Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5769–5773, August 1988
Biochemistry

Stable expression of rat cytochrome P-450IIB1 cDNA in Chinese hamster cells (V79) and metabolic activation of aflatoxin B_1

(gene transfer/monooxygenases/rat P-450IIB1 gene expression/test system for mutagens and carcinogens)

JOHANNES DOEHMER*†, SATISH DOGRA*, THOMAS FRIEDBERG*, SOLANGE MONIER‡, MILTON ADESNIK§, HANSRUEDI GLATT*, AND FRANZ OESCH*

*Institut für Toxikologie, Universität Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz, Federal Republic of Germany; §Department of Cell Biology and the Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016; and ‡Institut d'Embryologie du Centre National de la Recherche Scientifique et du College de France, Nogent-sur-Marne, France

Communicated by Ronald W. Estabrook, March 25, 1988 (received for review December 6, 1987)

ABSTRACT **V79 Chinese hamster fibroblasts are widely used for mutagenicity testing but have the serious limitation that they do not express cytochromes P-450, which are needed for the activation of many promutagens to mutagenic metabolites. A full-length cDNA clone encoding the monooxygenase cytochrome P-450IIB1 under control of the simian virus 40 early promoter was constructed and cointroduced with the selection marker neomycin phosphotransferase (conferring resistance to G418) into V79 Chinese hamster cells. G418-resistant cells were selected, established as cell lines, and tested for cytochrome P-450IIB1 expression and enzymatic activity. Two cell lines (SD1 and SD3) were found that stably produce cytochrome P-450IIB1. Although purified cytochromes P-450 possess monooxygenase activity only after reconstitution with cytochrome P-450 reductase and phospholipid, the gene product of the construct exhibited this activity. This implies that the gene product is intracellularly localized in a way that allows access to the required components. If compared with V79 cells, the mutation rate for the hypoxanthine phosphoribosyltransferase (*HPRT*) locus in SD1 cells is markedly increased when exposed to aflatoxin B_1, which is activated by this enzyme.**

The microsomal cytochrome P-450 monooxygenase enzyme system is involved in the oxidation of a wide variety of structurally unrelated compounds. This includes endogenous substrates such as steroids, fatty acids, and exogenous pharmaceutical agents; chemical carcinogens; and other lipophilic xenobiotics (1). In terms of broad substrate specificity leading to the biotransformation of xenobiotics, this enzyme system is considered to be equally as important in handling xenobiotics as the immune system is in handling pathogens (2). Paradoxically, monooxygenases may turn xenobiotics into chemically highly reactive metabolites, which then may exert mutagenic, carcinogenic, and cytotoxic activities. It is of fundamental toxicological interest to identify those compounds that may be metabolized to mutagens and carcinogens and those conditions that control the toxification and detoxification reactions. For this purpose, a variety of test systems combining a toxicological end point with an appropriate xenobiotic-metabolizing system have been developed (1). Systems with bacteria or cell lines as indicator organisms appear particularly convenient and efficient for the detection of biologically active compounds. However, they have the shortcoming of having very limited xenobiotic metabolizing activities. In mammalian cells, the expression of cytochromes P-450 is rapidly decreased or even completely lost during culture (3). This is true, in particular, for well proliferating cells as required for mutagenicity studies. Therefore, exogenous metabolizing systems, such as host animals, freshly isolated intact cells, subcellular tissue preparation, and purified enzymes are used. This not only renders the test more difficult, but also implies the risk that active metabolites that are unable to penetrate cell membranes are not detected (4).

Nowadays, cloning of genes and gene transfer are well established procedures that can readily be applied to overcome the problems described above and to set up cell lines with specific functions, such as expression of monooxygenases. A stable cell line would be an advantage, because, first, a stably expressing cell line would substantially facilitate toxicological test systems and allow repeatable measurements under highly defined conditions. Second, a test system based on a genetically engineered cell is biologically relevant as the place of generation and action of newly generated metabolites is the same without being separated by the cellular membrane. Third, genes for a single cytochrome P-450 or a defined set of genes for xenobiotic-metabolizing enzymes could be introduced and, therefore, changes in metabolism and toxicological response could be attributed to specific enzyme(s), thus defining the role of the enzyme(s) in toxification and detoxification processes.

Several genes encoding monooxygenases have been cloned, described, and analyzed (5, 6). A few attempts have been undertaken to establish cell lines with xenobiotic-metabolizing enzymes. Montisano and Hankinson (7) transferred uncloned and unselected genomic DNA into mouse Hepa-1 cells and succeeded in selecting for a cell line producing cytochrome P_1-450 (new nomenclature: P-450IA1) (8). Monkey kidney cells (Cos 1) were shown to be capable of expressing and producing bovine 17α-hydroxylase (P-450XVIIA1) transiently after gene transfer of a full-length cDNA in a simian virus 40 (SV40) expression vector (9). Recently, yeast cells were successfully transformed with a yeast expression vector containing the mouse cytochrome P_1-450 (P-450IA1) (10) and cytochrome P-450$_d$ (P-450IA2) (11). Transient expression of cytochromes P_1-450 (P-450IA1) and P_3-450 (P-450IA2) in various mammalian cells was described recently by Battula *et al.* (12).

Here we report on two V79-derived cell lines stably expressing an enzymatically active monooxygenase rat cytochrome P-450IIB1, which is the major isozyme of the phenobarbital-inducible P-450 subfamily. A full-length cDNA under control of the SV40 early promoter was delivered to Chinese hamster cells (V79). This cell line was chosen as recipient for two reasons. They are the cells that are most widely used in mutagenicity studies and they completely lack cytochrome P-450-dependent enzyme activities (13, 14). Nevertheless, V79 cells contain NADPH-dependent cytochrome P-450 reductase, an enzyme required for cytochrome

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: SV40, simian virus 40.

†To whom reprint requests should be addressed.

P-450 to be operative as monooxygenase. With cytochrome *c* as the substrate for cytochrome P-450 reductase, V79 cells showed an activity of ≈3000 pmol per mg of total protein per min, while freshly isolated hepatocytes had an activity of 42,000 pmol per mg of total protein per min (15).

The usefulness of these cell lines in mutagenicity evaluation studies was demonstrated by using the potent hepatocarcinogen aflatoxin B_1.

MATERIALS AND METHODS

Reconstruction of a Complete P-450 cDNA. This plasmid, pSP450, which contains the entire coding region of the cytochrome P-450IIB1 cDNA, most of the 5′ untranslated region, and a small portion of the 3′ untranslated region in the plasmid vector pSP64, was obtained by ligation of nonoverlapping portions of two partial cDNA clones (p5′-2 and pF6) isolated from a cDNA library (16) and a portion of a rat genomic clone (pR11) containing the first exon of the P-450IIB2 gene (17). This part of the P-450IIB2 sequence is identical with the P-450IIB1 sequence (18). For this construction (Fig. 1), an *Acc* I/*Pst* I fragment of pR11, representing sequences within the first exon, was cloned in the corresponding sites of the vector pUC8 to obtain the plasmid pg5′. This was cut with *Pst* I and ligated to a *Pst* I fragment of p5′-2 to generate the plasmid p5′ half. The *Pst* I sites at the 5′ end of the insert in p5′-2 and at the 3′ end of the insert in pg5′ correspond to the *Pst* I site at nucleotide 164 of the P-450IIB1 cDNA (19). The *Acc* I (*Hin*cII) site in the p5′ half was replaced by a *Hin*dIII site by addition of *Hin*dIII linkers to blunt ends generated by *Hin*cII cleavage. The *Hae* III fragment (base pair 790–1548) of pF6, containing the 3′ half of the coding region of P-450IIB1 and 41 base pairs (bp) of 3′ untranslated sequence, was cloned into the *Hin*cII site of the plasmid vector pUC8PA (20) to give the subclone p3′ half. The plasmid pSP450, containing the full-length P-450IIB1 cDNA, was obtained by three-way ligation of the pSP64 plasmid vector cut with *Hin*dIII and *Bam*HI, the *Hin*dIII/*Fsp* I fragment of the p5′ half, and the *Fsp* I/*Bam*HI fragment of the p3′ half. pSP450 contains, just downstream from the P-450 insert, a small segment of the SV40 genome, which includes the poly(A)$_n$ addition signals for the SV40 viral genes.

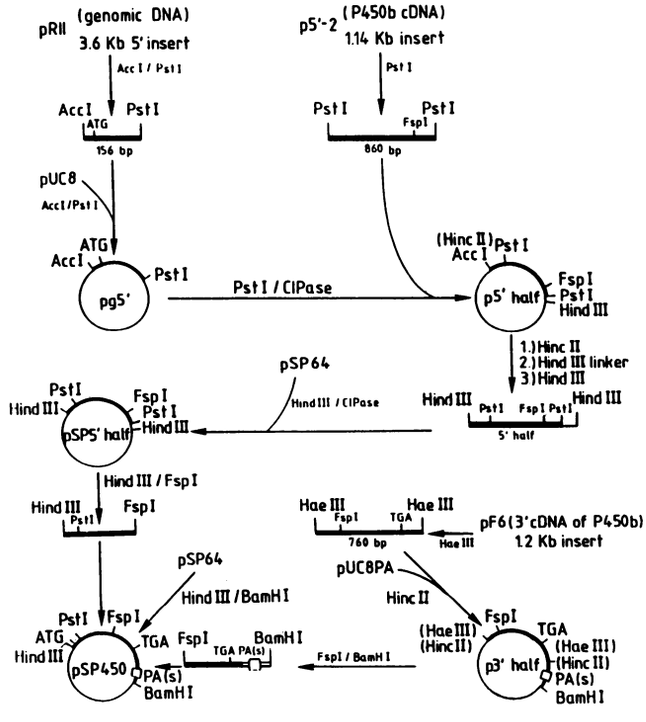

FIG. 1. Construction of full-length P-450IIB1 and recombination with the SV40 polyadenylylation signal [PA(s)]. kb, Kilobases.

Cloning of P-450IIB1 cDNA into the pSV2 Vector. The plasmid pSV450 (Fig. 2*A*) contains a full-length P-450 cDNA whose transcription is controlled by the SV40 early promoter. It was obtained by a trimolecular ligation involving the pSV2 vector (21) cleaved with *Hin*dIII and *Bam*HI, and the *Hin*dIII/*Fsp* I and *Fsp* I/*Bam*HI fragments of pSP450. The *Fsp* I/*Bam*HI fragment restores the SV40 polyadenylylation signal, which is removed in the cleavage of pSV2.

Cell Culture. V79 Chinese hamster cells were maintained in Dulbecco–Vogt's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were free of mycoplasma contamination (22).

Transfection of Cells. Transfection was carried out essentially as described by Graham and van der Eb (23) and by Stow and Wilkie (24). Briefly, 20 μg of purified and *Eco*RI linearized plasmid pSV450 DNA and 1 μg of purified and *Eco*RI linearized plasmid pMTneo342-2 DNA were diluted into 1000 μl of Hepes-buffered saline (137 mM NaCl/6 mM dextrose/5 mM KCl/0.7 mM Na_2HPO_4/20 mM Hepes, adjusted to pH 7.05 with 0.5 M NaOH). The DNA was precipitated by addition of 52.6 μl of 2.5 M $CaCl_2$ (final concentration, 125 mM), and the mixture was left at room temperature for 25 min and then added to 1×10^6 V79 cells in a 60-mm Petri dish. After 4 hr of incubation, the cells were treated with 15% (vol/vol) glycerol in DMEM for 2 min and then washed twice with DMEM. After overnight incubation at 37°C in DMEM/10% fetal calf serum, cultures were split into three 80-mm Petri dishes. G418 was added to the medium 2 days after splitting. Colonies appeared ≈2 weeks after the addition of G418 and were picked after 3 weeks by cloning cylinders and grown in mass culture for further studies.

Nucleic Acid Analysis. High molecular weight DNA from cells was prepared and analyzed by the Southern blot transfer procedure (25). Briefly, 10 μg of genomic DNA, cleaved with *Eco*RI, was electrophoresed in 0.7% agarose gel and transferred to nitrocellulose filters. Blotted DNA was hybridized to the ^{32}P-labeled *Hin*dIII/*Bam*HI fragment of pSV450 encoding the 5′ half of the P-450 gene. Hybridizations were

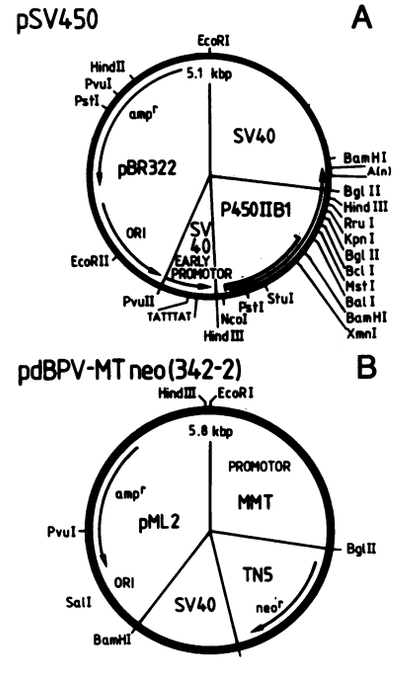

FIG. 2. Restriction maps of plasmids pSV450 and pMTneo342.

performed in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/50% formamide/1× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/100 μg of salmon sperm DNA per ml at 45°C for 18 hr. Filters were washed in 2× SSC and 0.1% $NaDodSO_4$ for 5 min with two changes and in 0.1× SSC/0.1% $NaDodSO_4$ for 15 min at 60°C with two changes. Fluorography was carried out by exposure of Kodak X-Omat film to dried filters at −70°C, in conjunction with intensifying screens.

Total RNA of SD1 cells was prepared by homogenization of liquid nitrogen frozen cells in 6 M guanidium HCl/1 mM dithiothreitol/20 mM sodium acetate, pH 7.0. The cell homogenate was purified by low speed centrifugation (2000 × *g*) for 30 min. The supernatant was layered onto a 10-ml cushion of 5.7 M CsCl and centrifuged in an SW28 rotor at 28,000 rpm for 18 hr (26). Pelleted RNA was dissolved in H_2O, ethanol precipitated, and resuspended in H_2O at a concentration of 1 μg/μl.

Total RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and blotted onto nitrocellulose filters (Schleicher & Schüll). Hybridization conditions were as described for Southern blotting. Total RNA of Aroclor-1254 (gift from Bayer AG, Leverkusen, F.R.G.) treated rat liver was prepared as described above.

Protein Analysis. The presence of P-450IIB1 in the cells was detected by electrophoretic separation of cellular homogenate ($NaDodSO_4$/PAGE) and immunological detection (immunoblotting) as follows. $NaDodSO_4$/PAGE was performed according to Laemmli (27), with the following modifications. The stacking gel was 5% acrylamide and 0.1% bisacrylamide in 0.05 M Tris phosphate (pH 6.7) and 0.1% $NaDodSO_4$. The separating gel was 10% acrylamide and 0.2% bisacrylamide in 0.38 M Tris·HCl (pH 8.9) and 0.1% $NaDodSO_4$. Transfer of proteins to nitrocellulose sheets and immunological detection of proteins on nitrocellulose was carried out as described (28). Peroxidase staining was done with diaminobenzidine and H_2O_2 as substrates. As first antibody in the immunoblotting experiments, anti-cytochrome P-450IIB1 IgG was used (29).

Enzyme Assays. Enzymatic activity was measured by cytochrome P-450IIB1-dependent O-dealkylation of a 7-pentoxyresorufin (Boehringer Mannheim) assay mixture. Cells from two confluent 75-cm^2 flasks were washed with phosphate-buffered saline (PBS) and removed by trypsin, harvested by centrifugation (100 × *g*) for 10 min, washed with PBS, and resuspended in 1 ml of PBS. Intact hepatocytes were isolated from Aroclor-1254 and corn oil-treated rats by the two-step collagenase perfusion technique (30). Aroclor-1254 in corn oil was injected i.p. at 500 mg per kg of body weight 5 days before the animals were sacrificed. Hepatocytes and cultivated cells in PBS were disrupted with a sonifier cell disruptor (Branson-B15) to yield cell homogenate. Total protein content was determined with a Bio-Rad protein assay kit using bovine serum albumin as standard. The O-dealkylation of 7-pentoxyresorufin was measured essentially as described by Lubet *et al.* (31). A 2-ml reaction mixture consisted of 50 mM Tris·HCl, pH 7.5/25 mM $MgCl_2$/10 μl of substrate (10 μM). Between 50 and 300 μl of homogenate was added. The reaction was started by the addition of 12.5 μM NADPH. The dealkylation of 7-pentoxyresorufin was monitored by recording the increase in the relative fluorescence of the product of this reaction (resorufin) as a function of time. Measurements were carried out on an Aminco-SPF 500 spectrophotofluorimeter with excitation at 522 nm and emission at 586 nm. Purified resorufin, kindly provided by Boehringer Ingelheim, served as standard.

Aryl hydrocarbon hydroxylase activity was determined by measuring the fluorescence of the hydroxylated metabolites of benzo[*a*]pyrene in an Aminco-SPF 500 spectrophotofluorimeter with excitation at 396 nm and emission at 522 nm (32); 3-OH benzo[*a*]pyrene was used as reference. The reaction mixture (1.0 ml) contained 50 μmol of Tris·HCl (pH 7.5), 0.3 μmol of $MgCl_2$, 0.6 μmol of NADPH, 100 nmol of benzo[*a*]pyrene, and 200–800 μg of cell homogenate. Aryl hydrocarbon hydroxylase activity was expressed as pmol of 3-OH-benzo[*a*]pyrene formed per mg of protein per min. As a positive control, aryl hydrocarbon hydroxylase activity was also determined in control hepatocytes and hepatocytes prepared from Aroclor-1254-treated rats.

Mutagenicity Test. On day 1, 1.5×10^6 cells were seeded with 30 ml of medium in 15-cm Petri dishes. After 18 hr, the test compound or the solvent only (60 μl) was added. Twenty-four hours later, the exposure was terminated by a change of the medium. On day 4, the cells were detached by treating with trypsin. Solvent control cultures yielded $\approx 8 \times 10^7$ cells and mutagen-treated cultures yielded $5–8 \times 10^7$ cells, indicating at most low cytotoxicity of the treatment. The cells were then subcultured at a density of 3×10^6 cells per 15-cm Petri dish. On day 8, they were subcultured again at a density of 10^6 cells per 15-cm Petri dish in medium containing 6-thioguanine (7 μg/ml) to determine the number of mutants (six replicate plates). The cloning efficiency was determined by plating 100 cells with 5 ml of 6-thioguanine-free medium in 6-cm Petri dishes (three replicate plates). The colonies were counted after incubating for 9 days (cloning efficiency plates) and 10 days (selection plates). The coefficient of variation in the number of colonies between replicate plates was 7–25%.

RESULTS

Reconstruction of a Complete P-450 cDNA. A full-length cDNA encoding the rat cytochrome P-450IIB1 was reconstructed starting from three different DNA fragments (Fig. 1): the *Acc* I/*Pst* I fragment (plasmid pR11) containing the ATG start site and the first 156 bp of the first exon, the *Pst* I/*Pst* I fragment (plasmid p5′-2) containing 860 bp of the coding sequence, the *Hae* III/*Hae* III fragment (plasmid pF6) containing 760 bp that overlap with the *Pst* I/*Pst* I fragment, and the TGA stop site and 3′ untranslated region.

Insertion of Reconstructed P-450IIB1 into the Expression Vector pSV2. The full-length P-450IIB1 cDNA was recombined with the pSV2 vector and placed under the control of the SV40 early promoter. The resulting plasmid pSV450 is shown in Fig. 2*A*.

Construction of V79 Cells with Recombinant Plasmid pSV450. The plasmid pSV450 (Fig. 2*A*) was transfected into V79 cells by the calcium phosphate procedure (23, 24). The neomycin phosphotransferase gene, under the control of the mouse metallothionein promoter in plasmid pMTneo(342-12) (33), served as a selection marker. A part of this plasmid containing the genomic DNA of bovine papilloma virus was deleted with *Bam*HI. The remaining part of the plasmid (pMT342-2) was subcloned prior to use in transfection experiments (Fig. 2*B*).

Plasmid pSV450 and pMTneo342-2 were linearized with *Eco*RI before transfection.

G418-resistant colonies appeared between 10 and 14 days after transfection. Seven colonies were picked with cloning cylinders and grown in mass culture for further studies.

To study the presence and organization of pSV450 DNA in the G418-selected cells, cellular clones were grown and their DNA was analyzed by Southern blot hybridization (Fig. 3). Chromosomal DNA was digested with *Eco*RI and hybridized to a P-450IIB1-specific probe (*Hin*dIII/*Bam*HI fragment shown as a dark line in Fig. 2*A*). No hybridizing fragments were detected in the chromosomal DNA of V79 cells (Fig. 3, lane b). Strongly hybridizing fragments were detected in *Eco*RI-digested chromosomal DNA of cell lines SD1, -3, -4,

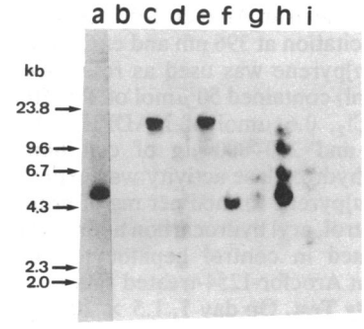

FIG. 3. Southern blot of *Eco*RI-digested chromosomal DNA. DNA was hybridized to the *Hin*dIII/*Bam*HI fragment of the P-450IIB1 DNA. Lanes: a, *Eco*RI linearized plasmid pSV450; b, cell line V79; c–i, cell lines SD1, -2, -3, -4, -5, -6, and -7. kb, Kilobases.

and -6. Some weakly hybridizing fragments were visible in the digested DNA of cell lines SD2, -3, -5, −6, and -7, suggesting that these DNA fragments contained shortened P-450IIB1 DNA. The strongly hybridizing DNA fragments of cell lines SD4 and SD6 were slightly smaller than the *Eco*RI-digested plasmid pSV450 DNA, which should be the minimum size of an intact integrated copy but might nevertheless contain a deleted pSV450 DNA. Digested chromosomal DNA of cell lines SD1 and SD3 only contained a strongly hybridizing DNA fragment, which was larger than the pSV450 *Eco*RI linearized plasmid DNA.

Expression of the P450IIB1 Gene. To test whether the integrated P-450IIB1 gene is expressed, total mRNA was isolated, electrophoresed on a 0.7% agarose gel under denaturing conditions, and hybridized to the pSV450 *Hin*dIII/*Bam*HI fragment (Fig. 4). Cell line SD1 contained reacting mRNA (Fig. 4, lane d). Reacting mRNA in total RNA isolated from treated rat liver is shown as a reference (lane a). SD2 and V79 cells did not contain hybridizing mRNA (lanes b and c).

Cell lysates were separated on $NaDodSO_4$/polyacrylamide gel, transferred to a nitrocellulose filter, and probed with a polyclonal antibody directed against P-450IIB1 (immunoblotting). Cell line SD1 was found to contain immunoreacting protein comigrating with purified P-450IIB1 (Fig. 5, lane e). All other cell lines, especially the parental cell line V79, did not contain immunoreactive material (lanes a, b, d, and f). For comparison, microsomes of Aroclor-1254-induced parenchymal liver cells (lanes i and j), which contained the cytochrome P-450IIB1 and P-450IIB2, were tested in parallel. Because of their high degree of structural homology, both isozymes crossreacted with the antibody. A third as yet unidentified isozyme form was detected that is structurally related to P-450IIB1 and P-450IIB2. Also, cell line SD3 was found to be positive in immunoblotting (data not shown).

Enzymatic Characterization of the P-450IIB1 Gene Product. Enzymatic activity in SD1 cells was tested by O-dealkylation of 7-pentoxyresorufin and compared to activities found in hepatocytes isolated from Aroclor-1254-pretreated as well as untreated rats (Table 1). Activity in SD1 cell lysate was between 3 and 4 times higher than in cell lysate of hepatocytes

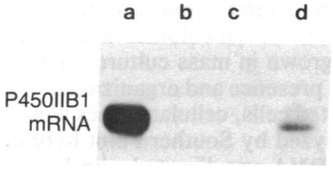

FIG. 4. RNA blot of P-450IIB1 mRNA. Total RNA was hybridized to the *Hin*dIII/*Bam*HI fragment of the P-450IIB1 DNA. Lanes: a, 10 μg of total RNA from hepatocytes of Aroclor-1254-treated liver; b, 10 μg of total RNA from V79 cells; c, 10 μg of total RNA from SD2 cells; d, 10 μg of total RNA from SD1 cells.

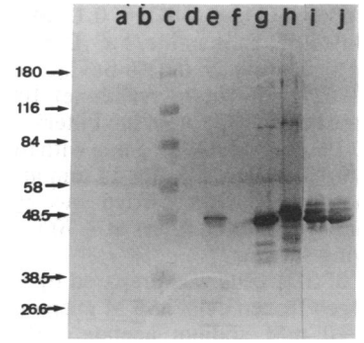

FIG. 5. Immunoblot of P-450IIB1 protein. Sonicated cell lysate (100 μg of total protein) in SD2 (lane a), SD4 (lane b), SD1 (lane e), and V79 (lane f). Purified P-450 (1 μg) in lane g, P-450IIB1; in lane h, P-450IIB2. Microsomal preparation from parenchymal liver cells of Aroclor-1254-treated animals in lane i (50 μg of total protein) and lane j (25 μg of total protein). Lane e, protein size marker; lane d, void.

from untreated rats and was 5 times lower than in the cell lysate of hepatocytes from Aroclor-1254-pretreated rats. No activity was detected in V79 cells. Enzymatic activity in cell line SD3 was found to be similar to that in SD1 (data not shown).

No detectable aryl hydrocarbon hydroxylase activity was found in V79 or SD-1 cells. The specific aryl hydrocarbon hydroxylase activity in control hepatocytes and in hepatocytes from Aroclor-1254-treated rats was found to be 52–70 and 280–380 pmol per mg of cell lysate, respectively.

SD1 and SD3 cell lines were checked periodically for enzyme activity over 4 months. No change in activity was observed.

Mutagenicity Test with Aflatoxin B_1. Aflatoxin B_1 needs metabolic activation by cytochrome P-450 to be mutagenic (34, 35). SD1 cells were exposed to aflatoxin B_1 for 24 hr at concentrations of 1, 3, 10, 20, and 30 μM and after an expression period of 6 days, cultivated in the presence of 6-thioguanine. Colonies of hypoxanthine phosphoribosyltransferase-negative ($HPRT^-$) cells were scored after 10 days (Table 2). SD1 and V79 cells showed similar spontaneous mutation frequencies (18 colonies per 10^6 cells). After exposure to aflatoxin B_1 the mutation frequency in V79 cells marginally changed, whereas the mutation frequency in SD1 cells increased ≈4-fold in a dose-dependent manner. When exposed to the direct mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, the mutation frequency increased in V79 and SD1 cells alike, to 248 colonies and 229 colonies per 10^6 cells, respectively.

DISCUSSION

The genetically engineered cell lines SD1 and SD3 stably produce cytochrome P-450IIB1. It is enzymatically active as shown by O-dealkylation of the test substrate 7-pentoxyresorufin, which is a specific substrate for P-450IIB1 (31).

Table 1. Enzymatic activity in SD1 cells and hepatocytes of Aroclor-1254-pretreated and untreated Sprague–Dawley rats

Cells	Specific activity, $pmol \cdot mg^{-1} \cdot min^{-1}$
SD1	36.2 ± 3.4
Hepatocytes from untreated rats	10.6 ± 0.9
Hepatocytes from pretreated rats	174.6 ± 44.6
V79	ND (<0.1)

Specific activity denotes picomoles of resorufin produced from 7-pentoxyresorufin per mg of total protein per minute. Purified resorufin (Boehringer Ingelheim) served as standard. ND, nondetectable.

Table 2. Mutagenicity test with aflatoxin B_1 and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)

Cell line	Mutagen	Frequency of 6-thioguanine-resistant cells × 10^6
V79	None	18.3
	None	18.8
	MNNG (1 μM)	248.7
	Aflatoxin B_1 (1 μM)	26.0
	Aflatoxin B_1 (3 μM)	15.7
	Aflatoxin B_1 (10 μM)	20.2
	Aflatoxin B_1 (20 μM)	19.8
	Aflatoxin B_1 (30 μM)	32.8
SD1	None	18.0
	None	17.7
	MNNG (1 μM)	229.4
	Aflatoxin B_1 (1 μM)	27.9
	Aflatoxin B_1 (3 μM)	Contaminated
	Aflatoxin B_1 (10 μM)	31.8
	Aflatoxin B_1 (20 μM)	48.7
	Aflatoxin B_1 (30 μM)	79.5

According to the Spearman's rank correlation test, the effect of aflatoxin B_1 was statistically significant in SD1 cells ($P < 0.005$), but not in V79 cells ($P > 0.1$).

Compounds can be metabolized into their mutagenic form as was demonstrated with aflatoxin B_1. These cell lines therefore mimic a natural situation in a liver cell with respect to one specific liver function; i.e., a potential mutagen is metabolized by the newly introduced monooxygenase, implying that the heme group is incorporated into the cytochrome and that it is cellularly organized in a way that allows reduction by NADPH-dependent cytochrome P-450 reductase.

These newly established cell lines will serve as a valuable tool in toxicological evaluations. A detailed test protocol for aflatoxin B_1 as well as other substances will be published elsewhere. In this context, it is worth mentioning that V79 cells were found to contain as much glutathione *S*-transferase activity as determined with 1-chloro-2,4-dinitrobenzene as substrate (637 nmol per mg of total protein per min) as hepatocytes (14), which is one of the most important detoxifying enzymes for the coupling of highly reactive molecules—e.g., the epoxide form of aflatoxin B_1. Other cell lines were found to contain less glutathione transferase—e.g., BALB 3T3 [231 nmol per mg of total protein per min (15)]. These data are in agreement with those reported by Wiebel *et al.* (36). Even though a relatively high content of this inactivating enzyme is present in V79 cells, aflatoxin B_1 was mutagenic in SD1 cells (Table 2). But our studies do not allow the conclusion that the cytochrome P-450IIB1 is the major or exclusive monooxygenase for the metabolic activation of aflatoxin B_1. This can only be deduced from comparative studies (35, 37). The choice to use aflatoxin B_1 in our mutagenicity studies was tentative to demonstrate the feasibility of cell lines SD1 and SD3 in general.

Cell lines SD1 and SD3 may serve as recipient cells in secondary transfection experiments for other genes that encode xenobiotic-metabolizing enzymes (e.g., epoxide hydrolase) to build up more complex metabolic pathways for xenobiotics and for the study of the effects of phase II reactions on mutagenicity as well.

In addition, cell lines SD1 and SD3 may serve as a unique source for purification of cytochrome P-450IIB1.

We thank our colleagues for valuable discussions, Mrs. Angelika Götz and Mrs. Karin Pauly for excellent technical assistance, Mrs. I. Böhm for typing the manuscript. We thank especially Dr. David Waxman for the generous gift of polyclonal antibodies against P-450IIB1 (P-450IIB4), purified P-450IIB1 (P-450PB4), and P-450IIB2 (P-450PB5); K. Marx for supplying resorufin (Boehringer Ingelheim) as reference standard; and Dr. Pablo Steinberg for providing hepatocytes. This work was supported by a fellowship from the Alexander von Humboldt Stiftung to S.D. and the Deutsche Forschungsgemeinschaft, SFB 302 "Kontrollfaktoren der Tumorentstehung", and by Grant GM 30701 from the National Institutes of Medical Sciences to M.A.

1. Pelkonen, O. & Nebert, D. W. (1982) *Pharmacol. Rev.* **34,** 189–222.
2. Marx, J. L. (1985) *Science* **228,** 975–976.
3. Jefferson, D. M., Clayton, D. F., Darnell, J. E., Jr., & Reid, L. M. (1984) *Mol. Cell. Biol.* **4,** 1929–1934.
4. Turchi, G., Glatt, H. R., Doerjer, G. & Oesch, F. (1987) *Mutat. Res.* **190,** 31–34.
5. Adesnik, M. & Atchison, M. (1985) *CRC Crit. Rev. Biochem.* **19,** 247–305.
6. Nebert, D. W. & Gonzalez, F. J. (1987) *Annu. Rev. Biochem.* **56,** 949–993.
7. Montisano, D. F. & Hankinson, O. (1985) *Mol. Cell. Biol.* **5,** 698–704.
8. Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1987) *DNA* **6,** 1–11.
9. Zuber, M. Y., Simpson, E. R. & Waterman, M. R. (1986) *Science* **234,** 1258–1261.
10. Kimura, S., Smith, H. H., Hankinson, O. & Nebert, D. W. (1987) *EMBO J.* **6,** 1929–1933.
11. Shimizu, T., Sogawa, K., Fujii-Kuriyama, Y., Takahashi, M., Ogoma, Y. & Hatano, M. (1986) *FEBS Lett.* **207,** 217–221.
12. Battula, N., Sagara, J. & Gelboin, H. V. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4073–4077.
13. Bradley, M. O., Bhuygan, B., Francis, M. C., Langenbach, R., Peterson, A. & Huberman, E. (1981) *Mutat. Res.* **87,** 81–142.
14. Platt, K. L., Utesch, D., Gemperlein-Mertes, I., Glatt, H. R. & Oesch, F. (1986) *Food Chem. Toxicol.* **24,** 721–729.
15. Gemperlein, I. (1986) Ph.D. thesis (Johannes Gutenberg-Universität, Mainz, F.R.G.).
16. Friedberg, T., Waxman, D. J., Atchison, M., Kumar, A., Haaparanta, T., Raphael, C. & Adesnik, M. (1986) *Biochemistry* **25,** 7975–7983.
17. Rivkin, E. (1984) Ph.D. thesis (New York Univ., New York).
18. Mizukami, Y., Sogawa, K., Suwa, Y., Muramatsu, M. & Fujii-Kuriyama, Y. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3958–3962.
19. Fujii-Kuriyama, Y., Mizukami, Y., Kawajiri, K., Sogawa, K. & Muramatsu, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2793–2797.
20. Rizzolo, L. J., Finidori, J., Gonzalez, M. A., Ivanov, I. E., Adesnik, M. & Sabatini, D. D. (1985) *J. Cell Biol.* **101,** 1351–1362.
21. Mulligan, R. C. & Berg, P. (1980) *Science* **209,** 1422–1427.
22. Russel, W. C., Newman, C. & Williamson, D. H. (1975) *Nature (London)* **253,** 461–462.
23. Graham, F. L. & van der Eb, A. (1973) *J. Virol.* **52,** 456–467.
24. Stow, N. D. & Wilkie, N. M. (1976) *J. Gen. Virol.* **33,** 447–458.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
26. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
27. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
28. Burnette, N. (1981) *Anal. Biochem.* **112,** 195–203.
29. Waxman, D. J. & Walsh, C. (1982) *J. Biol. Chem.* **257,** 10446–10457.
30. Seglen, P. O. (1973) *Exp. Cell Res.* **82,** 391–398.
31. Lubet, R. A., Nims, R. W., Mayer, R. T., Cameron, H. W. & Schechtman, L. M. (1985) *Mutat. Res.* **142,** 127–131.
32. Nebert, D. W. & Gelboin, H. V. (1968) *J. Biol. Chem.* **243,** 6242–6249.
33. Law, M. F., Byrne, J. C. & Howley, P. M. (1983) *Mol. Cell. Biol.* **3,** 2110–2115.
34. Neumann, H. G. (1984) in *Biochemical Basis of Chemical Carcinogenesis*, eds. Greim, H., Jung, R., Kramer, M., Marquardt, H. & Oesch, F. (Raven, New York), pp. 33–45.
35. Ishii, K., Maeda, K., Kamataki, T. & Kato, R. (1986) *Mutat. Res.* **174,** 85–88.
36. Wiebel, F. J., Singh, J., Schindler, E. & Summer, K. H. (1980) *Toxicology* **17,** 123–126.
37. Shimada, T., Nakamura, S., Imaoka, S. & Funae, Y. (1987) *Toxicol. Appl. Pharmacol.* **91,** 13–21.