*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 848–852, February 1994
Cell Biology

# Liver fatty acid-binding protein: Specific mediator of the mitogenesis induced by two classes of carcinogenic peroxisome proliferators

(fatty acids/liver carcinogenesis/transfected hepatoma cells)

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*Communicated by Sidney Weinhouse, October 12, 1993 (received for review August 5, 1993)*

ABSTRACT **Peroxisome proliferators (PP) are a diverse group of chemicals that induce dramatic increases in peroxisomes in rodent hepatocytes, followed by hypertrophy, hepatomegaly, alterations in lipid metabolism, mitogenesis, and finally hepatocarcinomas. Termed nongenotoxic carcinogens, they do not interact with DNA, are not mutagenic in bacterial assays, and fail to elicit many of the phenotypes associated with classic genotoxic carcinogens. We report here that the mitogenesis induced by the major PP class, the amphipathic carboxylates, and by the tetrazole-substituted acetophenones specifically requires liver fatty acid-binding protein (L-FABP) in cultured rat hepatoma cells transfected with the sense cDNA of L-FABP, in contrast to L-FABP-nonexpressing cells transfected with its antisense cDNA. The mitogenic actions of L-FABP were protein-specific, inasmuch as no other protein in the nonexpressing cells could act like L-FABP. L-FABP was previously shown not only (*i*) to interact covalently with metabolites of the two genotoxic carcinogens 2-acetylaminofluorene and aminoazo dyes during liver carcinogenesis, but also (*ii*) to bind noncovalently the two classes of PP *in vitro* with avidities that correlate with their abilities to elicit peroxisomal enzymatic responses, and (*iii*) together with unsaturated fatty acids, especially linoleic acid, to promote multiplication of the transfected hepatoma cells in culture. The convergence of the two types of genotoxic carcinogens with the two classes of PP nongenotoxic carcinogens, and also with unsaturated fatty acids, at L-FABP actions in inducing mitogenesis allows the following hypothesis. During tumor promotion of carcinogenesis *in vivo*, these groups of genotoxic and nongenotoxic carcinogens act on the normal process by which L-FABP, functioning as a specific receptor of unsaturated fatty acids or their metabolites, promotes hepatocyte proliferation.**

Induced cell proliferation (mitogenesis) is increasingly thought to play a key role in carcinogenesis in animals and humans (1–3). The actions of the peroxisome proliferators (PP) have been revealing. This diverse group of chemicals induces dramatic increases in peroxisomes in rodent hepatocytes, followed by hypertrophy, hepatomegaly, alterations in lipid metabolism, mitogenesis, and finally hepatocarcinomas. Termed nongenotoxic carcinogens, they do not interact with DNA, are not mutagenic in bacterial assays, and fail to elicit many phenotypes associated with classic genotoxic carcinogens. Considerable interest surrounds the unknown intracellular mediators and the mechanisms of the induced mitogenesis and carcinogenesis (1–7).

Liver fatty acid-binding protein (L-FABP), a cytoplasmic 14-kDa protein previously termed Z protein, is conventionally considered an intracellular carrier of fatty acids in hepatocytes (8–12). Multiple associations have also connected the protein to mitogenesis of rat hepatocytes. Thus, L-FABP expression is markedly elevated in the cytoplasm of hepatocytes during mitosis in normal and regenerating rat livers (13–15) and throughout the cell cycle in hyperplastic hepatocytes and hepatocarcinomas produced in rats by ingestion of the genotoxic liver carcinogens 2-acetylaminofluorene (*N*-2-fluorenylacetamide) and aminoazo dyes (13, 16). In addition, L-FABP binds many ligands of potential importance in the modulation of cell multiplication, including four types of liver carcinogens—i.e., genotoxic metabolites of both 2-acetylaminofluorene and aminoazo dyes during hepatocarcinogenesis (17, 18) and two classes of nongenotoxic PP carcinogens *in vitro* (19–22)—the essential fatty acids linoleic acid and arachidonic acid (8–12), prostaglandin $E_1$ (23), growth modulatory hydroxy and hydroperoxy metabolites of arachidonic acid (24), growth inhibitory prostaglandins (25), mitogenic lysophosphatidic acids (26), lysophosphatidylcholines (27), antiproliferative and anticancer selenium (28), retinyl palmitate (29), and heme (30).

PP differ in their abilities to elicit peroxisomal responses (e.g., elevated peroxisomal β-oxidation of fatty acids), carcinogenicities in rodents, and avidities for L-FABP *in vitro*. Among the synthetic PP, the largest group consists of the amphipathic carboxylates, such as Wy-14,643, bezafibrate, ciprofibrate, nafenopin, fenofibrate, and clofibric acid (see formulas in Fig. 1). Fenofibrate, an ester, is thought to be hydrolyzed to the amphipathic carboxylic acid in cells. These compounds are strong to moderate PP and hepatocarcinogens in rats and mice (4–7). Brandes *et al.* (19) initially reported that bezafibrate binds to L-FABP. Eacho and coworkers (21, 22) then found a correlation between the different avidities of both amphipathic carboxylates and four closely similar tetrazole-substituted acetophenones in binding to L-FABP *in vitro* and their diverse abilities to induce a peroxisomal enzymatic response. Among the amphipathic carboxylates, the strong carcinogen Wy-14,643 was the most potent PP and most avid binder to L-FABP, followed in decreasing rank order by bezafibrate, ciprofibrate, and clofibric acid. Among the acetophenones, the rank order of both L-FABP binding and peroxisomal response was LY189585 ≥ LY171883 ≫ LY213768 ≥ LY163443 (see formulas in Fig. 2). LY171883, which is a leukotriene $D_4$ antagonist, increases the incidence of hepatocellular carcinomas in female B6C3 $F_1$ mice (31).

Whether the interactions of the many ligands with L-FABP actually modulate the mitogenesis of hepatocytes has been unknown. Therefore, we had derived stable cell lines (32) containing the sense and antisense cDNAs of rat L-FABP in

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Abbreviations: PP, peroxisome proliferator(s); L-FABP, liver fatty acid-binding protein.

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a subline of rat hepatoma HTC cells that do not endogenously express L-FABP RNA or its protein. The cells transfected with the L-FABP sense cDNA express the recombinant L-FABP RNA and protein, whereas the cells transfected with the antisense cDNA do not. This technique provides a zero-background homologous cell model of L-FABP expression suitable for controlled studies of the intracellular roles of L-FABP. With this system, unsaturated fatty acids, especially linoleic acid, were found to cooperate with L-FABP in promoting DNA synthesis and cell multiplication (32–34). This cell model has now been applied to a study of the role of L-FABP in PP-induced mitogenesis. We report here that the mitogenesis induced by the major PP class, the amphipathic carboxylates, and by the tetrazole-substituted acetophenones specifically requires L-FABP in the rat hepatoma cells transfected with the sense cDNA of L-FABP. The convergence of actions of certain genotoxic carcinogens, these nongenotoxic carcinogens, and fatty acids at L-FABP in induction of mitogenesis implies that, in these cases, both types of carcinogens act on the normal process by which L-FABP promotes the multiplication of hepatocytes.

## MATERIALS AND METHODS

**Cells.** Rat hepatoma HTC-$R_3T_2$ cells that do not contain L-FABP mRNA or protein had been transfected with sense and antisense L-FABP cDNA (32); expression of the transfected L-FABP cDNA was under the promoter control of the long terminal repeat of Rous sarcoma virus. The cloned transfected cell lines were routinely passaged in RPMI 1640 medium/10% calf serum (both from GIBCO)/sodium bicarbonate (2 g/liter)/penicillin (50 units/ml)/streptomycin sulfate (50 μg/ml)/kanamycin sulfate (100 μg/ml) at 37°C in a humidified atmosphere of 5% $CO_2$. HTC-Z-S7 cells express L-FABP at a level previously found to be virtually unchanged during 25 passages. HTC-Z-A1 cells, which were transfected with antisense L-FABP cDNA, do not express L-FABP (32). All cells were mycoplasma-free in tests that used fluorescent staining.

**Measurements of Cell Multiplication.** Growing S7 and A1 cells ($4.0 \times 10^4$), previously maintained in RPMI 1640 medium/10% calf serum, were seeded at day 0 in 7.0 ml of that medium/1% calf serum with or without PP in 25-$cm^2$ flasks (Corning) as triplicate or quadruplicate cultures. That medium supports continuous cell multiplication (32–34). At days 1 and 7, the latter at near confluency, cells were virtually all attached to the substrate, treated with trypsin, harvested, and counted in a Coulter Counter. Analyses of the cell viability of the L-FABP-expressing and -nonexpressing cells without or with treatment with $10^{-7}$ M Wy-14,643 or $10^{-8}$ M fenofibrate for 1 and 7 days indicated that >97% of the trypsinized cells excluded trypan blue.

**PP.** Wy-14,643 was a product of Chemsyn Science Laboratories, Lenexa, KS. Clofibric acid, di(2-ethylhexyl)phthalate, and di(2-ethylhexyl)adipate were from Sigma. Nafenopin was donated by CIBA–Geigy, and fenofibrate was given by Michael Arand (Institute of Toxicology, University of Mainz, Mainz, Germany). LY189585, LY171883, LY213768, and LY163443 were donated by Eli Lilly, and mono(2-ethylhexyl)phthalate was donated by Per Garberg (National Institute of Occupational Health, Solna, Sweden). The monoester was purified further by HPLC, and the structure was verified by means of IR and NMR.

## RESULTS

**Mitogenesis Induced by PP on the Background of Hepatoma Cell Multiplication.** As immortal hepatoma cell lines, the L-FABP-expressing S7 cells and L-FABP-nonexpressing A1 cells grew continually in number both with and without PP. However, only the L-FABP-expressing cells were induced by amphipathic carboxylate and tetrazole-substituted acetophenone PP to proliferate additionally. Throughout the study, changes in cell numbers from day 1 to day 7 were compared in the presence vs. the absence of PP in L-FABP-expressing and L-FABP-nonexpressing cell cultures. In all cases, the PP increased the number of L-FABP-expressing cells, whereas the number of L-FABP-nonexpressing cells was virtually unaffected or relatively reduced. Fig. 3 shows the increases in cell numbers over day 1–day 7 from the combination of background multiplication of cell lines and PP-induced mitogenesis. In contrast, Figs. 1, 2, and 4 deal only with the latter—i.e., the mitogenesis induced by the PP and its dependence on L-FABP. The methods of computations of both types of cell multiplication are indicated in the figure legends.

**Mitogenesis Induced by Amphipathic Carboxylates.** Induction of mitogenesis by the amphipathic carboxylic PP required L-FABP expression. Wy-14,643, the most potent inducer of peroxisomal β-oxidation (4–7, 21), the most avid binder to L-FABP *in vitro* (21), and a strong liver carcinogen (4–7), was the best inducer of mitogenesis of all examined PP. Further, the cell proliferation was most attributable to L-FABP presence. Fig. 1*A* shows that Wy-14,643 at $10^{-7}$ M brought about the greatest elevation in number of transfected L-FABP-expressing cells; matching treatment of the L-FABP-nonexpressing cells elicited virtually no change or was inhibitory. Daily measurements for 7 days revealed that after a 3-day lag, Wy-14,643 steadily increased only the number of L-FABP-expressing cells (Fig. 4).

Likewise, in decreasing rank order, nafenopin, clofibric acid, and fenofibrate stimulated mitogenesis of the L-FABP-expressing cells, whereas the L-FABP-nonexpressing cells were inhibited. Nafenopin at $10^{-8}$ M elevated the number of L-FABP-expressing cells by 36% above that of the cultures without PP, which was 43% greater than with the L-FABP-nonexpressing cells (Fig. 1*B*). The corresponding increases were 26% and 38% by clofibric acid (Fig. 1*C*) and 23% and 35% by fenofibrate (Fig. 1*D*). The last position of fenofibrate in the order is consistent with the assumed need of this ester to be hydrolyzed to the amphipathic carboxylic acid.

Similar but lower responses to Wy-14,643 were shown by other clones of transfected L-FABP-expressing and -nonexpressing hepatoma cell lines that have been reported (32–34). At $10^{-8}$ M Wy-14,643, the number of L-FABP-expressing S9 cells increased by a maximum of 22%, whereas nonexpressing A2 cells were inhibited by 12%—i.e., a difference of 34%. Also, in $10^{-7}$ M Wy-14,643, L-FABP-expressing S12 cells grew in number by 12%, and nonexpressing A4 cells decreased by 8%—i.e., a 20% difference. The lower mitogenic stimulations of S9 and S12 cells compared with S7 cells suggest deficiencies in the induction and multistep process of mitogenesis in the S9 and S12 cells.

**Mitogenesis Induced by Tetrazole-Substituted Acetophenones.** Because of the close similarity of the chemical structures of the four tetrazole-substituted acetophenones, shown in Fig. 2, and their diverse abilities to bind to L-FABP *in vitro* and to induce peroxisomal enzymatic responses, examination of the abilities of the four compounds to stimulate cell multiplication constituted a particularly stringent test of the correlation between presence of L-FABP and induction of mitogenesis by PP. Fig. 2 (see arrows) shows that the only structural difference between the moderately active LY171883 and the essentially inactive LY213768 is the presence of the acidic hydrogen on the tetrazole ring in the active species and the replacement of that hydrogen by a methyl group in the inactive molecule. Likewise, the only difference between the moderately active LY189585 and the virtually inactive LY163443 is the meta vs. the para position of their methylene-tetrazole ring substituent, resulting in an ability of

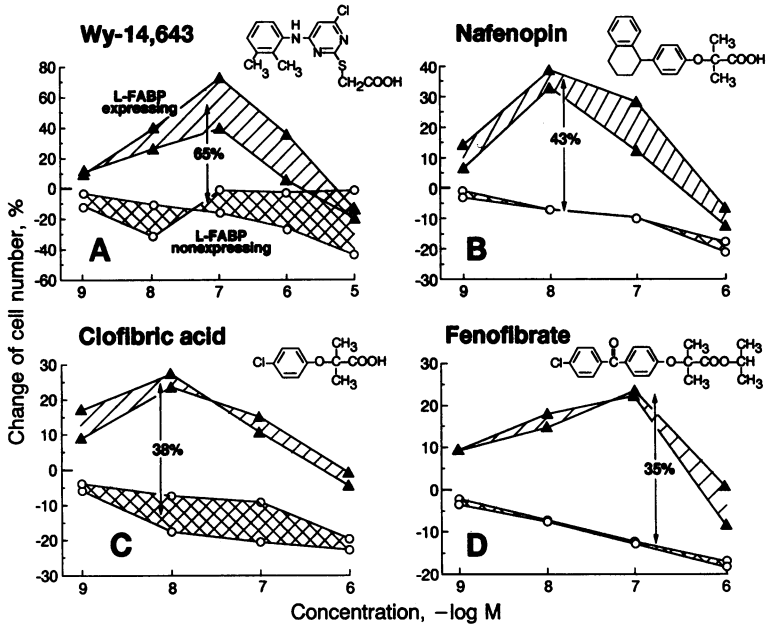


FIG. 1. Mitogenesis of L-FABP-expressing hepatoma cells induced by four amphipathic carboxylic PP. Changes in cell numbers from day 1 to day 7 by different PP concentrations, relative to PP absence, in two series of experiments are shown. ▲, L-FABP-expressing S7 cells; ○, L-FABP-nonexpressing A1 cells. Percentage changes of cell numbers (ordinates) were computed as follows: [(number$^{day\ 7}$ − number$^{day\ 1}$)/number$^{day\ 1}$] with (A) and without (B) PP, and then [(A − B)/B] × 100.

the active meta molecule to assume a bent configuration when bound in L-FABP (21, 22). Despite these small differences, the four compounds differ quantitatively in their peroxisomal enzymatic responses and their affinities for L-FABP *in vitro*. Cannon and Eacho (21) reported that the rank order of both properties was LY189585 ≥ LY171883 ≫ LY213768 ≥ LY163443. It is noteworthy that the requirements for the acidic hydrogen and ability to assume the bent molecular configuration are both properties that appear to be needed by fatty acids in binding to L-FABP (22).

In the present study spanning $10^{-6}$ M–$10^{-9}$ M concentrations in two experimental sets, the four acetophenones at $10^{-7}$ M exhibited a range of moderate to insignificant stimulations of mitogenesis of the L-FABP-expressing cells and, in contrast, an inhibition or no effect on cell number in the nonexpressing cells. The mitogenic responses were, respectively, 24% vs. −9% with LY189585 (Fig. 2*A*), 16% vs. −19% with LY171883 (Fig. 2*B*), 16% vs. −9% with LY213768 (Fig. 2*C*), and 15% vs. 2% with LY163443 (Fig. 2*D*). The differential responses to the last two compounds by the two cell types appear of little or no significance. The rank order of the inductions of mitogenesis by the acetophenones agreed essentially with those of their reported different affinities for L-FABP *in vitro* and their diverse abilities to induce a peroxisomal enzymatic response. Thus, the correlation between induction of mitogenesis and the presence of L-FABP appears to apply also to the tetrazole-substituted acetophenones. However, the differences in the mitogenic responses were smaller than with the amphipathic carboxylates, presumably reflecting the closer resemblance of the latter to long-chain fatty acids, which are also amphipathic carboxylates.

**Other PP.** PP of other classes were also similarly examined at $10^{-6}$ M–$10^{-9}$ M concentrations in two experimental sets (data not shown). At $10^{-8}$ M and $10^{-7}$ M, these PP exhibited either little or no stimulatory activity or were less inhibitory to the L-FABP-expressing cells than to the nonexpressing cells. The PP were the diethanol, tiadenol (14% vs. −8%, respectively), and the three weakly active esters, mono(2-ethylhexyl)phthalate (−15% vs. −23%), di(2-ethylhexyl)-

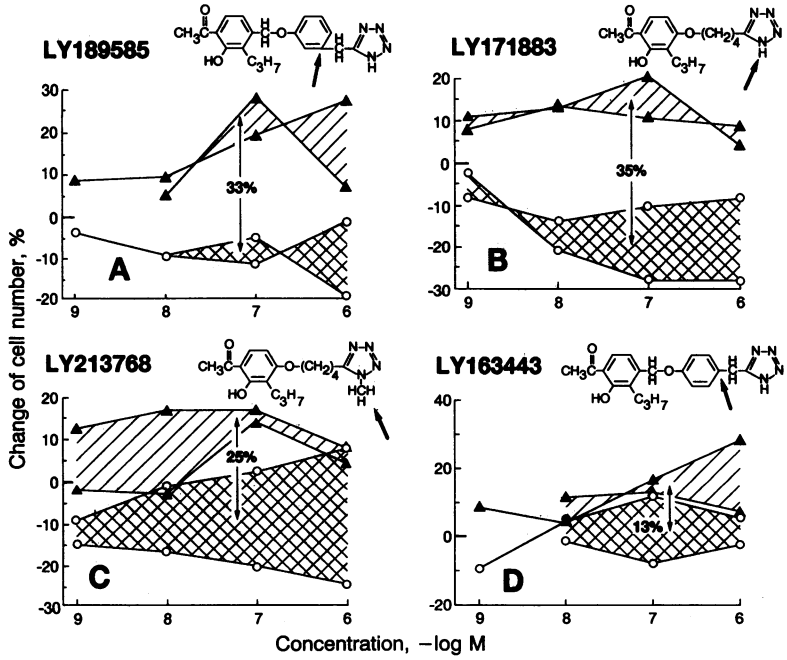


FIG. 2. Mitogenesis of L-FABP-expressing hepatoma cells induced by tetrazole-substituted acetophenones. The cells, protocol, computations, and symbols are as in Fig. 1 legend. Arrows point to the small structural differences between the moderately active LY171883 vs. the essentially inactive LY213768 and between the moderately active LY189585 vs. the virtually inactive LY163443.

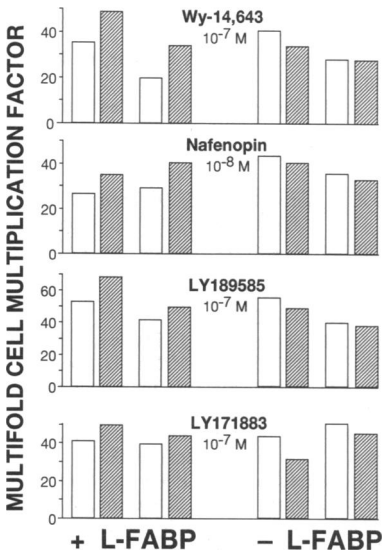


FIG. 3. Mitogenesis induced by PP in L-FABP-expressing cells and the absence thereof in L-FABP-nonexpressing cells. Multifold increases in cell numbers are shown—i.e., total of background multiplications as hepatoma cell lines and PP-induced mitogenesis. In duplicate experiments L-FABP-expressing S7 cells (+ L-FABP) and L-FABP-nonexpressing A1 cells (– L-FABP) were exposed for 7 days to the presence (hatched bars) vs. the absence (open bars) of four PP at their most active concentrations (Figs. 1 and 2). All cells increased in number; however, the PP induced cell proliferations only in the L-FABP-expressing cells. Cell multiplication factors (ordinates) were computed from cell numbers at day 1–day 7 as for A and B in Fig. 1 legend.

phthalate (−8% vs. −21%), and di(2-ethylhexyl)adipate (13% vs. −11%). The relative nonresponsiveness of the transfected hepatoma cells to the diethanol and these ester PP may reflect an inability of the cultured cells to metabolize these compounds to their active proximal forms (35), or for other reasons an insensitivity of the cells to these PP, or a noninvolvement of L-FABP in the actions of these PP.

## DISCUSSION

Mitogenesis induced by the major class of PP—i.e., the amphipathic carboxylates—and by the tetrazole-substituted acetophenones requires L-FABP in rat hepatoma cells transfected with the sense cDNA of L-FABP. L-FABP not only directly binds these PP *in vitro* with avidities that correlate with their abilities to elicit peroxisomal enzymatic responses (21, 22), but it also interacts with metabolites of two genotoxic carcinogens during liver carcinogenesis (17, 18) and

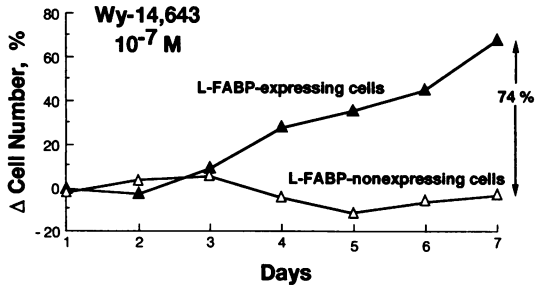


FIG. 4. Daily changes in relative numbers of transfected L-FABP-expressing S7 hepatoma cells and L-FABP-nonexpressing A1 hepatoma cells with Wy-14,643 at $10^{-7}$ M. Growing cells were treated and assayed as in Fig. 1 legend, except that $4 \times 10^4$ cells were seeded in 2.5 ml of medium in triplicate in 35-mm culture dishes. Data from days 7 and 1 are included in Fig. 1*A*.

together with unsaturated fatty acids, especially linoleic acid, promotes growth of the transfected hepatoma cells in culture (32–34). The convergence of these genotoxic and nongenotoxic carcinogens and fatty acids at L-FABP actions in inductions of mitogenesis implies that both types of carcinogen act on the normal process by which L-FABP, together with unsaturated fatty acids or their metabolites, promotes the multiplication of hepatocytes.

The mitogenesis induced by the two PP classes and by unsaturated fatty acids (32–34) occurred above a background of continuous cell multiplication characteristic of immortal cell lines. It is reasonable to assume that PP and the unsaturated fatty acids, acting in conjunction with L-FABP, exert greater relative effects on the rates of cell multiplication in normal and nontransformed rat hepatocytes.

The synergy between the actions of L-FABP and amphipathic carboxylate PP and acetophenones in induction of mitogenesis was protein-specific, in that no other protein in the L-FABP-nonexpressing cells could duplicate L-FABP actions. Previously, a 70-kDa subunit of a rat liver protein dimer, termed PP-binding protein, which is homologous to heat-shock protein hsp72, was isolated from rat liver by nafenopin-, clofibric acid-, and ciprofibrate-affinity chromatographies (36–38). Unlike L-FABP, which is mainly present in liver (8–12) and which binds the potent Wy-14,643 with highest relative avidity (21), the PP-binding protein is expressed ubiquitously at high levels, does not bind Wy-14,643 (36–38), and has not been shown to bind directly any PP. The properties of L-FABP appear thus to be uniquely consistent with those of a specific intracellular receptor mediator of the inductions of mitogenesis by unsaturated fatty acids and by the amphipathic carboxylic PP and tetrazole-substituted acetophenones.

A hypothesis to explain the multiple gene activations involved in the pleiotropic biochemical responses to PP has gained substantial support from the discovery by Green and coworkers (39–41) of the mouse PP-activated receptor, which is a member of the superfamily of steroid hormone nuclear receptors. The receptor is a ligand-activated transcription factor that can bind to a specific DNA sequence, the PP-response element, situated upstream of the rat acyl-CoA oxidase gene. Structure–activity relationships of different synthetic activators of the nuclear receptor correlate with their abilities to induce peroxisomal biochemical responses (e.g., fatty acid $\beta$-oxidation) (39–41). Furthermore, not only do synthetic PP activate the receptor but so also do a wide variety of fatty acids, especially polyunsaturated fatty acids (42). In addition, the PP-activated receptor and the retinoid X receptor $\alpha$ of the ligand 9-*cis*-retinoic acid (an all-*trans*-retinoic acid metabolite) form a heterodimer that binds specifically to the PP-response element, indicating convergence of signaling pathways in the regulation of peroxisomal fatty acid $\beta$-oxidation by fatty acids, retinoids, and PP (43–45). However, no direct binding of PP or fatty acid to the nuclear receptor has been demonstrated thus far. Nor has there been indication that the PP receptor is involved in the promotion of mitogenesis.

Evidence has also been presented in support of the hypothesis by Sharma *et al.* (46) and Lock *et al.* (6) that the induction of cytochrome P450IVA1 by PP increases the $\omega$-hydroxylation of fatty acids, leading to fatty dicarboxylic acids that, in turn, cause peroxisome proliferation (6, 46, 47). Whether the dicarboxylic acids interact with a protein receptor in hepatocytes is unknown. Our findings that promotion of cell multiplication by Wy-14,643 involves a 3-day lag (Fig. 4) and that L-FABP is required for the induction of mitogenesis together raise the question whether L-FABP operates as a specific cytoplasmic carrier of both fatty acids and PP before their conversion to active metabolites or

before activation of the nuclear PP-activated receptor or another cellular target in the induction of mitogenesis.

The discovery that L-FABP, the target protein of the two genotoxic carcinogens 2-acetylaminofluorene and aminoazo dyes, is required for mitogenesis induced by two classes of nongenotoxic PP clearly indicates a commonality in the processes by which both types of carcinogens promote hepatocyte multiplication. (*i*) According to one hypothesis, PP may act as tumor initiators through oxidative DNA damage from increased levels of active oxygen from peroxisomal β-oxidation of fatty acids (4, 48). (*ii*) Alternatively, PP may operate as tumor promoters of spontaneous preneoplastic hepatic lesions (49–51). In both mechanisms, differential mitogenesis of transformed hepatocytes has been proposed to be at the crux of the oncogenesis by genotoxic and nongenotoxic carcinogens (49–51). Our findings that L-FABP is required for the mitogenesis induced by two classes of carcinogenic PP, the previously reported associations between L-FABP and multiplication of normal and carcinogen-exposed hepatocytes *in vivo*, and the demonstration that L-FABP acting with unsaturated fatty acids promotes proliferation of transfected hepatoma cells *in vitro* (32–34) all collectively imply that L-FABP mediates the mitogenesis of hepatocytes induced by unsaturated fatty acids and by these nongenotoxic PP and genotoxic carcinogens. Thus, these PP act as fraudulent fatty acids in binding to L-FABP. On the basis of the correlation between peroxisomal enzymatic response and the *in vitro* binding of PP to L-FABP, Eacho and coworkers (21, 22) proposed that L-FABP and a perturbation of fatty acid metabolism may jointly be involved in the pleiotropic biochemical responses to PP. More recently, Issemann *et al.* (52) hypothesized that PP may operate by binding to L-FABP, thereby displacing fatty acids that, in turn, activate the nuclear PP receptor. Induction of cell multiplication and oncogenesis were not addressed in either proposal. The present observed lack of mitogenesis by the PP in the L-FABP-nonexpressing cells appears to argue against a passive role of L-FABP as primarily a reservoir of fatty acids in the induction of mitogenesis by the PP. Rather, the evidence here and previously assembled implies that during hepatic tumor promotion certain genotoxic and nongenotoxic carcinogens may act on the normal process by which L-FABP, functioning as a specific receptor of unsaturated fatty acids or their metabolites, promotes the multiplication of hepatocytes.

We thank Dr. Jerome J. Freed and Sharon Howard for generous cooperation, Dr. Michael Arand for fenofibrate, CIBA-Geigy for nafenopin, Dr. Patrick I. Eacho and Eli Lilly and Co. for the LY compounds, Dr. Per Garberg for mono(2-ethylhexyl)phthalate, and Cynthia B. Myers for additional purification and analyses of the last compound. This work was supported, in part, by National Institutes of Health Grant CA-05945, institutional grants CA-06927 and RR-05539 from the National Institutes of Health, and an appropriation from the Commonwealth of Pennsylvania.

1. Ames, B. N. & Gold, L. S. (1990) *Science* **249**, 970–971.
2. Cohen, S. M. & Ellwein, L. B. (1990) *Science* **249**, 1007–1011.
3. Butterworth, B. E. & Slaga, T., eds. (1991) in *Chemically Induced Cell Proliferation: Implications for Risk Assessment* (Wiley/Liss, New York).
4. Reddy, J. K. & Lalwani, N. D. (1983) *CRC Crit. Rev. Toxicol.* **12**, 1–87.
5. Hawkins, J. M., Jones, W. E., Bonner, F. W. & Gibson, G. G. (1987) *Drug Metab. Rev.* **18**, 441–515.
6. Lock, E. A., Mitchell, A. M. & Elcombe, C. R. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29**, 145–163.
7. Gibson, G. & Lake, B., eds. (1993) in *Peroxisomes: Biology and Importance in Toxicology and Medicine* (Taylor & Francis, London).
8. Sweetser, D. A., Heuckeroth, R. O. & Gordon, J. I. (1987) *Annu. Rev. Nutr.* **7**, 337–359.
9. Bass, N. M. (1988) *Int. Rev. Cytol.* **111**, 143–184.
10. Matarese, V., Stone, R. L., Waggoner, D. W. & Bernlohr, D. A. (1989) *Prog. Lipid Res.* **28**, 245–272.
11. Kaikaus, R. M., Bass, N. M. & Ockner, R. K. (1990) *Experientia* **46**, 617–630.
12. Veerkamp, J. H., Peeters, R. A. & Maatman, R. G. H. J. (1991) *Biochim. Biophys. Acta* **1081**, 1–24.
13. Custer, R. P. & Sorof, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6738–6742.
14. Custer, R. P. & Sorof, S. (1985) *Differentiation* **30**, 176–181.
15. Sweetser, D. A., Birkenmeyer, E. H., Hoppe, P. C., McKeel, D. W. & Gordon, J. I. (1988) *Genes Dev.* **2**, 1318–1332.
16. Sorof, S. & Custer, R. P. (1987) *Cancer Res.* **47**, 210–220.
17. Ketterer, B., Tipping, E., Hackney, J. F. & Beale, D. (1976) *Biochem. J.* **155**, 511–521.
18. Blackburn, G. R., Schnabel, S. J., Danley, J. M., Hogue-Angeletti, R. A. & Sorof, S. (1982) *Cancer Res.* **42**, 4664–4672.
19. Brandes, R., Kaikaus, R. M., Lysenko, N., Ockner, R. K. & Bass, N. M. (1990) *Biochim. Biophys. Acta* **1034**, 53–61.
20. Kanda, T., Ono, T., Matsubara, Y. & Muto, T. (1990) *Biochem. Biophys. Res. Commun.* **168**, 1053–1058.
21. Cannon, J. R. & Eacho, P. I. (1991) *Biochem. J.* **280**, 387–391.
22. Eacho, P. I., Foxworthy, P. S. & Herron, D. K. (1993) in *Peroxisomes: Biology and Importance in Toxicology and Medicine*, eds. Gibson, G. & Lake, B. (Taylor & Francis, London), pp. 343–372.
23. Dutta-Roy, A. K., Gopalswamy, N. & Trulzsch, D. V. (1987) *Eur. J. Biochem.* **162**, 615–619.
24. Raza, H., Pongubala, J. R. & Sorof, S. (1989) *Biochem. Biophys. Res. Commun.* **161**, 448–455.
25. Khan, S. H. & Sorof, S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9401–9405.
26. Vancura, A. & Haldar, D. (1992) *J. Biol. Chem.* **267**, 14353–14359.
27. Burrier, R. E. & Brecher, P. (1986) *Biochim. Biophys. Acta* **879**, 229–239.
28. Bonsal, M. P., Cook, R. G., Danielson, K. G. & Medina, D. (1989) *J. Biol. Chem.* **264**, 13780–13784.
29. Fukai, F., Kase, T., Shidotani, T., Nagai, T. & Katayama, T. (1987) *Biochem. Biophys. Res. Commun.* **147**, 899–903.
30. Liem, H. H., Grasso, J. A., Vincent, S. H. & Muller-Eberhard, U. (1990) *Biochem. Biophys. Res. Commun.* **167**, 528–534.
31. Bendele, A. M., Hoover, D. M., Van Lier, R. B. L., Foxworthy, P. S. & Eacho, P. I. (1990) *Fundam. Appl. Pharmacol.* **15**, 676–682.
32. Keler, T., Barker, C. S. & Sorof, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4830–4834.
33. Keler, T., Barker, C. & Sorof, S. (1993) in *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury*, eds. Nigam, S., Honn, K. V., Marnett, L. J. & Walden, T. L., Jr. (Kluwer, Dordrecht, The Netherlands), pp. 435–438.
34. Keler, T. & Sorof, S. (1993) *J. Cell. Physiol.* **157**, 33–40.
35. Mitchell, A. M., Lhuguenot, J.-C., Bridges, J. W. & Elcombe, C. R. (1985) *Toxicol. Appl. Pharmacol.* **80**, 23–32.
36. Lalwani, N. D., Fahl, W. E. & Reddy, J. K. (1983) *Biochem. Biophys. Res. Commun.* **116**, 388–393.
37. Lalwani, N. D., Alvares, K., Reddy, M. K., Reddy, M. N., Parikh, I. & Reddy, J. K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5242–5246.
38. Alvares, K., Carrillo, A., Yuan, P. M., Kawano, H., Morimoto, R. I. & Reddy, J. K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5293–5297.
39. Issemann, I. & Green, S. (1990) *Nature (London)* **347**, 645–650.
40. Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L. & Green, S. (1992) *EMBO J.* **11**, 433–439.
41. Green, S. (1992) *Cancer Surv.* **14**, 221–232.
42. Göttlicher, M., Widmark, E., Li, Q. & Gustafson, J. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4653–4657.
43. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A. & Evans, R. (1992) *Nature (London)* **358**, 771–774.
44. Bardot, O., Alridge, T. C., Latruffe, N. & Green, S. (1993) *Biochem. Biophys. Res. Commun.* **192**, 37–45.
45. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K. & Wahli, W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2160–2164.
46. Sharma, R., Lake, B. G., Foster, J. & Gibson, G. G. (1988) *Biochem. Pharmacol.* **37**, 1193–1201.
47. Ortiz de Montellano, P. R., Chan, W. K., Tuck, S. F., Kaikaus, R. M., Bass, N. M. & Peterson, J. A. (1992) *FASEB J.* **6**, 695–699.
48. Rao, M. S. & Reddy, J. K. (1987) *Carcinogenesis* **8**, 631–636.
49. Schulte-Hermann, R., Timmermann-Trosiener, I. & Schuppler, J. (1983) *Cancer Res.* **43**, 839–844.
50. Marsman, D. S., Cattley, R. C., Conway, J. G. & Popp, J. A. (1988) *Cancer Res.* **48**, 6739–6744.
51. Cattley, R. C. & Popp, J. A. (1989) *Cancer Res.* **49**, 3246–3251.
52. Issemann, I., Prince, R., Tugwood, J. & Green, S. (1992) *Biochem. Soc. Trans.* **20**, 824–827.