Identification of Nuclear Receptor Corepressor as a Peroxisome Proliferator-activated Receptor α Interacting Protein*

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Nuclear receptor corepressor (NCoR) was demonstrated to interact strongly with peroxisome proliferator-activated receptor α (PPARα), and PPARα ligands suppressed this interaction. In contrast to the interaction of PPARα with the coactivator protein, p300, association of the receptor with NCoR did not require any part of the PPARα ligand binding domain. NCoR was found to suppress PPARα-dependent transcriptional activation in the context of a PPARα-retinoid X receptor α (RXRα) heterodimeric complex bound to a peroxisome proliferator-responsive element in human embryonic kidney 293 cells. This repression was reversed agonists of either receptor demonstrating a functional interaction between NCoR and PPARα-RXRα heterodimeric complexes in mammalian cells. NCoR appears to influence PPARα signaling pathways and, therefore, may modulate tissue responsiveness to peroxisome proliferators.

Members of the steroid/thyroid hormone receptor superfamily function by binding to specific DNA response elements within the regulatory regions of target genes and modulating expression of these genes at the transcriptional level (1–3). Regulation of target gene expression mediated by nuclear receptors may occur in response to activation of the receptors by ligands (4) or by phosphorylation (5) or by a combination of both events (5).

The mammalian PPARα family is composed of at least three genetically and pharmacologically distinct subtypes, PPARα, -γ, and -β/δ (also referred to as NUCI; reviewed in Refs. 6 and 7). The primary physiological roles for the α and γ subtypes of PPAR appear to be regulation of lipid metabolism and adipogenesis, respectively, and both subtypes have been implicated in modulating inflammatory responses (8–12). A physiological role for PPARβ/δ has not been elucidated but this receptor subtype is expressed ubiquitously in the mouse, possibly suggesting a more general function (13).

Retinoid X receptors (RXRs) serve as obligate heterodimeric partners for all PPAR subtypes, and the PPAR-RXR heterodimeric complex binds most efficiently to degenerate direct repeats of the hexameric nucleotide sequence, AGGTCA, separated by 1 base pair (DR1; Refs. 14–17). PPAR-RXR heterodimeric binding sites, known as peroxisome proliferator response elements (PPREs), have been identified within the regulatory regions of several genes that encode proteins implicated in lipid metabolism (8, 9, 14, 16, 18) and adipocyte function (19, 20).

PPARα was initially identified in a search for novel superfamily members and was shown to be activated by a group of compounds known to elicit proliferation of hepatic peroxisomes in rodents (21). Structurally diverse peroxisome proliferators include phthalate ester plasticizers (di-2-ethylhexyl)-phthalate), herbicides (2,4,5-trichlorophenoxyacetic acid), and several fibrin anti-hyperlipidemic agents (WY-14, 643, clofibric acid, gemfibrozil; Ref. 22). Additional compounds have since been shown to activate PPARα, including the fatty acid, arachidonic acid (23), and the corresponding synthetic analog 5,8,11,14-eicosatetraenoic acid (ETYA; Ref. 24), the leukotriene D4 antagonist, LY-171883 (25), and the arachidonic acid derivative, leukotriene B4, which has been proposed to be an endogenous ligand for PPARα (12).

In addition to activating expression of target genes upon binding cognate ligands, receptors for all-trans retinoic acid (RAR) and thyroid hormone (TR) actively repress genes in the absence of ligand (26, 27). The related corepressor proteins, NCoR and silencing mediator of retinoid and thyroid hormone receptors (SMRT), have been demonstrated to interact with and mediate the repression functions of unliganded RAR and TR (28, 29). NCoR and SMRT are associated with a multiprotein corepressor complex that minimally contains Sin3a and the histone deacetylase, HDAC1/Rpd3 (30, 31). Ligand binding by RAR and TR promotes dissociation of the receptor-corepressor complex (28, 29) and subsequent interaction of the receptor with one or more coactivator proteins that possess intrinsic histone acetyltransferase activity (32–35). Several nuclear receptor-associated coactivator proteins that exhibit histone acetyltransferase activity have been identified including p300/CREB binding protein (CBP; Refs. 35 and 36), steroid receptor coactivator-1 (SRC-1; Ref. 37), and p300/CREB-interacting protein (p/CIP; Ref. 34). These findings, when considered together with previous observations that hyperacetylated histones are
associated with actively transcribed chromatin (reviewed in Ref. 38), offer a molecular explanation for ligand-dependent transcriptional modulation by nuclear receptors. Thus, ligand binding may serve as a molecular switch between transcriptional repression and activation by promoting exchange of a receptor-associated deacetylase-containing corepressor complex with that of a histone acetyltransferase-containing coactivator complex.

PPAR-dependent transcriptional activation of many genes is well documented, and direct, ligand-enhanced interactions between PPARs and the coactivators, p300/CPB (39), SRC-1 (39–41), PPAR-binding protein (PBP; Ref. 42), and PGC-1 (43) are thought to play a role in such activation. In contrast, PPAR-mediated transcriptional repression of target genes, as observed for RAR and TR (see above), is relatively unexplored or at best controversial. PPARγ has been shown to interact in solution with the corepressors, NCoR and SMRT, but weakly if at all when bound to DNA, possibly suggesting that neither of these corepressors mediate putative PPAR-dependent gene repression (44). However, Lavinsky and co-workers (45) demonstrated SMRT-dependent gene repression mediated by a phosphorylated form of PPARγ. Such findings illustrate the need for a more complete mechanistic understanding of potential PPAR-dependent repression of gene expression. We report here the isolation of NCoR from a yeast two-hybrid screen using PPARα as a bait. We describe results from studies in yeast, in mammalian cells, and in vitro that were conducted to characterize PPARs-NCoR interactions and to examine the influence of PPAR ligands upon such interactions.

MATERIALS AND METHODS

Plasmids and Receptor Constructs—Plasmids encoding the receptors described below were used either directly or as templates for polymerase chain reaction to assemble all constructs described herein using standard techniques. All plasmids were kind gifts from the following individuals: mouse PPARα (21) from Drs. S. Green and J. D. Tugwood (Zeneca, Macclesfield, United Kingdom); mouse RXRa (RXRa; Ref. 46) and human RXRa (RXRa; Ref. 47) from Drs. P. Kastner, A. Krust and P. Chambon (Institut de Génétique et de Biologie Moleculaire et Cellulaire, Illkirch, France); human p300 (48) from Dr. D. Livingston (Dana Farber Cancer Institute, Boston, MA), NCoR (29) from Dr. T. Heinzel (German Cancer Research Center, Heidelberg, Germany), SMRT (28) from Dr. R. Evans (Salk Institute, La Jolla, CA). The integrity of all constructs was verified by restriction digest and/or sequence analysis.

The parental bait vector for the yeast two-hybrid screen (pBTM16) and the yeast reporter strain L40 (Refs. 49 and 50, respectively) were kind gifts from Drs. R. Losson and P. Chambon (Institut de Génétique et de Biologie Moleculaire et Cellulaire, Illkirch, France). The yeast expression vectors encoding p300 amino acids 39–221 and the following amino acids of the indicated receptors have been described previously (39): PPARα (91–468 of mPPARα), PPARα D/E (166–468 of mPPARα), RXRa (132–467 of mRXRa), and RXRa (90–454 of hRXRa). PPARα (91–447 of mPPARα), PPARαΔ425 (amino acids 91–424 of mPPARα), PPARαΔ465 (amino acids 91–421 of mPPARα), and PPARα (282–468 of mPPARα), and identical fragments of all receptors and mutants thereof listed above were subcloned in pTLL (46) and have been described previously (39, 51). PPARαΔ288 (amino acids 91–287 of mPPARα), PPARαΔ227 (amino acids 91–226 of mPPARα), PPARαΔ202 (amino acids 91–201 of mPPARα), and PPARαΔ180 (amino acids 91–179 of mPPARα) were constructed by polymerase chain reaction amplification of the indicated receptor regions with primers containing appropriate restriction sites for insertion into pTLL. Receptor proteins and derivatives thereof were expressed by in vitro transcription/translation for use in GST pulldown experiments as described previously (39, 51). Note that all PPARα constructs encode receptors truncated in the amino-terminal (ΔAAB).

GSTp300 has been described previously as GSTp300 (39–221; Ref. 21). GST-NCoR (ΔID II) and GST-NCoR, encoding GST fusions with NCoR amino acids 2218–2381 and 2110–2453, respectively, were prepared by polymerase chain reaction amplification with primers containing BamHI and EcoRI sites using the original pACT2/NCoR plasmid isolated in the yeast two-hybrid screen as a template. The resulting fragments were digested and subcloned into BamHI/EcoRI-digested pGEX2T (Amersham Pharmacia Biotech). A PPARαΔ132–467-CAT reporter construct was prepared by insertion of a multimerized acyl-CoA oxidase PPRE (8) into the XhoI site of pBL2CAT2 (52). Additional information concerning any of the above constructs described herein can be obtained upon request.


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FIG. 1. Isolation of NCoR as a PPARα-interacting protein. Carboxyl-terminal NCoR amino acid sequence. The encoded fragment isolated in the screen is indicated by bold, underlined text (amino acids Lys3116–Asp3453 of NCoR; Ref. 29). Previously identified nuclear receptor interaction domains, ID I (Asp2237–Met2300) and ID II (Arg2062–Ser2145), are delineated by boxed amino acids (see Ref. 54). Note that amino acids Lys3116–Asp3453 of NCoR are identical to Lys2110–Asp2453 of the originally reported RXR-interacting protein (RIP) 13 isolate (55). For clarity we have referred to our clone throughout the text as NCoR amino acids 2110–2453.

RESULTS

A previously described yeast two-hybrid system (49) was used to isolate a carboxyl-terminal NCoR (29) fragment as a PPARα-interacting protein from an oligo(dT)-primed, mouse brain cDNA library (NCoR amino acids 2110–2453, Fig. 1). Previous studies have identified two domains, ID I and ID II, that mediate interactions between NCoR and nuclear receptors
were carried out three (RAR (amino acids 2110–2453) fusion (GAD/NCoR) and examined for the ability to activate an integrated LexA-responsive lacZ reporter in the yeast strain L40 (49). Ligand-dependent interactions were examined in the presence of 100 μM WY-14,643, 1 μM 9-cis-RA, or vehicle where indicated. At least three independent transformants were assayed for each experiment and the results shown represent the mean ± S.E. for each determination. No reporter activity was detected when the GAD/NCoR fusion was coexpressed with either LexA DBD alone or LexA DBD fused to an unrelated protein (data not shown). Statistical significance at the 99% (p < 0.01) confidence level is indicated by ** symbols for ligand versus vehicle treatment. B, in vitro protein-protein interactions between NCoR and nuclear receptors. GST alone or GST fused to NCoR amino acids 2110–2453 (GST/NCoR) were bound to glutathione-Sepharose and used as an affinity matrix to examine interactions with GST/NCoR in vitro translated [35S]methionine-labeled PPARα and RXRa in the absence and presence of ligand (100 μM 9-cis-RA, 1 μM 9-cis-RA, or vehicle where indicated). Input lanes (IN) represent 25% of receptor preparations used in binding reactions for the indicated samples. Note that the signals present in lanes 7–9 were obtained by increased exposure time relative to those in all other lanes. All receptors preparations expressed in yeast or translated in vitro were truncated in the amino-terminal (ΔAB) region (see “Materials and Methods”). Shown in both A and B are representative experiments that were carried out three (RARy-NCoR and RXRa/NCoR) to eight (PPARα-NCoR) times.

(29, 54). The NCoR fragment isolated in this screen encompasses the last 33 amino acids of ID II and the entirety of ID I (Fig. 1).

PPARα and interaction domains of the coactivators, p300 and SRC-1, exhibit strong ligand-independent association when examined in a yeast two-hybrid system (39). When examined in vitro, however, these interactions are strictly ligand-dependent, suggesting that yeast may contain endogenous PPARα activating ligands (39). The isolation of NCoR in a yeast two-hybrid screen was therefore unexpected as we (39) and others had hypothesized that PPARα existed in a liganded state in yeast that may not facilitate receptor interactions with potential corepressor proteins as has been observed for other liganded nuclear receptors (28, 29). Toward the goal of understanding the influence of NCoR on PPARα signaling mechanisms, we chose to conduct a more thorough analysis of the interaction between these two proteins.

**Fig. 2. Interactions between NCoR and PPARα, RARy, and RXRa.** A, interactions between NCoR and nuclear receptors in yeast. LexA DBD/receptor fusions were coexpressed with a GAL4 AD/NCoR (amino acids 2110–2453) fusion (GAD/NCoR) and examined for the ability to activate an integrated LexA-responsive lacZ reporter in the yeast strain L40 (49). Ligand-dependent interactions were examined in the presence of 100 μM WY-14,643, 1 μM 9-cis-RA, or vehicle where indicated. At least three independent transformants were assayed for each experiment and the results shown represent the mean ± S.E. for each determination. No reporter activity was detected when the GAD/NCoR fusion was coexpressed with either LexA DBD alone or LexA DBD fused to an unrelated protein (data not shown). Statistical significance at the 99% (p < 0.01) confidence level is indicated by ** symbols for ligand versus vehicle treatment. B, in vitro protein-protein interactions between NCoR and nuclear receptors. GST alone or GST fused to NCoR amino acids 2110–2453 (GST/NCoR) were bound to glutathione-Sepharose and used as an affinity matrix to examine interactions with GST/NCoR in vitro translated [35S]methionine-labeled PPARα and RXRa in the absence and presence of ligand (100 μM 9-cis-RA, 1 μM 9-cis-RA, or vehicle where indicated). Input lanes (IN) represent 25% of receptor preparations used in binding reactions for the indicated samples. Note that the signals present in lanes 7–9 were obtained by increased exposure time relative to those in all other lanes. All receptors preparations expressed in yeast or translated in vitro were truncated in the amino-terminal (ΔAB) region (see “Materials and Methods”). Shown in both A and B are representative experiments that were carried out three (RARy-NCoR and RXRa/NCoR) to eight (PPARα-NCoR) times.

**Fig. 3. Influence of PPAR ligands on NCoR-PPARα interactions.** A, ligand-dependent dissociation of NCoR and PPARα in yeast. PPARα interaction with NCoR was examined in the presence of 1 μM 9-cis-RA, 100 μM troglitazone, 1 mM clofibrate, 100 μM LY-171883, 100 μM ETYA, 100 μM WY-14,643 or vehicle where indicated. Statistical significance at the 99% (p < 0.01) confidence level is indicated by ** symbols for ligand versus vehicle treatment. Each determination represents the mean ± S.E. of three independent experiments. B, WY-14,643 dose-response curve for dissociation of NCoR-PPARα in yeast. PPARα interaction with NCoR was examined in the presence of increasing concentrations of WY-14,643 (1–10,000 nM in one log unit increments) or vehicle. Each point represents the mean ± S.E. of four independent experiments. The theoretical curve shown was obtained by fitting the data using an iterative, curve-fitting routine (GraphPad Prism), which yielded an IC50 of 134 nM.
PPARα was WY-14,643, and this effect was dose-dependent with an apparent IC₅₀ of 134 nM (Fig. 3B).

NCoR was WY-14,643, and this effect was dose-dependent with an apparent IC₅₀ of 134 nM (Fig. 3B).

NCOR and p300 Require Distinct PPARα Regions for Interaction—To determine which regions of PPARα are required for association with NCOR, we examined GST/NCOR interactions with several carboxyl- and amino-terminal PPARα truncation mutants in vitro using standard GST pulldown methodology (Fig. 4A). The results of these in vitro studies are depicted schematically in Fig. 4A. As shown above, PPARα interacted strongly with both GST/NCOR and GST/p300 in vitro (Fig. 4B, lanes 4–7). The former interaction was only modestly inhibited by ligand, while the latter interaction was strictly ligand-dependent. In contrast, PPARαΔ448, which lacks 21 carboxy-terminal amino acids, efficiently interacted with GST/NCOR but ligand-dependent interaction between this truncation mutant and GST/p300 was abolished (Fig. 4B, lanes 11–14). The hinge/LBD region of PPARα, PPARα D/E, interacted in vitro with GST/NCOR and GST/p300 in a manner similar to that of PPARα (Fig. 4B, lanes 18–21), although the WY-14,643-induced dissociation of receptor-GST/NCOR complexes was more apparent (lanes 18–19). In contrast, PPARα E, which lacks residues contained within the hinge region of PPARα (amino acids 166–281), did not interact with either GST/NCOR or GST/p300 (Fig. 4B, lanes 25–28). These results demonstrate that both NCOR and p300 require the hinge region of the receptor for efficient interaction, while only the latter requires an intact PPARα LBD. Therefore, the regions of PPARα required for interaction with NCOR and p300 are partially overlapping but largely distinct.

The carboxyl-terminal PPARα truncation mutants, PPARαΔ425, PPARαΔ288, PPARαΔ227, and PPARαΔ202, all interacted efficiently with GST/NCOR (Fig. 4C, lanes 3, 6, 9, and 12). PPARαΔ180, which lacks the entire LBD, also interacted with GST/NCOR (Fig. 4C, lane 15). PPARα amino acids 166–179 within the hinge region are common to all receptor proteins observed to interact with GST/NCOR, suggesting that these residues may play a role in PPARα interactions with NCOR. However, this isolated region of the receptor did not interact with NCOR either in yeast or in vitro under a variety of conditions (data not shown), suggesting that this region of PPARα is necessary but not sufficient to mediate interaction with NCOR.

NCOR ID II Is Not Necessary for PPARα-NCOR Interactions—Protein-protein interaction studies carried out with a GST/NCOR fusion protein lacking the entire ID II, GST/NCOR (ΔID II), demonstrated that the ID II region of NCOR was not required for interaction with PPARα (Fig. 5). As observed for interactions between PPARα and GST/NCOR (amino acids 2110–2453, see Fig. 2B, lanes 2 and 3), WY-14,643 did not significantly affect receptor-NCOR interactions in vitro (Fig. 5, lanes 4 and 5).

PPARα Ligands Promote Both PPARα-NCOR Dissociation and PPARα-p300 Association—The yeast two-hybrid system was used to compare the influence of several PPAR ligands on receptor interactions with the corepressor, NCOR (amino acids 2110–2453), and the coactivator, p300 (amino acids 39–221). PPARα D/E was used as the receptor component in this series of experiments because we previously observed a readily detectable WY-14,643-enhanced interaction in yeast between the D/E region of PPARα and p300 amino acids 39–221 (39). As observed for yeast two-hybrid analyses using PPARα (see Fig. 3), WY-14,643, but not troglitazone, clofibrate, LY-171883, or ETYA, significantly reduced the interaction between PPARα D/E and NCOR (Fig. 6A). Similarly, troglitazone, clofibrate and LY-171883 had no significant influence on the strong ligand-independent PPARα D/E interaction with p300, while both ETYA and WY-14,643 modestly, but significantly, enhanced this interaction (Fig. 6A). Thus, while both ETYA and WY-14,643 promoted p300-PPARα interactions in yeast and in vitro, only WY-14,643 was observed to induce dissociation of NCOR-PPARα complexes, and this was only significantly ap-
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parent in yeast.

To confirm the observed interactions in yeast, in vitro protein-protein interaction assays were conducted as described above. GST/NCoR (amino acids 2110–2453) and GST/p300 (amino acids 39–211) were examined for interaction with both PPARα D/E and both GST/NCoR and GST/p300 in the absence and presence of several, above-mentioned PPAR ligands. None of the ligands tested significantly affected the in vitro interaction of NCoR with either PPARα D/E (Fig. 6B) or PPARα (Fig. 6C). In contrast, in vitro association between GST/p300 and both PPARα D/E (Fig. 6B) and PPARα (Fig. 6C) was observed only in the presence of ETYA or WY-14,643 (lanes 18 and 19, respectively).

NCoR Represses PPARα/RXRα-mediated Transcriptional Activation from a PPRE in HEK293 Cells—Cotransfection experiments in HEK293 cells were conducted to determine the physiological significance of the strong interaction between PPARα and NCoR observed in yeast and in vitro. The reporter construct used for these studies, (PPRE) 3-tk-CAT, exhibited low basal or ligand-stimulated activity in the absence of cotransfected receptor (Fig. 7, lanes 1–6). However, cotransfection of PPARα and RXRα resulted in a strong, constitutive activity of this reporter that was only minimally stimulated by PPARα (WY-14,643 and ETYA) or RXRα (9-cis-RA) agonists or both types of ligands together (Fig. 7, lanes 7–12). Cotransfection of full-length NCoR (2110–2453) and p300 (amino acids 39–221) in yeast. As-

|Vehicle| WY-14,643 (10 μM)| ETYA (10 μM) |
|-------|-----------------|-------------|
|No Receptors| NCoR only| PPARα NCoR| RXRα NCoR| PPARα+RXRα NCoR|
|EMPTY VECTOR|**|**|**|**|
|VECTOR + NCoR|**|**|**|**|
|VECTOR + p300|**|**|**|**|

**Fig. 7. Effect of NCoR on transcriptional activation mediated by PPARα-RXRα heterodimeric complexes in HEK293 cells.** HEK293 cells were cotransfected with 2 μg of the (PPRE) 3-tk-CAT reporter plasmid and, where indicated, expression vectors for full-length NCoR (2 μg) and PPARα/RXRα (0.5 μg of each). Cells were treated with the indicated concentrations of WY-14,643, 9-cis-RA (9cRA), or ETYA for 24 h. Extracts were normalized for transfection efficiency by cotransfection with a β-galactosidase expression vector (pCMV-Sport-βgal; Life Technologies, Inc.), and the chloramphenicol acetyltransferase (CAT) activity was determined in the presence [14C]chloramphenicol and acetyl-CoA using standard methodology. Acetylated and unacetylated [14C]chloramphenicol were separated by thin layer chromatography and visualized by autoradiography. Shown is a representative experiment that was replicated four times.

**Fig. 6. Influence of ligands on NCoR and p300 interactions with PPARα.** A, interactions between PPARα D/E and both NCoR (amino acids 2110–2453) and p300 (amino acids 39–211) in yeast. Assays were conducted as described in Fig. 3A. Each determination represents the mean ± S.E. of three independent experiments. Statistical significance at the 95% (p < 0.05) and 99% (p < 0.01) confidence levels are indicated by * and ** symbols, respectively, for ligand versus vehicle treatment. B, in vitro protein-protein interactions between PPARα D/E and both GST/NCoR (2110–2453) and GST/p300 (39–221). Assays were carried out as described in the legend to Fig. 2B, and ligand concentrations were the same as those indicated in Fig. 3A. C, in vitro protein-protein interactions between PPARα and both GST/NCoR and GST/p300. Assays were carried out as described above (Fig. 6B). Shown in B and C are representative GST pulldown experiments that were replicated four to six times.

**DISCUSSION**

We have demonstrated that PPARα interacts strongly with NCoR, and the PPARα ligand, WY-14,643, inhibits this interaction in yeast and in mammalian cells. The PPARα ligand, ETYA, also inhibits PPARα/NCoR interaction in mammalian...
that treatment of transfected HEK293 cells with ETYA acti-
possibility that a combination of these two possibilities may be
mammalian cells.

hornous agonists in yeast that may disfavor PPAR
a
interaction. These hypotheses may provide an explanation for
how NCoR was unexpectedly isolated as a PPARα-interacting
protein in our yeast two-hybrid screen.

Previously we demonstrated that the coactivators, p300 and
SRC-1, require 21 carboxyl-terminal residues of the PPARα
LBD for interaction with PPARα (39). By analogy with other
nuclear receptors, this region of PPARα is predicted to contain
the putative core of the ligand dependent-transcriptional acti-
vation function (57). We demonstrate herein that the PPARα
truncation mutant, PPARαΔ448, which lacks the 21 carboxy-
terminal amino acids encompassing the putative AF-2 core,
interacts with NCoR but not p300. Therefore, the corepressor,
NCoR, and the coactivators, p300 and SRC-1, appear to inter-
act with the receptor in mechanistically distinct manners that
utilize different regions of PPARα as protein-protein interac-
tion surfaces. However, simultaneous interaction between
the receptor and both NCoR and p300/SRC-1 are unlikely, be-
causethat both types of interaction require common amino acid residues
within the hinge region of the receptor.

deletion of the hinge region of PPARα (amino acids 166–281)
abolished NCoR-PPARα interaction, and amino acids 166–179
within the amino-terminal portion of the PPARα hinge region
were common to all receptor fragments that exhibited interac-
tion with NCoR. The PPARα mutant, PPARαA180 (amino acids
91–179), which interacted efficiently with NCoR, lacks the
entirety of both the putative CoR box (29) and the ligand
binding domain. These results suggest that PPARα amino ac-
ids 166–179 may mediate interactions with NCoR. However,
extensive analyses in yeast and in vitro have failed to demon-
strate that PPARα 166–179 are sufficient to mediate interac-
tion with NCoR (data not shown), possibly indicating that
additional contacts are required for efficient interaction. None-
theless, our results suggest that PPARα likely contains a NCoR
interaction surface that is clearly not contained within the LBD
of the receptor and, thus, may be distinct from that present in
eitherRAR or TR (28, 29).

Zamir and collaborators (44) have shown that PPARγ can
interact with the corepressors, SMRT and NCoR, in solution,
but weakly if at all when bound to DNA. Similarly, we were
unable to demonstrate the formation of a DNA bound
PPARα-RXRα-NCoR complex in vitro (data not shown). How-
ever, in contrast to the findings of Zamir and colleagues (44)
who observed no corepressor-dependent PPARγ-mediated
repression, our transient transfection studies clearly dem-
strate a functional interaction between a PPRE-bound,
PPARα-RXRα complex and NCoR. Such discrepant results
could simply be a result of either differing PPAR subtypes (α
versus γ) or cell lines (HEK 293 versus 293T) or a combina-
tion of these two possibilities. The inability to observe a DNA-bound
PPARα-RXRα-NCoR complex in vitro may be due to an inher-
ent instability of such complexes, and indeed, other groups have
reported that cross-linking reagents are required to stabilize
similar complexes in vitro (58). NCoR clearly associates with
and represses the transcriptional activity of PPRE-bound,
PPARα-RXRα heterodimeric complexes in HEK293 (Fig. 7).
However, we cannot exclude the possibility that additional nucle-
lar factor(s) present in HEK293 cells, but lacking in vitro, are
required for the assembly of a DNA-bound, PPARα-RXRα-NCoR
complex.

Finally, it is conceivable that interaction of NCoR or SMRT
with either PPARα or PPARα-RXRα complexes may influence
other signaling pathways by titration of limiting amounts of
these corepressors. This form of receptor cross-talk may serve
to relieve transcriptional repression mediated by other nuclear
receptors, such as RAR, TR, or Rev-Erb, that utilize common
corepressors. Results presented herein raise the possibility
that PPAR interactions with corepressors in solution or on DNA may play a prominent role in regulating PPAR-dependent transcriptional regulation of target genes.

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