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SHORT COMMUNICATION

Identification of *CYP2B14P* and *CYP2B16P*, Two Apparent Pseudogenes in the Rat Cytochrome P450 2B (*CYP2B*) Subfamily

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ABSTRACT. Cytochrome P450 2B3 (CYP2B3) is a member of the CYP2B subfamily and is present constitutively in rat liver. During cloning of the *CYP2B3* gene, several variant genomic inserts were isolated, exon-containing fragments of which were identified by hybridizing to CYP2B3 cDNA or exonic probes. Nucleotide sequence analysis of one set of variant inserts showed them to carry exon 1 and the 5′-flanking region of a gene designated *CYP2B14P*. The last codon of *CYP2B14P* exon 1 is a TAG translation stop codon, and thus *CYP2B14P* is a pseudogene. Three other inserts covering 34 kb of genomic sequence together carried 9 exons and the 5′- and 3′-flanking regions of the gene designated *CYP2B16P*. The 5′-splice site of *CYP2B16P* intron 1 is inactivated by the replacement of G by T in the normally invariant GT dinucleotide. Thus, *CYP2B16P* also has the characteristics of a pseudogene. The identification of *CYP2B14P* and *CYP2B16P* brings to at least seven the number of genes or pseudogenes shown by nucleotide sequence analysis to belong to the rat *CYP2B* subfamily. BIOCHEM PHARMACOL 52;6:963–965, 1996.

KEY WORDS. pseudogenes; phenobarbital; induction; *CYP2B* subfamily; cytochrome P450; constitutive

The CYP† superfamily of hemoproteins is involved in the oxidation of endogenous substrates such as steroids and fatty acids, as well as exogenous substrates such as drugs, mutagens, and carcinogens [1, 2]. A characteristic CYP feature is the presence of forms inducible by xenobiotics such as PB or methylcholanthrene, coexisting with constitutive forms [2]. Mammalian CYP proteins are classified according to their amino acid sequences into 14 families and 26 subfamilies [3].

The two major hepatic forms of the rat CYP2B subfamily, CYP2B1 and CYP2B2, are strongly induced by PB [4]. We recently isolated and characterized a third gene in the *CYP2B* subfamily, *CYP2B3* [5], which like *CYP2B1* and *CYP2B2* [6, 7] had 9 exons and 8 introns. *CYP2B3* is transcribed into a constitutive hepatic mRNA encoding a protein the deduced 491-amino acid sequence of which has 77% identity with the CYP2B1/CYP2B2 proteins [8, 9]. While cloning *CYP2B3*, we isolated several variant genomic inserts with restriction patterns different from those corresponding to *CYP2B3*, suggesting the existence of new genes [5]. We report here the further characterization of these inserts, which led to the identification of two apparent pseudogenes, *CYP2B14P* and *CYP2B16P*.

MATERIALS AND METHODS

Structure of CYP2B14P *and* CYP2B16P *Genomic Inserts*

To clone and characterize *CYP2B3*, a λEMBL4 rat genomic library was screened with probes from the 5′, central, and 3′ portions of the CYP2B3 cDNA [5]. Five variant inserts were obtained from the screen with the 5′ CYP2B3 cDNA probe, all of which hybridized with *CYP2B3* exon 1 probes, but not with probes for other *CYP2B3* exons. Screening with the central *CYP2B3* probe yielded B18, a variant insert that hybridized with probes for *CYP2B3* exons 2 to 6. Finally, B2, a variant insert hybridizing with probes for *CYP2B3* exons 7 to 9, was isolated by screening with the 3′ *CYP2B3* probe.

RESULTS AND DISCUSSION

Restriction mapping showed that the five 5′ inserts overlapped each other. They all carried the 5′-flanking region, exon 1, and part of intron 1 of a *CYP2B* gene later designated *CYP2B14P* (Fig. 1A). B18 and B2 were also found to overlap. Together they carried exons 2 to 9 and the 3′-flanking region of a *CYP2B* gene later designated *CYP2B16P* (Fig. 1B). However, no overlap was found be-

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† *Abbreviations:* CYP, cytochrome P450; and PB, phenobarbital.

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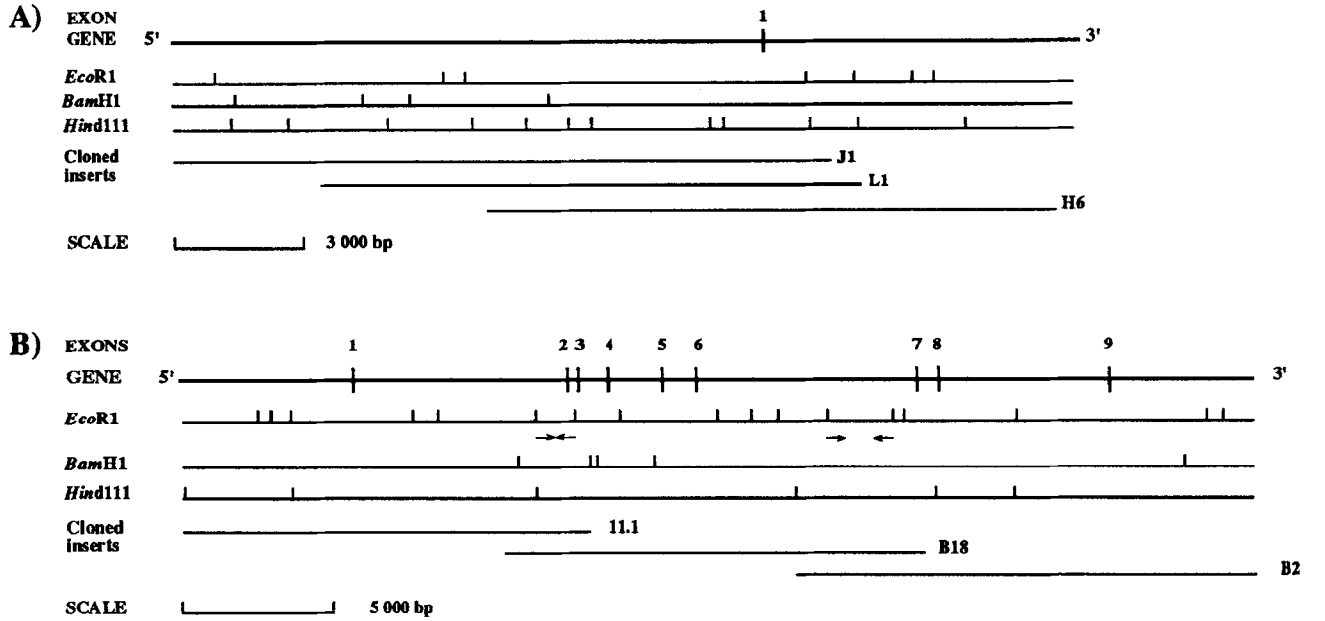

FIG. 1. Restriction maps of *CYP2B14P* (A) and *CYP2B16P* (B) including the lengths of representative cloned inserts. Methods of screening and analysis of genomic clones as well as restriction mapping and DNA sequencing have been described [5]. In addition to the 5', central, and 3' CYP2B3 cDNA probes described previously [5], a 450-bp AccI genomic fragment of insert B18 from *CYP2B16P* intron 1 was used to screen the genomic library to obtain clone 11-1. The overlaps in *CYP2B16P* introns 1 and 6 were confirmed by sequence analysis (see horizontal arrows in the figure). Nucleotide sequences were analysed using the University of Wisconsin GCG programs [10].

tween the five 5' inserts and B18. Be rescreening the genomic library with a *CYP2B16P* intron 1 probe, we isolated a clone the insert of which (11-1) overlapped with B18 but not with the 5' inserts. Furthermore, 11-1 carried exons 1, 2, and 3 as well as the 5'-flanking region of *CYP2B16P* (Fig. 1B), thus demonstrating that *CYP2B14P* and *CYP2B16P* are indeed two distinct genes. Insofar as comparisons are possible, the *CYP2B14P* and *CYP2B16P* restriction maps appear to be different from those reported for other cloned rat *CYP2B* genes [5–7, 11–13].

Sequence Analysis of CYP2B14P *and* CYP2B16P

By DNA sequence analysis, the last codon of *CYP2B14P* exon 1 was found to be a TAG stop codon, the presence of which was confirmed in three independently isolated cloned inserts. This indicates that *CYP2B14P* is a pseudogene. The nucleotide sequence of *CYP2B16P* exons 1 to 9, the intronic boundaries, and the 5'- and 3'-flanking regions were determined. The eight introns were found to be inserted at positions precisely equivalent to those of other CYP family 2 genes [5–7, 14, 15], except that the first codon of *CYP2B16P* exon 6 is deleted. The 5'-splice site of *CYP2B16P* intron 1 is abolished by the replacement of G by T in the normally invariant GT dinucleotide. Mutational replacement of this G by T or A completely inactivates the 5'-splice site of the human β-globin gene [16, 17], and no functional 5'-splice sites beginning with TT were found among more than 7500 natural splice sites examined [18, 19]. This indicates that *CYP2B16P* cannot direct the synthesis of a typical CYP2B protein and thus has the properties of a pseudogene, although the formal possibility that a nearby cryptic splice site may be functional cannot be excluded. The Genbank accession numbers for *CYP2B14P* and *CYP2B16P* are U33540, U33541, U33542, U33543, U33544, U33545, and U33546.

The nine *CYP2B16P* exons and the single cloned *CYP2B14P* exon define open reading frames encoding virtual proteins of 490 and 56 amino acids, respectively. The virtual CYP2B16P protein shares 81% identity with CYP2B1 and 75% with CYP2B3, while the virtual peptide encoded by *CYP2B14P* shares 70, 82, and 77% identity with the peptides encoded by exon 1 of *CYP2B1*, *CYP2B3*, and *CYP2B16P*, respectively. Thus, *CYP2B14P* and *CYP2B16P* are new members of the *CYP2B* subfamily.

Previous work led to the cloning and characterization at the DNA sequence level of all nine exons of four rat *CYP2B* genes [*CYP2B1* [6], *CYP2B2* [7, 12], *CYP2B3* [5, 20], and *CYP2B15* [21] (which appears to be the same as the gene designated CYP2B8 by Giachelli *et al.* [13])] as well as two gene fragments (*CYP2B12* [12, 22] and *gene 3* of Atchison and Adesnik [12, 20]). By sequence, *CYP2B16P* is different from all six of these, and *CYP2B14P* is different from at least five (since their cloned portions do not overlap, *CYP2B14P* and *gene 3* may be the same gene). Thus, the present cloning of *CYP2B14P* and *CYP2B16P* brings to at least seven the number of sequenced rat *CYP2B* genes or gene fragments. Other rat *CYP2B* genes have been cloned [11, 13] but have yet to be characterized.

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