GLIA: 4:124–137 (1991)

Glial Cell Lineage in the Cerebral Cortex: A Review and Synthesis

RICHARD S. CAMERON AND PASKO RAKIC
Section of Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06510

KEY WORDS Gliogenesis, Astrocytes, Oligodendrocytes, Radial glial cells, Cell Differentiation

ABSTRACT The present review is focused on the cell lineage relationships underlying gliogenesis in the cerebral cortex. Studies conducted both in vivo and in vitro suggest that the process of cortical gliogenesis involves a hierarchy of progressively restricted progenitor cell pools. In the cerebral cortex, as well as other areas of the central nervous system, glial cells differentiate from one another through a series of steps that can be defined at molecular, structural, and functional levels. Although the precise timing, sequence, and diversity of the steps involved in cortical gliogenesis are still not fully defined, the emerging picture suggests that both cell lineage and cell-cell interactions play a synergetic role in the determination and maintenance of the proper blend of glial cells in the cerebrum.

INTRODUCTION

It is well established that both neuronal and glial cells in the mammalian brain are derived from the neuroepithelium of the primitive neural tube. However, the molecular and cellular mechanisms by which neuroepithelial progenitor cells generate the diversity and multiplicity of neural cell types are still obscure. This may be illustrated, in part, by considering the conflicting interpretations on the developmental history of progenitor cells in the central nervous system. In a classic series of studies on human embryos, His (1889) proposed that the germinal epithelium, situated near the lateral ventricular surface of the developing cerebral wall, comprised two classes of precursor cells: one that produced neuronal cells (Keimzellen) and another that generated glial cells (Spongioblasten). In contrast, Schaper (1897) suggested that the germinal zone consisted of a homogeneous proliferative precursor cell population that differentiated into both neuronal and glial cells.

The concept of ventricular cell homogeneity gained support from histological studies carried out by Sauer (1935), whereby morphological (cell shape) differences between ventricular cells were explained by differential nuclear positioning during the mitotic cycle. Subsequent histochemical analyses (Sauer and Chittenden, 1959), electron microscopic observations (Fujita and Fujita, 1963, 1964; Hinds and Ruffet, 1971), and [^{3}H]-thymidine autoradiographic studies (Fujita, 1963; Sidman et al., 1959) supported further the concept of a functionally homogeneous ventricular cell population. In addition, studies of the time of cell origin, revealing few labeled glial cells in adult animals that had been exposed to [^{3}H]-thymidine as embryos, were interpretated to suggest a sequential, rather than concurrent, generation of neuronal and glial cells (Fujita, 1963, 1966); thus, implying that a homogeneous cell population comprised the proliferative ventricular zone.

The present review is focused on the cell lineage relationships underlying gliogenesis in the mammalian cerebral cortex, a neural area that comprises the largest cellular mass in the mammalian brain. An emphasis has been placed on defining the cell populations that participate in the development and emergence of distinct macroglial cell lines. As shown schematically in Figure 1, the process of cortical gliogenesis, as recently suggested for neural crest cell derivatives (Anderson, 1989), appears to involve a complex hierarchial progression of restricted progenitor or "stem" cell pools and may

Address reprint requests to Pasko Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

be considered, in part, analogous to the process of hematopoiesis (Metcalf, 1989; Quesenberry and Levitt, 1979a,b). Conceptually, four principal progenitor or "stem" cell pools (boldfaced in Fig. 1) appear to be encountered at early stages of cortical gliogenesis: 1) multipotential progenitor cells, 2) bipotential progenitor cells, and lastly 3) glial and neuronal lineage-restricted progenitor cells. Within the glial cell family, specific progenitor cell populations are evident for each individual macroglial cell lineage, namely, type-1 astrocyte, type-2 astrocyte, and oligodendrocyte.

In the following sections, we discuss the evidence that allows for an identification (or proposal) of distinct progenitor cell populations that participate in the development of the glial cell populations that are observed in the mammalian cerebral cortex. It is worthwhile pointing out, however, that construction of a cerebral cortical glial cell lineage diagram has necessitated the usage of a great deal of relevant data and insights obtained from cell lineage analyses carried out in noncerebral cortical areas as well as in nonmammalian species, both in vitro and in vivo.

HEIRARCHY OF EARLY PROGENITOR CELL POOLS

Multipotential "Stem" Cell

At present, a multipotent stem cell population in the mammalian cerebrum can be defined only operationally as a kinetic entity possessing self-renewal capability. Evidence implying the presence of a population of multipotent stem cells has been obtained in vitro (Temple, 1989). Following clonal expansion of single cells obtained from embryonic day 13.5–14.5 rat forebrain, three distinct types of clonal cell colonies were identified on the basis of cell progeny composition and number. The data obtained are consistent with the presence of three populations of mitotic progenitor cells in embryonic rat forebrain: 1) a progenitor cell that produces a large cell population of terminally differentiated glial and neuronal cells, as well as a mitotic cell population morphologically similar to the initial founder cell; 2) a progenitor cell that yields terminally differentiated cell progeny consisting of both neuronal and glial cells; and 3) a progenitor cell that gives rise to terminally differentiated cell progeny composed of a single phenotype—either glial or neuronal. As identified by the property of self-renewal, only the cell population described initially may be considered to have been derived from a multipotent stem cell. Further proof, however, will require the demonstration that secondary cultures derived by subcloning are capable of indefinite cell colony formation. Characteristics of the second and third cell populations are consistent with the presence in cerebral cortex of both bipotential (see the following section) and cell lineage-restricted progenitor (see the second section following) cells, respectively.

In contrast to the multipotential stem cell populations that characterize rapidly regenerating tissues in the adult (e.g., hematopoietic, epidermal, and gastrointestinal epithelia) or quiescent tissues that retain regenerative potential indefinitely (e.g., liver), CNS multipotential "stem" cells are likely to be present only during embryogenesis. Two lines of evidence concur with this interpretation: first, the multipotential stem cell niche, the embryonic ventricular zone, is transient, disappearing by early postnatal life; and second, the adult CNS comprises terminally differentiated cells that do not express proliferative capabilities (Rakic, 1985). Consequently, the CNS multipotential "stem" cell may be considered a *finite* cell population that eventually loses any self-renewal function by forming two committed bipotential precursor cells. In distinction to mammals, however, both neuronal and glial cells in the avian brain are generated from a common precursor that is present, evidently, throughout the entire life span (Alvarez-Buylla et al., 1990). Thus, the avian brain appears to maintain a cellular compartment that can be defined according to the attributes expressed by classical stem cells (Hall and Watt, 1989; Lajtha, 1979).

Multipotential progenitor cells in the mammalian forebrain can be inferred to yield other multipotential progenitor cells at developmental time periods prior to the rapid amplification in cell number that is seen to accompany cerebral cortical neurogenesis (cortical neurogenesis begins at approximately embryonic day 16 in rat, Frederiksen and McKay, 1988; and at embryonic day 40 in the rhesus monkey and human; Rakic, 1974, 1988). However, it is not known whether the multipotential stem cell can undergo asymmetric as well as symmetric division. The former process would lead to the generation a bipotential progenitor cell population while maintaining a population of multipotential "stem" cells.

Bipotential (Glial/Neuronal) Progenitor Cell

The bipotential progenitor cell is, in principle, functionally similar to the multipotent "stem" cell because it can generate progeny that will ultimately comprise differentiated neuronal and glial cells. However, it differs critically by having a limited or transient proliferative capacity. Evidence for the presence of a discrete bipotential progenitor cell population in the cerebral cortex has been obtained both in vivo and in vitro from several distinct, but complementary, lines of research: cell colony-forming assays, recombinant retroviral infection, and oncogene transduction.

Replication-defective, recombinant retroviral vectors, constructed to carry a histochemically detectable reporter gene (e.g., B-galactosidase gene of *Escherichia coli*), have been used to mark proliferative progenitor cell populations in vivo (Price, 1987). The observed presence of clonal cohorts comprising both glial and neuronal cells implies, but does not prove definitively, the in vivo existence of a bipotential precursor cell

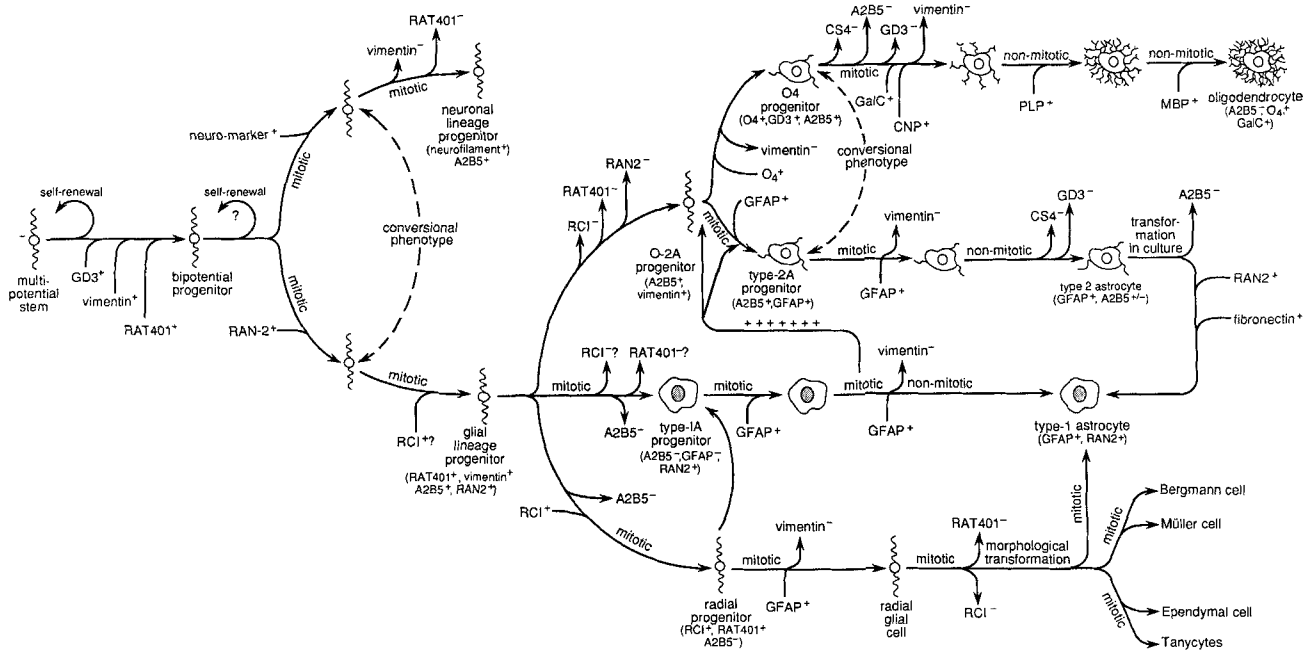

Fig. 1. Schematic circuit diagram of the possible glial cell lineages that occur during the development of the cerebral cortex. The acquisition or loss of antigenic components that accompany the progressive maturation of individual cell types are indicated by arrows with (+) or (−) signs. Further explanation and designation of the various progenitor and glial cell forms is provided in the text.

population (Turner and Cepko, 1987; Walsh and Cepko, 1988; Price and Thurlow, 1988; Galileo et al., 1990; Leber et al., 1990; Turner et al., 1990). Consistent with the evidence obtained from clonal microculture studies (Temple, 1989), the "retroviral-labeled" bipotential precursor cell population appears to be committed to terminal cell differentiation. Thus, in the absence of any self-renewal capability, the limited proliferative capacity serves to define the bipotential progenitor cell as a transient, amplifying cell population.

Assuming that the identified clonal cell populations are representative of the cellular composition of the ventricular surface, then the bipotential progenitor cell may be taken to comprise a minor percentage of the total cortical progenitor cell population (2% of identified cell colonies, Luskin et al., 1988; for a possible exception see Price and Thurlow, 1988). However, this conclusion contrasts with the greater number of bipotential progenitor cells identified from similar analyses of chick neural tissues: optic tectum, telencephalon, and spinal cord (Galileo et al., 1990; Gray et al., 1988; Leber et al., 1990). Since the reason for the difference between species is unclear, retroviral-infection analyses need to be extended in order to determine if a cortical bipotential progenitor cell is absent, to a great degree, at all developmental time periods, or whether the existing data are a consequence of retroviral infection at a time period in which the cortical bipotential cell pool has been depleted previously.

Additionally, the available data cannot rule out the possibility that the cortical bipotential progenitor cell is induced to generate neuronal, rather than glial, lineage-restricted progenitor cells by cues present in the local milieu at early time points. Such an interpretation would be in agreement with the conclusions reached following similar cell lineage analyses of retina (Turner and Cepko, 1987; Holt et al., 1988; Wetts and Fraser, 1988; Adler and Hatlee, 1989; Turner et al., 1990) and with the larger percentage (22%) of mixed glial-neuronal cell colonies observed in clonal cortical cell microcultures (Temple, 1989). The concept of an environmentally cued manipulation of cell phenotype is further supported by analyses of isolated optic nerve cells, whereby the neuronal cell potentiality developed in vitro is restricted to glial cell lineage(s) in vivo (Giess et al., 1990; Omlin and Waldemyer, 1989). It is worth noting that retroviral cell lineage analysis documents the ultimate fate, but not the developmental potential, of clonally related cells. In contrast, tissue culture manipulations reflect the range of cell differentiation potential, but possibly not the correlative cell fate in vivo.

Recombinant retroviral vector-mediated oncogene transduction of embryonic neuroepithelial cells has provided a means for establishing permanent cell populations that differentiate toward a stable neuronal or glial cell phenotype according to the tissue culture conditions employed (Bartlett et al., 1988; Frederiksen et al., 1988; Bernard et al., 1989; Ryder et al., 1990; Evrard et al., 1990). The validity of this "immortalization" approach relies critically on the largely unproven assumption

that immortalized cell lines in vitro retain the antigenic and biochemical characteristics representative of their in vivo history. As a first approximation, however, the success in obtaining cells of bipotential character correlates with the developmental time window and brain region where gliogenesis and neurogenesis are simultaneous, rather than sequential, events.

Immortalized bipotential progenitor cells display heterogeneous morphologies in vitro. However, the significance of the observed morphology is obscure as the cerebral cortical bipotential progenitor cell has not been described in vivo (for a possible description, see Liepelt et al., 1990). Bipotential progenitor cells evidently express a novel (class VI) intermediate filament protein, nestin (Lendahal et al., 1990), a polypeptide previously defined antigenically as Rat-401 (Hockfield and McKay, 1985). The cellular distribution of nestin observed during neural development (Frederiksen and McKay, 1988) and the presence of nestin in immortalized bipotential cells (Frederiksen et al., 1988; McKay, 1989) are evidence in accordance with this interpretation. Vimentin, detected antigenically in undifferentiated neuroepithelial cells (Tapscott et al., 1981a; Houle and Fedoroff, 1983) and in both neurons and radial glial cells during early stages of their differentiation (Cochard and Paulin, 1984; Bignami et al., 1982; Frederiksen et al., 1988; Culican et al., 1990), may be considered a coexpressed intermediate filament protein. Additional antigenic constituents of the bipotential progenitor cell appear to be the ganglioside G_{D3} (Goldman et al., 1984; Rosner et al., 1985) and the ganglioside series recognized by the monoclonal antibody A2B5 (Eisenbarth et al., 1979; Fredman et al., 1984; Frederiksen and McKay, 1988).

Of note, the antigens G_{D3}, Rat-401, and vimentin are detected immunologically in most, if not all, cells subsequent to the formation of the neural tube (Goldman et al., 1984; Rosner et al., 1985; Frederiksen and McKay, 1988; Cochard and Paulin, 1984). Consequently, these antigens may also be expressed by the multipotential progenitor cell. However, as the existence of a multipotential progenitor cell in vivo has not been proved, the initial placement of these antigens in Figure 1 is only tentative.

Glial or Neuronal Lineage-Restricted Progenitor Cell

The integration of a glial or neuronal cell identity upon transitional cell intermediates present between the initial bipotential cell and either glial or neuronal lineage-restricted cell populations may not be instantaneous. Thus, in the absence of definitive or contradictory evidence, the amplifying transitional cell population is schematized to show a progressive commitment in cell phenotype (Fig. 1). However, it is not clear at what maturational stage an intermediate transitional cell becomes irreversibly committed to a glial or neuronal cell differentiation program. Moreover, whether intermediate transitional cells in the cerebral cortex possess the ability to elect either a glial or a neuronal cell lineage in response to available environmental cues is unclear (McKay, 1989).

From the glial lineage-restricted progenitor cell, cortical glial cell sublineages diverge into independent pathways of development (Fig. 1). Although previous morphological and immunohistochemical analyses of the embryonic primate telencephalon (Levitt et al., 1981, 1983) and dentate gyrus (Eckenhoff and Rakic, 1984, 1988) suggested the presence of co-existing proliferative glial and neuronal progenitor cell pools, evidence for the presence of a discrete multipotential glial lineage-restricted progenitor cell population has been obtained only recently. Following retroviral infection of chick spinal cord, Sanes and colleagues (Leber et al., 1990) have identified clonally related cell populations composed of both astrocytes and, tentatively, oligodendrocytes. Additional cell lineage analyses suggest that a similar mechanism for glial cell diversification may be operative in the mammalian forebrain (Ryder et al., 1988; see also Price and Thurlow, 1988 for a possible equivalent result).

The acquisition of the glial cell specific antigen Ran-2 (Bartlett et al., 1981), expressed at embryonic day 11 (Abney et al., 1981), may provide an initial indication of the segregation of glial and neuronal cell lineages (Fig. 1). In accordance with their selective detection in subsequent glial cell sublineages, the antigens vimentin and Rat-401 are likely to be expressed by the glial lineage-restricted progenitor cell. Although available evidence is insufficient to warrant a definitive conclusion, the constellation of A2B5 and G_{D3} antigens may also be expressed by the glial lineage-restricted progenitor cell.

Analogous to the developmental potentials expressed by the glial lineage-restricted progenitor cell, a single multipotential neuronal progenitor cell may give rise to a clonal cohort comprising neuronal cells that occupy multiple cortical laminae and display diverse morphologies and chemical phenotypes (reviewed in McConnell, 1989). However, in contrast to results obtained for the neuronal progenitor cells of the peripheral nervous system (e.g., DiCicco-Bloom et al., 1990 and references within), polypeptides that selectively characterize proliferative neuronal lineage-restricted cell precursors in the cortex have not been identified. At present, neuronal cell-specific forms of polypeptides that mediate physiologic functions common to most eukaryotic cells merit further analysis as selective phenotypic markers for replicating cortical neuronal lineage-restricted cell precursors: for example, the high-molecular-weight clathrin light chain (Wong et al., 1990) and the $\alpha 3$ subunit of the Na^+,K^+-ATPase (R. Levenson, unpublished).

Immunocytochemical analyses of developing and adult neural tissues indicate that post-mitotic neurons terminate the synthesis of the antigens G_{D3}, vimentin, and Rat-401 (Rosner et al., 1985; Tapscott et al., 1981a; Hockfield and McKay, 1985; Frederiksen and McKay, 1988). Present evidence suggests that the loss of these antigens occurs at the penultimate and/or terminal

mitotic division of the neuronal progenitor cell: a mitotic division that coincides with the initial expression of neuronal cell-specific cytoskeletal components such as the neurofilament protein (Tapscott et al., 1981a,b; Bennett and DiLullo, 1985a,b) and the class III B-tubulin isotype (Lee et al., 1990).

Finally, a further issue that should be raised in the context of progenitor cells is the heterogeneous size of the clonal cell colonies observed, both in vitro and in vivo. Although the phenomenon of programmed cell death can not be excluded, the number of cell progeny detected in cell colony-forming assays is likely to reflect the proliferative capacity of the progenitor cell at the time of isolation or infection (Vaysse and Goldman, 1990; Temple, 1989; Gray et al., 1988). In contrast to the results obtained for mammalian retina and for several chick neural tissues, the cohort size determined for neuronal cell colonies identified in the cerebral cortex is, in general, small (average 2–3 cells). Consequently, the number of cell divisions that intervene between neuronal cell lineage commitment and terminal mitotic division in the cerebral cortex may also be minimal. This stands in distinction to the considerable proliferative capacity observed for cortical glial cell colonies.

SUBLINEAGE-SPECIFIC PRECURSOR AND DIFFERENTIATED CELL POOLS

Radial Glial Progenitor Cell Pool

Radial glial cells are characterized by an elongated form that during cerebral cortical development radially spans the entire cerebral wall, from the ventricular to the pial surface. Several lines of evidence, obtained from both in vivo and in vitro analyses, indicate that contact guidance interaction between migrating post-mitotic neurons and the surface of neighboring radial glial cells plays a critical role in organizing the spatial arrangement of the developing cerebral and cerebellar cortices (Rakic, 1972, 1981, 1988; Schmechel and Rakic, 1979b; Hatten, 1990; Edwards et al., 1990).

Progenitor-like or primary radial glial cells, which in nonprimates do not express the glial fibrillary acidic protein (GFAP), are the earliest of the telencephalic glial cell sublineages to be identified by morphological and immunological criteria. This system of radial glial cells appears to be assembled in the telencephalon prior to the earliest migratory wave of post-mitotic neurons (Levitt et al., 1981, 1983; Richmann et al., 1987; Gadisseux et al., 1989).

In nonprimates, emergence of the radial glial cell lineage and differentiation from radially organized primitive neuroepithelial cells and committed neuronal cell precursors appears to be indicated by the expression of the radial glial cell-specific antigens RC1 and RC2 (Misson et al., 1988a,b; Edwards et al., 1990). Consistent with this interpretation are two lines of evidence: first, the RC1 epitope, in contrast to the antigens Rat-401 and vimentin, is not detected in cells immunopositive for neurofilament (Frederiksen and McKay, 1988; Culican et al., 1990); and second, the proliferative pool for RC1 and/or RC2 positive cells in the ventricular zone is in contrast to a larger proliferative pool of RC1 and RC2 negative (presumably neuronal) cells (Misson et al., 1988a,b; Edwards et al., 1990). In primates, two classes of mitotic cells, neuronal and radial glial, can be distinguished according to GFAP immunoreactivity. These two cell populations continue to coexist during the entire period of corticogenesis; however, their proportion changes in harmony with the appearance of increasing increments of neuronal and astroglial cells (Levitt et al., 1983). The original concept of two separate precursor cell lines and two alternative morphogenetic pathway for production of astrocytes is illustrated schematically in Figure 2.

At present, it is unclear if the primary radial glial cell can be equated to the radially-oriented glial lineage-restricted progenitor cell. Whether the RC1 and RC2 antigens are expressed in the multipotential glial progenitor cell as well as primary radial glial cells is unknown. The former would predict the loss of the RC1 and RC2 antigens during subsequent glial cell progression into the type-1 and/or O-2A glial progenitor cell pools (see Fig. 1 and the second next section). The questionable placement of the acquisition of either the RC1/RC2 antigen is reflected, therefore, in the cell lineage diagram (Fig. 1). That the population of primary radial glial cells possess extensive proliferative capability has been shown by the use of [^{3}H]-thymidine alone (Schmechel and Rakic, 1979a) or in combination with antibodies directed against either Rat-401 (Hockfield and McKay, 1985), RC1/RC2 (Misson et al., 1988a,b), or GFAP (Levitt et al., 1981).

Maturation of Radial Glial Cell Lineage

In nonprimates, the antigenic phenotype of the primary radial glial cell (RC1$^+$, RC2$^+$, vimentin$^+$, Rat-401$^+$, Ran-2$^+$) is remodeled during development of a secondary phenotype as revealed by a substitution in the intermediate filament protein composition; vimentin to GFAP (Pixley and De Vellis, 1984; Rickmann et al., 1987; Voigt, 1989). At present, studies have not been carried out to ascertain whether vimentin expression precedes GFAP expression in radial glial cells of primates. Glial fibrillary acidic protein has been detected in glial cells of all vertebrate species, exclusive of cyclostomes, and provides a definitive marker of the astrocytic cell phenotype (Onteniente et al., 1983; Dahl et al., 1985).

In contrast to nonmammalian species (e.g., King, 1966) radial glial cells constitute a transient cell population in the mammalian telencephalon, disappearing, for the most part, at the end of neuronal cell migration (Rakic, 1972; Schmechel and Rakic, 1979a,b). Considerable analyses of transitional forms of radial glial cells in Golgi-stained and GFAP-immunolabeled sections

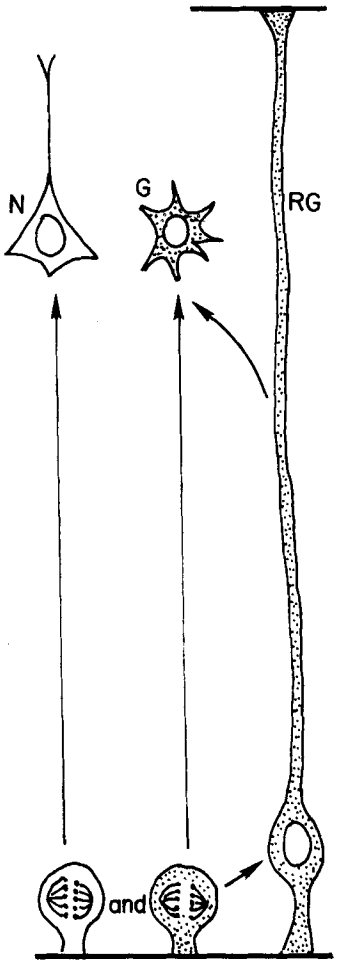

Fig. 2. Diagram of dual cell-line and morphogenetic-pathway concept. On the basis of a series of immunocytochemical analyses of gliogenesis in the fetal monkey cerebrum (Levitt et al., 1981, 1983; Eckenhoff and Rakic, 1983), it appears that both GFAP-positive (empty box) and GFAP-negative (dotted box) dividing cells coexist throughout the period of neurogenesis. The GFAP-positive cells initially produce radial glial (RG) cells; later, either directly or indirectly ( ), they generate astrocytes (G) and various specialized astrocyte-like cells. N, neuron. (From Rakic, 1981.)

prepared from cerebral cortex implied that radial glial cells were transformed into fibrillary astrocytes and/or protoplasmic astrocytes (Choi and Lapham, 1978; Schmechel and Rakic, 1979a; Levitt and Rakic, 1980; Rakic, 1984; Rickmann et al., 1987). In concordance with this interpretation, the timetable of the disappearance of radial glial cells in neocortex, hippocampus, and cerebellum of developing rhesus monkey correlates with the initial appearance of cortical astrocytes (Rakic, 1971; Schmechel and Rakic, 1979a; Eckenhoff and Rakic, 1984). On the basis of information obtained from Golgi preparations, Figure 3 provides a semidiagramatic sequence of the morphogenetic transformations that lead to the formation of astrocytic cell forms observed in the adult neocortex, cerebellar cortex, and hippocampus. Initially, the only glial cell elements present in all three regions are radial glial cells (Fig. 3A–C). However, only in the primate telencephalon do radial glial cell fibers attain enormous lengths, reaching several centimeters in midterm human fetus (Rakic, 1984). In the monkey, transitional forms between radial glial cells and astrocytes are first observed around E65 (Schmechel and Rakic, 1979b), a time period when the somata of some radial glial cells detach from the ventricular surface and become displaced to the intermediate zone (Fig. 3). Subsequently, cell forms of some radial glial cells may move to various depths of the cerebral wall and acquire typical, bushy astrocyte-like appendages. Recently, the morphological transformation of radial glial cells into mature astrocytic cell classes has been confirmed following the uptake of fluorescent dyes by embryonic radial glial cells in vivo (Voigt, 1989).

Little is known, however, about the cellular or the molecular events that underlie the observed morphological transformation of radial glial cells. In primary cultures of astrocytes, neuronal cells have been shown to exert an inhibitory effect on glial cell proliferation and, additionally, appear to regulate changes in astroglial cell shape from epithelial-like to radial or stellate (Sobue and Pleasure, 1984; Hatten, 1985, 1987; Ard and Bunge, 1988; Gasser and Hatten, 1990; Culican et al., 1990). Interestingly, in rhesus monkey a cessation in the mitotic activity of many radial glial cells accompanies the entire period of neuronal cell migration, which in these species approaches two months (Schmechel and Rakic, 1979b). As shown in Figure 2, subsequent to the completion of neuronal cell migration, radial glial cells reenter the mitotic cycle, and many simultaneously undergo a transformation into astrocytes.

The morphological transformation of GFAP-positive radial glial cells into classical astrocytic cell forms, as well as alternative radial cell forms (see below), appears to coincide with the loss of RC1, RC2, and Rat-401 antigens (the antigens are not expressed in the adult CNS; Hockfield and McKay, 1985; Misson et al., 1988a,b; Edwards et al., 1990). Although a correlative analysis of antigenic and morphologic transformation in vivo has been reported only preliminarily (Caviness et al., 1989), in vitro studies carried out by Culican et al. (1990) demonstrate that the morphological transformation of radial glial cells occurs concomitantly with a gradual acquisition of GFAP immunoreactivity and with a corresponding loss of RC1 immunoreactivity.

Ependymal cells, tanycytes, cerebellar Bergmann glial cells, and retinal Müller cells share many morphological, immunological, and biochemical features with cortical radial glial cells (e.g., Bartlett et al., 1981; Robinson and Dreher, 1990; Edwards et al., 1990; reviews in Fedoroff and Vernadakis, 1986). Accordingly, these cells may be considered morphologically and biochemically divergent forms of embryonic radial glial cells that continue to be maintained in the adult mammalian brain. As suggested recently, a continued cell association with a selective microenvironment may allow for the maintenance of a modified radial glial cell form in the adult (Reichenbach, 1989). Therefore, both cell-cell interactions and cell lineages are likely to deter-

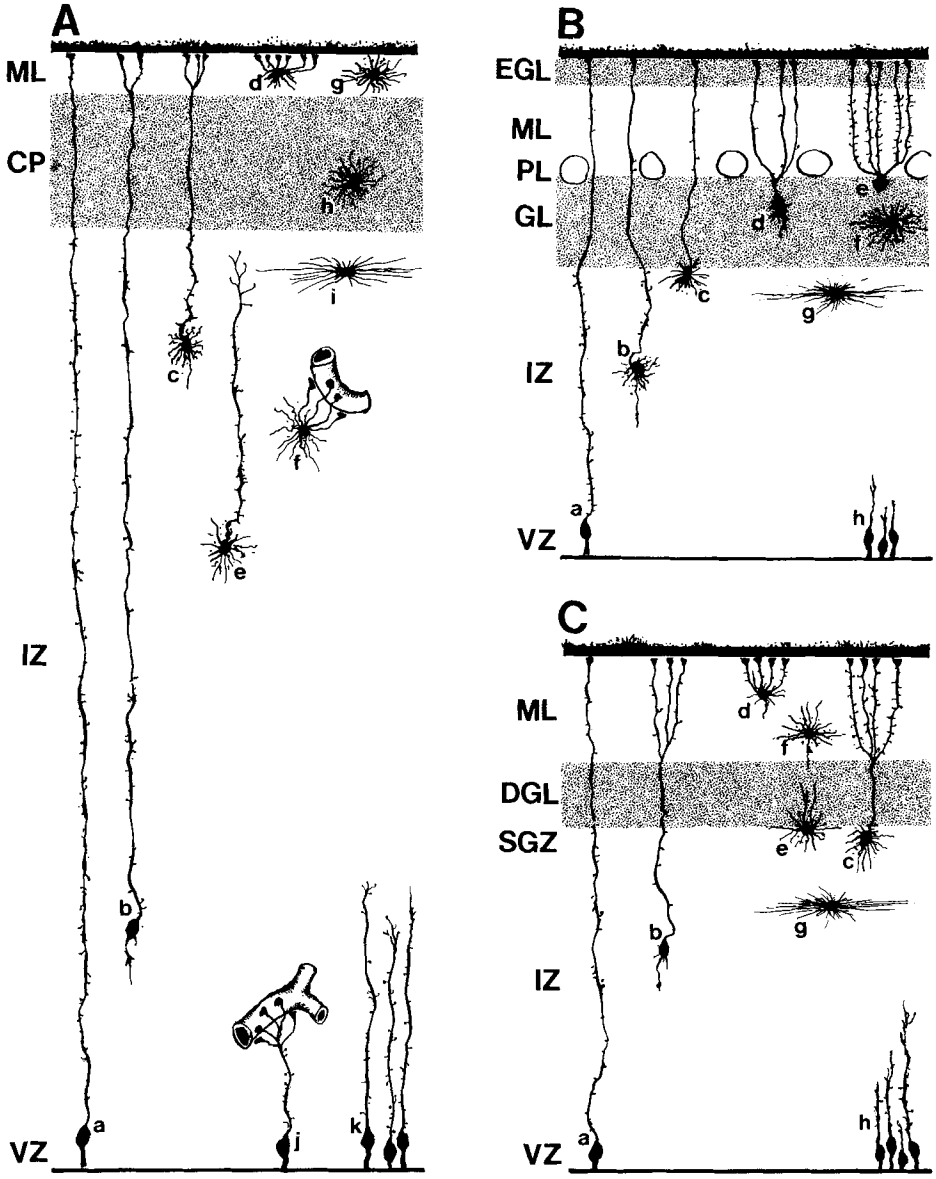

Fig. 3. Semischematic diagram illustrating morphogenetic transformation of fetal radial glial cells into various astrocytic forms in the cerebral hemisphere [A], cerebellar hemisphere [B], and dentate gyrus of the hippocampus [C]. CP, cerebral cortical plate; EGL, external granule layer; GL, granular layer (internal) of the cerebellum; DGL, dentate gyrus-granular layer of the hippocampal region; IZ, intermediate zone; ML, molecular layer; PL, Purkinje cell layer; SGZ, subgranular zone of the dentate gyrus; VZ, ventricular zone. Further explanation and designation of various transitional cell forms (a–k) is provided in the text (from Rakic, 1984).

mine the fate of radial glial cells and their quantitative contribution to the astrocyte populations.

Type-1 Astrocyte Progenitor Cell Pool

Mixed cell cultures prepared from developing cerebral or cerebellar cortices comprise two classes of astrocytes, which can be distinguished by morphologic, physiologic, and antigenic features. Astrocytes and oligodendrocytes develop in dissociated cell cultures of embryonic brain according to an in vivo schedule: type-1 astrocytes, immunopositive for GFAP, are detected in rat brain cell suspensions at embryonic day 15/16 and precede the appearance of oligodendrocytes at approximately birth, and type-2 astrocytes at postnatal day 6/8 (Abney et al., 1981; Williams et al., 1985; Lillien et al., 1990b). Antigenic pulse-label/chase and complement dependent lysis analyses indicate that type-1 and type-2 astrocytes are not interconvertable, thus, implying two distinct astrocyte lineages (Raff et al., 1983a,b; 1984). Type-1 astrocytes, found predominantly in cultures of grey matter, are considered to approximate morphologically and antigenically the protoplasmic astrocyte in vivo (Miller and Raff, 1984).

The type-1 precursor cell in vitro assumes a flattened, epithelial-like morphology, proliferates extensively in response to undefined growth factors (pituitary extracts), and has been identified primarily as comprising the antigenic phenotype Ran-2$^+$, GFAP$^-$. Additional antigenic characteristics that have been determined for the type-1 astrocyte progenitor cell may be listed as follows: A2B5$^-$ (5% are A2B5$^+$), fibronectin$^-$, vimentin$^+$, growth-associated protein 43$^+$ (Abney et al., 1981; Raff et al., 1983a, 1984; da Cunha and Vitkovic, 1990). An immortalized subclonal cell line, described preliminarily (Geller and Dubois-Dalcq, 1988), appears to possess antigenic and morphologic features anticipated of the type-1 astrocyte progenitor cell and may, therefore, provide a useful cell prototype for further developmental and functional analyses.

In principle, the type-1 astrocyte progenitor cell population is derived directly by a cellular transformation of the ill-defined radially oriented glial lineage-restricted progenitor cell. Preliminary supportive evidence for this cellular origin are several fold, although data are insufficient to exclude a potential relationship between the progenitor-like or primary radial glial cell and the type-1 astrocyte progenitor cell (indicated by an arrow connecting the two cell types in Fig. 1).

First, although the antigenic phenotype characteristic of the type-1 progenitor cell (Ran-2$^+$, GFAP$^-$) is detected at a time that coincides with the appearance of primary radial glial cells (embryonic day 11, Abney et al., 1981; Bartlett et al., 1981), a reevaluation of cell identity is warranted in order to distinguish between the two possible progenitor cell populations. Available analyses are insufficient to ascertain whether the type-1 astrocytic progenitor cell expresses the RC1, RC2, and Rat-401 antigens, although it is of potential significance that a proliferating population of Rat-401-immunonegative cells can be identified in the neuroepithelium of the rat cerebral cortex after embryonic day 12 (Frederiksen and McKay, 1988). As currently viewed, the RC1, RC2, and Rat-401 antigens are considered to mark selectively the radial glial cell population. At present, a possible distinction in the progenitor cell populations may be inferred from immunolabeling analyses carried out with antibodies directed to cytotacting, an extracellular matrix molecule synthesized by cortical radial glial cells (Steindler et al., 1989; Crossin et al., 1989; Prieto et al., 1990) but not by type-1 astrocyte progenitor cells (ffrench-Constant and Raff, 1986b; ffrench-Constant et al., 1986).

Second, radial glial cells cultured in the absence of neurons assume a morphology that is reminiscent of the epithelial-like morphology displayed by most type-1 astrocytes in vitro (Hatten, 1985, 1987; Gasser and Hatten, 1990). Although these epithelial-like radial glial cells eventually acquire GFAP immunoreactivity (Culican et al., 1990), they continue to express the RC1 and Rat-401 antigens, at least on the time scale analyzed (Wilkie and Lauder, 1988; Culican et al., 1990; Cameron and Rakic, unpublished; see also Frederiksen and McKay, 1988). In contrast, GFAP-immunonegative radial glial cells cultured in the presence of neuronal cell contact develop a stellate morphology, which is characteristic of the mature type-1 astrocyte both in vitro and in vivo (Meller, 1987; Ard and Bunge, 1988). Yet, they acquire increasing levels of GFAP immunoreactivity while becoming RC1-immunonegative, thereby, mimicking the cellular transformation of radial glial cells into type-1 astrocytes that has been observed in vivo (Culican et al., 1990).

Maturation of Type-1 Astrocyte Cell Lineage

Differentiated type-1 astrocytes may be identified according to the following antigenic phenotype: RC1−, RC2−, Rat-401−, growth-associated protein 43−, cytotactin− (NSP-4 monoclonal antibody), vimentin−, chondroitin 4-sulfate−, tetanus toxin receptor−, platelet-derived growth factor receptor−, G_{D3}− (<2% are G_{D3}+), A2B5− (<5% are A2B5+), fibronectin+, laminin+, GFAP+, and Ran-2+ (Liesi et al., 1983, 1986; ffrench-Constant and Raff, 1986b; ffrench-Constant et al., 1986; Behar et al., 1988; Aloisi et al., 1988b; Price and Hynes, 1988; Ard and Bunge, 1988; Gallo et al., 1987, 1990; da Cunha and Vitkovic, 1990; Vaysse and Goldman, 1990).

Although considered a single cell type morphologically, accumulated data suggest that type-1 astrocytes obtained from anatomically distinct regions of the CNS display a heterogeneous subset of functional parameters (reviewed in Hansson, 1988; Wilkin et al., 1990). It is unclear, however, whether the implied compositional heterogeneity reflects a flexible amplification of polypeptide components common to a prototypic type-1 astrocyte, or if the compositional heterogeneity is detected secondarily to the development of multiple type-1 astrocytic cell lineages.

Differentiated type-1 astrocytes appear to contribute to regulatory mechanisms that underlie the development of the O-2A cell lineage (see the following section; Fig. 1). Type-1 astrocytes obtained from cerebral cortex have been shown to secrete a factor, similar or identical to platelet-derived growth factor (PDGF) AA homodimers. Although the cellular functions mediated by PDGF are likely to be more complex than were considered initially (e.g., see Bögler et al., 1990), PDGF evidently promotes the proliferation and survival of the oligodendrocyte/type-2 astrocyte progenitor cell in vitro (Noble et al., 1988; Raff et al., 1988; Richardson et al., 1988; Geller and Dubois-Dalcq, 1988; Pringle et al., 1989). PDGF is evidently not a mitogen for neuronal cells (Besnard et al., 1987), nor does PDGF function as a feedback regulator (positively or negatively) of type-1 astrocyte proliferation (Hart et al., 1989). Type-1 astrocytes also appear to initiate, in part, the entrance of O-2A progenitor cells into the type-2 astrocyte sublineage by synthesis and secretion of a factor similar or identical to ciliary neurotrophic factor (see the second section following; Lillien and Raff, 1990a,b).

Oligodendrocyte/Type-2 Astrocyte Progenitor Cell Pool

Oligodendrocyte/type-2 (O-2A) astrocyte progenitor cells isolated from newborn rodent cerebellar (Levi et al., 1986) and cerebral cortices (Behar et al., 1988; Levine, 1989; Ingraham and McCarthy, 1989) exhibit, for the most part, analogous developmental and antigenic features described for the O-2A progenitor cell population obtained from the rat optic nerve (reviewed in Raff, 1989). In culture, O-2A progenitor cell has a bipolar, process-bearing morphology and, traditionally, has been identified according to the antigenic phenotypic A2B5+, vimentin+, GFAP−, and Ran 2− (Raff, 1989). Further antigenic analyses indicate that the O-2A progenitor cell displays the following additional phenotype: alpha form of platelet derived growth factor receptor+, tetanus toxin receptor+, chondroitin 4-sulfate+ (CS4), G_{D3}+, J1/cytotactin/tenascin/ NSP4+, and growth-associated protein 43+ (Raff et al., 1983a,b, 1984; Williams et al., 1985; Behar et al., 1988; Hart et al., 1989; Gallo et al., 1990; da Cunha and Vitkovic, 1990; Lee et al., 1990).

Cerebral O-2A progenitor cells apparently originate from the subventricular zone (LeVine and Goldman, 1988a,b), and cerebellar O-2A progenitor cell appear to arise from subependymal layers of the 4th ventricle (Curtis et al., 1988; Reynolds and Wilkin, 1988). Cell proliferation and early stages of oligodendrocytic cell differentiation evidently take place prior to their active migration into subcortical white matter. As such, cortical O-2A progenitor cells mimick the migratory behavior demonstrated for O-2A progenitor cells isolated from optic nerve (Small et al., 1987). In spinal cord, available evidence is consistent with the possibility that the O-2A progenitor cell (or the oligodendrocyte precursor equivalent) arises by cellular transformation of primary radially oriented glial cells (Choi et al., 1983; Hirano and Goldman, 1988; see also Godfraind et al., 1989). Identification, in chick spinal cord, of clonal cell cohorts comprising both astrocytes and oligodendrocytes (Leber et al., 1990) would appear to support the former morphological evidence. However, it remains to be determined if radially oriented cerebral cortical glial lineage-restricted progenitor cells possess the same developmental potential regarding the genesis of cortical oligodendrocytes. Analyses carried out in vitro indicate that glial cell progression into the cortical O-2A lineage represents an irreversible commitment (Vaysse and Goldman, 1990).

The finding that a percentage of isolated cortical O-2A-like progenitor cells (O4-cells, see the next section) remain quiescent in tissue culture implies that a stable population of O-2A-like progenitor cells may be retained in the adult cerebral cortex (Gard and Pffeiffer, 1989; see also Norton et al., 1988 and Norton and Farooq, 1989). This suggestion is in agreement with previous autoradiographic analyses of [^{3}H]-thymidine uptake in adult animals (Rakic, 1985; McCarthy and Leblond, 1988). Of note, adult O-2A progenitor cells, which have been isolated from adult rat optic nerve, express several phenotypic features (e.g., antigenic phenotype; A2B5+, O4+, and vimentin−) that serve to distinguish them from the perinatal O-2A progenitor cell (ffrench-Constant and Raff, 1986a; Wolswijk and Noble, 1989). Cells comprising the antigenic phenotype of the O-2A progenitor cell have been identified also in adult cerebellar cortex and, likely, cerebral cortex (Levine, 1989). From a functional viewpoint, however, it remains unclear whether adult O-2A, rather than perinatal O-2A, progenitor cells form the proliferative pool of oligodendrocyte precursors that have been observed during studies of adult CNS demyelination/remyelination (Godfraind et al., 1989; Armstrong et al., 1990). At the very least, the coexistence of distinct adult and perinatal O-2A progenitor cells during early optic nerve development, in vivo and in vitro, suggests that the classical O-2A progenitor cell is likely to comprise a phenotypically diverse, functionally related family of progenitor cells rather than a single cell type (Wolswijk et al., 1990).

Maturation of the O-2A Cell Lineage

The oligodendrocyte

In the absence of inducing factors for astrocytic cell differentiation in vitro, the proliferative pool of cortical bipolar O-2A progenitor cells (antigenically A2B5+, vimentin+) differentiate constitutively into cells of the oligodendrocyte lineage (reviewed in Lillien and Raff, 1990b). Progressive stages of subsequent oligodendrocyte development are distinguishable on the basis of the sequential expression of antigenic markers and by changes in physiologic and morphologic features. Isolated cortical O-2A progenitor cells differentiate on a time schedule in vitro that approximates the time schedule of myelinogenic activity observed in vivo (Knapp et al., 1987; Gard and Pfeiffer, 1989).

The earliest cell type in the cortical oligodendrocyte lineage is identified by the immunocytochemical detection of the O4 antigen (Sommer and Schachner, 1981, 1982; Schachner et al., 1981; Dubois-Dalcq, 1987; Levi et al., 1987; Aloisi et al., 1988a,b). Homogeneous O4-cell populations, obtained from both cerebral and cerebellar cortices (Trotter and Schachner, 1989; Gard and Pfeiffer, 1989), display, to a great degree, physiologic and antigenic properties analogous to those discerned for the O-2A progenitor cell (Behar et al., 1988; Sontheimer et al., 1989; Gard and Pfeiffer, 1989). As indicated by the conversional phenotype arrow in Figure 1, a variable proportion of cortical O4-cells retain the phenotypic plasticity of the O-2A progenitor cell and can differentiate, under appropriate culture conditions, into the type-2 astrocyte (see the next section; Levi et al., 1987; Aloisi et al., 1988a; Trotter and Schachner, 1989). Significantly, cells comprising a combined oligodendrocyte-astrocyte phenotype (GFAP+, O4+) are observed, both in vivo and in vitro, as a proliferative cell population

following virally induced demyelination (Godfraind et al., 1989; Armstrong et al., 1990).

The near simultaneous appearance of galactocerebroside (GalC; Raff et al., 1978) and 2′, 3′-cyclic nucleotide 3′-phosphohydrolase (CNP; Bansal and Pfeiffer, 1985), determined enzymatically or antigenically, denotes the second stage of oligodendrocyte cell maturation in the cerebral cortex. In comparison to the modest changes undergone by O4-cells, GalC/CNP cells have been remodeled extensively. Morphologically, GalC/CNP cells are characterized by an increased cell size and by an expansion in the number of cell processes. The antigens G_{D3}, A2B5, tetanus toxin receptor, chondroitin 4-sulfate (CS4), and vimentin are not detectable, for the most part, immunocytochemically (Raff et al., 1984; Dubois-Dalqc, 1987; Curtis et al., 1988; Gard and Pfeiffer, 1989; Gallo et al., 1990). Additionally, changes in ion channel properties are evident (Sontheimer et al., 1989, Barres et al., 1990; Verkhratsky et al., 1990). Acquisition of GalC/CNP immunoreactivity coincides with the cessation of proliferative activity, thus suggesting that the appearance of both antigens may be correlated to a terminal progression into the oligodendrocytic cell lineage (Gard and Pfeiffer, 1989).

Further maturational stages in the cortical oligodendrocytic cell are indicated by the sequential appearance of the proteolipid protein, myelin-associated glycoprotein, and myelin basic protein, respectively (Gard and Pfeiffer, 1989). At least in vitro, the growth-associated protein 43 appears to be expressed constitutively at all stages of oligodendrocyte development (da Cunha and Vitkovic, 1990).

The type-2 astrocyte

Cell egression from the O-2A progenitor (and/or O4) cell pool and subsequent entrance into the type-2 astrocytic cell lineage is signified initially by the expression of low levels of GFAP (Fig. 1). In contrast to the autonomous progression of O-2A progenitor cells into the oligodendrocytic cell lineage, the development of a stable population of type-2 astrocytes in serum-free optic nerve cultures requires the presence of both diffusible (ciliary neurotropic factor) and nondiffusible (extracellular matrix components) inducing factors (Lillien et al., 1990; Lillien and Raff, 1990b). Alternatively, constitutive and stable induction of type-2 astrocyte differentiation can be achieved by O-2A progenitor cell growth in culture medium containing 10 to 20% fetal calf serum. Preliminary analyses indicate that a similar developmental program operates in serum-free cultures prepared from cerebral cortex (Lillien et al., 1988).

Homogeneous cell populations of type-2 astrocytes have been obtained by subculturing mixed cortical glial cell dissociates in the presence of fetal calf serum (Aloisi et al., 1988a). Process-bearing, stellate type-2 astrocytes obtained from cerebral cortex display an antigenic composition [chondroitin 4-sulfate+, G_{D3}+, A2B5+, GFAP+, cytotactin+ (NSP-4), growth-associated protein 43+, laminin +/−, fibronectin−] similar to that described for the type-2 astrocyte identified in cell cultures of optic nerve (Raff et al., 1983a,b) and cerebellum (Levi et al., 1986, 1987; Gallo et al., 1987, 1990). Antigenically, loss of G_{D3} and chondroitin 4-sulfate accompanies maturation of type-2 astocytes, and although developing type-2 astrocytes retain proliferative cabability, mature type-2 astrocytes are postmitotic (Lillien and Raff, 1990a). Functionally, type-2 astrocytes are considered to approximate the fibrous astrocyte (perinodal astrocyte; Black and Waxman, 1988) of myelinated fiber tracts (ffrench-Constant and Raff, 1986b; ffrench-Constant et al., 1986; Miller and Raff, 1984; Miller et al., 1985).

On several accounts, however, features of type-2 astrocyte development determined in vitro have proven enigmatic in establishing a correlate of the type-2 astrocyte in vivo, both in terms of proposed location and possible function. For example, the type-2 astrocyte phenotype is transient in vitro. In long-term tissue culture, loss of the phenotypic features indicative of the mature type-2 astrocyte coincide with the progressive acquisition of morphologic (stellate to epithelial-like), antigenic (loss of the A2B5, and gain of fibronectin and Ran-2 antigens) and functional (loss of the high affinity GABA uptake mechanism, change in neuronal-like to non-neuronal channel properties) features characteristic of the type-1 astrocyte (Aloisi et al., 1988a; Barres et al., 1990; Lillien and Raff, 1990a). Further, cells that co-express the ganglioside G_{D3} and GFAP (an antigenic phenotype which defines the type-2 astrocyte) have not been identified in intact tissue sections prepared from cerebral or cerebellar cortices (Curtis, et al., 1988; Goldman et al., 1986). Indeed, G_{D3}-immunopositive cells in vivo and evidently in vitro act only as oligodendrocyte precursors (LeVine and Goldman, 1988b; Vaysse and Goldman, 1990). In the same context, A2B5 immunoreactivity (also an antigenic marker of the type-2 astrocyte) has not been detected on putative type-2 astrocyte perinodal process in vivo, although the perinodal processes can be immunolabeled with other type-2 astrocyte immunomarkers, NSP-4 and HNK-1 (ffrench-Constant and Raff, 1986b; ffrench-Constant et al., 1986). Finally, a combined immunocytochemical and [^{3}H]-thymidine autoradiographic analysis (Skoff, 1990) has not revealed a proliferative pool of astrocytes that would correspond to the generative time window of optic nerve type-2 astrocytes as described by Miller et al. (1985). In this regard, it is interesting that the in vitro population of type-2 astrocytes quantitatively excedes by far the number of potential type-2 astrocytes identified in vivo.

CONCLUDING REMARKS - FUTURE PERSPECTIVE

In spite of considerable progress, the study of cell lineage in the mammalian cerebral cortex is in its infancy. As judged by the findings reviewed here, recent analyses have provided a basic "circuit diagram" of

cortical glial cell development. However, numerous unsolved problems remain, and major hypotheses concerning cortical glial cell differentiation have not been tested critically, particularly at early developmental timepoints. Thus, it seems that the focus of future research will likely involve studies in at least four general areas. First, the identification of cell pedigrees with retroviral gene transfer need to be combined with the use of markers specific for both a cell class and for a cell phenotype. Such analyses may be anticipated to provide a means of determining whether the extent of cell diversification seen in the adult cerebral cortex reflects an actual heterogeneity in cell lineage or the acquisition of a phenotype induced by an interaction with the local environment. Second, at present, cellular analyses of CNS development have been limited largely to the identification and characterization of polypeptides that identify differentiated glial and/or neuronal cell populations. This limitation emphasizes a continued need for the development of immunological tools and cellular assays for the identification and characterization of cerebral cortical progenitor cells. Third, on the basis of results obtained for other tissues, the regulation of cell proliferation and differentiation events for each CNS progenitor cell population is likely to be dependent on an interacting network of growth factors and associated receptor components coupled to distinct second messenger systems (Melton and Whitman, 1989; Metcalf, 1989; Whetton and Dexter, 1989; Ullrich and Schlessinger, 1990). However, to date, growth factors that participate in the specification and maintenance of individual cortical glial cell lineages remain largely unidentified. Finally, the regulation of progenitor cell commitment to specific cell lineages ultimately lies at the level of control of differential gene transcription. As exemplified by polypeptides of the MyoD family (Olson, 1990), a principal mechanism of transcriptional control is achieved through the interaction of a variety of DNA-binding trans-acting factors with multiple cis-regulatory promoter elements (Mitchell and Tjian, 1989; Latchman, 1990). The isolation and characterization of neural cell-specific DNA binding polypeptides may provide an initial means of identifying these critical regulatory polypeptides.

ACKNOWLEDGMENTS

The authors are grateful to Sharon Schmiedel for the illustration of Figure 1 and to Dr. Patricia Cameron, Dr. David Kornack, Dr. Douglas Meinecke, Dr. Claudia Toomim, and Dr. Kenneth Wikler for review of the manuscript, and particularly to Dr. Susan Hockfield for thoughtful discussions throughout.

REFERENCES

Abney, E.R., Bartlett, P.P., and Raff, M.C. (1981) Astrocytes, ependymal cells, and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain. *Dev. Biol.*, 83:301–310.

Adler, R. and Hatlee, M. (1989) Plasticity and differentiation of embryonic retinal cells after terminal mitosis. *Science*, 243:391–393.

Aloisi, F., Agresti, C., and Levi, G. (1988a) Establishment characterization, and evolution of cultures enriched in type-2 astrocytes. *J. Neurosci. Res.*, 21:188–198.

Aloisi, F., Agresti, C., D'Urso, D., and Levi, G. (1988b) Differentiation of bipotential glial precursors into oligodendrocytes is promoted by interaction with type-1 astrocytes in cerebellar cultures. *Proc. Natl. Acad. Sci. U.S.A.*, 85:6167–6171.

Alvarez-Buylla, A., Theelen, M., and Nottebohm, F. (1990) Proliferation "hot spots" in adult avain ventricular zone reveal radial cell divisions. *Neuron*, 5:101–109.

Armstrong, R., Friedrich, V.L., Jr., Holmes, K.V., and Dubois-Dalcq, M. (1990) *In vitro* analysis of the oligodendrocyte lineage in mice during demyelination and remyelination. *J. Cell Biol.*, 111:1183–1195.

Anderson, D.J. (1989) The neural crest cell lineage problem: Neuropoiesis? *Neuron*, 3:1–12.

Ard, M.D. and Bunge, R.P. (1988) Heparin sulfate proteoglycan and laminin immunoreactivity on cultured astrocytes; relationship to differentiation and neurite growth. *J. Neurosci.*, 8:2844–2858.

Bansal, R. and Pfeiffer, S.E. (1985) Developmental expression of 2′, 3′-cyclic nucleotide 3′-phosphohydrolase in dissociated fetal rat brain cultures and rat brain. *J. Neurosci. Res.*, 14:21–34.

Barres, B.A., Chun, L.L.Y., and Corey, D.P. (1990) Ion channels in Vertebrate Glia. *Annu. Rev. Neurosci.*, 13:441–474.

Bartlett, P.P., Noble, M.D., Pruss, R.M., Raff, M.C., Rattray, S., and Williams, C.A. (1981) Rat neural antigen-2 (Ran-2): A cell surface antigen on astrocytes, ependymal cells, Muller cells and leptomeninges defined by a monoclonal antibody. *Brain Res.*, 204: 339–351.

Bartlett, P.F., Reid, H.H., Bailey, K.A., and Bernard, O. (1988) Immortalization of mouse neural precursor cells by the c-myc oncogene. *Proc. Natl. Acad. Sci. U.S.A.*, 85:3255–3259.

Behar, T., McMorris, F.A., Novotny, E.A., Barker, J.L., and Dubois-Dalcq, M. (1988) Growth and differentiation properties of O-2A progenitors purified from rat cerebral hemispheres. *J. Neurosci. Res.*, 21:168–180.

Bennett, G.S. and DiLullo, C. (1985a) Transient expression of a neurofilament protein by replicating neuroepithelial cells of the embryonic chick brain. *Dev. Biol.*, 107:107–127.

Bennett, G.S. and DiLullo, C. (1985b) Expression of a neurofilament protein by the precursors of a subpopulation of ventral spinal cord neurons. *Dev. Biol.*, 107:94–106.

Bernard, O., Reid, H.H., and Bartlett, P.F. (1989) Role of the c-myc and the N-myc proto-oncogenes in the immortalization of neural precursors. *J. Neurosci. Res.*, 24:9–20.

Besnard, F., Perrand, F., Sensenbrenner, M., and Labordette, G. (1987) Platelet derived growth factor is a mitogen for glial but not for neuronal rat brain cells in vitro. *Neurosci. Lett.*:73:287–292.

Bignami, A., Raju, T., and Dahl, D. (1982) Localization of vimentin, the nonspecific intermediate filament protein in embryonal glia and in early differentiating neurons. *Dev. Biol.*, 91:286–295.

Black, J.A. and Waxman, S.G. (1988) The perinodal astrocyte. *Glia*, 1:169–183.

Bögler, O., Wren, D., Barnett, S.C., Land, H., and Noble, M. (1990) Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proc. Natl. Acad. Sci. U.S.A.*, 87:6368–6372.

Caviness, V.S., Jr., Misson, J.-P., Takahashj, T., and Crandall, J. (1989) Astroglial transformations in the developing murine cerebral wall. *Soc. Neurosci. Abstr.*, 15:328.

Choi, B.H. and Lapham, L.W. (1978) Radial glia in the human fetal cerebrum: A combined Golgi, immunofluorescent and electron microscopic study. *Brain Res.*, 148:295–311.

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

Cochard, P. and Paulin, D. (1984) Initial expression of neurofilaments and vimentin in the central and peripheral nervous system of the mouse embryo in vivo. *J. Neurosci.*, 8:2080–2094.

Crossin, K.L., Hoffman, S., Tan, S.-S., and Edelman, G.M. (1989) Cytotactin and its proteoglycan ligand mark structural and functional boundaries in somatosensory cortex of the early postnatal mouse. *Dev. Biol.*, 136:381–392.

Culican, S.M., Baumrind, N.L., Yamamoto, M., and Pearlman, A.L. (1990) Cortical radial glia: Identification in tissue culture and evidence for their transformation to astrocytes. *J. Neurosci.*, 10:684–692.

Curtis, R., Cohen, J., Fok-Seang, J., Hanley, M.R., Gregson, N.A., Reynolds, R., and Wilkin, G.P. (1988) Development of macroglial cells in rat cerebellum. I. Use of antibodies to follow early in vivo

development and migration of oligodendrocytes. *J. Neurocytol.*, 17:43–54.
da Cunha, A. and Vitkovic, L. (1990) Regulation of immunoreactive GAP-43 expression in rat cortical macroglia is cell type specific. *J. Cell Biol.*, 111:209–215.
Dahl, D., Crosby, C.J., Sethi, J.S., and Bignami, A. (1985) Glial fibrillary acidic (GFA) protein in vertebrates: Immunofluorescence and immunoblotting study with monoclonal and polyclonal antibodies. *J. Comp. Neurol.*, 239:75–88.
DiCicco-Bloom, E., Townes-Anderson, E., and Black, I.B. (1990) Neuroblast mitosis in dissociated culture: Regulation and relationship to differentiation. *J. Cell Biol.*, 110:2073–2086.
Dubois-Dalcq, M. (1987) Characterization of a slowly proliferative cell along the oligodendrocyte differentiation pathway. *EMBO J.*, 6:2587–2595.
Eckenhoff, M.F. and Rakic, P. (1984) Radial organization of the hippocampal dentate gyrus: A Golgi, ultrastructural and immunocytochemical analysis in the developing rhesus monkey. *J. Comp. Neurol.*, 223:1–21.
Eckenhoff, M.F. and Rakic, P. (1988) Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. *J. Neurosci.*, 8:2729–2747.
Edwards, M.A., Yamamoto, M., and Caviness, V.S., Jr. (1990) Organization of radial glial and related cells in the developing murine CNS: An analysis based upon a new monoclonal antibody marker. *Neuroscience*, 36:121–144.
Eisenbarth, G.S., Walsh, F.S., and Nirenberg, M. (1979) Monoclonal antibody to a plasma membrane antigen of neurons. *Proc. Natl. Acad. Sci. U.S.A.*, 76:4913–4917.
Evrard, C., Borde, I., Marin, P., Galiana, E., Premont, J., Gros, F., and Rouget, P. (1990) Immortalization of bipotential and plastic glioneuronal precursor cells. *Proc. Natl. Acad. Sci. U.S.A.*, 87: 3026–3066.
Fedoroff, S. and Vernadakis, A. (eds.) (1986) *Astrocytes: Development, Morphology, and Regional Specialization of Astrocytes*, Vol. 1. Academic Press, New York.
ffrench-Constant, C. and Raff, M.C. (1986a) Proliferating bipotential glial progenitor cells in the adult rat optic nerve. *Nature*, 319: 499–502.
ffrench-Constant, C. and Raff, M.C. (1986b) The oligodendrocyte-type 2 astrocyte cell lineage is specialized for myelination. *Nature*, 323:335–338.
ffrench-Constant, C., Miller, R.H., Kruse, J., Schachner, M., and Raff, M.C. (1986) Molecular specialization of astrocyte processes at nodes of Ranvier in rat optic nerve. *J. Cell Biol.*, 102:844–852.
Frederiksen, K., and McKay, R.D.G. (1988) Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. *J. Neurosci.*, 8:1144–1151.
Frederiksen, K., Jat, P.S., Valtz, N., Levy, D., and McKay, R. (1988) Immortalization of precursor cells from the mammalian CNS. *Neuron*, 1:439–448.
Fredman, P., Magnani, J.L., Nirenberg, M., and Ginsberg, V. (1984) Monoclonal antibody A2B5 reacts with many gangliosides in neuronal tissue. *Arch. Biochem. Biophys.*, 233:661–666.
Fujita, H. and Fujita, S. (1963) Electron microscopic studies on neuroblast differentiation in the central nervous system of domestic fowl. *Z. Zellforsch.*, 60:463–478.
Fujita, H. and Fujita, S. (1964) Electron microscopic studies on the differentiation of the ependymalm cells and the glioblast in the spinal cord of domestic fowl. *Z. Zellforsch*, 64:262–272.
Fujita, S. (1963) The matrix cell and cytogenesis in the developing central nervous system. *J. Comp. Neurol.*, 120:37–42.
Fujita, S. (1966) Application of light and electron microscopic autoradiography to the study of cytogenesis of the forebrain. In: *Evolution of the Forebrain.*, R. Hassler and H. Stephan, eds. Georg Thieme, Stuttgart, pp. 180–196.
Gadisseux, J.F., Evrard, P., Misson, J.P., and Caviness, V.S. (1989) Dynamic structure of the radial glial fiber system of the developing murine cerebral wall: An immunocytochemical analysis. *Dev. Brain. Res.*, 50:55–67.
Galileo, D.S., Gray, G.E., Owens, G.C., Majors, J., and Sanes, J.R. (1990) Neurons and glia arise from a common progenitor in chick optic tectum: Demonstration with two retroviruses and cell type-specific antibodies. *Proc. Natl. Acad. Sci. U.S.A.*, 87:458–462.
Gallo, V., Bertolotto, A., Levi, G. (1987) The proteoglycan chondroitin sulfate is present in a subpopulation of cultured astrocytes and in their precursors. *Dev. Biol.*, 123:282–285.
Gallo, V. and Bertolotto, A. (1990) Extracellular matrix of cultured glial cells: Selective expression of chondroitin 4-sulfate by type-2 astrocyte and their progenitors. *Exp. Cell Res.*, 187:211–223.
Gard, A.L. and Pfeiffer, S.E. (1989) Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: Myelinogenic potential in a defined environment. *Development*, 106:119–132.
Gasser, U.E. and Hatten, M.E. (1990) Neuron-glia interactions of rat hippocampal cells in vitro: Glial-guided neuronal migration and neuronal regulation of glial differentiation. *J. Neurosci.*, 10:1276–1285.
Geller, H.M. and Dubois-Dalcq, M. (1988) Antigenic and functional characterization of a rat central nervous system-derived cell line immortalized by a retroviral vector. *J. Cell Biol.*, 107:1977–1986.
Giess, M.-C., Cochard, P., and Duprat, A.-M. (1990) Neuronal potentialities of cells in the optic nerve of the chicken embryo are revealed in culture. *Proc. Natl. Acad. Sci. U.S.A.*, 87:1643–1647.
Godfraind, C., Friedrich, V.L., Holmes, K.V., and Dubois-Dalcq, M. (1989) In vivo analysis of glial cell phenotypes during a viral demyelinating disease in mice. *J. Cell Biol.*, 109:2405–2416.
Goldman, J.E., Hirano, M., Yu, R.K., and Seyfried, T.N. (1984) G_{D3} ganglioside is a glycolipid of immature neuroectodermal cells. *J. Neuroimmunol.*, 7:179–192.
Goldman, J.E., Geier, S.S., and Hirano, M. (1986) Differentiation of astrocytes and oligodendrocytes from germinal matrix cells in primary culture. *J. Neurosci.*, 6:52–60.
Gray, G.E., Glover, J.C., Majors, J., and Sanes, J.R. (1988) Radial arrangement of clonally related cells in the chicken optic tectum: lineage analysis with a recombinant retrovirus. *Proc. Natl. Acad. Sci. U.S.A.*, 85:7356–7360.
Hall, P.A. and Watt, F.M. (1989) Stem cells: the generation and maintenance of cellular diversity. *Development*, 106:619–633.
Hansson, E. (1988) Astroglia from defined brain regions as studied with primary cultures. *Prog. Neurobiol.*, 30:369–397.
Hart, I.K., Richardson, W.D., Heldin, C.-H., Westermark, B., and Raff, M.C. (1989) PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. *Development*, 105:595–603.
Hatten, M.E. (1985) Neuronal regulation of astroglial morphology and proliferation in vitro. *J. Cell Biol.*, 100:384–396.
Hatten, M. E. (1987) Neuronal inhibition of astroglial cell proliferation is membrane mediated. *J. Cell Biol.*, 104:1353–1360.
Hatten, M.E. (1990) Riding the glial monorail: A common mechanism for glial-guided neuronal migration in different regions of the developing brain. *Trends Neurosci.*, 13:179–184.
Hinds, J.W. and Ruffett, T.L. (1971) Cell proliferation in the neural tube: An electron microscopic and Golgi analysis in the mouse cerebral vesicle. *Z. Zellforsch. Mikrosk. Anat.*, 115:226–264.
Hirano, M. and Goldman, J.E. (1988) Gliogenesis in rat spinal cord: Evidence for origin of astrocytes and oligodendrocytes from radial precursors. *J. Neurosci. Res.*, 21:155–167.
His, W. (1889) Die Neuroblasten und deren Entstehung im embryonalen Mark. Abhandl. *Math. Phys. Kl. Konigl Sachs. Ges. Wiss.*, 26:313–372.
Hockfield, S. and McKay, R.D.G. (1985) Identification of major classes in the developing mammalian nervous system. *J. Neurosci.*, 5:3310–3328.
Holt, C.E., Bertsch, T.W., Ellis, H.M., and Harris, W.A. (1988) Cellular determination in the Xenopus retina is independent of lineage and birth date. *Neuron*, 1:15–26.
Houle, J. and Fedoroff, S. (1983) Temporal relationship between the appearance of vimentin and neural tube development. *Dev. Brain Res.*, 9:189–195.
Ingraham, C.A. and McCarthy, K.D. (1989) Plasticity of process-bearing glial cell cultures from neonatal rat cerebral cortical tissue. *J. Neurosci.*, 9:63–69.
King, J.S. (1966) A comparative investigation of neuroglia in representative veretebrates: A silver carbonate study. *J. Morphol.*, 119:435–466.
Knapp, P.E., Bartlett, W.P., and Skoff, R.P. (1987) Cultured oligodendrocytes mimic in vivo phenotypic characteristics: Cell shape, expression of myelin-specific antigens, and membrane production. *Dev. Biol.*, 120:356–365.
Lajtha, L.G. (1979) Stem cell concepts. *Differentiation*, 14:23–34.
Latchman, D.S. (1990) Eukaryotic transcription factors. *Biochem J.*, 270:281–298.
Leber, S.M., Breedlove, S.M., and Sanes, J.R. (1990) Lineage, arrangement, and death of clonally related motoneurons in chick spinal cord. *J. Neurosci.*, 10:2451–2462.
Lee, K.-H., Bowen-Pope, D.F., and Reed, R.R. (1990) Isolation and characterization of the α platelet-derived growth factor receptor from rat olfactory epithelium. *Mol. Cell Biol.*, 10:2237–2246.
Lee, M.K., Tuttle, J.B., Rebhun, L.I., Cleveland, D.W., and Frankfurter, A. (1990) The expression and posttranslational modification of a neuron-specific B-tubulin isotype during chick embryogenesis. *Cell Motil. Cytoskel.*, 17:118–132.
Lendahl, U., Zimmerman, L.B., and McKay, R.D.G. (1990) CNS stem cells express a new class of intermediate filament protein. *Cell*, 60:585–595.

Levi, G., Aloisi, F., and Wilkins, G.P. (1987) Differentiation of cerebellar bipotential glial precursors into oligodendrocytes in primary culture: Developmental profile of surface antigens and mitotic activity. *J. Neurosci. Res.*, 18:407–417.

Levi, G., Gallo, V., and Ciotti, M.T. (1986) Bipotential precursors of putative fibrous astrocytes and oligodendrocytes in rat cerebellar cultures express distinct surface features and "neuron-like" gamma-aminobutyric acid transport. *Proc. Natl. Acad. Sci. U.S.A.*, 83:1504–1508.

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

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

Levine, J. (1989) Neuronal influences on glial progenitor cell development. *Neuron*, 3:103–113.

Levitt, P. and Rakic, P. (1980) Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing rhesus monkey brain. *J. Comp. Neurol.*, 193:815–840.

Levitt, P., Cooper, M.L., and Rakic, P. (1981) Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: An ultrastructural immunoperoxidase analysis. *J. Neurosci.*, 1:27–39.

Levitt, P., Cooper, M.L., and Rakic, P. (1983) Early divergence and changing proportions of neuronal and glial precursor cells in the primate cerebral ventricular zone. *Dev. Biol.*, 96:472–484.

Liepelt, U., Kindler-Rohrborn, A., Lennartz, K., Reinardt-Maelicke, S., and Rajewsky, M.F. (1990) Differentiation potential of a monoclonal antibody-defined neural progenitor cell population isolated from prenatal rat brain by fluorescence-activated cell sorting. *Dev. Brain Res.*, 51:267–278.

Liesi, P., Dahl, D., and Vaheri, A. (1983) Laminin is produced by early rat astrocytes in primary culture. *J. Cell Biol.*, 96:920–924.

Liesi, P., Kirkwood, T., and Vaheri, A. (1986) Fibronectin is expressed by astrocytes cultured from embryonic and early postnatal rat brain. *Exp. Cell Res.*, 163:175–185.

Lillien, L.E., Sendtner, M., Rohrer, H., Hughes, S.M., and Raff, M.C. (1988) Type-2 astrocyte development in rat brain cultures is initiated by a CNTF-like protein produced by type-1 astrocytes. *Neuron*, 1:485–494.

Lillien, L.E. and Raff, M.C. (1990a) Analysis of the cell-cell interaction that control type-2 astrocyte development in vitro. *Neuron*, 4:525–534.

Lillien, L.E. and Raff, M.C. (1990b) Differentiation signals in the CNS: Type-2 astrocyte development in vitro as a model system. *Neuron*, 5:111–119.

Lillien, L.E., Sendtner, M., and Raff, M.C. (1990) Extracellular matrix-associated molecules collaborate with ciliary neurotropic factor to induce type-2 astrocyte development. *J. Cell Biol.*, 111:635–644.

Luskin, M.B., Pearlman, A.L., and Sanes, J.R. (1988) Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. *Neuron*, 1:635–647.

McCarthy, G.F. and Leblond, C.P. (1988) Radioautographic evidence for slow astrocyte turnover and modest oligodendrocyte production in the corpus callosum of adult mice infused with ^{3}H-thymidine. *J. Comp. Neurol.*, 271:589–603.

McConnell, S.K. (1989) The determination of neuronal fate in the cerebral cortex. *Trends Neurosci.*, 12:342–349.

McKay, R.D.G. (1989) The origins of cellular diversity in the mammalian central nervous system. *Cell*, 58:815–821.

Meller, K. (1987) Gradient isolation of glial cells: Evidence that flat epithelial cells are astroglial cell precursors. *Cell Tissue Res.*, 249:79–88.

Melton, D.A. and Whitman, M. (1989) Growth factors in early embryogenesis. *Annu. Rev. Cell Biol.*, 5:93–117.

Metcalf, D. (1989) The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature*, 339:27–30.

Miller, R.H. and Raff, M.C. (1984) Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. *J. Neurosci.*, 4:585–592.

Miller, R.H., David, S., Patel, R., Abney, E.R., and Raff, M.C. (1985) A quantitative immunohistochemical study of macroglial cell development in the rat optic nerve: In vivo evidence for two distinct astrocyte lineages. *Dev. Biol.*, 111:35–41.

Misson, J.-P., Edwards, M.A., Yamamoto, M., and Caviness, V.S. (1988a) Mitotic cycling of radial glial cells of the fetal murine cerebral wall: A combined autoradiographic and immunohistochemical study. *Dev. Brain Res.*, 38:183–190.

Misson, J.-P., Edwards, M.A., Yamamoto, M., and Caviness, V.S. (1988b) Identification of radial glial cells within the developing murine central nervous system: Studies based upon a new immunohistochemical marker. *Dev. Brain Res.*, 44:95–108.

Mitchell, P.J. and Tjian, R. (1989) Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science*, 245:371–378.

Noble, M., Murray, K., Stroobant, P., Waterfield, M.D., and Riddle, P. (1988) Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/Type-2 astrocyte progenitor cell. *Nature*, 333:560–562.

Norton, W.T. and Farooq, M. (1989) Astrocytes cultured from mature brain derive from glial precursor cells. *J. Neurosci.*, 9:769–775.

Norton, W.T., Farooq, M., Chiu, F.-C., and Bottenstein, J.E. (1988) Pure astrocyte cultures derived from cells isolated from mature brain. *Glia*, 1:403–414.

Olson, E.N. (1990) MyoD family: A paradigm for development. *Genes Dev.*, 4:1454–1461.

Omlin, F.X. and Waldmeyer, J. (1989) Differentiation of neuron-like cells in cultured rat optic nerves: A neuron or common neuron-glia progenitor. *Dev. Biol.*, 133:247–253.

Onteniente, B., Kimura, H., and Maeda, T. (1983) Comparative study of the glial fibrillary protein in vertebrates by PAP immunohistochemistry. *J. Comp. Neurol.*, 215:427–436.

Pixley, S.K.R. and De Vellis, J. (1984) Transition between immature radial glia and mature astrocytes studied with a monoclonal antibody to vimentin. *Dev. Brain Res.*, 15:201–209.

Price, J. (1987) Retrovirus and the study of cell lineage. *Development*, 101:409–419.

Price, J. and Hynes, R.D. (1988) Astrocytes in culture synthesize and secrete a varient form of fibronectin. *J. Neurosci.*, 5:2205–2211.

Price, J. and Thurlow, L. (1988) Cell lineage in the rat cerebral cortex: A study using retroviral-mediated gene transfer. *Development*, 104:473–482.

Prieto, A.L., Jones, F.S., Cunningham, B.A., Crossin, K.L., and Edelman, G.M. (1990) Localization during development of alternatively spliced forms of cytotactin mRNA by in situ hybridization. *J. Cell Biol.*, 111:685–698.

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

Quesenberry, P. and Levitt, L. (1979a) Hematopoietic stem cells (first of three parts). *N. Engl. J. Med.*, 301:755–760.

Quesenberry, P. and Levitt, L. (1979b) Hematopoietic stem cells (second of three parts). *N. Engl. J. Med.*, 301:819–823.

Raff, M.C. (1989) Glial cell diversification in the rat optic nerve. *Science*, 243:1450–1455.

Raff, M.C., Abney, E.R., Cohen, J., Lindsay, R., and Noble, M. (1983a) Two types of astrocytes in cultures of developing rat white matter: Differences in morphology, surface gangliosides, and growth characteristics. *J. Neurosci.*, 6:1289–1300.

Raff, M.C., Abney, E.R., and Miller, R.H. (1984) Two glial cell lineages diverge prenatally in rat optic nerve. *Dev. Biol.*, 106:53–60.

Raff, M.C., Miller, R.H., and Noble, M. (1983b) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. *Nature*, 303:390–396.

Raff, M.C., Mirsky, R., Fields, K.L., Lisak, R.P., Dorfman, S.H., Silberberg, D.H., Gregson, N.A., Liebowitz, S., and Kennedy, M.C. (1978) Galactocerebroside is a specific cell surface antigenic marker for oligodendrocytes in culture. *Nature*, 274:813–816.

Raff, M.C., Lillien, L.E., Richardson, W.D., Burne, J.F., and Noble, M.D. (1988) Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature*, 333:562–565.

Rakic, P. (1971) Neuron-glia relationship during granule cell migration in developing cerebellar cortex: A Golgi and electron microscopic study in Macacus rhesus. *J. Comp. Neurol.*, 141:283–312.

Rakic, P. (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.*, 145:61–84.

Rakic, P. (1974) Neurons in the monkey visual cortex: Systematic relation between time of origin and eventual disposition. *Science*, 183:425–427.

Rakic, P. (1981) Neuron-glia interaction during brain development. *Trends Neurosci.*, 4:184–187.

Rakic, P. (1984) Emergence of Neuronal and glial cell lineages in primate brain. In: *Cellular and Molecular Biology of Neuronal Development*. I.B. Black, ed. Plenum Press, New York, pp. 29–50.

Rakic, P. (1985) Limits of neurogenesis in primates. *Science*, 227:1054–1056.

Rakic, P. (1988) Specification of cerebral cortical areas. *Science*, 241:170–176.

Reichenbach, A. (1989) Attempt to classify glial cells by means of their

process specialization using the rabbit Muller cell as an example of cytotopographic specialization of glial cells. *Glia*, 2:250–259.
Reynolds, R. and Wilkin, G.P. (1988) Development of macroglial cells in rat cerebellum. II. An in situ immunohistochemical study of oligodendroglial lineage from precursor to mature myelinating cell. *Development*, 102:409–425.
Richardson, W.D., Pringle, N., Mosley, M.J., Westermark, B., and Dubois-Dalcq, M. (1988) A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell*, 53:309–319.
Richmann, M., Amaral, D.G., and Cowan, W.M. (1987) Organization of radial glial cells during the development of the rat dentate gyrus. *J. Comp. Neurol.*, 264:449–479.
Robinson, S.R. and Dreher, Z. (1990) Muller cells in adult rabbit retinae: Morphology, distribution and implications for function and development. *J. Comp. Neurol.*, 292:178–192.
Rosner, H., Al-Aqtum, M., and Henke-Fahle, S. (1985) Developmental expression of G_{D3} and polysialoganglioside in embryonic chicken nervous tissue reacting with monoclonal antiganglioside antibodies. *Dev. Brain Res.*, 18:85–95.
Ryder, E.F., Walsh, C., and Cepko, C.L. (1988) A common precursor for astrocytes and oligodendrocytes. *Soc. Neurosci. Abstr.*, 14:892.
Ryder, E.F., Snyder, E.Y., and Cepko, C.L. (1990) Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J. Neurobiol.*, 21:356–375.
Sauer, F.C. (1935) The cellular structure of the neural tube. *J. Comp. Neurol.*, 63:13–23.
Sauer, M.E. and Chittenden, A.C. (1959) Deoxyribonucleic acid content of cell nuclei in the neural tube of the chick embryo: Evidence for intermitotic migration of nuclei. *Exp. Cell Res.*, 16:1–6.
Schachner, M., Kim, S.K., and Zehnle, R. (1981) Developmental expression in central and peripheral nervous system of oligodendrocyte cell surface antigens (O antigens) recognized by monoclonal antibodies. *Dev. Biol.*, 83:328–338.
Schaper, A. (1897) The earliest differentiation in the central nervous system of vertebrates. *Science*, 5:430–431.
Schmechel, D.E. and Rakic, P. (1979a) Arrested proliferation of radial glial cells during midgestation in rhesus monkey. *Nature*, 277:303–305.
Schmechel, D.E. and Rakic, P. (1979b) A Golgi study of radial glial cells in developing monkey telencephalon: Morphogenesis and transformation into astrocytes. *Anat. Embryol.*, 156:115–152.
Sidman, R.L., Maile, I.L., and Feder, N. (1959) Cell proliferation in the primiative ependymal zone: An autoradiographic study of histogenesis in the nervous system. *Exp. Neurol.*, 1:322–333.
Skoff, R.P. (1990) Gliogenesis in rat optic nerve: Astrocytes are generated in a single wave before oligodendrocytes. *Dev. Biol.*, 139:149–168.
Small, R.K., Riddle, P., and Noble, M. (1987) Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature*, 328:155–157.
Sobue, G. and Pleasure, D. (1984) Astroglia proliferation and phenotype are modulated by neuronal plasma membrane. *Brain Res.*, 213:351–364.
Sommer, I. and Schachner, M. (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: An immunocytological study in the central nervous system. *Dev. Biol.*, 83:311–327.
Sommer, I. and Schachner, M. (1982) Cell that are O4 antigen-positive and O1 antigen-negative differentiate into O1 antigen-positive oligodendrocytes. *Neurosci. Lett.*, 29:183–188.
Sontheimer, H., Trotter, J., Schachner, M., and Kettenmann, H. (1989) Channel expression correlates with differentiation stage during the development of oligodendrocytes from their precursor cells in culture. *Neuron*, 2:1135–1145.
Steindler, D.A., Cooper, N.G.F., Faissner, A., and Schachner, M. (1989) Boundaries defined by adhesion molecules during development of the cerebral cortex: The J1/tenascin glycoprotein in the mouse somatosensory cortical barrel field. *Dev. Biol.*, 131:243–260.
Tapscott, S.J., Bennett, G.S., Toyama, Y., Kleinbart, F., and Holtzer, H. (1981a) Intermediate filament proteins in the developing chick spinal cord. *Dev. Biol.*, 86:40–54.
Tapscott, S.J., Bennett, G.S., and Holtzer, H. (1981b) Neuronal precursor cells in the chick neural tube express neurofilament proteins. *Nature*, 292:836–838.
Temple, S. (1989) Division and differentiation of isolated CNS blast cells in microculture. *Nature*, 340:471–473.
Trotter, J. and Schachner, M. (1989) Cells positive for the O4 surface antigen isolated by cell sorting are able to differentiate into astrocytes or oligodendrocytes. *Dev. Brain Res.*, 46:115–122.
Turner, D.L. and Cepko, C.L. (1987) A common progenitor for neurons and glia persists in rat retina late in development. *Nature*, 328:131–136.
Turner, D.L., Snyder, E.Y., and Cepko, C.L. (1990) Lineage-independent determination of cell type in embryonic mouse retina. *Neuron*, 4:833–845.
Ullrich, A. and Schlessinger, J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell*, 61:203–212.
Vaysse, P.J.-J. and Goldman, J.E. (1990) A clonal analysis of glial lineages in neonatal forebrain development in vitro. *Neuron*, 5:227–235.
Verkhratsky, A.N., Trotter, J., and Kettenmann, H. (1990) Cultured glial precursor cells from mouse cortex express two types of calcium currents. *Neurosci. Lett.*, 112:194–198.
Voigt, T. (1989) Development of glial cells in the cerebral wall of ferrets: Direct tracing of their transformation from radial glial into astrocytes. *J. Comp. Neurol.*, 289:74–88.
Walsh, C. and Cepko, C.L. (1988) Clonally related cortical cells show several migration patterns. *Science*, 241:1342–1345.
Wetts, R. and Fraser, S.E. (1988) Multipotent precursors can give rise to all major cell types of the frog retina. *Science*, 239:1142–1145.
Whetton, A.D. and Dexter, T.M. (1989) Myeloid haemopoietic growth factors. *Biochim. Biophys. Acta*, 989:111–132.
Wilkin, G.P., Marriott, D.R., and Cholewinski, A.J. (1990) Astrocyte heterogeneity. *Trends Neurosci.*, 13:43–46.
Williams, B.P., Abney, E.R., and Raff, M.C. (1985) Macroglial cell development in embryonic rat brain: Studies using monoclonal antibodies, fluorescence activated cell sorting, and cell culture. *Dev. Biol.*, 112:126–134.
Wilkie, M.B. and Lauder, J.M. (1988) Simple method for the culture of glial cells from embryonic rat brain: Implications for regional heterogeneity and the radial glial lineage. *J. Neurosci. Res.*, 21:220–225.
Wolswijk, G. and Noble, M. (1989) Identification of an adult-specific glial progenitor cell. *Development*, 105:387–400.
Wolswijk, G., Riddle, P.N., and Noble, M. (1990) Coexistence of perinatal and adult forms of a glial progenitor cell during development of the rat optic nerve. *Development*, 109:691–698.
Wong, D.H., Ignatius, M.J., Parosky, G., Parham, P., Trojanowski, J.Q., and Brodsky, F.M. (1990) Neuron-specific expression of high-molecular-weight clathrin light chain. *J. Neurosci.*, 10:3025–3031.