Three proximal regulatory elements, AIB, AIC, and AID, of the apoA-I gene are necessary and sufficient for its hepatic expression in vivo and in vitro. DNA binding and competition assays showed that elements AIB and AID contain hormone response elements composed of imperfect direct repeats that support the binding of the hepatic nuclear factor-4, other nuclear orphan receptors, and the ligand-dependent nuclear receptors retinoic X receptor (RXRα), RXRα/RARα, and RXRα/T3Rβ. Substitution mutations on repeats 1 and 2 in the hormone response sites of elements AIB and AID, respectively, abolished the binding of all nuclear receptors and reduced promoter activity to background levels, indicating the importance of both hormone response elements for the hepatic expression of the apoA-I gene. Cotransfection experiments in HepG2 cells with normal and mutated promoter constructs and plasmids expressing nuclear hormone receptors showed that RXRα homo- and heterodimers transactivated the wild type promoter 150% of control, in the presence of 9-cis-retinoic acid (RA), whereas RXRα/T3Rβ heterodimers repressed transcription to 60% of control, in the presence of T3. RXRα/RARα and hepatic nuclear factor-4 did not affect the transcription, driven by the proximal apoA-I promoter. Potassium permanganate and dimethyl sulfoxide interference experiments showed that RXRα homodimers, RXRα/RARα, and RXRα/T3Rβ heterodimers participate in protein-DNA interactions with 12, 13, and 11 out of the 14 nucleotides, respectively, that span repeats 1 and 2 and the spacer region separating them on the hormone response element of apoA-I. The binding of RXRα homodimers and RXRα/T3Rβ heterodimers is associated with ligand-dependent activation by 9-cis-RA or repression by T3 upon deletion or mutation of repeat 1, homodimeric binding of RXRα is lost whereas heterodimeric binding is retained. This heterodimeric binding to the mutated element AID is mediated solely by interactions with repeat 2 and one adjacent nucleotide and is confined to a heptameric core recognition motif. The interactions of the RXRα heterodimers with repeat 2 are associated with low levels of ligand-independent transcriptional activity. The findings suggest that the specific types of homo- and heterodimers of nuclear hormone receptors occupying the hormone response elements of apoA-I and the availability of the ligand may play an important role in the transcriptional regulation of the human apoA-I gene.

Epidemiological data and transgenic animal experiments have shown that increased apoA-I and high density lipoprotein levels are protective against atherosclerosis (1, 2). Thus the mechanisms which regulate the synthesis of apoA-I are important. Previous studies have shown that the regulatory elements AIB (−128/−77), AIC (−175/−148), and AID (−220/−190) are sufficient for hepatic expression of the human apoA-I gene in tissue culture (3, 4) and in transgenic mice (5). It has been shown that the regulatory element AIC is recognized by heat stable factors related to CCAAT/enhancer-binding protein (C/EBP) which act as positive regulators and by heat labile activities, one of which acts as a negative regulator (4). In addition, the regulatory element AID contains a hormone response element (HRE) that is recognized by ARP-1, a transcriptional repressor of apoA-I (6), hepatic nuclear factor-4 (HNF-4) (7), RXRα homodimers, RXRα/RARα, RXRα/ARP-1 (8, 9), and RXRα/PPAR heterodimers (10). All these factors are members of the steroid/thyroid receptor superfamily, that includes receptors for steroids, thyroid hormone, and retinoic acids as well as orphan nuclear receptors with unidentified ligands. In the present study we have demonstrated the existence of an additional HRE on the regulatory element AIB between nucleotides −132/−119 and we have investigated the role of both HREs and of different types of hormone nuclear receptors on the transcriptional regulation of the human apoA-I gene. We demonstrate that both HREs are recognized by homodimers of HNF-4, ARP-1, EAR-2, EAR-3, and RXRα as well as heterodimers of RXRα with RARα or T3Rβ, and that both elements are essential for the hepatic expression of the apoA-I gene. Binding of the RXRα homodimers on the HRE of element AID requires direct repeats 1 and 2 and leads to ligand-dependent transcriptional activation whereas binding of the RXRα/RARα and RXRα/T3Rβ heterodimers on this HRE may occur on direct repeats 1 and 2 or only on repeat 2. When bound on both repeats 1 and 2, the RXRα/T3Rβ heterodimers repress transcription in the presence of T3 whereas the RXRα/RARα heterodimers and HNF-4 do not affect the transcription. In addition, binding of the RXRα heterodimers to only one repeat on the HRE of element AID is associated with low levels of ligand-independent transcriptional activity. The findings demonstrate that hormone nuclear receptors can modulate the transcription of the human apoA-I gene and thus may affect plasma apoA-I and high density lipoprotein levels.

MATERIALS AND METHODS

T3, DNA ligase, polynucleotide kinase, Vent polymerase, and restriction enzymes were purchased from New England Biolabs. Transforma-
tion competent bacterial HB101 cells were purchased from Life Technologies, Inc. [γ-32P]ATP (5000 Ci/mmol), [α-32P]dCTP, [3H]acetyl coenzyme A (200 mCi/mmol), [α-32P]P[35S]TP (4000 Ci/mmol), and E. coli fluorocellulose fluid were purchased from DuPont NEN. Chloroampholine was purchased from Sigma. Reagents for automated DNA synthesis were purchased from Applied Biosystems, Inc. The sequencing reagents for the pUC19 vector from S. Biotechnology Corp. were purchased from J. T. Baker Chemical Co. Bacto-tryptone and Bacto-yeast extract were purchased from Difco. O-Nitrophenyl-β-D-galactopyranoside was purchased from Sigma. Double-stranded poly(dI-dC) was purchased from Pharmacia LKB Biotechnology Inc. Acrylamide, sodium dodecyl sulfate (SDS), urea, Tris, and the anti-Flag antibody were purchased from Pierce. Other reagents used were purchased from Pharmacia LKB Biotechnology Inc. Acrylamide, sodium dodecyl sulfate (SDS), urea, Tris, and the anti-Flag antibody were purchased from Pierce.

Plasmid Constructions and CAT Assays—The apoA-I promoter region was derived from an apoA-I genomic clone as described previously (11). The proximal promoter region spanning nucleotides −264/+5 was subcloned into the Asp718 and SmaI restriction sites of the pUC7-CAT plasmid to produce the CAT derivative designated −264/+5apoA-I-CAT (4). This reporter construct was used to create mutated apoA-I promoter constructs. The mutated −264/+5 CAT constructs containing deletions or nucleotide substitutions in elements AIB and AID were generated by amplification of the parent pUC7−264/+5 apoA-I CAT construct (4). For example, in order to generate the mutation AIDM1, the region upstream of nucleotide −186 was amplified by PCR using the PCR-264AI 5′ primer which contains an Asp718 and EcoRV restriction sites and the mutagenic AIDM1 primer (Table I). The region downstream of nucleotide −225 was amplified by using the AID1C mutagenic primer and the PCGR-19AI 3′ primer which contains an SspI restriction site. An aliquot containing 5% of the two amplified regions was used as a template for further amplification by the 5′ and 3′ primers PCR-264AI and PCR-19AI. The amplified DNA was digested with Asp718 and SspI and cloned into the Asp718 and SmaI sites of pUC7-CAT (4). The remaining mutations were constructed in a similar manner using the amplification primers shown in Table I. PCR reactions were performed using the Perkin Elmer automated thermocycler according to the manufacturer’s specifications. The sequence of the DNA fragment was determined by DNA sequencing. The deoxyribonucleotides used were synthesized by the solid-phase phosphite triester method using an automatic AB-380B oligonucleotide synthesizer.

Preparation of Nuclear Extracts and Whole Cell Extracts from Transfected COS-1 Cells—Nuclear extracts were prepared from livers of 10 rats (approximately 120 g of liver) as described (12). Extracts from COS-1 cells transfected with the pMT2 vector carrying full-length cDNAs for HNF-4, ARP-1, EAR-2, EAR-3, and RXRα and RXRβ were prepared as described (6). Similarly prepared were extracts for COS-1 cells transfected with the pSGS expression vector carrying a flag epitope fused to the NH2-terminal of TpR8.

Gel Electrophoretic Mobility Shift Assay—This analysis was performed using either crude hepatic nuclear extracts or COS-1 whole cell extracts, unless otherwise stated (13). Complexes were visualized by autoradiography.

RESULTS

Binding of Orphan and Ligand-dependent Nuclear Receptors to the Regulatory Elements AIB and AID of the Human ApoA-I: Effect of Promoter Mutations on Binding

The regulatory element AID (−220/−190) contains sequences that show high similarity with an AGG/TTCG motif (half-site) found in HREs on the promoter regions of a variety of genes (15–17). Examination of the HRE on element AID showed the presence of three putative direct repeats between nucleotides −190 to −210, whereas the element AIB contains two putative direct repeats between nucleotides −132 to −119 (Fig. 1A). DNA binding assays have shown that both regulatory elements AIB and AID can support the homodimeric binding of RXRα and RXRβ (Fig. 1B). RXRα and RXRβ heterodimers bound to oligonucleotides containing 5′ and 7,5, and 10 milliunits of purified β-galactosidase allowed the conversion of the OD units of the different samples into β-galactosidase units.
elements AIB and AID, designated AIBM, AIDM, AIDM1, AIDM2, AIDM3, AIDM4, and AIDM5 (Table II). AIBM and AIDM mutations span part of both repeats of element AIB or only the second repeat of element AID, respectively. Mutation AIDM1 is localized upstream of the HRE on element AID, whereas mutation AIDM2 contains nucleotide substitutions in repeat 1. Finally, the AIDM3 and AIDM4 mutations contain substitutions in the putative repeat 2, and the AIDM5 mutation contains substitutions in the putative repeat 3. We also generated two deletion mutations, one of repeat 1 and one of putative repeat 3, in the HRE of element AID, which were designated AIDMREP1 and AIDMREP3, respectively. The oligonucleotides containing these mutations, shown in Table II, were tested in DNA binding and competition experiments. These analyses showed that the AIDM1 mutation had no effect on the binding of rat hepatic nuclear extracts (compare lane 1 with lane 7 of Fig. 2A). The AIDM2 mutation increased substantially the binding of the slower migrating activity present in the rat liver nuclei (compare lane 1 with lane 9 of Fig. 2A). The AIDM3 and AIDM4 mutations did not bind substantially or compete for the binding of hepatic nuclear activities to element AID (Fig. 2A, lanes 5, 6, 11, and 13). The DNA-protein complexes formed with AIDM4 are not competed out by the wild type AID sequences (data not shown). Thus, these complexes must originate from the binding to the mutated probe of activities unrelated to nuclear hormone receptors which do not normally bind to the wild type probe. The AIBM mutation and the AIDM mutation which altered drastically the HREs of element AIB and AID, respectively, abolished the binding of all nuclear activities to element AID (Fig. 2D, lanes 8 and 16), whereas deletion or mutation of the putative repeat 3 and deletion of repeat 1 (AIDMREP3, AIDM5, and AIDMREP1) did not affect qualitatively the binding of hepatic nuclear activities to this site (Fig. 2, E and F).

We have also tested the effects of the deletion and oligonucleotide substitution mutations within the two regulatory elements AID and AIB (Table II) on the binding of orphan and ligand-dependent nuclear receptors. This analysis showed that the AIDM1 mutation did not affect the binding of the homodimers of the orphan receptors HNF-4, ARP-1, EAR-2, and EAR-3 (Fig. 2B). The remainder of the mutations affected the binding of the orphan receptors differentially. Binding of HNF-4 was moderately affected by changes in repeat 1 (AIDM2 mutation) and it was greatly affected by changes in the first half of repeat 2 (AIDM3 mutation). Finally, binding was dimin-
The role of the hormone response elements in apoA-I gene transcription

Wild-type and mutated oligonucleotide sequences used in the DNA binding assays

Mutated sequences are in bold and underlined. The putative repeats of the HREs are indicated as in Fig. 1A.

Table I

| Name | Sequence | Repeat 1 | Repeat 2 |
|------|----------|----------|----------|
| AIDM1 C | CCTCCGCCGAAA GCATGACCCCTTGACCCCCCTGCAG -186 | -226 | |
| AIDM1 NC | CTCAAAGGGCAGGCAAGGGGAGGCTTGACCCCCCTGCAG -186 | -186 | |
| AIDM2 C | CCTCCGCCGACGCACGTGGACCCCCCTGCAG -186 | -226 | |
| AIDM2 NC | TCCAGGGGACAGGGAAGGCTTGACCCCCCTGCAG -186 | -186 | |
| AIDM3 C | CCTCCGCCGCACACGTGGAGGCCCTGCAG -186 | -226 | |
| AIDM3 NC | TCCAGGGGAAGGCAAGGGGAGGCTTGACCCCCCTGCAG -186 | -186 | |
| AIDM4 C | CCTCCGCCGACGCACGTGGACCCCCCTGCAG -186 | -226 | |
| AIDM4 NC | TCCAGGGGACAGGGAAGGCTTGACCCCCCTGCAG -186 | -186 | |
| AIDM5 C | CCGCAGGACCTGAGCCCTTGAGCCTGACCCCCCTGCAG -186 | -221 | |
| AIDM5 NC | CAGGGTGGTCAAGGGGAGGCTTGACCCCCCTGCAG -186 | -189 | |

ΔREP1

| Name | Sequence | Repeat 1 | Repeat 2 |
|------|----------|----------|----------|
| ΔREP1 | CTCCGGCAGGGAAGGCTTGACCCCCCTGCAG -186 | -223 | |
| ΔREP3C | CTGGCAGGGAAGGCTTGACCCCCCTGCAG -186 | -186 | |

PCR-19AI

| Name | Sequence | Repeat 1 | Repeat 2 |
|------|----------|----------|----------|
| 3' Primer | AATTAAAAATATTTGCTCTAACTGACCCGACCTGCTTCGCA | ATTTAAAAATATTTGCTCTAACTGACCCGACCTGCTTCGCA | -19 | |

PCR-264AI

| Name | Sequence | Repeat 1 | Repeat 2 |
|------|----------|----------|----------|
| 5' Primer | GATATCGGTACCGACCCCACCCGGGAGACCTGCAAGGCTGCAGACACTC | -140 GACAGAGCTGAGATATCTCAGCTCTGTCCCTGGG | -264 | |

Table II

Wild-type and mutated oligonucleotide sequences used in the DNA binding assays

Mutated sequences are in bold and underlined. The putative repeats of the HREs are indicated as in Fig. 1A.

| Name | Sequence |
|------|----------|
| AIB wt | GACAGAGCTGGAGATATCTCAGCTCTGTCCCTGGG |
| AIBM | GACAGAGCTGGAGATATCTCAGCTCTGTCCCTGGG |
| AID wt | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM1 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM2 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM3 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM4 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM5 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| ΔREP1 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| ΔREP3 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |

Role of the hormone response elements in apoA-I gene transcription

Wild-type and mutated oligonucleotide sequences used in the DNA binding assays

Mutated sequences are in bold and underlined. The putative repeats of the HREs are indicated as in Fig. 1A.

| Name | Sequence |
|------|----------|
| AIBM | GACAGAGCTGGAGATATCTCAGCTCTGTCCCTGGG |
| AIBM | GACAGAGCTGGAGATATCTCAGCTCTGTCCCTGGG |
| AIDM1 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM2 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM3 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM4 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| AIDM5 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| ΔREP1 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |
| ΔREP3 | CCTCCGCCGACGCACGTGGACCCCCCTGCAG |

Deletion of repeat 1 of element AID (ΔREP1) reduced the binding of HNF-4 but did not affect qualitatively the binding of the other orphan nuclear receptors. DNA mobility was affected considerably by changes in repeat 2. The analysis with the ligand-dependent nuclear receptors showed that the AIDM2 and AIDM4 mutations which affect the first repeat and the AIDM3 and AIDM4 mutations which affect the second repeat in the HRE of element AID abolished the binding of RXRα homodimers (Fig. 2, C and E). The AIDM4 mutations affected mainly the binding of RXRα/RXRα heterodimers. The AIDM2, AIDM3, and AIDM4 mutations decreased the binding of RXRα/RXRβ heterodimers. Deletion of repeat 1 of element AID (ΔREP1) did not affect the qualitative binding of RXRα/RXRα and RXRα/RXRβ heterodimers. Deletion or mutations in the third repeat of the element AID (AIDM5 and ΔREP3) did not affect qualitatively the binding of RXRα homo- and heterodimers (Fig. 2, E and F). In addition, the AIDM2 mutation formed a DNA-protein complex of higher mobility than the complex formed with the wild type oligonucleotide or those carrying the AIDM3 and AIDM4 mutations
Finally, the AIBM and AIDM mutations, which altered drastically the HREs of the regulatory elements AIB and AID, respectively, eliminated the binding of all combinations of ligand-dependent nuclear receptors to the mutated probe (Fig. 2D).

In summary, the findings of Fig. 2, A-F, demonstrate that binding of RXRα homodimers requires intact repeats 1 and 2 in the HRE of element AID, whereas binding of RXRα/RXRα and RXRα/RARα/Rb heterodimers can still occur when short mutations are introduced in either repeat 1 or repeat 2. Similarly, the binding of the orphan nuclear receptors is differentially affected by the various mutations. Binding of HNF-4 requires the intact second part of repeat 2, whereas binding of EAR-2 and EAR-3 requires the intact first part of repeat 2 of element AID. Additionally, binding of ARP-1 is affected only by extensive mutagenesis of repeat 2. Finally, the binding of crude rat liver nuclear extracts parallels that of HNF-4.

Contribution of the HREs to the Strength of the ApoA-I Promoter in HepG2 Cells

To assess the importance of the two HREs for the apoA-I promoter strength we introduced the mutations of Table II in the apoA-I promoter and generated mutant −264/−5 A-I CAT constructs. Transient transfection assays in HepG2 cells showed that drastic mutations in the regulatory element AIB (AIBM mutation) or the regulatory element AID (AIDM mutation) or both elements (AIBM2, AIDM, and AIDM4) probes. Panel D, binding of rat liver nuclear extracts, orphan and ligand-dependent nuclear receptors to probes having deletions of either the putative repeat 3 (A1DREP3) or repeat 1 (A1DREP1) of element AID. Panel F, binding of rat liver nuclear extracts, orphan, and ligand-dependent nuclear receptors to a probe having nucleotide substitution in the putative repeat 3 (AIDM5) of element AID.
Role of the Hormone Response Elements in ApoA-I Gene Transcription

The KMnO₄ and dimethyl sulfate modification analysis was also utilized to determine the mode of binding of RXRα/RARα or RXRα/T₃Rβ heterodimers on the wild type element AID. The analysis with the RXRα/RARα heterodimers showed that all the nucleotides of repeats 1 and 2, which participate in DNA-protein interactions with the RXRα homodimers also participate in DNA-protein interactions with the RXRα/RARα heterodimers. In addition, nucleotide 4 of the noncoding strand of repeat 1 participates in weak DNA-protein interactions with the RXRα/RARα heterodimers (Fig. 4, D-F). Overall 13 of the 14 nucleotides that form repeats 1 and 2 and the putative spacer region between them participate in interactions with this heterodimer. Six of the nucleotides are in repeat 1, six in repeat 2, and one in the spacer region between repeats 1 and 2. Ten of the nucleotides participate in strong, and three participate in weak DNA-protein interactions. The nucleotides which participate in weak interactions are nucleotides 3 and 4 of the noncoding strand and nucleotide –1 of the coding strand. Repeat 3 is not involved in the binding of RXRα/RARα heterodimers (Fig. 4F). The analysis with the RXRα/T₃Rβ heterodimers showed that all but one of the nucleotides of repeats 1 and 2 which participate in DNA-protein interactions with the RXRα homodimers also participate in interactions with the RXRα/T₃Rβ heterodimers. The oligonucleotide which does not participate in DNA-protein interactions with the RXRα/T₃Rβ heterodimers is nucleotide 3 of the noncoding strand of repeat 1 (Fig. 4, G and H). Overall, four nucleotides of repeat 1 and seven nucleotides of repeat 2 and the putative spacer region between them participate in DNA-protein interactions with the RXRα/T₃Rβ heterodimers. Six of the nucleotides participate in strong and the remaining five in weak DNA-protein interactions. The nucleotides which participate in strong interactions are nucleotides 5 of repeat 1 and 5 and 6 of repeat 2 of the coding strand and nucleotides 1, 2, and 3 of repeat 2 of the noncoding strand. The remaining nucleotides as well as the nucleotide –1 in the putative spacer region between repeats 1 and 2 participate in weak DNA-protein interactions. Residues of the putative repeat 3 are not involved in the binding of the RXRα/T₃Rβ heterodimers (Fig. 4, G-I).

Mode of Binding of the RXRα/RARα and the RXRα/T₃Rβ Heterodimers on the Regulatory Element AID Carrying Mutation or Deletion of Repeat 1, as Determined by KMnO₄ and Dimethyl Sulfate Interference Assays

As shown in Fig. 2, C and E, mutation or deletion of the first repeat in the HRE of the regulatory element AID (AIDM2 and AIDΔREP1 mutations) prevents the binding of RXRα homodimers but allows the binding of RXRα/RARα and RXRα/T₃Rβ heterodimers. To investigate further the mode of binding of the heterodimers to the mutated sequence we performed KMnO₄ and dimethyl sulfate interference analysis using the oligonucleotides AIDM2 and AIDΔREP1 as probes (Table I). The KMnO₄ and dimethyl sulfate modification pattern of the RXRα/RARα heterodimers with the mutated AIDM2 probe is shown in Fig. 5, A and B, respectively, and summarized in Fig. 5C. This analysis showed that the binding of the RXRα/RARα heterodimers to the mutated AIDM2 probe is confined to a heptameric core recognition motif. The seven oligonucleotides which participate in DNA-protein interactions are the six nucleotides of repeat 1 and the nucleotide –1 localized in the putative spacer region between repeats 1 and 2. Nucleotides 5 and 6 of the coding strand and nucleotide 4 of the noncoding strand participate in strong, and the remaining in weak, DNA-protein interactions with the RXRα/RARα heterodimers.

The KMnO₄ and dimethyl sulfate modification pattern of the RXRα/T₃Rβ heterodimers with the mutated AIDM2 probe is...
shown in Fig. 5, and E, respectively, and summarized in Fig. 5F. This analysis showed that the binding of the RXRα/T3Rβ heterodimers to the mutated AIDM2 probe is confined to a heptameric core recognition motif. The seven nucleotides of the coding and noncoding strand which participate in DNA-protein interactions are indicated by an asterisk (*).
residues, nucleotides 5 and 6 of the coding strand of repeat 2, participate in strong DNA-protein interactions and the remaining in weak interactions. The putative repeat 3 does not participate in any DNA-protein interactions with the RXR<sub>a</sub>/T<sub>3R</sub><sub>b</sub> heterodimers. The KMnO<sub>4</sub> and dimethyl sulfate modification pattern of the RXR<sub>a</sub>/RAR<sub>a</sub> heterodimers with the mutated element AIDM2 or A1REP1 as probes (Table II). The RXR<sub>a</sub>/RAR<sub>a</sub> and RXR<sub>a</sub>/T<sub>3R</sub><sub>b</sub> heterodimers were produced by expression of the corresponding cDNAs in COS-1 cells. The KMnO<sub>4</sub> and dimethyl sulfate modification pattern of RXR<sub>a</sub>/RAR<sub>a</sub> heterodimers with the coding and noncoding strand of element AIDM2 is shown in Panels A and B, respectively. Panel C is a summary of the interference pattern deduced from the findings of Panels A and B. The KMnO<sub>4</sub> and dimethyl sulfate modification pattern of RXR<sub>a</sub>/T<sub>3R</sub><sub>b</sub> heterodimers with the coding and noncoding strand is shown in Panels D and E, respectively. Panel F is a summary of the interference pattern deduced from the findings of Panels D and E. The KMnO<sub>4</sub> and dimethyl sulfate modification pattern of RXR<sub>a</sub>/RAR<sub>a</sub> heterodimers with the coding and noncoding strand of the A1REP1 probe is shown in Panels G and H. Panel I is a summary of the interference pattern deduced from the findings of Panels G and H. F indicates the free probe, and B indicates the probe recovered from the DNA-protein complex after chemical treatment. Strong interactions are illustrated with ovals for the RXR<sub>a</sub>/RAR<sub>a</sub> heterodimers, and diamonds for the RXR<sub>a</sub>/T<sub>3R</sub><sub>b</sub> heterodimers. Weak interactions are illustrated with small ovals or diamonds. The nucleotide sequence of the coding and noncoding strand of the mutated element AID is indicated on each side of Panels A, B, D, E, G, H, and I. Nucleotides which participate in DNA-protein interactions are indicated by an asterisk (*).
The interaction of the RXR heterodimers with the regulatory element AID. The figure is deduced from the data of Figs. 4 and 5.

**A**

![Diagram](image)

**B**

![Diagram](image)

Fig. 6. Panel A, schematic representation of the nucleotides in the HRE of regulatory element AID which participate in DNA-protein interactions with the RXRα homodimers and the RXRα/RARα or RXRα/T₃Rβ heterodimers. Panel B, schematic representation of the mode of interaction of the RXRα homodimers and RXRα/RARα and RXRα/T₃Rβ heterodimers with the normal and the mutated HREs of the regulatory element AID. The figure is deduced from the data of Figs. 4 and 5.

AID/REP1 probe is shown in Fig. 5, G and H, and summarized in Fig. 5I. The pattern is similar to that obtained with the AIDM2 probe which carries mutations in the first repeat. The only exception is that nucleotide 5 on the coding strand of the putative third repeat also participates in weak DNA-protein interactions.

The combined data of Figs. 4, A-I, and 5, A-I, indicate that a heptameric core recognition motif is the minimum sequence required for the binding of RXRα/RARα and RXRα/T₃Rβ heterodimers to the regulatory element AID. The binding of the RXRα homodimers requires the presence of both direct repeats of element AID. Finally, the putative repeat 3 has minimal participation in DNA-protein interactions with homo- or heterodimers of RXRα. Fig. 6A shows a summary of all the nucleotides which participate in DNA-protein interactions with the various combinations of the ligand-dependent nuclear receptors. Fig. 6B summarizes the mode of binding of the ligand-dependent nuclear receptors RXRα, RXRα/RARα, and RXRα/T₃Rβ on the wild type and the mutated regulatory element AID of the human apoA-I promoter. This summary is based on the DNA binding data of Figs. 1 and 2 and K/MnO₄ and dimethyl sulfate interference analyses pattern of Figs. 4, A-I, and 5, A-I.

**Effect of Ligand-dependent Nuclear Receptors and HNF-4 on the Apo-A-I Promoter Strength**

Transactivation by RXRα Homodimers in the Presence of 9-cis-RA—We have performed cotransfection titration experiments in HepG2 cells with the wild type or mutated —264/+5 apoA-I CAT constructs and plasmids expressing various combinations of ligand-dependent nuclear receptors in the presence or absence of their corresponding ligands. The experiments with RXRα were performed in the presence or absence of its ligand 10⁻⁶ M 9-cis-RA and increasing amounts of an RXRα expression plasmid, ranging from 50 to 750 ng. This analysis showed that cotransfection with RXRα transactivated moderately (1.5-fold) the apoA-I promoter, in the presence of its ligand 9-cis-RA. Optimal ligand-dependent transactivation was observed in the range of 100–250 ng of plasmid. In the absence of exogenously added ligand there was no significant transactivation in this range of concentrations whereas at higher concentrations there was a trend toward transcriptional repression (Fig. 7A).

Lack of Transactivation by RXRα or RARα Heterodimers—The experiments involving RXRα/RARα heterodimers were performed with constant amounts (100 ng) of RXRα expression plasmid and increasing amounts of RARα, ranging from 50 to 500 ng. It is expected that the higher RARα concentrations will favor the formation of heterodimers rather than homodimers of RXRα. The experiments were performed in the absence of any ligand or in the presence of 10⁻⁶ M 9-cis-RA or 10⁻⁶ M all-trans-RA. This analysis showed that the RXRα/RARα heterodimers did not transactivate significantly the apoA-I promoter in the presence of any of the ligands. In the absence of both ligands and at higher concentrations of RARα, there was a trend toward transcriptional repression (Fig. 7B). The findings suggest that the RXRα/RARα heterodimers abolished the 1.5-fold transactivation achieved by RXRα homodimers in the presence of 9-cis-RA.

Repression of Transactivation by RXRα/T₃Rβ Heterodimers in the Presence of T₃—The experiments involving RXRα/T₃Rβ heterodimers were performed with constant amounts of RXRα (100 µg) and increasing amounts of T₃Rβ, ranging from 50 to 500 ng for the reasons described above. The experiments were performed in the absence of any ligand or in the presence of either 10⁻⁶ M 9-cis-RA or 10⁻⁷ M T₃. This analysis showed that the RXRα/T₃Rβ heterodimers repressed transcription to 60% of control in the presence of T₃. In the presence of 9-cis-RA, cotransfection with 50 ng of T₃Rβ and 100 ng of RXRα expression plasmids caused a 1.5-fold increase in transcription, similar to the increase observed with the RXRα homodimers (Fig. 7A). Most likely, this increase is the result of the formation of RXRα homodimers, promoted by the higher concentration of RXRα as compared to the T₃Rβ vector, in the presence of 9-cis-RA. When the two receptor-expressing plasmids were used in equal concentration (100 ng), this increase was no longer apparent.

Lack of Transactivation by HNF-4 Homodimers—Cotransfection experiments in HepG2 cells were also performed with plasmids expressing HNF-4. This analysis showed that low concentrations (25–100 ng) of HNF-4 did not increase the hepatic expression of apoA-I beyond the levels of expression achieved in the absence of exogenously added HNF-4. Higher concentrations of HNF-4 resulted in a gradual repression of
transcription which reached 75% of the control value at 750 ng of HNF-4 expression plasmid (Fig. 7D).

Effect of Selected Mutations within the Repeats of the Regulatory Element AID on the ApoA-I Promoter Strength and Its Transactivation by HNF-4 and the Ligand-dependent Nuclear Receptors

Cotransfection experiments with HNF-4 and the AIDM1 to AIDM4 mutated promoter constructs showed that the apoA-I promoter strength remained similar in the absence and presence of HNF-4 (Fig. 8A). This indicates that diminished binding of HNF-4 to the regulatory element AID impairs the promoter strength despite the fact that other nuclear receptors may still bind to this element. Similar cotransfection experiments were performed in HepG2 cells using the AIDM2, AIΔREP1, and AIΔREP3 mutated promoter constructs and combinations of plasmids expressing RXRα, RARα, or T3Rβ. This analysis showed that the promoter which lacks repeat 3 (AIΔREP3) behaves in all cases similarly to the wild type promoter (data not shown). The promoter which lacks repeat 1 (AIΔREP1) had 30–35% activity and was not affected by RXRα in the presence or absence of 9-cis-RA. Interestingly the promoter which carries a mutation in the first repeat of element AID (AIDM2) was transactivated 2-fold by RXRα/RARα heterodimers in the presence or absence of any of the two ligands (Fig. 8C). Finally, cotransfection experiments with RXRα/T3Rβ heterodimers showed that the promoter which lacks the first repeat had the same 30–35% activity and was not affected by the RXRα/T3Rβ heterodimers and their ligands. The promoter which carries a mutation in the first repeat of element AID (AIDM2) was transactivated 1.3-fold by RXRα/T3Rβ heterodimers in the absence of any of the two ligands, was transactivated 2.1-fold in the presence of 9-cis-RA, and was not affected by T3 (Fig. 8D). It should be noted that binding of the RXRα/T3Rβ heterodimers to this mutated promoter sequence generates a slow migrating DNA-protein complex (Fig. 2C).

The combined data of Figs. 4, 5, and 8 indicate that binding of RXRα/RARα and RXRα/T3Rβ heterodimers to repeat 2 of element AID is associated with low levels of ligand-independent transcriptional activity whereas binding of homo- or heterodimers of RXRα to repeats 1 and 2 can lead to ligand-dependent activation or repression of transcription.

**DISCUSSION**

The Two Proximal HREs of the Human ApoA-I Promoter Are Essential for Hepatic Expression—The present study has focused on the functional significance of the regulatory elements AID and AIB of apoA-I, and the potential contribution of hormone nuclear receptors to the transcriptional activation of this promoter in hepatic cells. Sequence comparisons showed that the regulatory elements AID and AIB contain sequences with high similarity to an AGG/TTCA motif found on the promoter sites of genes responsive to members of the steroid/thyroid receptor superfamily (15, 16, 18). The HRE present on element
Role of the Hormone Response Elements in ApoA-I Gene Transcription

AID is composed of three putative direct repeats with the sequence A/GGG/TTCA on the noncoding strand, whereas the HRE on element AIB is composed of two putative direct repeats with the sequence A/GGT/ATCA on the noncoding strand. In both cases there is a 1 to 2-nucleotide spacer region between the repeats. Drastic mutagenesis which altered either part of both repeats in the HRE of element AIB or repeat 2 and the adjacent spacer region in the HRE of element AID, eliminated the binding of hepatic activities present in rat liver nuclei and reduced the promoter strength to approximately 5-7% of control. These findings suggest that both HREs are essential for optimal hepatic expression of the apoA-I gene and that the factors which occupy them act synergistically to increase transcription.

The HREs on the Regulatory Elements AID and AIB of Apo-A-I Are the Binding Sites of Orphan and Ligand-dependent Nuclear Receptors: Mutations in the HRE of Element AID Affect Differently the Binding of the Orphan and Ligand-dependent Nuclear Receptors—Previous studies have identified some of the factors occupying the regulatory element AID (3, 4, 6). In the present study we demonstrate that both regulatory elements AIB and AID of apo-A-I are the binding sites of the orphan nuclear receptors HNF-4, ARP-1, EAR-2, and EAR-3, as well as of homodimers of RXRα and heterodimers of RXRα with RARα or T3Rβ. Binding of the RXRα heterodimers was also verified by supershift assays. We did not observe binding of RAR homodimers as suggested previously (8) as well as binding of RARα/T3Rβ heterodimers shown previously to recognize the direct repeat of myosin heavy chain (18) or monomers of homodimers of T3Rβ shown previously to recognize inverted or palindromic repeats (17, 19).

Drastic mutations on the second repeat in the HRE of element AID which eliminated the binding of orphan and ligand-dependent nuclear receptors also diminished the apoA-I promoter strength and its ability to be transactivated or repressed by them. On the other hand, limited mutations in repeat 2 (AIDM3 and AIDM4) which reduced the promoter strength to 30 and 15% of control, respectively, resulted in the diminished binding of liver nuclear extracts and of HNF-4 as well as other orphan receptors and ligand-dependent nuclear receptors to the mutated sites. The findings suggest that an intact repeat 2 in the HRE of element AID is required for optimal hepatic expression of the apoA-I gene. Interestingly, certain mutations which diminished the binding of HNF-4 did not affect considerably the binding of ARP-1, and of EAR-2 and EAR-3 to the mutated probes. ARP-1, EAR-2, and EAR-3 which usually act as repressors, exhibit a wider tissue distribution than HNF-4 but are also expressed, at lower concentrations, in liver and intestinal cells as compared to HNF-4 (20). The promoter mutations which allow preferential binding of ARP-1, EAR-2, and EAR-3 but diminish binding of liver nuclear extracts and HNF-4 decreased substantially the apoA-I promoter strength, thus supporting further the role of HNF-4 as a positive regulator of the hepatic expression of the human apoA-I gene. The mutagenesis analysis also established that the binding RXRα homodimers have a strict requirement for intact repeats 1 and 2 in the HRE of element AID. On the other hand, binding of the RXRα/RARα heterodimers is affected mostly by alterations in repeat 2, and binding of the RXRα/T3Rβ heterodimers is affected by alterations in both repeats 1 and 2 of this HRE.

Interestingly, mutations in repeat 1 (AIDM2) of element AID produced a slower migrating DNA-RXRα/RARα complex, compared to that formed with the wild type or other mutated AID probes. The mobility of this complex is similar to that formed with hepatic nuclear extracts using the same mutated probe. The origin of this complex remains unclear. DNA binding assays with the wild type element AID and 1:1 ratio of COS-1 extracts enriched in RXRα or T3Rβ have provided a tentative explanation for the observed higher mobility DNA-RXRα/T3Rβ
Interestingly, the RXR strand of element AID which does not participate appreciably contrast, the putative repeat 3 of the HRE on the noncoding strand of element AIB are preceded by G and A, respectively. In tide. Similarly repeats 1 and 2 of the HRE on the noncoding noncoding strand of element AID are preceded by a G nucleotide—shown in Fig. 1 containing either an A or a G immediately upstream of the RXR strand. We have found that supplementation of the COS-1 extracts with 9-cis-RA favors the formation of both the higher RXR homodimeric complex and a RXR heterodimeric complex that displays intermediate mobility between the high and the low DNA-RXR/T3Rβ complex. In contrast, supplementation of the COS-1 extract mixed with T3 favors the formation of the faster migrating complex (data not shown). Thus, it is possible that the fast-migrating complex may represent a DNA-RXRα/T3Rβ dimer which is accessible to T3 (21) and the slow-migrating complex may represent a higher order complex between RXRα/T3Rβ and a third protein, and that the formation of this complex is enhanced by the presence of 9-cis-RA (22, 23).

Nucleotides in the HRE of Element AID Which Participate in Protein-DNA Interactions with Homo- and Heterodimers of RXR/α: Spacing Requirements for Binding to AID and Ability of RXRα/RARα and RXRα/T3Rβ Heterodimers to Bind to a Hepaticore Motif—A series of KMNα and dimethyl sulfate interference experiments using normal and mutated AID sequences as probes demonstrated that the RXRα homodimers and the RXRα/RARα and RXRα/T3Rβ heterodimers share almost identical contact points with the direct repeat 1 and 2 in the HRE of element AID. These interactions involve four or five nucleotides of repeat 1, six nucleotides of repeat 2, and one nucleotide in the putative spacer region between repeat 1 and repeat 2. The putative repeat 3 is not involved in binding of the RXRα heterodimers and shows only a weak contact point with the RXRα homodimer. Overall, the RXRα homodimers and the RXRα/RARα heterodimers appear to bind more strongly to the intact HRE of element AID than the RXRα/T3Rβ. The RXRα homodimers have 11 strong and 2 weak DNA-protein contact points, the RXRα/RARα heterodimers have 10 strong and 3 weak DNA-protein contact points and the RXRα/T3Rβ heterodimers have 6 strong and 5 weak DNA-protein contact points.

An important feature of the HREs is the number of nucleotides separating the two repeats (spacer region). It has been proposed that spacing determines the type of homo- or heterodimers of receptors that bind to an HRE (15, 16, 24). It has been suggested that RXR homodimers require direct repeats with a spacing of one nucleotide (DR1) for binding (25–28), RXR/RAR heterodimers can bind to DR1, DR2, or DR5s (28–31), whereas RXR/T3R heterodimers prefer DR4s for binding (16, 32, 33). Nevertheless, exceptions to this rule have been noted (15, 18). Repeats 1 and 2 on element AID of apoA-I have been classified as a DR2, whereas repeats 2 and 3 can be classified as a DR1 (15). Similarly, repeats 1 and 2 of element AIB can be classified as a DR1. As shown in this study, contrary to the rule, the HREs of apoA-I are recognized both by homodimers of RXRα as well as heterodimers of RXRα with RARα or T3Rβ. Furthermore, the nucleotides which participate in the binding of these homo- and heterodimers are nearly identical. It has been shown that binding of homodimeric RXRα is dramatically influenced by the nature of the nucleotide preceding both AGG/TTCA motifs (34). According to the data, RXRα homodimers preferentially interact with direct repeats containing either an A or a G immediately upstream of the AGG/TTCA motif, whereas repeats which contain either a T or a C at the same position have greatly reduced binding (34). As shown in Fig. 1A, both repeat 1 and repeat 2 in the HRE on the noncoding strand of element AID are preceded by a G nucleotide. Similarly repeats 1 and 2 of the HRE on the noncoding strand of element AIB are preceded by G and A, respectively. In contrast, the putative repeat 3 of the HRE on the noncoding strand of element AID which does not participate appreciably in protein-DNA interactions is preceded by a C nucleotide. Interestingly, the RXRα/RARα and RXRα/T3Rβ heterodimers also have the ability to interact with repeat 2 and one or two neighboring nucleotides, suggesting that their minimum requirement for binding to this HRE is a heptameric core recognition motif rather than a hexameric motif, as suggested previously (18). In this latter case, one should not exclude the possibility of extensive nonspecific but nevertheless stabilizing interactions of the heterodimers with the phosphate backbone of the mutated probe (35, 36).

Modulation of ApoA-I Promoter Strength by the Combination of Homo- and Heterodimers of Orphan and Ligand-dependent Nuclear Receptors Occupying the HREs—To ascertain the effects of HNF-4 and RXRα homo- and heterodimers we performed cotransfection titration experiments using different combinations of nuclear receptor plasmids and apoA-I promoter CAT plasmids. The titration experiments were essential in order to establish the minimum concentration of expression vector required for optimal activation or repression of transcription. This approach enabled us to establish 9-cis-RA dependent activation of the apoA-I promoter by RXRα homodimers and T3-dependent repression by RXRα/T3Rβ heterodimers. Optimal activation or repression was achieved at concentrations of expression vector in the range of 100–250 ng. In this range of concentrations, the RXRα/RARα heterodimers and HNF-4 homodimers did not affect significantly the apoA-I promoter strength. At higher concentrations, both the HNF-4 and the RXRα/RARα heterodimers showed a trend toward transcriptional repression. The optimal apoA-I transactivation obtained, in the context of the proximal (−264/+5) apoA-I promoter, by RXRα homodimers was 1.5-fold and the repression by RXRα/T3Rβ heterodimers was 60% of control.

It has been shown previously that the HRE in element AID is a RXR response element (8, 9, 19). This element, when placed in front of a minimal promoter was transactivated 12-fold by RXRα, in the presence of 9-cis-RA in CV-1 cells (19) and 8-fold by all-trans-RA in HepG2 cells (9). The minimal thymidine kinase promoter carrying the HRE of element AID was transactivated 10-fold by the RXRα/RARα heterodimers, in the presence of all-trans-RA, and 25-fold in the presence of 9-cis-RA in CV-1 cells (19). HREs from other promoters linked to the minimal thymidine kinase promoter could also confer transactivation of the heterologous promoter by RXRα/RARα heterodimers, whereas larger promoter constructs containing other HREs could be either activated or repressed by RXRα/RARα heterodimers (19). In general, it has been proposed that the RXR/RAR complexes can function both as repressors or activators of gene expression, depending upon the nature of the direct repeats. Repression was generally observed by DR1s, whereas activation can be observed by certain DR1 as well as by DR2 and DR5 motifs (22, 23, 28, 29). Finally the minimal thymidine kinase promoter under the control of the HRE of element AID was not transactivated by RXR/T3R heterodimers. These heterodimers in the presence of T3 (37) were shown previously to bind mainly to DR4 motifs (38–40) and to activate transcription in the presence of T3.

Our findings show that the extent of transactivation observed using minimal promoters carrying the HRE of element AID are much greater than the extent of transactivation observed by the RXRα homodimers and the RXRα/RARα heterodimers, when this HRE exists in the context of the proximal human apoA-I promoter (−264/+5). Consistent with these findings, a recent study has shown that retinoids can increase 100 to 150% above control the apoA-I promoter activity and by 25 to 30% the apoA-I mRNA and protein in HepG2 cells (41). Similar to other promoter systems we suggest that in the context of the entire apoA-I promoter the nuclear hormone receptors which occupy elements AIB and AID form stereospecific DNA-protein interactions.
Role of the Hormone Response Elements in ApoA-I Gene Transcription

complexes which interact directly or indirectly via the TATA box binding protein associated factors with the proteins of the basal transcription system (42, 43). Other proteins bound to the proximal apoA-I promoter may help to orient properly the nuclear receptor molecules bound to the two sites and optimize their interactions as well as their interactions with the proteins of the basal transcriptional machinery. It is expected that the complex formed with the heterologous promoters which contain the HRE are different than the complexes formed with the intact apoA-I promoter and this may explain the relatively large increase in transactivation observed in the heterologous promoter constructs. We must also point out that the minimal reporter constructs containing the HREs utilized in the previous studies have very low levels of promoter activity. Thus the 8- to 15-fold transactivation achieved by the RXRα homodimers and RXRα/RXRα heterodimers using the heterologous promoters is low compared to the activity of the intact apoA-I promoter. Previous studies have established that the proximal promoter region is sufficient for hepatic transcription both in vivo (5) and in vitro (3, 4), therefore the transactivation levels observed in this study using the proximal apoA-I promoter by homo- and heterodimers of RXRα in hepatic cells, reflect more closely the physiological situation.

Alterations in the HRE of Element AID Affect the Ability of the Homo- and Heterodimers of RXRα to Transactivate the ApoA-I Promoter—Another strong indication that the ability of the RXRα homo- and heterodimers to activate or repress transcription depends on their precise protein-DNA interactions with the intact apoA-I promoter came from transactivation studies of mutated promoters. This analysis showed that the promoter which lacks repeat 3 could be transactivated or repressed by RXRα homo- and heterodimers in the presence of their ligand to the same extent as the wild type promoter. The findings suggest that deletion of repeat 3 did not affect the optimal DNA-protein interactions of these receptor combinations on the apoA-I promoter and their ability to be activated by their ligands. These findings are consistent with the dimethyl sulfate and KMnO4 interference experiments which showed that the putative third repeat of the HRE of element AID participates minimally in DNA-protein interactions only with RXRα homodimers. On the other hand, a mutated promoter which lacks repeat 1 lost its ability to respond to the RXRα homo- and heterodimers in the presence or absence of their ligands. The KMnO4 and dimethyl sulfate interference studies showed that deletion of repeat 1 resulted in association of the RXRα heterodimers with a motif which consists of repeat 2 and one or two neighboring nucleotides. Thus, it is possible that this generalized lack of responsiveness to the different hormonal signals may reflect altered DNA-protein and protein-protein interactions between the nuclear hormone receptors, other factors (22, 23), and/or the proteins of the basal transcription system (44, 45). An interesting pattern of transactivation was observed with the promoter carrying mutations in repeat 1 of element AID (AIDM2). This mutation eliminated binding of the RXRα homodimers but permitted binding of the RXRα/RXRα and RXRα/T3Rβ heterodimers as demonstrated by the DNA binding and interference assays. This promoter was transactivated 2-fold by RXRα and RXRα/RXRα heterodimers, in a ligand-independent fashion. Since RXRα homodimers do not bind to this promoter, most likely the observed transactivation in both cases is mediated by RXRα/RXRα heterodimers formed with the endogenous RXRα present in HepG2 cells or the exogenously added RXRα, respectively. The observed ligand-independent transactivation by the heterodimers may be caused by conformational changes induced upon its binding to the heptamer motif which consists of repeat 2 and one neighboring nucleotide. Such conformational change may be caused by the altered DNA-protein interactions observed by in vitro KMnO4 and dimethyl sulfate studies as well as possible altered interactions with the phosphate groups of the backbone. This may explain the fact that the promoter which lacks repeat 1 (AIDRE1) does not respond to heterodimers of RXRα in the presence or absence of their ligands. The mutated promoter in repeat 1 (AIDM2) was activated by RXRα/T3Rβ heterodimers in the presence of 9-cis-RA but was not affected by T3. The lack of the T3-mediated repression may again reflect conformational changes of the RXRα/T3Rβ heterodimers induced by altered DNA-protein and protein-protein interactions, which may relieve the transcriptional repression. As discussed, binding of the RXRα/T3Rβ heterodimers to a probe carrying mutations in repeat 1 generated a slower migrating DNA-protein complex as compared to the complex formed with the wild type probe.

The present as well as previous studies demonstrate that a large number of transcription factors that belong to the steroid/thyroid receptor superfamily bind to the HREs on the regulatory elements A1B and AID of apoA-I promoter. It is believed that the major role of RXR is to modulate a number of different hormonal signaling pathways through the formation of heterodimers. In addition, RXR has the ability to form homodimers that respond to 9-cis-RA. The dual function of RXR would always be under the tight control of intracellular signals such as the retinoids, thyroid hormone, or other unidentified ligands, that will ensure the activation of one or more specific signaling pathways. Regarding apoA-I gene regulation the present study indicates that increase in the concentration of RXRα and 9-cis-RA will accelerate transcription. On the other hand, at a given RXR concentration, an increase in the concentration of T3Rβ and T3 or an increase in ARP-1, EAR-2, and EAR-3 will repress transcription. Repression in transcription may occur either by sequestering RXR or by displacing the binding of other positive regulators (46). Finally, an increase in the concentration of RXRα and all-trans-RA or of HNF-4 will lead to intermediate levels of transcription. Overall, the specific types of homo- and heterodimers which can occupy the HREs of apoA-I and the extent of transcriptional activation in the cell nucleus may determine the extent of transcriptional activation of the human apoA-I promoter.

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Binding Specificity and Modulation of the ApoA-I Promoter Activity by Homo- and Heterodimers of Nuclear Receptors

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