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Cloning of cDNA encoding steroid 11β-hydroxylase (P450c11)

(congenital adrenal hyperplasia/chromosomal localization/cytochrome P-450/inborn error of metabolism)

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ABSTRACT **We have isolated bovine and human adrenal cDNA clones encoding the adrenal cytochrome P-450 specific for 11β-hydroxylation (P450c11). A bovine adrenal cDNA library constructed in the bacteriophage λ vector gt10 was probed with a previously isolated cDNA clone corresponding to part of the 3′ untranslated region of the 4.2-kilobase (kb) mRNA encoding P450c11. Several clones with 3.2-kb cDNA inserts were isolated. Sequence analysis showed that they overlapped the original probe by 300 base pairs (bp). Combined cDNA and RNA sequence data demonstrated a continuous open reading frame of 1509 bases. P450c11 is predicted to contain 479 amino acid residues in the mature protein in addition to a 24-residue amino-terminal mitochondrial signal sequence. A bovine clone was used to isolate a homologous clone with a 3.5-kb insert from a human adrenal cDNA library. A region of 1100 bp was 81% homologous to 769 bp of the coding sequence of the bovine cDNA except for a 400-bp segment presumed to be an unprocessed intron. Hybridization of the human cDNA to DNA from a panel of human–rodent somatic cell hybrid lines and *in situ* hybridization to metaphase spreads of human chromosomes localized the gene to the middle of the long arm of chromosome 8. These data should be useful in developing reagents for heterozygote detection and prenatal diagnosis of 11β-hydroxylase deficiency, the second most frequent cause of congenital adrenal hyperplasia.**

Congenital adrenal hyperplasia is a disease caused by any of several defects in the adrenal biosynthetic pathways to cortisol and other steroids. Cortisol is normally synthesized from cholesterol in the adrenal cortex in five enzymatic steps: the cholesterol side chain is cleaved to form pregnenolone, which is dehydrogenated at the 3β position to yield progesterone; three successive hydroxylations at the 17α, 21, and 11β positions yield cortisol. In 90–95% of congenital adrenal hyperplasia patients, 21-hydroxylation is impaired. The molecular genetic basis of 21-hydroxylase deficiency has been extensively studied (1).

In 5–8% of reported cases of congenital adrenal hyperplasia, 11β-hydroxylation is defective, so that 11-deoxycortisol cannot be converted to cortisol, and, usually, deoxycortisol is not metabolized to corticosterone (2, 3). Blood levels of these precursors are elevated in the untreated state. Accumulated precursors are shunted into the androgen biosynthetic pathway, causing symptoms of androgen excess including disordered sexual differentiation and accelerated somatic growth. Elevated levels of deoxycortisol or other metabolites with mineralocorticoid activity may cause hypokalemia and hypertension.

Steroid 11β-hydroxylation takes place in mitochondria of cells in the adrenal cortex. It requires a substrate-specific cytochrome P-450 (P450c11), which is a heme-containing protein with a molecular mass of ≈47 kDa (4), and an accompanying NADPH-dependent redox system without substrate specificity that consists of a flavoprotein, adrenodoxin reductase, and adrenodoxin, an iron/sulfur protein.

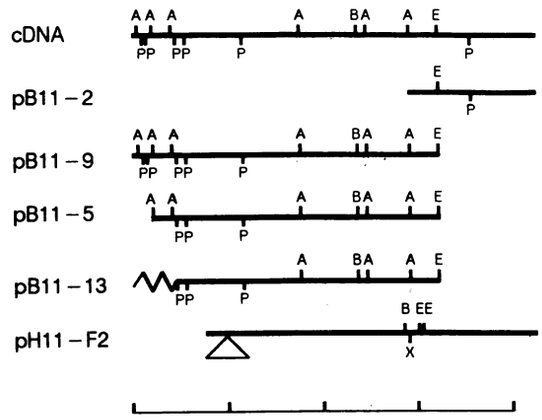

FIG. 1. Restriction maps of cDNA clones encoding P450c11. The deduced restriction map of full-length bovine cDNA is shown at the top of the figure. Clones pB11β-2, -5, -9, and -13 are bovine cDNA clones, and pH11β-F2 is a human cDNA clone. A, *Apa* I; B, *Bam*HI; E, *Eco*RI; P, *Pst* I; X, *Xho* I (*Apa* I and *Pst* I sites in pH11β-F2 are not shown). Clone pB11β-13 contains a second insert at its 5′ end that does not encode part of P450c11 (jagged line). Clone pH11β-F2 contains an unspliced intron (triangle). Scale is marked in kb.

As a first step in the molecular analysis of steroid 11β-hydroxylase (EC 1.14.15.4) deficiency, we have isolated bovine and human cDNA clones encoding P450c11. The gene encoding P450c11 is present in a single copy in the human genome and is located on the long arm of human chromosome 8.‖

METHODS AND MATERIALS

Enzymes and related reagents were obtained from International Biotechnologies (New Haven, CT), Boehringer Mannheim, Pharmacia, or Bethesda Research Laboratories. Bacteriophage gt10 DNA and packaging extracts were purchased from Stratagene (San Diego, CA). Radioactive precursors were purchased from New England Nuclear.

Clone pB11β-2 contains a 1.3-kilobase (kb) insert corresponding to the 3′ end of P450c11 mRNA, including the poly(A) tail (5).

Construction and Screening of a Bovine Adrenal cDNA Library. Bovine adrenal mRNA was prepared as described and size-fractionated by sedimentation on a sucrose gradient

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‖The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02985).

xxxxAGGATGGCACTGTGG
tcctaccgtgacacc

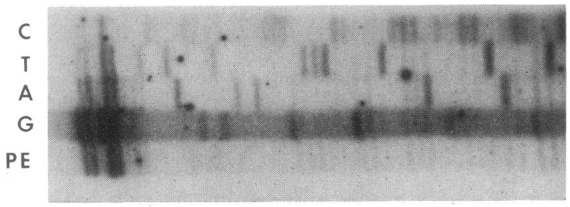

FIG. 2. Primer extension and RNA sequencing of the 5′ terminus. A primer corresponding to the complementary sequence of codons 9–15 was used. The antisense sequence, as read from the ladder, is displayed in lowercase letters. The derived sense sequence is displayed in uppercase letters. Only the area of sequence divergence (see text) is displayed. PE lane represents a primer extension without dideoxynucleotides.

(6). Aliquots of fractions were analyzed by RNA blot hybridization (7) with pB11β-2 DNA, which had been radioactively labeled with [^{32}P]dATP by nick-translation (8). Fractions containing a 4.2-kb mRNA species that hybridized with pB11β-2 were pooled.

Fractionated mRNA was reverse-transcribed into cDNA as described (9) and used to construct a library in the bacteriophage λ vector, gt10, according to published procedures (10). A portion of the library (400,000 plaques) was grown in liquid culture using the bacterial strain C600; phage DNA was prepared (11) and digested with *Eco*RI and size-fractionated by electrophoresis in agarose. The 3- to 4.2-kb fraction was recovered by binding to glass powder (12) and was ligated to the plasmid vector pIBI24 (International Biotechnologies), which had been digested with *Eco*RI and treated with alkaline phosphatase. Competent DH-1 cells (13) were transformed with the ligated plasmids.

Ampicillin-resistant colonies were cultured on nitrocellulose filters (Millipore) and replicated on duplicate filters for colony hybridization (14) using a 600-base-pair (bp) *Pst* I insert fragment of pB11β-2 or a 650-bp *Pst* I fragment of pB11β-13 (see below) as a probe. Plasmid DNA was prepared

```
    -24                                                                                       -1  1
    Met Ala Leu Trp Ala Lys Ala Arg Val Arg Met Ala Gly Pro Trp Leu Ser Leu His Glu Ala Arg Leu Leu|Gly Thr Arg Gly Ala     5
AGG ATG GCA CTG TGG GCA AAG GCC AGG GTG CGG ATG GCA GGG CCC TGG CTG TCC CTG CAT GAG GCA CGC CTA CTG|GGC ACA AGA GGT GCT    90

Ala Ala Pro Lys Ala Val Leu Pro Phe Glu Ala Met Pro Arg Cys Pro Gly Asn Lys Trp Met Arg Met Leu Gln Ile Trp Lys Glu Gln    35
GCA GCC CCC AAG GCG GTG CTG CCC TTC GAA GCC ATG CCC CGG TGT CCT GGC AAC AAG TGG ATG CGG ATG CTG CAG ATC TGG AAG GAG CAG   180

Gly Ser Glu Asn Met His Leu Asp Met His Gln Thr Phe Gln Glu Leu Gly Pro Ile Phe Arg Tyr Asp Val Gly Gly Arg His Met Val    65
GGT TCT GAG AAC ATG CAC TTG GAC ATG CAT CAG ACC TTC CAG GAG CTG GGG CCC ATT TTC AGG TAC GAC GTG GGA GGG AGA CAC ATG GTG   270

Phe Val Met Leu Pro Glu Asp Val Glu Arg Leu Gln Gln Ala Asp Ser His His Pro Gln Arg Met Ile Leu Glu Pro Trp Leu Ala Tyr    95
TTC GTG ATG CTG CCC GAG GAC GTG GAG AGG CTG CAG CAG GCG GAC AGC CAT CAC CCC CAG CGG ATG ATC CTG GAG CCC TGG CTG GCC TAC   360

Arg Gln Ala Arg Gly His Lys Cys Gly Val Phe Leu Leu Asn Gly Pro Gln Trp Arg Leu Asp Arg Leu Arg Leu Asn Pro Asp Val Leu   125
CGA CAG GCT CGC GGG CAC AAG TGT GGC GTG TTC TTG CTC AAC GGG CCC CAG TGG CGT TTG GAC CGA CTG CGG CTG AAC CCA GAC GTC CTC   450

Ser Leu Pro Ala Leu Gln Lys Tyr Thr Pro Leu Val Asp Gly Val Ala Arg Asp Phe Ser Gln Thr Leu Lys Ala Arg Val Leu Gln Asn   155
TCG CTG CCA GCC CTG CAG AAG TAC ACG CCC TTG GTG GAT GGC GTG GCC AGG GAC TTC TCC CAG ACC CTG AAG GCG AGG GTG CTG CAG AAT   540

Ala Arg Gly Ser Leu Thr Leu Asp Ile Ala Pro Arg Leu Phe Arg Tyr Thr Ile Glu Ala Ser Thr Leu Val Leu Tyr Gly Glu Arg Leu   185
GCT CGG GGG AGT CTG ACC CTG GAC ATC GCG CCC AGG CTC TTC CGC TAC ACC ATC GAA GCC AGC ACC TTA GTC CTT TAC GGG GAG CGG CTT   630

Gly Leu Leu Thr Gln Gln Pro Asn Pro Asp Ser Leu Asn Phe Ile His Ala Leu Glu Ala Met Leu Lys Ser Thr Val Gln Leu Met Phe   215
GGC CTT TTG ACC CAG CAA CCA AAC CCT GAC AGC CTG AAC TTT ATC CAC GCG CTG GAG GCC ATG TTG AAG TCC ACC GTG CAG CTC ATG TTT   720
  - TGG --- G-- G-- GCC --- -GT T-- -C- --- --- --- --C C-- --T --C A-- --- -T- --- --C --A --- --- --C --- --- --- --C

Val Pro Arg Arg Leu Ser Arg Trp Met Ser Thr Asn Met Trp Arg Glu His Phe Glu Ala Trp Asp Tyr Ile Phe Gln Tyr Ala Asn Arg   245
GTG CCC AGG CGC CTG TCA CGG TGG ATG AGC ACC AAC ATG TGG AGG GAG CAC TTT GAG GCC TGG GAC TAC ATC TTC CAG TAT GCC AAC AGA   810
A-- --- --- A-- --- --T --C --- -CC --- C-- --G G-- --- -A- --- --- --- --- --- --- --- -G- --- --- --- --C -G- G-- -AC

Ala Ile Gln Arg Ile Tyr Gln Glu Leu Ala Leu Gly His Pro Trp His Tyr Ser Gly Ile Val Ala Glu Leu Leu Met Arg Ala Asp Met   275
GCC ATC CAG AGA ATC TAT CAG GAG CTG GCC CTC GGC CAC CCG TGG CAC TAC AGC GGC ATC GTG GCA GAG CTG CTG ATG CGA GCA GAC ATG   900
TGT --- --- -A- --- --- --- --A --- --- AA- A-- -G- --T CAA --G --- -C- A-- --- --- --G --- --C --- T-- AAT --G --A C--

Thr Leu Asp Thr Ile Lys Ala Asn Thr Ile Asp Leu Thr Ala Gly Ser Val Asp Thr Thr Ala Phe Pro Leu Leu Met Thr Leu Phe Glu   305
ACC CTG GAT ACC ATC AAG GCC AAC ACG ATC GAC CTC ACT GCT GGG AGT GTG GAC ACG ACA GCC TTC CCC TTG CTG ATG ACT CTC TTT GAG   990
T-G -CA --- G-- --- --- --- --C TGT --G --A --- --- --A --- --C --- --- -G- --G -TG --T --- --- --- --- --G --- --- ---

Leu Ala Arg Asn Pro Glu Val Gln Gln Ala Val Arg Gln Glu Ser Leu Val Ala Glu Ala Arg Ile Ser Glu Asn Pro Gln Arg Ala Ile   335
CTG GCT CGG AAC CCG GAG GTG CAG CAG GCG GTG CGC CAG GAG AGC CTG GTG GCT GAG GCC CGG ATC TCA GAA AAT CCC CAG AGG GCC ATC  1030
--- --- --- --- --C A-C --- --- --- --C C-- --- --- --- --- --- -CC --C -CA --- A-C --- --- --- C-- --- --- -A- --A -C-

Thr Glu Leu Pro Leu Leu Arg Ala Ala Leu Lys Glu Thr Leu Arg Leu Tyr Pro Val Gly Ile Thr Leu Glu Arg Glu Val Ser Ser Asp   365
ACG GAG CTG CCT TTG CTG CGG GCG GCC CTC AAG GAG ACC TTG AGG CTG TAC CCC GTG GGT ATC ACC TTG GAG CGG GAA GTG AGC TCG GAC  1170
--C --- --- --C C-- --- --T --- --- --- --- --- --- --- C-- --C --- --T --- --- C-G -TT C-- --- --A -TG --- --- --A ---

Leu Val Leu Gln Asn Tyr His Ile Pro Ala Gly Thr Leu Val Lys Val Leu Leu Tyr Ser Leu Gly Arg Asn Pro Ala Val Phe Ala Arg   395
CTG GTG CTG CAG AAC TAC CAC ATC CCA GCT GGG ACG CTG GTG AAG GTC CTA CTC TAT TCC CTG GGT CGA AAC CCC GCC GTG TTC GCC AGG  1260
T-- --- --T --- --- --- --- --- --- --- --- --A T-- --- CGC --G T-C --- --C --T --- --- --C --- --- --- T-- --- C-G ---

Pro Glu Ser Tyr His Pro Gln Arg Trp Leu Asp Arg Gln Gly Ser Gly Ser Arg Phe Pro His Leu Ala|Phe Gly Phe Gly Val Arg Gln   425
CCT GAG AGC TAT CAC CCC CAG CGC TGG CTG GAC CGC CAG GGC TCT GGA AGC AGA TTC CCG CAC CTG GCC|TTT GGC TTT GGC GTA CGC CAG  1350
--- --- C-- --- A-- --- --- --- --- --A --- AT- A-- --- --C --C --G -AC --- TAC --- G-- C--|--- --- --- --- A-G --- ---

Cys Leu Gly Arg Arg Val Ala Glu Val Glu Met Leu Leu|Leu Leu His His Val Leu Lys Asn Phe Leu Val Glu Thr Leu Glu Gln Glu   455
TGC CTG GGG CGG CGC GTG GCT GAG GTG GAG ATG CTG CTC|CTG CTG CAC CAT GTG CTG AAG AAC TTC CTG GTG GAG ACA CTG GAG CAA GAG  1440
--- --T --- --- --- C-- --A --- -CA --T --- --- --G|--- --- --G ---
                                                                              479
Asp Ile Lys Met Val Tyr Arg Phe Ile Leu Met Pro Ser Thr Leu Pro Leu Phe Thr Phe Arg Ala Ile Gln ***
GAC ATA AAG ATG GTC TAC CGC TTC ATA CTG ATG CCC TCC ACC CTG CCC CTC TTC ACC TTC CGG GCC ATC CAG TAG TCGTGTCAGCACACTGCCG 1534

------------------GACGGACGGAACCATGTATACCTGTGGCGCTTCATGCTGAGATGGCAAGAAGCCAACACAACATAGGAAGCCCACCCAGTTAATAAATACCATTAACAAT
```

FIG. 3. Sequence analysis of cDNA clones. Combined nucleotide sequence data from bovine clones pB11β-2, -5, -9, and -13 as well as from RNA sequencing (nucleotides 1–75) are displayed along with an amino acid translation. Approximately 2600 bp of sequence in the 3′ untranslated region (dashed line) are not shown but are available through GenBank (J02985). It is predicted that the protein is cleaved in mitochondria between amino acid residues −1 and 1; the sequence of the mature porcine enzyme (positions 1–20) is *Gly-Thr*-Ser-Ala-*Ala*-Leu-*Ala-Pro-Lys-Ala-Val-Leu-Pro-Phe*-Asn-*Ala*-Ile-*Pro*-Gln-*Cys* (matching residues are italicized). The cysteine-containing HR2 peptide is boxed. The polyadenylylation signal is indicated by underlining; the poly(A) tail is not shown. A partial nucleotide sequence of the human cDNA clone pH11β-F2 is displayed below the bovine sequence starting at nucleotide 634. Matching bases are indicated by dashed lines. The location of an unprocessed intron in pH11β-F2 (not shown) is marked by an arrow.

from positively hybridizing clones by the rapid-boil technique (15).

Isolation of Human cDNA. A human fetal adrenal cDNA library was a gift of David Russell (16). The library was size-fractionated as described by Okayama and Berg (17), except that plasmid DNA was linearized with restriction endonucleases *Sal* I or *Cla* I. Plasmids with inserts >3 kb were recovered, recircularized, and used to transform competent DH-1 cells. Clones were screened by colony hybridization with a 2.5-kb *Bam*HI/*Eco*RI fragment of pB11β-13.

Nucleotide Sequence Analysis. Sequencing was performed by the chain-termination method using dATP[^{35}S] (18). Bacteriophage M13mp19 subclones were generated by sequential deletion (19), or restriction fragments were subcloned and sequenced directly from plasmid DNA (20). Some sequences were obtained using specific oligonucleotide primers (synthesized by the Department of Microbiology, Cornell University). RNA sequencing was performed as described in procedure 2 of Geliebter *et al.* (21).

Southern Blot Analysis. High molecular weight DNA was isolated as described (22) from a previously characterized panel of human–rodent hybrid cell lines (23). Twenty micrograms from each cell line was digested overnight with *Eco*RI at 5 units per μg of DNA. Digests were subjected to electrophoresis through 0.7% agarose gels and processed for blotting and hybridization as described (24, 25). DNA probes were labeled by nick-translation to specific activities >1 × 10^8 dpm/μg. Final stringent washes were performed at 65°C in 0.03 M NaCl/0.003 M sodium citrate/0.5% NaDodSO$_4$.

***In Situ* Hybridization to Metaphase Chromosomes.** Hybridization was performed as described (26) using plasmid DNA labeled by nick-translation with [^{125}I]dCTP to 2 × 10^8 dpm/μg. Slides were coated with Kodak emulsion NTB2 and exposed for 1 week. Positions of silver grains were determined relative to banding patterns produced by staining with either Giemsa or quinacrine.

RESULTS

Bovine cDNA Clones Encoding P450c11. Due to the presence of a long 3′ untranslated region, the mRNA encoding P450c11 is ≈4.2 kb long, although the coding region is only 1.5 kb long. In general, sequences of corresponding cDNAs from different species are poorly conserved in 3′ untranslated regions compared to coding regions. In fact, we failed to isolate a human cDNA clone when we used pB11β-2, which consists solely of part of the 3′ untranslated region, as a probe (unpublished observations). For this reason, pB11β-2 was used to isolate a full-length bovine cDNA clone from a size-fractionated cDNA sublibrary.

Three clones with inserts of ≈3.2 kb were isolated: pB11β-5, -9, and -13. They had identical restriction maps (Fig. 1) except at the 5′ end. Comparison of the DNA sequences of pB11β-2 and -13 revealed a 300-bp region of overlap between the 5′ end of pB11β-2 and the 3′ end of pB11β-13 in which the sequences of the two clones were identical. There is an *Eco*RI site 300 bp from the 5′ end of the insert of pB11β-2, and the region 3′ to this site in pB11β-13 was lost during *Eco*RI digestion of phage DNA to release the cDNA insert.

Sequence analysis of pB11β-13 suggested that it did not contain a full-length transcript of P450c11 mRNA but instead had ≈500 bp at the 5′ end without homology to any known cytochrome P-450 (unpublished observations). Therefore, additional data were obtained from pB11β-5 and -9. To confirm the sequence of the 5′ end, RNA sequencing was performed by primer extension of an oligonucleotide complementary to codons 9–15 (Fig. 2). Comparison to the RNA sequence showed that pB11β-9 contained 13 bases divergent from the RNA at the 5′ end (AGCCGGGATTCCG for the cDNA instead of AGGATGGCACTGT). Whether these bases represent heterogeneity of the mRNA or a cloning artefact has not been determined, although heterogeneity at the amino terminus of the porcine enzyme has been reported (4).

Combined sequence data (Fig. 3) demonstrate an open reading frame of 1509 bp. Although the 5′ untranslated region is very short (6–10 bases), the first ATG is probably the initiation codon since it lies in a strong context for initiation of translation with an A at the -3 and a G at the +4 positions (27). The protein is predicted to contain 503 amino acid residues, with the amino-terminal 24 residues being cleaved in mitochondria to yield a mature protein of 479 residues.

Isolation of Human cDNA Encoding P450c11. A clone with an insert of 3.5 kb (pH11β-F2) was isolated. As the human cDNA library was prepared by the Okayama–Berg protocol (16, 17), all clones carry the complete 3′ end of the corresponding mRNAs. There is an 1100-bp segment at the 5′ end of the insert of pH11β-F2, which is homologous to the coding region of the bovine cDNA, beginning 600 bp downstream of the first ATG. This segment includes a 400-bp region that has no counterpart in the bovine coding sequence and that begins and ends with canonical splice donor and acceptor sequences (unpublished observations). It presumably corresponds to an unspliced intron, since another human cDNA clone (pH11β-E3) contains sequences 5′ of the presumed splice site and does not include this region (unpublished observations). If the intron is ignored, the open reading frame of pH11β-F2 is 80% homologous over 769 bp to the corresponding region of bovine cDNA at the nucleotide level. The predicted amino acid sequence of pH11β-F2 is 71% homologous to the bovine sequence over 256 residues. Efforts to extend the sequence in the 5′ direction by repeated screening of the library were unsuccessful.

Chromosomal Localization. Southern blots using 5′ and 3′ fragments of the cDNA suggest that the gene encoding P450c11 [we propose that this gene be termed *OH11*; another suggested nomenclature is P450XIA (28)] is probably present as a single copy in the haploid human genome (unpublished observations). DNA samples from a panel of 28 human–mouse and human–hamster somatic cell hybrid lines were digested with *Eco*RI and analyzed by Southern blot hybridization using pH11β-F2 (Fig. 4, Table 1). The hybridization pattern corresponding to the human *OH11* gene was detected in a distribution among the cell lines corresponding most closely to that of chromosome 8. Twenty-seven of the 28 cell lines yielded signals concordant with the presence or absence of this chromosome. The one discordant cell line, in which a predicted positive hybridization signal was not observed,

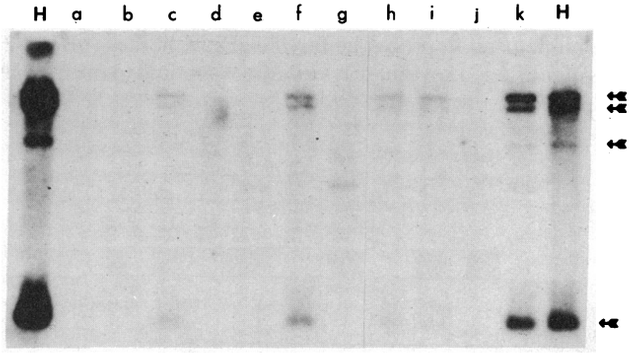

FIG. 4. Hybridization of human–rodent hybrid cell lines to pH11β-F2. DNA samples were digested with restriction endonuclease *Eco*RI and processed as described. Lanes: a, cell line 12 in Table 1; b, hamster cell line; c–g, lines 10–6 in Table 1; h–k, lines 4–1 in Table 1. Lanes H, human cell line controls. Arrows indicate the bands derived from human DNA.

Table 1. Chromosomal location of the *OH11* gene by hybridization to DNA samples from human–rodent somatic cell hybrid lines

Cell line	Hybrid-ization	Human chromosome 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Translocation
1	+	−	+	+	+	−	−	+	+	−	−	+	−	+	−	+	+	+	+	+	−	+	+	+	+	
2	−	+	−	+	+	+	+	−	−	−	−	−	−	−	*	−	−	−	+	−	−	+	−	−	−	
3	+	+	−	+	−	+	+	+	+	+	−	*	+	−	+	*	−	−	+	−	−	−	+	*	−	Xp+
4	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	*	−	Xp+
5	+	+	−	*	+	+	−	−	+	−	+	−	*	−	−	−	−	−	−	+	−	+	−	*	−	Xq+, fragment
6	−	−	−	*	+	+	+	+	−	−	+	−	+	+	+	−	+	−	−	−	+	+	+	*	−	3q−
7	+	−	−	+	+	−	−	+	+	−	−	−	−	+	+	+	−	−	+	−	−	−	+	*	−	Xp−
8	−	−	−	*	−	+	*	*	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	6q−
9	−	−	+	*	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	+	−	−	−	3p−
10	+	+	−	+	+	−	+	−	+	−	+	−	+	−	+	−	−	+	−	−	−	+	+	+	+	
11	+	+	−	+	+	−	−	−	+	−	+	−	+	−	+	−	−	+	−	−	−	+	+	+	+	
12	−	−	−	+	+	−	−	+	−	−	−	+	−	−	−	+	+	−	+	−	*	+	+	*	−	Xq−
13	−	*	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	*	−	1q−
14	−	−	−	+	+	−	−	*	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	7q
15	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	7q
16	−	*	−	+	−	*	+	*	−	−	*	−	−	−	*	−	−	+	−	*	−	+	+	*	−	7p
17	−	−	−	−	−	+	*	*	−	−	−	−	+	−	−	+	+	+	+	−	−	+	−	+	−	7p+
18	−	−	−	−	−	−	*	*	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	6q−
19	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
20	−	−	−	*	+	−	−	*	−	−	−	−	+	+	+	*	−	−	−	−	−	−	−	*	−	3q−, 7q−
21	+	+	−	+	+	−	−	*	+	−	−	−	+	+	+	*	−	−	+	−	−	−	−	+	−	15q
22	−	−	−	−	−	−	+	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Fragment
23	−	−	−	−	−	+	*	*	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	6q−
24	+	+	−	+	+	+	*	+	+	−	−	+	−	+	−	+	+	−	*	+	+	+	−	+	−	18p+
25	−	*	−	+	+	+	*	−	−	−	−	−	+	*	+	+	−	−	*	−	+	−	+	+	−	6q−, 1q−
26	−	−	−	+	−	+	*	−	+	−	−	−	+	+	+	+	+	+	*	−	+	+	+	−	−	6q−
27	−	−	*	−	−	−	*	−	−	−	−	−	−	+	+	+	−	−	+	+	+	+	+	−	−	2p−
28	−	−	*	−	−	−	*	−	−	−	−	−	−	+	+	+	−	−	+	−	−	+	−	−	−	6p+
No. of discordant lines		6	11	10	8	13	18	16	1	9	7	6	9	12	14	10	10	9	12	7	12	16	11	9	6	

↑

The chromosomal content of the hybrid cell lines was determined by cytogenetic and isoenzyme analysis. +, Chromosome detected; −, chromosome not detected; *, chromosome fragment detected. The distribution of positive hybridization is most consistent with a location on chromosome 8, indicated by an arrow.

may not have contained all of chromosome 8, or the chromosome may have been lost during propagation.

To confirm and extend these results, pH11β-F2 was hybridized *in situ* to spreads of metaphase chromosomes from normal human leukocytes (Fig. 5). Approximately 50% of total silver grains were observed overlying chromosome 8. The grains were concentrated over a small region in the middle of the long arm of chromosome 8. The peak distribution was seen at 8q21, as defined by banding with either Giemsa (40 metaphases) or quinacrine (12 metaphases).

DISCUSSION

The clone used to probe the bovine cDNA library (pB11β-2) was identified in several ways as corresponding to the mRNA encoding P450c11 (5). The mRNA corresponding to the clone was inducible by corticotropin, was found only in the adrenal gland, and, when hybrid-selected and translated *in vitro*, encoded a protein that could be immunoprecipitated by a monospecific antiserum to P450c11. The bovine cDNA clones pB11β-5, -9, and -13 correspond to the same mRNA as pB11β-2, as determined by the presence of 300 bp of identical sequence in the region of overlap. The coding region deduced by analysis of these clones encodes a protein of the appropriate size for P450c11 [48 kDa predicted for the mature protein vs. 47.5 kDa reported (4)]. It includes the cysteine-containing (Cys-426) HR-2 region characteristic of P-450 enzymes that is thought to interact with the heme prosthetic group. A segment corresponding to the amino terminus of the mature P450c11 protein is also observed. Thus, it is concluded that these clones encode P450c11.

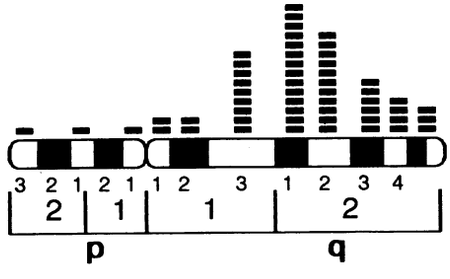

FIG. 5. *In situ* chromosomal localization of the human 11β-hydroxylase gene *OH11*. Of 51 metaphase cells examined, 54 grains were located on chromosome 8. The distribution of grains along chromosome 8 (shown) was centered on 8q21, with 34 grains (63%) located on q13–q22.

P450c11 is a mitochondrial protein synthesized from a nuclear transcript. It therefore has an amino-terminal signal peptide that directs the nascent protein to the mitochondria and is then cleaved to yield the mature protein. The signal peptide of P450c11 is predicted to contain 24 amino acid residues, as compared to the 39 residues of P450scc (cholesterol desmolase), which is also a mitochondrial P-450 enzyme (29). Functional mitochondrial signal peptides of about this length have been reported (30). The predicted amino acid sequence of P450c11 is 40% homologous to the sequence of P450scc but only 22% homologous to the microsomal enzyme P450c21 (steroid 21-hydroxylase). This suggests that the mitochondrial P-450 enzymes are relatively closely related evolutionarily within the cytochrome P-450 superfamily (28).

The partial coding region of the human cDNA clone pH11β-F2 is 80% homologous to the corresponding region of pB11β-13 in nucleotide sequence and 71% in predicted amino acid sequence. This degree of sequence conservation is similar to that observed for other P-450 genes, such as those encoding P450c21 (31, 32), and pH11β-F2 appears to hybridize to a single gene in humans, suggesting that it indeed encodes P450c11 and not a related protein.

The human cDNA clone encoding P450c11 was isolated to serve as a reagent for analyzing the inherited disorder of cortisol biosynthesis, congenital adrenal hyperplasia due to 11β-hydroxylase deficiency. This disease is inherited as a monogenic autosomal recessive trait that, unlike 21-hydroxylase deficiency, is not linked to the *HLA* complex on chromosome 6 (33). The presence of a single copy of the structural gene for P450c11 (*OH11*) on chromosome 8q is consistent with this mode of inheritance. Notable genes in this region include the *MYC* and *MOS* cellular oncogenes and genes encoding glutamic pyruvate transaminase, thyroglobulin, and the β polypeptide of DNA polymerase (34). Further studies will be required to establish a linkage map of *OH11* in relation to these genes. The other P-450 enzymes involved in cortisol biosynthesis, P450scc and P450c17, have been mapped to human chromosomes 15 (35) and 10 (36), respectively. Thus, despite common regulation of transcription by corticotropin, there is no clustering of the genes encoding adrenal cytochromes P-450.

Early prenatal diagnosis of 11β-hydroxylase deficiency by analysis of DNA, obtained by chorionic villus biopsy, would improve counseling and permit prenatal therapy. Such therapy consists of administering dexamethasone to mothers of affected female fetuses, suppressing the fetal adrenal gland and possibly preventing masculinization of the external genitalia (37). Otherwise, masculinized females require surgery to reconstruct functional external genitalia if they are not to forfeit their reproductive potential. The reagents and linkage data presented here may be useful for this purpose.

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