Biggin, M. D., Gibson, T. J., & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3963-3965.
Birnstiel, M. L., Busslinger, M., & Strub, K. (1985) *Cell (Cambridge, Mass.) 41*, 349-359.
Chatterjee, B., & Roy, A. K. (1980) *J. Biol. Chem. 255*, 11607-11613.
Chatterjee, B., Demyan, W. F., Lalwani, N. D., Reddy, J. K., & Roy, A. K. (1983) *Biochem. J. 214*, 879-883.
Chatterjee, B., Majumdar, D., Ozbilen, O., Murty, C. V. R., & Roy, A. K. (1987a) *J. Biol. Chem. 262*, 822-825.
Chatterjee, B., Murty, C. V. R., Olson, M. J., & Roy, A. K. (1987b) *Eur. J. Biochem. 166*, 273-278.
Deininger, P. L. (1983) *Anal. Biochem. 129*, 216-223.
Farrell, S. O., & Bieber, L. L. (1983) *Arch. Biochem. Biophys. 222*, 123-132.
Farrell, S. O., Fiol, C. J., Reddy, J. K., & Bieber, L. L. (1984) *J. Biol. Chem. 259*, 13089-13095.
Feinberg, A. P., & Vogelstein, B. (1983) *Anal. Biochem. 132*, 6-13.
Fujiki, Y., Rachubinski, R. A., Dehesa, A. Z., & Lazarow, P. B. (1986) *J. Biol. Chem. 261*, 15787-15793.
Furuta, S., Hayashi, H., Hijikata, M., Miyazawa, S., Osumi, T., & Hashimoto, T. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 313-317.
Hashimoto, T. (1987) in *Peroxisomes in Biology and Medicine* (Fahimi, H. D., & Sies, H., Eds.) pp 97-104, Springer-Verlag, New York.
Kozak, M. (1984) *Nucleic Acids Res. 12*, 857-872.
Lazarow, P. B., & Fujiki, Y. (1985) *Annu. Rev. Cell Biol. 1*, 489-530.
Maniatis, T., Fritsch, E. T., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
Markwell, M. A. K., McGaroarly, E. J., Bieber, L. L., & Tolbert, N. E. (1973) *J. Biol. Chem. 248*, 3426-3432.
Miyazawa, S., Ozasa, H., Osumi, T., & Hashimoto, T. (1983) *J. Biochem. (Tokyo) 94*, 529-542.
Osumi, T., Ozasa, H., & Hashimoto, T. (1984) *J. Biol. Chem. 259*, 2031-2034.
Osumi, T., Ishi, N., Hijiakta, M., Kamijo, K., Ozasa, H., Furutua, S., Mayazawa, S., Kondo, K., Inone, K., Kagamiyama, H., & Hashimoto, T. (1985) *J. Biol. Chem. 260*, 8905-8910.
Proudfoot, N. J., & Brownlee, G. G. (1976) *Nature (London) 263*, 211-214.
Rachubinski, R. A., Fijiki, Y., & Lazarow, P. B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3973-3977.
Reddy, J. K., Goel, S. K., Nemali, M. R., Carrino, J. J., Laffler, T. G., Reddy, M. K., Sperbeck, S. J., Osumi, T., Hashimoto, T., Lalwani, N. D., & Rao, M. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1747-1751.
Rosenfeld, G. C., Comstock, J. P., Means, A. R., & O'Malley, B. W. (1972) *Biochem. Biophys. Res. Commun. 46*, 1695-1703.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Solberg, H. E. (1972) *Biochim. Biophys. Acta 280*, 422-433.
Staden, R. (1982) *Nucleic Acids Res. 10*, 4731-4751.
Young, R. A., & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1194-1198.

Human Ethanol-Inducible P450IIE1: Complete Gene Sequence, Promoter Characterization, Chromosome Mapping, and cDNA-Directed Expression[†,‡]

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ABSTRACT: The human P450IIE1 gene, coding for an ethanol-inducible nitrosamine-metabolizing P-450, was isolated from a λEMBL3 genomic library and completely sequenced. The human gene spanned 11 413 base pairs and contained nine exons and a typical TATA box. Upstream and downstream DNAs of 2788 and 559 base pairs were also sequenced and compared to the rat gene. Significant areas of sequence similarity were observed within 140 base pairs upstream of the transcription start site in the rat and human genes. Human DNA 539 base pairs upstream of the transcription start site was inserted into the expression vector pSVOALΔ5′, and luciferase activity was detected when the constructs were introduced into a rat hepatoma cell line. The activity was over 100-fold lower than that of pRSVL, a Rous sarcoma virus LTR-driven luciferase gene. By use of panels of rodent–human cell hybrids, the gene was mapped to chromosome 10 (CYP2E locus). A full-length cDNA, constructed with the first exon of the genomic clone and a partial cDNA clone, was expressed in COS cells and found to code for *N*-nitrosodimethylamine demethylase activity.

The cytochrome P-450 gene superfamily consists of at least ten families of enzymes that share several common features among which is the presence of a noncovalently bound heme associated with a conserved cysteine-containing peptide near the carboxy terminus of the enzyme. The P-450s all contain about 500 amino acid residues and a noncleaved signal sequence and are intrinsically bound to endoplasmic reticulum membranes. These enzymes carry out a myriad of diverse biotransformations including both anabolic and catabolic reactions. The enzymology (Waxman, 1986; Black & Coon,

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1986) and molecular biology (Nebert & Gonzalez, 1987) of P-450s have been reviewed.

P450IIE1 is an unusual enzyme since it is involved in metabolism of both endogenous substances and foreign compounds such as drugs and carcinogens. In rodents, IIE1 was shown to be both a substrate-inducible acetone and acetol monooxygenase that may be involved in gluconeogenesis (Casazza et al., 1984; Koop & Casazza, 1985) and an efficient *N*-nitrosodimethylamine demethylase (Tu & Yang, 1983; Yang et al., 1985a,b; Patten et al., 1986; Thomas et al., 1987).

Rat IIE1 is under both transcriptional and posttranscriptional control. The 5-fold increase in IIE1 protein levels after administration of ethanol, acetone, and other substances is not associated with an increase in IIE1 mRNA levels (Song et al., 1986; Hong et al., 1987). In contrast, when rats are starved or made diabetic, the level of IIE1 mRNA is increased severalfold (Song et al., 1987; Hong et al., 1987). However, this increase is the result of posttranscriptional mRNA stabilization (Song et al., 1987). During development, the IIE1 gene becomes transcriptionally activated immediately after birth and is maximally expressed within 1 week post partum (Song et al., 1986, 1987; Hong et al., 1987; Umeno et al., 1988).

In the present paper we describe the complete sequence of the human IIE1 gene and analysis and comparison of the rat and human IIE1 promoters. We also determined the chromosome location of the human IIE1 gene and expressed the IIE1 cDNA in COS cells.

Materials and Methods

Materials. The luciferase expression vectors pSVOALΔ5′ and pRSVL were generously provided by Dr. Suresh Subramani, Department of Biology, University of California, San Diego. The expression vector p91023(B) was provided by Dr. Ronald J. Kaufman of Genetics Institute, Boston, MA.

Cloning and Sequence of the IIE1 Gene. A λEMBL3 library was prepared from a single adult human liver. DNA was isolated, partially digested with *Sau*3A, and size fractionated on a 5–20% NaCl gradient, and the 15–20-kbp fragments were ligated to *Bam*H1-digested λEMBl3 arms. The ligated DNA was packaged (Gigapak Gold, Vector Cloning Systems), and the recombinant phage were plated on *Escherichia coli* K802. Phage were screened with [α-^{32}P]IIE1 cDNA labeled by nick translation (BRL, Inc.) with [α-^{32}P]-dCTP (3000 Ci/mmol, Amersham, Inc.). Phage clones were amplified on *E. coli* LE392, and DNA was isolated from CsCl-handed phage particles. *Eco*RI fragments from purified phage clones were subcloned into pUC9 and sequenced by shotgun cloning into m13 (Deininger, 1983) in conjunction with the dideoxy chain termination method (Sanger et al., 1977). Each base was sequenced an average of five times and at least once in both directions. Sequence data were assembled with the Beckman Microgenie program.

Primer Extension and S1 Nuclease Mapping. The transcription start site was determined by both S1 mapping and primer extension analyses. A 386-bp fragment encompassing the first exon and upstream DNA was isolated from a genomic subclone with the enzymes *Nhe*I and *Xmn*I which cleave at positions −217 and +169, respectively (Figure 2). The fragment was 5′ end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (5000 Ci/mmol, Amersham Corp.). S1 nuclease mapping was then performed as described (Sharp et al., 1980) with human liver poly(A) RNA. A sequencing reaction was also performed on the 386-bp fragment according to the chemical cleavage procedure of Maxam and Gilbert (1980). An *Eco*RI (position −539, Figure 2) to *Xmn*I fragment was first 5′ end labeled prior to gel purification of the *Nhe*I–*Xmn*I

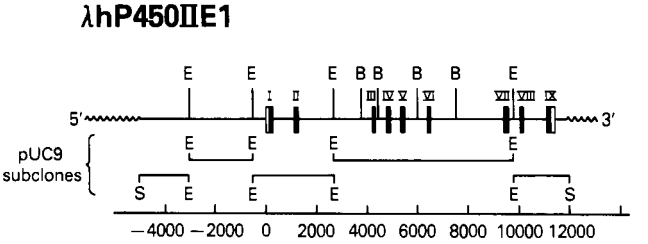


FIGURE 1: Restriction map of the human IIE1 gene. The map was generated by single and double restriction enzyme digestions with *Eco*RI (E), *Bam*HI (B), and *Sal*1 (S).

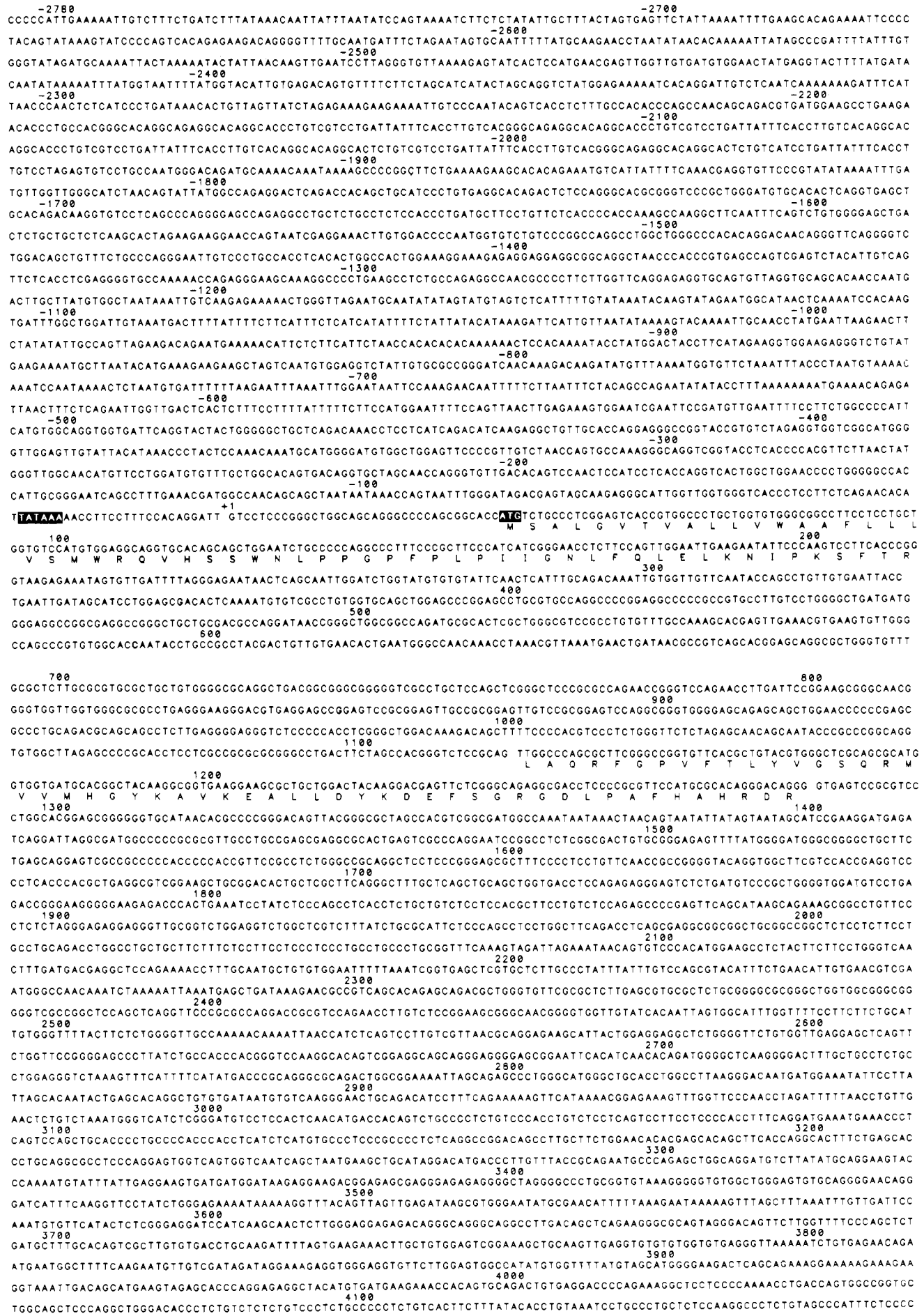
fragment. Primer extension analysis was carried out with a 25-mer oligonucleotide synthesized on an Applied Biosystems Model 380B synthesizer. The oligonucleotide corresponded to positions +144 to +169 (Figure 2). The primer was annealed to 10 μg of human poly(A) RNA and extended with AMV reverse transcriptase. The sequencing reaction, S1 protected fragments, and primer extended material were subjected to electrophoresis on a 15% polyacrylamide–8.3 M urea sequencing gel.

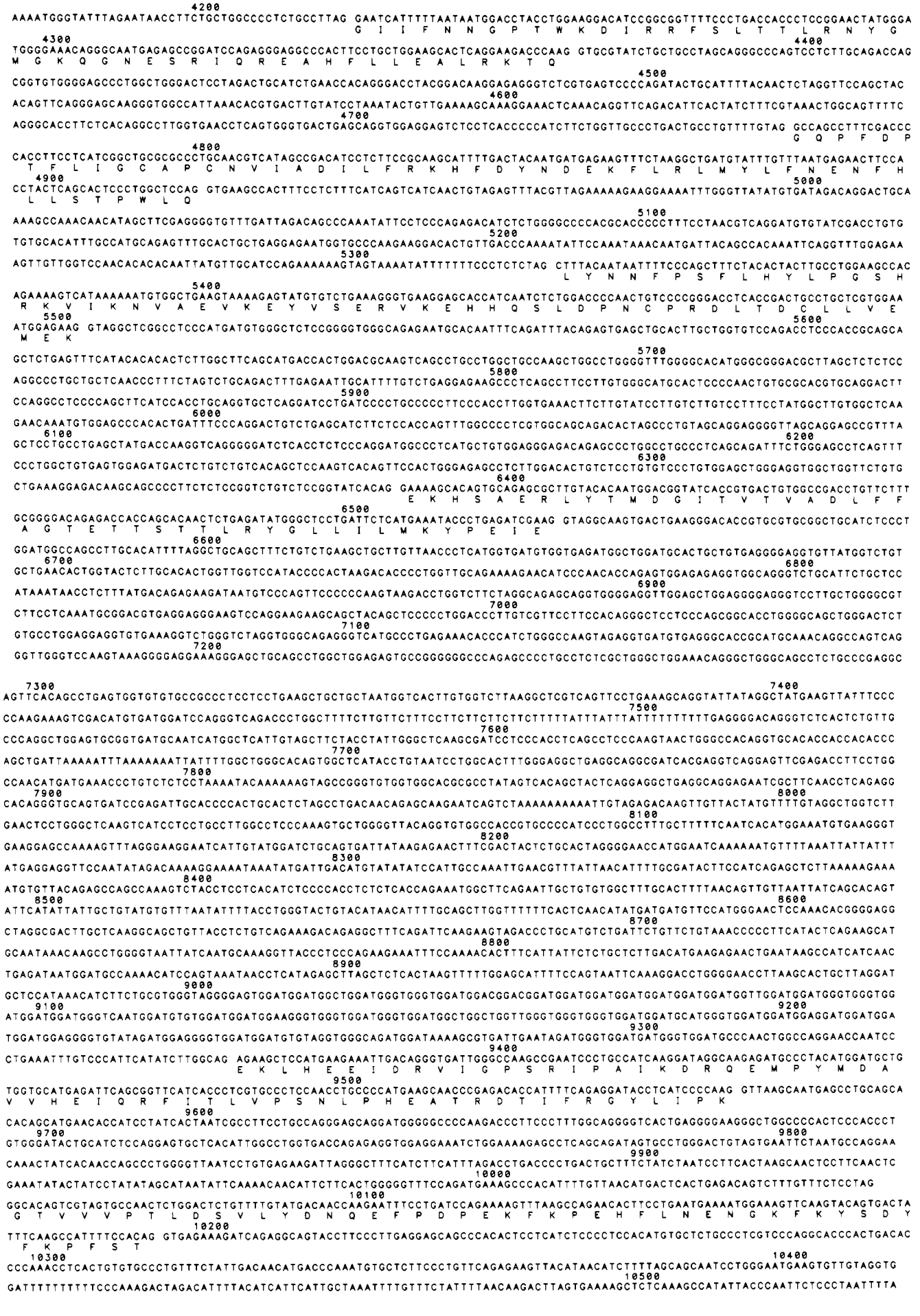
IIE1 Promoter Luciferase Expression. The human IIE1 promoter region was excised from a genomic subclone with *Eco*RI (position −539, Figure 2) and *Sma*1 (position +8, Figure 2), treated with Klenow fragment, ligated with *Hin*dIII linkers, and introduced into pUC9. The human IIE1 promoter fragment was then excised from pUC9 and ligated to pSVOALΔ5′, the promoter-lacking luciferase expression vector (de Wet et al., 1987). Plasmids were purified by two CsCl gradients and transfected into the rat hepatoma cell line McA-RH7777 (ATCC CRL 1601) by use of the calcium phosphate procedure (Graham & van der Eb, 1973) in conjunction with a glycerol shock (Parker & Stark, 1979). Luciferase activities were measured by use of an LKB luminometer.

Chromosome Mapping. Characterization of the hybrid cell lines used in this paper has been detailed elsewhere (McBride et al., 1982a,b,c, 1986). DNA from panels of hybrid cell lines was digested with *Eco*RI, electrophoretically resolved on agarose gels, and blotted to nylon membranes. Hybridization and washing were performed as described (McBride et al., 1986).

Expression of the IIE1 cDNA in COS Cells. A full-length cDNA for human IIE1 was constructed by joining the portion of the first exon of the IIE1 gene to a partial cDNA clone that lacked the coding region for four amino acids at its 5′ end (Song et al., 1986). This was accomplished by use of a unique *Xmn*I site in the cDNA (position +169, Figure 2) and an *Eco*RI site upstream of the gene's start site (position −539, Figure 2). The *Eco*RI/*Xmn*I fragment was ligated to the partial IIE1 cDNA clone at the cDNA's *Xmn*I site, and the upstream region of this construct was removed by digestion with *Bgl*I (position +29, Figure 2). After treatment with T4 DNA polymerase and addition of *Eco*RI linkers, the final cDNA containing the complete coding information for IIE1 was inserted into the *Eco*RI site of p91023(B) (Wong et al., 1985). The construct, designated p91023(B)-IIE1, was transfected into COS 1 cells as described (Luthman & Magnusson, 1983). Expression was quantitated by immunoblot analysis (Towbin et al., 1979) using anti-rat IIE1 (Song et al., 1987), and *N*-nitrosodimethylamine demethylase assays were performed as previously outlined (Yoo et al., 1987).

Other Methods. Southern blotting (Southern, 1975) was performed by the alkaline transfer method (Reed & Mann, 1985). DNA was digested, electrophoretically resolved on 1% agarose gels, depurinated, and transferred to nylon membranes





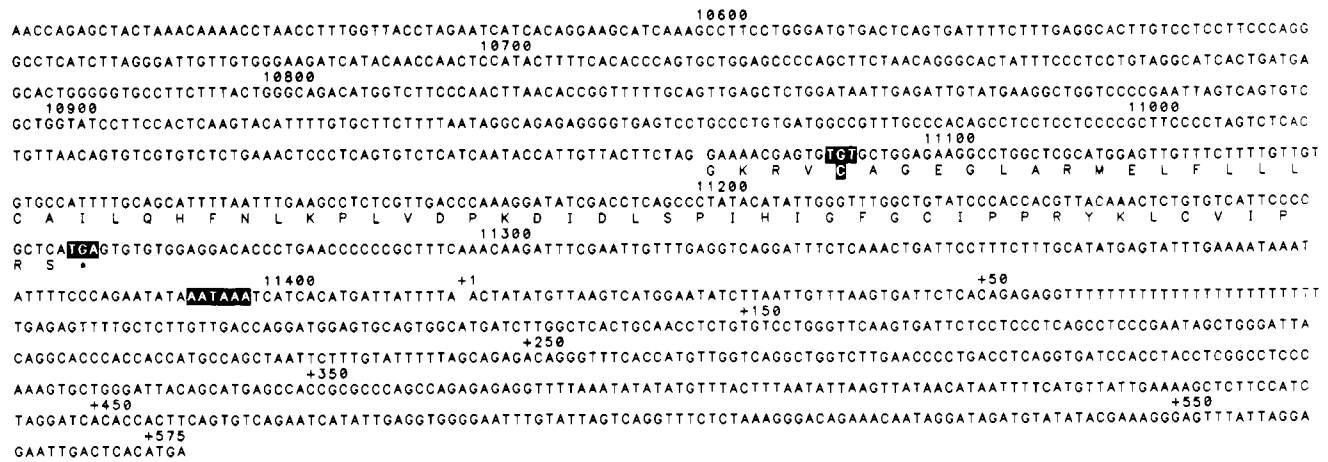


FIGURE 2: Sequence of the human IIE1 gene. The complete sequence of the structural gene and flanking regions is displayed. The mRNA cap site, determined by S1 mapping and primer extension, is designated +1. DNA upstream of the cap site and downstream of the mRNA poly(A) addition site is numbered beginning with −1 and +1, respectively. The TATA box, initiator methionine, heme binding cystine, termination codon, and consensus poly(A) addition signal are outlined by black boxes.

Table I: Comparison of the Exons and Introns of the Rat and Human IIE1 Genes

	percent similarity[a]		percent similarity[a]	intron size (bp) human	intron size (bp) rat
5′ flanking					
−1500 to −1000	37				
−1000 to −1	50				
exon		intron			
I	75	1	34	904	761
II	75	2	36	2938	3405
III	78	3	30	388	305
IV	82	4	33	406	325
V	75	5	40	881	788
VI	84	6	32	2833	1873
VII	83	7	48	499	496
VIII	84	8	25	882	776
IX	76 (coding)				
3′ flanking					
+1 to +500	25				

[a] Percent similarity was determined with the NUCALN program of Wilbur and Lipman (1983). Exon IX similarity was computed through the coding region only. The 3′ noncoding region showed no significant similarity.

Table II: Expression of the Human IIE1 Gene Promoter Linked to the Luciferase cDNA[a]

	mV/mg expt 1	mV/mg expt 2
phIIE1L (−539)	1.25	2.38
pRSVL	333	250

[a] Supercoiled plasmid DNAs were transfected onto McA-RH7777 cells, and sonicated cell extracts were assayed after 72 h of incubation. Results are the average of two transfection experiments. Values ranged about 20% between duplicate transfection in the same experiment. In each experiment a different stock of cells and a different plasmid preparation was used. Results are expressed as millivolts per milligram of cellular protein. The vector pSVOALΔ5′ produced no detectable luciferase activity, similar to that reported by de Wet et al. (1987).

(Biotrace RP, Gelman Sciences). Membranes were prehybridized and hybridized at 50 °C in Hybrisol (Oncor) with nick translated probes. Washing was performed at 65 °C in 0.3 M NaCl, 30 mM sodium citrate, and 0.2% $NaDodSO_4$. Under these conditions only sequences with less than 20% divergence are detected. Protein electrophoresis was performed as described by Laemmli (1970). Western blotting was carried out by the method of Towbin et al. (1979), as previously outlined (Song et al., 1987).

Results and Discussion

Isolation and Sequence of the IIE1 Genomic Clone. A human liver DNA λEMBL3 library was screened with the IIE1 cDNA probe, and several clones were isolated. A map of the clone containing the complete IIE1 gene and the restriction enzyme sites used for subcloning and sequencing are displayed in Figure 1. The clone, designated hP450IIE1, contained an insert of approximately 17 kbp. The complete sequence of the human IIE1 gene was determined by shotgun DNA sequencing (Figure 2). The gene spanned 11 413 bp from the start site to the poly(A) addition site (determined from the cDNA sequence). DNA of 2788 and 559 bp, upstream and downstream, respectively, was also sequenced. Comparison of the human and rat introns revealed that several are of similar size but share much less nucleotide similarities than the exons (Table I). Only intron 6 was substantially larger in the human gene. Intron 7 had the highest level of similarity at 48% whereas intron 3 was only 30% similar. In general the 3′ half of the IIE1 gene is more similar in rat and human. All intron–exon boundaries were precisely conserved between the two species.

Determination of the Transcription Start Site. The transcription start site was determined by both S1 mapping and primer extension (Figure 3). Both procedures produced fragments of identical sizes that corresponded to a G polymerase start site when compared with a sequencing ladder of the fragment used for S1 protection. This start site was located 23 bp downstream from a TATA box (Figure 3). A CCATT box was also detected at −144 bp from the start site. The 5′ untranslated region of the mRNA was only 36 nucleotides.

Comparison of Rat and Human IIE1 Upstream DNA Sequences. Alignment of rat and human upstream DNA revealed 50% overall nucleotide similarity within 1000 bp of the transcription start site (Table I). This amount of similarity is significantly more than the 34% and 37% similarity detected in the first introns and −1000- to −1500-bp region, respectively. More significant similarities were observed within 150 bp of the polymerase start site. Two regions, arbitrarily designated region I and region II, shared 80% and 94% nucleotide similarities, respectively (Figure 4). The significance of these highly similar segments, however, is still unclear.

Analysis of the Human IIE1 Upstream DNA by a Promoter Expression Vector. To determine if the DNA immediately upstream of the start site in the human gene was competent

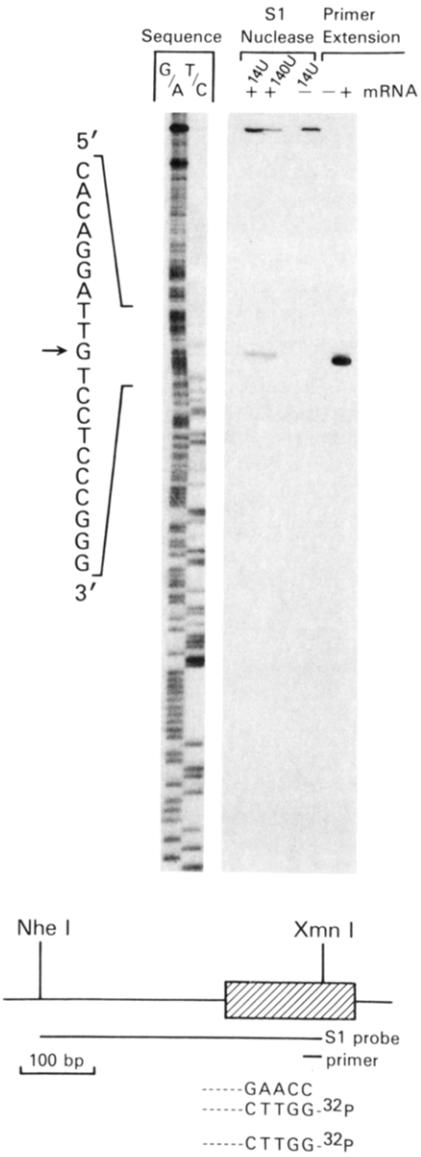


FIGURE 3: Determination of the IIE1 mRNA cap site. S1 nuclease mapping and primer extension were carried out on human liver poly(A) RNA. The resultant fragments were resolved on a 15% polyacrylamide–8.3 M urea gel. A DNA sequence ladder generated by chemical cleavage of the S1 mapping probe was electrophoresed on the same gel. The 5′ end of this fragment is coincident with the 5′ end of the primer used for primer extension (illustrated at the bottom of the figure). The precise fragment lengths were identified on a separate lower percentage gel and correspond to the G residue demarcated at the left of the figure.

to drive a promoter-lacking expression vector, it was inserted into pSVOALΔ5′ (de Wet et al., 1987). The human promoter construct, designated hIIE1L (−539), expressed luciferase activity when transfected into the rat hepatoma cell line McA-RH7777 (ATCC CRL 1601) (Table II); however, the activity was 100–300-fold less than that of pRSVL (de Wet et al., 1987). The low activity of the IIE1 promoter reflects the fact that the endogenous IIE1 gene is expressed at a level considerably less than that observed in rat liver.[1] Further experimentation will be required to determine the DNA elements necessary for efficient promoter activity.

Chromosome Mapping of the IIE1 Gene. The chromosome location of the IIE1 gene was determined according to the somatic cell hybrid strategy. Human–rodent hybrid cells

[1] T. Aoyama, S. Kimura, H. V. Gelboin, and F. J. Gonzalez, unpublished results.

Table III: Segregation of the IIE1 Gene with Individual Human Chromosomes[a]

human chromosome	gene/chromosome +/+	+/−	−/+	−/−	% discordancy
1	6	6	15	50	27
2	2	10	13	52	30
3	5	7	19	46	34
4	7	5	29	39	44
5	6	6	12	53	23
6	8	4	27	38	40
7	2	10	24	41	44
8	8	4	15	47	26
9	3	9	12	53	27
10	12	0	0	65	0
11	6	6	16	49	29
12	7	5	15	50	26
13	2	9	20	42	40
14	7	5	31	34	47
15	4	8	29	36	48
16	7	5	15	50	26
17	9	3	39	26	55
18	4	8	33	32	53
19	7	5	14	51	25
20	8	4	21	44	32
21	8	4	40	25	57
22	5	7	14	51	27
X	6	6	35	30	53

[a] The human IIE1 gene was detected as 3.0, 6.0, and 7.0-kbp hybridizing bands in *Eco*RI digests of somatic cell hybrid DNAs after Southern hybridization with a 1.6-kbp cDNA probe. Detection of the human IIE1 gene is correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy represents the presence of the gene in the absence of the chromosome or the absence of the chromosome despite the presence of the gene, and the sum of these numbers divided by total hybrids examined (× 100) represents percent discordancy. The human–hamster hybrids consisted of 18 primary hybrids and 15 subclones (7 positives of 33 total), and the human–mouse hybrids included 13 primary clones and 31 subclones (5 positives of 44 total).

containing one or more human chromosomes in a background of rodent chromosomes were analyzed by Southern blotting with the IIE1 cDNA probe. Human specific fragments of 7, 6, and 3 kbp were detected in several of the hybrid cell lines (data not shown). Under our hybridization and washing conditions, endogenous hamster IIE1 gene sequences were not detected while 10- and 3.2-kbp bands were observed in mouse DNA. The human IIE1 sequences were found only in those hybrids containing human chromosome 10, while in hybrids lacking this chromosome the human-specific fragments were not seen. The results, summarized in Table III, demonstrate a complete concordance of the presence of the IIE1 gene with chromosome 10. Recently, a member of the P450IIC gene subfamily was mapped to chromosome 10 (Okino et al., 1987). In addition, the gene coding for a P-450 involved in steroid biosynthesis (P450XVII) is located on chromosome 10 (Matteson et al., 1986). It appears, therefore, that several P-450 genes exist on this chromosome. The genes, coding for drug- and carcinogen-metabolizing P-450s, might be involved with or linked to the recently described multiple endocrine neoplasia type 2A (MEN2A) on chromosome 10 (Mathew et al., 1987; Simpson et al., 1987). Other genes in the P450II family, however, are on different chromosomes. For instance, the human IIA gene subfamily is on chromosome 19 (Phillips et al., 1985), and the IID subfamily is located on chromosome 22 (Gonzalez et al., 1988).

cDNA-Directed Expression of IIE1. Complementary DNA expression was performed to determine whether the human IIE1 had similar enzymatic characteristics to its extensively studied rodent counterparts. A portion of the first exon of the IIE1 gene was joined to a partial IIE1 cDNA (Song et al.,

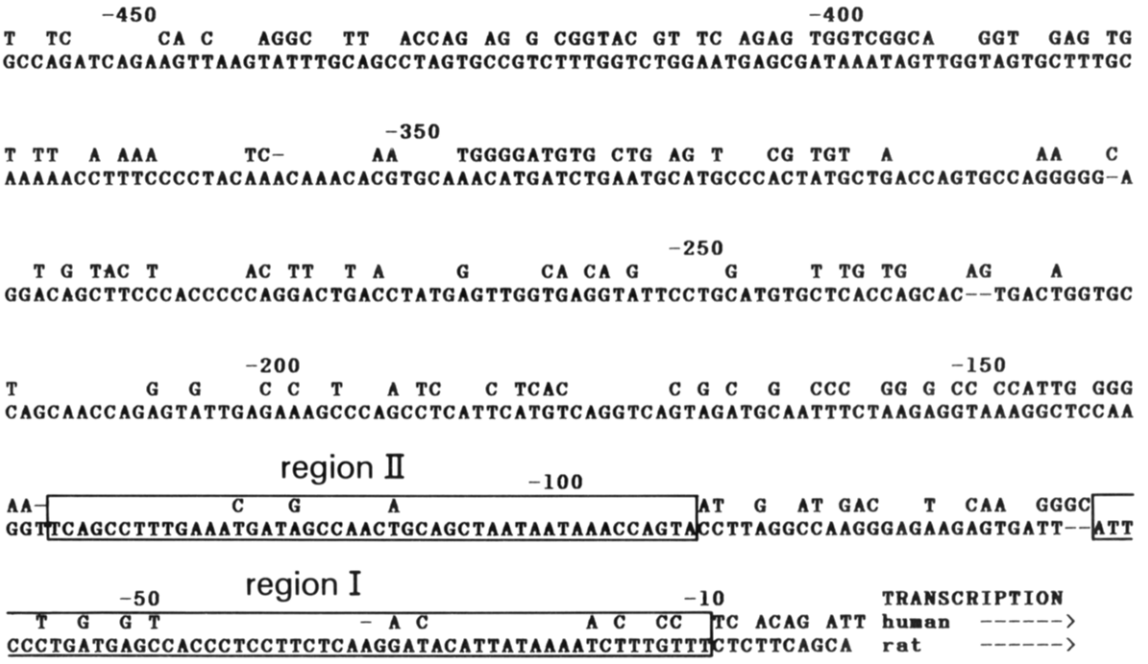


FIGURE 4: Alignment of rat and human IIE1 gene upstream DNA. Nucleotide alignment was performed with the program described by Wilbur and Lipman (1983). The rat sequence on the lower line is continuous and numbered as described in Umeno et al. (1988). Only those bases that differ between human and rat are displayed in the human sequence in the top line.

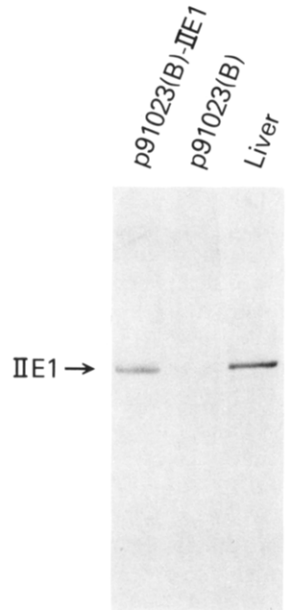


FIGURE 5: Expression of the IIE1 cDNA in COS cells. The IIE1 cDNA was inserted into p91023(B) and transfected into COS cells. Production of IIE1 protein was monitored by Western blot analysis. Total COS cell protein (100 μg) and human liver microsomal protein (10 μg) were analyzed for IIE1 by Western blotting using polyclonal antibody to rat IIE1.

1986), and the cDNA containing the complete amino acid coding sequences was inserted into the expression vector p91023(B) (Wong et al., 1985). Western immunoblot analysis confirmed that the IIE1 protein was expressed in COS cells transfected with the IIE1 cDNA, and its mobility was identical with that of the IIE1 protein detected in human liver microsomes (Figure 5). IIE1 was not detected in COS cells transfected with vector alone. Only those cells which were transfected with the IIE1 cDNA expressed *N*-nitrosodimethylamine demethylase activity (Table IV). Addition of purified rat NADPH–P-450 oxidoreductase further enhanced the activity of the IIE1-transfected cells 1.5-fold. These results conclusively demonstrated that human IIE1 possesses nitrosamine-metabolizing activity, similar to its rodent counterpart.

Table IV: *N*-Nitrosodimethylamine Demethylase Activities in COS Cells Transfected with p91023(B)-IIE1[a]

	transfected construct	specific activity [pmol min^{-1} (mg of protein)$^{-1}$][b]
experiment 1	p91023(B)	ND
	p91023(B)-IIE1	8.0
experiment 2	p91023(B)	ND
	p91023(B)-IIE1	8.4
experiment 3	p91023(B)	ND
	p91023(B)-IIE1	8.3 (12.6)

[a] COS cell microsomes were isolated from cultures transfected with p91023(B) vector alone or p91023(B)-IIE1 containing the coding region of the IIE1 cDNA. [b] ND, no activity was detected above background. The number in parentheses indicates the specific activity after a 15-min preincubation of microsomes with 1.0 kilounit of rat NADPH–P-450 oxidoreductase at 37 °C prior to addition of substrate. The substrate and protein concentrations were 40 μM and 480–640 μg/mL, respectively. Incubations were carried out for 20 min.

Registry No. P-450IIE1, 117039-30-2; human P-450IIE1 gene, 117039-31-3; human P-450IIE1 mRNA, 117039-32-4; cytochrome P-450, 9035-51-2; *N*-nitrosodimethylamine demethylase, 69772-95-8; ethanol, 64-17-5.

REFERENCES

Black, S., & Coon, M. (1986) in *Cytochrome P450: Structure, Mechanism and Biochemistry* (Ortiz de Montellano, P. R., Ed.) pp 161–216, Plenum, New York.

Casazza, J. P., Felver, M. E., & Veech, R. L. (1984) *J. Biol. Chem. 259*, 231–236.

Deininger, P. L. (1983) *Anal. Biochem. 129*, 216–223.

de Wet, J. R., Wood, K. V., De Luca, M., Helinski, D. R., & Subramani, S. (1987) *Mol. Cell. Biol. 7*, 725–737.

Gonzalez, F. J., Vilbois, F., Hardwick, J. P., McBride, O. W., Nebert, D. W., Gelboin, H. V., & Meyer, V. A. (1988) *Genomics 2*, 174–179.

Graham, F. L., & van der Eb, A. J. (1973) *J. Virol. 52*, 456–467.

Hong, J., Pan, J., Gonzalez, F. J., Gelboin, H. V., & Yang, C. S. (1987) *Biochem. Biophys. Res. Commun. 142*, 1077–1083.

Koop, D. R., & Casazza, J. P. (1985) *J. Biol. Chem. 260*, 13607–13612.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Luthman, H., & Magnusson, G. (1983) *Nucleic Acids Res. 11*, 1295–1305.
Mathew, C. G. P., Chin, K. S., Easton, D. F., Thorpe, K., Caster, C., Liou, G. I., Fong, S.-L., Bridges, C. D. B., Haak, H., Nieuwenhuijzen Kruseman, A. C., Schifter, S., Hansen, H. H., Telenius, H., Telenius-Berg, M., & Ponder, B. A. J. (1987) *Nature (London) 328*, 527–528.
Matteson, K. Y., Picado-Leonard, J., Chung, B.-C., Mohandas, T. K., & Miller, W. L. (1986) *J. Clin. Endocrinol. Metab. 63*, 789–791.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.
McBride, O. W., Battey, J., Hollis, G. F., Swan, D. C., Siebenlist, U., & Leder, P. (1982a) *Nucleic Acids Res. 10*, 8155–8170.
McBride, O. W., Hieter, P. A., Hollis, G. F., Swan, D. C., Otey, M. C., & Leder, P. (1982b) *J. Exp. Med. 155*, 1480–1490.
McBride, O. W., Swan, D. C., Santos, E., Barbacid, M., Tronick, S. R., & Aaronson, S. A. (1982c) *Nature (London) 300*, 773–774.
McBride, O. W., Merry, D., & Givol, D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 130–134.
Nebert, D. W., & Gonzalez, F. J. (1987) *Annu. Rev. Biochem. 56*, 945–993.
Okino, S. T., Quattrochi, L. C., Pendurthi, U. R., McBride, O. W., & Tukey, R. H. (1987) *J. Biol. Chem. 262*, 16072–16079.
Parker, B., & Stark, G. (1979) *J. Virol. 31*, 360–369.
Patten, C. J., Ning, S. M., Lu, A. Y. A., & Yang, C. S. (1986) *Arch. Biochem. Biophys. 251*, 629–638.
Reed, K. C., & Mann, D. A. (1985) *Nucleic Acids Res. 13*, 7207–7221.
Sanger, F., Niklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Sharp, P. A., Bork, A. J., & Berget, S. M. (1980) *Methods Enzymol. 65*, 750–768.
Simpson, N. E., Kidd, K. K., Goodfellow, P. J., McDermid, H., Myers, S., Kidd, J. R., Jackson, C. E., Duncan, A. M. V., Farrer, L. A., Brasch, K., Castiglione, C., Genel, M., Gertner, J., Greenberg, C. R., Gusella, J. F., Holden, J. J. A., & White, B. N. (1987) *Nature (London) 328*, 528–530.
Sinclair, J., Cornell, N. W., Zaitlin, L., & Hansch, C. (1986) *Biochem. Pharmacol. 35*, 707–710.
Song, B.-J., Gelboin, H. V., Park, S.-S., Yang, C. S., & Gonzalez, F. J. (1986) *J. Biol. Chem. 261*, 16689–16697.
Song, B. J., Matsunaga, T., Hardwick, J. P., Park, S.-S., Veech, R. L., Yang, C. S., Gelboin, H. V., & Gonzalez, F. J. (1987) *Mol. Endocrinol. 1*, 542–547.
Southern, E. M. (1975) *J. Mol. Biol. 98*, 503–517.
Thomas, P. E., Bandiera, S., Maines, S. L., Ryan, D. E., & Levin, W. (1987) *Biochemistry 26*, 2280–2289.
Towbin, H., Staehlin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4355.
Tu, Y. Y., & Yang, C. S. (1983) *Cancer Res. 42*, 623–629.
Umeno, M., Song, B. J., Kozak, C., Gelboin, H. V., & Gonzalez, F. J. (1988) *J. Biol. Chem. 263*, 4956–4962.
Waxman, D. J. (1986) in *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ortiz de Montellano, P. R., Ed.) pp 525–539, Plenum, New York.
Wilbur, W. J., & Lipman, D. J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 726–730.
Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A., & Clark, S. C. (1985) *Science (Washington, D.C.) 228*, 810–815.
Yang, C. S., Koop, D. R., Wang, T., & Coon, M. J. (1985a) *Biochem. Biophys. Res. Commun. 128*, 1007–1013.
Yang, C. S., Tu, Y. Y., Koop, D. R., & Coon, M. J. (1985b) *Cancer Res. 45*, 1140–1145.
Yoo, J. S. H., Chung, R., Patten, C., Wade, D., & Yang, C. S. (1987) *Cancer Res. 47*, 3378–3383.