The Orphan Nuclear Hormone Receptor LXRα Interacts with the Peroxisome Proliferator-activated Receptor and Inhibits Peroxisome Proliferator Signaling*

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The yeast two-hybrid system was used to isolate novel cellular factors that interact with the mouse peroxisome proliferator-activated receptor α (PPARα). One of the interacting clones isolated encoded LXRα, a recently described human orphan nuclear hormone receptor. LXRα bound directly to PPARα, as well as to the common heterodimerization partner 9-cis-retinoic acid receptor (RXRα). LXRα did not form a DNA binding complex with PPARα on synthetic hormone response elements composed of direct repeats of the TGACCT consensus half-site or on naturally occurring peroxisome proliferator response elements (PPREs) or LXRα response elements. However, LXRα inhibited binding of PPARα/RXRα heterodimers to PPREs, and coexpression of LXRα in mammalian cells antagonized peroxisome proliferator signaling mediated by PPARα/RXRα in vivo. These findings identify a novel partner for PPARα and suggest that LXRα plays a role in modulating PPARα signaling pathways in the cell.

Peroxisome proliferator-activated receptors (PPARs)1 are re-
cently described members of the ligand-activated nuclear hormone receptor superfamily, which includes receptors for steroids, vitamin D, and thyroid and retinoid hormones (1). PPARs have been shown to regulate a broad spectrum of genes involved in lipid metabolism, cellular growth, and differentiation (2). Consequently, there is a great deal of interest in understanding their specificity and mechanisms of action. 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 (3, 4). Subsequently, PPARs were shown to be differentially activated by a variety of long chain fatty acids and lipid-like compounds (5), suggesting that fatty acids or fatty acid derivatives serve as physiological activators. PPARs exist in a variety of pharmacologically distinct subtypes and isoforms that are differentially expressed and which mediate distinct patterns of tissue-specific gene expression (4, 6–8). For example, mouse (m) PPARα triggers adipogenesis in cultured cells (8) and is selectively activated by 15-deoxy-Δ12,14-prostaglandin J2, a recently identified high affinity ligand of this PPAR subtype (9, 10).

PPARs activate expression of target genes by recognizing peroxisome proliferator response elements (PPREs) composed of TGAACAT-related direct repeats that are spaced by one nucleotide (DR1) (11, 12). Specific DNA binding is manifested through heterodimerization with the 9-cis-retinoic acid receptor, RXRα (13, 14), another member of the nuclear hormone receptor superfamily that also serves as a heterodimerization partner for thyroid hormone, retinoic acid, and vitamin D receptors (1). The involvement of PPARs in multiple and diverse cellular functions suggests that these receptors may be integrated with other cellular signaling pathways, in addition to the well characterized RXRα pathway. Indeed, the reciprocal modulation of thyroid hormone and peroxisome proliferator-responsive genes through cross-talk between thyroid hormone receptors and PPARs has recently been demonstrated (15–17). Moreover, it has been reported that rat PPARα heterodimerizes with the thyroid hormone receptor (18), although this conclusion remains controversial (15). Unraveling the pleiotropic functions of PPARs requires identification of the full spectrum of factors that interact with PPARs. In this report, we used the yeast two-hybrid system (19) to isolate novel factors that interact with mPPARα. One mPPARα-interacting factor isolated was identified as LXRα, a recently described human orphan nuclear hormone receptor that appears to be involved in a novel retinoid signaling pathway (20). LXRα inhibited the binding of mPPARα/RXRα to PPREs in vitro and antagonized transcriptional activation by mPPARα in vivo. Our findings demonstrate that nuclear receptors other than RXRα bind directly to PPARα and may play a role in modulating the cellular functions of this receptor.

MATERIALS AND METHODS

Two-hybrid Library Screening—Yeast two-hybrid vectors expressing full-length human RXRα and mPPARα as fusions to the GAL4 DNA-binding domain (GBD-RXRα and GBD-mPPARα, respectively) and RXRα fused to the GAL4 activation domain (GAD-RXRα) have been described (21). GBD-mPPAR was not suitable for two-hybrid library screening, since it induced a low level of constitutive activity of the β-galactosidase reporter gene when expressed alone in yeast (21). We

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1 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; mPPAR, mouse PPAR; GBD, GAL4 DNA-binding domain; GAD, GAL4 activation domain; BSA, bovine serum albumin; AOX, fatty acyl-CoA oxidase; DR, direct repeat; HD, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; kbp, kilobase pair; LXRα, LXRα response element; MBP, maltose-binding protein; PPRE, peroxi-
therefore constructed a modified vector, GBD-NmPPAR (missing the amino-terminal 83 amino acid codons of mPPARα) that was devoid of this intrinsic activation function but remained capable of interacting with RXRα (see Table I). This derivative was used as bait in the two-hybrid system. Saccharomyces cerevisiae strain H7c7c (MATa, ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3, 112, gal-4-524, leu2-3, 112, his3-ΔCysC14, lys2-188, his3-ΔCysC14) harboring GBD-NmPPAR was subjected to electroporation (22) with a HeLa cell cDNA library constructed in the GAD vector pGADGH (Clontech). Transformsants were plated onto synthetic complete media plates lacking histidine, leucine, and tryptophan and containing 25 mg 3-amino-1,2,4-triazole. His+, leu+, trp+ colonies were assayed for expression of mPPARα reporter gene by agarose overlay assay using 0.2% (w/v) Triton X-100 as a permeabilization agent. Library plasmids were rescued by electroporation into Escherichia coli ElectroMAX DH10B (Life Technologies, Inc.), and isolated plasmids were used to transform S. cerevisiae H7c7c. Candidate clones were tested for interaction against GBD-NmPPAR and to GBD-RXRα and also to irrelevant fusion proteins and the empty fusion vector so as to eliminate false positives (23) and to ensure that the his+ and β-galactosidase phenotypes were dependent on the presence of both the respective GAD-cDNA library vectors and GBD-NmPPAR. Positive clones were recovered and subjected to partial 5′- and 3′-sequence analysis. Two clones contained the same partial cDNA that encoded a novel member of the nuclear hormone receptor superfamly, subsequently shown to be nearly identical to the recently described human LXRα (20). One of these clones (GAD-SM1; encoding amino acid residues 61–447 of LXRα) was selected for further analysis. The remaining positive clones were not related to LXRα or to other nuclear receptors and will be the subject of future studies.

Cloning a Full-length LXRα cDNA—The 8.1-kilobase pair (kbp) insert of GAD-SM1 was labeled with [γ-32P]dATP by random priming and used to probe, under high stringency conditions, a 5′-stretch ofgt11 human liver cDNA library (Stratagene). The largest hybridizing clone contained a 1.7-kbp insert. The cDNA was sequenced in both directions and shown to contain the entire 447-amino acid long open reading frame of LXRα (20). The 1.2-kilobase pair (kbp) insert of GAD-SM1 was labeled with (α-32P)dATP by random priming and used to probe, under high stringency conditions, a 5′-stretch ofgt11 human liver cDNA library (Stratagene). The largest hybridizing clone contained a 1.7-kbp insert. The cDNA was sequenced in both directions and shown to contain the entire 447-amino acid long open reading frame corresponding to the published sequence of LXRα (20).

In Vitro Transcription/Translation—in vitro expression vectors for mPPARα and human RXRα were described (14). The entire open reading frame of LXRα was amplified from the human liver cDNA library by the polymerase chain reaction (forward primer, 5′-GGCCGGATCCGTCGACATCGTCTGTTGCGGGG; reverse primer, 5′-GGCCGGATCCGATATTTTCATCTTGCACTCCAGATC; initiator codon is underlined) and cloned into the BglII site of the SP6 transcription vector pSPUTK. Transcription of the different cDNAs followed by translation in rabbit reticulocyte lysates was performed using a coupled system (Promega), as described previously (14, 24).

Protein Binding Assays—Maltose-binding protein (MBP)-mPPARα and MBP-RXRα fusion protein expression vectors were constructed in pMAL-2c (New England Biolabs), and the fusion proteins were purified by affinity chromatography according to the manufacturer’s instructions. Purified MBP-RXRα, MBP-mPPARα, and MBP proteins (2 mg/ml in column buffer (20 mm Tris-HCl (pH 7.4), 200 mm NaCl, 1 mm EDTA, 1 mm dithiothreitol, 20% (v/v) glycerol)) were adsorbed to amylose resin (4.5 mmol settled resin) and resuspended as a 50% slurry in column buffer. Twenty μl of slurry was adjusted to 500 μl with column buffer containing 0.2% (v/v) Nonidet P-40 and 2% bovine serum albumin (BSA) and incubated with 1–5 μl of programmed reticulocyte lysate for 30 min at room temperature with continuous mixing. Beads were washed sequentially with 20 volumes of column buffer containing 0.2% (v/v) Nonidet P-40 and 2% BSA; column buffer containing 0.1% (v/v) Nonidet P-40 and 2% BSA; and column buffer alone. Bound material was eluted from the beads in SDS sample buffer and analyzed by SDS–polyacrylamide gel electrophoresis.

Gel Retardation Analysis—Electrophoretic mobility shift assays were carried out as before (14, 24). Synthetic oligonucleotide probes corresponding to the rat fatty acyl-CoA oxidase (AOx) and enoyl-CoA hydratase 3-hydroxyacyl-CoA dehydrogenase (HD) PPPRs have been described (14). The following double-stranded oligonucleotides containing TGAACCT direct repeats spaced by 0–5 nucleotides (DR0–DR5) were synthesized (for each, only the top strand is shown; TGACCT direct repeats are underlined; single-stranded spatCAG direct repeats are italicized). DR0, gattGTCACGTCGCACGTCGCACGTCG; DR1, gattGTCACGTCGCACGTCGCACGTCG; DR2, gattGTCACGTCGCACGTCGCACGTCG; DR3, gattGTCACGTCGCACGTCGCACGTCG; DR4, gattGTCACGTCGCACGTCGCACGTCG; DR5, gattGTCACGTCGCACGTCGCACGTCG. The LXRα response element (LXRE) (20) was synthesized by annealing 5′-GGTCCTGCAGTTCCAGGTGTTATGAATGTCACAG- and the comple-
that is missing 60 amino-terminal amino acids. This derivative retains the ability to interact with RXRα (lane 5) and mPPARα (not shown).

**Figure 1.** LXRα binds to DR4 and LXRE in vitro. Bound radiolabeled protein was analyzed by polyacrylamide gel electrophoresis. Labeled luciferase was used as a negative control. NARIP1 in panel b is a truncated version of LXRα that is missing 60 amino-terminal amino acids. This derivative retains the ability to interact with RXRα (lane 5) and mPPARα (not shown).

**Figure 2.** LXRα binds cooperatively with RXRα but not with mPPARα to synthetic or natural response elements. Electrophoretic mobility shift assays were carried out by incubating in vitro synthesized LXRα, mPPARα, and RXRα (1 μl of programmed reticulocyte lysate), as indicated, with synthetic DR0–DR5 direct repeat response element, AOx-PPRE, HD-PPRE, or LXRE probes (panel a). Panel b, the HD- and AOx-PPREs were incubated with constant amounts of RXRα and mPPARα (1 μl of programmed reticulocyte lysate each) and increasing amounts of LXRα (0.5–4 μl of programmed reticulocyte lysate). Protein concentration in each reaction was normalized with unprogrammed reticulocyte lysate as appropriate.

**Figure 3.** LXRα antagonizes peroxisome proliferator-mediated signaling in vivo. To investigate the effect of LXRα on peroxisome proliferator-mediated signaling in vivo, a luciferase reporter plasmid containing the AOx-PPRE (pAOx(X2)Luc) was cotransfected along with LXRα, RXRα, and mPPARα expression vectors into BSC40 cells, and luciferase activity was monitored. LXRα and mPPARα individually or in combination had little effect on reporter gene expression either in the presence or absence of the peroxisome proliferator Wy-14,643 (Fig. 3a). The LXRE ligand 9-cis-retinoic acid (not shown). However, LXRα potently inhibited induction mediated by mPPARα/RXRα. As shown in Fig. 3b, cotransfection with increasing amounts of the LXRα expression plasmid led to a progressive reduction in drug-independent and -dependent induction of the AOx-PPRE reporter gene construct by mPPARα/RXRα. The inclusion of 9-cis-retinoic acid in the transfections along with Wy-14,643 had little effect on LXRα-mediated repression.
controls we demonstrated that LXRα is a negative regulator of PPAR-mediated activation of peroxisome proliferator-responsive genes. Whether PPAR/LXRα heterodimers may also positively regulate gene expression awaits the definition of natural high affinity binding sites and the identification of potential LXRα ligands and target genes.

In summary, we have shown that LXRα can interact directly with other members of the nuclear hormone receptor superfamily in addition to RXRα, suggesting that combinatorial receptor interactions involving PPARGs are more extensive than previously anticipated. Moreover, our findings indicate that both LXRα and PPARGs play a broader physiological role in the convergence of distinct receptor signaling pathways. Since LXRα binds to the two identified components that are necessary for peroxisome proliferator responsiveness, there is potential for complex and diverse effects on both retinoid and peroxisome proliferator signaling pathways. The physiological importance of LXRα and related receptors in PPAR signaling is not known at present. Thus far, we have demonstrated that LXRα is a negative regulator of PPARG-mediated activation of peroxisome proliferator-responsive genes. Whether PPAR/LXRα heterodimers may also positively regulate gene expression awaits the definition of natural high affinity binding sites and the identification of potential LXRα ligands and target genes.

REFERENCES
1. Tsai, M.-j., and O'Malley, B. W. (1994) Annu. Rev. Biochem. 63, 451–486
2. Green, S., and Wahl, W. (1994) Mol. Cell. Endocrinol. 100, 149–153
3. Issmann, I., and Green, S. (1990) Nature 347, 645–650
4. Dreyer, C., Krey, G., Keller, H., Givel, F., Hefternen, G., and Wahl, W. (1992) Cell 68, 879–887
5. Göttlicher, M., Widmark, E., Li, Q., and Gustafsson, J.-Å. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 4653–4657
6. Chen, F., Law, S. W., and O'Malley, B. W. (1993) Biochem. Biophys. Res. Commun. 196, 671–677
7. Kliewer, S. A., Forman, B., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7355–7359
8. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) Cell 79, 1147–1156
9. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) Cell 83, 823–832
10. Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I., Morris, D. C., and Lehman, J. M. (1995) Cell 83, 813–819
11. Tugwood, J., Issmann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L., and Green, S. (1992) EMBO J. 11, 433–440
12. Zhang, B., Marcus, S. L., Sajjadi, F. G., Alvares, K., Reddy, J. K., Subramani, S., Rachubinski, R. A., and Capone, J. J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7541–7545
13. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) Nature 358, 771–774
14. Marcus, S. L., Miyata, K. S., Zhang, B., Subramani, S., Rachubinski, R. A., and Capone, J. P. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 5723–5727
15. Juge-Aubry, C. E., Gorla-Bajszczak, A., Pernin, A., Lemberger, T., Wahl, W., Burger, A. G., and Meier, C. A. (1995) J. Biol. Chem. 270, 11593–11597
16. Hunter, J., Kassam, A., Winrow, C. J., Rachubinski, R. A., and Capone, J. P. (1996) Mol. Cell. Endocrinol. 116, 213–221
17. Chu, R. Y., Madison, L. D., Lin, Y. L., Koop, P., Rao, M. S., Jameson, J. L., and Reddy, J. K. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 11593–11597
18. Bogazzi, F., Hudson, L. D., and Nikodem, V. M. (1994) J. Biol. Chem. 269, 11683–11686
19. Fields, S., and Song, O. (1989) Nature 340, 245–246
20. Willy, R. J., Umesono, K., Ong, E. S., Evans, R. M., Heyman, R. A., and Mangelsdorf, D. J. (1995) Genes & Dev. 9, 1033–1045
21. Miyata, K. S., McCaw, S., Marcus, S. L., Rachubinski, R. A., and Capone, J. P. (1994) Gene (Amst.) 149, 327–330
22. Ausubel, G. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1995) Current Protocols in Molecular Biology, Vol. 2, John Wiley and Sons, Inc., New York
23. Bartel, P., Cheng, C.-T., Stenglanl, R., and Fields, S. (1993) BioTechniques 14, 920–924
24. Maitta, K. S., Zhang, B., Marcus, S. L., Capone, J. P., and Rachubinski, R. A. (1993) J. Biol. Chem. 268, 19169–19172
25. Apfel, R., Benbrook, D., Lenhard, E., Ortiz, M. A., Salibert, G., and Pfahl, M. (1994) Mol. Cell. Biol. 14, 7025–7035
26. Shinar, D. M., Endo, N., Rutledge, S., Yovel, R., Rodan, G. A., and Schmidt, K. A. (1994) Gene (Amst.) 147, 273–276
27. Song, C., Kakonis, J. M., Hirspalka, R. A., and Liao, S. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 10808–10813
28. Leblanc, B. P., and Stunnenberg, H. G. (1995) Genes & Dev. 9, 1811–1816

Fig. 3. LXRα antagonizes mPPARα RXRα-mediated transcriptional activation by peroxisome proliferators. Panel a, the pAOx(X2)luc reporter plasmid was cotransfected into BSC40 cells with LXRα, mPPARα, and RXRα expression plasmids (0.5 μg each) in the absence or presence of the peroxisome proliferator Wy-14,643, as indicated, and luciferase activity was measured. Panel b, effect of increasing amounts of LXRα expression vector in the presence of constant amounts of mPPARα and RXRα expression vectors, with and without 9-cis-retinoic acid and Wy-14,643. Panel c, activation of a DR4 reporter gene construct by LXRα/RXRα. Transfections were carried out with LXRα and RXRα expression plasmids and with luciferase reporter gene constructs that contained a synthetic DR4 element (pDR4(X2)luc) or a DR1 element (pDR1(X2)luc), as indicated. Luciferase activity (± S.D.) in panels a and b is the average (corrected against the β-galactosidase internal reference) from three independent transfections, each carried out in duplicate. The values shown were normalized to the values obtained with cotransfected RXRα and mPPARα expression plasmids in the presence of Wy-14,643, which was taken as 100% in panel c. The values are from duplicate transfections (values did not vary by more than 15%) and were normalized to the value obtained with the respective reporter gene alone, which was taken as 1.

Fig. 3a.png

Fig. 3b.png

Fig. 3c.png
The Orphan Nuclear Hormone Receptor LXR Interacts with the Peroxisome Proliferator-activated Receptor and Inhibits Peroxisome Proliferator Signaling

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