

Identification of ARA70 as a Ligand-enhanced Coactivator for the Peroxisome Proliferator-activated Receptor γ *

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Cynthia A. Heinlein[‡], Huei-Ju Ting[‡], Shuyuan Yeh[‡], and Chawnshang Chang[§]

From the George Whipple Laboratory for Cancer Research, Departments of Pathology, Urology, and Radiation Oncology, University of Rochester Medical Center, Rochester, New York, 14642

In an effort to understand transcriptional regulation by the peroxisome proliferator-activated receptor γ (PPAR γ), we have investigated its potential interaction with coregulators and have identified ARA70 as a ligand-enhanced coactivator. ARA70 was initially described as a coactivator for the androgen receptor (AR) and is expressed in a range of tissues including adipose tissue (Yeh, S., and Chang, C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 5517–5521). Here we show that ARA70 and PPAR γ specifically interact by coimmunoprecipitation and in a mammalian two-hybrid assay. PPAR γ and ARA70 interact in the absence of the PPAR γ ligand 15-deoxy- Δ 12,14-prostaglandin J2, although the addition of exogenous ligand enhances this interaction. Similarly, in transient transfection of DU145 cells, cotransfection of PPAR γ and ARA70 induces transcription from reporter constructs driven by either three copies of an isolated PPAR response element or the natural promoter of the adipocyte fatty acid-binding protein 2 in the absence of exogenous 15-deoxy- Δ 12,14-prostaglandin J2. However, this PPAR γ -ARA70 transactivation is enhanced by the addition of ligand. Thus, ARA70 can function as a ligand-enhanced coactivator of PPAR γ . Finally, we show that AR can squelch PPAR γ -ARA70 transactivation, which suggests that cross-talk may occur between PPAR γ - and AR-mediated responses in adipocytes.

The peroxisome proliferator-activated receptors (PPARs)¹ are structurally similar members of the nuclear hormone receptor superfamily that become transcriptionally active in response to a diverse group of chemical compounds, including fibrate hyperlipidemic drugs, thiazolidinedione anti-diabetic drugs, arachidonic acid derivatives, fatty acids, and peroxisome-proliferating chemicals (reviewed in Ref. 2). These recep-

tors are considered to play key roles in lipid metabolism and storage (3). The three mammalian PPAR members (α , γ , and δ) are each encoded by a separate gene and show a distinct but overlapping tissue distribution (4). PPAR α is highly expressed in the liver, kidney, and adrenal gland and has been shown to regulate expression of genes involved in hepatic lipid metabolism, including the P450 fatty acid ω -hydroxylase gene and genes involved in the peroxisomal β -oxidation pathway (5, 6). PPAR δ (also referred to as NUC1 or FAAR) is expressed ubiquitously and has been reported to repress transcription by PPAR α and the thyroid hormone receptor (7). PPAR γ exists as two isoforms encoded by a single gene, PPAR γ 1 and PPAR γ 2 (8). PPAR γ 2 is transcribed from an alternative promoter and is 30 amino acids longer than PPAR γ 1. Although PPAR γ 2 is the predominant isoform in adipocytes (8), PPAR γ 1 is also expressed in a number of other tissues including the adrenal gland and spleen (4). Consistent with its adipocyte localization, PPAR γ is involved in the regulation of adipocyte-specific genes, including the adipocyte fatty acid binding protein 2 (ap2) (8). PPAR γ is also implicated in adipogenesis (9). Stable transfection of PPAR γ 1 or PPAR γ 2 into fibroblasts induces them to differentiate into adipocytes (3, 8). Ligands identified for PPAR γ include the insulin-sensitizing thiazolidinedione anti-diabetic drugs and the naturally occurring prostaglandin derivative, 15-deoxy- Δ 12,14-prostaglandin J2 (15dJ2), each of which can promote adipocyte differentiation (10–12). In addition to its role in adipocyte differentiation and function, PPAR γ has recently been implicated in a number of pathological conditions including atherosclerosis and colorectal cancer (13–15).

The PPAR isoforms regulate target gene transcription through binding to PPAR response elements (PPREs), a DR1-type hormone response element. This binding is enhanced by heterodimerization to the retinoid X receptor (RXR) (16, 17). The PPAR-RXR heterodimer is responsive to both PPAR ligands and the RXR ligand 9-*cis*-retinoic acid (17, 18). Functional PPREs have been identified in the promoters of a number of genes including acyl-CoA oxidase (6), phosphoenolpyruvate carboxykinase (19), CYP4A6 (5), lipoprotein lipase (20), and ap2 (8).

Transcriptional activation or repression by nuclear hormone receptors can be augmented by transcriptional coactivators and corepressors, which can serve as a bridge between the nuclear receptor and the basal transcriptional machinery. A number of coactivators, including ARA70 (1), RIP140 (21), CBP/p300 (22, 23), TIF2 (24), SRC-1 (25), and ARA54 (26) have recently been identified as interacting with one or more nuclear hormone receptors. These interactions are typically dependent on the presence of ligand. Recently a number of cofactors for the PPAR isoforms have been identified. SRC-1, PBP, CBP, and PGC-1 (27–30) have been shown to interact with PPAR γ , and p300 has been shown to be a coactivator for PPAR α (31). The rat dUTPase is a potential corepressor for all the PPAR isoforms

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[‡] These authors contributed equally to this work.

[§] To whom correspondence should be addressed: George Whipple Laboratory for Cancer Research, Dept. of Pathology, Urology, and Biochemistry, University of Rochester Medical Center, Box 626, 601 Elmwood Ave., Rochester, NY, 14642. Tel.: 716-273-4500; Fax: 716-756-4133; E-mail: chang@pathology.rochester.edu.

¹ The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; mPPAR, mouse PPAR; DBD, DNA binding domain; 15dJ2, 15-deoxy- Δ 12,14 prostaglandin J2; AR, androgen receptor; hAR, human AR; RXR, retinoid X receptor; SRC-1, steroid receptor coactivator 1; ap2, adipocyte fatty acid-binding protein 2; DHT, dihydroxytestosterone; CAT, chloramphenicol acetyltransferase; BES, 2-[bis(2-hydroxyethyl)-amino]ethanesulfonic acid; TK, thymidine kinase; CBP, cAMP response element-binding protein-binding protein; PBP, PPAR γ -binding protein.

(32). To further understand transcriptional regulation by PPAR γ , we have investigated the potential interaction between PPAR γ and ARA70. ARA70 was originally described as a coactivator of the androgen receptor and shows a broad tissue distribution of expression, including adipose tissue (1). Here we present evidence to demonstrate that ARA70 can induce PPAR γ target genes through a PPAR γ -ARA70 complex. We also show that PPAR γ - and AR-mediated pathways may be linked through their common use of ARA70. Unlike previously described receptor-coactivator interactions, ARA70 can confer transcriptional activity to PPAR γ in the absence of ligand, although the presence of ligand enhances PPAR γ -ARA70 transactivation.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmid expressing the GAL4-DNA binding domain (DBD) and the mPPAR γ ligand binding domain (pGAL4DBD-mPPAR γ) was constructed by inserting the mouse PPAR γ 1 ligand binding domain (from amino acids 162–475), isolated as a *Scal/BamHI* fragment from pGBTmPPAR γ 1 in-frame into pCMXGal4 DBD. The plasmid expressing VP16-ARA70 was constructed by inserting a fragment of ARA70 cDNA encoding amino acids 1–401 into the VP16 activation domain plasmid pCMX-VP16. The site-directed mutagenesis was generated by using the following four primers: 5'-CCGGAATTCTCAGTCCACCAAGGTCT-3', 5'-GCTCTACTCGGCAGCGGGCCAGTTCAATTG-3', 5'-GAACTGGCCCGCTGCCGAGTAGAGCGCTG-3', 5'-CGCGGATCCCTCTACCTTACATGGGTC-3'. Mutagenesis was carried out on the ARA70 cDNA fragment encoding amino acids 1–401 by polymerase chain reaction (33). The mutated fragment was then reinserted in-frame into pCMX-VP16. The construction of pSG5-PPAR was done by inserting the Asp718-*SalI* fragment from pGBTmPPAR γ 1 into pSG5.

Coimmunoprecipitation—Recombinant mPPAR γ 1, RXR α , AR, and ARA70 were expressed by the TNT Coupled Reticulocyte Lysate System (Promega) incorporating [³⁵S]methionine according to the manufacturer's instructions. Fifteen μ l of labeled receptors were mixed with 50 μ l of ARA70 and incubated with 15–20 μ l of antibody to PPAR γ (Santa Cruz), RXR α (Santa Cruz), or with the AR antibody NH27 (34). Proteins and antibody were incubated in 70 μ l of HC400 (20 mM Hepes-KOH, pH 7.9, 400 mM KCl, 0.2 mM EDTA, 20% glycerol, 0.1 mg/ml bovine serum albumin) for 1 h at 4 °C before adding 10 μ l of protein A-Sepharose beads (Amersham Pharmacia Biotech). DHT was added to the AR + ARA70 reaction at a final concentration of 100 nM. The reaction was then incubated while rocking overnight at 4 °C. Immunoprecipitated complexes were collected by centrifugation at 2000 rpm at 4 °C for 1 min. The pelleted beads were washed three times with HC400, four times with 10 mM K₃PO₄, pH 8.0, 0.1 M KCl, and then mixed with SDS sample buffer, boiled, and separated by 8% SDS-polyacrylamide gel electrophoresis.

Cell Culture and Transfection—Human prostate DU145 cells were grown in Dulbecco's minimal essential medium containing 5% fetal calf serum at 37 °C. The cells were transfected by modified BES-calcium phosphate procedure (25). Cells were plated 4 × 10⁵/60-mm Petri dish 1 day before transfection. Transfection medium contained a constant amount of reporter plasmid and indicated amounts of pSG5-mPPAR γ , pSG5-hRXR α , and pSG5-hARA70 using pSG5 as a carrier to provide equal amounts of transfected DNA. One h before transfection, the medium was changed to Dulbecco's minimal essential medium with 5% charcoal-stripped fetal calf serum, and the medium was changed again 20 h post-transfection and treated with steroid hormone or 15dJ2 for another 14–16 h. Cell extracts were prepared and assayed for CAT or luciferase activity (Promega) and normalized against β -galactosidase or *Renilla* luciferase activity as indicated. All data were the average results from three to six independent experiments.

RESULTS

Interaction of ARA70 with PPAR γ —To investigate the potential interaction between ARA70 and PPAR γ , we first analyzed their interaction in a mammalian two-hybrid assay in DU145 cells. DU145 cells were used because they do not express AR, and the subline in our hands shows a low level of endogenous ARA70 activity to coactivate AR-mediated transcription. The ligand binding domain of AR, PPAR γ , and RXR were fused to the GAL4 DNA binding domain (GAL4DBD). These were co-transfected with a vector expressing the VP16 activation do-

main alone or linked with ARA70. In agreement with our previous results (1), coexpression of GAL4DBD-AR with VP16-ARA70 induced CAT activity by 11-fold in the presence of DHT (Fig. 1A, lanes 7 and 8). ARA70 is also able to interact with RXR in the presence of its ligand, 9-*cis*-retinoic acid. In contrast, GAL4DBD-PPAR γ cotransfected with VP16-ARA70, did not require the presence of ligand to induce CAT activity. In this case, CAT activity was induced 36-fold in the absence of the PPAR γ ligand 15dJ2 (10, 11) and 52-fold in the presence of exogenous ligand (Fig. 1A, lanes 4 and 10). It has previously been reported that SRC-1 is a ligand-dependent cofactor of PPAR γ (28). Here we also find that VP16-SRC1 interacts with GAL4DBD-PPAR γ to induce luciferase activity. A slight interaction between SRC-1 and PPAR γ is observed in the absence of ligand, but this is significantly weaker than the ligand-independent activation seen with ARA70 (Fig. 1B, lanes 2 and 3). In the presence of 15dJ2, the interaction between PPAR γ and ARA70 is more than twice that of PPAR γ and SRC-1 (Fig. 1B, lanes 5 and 6).

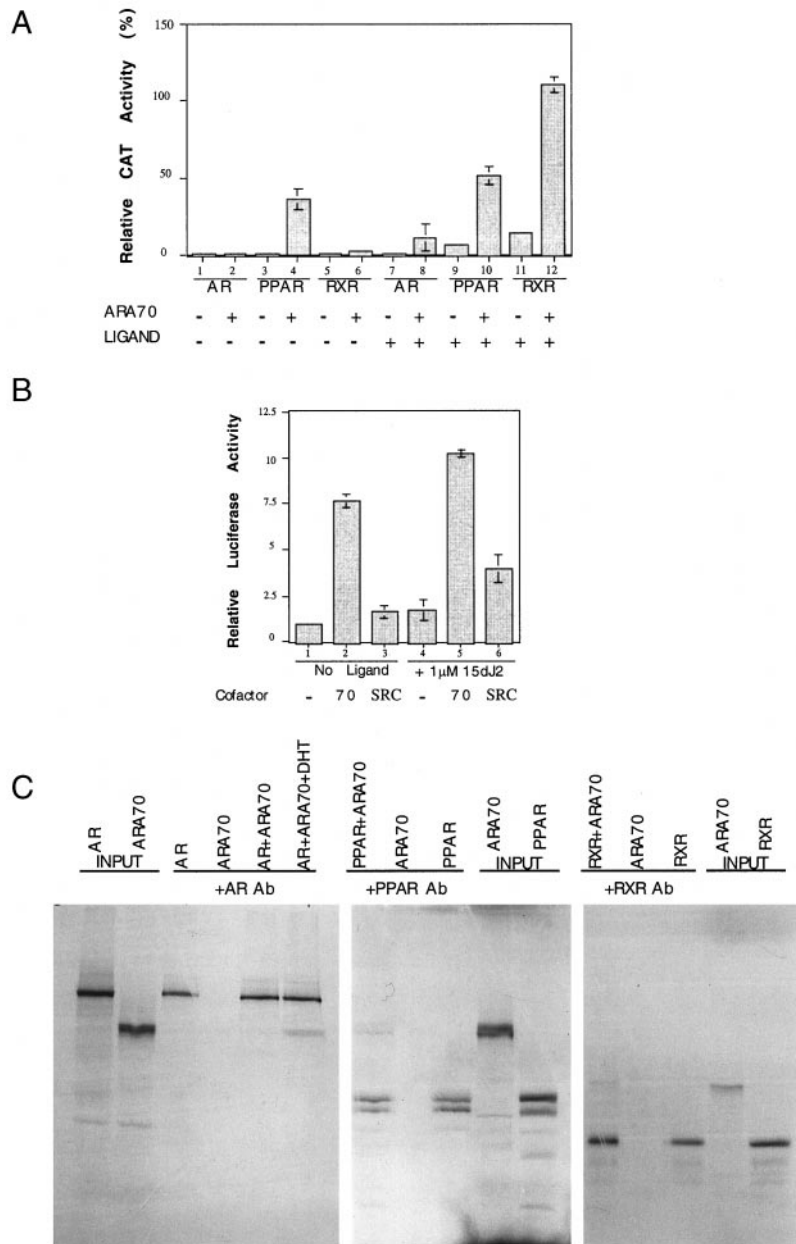
Interaction between PPAR γ and RXR with ARA70 was further confirmed by coimmunoprecipitation. The receptors and ARA70 were expressed *in vitro* using rabbit reticulocyte lysate and coimmunoprecipitated using antibodies specific for AR, RXR, or PPAR γ as indicated (Fig. 1C). Although AR coimmunoprecipitated ARA70 only in the presence of DHT, PPAR γ and RXR were able to bind ARA70 even in the absence of their respective ligands.

Site-directed Mutation of ARA70 Attenuates Its Interaction with PPAR γ and RXR—A number of nuclear receptor coactivators contain a LXXLL motif (or NR box) that is considered to play an important role in nuclear receptor-coactivator interaction (35, 36). ARA70 contains one NR box (LYSL) at amino acids 92–96 (Fig. 2A). To investigate whether the ARA70 NR box influences the PPAR γ -ARA70 and RXR-ARA70 interactions, we mutated this region. Mutation of the leucine doublet to alanines (LYSL → LYSA) of ARA70 (mtARA70) was tested in a mammalian two-hybrid assay in DU145 cells. The VP16-mtARA70 showed reduced transactivation of both GAL4DBD-RXR and GAL4DBD-PPAR γ (Fig. 2B). In the presence of the appropriate ligand, the VP16-mtARA70 significantly reduced the transactivation of both receptors compared with the wild type ARA70. In the case of GAL4DBD-PPAR γ , the mutant ARA70 also reduced transactivation in the absence of ligand (Fig. 2B, lanes 3 and 6). This demonstrates the importance of the ARA70 NR box in the interaction of ARA70 with both PPAR γ and RXR.

ARA70 Enhances the Transcriptional Activity of PPAR γ —To investigate the functional relevance of the interaction between PPAR γ and ARA70, we coexpressed PPAR γ and ARA70 in DU145 cells. We examined PPAR γ -mediated transcriptional activity on two luciferase reporter genes, one driven by three copies of a PPRE linked to a TK promoter (Fig. 3) and another containing 5.4 kilobases of the ap2 gene promoter (Fig. 4). The addition of ARA70 increases the PPAR γ -mediated transcription of both reporter constructs. The transfection of ARA70 with PPAR γ alone or with PPAR γ and RXR did not show significant differences, probably because of abundant endogenous RXR expression in DU145 cells. RXR, like PPAR, binds to a DR-1-type response element and therefore in theory could activate our reporter constructs. Transfection of RXR with ARA70 or ARA70 alone did not result in reporter gene expression, showing that both reporter genes are activated through PPAR γ . In agreement with previous reports (17, 37), we show that transcriptional activation occurs in response to either 15dJ2 or 9-*cis*-retinoic acid and that maximal transcription by PPAR γ -RXR occurs in the presence of both ligands (Fig. 3). In

FIG. 1. Interaction of ARA70 with hAR, mPPAR γ 1, and hRXR α in ligand-dependent or -independent manner.

A, DU145 cells were transfected with an expression vector expressing a chimeric protein consisting of the GAL4 DBD linked the hAR, mPPAR γ 1 or hRXR α nuclear receptor ligand binding domain (2 μ g). These cells were cotransfected with a vector expressing VP16 activation domain alone or linked to the N-terminal 401 amino acids of ARA70 (4 μ g). Plates were also transfected with the pG5-CAT reporter (3.5 μ g) and pCMV- β -galactosidase as the internal control. After 24 h of transfection, cells were treated with ethanol or ligand for AR, PPAR γ 1, or RXR, that is, 1 nM 5 α -DHT, 1 μ M 15dJ2, and 1 μ M 9-*cis*-retinoic acid, respectively. Cells were harvested 24 h after treatment of ligands for determination of β -galactosidase and CAT activity. The values are the means \pm S.D. of three experiments. **B**, DU145 cells were cotransfected with pGAL4 DBD-mPPAR γ (2 μ g), the pG5-tk-luciferase reporter plasmid (2.5 μ g), and 4 μ g of the VP16 activation domain alone or linked to either the N terminus of ARA70 (70) or the C terminus of SRC-1 (SRC). Transfected cells were either mock-treated with ethanol or with 1 μ M 15dJ2 as indicated. *Renilla* luciferase activity from 2 μ g of transfected pRL-tk-luciferase was used as an internal control. The activity of pG5-tk-luciferase when cotransfected with GVAL4DBD-mPPAR γ , and the VP16 activation domain was set as one. The results are the mean \pm S.D. of three experiments. **C**, TNT-translated (Promega) [³⁵S]methionine-labeled ARA70 full-length protein, hAR, mPPAR γ 1, and hRXR α were incubated with the appropriate receptor antibody and protein A-Sepharose beads as described under "Experimental Procedures." Input receptor-loaded represents 1/3 of that used in the immunoprecipitation reaction, and input ARA70-loaded represents 1/10 of the final used.



the presence of both ligands, PPAR γ -RXR-ARA70 elicits a stronger transcriptional response than PPAR γ -RXR-SRC-1 (Fig. 4). Consistent with the mammalian two-hybrid assay and the coimmunoprecipitation data, we observe that ARA70 can enhance PPAR γ -mediated expression in the absence of exogenous ligand, although this expression is significantly less than that seen when ligand is added (Figs. 3A and 4A). This is potentially because of the presence of endogenous ligand, as has been suggested for PPAR γ transactivation by SRC-1 observed in the absence of added ligand (28). However, transfection of PPAR γ with SRC-1 in the absence of ligand in our hands shows only background transcriptional activity (Fig. 4, lane 3), whereas cotransfection of PPAR γ -RXR with SRC-1 shows ligand-independent activation comparable with that observed for ARA70 (Fig. 4, lane 6). It is unclear whether this indicates an absence of endogenous ligand in our system or an unanticipated regulatory affect of overexpressed RXR with SRC-1 on the ap2 promoter. It has recently been suggested that PPAR γ 1 and PPAR γ 2 may have different activation capabilities, which is determined by their different N termini (38). We find that

ARA70 shows similar increases in transcription with both PPAR γ isoforms (data not shown).

AR Can Squelch ARA70 from PPAR γ —In a previous report (1), we demonstrated that ARA70 and AR physically interact and that ARA70 can function as an androgen-dependent coactivator for AR. We were interested to know if AR and PPAR γ could compete for ARA70. Cotransfection of PPAR γ , RXR, and AR does not significantly influence PPAR γ -RXR transcription (Fig. 5). However, AR is able to significantly reduce PPAR γ -RXR-ARA70 transcriptional activity even in the presence of 15dJ2 and 9-*cis*-retinoic acid (Fig. 5, lanes 15 and 16). Potentially, cross-talk between AR and PPAR γ could occur through their functional association with ARA70.

DISCUSSION

Previously, we have demonstrated that ARA70 is a relatively specific transcriptional coactivator of the androgen receptor that shows only a very marginal induction of transcriptional activity by the estrogen, glucocorticoid, and progesterone receptors in DU145 cells (1). Here we show that ARA70 is also a

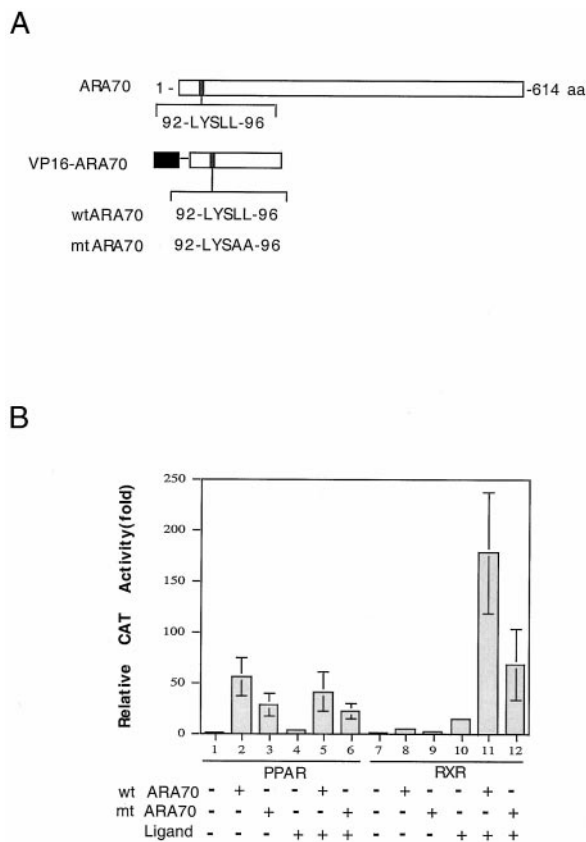


FIG. 2. Site-directed mutation of ARA70 attenuate the interaction with nuclear receptors. *A*, site-directed mutation of ARA70 was generated by polymerase chain reaction ("Experimental Procedures") to mutate LYSSL, the single LXXLL motif of ARA70 located at 92–96 amino acids, to LYSAA. This fragment, containing 401 amino acids of N-terminal ARA70, was then fused to VP16 for a mammalian two-hybrid assay. *B*, DU145 cells were cotransfected with pGAL4DBD-hAR, -mPPAR γ 1, or -hRXR as described in Fig. 1*A*. Cells were cotransfected with VP16-ARA70 (*wt* ARA70), the mutated ARA70 construct (*mt* ARA70), or the VP activation domain alone. After 24 h, cells were treated with ethanol or ligand for AR, PPAR γ 1, or RXR, that is, 1 nM 5 α -DHT, 1 μ M 15dJ2, and 1 μ M 9-*cis*-retinoic acid, respectively. Cells were harvested after 16 h of treatment with ligand and assayed for β -galactosidase activity and CAT activity.

coactivator of PPAR γ . Unlike the AR-ARA70 interaction, which requires the presence of androgen, the PPAR γ -RXR-ARA70 interaction occurs in the absence of exogenous ligand as determined by a mammalian two-hybrid assay and by coimmunoprecipitation. Cotransfection of PPAR γ and ARA70 also results in low level constitutive expression from an ap2 promoter and an isolated PPRE-driven construct. However, in both the mammalian two-hybrid system and the transfection studies, the transcriptional activation of the reporter genes could be further enhanced by the addition of the appropriate ligands, demonstrating that the binding of ligand is still necessary for maximal transcriptional response. Interestingly, in the presence of ligand, ARA70 mediates a greater increase in transcriptional activity than SRC-1, suggesting that in adipose tissue it may be the more important coactivator of PPAR γ . Recently, CBP and PBP have been identified as cofactors of PPAR γ (27, 30). CBP and PBP demonstrate ligand-independent physical interaction with PPAR γ but require ligand to show a transcriptional effect. This suggests that although unliganded PPAR γ may be permissive to some cofactor interactions, the nature of its interaction with ARA70 is different in that it allows constitutive transcription.

Ligand-independent activation by PPAR γ in the presence of a cofactor has also been observed for SRC-1 (28). It is possible

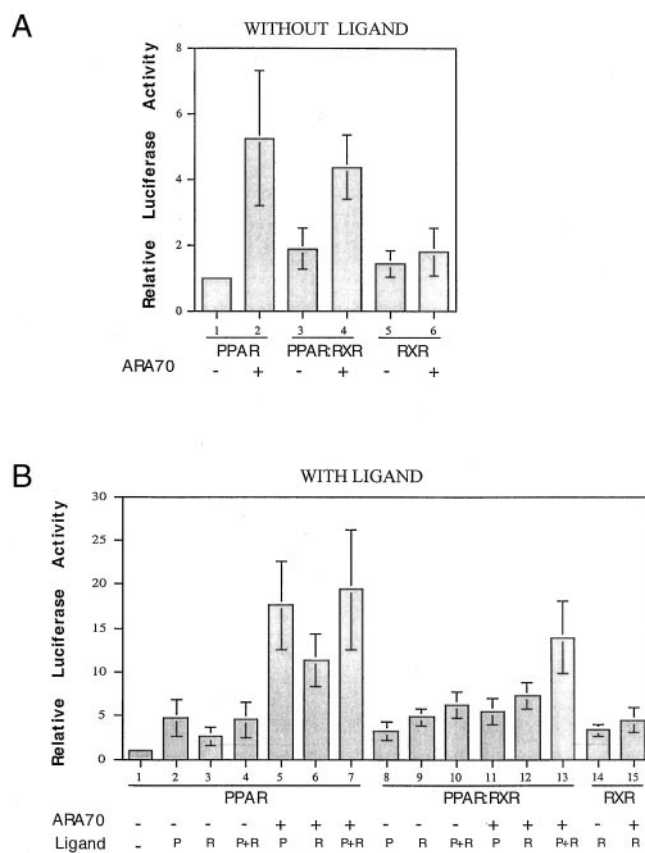


FIG. 3. ARA70 increases PPAR γ -mediated transactivation of the (PPRE) $_3$ -tk-luciferase reporter construct in DU145 cells. *A*, DU145 cells were cotransfected with 3 μ g (PPRE) $_3$ -tk-luciferase, 1 μ g of pSG5-mPPAR γ (PPAR), 0.5 μ g pSG5-mPPAR γ and 0.5 μ g pSG5-hRXR α (PPAR:RXR), or 1 μ g of pSG5-hRXR α (RXR). pSG5-hARA70 was transfected at 5 μ g, and pSG5 was used to provide equal amounts of transfected DNA. Cells were mock-treated with ethanol. *B*, DU145 cells were transfected as in *A* but were treated with 3 μ M 15dJ2 (P), 1 μ M 9-*cis*-retinoic acid (R) or both (P+R). All ligands were dissolved in ethanol. Transfection efficiency was normalized against the internal control of *Renilla* luciferase activity. The activity of (PPRE) $_3$ -tk-luciferase transfected with pSG5-mPPAR γ and mock-treated with ethanol was taken as one. Results are the mean \pm S.D. of three to six independent experiments.

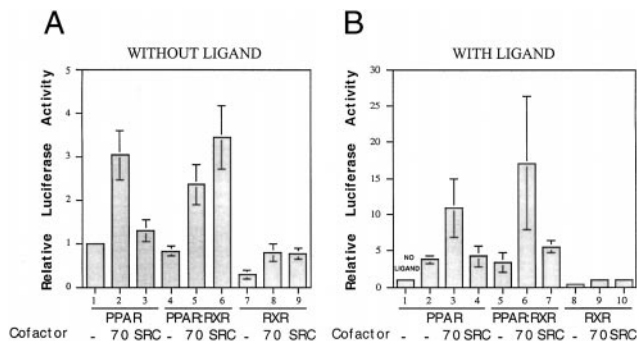


FIG. 4. ARA70 and SRC-1 increase the PPAR γ -mediated transcription of the ap2(-5.4)-luciferase reporter construct. *A*, DU145 cells were cotransfected as in Fig. 3*A* using 3 μ g of the ap2(-5.4)-luciferase reporter construct and 5 μ g of pSG5-hARA70 or pSG5SRC-1 as indicated. Cells were mock-treated with ethanol. *B*, DU145 cells were transfected as in Fig. 3*B* using 5 μ g of pSG5-hARA70 or pSG5SRC-1 as indicated with the ap2(-5.4)-luciferase reporter gene. Results are the mean \pm S.D. of three to six independent experiments.

that these effects are due to the presence of an endogenous ligand. However, transfection of PPAR γ and SRC-1 in our system does not lead to transcription above background, suggesting that ARA70 alone induces a low level of PPAR γ -medi-

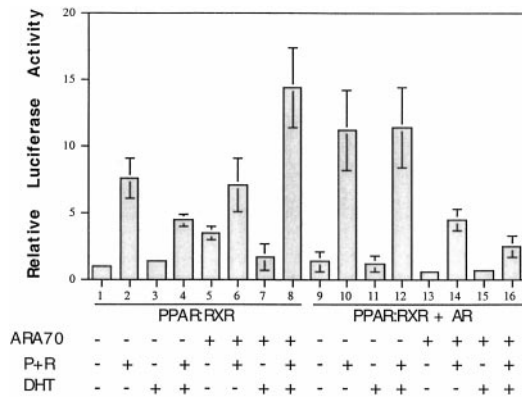


FIG. 5. Effect of AR on the PPAR γ (PPRE)x3-tk-luciferase reporter in the presence or absence of androgen. DU145 cells were cotransfected with 3 μ g of (PPRE)x3-tk-luciferase reporter, 0.5 μ g each of pSG5-mPPAR γ and pSG5-hRXR α , 5 μ g of pSG5-hARA70, and 1 μ g pSG5-AR as indicated. Cells were either mock-treated with ethanol, with 3 μ M 15dJ2 and 1 μ M 9-*cis*-retinoic acid (P+R), or with 10 nM DHT as indicated. The data are the mean \pm S.D. of three independent experiments. P+R, 9-*cis*-retinoic acid and 15dJ2.

ated transcription that is further enhanced in the presence of ligand. Ligand-independent transcription is not seen between AR and ARA70 (1), suggesting that the manner in which ARA70 interacts with PPAR γ and AR may be different. ARA70 may have a limited ability to stabilize the AF-2 helix of PPAR γ in an "active" conformation (similar to a liganded receptor) to allow transcription to occur but may be unable to do this with AR. Ligand-independent activation has been shown for several nonmutated steroid receptors. Both the estrogen and progesterone receptors can induce transcription in the absence of ligand when phosphorylated (39, 40). Alternatively, the unliganded receptor may bind to a transcriptional repressor and allow constitutive expression, as is the case when the thyroid hormone receptor binds p53 to reverse repression in some cell types (41). The role of phosphorylation of PPAR γ via the mitogen-activated protein kinase pathway is unclear, with some authors showing phospho-PPAR γ having a negative transcriptional effect (42) and others showing phospho-PPAR γ can activate transcription in the absence of endogenous ligand (43). However, in DU145 cells, transfection of PPAR γ carrying a mutated mitogen-activated protein kinase target site or incubation with inhibitors of the mitogen-activated protein kinase pathway does not alter the ligand-independent activation of PPAR γ in the presence of ARA70, but it does decrease the ligand enhanced effect.² The possibility that ARA70 itself may possess kinase activity is currently being investigated. It is possible that the PPAR γ -ARA70 complex may bind to a repressor and thus allow constitutive expression of our reporter constructs. COUP-TF has been identified as a potential repressor of some PPAR γ target genes and could possibly be subject to this interaction (2, 44).

ARA70 contains a single LXXLL motif at amino acids 92–96. This motif has been identified in several nuclear receptor coactivators as being involved in the interaction with the receptor AF2 domain. For example, mutation of the four LXXLL boxes of SRC-1 abolished its ability to interact with the estrogen receptor (35), and the crystal structure of PPAR γ with SRC-1 has been determined (45). The importance of this motif in PPAR γ -ARA70 and RXR-ARA70 interactions was confirmed by mutating the conserved leucine doublet to alanines (LYSLL \rightarrow LY-SAA). The mutant ARA70 showed a decreased interaction with both PPAR and RXR in a mammalian two-hybrid assay. In the

case of PPAR γ , the mutant ARA70 showed a decreased interaction in the presence and absence of 15dJ2. This suggests that ligand-dependent and -independent interactions between PPAR γ and ARA70 both occur through the same ARA70 domain. However, more detailed interaction data between PPAR γ and ARA70 is needed to confirm this possibility.

The heterodimeric partner of PPAR γ , RXR, also interacts with ARA70 but in a ligand-dependent manner. PPAR γ -RXR-mediated transcription can be activated by the addition of RXR ligands (Fig. 3 and Ref. 28). It is therefore possible that *in vivo* PPAR γ and RXR may independently recruit ARA70 to contribute to transactivation by the heterodimer, as has been reported for SRC-1 (28). Whether ARA70 can enhance transactivation by RXR-RAR heterodimers is currently under investigation.

The relative specificity of ARA70 could allow cross-talk between PPAR γ and AR in tissues where they are coexpressed with ARA70, as is the case in adipocytes. We have shown that when PPAR and AR are cotransfected with ARA70, DHT treatment reduces the expression of a PPAR γ reporter gene. This may indicate that liganded AR is able to compete with PPAR γ for ARA70 to differentially regulate their respective target genes. PPAR γ and AR are both known to mediate adipose tissue metabolism and have both been implicated in relation to insulin sensitivity. It has been demonstrated that activators of PPARs, and particularly of PPAR γ , promote adipocyte differentiation in preadipocytes (46) and in the multipotential cell line C3H10T1/2 (10). Stable transfection of PPAR γ can induce differentiation of fibroblastic NIH 3T3 cells to adipocytes in the presence of PPAR γ ligands (9, 11). PPAR γ is also known to transcriptionally activate a number of adipocyte-expressed genes including lipoprotein lipase (20) and ap2 (8). Testosterone affects the regulation of lipolysis by increasing β -adrenoceptors and regulating adenylate cyclase activity (47). Testosterone in some studies downregulates lipoprotein lipase (48–50). The differential expression of lipoprotein lipase may contribute to gender-specific regional fat distribution (47, 48). The thiazolidinedione class of drugs increase insulin sensitivity in patients with noninsulin-dependent diabetes and are known to be PPAR γ ligands (11, 12), although the mechanism of this affect is unknown. Aberrant levels of testosterone have been associated with insulin resistance. Female rats treated with testosterone or human females with polycystic ovary syndrome (which results in higher levels of circulating androgens) show an increased incidence of insulin resistance (51, 52). In males, low levels of androgens have been correlated with insulin resistance. Castrated male rats or hypogonadal men have increased insulin resistance, which can be improved by low level testosterone treatment (49, 50). That PPAR γ and AR share a coactivator suggests that cross-talk between the two receptor-mediated pathways may occur. However, further investigation is necessary to establish the degree of this possible interaction *in vivo*.

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