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MYELIN BASIC PROTEIN AND THE STABILITY OF THE MULTILAMELLAR MYELIN STRUCTURE

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Summary

A purified myelin fraction was prepared from myelin deficient (mld) mutant mice and normal littermates (20 to 25 days old). Mld brains contained only 5 % of the amount of myelin present in controls. Myelin isolated from mld brains had a normal lipid and protein content. The lipid composition of mld myelin was also normal. Myelin basic protein (MBP) was nearly missing in mld myelin. At the electron microscope, in mld myelin, the major dense line was often missing and the myelin lamellae were uncompacted. The high specific radioactivity of the myelin associated glycoprotein and sulfatides, two myelin constituents which are not affected by the mutation, indicate a high turnover rate of myelin membranes in the mld mutant. Furthermore, the presence of increased amounts of cholesterol esters in mld brains suggests that myelin in mld mutants is unstable. We propose that MBP, which is responsible in the central nervous system for myelin compaction, is responsible for the stability of this membrane.

The myelin sheath is a modified and highly specialized plasma membrane which is wrapped around a portion of the axon like a spiral (Fig. 1). The myelin membrane is an extension of the oligodendrocyte plasma membrane in the central nervous system (CNS). During the process of myelin formation, the cytoplasm is extruded and the cellular leaflets are fused to form the major dense line (Fig. 2). The external surfaces come together and form the intraperiod line (also called minor dense line). For a review see ref. 11. Myelin acts as an insulator and is responsible for the saltatory conduction which allows action potential to propagate faster in smaller axons than in unmyelinated fibers. Therefore, myelination is a major evolutionary breakthrough toward a miniaturized nervous network. Furthermore, saltatory conduction requires only a fraction of the energy necessary for a continuous sequential depolarization.

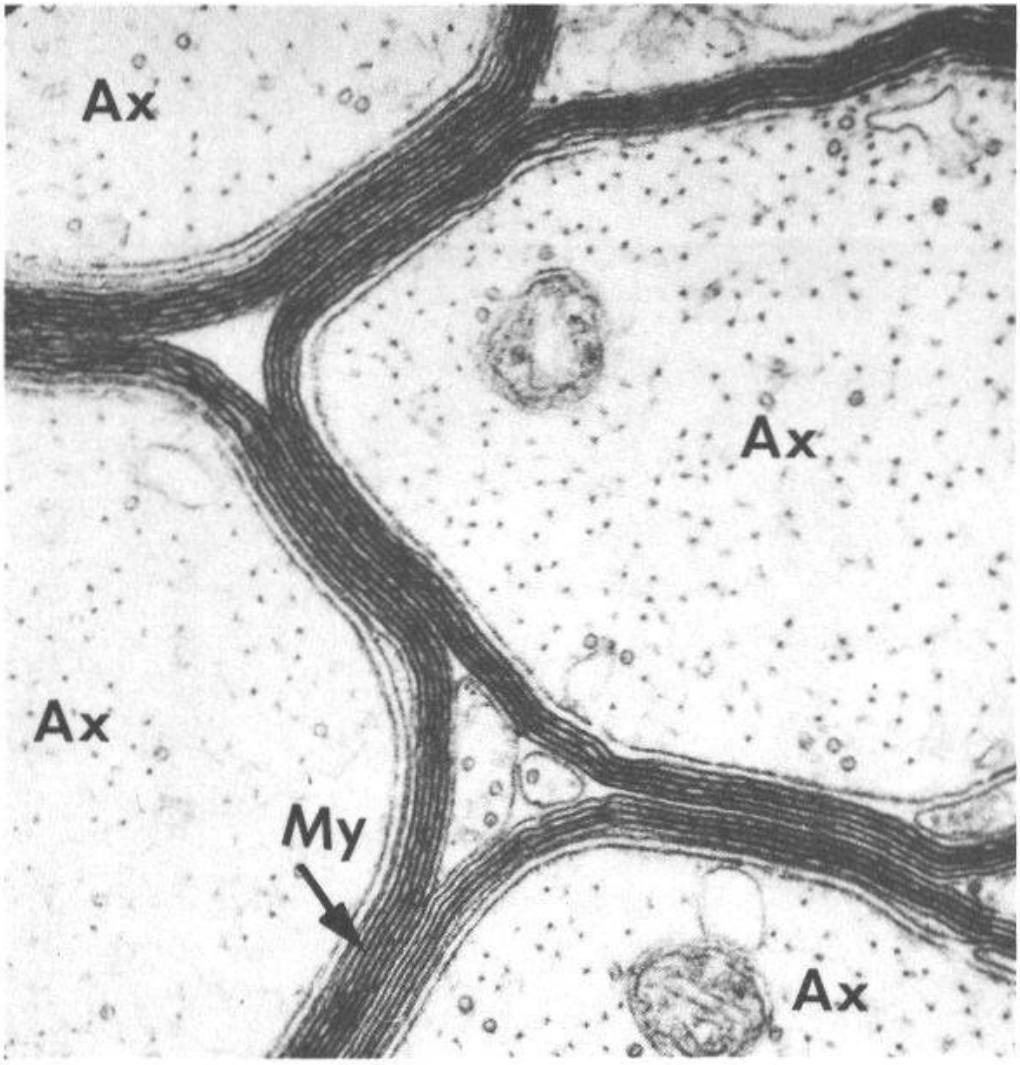

Fig. 1. Electron micrograph of a cross-section through 4 myelinated axons (Ax). The multilamellar structure of the myelin sheath (My) is easy to recognize. In the CNS, the myelin sheath is not surrounded by the cytoplasm of the glial cell like in the PNS. Therefore, the myelin sheath from two adjacent axons can make direct contact. On this micrograph only the major dense line of myelin can be observed. Magnification ca. X 120,000 (Courtesy of Dr H. de F. Webster).

Such a complex organization like the myelin sheath must result from a progressive and sequential process in which the interaction between the axon, the oligodendrocyte (Schwann cell in the peripheral nervous system) and the environment modulates the expression of a basic genetic program. In the human, hereditary neurological disorders reveal that several genes are essential for normal myelin formation. Therefore, the study of mutations affecting myelination should provide improved understanding of the assembly and maintenance of myelin. Murine mutants, unlike human disorders have a homogeneous genetic background and can be studied in a controlled environment. Since a great number of animals can be bred

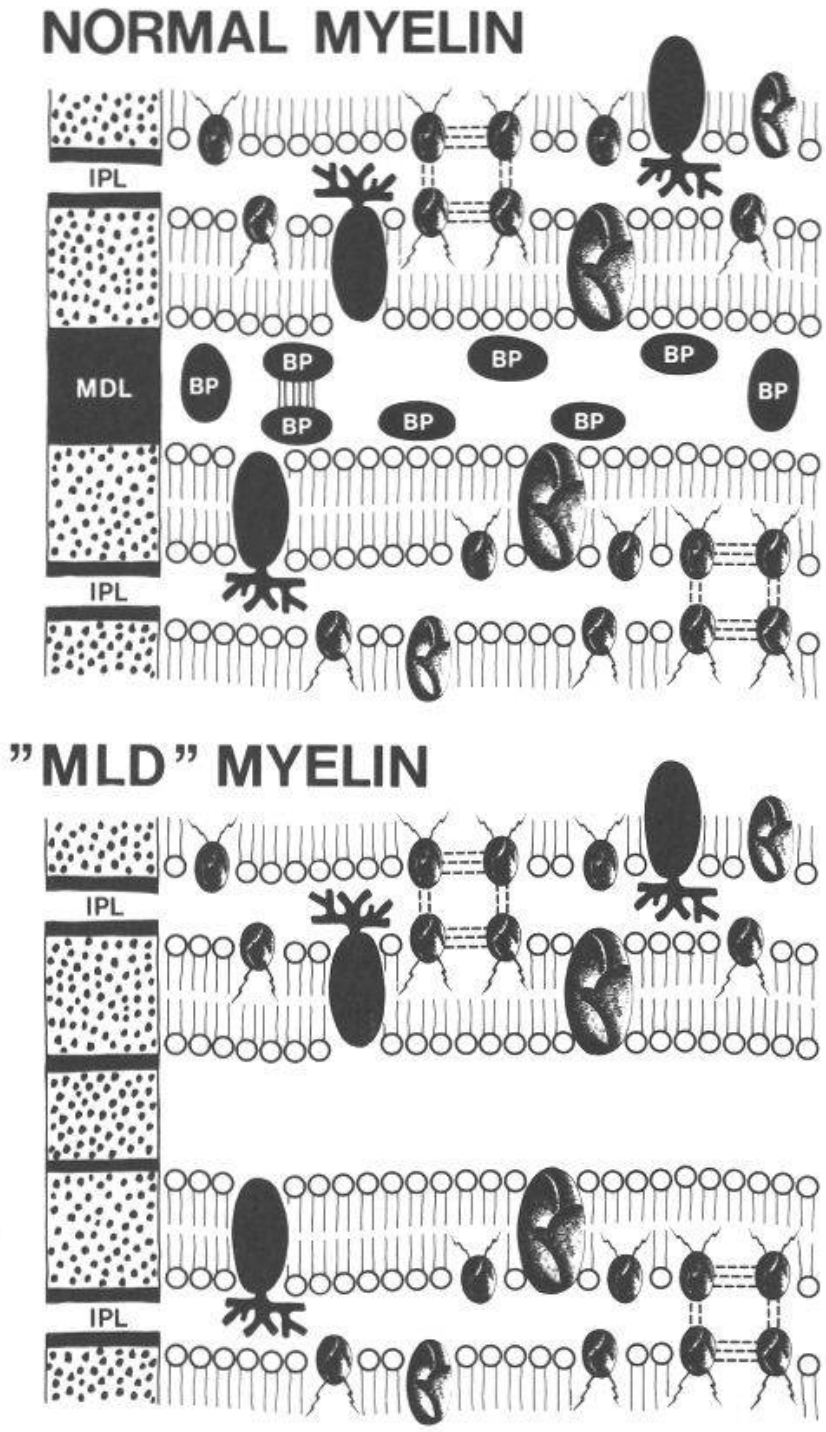

Fig. 2. Schematic representation at the molecular level of a myelin lamella in normal and mld CNS. On the left part of the figure, a schematic enlargement of the myelin lamella at the electron microscope is presented. BP, myelin basic protein forms the major dense line. Several myelin proteins (dark ovoids) are inserted into the lipid bilayer (open circles). In mld, myelin basic proteins and the major dense line (MDL) are practically missing. IPL, intraperiod line. The molecular organization of the myelin membrane was inspired from P.E. Braun (In P. Morell, ed. Myelin, Plenum Press, 1977, p. 110).

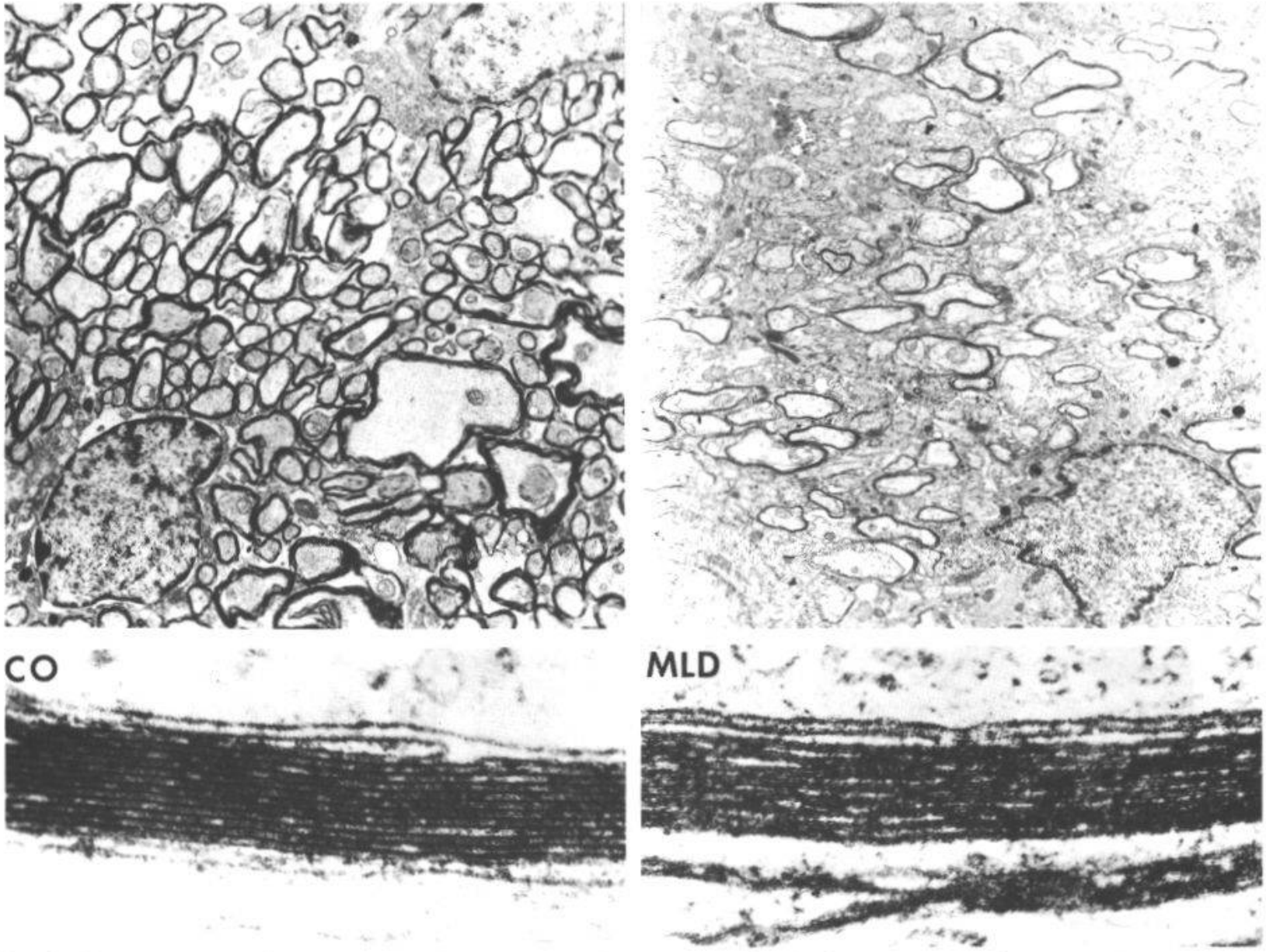

Fig. 3. Electron micrographs of control (left) and mld (right) optic nerves (33 days). Cross-sectioned axons of different diameters are surrounded by a dark ring, the myelin sheath. In mld optic nerves, very few axons are myelinated and the myelin sheath appears very thin. Lower part of the figure, high power magnification of control (left) and mld (right) myelin. In control myelin, the sheath is well compacted and the major and minor dense lines are easily identified. In contrast, mld myelin is poorly compacted and only the minor dense line seems present. Magnifications, X 8,400 and X 125,000. These micrographs were prepared by Dr R.L. Friede (ref. 7).

readily, sequential analyses allow a dynamic examination of myelination processes.

Myelin has a high lipid content and myelin fragments can be isolated as a highly purified fraction from other cellular particles, by ultracentrifugation of brain homogenates on a sucrose gradient.

CNS myelin has a relatively simple protein pattern (Fig. 4) when compared to other plasma membranes. Myelin basic proteins (MBP) account for 30 % of the total myelin proteins. Several structurally related forms of MBP have been identified in mouse brain, their molecular weights range from 21.5 KD (Kilo Daltons) to 14 KD. The major components are called large (LBP) and small (SBP) myelin basic protein. MBP is the factor responsible for experimental allergic encephalomyelitis (EAE) and consists of a single highly basic polypeptide chain of approximately 170 amino acid residues.

In this paper, I would like to present several pieces of evidence indicating that MBP is directly involved in the compaction of the multilayered structure and plays an important role to maintain the stability of the myelin sheath.

For this study, myelin deficient (mld) mutant mice were used. The CNS of mld mutants has a dramatic myelin deficit (ref. 7). The few myelinated axons present in mld brains have a thin and poorly compacted myelin sheath with an absence of the major dense line (Fig. 3).

In CNS myelin from young mld mice, the two myelin basic components (LBP and SBP) are practically missing, whereas the Wolfgram protein is increased (Fig. 4). A fast migrating peptide (12 KD) was increased in mld myelin. Using the immuno-blotting technique this "X" band did not bind antibodies against MBP. Additional work is currently in progress in our laboratory to characterize this small peptide. The lipid composition of mld myelin is normal. Myelin fractions were purified from mld brains and examined at the electron microscope. In mld myelin, fewer multilayered structures were present than in control myelin and the major dense line was difficult to identify and hazy. These results indicate that MBP is localized at the major dense line, as suggested by other studies (ref. 3-5, 13, 14).

A similar situation was also reported in another mutant, the shiverer mouse (ref. 1) and the two mutations were recently compared in some details (ref. 9). In this disposition MBP could be classified as an extrinsic or peripheral membrane protein since the major dense line is formed by the fusion of the apposed cytoplasmic surface of the oligodendroglial plasma membrane (Fig. 2). Therefore, the possible function of the protein could be the compaction of the myelin sheath. These indirect evidences were confirmed very recently by Dr F. Omlin, a young Swiss neurobiologist working in Dr H. de F. Webster's laboratory at the National Institutes of Health in Bethesda, Maryland (U.S.A.) who showed for the first time convincingly that MBP is localized at the major dense line of myelin (ref. 12).

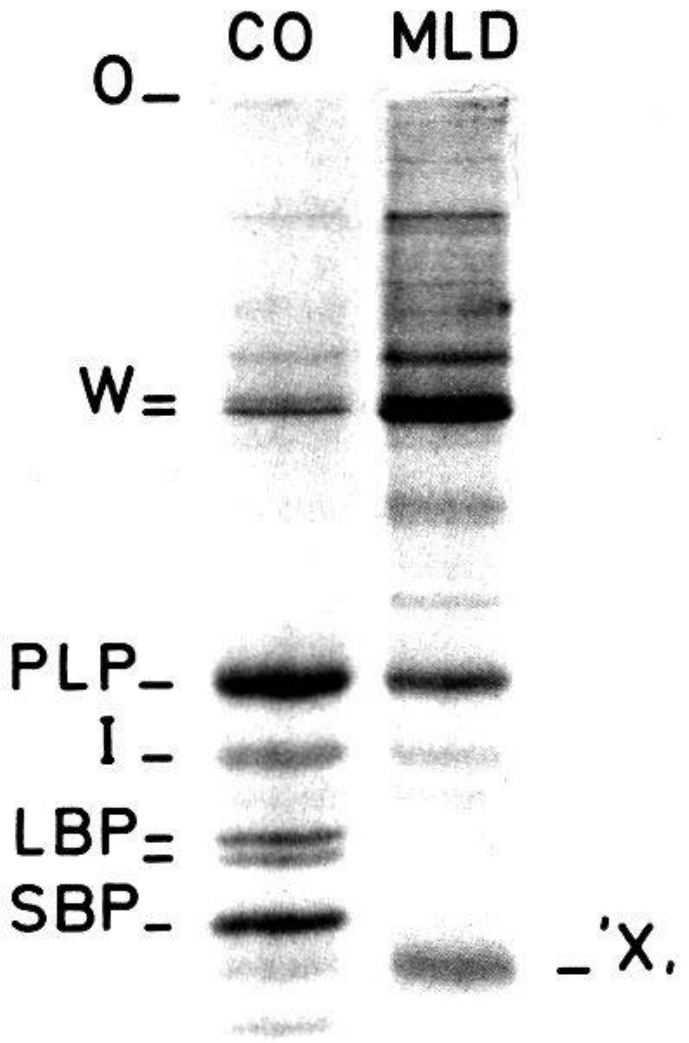

Fig. 4. Proteins from control (CO) and mld CNS myelin stained with Fast Green after separation by electrophoresis on a 12.5 % SDS-polyacrylamide slab gel. O, origin of the migration. W, Wolfgram protein doublet. PLP, proteolipid protein. I, intermediate protein. LBP, large components of myelin basic protein. SBP, small component of myelin basic protein. "X", 12 KD band, increased in mld myelin.

In the absence of MBP, what is the stability of the uncompacted myelin sheath? Four pieces of evidence indicate that myelin lamellae in the absence of MBP are unstable (ref. 10):

1. While only traces of cholesterol esters could be detected in lipid extracts from control brains, in mld brains we found increased amounts of cholesterol esters. This is a commonly accepted sign that active demyelination is occurring in the CNS.
2. While the incorporation of (^{3}H) fucose into whole brain glycoproteins was similar in young mld and control mice, the specific radioactivity of the myelin associated glyco-

protein (MAG) was 12 times higher in mld than in control myelin. Since MAG concentrations in mld myelin are normal, these results suggest that MAG has an extremely high turnover rate in mld myelin.

3. Furthermore, the specific radioactivity of (^{35}S) sulfatides in myelin was also increased in mld myelin, whereas the sulfatide content of myelin was normal, indicating an increased turnover rate of this myelin constituent in mld brains.
4. At 60 days of age, the concentration of MBP increases and its concentration in purified myelin at 135 days of age is practically normal (ref. 2). Myelin components have normally a very slow turnover rate and this normalization can only be explained by the deposition of a newly formed membrane which is not diluted in the pool of preexisting abnormal myelin.

   These preliminary results are confirmed by a comparative study performed in the normally myelinated peripheral nerves of the same mutants (ref. 8) in which MBP reaches approximately 50 % of control values. In mld PNS, newly formed myelin membranes with a normal MBP content are diluted by the normal amounts of myelin devoid of MBP. Previous studies indicate that, in the PNS, myelin can be assembled in the absence of MBP, and another protein PO, which represents 60 % of the total protein content in control and mld PNS myelin could be responsible for the formation of the major dense line (ref. 6,8).

In conclusion, these results indicate that myelin in young mld brains has a high turnover rate. This instability prevents from the deposition of myelin in mld brains. Therefore, we propose that myelin compaction (the linkage between the cytoplasmic faces of the modified oligodendroglial plasma membrane), which is performed in the CNS by MBP, is a prerequisite for the stability of the membrane.

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