Abstract. Quaking is an autosomal recessive hypo/dysmyelinating mutant mouse which has a 1-Mbp deletion on chromosome 17. The mutation exhibits pleiotropy and does not include genes encoding characterized myelin proteins. The levels of the 67-kD isoform of the myelin-associated glycoprotein (S-MAG) relative to those of the 72-kD isoform (L-MAG) are increased in the quaking CNS, but not in other dysmyelinating mutants. Abnormal expression of MAG isoforms in quaking may result from altered transcription of the MAG gene or from abnormal sorting, transport, or targeting of L-MAG or S-MAG. To test these hypotheses, we have determined the distribution of L-MAG and S-MAG in cervical spinal cord of 7-, 14-, 21-, 28-, and 35-d-old quaking mice. In 7-d-old quaking and control spinal cord, L- and S-MAG was detectable in periaxonal regions of myelinated fibers and in the perinuclear cytoplasm of oligodendrocytes. Between 7 and 35 d, L-MAG was removed from the periaxonal membrane of quaking but not control mice. Compared to control mice, a significant increase in MAG labeling of endosomes occurred within oligodendrocyte cytoplasm of 35-d-old quaking mice. S-MAG remained in periaxonal membranes of both quaking and control mice. Analysis of the cytoplasmic domain of L-MAG identifies amino acid motifs at tyrosine 35 and tyrosine 65 which meet the criteria for "tyrosine internalization signals" that direct transmembrane glycoproteins into the endocytic pathway. These results establish that L-MAG is selectively removed from the periaxonal membrane of CNS-myelinated fibers by receptor-mediated endocytosis. The loss of L-MAG from quaking periaxonal membranes results from increased endocytosis of L-MAG and possibly a decrease in L-MAG production.
COOH terminus that are not present on L-MAG (Arquint et al., 1987; Lai et al., 1987; Salzer et al., 1987). L-MAG has several putative phosphorylation sites, including a tyrosine, that are not present in the S-MAG isoform. Tyrosine phosphorylation is important in intracellular signalling, and recently 

**Materials and Methods**

**Tissue**

Quaking mice (C57BLJ-6[qk,qk]) were obtained from Jackson Laboratory (Bar Harbor, ME). Controls were (qk,-). 7-, 14-, 21-, 28-, and 35-d-old quaking and control mice were anesthetized and perfused through the heart with 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry or 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for light microscopic immunocytochemistry or 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for light microscopic immunocytochemistry or 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry. The tissue was postfixied overnight and the cervical spinal cord was removed by dissection.

**Immunocytochemistry**

The cervical spinal cord was cryoprotected overnight in 20% glycerol in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry or 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry. The tissue was postfixied overnight and the cervical spinal cord was removed by dissection. 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry or 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M Sorensen's phosphate buffer for electron microscopic immunocytochemistry. The tissue was postfixied overnight and the cervical spinal cord was removed by dissection. Were cut with a freezing-sliding microtome (Microm, Heidelberg, Germany). Sections processed for avidin-biotin complex (ABC) staining were pre-treated with 1% H₂O₂ in 10% Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 30 min and incubated in the following solutions: 3% normal goat serum for 30 min at 22°C; primary antibodies for 14 h at 4°C; biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA) diluted 1:500 for 30 min at 22°C; enzyme (HRP)-linked biotin (1:1,000) and avidin (1:1,000) (Vector Laboratories) in PBS for 1 h; diaminobenzidine/hydrogen peroxide for 8 min; and 0.08% osmium tetroxide for 2 min. Sections double-stained by immunofluorescence procedures were treated with 10% Triton X-100 for 30 min, incubated in 3% normal goat serum for 30 min, and incubated in primary antibodies for 14 h. Fluorescein- and rhodamine-conjugated secondary antibodies (diluted 1:100; Jackson Laboratories, West Grove, PA) were applied for 1 h. Sections were mounted in a Mowiol-based (Calbiochem-Behring Corp., San Diego, CA) mounting medium containing 0.1% para-phenylene-diamine-hydrochloride.

**Antibodies**

Antibodies for L- and S-MAG have been described elsewhere (Fujita et al. 1990). PLP antibody (mouse) was kindly provided by Dr. N. Groome (Brooke's University, Oxford, UK). The rabbit anti-MBP antibody was purchased from Dako (Copenhagen, Denmark).

**Electron Microscopic Immunocytochemistry**

Ventrolateral white matter tract segments were dissected from cervical spinal cord specimens and infiltrated with 2.3 M sucrose/30% polyvinyl pyrrolidone, placed on specimen stubs, and frozen in liquid nitrogen. Ultrathin frozen sections (~120-nm thick) were cut on glass knives in an ultracytometric microscope with cold-tipped diamond knife (Leitz Wetzlar, Heidelberg, Germany). Confocal images represented optical sections (~0.35-μm axial resolution. Final images represented an average of 32 line scans of the chosen fields (line averaging). Fluorescence in the red (rhodamine) and green (fluorescein) channels was collected simultaneously. In the red/green merged images, areas of colocalization are yellow. Images were photographed with a Leitz Aristoplan confocal laser scanning microscope.
Figure 1. Comparison of the distribution of S-MAG (A, B, E, and F) and L-MAG (C, D, G, and H) in control (A, C, E, and G) and quaking (B, D, F, and H) spinal cord at 7- (A–D) and 35- (E–F) d of age. The number of S- and L-MAG–positive myelin internodes are reduced in quaking spinal cord at 7 d of age. At 35 d of age, S-MAG immunoreactivity is similar in control (E) and quaking (F) spinal cords, while L-MAG immunoreactivity is greatly reduced in quaking (H) when compared to control (G). Arrowheads point to MAG-positive oligodendrocyte perinuclear cytoplasm. Bars, 125 μm.

rowheads). The abundant myelinated fiber staining obscures oligodendrocyte staining in white matter tracts. Compared to control mice, the amount of S- (Fig. 1 B) and L-MAG (Fig. 1 D) immunoreactivity was reduced in 7-d-old quaking spinal cord. Reduced MAG immunoreactivity in quaking spinal cord was due to fewer fibers stained. L- and S-MAG antibodies appear to stain the same number of fibers in 7-d-old quaking spinal cord. Oligodendrocyte perinuclear cytoplasm in sections from 7-d-old quaking spinal cord was stained more intensely for S-MAG (Fig. 1 B, arrowheads) than for L-MAG (Fig. 1 D, arrowheads). This difference was not apparent in sections from 7-d-old control mice.
In sections from 35-d-old control spinal cord, S-MAG (Fig. 1 E) and L-MAG (Fig. 1 G) antibodies stained myelinated fibers in white and gray matter. The fibers were more abundant and larger than those stained at 7 d. L- and S-MAG immunoreactivity was not prominent in oligodendrocyte perinuclear cytoplasm in spinal cord sections from 35-d-old control mice. The amount of S-MAG immunoreactivity increased in quaking at 14, 21, 28, and 35 d, so that in 35-d-old quaking animals (Fig. 1 F) the number of periaxonal rings of S-MAG staining was similar to the number in control sections (Fig. 1 E). In sections from 35-d-old mice, staining of oligodendrocyte perinuclear cytoplasm by S-MAG antibodies (Fig. 1 F, arrowheads) was more apparent in quaking than in control. Between 14 and 35 d of age, the amount of L-MAG immunoreactivity progressively decreased in quaking animals (Fig. 1 H), while it increased in controls (Fig. 1 G).

When sections from 35-d-old spinal cords were viewed at higher magnification, L-MAG staining surrounded all myelinated axons in control mice (Fig. 2 A, arrows) and only a few axons in quaking mice (Fig. 2 B, arrow). Numerous dots of L-MAG staining were present in the white matter (Fig. 2 B, arrowheads) and along axons in gray matter in 35-d-old quaking spinal cord. Similar dots of staining were not detected with S-MAG antibodies (data not shown). In sections from 35-d-old quaking mice, the perinuclear cytoplasm of a few oligodendrocytes was stained by L-MAG antibodies (Fig. 2 B, open arrow). L-MAG staining of oligodendrocyte cytoplasm was weak or absent in 35-d-old control animals.

**Distribution of PLP and MBP in Quaking and Control Spinal Cord**

To determine if the distributions of other myelin proteins were changed in quaking spinal cord, sections from 7-, 14-, 21-, 28-, and 35-d-old animals were immunostained with PLP and MBP antibodies. Fewer and thinner myelin sheaths were stained with the MBP and PLP antibodies in 7-d-old quaking spinal cord when compared to 7-d-old controls (data not shown), reflecting the hypomyelination in quaking. Between 14 and 35 d, the number of myelinated fibers, and the thickness of PLP and MBP stained myelin sheaths in quaking spinal cord increased at a pace similar to that observed in control mice. At all ages analyzed, dots or blebs of MBP and PLP staining were abundant along myelin internodes in quaking (Fig. 3, B and D, arrowheads), but not in control animals (Fig. 3, A and C). Oligodendrocyte perinuclear cytoplasm was not stained intensely by PLP or MBP antibodies at any of the timepoints tested (Fig. 3, A–D).

Double-labeling immunofluorescence and confocal microscopy were used to determine if the dots of staining detected in quaking spinal cord with MBP, PLP, and L-MAG antibodies represented the same, or different structures. In sections from 7-d-old mice, MBP and L-MAG immunoreactivity co-localized in regions of compact myelin in both control (not shown) and quaking (Fig. 4, A–C) mice. In addition, many quaking fibers had regions of MBP and PLP staining which blebbled from the normal contour of the myelin internode (Fig. 4 C, arrow). These structures were continuous with the myelin sheath and represent the redundant loops of myelin identified in previous electron microscopic studies of quaking (Wisniewski et al., 1971; Watanabe et al., 1972). In spinal cord sections from 7-d-old quaking mice double labeled with PLP and MAG antibodies, PLP staining was present in compact myelin and in redundant loops of myelin (Fig. 4 D), while L-MAG immunoreactivity was continuous and in close apposition to the axon (Fig. 4 E). L-MAG immunoreactivity did not colocalize with PLP in the redundant loops of myelin (Fig. 4 F, arrow). Occasional dots of L-MAG staining (Fig. 4, E and F, arrowheads) were separated from the periaxonal region.

In transverse spinal cord sections from 35-d-old quaking mice, PLP-positive myelin sheaths were abundant (Fig. 4 G), while only a few axons were surrounded by L-MAG staining (Fig. 4 H, arrowheads). Dots of L-MAG staining were scattered throughout the white matter (Fig. 4 H). In double-labeled sections, very little or no PLP immunoreactivity was detected around axons containing periaxonal L-MAG staining (Fig. 4 I, arrowheads), indicating that very little or no compact myelin was present around these axons. When S-MAG and PLP were colocalized in 35-d-old quaking spinal cord, S-MAG (Fig. 4 J) and PLP (Fig. 4 K) were detected in all myelinated fibers (Fig. 4 L). The distribution of S-MAG in quaking spinal cord was similar to that in control mice.

**Figure 2.** Distribution of L-MAG immunoreactivity in 30-μm-thick sections of 35-d-old control (A) and quaking (B) spinal cords. In control spinal cords (A), L-MAG immunoreactivity surrounds the perimeter of many axons while oligodendrocyte immunoreactivity is weak or absent. In quaking spinal cord, periaxonal L-MAG immunoreactivity is rare (B, arrow) and L-MAG immunopositive oligodendrocytes are occasionally present (D, open arrow). Intense dots of L-MAG immunoreactivity (B, arrowheads) are abundant in quaking but not control spinal cord. Bars, 25 μm.
Figure 3. Immunocytochemical distribution of PLP (A and B) and MBP (C and D) in 35-d-old control (A and C) and quaking (B and D) spinal cord. PLP and MBP immunoreactivity is confined to myelin internodes in both control and quaking sections. In addition, quaking fibers have dots or blebs of immunoreactivity (B and D, arrowheads) attached to myelin internodes. Bars, 25 μm.

Subcellular Distribution of MAG

To characterize the subcellular distribution of MAG in quaking spinal cord, ultrathin cryosections were stained by immunogold procedures (Fig. 5). Because L- and S-MAG specific antibodies did not label glutaraldehyde fixed sections, a monoclonal antibody directed against an epitope common to both MAG isoforms (B11F7) was used. In MAG-stained ultrathin cryosections of spinal cord from 7-, 14-, and 35-d-old quaking animals, gold particles were enriched in periaxonal regions of myelinated fibers and in large membrane-bound organelles that contained internal membranes (Fig. 5, A–F). These organelles had the ultrastructural characteristics of endosomes. MAG-positive endosomes were found in oligodendrocyte cytoplasm of inner (Fig. 5, A and B) and outer (Fig. 5 C) tongue processes (Fig. 5 C), in redundant loops of myelin (Fig. 5 D), in oligodendrocyte processes that extended to myelin internodes (Fig. 5 E), and in oligodendrocyte perinuclear cytoplasm (Fig. 5 F). Consistent with a previous study of rat spinal cord (Trapp et al., 1989), MAG was enriched in oligodendrocyte endosomes in control mice during early (7 d) but not late (35 d or older) stages of myelination. Quantitation of gold particles within endosomes in 35-d-old spinal cord (Table I) detected a significant increase \( P < 0.0001 \) in MAG labeling of endosomes in quaking compared to control mice. Therefore, a major difference in our analysis of 35-d-old mice was the enrichment of MAG in endosomes of quaking but not of control oligodendrocytes. Gold particles were also enriched in oligodendrocyte perinuclear cytoplasm (Fig. 5 F) and over periaxonal membranes of myelin internodes in both quaking (Fig. 5, A–D, arrowheads) and control animals at all timepoints studied. In PLP stained cryosections, gold particles were detected over myelin sheaths and redundant loops of myelin, but were rare in oligodendrocyte cytoplasm and processes. PLP and MBP were not enriched in endosomes in quaking or control spinal cord (data not shown).

Discussion

Previous studies indicated that, in comparison to control mice, the mRNA for S-MAG was substantially overexpressed relative to that for L-MAG in quaking brain (Frail et al., 1985; Fujita et al., 1988). However, subsequent reports in which expression of the L- and S-MAG proteins was monitored by Western blotting reached different conclusions. One report indicated that L-MAG was barely detectable in developing and adult quaking mice (Fujita et al., 1990), while another detected equal amounts of L- and S-MAG in adult mice (Braun et al., 1990). To clarify these apparent discrepancies, the present immunocytochemical study was undertaken in parallel with a biochemical investigation of MAG expression in spinal cords from quaking mice (Bartoszewicz et al., 1994). Both approaches detected
Figure 4. Confocal images of sections (30-μm thick) from 7- (A–F) and 35-d-old (G–L) quaking spinal cord. In a 7-d-old section PLP (A, green) and MBP (B, red) colocalize in compact myelin and in a redundant loop of myelin (C, arrow). Redundant loops of myelin (F, arrow) are labeled by PLP (D, arrow) but not by L-MAG antibodies (E). L-MAG antibodies stain periaxonal regions of myelin internodes (E) and also produce dots of staining which are not connected to the periaxonal region of the myelinated fibers (E and F, arrowheads). In sections from 35-d-old quaking labeled with PLP (G) and L-MAG (H) antibodies, many myelin sheaths are stained with PLP (G and I, arrows) while comparatively few periaxonal rings of L-MAG staining are detected (H and I, arrowheads). Axons surrounded...
significant L-MAG in young quaking mice and a dramatic decrease in L-MAG with maturation. Immunocytochemical observations established that L-MAG is present around most axons during the initial stages of myelination in quaking spinal cord. By 35 days of age, however, periax- 
onal L-MAG is absent from most myelinated fibers. Our ultrastructural studies indicate that periaxonal L-MAG is removed by endocytosis and not replaced. In addition, biochemical studies have demonstrated that both L- and S-MAG are abnormally glycosylated in quaking mutants (Bartoszewicz et al., 1994). Overall, our combined immu-
ocytochemical and biochemical studies indicate that the quaking mutation leads to an under expression of L-MAG that becomes more severe with maturation, abnormalities in the posttranslational glycosylation of MAG, and the eventual depletion of L-MAG from the periaxonal mem-

**MAG Isoforms May Have Different Functions**

Several adhesion molecules in the central nervous system, including MAG, undergo a developmentally regulated, al-
ternative splicing of cytoplasmically disposed carboxy ter-

al amino acids (Frael et al., 1984; Tropak et al., 1988; Ny-

broe et al., 1990; Rutishauser, 1990; Pedraza et al., 1991). L-MAG is the predominant MAG isoform in the CNS during early stages of myelination, suggesting that the ad-
tional amino acids of L-MAG may have unique functions during initial stages of oligodendrocyte—axon interactions (Frael et al., 1984; Tropak et al., 1988; Pedraza et al., 1991). These interactions could involve a role for L-MAG in ax-
onal recognition, axonal ensheathment, regulation of mye-
lent sheath thickness, and signaling to the oligodendrocyte to regulate the number of axons it associates with. The func-
tions of MAG isofoms may be reflected in their distribu-
tion within oligodendrocytes or their myelin sheaths. Results from the present study establish that both L- and S-MAG are present around the entire perimeter of the periaxonal membrane of CNS myelin sheaths. Previous studies had sug-
gested that L-MAG, but not S-MAG, was endocytosed from periaxonal membranes during early stages of CNS mye-
elination, suggesting an L-MAG specific function in an end-
cytic pathway (Trapp et al., 1989). The results of this study indicate that L-MAG is endocy-
tosed from the periaxonal membrane to oligodendrocyte endosomes. These endosomes were evident as small dots of L-MAG staining in light microscopic immunocyto-

tical studies (Fig. 5, Table I). The pronounced loss of L-MAG periaxonal immunoreactivity concomitant with an accumulation of MAG in endosomes indicates that MAG is degraded in the endosomal—lysosomal system.

Receptor-mediated endocytosis is initiated by the inter-
a
duction of a receptor with its ligand. Specific amino acid se-
quencies signal internalization of transmembrane glycopro-
tins in an energy-dependent ligand process (Sandoval et al., 1994). Endocytic signals usually reside in the cytoplasmic domain of the receptor. A tyrosine internalization sig-
nal, which is necessary and sufficient for rapid internaliza-
tion through clathrin-coated pits, has been identified on a number of transmembrane glycoproteins, including the poly-


globulin receptor and the low density lipoprotein (LDL) receptor (Kistakis et al., 1990). This internalization signal is formed by a motif of 8 to 10 cytoplasmic amino acids with a central tyrosine which is flanked by amino acids which are basic or polar or that prefer a random coil con-
f


**Table I. Endosomes in 35-d-old Quaking Spinal Cord Have Significantly More MAG Than Endosomes in 35-d-old Control Spinal Cord**

<table>
<thead>
<tr>
<th>Control mice</th>
<th>Quaking mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of endosomes examined</td>
<td>61</td>
</tr>
<tr>
<td>Gold particles per endosome (mean ± SD)</td>
<td>0.79 ± 1.22</td>
</tr>
</tbody>
</table>

*P < 0.0001

Ultrathin cryosections of ventral lateral spinal cord were labeled with MAG antibo-
dies and examined in an electron microscope. Endosomes associated with myelinated fibers or oligodendrocytes were identified and photographed. The number of gold par-
ticles in each endosome was quantitated and a students t-test was performed. Com-
pared to control mice, the quaking mouse had a significant increase in MAG labeling of endosomes (P > 0.0001).

**L-MAG Is Internalized through Receptor-mediated Endocytosis**

Receptor-mediated endocytosis (RME) is a constitutive pro-
cess that occurs in most eukaryotic cells. This process in-
ternalizes cell surface receptors and their ligands and it

by rings of L-MAG staining are in early stages of myelination as very little or no PLP immunoreactivity is detected around L-MAG-po-


give periaxonal membranes (I, arrowheads). In contrast, PLP (J) and S-MAG (K) colocalize (L) in myelinated fibers in 35-d-old quak-

ing spinal cord. Bars, (A–F) 5 μm; (G–L) 10 μm.
motif at Y35 (Table II). Tyrosine 35 is at the splice site which distinguishes L- and S-MAG, and therefore the Y35 signal is not identical in L- and S-MAG. Whether endocytosis of L-MAG requires the motif at Y35, Y65, or both, remains to be established. Receptor-mediated endocytosis has also been linked with tyrosine phosphorylation (Sibley et al., 1987). Tyrosine phosphorylation plays an important role in regulation of cell differentiation and growth. Recent studies indicated that L-MAG co-immunoprecipitated with fyn, a nonreceptor-type tyrosine kinase of the src family that is activated during early stages of myelination (Umemori et al., 1994). Antibodies binding to the extracellular domain of L-MAG, but not S-MAG, resulted in rapid increases in fyn tyrosine kinase activity (Umemori et al., 1994). These observations potentially link L-MAG to intercellular signaling pathways, and raises the possible connection between fyn tyrosine phosphorylation and endocytosis of L-MAG. An important role for fyn in CNS myelination is indicated by defective myelination in fyn knock-out mice (Umemori et al., 1994).

**Receptor-mediated Endocytosis of L-MAG and the Quaking Phenotype**

The selective removal of L-MAG from the periaxonal membrane in quaking mice may contribute to their hypo- and
Table II. Identification of Tyrosine Internalization Signals in the Cytoplasmic Domain of L-MAG

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>Tyrosine position</th>
<th>Length of tail</th>
<th>Amino acid sequence</th>
<th>Polar or Positively Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-MAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R I S G A P D K Y E</td>
<td>35</td>
<td>+3</td>
<td>R I S G A P D K Y E</td>
<td>+3</td>
</tr>
<tr>
<td>S-MAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R I S G A P D K Y E</td>
<td>35</td>
<td>+3</td>
<td>R I S G A P D K Y E</td>
<td>+3</td>
</tr>
<tr>
<td>L-MAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G K R P T K D S Y T L T</td>
<td>65</td>
<td></td>
<td>G K R P T K D S Y T L T</td>
<td></td>
</tr>
</tbody>
</table>

Sequences are oriented from NH2 to COOH terminus. L-MAG contains two motifs at tyrosine position 35 and 65, S-MAG contains one motif at tyrosine 35. The endocytosis motif consists of a tyrosine residue which is flanked by amino acids that prefer a random coil conformation and/or that are polar or positively charged. Amino acids which meet these criteria are underlined.

Dysmyelinating phenotype. L-MAG is detectable in most quaking CNS periaxonal membranes during axonal ensheathment. Quantitative western blot assays indicate that L-MAG levels are dramatically reduced (Fujita et al., 1990; Bartoszewicz et al., 1994). The deficiency in L-MAG occurs during the most rapid stages of myelination, 21–35 d. Abnormal glycosylation of L-MAG may also contribute to the hypomyelination and formation of redundant loops of compact myelin. Results of the present study also indicate that the deficit in replacing L-MAG in the periaxonal membrane is regulated developmentally for individual oligodendrocytes. This is based on the presence of occasional L-MAG–positive fibers in 35-d-old quaking spinal cord. These oligodendrocytes are in early stages of myelination or remyelination because their fibers were L-MAG positive and PLP negative. While fibers in early stages of myelination occur in 35-d-old quaking spinal cord, their numbers are not sufficient to influence our quantitative analysis of MAG-labeled endosomes. MAG-labeled endosomes associated with axon ensheathment or initial spiral wrapping of compact myelin were not found in 35-d-old quaking ultrathin cryosections.

An important unanswered question is how the quaking mutation affects the developmental program of L-MAG expression. Endocytosis of L-MAG occurs in normal mammalian CNS at early stages of myelination (Trapp et al., 1989). The amino acid sequence of MAG is not mutated in quaking mice. Initial characterization of the quaking mutation has identified a large deletion (1-Mbp) on the proximal end of chromosome seventeen (Eversole et al., 1992), which may account for the phenotypes affecting multiple organs (i.e., brain and testes). The mutation could affect processing of MAG transcripts, post-translational modifications of MAG, or targeting of L-MAG to the periaxonal membrane. Based on the low levels of L-MAG mRNA and the failure of our ultrastructural immunocytochemical studies to detect a build-up of L-MAG in RER and Golgi membranes or mistargeting of L-MAG to surface membranes other than the periaxonal membranes, it is likely that the mutation affects processing or turnover of MAG transcripts. This hypothesis is supported by recent studies which indicate that the quaking deletion includes a multifunctional gene which is involved in RNA metabolism (Artzt, K., personal communication).

Our immunocytochemical studies failed to detect alterations in the distributions of myelin basic protein or proteolipid protein in quaking CNS. MBP and PLP were not enriched in endosomes in quaking or control CNS, both were present in the redundant loops of myelin, and neither accumulated in the oligodendrocyte perinuclear cytoplasm or cytoplasm associated with redundant myelin loops. The quaking mutation does not appear to affect the processing and targeting of MBP and PLP in myelinating oligodendrocytes. An increase in the 21-, 18-, and 17-kD MBP isoforms relative to the 14-kD isoform has been reported in quaking CNS myelin (Inuzuka et al., 1987). This likely reflects the immature nature of quaking myelin rather than defective splicing of MBP transcripts.

Conclusion

This study has described a selective abnormality in the distribution of L-MAG in the quaking central nervous system. Our studies have demonstrated that L-MAG is inserted into the periaxonal membrane during early stages of myelination and then removed by receptor-mediated endocytosis and not replaced. We have also identified “tyrosine internalization signals” in L-MAG. The distribution of S-MAG is identical in control and quaking CNS. The removal of L-MAG from the periaxonal membrane of quaking myelinating fibers may disturb axon/oligodendrocyte signaling and contribute to the hypo/dysmyelination.

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