Identification of a New P450 Expressed in Human Lung: Complete cDNA Sequence, cDNA-Directed Expression, and Chromosome Mapping†

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ABSTRACT: A cDNA coding for a P450 expressed in human lung was isolated from a λgt11 library constructed from human lung mRNA using a cDNA probe to rat P450 IVA1. The cDNA-deduced amino acid sequence of this P450, designated IVB1, consisted of 511 amino acids and had a calculated molecular weight of 59 558. The IVB1 amino acid sequence bore 51%, 53%, and 52% similarities to rat IVA1, IVA2, and rabbit $P450_{p-2}$, respectively. Comparison of the primary amino acid sequence of human IVB1 with rat IVA and rabbit p-2 P450 sequences revealed a region of absolute sequence identity of 17 amino acids between residues 304 and 320. However, the functional significance of this conserved sequence is unknown. Human IVB1 also appears to be related to P450 isozyme 5 that has been extensively characterized in rabbits. The IVB1 cDNA was inserted into a vaccinia virus expression vector and the enzyme expressed in human cell lines. The expressed enzyme had an absorption spectrum with a λ_{max} at 450 nm when reduced and complexed with carbon monoxide, typical of other cytochrome P450s. Unlike rabbit P450 isozyme 5, however, human IVB1 was unable to activate the promutagen 2-aminofluorene. Human lung microsomal P450s were also unable to metabolize this compound despite the presence of IVB1 mRNA in three out of four human lungs analyzed. In contrast to its expression in lung, IVB1 mRNA was undetectable in livers from 14 individuals, including those from which the lungs were derived. IVB1-related mRNA was also expressed in rat lung and was undetectable in untreated rat liver. In addition, this mRNA was abundant in both rat kidney and intestine. Human chromosome mapping, determined by using the somatic cell hybrid strategy, allowed the assignment of the IVB subfamily to chromosome 1 (1p12-p34). Two restriction fragment length polymorphisms were identified by *Hin*dIII and *Taq*I that were linked to the IVB1 gene.

P450s are the terminal components of the microsomal mixed-function monooxidase system. These enzymes have been grouped into a superfamily consisting of nine families in mammals (Nebert et al., 1989). Five of these families, XI, XVII, XIX, XXI, and XXVI, are involved in steroid biosynthetic processes whereas the remaining families, I, II, III, and IV, catalyze the oxidation of fatty acids, drugs, and carcinogens [reviewed in Guengerich (1987) and Gonzalez (1988)]. In these latter four families, it appears that a tremendous variability exists between species in the number of P450 genes and the substrate specificities of individual P450 forms (Gonzalez, 1989). On the basis of these findings, it is difficult, in many cases, to extrapolate toxicology and carcinogenicity studies from rodents to man. Thus, species differences underscore the necessity to directly study human P450s.

Human P450s have been studied by purifying these proteins from human liver and preparing polyclonal antibodies [reviewed in Distlerath and Guengerich (1987)]. In addition, cDNAs have been isolated from human liver libraries [reviewed in Gonzalez (1988)]. By use of cDNA probes, mutations in alleles of a P450 gene, P450 IID1 (Gonzalez et al., 1988; Skoda et al., 1988), were uncovered that account for a common drug oxidation polymorphism. It therefore seems plausible that other P450 gene defects may exist in the human population and possibly some of these may be associated with carcinogen-activating forms of P450. We are particularly interested in examining P450s expressed in human lung, a target of cigarette-induced cancer. To this end, we used a rodent cDNA probe to isolate a unique P450 cDNA from a human lung λgt11 library.

In the present report, we describe the characterization of a new human P450 cDNA, designated IVB1,[1] that is a member of P450 gene family IV. The IVB1 gene was localized to human chromosome 1 and was found to be expressed in human and rat lung and not in liver. We present evidence that IVB1 likely corresponds to the human counterpart of rabbit P450 isozyme 5 that is involved in the metabolism and activation of the promutagen 2-aminofluorene (Gasser & Philpot, 1987). The human IVB1 was also produced via cDNA-directed expression in vaccinia virus but, unlike the rabbit enzyme, could not activate this compound to a mutagenic metabolite.

Materials and Methods

Materials. Lung and liver specimens were obtained from the University of Miami Kidney Transplant Unit. *Eco*RI linkers, Sepharose CL-4B, and CsTFA were obtained from PL-Pharmacia. Packaging extract was purchased from Stratagene Cloning Systems. All other enzymes were purchased from Bethesda Research Laboratories, New England Biolabs, and International Biotechnologies, Inc. Sprague-Dawley rats were obtained from the NIH small animals repository. The vaccinia virus recombination vector pSC11, wild-type vaccinia virus strain WR, and TK$^-$ 143 cells were kindly provided by Dr. Bernard Moss of the National Institute of Allergy and Infectious Diseases.

† The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02871.

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[1] The human IVB1 cDNA and gene were named according to the nomenclature system of Nebert et al. (1989).

Cloning and Sequencing of the IVB1 cDNA. Total RNA was isolated from human tissue by a modification of the method of Chirgwin et al. (1979). Tissue was emulsified by polytron treatment in 6 M guanidine thiocyanate. Solubilized tissue was then layered over a cushion of CsTFA and centrifuged at 15 °C for 16 h at 25000 rpm using a Beckman SW 28 rotor. The RNA pellet was dissolved in sterile H_2O and extracted twice with a 1:1 emulsion of phenol and $CHCl_3$. The RNA was then collected by ethanol precipitation. Poly(A) RNA was selected by oligo(dT) chromatography (Aviv & Leder, 1972) and used as a template to construct a cDNA library in λgt11 (Young & Davis, 1983) according to a procedure similar to that described by Watson and Jackson (1985). Double-stranded cDNA was synthesized by using a kit supplied by Bethesda Research Laboratories. The DNA was blunt-ended by treatment with T4 DNA polymerase, methylated with *Eco*RI methylase, and ligated with phosphorylated *Eco*RI linkers. The ligated DNA was digested with *Eco*RI and size-fractionated on a Sepharose CL-4B column. DNA of greater than 1 kbp was ligated to *Eco*RI-digested, phosphatase-treated λgt11 DNA. Ligated DNA was then packaged and plated on *Escherichia coli* Y1088. The libraries were screened by using nick-translated rat P-$450_{LA\omega}$ (IVA1) probe (Hardwick et al., 1987) and plaque hybridization. The clones with the largest inserts were processed further. The cDNAs were subcloned into pUC9 and sequenced by using the shotgun cloning (Deininger, 1983) and dideoxy sequencing (Sanger et al., 1977) methods. Each base was sequenced at least twice in each direction. Sequence data were compiled and analyzed by using the Beckman Microgenie program.

Chromosome Localization. The somatic cell hybrid mapping strategy was used to localize the IVB1 gene. Isolation and characterization of the human–mouse and human–hamster cell lines have been described (McBride et al., 1982a–c). Briefly, human cells were fused with mutant mouse or hamster fibroblasts, and the resulting hybrid cells were isolated on selective media. Hybrid cell lines were established after subcloning that contained a subset of human chromosomes in a background of rodent chromosomes. The human chromosome content of each cell line was determined by isozyme analysis and sometimes karyotyping. DNAs were isolated from the same expanded cell populations used for characterization of human chromosome content. These DNAs were electrophoresed in agarose gels after digestion with restriction enzymes and blotted to nylon membranes, and the membranes were hybridized with nick-translated IVB1 cDNA insert as described (McBride et al., 1986).

Expression of the IVB1 cDNA. The IVB1 cDNA was expressed by using the vaccinia virus expression system. Briefly, the IVB1 cDNA was inserted into the recombination vector pSC11 (Chakrabarti et al., 1985). The pSC11 plasmid containing the IVB1 cDNA was then allowed to integrate into the vaccinia virus genome, and recombinants were selected as described (Macket et al., 1984; Chakrabarti et al., 1985). The recombinant virus was then used to infect TK^- 143 cells, and P450 spectral analysis was carried out as described (Omura & Sato, 1966). Cells were dissolved in 0.1 M sodium phosphate, pH 7.2, containing 20% w/v glycerol and 0.3% w/v Emulgen 913, and insoluble material was removed by centrifugation at 10000*g* for 10 min at 4 °C. The protein suspension was divided into two cuvettes, the sample cuvette was gently bubbled with carbon monoxide gas, and then a few crystals of solid dithionite were added to both the sample and reference cuvettes. A difference spectrum was recorded with an Aminco DW-2000 spectrophotometer. For enzyme assays, recombinant vaccinia virus was used to infect Hep G2 cells. These cells were found to have high levels of endogenous NADPH–P450 oxidoreductase and cytochrome b_5. Cell lysates were prepared by brief sonication, and enzyme activities were measured in the presence of NADPH. Aryl hydrocarbon hydroxylase (Nebert & Gelboin, 1968), 7-ethoxycoumarin *O*-deethylase (Greenlee & Poland, 1978), lauric acid hydroxylase (Orton & Parker, 1982), and 2-aminofluorene *N*-hydroxylase (Belanger et al., 1981) activities were determined as described. These assays were performed in a 2-mL incubation mixture consisting of 50 mM sodium phosphate pH 7.25, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM NADPH, 200–500 μM substrate, and 1–5 mg of cellular protein (15–75 pmol of P450). Reactions were carried out at 37 °C for 20 min. Mutagenesis assays were performed as previously outlined (Ames et al., 1975; Raineri et al., 1981) using 3 mg of vaccinia-infected cell lysate protein that was prepared by sonicating cells in 0.1 M sodium phosphate, pH 7.2, and removing nuclei and heavy debris by centrifugation at 10000*g* for 10 min at 4 °C.

Other Procedures. Rat liver, lung, kidney, and intestine RNAs were prepared as described above for human RNA except fresh tissue was used. RNA was analyzed by electrophoresis on 2.2 M formaldehyde–1% agarose (Lehrach et al., 1977), blotted to a Nytran membrane, and hybridized with the IVB1 cDNA clone by the method of Church and Gilbert (1984).

Results

Isolation and Sequence of the IVB1 cDNA. A λgt11 library was constructed from human lung mRNA. The library was screened without amplification using the rat IVA1 cDNA as a probe. Several weakly reacting plaques were identified and purified. The phage containing the largest insert was processed further and completely sequenced. The lung cDNA, designated IVB1, contained 2083 bp and an open-reading frame of 511 amino acids coding for a protein of calculated M_r 59558 (Figure 1). A typical poly(A) addition signal was detected 15–20 bp upstream of the poly(A) tail. The IVB1 mRNA contains an untranslated 3′ end of 535 nucleotides, and the cDNA possesses 12 bp of the 5′ untranslated region of the mRNA.

General Characteristics of the IVB1 Protein and Comparison with Other P450s. The IVB1 amino acid sequence had the expected hydrophobic amino-terminal peptide and the conserved Cys near the carboxy end of the protein. This residue serves as the fifth thiolate ligand to the heme iron at the enzyme's active site (Gotoh & Fujii-Kuriyama, 1988) and is surrounded by several residues that are highly conserved among all P450s including bacterial, yeast, and mammalian microsomal and mitochondrial P450s. Relative to the Cys, located at position 453 along the primary sequence, are the conserved Phe-446, Gly-449, Arg-451, Gly-455, Ala-459, and Leu-471. Several other residues in this region show striking conservative substitutions (Gotoh & Fujii-Kuriyama, 1988).

When the amino acid sequences of other members of the P450 IV gene family were compared with human IVB1, overall sequence similarities of 51%, 53%, and 52% were calculated for rat IVA1 (Hardwick et al., 1987), rat IVA2 (Kimura et al., 1989), and rabbit p-2 (Matsubara et al., 1987), respectively. These results are consistent with the assignment of human lung IVB1 to gene subfamily IVB (Nebert et al., 1989). Most of the amino acid substitutions between the IVB1 and the other IVA sequences were randomly dispersed although a greater similarity was noted in the carboxy halves of proteins (Figure 2). Most noteworthy was a region of absolute identity

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GGAACTGCAACCATGGTGCCCAGCTTCCTCTCCCTGAGCTTCTCCTCCTTGGGCCTGTGGGCTTCTGGGCTGATCTTGGTCTTAGGCTTTCTCAAGCTCATCCACCTGCTGCTGCGGAGG  120
        MetValProSerPheLeuSerLeuSerPheSerSerLeuGlyLeuTrpAlaSerGlyLeuIleLeuValLeuGlyPheLeuLysLeuIleHisLeuLeuLeuArgArg  36

CGAACGTTGGCTAAGGCTATGGACAAATTCCCAGGGCCTCCCACCCACTGGCTTTTTGGACATGCCCTCGAGATCCAGGAGACGGGGAGCCTGGACAAAGTGGTGTCCTGGGCCCACCAG  240
ArgThrLeuAlaLysAlaMetAspLysProGlyProProThrHisTrpLeuPheGlyHisAlaLeuGluIleGlnGluThrGlySerLeuAspLysValValSerTrpAlaHisGln  76

TTCCCGTATGCCCACCCACTCTGGTTCGGACAGTTCATTGGCTTCCTGAACATCTATGAGCCTGACTATGCCAAAGCTGTGTACAGCCGTGGGGACCCTAAGGCCCCTGATGTGTATGAC  360
PheProTyrAlaHisProLeuTrpPheGlyGlnPheIleGlyPheLeuAsnIleTyrGluProAspTyrAlaLysAlaValTyrSerArgGlyAspProLysAlaProAspValTyrAsp  116

TTCTTCCTCCAGTGGATTGGGAGAGGCCTGCTGGTTCTTGAGGGGCCCAAGTGGTTGCAGCACCGCAAGCTGCTCACACCTGGCTTTCATTATGATGTGCTGAAGCCCTATGTGGCCGTG  480
PhePheLeuGlnTrpIleGlyArgGlyLeuLeuValLeuGluGlyProLysTrpLeuGlnHisArgLysLeuLeuThrProGlyPheHisTyrAspValLeuLysProTyrValAlaVal  156

TTCACTGAGTCTACACGTATCATGCTGGACAAGTGGGAAGAGAAAGCTCGGGAGGGTAAGTCCTTTGACATCTTCTGCGATGTGGGTCACATGGCGCTGAACACACTCATGAAGTGCACC  600
PheThrGluSerThrArgIleMetLeuAspLysTrpGluGluLysAlaArgGluGlyLysSerPheAspIlePheCysAspValGlyHisMetAlaLeuAsnThrLeuMetLysCysThr  196

TTTGGAAGAGGAGACACCGGCCTGGGCCACAGGGACAGCAGCTACTACCTTGCAGTCAGCGATCTCACTCTGTTGATGCAGCAGCGCCTTGTGTCCTTCCAGTACCATAATGACTTCATC  720
PheGlyArgGlyAspThrGlyLeuGlyHisArgAspSerSerTyrTyrLeuAlaValSerAspLeuThrLeuLeuMetGlnGlnArgLeuValSerPheGlnTyrHisAsnAspPheIle  236

TACTGGCTCACCCCACATGGCCGCCGCTTCCTGCGGGCCTGCCAGGTGGCCCATGACCATACAGACCAGGTCATCAGGGAGCGGAAGGCAGCCCTGCAGGATGAGAAGGTGCGGAAGAAG  840
TyrTrpLeuThrProHisGlyArgArgPheLeuArgAlaCysGlnValAlaHisAspHisThrAspGlnValIleArgGluArgLysAlaAlaLeuGlnAspGluLysValArgLysLys  276

ATCCAGAACCGGAGGCACCTGGACTTCCTGGACATTCTCCTGGGTGCCCGGGATGAAGATGACATCAAACTGTCAGATGCAGACCTCCGGGCTGAAGTGGACACATTCATGTTTGAAGGC  960
IleGlnAsnArgArgHisLeuAspPheLeuAspIleLeuLeuGlyAlaArgAspGluAspAspIleLeuSerLeuSerAspAlaAspLeuArgAlaGluValAspThrPheMetPheGluGly  316

CATGACACCACCACCAGTGGTATCTCCTGGTTTCTCTACTGCATGGCCCTGTACCCTGAGCACCAGCATCGTTGTAGAGAGGAGGTCCGCGAGATCCTAGGGGACCAGGACTTCTTCCAG  1080
HisAspThrThrThrSerGlyIleSerTrpPheLeuTyrCysMetAlaLeuTyrProGluHisGlnHisArgCysArgGluGluValArgGluIleLeuGlyAspGlnAspPhePheGln  356

TGGGATGATCTGGGCAAAATGACTTATCTGACCATGTGCATCAAGGAGAGCTTCCGCCTCTACCCACCTGTGCCCCAGGTGTACCGCCAGCTCAGCAAGCCTGTCACCTTTGTGGATGGC  1200
TrpAspAspLeuGlyLysMetThrTyrLeuThrMetCysIleLysGluSerPheArgLeuTyrProProValProGlnValTyrArgGlnLeuSerLysProValThrPheValAspGly  396

CGGTCTCTACCTGCAGGAAGCCTGATCTCTATGCATATCTATGCCCTCCATAGGAACAGTGCTGTATGGCCCGACCCTGAGGTCTTTGACTCTCTGCGCTTTTCCACTGAGAATGCATCC  1320
ArgSerLeuProAlaGlySerLeuIleSerMetHisIleTyrAlaLeuHisArgAsnSerAlaValTrpProAspProGluValPheAspSerLeuArgPheSerThrGluAsnAlaSer  436

AAACGCCATCCCTTTGCCTTTATGCCCTTCTCTGCTGGGCCCAGGAACTGCATTGGGCAGCAGTTTGCCATGAGTGAGATGAAGGTGGTCACAGCCATGTGCTTGCTCCGCTTTGAGTTC  1440
LysArgHisProPheAlaPheMetProPheSerAlaGlyProArgAsnCysIleGlyGlnGlnPheAlaMetSerGluMetLysValValThrAlaMetCysLeuLeuArgPheGluPhe  476

TCTCTGGACCCCTCACGGCTGCCCATCAAGATGCCCCAGCTTGTCCTGCGCTCCAAGAATGGCTTTCACCTCCACCTGAAGCCACTGGGCCCTGGGTCTGGGAAGTAGCTCTGATGAGAA  1560
SerLeuAspProSerArgLeuProIleLysMetProGlnLeuValLeuArgSerLysAsnGlyPheHisLeuHisLeuLysProLeuGlyProGlySerGlyLys *  511

TGGGGTCCCAGATGGCTCAGGCTGTGACCTCCCTGGGCACCACCCTCCCCAGGCTGGGTGTGGAGGAGTTGGGGCCCCCTGCCTTCAGGAGGCTTGTAGTTTAGAAGGGAAGTAGGCATT  1680

ACCATAGACGACTCCTAGAGGACAGTGCTATGTAAAAATGTGTGTCTATAAATGTTTATCATGCATGTATTCTAGAGCTCATTCATTTATTCAACAAACATTTGGTGAGCACCTATTTCG  1800

GTTCGAGAAACTTCATTTATCTCCTATAATTGGCAAACTTAAAAATGCAGCAGAAACTTACATTCCAACCTTAGAGACTCATAGTGAGCACAAGGAAAGTTTTGCCCTGAGATTCATGGT  1920

TATGGCTGGGTACCACCAAATAGAAGAATGGCTTAGGGGAGTGCCCCTTCACTGAGATGTGTTTCTTTGTTGAACTTTGTGTGTGTGTGTTTAGAATATAACAGACATAAGAAAAAATTA  2040

CCTAAATGAAGACTGTACAAAATAATAAATAATTCTGAAGCAGAn  2083
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FIGURE 1: cDNA and deduced amino acid sequence of human IVB1. The complete nucleotide and amino acid sequences are presented. The consensus poly(A) addition signal is underlined. The termination codon is indicated by an asterisk.

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IVB1       MVPSFLSLSFSSLGLWASGLILVLGFLKLIHLLLRRRTLAKAMDKFPGPPTHWLFGHALEIQETGSLDKVVSWAHQFPYAHPLWFGQFIGFLNIYEPDYAKAVYSRGDPKAPDVY     115
IVA1   MSVSALSSTRFTG I GFLQV  V G L LLV AVQFY Q QW L  FQQ  S  F  F   K-QF GDKE QQIMTCVEN  S F R  WGSKAY IV D   M VILG S    NG     119
IVA2   MGFSVFS TRSLDGV GFFQG FL S F VLF AVQFY   QW L  LE   ST S   W  N KDR ---FQQ LT VEK  G CLQ LSGSTARVLL D   V V LG S   P---    117
p-2      MALS TR PG L G LQV AL G L LL  AAQ Y H QW LR LQQ  C  F   L  SR F NDQE ERIQK VEK  G C W LSGNKAR LV D   L VILG S     RN     117

       DFFLQWIGRGLLVLEGPKWLQHRKLLTPGFHYDVLKPYVAVFTESTRIMLDKWEEKAREGKSFDIFCDVGHMALNTLMKCTFG-R-GDTGLGHRDSSYYLAVSDLTLLMQQRLVSFQYHND    234
       RLLAP   Y   L N QP F   RM   A    I     KNMAD I L      QL GQDS IE  QHISL T D V   A S-HN SVQVDGNYK  IQ IGN ND FHS VRNIFHQ     239
       QSLAP   Y   L N K  F   RM   A    I     KIMAD VS       KLDDQDHPLE  HY SL T D V   A S-HQ SVQ DVNSR  TK  E  NN IFF VR AF G S   237
       KLMTP   Y   L D QT F   RM   A    I     GLMVD VQ    R  QLISQDS LE  QH SL T D I   A SYQ- SVQ DRNSH  IQ IN  NN VFY ARNVFHQS    237

       FIYWLTPHGRRFLRACQVAHDHTDQVIRERKAALQDEKVRKKIQNRRHLDFLDILLGARDEDDIKLSDADLRAEVDTFMFEGHDTTTSGISWFLYCMALYPEHQHRCREEVREILGDQDF    354
       T  NFSSN HL N    L      G  KL  DQ  NAGELE VKKK R         L  M NGDS   K                A  V  IF AL TH K  Q      QSV   GSS   359
       I  NMSSD  LSR    I  E   G  KT   Q  N EELQ ARKK           F KM  GKS   E                A     VF AL TH    E      QS    GTS   357
        L R S E  L H    L  E   R  QQ   Q  Q GELE VRRK R      V  F KM NGSS   Q                A  V  IF AL TH           IQGL   GAS   357

       FQWDDLGKMTYLTMCIKESFRLYPPVPQVYRQLSKPVTFVDGRSLPAGSLISMHIYALHRNSAVWPDPEVFDSLRFSTENASKRHPFAFMPFSAGPRNCIGQQFAMSEMKVVTAMCLLRF    474
       IT  H DQIP T      AL       GIV E  TS   P      K IQVTLS  G  H PK   N     PS  APDSP--  SHS L   G A     K         IV LT       477
       VT  H DQ P T      AL   S   S S E  S   P    I K IRVTIL  G  H PSY  N K   PS   PDSP--  SH YL   G A     K    N L  AV LT       475
       IT EH DQ P T      AL       S T        P      K VILFLS  G  Y PK  QN     PF  APDS YHS --  L   G A     K    R L  AV LT V     475

       EFSLDPSRLPIKMPQLVLRSKNGFHLHLKPLGPGSGK*                                                                                      511
        LLP  TKV  PL R   K    IY Y  K H*                                                                                          509
        LLP  T I VP  R   K    I  R  K R*                                                                                          507
        LLP  T I  PIARV  K    I  R RK H*                                                                                          507
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FIGURE 2: Alignment of the IVB1 amino acid sequence with the rat IVA1 and IVA2 and rabbit $P450_{p-2}$ sequences. Alignments were performed pairwise by using the FASTA program of Pearson and Lipman (1988). Asterisks indicate the end of the sequences. Gaps are introduced in rat IVA1, IVA2, and rabbit $P450_{p-2}$ sequences at amino acid residues that are the same as in IVB1 and dashes are introduced to maximize alignments.

between all 4 sequences, consisting of 17 amino acids located at positions 304–320 along the IVB1 sequence (Figure 2). Although it is possible that this segment was the result of gene conversion, the fact that it is conserved in three species and two subfamilies makes this possibility unlikely. The 17 amino acid cluster may have been functionally conserved as a portion of the protein that is associated with the active site of the P450. For instance, these amino acids may form a pocket for substrate binding. In this connection, it is noteworthy that a Thr residue located at amino acid position 301 in two rabbit P450s in the II family appears to be involved in substrate binding (Imai & Nakamura, 1988). Changing this residue to a His results in abolition of enzymatic activity and lack of detectable substrate binding; otherwise, the mutant P450 appears to possess heme in its normal configuration. On the basis of work using cDNA expression and chimeric enzyme construction, it was also proposed that the middle third of the P450 confers substrate specificity (Sakaki et al., 1987). The role of these 17 conserved residues in catalytic activity of the P450 IV enzymes awaits further experimentation.

The primary amino acid sequence of human IVB1 displayed high 84% similarity with the recently published cDNA-deduced sequence of rabbit P450 isozyme 5 (Gasser & Philpot, 1989). The 17 amino acid cluster was also absolutely conserved between these proteins. This high degree of sequence similarity suggests quite strongly that human IVB1 is the orthologue of rabbit form 5, that has been extensively studied by Philpot and co-workers (Robertson et al., 1983; Parandoosh et al., 1987; Vanderslice et al., 1985, 1987; Gasser & Philpot, 1987). This is supported by calculated amino acid similarities between other known P450 orthologues between rabbit and man (Nebert et al., 1987).

Chromosomal Localization of the IVB1 Gene. The somatic cell hybrid mapping strategy was used to localize the IVB1

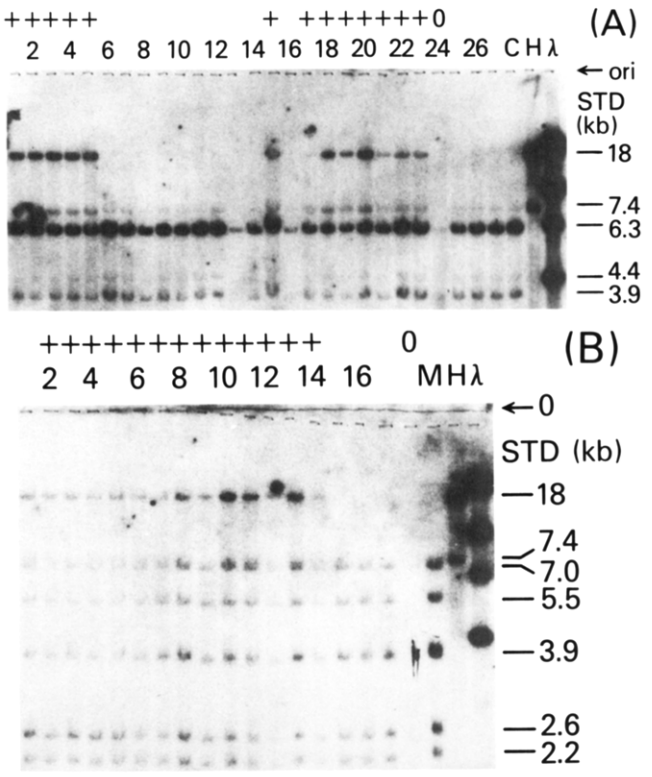

FIGURE 3: Hybridization of representative *Eco*RI-digested human–hamster (A) and human–mouse (B) somatic cell hybrid DNAs with 1450 bp 5′ human P450 IVB1 cDNA probe. A different hybrid cell DNA is present in each lane; parental Chinese hamster (C) and mouse (M) and human placental (H) DNAs are also shown. *Hin*dIII-digested λDNA markers are shown (λ). The size of hybridizing sequences is indicated (right margin). The presence of the 18 kbp hybridizing human sequence is indicated above the lanes (+); the absence of DNA or presence of degraded DNA in a lane is indicated by the symbol 0.

gene. The human–rodent cell hybrid lines and mapping procedures have been described (McBride et al., 1982a–c, 1986). This involved Southern analysis of *Eco*RI-digested, size-fractionated, hybrid cell DNAs which have been used previously to map genes on each human chromosome. The enzyme *Eco*RI was found to produce a human-specific 18 kbp IVB1 fragment that was resolved from the mouse and Chinese hamster parental cell line fragments (Figure 3). A second weakly hybridizing human band of 7.4 kbp appeared to cosegregate with the 18 kbp band, but it could not be definitively localized due to overlap with cross-hybridizing rodent sequences. A total of 14 mouse–human and 27 Chinese hamster–human primary hybrids and 49 subclones were analyzed as shown in Figure 3 and Table I. The 18 kbp human band segregated discordantly with all human chromosomes except chromosome 1 (Table I). Every cell line that contained chromosome 1 contained the 18 kbp band, and every cell line that lacked chromosome 1 lacked the 18 kbp band. These results allow unambiguous assignment of the IVB1 gene to this chromosome. One human–mouse and two human–hamster hybrids, containing spontaneous breaks involving chromosome 1 with retention of the short arm, all retained the human IVB1 gene. In one of these hybrids, the break occurred in the short arm distal to N-RAS (1p12) whereas the break occurred in the proximal long arm in the other two cases. The human IVB1 gene was not present in two other hybrids containing a 1p34-pter translocation chromosome. These results permit regional localization of the IVB1 gene to 1p12-p34. This locus is named *CYP4B* according to the recently described P450 gene nomenclature report (Nebert et al., 1989) and is

Table I: Segregation of the IVB1 Gene with Human Chromosome 1[a]

human chromosome	gene chromosome +/+	+/−	−/+	−/−	% discordancy
1	31	0	0	59	0[b]
2	23	8	3	56	12
3	20	11	15	44	29
4	28	3	30	29	37
5	24	7	7	52	16
6	31	0	17	42	19
7	21	10	16	43	29
8	20	11	17	42	31
9	22	9	9	50	20
10	15	16	5	54	23
11	19	12	11	48	26
12	16	15	12	47	30
13	18	13	13	46	29
14	19	12	20	39	24
15	21	10	22	37	36
16	16	15	19	40	38
17	26	5	26	33	34
18	21	10	26	33	40
19	24	7	3	56	11
20	26	5	15	44	22
21	22	9	37	22	51
22	17	14	11	48	28
X	20	11	25	34	40

[a] The IVB1 gene(s) was(were) detected as an 18 kbp hybridizing sequence in *Eco*RI digests of human–rodent hybrid cell DNAs after Southern hybridization with a 1450 bp 5′ human cDNA probe. This band was well resolved from 2.2, 2.6, 3.9, 5.5, and 7.0 kbp mouse and 3.9, 4.4, and 6.3 kbp hamster cross-hybridizing sequences. A very weakly hybridizing 7.4 kbp human band appeared to cosegregate with the larger human band, but the low intensity of hybridization and the overlap with a rodent band of similar size precluded unambiguous chromosomal assignment for this band. Detection of the gene is correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy represents presence of the gene in the absence of the chromosome (+/−) or absence of the gene despite the presence of the chromosomes (−/+), and the sum of these numbers divided by the total number of hybrids examined × 100 represents percent discordancy. The human–hamster hybrids contained 27 primary hybrids and 13 subclones (14 positives of 40 total), and the human–mouse hybrids consisted of 14 primary clones and 36 subclones (17 positives of 50 total). [b] Analysis of several hybrids containing breaks or translocations involving human chromosome 1 permitted regional localization of the IVB1 gene to 1p12–p34.

the first P450 locus yet localized to human chromosome 1. These results also indicate that the IVB subfamily may contain only one or two genes since only 7.4 and 18 kbp fragments were detected.

Restriction Fragment Length Polymorphisms (RFLP) in the CYP4B Gene. Simple two-allele polymorphisms (RFLPs), due to the presence or absence of single restriction sites, were detected in both *Hin*dIII and *Taq*I digests of DNA isolated from normal individuals after Southern hybridization with the IVB1 cDNA probe (Figure 4). Thirty-nine individuals were examined, and polymorphic fragments of 12.3 and 7.3 kbp (A2) were detected with *Hin*dIII that are allelic with a 20 kbp fragment (A1). With *Taq*I, allelic fragments of 11.2 kbp (B2) and 14.4 kbp (B1) were detected (Figure 4). The allele frequencies were A1:A2 = 0.83:0.17 and B1:B2 = 0.92:0.08. No linkage disequilibrium was detected between these two polymorphisms. These RFLPs will permit further localization of the IVB1 gene by genetic linkage analysis in families. No RFLPs were detected (10 individuals examined) with *Eco*RI, *Bam*HI, *Sac*I, *Xba*I, *Pst*I, *Pvu*II, *Eco*RV, *Bgl*II, *Msp*I, or *Kpn*I.

cDNA-Directed Expression of IVB1. The IVB1 cDNA was inserted into vaccinia virus and expressed in human TK$^-$ cells. Infection of these cells with the IVB1 recombinant virus

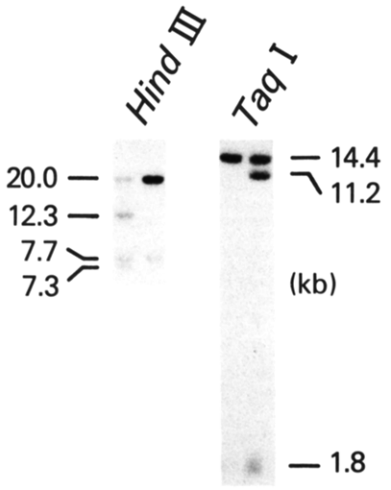

FIGURE 4: Detection of RFLPs at the *CYP4B* locus on chromosome 1. Aliquots (10 μg) of DNA isolated from the peripheral leukocytes of 39 normal, unrelated individuals were digested to completion with *Hin*dIII or *Taq*I, fractionated by gel electrophoresis, and analyzed by Southern hybridization with the IVB1 cDNA probe followed by autoradiography. In *Hin*dIII digests, 7.3 and 12.3 kbp (A2) bands are allelic with a 20 kbp band (A1), and a 7.7 kbp constant band is also observed. A 14.4 kbp band (B1) is allelic with an 11.2 kbp band (B2) in *Taq*I digests; the 1.8 kbp band is invariant. No individuals homozygous for A2 or B2 were observed; the hybridization patterns of heterozygotes are shown in the outside lanes. The allele frequences (39 individuals) were A1:A2 = 0.83:0.17 and B1:B2 = 0.92:0.08.

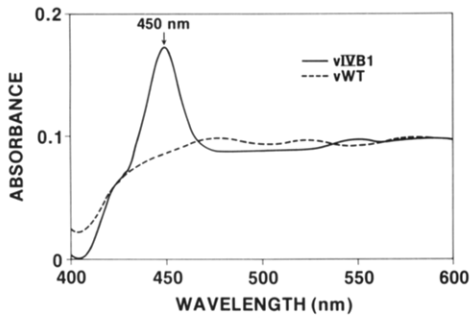

FIGURE 5: Spectral analysis of cDNA-expressed IVB1. TK⁻ 143 cells were infected with recombinant vaccinia virus expressing IVB1 (vIVB1) or wild-type vaccinia virus (vWT) and harvested after 48 h. Spectra were measured from 41 and 37 mg of solubilized protein from vIVB1- and vWT-infected cells, respectively, as described under Materials and Methods.

(vIVB1) resulted in the production of protein that when reduced and complexed with carbon monoxide displayed an absorption peak with a λ_{max} of 450 nm (Figure 5). Cells infected with wild-type virus (vWT) did not contain any measurable P450. These spectra results are typical of P450 and demonstrate that the human IVB1 cDNA is capable of producing a spectrally active enzyme. Even though the rat IVA1 cDNA was used to isolate this human lung cDNA, antibody against the rat enzyme (Hardwick et al., 1987) was unable to recognize human IVB1 on Western immunoblots. In any case, in view of the fact that the expressed IVB1 P450 is spectrally active and shares high amino acid similarity and regulatory aspects similar to P450 isozyme 5, we examined its activity toward substrates known to be suitable for isozyme 5, such as 2-aminofluorene (Vanderslice et al., 1987) and lauric acid (Williams et al., 1984). Vaccinia-expressed IVB1 (45 pmol) was unable to produce the *N*-hydroxy metabolite of 2-aminofluorene. In contrast, the same amount of human IA2, expressed by using vaccinia virus, was able to catalyze 2-aminofluorene N-hydroxylation.[2] This metabolite, however,

Table II: Mutagen Activation by cDNA-Expressed Human P450s IVB1 and IA2[a]

	revertants/mg of cell lysate		
expt	vWT	vIA2	vIVB1
1	10	4698	10
2	8	4176	9

[a] Total cell lysate (3 mg of protein) of Hep G2 cells, infected with wild-type vaccinia (vWT) and vaccinia expressing IA2 (vIA2) and IVB1 (vIVB1), was incubated with 5 μg of 2-aminofluorene in the presence of *Salmonella typhimurium* strain TA-98, and histidine revertants were scored on selective media. The duplicate results are presented as revertants/mg of cell lysate protein. Each sample contained 40–50 pmol of vaccinia-expressed P450 as measured spectrophotometrically (Omura & Sato, 1966).

Table III: Mutagen Activation by Human Lung and Liver S9[a]

	revertants/mg of protein					
	lung S9				liver S9	
control	1	2	3	4	1	2
10	12	11	13	10	222	343
9	15	8	13	10	242	313

[a] Tissue was homogenized in 0.05 M sodium phosphate, pH 7.25, and 0.14 M NaCl. The samples were centrifuged at 10000*g* for 10 min at 4 °C, and supernatant fractions were assayed by using the Ames test as described in the legend to Table II. Results are duplicate determinations.

is highly reactive, so the possibility exists that the assay was not sensitive enough to detect low levels of human IVB1 activity. We therefore used the Ames mutagenesis assay to establish whether IVB1 could activate 2-aminofluorene to its mutagenic metabolite. IVB1 was unable to promote the production of histidine revertants over background levels as assessed by using cell lysates from IVB1 recombinant vaccinia-infected cells (Table II). Vaccinia-expressed IA2, on the other hand, was able to activate this promutagen. IA2 is known to be expressed in liver but not in extrahepatic tissue (Gonzalez, 1988), whereas IVB1 is selectively expressed in human lung and perhaps other nonhepatic tissues (see below). We therefore examined four human lung specimens and two liver specimens for 2-aminofluorene activation. Both liver S9 preparations displayed significant activities toward 2-aminofluorene activation whereas the lung preparations were totally inactive (Table III). These results contrast those of rabbit lung samples which are quite active in 2-aminofluorene metabolism and activation (Robertson et al., 1983). These results further support the contention that human IVB1 does not metabolize this potent mutagen. Lauric acid hydroxylase activity, previously associated with rabbit P450 (LM_5) (presumably identical with isozyme 5), was also not catalyzed by our expressed IVB1 P450 whereas vaccinia-expressed rat IVA1 very efficiently catalyzed ω and ω-1 hydroxylation of this substrate.[3] We were also unable to detect benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities. These activities were, however, catalyzed by cDNA-expressed mouse IA1 (Aoyama et al., 1989).

Expression of the IVB Gene. To analyze the expression of the IVB1 gene, Northern blotting was performed on livers and lungs of four individuals. Three of the human lung samples had mRNAs of about 2.3 kb (Figure 6). This size is predicted from the cDNA sequence (Figure 1) assuming a poly(A) tail of 200–300 nucleotides. In one lung sample, only a small amount of IVB1 mRNA was detected. This lung sample did,

[2] P. T. Nhamburo and F. J. Gonzalez, unpublished results.

[3] T. Aoyama, J. P. Hardwick, and F. J. Gonzalez, unpublished results.

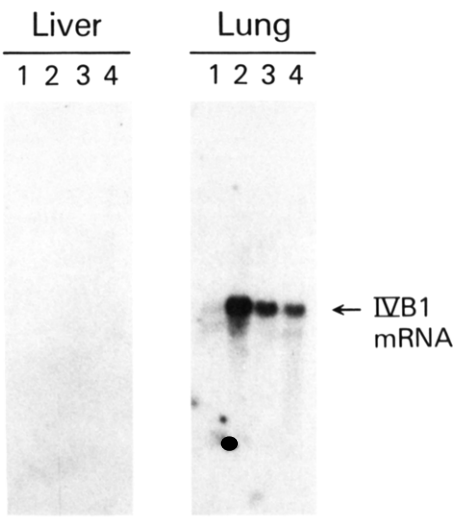

FIGURE 6: Analysis of IVB1 mRNA in human lung and liver. Total RNA (10 μg) was electrophoresed, transferred to nylon filters, and hybridized with nick-translated IVB1 cDNA. The filters were exposed to autoradiographic film for 24 h. The mRNA size was estimated in a separate experiment using an RNA ladder obtained from Bethesda Research Laboratories.

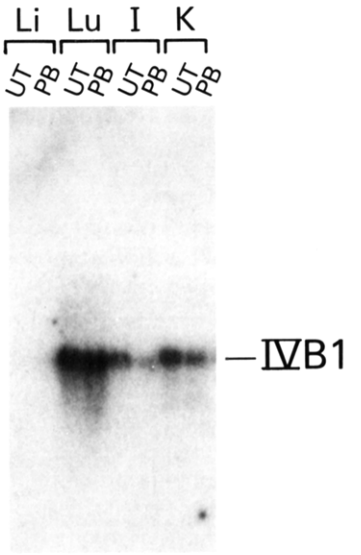

FIGURE 7: Analysis of IVB1 mRNA in rat tissues. Total RNA (10 μg) from untreated (UT) and phenobarbital-treated (PB) adult male rats (an intraperitoneal injection of 100 mg/kg body weight 48 h prior to killing) was subjected to Northern blotting analysis using the human IVB1 cDNA probe. RNA was isolated from liver (Li), lung (Lu), intestine (I), and kidney (K).

however, contain levels of actin mRNA comparable to the other three specimens.[2] The low-level expression of IVB1 mRNA in lung sample 1 could be due to either a genetic deficiency or a regulatory difference. For example, three of the lung specimens could have been induced for IVB1 mRNA, although the kidney donors from which these lungs were derived were not receiving any known P450 inducers. In contrast to the results in lung, human livers were totally devoid of IVB1 or related mRNAs. Analysis of a total of 14 human liver specimens for IVB1 mRNA did not reveal the presence of this mRNA, even after long exposure of the filters to X-ray film. These results indicate that the IVB1 gene is selectively expressed in human lung and is present at very low levels or is absent from the liver.

Expression of the IVB1 gene was also examined in rats. RNA was isolated from four different tissues of untreated and phenobarbital-treated adult male rats and subjected to Northern blotting analysis using the human IVB1 cDNA as a probe. IVB1-related RNA was detected in lung, intestine, and kidney, and its level was not induced by phenobarbital treatment (Figure 7). In fact, a slight decrease was noted in both intestine and kidney. IVB1 mRNA was not detected in untreated or phenobarbital-treated rat liver. The expression of IVB1-related mRNA in rat lung but not in liver agrees with the results of isozyme 5 protein levels in rat (Vanderslice et al., 1987).

Discussion

In the current report, we describe the primary sequence of a novel human P450 that is expressed in lung but whose mRNA is not detectable in liver. At present, however, we cannot rule out the possibility that IVB1 mRNA is expressed in human liver but below the level of sensitivity of our assay. Another possibility is that the IVB1 gene is not constitutively expressed in this tissue but is inducible by drugs or other agents. Indeed, the rabbit counterpart of human IVB1 is inducible in liver by phenobarbital although it is also detected in untreated animals (Robertson et al., 1983; see below). Unfortunately, induction studies are not feasible in man.

The IVB1 is a member of the P450 IV family and was isolated by low-stringency screening of extrahepatic tissues with the rat IVA1 probe. We could not detect any IVA mRNA in our human lung samples nor could we isolate any IVA cDNA clones from our lung libraries. A IVA cDNA has, however, been isolated from the lungs of progesterone-treated rabbits (Matsubara et al., 1987). This clone, designated $P450_{p-2}$, corresponds to a P450 prostaglandin hydroxylase that is induced in lungs of progesterone-treated (Yamamoto et al., 1984) and pregnant rabbits (Williams et al., 1984). We do not know if any IVA genes are expressed in lungs of pregnant women, and indeed this would be a very difficult question to answer unless a noninvasive test could be developed for these gene products. Interestingly, in lungs of pregnant rats, no IVA mRNA has been detected on Northern blots.[2]

On the basis of high amino acid sequence similarities, IVB1 appears to be the human orthologue of rabbit P450 isozyme 5 that has been extensively studied by Philpot and co-workers (Robertson et al., 1983; Parandoosh et al., 1987; Vanderslice et al., 1985, 1987; Gasser & Philpot, 1987). At present, it cannot be ruled out whether two or more genes exist in the IVB subfamily since 7.4 and 18 kbp exist in man (Figure 3). In rabbits and several other species, only a single protein has been detected on immunoblots (Vanderslice et al., 1987).

Isozyme 5 was first identified in both the liver and lung of untreated rabbits, and it is induced by phenobarbital only in liver (Robertson et al., 1983; Parandoosh et al., 1987). In mouse, rat, pig, and monkey, on the other hand, immunochemically detectable isozyme 5 was also found in lung but was undetectable in liver (Vanderslice et al., 1987). These studies are consistent with our findings in rat using the human IVB1 cDNA as a probe. The putative rat IVB1 mRNA was detected in lung, intestine, and kidney but was absent or in very low content in liver of untreated rats. Similarly, IVB1 mRNA was not found in several human liver specimens. Expression of IVB1 in rabbit but not rat or human liver suggests an interesting species difference in tissue-specific expression of this gene.

Our data also indicate a potential species difference in catalytic activities of IVB1. Isozyme 5 is capable of N-hydroxylating 2-aminofluorene (Vanderslice et al., 1987) and of activating this compound to mutagenic metabolites (Robertson et al., 1983; Vanderslice et al., 1987). Human IVB1,

expressed via vaccinia virus, is incapable of either producing the N-hydroxylated metabolite or activating 2-aminofluorene in the Ames test. In contrast, vaccinia-expressed human IA2 readily activates this promutagen. The lack of 2-aminofluorene N-hydroxylation activity in three human lung microsome samples, derived from lung specimens expressing ample IVB1 mRNAs, also supports the possibility that human IVB1 is catalytically dissimilar to rabbit isozyme 5.

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