring cells. As a result, detection of the β-galactosidase provides a permanent marker of the original infected cell and its descendants. Such retroviruses provide a powerful system for the analysis of cell migration in neural development. Following injection into the SVZ, migration of labelled cells occurred into both white and grey matter of the ipsilateral hemisphere, although the majority of labelled cells were present in white matter. Many of the labelled cells in both white and grey matter were oligodendrocytes, confirming directly the migration of cells of the oligodendroglial lineage from the SVZ.

A different approach to labelling taken by Warf et al. (1991) shows migration of oligodendroglial cells in the spinal cord. Having demonstrated that oligodendrocyte precursors first appeared in the ventral spinal cord, they labelled cells within this region using DiI. DiI-labelled oligodendrocytes appeared in the dorsal spinal cord within 18 hours, reflecting the dorsal migration of oligodendroglial cells.

THE IDENTITY OF THE MIGRATORY CELL TYPE

Taken together, these studies provide excellent evidence for migration during oligodendrocyte development. This in turn raises the question as to which cell type is responsible for the observed migration. This question has been addressed in both in vitro and in vivo studies. These studies follow from a cell culture analysis of oligodendrocyte development (for reviews see Raff, 1989; Bansal et al., 1992) that has characterised the expression of various cell-surface markers on the different cell types in the oligodendrocyte lineage. Four major stages of oligodendrocyte differentiation have been defined clearly, and are summarised in Fig. 1. A number of different experimental approaches suggest that the migratory cell is the first of these stages, the O-2A progenitor cell. The O-2A progenitor cell is so called because it also gives rise to a specific type of astrocyte in cell culture (Raff et al., 1983); however there is as yet no convincing evidence that such astrocytes are formed in normal development and the bipotential nature of the oligodendrocyte precursor remains unproven (Fulton et al., 1992; Noble, 1991).

Whatever the controversy concerning astrocyte development, studies on the expression in vivo of many of the different cell surface markers expressed during development of oligodendrocytes in vitro allow the location of oligodendrocyte precursors within developing tissue to be identified and provide evidence for the migration of these cells. LeVine and Goldman (1988a,b) used markers whose expression overlaps temporally during oligodendrocyte maturation to examine oligodendroglial cells arising within the rat SVZ. They found that antibodies against the GD3 ganglioside, expressed in early oligodendrocyte precursors, labels cells within the SVZ while later markers labelled some SVZ cells as well as cells in surrounding white and grey matter, consistent with the migration and coincident differentiation of oligodendrocyte precursors from the SVZ. Curtis et al. (1988) and Reynolds and Wilkin (1988) used a similar approach to examine the developing rat cerebellum. They also observed progressive maturation associated with apparent migration; GD3 was present on cells in the superior medullary vellum at the base of the cerebellum, and at positions intermediate between this zone and the final destination of oligodendrocytes within the cerebellum. Later

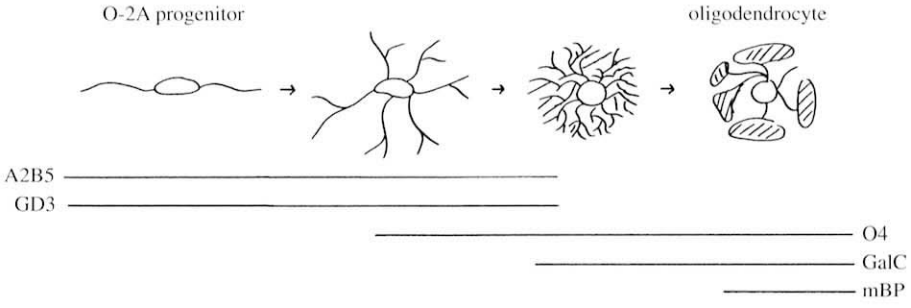

Fig. 1. A combination of cellular morphology and cell-surface markers allow oligodendrocyte differentiation to be subdivided into a number of distinct stages, four of which are shown here. Note that the O-2A progenitor cell appears to be responsible for the majority of the migration of this lineage in vivo.

markers, however, were only expressed on cells that had reached their final destination within the cerebellum.

A major disadvantage of the approach used in these studies is that the transient expression of markers during oligodendrocyte differentiation makes it impossible to follow cells using one single marker throughout the putative migration. An ideal marker for such studies would be one expressed during all different stages prior to oligodendrocyte maturation. The closest we have to such a marker at present is the PDGF α receptor mRNA (Pringle et al., 1992) and studies with this marker also support the hypothesis that oligodendrocyte precursors undergo migration. Initially, labelled cells are only seen in a longitudinal band of cells in the ventral region of the ventricular zone in the spinal cord and also in the ventricular and subventricular zones of the developing brain (Pringle and Richardson, 1993). Subsequently, however, labelled cells are seen throughout the spinal cord, forebrain and midbrain. Whilst some labelled cells early in development may be neurones as well as oligodendrocyte precursors, the pattern of dispersion seen in these studies at a time later in development when PDGF α receptor mRNA appears to be specific to the oligodendrocyte lineage is best explained by the migration of the oligodendrocyte precursors.

A second line of evidence for the migration of O-2A progenitor cells comes from in vitro studies on dissociated cell populations. Small et al. (1987) analysed the migratory behaviour of oligodendrocytes and their precursors with time lapse microscopy. They found the O-2A progenitor cell to be highly motile, while the mature oligodendrocyte did not migrate. Further studies have confirmed this observation and shown the growth factor PDGF to enhance migration of the O-2A progenitor cells (Noble et al. 1988; Armstrong et al., 1990).

Perhaps the most convincing evidence for the identity of the migratory cell as the O-2A progenitor comes from an ingenious in vivo study taking advantage of both the ability to isolate different stages of oligodendrocyte precursors and the *shiverer* host model described above. Warrington et al. (1993) purified populations of immature O4– O-2A progenitors, intermediate O4+ cells (see Fig. 1) and GalC+ oligodendrocytes. When these different populations were transplanted into neonatal *shiverer* mice, extensive migration was seen only in the group transplanted with the immature O-2A progenitors. Animals transplanted with the more mature cells showed smaller areas of myelin that were close to the site of the transplant, consistent with a diminished capacity for migration of these more mature cell types.

PATHWAYS OF O-2A PROGENITOR CELL MIGRATION

The observation that the O-2A progenitor cell is migratory, and has the ability to migrate extensively in neonatal brain, raises the question as to whether this migration is restricted in any way. Clearly, such a restriction could be an important mechanism in determining the final distribution of myelin in the CNS. At first sight, the finding of Levison and Goldman (1993) that oligodendrocytes are found in both white and grey matter following labelling of cells within the SVZ would suggest that migration is widespread, with formation of myelin being regulated by other mechanisms. Two potential mechanisms would be the regulation of myelin formation by the different environments and differing degrees of cell death in white and grey matter. Recent work by Barres et al. (1992) has shown cell death to be extensive in the oligodendrocyte lineage, with 50% of newly born oligodendrocytes dying in the normal optic nerve during development. This cell death arises as a result of limited levels of survival factors in the nerve. As a result, regulation of programmed cell death by differing levels of survival factors could certainly provide a mechanism for regulating oligodendrocyte numbers in different environments.

There is, however, evidence for the restriction of migrating oligodendrocyte precursors. The transplantation study of Gansmuller et al. (1991), in which the location of transplanted cells was analysed at different time points following injection, showed that the majority of labelled cells were located in developing white matter tracts during migration. As these migratory cells are likely to be immature precursors, this observation suggests that these O-2A progenitor cells are migrating preferentially in developing white matter tracts in *shiverer* hosts. More studies are required to determine the precise pathways of migration during normal development.

In at least one area of the developing CNS there is direct evidence for a distinct barrier to migration. O-2A progenitor cells migrate along the optic nerve from the chiasm. In support of this, both cell culture studies and direct visualisation of putative O-2A progenitor cells with probes against the PDGF α receptor mRNA show a gradient of progenitor cells, highest at the chiasm, in the embryonic nerve (Small et al., 1987; Mudhar et al., 1993). This gradient disappears in the early postnatal animal, consistent with the completion of population of the nerve by migrating precursors. However, in the rat these cells do not enter the retina and myelinate the retinal ganglion axon cells in the nerve fibre layer - they appear to be prevented from so doing by a barrier at the lamina cribrosa, the region of the nerve behind the eye where the nerve pierces the sclera (ffrench-Constant et al., 1988). As retinal ganglion axons are myelinated in the optic nerve it seems unlikely that this restriction of myelination is mediated by molecules on the axon surface. This was shown directly by a transplantation experiment by Perry and Lund (1990) in which retinae were transplanted into the brain. In these retinae, myelination was observed around the retinal ganglion cell axons in the nerve fibre, showing that the axons themselves do not inhibit myelination.

Based on the observation that radially orientated astrocytes were present in the lamina cribrosa region of the rat optic nerve, but not in the rabbit optic nerve in which migrating precursor cells do enter the retina and form myelin, ffrench-Constant et al. (1988) proposed that the astrocytes in the rat lamina cribrosa formed a barrier to O-2A progenitor cell migration. Why these astrocytes should form such a barrier is unknown; however, Perry and Lund (1990) observed that the blood brain barrier, which is leaky in the region of the lamina cribrosa, is also leaky in other areas of the brain where myelination is sparse such as the median eminence, pituitary stalk and subfornical organs. As

there is no evidence for specialised astrocytes in these regions, they suggested that plasma proteins might be responsible for preventing migration, either by inhibition of movement or alternatively by inducing differentiation of the O-2A progenitor cell into non-migratory astrocytes. Their hypothesis does not, however, explain the myelination observed in the rabbit retina. The blood-brain barrier at the retinal end of the rabbit optic nerve is believed to be permeable (Flage, 1977) but O-2A progenitor cells must migrate through this region to reach the retina.

CONTROL OF MIGRATION

The directed nature of O-2A progenitor cell migration raises the question as to the molecular mechanisms responsible both for the preferential migration in developing white matter tracts and for the barrier(s) to migration. Interactions between the cell and the extracellular matrix (ECM) play an important role in migration elsewhere in development (Bard, 1990). ECM molecules are therefore attractive candidates for controlling O-2A progenitor cell migration. Studies on the distribution of ECM molecules in the migratory pathways of these cells, however, show a rather different pattern of ECM molecules than those found in association with migration during early embryogenesis. Fibronectin, for example, is present in the developing CNS early in development but appears to be absent from O-2A progenitor cell migratory pathways later in development (Stewart and Pearlman, 1987; Sheppard et al., 1991). A similar situation exists for laminin, which is present in putative white matter tracts such as the optic nerve during the period of axon outgrowth in embryogenesis but is found at very much lower levels during the later postnatal glial cell migration (McLoon et al., 1988).

In addition to laminin, two other ECM molecules have been described in developing white matter tracts at the time of O-2A progenitor cell migration; tenascin (Bartsch et al., 1992a,b) and thrombospondin (O'Shea et al., 1990). Tenascin (also called cytotactin or hexabrachion) is a 200-220×10^3 M_r glycoprotein found as hexamers (Chiquet et al., 1991; Erickson and Bourdon, 1989), while thrombospondin is a 180×10^3 M_r glycoprotein found as trimers (Bornstein, 1992). Both have been implicated in neuronal cell migration elsewhere in the CNS; the migration of granule cells on explants of cerebellar slices can be blocked by antibodies against these two ECM molecules (O'Shea et al., 1990; Chuong et al., 1987). It seems plausible, therefore, that these molecules will play a role in O-2A progenitor cell migration. However any studies examining this hypothesis must consider recent work showing considerable molecular heterogeneity of these molecules. Thrombospondins comprise a multigene family with at least five forms presently recognised (Adams and Lawler, 1993). Tenascin can be found in a number of forms as a result of alternative splicing (Siri et al., 1991). In addition, there appear to be other tenascin-like genes suggesting that the tenascins may also form a multigene family (Bristow et al., 1993). At least in the case of tenascin there is evidence to suggest that the different molecular forms may have different properties. Splicing occurs within a region of the molecule containing a series of fibronectin-like type III repeats, and a recombinant fragment of tenascin corresponding to the spliced repeats diminished cell adhesion in endothelial cells (Murphy-Ullrich et al., 1991). Inclusion of this spliced region could therefore make tenascin less adhesive, and such differences could have important consequences for the behaviour of migrating cells. As a result, it will be important to determine the precise form of molecule present before drawing conclusions as to function.

The apparent paucity of ECM molecules in the pathways of O-2A progenitor cell migration may reflect a lack of appropriate studies. It seems likely that novel members of multigene families or different classes of molecules such as the proteoglycans will be found in the pathways. Equally, however, it may be that cell-cell interactions rather than cell-matrix interactions predominate. A striking feature of the developing CNS is the density of the tissue and the lack of extracellular space as compared to many other developing systems. As a result one might expect less ECM to be present and for cell interactions to be regulated by cell surface molecules. One obvious possibility is that O-2A progenitor cell migration is influenced by molecules on the surface of the exposed axons prior to myelination. Axonal molecules of the immunoglobulin superfamily play an important role in the adhesive interactions during axon outgrowth earlier in development (Rathjen and Jessell, 1991). It seems very likely, therefore, that such molecules may influence migration itself.

A number of features make O-2A progenitor cell

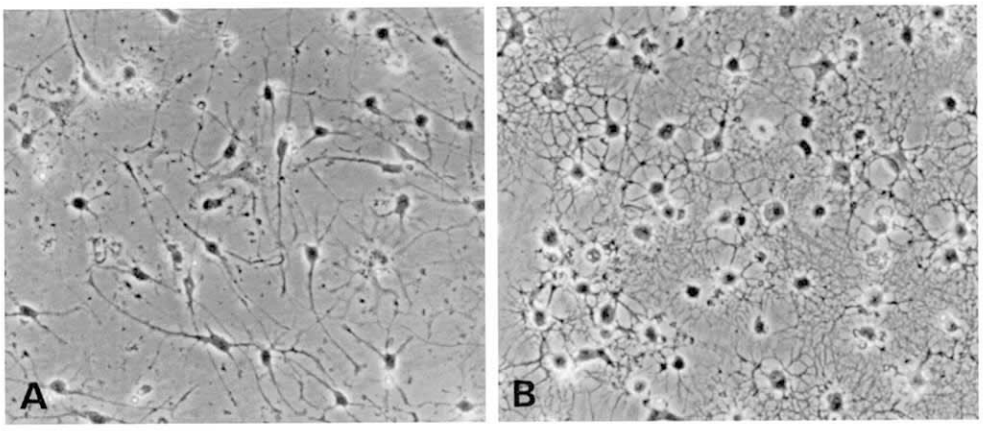

Fig. 2. O-2A progenitor cells prepared according to the shake-off protocol of McCarthy and de Vellis (1980). A shows the cells soon after initial plating. If the cells are left in culture without growth factors to stimulate division they will differentiate into oligodendrocytes. This is shown in B; note the complex multipolar morphology of the cells.

migration a particularly accessible system for experiments addressing these potential molecular mechanisms.

(1) Large numbers of O-2A progenitor cells can be obtained in cell culture. A simple technique was described by McCarthy and de Vellis (1980); cultures of neonatal rat or mouse brain are grown for 7-10 days and then shaken to remove the O-2A progenitor cells that grow on top of the cell monolayer. This technique produces large numbers of O-2A progenitor cells (see Fig. 2), although there is a significant degree of contamination with other cell types. More recently, it has been established that O-2A progenitor cells can be driven into apparently indefinite division by a combination of the growth factors PDGF and FGF (Bogler et al. 1990). As a result, it is possible to obtain large numbers of almost completely pure cell populations for analysis. This in turn allows reductionist experiments that define the response of the cells to different growth factors and substrate molecules.

(2) The identification of stage-specific markers allows the purification by immunopanning or FACS sorting of cells from each different stage. The properties of these different stages can then be analysed separately and, as shown by Warrington et al. (1993), there may be important differences.

(3) The ability of transplanted O-2A progenitor cells to migrate and form new myelin allows migration to be analysed in vivo. Manipulation of these precursor cells prior to transplantation so as to alter expression of cell surface or extracellular matrix molecules will allow potential roles of these molecules, suggested by the simple cell culture experiments, to be analysed in vivo.

(4) A cell line (termed CG4) has been described and shows many of the features of primary O-2A progenitor cells (Louis et al., 1992). These cells are motile in culture (unpublished observations) and it seems certain that this and other cell lines will simplify the application of molecular biology techniques to the question of O-2A progenitor cell migration and differentiation.

In collaboration with Andreas Faissner (Heidelberg), we have taken advantage of the ability to obtain pure O-2A progenitor cells to examine the molecular nature of the barrier at the lamina cribrosa in the rat. We have focused on tenascin as this molecule is enriched at the lamina cribrosa in the adult nerve (Bartsch et al., 1992) in this region prior to the arrival of migrating O-2A progenitor cells (Bartsch, Faissner, Trotter, Dorries, Bartsch, Mohajeri and Schachner, personal communication). We find that tenascin is an anti-adhesive substrate for purified O-2A progenitor cells; these cells will not adhere to a stripe of tenascin present on an adhesive poly-D-lysine substrate (Fig. 3). Such an anti-adhesive effect of tenascin has been described in a wide range of different cell types (Chiquet-Ehrismann, 1991). By using time lapse microscopy we have begun to analyse the effect of tenascin substrates on migration. High concentrations (40 μg/ml) of tenascin inhibit migration whilst lower concentrations show no such inhibitory effect (Fig. 4).

These preliminary experiments suggest that tenascin might contribute to the barrier to O-2A progenitor cell migration at the lamina cribrosa by inhibiting migration through the region. This attractive hypothesis has also been suggested by other workers (Bartsch, Faissner, Trotter, Dorries, Bartsch, Mohajeri and Schachner, personal communication) and is also supported by work on different developmental systems. Tenascin restricts mesodermal cell migration during amphibian gastrulation (Riou et al., 1990) and neural crest cell migration on fibronectin substrates (Tan et al., 1987). A barrier function for tenascin in preventing inappropriate migration has also been suggested in two other areas of the CNS. Tenascin may define the boundaries of the vibrissae-related whisker barrels in the mouse somatosensory cortex (Steindler et al., 1989) and prevent the formation of myelinated fibres in the molecular layer of the mouse cerebellum (Chuong et al., 1987).

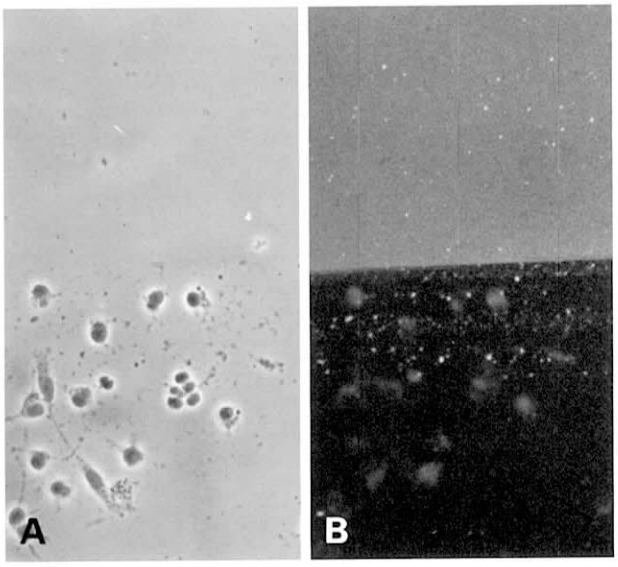

Fig. 3. O-2A progenitor cells in cell culture do not adhere to tenascin. (A) Phase contrast. (B) Immunofluorescence with anti-tenascin antibodies, showing the border of a stripe of tenascin. Note that the progenitor cells do not adhere to the tenascin.

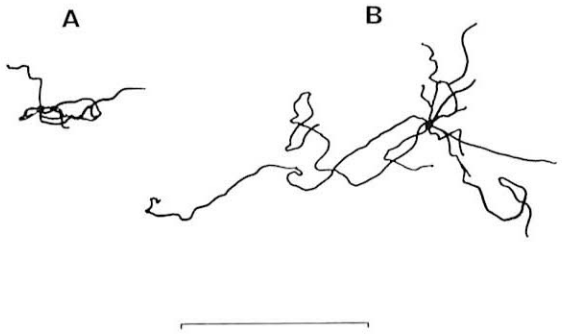

Fig. 4. O-2A progenitor cells purified from postnatal rat optic nerve plated on poly-D-lysine substrates with (A) and without (B) 40 μg/ml tenascin. Migration over 24 hours is shown as if all cells started from a single point; note the reduced movement on the tenascin-containing substrate. Scale bar, 200 μm.

CONCLUSIONS

O-2A progenitor cell migration represents a highly accessible system in which to study migration within the CNS. The

elucidation of the mechanisms responsible will contribute to our knowledge of the development of the CNS. It will also be of great value to those interested in the repair of the damaged CNS. The human disease multiple sclerosis results from patches of demyelination that fail to repair by remyelination. Groves et al. (1993) have shown that O-2A progenitor cells injected into a demyelinated lesion in the rat spinal cord will form new myelin sheaths. This raises the possibility that increasing the entry of new O-2A progenitor cells into a demyelinated region, either from adjacent tissue or by transplantation, might be an effective therapy in multiple sclerosis. A better understanding of migration may suggest strategies for achieving this by enhancing migration of endogenous or transplanted cells in the damaged nervous system.

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