Expression of Myelin Basic Protein Genes in the Developing Mouse Brain


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Myelination is a major biological event during the early postnatal development of the mouse brain, yet relatively little is known about the mechanism and regulation of myelin assembly. In view of the importance of the myelin sheath in the maintenance of a functionally normal brain, and because of the wide variety of neurological diseases and nutritional deficiencies that result in hypomyelination, studies into the mechanism by which the membrane is formed are particularly important.

Current understanding of the synthesis of myelin proteins and their assembly into the membrane is very incomplete, and research in this area is only at its earliest stages. The overall process by which proteins are synthesized and assembled into myelin may arbitrarily be divided into three rather broad stages: (a) the synthesis and processing of the mRNAs which code for the myelin proteins; (b) the translation of these mRNAs on ribosomes and subsequent posttranslational modification of the myelin proteins; and (c) the intracellular transport and assembly of these proteins into myelin.

MYELIN PROTEINS

The myelin basic protein (MBP) is one of the macromolecular constituents of myelin. It can be considered a peripheral membrane protein because of its particular topography within the myelin membrane and its solubility properties (1,2). It is a highly basic hydrophilic protein, in marked contrast to the other major myelin protein, the proteolipid protein, which is extremely hydrophobic. The MBP was first isolated from guinea pig brain (3), and its localization in the myelin sheath was subsequently established by a number of biochemical and immunocytotoxic chemical techniques. The protein has been isolated from a number of species and its complete primary sequence has been determined (4). The MBP from most species contains approximately 170 amino acid residues and has a molecular weight of 18,400. Table 1 summarizes some of the characteristics of the major myelin proteins.
**TABLE 1. Properties of the major myelin proteins**

<table>
<thead>
<tr>
<th>Type</th>
<th>Molecular weight</th>
<th>Primary sequence</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteolipid protein</td>
<td>Integral</td>
<td>30 kdalton</td>
<td>known</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Extremely hydrophobic; contains 2%–4% covalently bound fatty acid</td>
</tr>
<tr>
<td>Myelin basic protein(s)</td>
<td>Peripheral</td>
<td>18.5 kdalton</td>
<td>known</td>
</tr>
<tr>
<td>major forms</td>
<td></td>
<td>14 kdalton</td>
<td>Hydrophilic; pI greater than 10.8; highly encephalitogenic</td>
</tr>
<tr>
<td>minor forms</td>
<td></td>
<td>21.5 kdalton</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 kdalton</td>
<td></td>
</tr>
</tbody>
</table>

*FIG. 1. Immunoblot of mouse myelin showing the four myelin basic proteins (MBPs). Two preparations of myelin were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, treated with anti-MBP; and the antibody-MBP complexes were visualized by immunoperoxidase staining. The presence of the 17K and 21.5K minor forms of the MBP are evident in addition to the two major forms of the protein (14K and 18.5K).*
FIG. 2. Proposed structural relationship among the four mouse myelin basic proteins (MBPs) showing the positions of the two additional sequences in the N-terminal and C-terminal halves of the molecule. The 21.5K MBP contains both insertions. The 18.5K and 17K MBPs are each missing one of the two insertions. The 14K MBP is missing both insertions. The numbers refer to the residue numbers in the 14K MBP.

A number of species in the mammalian suborders *Myomorpha* and *Sciuromorpha* (which include the rat and mouse) contain two major MBPs which have been designated 18.5K and 14K, reflecting their apparent molecular weights (5). The rat 18.5K protein has been found similar in size, chromatographic properties, amino acid composition, and encephalitogenic activity to the single MBP found in other species, such as man and ox (6). The rat 14K protein has been isolated and sequenced, and it appears to be identical to the rat 18.5K protein except for a deletion within the interior of the molecule. The mouse 14K and 18.5K MBPs appear to bear the same structural relationship to each other, as do the rat proteins. In rats and mice the 14K MBP predominates over the 18.5K MBP by a ratio of 2–3:1 (7).

Two other low molecular weight MBPs can be detected in immunoblots of myelin (Fig. 1). These two MBPs, first described by Barbarese et al. (8), are immunologically related to the two major forms of the mouse basic proteins (i.e., 14K and 18.5K) and have apparent molecular weights of approximately 21,500 and 17,000. They appear to be structurally related to the 14K and 18.5K basic proteins, having an extra sequence of approximately 3,000 daltons in the N-terminal half of the molecule. Until recently, the exact position of this sequence was unclear; however, it has now been localized between residues 57 and 58 in sheep 21.5K MBP (9). Thus the four MBPs appear to be related through the 14K MBP primary sequence which is common to all four proteins, and they differ from each other by the presence or absence of one or two additional sequences inserted within the interior of the molecule (Fig. 2).

**SYNTHESIS AND ASSEMBLY OF PROTEINS INTO MYELIN**

In the developing mouse brain, expression of the two major forms of the MBP occurs synchronously and reaches a maximum at 18 days of age (10). Maximal ex-
TABLE 2. Cell biological characteristics of myelin protein assembly

<table>
<thead>
<tr>
<th>Myelin basic proteins</th>
<th>Proteolipid protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leader sequence</td>
<td>No</td>
</tr>
<tr>
<td>Site of synthesis</td>
<td>Free polysomes</td>
</tr>
<tr>
<td>Transport mechanism</td>
<td>Unknown: possibly by ribosome movement, vesicles, or microtubules</td>
</tr>
<tr>
<td>Movement</td>
<td>Bound ribosomes</td>
</tr>
<tr>
<td>Order of assembly</td>
<td>14 and 18.5K MBPs before the proteolipid protein</td>
</tr>
<tr>
<td></td>
<td>Golgi involved</td>
</tr>
</tbody>
</table>

FIG. 3. Autoradiogram of myelin basic proteins (MBPs) synthesized in vitro using mRNA isolated from either guinea pig (GP) or mouse (M) brain poly A(+) mRNA. Messenger RNA was isolated from either fetal guinea pig brain or 18 day old mouse brain and translated in reticulocyte lysates. Newly synthesized MBP labeled with $^{35}$S-methionine was immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis. The autoradiogram shows that guinea pig brain synthesizes the two high molecular weight forms of the MBP (i.e., 18.5K and 21.5K) in contrast to the four forms synthesized by mouse brain.
pression of the other major myelin protein, the proteolipid protein, occurs several days later, suggesting that the "turning on" of the major myelin protein genes may be staggered (10).

Studies from our laboratory with isolated polyribosomes have shown that the predominant sites of synthesis of the MBPs are the free ribosomes in the oligodendrocyte (11). These studies have been confirmed elsewhere, and it has also been found that the proteolipid protein is synthesized on ribosomes bound to the endoplasmic reticulum (12). The proteolipid protein is synthesized without a leader sequence and it appears to be cotranslationally inserted into the endoplasmic reticulum (12-14). All the available biochemical and immunohistochemical data indicate that the basic proteins and proteolipid protein follow separate intracellular routes from their sites of synthesis to their assembly into the growing myelin membrane (15-19). Transport of the proteolipid protein probably involves the Golgi bodies, and transport of the basic proteins probably occurs through the cytosol by some other mechanism. Table 2 lists some of the cell biological characteristics of the major classes of myelin proteins.

When the 17K and 21.5K MBPs were first discovered there was a possibility that these proteins might be metabolic precursors of the quantitatively more important 14K and 18.5K MBPs. We undertook a study to determine whether or not this was the case, and it became apparent that the four MBPs were coded for by four separate mRNAs and that the four basic proteins were synthesized without leader sequences (20). The synthesis of all four mouse MBPs could be detected in mRNA-stimulated reticulocyte lysates which had been shown to contain no processing activity; and the kinetics of labeling of the four mouse MBPs did not support the concept of a precursor-product relationship among any of the MBPs. Figure 3 shows the anti-MBP immunoprecipitated products of a reticulocyte lysate stimulated with guinea pig and mouse polysomal mRNA. Note that the complete proteins are synthesized and that the guinea pig synthesizes only the two higher molecular weight forms of the MBP, whereas the mouse synthesizes all four forms.

CHARACTERIZATION OF THE MBP mRNA

Using two families of synthetic oligonucleotide probes corresponding to two regions of the MBP, Zeller et al. (21) were able to prepare and screen for a cDNA common to all four mouse MBP mRNAs. These two families of oligonucleotide probes corresponded to two different regions of the MBP. One family was used as a primer to prepare cDNAs which were cloned into pBR322, and both families were used to screen for cDNA clones containing an MBP insert. One clone, designated NZ11, contained 94 nucleotides and its sequence was found to correspond to residues 60 to 93 of the MBP. The clone was identified as an authentic MBP mRNA segment from its nucleotide sequence and from its ability to selectively hybridize to MBP mRNA using hybrid selection techniques.

This clone has been used to characterize the MBP mRNAs from mouse and
FIG. 4. Autoradiogram showing the inhibition of the cell-free synthesis of the four mouse myelin basic proteins (MBPs) with increasing concentrations of the "cap" inhibitor, m7GTP. Reticulocyte lysates were programmed with 18 day old mouse brain poly A(+) mRNA, and MBPs were isolated by immunoprecipitation. The STD lane shows the migration of 125I-labeled 14K and 18.5K mouse MBPs.

guinea pig brain (21,22). Northern blot analysis of 18 day old mouse poly A(+) mRNAs vary in size from approximately 2,100 to 2,400 nucleotides. Parallel Northern analysis of fetal guinea pig brain poly A(+) mRNA revealed a narrower band of MBP mRNA corresponding to the higher molecular weight half of the mouse MBP mRNA band. These data are consistent with the absence of the 14K and 17K MBPs in the guinea pig brain. Thus the mRNAs for the four mouse MBPs are of similar length and are encompassed within a range of 2,100 to 2,400 nucleotides. Since the coding region for the largest MBP (21.5K) would be approximately 600 nucleotides then, as has also been demonstrated from sequence data, there are large untranslated regions present in these mRNAs.

It has been known for some time that the MBP mRNAs are polyadenylated and it is now clear that they are very large. Another piece of information about mRNA structure that could have important regulatory implications is whether or not the mRNA contains a "cap" at its 5' end. Cap structures are 7-methyl guanosine residues linked from their 5' hydroxyl to the 5' hydroxyl group of the mRNA by a triphosphate bridge. One method commonly used to determine whether a mRNA is capped is to examine the ability of cap inhibitors, such as 7-methyl guanosine tri-
phosphate, to inhibit the translation of that message. In cell-free translation experiments with brain mRNA-stimulated reticulocyte lysates the cap analog, m7GTP, significantly inhibited 35S-methionine incorporation in the four mouse MBPs. At the highest concentration examined, inhibition by 70% or more was observed (Fig. 4). The 14K mRNA appeared to be most inhibited by the cap analog and the 21.5K MBP mRNA least inhibited, but all four MBP mRNAs were substantially inhibited suggesting that all four MBP mRNAs are capped.

In summary, all the studies performed to date indicate that the various forms of the MBPs are coded for by separate mRNAs; that these mRNAs are polyadenylated at their 3’ ends and capped at their 5’ ends; that they are very large (2,100–2,400 nucleotides) considering the sizes of the proteins they code for; and that they contain substantial lengths of untranslated sequence. The rat 14K MBP mRNA has been cloned by Roach et al. (23) and its structure corresponds to that predicted by our results. It also supports the finding that the MBPs are the product of separate mRNAs.

EXPRESSION OF MBPs IN NORMAL AND MUTANT MICE

The mouse cDNA probe was also used in Northern and dot blot analyses to titrate the concentration of MBP mRNA in total polyribosomes isolated from the developing brains of normal and dysmyelinating mutant mice (22). Northern blots on mRNA isolated from 18 day old normal and mutant mice suggested that the three mutants, jimpy, quaking, and shiverer, contained greatly differing levels of MBP mRNA, although MBP mRNAs of apparently normal size were found to be associated with quaking and jimpy polyribosomes. Quaking polysomes contained much more MBP mRNA than jimpy, and virtually no MBP mRNA was detected in shiverer polysomes.

The concentrations of MBP mRNA were estimated by dot blot analysis (21,22) and maximal levels of MBP mRNA were observed to occur at 18 days in the normal developing mouse brain, in agreement with earlier in vivo synthesis studies (24). These studies were extended to the three hypomyelinating mutants, quaking (qk/qk), jimpy (jp/Y), and shiverer (sh/shi), as well as heterozygote controls. A method was developed for quantifying the levels of MBP mRNA from dot blots which correlated well with estimates made from Northern blots (22). Figure 5 shows the levels of MBP mRNA present in C57BL/6J and mutant brains from 12 to 27 days postpartum. These studies were begun at 12 days, the earliest age at which clinical signs allowed definitive identification of the mutations. The data are presented relative to the levels of MBP mRNA present in 18 day old C57BL/6J control mice. Very low levels of MBP mRNAs were observed in shi/shi brain polyribosomes throughout early postnatal development, consistent with reports that there may be a deletion in the MBP gene in this mutant (22). Accumulation of MBP mRNAs in the brain polyribosomes of quaking homozygotes and heterozygotes (data not shown) was delayed by several days. In these animals, whereas MBP mRNA levels were below normal between 12 and 18 days, normal levels of message were found in polyribosomes
FIG. 5. Levels of myelin basic protein (MBP) mRNA in the developing brains of normal and mutant mice determined by dot blot analysis. Closed circles, normal C57BL/6J mice; open circles, quaking mice; closed squares, jimpy mice; closed triangles, shiverer mice. All results are presented as percent of 18 day old C57BL/6J MBP mRNA levels.

STRATEGY

FIG. 6. Outline of the strategy used for sequencing the 1.85 kb mouse MBP cDNA clone. S and R refer to the Sau 3a and Eco RI restriction sites, respectively. Arrows indicate regions sequenced and in areas where arrows overlap both strands have been sequenced.
between 21 and 27 days. MBP mRNA levels remained well below control levels in
jp/Y polyribosomes throughout early postnatal development. However, the levels
did fluctuate slightly and peaked at 15 days in both jp/Y and jp/+ brains, three days
earlier than in normal mice. Thus the expression of MBP is affected in all the mu-
tants. In jimpy and quaking animals, the phenomenon is age dependent such that
these mutants exhibit developmental patterns of MBP expression which are different
from control mice as well as from each other. Thus the pattern of MBP gene expres-
sion in these mutants is more complicated than expected.

Although synthesis occurs in quaking mutants at all ages at a significant rate, very
little of the newly synthesized MBP is incorporated into myelin (25-27), whereas in
jimpy mutants not only is there reduced synthesis of the basic proteins, but there is
also incomplete incorporation into myelin of those that are synthesized (22,27).
Thus in these two mutants the expression of MBP is affected and in addition the as-
sembly of the basic proteins into myelin appears to be blocked.

ISOLATION AND PARTIAL SEQUENCE OF A cDNA OF A
MOUSE MBP mRNA

An examination of the expression of the multiple forms of MBP has generated
a number of questions which are actively being explored in many laboratories. For
example, what exactly is the structural relationship among the four mouse myelin
basic proteins? How many genes code for the MBPs exist, and what, if any, MBP
mRNAs are derived by differential splicing of a common primary transcript? One
approach has been to attempt to clone all four mouse MBP mRNAs and determine
their primary sequence. We have prepared an ‘expression’ cDNA library in λgt11
which we have screened with both the mouse cDNA probe and with polyclonal anti-
bodies directed against the MBP. The purpose of these studies has been to isolate
and sequence the cDNAs corresponding to each of the four mouse MBPs in order to
(a) establish the structure of the four MBP mRNAs, (b) unambiguously establish the
structural relationships among the four mouse proteins, and (c) provide additional
probes with which to study MBP mRNA metabolism in normal and mutant mice.

λgt11 libraries are prepared by inserting copies of cDNAs of mRNAs, in this case
18 day old mouse polysomal poly A(+) mRNA into λgt11 which contains the lac Z
gene. This gene provides a promoter site such that when bacterial cells are infected
with this phage a hybrid protein is made which consists of a portion of the lac Z gene
product plus the protein of interest (28). The plaques containing a cDNA copy of
this protein can then be detected by screening the library with an appropriate an-
tibody.

Using the small NZ111 probe and this immunological approach we screened a
λgt11 library and obtained a large number of clones varying in length from 0.44 to
3.7 Kb. We chose one of these clones (1.85 Kb) for further characterization and as-
certained through a comparison of restriction maps and preliminary sequencing data
that it probably was not a mRNA corresponding to the mouse 14K MBP. It was
clear that the clone was not full length because of its size, and it appeared to begin
(at its 5’ end) within the NZ111 region of the coding region. Restriction maps indi-
FIG. 7. A comparison of the mouse and rat MBP mRNA sequences. Asterisks refer to nucleotides that differ between the two species. The rat 14K sequence was obtained from Roach et al. (23) and the mouse NZ111 sequence was taken from Zeller et al. (21). The Sau 3a restriction sites in the mouse sequence have been underlined.

cated three Sau 3a sites, so the clone was digested with Sau 3a and the fragments were cloned into M13 for sequencing. One of the Sau 3a sites was just inside the coding region, as predicted from the rat 14K MBP sequence, but another site, unique to this mouse MBP mRNA, was found downstream from the coding region. Figure 6 illustrates the sequencing strategy. The arrows indicate the directions of sequencing and the regions where both strands were sequenced.

The sequence of this clone indicated that it was part of either the 18.5K or the 21.5K MBP mRNAs because it contained the extra sequence found in these two
proteins. Approximately 450 bases were sequenced including all of the available coding region and part of the noncoding region. A comparison of the mouse sequence with the rat 14K MBP mRNA sequence (Fig. 7) indicated a high degree of homology with only 15 base substitutions and 7 deleted nucleotides within the 323 bases common to both mRNAs. There was a perfect correlation of the sequence of this clone with the smaller NZ111 sequence. The two Sau 3a sites are underlined in Fig. 7.

In Fig. 8 the sequence of the mouse MBP protein is compared to that of the rat 14K MBP obtained through its cDNA sequence (23) and to the bovine (29) and human (30) 18.5K MBPs obtained by conventional protein sequencing techniques. In the region sequenced, the mouse protein contains a His-Gly sequence at residues 78

<table>
<thead>
<tr>
<th>Mouse 18.5K/21.5K</th>
<th>Tyr Gly Ser Leu Pro Glu Lys Ser Gin Gin His Gly Arg Thr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 14K</td>
<td>Tyr Gly Ser Leu Pro Glu Lys Ser Gin - - Arg Thr</td>
</tr>
<tr>
<td>Bovine</td>
<td>Tyr Gly Ser Leu Pro Glu Lys Ala Gin Gly His Arg Pro</td>
</tr>
<tr>
<td>Human</td>
<td>Tyr Gly Ser Leu Pro Glu Lys Ala - His Gly Arg Thr</td>
</tr>
<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val</td>
</tr>
<tr>
<td>Rat 14K</td>
<td>Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val</td>
</tr>
<tr>
<td>Bovine</td>
<td>Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val</td>
</tr>
<tr>
<td>Human</td>
<td>Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val</td>
</tr>
<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Thr Pro Arg Thr Pro Pro Ser Gin Gly Lys Gly Arg Gly</td>
</tr>
<tr>
<td>Rat 14K</td>
<td>Thr Pro Arg Thr Pro Pro Ser Gin Gly Lys Gly Arg Gly</td>
</tr>
<tr>
<td>Bovine</td>
<td>Thr Pro Arg Thr Pro Pro Ser Gin Gly Lys Gly Arg Gly</td>
</tr>
<tr>
<td>Human</td>
<td>Thr Pro Arg Thr Pro Pro Ser Gin Gly Lys Gly Arg Gly</td>
</tr>
<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gin</td>
</tr>
<tr>
<td>Rat 14K</td>
<td>Leu Ser Leu Ser Arg Phe Ser Trp - - - - - - -</td>
</tr>
<tr>
<td>Bovine</td>
<td>Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gin</td>
</tr>
<tr>
<td>Human</td>
<td>Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gin</td>
</tr>
<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser</td>
</tr>
<tr>
<td>Rat 14K</td>
<td>- - - - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>Bovine</td>
<td>Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser</td>
</tr>
<tr>
<td>Human</td>
<td>Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser</td>
</tr>
<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Ala His Lys Gly Phe Lys Gly Ala Tyr Asp Ala Gin Gly Thr</td>
</tr>
<tr>
<td>Rat 14K</td>
<td>- - - - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>Bovine</td>
<td>Ala His Lys Gly Leu Lys Gly His - Asp Ala Gin Gly Thr</td>
</tr>
<tr>
<td>Human</td>
<td>Ala His Lys Gly Phe Lys Gly Val - Asp Ala Gin Gly Thr</td>
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<tr>
<td>Mouse 18.5K/21.5K</td>
<td>Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp Ser Arg</td>
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<td>Rat 14K</td>
<td>- - - - - - - - - - - - - - - - - - -</td>
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<td>Bovine</td>
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</tr>
<tr>
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<tr>
<td>Bovine</td>
<td>Ser Gin Ser Pro Met Ala Arg Arg</td>
</tr>
<tr>
<td>Human</td>
<td>Ser Gin Ser Pro Met Ala Arg Arg</td>
</tr>
</tbody>
</table>

FIG. 8. A comparison of the protein sequence of the mouse 18.5K myelin basic protein (MBP), deduced from its nucleotide sequence, with the rat 14K MBP and the bovine and human 18.5K MBPs. The rat 14K MBP sequence was deduced from its mRNA sequence (23), and the bovine (29) and human (30) sequences are those published using conventional protein sequencing techniques. The regions where the sequence of the mouse protein differs from the sequences of one or more of the other proteins have been blocked in.
to 79 which is missing in the rat 14K MBP, and a methionine in place of an isoleu-
cine near the C-terminus of the molecule. Within the sequence which is missing
from the rat 14K MBP the mouse protein has four substitutions which distinguish it
from the bovine and human proteins. This cDNA has been used to analyze the other
cDNA clones by Southern analysis and several of these others appear to represent
different mRNAs.

Information about the structure of the MBP gene is itself now beginning to
emerge. It appears to be very large (>30 Kb) and to contain a number of relatively
small exons (perhaps 7–9) distributed among some very large introns (31–33). It is
not yet clear how many MBP genes there are in the mouse or whether some of the
exons may appear as sequences in mRNAs which code for proteins other than the
MBPs in brain (34).

SUMMARY

In summary, studies on the expression of myelin proteins at the molecular biolog-
ical level in normal brain are now beginning and it is possible to answer some funda-
mental molecular biological questions about the nature of the myelinogenesis defect
in the dysmyelinating mutants for the first time since they were discovered. It seems
clear that the mechanisms governing the expression of the MBPs will prove to be
complex and that it is possible that an examination of this system may provide us
with important insights into brain genetic regulatory mechanisms.

ACKNOWLEDGMENTS

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DISCUSSION

*Dr. Mariani:* Are there genetic defects in the human that lead to abnormality in MBP? And is it possible to study such defects using molecular genetics, taking advantage of the homologous nature of the gene?

*Dr. Campagnoni:* To my knowledge there are no human diseases which involve the basic protein gene, but that does not mean we will not continue to look.

*Dr. Caviness:* Do you have any thoughts about why the MBP is not inserted in the myelin structure? Could this have to do with the posttranslational history of the MBP itself in relation to the mutation?

*Dr. Campagnoni:* That is one possibility. To start with, the mechanism by which the basic protein (which is synthesized on free ribosomes in the cytosol) is transported to the site of myelin assembly is simply unknown. It could possibly be related to posttranslational modifications such as methylation or phosphorylation. However, we have measured the activity of MBP-specific methylase in the brain and this appears perfectly normal in the quaking mutant where assembly is affected, though it is abnormal in the jimpy mouse. A more fundamental question is how the molecule is transported. Is the MBP inserted into small vesicles, or do the ribosomes move? There are two main theories so far. One is from Colman in Sabatini's laboratory who believes that the ribosomes actually move down the process and deposit the MBP in the region where myelin is assembled, after which it simply diffuses to the cytoplasmic surface of the plasma membrane which eventually becomes myelin. This is a testable hypothesis using *in situ* hybridization coupled with immunohistochemistry, and we are doing these experiments now. What you might expect if the ribosomes are moving is to see both the protein and the message moving together down the process. If the ribosomes do not move you would expect to see the protein move but the message stay behind in the cytosol of the oligodendrocyte. The second possibility that has been suggested is that there are vesicles which shuttle the protein back and forth, though I do not know of any other example where cytoplasmic protein is transported by a vesicular mechanism. One other possibility is that there is a specific transport molecule.

*Dr. Guénét:* Many countries continue to prepare rabies vaccine on baby mouse brain, though the basic protein may produce an immune encephalopathy. Do you think it would be a good idea to use a brain source such as shiverer or jimpy mutants?

*Dr. Campagnoni:* You do not really begin to see an accumulation of much MBP until about 8 to 10 days in the normal baby mouse. You can detect it immunocytochemically just after birth, but quantitatively it does not amount to very much; furthermore, mouse protein is not as encephalopathogenic as some other proteins, so on the whole I do not think it would be worthwhile to do as you suggest.

*Dr. Sotelo:* I should like to know whether the gene for MBP is the same in oligodendrocytes as it is in Schwann cells, since in many of these dysmyelinated mutant mice the peripheral nervous system seems to be totally normal.
Dr. Campagnoni: The same gene should be present in all cells and presumably there is a small amount of the basic protein made in peripheral nerve myelin. Whether the mechanisms which govern it and its expression in the Schwann cell and the oligodendrocyte are exactly the same I am not sure.

Dr. Caviness: Dr Sotelo's question is very interesting because is it not true that the shiverer mutant has normal myelination with MBP in the peripheral nervous system?

Dr. Campagnoni: It has a normal myelin-like structure but it has no MBP. The potential difference across the unit membrane is little different from normal, but there is no basic protein there.