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Cross-Talk between Peroxisome Proliferator-Activated Receptor (PPAR) α and Liver X Receptor (LXR) in Nutritional Regulation of Fatty Acid Metabolism. I. PPARs Suppress Sterol Regulatory Element Binding Protein-1c Promoter through Inhibition of LXR Signaling

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Liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) are members of nuclear receptors that form obligate heterodimers with retinoid X receptors (RXRs). These nuclear receptors play crucial roles in the regulation of fatty acid metabolism: LXRs activate expression of sterol regulatory element-binding protein 1c (SREBP-1c), a dominant lipogenic gene regulator, whereas PPARα promotes fatty acid β-oxidation genes. In the current study, effects of PPARs on the LXR-SREBP-1c pathway were investigated. Luciferase assays in human embryonic kidney 293 cells showed that overexpression of PPARα and γ dose-dependently inhibited SREBP-1c promoter activity induced by LXR. Deletion and mutation studies demonstrated that the two LXR response elements (LXREs) in the SREBP-1c promoter region are responsible for this inhibitory effect of PPARs. Gel shift assays indicated that PPARs reduce binding of LXR/RXR to LXRE. PPARα-selective agonist enhanced these inhibitory effects. Supplementation with RXR attenuated these inhibitions by PPARs in luciferase and gel shift assays, implicating receptor interaction among LXR, PPAR, and RXR as a plausible mechanism. Competition of PPARα ligand with LXR ligand was observed in LXR/RXR binding to LXRE in gel shift assay, in LXR/RXR formation in nuclear extracts by coimmunoprecipitation, and in gene expression of SREBP-1c by Northern blot analysis of rat primary hepatocytes and mouse liver RNA. These data suggest that PPARα activation can suppress LXR-SREBP-1c pathway through reduction of LXR/RXR formation, proposing a novel transcription factor cross-talk between LXR and PPARα in hepatic lipid homeostasis. (*Molecular Endocrinology* 17: 1240–1254, 2003)

STEROL REGULATORY ELEMENT (SRE)-binding proteins (SREBPs) are membrane-bound transcription factors that belong to the basic helix-loop-helix leucine zipper family (1–3). Through sterol-regulated cleavage, SREBP enters the nucleus and activates the transcription of genes involved in cholesterol and fatty acid synthesis by binding to a SRE or its related sequences including SRE-like sequences and E-boxes, within their promoter regions (4, 5). There are three forms of SREBP: SREBP-1a and -1c (also known as adipocyte determination and differentiation 1, ADD1) and -2 (6–8). Most organs, including the liver and adipose tissue, express predominantly SREBP-2 and the -1c isoform of SREBP-1 (9). Recent *in vivo* studies demonstrated that SREBP-1c plays a crucial role in the dietary regulation of most hepatic lipogenic genes, whereas SREBP-2 is actively in-

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACO, acyl-CoA oxidase; 36B4, acidic ribosomal phosphoprotein PO; 9CRA, 9-*cis*-retinoic acid; CBP, cAMP response element binding protein-binding protein; CMV, cytomegalovirus; CoA, coenzyme A; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; FXR, farnesoid X receptor; β-gal, β-galactosidase; HEK, human embryonic kidney; Luc, luciferase; LXR, liver X receptor; LXRE, liver X receptor response element; m, mouse; Pio, pioglitazone; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator responsive element; pSV, simian virus 40 promoter plasmid; 22RHC, 22(R)-hydroxycholesterol; RXR, retinoid X receptor; SRE, sterol regulatory element; SREBP-1c, sterol regulatory element-binding protein 1c; TK, thymidine kinase; Wy, Wy14,643.

volved in the transcription of cholesterogenic enzymes (10–17). SREBP-1c seems to control hepatic lipogenic enzymes through changing its mRNA level in a process that appears to be highly related to glucose/insulin signaling (18).

Liver X receptors (LXRs) belong to a subclass of nuclear hormone receptors that form obligate heterodimers with retinoid X receptors (RXR) and are activated by oxysterols (19–22). There have been two subtypes of LXRs identified: LXRα and LXRβ. LXRα is expressed in liver, spleen, kidney, adipose, and small intestine (23), whereas LXRβ is ubiquitously expressed (24). LXRs have been established to regulate intracellular cholesterol levels by transactivating the expression of cholesterol 7α-hydroxylase (21, 22, 25), cholesterol ester transfer protein (26), and ATP-binding cassette transporter A1 (ABCA1), which modulates cholesterol efflux and mediates reverse cholesterol transport from peripheral tissues. LXR/RXR also appears to be involved in cholesterol absorption in intestine (27). Furthermore, LXR/RXR was recently identified as a dominant activator of SREBP-1c promoter (28, 29), implicating a new link between cholesterol and fatty acid metabolism.

Peroxisome proliferator-activated receptors (PPARs) belong to a ligand-activated nuclear hormone receptor superfamily and are known to regulate the expression of numerous genes involved in fatty acid metabolism and adipocyte differentiation (30, 31). PPARα is primarily expressed in the liver, in which it has been shown to promote β-oxidation of fatty acids (30). PPARγ is mainly expressed in adipose tissue (32), where it has been shown to be an essential component of the adipocyte differentiation program (33); and in macrophages, where it modulates differentiation and cytokine production (34–36). PPARs were originally identified as factors that mediate transcriptional responses to peroxisome proliferators, a broad class of xenobiotic chemicals that include fibrate hypolipidemic drugs and other nongenotoxic rodent hepatocarcinogens (37, 38). Subsequently, PPARs were shown to be differentially activated by a variety of saturated or unsaturated long chain fatty acids and lipid-like compounds (39–43), suggesting that fatty acids or fatty acid derivatives serve as physiological activators.

The roles of these nutritional transcription factors in whole body physiology and metabolism can be best illustrated by comparing two opposite nutritional states: fasted and refed states. In the fasted liver, fatty acids are oxidized to acetyl-coenzyme A (CoA) and subsequently to ketone bodies. PPARα, plays a major role in both processes, which was confirmed by observations in PPARα-null mice (44, 45). In contrast, expression of SREBP-1c is reduced during fasting. In the refed state, lipogenesis is induced through increased amount of SREBP-1, whereas PPARα is decreased. This coordinated reciprocal regulation of the two transcription factors is key to nutritional regulation of fatty acids and triglycerides as energy storage system and implicates the presence of a cross-talk between these factors. The involvement of LXRs and PPARs in multiple and diverse cellular functions on the nutritional regulation of fatty acid metabolism suggests that these receptors may be integrated with other cellular signaling pathways, in addition to the well-characterized RXR pathway. Indeed, the reciprocal modulation of thyroid hormone and peroxisome proliferator-responsive genes through cross-talk between thyroid hormone receptors (TRs) and PPARs has been demonstrated (46, 47). Moreover, it is reported that LXRα interacts with PPARα and inhibits peroxisome proliferator signaling (48).

In the current study, we analyzed effects of PPARs on the LXR-SREBP-1c system. The results demonstrate that activation of PPARα represses LXR signaling through reduction of LXR/RXR heterodimerization in the liver. Taken together with the accompanying paper (49) describing LXR suppression of the PPARα signaling, we propose a novel aspect of nutritional regulation with these mutual interactions forming a network of transcription factors regulating fatty acid metabolism.

RESULTS

PPARs Suppress SREBP-1c Promoter Activity through LXR Response Elements (LXREs)

The SREBP-1c promoter contains two LXREs and is activated by overexpression of LXRα, β, and/or addition of an LXR agonist, 22(R)-hydroxycholesterol (22RHC) (Fig. 1) as previously reported (29). As shown in luciferase (Luc) reporter gene assays (Fig. 1), this LXR activation of SREBP-1c promoter (2.6 kb) is efficiently suppressed by cotransfection of PPARα or γ. Even without LXR activation, overexpression of PPAR substantially decreased the basal activity of the SREBP-1c promoter. PPAR inhibition was observed in both HepG2 cells, a liver cell line, and in human embryonic kidney (HEK) 293 cells. HEK293 cells were used for the experiments thereafter. Expression level of transfected PPARα or LXR gene in HEK293 cells were roughly comparable to that in mouse liver as estimated by Northern blotting, and thus was within a physiological range (data not shown). To locate *cis*-element(s) responsible for this inhibitory effect in the SREBP-1c promoter, sequential deletion constructs of SREBP-1c promoter-Luc were estimated in light of PPAR repression of LXR activation (Fig. 2). The inhibitory effect of PPAR on both basal and LXRα-induced activity was partially impaired by deletion of upstream LXRE (LXREa) of the two LXR binding sites and was completely abolished in the absence of both LXREs (LXREa and b). These results suggest that PPAR overexpression cancels out LXR activation of the SREBP-1c promoter through the two LXREs.

To further investigate the effects of PPARs on these LXREs in the SREBP-1c promoter, an LXRE enhancer

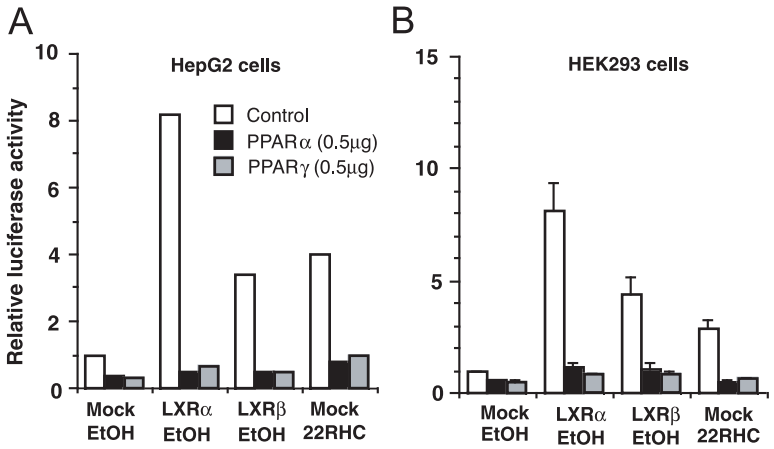

Fig. 1. PPARs Suppress SREBP-1c Promoter Activity in HepG2 and HEK293 Cells

A Luc reporter gene containing the mouse SREBP-1c promoter (2.6 kb); pBP1c2600-Luc was cotransfected in HepG2 (A) and HEK293 (B) cells with pCMV-LXR (0.1 μg), CMV-PPARα or γ (0.5 μg) or an empty vector CMV-7 as a control, and pSV-β-gal as a reference plasmid. 22RHC (10 μM), or ethanol (EtOH) as a control was added to the cells after transfection in medium with 10% fetal bovine serum 24 h before the assay. After incubation, Luc activity was measured and normalized to β-gal activity. The relative fold change in Luc activity as compared with a mock-transfected control is shown (means ± SD, n = 3).

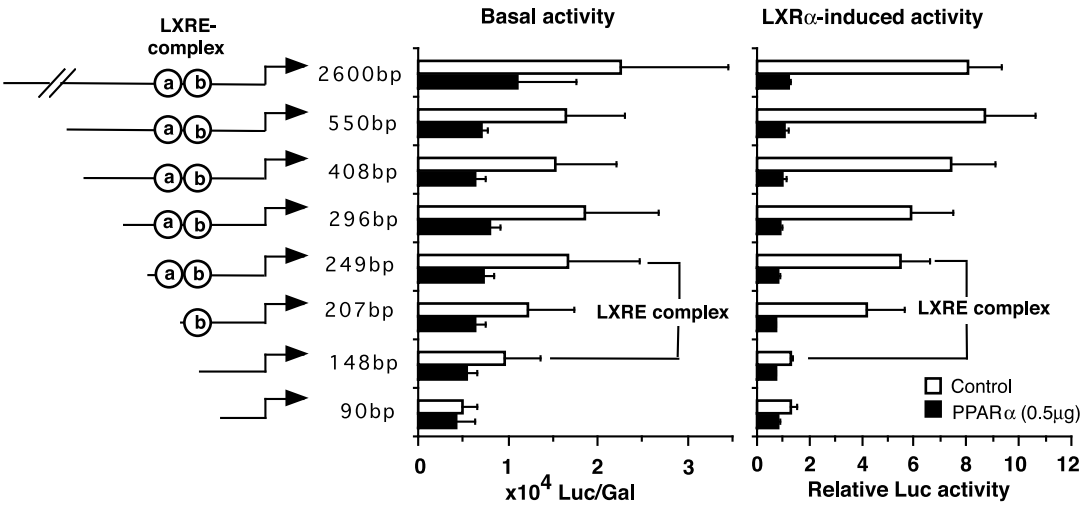

Fig. 2. Identification of a PPAR-Suppressive Region in the SREBP-1c Promoter by Deletion Analysis

SREBP-1c promoter Luc reporters of various lengths were constructed (*left panel*). The HEK293 cells were transfected with each reporter plasmid, pCMV-LXRα, pCMV-PPARα, and reference plasmid, pSV-β-gal. After incubation, Luc activity was measured and normalized to β-gal activity. The effect of PPARα in each reporter construct without LXRα coexpression (basal activity) is expressed as normalized Luc activity (means ± SD, n = 3) (*middle panel*). The data from LXRα coexpression (0.1 μg pCMV-LXRα, LXRα-induced activity) are shown as fold-change relative to mock transfected control (means ± SD, n = 3) (*right panel*).

construct (LXRE-Luc) was used. This construct was shown to be activated by LXRα, or β, or an LXR ligand in a very similar fashion to the native SREBP-1c promoter Luc. Reflecting the observation from the 2.6-kb SREBP-1c promoter, LXRE-Luc showed a very similar repression pattern by either PPARα or γ coexpression (Fig. 3A). When either LXREa or b was disrupted by positionall mutation in LXRE-Luc, the inhibitory effect of PPAR was partially impaired. Mutation of both LXREs abolished the PPAR inhibition of LXR activation. Figure 3B shows that the LXR (0.1 μg DNA)-induced LXRE-Luc activity is inhibited by PPARα and γ overexpression in a dose-dependent manner. Fifty percent inhibition was observed at 0.025 μg DNA of PPAR. We have previously shown that the SREBP-1c promoter contains an SRE, an activation site for SREBPs, mediating an auto-loop activation of the SREBP-1c-lipogenic gene expression system (50). The 90-bp SREBP-1c promoter construct containing the SRE, but not the upstream LXREs was activated by coexpression of nuclear SREBP-1a or -1c, but not by LXR. The activity of this reporter construct was not

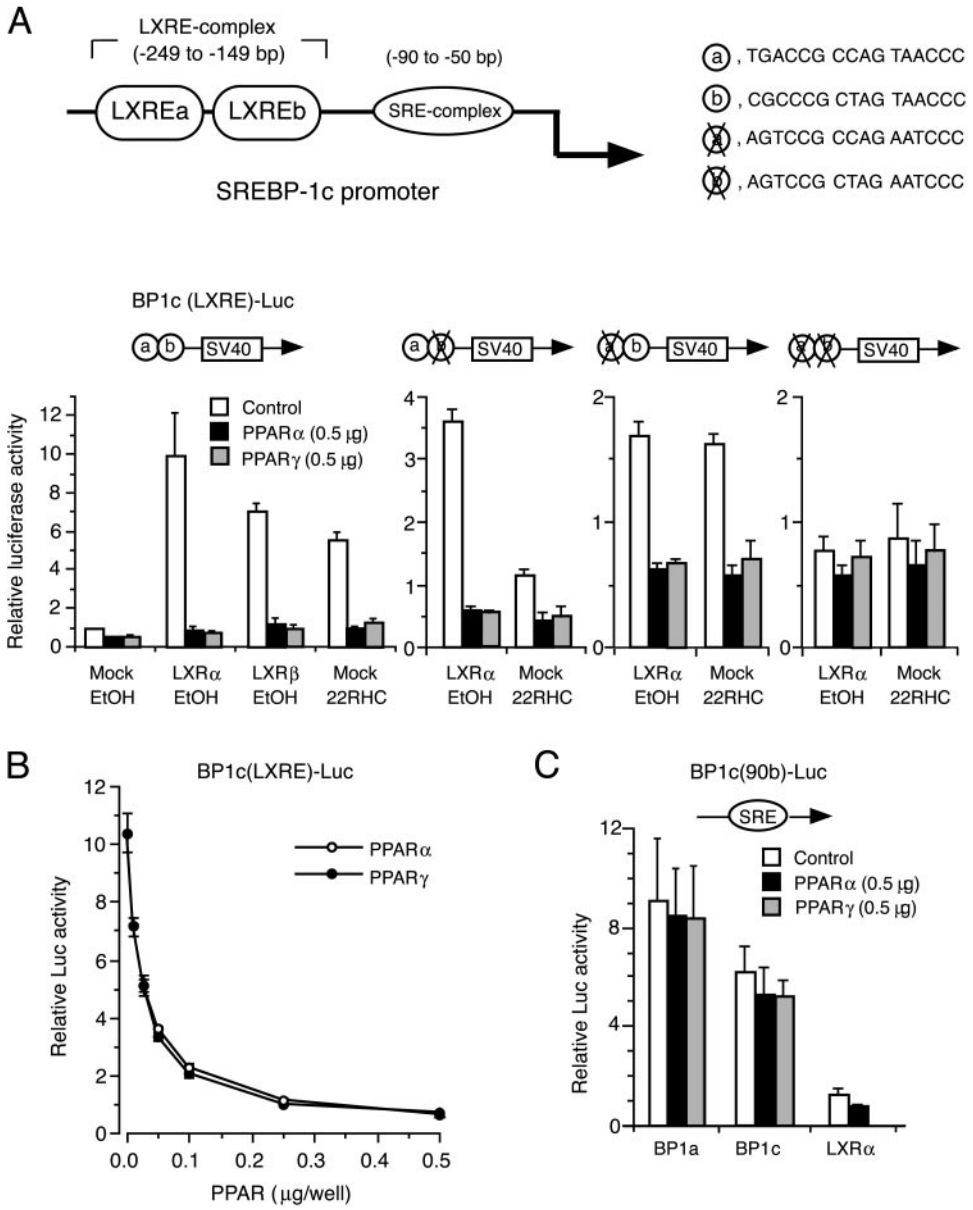

Fig. 3. Inhibitory Effect of PPARs on SREBP-1c Promoter Activity Is Mediated by the LXRE Complex in the SREBP-1c Promoter

A, The LXRE complex containing two LXREs (LXREa and b) was located at −249 to −148 bp in the SREBP-1c promoter as described previously (29). The LXRE complex in the SREBP-1c promoter was fused to a luciferase reporter plasmid, which contained a simian virus 40 promoter (pGL2 promoter vector). This enhancer construct [pBP1c(LXRE)-Luc] or the indicated mutant construct was cotransfected into HEK293 cells with pCMV-LXRα, β (0.1 μg, each), pCMV-PPARα, γ (0.5 μg, each), or an empty vector, CMV-7 as a control and pSV-β-gal as a reference plasmid. B, Dose-dependent suppression of the LXRE complex enhancer in the SREBP-1c promoter by PPARs. pBP1c(LXRE)-Luc and pSV-β-gal were cotransfected into HEK293 cells with pCMV-LXRα, pCMV-PPARα, pCMV-PPARγ or an empty vector, CMV-7 as a control. After the 24-h incubation, Luc activity was measured and normalized to β-gal activity. The fold change by PPARs in the Luc activity (means ± SD, n = 3) as compared with the LXRα (0.1 μg)-induced control is shown. C, pBP1c(90b)-Luc, which contained SRE-complex but not LXRE-complex, was cotransfected into HEK293 cells with pCMV-SREBP-1a, pCMV-SREBP-1c, pCMV-LXRα, or an empty vector, CMV-7 in the presence or absence of pCMV-PPARα, or pCMV-PPARγ as a control and pSV-β-gal as a reference plasmid. 22RHC (10 μM), or ethanol (EtOH) as a control was added to the cells after transfection in medium with 10% fetal bovine serum 24 h before the assay. After incubation, Luc activity was measured and normalized by β-gal activity. The fold change by LXRs or their ligands in the Luc activity (means ± SD, n = 3) as compared with the respective control is shown.

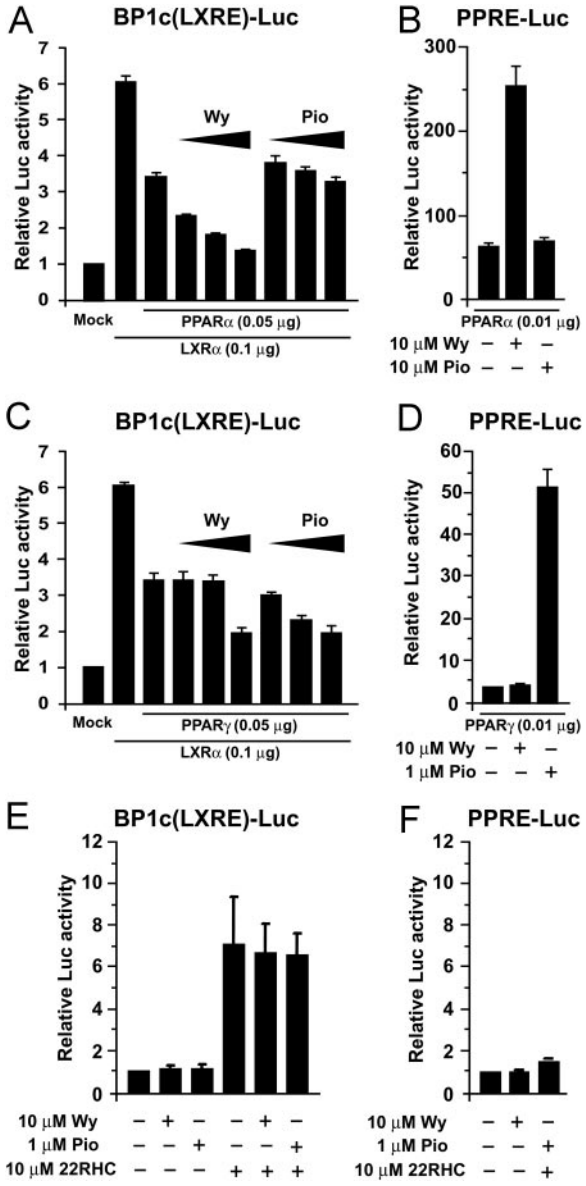

Fig. 4. Suppression of the SREBP-1c Promoter (LXRE Complex Luc) Activity by PPAR Ligand-Activation of PPARs

Activation of BP1c(LXRE)-Luc by LXR was competed with ligand activation of PPARα (A) or PPARγ (C). Ligand-specific activation of PPARα (B) or PPARγ (D) was confirmed by PPRE-Luc. Effects of ligands in the absence of receptor cotransfection on BP1c(LXRE)-Luc (E) and PPRE-Luc (F) were also estimated. A, pBP1c(LXRE)-Luc (0.25 μg) was cotransfected into HEK293 cells with pCMV-LXRα (0.1 μg), pCMV-PPARα (0.05 μg), and pSV-β-gal (0.25 μg) as a reference plasmid. A PPARα pharmacological ligand; Wy (1, 10, 100 μM), a PPARγ pharmacological ligand; Pio (1, 10, 100 μM), or DMSO was added to the cells after transfection. B, pPPRE-Luc (0.25 μg) was cotransfected into HEK293 cells with pCMV-PPARα (0.01 μg), and pSV-β-gal (0.25 μg). Wy (10 μM), Pio (10 μM), or DMSO was added to the cells without 22RHC after transfection. C, pBP1c(LXRE)-Luc (0.25 μg) was cotransfected into HEK293 cells with pCMV-LXRα (0.1 μg), pCMV-PPARγ (0.05 μg), and pSV-β-gal (0.25 μg). Wy (1, 10, 100 μM), Pio (1, 10, 100 μM), or DMSO was added to the cells without 22RHC after transfection. D, pPPRE-Luc (0.25 μg) was cotransfected into HEK293 cells with pCMV-PPARγ (0.01 μg), and pSV-β-gal (0.25 μg). Wy (10 μM), Pio (1 μM), or DMSO was added to the cells without 22RHC after transfection. E, pBP1c(LXRE)-Luc (0.25 μg) and pSV-β-gal (0.25 μg) were cotransfected into HEK293 cells without pCMV-LXRα. Wy (10 μM), Pio (1 μM), or DMSO (and/or ethanol) was added to the cells with or without 22RHC (10 μM) after transfection. F, pPPRE-Luc (0.25 μg) and pSV-β-gal (0.25 μg) were cotransfected into HEK293 cells without pCMV-LXRα and pCMV-PPARα or γ. Wy (10 μM), Pio (1 μM), or DMSO (and/or ethanol) was added to the cells with or without 22RHC (10 μM) after transfection. After incubation for 24 h, Luc activity (means $\pm$ SD, n = 3) was measured and normalized to β-gal activity.

changed by PPARα or γ coexpression (Fig. 3C), indicating that PPAR does not affect SREBP activation of the SREBP-1c promoter through SRE.

PPAR Agonists Enhance PPARs Repression of SREBP-1c Promoter Activity

Activities of PPARs are usually thought to depend upon the presence of PPAR agonists. We estimated the effects of pharmacological PPAR agonists on PPAR inhibition of LXRE-Luc. We used Wy14,643 (Wy), a specific agonist for PPARα, and pioglitazone (Pio) for PPARγ. Their specific actions on PPRE-Luc were shown in Fig. 4, B and D. The suppression of LXRE-Luc activity was enhanced by addition of each PPAR specific agonist in a dose-dependent manner (Fig. 4, A and C). Without overexpression of PPARs, PPARα and γ agonists had no effect on LXRE-Luc activity induced by LXRα activation (Fig. 4E). As was expected, PPARα and γ agonists did not influence PPRE-Luc activity without overexpression of PPARα and γ, respectively (Fig. 4F). These results are presumably due to lack of endogenous PPAR expression in HEK293 cells (data not shown).

To examine this suppressive action with endogenous nuclear receptors, we performed reporter assay using rat primary hepatocytes. An LXR ligand, T0901317 highly induced LXRE luciferase activity in these cells. Wy dose dependently suppressed this induced activity (Fig. 5A). Thus, PPARα interference of the LXRα-SREBP-1c pathway could be observed under physiological expression levels of these receptors. Meanwhile, Wy alone slightly increased basal LXRE Luc activity, presumably due to a slight induction of endogenous LXRα expression, to lesser extent, but consistent with previous reports in macrophages (51–53). We also examined the reciprocal effects of the PPAR ligand with a dual reporter system using SREBP-1c(LXRE)-renilla and PPRE-firefly luciferases in the same setting of HEK 293 cells. After transfection of both PPARα and LXRα, addition of Wy increased PPRE-firefly Luc activity and simultaneously decreased BP1c(LXRE)-renilla Luc activity (Fig. 5, B and C). Conversely, addition of T0901713 increased SREBP1c(LXRE) renilla and decreased PPRE-firefly.

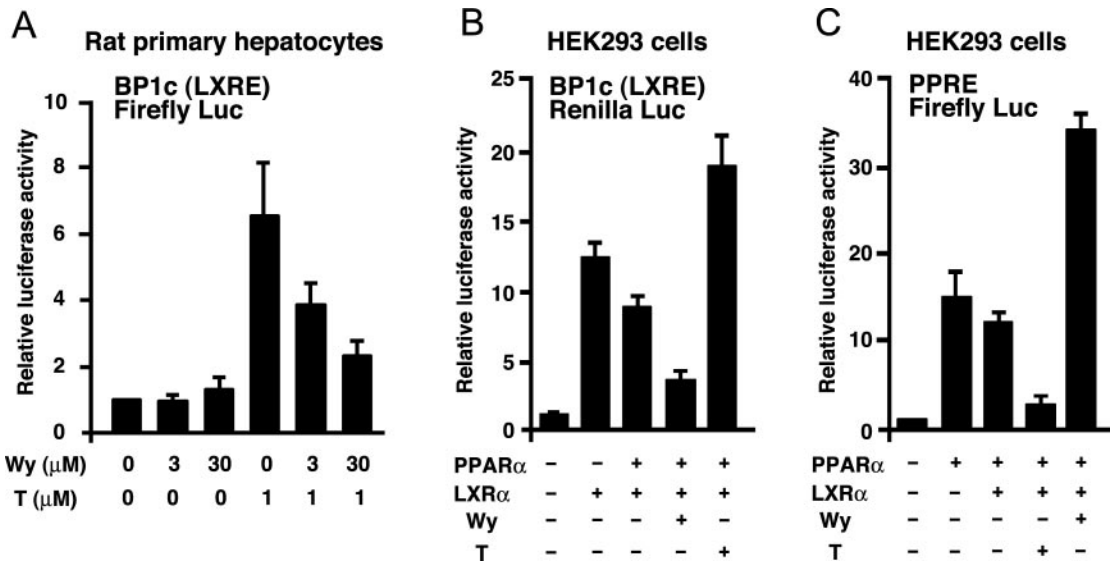

Fig. 5. Effect of PPARα Ligand on the SREBP-1c Promoter (LXRE Complex Luc) Activity in Rat Primary Hepatocytes (A) and on Dual Reporter Assays for LXRE (B) and PPRE (C) in HEK293 Cells

A, Reporter plasmid, pBP1c(LXRE)-firefly Luc, and reference plasmid, pSV-renilla Luc, were transfected into rat primary hepatocytes. T0901317 and/or Wy were added to the cells after transfection of pBP1c(LXRE)-Luc and pSV-renilla Luc 24 h prior to the assay. After incubation, firefly Luc activity was measured and normalized to renilla Luc activity. B and C, Dual reporters assay was performed with pBP1c(LXRE)-renilla Luc, pACO-PPRE firefly Luc, and pSV-β-gal that were cotransfected into HEK293 cells with pCMV-LXRα (0.25 μg) and/or pCMV-PPARα (0.25 μg). The cells were cultured with T0901317 or Wy for 24 h after transfection. After incubation, firefly and renilla Luc activities were measured and normalized to β-gal activity. The fold change by their ligands in the Luc activity (means $\pm$ SD, n = 3) as compared with the control is shown.

These results from dual reporter system confirmed consistency of the data from individual reporter system, providing further evidence for the possibility of cross-talk between PPAR and LXR signaling in the cultured cells [see the accompanying paper (49)].

Because PPARs and LXR share RXR as an obligate heterodimer partner, it is conceivable that PPAR repression of LXR activation on the LXRE-containing promoter is mediated through reduction of LXR/RXR heterodimers when RXR levels are limiting. To explore this possibility, the effect of RXR overexpression and/or addition to an RXR ligand [9-*cis*-retinoic acid (9CRA)] was estimated (Fig. 6). Under basal conditions of LXRE-Luc without LXR overexpression, coexpression of PPARα or γ caused approximately a 50% reduction. This PPAR inhibition was essentially abolished by coexpression of the same amount of RXR. Although LXRα (0.1 μg) coexpression induced LXRE-Luc activity by 6-fold, the percent inhibition by PPARs was more prominent (65%) than that without LXR overexpression. RXR overexpression also increased LXRE-Luc activity, and dose dependently suppressed PPAR inhibition, although the percent restoration was slightly less than in the absence of LXR overexpression. When an RXR ligand, 9CRA was added, LXRE-Luc was activated robustly because of LXR/RXR activation. Even in this condition, PPARα and γ overexpression efficiently suppressed its activity. Overexpression of RXR substantially, although not completely, restored the activity. These data suggested that PPAR inhibition of LXR activated SREBP1c-LXRE-luc is at least partly due to RXR competition.

RXR competition as possible mechanism for PPAR inhibition of LXR signaling prompted us to study the effects of TR and farnesoid X receptor (FXR), other RXR heterodimer partners. Whereas each nuclear receptor was active for luciferase reporter containing its own target *cis*-element (Fig. 7A), PPARα was the most effective at inhibiting LXR induced-SREBP1c promoter activity (Fig. 7B), highlighting the importance of the cross-talk between PPAR α and LXR. TRβ has some inhibitory effect. Inhibition by FXR was barely detectable. Although recruitment of coactivators is important for activation of receptors (54) including PPARs, overexpression of neither cAMP response element binding protein-binding protein (CBP) nor p300 (data not shown) restored PPARα suppression of LXRα-induced LXRE-Luc activity (Fig. 7C).

PPARs Inhibit LXRα/RXR Binding to LXRE of SREBP-1c Promoter

The PPAR inhibition of LXR activation observed in reporter assays was also evaluated in gel mobility shift assays (Fig. 8). The LXRE DNA probe from the SREBP-1c promoter was shifted by coincubation with recombinant LXR and RXR. This LXR/RXR binding to LXRE was specific and required RXR because none

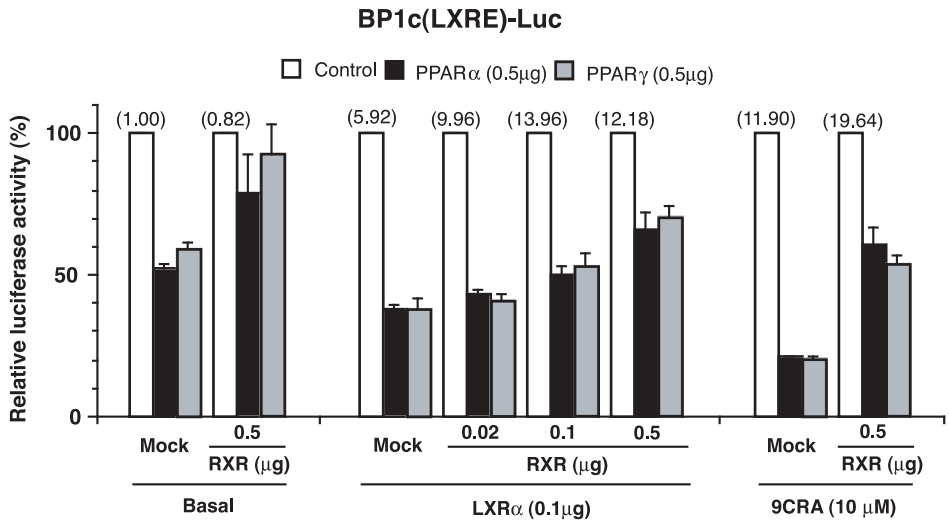

Fig. 6. Inhibitory Effects of PPARs on the SREBP-1c Promoter (LXRE Complex Luc) Activity Are Restored by Overexpression of RXR

pBP1c(LXRE)-Luc was cotransfected into HEK293 cells with pCMV-LXRα (0.1 μg), PPARα (0.01 μg), PPARγ (0.01 μg), pCMV-RXR (0.02, 0.1, and 0.5 μg), and pSV-β-gal as a reference plasmid. 9CRA (10 μM) was added to the cells after transfection. After incubation for 24 h, Luc activity was measured and normalized by β-gal activity. The percent inhibition of Luc activity by PPARα or γ (0.5 μg) as compared with the mock, LXRα (0.1 μg)-, or 9CRA (10 μM)-induced controls is shown (means $\pm$ SD, n = 3). The number in *parentheses* indicates the fold induction by LXRα (0.1 μg) or 9CRA (10 μM) in Luc activity as compared with basal BP1c(LXRE)-Luc activity.

of LXRs alone, PPARα/RXR, nor PPARα/LXR bound LXRE (Fig. 8A). Figure 8B shows that addition of PPARα or γ to the incubation diminished the shifted signal in a dose-dependent manner. A 4-fold increase in amount of PPAR compared with LXR nearly abolished the shifted signal. Addition of PPAR agonists (fenofibrate, Wy, or Pio) essentially did not change LXR/RXR binding to LXRE without PPARα or γ. However, these ligands slightly enhanced the inhibitory effect of PPAR on the binding of LXR to LXRE. As shown in Fig. 8C, supplementation with RXR, did not change the basal binding of LXR/RXR to the LXRE, suggesting that the amount of RXR added in the basal condition of this assay was sufficient to form an LXR/RXR heterodimer. The decreased LXR/RXR binding to the LXRE by PPAR was completely restored by further addition of RXR. The data suggest that PPAR suppression of LXR-activated SREBP-1c promoter, as observed in the reporter assays, was mediated through inhibiting LXR/RXR binding to the LXRE, which was presumably caused by reduction of LXR/RXR heterodimer formation.

PPARα Activation Represses LXR Agonist-Induced SREBP-1c Expression

To further evaluate the *in vivo* physiological significance of PPARα interference with LXR signaling, we studied hepatic gene expression in mice treated with the PPARα ligand and/or the LXR ligand. The mice were fasted to induce hepatic endogenous PPARα. The activation of LXR by T0901317 was confirmed by observational increases in SREBP-1 and ATP-binding cassette transporter A1 (ABCA1) mRNA levels, both of which are well-known LXR target genes. Wy alone minimally increased basal ABCA1 mRNA, which is consistent with reports of PPAR-LXR-ABCA1 pathway (51, 52). The hepatic mRNA levels of ABCA1 and SREBP1 induced by the LXR ligand was suppressed by Wy (Fig. 9A). Coimmunoprecipitation experiments were performed to estimate LXRα/RXR heterodimers in hepatic nuclear extracts. As a control experiment, LXRα antibody was shown to coimmunoprecipitate successfully the *in vitro* translated LXRα/RXR proteins as detected by RXR antibody (Fig. 9B). These assays demonstrated that the appreciable increase in amount of LXRα/RXR heterodimers in hepatic nuclear extracts that was observed in the mice treated with T0901317 was completely suppressed by concomitant Wy treatment (Fig. 9C). These results suggest that PPARα agonist could cancel hepatic LXR ligand-induced SREBP-1c gene induction via reduction of nuclear LXR/RXR formation. We confirmed that PPARα/RXR complex formation was enhanced by PPARα agonist using coimmunoprecipitation of *in vitro* transcription/translation protein (Fig. 9D), as was previously reported (41). Effects of ligand activation of PPARα on LXR/RXR binding to LXRE was also assessed by gel mobility shift assays using hepatic nuclear extracts from fasted mice as physiological receptors. As shown in Fig. 9E, the signal was enhanced by T0901317, and this induction was canceled by further addition of Wy.

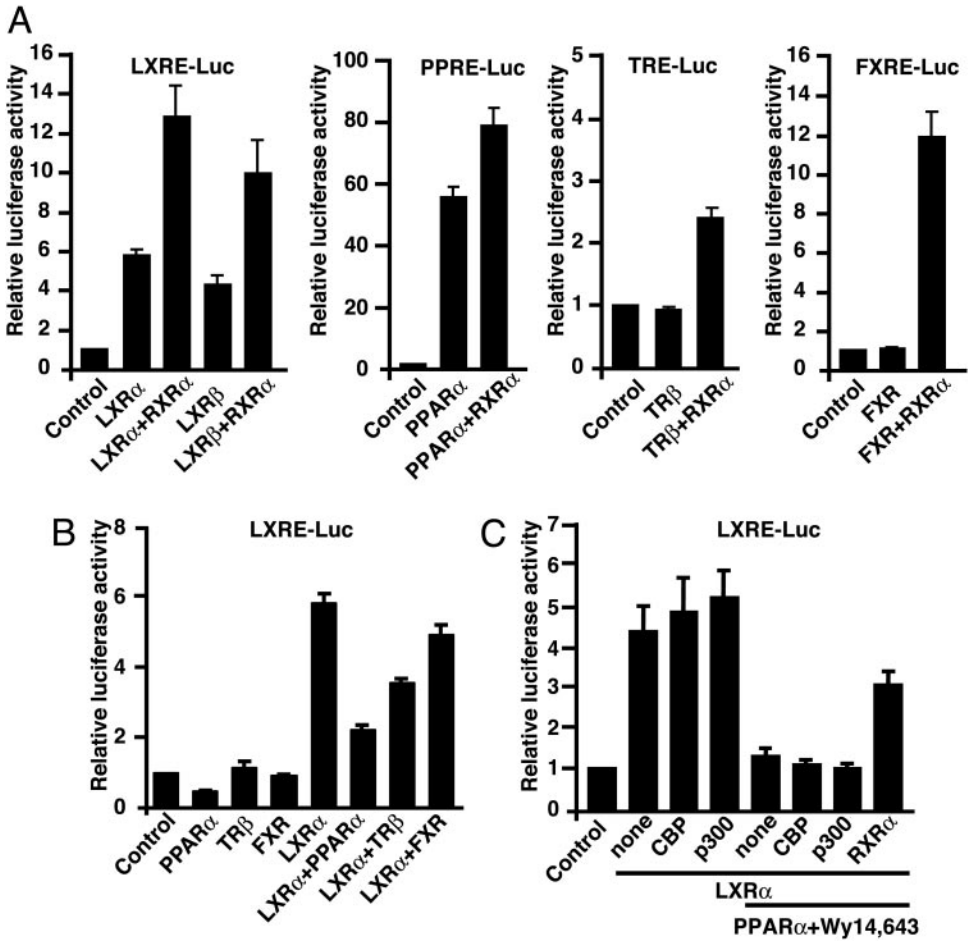

Fig. 7. Effects of RXR Heterodimers and CBP/p300 on SREBP-1c Promoter Activity

HEK293 were grown at 37 C in an atmosphere of 5% CO_2 in DMEM supplemented with 10% FBS. Transfection studies were carried out with cells plated on 12-well plates. A, pPPRE-Luc [(ACO-PPRE)3-TK-Luc], pLXRE-Luc (BP1c-LXREb-Luc), pTRE-Luc [(DR4)2-Ld40-Luc], or FXRE-Luc (human ileal bile acid-binding protein promoter-Luc (−862/+ 30-Luc, relative translation start site) and pSV-renilla Luc were cotransfected into HEK293 cells with indicated expression plasmids (0.25 μg): pCMV-LXRα, pCMV-LXRβ, pCMV-PPARα, pCMV7-TRβ, pCMV-FXR, and/or pCMV-RXRα (0.25 μg). After incubation for 24 h, Luc activity was measured and normalized to renilla Luc activity. B, pBP1c(LXRE)-Luc and pSV-renilla Luc were cotransfected into HEK293 cells with indicated expression CMV-promoter plasmid (0.25 μg). C, pBP1c(LXRE)-Luc and pSV-renilla Luc were cotransfected into HEK293 cells with indicated expression plasmids; pCMV-LXRα (0.25 μg), pCMV-PPARα (0.25 μg), pCMV-RXRα (0.5 μg), pCMV-CBP (0.5 μg), and pCMV-p300 (0.5 μg). Cells were treated with or without 3 μM Wy. After incubation for 24 h, Luc activity was measured and normalized to renilla Luc activity. The fold change in the Luc activity as compared with the control is shown (means ± SD, n = 3).

Studies with receptor antibodies and T0901317 indicated that shifted band of LXRE probe incubated with nuclear extracts was mainly caused by LXR (Fig. 9E), although the complex contain some other proteins. The data also suggest that the PPARα agonist inhibits LXR ligand-induced LXR/RXR binding to LXRE in a competitive manner.

The observational inhibition of SREBP-1c gene expression by PPARα activation in transfection studies and mouse livers was also estimated in rat primary hepatocytes. As evaluated by Northern blot analysis, the hepatocytes showed consistent PPARα activation by Wy (Fig. 10A). Addition of Wy to the medium caused dose-dependent increases in PPARα target gene, mHMG-CoA synthase, and acyl-CoA oxidase (ACO). LXRα expression was induced as previously described (51–53) but only modestly. PPARα gene was also significantly induced by Wy owing to autoregulation as was recently described (55). As shown in Fig. 10B, when the cells were incubated with T0901317, SREBP-1c expression was robustly induced through LXR activation. This induction was suppressed in a dose-dependent manner by coincubation with Wy (Fig. 10B). The inhibitory effect of Wy was also observed with cycloheximide treatment, suggesting that this action might be independent of *de novo* protein synthesis (Fig. 10C). These data suggest that suppression of the LXR-SREBP1c pathway by the PPARα ligand is mediated through its PPARα activation and is more prominent under conditions of LXR activation.

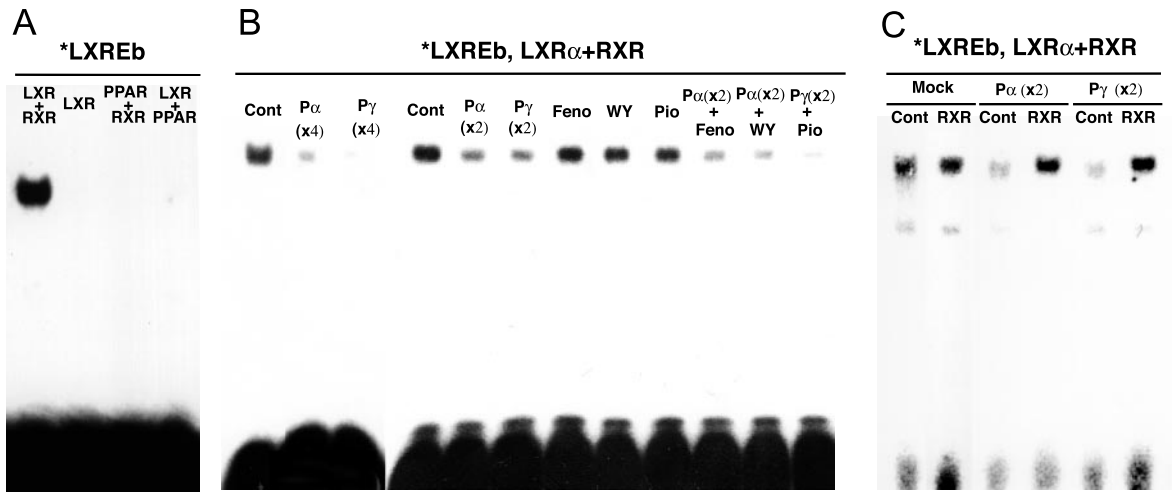

Fig. 8. PPARs Inhibit LXR/RXR Binding to LXREs in the SREBP1c-Promoter in Gel Mobility Shift Assay

A, PPAR/RXR or PPAR/LXR had no ability to bind to LXRE. ^{32}P-labeled LXREb in the SREBP-1c promoter was incubated with *in vitro* synthesized LXRα, PPARα, and/or RXRα (1.5 μl of programmed reticulocyte lysate) as indicated for 30 min on ice. The DNA-protein complexes were resolved on a 4.6% PAGE. B, PPARs and their activators inhibited LXR/RXR binding to LXRE. Complexes of LXR/RXR-LXREb were incubated with 2- or 4-fold higher amounts of PPARα or PPARγ in the absence or presence of fenofibric acid (Feno, 100 μM), Wy (100 μM), or Pio (10 μM). C, PPARα or PPARγ inhibition of LXR/RXR binding to LXREb as observed in panel B was restored by addition of a 4-fold higher amount of RXR.

DISCUSSION

The current studies demonstrate that PPARα activation suppresses LXR-mediated SREBP-1c gene expression. Evidence for this was first shown in luciferase assays in cultured cells and was confirmed in rat primary hepatocyte cultures and mouse livers. Overexpression of PPARs represses LXR/RXR activation of LXRE containing promoters such as the SREBP-1c promoter. This inhibitory effect was enhanced by addition of PPAR ligands. In the liver nuclei, addition of PPARα agonist enhanced binding of PPARα to RXR, decreased the amount of LXR/RXR heterodimers, leading to suppression of LXR ligand-activated SREBP-1c expression. RXR supplementation experiments suggest that the mechanism for PPAR inhibition of LXR/RXR activity could be at least partly RXR competition between PPAR and LXR. Involvement of RXR competition in the regulation of activities of nuclear receptors has been well established for PPAR and TR (46, 47, 56). It has been also reported that LXRα interacts with PPARα and inhibits peroxisome proliferator signaling (48). As described in the accompanying paper (49), PPARα heterodimerizes with both LXRα and β as efficiently as with RXR. Because PPARα/LXRα (β) cannot bind to LXRE, this complex formation could interfere with the formation of LXRα/RXR and activation of LXRE containing promoters by LXRα/RXR. Therefore, as schematized in Fig. 11, it is possible that abundant PPARα can absorb LXR as well as RXR, resulting in inhibition of LXR/RXR formation and SREBP-1c promoter activation. Reduction of hepatic nuclear LXR/RXR from fasted mice doubly treated with LXR and PPARα ligands supports this hypothesis. Whether the dominant mechanism for PPAR inhibition of SREBP-1c expression is RXR competition between PPAR and LXR, or LXR competition between PPARα and RXR is currently unknown. The amounts of three nuclear receptor proteins in the nucleus, concentration of each ligand, and affinity for one another, possible involvement of other nuclear receptors and cofactors will all be involved in determining the overall effect. Further studies will be needed to determine the precise mutual interactions in other combinations of nuclear receptors.

LXRα expression was recently shown to be regulated by PPAR (51–53). PPAR enhances cholesterol efflux in macrophages via PPAR-LXRα-ABCA1 pathway, and a combination treatment with both PPAR and LXR agonists result in a further induction in ABCA1 mRNA in macrophages (51, 52). We also observed a relatively small PPARα ligand-gene induction of LXR. We propose that the PPAR-LXRα-ABCA1 pathway has been established in macrophages, PPAR-LXR-SREBP1c pathway may not be very potent in liver. Lack of hepatic SREBP-1c induction after adenoviral overexpression of PPARγ supports this (57). Each receptor, ligand, and cofactor concentration could be important for this tissue specificity of cross-talk of transcription factors.

The physiological relevance of the cross-talk between PPARα and LXR could be extended to nutritional regulation of energy metabolism by a mutual interaction between PPARα and SREBP-1c, whose expression are dominated by LXR. Hepatic fatty acid degradation is activated in an energy-depleted state such as fasting to produce alternate energy substrates

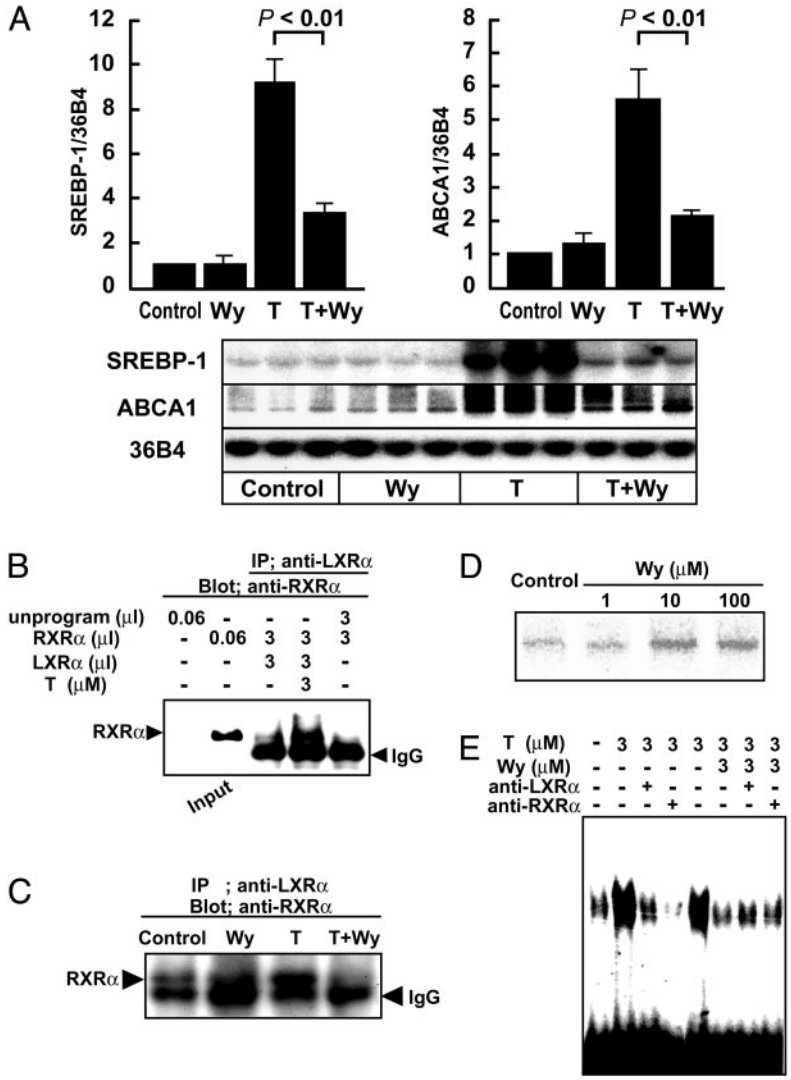

Fig. 9. Inhibition by PPARα Ligand of Gene Induction of LXR Target Genes in Mouse Livers (A), LXR Ligand-Induced LXR/RXR Heterodimer Formation (B and C), and Binding to LXRE (D)

A, Mice (n = 3) were fasted and treated with T0901317 (T, 50 mg/kg), Wy (50 mg/kg), or both agonists for 18 h. Total RNA was isolated from the livers of mice and subjected to Northern blot analysis with the indicated cDNA probes. Fold increases of expression relative to corresponding with vehicle-treated controls are shown. Data are means ± SEM. The statistical significance of differences between T0901317-treated and both T0901317- and Wy-treated mice was assessed with the Sheffé test. B, Confirmation of immunoprecipitation of LXRα/RXRα heterodimer by anti-LXRα antibody. *In vitro* translated RXRα and LXRα proteins were incubated with or without T0901317, and the reaction mixtures were incubated with anti-LXRα antibody (H-144 sc-13068, Santa Cruz Biotechnology, Inc.), followed by binding to protein G-sepharose. The precipitations were washed and subjected to immunoblotting with an anti-RXRα antibody (D-20, sc-553, Santa Cruz Biotechnology, Inc.). C, Wy inhibited T0901317-induced LXR/RXRα heterodimer formation in the liver from C57BL6 mice. Mice were fasted and treated with T0901317 (50 mg/kg), Wy (50 mg/kg), or both agonists for 18 h. Hepatic nuclear extracts were prepared from each group. Nuclear extracts (equalized by protein concentrations) were subjected to immunoprecipitation using anti-LXRα antibody (H-144 *sc*-13068, Santa Cruz Biotechnology, Inc.) coupled to protein G-sepharose beads. Immunoprecipitates were subjected to SDS-PAGE, and immunoblotted with an anti-RXRα antibody (D-20 *sc*-553, Santa Cruz Biotechnology, Inc.). *Arrowheads* represent the RXRα or IgG signal. D, Wy potentiates PPARα binding to RXRα. Coimmunoprecipitation of unlabeled RXRα and ^{35}S-labeled PPARα with an anti-RXRα antibody in the absence or presence of Wy. After immunoprecipitation with protein G-sepharose beads, samples were run on SDS-PAGE. T0901317 induced LXR/RXRα binding to LXRE was inhibited by addition of Wy. ^{32}P-labeled SREBP1c-LXRE probe and hepatic nuclear extracts from fasted mice were incubated with T0901317 and/or Wy. The DNA-protein complexes were resolved on a 4.6% PAGE. E, T0901317 induced-proteins/LXRE complexes were reduced by an anti-LXRα antibody (H-144 sc-13068, Santa Cruz Biotechnology, Inc.) or anti-RXRα antibody (D-20 sc-553, Santa Cruz Biotechnology, Inc.). ^{32}P-labeled SREBP1c-LXRE probe and hepatic nuclear extracts (1 μg) from fasted mice were incubated with 20 μl buffer (buffer A) containing 10 mM HEPES (pH 7.6), 50 mM KCl, 0.05 mM EDTA, 2.5 mM $MgCl_2$, 8.5% glycerol, 1 mM dithiothreitol, 0.1% Triton X-100, and 1 mg/ml nonfat milk for 1 h on ice. The DNA-protein complexes were resolved in a 4.6% PAGE.

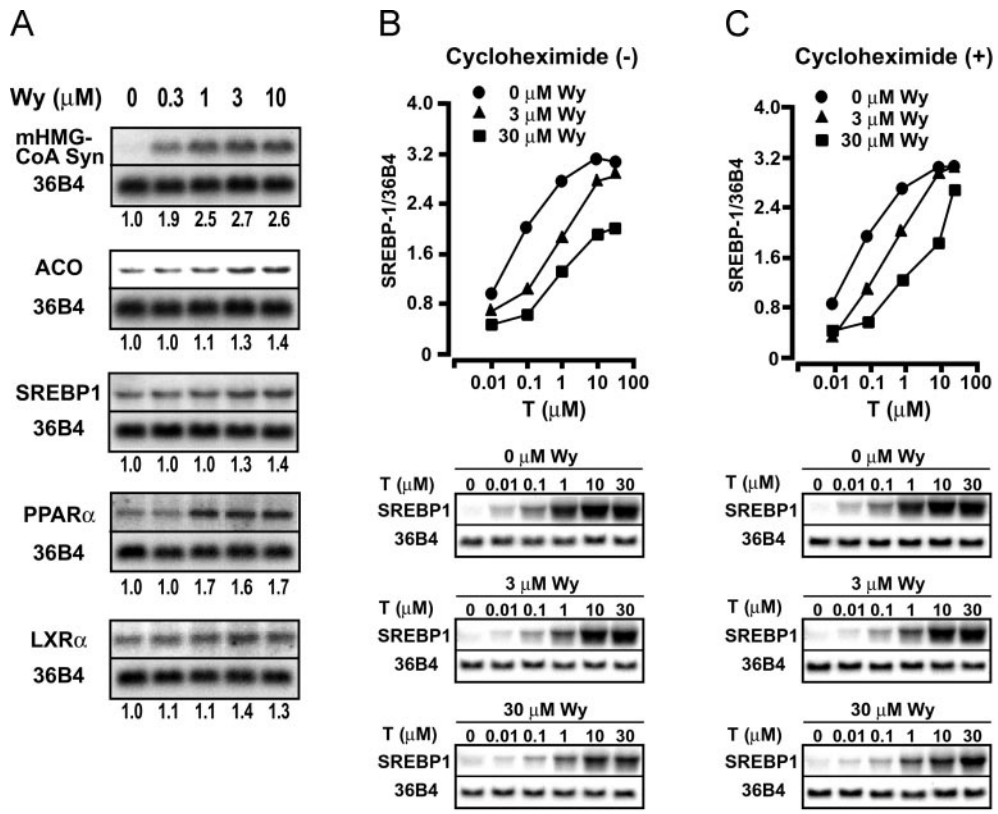

Fig. 10. Ligand-Activation of PPARα Suppresses LXR Ligand Induced-SREBP1 Gene Expression in Primary Rat Hepatocytes

A, Wy induced gene expression of mHMG-CoA Syn, ACO, SREBP-1, PPARα, and LXRα. Rat primary hepatoctyes were prepared as described in *Materials and Methods*. The cells were incubated with the indicated concentration of Wy for 24 h. B, Wy suppressed induction of SREBP-1 gene expression by T0901317 (T) treatment. The hepatocytes were incubated with the indicated concentration of T0901317 in the absence or presence of Wy for 24 h. C, Cycloheximide had no effect in the inhibition of Wy on T0901317 induced-SREBP-1 gene expression. Total RNA was extracted and Northern blot analysis was performed with the indicated cDNA probes. Fold changes of expression relative to corresponding with vehicle-treated controls are shown.

such as ketone bodies. PPARα activation plays a key role in this adaptic gene induction (44, 45). As has been shown for other nuclear receptors, PPARα activation requires binding of its ligands, one of which could be polyunsaturated fatty acids, presumably lipolyzed from adipose tissues. More importantly, we (12) and other group (44) have shown that hepatic PPARα expression is nutritionally regulated. Hepatic PPARα level is induced by fasting and suppressed by refeeding. This nutritional regulation of PPARα expression suggests that changes in the amount of PPAR protein could also control its downstream genes of lipid oxidation as well as changes in the ligand concentration. In a fasted state, lipogenesis should be declined in a coordinated fashion with activation of fatty acid degradation. Marked induction of PPARα in the livers of fasted mice might play a role in an efficient suppression of lipogenic genes by inhibition of SREBP-1c gene expression through a mechanism that is proposed in the current study. Consistently, it has recently been reported that PPARα-null mice show dysregulation of hepatic lipogenic genes (58). Polyunsaturated fatty acid could contribute to this reciprocal energy regulation by PPARα and SREBP-1c through activation of PPARα ligands and direct inhibition of SREBP-1c as recently reported (59).

In the current study, we also observed similar inhibitory effect by overexpression of PPARγ on SREBP-1c expression. In a regular nutritional state, PPARγ expression is extremely low in the liver, and contribution of PPARγ expression to hepatic regulation of SREBP-1c is unlikely. In contrast, PPARγ is highly induced and involved in adipogenesis. Previous reports suggest that the role of adipocyte determination and differentiation 1 (ADD1)/SREBP-1c in the adipose tissue is positioned upstream of PPARγ activation through two different mechanisms: ligand production (60) and direct induction (61). PPARγ has recently been reported to activate LXRα gene expression (52), which could in turn activate SREBP-1c gene expression, leading to a speculation that these three factors could form an auto-loop activation in adipogenesis. PPARγ inhibition of SREBP-1c in the current study could antagonize this potential auto-loop and be involved in the complex transcriptional cross-talk among PPARγ, SREBP-1c, and LXR in the adipocytes.

Taken together with the accompanying paper (49) showing LXR inhibition of PPARα signaling, we show

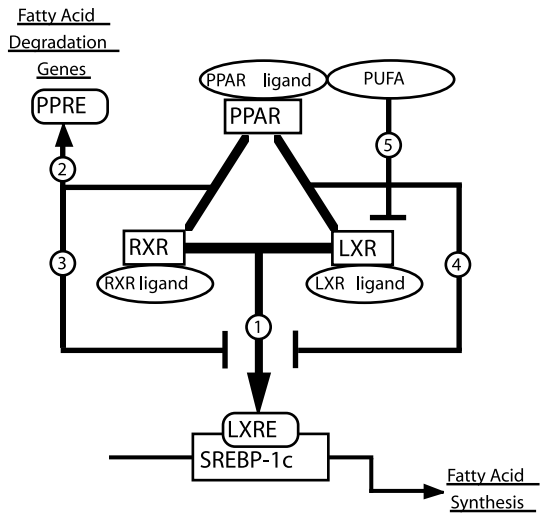

Fig. 11. Mechanism by which PPAR Suppresses the SREBP-1c Promoter Activity through Interfering with LXR-RXR.

1, LXR/RXR has been shown to bind and activate LXREs in the SREBP-1c promoter. 2, Reciprocally, PPARα/RXR activates gene expression crucial for lipid degradation through PPREs. 3 and 4, The current study proposes interference of LXR/RXR signaling by PPAR/RXR and PPAR/LXR. 5, PUFA (polyunsaturated fatty acid), representative PPARα ligands, can suppress LXR activation independently of PPARα (59, 64). The accompanying paper (49) describes LXR suppress PPARα-targeted gene expression crucial for lipid catabolism by inhibiting PPAR-RXR binding to the PPRE [see Fig. 12 of the accompanying paper (49)].

the mutual interaction between PPARα and LXRs in the reciprocal regulation of PPARα and SREBP-1c target genes. The data suggest that cross-talk of these nuclear factors could play crucial roles in nutritional regulation of fatty acid metabolism (see Fig. 11). However, as recent studies show that there could be a global and complex network of nutritional transcriptional factors, further studies are needed to evaluate the physiological relevance of the cross-talk between these and other nuclear transcription factors.

MATERIALS AND METHODS

Materials

Anti-RXRα (D-20, sc-553) and LXRα (H-144, sc-13068) antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), 22RHC, 9CRA, and Wy were purchased from Sigma (St. Louis, MO), Redivue [α-^{32}P]deoxy-CTP (6000 Ci/mmol) from Amersham Biosciences Inc. (Amersham, Buckinghamshire, UK), and restriction enzymes from New England Biolabs (Boston, MA). Fenofibric acid and Pio were provided by Laboratories Fournier (Paris, France), and Takeda Pharmaceutical (Osaka, Japan), respectively.

Plasmid

Luc gene constructs containing a 2.6-kb fragment of the mouse SREBP-1c promoter (pBP1c2600-Luc), other SREBP-1c promoter luciferase constructs, and expression plasmids, pCMV (cytomegalovirus)-mLXRα and pCMV-mLXRβ were prepared as previously described (29). Expression plasmids, pCMV-PPARα and pCMV-PPARγ were prepared by subcloning PCR products from mouse liver cDNA into CMV-7. CMV-T7 promoter expression plasmid of human RXRα (pCMV-RXR) was a kind gift from Dr. D. J. Mangelsdorf (University of Texas Southwestern Medical Center, Dallas, TX). pCMV-FXR, pCMX-TRβ, and pCMV-CBP were from Dr. H. Fujii (Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan), Dr. R. M. Evans (The Salk Institute for Biological Studies, La Jolla, CA), and Dr. T. Nakajima (St. Marianna University School of Medicine, Kawasaki, Japan), respectively.

Transfections and Luciferase Assays

HEK293 and HepG2 cells were grown at 37 C in an atmosphere of 5% CO_2 in DMEM containing 25 mM glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate supplemented with 10% fetal bovine serum (FBS). Transfection studies were carried out with cells plated on 12-well plates as previously described (50). The indicated amount of each expression plasmid was transfected simultaneously with a Luc reporter plasmid (0.25 μg) and pSV (simian virus 40)-β-galactosidase (β-gal) (0.2–0.4 μg) or pSV-renilla Luc (0.05 μg). The total amount of DNA in each transfection was adjusted to 1.5 μg/well with pCMV7. 22RHC was dissolved in ethanol and PPAR ligands in dimethylsulfoxide (DMSO). Each agent was added to the cells immediately after transfection in DMEM with 10% FBS, and incubated for 24 h. After incubation, the amount of Luc activity in transfectants was measured and normalized to the amount of β-gal or renilla Luc activity as measured by standard kits (Promega, Madison, WI). pBP1c(LXRE)-firefly Luc (1.5 μg) and pSV-renilla Luc (0.5 μg) were transfected into rat primary hepatocytes by using lipofectin reagent (Invitrogen). After incubation for 24 h, the amount of firefly Luc activity in transfectants was measured and normalized to the amount of renilla Luc activity. Dual reporter assay was performed with pBP1c(LXRE)-firefly Luc (0.25 μg), PPRE-renilla Luc (0.25 μg), and pSV-β-gal (0.2 μg).

Gel Mobility Shift Assays

Gel mobility shift assays were performed as previously described (29). Briefly, the entire open reading frames of mouse (m) LXRα and mPPARα were amplified from the pCMV-LXRα and pCMV-mPPARα by PCR (forward primers, 5′-TTGGTAATGTCCAGGG and 5′-GCCATACACTTGAGTGACAAT; reverse primers, 5′-CTTCCAAGGCCAGGAGA and 5′-AGATCAGTACATGTCTCTGTAGA) and cloned into the *Eco*RI and *Not*I sites, and *Sal*I and *Not*I sites of the pBluescript II SK plasmid, respectively. mLXRα, mPPARα, and human (h) RXR proteins were generated from the expression vectors using a coupled *in vitro* transcription/translation system (Promega). Double-stranded oligonucleotides used in gel shift assays were prepared by annealing both strands of the LXREb in the LXRE complex of the SREBP-1c promoter (29). These were then labeled with [α-^{32}P]deoxy-CTP by Klenow enzyme, followed by purification on Sephadex G50 columns. The labeled probes (30,000–100,000 cpm) were incubated with nuclear receptor lysates (1–1.5 μl) or hepatic nuclear extract (1 μg) in a mixture (20 μl) containing 10 mM Tris-HCl, pH 7.6; 50 mM KCl; 0.05 mM EDTA; 2.5 mM $MgCl_2$; 8.5% glycerol; 1 mM dithiothreitol; 0.5 μg/ml poly (deoxyinosine-deoxycytidine), 0.1% Triton X-100; and 1 mg/ml nonfat milk for 30 min on ice. The DNA-protein complexes were resolved on a 4.6% PAGE at 140 V for 1 h at 4 C. Gel were dried and exposed to BAS2000 filters with BAStation software (Fuji Photo Film, Kanagawa, Japan).

Animals

Male mice (C57BL/6J) were obtained from Charles River Japan (Yokohama, Japan). All mice were given a standard diet and tap water *ad libitum*. All institutional guidelines for animal care and use were applied in this study. Vehicle (0.5% carboxymethyl-cellulose) T0901317 (50 mg/kg), Wy (50 mg/kg), or both their agonists was orally administered to the mice before 18 h fasting. For fasting and refeeding treatment, mice were fasted for 24 h and fed a high sucrose/fat free diet for 12 h as described (13). Hepatic nuclear extracts was prepared from the livers as previously described (62).

Coimmunoprecipitation of Receptors

In vitro translated [^{35}S]-methionine-labeled receptors with unlabeled receptors or hepatic nuclear extracts from ligand-treated mice were brought to a final volume of 20 μl with buffer containing 10 mM HEPES (pH 7.6), 50 mM KCl, 0.05 mM EDTA, 2.5 mM $MgCl_2$, 8.5% glycerol, 1 mM dithiothreitol, 0.1% Triton X-100, and 1 mg/ml nonfat milk for 2 h at 4 C and incubated with 10 μl of anti-PPARα (H-98 sc-9000, Santa Cruz Biotechnology, Inc.) or anti-LXRα (H-144 sc-13068, Santa Cruz Biotechnology, Inc.) polyclonal antibody binding to protein G-sepharose for overnight at 4 C. The precipitations were washed with PBS containing 0.2% Tween-20 and 3% BSA. After microcentrifugation, the pellet was washed four times with 1 ml of ice-cold PBS containing 0.2% Tween-20. Twenty microliters of SDS-PAGE sample buffer were added to the final pellet and boiled for 5 min at 95 C. The supernatant was subjected to electrophoresis on 8 or 10% SDS-PAGE.

Hepatocyte Isolation and Culture

Primary hepatocytes were isolated from male Sprague-Dawley rats (160–180 g; Japan Clea, Tokyo, Japan) using the collagenase perfusion method as described previously (63). The viability of isolated cells was over 90% as determined by the trypan blue. Cells were resuspended in DMEM containing 100 U/ml penicillin and 100 μg/ml streptomycin sulfate supplemented with 5% FBS, seeded on collagen-coated dishes 100 mm at a final density of 4×10^4 cells/cm^2. After an attachment for 4 h, cells were cultured with medium containing the indicated agonists with or without 5 μM cycloheximide for 24 h.

Northern Blot Analysis

Total RNA was extracted using TRIZOL Reagent (Invitrogen Corp., Carlsbad, CA). Equal aliquots of total RNA from mice in each group were pooled (total 10 μg), subjected to formalin-denatured agarose electrophoresis, and transferred to nylon membrane (Hybond N, Amersham Pharmacia Biotech, Uppsala, Sweden). Blot hybridization was performed with the cDNA probes labeled with [α-^{32}P]CTP (6000 Ci/mmol) using the Megaprime DNA Labeling System (Amersham Biosciences Inc.). The cDNA probes for SREBP-1, ACO, PPARα, LXRα and β, ABCA1, and acidic ribosomal phosphoprotein PO (36B4) were prepared as previously described (12, 29). The cDNA probes for mHMG-CoA Syn were provided by Kyorin Pharmaceutical Co. Ltd. (Tochigi, Japan). Each signal was analyzed with BAS2000 and BAStation software (Fuji Photo Film).

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T.Y. and T.I. equally contributed to this work.

REFERENCES

1. Brown MS, Goldstein JL 1997 The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340
2. Brown MS, Goldstein JL 1999 A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc Natl Acad Sci USA 96:11041–11048
3. Brown MS, Ye J, Rawson RB, Goldstein JL 2000 Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell 100:391–398
4. Briggs MR, Yokoyama C, Wang X, Brown MS, Goldstein JL 1993 Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. J Biol Chem 268:14490–14496
5. Kim JB, Spotts GD, Halvorsen YD, Shih HM, Ellenberger T, Towle HC, Spiegelman BM 1995 Dual DNA binding specificity of ADD1/SREBP1 controlled by a single amino acid in the basic helix-loop-helix domain. Mol Cell Biol 15:2582–2588
6. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL, Brown MS 1993 SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 75:187–197
7. Tontonoz P, Kim JB, Graves RA, Spiegelman BM 1993 ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol Cell Biol 13:4753–4759
8. Hua X, Yokoyama C, Wu J, Briggs MR, Brown MS, Goldstein JL, Wang X 1993 SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc Natl Acad Sci USA 90:11603–11607
9. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS 1997 Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest 99:838–845
10. Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL, Shimano H 1998 Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J Clin Invest 101:2331–2339
11. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL 1996 Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest 98:1575–1584
12. Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N 1999 Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 274:35832–35839

13. Horton JD, Bashmakov Y, Shimomura I, Shimano H 1998 Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc Natl Acad Sci USA 95:5987–5992
14. Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Shionoiri F, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N 1999 A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 274:35840–35844
15. Xu J, Nakamura MT, Cho HP, Clarke SD 1999 Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem 274:23577–23583
16. Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman BM 1998 Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J Clin Invest 101:1–9
17. Kim HJ, Takahashi M, Ezaki O 1999 Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. J Biol Chem 274:25892–25898
18. Osborne TF 2000 Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J Biol Chem 275:32379–32382
19. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P 1995 The nuclear receptor superfamily: the second decade. Cell 83:835–839
20. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ 1996 An oxysterol signalling pathway mediated by the nuclear receptor LXR α. Nature 383:728–731
21. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, Willson TM 1997 Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J Biol Chem 272:3137–3140
22. Russell DW 1999 Nuclear orphan receptors control cholesterol catabolism. Cell 97:539–542
23. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ 1995 LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 9:1033–1045
24. Song C, Kokontis JM, Hiipakka RA, Liao S 1994 Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. Proc Natl Acad Sci USA 91:10809–10813
25. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, Mangelsdorf DJ 1998 Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR α. Cell 93:693–704
26. Luo Y, Tall AR 2000 Sterol upregulation of human CETP expression *in vitro* and in transgenic mice by an LXR element. J Clin Invest 105:513–520
27. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietschy JM, Mangelsdorf DJ 2000 Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289:1524–1529
28. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ 2000 Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Genes Dev 14: 2819–2830
29. Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada N 2001 Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 21:2991–3000
30. Schoonjans K, Staels B, Auwerx J 1996 The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta 1302:93–109
31. Willson TM, Brown PJ, Sternbach DD, Henke BR 2000 The PPARs: from orphan receptors to drug discovery. J Med Chem 43:527–550
32. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W 1996 Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. Endocrinology 137: 354–366
33. Tontonoz P, Hu E, Spiegelman BM 1994 Stimulation of adipogenesis in fibroblasts by PPAR γ2, a lipid-activated transcription factor. Cell 79:1147–1156
34. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK 1998 The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature 391:79–82
35. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM 1998 PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93:241–252
36. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM 1998 Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 93:229–240
37. Issemann I, Green S 1990 Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645–650
38. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W 1992 Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68:879–887
39. Gottlicher M, Widmark E, Li Q, Gustafsson JA 1992 Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. Proc Natl Acad Sci USA 89:4653–4657
40. Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W 1993 Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. Proc Natl Acad Sci USA 90:2160–2164
41. Forman BM, Chen J, Evans RM 1997 Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci USA 94:4312–4317
42. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM 1997 Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc Natl Acad Sci USA 94:4318–4323
43. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W 1997 Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol Endocrinol 11:779–791
44. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W 1999 Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. J Clin Invest 103:1489–1498
45. Leone TC, Weinheimer CJ, Kelly DP 1999 A critical role for the peroxisome proliferator-activated receptor alpha (PPARα) in the cellular fasting response: the PPARα-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci USA 96:7473–7478

46. Juge-Aubry CE, Gorla-Bajszczak A, Pernin A, Lemberger T, Wahli W, Burger AG, Meier CA 1995 Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. J Biol Chem 270:18117–18122
47. Chu R, Madison LD, Lin Y, Kopp P, Rao MS, Jameson JL, Reddy JK 1995 Thyroid hormone (T3) inhibits ciprofibrate-induced transcription of genes encoding beta-oxidation enzymes: cross talk between peroxisome proliferator and T3 signaling pathways. Proc Natl Acad Sci USA 92:11593–11597
48. Miyata KS, McCaw SE, Patel HV, Rachubinski RA, Capone JP 1996 The orphan nuclear hormone receptor LXR α interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. J Biol Chem 271:9189–9192
49. Ide T, Shimano H, Yoshikawa T, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Yatoh S, Iizuka Y, Tomita S, Ohashi K, Takahashi A, Sone H, Gotoda T, Osuga J-i, Ishibashi S, Yamada N 2003 Cross-talk between peroxisome proliferator-activated receptor (PPAR) α and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. II. LXRs suppress lipid degradation gene promoters through inhibition of PPAR signaling. Endocrinology 144:1255–1267
50. Amemiya-Kudo M, Shimano H, Yoshikawa T, Yahagi N, Hasty AH, Okazaki H, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Sato R, Kimura S, Ishibashi S, Yamada N 2000 Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J Biol Chem 275:31078–1085
51. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart JC, Clavey V, Staels B 2001 PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 7:53–58
52. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P 2001 A PPAR γ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 7:161–171
53. Tobin KA, Steineger HH, Alberti S, Spydevold O, Auwerx J, Gustafsson JA, Nebb HI 2000 Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-α. Mol Endocrinol 14:741–752
54. McKenna NJ, O'Malley BW 2002 Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 108:465–474
55. Pineda Torra I, Jamshidi Y, Flavell DM, Fruchart JC, Staels B 2002 Characterization of the human PPARα promoter: identification of a functional nuclear receptor response element. Mol Endocrinol 16:1013–1028
56. Hunter J, Kassam A, Winrow CJ, Rachubinski RA, Capone JP 1996 Crosstalk between the thyroid hormone and peroxisome proliferator-activated receptors in regulating peroxisome proliferator-responsive genes. Mol Cell Endocrinol 116:213–221
57. Yu S, Matsusue K, Kashireddy P, Cao WQ, Yeldandi V, Yeldandi AV, Rao MS, Gonzalez FJ, Reddy JK 2003 Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to PPARγ 1 overexpression. J Biol Chem 278:498–505
58. Patel DD, Knight BL, Wiggins D, Humphreys SM, Gibbons GF 2001 Disturbances in the normal regulation of SREBP-sensitive genes in PPAR α-deficient mice. J Lipid Res 42:328–337
59. Yoshikawa T, Shimano H, Yahagi N, Ide T, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Tomita S, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Takahashi A, Sone H, Osuga Ji J, Gotoda T, Ishibashi S, Yamada N 2002 Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. J Biol Chem 277:1705–1711
60. Kim JB, Wright HM, Wright M, Spiegelman BM 1998 ADD1/SREBP1 activates PPARγ through the production of endogenous ligand. Proc Natl Acad Sci USA 95: 4333–4337
61. Fajas L, Schoonjans K, Gelman L, Kim JB, Najib J, Martin G, Fruchart JC, Briggs M, Spiegelman BM, Auwerx J 1999 Regulation of peroxisome proliferator-activated receptor γ expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. Mol Cell Biol 19:5495–5503
62. Sheng Z, Otani H, Brown MS, Goldstein JL 1995 Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. Proc Natl Acad Sci USA 92:935–938
63. Massague J, Guinovart JJ 1977 Insulin control of rat hepatocyte glycogen synthase and phosphorylase in the absence of glucose. FEBS Lett 82:317–320
64. Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, Goldstein JL, Brown MS 2001 Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci USA 98:6027–6032