Sequence and gene expression of rabbit cytochrome P450 IIC16: comparison to highly related family members

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ABSTRACT

Cytochrome P450s are heme containing proteins which evolved from an ancestral gene(s) to form a large superfamily of enzymes. We have isolated a unique cDNA from the rabbit P450 IIC subfamily, IIC16, which is 2028 bp in length. Nucleotide sequence determination indicated an ATG start codon 66 bp from the 5' end of the molecule, and an open reading frame coding for a protein of 487 amino acids. P450 IIC16 protein is $\geq 90\%$ identical in sequence to rabbit P450 IIC4, IIC5, and to the partial sequence available for IIC15. Northern and slot blot experiments demonstrated that the P450 IIC16 gene is expressed constitutively in liver, lung, testes, and kidney, and is inducible by phenobarbital in each tissue with the exception of the kidney, where mRNA levels are repressed. Alignment analysis of eight rabbit P450 IIC proteins revealed conserved and variable regions common to all IIC enzymes, and specific areas are suggested which may be important with respect to structure and function.

INTRODUCTION

Cytochrome P450s have been identified in procaryote and eucaryote organisms, implying the existence of an ancestral precursor gene approximately 1.4 billion years ago (1). Through duplication events the putative precursor gene has expanded to include a large superfamily which is currently subdivided based on protein sequence comparisons into eight vertebrate families (2). The total number of cytochrome P450 genes within a mammalian species is not known, but estimates of greater than 50 have been suggested (3,4). The extensive array of cytochrome P450 genes which have evolved must certainly be a consequence of the essential and diverse metabolic functions of these enzymes.

The mammalian P450 cytochrome IIC¹ genes are of special interest for several reasons. Southern blot data from studies with rat (5), rabbit (6,7), and human (8) DNA indicate that the cytochrome P450 IIC subfamily contains multiple members in each species. The genes in this group are usually expressed constitutively, but the expression patterns can be modulated by a variety of factors. In addition to developmental regulation (5), certain genes such as P450 IIC1, IIC2 (9), IIC4 (10), IIC6 (11), and form 1a (6) are inducible by phenobarbital (PB)². In

contrast, rabbit cytochrome P450 IIC5 (7), which catalyzes an unusual hepatic 21-hydroxylation of progesterone (12) and is 94% identical to IIC4 (13) in amino acid sequence, is not inducible by PB (14). Also striking are the rat cytochrome P450 genes IIC11 (15), IIC12 (16), and IIC13 (17), which are expressed in a sex-dependent manner in adult animals. The human P450 genes IIC8 (8) and IIC10 (18) have been characterized, and the latter has been shown to be polymorphic (19).

Previously, we designed a consensus oligomer (the PB mer), targeted to a highly conserved nucleotide sequence in the P450 IIC and IIB subfamilies, and demonstrated its utility for the isolation of cytochrome P450 cDNAs (6). The PB-mer facilitated the identification of a novel cDNA clone, termed P450 1a, which contained partial coding sequence for a cytochrome P450 in the rabbit IIC subfamily (6). Here we report the isolation and sequence of a full-length cDNA coding for P450 1a (now designated P450 IIC16). The constitutive and phenobarbital-induced gene expression patterns of cytochrome P450 IIC16 in rabbit liver and various extrahepatic organs are determined. The amino acid sequence of eight rabbit P450 IIC proteins is compared, and regions likely to be involved in enzyme structure and function are identified.

MATERIALS AND METHODS

Radiolabeled hybridization probes

A 3'-noncoding fragment generated by restriction digestion with BglII and SspI was excised from pP450 1a (6). The 300 bp fragment had no significant sequence homology (<50%) with any reported cytochrome P450 sequence, or any other DNA sequence contained in the GenBank data base (release 61). Radiolabeling was accomplished as described (20,21).

The PB-mer, an 18 base oligomer with redundancies at 3 positions, 5'-TCC TG[T/C] [T/G]GA GAA [G/A]GG CAT-3', is complimentary to a conserved sequence with known occurrence in 15 distinct P450 IIB and IIC mRNAs of rat, rabbit, and human. This oligomer was used for the second round of library screening and also as a sequencing primer. A 20 base oligomer, 5'-CAC CTC TAG CGG CGC AAT AC-3', with complimentary sequence to mammalian 18s ribosomal RNA was synthesized as described (22) and employed as a hybridization probe to standardize slot blot results. Oligomers used as hybridization

¹The nomenclature for the members of the P450 gene superfamily is as proposed by Nebert, *et al.* (2).

²The abbreviations used are PB, phenobarbital; SSC, standard sodium citrate; SDS, sodium dodecyl sulfate.

probes were end-labeled with T₄ kinase and ³²P as described previously (6).

Identification and characterization of P450 IIC16

A liver cDNA library in lambda gt11, prepared from adult male and female New Zealand White rabbit RNAs (Clontech Laboratories, Inc., Palo Alto, CA), was used. 350,000 plaques were screened for the presence of P450 IIC16 insert DNA using the pP450 1a BglII/SspI fragment. Approximately 150 positive hybridization signals were obtained. Of these, six plaques were rescreened at low density with the PB-mer. DNA from two plaques was digested with EcoRI and subcloned into pUC13. Insert DNA was sequenced in these plasmids using Sequenase (United States Biochemical, Cleveland, OH) and universal forward and reverse pUC sequencing primers. Additional primers were synthesized based on sequence derived from the insert DNA and used to facilitate sequence analysis of both strands. One of the two isolates appeared to be full-length and was sequenced at least three times on both strands. The other isolate contained identical DNA sequence through the regions of comparison.

RNA blot analysis

RNA was isolated (22) from 2 sets of male New Zealand White rabbits (2/group in one set; 3/group in the other). For Northern blot analysis, 20 µg of total RNA from the livers of control or PB-treated rabbits was electrophoresed in a 1.15% agarose formaldehyde gel and transferred to Genescreen Plus nylon membrane as per manufacturer's instructions (Dupont, Boston, MA). An RNA ladder (BRL, Gaithersburg, MD) was used as a molecular size standard.

Various concentrations of total RNA isolated from liver, lung, kidney, and testes also were slot-blotted (Schleicher & Schuell, Inc., Keene, NH) on a nylon membrane. A radiolabeled BglII/SspI fragment, isolated from a cDNA for cytochrome P450 IIC16, was used as a specific probe. Membranes were washed at a final stringency of 0.1× SSC/ 0.1% SDS at 50°C, and exposed for 3.5 days with 2 intensifying screens. Densitometric analysis of the autoradiogram was used to assess relative levels of cytochrome P450 IIC16 mRNA.

Subsequently, membranes were dehybridized with 0.05× SSC/0.1% SDS at 95°C, 3× for 10 min each. Blots were then hybridized to the rRNA oligomer at low specific activity (50,000 dpm/pmol/ml of hybridization solution) at 54°C as previously described (22). Membranes were washed in 5× SSC/0.1% SDS at 54°C, and exposed for four hours with one intensifying screen.

Computer analysis

Nucleotide sequences were compiled, and protein sequences deduced, using GenePro Software programs (Riverside Scientific, Seattle, WA). Analyses of slot blots were performed using a model GS300 scanning densitometer with GS350 software (Hoeffer Scientific Instruments, San Francisco, CA). A matrix procedure, CLUSTAL (23), was used to compare nucleotide and protein sequences from the P450 IIC subfamily.

All other materials and methods were as described previously (6,22).

RESULTS AND DISCUSSION

Cytochrome P450 IIC16 Isolation and Description

A ³²P labeled BglII/SspI 3'-noncoding fragment from pP450-1a (6), highly specific for this clone, was used to screen a rabbit

CGGCATCGGT	ACCAATAAAG	ATAGACAGTG	CTACTCAGAA	ATCCAAGAAA	50
ATGGTGGGAG	AAATAATGGA	TCCAGTTGTG	GTCTCGGTGT	TGGGTCTCTG	100
CTGTTTGCTT	CTCCTTTCAC	ACTGGAAGCA	AAATTCGGG	AGGGGGAAGC	150
TCCTTCCCGG	CCCCACTCCT	TTCCCCATTA	TTGGAATAT	TCTCCAGATA	200
GATGCTAAGG	ACATCAGCAA	ATCCCTAACT	AAGTCTCAG	AACGCTATGG	250
CCCCGTGTTC	ACTGTGTATC	TGGGCATGAA	GCCCGCTGTA	GTGCTGCATG	300
GATACCAGGC	AGTGAAGGAG	GCCCTGGTGG	ATCTTGGAGA	GGAGTTTGCT	350
GGAAGAGGCA	GTTTTCCTAT	GCTTGATAAA	GTTAGTAAGG	GACTCGGAAT	400
CGTTTTCACC	AATGGAAGA	GATGGAAGA	GATCCGCGCG	TTCTCGCTCA	450
TGACCCTGCG	GAATTTCCGG	ATGGGGAAGA	GGAGCATTTA	GGACCGAGTT	500
CAAGAGGAGG	CCCGCTGCCT	GCTGGAGGAG	CTGAGAAAA	CCAACGCCTC	550
ACCCTGTGAT	CCCACCTTTA	TCTTGGGCTG	TGCTCCCTGC	AATGTGATCT	600
GCTCCATTAT	TTTCCATAAT	CGCTTTGATT	ATAAGATGA	GGAGTTTCTT	650
AAACTATTGG	AAAAATTCAA	TGAAAAATGT	AGGATTCTGA	GTCTCCCATG	700
GTTGCAGGTC	TGCAATAATT	TCCCTGCTCT	TATTTGATTAC	TTACCAAGAA	750
GTCAATAAGC	CTTACTAAAG	AATCTGATTT	ATGTGAAAA	TTTTATTATG	800
GAGAAGTGA	AGGAACACCA	AAAAATTCCTG	ATGCTTAACA	ATCCTCGGGA	850
CTTTATAGAT	TGTTTCTTGA	TCAAAATGGA	GCAGGAAAA	CATTGTGGAGT	900
TCACTCTTGA	AAGCTTGGTA	ACCACCTGCT	TTGATTGTGT	TGGAGCTGGG	950
ACTGAGACAA	CGAGCACAAC	GCTGAGATAC	TCCTCTCTGA	TCCCTCTGAA	1000
GCACCCGAGG	GTGCGAGATA	AAGTGCAGGA	GGAGATTGAG	CGTGTGATTG	1050
GCAGGCACCG	GAGCCCTGCG	ATGCAGGACA	GGAGCCGCAT	GCCTTACACA	1100
GATGCCGTAA	TACATGAGAT	CCAGAGATTG	ATTGACCTGG	TCCCAATATA	1150
TCTGCCCCAC	ACAGTGACCC	GTGACATTAA	ATTCAGAAAC	TACTTTATCC	1200
CCAAGGGTAC	GGACATCATG	ACATCACTGA	CATCCGTGCT	ACATGATGAA	1250
AAAGGCTTTC	CTAACCCAAA	GGTATTGTAC	CCCTGGACAT	TTCTGGATGA	1300
GAGTGGCAAC	TTCAAGAAAG	GTGACTACTT	CATGCTTTTC	TACGACGAGA	1350
AACGGATCTG	TGTGGGAGAG	GCCCTGGCCC	GCATGGAGCT	GTCTTTGTTC	1400
CTGACCTCCA	TTTTCAGAAA	CTTTAACTGT	CAATCTCTGG	TTGAGCCAAA	1450
GGACCTGGAC	ATCACTGCAG	TTCTCAATGG	ATTGTTTCTT	GTGCCACCTT	1500
CGTTCCAGCT	CTGCTTCATT	CCTGTTTGA	AAGGAGCAGA	CTGGCTTCTA	1550
CTGTGCCATC	ATTTCAAAAG	CATTGGCCAT	CACCTTACTG	CATTGTGAGC	1600
ACTTCTTTAA	CTTTTCTCAC	ATCTTACTAT	TCCTTTAAGA	TCTAGTGAAA	1650
ACCTAACTTC	TGTGGGTGAT	CCCCTGAGAC	TGCTTCCCTT	GACCATGCAA	1700
GAGGTAGAGA	GGGCATGGCA	AGCCATGGTC	CTGGGAGGGA	CCCCACAGCC	1750
TGGCTGCTGG	CAGGTGGCGG	GACCCAGGCA	CATTTCTCTC	CTGGCTTCCC	1800
TGTCAGGTAA	ACTGCTCCTA	GCTGTGTCCA	AAGCCCATCA	AGAAAGCTAC	1850
CGTAGGCTAT	GTGACCTTCA	AGATGATTGT	AGGAGCATAT	CAGTACCAAT	1900
ATTGCTCTTA	TCCTATAGAA	TTAGTATGCG	CCTGAATTAG	TTACACCTTT	1950
TCCTGCTGCC	CTTTAGAAAG	TGTGATGCTC	CATTAAATAA	GTGGATGCAT	2000
TCACTGAAAA	AAAAAAAAAA	AAAAAAAAAA			2028

Fig. 1. Nucleotide sequence of rabbit P450 IIC16. Initiation and stop codons are indicated by double underline. Nucleotides 66–1526 encode an open reading frame for 487 amino acids. A typical polyadenylation signal, 22 bases from the beginning of the poly A tail, is single underlined. The sequence of the PB-mer, used for the isolation of pP450-1a (6) as well as for sequencing, is in bold type.

liver cDNA library. DNA from two positive clones was isolated, subcloned into pUC13, and subjected to dideoxy chain termination sequence analysis (24). Both clones were found to encode cytochrome P450 IIC16; one cDNA extended approximately 350 base pairs farther 5' and its deduced amino acid sequence encoded a protein extending to the amino terminal methionine.

The nucleotide sequence determined for the cytochrome P450 IIC16 cDNA³ was 2,028 base pairs long and is shown in Fig. 1. The translational start site codon (ATG) was positioned 66 bp from the 5' end of the DNA molecule. An open reading frame, coding for 487 amino acids, was followed by a typical termination codon (TGA) at base 1527. An unusually long 3' noncoding region of more than 500 bases terminated with a polyadenylation signal (AATAAA) 22 bases upstream from the beginning of the poly A tail. This noncoding sequence was longer and demonstrated little sequence similarity to the structurally similar cytochrome P450 mRNAs, IIC4 (13), IIC5 (7), and IIC15 (25). The presence of unique 3' sequence in the P450 IIC16 mRNA provides evidence that a distinct gene, not an allelic variant, codes for this protein.

Rabbit Cytochrome P450 IIC Protein Comparisons

As predicted earlier (6), the deduced amino acid sequence of the cytochrome P450 IIC16 cDNA shares marked similarity to other rabbit P450 sequences. P450 IIC4 (13) and IIC5 (7), as well as

³This sequence has been submitted GenBank and is assigned accession Number M29968.

Fig. 2. Alignment of rabbit P450 IIC proteins. Beginning with the top line of the Fig., P450 IIC3 (26,28), IIC2 (26,29), IIC1 (26), are aligned with IIC14 (27), the reference sequence. Line 5 displays the deduced amino acid sequence of P450 IIC16, the reference for IIC5 (7), IIC4 (13), and IIC15 (25). Only amino acids differing from the reference sequences are shown. The sequences for IIC1 and IIC15 are not complete, and alignment begins with the designation '>'. Maximal alignment between all sequences is achieved by the insertion of a 1 or 3 residue gap in the sequence of P450 IIC3 or IIC16, IIC5, IIC4 and IIC15, respectively. Invariant residues between all proteins are indicated by bold type. Regions of high conservation are shown with light grey. Areas of high variability are shaded in dark grey.

the partial sequence for a recently reported rabbit P450, IIC15 (25) share at least 90% identity with IIC16 in amino acid sequence.

These four proteins can be aligned, with no gaps, and each is predicted to be 487 amino acids long (Fig. 2, lower four lines). Cytochrome P450 IIC16 was arbitrarily chosen as the reference protein sequence for comparison to the other two full-length sequences. Only residues differing from P450 IIC16 are shown below that sequence; alignment with cytochrome P450 IIC15 begins at amino acid 111.

Four other rabbit cytochrome P450 IIC cDNA sequences have been reported (26,27). Utilizing protein sequence data derived for P450 IIC3 (28) and genomic sequence for P450 IIC2 (29), the complete sequence for three of these proteins is available. The top four lines of Fig. 2 presents the alignment of these four proteins; cytochrome P450 IIC14 (27) was selected as a reference sequence. Cytochrome P450 IIC3 is the most dissimilar protein with only 65–66% similarity to the other three enzymes. Of note, there is greater variability in this group of proteins (ranging from 65–83%) compared to the four P450 IIC proteins presented in the bottom 4 lines.

The eight rabbit cytochrome P450 proteins displayed in Fig. 3 can be grouped into two distinct sets based upon similarities.

In one group (top 4 lines) three of the proteins are 490 amino acids in length, while the fourth (IIC3) is 489. In the second set (bottom 4 lines) each sequence is predicted to contain 487 residues. A gap inserted beginning at residue 276/277 permits optimal alignment of all eight proteins. The amino acids shown in Fig. 2 which are invariant in the known sequence of all eight proteins are in bold type. There are a total of 242 such residues, yielding a remarkable 50% absolute conservation.

The close similarity between rabbit P450 IIC proteins suggests that relatively recent gene duplication events gave rise to these enzymes. The fact that P450 IIC proteins 487 amino acids in length have yet to be characterized other species suggests that orthologous proteins may not exist in rats or humans.

Each of the rabbit cytochrome P450 IIC proteins contains approximately 20 N-terminal hydrophobic residues which probably act as a noncleaved leader sequence for insertion into the microsomal membrane (30). In addition, there is strong conservation of some amino acids immediately following the putative membrane insertion residues. Notably, there are 5 proline residues absolutely conserved from positions 30–37. Furthermore, the analogous positions in all P450 IIC proteins are proline residues. Proline is a helix-terminating amino acid (31) and is unlikely to occur in a membrane environment. These

Fig.3. Gene expression analysis of rabbit P450 IIC16. Panel A. Twenty µg of total RNA isolated from untreated (C) or phenobarbital-exposed rabbits was electrophoresed in a 1.15% formaldehyde gel and transferred to a nylon membrane. Probing with a BglII/SspI fragment isolated from a P450 IIC16 cDNA identified a single 2.2 kb band in control rabbits which was induced following phenobarbital treatment. Panel B. Total RNA from control and phenobarbital-treated rabbit liver, lung, kidney, and testes was applied to a slot blot and hybridized to the BglII/SspI P450 IIC16 probe. The amounts applied were 5, 10, or 20 µg for liver, lung, and kidney tissue; ten µg of testes RNA was blotted. Panel C. The blot shown in Panel B was dehybridized and re-probed with an oligomer complementary to mammalian 18S rRNA. Yeast tRNA at 5, 10, or 20 µg was employed as a negative control in Panels B and C.

observations argue that cytochrome P450 proteins contain only one N-terminal membrane-spanning domain, as has been proposed for microsomal cytochrome P450 IIB1 (32,33).

Other regions in the rabbit P450 IIC protein sequences demonstrate particularly enhanced conservation, and it is possible that these areas participate in functions common to P450 enzymes. Amino acids 404/407–447/450 (light grey shading in Fig. 2) are 82% invariant, and surround a cysteine residue which is absolutely conserved in each eucaryote cytochrome P450 examined (3,34). This cysteinyl fragment acts as the fifth axial ligand to the prosthetic heme moiety (35), critical for the function of P450 proteins.

Association with the electron donor NADPH-cytochrome P450 reductase is another feature common to eucaryotic microsomal cytochrome P450 proteins (35). Although residues important for the interaction between cytochrome P450 and reductase have not been identified, it is likely they will be reflected as conserved sequence. Two such segments are identifiable in the rabbit P450 IIC proteins and one or both of these may participate in P450 reductase interaction. The first region, encompassing amino acids 120–192, is highly conserved with all but six residues being either absolutely retained or conservatively substituted. A second region, from residues 320/323 to 354/357, contains very little absolute variation (<17%). The importance of this region is

further suggested by a complete conservation of 66% of these amino acids when all reported P450 IIC proteins (2) are aligned (data not shown). Based upon alpha-helical comparisons between mammalian P450s and bacterial P450_{cam} (P450 CI), it was suggested that regions analogous to those identified above in the rabbit P450 IIC proteins may represent binding sites for cytochrome P450 reductase (36).

Rabbit P450 IIC protein sequences also demonstrate hypervariable areas which may be important for interaction of enzyme and substrate. The x-ray crystallographic structure of bacterial P450_{cam} has been determined, and two regions important for substrate binding have been identified in this enzyme (37,38). This information permits a comparison to analogous segments of the rabbit P450 IIC proteins. Poulos and coworkers identified two residues (Tyr 96 and Val 295) in P450_{cam} which they suggested were critical for interaction with substrate (37). Furthermore, they speculated that residues surrounding these amino acids would be expected to be highly variable in different P450 enzymes in order to accommodate a variety of substrates (37). The two areas surrounding the analogous residues in the rabbit P450 IIC proteins are indicated with dark shading in Fig. 2. Region I, extends from amino acids 98–119 (for Tyr 96) and region II encompasses residues 358/361–377/380 (for Val 295). Clearly, these segments are hypervariable compared to other areas of the proteins.

The significance of region I for substrate interaction has been substantiated by two recent reports. Kronbach *et al.* (39) substituted for amino acids 113, 115, and 118 in P450 IIC4 and showed that these changes were sufficient to alter the substrate specificity normally associated with this enzyme. Region I was also shown to be important as a substrate binding site for P450 IIC2. Employing chimeric expression constructs with fragment substitution of IIC14 for IIC2 at residues 90–125, it was determined that reactivity with normal substrate was completely abolished (40).

An third region of considerable variation in the eight rabbit P450 IIC proteins in the vicinity of amino acids 193–249 is also apparent. Employing chimeric cDNA constructs, Imai and coworkers demonstrated that this general region, encompassing amino acid residues 211–262 of P450 IIC2, is important, though not essential for substrate binding (40,41). The three regions of hypervariability identified in the rabbit P450 IIC proteins probably interact together in three dimensional space and permit substrate association with the enzyme active site.

Expression of Rabbit Cytochrome P450 IIC Genes

Cytochrome P450 IIC16 gene expression was examined in hepatic tissue using Northern blot analysis. Cytochrome P450 IIC16 was expressed constitutively, and induced following exposure to PB (Fig. 3a). These results are in confirmation with our previous observations (6). Using an RNA ladder as the molecular size standard in the present study, we estimate the size of the *in vivo* mRNA transcript to be 2.2 kb.

Total cellular RNA was applied to slot blots and used to evaluate cytochrome P450 IIC16 gene transcription in liver, lung, kidney, and testes. Fig. 3b shows that in every organ examined, the gene for cytochrome P450 IIC16 is expressed constitutively. Furthermore, following treatment with PB, cytochrome P450 IIC16 mRNA is modulated differentially. In liver, lung, and testes PB treatment results in increased levels of cytochrome P450 IIC16 mRNA. However, exposure to PB repressed P450 IIC16 mRNA expression in the kidney.

An oligomer probe complimentary to 18s rRNA was used to normalize any irregularities of loading the RNA slot blot (Fig 3c). With densitometric analyses, the autoradiographic signal areas in Fig. 3b were standardized and the relative P450 IIC16 mRNA content of each organ was quantified. In control animals RNA expression was greatest in liver followed by kidney, testes and lung, respectively. Following treatment with PB, P450 IIC16 mRNA levels increased approximately 6.4 fold in liver, 1.8 fold in lung, and 4.1 fold in testes. In the kidney P450 IIC16 mRNA levels decreased by a factor of 0.6 following PB exposure.

These data indicate that P450 IIC16 gene regulation is distinct and more complex in comparison to the cytochrome P450 IIC4 and IIC5 genes. For example, P450 IIC5 is not expressed in kidney (42), nor is it PB inducible in liver (14). Although inducible by PB in rabbit liver, P450 IIC4 mRNA is not detectable in lung, kidney (10) or testes⁴ either before or following PB treatment. The regulatory features which account for the unique expression patterns observed for these very similar P450 proteins remain to be determined.

The genetic expression of cytochrome P450 IIC16 can also be contrasted with other rabbit IIC proteins, which have been shown to be differentially regulated. P450 IIC1 is not constitutively expressed in hepatic tissue but is inducible by PB (9). P450 IIC3 mRNA is present in control rabbits, however there is no induction following PB exposure (9). In contrast, P450 IIC2 is both constitutively expressed and PB-inducible in hepatic and kidney tissue (9). None of these genes appears to be expressed in pulmonary tissues of control or PB-treated rabbits (9). Of the rabbit cytochrome P450 IIC genes examined, only IIC16 has been shown to be expressed in lung and testes, where it is also inducible by PB.

The metabolic function of P450 IIC16 was not characterized in this study. However, two lines of evidence suggest that P450 IIC16 does not catalyze the liver microsomal 21-hydroxylation of progesterone as does the very similar protein IIC5 (12). First, it has been reported that the rate of this enzymatic activity is not substantially affected by PB (43), yet P450 IIC16 mRNA is markedly inducible by PB. Also, two of the three critical amino acids (i.e. positions 113 and 115) that apparently confer substrate specificity to P450 IIC5 are substituted with residues identical to those found in IIC4, and the latter isozyme has virtually no liver progesterone 21-hydroxylase activity (39).

In summary, the data presented enabled characterization of a novel cDNA clone for a rabbit cytochrome P450, IIC16. The isolation was achieved using a consensus oligomer probe, targeted for conserved sequence in the P450 IIC and IIB subfamilies. The P450 IIC16 gene was expressed in each organ examined (liver, lung, testes, and kidney), but the respective mRNA levels were differentially modulated in the organs following exposure to PB. Comparison of highly related members in the rabbit P450 IIC subfamily identified regions likely to be important for enzyme structure and function. *In vitro* expression studies coupled with *in situ* mutagenesis experiments will enable questions of substrate specificity and the importance of conserved domains in P450 IIC16 to be addressed.

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