Clofibrate-inducible rat hepatic P450s IVA1 and IVA3 catalyze the ω- and (ω-1)-hydroxylation of fatty acids and the ω-hydroxylation of prostaglandins E_1 and $F_{2\alpha}$

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Abstract Cytochromes P450IVA1 and IVA3 display 72% amino acid sequence similarity and are expressed in livers of rats treated with the hypolipidemic drug clofibrate. The catalytic activities of IVA1 and IVA3 were examined by cDNA-directed expression using vaccinia virus. cDNA-expressed IVA1 and IVA3 had relative M_rs of 51,500 and 52,000, respectively, on SDS-polyacrylamide gels. Both enzymes displayed reduced, CO-bound absorption spectra with λ_{max} of 452.5 nm. IVA1 and IVA3 hydroxylated lauric acid at the ω and ω-1 positions with equivalent ω/ω-1 ratios of about 12.5. IVA1 had a substrate turnover of 21 min^{-1} which was about fourfold higher than that of IVA3. The ω and ω-1 hydroxylation of palmitic acid was also catalyzed by these P450s with combined turnover numbers for both metabolites of 45 min^{-1} or 18 min^{-1} for IVA1 and IVA3, respectively. The ω/ω-1 oxidation ratio of IVA1 for palmitate was 1.25 which was almost fourfold higher than that obtained for IVA3. These enzymes also catalyzed ω oxidation of the physiologically important eicosanoids prostaglandins E_1 and $F_{2\alpha}$ with turnover numbers of about one-tenth those calculated for fatty acid oxidations. No ω-1 hydroxy metabolites were produced. These studies indicate that the P450 enzymes IVA1 and IVA3 are able to catalyze the oxidations of both fatty acids and prostaglandins. —**Aoyama, T., J. P. Hardwick, S. Imaoka, Y. Funae, H. V. Gelboin, and F. J. Gonzalez.** Clofibrate-inducible rat hepatic P450s IVA1 and IVA3 catalyze the ω- and (ω-1)-hydroxylation of fatty acids and the ω-hydroxylation of prostaglandins E_1 and $F_{2\alpha}$. *J. Lipid Res.* 1990. **31:** 1477–1482.

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Administration of the hypolipidemic drug clofibrate to rats results in the proliferation of peroxisomes and endoplasmic reticulum and the induction of a number of peroxisomal enzymes and a few specific forms of cytochrome P450s (1–4). This induction process is mediated, in part, through a transcriptional mechanism (5, 6).

A P450, designated $P450_{LA\omega}$ or IVA1[1] has been purified and found to catalyze the hydroxylation of lauric acid (4, 6). By use of antibody against IVA1, two proteins with relative M_rs of 52,000 and 51,500 have been detected on Western immunoblots (6, 7), the latter of which corresponds to IVA1. A cDNA to IVA1 mRNA was sequenced and found to encode a P450 with the expected lauric acid hydroxylase activity (6). The nature of the second 52,000 dalton immunorelated protein has remained elusive.

Recently, two additional genes in the IVA subfamily, designated IVA2 and IVA3, were identified (8, 9). By use of oligonucleotides specific for each mRNA, it was determined that all three IVA genes are induced in liver by clofibrate (9). The IVA2 mRNA is constitutively expressed in kidney while the IVA1 and IVA3 mRNAs were present at low levels and inducible by clofibrate in this organ. Based on similarities in amino terminal protein sequence, it appeared that a P450 purified from livers of diabetic rats, designated P450 DM-2, that catalyzes lauric acid hydroxylation, is equivalent to IVA3 (10).

In the present study we used vaccinia virus to carry out expression of the IVA3 cDNA. The expressed protein was found to react with antibody against P450 K-5, to have a relative M_r of 52,000 on SDS-polyacrylamide gels, and to catalyze the ω- and (ω-1)-hydroxylation of lauric acid and palmitic acid and the ω-hydroxylation of prostaglandins E_1 and $F_{2\alpha}$.

Abbreviations: PBS, phosphate-buffered saline; HPLC, high performance liquid chromatography.

[1]The nomenclature used in this report is that described by Nebert et al. (30).

MATERIALS AND METHODS

Materials

Vaccinia virus strain WR, pSC11 and human TK$^-$ 143 cells were obtained from Dr. Bernard Moss at the National Institutes of Health. HepG2 (ATCC GB 8095) and CV-1 cells were purchased from the American Type Culture Collection. [1-^{14}C]Lauric acid (30 mCi/mmol, CFA.106), [1-^{14}C]palmitic acid (60 mCi/mmol, CFA.23) [5,6-^{3}H(N)]prostaglandin E_1 (50 mCi/mmol, CFA.567), and [1-^{14}C]prostaglandin $F_{2\alpha}$ (50 mCi/mmol, CFA.571) were obtained from Amersham Corp. Unlabeled fatty acids and prostaglandins were purchased from Sigma.

cDNA expression using vaccinia virus

The IVA3 cDNA, consisting of two Eco RI fragments in pUC9, was excised by partially digesting the plasmid with Eco RI. The IVA1 cDNA was removed from pUC9 using a complete digestion with Eco RI. The inserts were made blunt ended with DNA polymerase Klenow fragment and inserted into Sma I-digested and alkaline phosphatase-treated pSC11, the vaccinia virus transfer plasmid (11). Recombinant vaccinia viruses containing IVA1 (vIVA1) and IVA3 (vIVA3) were constructed as described by Mackett, Smith, and Moss (12) as outlined earlier (13). vIVA1 and vIVA3 were used to infect HepG2 cells. Twenty four hours after infection, cells were harvested, washed in PBS (Biofluids, Rockville, MD), and resuspended in PBS at 4°C (1 ml/10^6 cells). Cells were lysed by brief sonication and nuclei were sedimented by centrifugation at 5,000 *g* for 10 min. The supernatant was centrifuged at 100,000 *g* for 60 min to sediment microsomal membrane fractions. The membranes were resuspended in 100 mM sodium phosphate, pH 7.25. Microsomes were isolated from Sprague-Dawley rats treated 24 h with 400 mg/kg sodium clofibrate as described earlier (6).

For spectral analysis, total cell protein was solubilized using Emulgen 913 (final concentration of 0.35% w/v). The soluble protein was divided into two cuvettes, CO gas was gently bubbled into the sample cuvette for 15 sec, and a few crystals of sodium dithionite were added to both sample and reference cuvettes. Spectra were immediately recorded using an Aminco DW-2000 spectrophotometer. P450 contents were calculated as described (14) using an extinction coefficient of 91 mM^{-1}.

Western immunoblotting was performed as described previously (15) using SDS-containing 7.5% polyacrylamide gels (16). Blots were developed using either rabbit antibody against IVA1 (6) or against P450 K-5 (10) and alkaline phosphatase-conjugated goat anti-rabbit IgG (KPL, Gaithersburg, MD).

Fatty acid hydroxylase assays using [^{14}C]lauric acid and [^{14}C]palmitic acid were carried out as described by Aoyama and Sato (17). Briefly, microsomes (1–3 mg) containing from 45–135 pmol P450 were incubated with 1×10^5 dpm ^{14}C-labeled fatty acid (final concentration 100 μM), 0.3 mM NADPH in 100 mM potassium phosphate buffer, pH 7.25, at 37°C with constant agitation for 20 min in a final reaction volume of 1 ml. The fatty acids were extracted as described (17), conjugated with *p*-bromophenacyl bromide and subjected to HPLC analysis and liquid scintillation counting. The ω and ω-1 peaks of hydroxy metabolites for palmitic acid and lauric acid were separated by about 10 min. Prostaglandin hydroxyalse assays were carried out essentially as described (17) except the hydroxylated metabolites were conjugated with 9-anthryldiazomethane prior to HPLC.

7-Ethoxy- and 7-propoxycoumarin (18) and 7-ethoxy- and 7-pentoxyresorufin (19) dealkylase assays were performed by published procedures. Testosterone hydroxylase assays were carried out as detailed previously (20).

RESULTS

Recombinant vaccinia viruses vIVA1 and vIVA3 and wild type vaccinia vWT were used to infect HepG2 cells and the levels of expression of P450 in infected cells were quantified by spectral analysis. Typical reduced and CO-bound P-450 Soret bands, with λ_{max} of 452.5 nm were detected in cells infected with either recombinant virus (**Fig. 1**). Lysate from cells infected with vWT produced no peak around 450 nm and a small peak at between 420 and 430 nm. The levels of expression of IVA1 and IVA3 ranged from 13.2 to 14.7 pmol/mg cell lysate protein.

Western immunoblot analysis was also carried out on cell lysate from vIVA1- and vIVA3-infected cells, in parallel with microsomal membrane protein isolated from livers of rats treated with clofibrate. Two proteins with relative M_rs of 52,000 and 51,500 were detected in liver microsomes using antibody against IVA1 (**Fig. 2**). This antibody reacted strongly with a protein of relative M_r

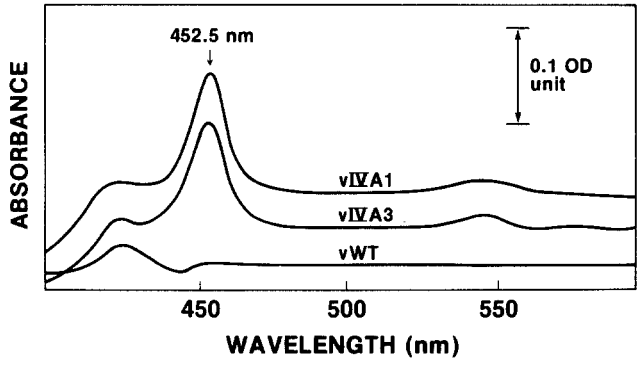

Fig. 1. Spectral analysis of P450 in vIVA1-, vIVA3-, and vWT-infected cells. Cells were infected for 24 h and analyzed by difference spectra for the presence of P450. Approximately 35 mg of total cell protein was used in each analysis.

TABLE 1. Fatty acid hydroxylations catalyzed by vaccinia virus-expressed IVA1 and IVA3

	Catalytic Activity (nmol/min per nmol P450)					
	Palmitate			Laurate		
P450	15-OH[a]	16-OH[b]	(16-OH/15-OH)	11-OH[a]	12-OH[a]	(12-OH/11-OH)
vIVA1	19.8 ± 3.2	24.8 ± 4.0	(1.25)	1.7 ± 0.3	19.4 ± 2.9	(11)
vIVA3	3.2 ± 0.6	14.6 ± 2.6	(4.5)	0.4 ± 0.1	4.9 ± 1.0	(13)
vWT	<0.05	<0.05		<0.05	<0.05	

HepG2 cells were infected with virus and microsomal membranes were isolated 24 h after infection. Fatty acid hydroxylase assays were carried out and the results are expressed as mean ± standard deviation of four determinations.
[a] $P < 0.001$.
[b] $P < 0.01$.

51,500 from vIVA1-infected cells and weakly with an M_r 52,000 protein from vIVA3-infected cells. Qualitatively similar results were obtained using antibody against P450 IVA2 and IVA3[2] except that the 52,000 dalton protein from vIVA3-infected cells was more heavily stained than was the 51,500 dalton protein from vIVA1-infected cells.

Next, the ability of lysate from vIVA1- and vIVA3-infected cells to catalyze fatty acid hydroxylation was examined. Both IVA1 and IVA3 were able to hydroxylate palmitate and laurate (**Table 1**). The highest turnover number of 24 min^{-1} was obtained with 16-hydroxylation (ω-position) of palmitate by IVA1. This enzyme also catalyzed hydroxylation at the 15 position (ω-1) at a similar rate of about 20 min^{-1}. IVA3 hydroxylated palmitate at a rate of about 40% that of IVA1 with a preponderance of hydroxylation at the ω position. Laurate was hydroxylated by both P450s at a rate of about one half that found with the longer chain fatty acid palmitate (Table 1). The ratios of ω- to (ω-1)-hydroxylation were 11 and 13 for IVA1- and IVA3-catalyzed laurate hydroxylations, respectively.

The abilities of IVA1 and IVA3 to hydroxylate prostaglandins E_1 and $F_{2\alpha}$ were also examined. Both the eicosanoids were hydroxylated only at their ω positions by IVA1 and IVA3 (**Table 2**). IVA1 was about twofold more active than IVA3. Neither expressed enzyme was able to catalyze metabolism of the common P450 substrates, 7-ethoxycoumarin, 7-propoxycoumarin, 7-ethoxyresorufin, 7-pentoxyresorufin, and testosterone.

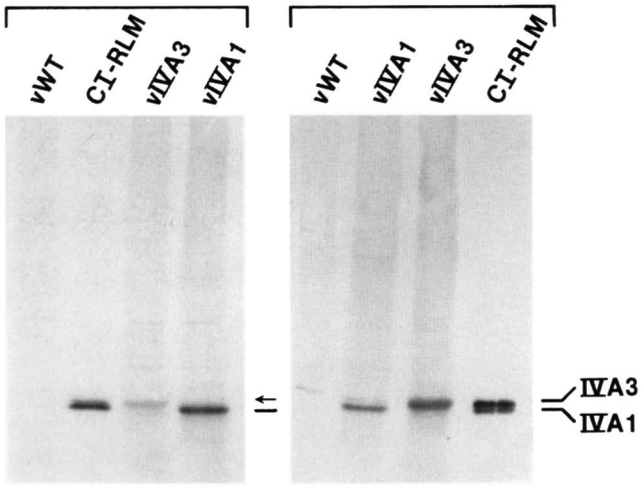

Fig. 2. Western immunoblotting of cell lysate from vIVA1- and vIVA3-infected cells and microsomes from clofibrate-treated rats. Total cell lysate protein (50 μg) from vaccinia virus-infected cells and rat liver microsomal protein (3 μg) from clofibrate-induced rats (CI-RLM) were subjected to electrophoresis and Western blotting. The blots were developed with antibody against IVA1 (left panel) and P450 K-5 (right panel).

DISCUSSION

The rat clofibrate-inducible IVA3 P450 was expressed using vaccinia virus and found to encode a protein of relative M_r 52,000 having a reduced CO-bound difference spectrum with a λ_{max} of 452.5 nm. This enzyme also catalyzes the ω- and (ω-1)-hydroxylation of the fatty acids laurate and palmitate and the ω-hydroxylation of prostaglandins E_1 and $F_{2\alpha}$. IVA3 protein reacts quite strongly with antibody generated against P450 K-5 (10), a P450 that was purified from rat kidney. This antibody reacts equally well with a hepatic P450 purified from the livers of diabetic rats, designated P450 DM-2. In fact, cDNA-expressed IVA3 comigrated with P450 DM-2 on SDS-polyacrylamide gels (Aoyama, T., J. P. Hardwick, S. Imaoka, Y. Funae, H. V. Gelboin, and F. J. Gonzalez, unpublished results). The limited amino terminal sequence data on P450 DM-2 (10) also agree with the cDNA-deduced sequence of IVA3 as previously noted (9). These data strongly suggest that the IVA3 cDNA encodes a P450 identical to P450 DM-2 and suggest that the second lower mobility 52,000 dalton protein previously detected on Western blots using antibody against IVA1 (6) is due to

[2]Since IVA2 and IVA3 display 96% amino acid sequence similarity, antibody against IVA2 (P450 K-5) would be expected to yield the same results as antibody against IVA3.

TABLE 2. Prostaglandins E_1 and $F_{2\alpha}$ hydroxylations catalyzed by vaccinia virus-expressed IVA1 and IVA3

	Catalytic Activity (nmol/min per nmol P450)			
	Prostaglandin E_1		Prostaglandin $F_{2\alpha}$	
P450	19-OH	20-OH[a]	19-OH	20-OH[b]
vIVA1	<0.05	1.20 ± 0.2	<0.05	0.74 ± 0.3
vIVA3	<0.05	0.50 ± 0.1	<0.05	0.34 ± 0.1

HepG2 cells were infected with virus and microsomal membranes were isolated 24 h after infection. Prostaglandin hydroxylase assays were carried out and the results are expressed as mean ± standard deviation of three determinations.

[a] $P < 0.001$.

[b] $P < 0.05$.

IVA3. It should be noted, however, that a third P450, designated IVA2, has been identified from sequence of a genomic clone that displays a high 96% sequence similarity with IVA3 (8). This P450 is expressed constitutively in kidney and is induced in liver by clofibrate. It apparently corresponds to P450 k-5 (21). This latter protein comigrates with cDNA-expressed IVA1 on SDS-containing polyacrylamide gels despite its higher sequence similarity with IVA3. This is rather surprising since the IVA2 protein sequence is more similar to IVA3 (96%) than to IVA1 (72%) (9).

P450 IVA1 hydroxylates palmitate and laurate at both the ω and ω-1 positions. The ω/ω-1 ratio, however, is about ninefold greater for laurate hydroxylation. The ω/ω-1 ratios for laurate and palmitate hydroxylation by IVA3 are 13 and 4.5, respectively, only a threefold difference. These data indicate that IVA3 has a preference for hydroxylation of palmitate at the ω position as compared to IVA1. Early studies also demonstrated a marked effect of fatty acid chain lengths on the rate of ω and (ω-1)-hydroxylation by a kidney P450 (22, 23). In addition, maximal catalytic activities were obtained with the 12-carbon chain laurate and activities decreased when the carbon chain length either increased or decreased. In contrast, IVA1 and IVA3 hydroxylated the 16-carbon palmitate two- to threefold faster than laurate. It should be noted that several studies using purified kidney P450s reported ω/ω-1 ratios for laurate hydroxylation that differed significantly from our values (6, 21, 24). This may be due to differences in assays since these studies used a gas chromatography-based procedure (25) to separate the two hydroxy derivatives, whereas we used HPLC in which the two metabolites are resolved by more than 10 min on the C_{18} column (17). In any case, our studies clearly demonstrate that IVA3 possesses the ability to hydroxylate laurate and palmitate similar to IVA1. The absolute activities and ratios of ω- and (ω-1)-hydroxy metabolites vary significantly between the two enzymes which may reflect differences in binding or positioning of the substrates at the active sites of these P450s. Still, it is surprising that IVA1 and IVA3, sharing only 72% sequence identity, display such similar substrate preferences.

Both IVA1 and IVA3 were also able to hydroxylate prostaglandins E_1 and $F_{2\alpha}$, albeit only at their ω positions and at rates considerably lower than those for fatty acid oxidations. These hydroxylations probably abolish their biological activities. However, it is unknown whether IVA1 and IVA3 play physiological roles in regulating the degradation of these compounds.

In summary, recent studies at the molecular level have resulted in a clearer understanding of the structure, regulation, and tissue-specific expression substrate specificities of P450s in the IVA subfamily. A P450 capable of ω and ω-1 hydroxylation of fatty acids has been purified from untreated rat kidney by two groups (21, 24, 26). As noted earlier, based on limited amino acid sequence similarities, this appears to correspond to IVA2, a P450 whose mRNA is constitutively expressed in rat kidney (9). A P450 with structural and catalytic similarities to P450 K-5 is also found in human kidney (26). Other fatty acid hydroxylases have been purified from kidney, for example a form designated P450 K-1 (24) which is apparently identical to P450 K-4 (26). This form is capable of both fatty acid and prostglandins A_1 and A_2 oxidation. On the other hand, the purified form corresponding to IVA2 apparently does not metabolize the latter substrates (24). It is unclear whether this enzyme will hydroxylate prostaglandins E_1 and $F_{2\alpha}$. Based on the high structural similarities of IVA2 to IVA3 we would predict that indeed it would. Unfortunately the IVA2 cDNA has not been isolated. We have, however, isolated several cDNAs from a commercially available rat kidney library (Clonetech Laboratories Inc. Palo Alto, CA., Catalog number RL 10076) that all corresponded to IVA3 (Aoyama, T., J. P. Hardwick, S. Imaoka, Y. Funae, H. V. Gelboin, and F. J. Gonzalez, unpublished results). These data would indicate strain differences in the expression of the IVA2 and IVA3 genes in which some strains constitutively express the IVA3 gene. Strain differences in P450 expression are not uncommon (27). Again, these data would suggest

functional and/or enzymological similarities between IVA2 and IVA3. It is also noteworthy that IVA1 and IVA3, whose mRNAs are expressed at low levels in kidneys of our colony of untreated rats, are highly inducible by clofibrate in this tissue (9).

Messenger RNAs encoding the three members of the IVA subfamily are expressed in liver at very low levels unless rats are treated with inducing agents such as clofibrate (9). Therefore, fatty acid hydroxylases expressed in untreated rats (28) are likely encoded by other constitutively expressed P450s genes. The role of fatty acid hydroxylases induced by hypolipidemic agents is still unclear since these enzymes only participate to a minor degree in the clearance of plasma lipid by these drugs (29). Understanding of the precise physiological role of the IVA P450s awaits further experimentation.

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REFERENCES

1. Reddy, J. K., and N. D. Lalwani. 1983. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* **12:** 1–58.
2. Lazarow, P. B., and C. de Duve. 1976. A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA.* **73:** 2043–2046.
3. Furuta, S., S. Miyazawa, and T. Hashimoto. 1982. Biosynthesis of enzymes of peroxisomal β-oxidation. *J. Biochem. (Tokyo)* **92:** 319–326.
4. Tamburini, P. P., H. A. Masson, S. K. Bains, R. J. Makowski, B. Morris, and G. G. Gibson. 1984. Multiple forms of hepatic cytochrome P-450: purification, characterization and comparison of a novel clofibrate-induced isozyme with other major forms of cytochrome P-450. *Eur. J. Biochem.* **139:** 245–246.
5. Reddy, J. K., S. K. Goel, M. R. Nemali, J. J. Carrino, T. G. Laffler, M. K. Reddy, S. J. Sperbeck, T. Osumi, T. Hashimoto, N. D. Lalwani, and M. S. Rao. 1986. Transcriptional regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc. Natl. Acad. Sci. USA.* **83:** 1747–1751.
6. Hardwick, J. P., B. J. Song, E. Huberman, and F. J. Gonzalez. 1987. Isolation, complimentary DNA sequence, and regulation of rat hepatic lauric acid ω-hydroxylase (cytochrome P-450$_{LA\omega}$): identification of a new cytochrome P-450 gene family. *J. Biol. Chem.* **262:** 801–810.
7. Sharma, R. K., B. G. Lake, R. Makovski, T. Bradshaw, D. Earnshaw, J. W. Dale, and G. G. Gibson. 1989. Differential induction of peroxisomal and microsomal fatty acid oxidizing enzymes by peroxisome proliferators in rat liver and kidney. *Eur. J. Biochem.* **184:** 69–78.
8. Kimura, S., N. Hanioka, E. Matsunaga, and F. J. Gonzalez. 1989. The rat clofibrate-inducible *CYP4A* gene subfamily I. Complete intron and exon sequence of the *CYP4A1* and *CYP4A2* genes, unique exon organization, and identification of a conserved 19-bp upstream element. *DNA.* **8:** 503–516.
9. Kimura, S., J. P. Harwick, C. A. Kozak, and F. J. Gonzalez. 1989. The rat clofibrate-inducible *CYP4A* subfamily II. cDNA sequence of IVA3, mapping of the *Cyp4a* locus to mouse chromosome 4, and coordinate and tissue-specific regulation of the *CYP4A* genes. *DNA.* **8:** 517–525.
10. Imaoka, S., H. Shimojo, and Y. Funae. 1988. Induction of renal cytochrome P-450 in hepatic microsomes of diabetic rats. *Biochem. Biophys. Res. Commun.* **152:** 680–687.
11. Chakrabarti, S., K. Breckling, and B. Moss. 1985. Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. *Mol. Cell. Biol.* **5:** 3403–3409.
12. Mackett, M., G. L. Smith, and B. Moss. 1984. Method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. *J. Virol.* **49:** 857–864.
13. Aoyama, T., S. Yamano, D. J. Waxman, D. P. Lapenson, U. A. Meyer, V. Fisher, R. Tyndale, T. Inaba, W. Kalow, H. V. Gelboin, and F. J. Gonzalez. 1989. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver: cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J. Biol. Chem.* **264:** 10388–10395.
14. Omura, T., and R. Sato. 1964. The carbon monoxide binding pigment of liver microsomes II. Solubilization, purification, and properties. *J. Biol. Chem.* **239:** 2379–2385.
15. Towbin, H., T. Staelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc. Natl. Acad. Sci. USA.* **76:** 4350–4355.
16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (London)* **227:** 680–685.
17. Aoyama, T., and R. Sato. 1988. High pressure liquid chromatography methods for separation of ω- and (ω-1)-hydroxy fatty acids: their applications to microsomal fatty acid ω-oxidation. *Anal. Biochem.* **170:** 73–82.
18. Greenlee, W. F., and A. Poland. 1978. An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Pharmacol. Exp. Ther.* **205:** 596–608.
19. Burke, M. D., and R. T. Mayer. 1974. Ethoxyresorufin: direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Dispos.* **2:** 583–588.
20. Wood, A. W., D. E. Ryan, P. E. Thomas, and W. Levin. 1983. Regio- and stereoselective metabolism of two C_{19} steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. *J. Biol. Chem.* **258:** 8839–8847.
21. Imaoka, S., and Y. Funae. 1986. Purification and NH_2-terminal sequence of cytochrome P-450 from kidney microsomes of untreated male rats. *Biochem. Biophys. Res. Commun.* **141:** 711–717.
22. Ellin, A., S. V. Jakobsson, J. B. Schenkman, and S. Orrenius. 1972. Cytochrome P450k of rat kidney cortex microsomes: its involvement in fatty acid ω- and (ω-1)-hydroxylation. *Arch. Biochem. Biophys.* **150:** 64–71.
23. Ellin, A., S. Orrenius, A. Pilotti, and G. G. Swahn. 1973. Cytochrome P-450k of rat kidney cortex microsomes: further studies on its interaction with fatty acids. *Arch. Biochem. Biophys.* **158:** 597–604.
24. Yoshimoto, M., E. Kusunose, S. Yamamoto, M. Maekawa, and M. Kusunose. 1986. Purification and characterization

of two forms of cytochrome P-450 from rat kidney cortex microsomes. *Biochem. Int.* **13:** 749–755.
25. Kusunose, M., E. Kusunose, and M. J. Coon. 1964. Enzymatic ω-hydroxylation of fatty acids. I. Products of octanoiate, decanoate, and laurate oxidation. *J. Biol. Chem.* **239:** 1374–1380.
26. Imaoka, S., K. Nagashima, and Y. Funae. 1990. Characterization of three cytochrome P450s purified from renal microsomes of untreated male rats and comparison with human renal cytochrome P450. *Arch. Biochem. Biophys.* **276:** 473–480.
27. Gonzalez, F. J. 1988. The molecular biology of cytochrome P450s. *Pharmacol. Rev.* **40:** 243–288.
28. Ortiz de Montellano, P. R., and N. O. Reich. 1984. Specific inactivation of hepatic fatty acid hydroxylases by acetylenic fatty acids. *J. Biol. Chem.* **259:** 4136–4141.
29. Reich, N. O., and P. R. Ortiz de Montellano. 1986. Dissociation of increased lauric acid ω-hydroxylase from the antilipidemic action of clofibrate. *Biochem. Pharmacol.* **35:** 1227–1233.
30. Nebert, D. W., D. R. Nelson, M. Adesnik, M. J. Coon, R. W. Estabrook, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, B. Kemper, W. Levin, I. R. Phillips, R. Sato, and M. R. Waterman. 1989. The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA.* **8:** 1–13.