0888-8809/03/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 17(7):1255–1267

doi: 10.1210/me.2002-0191

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

TOMOHIRO IDE, HITOSHI SHIMANO, TOMOHIRO YOSHIKAWA, NAOYA YAHAGI, MICHIYO AMEMIYA-KUDO, TAKASHI MATSUZAKA, MASANORI NAKAKUKI, SHIGERU YATOH, YOKO IIZUKA, SACHIKO TOMITA, KEN OHASHI, AKIMITSU TAKAHASHI, HIROHITO SONE, TAKANARI GOTODA, JUN-ICHI OSUGA, SHUN ISHIBASHI, AND NOBUHIRO YAMADA

Department of Internal Medicine (T.I., H.S., T.M., M.N., S.Y., A.T., H.S., N.S.), Institute of Clinical Medicine, University of Tsukuba, Ibaraki 305-8575, Japan; and Department of Metabolic Diseases (T.Y., N.Y., M.A.-K., Y.I., S.T., K.O., T.G., J.O., S.I.), Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113-8655, Japan

Fatty acid metabolism is transcriptionally regulated by two reciprocal systems: peroxisome proliferator-activated receptor (PPAR) α controls fatty acid degradation, whereas sterol regulatory element-binding protein-1c activated by liver X receptor (LXR) regulates fatty acid synthesis. To explore potential interactions between LXR and PPAR, the effect of LXR activation on PPARα signaling was investigated. In luciferase reporter gene assays, overexpression of LXRα or β suppressed PPARα-induced peroxisome proliferator response element-luciferase activity in a dose-dependent manner. LXR agonists, T0901317 and 22(R)-hydroxycholesterol, dose dependently enhanced the suppressive effects of LXRs. Gel shift assays demonstrated that LXR reduced binding of PPARα/retinoid X receptor (RXR) α to peroxisome proliferator response element. Addition of increasing amounts of RXRα restored these inhibitory effects in both luciferase and gel shift assays, suggesting the presence of RXRα competition. *In vitro* protein binding assays demonstrated that activation of LXR by an LXR agonist promoted formation of LXR/RXRα and, more importantly, LXR/PPARα heterodimers, leading to a reduction of PPARα/RXRα formation. Supportively, *in vivo* administration of the LXR ligand to mice and rat primary hepatocytes substantially decreased hepatic mRNA levels of PPARα-targeted genes in both basal and PPARα agonist-induced conditions. The amount of nuclear PPARα/RXR heterodimers in the mouse livers was induced by treatment with PPARα ligand, and was suppressed by superimposed LXR ligand. Taken together with data from the accompanying paper (Yoshikawa, T., T. Ide, H. Shimano, N. Yahagi, M. Amemiya-Kudo, T. Matsuzaka, S. Yatoh, T. Kitamine, H. Okazaki, Y. Tamura, M. Sekiya, A. Takahashi, A. H. Hasty, R. Sato, H. Sone, J. Osuga, S. Ishibashi, and N. Yamada, *Endocrinology* 144:1240–1254) describing PPARα suppression of the LXR-sterol regulatory element-binding protein-1c pathway, we propose the presence of an intricate network of nutritional transcription factors with mutual interactions, resulting in efficient reciprocal regulation of lipid degradation and lipogenesis. (*Molecular Endocrinology* 17: 1255–1267, 2003)

RECENT PROGRESS IN studying transcriptional regulation of lipid catabolism has unveiled the functions of orphan nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs), whereas roles by sterol regulatory element-binding protein (SREBP) family have been established as lipid synthetic regulators. PPARs are members of a nuclear receptor superfamily and are

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACO, acyl-CoA oxidase; 36B4, acidic ribosomal phosphoprotein PO; CBP, cAMP-response element binding protein-binding protein; CMV, cytomegalovirus; CoA, coenzyme A; CPTI, L-carnitine palmitoyltransferase; 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; mHMG-CoA syn, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase; PGC-1, peroxisome proliferator-activated receptor γ coactivator-1; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator responsive element; pSV, simian virus 40 promoter plasmid; 22RHC, 22(R)-hydroxycholesterol; RXR, retinoid X receptor; SREBP-1c, sterol regulatory element-binding protein 1c; TK, thymidine kinase; TR, thyroid hormone receptor; VDR, vitamin D3 receptor.

structurally related to other members such as thyroid hormone receptor (TR) and vitamin D3 receptor (VDR) (1). PPARs are known to regulate expression of numerous genes involved in fatty acid metabolism and adipocyte differentiation (2, 3). PPARα is abundantly expressed in tissues, which have high lipid catabolic activity, such as liver, kidney, heart, skeletal muscle, and brown adipose tissue (4, 5). PPARα is activated by fatty acids, eicosanoids, and fibrates, a known class of hypolipidemic drugs. Like other nuclear receptors such as: TR, VDR, and retinoic acid receptor (RAR) (6–9), PPARα forms a heterodimer with RXRα, which enhances its binding to DNA sequence elements termed peroxisome proliferator response elements (PPRE) (10–12). PPREs have been recently identified in the 5′-flanking sequences of genes involved in lipid degradation such as the ACO [acyl-coenzyme A (CoA) oxidase] (13), mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoA Syn) (14), L-carnitine palmitoyltransferase (CPTI) (15), and L-fatty acid binding protein genes (16). Studies using PPARα-deficient mice have established that PPARα plays a crucial role in fatty acid degradation (17–19).

LXRs (LXRα/NR1H3 and LXRβ/NR1H2) were identified as orphan nuclear receptors and are now thought to regulate the metabolism of several important lipids, including cholesterol and bile acid (20, 21). LXRs regulate intracellular cholesterol levels by induction of the gene expression of cholesterol 7α-hydroxylase (22, 23), which is the rate-limiting enzyme of the classic bile acid synthesis pathway, and ATP-binding cassette transporter A1 (ABCA1) (24), which modulates apolipoprotein mediated-efflux of cholesterol. Further evidence supporting an important role of LXRα in lipid homeostasis is provided by the loss of capacity to regulate catabolism of dietary cholesterol in LXRα deficient mice, an effect for which the isoform, LXRβ, could not compensate (25). Differences in the physiological functions between LXRα and LXRβ including target and tissue specificity have been suggested (26). LXRβ is ubiquitously expressed, whereas LXRα is restricted to metabolically active tissues, such as liver, kidney, intestines, and adrenal glands (27, 28). Recently, we (29) and others (30) reported that both LXR α and β are dominant activators for SREBP-1c/adipocyte determination and differentiation 1 (ADD1). Previous *in vivo* studies established that SREBP-1c plays a crucial role in the dietary regulation of most hepatic genes of fatty acid synthetic enzymes (31–34). Therefore, LXRs could be also important in fatty acid synthesis (35).

As PPARα and LXR-SREBP-1c are reciprocal regulators for fatty acid metabolism, nuclear receptors could interact with each other as was previously observed in a cross-talk between PPARα and TR (36, 37). The current study examined the effects of LXR activation on PPARα signaling. The results demonstrate that LXRα ligand activation represses PPARα signaling through reduction of stimulated-PPARα/RXR heterodimerization in the liver. Taken together with the accompanying paper (38) describing PPAR suppression of the LXR-SREBP-1c pathway, we propose a novel aspect of nutritional regulation with these mutual interactions forming a network of transcription factors regulating fatty acid metabolism.

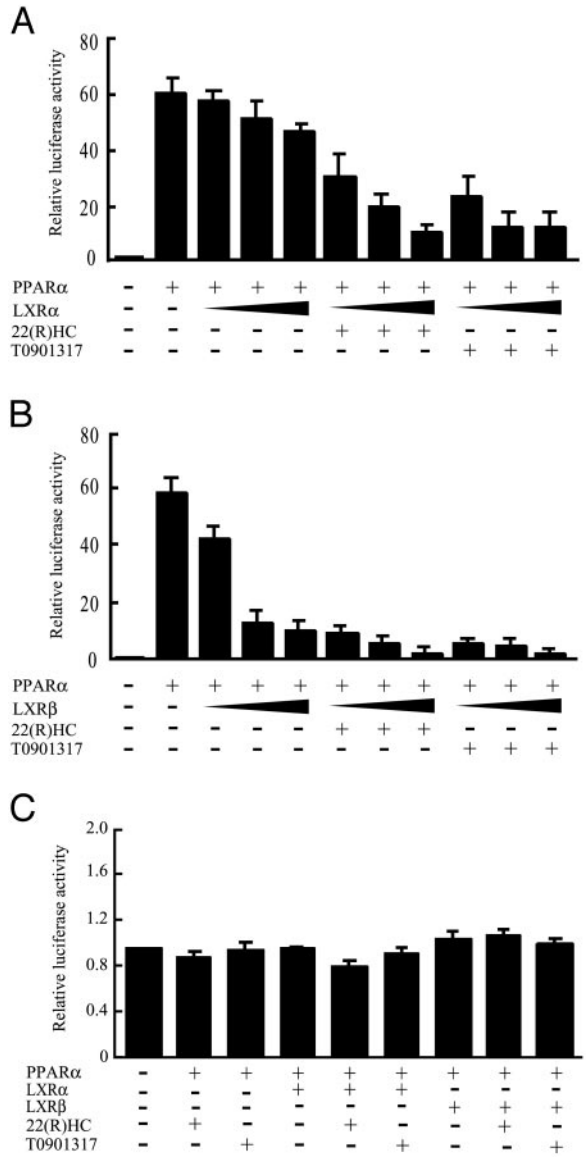

Fig. 1. Inhibition of PPARα-Induced PPRE-Luc Activity by LXRα and β

HEK293 cells were transfected with a reporter plasmid (0.25 μg): pPPRE-Luc (A and B) or constitutive Luc plasmid, pTK-Luc (C), expression plasmids (0.05 μg): CMV-mPPARα with CMV-mLXRα (A) or CMV-mLXRβ (B), and a reference plasmid (0.2 μg): pSV-β-gal. Cells were treated with either ethanol as vehicle, 3 μM of 22(R)HC or T0901317. All Luc activities were corrected for transfection efficiency by measuring β-gal activities. Results are means ± SE from three independent experiments.

RESULTS

LXR Activation Represses PPARα-Mediated Transactivation

To estimate the effect of LXR/RXRα activation on PPARα signaling, transfection studies with human embryonic kidney (HEK) 293 cells were performed using luciferase (Luc) reporter gene assays containing a PPRE from the ACO gene promoter [pPPRE-Luc (PPRE-Luc plasmid)], a representative PPARα target. As shown in Fig. 1, the Luc activity of PPRE-Luc was markedly (60-fold) induced by cotransfection of PPARα due to lack of endogenous PPAR expression in HEK293 cells (data not shown). The PPARα-inducible expression of Luc activity was slightly, but dose dependently suppressed by coexpression of LXRα (Fig. 1A) (50% inhibition at a DNA dose ratio of 1:10). 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). This observation is consistent with a previous report describing LXR interaction with PPARα signaling (39). More interestingly, addition of LXR ligands such as 22RHC and T0901317 markedly enhanced the inhibitory effects of LXRα. LXRβ alone, without an LXR ligand, substantially inhibit PPARα activation of PPRE-Luc in a dose-dependent manner (Fig. 1B). A 50% inhibition was observed at a DNA ratio of 1:5. Additional LXR ligands further augmented the inhibitory effect of LXRβ. These effects by LXRs and LXR ligands are not due to direct inhibition of Luc activity because Luc activity from constitutive expression of Luc gene by TK promoter-Luc construct was not affected by LXR coexpression, by addition of LXR ligand, or a combination of both (Fig. 1C). These data demonstrate that LXRs and their ligands inhibit PPAR signaling with overexpressing nuclear receptors system.

LXR Cannot Bind to PPRE, but Inhibits PPARα/RXRα Binding to PPRE

The inhibitory effect of LXR was estimated in the light of the dose dependency of LXR ligands in Fig. 2. As a positive control, a dose-response curve of LXRE (LXR response element)-Luc activation by T0901317 and 22RHC with coexpression of LXRα is shown in the *upper panel*, which is consistent with previous data (29). With a similar efficiency, these LXR agonists inhibited PPARα activation of PPRE-Luc, making both curves a mirror image of each other. LXRβ coexpression gave similar results with a slightly higher efficiency.

To further investigate the mechanism for inhibition of PPARα by LXR, gel mobility shift assays were performed. The binding specificity of LXR/RXRα to LXRE, and PPARα/RXRα to PPRE was confirmed in the experiments shown in Fig. 3, A and B. RXRα is required for specific binding of both LXR and PPAR. Neither LXRα/RXRα nor LXRβ/RXRα bound to PPRE within the sensitivity of this assay. Coincubation of PPARα and LXRα or β essentially caused no shift using PPRE probe. These data exclude the possibility that LXR/RXRα competes with PPARα/RXRα binding to a response element. Next, PPARα/RXRα binding to PPRE was estimated in the presence of LXR and/or its ligand (Fig. 4). PPARα/RXRα binding to PPRE was partially inhibited by addition of LXR protein. LXR ligand en-

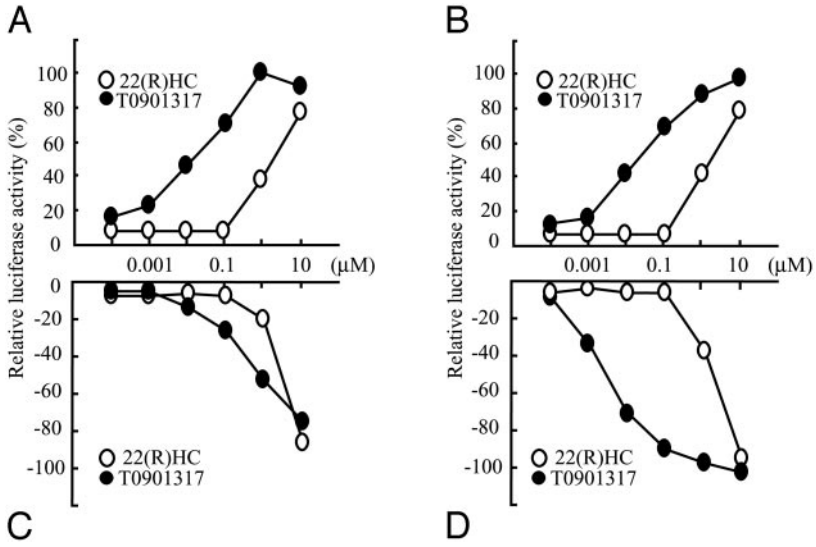

Fig. 2. Dose-Dependent Reversal Effects of LXR Ligands on LXR- and PPARα-Mediated Transactivation

HEK293 cells were transfected with 0.25 μg of pLXRE-Luc (A and B) or pPPRE-Luc (C and D) as a reporter plasmid, 0.05 μg of pCMVmLXRα (A and C), pCMVmLXRβ (B and D) as an expression plasmid and pSV-β-gal as a reference plasmid were cotransfected in the absence (A and B) or presence (C and D) of 0.05 μg of pCMV-mPPARα. Cells were treated with either ethanol as vehicle or the indicated concentrations of 22(R)HC or T0901317. All Luc activities were corrected for transfection efficiency by measuring β-gal activities.

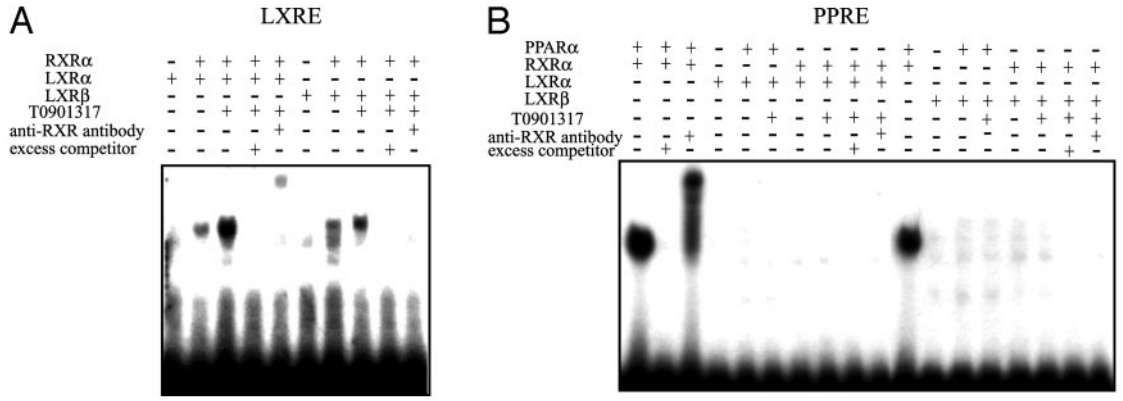

Fig. 3. No Binding of LXRα or β to the PPRE

Gel shift analysis was performed using *in vitro* translated receptor proteins as described in *Materials and Methods* with an LXR ligand (3 μM T0901317). *In vitro* translated LXRα or β (2 μl), PPARα (1 μl), and/or RXRα (1 μl) as indicated were incubated with ^{32}P-labeled SREBP1c-LXRE (A) or ^{32}P-labeled ACO-PPRE (B), and resolved on a 4.5% PAGE. Excess competitor was in 50-fold molar excess of unlabeled LXRE or PPRE.

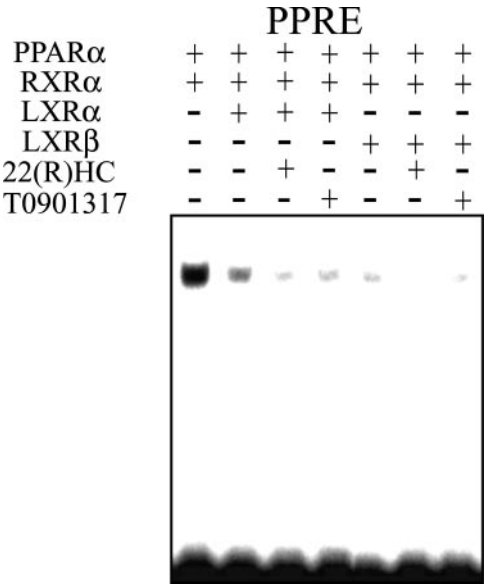

Fig. 4. LXRα and β Inhibition of PPARα/RXRα Binding to the PPRE

Gel shift analysis was performed using *in vitro* translated receptor proteins as indicated with LXR ligands as indicated. *In vitro* translated PPARα (1 μl) and RXRα (1 μl), with or without LXRα or β (4 μl) in the presence or absence of LXR ligands [10 μM 22(R)HC or T0901317] were incubated with ^{32}P-labeled ACO-PPRE, and resolved on a 4.5% PAGE.

hanced this inhibition. These data are consistent with results from reporter assays, suggesting that suppression of PPARα activity by LXR is mediated at least partly through inhibition of PPARα/RXRα binding to PPRE.

RXR Restores LXR Repression of PPARα/RXRα-Mediated Transactivation

Because PPARα and LXRs share RXRα as a heterodimer partner for their specific functions, it is conceivable that LXR inhibition of PPARα activation on PPRE-Luc is mediated through competition for RXRα when RXRα is in limiting amounts in cultured cells. Thus, the effect of supplementation with RXRα on the LXR inhibition of PPRE-Luc activity was evaluated (Fig. 5). By cotransfection of an increasing amount of RXRα, PPARα-activated PPRE-Luc activity was enhanced, indicating that the amount of RXRα is not saturated for PPARα/RXRα activation (Fig. 5A). Figure 5B shows effect of each amount of RXRα on the percent inhibition of PPARα-induced PPRE-Luc values by LXRα activation (LXRα coexpression with or without an LXR ligand, T0901317 or 22RHC). The inhibitory efficiency of LXR activation was attenuated by coexpressed RXRα in a dose-dependent manner (Fig. 5B). Similar results were obtained in the repression by LXRβ (Fig. 5C).

Next, we compared the inhibitory action of LXR on PPAR signaling with other RXR heterodimer partners, TR and farnesoid X receptor (FXR) (Fig. 6A). TRβ strongly suppressed PPRE-Luc activity, which is consistent with a previous report (37). Inhibition by FXR was modest. The ability of inhibitory action on PPARα-mediated transactivation appears to be different among LXR, TRβ, and FXR, suggesting their constitutive activity based on levels of intracellular natural ligands may influence cross-talk in heterodimeric receptors with RXRα.

cAMP response element binding protein-binding protein (CBP)/p300 is known to be involved in ligand-dependent activation of PPARs (40–42). However, unlike RXR, overexpression of CBP/p300 did not restore on LXR suppression of PPARα-induced PPRE-Luc activity (Fig. 6B).

The RXR restoration of LXR-dependent suppression was also evaluated in gel mobility shift assays. Without LXR, the signal of PPRE shifted by PPARα was enhanced with increasing amounts of RXRα, suggesting that the amount of RXRα is a limiting factor in the

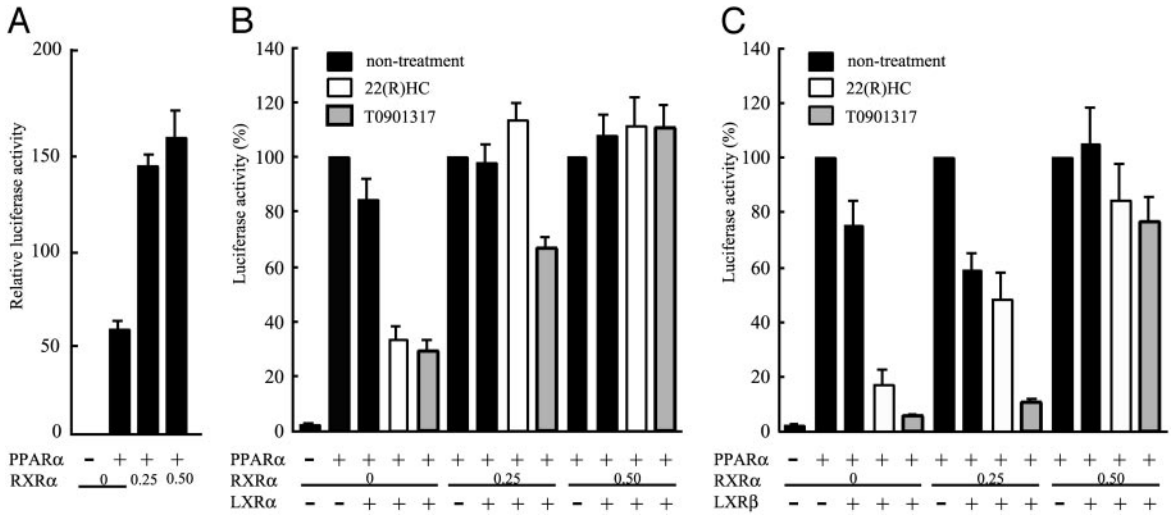

Fig. 5. Effect of Supplementation with RXRα on LXR Inhibition of PPARα/RXRα-Induced PPRE-Luc Activity

HEK293 cells were transfected with pPPRE-Luc as reporter plasmid (0.25 μg), pCMVmPPARα (0.05 μg), the indicated amount of pCMV-RXRα as expression plasmids and SV-β-gal (0.2 μg) as a reference plasmid were cotransfected (A). Inhibition of PPARα-mediated PPRE-Luc activation by LXR was evoked by cotransfection with CMV-mLXRα (B) or CMV-mLXRβ (C) (0.05 μg). RXR-restoration was shown by further cotransfection with the indicated amount of pCMV-RXRα (B and C). Cells were treated with either ethanol as vehicle, 22(R)HC or T0901317 (3 μM). All Luc activities were corrected for transfection efficiency by measuring β-gal activities. Results are means ± SE from three independent experiments.

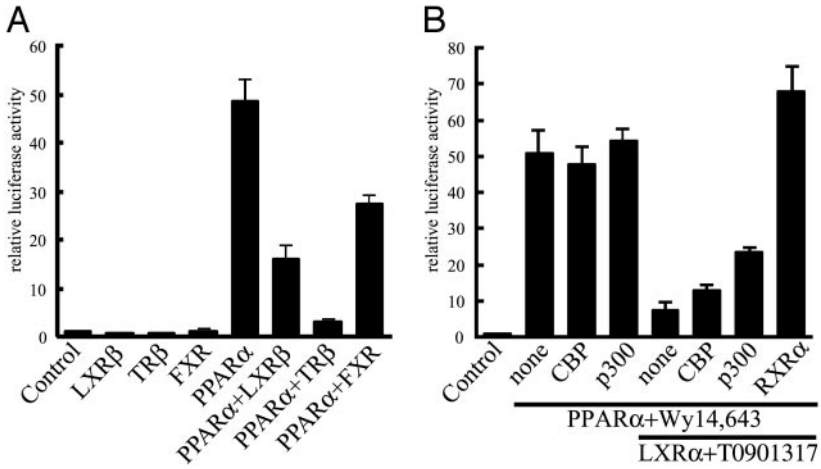

Fig. 6. Effects of RXR Heterodimers (A) and CBP/p300 (B) on PPRE-Luc Activity

A, pPPRE-Luc and pSV-renilla Luc were cotransfected into HEK293 cells with indicated expression plasmids: pCMV-mPPARα (0.25 μg), pCMV-LXRβ (0.25 μg), pCMV-TRβ (0.25 μg), and pCMV-FXR (0.25 μg). After incubation for 24 h, Luc activity was measured and normalized to renilla Luc activity. B, pPPRE-Luc and pSV-renilla Luc were cotransfected into HEK293 cells with indicated expression plasmids: pCMV-PPARα (0.25 μg), pCMV-LXRα (0.25 μg), pCMV-CBP (0.5 μg), pCMV-p300 (0.5 μg), and pCMV-RXRα (0.5 μg). Cells were treated with or without 3 μM Wy14,643 and/or 3 μM T0901317. After incubation for 24 h, Luc activity was measured and normalized to renilla Luc activity. All results are means ± SE from three independent experiments.

range of PPARα/RXRα ratio used here (Fig. 7A). With the lowest amount of RXRα, addition of LXRs and T0901317 caused a significant decrease in PPARα/RXRα binding to PPRE (Fig. 7, A and B). The inhibitory effect of LXRα activation was abolished with increasing concentrations of RXRα. In the case of LXRβ, RXR restoration of the binding was partial even at the highest amount, reflecting stronger LXRβ inhibitory effect. These data are consistent with the results from cell reporter assays (Fig. 5), providing additional evidence for the hypothesis that LXR inhibits PPARα activation by competing with it for heterodimerization with RXRα.

LXR Can Bind to PPARα and Inhibits Formation of PPARα/RXRα Heterodimer

To explore the heterodimerization of the three nuclear receptors, mutual protein-protein interactions were in-

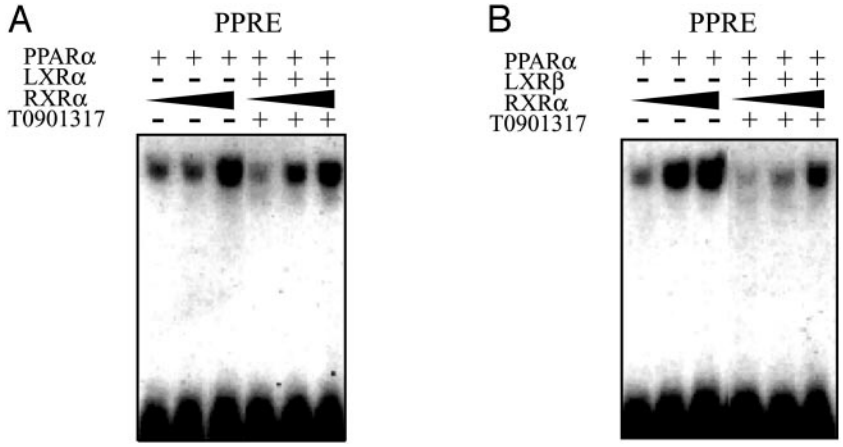

Fig. 7. Effect of Supplementation with RXRα on LXR Inhibition of PPARα/RXRα Binding to PPRE

Gel shift analysis was performed using *in vitro* translated receptor proteins as indicated. *In vitro* translated PPARα (1 μl), RXRα (1, 2, and 4 μl), and LXRα (2 μl, A) or LXR β (2 μl, B) were incubated with ^{32}P-labeled PPRE in the absence or presence of T0901317 (3 μM) and resolved on a 4.5% PAGE.

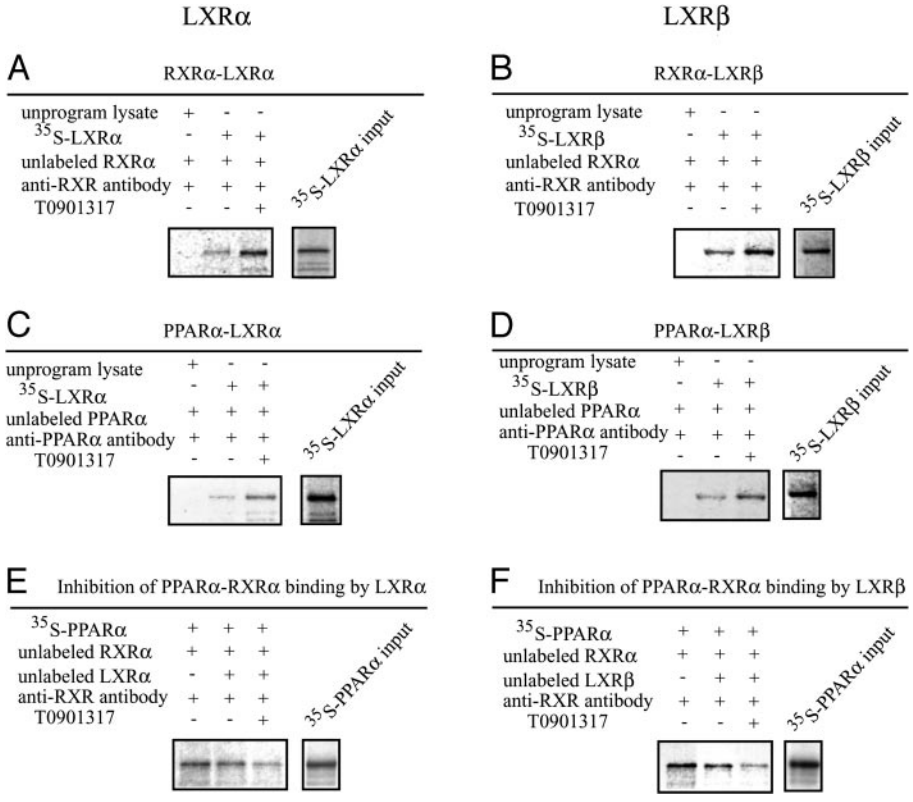

Fig. 8. LXR Inhibits PPARα/RXRα Heterodimerization by the Formation of LXR/RXRα and LXR/PPARα

In vitro translated receptor proteins as indicated were used for protein-protein interaction assay. Indicated proteins were used in the absence or presence of T0901317. Coimmunoprecipitation of the unlabeled RXRα and ^{35}S-labeled LXRα (A) or β (B) with an anti-RXR antibody; unlabeled PPARα, and ^{35}S-labeled LXRα (C) or β (D); ^{35}S-labeled PPARα, unlabeled RXRα, and unlabeled LXRα (E) or β (F). After precipitation with protein G-sepharose, samples were run on SDS-PAGE.

vestigated (Fig. 8). First, LXRα protein was labeled with ^{35}S-methionine, incubated with unlabeled RXRα protein, and precipitated with anti-RXRα antibody. Resolution of immunocomplex on SDS-PAGE detected labeled LXRα, indicating formation of an LXRα/RXRα heterodimer. The complex formation was markedly enhanced by addition of T0901317, suggesting that LXR ligand increases affinity for LXRα/RXRα het-

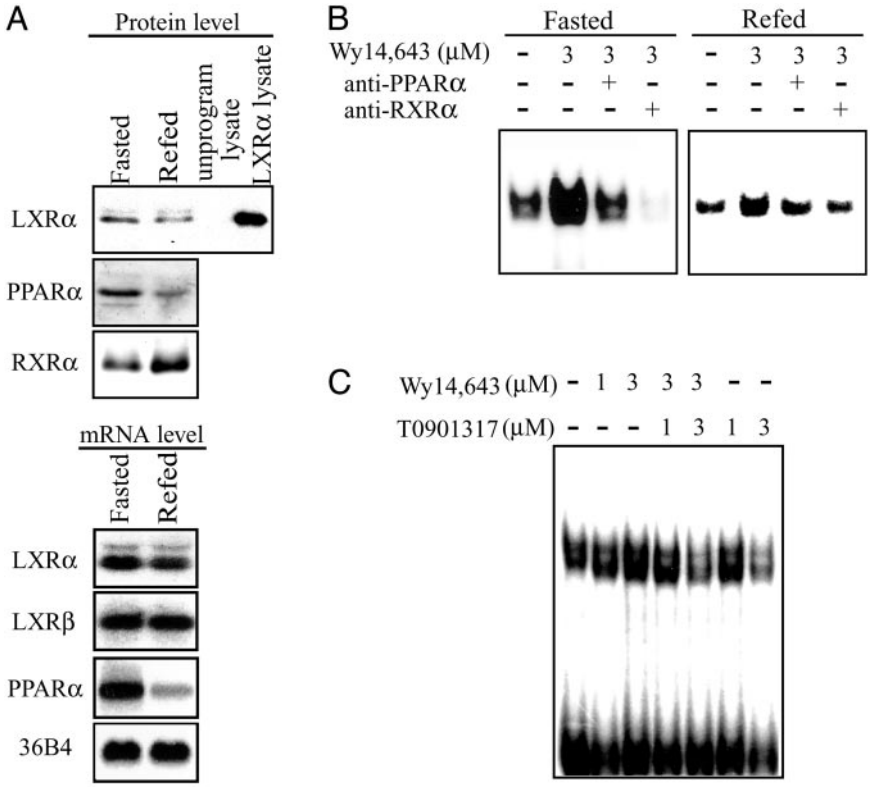

Fig. 9. PPARα Activator Inhibits LXR Ligand-Induced LXR/RXRα Binding to PPRE in Hepatic Nuclear Extracts

A, Protein and mRNA levels of PPARα, LXRα, and RXRα in hepatic nuclear extracts from fasted or refed mice. For fasting and refeeding treatment, mice were fasted for 24 h and fed a high sucrose/fat-free diet for 12 h. Equal protein amounts (20 μg) in nuclear extracts were subjected to SDS-PAGE, and immunoblotted with an anti-PPARα, anti-LXRα, or anti-RXRα antibody. Total RNA was isolated from the livers of mice from each group, pooled, and subjected to Northern blot analysis with the indicated cDNA probes. B, Confirmation of presence of PPARα in Wy14,643 induced-complexes of nuclear proteins/ACO-PPRE in nuclear extracts from fasted mice. The ^{32}P-labeled ACO-PPRE probe and hepatic nuclear extracts (1 mg) from fasted mice were incubated with Wy14,643 in the presence or absence of anti-PPARα (H-98, sc-9000, Santa Cruz Biotechnology Inc.) or anti-RXRa (D-20, sc-553, Santa Cruz Biotechnology Inc.) antibody. The DNA-protein complexes were resolved in a 4.5% PAGE. C, Wy14,643 induced-PPARα/RXRα binding to PPRE was inhibited by addition of T0901317. ^{32}P-labeled ACO-PPRE probe and hepatic nuclear extracts from fasted mice were incubated with Wy14,643 and/or T0901317. The DNA-protein complexes were resolved in a 4.5% PAGE.

erodimerization (Fig. 8A). We also tested the possibility of formation of PPARα/LXRα heterodimers, as was previously described (39). Labeled LXRα was incubated with PPARα and immunoprecipitated with anti-PPARα antibody. PPARα/LXRα complex was detected with an intensity comparable to LXRα/RXRα, suggesting that LXRα can heterodimerize with PPARα with similar efficiency to RXRα (Fig. 8C). T0901317 enhanced this interaction. LXRβ gave very similar results (Fig. 8, B and D). The gel shift assay (Fig. 3B) showed no retardation of the PPRE probe in the presence of both PPARα and LXRα, indicating that PPARα/LXRα heterodimers do not bind to PPRE. Therefore, LXRα could show inhibition of PPARα signaling by forming not only LXRα/RXRα, but also PPARα/LXRα in a competition with functional PPARα/RXR formation. To show this, effects of LXRs on PPARα/RXRα interaction were estimated in this protein interaction assay. PPARα protein was labeled, incubated with RXRα in the presence or absence of LXRα, precipitated with anti-RXRα antibody, and run on gels. PPARα/RXRα heterodimer formation was inhibited by addition of LXRα or LXRβ with T0901317 (Fig. 8, E and F), suggesting that PPARα/LXR as well as LXR/RXRα could prevent formation of PPARα/RXRα and participate in repressing PPARα signaling.

LXR Activation Inhibits PPARα/RXRα Binding to PPRE in Hepatic Nuclear Extracts

To examine suppressive action of LXR on PPAR/RXR binding to PPRE in a more physiological condition, gel mobility shift assays were performed using hepatic nuclear extracts from fasted mice. In nuclear extracts from fasted mice, LXRα and RXRα were abundant on immunoblot analysis, although PPARα was decreased after refeeding (Fig. 9A) as well as at mRNA level. Gel shift mobility assays demonstrated that the PPRE probe was shifted by coincubation with hepatic nuclear extracts. The signal of the complexes was stron-

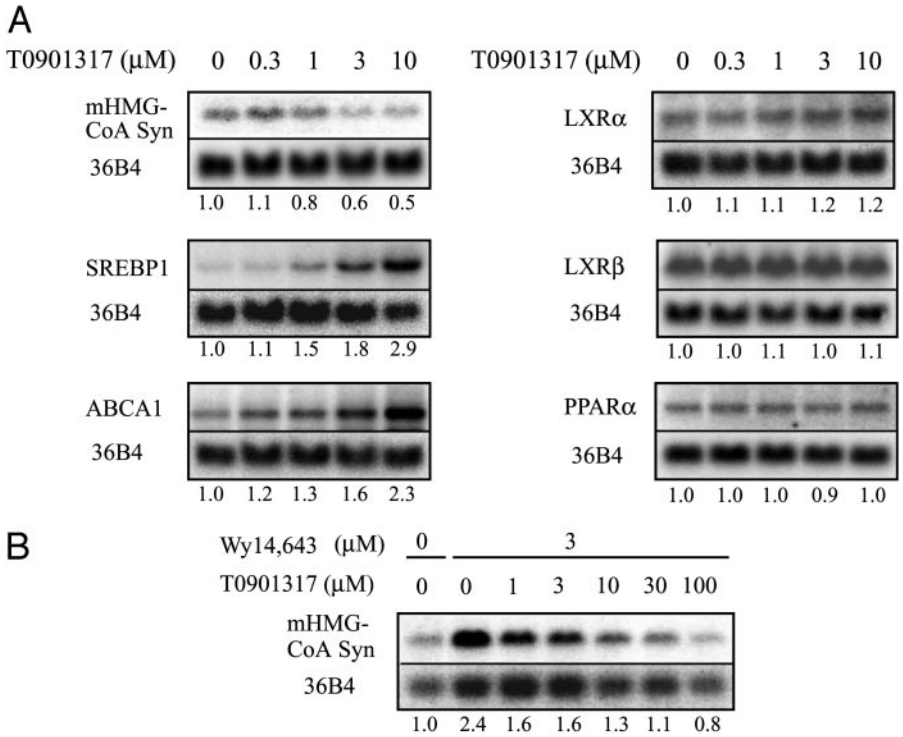

Fig. 10. LXR Ligand Suppresses PPARα-Target Gene Expression in Rat Primary Hepatocytes

A, T0901317 inhibited gene expression of mHMG-CoA Syn in rat primary hepatoctyes. The cells were incubated with the indicated concentration of T0901317 for 24 h. B, T0901317 suppressed induction of mHMG-CoA Syn gene expression by Wy14,643 treatment. The hepatocytes were incubated with the indicated concentration of T0901317 in the absence or presence of Wy14643 for 24 h. Total RNA (10 μg) was extracted and Northern blot analysis was performed with the indicated cDNA probes. Fold changes of expression relative to corresponding with controls are shown.

ger from fasted mice than in a refed state, was enhanced by addition of Wy14,643 and declined after addition of anti-PPARα or anti-RXRα antibody, confirming the presence of PPARα/RXRα in the hepatic nuclear extracts from fasted mice and its specific binding to PPRE (Fig. 9B). The PPRE complex also contained a small amount of unidentified RXR heterodimer(s). T0901713 suppressed the signal of the PPARα/RXRα-PPRE complex induced by Wy14,643 (Fig. 9C). Thus, these data indicate that LXR ligand suppressed PPARα ligand-induced PPARα/RXRα-PPRE complexes in a condition with physiological levels and spectrum of various nuclear receptors.

LXR Activation Represses PPARα Target Gene in Primary Hepatocytes and Mouse Livers

To extend these findings to a system with more physiological relevance, LXR interference with PPARα signaling was estimated using their ligands in rat primary hepatocytes. Hepatocytes were incubated with increasing amounts of T0901317 and expression of related target genes was estimated by Northern blot analysis (Fig. 10). LXR activation was confirmed by a dose-dependent increases in SREBP-1c and ABCA1 mRNA levels (Fig. 10A). LXR ligand dose dependently suppressed a representative PPARα gene, mHMG-CoA syn mRNA level. In the presence of Wy14,643 at a concentration sufficient to induce mHMG-CoA syn, a clear dose-dependent suppression by T0901317 was still observed (Fig. 10B). These data demonstrate that LXR activation by its ligand suppresses PPARα-target genes, in a competitive manner with PPARα activation, supporting LXR interference with PPARα signaling in primary hepatocytes. To further evaluate the *in vivo* physiological significance of LXR interference to PPARα-mediated transactivation, similar conditions in mouse livers were recreated. Mice were fasted to induce hepatic endogenous PPARα and its downstream genes (17, 31) and were treated with T0901317 to activate LXR. Various PPARα-target genes in the livers such as mHMG CoA Syn, ACO, CPTI, and cytochrome P450A2 were estimated by Northern blot analysis. Wy14,643 was also administered to fasted mice as a positive control for activation of PPARα target genes. As shown in Fig. 11A, hepatic mRNA levels of these PPARα-regulated genes from T0901317 treated animals were considerably decreased, whereas Wy14643 treatment increased expression of these genes. The activation of LXR by T0901317 was confirmed by observational increases in SREBP-1 and ABCA1 mRNA levels, both of which are well-known LXR target genes (Fig. 11B). These data demonstrate that LXR activation

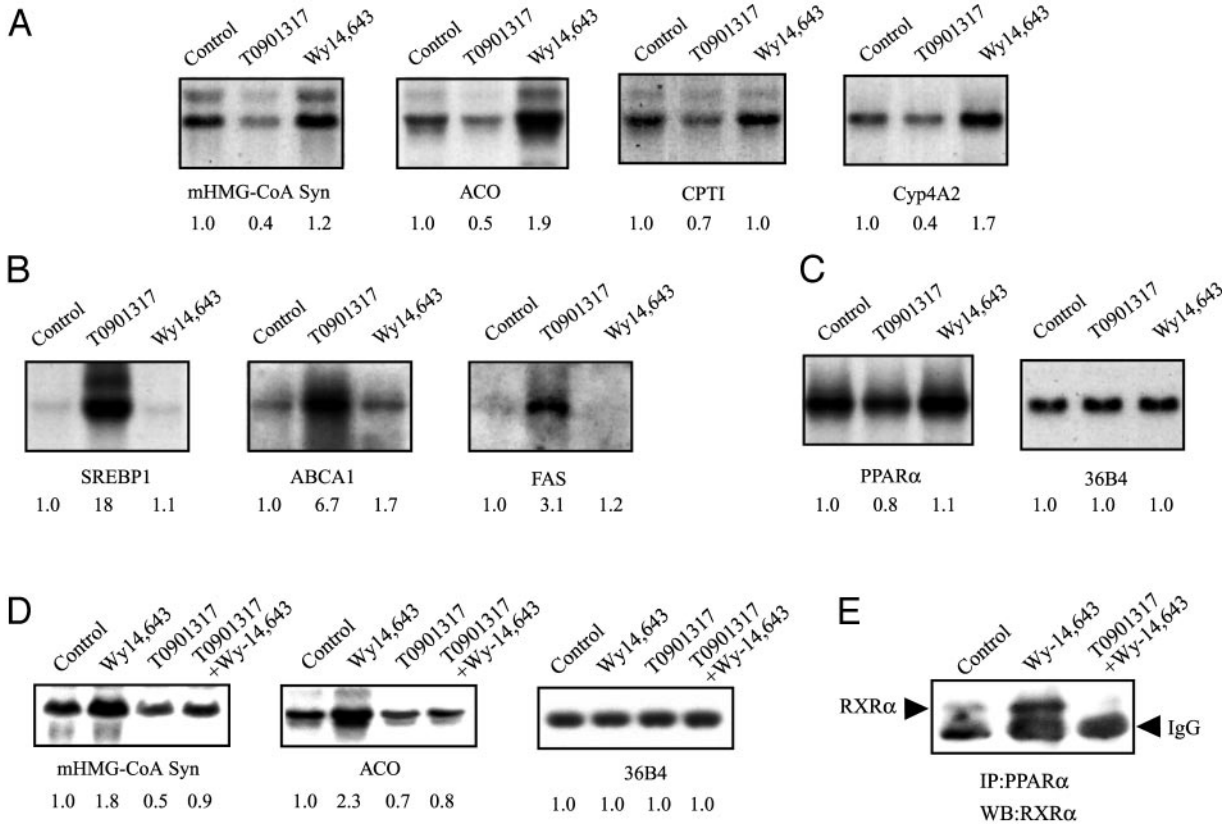

Fig. 11. Effect of LXR Ligand on PPARα Target Gene Expression in the Livers of Fasted Mouse

A–C, Mice (n = 5) were fasted and treated with T0901317 (50 mg/kg) or Wy14,643 (50 mg/kg) for 18 h. D, Mice (n = 3) were fasted and treated with T0901317 (50 mg/kg), Wy14,643 (50 mg/kg), or both these ligands for 18 h. Total RNA was isolated from the livers of mice from each group, pooled, and subjected to northern blot analysis with the indicated cDNA probes. Fold increases of expression relative to corresponding with vehicle-treated controls are shown. E, T0901317 inhibited Wy14,643-induced LXR/RXRα heterodimer formation in the liver from C57BL6 mice. Hepatic nuclear extracts were prepared from each group. Equal protein amounts of nuclear extracts were subjected to immunoprecipitation by using anti-PPARα antibody, which had been coupled to protein G-sepharose beads. Immunoprecipitates were subjected to SDS-PAGE, and immunoblotted with an anti-RXRα antibody. *Arrowheads* represent RXRα or IgG signal.

by LXR ligand can affect PPARα signaling in mouse livers. In contrast, Wy14,643 alone slightly increased ABCA1 mRNA (Fig. 11B), which is consistent with recently reports of PPAR-LXR-ABCA1 pathway in macrophages (43–45). Moreover, we studied LXR interference in mice treated with both PPARα and LXR ligands. As estimated by Northern blot analysis of mHMG-CoA syn and ACO mRNA levels, these PPARα-target genes were suppressed by T0901317 in both Wy14,643-induced and untreated conditions (Fig. 11D). Immunoprecipitation assays of liver nuclear extracts from these mice demonstrated that PPARα/RXRα heterodimers were increased by Wy14,643 treatment and this induction was suppressed by T0901317. These results suggested that LXRα ligand could repress hepatic expression of the PPARα target genes via reduction of PPARα/RXRα formation in liver nucleus (Fig. 11E), extending the hypothesis from transfection studies to *in vivo* conditions.

DISCUSSION

The current studies demonstrate that LXR activation by either overexpression of LXR or its ligand activation causes suppression of PPARα signaling. The Luc, gel shift, and protein-protein binding assays show that LXR activation inhibits formation of PPARα/RXRα, and thus, their binding to PPRE. RXRα supplementation restored this LXR inhibition of PPAR action, indicating that the mechanism could be at least partially explained by RXRα competition between PPAR and LXR. Protein-protein interaction experiments established the formation of PPARα/LXRα and PPARα/LXRβ as well as LXR/RXR heterodimers. Direct binding between PPARα and LXRα has been found in a two-hybrid system, and involvement of LXRα in PPAR signaling has been implicated (39). We further found that PPARα/LXR formation is enhanced by LXR ligand. PPARα/LXR complex cannot bind to either PPRE or LXRE. Therefore, this heterodimer can interfere with

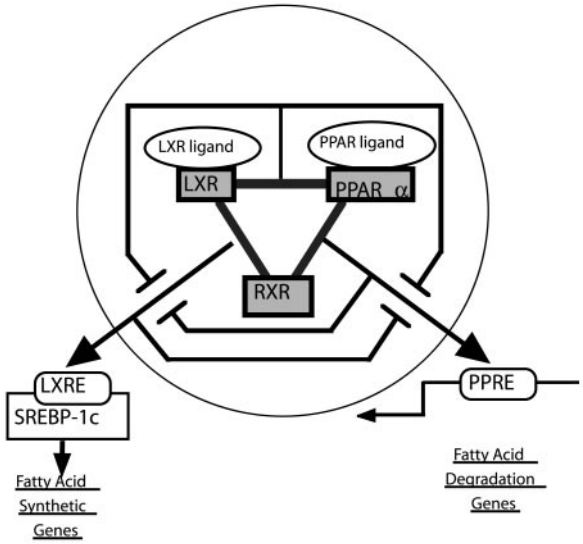

Fig. 12. Mutual Interactions between PPARα and LXR-SREBP-1c in Reciprocal Regulation of Fatty Acid Metabolism

Current cross-talk of PPARα, LXR, and SREBP-1c in nutritional regulation is schematized, based upon the current study and the accompanying paper (38).

both PPAR and LXR action and can contribute to PPAR inhibition of the LXR-SREBP-1c system as proposed in the accompanying paper (38) as well as LXR inhibition of PPAR signaling as described in the current study. Consistently, the combined treatment with both PPARα and LXR agonists resulted in simultaneous reduction of both PPARα/RXRα and LXR/RXRα [Fig. 11E in this paper and Fig. 9B in the accompanying paper (38)], implicating an important contribution of PPARα/LXR formation in reciprocal inhibition of LXR and PPARα signaling. This can also explain the restoration by supplementation with RXR, not only by providing sufficient RXR for functional RXR heterodimers, but also by preventing nonfunctional PPARα/LXR formation.

LXR inhibition of PPARα signaling observed from transfection studies with cultured cells is likely to be extendable to the *in vivo* regulation of the hepatic energy metabolism. It is noteworthy that administration of LXR agonist reduced hepatic nuclear PPARα/RXR and impaired expression of hepatic fatty acid degradation enzyme genes both in rat primary hepatocytes and livers of fasted mice (Figs. 10 and 11). In a fasted state, hepatic PPARα expression is highly induced and fatty acids are recruited as ligands for PPARα activation (17, 18). Nutritional condition does not markedly change the expression of hepatic LXRα (29). Therefore, LXR ligand can activate hepatic LXRα and cause cross-talk with PPARα/RXR leading to suppression of expression of PPARα target genes involved in lipid degradation. These data demonstrate that LXR interference with PPAR signaling can occur *in vivo*. In the accompanying paper (38), we observed a mirror image observation, that PPARα activation inhibits ligand-induced LXR signaling in hepatocytes.

The triangle relationship among LXR, PPAR, and RXR could be crucial for mutual regulation of both LXR and PPAR activities, and thus nutritional regulation of their downstream genes. PPARα is involved in fatty acid degradation as an adaptic control of energy depletion. Meanwhile, SREBP-1c, whose expression is dominated by LXR is involved in fatty acid synthesis for storage of excess energy. These opposite nutritional regulators are reciprocally up- and down-regulated depending upon energy states. As summarized in Fig. 12, our findings suggest that the mutual suppression efficiently facilitates these reciprocal actions of PPARα and LXR-SREBP-1c systems. These studies should open up a new paradigm of a novel cross-talk of nutritional transcription factors in energy metabolism where the nuclear concentrations of each receptor and ligand are crucial for nutritional regulation for fatty acid metabolism. It is also important to investigate whether the cross-talk of these receptors involves a potential competition for their coactivators such as CBP/p300 (42, 46, 47), steroid receptor coactivator-1 (48–50), and PPARγ coactivator-1 (51, 52). Further studies should focus on the precise mechanisms for this network.

MATERIALS AND METHODS

Materials

Anti-PPARα sc-9000, RXRα D20 sc-553, and LXRα C-19 sc-1201 antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), Redivue [α-^{32}P]deoxy-CTP (6,000 Ci/mmol) and Protein G Sepharose from Amersham Biosciences Inc. (Uppsala, Sweden), *in vitro* transcription and translation kit (TNT Coupled Reticulocyte Lysate System) from Promega (Madison, WI), and other chemicals from Sigma (St. Louis, MO). T0901317 (*N*-methyl-*N*-[4-(2, 2, 2-trifluoro-1-hydroxy-1-trifluoromethylethyl)-phenyl]-benzenesulfonamide) was provided by Kyorin Pharmaceutical Co. Ltd. (Tochigi, Japan).

Plasmids

Cytomegalovirus (CMV) promoter expression plasmid of human RXRα and the Luc reporter gene construct, PPRE from ACO gene promoter fused upstream from thymidine kinase (TK) promoter (pPPRE-Luc), and pCMV-RXR were kind gifts 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. The LXRE of SREBP-1c promoter-Luc construct (pLXRE-Luc), pCMV-mLXRα and pCMV-mLXRβ were prepared as previously described (29). CMV or T7 promoter expression plasmid of mouse (m) PPARα (1–468, amino acid) (16) was prepared by PCR, and the cDNA was introduced into pCMV7 or pBluescript II SK vector, respectively. T7 promoter expression plasmids of mLXRα and mLXRβ were prepared using pBluescript II SK vector.

Transfections and Luc Assays

HEK293 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 (29). The indicated amount of each expression plasmid was transfected simultaneously with a Luc reporter plasmid (0.25 μg) and simian virus 40-β-galactosidase (β-gal) plasmid (0.2 μg) or (0.05 μg). The total amount of DNA in each transfection was adjusted to 1.5 μg/well with the vector DNA, pCMV7. 22RHC and T0901713 were dissolved in ethanol. Each agent was treated 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 activity.

Gel Mobility Shift Assays

Gel mobility shift assays were performed as previously described (53). Briefly, mLXRα, mLXRβ, mPPARα, and human (h) RXRα proteins were generated from the expression vectors using a coupled *in vitro* transcription/translation system (Promega). Double-stranded oligonucleotides used in gel mobility shift assays were the LXREb of the SREBP-1c promoter (29) and PPRE of the ACO promoter (16). 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 lysates (1 to 4 μl), or hepatic nuclear extract (1 μg) in a mixture (20 μl) 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, 0.4 μg/ml poly(deoxyinosine-deoxycytidine), 0.1% Triton X-100, and 1 mg/ml nonfat milk for 60 min on ice. The DNA-protein complexes were resolved on a 4.5% PAGE at 100 V for 2 h at 4 C. Gels were dried and exposed to the filter of BAS2000 with BAStation software (Fuji Photo Film, Kanagawa, Japan).

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 or 200 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 rabbit or goat polyclonal antibodies 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 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 (54). 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 for 24 h.

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), Wy14,643 (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 (55). Hepatic nuclear protein were prepared from the livers as previously described (56), were subjected to immunoblotting with the anti-PPARα, anti-LXRα, or anti RXRα antibodies.

Northern Blot Analysis

Total RNA was extracted from livers and rat primary hepatocytes 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, fatty acid synthase, ACO, cytochrome P450A2, PPARα, LXRα and β, ABCA1 and acidic ribosomal phosphoprotein PO (36B4) were prepared as previously described (29, 31). The cDNA probes for L-FABP, CPTI, and mHMG-CoA Syn were provided by Kyorin Pharmaceutical Co. LTD. Each signal was analyzed with BAS2000 and BAStation software (Fuji Photo Film).

Acknowledgments

We are grateful to A. H. Hasty for critical reading of the manuscript. We also thank T. Kitamine, H. Okazaki, Y. Tamura, and M. Sekiya for helpful discussion.

Received May 23, 2002. Accepted April 14, 2003.

Address all correspondence and requests for reprints to: Hitoshi Shimano, M.D., Ph.D., Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. E-mail: shimano-tky@umin.ac.jp.

This study was supported by the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research.

T.I. and H.S. equally contributed to this work.

REFERENCES

1. Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D 1992 Evolution of the nuclear receptor gene superfamily. EMBO J 11:1003–1013
2. 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
3. Willson TM, Brown PJ, Sternbach DD, Henke BR 2000 The PPARs: from orphan receptors to drug discovery. J Med Chem 43:527–550
4. 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
5. Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, Wahli W 1993 Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). Biol Cell 77:67–76
6. Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Naar AM, Kim SY, Boutin JM, Glass CK, Rosenfeld MG 1991 RXR β: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell 67:1251–1266
7. Kliewer SA, Umesono K, Mangelsdorf DJ, Evans RM 1992 Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. Nature 355:446–449
8. Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen JY, Staub A, Garnier JM, Mader S, Chambon P 1992 Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. Cell 68:377–395
9. Zhang XK, Hoffmann B, Tran PB, Graupner G, Pfahl M 1992 Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. Nature 355: 441–446
10. Forman BM, Chen J, Evans RM 1997 Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. Proc Natl Acad Sci USA 94:4312–4317
11. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM 1992 Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 358: 771–774
12. Gearing KL, Gottlicher M, Teboul M, Widmark E, Gustafsson JA 1993 Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor. Proc Natl Acad Sci USA 90:1440–1444
13. Osumi T, Wen JK, Hashimoto T 1991 Two cis-acting regulatory sequences in the peroxisome proliferator-responsive enhancer region of rat acyl-CoA oxidase gene. Biochem Biophys Res Commun 175:866–871
14. Rodriguez JC, Gil-Gomez G, Hegardt FG, Haro D 1994 Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J Biol Chem 269: 18767–18772
15. Louet JF, Chatelain F, Decaux JF, Park EA, Kohl C, Pineau T, Girard J, Pegorier JP 2001 Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor α (PPARα)-independent pathway. Biochem J 354:189–197
16. Issemann I, Green S 1990 Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645–650
17. 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
18. Leone TC, Weinheimer CJ, Kelly DP 1999 A critical role for the peroxisome proliferator-activated receptor α (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
19. Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T 1998 Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J Biol Chem 273:29577–29585
20. 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
21. 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
22. 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
23. Russell DW 1999 Nuclear orphan receptors control cholesterol catabolism. Cell 97:539–542
24. 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
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. Zhang Y, Repa JJ, Gauthier K, Mangelsdorf DJ 2001 Regulation of lipoprotein lipase by the oxysterol receptors, LXRα and LXRβ. J Biol Chem 276:43018–43024
27. Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M 1994 A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. Mol Cell Biol 14:7025–7035
28. Shinar DM, Endo N, Rutledge SJ, Vogel R, Rodan GA, Schmidt A 1994 NER, a new member of the gene family encoding the human steroid hormone nuclear receptor. Gene 147:273–276
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. 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
31. 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
32. 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
33. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL 1997 Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 99:846–854
34. Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD 1998 Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J Biol Chem 273:35299–35306
35. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B 2000 Role of LXRs in control of lipogenesis. Genes Dev 14:2831–2838

36. 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 β-oxidation enzymes: cross talk between peroxisome proliferator and T3 signaling pathways. Proc Natl Acad Sci USA 92:11593–11597
37. 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
38. Yoshikawa T, Ide T, Shimano H, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Yatoh S, Kitamine T, Okazaki H, Tamura Y, Sekiya M, Takahashi A, Hasty AH, Sato R, Sone H, 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. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. Endocrinology 144:1240–1254
39. 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
40. McKenna NJ, O'Malley BW 2002 Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 108:465–474
41. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV 1998 Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. Nature 395:137–143
42. Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, Leid M 1997 p300 functions as a coactivator for the peroxisome proliferator-activated receptor α. J Biol Chem 272:33435–33443
43. 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
44. 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
45. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, Tontonoz P 2000 Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR α. Proc Natl Acad Sci USA 97:12097–12102
46. Gelman L, Zhou G, Fajas L, Raspe E, Fruchart JC, Auwerx J 1999 p300 interacts with the N- and C-terminal part of PPARγ2 in a ligand-independent and -dependent manner, respectively. J Biol Chem 274:7681–7688
47. Schulman IG, Shao G, Heyman RA 1998 Transactivation by retinoid X receptor-peroxisome proliferator-activated receptor γ (PPARγ) heterodimers: intermolecular synergy requires only the PPARγ hormone-dependent activation function. Mol Cell Biol 18:3483–3494
48. Westin S, Kurokawa R, Nolte RT, Wisely GB, McInerney EM, Rose DW, Milburn MV, Rosenfeld MG, Glass CK 1998 Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. Nature 395: 199–202
49. 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
50. Zhu Y, Qi C, Calandra C, Rao MS, Reddy JK 1996 Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor γ. Gene Expr 6:185–195
51. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829–839
52. Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman BM 1999 Activation of PPARγ coactivator-1 through transcription factor docking. Science 286: 1368–1371
53. 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 275:31078–31085
54. Massague J, Guinovart JJ 1977 Insulin control of rat hepatocyte glycogen synthase and phosphorylase in the absence of glucose. FEBS Lett 82:317–320
55. 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
56. 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