Pergamon

0006-2952(95)00200-6

Biochemical Pharmacology, Vol. 50, No. 6, pp. 781–785, 1995.

0006-2952/95 $9.50 + 0.00

INDUCTION OF CYTOCHROME P-4502B1-RELATED MOUSE CYTOCHROME P-450 AND REGULATION OF ITS EXPRESSION BY EPIDERMAL GROWTH FACTOR/TRANSFORMING GROWTH FACTOR α IN PRIMARY HEPATOCYTE CULTURE

JIRI AUBRECHT,*† KAREN I. HIRSCH-ERNST,*
VOLKER BECKER-RABBENSTEIN,* GEORG FRIEDRICH KAHL,*
HISAAKI TANIGUCHI‡ and MARTIN W. HÖHNE*

*Institute of Pharmacology and Toxicology, University of Göttingen, D-37075 Göttingen, Germany; and
‡Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-11, Japan

(*Received* 20 *January* 1995; *accepted* 25 *April* 1995)

Abstract—Phenobarbital-dependent induction of mouse cytochrome P-450 (Cyp) orthologous to rat CYP2B1 and its modulation by hepatotrophic growth factors were examined in primary hepatocyte cultures. Compared to rat hepatocytes, induction in mouse hepatocytes was more rapid and effective. Ligands of the EGF receptor, epidermal growth factor, and transforming growth factor α inhibited induction on the basis of protein expression and CYP2B-associated 7-pentoxyresorufin-O-depentylase activity. Furthermore, EGF led to repression of accumulation of corresponding mRNA under phenobarbital, an effect not blocked by inhibition of protein synthesis under cycloheximide. Ligands of the EGF receptor may contribute towards the decrease in hepatic CYP expression observed during (pre)neoplastic development and regeneration.

Key words: hepatocyte culture; phenobarbital; epidermal growth factor; transforming growth factor α; cytochrome P-450; gene expression

During the process of chemically induced hepatocarcinogenesis in rodents the appearance of phenotypically altered cell populations is observed [1]. These focally growing lesions, believed to comprise potential progenitor cells of hepatocellular carcinoma, have been shown to exhibit changes in activities of enzymes associated with metabolism of xenobiotics [1, 2]; levels of CYP,§ isoenzymes of which are involved in activation of xenobiotics to reactive species, are often found to be decreased [2]. A continuous reduction in basal expression and in induction levels of individual isoenzymes such as CYP1A1, CYP1A2, and CYP2B1 [2, 3] can occur during progressive stages of hepatocarcinogenesis, in the case of CYP2B1 sometimes following an initial increase [3].

That some phenotypic alterations may be linked to a proliferative state of the hepatocyte is indicated by the observation that a transient decrease in cytochrome P-450 (coinciding with peak DNA synthesis) occurs during liver regeneration following partial hepatectomy [4, 5]. The hepatic growth factor TGF-α is thought to play an important role in liver regeneration and in autocrine/paracrine stimulation of (pre)neoplastic growth. TGF-α mRNA expression has been shown to be increased in rat liver following partial hepatectomy [6] and in preneoplastic liver foci [7]. Furthermore, an elevated expression of TGF-α has been demonstrated immunohistochemically in human hepatocellular carcinoma tissue [8]. We have previously shown that TGF-α and EGF, both ligands of the EGF receptor [6], repress methylcholanthrene-mediated induction of mouse Cyp1a-1 *in vitro* [9], suggesting that the decrease in CYP expression during carcinogenesis and regeneration may be mediated by a ligand of the EGF receptor.

The present study demonstrates that phenobarbital-inducible isoforms of the CYP2B subfamily are also subject to repression of induction by ligands of the EGF receptor in rodents, although the mechanism of phenobarbital-mediated induction [10] is distinct from the pathway of CYP1A1 induction via the Ah receptor [11].

MATERIALS AND METHODS

Cell culture

Hepatocytes were isolated from adult male animals (C57BL/6J mice and Wistar rats) by collagenase perfusion [12], resuspended in MX-83 medium [13] lacking arginine but containing 1 μM insulin, 20 μM hydrocortisone and 10% fetal calf serum, and seeded on culture dishes at a density of 8.6×10^4 cells/cm^2. Following an initial attachment period, the medium was exchanged to fresh MX-83 medium without serum [14]. In experiments conducted to distinguish the effect exerted by EGF-TGF-α from a possible insulin effect, insulin was omitted from the medium as indicated in Results and Discussion. The medium was changed twice daily. Mouse hepatocytes were maintained on untreated plastic dishes (Nunc, Wiesbaden, Germany), whereas rat hepatocytes were cultivated on dishes coated with 4.1 μg/cm^2 type I collagen. Collagen, bovine insulin, and murine

Corresponding author: K. I. Hirsch-Ernst, Institute of Pharmacology and Toxicology, University of Göttingen, Robert-Koch-Strasse 40, D-37075 Göttingen, Germany. Tel. +49-551-395306; FAX +49-551-399552.

† Present address: Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA, U.S.A.

§ Abbreviations: CYP, cytochrome P-450; EGF, epidermal growth factor; IGF-I, insulin-like growth factor I; PROD, 7-pentoxyresorufin-O-depentylase; TGF-α, transforming growth factor α.

EGF were purchased from Boehringer Mannheim, Germany; recombinant human TGF-α and insulin-like growth factor (IGF-I) were obtained from Bissendorf (Hannover, Germany).

In vivo *induction of CYP2B*

Animals were treated with phenobarbital (Na-salt, Serva, Heidelberg, Germany, 20 mg/ml saline, 80 mg/kg body weight) by three i. p. injections administered at intervals of 24 hours.

Immunoblot analysis

Hepatocyte microsomes were obtained by differential centrifugation [15], and their protein content determined according to ref. [16]. For immunoblot analyses, samples of 100 μg of microsomal protein were separated by electrophoresis through 10% SDS-polyacrylamide gels [17], and transferred to nitrocellulose membranes (Amersham, Braunschweig, Germany) by semi-dry blotting [18]. Autoradiographic detection of CYP2B1-related mouse protein was facilitated by use of a primary antibody against rat CYP2B1 in a dilution of 1:500 and secondary ^{125}I-labeled Ig-F(ab')$_2$ fragments against rabbit Ig (Amersham). The primary antibody was raised in rabbits using rat CYP2B1 isolated from phenobarbital-induced rat liver microsomes and affinity-purified by antigen-coupled Sepharose 4B (Pharmacia, Freiburg, Germany) as described [19].

Assessment of CYP content and CYP2B-associated activity

7-Pentoxyresorufin-O-depentylase (PROD) activity was measured fluorimetrically in microsomal samples containing 150 μg of protein according to ref. [20], and total CYP content in microsomes determined by CO difference spectroscopy [21].

RNA blot analysis

Total RNA was isolated by guanidinium thiocyanate-phenol extraction [22] and subjected to electrophoresis through formaldehyde-agarose gels. Subsequently, RNA transfer to nylon membranes (Amersham) was performed as described previously [14]. Equal loading of lanes (15 μg/lane) was ensured by evaluation of ethidium bromide gel staining. RNA blots were hybridized to a ^{32}P-labeled rat *CYP2B1* (exon 7) oligonucleotide probe [23] that had been 5'-end-labeled by T4-polynucleotide kinase [23].

RESULTS AND DISCUSSION

The usefulness of primary rat hepatocyte cultures in studying regulation of CYP2B1 gene expression is impeded by the fact that maintenance in culture is often accompanied by a progressive loss of responsiveness of the rat *CYP2B1* gene to induction by phenobarbital [10]. The present study, constituting the first demonstration of induction of CYP2B1-related mouse mRNA *in vitro*, shows that phenobarbital-dependent induction of mouse Cyp2b mRNA in hepatocytes cultivated in serum-free MX-83 medium is achieved at levels equivalent to those obtained during induction *in vivo* (Fig. 1). Mouse Cyp2b mRNA induced in the course of phenobarbital treatment comigrated with rat CYP2B1 mRNA (data not shown). Nevertheless, hybridization to the rat *CYP2B1* specific oligonucleotide probe [23] employed in the present

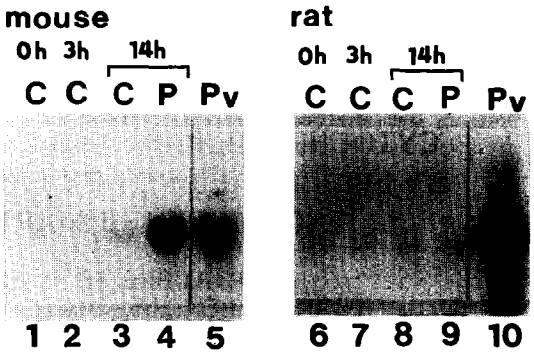

Fig. 1. Expression of CYP2B1-related mouse cytochrome P-450 mRNA and of rat CYP2B1 mRNA *in vivo* and *in vitro* as determined in RNA blot analysis. Animals were subjected to *in vivo* induction under phenobarbital for 3 days ("Pv"). Mouse (lanes 1–4) and rat hepatocytes (lanes 6–9) were cultivated for the periods of time indicated (0–14 hours) with 1.5 mM phenobarbital ("P") or in absence of an inducer ("C").

study does not allow exact specification of the individual mouse *Cyp2b* mRNA expressed during culture, as at least two *Cyp2b* genes of the mouse share the sequence complementary to the rat *CYP2B1* antisense probe. The *16-αoh-b* gene [24], redesignated as mouse *Cyp2b-13* [25], bears this sequence in its exon 7 region as well as cDNA clones pf3/46 (nucleotides 979–996) [26], which have been designated as mouse *Cyp2b-10* [25], and whose protein product has been purified from mouse liver subsequent to phenobarbital induction [27].

We have previously shown that maximal induction of rat CYP2B1 mRNA in hepatocytes cultivated in serum-free MX-83 medium does not exceed 15–20% of mRNA levels observed in the course of induction *in vivo* [14]. It seems unlikely that differences in the extent of mRNA accumulation in rat and mouse *in vitro* systems might be attributed to the use of differing culture substrates, since expression levels of CYP2B1-related mRNA under phenobarbital in mouse hepatocytes were not affected by the fact that cells were grown on collagen or on uncoated plastic dishes (data not shown). Furthermore, induction of CYP2B-associated PROD activity in rat hepatocytes grown on plastic does not significantly differ from activity in cells maintained on collagen [14].

After 14 hours of incubation with phenobarbital, maximal levels of CYP2B1-related mRNA were observed in mouse hepatocytes (Fig. 1), whereas rat CYP2B1 mRNA levels had not yet increased (Fig. 1). Peak expression of rat CYP2B1 mRNA was shown to occur after approximately 72 hours of incubation [14]. Although inhibition of ongoing protein synthesis by cycloheximide is known to block increase in CYP2B mRNA under phenobarbital in rat hepatocyte culture [28], concurrent incubation of mouse hepatocytes with cycloheximide and phenobarbital did not lead to a block of expression of CYP2B1-related mRNA (Fig. 2). Differences not only in kinetics of induction but also in responsiveness to cycloheximide indicate that species-specific mechanisms mediating accumulation of mRNA in response to phenobarbital might be crucial in mice in comparison to rats. Induction of CYP2B1/2 by phenobarbital in rats has been attributed to transcriptional activation [10]; whether stabilization of mRNA might contribute to accumulation of related mRNA in mice remains to be elucidated.

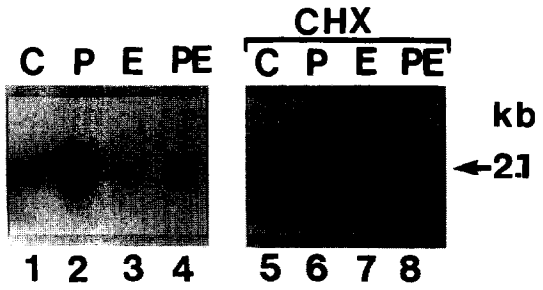

Fig. 2. Effect of cycloheximide on modulation of CYP2B1-related mRNA expression in primary mouse hepatocytes. Total RNA was extracted from hepatocytes maintained in MX-83 medium supplemented with 1.5 mM phenobarbital ("P"), 16 nM EGF ("E"), or with both ("PE") for 14 hours. Lanes 5–8 denote expression in cells concomitantly treated with 35 μM cycloheximide.

A polyclonal antibody raised against rat CYP2B1 was used for immunodetection of CYP2B1-related mouse protein. In the course of incubation of mouse hepatocytes with phenobarbital, maximal levels of mouse Cyp2b protein were observed after 3–4 days (Fig. 3A). The two protein species cross reacting with the primary antibody exhibited approximate molecular weights of 56 and 52 kDa, respectively. The amount of immunodetectable protein after 72 hours of induction was similar to that observed during *in vivo* induction (Table 1). Cross reactivity of monoclonal antibodies directed against rat CYP2B1 epitopes with human CYP2E1 has been described [29]. As species boundaries were crossed in immunodetection of CYP2B1-related mouse protein, the possibility must be considered that the primary antibody

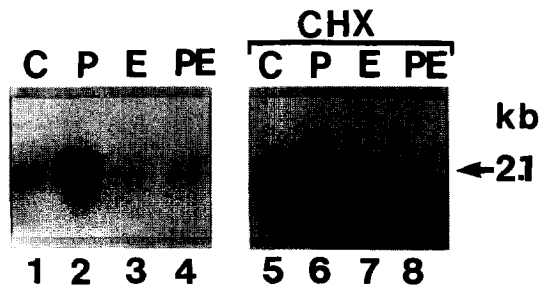

Fig. 3. Expression of immunodetectable Cyp2b protein in mouse hepatocytes under phenobarbital induction and in the presence of growth factors. Protein blot of microsomal fractions developed for immunoreactivity with a primary antibody raised against rat CYP2B1. (A) Cells were cultivated in MX-83 medium supplemented with 20 μM hydrocortisone and 1.5 mM phenobarbital for 0 hours (lane 1), 1 day (lane 2), 2 days (lane 3), 3 days (lane 4), 4 days (lane 5), and 5 days (lane 6). (B) Hepatocytes were cultivated in MX-83 medium containing phenobarbital and hydrocortisone without additional growth factors (lane 5) or supplemented with 16 nM concentrations of individual growth factors (lanes 1–4) for three days. Lane 6: 3-day culture control without phenobarbital; Ins = insulin.

used in the present study might also recognize (a) phenobarbital-inducible mouse Cyp isoform(s) apart from the Cyp2b subfamily. Nevertheless, elevation of Cyp2b-associated PROD activity in phenobarbital-treated mouse hepatocytes (Table 1) is consistent with the interpretation that phenobarbital led to induction of functional Cyp2b protein *in vitro*. The phenobarbital-dependent increase in PROD activity in mouse hepatocytes analysed after a culture period of 72 hours amounted to approximately 55–60% of the level achieved by induction *in vivo* (Table 1).

In addition to responsiveness to various xenobiotics, expression of individual cytochrome P-450 enzymes may be subject to regulation by endogenous substances. Repression of rat CYP2B1/2 induction *in vitro* has been shown to be elicited by growth hormone [30] or by interleukin 6, a mediator of hepatic acute phase reaction [31]. In the present study we examined the effects of EGF and TGF-α on induction of rat CYP2B1 and on corresponding mouse isoform(s).

As determined by immunoblot analyses, concurrent treatment of mouse hepatocytes with 100 ng EGF/ml (16 nM EGF) for 72 hours led to repression of accumulation of CYP2B1-related protein expression (Fig. 3B, lane 3) in comparison to control cultures treated with phenobarbital alone (Fig. 3B, lane 5). Both immunodetectable protein species were affected. TGF-α at the same concentration exerted the same effect on protein accumulation as did EGF (Fig. 3B, lane 4), consistent with the fact that TGF-α and EGF both bind to the same receptor [6]. Repression of induction by EGF/TGF-α was also apparent on the functional level determined as PROD activity (Table 1), whereas enzyme activity was not affected by other growth factors examined. Although equimolar concentrations (16 nM) of insulin and EGF have been shown to be potent in induction of expression of "early response" genes such as c-*fos*, c-*jun*, and c-*myc*, and to stimulate reinitiation of DNA synthesis in primary mouse hepatocytes subjected to growth arrest [9], insulin failed to repress induction of CYP2B1-related protein expression in the mouse (Fig. 3B, lane 1), indicating that EGF/TGF-α dependent repression is not linked to cell cycle progression or stimulation of DNA synthesis *per se*. IGF-I also proved ineffective in inhibiting induction (Fig. 3B, lane 2), consistent with previous findings whereby the repression of CYP2B1/2 induction in rats by growth hormone is not mediated by IGF-I [30]. EGF furthermore inhibited induction in hepatocytes of both mice and rats on the mRNA level (Fig. 4). Repression of mRNA accumulation and inhibition of PROD activity occurred in a similar dose-dependent manner in both species, with a maximal effect being elicited by 10–100 ng EGF/ml (Figs. 4 and 5).

As shown in Fig. 2, inhibition of ongoing protein synthesis by cycloheximide did not influence EGF-dependent repression of mRNA induction, indicating that *de novo* or continuous synthesis of a labile repressor protein is not crucial in mediating repression by EGF. It therefore seems likely that the EGF effect might be exerted via posttranslational modifications of pre-existing proteins (e.g., phosphorylation/dephosphorylation). Whether the EGF/TGF-α-mediated effect is founded on a transcriptional block or on the destabilization of mRNA, both resulting in a decrease in mRNA levels, remains to be determined. Modulation of mouse Cyp1a-1 expression by EGF/TGF-α has been described previously [9].

Table 1. Influence of phenobarbital and growth factors on levels of immunodetectable Cyp2b protein and enzyme activities

	Total Cyp content (nmol/mg)	Immunodetectable Cyp2b protein in %	PROD activity (nmol/min/mg)
Mouse liver			
control	0.69 ± 0.18	85 ± 29	0.014 ± 0.005
72 h Pb	1.54 ± 0.39	313 ± 74	0.793 ± 0.015
Mouse hepatocytes			
0 h, control	0.83 ± 0.24	100 ± 21	0.015 ± 0.008
72 h, control	0.17 ± 0.06	42 ± 13	0.000
72 h, Pb	0.58 ± 0.11	455 ± 92	0.462 ± 0.010
72 h, EGF/Pb	n.d.	88 ± 16	0.018 ± 0.002
72 h, TGF-α/Pb	n.d.	94 ± 22	0.016 ± 0.006
72 h, Ins/Pb	n.d.	416 ± 88	0.422 ± 0.041
72 h, IGF-I/Pb	n.d.	419 ± 96	0.427 ± 0.063

Total Cyp content is expressed as nmol Cyp/mg microsomal protein, PROD activity as nanomoles resorufin formed per minute per milligram microsomal protein. Levels of immunodetectable protein were determined by scintillation counting of total Cyp protein bands excised from immunoblots, and are expressed as % of levels observed in the 0-hr control *in vitro*. In order to achieve *in vivo* induction, animals were treated with phenobarbital ("Pb") as described in Materials and Methods. Hepatocytes were cultivated with phenobarbital or without (control) in the presence of growth factors (at 16 nM concentrations each) as indicated. Results are presented as mean values ± *SD* obtained from three or more independent experiments; n.d. = not determined.

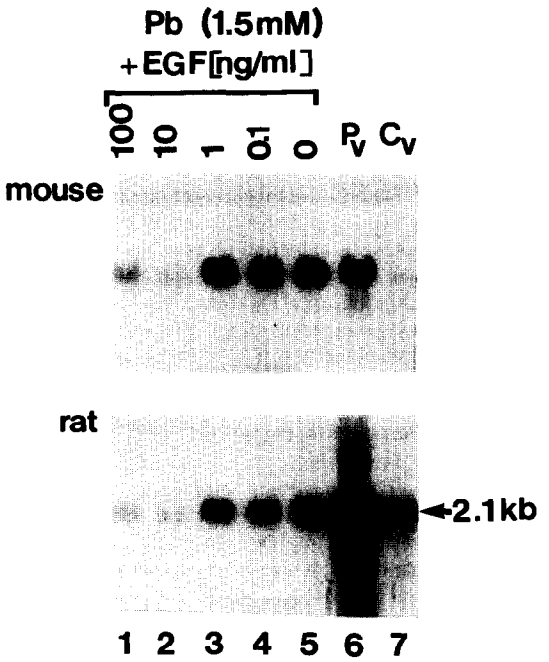

Fig. 4. Suppression of phenobarbital-dependent induction of rat CYP2B1 and related mouse cytochrome P-450 EGF. RNA blot analysis; lanes 1–5: mouse and rat hepatocytes cultivated in the presence of phenobarbital ("Pb") and of increasing concentrations of EGF (0–100 ng/ml = 0–16 nM) for 72 hours. MRNA expression in animals having received phenobarbital injections ("Pv") or saline ("Cv") on three consecutive days is shown in lanes 6 and 7, respectively.

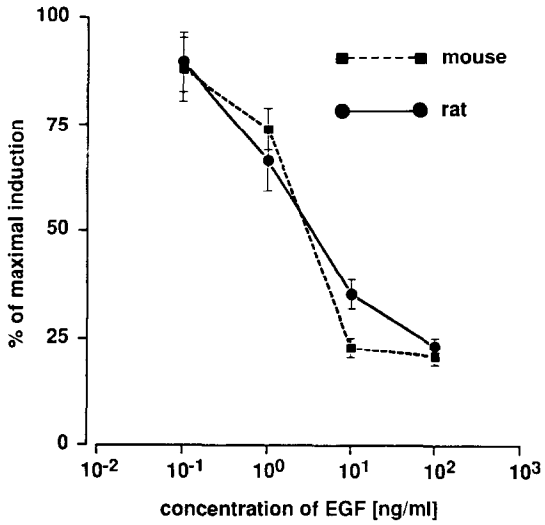

Fig. 5. Influence of EGF concentration on induction of relative PROD activity in mouse and rat hepatocytes cultivated for 72 hours. Results represent mean values ± *SD* of three or more independent experiments performed in triplicate.

Since EGF and TGF-α also affect induction of other, differently regulated cytochrome P-450s as demonstrated in the present study, it appears unlikely that a CYP1A1-specific mechanism should be involved in repression, but rather one common to those cytochrome P-450s examined so far. Repression of individual cytochrome P-450s by ligands of the EGF-receptor may be of significance *in vivo* in that the autocrine/paracrine production of TGF-α observed during stages of liver differentiation (e.g., following partial hepatectomy) or during preneoplastic/neoplastic development might contribute towards a loss of cytochrome P-450 expression associated with these processes.

Acknowledgements— The authors are indebted to Dr. M. Höhne for his outstanding contribution to the project (deceased 30 May 1993). Financial support was received from the "Deutsche Forschungsgemeinschaft" (DFG grant No. Ho 1105/1-2 and SFB 402 A2).

REFERENCES

1. Farber E, The sequential analysis of liver cancer induction. *Biochim Biophys Acta* **605:** 149–166, 1980.
2. Hanigan MH and Pitot HC, Activities of benzphetamine N-demethylase and aryl hydrocarbon hydroxylase in cells isolated from γ-glutamyl transpeptidase-positive foci and surrounding liver. *J Natl Cancer Inst* **75:** 1107–1112, 1985.
3. Kunz HW, Buchmann A, Schwarz M, Schmitt R, Kuhlmann WD, Wolf CR and Oesch F, Expression and inducibility of drug-metabolizing enzymes in preneoplastic and neoplastic lesions of rat liver during nitrosamine-induced hepatocarcinogenesis. *Arch Toxicol* **60:** 198–203, 1987.
4. Klinger W and Karge E, Interaction of induction, ontogenetic development and liver regeneration on the monooxygenase level. *Exp Pathol* **31:** 117–124, 1987.
5. Marie IJ, Dalet C, Blanchard J-M, Astre C, Szawlowski A, Saint Aubert B, Joyeux H and Maurel P, Inhibition of cytochrome P-450p (P450IIIA1) gene expression during liver regeneration from two-thirds hepatectomy in the rat. *Biochem Pharmacol* **37:** 3515–3521, 1988.
6. Mead JE and Fausto N, Transforming growth factor alpha may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* **86:** 1558–1562, 1989.
7. Evarts RP, Nakatsukasa H, Marsden ER, Hu Z and Thorgeirsson SS, Expression of transforming growth factor-alpha in regenerating liver and during hepatic differentiation. *Mol Carcinogenesis,* **5:** 25–31, 1992.
8. Collier JD, Guo K, Gullick WJ, Bassendine MF and Burt AD, Expression of transforming growth factor alpha in human hepatocellular carcinoma. *Liver* **13:** 151–155, 1993.
9. Höhne M, Becker-Rabbenstein V, Kahl GF and Taniguchi H, Regulation of cytochrome P-450 CYP1A1 gene expression and protooncogene expression by growth factors in primary hepatocytes. *FEBS Lett* **273:** 219–222, 1990.
10. Waxman DJ and Azaroff L, Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J* **281:** 577–592, 1992.
11. Hankinson O, Research on the aryl hydrocarbon (dioxin) receptor is primed to take off. *Arch Biochem Biophys* **300:** 1–5, 1993.
12. Seglen PO, Preparation of isolated rat liver cells. In: *Methods in Cell Biology,* vol 13 (Ed. Prescott DM), pp. 29–83. Academic Press, New York, 1976.
13. Hoffmann B, Piasecki A and Paul D, Proliferation of fetal rat hepatocytes in response to growth factors and hormones in primary culture. *J Cell Physiol* **139:** 654–662, 1989.
14. Aubrecht J, Kahl GF and Höhne MW, Regulation of fibronectin mRNA expression in primary hepatocytes in response to EGF and phenobarbital. *Biochem Biophys Res Commun* **190:** 1023–1028, 1993.
15. Dallner G, Isolation of microsomal subfractions by use of density gradients. *Meth Enzymol* **52:** 71–83, 1978.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
17. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685, 1970.
18. Kyhse-Andersen J, Electroblotting of multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Methods* **10:** 203–209, 1984.
19. Pyerin W and Taniguchi H, Phosphorylation of hepatic phenobarbital-inducible cytochrome P-450. *EMBO J* **8:** 3003–3010, 1989.
20. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolff T and Guengerich FP, Dealkylation of pentoxyresorufin: A rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238:** 43–48, 1985.
21. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239:** 2370–2378, 1964.
22. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
23. Omiecinski CJ, Walz FG, Jr and Vlasuk GP, Phenobarbital induction of rat liver cytochromes P-450b and P-450e. *J Biol Chem* **260:** 3247–3250, 1985.
24. Lakso M, Masaki R, Noshiro M and Negishi M, Structures and characterization of sex-specific mouse cytochrome P-450 genes as members within a large family. *Eur J Biochem* **195:** 477–486, 1991.
25. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW, The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* **12:** 1–51, 1993.
26. Noshiro M, Lakso M, Kawajiri K and Negishi M, Rip Locus: Regulation of female-specific isozyme (I-P-$450_{16\alpha}$) of testosterone 16α-hydroxylase in mouse liver, chromosome localization, and cloning of P-450 cDNA. *Biochemistry* **27:** 6434–6443, 1988.
27. Mäenpää J, Rane A, Raunio H, Honkakoski P and Pelkonen O, Cytochrome P450 isoforms in human fetal tissues related to phenobarbital-inducible forms in the mouse. *Biochem Pharmacol* **45:** 899–907, 1993.
28. Burger H-J, Schuetz EG, Schuetz JD and Guzelian PS, Divergent effects of cycloheximide on the induction of class II and class III cytochrome P450 mRNAs in cultures of adult rat hepatocytes. *Arch Biochem Biophys* **281:** 204–211, 1990.
29. Wrighton SA, Vandenbranden M, Becker GW, Black SD and Thomas PE, Two monoclonal antibodies recognizing different epitopes on rat cytochrome IIB1 react with human IIE1. *Mol Pharmacol* **41:** 76–82, 1992.
30. Schuetz EG, Schuetz JD, May B and Guzelian PS, Regulation of cytochrome P-450b/e and P-450p gene expression by growth hormone in adult rat hepatocytes cultured on a reconstituted basement membrane. *J Biol Chem* **265:** 1188–1192, 1990.
31. Williams JF, Bement WJ, Sinclair JF and Sinclair PR, Effect of interleukin 6 on phenobarbital induction of cytochrome P-450IIB in cultured rat hepatocytes. *Biochem Biophys Res Commun* **178:** 1049–1055, 1991.