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Biochemistry

Cytochrome b_5 potentiation of cytochrome P-450 catalytic activity demonstrated by a vaccinia virus-mediated *in situ* reconstitution system

(cDNA expression/electron transfer/*p*-nitrophenetole deethylation)

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ABSTRACT **A cDNA containing the full coding region of human cytochrome b_5 was inserted into a vaccinia virus cDNA expression vector. Infection of human thymidine kinase-minus (TK^-) 143 cells in culture with this recombinant virus resulted in production of 0.3 nmol of cytochrome b_5 per mg of cell lysate protein. The expressed cytochrome had a reduced difference spectrum with a Soret peak at 424 nm, typical of pure cytochrome b_5. TK^- 143 cells have little detectable endogenous cytochrome b_5, cytochrome P-450 (P450), and NADPH–P450 oxidoreductase. To test whether cytochrome b_5 potentiated mixed-function monooxygenation *in situ*, these cells were coinfected with three recombinant vaccinia viruses individually carrying cDNAs encoding cytochrome b_5, NADPH–P450 oxidoreductase, and P450 form IIB1. These triple-virus-infected cells were compared to cells infected with the P450IIB1 and NADPH–P450 oxidoreductase recombinant viruses with respect to P450IIB1-catalyzed monooxygenase activities. Cytochrome b_5 specifically augmented the deethylation of *p*-nitrophenetole in microsomal membrane fractions of infected cells or when substrate was incubated directly with cells *in situ*. No significant increases were seen with P450IIB1-catalyzed testosterone, 7-ethoxycoumarin, or 7-pentoxyresorufin oxidations. These data demonstrate that cytochrome b_5 is capable of specifically augmenting monooxygenase activities in intact cells.**

Multiple forms of cytochrome P-450 (P450) and a single flavoprotein NADPH–P450 oxidoreductase constitute the microsomal mixed-function monooxygenase system. This system is capable of oxidizing numerous structurally diverse chemicals, including drugs, carcinogens, environmental chemicals, steroids, and fatty acids. The P450 reaction requires two electrons and a molecule of O_2 for substrate oxygenation and water formation. Electrons from NADPH are transferred to P450 individually at different points of the catalytic cycle by the NADPH–P450 oxidoreductase (for a review, see refs. 1 and 2). In certain instances cytochrome b_5 (Cyt b_5) is also capable of transferring electrons directly to P450 (3, 4). *In vitro* reconstitution studies established that Cyt b_5 potentiated monooxygenase reactions, and this potentiation was both P450 form-specific and dependent on the substrate (5–12). In some cases, the oxidation of a particular substrate is markedly increased in the presence of Cyt b_5. Examples are the oxidation of halothane (13, 14), demethylation of *p*-nitroanisole (12, 13, 15), deethylation of *p*-nitrophenetole (16), and demethylation of *N*-nitrosodimethylamine (11). Of interest is the fact that the oxidation of some substrates by a particular form of P450 is not stimulated by Cyt b_5, whereas oxidations of other substrates are enhanced (8–16). In addition, oxidation of a specific substrate by one P450 form can be stimulated by Cyt b_5, while oxidation of the same substrate by another P450 form is unaffected by Cyt b_5 (12, 15). The stimulatory effects of Cyt b_5 also have been demonstrated in intact microsomes by using antibodies generated against purified Cyt b_5 (17, 18).

It remains to be established how Cyt b_5 stimulates the monooxygenation of certain substrates and not others for a specific form of P450 or why the rate of oxidation of a substrate is potentiated by Cyt b_5, with one P450 form but not with others. The effect of Cyt b_5 does not appear to be mediated by increasing the rate of interaction of the substrate with the P450 active site (19). In fact, most studies suggest that with certain P450 forms and certain substrates, Cyt b_5 more rapidly supplies electrons to the intermediate oxygenated–ferrous–P450–substrate complex than does NADPH–P450 oxidoreductase (8, 9, 15, 20, 21). This is accompanied by a decrease in H_2O_2 formation by the enzyme (15, 21). As a consequence, with reactions catalyzed by certain P450 forms in the absence of Cyt b_5, excess O_2^- and H_2O_2 can be generated that could have detrimental consequences in the intact cell. With other P450 forms, however, Cyt b_5 stimulates monooxygenase activity in the absence of a decrease in peroxide formation (15). It should be noted, however, that all prior studies to date on the role and mechanism of Cyt b_5 in P450-catalyzed monooxygenation have been based solely on *in vitro* experimentation using both reconstitution systems and microsome-immunoinhibition experiments. In fact, results from studies using reconstitution systems are highly dependent on the contents of the reaction mixtures, such as the levels of NADPH–P450 oxidoreductase used and the lipid composition (22, 23). In some cases, P450s exhibit activities in reconstituted systems that are not expected from their activities in microsomal membranes (24, 25). The question still remained whether Cyt b_5 actually stimulates oxidation of certain substrates in the intact cell.

Recently, we have used vaccinia virus to express cDNAs encoding P450s and NADPH–P450 oxidoreductase (26). This cDNA expression system is quite suitable for studying the enzymology of P450s because it can be used with any mammalian cell; especially useful are cells, such as thymidine kinase-minus (TK^-) 143 (26), that have low levels of the monooxygenase components. Rather high levels of expression can be achieved so that spectral analysis can be carried out easily. In addition, multiple recombinant vaccinia viruses can be used to infect a single cell. The latter property was exploited to determine the optimum ratios of NADPH–P450 oxidoreductase to P450 needed for maximal mixed-function monooxygenase activity in intact intracellular membranes

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Abbreviations: P450, cytochrome P-450; Cyt b_5, cytochrome b_5; TK, thymidine kinase; vWT, wild-type vaccinia virus.

(26). In effect, we have reconstituted a monooxygenation system in an intact cell through the simultaneous infection with two or more recombinant vaccinia viruses.

In the present report, we describe the expression of human Cyt b_5 cDNA (27). Expression of multiple recombinant vaccinia viruses in the same cell established that Cyt b_5 potentiates the deethylation of *p*-nitrophenetole by rat cDNA-expressed P450 form IIB1 but has no effect on several other P450IIB1-catalyzed monooxygenase activities tested.

MATERIALS AND METHODS

The human Cyt b_5 cDNA was isolated from a liver λgt11 library by using polyclonal antibody against the purified rat cytochrome (27). The cDNA was inserted into the plasmid pSC11 (28), and the recombinant plasmid was introduced into vaccinia virus as described (29). Recombinant vaccinia viruses containing the human NADPH–P450 oxidoreductase (26) and rat P450IIB1 (30) cDNAs were described in earlier reports. Recombinant vaccinia viruses containing Cyt b_5, NADPH–P450 oxidoreductase, and P450IIB1 were designated vb_5, vOR, and vIIB1, respectively.

Human TK^- 143 cells (28, 29) were used as recipients for the vaccinia expression experiments. Expression of Cyt b_5 was verified by immunoblot (Western) analysis (31) with sodium dodecyl sulfate (SDS)-containing 12% polyacrylamide gels (32). The level of expression of Cyt b_5 (33) and P450 (34) were determined by monitoring difference spectra of reduced vs. oxidized and reduced vs. reduced CO-bound pigment, respectively. The Cyt b_5 content was quantified from the ferrous form of the cytochrome by using the extinction coefficient of $\Delta\varepsilon(\text{reduced–oxidized})_{423-409}$ = 185 $mM^{-1}\cdot cm^{-1}$ (34). P450 levels were quantitated by using an extinction coefficient of the ferrous form complexed with CO of 91 $mM^{-1}\cdot cm^{-1}$. To determine the level of Cyt b_5 expression in cells that had been infected with vb_5, vOR, and vIIB1, reduced difference spectral analysis with an extinction coefficient $\Delta\varepsilon_{423-500}$ = 171 $mM^{-1}\cdot cm^{-1}$ (34). Membrane fractions of cells infected with vIIB1 and vOR only were used in the reference cuvettes. All spectra were recorded with an Aminco DW-2000 spectrophotometer and accompanying software. Enzyme activity measurements were carried out on microsome fractions prepared by brief sonication and differential centrifugation (30). Testosterone hydroxylase assays were performed using a modification of the method described by Wood *et al.* (35). 7-Ethoxycoumarin O-deethylation (36), 7-pentoxyresorufin O-dealkylation (37), and *p*-nitrophenetole deethylation (38) assays were carried out by published procedures. NADPH–P450 oxidoreductase was assayed by measuring the oxidation of NADPH at 340 nm with Cyt *c* as an electron acceptor (39). NADH–Cyt b_5 oxidoreductase was assayed in cells infected with vb_5 by adding NADH to isolated microsomal membranes and monitoring the reduction of Cyt b_5 by following difference spectra at 424 nm.

p-Nitrophenetole metabolism was analyzed *in situ* in cells plated on 75-cm^2 flasks in 15 ml of medium lacking phenol red. Cells were infected with recombinant vaccinia viruses as described above, and 18 hr after infection, *p*-nitrophenetole was added to the medium to a final concentration of 0.4 mM. The production of the product *p*-nitrophenol was measured in medium withdrawn from the cells at various times after addition of substrate.

RESULTS

Isolation and Expression of a Human Cyt b_5 cDNA. The Cyt b_5 cDNA was inserted into vaccinia virus, and the virus was used to infect human TK^- 143 cells. Western blotting of lysate from vaccinia-infected cells revealed a protein with an apparent molecular mass of 17 kDa that comigrated with a

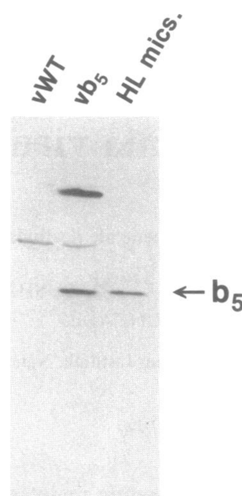

FIG. 1. Western blot analysis of Cyt b_5 expression in cells infected with wild-type vaccinia virus (vWT) and recombinant vaccinia virus containing Cyt b_5 cDNA designated vb_5. Cell lysate protein (20 μg) and human liver microsomal protein (10 μg), designated HL mics., were subjected to electrophoresis and transferred to nitrocellulose paper. The blot was developed with rabbit antisera against rat Cyt b_5 and alkaline phosphatase-conjugated goat anti-rabbit IgG.

protein detected in human liver microsomes (Fig. 1). This protein was not found in lysates of cells infected with vWT although a second lower mobility protein was seen that was also in vb_5-infected cells. The nature of this cross-reactive protein is unknown. In addition to the 17-kDa protein, which appears to correspond to mature monomeric Cyt b_5, we also detected an immunochemically related larger protein of about 60 kDa in vb_5-infected cells. This protein, which is not seen in vWT-infected cells or human liver, may be a polymer or aggregate of Cyt b_5 or may represent the result of a covalent coupling of expressed Cyt b_5 to another cellular protein.

To determine if the expressed Cyt b_5 yielded a ferrous difference spectra typical of the purified cytochrome, cell lysates from vb_5-infected cells were analyzed. Samples prepared from cells infected with vWT contained a small spectral peak at 428 nm with a trough at 417 nm when reduced with dithionite (Fig. 2). Cells infected with vb_5, on the other hand, contained a large peak at 424 nm with a trough at 410 nm. Two additional peaks were detected at about 530 and 560 nm that correspond to β and α spectral bands, respectively. This difference spectra, obtained with vb_5-infected cells, is typical of the purified Cyt b_5 heme protein (33). By harvesting cells at various times after infection with vb_5, we determined that maximal levels of the cytochrome were synthesized at 24 hr and that these levels remained up to 4 days after infection.

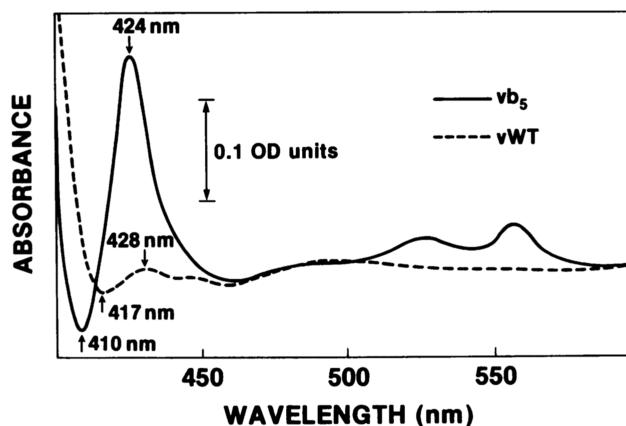

FIG. 2. Difference spectra of the ferrous form of Cyt b_5. Sonicated lysate protein (25 mg) from cells infected with vWT and vb_5 were dissolved in 0.1 M sodium phosphate buffer (pH 7.25) containing 0.3% Emulgen 913 and 20% glycerol. The samples were divided into two cuvettes; the sample cuvette was treated with a few crystals of sodium dithionite, and difference spectra were recorded with an Aminco DW-2000 spectrophotometer.

The cellular content of Cyt b_5 was calculated to be 0.31 nmol/mg of cell lysate protein.

Coexpression of Cyt b_5, NADPH–P450 Oxidoreductase, and P450IIB1 in TK$^-$ 143 Cells. To assess whether Cyt b_5 could potentiate the activity of rat P450IIB1, we coinfected TK$^-$ 143 cells with vb$_5$ and vIIB1. Since these cells have very low levels of NADPH–P450 oxidoreductase (26), we also included vOR in the infection. Virus titers were first determined in CV-1 cells by using the plaque-forming assay with trypan blue (29). Preliminary experiments were undertaken with various ratios of these viruses to determine the optimum ratio to achieve about a 1:1 molar ratio of P450IIB1/Cyt b_5. The results obtained with optimum virus ratios are displayed in Fig. 3 and Table 1. When using a ratio of multiplicity of infections of 30:20:10 for vIIB1/vOR/vb$_5$, the molar amounts of P450IIB1 and Cyt b_5 produced were similar. In cells infected with only vIIB1 and vOR, the levels of the expressed enzymes were about 20–23% higher than those of the corresponding enzymes produced in cells infected with all three viruses (Fig. 3*A* and Table 1). Cyt b_5 levels were quantified by obtaining difference spectra that compared

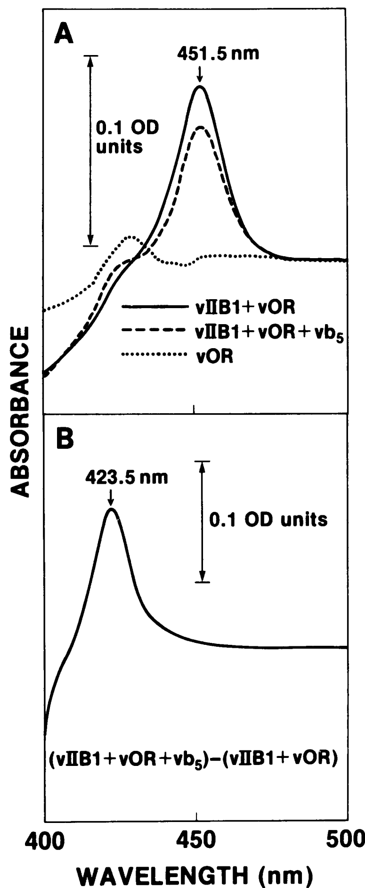

FIG. 3. Quantitation of P450IIB1 content (*A*) and Cyt b_5 content (*B*) in microsomal membrane fractions of cells infected with various combinations of recombinant vaccinia viruses designated vb$_5$, vOR, and vIIB1 (see *Materials and Methods*). To measure P450IIB1 contents, microsomal membrane fractions (16 mg of protein) were prepared, and spectra were measured as described in the legend to Fig. 2. The fractions were divided into two cuvettes, and the sample cuvette was gently bubbled with CO for 15 sec. Both the sample and reference cuvettes were treated with a few crystals of sodium dithionite, and difference spectra were recorded (*A*). Cyt b_5 content was determined as described in Fig. 2 except that the microsomal membrane fraction obtained from the vIIB1/vOR/vb$_5$-infected cells was placed in the sample cuvette, and the fraction obtained from the vIIB1/vOR-infected cells was placed in the reference cuvette for recording of Cyt b_5-specific difference spectra (*B*).

Table 1. Quantitation of P450, Cyt b_5, and NADPH–P450 oxidoreductase (OR) in vaccinia virus-infected cells

Virus (moi)	P450, pmol/mg	Cyt b_5, pmol/mg	OR, milliunit/mg
vIIB1 (30) + vOR (20) + vb$_5$ (10)	47	42	111
vIIB1 (30) + vOR (20)	61	<0.2	135
vOR (50)	<0.2	<0.2	212
vWT (50)	<0.2	<0.2	9

TK$^-$ 143 cells were infected with recombinant vaccinia viruses as indicated and harvested 48 hr after infection, and microsomal membrane fractions were prepared. The ratios of viruses in multiplicity of infection (moi) are indicated in parentheses. Quantitation of P450 was carried out by difference spectra of the ferrous–CO-bound form of the cytochrome. Cyt b_5 content was determined by measuring the difference between absorption of the ferrous cytochrome in cells infected with three recombinant viruses and cells infected with two recombinant viruses (minus vb$_5$). The extinction coefficients are listed in the text. OR activities were determined as described (39); 1 pmol of OR corresponds to 3 milliunits of activity (26). The ratio of P450IIB1/OR is optimal for maximal monooxygenase activity, analyzed as described (26).

cells infected with vIIB1, vOR, and vb$_5$ with cells infected with only vIIB1 and vOR, after reduction with dithionite. The level of Cyt b_5 expression was similar to that of P450IIB1 (Fig. 3*B* and Table 1), and the levels of NADPH–P450 oxidoreductase were sufficient to support the monooxygenase activities of P450IIB1. It should be noted that Cyt b_5 expression is considerably lower in microsomal membranes of cells coinfected with three recombinant viruses than in the total cell lysate protein from cells infected with vb$_5$ alone (Fig. 2). This could be due to more binding sites for the cytochrome in total cell membranes, competition of infectivity of the multiple recombinant vaccinia, competition for microsome binding sites of Cyt b_5 with the other expressed membrane proteins, or a combination of these factors.

Effects of Cyt b_5 on P450IIB1-Catalyzed Monooxygenase Activities. Membrane fractions containing cDNA-expressed P450IIB1 and NADPH–P450 oxidoreductase with and with-

Table 2. Role of Cyt b_5 in the monooxygenase activities catalyzed by P450IIB1

		Catalytic activity, nmol/min per nmol of P450	
Substrate	Product	P450IIB1 + Cyt b_5	P450IIB1
7-Ethoxy-coumarin	7-Hydroxycoumarin	11.2 ± 0.4	12.3 ± 0.4
7-Pentoxy-resorufin	Resorufin	13.6 ± 0.4	11.8 ± 0.3
Testosterone	16β-Hydroxy-17-ketotestosterone	4.3 ± 0.6	3.6 ± 0.6
	16α-Hydroxytestosterone	11.5 ± 1.4	9.9 ± 1.4
	16β-Hydroxytestosterone	10.7 ± 1.2	8.7 ± 1.4
	17-Ketotestosterone	5.8 ± 0.7	4.9 ± 0.8
p-Nitro-phenetole	*p*-Nitrophenol	9.6 ± 0.8	1.4 ± 0.1

Microsomal membrane fractions from vaccinia virus-infected cells were quantified for levels of Cyt b_5 and P450IIB1 as described in Fig. 3 and Table 1 and then were assayed for monooxygenase activities. Results are averages ± SD of three experiments. Only trace metabolism of 7-ethoxycoumarin and 7-pentoxyresorufin was observed in cells infected with vWT. However, since we could not detect P450 in these cells, the catalytic activities or turnover numbers were not quantitated. No testosterone or *p*-nitrophenetole metabolism was observed in control cells.

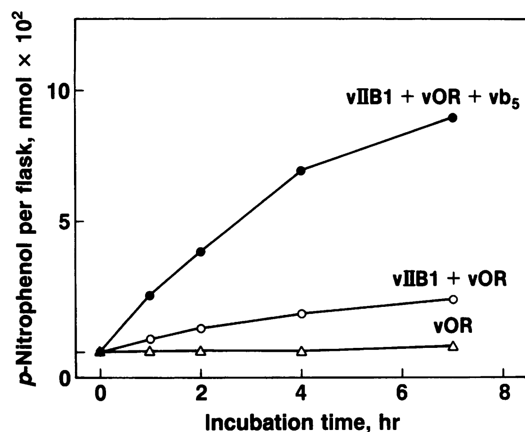

FIG. 4. Metabolism of *p*-nitrophenetole *in situ* by cells infected with recombinant vaccinia viruses. Cells were infected with the various recombinant viruses shown above each curve in the figure. Eighteen hours after infection, *p*-nitrophenetole was added to a final concentration of 0.4 mM, and the nmol of *p*-nitrophenol produced per flask was determined as a function of time. The results are the average of three flasks per time point.

out cDNA-expressed Cyt b_5 were analyzed for activities toward four substrates. Inclusion of Cyt b_5 did not affect 7-ethoxycoumarin O-deethylase and 7-pentoxyresorufin O-dealkylase activities catalyzed by P450IIB1 (Table 2). We also analyzed testosterone metabolism, since P450IIB1 is able to hydroxylate this substrate at numerous positions, with the major metabolites resulting from hydroxylations at the 16α, 16β, and 17 positions. The 17-keto testosterone metabolite is further hydroxylated at the 16β position. Inclusion of Cyt b_5 resulted in a modest 15% increase in testosterone hydroxylation at all positions (Table 2). When *p*-nitrophenetole deethylation was examined, Cyt b_5 was found to stimulate activity 7-fold. In the absence of P450IIB1, membranes containing Cyt b_5 and NADPH-P450 oxidoreductase were catalytically inactive toward all substrates. Further, when membrane fractions from cells infected individually with vb$_5$, vIIB1, and vOR were combined and assayed, no increase in activity over that expected from membranes of vIIB1-infected cells was observed for any substrate. These results establish that the three monooxygenase components must be within the same membrane for electron transfer and substrate oxidation to occur.

The vaccinia virus-infected cells were used also to determine whether the oxidation of *p*-nitrophenetole is stimulated by Cyt b_5 *in situ*. Cells infected with vIIB1 and vOR and cells infected with vIIB1, vOR, and vb$_5$ were incubated with the inclusion of *p*-nitrophenetole in the medium. The rate of accumulation of the product *p*-nitrophenol was quantified and found to be 5-fold higher in the medium of cells expressing Cyt b_5 and linear for up to 5 hr after addition of substrate (Fig. 4). In contrast, metabolism of 7-ethoxycoumarin in intact cells was not enhanced by the presence of Cyt b_5. Based on the known amount of P450IIB1 in a 75-cm^2 flask, calculated from spectral data, we estimated that the turnover of *p*-nitrophenetole in cells containing both P450IIB1 and Cyt b_5 was about 15 nmol·min^{-1}·(nmol of P450IIB1)$^{-1}$. This value is about 56% higher than that obtained in the *in vitro* incubations. These results confirm that Cyt b_5 can enhance the rate of metabolism of certain P450 substrates in intact cells.

DISCUSSION

Cyt b_5 is known to be involved in cholesterol (40) and fatty acid (41) biosynthetic pathways. This occurs through the transfer of electrons from NADH via the flavoprotein NADH–Cyt b_5 oxidoreductase. As noted in the Introduction, numerous studies have documented the potentiation effect and mechanism of Cyt b_5 in certain monooxygenase activities. However, these studies relied on *in vitro* reconstituted systems. The studies reported herein directly demonstrate that Cyt b_5 can increase monooxygenase activities in intact cells. A 5-fold to 7-fold increase in *p*-nitrophenetole deethylation was detected in microsomes and intact cells when Cyt b_5 was concurrently expressed with NADPH–P450 oxidoreductase and P450IIB1. We also detected a low-level activity in microsomes and cells only expressing P450IIB1 in the absence of Cyt b_5, indicating that the latter cytochrome is not absolutely required for oxidation of this substrate. These data are in agreement with *in vitro* reconstitution studies using rat P450IIB1 (16) and the rabbit P450LM2 (15). It should be interesting to determine whether H_2O_2 formation is decreased by Cyt b_5 incorporation in the *in situ* reconstitution system similar to that observed *in vitro* (15, 21). The role of NADH and NADH–Cyt b_5 oxidoreductase in the intact cell should also be addressed. Indeed, these studies have implications for *in vivo* hydrogen peroxide formation during drug oxidations.

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1. Sligar, S. G. & Gunsalus, I. C. (1977) *Adv. Enzymol.* **47,** 1–44.
2. White, R. E. & Coon, M. J. (1980) *Annu. Rev. Biochem.* **49,** 315–356.
3. Hildebrant, A. & Estabrook, R. W. (1971) *Arch. Biochem. Biophys.* **143,** 66–79.
4. Estabrook, R. W. (1984) in *Drug Metabolism and Drug Toxicity*, eds. Mitchell, J. R. & Horning, M. E. (Raven, New York), pp. 1–20.
5. Jansson, I. & Schenkman, J. B. (1973) *Mol. Pharmacol.* **9,** 840–841.
6. Hrycay, E. G. & Estabrook, R. W. (1974) *Biochim. Biophys. Acta* **60,** 771–778.
7. Sasame, H. A., Thorgeirsson, S. S., Mitchell, J. R. & Gillette, J. (1974) *Life Sci.* **14,** 35–46.
8. Lu, A. Y. H., Levin, W., Selander, H. & Jerina, D. M. (1974) *Biochem. Biophys. Res. Commun.* **61,** 1348–1355.
9. Ingleman-Sundberg, M. & Johansson, I. (1980) *Biochem. Biophys. Res. Commun.* **97,** 582–589.
10. Imai, Y. (1981) *J. Biochem.* **89,** 351–362.
11. Patten, C. J., Ning, S. M., Lu, A. Y. H. & Yang, C. S. (1986) *Arch. Biochem. Biophys.* **251,** 629–638.
12. Jansson, I., Tamburini, P. P., Favreau, L. V. & Schenkman, J. B. (1985) *Drug Metab. Dispos.* **13,** 453–458.
13. Tamura, S., Kawata, S., Sugiyama, T. & Tarui, S. (1987) *Biochim. Biophys. Acta* **926,** 231–238.
14. Gruenke, L. D., Konopka, K., Koop, D. R. & Waskell, L. A. (1988) *J. Pharmacol. Exp. Ther.* **246,** 454–459.
15. Jansson, I. & Schenkman, J. B. (1987) *Drug. Metab. Dispos.* **15,** 344–348.
16. Kuwahara, S., Harada, N., Omura, T. & Mannering, G. (1982) in *Microsomes and Drug Oxidations*, eds. Sato, R. & Kato, R. (Japan Sci. Soc., Tokyo), pp. 89–90.
17. Noshiro, M., Harada, N. & Omura, T. (1980) *J. Biochem.* **88,** 1521–1535.
18. Noshiro, M., Ullrich, V. & Omura, T. (1981) *Eur. J. Biochem.* **116,** 521–526.
19. Lipka, J. J. & Waskell, L. A. (1989) *Arch. Biochem. Biophys.* **268,** 153–160.
20. Pompon, D. & Coon, M. J. (1984) *J. Biol. Chem.* **259,** 15377–15385.
21. Gorsky, L. D. & Coon, M. J. (1986) *Drug Metab. Dispos.* **14,** 89–96.
22. Kaminsky, L. S. & Guengerich, F. P. (1986) *Eur. J. Biochem.* **149,** 479–487.
23. Yamazoe, Y., Murayama, N., Shimada, M., Yamauchi, K., Nagata, K., Imaoka, S., Funae, Y. & Kato, R. (1988) *J. Biochem.* **104,** 785–790.
24. Christou, M., Mitchell, M. J., Jovanovich, M. C., Wilson, N. M. & Jefcoate, C. R. (1989) *Arch. Biochem. Biophys.* **270,** 162–172.
25. Ryan, D. E. & Levin, W. (1989) *Pharmacol. Ther.* **45,** 153–239.
26. Yamano, S., Aoyama, T., McBride, O. W., Hardwick, J. P.,

Gelboin, H. V. & Gonzalez, F. J. (1989) *Mol. Pharmacol.* **35,** 83–88.
27. Miyata, M., Nagata, K., Yamazoe, Y. & Kato, R. (1989) *Pharmacol. Res.* **21,** 513–520.
28. Chakrabarti, S., Brechling, K. & Moss, B. (1985) *Mol. Cell. Biol.* **5,** 3403–3409.
29. Mackett, M., Smith, G. L. & Moss, B. (1984) *J. Virol.* **49,** 857–864.
30. Aoyama, T., Korzekwa, K., Nagata, K., Adesnik, M., Reiss, H., Lapenson, D. P., Gillette, J., Gelboin, H. V., Waxman, D. J. & Gonzalez, F. J. (1989) *J. Biol. Chem.* **264,** 21327–21333.
31. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
32. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
33. Spatz, L. & Strittmatter, P. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 1042–1046.
34. Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239,** 2370–2378.
35. Wood, A. W., Ryan, D. E., Thomas, P. E. & Levin, W. (1983) *J. Biol. Chem.* **258,** 8839–8847.
36. Greenlee, W. F. & Poland, A. (1978) *J. Pharmacol. Exp. Ther.* **205,** 596–608.
37. Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V. & Kaminsky, L. S. (1982) *Biochemistry* **21,** 6019–6030.
38. Shigematsu, H., Yamano, S. & Yoshimura, H. (1976) *Arch. Biochem. Biophys.* **173,** 178–186.
39. Yasukochi, Y. & Masters, B. S. S. (1976) *J. Biol. Chem.* **251,** 5337–5344.
40. Fukushima, H., Grinstead, G. F. & Gaylor, J. L. (1981) *J. Biol. Chem.* **256,** 4822–4826.
41. Keyes, S. R., Alfano, J. H., Jansson, I. & Cinti, D. L. (1979) *J. Biol. Chem.* **254,** 7778–7784.