Presence of the Myelin-associated Glycoprotein Correlates with Alterations in the Periodicity of Peripheral Myelin

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ABSTRACT The myelin-associated glycoprotein (MAG) is an integral membrane protein (≃100,000 mol wt) which is a minor component of purified peripheral nervous system (PNS) myelin. In the present study, MAG was localized immunocytochemically in 1-μm thick Epon sections of 7-d and adult rat peripheral nerves, and its localization was compared to that of the major structural protein (Po) of PNS myelin. To determine more precisely the localization of MAG, immunostained areas in 1 μm sections were traced on electron micrographs of identical areas from adjacently cut thin sections. MAG was localized in periaxonal membranes, Schmidt-Lantermann incisures, paranodal membranes, and the outer mesaxon of PNS myelin sheaths. Compact regions of PNS myelin did not react with MAG antiserum. The results demonstrate MAG's presence in "semi-compact" Schwann cell or myelin membranes that have a gap of 12–14 nm between extracellular leaflets and a spacing of 5 nm or more between cytoplasmic leaflets. In compact regions of the myelin sheath which do not contain MAG, the cytoplasmic leaflets are "fused" and form the major dense line, whereas the extracellular leaflets are separated by a 2.0 nm gap appearing as paired minor dense lines. Thus, it is proposed that MAG plays a role in maintaining the periaxonal space, Schmidt-Lantermann incisures, paranodal myelin loops, and outer mesaxon by preventing "complete" compaction of Schwann cell and myelin membranes. The presence of MAG in these locations also suggests that MAG may serve a function in regulating myelination in the PNS.

Myelin-associated glycoprotein (MAG) is an integral membrane glycoprotein (≃100,000 mol wt) which is quantitatively a minor component of purified CNS and PNS myelin. Its presence in purified CNS myelin was first demonstrated by radioactive labeling with sugar precursors (10). Subfractionation experiments suggested that MAG was not present in compact CNS myelin but was concentrated in closely associated oligodendroglial membranes (11). This hypothesis was confirmed by immunocytochemical studies (15). When sections of developing rat brain were treated with MAG antiserum, oligodendrocytes, and myelin sheaths were selectively stained. When compact myelin sheaths grew in thickness, only periaxonal portions of the myelin sheaths were stained. Compact myelin lamellae did not react with MAG antiserum. An unexpected finding in the immunocytochemical study (15) was the staining for MAG in PNS myelin sheaths, because previous biochemical studies had not detected MAG in PNS myelin (3). However, reexamination with biochemical and immunochemical techniques has now also revealed the presence of MAG in the PNS (4). In the PNS, MAG antiserum stained periaxonal membranes, Schmidt-Lantermann incisures, and paranodal portions of PNS myelin sheaths (14, 15).

Integral membrane glycoproteins are believed to be involved in cell-cell recognition and in interactions between cell membranes (5). The periaxonal localization of MAG in CNS and PNS myelin sheaths suggests a possible role for this glycoprotein in glia-axon interactions. The present study describes the immunocytochemical localization of MAG in 1-μm thick Epon sections of developing and adult rat peripheral nerves. MAG localization is compared to that of the major structural protein (Po) of PNS myelin. To determine more precisely the localization of MAG, immunostained areas in 1-μm thick Epon sections were tracted on electron micrographs of adjacent thin sections.

MATERIALS AND METHODS

Tissue

Seven-day-old and adult Sprague-Dawley rats were fixed by intracardiac perfusion with a solution containing 1.5% glutaraldehyde and 0.5% paraformaldehyde in 0.08 M Sorensen's buffer. Segments from the trigeminal and sciatic nerves were removed and placed in the fixative for an additional hour. To obtain better fixation of adult sciatic nerves, additional animals were perfused through the abdominal aorta. The tissue was postfixed in 2% osmium tetroxide, dehydrated in ethanol, and embedded in Epon. 1 μm-thick sections were cut with glass

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knives, mounted on glass slides, and encircled with a diamond scribe. Six serial sections were cut from the 7-d-old and adult nerves. Even numbered sections were stained immunocytochemically with MAG antiserum; the others were stained with Po antiserum. From the 7-d trigeminal nerve, additional 1-μm thick sections were cut adjacent to thin sections with silver interference colors. The 1-μm thick sections were stained with MAG antiserum. The thin sections were mounted on formvar-coated slot grids, stained with uranyl acetate and lead citrate, and examined in a Philips 400 electron microscope. Areas of MAG staining in 1-μm thick sections were photographed, and their negative images were enlarged and compared to the fine structure in electron micrographs from identical areas of the adjacently cut thin sections.

Immunostaining Procedure

1-μm thick Epon sections mounted on glass slides were placed in a 60–80°C oven for 24–48 h. Epon was removed from the sections by sodium ethoxide as previously described (18). The slides were then placed in 0.2% hydrogen peroxide for 5 min, rinsed in 0.5 M Tris buffer, and stained with a (1:250) dilution of MAG antiserum or a (1:500) dilution of Po antiserum by the peroxidase-antiperoxidase (PAP) method as previously described (18). All sections were examined microscopically with bright-field illumination.

MAG and Po antisera were prepared in rabbits. The purity of the MAG and Po proteins used in preparing the antisera and the immunological and immunocytochemical specificity of these antisera have been described (6, 13, 15, 17, 18).

RESULTS

In 1-μm thick sections of 7-d trigeminal nerves treated with Po antiserum, compact myelin sheaths were intensely stained (Fig. 1). Schmidt-Lantermann incisures remained unstained, and thin myelin sheaths stained with less intensity than thick sheaths. When adjacent sections were treated with MAG antiserum (Fig. 2), compact regions of myelin sheaths were not stained. A continuous narrow ring of MAG staining was present on the inner margin of all compact myelin sheaths. Part of the external margins of compact myelin sheaths were also stained. This staining consisted of a small granule or a narrow band which partially surrounded the fiber. In addition to the narrow band of MAG staining on the inner and outer margins of the myelin sheaths, occasional fibers were surrounded by thick intense bands of staining. These bands partially or totally surrounded the axons, and they were often separated from the periaxonal ring of staining by unstained areas. Comparison of MAG and Po staining in adjacent 1-μm sections demonstrated that many of the thick bands which stained intensely for MAG represented Schmidt-Lantermann incisures or paranodal regions of myelin sheaths. The Schmidt-Lantermann incisures and paranodal myelin loops not stained by Po antiserum were stained intensely by MAG antiserum. Perinuclear areas of Schwann cell cytoplasm that were stained by Po antiserum (18) were not stained by MAG antiserum. Schwann cell cytoplasm and periaxonal regions of unmyelinated fibers were not stained by either Po or MAG antisera.

In sections of adult sciatic nerves, Po antiserum stained compact myelin intensely and uniformly, whereas Schmidt-Lantermann incisures remained unstained (Fig. 3). In adjacent sections treated with MAG antiserum (Fig. 4), compact regions of myelin sheaths did not stain. All compact myelin sheaths contained a thin ring of periaxonal staining. The staining on the outer margins of the myelin sheaths was restricted to a small granule. Intense staining of Schmidt-Lantermann incisures and paranodal myelin was also present in adult nerves.

The distribution of MAG staining in 7-d trigeminal nerves was traced on electron micrographs of the same fibers in adjacent thin sections (Figs. 5 and 6). This comparison showed conclusively that all Schmidt-Lantermann incisures and paranodal regions examined were stained intensely by MAG antiserum. The comparison also demonstrated that the thin band and granule of MAG staining on the outer margin of the myelin sheath corresponded to the outer mesaxon (Fig. 6).

DISCUSSION

The immunocytochemical localization of MAG in Epon sections of rat peripheral nerve has confirmed and extended earlier findings of MAG in periaxonal myelin membranes, Schmidt-Lantermann incisures, and paranodal myelin loops (14, 15). In addition, it has demonstrated the presence of MAG in the outer mesaxon of PNS myelin sheaths (Fig. 7).

Comparison of MAG staining in 1-μm sections to the fine structure in adjacently cut thin sections has permitted ultrastructural analysis of areas stained by MAG antiserum. There was a strict correlation between the presence of MAG and the periodicity of the Schwann cell or myelin membranes (Table I). MAG is present in "semi-compact" Schwann cell or myelin membranes that have a gap of 5 nm or more between cytoplasmic leaflets and a spacing of 12–14 nm between extracellular leaflets (7). In compact regions of the myelin sheath that do not contain MAG, the cytoplasmic leaflets of myelin membranes appear "fused" and form the major dense line, whereas the extracellular leaflets are separated by a 2.0 nm gap, appearing as paired minor dense lines (7). Thus, it is proposed that MAG plays a structural role in maintaining the periaxonal space, Schmidt-Lantermann incisures, paranodal myelin loops, and the outer mesaxons by preventing "complete" compaction of their membranes. It has been concluded from ultrastructural analysis of developing rat peripheral nerve that growth of the myelin spiral occurs by insertion of molecules exposed to Schwann cell cytoplasm (19). Therefore, the presence of MAG in these locations also suggests that MAG may serve a function in regulating myelination in the PNS.

From the usual orientation of glycoproteins in cell surface membranes, the carbohydrate moieties of MAG would be expected to be on the extracellular side of the Schwann cell or myelin membranes. Because MAG contains ~30% sugar (2), the bulk and polarity of these oligosaccharide moieties could account for the 10–12 nm spacing of the extracellular leaflets or intraperiod lines of MAG containing membranes when compared to the 2.0 nm spacing of compact PNS myelin. Similarly, the location of Po proteins' sugar moieties on the extracellular side of the intraperiod line of PNS myelin (8, 9, 21) has been proposed to account, in part, for the greater periodicity of compact PNS myelin when compared to CNS myelin, which contains the highly hydrophobic proteolipid protein as a major structural protein (12, 21).

The cytoplasmic sides of MAG-containing membranes are not fused to form a major dense line and the amount of cytoplasm between these membranes varies extensively. The lower limit of 5 nm occurs in the periaxonal membranes of adult fibers (7). Much larger areas of cytoplasm occur between cytoplasmic sides of Schmidt-Lantermann incisure, lateral loops, and outer mesaxon membranes (7). If MAG is a transmembrane protein, the unglycosylated portion would be located on the cytoplasmic side of the membrane (5). This part of the MAG molecule may have a direct effect in preventing the fusion of the cytoplasmic side of the membrane; or indirectly MAG may mask other molecules such as Po and basic protein, which could be responsible for the fusion. The separation of the cytoplasmic sides of MAG-containing membranes provides a pathway through which cytoplasm on the outside of the myelin sheath is confluent with that on the inside via the Schmidt-Lantermann incisures and paranodal myelin loops

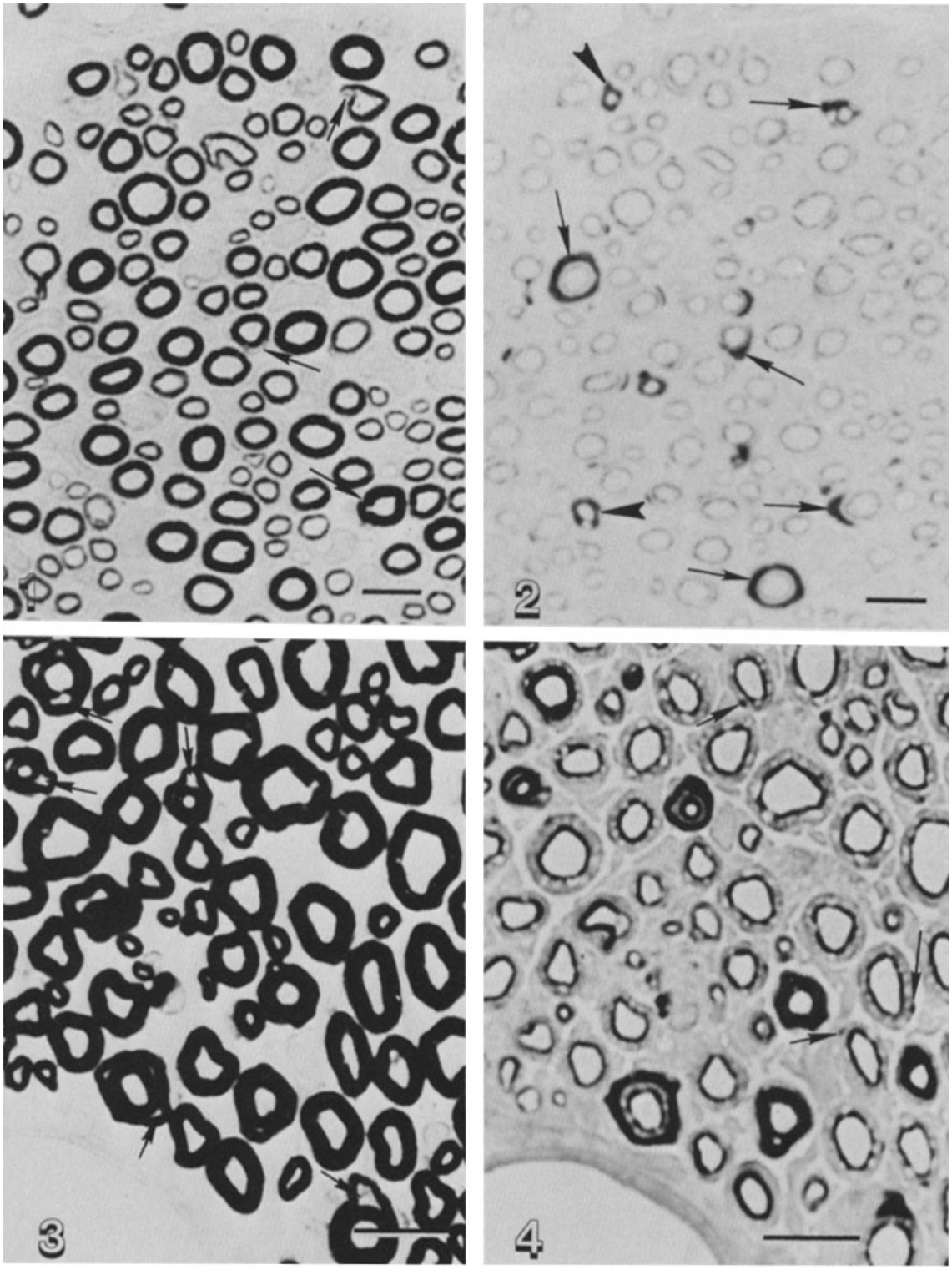

FIGURE 1 Transverse section of 7-d trigeminal nerve stained with Po antiserum (1:500). thick myelin sheaths are intensely stained; thinner sheaths are surrounded by less intense staining. Areas of Schmidt-Lantermann incisures (arrows) are unstained. Bright field. Bar, 10 μm. × 950.

FIGURE 2 This section is adjacent to that shown in Fig. 1; it was stained with MAG antiserum (1:250). All myelinated axons are surrounded by a thin ring of periaxonal staining. Portions of the external margins of compact myelin sheaths contain a granule or band of staining that partially surrounds the fiber. Areas of Schmidt-Lantermann incisures (arrows) and paranodal regions of myelin sheaths (arrowhead) are intensely stained. Bright field. Bar, 10 μm. × 950.

FIGURE 3 Transverse section, adult sciatic nerve, stained with Po antiserum (1:500). Myelin sheaths are intensely stained. Schmidt-Lantermann incisures are unstained (arrows). Bright field. Bar, 10 μm. × 1,500.

FIGURE 4 This section is adjacent to that shown in Fig. 3; it was stained with MAG antiserum (1:250). All myelinated axons are surrounded by a thin ring of periaxonal staining. Outer margins of myelin sheaths contain a single granule of staining (arrows). Compact portions of myelin sheaths are unstained. Schmidt-Lantermann incisures are intensely stained. Bright field. Bar, 10 μm. × 1,500.

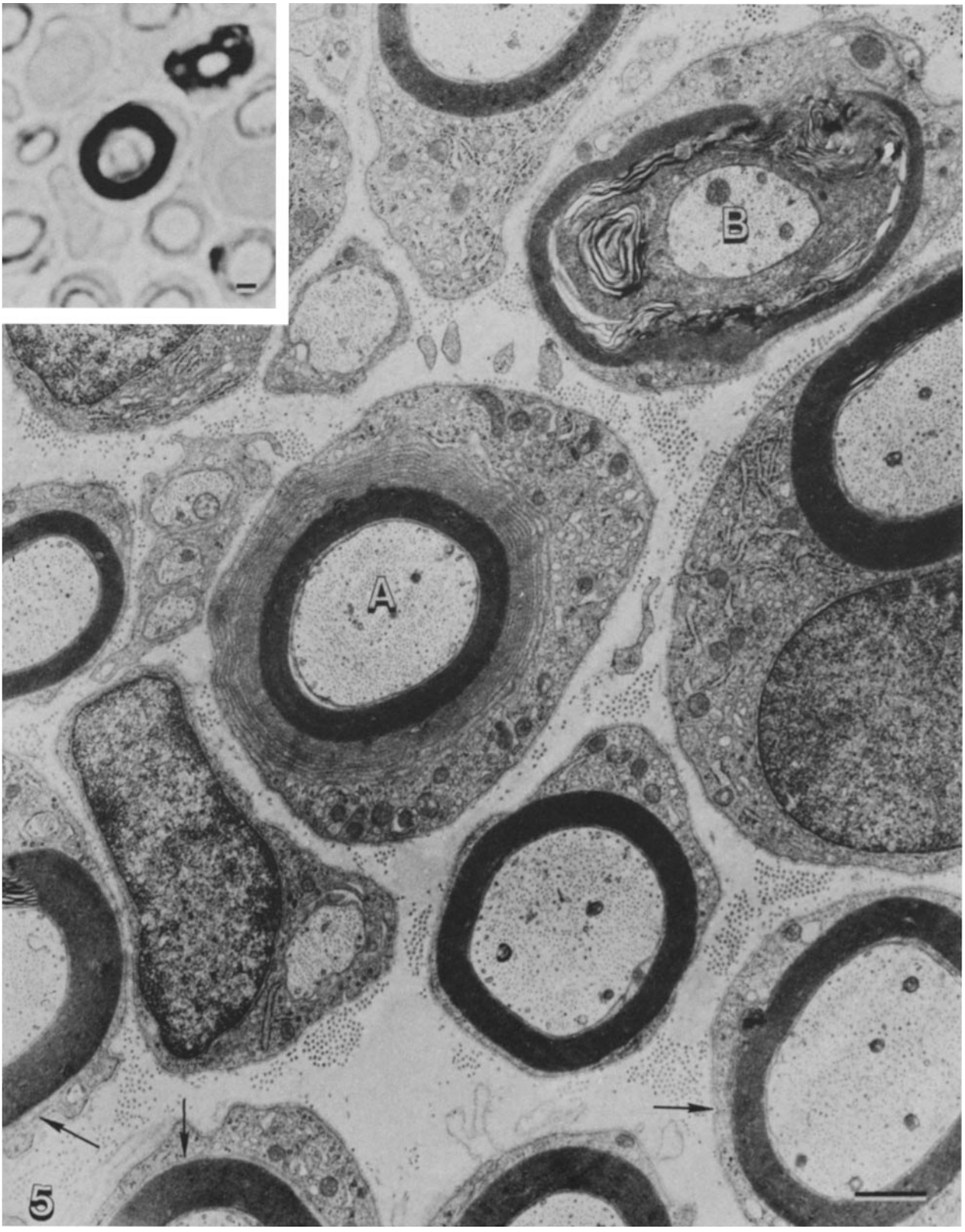

FIGURE 5 Electron micrograph of 7-d trigeminal nerve. The insert shows the same area in an adjacent 1-μm section that was stained with MAG antiserum. Fiber *A* is surrounded by a Schmidt-Lantermann incisure which is intensely stained by MAG antiserum. Compact portions of the myelin sheath are unstained. Periaxonal ring of staining is also present. Fiber *B* is surrounded by paranodal myelin loops that are intensely stained by MAG antiserum. Outer mesaxons (arrows) are also stained by MAG antiserum. Bar, 1 μm. EM; × 12,250, insert; × 3,200.

(Fig. 7). These cytoplasmic channels may facilitate the transport of metabolites and degradation products throughout the myelin sheath.

Recent experiments have indicated that Schwann cells require a signal from the axon to produce myelin (1, 16). Because membrane glycoproteins are believed to be involved in recog-

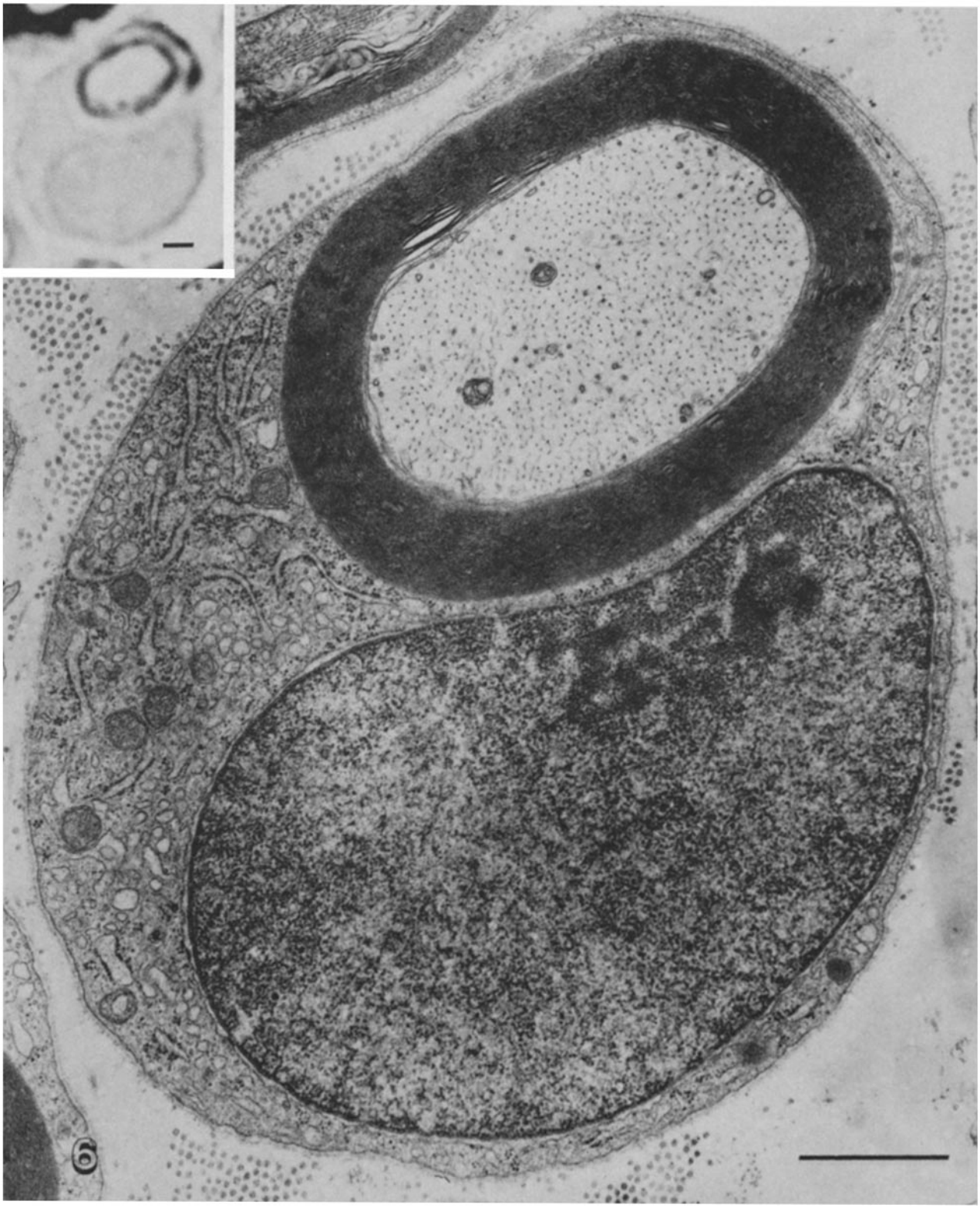

FIGURE 6 Electron micrograph of a myelinated fiber from a 7-d trigeminal nerve. The insert shows the same fiber in an adjacent 1-μm section that was stained by MAG antiserum. MAG staining is restricted to a periaxonal ring and to the outer mesaxon. Bar, 1 μm. EM; × 25,500, insert; × 5,000.

nition and cell-cell interactions, the periaxonal location of MAG is consistent with the hypothesis that MAG may be involved in glial-axonal interactions (12, 20). What role MAG may play in the initiation of myelination in either the central or peripheral nervous system remains to be clarified. The data presented in this manuscript support a structural role for MAG in maintaining the periodicity of discrete regions of Schwann cell and PNS myelin membranes.

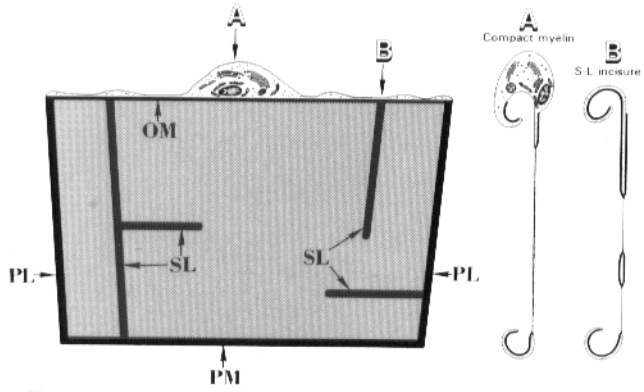

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FIGURE 7 Diagrammatic representation of MAG localization in *face* and transverse views of the "unrolled" myelin sheath. In the face view, the dark bands represent areas stained by MAG antiserum (*OM*-outer mesaxon, *SL*-Schmidt-Lantermann incisures, *PL*-paranodal loops, *PM*-periaxonal membranes). The stippled areas represent compact regions of the myelin sheath. Transverse views *A* and *B* correspond to levels shown in the *face* view. Cytoplasmic side of MAG containing membranes (thick lines) are separated by Schwann cell cytoplasm which is confluent with that of the cell soma.

TABLE I

Membrane Periodicity and Immunocytochemical Localization of MAG in PNS Myelin

	MAG	No MAG
Membrane periodicity		
Separation of:		
Extracellular leaflets	12–14 nm	1.5–2.5 nm
Cytoplasmic leaflets	>5 nm	"Fused"
Location	Periaxonal Schmidt-Lantermann incisures Paranodal loops Outer mesaxon	Compact myelin

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