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Monoclonal Antibody-Directed Determination of Cytochrome P-450 Types Expressed in a Human Lymphoblastoid Cell Line

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SUMMARY

Cytochrome P-450-dependent aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase activities of a cloned line of human lymphoblastoid AHH-1 cells are inhibited by a monoclonal antibody (MAb 1-7-1) prepared to a 3-methylcholanthrene-induced rat liver cytochrome P-450. The monoclonal antibody inhibition determined that a single MAb 1-7-1-sensitive type of cytochrome P-450 is responsible for all of AHH expression in both the basal and benz[*a*]anthracene-induced cells. Partial inhibition by the MAb 1-7-1, however, indicates that at least two forms of cytochrome P-450 catalyze 7-ethoxycoumarin *O*-deethylase in both the basal and the induced cells, one form of which is identical to the MAb-sensitive cytochrome P-450 responsible for all of the AHH. Thus, a single cloned cell line is capable of expressing two classes of cytochromes P-450, and the observed multiplicity of cytochrome P-450 in animal tissues does not necessarily depend on cell heterogeneity. A sensitive MAb 1-7-1-based radioimmunoassay also directly demonstrates the presence in these cells of a MAb 1-7-1-specific type of cytochrome P-450 as well as its elevation in the induced cells. These MAb-based methods thus can determine the contribution of specific MAb-defined types of cytochromes P-450 to the cellular metabolism of specific xenobiotics.

INTRODUCTION

The cytochromes P-450 metabolize a variety of xenobiotic and endobiotic compounds, including carcinogens, drugs, and steroids (1–5). There is a large multiplicity of cytochrome P-450 forms (6) which catalyze reactions that convert these substrates to numerous products, including detoxified metabolites and toxic or carcinogenic compounds. Since the individual cytochrome P-450 isozymes display unique yet overlapping substrate specificity as well as selectivity, the type and amount of cytochromes P-450 present in a tissue may control the choice of alternative metabolic pathways to detoxification or activation of products. The multiplicity of cytochromes P-450 has hindered progress in the determination of the phenotype, or distribution of isozymes, by conventional methods. The capability to detect and measure individual cytochromes P-450 would make feasible a variety of studies, including relating cytochrome P-450 phenotype to metabolism of specific substrates to particular metabolites, and perhaps to individual susceptibility to carcinogens and individual variation in drug metabolism (7).

As a new approach to the problem of cytochrome P-450 multiplicity, panels of MAbs[1] to cytochromes P-450 have been prepared (8–14). As highly specific probes for cytochromes P-450, MAbs can be used to define immunochemically these isozymes based on their epitope content. An MAb probe thus interacts with "types" or "classes" of cytochrome P-450, which may include either a single isozyme or multiple isozymes with a common epitope. The MAbs have been proven useful for the detection and identification of cytochromes P-450 by enzyme inhibition assay (15–17), RIA (18, 19) and immunopurification of specific cytochromes P-450 (19–21).

The MAb approach as applied to human tissues (lymphocytes, monocytes, placenta, liver) has detected intertissue and interindividual differences in cytochrome P-450 content (15, 17). Multiple MAb-specific cytochromes P-450 have previously been found within individual tissues and cells in cultures, including lymphocytes (15). The cells examined, however, typically consist of populations which are heterogeneous in their cytochrome P-450 content. Buffalo rat liver cells, for example, may be subcloned into cell strains with different AHH activities (22). A finding of multiple cytochromes P-450 within a parent population of cells does not therefore reveal whether the observed spectrum of isozymes is characteristic of the entire population, or whether individual subclones produce different profiles of cytochrome P-450 isozymes. Examination of an individual cloned cell line

[1] The abbreviations used are: MAb, monoclonal antibody; AHH, aryl hydrocarbon hydroxylase; ECD, 7-ethoxycoumarin *O*-deethylase; BA, benz[*a*]anthracene; RIA, radioimmunoassay; MC, 3-methylcholanthrene.

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should reveal whether (*a*) it expresses either unique or multiple forms of cytochrome P-450, and (*b*) induction increases production of a constitutive cytochrome P-450, or initiates expression of a nonconstitutive cytochrome P-450.

One approach to analysis of the cytochrome P-450 isozymes in a cell line is by reaction phenotyping, which is a characterization of their catalytic activities with respect to inhibition by a MAb. If the observed activities all derive from a single MAb-inhibitable type of cytochrome P-450, which may include one or more isozymes, the MAb should similarly inhibit all these activities. But if a cloned cell expresses multiple types of cytochrome P-450, each with a different substrate and product specificity, an MAb may interact with only some forms but not others and variable degrees of inhibition will be observed.

The AHH-1 cell line, recently derived as a subclone of RPMI-1788 lymphoblasts (23), possesses a basal AHH activity that is induced by polycyclic hydrocarbons. We have examined the inhibition of cytochrome P-450-dependent enzyme activity by MAb 1-7-1, made to 3-methylcholanthrene-induced rat liver cytochrome P-450. This MAb has been especially well characterized in numerous studies. It specifically inhibits purified MC-inducible, AHH-active cytochrome P-450 from rat liver (12) as well as microsomes from MC-treated rats (12, 16), while no inhibition is observed on microsomes from untreated or phenobarbital-treated rats (12, 16). These results demonstrate that in rat liver only MC pretreatment results in the presence of MAb-sensitive AHH- and ECD-active cytochromes P-450. It also demonstrates that, in the livers of untreated rats and mice, the AHH and ECD activities are a result of cytochromes P-450 that are insensitive to MAb 1-7-1 and are thus lacking the MAb-sensitive epitope. This MAb has also been shown to be specific for MC-inducible cytochrome P-450 in RIA studies (18, 19) of animal tissue microsomes. In addition, amino acid sequence analysis of rat hepatic cytochromes P-450 immunopurified with MAb 1-7-1 indicates that it specifically recognizes the P-450c and P-450d isozymes (21). Other studies with human tissues (15, 17) and extrahepatic animal tissues (16) have shown the presence of MAb 1-7-1-specific cytochromes P-450 with AHH and ECD activities in uninduced as well as MC-induced animals.

Enzyme inhibition assays of AHH and ECD have been performed to characterize the cytochromes P-450 responsible for these activities in AHH-1 cells with respect to sensitivity to MAb 1-7-1. In addition, we measured the cytochrome P-450 content of AHH-1 cells with an RIA that is based on MAb 1-7-1. We report that these two types of analyses, enzyme inhibition and RIA, yield consistent and complementary results for AHH-1 cells. The two methodologies are rapid, efficient, and require small amounts of tissues or cells. When coupled together, such MAb-based analyses provide a novel, powerful approach with which to examine human tissues and cells for cytochrome P-450 content, regulation, and synthesis.

MATERIALS AND METHODS

AHH-1 cells were subcloned from the parent RPMI-1788 lymphoblasts and selected for their inducibility for AHH by polycyclic hydrocarbons (23). The cells were cultured in RPMI medium 1640 supplemented with 5% horse serum (both from GIBCO), and were maintained in constant exponential growth by daily dilution to 4×10^5 cells/ml. They were induced by treatment with 2 μg/ml BA (Sigma) for 24 hr prior to harvest.

Standard methods were employed for measurement of AHH (24) and ECD (25) activities. For the activity assays, cells were centrifuged, washed with phosphate-buffered saline, and resuspended in 0.05 M Tris-HCl (pH 8.5) to 10^6 cells/ml. They were then disrupted with a glass/Teflon homogenizer. A volume of homogenate equivalent to 1–2 $\times 10^7$ cells was typically assayed in a total volume of 1.0 ml of the standard assay mixtures (24, 25). MAb 1-7-1 to rat liver 3-methylcholanthrene-induced cytochrome P-450 was prepared (12) and purified from ascites fluid (19, 20). For MAb-inhibition studies, 0.1 ml of the appropriate concentration of MAb in phosphate-buffered saline was preincubated with the cell homogenate for 15 min at 23° prior to initiation of the reactions. As a control, each assay was carried out with an anti-lysozyme MAb, obtained from Dr. S. Smith-Gill (National Cancer Institute).

The data with MAb 1-7-1 are reported relative to those of the antilysozyme MAb; the latter had no significant effect on enzyme activities. The RIA procedure was essentially as previously described (18), except that [^{3}H]MAb 1-7-1 was used. This was prepared by reductive methylation with NaB^3H_4 (New England Nuclear) (26) to a specific activity of 1.9×10^6 cpm/μg. The microtiter wells were coated with 1 μg of liver microsomes from MC-treated rats (specific P-450 content of 1.5 nmol/mg). [^{3}H]MAb 1-7-1 (3500 cpm) was then added along with several different amounts of AHH-1 homogenate. Data are presented as per cent inhibition of binding by [^{3}H]MAb 1-7-1 to the microtiter wells.

RESULTS

Treatment of AHH-1 cells with BA induced both AHH and ECD activities. The AHH activity increased 7-fold. Values of 0.046 ± 0.012 and 0.328 ± 0.047 pmol/min/10^6 cells were found for control and induced cells, respectively. The degree of induction of ECD activity was much less. Values of 17 ± 7 and 39 ± 3 fmol/min/10^6 cells were obtained for control and induced cells, respectively. The effect of MAb 1-7-1 on these activities is illustrated by the experiment in Fig. 1. The measured activity as well as the per cent residual activity in the presence of different amounts of MAb is presented in order to facilitate comparison between the various samples.

Fig. 1 clearly shows that both AHH and ECD are inhibited in both control and BA-induced cells. AHH inhibition (Fig. 1A) is virtually complete, indicating that this activity derives entirely from cytochromes P-450 that contain the epitope recognized by MAb 1-7-1. The observation that AHH is completely inhibited in induced as well as in control cells demonstrates that the AHH-active cytochromes P-450 induced by BA are of the same type (MAb 1-7-1-sensitive) as the AHH-active cytochromes P-450 present in basal cells.

Inhibition of ECD was observed for both control and induced cells (Fig. 1B), indicating that both contain ECD-active cytochrome P-450 that contains the epitope for, and is inhibited by, MAb 1-7-1. However, since inhibition of ECD activity is only partial, even at the highest level of added MAb (100 μg), both control and induced cells also contain an additional type of cyto-

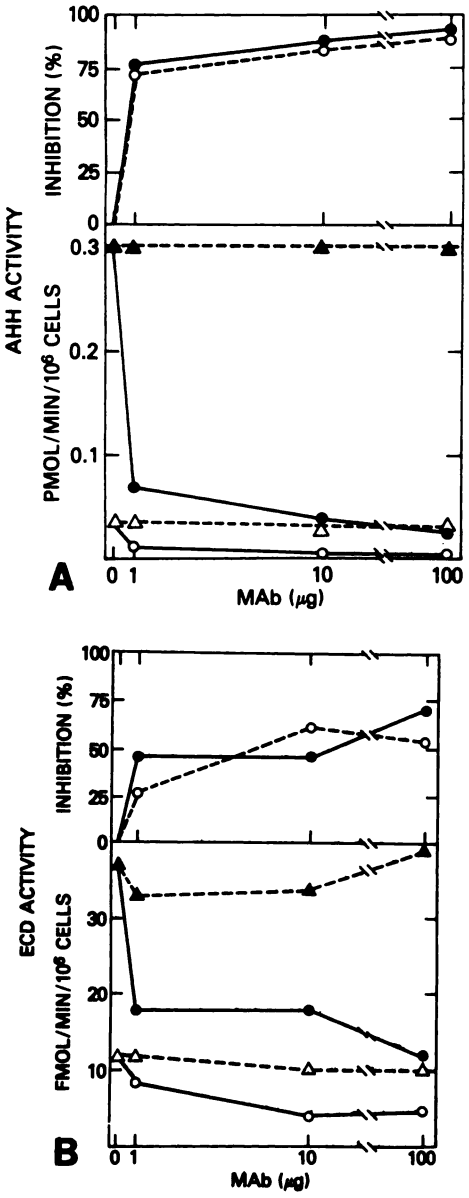

FIG. 1. *Inhibition of (A) AHH and (B) ECD activity by AHH-1 cells by MAb 1-7-1*

Bottom graphs: activity of basal cells in the presence of MAb 1-7-1 (○) and control MAb (△); activity of BA-induced cells in the presence of MAb 1-7-1 (●) and control MAb (▲). Top graphs are the per cent inhibition by MAb 1-7-1 of basal cells (○) and BA-induced cells (●).

chrome P-450 that is ECD-active but is not inhibited by MAb 1-7-1. The inhibition profiles for both control and induced cells are similar, suggesting that BA treatment elevates the levels of both types of ECD-active cytochromes P-450, MAb-sensitive and MAb-insensitive, to a similar extent.

The cytochromes P-450 in AHH-1 cells can be defined and distinguished on the basis of their interaction with MAb 1-7-1 as determined by the influence of this MAb on two cytochrome P-450-dependent catalytic activities. AHH activity derives entirely from MAb-specific cytochrome P-450, while ECD derives partially (70%) from MAb-specific cytochrome P-450 and partially (30%) from MAb-insensitive cytochrome P-450. Whether the AHH and inhibitable ECD activities derive from the same cytochrome P-450 is unknown, although the cytochromes P-450 responsible for both these activities are of the same type, i.e., those which contain an epitope specific for MAb 1-7-1. The results with AHH-1 cells are qualitatively similar to those previously obtained with human lymphocytes (15). With the latter, however, AHH was inhibited 18–78% among lymphocytes from different individuals, indicating individual variation in AHH expression. The more complete inhibition observed with AHH-1 cells may result from greater homogeneity of cytochrome P-450 in this cloned line of cells. AHH-1 cells may thus provide a model system for characterizing cytochrome P-450 content, regulation, and synthesis in human cells.

Complementary to the enzyme inhibition experiments, the cytochrome P-450 content of both basal and BA-induced cells was independently evaluated by competitive RIA with [^{3}H]MAb 1-7-1. The standard competition curve with various amounts of rat microsomes from MC-induced rats is plotted in Fig. 2. The level of MAb-specific cytochrome P-450 in homogenized AHH-1 cells is related to the degree of inhibition of [^{3}H]MAb 1-7-1 binding to the microtiter wells. The curve for induced cells is considerably shifted to the left of the curve for basal cells, indicating higher levels of MAb-specific cytochrome P-450. The RIA detects about a 3-fold elevation in the level of this cytochrome P-450 upon BA treatment. The degree of elevation is comparable, but not identical, to the observed degree of induction of catalytic activities. The relationship between cytochrome P-450 levels as measured enzymatically and by RIA is not direct, since RIA may detect MAb-specific cytochromes P-450 with neither AHH nor ECD activity. In addition, ECD activity partially derives from cyto-

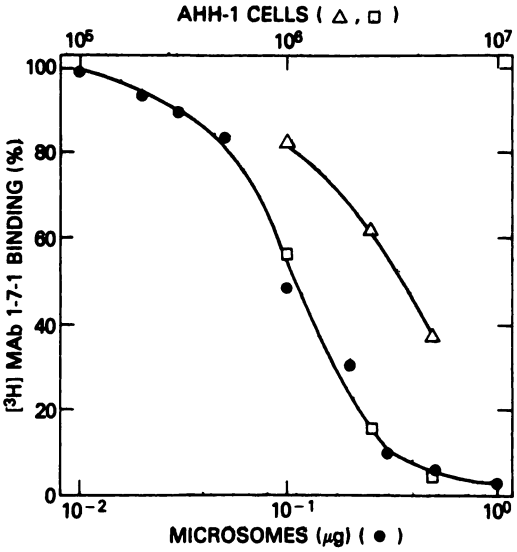

FIG. 2. *Competitive RIA of AHH-1 cells*

Microtiter wells were coated with liver microsomes from MC-induced rats and the percentage of maximal binding of [^{3}H]MAb 1-7-1 to the wells was measured in the presence of varying amounts of rat liver microsomes (●) (lower abscissa), and basal (△) or BA-induced (□) cells (upper abscissa).

chromes P-450 that are distinct from the MAb-specific form and hence not measurable by the RIA.

DISCUSSION

The multiplicity of cytochromes P-450 has been demonstrated in numerous species and tissues by several criteria, including spectral, electrophoretic, catalytic, and immunologic properties (6). Additional isozymes are continually being discovered and subjected to these analyses while cloned genes are being isolated and sequenced (27–29). An individual cell therefore has the potential to express a number of cytochromes P-450, but it is at present unclear whether individual cells in a population express only a single cytochrome P-450 or whether they express a range of isozymes. The cytochrome P-450 phenotype of a cell depends on both genetic and environmental factors such as sex, nutritional and developmental state of the organism, and exposure to drugs or xenobiotics. Responsiveness to such a wide variety of factors and stimuli requires complex regulatory mechanisms that are poorly characterized at present. Cloned cell lines in a controlled environment provide a system with relatively uniform properties for studying the cellular and molecular aspects of cytochrome P-450 regulation. The AHH-1 cell line may be a suitable model for such studies using MAbs to detect and distinguish various cytochrome P-450 isozymes under various experimental conditions. This report exemplifies such a study in that it demonstrates that (*a*) AHH-1 cells express only one type of MAb-defined class of cytochrome P-450 that is responsible for all of the AHH activity in both basal and induced cells and (*b*) AHH-1 cells express two MAb-defined classes of cytochrome P-450 (sensitive and insensitive), responsible for ECD and that both classes are present in both basal and BA-induced cells.

As shown in this report, RIA and enzyme inhibition studies using MAbs yield consistent and complementary information on cellular cytochrome P-450 content. The methodologies described with MAb 1-7-1 can be extended to include other MAbs, and thus to characterize the entire range of cellular cytochromes P-450 on the basis of their interaction with a panel of MAbs. In addition to examining MAb-based RIAs and the effect of MAbs on catalytic activities, MAb-based immunopurifications (19–21) may also be performed. Such a multidimensional approach provides a phenotype of cytochrome P-450 in human tissues and cells which may be useful in relating the expression of specific cytochrome P-450 isozymes to individual susceptibility to carcinogenesis and sensitivity to certain drugs. The analyses carried out with AHH-1 cells can thus be applied to phenotyping of lymphocytes, which are readily available human cells, since the methodologies presented in this report are easily adapted for carrying out such studies on suitable human populations.

REFERENCES

1. Conney, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19**:317–366 (1967).
2. Conney, A. H., and J. J. Burns. Metabolic interactions among environmental chemicals and drugs. *Science* **178**:576–586 (1972).
3. Gillette, J. R., D. C. Davis, and H. A. Sasame. Cytochrome P-450 and its role in drug metabolism. *Annu. Rev. Pharmacol.* **12**:57–84 (1972).
4. Gelboin, H. V. Benzo[a]pyrene metabolism activation, and carcinogenesis: role of mixed-function oxidases and related enzymes. *Physiol. Rev.* **60**:1107–1166 (1980).
5. Sato, R. and R. Kato (eds.). *Microsomes, Drug Oxidations, and Drug Toxicity*, Japan Scientific Societies Press, Tokyo (1982).
6. Lu, A. Y. H., and S. B. West. Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.* **31**:277–295 (1980).
7. Gelboin, H. V. Carcinogens, drugs, and cytochromes P-450: a retrospective. *N. Engl. J. Med.* **309**:105–107 (1983).
8. Park, S. S., A. V. Persson, M. J. Coon, and H. V. Gelboin. Monoclonal antibodies to rabbit liver cytochrome P-450$_{LM2}$. *FEBS Lett.* **116**:231–235 (1980).
9. Park, S. S., S. J. Cha, H. Miller, A. V. Persson, M. J. Coon, and H. V. Gelboin. Monoclonal antibodies to rabbit liver cytochrome P-450$_{LM2}$ and P-450$_{LM4}$. *Mol. Pharmacol.* **21**:248–258 (1982).
10. Boobis, A. R., M. B. Slade, C. Stern, K. M. Lewis, and D. S. Davies. Monoclonal antibodies to rabbit liver cytochromes P-448. *Life Sci.* **29**:1443–1448 (1981).
11. Lewis, K. H., A. R. Boobis, M. B. Slade, and D. S. Davies. Immunopurification of cytochrome P-448 from microsomal fractions of rabbit liver with retention of metabolic activity. *Biochem. Pharmacol.* **31**:1815–1817 (1982).
12. Park, S. S., T. Fujino, D. West, F. P. Guengerich, and H. V. Gelboin. Monoclonal antibodies that inhibit enzyme activity of 3-methylcholanthrene induced cytochrome P-450. *Cancer Res.* **42**:1798–1808 (1982).
13. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Characterization of nine monoclonal antibodies against rat hepatic cytochrome P-450c. *J. Biol. Chem.* **259**:3890–3899 (1984).
14. Park, S. S., T. Fujino, H. Miller, F. P. Guengerich, and H. V. Gelboin. Monoclonal antibodies to phenobarbital induced rat liver cytochrome P-450. *Biochem. Pharmacol.* **33**:2071–2081 (1984).
15. Fujino, T., S. S. Park, D. West, and H. V. Gelboin. Phenotyping of cytochromes P-450 in human tissues with monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* **79**:3682–3686 (1982).
16. Fujino, T., D. West, S. S. Park, and H. V. Gelboin. Monoclonal antibody-directed phenotyping of cytochrome P-450-dependent aryl hydrocarbon hydroxylase and 7-ethoxycoumarin deethylase in mammalian tissues. *J. Biol. Chem.* **259**:9044–9050 (1984).
17. Fujino, T., K. Gottlieb, D. K. Manchester, S. S. Park, D. West, H. L. Gurtoo, and H. V. Gelboin. Monoclonal antibody phenotyping of inter-individual differences in cytochrome P-450 dependent reactions of single and twin human placenta. *Cancer Res.* **44**:3916–3923 (1984).
18. Song, B. J., T. Fujino, S. S. Park, F. K. Friedman, and H. V. Gelboin. Monoclonal antibody-directed radioimmunoassay of specific cytochromes P-450. *J. Biol. Chem.* **259**:1394–1397 (1984).
19. Cheng, K.-C., F. K. Friedman, B. J. Song, S. S. Park, and H. V. Gelboin. Detection and purification of cytochromes P-450 in animal tissues with monoclonal antibodies. *J. Biol. Chem.* **259**:12279–12284 (1984).
20. Friedman, F. K., R. C. Robinson, S. S. Park, and H. V. Gelboin. Monoclonal antibody-directed immunopurification and identification of cytochromes P-450. *Biochem. Biophys. Res. Commun.* **116**:859–865 (1983).
21. Cheng, K.-C., H. C. Krutzsch, S. S. Park, P. H. Grantham, H. V. Gelboin, and F. K. Friedman. Amino-terminal sequence analysis of six cytochrome P-450 isozymes purified by monoclonal antibody directed immunopurification. *Biochem. Biophys. Res. Commun.* **123**:1201–1208 (1984).
22. Whitlock, J. P., H. V. Gelboin, and H. G. Coon. Variation in aryl hydrocarbon benzo[a]pyrene hydroxylase activity in heteroploid and predominantly diploid rat liver cells in culture. *J. Cell Biol.* **70**:217–225 (1976).
23. Crespi, C. L., J. D. Altman, and M. A. Marletta. Xenobiotic metabolism and mutation in a human lymphoblastoid line. *Chem. Biol. Interact.* (in press).
24. Nebert, D. W., and H. V. Gelboin. Substrate-inducible microsomal aryl hydrocarbon hydroxylase in mammalian cell culture. 1. Assay and properties of induced enzyme. *J. Biol. Chem.* **243**:6242–6249 (1968).
25. Greenlee, W. F., and A. Poland. 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**:596–605 (1978).
26. Tack, B. F., J. Dean, D. Eilat, P. E. Lorenz, and A. N. Schechter. Tritium labeling of proteins to high specific radioactivity by reductive methylation. *J. Biol. Chem.* **255**:8842–8847 (1980).
27. Nebert, D. W., and M. Negishi. Multiple forms of cytochrome P-450 and the importance of molecular biology and evolution. *Biochem. Pharmacol.* **31**:2311–2317 (1982).
28. Fujii-Kuriyama, Y., Y. Mizukami, K. Kawajiri, K. Sogawa, and M. Muramatsu. Primary structure of a cytochrome P-450: coding nucleotide sequence of phenobarbital-inducible cytochrome P-450 cDNA from rat liver. *Proc. Natl. Acad. Sci. USA* **79**:2793–2797 (1982).
29. Kawajiri, K., O. Gotoh, K. Sogawa, Y. Tagashira, M. Muramatsu, and Y. Fujii-Kuriyama. Coding nucleotide sequence of 3-methylcholanthrene-inducible cytochrome P-450 cDNA from rat liver. *Proc. Natl. Acad. Sci. USA* **81**:1649–1653 (1984).

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