Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 18–22, January 1984
Biochemistry

Characterization of mouse myelin basic protein messenger RNAs with a myelin basic protein cDNA clone

(multiple mRNAs/RNA blot analysis/cDNA cloning)

NANCY K. ZELLER*, MARKUS J. HUNKELER†, ANTHONY T. CAMPAGNONI†, JUDY SPRAGUE*, AND ROBERT A. LAZZARINI*

*Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20205; and †Chemistry Department, University of Maryland, College Park, MD 20740

Communicated by D. Carleton Gajdusek, August 29, 1983

ABSTRACT **Using a family of synthetic tetradecamer oligonucleotides as a primer for cDNA synthesis and a second family of tetradecamers as a hybridization probe, we have prepared and isolated a cDNA clone of mouse myelin basic protein (MBP). The clone, pNZ111, corresponds to the region of the mRNA that codes for an amino acid sequence present in all four major forms of MBP. The relative abundance of MBP mRNA, estimated by dot blot hybridization, increased with the age of the mouse to a maximum at 18 days, then decreased to about one-fourth of that amount at later ages. Mouse MBP mRNAs, selected by their ability to hybridize to the clone, translate into the four forms of myelin basic protein. In RNA blot analyses, pNZ111 hybridized to multiple species of mouse mRNA. The predominant hybridization is to a broad band of RNAs ranging in length from 2,350 to 2,100 bases. These mRNA species are extremely long, considering that the largest MBP could be encoded by approximately 600 bases. In addition to these, there are also minor bands that hybridize with pNZ111, including a band of 4,100 bases and smaller ones of 1,900, 1,500, and 1,200 bases.**

Myelin is a specialized membrane that ensheaths nerve cell axons and facilitates the saltatory conduction of nerve impulses (see ref. 1 for a general review). It is produced by oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system in a tightly orchestrated process presumably stimulated by neuronal-glial signals (2–5). Large amounts of the membrane are produced relative to the size of the myelin-forming cell. The process involves the synthesis of myelin-specific proteins followed by their integration into the growing membrane. During early myelinogenesis, membranes are formed that are loosely whorled around the axon. As myelin maturation proceeds, compaction of these membranes occurs to form the tight multilamellar structure characteristic of myelin.

The myelin basic protein (MBP) constitutes about 33% of the protein of the myelin sheath in the central nervous system and 1–10% in the peripheral (6, 7). In most species, the predominant form of the MBP has a molecular mass of 18.5 kilodaltons (kDa), and the primary sequence of this protein is well conserved in animals as distantly related as the chicken, human, and rat (8–10). Two major forms of MBP predominate in rats and mice: one of these corresponds to the single major protein found in other species (18.5 kDa) and the other has a molecular mass of 14 kDa. In rats, the 14-kDa MBP is identical in sequence to the 18.5-kDa MBP, except for an internal deletion of residues 118–158 [numbered according to Martenson (11)] near the carboxyl terminus of the protein (10, 12). Two quantitatively minor forms of MBP have been found in rats and mice—a 21.5-kDa protein that is presumably identical to the 18.5-kDa MBP, except for the inclusion of a 25–30 amino acid sequence in the NH_2-terminal half of the molecule and a 17-kDa MBP that bears the same relationship to the 14-kDa MBP (13).

Myelination occurs postnatally in mouse brain beginning 8–10 days postpartum and continuing actively for 7–10 wk, with the maximal rate of myelin deposition occurring at about 18 days (4). Maximal synthesis of the 18.5-kDa and 14-kDa MBPs occurs at 18 days *in vivo* and coincides closely with the peak of myelin synthesis (14). During myelin maturation in the mouse, the proportions of the four MBPs in the membrane change (15–17) and this may be due to alterations in the relative rates of synthesis of the four proteins with age. With maturation, the proportion of the two minor MBPs (i.e., the 21.5 kDa and 17 kDa in myelin falls relative to the 18.5-kDa proteins and the 14-kDa/18.5-kDa MBP ratio increases dramatically. This latter change has been correlated with the relative rate of synthesis of these two proteins *in vivo* (14).

Recently, Carson *et al.* (18) have identified trace amounts of larger forms of MBP that appear during the early stages of myelination. Identification of these larger forms has depended on immunoreagents and the relationships of the amino acid sequences to the major MBPs have, as yet, not been determined.

We have recently initiated a study of the component molecular events that culminate in the formation of compact myelin. The initial focus of this study is the interrelationships of the four MBPs and the developmental program that controls their synthesis and accumulation into myelin. In the present communication, we report the isolation of a cDNA clone of MBP mRNA by using synthesized families of tetradecamer oligodeoxyribonucleotides as primers for cDNA synthesis and as probes to identify the desired clone. The cDNA clone specifies a sequence found in the four predominant MBPs and has been used to analyze the complexity, size, and cellular distribution of MBP mRNAs.

MATERIALS AND METHODS

Materials. The two families of tetradecamer oliogonucleotides, MBP 82 and MBP 89, were obtained from P-L Biochemicals. Restriction enzymes and polynucleotide kinase were obtained from either Bethesda Research Laboratories or New England BioLabs. The Klenow fragment of *Escherichia coli* DNA polymerase I was obtained from Boehringer Mannheim. S1 nuclease was obtained from Miles. Avian myeloblastosis virus reverse transcriptase was obtained from Life Sciences (St. Petersburg, FL). Restriction endonuclease digestions were carried out under the conditions suggested by the producer or by Davis *et al.* (19).

mRNA Preparation. Total RNA was prepared by directly homogenizing either fresh or liquid nitrogen-frozen brain in a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MBP, myelin basic protein; kDa, kilodalton(s).

1:1 mixture of water-saturated phenol and 0.1 M Tris·HCl, pH 9.0/1 mM EDTA/0.5% $NaDodSO_4$ (20) for 20 sec with a high-speed electric tissue homogenizer. After serial phenol extractions, the RNA was ethanol precipitated and separated from contaminating DNA by sedimentation through cesium chloride. Polyadenylylated RNA was separated from total RNA by passage over columns of oligo(dT)-cellulose (P-L Biochemicals) (21).

In some experiments, mRNA was isolated from a purified polysomal fraction of mouse brain homogenate by using the method previously described (22). This postnuclear polysomal polyadenylylated RNA was used in all the hybridization experiments.

Isolation and Screening of cDNA Clones. cDNA reverse transcripts of polyadenylylated RNA from 15-day-old mouse brain were prepared by using the family of tetradecamers, MBP 89, as primers at primer/polyadenylylated RNA ratio of 200 pmol/μg. The reaction conditions were essentially those previously described by Sprague *et al.* (23) but with the inclusion of 800 units of RNasin per ml to inhibit ribonuclease. Double-stranded cDNA was synthesized under the same general conditions but without primer or RNA. Double-stranded cDNA was trimmed with S1 nuclease and tailed with oligo(dC) acid as described (24, 25). The dC-tailed cDNA was hybridized to pBR322 that had been linearized with endonuclease *Pst* I and tailed with deoxyguanosine residues (New England Nuclear). The hybridized mixture was used to transform *E. coli* HB101 that had been made competent as described by Kushner (26). Transformed cells were plated on LB medium containing 10 μg of tetracycline per ml. Individual colonies were picked in duplicate onto nitrocellulose filters supported on tetracycline-containing LB agar and allowed to grow for 8 hr at 37°C. Nitrocellulose filters were then transferred to LB agar containing chloramphenicol at 170 μg/ml and incubated overnight to amplify the plasmids. Filters were prepared for hybridization as described by Gergen *et al.* (27). The filters were incubated for 3–4 hr at 42°C, and the colony debris was carefully removed before hybridization with ^{32}P-labeled oligonucleotides at 37°C (28, 29). The conditions for hybridization and the removal of unhybridized probe were as described by Wallace *et al.* (28, 29).

Nucleotide Sequence Determination. Plasmid DNA obtained from recombinant clones was digested with *Pst* I and the 3′ termini of both the plasmid and insert DNA strands were labeled with cordycepin 5′-[^{32}P]triphosphate by the method of Tu and Cohen (30). Labeled DNAs were cut with a second restriction endonuclease, and the uniquely labeled fragments were separated by polyacrylamide gel electrophoresis. The labeled fragments were recovered from the gel and their sequences were determined by the method of Maxam and Gilbert (31).

Hybrid Selection of MBP mRNA. Mouse brain polyadenylylated RNA was hybridized to linearized pNZ111 DNA immobilized on nitrocellulose as described by Patterson and Roberts (32). Unbound RNA was removed by washing the nitrocellulose two times with 2× NaCl/Cit/2× NaCl/Cit = 3 M NaCl/0.3 M Na citrate) at 25°C and four times at 52°C with 0.1× NaCl/Cit/2 mM EDTA. The hybridized RNA was eluted from the nitrocellulose-bound pNZ111 by boiling for 2 min in 10 mM Tris·HCl/2 mM EDTA, pH 7.4, as described by Patterson and Roberts (32) and translated in a reticulocyte lysate system as described by Yu and Campagnoni (22). Portions of the translation product were immunoprecipitated with anti-MBP antibody (33). Both unfractionated and immunoprecipitated translation products were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis (34). The radiolabeled proteins were visualized by fluorography.

Hybridization of Immobilized mRNA (RNA Blot Analyses). Five-microgram samples of polyadenylylated RNA were separated by electrophoresis in horizontal 1% agarose/3% formaldehyde slab gels (35). After electrophoresis, the RNA was transferred to a nylon membrane ('Zeta-Probe'; Bio-Rad) by electrophoresis. The nucleic acids were fixed to the membrane by drying at reduced pressure for 2 hr at 80°C. The membranes were prehybridized for 4 hr and hybridized overnight at 42°C under the conditions suggested by Bio-Rad. The pNZ111 clone was cut and labeled with [α-^{32}P]-dNTPs by filling in the 3′ recessed ends, and then purified on a gel. Approximately 2×10^6 cpm of purified insert was used to hybridize with each blot.

Dot hybridizations with polyadenylylated RNA were done on nitrocellulose membranes as described by Thomas (36) using 1×10^6 cpm of ^{32}P-labeled NZ111. Serial 1:2 dilutions were made of the samples starting with 1 μg.

RESULTS

Preparation and Selection of the MBP cDNA Clone. Our cloning strategy was an adaptation of one described by Agarwal and colleagues (37) and took advantage of the known amino acid sequences of MBPs from several species. Using the MBP amino acid sequence as a guide, we synthesized two families of tetradecamer oligonucleotides complementary to all possible sequences in two specific regions of the MBP mRNA. These regions, corresponding to amino acids 82–86 and 89–92 of MBP, had minimal degeneracy in the amino acid code, thereby reducing the number of oligonucleotide sequences necessary to cover all possibilities to 8 and 16 sequences, respectively.

Initial efforts to obtain a MBP clone used only the MBP-82 oligonucleotide as a primer for cDNA synthesis and as a probe to identify recombinant clones. The sequences of 35 clones that hybridized with MBP-82 were determined, but none of the sequences adjacent to the primer region corresponded to those expected of authentic MBP cDNA. To overcome this lack of specificity and to reduce the number of false positives, we used two families of oligonucleotides, one as the primer for cDNA synthesis and a second as a hybridization probe. As shown in Fig. 1, the primer and probe oligonucleotides corresponded to adjacent regions of the MBP mRNA, so that even very small MBP cDNA clones would be identified. Six hundred recombinant clones, prepared by using MBP-89 as a primer, were tested for their ability to hybridize with ^{32}P-labeled MBP-89 and ^{32}P-labeled MBP-82 tetradecamer families. Two clones hybridized with both probes. Dot hybridization of DNA isolated from the two recombinants confirmed that they contained sequences hybridizing to MBP-82 and MBP-89, although the A+T-rich MBP 89 gave a weaker signal. Restriction endonuclease analysis revealed that one of the clones had lost an expected *Pst* I site and it was not further studied. The sequence of the other clone, pNZ111, was determined and shown to consist of 94 nucleotides coding for the region of MBP spanning amino acids 60–91, including the region analyzed by Burgess *et al.* (38). The position of the sequence within the 18.5-kDa MBP is diagrammed in Fig. 1. A sequence of one MBP-89 and one MBP-82 oligonucleotide is present in the clone, indicating that MBP-89 did indeed act as a specific primer for cDNA synthesis using MBP-specific mRNA as a template.

pNZ111 Hybrid Selects mRNAs Coding for Four Myelin Basic Proteins. MBP mRNAs were purified by hybrid selection to pNZ111 denatured DNA that had been immobilized on nitrocellulose. Extraneous mRNAs were removed by extensive washing with 0.1× NaCl/Cit at 52°C. Specifically bound mRNAs were eluted and used to program a reticulocyte lysate protein synthesis reaction. The results of two such experiments are shown in Fig. 2. pNZ111 selected mRNAs coding for all four myelin basic protein species. Note that the principal products of synthesis in the total trichloroacetic acid precipitate of the hybrid-selected mRNA-programmed

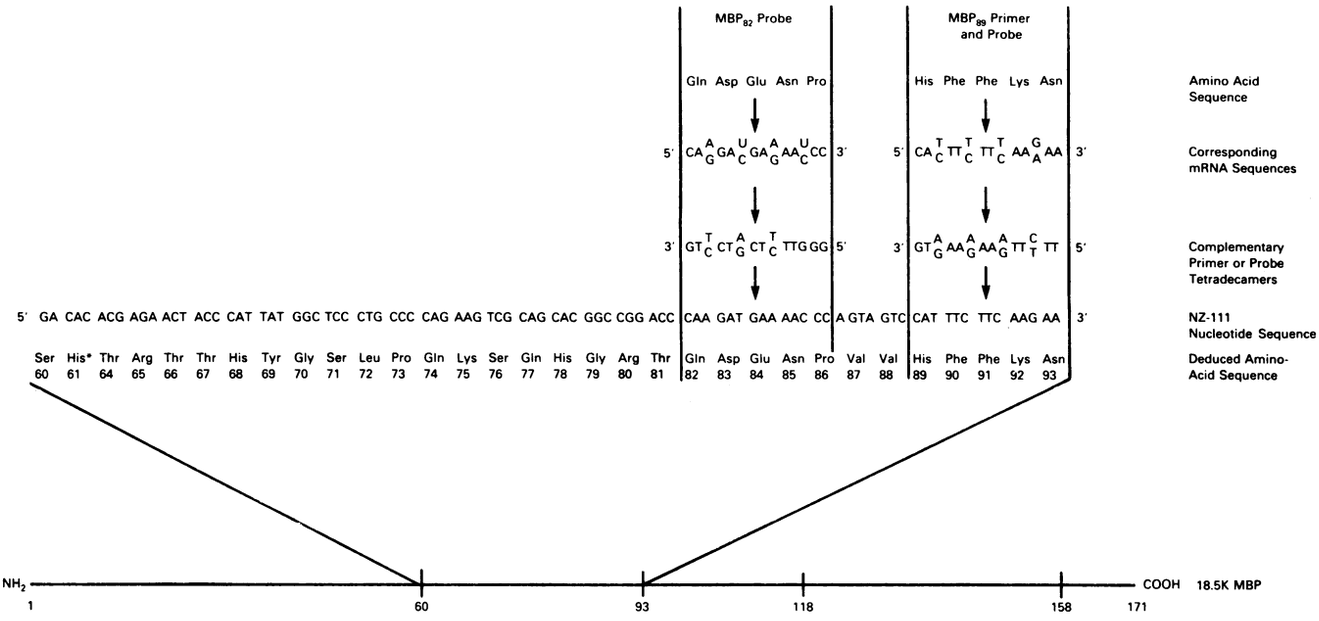

FIG. 1. Schematic representation of the MBP amino acid sequence, the pNZ111 nucleotide sequence, and the probes and primers used to prepare the clone. Stacked double letters in the primer and probe sequences denote alternative bases for those positions due to the degeneracy in the amino acid code. The number of sequences present in MBP-82 was reduced by allowing for a possible G·T base pair in the third position from the 5' end of the probe.
*Amino acid position numbers 62 and 63 have been deleted in keeping with the numbering system of Martenson (11).

incubations were the four MBPs. These were identified not only by their comigration with ^{125}I-labeled authentic MBP but also by immunoprecipitation with affinity-purified antibody. In addition to the four MBPs, two minor products that crossreacted with anti-MBP were synthesized from pNZ111-selected mRNA—one slightly larger than the 18.5-kDa MBP and one smaller than the 17-kDa protein. It is not clear whether these are degradation products of larger MBPs or whether they are other minor forms of the MBP such as those described by Greenfield *et al.* (39).

Developmental Expression of MBP mRNA. The concentration of MBP-specific mRNA was estimated during early postnatal brain development when the period of most active myelination occurs in the mouse. Dot blot hybridization of brain polyadenylylated RNA with radiolabeled pNZ111 was used to identify both functional and nonfunctional mRNA. Equal amounts of brain mRNA extracted from postnuclear supernatants of mice of different ages were applied to nitrocellulose in serial 1:2 dilutions. As shown in Fig. 3, the labeled recombinant DNA hybridized with mRNA from mouse brain but not with that from mouse liver. The amount of mRNA per μg of total mRNA that hybridizes with pNZ111 increases steadily from 4 days after birth, when it is first detected, to 18 days after birth. The total MBP mRNA content decreases thereafter, but some MBP mRNA was still present in 5-month-old mouse brain.

Multiple Species of MBP Specific RNAs. Recently, Yu and Campagnoni (22) and Colman *et al.* (40) have presented evidence indicating that the multiple forms of MBP are independently synthesized and are not related to one another by post-translational modification and proteolysis. These re-

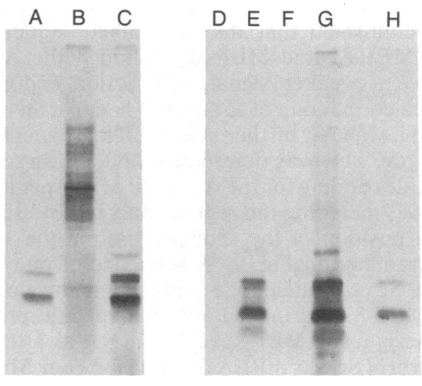

FIG. 2. *In vitro* translation of pNZ111-selected brain mRNAs. A reticulocyte lysate system containing [^{35}S]methionine was programmed with either hybrid-selected or total brain mRNA. The products were either directly fractionated by NaDodSO$_4$ gel electrophoresis or immunoprecipitated and then electrophoresed. Lanes: A and H, ^{125}I-labeled MBP standards; B, unselected brain mRNA; C and G, hybrid-selected mRNA; E, hybrid-selected immunoprecipitated mRNA; D and F, products of a reticulocyte lysate system without added mRNA were immunoprecipitated and then electrophoresed (lane D) or were directly electrophoresed (lane F).

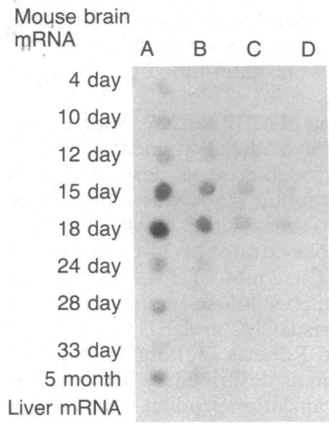

FIG. 3. Estimation of MBP mRNA by dot blot hybridization to ^{32}P-labeled pNZ111. Serial 1:2 dilutions of polyadenylylated RNA isolated from brain and liver of mice of different ages were spotted onto nitrocellulose membranes and hybridized with ^{32}P-pNZ111. Lanes: A–D, 500, 250, 125, and 62.5 ng of RNA, respectively.

sults predict multiple mRNA species for MBP. This possibility was investigated by electrophoretically fractionating mouse brain polyadenylylated mRNA, transferring the RNAs to membranes, and then hybridizing the membrane-bound mRNA with ^{32}P-labeled pNZ111. As shown in Fig. 4, the most intense band of hybridization to ^{32}P-labeled pNZ111 corresponded to mRNAs of 2,100–2,350 nucleotides. The width of this band strongly suggests that it contains several species of mRNA. In addition, there are other species of mRNA that hybridized to the probe. Their estimated lengths are 4,100, 1,900, 1,500, and 1,200 bases. The possibility that these RNAs are only distantly related to the myelin basic protein sequence and do not form perfect duplexes with the probe was investigated by increasing the stringency of the post-hybridization washes to 0.2× NaCl/Cit at 65°C. The fact that the hybridization pattern remained the same even at this high stringency (unpublished observations) strongly indicates that the mRNA species are closely related to the sequence of the probe.

Guinea pig and chicken brain mRNA also hybridized with pNZ111, as would be expected because MBP is a highly conserved protein. In each case, several mRNA species, ranging in size from 1,900 to 4,100 bases, were identified by hybridization. These results indicate that the multiplicity of MBP mRNAs is widespread and not limited to those animal species having four major MBPs because guinea pig possesses only the 18.5-kDa and, possibly, the 21.5-kDa form(s) (12, 13).

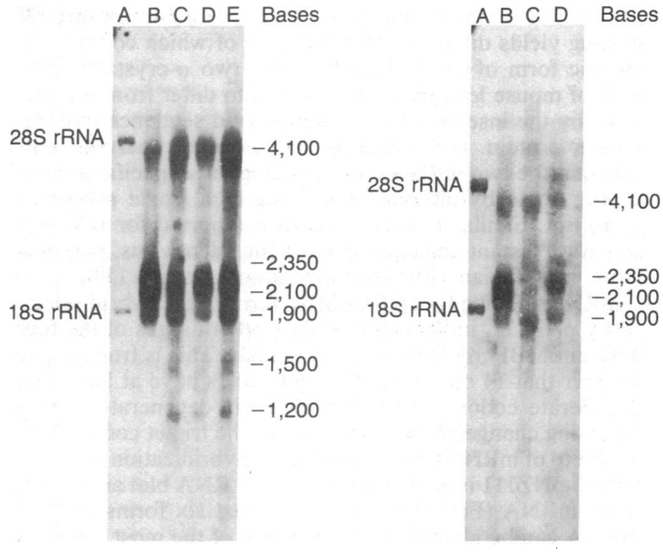

FIG. 4. RNA blot analysis of polyadenylylated RNA hybridizing to pNZ111. *(Left)* Polyadenylylated RNAs isolated from brains of mice of various ages were fractionated by electrophoresis in formaldehyde/agarose gels. Samples were 5 μg of 14-day-old (lane B), 3 μg of 18-day-old (lane C), 6.3 μg of 24-day-old (lane D), and 9.4 μg of 33-day-old (lane E) mouse brain polyadenylylated RNA. 28S [^{14}C]-rRNA (5,000 bases) and 18S [^{14}C]rRNA (1,950 bases) were used as size standards. *(Right)* Comparison of rRNA standards (lane A), and 5 μg of polyadenylylated RNAs from 18-day-old mouse (lane B), 18-day fetal chicken (lane B; ref. 41), and 56-day fetal guinea pig (lane D; ref. 42).

DISCUSSION

A variety of cloning strategies with synthetic oligonucleotides have been developed and have proven useful. Most frequently these have involved direct use of a synthetic oligonucleotide as a probe or for the preparation of a larger, more-specific hybridization probe by primer extension on a mRNA template. Both types of probes are then used to identify specific colonies from oligo(dT)-primed cDNA libraries. We have taken a different tack to arrive at the specificity necessary for the selection of a desired clone. One set of oligomers was used as a primer for cDNA cloning and a second set was used as a hybridization probe to identify the colony. The desired colony was selected from those that hybridized with both the primer oligomer and the probe oligomer. The authenticity of the clone was ultimately shown by determining its sequence. Our earlier attempts to use one oligomer family as both primer and probe were unsuccessful because of lack of specificity. In those experiments, only 35 out of 600 colonies were the result of specific priming on the mRNA templates. Moreover, sequence analysis of the 35 showed that, although all contained the correct primer sequences, none were MBP cDNA. When the procedure was modified to include two different oligomers, two out of 600 clones were obtained that specifically hybridized to both. One of these, pNZ111, was shown to be a MBP cDNA. These results support the idea that the use of multiple oligomers greatly increases the ease and likelihood of obtaining the desired cDNA clone (43, 44).

pNZ111 is a small DNA clone, containing 92 base pairs of MBP-specific information. Its small size is likely attributed to a number of factors. First, we have used two different oligomers that correspond to regions of the MBP mRNA separated by only 132 nucleotides. Thus, clones containing small cDNAs, which are likely to be abundant, are identified by our method. Second, specific precautions were not taken to reduce secondary structure in the template RNA. Other investigators have found that the ability of synthetic oligonucleotides to act as primers for DNA synthesis varies greatly, suggesting that RNA template secondary structure may affect the length of the primer extended DNA (37, 43, 45, 46).

The shortness of the cDNA in pNZ111 has not diminished its usefulness in analyzing MBP mRNAs. pNZ111 was used to detect developmental changes in MBP gene expression, as shown in Fig. 3. MBP mRNAs could be detected in 4-day-old mouse brain samples, and the amount of MBP-specific mRNA increased 8-fold by 18 days of age. Thereafter, the MBP mRNA content diminished but it was still detectable in 5-month-old brain mRNA. These results correlate well with the finding that the maximal rate of MBP synthesis occurs at 18 days *in vivo* (14) and that the amount of functional (translatable) MBP mRNA peaks at the same age (18). They are also in accord with immunohistochemical studies of Sternberger *et al.* (47), showing that MBP is present in certain regions of the central nervous system at birth. MBP accumulates in the cytoplasm of cells in these regions until active myelination begins between the 7th and the 12th day of life (47). Myelin continues to accumulate at a slow rate throughout the life of the animal (4, 5), suggesting that MBP mRNA is present in the brain albeit at a diminished amount throughout the life of the animal.

Hybrid selection with immobilized pNZ111 yielded mRNAs that were translated in a reticulocyte lysate system into the four predominant forms of MBP, 21.5, 18.5, 17, and 14 kDa (Fig. 3). The larger forms of MBP are believed to differ from the 14-kDa form by the insertion of a 40-amino acid sequence near the carboxyl terminus or a 25- to 35-amino acid sequence near the amino terminus or both (13). However, although the sequences of the 18.5-kDa and the 14-kDa MBP have been determined, the structures of the other two can only be considered tentative. The origin of the multiplicity of the MBP forms is still obscure. Experiments performed in several laboratories argue against a precursor–product relationship among the various MBPs (22, 40). Two other possible explanations are obvious. The first is that there may exist a MBP gene family—multiple genes, each of which codes for a specific MBP, in analogy to the genes specifying many abundant proteins (48). The second possi-

bility is that a single gene with alternative modes of mRNA splicing yields different mRNAs, each of which codes for a specific form of MBP. Recently, the two α-crystallin proteins of mouse lens have been shown to differ from one another by the insertion of a 22-amino acid sequence into the smaller protein. Hybridization and sequence analyses indicate that the two mRNAs, each coding for a specific α-crystalline, arose by alternative splicing of a single precursor transcript. Similar results have been reported for IgM (49) and more distant analogies exist in the adenovirus, polyoma virus, and Simian virus 40 transcription systems (50).

mRNAs coding for all four MBP forms were hybrid selected by pNZ111, indicating that the codon usages of the four different MBP mRNAs are very similar. This is true despite the fact that 14 out of the 31 amino acids have at least four degenerate codons and that 6 have six degenerate codons involving changes in two positions of the triplet codon. That a variety of mRNAs were selected by hybridization to immobilized pNZ111 is also indicated by the RNA blot analysis of brain mRNA (Fig. 4), in which at least six forms of MBP mRNA can be identified. The breadth of the most intensely hybridizing RNA band suggests that the major 2,100- to 2,350-base-pair material may contain multiple species of RNA. The fact that guinea pig and chicken brain MBP mRNAs also contain multiple species that hybridized to pNZ111 underscores the conclusion that the multiplicity of MBP mRNAs is a common feature and not restricted to animal species possessing the four major MBPs. The sizes of these messages are very large considering that the 21.5-kDa MBP could be coded for by a sequence of about 600 nucleotides. Thus, these RNAs may consist of a sizeable amount of nontranslatable sequence. In addition to the broad band, there is a mRNA of 4,100 nucleotides and some smaller minor bands, possibly degradation products, that hybridized with pNZ111. A definitive correlation between the mRNA species and the proteins encoded by them must await systematic testing of the separated mRNAs in a cell-free translation system.

We wish to express our gratitude to Yie-Tah Yu for her assistance in the early part of these studies. This work was supported in part by Public Health Service Grant NS15469 to A.T.C.

1. Morell, P., ed. (1977) *Myelin* (Plenum, New York).
2. Bunge, R. P. (1970) in *The Neurosciences: Second Study Program*, ed. Schmitt, F. O. (Rockefeller Univ. Press, New York), pp. 782–797.
3. Peters, A. & Vaughn, J. E. (1970) in *Myelination*, eds. Davison, A. N. & Peters, A. (Thomas, Springfield, IL), pp. 3–79.
4. Norton, W. T. (1977) in *Myelin*, ed. Morell, P. (Plenum, New York), pp. 161–199.
5. Morell, P., Greenfield, S., Constantino-Cerrarini, E. & Wisniewski, H. (1972) *J. Neurochem.* **19,** 2545–2554.
6. Sabri, M. I., Bone, A. H. & Davison, A. N. (1974) *Biochem. J.* **142,** 499–507.
7. Benjamins, J. A. & Morell, P. (1978) *Neurochem. Res.* **3,** 137–174.
8. Mendz, G. L., Moore, W. J. & Martenson, R. E. (1983) *Biochim. Biophys. Acta* **742,** 215–223.
9. Carnegie, P. R. (1971) *Biochem. J.* **123,** 57–67.
10. Dunkley, P. R. & Carnegie, P. R. (1974) *Biochem. J.* **141,** 243–255.
11. Martenson, R. E. (1981) *J. Neurochem.* **36,** 1543–1560.
12. Martenson, R. E., Deibler, G. E. & Kies, M. W. (1971) *J. Neurochem.* **18,** 2427–2433.
13. Barbarese, E., Braun, P. E. & Carson, J. H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3360–3364.
14. Campagnoni, C. W., Carey, G. D. & Campagnoni, A. T. (1978) *Arch. Biochem. Biophys.* **190,** 118–125.
15. Carey, G. D. & Campagnoni, A. T. (1979) *J. Neurochem.* **33,** 81–87.
16. Magno-Sumbilla, C. & Campagnoni, A. T. (1977) *Brain Res.* **126,** 131–148.
17. Barbarese, E., Carson, J. H. & Braun, P. E. (1978) *J. Neurochem.* **31,** 779–782.
18. Carson, J. H., Nielson, M. L. & Barbarese, E. (1983) *Dev. Biol.* **96,** 485–492.
19. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Edmonds, M. & Caramela, M. G. (1969) *J. Biol. Chem.* **244,** 1314–1324.
21. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
22. Yu, Y.-T. & Campagnoni, A. T. (1982) *J. Neurochem.* **39,** 1559–1568.
23. Sprague, J., Condra, J. H., Arnheiter, H. & Lazzarini, R. A. (1983) *J. Virol.* **45,** 773–781.
24. Wahli, W., Ryffel, G. U., Wyler, T., Jaggi, R. B., Weber, R. & David, I. B. (1978) *Dev. Biol.* **67,** 371–383.
25. Deng, G.-R. & Wu, R. (1981) *Nucleic Acids Res.* **9,** 4173–4188.
26. Kushner, S. R. (1978) in *Genetic Engineering*, eds. Boyer, H. W. & Nicosia, S. (Elsevier/North-Holland, Amsterdam), pp. 17–23.
27. Gergen, J. P., Stern, R. H. & Wensink, P. C. (1979) *Nucleic Acids Res.* **7,** 2115–2136.
28. Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J., Hirose, T. & Itakura, K. (1979) *Nucleic Acids Res.* **6,** 3543–3557.
29. Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H. & Itakura, K. (1981) *Nucleic Acids Res.* **9,** 879–894.
30. Tu, C.-P. D. & Cohen, S. N. (1980) *Gene* **10,** 177–183.
31. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
32. Patterson, B. M. & Roberts, B. E. (1981) in *Gene Amplification and Analysis*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, Amsterdam), Vol. 2, pp. 417–437.
33. Reidl, L. S., Campagnoni, C. W. & Campagnoni, A. T. (1981) *J. Neurochem.* **37,** 373–380.
34. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
35. Derman, E., Krauter K., Walling, L., Weinberger, C., Ray, M. & Darnell, J. E., Jr. (1981) *Cell* **23,** 731–739.
36. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
37. Noyes, B. E., Mevarech, M., Stein, R. & Agarwal, K. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1770–1774.
38. Burgess, K. T., Bernard, C. C. A. & Carnegie, P. R. (1978) *Adv. Exp. Med. Biol.* **100,** 303–305.
39. Greenfield, S., Gantt, G., Weise, M., Brostoff, S. W. & Hogan, E. L. (1983) *Trans. Am. Soc. Neurochem.* **14,** 128 (abstr.)
40. Colman, D. R., Kreibich, G., Frey, A. B. & Sabatini, D. D. (1982) *J. Cell Biol.* **95,** 598–608.
41. Kurihara, T. & Tsukada, Y. (1968) *J. Neurochem.* **15,** 827–832.
42. Dobbing, J. & Sands, J. (1970) *Brain Res.* **17,** 115–123.
43. Edlund, T., Ny, T., Ranby, M., Heden, L., Palm, G., Holmgren, E. & Josephson, S. (1982) *Proc. Natl. Acad. Sci. USA* **80,** 349–352.
44. Singer-Sam, J., Simmer, R. L., Keith, D. H., Shively, L., Teplitz, M., Itakura, K., Gartler, S. & Riggs, A. D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 802–806.
45. Chan, S. J., Noyes, B. E., Agarwal, K. L. & Steiner, D. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5036–5040.
46. Das, H. K., Biro, P. A., Cohen, S. N., Erlich, H. A., von Gabain, A., Lawrance, S. K., Lemaux, P. G., McDevitt, H. O., Peterlin, B. M., Schulz, M., Sood, A. K. & Weissman, S. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1531–1535.
47. Sternberger, N. H., Itoyama, Y., Kies, M. W. & Webster, H. deF. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2521–2524.
48. Lewin, B., ed. (1983) *Genes* (Wiley, New York).
49. Liu, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. (1980) *Science* **209,** 1348–1353.
50. Tooze, J., ed. (1981) *DNA Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).