COMMENTARY

Characteristics of cells that give rise to the central nervous system

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Introduction

Neural induction by the axial mesoderm designates a population of embryonic ectoderm cells to give rise to the central nervous system (CNS). The cells of the neural rudiment are overtly similar and are distributed in a single cell layer that folds up to become the neural tube. The nuclei of the neural tube cells undergo a 'to and fro' migration in synchrony with their cell cycles, and the mitotic phase occurs at the border of the neural tube lumen that comprises the early ventricular system (Sauer, 1935). As development proceeds, neurons and glioblasts migrate away from this ventricular zone and begin to form the mature CNS. Different classes of CNS cells arise on well-defined time-schedules (see Jacobson, 1978, for a review) and the precise timing of cell differentiation is likely to be important in generating CNS cytoarchitecture and appropriate cell–cell interactions.

One of the most intriguing questions in developmental biology is how do the early neuroectodermal cells generate the vast diversity of neuronal and glial cell types? In order to understand this process we have studied CNS progenitor cells, the immature cells that give rise to the final CNS cell complement. Because CNS progenitor cells are transient and exist in a complex and relatively inaccessible cellular environment – the developing CNS – the following basic questions about the biology of these cells are only partially answered. (1) What are the phenotypes of CNS progenitor cells? (2) What factors control the generation of diverse cell types from CNS progenitor cells? (3) What factors control CNS progenitor cell division? (4) What factors control the timing of CNS progenitor cell differentiation?

Recent advances in the ability to identify and follow CNS progenitor cells *in vivo* and *in vitro* are providing new information about the characteristics of CNS progenitor cells. This paper reviews our present understanding of the characteristics of CNS progenitor cells, concentrating on the four questions stated above, and points out directions that this field is taking.

Are there different types of CNS progenitor cells: what are their phenotypes?

Probably all the neurons and at least some early glioblasts are generated in the germinal zones of the developing nervous system. These are areas of dividing cells that are located in characteristic places in the developing CNS. The ventricular zone is the major germinal zone, running the length of the neural tube. Are there different types of progenitor cells in the CNS germinal zones, or are the cells equivalent? I am going to describe briefly some CNS progenitor cells that have been identified: (1) by studying the appearance of cells in germinal zones *in vivo*; (2) by following the development of neural cells *in vitro*; and (3) by analysing the clonal progeny of progenitor cells marked *in vivo*. Although the list of identified CNS progenitor cells is short, it suggests that there are distinct types of CNS progenitor cell with differing developmental potentials. At this stage it is not clear how the identified CNS progenitor cells relate to one another; probably more progenitor cell types need to be studied to clarify this issue. This is discussed more fully below.

Appearance of cells in germinal zones in vivo

In vivo, ventricular zone cells are columnar, joined at the ventricular margin and span the width of the developing CNS to contact the pial margin, except during mitosis when they round up at the ventricular surface. Ventricular zone cells have been shown to express a number of molecules, including the adhesion molecules N-CAM (see Rutishauser and Jessell, 1988), N-cadherin (Hatta *et al.* 1987), fibronectin and the fibronectin receptor (Stallcup *et al.* 1989), the gangliosides GD3 (Goldman *et al.* 1984) and D1.1 (Levine *et al.* 1984) and the intermediate filament proteins vimentin (Traub, 1985) and nestin (Hockfield and McKay, 1985; Lendahl *et al.* 1990). Cells of the ventricular zone have been described as 'overtly homogeneous', i.e. having no obvious detectable differences that might indicate that there are sub-types of progenitor cells (Fujita, 1963; Jacobson, 1978). However, in the monkey, antibodies to glial fibrillary acidic protein (GFAP), label some cerebral cortical ventricular zone cells but not others. The expression of GFAP is diagnostic for astrocytes, and the fact that a sub-population of ventricular zone cells express GFAP led to the suggestion that there are distinct neuronal and glial precursor cells in the monkey cerebral cortical ventricular zone (Levitt *et al.* 1981).

One of the earliest cell types to emerge from the ventricular zone is the radial glial cell, a transitory, elongated cell that spans the width of the neural tube and guides neurons during their migrations from the ventricular zone. Radial glial cells have been shown to divide and to exhibit the to and fro nuclear migration of ventricular zone cells during division (Misson *et al.* 1988). During development, cells arise that are intermediate in morphology between radial glial cells and astrocytes, sugges-

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ting that radial glial cells are progenitor cells for astrocytes (Schmechel and Rakic, 1979). More recently, evidence has been presented that radial glial cells may also be precursors for neurons (Frederiksen and McKay, 1988; McKay, 1989). An antibody, 'Rat 401', labels a new intermediate filament protein 'nestin' in neuroepithelial cells (Lendahl *et al.* 1990). Rat 401 begins to be expressed in the developing rat nervous system at around E10. By E11–12 approximately 98% of neural cells express this antibody. After E12 the proportion of cells expressing Rat 401 declines, and the fall correlates with the differentiation of neurons (Hockfield and McKay, 1985; Frederiksen and McKay, 1988). These results suggest that neuronal progenitor cells are Rat 401 positive. As Rat 401 is expressed in cells with the morphology of radial glial cells (Hockfield and McKay, 1985), there is the possibility that radial glial cells are neuronal progenitor cells. However, this depends on the relative proportion of Rat 401-positive cells that are radial glial cells, and this may be difficult to ascertain without a specific marker for radial glial cells.

Development of neural cells in vitro

Cell culture systems allow progenitor cells to be identified and followed as they divide and differentiate, which is not yet possible in the complex cellular environment of the developing mammalian CNS. Recently a culture method was described that permits the clonal analysis of neuronal and glial progenitor cells from a number of regions of embryonic rat forebrain (Temple, 1989). Clones from the developing septal region have been described in the most detail.

At embryonic day 13.5–14.5 (E13.5–14.5), the septal region of the rat forebrain contains a part of the ventricular zone that continues to generate neurons and glial precursor cells for at least 7 days (Bayer, 1979*a*,*b*). When the E13.5–14.5 septal region is dissociated and single cells are plated into culture wells, blast cells can be identified and followed as they give rise to clones of neurons and glial cells (Temple, 1989).

The morphology of septal blast (progenitor) cells in monolayer culture is clearly distinct from that of neurons and glial cells. The septal blast cells are flattened, often have a triangular or box-like profile and usually have prominent phase-dark granules in the cytoplasm. Although these cells are unlike mature CNS cells in culture, they bare a distinct resemblance to other neuroectodermal cells in culture, such as those from chick (Keane *et al.* 1984).

Three major types of progenitor cell are consistently identified in E13.5–14.5 septal cultures. One appears to have a restricted capacity for division and differentiation, giving rise to small, pure clones of either neurons or glial cells. The second also has a limited capacity for division, but is multipotential, giving rise to both neurons and glial cells. The third type appears to have more extensive proliferative capacity and to be multipotential, as it gives rise to large, mixed clones of neurons and glial cells. This latter type of progenitor cell may be a stem cell (defined as a cell that is capable of asymmetric divisions giving rise to a differentiated cell and another stem cell), as the large clones still contained cells with the phenotype of progenitor cells, even after long periods of culture.

No obvious morphological differences were detected that might distinguish these three types of septal progenitor cells, re-affirming that the similar morphology of ventricular zone cells might belie differences in their developmental potentials. These septal blast cells also do not stain with anti-GFAP; perhaps the expression of GFAP in progenitor cells varies between species or in different regions of the ventricular zone. Markers that might define the different types of septal progenitor cell remain to be identified.

Neuronal progenitor cells have also been identified in cultures of the mammalian olfactory epithelium. This epithelium gives rise to a single type of neuron, the olfactory receptor neuron, and it has the ability to regenerate neurons throughout adult life (Graziadei and Monti Graziadei, 1978). In culture, flat olfactory epithelial cells from postnatal rats can give rise to rounded neuronal precursor cells that divide and produce post-mitotic neurons exhibiting action potentials (Schubert *et al.* 1985; Calof and Chikaraishi, 1989). In the mouse, the flat epithelial cells express keratin but not N-CAM, the rounded neuronal precursor cells do not express either marker, and the differentiating olfactory neurons gain N-CAM, Gap-43 and OMP (a cytoplasmic protein characteristic of mature olfactory neurons; Calof and Chikaraishi, 1989).

Two major types of glial progenitor cell have been identified in CNS cultures. One is the O-2A progenitor cell that gives rise to two types of glial cell in cultures of optic nerve: oligodendrocytes and a fibrous 'type-2' astrocyte (for review, see Raff, 1989). An analogous cell type has been described in cultures of rat cerebral cortex (Ingraham and McCarthy, 1989) and cerebellum (Levi *et al.* 1986; Levine and Stallcup, 1987). The O-2A progenitor cell is bipolar and expresses a surface ganglioside recognised by the antibody A2B5. There is evidence that the O-2A progenitor cell migrates into the optic nerve during development (Small *et al.* 1987). It has been suggested that the majority of glial cells originate from glioblasts that are born in the ventricular zone but then migrate away from the germinal zone and populate areas of the CNS by dividing *in situ* as the region develops (see Jacobson, 1978). It is possible that the O-2A progenitor cell is such a migratory glioblast.

Recently, an adult form of the O-2A progenitor cell has been described that differs from the O-2A progenitor cell present during development in terms of its morphology, antigenic phenotype, slower cell cycle time, slower migration rate and more rapid differentiation into either astrocytes or oligodendrocytes *in vitro* (Wolswijk and Noble, 1989).

The other major type of glial progenitor cell identified in CNS cultures is the precursor to the flattened 'type-1' astrocyte. Type-1 astrocytes are common in cultures from a variety of CNS regions and precursors for these cells appear to be flattened, GFAP-negative cells (Fischer *et al.* 1982; Goldman *et al.* 1986; Norton and Farooq, 1989) that in the rat express the ganglioside GD3 (Goldman *et al.* 1986).

Analysis of clonal progeny of progenitor cells marked in vivo

In the past few years new methods of cell marking, especially with stable genetic markers, have been applied to the study of CNS development. It is now possible to mark CNS precursor cells *in vivo* with genes carried by retroviral vectors (reviewed by Price, 1987; Cepko, 1988; Sanes, 1989). The marker gene is passed on to the blast cell progeny and the resultant clone can be visualised by histochemical detection of the marker gene product. The major types of progenitor cells that have been described in these studies are outlined in this section.

In the rodent and amphibian retina the precursor cell population appears to be multipotential even until late stages of development (Turner and Cepko, 1987; Wetts and Fraser, 1988; Holt *et al.* 1988). There is no clear evidence for retinal progenitor cells with restricted developmental potentials, such as 'rod cell progenitors' or 'ganglion cell progenitors', instead clones are very heterogeneous in terms of cell number and the types of cells they contain. In the rodent cerebral cortex (Luskin *et al.* 1988; Price and Thurlow, 1988; Walsh and Cepko, 1988) the progenitor population at around E14 appears to contain progenitor cells that are somewhat developmentally restricted, at least in terms of neuronal/glial lineages, as the majority of clones are purely neuronal or glial in their composition. The neuronal clones are, however, heterogeneous in containing a number of types of neuron, i.e. there is still no evidence for neuronal progenitor cells in the cerebral cortex that are restricted to give rise to just one or two neuronal types. In the chick tectum at E3 there is evidence both for progenitor cells that are restricted to give rise to only neurons or glial cells and for progenitor cells that can give rise to neurons and glia. Again, most clones in the optic tectum contain more than one type of neuron (Gray *et al.* 1988; Galileo *et al.* 1990).

In summary, although ventricular zone cells *in vivo* have similar morphologies, it is clear from a variety of approaches that progenitor cells identified in particular CNS areas and at particular developmental ages have distinguishing characteristics. This suggests that there are different types of CNS progenitor cell with differing developmental potentials in CNS germinal zones. How do CNS regions acquire appropriate populations of progenitor cells? What are the lineage relationships of CNS progenitor cells in a given CNS region and what factors control the choices in these lineage pathways? These issues will be discussed in the next section.

What factors control the generation of diverse cell types from CNS progenitor cells?

There is evidence that the regional differences between areas of the brain are established very early, perhaps at the same time as, or shortly after, neural induction (see Slack, 1983; Saxen, 1982–83). The mechanisms underlying this process are not yet understood. The lineage relationships of cells within a developing CNS area are beginning to be described, but not enough information is available to make more than speculations about associations between progenitor cells. The fact that there are some progenitor cells that can give rise to both neurons and glial cells and others that can give rise to only neurons or glial cells raises the possibility that the former cell type gives rise to the latter two cell types. This hypothesis seems especially plausible in areas such as the E13.5–14.5 rat septal region and the E3 chick optic tectum where there is evidence for the co-existence of multipotential, neuronal/glial progenitor cells and 'restricted' neuronal or glial progenitor cells. There is additional evidence that multipotential CNS progenitor cells may give rise to neuronal or glial progenitor cells, from analysis of septal clone composition. Because neurons are post-mitotic (i.e. they cannot divide), their numbers in clones reflect the proliferative patterns of the neuroblasts that gave rise to them. In mixed septal clones from multipotential, neuronal/glial progenitor cells, neuronal numbers cluster around a factor of 2, e.g. (2, 4, 8, 16) suggesting that they arose from symmetric, proliferative divisions. That is, it is likely that at some point in the development of the clone, a restricted progenitor cell was produced that gave rise to neurons only. There is evidence for stem cells releasing progenitor cells with limited proliferative capacities in a number of other vertebrate systems, including the myogenic lineage (Quinn *et al.* 1985), the erythroid lineage (Gusella *et al.* 1976) and the *Xenopus* lateral line system (Winklbauer and Hausen, 1983).

In the rodent cerebral cortex there is no evidence as yet for multipotential, neuronal/glial progenitor cells. It is possible that in this area these two lineages diverged prior to the earliest time studied. Alternatively, there may not be a common progenitor for neurons and glial cells in the cerebral cortical ventricular zone.

Although this scheme of restriction of developmental potential according to cell type may account for the divergence of neurons and glial cells in the CNS, it does not appear to account for the generation of different types of neurons. In all CNS areas studied so far, clones that contain neurons are in general heterogeneous; there is no evidence for neuronal progenitor cells that are only able to give rise to one or two cell types. Another mechanism must be proposed to account for the generation of different classes of CNS neurons. One prominent current hypothesis is that the environment is the key to neuronal determination. This mechanism was proposed to account for retinal development, and is discussed below.

In the vertebrate retina, multipotential progenitor cells for neurons and Muller glia are present even up to postnatal ages. The lack of consistent patterns in retrovirally marked clonal progeny has led to the hypothesis that some multipotential CNS progenitor cells, such as those in the retina, give rise to different cell types according to the demands of the environment (Turner and Cepko, 1987; Wetts and Fraser, 1988; Holt *et al.* 1988; Price, 1989). The development of the vertebrate retina has been likened to that of *Drosophila*, in which undetermined ommatidial precursors are drawn into an orderly crystalline array of cells and their final position leads to commitment down a particular lineage pathway (reviewed by Ready, 1989). In this analogy, vertebrate multipotential progenitor cells would throw off undetermined retinal precursor cells that would migrate away from the germinal zone and differentiate according to the final position they attain in the emerging retinal layers. Although in this model the environment acts on the cell after migration, it is possible that it acts before migration, even before the final mitosis. There is evidence that at least some retinal cell types are determined before they migrate to their final positions; for example, the antibody HCP-1 labels amacrine cells in the retina and is expressed on pre-migratory amacrine cells (Barnstable *et al.* 1985).

At this point I will discuss further the environmental control of CNS cell fate. Our concept of cell-intrinsic mechanisms of determination are based largely on studies of invertebrate systems, such as the nematode worm *Caenorhabditis elegans* (review, Sternberg and Horvitz, 1984). The signature of cell-intrinsic mechanisms that regulate cell determination in these systems is the reiterated lineage pattern. That is, the same patterns of division are seen to lead to the same cell in different individuals or in repeated segments of the same organism. There is no evidence of reiterated patterns of progeny in the retroviral studies of vertebrate CNS; the marked heterogeneity of CNS clones has thus been the prime piece of evidence that led researchers away from the idea that a

cell-intrinsic mechanism could be at work. However, it is possible that cell intrinsic mechanisms of cell determination in vertebrate systems, where there are orders of magnitude more cells, may be different from those of invertebrate systems and may be compatible with the observation of heterogeneous clones. For example, let us say that during retinal development there are multipotential progenitor cells that can give rise to all the retinal cell types, but the probability of giving rise to a particular cell type changes with time according to a cell-intrinsic mechanism. Early in development, the probability of giving rise to ganglion cells would be highest and to photoreceptor cells lowest, but as development proceeds the reverse would become true. In such a system, individual multipotential progenitor cells would give rise to heterogeneous clones according to the time-dependance of the cell-intrinsic, stochastic process, but the overall progenitor population will show an ordered restriction in developmental potential. Models based on stochastic controls of cell proliferation have been put forward to explain the senescence of fibroblast cultures (Smith and Whitney, 1980) and, to speculate further, if a stochastic mechanism also controlled some aspects of cell division potential, the eventual decay that occurs in most CNS germinal zones could be explained. Clearly, it is possible that there are CNS progenitor cell types that are intrinsically restricted in their developmental potentials and proliferative capacities, even though they give rise to heterogeneous clones. It will be interesting to examine whether CNS progenitor cell clones grown in standardised culture environments are heterogeneous or homogeneous in the types of neurons they contain.

A purely cell-intrinsic mechanism for controlling CNS cell fate seems unlikely as there are clear examples of the ability of the developing CNS to compensate for experimentally induced deficits. For example, in the frog, depletion of central retinal dopaminergic amacrine cells after application of 6-hydroxydopamine, leads to a compensatory increased production of dopaminergic amacrine cells from the ciliary margin, suggesting that retinal precursor cells are able to respond to environmental cues that regulate final cell number (Reh and Tully, 1976). There is also evidence from culture studies that the environment can influence the fate of CNS progenitor cells. For example, the O-2A progenitor cell gives rise to oligodendrocytes in serum-free medium, but is stimulated to give rise to type-2 astrocytes if exposed to 10% foetal calf serum (Raff *et al.* 1983) or to the protein ciliary neurotrophic factor (CNTF) (Hughes *et al.* 1988; Lillien *et al.* 1988). Also, rat embryonic retinal progenitor cells exposed to an excess of neonatal retinal cells over-produce photoreceptor cells (cells that are normally produced after birth) (Watanabe and Raff, 1990). Furthermore, it is important to remember that while cell culture experiments may show that a progenitor cell has a particular intrinsic potential, *in vivo* this potential may be influenced by environmental factors. I would favour a model that includes stem cells, restricted progenitor cells and also environmental responsiveness. Different areas of the brain, or different steps in lineage pathways, might have different levels of environmental responsiveness (see Harris and Holt, 1990).

What factors control CNS progenitor cell division?

A number of experiments suggest that cell–cell interactions are important in regulating the proliferation of some CNS progenitor cells. For example, in the mammalian cerebellum several lines of evidence suggest that Purkinje cells regulate the proliferation of external granule cells, the precursors of cerebellar granule cells (reviewed by Williams and Herrup, 1988). Another example comes from an intriguing experiment in which segments of quail hindgut were implanted between the neural tube and somites in chick embryos. In these embryos, the neural tube cells close to the hindgut explant were stimulated to proliferate, showing that neuroepithelial cells are responsive to soluble mitogens (Rothman *et al.* 1987).

The O-2A progenitor cell also requires an environmental stimulus for cell division. Noble and Murray (1984) showed that type-1 astrocytes release a mitogen for O-2A progenitor cells. The mitogen was later identified as platelet-derived growth factor (PDGF) (Richardson *et al.* 1988; Raff *et al.* 1988).

There is evidence that septal progenitor cells also respond to a soluble mitogen. I found that when single E13.5–14.5 septal cells are plated in wells containing medium with 10% fetal calf serum (FCS), the cells survive for long periods but do not divide more than once. If, however, the single cells are plated into wells containing 10% FCS that has been conditioned by embryonic forebrain culture growing on the walls of the wells, then progenitor cells will divide and give rise to CNS clones. This suggests that there is a soluble mitogenic activity released by embryonic forebrain cells that acts on septal blast cells; however, the identity of the mitogenic activity is not known.

Although a mitogen may be needed for division of CNS progenitor cells, the presence of mitogen may not be the only factor regulating division. For example, individual O-2A progenitor cells grown in standardised culture conditions have heterogeneous proliferative capacities. When sister O-2A progenitor cells were plated into separate culture wells they gave rise to clones of similar size, suggesting that O-2A progenitor cells have intrinsic controls that regulate their ability to respond to mitogen (Temple and Raff, 1986). I observed a similar result when single septal cells were plated in standardised culture conditions: clones were heterogeneous in size, suggesting that there may be cell-intrisic controls on septal progenitor cell proliferation (Temple, 1989).

What factors control the timing of differentiation of CNS progenitor cells?

When cells of the embryonic brain are grown in monolayer culture, major classes of CNS cells arise on the same time-course as they do *in vivo* (Abney *et al.* 1981). This suggests that the timing of differentiation of some CNS progenitor cells does not depend on the normal cytoarchitecture of the developing CNS. This has been clearly shown for the O-2A progenitor cell, which can give rise to oligodendrocytes on the same time-course in monolayer culture as it does *in vivo*. The proliferation of the O-2A progenitor cell, driven by mitogen, is required for the normal timing of oligodendrocyte differentiation (Raff *et al.* 1985; Raff *et al.* 1988). There is evidence that cell-intrinsic controls on cell division may be central to the timing mechanism: the behavior of single O-2A progenitor cells is consistent with them having an internal clock that measures time by counting cell divisions (Temple and Raff, 1986; Raff *et al.* 1988).

In contrast, the timing of differentiation of retinal neurons has not yet been reconstituted in monolayer cultures. When retinal cells are grown in monolayer culture they differentiate prematurely and give rise to different cell types depending on, and appropriate to, the age of the animal from which they had been taken: embryonic retinal precursor cells produce ganglion cells rather than rods, neonatal precursors produce rods rather than ganglion cells (Reh and Klajavin, 1989). This suggests that retinal progenitor cells respond to temporally regulated factors present in the developing retina prior to dissociation, but that the dissociated cells are unable to reconstruct the normal sequence of development in the monolayer culture. If, however, retinal cells are grown in aggregate or explant cultures that maintain some aspects of the *in vivo* retinal cytoarchitecture, retinal cell types differentiate in the same sequence as they do *in vivo* (Sparrow *et al.* 1990). This suggests that cell interactions are important to the timing of retinal cell differentiation, but there may also be a cell-intrinsic component to the mechanism. Exposing embryonic retinal precursor cells to neonatal retinal cells in aggregate cultures does stimulate the production of rods, demonstrating that the embryonic progenitor cells are environmentally responsive. However, the timing of rod cell differentiation is not altered from normal (Watanabe and Raff, 1990). Perhaps, as suggested for the timing of differentiation of O-2A progenitor cells, the environmental factors that regulate timing of neuronal differentiation in the retina are permissive, allowing the expression of a cell-intrinsic clock. To speculate further, rodent retinal cells do not continue to proliferate in these monolayer cultures (Reh and Kljavin, 1989; Sparrow *et al.* 1990), whereas they do in these explant cultures; perhaps the maintenance of progenitor cell division may also be key for the normal timing of differentiation of retinal cells.

What are the next steps in our study of CNS progenitor cells?

Now that CNS progenitor cells and their progeny are becoming accessible to study *in vitro* and *in vivo*, we are in a position to characterize progenitor cells over the span of developmental time and in different CNS regions. It seems likely that a large number of progenitor cell types will emerge, and it will be an exciting challenge to characterize these cells. Clonal development in culture can be followed over time to reveal the precise lineage relationships of cells in a clone and to provide insight into the behavior of CNS progenitor cells as they divide and differentiate. It should be possible to define mitogens for CNS progenitor cells and the cells that produce these mitogens, and to investigate environmental and cell-intrinsic factors that regulate lineage choices. Just as the isolation of hemopoietic progenitor cells was pivotal to elucidating blood cell lineages and the factors regulating the generation of blood cells, we hope that the isolation and characterization of CNS progenitor cells will lead to a greater understanding of how the early neuroectoderm cells generate the vast wealth of CNS cell diversity.

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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. *Devl Biol.* **83**, 301–310.

Barnstable, C. J., Hofstein, R. and Akagawa, K. (1985). A marker of early amacrine cell development in rat retina. *Devl Brain Res.* **20**, 286–290.

Bayer, S. A. (1979*a*). The development of the septal region in the rat. I. Neurogenesis examined with ^{3}H-Thymidine autoradiography. *J. comp. Neurol.* **183**, 89–106.

Bayer, S. A. (1979*b*). The development of the septal region in the rat. II. Morphogenesis in normal and X-irradiated embryos. *J. comp. Neurol.* **183**, 107–120.

Calof, A. L. and Chikaraishi, D. M. (1989). Analysis of neurogenesis in a mammalian neuroepithelium: Proliferation and differentiation of an olfactory neuron precursor *in vitro*. *Neuron* **3**, 115–127.

Cepko, C. (1988). Retrovirus vectors and their applications in neurobiology. *Neuron* **1**, 345–353.

Fischer, G., Levtz, A. and Schachner, M. (1982). Cultivation of immature astrocytes of mouse cerebellum in a serum-free, hormonally defined medium. Appearance of the mature astrocyte phenotype after addition of serum. *Neurosci. Lett.* **29**, 297–302.

Frederiksen, K. and McKay, R. D. G. (1988). Proliferation and differentiation of rat neuroepithelial precursor cells *in vivo*. *J. Neurosci.* **8**(4), 1144–1151.

Fujita, S. (1963). The matrix cell and cytogenesis in the developing central nervous system. *J. comp. Neurol.* **120**, 37–42.

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 chicken optic tectum: Demonstration with two retroviruses and cell type specific antibodies. *Proc. natn. Acad. Sci. U.S.A.* **87**, 458–462.

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.

Goldman, J. E., Hirano, M., Yu, R. K. and Seyfried, T. N. (1984). GD3 ganglioside is a glycolipid characteristic of immature neuroectodermal cells. *J. Neuroimmun.* **7**, 179–192.

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. natn. Acad. Sci. U.S.A.* **85**, 7356–7360.

Graziadei, P. P. C. and Monti Graziadei, G. A. (1978). Continuous nerve cell renewal in the olfactory system. In *Handbook of Sensory Physiology*, vol. IX: *Development of Sensory Systems* (ed. M. Jacobson), pp. 55–83. New York: Springer-Verlag.

Gusella, J., Geller, R., Clarke, B., Weeks, V. and Houseman, D. (1976). Commitment to erythroid differentiation by Friend erythroleukemia cells: A stochastic analysis. *Cell* **9**, 221–229.

Harris, W. A. and Holt, C. E. (1990). Early events in the embryogenesis of the vertebrate visual system: Cellular determination and pathfinding. *A. Rev. Neurosci.* **13**, 155–167.

Hatta, K., Tagaki, S., Fugisawa, H. and Takeichi, M. (1987). Spatial and temporal expression pattern of N-cadherin cell adhesion molecules correlated with morphogenetic processes of chick embryos. *Devl Biol.* **120**, 215–227.

Hockfield, S. and McKay, R. D. G. (1985). Identification of major cell 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.

Hughes, S. M., Lillien, L. E., Raff, M. C., Rohrer, H. and Sendtner, M. (1988). Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. *Nature* **335**, 70–73.

Ingraham, C. A. and McCarthy, K. D. (1989). Plasticity of process-bearing glial cell cultures from neonatal rat cerebral cortical tissue. *J. Neurosci.* **9**(1), 63–69.

Jacobson, M. (1978). *Developmental Neurobiology*, Plenum Press, New York.

Keane, R. W., Libsich, L. A. and Brugge, J. S. (1984). Differentiation and transformation of neural plate cells. *Devl Biol.* **103**, 38–52.

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., 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 GABA transport. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1504–1508.

Levine, J. M., Beasley, L. and Stallcup, W. B. (1984). The D1.1 antigen: A cell surface marker for the germinal cells of the central nervous system. *J. Neurosci.* **4**, 820–831.

LEVINE, J. M. AND STALLCUP, W. B. (1987). Plasticity of developing cerebellar cells *in vitro* studied with antibodies against the NG2 antigen. *J. Neurosci.* **7(9)**, 2721–2731.

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(1)**, 27–39

LILLIEN, L. E., SENDTNER, M., ROHRER, H. 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.

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.

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

MISSON, J.-P., EDWARDS, M. A., YAMAMOTO, M. AND CAVINESS, V. S. JR (1988). Mitotic cycling of radial glial cells of the fetal murine cerebral wall: a combined autoradiographic and immunohistochemical study. *Devl Brain Res.* **38**, 183–190.

NOBLE, M. AND MURRAY, K. (1984). Purified astrocytes promote the *in vitro* division of a bipotential glial progenitor cell. *EMBO J.* **3**, 2243–2247.

NORTON, W. T. AND FAROOQ, M. (1989). Astrocytes cultured from mature brain derive from glial precursor cells. *J. Neurosci.* **9(3)**, 769–775.

PRICE, J. (1987). Retroviruses and the study of cell lineage. *Development* **101**, 409–419.

PRICE, J. (1989). When are neurons specified? *Trends Neurosci.* **12 (8)**, 276–278.

PRICE, J. AND THURLOW, L. (1988). Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. *Development* **104**, 473–482.

QUINN, L. S., HOLZER, H. AND NAMEROFF, M. (1985). Generation of chick skeletal muscle cells in groups of 16 from stem cells. *Nature* **313**, 692–694.

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

RAFF, M. C., ABNEY, E. R. AND FOK-SEANG, J. (1985). Reconstruction of a developmental clock *in vitro*: a critical role for astrocytes in the timing of oligodendrocyte differentiation. *Cell* **42**, 61–69.

RAFF, M. C., LILLIEN, L. E., RICHARDSON, W. D., BURNES, 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.

RAFF, M. C., MILLER, R. H. AND NOBLE, M. (1983). A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature* **303**, 390–396.

READY, D. F. (1989). A multifaceted approach to neural development. *Trends Neurosci.* **12**, 102–109.

REH, T. AND KLJAVIN, I. J. (1989). Age of differentiation determines rat retinal germinal cell phenotype: Induction of differentiation by dissociation. *J. Neurosci.* **9(12)**, 4179–4189.

RICHARDSON, W. D., PRINGLE, N., MOSLEY, M., WESTERMARK, M. AND DUBOIS-DALCQ, M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell* **53**, 309–319.

ROTHMAN, T. P., GERSHON, M. D., FONTAINE-PERUS, M. C. AND LE DOUARIN, N. M. (1987). The effects of back-transplants of the embryonic gut wall on the growth of the neural tube. *Devl Biol.* **124**, 331–346.

RUTISHAUSER, U. AND JESSELL, T. M. (1988). Cell adhesion molecules in Vertebrate neural development *Physiol. Rev.* **68**, 819–857.

SANES, J. R. (1989). Analysing cell lineage with a recombinant retrovirus. *Trends Neurosci.* **12(1)**, 21–28

SAUER, F. C. (1935). Mitosis in the neural tube. *J. comp. Neurol.* **62**, 377–405.

SAXEN, L. (1982–83). Neural induction. *Internat. J. Neurol.* **16–17**, 155–166.

SCHMECHEL, D. E. AND RAKIC, P. (1979). A golgi study of radial glial cells in the developing monkey telencephalon: morphogenesis and transformation into astrocytes. *Anat. Embryol.* **156**, 115–152.

SCHUBERT, D., STALLCUP, W., LACORBIERE, M., KIDOKORO, Y. AND ORGEL, L. (1985). Ontogeny of electrically excitable cells in cultured olfactory epithelium. *Proc. natn. Acad. Sci. U.S.A.* **82**, 7782–7786.

SLACK, J. M. W. (1983). *From Egg to Embryo.* Cambridge University Press.

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.

SMITH, J. R. AND WHITNEY, R. G. (1980). Intraclonal variation in proliferative potential of human diploid fibroblasts: stochastic mechanism for aging. *Science* **207**, 82–84.

SPARROW, J. R., HICKS, D. AND BARNSTABLE, C. J. (1990). Cell commitment and differentiation in explants of embryonic rat neural retina. Comparison with the developmental potential of dissociated retina. *Devl Brain Res.* **51**, 69–84.

STALLCUP, W. B., PYTELA, R. AND RUOSLAHTI, E. (1989). A neuroectoderm-associated ganglioside participates in fibronectin receptor-mediated adhesion of germinal cells to fibronectin. *Devl Biol.* **132**, 212–229.

STERNBERG, P. W. AND HORVITZ, H. R. (1984). The genetic control of cell lineage during nematode development. *A. Rev. Genet.* **18**, 489–524.

TEMPLE, S. (1989). Division and differentiation of isolated CNS blast cells in microculture. *Nature* **340**, 471–473.

TEMPLE, S. AND RAFF, M. C. (1986). Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. *Cell* **44**, 773–779

TRAUB, N. (1985). *Intermediate Filaments: A Review.* Berlin: Springer-Verlag.

TURNER, D. L. AND CEPKO, C. L. (1987). A common progenitor for neurons and glial persists in rat retina late in development. *Nature* **238**, 131–136.

WALSH, C. AND CEPKO, C. L. (1988). Clonally related cortical cells show several migration patterns. *Science* **241**, 1342–1345.

WATANABE, T. AND RAFF, M. C. (1990). Rod photoreceptor development *in vitro*. Intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. *Neuron* **2**, 461–467.

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.

WILLIAMS, R. W. AND HERRUP, K. (1988). The control of neuron number. *A. Rev. Neurosci.* **11**, 423–453.

WINKLBAUER, R. AND HAUSEN, P. (1983). Development of the lateral line system in *Xenopus laevis*. III Cell multiplication and organ formation in the supraorbital system. *J. Embryol. exp. Morph.* **76**, 283–296.

WOLSWIJK, G. AND NOBLE, M. (1989). Identification of an adult-specific glial progenitor cell. *Development* **105(2)**, 387–400.