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Induction and Repression of Peroxisome Proliferator–Activated Receptor α Transcription by Coregulator ARA70

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In an effort to understand transcriptional regulation by the peroxisome proliferator–activated receptor α (PPARα), we investigated the ability of a number of transcriptional coactivators to enhance PPARα:retinoic acid receptor (RXR) mediated transcription. We identified ARA70, a coactivator of the androgen receptor and PPARγ, as a ligand-enhanced coactivator of PPARα in the prostate cancer cell line DU145. In prostate cancer cells, ARA70 demonstrated the strongest enhancement of PPARα transcription among the coactivators examined. Mutation of the N-terminal of the PPARα ligand-binding domain dramatically reduced the ability of ARA70 to enhance PPARα:RXR transcription. ARA70 was able to physically interact with both the wild-type and mutant PPARα as determined by coimmunoprecipitation. However, in the adrenal cell line Y1, ARA70 behaved as a repressor of PPARα while still able to coactivate PPARγ.

Key Words: Peroxisome proliferator–activated receptor α; coactivator; androgen receptor; prostate cancer.

Introduction

The peroxisome proliferator–activated receptors (PPARs) are a family of three ligand-inducible nuclear receptors (PPARα, -PPARβ, and PPARγ), with each member demonstrating a unique tissue distribution and ligand specificity (reviewed in ref. *1*). PPARα is highly expressed in the liver, kidney, and adrenal gland as well as the male and female genital systems *(2,3)*. PPARγ is expressed in adipocytes, where it has been implicated in adipogenesis *(4)* and the regulation of adipocyte-specific genes such as adipocyte fatty acid binding protein 2 *(5)*. PPARγ also shows a high level of expression in the adrenal gland and spleen *(2)*. PPARβ (also referred to as PPARδ or NUC1) is ubiquitously expressed in the mouse and rat *(2,3,6)*. While the regulatory role of PPARβ is unclear, it has been reported to repress PPARα and thyroid hormone receptor–mediated transcription *(7)*. The ligands of PPARα include the fibrate hyperlipidemic drugs (including WY14,643 and clorfibric acid) and leukotriene B_4 *(8,9)*. PPARγ ligands include the thiazolidinedione antidiabetic drugs *(10)* and the prostaglandin derivative 15-deoxy-Δ12,14 prostaglandin J2 (15dJ2) *(11,12)*. Fatty acids and eicosanoids are also capable of binding to and activating PPAR-mediated transcription *(1,13)*.

As with PPARγ, PPARα is considered to play a key role in lipid metabolism and storage and in inflammatory response *(8,9,14)*. PPARα has been shown to regulate the expression of genes involved in the peroxisomal-oxidation pathway *(15)* and genes involved in hepatic lipid metabolism, including the P450 fatty acid ω-hydroxylase gene *(16)*. In addition, PPARα is involved in the regulation of the steroid-metabolizing enzymes 17β-hydroxysteroid dehydrogenase type IV and 3α-hydroxysteroid dehydrogenase *(17–19)*. PPARα and PPARγ have also been implicated in a number of pathologic conditions. PPARγ may contribute to the progression of athlerosclerosis through binding oxidized low-density lipoprotein *(20)*. While PPARγ also appears to be involved in colorectal cancer, it is unclear whether it acts to promote or reduce colon polyp formation *(21,22)*. Elevation of PPARα expression has been found in prostate cancer samples, and the degree of expression correlates with tumor grade and stage *(23)*. Ligands of both PPARγ and PPARα, however, appear to induce cytostasis of prostate cancer cells in vitro and in in vivo model systems *(24–26)*.

The PPAR isoforms regulate gene transcription through heterodimerization with 9-*cis*-retinoic acid receptor (RXR) and binding to a DR1-type PPAR response element *(27,28)*. The PPAR:RXR heterodimer responds to the RXR ligand 9-*cis*-retinoic acid as well as the PPAR ligands [28, 29]. As with other members of the nuclear receptor superfamily, PPAR:RXR transcription is modulated through transcriptional coactivators and corepressors. Coactivators may enhance the transcriptional activity of nuclear receptors through multiple mechanisms, including interaction with the basal transcriptional machinery, recruitment of chromatin-modifying complexes, modulation of receptor ligand-binding affinity, and facilitation of nuclear translocation of

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the receptor *(30,31)*. One category of transcriptional corepressors, including NCoR and SMRT, is associated with a multiprotein corepressor complex that includes histone deacetylase *(32–34)*. On ligand binding, the repressor complex dissociates from the receptor, allowing receptor interaction with coactivator complexes *(35,36)*. By contrast, the coregulator RIP140 has been shown to repress RXR:RAR transcription in the presence of retinoids, suggesting an alternative mechanism of corepression *(37)*. NCoR and RIP140 have been identified as corepressors of PPARα *(38,39)*. A number of coactivators, including SRC-1, CBP/p300, PGC-1, PGC-2, and PBP/TRIP220/GRIP230, have been shown to enhance the transcription of PPARα and/or PPARγ as well as one or more other nuclear receptors (ref. *1* and references therein).

Recently, we identified the androgen receptor (AR) coactivator ARA70 *(40)* as a coactivator of PPARγ *(41)*. ARA70 was originally isolated as an AR-interacting protein that enhances AR transcription, with a significantly weaker effect on other steroid receptors such as glucocorticoid receptor (GR), estrogen receptor (ER), and progesterone receptor (PR) *(40,42)*. To determine whether ARA70 and other AR coactivators are also PPARα coactivators, we investigated whether ARA70, ARA55 *(43)*, ARA54 *(44)*, and Rb *(45)* could enhance PPARα-mediated transcription. Here, we show that ARA70 is able to interact with PPARα and enhance PPARα target gene transcription in the prostate cancer line DU145. In comparison with ARA70, the other coregulators examined provided an only minimal effect on PPARα transcriptional activation. However, in adrenal cells, ARA70 behaved as a repressor of PPARα while still able to coactivate PPARγ.

Results

ARA70 Enhances Transcriptional Activity of PPARα

ARA70 was initially identified as a coactivator of AR *(40)* and has recently been described as a coactivator of PPARγ *(41)*. To investigate whether other AR coactivators are also capable of enhancing transactivation of PPARγ and PPARα, we coexpressed PPARγ or PPARα with ARA70, ARA55, ARA54, SRC-1, and Rb in DU145 cells. The prostate cell line DU145 was chosen because of the low activity of AR transcription in the absence of exogenous AR coactivators. Additionally, PPARα ligands have been demonstrated to induce cytostasis and alter the expression of PPARα-responsive genes in prostate cancer cells *(18,25)*. In agreement with previously reported results *(41)*, ARA70 more strongly enhanced PPARγ-mediated transcription than SRC-1 in DU145 cells under these conditions (Fig. 1A, lanes 16 and 22). SRC-1, ARA54, and Rb all showed a similar degree of ligand-enhanced transactivation of PPARγ, while ARA55 showed the least enhancement. ARA70 also demonstrated the strongest transactivation of PPARα in this cell line (Fig. 1B). In contrast to PPARγ transactivation, SRC-1 provided only weak enhancement of PPARα transcription, and ARA55, ARA54, and Rb had a negligible transcriptional effect. PPARα:RXR transcription was enhanced fourfold more by ARA70 than SRC-1 (Fig. 1B, lanes 16 and 22), suggesting that in the context of DU145 prostate cancer cells, ARA70 may be a more significant mediator of PPARα transcription than SRC-1. Transfection of PPARα or PPARγ alone or with RXR did not show significant transcriptional differences, probably owing to the presence of abundant endogenous RXR in DU145 cells *(41)*. PPAR and RXR both bind to DR1-type response elements; however, transfection of RXR in the absence of PPAR did not result in reporter gene expression.

Point Mutation of PPARα Ligand-Binding Domain Attenuates ARA70 Coactivation

The PPARα mutant PPARα-G differs from the wild-type (WT) receptor by a single amino acid substitution (Glu282 to glycine) at the beginning of the ligand-binding domain (LBD) *(16)*. This mutation was originally characterized as demonstrating a lower level of background transcriptional activation in the absence of ligand in RK13, HepG2, and COS-1 cells but retains the ability to be fully induced by WT-14643 *(16,46,47)*. PPARα-G displays protein expression levels, protein turnover, nuclear localization, and DNA-binding characteristics similar to those of PPARα *(47)*. The reduced basal transcriptional activity of the PPARα-G mutant suggests that it may have an altered ability to bind activators or has an altered interaction with other aspects of the transcriptional machinery *(47)*. Although the ligand-induced transcriptional activation of PPARα-G and PPARα is comparable (Figs. 1B and 2), PPARα-G shows a reduced level of transactivation in the presence of ARA70 as compared to the WT receptor (Fig. 2). Whereas ARA70 enhances PPARα:RXR transcription by 6-fold (Fig. 1B, lanes 14 and 16), ARA70 only enhances PPARα-G:RXR-mediated transcription by 2.5-fold (Fig. 2, lanes 14 and 16). PPARα:RXR transcription is most strongly enhanced by ARA70 (Fig. 1), whereas PPARα-G:RXR transcription is enhanced to an approximately equal degree by all coactivators examined (Fig. 2).

ARA70 Interacts with PPARα and PPARα-G In Vitro

To confirm the observed enhancement of PPARα transactivation by ARA70 owing to direct interaction, coimmunoprecipitation assays were conducted. We have previously demonstrated that ARA70 interacts directly with PPARγ and RXR *(41)*, and it is possible that ARA70-mediated enhancement of PPARα:RXR transcription could occur through RXR-ARA70 interaction. In vitro–expressed ARA70 and PPARα or PPARα-G were incubated with an anti-PPARα antibody as described in Materials and Methods. As shown in Fig. 3, ARA70 was able to interact with both PPARα and PPARα-G, and this interaction was not influenced by the addition of WY14,643.

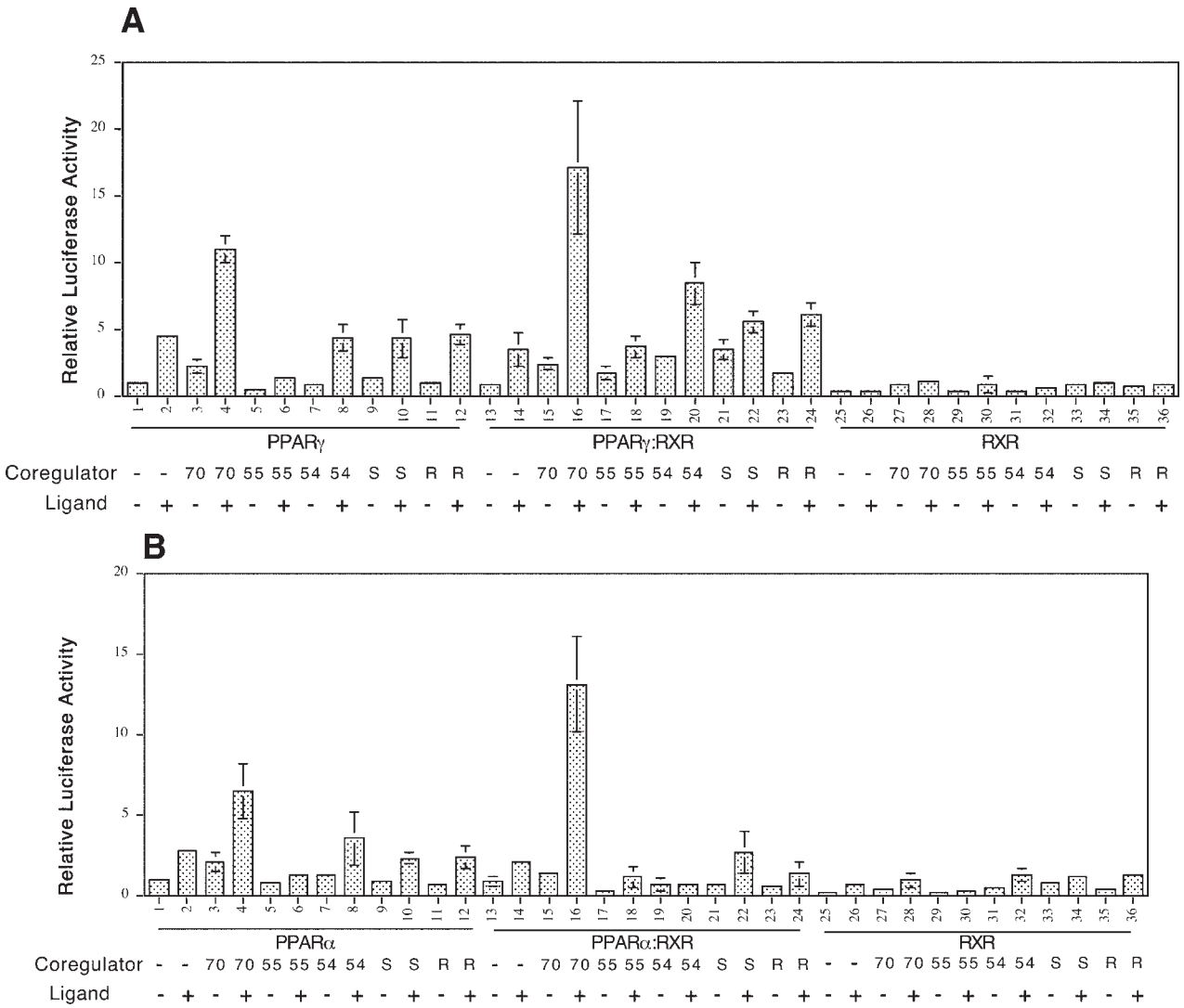

Fig. 1. Comparison of effect of various coactivators on transcriptional activity of PPARγ and PPARα in DU145 cells. (**A**) DU145 cells were cotransfected with 3 μg of (PPRE)x3-tk-luciferase, 1 μg of pSG5-PPARγ (PPARγ), 0.5 μg of pSG5-PPARγ, and 0.5 μg of pSG5-RXRα (PPARγ:RXR), or 1 μg of pSG5-RXRα (RXR). Five micrograms of pSG5-ARA70 (70), pSG5-ARA55 (55), pSG5-ARA54 (54), pSG5-SRC-1 (S), or pSG5-Rb (R) was transfected as indicated, and pSG5 was used to provide equal amounts of transfected DNA. Cells were mock treated with ethanol (–) or 3 μ*M* 15dJ2 (PPARγ), 1 μ*M* 9-*cis*-retinoic acid (RXR), or both (PPARγ:RXR). The activity of (PPRE)x3-tk-luciferase transfected with pSG5-PPARγ and mock treated with ethanol was taken as one. (**B**) DU145 cells were transfected as in (A) except that pSG5-PPARα was substituted for PPARγ. Cells were mock treated with ethanol (–) or 20 μ*M* WY14,643 (PPARα), 1 μ*M* 9-*cis*-retinoic acid (RXR), or both 20 μ*M* WY14,643 and 1 μ*M* 9-*cis*-retinoic acid (PPARα:RXR). All ligands were dissolved in ethanol. Transfection efficiency was normalized against the internal control of *Renilla* luciferase activity. Data are the mean ± SD of three to five independent experiments.

AR Can Compete ARA70 Away from PPARα and PPARα-G

In a previous report, we demonstrated that AR can squelch ARA70 from PPARγ *(41)*. To determine whether a similar effect is observed for PPARα, we cotransfected PPARα, RXR, and AR in DU145 cells (Fig. 4A). The presence of AR does not influence PPARα:RXR transcription in the absence of transfected coregulator. However, AR is able to abolish the ability of ARA70 to enhance PPARα:RXR transactivation in the presence of both WY14,643 and 9-*cis*-retinoic acid (Fig. 4A, lanes 13–16). A similar effect is observed for PPARα-G (Fig. 4B). These experiments suggest that ARA70 has a higher affinity for AR and that in ARA70-positive cell types that express comparable levels of PPARα, RXR, and AR, PPARα may be forced to use a different and possibly less potent coregulator than ARA70.

ARA70 Functions as Both Coactivator and Repressor in Adrenal Y1 Cells

The adrenal gland expresses AR, ARA70, and all three PPAR isoforms *(2,40,48)*. To investigate the role of ARA70 in adrenal cells, we transfected Y1 cells with PPARα, PPARγ, or AR and monitored transcription using a (PPRE)x3-tk-luci-

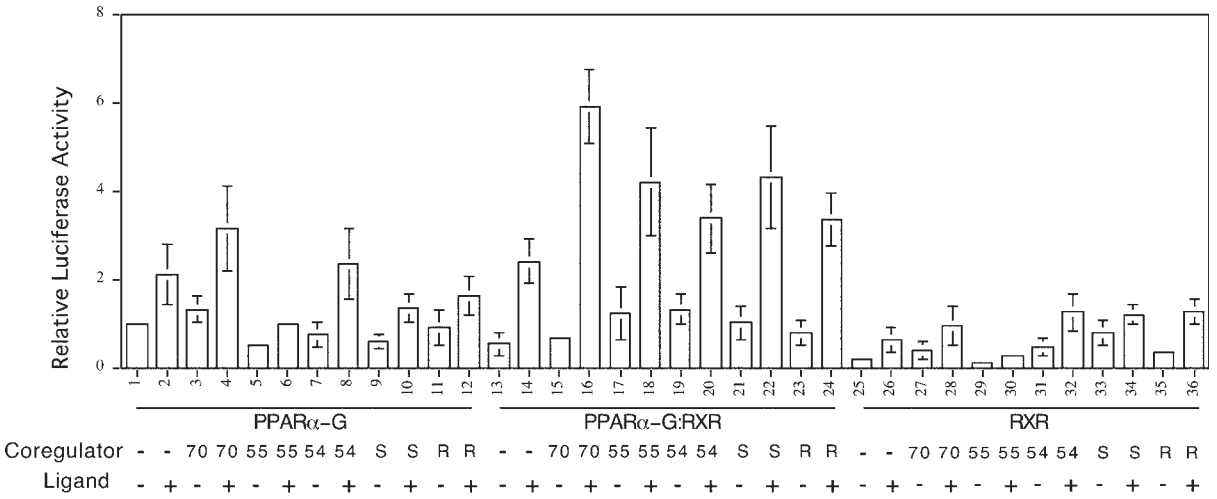

Fig. 2. Comparison of transcriptional coactivators on PPARα-G LBD mutant. DU145 cells were transfected with 3 μg of (PPRE)x3-tk-luciferase, 1 μg of pSG5-PPARα-G (PPARα-G), 0.5 μg of pSG5-PPARα-G and 0.5 μg of pSG5-RXRα (PPARα-G:RXR), or 1 μg of pSG5-RXRα (RXR). Five micrograms of pSG5-ARA70 (70), pSG5-ARA55 (55), pSG5-ARA54 (54), pSG5-SRC-1 (S), or pSG5-Rb (R) was transfected as indicated, and pSG5 was used to provide equal amounts of transfected DNA. Cells were mock treated with ethanol (–) or 20 μ*M* WY14,643 (PPARα-G), 1 μ*M* 9-*cis*-retinoic acid (RXR), or both 20 μ*M* WY14,643 and 1 μ*M* 9-*cis*-retinoic acid (PPARα-G:RXR). All ligands were dissolved in ethanol. Transfection efficiency was normalized against the internal control of *Renilla* luciferase activity. Data are the mean ± SD of three to five independent experiments.

ferase or MMTV-luciferase reporter gene (Fig. 5). Cotransfection of AR and ARA70 showed that in Y1 cells, ARA70 provided a moderate enhancement of AR-mediated transcription while SRC-1 enhanced AR transcription by approximately twofold (Fig. 5C, lanes 4 and 8). This is in contrast to the transcriptional effect in DU145 cells where ARA70 is a stronger AR transcriptional coactivator than SRC-1 *(44, 49)*. Similarly, PPARγ:RXR transcription was enhanced by ARA70 in Y1 cells and transfection of an ARA70 antisense construct reduced this effect (Fig. 5A, lanes 4 and 6). Cotransfection of SRC-1 mediated a greater increase in PPARγ:RXR transcription in Y1 cells than ARA70 (Fig. 5A, lanes 4 and 8). In contrast to AR and PPARγ, PPARα:RXR transcription was inhibited by ARA70 in Y1 cells, and this repression was alleviated by transfection of ARA70 antisense (Fig. 5B). SRC-1 enhanced PPARα:RXR transcription to approximately the same degree as in DU145 cells (Fig. 1B).

Discussion

ARA70 provides a stronger enhancement of AR and PPARγ transcription compared to the relatively weak effect on ER, GR, and PR in DU145 cells *(40,41)*. We showed that ARA70 is a dichotomous regulator of PPARα. In prostate cells, ARA70 enhanced PPARα:RXR-mediated transcription to a greater degree than SRC-1 under the transfection conditions used. The prostate coactivators ARA54 and ARA55 had a moderate or no effect on PPARα:RXR transactivation, respectively. In the case of PPARγ, ARA70 was also a stronger coactivator than SRC-1. However, ARA54, SRC-1, and Rb were more potent coactivators of PPARγ:RXR than PPARα:RXR. These results suggest that in prostate cancer cells, ARA70 may have a stronger transcriptional effect on PPARα and PPARγ than SRC-1.

The interaction of coactivators with nuclear receptors typically occurs through helices 3–5 and 12 of the nuclear receptor LBD and with the C-terminal hinge region *(50–52)*. The PPARα LBD extends from amino acids 281–468 *(1)*, and the mutant PPARα-G contains a Glu-Gly substitution at amino acid 282 *(16)*. Although PPARα-G had a response to ligand similar to that of the WT receptor (Figs. 1B and 2), we observed that PPARα-G showed an altered response to coactivators compared to the WT receptor in DU145 cells. Most notably, ARA70 provided less than half the transcriptional enhancement for PPARα-G:RXR than PPARα:RXR. However, ARA55, ARA54, SRC-1, and Rb all enhanced PPARα-G transactivation to a similar degree and almost to the level of ARA70. This is in contrast to the nonmutated receptor, for which there is a substantial difference between ARA70 and the other coactivators examined. ARA70 appears to interact equally with PPARα and PPARα-G as determined by coimmunoprecipitation, suggesting that one effect of the PPARα-G mutation may be to alter the ability of PPARα-G:RXR-ARA70 to interact with the general transcriptional machinery. The PPARα-G mutation, however, broadens the ability of the receptor to interact with other coactivators in a ligand-dependent manner. It is still unclear if this alteration in coactivator enhancement is owing to WY14,643 interacting with the ligand-binding pocket differently, thus changing the coactivator interaction surface, or if the mutation acts independent of ligand binding.

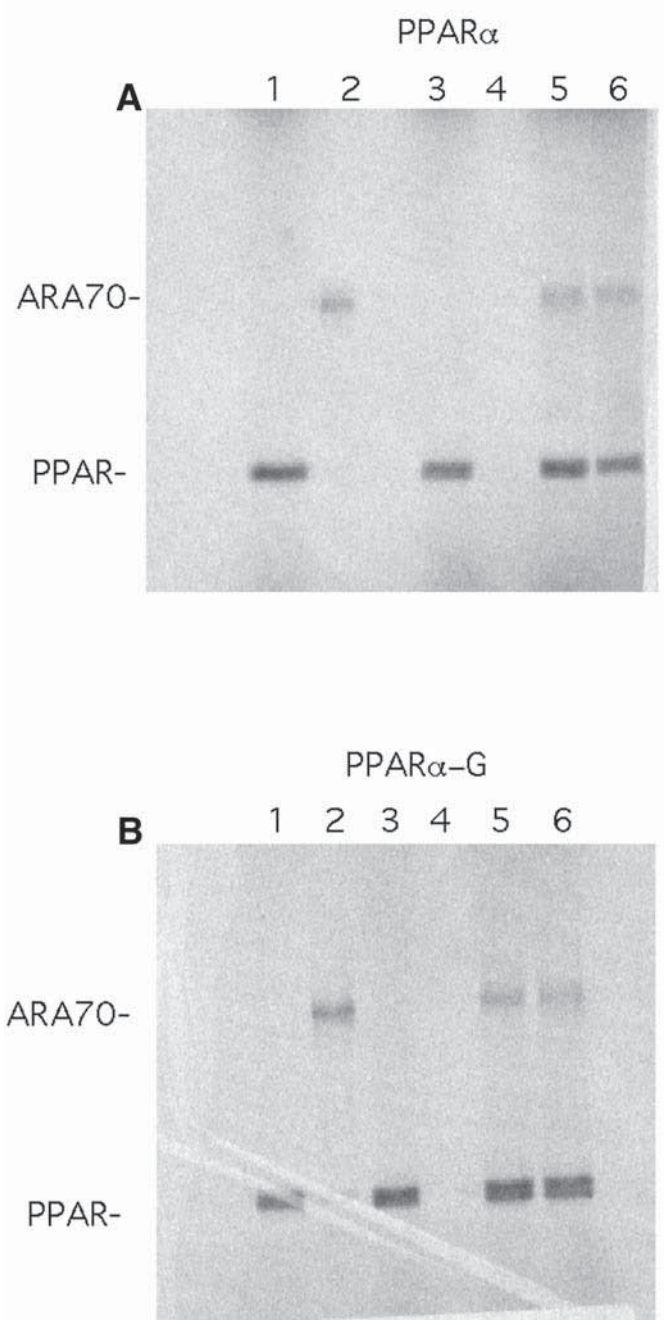

Fig. 3. Coimmunoprecipitation of ARA70 with PPARα and PPARα-G. TNT-translated (Promega) ^{35}S-methionine-labeled ARA70, PPARα (**A**), or PPARα-G (**B**) was incubated with PPARα antibody and protein A Sepharose beads as described in Materials and Methods. Lane 1, input receptor (represents one-third of that used in the immunoprecipitation reaction); lane 2, input ARA70 (represents one-fifteenth of the final used); lane 3, receptor alone with anti-PPARα antibody; lane 4, ARA70 alone with anti-PPARα antibody; lane 5, PPARα and ARA70 coimmunoprecipitated in the absence of PPARα ligand; lane 6, PPARα and ARA70 coimmunoprecipitated in the presence of 70 μ*M* WY14,643.

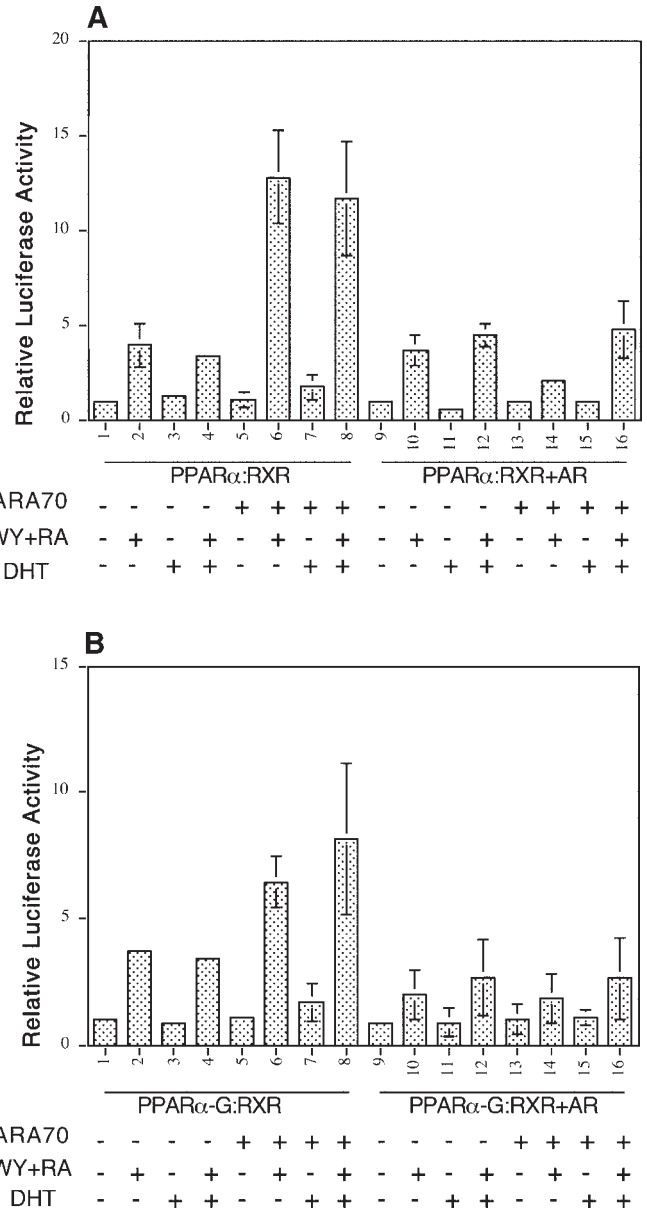

Fig. 4. Effect of AR on PPARα:RXR and PPARα-G:RXR transactivation in presence or absence of androgen. DU145 cells were transfected with 0.5 μg each of pSG5-PPARα (**A**) or pSG5-PPARα-G (**B**) and pSG5-RXRα with 3 μg of (PPRE)x3-tk-luciferase reporter, 5 μg of pSG5-ARA70, and 1 μg of pSG5-AR as indicated. Cells were treated with vehicle, 20 μ*M* WY14,643, and 1 μ*M* 9-*cis*-retinoic acid (WY+RA), or with 10 n*M* dihydrotestosterone (DHT) as indicated. Data are the mean ± SD of three to four independent experiments.

AR is able to compete ARA70 away from both PPARγ:RXR *(41)* and PPARα:RXR in prostate DU145 cells (Fig. 4). While the functional consequences of this competition require further investigation, it is of possible relevance in prostate cancer. PPARα expression is elevated in prostate cancer, and the level of PPARα protein increases with prostate cancer grade and stage *(23)*. The human prostate cancer cell lines DU145, PC3, and LNCaP express endogenous PPARα and PPARγ *(18,25,26)*. Activators of PPARα have been shown to induce cytostasis in PC3 cells *(25)*. Ligands of PPARγ reduce the secretion of the prostate cancer marker

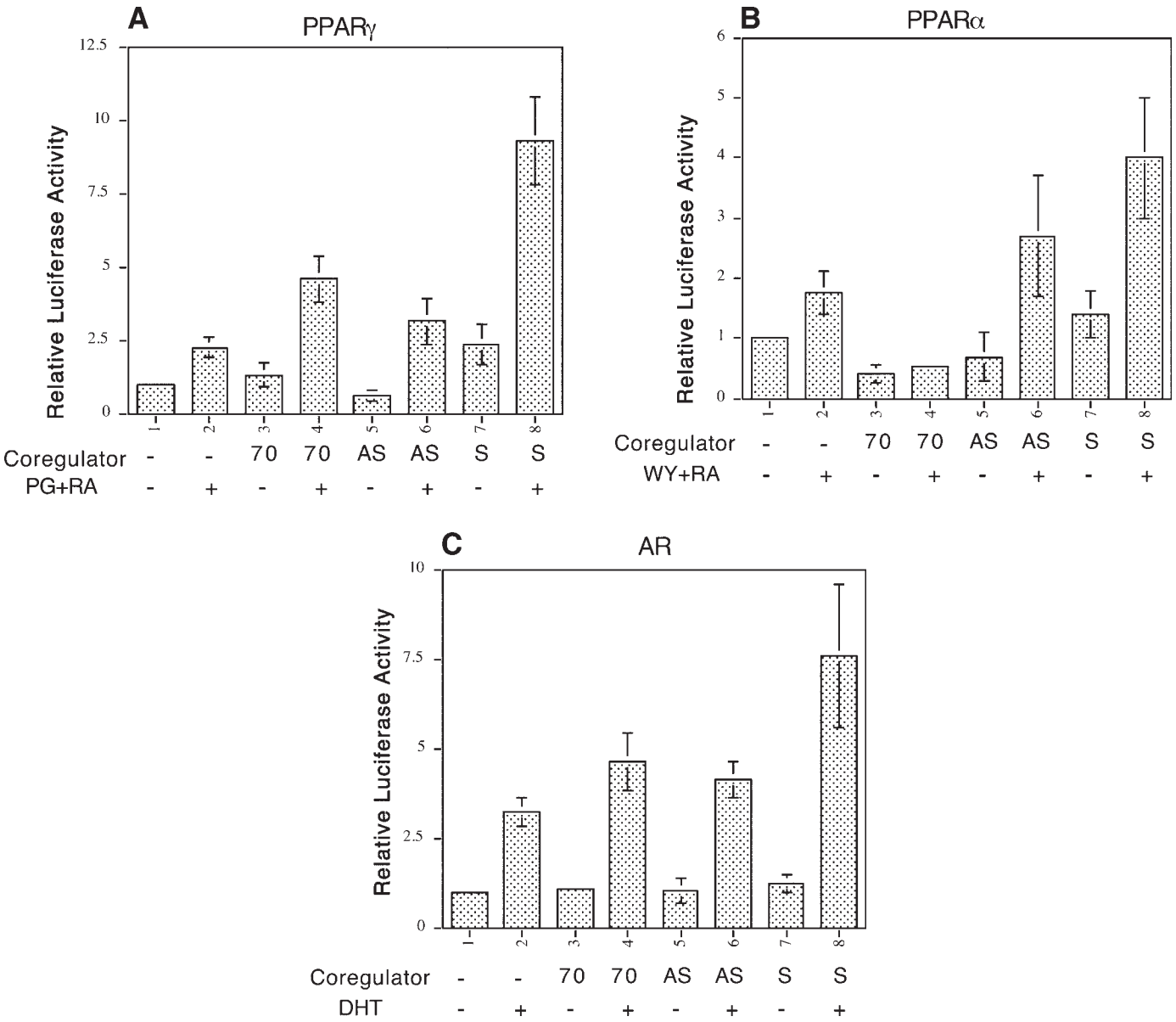

Fig. 5. ARA70 can function as both coactivator and corepressor in Y1 adrenal cells. (**A**) Y1 cells were cotransfected with 0.5 μg of pSG5-RXRα, 0.5 μg of pSG5-PPARγ, and 5 μg of pSG5-ARA70 (70), pSG5-SRC-1 (S), or both pSG5-ARA70 and pSG5ARA70AS (AS) as indicated. The pSG5ARA70AS plasmid expresses the antisense of ARA70 (plasmid construction is described in the Materials and Methods). Transcription was assayed using 3 μg of the (PPRE)x3-tk-luciferase reporter. After transfection, cells were treated with vehicle or 3 μ*M* 15dJ2 and 1 μ*M* 9-*cis*-retinoic acid (PG+RA) as described in Materials and Methods. (**B**) Cells were cotransfected with 0.5 μg of pSG5-RXRα and 0.5 μg of pSG5-PPARα and 5 μg of the coactivator constructs as in (A). After 20 h, cells were mock treated with ethanol or with 20 μ*M* WY14,643 and 1 μ*M* 9-*cis*-retinoic acid (WY+RA) (C) Y1 cells were cotransfected with 1 μg of pSG5-AR, 3 μg of MMTV-tk-luciferase, and 5 μg of coactivator constructs as in (A). After 20 h, cells were mock treated with ethanol or 10 n*M* DHT. Cells were harvested and luciferase activity was assayed as described in Materials and Methods. Data are the mean ± SD of three to four independent experiments.

prostate-specific antigen in LNCaP cells, reduce prostate tumor growth in nude mice, and increase necrosis of surgically obtained human prostate tumors *(26)*. By comparison, androgen-bound AR is a major contributor to prostate tumor proliferation *(53,54)*. It is possible that in proliferating prostate cancer cells, ARA70 associates with AR and transactivation by PPAR is reduced because PPARα and PPARγ are forced to use less potent transcriptional coactivators. The standard treatment of prostate cancer patients—the reduction of circulating androgen levels by chemical or surgical castration—would be predicted to increase the available ARA70 from unliganded AR. Supplying PPARγ and/or PPARα activators, in combination with androgen ablation, could potentially allow ARA70 to enhance PPAR transcription above that of less potent PPAR coactivators to facilitate the cytostatic/necrotic effect. However, a more detailed

analysis of the role of ARA70 with PPARα and PPARγ in prostate tumor cells is necessary to confirm this hypothesis.

While ARA70 is a transcriptional coactivator of AR, PPARα, and PPARγ in human prostate cancer cells, it functions as a repressor of PPARα in adrenal Y1 cells. Because ARA70 is still able to enhance PPARγ transactivation in Y1 cells, it is unlikely that this effect is owing solely to a modification of ARA70 that confers a repressive effect. Phosphorylation of PPARγ in response to insulin in adipocytes has been reported to result in transcriptional inhibition *(55)*. However, phosphorylation of PPARα via the mitogen-activated protein kinase (MAPK) pathway has been reported to enhance PPARα transcriptional activity *(56,57)*. PPARα positively responds to ligand treatment and it is coactivated by SRC-1 in Y1 cells, suggesting that PPARα is not modified in a manner that generally inhibits its transcriptional activity. These results suggest that protein modification and/or interaction in Y1 cells occurs that is specific for the PPARα:RXR-ARA70 complex (and not the PPARγ-containing complex), which inhibits PPARα-mediated transcription.

These observations are similar to those described for the Wilms tumor gene product WT1 and p53. Interaction of DNA-bound WT1 with non-DNA-bound p53 inhibits WT1-mediated transcription in the kidney cell line, BHK *(58)*. However, in the colorectal carcinoma cell line RKO, p53 enhances WT1-mediated transcription in the absence of a p53 DNA-binding site *(59)*. Coimmunoprecipitation and gel filtration of the WT1-p53 complex indicates that these proteins are found in complexes ranging from 100 to 669 kDa *(58)*, and the dichotomous transcriptional activity may be owing to the cell type–specific or physiologic status–dependent composition of this complex *(59)*.

A similar situation may exist for ARA70 and PPARα. An additional bridging protein or complex of proteins may be found in Y1 cells that interacts with PPARα:RXR-ARA70 to repress PPARα transcription. Alternatively, phosphorylation of ARA70 may alter its ability to function as a coactivator in Y1 cells. Ko et al. *(60)* recently reported the isolation of the steroid receptor coactivator GT198, which is differentially regulated by phosphorylation. When GT198 is phosphorylated by protein kinase C, it functions to enhance steroid receptor transcription. However, MAPK phosphorylation of GT198 causes it to function as a corepressor *(60)*.

It is possible that differential endogenous phosphorylation of ARA70 in prostate and adrenal cell lines contributes to the altered effect of ARA70 on PPARα transcription. The potential phosphorylative regulation of ARA70 and the isolation of its potential interaction partners is currently under investigation. It is also possible that the differential ability of ARA70 to modulate PPARα transcription in adrenal cells reflects a species difference in ARA70:PPARα function since the DU145 prostate cells used in our study are of human origin and the Y1 adrenal cell line is derived from the mouse. Although species-specific differences in receptor or coregulator phosphorylation or multiprotein complex formation cannot be completely excluded, our preliminary results using the human NCI H295 adrenal cell line suggest that ARA70 also functions as a PPARα repressor in this cell line. Further studies will help clarify the role of ARA70 in the transcriptional control of different members of the nuclear receptor superfamily.

Materials and Methods

Plasmids

The pSG5-PPARα-G plasmid was constructed from pCMV-PPARα-G (kindly provided by Dr. E. F. Johnson, The Scripps Institute, La Jolla, CA). The PPARα-G *Xba*I-*Bam*HI fragment was inserted into the *Eco*RI site of pSG5. The pSG5- ARA70AS plasmid was constructed by inserting the *Bam*HI fragment of the ARA70 cDNA encoding amino acids 1–401 into the *Bam*HI site of pSG5 in the antisense orientation (unpublished observations). The pSG5-PPARα plasmid was kindly provided by Dr. S. Green (Zeneca, UK).

Coimmunoprecipitation

Recombinant mPPARα, mPPARα-G, and full-length ARA70 were expressed using the TNT Coupled Reticulocyte Lysate System (Promega, Madison, WI) incorporating ^{35}S-methionine according to the manufacturer's instructions. Fifteen microliters of labeled receptors was mixed with 15 µL of ARA70 and incubated with 5 µL of the PPARα antibody PA3-820 (Affinity Bioreagents). Proteins and antibody were incubated in 20 µL of HC400 (20 m*M* HEPES-KOH, pH 7.9; 400 m*M* KCl; 0.2 m*M* EDTA; 20% glycerol; 0.1 mg/mL of bovine serum albumin) for 1 h at 4ºC prior to adding 10 µL of protein A Sepharose beads (Pharmacia). Wy14,643 was added to the precipitation reaction at a final concentration of 70 µ*M*. The reaction was then incubated while rocking overnight at 4ºC. Immunoprecipitated complexes were collected by centrifuging at 2000 rpm at 4ºC for 1 min. The pelleted beads were washed three times with HC400 with 0.1% NP40 and two times with 10 m*M* KPO_4 (pH 8.0)/0.1 *M* KCl with 0.1% NP40, mixed with sodium dodecyl sulfate (SDS) sample buffer, boiled, and then separated on a 6% SDS-polyacrylamide gel electrophoresis gel.

Cell Culture and Transfection

Human prostate DU145 cells were grown in Dulbecco's minimal essential medium (DMEM) containing 10% fetal bovine serum (FBS) at 37ºC. Y-1 adrenal tumor cells (American Type Culture Collection) were maintained in Ham's F10 medium (Life Technologies) supplemented with 15% horse serum and 2.5% FBS. The cells were transfected by modified BES-calcium phosphate procedure *(40,41)*. Y1 cells were 15% confluent when transfected. Transfection medium contained a constant amount of reporter plasmid and indicated amounts of receptor and coactivator constructs, using pSG5 as a carrier to provide equal amounts of transfected DNA. One hour before transfection, fresh culture

medium was added to Y1 cells and the medium for DU145 was changed to DMEM with 10% charcoal-stripped FBS. The medium was changed again 20 h posttransfection and treated for 14–16 h with 20 μ*M* WY14,643, 3 μ*M* 15dJ2, 1 μ*M* 9-*cis*-retinoic acid, or 10 n*M* DHT as indicated in the figure legends. Cell extracts were prepared and assayed for firefly luciferase activity (Promega) and normalized against *Renilla* luciferase activity (Promega). All data were the mean ± SD of at least three independent experiments.

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