Glial Cell Diversification in the Rat Optic Nerve

MARTIN C. RAFF

A central challenge in developmental neurobiology is to understand how an apparently homogeneous population of neuroepithelial cells in the early mammalian embryo gives rise to the great diversity of nerve cells (neurons) and supporting cells (glial cells) in the mature central nervous system. Because the optic nerve is one of the simplest parts of the central nervous system, containing several types of glial cells but no intrinsic neurons, it is an attractive place to investigate how neuroepithelial cells diversify. Studies of developing rat optic nerve cells in culture suggest that both cell-cell interactions and intrinsic cellular programs play important parts in glial cell diversification.

HOW CAN WE HOPE TO UNDERSTAND THE DEVELOPMENT of our own central nervous system (CNS), the most complex system of cells known? Despite its bewildering complexity, the mammalian CNS develops from a simple epithelial tube, called the neural tube, which is initially composed of a single layer of neuroepithelial cells that all look alike. In trying to understand how the neural tube develops into the CNS, it is helpful to divide the problem into two parts: (i) The cell diversification problem: how does the morphologically homogeneous population of neuroepithelial cells give rise to the diverse array of neurons and glial cells of the mature CNS? (ii) The morphogenesis problem: how do the neurons form the highly ordered layers, nuclei, and synaptically connected networks that are the hallmarks of CNS organization?

My colleagues and I have been investigating the cell diversification problem in the rat CNS. Because the genetic approaches that have been so useful in studying cell diversification in *Drosophila* (*1*) and *Caenorhabditis elegans* (*2*) cannot be applied to mammals, we have used a cell-biological approach that combines three strategies. First, to simplify the problem, we have studied one of the simplest parts of the CNS, the optic nerve, which develops from an extension of the neural tube called the optic stalk. Second, to gain access to the process of cell diversification in order to experimentally manipulate it, we have studied the process in cultures of dissociated cells prepared from developing rat optic nerve. Third, to help overcome the problem of cell identification in culture, we have used antibodies to distinguish (and in some cases to manipulate) specific cell types and their precursors. In this way we have shown that both cell-cell interactions and intrinsic cellular programs contribute to the diversification process, and we have begun to identify the signaling molecules that mediate some of the cell-cell interactions.

The author is in the Medical Research Council Developmental Neurobiology Program, Biology Department, Medawar Building, University College London, London WC1E 6BT, United Kingdom.

Three Types of Optic Nerve Glial Cells

The optic nerve is simple because it does not contain any neuronal cell bodies. It does, however, contain the long nerve processes (axons) of retinal ganglion neurons that project from the eye to the brain. Two major classes of glial cells, called oligodendrocytes and astrocytes, structurally and functionally support the axons in the nerve. The oligodendrocytes extend processes that wrap concentrically around the axons to form an insulating myelin sheath. The myelin sheath is interrupted at regular intervals by gaps, called nodes of Ranvier, where axonal electrical excitation is confined; the nerve impulse (action potential) travels along the axon by jumping from node to node, which greatly increases the rate and efficiency of nerve impulse propagation. The functions of the astrocytes are less clear, although it is known that they extend processes to the surface of the nerve (forming a glial limiting sheath), to blood vessels (forming a perivascular sheath), and to nodes of Ranvier. There are other cell types in the nerve, mainly meningeal cells that surround the nerve, macrophages (called microglial cells) (*3*), and cells associated with blood vessels, but none of these develop from the neuroepithelial cells of the neural tube and therefore need not concern us here.

Until recently, it was assumed that there was only one type of astrocyte in the rat optic nerve (*4, 5*). Cultures of the developing optic nerve, however, contain two types of astrocytes, which can be distinguished by morphology, antigenic phenotype, and response to growth factors (*6*); for simplicity, they have been called type-1 and type-2 astrocytes. Immunofluorescence micrographs of the three types of differentiated glial cells in such cultures are shown in Fig. 1. In studies in which antibodies were used to distinguish the three glial cell types in cell suspensions prepared from optic nerves of rats of different ages, it was found that type-1 astrocytes first appear at embryonic day 16 (E16), oligodendrocytes on the day of birth (which usually occurs at E21), and type-2 astrocytes at the beginning of the second postnatal week (*7*).

What do the two types of astrocytes in vitro correspond to in vivo? The answer has been elusive. In particular, it has been difficult to identify type-2 astrocytes in the intact optic nerve (*8*). A clue was provided by immunohistochemical studies with several antibodies that recognize an approximately 180-kD protein in immunoblots of extracts of adult optic nerve (*9*). These antibodies, which include the HNK-1 (*10*) and NSP-4 (*11*) monoclonal antibodies and rabbit antibody to J1 (*12*), preferentially label astrocyte processes associated with nodes of Ranvier in frozen sections of adult optic nerve; in cultures of neonatal optic nerve cells, they label type-2 but not type-

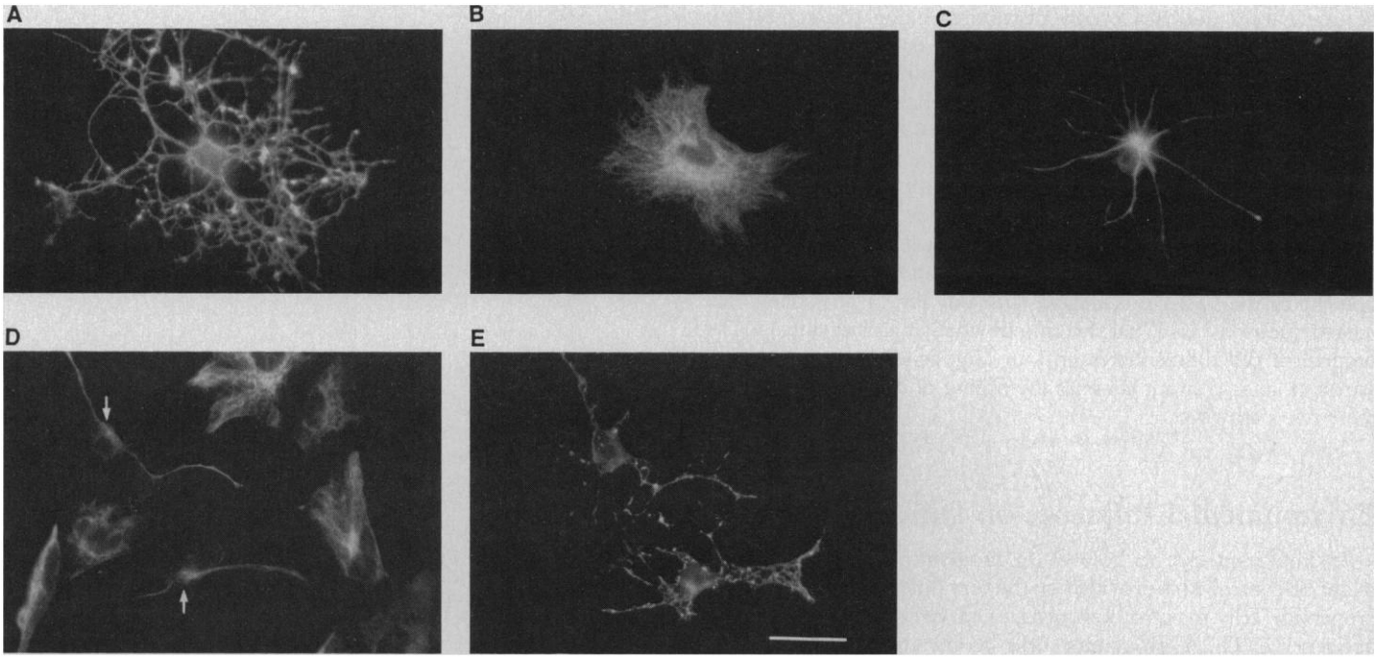

Fig. 1. Immunofluorescence micrographs of three types of glial cells in cultures of newborn rat optic nerve, stained with the antibodies most commonly used to distinguish them. (**A**) An oligodendrocyte stained on its surface with antibody to galactocerebroside, which is a major myelin glycolipid that is made only by oligodendrocytes in the CNS (*48*). (**B**) A type-1 astrocyte stained with antiserum to GFAP, which is a major subunit of glial filaments, a class of intermediate filaments found only in astrocytes in the rat CNS (*32*). (**C**) A type-2 astrocyte stained with antiserum to GFAP. (**D**) Type-1 and type-2 astrocytes stained with antiserum to GFAP. (**E**) The same cells as in (D), stained on their surface with the A2B5 monoclonal antibody, which recognizes specific gangliosides (*49*). The A2B5 antibody labels the two type-2 astrocytes [arrows in (D)] but not the type-1 astrocytes (*6*). Scale bar, 40 μm.

1 astrocytes (*13*). These findings suggest that type-2 astrocytes in vivo might specifically extend processes to nodes of Ranvier. This hypothesis has received support from studies in which individual glial cells in the optic nerve are visualized either by impregnating them with silver or by injecting them with horseradish peroxidase. With both techniques, two types of astrocyte-like glial cells are seen: one extends primarily sheetlike radial processes that terminate mainly on blood vessels or at the surface of the nerve, whereas the other extends primarily fine longitudinal processes that terminate at nodes of Ranvier (*14*). In silver-impregnated preparations, the cells with primarily longitudinal processes do not appear until the second postnatal week, whereas those with primarily radial processes are already present at birth (*14*), suggesting that these two kinds of glial cells in vivo correspond to type-2 and type-1 astrocytes, respectively, in vitro. Our current view of the organization of glial cells in the optic nerve, based on these findings, is illustrated schematically in Fig. 2.

Two Glial Cell Lineages

A major challenge to those studying the cell diversification problem is to understand how developing cells choose between alternative fates. Why does one neuroepithelial cell become an astrocyte and another an oligodendrocyte, for example? To address this type of question, one first needs to determine the genealogical relationships among the various differentiated cell types in order to know what the developmental choice points are. This is why developmental biologists pay so much attention to cell lineage.

With the use of antibodies against cell-surface antigens to eliminate (*15*) or pulse label (*15, 16*) specific cells in developing optic nerve cultures, it has been shown that the three types of glial cells in such cultures arise from two distinct lineages. Oligodendrocytes and type-2 astrocytes develop from a common, bipotential O-2A progenitor cell (*15*), whereas type-1 astrocytes develop from a different precursor cell (*16*). Both kinds of precursor cells proliferate before they differentiate. Type-1 astrocytes continue to divide for at least a week or so after they develop, whereas oligodendrocytes (*5*), and probably type-2 astrocytes (*7*), divide infrequently, if at all, after they are formed.

Initially, it was surprising to find that the two types of astrocytes arise from different precursor cells and that type-2 astrocytes develop from the same precursor cells that give rise to oligodendrocytes. The recent evidence that putative type-2 astrocytes extend processes exclusively to nodes of Ranvier (*14*), however, suggests that the O-2A cell lineage might be specialized for myelination (*13*), which would provide a link between function and genealogy. Nonetheless, it is important that these lineage relationships be confirmed in vivo.

Migration of O-2A Progenitor Cells

There is circumstantial evidence that O-2A progenitor cells do not develop from the neuroepithelial cells of the optic stalk but instead migrate into the developing optic nerve from the brain. First, oligodendrocytes or their precursors have been reported to migrate substantial distances into normal CNS tissue from both CNS transplants in vivo (*17*) and CNS explants in vitro (*18*). Second, time-lapse microcinematographic studies of neonatal optic nerve cells in culture suggest that it is the O-2A progenitor cells, rather than the oligodendrocytes, that are migratory. In such cultures, progenitor cells actively migrate until they differentiate into oligodendrocytes, at which point locomotion stops (*19*). Third, in E16 rats, O-2A progenitor cells are found at the brain end but not at the eye end of the optic nerve; at birth, they are found at the eye end, but in small numbers compared to the brain end; only by the second

postnatal week are they evenly distributed along the nerve (*19*). Since type-1 astrocytes first appear at the eye end (*19*), it is unlikely that the prolonged gradient of O-2A progenitor cells in the opposite direction reflects a gradient of neuroepithelial cell differentiation along the developing nerve; it probably reflects a migration of O-2A progenitor cells down the nerve, from the brain toward the eye.

If O-2A progenitor cells migrate into the developing nerve to produce oligodendrocytes and type-2 astrocytes, then the neuroepithelial cells of the optic stalk presumably give rise only to type-1 astrocytes. We have, therefore, focused our attention on the development of the O-2A cell lineage with the aim of answering two related questions: (i) What determines whether an individual O-2A progenitor cell differentiates into an oligodendrocyte or a type-2 astrocyte, and (ii) what controls the timing of differentiation along these two pathways?

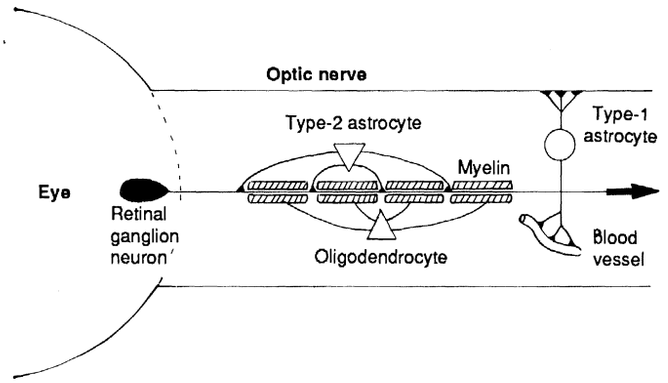

Fig. 2. A tentative model of how the three types of glial cells are arranged in the adult rat optic nerve. Type-1 astrocytes form the glial-limiting sheath at the surface of the nerve and extend processes that terminate on blood vessels (*14*), where they are thought to induce the underlying endothelial cells to form the blood-brain barrier (*50*). Putative type-2 astrocytes seem to extend processes exclusively to nodes of Ranvier (*14*), but their function is not known. At least some type-1 astrocytes also contact nodes of Ranvier (*51*). Contrary to our original proposal that type-1 astrocytes correspond to protoplasmic astrocytes and that type-2 astrocytes correspond to fibrous astrocytes (*52*), the model shown implies that type-1 astrocytes correspond to fibrous astrocytes, whereas type-2 astrocytes represent a novel type of glial cell (*8*).

Environmental Influence on Differentiation

An important clue to how O-2A progenitor cell differentiation might be controlled is provided by the very different behavior of the progenitor cells in vitro compared to in vivo. In the developing optic nerve, O-2A progenitor cells proliferate and give rise to postmitotic oligodendrocytes beginning on the day of birth (*7*), and new oligodendrocytes develop from dividing progenitor cells for at least the next 2 weeks (*20*). Although less is known about type-2 astrocyte development in vivo, it is thought that they begin to develop from dividing progenitor cells beginning in the second postnatal week (*7*). In contrast, when dissociated from an embryonic or newborn optic nerve and placed in culture, O-2A progenitor cells prematurely stop dividing and differentiate within 2 days. If these cells are cultured in 10% fetal calf serum (FCS) they become type-2 astrocytes, whereas if they are cultured in the absence of FCS they become oligodendrocytes (*15, 21*). Clearly, environmental conditions can dramatically influence both the timing and direction of O-2A progenitor cell differentiation. Moreover, the conditions in culture must be different in this respect from those in the developing nerve. By analyzing these differences we have begun to understand how O-2A progenitor cell differentiation is normally controlled.

Oligodendrocyte Differentiation

If a single O-2A progenitor cell is cultured alone in a microwell in ≤1% FCS, it stops dividing and differentiates into an oligodendrocyte within 2 days (*22*). This suggests that oligodendrocyte differentiation is the constitutive pathway of O-2A progenitor cell development, which is automatically triggered when the cell is deprived of signals from other cells. Which cells are responsible for keeping the O-2A progenitor cells proliferating in the developing optic nerve and for preventing the progenitor cells from differentiating prematurely? Retinal ganglion cell axons, which are present in the nerve but not in cultures of optic nerve cells, are an obvious possibility. They seem not to be responsible, however, because if an optic nerve in a newborn rat is cut just behind the eye, the axons in the nerve degenerate but the O-2A progenitors continue to proliferate and do not differentiate prematurely (*23*).

Role of type-1 astrocytes. Experiments in vitro suggest that type-1 astrocytes, the first glial cells to differentiate in the developing optic nerve, have a major influence on O-2A progenitor cell proliferation and differentiation. Cultures of purified type-1 astrocytes (*24*) secrete growth factors that keep O-2A progenitor cells proliferating and prevent their premature differentiation in vitro (*25*). Moreover, the normal timing of oligodendrocyte development can be reconstituted in vitro if O-2A progenitor cells from embryonic optic nerve are cultured with an excess of purified type-1 astrocytes or in medium conditioned by type-1 astrocytes (*26, 27*). In such cultures, the progenitor cells give rise to oligodendrocytes beginning on the equivalent of the day of birth, and new oligodendrocytes develop from dividing progenitor cells for at least 2 weeks, just as in vivo (*27*).

Role of PDGF. Accumulating evidence suggests that platelet-derived growth factor (PDGF) is a crucial growth factor that type-1 astrocytes secrete to stimulate O-2A progenitor cells to proliferate. First, purified PDGF stimulates the proliferation of O-2A progenitor cells in culture and prevents them from differentiating prematurely into oligodendrocytes (*28*). Second, when type-1 astrocyte–conditioned medium (ACM) is fractionated by gel filtration, the mitogenic activity for O-2A progenitor cells is found in the same fractions as radiolabeled PDGF (*29*). Third, cultures of purified type-1 astrocytes secrete PDGF and contain messenger RNA encoding the PDGF A chain (*29*). Fourth, antibodies to PDGF inhibit the ability of ACM to stimulate O-2A progenitor cell proliferation in vitro (*29*). Although PDGF has yet to be shown to stimulate O-2A progenitor cells to proliferate in vivo, it is apparently present in the developing optic nerve; extracts of developing optic nerve stimulate O-2A progenitor cells to divide in culture and most of this activity is inhibited by antibodies to PDGF (*30*).

The ability of type-1 astrocytes to reconstitute the normal timing of oligodendrocyte development in cultures of embryonic optic nerve cells also depends on the secretion of PDGF. PDGF can replace exogenous type-1 astrocytes or ACM in reconstituting the normal timing of oligodendrocyte development in these cultures, and antibodies to PDGF neutralize the ability of ACM to reconstitute normal timing (*30*).

An intrinsic clock in the O-2A progenitor cell. What timing mechanism ensures that the first oligodendrocytes appear on the day of birth or at the equivalent time in vitro? It is unlikely that the mechanism depends on an oligodendrocyte differentiation–inducing signal that becomes available at this time because, as already mentioned, an O-2A progenitor cell automatically differentiates into an oligodendrocyte when cultured alone in a microwell (*22*); it seems that an inducing signal is not required. It is unlikely that PDGF becomes

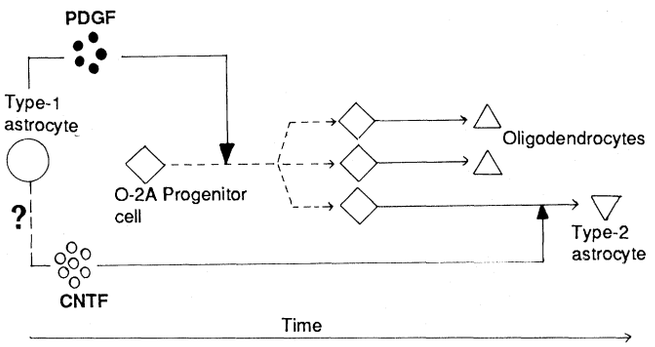

Fig. 3. How the timing and direction of O-2A progenitor cell differentiation are thought to be controlled. Type-1 astrocytes, the first glial cells to develop in the rat optic nerve, secrete PDGF, which stimulates O-2A progenitor cells to proliferate until an intrinsic timing mechanism in the progenitor cell initiates the process that leads to oligodendrocyte differentiation. The first cells differentiate into oligodendrocytes on the day of birth. Beginning in the second postnatal week, a CNTF-like protein, which might also be made by type-1 astrocytes (?), is produced in large amounts; it acts on residual proliferating progenitor cells to initiate their differentiation into type-2 astrocytes.

limiting at this time, because adding excessive amounts of PDGF to cultures of embryonic optic nerve cells does not alter the timing of oligodendrocyte development (*30*).

It seems that the role of PDGF in the timing process is to keep each O-2A progenitor cell and its progeny dividing until an intrinsic clock in these cells initiates the process that leads to oligodendrocyte differentiation (*27*). The strongest evidence for such an intrinsic timing mechanism comes from clonal analyses of oligodendrocyte development, either in microcultures of single O-2A progenitor cells (*31*) or in bulk cultures where individual clones of progenitor cells are followed by time-lapse microcinematography (*30*). These studies show that the two daughters of a neonatal progenitor cell usually differentiate more or less synchronously after the same number of divisions when stimulated to proliferate by type-1 astrocytes or PDGF. These findings are consistent with a timing mechanism that counts cell divisions (*27, 31*), but they do not exclude other mechanisms.

Only a small proportion of the O-2A progenitor cells in the developing optic nerve differentiates into oligodendrocytes on the day of birth; many more do so in the days that follow (*7, 27*). Thus the clocks in O-2A progenitor cells are not all synchronized to go off at the same time. This heterogeneity has been directly demonstrated by the same type of clonal analysis just described: when O-2A progenitor cells in cultures of newborn optic nerve cells are stimulated to proliferate by PDGF or type-1 astrocytes, some divide only once before the daughter cells differentiate into oligodendrocytes, whereas others divide up to eight times before differentiation occurs (*30, 31*). A simple hypothesis to account for this heterogeneity is that O-2A progenitor cells are produced continually from preprogenitor cells during development, so that the population of progenitor cells in the developing nerve at any one time consists of cells of differing maturity (*31*). The location and properties of the putative preprogenitor cells are unknown.

The nature of the clock in the O-2A progenitor cell is also unknown. It is clear that oligodendrocyte differentiation is associated with withdrawal from the cell cycle (*20, 25*), but the relationship between the two processes is uncertain. The clock might primarily control the onset of oligodendrocyte differentiation, with the cessation of proliferation occurring as a consequence. Alternatively, the clock might primarily control the onset of unresponsiveness to PDGF, with oligodendrocyte differentiation occurring as a result of withdrawal from the cell cycle. The latter possibility is more attractive as it would most simply explain why O-2A progenitor cells differentiate prematurely when deprived of PDGF (*30*). One possibility is that some molecule in the progenitor cell that is required for the proliferative response to PDGF decreases with time or with each cell division until its concentration falls below threshold; the cell would then stop dividing and, as a consequence, differentiate into an oligodendrocyte.

Type-2 Astrocyte Differentiation

Unlike oligodendrocyte differentiation, type-2 astrocyte differentiation does not occur when O-2A progenitor cells are cultured alone in serum-free medium (*15, 22*). Progenitor cells can be induced to differentiate prematurely into type-2 astrocytes, however, if they are cultured in 10 to 20% FCS (*15, 22*). On the basis of these findings, it was postulated that, whereas oligodendrocyte differentiation is the constitutive pathway of O-2A progenitor cell development, type-2 astrocyte differentiation is an induced pathway. It was also postulated that FCS mimics the effect of an endogenous inducer that does not appear in effective concentration in the developing optic nerve until the second postnatal week (*27*).

To test this hypothesis, extracts of developing rat optic nerve were analyzed (in the absence of FCS) for their ability to induce O-2A progenitor cells in vitro to express an astrocyte-specific molecule, glial fibrillary acidic protein (GFAP) (*32*), as a measure of type-2 astrocyte differentiation. Whereas extracts of an optic nerve from a 3-week-old rat were found to have such GFAP-inducing activity, extracts from the nerve of a 1-week-old animal (a time when type-2 astrocyte development has not yet begun) had about 50 times less (*33*). Biochemical studies suggest that the active factor in the nerve extract is a 20- to 25-kD protein (*33*). Since the protein can act on O-2A progenitor cells dissociated from E17 optic nerve (*33*), which is more than 10 days before these cells normally develop into type-2 astrocytes (*7*), it seems that it is the increase in the inducing protein, rather than the onset of progenitor cell responsiveness to it, that is responsible for timing type-2 astrocyte differentiation in the developing nerve.

Role of ciliary neurotrophic factor (CNTF). CNTF was first identified as an activity in culture medium conditioned by heart cells that would keep neurons from the chick ciliary ganglion (a parasympathetic ganglion) alive in vitro (*34*). It was later purified from chick eye (*35*) and rat sciatic nerve (*36*) and shown to be a 20- to 25-kD acidic protein. Several lines of evidence suggest that the type-2 astrocyte–inducing protein in extracts of rat optic nerve is either CNTF or a closely related protein. First, CNTF and the inducing protein are similar in size and have a similar tissue distribution (*37*). Second, a low concentration ($\sim 10^{-11} M$) of CNTF, purified to homogeneity from rat sciatic nerve, induces O-2A progenitor cells to express GFAP in culture (*37, 38*). The biological effect of CNTF on O-2A progenitor cells in vitro is indistinguishable from that of optic nerve extract, and when optimal concentrations of CNTF are used, optic nerve extract has no additional effect (*37*). Third, optic nerve extract promotes the survival of chick ciliary ganglion neurons in culture in a similar dose range as it induces GFAP expression in O-2A progenitor cells; whereas extracts of optic nerve from a 3-week-old rat are highly active in promoting neuronal survival, extracts of nerve from a 1-week-old animal are much less effective, just as in the GFAP-induction assay (*37*). Fourth, when optic nerve extract is subjected to the same purification scheme used to purify CNTF from rat sciatic nerve, both ciliary neurotrophic and GFAP-inducing activities are highly and similarly enriched (*37*).

Although CNTF and optic nerve extract can induce O-2A

progenitor cells to express GFAP prematurely, the effect is transient, even if they are added repeatedly: the effect is maximal at 24 hours and gone by 3 days (*37, 38*). The induced cells apparently lose GFAP and go on to become oligodendrocytes (*39*). It seems that CNTF can initiate type-2 astrocyte differentiation but other signals are required to drive the process to completion. Moreover, CNTF and optic nerve extract only induce 20 to 30% of the O-2A progenitor cells in the optic nerve of the newborn to express GFAP in culture (*33, 37, 38*). It is not clear in what other respects the inducible and noninducible populations differ.

It is unlikely that the component in FCS that induces type-2 astrocyte differentiation in culture is CNTF. CNTF acts quickly to induce about a third of the O-2A progenitor cells in the optic nerve of the newborn to express GFAP transiently, whereas FCS acts more slowly and induces virtually all of the progenitor cells to express GFAP, and the induction is long-lasting (*15, 21, 33*). Moreover, whereas the CNTF-like molecule in optic nerve extract seems to act directly on O-2A progenitor cells to induce GFAP expression (*33*), at least part of the induction by FCS is apparently indirect (*22*).

Which cells in the optic nerve make CNTF? Of the major cell types found in the nerve, only type-1 astrocytes release CNTF-like molecules into the medium when they are cultured as enriched populations, and these cells do so only after they are injured, either by scratching the culture or by passaging the cells (*38, 40*). These findings raise the possibility that type-1 astrocytes might be the normal source of CNTF in the optic nerve, in which case type-1 astrocytes would play a crucial part in the timing of both oligodendrocyte and type-2 astrocyte differentiation (Fig. 3). The factors that normally regulate the production and release of CNTF are unknown.

Glial Cell Diversification Can Occur Independently of CNS Morphogenesis

The O-2A lineage cells are not confined to the optic nerve. They are present throughout the CNS, wherever axons are myelinated. It is not surprising, therefore, that O-2A progenitor–like cells are found in cultures of developing brain (*38, 41*) and cerebellum (*42*). The mechanisms that regulate O-2A progenitor cell differentiation in brain cultures are apparently similar to the mechanisms operating in the optic nerve. When perinatal brain cells are cultured in serum-free medium, oligodendrocytes and type-2 astrocytes develop on the same schedule as they do in the developing brain, which is virtually identical to the schedule in the optic nerve. At the time type-2 astrocytes begin to develop in these cultures, a CNTF-like protein appears in the culture medium that can induce O-2A progenitor cells in vitro to express GFAP prematurely (*38*).

The O-2A lineage cells are not the only glial cells that develop on schedule in dissociated cell cultures of embryonic rat brain. Type-1–like astrocytes and ependymal cells (the ciliated cells that line the fluid-filled ventricular cavities in the brain) also develop on the same schedule in vitro as they do in vivo (*43*). Glial cells develop on schedule, even when the cultures are prepared from 10-day-old embryos (*43*). This surprising observation suggests that the mechanisms controlling glial cell diversification from E10 onward operate independently of CNS morphogenesis.

Mammalian CNS Cell Diversification in Perspective

Vertebrate CNS development begins with neural induction, in which the mesoderm induces an overlying region of ectoderm to become neuroectoderm. The neuroectoderm (called the neural plate) then rolls up along the length of the embryo and pinches off to form the neural tube. An early step in CNS cell diversification presumably involves the subdivision of the continuous neuroepithelium of the neural plate or tube into regions that will later give rise to the characteristic sets of cells of the retina, forebrain, midbrain, hindbrain, and so on. This process might be analogous to dividing the blastoderm of a *Drosophila* embryo into segments or parasegments (*44*). Unlike segmentation in *Drosophila*, however, little is known about the mechanisms involved in the regionalization of the early neuroepithelium.

The studies I have reviewed here on glial cell diversification in the rat optic nerve are concerned with later steps in CNS cell diversification. By the time we begin to study them, apparently the cells of the optic stalk are committed to forming type-1 astrocytes (*19*), while the O-2A progenitor cells are restricted to becoming either oligodendrocytes or type-2 astrocytes (*16*). As discussed, the timing and direction of O-2A progenitor cell differentiation depends partly on an intrinsic program in the progenitor cell and partly on interactions with other cells, especially type-1 astrocytes; a clear role for neurons in the development of O-2A progenitor cells has yet to be demonstrated, which is surprising because these are the cells that the O-2A cell lineage seems to have evolved to serve. Although more is known about cell diversification in the optic nerve than in any other part of the mammalian CNS, there is still much to learn about this relatively simple system of cells. The intracellular events responsible for the proliferation or differentiation of O-2A progenitor cells, for example, remain to be explored.

Until recently, cell diversification in the more complex parts of the mammalian CNS was largely inaccessible to study, primarily because it was not possible to determine directly the lineage relationships between the various cell types. The advent of retrovirus-mediated gene transfer as a method of cell lineage analysis (*45*) promises to revolutionize the study of cell diversification in higher vertebrates, not only in the CNS, but in other organs as well. In the rat retina, for example, this approach has provided evidence that the various types of neurons and the major class of glial cells (Müller cells) arise from a single lineage (*46*); experiments in the frog retina, in which a tracer molecule is injected into a single cell, support this conclusion (*47*). In vitro analyses of the kind discussed in this review are likely to play an important part in future studies aimed at determining how neuroepithelial cells in the developing retina choose between multiple fates.

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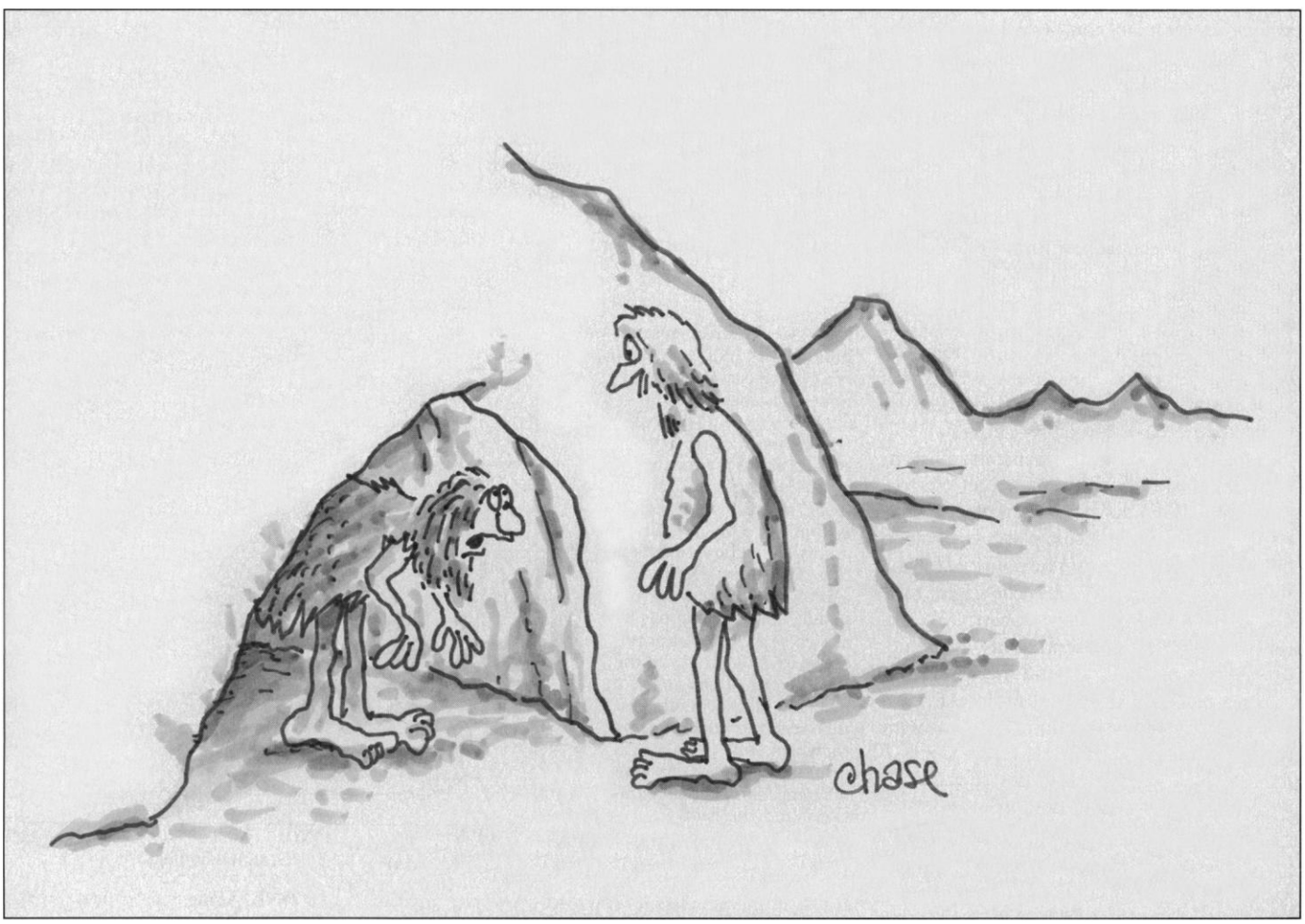

"I tried standing erect, but I kept banging my head!"