Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9746–9750, December 1990
Biochemistry

A different cytochrome P450 form is induced in primary cultures of rat hepatocytes

(cDNA sequence)

YOSHIKAZU EMI, CHIKAYUKI CHIJIIWA, AND TSUNEO OMURA

Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Higashi-ku, Fukuoka, Fukuoka 812, Japan

Communicated by Ronald W. Estabrook, September 4, 1990 (received for review June 10, 1990)

ABSTRACT **A 49-kDa protein (P49) was discovered in the primary cultures of rat hepatocytes. P49 cross-reacted with the antibodies against purified P450IIC11 [formerly P-450(M-1)]. P49 was located in microsomes and highly induced after plating of isolated hepatocytes on collagen-coated culture dishes. To characterize P49, cDNA clones were screened from a rat liver λgt11 expression library. From sequence analysis of the cloned cDNAs, the amino acid sequence of P49 was deduced, and the protein was identified as a previously uncharacterized form of cytochrome P450. P49 consists of 489 amino acids and shows ≈60% similarity with the members of class IIC subfamily of rat cytochrome P450, such as P450IIC11 and P450IIC12 [formerly P-450(F-1)]. RNA blot analysis indicates that the mRNA translating P49 was induced ≈20- to 30-fold at 70 hr in the primary cultures compared with the liver of adult rats. Induction of P49 was not affected by density of the plated cells and the presence or absence of several hormones, serum, or antibiotics in the culture medium. On the other hand, lower induction of P49 was seen when the hepatocytes were cultured on Matrigel-coated plates. Expression of P49 mRNA was low in the liver of adult rats and was not detectable in the livers of 1- and 2-week-old male and female rats. P49 is an additional form of cytochrome P450, which is induced in the primary culture of rat hepatocytes.**

Primary culture of hepatocytes is a convenient experimental system by which to investigate various liver functions under conditions free from physiological regulations. However, many liver-specific proteins, including cytochrome P450, decrease within a few days in this primary culture. Decrease of liver-specific proteins is mainly due to transcriptional block (1). On the other hand, expressions of a few enzymes, such as heme oxygenase, are highly elevated in primary cultures (2, 3). Synthesis of cytoskeletal proteins, such as α-tubulin and β-actin, are also highly stimulated in primary cultures, but their expressions are lowered when the hepatocytes are plated on certain types of extracellular matrices (4, 5).

Hepatic microsomal cytochrome P450 consists of various forms and catalyzes oxidative metabolism of a wide variety of xenobiotic and endogenous substrates. Under conventional culture conditions, cytochrome P450 of the hepatocytes declines rapidly to low levels in a few days of culture (6–8). A few forms of cytochrome P450, such as P450IA1 (formerly P_1-450) (9, 10) and P450IIIA1 (formerly P-450_p) (11), are induced in the primary cultures with suitable inducers. Recently Guzelian and coworkers (3) developed a culture system using Matrigel for induction of cytochrome P450 in cultured hepatocytes. When rat hepatocytes were cultured on a Matrigel-coated dish and treated with phenobarbital, induced expression of P450IIB1 (formerly P-450_b) and P450IIB2 (formerly P-450_e) was observed. These are two major phenobarbital-inducible forms of cytochrome P450. However, constitutive forms of cytochrome P450, including P450IIC11 [formerly P-450(M-1)] could not be maintained in the cultured cells, even when plated on Matrigel (12).

We examined the changes in synthesis of several forms of cytochrome P450 in the primary cultures of rat hepatocytes. When immunoblot analysis of cytochrome P450 in cultured hepatocytes was performed by using polyclonal antibodies, one lot of the antibodies prepared against purified P450IIC11 detected a 49-kDa protein in addition to P450IIC11. The 49-kDa protein (P49) was slightly expressed in the liver of adult rat and highly induced in the primary cultures of hepatocytes. To characterize P49, the cDNA encoding P49 was cloned.* Amino acid sequence deduced from the nucleotide sequence was compared with various forms of cytochrome P450, and P49 was identified as a distinct form of cytochrome P450. Culture conditions that affected the expression of P49 mRNA were investigated. Tissue-specific and age-dependent expression of P49 were also examined.

MATERIALS AND METHODS

Primary Culture of Rat Hepatocytes. Male Sprague–Dawley rats weighing 150–200 g were used in all experiments. The hepatocytes were prepared by an *in situ* collagenase perfusion method based on that of Seglen (13). The isolated hepatocytes were suspended at 5×10^5 cells per ml with Eagle's minimal essential medium supplemented with 10% bovine serum, 10 μM dexamethasone, insulin at 10 μg/ml, kanamycin at 60 μg/ml, penicillin at 10 units/ml, streptomycin at 100 μg/ml, and amphotericin B at 0.25 μg/ml. Ten-milliliter portions of the cell suspension were plated onto type I collagen-coated culture dishes (10 cm in diameter) and incubated at 37°C in 95% air/5% CO_2. In some experiments, cells were plated onto the dishes coated with Matrigel (Collaborative Research); 6–8 hr after plating, the culture medium was changed, and it was changed daily thereafter.

Preparation of Cell Extracts and Immunoblot Analysis. Cultured hepatocytes were washed once with saline containing 20 mM phosphate buffered saline, pH 7.5, scraped off from two dishes with a rubber policeman, and then washed once with ice-cold phosphate-buffered saline by centrifugation ($50 \times g$ for 2 min). The pelleted cells were suspended in 200 μl of STE buffer (0.25 M sucrose/20 mM Tris·HCl/1 mM EDTA, pH 7.4) containing 5 mM phenylmethylsulfonyl fluoride, leupeptin at 5 μg/ml, and pepstatin at 5 μg/ml. The cells were disrupted by sonication (5 sec, 5 times), and the disrupted cells were centrifuged in a microcentrifuge at

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.

Abbreviations: P450s are classified according to the recommended nomenclature system (34) as follows: P450IIC11, P-450(M-1); P450IIC12, P-450(F-1) or P-$450_{15\beta}$; P450IIB1, P-450_b; P450IIB2, P-450_c; P450IA1, P-450_c; P450IIIA1, P-450_p; P450IIE1, P-450_j.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M58041).

12,000 × *g* for 20 min. The supernatant was used as the cell extract.

Antibodies against purified P450IIC11 were prepared as described (14, 15). Two different lots of the antibodies, one reacting with both P450IIC11 and P49 and another reacting only with P450IIC11, were used. Forty micrograms of the cell extracts was electrophoresed with SDS/polyacrylamide gel (16) and transferred onto nitrocellulose filter (17). The protein bands treated with anti-P450IIC11 IgG were visualized by alkaline phosphatase-conjugated anti-rabbit IgG in the presence of nitroblue tetrazolium salt and 5-bromo-4-chloroindolyl phosphate. Intensity of the bands were determined by densitometry using a Shimadzu model CS-930 chromatoscanner.

Isolation and Characterization of cDNA Clones. Hepatocytes recovered from five culture dishes were suspended in 500 μl of STE buffer, and total RNA was extracted by the SDS/phenol method, as described (18). The recovery of total RNA was 0.3–0.5 mg. Total RNA of rat livers was prepared as reported (15).

All manipulations of recombinant DNA were as described (19). Double-stranded cDNA was synthesized from poly(A)$^+$ RNA prepared from the livers of male rats and ligated into λgt11 with *Eco*RI linkers. The library was screened by using two different lots of antibodies against P450IIC11. The concentration of the primary antibodies was 20 μg/ml in PBS/0.1% Triton X-100/0.1% nonfat dried milk. Proteins in the plaques on LB plates were transferred to nitrocellulose filters in the presence of isopropyl β-D-thiogalactoside, and the plaques that reacted with the antibodies were selected and further screened. DNAs from the cloned phage plaques were purified from the plate lysates (20). Sequencing of single-stranded DNA was performed by the dideoxynucleotide chain-termination method (21).

Total RNAs prepared from cultured hepatocytes and rat livers were electrophoretically separated in a formaldehyde-containing agarose gel and transferred to nitrocellulose filters. Specific RNA was hybridized with ^{32}P-labeled DNA fragment at 60°C in 4× SSC (1×SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7) containing 10 mM EDTA and salmon testis DNA at 25 μg/ml. The filter was washed two times with 0.1× SSC/0.1% SDS at 65°C for 30 min and autoradiographed. Intensities of the bands on the autoradiogram were determined by densitometric assay. cDNAs of P450IIC11 and carboxyesterase E1 had been cloned (22, 23).

RESULTS

Detection of the 49-kDa Protein in Primary Cultures of Rat Hepatocytes. The extracts of cultured hepatocytes at various time points were analyzed by immunoblotting by using two lots of antibodies against purified P450IIC11. Fig. 1 shows that one lot of antibodies (lot 1) reacted with only P450IIC11, but another lot (lot 2) reacted with an extra protein band of 49 kDa (P49) in addition to P450IIC11. P49 was only slightly expressed in liver, and it increased rapidly after plating of the hepatocytes. Maximum induction of P49, which was ≈15-fold in comparison with time 0, was seen at ≈72 hr (Fig. 1*B*). On the other hand, P450IIC11 gradually decreased in the cultured hepatocytes, although it was maintained at nearly the same level as time 0 in some experiments. Induction of P49 was constantly observed, irrespective of maintenance of P450IIC11 in the primary cultures (Fig. 1 *B* and *C*), indicating that P49 was not a degradation product of P450IIC11. P49 was recovered in the microsomal fraction when the cell extract was ultracentrifuged.

Cloning and Sequencing of P49 cDNA. Because P49 was also detected in the livers of adult rats, a cDNA library was prepared from the hepatic mRNA of male rats. The λgt11

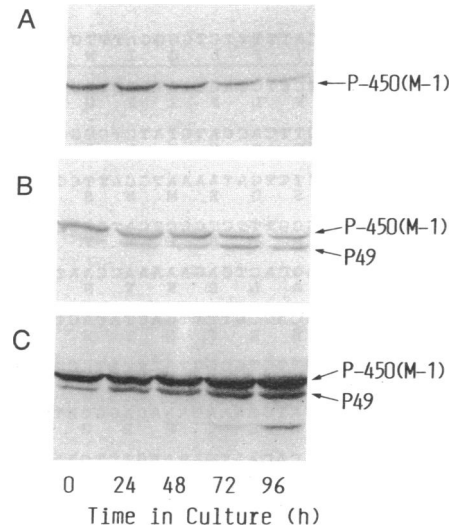

FIG. 1. Detection of P49 in primary cultures of rat hepatocytes. Hepatocytes were plated at time 0 and harvested at 24, 48, 72, and 96 hr after plating. Forty micrograms of cell extracts was analyzed by immunoblotting with two lots of antibodies against purified P450IIC11 [P-450(M-1)]. (*A* and *B*) Experiments using lot 1 and lot 2 antibodies, respectively. (*C*) Results of another experiment using lot 2 antibodies.

cDNA library was screened by using the two lots of P450IIC11 antibodies (Fig. 1, lots 1 and 2). From 6 × 10^5 recombinant phages, 239 plaques, which were supposed to have an inserted cDNA encoding P450IIC11, reacted with both lots of the antibodies, and 36 plaques reacted only with lot 2 antibodies. The latter 36 plaques were screened further by using lot 2 antibodies, and finally 14 recombinant phages were obtained. A recombinant phage (λP49-A4), which had the longest cDNA insert of ≈1.5 kilobases (kb) consisting of two *Eco*RI fragments (0.3 plus 1.2 kb), was isolated.

The two fragments of the *Eco*RI-digested cDNA insert were subcloned into pUC19 separately and digested with various restriction endonucleases to obtain a restriction map (Fig. 2). The fragment containing the internal *Eco*RI site of the cDNA was obtained from the *Sph* I fragment of λP49-A4 DNA. From sequence analysis, the 0.3-kb and 1.2-kb fragments were found to code N-terminal and C-terminal portions of P49, respectively, but the 0.3-kb fragment lacked an initiation ATG codon. The cDNA library was further screened by using the 0.3-kb fragment as probe to obtain a full-length cDNA. From 2 × 10^5 recombinant phages, 32 positive plaques were obtained. λP49-D2 in Fig. 2 had the longest cDNA insert of ≈1.7 kb (0.4 plus 1.3 kb). The nucleotide sequence showed that the inserted cDNA included 1706 base pairs and had an open reading frame coding 489 amino acids with a molecular size of ≈56 kDa (Fig. 3). However, a poly(A) tail was absent from the cloned cDNA.

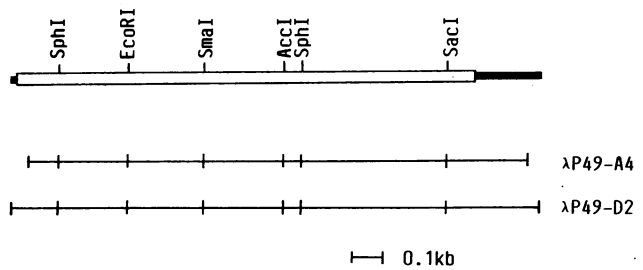

FIG. 2. Restriction map of P49 cDNA clones. Open box at top shows P49 encoding region. Lower two lines show the restriction maps of two P49 cDNA clones.

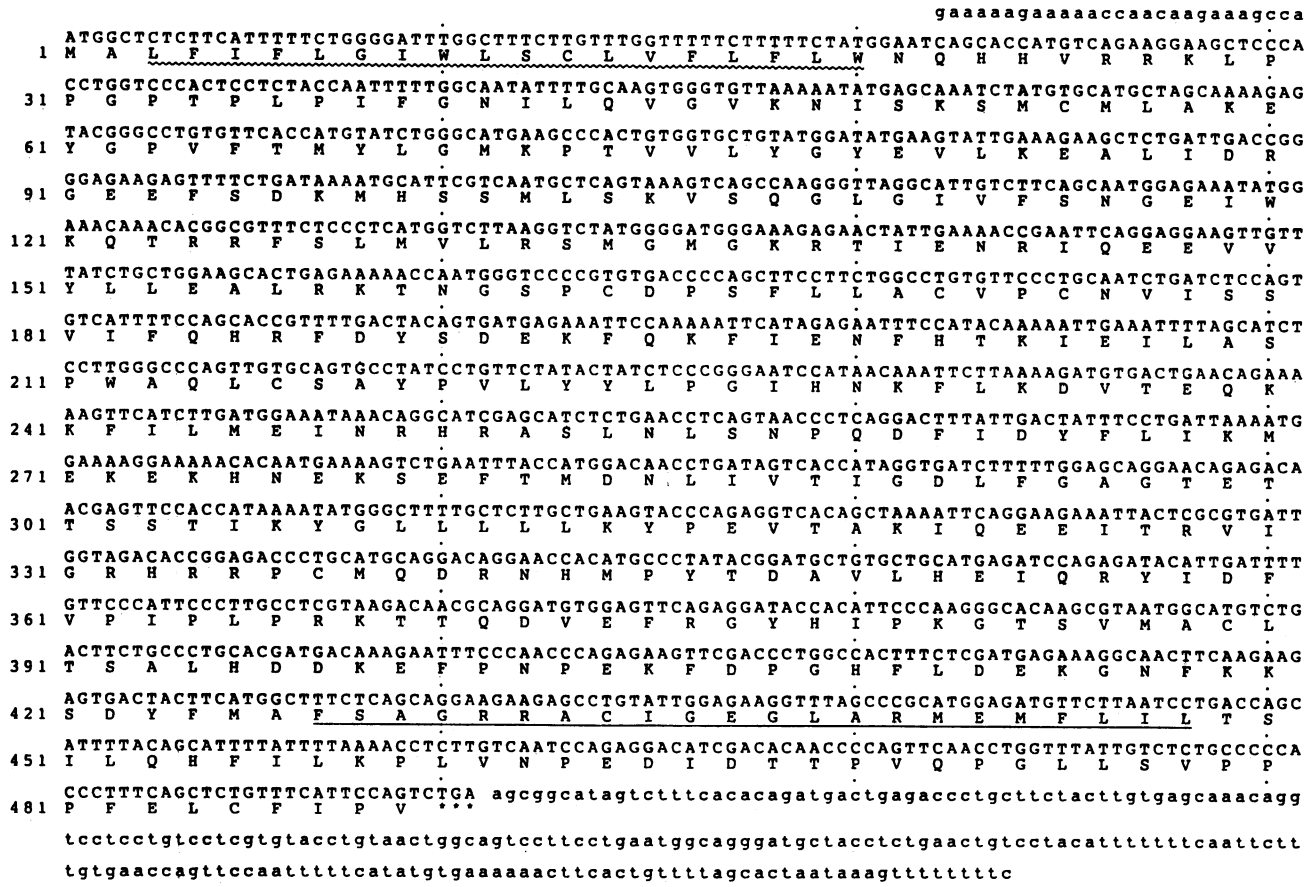

FIG. 3. Nucleotide sequence and deduced amino acid sequence of P49 (P49 cDNA in λP49-D2). The sequence underlined with a straight line is the putative heme-binding site, and that underlined with a wavy line is the N-terminal hydrophobic stretch necessary for membrane anchoring. The termination codon is indicated by stars.

Amino Acid Sequence of P49. The amino acid sequence of P49 deduced from the nucleotide sequence of λP49-D2 was compared with P450IIC11 (22). A homology of 61% between P49 and P450IIC11 was obtained. When compared with other forms of cytochrome P450, a maximum homology of 65% was obtained with rabbit P450IIC3 (formerly P-450$_{3b}$) (24). Moreover, P49 had the consensus amino acid sequence of the heme-binding site of cytochrome P450, HR2 (25) (Fig. 3, underlined sequence). The initiator methionine was followed by a stretch of hydrophobic residues (Fig. 3, wavy line). Such a hydrophobic stretch at the N-terminal portion is always found in microsomal forms of cytochrome P450 and is essential for their anchoring to microsomal membrane (26, 27).

Comparison of P49 with various forms of rat cytochrome P450 indicated that P49 is a previously uncharacterized form of cytochrome P450 belonging to the IIC subfamily. P49 showed 59% homology with P450IIC12 (formerly P-450$_{15\beta}$) (28), which is a female-specific form and developmentally regulated in rat liver as P450IIC11. P49 had 53% homology with P450IIE1 (formerly P-450$_j$) (29), an ethanol-inducible form, 46% homology with P450IIB1 (P-450$_b$) (30), a phenobarbital-inducible form, and 28% homology with P450IA1 (P-450$_c$) (31), a 3-methylcholanthrene-inducible form. Homologies with mitochondrial and bacterial forms of cytochrome P450 were lower. We conclude that P49 is another member of P450IIC subfamily, which is highly induced in the primary cultures of rat hepatocytes.

Expression of P49 in Cultured Hepatocytes. Total RNAs were prepared from the cultured hepatocytes at various time points after plating and analyzed by RNA blotting. Fig. 4 shows that the mRNA translating P49 increased ≈20-fold in the cultured hepatocytes as compared with the time 0 value based on the densitometric assay. On the other hand, the mRNAs translating P450IIC11 and carboxyesterase E1, two other microsomal proteins, rapidly decreased after plating of the cells.

When the cultured rat hepatocytes are treated with dexamethasone, P450IIIA1 is highly induced in microsomes of the cultured cells (11). However, induction of P49 was seen whether dexamethasone was present or not in the culture medium (data not shown). Neither cell density of the cultures nor additives to the culture medium, including antibiotics,

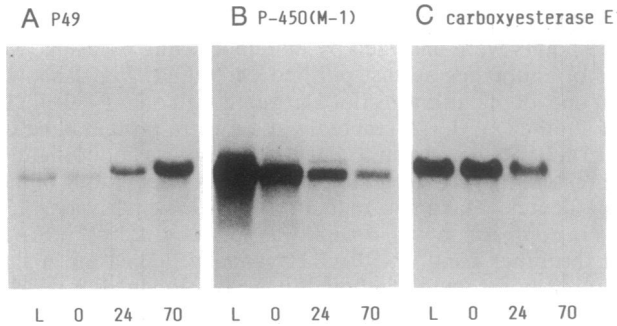

FIG. 4. Northern blot analysis of mRNAs of P49 and two other microsomal proteins in primary cultures of rat hepatocytes. Hepatocytes were plated at time 0 and harvested at 24 and 70 hr after plating. Fifteen micrograms of total RNA prepared from the livers of untreated male rats and cultured cells was hybridized with ^{32}P-labeled cDNA probes. (*A*) P49. (*B*) P450IIC11 [P-450(M-1)]. (*C*) Carboxyesterase E1. Culture hours are indicated for each lane; L, liver RNA.

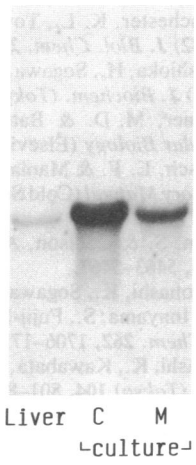

FIG. 5. Effects of extracellular matrices on induction of P49 mRNA in primary cultures of rat hepatocytes. Hepatocytes were plated onto type I collagen (lane C)- and Matrigel (lane M)-coated dishes and harvested at 70 hr after plating. Fifteen micrograms of total RNA prepared from the livers of male rats and the cultured hepatocytes was analyzed by Northern blotting with a ^{32}P-labeled P49 cDNA probe.

phenol red, insulin, and bovine serum, had any effect on induction of P49 mRNA (data not shown). When rat serum was added to the medium as a substitute for bovine serum, P49 expression was not affected (data not shown). However, plating of the hepatocytes on Matrigel, an extracellular matrix prepared from Engelbreth-Holm–Swarm (EHS) sarcoma, affected the induction of P49 mRNA. As shown in Fig. 5, P49 mRNA was induced ≈30-fold in the hepatocytes cultured on type I collagen-coated plates, whereas the induction was only ≈4-fold when the cells were cultured on Matrigel-coated plates.

Expression of P49 in Rat Liver. Total RNAs prepared from the livers of untreated, phenobarbital-treated, and methylcholanthrene-treated adult rats were analyzed by Northern blotting. Fig. 6*A* shows that expression of the mRNA translating P49 in the liver of untreated adult rats was much lower than in the cultured hepatocytes. Male rats expressed more P49 compared with female rats. Phenobarbital and methylcholanthrene administration did not affect expression of the mRNA in either sex. Developmental expression of P49 was also examined in the livers of male and female rats (Fig. 6*B*). P49 mRNA was not detected in the livers of newborn rats (1 and 2 weeks of age) in either sex; elevated levels of expression were seen after 4 weeks of age. However, the levels of P49 mRNA expression in the livers of adult rats were still very low as compared with those in primary cultures.

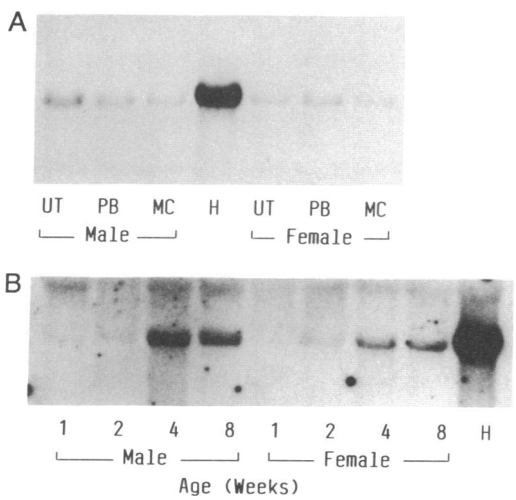

FIG. 6. P49 mRNA in livers of male and female rats. Fifteen micrograms of total RNA from rat livers was analyzed by Northern blotting with ^{32}P-labeled P49 cDNA as probe. Total RNA from 70-hr cultured hepatocytes is a positive control in lane H. (*A*) UT, untreated; PB, phenobarbital-treated; and MC, 3-methylcholanthrene-treated rats. (*B*) Total RNA from male and female rats at 1, 2, 4, and 8 weeks of age was analyzed.

To investigate whether the expression of P49 mRNA is influenced by the proliferation of hepatocytes or not, total RNA was extracted from the livers of partially hepatectomized rats and analyzed by Northern blotting. On 4–7 days after the operation, expression of P49 mRNA was not significantly affected in the regenerating livers (data not shown). Expression of P49 mRNA was also examined in various rat tissues—brain, lung, heart, kidney, spleen, intestine, adipose tissue, and testis—but only in liver was the mRNA detected (data not shown).

DISCUSSION

When rat hepatocytes are transferred to primary cultures, the composition of the proteins of the cultured cells is significantly altered, and many liver-specific functions decline sharply (1). Cellular content of cytochrome P450, which catalyzes the oxygenation of various endogenous and exogenous substrates in the liver, also decreases rapidly in these primary cultures (6–8).

As P450IIC11 is one of the major components of cytochrome P450 in the liver of male rats, we examined its alteration in cultured hepatocytes. We found that one lot of antibodies against P450IIC11 recognized an additional protein band of 49 kDa (P49) and that this additional protein component increased sharply after plating of the hepatocytes. To characterize P49, cDNA clones encoding P49 were isolated from a λgt11 library of rat liver cDNA; we obtained a cDNA clone containing an open reading frame for 489 amino acids. When the mRNA of the cultured hepatocytes was analyzed by Northern blotting, P49 mRNA was induced ≈20- to 30-fold at 70 hr after plating of cells as compared with time 0. The increase of P49 in cultured hepatocytes paralleled the increase of its mRNA.

P49 seems to be a distinctive member of cytochrome P450IIC subfamily because its amino acid sequence was highly homologous with P450s of IIC subfamily, including P450IIC11 and other members of the subfamily. As the primary structure of P49 resembles those of sex-specific forms of cytochrome P450 that catalyze hydroxylation of various steroid hormones, it is likely that P49 also participates in the metabolism of such steroid substrates. However, the physiological significance of the rapid increase of P49 in cultured hepatocytes is yet to be elucidated.

The density of plated cells is known to alter expression of certain proteins. Secretion of serum albumin and the enzyme for DNA synthesis are elevated at low cell density, whereas succinate dehydrogenase and malic enzyme are elevated at high cell density (32). However, the same level of P49 expression was seen in both high and low cell densities. When cultured hepatocytes are treated with dexamethasone, several proteins including P450IIIA1 (11) and tryptophane 2,3-dioxygenase (33) are induced. However, induction of P49 was constantly observed with or without dexamethasone in the culture medium. Expression of P49 was not affected by various compounds added to the culture medium, such as serum, antibiotics, and insulin.

The only culture condition in which P49 induction was reduced was plating on Matrigel-coated dishes. Gene expression in cultured hepatocytes is known to be altered by the various extracellular matrices on which cells are plated (3–5). Because Matrigel contains various extracellular matrices, the

expression of the P49-encoding gene in the hepatocytes seems affected by contact with some of the extracellular matrix components. The precise mechanism responsible for induction of P49 in the cultured hepatocytes remains to be clarified.

We thank Dr. O. Gotoh (Saitama Cancer Center Research Institute) for estimating the homology of P49 with other forms of cytochrome P450. Helpful suggestions and advice were provided by Dr. K. Mihara and Dr. A. Ito (Kyushu University). This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

1. Clayton, D. F. & Darnell, J. E. (1983) *Mol. Cell. Biol.* **3,** 1552–1561.
2. Paine, A. J. & Legg, R. F. (1978) *Biochem. Biophys. Res. Commun.* **81,** 672–679.
3. Schuetz, E. G., Li, D., Omiecinski, C. J., Muller-Eberhard, V., Kleinman, H. K., Elswick, B. & Guzelian, P. S. (1988) *J. Cell Physiol.* **134,** 309–323.
4. Reid, L. M., Narita, M., Fujita, M., Murray, Z., Liverpool, C. & Rosenberg, L. (1986) in *Isolated and Cultured Hepatocytes*, eds. Guillouzo, A. & Guguen-Guillouzo, C. (Libbey, Paris), pp. 225–258.
5. Fujita, M., Spray, D. C., Choi, H., Saez, H. C., Rosenberg, L. C. & Reid, L. M. (1987) *Hepatology* **7,** 1s–9s.
6. Guzelian, P. S., Bissell, D. M. & Meyer, U. A. (1977) *Gastroenterology* **72,** 1232–1239.
7. Fahl, W. E., Michalopoulos, G., Sattler, G. L., Jefcoate, C. R. & Pitot, H. C. (1979) *Arch. Biochem. Biophys.* **192,** 61–72.
8. Evarts, R. P., Marsden, E. & Thorgeirsson, S. S. (1984) *Biochem. Pharmacol.* **33,** 565–569.
9. Nebert, D. W. & Gelboin, H. V. (1968) *J. Biol. Chem.* **243,** 6242–6249.
10. Nebert, D. W. & Gielen, J. E. (1971) *J. Biol. Chem.* **246,** 5199–5206.
11. Elshourbagy, N. A., Barwick, J. L. & Guzelian, P. S. (1981) *J. Biol. Chem.* **256,** 6060–6068.
12. Guzelian, P. S., Li, D., Schuetz, E. G., Thomas, P., Levin, W., Mode, A. & Gustafsson, J.-Å. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9783–9787.
13. Seglen, P. O. (1973) *Exp. Cell Res.* **82,** 391–398.
14. Matsumoto, T., Emi, Y., Kawabata, S. & Omura, T. (1986) *J. Biochem. (Tokyo)* **100,** 1359–1371.
15. Emi, Y. & Omura, T. (1988) *J. Biochem. (Tokyo)* **104,** 40–43.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Nielsen, P. J., Manchester, K. L., Towbin, H., Gordon, P. J. & Thomas, G. (1982) *J. Biol. Chem.* **257,** 12316–12321.
18. Morohashi, K., Yoshioka, H., Sogawa, K., Fujii-Kuriyama, Y. & Omura, T. (1984) *J. Biochem. (Tokyo)* **95,** 949–957.
19. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, Amsterdam).
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Yoshioka, H., Morohashi, K., Sogawa, K., Miyata, T., Kawajiri, K., Hirose, T., Inayama, S., Fujii-Kuriyama, Y. & Omura, T. (1987) *J. Biol. Chem.* **262,** 1706–1711.
23. Takagi, Y., Morohashi, K., Kawabata, S., Go, M. & Omura, T. (1988) *J. Biochem. (Tokyo)* **104,** 801–806.
24. Ozols, J., Heinemann, F. S. & Johnson, E. F. (1985) *J. Biol. Chem.* **260,** 5427–5434.
25. Gotoh, O., Tagashira, Y., Iizuka, T. & Fujii-Kuriyama, Y. (1983) *J. Biochem. (Tokyo)* **93,** 807–817.
26. Sakaguchi, M., Mihara, K. & Sato, R. (1987) *EMBO J.* **6,** 2425–2431.
27. Sato, T., Sakaguchi, M., Mihara, K. & Omura, T. (1990) *EMBO J.* **9,** 2391–2397.
28. Mode, A., Zaphiropoulos, P., Strom, A., MacGeoch, C., Moller, C., Morgan, E. & Gustafsson, J.-Å. (1988) in *Microsomes and Drug Oxidations*, eds. Miners, J. O., Birkett, D. J., Drew, R., May, B. K. & McManus, M. E. (Taylor & Francis, London), pp. 131–139.
29. Song, B. J., Gelboin, H. V., Park, S. S., Yang, C. S. & Gonzalez, F. J. (1986) *J. Biol. Chem.* **261,** 16689–16697.
30. Fujii-Kuriyama, Y., Mizukami, Y., Kawajiri, K., Sogawa, K. & Muramatsu, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2793–2797.
31. Yabusaki, Y., Shimizu, M., Murakami, H., Nakamura, K., Oeda, K. & Ohkawa, H. (1984) *Nucleic Acids Res.* **12,** 2929–2938.
32. Guguen-Guillouzo, C. (1986) in *Isolated and Cultured Hepatocytes*, eds. Guillouzo, A. & Guguen-Guillouzo, C. (Libbey, Paris), pp. 259–284.
33. Nakamura, T., Shinno, H. & Ichihara, A. (1980) *J. Biol. Chem.* **255,** 7533–7535.
34. 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.