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Characterization of Myelin Proteolipid mRNAs in Normal and Jimpy Mice

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A clone specific for the rat myelin proteolipid protein (PLP) was isolated from a cDNA library made in pUC18 from 17-day-old rat brain stem mRNA. This clone corresponded to the carboxyl-terminal third of the PLP-coding region. The clone was used to identify PLP-specific mRNAs in mouse brain and to establish the time course of PLP mRNA expression during mouse brain development. Three PLP-specific mRNAs were seen, approximately 1,500, 2,400, and 3,200 bases in length, of which the largest was the most abundant. During brain development, the maximal period of PLP mRNA expression was from 14 to 25 days of age, and this was a similar time course to that for myelin basic protein mRNA expression. When the jimpy mouse, an X-linked dysmyelination mutant, was studied for PLP mRNA expression, low levels of PLP mRNA were seen which were approximately 5% of wild-type levels at 20 days of age. When jimpy brain RNA was analyzed by Northern blotting, the PLP-specific mRNA was shown to be 100 to 200 bases shorter than the wild-type PLP-specific mRNA. This size difference was seen in the two major PLP mRNAs, and it did not result from a loss of polyadenylation of these mRNAs.

The biosynthesis of the myelin sheath is a crucial part of nervous system development, and as a result, the developmental expression of the myelin protein genes is highly regulated. The normal pattern of myelin protein expression in rodents indicates that little myelin protein expression occurs prenatally or in neonatal animals (2, 5, 6, 8, 23, 26, 28) and that myelin protein synthesis and accumulation increase significantly in the brain at about 10 days after birth, reaching a peak between 15 and 22 days after birth (2, 5, 6, 8, 23).

Central nervous system myelin contains two major classes of proteins, myelin basic protein (MBP) and proteolipid protein (PLP), which together constitute as much as 80% of the total myelin protein (15, 30). The four MBPs that are present in rodent myelin have homologous amino acid sequences, including identical amino- and carboxyl-terminal sequences but internal segments of unique amino acid sequence (3). They are translated from four different mRNAs (50), and the four MBP mRNAs can be produced from a single MBP gene by alternative mRNA splicing (12, 44). Two PLPs are present in myelin, the major PLP, which has an apparent molecular weight of 25,000, and the closely related proteolipid DM20 (1), which has an apparent molecular weight of 20,000. PLP and DM20 may have a relationship similar to that found among the four MBPs, since they have identical amino- and carboxyl-terminal sequences (46), and they are very closely related by amino acid composition and by antigenic characteristics. The expression of these myelin proteins correlates closely with the formation of myelin in the developing rat brain, and mouse mutants that are defective in myelin formation show lowered levels of these myelin proteins (4, 14, 20, 25).

With the isolation of MBP cDNA clones (33, 51), it has been possible to investigate the regulation of MBP gene expression and the genetic deficiency in the shiverer mouse myelination mutant (12, 34, 44). Several groups have recently isolated cDNA clones for the other major myelin protein, PLP, and have studied PLP mRNAs in bovine and rat tissue (11, 13, 27, 29). The current studies were undertaken to isolate PLP cDNA clones for studies on the developmental regulation of PLP expression in mice. We focused on PLP expression in normal and jimpy mice because it has recently been demonstrated that the PLP gene and the jimpy gene map to comparable regions of the human and mouse X chromosome, respectively (47). Since this region of the X chromosome is highly conserved between mice and humans, the possibility exists that the PLP gene is the locus of the jimpy mutation.

(Preliminary reports of this work have been presented elsewhere [13, 16].)

MATERIALS AND METHODS

Animals. Rats were obtained from Holtzman Animals (Madison, Wis.). BALB/cByJ mice were obtained from The Jackson Laboratory (Bar Harbor, Maine) and bred in the Animal Care Facility at Louisiana State University Medical Center (LSUMC). A colony of jimpy mutant mice was established at LSUMC from breeder pairs provided by Susan Billings-Gagliardi and Merrill K. Wolf, University of Massachusetts, Worcester. These breeder pairs consisted of heterozygous *jp* carrier females (*Tajp*/++) and (C57BL/6J × C3H/HeJ)F_1 hybrid males. Since the *jp* mutation was maintained on the (C57BL/6J × C3H/HeJ)F_1 background, C57BL/6J (B6) and C3H/HeJ (C3H) mice were also obtained from The Jackson Laboratory and bred as a hybrid strain at LSUMC.

Isolation of RNA. For the cloning experiments, poly(A)$^+$ RNA for cloning was isolated from 17-day-old rat brain stem with guanidinium isothiocyanate (9). An alternative procedure used for the total RNA preparations in the expression studies involved tissue homogenization and digestion with

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175  ile tyr phe asn thr trp thr thr cys gln ser ile ala phe pro ser lys thr ser ala
  1  ATT TAC TTC AAT ACC TGG ACC ACC TGC CAG TCT ATT GCC TTC CCT AGC AAG ACC TCT GCC
           T       C                                   T GC    C                   A

195  ser ile gly ser leu cys ala asp ala arg met tyr gly val leu pro trp asn ala phe
 61  AGT ATA GGC AGT CTC TGC GCT GAT GCC AGA ATG TAT GGT GTT CTC CCA TGG AAT GCT TTT
                 C        T                                                      C

215  pro gly lys val cys gly ser asn leu leu ser ile cys lys thr ala glu phe gln met
121  CCT GGC AAG GTT TGT GGC TCC AAC CTT CTG TCC ATC TGC AAA ACA GCC GAG TTC CAA ATG
                   G                                          T

235  thr phe his leu phe ile ala ala phe val gly ala ala ala thr leu val ser leu leu
181  ACC TTC CAC CTG TTT ATT GCT GCA TTT GTG GGT GCT GCA GCC ACA CTA GTT TCC CTG CTC
               T                    G           G                   G   G

255  thr phe met ile ala ala thr tyr asn phe ala val leu lys leu
241  ACC TTC ATG ATT GCT GCC ACT TAC AAC TTT GCC GTC CTT AAA CTC ACG GG            290
                                                    G          T
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FIG. 1. Sequence of a partial PLP cDNA clone. The sequence of this PLP cDNA clone (pJD13A) is presented along with the predicted amino acid translation. The numbering of the DNA sequence begins at the first base that was determined. The numbering of the amino acid sequence is taken from the protein sequence of the bovine PLP (41). Nucleotides that differ between the rat and the bovine cDNA sequences are underlined, and the nucleotide found in the bovine sequence (29) is listed below each underlined nucleotide. The underlined amino acids represent amino acids that differ between the rat and the bovine protein sequences.

proteinase K in the presence of sodium dodecyl sulfate followed by a DNase I treatment, phenol-chloroform extractions, and ethanol precipitation (17). The poly(A)$^+$ RNA fraction was then isolated by two cycles of oligo(dT)-cellulose chromatography and ethanol precipitation.

cDNA cloning and screening. Double-stranded cDNA was synthesized by a modification of standard procedures with poly(A)$^+$ RNA (24). Calf thymus oligonucleotide primers (0.1 μg/ml) were used to randomly prime the first-strand cDNA synthesis. The second strand of the cDNA was synthesized with the Klenow fragment of DNA polymerase I (24). The double-stranded cDNA was treated with S1 nuclease and C tailed; pUC18 was cleaved with *Pst*I, G tailed, and annealed to the cDNA (24). Samples of this mixture were transfected into *Escherichia coli* JM83 by the high-efficiency technique of Hanahan (18). A total of 20,000 individual clones containing inserts (as determined by blue/white selection in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactosidase) were scraped from the plates and stored.

The library was screened by plating out 15,000 recombinants onto nitrocellulose filters. The growth and replica plating of the library were as described previously (24, 49). The screening was carried out in duplicate by the procedure of Woods (49). The first probe used was a 20-base oligonucleotide synthesized (43) with all 256 possible sequences represented by the sequence 5′-CCNGG$^{A}_{G}$AANGC$^{A}_{G}$TTC CANGG. Filters were hybridized at 40°C and washed at a final temperature of 50°C. Autoradiographic signals were obtained overnight with a Du Pont Cronex Lightning-Plus intensifying screen at −70°C.

Thirty-nine clones that hybridized on both duplicate filters were rescreened for confirmation, and an additional screening was carried out with a second oligonucleotide. This 17-base oligonucleotide consisted of a mixture of the 32 possible permutations of the sequence TA$^{C}_{T}$TT$^{C}_{T}$ AA$^{C}_{T}$ACNTGGAC. For this oligonucleotide mixture, the hybridization temperature was 37°C with a 47°C final wash. Twenty of the original clones showed positive hyridizations with both oligonucleotide mixtures, and one that hybridized well with both oligonucleotides was replated to isolate individual colonies for plasmid DNA preparation (24).

DNA sequence analysis. The cDNA clone chosen for further studies, pJD13A, contained an insert about 350 bases in length. This insert was sequenced by cleaving the insert out of pUC18 with *Bam*HI and *Hin*dIII and isolating the insert fragment from an agarose gel. These ends were made blunt and then cleaved near one end of the cDNA insert with *Rsa*I. The resulting fragments were then blunt-end cloned into M13mp18 cleaved with *Sma*I. Clones were picked and sequenced by standard dideoxy techniques (37). By sequencing recombinants with the insert in opposite orientations in the M13 vector, sequence was obtained on both strands of the insert.

Hybridization analyses. Dot-blot analyses were carried out essentially by the method of Thomas (45). RNA was applied to the nitrocellulose filter in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The insert was cut from pJD13A with *Pst*I and purified by agarose gel electrophoresis. The purified insert was nick translated with [α-^{32}P]dATP (32), and 10^6 cpm of probe per ml of hybridization buffer were incubated with the blot for 48 h at 37°C with a final wash at 53°C (45).

For Northern blot analysis of the RNA samples, the RNA was electrophoresed in denaturing agarose-formaldehyde gels and then transferred to nitrocellulose in 20× SSC (36). Ethidium bromide-stained rRNA species were used as size markers (24). Hybridizations and washes were carried out exactly as for the dot blots described above (45).

For analysis of genomic DNA by Southern blotting (39), 5 μg of mouse DNA was cleaved with specific restriction enzymes. The DNA fragments were separated by electrophoresis on a 1% agarose gel and transferred to nitrocellulose in 10× SSC. The hybridization to the filters was carried out with 2×10^6 cpm of labeled probe per ml of hybridization buffer as described above. Hybridization conditions were as previously described (45), with a final wash in 0.1× SSC at 51°C. Autoradiography was carried out at −70°C with two Du Pont Cronex Lightning-Plus intensifying screens.

RESULTS

Cloning of PLP cDNA. Because the entire amino acid sequence of the bovine PLP (22, 40–42) and portions of the

rat PLP (31) had been reported previously, it was possible to predict several oligonucleotide mixtures that would contain homology to the rat PLP mRNA. Two such oligonucleotides were selected from the middle of the protein-coding sequence to screen a rat brain stem cDNA library by hybridization. The identity of PLP-positive clones was then confirmed by sequence analysis with comparisons to the known PLP cDNA and amino acid sequences. The two oligonucleotides that were chosen to screen the library coded for amino acid sequences 176 to 181 and 210 to 216 (Fig. 1). The nucleotide sequence of pJD13A was identical to nucleotides 649 to 938 of the full-length rat cDNA isolated by Milner et al. (27) except for a substitution of a C for a T at nucleotide 287 of our sequence. Since our cDNA library was synthesized with a random primer, it is possible that this substitution, which is near the end of the cDNA, represents a mismatch between the primer and the mRNA during cDNA synthesis. We believe this to be likely because this nucleotide alteration would result in a substitution of a threonine for a methionine at amino acid residue 270 in the protein, and protein sequence data do not indicate such a substitution. In addition to this nucleotide change, there were 18 nucleotide differences between this rat cDNA and the bovine cDNA isolated by Naismith et al. (29). The predicted translation of the sequence of the cDNA clone pJD13A agreed with the carboxyl-terminal third of the bovine PLP amino acid sequence derived from the bovine cDNA sequence (amino acids 175 to 269, Fig. 1) with the exception of two amino acid residues.

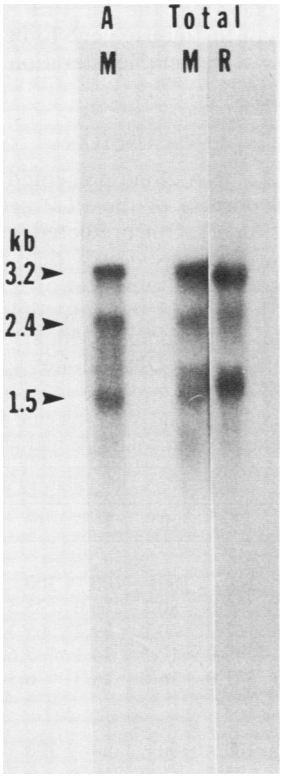

FIG. 2. Analysis of PLP-specific brain RNAs. RNA samples were electrophoresed in agarose-formaldehyde gels (17), blotted onto nitrocellulose, hybridized with ^{32}P-labeled PLP cDNA probe, and autoradiographed. Poly(A)$^+$ mouse brain RNA (A, 2 μg) and total brain RNA (20 μg) from mice (M) or rats (R) were analyzed. Samples were run on the same gel, and the sizes of the three mouse mRNAs were calculated by using four RNAs as standards, the two rRNAs and the two rat PLP mRNAs, whose sizes are known from their sequences (27). kb, Kilobases.

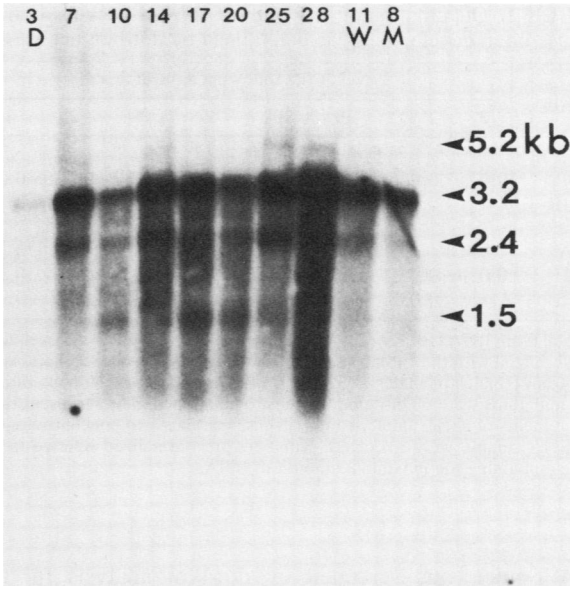

FIG. 3. Analysis of PLP-specific brain mRNAs during mouse brain development. Poly(A)$^+$ RNA samples (2 μg) from BALB/cByJ mice were studied as described in the legend to Fig. 2. RNA was from 3-, 7-, 10-, 14-, 17-, 20-, 25-, and 28-day-old mice and from 11-week- and 8-month-old mice. kb, Kilobases; D, day; W, week; M, month.

PLP mRNA expression in normal mice. The rat PLP cDNA was used in studies on PLP mRNA expression in rats and mice. When rat brain RNA was screened by Northern analysis, two major mRNAs approximately 1,600 and 3,200 nucleotides in length were identified (Fig. 2), as has been determined by other investigators (27, 29). In addition, a faint band approximately 2,400 nucleotides in length was occasionally observed. In the mouse, the major PLP mRNAs were 2,400 and 3,200 nucleotides in length, with a minor mRNA of approximately 1,500 nucleotides. This small mRNA appeared to be somewhat shorter than the small rat PLP mRNA, and in some samples two PLP mRNAs appeared in this size range.

Poly(A)$^+$ brain RNA was obtained from BALB/cByJ mice of different ages, from 3 days to 8 months. Even at 3 days of age, the two major PLP mRNAs were seen. These increased in concentration, reaching a broad peak of maximal expression between 14 and 28 days of age, coinciding with the period of maximal myelin formation (2, 15, 26, 28, 30) (Fig. 3). In many poly(A)$^+$ RNA samples, a fourth mRNA was identified, approximately 5,200 bases in length. This RNA was generally not detected when total RNA samples were analyzed. As in the rat, significant levels of PLP mRNA were still detectable in mouse brain at 8 months of age.

Since the jimpy mutant mice to be studied in subsequent experiments were maintained on a B6 × C3H hybrid genetic background, age studies were conducted for PLP and MBP mRNA expression in normal hybrid mice. When screened by Northern blot analysis, total brain RNA from these mice contained the three major PLP RNAs, 3,200, 2,400, and 1,500 nucleotides in length. Brain total RNA from (B6 ×

TABLE 1. Quantitation of PLP and MBP mRNA levels during mouse brain development, as determined by dot-blot analysis[a]

Protein	Level at the following days of age:							
	3	7	10	14	17	20	25	60
+ +/+ +								
PLP	0.025	0.066	0.200	0.294	0.464	0.289	0.535	0.242
MBP		0.071	0.218	0.189	0.479	0.806	0.477	0.277
+ +/Y								
PLP	0.029	0.051	0.159	0.563	0.424	0.599	0.394	0.130
MBP		0.038	0.131	0.353	0.411	0.724	1.00	0.282

[a] Total brain RNA (10, 5, or 2.5 μg per sample) was applied to nitrocellulose and hybridized with the ^{32}P-labeled PLP or MBP cDNA probe. Samples were taken from females or males at 3, 7, 10, 14, 17, 20, 25, and 60 days of age. Autoradiograms were quantitated by densitometry. Numbers represent three measurements of each sample, and in all cases at least two concentrations of each RNA sample were averaged. Numbers are normalized relative to the sample containing the maximum amount of RNA.

C3H)F_1 mice was screened by dot-blot analysis for the presence of both PLP and MBP mRNAs. A comparison of these dot blots indicated that PLP mRNA levels increased at approximately the same time during brain development as did MBP mRNA levels (Table 1).

PLP mRNA expression in jimpy mice. A comparison was made of PLP and MBP mRNA levels in wild-type and jimpy mouse brain. In young mice (7 to 10 days old), both PLP and MBP mRNA levels were low, and in jimpy mice they were reduced only slightly relative to normal animals. At 7 days of age, the levels of PLP mRNA in hemizygous jimpy males were reduced to approximately 40 to 50% of wild type (Table 2). In normal animals, PLP and MBP mRNA levels increased significantly between 10 and 14 days of age (Table 1), but the jimpy mice maintained the same low level of mRNA until 20 days of age, after which PLP and MBP mRNA levels dropped. Thus, when compared with wild-type male or female mRNA levels, the levels of PLP and MBP RNAs were significantly reduced in 14- to 20-day-old hemizygous jimpy males (Table 2). For example, at 20 days, the levels dropped to 5 to 6% for PLP and MBP RNAs. Comparable results were obtained from two sets of dot blots containing RNA from two separate sets of individual normal and jimpy animals. At all ages, PLP and MBP mRNA levels were also reduced in heterozygous females. From 10 to 25 days of age, PLP mRNA levels were approximately 50 to 75% of wild type. MBP mRNA levels in heterozygous females varied significantly during this period when compared with levels in wild-type females.

When these RNAs were analyzed by Northern blotting, it was apparent that in 7-day-old animals there was only a small difference in the level of PLP mRNA between normal and jimpy animals (Fig. 4). The most important aspect of this Northern blot was the observation that the PLP mRNA in the jimpy mice was somewhat shorter than the wild-type PLP mRNA. This size difference was quite small and was calculated on the basis of rRNA markers to be approximately 100 to 200 bases in length. This size difference was hard to measure in animals over 14 days of age, because the intensity of the wild-type mRNA signal broadened the wild-type band significantly, but it could be seen most distinctly when comparing the wild-type mRNA at 7 and 10 days with that for the *Tajp*/Y and +*jp*/Y animals at all ages.

It was necessary to determine whether this size difference might result from a loss of PLP mRNA polyadenylation. Poly(A)$^+$ and nonpoly(A)$^+$ RNAs from jimpy mouse brain were therefore screened with the PLP cDNA probe (Fig. 5). No PLP mRNA was found in the nonpoly(A)$^+$ RNA in jimpy mice after a 2-week film exposure, indicating that the size difference between the normal and jimpy PLP RNA did not result simply from a loss of polyadenylation on the PLP mRNA. It is, however, possible that if a 100 to 200 base segment of a very long poly(A)$^+$ tail was lost, the PLP-specific RNA would not lose its capacity to bind to oligo(dT)-cellulose.

Southern analysis of jimpy DNA. To investigate whether the alteration in the PLP mRNA in jimpy mice was the result of an altered PLP gene in these mice, we analyzed jimpy mouse liver DNA by Southern blotting, using four different restriction enzymes, *Pst*I, *Bam*HI, *Eco*RI, and *Hin*dIII. In this experiment, a full-length rat PLP cDNA probe was used (obtained from J. Gregor Sutcliffe and Robert Milner). A single band was seen for both the *Bam*HI and *Eco*RI digests of mouse DNA, two bands for *Hin*dIII, and four bands for *Pst*I (data not shown). In no case was a change in the pattern of restriction fragments seen between normal and jimpy mice. This is not too surprising since a small deletion (less than 200 nucleotides) might not be detectable by Southern blotting when large restriction fragments are being analyzed, e.g., 9-kilobase fragments. Furthermore, it is possible that the alteration in the PLP gene could be a point mutation, which might not be detected by Southern blotting under any conditions. It is, however, possible that differences between the mouse PLP gene and the rat PLP cDNA cause this Southern analysis of the mouse gene to be incomplete, thus masking an otherwise detectable alteration in the mouse PLP gene in the jimpy mice.

DISCUSSION

The partial PLP cDNA clone presented here encodes the carboxyl-terminal portion of the PLP protein from amino acids 175 to 269 (Fig. 1). Our predicted sequence is in very good agreement with the previously determined portions of rat PLP sequence and with other rat cDNA clones (11, 27). It is of interest that, as in the rat, multiple PLP-specific mRNAs are found in the mouse. Milner et al. (27) isolated and sequenced full-length cDNA clones specific for the two

TABLE 2. Levels of PLP- and MBP-specific RNA during mouse brain development in wild-type and jimpy mice[a]

Protein	Level (%) at the following days of age:							
	3	7	10	14	17	20	25	60
PLP								
+ +/+ +	100	100	100	100	100	100	100	100
+ +/Y	130.2	70.0	80.1	218.0	75.5	79.1	62.0	32.0
Tajp/+ +	119.6	67.2	60.0	79.2	65.3	53.8	63.0	36.8
Tajp/Y	89.1	48.9	12.5	14.1	7.6	5.4	5.6	—
+*jp*/Y	—[b]	41.0	16.8	16.3	6.3	4.0	—	—
MBP								
+ +/+ +		100	100	100	100	100	100	100
+ +/Y		73.3	66.9	216.7	90.7	91.8	207.3	41.9
Tajp/+ +		81.9	58.2	122	87.6	56.4	83.2	47.4
Tajp/Y		56.4	11.5	16.1	10.0	5.7	7.9	—
+*jp*/Y		150.7	24.1	29.2	7.9	3.3	—	—

[a] Samples were analyzed as in Table 1 from wild-type females (+ +/+ +), wild-type males (+ +/Y and *tjp*/Y), heterozygous females (*Tajp*/+ +), and affected jimpy males (*Tajp*/Y). Animals were 3, 7, 10, 14, 17, 20, 25, or 60 days of age. Blots were incubated with ^{32}P-labeled PLP or MBP probe, and autoradiograms were quantitated by densitometry as in Table 1.

[b] No animals were available at these time points.

major size classes of rat PLP-specific mRNA, and they demonstrated that these cDNAs were identical in both their protein-coding sequences and their 5'-noncoding sequences. The 3'-noncoding segments that immediately follow the coding region of the two PLP-specific cDNAs were also identical, but the 1,600-nucleotide PLP-specific cDNA ended at a polyadenylation signal at positions 1383 to 1388. In contrast, the sequence of the 3,200-nucleotide cDNA continued through the potential polyadenylation signal, and after another 1,500 nucleotides of 3'-noncoding region, polyadenylation occurred. Thus, the nucleotide sequences of both cDNAs were identical for close to 1,400 nucleotides. These investigators noted the presence of a third polyadenylation signal within the sequence of the 3,200-nucleotide rat PLP cDNA which could potentially produce a third PLP-specific mRNA of approximately 2,400 nucleotides. Since this is the size of a major mouse mRNA, and the other mouse mRNAs are of similar size to the rat PLP mRNAs, it may well be that these mouse PLP-specific mRNAs are related in a manner similar to that of the rat PLP mRNAs. However, the short mouse PLP mRNA is somewhat smaller than the short rat PLP mRNA, by approximately 100 nucleotides, and it is possible that a direct correlation between the multiple rat and mouse PLP-specific mRNAs is premature.

The developmental time course of expression of the PLP mRNAs shows a high degree of regulation. The levels of mRNA in the brain are low at 3 days of age, and they reach a peak over a broad period from about 14 to 25 days. This closely correlates with the time of active myelination and the most active period of PLP synthesis (5). Even the adult mouse maintains approximately one-fourth to one-third of the maximal PLP mRNA levels, suggesting that myelin protein synthesis and replacement is continuing in the adult.

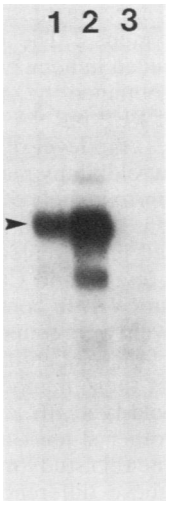

FIG. 5. Analysis of PLP-specific RNA in poly(A)$^+$ and nonpoly(A)$^+$ RNA from jimpy mice. RNA samples were analyzed as described in the legend to Fig. 2, using ^{32}P-labeled PLP probe. Total jimpy brain RNA (20 μg, lane 1), poly(A)$^+$ jimpy brain RNA (3 μg, lane 2), and nonpoly(A)$^+$ jimpy brain RNA (20 μg, lane 3) were studied. Nonpoly(A)$^+$ jimpy brain RNA consisted of RNA that did not bind to oligo(dT)-cellulose. Arrow: Largest PLP mRNA.

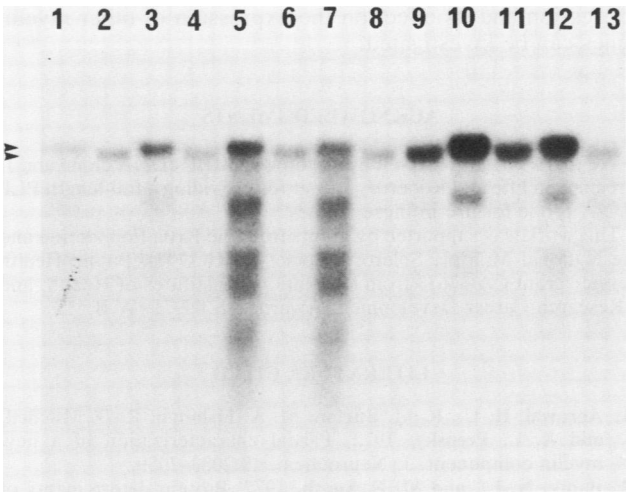

FIG. 4. Analysis of PLP-specific RNAs in wild-type and jimpy mice. RNA samples were analyzed as described in the legend to Fig. 2, using ^{32}P-labeled PLP probe. Samples were obtained from 7-day-old (lanes 1 to 3), 10-day-old (lanes 4 to 8), or 17-day-old (lanes 9 to 13) mice. RNA samples from wild-type mice (lanes 1, 3, 5, 7, 10, and 12), *Tajp*/Y mice (lanes 4, 8, 9, and 13), or *+jp*/Y mice that had lost the *Ta* coat color marker (lanes 2, 6, and 11) were studied. The upper arrowhead indicates the position of the 3,200-nucleotide PLP-specific mRNA found in wild-type mice, and the lower arrowhead indicates the position of the large PLP-specific mRNA found in *Tajp*/Y or *+jp*/Y mice. Lanes 1 to 9, 11, and 13 contained 20 μg of total brain RNA. Lanes 10 and 12 contained 3 μg of total brain RNA plus 17 μg of total liver RNA, which has no PLP-specific RNA. When larger amounts of wild-type brain RNA from 17-day-old animals were analyzed, the signal was too strong, and no size comparison could be made with the *Tajp*/Y samples. These samples were diluted with liver RNA so that all samples contained a total of 20 μg of RNA.

Quantitation of PLP mRNA levels by dot-blot hybridization is somewhat complicated by the presence of multiple PLP mRNAs. However, since both rat PLP mRNAs have identical protein-coding regions, they can presumably both be translated into protein. Thus, assuming that such a relationship exists among the multiple mouse mRNAs, the total amount of RNA capable of being translated into PLP protein was quantitated in these dot blots.

The observation that the peak of PLP mRNA occurred at the same time as or before the peak of MBP mRNA expression is comparable to the data of Naismith et al. (29) on the expression of PLP and MBP mRNAs in the developing rat nervous system. It is of interest that these data contrast with observations on the active synthesis of the two proteins in brain-slice experiments. The peak of active synthesis of MBP occurs between 16 and 18 days of age in the mouse, and the peak of active synthesis of PLP occurs at approximately 22 days of age (5). This apparent discrepancy may result from the fact that different methodologies were utilized in the two sets of experiments. On the other hand, it is also possible that certain aspects of MBP and PLP transcription and translation are regulated in different ways, perhaps producing a relatively high level of PLP mRNA before active protein synthesis begins.

The PLP gene was recently mapped to region Xq13-Xq22, in the middle of the long arm of the human X chromosome. This region of the X chromosome is highly conserved between humans and mice, and in the mouse, this region encompasses the mouse jimpy locus (47). Since these data suggested that the PLP gene was altered in the jimpy mouse

mutant, we conducted experiments to investigate PLP expression in the jimpy mouse. The observation that PLP mRNA levels were reduced in hemizygous jimpy males was consistent with data obtained by Sorg et al. (38) who quantitated the level of PLP mRNA in cell-free translation studies. In those studies, the level of PLP mRNA was 3 to 12% of normal in 21-day-old jimpy males, and in the current studies, we detected approximately 5% of normal in 20-day-old jimpy males. When Roth et al. (35) measured MBP mRNA levels in affected jimpy males, they found approximately 25% of control levels, and Carnow et al. (7) found normal levels of MBP mRNA. In contrast, only 6 to 16% of normal MBP mRNA levels were found in *Tajp*/Y mice in the current studies. Some of these quantitative differences might result from the fact that these numbers were calculated by comparison with the wild-type B6 × C3H females at each time point or that the other studies measured the levels of translatable mRNA while this study measured total RNA. It is also possible that these differences result from strain differences, since the present studies were conducted with jimpy mice on the B6 × C3H hybrid strain background, and the other studies all used jimpy mice on the B6 × CBA background. Wolf et al. (48) have shown that the jimpy mutation is expressed to a different degree when present in mice with different genetic backgrounds. In particular, they demonstrated that when the jimpy mutation is present in mice of the B6 × C3H hybrid strain, the dysmyelination is enhanced relative to its expression in mice of the B6 × CBA genetic background.

The data obtained on PLP and MBP mRNA levels in heterozygous females indicated an approximate 40% reduction in the amount of PLP mRNA in heterozygous *Tajp* females and a smaller, variable reduction in the amount of MBP mRNA. This is consistent with other studies (21, 35, 38) indicating that in *Tajp* heterozygous females, the amounts of these proteins or their mRNAs are reduced. Sorg et al. (38) reported an approximate 25% reduction in the amount of MBP mRNA in heterozygous females. Although that study indicated only a slight reduction in PLP mRNA in heterozygous females, Kerner and Carson (21) reported a reduction of approximately 50% of the normal amount of PLP in heterozygous females, as measured by immunoblotting. Kerner and Carson (21) suggested that as a result of X chromosome inactivation, half of the oligodendrocytes are phenotypically jimpy and half are phenotypically wild type. Since the PLP gene is on the X chromosome, studies of PLP expression in such heterozygous animals might provide information on the regulation of myelin gene expression.

The possibility that the major PLP mRNAs in jimpy mice are altered in size is consistent with the proposal of Willard and Riordan (47) that the PLP gene is the locus of the jimpy mutation. In contrast to the major deletion of the basic protein gene in the shiverer mouse mutant (33, 34), there is apparently only a slight change in the PLP gene in the jimpy mouse, which was undetectable by Southern blot analysis with our methodology. Hoffman-Chudzick et al. (19) have also reported that no major structural alteration of the PLP gene was detected by Southern analysis in the jimpy mouse with their human PLP cDNA probe. In long exposures of Northern blots such as the one seen in Fig. 4, the three major PLP-specific mRNAs were seen. The amounts of the two smaller mRNAs appeared to be somewhat lower in jimpy brain, relative to the large PLP mRNA, when compared with wild-type brain (compare lanes 9 and 11 with lanes 5 and 7 in Fig. 4), and it was not possible to accurately measure the size of the smallest PLP mRNA because of its low concentration. However, both of the larger PLP mRNAs (3,200 and 2,400 nucleotides) appeared to be slightly altered in size. Since the change in PLP mRNA size in jimpy brain was apparently not the result of a loss of polyadenylation and since it was seen in the two major mouse PLP mRNAs, it is possible that it represents a change in the first 2,200 nucleotides of the PLP mRNA. To assess possible changes in the 5′-noncoding region of the PLP mRNA, we carried out S1 protection studies on PLP mRNA from both wild-type and jimpy mouse brain. A probe spanning the first exon from a mouse PLP genomic clone was annealed to total mouse brain stem mRNA. A slightly heterogeneous pattern of fragment lengths for the 5′-noncoding region was detected (presumably from different cap site usage), but the pattern was identical between wild-type and jimpy mRNA (data not shown). Since the region probed covers the entire 5′-noncoding region of the mRNA, these data indicate that the size difference between wild-type and jimpy PLP mRNA is unlikely to result from an alteration in that region of the mRNA. Studies to establish the exact site of alteration are in progress.

It is clearly of some interest to investigate PLP expression and gene structure in other X-linked myelination disorders. For example, the X-linked myelin-deficient rat (10) may have an altered PLP gene. Hoffman-Chudzik et al. (19) recently screened four patients with Pelizaeus-Merzbacher disease, an X-linked human hypomyelination disorder causing severe neurological problems, and they observed an alteration in the DNA restriction fragments from one patient. It is thus possible that by studying an altered PLP gene in several different species, many aspects of the regulation of PLP expression and its effect on the expression of other myelin genes can be investigated.

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