

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

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ABSTRACT The myelin-associated glycoprotein (MAG) is an integral membrane glycoprotein that is located in the periaxonal membrane of myelin-forming Schwann cells. On the basis of this localization, it has been hypothesized that MAG plays a structural role in (a) forming and maintaining contact between myelinating Schwann cells and the axon (the 12-14-nm periaxonal space) and (b) maintaining the Schwann cell periaxonal cytoplasmic collar of myelinated fibers. To test this hypothesis, we have determined the immunocytochemical localization of MAG in the L₄ ventral roots from 11-mo-old quaking mice. These roots display various stages in the association of remyelinating Schwann cells with axons, and abnormalities including loss of the Schwann cell periaxonal cytoplasmic collar and dilation of the periaxonal space of myelinated fibers. Therefore, this mutant provides distinct opportunities to observe the relationships between MAG and (a) the formation of the periaxonal space during remyelination and (b) the maintenance of the periaxonal space and Schwann cell periaxonal cytoplasmic collar in myelinated fibers. During association of remyelinating Schwann cells and axons, MAG was detected in Schwann cell adaxonal membranes that apposed the axolemma by 12-14 nm. Schwann cell plasma membranes separated from the axolemma by distances >12-14 nm did not react with MAG antiserum. MAG was present in adaxonal Schwann cell membranes that apposed the axolemma by 12-14 nm but only partially surrounded the axon and, therefore, may be actively involved in the ensheathment of axons by remyelinating Schwann cells. To test the dual role of MAG in maintaining the periaxonal space and Schwann cell periaxonal cytoplasmic collar of myelinated fibers, we determined the immunocytochemical localization of MAG in myelinated quaking fibers that displayed pathological alterations of these structures. Where Schwann cell periaxonal membranes were not stained by MAG antiserum, the cytoplasmic side of the periaxonal membrane was "fused" with the cytoplasmic side of the inner compact myelin lamella and formed a major dense line. This loss of MAG and the Schwann cell periaxonal cytoplasmic collar usually resulted in enlargement of the 12-14-nm periaxonal space and ruffling of the apposing axolemma. In myelinated fibers, there was a strict correlation between the presence of MAG in the Schwann cell periaxonal membrane and (a) maintenance of the 12-14-nm periaxonal space, and (b) presence of the Schwann cell periaxonal cytoplasmic collar. These results support a dual structural role for MAG in forming and maintaining the 12-14-nm periaxonal space and Schwann cell periaxonal cytoplasmic collar of myelinated fibers.

The extracellular leaflet of the intranodal periaxonal membrane of myelinating Schwann cells is separated from the extracellular leaflet of the axolemma by a 12–14-nm gap or periaxonal space (18). A collar of Schwann cell cytoplasm completely surrounds the periaxonal space and separates the cytoplasmic leaflet of the periaxonal membrane from compact myelin. This periaxonal space and cytoplasmic collar are maintained in all myelinated fibers from normal peripheral nerve. The periaxonal space also exhibits a remarkable resistance to change during a number of pathological conditions that result in axonal swelling (11, 23), axonal shrinkage (11), or swelling of the Schwann cell periaxonal cytoplasmic collar (10, 11). In extreme stages of axonal or Schwann cell destruction the periaxonal space is not maintained. When this 12–14-nm gap between Schwann cell and axon is lost, the cytoplasmic leaflet of the Schwann cell periaxonal membrane “fuses” with the cytoplasmic leaflet of the inner compact myelin lamella to form a major dense line (9). Therefore, the maintenance of the periaxonal space and the Schwann cell periaxonal cytoplasmic collar appear to be structurally related and may be mediated by an intrinsic component of the Schwann cell periaxonal membrane. The myelin-associated glycoprotein (MAG)¹ is an integral membrane glycoprotein ($\approx 100,000$ mol wt) which is present in the periaxonal membranes of myelinating Schwann cells (24, 28, 30). The biochemical properties and immunocytochemical localization of MAG are consistent with the hypothesis that MAG plays a role in maintaining the periaxonal space and Schwann cell periaxonal cytoplasmic collar (6, 17, 19, 28, 30).

One way of determining if MAG has a functional role in forming and maintaining the periaxonal space and Schwann cell periaxonal cytoplasmic collar is to determine the immunocytochemical localization of MAG during the formation and breakdown of these structures. Suzuki and Nagara (25) have reported ultrastructural abnormalities in the ventral roots from adult quaking mice which include hypomyelination, a slowly progressing demyelination and remyelination, axonal vacuolation, and loss of the Schwann cell periaxonal collar and dilation of the periaxonal space in myelinated fibers. The present study was designed to determine the immunocytochemical localization of MAG in ventral roots from the 11-mo-old quaking mouse with emphasis on the role of MAG in forming the periaxonal space during remyelination and in maintaining this space and the Schwann cell periaxonal cytoplasmic collar in myelinated fibers. Our results support the hypothesis that MAG plays a role in forming and maintaining the periaxonal space and Schwann cell periaxonal cytoplasmic collar in myelinated fibers in the peripheral nervous system. Preliminary reports of this work have appeared in abstract form (31, 32).

MATERIALS AND METHODS

Tissue: Quaking mice (C57BL/6 [qk, qk]) were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained at Albert Einstein College of Medicine. 11-mo-old quaking mice were perfused with 5% glutaraldehyde in 0.08 M phosphate buffer (pH 7.4) for 10 min. The L₄ ventral roots were removed, fixed for an additional hour, postfixed in Dalton's fixative, dehydrated through a graded series of ethanols, and embedded in Epon. 1- μ m-thick sections were cut with glass knives, mounted on glass slides, stained with toluidine blue, and examined by light microscopy to confirm previously described pathologies (25). Additional 1- μ m sections of the entire ventral root were cut adjacent to thin sections with silver interference colors. The 1- μ m sections were stained

immunocytochemically 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- μ m sections were photographed and their negative images were enlarged and compared with the fine structure in electron micrographs from identical areas of the adjacently cut thin sections.

Immunostaining Procedure: 1- μ m-thick Epon sections mounted on glass slides were placed in a 60°C oven for at least 24 h. Epon and osmium was removed from the sections as previously described (27, 28, 30). The sections were then stained with a (1:250) dilution of MAG antiserum by the peroxidase-antiperoxidase method as previously described (28, 30). Sections were examined microscopically with bright-field illumination.

MAG antiserum was prepared in rabbits. The purity of MAG used in preparing this antiserum and the immunological and immunocytochemical specificity of this antiserum have been described (6, 14, 20, 24, 28, 30).

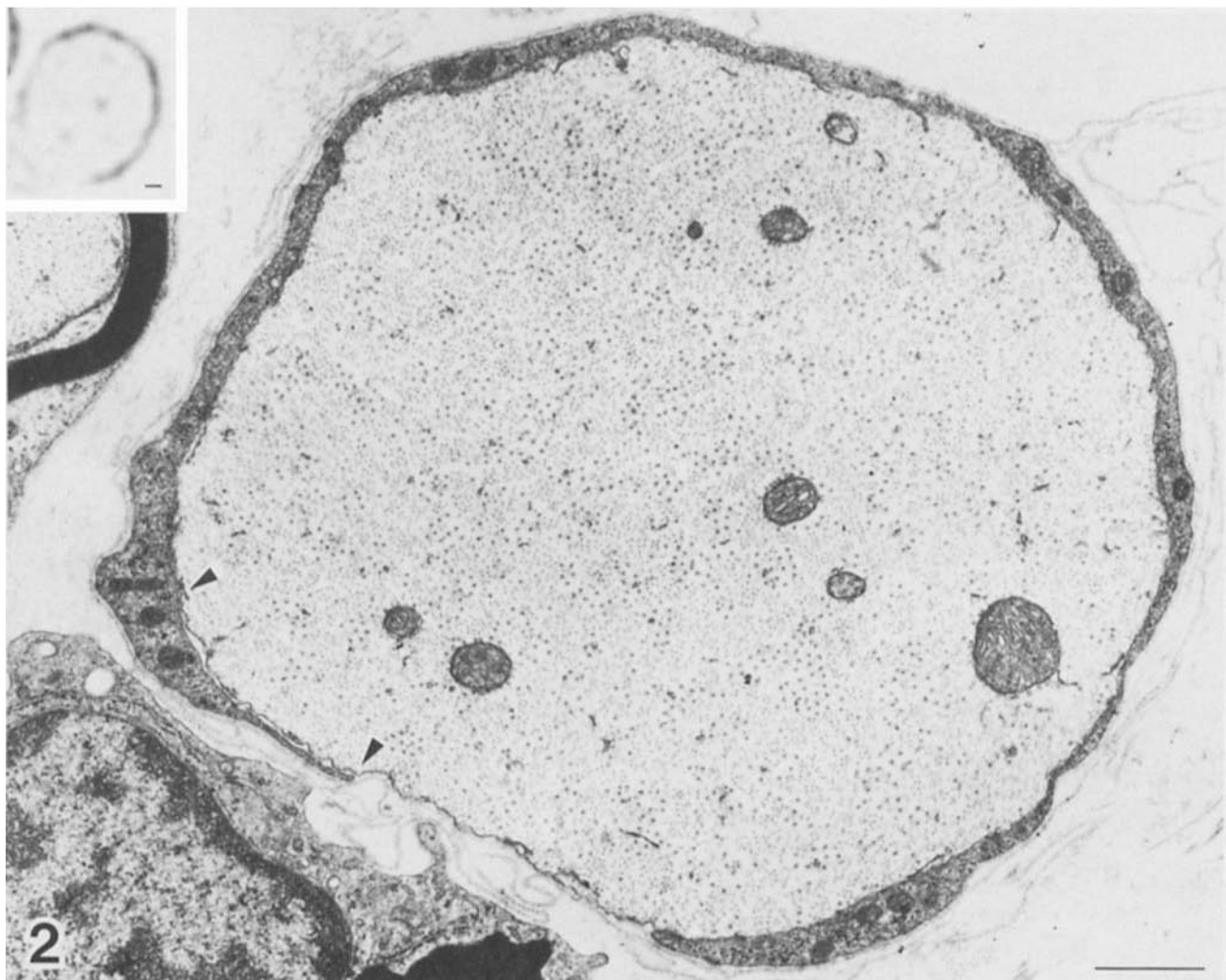
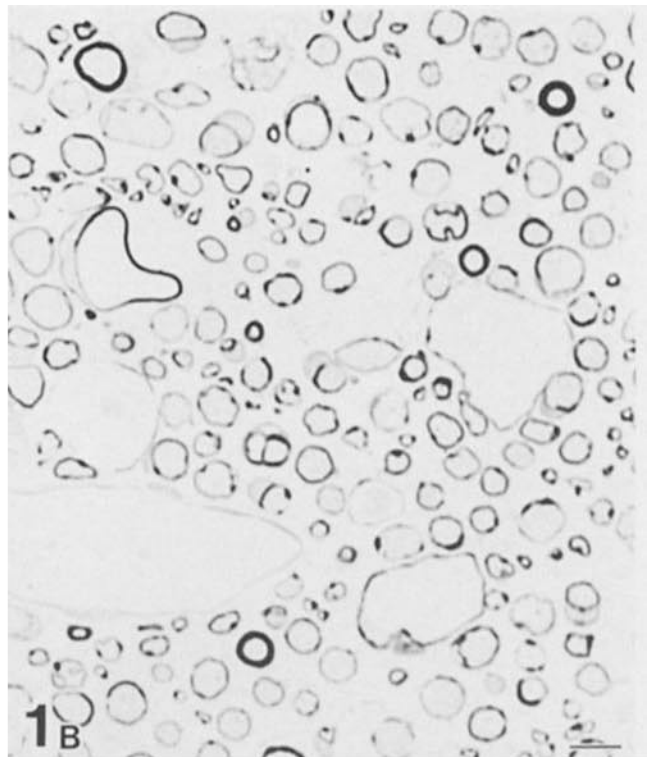
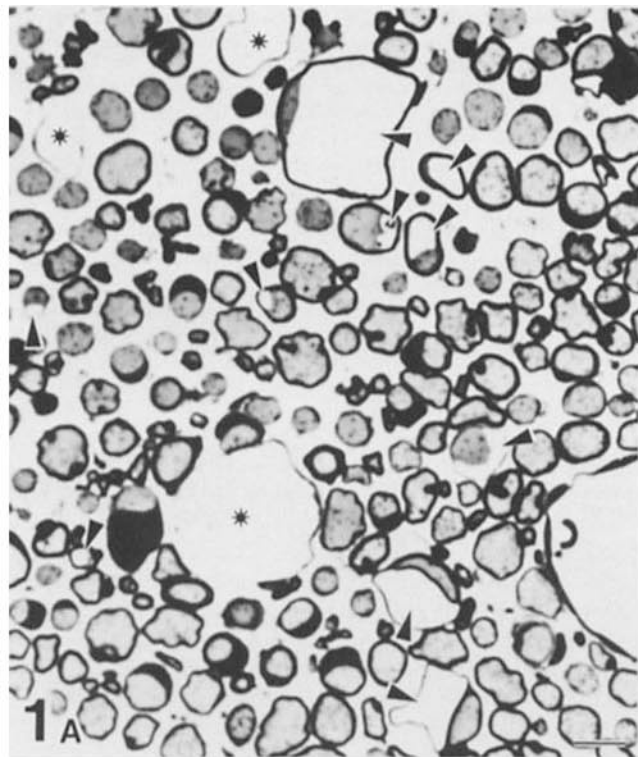
RESULTS

Light and electron microscopic alterations in myelin sheaths and axons from the ventral roots of quaking mice have been described in detail (25, 26). Fig. 1A is a light micrograph of a toluidine blue-stained 1- μ m-thick Epon section from the L₄ quaking ventral root. Many of the pathological features described previously (25, 26) such as naked axons, hypomyelination, intramyelinic or axonal vacuoles, and focal dilation of periaxonal spaces are apparent. Many axons appear to be surrounded by thin Schwann cell processes. Axonal diameters appear to be similar to those found in control animals. Demyelination and remyelination are apparent, although this appears to be a slowly progressing process and onion bulbs are not a prominent feature. When a similar section (Fig. 1B) was stained immunocytochemically with MAG antiserum, axons were surrounded by complete or incomplete rings of immunoprecipitate which varied in thickness and density. Immunoprecipitate was also present to various degrees around fibers displaying axonal and intramyelinic vacuoles. To determine more precisely the significance of the MAG immunostaining, we compared immunostained areas in 1- μ m-thick sections with the ultrastructure in adjacently cut thin sections.

Formation of the Periaxonal Space during Remyelination

Utilizing the thick-thin comparison, we detected the presence of MAG in Schwann cell periaxonal membranes during the initial association of Schwann cells with axons during remyelination. When a Schwann cell associates with an axon, but only partially surrounds its circumference, MAG antiserum stains the periaxonal membranes that appose the axolemma by 12–14 nm (Fig. 2). Regions of the Schwann cell plasma membrane that are separated from the axolemma by a distance >12 –14 nm do not react with MAG antiserum (Fig. 2; arrowheads). For remyelination to occur in the peripheral nervous system, Schwann cells must migrate to and surround demyelinated axons. If a basal lamina surrounds the plasma membrane of these Schwann cells, it must be removed or displaced from that portion of the membrane that will directly appose the axon and form the periaxonal space. In regions where a basal lamina is present, the 12–14-nm periaxonal space is not formed (Fig. 3; right inset) and the Schwann cell plasma membrane is not stained by MAG antiserum (Fig. 3). When a Schwann cell process totally encircles an axon, the periaxonal membrane is stained by MAG antiserum where it apposes the axolemma by 12–14 nm (Fig. 4). Regions of the Schwann cell periaxonal membrane separated from the axon by distances >12 –14 nm do

¹ *Abbreviation used in this paper:* MAG, myelin-associated glycoprotein.



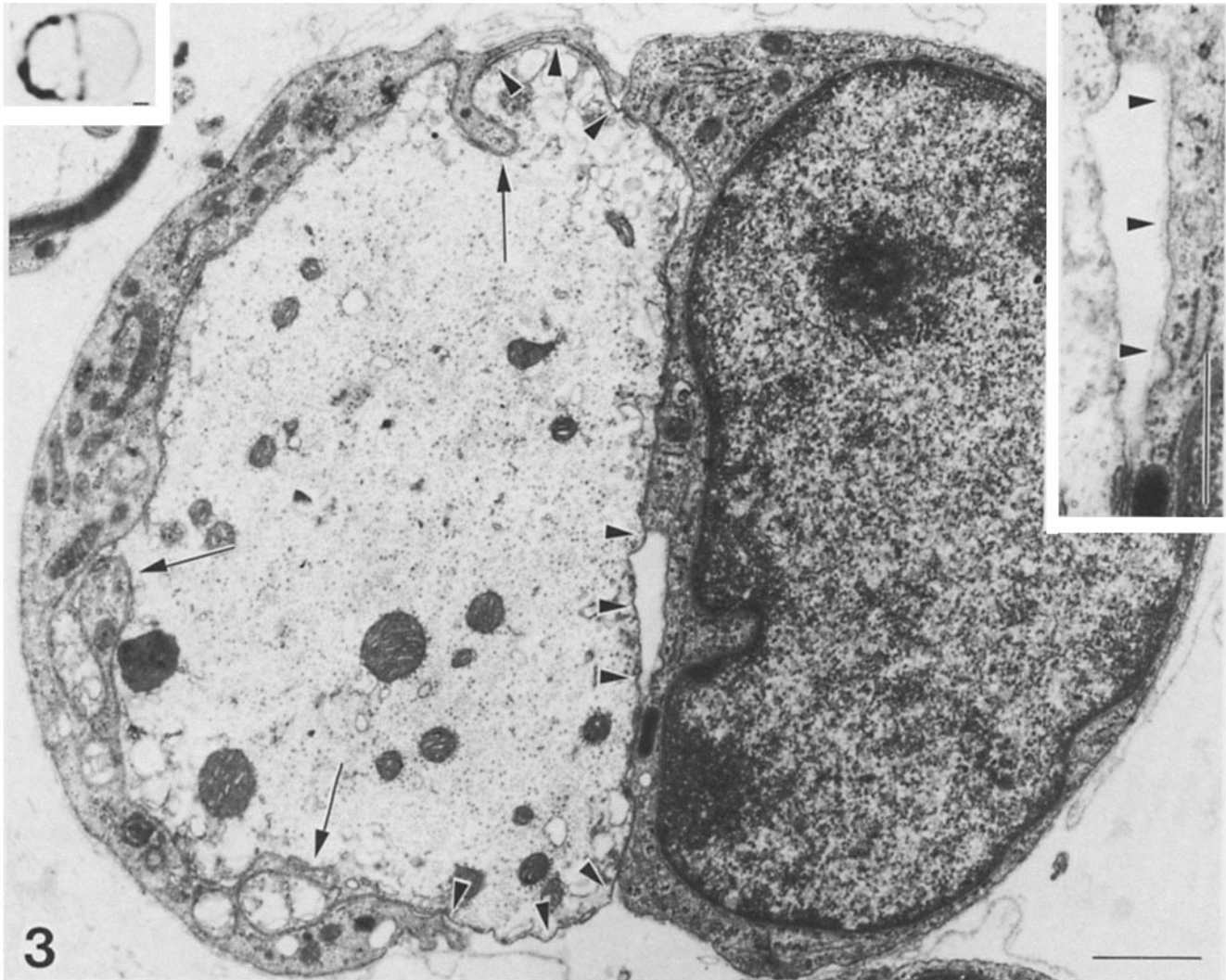


FIGURE 3 Electron micrograph of an axon partially surrounded by Schwann cell and process. The inset at left shows the same fiber in an adjacent 1- μ m section that was stained by MAG antiserum. The periaxonal region of this fiber is stained by MAG antiserum where the Schwann cell plasma membrane apposes the axolemma by 12–14 nm. Increases in the thickness of the periaxonal staining are due to overlapping periaxonal spaces produced by the ingrowth of Schwann cell processes into the axon (arrows). Periaxonal regions where the axolemma is unapposed by Schwann cell membrane and regions where Schwann cell-axon apposition is >12–14 nm (arrowheads) are not stained by MAG antiserum in the adjacent 1- μ m section. The Schwann cell plasma membrane in one of these MAG-negative regions is covered by a basal lamina (right inset, arrowheads). 11-mo-old quaking L₄ ventral root. Bars, 1 μ m (large panel and left inset); 0.5 μ m (right inset). \times 15,500 (large panel); \times 2,000 (left inset); \times 42,300 (right inset).

not react with MAG antiserum (Fig. 4). When the 12–14-nm periaxonal space is formed around the entire circumference of an axon, a mesaxon is formed and the periaxonal MAG

staining surrounds the entire axon (Fig. 5). The periaxonal ring of MAG staining is thicker and denser in the region of the mesaxon (Figs. 4 and 5; arrows).

FIGURE 1 (A) 1- μ m-thick toluidine blue-stained Epon section of the L₄ ventral root from an 11-mo-old quaking mouse. Markedly hypomyelinated and occasional nonmyelinated fibers are present. Axonal vacuoles (asterisks) and focal dilation of periaxonal spaces and/or intramyelinic vacuoles (arrowheads) are common. (B) Similar 1- μ m section stained immunocytochemically with MAG antiserum. Axons are surrounded by complete or incomplete rings of immunoprecipitate that vary in thickness and density. Immunostaining is also present to various degrees around fibers displaying either axonal or intramyelinic vacuoles. Bars, 10 μ m. \times 675.

FIGURE 2 Electron micrograph of an axon partially surrounded by a Schwann cell process. The inset shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. Portions of the Schwann cell plasma membrane separated from the axolemma by 12–14 nm are stained by MAG antiserum in the adjacent 1- μ m section. The Schwann cell plasma membrane does not react with MAG antiserum when it is separated from the axolemma by distances >12–14 nm (small area between arrowheads). 11-mo-old quaking L₄ ventral root. Bars, 1 μ m. \times 15,500; \times 2,300 (inset).

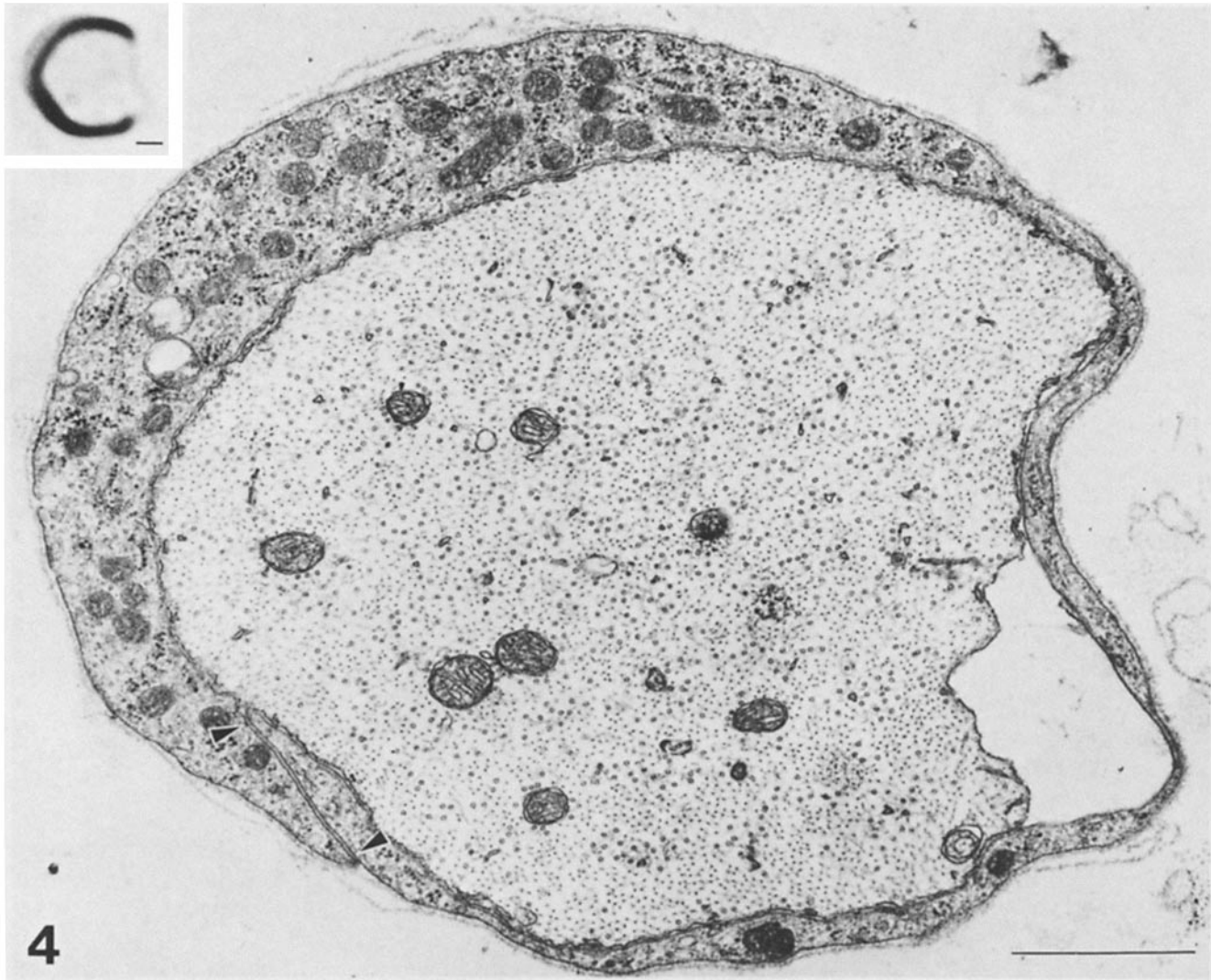


FIGURE 4 Electron micrograph of an axon surrounded by a Schwann cell process. The inset shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. MAG staining is restricted to regions where the 12–14-nm periaxonal space has formed. The thickness and density of the periaxonal MAG staining is greater in the region of the mesaxon (arrowheads). 11-month-old quaking L₄ ventral root. Bars, 1 μ m. \times 26,500; \times 3,700 (inset).

Breakdown of the Periaxonal Space and Schwann Cell Periaxonal Cytoplasmic Collar in Myelinated Fibers

In electron micrographs of myelinated fibers from the peripheral nervous system, the extracellular leaflet of the intranodal Schwann cell periaxonal membrane is separated from the axolemma by a 12–14-nm gap or periaxonal space, and a collar of Schwann cell cytoplasm completely separates the cytoplasmic leaflet of the periaxonal membrane from compact myelin (Fig. 6*A*). Myelinated fibers in the L₄ ventral roots of quaking mice often display a dilation of the periaxonal space which is accompanied by a loss of the Schwann cell periaxonal cytoplasmic collar (Fig. 6*B*). To determine if MAG plays a dual structural role in maintaining the periaxonal space and Schwann cell periaxonal cytoplasmic collar of myelinated fibers, MAG was localized immunocytochemically in myelinated quaking fibers that display breakdown of the periaxonal space and/or loss of the Schwann cell periaxonal cytoplasmic collar. Fig. 7 shows one such fiber that has a focal splitting and vacuolation of the myelin sheath similar to that described

in hexachlorophene intoxication (15, 35). In addition, a portion of the periaxonal space is enlarged. In the adjacent 1- μ m section, MAG antiserum stains the Schwann cell periaxonal membrane where the 12–14-nm periaxonal space and Schwann cell periaxonal cytoplasmic collar are maintained, but does not react with the periaxonal membrane in the region of enlargement of the periaxonal space. The length of the MAG-negative area in the 1- μ m section is greater than the area displaying an enlarged periaxonal space in the electron micrograph (Fig. 7*A*). Examination of this MAG-negative periaxonal region at higher magnification showed the cytoplasmic side of the Schwann cell periaxonal membrane fusing with the cytoplasmic side of the inner compact myelin lamella at points distal to the periaxonal space enlargement (Fig. 7, *C* and *D*; arrowheads). The MAG-negative area in the 1- μ m section is identical to the area in which the Schwann cell periaxonal cytoplasmic collar is absent (Fig. 7*A*; arrowheads). The compact myelin membranes surrounding or within the vacuole in Fig. 7*A* do not react with MAG antiserum, although two Schmidt-Lanterman incisures (Fig. 7*A*; arrows) shown at higher magnification in Fig. 7, *B* and *E* are stained intensely.

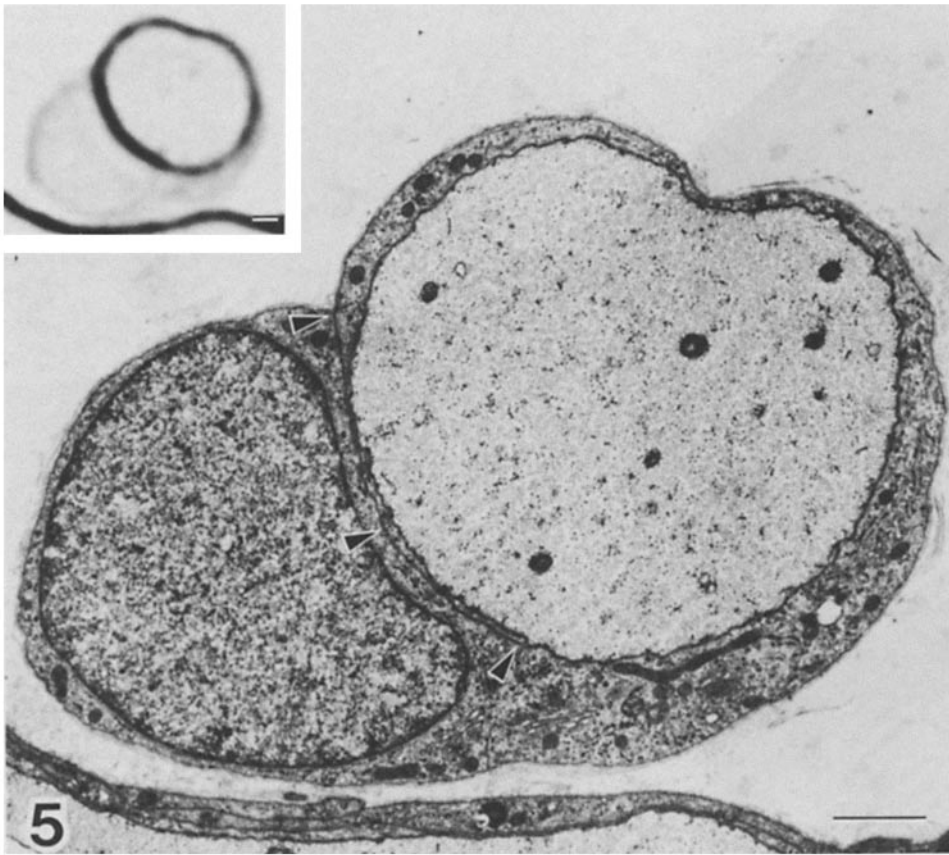


FIGURE 5 Electron micrograph of an axon surrounded by a Schwann cell. The inset shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. The 12–14-nm periaxonal space and MAG staining surrounds the entire circumference of this axon. The periaxonal ring of MAG staining is thicker and denser in the region of the mesaxon (arrowheads). 11-mo-old quaking L_4 ventral root. Bars, 1 μ m. $\times 12,000$; $\times 3,300$ (inset).

Approximately 50% of the periaxonal space is enlarged in the fiber in Fig. 8A. In the adjacent 1- μ m section, MAG staining is restricted to the periaxonal membranes which maintain a normal periaxonal space and periaxonal cytoplasmic collar. MAG staining and the inner collar of Schwann cell periaxonal cytoplasm are lost where the periaxonal space is enlarged (Fig. 8B). MAG immunoprecipitate surrounds ~50% of the fiber in the 1- μ m section of Fig. 9A. In the adjacent electron micrograph, the 12–14-nm periaxonal space and Schwann cell periaxonal cytoplasmic collar are present in the region stained by MAG antiserum. The periaxonal space does not appear enlarged in the MAG-negative region (Fig. 9A). Analysis of this MAG-negative region at higher magnification (Fig. 9, B and C) demonstrated that the cytoplasmic side of the periaxonal membrane had fused with the cytoplasmic side of the inner compact myelin lamella to form a major dense line (arrowheads). The axolemma in the MAG-negative regions of Fig. 9A apposes the compact myelin sheath by distances ≤ 12 –14 nm. Therefore, the loss of the Schwann cell periaxonal cytoplasmic collar appears to be the initial morphological response to the absence of MAG in the Schwann cell periaxonal membrane of quaking mice. Enlargement of the periaxonal space may be a secondary response which requires axonal shrinkage or swelling of the myelin sheath. In extreme cases, quaking axons can be totally separated from a surrounding myelin sheath (Fig. 10). In the adjacent, 1- μ m section the surrounding myelin sheath does not react with MAG antiserum except for a small dot of staining (arrowhead) which represents the outer mesaxon (Fig. 10; right inset). The collar of Schwann cell periaxonal cytoplasm is absent from the entire myelin sheath.

DISCUSSION

The ventral roots of quaking mice provide an excellent model for investigating the role of MAG in the formation and maintenance of the periaxonal space and the Schwann cell periaxonal cytoplasmic collar. The various pathologies associated with the hypomyelination and the slowly progressing demyelination and remyelination of fibers with large axonal diameters permit resolution of MAG immunostaining at the light microscopic level. The fine structure of MAG containing membranes can be determined by thick-thin comparison. The micrographs in this study are not unique and were chosen to represent stages during the formation and breakdown of the periaxonal space. Many axons in these quaking roots are surrounded by ultrastructurally normal myelin sheaths and serve as internal controls for MAG immunostaining.

Our results demonstrate the presence of MAG in Schwann cell periaxonal membranes during the initial association of remyelinating Schwann cells and axons. MAG may be actively involved in the initial ensheathment of axons by remyelinating Schwann cell membranes as it is present in adaxonal Schwann cell plasma membranes that appose the axolemma by 12–14 nm but only partially surround the axon (Figs. 2–4). Portions of adaxonal Schwann cell plasma membranes that appose the axolemma by distances >12 –14 nms do not react with MAG antiserum. Once the mesaxon is formed, MAG is present in only those periaxonal membranes that appose the axolemma by 12–14 nm (Fig. 4). MAG is also present in the mesaxon membranes that spirally wrap around the axon and the extracellular leaflets of these membranes are separated by 12–14 nm (28, 29). Increases in the width of the

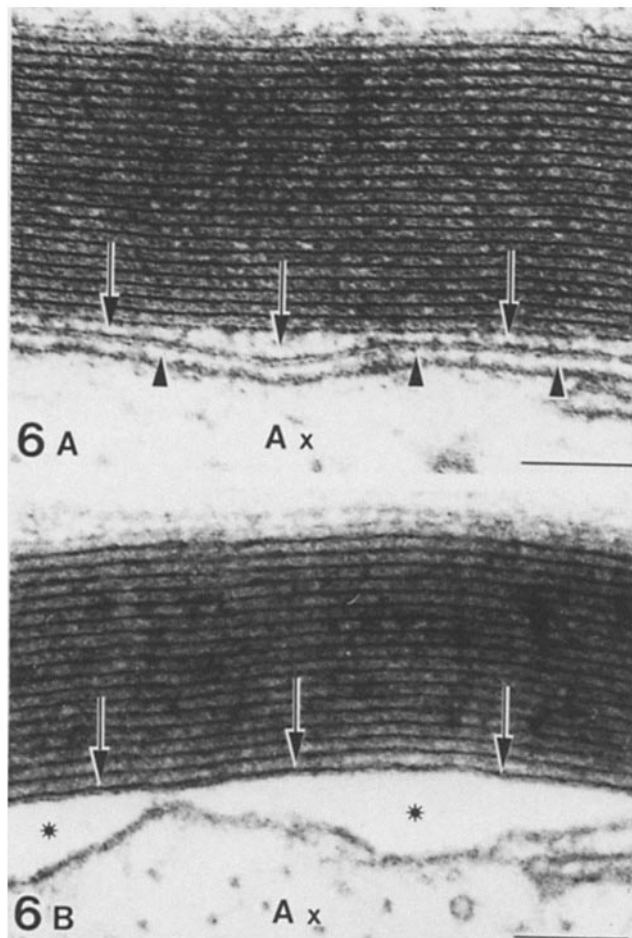


FIGURE 6 Electron micrographs of myelinated fibers in the L₄ ventral root from an 11-mo-old quaking mouse. (A) Normally myelinated fiber. The extracellular leaflet of the periaxonal membrane is separated from the extracellular leaflet of the axolemma by a 12–14-nm gap or periaxonal space (arrowheads). The Schwann cell periaxonal cytoplasmic collar (arrows) separates the cytoplasmic leaflet of the periaxonal membrane from the cytoplasmic leaflet of the inner compact myelin lamellae. (B) Myelinated fiber displaying a dilation of the periaxonal space (asterisks). The cytoplasmic leaflet of the periaxonal membrane has “fused” with the cytoplasmic side of the inner compact myelin lamella to form a major dense line (arrows). Ax, axon. Bars, 0.1 μ m. \times 150,000.

ring of periaxonal MAG staining are always due to the presence of additional Schwann cell membranes whose extracellular leaflets are apposed by 12–14 nm (Figs. 3–6 and 8–10) (29).

Before the plasma membrane of remyelinating Schwann cells can appose the axolemma by a 12–14-nm gap, it must be free of a basal lamina and contain MAG (Fig. 3). The plasma membrane of many of the supernumerary Schwann cells not associated with axons in these quaking roots were surrounded, at least in part, by a basal lamina. Tissue culture studies have demonstrated that generation, but not persistence, of Schwann cell basal lamina requires axonal contact (2). It could not be determined whether the basal lamina surrounding these remyelinating Schwann cells (Fig. 3) was newly synthesized or present prior to the reassociation of the Schwann cell and axon. In any event, Fig. 3 supports and extends the hypothesis (1, 3) that the basal lamina plays a role

in polarizing the Schwann cell surface into regions (adaxonal, basal lamina-free, MAG-containing) that can and regions (basal lamina-containing, MAG-negative) that cannot interact with axons during ensheathment.

Adhesion of the Schwann cell periaxonal membrane to the axolemma (formation of the periaxonal space) appears to be mediated, at least in part, by MAG and is a prerequisite for formation of the myelin sheath. Once the periaxonal space is formed, it demonstrates a remarkable resistance to change during pathological alterations resulting in axonal swelling, axonal shrinkage, or swelling of the Schwann cell periaxonal cytoplasmic collar (reviewed in reference 9). Markedly distorted periaxonal complexes within axonal swellings produced by chronic B, B'-iminodipropionitrile intoxication contain MAG and the 12–14-nm spacing (30). Therefore, MAG appears to play a crucial role in maintaining contact at a defined distance between the major interface of myelinating Schwann cells and axons. Breakdown of the periaxonal space of myelinated fibers only occurs in rare cases of axonal and myelin pathology (9). The quaking L₄ ventral root is one such case and provides a distinct opportunity to investigate the relationship of MAG to the maintenance of the periaxonal space and Schwann cell periaxonal cytoplasmic collar of myelinated fibers.

In myelinated fibers, a strict correlation exists between (a) maintenance of the periaxonal space, (b) presence of the Schwann cell periaxonal cytoplasmic collar, and (c) presence of MAG in the periaxonal membrane. Therefore, it is reasonable to suggest that maintenance of the periaxonal space and the Schwann cell periaxonal cytoplasmic collar is mediated at least in part by MAG, an intrinsic component of the Schwann cell periaxonal membrane. Dilation of the periaxonal space and loss of the Schwann cell periaxonal cytoplasmic collar in MAG-negative periaxonal regions (Figs. 7, 8, and 10) strongly support this hypothesis. Occasionally, MAG-negative periaxonal regions demonstrate a loss of the Schwann cell periaxonal cytoplasmic collar with compression or no change in the 12–14-nm periaxonal space (Figs. 7 and 9). The loss of the Schwann cell periaxonal cytoplasmic collar appears to be the initial response to the absence of MAG in the Schwann cell periaxonal membrane of quaking mice. Enlargement of the periaxonal space may be a secondary response which requires axonal shrinkage or swelling of the myelin sheath. The absence of MAG and enlargement of the periaxonal space results in a ruffled appearance of the apposing axolemma (Figs. 8 and 10). The axolemma also has a ruffled appearance in regions where the periaxonal space has not yet formed during remyelination (Figs. 2–4). Therefore, the presence of MAG in the periaxonal membrane may have a stabilizing affect on the contour of the axolemma.

The periaxonal localization of MAG has been challenged by recent immunocytochemical studies of central and peripheral nervous system myelinated fibers (5, 34). These studies have used the avidin-biotin peroxidase method to stain H₂O₂ “etched” thin sections. The preparations suggested that MAG is present in compact myelin, but not in periaxonal membranes. The results of the present study add additional support to light (12, 13, 22, 24, 28–30, 35) and electron microscopic (29) observations demonstrating the presence of MAG in periaxonal membranes and the absence of MAG in compact myelin. Since the results presented here and those referenced above are mutually exclusive of those of Webster and co-workers (5, 34), we believe that the methods used by the latter

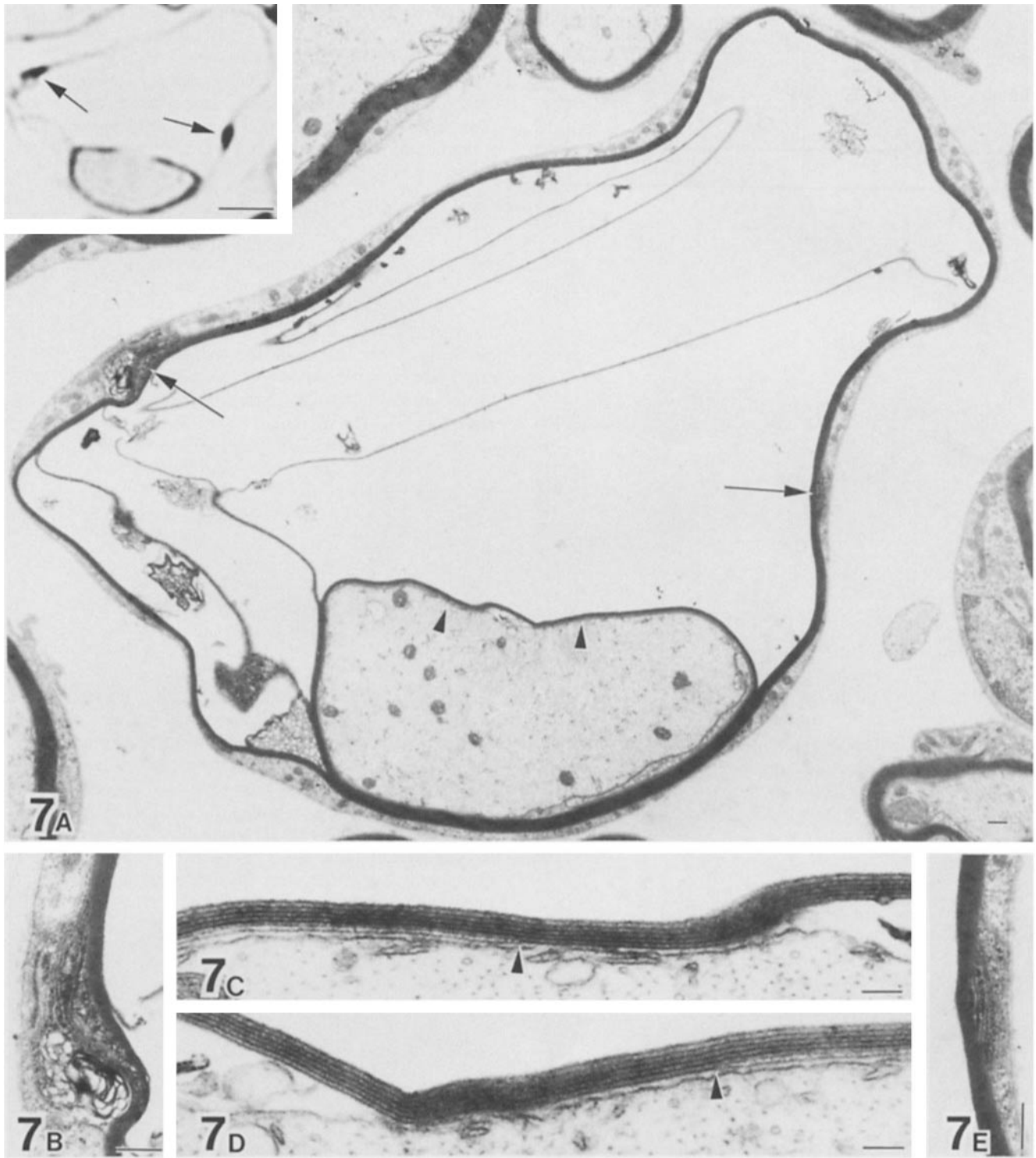


FIGURE 7 Electron micrograph of a myelinated fiber from the L₄ ventral root of an 11-mo-old quaking mouse (A). The light micrograph (*inset*) shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. A focal splitting and vacuolization of the myelin sheath has occurred. A portion of the periaxonal space is enlarged. MAG antiserum stains the periaxonal membrane where the 12-14- μ m periaxonal space and Schwann cell periaxonal cytoplasmic collar is maintained. MAG antiserum does not react with the periaxonal membrane in the region of periaxonal space enlargement (A, arrowheads). In this MAG-negative periaxonal region, the cytoplasmic side for the Schwann cell periaxonal membrane has fused with the cytoplasmic side of the inner compact myelin lamella to form a major dense line (C and D, arrowheads). The length of the MAG-negative area in the 1- μ m section is proportional to the area where the Schwann cell periaxonal cytoplasmic collar is absent (A, arrowheads). MAG antiserum does not react with the compact myelin membranes surrounding or within the vacuole, although two Schmidt-Lanterman incisures (B and E) are stained intensely (A, arrows). Bars, 1 μ m; 10 μ m (*inset*). \times 3,100 (A); \times 7,750 (B and E); \times 69,000 (C and D); \times 920 (*inset*).

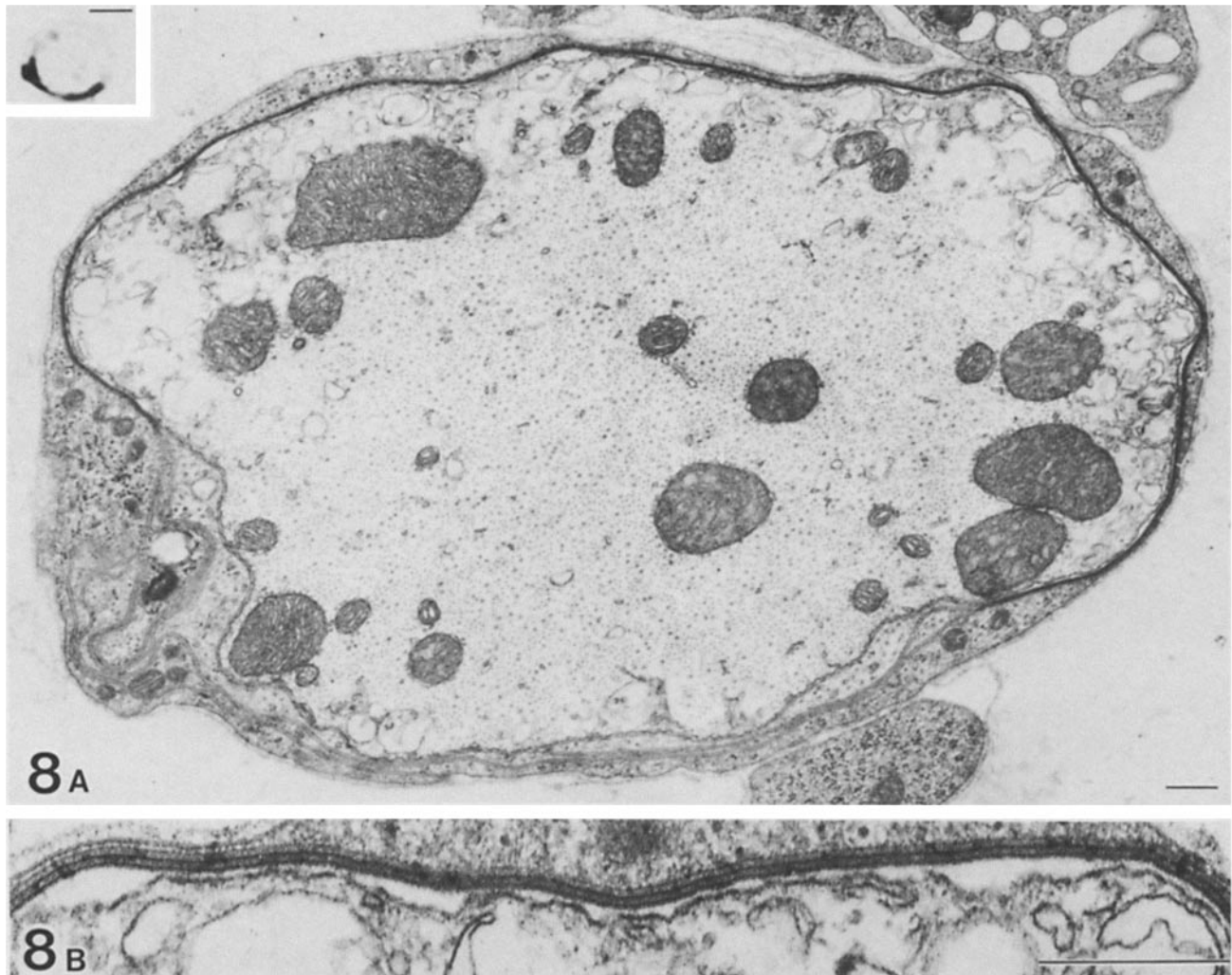


FIGURE 8 Electron micrograph of a myelinated fiber from the L₄ ventral root of an 11-mo-old quaking mouse (A). The inset shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. The 12–14-nm periaxonal space and Schwann cell cytoplasmic collar are present in the region stained by MAG antiserum. The 12–14-nm periaxonal space and the Schwann cell periaxonal cytoplasmic collar is absent (B) in the MAG-negative region. Bars, 1 μ m. 10 μ m (inset). \times 7,100 (A); \times 27,000 (B); \times 600 (inset).

authors do not demonstrate the true localization of MAG. This issue is discussed in detail elsewhere (29).

A hypothetical model indicating how the chemical properties of MAG may be responsible for maintaining the 12–14-nm periaxonal space and the periaxonal cytoplasmic collar of myelinating Schwann cells is presented in Fig. 11. MAG is a large molecule with an apparent molecular weight (M_r) of \sim 100,000. It is 30% carbohydrate by weight and contains a mixture of complex and smaller oligosaccharide moieties (19). Since the M_r of the polypeptide is \sim 70,000, the molecule contains about 600 amino acids. The molecule is highly negatively charged because of sialic acid and sulfate residues, as well as an excess of acidic over basic amino acids. The model in Fig. 11 assumes a number of similarities between MAG and other well-characterized integral membrane glycoproteins (21). Thus MAG is likely to be divided into at least three domains: (a) a heavily glycosylated domain on the extracellular surface of the Schwann cell plasma membrane and, therefore, in the periaxonal space; (b) at least one hydrophobic region which spans the lipid bilayer of the membrane; and (c) a polypeptide domain including the carboxy terminus

on the cytoplasmic side of the Schwann cell membrane. This postulated orientation of the MAG molecule in the Schwann cell plasmalemma is consistent with biosynthetic mechanisms of co-translational insertion of integral membrane glycoproteins into the membranes of the rough endoplasmic reticulum (21).

The model makes the assumption that about half of the polypeptide of MAG is in the periaxonal space and is heavily glycosylated. This domain of the molecule would consist of approximately equal amounts of polypeptide and carbohydrate. Fig. 11 illustrates our hypothesis (28) of how the bulk and polarity of this part of the MAG molecule could prevent very close contact of the Schwann cell plasmalemma with the axolemma and actually stabilize the 12–14-nm space between the two membranes. The correlation between the formation and maintenance of this periaxonal space and the presence of MAG observed in ventral roots of quaking mice supports this hypothesis. The space could be maintained nonspecifically simply by the large size of this domain of the MAG molecule, or there may be specific parts of MAG that interact with sites on the axolemma as suggested by Fig. 11. The parts of MAG

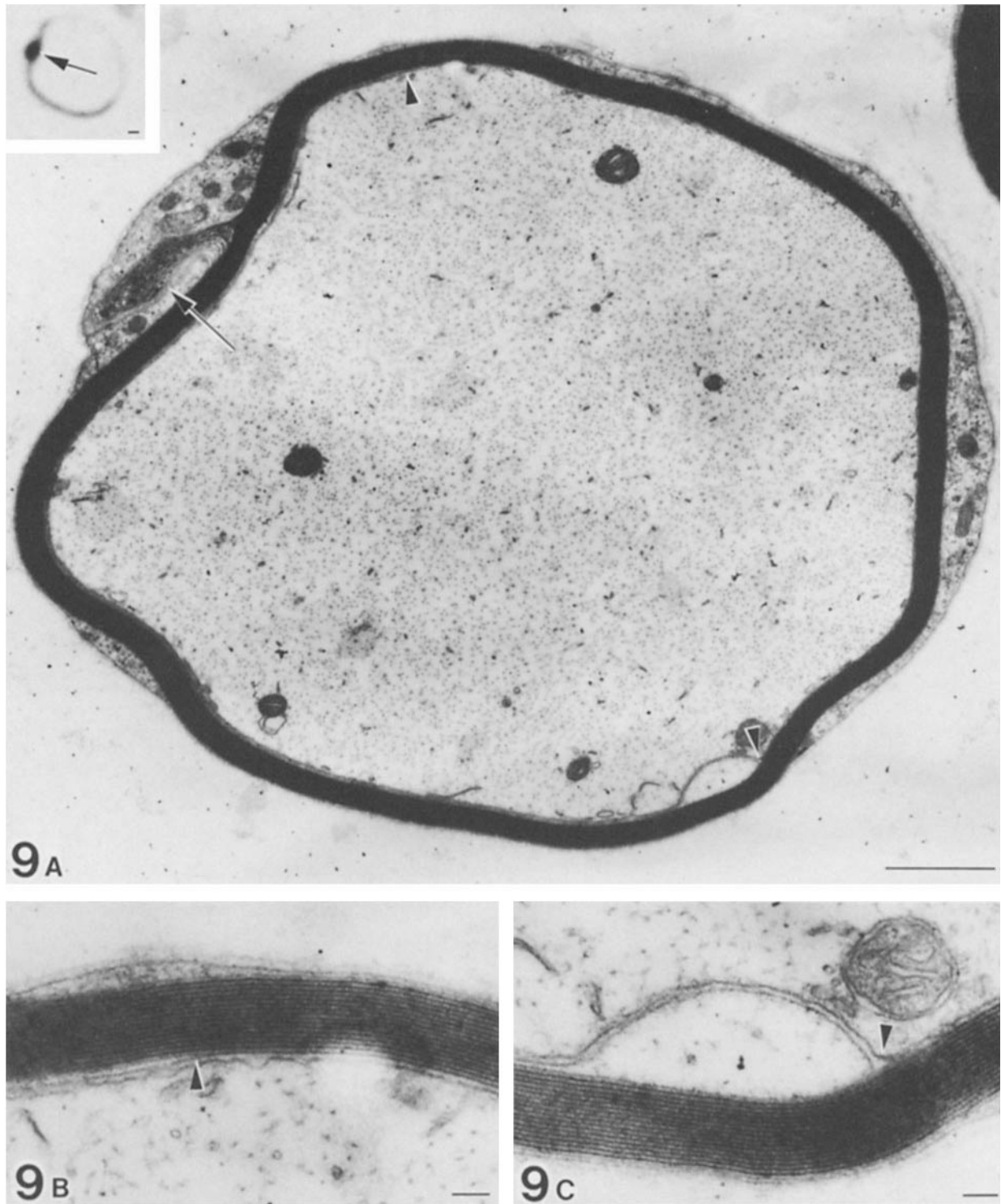


FIGURE 9 Electron micrograph of a myelinated fiber from the L₄ ventral root of an 11-mo-old quaking mouse (A). The inset shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. The 12–14-nm periaxonal space and the Schwann cell periaxonal cytoplasmic collar is maintained in the region stained by MAG antiserum. In the MAG-negative region, the Schwann cell periaxonal cytoplasmic collar is not present (B and C, arrowheads), whereas the 12–14-nm periaxonal space is maintained or compressed. A Schmidt-Lanterman incisure (arrows) is intensely stained by MAG antiserum (A). Bars, 1 μ m. \times 17,900 (A); \times 59,800 (B and C); \times 1,500 (inset).

that bind to the axolemma could either be carbohydrate residues (as represented in the figure) or discrete regions of the MAG polypeptide. Since the length of the MAG molecule is sufficient to transverse the 12–14-nm periaxonal space

numerous times if extended, Fig. 11 shows it as binding to sites represented by the shaded rectangles on the surfaces of both the axolemma and the Schwann cell plasma membrane. The nature of the sites on the axolemma and the Schwann

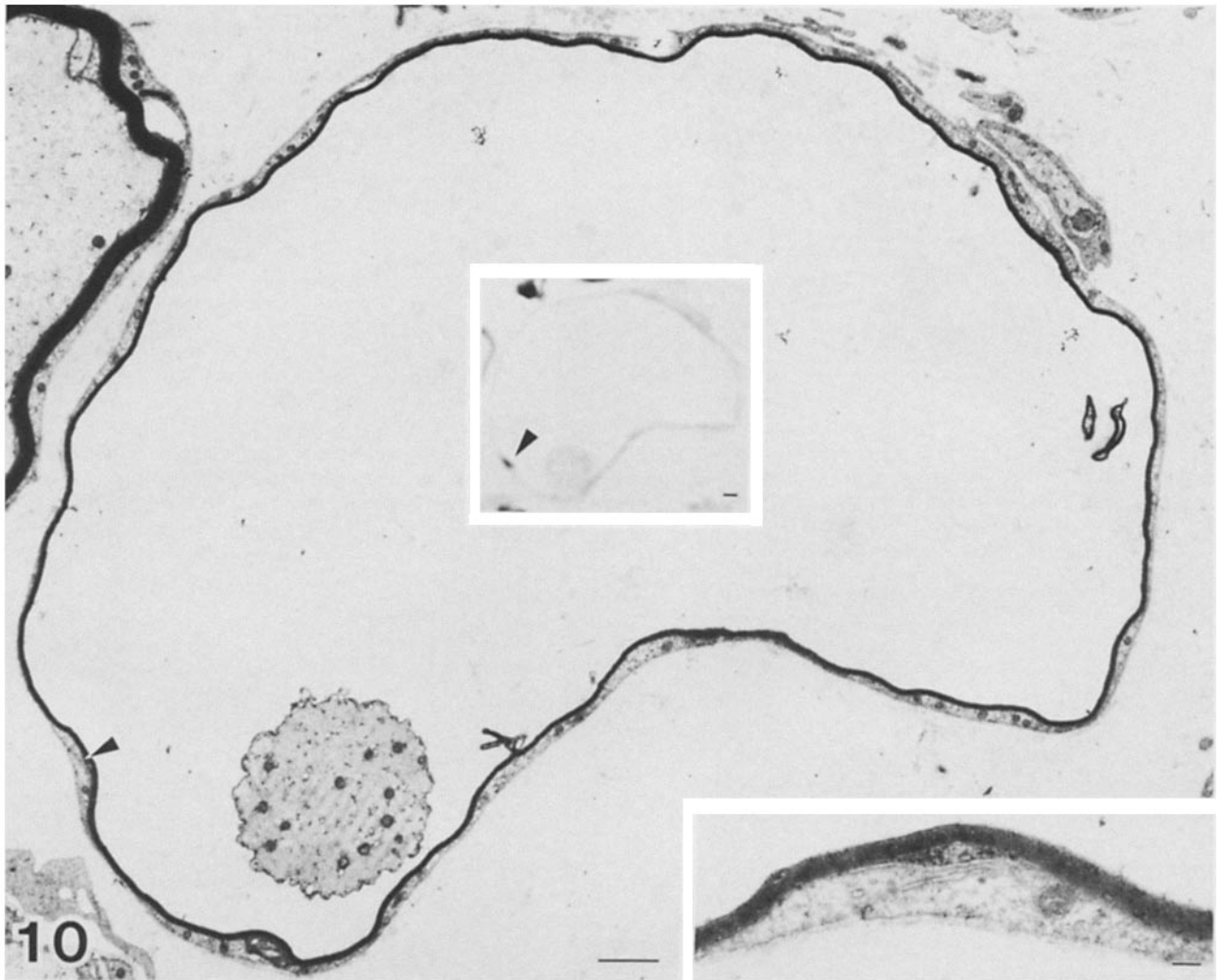


FIGURE 10 Electron micrograph from the L₄ ventral root of an 11-mo-old quaking mouse. The myelin sheath is swollen and totally separated from the axon. The light microscopic inset (*lower right*) shows the same fiber in the adjacent 1- μ m section that was stained by MAG antiserum. The swollen myelin sheath does not react with MAG antiserum except for a small dot (arrowhead in central inset) which represents the outer mesaxon. The Schwann cell periaxonal cytoplasmic collar is absent from the entire myelin sheath. Bars, 1 μ m. \times 8,500; \times 1,900 (*central inset*); \times 39,700 (*right inset*).

cell may be identical or different. However, the advantage of postulating sites on both membranes is that it would help explain how MAG could function to maintain and stabilize both the 12–14-nm periaxonal space between Schwann cell and axon and the 12–14-nm spaces between adjacent Schwann cell membranes in the outer mesaxon, Schmidt-Lanterman incisures, and lateral loops which have also been demonstrated to contain MAG (28). During the process of myelination, the Schwann cell must first make contact with and encircle the axon, following which the Schwann cell continues to grow around the axon forming the spiraled mesaxon. In the latter process, the layered Schwann cell surface membranes are separated from each other by a 12–14-nm space. If MAG binds to sites on both the axolemma and the Schwann cell surface membrane, it could mediate membrane-membrane interactions in both phases of Schwann cell growth. However, before the Schwann cell membranes could be converted to compact myelin, the bulky MAG molecules would have to be removed by degradation or lateral diffusion.

By analogy with other integral membrane proteins, MAG

is postulated to be a transmembrane protein with a hydrophobic domain in the conformation of an α -helix crossing the lipid bilayer. MAG has a relatively high content of nonpolar amino acids which is comparable to that in other membrane glycoproteins (19). For simplicity, the diagram in Fig. 11 shows only one transmembrane domain. However, since it takes only 32 amino acids to cross a lipid bilayer, MAG could easily cross the Schwann cell membrane several times.

This immunocytochemical study of pathological changes in quaking mutants revealed a strict correlation between the absence of MAG in the periaxonal Schwann cell membrane and the fusion of this membrane with the inner lamella of compact myelin to form a major dense line. This correlation supports our hypothesis that the cytoplasmic domain of the MAG polypeptide could function directly or indirectly to maintain the inner cytoplasmic collar of Schwann cells under normal circumstances (28). Loss of this cytoplasmic collar in areas of enlargement of the periaxonal space has also been described in a mutant hamster with hind leg paralysis (9, 11). How the MAG molecule could function to maintain the cytoplasmic collar is not known. Recently, actin (36) and

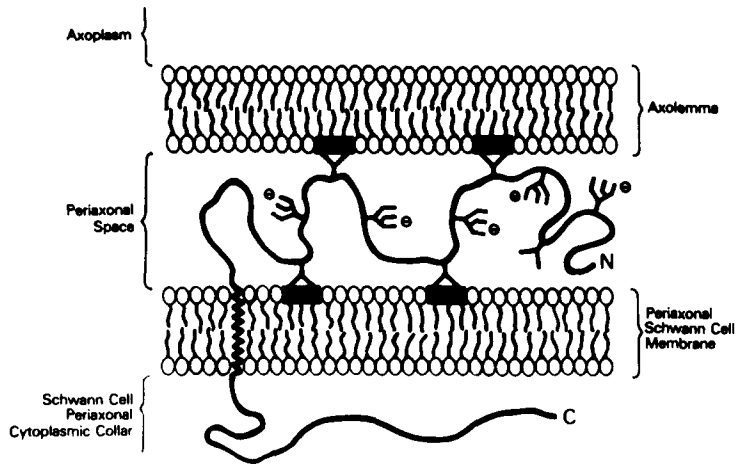


FIGURE 11 A schematic representation of how MAG may be oriented in the Schwann cell plasma membrane and function in maintaining the periaxonal space and the periaxonal cytoplasmic collar of the myelinating Schwann cell. By analogy with other integral membrane glycoproteins, MAG is postulated to be a transmembrane protein with the glycosylated amino terminal end of the molecule on the extracellular surface of the Schwann cell and the carboxy terminus in the Schwann cell cytoplasm. The three-pronged structures on the glycosylated domain of the molecule represent large, negatively charged, complex oligosaccharides, while the Y-shaped structures represent smaller, simpler oligosaccharides. The glycosylated domain of the MAG molecule may stabilize the 12–14-nm periaxonal space in a nonspecific way solely as a result of its bulk and polarity, or it may interact with specific sites (represented by the shaded rectangles) on the axon and Schwann cell surface membranes. These

hypothetical binding sites could be specific proteins that recognize oligosaccharide (as depicted in the figure) or polypeptide determinants on the MAG molecule. Alternatively, there could be amino acid sequences in MAG that specifically bind to protein, carbohydrate, or lipid determinants on the axon and Schwann cell surfaces. The carboxy terminal domain of MAG may help maintain the cytoplasmic Schwann cell collar by interaction with cytoskeletal or other components in the cytoplasm. See the text for additional comments regarding this hypothesis.

fodrin (8) have been localized in the periaxonal region of myelin-forming Schwann cells. It is well-known that components of cell surface membranes, including glycoproteins functioning in adhesion, can be linked to the cellular cytoskeleton (4, 7, 8). The results of the present study suggest an adhesion role for MAG. Therefore, it seems reasonable to suggest that coordination of Schwann cell movement and growth with axonal contact and membrane layering could be mediated by a link between MAG and the cytoskeleton.

The model illustrated in Fig. 11 is speculative at this time, but it is consistent with what is known about membrane glycoproteins in general and is supported by this and other immunocytochemical and biochemical investigations of MAG. Hopefully, it will act as a working hypothesis to suggest future experiments as the chemistry and function of this molecule is worked out in greater detail.

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