SHORT COMMUNICATION

Isolation, Characterization, and Chromosomal Mapping of Mouse P450 17α-Hydroxylase/C17-20 Lyase

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Cytochrome P450 17α-hydroxylase/C17-20 lyase (P45017α) catalyzes the conversion of C-21 steroids to C-19 steroids in gonads. A full-length mouse cDNA encoding P45017α was isolated from a mouse Leydig cell library and characterized by restriction mapping and sequencing. The predicted amino acid sequence has 83% homology to rat, 66% homology to human, and 62% homology to bovine P45017α amino acid sequences. The protein is 507 amino acids in length, which is 1 amino acid shorter than the human protein and 2 amino acids shorter than the bovine protein. The structural gene encoding P45017α (Cyp17) was localized utilizing an interspecific testcross to mouse chromosome 19, distal to Got-1. Another cytochrome P450, P4502c (Cyp2c), also is located at the distal end of chromosome 19. CYP17, CYP2c, and GOT1 have been mapped to human chromosome 10, with CYP2C and GOT1 mapped to the distal region, q24.3 and q25.3, respectively. The data in the present study indicate conserved syntenic loci on mouse chromosome 19 and human chromosome 10 and predict that the structural gene encoding P45017α will be found distal to GOT1 on human chromosome 10.

The biosynthesis of testosterone in Leydig cells of the testis involves the action of two cytochrome P450 enzymes, cholesterol side-chain cleavage (P450sec) and 17α-hydroxylase/C17-20 lyase (P45017α), (Payne, 1990). The initial and rate-limiting step in the biosynthesis of steroid hormones is catalyzed by P450sec, an inner mitochondrial enzyme that cleaves the side chain of cholesterol to yield the C-21 steroid, pregnenolone. Synthesis of androgens from C-21 precursors requires the activities of P45017α, which is associated with the smooth endoplasmic reticulum. P45017α is a single protein that catalyzes two distinct reactions, the hydroxylation of the C-21 steroid progesterone or pregnenolone (17α-hydroxylase activity), followed by cleavage of the two-carbon side chain (C17-20 lyase activity) to yield the C-19 steroid androstenedione or dehydroepiandrosterone, respectively (Fevold et al., 1989). Androstenedione is the immediate precursor of testosterone. Previous studies from this laboratory demonstrated that the expression of these two P450 enzymes is differentially regulated in mouse Leydig cells (Anakwe and Payne, 1987; Payne, 1990). P450sec is constitutively expressed. Treatment of Leydig cell cultures with cAMP increases steady-state levels of P450sec mRNA and protein synthesis. In contrast, cAMP is obligatory for de novo synthesis of P45017α (Hales et al., 1987; Anakwe and Payne, 1987) and accumulation of P45017α mRNA (Payne and Sha, unpublished data). We reported previously that the structural gene for P450sec (Cyp11a) is located on mouse chromosome 9, closely linked to two other genes expressing P450 enzymes, Cyp19, the structural gene that encodes P450 aromatase, and Cyp1, the gene that expresses aryl hydrocarbon hydroxylase (Youngblood et al., 1989). In a subsequent study we demonstrated that quantitative differences in the amount of Leydig cell P450sec enzyme protein are regulated by either the structural gene Cyp11a or a closely linked regulatory gene (Nolan and Payne, 1990).

To further our knowledge of the genetic and hormonal regulation of P45017α expression in Leydig cells, a mouse Leydig cell λgt11 library (Bain and Payne, unpublished data) was screened with a full-length rat P45017α cDNA obtained from Dr. Richard Fevold (Fevold et al., 1989). A clone containing a 1.7-kb insert was isolated and subcloned into Bluescript for further analysis. Figure 1 shows the restriction map of the...
FIG. 1. Partial restriction map and sequencing strategy of mouse P450, cDNA. The solid bar represents the coding sequences. The open bars at each end represent the 5' and 3' untranslated regions. Arrows represent regions sequenced using double-stranded dideoxy sequencing of fragments cloned into Bluescript. Sequences represented by the dashed arrow were obtained using a synthetic oligonucleotide as a primer. Restriction endonuclease abbreviations: P, PvuII; K, KpnI; E, EcoRI; B, BamHI; S, SacI; and Bc, BclI.

mouse P450, and the strategy used for sequencing. The complete nucleotide sequence and the predicted amino acid sequence are given in Fig. 2. The full-length P450, clone contains an open reading frame of 1521 nucleotides, 32 5' untranslated nucleotides, and 143 3' untranslated nucleotides. A putative poly(A) addition signal is located 11 nucleotides upstream of a 17-nucleotide-long poly(A) tail. The predicted protein is 507 amino acids in length.

In Fig. 3, the complete amino acid sequence of mouse P450, is compared with that of rat (Fevold et al., 1989), human (Chung et al., 1987), and bovine (Zuber et al., 1986). The mouse and rat amino acid sequences are 83% identical, while the human (66% identical) and bovine (62% identical) amino acid sequences are considerably less homologous to the mouse sequence. The mouse and rat sequences contain one less amino acid than the human and two less amino acids than the bovine. Regions of high homology common to members of the P450 gene family are shown boxed in Fig. 3. They are the putative heme binding region (aa 434-454) (Gotoh et al., 1983); the \( \alpha \)1s tridecapetide sequence (aa 343-372) (Ozols et al., 1985), which may play a role in substrate specific-

<table>
<thead>
<tr>
<th>Locus</th>
<th>Emu-22</th>
<th>Got-1</th>
<th>Cyp17</th>
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<tr>
<td></td>
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<td></td>
<td>Number</td>
</tr>
<tr>
<td>Emu-22 scored</td>
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<tr>
<td>C (^a)</td>
<td>C</td>
<td>C</td>
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<td>M (^b)</td>
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<td>Total</td>
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\(^a\) Segregation data for Emu-22 and Got-1 were reported previously (15).

\(^b\) Alleles inherited from the CAST/Ei and MEV strains are denoted by the letters C and M, respectively.

\(^*\) An \( x \) is used to denote regions of recombination.

**TABLE 1**

Linkage of Cyp17 to Emu-22 and Got-1 on Chromosome 19 in CAST/Ei x MEV Testcross

<table>
<thead>
<tr>
<th>Emu-22</th>
<th>Got-1</th>
<th>Cyp17</th>
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\(0.213 \pm 0.047\) (Recombinants/total)

\(0.064 \pm 0.025\) (Recombination frequency ± standard error)
FIG. 2. Nucleotide and predicted amino acid sequence of mouse P450.

The putative poly(A) addition signal is underlined. Numbers on the left and under the line indicate the amino acid position. Numbers on the right, above the line and in parentheses, indicate the nucleotide position.
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FIG. 3. Comparison of the predicted amino acid sequences of mouse, rat, human, and bovine P450. Boxed regions indicate conserved sequences common to members of the P450 gene family: the N-terminal hydrophobic region, the unique P450 sequence (amino acids 296-319); the Glycine-rich region (343-372), and the heme binding region (434-454). Diamonds (●) indicate sites where comparable amino acids are absent. Dashes (—) indicate identity.
found in the homologous region of human chromosome 10 close to GOT1.

ACKNOWLEDGMENTS

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REFERENCES


