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Cellular localization of 1B236/myelin-associated glycoprotein mRNA during rat brain development

(oligodendrocytes/myelinogenesis/neurons/cell adhesion molecule/cell type-specific gene expression)

GERALD A. HIGGINS*†, HARTWIG SCHMALE‡§, FLOYD E. BLOOM‡, MICHAEL C. WILSON†, AND ROBERT J. MILNER‡¶

*Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642; and †Department of Molecular Biology and ‡Division of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037

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ABSTRACT **The protein encoded by the rat brain cDNA 1B236 has been shown to be identical to myelin-associated glycoprotein (MAG). In this report we describe the cellular distribution of 1B236/MAG mRNA transcripts in rat brain by using *in situ* hybridization. At postnatal day 20, large numbers of 1B236/MAG mRNA-containing oligodendrocytes are concentrated in myelinated fiber tracts and throughout gray matter regions. The presence of high levels of 1B236/MAG mRNA within oligodendrocytes at postnatal day 20 is consistent with the proposed role of MAG in formation of the myelin sheath during development. In the adult brain, our results suggest that not only is 1B236/MAG mRNA expressed at reduced levels within oligodendrocytes but also 1B236/MAG or a 1B236/MAG-like mRNA is present within neurons. This localization is consistent with the results of previous immunocytochemical studies using antibodies against the 1B236 protein. The apparent developmental profile of 1B236/MAG mRNA with different cell-type-specific patterns of expression suggests that oligodendrocytes and neurons employ different mechanisms for regulating the same gene. Thus, different cell types may use a similar cell adhesion molecule both during myelinogenesis and in the mature nervous system.**

Myelin-associated glycoprotein (MAG), a constituent of myelin in both peripheral and central nervous systems (CNS), appears to act as a cell adhesion molecule between glia and neurons (1, 2) and may be involved in formation of the periaxonal space (3). Molecular cloning studies (4–6) have shown that MAG is identical to the rat brain protein 1B236, previously defined by characterization of "brain-specific" mRNAs (7). The single 1B236/MAG gene gives rise to several mRNAs by alternative RNA splicing (4); the omission or inclusion of exon 12 of the gene results in mRNAs encoding polypeptides with different carboxyl-terminal sequences and molecular masses of 72 and 67 kDa, respectively (4, 5). During development, 1B236/MAG accumulates in the rat CNS until approximately postnatal day (PD) 20, by which time myelination has largely been completed (1). Similarly, the steady-state level of 1B236 mRNA increases until PD 15–25 and drops in the adult to 20% of the maximal levels attained during development (8). This developmental change in 1B236/MAG abundance appears to be accompanied by a transition in expression of the alternatively spliced mRNAs and their products: the 72-kDa form is expressed during postnatal development and myelinogenesis, whereas the 67-kDa form predominates in the adult (4, 9).

Immunocytochemistry has been used to demonstrate the presence of MAG within oligodendrocytes in both developing and adult CNS (3, 10), consistent with its proposed role in formation of the myelin sheath. However, anti-MAG antibodies have been reported to stain a variety of other cell types (11–15), including neurons (16, 17). Using antibodies generated against synthetic peptides derived from the 1B236 cDNA sequence, we have also detected 1B236/MAG immunoreactivity within subpopulations of neurons in the adult rat CNS (7, 18). These results suggest that the 1B236/MAG gene product may be expressed in cells other than oligodendrocytes and may play a role in the functions of mature neurons.

As an alternative approach to resolve the differences in MAG and 1B236 location reported in immunocytochemical studies, we have used *in situ* hybridization to localize sites of 1B236/MAG biosynthesis during postnatal development and in the adult rat brain. The early postnatal peak of 1B236/MAG mRNA expression in oligodendrocytes coincides with myelinogenesis. In contrast, in the adult 1B236/MAG mRNA is expressed in oligodendrocytes and in a discrete set of neurons that are widely distributed in the brain. Thus, this mRNA is apparently expressed in oligodendrocytes and in neurons, but with different developmental time courses of expression, suggesting cell-type-specific developmental regulation of 1B236/MAG gene expression. A preliminary report of these findings has been published (19).

MATERIALS AND METHODS

Tissue Preparation. Anesthetized PD-20 or adult male Sprague–Dawley rats (Charles River Breeding Laboratories) were perfused transcardially with 4% (wt/vol) paraformaldehyde in 0.15 M sodium phosphate (pH 7.4), and the brains were prepared as described (20, 21).

RNA Probes. cDNA fragments corresponding to 5′ and 3′ portions of the coding region of p1B236 (5, 8) and to the coding region of proteolipid protein (PLP) (22) were subcloned into pSP65 or pGEM-4 plasmid vectors (Promega). Radiolabeled antisense RNA probes were generated by transcription by SP6 polymerase in the presence of uridine 5′-[α-[^{35}S]thio]triphosphate (21, 23).

***In Situ* Hybridization.** Coronal sections (10–20 μm) were collected on slides and stored at 4°C for no more than 2–3 days before hybridization. The sections were pretreated and hybridized with radiolabeled RNA probes as described, with minor modifications (21). After hybridization overnight at 48°C, coverslips were removed in 4× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) containing 300 mM 2-mercaptoethenol. The sections were rinsed in 4×

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Abbreviations: MAG, myelin-associated glycoprotein; PD, postnatal day; PLP, proteolipid protein; CNS, central nervous system.

§Present address: Institut fur Zellbiochemie und Klinische Neurobiologie, Universitat Hamburg, Martinistrasse 52, 2 Hamburg 20, F.R.G.

¶To whom reprint requests should be addressed at: Scripps Clinic BCR1, 10666 North Torrey Pines Road, La Jolla, CA 92037.

SSC without 2-mercaptoethenol, digested with pancreatic RNase at 50 μg/ml in 0.5 M NaCl/10 mM Tris·HCl, pH 8/1 mM EDTA for 30 min at 37°C, incubated in the same buffer without RNase at 37°C, and washed in 0.4× SSC at 42°C. The slides were air-dried and exposed to x-ray film (DuPont Cronex 5) for 12–24 hr at room temperature. The slides were then dipped in emulsion (Kodak NTB2), exposed for 2–5 days, and developed for autoradiography.

RESULTS

To determine the cellular distribution of 1B236/MAG mRNA during the development of the rat CNS and to correlate the expression with previous immunocytochemical mapping of 1B236 peptide distribution in the developing (8) and adult (18) rat, we used *in situ* hybridization for localization of 1B236/MAG transcripts in PD 20 and adult brains. PD 20 was chosen because 1B236/MAG mRNA abundance is highest during this period of active CNS myelinogenesis (8). Single-stranded, ^{35}S-labeled RNA probes were used for *in situ* mRNA hybridization because they offer high sensitivity and allow resolution of single cells (21, 23). Two RNA probes were used, corresponding to the 5′ and 3′ portions of the open reading frame of 1B236/MAG mRNA (4), and thus both probes should recognize all known species of 1B236/MAG mRNA.

The highest levels of 1B236/MAG mRNA were observed in PD-20 brain. At this age, the mRNA is concentrated in myelinated fiber tracts and white matter regions, such as the lateral olfactory tract, anterior commissure, optic chiasm and tract, corpus callosum, internal and external capsule, fornix, stria medullaris, mammillothalamic tracts, white matter of the cerebellum, and pyramidal tract (Fig. 1). At higher magnification, the distribution of 1B236/MAG-positive cells is consistent with expression in oligodendrocytes. For example, 1B236/MAG mRNA is present within cells of the anterior commissure and fornix (Fig. 2 *A–C*). In the striatum, 1B236/MAG hybridization appears to be predominately located in structures corresponding to the striatal fiber bundles (Fig. 2 *D* and *E*). In the cerebellum, 1B236/MAG mRNA is concentrated in the white matter, with a few scattered oligodendrocytes in the granule cell layer (Fig. 2*F*).

In the adult rat brain, both neurons and oligodendrocytes appear to express 1B236/MAG mRNA. However, the overall abundance of the mRNA, as determined by *in situ* hybridization, is greatly reduced compared to the younger age. The striking difference between the distribution of 1B236/MAG mRNA-containing cells observed during early postnatal development and in the adult rat CNS can be observed on x-ray film images of matched coronal sections of PD-20 versus adult rat brain (Fig. 3), which show a shift in expression from white matter areas in the young rat to a more dispersed

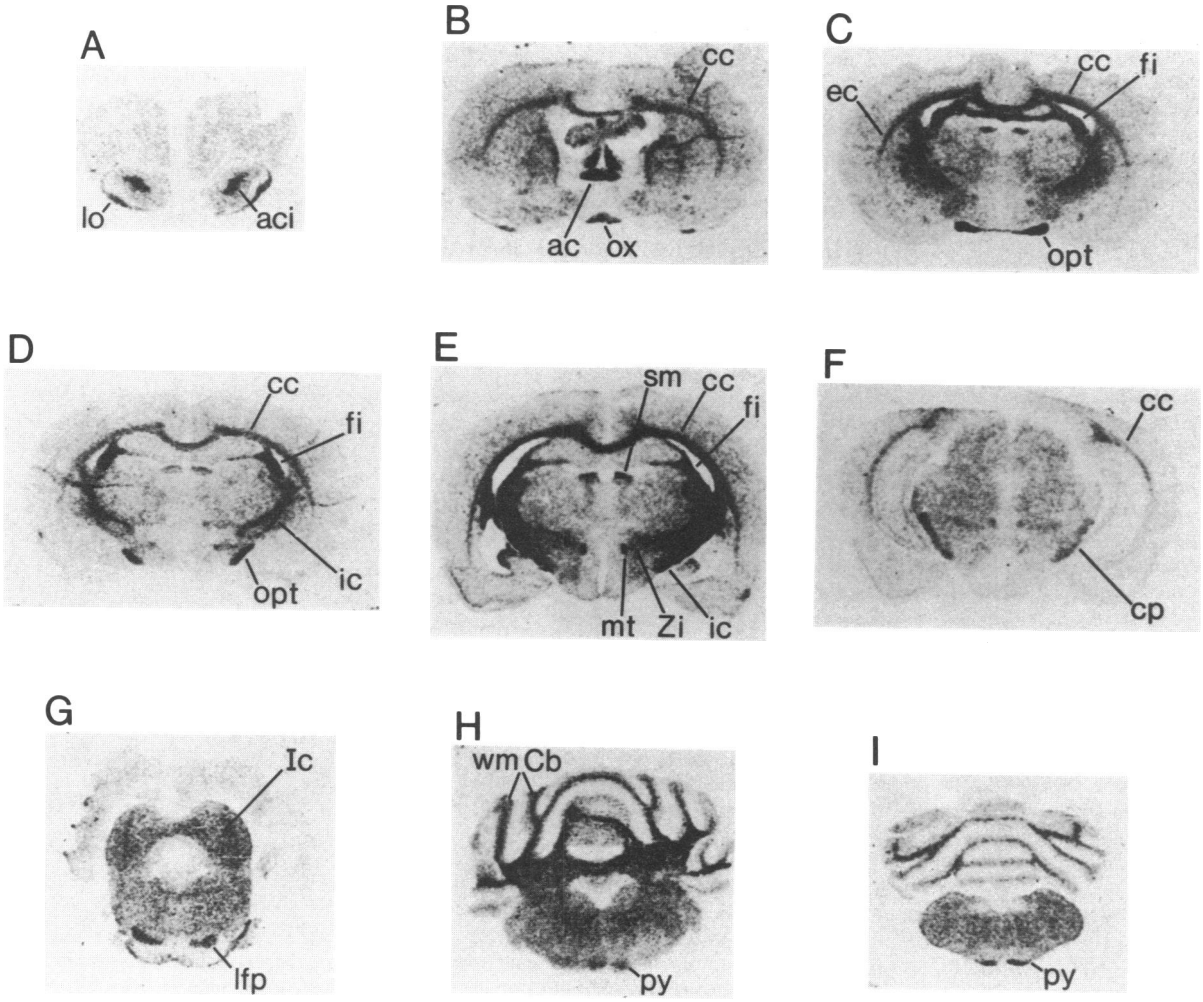

FIG. 1. X-ray film images of 1B236/MAG mRNA hybridization within various coronal sections of a PD-20 rat brain (*A–I*). Note the "myelin-like" pattern of 1B236/MAG mRNA distribution. ac, anterior commissure; aci, ac, intrabulbar portion; cc, corpus callosum; cp, caudate-putamen; ec, external capsule; fi, fimbria; ic, internal capsule; Ic, inferior colliculus; lfp, longitudinal fibers of the pons; lo, lateral olfactory tract; mt, mammillothalamic tract; opt, optic tract; ox, optic chiasm; py, pyramidal tract; sm, stria medullaris; wmCB, white matter of the cerebellum; Zi, zona incerta. (×2.45.)

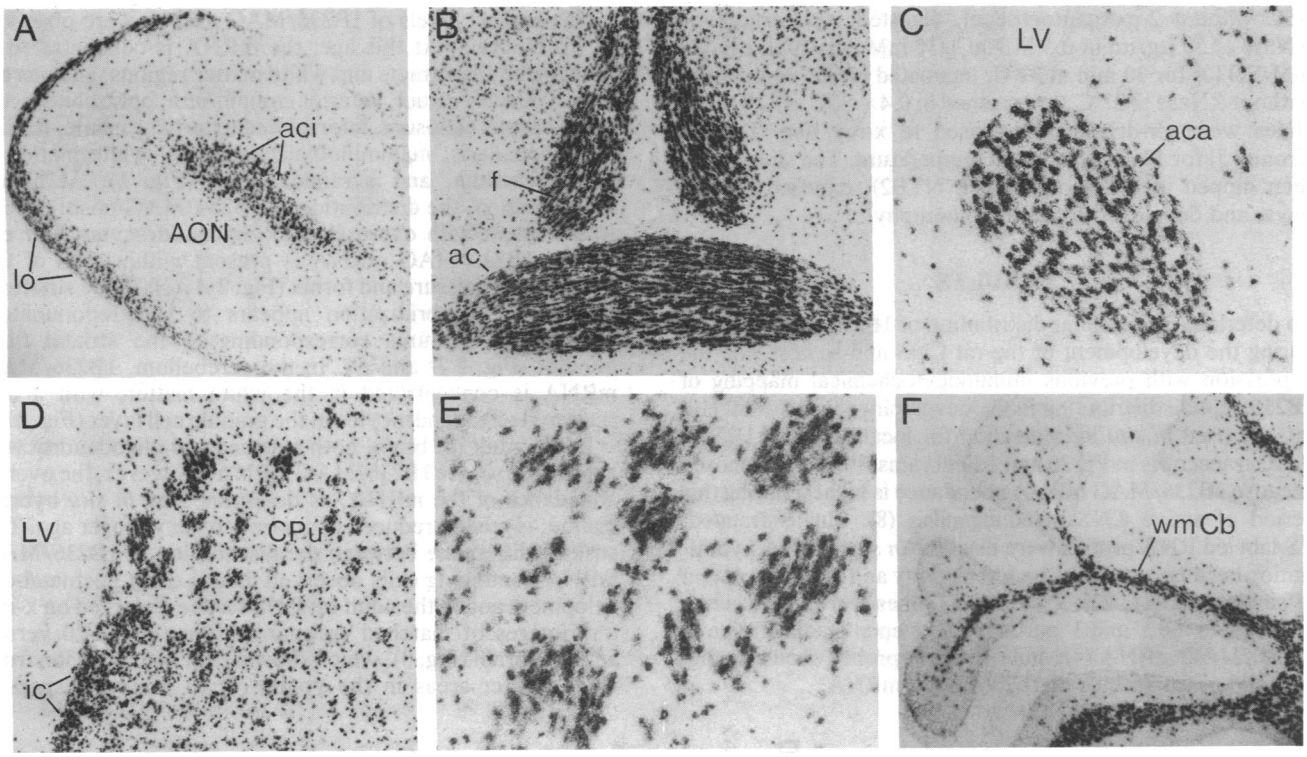

FIG. 2. *In situ* hybridization of 1B236/MAG mRNA in myelinating fiber tracts of a PD-20 rat brain. (*A*) In the olfactory peduncle hybridization is evident in the lateral olfactory tract (lo) and intrabulbar division of the anterior commissure (aci), but only scattered oligodendrocytes are present in the gray matter of the anterior olfactory nucleus (AON). (*B*) Intense hybridization is present at the decussation of the anterior commissure (ac) and within the descending limbs of the fornix (f). (*C*) 1B236/MAG-positive oligodendrocytes in the anterior division of the anterior commissure (aca) adjacent to the lateral ventricle (LV). (*D* and *E*) Hybridization in the internal capsule (ic) and in the caudate-putamen (CPu) within the striate fiber bundles. (*F*) Hybridization within oligodendrocytes of the white matter of the cerebellum (wmCb). (Bright-field photomicrographs of coronal sections processed for emulsion autoradiography. For *A* and *F*, ×9.35; for *B* and *D*, ×27.50; for *C* and *E*, ×110.)

distribution in the adult. A reduction in 1B236/MAG mRNA abundance is obvious in myelinated fiber tracts in the adult CNS. For example, at PD 20, the optic chiasm contains many 1B236/MAG-positive oligodendrocytes, determined by hybridization with 1B236 probes that detect either 5′ or 3′ regions of 1B236/MAG mRNA (Fig. 4 *A* and *B*) and by

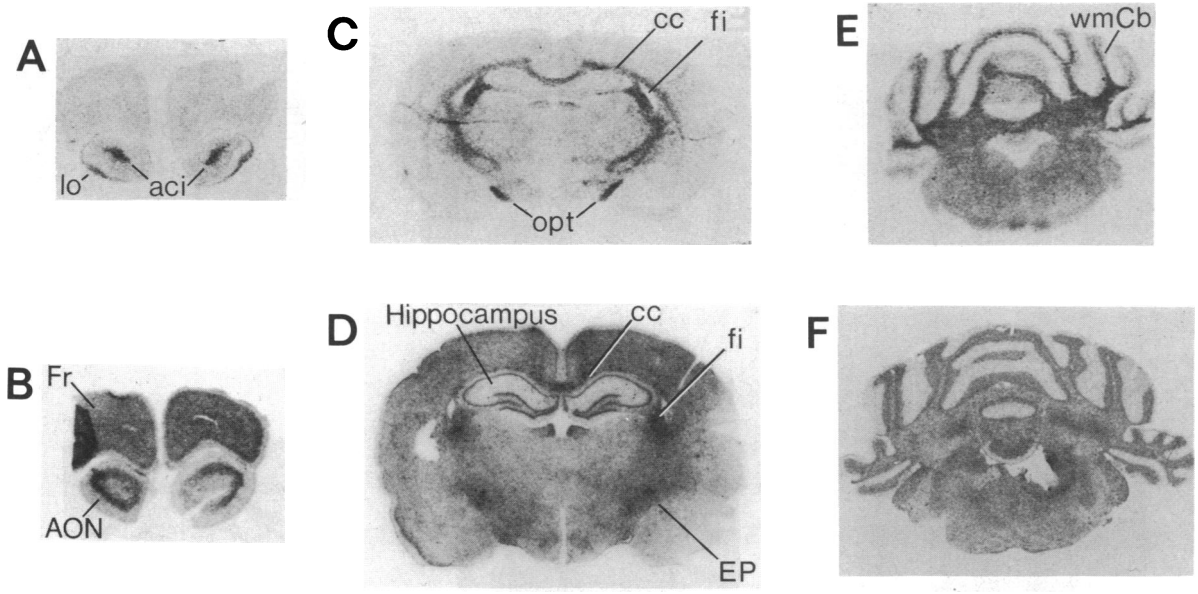

FIG. 3. Developmental shift in 1B236/MAG mRNA expression from white matter at PD 20 to largely neuronal expression in the adult. (*A* and *B*) Level through the rostral pole of the telencephalon showing hybridization in white matter structures, such as the intrabulbar division of the anterior commissure (aci) and lateral olfactory tract (lo), at PD 20, and in gray matter structures, such as frontal cortex (Fr) and anterior olfactory nucleus (AON), in the adult. (*C* and *D*) Mid-telencephalon showing transition from expression in myelinated fiber tracts to mixed white and gray matter hybridization. Note the emergence of neuronal hybridization in the hippocampal formation and endopeduncular nucleus (EP), retention of hybridization in the corpus collosum (cc) and fimbria of the fornix (fi), and loss of signal in the optic tract (opt) in the adult. (*E* and *F*) Sections through the caudal pons showing loss of 1B236/MAG hybridization in the white matter of the cerebellum (wmCb) during development. (X-ray film images of coronal section hybridized with ^{35}S-labeled RNA probes, modified from ref. 19. ×2.75.)

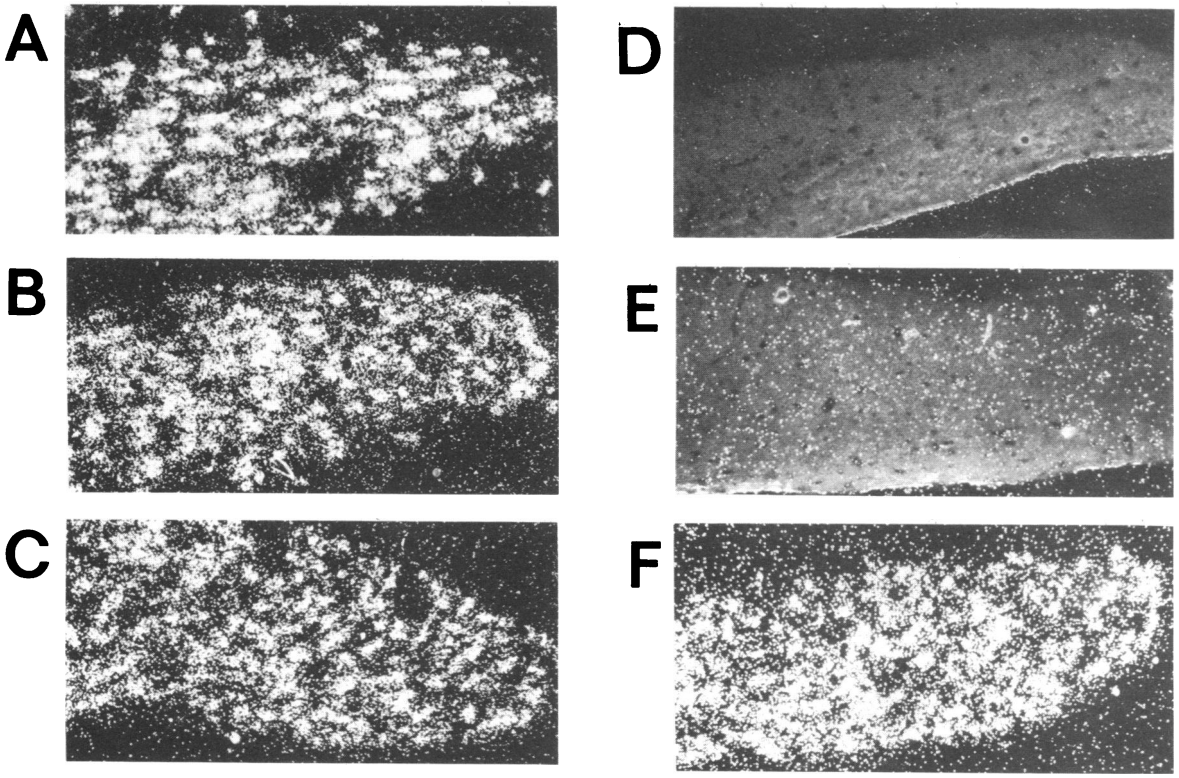

FIG. 4. Developmental loss of 1B236/MAG mRNA expression in the optic chiasm. Tissue sections were hybridized with probes complementary to the 3′ end of the coding region of 1B236/MAG mRNA (*A* and *D*), the 5′ portion of 1B236/MAG mRNA (*B* and *E*), and PLP mRNA (*C* and *F*). At PD 20, all probes appear to hybridize to oligodendrocytes in the optic chiasm. In the adult, 1B236/MAG mRNA is no longer evident in these cells of the mature optic chiasm using either of the probes (*D* and *E*), but PLP is still expressed in oligodendrocytes (*C* and *F*). (Dark-field photomicrographs of emulsion autoradiographic material. ×280.)

hybridization to PLP, a marker of oligodendrocytes (Fig. 4*C*). In contrast, in the optic chiasm of adult rat brain, oligodendrocytic hybridization was observed with the PLP probe (Fig. 4*F*), but no hybridization was observed with either of the 1B236/MAG probes (Fig. 4 *D* and *E*).

In the adult rat brain, although the overall abundance of the mRNA is greatly reduced compared to PD-20 brain, we have been able to identify 1B236/MAG mRNA within cell types that resemble neurons. Thus, significant hybridization can be observed in large-sized cells with Nissl-staining profiles characteristic of neurons (Fig. 5). The distribution of neuronal 1B236/MAG hybridization in the adult is in general agreement with previous immunocytochemical findings using antibodies directed against 1B236 synthetic peptides (7, 18), including expression within neuronal cell groups such as the medial trapezoid nucleus and pontine nuclei and within the olfactory bulb and cingulate cortex.

DISCUSSION

We have examined the expression of the 1B236/MAG gene during the development of the rat CNS and showed that there is a shift in the abundance of the mRNA in different cell types in developing and adult animals. At PD 20, during the period in which 1B236/MAG mRNA is present at highest abundance (8), the predominant site of expression is within oligodendrocytes undergoing myelinogenesis. In the adult CNS, when the steady-state level of the mRNA is diminished (8), transcripts of the 1B236/MAG gene appear to be widely expressed by both neurons and oligodendrocytes.

The presence of 1B236/MAG mRNA within neurons of the adult brain is consistent with our previous immunocytochemical studies using antibodies against the peptides derived from the nucleotide sequence of 1B236 cDNA, which has now been established to encode MAG (4–6). In those studies, antibodies against three nonoverlapping 1B236 peptides gave essentially identical patterns of immunoreactivity in discrete populations of neurons throughout the neuraxis of the adult rat (18). The localization of the detected immunoreactivity in neuronal cell bodies, axons, and dendrites was verified by electron microscopy (18).

Our observation of the neuronal expression of 1B236 protein in the adult rat brain contrasts to previous reports of immunohistochemical and radioimmunoassay analyses indicating MAG immunoreactivity was limited to oligodendrocytes and myelin sheaths (10, 24). In the histochemical studies, MAG expression in neurons of the adult CNS may not have been readily detected, possibly because of differences in the conditions used for tissue fixation. To define precisely 1B236/MAG expression in the CNS, we have used polyclonal and monoclonal antibodies directed against purified, intact MAG under the conditions used for anti-1B236 antibodies (F.E.B., E. L. F. Battenberg, and R.J.M., unpublished results). We have observed patterns of MAG immunoreactivity in neurons similar to those described for 1B236, at the light and electron microscopic levels, and consistent with the *in situ* hybridization results reported here.

Although the distribution of 1B236/MAG transcripts appear widespread in adult neurons, it is unlikely that this pattern results from nonspecific hybridization of the antisense probe to ubiquitous rRNA sequences (25). Such nonspecific binding has been demonstrated to result in a "Nissl-like" pattern that reflects the high cell density of neuronal populations within regions of the CNS such as the dentate gyrus, the habenula, and the primary olfactory and piriform cortices and within the granule cell layer of the cerebellum (20). However, the hybridization of the 1B236/MAG probe indicates expression within neurons of the medial trapezoid nucleus and pons, areas that do not show a significant nonspecific signal after *in situ* hybridization. An alternative

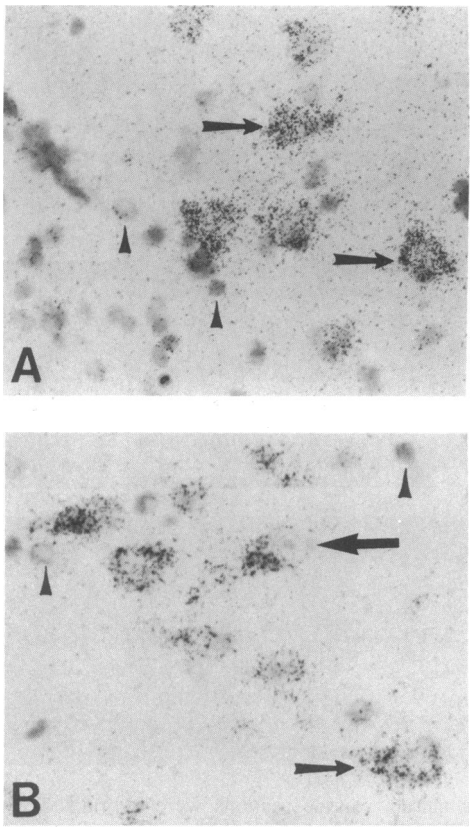

FIG. 5. 1B236/MAG mRNA hybridization within neurons of adult rat brain. Tissue sections were counter-stained with cresyl violet. Horizontal arrows indicate putative neuronal profiles of large-sized cells with cytoplasmic processes, vertical arrowheads indicate smaller-sized glia that do not hybridize with the 1B236/MAG probe. Large arrow in *B* indicates cell with nucleolus characteristic of a neuron. (*A*) Pons. (*B*) Medial trapezoid nucleus. (×320.)

possibility is that the probes cross-hybridize to a 1B236/MAG-like mRNA, potentially encoding a related member of the immunoglobulin superfamily. No such mRNAs have been detected by hybridization to size-fractionated RNA on Northern blots (4, 7, 8), and probes directed against both 5′ and 3′ portions of 1B236/MAG mRNA show the same pattern of *in situ* hybridization supporting both the specificity of the hybridization and the unlikelihood of cross-hybridization to a similar sequence expressed in adult neurons. The experiments described here do not address the cell specificity of expression of the alternatively spliced 1B236/MAG mRNAs. In preliminary studies oligonucleotide probes that distinguish the alternative mRNAs appear to hybridize to both neurons and oligodendrocytes.

1B236/MAG mRNA hybridization is largely confined to cytoplasmic regions in close proximity to the nucleus in both oligodendrocytes and neurons. A similar distribution was found for PLP mRNA in oligodendrocytes, as described (26). Therefore, 1B236/MAG transcripts appear not to be transported from the cell body to distal fibers undergoing active myelinogenesis, as has been described for myelin basic protein mRNA (26). In contrast to the expression of PLP, which is maintained at high abundance in adult oligodendrocytes, the expression of 1B236/MAG transcripts in oligodendrocytes occurs abundantly during active myelinogenesis but is barely detectable in the adult (Fig. 4). This suggests that the 1B236/MAG protein is, in this cell type, predominantly involved with the formation of myelin during development, rather than the maintenance of myelin structure in the adult.

Recent studies suggest that 1B236/MAG contains a number of functional domains that can interact with cell surface molecules and constituents of the extracellular matrix (4, 5) and shares these properties with adhesion molecules such as vitronectin and the neural cell adhesion molecule (N-CAM). It has been shown that 1B236/MAG can bind constituents of the basal lamina, such as heparin and several forms of collagen (27), and may mediate oligodendrocyte–oligodendrocyte and oligodendrocyte–neuron interactions (2). The neuronal expression of 1B236/MAG described here suggests that 1B236/MAG may not only function as an adhesion molecule during myelination but could serve potentially similar adhesive functions between neurons or in neuron–substrate interactions.

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