

# Distribution of the Myelin-associated Glycoprotein and P<sub>0</sub> Protein during Myelin Compaction in Quaking Mouse Peripheral Nerve

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**Abstract.** Ultrastructural studies have shown that during early stages of Schwann cell myelination mesaxon membranes are converted to compact myelin lamellae. The distinct changes that occur in the spacing of these Schwann cell membranes are likely to be mediated by the redistribution of (a) the myelin-associated glycoprotein, a major structural protein of mesaxon membranes; and (b) P<sub>0</sub> protein, the major structural protein of compact myelin. To test this hypothesis, the immunocytochemical distribution of these two proteins was determined in serial 1- $\mu$ m-thick Epon sections of ventral roots from quaking mice and compared to the

ultrastructure of identical areas in an adjacent thin section. Ventral roots of this hypomyelinating mouse mutant were studied because many fibers have a deficit in converting mesaxon membranes to compact myelin. The results indicated that conversion of mesaxon membranes to compact myelin involves the insertion of P<sub>0</sub> protein into and the removal of the myelin-associated glycoprotein from mesaxon membranes. The failure of some quaking mouse Schwann cells to form compact myelin appears to result from an inability to remove the myelin-associated glycoprotein from their mesaxon membranes.

**M**YELIN sheaths in the peripheral nervous system (PNS)<sup>1</sup> are formed by Schwann cells in a systematic and predictable manner that has been described in detail in ultrastructural studies (for review see Webster and Favilla [34]). The biochemical composition of mammalian PNS myelin is also well characterized (for review see Lees and Brostoff [15]). Nevertheless, little is known about how myelination occurs at the molecular level. Correlating the biochemical and molecular properties of myelin proteins with their immunocytochemical localization in ultrastructurally distinct myelin membranes can provide clues to their possible role in formation and maintenance of the myelin sheath. This approach has provided evidence that the myelin-associated glycoprotein (MAG) is involved in maintaining the membrane periodicity of periaxonal membranes, Schmidt-Lanterman incisures, paranodal myelin loops, and mesaxon membranes of PNS myelinated fibers (27). Similarly, in pathological conditions, correlations between the absence of a myelin protein and ultrastructural alterations in myelin membranes have strengthened the hypotheses that myelin basic protein maintains the major dense line of central nervous system (CNS) myelin (12), that proteolipid protein maintains the intraperiod line of CNS myelin (3), and that MAG participates in the formation and maintenance of the periaxonal space and periaxonal cytoplasm of myelinating Schwann cells (32).

1. *Abbreviations used in this paper:* CNS, central nervous system; MAG, myelin-associated glycoprotein; PNS, peripheral nervous system.

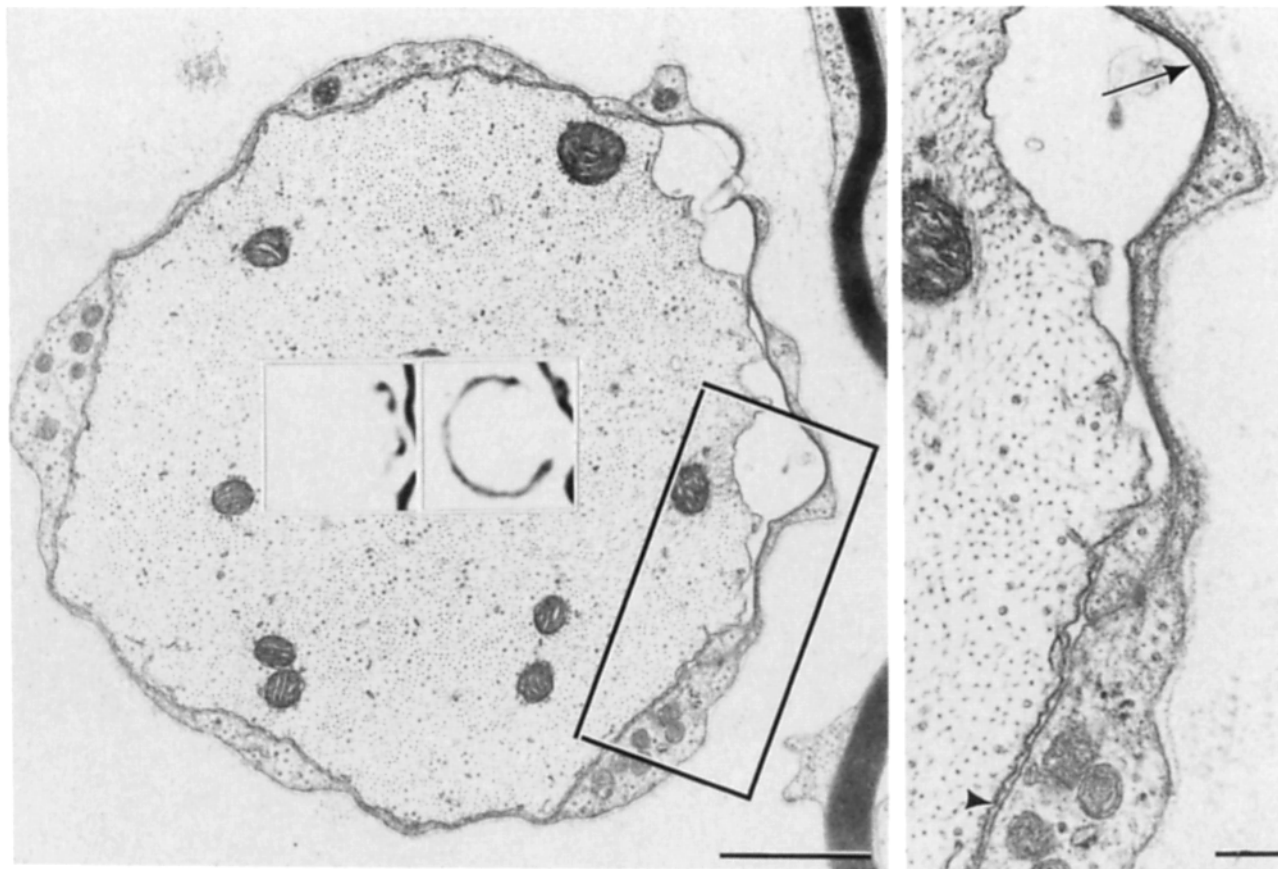
The purpose of the present study was to compare the localization of two myelin-specific integral membrane glycoproteins (P<sub>0</sub> and MAG) during Schwann cell remyelination in ventral roots from 11-mo-old quaking mice. Fibers in these ventral roots undergo a chronic, slowly progressive demyelination and remyelination that results in a generalized hypomyelination (25, 32). The present studies focused on early stages of myelin formation that included expansion of mesaxon membranes and their subsequent conversion to compact myelin for the following reasons: (a) many Schwann cells in these roots do not convert their mesaxon membranes to compact myelin; (b) MAG is a major structural protein of mesaxon membranes but it is not present in compact myelin where P<sub>0</sub> protein is the major structural protein; and (c) mesaxon membranes and compact myelin have distinct and different periodicities.

The results suggest that the conversion of mesaxon membranes to compact myelin involves the insertion of P<sub>0</sub> protein into and the removal of MAG from mesaxon membranes. The failure of some quaking mouse Schwann cells to convert mesaxon membranes to compact myelin appears to involve their inability to remove MAG from mesaxon membranes.

## Materials and Methods

### Tissue

11-mo-old quaking mice (C57 BLJ-6 [qk, qk]) were obtained from Jackson Laboratory (Bar Harbor, ME) and perfused with 5% glutaraldehyde in 0.08



**Figure 1.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacently cut, 1- $\mu$ m sections that were stained by P<sub>0</sub> (left inset) and MAG (right inset) antisera. The region of this fiber where a single compact myelin lamella had formed (right panel, enlargement of boxed area) (arrow) was stained only by P<sub>0</sub> antiserum. The remaining periaxonal region had a normal periaxonal space (arrowhead) and was stained only by MAG antiserum. Bars: (left) 1  $\mu$ m; (right) 0.2  $\mu$ m.

M phosphate buffer (pH 7.4) for 10 min. The L<sub>4</sub> 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. Two serial 1- $\mu$ m-thick sections of the entire ventral root were cut adjacent to a thin section with silver interference colors. The 1- $\mu$ m-thick section adjacent to the thin section was stained with P<sub>0</sub> antiserum; the other 1- $\mu$ m-thick section was stained with MAG antiserum. The thin section was mounted on Formvar-coated slot grids, stained with uranyl acetate and lead citrate, and examined in an electron microscope (model H-600; Hitachi Ltd., Tokyo, Japan). Areas of P<sub>0</sub> and MAG staining in the 1- $\mu$ m sections were photographed and their negative images were enlarged and compared with the fine structure in electron micrographs from identical areas of the adjacent thin section. This procedure allows the comparison of the immunocytochemical localization of P<sub>0</sub> and MAG with the fine structure of individual nerve fibers.

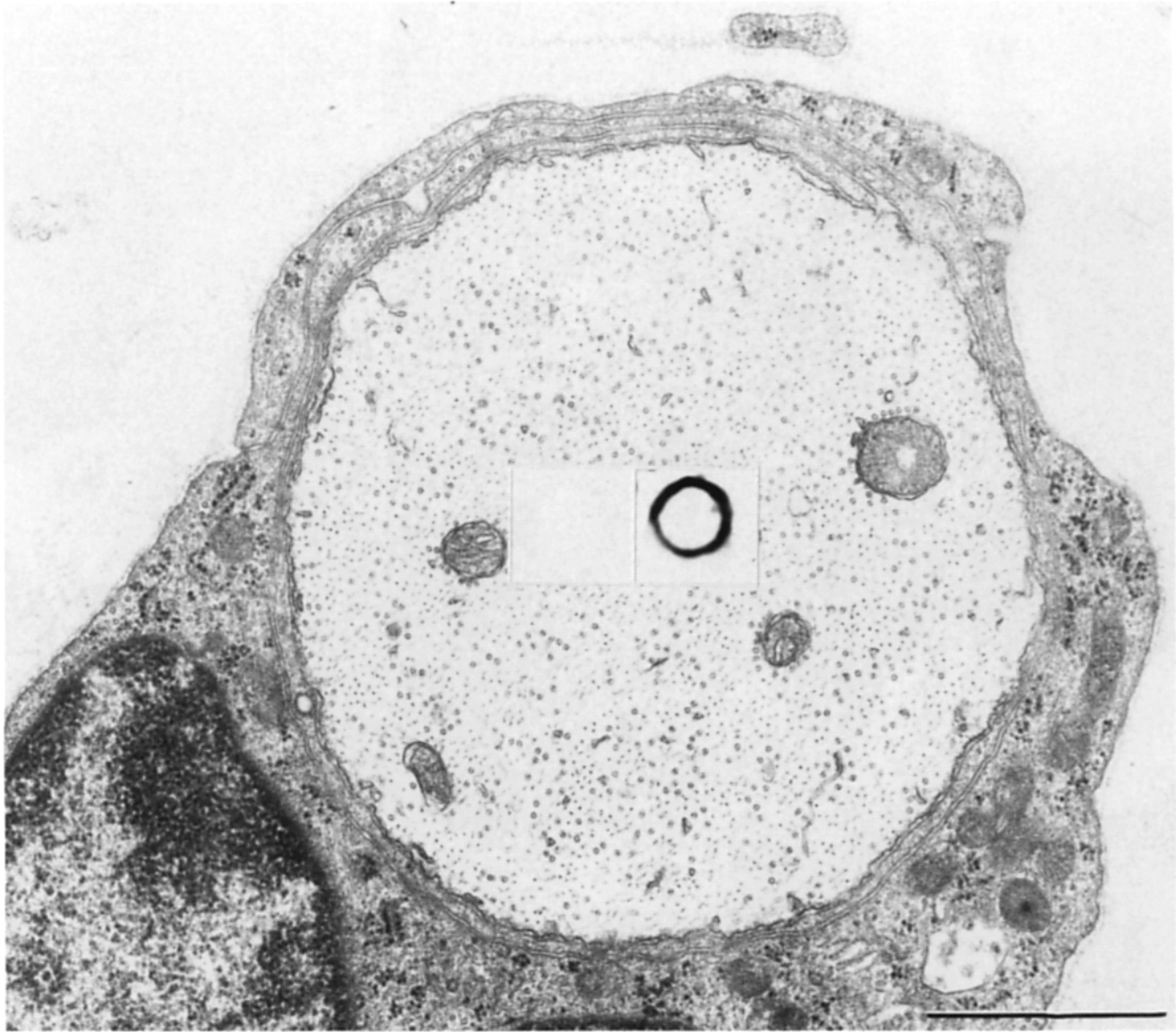
### Immunostaining Procedure

1- $\mu$ m-thick Epon sections mounted on glass slides were placed in a 60°C oven for  $\geq 24$  h. Epon and osmium were removed as previously described (27, 28, 30, 32). The sections were then stained with P<sub>0</sub> or MAG antiserum by the peroxidase-antiperoxidase method as previously described (27, 28, 30, 32). Sections were examined microscopically with brightfield illumination.

P<sub>0</sub> and MAG antisera were prepared in rabbits and used at a dilution of 1:250. The purity of antigens used in preparing these antisera and the immunological and immunocytochemical specificity of these antisera have been described for P<sub>0</sub> (27, 30, 31) and MAG (5, 11, 21, 24, 27, 32).

### Results

The immunocytochemical localization of MAG and P<sub>0</sub> protein in serial 1- $\mu$ m-thick Epon sections from the ventral roots of quaking mice was compared to the ultrastructure of identical areas in an adjacently cut thin section. With this approach, the distribution of MAG, P<sub>0</sub> protein, and ultrastructurally defined Schwann cell membranes can be compared in individual fibers over a 2- $\mu$ m distance. In all cases, P<sub>0</sub> protein was localized in the 1- $\mu$ m section immediately adjacent to the thin section. In the electron micrograph shown in Fig. 1, the Schwann cell process has formed 1.25 spiral turns around the axon. Where the axon is surrounded by the single Schwann cell process, the 12-14-nm periaxonal space has formed (Fig. 1, arrowhead). In the adjacent 1- $\mu$ m sections, this region was stained by MAG antiserum but it was not stained by P<sub>0</sub> antiserum. Where the Schwann cell process encircles the axon twice, the periaxonal space is not formed. In addition, the cytoplasmic leaflets of the inner Schwann cell process have fused to form a major dense line of compact myelin (Fig. 1, arrow). This region of the fiber was stained by P<sub>0</sub> antiserum but it was not stained by MAG antiserum in the adjacent 1- $\mu$ m sections.



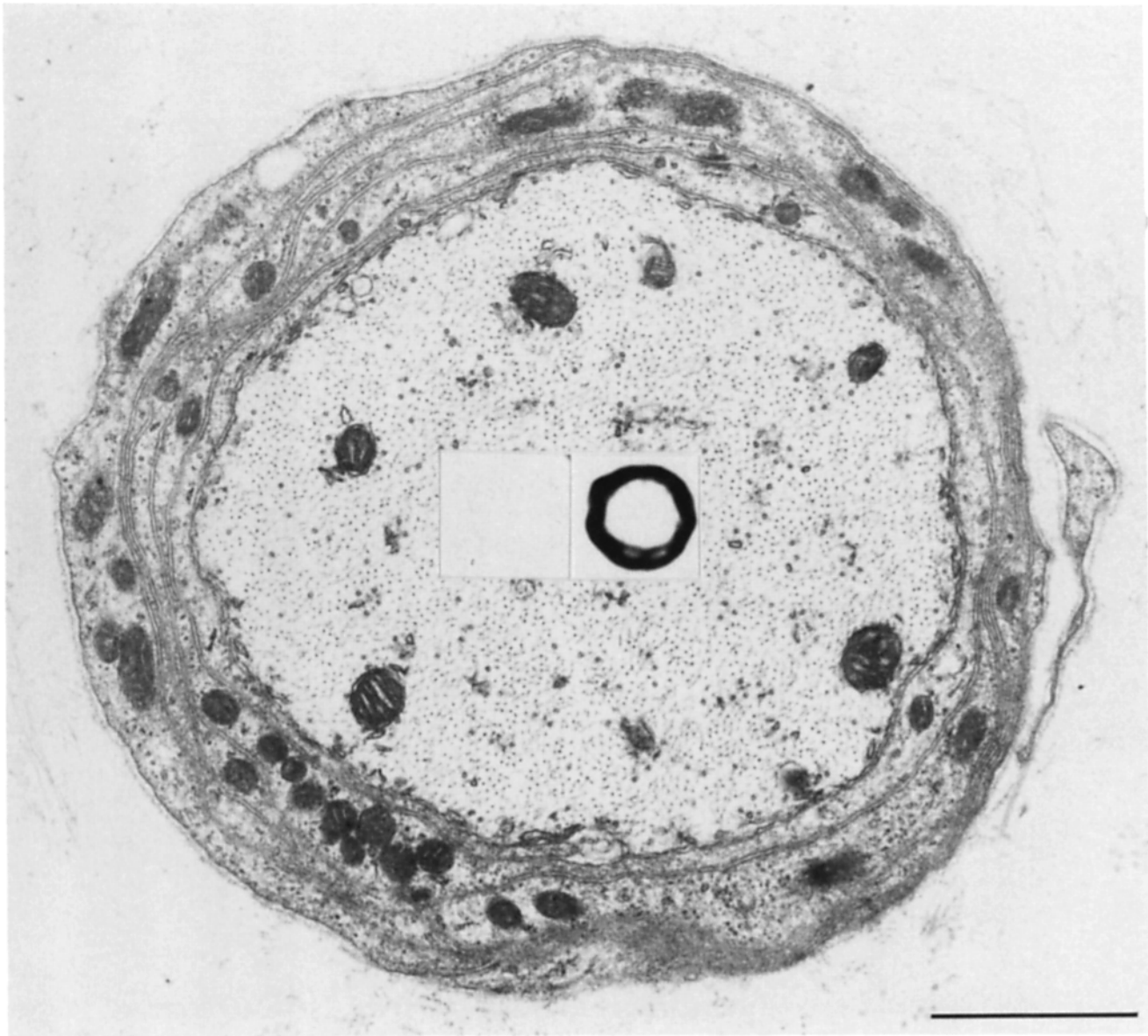
**Figure 2.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacently cut, 1- $\mu$ m sections that were stained by P<sub>0</sub> (*left inset*) and MAG (*right inset*) antisera. The mesaxon process which encircles the axon 2.3 times did not contain detectable levels of P<sub>0</sub> protein. In contrast, these membranes were stained intensely by MAG antiserum. Bar, 1  $\mu$ m.

After axonal ensheathment, myelination proceeds by spiral rotation of mesaxon membranes around the axon. The electron micrographs in Figs. 2 and 3 show mesaxon membranes encircling the axon  $\sim$ 2.3 and 5 times, respectively. In Fig. 2, mesaxon cytoplasm is relatively sparse and free from organelles such as mitochondria and microtubules. Mesaxon cytoplasm in Fig. 3 is more abundant and contained many mitochondria. In the adjacently cut 1- $\mu$ m sections, MAG antiserum intensely stained these membranes; in contrast, P<sub>0</sub> immunoreactivity was undetectable.

Analysis of electron micrographs of normally developing peripheral nerve has shown that once mesaxon membranes form several spiral turns, their spacing or periodicity changes to that of compact myelin (33). Many of the remye-

linating Schwann cells in quaking mice ventral roots do not convert their mesaxon membranes to compact myelin, resulting in an abundance of mesaxon wraps not found during normal myelination. For example, the mesaxon membranes in Figs. 4 and 5 have formed 10 spiral wraps that stain intensely with MAG antiserum in the adjacent 1- $\mu$ m sections. These mesaxon membranes are also stained with P<sub>0</sub> antiserum. In Fig. 4, the intensity of P<sub>0</sub> immunoreaction product is variable. In Fig. 5, P<sub>0</sub> staining showed little variability and was similar in intensity to that found over neighboring compact myelin sheaths. Mesaxon cytoplasm, sparse in Fig. 4, was more abundant in Fig. 5 and contained occasional mitochondria and microtubules.

Comparison of unstained axonal areas and areas occupied

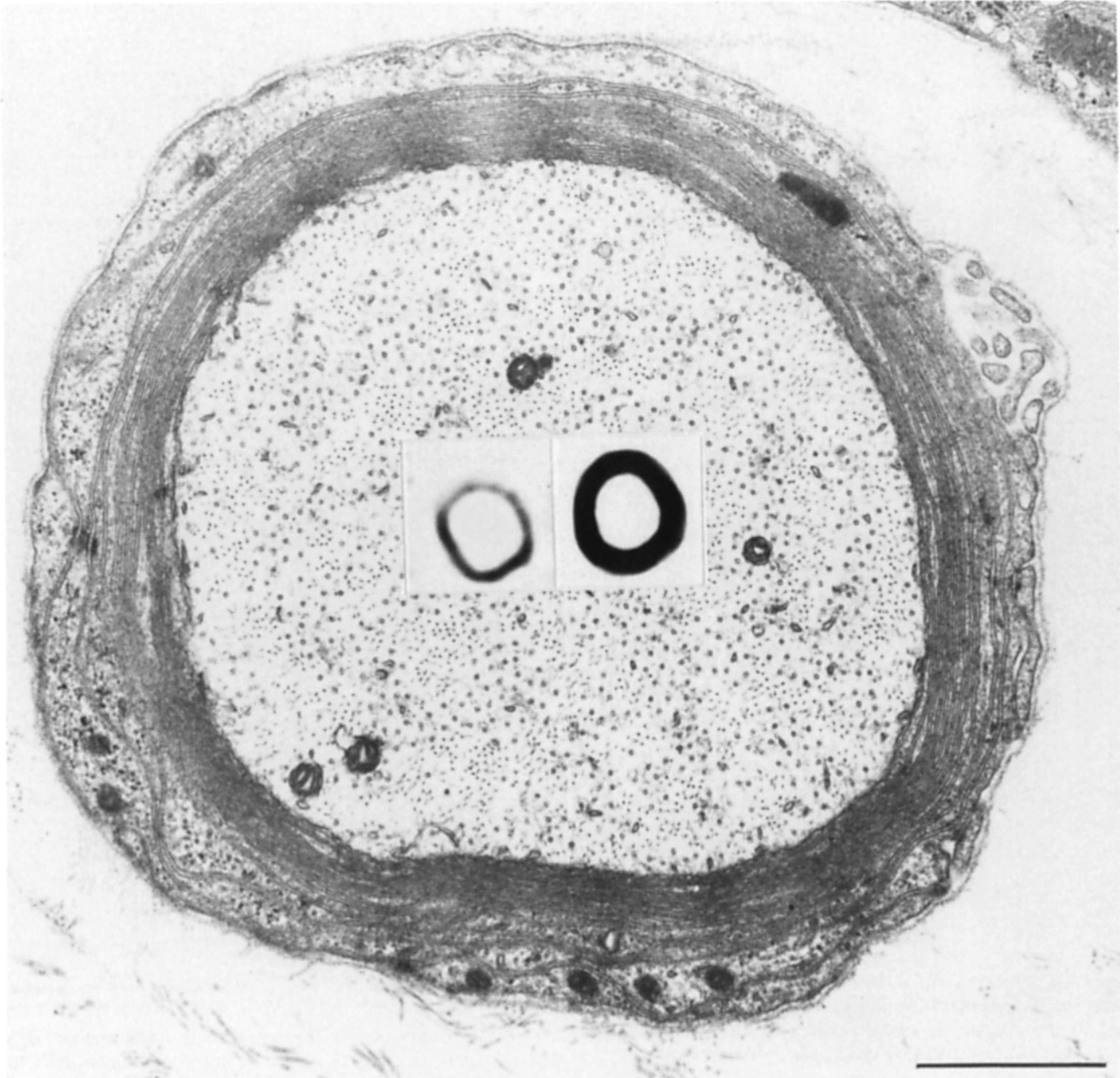


**Figure 3.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacently cut, 1- $\mu$ m sections that were stained by P<sub>0</sub> (left inset) and MAG (right inset) antisera. The mesaxon process which encircles the axon five times did not contain detectable levels of P<sub>0</sub> protein. In contrast, these membranes were stained intensely by MAG antiserum. Bar, 1  $\mu$ m.

by P<sub>0</sub> and MAG immunoreaction product in Fig. 5 indicated that MAG staining extended farther from the unstained axonal perimeter than did P<sub>0</sub> staining and that the band of MAG staining was 30–40% thicker than the band of P<sub>0</sub> staining. These observations suggested that P<sub>0</sub> protein was more concentrated in mesaxon membranes that were closest to the axon. Analysis of P<sub>0</sub> and MAG distribution in the thick-thin comparison of Fig. 6 showed this more clearly. The axon in Fig. 6 was surrounded by 11 mesaxon wraps. The inner eight wraps contained little mesaxon cytoplasm, tightly apposed the axon, and were stained by both P<sub>0</sub> and MAG antisera in the adjacent 1- $\mu$ m sections. The outer three wraps contained variable amounts of mesaxon cytoplasm and organelles. A segment of the outermost mesaxon wrap (Fig. 6,

*arrowheads*) was separated from the other mesaxon membranes by abundant Schwann cell cytoplasm. MAG immunoreactivity was present along the length of this segment. In contrast, only a short segment of this outer turn was stained by P<sub>0</sub> antiserum. The unstained area separating the MAG- and P<sub>0</sub>-stained portion of this outer mesaxon from the others was greater in the P<sub>0</sub>-stained section. This indicated that P<sub>0</sub> protein was more concentrated in mesaxon membranes that were closest to the axon.

Occasional axons in these quaking roots were surrounded by variable amounts of both compact myelin and noncompact Schwann cell membranes. For example, the right half of the axon in Fig. 7 was surrounded by compact myelin. The vast majority of the Schwann cell membranes on the left half



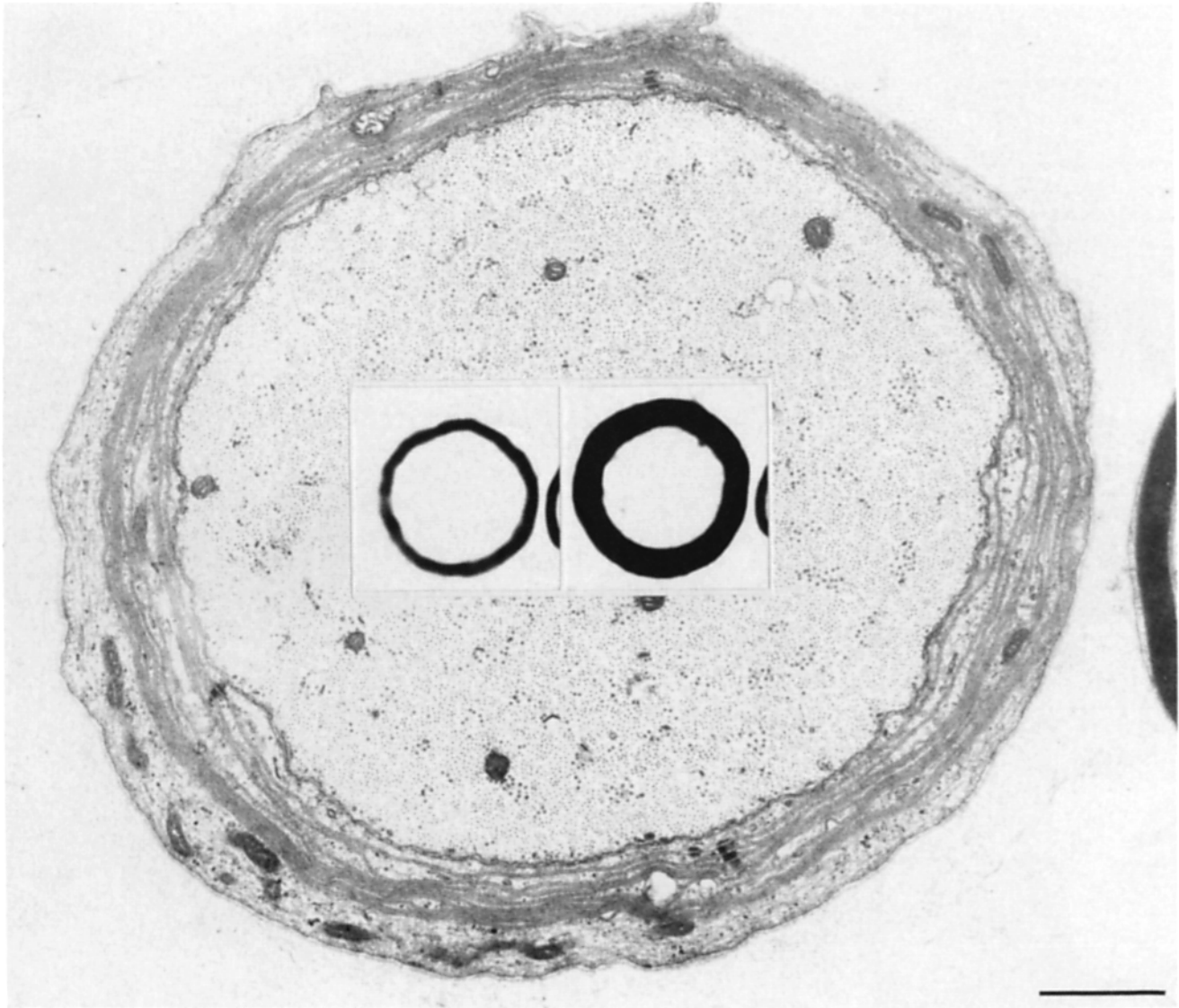
**Figure 4.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacent, 1- $\mu$ m sections that were stained by P<sub>0</sub> (left inset) and MAG (right inset) antisera. The mesaxon process which encircles the axon 10 times contained sparse Schwann cell cytoplasm and detectable levels of both P<sub>0</sub> protein and MAG. Compared to MAG staining, the ring of P<sub>0</sub> staining was thinner and varied in intensity. Bar, 1  $\mu$ m.

had the periodicity of mesaxon membranes that contained sparse cytoplasm. In the adjacent 1- $\mu$ m sections, a thick band of MAG staining was present over the mesaxon membranes. A thin band of MAG staining, similar to that present in neighboring fibers surrounded by totally compact myelin sheaths, was present in the region of compact myelin. In contrast, P<sub>0</sub> staining present over compact myelin and mesaxon membranes was similar in intensity and thickness. These observations indicate that MAG was present in mesaxon and periaxonal membranes but was not present in compact myelin. In contrast, P<sub>0</sub> protein was enriched in both compact myelin and mesaxon membranes.

### Discussion

After axonal ensheathment, PNS myelination normally proceeds by expansion of mesaxon membranes (34). A mesaxon can be considered as a Schwann cell process that spirally wraps around the axon by rotating upon itself. After several spiral turns, the cytoplasm between the mesaxon membranes disappears and the major dense line of compact myelin is formed. Simultaneously, the 12–14-nm spacing between the extracellular leaflets of apposing mesaxon membranes decreases to the 2-nm spacing of compact myelin.

Conversion of mesaxon membranes to compact myelin has



**Figure 5.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacently cut, 1- $\mu$ m sections that were stained by P<sub>0</sub> (*left inset*) and MAG (*right inset*) antisera. The mesaxon process which encircles the axon 10 times contained variable amounts of Schwann cell cytoplasm and detectable levels of both P<sub>0</sub> and MAG. The ring of P<sub>0</sub> staining was thinner than the ring of MAG staining. Bar, 1  $\mu$ m.

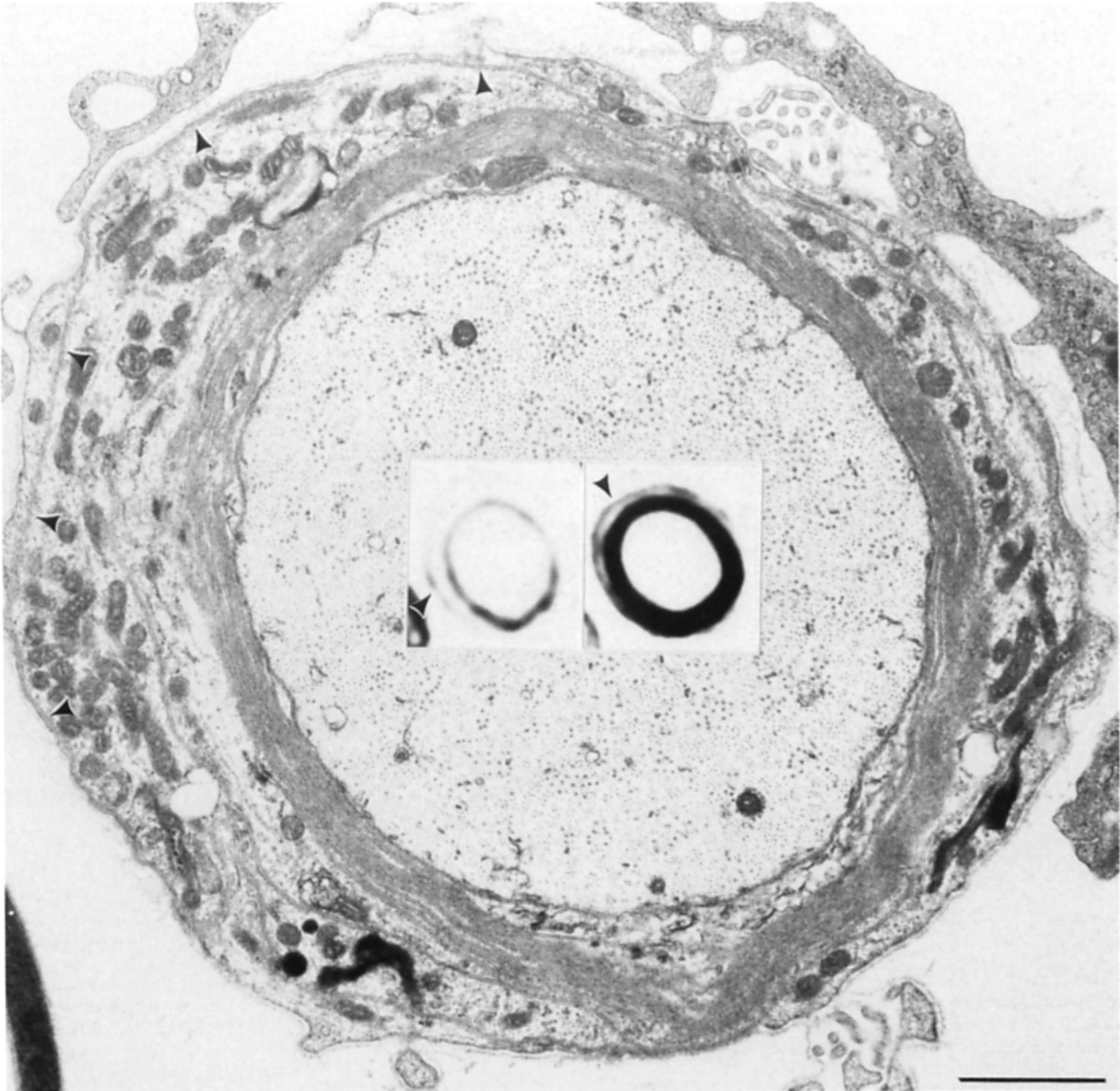
three requirements: the insertion of P<sub>0</sub> protein, the exclusion of Schwann cell cytoplasm, and the removal of MAG. The quaking mutation affects all three events. Each can be altered to various degrees within individual fibers. This suggests that these events are intricately related and likely to be dependent on a variety of feedback mechanisms. How these "events" relate to each other, to the pathology in the quaking mouse, and to the normal process of myelination is discussed below and schematically summarized in Fig. 8.

#### **Insertion of P<sub>0</sub> Protein**

P<sub>0</sub> protein has an *M<sub>r</sub>* of 28 kD, contains ~5% sugar, and comprises >50% of the total protein in myelin fractions isolated from peripheral nerve (8). The complete amino acid sequence of P<sub>0</sub> protein has been deduced from a cDNA clone (17). As the major structural protein of PNS myelin, P<sub>0</sub>

spans the lipid bilayer and is thought to function in stabilizing the apposition of both the extracellular and cytoplasmic membrane surfaces of compact myelin (12, 16, 17). The P<sub>0</sub> gene, recently isolated by Lemke et al. (18), is divided into six exons which can be segregated into extracellular, membrane-spanning, and cytoplasmic domains. The extracellular domain of P<sub>0</sub> is homologous to the variable region fold of immunoglobulins and has been proposed to stabilize the intraperiod line of compact PNS myelin by homotypic interactions (14, 18). Compared to that of MAG, the extracellular domain of P<sub>0</sub> protein is relatively small.

Mesaxon membranes are normally converted to compact myelin after a few wraps are formed. The lack of detectable P<sub>0</sub> immunoreaction product in mesaxon membranes that encircle the axon 2.3 and 5 times (Figs. 2 and 3) suggests that initial insertion and accumulation of P<sub>0</sub> protein into these quaking mouse mesaxons were delayed. The possibility that



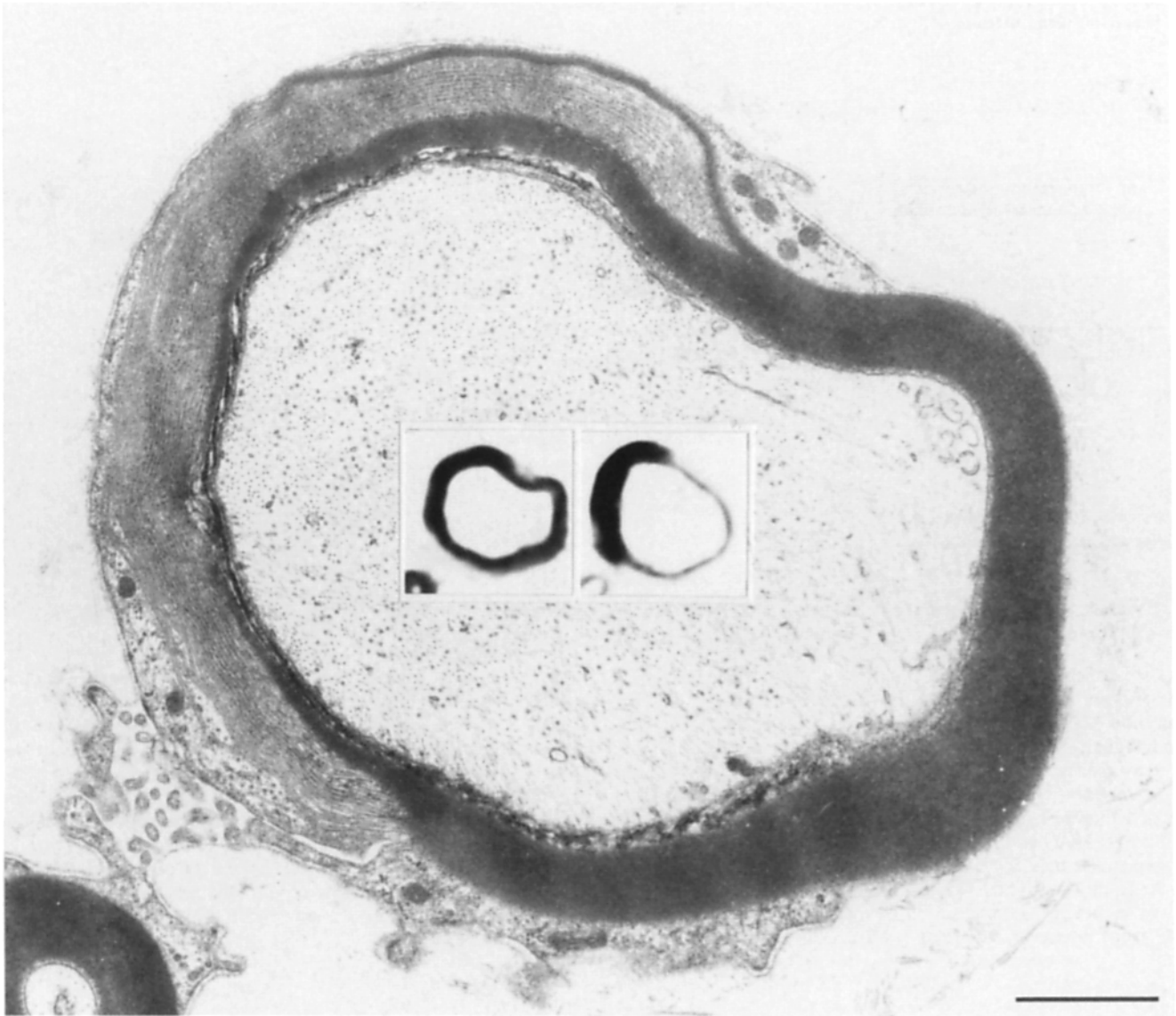
**Figure 6.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacently cut, 1- $\mu$ m sections that were stained by P<sub>0</sub> (left inset) and MAG (right inset) antisera. The mesaxon process encircles the axon 11 times. The eight inner processes closely appose the axon and contain detectable levels of both P<sub>0</sub> and MAG. The outer mesaxon process (arrowheads) contained MAG along its entire length (right inset, arrowhead). In contrast, P<sub>0</sub> protein was detectable over a short segment of the outer mesaxon process (left inset, arrowhead). Bar, 1  $\mu$ m.

these mesaxons contained P<sub>0</sub> protein at concentrations too low to be detected by immunocytochemical means cannot be excluded, but P<sub>0</sub> protein was clearly detectable in a single compact myelin lamella (Fig. 1) and in a single mesaxon membrane (Fig. 6, P<sub>0</sub> inset, arrowhead). In quaking mouse fibers containing multiple mesaxon wraps, P<sub>0</sub> protein appears to be concentrated within the inner wraps (Fig. 7). This is likely to occur during normal myelination since mesaxon membranes closest to the axon are the first to be converted to compact myelin (33) and the first to incorporate P<sub>0</sub> pro-

tein (7). Since the concentration of P<sub>0</sub> protein in mesaxon membranes of quaking mice can be similar to that detected in compact myelin (Fig. 5), and for reasons discussed below, alterations in P<sub>0</sub> protein expression do not appear to be the primary defect in these quaking fibers.

#### **Exclusion of Schwann Cell Cytoplasm**

Two types of cytoplasmic domains can be distinguished in quaking mesaxon membranes: organelle enriched and or-



**Figure 7.** Electron micrograph of a fiber from the L<sub>4</sub> ventral root of an 11-mo-old quaking mouse. The same fiber is shown in serial, adjacent, 1- $\mu$ m sections that were stained by P<sub>0</sub> (*left inset*) and MAG (*right inset*) antisera. The axon is partially surrounded by compact myelin lamellae (*right half*) that are contiguous with noncompact Schwann cell membranes (*left half*). The intensity and thickness of P<sub>0</sub> staining were similar over compact and noncompact membranes. Compared to MAG staining over noncompact membranes and P<sub>0</sub> staining over compact myelin, the band of MAG staining over compact myelin was thinner. Bar, 1  $\mu$ m.

ganelle free. Both areas persist in some fibers, whereas in others one predominates. The presence of organelles such as mitochondria and microtubules in quaking mouse mesaxon cytoplasm must inhibit formation of the major dense line. However, the presence of occasional organelles is likely to be a secondary response because many mesaxon wraps contained little cytoplasm, no organelles, and were enriched in P<sub>0</sub> protein. This observation is of particular interest since P<sub>0</sub> protein is thought to be responsible for maintaining the major dense line of compact PNS myelin (12, 17, 18).

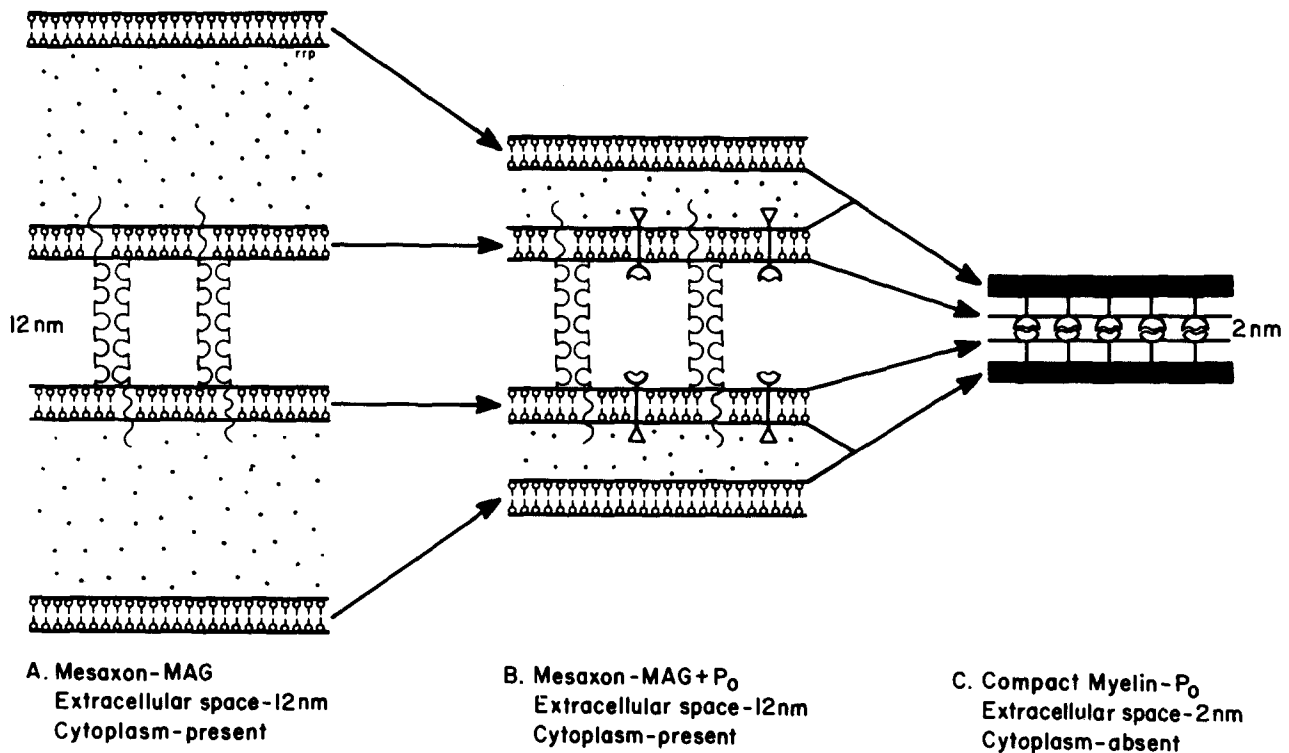
A striking feature of these quaking mesaxon membranes was the strict correlation between the presence of mesaxon cytoplasm and the persistence of the 12–14-nm spacing between their extracellular leaflets. Therefore, the two morphological markers for conversion of mesaxon membranes

to compact myelin, exclusion of mesaxon cytoplasm and a six- to sevenfold decrease in the spacing between extracellular leaflets, appear to be functionally related in quaking mouse fibers. This observation raises the possibility that a transmembrane constituent of mesaxon membranes plays a role in maintaining the extracellular spacing of mesaxon membranes and inhibiting the exclusion of mesaxon cytoplasm. Potential mechanisms controlling the removal of mesaxonal cytoplasm during normal myelination, and how this may be altered in quaking fibers, are discussed below.

#### **Removal of MAG**

MAG is a transmembrane protein that comprises  $\sim 0.1\%$  of the total protein in PNS myelin (20). MAG has an apparent





**Figure 8.** Schematic representation of the orientation and membrane distribution of P<sub>0</sub> protein and MAG during conversion of mesaxon membranes to compact myelin. MAG and P<sub>0</sub> are shown only in the inner two membrane bilayers. Initial mesaxon wraps contain MAG, but little P<sub>0</sub> protein (A). The bulk of MAG's extracellular domain maintains a constant spacing of 12–14 nm between apposing mesaxon membranes. As has been proposed for several immunoglobulin-related adhesion molecules, this may occur by homotypic interactions between apposing MAG molecules. As mesaxon membranes increase in length, P<sub>0</sub> protein is inserted (B). The spacing between the extracellular leaflets of apposing mesaxon membranes containing MAG and P<sub>0</sub> is dictated by the larger MAG molecule. The cytoplasmic domain of MAG inhibits fusion of the cytoplasmic leaflets of these P<sub>0</sub>-containing mesaxon membranes. When the concentration of P<sub>0</sub> protein is sufficient, MAG is removed from mesaxon membranes and compact myelin is formed (C). The extracellular spacing of compact myelin is depicted to be mediated via homotypic interactions between apposing P<sub>0</sub> molecules, as proposed by Lemke and colleagues (16–18). The close apposition of the cytoplasmic leaflets of compact myelin may occur by homotypic interactions between the cytoplasmic domain of P<sub>0</sub> or by heterotypic interactions involving P<sub>0</sub> and acidic lipids. The results of the present study indicated that the failure of quaking mesaxons to convert to compact myelin was a result of their inability to remove MAG.

*M<sub>r</sub>* of 100 kD and contains 30% carbohydrate. In addition to mesaxon membranes, MAG is located in periaxonal membranes, Schmidt-Lanterman incisures, and paranodal myelin loops (27). All MAG-containing membranes have two striking ultrastructural features: (a) their extracellular leaflets are separated by a 12–14-nm gap; and (b) Schwann cell cytoplasm underlies their cytoplasmic leaflets. Based on this correlation, it was proposed that MAG had a structural role in maintaining the periodicity of these membranes (27). The observation that maintenance of the periaxonal space and periaxonal Schwann cell cytoplasmic collar required detectable levels of MAG in periaxonal regions of myelinated fibers in quaking mouse roots provided additional support for this hypothesis (32). In the present study mesaxon membranes that failed to convert to compact myelin always contained MAG, had a 12–14-nm gap between their extracellular leaflets, and also had Schwann cell cytoplasm between their cytoplasmic leaflets. These correlations add further support for the dual structural role of MAG described above.

The amino acid sequence and putative orientation of MAG with respect to the membrane bilayer have recently been deduced from cDNA clones (1, 13, 22). The molecule is

postulated to have a large extracellular domain that contains five segments of internal homology and eight potential N-linked glycosylation sites, a single transmembrane domain, and a 100-amino acid cytoplasmic domain. Most important, these analyses showed homology between the extracellular domain of MAG and the variable region of immunoglobulins, a property shared by a growing number of neuronal and glial adhesion molecules (9, 14). This region of the molecule contains the tripeptide sequence Arg-Gly-Asp (RGD) which appears to be crucial in mediating interactions between transmembrane and extracellular proteins. The bulk of the extracellular domain of MAG and its shared homology with the immunoglobulin superfamily are consistent with the ability of this region of the molecule to maintain the 12–14-nm gap between apposing mesaxon membranes. This may occur by homotypic interactions between apposing MAG molecules as shown schematically in Fig. 8. Whereas the five immunoglobulin domains of MAG are arranged in a straight line in Fig. 8 (13), the tertiary structure of this region of the MAG molecule is unknown and it may fold back upon itself.

Because the bulk of the extracellular domain of MAG exceeds that of P<sub>0</sub> protein, mesaxon membranes containing

both molecules had the 12–14-nm spacing dictated by MAG. The presence of MAG in these mesaxon membranes also dictated the periodicity of their cytoplasmic leaflets in that they were always separated by a variable amount of Schwann cell cytoplasm. This suggests that the cytoplasmic domain of MAG inhibits formation of the major dense line of compact PNS myelin, an event thought to be mediated by the cytoplasmic domain of P<sub>0</sub> protein. Therefore, the failure to remove MAG from quaking mesaxon membranes is likely to account for the deficit in their conversion to compact myelin.

Removal of MAG from mesaxon membranes is probably mediated by the cytoplasmic domain of the molecule. The amino acid sequence of the cytoplasmic domain predicts several proteolytic cleavage sites (19). Proteolysis of this domain could result in release of the rest of the molecule from the membrane as is likely to happen to CNS MAG (20, 23). A more likely hypothesis would involve an interaction between the cytoplasmic domain of MAG and the Schwann cell cytoskeleton that permits lateral diffusion of MAG to the inner and/or outer mesaxon as compact myelin is formed. In support of this hypothesis, actin microfilaments have been shown to colocalize with MAG in PNS myelinated fibers (Trapp, B. D., S. B. Andrews, A. Wong, M. O'Connell, and J. W. Griffin; manuscript submitted for publication). The cytoplasmic domain of MAG contains several phosphorylation sites and shares homology with the cytoplasmic domain of integrin (22), a transmembrane protein that binds microfilaments intracellularly (26). If such an interaction exists, failure to convert mesaxon membranes to compact myelin could be due to (a) alterations in the cytoplasmic domain of quaking MAG, which has an apparent lower *M<sub>r</sub>* than does MAG from control mice (10); (b) alterations in Schwann cell cytoskeletal components; or (c) alterations in cellular regulatory mechanisms (i.e., pH, Ca<sup>2+</sup> concentration, phosphorylation) that govern cytoskeleton–membrane interactions. Since only a proportion of the Schwann cells in quaking mouse ventral roots fail to convert mesaxon membranes to compact myelin and because the MAG gene and the quaking mutation are on different chromosomes (2), alterations in regulatory mechanisms that govern removal of MAG appear to be the most plausible explanation. Failure to remove MAG from mesaxon membranes, therefore, is likely to be a secondary response to the primary quaking mutation.

Fig. 8, depicting homotypic interactions between MAG molecules present in apposing mesaxon membranes, is speculative at this time. Although recent experiments (4) did not show homotypic interactions between MAG molecules purified from the CNS, this does not necessarily rule out the possibility that PNS MAG molecules have the potential to interact homotypically under appropriate conditions. CNS MAG is enriched only in oligodendrocyte periaxonal membranes, and hence it does not have the opportunity to undergo homotypic interaction.

It should be stressed that the MAG sequence data discussed in the present manuscript were derived from cDNA clones isolated from the CNS. Although several studies have shown remarkable similarities between CNS and PNS MAG, such as similar peptide maps, cross-reactivity to antisera (5), and similar mRNA mobilities on agarose gels (22), minor differences may exist between the amino acid sequence or glycosylation of CNS and PNS MAG. Indeed, PNS MAG transcripts apparently do not undergo differential splicing as described for CNS MAG during development (6, 14, 22).

Cloning and sequencing of PNS MAG therefore may reveal other differences that contribute to the wider distribution of MAG in PNS myelin sheaths. In the PNS, a critical question is whether the molecule(s) to which MAG adheres are the same in apposing Schwann cell membranes and axolemma. Whereas it is possible that MAG interacts homotypically in apposing Schwann cell membranes, heterotypic interactions with axolemmal components must occur in both the CNS and PNS.

The author thanks Dr. Pamela Talalay for helpful discussion, Peter Hauer and Cindi Cootauco for preparing the micrographs, and Rod Graham for typing and editing the manuscript.

This work was supported by grant NS-22849 from the National Institutes of Health. Dr. Trapp is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society.

Received for publication 25 February 1988, and in revised form 25 April 1988.

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