The Myelin-associated Glycoprotein Is Enriched in Multivesicular Bodies and Periaxonal Membranes of Actively Myelinating Oligodendrocytes

Bruce D. Trapp, S. Brian Andrews,* Cindy Cootauco, and Richard Quarles‡

Department of Neurology, Neuromuscular Division, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and *Laboratory of Neurobiology and ‡Molecular and Cellular Neurobiology National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20892

Abstract. The myelin-associated glycoprotein (MAG) is a member of the immunoglobulin gene superfamily that is selectively expressed by myelin-forming cells. A developmentally regulated, alternative splicing of a single MAG transcript produces two MAG polypeptides (72 and 67 kD) in the central nervous system (CNS). MAG occurs predominantly as the 67-kD polypeptide in the peripheral nervous system (PNS). This study determined the subcellular localization of CNS MAG at different postnatal times when the 72-kD form (7-d) and 67-kD form (adult) are quantitatively abundant. These distributions were also compared to those of MAG in the PNS. In adult rat, MAG is selectively enriched in periaxonal membranes of CNS myelin internodes. This restricted distribution differs from that in PNS myelin internodes where MAG is also enriched in paranodal loops, Schmidt-Lanterman incisures, and mesaxon membranes. In 7-d-old rat CNS, MAG was associated with periaxonal membranes during axonal ensheathment and enriched in Golgi membranes and cytoplasmic organelles having the appearance of multivesicular bodies (MVBs). MAG-enriched MVBs were found in oligodendrocyte perinuclear regions, in processes extending to myelin internodes, and along the myelin internode in outer tongue processes and paranodal loops. MAG-enriched MVBs were not found in oligodendrocytes from adult animals or in myelinating Schwann cells. These findings raise the possibility that the 72-kD MAG polypeptide is associated with receptor-mediated endocytosis of components from the periaxonal space or axolemma during active stages of myelination.

For well over a decade, the myelin-associated glycoprotein (MAG)[1] has been proposed to play a role in maintaining contact between myelin-forming cells and axons. This function was first proposed on the basis of MAG's biochemical properties and enrichment in myelin subfractions (21, 22). This hypothesis was subsequently supported by several immunocytochemical studies, which demonstrated MAG's enrichment in periaxonal regions of central nervous system (CNS) and peripheral nervous system (PNS) myelin internodes (28), and which showed a strict correlation between the presence of MAG in periaxonal membranes and the formation and maintenance of the periaxonal space during Schwann cell remyelination in Quaking mice (33, 35).

Several laboratories have deduced the amino acid sequence of CNS MAG from cDNA clones (1, 14, 15, 26) and have identified MAG as a member of the immunoglobulin gene superfamily (37). Amino acid sequence homologies between the extracellular domains of MAG and those of other adhesion molecules such as the neuronal cell adhesion molecule (5) provided further support for MAG's potential role in cell–cell adhesion. In addition, in vitro assays demonstrated that an antibody directed against the extracellular domain of MAG could inhibit the adhesion of MAG-containing liposomes to axons, as well as the adhesion of oligodendrocyte perikarya to each other and to neurons (20).

However, adhesion to the axolemma cannot be the only function of MAG within the PNS, because MAG is enriched in membranes that are not involved in axon attachment, i.e., Schmidt-Lanterman incisures, paranodal loops, and outer and inner mesaxons (31, 33, 34). We have also considered the possibility that MAG has additional functions within the CNS. This was based in part on the observation that two developmentally regulated MAG polypeptides with molecular masses of 72 and 67 kD are derived from a primary RNA transcript by differential splicing (7, 14, 23, 26, 36). The mRNAs for these polypeptides differ by a 45-bp insert (exon 12) in the 3′ or carboxy terminal region. In the CNS, the 72-kD MAG polypeptide is the predominant form during active stages of myelination, whereas the 67-kD form predominates in the adult.

1. *Abbreviations used in this paper*: CNP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; CNS, central nervous system; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MVB, multivesicular body; PNS, peripheral nervous system.

This study compares the subcellular localization of MAG in mature CNS and PNS myelin internodes and demonstrates a broader distribution of MAG in the PNS than in the CNS. In addition, we have investigated the subcellular distribution of CNS MAG during stages of active myelination (7-d spinal cord) and of myelin maintenance (adult spinal cord). The results indicate that CNS MAG is enriched in multivesicular bodies (MVBs) that are found within most cytoplasmic domains of actively myelinating oligodendrocytes. MAG-containing MVBs are not prominent features in oligodendrocytes in adult animals or in myelinating Schwann cells. These studies raise the possibility that the 72-kD MAG polypeptide is associated with receptor-mediated endocytosis of components from the periaxonal space or axolemma during active stages of CNS myelination.

Materials and Methods

Light Microscopy

Adult and 7-d-old Sprague-Dawley rats were perfused with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M phosphate buffer. The cervical spinal cord and the sciatic nerve were removed, placed in fixative overnight, osmicated, and processed into Epon by standard procedures. Sections (1-μm-thick) were cut on an ultramicrotome, placed on glass slides, treated to remove Epon and osmium, and immunostained with MAG antiserum by the peroxidase-antiperoxidase procedure as described previously (31).

Electron Microscopic Immunocytochemistry

Adult and 7-d-old Sprague-Dawley rats were perfused as above. The cervical spinal cord and lumbar ventral roots were removed, cut into 0.5-cm-long segments, and placed in fixative overnight. The ventrolateral white matter tracts and adjoining grey matter (dissected from the spinal cord segments) and the peripheral nerve segments were infiltrated with 2.3 M sucrose and 30% polyvinyl pyrrolidone (29), placed on specimen stubs, and frozen in liquid nitrogen. Ultrathin frozen sections (~120 nm thick) were cut on glass knives in an ultracryomicrotome (Ultracut FC4; Reichert Scientific Instruments, Buffalo, NY) maintained at −110°C. The sections were transferred, thawed, washed, and stained by previously described modifications (34, 35) of standard methods (27, 29). After the immunostaining procedure, the grids were placed in 2.5% glutaraldehyde in PBS for 15 min and rinsed. The sections were then stained with neutral uranyl acetate followed by embedding in 1.3% methyl cellulose containing 0.3% uranyl acetate. Grids were examined in an Hitachi H-600 electron microscope.

Controls

Specificity of the immunogold procedure was demonstrated by positive controls. Cryosections were immunostained with antisera directed against myelin basic protein (MBP) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP).

Antibodies

The polyclonal antiserum used in this study is well-characterized: MAG (24, 33), MBP (4), and CNP (2, 32). MAG was localized at the electron microscopic level with a cocktail of two monoclonal antibodies (B1IF7 and D7E10) that are specific for the polypeptide of both CNS and PNS MAG (6). The myelin basic protein and CNP antibodies were polyclonal and affinity purified.

Results

MAG Distribution in Mature CNS and PNS Myelin Internodes

The distributions of MAG in 1-μm-thick Epon sections of adult rat spinal cord and sciatic nerve are compared in Fig. 1. In transverse orientation, myelinated fibers in both the CNS (Fig. 1 *A*) and PNS (Fig. 1 *B*) contained a periaxonal ring of MAG staining. A small intense dot of MAG immunoreactivity was often part of this periaxonal ring of staining (*arrowheads*, Fig. 1, *A* and *B*) in both CNS and PNS fibers. These dots represent the presence of MAG in apposing membranes of the inner mesaxon. In the CNS, MAG immunoreactivity was not apparent in other locations, except for occasional particulate staining of oligodendrocyte perinuclear cytoplasm. Some axons in the PNS were surrounded by thick bands of MAG reaction product (Fig. 1 *B*). In longitudinal orientation, PNS fibers clearly showed thick bands of MAG staining in paranodal loops (Fig. 1 *C, arrowheads*) and Schmidt-Lanterman incisures (Fig. 1 *C, arrows*). Particulate staining of Schwann cell perinuclear cytoplasm by MAG antiserum was not readily apparent.

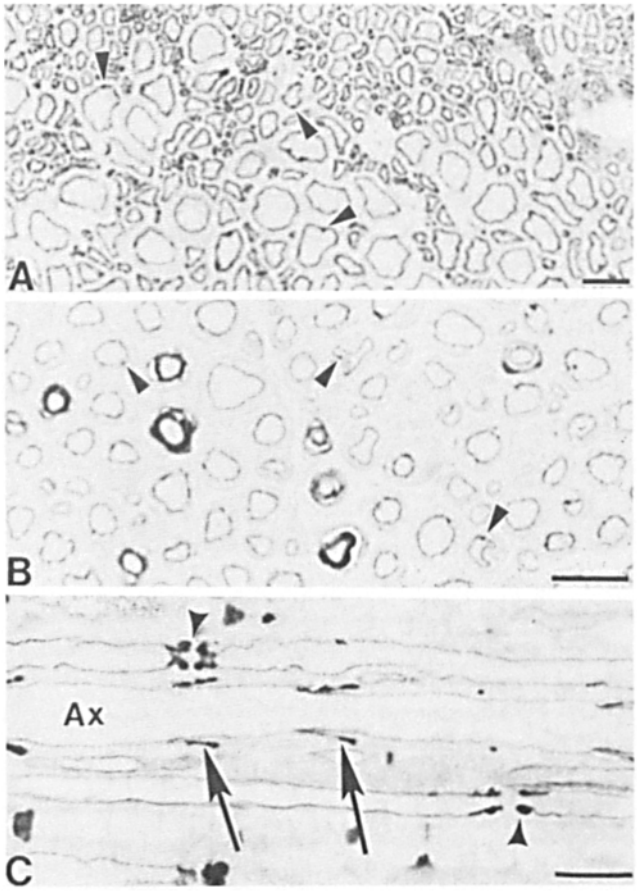


Figure 1. Distribution of MAG in 1-μm-thick Epon sections from adult rat spinal cord (*A*) and sciatic nerve (*B* and *C*). Periaxonal regions of CNS (*A*) and PNS (*B*) myelinated fibers are stained by MAG antiserum and often include an intense dot of staining (*A* and *B, arrowheads*). In addition, some PNS fibers have darker and thicker bands of staining (*B*) that represent enrichment in paranodal loops (*C, arrowheads*) and Schmidt-Lanterman incisures (*C, arrows*). *A* and *B*, cross sections; *C*, longitudinal section. Bars, 20 μm.

To characterize further the subcellular distributions of MAG in adult CNS and PNS myelin internodes, MAG was localized in ultrathin cryosections by immunogold procedures. The distribution of gold particles in Fig. 2 clearly shows that MAG is concentrated in periaxonal regions of

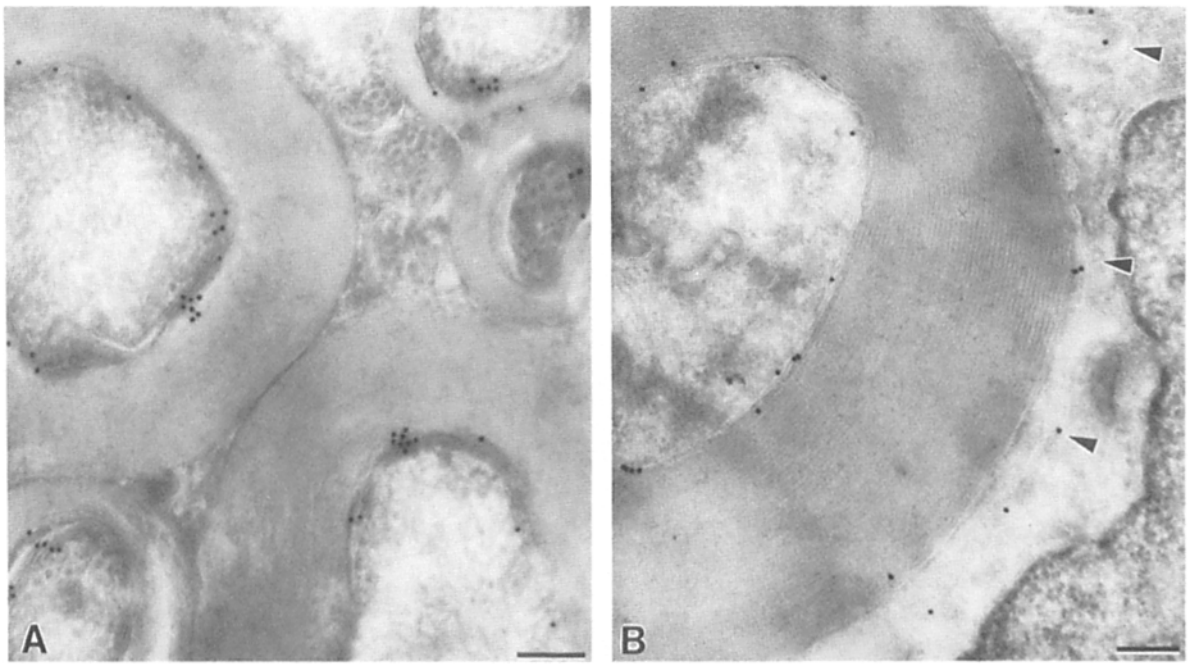


Figure 2. Distribution of MAG in ultrathin cryosections of CNS (*A*) and PNS myelinated (*B*) fibers from adult rats. Periaxonal regions of both CNS and PNS myelinated fibers are labeled by MAG antibodies. In the PNS (*B*) MAG antiserum also labels the membranes of the outer mesaxon (*arrowheads*). Bars, 0.2 μm.

both CNS and PNS myelin sheaths. Compact myelin and axoplasm contained few, if any, gold particles. However, gold particles show that MAG is also present in the outer mesaxon of PNS myelin sheaths (Fig. 2 *B*). This finding had been observed previously (31, 33) but is not always apparent in light micrographs of mature fibers. Gold particles were not associated consistently with oligodendrocyte membranes that were located at the outer perimeter of CNS myelinated fibers. However, occasional gold particles were located within the cytoplasmic domains of the outer loop and likely reflect MAG in transport between perinuclear and periaxonal regions.

Schmidt-Lanterman incisures, prominent structures in most PNS myelin internodes, were labeled by MAG antibodies in ultrathin cryosections (Fig. 3 *A*). Many of the larger CNS myelin sheaths contain oligodendrocyte cytoplasmic domains within regions of compact myelin. In MAG-stained electron micrographs, these CNS "incisure-like" structures were not labeled (Fig. 3 *B*).

PNS paranodal regions consistently contained gold particles (Fig. 3 *C*). These gold particles were very close to apposing Schwann cell membranes of the paranodal loops. In contrast, CNS paranodal regions contained few gold particles (Fig. 3 *D*), which, when present, were distributed diffusely within paranodal cytoplasm and likely reflect MAG in transit to periaxonal regions.

Controls

Several other myelin-specific proteins were localized and served as positive controls for immunolabeling procedures. Antiserum directed against myelin basic protein, a component of both CNS and PNS compact myelin, labeled this multilamellar membrane but did not react with membranes maintaining paranodal loops (Fig. 3 *E*). Antiserum specific for CNP, a protein shown previously to be localized in CNS paranodal loops at the light microscopic level (34), labeled CNS paranodal membranes in longitudinally oriented thin sections (Fig. 3 *F*). These results established that compact myelin and CNS paranodal membranes, two structures not labeled by MAG antibodies, do react with appropriate antisera.

Distribution of CNS MAG during Active Stages of CNS Myelination

Immunostaining of 1-μm-thick Epon sections of transversely oriented 7-d-old rat spinal cord with MAG antibodies (Fig. 4 *A*) gave staining patterns similar to those described previously in 20-μm-thick vibratome sections (28). MAG immunoreactivity was found within perinuclear cytoplasm of cells having the distribution and appearance of oligodendrocytes. This staining consisted of a less intense diffuse staining which was also present in thin processes extending toward developing myelin internodes, and more intense particulate staining which was most prominent in perinuclear cytoplasm and found occasionally within cellular processes extending toward myelin internodes. Small rings of MAG staining were scattered throughout the white matter tracts and indicate the presence of MAG along developing myelin internodes.

These light microscopic observations were extended to the subcellular level by using immunogold procedures and ultrathin cryosections (Fig. 4, *B–E* and Figs. 5–7). Because MAG has been proposed to be involved in oligodendrocyte–axon interactions, we were interested in whether it could be detected in oligodendrocyte processes during axonal ensheathment. Many small axons in these electron micrographs were surrounded by relatively electron-dense processes that had formed one or two spinal turns (Fig. 4 *B*). Gold particles representing MAG were abundant within these oligodendrocyte processes but were rarely found over axons, nuclei, or

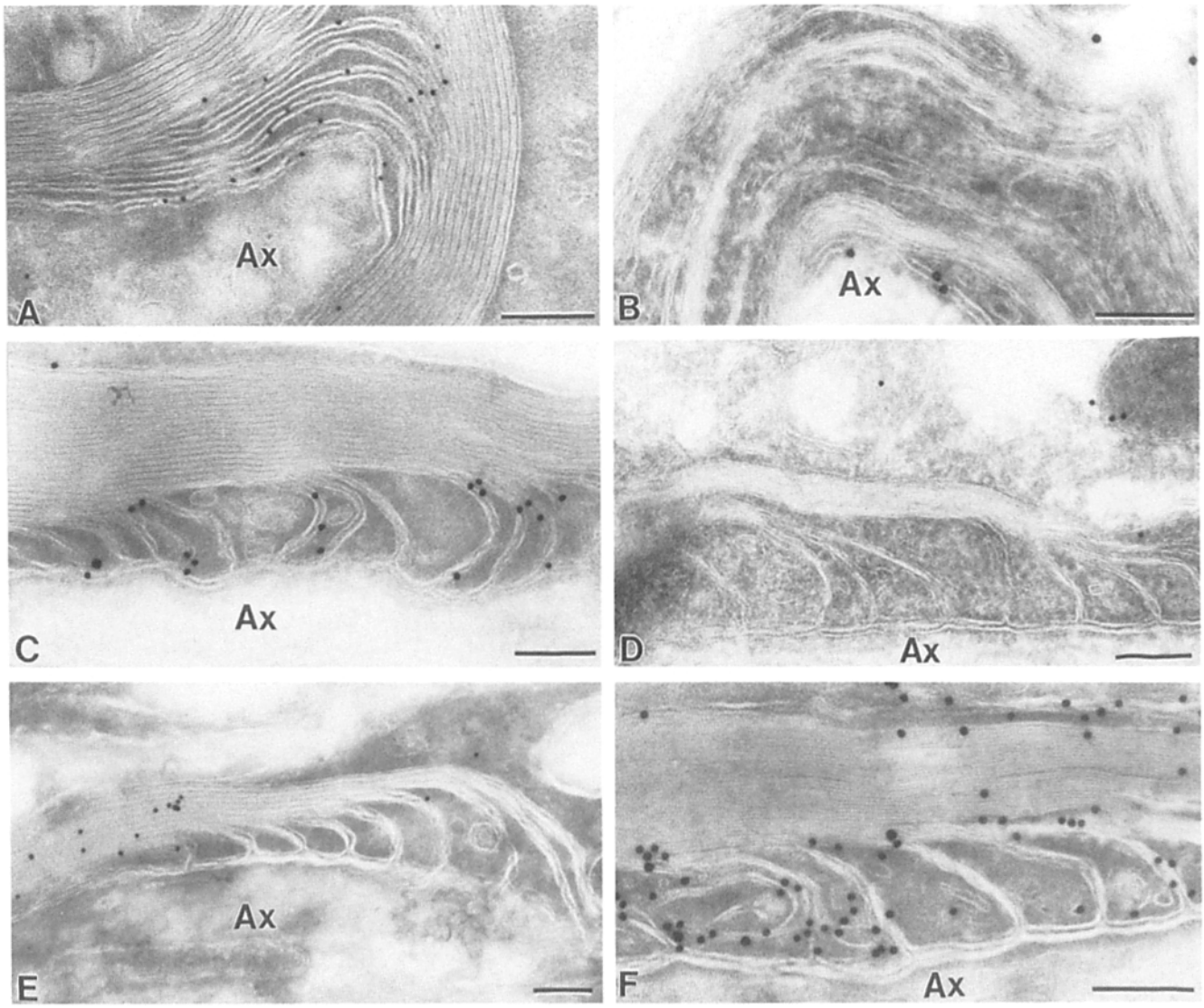
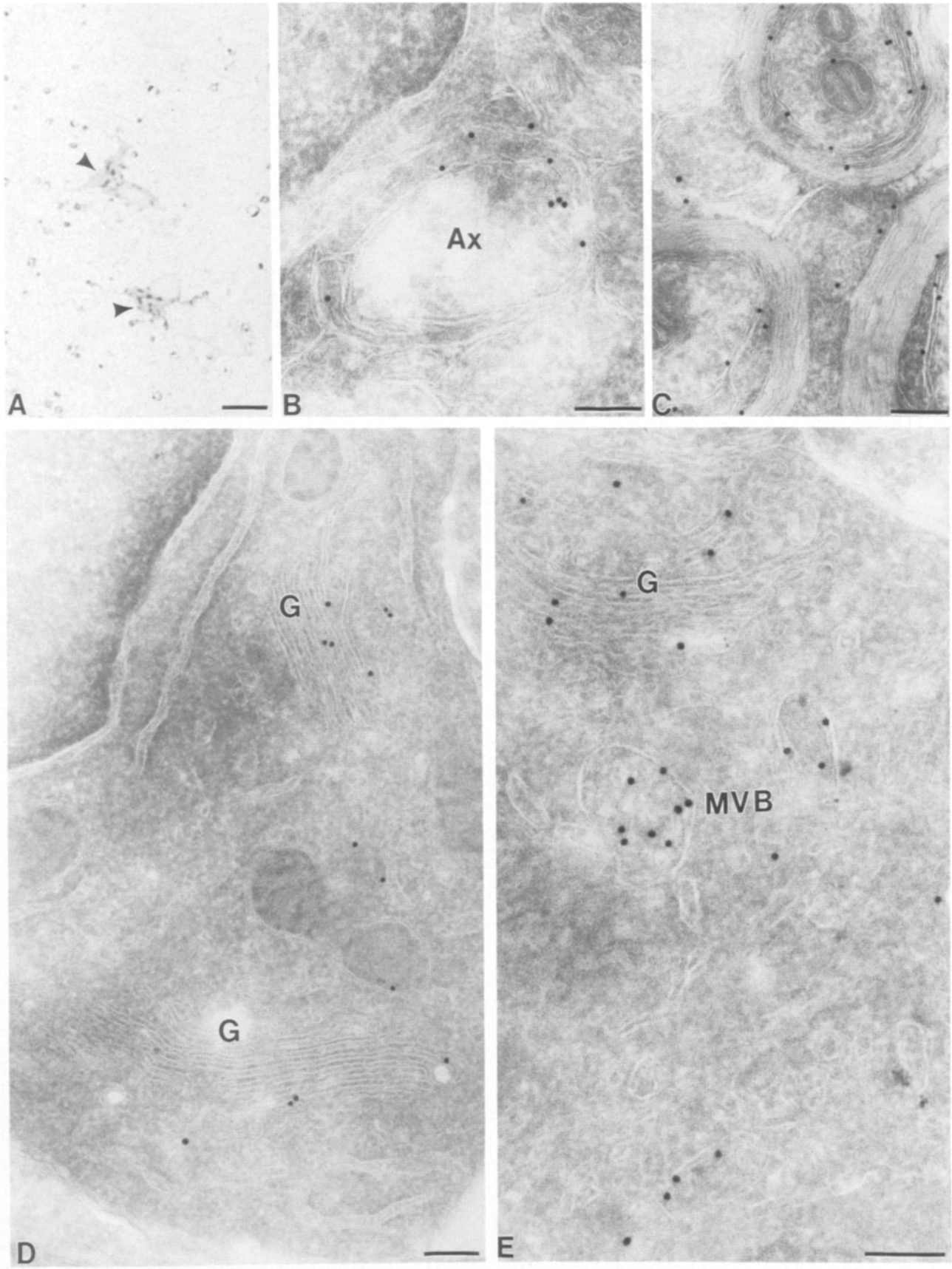


Figure 3. Comparison of MAG distribution in ultrathin cryosections from PNS (*A* and *C*) and CNS (*B* and *D*) myelin sheaths. MAG is readily detectable in Schmidt-Lanterman incisures (*A*) and paranodal regions (*C*) of PNS fibers but not in incisure-like structures (*B*) or paranodal membranes (*D*) in CNS fibers. As positive controls for immunogold procedures, MBP antiserum reacts with compact myelin (*E*) and CNP antibodies label paranodal membranes of CNS fibers (*F*). *Ax*, axon. Bars, 0.2 μm.

astrocytes. In micrographs of developing myelin sheaths, gold particles were abundant at the inner margins of developing myelin sheaths (Fig. 4 *C*), whereas few gold particles were found over compact myelin. Gold particles were consistently found within the cytoplasm of outer tongue processes.

In MAG-stained electron micrographs of oligodendrocyte perinuclear cytoplasm and processes, gold particles were associated with Golgi membranes (Fig. 4 *D*). Golgi profiles, most abundant in perinuclear cytoplasm, also extended into oligodendrocyte processes. In addition, relatively large membrane-bound organelles that often contained internal membranes were labeled intensely. These organelles had ultrastructural characteristics of MVBs. MAG-containing MVBs were abundant in oligodendrocyte perinuclear cytoplasm (Fig. 4 *E*) and in oligodendrocyte processes that extended to myelin internodes (Fig. 5 *A*). They were also found within the cytoplasm of outer tongue processes (Fig. 5 *B*), paranodal loops (Fig. 6 *B*), and, in rare instances, in periaxonal cytoplasm (data not shown). The labeling of Golgi membranes

Figure 4. Immunocytochemical localization of MAG in 7-d-old rat spinal cord. In 1-μm-thick Epon sections MAG antisera stains perinuclear cytoplasm of oligodendrocytes (*A*, *arrowheads*). Reaction product occurs as intense particulate staining and less intense diffuse staining that also extends into oligodendrocyte processes. Small rings of MAG staining are also present within the neuropil and represent the presence of MAG in developing myelin internodes (*B* and *C*). In electron micrographs of perinuclear cytoplasm (*D* and *E*), MAG is enriched in Golgi membranes (*G*) and in multivesicular bodies (*MVB*). Gold particles are also distributed randomly within oligodendrocyte cytoplasm and are likely to represent MAG in small vesicles. Bars: (*A*) 20 μm; (*B–E*) 0.2 μm.


A
B
Ax
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MVB

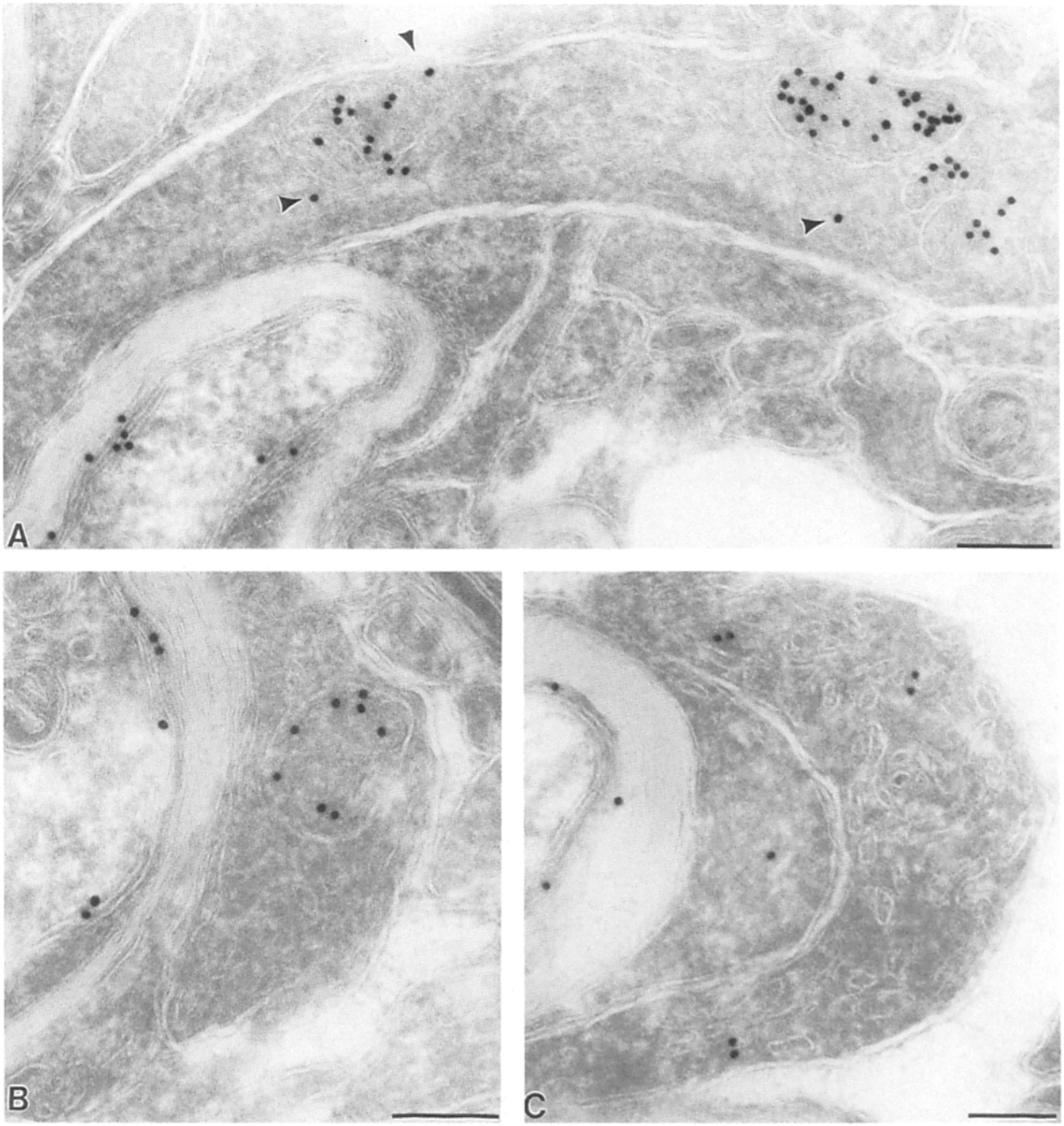

Figure 5. Immunocytochemical localization of MAG in ultrathin cryosections of 7-d-old rat spinal cord. MVBs are present in oligodendrocyte processes that extend to myelin internodes (*A*), and MVBs within outer tongue processes of myelin internodes (*B*) are enriched in MAG. Similar to perinuclear cytoplasm, gold particles are also distributed randomly within the cytoplasm of oligodendrocyte processes (*A*, *arrowheads*) and in outer tongue processes (*C*). Bars, 0.2 μm.

and these large MVBs corresponds to the intense particulate staining observed in light micrographs.

Gold particles were also distributed diffusely throughout all cytoplasmic domains of the oligodendrocyte. This diffuse cytoplasmic labeling was evident in electron micrographs of perinuclear regions (Fig. 4, *D* and *E*), processes extending to myelin internodes (Fig. 5 *A*), outer tongue processes (Fig. 5 *C*), paranodal loops (Fig. 6, *A* and *B*), and periaxonal regions of the myelin internode. This labeling likely corresponds to the less intense diffuse MAG staining found in oligodendrocyte perinuclear regions and processes in light micrographs and may represent the presence of MAG in small vesicles which are not always contrasted enough to visualize in cryosections.

To determine whether the enrichment of MAG in MVBs was regulated developmentally, we compared the distribution of MAG in electron micrographs from actively myelinating and adult animals. MAG-enriched MVBs were present within oligodendrocyte processes at the earliest stages of CNS myelination (Fig. 7 *A*). The axon in Fig. 7 *A* is covered

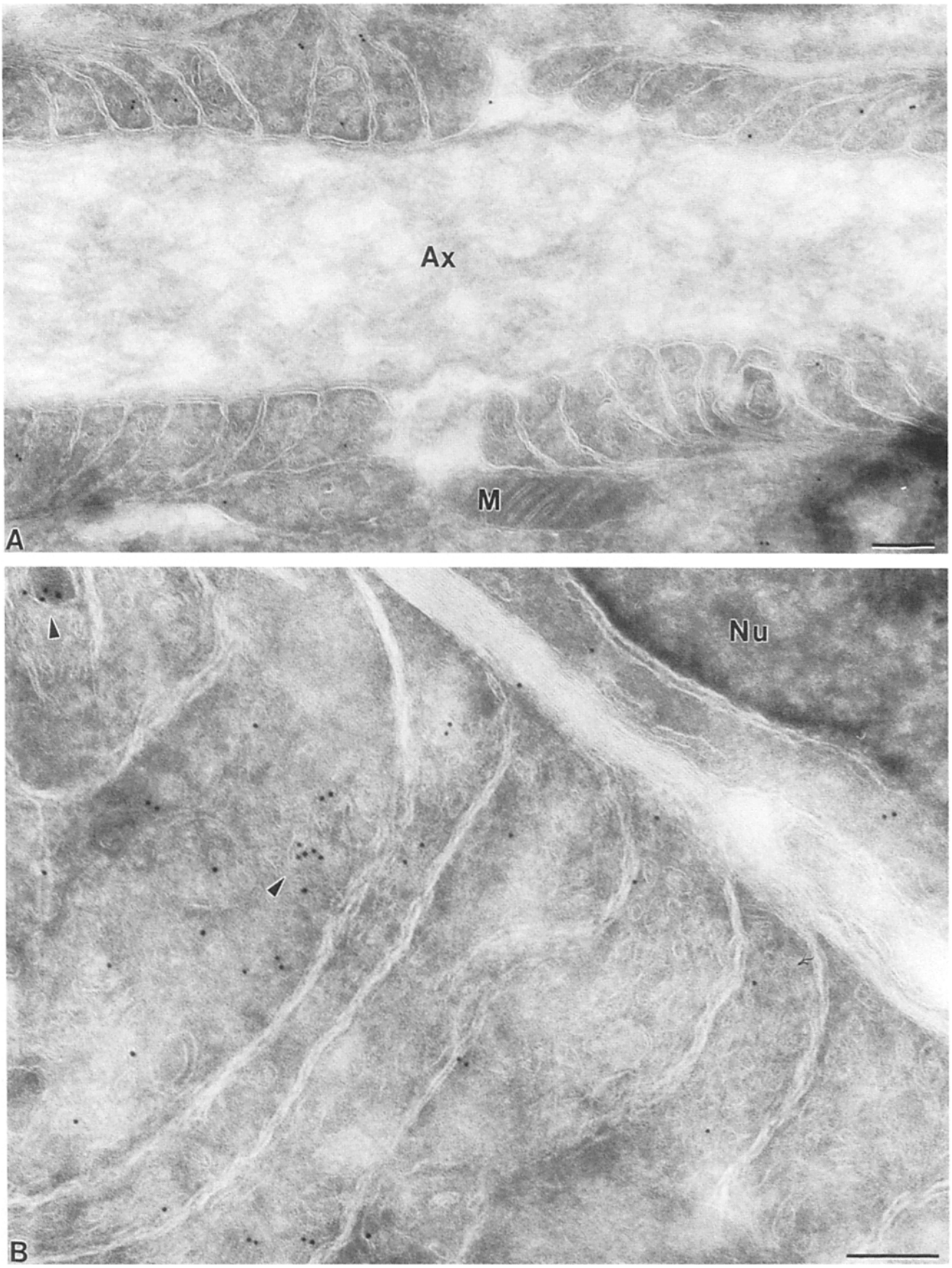

Figure 6. Immunocytochemical localization of MAG in paranodal cytoplasm from 7-d-old rat spinal cord. Majority of gold particles are distributed randomly; however, some clustering of gold particles in larger vesicles can be found (*B*, *arrowheads*). *Ax*, axon; *Nu*, nucleus; and *M*, mitochondria. Bars, 0.2 μm.

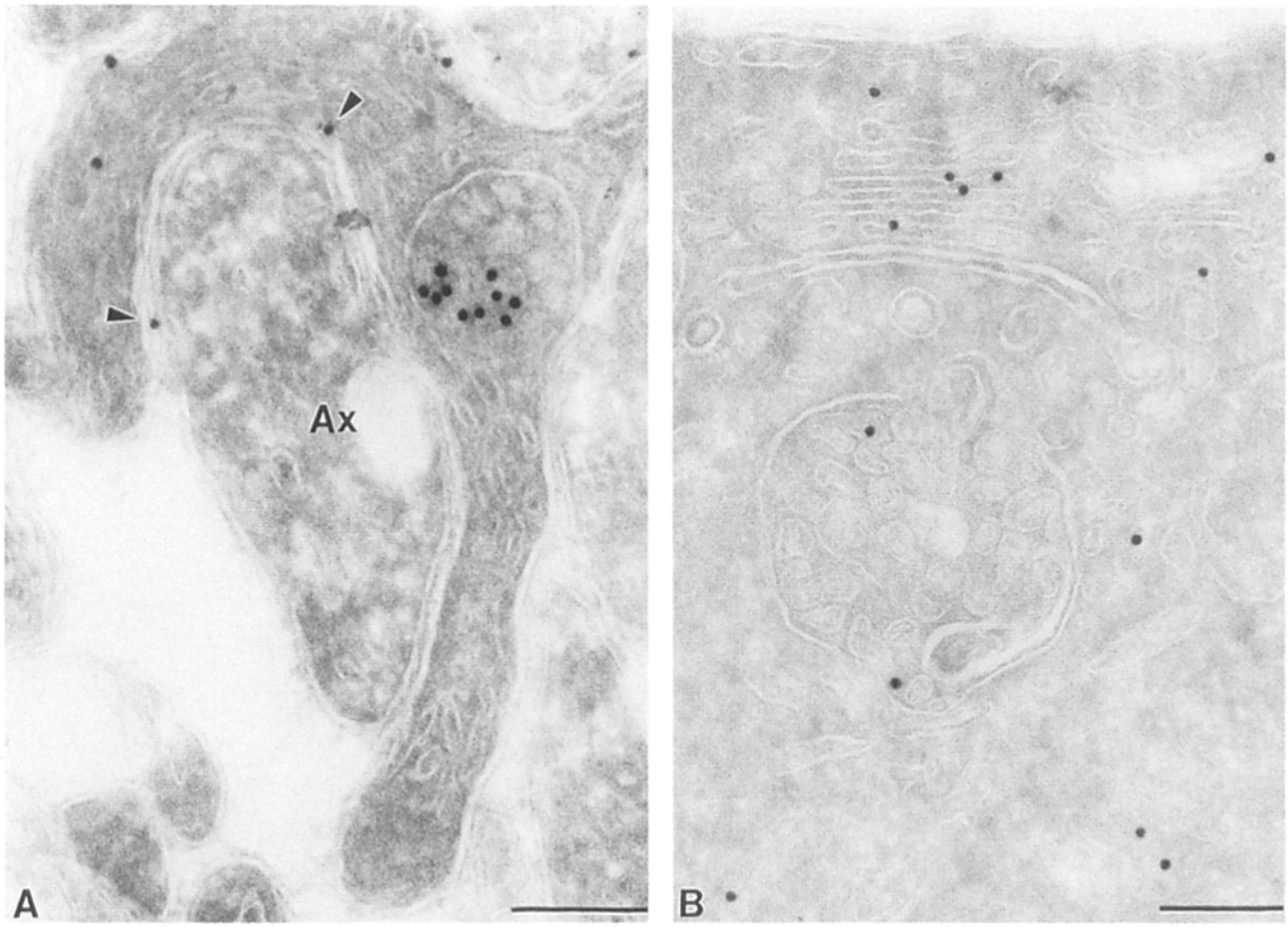

Figure 7. Immunocytochemical localization of MAG in ultrathin cryosections of 7-d-old (*A*) and adult (*B*) rat spinal cord. MAG-enriched MVBs and the presence of MAG in oligodendrocyte periaxonal membranes (*A*, *arrowheads*) are found at the earliest stages of CNS myelination. MVBs within oligodendrocytes from adult animals contain few gold particles (*B*). *Ax*, axon. Bars, 0.2 μm.

partially by a process that contains a MAG-labeled MVB. Gold particles (Fig. 7 *A*, *arrowheads*) were also found in close apposition to the periaxonal membrane of this process, indicating that MAG can be associated with oligodendrocyte membranes during their initial axonal ensheathment. MVBs were also found within the cytoplasm of oligodendrocytes from adult spinal cord. In contrast to actively myelinating oligodendrocytes, however, these MVBs were either not labeled or labeled slightly (one to two gold particles) (Fig. 7 *B*). The vast majority of label present in adult oligodendrocytes was associated with Golgi membranes or distributed diffusely throughout the cytoplasm (Fig. 7 *B*). These results indicated that association of MAG with MVBs is developmentally down-regulated but not eliminated in adult animals.

Discussion

Distribution of MAG in CNS and PNS Myelin Internodes

We have compared the subcellular distribution of MAG in the central and peripheral nervous system, using light and electron microscopic immunocytochemistry. The data confirm previous reports that MAG is enriched in periaxonal membranes of both CNS and PNS myelin internodes and in Schmidt-Lanterman incisures, paranodal loops and outer mesaxons of PNS myelin internodes (16, 28, 33–35). In addition, MAG is associated with oligodendrocyte processes during their initial ensheathment of CNS axons and enriched in multivesicular bodies located within actively myelinating oligodendrocytes. Our results also indicate that CNS MAG is not enriched in the plasma membrane of oligodendrocyte perikarya or processes that connect oligodendrocytes and myelin internodes. Unlike PNS myelin internodes, MAG is not enriched in membranes maintaining paranodal loops, incisure-like structures, or outer tongue processes of CNS myelin internodes.

The association of MAG with oligodendrocyte processes during initial axonal ensheathment, and its selective localization in mature CNS periaxonal membranes provide additional support for the participation of MAG in oligodendrocyte–axon interaction. The significant amino acid sequence homology between the extracellular domain of MAG and other cell adhesion and ligand-binding molecules also supports the notion that MAG participates in myelin-forming cell–axon contact. Confirmation of this hypothesis, however, awaits characterization of an axolemmal molecule that binds to MAG. The possibility that MAG serves as a membrane spacer that modulates cell adhesion would also be consistent with current data. The enrichment of MAG in the outer mesaxon, Schmidt-Lanterman incisures, and paranodal loops of PNS myelin internodes indicates that PNS MAG has addi-

tional functions unrelated to axonal contact. These are discussed in detail elsewhere (25, 30, 33).

Intracellular Transport of MAG in Oligodendrocytes

This study describes for the first time the enrichment of MAG in MVBs during active stages of CNS myelination. MAG-enriched MVBs were detected in oligodendrocyte perinuclear regions, processes extending to myelin internodes, outer tongue processes, and paranodal regions. Therefore, it is reasonable to expect that these structures are actively involved in the translocation of MAG between oligodendrocyte perikarya and periaxonal membranes. A major question is whether these MVBs are traveling in an anterograde or retrograde direction.

Anterograde Transport

Since little precedence exists for the delivery of plasma membrane component via MVBs, we assume that anterograde transport is unlikely, although it cannot presently be ruled out. The immense task of forming multiple myelin internodes simultaneously could benefit from the delivery of a bolus of MAG-containing vesicles to individual myelin internodes, and could also explain why MAG is not similarly enriched in MVBs during Schwann cell myelination. Given that MAG has a classical transmembrane amino acid sequence, it is reasonable to assume that MAG remains membrane-associated during its transport to periaxonal membranes. Thus, the less intense diffuse labeling of oligodendrocyte cytoplasm produced by MAG antibodies is likely to represent the presence of MAG in small vesicles. The anterograde transport of MAG to periaxonal membranes via small vesicles would be consistent with generally accepted mechanisms for transport of intrinsic membrane proteins from Golgi to cell surface. In addition, double-labeling EM studies have shown that the major structural proteins of myelin (proteolipid protein and MBP in the CNS; P_0 and MBP in the PNS) are not enriched in MVBs (Trapp, B. D., unpublished data). Antibodies directed against PLP and P_0, on the other hand, produce diffuse labeling of oligodendrocyte and Schwann cell cytoplasm, consistent with an association of these intrinsic membrane proteins with small vesicles during transport to myelin internodes.

Retrograde Transport: Are MAG-enriched MVBs Part of an Endocytic Pathway?

A wealth of literature associates multivesicular bodies with retrograde transport of membrane components from surface membranes via receptor-mediated endocytosis (3, 13). It is generally assumed that clathrin-coated pits and vesicles associated with or close to cellular plasma membranes serve as intermediates in such endocytosis. Once internalized, these vesicles lose their clathrin coat and enter a ubiquitous prelysosomal compartment referred to as endosomes (13), receptosomes (19), or compartment of uncoupling receptor and ligands (CURL) (9). MAG-enriched MVBs are ultrastructurally indistinguishable from these prelysosomal organelles described in a variety of cell types (9–12). Our data are most consistent, therefore, with the association of MAG with an endocytic pathway that originates in the periaxonal membrane of CNS myelin internodes and terminates in oligodendrocyte perinuclear cytoplasm. This hypothesis is supported by the abundant and consistent association of clathrin-coated pits with oligodendrocyte periaxonal membranes during active stages of myelination, and by enrichment of cathepsin D, a lysosomal enzyme, in some of the MAG-positive MVBs located in oligodendrocyte perinuclear regions (Trapp, B. D., unpublished observations).

If MAG is associated with an endocytic pathway, a critical question is whether it is actively involved as a receptor for components present in the axolemma/periaxonal space or passively involved as a structural protein of the periaxonal membrane without bound ligand. Several observations suggest that the 72-kD polypeptide of MAG is actively involved in receptor-mediated endocytosis. MAG has significant amino acid homologies with, and an overall structural similarity to, the polyimmunoglobulin receptor, another member of the immunoglobulin gene superfamily actively involved in an endocytic process (17). Significant enrichment of MAG within MVBs occurs in oligodendrocytes during active myelination when the majority of MAG has the 72-kD polypeptide. Similar enrichment did not occur in adult oligodendrocytes when the majority of MAG has the 67-kD polypeptide. EM studies with antibodies specific for the 72-kD MAG polypeptide (kindly provided by David Colman, Columbia University) confirmed its presence in MVBs during active CNS myelination (Trapp, B. D., unpublished observation). Whether the 67-kD polypeptide is also present within oligodendrocyte MVBs cannot be determined at present because specific antibodies are not available. However, MAG is not enriched in MVBs in actively myelinating Schwann cells where the 67-kD polypeptide represents at least 95% of the total at all ages of development (8, 36, 18). Since MAG-containing Schwann cell membranes contain numerous coated pits during active stages of myelination, this observation also suggests that the 67-kD MAG polypeptide is segregated from Schwann cell membranes during endocytosis.

It is generally accepted that axons modulate the process of myelination; i.e., length of myelin internode and number of spiral wraps. How this modulation occurs on a molecular level is unknown, although it is likely to be mediated by an interaction between the axolemma and the oligodendrocyte periaxonal membrane. The myelin-associated glycoprotein and coated pits are associated with oligodendrocyte periaxonal membranes from the onset of axonal ensheathment and therefore are candidates for transducing axonal influences. Regardless of the precise role of MAG, receptor-mediated endocytosis of an axonal component should be considered as one mechanism for modulating axonal influences on oligodendrocyte myelination.

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References

1. Arquint, M., J. Roder, L. S. Chia, J. Down, D. Wilkinson, H. Bayley, P. Braun, and R. Dunn. 1987. Molecular cloning and primary structure of myelin-associated glycoproteins. *Proc. Natl. Acad. Sci. USA.* 84: 600–604.

2. Bernier, L., F. Alvarez, E. M. Norgard, D. W. Raible, A. Mentaberry, J. G. Schembri, D. D. Sabatini, and D. R. Colman. 1987. Molecular cloning of a 2',3'-cyclic nucleotide 3'-phosphodiesterase: mRNAs with different 5' ends encode the same set of proteins in neurons and lymphoid tissues. *J. Neurosci.* 7:2703–2710.
3. Brown, M. S., R. G. W. Anderson, and J. L. Goldstein. 1983. Recycling receptors: the round-trip itinerary of migrant membrane proteins. *Cell.* 32:663–667.
4. Colman, D. R., G. Kreibich, A. B. Frey, and D. D. Sabatini. 1982. Synthesis and incorporation of myelin polypeptide into CNS myelin. *J. Cell Biol.* 95:598–608.
5. Cunningham, B. A., J. J. Hemperly, B. A. Murray, E. A. Prediger, R. Brackenberry, and G. M. Edelman. 1987. Neural adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation and alternative RNA splicing. *Science (Wash. DC).* 236:799–806.
6. Dobersen, M. J., J. A. Hammer, A. B. Noronha, T. D. MacIntosh, B. D. Trapp, R. O. Brady, and R. H. Quarles. 1985. Generation and characterization of monoclonal antibodies to the myelin-associated glycoprotein. *Neurochem. Res.* 10:423–437.
7. Frail, D. E., and P. E. Braun. 1984. Two developmentally regulated messenger RNAs differing in their coding region may exist for the myelin-associated glycoprotein. *J. Biol. Chem.* 259:14857–14862.
8. Frail, D. E., H. deF. Webster, and P. E. Braun. 1985. Developmental expression of myelin-associated glycoprotein in the peripheral nervous system is different from that in the central nervous system. *J. Neurochem.* 45:1308–1310.
9. Geuze, H. J., J. W. Slot, G. J. A. M. Strous, H. F. Lodish, and A. L. Schwartz. 1983. Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. *Cell.* 32:277–287.
10. Geuze, H. J., J. W. Slot, G. J. A. M. Strous, J. Peppard, K. Von Figura, A. Hasilik, and A. L. Schwartz. 1984. Intracellular receptor sorting during endocytosis: comparative immunoelectron microscopy of multiple receptors in rat liver. *Cell.* 37:195–204.
11. Geuze, H. J., J. W. Slot, and A. L. Schwartz. 1987. Membranes of sorting organelles display lateral heterogeneity in receptor distribution. *J. Cell Biol.* 104:1715–1723.
12. Geuze, H. J., W. Stoorvogel, G. J. Strous, J. W. Slot, J. E. Bleekemolen, and I. Mellman. 1988. Sorting of mannose 6-phosphate receptors and lysosomal membrane proteins in endocytic vesicles. *J. Cell Biol.* 107: 2491–2501.
13. Helenius, A., I. Mellman, D. Wall, and A. Hubbard. 1983. Endosomes. *Trend Biochem. Sci.* 7:245–250.
14. Lai, C., M. A. Brow, K.-A. Nave, A. B. Noronha, R. H. Quarles, F. E. Bloom, R. J. Milner, and J. G. Sutcliffe. 1987. Two forms of 1B236/myelin-associated glycoprotein (MAG), a cell adhesion molecule for postnatal neural development, are produced by alternative splicing. *Proc. Natl. Acad. Sci. USA.* 84:4337–4341.
15. Lai, C., J. B. Watson, F. L. Bloom, J. G. Sutcliffe, and R. J. Milner. 1988. Neural protein 1B236/myelin-associated glycoprotein (MAG) defines a subgroup of the immunoglobulin superfamily. *Immunol. Rev.* 100:127–149.
16. Martini, R., and M. Schachner. 1986. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. *J. Cell Biol.* 103:2439–2448.
17. Mostov, K. E., M. Friedlander, and G. Blobel. 1984. The receptor for trans-epithelial transport of IgA and IgM contains multiple immunoglobulin domains. *Nature (Lond.).* 308:37–43.
18. Noronha, A. B., J. A. Hammer, C. Lai, M. Kiel, R. J. Milner, J. G. Sutcliffe, and R. H. Quarles. 1989. Myelin-associated glycoprotein (MAG) and rat brain specific 1B236 protein: Mapping of epitopes and demonstration of immunological identity. *J. Mol. Neurosci.* 1:159–170.
19. Pastan, I., and M. C. Willingham. 1983. Receptor-mediated endocytosis: coated pits, receptosomes and the Golgi. *Trends Biochem. Sci.* 7:250–254.
20. Poltorak, M., R. Sadoul, G. Keilhauer, C. Landa, T. Fahrig, and M. Schachner. 1987. Myelin-associated glycoprotein, a member of the L2/HNK-1 family of neural cell adhesion molecules, is involved in neuron-oligodendrocyte and oligodendrocyte-oligodendrocyte interaction. *J. Cell Biol.* 105:1893–1899.
21. Quarles, R. H. 1979. Glycoproteins in myelin and myelin-related membranes. *In* Complex Carbohydrates of Nervous Tissue. R. U. Margolis and R. K. Margolis, editors. Plenum Publishing Corp., New York. 209–233.
22. Quarles, R. H., J. L. Everly, and R. O. Brady. 1973. Evidence for the close association of a glycoprotein with myelin. *J. Neurochem.* 21:1177–1191.
23. Quarles, R. H., J. L. Everly, and R. O. Brady. 1973. Myelin-associated glycoprotein: A developmental change. *Brain Res.* 58:506–509.
24. Quarles, R. H., D. Johnson, R. O. Brady, and N. H. Sternberger. 1981. Preparation and characterization of antisera to the myelin-associated glycoprotein. *Neurochem. Res.* 6:1115–1127.
25. Quarles, R. H., J. A. Hammer, and B. D. Trapp. 1989. The immunoglobulin gene superfamily and myelination. *In* Dynamic Interactions of Myelin Proteins. G. Hashim, editor. Alan R. Liss, Inc., New York. In press.
26. Salzer, J. L., W. P. Holmes, and D. R. Colman. 1987. The amino acid sequences of the myelin-associated glycoproteins: homology to the immunoglobulin gene superfamily. *J. Cell Biol.* 104:957–965.
27. Slot, J. W., and H. J. Geuze. 1984. Gold markers for single and double immunolabeling of ultrathin cryosections. *In* Immunolabeling for Electron Microscopy. J. M. Polak and I. M. Varndell, editors. Elsevier Science Publishing Co., Inc., New York 129–142.
28. Sternberger, N. H., R. H. Quarles, Y. Itoyama, and H. deF. Webster. 1979. Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rats. *Proc. Natl. Acad. Sci. USA.* 76:1510–1514.
29. Tokuyasu, K. T. 1986. Application of cryoultramicrotomy to immunocytochemistry. *J. Microsc. (Oxf.).* 143:139–149.
30. Trapp, B. D. 1988. Distribution of the myelin-associated glycoprotein and P_0 protein during myelin compaction in quaking mouse peripheral nerve. *J. Cell Biol.* 107:675–685.
31. Trapp, B. D., B. S. Andrews, A. Wong, M. O'Connell, and J. W. Griffin. 1989. Co-localization of the myelin-associated glycoprotein and the microfilament components, F-actin and spectrin, in Schwann cells of myelinated nerve fibres. *J. Neurocytol.* 18:47–60.
32. Trapp, B. D., L. Bernier, S. B. Andrews, and D. R. Colman. 1988. Cellular and subcellular distribution of 2',3'-cyclic nucleotide 3'-phosphodiesterase and its mRNA in the rat centeral nervous system. *J. Neurochem.* 51:859–68.
33. Trapp, B. D., and R. H. Quarles. 1982. Presence of the myelin-associated glycoprotein correlates with alterations in the periodicity of peripheral myelin. *J. Cell Biol.* 92:877–882.
34. Trapp, B. D., and R. H. Quarles. 1984. Immunocytochemical localization of the myelin-associated glycoprotein: fact or artifact? *J. Neuroimmunol.* 6:231–249.
35. Trapp, B. D., R. H. Quarles, and K. Suzuki. 1984. Immunocytochemical studies of quaking mice support a role for the myelin-associated glycoprotein in forming and maintaining the periaxonal space and periaxonal cytoplasmic collar of myelinating Schwann cells. *J. Cell Biol.* 99:594–606.
36. Tropak, M. B., P. W. Johnson, R. J. Dunn, and J. C. Roder. 1988. Differential splicing of MAG transcripts during CNS and PNS development. *Mol. Brain Res.* 4:143–155.
37. Williams, A. F. 1987. A year in the life of the immunoglobulin superfamily. *Immunol. Today.* 8:298–303.