Mini-Review

Radial Glia Phenotype: Origin, Regulation, and Transdifferentiation

Grazyna Chanas-Sacre,1 Bernard Rogister,1,2 Gustave Moonen,1,2 and Pierre Leprince1*
1Center for Cellular and Molecular Neurobiology, University of Lièges, Liège, Belgium
2Department of Neurology, University of Liège, Liège, Belgium

Radial glial cells play a major guidance role for migrating neurons during central nervous system (CNS) histogenesis but also play many other crucial roles in early brain development. Being among the earliest cells to differentiate in the early CNS, they provide support for neuronal migration during embryonic brain development; provide instructive and neurotrophic signals required for the survival, proliferation, and differentiation of neurons; and may be multipotential progenitor cells that give rise to various cell types, including neurons. Radial glial cells constitute a major cell type of the developing brain in numerous nonmammalian and mammalian vertebrates, increasing in complexity in parallel with the organization of the nervous tissue they help to build. In mammalian species, these cells transdifferentiate into astrocytes when neuronal migration is completed, whereas, in nonmammalian species, they persist into adulthood as a radial component of astroglia. Thus, our perception of radial glia may have to change from that of path-defining cells to that of specialized precursor cells transiently fulfilling a guidance role during brain histogenesis. In that respect, their apparent change of phenotype from radial fiber to astrocyte probably constitutes one of the most common transdifferentiation events in mammalian development. J. Neurosci. Res. 61:357–363, 2000. © 2000 Wiley-Liss, Inc.

Key words: RC2 antibody; GFAP; vimentin; Bergmann glia; astrocyte; neuronal migration

Identification and Organization of Radial Glial System

The existence during development of cells that have a general radial orientation (Ramon y Cajal, 1955) has been established in almost all regions of the developing nervous system. These cells exhibit a characteristic orientation perpendicular to the plane of the growing tissue. Their bipolar somas are located in the ventricular zone. Their short basal processes contact the ventricular surface and terminate by a “pied ventriculaire” or basal foot. The ascending processes elaborate the radial fibers spanning the whole thickness of the cerebral wall and terminate at the pial surface in a bifurcating end foot formation constituting the glial limiting membrane. These radial glial cells (also variously called epithelial cells, radial cells, fetal ependymal cells) establish a specific relationship between their radial fibers and migrating neurons (Rakic, 1972).

Immunocytochemical markers for glial cells have been used to label radial glia but are sometimes present only at late stages of differentiation [e.g. glial fibrillary acidic protein (GFAP) in rodents (Dahl et al., 1985)]. Other markers are not exclusively expressed by immature glia [e.g., vimentin found in some mature astrocytes of mammals and more frequently in lower vertebrates and invertebrates (Pixley and de Vellis, 1984; Meyer et al., 1989; Monzon-Mayor et al., 1990; Oudega and Marani, 1991), or nestin, present in radial glia as well as in multipotential neural stem cells (Hockfield and McKay, 1985; Frederiksen and McKay, 1988; Lendahl and McKay, 1990)]. Other proteins constitute more specific immunocytochemical markers of radial glial cells. Example of these are the antigens recognized by RC1 (Edwards et al., 1990) and RC2 (Misson et al., 1988a) antibodies and the brain lipid-binding protein (BLBP; Feng et al., 1994). RC2 appears to label only cells spanning the cortical mantle during neuronal migration and thus may be the best radial glia marker available (Misson et al., 1988a).

In mammals two main forms of radial glial cells are identified by RC2: bipolar radial glia, spanning the whole thickness of the cerebral wall and monopolar radial glia forms, which have not descending processes but only single process or multiple processes starting from their...
apical pole and terminating in end foot varicosities at the pial surface during the prenatal period (Misson et al., 1988a, 1991). The monopolar cells appear after the bipolar forms. After birth, their ascending process becomes more complex, no longer reaching the limiting membrane at the surface of the cerebrum (Misson et al., 1991).

The radial glia system is not static and its configuration changes during development to accommodate the extensive increases of both thickness and surface of the growing brain (Misson et al., 1988a; Kadhim et al., 1988; Gadisseux et al., 1992). Proliferation, extension of new fibers, organization into fascicles, and translocation of radial glia cell bodies close to novel secondary germinative zones are common features of the radial glia system across the whole developing brain (Misson et al., 1988b, 1991; Takahashi et al., 1990; Gressens and Evrard, 1993).

**RADIAL GLIAL CELLS ORIGIN AND IDENTITY**

To fulfill their role as guide for neuronal migration, radial glial cells must appear early and, indeed, are detected by the RC2 antibody as early as E9 in the pseudostratified neuroepithelium of the mouse spinal cord (Misson et al., 1988a). The generation of these cells from neuroepithelial cells of the primitive neural tube raises the question of their progenitor’s nature. Are the radial glial cells generated from multipotential progenitors or from cells already destined to only produce glial cells?

Studies of radial glial cells lineage by use of retroviral labeling techniques show that, in chicken optic tectum and mammalian striatum, radial glia arise from multipotent progenitors generating clones composed of radial glia, neurons, and astrocytes. These labeled clones generally do not contain more than one radial glial cell that guides the migration of its clonal relatives (Gray and Sanes, 1992; Halliday and Cepko, 1992). In the chick, new cells with distinct radial glia phenotype continue to be produced by multipotent progenitors later in development after the peak of neurogenesis (Gray and Sanes, 1992). This late appearance of a cell with radial glia phenotype could be understood by assuming that the common progenitors are the radial glial cells themselves that assume their differentiated radial glia phenotype only after giving birth to their sibling neurons and astrocytes. The asymmetrical mode of cell division of stem cells, by which a progenitor cell gives rise to a specified neuron or glial cell and another progenitor cell, has been shown to occur in radial glial cells (Kamei et al., 1998) and provides a means to generate clones with multiple cell types and only one radial glial cell. This could happen if, when ceasing to proliferate, the progenitor assumes the radial glia phenotype, as suggested by the analysis of the cell lineage in the chick optic tectum (Gray and Sanes, 1992). Another possibility is that in a first proliferative event an early radial glial cell divides symmetrically into two radial glia and one of them subsequently divides into multiple (non radial glia) progeny. In both cases of early or late generation of the radial glia member in a cell clone, the progenitor is a radial glial cell that assumes its differentiated state before or after producing its progeny. Exit of the proliferation cycle and entry into the differentiation process is a common event that is controlled in progenitor cells undergoing asymmetric division in the ventricular zone by the Notch/Delta signaling system (Artavanis-Tsakonas et al., 1995). Evidence for Notch presence in radial glia has been obtained recently, but no function for this signaling system in these cells has been demonstrated yet (Tanaka et al., 1999).

A recent study shows that, in mice, the expression of the RC2 antigen coincides exactly with that of proliferation markers and nestin in acutely dissociated cells from the telencephalon (Hartfuss et al., 1999), suggesting that all RC2-immunopositive cells have the characteristics of precursor cells. Part of these cells also expresses other radial glial markers such as BLBP and GLAST (Shibata et al., 1997) in a developmentally regulated manner, constituting several sets of radial glia-like precursor cells that differ in their antigenic profile. The possibility that such precursor cell subpopulations are committed to generate different neuronal or glial cell types is now under study by analysis of their progeny (Malatesta et al., 1999). Altogether, this study shows that all the early precursor cell types in mouse telencephalon are RC2-immunoreactive and thus may constitute a multipotential population that is progressively split into different compartments with altered expression of radial glia markers and possibly restricted cellular fates. In other words, primary radial glia may be multipotential progenitors becoming more restricted in their cellular fate during later phases of neurogenesis.

Three further arguments for a progenitor nature of radial glial cells have been proposed. One comes from studies of continued neurogenesis in the brain of adult canary. In some specific locations, radial glia proliferate actively in sites where new neurons are generated, suggesting that radial glia could be the source of these new neurons (Alvarez-Buylla et al., 1990). The second argument relates to the mode of proliferation of radial glia in both birds and mammals. During the cell cycle, their nuclei undergo an interkinetic vertical migration, which is a phenomenon unique to stem cells in all pseudostratified germinative epithelia throughout the body (Misson et al., 1988b). Finally, multipotential cell lines in vitro are labeled by RC1, a specific marker of radial glia (Blass-Kampmann et al., 1997).

**DO STEM CELLS BELONG TO THE RADIAL GLIA LINEAGE?**

The question then arises of the origin of neural stem cells that are found in various locations in the adult nervous system and have been shown to give rise in vitro to all the major cell types of the brain. According to the hypothesis that radial glia could be multipotential progenitor cells, could the adult neural stem cells originate from earlier radial glia and still express radial glia markers? Careful observations of the subventricular zone (SVZ) of the cerebral hemispheres where stem cells reside reveal the presence of nestin but not that of RC2 immunopositivity (Gates et al., 1995). Ventricular zone (VZ) subependymal cells (Chiasson et al., 1999) that are remnants of the highly
proliferative SVZ (Takahashi et al., 1995; Chiasson et al., 1999), and SVZ astrocytes (Doetsch et al., 1999) were identified as cells capable of giving rise to proliferative “neuropheres”, which represent the mode of growth of stem cells in vitro (Reynolds and Weiss, 1992). These two cell types have glial characteristics that could indicate a possible relationship with a radial glia lineage of a multipotential progenitor cell nature.

Astrocytes with stem cell characteristics appear to be more widespread during development. Indeed, even after multiple passages, astrocytes cultured from many different regions of perinatal brains give rise to neurospheres containing multipotential progenitors (Laywell et al., 1999). This ability is lost by astrocytes obtained from all brain regions (except from the SVZ) after postnatal day 11, at roughly the time when all radial glial cells have disappeared. Could this mean that radial glial cells are the ones that are multipotential precursor cells and that they loose this property in most regions when they have completed their transformation into astrocytes?

**RADIAL GLIAL CELLS LINEAGE AND TRANSDIFFERENTIATION**

In mammals, many radial glial cells seem to undergo a dramatic change in phenotype and function, transforming them into mature astrocytes in the perinatal period after completion of neuronal migration (Cameron and Racik, 1991; Misson et al., 1991). There are several arguments in favor of the radial glia-to-astrocyte transformation hypothesis. From an ontogenetic point of view, the period of diminution of the radial glial population coincides with the appearance of astrocytes (Choi and Lapham, 1978; Levitt and Racik, 1980). A second argument is the presence of cells displaying transitional, monopolar forms between bipolar radial glia and multipolar astrocytes (Pixley and McKay, 1984; Benjelloun-Touimi et al., 1985; Misson et al., 1988a; Marin-Padilla, 1995). Strong support for the transformation hypothesis is provided by a study of gial development in the newborn ferret demonstrating that a fluorescent dye applied to end feet of radial glial cells from the pial surface is found, several weeks later, within newly formed GFAP-positive astrocytes in the cortex (Voigt, 1989). Cell culture experiments in which transitional figures, in morphology and cytoskeletal composition, of radial glial cells acquiring astrocyte characteristics are found, also provide supporting evidence for the transformation hypothesis (Culican et al., 1990; Chanas-Sacre, submitted).

The transformation of radial glia into astrocytes constitutes a surprising event whereby a highly differentiated, functional cell is converted into another cell type with different morphology and functions. This corresponds to the process of transdifferentiation that is generally considered as a rare phenomenon occurring in development (Landis, 1990; Patapoutian et al., 1995) and in regeneration in response to injury (Brockes, 1994). Insofar as radial glial cells can be grown in vitro and their transformation into astrocytes can be observed and manipulated (Culican et al., 1990; Hunter and Hatten, 1995; Chanas-Sacre, submitted), they should provide abundant material to assess the extent of phenotypic alterations in their transdifferentiation process and to study the molecular events involved in the regulation of this process.

In rodents (Pixley and McKay, 1984; Sancho-Tello et al., 1995) and carnivores (Voigt, 1989) the change of morphology of radial glial cells is linked to a change in the expression of intermediate filament proteins. Vimentin is present prenatally in radial glial cells, whereas GFAP appears at a later fetal or early postnatal stage and is specific for astrocytes. Other components of the intermediate filaments such as antigens recognized by RC1 and RC2 and nestin are lost at the time of radial glia to astrocyte transformation (Hockfield and McKay, 1985; Misson et al., 1988a; Edwards et al., 1990; Kurtz et al., 1994; Chanas-Sacre, submitted).

Radial glia transformation into astrocyte is most complete in mammals, although this spares some radial glia. The Bergmann glia in the mouse postnatal cerebellum thus retain their elongated shape and palisadic organization while becoming GFAP-immunopositive at about the time when other astrocytes in the cerebellar white matter start to express this marker (Bovolenta et al., 1984). Bergmann glia constitute a particular radial glia population whose cell bodies are translocated postnatally from an initial periventricular location to the Purkinje cell layer, where their radial fibers serve as guide for the inward migration of granule neurons (Yuasa, 1996). This neuronal migration event ends when astrocytic generation from other source and dispersal into the white matter and internal granule layer is complete (Bovolenta et al., 1984). Bergmann glia are thus not necessary for astrocyte production in the cerebellum and may therefore skip the phenotypic transition into mature astrocytic cell that is prevalent elsewhere in the brain. Indeed, in addition to maintaining their radially elongated morphology, Bergmann glia also continue to express vimentin in the adult mouse (Bovolenta et al., 1984), thus preserving two characteristics of immature mammalian glial cells.

Do all radial glial cells that disappear at the end of the neuronal migration period transform into astrocytes? Astrocytic cell death occurs during normal central nervous system development, including dying GFAP-positive astrocytes in postnatal rat cortex (Soriano et al., 1993) and a large-scale astrocytic death during the first postnatal week in the mouse cerebellum (Krueger et al., 1995). In both cases, the cell death coincides with transformation of radial glia into astrocytes. However, in one study, no degenerating pyknotic nuclei were found associated with vimentin immunopositivity, indicating that only already mature astrocytes undergo apoptosis (Soriano et al., 1993).

Abnormal radial glia cell development and transformation have been investigated in *reeler* neurologic mutant mice in which the morphology and organization of radial glia are perturbed (Pinto-Lord et al., 1982; Yuasa et al., 1993). *Reeler* radial glia differentiate less extensively and disappear early in development, being replaced by astrocytes sooner than in normal cortex (Hunter-Schaeidel, 1997). The lack of transitional astroglial forms in *reeler* perinatal cortex, indicative of transdifferentiation of radial glia into astrocyte, may result from a large-scale cell death accompanied by premature as-
REGULATION OF RADIAL GLIAL PHENOTYPE

Despite their crucial role in CNS development, the mechanisms that regulate the identity and function of the radial glial cells are poorly understood. Cell autonomous mechanisms, cell–cell contacts, and soluble factors that play important roles in the development of the CNS and of glial cells in particular have been implicated in the regulation of the radial glia phenotype.

The transcription factor Pax6 is localized to a specific subclass of radial glia of the cerebral cortex but not of the basal telencephalon (Fig. 1A; Gotz et al., 1998). These cells are affected in the earliest stage of development in Pax6 mutant mice [small eye, Sey (Hill et al., 1991), Pax6 \(-/-\) (St. Onge et al., 1997)] in their morphology, tenascin-C expression, and cell cycle characteristics (Gotz et al., 1998). As the morphological alterations of radial glia appear to be cell autonomous, Pax6 may play an important role in the differentiation of cortical radial glia, thereby establishing regional differences with radial glial cells of other prospective brain region, e.g., the adjacent ganglionic eminence (Gotz et al., 1998).

Radial glia differentiation and transdifferentiation into astrocytes that normally occur at the end of the neuronal migration period are bidirectional. Indeed, grafting of embryonic Purkinje cells in the adult cerebellum or of embryonic neocortical neurons in adult somatosensory cortex induces the reexpression by host Bergmann glia or astrocytes of nestin and of the RC2 antigen, two elements of juvenile radial glial phenotype (Sotelo et al., 1994; Leavitt et al., 1999). Radial glia phenotype, including bipolar morphology and expression of RC2 antigen, can be reinduced, both in vitro and in vivo, in GFAP-positive adult cortical astrocytes in response to a diffusible factor released by embryonic forebrain (Fig. 1A; Hunter and Hatten, 1995). This glial differentiation signal, termed radializing factor (RF60), is a protein of about 60 kDa produced by embryonic neurons, whose expression is down-regulated perinatally, when the radial glial population decreases (Hunter and Hatten, 1995). In injured brain, infusion with RF60 limits the gliosis reaction (Hunter and Hatten, 1997) known to inhibit neuronal regeneration (Davies et al., 1997; Fitch and Silver, 1997; Ridet et al., 1997; Fitch et al., 1999). Although RF60 is present in embryonic reeler cortex, reeler astroglia are poorly responsive to it, suggesting an intrinsic defect in glial differentiation that could explain the alteration of radial glial cells in this mutant mouse (Hunter–Schaedle, 1997).

Embryonic Cajal–Retzius (CR) neurons responsible for the synthesis of the extracellular matrix protein Reelin that is mutated in reeler mice (Hirotosune et al., 1995; D’Arcangelo et al., 1997), induce via a diffusible factor the rejuvenation of Bergmann glia in adult cerebellum (Soriano et al., 1997). CR cells from reeler embryos also induce the radial glia phenotype (Soriano et al., 1997), but reeler radial glial cells are shorter and disorganized (Pinto–Lord et al., 1982) and transdifferentiate into astrocytes earlier in development (Hunter–Schaedle, 1997). Thus reelin could play an indirect role in the differentiation of radial glial phenotype, possibly by interfering with RF60 signaling (Fig. 1A).

The ability of adult astroglia to dedifferentiate and to assume properties of the radial glia phenotype is promising for CNS repair after injury. Indeed, grafted immature neurons are able to migrate long distances from their site...
of transplantation (Macklis, 1993; Sheen and Macklis, 1995; Leavitt et al., 1999) in a manner that is, at least partially, similar to radial glia-guided neuronal migration occurring during normal cortical development (Hatten, 1990, 1993; Leavitt et al., 1999). This suggests that such migratory event could be supported by astroglia reexpressing aspects of radial glia phenotype. Thus, embryonic neurons might be endowed with the ability to maintain radial glial cells and probably revert their differentiation into astrocytes, rendering adult neocortical environment permissive for migration of immature neurons (Sotelo et al., 1994; Soriano et al., 1997). Better understanding of the signals controlling the acquisition of the various glial phenotypes as well as the interactions between migrating neurons and glia is thus important for CNS repair.

Communication between migrating and differentiating neurons and radial glia is mediated by an increasing number of signaling systems that participate to the induction of the glial scaffold by young neurons. The neuregulins (NRGs), members of the EGF family of growth and differentiation factors, and their erbB receptors play a role in the neuronal migration program (Fig. 1A,B; Anton et al., 1997; Rio et al., 1997). In developing nervous system, NRGs are mostly expressed by neurons (Corfas et al., 1995), whereas radial glial cells express the cognate erbB receptors (Anton et al., 1997). The glial growth factor (GGF), a soluble form of neuregulin, participates in neuronal induction of radial glial morphology, elongation of glial fibers, expression of glial protein such as BLBP (Anton et al., 1997), and neuronal migration itself (Rio et al., 1997; Anton et al., 1997). Sonic hedgehog (Shh) signaling regulates the cerebellar development at multiple levels. Shh is produced by Purkinje neurons and induces the differentiation of Bergmann glia (Dahmane and Ruiz i Altaba, 1999). One of the effects of astrotactin, a neuronal cell surface glycoprotein that mediates the adhesion between radial glia and migrating neurons, is to maintain the differentiation of glial cells in vitro, inducing glial process extension and GFAP expression (Fig. 1A,B; Zheng et al., 1996). Specific domains that endow astrotactin with intracellular signaling abilities are found in its primary structure, which is unlike that of other cell adhesion or signaling molecule (Zheng et al., 1996). It combines EGF-like repeats found in signaling molecules such as Notch (Greenwald, 1994), fibronectin III repeats also present in axonal proteins of the immunoglobulin (Ig) superfamily, and the absence of IgG-like domains. A receptor for astrotactin is postulated for the glial surface but has not been identified yet. BLBP is induced in vitro in radial glia by neurons and is essential for the establishment and maintenance of radial glial fibers system during neuronal migration and, as a secondary effect, in regulation of neuronal differentiation (Fig. 1A,B; Feng et al., 1994; Kurtz et al., 1994; Feng and Heintz, 1995). How this protein mediates those effects is not known, but its low-molecular-weight hydrophobic ligand-binding property could endow it with the ability to interfere with intracellular signaling involving lipid derivative. Conversely, glia-derived signals regulate neuronal differentiation. Retinoic acid is thus produced during development by radial glia of the lateral ganglionic eminence and could influence the differentiation of the neurons that migrate along their fibers into the developing striatum (Fig. 1A; Toresson et al., 1999).

So far, none of the cell-derived factors that were found in vitro to influence the radial glia phenotype have been identified. Our studies demonstrate that the bidirectional character of the transformation of radial glia into astrocyte is controlled in vitro by factors contained in serum. Indeed the transition between RC2-positive cerebellar radial glia and stellate GFAP-positive astrocytes and its reversal can be induced, respectively, by withdrawal and addition of serum in the culture medium (Chanassacré, unpublished results). Serum-dependent effects on the stellation of astrocytes have been attributed to both thrombin and lysosphosphatidic acid, which are normal serum components (Suidan et al., 1997). Our recent results indicate that both of them are also sufficient to maintain the expression of the RC2 antigen in cultured cerebellar glial cells, making them potential inducers of the radial glia phenotype. Further studies are now needed to establish a physiological role for these agents in radial glia biology during normal nervous system development.

CONCLUSIONS

Our appreciation of the multiple facets of the migrating neurons–radial glia relationship (Hatten, 1999) is now becoming richer with the realization that the radial glial cells are progenitors for astrocytes and, possibly, for other cell types as well. The transient but extended presence of radial glia during nervous system development in higher vertebrates is instrumental in allowing the formation of multilayered brain structures. It should also be viewed as a major step in the generation of astrocytes, the most abundant cell type of the mammalian brain. We are only starting to discover the complexity of the interactions between migrating neurons and radial glia, which extend well beyond their guiding function. Radial glia phenotype is influenced by signaling systems that have been shown to regulate the production and fate determination of various cell types in the developing brain, such as sonic hedgehog, neuregulins, thrombin, and lysosphosphatidic acid. They also respond to signaling systems that so far seem to be restricted to radial glia, such as those involving BLBP and RF-60. Some of these signals have been shown to be released by neurons, but others may be released by different cell types, such as pial fibroblasts, or have no clearly identified cellular sources.

All these signaling processes have to be sorted out if one expects to design simple therapeutic schemes whereby radial glia fibers would be reinduced in adult brain in order to guide migration of grafted neurons. Overwhelming evidence has been found now that adult-derived neural stem cells that have been amplified in vitro can be grafted into brain, where they will assume various neural phenotypes and integrate into neuronal circuits (Yandava et al., 1999; Zhang et al., 1999). This opens vast possibilities in terms of repair of injured brain, which would be greatly improved if, at the same time, defined migration pathways
could be activated that would direct cell displacements to specific sites. It is through reversal of the radial glia to astrocyte transdifferentiation that such guides may be generated in adult brain, emphasizing the usefulness of characterizing this peculiar developmental event.

ACKNOWLEDGMENTS

P.L. and B.R. are supported by the FNRS, G.C.-S. is supported by the FRIA. We thank Dr. M. Götz for helpful suggestions.

REFERENCES


