Transactivation of the Human Apolipoprotein CII Promoter by Orphan and Ligand-dependent Nuclear Receptors

THE REGULATORY ELEMENT CIIC IS A THYROID HORMONE RESPONSE ELEMENT*

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The regulatory elements CIIC (−159/-116) and CIIB (−102/-81) of the apolipoprotein CII (apoCII) promoter have distinct specificities for orphan nuclear receptors (Vorgia, P., Zannis, V. I., and Kardassis, D. (1998) J. Biol. Chem. 273, 4188–4199). In this communication we investigated the contribution of ligand-dependent and orphan nuclear receptors on the transcriptional regulation of the human apoCII gene. It was found that element CIIC in addition to ARP-1 and EAR-2 binds RXRα/T3Rβ heterodimers strongly, whereas element CIIB binds hepatic nuclear factor 4 (HNF-4) exclusively. Binding is abolished by mutations that alter the HRE binding motifs.

Transient cotransfection experiments showed that in the presence of T3, RXRα/T3Rβ heterodimers transactivated the −205/+18 apoCII promoter 1.6- and 11-fold in HepG2 and COS-1 respectively. No transactivation was observed in the presence of 9-cis-retinoic acid. Transactivation requires the regulatory element CIIC, suggesting that this element contains a thyroid hormone response element. HNF-4 did not affect the apoCII promoter activity in HepG2 cells. However, mutations in the HNF-4 binding site on element CIIB and inhibition of HNF-4 synthesis in HepG2 cells by antisense HNF-4 constructs decreased the apoCII promoter activity to 25–40% of the control, indicating that HNF-4 is a positive regulator of the apoCII gene. ARP-1 repressed the −205/+18 apoCII promoter activity in HepG2 cells, indicating that the repression depends on the regulatory element CIIC. In contrast, combination of ARP-1 and HNF-4 transactivated different apoCII promoter segments as well as a minimal adenovirus major late promoter driven by the regulatory element CIIB. Mutagenesis or deletion of elements CIIB or CIIC established that the observed transactivation requires DNA binding of one of the two factors and may result from HNF-4-ARP-1 interactions that elicit the transactivation functions of HNF-4.

The combined data indicate that RXRα/T3Rβ in the presence of T3 and HNF-4 can upregulate the apoCII promoter activity by binding to the regulatory elements CIIC and CIIB, respectively. In addition, ARP-1 can either have inhibitory or stimulatory effects on the apoCII promoter activity via different mechanisms.

Plasma apolipoprotein CII (apoCII) is a potent activator of the lipoprotein lipase, has known protein and gene sequence, and plays an important role in the catabolism of triglyceride-rich lipoproteins (1–8).

We have shown recently that the 0.55-kilobase intergenic region between the apoCII and apoCIV genes is a strong cell type-specific promoter, and its activity is enhanced by hepatic control region 1 (9, 10). The apoCII promoter contains five footprints defined by hepatic nuclear extracts and designated CII-A (−74/~44), CII-B (−102/~81), CII-C (−159/~116), CII-D (−288/~265), and CII-E (−497/~462). An important role in apoCII gene regulation and transcriptional enhancement is mediated by two hormone response elements, which map within the footprinted regions CIIB (−102/~81) and CIIC (−159/~116) and have different specificities for orphan nuclear receptors. CIIC is recognized by ARP-1, EAR-2, but not HNF-4, whereas CIIB is recognized exclusively by HNF-4 (10).

Orphan nuclear receptors as well as receptors for retinoids and thyroids are members of a nuclear receptor superfamily that controls diverse biological functions including growth, development, and homeostasis (11–15). They recognize specific hexameric AG(G/T)TCA motifs with variations in sequence, spacing, and orientation, designated hormone response elements (HREs) (16–20). In the current study, we demonstrate that in the presence of T3, RXRα/T3Rβ heterodimers bind to a thyroid hormone response element (TRE) present in element CIIC and transactivate the human apoCII promoter. Binding of ARP-1 to the same site repressed the promoter activity. Furthermore, antisense methodologies and promoter mutagenesis established that HNF-4 is a positive activator required for optimal activity of the apoCII promoter in HepG2 cells. Finally, combination of ARP-1 and HNF-4 superactivate the apoCII promoter via novel mechanisms that may require interaction of the two factors on the apoCII promoter.

EXPERIMENTAL PROCEDURES

Materials—The sources of materials utilized have been described (10).

Plasmid Constructions—The construction of the apoCII promoter plasmids −545/+18 CII CAT, −388/+18 CII CAT, −388/+18 CII Bmut CAT, −388/+18 CII C/B mut CAT, −205/+18 CII CAT, −104/+18 CII CAT as well as the pUCSHCAT vector have been described previously (10, 21). Plasmids pMT2, pMT2-

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† The abbreviations used are: apoCII, apolipoprotein CII; apoCIV, apoCII pseudogene; HRE, hormone response element; TRE, thyroid hormone response element; hHNF-4, human hepatic nuclear factor 4; hEAR-2, human v-erbA-related factor 2; hARP-1, human apoA-I regulatory protein 1; CAT, chloramphenicol acetyltransferase; AdML, adenovirus major late promoter; hRXRα, human retinoid X receptor α; hT3Rβ, human thyroid hormone receptor β; hRARα, human retinoic acid receptor α; hPPARα, human peroxisome proliferator-activated receptor α; T3, triiodothyronine; CREB, cAMP response element binding protein.
Oligonucleotides used in polymerase chain reaction-based mutagenesis and in the DNA binding assays

| Primer   | Sequence                                           | Location                  |
|----------|----------------------------------------------------|---------------------------|
| CIIB     | 5′-AGCTTCTGGGGCGAGGCTTCCGA-3′                     | apoCII – 104/–83 coding   |
| CIIB mutant | 5′-AGCTTCTGGGGCGAGGCTTCCGAAGAAGT-3′             | apoCII – 104/–83 coding   |
| CIIC     | 5′-AGCTTCTGGGGCGAGGCTTCCGA-3′                     | apoCII – 161/–117 coding  |
| CIIC mutant | 5′-AGCTTCTGGGGCGAGGCTTCCGAAGAAGT-3′             | apoCII – 161/–117 coding  |
| HNF-4 RI | 5′-AGACAGCTCTGAGGCTTCCGA-3′                       | HNF-4 - 402/427 coding    |
| HNF-4 Xh | 5′-AGCTGAGGCGAGGATCTGACGTTGGTTCCCA-3′            | contains an EcoRI site    |
| Rev-5–26 | 5′-TCACAGAGAAGACAGCTATGACATGC-3′                  | HNF-4 - 1215/1290 noncoding: contains a XhoI site |
| CAT      | 5′-AGCATTTCCTGAGGCTTCCGA-3′                       | pUC                        |
|          |                                                    | CAT                        |

hHNF-4, pMT2-hARP-1, pMT2-hEAR-2, pMT2-hRXRα, pMT2-hRARα, pMT2-hT3Rβ, and pMT2-hPPARα were described previously (22, 23). The plasmid –205/+18 CII Cmut CAT was constructed as follows. The –388/+18 CII Cmut plasmid was digested by EcoRI, and the excised fragment corresponding to the apoCII promoter was transferred to the pMT2-hT3Rα, pMT2-hRARα, pMT2-hRXRα, and pMT2-hPPARα (22, 23). Eighteen h later, cells were fed with fresh medium and cultured for an additional 48 h. For the preparation of whole cell extracts, cells were washed 2× with phosphate-buffered saline and scraped into 1 ml of TEN buffer (40 mM Tris, pH 7.9, 1 mM EDTA, 150 mM NaCl). Cells were pelleted by low speed centrifugation, resuspended in 200 μl of WCE lysis buffer (500 mM KCl, 50 mM Heps, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 100 μM phenylmethysulfonyl fluoride, 10% glycerol), and lysed by three cycles of freeze-thawing. Undissolved material was removed by high speed centrifugation at 4 °C, and the supernatant was aliquoted and stored at –80 °C until use. The concentration of the proteins in the whole cell extracts was approximately 0.5–1 μg/ml.

**Transactivation Of The apoCII Promoter**

**RESULTS**

**Heterodimers of RXRα/T3Rβ Bind to a TRE on Element CIIC and Transactivate the Human apoCII Promoter in HepG2 and COS-1 Cells—DNA binding experiments were performed using oligonucleotide CIIC as a probe (Table I) and extracts from COS-1 cells expressing RXRα, RXRβ, T3Rβ, and PPARα. This analysis showed that element CIIC strongly binds RXRα/T3Rβ, less efficiently binds T3Rβ, and weakly binds RXRα/RXRα and RXRα/PPARα heterodimers (Fig. 1A–D). Element CIIC contains two direct repeats 5′ AGGGTC(A/G)AGGTTCA 3′ in the noncoding strand between nucleotides –140 to –155 separated by four spacer nucleotides (included in parentheses) (Fig. 1A). Mutations in both half repeats of element CIIC abolished the binding of orphan and ligand-dependent nuclear receptors to this site (Fig. 1A–D). Cotransfection experiments in HepG2 cells using the –205/+18 apoCII promoter mutated in element CIIC showed that this mutation reduced the apoCII promoter activity to approximately 40% of the control value (Fig. 2A).

Similar cotransfection experiments in HepG2 cells showed that the activity of the –205/+18 apoCII promoter was repressed by approximately 50% by RXRα/T3Rβ heterodimers in the absence of ligand (Fig. 2A). The promoter activity increased 3-fold by the addition of 10−7 M T3 and was 1.6-fold higher than the activity observed in the absence of both RXRα/T3Rβ heterodimers and T3 (Fig. 2A). The presence of T3, RXRα/T3Rβ heterodimers also transactivated a synthetic promoter containing a single copy of the element CIIC fused to the minimal AdML promoter 3-fold (Fig. 2B). A synthetic promoter under the control of a mutated version of the TRE found in the growth hormone promoter was transactivated 24.5-fold (Fig. 2B). This promoter is directed by an ideal DB1 direct repeat AGGGTCAGGTTCA motif and could be transactivated 21-fold by RXRα/T3Rβ heterodimers in the presence of T3 (28).
Fig. 1. Binding of hormone nuclear receptors to the regulatory element CIIC and the effect of mutations on receptor binding. Panel A, schematic representation of the apoCII promoter region relative to the apoEapoCI/apoCI/apoCIVapoCI gene cluster and the hepatic control region-1 and hepatic control region 2 on human chromosome 19 (Chr. 19) (7–9). Double arrows show the intergenic distances in kilobases. The lower part of the panel shows the position of the footprinted regions CIIA to CIIE as well as the mutations within the HREs of elements CIIB and CIIC, which affect the promoter strength and the binding of nuclear receptors. Panels B–D. DNA binding gel electrophoretic mobility shift assays using wild-type and mutated oligonucleotides CIIC (−151/−116) as probes and rat liver nuclear extracts or extracts of COS-1 cells expressing ARP-1, EAR-2, RXRβ, T3Rβ, and PPARα. A double-stranded oligonucleotide corresponding to the wild-type and mutated apoCII footprint CIIC shown in Table I was labeled at both ends with the Klenow fragment of the DNA polymerase I and [32P]dCTP and incubated with 4 μg of rat liver nuclear extracts or 1 μl of COS-1 extracts expressing nuclear receptors as indicated at the top of the figure, analyzed by native polyacrylamide gels and detected by autoradiography. Note strong binding of hRXRα/hT3Rβ heterodimers, moderate binding of T3Rβ, and weak binding of RXRα/RARα and RXRα/PPARα heterodimers (Panel B). Also note the loss of binding of orphan and ligand-dependent hormone nuclear receptors by mutations within the HRE of element CIIC (Panels C and D). kb, kilobases.

Fig. 2. Transactivation of the −205/+18 promoter and the CIIC AdML promoter in HepG2 or COS-1 cells. Panel A, HepG2 cells were transiently cotransfected with 3 μg of the −205/+18 CII CAT plasmids along with 2 μg of phosphoglycerate kinase-β-Gal plasmid and 1 μg of the indicated pMT2 hormone nuclear receptor vectors or the expression vector pMT2 alone. Forty h after transfection, cells were harvested, and the CAT activity was determined. The mean values (±S.E.) from at least two independent transfections performed in duplicate are presented in the form of bar graphs. Panel B, HepG2 cells were transiently cotransfected with 3 μg of the pCIIC AdML plasmid or the control growth hormone-TRE (GH-TRE) plasmid along with 2 μg of phosphoglycerate kinase-β-Gal plasmid and 1 μg of the indicated pMT2-hormone nuclear receptor plasmid or the expression vector pMT2 alone. Forty h after transfection, cells were harvested, and CAT activity was determined. The mean values (±S.E.) from at least two independent transfections performed in duplicate are presented in the form of bar graphs. Panel C, COS-1 cells were transiently cotransfected with 3 μg of the −250/+18 apoCII promoter along with 2 μg of phosphoglycerate kinase-β-Gal and 1 μg of the indicated pMT2 hormone nuclear receptor plasmid or the expression vector pMT2 alone. Forty h after transfection, cells were harvested, and CAT activity was determined. The mean values (±S.E.) from at least two independent transfections performed in duplicate are presented in the form of bar graphs.

The −205/+18 apoCII promoter was also transactivated 4-fold by RXRα/T3Rβ heterodimers in the presence of T3 in COS-1 cells (Fig. 2C). The promoter activity was unaffected by RXRα/T3Rβ heterodimers in the absence of T3 and by T3Rβ in the presence of T3. T3Rβ in the absence of T3 or RXRα/T3Rβ heterodimers in the presence of 9-cis-retinoic acid reduced the apoCII promoter activity in COS-1 cells by approximately 50%. The combined data of Figs. 1A–D and 2A–C indicate that the element CIIC is a functional TRE that confers T3-dependent transactivation of the apoCII promoter by RXRα/T3Rβ heterodimers.

HNF-4 Binds Exclusively to the Regulatory Element CIIB and Is Required for Optimal Activity of the apoCII Promoter in Cells of Hepatic Origin—A previous study showed that the regulatory element CIIB binds HNF-4 but not ARP-1 or EAR-2 (10). This element contains a direct AAGTCCTGGCCA repeat between nucleotides −87 to −98 of the noncoding strand without spacer nucleotides between the two half repeats (DR0). Mutations within the two half repeats (Fig. 1A) abolish the binding of HNF-4 to this site (Fig. 3A). DNA binding experiments using the oligonucleotide CIIB as probe (Table I) and extracts from COS-1 cells expressing different hormone nuclear receptors showed that homodimers of RXRα and heterodimers of RXRα with RAR, T3Rβ, and PPARα do not bind to this element, indicating that the HRE of element CIIB is an exclusive HNF-4 binding site (Fig. 3A).

We have shown recently that HNF-4 does not increase the apoCII promoter strength in HepG2 cells (10). On the other hand, mutations in element CIIB that abolish the binding of HNF-4 to this site reduced the apoCII promoter activity by 60% of the control (Fig. 3B). To assess the role and the importance of HNF-4 for the function of the apoCII promoter, we generated
cell lines expressing two antisense HNF-4 constructs or the empty vector. One of the constructs expresses the 402 to 1215 HNF-4 antisense sequence, whereas the other expresses the same sequence fused to the catalytic domain of the hammerhead ribozyme (29). Cotransfection experiments using the parental HepG2 and the stable HepG2 cell lines expressing the antisense constructs showed that the activity of the −545/+18 promoter decreased by approximately 60–75% of the control in HepG2 cells expressing the two antisense HNF-4 sequences. The activity of the Rous sarcoma virus promoter, which does not contain the HNF-4 binding site, was not affected. The activity of the promoter in HepG2 cell lines expressing the antisense constructs could be restored to the same level as the control by cotransfection with an HNF-4 expression vector (data not shown). The findings indicate that HNF-4 is an important regulator of the apoCII promoter activity and that its concentration is limited in the cell lines expressing the antisense HNF-4 constructs.

**ARP-1 Can Repress the apoCII Promoter Activity by Binding to the Regulatory Element CIIC.** Combination of ARP-1 and HNF-4 Superactivates the apoCII Promoter—Cotransfection of HepG2 cells with the −205/+18 apoCII promoter CAT construct along with an ARP-1 expression vector repressed the apoCII promoter activity by 50%. Repression could be reversed by cotransfection with HNF-4 (Fig. 4A). This result indicated that ARP-1 plays a negative role in apoCII gene regulation. ARP-1 was unable to repress the activity of the −104/+18 apoCII promoter, which lacks element CIIC in HepG2 cells, indicating that repression depends on the presence of the regulatory element CIIC (Fig. 4B). Unexpectedly, cotransfection of HepG2 cells with ARP-1 and HNF-4 transactivated the −205/+18 or the −104/+18 apoCII promoter 4.7- and 2.2-fold respectively, despite the fact that the activity of these promoters in HepG2 cells is not affected by HNF-4 (10) (Fig. 4A and B). In addition, the combination of ARP-1 and HNF-4 transactivated the synthetic CIIB-AdML promoter, which contains a single copy of element CIIB, whereas HNF-4 had no effect, and ARP-1 increased 2-fold the activity of this promoter (Fig. 4C). Transactivation occurs despite the fact that ARP-1 cannot bind to either the −104/+18 apoCII promoter or the CIIB AdML promoter. The findings suggest that the observed transactivation may be the result of direct protein-protein interactions, which increase the transactivation potential of one or both of these factors. The possibility that ARP-1 squelches negative regulators is less likely since element CIIB is an exclusive HNF-4 binding site. In addition, interactions of negative regulators such as ARP-1 with components of the basal transcription complex are expected to exert negative rather than positive effects on transcription (30). The positive effect of ARP-1 on the apoCII promoter activity was further demonstrated by cotransfection experiments in COS-1 cells that lack or contain very low amounts of endogenous HNF-4 and ARP-1. This analysis showed that HNF-4 transactivated the −104/+18 apoCII promoter 4-fold. The same promoter, in the presence of increasing concentrations of ARP-1, was transactivated up to 15-fold (Fig. 4D). Transactivation of the −205/+18 apoCII promoter by the combination of ARP-1 and HNF-4 also occurred when the regulatory element CIIB, which is the binding site of HNF-4, was mutated (Fig. 4E). The combined data of Fig. 4, D and E indicate putative HNF-4-ARP-1 interactions on the apoCII promoter when either one of the two factors is bound to the DNA. A schematic representation of the putative mechanisms of activation or repression of the apoCII promoter activity by HNF-4, ARP-1, and RXRα/T3Rβ heterodimers is shown in Fig. 5.

**DISCUSSION**

*The Regulatory Element CIIC Is a TRE—Thyroid hormone receptors recognize specific hexameric half repeat motifs AGi(G/T)C/TCA, preferably separated by four spacer nucleotides (16–20). The regulatory element CIIC, which is recognized by ARP-1 and EAR-2, contains two direct repeats with DR4 spacing on the noncoding strand between nucleotides −140 to −155. Previous studies have suggested that this motif is the preferred binding site of RXRα/T3Rβ heterodimers. Indeed, the DNA binding data confirmed that T3Rβ/RXRα heterodimers bind very strongly to this element. T3Rβ binds less efficiently, and homodimers of RXRα or heterodimers of RXRα with RARe and PPAR bind weakly, thus establishing that element CIIC contains a TRE. The functionality of this TRE was established with cotransfection experiments involving homologous and heterologous*
promoters. Ligand-dependent transactivation by RXRα/T3Rβ heterodimers was achieved with the wild-type −205/+18 apoCII promoter as well as with a synthetic AdML promoter under the control of the wild-type CIIIC TRE. Mutations in the TRE that prevented the binding of the nuclear receptors to this site abolished the transactivation. The transactivation achieved by the TRE in the context of the −205/+18 apoCII promoter and the minimal AdML promoter in HepG2 cells was 1.7- and 3-fold respectively. This 3-fold transactivation of the synthetic promoter by the CIIIC TRE is comparable with the 3.6-fold transactivation by RXRα/T3Rβ heterodimers of a synthetic promoter under the control of the growth hormone TRE. Similar to the CIIIC element, the growth hormone TRE contains a direct AGGTAA(GATC)AGGGAC repeat with DR4 spacing (28). Much greater promoter transactivations can be achieved by an ideal version of this element, which contains (AGGT(A/C)A)2 DR4 repeats (28). In contrast, in the absence of T3, the apoCII promoter activity was repressed. The 60% repression in apoCII promoter activity by the RXRα/T3Rβ heterodimer in the absence of T3 is in agreement with the recently proposed model for their mode of action. According to the proposed model, in the absence of ligand, the RXR/T3R heterodimers bind transcriptional co-repressors such as nuclear repressor corepressor Sin3 and N-CoR, which results in histone deacetylation and condensation of chromatin (32, 33). This leads to a repression of transcription. Binding of ligand to the heterodimer results in the displacement of the repressors by activators containing acetylase activity such as p300/CREB binding protein and P/CREB binding protein-associated factor. This leads to acetylation of histones, activation of chromatin, and gene transcrip-
Transactivation Of The apoCII Promoter

HNF-4 Is an Important Activator of the apoCII Promoter—The DNA binding data of this and a previous study (10) established that the HRE present in element CIIB recognizes HNF-4 exclusively but does not recognize other orphan or ligand-dependent nuclear receptors that bind to the regulatory element CIIC (Fig. 3A). The importance of HNF-4 for the function of the apoCII promoter could not be assessed by cotransfection experiments, possibly due to the saturating amounts of HNF-4 in HepG2 cells (10). In the current study, utilization of antisense methodologies very convincingly established that HNF-4 is an important activator of the apoCII promoter. It is interesting that both the mutagenesis of the DNA recognition motif of HNF-4 as well as the antisense HNF-4 constructs reduced the apoCII promoter activity by approximately 60 to 75% of the control. The finding suggests that HNF-4 contributes to optimal promoter strength, but other factors, such as CCAAT enhancer binding protein C/EBP or related activities that bind to element CIID, may account for the remaining 25 to 40% of the promoter activity in the absence of HNF-4. The fact that HNF-4 is an activator of the apoCII promoter strength is also supported by cotransfection experiments in COS-1 cells, where the promoter is transactivated 9-fold in the presence of HNF-4 (10). The preservation of partial promoter activity in the absence of HNF-4 also differentiates the apoCII promoter from the promoters of the apoA-I, CIII, and AIV gene cluster, where intact HREs are essential for promoter activity (23, 34).

ARP-1 May either Repress or Transactivate the apoCII Promoter Activity. Repression Requires Binding of ARP-1 to the TRE of Element CIIC. Transactivation Requires the Presence of HNF-4 and Is Independent of DNA Binding—The regulatory element CIIC that contains the TRE is required for optimal promoter activity, since mutagenesis of the TRE that abolishes binding of hormone nuclear receptors to this site reduces the apoCII promoter activity to approximately 40% of the control (Fig. 2A). On the other hand, this element is the binding site of orphan nuclear receptors ARP-1 and EAR-2. Previous studies have shown that ARP-1 and EAR-2 can usually but not always (36, 37) repress the promoter activity of other genes by competing for binding to the same HRE (38–43). In the case of RXRα and T3Rβ, transrepression may also be involved (30). The DNA binding data, in combination with the transactivation data of this study, established that the transcriptional repression is caused by the binding of ARP-1 to the regulatory element CIIC, which in addition to orphan nuclear receptors, strongly binds RXRα/T3Rβ heterodimers. In the absence of this element, the activity of the apoCII promoter is not affected by ARP-1.

It has been proposed that transcription factors bound to distal regulatory elements form a stereospecific DNA protein complex (44). This complex may interact directly or indirectly through the TATA box-binding protein-associated factors or transcriptional mediators/intermediary factors (45–48) with the factors of the basal transcription complex, thus leading to the transcriptional activation or repression of the target gene (Figs. 4 and 5). In the case of ARP-1, it has been suggested that its interactions with the basal transcription factor TFIIIB may freeze the pre-initiation complex in an inactive configuration and may account for the repressor activity of this factor (30). The repression of the −205/+18 apoCII promoter activity is reversed in the presence of excess HNF-4. Since ARP-1 and HNF-4 bind on distinct HREs on elements CIIC and CIIB, respectively (Figs. 1 and 3), reversion of the repression could be the result of favorable protein-protein interactions involving the two factors. Additional indirect evidence of this type of interaction was provided by the observation that the −104/+18 apoCII promoter, which lacks element CIIC, or a synthetic promoter, which contains a single copy of element CIIB, is greatly transactivated by combination of ARP-1 and HNF-4. Since these promoters cannot bind ARP-1 and ARP-1 cannot form heterodimers with HNF-4 (49), the observed potentiation of the transcriptional activity of HNF-4 could result from transient interactions of HNF-4 with ARP-1 on the apoCII promoter. Evidence in support of this type of interaction was obtained in recent studies involving the HNF-1 promoter. This promoter, which contains an exclusive HNF-4 binding site, could likewise be transactivated by combination of ARP-1 and HNF-4 (49). Direct interactions between chicken ovalbumin upstream promoter transcription factors I and II (EAR-3 and ARP-1) were demonstrated by in vitro protein-protein interaction experiments involving glutathione S-transferase fusion proteins (49). In addition, cell culture studies showed that truncated HNF-4 forms that lack the nuclear localization domain can be transported into the nucleus of COS-1 cells by cotransfection with ARP-1 or EAR-3 (49). The direct protein-protein interactions between these two factors involve residues 227 to 271 of HNF-4, and the formation of a functionally active complex between these two factors requires an intact activation domain (residues 130–368) of HNF-4 (49). Deletion of residues 354–368 of HNF-4 located within a region homologous to the activation function-2 domain found in other hormone receptors (50–52) abolished the synergistic activation of the target promoter by HNF-4 and ARP-1 or EAR-3 (49). Earlier studies established that the transcriptional activity of hormone nuclear receptors is modulated by direct protein–protein interactions involving transcription intermediary factors, which may act either as activators or repressors (45, 51–56). It has been proposed that association of ARP-1 or EAR-3 with HNF-4 bound to its cognate site on the promoters alters the conformation or the activation function-2 of HNF-4 or it may facilitate the formation of the pre-initiation complex (49).

The present study as well as previous studies have shown convincingly that HNF-4 is a positive transcriptional activator of a number of liver specific genes (34, 38, 43). HNF-4 can also synergize with a variety of other transcription factors such as C/ enhancer binding protein (57), c-AMP response element-binding protein (58), and HNF-1 (39) bound to their target sites. The current study shows that in promoters such as the apoCII, which contain exclusive ARP-1 binding sites, HNF-4 may also play the role of a transcriptional mediator. This HNF-4 function is the topic of ongoing research and may likewise involve interactions with ARP-1 dimers bound to DNA. Such interactions could allow utilization of the activation domain of HNF-4 bound to ARP-1 to drive transcription. Overall, the current study demonstrates that orphan as well as ligand-dependent nuclear receptors can modulate the apoCII gene transcription positively or negatively via different mechanisms. Thus different combinations of hormone nuclear receptors in hepatic cells and the availability of the ligands may affect the overall apoCII synthesis and plasma levels of apoCII and thus affect the catabolism of triglyceride-rich lipoproteins.
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