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Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta

(adrenal/steroid hormone/monooxygenase/DNA sequence/chromosome)

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ABSTRACT **Conversion of cholesterol to pregnenolone is mediated by P450scc [cholesterol, reduced-adrenal-ferrodoxin: oxygen oxidoreductase (side-chain-cleaving), EC 1.14.15.67]. RNA from several human adrenal samples was translated *in vitro* and immunoprecipitated with anti-bovine P450scc, indicating that P450scc mRNA represents about 0.5% of human adrenal mRNA in normal, hypertrophied, and malignant adrenals. A 1626-base-pair human adrenal P450scc cDNA was cloned in bacteriophage λgt10. Primer extension data indicated P450scc mRNA is about 1850 bases long and that all adrenal P450scc mRNA has the same 5′ end. A full-length clone containing 1821 bases was obtained from a human testis cDNA library to yield the complete sequence. The encoded human preP450scc contains 521 amino acids with a molecular weight of 60189.65. The testis and adrenal sequences were identical; the human cDNA and amino acid sequences are 82% and 72% homologous, respectively, with the bovine sequences. P450scc cDNA was used to probe DNA from a panel of mouse–human somatic cell hybrids, showing that the single human P450scc gene lies on chromosome 15. The human P450scc gene is expressed in the placenta in early and midgestation; primary cultures of placental tissue indicate P450scc mRNA accumulates in response to cyclic AMP.**

Conversion of cholesterol to pregnenolone is the first and rate-limiting step in the synthesis of all steroid hormones. This conversion entails three steps—20-hydroxylation, 22-hydroxylation, and cleavage of the C_{20}–C_{22} bond to produce pregnenolone and isocaproic acid. These three steps are mediated by a single mitochondrial cytochrome termed P450scc [cholesterol, reduced-adrenal-ferrodoxin:oxygen oxidoreductase (side-chain-cleaving), EC 1.14.15.67] (1–3). Deficient P450scc activity causes lipoid adrenal hyperplasia, a generally lethal disease (4). P450scc functions as the terminal oxidase in an electron transport chain consisting of adrenodoxin reductase, a flavoprotein accepting electrons from NADPH, and adrenodoxin, an iron–sulfur protein mediating electron transfer from adrenodoxin reductase to the P450 (5). Genes for several hepatic P450s and for bovine (6, 7) and human (8, 9) steroidogenic P450c21 have been sequenced. Complementary DNAs have been reported for bovine P450c21 (10), P450scc (11), and P450c17 (12), but few data exist about the sequences encoding the human enzymes or their human chromosomal locations.

P450scc mRNA accumulation is regulated hormonally (13–16) and in development (17), presumably due to increased transcription of the single P450scc gene (18). Similarly, gonadotropins and cyclic AMP stimulate P450scc mRNA accumulation in cultured human ovarian granulosa cells (16). Pregnenolone is also produced from cholesterol in the placenta (19, 20), but the nature of the enzyme(s) mediating placental progesterone synthesis is unclear (21).

We have cloned and sequenced full-length human P450scc cDNA and located the human P450scc gene on chromosome 15. This gene is expressed in the human placenta as early as wk 10 of gestation. In primary cultures of midterm human placenta, accumulation of P450scc mRNA is stimulated by cAMP.

MATERIALS AND METHODS

Adrenal RNA preparation, cell-free translation, immunoprecipitation, and $NaDodSO_4$ gel electrophoresis were done as described (22). Placental RNA was prepared by guanidine thiocyanate/LiCl extraction (23), and RNA from primary cultures was prepared as described (16). One 63-mer and three 72-mers corresponding to various regions of the bovine P450scc cDNA sequence (11) were produced using a noncommercial synthesizer (24); the sequences and hybridization characteristics of these oligonucleotides have been described (25). Dot and RNA blots were done as described (16). A 27-mer (see *Results*) was produced on an Applied Biosystems synthesizer. Oligonucleotides were end-labeled with [γ-^{32}P]ATP by polynucleotide kinase.

Cloned cDNA was cleaved from bacteriophage λgt10, inserted directly into plasmids pUC13 and pUC18, and appropriate fragments were cloned in M13mp10 and M13mp11 for dideoxy chain-termination sequencing (26). Primer extension was done exactly as described previously (7) using 10 μg of human adrenal poly(A)$^+$ RNA and 0.1 pmol of the ^{32}P-labeled 27-mer.

A panel of 15 clonal mouse–human somatic cell hybrid lines was isolated and characterized as described (27). Karyotype analysis was done on each hybrid clone when the cells were harvested for DNA extraction; at least 30 G-banded metaphases were photographed and analyzed for each hybrid clone. DNA was isolated (28), digested with *Hin*dIII, and displayed by agarose gel electrophoresis. Gels were blotted and probed with ^{32}P-labeled P450scc cDNA by Southern transfers (6).

Placental tissue was minced and cultured directly on Falcon plastic dishes in 45% medium 199/45% medium F12/10% fetal bovine serum/2 mM glutamine/50 μg of gentamycin per ml in a 5% CO_2/95% air atmosphere.

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Abbreviations: P450scc, cholesterol side-chain cleavage cytochrome P450; bp, base pair(s).

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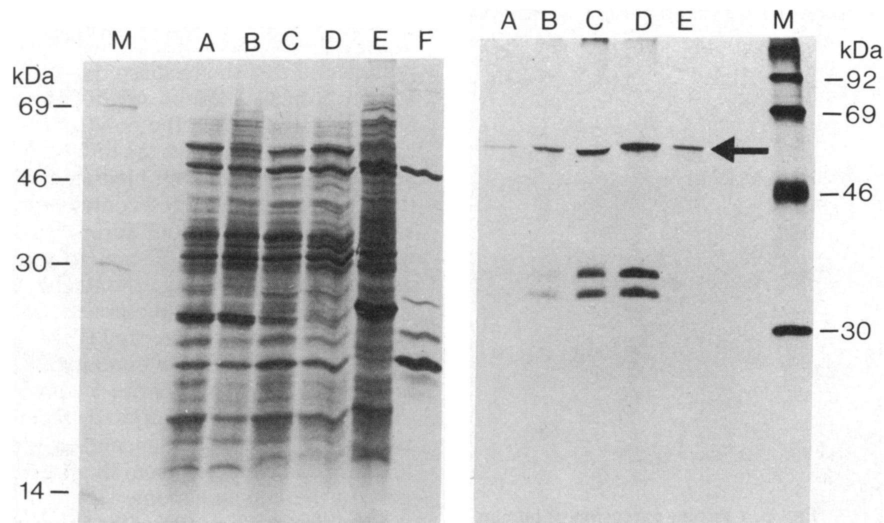

FIG. 1. Characterization of human P450scc mRNA by cell-free translation and immunoprecipitation. (*Left*) Autoradiogram of $NaDodSO_4$/polyacrylamide gel of total [^{35}S]methionine-labeled translation products encoded by adrenal poly(A)$^+$ RNAs from the following sources: lane A, male with adrenal hyperplasia due to Cushing disease that was unresponsive to transsphenoidal pituitary surgery and 3 mo of oral metyrapone therapy; lane B, female adrenal carcinoma; lanes C and D, normal male and female, respectively (cadaver kidney donors); lane E, male bovine adrenal; lane F, no RNA (translation products endogenous to the reticulocyte lysate); lane M, ^{14}C-labeled protein molecular markers: phosphorylase b, 92 kDa; bovine albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; and lysozyme, 14.3 kDa. (*Right*) Autoradiogram of $NaDodSO_4$ gel of [^{35}S]methionine-labeled translation products shown at left immunoprecipitated with rabbit anti-bovine P450scc (15). Lanes are designated as in *Left*. The P450scc band is indicated by the arrow.

RESULTS

The cell-free translation patterns (Fig. 1) of polyadenylylated RNA from the adrenal of a patient with Cushing disease, from an adrenal carcinoma, and from normal tissues are quite similar. Aliquots of these cell-free translations were immunoprecipitated with anti-P450scc (Fig. 1 *Right*). Because ACTH stimulates P450scc mRNA accumulation in cultured bovine adrenal cells (18), we had anticipated that RNA from the adrenal of the patient with Cushing disease would be relatively enriched in P450scc mRNA. All four RNAs gave similar patterns; the differences in intensities of the immunoprecipitated preP450scc bands reflect the amount of radioactivity loaded on the gel, not the relative abundance of P450scc mRNA in each RNA sample. Both bovine and human preP450scc mRNAs have similar gel mobilities of about 58 kDa, consistent with the 54.5-kDa figure determined previously by cell-free translation (29) and with the molecular weights of 60,322 for bovine (11) and 60,189.65 for human preP450scc determined from the amino acid sequences predicted by the cDNAs. The coprecipitating bands of 32 and 34 kDa seen in the human samples were not seen in the bovine samples run on the same gel or in other experiments (30); the nature of these bands is unknown. Based on the fraction of incorporated [^{35}S]methionine radioactivity found in the specific P450scc bands, human P450scc mRNA represents less than 0.5% of total human adrenal mRNA.

Identification and Sequence of P450scc cDNA. The construction of our human adrenal cDNA library in bacteriophage λgt10, the synthesis and characteristics of oligonucleotides SCC-1, 2, 3 and 4, and their use to identify the 818-base-pair (bp) human P450scc cDNA fragment λhSCC-36 have been described (25). A 159-bp *Eco*RI–*Pst* fragment from the 5′ end of that cDNA was used to reprobe the amplified cDNA library, identifying 30 putatively positive clones. These were then probed with oligonucleotide SCC-2, a 72-mer corresponding to the sequence encoding amino acids 181–204 of bovine P450scc (11, 25), and two positive clones were identified. The phage containing the longer insert was designated λhaSCC-71. The λhaSCC-71 cDNA was subcloned, mapped, and sequenced (Fig. 2). This cDNA contains an open reading frame encoding 464 amino acids of human P450scc; however, by analogy with the bovine sequence (11), it lacks the codons for the leader peptide and 17 amino-terminal amino acids.

We prepared a ^{32}P-labeled, 27-base oligonucleotide corre-

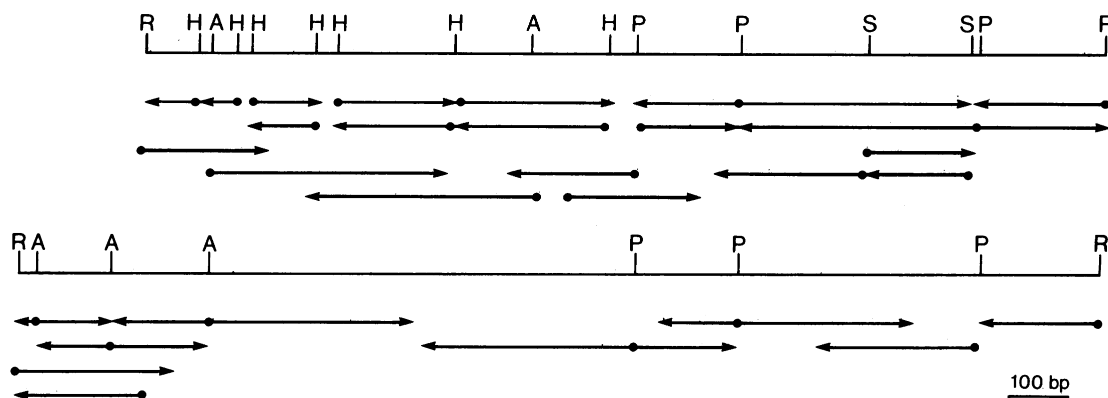

FIG. 2. Sequencing strategies. Only the restriction sites used in sequencing are shown. (*Upper*) Strategy for sequencing adrenal λhaSCC-71. (*Lower*) Strategy for sequencing testicular λhtSCC-2. Each sequencing reaction was performed at least twice.

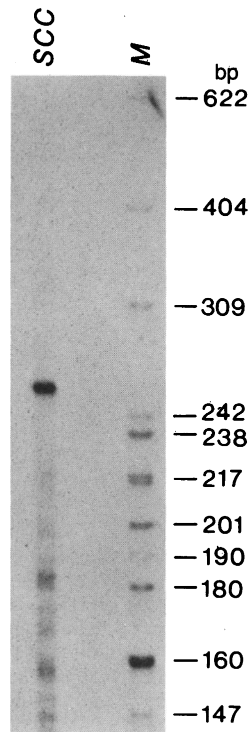

FIG. 3. Primer extension of human P450scc mRNA. RNA used in lane B of Fig. 1 was hybridized to ^{32}P-labeled synthetic 27-mer corresponding to the 5′ end of the λhaSCC-71 cDNA and used to initiate reverse transcription (lane SCC). M_r standards in bp are from ^{32}P-labeled *Hpa* II-cleaved pBR322 (lane M). Samples were run on 5% acrylamide/7 M urea gel.

sponding to the 5′ end of the λhaSCC-71 cDNA and hybridized it to the human adrenal polyadenylylated RNA used in Fig. 1, lane B. The 27-mer was used to prime reverse transcription of cDNA, and the primer-extended material was analyzed on a denaturing gel; this produced a sharp band of about 255 bases (including the 27-base primer) (Fig. 3). The presence of this single sharp band indicates that virtually all human adrenal P450scc mRNA molecules are about 1850 bases long [excluding the poly(A) tail] and have the same 5′ cap site, suggesting that the differential regulation by ACTH and angiotensin II is not mediated via alternative transcriptional start sites. Clones containing longer P450scc cDNA could not be found in our adrenal cDNA library. Therefore, we used the synthetic 27-mer to screen a human testicular cDNA library (K. Fong, Clontech). Several positive clones were identified and characterized, and the longest, designated λhtSCC-2, was sequenced (Figs. 2 and 4). Excluding the poly(A) tail, this clone contains 1821 bases encoding the entire preprotein, the entire 3′ untranslated region, and 44 bases of the 5′ untranslated region. The corresponding regions of the testicular and adrenal cDNA clones are identical, as expected from the presence of a single P450scc gene in the human genome (25).

Chromosomal Location of the Human P450scc Gene. Southern blots of human genomic DNA cleaved with five restriction endonucleases and probed either with the bovine-sequence P450scc oligonucleotides or with cDNA clone λhSCC-36 indicate the human genome contains a single P450scc gene (25); similar studies indicate the bovine genome also has a single P450scc gene (18). To determine the chromosomal location of this unique human gene for P450scc, we examined DNA from a panel of 15 mouse–human somatic cell hybrids using λhaSCC-71 cDNA as the probe. DNA from 7 of the 15 cell lines contained a 23-kb *Hin*dIII fragment hybridizing to P450scc cDNA (Fig. 5); correlation of known human chromosomal components of each cell line with positively hybridizing cell lines identifies chromosome 15 as carrier of the P450scc gene (Table 1). All other

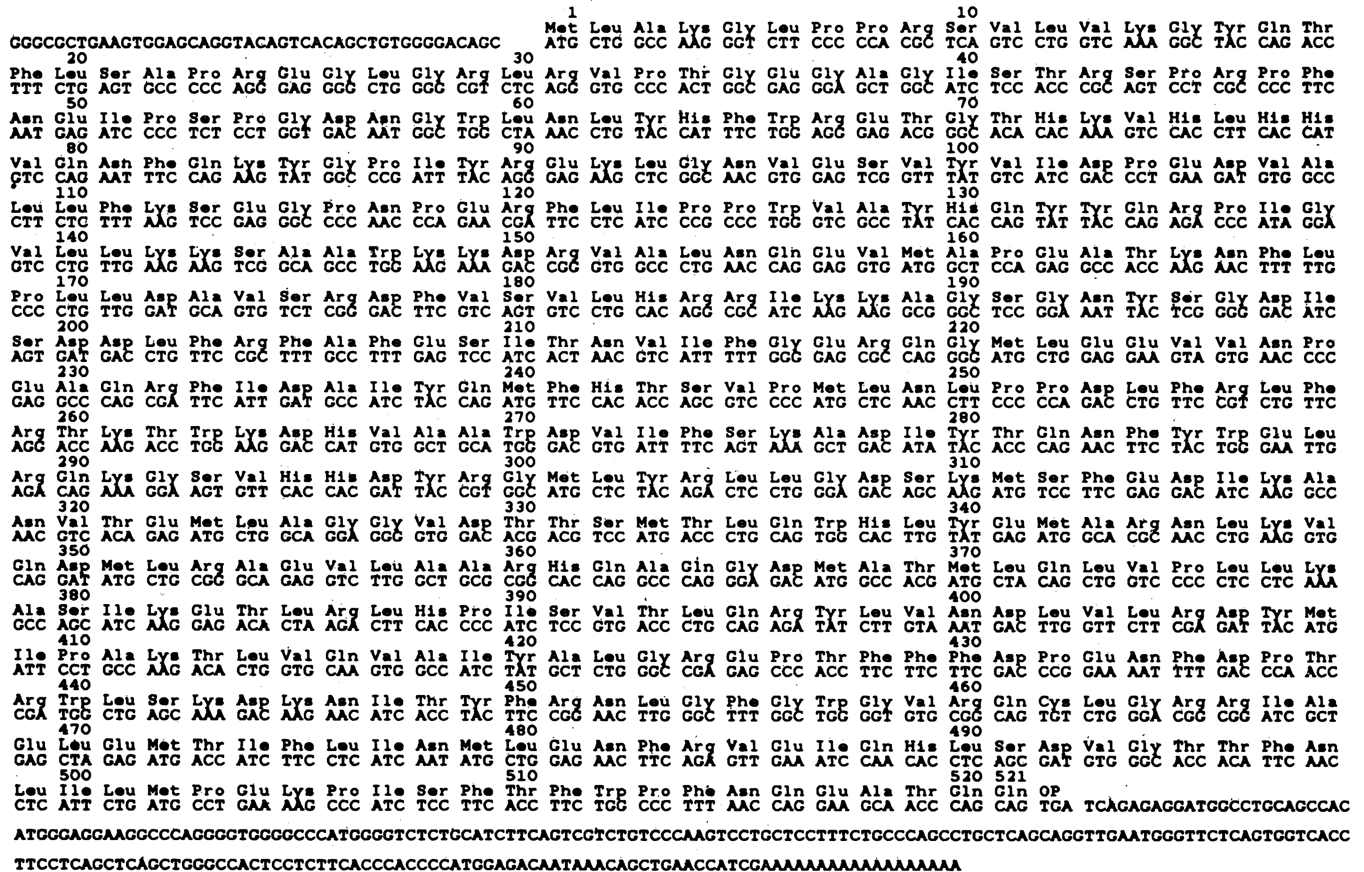

FIG. 4. Sequence of human P450scc cDNA. The amino acid sequence was deduced from the genetic code. Note the AATAAA sequence beginning 20 bases 5′ to the poly(A) region.

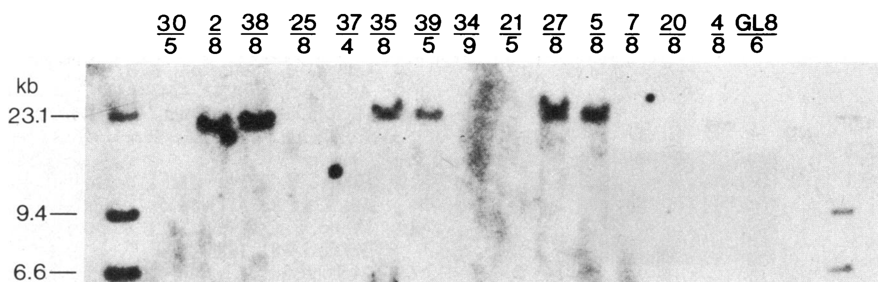

FIG. 5. Chromosomal location of the human P450scc gene. DNA from 15 mouse–human somatic cell hybrid lines was digested with *Hin*dIII, displayed by electrophoresis through 0.8% agarose, transferred to nitrocellulose, blotted, and probed with ^{32}P-labeled P450scc cDNA. The lanes are designated by the somatic cell hybrid. Note the faintly hybridizing band of lane 4/8. The gel is interpreted in Table 1. Lane M contains ^{32}P-labeled *Hin*dIII-cleaved bacteriophage λ as molecular weight markers (in kb).

chromosomes show 4 or more cell lines discordant with the P450scc pattern.

Placental Expression of P450scc. Blotting of RNA from human placentas at wk 10 and 25 of gestation suggests the relative abundance of P450scc mRNA is greater in early gestation (Fig. 6 *Left*). This placental P450scc mRNA can be regulated by cAMP; dot blots in Fig. 6 *Right* show that 4 days of primary culture significantly decreased the abundance of P450scc mRNA in wk 20 placenta, but 2 days treatment with 1 mM cAMP increased P450scc mRNA back to initial levels. P450scc mRNA is also regulated by cAMP in bovine (31) and human ovarian granulosa cells (16) and in bovine (32) and human adrenal cells (unpublished data; A. Di Blasio, R. Jaffe, R.V., and W.L.M.).

DISCUSSION

The coding sequence of the human P450scc cDNA is 82% homologous to the bovine sequence, while the amino acid sequences are 72% homologous. With the introduction of only seven gaps in the 3′-untranslated regions, the 214 bases in the human sequence are 69% homologous to the corresponding regions of the 264 base bovine 3′-untranslated region. The nucleotide differences between the two species appear to cluster nonrandomly. In the coding sequences, five regions totaling 47 bases (3% of the total number of bases) have 40 nucleotide changes (14% of the total of nucleotide changes; Table 2); the cluster of differences at amino acids 292–297 contains an additional codon not found in the bovine sequence. By contrast, five other regions encompassing 120 amino acids (23% of the total) have only five amino acid changes (3.4% of the total). This pattern and degree of homology is different from that seen for other bovine and human proteins such as growth hormone (33), prolactin (34), and pro-opiomelanocortin (35), a conservation that suggests these regions are important to enzymatic function.

The evolutionary divergence and chromosomal dispersion of the superfamily of P450 genes preceded mammalian speciation. P450s appear to exist in all eukaryotes, including yeast (36), and are also found in some prokaryotes (37, 38). Among the steroidogenic P450s, P450scc and P450c11 are

Table 1. Correlation of human chromosomes in hybrid cell lines and hybridization patterns of Fig. 5

	Cell line															
Chromosome	30/5	2/8	38/8	25/8	37/4	35/8	39/5	34/9	21/5	27/8	5/8	7/8	20/8	4/8	GL8/6	Discordant, no.
1	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	4
2	−	+	−	−	−	−	−	−	−	+	+	−	+	+	−	4
3	+	−	+	−	−	+	+	−	−	−	+	+	+	+	−	5
4	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	9
5	(+)	(+)	−	+	+	−	−	−	−	+	+	−	(+)	−	−	8
6	+	+	+	+	+	+	−	+	−	+	(+)	+	−	+	−	6
7	−	+	+	−	+	+	+	+	+	−	−	(−)	+	+	−	7
8	−	+	+	+	(+)	+	+	+	−	+	+	(+)	+	+	−	5
9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	8
10	+	−	+	+	−	−	−	+	+	−	−	−	−	+	−	9
11	−	(−)	−	+	+	−	−	+	−	+	−	+	+	−	−	10
12	(+)	+	+	+	+	+	−	−	+	+	−	(+)	+	−	−	9
13	+	−	+	−	−	−	−	−	−	−	(+)	−	+	+	−	6
14	+	+	+	−	+	+	−	+	+	−	+	+	+	+	−	8
15	−	+	+	−	−	+	+	−	−	+	+	−	(−)	+	−	0
16	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	7
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	7
18	+	+	+	−	−	+	−	−	−	+	−	−	+	+	−	4
19	−	+	+	(+)	−	−	−	(+)	+	−	+	−	−	+	−	6
20	(+)	−	−	−	+	−	−	+	+	+	−	+	+	+	−	10
21	+	−	−	+	+	−	−	−	−	+	+	−	+	−	−	9
22	+	+	+	−	+	−	−	(+)	−	−	+	−	+	−	−	8
X	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	8
Y	−	−	−	−	−	−	−	−	(+)	−	+	−	−	−	−	7

Human chromosomes (left) are scored against cell lines; number of discordances with the pattern in Fig. 5 appears at right. Note the absence of discordance for chromosome 15. Presence of a human chromosome in >30% of analyzed cells, +; 10–20%, (+); 5–9%, (−); and not detected, −. Hybrid clone GL8 selectively and exclusively retains translocation chromosome Xqter→Xq12::9p24→9qter.

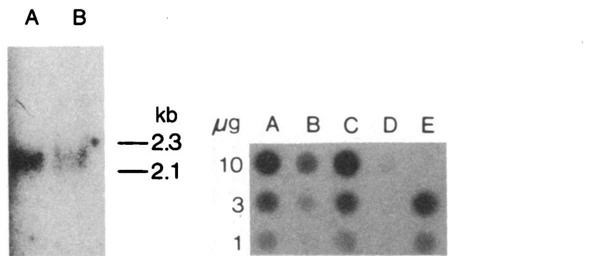

FIG. 6. Placental expression of human P450scc. (*Left*) Blot of total RNA prepared from human placentas at wk 10 of gestation (lane A) and wk 25 of gestation (lane B). Molecular size markers (in kb) are from *Hin*dIII-cut bacteriophage λ (data not shown). (*Right*) Dot blots of RNAs probed with P450scc cDNA. The top, middle, and bottom rows contain 10, 3, and 1 μg of RNA, respectively: 20-wk human placenta without tissue culture (column A); 20-wk placenta in primary culture for 4 days (column B); duplicate dish of placental cells cultured for 2 days without, then 2 days with 1 mM dibutyryl cAMP (column C); rat pituitary tumor cell line GH_3 (column D); and pig testis (column E; no 10-μg sample).

mitochondrial enzymes employing electron-transport intermediates different from the steroidogenic enzymes P450c17 and P450c21 found in the endoplasmic reticulum. The existing sequence data suggest the relationship between P450scc and P450c21 is quite distant; they are less homologous than microsomal P450c21 and the hepatic microsomal P450s induced by phenobarbital and by 3-methylcholanthrene (7). In view of this evolutionary distance among these various P450s, it is not surprising that their genes are widely dispersed among chromosomes. Thus, steroidogenic P450c21 is located on human chromosome 6 (39), phenobarbital-inducible P450pb is located on chromosome 19 (40), adrenal P450c17 is found on chromosome 10 (41), dioxin-inducible P450 is located on chromosome 15 (42), and P450scc is also on chromosome 15.

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Table 2. Clusters of nucleotide differences and amino acid identities in human and bovine P450scc

Clustered differences		Clustered homologies	
Amino acid location*	Nucleotide changes	Amino acid location*	Amino acid changes
21–24	7/8	50–68	0/19
42–44	6/7	81–108	3/28
193–195	6/7	321–344	1/24
292–297	13/16	374–398	0/25
307–309	8/9	450–473	1/24

*Locations refer to amino acid numbers shown in Fig. 4.

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