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Small basic proteins of myelin from central and peripheral nervous systems are encoded by the same gene

(cDNA cloning/mRNA levels/oligodendrocytes/Schwann cells)

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ABSTRACT **Peripheral nervous system (PNS) and central nervous system (CNS) rodent myelins, which are produced by different cell types, share common morphological and functional characteristics although their major integral membrane proteins are completely different. Both types of myelin however, contain sets of four myelin basic proteins (MBPs), which share similar immunochemical and electrophoretic properties. We have isolated and characterized cDNA clones corresponding to the rat mRNAs encoding the small MBPs (SMBPs) found in both CNS and PNS myelin. Sequence analysis of these clones indicate that SMBPs in both divisions of the nervous system are encoded by the same nucleotide sequences, which suggests that they are the products of the same gene expressed in both oligodendrocyte and Schwann cells. In dot-blot hybridization experiments with the CNS SMBP cDNA as a probe, it was shown that there is a 20-fold higher level of MBP mRNA in a CNS myelin fraction than in total brainstem mRNA. It also was found that in optic and sciatic nerves, which contain oligodendrocytes and Schwann cells respectively, there are higher levels (4-fold and 2-fold, respectively) of MBP mRNA than in brainstem. Blot-hybridization experiments showed that a probe derived from the coding region of the rat SMBP cDNA hybridizes to an homologous mRNA (≈2.6 kilobases) present in human optic nerve, which is not detectable with a probe derived from the 3′ untranslated region. This conservation of coding-region sequences is in accord with the highly homologous amino acid sequences reported for the MBPs in the two species.**

Myelin sheaths that envelope axons in the central nervous system (CNS) and the peripheral nervous system (PNS) are derived from the plasma membranes of specialized glial cells. In the CNS, the myelin-forming cell is the oligodendrocyte, a cell that during myelination sends out numerous cytoplasmic processes, each of which myelinates a single internodal segment. Thus, a single oligodendrocyte may support up to 50 internodes in different axons that may be some distance away from the cell body (1). By contrast, in the PNS, each internodal segment is myelinated by one Schwann cell, and this remains closely apposed to the myelinated axon.

Although PNS and CNS rodent myelins have very similar morphological features and functional characteristics, their major integral membrane proteins are different. However, both classes of myelin contain similar sets of four peripheral membrane polypeptides, the myelin basic proteins (MBPs). CNS and PNS MBPs have similar immunochemical and electrophoretic properties, although in the PNS the polypeptides are less abundant (2).

The four MBPs of the CNS show striking sequence relatedness. The two major ones (L and S, 18.5 kDa and 14 kDa, respectively) represent ≈90% of the total complement of MBPs and have the same amino acid sequence, except for an internal deletion of 40 amino acids near the carboxyl terminus of S (3). The two other species (21.5 kDa and 17 kDa) differ from the L and S proteins only in that they contain near their amino termini an additional polypeptide segment that is common to both (4). It has been shown that the CNS MBPs represent four distinct primary translation products, and from this it was inferred that they are encoded by four distinct mRNAs (5, 6). The precise genetic relationship between the four MBP polypeptides of the CNS has not been elucidated, nor is it known if the corresponding polypeptides found in CNS and PNS are products of the same gene.

We have isolated and characterized cDNA clones corresponding to the rat mRNAs encoding the small MBPs (SMBPs) of both CNS and PNS myelins and have studied the relationships between MBP and mRNAs in anatomically defined CNS tracts and PNS nerves. Our findings indicate that SMBPs in both divisions of the nervous system are identical polypeptides encoded by the same nucleotide sequence and strongly suggest that they are both products of a single gene that is expressed in both oligodendrocytes and Schwann cells.

MATERIALS AND METHODS

Cloning and Identification of MBP cDNA Clones. A cDNA library in *Escherichia coli* RR1 was prepared from rat brain myelin poly(A)$^+$ mRNA using dG·dC-tailing (7) and insertion into the *Pst* I site of pBR322 (8). Putative MBP clones were tentatively identified by a differential cDNA colony hybridization procedure. Specifically, myelin mRNA and total brain mRNA were preparatively fractionated on methylmercury-agarose gels, and those fractions enriched in translatable MBP mRNAs (2200 nucleotides long) were used as templates for the synthesis of ^{32}P-labeled cDNA probes. Clones that hybridized more efficiently to the probe obtained from myelin mRNA (pMBP1, -3, and -4) were tentatively identified as MBP clones. Definitive identification of these clones was obtained by a hybridization selection translation assay (see the legend to Fig. 1). Other procedures such as mRNA preparation, antibody production, *in vitro* protein synthesis, immunoprecipitation, and $NaDodSO_4$ gel electrophoresis were performed exactly as described (6, 9).

RESULTS

It was previously demonstrated by *in vitro* translation that mRNA isolated from a purified myelin fraction of rat brain

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Abbreviations: PNS, peripheral nervous system; CNS, central nervous system; MBP, myelin basic protein; SMBP, small MBP.

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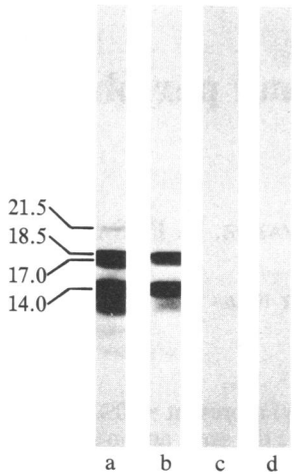

FIG. 1. pMBP1 hybridizes to several MBP mRNAs. Total brainstem poly(A)$^+$ mRNA (40 μg) was hybridized in a formamide-containing buffer (20 mM Pipes, pH 6.4/0.4 M NaCl/12 mM EDTA/0.1% $NaDodSO_4$/50% formamide) for 10 hr at 41°C (10) to 20 μg of putative MBP and control plasmid DNA immobilized on Millipore filters as described (10). After five washes (0.015 M NaCl/0.0015 M sodium citrate, pH 7, at 60°C for 10 min each), the bound mRNA was eluted by heating the filters (95°C) in water for 1 min. The eluted mRNA recovered by ethanol precipitation was translated in a wheat germ system containing [^{35}S]methionine. Translation products were immunoprecipitated with MBP antisera and analyzed by $NaDodSO_4$/PAGE and fluorography after a 1-day exposure. Four different plasmids were used for the selection assay: pMBP1 (lane a), pMBP3 (lane b), pBR322 (lane c), and a recombinant plasmid (pR17) that contains a 1.1-kilobase insert encoding part of rat liver cytochrome P-450e (11). In this fluorogram from a 7–17% gradient gel, the 17-kDa MBP band overlaps with the more intense and broader 18.5-kDa band.

contains a 20-fold higher level of MBP mRNAs than does total brainstem mRNA (6). A myelin cDNA library was constructed and putative MBP cDNA clones were selected by a differential hybridization screening procedure that took advantage of the enrichment of MBP mRNAs in the myelin fraction. Six clones showed a strong signal with a size-fractionated myelin mRNA probe and a much weaker one with a comparable brainstem probe. Four of these were found to contain MBP sequences by a hybridization-selection translation assay (illustrated for clones pMBP1 and pMBP3 in Fig. 1). A striking result of these experiments was that a single cloned cDNA was capable of selecting mRNAs encoding all four MBPs, indicating that homologous sequences are present in all of them. Three of the inserts in the four recombinant plasmids (PMBP1, -3, and -4) hybridized to each other (data not shown), and restriction maps (Fig. 2) suggested that these represented partially overlapping segments of a single sequence.

^{32}P-labeled pMBP1 DNA was used as a probe in blot-hybridization analysis to identify homologous mRNAs in total RNA extracts from optic nerve, a CNS tract, and from two peripheral nerves (trigeminal and sciatic). It was found that, even under high-stringency conditions, both PNS and CNS samples gave a single strong hybridization band (Fig. 3) that corresponded to mRNA molecules with a chain length of ≈2200 nucleotides. Much higher levels of the hybridizing RNAs were present in RNA from a brain myelin fraction (Fig. 3, lane a). The size of the hybridizing mRNA greatly exceeded that required to encode the relatively small MBP polypeptides but corresponded to the size expected from the electrophoretic mobility of the MBP mRNA assayed by *in vitro* translation (data not shown). It should be noted that the agarose gels used for these experiments could not separate mRNAs differing by only 100–200 nucleotides; therefore, one would not expect to find distinct bands for the four MBP mRNAs were these to contain untranslated regions of com-

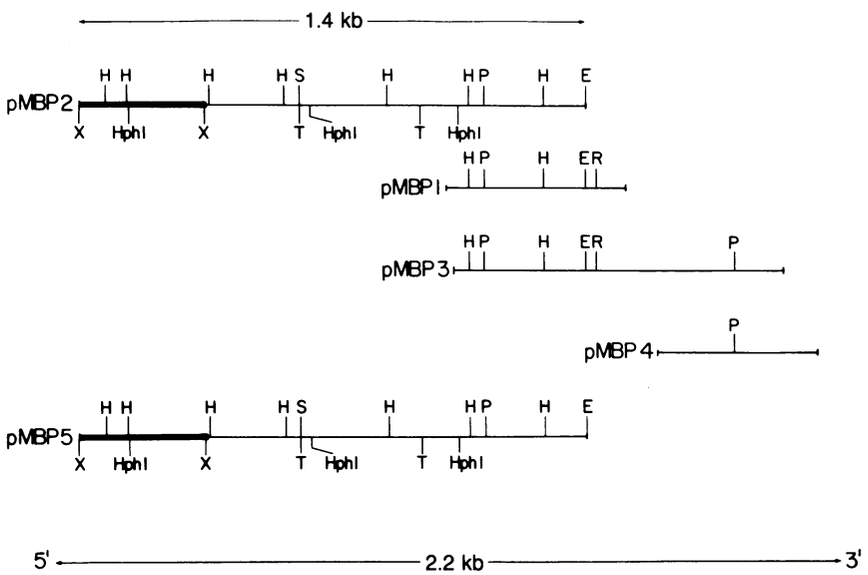

FIG. 2. Restriction maps of SMBP cDNA clones. Restriction maps of the various clones were obtained by standard procedures with the following restriction enzymes: *Pst* I (P), *Xho* II (X), *Hin*fI (H), *Rsa* I (R), *Sal* I (S), *Taq* I (T), *Eco*RI (E), and *Hph* I. The 5′–3′ orientation of the cloned insert in pMBP1 was determined by 5′-end-labeling (at the *Eco*RI site) the two *Eco*RI insert fragments and showing that only the larger fragment hybridized to brain mRNA. The relative orientations of the other clones were obtained by cross-hybridization between appropriate fragments of the various clones. cDNA clones pMBP2 and pMBP5 were obtained by cloning *Bam*HI/*Eco*RI fragments of cDNA (20 ng) prepared against total brainstem or sciatic nerve mRNA, respectively. After column (Sephadex G-50) chromatography of the restriction enzyme digests to eliminate small fragments, the cDNA was ligated to the *Bam*HI/*Eco*RI-cleaved pUC12 vector with phage T4 DNA ligase. Transformation of *E. coli* LE 392 with 1 ng of each recombinant mixture yielded about 5000 independent clones, which were replicated onto nitrocellulose filters and screened with an agarose gel-purified cDNA fragment of pMBP1 as a probe. In both cases, comparable numbers of clones hybridized to the probe. Analysis showed that these corresponded to recombinants containing a *Bam*HI/*Eco*RI fragment ≈1.4 kb long. The heavy lines denote the coding region in pMBP2 and pMBP5. kb, Kilobases.

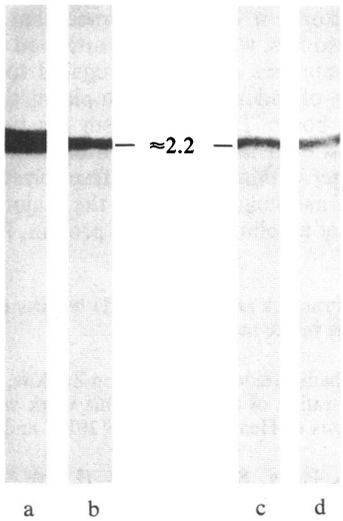

FIG. 3. Identical electrophoretic mobility of MBP mRNAs in the CNS and PNS: hybridization of pMBP1 to total RNA from different CNS and PNS components shows a single band at 2200 base pairs. Total RNAs (10 μg) prepared from a crude brain myelin fraction (lane a), optic (lane b), trigeminal (lane c), and sciatic (lane d) nerves were fractionated by electrophoresis in formaldehyde-containing 1.5% agarose gels (12). The RNA was transferred to GeneScreen membranes (New England Nuclear products) which were then probed with ^{32}P-labeled nick-translated pMBP1 DNA and washed as described in the GeneScreen manual. An additional final wash in 0.015 M NaCl/0.0015 M sodium citrate, pH 7, at 50°C for 30 min was included. The approximate molecular weight (in kilobases) of the hybridized RNA was estimated from its relative mobility by using rabbit reticulocyte rRNA standards localized by ethidium bromide staining.

parable lengths. An assessment of the relative abundance of MBP sequences in mRNA populations obtained from myelin, total brainstem, and different nerve tracts was obtained by dot-blot analysis (Fig. 4) with ^{32}P-labeled pMBP1 as the probe. This showed that, indeed, there is a 20-fold higher level of MBP mRNA in RNA extracted from a brain myelin fraction (Fig. 4, row b) than in total brainstem RNA (Fig. 4, row a). It also was observed that the optic nerve (Fig. 4, row

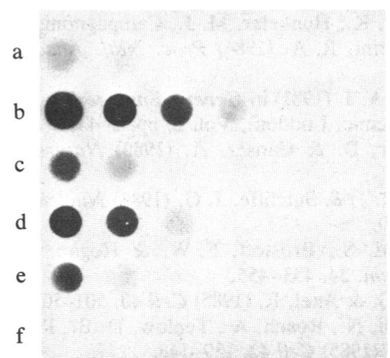

FIG. 4. Quantitation of hybridization of pMBP1 to total RNA from CNS and PNS. Samples from a dilution series of various total RNA preparations were bound to a nitrocellulose filter (Schleicher & Schuell, application update 372) using the Schleicher & Schuell dot-blot apparatus, and the filters were incubated with ^{32}P-labeled pMBP cDNA for hybridization as in Fig. 3. Consecutive dots in each row represent 2-fold serial dilutions. The different rows correspond to RNA from: brainstem (row a), myelin (row b), optic nerve (row c), trigeminal nerve (row d), sciatic nerve (row e), and human placenta (row f). The initial undiluted myelin RNA sample contained 2.5 μg, and all others, 5 μg. Autoradiography was carried out with an intensifier screen and Kodak XAR-5 film.

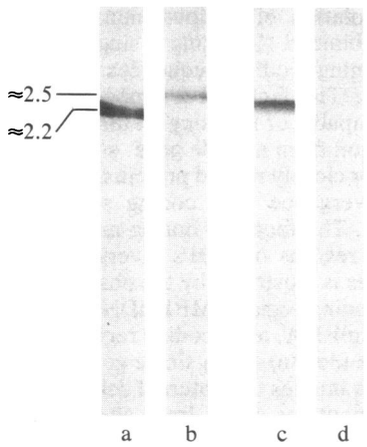

FIG. 5. Human MBP mRNA is recognized by a coding-region probe from rat SMBP cDNA. Blot hybridizations of RNA (5 μg) from rat brain (lanes a and c) or human optic chiasmas (lanes b and d) were probed with ^{32}P-labeled *Xho* II–*Xho* II (lanes a and c) or *Xho* II–*Eco*RI (lanes b and d) fragments of pMBP2, corresponding to the coding and noncoding regions, respectively, as described in the legend to Fig. 2. Sizes are shown in kilobases.

c), which is rich in oligodendrocytes, and the sciatic nerve (Fig. 4, row e), in which Schwann cells are abundant, contain higher levels (4-fold and 2-fold, respectively) of MBP mRNA than does total brainstem.

A comparison of the restriction map of pMBP1 (Fig. 2) with the sequence for CNS MBP cDNA reported by Roach *et al.* (13) indicated that pMBP1 corresponded to a sequence within the 3′ noncoding region of the SMBP mRNA. The presence of a *Bam*HI restriction site near the 5′ end of the coding region of the messenger (13) and an *Eco*RI site in the 3′ untranslated region of the cDNAs identified by us (Fig. 2, pMBP1 and pMBP3) led us to attempt to obtain large cDNA clones containing nearly the entire coding region of the SMBP mRNAs from a library constructed by cloning a *Bam*HI/*Eco*RI digest of brain myelin cDNA into the polylinker region of the vector pUC12 (14). We also searched for similar clones in a pUC8 library constructed using mRNA from sciatic nerve as a template. Both libraries were screened by using as a probe the larger *Pst* I segment contained in pMBP1. Comparable numbers of clones in both libraries hybridized to these probes, and several of these were shown to contain *Bam*HI–*Eco*RI inserts of identical electrophoretic mobility. Detailed restriction maps (15 different sites) of the inserts (Fig. 2) in clones from both sources (CNS, pMBP2; PNS, pMBP5) were indistinguishable, strongly suggesting that corresponding mRNAs were identical. The sequences of the coding regions within these two clones, extending from codons for amino acid residues 12 to 128 (carboxyl terminus) of the SMBP polypeptides, were determined (not shown), and both were found to be identical to the sequence previously reported for rat brain SMBP (13). In hybridization studies, the coding region of pMBP2 recognized a homologous mRNA present in human optic nerve that was not detectable when a probe containing only 3′ untranslated sequences was used (Fig. 5).

DISCUSSION

Previous studies on the sequence of the four MBP polypeptides found in rat CNS have shown that they differ only by the presence or absence of one or both of two internal peptide segments (4). The results in this paper show that mRNAs for the different MBP polypeptides can be isolated from total brain RNA by hybridization to a single cloned cDNA corresponding to 3′ untranslated sequences of the SMBP mRNA.

A similar isolation of all four mouse MBP mRNAs was previously obtained (15) using a single cloned cDNA fragment containing coding sequences common to all four polypeptides. The fact that a probe for the nontranslated region was capable of selecting all four messengers suggests their derivation from a single gene, since noncoding regions of mRNAs for closely related proteins generally show greater sequence divergence than coding segments of the same mRNAs (16). The fact that during mammalian evolution 3′ untranslated regions of MBPs diverged more rapidly than coding regions is illustrated by the observation that, whereas a rat brain coding region SMBP cDNA probe hybridized to human MBP mRNA, a noncoding region cDNA probe failed to do so. The idea (6) that a single gene encodes all four rat MBP mRNAs implies that internal deletions within the three smaller polypeptides result from alternative splicing pathways for a common primary transcript. The existence of a single gene for the four MBPs has been suggested previously from the relatively simple banding pattern in genomic Southern blots (13), which has been confirmed by us, using pMBP2 as a probe (data not shown). In addition, it has been shown that in the mouse mutant "Shiverer," a single mutation eliminates the expression of all four MBPs (17).

Blot-hybridization experiments indicated that both PNS and CNS mRNAs for the four rat MBPs are approximately 2200 nucleotides long and that the human CNS MBP mRNA is somewhat longer (≈2500 nucleotides). Contrary to a previous report (15), no additional bands of higher molecular weight were observed. The DNA restriction mapping and sequence data in this and previous work (13) show that the large size of the MBP mRNA can be accounted for by the long untranslated region at the 3′ end of the mRNA molecule. It has previously been noted that brain-specific mRNAs frequently contain long 3′ untranslated regions (18), but the physiological significance of this feature is not yet known.

CNS and PNS myelins are the products of oligodendrocytes and Schwann cells, respectively, and contain distinctly different major transmembrane proteins. However, it has long been known (2, 19) that similar MBPs are found in both CNS and PNS myelin sheaths, albeit at different concentrations. The data presented here show definitively that in both oligodendrocytes and Schwann cells, SMBPs are encoded by identical mRNAs, strongly suggesting that the same gene is expressed in both cell types. This is in accordance with the finding that the Shiverer mutation leads to the absence of the MBPs from both CNS and PNS myelin (17). The fact that the structure and function of PNS myelin appears not to be significantly affected in the "Shiverer" mutant probably reflects the capacity of P_0, an integral membrane protein found only in the PNS, to assume at least in part the function of the MBPs when these are absent (17, 20).

The availability of cloned cDNAs for the MBPs allowed us to demonstrate that there is a 20-fold higher level of the corresponding mRNAs in a myelin fraction than in total brain. The level of MBP mRNA in myelin is even 5-fold higher than in optic nerve, a CNS tract that is highly enriched in myelinating oligodendrocytes. Because the myelin fraction primarily contains oligodendrocyte processes surrounding myelinating axons, it can be assumed that the MBP-synthesizing polysomes, which are not attached to endoplasmic reticulum membranes (6), are segregated to those specific regions of the oligodendrocyte cytoplasm that may be far from the cell body. The mechanism for this segregation, which was first evident from the enrichment of translatable MBP messenger within the myelin fraction (6) as compared to the level of messenger encoding the major integral membrane protein of myelin (proteolipid protein, PLP), is not yet understood.

Note Added in Proof. A recent paper (21) reports the isolation of a single MBP gene in the mouse.

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