

Comparison of Genomic and cDNA Sequences of Guinea Pig CYP2B18 and Rat CYP2B2: Absence of a Phenobarbital-Responsive Enhancer Module in the Upstream Region of the *CYP2B18* Gene

Midori Yamamoto,¹ Masashi Mise,¹ Sanae Matsumoto,¹ Shoich Ito,¹ Noriko Gohyama,¹ Satoru Ishida,¹ Yasuhiro Sagara,² Curtis J. Omiecinski,³ Kazuta Oguri,⁴ and Hideyuki Yamada¹

¹Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; E-mail: yamada@xenoba.phar.kyushu-u.ac.jp

²Nakamura Gakuen University, 5-7-1 Befu, Johnan-ku, Fukuoka 814-0198, Japan

³Department of Veterinary Science, The Pennsylvania State University, University Park, PA 16802, USA

⁴School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino-Machi, Nobeoka 882-8508, Japan

Received 12 November 2003; revised 1 March 2004; accepted 6 March 2004

ABSTRACT: Potential mechanisms were investigated whereby *CYP2B18*, a cytochrome P450 gene exhibiting high constitutive expression but only low levels of phenobarbital-inducibility in the guinea pig liver, may be differentially regulated versus the highly inducible rat *CYP2B2* gene. To comparatively assess potential regulatory sequences associated with *CYP2B18*, a guinea pig genomic library was screened enabling isolation of the *CYP2B18* gene. The genomic screening process resulted in the identification of at least four closely-related *CYP2B18* genes, designated here as *CYP2B18A-D*. Of these isolates, *CYP2B18A* exhibited sequence identical to that of the *CYP2B18* cDNA. Further, the deduced amino acid sequence of the *CYP2B18* cDNA was identical to that of N-terminal and internally-derived peptide sequences obtained in this investigation from *CYP2B18* protein isolated from guinea pig liver. Genomic structural sequences were derived for *CYP2B18A*, together with the respective 5'-upstream and intronic regions of the gene. Comparison of the *CYP2B18A* and *CYP2B2* gene sequences revealed the lack of repetitive *LINE* gene sequences in *CYP2B18A*, putative silencing elements that effect neighboring genes, although these sequences were present in both 5'-upstream and 3'-downstream regions of *CYP2B2*. We determined that the phenobarbital-responsive enhancer module was absent from the 5'-upstream region as well as the intronic regions of *CYP2B18A* gene. We hypothesize that the compromised phenobarbital

inducibility of *CYP2B18A* stems from its lack of a functional phenobarbital responsive enhancer module.

© 2004 Wiley Periodicals, Inc. *J Biochem Mol Toxicol* 18:124–130, 2004; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.20016

KEYWORDS: CYP2B18; CYP2B2; CYP2B6; Gene Structure; Intronic Sequence; Phenobarbital-Responsive Enhancer Module

INTRODUCTION

Cytochromes P450 (P450) belonging to the CYP2B subfamily catalyze the hydroxylation of a large number of structurally diverse xenobiotics [1]. In the rat, constitutive expression of the *CYP2B1* and *CYP2B2* genes is extremely low, however the transcription of each gene is markedly activated following phenobarbital (PB) treatment [2]. The PB-responsive enhancer module, or unit (PBREM/PBRU), has been identified as a conserved region located upstream of PB-inducible genes such as the *CYP2B* subfamily [3–8] and functions to drive the induction process. A nuclear receptor heterodimer consisting of the constitutive androstane receptor (CAR) and retinoid X receptor alpha (RXR α) has been identified as playing a critical role in transactivating gene transcription via the PBREM [9]. In support of this concept, CAR-deficient mice lack PB-inducibility of *Cyp2b10* [10]. A recent study from our laboratory examined the Qdj:SD rat, a mutant strain that lacks responsiveness to PB-mediated CYP2B2 in-

Correspondence to: Hideyuki Yamada.

Contract Grant Sponsor: USPHS.

Contract Grant Number: GM32281 (CJO).

duction. We demonstrated that no mutations or deletions exist within the PBREM sequence in the *CYP2B2* gene of these animals [11]. This observation suggested a role of other region(s), distinct from the PBREM, that may also participate in the PB induction process. In this respect, several non-PBREM regions, such as RBP- κ /NF κ B, have been suggested to be involved in the regulation of *CYP2B* gene expression [12,13]. Further studies are needed to better elucidate the basis of these alternative regulatory schemes.

It is of interest that the constitutive and inducible nature of expression of the *CYP2B* subfamily varies greatly in different animal species. For example, in untreated guinea pigs, *CYP2B18* is one of the major forms of P450 expressed in the liver, in clear distinction to rat *CYP2B1/2* [14,15]. In further contrast, PB treatment of guinea pigs results in only a minor increase, less than two fold, of the specific content of liver microsomal *CYP2B18*, compared to the high levels of induction observed for the orthologous rat *CYP2B* members [14,15]. To help elucidate the mechanisms that underlie these differences in species expression and inducer responsiveness, we hypothesized that the respective regulatory region sequences may be divergent between the *CYP2B18* and *CYP2B2* genes. To explore this hypothesis, we undertook comparative sequencing analysis of the guinea pig *CYP2B18* and rat *CYP2B2* genes.

MATERIALS AND METHODS

Materials

A guinea pig liver cDNA library (cloning vector, λ gt11; Clontec Laboratories, Inc., Palo Alto, CA, USA) and genomic library (Stratagene, La Jolla, CA, USA) were obtained from the sources indicated. A cosmid clone (pWE'-39E) of *CYP2B2* gene was prepared as previously reported [16]. The *CYP2B2* cDNA was a gift from Dr. Frank J. Gonzalez (National Institute of Health, Bethesda, MD, USA). Hepatic *CYP2B18* protein was purified by the methods described elsewhere [14]. For library screenings, *CYP2B18* probe DNAs were labeled, either fluorescently for cDNA cloning, or with alkaline phosphatase for genomic DNA cloning, using commercial kits (Amersham Biosciences, Piscataway, NJ, USA). All other materials were of the reagent grade and obtained from standard commercial suppliers.

Determination of Internal Amino Acid Sequences of *CYP2B18* Protein

A purified protein specimen (30–45 μ g) was partially digested in either (i) 40 mM phosphate (pH 7.25)

containing 0.3 μ g α -chymotrypsin, 0.1% sodium dodecyl sulfate (SDS), and 15% glycerol or (ii) 0.15 M BrCN/70% formic acid. The peptides generated were separated by SDS-polyacrylamide gel electrophoresis, and the major bands subsequently transferred to a polyvinylidene difluoride membrane. Individual bands were then excised and subjected to analysis using an automated protein sequencer (PE Biosystems ABI473A protein sequencer).

Cloning of Guinea Pig Liver *CYP2B18* cDNA

The guinea pig liver cDNA library was screened by plaque hybridization using a fluorescently-labeled *CYP2B2* full-length cDNA probe. After screening approximately 1×10^5 plaques, three positive clones (pGP4, pGP9, and pGP10) were obtained. Each clone, together with respective restriction fragments yielded by EcoRI, Pst I, and Hind III digestion, were subcloned into an M13 phage vector for DNA sequencing. The sequence of the *CYP2B18A* cDNA is available in the DDBJ/GenBank/EMBL database under the accession number AB115744.

Cloning of *CYP2B18* Genes

A guinea pig gene library consisting of 7×10^6 plaques was screened by plaque hybridization with an alkaline phosphatase-labeled *CYP2B18* cDNA fragment (760 bp), spanning the 2nd through the 7th exon. The fragment was generated by polymerase-chain reaction (PCR) using *CYP2B18* cDNA (pGP10) as a template. Thirty-four positive clones were obtained. Because of the presence of multiple related genes (see Results section), a variable region located within exon 2 was PCR-amplified for each positive clone, and the resulting sequence information enabled identification of specific clones encoding the *CYP2B18* protein. The PCR reaction mixture (50 μ L) included template (1.5×10^6 λ FIXII phage), 5 pmol each of primer and 2.5 units DNA polymerase (LA-Taq, Takara Shuzo Co., Ltd., Shiga, Japan), and was cycled according to the following conditions: 95°C for 5 min, (98°C for 10 s and 68°C for 15 min) \times 30 cycles; followed by final extension (72°C for 10 min) and hold (4°C) steps. The primer sequences used were as follows: 5'-AGATTAATCCGGCAGC-GTCCGTCGTTG-3' (sense strand; targeted to exon 2) and 5'-AAGCAACCTGCAACGTATTGAGCGC-3' (reverse strand; targeted to exon 3). The screening analysis identified 4 of the 34 positive clones as *CYP2B18* cDNAs.

Determination of Nucleotide Sequence

The DNA inserts of 2 of the 4 genomic clones confirmed to code CYP2B18 protein, pGP18A1 (insert DNA size, 18.5 kbp) and pGP18A2 (insert size 18 kbp) were PCR-amplified, and the respective DNA sequences were determined by shot-gun methods. Briefly, three regions of the *CYP2B18* gene were amplified separately by PCR (PCR 1–4; Figure 1) with LA-Taq (Takara Shuzo Co., Ltd.) under the conditions suggested by the manufacturer. The PCR products were partially digested by sonication, and DNA fragments in the range of 1–3 kbp were extracted by polyacrylamide gel (3%) electrophoresis. The respective DNA fragments were subcloned into M13 vectors for sequencing. The sequence of another clone (pGP18B1; insert DNA size 21 kbp), selected randomly from remaining 30 positive clones, was analyzed similarly. The sequence for rat *CYP2B2* gene was also determined by the same methods as above. However, in this case, whole pWE'-39E cosmid DNA was prepared from transformed *E. coli* was fragmented without first extracting *CYP2B2* gene sequences, and respective fragments in the 1–3 kbp size range were subcloned into M13. Nucleotide sequences were determined by a dye-primer method using a PE Biosystems ABI373 DNA sequencer. The derived *CYP2B18* and *2B2* gene sequences were assembled by using ABI377 instrumentation assembly software. Sequence similarities between the *CYP2B* genes were analyzed by PipMaker (PIP; Percent Identity Plot) [17]. The sequence of the *CYP2B18A* gene (pGP18A1/18A2; accession number: AB115742) was registered in the DDBJ/GenBank/EMBL database together with the *CYP2B18B* gene (pGP18B1; AB115743).

RESULTS

Sequence of *CYP2B2* and Surrounding Genes

The nucleotide sequence of a cosmid clone of rat *CYP2B2*, pWE'-39E, was determined by a shotgun sequencing strategy. We derived 26 kbp of *CYP2B2* gene sequence, containing 7 kbp and 3 kbp of the 5'- and 3'-flanking regions, respectively, although the exact number of (G)GA repeats starting at approximately 420 bp downstream from the end of the 8th exon could not be determined. The 5'-flanking region upstream of -2.7 kbp was highly homologous with *LINE* (long interspersed repeated DNA element) [18] gene sequences, a characterized retrotransposon (Figure 2). High homology with *LINE* sequences also was identified in the 3'-flanking region of *CYP2B2* gene (Figure 2). It should be noted that the draft sequence from the rat genome project currently available through database resources (Pub-Med, NCBI) does not contain the majority of the *LINE* gene sequences from the 5'-upstream region of *CYP2B2*. The reason for this database omission is not known.

Cloning of *CYP2B18* cDNA

To enable sequencing the *CYP2B18* gene, we first screened a guinea pig liver λ gt11 cDNA library and obtained three positive clones that differed in size, but shared an identical sequence in their region of overlap (>400 bp). The combined sequence totaled 1906 bp and comprised an open reading frame except for a missing 196 bp region coding the N-terminal region. The nucleotide sequence for the missing N-terminal

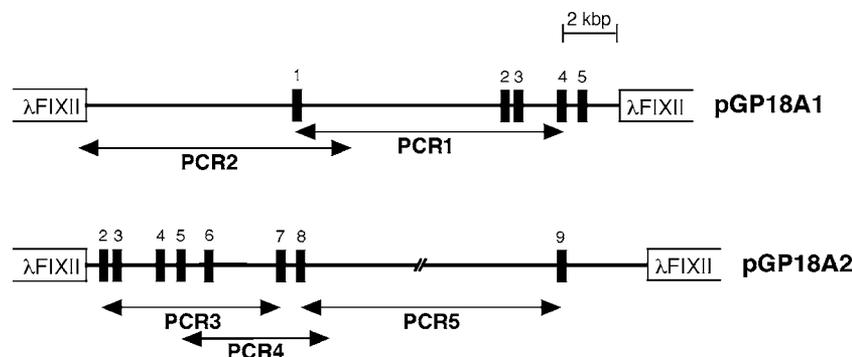


FIGURE 1. *CYP2B18A* clones isolated and the regions amplified by PCR for sequencing. Fragments (PCR1–4) of pGP18A1 and 18A2 were amplified by PCR, sequenced, and assembled. The PCR5 could not be amplified because of interference by a repeated sequence likely located in the 8th intron. Primers for amplification were as follows: PCR1, 5'-AGTCAGATAAGGACCATGGAGCTCAGC-3' (sense) and 5'-GAACAAGAAGAAGGTCAGACGATGACGG-3' (reverse); PCR2, 5'-AGATTAATCCGGCAGCGTCCGTCGTTG-3' (sense) and 5'-CCTAGTGGTACTCAAGATCCTGTCTAG-3' (reverse); PCR3, 5'-TGTCGAGAGCCTACATTTTC-3' (sense) and 5'-CCGACAGTAGACTCTAAG-3' (reverse); PCR4, 5'-TGAGCTTTTCTCCACCATCC-3' (sense) and 5'-AGCATCCATCAATCATCCGGCTCT-3' (reverse).

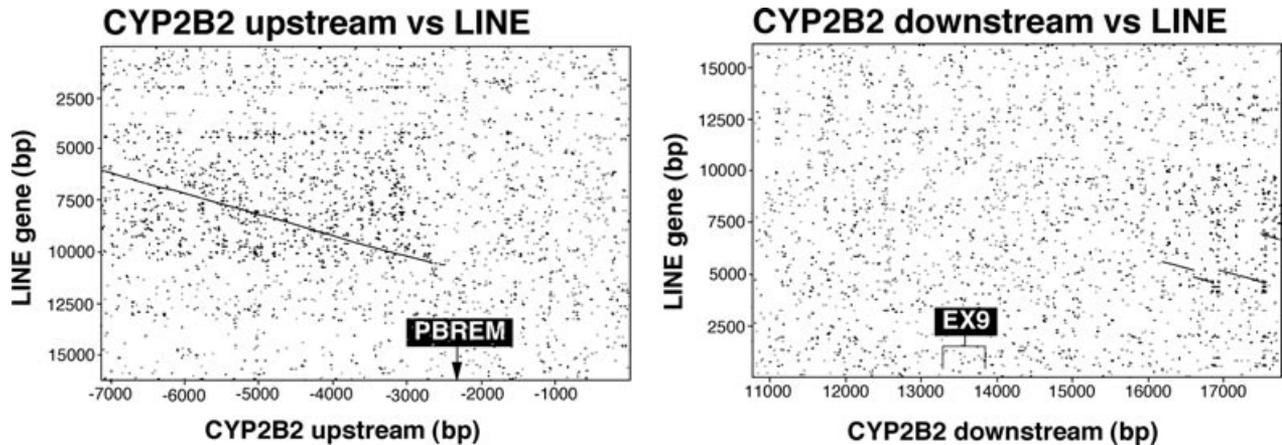


FIGURE 2. Sequence similarity between *CYP2B2* and *LINE* gene. The MacVector parameters used were as follows: window size, 20; minimum% score, 45; hash value, 6; strand, both strands; jump, 1. The *LINE* sequence was taken from Ref. [18].

region was derived from sequence of a *CYP2B18A* genomic clone, as discussed below. The deduced amino acid sequence consisted of 432 residues (Figure 3). The deduced internal peptide sequences as well as an N-terminal amino acid sequence derived independently (bold underline in Figure 3) were identical to those determined for the purified *CYP2B18* protein. The *CYP2B18* protein actually contained an additional C-terminal sequence that was absent in other member of this subfamily (Figure 3).

Sequence of *CYP2B18* Gene and the Comparison with Other Members of the *CYP2B* Subfamily

Screening of a guinea pig λ FIXII genomic library yielded 32 positive clones. Preliminary sequencing revealed that some of the clones possessed different exonic sequences compared with that of the cDNA structures described above, indicating that multiple *CYP2B18* genes existed in the guinea pig. Three (pGP18A1/pGP18A2 and pGP18B1) of 32 clones that possessed altered exonic sequences were sequenced further. The insert DNA regions of pGP18A1/pGP18A2 and pGP18B1 (21 kbp, *CYP2B18B*) clones were amplified by PCR, and sequenced by shotgun sequencing. The pGP18B1 clone contained approximately 2 kbp of 5'-flanking region and extended downstream through the middle of the 8th intron. The pGP18A1 + 18A2 contained approximately 4 kbp of 5'-flanking sequence and extended downstream through the middle of 6th intron. The exonic sequences of pGP18B1 were divergent from that of the *CYP2B18* cDNA (homology, 91%). Differences for the deduced amino acid residues were con-

firmed using protein sequencing procedures (Figure 3). Although the *CYP2B18B* (pGP18B1) gene had correct intron-exon junctions with no stop codons present in the exonic regions, we could not detect expression of its corresponding mRNA—despite examining RNA preparations from several tissues, including liver, by reverse transcriptase-coupled PCR. On the other hand, the exonic sequences of pGP18A1/18A2 were identical with those of *CYP2B18* cDNA. Consequently, we designated the gene encoding *CYP2B18* protein expressed in the guinea pig tissues as *CYP2B18A*. Comparison of the exonic sequences of the two genes revealed several regions exhibiting hypervariable base substitutions, including that of the 2nd exon. The latter region from each of the 32 clones was amplified subsequently by PCR, and comparatively sequenced. The results, presented in Figure 4, demonstrate four different sequence patterns, suggesting the presence of at least four closely related genes (*CYP2B18A-D*).

Sequence similarities between the *CYP2B2* and *CYP2B18A* genes were analyzed by PipMaker [17] and the results are shown in Figure 5A. The lack of sequence similarity in the distal upstream regions of the two genes (upstream of -2.5 kb) indicates that the presence of *LINE* gene sequence is likely specific for *CYP2B2*. It was noteworthy that the PBREM was completely absent in the 5'-upstream as well as intronic regions of the guinea pig *CYP2B18A* gene. In the comparison between *CYP2B18A* and human *CYP2B6* (Figure 5B), little homology was detected within their respective 5'-upstream regions. However, in contrast to the *CYP2B18A* vs *CYP2B2* comparison, highly homologous regions were present in the 1st and 3rd introns of *CYP2B18A* and *CYP2B6* genes, respectively.

<u>ATGGAGCTCAGTCTC</u> <u>CTCTTGTTCCTTGCT</u> <u>CTCCTACTGGGGCTC</u> <u>TTACTTCTGCTTTTC</u> <u>AAGGGCCACCCAAAG</u>	75
M E L S L L L F L A L L L G L L L L L F K G H P K	25
<u>GGCCATGGCAACCTC</u> <u>CCACGAGGCCACAT</u> <u>CCTCTGCCCTTCCTG</u> <u>GGGAACATTCTGCAG</u> <u>ATGAACAGAAAAGGC</u>	150
A H G N L P F G P H P L P F L G N I L Q M N R K G	50
<u>CTGTTCAATCCTTC</u> <u>CTGCAT</u> <u>TTCCGAGAG</u> <u>AAATACGGAGATGTG</u> <u>TTCACCGTGTACCTG</u> <u>GGCCAAGGCCAGTG</u>	225
L F K S F L Q F R E K Y G D V F T V Y L G P R P V	75
<u>GTGATGCTGTGGG</u> <u>GCAGAGACCATACGG</u> <u>GAGCCTTGGTGGAC</u> <u>CAAGCTGATCTTTT</u> <u>TCTGGCCGGGGATG</u>	300
V M L C G A E T I R E A L V D Q A D S F S G R G M	100
<u>ATTGCTGTCGCAGAG</u> <u>CCTACATTTTCAGGGA</u> <u>TATGGTGTGGTCTTT</u> <u>GCCAAATGGGGACCGC</u> <u>TGGAAGGCCCTTCGG</u>	375
I A V A E P T F Q G Y G V V F A N G D R W K A L R	125
<u>AGATTCCTTTGGCC</u> <u>ACCATGAGGGACTTT</u> <u>GGGATGGGAAAGCGG</u> <u>ACTGTGGAGGAGCGG</u> <u>ATTCAGGAGGAGCT</u>	450
R F S L A T M R D F G M G K R T V E E R I Q E E A	150
<u>CAGTGTCTGGTGGAG</u> <u>GAGATAAAGAACT</u> <u>AAGGGAGATCTTCTG</u> <u>GACCCCTTGTCTTTC</u> <u>TTCCAGTCTGCTACT</u>	525
Q C L V E E I K K S K G D L L D P L F F F Q S A T	175
<u>GCCAACATCATCTC</u> <u>TCTATTGTGTTTGA</u> <u>GAAACGCTTGGACTAC</u> <u>AAAGATCAGCAGTTC</u> <u>CTGAGGCTGCTGGAC</u>	600
A N I I C S I V F G E R F D Y K D Q Q F L R L L D	200
<u>CTGTTCTATCAGTCC</u> <u>TTTGCACTTGTGAGC</u> <u>TCCTATCCAGTCAG</u> <u>ATATTGAGCTTTTC</u> <u>TCCACCATCCTGAAG</u>	675
L F Y Q S F A L V S S L S S Q I F E L F S T I L K	225
<u>TACTTTCCTGGCCCA</u> <u>CACAGGGAAGTCTAC</u> <u>AAAAACCTGCAGGAA</u> <u>GTCACAGGTTTATT</u> <u>GGCCACAGGTTAGAG</u>	750
Y F P G P H R E V Y K N L Q E V N R F I G H R V E	250
<u>AAACACCGTGAACCT</u> <u>CTGGACCCAGCAAC</u> <u>CCTAGAGACTTCATT</u> <u>GATACCTTCCTGCTC</u> <u>CGCATGGACAAGAA</u>	825
K H R E T L D P S N P R D F I D T F L L R M D K E	275
<u>AAGTCTAACTCACAC</u> <u>ACCGAGTTCACCAC</u> <u>AAGAACCTCATCTG</u> <u>ACCTCACITTCCTC</u> <u>TTCTTGTCTGGCAG</u>	900
K S N S H T E F H H K N L I L T S L S L F F A G T	300
<u>GAGACCACAGCACA</u> <u>ACTTCCGCTATGGC</u> <u>TTCTGTTTTGCTC</u> <u>AAATACCCCATGTC</u> <u>ACAGAGAGATCCAA</u>	975
E T T S T T L R Y G F L F L L K Y P H V T E R V Q	325
<u>AAGGAAATGAACAA</u> <u>GTGATTGGCTCACAC</u> <u>CGCCAGCCAGCCTTG</u> <u>GATGATCGATCCAAA</u> <u>ATGCCATACACTGAG</u>	1050
K E I E Q V I G S H R Q P A L D D R S K M P Y T E	350
<u>GCTGTCATCTGTGAG</u> <u>ATTCAGAGATTTCGG</u> <u>GACCTGCTCCCATTT</u> <u>GGTTGGCCCCACATG</u> <u>GTCACCAAGACACC</u>	1125
A V I C E I Q R F A D L L P I G L P H M V T K D T	375
<u>CACTTCCGAGGGTTC</u> <u>TTTCATCCCTAAGGGC</u> <u>ACTGAAGTATACCCA</u> <u>CTCCTGTCCACTGCT</u> <u>CTCCATGACCCACAT</u>	1200
H F R G F F I P K G T E V Y P L L S T A L H D P H	400
<u>CACTTTGAAAAGCCA</u> <u>GAATTCCTTAACCCT</u> <u>GACCACTTCTGGAT</u> <u>GCTAAAGGGCACTG</u> <u>AAGAAGAATGAAGT</u>	1275
H F E K P E F F N P D H F L D A K G A L K K N E A	425
<u>TTTTATCCCTTCTCC</u> <u>ATGGGGAAGCGTATC</u> <u>TGCTTGGAGAAGGT</u> <u>GTTGCCCTGCAGAA</u> <u>TTGTTTCTCTCTTC</u>	1350
F I P F S M G K R I C L G E G V A R A E L F L F F	450
<u>ACCACATCCTTCAG</u> <u>AACTTCTCCATAGCG</u> <u>AGTCCAGTGGCTCCA</u> <u>GAGGATATTGACTTG</u> <u>ACACCAAGGAGCTT</u>	1425
T T I L Q N F S I A S P V A P E D I D L T P K E L	475
<u>GGTATTGGCAGAGTG</u> <u>CCCCAACATACCAG</u> <u>ATTTCCCTCCTGCCT</u> <u>CGCACAAATAGACTCA</u> <u>AGAGAGAAATAA</u>	1497
G I G R V P P T Y Q I S F L P R T I D S R E K	498

FIGURE 3. Nucleotide and deduced amino acid sequence of CYP2B18A cDNA. The nucleotide and the deduced amino acid sequences of CYP2B18A expressed in the tissues of guinea pigs [14,15] are shown in the first and second line, respectively. The amino acid residues of CYP2B18B (see text) that differ from CYP2B18A are shown in the third line. Thin underlining indicates the region where nucleotide sequence was determined from genomic DNA (*CYP2B18A* gene). The sequence of CYP2B18A without a thin underline (downstream from 197th base) was determined by sequencing the pGP4 (783–2,203 bp), pGP9 (727–1,830 bp), and pGP10 (197–1,293 bp) clones which contained the regions indicated, respectively. Deduced peptide sequences, indicated by bold underlines, of CYP2B18A cDNA were matched with the sequences determined from purified protein. The region coded in the 2nd exon is boxed. The C-terminal peptide specific for CYP2B18A as compared with the other members of this subfamily from other species is shown by a bracket.

DISCUSSION

In this study, we report for the first time on the existence of multiple guinea pig *CYP2B18* genes. Although the possibility that *CYP2B18B–D* are active genes can not be ruled out, the present results together with our previous data [14,15] strongly suggest that

CYP2B18A is the predominantly and perhaps solely expressed gene in this cluster, at least in liver. While the rat *CYP2B2* gene is located between *LINE* gene sequences, the *CYP2B18A* gene, which is highly expressed in the liver of untreated guinea pigs, does not contain corresponding *LINE* homologies. *LINE* gene sequences are located downstream of the rat *insulin-1* gene and have

2B18A	TCACCTGTGA	CCTGGGSCCA	AGGCCAETGG	TCATGCTGTG	TGGGGCAGAG	50
2B18B	TCACCTGTGA	CCTGGGSCCA	AGGCCAETGG	TCATGCTGTG	TGGGGCAGAG	50
2B18C	TCACCTGTGA	CCTGGGSCCA	AGGCCAETGG	TCATGCTGTG	TGGGGCAGAG	50
2B18D	TCACCTGTGA	CCTGGGSCCA	AGGCCAETGG	TCATGCTGTG	TGGGGCAGAG	50
2B18A	ACCATACGGG	AAGCCTTGGT	GGACCAAGCT	GATTCCTTTT	CTGGCCGGGG	100
2B18B	ACCATACGGG	AAGCCTTGGT	GGACCAAGCT	GATTCCTTTT	CTGGCCGGGG	100
2B18C	ACCATACGGG	AAGCCTTGGT	GGACCAAGCT	GATTCCTTTT	CTGGCCGGGG	100
2B18D	ACCATACGGG	AAGCCTTGGT	GGACCAAGCT	GATTCCTTTT	CTGGCCGGGG	100
2B18A	GATGATTGCT	GTCGCAGAGC	CTACATTTC	GGGATATG		138
2B18B	GATGATTGCT	ACCATAGAGT	CTACCTTCCA	GGGATATG		138
2B18C	GATGATTGCT	ACCATAGAGT	CTACCTTCCA	GGGATATG		138
2B18D	GATGATTGCT	ACCATAGAGT	CTACCTTCCA	GGGATATG		138

FIGURE 4. Sequence similarity of the 2nd exon between *CYP2B18A* and closely related genes.

been reported to function as a gene silencer in this context [19]. Therefore, it is possible that the low constitutive levels of rat *CYP2B2* may also be negatively regulated by the presence of *LINE* sequence elements.

Sequence similarity between human *CYP2B6* and guinea pig *CYP2B18A* within the 1st and 3rd introns was higher than that present between *CYP2B2* and *CYP2B18A*, suggesting that evolutionarily, the *CYP2B18A* gene more closely resembles *CYP2B6* than *CYP2B2*. This conclusion is also supported by higher similarity in cDNA sequence composition between *CYP2B18A* and *CYP2B6* (75%), as compared to *CYP2B18A* and *CYP2B2* (74%).

The number of putative regulatory motifs between the *CYP2B18A* and the *CYP2B2* genes was compared.

Since no PBREM was detected in the intronic sequences or the 3'-flanking region of the *CYP2B2* gene, it is likely that the PBREM at ~-2.3 kbp upstream in the *CYP2B2* 5'-flanking region is the sole contributor to this gene's PB-responsiveness. However, the absence of an identifiable PBREM motif within the *CYP2B18A* gene is consistent with the low PB-inducible nature of this gene in guinea pig liver [14,15], although nonresponsiveness of this gene to the stimulation by PB should be confirmed using reporter constructs. The glucocorticoid responsive element (GRE) neighboring the PBREM in *CYP2B2* gene has been suggested to play a role in maximizing PB responsiveness [5]. Our sequence analyses were unable to localize a corresponding GRE motif in the corresponding 5'-upstream region of *CYP2B18A*. We have reported that dexamethasone has no ability to increase the liver *CYP2B18A* as well as P450 content in guinea pig [15]. The lack of GRE in *CYP2B18A* gene seems to be consistent to this previous data.

Similarly, a RBP-Jκ/NF-κB site contained within the *CYP2B2* promoter region has been suggested to maintain this gene in a constitutively repressed state [12]; however, the presence of this latter motif could not be identified within the 5'-upstream or intronic sequences of *CYP2B18A*. Since both RBP-Jκ/NF-κB and *LINE* gene sequences have been suggested to function as negative regulators of transcription, it appears possible that the absence of these sequences in the *CYP2B18A* gene is at least in part responsible for directing a high constitutive level of gene expression, in vivo. However,

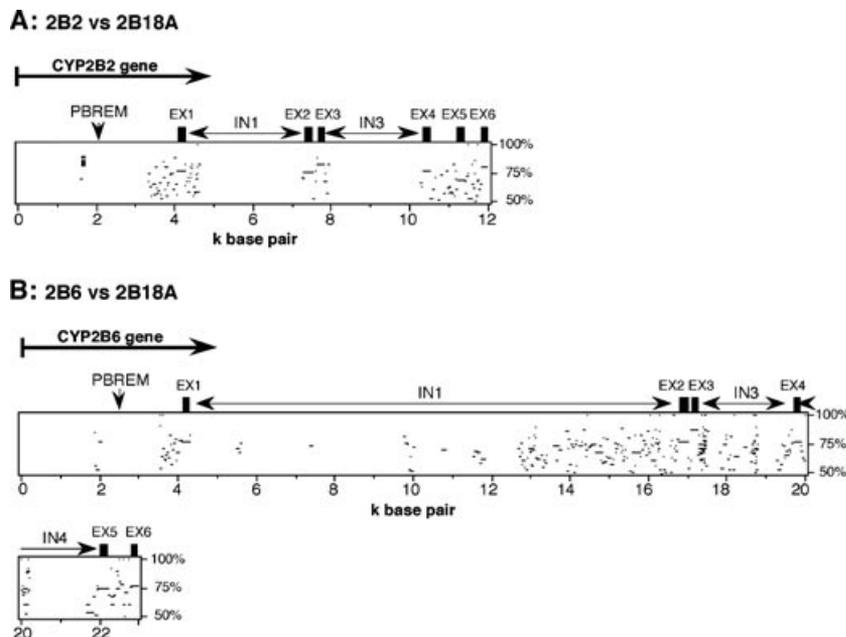


FIGURE 5. Percent identity plot between *CYP2B18A* and *CYP2B2/2B6* genes. Comparisons of 2B2 vs 2B18A (A) and 2B18A vs 2B6 (B) are shown. A reference gene, *CYP2B6*, was obtained from a database [BLAST, NCBI].

absence of *LINE* gene sequences around *CYP2B6* gene, the constitutive expression of which seems to be low, does not suggest that *LINE* gene is a sole regulator suppressing basal expression of *CYP2B* gene. It is likely that a complex interplay of regulatory regions combine to determine the constitutive and PB-inducible nature of *CYP2B* gene expression and that the sequence context of the *CYP2B* genes in different species serves to differentially modulate the respective gene expression character.

ACKNOWLEDGMENTS

We are indebted to Dr. Frank J. Gonzales, National Institute of Health, Bethesda, USA for donating *CYP2B2* cDNA. We thank Prof. Takeyoshi Miki, Fukuoka Dental College, Fukuoka 814-0193 for helpful advice for nucleotide sequencing.

REFERENCES

- Guengerich FP. Enzymology of rat liver cytochrome P-450. In: Guengerich FP, editor. *Mammalian Cytochromes P-450*. Boca Raton: CRC Press; 1987, Vol. 1, pp 1–54.
- Waxman DJ, Azaroff L. Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J* 1992;281:577–592.
- Trottier E, Belzil A, Stoltz C, Anderson A. Localization of a phenobarbital-responsive element (PBRE) in the 5'-flanking region of the rat *CYP2B2* gene. *Gene* 1995;158:263–268.
- Honkakoski P, Negishi M. Characterization of a phenobarbital-responsive enhancer module in mouse P450 *Cyp2b10* gene. *J Biol Chem* 1997;272:14943–14949.
- Stoltz C, Vachon MH, Trottier E, Dubois E, Paquet Y, Anderson A. The *CYP2B2* phenobarbital response unit contains an accessory factor element and a putative glucocorticoid response element essential for conferring maximal phenobarbital responsiveness. *J Biol Chem* 1998;273:8528–8536.
- Honkakoski P, Moore R, Washburn K, Negishi M. Activation by diverse xenochemicals of the 51-base pair phenobarbital-responsive enhancer module in the *CYP2B10* gene. *Mol Pharmacol* 1998;53:597–601.
- Honkakoski P, Zelko I, Sueyoshi T, Negishi M. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the *CYP2B* gene. *Mol Cell Biol* 1998;18:5652–5658.
- Sueyoshi T, Kawamoto T, Zeiko I, Honkakoski P, Negishi M. The repressed nuclear receptor CAR responds to phenobarbital in activating the human *CYP2B6* gene. *J Biol Chem* 1999;274:6043–6046.
- Muangmoonchai R, Smirlis D, Wong S-C, Edwards M, Phillips IR, Shephard EA. Xenobiotic induction of cytochrome P450 2B1 (*CYP2B1*) is mediated by the orphan nuclear receptor constitutive androstane receptor (CAR) and requires steroid co-activator 1 (SRC-1) and the transcription factor Sp1. *Biochem J* 2001;355:71–78.
- Wei P, Zhang J, Egan-Hafley M, Liang S, Moore DD. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* 2000;407:920–923.
- Yamada H, Matsunaga H, Tsuji K, Matsumoto S, Yamamoto M, Ishii Y, Omiecinski CJ, Oguri K. Sequence analyses of *CYP2B* genes and catalytic profiles for P450s in Qd;Sprague-Dawley rats that lack response to the phenobarbital-mediated induction of *CYP2B2*. *J Pharmacol Exp Ther* 2000;295:986–993.
- Lee SH, Wang X-L, DeJong J. Functional interactions between an atypical NF- κ B site from the rat *CYP2B1* promoter and the transcriptional repressor RBP-J κ /CBF1. *Nucleic Acid Res* 2000;28:2091–2098.
- Mani SA, Harish S, Vathsala PG, Rangarajan PN, Padmanaban G. Receptor-mediated gene delivery approach demonstrates the role of 5'-proximal DNA region in conferring phenobarbitone responsiveness to *CYP2B2* gene in rat liver in vivo. *Biochem Biophys Res Commun* 2000;268:734–739.
- Oguri K, Kaneko H, Tanimoto Y, Yamada H, Yoshimura H. A constitutive form of guinea pig liver cytochrome P450 closely related to phenobarbital inducible P450b(e). *Arch Biochem Biophys* 1991;287:105–111.
- Yamada H, Kaneko H, Takeuchi K, Oguri K, Yoshimura H. Tissue-specific expression, induction, and inhibition through metabolic intermediate-complex formation of guinea pig cytochrome P450 belonging to the *CYP2B* subfamily. *Arch Biochem Biophys* 1992;299:248–254.
- Ramsden R, Sommer KM, Omiecinski CJ. Phenobarbital induction and tissue-specific expression of the rat *CYP2B2* gene in transgenic mice. *J Biol Chem* 1993;268:21722–21726.
- Schwartz S, Zhang Z, Frazer KA, Smit A, Riemer C, Bouck J, Gibbs R, Hardison R, Miller W, PipMaker—a web server for aligning two genomic DNA sequences. *Genome Res* 2000;10:577–586.
- Schmitz E, Mohr E, Richter D. Rat vasopressin and oxytocin are linked by a long interspersed repeated DNA element (LINE): Sequence and transcriptional analysis of LINE. *DNA Cell Biol* 1991;10:81–91.
- Laimins L, Holmgren-König M, Khoury G. Transcriptional “silencer” element in rat repetitive sequences associated with rat insulin 1 gene locus. *Proc Natl Acad Sci USA* 1986;83:3151–3155.