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What Are the Characteristics of Cycling Cells in the Adult Central Nervous System?

James E. Goldman*

Department of Pathology and the Center for Neurobiology and Behavior, Columbia University, New York, New York 10032

Abstract Many regions of the adult central nervous system contain cycling cells. Such cells comprise a relatively small fraction of the total population of the CNS. Work over decades has attempted to determine the normal fates of these cells and their fates under pathological conditions. The recent interest in ''stem'' cells and ''progenitors'' in the adult CNS has sparked a much revived exploration into the nature of these cells and in the signals by which they may be induced to differentiate into mature neurons or glia. This population has not yet been fully characterized, although it has become clear that this is a heterogeneous group of cells, differing in morphology, antigen expression, migratory capacity, and potential fates. J. Cell. Biochem. 88: 20–23, 2003.

Key words: progenitors; stem cells; neural progenitors

Recent attention has focused on the ability of the mammalian CNS to generate new interneurons in the dentate gyrus of the hippocampus and in the olfactory bulb well into adult life. The immediate source of these neurons are populations of dividing cells in the subgranular layer of the hippocampus and the subventricular zone, respectively [summarized in Seri and Alvarez-Buylla, 2002]. The possibility of neurogenesis in other areas of the adult brain, such as the cerebral cortex, has generated a thriving controversy, in part because of differences in the techniques of labeling and identifying specific cell types and in the interpretations of the results [see Gould and Gross, 2002; Rakic, 2002].

DIVIDING CELLS IN THE ADULT CNS

The initial studies that discovered cycling cells in the adult CNS used ^{3}H-thymidine as a marker for DNA synthesis, concluding that dividing cells gave rise to postmitotic (thus mature) neurons and glia [see e.g., Smart and Leblond, 1961; Altman, 1969; Kaplan and Hinds, 1977; Paterson, 1983; McCarthy and Leblond, 1988]. Such cells are distributed widely throughout the CNS, both in gray and white matter, although concentrated particularly in the residual subventricular zone around the lateral ventricles and in the subgranular zone of the hippocampus. The labeled cells were categorized in autoradiograms by their nuclear morphology at the light microscopic level and by the fine details of their nuclei and cytoplasm at the electron microscopic level. Some labeled cells were identified as neurons, astrocytes, and oligodendrocytes, and in general this identification was not problematic. However, many of the cycling cells did not easily fit into a mature category, and thus their nature was unclear. Although mitotic labeling, with thymidine or BrdU, is a powerful method for finding and visualizing dividing cells, it does have limitations. For one thing, cells that continue to divide over and over again after being initially labeled will eventually lose the label by dilution. Thus, the fates of these dividing cells cannot be followed. In addition, it is difficult to follow cell migration by mitotic labeling. Thus, if one finds a labeled cell in one specific area of the CNS (such as the neocortex), one has to decide whether it divided at some distance from that area and migrated in or whether it represents a

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*Correspondence to: James E. Goldman, Department of Pathology and the Center for Neurobiology and Behavior, Columbia University, NY, NY 10032. E-mail: jeg5@columbia.edu

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dividing cell resident to that area. This can be a difficult, or impossible, distinction (see below for a discussion of heritable markers to follow cell migration). Finally, immature cells that were not proliferating at the time of thymidine labeling and either did not require proliferation to acquire mature characteristics or divided when the thymidine was no longer available would not be detected. Whether such cells exist is not clear.

WHERE DO NEW NEURONS AND GLIA COME FROM IN THE ADULT CNS?

In one setting, neuroblasts are generated at one site and then migrate to another site, where they fully differentiate and integrate into the existing neural circuitry. Thus, in the olfactory bulb, dividing neuronal progenitors are generated in the subventricular zone around the lateral ventricle and then migrate rostrally into the bulb. This migratory pathway is established in the embryonic period and is maintained throughout life [Luskin, 1993; Doetsch and Alvarez-Buylla, 1996]. Alvarez-Buylla and colleagues have argued that these neuroblasts originate in a population within the adult subventricular zone that has some characteristics of astrocytes, such as the expression of the intermediate filament, glial fibrillary acidic protein [Alvarez-Buylla and Garcia-Verdugo, 2002].

In another setting, neurons are generated more locally, and have to migrate a short distance to intercalate into local circuits. Thus, dividing progenitors in the hilum of the dentate gyrus must move only microns to reach the granule cell layer. In this region, the progenitors are also generated from cells that share characteristics with astrocytes [Seri et al., 2001].

In addition, recent studies that potential neurons, and indeed multipotent cells, exist in many brain regions in addition to the SVZ and hippocampus, including neocortex, amygdala, striatum, retina, and spinal cord [e.g., Kirschenbaum et al., 1994; Weiss et al., 1996; Marmur et al., 1998; Johansson et al., 1999; Palmer et al., 1999; Tropepe et al., 2000; Horner et al., 2000; Arsenijevic et al., 2001]. In most of the studies, the presence of these immature cells has been inferred retroactively, i.e., by finding some, as yet unidentified, population(s) that under the influence of growth factors, principally epidermal growth factor and basic fibroblast growth factor, are able to generate neurons and glia. Identifying the nature of these cells in vivo (i.e., prior to their differentiation into mature elements) has been more difficult. It is also not clear that all of such cells were dividing in vivo, prior to removal from the CNS.

Two strategies have emerged to characterize the nature of the immature cells: direct cell isolation and cell fate tracing with heritable markers. In the first, cells are isolated from the CNS, using dissection of specific regions and enzymatic and mechanical dissociation, followed by gradient centrifugation [Vick et al., 1990; Armstrong et al., 1992; Palmer et al., 1999; Roy et al., 1999; Gensert and Goldman, 2001]. Theo Palmer's work and ours shows that this procedure leads to a great enrichment for cells that had been cycling in vivo, as determined by labeling with BrdU prior to cell isolation, and does not isolate mature neurons, astrocytes, or oligodendrocytes [Gensert and Goldman, 2001]. Cells can be directly analyzed [see for e.g., Mason and Goldman, 2002] or cultured under a variety of conditions. Steven Goldman and colleagues have further added a step in which the isolated cells are infected with adenoviruses that encode green fluorescent protein (GFP) under the control of a promoter expressed by immature neural cells (such as nestin or musashi) or early oligodendrocytes (CNPase). The fluorescent population can then be isolated by fluorescence activated cell sorting [Roy et al., 1999; summarized in Goldman et al., 2002].

Immature cells taken from mature white matter or cortex represent a heterogeneous population, many of which will acquire in culture the characteristics of oligodendrocytes or astrocytes within a short time [Roy et al., 1999; Gensert and Goldman, 2001]. In addition, several studies also indicate that neurons can be generated, at least in culture, from immature cells in adult white matter, cells that had previously thought to belong to glial lineages [Roy et al., 1999; Kondo and Raff, 2000]. These cells are unlikely to be identical to the "astrocytic" stem cells in the SVZ and hippocampus. For one thing, isolated cells do not express GFAP. In contrast, in our studies, cycling cells that express the early oligodendrocyte marker, O4, as well as cells that express an earlier, less specific lineage marker, A2B5, can be isolated

from both white matter and cortex of the adult rat [Gensert and Goldman, 2001] and then rapidly induced by bFGF to acquire neuronal characteristics (Mason, Lin, Ventura, Goldman, unpublished data).

The second strategy, cell fate mapping with heritable markers, has utilized replication-deficient retroviruses to introduce reporters or other genes of interest into dividing cells so that one can follow all of the progeny of a cell that has integrated the retrovirus into its genome [see for example, Walsh and Cepko, 1992; Luskin, 1993]. Furthermore, if one can control the specific region into which a retrovirus is placed, and thus label cells in a circumscribed volume, one can then trace the migration of those cells to other regions of the CNS (even imaging migration, as in neonatal rat forebrain; [Kakita and Goldman, 1999]). This approach has been used extensively in embryonic and neonatal CNS, but less so in the adult brain.

One study labeling dividing cells in adult rat white matter concluded that they do not migrate into neighboring regions or differentiate into mature neurons or glia under normal conditions [Gensert and Goldman, 1996]. However, after a demyelinating lesion, they will develop into myelinating oligodendrocytes [Gensert and Goldman, 1997]. Thus, the pathological condition induces these cells to differentiate into oligodendrocytes, or removes whatever constraints may normally inhibit their differentiation. Little is known of the molecular controls that keep these cells immature and mitotically active. Examining the interactions of immature cells with their mature neighbors might reveal cell–cell contacts that inhibit differentiation. Alternatively, in the case of oligodendrocytes, the levels of mitogens might be high enough to allow some cells to continue cycling, but under normal conditions, there would be no myelinatable axons available. Demyelination would then allow contact between immature cells and axons, resulting in differentiation signals for the glial cells.

ARE PROGENITORS ABLE TO MIGRATE THROUGH THE CNS SO THAT THEY CAN PROVIDE NEW NEURONS AND GLIA AT SITES DISTANT FROM THEIR ORIGINS?

With the exception of the olfactory tract, there has been no unequivocal data that endogenous progenitors migrate to distant sites to colonize the adult brain. In the olfactory tract (rostral migratory stream, RMS) a specialized route is maintained throughout adult life. However, no such routes appear to exist in other regions of the CNS. For example, the radial glia along which progenitors migrate during development have largely disappeared by adult life, either by conversion to astrocytes or by cell death, in most regions of the CNS. Where they have remained, they may themselves generate neurons or they may not be accessible to progenitors or they may not be any progenitors that are situated to take advantage of a migratory path. In the cerebellum, for instance, Bergmann glia persist throughout life, but the immature cells of the external germinative layer eventually disappear [Altman and Bayer, 1996].

In the telencephalon, progenitors in the SVZ migrate into the cortex for about the first 2 weeks of life in the rat CNS, but thereafter do not appear to do so [Levison et al., 1993], perhaps because radial glial tracks into the cortex have disappeared. At that time, however, progenitors still migrate from the SVZ into and along the white matter.

We should not rule out the possibility of progenitor migration through the CNS, especially in pathological circumstances. Magavi et al. [2000] have inferred that cells of the SVZ emigrate therefrom into the overlying neocortex after death of layer 6 cortico-thalamic projection neurons since they observed cells expressing doublecortin and BrdU. While this study did not directly visualize migration, the findings are consistent with their hypothesis. Nait-Oumesmar et al. [1999] have presented observations consistent with migrating progenitors in the anterior SVZ being diverted and migrating dorsally by a demyelinating lesion in the overlying callosum. Perhaps immature cells will migrate better in a pathological environment [Magavi and Macklis, 2002], possibly because the edema in the tissue will increase the extracellular space or because damaged tissues secrete chemotropic agents or migration-friendly extracellular matrix.

REFERENCES

Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. J Comp Neurol 137:433–457.

Altman J, Bayer SA. 1996. Development of the cerebellar system. Boca Raton: CRC Press.

Alvarez-Buylla A, Garcia-Verdugo JM. 2002. Neurogenesis in adult subventricular zone. J Neurosci 22:629–634.

Armstrong RC, Dorn HH, Kufta CV, Friedman E, Dubois-Dalcq ME. 1992. Pre-oligodendrocytes from adult human CNS. J Neurosci 12:1538–1547.

Arsenijevic Y, Villemure J-G, Brunet J-F, Bloch JJ, Deglon N, Kostic C, Zurn A, Aebischer P. 2001. Isolation of multipotent neural precursors residing in the cortex of the adult human brain. Exp Neurol 170:48–62.

Doetsch F, Alvarez-Buylla A. 1996. Network of tangential pathways for neuronal migration in adult mammalian brain. Proc Natl Acad Sci USA 93:14895–14900.

Gensert JM, Goldman JE. 1996. In vivo characterization of endogenous proliferating cells in adult rat subcortical white matter. Glia 17:39–51.

Gensert JM, Goldman JE. 1997. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197–203.

Gensert JM, Goldman JE. 2001. Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. J Neurobiol 48:75–86.

Goldman SA, Benraiss A, Chmielnicki E, Keyoung HM, Nunes M, Roy NS, Samdani A, Windrem M. 2002. Isolation and induction of adult neural progenitor cells. Clin Neurosci Res 2:70–79.

Gould E, Gross CG. 2002. Neurogenesis in adult mammals: Some progress and problems. J Neurosci 22:619–623.

Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci 20:2218–2228.

Johansson CV, Svensson M, Wallstedt L, Janson AM, Frisen J. 1999. Neural stem cells in the adult human brain. Exp Cell Res 253:733–736.

Kakita A, Goldman JE. 1999. Patterns and dynamics of SVZ cell migration in the postnatal forebrain: monitoring living progenitors in slice preparations. Neuron 23:461–472.

Kaplan MS, Hinds JW. 1977. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197:1092–1094.

Kirschenbaum B, Nedergaard M, Preuss A, Barami K, Fraser RAR, Goldman SA. 1994. In vitro neuronal production and differentiation by precursor cells derived from the adult forebrain. Cereb Cortex 6:576–589.

Kondo T, Raff M. 2000. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. Science 2898:1754–1757.

Levison SL, Young GM, Goldman JE. 1999. Cycling cells in the adult rat neocortex preferentially generate oligodendrocytes. J Neurosci Res 57:435–446.

Levison SW, Chuang C, Abramson B, Goldman JE. 1993. The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. Development 119:611–623.

Luskin MB. 1993. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173–189.

Magavi SS, Leavitt BR, Macklis JD. 2000. Induction of neurogenesis in the neocortex of adult mice. Nature 405: 951–955.

Magavi SS, Macklis JD. 2002. Manipulation of neural precursors in situ toward induction of neurogenesis in the adult brain: potential and limitations. Clin Neurosci Res 2:40–57.

Marmur R, Mabie PC, Gokhan S, Song Q, Kessler JA, Mehler MF. 1998. Isolation and developmental characterization of cerebral cortical multipotent progenitors. Dev Biol 204:577–591.

Mason JL, Goldman JE. 2002. A2B5+ and O4+ cycling progenitors in the adult forebrain white matter respond differentially to PDGF-AA, FGF-2, and IGF-1. Mol Cell Neurosci 20:30–42.

McCarthy GF, Leblond CP. 1988. Radioautographic evidence for slow astrocyte turnover and modest oligodendrocyte production in the corpus callosum of adult mice infused with 3H-thymidine. J Comp Neurol 271:589–603.

Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Baron-Van Evercooren A. 1999. Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. Eur J Neurosci 11:4357–4366.

Palmer TD, Markakis EA, Willhoite AR, Safar R, Gage FH. 1999. Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. J Neurosci 19:8487–8497.

Paterson JA. 1983. Dividing and newly produced cells in the corpus callosum of adult mouse cerebrum as detected by light microscopic radioautography. Anat Anz, Jena 153:149–168.

Rakic P. 2002. Adult neurogenesis in mammals: An identity crisis. J Neurosci 22:614–618.

Roy NS, Wang S, Harrison-Restelli C, Benraiss A, Fraser RA, Gravel M, Braun PE, Goldman SA. 1999. Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. J Neurosci 19:9986–9995.

Seri B, Alvarez-Buylla A. 2002. Neural stem cells and the regulation of neurogenesis in the adult hippocampus. Clin Neurosci Res 2:11–16.

Seri B, Garcia-Verdugo JM, McEven BS, Alvarez-Buylla A. 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 21:7153–7160.

Smart I, Leblond CP. 1961. Evidence for division and transformation of neuroglia cells in mouse brain, as derived from radioautography after injection of thymidine-H3. J Comp Neurol 116:349–367.

Tropepe V, Coles BL, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR. 2000. Retinal stem cells in the adult mammalian eye. Science 287:2032–2036.

Vick RS, Chen S-J, DeVries GH. 1990. Isolation, culture and characterization of adult rat oligodendrocytes. J Neurosci Res 25:524–534.

Walsh CA, Cepko CL. 1992. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. Science 255:434–440.

Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson A, Reynolds BA. 1996. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 16:7599–7609.