Volume 13 Number 20 1985 Nucleic Acids Research

Study of the expression of myelin proteolipid protein (lipophilin) using a cloned complementary DNA

A.L.Naismith[1], E.Hoffman-Chudzik[1], L.-C.Tsui[2] and J.R.Riordan[1]

[1]Departments of Biochemistry and Clinical Biochemistry, [2]Department of Medical Genetics and Medical Biophysics, University of Toronto, Toronto M5S 1A8, and [1,2]The Research Institute, Hospital for Sick Children, Toronto M5G 1X8, Canada

Received 20 May 1985; Revised 6 September 1985; Accepted 9 September 1985

ABSTRACT

We have prepared a λgt10 cDNA library with the mRNA isolated from fetal calf brains which were actively myelinating. Using two oligonucleotides made according to the known amino acid sequence of myelin proteolipid protein (PLP or lipophilin), we have isolated several cDNA clones for this major intrinsic membrane protein of myelin. One of these clones, designated as pLP1, is found to contain 444 bp of coding sequence for the C-terminal half of PLP and 486 bp of 3' untranslated sequence. Using pLP1 as a hybridization probe, we have studied the regulation of PLP at the mRNA level during rat brain development. Our results show that the relative amounts of mRNA for PLP and that for the major extrinsic protein of the myelin membrane, myelin basic protein, increase coordinately during the course of myelination in the brain.

INTRODUCTION

Myelin of the central nervous system contains two major and several minor membrane proteins (1). A considerable amount of information has been accumulated in understanding the importance of these proteins in nervous function. The two abundant ones, the extrinsic myelin basic protein (MBP) and the intrinsic proteolipid (PLP) or lipophilin (2), have been most extensively investigated. Studies have provided the complete primary structures of these two proteins (3-5,26), considerable insight into the arrangement of these proteins in association with the myelin membrane (1,6) and interesting suggestions as to their possible functions (7,8). However, little is known about the regulation of MBP and PLP synthesis and control of gene expression during myelination. In addition, several genetically determined disorders affecting myelin are known in both animals and man (9). To understand the basic defect in these diseases and the regulation of MBP and PLP expression, studies have been initiated to examine these proteins at the gene level. Recently the isolation of

cDNA clones for MBP have been reported (10-12). We here describe the molecular cloning of a complementary DNA (cDNA) for PLP and provide preliminary evidence that the two proteins are coordinately regulated at the mRNA level during development.

MATERIALS AND METHODS

RNA Isolation

Total RNA was isolated from third trimester fetal calf or postnatal rat brains using the procedure described by Chirgwin et al (13). Poly A^+ RNA was selected from total RNA preparations by chromatography on oligo dT-cellulose (Type 3, Collaborative Research) according to Aviv and Leder (14).

cDNA Library Construction

cDNA was synthesized and cloned in the bacteriophage vector λgt10 following the procedures of Huynh et al (15). Briefly, single stranded cDNA was synthesized from 1 μg of poly A^+ RNA using oligo-dT as primer and AMV reverse transciptase (Life Science). The second DNA strand was synthesized using DNA polymerase I (prepared from E. coli 594 according to reference 16) by formation of a hairpin which was subsequently cleaved and removed by using nuclease S1 (Boehringer Mannheim). Following methylation with EcoRI methylase (prepared from E. coli RY13 as in reference 17) and filling of staggered ends with DNA polymerase I, the DNA was ligated to synthetic EcoRI linkers (Collaborative Research). After cleavage with EcoRI the cDNA was size fractionated on Sephacryl S-1000 (Pharmacia). Species constituting the leading edge of the cDNA peak were ligated into the EcoRI site of λgt10. The DNA was packaged *in vitro* and the resulting phages plated with *Escherichia coli* Y1073 cells. (Phage lacking inserts do not form plaques on Y1073). A cDNA library of approximately 1.5×10^5 independent λgt10 recombinant phages were obtained.

Oligonucleotide screening of recombinant phage

Two mixtures of oligonucleotide probes were used for the screening of PLP-specific cDNA (see below). They were synthesized by the solid phase phosphoramidite method (18) and purified by gel filtration and polyacrylamide gel electrophoresis. For hybridization screening of the cDNA library, the oligonucleotides were end-labelled using [γ^{32}P]-ATP (New England Nuclear) and T4 polynucleotide kinase (Pharmacia P-L Biochemicals).

DNA from individual bacteriophage plaques was transferred to nitrocellulose filters according to the method of Benton and Davis (18). After prehybridization (4 x 90min) in 5 x SSC (1 x SSC is 0.15M sodium chloride and 0.015M sodium citrate), 5 x Denhardt's solution (1 x Denhardt's is 0.02% Ficoll 400,000, 0.02% polyvinyl pyrrolidone 360,000 and 0.02% bovine serum albumin) and 0.05 % sodium pyrophosphate at 32°, the filters were hybridized to each of the ^{32}P-labelled mixed oligonucleotide probes (2 x 10^6 cpm/ml) for 60 h at 32° in the same solution. The filters were then washed 3 times, 15 min each, at 32° in 5 x SSC and 0.05 % sodium pyrophosphate.

The recombinant phages which hybridized with both mixed oligonucleotide probes were purified and the inserts in these phages were subcloned in the plasmid pUC9 (20) for restriction enzyme mapping and DNA sequencing analyses.

DNA Sequencing

The cDNA inserts were excised from the pUC vectors using appropiate restriction enzymes, purified by polyacrylamide gel electrophoresis, end-labelled and subjected to DNA sequencing analysis using the chemical cleavage method of Maxam and Gilbert (21).

Blot Hybridization of DNA and RNA

Bovine, human and rat DNA was isolated from cultured cells or indicated tissues using standard procedures (22). DNA samples were digested with restriction endonucleases under conditions recommended by the suppliers and subjected to agarose gel electrophoresis (23). Blot-hybridization analyses of DNA and RNA were performed as described by Southern (24) and Thomas (25), respectively.

RESULTS

Isolation and Characterization of PLP cDNA Clones

To facilitate isolation of PLP-specific cDNA clones, synthetic oligonucleotides corresponding to two selected pentapeptides were prepared (Figure 1). These two regions were chosen on the basis of minimal codon redundancy upon reverse translation of the known amino acid sequence of bovine PLP (1). Accordingly, mixtures of 8 different 14-mers corresponding to residues 176-180 and 32 different 15-mers corresponding to residues 231-235 were end-labelled and used sequentially to screen the fetal bovine brain cDNA library. Twenty-four "positive" clones were obtained using the "231-235" mixed probe but only 7 of them were also found to hydridize with the "176-180"

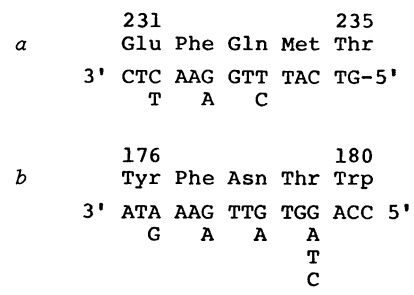
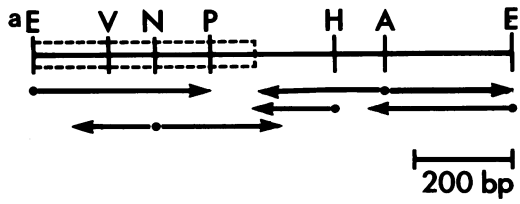


Fig 1. Synthetic oligonucleotides used for probing cloned cDNA for lipophilin. All possible sequences complimentary to the coding sequences for the peptides shown are represented.

mixed probe. All seven clones were isolated and the size of their inserts estimated by EcoRI digestion and gel electrophoresis. Two of these contained inserts of approximately 850 base pairs (bp) in size and the other five, approximately 950 bp. Results of restriction enzyme mapping and blot-hybridization analysis of the above cDNA clones revealed that all 7 clones were closely related, if not sibs. The longer inserts were found to contain sequences extending approximately 100 nucleotides further in the 5' direction than the shorter ones. Therefore, one of the longer cDNA inserts was subcloned into pUC9 and the resulting plasmid, designated as pLP1, was used for further studies described below.

Figure 2a depicts the restriction map of the insert in pLP1 as well as the stategy for DNA sequencing. The nucleotide sequence of pLP1 and its relationship to the known amino acid sequence of the protein is shown in Figure 2b. Nucleotides 1 to 444 correspond exactly with the amino acid sequence from his-129 to the C-terminal phe-276 with only one exception: The DNA sequence predicts a leucine at position 254 rather than valine which was determined by amino acid sequencing (26). It is possible that this val->leu change is due to a G->C transversion at base position 1 of the corresponding codon. Since valine and leucine are both hydrophobic residues, this conserved amino acid substitution presumably does not affect the general properties of the protein. However, this almost perfect match between the amino acid sequence of PLP and that predicted for pLP1 clearly indicates that pLP1 is a cDNA for PLP. In addition, a termination codon (TGA) is present immediately after that for the C-terminal residue phe-276,



b

```
                                10                  20                  30
    GlyLeuLeuGluCysCysAlaArgCysLeuValGlyAlaProPheAlaSerLeuValAlaThrGlyLeuCysPhePheGlyValAlaLeu

                                40                  50                  60
    PheCysGlyCysGlyHisGluAlaLeuThrGlyThrGluLysLeuIleGluThrTyrPheSerLysAsnTyrGlnAspTyrGluTyrLeu

                                70                  80                  90
    IleAsnValIleHisAlaPheGlnTyrValIleTyrGlyThrAlaSerPhePheLeuTyrGlyAlaLeuLeuAlaTyrGlyPhe

                               100                 110                 120
    TyrThrThrGlyAlaValArgGlnIlePheGlyAspTyrLysThrThrIleCysGlyLysGlyLeuSerAlaThrValThrGlyGlyGln


                               130                 140                 150
    LysGlyArgGlySerArgGlyGlnHisGlnAlaHisSerLeuGluArgValCysHisCysLeuGlyLysTrpLeuGlyHisProAspLys
                          CATCAAGCTCATTCTTTGGAGCGGGTGTGTCATTGTTTGGGAAAATGGCTAGGACATCCCGACAAG    66

                               160                 170                 180
    PheValGlyIleThrTyrAlaLeuThrValValTrpLeuLeuValPheAlaCysSerAlaValProValTyrIleTyrPheAsnThrTrp
67  TTTGTGGGCATCACCTATGCCCTGACCGTTGTGTGGCTCCTNGTGTTTGCCTGCTCTGCTGTGCCTGTATACATTTATTTCAACACCTGG   156

                               190                 200                 210
    ThrThrCysGlnSerIleAlaAlaProSerLysThrSerAlaSerIleGlyThrLeuCysAlaAspAlaArgMetTyrGlyValLeuPro
157 ACCACCTGCCAGTCTATTGCTGCCCCCAGCAAGACCTCTGCAAGTATAGGCACTCTCTGTGCTGATGCCAGAATGTATGGTGTTCTCCCA   246

                               220                 230                 240
    TrpAsnAlaProGlyLysValCysGlySerAsnLeuLeuSerIleCysLysThrAlaGluPheGlnMetThrPheHisLeuPheIle
247 TGGAATGCTTTCCCTGGCAAGGTGTGTGGCTCCAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCCAAATGACCTTCCATCTGTTTATT   336

                               250                 260                 270
    AlaAlaPheValGlyAlaAlaAlaThrLeuValSerLeuLeuThrPheMetIleAlaAlaThrTyrAsnPheAlaValLeuLysLeuMet
337 GCTGCGTTTGTGGGGGCTGCAGCCACACTGGTGTCCCTGCTCACCTTCATGATTGCTGCCACTTACAACTTTGCCGTCCTGAAACTCATG   426

                276
    GlyArgGlyThrLysPheEnd
427 GGCCGAGGCACCAAGTTCTGATCTCCTGTAGAAATTTCCCTTTCTCTAATAGCGAGGCTCTAACCACACAGTCCACAGTGCTGCGTCTCC   516

517 CATGTTAACTCTTTGCCTTTGCTACTGACTGGCCCTCTTCTTACTTGACGAGTGTAACAAGAAAGGAGAGTCTCACGGTGATTAATGTCT   606

607 CTGCGGACTCTCCCCTCTTACGTACCTCTTTTAGTCATTTTGCTTCATAGCTGGTTCCTGCTAGAAATGGGAAATGCTTAAAGATGGTGA   696

697 CTCCCCAACTGCAAGTCACAAAGAAGTGGAGGCTCTCATTCAATTTTCAAGCATCTCCCGAGGACNAGGAAGTGTTTTCTTCTCAAAGGG   786

787 CACTTCCACTGATGAAAACAAAGTGGAAAGAAAGATGTTCAGGTAGAGAGAAGGGAGGCACCTGTCCCTGTTGCCATCTTGGGAGCCAAA   876

877 TATATTCTCTTTGGTGTACAAAACCGAGAACTCACTCCAGTCTCCCTAGTACC 930
```

Fig 2a. Restriction map of the 0.93 kb cDNA insert in the phage λLP1. The boxed-in portion corresponds to the C-terminal 147 amino acids of bovine lipophilin. Arrows indicate sequences read. E, EcoRI; V, AvaII; N, NcoI; P, Pst I; H, HinfI; A, AvaI.

Fig 2b. Nucleotide sequence of bovine lipophilin cDNA pLP1 alligned to the amino acid sequence of the protein. The sequences corresponding to the oligonucleotides used for the screening are underlined.

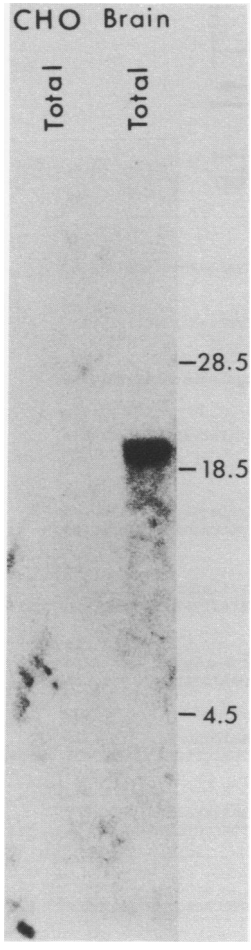


Fig 3. Blot hybridization analysis of total RNA (15 µg) from Chinese hamster ovary (CHO) cells and third trimester fetal bovine brain using pLP1 as probe. The positions of the ribosomal bands are indicated.

suggesting that the protein is not processed at its C-terminus. Furthermore, since neither a polyadenylate track nor polyadenlate addition sequence is found at the 3' end of this cDNA clone, the 3' untranslated region of a PLP message is probably much longer than 500 bp in length (see below).

Detection of PLP-specific RNA and DNA Sequences

To characterize the mRNA coding for bovine lipophilin, gel blot-hybridization analysis of RNA from third trimester brain was performed using radioactively labelled pLP1 as a probe. Fig 3 shows the specific hybridization of a single RNA band of approximately 3.3 kilobases (kb) in size to the probe, indicating the presence of a

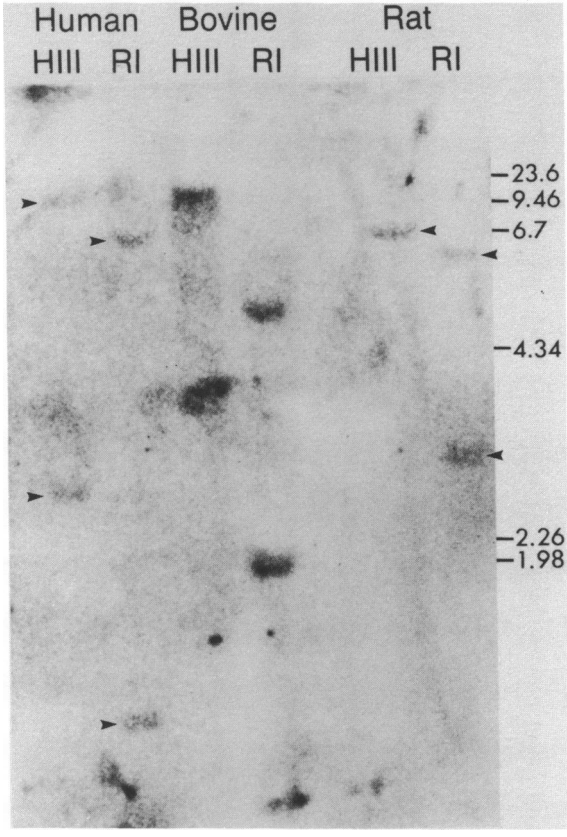


Fig 4. Blot hybridization analysis of bovine, human and rat DNAs. 10 µg of each were digested with Hind III (HIII) or EcoRI (RI), electrophoresed on a 1% agarose gel, transferred to nitrocellulose and probed with pLP1. The positions of Hind III digested lambda phage fragments are indicated.

single PLP mRNA species in the developing calf brain. Consistent with PLP being a glial cell specific protein, no PLP-hybridizing bands could be detected in the RNA prepared from Chinese hamster ovary (CHO) cells (Figure 3).

As a next prerequisite to expression studies, the genomic organization of the PLP gene was examined by gel blot-hybridization analysis. As shown in Figure 4, two Hind III fragments (14 kb and 2.8 kb) and two Eco RI fragments (4.5 kb and 2.2 kb) were detected in the bovine brain DNA samples by using pLP1 as a hybridization probe. Identical hybridizing fragments were obtained when calf thymus DNA was

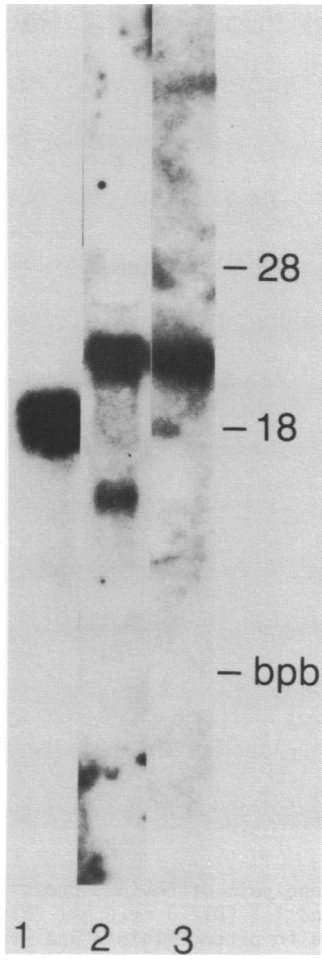


Fig 5. Blot hybridization analysis of total RNA (15 µg) from fetal bovine brain and 18 day postnatal rat brain. Lane 1, bovine RNA probed with myelin basic protein cDNA, pMBP1 (10); lane 2, rat RNA probed with lipophilin cDNA, pLP1; lane3, bovine RNA probed with pLP1. Positions of the 28 and 18S ribosomal bands and bromphenol blue marker are indicated.

used (data not shown). Restriction analysis using 8 other enzymes also showed a simple pattern consisting of one or few hybridizing bands (data not shown). The bovine PLP cDNA was then used as a probe to study the corresponding genomic sequences in other mammalian species. As would be expected from the high degree of protein sequence homology among different species (1) the bovine cDNA probe was found to

hybridize to restriction fragments of rat and human DNA (Figure 4). Two Hind III bands (11.5 kb and 2.5 kb) and two Eco RI bands (6.2 kb and 1.5 kb) were observed in the human whereas one Hind III fragment (6.7 kb) and two Eco RI fragments (6.2 kb and 1.5 kb) were detected in the rat. Based on the size of the cDNA probe and the size and number of hybridizing restricted genomic fragments, it is likely that there is only a single corresponding PLP gene with a small number of introns in all three animal species studied - bovine, rat and human.

Developmental regulation of Lipophilin gene expression

Previous studies from other laboratories have demonstrated a close correlation between myelination and synthesis of PLP (27). Since the time course of myelination is well established in rat, rat brains have been particularly useful for studies on developmental regulation of PLP expression. Rat PLP is first detected in myelin at approximately 10 days after birth and the maximal rate of synthesis is detected at about 24 days. It is thus of interest to study the expression of PLP at the mRNA level.

The bovine cDNA probe pLP1 was first used to hybridize to total rat brain RNA (Figure 5, lane 2). A prominent RNA species of approximately 3.3 kb in size, identical to that of the calf (lane 3), was readily detectable in the rat brain. This species was considered to be the corresponding PLP mRNA. However, an additional minor RNA species of approximately 1.4 kb in size was also detected. The relative intensity of the 1.4 kb band to that of the 3.3 kb varied slightly from experiment to experiment (data not shown).

To obtain some preliminary information about the developmental regulation of PLP expression at the transcriptional level, we used the bovine PLP cDNA probe to estimate the steady state levels of the rat mRNA species during development. Nucleic acid hybridization analysis was performed to compare the amount of lipophilin mRNA in the brains of rats from the first day of birth through the major period of myelination (Figure 6). Fifteen µg of total brain RNA from animals of each age were applied to a nitrocellulose sheet with the aid of to a slot blot apparatus and probed with radioactive pLP1. The relative amount of pLP1-hybridizing material in each slot was then estimated by densitometer scanning of the autoradiogram as shown in Figure 6.

The result of the above analysis shows that PLP-specific mRNA was not present in any detectable quantities in rat brains at birth nor at

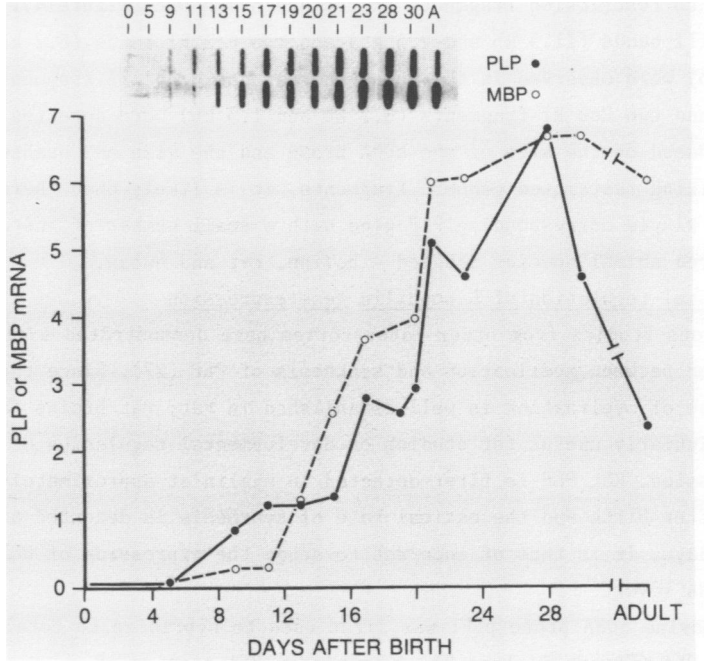


Fig 6. Slot blot hybridization analysis of total RNA (15 μg) from rat brains at various stages of development. The nitrocellulose strip with RNAs indicated was first probed with pLP1, deprobed (25) and reprobed with pMBP1. The relative intensities of each hybridizing slot were measured by scanning of the autoradiogram using a Joyce-Loebel Chromascan 3.

five days after birth. However, by 9 days a significant hybridization signal was clearly noticeable. The amount of hybridizing material continues to increase after this point until day 28 at which time the level of PLP-specific mRNA is approximately seven times of that at day 9. After day 28 the amount of PLP message decreases substantially. In the adult rat the PLP mRNA level is about one third of that at day 28.

These results are therefore in good agreement with the previous observations on the accumulation of PLP during brain development (27), suggesting that PLP synthesis is at least in part regulated at the transcriptional level. To further understand the regulation of gene expression during myelination, we determined the mRNA level for MBP, the other major myelin protein, during the course of myelination. Figure 5 shows that there is a single species of MBP mRNA of 2.1 kb in

size in the rat brain detected by a MBP cDNA probe (pMBP1; gift of Roach et al) as previously described (10). This cDNA was used to follow the level of MBP mRNA in rat brains of various ages.

The PLP cDNA probe was removed from the nitrocelluose filter of the previous experiment and the filter reprobed with radioactive MBP cDNA. The amount of MBP-hybridizing material was then estimated from the autoradiogram by densitometer scanning. Figure 6 shows that MBP messages are first detectable at 9 days, the same as that for PLP. Furthermore, the accumulation of MBP mRNA also follows a very similar time course as that of the PLP message. Significant quantitative difference is found only in the adult brain. A high level of MBP mRNA is present in the adult whereas the amount of PLP messages have markedly reduced. Hence, while the onset of MBP and PLP transcription appears to be coordinately regulated at early stages of development, there is apparently independent control of the expression of these two genes in adult animals. This may be necessary since it has been suggested that MBP plays a role in the compaction of successive myelin layers (28) which continues into early adulthood (29).

DISCUSSION

By screening a cDNA library prepared from the mRNA of calf brain at the time of peak myelination, we have isolated a 930 bp lipophilin cDNA which contains 444 bp of coding sequence corresponding to the C-terminal half of the protein and 486 bp of 3' untranslated sequence. This PLP cDNA detects a single mRNA species of approximately 3.3 kb in the calf brain. Therefore, the present cDNA clone for PLP, pLP1, is clearly much less than full length. In addition, since only 828 nucleotides are required to encode a PLP polypeptide of 276 amino acid residues, there must be a large portion of untranslated sequence in the PLP mRNA. It is also expected that the primary translation product of PLP contains a leader peptide since the protein is synthesized on membrane-bound polysomes (30). Preliminary analysis of additional cDNA clones isolated from a second cDNA library has suggested the presence of as much as 2 kb of 3' untranslated sequence (data not shown). In this regard, it is of interest to note that we have detected in the rat brain an additional PLP-specific RNA species, the size of which is approximately 1.4 kb, about half of that of the full-size mRNA. Since the relative level of this 1.4 kb RNA species varies in different

samples derived from animals of the same age (data not shown), it is possible that it represents a partial degradation product of the full-size transcript. However, it is not known at this moment whether this smaller RNA species is still capable of directing PLP synthesis.

The availability of cDNA clones for both PLP (this study) and MBP (10-12) has provided an opportunity for studying the developmental regulation of the genes that encode the two major membrane proteins in myelin. Our results show that the onset of transcription for both genes appears to be coordinately regulated during development. Both messages are first detectible at 9 days after birth and then continue to accumulate until approximately 30 days; after this time, however, the amount of PLP message declines more rapidly than that of MBP mRNA. This may reflect either a differential reduction in the transcription rate or in the stability of the messages for the two proteins. Interestingly, this difference in mRNA levels correlates well with reports that MBP turns over more rapidly than PLP (31,32).

REFERENCES

1. Lees, M.B. and Brostoff, S.W. (1984) in Myelin, 2nd Edition, ed. P. Morell (Plenum Press, New York), Chapter 6, pp 197-221.
2. Boggs,J.M., Moscarello, M.A. and Papahadjopoulos, D. (1982) in Lipid-Protein Interactions. eds. P. Jost and O.H. Griffith, (Academic Press, New York).
3. Eylar, E.H. (1972) in Functional and Structural Proteins of the Nervous System, eds. A. Davison, P. Mandel and I. Morgan (Plenum Press, New York) pp 215-240.
4. Lees, M.B., Chao, B., Lin, L.H., Samiullah, M. and Laursen, R. (1983) Arch. Biochem. Biophys. 226:643-656.
5. Jolles,J.,Nussbaum, M.-L. and Jolles, P. (1983) Biochim. Biophys. Acta 742:33-38.
6. Laursen, R.L., Samiullah, M. and Lees,M. (1983) FEBS Lett 161: 71-74.
7. Lin, L-F.H. and Lees, M.B. (1982) Proc. Natl. Acad. Sci. U.S.A. 79:941-945.
8. Boggs, J.M. and Moscarello, M.A. (1978) Biochim. Biophys. Acta 515:1-21.
9. Raine, C.E. (1984) in: Myelin, 2nd Edition, ed. P. Morell (Plenum Press, N.Y.), Chapter 8, pp 259-310.
10. Roach, A., Boylan, K., Horvath, S., Prusiner, S.B. and Hood, L.E. (1983) Cell 34:799-806.
11. Zeller, N.K., Sprague, J.A., Lazzarini, R.A., Yu, Y-T. and Campagnoni, A.T. (1983) Trans. Am. Soc. Neurochem. 14:255.
12. Amorese, P. A., Ellis, S. B., Harphold, M. M. and Linthicum, D. S. (1983) Trans. Am. Soc. Neurochem. 14:165.
13. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18:5294-5299.
14. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. U.S.A. 69:1408-1412.

15. Huynh, T.V., Young, R.A. and Davis, R.W. (1984) in DNA Cloning: A Practical Approach. ed. D. Glover (IRL Press, Oxford).
16. Davis, R. W., Botstein, D. and Roth, J. R. (1980) Advanced Bacterial Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
17. Modrich, P. and Zabel, D. (1976) J. Biol. Chem. 251:5866-5874.
18. Beaucage, S.L. and Carruthers, M.H. (1981) Tet. Lett. 22:1859-1862.
19. Benton, W.D. and Davis, R.W. (1977) Science 196:180-182.
20. Vieira, J. and Messing, J. (1982) Gene 19:259-268.
21. Maxam, A.M. and Gilbert, W. (1980) Meth. Enzymol. 65:499-560.
22. Kaplan, B.B., Schacter, B., Osterburg, H.H., de Vellis, J.C. and Finch, C.E. (1978) Biochemistry 17:5516-5524.
23. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
24. Southern, E.M. (1975) J. Mol. Biol. 98:503-517.
25. Thomas, P.S. (1983) in Meth. Enzymol. eds. R. Wu, L. Grossman and K. Moldave (Academic Press, New York) pp 255-266.
26. Stoffel, W., Hillen, H., Schroder, W. and Deutzmann, R. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363:1297-1407.
27. Benjamins, J. A. and Smith, M. E. (1984) in Myelin. 2nd Edition, ed. P. Morell (Plenum Press, New York), Chapter 7, pp 225-258.
28. Brady, G. W., Murthy, N. S., Fein, D. B., Wood, D. D. and Moscarello, M. A. (1981) Biophys. J. 34: 345-350.
29. Chia, L. S., Thompson, J. E. and Moscarello, M A.(1983) FEBS Letters 157: 155-158.
30. Colman, D.R., Kreibich, G., Frey, A.B. and Sabatini, D.D. (1982) J. Cell Biol. 95:598-608.
31. Sabri, M.I., Bone, A. H. and Davison, A.N. (1974) Biochem J. 142:499-507.
32. Lajtha, A., Toth, J., Fujimoto, K. and Agrawal, H. C. (1977) Biochem. J.164:323-329.