Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TF) Binds to a Peroxisome Proliferator-responsive Element and Antagonizes Peroxisome Proliferator-mediated Signaling*  

(Received for publication, June 10, 1993, and in revised form, June 30, 1993)  
Kenji S. Miyata*, Baowei Zhang†, Sandra L. Marcus*, John F. Capone†, and Richard A. Bachubinski†  
From the Department of Biochemistry, McMaster University, Hamilton, L8N 3Z5 Ontario, Canada  

Peroxisomes form a family of diverse xenobiotic compounds that includes hydrolipidogenic agents, herbicides, and plasticizers. These compounds activate transcription of a subset of nuclear genes including those encoding peroxisomal fatty acid β-oxidation enzymes, whose elevated activities can lead to hepatocarcinogenesis. Induction of the genes encoding fatty acyl-CoA oxidase and hydratase-dehydrogenase, the first and second enzymes of the pathway, is mediated by peroxisome proliferator-activated nuclear receptors (PPARs) that bind to upstream responsive elements (PPREs) through heterodimerization with retinoid X receptors. We demonstrate that the chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1), another member of the nuclear hormone receptor superfamily, binds to the hydratase-dehydrogenase PPRE in vitro and in vivo and antagonizes PPAR-dependent signaling. These data suggest that members of the COUP-TF family play a role in modulating receptor-mediated activation of peroxisome proliferator-responsive genes.

Peroxisomes are essential for normal lipid homeostasis (1, 2). A number of xenobiotics, including amphipathic carboxylates used in the treatment of hyperlipidemia, induce peroxisome proliferation and ultimately hepatocarcinogenesis in rodents (3). These so-called peroxisome proliferators are nongenotoxic carcinogens that promote tumor growth apparently by modulating genes involved in cell growth and differentiation (4, 5).

* This work was supported in part by grants from the Heart and Stroke Foundation of Ontario (to J. P. C. and R. A. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† These authors should be considered equivalent first authors.
§ Recipient of a Medical Research Council of Canada scholarship.
¶ National Cancer Institute of Canada Senior Scientist. To whom correspondence should be addressed. Tel.: 416-525-9140 (ext. 2774); Fax: 416-522-9033.
∥ Medical Research Council of Canada Scientist. To whom correspondence should be addressed. Tel.: 416-525-9140 (ext. 2316); Fax: 416-522-9033.

The administration of peroxisome proliferators leads to the coordinated transcriptional induction of the nuclear genes encoding the enzymes of the peroxisomal β-oxidation pathway: fatty acyl-CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (HD),1 and 3-ketoacyl-CoA thiolase (6–8). cis-Acting peroxisome proliferator-responsive elements (PPREs) have been identified in the 5'-flanking regions of the genes encoding fatty acyl-CoA oxidase and HD (9–12). Both PPREs contain direct repeats of the sequence TGACCT, which is the consensus binding site for several members of the nuclear hormone receptor superfamily (13, 14), including the vitamin D3, thyroid, and retinoic acid receptors. These receptors bind to their cognate sites through cooperativity with the retinoid X receptor (RXR) (15–17). Signal transduction by peroxisome proliferators is mediated by a similar mechanism involving ligand-activated receptors called peroxisome proliferator-activated receptors (PPARs) that belong to this family of transcription factors (18–21). PPARs bind cooperatively to PPREs through heterodimerization with the 9-cis-retinoic acid receptor, RXRo (12, 22–26).

The transcriptional activation mediated by steroid receptors is subject to negative regulation. For example, the chicken ovalbumin upstream promoter-transcription factors (COUP-TFs), which are orphan members of the nuclear hormone receptor superfamily, have been shown to repress hormonal induction of vitamin D3, thyroid, and retinoic acid receptor target genes by competing with these receptors for their binding sites (27, 28) and by heteromerization with RXR (28). COUP-TFs bind as homodimers to diverse response elements consisting of TGACCT repeats which exhibit wide variation in spacing and orientation (27); however, they have the greatest affinity for direct repeats separated by 1 base pair (DR1). The HD PPRE consists of three imperfect direct repeats of the TGACCT motif separated by 2 base pairs (DR2) and 1 base pair (DR1), respectively (Fig. 1A; Refs. 11 and 12). Herein we show that human (h) COUP-TF1 (which is identical to the human orphan receptor Ear3; Ref. 29) binds to the HD PPRE in vitro and represses peroxisome proliferator induction mediated by PPARs in vivo.

MATERIALS AND METHODS

Cells—Rat hepatoma H4IIEC3 cells were cultured as monolayers in Dulbecco’s modified Eagle’s medium containing 10% horse serum and 5% fetal bovine serum. HeLa cells were maintained in suspension in Joklik’s modified medium plus 5% fetal bovine serum. BSC40 cells were maintained in Dulbecco’s modified Eagle’s medium plus 10% calf serum. Plasmids—pCP15 Luc is a luciferase expression vector containing the minimal promoter for the gene encoding rat liver carbamoyl phosphate synthetase (11). Plasmids for expression in BSC40 cells were constructed by cloning an oligonucleotide corresponding to the HD PPRE (5′-CATCTTCTTGGATCTGCCATTGCCATACATCAAATGTTTGA and its complement 5′-GAAATGGATTTCTTGAAATGATCTGAAATGTTTGA) into the unique BamHI site of the chicken ovalbumin upstream promoter transcription factor (COUP-TF1), another member of the nuclear hormone receptor superfamily, binds to the hydratase-dehydrogenase PPRE in vitro and in vivo and antagonizes PPAR-dependent signaling. These data suggest that members of the COUP-TF family play a role in modulating receptor-mediated activation of peroxisome proliferator-responsive genes.

The abbreviations used are: HD, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; COUP-TF, chicken ovalbumin upstream promoter transcription factor; EMSA, electrophoretic mobility shift analysis; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-responsive element; RXR, retinoid X receptor; RXRo, 9-cis-retinoic acid receptor; r, rat; h, human.

19169
been described (26). A plasmid expressing hCOUP-TF1 was constructed by ligating the HindIII/XbaI fragment from pC0UP-TF1 (27) into the corresponding sites of pRcCMV (Invitrogen). A plasmid encoding a truncated form of hCOUP-TF1 (cCOUP-TF1; Ref. 27) was constructed by cloning the Smal/AarII fragment from pC0UP-TF1 into the EcoRV and AarII sites of pEGEM-SZ(+) (Promega). Transcription and translation of this plasmid in vitro generated a truncated hCOUP-TF1 protein lacking 51 amino acids at its amino terminus.

Transfections and Measurement of Luciferase Activity—Transfections of H4IIEC3 cells were done by the calcium phosphate method followed by a dimethyl sulfoxide shock (11). BSC40 cells were transfected similarly except that cells were incubated for 24 h before and during transfection in medium without phenol red and containing 5% charcoal-stripped fetal bovine serum (26). Transfections typically contained 5 µg of a reporter gene construct and, where indicated, 2 µg of rPPAR, 2 µg of RXRa, and 0.4 µg of hCOUP-TF1 expression plasmids. Effector plasmid dosage was kept constant by the addition of appropriate amounts of the corresponding empty expression vector (pSG5 or pRcCMV). The total amount of DNA was kept at 20 µg with sonicated salmon sperm DNA.

Measurement of Luciferase Activity—Transfections typically contained 48 h post-transfection, and luciferase activity was measured.

Electrophoretic Mobility Shift Analysis—Nuclear extracts from HeLa cells were prepared from cell suspensions (30). Nuclear extracts were prepared from monolayer cultures of H4IIEC3, BSC40, and BSC40 cells transfected with various expression plasmids as described (30, 31). EMSA was performed as described (11, 26). DNA binding reactions were analyzed by electrophoresis at 4°C on prerun 3.5% polyacrylamide/20% methylenebisacrylamide as indicated. The brackets indicate novel complexes of reduced mobility generated in the presence of anti-hCOUP-TF1 serum. Where indicated, 0.25 µl of preimmune serum (PI) or of anti-hCOUP-TF1 serum was added to the binding reactions, which were then preincubated for 5 min prior to addition of probe.

RESULTS AND DISCUSSION

The HD PPRE consists of three imperfect direct repeats of the consensus nuclear hormone binding motif TGACCT separated by 2 base pairs (DR2) and 1 base pair (DR1), respectively (Fig. 1A). We have demonstrated that an oligonucleotide corresponding to the HD PPRE interacts with cellular factors present in H4IIEC3 cells, a rat hepatoma cell line responsive to peroxisome proliferators (11, 25), and that the PPRE-binding proteins include rPPAR and RXRa (26). To explore whether members of the COUP-TF family are present in these complexes, EMSA was carried out with radiolabeled HD PPRE probe and nuclear extracts prepared from H4IIEC3 cells in the presence of antibody to hCOUP-TF1 (hereafter called COUP-TF1). This antibody recognizes both COUP-TF1 and a related receptor COUP-TF2 (27), which is also called ARP-1 (32). As shown in Fig. 1B, inclusion of anti-hCOUP-TF1 resulted in the formation of supershifted complexes (lane f), demonstrating that the PPRE-binding proteins include COUP-TF-related factors. The major protein-DNA complex formed between HD PPRE and nuclear extracts of HeLa cells was almost quantitatively supershifted with anti-hCOUP-TF1 (lane c). Therefore, COUP-TF-related factors in both rat hepatoma and HeLa nuclear extracts bind to the HD PPRE.

To determine whether COUP-TF1 could bind directly to the HD PPRE, EMSA was carried out with in vitro translated receptor. As shown in Fig. 2A, in vitro translated COUP-TF1 bound to the HD PPRE (lane e; the identity of COUP-TF1 in these complexes was established using anti-hCOUP-TF1 serum; data not presented). COUP-TF1 also bound to the PPRE of the gene encoding fatty acyl-CoA oxidase (data not shown).

Several distinct PPARs bind to the HD PPRE cooperatively through heteromerization with RXRa (26). To determine the DNA sequence requirements for binding COUP-TF1 versus the cooperative binding of PPAR and RXRa on the HD PPRE, we used oligonucleotides in which each of the core TGACCT motifs was individually mutated. Fig. 2B shows that mutation of the first repeat (M4) had no effect on binding of in vitro translated rPPAR/RXRa (lane c) but completely abolished COUP-TF1 binding (lane d). A single mutation in the second repeat (M3) reduced significantly binding of both rPPAR/RXRa (lane e) and COUP-TF1 (lane f). Mutation of the third repeat (M5) inhibited rPPAR/RXRa binding (lane g) but did not affect COUP-TF1 binding (lane h). Therefore, COUP-TF1 and rPPAR/RXRa recognize distinct, yet overlapping, core elements within the HD PPRE, with the first two repeats being the important determinants for COUP-TF1 binding and the second and third repeats being necessary for rPPAR/RXRa binding. The DR2 spacing of the first two repeats is not necessary for COUP-TF1 binding, as an oligonucleotide (M6) in which the DR2 was converted to a DR1 bound COUP-TF1 strongly (lane j). Therefore, the primary sequence of the first two repeats, and not their relative spacing, are important for binding of COUP-TF1.

The mutated PPREs were assessed for their ability to confer peroxisome proliferator responsiveness onto a luciferase reporter gene in transient transfection assays of rat H4IIEC3 cells. As shown in Fig. 3, the activities of the wild-type HD PPRE reporter constructs pHD(X1)luc and pHMX3)luc were induced approximately 3- and 10-fold, respectively, in the presence of the peroxisome proliferator ciprofibrate, similar to what has been shown previously (25). Reporter constructs containing either one copy or three tandem copies of the different mutant
PPREs were unresponsive to ciprofibrate. These results extend our previous observation (25) that multimerization of the wild-type HD PPRE serves only to amplify the behavior of the response element to ciprofibrate and has little effect on basal transcription with either the wild-type or mutant HD PPREs M3, M4, M5, and M6 were individually cloned as single copies (X1) or as three direct tandem copies (X3) upstream of reporter plasmid pCPSluc and transfected into H4IIEC3 cells, which were subsequently treated with dimethyl sulfoxide alone or with the peroxisome proliferator ciprofibrate. The values were normalized to the activity of control transfections done with pCPSluc in the absence of ciprofibrate, which was taken as 1. The values are from two independent transfections done in duplicate and did not vary by more than 15%.

We wished to determine whether COUP-TF1 could down-regulate PPAR/RXRα-mediated transactivation in vivo. The pHDX3luc reporter plasmid was cotransfected with expression vectors for rPPAR, RXRa, and COUP-TF1 into BSC40 cells. BSC40 cells are unresponsive to peroxisome proliferators in the absence of co-transfected PPAR and RXRa (Fig. 5) and do not contain factors that bind to the HD PPRE (11). A 10-fold induction in luciferase activity was observed in the presence of both rPPAR and RXRa, which was further increased by the addition of the ciprofibrate. Addition of increasing amounts of COUP-TF1 expression vector resulted in the progressive repression of rPPAR/RXRα-mediated induction with pHDX3luc. Repression of induction was specific, as the transfection of COUP-TF1 expression vector alone had no effect on the basal
were incubated as indicated with the wild-type HD PPRE probe and vitro analyzed by EMSA. The COUP-TF1 heterodimer.

indicated. The values shown are relative activities from repeat transfections done in duplicate and were normalized to the value obtained for the presence of cotransfected plasmid expressing COUP-TF1 and anti-COUP-TF1 serum. The antagonistic peroxisome-mediated signaling by ciprofibrate (data not shown), in agreement with the results of Fig. 4, suggests that COUP-TF1-mediated antagonism occurs via competition with PPAR/RXRα for occupancy of the DNA target site on the HD PPRE.

The promiscuous binding of members of the COUP-TF receptor family to diverse response elements composed of TGACCT repeats, with its consequences on transcriptional induction mediated by cognate receptors, has positioned COUP-TFs as central regulators of hormone-responsive networks. COUP-TFs have been shown to play an important role in the modulation of expression of apolipoprotein genes, whose products are involved in lipid homeostasis (34). Our demonstration that COUP-TF1 binds to a PPRE and antagonizes PPAR-mediated signaling implicates members of the COUP-TF family as playing a role in lipid homeostasis also through the modulation of peroxisome-mediated metabolism of fatty acids and in the cellular response to peroxisome proliferators and other xenobiotic compounds.

Acknowledgments—We thank Dr. M.-J. Tasi for the gifts of pCOUP-TF1 and anti-hCOUP-TF1 serum, Dr. F. M. Sladek for the gift of HNF4 cDNA, Dr. D. Noeman for the gift of rPPAR cDNA, and Dr. R. Evans for the gift of RXRα cDNA. Ciprofibrate was a kind gift from Sterling Drug. We greatly appreciate the comments of Dr. S. Subramani on the manuscript.

REFERENCES

1. Lazarow, P. B., and Fajiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489–530
2. Vamecq, J., and Draye, J. P. (1989) Essays Biochem. 24, 115–225
3. Rao, M. S., and Reddy, J. K. (1991) Environ. Health Perspect. 93, 205–209
4. Cattley, R. C., and Popp, J. A. (1989) Cancer Res. 49, 3246–3251
5. Marsmann, D. Z., Cattley, R. C., Conway, J. G., and Popp, J. A. (1988) Cancer Res. 48, 6739–6744
6. Furutsu, S., Miyazawa, S., and Hashimoto, T. (1986) J. Biochem. (Tokyo) 99, 123–129
7. Reddy, J. K., Goel, S. K., Nemali, M. R., Carrino, J. J., Laffer, T. G., Reddy, M. K., Sperbeck, S. J., Osuni, T., Hashimoto, T., Laivanwi, N. D., and Rao, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1747–1751
8. Sharma, R. K., Lake, B. G., Makowski, R., Bradshaw, T., Earnshaw, D., Dale, J. W., and Gibson, G. G. (1988) Eur. J. Biochem. 184, 69–78
9. Osuni, T., Wen, J.-K., and Hashimoto, T. (1991) Biochem. Biophys. Res. Commun. 175, 866–871
10. Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L., and Green, S. (1992) EMBO J. 11, 433–439
11. Zhang, B., Marcus, S. L., Sagiadi, F. G., Alred, K., Reddy, J. K., Subramani, S., Rachubinski, R. A., and Capone, J. P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7541–7545
12. Bardot, O., Aldridge, T. C., Latruffe, N., and Green, S. (1993) Biochem. Biophys. Res. Commun. 192, 37–45
13. Evans, R. (1988) Science 240, 889–895
14. Umesono, K., Murakami, K. R., Thompson, C. C., and Evans, R. M. (1991) Cell 65, 1255–1266
15. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Nätär, A. M., Kim, S. Y., Boutin, J.-M., Glass, C. K., and Rosenfeld, M. G. (1991) Cell 67, 1251–1263
16. Leid, M., Kastner, P., Lyons, R., Nakahashi, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S., and Chambon, P. (1992) Cell 68, 377–395
17. Kluwe, S. A., Umesono, K., Mangelsdorf, D. J., and Evans, R. M. (1992) Nature 355, 445–449
18. Issmann, I. and Green, S. (1990) Nature 347, 645–650
19. Dreyer, C., Krey, G., Keller, H., Givel, H., Helftenbein, G., and Wahli, W. (1992) Cell 68, 879–887
20. Göttlicher, M., Widmark, E., Li, Q., and Gustafsson, J.-Å. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4653–4657
21. Schmidt, A., Endo, N., Rutledge, J. S., Vogel, R., Shinar, D., and Rodan, G. A. (1992) Mol. Endocrinol. 6, 1634–1641
22. Kluwe, S. A., Umesono, K., Noeman, D. J., Heyman, R. A., and Evans, R. M. (1992) Nature 358, 771–774
23. Geerin, K. L., Götlichler, M., Teboul, M., Widmark, E., and Gustafsson, J.-Å. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1440–1444
24. Keller, H., Dreyer, C., Medin, J., Mahfoudsi, A., Otsa, K., and Wahli, W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2160–2164
25. Zhang, B., Marcus, S. L., Miyata, K. S., Subramani, S., Capone, J. P., and Rachubinski, R. A. (1993) J. Biol. Chem. 268, 12939–12945
26. 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
27. Cooney, A. J., Tsai, S. Y., O'Malley, B. W., and Tsai, M.-J. (1992) Mol. Cell. Biol. 12, 4153–4163
28. Cooney, A. J., Leng, X., Tsai, S. Y., O'Malley, B. W., and Tsai, M.-J. (1993) J. Biol. Chem. 268, 4102–4106
29. Miyazima, N., Kadowaki, Y., Fukushima, S., Shimizu, S., Semb, K., Yamashita, Y., Matsubara, K., Toyoshima, K., and Yamamoto, T. (1988) Nucleic Acids Res. 16, 11037–11044
30. Dingam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 4257–4261
31. Andrews, N. C., and Faller, D. V. (1991) Nucleic Acids Res. 19, 2499
32. Ladiges, J. A. A., and Karathanasis, S. K. (1991) Science 251, 561–565
33. Sladek, F. M., Zhong, W., Lai, E., and Darnell, J. E. Jr., (1990) Genes Dev. 4, 2355–2365
34. Ladiges, J. A. A., Hadzhilou-Cladaras, M., Kardasis, D., Cardot, P., Cheng, J., Zannis, V., and Cladaras, C. (1992) J. Biol. Chem. 267, 18549–18560

Fig. 4. COUP-TF1 does not form DNA-binding heteromers. In vitro translated RXRα, rPPAR, HNF4, COUP-TF1, and tCOUP-TF1 were incubated as indicated with the wild-type HD PPRE probe and analyzed by EMSA. The arrow indicates the complex formed by a COUP-TF1-tCOUP-TF1 heterodimer.

Fig. 5. COUP-TF1 antagonizes peroxisome proliferator-mediated signaling. pHDX3Luc was transfected into BSC40 cells along with effector plasmids expressing rPPAR, RXRα, and COUP-TF1, as indicated. The values shown are relative activities from repeat transfections done in duplicate and were normalized to the value obtained for ciprofibrate-treated cells cotransfected with rPPAR and RXRα expression plasmids, which was taken as 100%. The numbers for COUP-TF1 refer to the amount (in μg) of cotransfected plasmid expressing COUP-TF1.

activity of pHDX3Luc. Expression of reporter constructs containing mutant HD PPREs was not stimulated by cotransfection with plasmids expressing rPPAR and RXRα in either the presence or absence of ciprofibrate (data not shown), in agreement with the transfection results presented in Fig. 3. Therefore, COUP-TF1 antagonizes PPAR-mediated peroxisome proliferator signaling in vitro. This finding, combined with the results of Fig. 4, suggests that COUP-TF1-mediated antagonism occurs via competition with PPAR/RXRα for occupancy of the DNA target site on the HD PPRE.

The promiscuous binding of members of the COUP-TF receptor family to diverse response elements composed of TGACCT