Molecular Biology of Myelin Proteins from the Central Nervous System

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Myelinogenesis is a complex, developmentally regulated, neurobiological process involving the coordinated expression of myelin protein genes and the genes for several enzymes in biosynthetic pathways leading to the synthesis of myelin-specific lipids. In the past several years, the value of this neurobiological system for molecular genetic and cell biological studies has become increasingly recognized, and the genes encoding four of the myelin proteins have been isolated and their structures determined. Through alternative splicing mechanisms, each of these genes produces a family of mRNA transcripts that encode two or more polypeptide isoforms. In several cases, the production of the alternatively spliced transcripts is developmentally regulated, leading to changes in the relative proportions of isoforms synthesized, which appear to correspond to developmental changes in the ratios of those isoforms in the myelin membrane. Although the myelin protein composition initially was thought to be relatively simple, recent molecular biological studies have shown that the numbers of major myelin protein isoforms, and the regulation of their expression, are far more complex than originally expected. Nonetheless, in the past several years several important advances have been made in our understanding of the myelin protein genes, their structures, and their expression in several species and in dysmyelinating mutant mice.

Chromosomal assignments of three myelin protein genes have now been made in the human, the mouse, or both, and abnormalities in two separate genes appear to be responsible for the genetic defects in the murine dysmyelinating mutants shiverer and jimpy. Production of transgenic shiverer mice with one or two normal myelin basic protein (MBP) genes appears to correct many of the clinical and biological abnormalities associated with the defect. Even though the molecular basis of the mutation has been established in the case of shiverer and jimpy, most of the dysmyelinating mutants appear to exhibit pleiotropy with respect to the expression of other myelin protein genes and some enzyme activities associated with the metabolism of myelin lipids.

The major myelin proteins, constituting ~80% of the protein content of myelin, fall into two classes—the MBPs and the proteolipid proteins. Two less abundant myelin proteins about which molecular biological information is rapidly emerging are the myelin-associated glycoprotein (MAG) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP). In this short review, the molecular biology of each of these four proteins in the CNS will be discussed separately with respect to their genes and their expression in normal and mutant mice. The reader is referred to more extensive reviews for further information about the metabolism of these proteins in vivo and in vitro (Benjamins, 1984; Campagnoni and Macklin, 1988; Lees and Macklin, 1988).

MYELIN BASIC PROTEINS

The MBPs are a class of myelin proteins consisting of multiple polypeptide chains varying in molecular mass from 14 to 21.5 kilodaltons (kDa), depending on the species. At least six isoforms have been identified in mice (deFerra et al., 1985; Newman et al., 1987a,b) and four in humans (Kamholz et al., 1986; Roth et al., 1986, 1987). The MBPs are hydrophilic, extrinsic membrane proteins with isoelectric points...
higher than most of the histones. MBPs are generally believed to be located at the major dense line of myelin, which is formed during myelinogenesis by apposition of the cytoplasmic surfaces of the oligodendroglial plasma membrane as it wraps around an axonal segment. The MBPs undergo several post-translational modifications, including N-terminal acetylation, phosphorylation, and methylation. Although the physiological significance of these post-translational modifications has yet to be demonstrated, indirect evidence suggests that methylation of MBP may be important for compaction of the membrane during myelin maturation (Scott et al., 1981; Small et al., 1981; Amur et al., 1986).

### MBP gene structure

The mouse MBP gene is >30 kilobases (kb) in length (see Fig. 1), consisting of seven exons, some of which are as small as 33 base pairs (bp), which are interrupted by introns that can be quite large (deFerra et al., 1985; Takahashi et al., 1985). Southern blot analysis indicates the presence of only a single gene in the mouse (deFerra et al., 1985; Takahashi et al., 1985) and in the human (Kamholz et al., 1986). The MBP gene has been assigned to the distal end of chromosome 18 in both the mouse (Roach et al., 1985) and human (Saxe et al., 1985; Sparkes et al., 1987) by both in situ hybridization and somatic cell hybridization techniques.

![Diagram of MBP gene structure](image)

**Fig. 1.** Expression of the MBP gene, illustrating the production of the MBP mRNAs for which structures are known and the MBP isoforms for which they code. The borders of the coding region of the gene are indicated by the dotted lines shown in exon 1 (5' end) and exon 7 (3' end). The three exons involved in alternative splicing are cross-hatched (exons 2, 5, and 6), and their presence is indicated by a box in each of the mRNA representations. The numbers above the mRNAs indicate the presence of those exons in the mRNA. The sizes of the MBP isoforms for which the mRNAs code are given in parentheses beside each mRNA. The translation products of the mRNAs are presented in the bottom panel. Residues are identified according to the numbering of the 21.5-kDa MBP isoforms. The polypeptide regions that correspond to the alternatively spliced exons are given, presented as heavy lines along with the numbers of the N-terminal and C-terminal residues. Missing polypeptide segments are indicated by nonconsecutive adjacent numbers.
Alternative splicing and the origin of MBP isoforms

Cell-free translations of mouse and rat brain mRNA proved that the multiple isoforms of the murine MBPs were the products of separate mRNAs rather than posttranslational processing events (Colman et al., 1982; Yu and Campagnoni, 1982). To determine whether or not a single gene encoded the multiple MBP mRNAs, and also to determine the structural relationship among the MBP isoforms, several laboratories isolated cDNAs corresponding to mouse and rat MBP mRNAs (Roach et al., 1983; Zeller et al., 1984; Mentaberry et al., 1986; Roth et al., 1986). These studies revealed the existence of a wider variety of MBP isoforms than previously was thought to exist in the mouse and human (Roach et al., 1983; deFerra et al., 1985; Kamholz et al., 1986; Roth et al., 1986, 1987; Newman et al., 1987a). For example, cDNAs of mRNAs encoding five forms of the mouse MBP and four forms of the human MBP have now been isolated. The five mouse MBP cDNAs encode MBP isoforms with molecular masses of ~21.5, 18.5, 17, and 14 kDa and include cDNAs that encode two different 17-kDa MBP isoforms. The structures of these five MBP isoforms are shown diagrammatically in Fig. 1. From the proportion of the two 17-kDa MBP cDNA clones isolated from one cDNA library, it appears that one of the 17-kDa MBP mRNAs, i.e., that missing exon 6, is present in much greater abundance than the other (missing exons 2 and 5) in 18-day-old mouse brain (Newman et al., 1987a).

Alternative splicing accounts for the production of the multiple MBP mRNAs (see Fig. 1). The five mouse MBP cDNAs reported to date appear to be derived by the alternative splicing of exons 2, 5, and 6 of the gene. Four MBP cDNAs encoding human MBP isoforms with molecular masses of ~21.5, 20, 18.5, and 17 kDa have been isolated from fetal spinal cord and newborn brain cDNA libraries (Kamholz et al., 1986; Roth et al., 1986, 1987). The mRNAs represented by these cDNAs are apparently derived by alternative splicing of exons 2 and 5 of the MBP gene primary transcript. The human 17-kDa MBP mRNA is analogous to that present in 18-day-old mouse brain in only minor amounts, i.e., missing exons 2 and 5. Although a cDNA encoding a mouse 20-kDa MBP has not yet been isolated, a sixth mouse MBP isoform with a molecular mass of 20 kDa (missing the peptide sequence encoded by exon 5) has been detected in immunoblots of mouse CNS. Thus, to date, it appears that there are at least six MBP isoforms expressed in the mouse and four isoforms expressed in the human. There is, as yet, no evidence that exon 6 is alternatively spliced in the human, in contrast to the mouse, where it appears that at least three exons, i.e., exons 2, 5, and 6, can be alternatively spliced. This putative difference in the MBP mRNA splicing pathways of the mouse and human is noteworthy, because it provides an example of a difference in the alternative splicing pathway of the same gene between species.

The MBP mRNAs contain a relatively short 5' untranslated region (<48 nucleotides) and a very long 3' untranslated region (>1 kb) and migrate as a broad band at ~2.0–2.4 kb on Northern blots. Two polyadenylation signals are found near the 3' ends of mouse and human MBP mRNAs, and in humans both appear to be used, resulting in mRNAs that have slightly different 3' ends (Roth et al., 1987). In the 5' untranslated region, there is a second AUG codon five bases upstream of the initiator codon, which is immediately followed by a termination codon. This structural feature, typical of poorly initiated mRNAs, has been postulated to explain the poorer in vitro translation initiation efficiencies exhibited by MBP mRNAs relative to brain mRNAs as a whole (Campagnoni et al., 1987a).

Developmental expression of the MBPs

Expression of the MBP gene can be detected as early as 2 days after birth in the mouse, with maximal expression occurring between 16 and 20 days postnatally, as determined by various methods (Campagnoni et al., 1978; Carson et al., 1983; Zeller et al., 1984; Roth et al., 1985; Gardinier et al., 1986; Miskimins and Yu, 1986; Sorg et al., 1987). It has been known for a decade that the synthesis of the 14-kDa mouse MBP increases dramatically with development relative to the synthesis of the 18.5-kDa MBP (Campagnoni et al., 1978) and that this is reflected in an increase in the proportion of the 14-kDa MBP in the myelin membrane with maturation (Einstein et al., 1970; Morell et al., 1972; Adams and Osborne, 1973; Magno-Sumbilla and Campagnoni, 1977). Thus, it has been established for some time that there is a form of developmental regulation over the expression of the MBP gene, although its nature and complexity were far from appreciated.

Analysis of isolated mouse myelin indicates that at younger ages, e.g., 15 days, the membrane is proportionately richer in the 21.5- and 17-kDa MBPs relative to the other MBP isoforms than at older ages (Barbarese et al., 1978). Furthermore, immunoblots of whole brain for measuring the individual MBP isoforms and cell-free synthesis studies of brain mRNA for measuring the levels of individual MBP mRNAs indicate that each isoform exhibits its own developmental pattern of expression and accumulation (Carson et al., 1983). In general, it appears that the 21.5-kDa MBP is expressed to a greater extent during early brain development and that it is found in proportionately higher concentrations in myelin isolated from the brains of young rodents. With age, the proportion of the 21.5- and 17-kDa MBPs falls relative to that of the 18.5- and 14-kDa isoforms. It should be noted that all information to date about the metabolism of the 17-kDa MBP in the mouse reflects the mixture of the two 17-kDa MBP isoforms.
Recent data suggest that the mouse spinal cord may not undergo the developmental changes in MBP isoforms observed in whole brain (Newman et al., 1987b). Immunobots of spinal cords isolated from mice 2-24 days of age did not appear to exhibit the characteristic changes in the proportion of MBP isoforms noted during development in the mouse brain. Thus, there may be region-specific differences in the developmental expression of the MBP gene in the CNS. It has also been noted that the 20-kDa isofrom is present in high concentrations in the human fetal spinal cord (Kronquist et al., 1987; Roth et al., 1987), but its presence has not been noted in newborn human brain (Kamholz et al., 1986). Similarly, the presence of the 20-kDa MBP has been observed in immunobots of mouse spinal cord, but the protein is barely detectable, if at all, in mouse brain. No extensive data are available on the developmental expression of this isofrom in either the mouse or human. These observations underscore the possibility of region-specific expression of the MBP gene. There have been several reports in the literature of uncharacterized polypeptides that are immunologically related to MBP. For example, in rat brain, Agrawal et al. (1986) have identified a 23-kDa phosphorylated protein that is immunologically related to MBP, and immunobots of whole mouse brain homogenate with both polyclonal (Carson et al., 1983) and monoclonal (Bansal et al., 1987) anti-MBP antibodies indicate the presence of higher-molecular-weight immunoreactive proteins. The metabolic and genetic relationships among all these proteins and the more well-characterized MBP isoforms are not clear at present.

**MBP gene expression in the shiverer and shi<sup>md</sup> dysmyelinating mouse mutants**

The two autosomal, recessive murine mutations, shiverer and its allele shi<sup>md</sup> (Doolittle and Schweikart, 1977; Doolittle et al., 1981), map to the distal end of mouse chromosome 18, in the same region as the MBP gene (Roach et al., 1985; Sidman et al., 1985). Mice bearing these mutations begin to exhibit tremors at ~12 days after birth, and these manifestations become progressively worse until the animals die between 90 and 150 days of age (Chernoff, 1981). The CNS of these animals is severely hypomyelinated.

In shiverer mice, MBPs (Bourre et al., 1980; Barbarese et al., 1983) and MBP mRNA (Roach et al., 1983; Campagnoni et al., 1984; Roth et al., 1985; Sorg et al., 1987) have been shown to be essentially absent. The molecular defect in the shiverer mutant appears to be a deletion of exons 3-7 of the MBP gene (Kimura et al., 1985; Roach et al., 1985; Molineaux et al., 1986), and this explains the absence of MBP expression in the mutant. Readhead et al. (1987) have produced transgenic shiverer mice carrying one or two copies of the MBP transgene. Clinical signs of the disorder were largely eliminated in the homozygous transgenic mice, although the levels of MBP mRNA were only ~25% of normal.

The level of MBP expression in shi<sup>md</sup> mouse brains is extremely low in 18-day-old animals (3% of normal), but it increases to ~10% of control at 90 days (Ginalska-Winkelmann et al., 1983; Campagnoni et al., 1984; Roth et al., 1986; Okano et al., 1987; Popko et al., 1987). The MBP mRNA produced in the shi<sup>md</sup> mouse appears to be of normal size. Popko et al. (1987) have found that, in this mutant, there appears to be a duplication of the MBP gene. One gene appears to be normal, and the other appears to be abnormal, having an inversion of a length of DNA. This genetic abnormality results in a severe depression of expression of the MBP gene in shi<sup>md</sup> mice.

Although the primary defect in the shiverer mutant involves the MBP gene, the expression of the 30-kDa proteolipid protein mRNA (PLP mRNA) has also been reported to be reduced significantly (Sorg et al., 1986, 1987). In shiverer brains, the levels of PLP mRNA in polysomes and nuclei were only ~30-55% of control between 15 and 27 days postpartum. Thus, the deletion of a portion of the MBP gene has a major effect on the expression of the gene encoding the proteolipid proteins (PLP gene) in the shiverer mouse.

**MYELIN PROTEOLIPID PROTEINS**

In addition to the MBPs, the other major class of myelin proteins are the proteolipids. It is generally believed that the major biological role of both classes of myelin proteins is to maintain the structure of the membrane. The proteolipids, constituting ~50% of the total protein content of myelin (Eng et al., 1968), are integral membrane proteins. They are extremely hydrophobic and aggregate relatively easily under various experimental conditions (Agrawal and Hartman, 1980; Lees, 1982). The principal posttranslational modification of the 30-kDa myelin proteolipid protein (PLP) is fatty acylation, which contributes even further to the hydrophobicity. It has been estimated that the myelin PLP contains ~2-4% by weight fatty acid covalently attached to the apoprotein.

The myelin proteolipid proteins have been shown to exist in at least two isoforms, the most well characterized of which are the PLP (molecular mass = 30 kDa) and the DM20 protein (molecular mass = 25 kDa). The structural relationship between these proteins has only recently been established through cDNA analysis. Various workers have observed the presence of other lower-molecular-weight proteolipid proteins, apparently related to the PLP, in proteolipid preparations isolated from brain (Chan and Lees, 1974; Macklin et al., 1983/84; Lepage et al., 1986) and synthesized in vitro with brain mRNAs (Sorg et al., 1986). The structural relationship of these to the PLP and DM20 is only now beginning to emerge, but
it has not yet been defined precisely (Lepage et al., 1986).

**Myelin PLP gene structure**

The myelin PLP gene has been isolated and characterized in the human (Diehl et al., 1986) and mouse (Macklin et al., 1987a,b). Like the basic protein gene, it consists of seven exons, but it is smaller, ~17 kb in length (Fig. 2). The PLP gene has been assigned to the X chromosome in the human and mouse using somatic cell hybridization (Willard and Riordan, 1985) and chromosomal in situ hybridization techniques (Mattei et al., 1986).

**Structure of the PLP mRNAs and the relationship of DM20 to PLP**

Proteolipid protein cDNAs have been isolated from several species, including the rat (Dautigny et al., 1985; Milner et al., 1985; Gardinier et al., 1986), mouse (Nave et al., 1986; Hudson et al., 1987; Sorg et al., 1987), cow (Naismith et al., 1985), and human (Fahim and Riordan, 1986; Kronquist et al., 1987). The PLP mRNA is heterogeneous, consisting of a family of messages with lengths of ~1.5–1.6, 2.4–2.6, and 3.0–3.4 kb. In the rat, full-length 1.6- and 3.2-kb PLP cDNAs have been cloned, and they are identical except for the lengths of their 3′ untranslated regions (Milner et al., 1985). The 1.6 kb PLP mRNA arises presumably through the utilization of an alternative polyadenylation signal in the 3′ untranslated region. Such a signal is present at the same location in human PLP gene (Kronquist et al., 1987), but little 1.6-kb PLP mRNA is detectable in this species (Campagnoni et al., 1987b; Kronquist et al., 1987) for the mouse, the major forms of the PLP mRNA are 2.4–2.6 and 3.0–3.4 kb in length (Gardinier et al., 1986; Nave et al., 1986; Sorg et al., 1987), and 1.6-kb PLP mRNA is present in lesser amounts. Thus, the relative proportion of the members of the PLP family of mRNAs appears to differ from species to species, a finding suggesting that some factor addition to the simple presence of a polyadenylation signal may be important in determining the sites of polyadenylation.

Like the MBP isoforms, the DM20 protein appears to be the product of an alternatively spliced mRNA. Nave et al. (1987a) have recently isolated near full-length cDNA of the 2.4-kb mouse mRNA that encodes the DM20 protein. The sequence of cDNA is identical to the PLP mRNA except for deletion of 105 bp within the coding region of the molecule, corresponding to amino acid residues 116–150 of the PLP. This arises through the utilization of a cryptic splice site within the third exon of the gene, such that the resultant mRNA is missing a nucleotide sequence corresponding to about half of exon 3 (see Fig. 3). Macklin et al. (1987a,b) detected the presence of such a mRNA through nuclease protection experiments. These findings complement and prove the earlier cell-free pr

**FIG. 2.** The PLP gene and its products in normal and jimpy mice. The dotted line in exon 3 refers to the cryptic splice site used for production of the DM20 protein. The dotted lines in exon 7 refer to the polyadenylation sites that can be used for producing PLP mRNA species of differing sizes. The wavy line in the jimpy PLP and DM20 protein refers to the altered C-terminal region produced when exon is spliced to exon 7 in the jimpy mutant.

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synthesis work indicating that the two proteolipids were the products of separate mRNAs (Dautigny et al., 1983; Sorg et al., 1986) and the peptide mapping and immunological data predicting that the DM20 was identical to the PLP except for a deletion of ~40 amino acid residues (Trifilieff et al., 1985, 1986).

In addition to the PLP and DM20, the presence of other brain proteolipid polypeptides has been reported in mouse, rat, and bovine brain (Nussbaum and Mandel, 1973; Chan and Lees, 1974; Lerner et al., 1974; Campagnoni et al., 1976; Lepage et al., 1986). Lepage et al. (1986) have characterized two proteolipid polypeptides with apparent molecular masses of 14 and 16 kDa using fast atom bombardment-mass spectrometry. These two polypeptides share some, but not all, peptide sequences in common with the myelin proteolipids. Immunoblot data have also revealed the presence of lower-molecular-weight proteolipids in rat brain that cross-react with anti-PLP antisera (Macklin and Weill, 1985; Sorg et al., 1987). In general, the developmental pattern of expression of myelin proteolipids is different from that of the MBPs. Whereas the latter peaks at 16–20 days postpartum and then declines somewhat in mice, PLP and DM20 expression peaks a few days later and remains relatively high. The in vivo synthesis of myelin proteolipid proteins and the levels of polysomal PLP mRNA have been reported to peak a few days later than MBP synthesis in the mouse brain, i.e., 21–22 days for PLP vs. 18 days for MBPs (Campagnoni and Hunkeler, 1980; Sorg et al., 1987). The slight difference in the peak expression of PLP and MBPs has been noted in several species and CNS regions (Tennekoon et al., 1977; Macklin and Weill, 1985; Monge et al., 1986; Kronquist et al., 1987).

The myelin PLP and DM20 protein have been identified in several species (Lees and Macklin, 1987), and recent immunoblot studies have shown that expression of the DM20 protein seems to precede that of the PLP in the developing human spinal cord (Kronquist et al., 1987) and in the developing mouse brain (Gardinier and Macklin, 1988). Quantitatively, the DM20 isof orm is present at much lower levels than the PLP in the adult brain (10–20% of PLP), but at 18 weeks after conception, it is the principal, if not the only, isof orm present in the human spinal cord. Thus, like the MBP and MAG isof orms, expression of the PLP isof orm appears to be developmentally regulated, undoubtedly at the level of the splicing of the primary gene transcript.

Developmental expression of the myelin proteolipids

Like MBPs, the expression of the myelin proteolipids can be observed in the brain as early as birth in rats and mice using various different methods to detect either PLP mRNAs or their polypeptides (Macklin and Weill, 1985; Monge et al., 1986; Sorg et al., 1987). In general, the developmental pattern of expression of myelin proteolipids is different from that of the MBPs. Whereas the latter peaks at 16–20 days postpartum and then declines somewhat in mice, PLP and DM20 expression peaks a few days later and remains relatively high. The in vivo synthesis of myelin proteolipid proteins and the levels of polysomal PLP mRNA have been reported to peak a few days later than MBP synthesis in the mouse brain, i.e., 21–22 days for PLP vs. 18 days for MBPs (Campagnoni and Hunkeler, 1980; Sorg et al., 1987). The slight difference in the peak expression of PLP and MBPs has been noted in several species and CNS regions (Tennekoon et al., 1977; Macklin and Weill, 1985; Monge et al., 1986; Kronquist et al., 1987).
Myelin proteolipid protein gene expression in the jimpy and myelin synthesis-deficient (jpmd) dysmyelinating mouse mutants

The jimpy mutation and its allele jpmd (Meier and MacPike, 1970; Eicher and Hoppe, 1973) are recessive X-linked mutations. The mice exhibit tremors beginning at approximately postnatal day 11, which become progressively worse until the animals die at ~25-30 days of age. Both disorders appear to affect only the CNS, with no apparent involvement of the PNS (Billings-Gagliardi and Adcock, 1981). Histologically, there is almost a total lack of myelin and a significant reduction in oligodendrocyte number, presumably the result of a reduced oligodendrocyte lifespan (Sidman et al., 1964; Knapp et al., 1986). Jimpy animals appear to have a more severe disease, and they also have approximately one-half the myelin of jpm animals (Billings-Gagliardi et al., 1980; Wolf et al., 1983).

One of the first myelin protein deficits noted in jimpy brains was the virtual absence of PLP (Nussbaum and Mandel, 1973; Lerner et al., 1974). When more extensive studies were conducted using immunoblotting to identify PLP and DM20, neither was observed, a result indicating that the PLP was present at <0.5% of normal, if at all (Sorg et al., 1986; Yanagisawa and Quarles, 1986).

The PLP gene has been mapped to the jimpy locus by several groups (Willard and Riordan, 1985; Dautigny et al., 1986; Diehl et al., 1986; Hudson et al., 1987), and the genetic defect in jimpy animals has now been established as a point mutation within the splice acceptor sequence of exon 5 of the mouse PLP gene (Macklin et al., 1987a; Nave et al., 1987b). This results in an altered PLP mRNA in jimpy animals, which has an internal deletion of 74 nucleotides (Gardinier et al., 1986; Morello et al., 1986; Nave et al., 1986; Hudson et al., 1987). This deletion in the PLP mRNA causes a frameshift near the end of the coding region in the message, which results in a PLP with an altered C-terminal region in jimpy mice. The PLP translated from this mRNA in jimpy is identical to "normal" PLP for the first 206 amino acids, but the last 70 amino acids of the normal protein are replaced with a sequence of 36 different amino acids in the mutant.

The molecular defect in jpmd mice has not yet been established. This mutant has been reported to have reduced levels of PLP mRNA, but there was no evidence of any major structural alteration in the PLP message (Hudson et al., 1987). Gardinier and Macklin (1988) have observed that the smaller PLP mRNAs in jpmd brain are reduced in content to a greater extent than the 3,200 nucleotide mRNA. In this study, no major structural alteration of the PLP gene in jpmd mice was identified by Southern analysis, but the experiments would not identify point mutations or alterations in the upstream regulatory elements of the gene. Immunoblot analysis indicated that both PLP and DM20 were expressed in jpmd brain; however, at all ages up to 23 days, DM20 was present at equivalent or greater levels than PLP. This suggests that some aspect of the regulation of the alternative splicing of these two mRNAs is affected in this mutant.

Just as with shiverer, the jimpy mutation also appears to exhibit pleiotropy with respect to the expression of myelin protein genes other than the PLP gene. Accumulation of MBPs is significantly reduced in jimpy brains (Campagnoni et al., 1972; Barbarese et al., 1978; Bourre et al., 1980; Delassale et al., 1981; Kerner and Carson, 1984). Estimates for the deficit range from 92 to 98% in different regions of the jimpy brain (Delassale et al., 1981; Kerner and Carson, 1984), whereas no reduction in MBP content has been reported in mouse sciatic nerve (Jacque et al., 1983). This suggests that there may be differences in the regulation of the MBP gene in jimpy oligodendrocytes vs. Schwann cells.

Several studies on MBP gene expression in jimpy mice from one laboratory indicated that the levels of both polysomal and nuclear MBP mRNA were significantly reduced in the mutant brain, to ~26% of normal (Campagnoni et al., 1984; Roth et al., 1985; Sorg et al., 1986, 1987). In these studies, MBP mRNAs levels were determined by cell-free translation, Northern blot analysis, and dot blot analysis. A much greater reduction was noted in content of the 14-kDa MBP mRNA than that of the other three MBP mRNAs (Campagnoni et al., 1984). However, Carnow et al. (1984) reported normal levels of total MBP mRNA in both jimpy and jpmd mice when the content was measured by in vitro translation of polysomal RNA. Despite differences in MBP mRNA levels measured in these studies, in all cases, the level of detectable protein was significantly below the level of detectable mRNA, a finding suggesting that although the MBPs may be synthesized to some extent, the amount that accumulates is quite low. These data suggest that MBP that is not inserted into an appropriate membrane is rapidly turned over.

The expression of other myelin protein genes is also altered in jimpy mice. Immunoblot analysis indicates that the MAG protein level in jimpy brains is ~5% of normal at 20 days of age (Yanagisawa and Quarles, 1986). The MAG mRNA levels in jimpy mice have been estimated to be <10% of normal by in vitro translation of brain mRNA (Frail and Braun, 1985). A reduction in CNP activity was one of the earliest deficits observed in jimpy brains (Kurihara et al., 1969; Sarlieve et al., 1976), and CNP enzyme levels range from 7 to 25% of normal, depending on the region of the CNS examined (Mikoshiba et al., 1985). However, it is not yet clear whether this reduction in enzymatic activity is due to reduced expression of the gene or some other factor.
Undoubtedly, a part of the apparent pleiotropy observed in jimpy mice is due to the reduction in level of oligodendrocytes through premature cell death and the consequent reduction in content of all oligodendrocyte-specific products. However, comparison of the reductions estimated from morphological studies, often on limited brain regions, with the biochemical data obtained from whole brain has not been possible. However, through in situ hybridization procedures, direct measurement of the numbers of copies of each of the individual myelin protein mRNAs per cell for determining if there is down-regulation of the MBP, MAG, and CNP genes in individual jimpy oligodendrocytes should be possible.

**MYELIN-ASSOCIATED GLYCOPROTEIN**

The major myelin glycoprotein, constituting ~1% of the total myelin protein, is MAG (Quarles, 1979). Approximately 30% of MAG is carbohydrate, and the fully glycosylated protein has an apparent molecular mass of ~100 kDa on sodium dodecylsulfate (SDS) gels (Quarles et al., 1983). MAG exists in two polypeptide isoforms with apparent molecular masses of 72 and 67 kDa (Frail and Braun, 1984), although their actual masses, determined recently from sequence analysis of the cDNAs encoding them, are 69.3 and 64.2 kDa (Salzer et al., 1986). Cell-free translation studies have shown that each of these isoforms is encoded by a separate mRNA (Frail and Braun, 1984; Matthieu et al., 1986), and cDNAs encoding each of them have recently been isolated.

In myelin, a significant portion of the MAG is exposed to the extracellular surface of the unit bilayer (Poduslo et al., 1976). Of particular interest is that MAG shares a carbohydrate determinant with several molecules proposed to mediate cell-cell interactions in the nervous system, such as N-CAM, L1, J1, and ependymins (McGarry et al., 1983; Kruse et al., 1984, 1985; Shashoua et al., 1986; Holley and Yu, 1987; Salzer et al., 1987). The physiological role of MAG in myelin is not clear, but on the basis of immunohistochemical data localizing it to periaxonal regions (Sternberger et al., 1979; Trapp and Quarles, 1982; Trapp et al., 1984; Martini and Schachner, 1986), it has been proposed to be involved in the association of the myelin membrane with the axon (Quarles, 1983/84). Recent sequence data showing that it is related in structure to cell adhesion molecules (Salzer et al., 1987) are consistent with this hypothesis. This interpretation has been challenged, however, based on contradictory findings indicating that MAG is localized primarily within the myelin lamellae rather than in the periaxonal regions (Webster et al., 1983).

The MAG gene and the origin of the mRNAs for the two MAG isoforms

The MAG gene, which appears to be part of an immunoglobulin gene superfamily (Hunkapiller and Hood, 1986), is ~16 kb in length, contains 13 exons (Fig. 3), and maps to mouse chromosome 7 (Sutcliffe, 1987). The mRNA encoding the 72-kDa MAG polypeptide is composed of all the exons of the gene except exon 12, and the mRNA encoding the 67-kDa MAG is composed of all the exons of the gene except exon 2 (Lai et al., 1987). Because exon 12 contains a termination signal that creates a shorter coding region in the mRNA than would be present in its absence, translation of this message yields the smaller, 67-kDa MAG polypeptide (Lai et al., 1987; Salzer et al., 1987). Thus, the MAG gene, like the MBP and PLP genes, expresses a multiplicity of mRNAs through alternative splicing mechanisms that encode more than one isoform of each protein.

Transcription of the MAG gene appears to result in the production of several distinct MAG mRNAs of ~2,500 nucleotides in length, apparently produced by alternative splicing and the use of at least two polyadenylation sites (Sutcliffe, 1987). A minor band of ~3,000 nucleotides has also been observed on Northern blots (Sutcliffe et al., 1983; Salzer et al., 1987). Thus, the MAG gene, like the MBP and PLP genes, expresses a multiplicity of mRNAs through alternative splicing mechanisms that encode more than one isoform of each protein.

The MAG isoforms and their developmental expression

The first MAG cDNA was isolated from a rat brain cDNA library by Sutcliffe et al. (1983) as an unidentified, brain-specific cDNA with the designation 1B236. This cDNA was subsequently shown to encode MAG (Arquint et al., 1987; Lai et al., 1987; Salzer et al., 1987). From the deduced amino acid sequences of MAG cDNA clones isolated independently in several laboratories, it appears that the two MAG isoforms differ in their C-termini as a result of the alternative splicing patterns that produce their mRNAs (Sutcliffe et al., 1983; Arquint et al., 1987; Lai et al., 1987; Salzer et al., 1987).

Both cell-free translation studies and S1 nuclease protection experiments indicate that the two MAG mRNAs have different developmental patterns of expression in rats and mice. In cell-free translations of 15–18-day-old mouse brain mRNA, the 72-kDa MAG apoprotein was the predominant immunoprecipitated product, whereas in translations programmed with brain mRNA from older ages (>50 days), the 67-kDa MAG apoprotein was the predominant immunoprecipitated product (Frail and Braun, 1984; Matthieu et al., 1986). In S1 nuclease protection studies designed to detect the proportion of the two MAG transcripts in the rat hindbrain (Lai et al., 1987), the 72-kDa MAG mRNA appeared more abundant at earlier ages, increased to its highest level at 17–29 days, and decreased in content by day 50. In contrast, the 67-kDa MAG mRNA was present at low levels in the younger ages and increased in con-
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...tency continuously to 50 days when it became the major MAG mRNA in the hindbrain.

It has been known for some time that there is a developmental shift in the glycosylated form of MAG in rats by in vivo fucose labeling and SDS-polyacrylamide gel electrophoresis (PAGE) of myelin (Matthieu et al., 1974). It has been consistently observed that, in rats, glycosylated MAG in immature myelin has a higher apparent molecular mass than in mature myelin, and this developmental shift has been also been observed in gerbils and hamsters, but not in mice. Even though there is a clear developmental shift in the MAG apoprotein isoform expressed, the ultimate size of the glycosylated protein, as determined by fucose labeling and SDS-PAGE, is very similar for both apoproteins. Whether or not the developmental shift observed in glycosylated MAGs in the rat reflects differences in the glycosylation of the two apoproteins or two different levels of glycosylation for each of the apoproteins remains to be established.

Expression of MAG in dysmyelinating mutant mice

The expression of MAG has been studied in both jimpy and quaking mice. As indicated above, the expression of MAG in jimpy mice is severely depressed, although there appears to be no effect on the proportion of the two MAG mRNAs expressed at 15 and 20 days after birth (Frail and Braun, 1985). The expression of MAG has also been studied in the quaking mouse, and it appears its glycosylation is altered in this mutant. Matthieu et al. (1974) have found that 30-day-old quaking mice express a slightly larger form of MAG, which contains much less fucose than normal MAG. This and another minor form of slightly lower molecular mass (Inuzuki et al., 1987) may be unique to quaking mice. Although normal amounts of MAG have been seen in the PNS, these altered forms of MAG are apparently the mature form of MAG in quaking PNS (Inuzuki et al., 1987). The expression of the mRNAs encoding the two MAG isoforms also appears to be altered in quaking mice. The predominant MAG mRNA at 15 and 25 days of age in quaking mice is the p67 MAG mRNA, which is usually predominant only at older ages in normal animals (Frail and Braun, 1984).

2',3'-Cyclic Nucleotide 3'-Phosphodiesterase

The enzyme CNP (EC 3.1.4.37) catalyzes the hydrolysis of several 2',3'-cyclic nucleoside monophosphates. Its activity is very high in the CNS, where it has been shown to be associated with myelin (Kurihara and Tsukada, 1967, 1968; Olafson et al., 1969). The enzyme has been shown to be a component of the "Wolfgram" fraction, a heterogeneous group of high-molecular-weight myelin proteins, originally obtained by fractionation of myelin (Wolfgram, 1966). The purified enzyme consists of two polypeptide chains (Drummond, 1979; Sprinkle et al., 1980) that appear to share identical molecular, chemical, and immunological characteristics with the enzyme (see Lees and Sapirstein, 1983). The two isoforms of CNP have been reported to have molecular masses between 44 and 48 kDa, depending on the species (Karin and Waehneldt, 1985), and they are apparently the products of separate, alternatively spliced mRNAs (Bernier et al., 1987). Two CNP bands have been observed in immunoblots of myelin from the mouse, rat, human, and pig, and their sizes appear to differ slightly among species (Karin and Waehneldt, 1985). No major difference has been reported in their developmental expression, relative to each other, although this has not been examined extensively (Waehneldt, 1975).

An early study comparing CNP activity and MBP levels (Sprinkle et al., 1978) suggested that the two proteins had identical developmental profiles in the rat. In the developing chick brain, the appearance of CNP enzyme activity has been reported to lag behind MBP and PLP accumulation as determined by immunoblot analysis (Macklin and Weill, 1985). Recently, Monge et al. (1986) reported the immunohistochemical detection of Wolfgram proteins (including CNP) 4 days before the appearance of MBP in the developing mouse brain. This group has also detected CNP mRNA during embryonic development in the rat brain (Kanfer et al., submitted for publication). Their studies show that although there is variability in the temporal expression of mRNAs for MBP, PLP, and CNP among brain regions, most regions had peaks of CNP mRNA levels somewhat earlier than those for MBP.

Less is known about the molecular biology of CNP than the other myelin proteins. Very recently, a number of studies have reported the isolation of cDNAs encoding the bovine (Kurihara et al., 1987) and rat (Bernier et al., 1987) enzymes. The clones from the two species have some homology, but they appear to encode enzymes with significantly different amino acid sequences. Kurihara et al. (1987) isolated two partial-length cDNAs making up a full-length bovine cDNA of 2,305 bp. Subclones of the bovine cDNAs, including the coding region, hybridized to a bovine cerebellar mRNA of ~2,600 nucleotides but not to any mRNA present from rat brain (Kurihara et al., 1987). The bovine cDNAs encoded a protein of 400 amino acids, including the initiator methionine, with a molecular mass of ~45 kDa. Identification of the clone as a CNP cDNA was established by sequence homology of a portion of the coding region with a 236-amino acid fragment of the enzyme that had been isolated and sequenced by the authors.

Bernier et al. (1987) isolated a 2.6-kb rat CNP cDNA clone encoding a 46-kDa polypeptide. Translation of the mRNA transcription product of the insert subcloned into a riboprobe vector indicated that
the clone encodes the smaller of the two CNP polypeptides found in rat brain. Analysis of the deduced primary structure of the polypeptide indicates that it contains a polypeptide segment with homology to cyclic AMP binding sites found in several other proteins. The rat probe hybridized to mRNAs of ~2,400 and 2,800 nucleotides in brain and sciatic nerve and to an mRNA of ~2,600 nucleotides in thymus. A 373-bp segment from the 5′ end of the clone hybridized only with the 2,800-nucleotide mRNA, a finding suggesting that not all CNP mRNAs share the same 5′ ends. Bernier et al. (1987) have suggested that there is a single CNP gene that can be alternatively spliced to produce the various mRNA transcripts. Thus, like MBP, PLP, and MAG, CNP also appears to exist in multiple isoforms that are the products of alternatively spliced mRNAs.

THE QUAKING MUTANT

Although the molecular defect has not yet been defined in the quaking mouse mutant, several studies have been carried out on the synthesis and metabolism of the myelin proteins in quaking brain. This autosomal, recessive mutation maps to mouse chromosome 17, which rules out the possibility that the defect involves a mutation in the structural genes of MBP, MAG, or PLP. Yet, the expression of each of these genes has been shown to be affected in this mutant.

An interesting feature of the dysfunction in quaking is that there appears to be a caudal-rostral gradient in the severity of the CNS dysmyelination, with the spinal cord being less affected than the anterior commissure (Wisniewski and Morell, 1971; Fried- rich, 1974). Overall, the yield of myelin from adult quaking brain is only 5–10% of normal (Greenfield et al., 1971).

Several studies indicate that the steady-state levels of both PLP and MBP, especially the 14-kDa isoform, are fairly low in the whole brains and the isolated myelin of quaking animals (Nussbaum and Mandel, 1973; Lerner et al., 1974; Fagg, 1979; Delasale et al., 1981; Jacque et al., 1983; Sorg et al., 1986). Recently, Sorg et al. (1986, 1987) have reported significantly reduced levels of PLP mRNA in quaking brains, as measured by cell-free translation of brain mRNA and by Northern and dot blot analysis. These studies are at variance with the earlier in vivo studies of Greenfield et al. (1979), which indicated that PLP synthesis was normal in quaking brain.

After ~3 weeks of age, even though the steady-state levels of MBP are only 5–20% of normal, the levels of MBP mRNA and the synthesis of MBP appear to be close to normal in quaking brains (Campagnoni et al., 1984; Carnow et al., 1984; Roth et al., 1985; Hudson et al., 1987; Sorg et al., 1987). These results are consistent with the hypothesis, first postulated by Hogan and co-workers based on their in vivo studies, that quaking mice are defective in their ability to incorporate newly synthesized MBPs into myelin (Brostoff et al., 1977; Greenfield et al., 1977). However, expression of MBP mRNA appears to be somewhat complex, because at <15–20 days of age, the levels of MBP mRNA seem to be lower in quaking brains than in age-matched controls (Sorg et al., 1987). Thus, the developmental pattern of MBP mRNA expression also appears to be altered in the quaking brain.

As indicated previously, MAG expression in quaking mouse is also abnormal, and CNP activity is quite low (Kurihara et al., 1970; Mikoshiba et al., 1979). Thus, the quaking mutation seems to affect the developmental expression of several myelin proteins, an observation suggesting that their expression is interrelated in a way that is not clearly understood.

SUMMARY

Within the past several years, several of the genes coding for the major myelin proteins have been isolated, characterized, and mapped to specific chromosomes. In all cases, it has been clearly established that these proteins exist as multiple isoforms, and their structures have been established through an analysis of the cDNA clones encoding them. In each case, the isoforms appear to arise through the translation of individual mRNAs produced by alternative splicing of the primary transcript of a single gene. In several cases, the expression of the individual isoforms appears to be developmentally and/or regionally regulated, probably at the level of the splicing of the primary transcript. In the case of the dysmyelinating mutants shiverer and jimpy, the molecular defects involve the MBP gene and PLP gene, respectively; most of the dysmyelinating mutants, including those in which the genetic defect is established, appear to exhibit pleiotropy with respect to the expression of other myelin protein genes.

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REFERENCES


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The development of myelin in the central nervous system is a complex process that involves multiple cellular and molecular mechanisms. Myelin is a lipid-rich, proteinaceous sheath that ensheathes nerve fibers, providing insulation and support. The synthesis and expression of myelin proteins are critical for proper neural function. Understanding the molecular biology of myelin proteins is essential for elucidating the mechanisms underlying neurological disorders.


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