MOLECULAR AND CELLULAR BIOLOGY, Aug. 1992, p. 3380–3389
0270-7306/92/083380-10$02.00/0

Vol. 12, No. 8

Repression by ARP-1 Sensitizes Apolipoprotein AI Gene Responsiveness to RXRα and Retinoic Acid

RUSSELL L. WIDOM,† MYUNGCHULL RHEE,‡ AND SOTIRIOS K. KARATHANASIS§*

Laboratory of Molecular and Cellular Cardiology, Department of Cardiology, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, 02115

Received 14 February 1992/Accepted 30 April 1992

The gene coding for apolipoprotein AI (apoAI), a lipid binding protein involved in the transport of cholesterol and other lipids in the plasma, is expressed in mammals predominantly in the liver and the intestine. Liver-specific expression is controlled by synergistic interactions between transcription factors bound to three separate sites, sites A (−214 to −192), B (−169 to −146), and C (−134 to −119), within a powerful liver-specific enhancer located between nucleotides −222 and −110 upstream of the apoAI gene transcription start site (+1). Previous studies in our laboratory have shown that ARP-1, a member of the nuclear receptor superfamily whose ligand is unknown (orphan receptor), binds to site A and represses transcription of the apoAI gene in liver cells. In a more recent series of experiments, we found that site A is a retinoic acid (RA) response element that responds preferentially to the recently identified RA-responsive receptor RXRα over the previously characterized RA receptors RARα and RARβ. In this study we investigated the combined effects of ARP-1 and RXRα on apoAI gene expression in liver cells. Transient transfection assays showed that site A is necessary and sufficient for RXRα-mediated transactivation of the apoAI gene basal promoter in human hepatoma HepG2 cells in the presence of RA and that this transactivation is abolished by increasing amounts of cotransfected ARP-1. Electrophoretic mobility shift assays and subsequent Scatchard analysis of the data revealed that ARP-1 and RXRα bind to site A with similar affinities. These assays also revealed that ARP-1 and RXRα bind to site A as heterodimers with an affinity approximately 10 times greater than that of either ARP-1 or RXRα alone. Further transfection assays in HepG2 cells, using as a reporter a construct containing the apoAI gene basal promoter and its upstream regulatory elements (including site A) in their natural context, revealed that RXRα has very little effect on the levels of expression regardless of the presence or absence of RA. However, while ARP-1 alone or ARP-1 and RXRα together dramatically repress expression in the absence of RA, the repression by ARP-1 and RXRα together, but not ARP-1 alone, is almost completely alleviated in the presence of RA. These results indicate that transcriptional repression by ARP-1 sensitizes apoAI gene responsiveness to RXRα and RA and suggest that the magnitude of this responsiveness is regulated by the intracellular ratio of ARP-1 to RXRα. These observations raise the possibility that transcriptional repression is a general mechanism for switching gene transcription between alternative transcription activation pathways.

A large body of epidemiologic, genetic, pharmacologic, and biochemical evidence suggests that high-density lipoprotein (HDL) levels in plasma play an important role in regulation of cellular cholesterol homeostasis and atherosclerosis progression and regression. Although changes in plasma HDL levels have been associated with a diverse number of dietary, hormonal, and stress-related stimuli, there is very little information relevant to the molecular basis of these effects. Either or both processes involved in HDL biogenesis or catabolism could be influenced by these stimuli (for a review, see reference 7).

Our laboratory has been focusing on factors influencing the expression of the gene coding for apolipoprotein AI (apoAI), the major protein constituent of plasma HDL. In mammals, the apoAI gene is expressed predominantly in the liver and the intestine. Liver-specific expression is controlled by a powerful liver-specific enhancer located between nucleotides −222 and −110 upstream of the apoAI gene transcription start site (+1) (27, 36). It has been previously shown that in liver cells this enhancer is occupied by at least three transcription factors that bind to three distinct sites, sites A (−214 to −192), B (−169 to −146), and C (−134 to −119), and that maximal transcriptional activity of this enhancer depends on synergistic interactions between these transcription factors (36). Recent cloning and characterization of one of the proteins that bind to site A revealed that this protein, which was named apoAI regulatory protein 1 (ARP-1), is a novel member of the steroid-thyroid hormone receptor superfamily of ligand-dependent transcription factors and raised the possibility that apoAI gene regulation is accomplished, at least in part, by mechanisms similar to those regulating genes responsive to these hormones (11). However, cotransfection experiments indicated that overexpression of ARP-1 represses the expression of the apoAI gene in human hepatoma HepG2 cells (11). Although the possibility that ARP-1 in the presence of an appropriate ligand could activate apoAI gene expression has not been excluded, other evidence such as characterization of DNA-protein complexes formed with site A and liver nuclear extracts by using antibodies that immunoreact with ARP-1 indicated that liver cells contain in addition to ARP-1 other proteins that also bind to site A (35). A recent attempt to clone cDNAs for such proteins by screening a human liver

* Corresponding author.
† Present address: Department of Pediatrics, Boston University School of Medicine, Boston, MA 02118.
‡ Present address: Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115.
§ Present address: Department of Cardiovascular Molecular Biology, Lederle Laboratories, Pearl River, NY 10965.

cDNA library with a DNA probe corresponding to the ARP-1 DNA binding domain resulted in the cloning of another member of the steroid-thyroid hormone receptor superfamily (RXRα) that functions as a transcription activator in the presence of retinoic acid (RA) (24). It has been previously shown that RXRα binds to site A and activates nearby basal promoters in response to RA (24).

This report shows that the site A in the context of the basal apoAI gene promoter functions as a RA responsive element in the presence of RXRα and RA and that ARP-1 antagonizes this RXRα- and RA-dependent transcription activation. In addition, it is shown that ARP-1 and RXRα bind to site A with similar affinities and that RXRα/ARP-1 heterodimers bind to site A with an affinity approximately 10 times greater than that of either ARP-1 or RXRα alone. Furthermore, it is shown that repression of apoAI gene expression in HepG2 cells by ARP-1 is overcome by overexpression of RXRα in the presence of RA. These results indicate that regulation of apoAI gene expression is controlled by the balance of the intracellular levels of ARP-1 and RXRα and suggest that physiological signals relevant to vitamin A metabolism play an important role in regulation of plasma apoAI and HDL levels and the atherosclerosis process. In addition, these findings suggest that repression of the apoAI gene by ARP-1 and other orphan receptors plays a fundamental role in facilitating switching between alternative transcription activation pathways, at least one of which is sensitive to RA signaling.

MATERIALS AND METHODS

Plasmid constructions and propagation. General methods for cloning and restriction enzyme digestion were as previously described (26). Plasmids were propagated in *Escherichia coli* HB101 and were isolated by alkaline lysis and purified on Qiagen tip 500 columns as described by the manufacturer (Qiagen).

Chloramphenicol acetyltransferase (CAT) reporter constructs −41AI.CAT and −256AI.CAT were previously described (27). Plasmids [A]−41AI.CAT, [2XA]−41AI.CAT, and [3XA]−41AI.CAT were constructed by ligation of an oligonucleotide containing the apoAI enhancer site A [oligo A (36)] with 5′ GATC overhanging ends into a *Bam*HI site at the −41 position of −41AI.CAT (27). Plasmid [Amut]−41AI.CAT was constructed by cloning the mutated version of oligo A (oligo Amut [36]) into the −41AI.CAT construct as described above. The constructs pMT2-ARP-1, pMT2-RXRα, pMT2-RARα, and pMT2-RARβ, which express ARP-1, RXRα, RARα, and RARβ, respectively, have been previously described (11, 24). A vector which expresses an amino terminus deletion derivative of ARP-1, pMT2-ARP-ΔA1, was constructed by ligating the *Eco*RI insert from a previously described pGEM-4 clone (ΔA1 [11]) into the *Eco*RI site of pMT2.

The control vector pMT2-UT containing an approximately 500-bp *Eco*RI fragment from the 3′ untranslated region of the human growth hormone gene in the plasmid PLEN4S (29) has been previously described (18).

Cell culture and transient transfection assays. Human hepatoma HepG2 cells grown in Dulbecco modified Eagle medium (GIBCO) supplemented with 10% fetal calf serum (Sigma) were seeded at 2×10^6 cells per 100-mm dish 24 h prior to transfection by calcium phosphate coprecipitation as previously described (27). A total of 28 μg of plasmid DNA, consisting of 10 μg of CAT reporter, variable amounts of receptor expression vector made up to 15 μg with control vector pMT2-UT and 3 μg of pRSV-β-gal (4) which was included as an internal control for differences in transfection efficiency, was used per transfection. After glycerol shock, the medium was replaced with Dulbecco modified Eagle medium supplemented with 10% fetal calf serum that had been treated with dextran-coated charcoal as previously described (13) to remove low-molecular-weight ligands. Next, RA from a 10^{-3} M stock in dimethyl sulfoxide was added to a final concentration of 10^{-6} M. Control dishes were treated with an equal volume of dimethyl sulfoxide. Cells were harvested 48 h later and assayed for CAT and β-galactosidase (β-Gal) activity as previously described (27).

EMSA and determination of dissociation constants. To make whole-cell extracts for electrophoretic mobility shift assays (EMSA), Cos-1 cells maintained in Dulbecco modified Eagle medium plus 10% heat-inactivated (55°C, 1 h) fetal calf serum were seeded at 10^6 cells per 100-mm dish 24 h prior to transfection and transfected with 10 μg of pMT2-ARP-1, pMT2-RXRα, pMT2-RARα, pMT2-RARβ, or pMT2-ARPΔA1 by the DEAE-dextran procedure essentially as previously described (9). After 48 h, cells were scraped from the dishes, pelleted by centrifugation, and resuspended in a buffer containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.8), 100 mM KCl, 20% glycerol, 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol (50 μl per dish). Cell extracts were produced by three freeze-thaw cycles and were stored at −70°C. EMSA was performed as described previously (18, 36) by using as a probe the oligo A described previously (36), which was radiolabeled by using T4 polynucleotide kinase and [γ-^{32}P]ATP.

To determine dissociation constants (K_d) of RXRα and ARP-1 proteins produced in Cos-1 cells, EMSA was performed with fixed amounts of proteins and increasing amounts of oligo A probe. After electrophoresis, the gels were fixed, dried, and exposed to X-ray film. Areas of the gel corresponding to protein bound and free probe were excised and counted by liquid scintillation, and the radioactivity values were plotted by the method of Scatchard (28). A control lane was included in each gel to measure background and to correct for radioactivity quenching of the probe by the gel (18).

RESULTS

RXRα transactivates the apoAI gene basal promoter in liver cells. Recent studies in our laboratory have shown that in CV-1 cells, RXRα strongly stimulates transcription from site A cloned upstream of the herpes simplex virus thymidine kinase gene promoter in response to RA (24). In the current study the ability of RXRα to stimulate transcription from site A cloned upstream of the apoAI gene basal promoter was examined. The human hepatoma cell line HepG2 was chosen for these experiments because, unlike CV-1 cells, HepG2 cells express the apoAI gene and contain factors which activate transcription from site A in the context of the apoAI gene enhancer (27, 36). Single or multiple copies of oligo A (36) were inserted proximal to the apoAI basal promoter in a previously described vector containing the −41 to +397 apoAI gene region fused to the bacterial CAT gene (−41AI.CAT [27]). The resulting constructs were cotransfected into HepG2 cells with vectors expressing RXRα (pMT2-RXRα [24]) and β-Gal (pRSV-β-gal [4]) in the presence or absence of RA. CAT enzymatic activity in extracts from these cells was determined and normalized with respect to the corresponding β-Gal activity. The results, expressed as bar

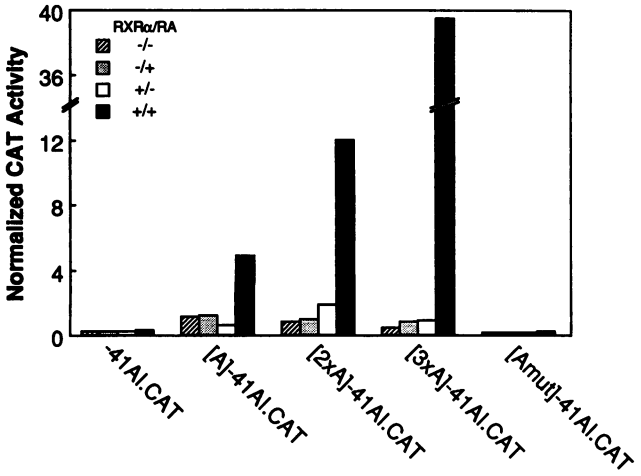

FIG. 1. Transactivation of the basal apoAI gene promoter by RXRα and RA. The indicated CAT reporter constructs, containing apoAI gene sequences from −41 to +397 without (−41AI.CAT) or with one or multiple copies of site A or mutated site A (Amut) cloned in the −41 position, were transfected into HepG2 cells in the presence or absence of 5 μg of the RXRα expression vector pMT2-RXRα. Transfected cells were then treated with 10^{-6} M RA or dimethyl sulfoxide as described in Materials and Methods. Four values, representing transfections done without RA or pMT2-RXRα (−/−), with RA but without pMT2-RXRα (−/+), with pMT2-RXRα but without RA (+/−), or with both RA and pMT2-RXRα (+/+), are shown for each construct. The values shown are the ratios of CAT enzymatic activity to β-Gal activity in cell extracts (normalized CAT activity) and represent the averages of at least three experiments.

graphs in Fig. 1, show that in the presence of both RXRα and RA the constructs containing one ([A]−41AI.CAT), two ([2xA]−41AI.CAT), and three ([3xA]−41AI.CAT) copies of site A express 8, 20, and 45 times, respectively, more CAT activity than the parental vector containing the apoAI basal promoter alone. This enhancement in expression was dependent on the simultaneous presence of both RXRα and RA, because the presence of either alone did not significantly enhance expression of any of the constructs tested (Fig. 1).

The ability of site A to confer RXRα and RA transcriptional stimulation of the apoAI gene basal promoter together with the observation that RXRα binds to site A with relatively high affinity (see below) suggested that binding of RXRα to site A is required for this transcriptional stimulation. To further explore this possibility a previously described mutated version of site A (oligo Amut [36]) that fails to bind nuclear proteins from HepG2 cells (36) or RXRα produced by transient transfection of pMT2-RXRα into Cos-1 cells (data not shown) was cloned in the −41AI.CAT vector, and the resulting construct was tested for its transcriptional activity as described above. The results (Fig. 1) show clearly that this version of site A is completely incapable of stimulating the apoAI basal promoter in response to RXRα and RA. It is therefore concluded that in liver (HepG2) cells, RXRα binds to site A and activates the apoAI basal promoter in response to treatment of the cells with RA.

ARP-1 inhibits RXRα- and RA-mediated transactivation from site A. Because ARP-1 binds to site A (11) with relatively high affinity (see below) and represses transcription of the apoAI gene in liver cells (11) and since ARP-1 and RXRα are both expressed in hepatocytes (16, 18, 25), it was of interest to determine the combined effects of ARP-1 and RXRα on the expression of site A containing apoAI gene basal promoter constructs. For these experiments the construct [3xA]−41AI.CAT was cotransfected with constant amounts of the RXRα expression vector pMT2-RXRα and the control vector pRSV-βgal and increasing amounts of an ARP-1 expression vector (pMT2-ARP-1 [11, 18]) into HepG2 cells in the presence or absence of RA. Normalized CAT activity in these cells was plotted against the amounts of pMT2-ARP-1 expression vector used in the cotransfection mixtures (Fig. 2). As shown in Fig. 2, in the absence of RA, RXRα alone did not stimulate CAT expression, as expected, and the presence of increasing amounts of ARP-1 together with RXRα did not alter this pattern of expression (Fig. 2A, filled circles). However, the substantial stimulation of expression mediated by RXRα in the presence of RA was progressively inhibited by increasing amounts of ARP-1 (Fig. 2A, open circles). ARP-1 alone in the presence or absence of RA did not stimulate expression (data not shown). It is of interest that the RXRα- and RA-mediated transcriptional stimulation was inhibited by 50% with only one-fifth the amount of ARP-1 expression vector compared with the amount of the RXRα expression vector used for cotransfection (i.e., at approximately 50% inhibition 1 μg of pMT2-ARP-1/5 μg of pMT2-RXRα = 1/5 [Fig. 2A]). Several observations suggest that the effectiveness of ARP-1 in inhibiting RXRα- and RA-mediated transactivation is not due to more efficient expression of ARP-1 from the pMT2-ARP-1 vector compared with the expression of RXRα from the pMT2-RXRα vector. First, both these expression vectors were constructed by cloning the corresponding cDNAs into the same parental vector, pMT2 (11, 18, 24). Second, as shown below, 50% inhibition of the RXRα- and RA-dependent transcription of the apoAI gene basal promoter under the control of its natural upstream regulatory elements requires an amount of ARP-1 expression vector approximately equivalent to the RXRα expression vector. Finally, titration of the amount of pMT2-RXRα expression vector required for maximal expression of the construct [3xA]−41AI.CAT in HepG2 cells in the presence of RA showed that amounts greater than 2.5 μg are saturating (Fig. 2B, open circles) and that cotransfection of only 1 μg of the pMT2-ARP-1 expression vector inhibits expression by 50% regardless of the amount of pMT2-RXRα expression vector in the cotransfection mixture (Fig. 2B, filled circles). Cotransfection with even 10 μg of pMT2-RXRα expression vector was not sufficient to overcome this inhibitory effect of ARP-1 (data not shown). It is therefore concluded that the RXRα- and RA-mediated transcription activation of the apoAI basal promoter from site A is efficiently inhibited by ARP-1 and that the absolute level of RXRα- and RA-dependent transactivation is determined by the intracellular ratio of ARP-1 to RXRα. These observations provide strong support for the concept that the apoAI gene site A is a conditional RA response element; it responds to RXRα and RA when the intracellular concentration of ARP-1 is low but loses responsiveness when the ARP-1 concentration is high.

ARP-1 and RXRα bind to site A with similar affinities. The effectiveness of ARP-1 in inhibiting the RXRα- and RA-mediated transactivation of the apoAI gene basal promoter from site A could be due to a higher binding affinity of ARP-1 for site A compared with the affinity of RXRα for the same site. To examine this possibility directly, the binding of Cos-1 cell-produced ARP-1 or RXRα to oligo A as a function of oligonucleotide concentration was determined by EMSA. Figures 3A and B show typical patterns of DNA-protein complexes (retardation complexes) formed with increasing

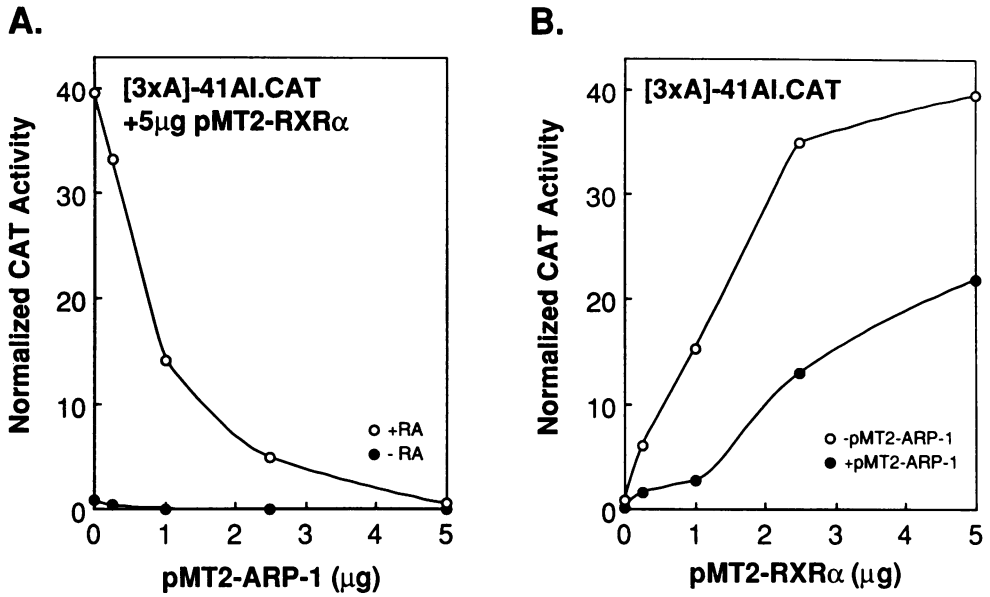

FIG. 2. ARP-1 antagonizes RXRα and RA transactivation of the apoAI gene basal promoter. The plasmid [3XA]-41AI.CAT was transiently transfected into HepG2 cells either with 5 μg of pMT2-RXRα and the indicated amounts of the ARP-1 expression vector pMT2-ARP-1 in the presence (open circles) or absence (filled circles) of 10^{-6} M RA (A) or with the indicated amounts of pMT2-RXRα in the presence of 10^{-6} M RA and in the absence (open circles) or presence (filled circles) of 1 μg of pMT2-ARP-1 (B). Normalized CAT activity is defined in the legend to Fig. 1.

amounts (lanes from left to right) of oligo A and constant amounts of RXRα and ARP-1, respectively. The amounts of oligo A in these complexes (Bound) were determined and plotted against the corresponding amounts of nonbound (Free) oligo A in each lane (Fig. 3C). Subsequent Scatchard analysis (28) of these data (Fig. 3C, insert) revealed that under these conditions the dissociation constants (K_d) for binding of ARP-1 and RXRα to site A are 5.2 and 8.8 nM, respectively. It is therefore concluded that the effectiveness of ARP-1 in inhibiting the RXRα- and RA-mediated transactivation of the basal apoAI gene promoter from site A is unlikely to be due to a greater affinity of ARP-1 for the same site. Thus, these observations suggest that ARP-1 inhibits RXRα- and RA-mediated transactivation by other mechanisms in addition to competition with RXRα for binding to site A.

RXRα/ARP-1, RXRα/RARα, and RXRα/RARβ heterodimers bind to site A. To further explore the mechanisms by which ARP-1 inhibits RXRα- and RA-mediated transactivation of the apoAI gene basal promoter from site A, the binding of ARP-1 or RXRα alone and ARP-1 and RXRα together on site A was investigated by EMSA. To facilitate discrimination between different DNA-protein complexes, the N-terminal domain of ARP-1 flanking its DNA binding domain was deleted and the resulting truncated version of ARP-1 (ARPΔA1) was produced in Cos-1 cells. Control experiments showed that this version of ARP-1 binds to site A with a K_d comparable to that for binding of the intact ARP-1 to the same site (data not shown). Figure 4 shows that, consistent with previous observations (11, 24), oligo A forms slowly migrating retardation complexes with Cos-1 cell-produced RXRα (lane 1) and faster migrating retardation complexes with ARPΔA1 (lane 2). We have shown previously that in vitro-translated RXRα does not bind to site A in the absence of exogenously added Cos-1 whole-cell extracts (24). Cos-1 cell-produced RXRα, on the other hand, binds both to site A (24) and to a RA response element from the 5′ flanking region of the RARβ gene (βRARE) (14). Since most of Cos-1 cell-produced RXRα bound to βRARE is in the form of heterodimers with intracellular RARα and RARγ (14), we considered it possible that the retardation complexes formed with oligo A and Cos-1 cell-produced RXRα could also be due to such heterodimers. As shown in Fig. 4, although Cos-1 cell-produced RARα and RARβ, under these experimental conditions, do not bind by themselves to oligo A (lanes 3 and 5), they dramatically stimulate oligo A binding activity when they are mixed with Cos-1 cell-produced RXRα (lanes 4 and 6). However, the electrophoretic mobility of these retardation complexes is significantly greater than those formed with RXRα alone (compare lane 1 with lanes 4 and 6). Control experiments showed that although addition of Cos-1 whole-cell extracts to Cos-1 cell-produced RARα or RARβ stimulates their binding to oligo A, the migration of the resulting retardation complexes is greater than those formed in the presence of RXRα (24; data not shown). Therefore, it appears that although RXRα/RARα and RXRα/RARβ heterodimers both bind efficiently to site A, they cannot account for the retardation complexes formed with RXRα alone. This suggests that the retardation complexes formed with RXRα alone (lane 1) are due either to RXRα alone or to heterodimers of RXRα with some other intracellular protein(s) other than RARα or RARγ.

The retardation complexes of oligo A with Cos-1 cell-produced ARPΔA1, on the other hand, are due to ARPΔA1 homodimers (11). The efficiency of formation of these homodimers, however, appears to be influenced by factors present in Cos-1 whole-cell extracts. Specifically, as shown in Fig. 4, Cos-1 whole-cell extracts, although they lack any intrinsic oligo A binding activity (lane 7), stimulate significantly the binding activity of Cos-1 cell-produced ARPΔA1 (compare lanes 2 and 8). Similar results have also been obtained by using Cos-1 cell-produced intact ARP-1 instead

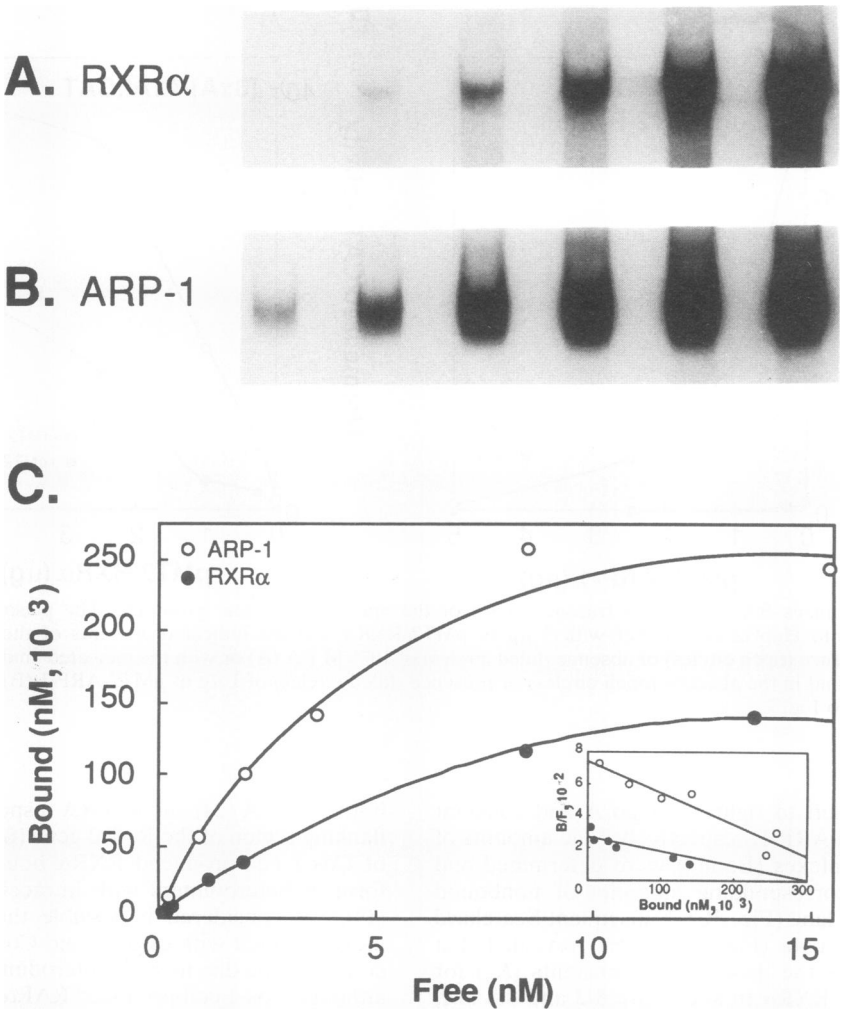

FIG. 3. ARP-1 and RXRα bind to site A with similar affinities. Autoradiograms from EMSA showing retardation complexes formed with increasing amounts (left to right) of radiolabeled oligo A probe and fixed amounts of whole-cell extracts from Cos-1 cells transiently transfected with pMT2-RXRα (RXRα, 1-μl extract [A]) or pMT2-ARP-1 (ARP-1, 0.4-μl extract [B]). The DNA amounts in these retardation complexes were quantitated as described in Materials and Methods and used to construct saturation curves showing binding of RXRα (filled circles) and ARP-1 (open circles) as a function of DNA concentration (C). These data were subsequently used to construct Scatchard plots (inset in panel C).

of ARPΔA1 (data not shown). The nature of this stimulatory activity is unknown, and it is the subject of current investigations. When the same amounts of Cos-1 cell extracts used in lanes 1 and 2 were first mixed and then used for EMSA, an additional retardation complex with intermediate electrophoretic mobility was also formed (lane 9). Control experiments in which similar amounts of untransfected or mock-transfected Cos-1 cell extracts were mixed with either ARPΔA1 (lane 8) or RXRα (data not shown) indicated that formation of retardation complexes with the intermediate electrophoretic mobility requires the presence of both ARPΔA1 and RXRα. Furthermore, mixing of Cos-1 cell extracts containing the intact ARP-1 with Cos-1 cell extracts containing an RXRα truncation mutant analogous to ARPΔA1 also resulted in formation of retardation complexes with intermediate electrophoretic mobility (data not shown). It therefore appears that ARP-1 and RXRα form heteromeric complexes on site A and that this heteromerization does not require the presence of the N-terminal regions flanking their DNA binding domains. These results indicate that in addition to ARP-1 and RXRα, site A also binds various heterodimeric versions of RXRα with other members of the steroid-thyroid hormone receptor superfamily such as RXRα/RARα, RXRα/RARβ, and RXRα/ARP-1 heterodimers. This raises the possibility that RXRα/ARP-1 heterodimers participate in the ARP-1-mediated inhibition of the RXRα- and RA-dependent transactivation of the apoAI gene basal promoter from site A. In addition, these findings strongly suggest that diverse signaling pathways influencing apoAI gene expression converge onto site A.

RXRα/ARP-1 heterodimers bind to site A with an affinity greater than either ARP-1 or RXRα alone. An additional consistent and reproducible finding in the experiments whose results are shown in Fig. 4 was an apparent increase in the total amount of DNA binding activity in ARPΔA1 and RXRα mixtures compared to the amount of binding activity in either ARPΔA1 or RXRα alone. For example, although the same amount of Cos-1 cell-produced ARPΔA1 was used in lanes 2 and 9, the amount of binding activity corresponding to ARPΔA1 in ARPΔA1- and RXRα/ARPΔA1-containing

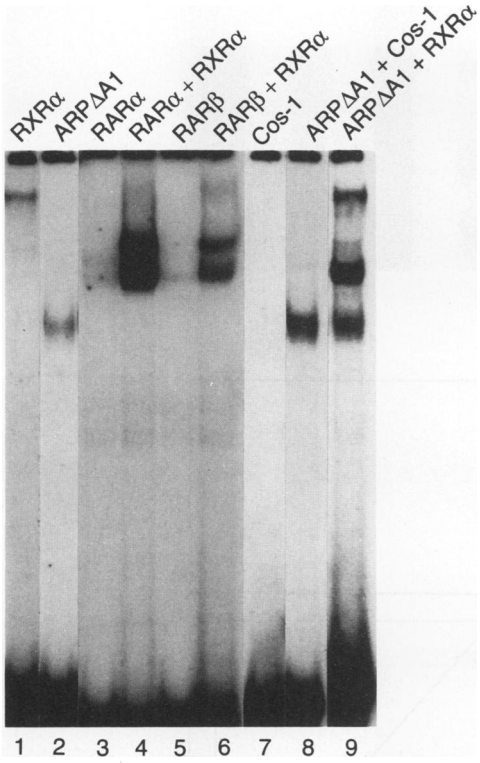

FIG. 4. RXRα/ARP-1, RXRα/RARα, and RXRα/RARβ heterodimers bind to site A. Autoradiogram from EMSA analysis with 1 ng of ^{32}P-labeled oligo A and whole-cell extracts from untransfected control Cos-1 cells (Cos-1) and Cos-1 cells transiently transfected with the expression vectors pMT2-RXRα (RXRα), pMT2-ARPΔA1 (ARPΔA1), pMT2-RARα (RARα), and pMT2-RARβ (RARβ). The volume of extracts used were as follows: 1 μl of Cos-1, RXRα, RARα, and RARβ and 0.25 μl of ARPΔA1.

complexes in lane 9 is significantly greater than the amount of binding activity corresponding to ARPΔA1 in lane 2. Although the increased binding activity in the ARPΔA1-containing complexes is most likely due to the stimulatory activity for ARP-1 binding discussed above, which may be present in the Cos-1 cell extracts containing RXRα, the increased ARPΔA1 binding activity in RXRα/ARPΔA1 heterodimers suggests that these heterodimers bind to site A more efficiently than either ARPΔA1 or RXRα alone. The mechanism for the increased efficiency of binding of RXRα/ARPΔA1 heterodimers to site A was investigated by determining the K_d for their binding to oligo A. Specifically, Cos-1 cell-produced ARPΔA1 and RXRα were mixed, and the binding of RXRα/ARPΔA1 heterodimers to oligo A in these mixtures as a function of oligonucleotide concentration was determined by EMSA. Figure 5A shows typical patterns of retardation complexes formed with increasing amounts (lanes from left to right) of oligo A and a constant amount of an ARPΔA1- and RXRα-containing mixture. Complexes due to RXRα, ARPΔA1 homodimers, and RXRα/ARPΔA1 heterodimers are indicated in Fig. 5A. The amounts of oligo A in RXRα/ARPΔA1 heterodimeric complexes (Bound) were determined and plotted against the corresponding amounts of nonbound (Free) oligo A in each lane (Fig. 5B). Subsequent Scatchard analysis (28) of these data (see insert in Fig. 5B), reveals that the K_d for binding of these heterodimers to oligo A is 0.6 nM. These data, taken together with those in the previous section, indicate that RXRα/ARP-1 heterodimers bind to site A with an affinity (K_d = 0.6 nM) approximately 10 times greater than the affinity of binding of either ARP-1 (K_d = 5.2 nM) or RXRα (K_d = 8.8 nM) alone for the same site.

Regulation of the apoAI gene by RA. The observations that site A in the context of the apoAI basal promoter is a powerful RA response element and that ARP-1, RXRα, and RXRα/ARP-1 heterodimers can bind to site A, taken together with the previous observation that maximal expression of the apoAI gene in liver cells is greatly dependent on synergistic interactions between transcription factors bound to site A and transcription factors bound to other nearby sites (i.e., sites B and C [see Introduction and reference 36]), provided a compelling reason to examine whether ARP-1, RXRα, or RXRα/ARP-1 heterodimers can cooperate with proteins bound to these nearby sites for stimulation of the apoAI gene promoter in liver cells. In previous transient transfection experiments, it was observed that the expression of a construct containing the apoAI gene basal promoter and its upstream regulatory elements, including site A, in their natural context (construct −256AI.CAT [27]) in HepG2 cells is dramatically repressed by cotransfection with increasing amounts of the ARP-1 expression vector pMT2-ARP-1 (11). Similar experiments using the RXRα expression vector pMT2-RXRα instead of pMT2-ARP-1 did not result in significant activation or inhibition of expression of the −256AI.CAT construct irrespective of the presence or absence of RA (Fig. 6A). In an attempt to determine whether RXRα/ARP-1 heterodimers bound to site A can participate in synergistic interactions with the transcription factors bound to the other sites in the apoAI gene upstream regulatory region, the −256AI.CAT construct was cotransfected with a constant amount of the RXRα expression vector pMT2-RXRα (5 μg), a constant amount of the control vector pRSV-β-gal (3 μg) and variable amounts of the ARP-1 expression vector pMT2-ARP-1 into HepG2 cells in the presence or absence of RA. The CAT activities in these cells were determined, normalized by using the corresponding β-Gal activities and plotted against the amounts of cotransfected pMT2-ARP-1 vector. The results in Fig. 6A show that in the absence of RA, increasing amounts of pMT2-ARP-1 progressively repress the CAT activity expressed by the −256AI.CAT construct (Fig. 6A, filled circles). This pattern of repression is very similar to that observed when the ARP-1 expression vector alone was used in similar cotransfection experiments (11). In the presence of RA, however, this repression was largely alleviated (Fig. 6A, open circles). Alleviation of repression was dependent on the presence of RXRα, because it was not observed in control cotransfections with pMT2-ARP-1 alone in the presence of RA (data not shown). Although this alleviation of repression is eventually overcome by cotransfection with very large amounts of pMT2-ARP-1 (Fig. 6A and data not shown), it is important to note that, as mentioned previously, this RXRα-mediated transcription activation of the apoAI gene is inhibited by only 50% with an amount of ARP-1 expression vector approximately equal to the amount of the RXRα expression vector used in these cotransfections (i.e., at approximately 50% inhibition 5 μg of pMT2-ARP-1/5 μg of pMT2-RXRα = 1 [Fig. 6A]). This observation is in clear contradiction with the observation that the RXRα- and RA-mediated transactivation of the apoAI gene basal promoter from site A is abolished when equivalent amounts of ARP-1 and RXRα expression vectors are used in similar cotransfection experiments (Fig. 2A) and raises the possibility that the activity of

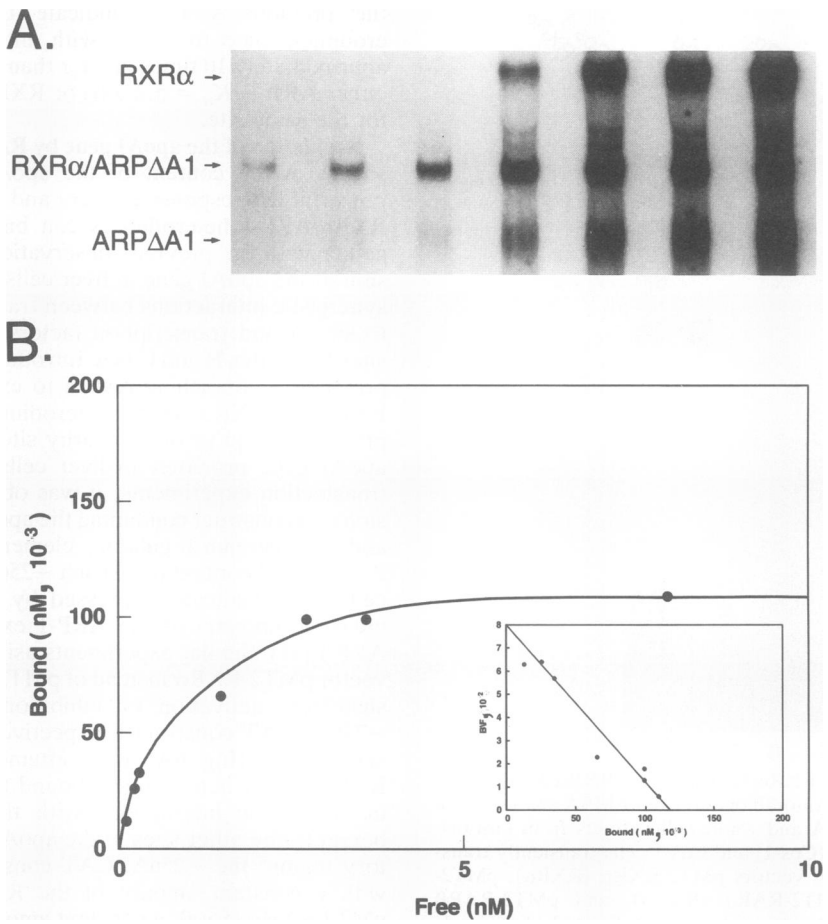

FIG. 5. RXRα/ARP-1 heterodimers bind to site A with high affinity. Autoradiogram showing retardation complexes formed with increasing amounts (left to right) of ^{32}P-labeled oligo A and fixed amounts of a mixture of whole-cell extracts from Cos-1 cells transiently transfected with the pMT2-RXRα and the pMT2-ARPΔA1 expression vectors. The mixture contained 1-μl extracts from the pMT2-RXRα-transfected cells and 0.25-μl extracts from the pMT2-ARPΔA1-transfected cells. Complexes due to RXRα, RXRα/ARPΔA1 heterodimers, and ARPΔA1 homodimers are indicated by arrows (A). The DNA amounts in the retardation complexes containing RXRα/ARPΔA1 heterodimers were determined and used to construct a binding saturation curve (B) and Scatchard plot (insert in panel B) as described in the legend to Fig. 3.

RXRα/ARP-1 heterodimers is influenced by the promoter context in which site A is located (see Discussion).

The direct involvement of RA in transactivation of the apoAI gene under these conditions becomes evident by replotting the data in Fig. 6A so that the activity of the −256AI.CAT construct in the presence and absence of RA is expressed as RA-dependent fold-activation (Fig. 6B). As shown in Fig. 6B, increasing amounts of ARP-1 expression vector promote an increased sensitization of the −256A I.CAT construct to respond to RA when RXRα is present in excess. It is therefore concluded that ARP-1 repression sensitizes transactivation of the apoAI gene by RXRα in the presence of RA and that the extent of this sensitization is controlled by the intracellular ratio of ARP-1 and RXRα. In addition, these results taken together with the results in the previous section raise the possibility that RXRα or RXRα/ARP-1 heterodimers or both, when bound to site A in the presence of RA, can participate in stimulation of apoAI gene transcription in HepG2 cells by cooperating with transcription factors bound to other regulatory elements in the apoAI gene 5′ flanking region (see also Discussion).

DISCUSSION

Although several previous studies have shown that variations in apoAI gene expression in response to developmental (17, 22), differentiation (2, 32), and hormonal (1, 34) factors and various pharmacologic (33) and toxic (21) substances can be accounted for, at least in part, by alterations in the apoAI gene transcription rates, neither the precise nature of signals modulating transcription nor the relevant signaling mechanisms are clearly understood. The current study shows that at least one of the mechanisms by which apoAI gene transcription is modulated involves ARP-1 repression-mediated transcriptional sensitization of the apoAI gene to RXRα and RA.

The apoAI gene site A is a conditional RA response element. The results show that RXRα and ARP-1 bind to the apoAI gene site A with similar affinities and that while RXRα in the presence of RA transactivates the apoAI gene basal promoter from site A, ARP-1 antagonizes this transactivation. Although the most straightforward interpretation of these results is that RXRα and ARP-1 compete for binding to site A and that while binding of RXRα in the presence of RA

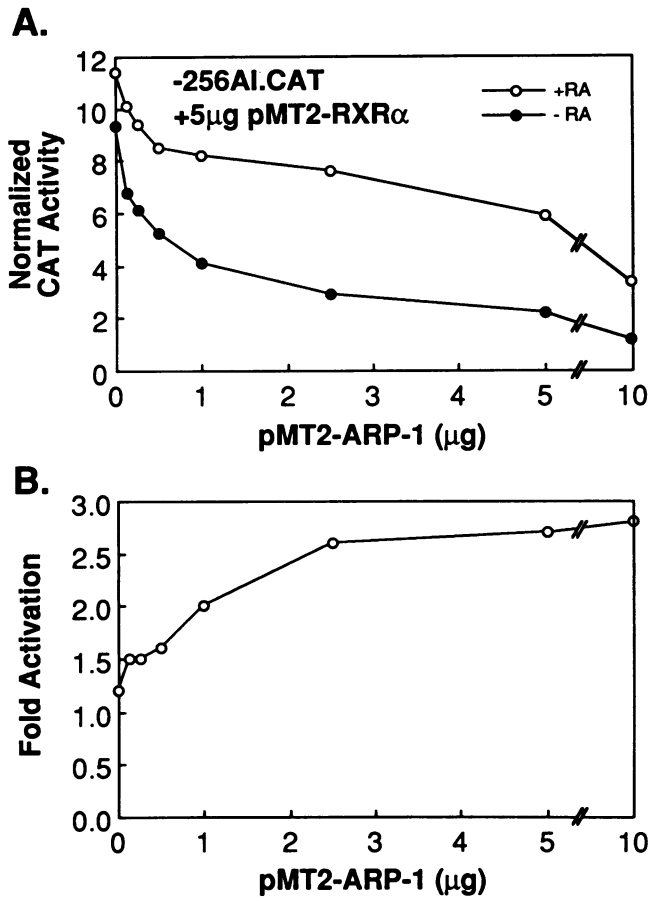

FIG. 6. RXRα and RA alleviate ARP-1-mediated repression of the apoAI gene. The CAT reporter construct −256AI.CAT was cotransfected into HepG2 cells with 5 μg of pMT2-RXRα and increasing amounts of the ARP-1 expression vector pMT2-ARP-1 as indicated. Normalized CAT activity, defined in the legend to Fig. 1, is shown in the presence (open circles) or absence (filled circles) of 10^{-6} M RA (A). Data from panel A were plotted to indicate the fold activation by RA (ratio of CAT activity in the presence versus absence of RA) as a function of the amounts of pMT2-ARP-1 used for cotransfection (B).

transactivates the basal promoter, binding of ARP-1 does not; it is important to emphasize that RXRα/ARP-1 heterodimers also bind to site A with an affinity (K_d = 0.6 nM) approximately 10 times greater than the affinity of either ARP-1 (K_d = 5.2 nM) or RXRα (K_d = 8.8 nM) alone. Furthermore, under cotransfection conditions favoring a greater intracellular concentration of RXRα/ARP-1 heterodimers over the concentration of ARP-1, the expression of the site A containing basal apoAI promoter in response to RXRα and RA is significantly reduced compared with its expression in the absence of ARP-1. It therefore appears that neither ARP-1 nor RXRα/ARP-1 heterodimers can transactivate the apoAI gene basal promoter from site A in response to RA. Taken together, these observations strongly suggest that site A is a conditional RA response element; it responds to RXRα and RA only when the intracellular ARP-1 concentration is low but loses responsiveness when the ARP-1 concentration is high. The recent finding that the ligand for RXRα is not RA but an intracellularly generated stereoisomeric metabolite of RA, 9-*cis*-RA (6, 15), suggests that RXRα/ARP-1 heterodimerization modifies the RXRα conformation to a form that either does not bind 9-*cis*-RA or does not conform to a structure appropriate for transactivation of the basal transcription machinery in response to binding of 9-*cis*-RA. The inability of RXRα/ARP-1 heterodimers to respond to 9-*cis*-RA and the observation that the ligand responsiveness of RXRα can be redirected by formation of heterodimers with the thyroid hormone receptor, vitamin D_3 receptor, and RA receptor α (RARα) which respond to thyroid hormone, vitamin D_3, and RA, respectively (10, 14, 37, 38), raise the possibility of the existence of an as yet unidentified ligand for RXRα/ARP-1 heterodimers.

Diverse signaling pathways converge onto site A. The importance of site A in apoAI gene regulation was initially revealed by deletion and point mutation experiments indicating that its occupation by nuclear proteins is essential for maximal expression of the apoAI gene in liver (HepG2) and intestinal (Caco2) cells (27, 36). Although the identities of proteins that bind to site A in these cells are still unclear, the data in the current study and other published and unpublished observations indicate that many different transcriptional regulators of the steroid-thyroid receptor superfamily bind to site A with relatively high affinities. The functional consequences of binding of these proteins on the expression of the apoAI gene basal promoter from site A vary, from constitutive activation by the hepatocyte nuclear factor 4 (HNF4 [35]) and inducible activation by RXRα and RXRα/RARα heterodimers in the presence of RA (35; this study) to repression of either constitutive or inducible activation by ARP-1, ear3-COUP-TF, and ARP-1/ear3-COUP-TF, and RXRα/ARP-1 or RXRα/TR heterodimers (35; this study). Clearly, these observations together with the heterodimerization-dependent fluctuation of the intracellular levels of these factors (10, 14, 37, 38; this study) provide a strong basis for the concept that site A is at least one of the points of integration of many diverse signaling pathways influencing apoAI gene expression. It is therefore very likely that the intracellular balance of these transcription factors ultimately determines the level of apoAI gene responsiveness to specific signals.

Repression as a mechanism for switching between alternative transcription-activating pathways. The discussion up to this point was restricted to findings in experiments using as a reporter the apoAI gene basal promoter (TATA box) under the control of site A. It is, however, becoming increasingly apparent that the promoter context around a site for binding of one or more transcription factors determines both transacting factor binding selectivity and its transcriptional activating or repressing capacity (for a review, see reference 12). This may be of particular relevance to the observations that, while RXRα and RA are powerful transactivators of site A in the context of the apoAI basal promoter, they have very little impact on the activity of site A within the natural context of sequences in the 5′ flanking region of the apoAI gene. One explanation for these observations is that in HepG2 cells site A is occupied by an endogenous activator(s) which interacts cooperatively with transacting factors bound to other nearby sites leading to formation of stable template-committed protein complexes that are refractory to RXRα and RA. This mechanism is analogous to that proposed to explain the inability of the transactivator SRF to stimulate transcription from preformed preinitiation complexes on the c-*fos* and adenovirus major late promoters (39). However, when it is considered in the context of the role of site A as a target for binding of several different transcription factors that influence apoAI gene expression in

response to diverse signals, this mechanism poses the paradox of how commitment for sustained responsiveness to a given signal (i.e., refractiveness to other signals) and promiscuity for responsiveness to other signals can both be accomplished through a single site. Resolution of this paradox may be provided by the data showing that although site A, within the natural context of the apoAI 5′ flanking sequences, is refractory to transactivation by RXRα and RA, prior repression of the apoAI gene by ARP-1 results in full transactivation by RXRα and RA. This observation strongly suggests that replacement of a putative endogenous activator(s) bound to site A by ARP-1 is a prerequisite step in RXRα binding and transactivation in response to RA. It is therefore conceivable that ARP-1 repression uncouples the endogenous activator(s) bound to site A from its interactions with other factors bound to nearby sites, thus converting endogenous transcriptional complexes into a form accessible to RXRα. ARP-1 could therefore play a fundamental role in switching the endogenous transcription activation pathway which is independent of RXRα and RA into a different pathway which depends on RXRα and RA.

The current data do not allow discrimination between the possibilities that replacement of the putative endogenous activator(s) by RXRα or RXRα/ARP-1 heterodimers is responsible for the RA-dependent transactivation of the ARP-1-repressed apoAI gene. In fact, since these heterodimers bind to site A with an affinity 10 times greater than that of RXRα, it would seem most likely that the endogenous activator(s) is replaced by RXRα/ARP-1 heterodimers rather than RXRα. This implies that RXRα/ARP-1 heterodimers bound to site A in the context of the natural apoAI gene 5′ regulatory sequences could function as RA-responsive transactivators. This is in contrast to the observation that these heterodimers in the presence of RA are incapable of transactivating the apoAI gene basal promoter from site A and raises the possibility that protein-protein interactions between different transcription factors in the apoAI gene natural promoter context induce responsiveness of these heterodimers to RA. It should, however, be noted that it is also possible that neither RXRα nor RXRα/ARP-1 heterodimers are involved in RA transactivation of the ARP-1-repressed apoAI gene but that RXRα heterodimerizes with RARβ which is induced by RA in HepG2 cells (3) and that these heterodimers mediate this RA-dependent effect on apoAI gene transcription.

Retinoids and atherosclerosis. As mentioned in the Introduction, apoAI is the major protein constituent of plasma HDL. Numerous epidemiologic, genetic, and biochemical studies have provided strong support for the concept that high plasma HDL concentrations protect against premature atherosclerosis (for a review, see references 7 and 19). Thus, the observation that there is a direct correlation between apoAI and plasma HDL levels and hepatic apoAI mRNA concentrations (30, 31) and the results in the current study showing that RA transactivates apoAI gene expression raise the possibility that RA and other vitamin A metabolites play an important role in atherosclerosis prevention. In this context it is interesting that recent studies show that ingestion of β-carotene, which serves either directly or indirectly through conversion to retinal as a precursor of RA (20), increases plasma HDL levels (23). Similarly, in preliminary studies we have observed that rabbits ingesting RA show significant increases in plasma HDL cholesterol and apoAI plasma levels (8). These observations, together with recent epidemiologic data suggesting that β-carotene therapy may reduce the risk for myocardial infarction and cardiac death (5), raise the possibility that apoAI gene upregulation through the RA-signaling pathway described in this report provides a physiological mechanism for atherosclerosis prevention.

ADDENDUM

While this work was under review, a different group of investigators reported that COUP-TF, a transcription factor very similar to ARP-1 (see reference 11), also represses RXRα- and RA-mediated transactivation of an RXRα response element from the CRBPII gene and that COUP-TF and RXRα form stable heterodimers in vitro (9a).

ACKNOWLEDGMENTS

We thank Patricia Costa-Giomi for critically reading the manuscript; Yisheng Wang, Patricia Costa-Giomi, Andrew Katocs, and Elwood Largis for permitting referencing of some of their unpublished results; and Barbara Elliott and Liisa Warren for typing and editing the manuscript.

This research was supported by grants from the National Institutes of Health (HL32032) and the American Heart Association (880640). During the course of these studies, S.K.K. was supported by an Established Investigator Award from the American Heart Association.

REFERENCES

1. **Apostolopoulos, J. J., M. J. La Scala, and G. J. Howlett.** 1988. The effect of triiodothyronine on rat apolipoprotein A-I and A-IV gene transcription. Biochem. Biophys. Res. Commun. **154:**997–1002.
2. **Basheeruddin, K., P. Stein, S. Strickland, and D. L. Williams.** 1987. Expression of the murine apolipoprotein E gene is coupled to the differentiated state of F9 embryonal carcinoma cells. Biochemistry **84:**709–713.
3. **de The, H., A. Marchio, P. Tiollais, and A. Dejean.** 1989. Differential expression and ligand regulation of the retinoic acid receptor α and β genes. EMBO J. **8:**429–433.
4. **Edlund, T., M. D. Walker, P. J. Barr, and W. J. Rutter.** 1985. Cell specific expression of the rat insulin gene: evidence for role of two distinct 5′ flanking elements. Science **230:**912–916.
5. **Gaziano, J. M., J. E. Manson, P. M. Ridker, J. E. Buring, and C. H. Hennekens.** 1990. Beta carotene therapy for chronic stable angina. Circulation **82**(Suppl. III):201.
6. **Heyman, R. A., D. J. Mangelsdorf, J. A. Dyck, R. B. Stein, G. Eichele, R. M. Evans, and C. Thaller.** 1992. 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell **68:**397–406.
7. **Karathanasis, S. K.** 1992. Lipoprotein metabolism: high-density lipoprotein. Monogr. Hum. Genet. **14:**140–171.
8. **Katocs, A. S., Jr., E. E. Largis, P. Costa-Giomi, and S. K. Karathanasis.** Unpublished data.
9. **Kaufman, R. J.** 1990. Vectors used for expression in mammalian cells. Methods Enzymol. **185:**487–511.

9a. **Kliewer, S. A., K. Umesono, R. A. Hayman, D. J. Mangelsdorf, J. A. Dyck, and R. M. Evans.** 1992. Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling. Proc. Natl. Acad. Sci. USA **89:**1448–1452.

10. **Kliewer, S. A., K. Umesono, D. J. Mangelsdorf, and R. M. Evans.** 1992. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. Nature (London) **355:**446–449.
11. **Ladias, J. A. A., and S. K. Karathanasis.** 1991. Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. Science **251:**561–565.
12. **Lamb, P., and S. L. McKnight.** 1991. Diversity and specificity in transcriptional regulation: the benefits of heterotypic dimerization. Trends Biochem. Sci. **16:**417–422.
13. **Leake, R. E., R. I. Freshney, and I. Munir.** 1987. Steroid response in vivo and in vitro, p. 205–218. *In* B. Green and R. E. Leake (ed.), Steroid hormones: a practical approach. IRL

Press, Washington, D.C.
14. **Leid, M., P. Kastner, R. Lyons, H. Nakshatri, M. Saunders, T. Zacharewski, J.-Y. Chen, A. Staub, J.-M. Garnier, S. Mader, and P. Chambon.** 1992. Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. Cell **68**:377–395.
15. **Levin, A. A., L. J. Sturzenbecker, S. Kazmer, T. Bosakowski, C. Huselton, G. Allenby, J. Speck, C. I. Kratzeisen, M. Rosenberger, A. Lovey, and J. F. Grippo.** 1992. 9-cis retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature (London) **355**:359–361.
16. **Mangelsdorf, D. J., E. S. Ong, J. A. Dyck, and R. M. Evans.** 1990. Nuclear receptor that identifies a novel retinoic acid response pathway. Nature (London) **345**:224–229.
17. **Meehan, R. R., D. P. Barlow, R. E. Hill, B. L. M. Hogan, and N. D. Hastie.** 1984. Pattern of serum protein gene expression in mouse visceral yolk sac and foetal liver. EMBO J. **3**:1881–1885.
18. **Mietus-Snyder, M., F. M. Sladek, G. S. Ginsburg, C. F. Kuo, J. A. A. Ladias, J. E. Darnell, Jr., and S. K. Karathanasis.** 1992. Antagonism between apolipoprotein AI regulatory protein 1, Ear3/COUP-TF, and hepatocyte nuclear factor 4 modulates apolipoprotein CIII gene expression in liver and intestinal cells. Mol. Cell. Biol. **12**:1708–1718.
19. **Miller, E. M.** 1987. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am. Heart J. **113**:589–597.
20. **Napoli, J. L., and K. R. Race.** 1988. Biogenesis of retinoic acid from β-carotene. J. Biol. Chem. **263**:17372–17377.
21. **Panduro, A., Y.-C. Lin-Lee, L. Chan, and D. A. Shafritz.** 1990. Transcriptional and posttranscriptional regulation of apolipoprotein E, A-I, and A-II gene expression in normal rat liver and during several pathophysiologic states. Biochemistry **29**:8430–8435.
22. **Panduro, A., F. Shalaby, and D. A. Shafritz.** 1987. Changing patterns of transcriptional and post-transcriptional control of liver-specific gene expression during rat development. Genes Dev. **1**:1172–1182.
23. **Ringer, T. V., M. J. DeLoof, G. E. Winterrowd, S. F. Francom, S. K. Gaylor, J. A. Ryan, M. E. Sanders, and G. S. Hughes.** 1991. Beta-carotene's effects on serum lipoproteins and immunologic indices in humans. Am. J. Clin. Nutr. **53**:688–694.
24. **Rottman, J. N., R. L. Widom, B. Nadal-Ginard, V. Mahdavi, and S. K. Karathanasis.** 1991. A retinoic acid response element in the apolipoprotein AI gene distinguishes between two different retinoic acid response pathways. Mol. Cell. Biol. **11**:3814–3820.
25. **Rowe, A., N. S. C. Eager, and P. M. Brickell.** 1991. A member of the RXR nuclear receptor family is expressed in neural-crest-derived cells of the developing chick peripheral nervous system. Development **111**:771–778.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Sastry, K. N., U. Seedorf, and S. K. Karathanasis.** 1988. Different *cis*-acting elements control expression of the human apolipoprotein AI gene in different cell types. Mol. Cell. Biol. **8**:605–614.
28. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
29. **Sladek, F. M., W. Zhong, E. Lai, and J. E. Darnell, Jr.** 1990. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. Genes Dev. **4**:2353–2365.
30. **Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams.** 1988. Apolipoprotein (Apo) A-I production and mRNA abundance explain plasma apoA-I and high density lipoprotein differences between two nonhuman primate species with high and low susceptibilities to diet-induced hypercholesterolemia. J. Biol. Chem. **263**:5183–5189.
31. **Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams.** 1989. Differential effects of dietary fat on the tissue-specific expression of the apolipoprotein A-I gene: relationship to plasma concentration of high density lipoproteins. J. Lipid Res. **30**:1379–1403.
32. **Spiegelberg, T., and J. O. Bishop.** 1988. Tissue-specific gene expression in mouse hepatocytes cultured in growth-restricting medium. Mol. Cell. Biol. **8**:3338–3344.
33. **Staels, B., A. van Tol, T. Andreu, and J. Auwerx.** 1992. Fibrates influence the expression of genes involved in lipoprotein metabolism in a tissue-selective manner in the rat. Arterioscler. Thromb. **12**:286–294.
34. **Strobl, W., N. L. Gorder, Y.-C. Lin-Lee, A. M. Gotto, Jr., and W. Patsch.** 1990. Role of thyroid hormones in apolipoprotein A-I gene expression in rat liver. J. Clin. Invest. **85**:659–667.
35. **Wang, Y., P. Costa-Giomi, and S. K. Karathanasis.** Unpublished data.
36. **Widom, R. L., J. A. A. Ladias, S. Kouidou, and S. K. Karathanasis.** 1991. Synergistic interactions between transcription factors control expression of the apolipoprotein AI gene in liver cells. Mol. Cell. Biol. **11**:677–687.
37. **Yu, V. C., C. Delsert, B. Andersen, J. M. Holloway, O. Devary, A. M. Naar, S. Y. Kim, J.-M. Boutin, C. K. Glass, and M. G. Rosenfeld.** 1991. RXRβ: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell **67**:1251–1266.
38. **Zhang, X.-K., B. Hoffmann, P. B.-V. Tran, G. Graupner, and M. Pfahl.** 1992. Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. Nature (London) **355**:441–446.
39. **Zhu, H., A. L. Roy, R. G. Roeder, and R. Prywes.** 1991. Serum response factor affects preinitiation complex formation by TFIID in vitro. New Biol. **3**:455–464.