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Cloning and Characterization of a cDNA Encoding a Novel Fatty Acid Binding Protein from Rat Brain

*Ellen Bennett, †Kaye L. Stenvers, †‡§P. Kay Lund, and *†§||Brian Popko

**Brain and Development Research Center, †Curriculum in Neurobiology, Departments of ‡Physiology and ||Biochemistry & Biophysics, and §Program in Molecular Biology and Biotechnology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.*

Abstract: We have adopted a polymerase chain reaction approach to identify and clone a cDNA that contains the complete coding sequence of a novel fatty acid binding protein (FABP) from a rat brain λgt10 library. Sequencing of the brain FABP (B-FABP) cDNA revealed an open reading frame coding for a protein with 132 amino acids and a predicted size of ~15,000 Da. This putative protein shares extensive sequence homology with other members of the FABP family. Northern blot analysis using the B-FABP cDNA as a probe established the presence of an abundant mRNA ~0.8 kb long in rat brain and in the MOCH-1 oligodendrocyte cell line. This transcript was also present in rat liver but not in other tissues examined. A developmental profile of this mRNA in rat brain demonstrated detectable expression in 15-day-old embryos with levels peaking in 1-day postnatal neonates and declining thereafter, reaching a low steady-state level at 3 weeks of age. In situ hybridization histochemistry revealed B-FABP mRNA in various brain regions, with the highest levels in fiber tracts. The B-FABP message was also detected at a lower level in several gray matter regions. The cloning approach used in this study would likely be useful in the identification and isolation of FABP-encoding genes from other tissues and species. **Key Words:** Fatty acid binding protein—MOCH-1 cells—Myelin—cDNA—Cloning—Development—In situ hybridization.
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Fatty acid binding proteins (FABPs) are a family of highly homologous cytosolic proteins that were first identified in rat intestinal mucosal cytosol (Ockner et al., 1972). They represent a group of distinct proteins with several common features, including their relative small size (14–16 kDa), their high concentration (representing up to 5% of cytosolic protein), and their ability to bind long-chain fatty acids and their CoA derivatives, as well as other small hydrophobic ligands (reviewed by Veerkamp et al., 1991). X-ray crystallography studies have revealed that these proteins consist of two layers of β-sheet structure surrounding a hydrophobic pocket occupied by a fatty acid ligand. In vitro binding studies have established that these proteins can bind fatty acid molecules with specificity (Ong and Chytil, 1978; Uyemura et al., 1984; Matarese and Bernlohr, 1988; Schoentgen et al., 1990); however, their natural ligands have not yet been established. The precise role these proteins play in the cell remains largely unknown, although several functions have been postulated (reviewed by Ockner, 1990; Veerkamp et al., 1991). They may serve as intracellular carriers, shuttling poorly soluble fatty acids from their site of synthesis or cell entry to target organelles where they participate in specific metabolic processes (Gangl and Ockner, 1975). Alternatively, it has been suggested that FABPs may serve to modulate the activity of enzymes involved in the intracellular metabolism of fatty acids (Fournier and Richard, 1990). It is also conceivable that FABPs provide a general maintenance service by protecting cellular enzymes and membranes from the detrimental effects of long-chain fatty acids and their acyl-CoA derivatives.

The myelin protein P_2 belongs to the family of FABPs (Narayanan et al., 1988, 1991). P_2 is found predominantly in myelin of the PNS and to a lesser extent in the caudal portion of the CNS (Greenfield et al., 1973; Trapp et al., 1983, 1984). Myelinating cells synthesize and transport to their plasma membrane extremely large quantities of lipid, suggesting that proteins responsible for binding fatty acids may play a key role in the myelination process. FABPs have been previously isolated from the cytosol fraction of rat and bovine brain (Bass et al., 1984; Senjo et al., 1985;

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Address correspondence and reprint requests to Dr. B. Popko at Brain and Development Research Center, CB# 7250, Room 320 BSRC, University of North Carolina, Chapel Hill, NC 27599-7250, U.S.A.

The GenBank accession number for the sequence reported in this article is U02096.

Abbreviations used: B-FABP, brain fatty acid binding protein; FABP, fatty acid binding protein; PCR, polymerase chain reaction; SSC, saline–sodium citrate.

Schoentgen et al., 1989, 1990). The complete amino acid sequence has been determined for bovine brain FABP (B-FABP) and shown to exhibit some microheterogeneity, suggesting the existence of multiple B-FABPs (Schoentgen et al., 1989, 1990).

To gain a better understanding of the functional properties of FABPs and their possible role in CNS myelination, we set out to clone FABP-encoding genes from the rat brain. The approach we have adopted to clone these genes exploits the high amino acid sequence homology observed among the FABP family members and uses the polymerase chain reaction (PCR), a technique that has provided a powerful means by which to clone members of multigene families (for examples, see Lee et al., 1988; Wilks, 1989; Lai and Lemke, 1991; Fremeau et al., 1992). We report here the isolation and nucleotide sequence of a novel FABP cDNA that is expressed in rat brain and in a mouse oligodendroglioma-derived tumor cell line (MOCH-1 cells) (Hayes et al., 1992) and to a lesser extent in rat liver. Expression was undetectable in rat heart, kidney, lung, spleen, thymus, and sciatic nerve. A developmental profile performed on rat brain demonstrated high levels of expression in 15-day-old embryos with mRNA levels peaking in 1-day-old neonates and declining thereafter. An in situ hybridization histochemistry analysis of neonatal rat brain has revealed abundant B-FABP mRNA levels in major fiber tracts, as well as lower levels in gray matter regions.

MATERIALS AND METHODS

Reverse transcription/PCR: cloning and sequencing of PCR products

Poly(A)$^+$ mRNA (300 ng) from 18-day-old rat brain was reverse-transcribed using murine leukemia virus reverse transcriptase (BRL) and 100 pmol of random hexamer primers. The mRNA and primer mixture was first denatured for 3 min at 95°C and cooled on ice for 2 min followed by addition of 1.5 m*M* $MgCl_2$, 0.625 m*M* deoxynucleotide triphosphates, 10 m*M* dithiothreitol, 20 units of RNasin (Promega), and 200 units of reverse transcriptase in a final volume of 20 μl. The reaction was incubated for 10 min at room temperature, 45 min at 42°C, and 5 min at 95°C. PCR was performed using 1-μl samples of a 10-fold dilution of the cDNA in a 100-μl volume containing a final concentration of 1.5 m*M* $MgCl_2$, 0.2 m*M* deoxynucleotide triphosphates, 2.5 units of *Taq* I polymerase (Boehringer Mannheim), and 4 μg of each degenerate oligonucleotide primer (Fig. 1). PCR parameters included an initial 6-min denaturation step performed at 94°C followed by 35 cycles of amplification beginning with a 2-min annealing at 55°C, a 3-min extension at 72°C, and a 1-min denaturation at 94°C. The reaction was terminated with a final 2-min annealing step at 55°C followed by a 10-min extension at 72°C. Amplified DNA (170-bp fragments) was cloned in pUC19 using the *Bam*HI and *Hin*dIII restriction enzyme sites included in the degenerative PCR primers. Miniprep DNA of the subclones was sequenced using the Sequenase (U.S. Biochemicals) dideoxy chain termination method.

Screening of λgt10 cDNA library and characterization of cDNA clones

The PCR clones described above were radioactively labeled and used as probes to screen ~2×10^5 plaques of a random-primed λgt10 cDNA library generated from mRNA isolated from the brains of 3-week-old rats. Primary plaques were picked and used to generate secondary plaques, which were selected, transferred to 100 μl of D_2O, and incubated for 2 h at 37°C. Five microliters of each secondary phage stock was used in PCR assays to determine the DNA insert size using the buffer conditions previously described with 500 ng of oligonucleotide primers λgt1031 and λgt1032 (New England Biolabs), which flank the unique *Eco*RI cloning site of λgt10. The amplified DNA products were size-fractionated on 0.8% (wt/vol) agarose gels and visualized by ethidium bromide staining. The PCR products from several secondary plaques of each positive clone were subcloned into the TA cloning vector PCRII (Invitrogen) and sequenced.

Sequence comparison

The comparison of our sequence data with the nucleotide and protein data banks was performed at the National Center for Biotechnology Information using the BLAST network service (Altschul et al., 1990). Nucleotide and amino acid manipulations and alignments were carried out using various programs available in the PC/Gene package (Intelligenetics).

RNA isolation and northern blot analysis

RNA was isolated from rat tissue according to the procedure of Chirgwin et al. (1979), and northern blots were carried out as previously described (Popko et al., 1987; Stahl et al., 1990). Probes for blot hybridizations were prepared using PCR according to the technique of Jansen and Ledley (1989). To control for pipetting and RNA transfer variability, the filters were stripped and hybridized with a radiolabeled probe specific for 18S ribosomal RNA (Stahl et al., 1990). Autoradiograms were digitized and quantified using the MCID M1 system (Imaging Research, St. Catharines, Ontario, Canada) running on a 486 ISA computer.

Southern blot analysis

DNA was isolated from spleen as described by Strauss (1987). DNA samples (5 μg) were digested with appropriate restriction enzymes and electrophoresed through 1% agarose gels. DNA was transferred to ZetaProbe (Bio-Rad, Melville, NY, U.S.A.) membranes overnight in 0.4 *M* NaOH (Reed and Mann, 1985).

In situ hybridization histochemistry

RNA probes were generated essentially as described (Fremeau and Popko, 1990; Stenvers et al., 1994). Sense and antisense radiolabeled B-FABP RNA was synthesized with the prokaryotic T7 and Sp6 RNA polymerases, respectively, using the corresponding promoter sites present in the PCRII cloning vector (Invitrogen). In situ hybridizations were carried out as described by Stenvers et al. (1994) on 12-μm cryostat-cut sections of an 11-day postnatal rat brain. Sections were fixed at room temperature for 30 min in 4% paraformaldehyde, washed in phosphate-buffered saline, and treated with proteinase K (0.5 μg/ml) at 37°C for 10 min. An acetylation step was performed with 0.1 *M* triethylammonium hydrochloride (pH 8.0) and 0.25% (vol/vol) acetic anhydride. Following dehydration in graded alcohols and

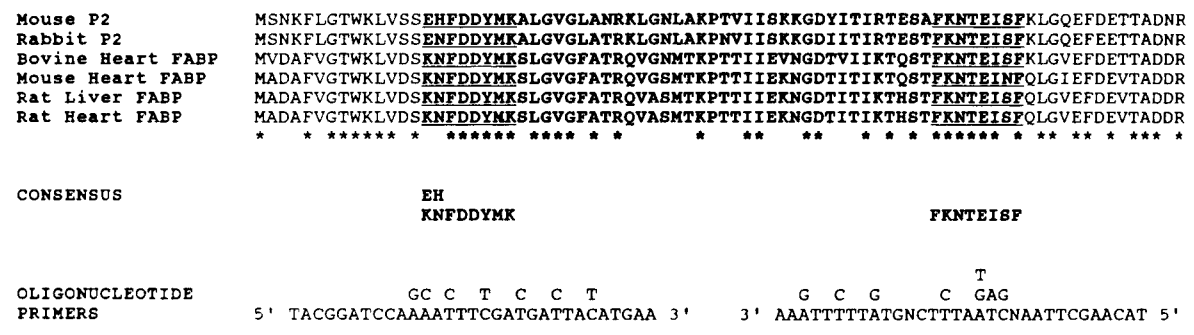

FIG. 1. Sequence and position of degenerative FABP primers. Partial sequences of the FABP members used to design the degenerative PCR primers are shown: mouse P_2 (Narayanan et al., 1991), rabbit P_2 (Narayanan et al., 1988), bovine heart FABP (Billich et al., 1988), mouse heart (Tweedie and Edwards, 1989), rat liver FABP (Gordon et al., 1983), and rat heart FABP (Heuckeroth et al., 1987). The consensus amino acid sequence used for the design of the degenerative primers is shown. The sequence of the degenerative primers is also presented. The three 5′-most nucleotides of each primer are included as spacer sequences. To facilitate the cloning of PCR products the spacer sequences are followed by a *Bam*HI restriction enzyme site in the 5′ oligonucleotide primer and a *Hin*dIII restriction enzyme site in the 3′ oligonucleotide primer.

air-drying, sections were hybridized for 18 h at 55°C in 75% formamide, 10% dextran sulfate, 3× saline–sodium citrate (SSC; 0.15 *M* NaCl and 0.015 *M* sodium citrate, pH 7.0), 50 m*M* sodium phosphate (pH 7.4), 0.02% polyvinylpyrolidone, 0.02% Ficoll, 0.02% bovine serum albumin, 10 m*M* dithiothreitol, 0.1 mg/ml of yeast tRNA, and $1-2 \times 10^6$ cpm of radiolabeled RNA probe. After hybridization, slides were washed in 2× SSC at 55°C. Sections were incubated in 200 μg/ml of RNase A for 30 min at 37°C, and then washed in decreasing concentrations of SSC at 55°C, followed by a final 1-h wash in 0.5× SSC at 55°C. Sections were dehydrated, air-dried, and exposed to Hyperfilm β-Max (Amersham Corp., Arlington Heights, IL, U.S.A.) for 2 days. Slides were then dipped in Kodak NTB-2 nuclear emulsion (Eastman Kodak Co., Rochester, NY, U.S.A.) for 2–4 weeks.

RESULTS

Identification of a novel FABP from rat brain

We used PCR to amplify FABP-related sequences located between the degenerate oligonucleotide primers shown in Fig. 1. These primers were designed based on homologous regions of protein sequence observed among six FABPs differing in both the origin of tissue from which the proteins were isolated and the species. The primers were selected because they flank a stretch of FABP sequence corresponding to a more divergent stretch of amino acids, thereby increasing the likelihood of identifying novel clones. The upstream primer was derived from an amino acid sequence encoded for by the first exon of FABP genes, and the downstream primer was based on sequences present in the second exon (Veerkamp et al., 1991). Amplification of contaminating genomic DNA would thus result in a DNA fragment in excess of 1 kb, which is easily distinguished from the 170-bp PCR product that is expected when the primers amplify the appropriate stretch of FABP cDNA.

The substrate used for amplification was cDNA generated by reverse transcription of poly(A)$^+$ mRNA isolated from the brains of 3-week-old rats. Amplified products were size-fractionated on agarose gels, and appropriately sized products (~170 bp) were subcloned into pUC19 and sequenced. Sequence analysis of 15 PCR product subclones revealed nine whose sequence was identical to the corresponding domain of rat heart FABP, which had previously been shown to be expressed in rat brain (Heuckeroth et al., 1987). These clones detected an mRNA transcript ~0.8 kb long in brain, heart, and kidney RNA but not in liver, lung, spleen, or thymus RNA (data not shown). These results parallel the tissue expression profile previously described for rat heart FABP (Heuckeroth et al., 1987), strengthening the evidence that these partial cDNA clones correspond to the rat heart FABP. Consequently, these clones were not examined further. The remaining six subclones, while identical to one another and exhibiting some homology to previously identified FABPs, appeared to be derived from a novel FABP mRNA, which we refer to as B-FABP.

Isolation and characterization of a B-FABP cDNA clone

To obtain the full-length cDNA sequence corresponding to the novel B-FABP clone described above, a λgt10 cDNA library constructed from 3-week-old rat brain mRNA was screened with the 170-bp unique DNA fragment as described in Materials and Methods. Several positive clones were identified, and the complete sequence of one, B-FABP#53, that contained the entire protein encoding domain is shown in Fig. 2. This cDNA contains an open reading frame capable of encoding a protein of 132 amino acids with a predicted molecular size of slightly less than 15,000 Da and an estimated isoelectric point of 5.35. Although this protein lacks the consensus FABP signature sequence, according to the PROSITE program of PC/GENE (data not shown), it shares extensive sequence homology with other members of the FABP family (Fig. 3). B-

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5'   cggagttttgtgaattacggtagtgggtaagacctgagttcctccagctcctacctactttca

gctgactagaagggcaagg ATG  GTA  GAT  GCT  TTC  TGT  GCC  ACC  TGG  AAG
                    Met  Val  Asp  Ala  Phe  Cys  Ala  Thr  Trp  Lys

CTG  ACA  GAC  AGC  CAG  AAT  TTT  GAT  GAG  TAC  ATG  AAG  GCT  CTG
Leu  Thr  Asp  Ser  Gln  Asn  Phe  Asp  Glu  Tyr  Met  Lys  Ala  Leu

GGC  GTG  GGC  TTT  GCC  ACT  AGG  CAA  GTG  GGA  AAT  GTG  ACC  AAA
Gly  Val  Gly  Phe  Ala  Thr  Arg  Gln  Val  Gly  Asn  Val  Thr  Lys

CCA  ACG  GTG  ATT  ATC  AGT  CAG  GAA  GGC  GGC  AAA  GTG  GTG  ATC
Pro  Thr  Val  Ile  Ile  Ser  Gln  Glu  Gly  Gly  Lys  Val  Val  Ile

CGG  ACA  CAA  TGC  ACA  TTC  AAG  AAC  ACA  GAG  ATC  AGT  TTC  GGA
Arg  Thr  Gln  Cys  Thr  Phe  Lys  Asn  Thr  Glu  Ile  Ser  Phe  Gly

GAA  GAG  TTT  GAG  GAA  ACA  AGC  ATA  GAT  GAC  AGG  AAC  TGT  AAG
Glu  Glu  Phe  Glu  Glu  Thr  Ser  Ile  Asp  Asp  Arg  Asn  Cys  Lys

TCT  GTG  ATT  CGG  TTG  GAT  GGA  GAC  AAG  CTG  ATT  CAT  GTG  CAG
Ser  Val  Ile  Arg  Leu  Asp  Gly  Asp  Lys  Leu  Ile  His  Val  Gln

AAG  TGG  GAT  GGC  AAA  GAA  ACA  AAT  TGT  GTC  AGA  GAA  ATT  AAG
Lys  Trp  Asp  Gly  Lys  Glu  Thr  Asn  Cys  Val  Arg  Glu  Ile  Lys

GAT  GGC  AAA  ATG  GTT  GTG  ACT  CTC  ACC  TTT  GGG  GAT  GTC  GTT
Asp  Gly  Lys  Met  Val  Val  Thr  Leu  Thr  Phe  Gly  Asp  Val  Val

GCT  GTT  CGC  TGT  TAT  GAA  AAG  GCA  TAG  aagaaatgccgggtcttttgaaa
Ala  Val  Arg  Cys  Tyr  Glu  Lys  Ala  Ter

acttggtttcctgaagttccagagttatccagctactgaagcactggtcactaattataagtttgtct

ttggtgtggaggcgaaaattataatttaagg    3'
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FIG. 2. Nucleotide and deduced amino acid sequence of B-FABP. The 604 nucleotides of the longest cDNA clone obtained corresponding to B-FABP are shown. The deduced amino acid sequence is presented below the nucleotide sequence.

FABP exhibits 65% sequence identity with the rat heart FABP, 70% identity with the bovine B-FABP, and 55% identity with the mouse P_2 myelin protein. As with other FABP members, B-FABP does not encode an apparent membrane insertion signal sequence or putative membrane-spanning domains. Within the deduced B-FABP protein there are two potential protein kinase C phosphorylation sites at amino acids 8 and 57; three potential casein kinase II phosphorylation sites at amino acids 74, 75, and 119; and a single *N*-myristoylation site at amino acid 25 (data not shown). The functional significance of these sites remains to be determined.

The B-FABP cDNA recognizes a single-copy gene in rat DNA

Because of the high amino acid sequence homology of the FABP members, we examined whether the B-FABP cDNA recognizes an individual gene or multiple DNA segments in the rat genome. As demonstrated in the Southern blot presented in Fig. 4, the B-FABP cDNA detects individual restriction fragments in rat

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Rat Brain FABP      MVDAFCATWKLTDSQNFDEYMKALGVGFATRQVGNVTKPTVIISQEGGKV   50
Bovine Brain FABP   MVDAFVGTWKLTESQNFDEYMKSLGVGFATRQVGNMTKPTLIISVNGDTE   50
Rat Heart FABP      MADAFVGTWKLVDSKNFDDYMKSLGVGFATRQVASMTKPTTIIEKNGDTI   50
Mouse P2            MSNKFLGTWKLVSSEHFDDYMKALGVGLANRKLGNLAKPTVIISKKGDYI   50
                    * - * -*****--*--**-***-****-*-*------*** **- -*-

Rat Brain FABP      VIRTQCTFKNTEISFQLGEEFEETSIDDRNCKSVIRLDGDKLIHVQKWDG  100
Bovine Brain FABP   IIKTQSTFKNTEISFHLGEEFDDTTADDQKVKSIVTLDGGKLVHVQKWDG  100
Rat Heart           TIKTHSTFKNTEISFQLGVEFDEVTADDRKVKSVVTLDGGKLVHVQKWDG  100
Mouse P2            TIRTESAFKNTEISFKLGQEFDETTADNRKAKSIVTLERGSLKQVQKWDG  100
                    -*-*---*********-** **----- *--- **-- *- --* -******

Rat Brain FABP      KETNCVREIKDGKMVVTLTFGDVVAVRCYEKA   132
Bovine Brain FABP   QESSLVREMVAGKLILTLTHGDVVAVRHYEK-   131
Rat Heart FABP      QETTLTRELSDGKLILTLTHGNVVSTRTYEK-   131
Mouse P2            KETAIRRTLLDGRMVVECIMKGVVCTRIYEKV   132
                    -*--  *-- -*----- -  -** -* ***
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FIG. 3. Comparison of the B-FABP amino acid sequence with other FABP family members. The amino acid sequences of rat B-FABP, bovine B-FABP (Schoentgen et al., 1990), rat heart FABP (Heuckeroth et al., 1987), and mouse P_2 (Narayanan et al., 1991) were compared using the CLUSTAL program of PC/GENE. Stars indicate amino acid identity in all four molecules, and dashes indicate positions of well-conserved amino acids.

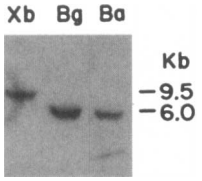

FIG. 4. Southern blot of FABP sequences in rat DNA. DNA samples (5 μg) were digested with the indicated restriction enzymes [*Xba*I (Xb), *Bgl*II (Bg), and *Bam*HI (Ba)], electrophoresed through agarose, transferred to nylon filter paper, and hybridized to a PCR-labeled B-FABP cDNA probe.

DNA, indicating that B-FABP is a single-copy gene. Moreover, the relatively small size of these restriction fragments suggests that the genomic organization of the B-FABP gene is simple.

B-FABP mRNA expression

To determine the tissue distribution of B-FABP mRNA, total RNA from various rat tissues was examined for the B-FABP transcript. B-FABP mRNA was detected in brain and liver RNA but not in heart, kidney, lung, spleen, thymus, or sciatic nerve RNA (Fig. 5). The B-FABP transcript was also present in RNA from the MOCH-1 mouse oligodendrocyte cell line, suggesting a potential cell of origin for the brain expression.

To characterize further B-FABP expression in rat brains the presence of this transcript was examined in total RNA isolated from rat brain at several developmental time points (Fig. 6). B-FABP mRNA was abundant in day 15 embryos, peaked in day 1 neonates, and declined sharply thereafter, reaching low steady-state levels in 3-week-old animals.

The regional and cellular distribution of the B-FABP message was examined using in situ hybridization histochemistry. As demonstrated in the autoradiograms presented in Fig. 7, B-FABP mRNA expression was widespread in the 11-day-old rat brain. The highest levels of expression appeared to be in fiber tracts, including the optic nerve, corpus callosum, external and internal capsule, fimbria, cingulum, and lateral olfactory tract. Expression was also detected, to a lesser degree, in most gray matter areas, including the anterior olfactory nuclei, striatum, hippocampal formation, thalamus, and cerebral cortex.

The B-FABP message was abundant in the ependymal and subependymal cell layers of the lateral ventricle (Fig. 8A). The subependymal layer has been described as a major site of gliogenesis in the developing rat forebrain (Privat and Leblond, 1972), suggesting that B-FABP may be a predominate transcript in immature or differentiating glia. B-FABP mRNA was also detected in the granule cell layer of the dentate gyrus (Fig. 8B). This region has been shown to be undergoing neurogenesis at the developmental period examined here (Altman and Bayer, 1990). B-FABP mRNA appeared to be present in the immature neurons within the subgranular zone described by Altman and Bayer (1990) but not in the mature granule cells of this region.

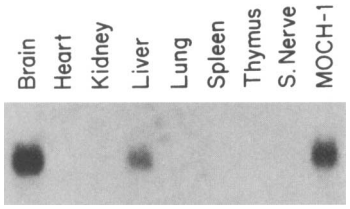

FIG. 5. Tissue distribution of B-FABP mRNA. Total RNA samples (10 μg) isolated from the indicated rat tissues and the MOCH-1 cell line were examined for B-FABP mRNA expression. The ages of the animals from which the tissues were isolated are 7 (brain), 32 (heart), 25 (kidney, lung, spleen, and thymus), 21 (liver), and 65 [sciatic (S.) nerve] days.

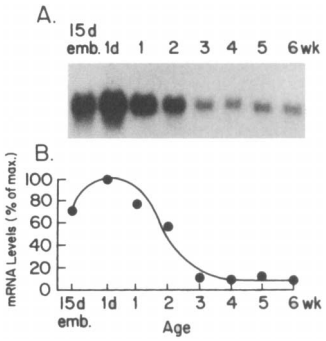

FIG. 6. Developmental expression of B-FABP mRNA in rat brain. **A:** Total RNA samples (10 μg) isolated from rat brain at the indicated developmental time points (15-day embryo, 1-day postnatal neonate, and 1–6 weeks) were examined for B-FABP expression. **B:** The nylon filter shown in A was stripped and hybridized with a probe specific to 18S ribosomal RNA to correct for variations in the amount of RNA loaded to the individual lanes. Signal intensities on each filter were digitized and quantitated as described in Materials and Methods.

DISCUSSION

We have used PCR with degenerate oligonucleotide primers, which were designed based on regions of amino acid sequence homology present in the FABP family, to isolate a novel FABP cDNA that is abundantly expressed in developing rat brain. Using this approach we have also identified the rat heart FABP, which had previously been shown to be expressed in brain (Heuckeroth et al., 1987). These were the only two B-FABP cDNAs identified of 15 PCR-derived clones that were sequenced. Possibly, these are the only FABPs expressed at significant levels in 3-week-old rat brain, the time point used for mRNA isolation. Alternatively, other FABPs might be present that are not amplified efficiently with the degenerative primers used in this study. The PCR primers and cloning approach used here should be of use in the identification and isolation of FABP-encoding genes from other tissues and organisms.

It is interesting that the developmental expression pattern of B-FABP and heart FABP in rat brain appears

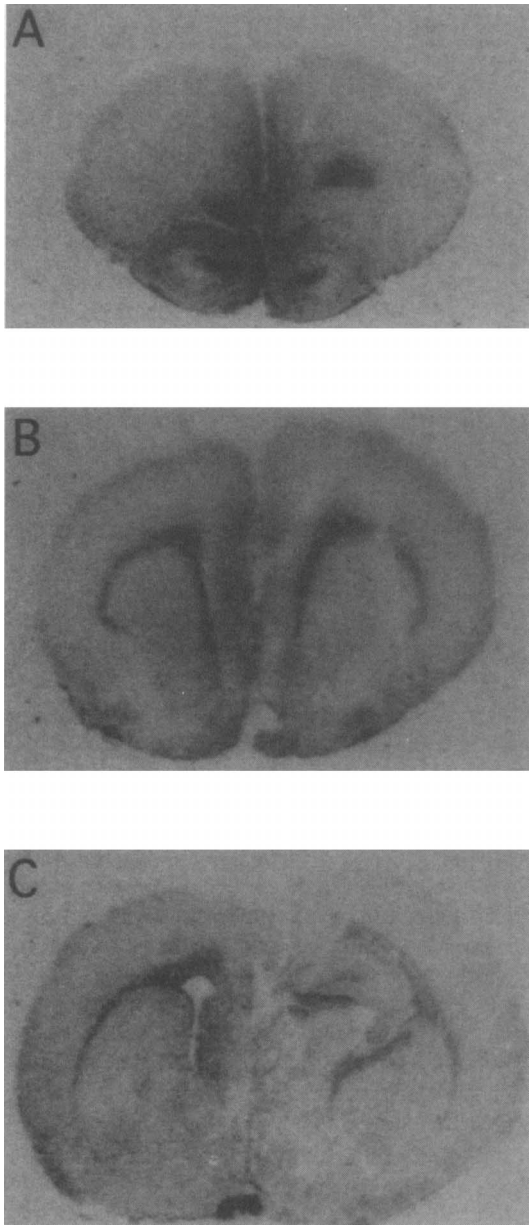

FIG. 7. B-FABP mRNA localization in 11-day-old rat brain, as determined by in situ hybridization histochemistry. **A–C:** Autoradiograms of three coronal sections from infant rat forebrain, progressing from the level of the anterior olfactory nuclei to the level of the dorsal hippocampus. B-FABP antisense probe hybridization was strongest within the fiber tracts, but lower levels of expression were seen in most gray matter regions. See text for details.

to be complementary. As shown here, B-FABP expression peaks relatively early in brain development and decreases to a low steady-state level by the third postnatal week. Heuckeroth et al. (1987) demonstrated that heart FABP expression is low early in brain development and reaches peak levels following weaning. At peak levels of synthesis, the absolute amount of B-FABP mRNA, however, appears to be much greater than that of heart FABP mRNA in rat brain (E.B. and B.P., unpublished data). The regional and cellular distribution of the heart FABP mRNA in brain has not been characterized. Possibly, B-FABP and heart FABP perform similar roles in the development and functioning of the CNS.

One of the goals of this study was to identify a FABP member expressed in the CNS that might serve an analogous function of the P_2 protein, which is a FABP found most predominantly in PNS myelin (reviewed by Martenson and Uyemura, 1992). P_2 is a basic protein that in addition to being found in the cytoplasm of myelinating Schwann cells is also detected within the multilayered myelin sheath. P_2 is believed to play a role in the transport of newly synthesized lipids to the multilayered structure. If such proteins are essential to the myelination process, we speculated that a similar FABP might also be expressed by oligodendrocytes. It is interesting that the novel B-FABP that we isolated in this study is abundantly expressed in the MOCH-1 mouse oligodendrocyte cell line, whereas the heart FABP transcript is not detected in MOCH-1 cells. Moreover, in situ hybridization histochemistry revealed abundant levels of B-FABP mRNA in the major fiber tracts of the developing brain. Nevertheless, several characteristics of B-FABP distinguish it from the P_2 protein. The developmental time courses of PNS myelination and P_2 expression in sciatic nerve are similar (Narayanan et al., 1988). In contrast, peak levels of B-FABP synthesis occur before the period of maximal myelination of the CNS. Moreover, although B-FABP shares limited homology with the P_2 protein (55% at the amino acid level), the two proteins have distinct biochemical properties. P_2 is enriched in positively charged amino acids and has an estimated isoelectric point of 10.22. The basic nature of P_2 is thought to facilitate its interactions with the negatively charged cytoplasmic face of the myelinating cell and thereby determine, at least in part, its position in the myelin sheath. The more acidic isoelectric point of B-FABP (5.25) is similar to that of the majority of FABP members. Therefore, although B-FABP is abundantly expressed in the MOCH-1 oligodendrocyte cell line, it appears unlikely that this protein is the functional equivalent of P_2. The availability of antibodies to B-FABP will allow us to determine the subcellular localization of this protein in vivo and facilitate the elucidation of the potential role B-FABP plays in CNS myelination.

Recently, a cDNA encoding a novel FABP has been isolated from a cDNA library generated from chicken retina (Godbout, 1993). This protein is 86% homologous with B-FABP, suggesting that the two proteins may be homologues. Nevertheless, the expression pattern of these genes is distinct. In addition to being expressed in the retina and brain, the chicken FABP is abundantly expressed in kidney but not in liver (Godbout, 1993). Although we have not examined the

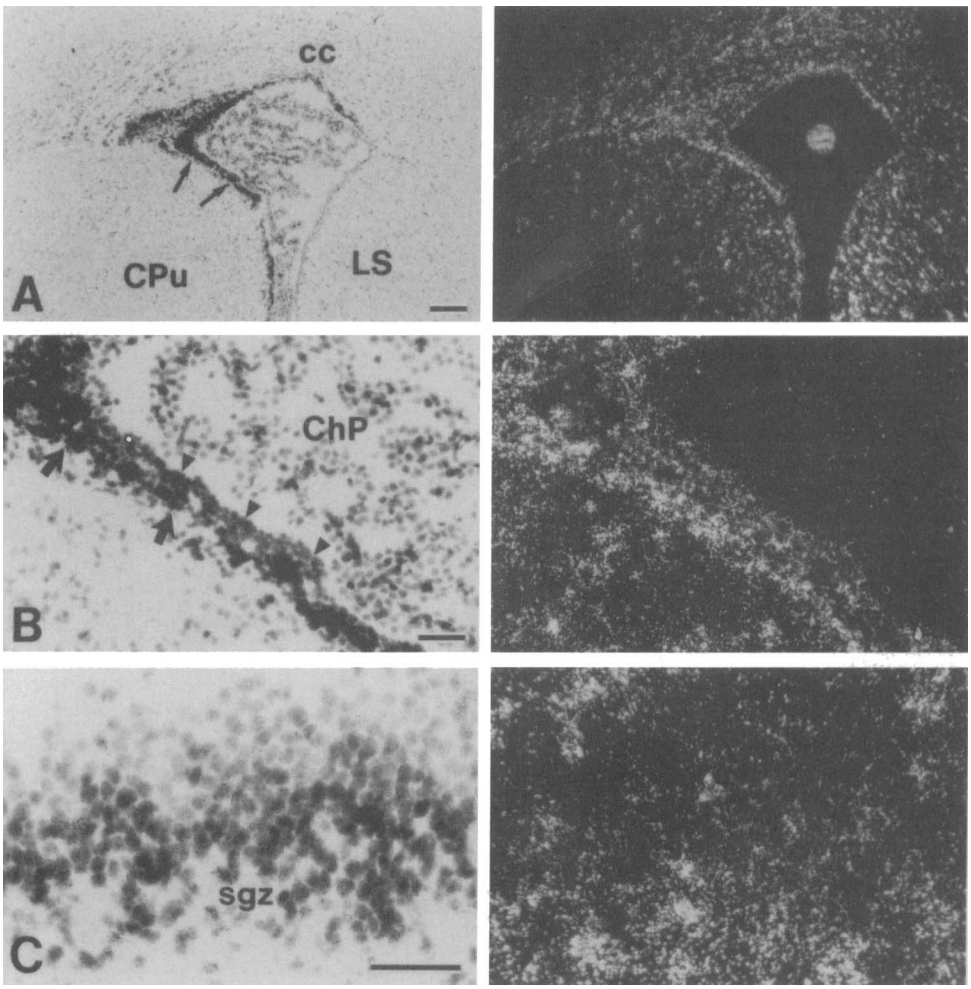

FIG. 8. Paired light- and dark-field photomicrographs of coronal sections through the infant rat forebrain show the cellular localization of B-FABP mRNA. **A:** Low-power photomicrographs through the lateral ventricle show strong hybridization in both white and gray matter areas. cc, corpus callosum; CPu, caudate putamen; LS, lateral septal nuclei. Arrows indicate the cell layers depicted in **B,** in which high-power photomicrographs of the ependymal (arrowheads) and subependymal (arrows) cell layers lining the lateral ventricle show strong hybridization in both layers. Note the absence of hybridization within the choriod plexus (ChP). **C:** High-power photomicrographs show silver grains localized over primitive neurons in the subgranular zone (sgz) of the dentate gyrus but not over the paler-staining mature granule cells. Bar = 200 (A), 100 (B), or 50 (C) μm.

retina for B-FABP expression, B-FABP mRNA is not detectable in rat kidney but is present in liver. Moreover, the developmental profile of expression of the these FABP genes in brain is different. The peak period of expression of the chicken FABP occurs significantly later in development than B-FABP. Whether these differences in expression represent distinct functional properties of these proteins awaits further study.

Because oligodendroglia are rare in prenatal rodent brains, the abundant B-FABP expression detected in the brains of 15-day embryos suggests that this gene is expressed in cells other than oligodendrocytes, at least early in CNS development. Possibly this expression occurs in oligodendroglial precursors or in a completely unrelated cell type. In situ hybridization histochemistry revealed abundant B-FABP mRNA expression in white matter tracts in an 11-day-old rat brain. Moreover, B-FABP mRNA was abundant in brain regions thought to be enriched in developing glia (subependymal layer). The cellular localization studies also suggest that B-FABP mRNA is present in gray matter regions. It is interesting that B-FABP expression was detected in the granule cell layer of the dentate gyrus in cells thought to represent developing neurons. The developmental pattern of expression of B-FABP mRNA presented in Fig. 6 and the in situ hybridization data shown in Figs. 7 and 8 suggest that B-FABP expression may be highly enriched in developing glia and neuronal cells. It is interesting that the liver FABP has recently been implicated as a positive effector molecule in the cellular response to certain mitogens (Khan and Sorof, 1994). Possibly, B-FABP plays a similar role in neuronal and glial cell differentiation. A detailed developmental in situ hybridization analysis

of B-FABP expression would likely provide additional insight into the potential function of this protein.

In summary, we have isolated a novel FABP-encoding gene that is abundantly expressed in rat brain. Although this protein is expressed in the MOCH-1 oligodendrocyte cell line, suggesting a possible function in the myelination process, this protein does not share extensive amino acid sequence or biochemical similarities with P_2, the FABP protein that is present in peripheral myelin. Moreover, during the development of the rat CNS, B-FABP is expressed most abundantly before the period of maximal myelination. The in situ hybridization data suggest a possible role of B-FABP in glial and neuronal cell precursors. The elucidation of the role B-FABP plays in CNS development and function awaits further molecular and biochemical characterizations.

Note added in proof: Feng et al. have recently reported the isolation of the apparent mouse homologue of the rat B-FABP cDNA reported here [Feng L., Hatten M. E., and Heintz N. (1994) Brain lipid-binding protein (BLBP): a novel signaling system in the developing mammalian CNS. *Neuron* **12,** 895–908].

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