Estrogen Regulation of the Apolipoprotein AI Gene Promoter through Transcription Cofactor Sharing*

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Estrogen replacement therapy increases plasma concentrations of high density lipoprotein and its major protein constituent, apolipoprotein AI (apoAI). Studies with animal model systems, however, suggest opposite effects. In HepG2 cells stably expressing estrogen receptor α (ERα), 17β-estradiol (E2) potently inhibited apoAI mRNA steady state levels. ApoAI promoter deletion mapping experiments indicated that ERα plus E2 inhibited apoAI activity through the liver-specific enhancer. Although the ERα DNA binding domain was essential but not sufficient for apoAI enhancer inhibition, ERα binding to the apoAI enhancer could not be detected by electrophoretic mobility shift assays. Western blotting and cotransfection assays showed that ERα plus E2 did not influence the abundance or the activity of the hepatocyte-enriched factors HNF-3β and HNF-4, two transcription factors essential for apoAI enhancer function. Expression of the ERα coactivator RIP140 dramatically repressed apoAI enhancer function in cotransfection experiments, suggesting that RIP140 may also function as a coactivator on the apoAI enhancer. Moreover, estrogen regulation of apoAI enhancer activity was dependent upon the balance between ERα and RIP140 levels. At low ratios of RIP140 to ERα, E2 repressed apoAI enhancer activity, whereas at high ratios this repression was reversed. Regulation of the apoAI gene by estrogen may thus vary in direction and magnitude depending not only on the presence of ERα and E2 but also upon the intracellular balance of ERα and coactivators utilized by ERα and the apoAI enhancer.

Apolipoprotein AI (apoAI) is the major protein constituent of plasma high density lipoproteins (HDLs), a class of lipoproteins thought to play a major role in protection against atherosclerosis (reviewed in Ref. 1). Because plasma HDL levels are correlated with plasma apoAI and liver apoAI mRNA levels (2), it is thought that factors affecting apoAI gene expression play an important role in atherosclerosis susceptibility. Although a number of pharmacological, dietary, and physiological factors affect apoAI and HDL plasma levels (3–8), the underlying molecular mechanisms remain obscure. For example, numerous observational studies and a recent randomized trial have shown that estrogen replacement therapy increases apoAI and HDL plasma levels (9–11). However, the underlying molecular mechanism(s) remains controversial. Both increased production of apoAI (12, 13) and reduced HDL catabolism (14) have been suggested as potential mechanisms.

Work with animals has further complicated the issue. In cynomolgus monkeys, ethinyl estradiol or conjugated equine estrogen markedly reduces apoAI and HDL plasma levels (15, 16). Similarly, ovariectomy increases hepatic apoAI mRNA levels in rats, further supporting the concept that estrogen may repress apoAI gene expression (17). Moreover, the ethinyl estradiol-induced increases in apoAI mRNA levels in these animals appears to occur via indirect dietary effects due to hormone treatment (17). Further, estrogen-induced increases in apoAI transcription rates in rats are dependent on the strains used (6).

Liver-specific expression of the apoAI gene is conferred by a powerful hepatocyte-specific enhancer located in the nucleotide region −220 to −110 upstream of the apoAI transcriptional start site (18). The activity of the enhancer depends on synergistic interactions between transcription factors bound to three distinct sites: A (−214 to −192), B (−169 to −146), and C (−134 to −119) within the enhancer (18, 19). Sites A and C bind various members of the nuclear receptor superfamily including the hepatocyte nuclear factor 4 (HNF-4) (20–22), retinoid X receptor α (23), and apolipoprotein AI regulatory protein-1 (24, 25). Site B binds members of the hepatocyte nuclear factor 3 family, HNF-3 (19, 26). Synergy between these factors during enhancer activation appears to involve interactions with uncharacterized transcription auxiliary factors (18, 26). Recent evidence suggests that one or more of these factors are regulated by estrogen in heterologous nonhepatic cells (26).

The actions of estrogen are mediated primarily by the estrogen receptors (ERs) α (27) and β (28, 29); however, only ERα is expressed in the liver (28, 29). Estrogen signal transduction involves high affinity binding to intracellular ERs, ligand-induced conformational changes of ERs leading to the recruitment of transcriptional auxiliary factors, binding of ERs to estrogen response elements (EREs) in gene promoters, and regulation of transcriptional activity in conjunction with other transcription factors bound to their cognate sites in the promoter. Recent efforts to characterize ER transcription auxiliary factors have led to the identification of a growing number of coactivators such as Trip/Sug1 (30, 31), ERAP140 and ERAP160 (32), RIP140 (33), TIF1 (34), and SRC-1 (35, 36). Although all of these proteins bind ERα in a ligand-dependent fashion, the mechanisms by which they modulate ER signaling and “cross-talk” with other signal transduction pathways is not understood. Recent findings indicate that the related coactivators p300 (37) and CBP (38) are also involved in ERα function and serve as a signal integrator for several hormone-dependent...
and hormone-independent signal transduction pathways (Refs. 36, 39, and 40 and reviewed in Ref. 41). Additional pathways of liganded ER action involve recruitment of ERα to gene promoters lacking EREs via protein-protein interactions with promoter-bound transcription factors (42–45) and activation of the mitogen-activated protein kinase pathway (46, 47).

This report shows that ERα and 17β-estradiol repress apoAI promoter activity in human hepatoma HepG2 cells. This effect appears to be due to ERα partitioning of coactivators required for apoAI enhancer function in liver cells. The data suggest that RIP140 may play an important role in apoAI enhancer function and ERα-mediated repression. We propose that estrogen effects on apoAI gene expression vary in direction and magnitude depending upon the balance of coactivators shared by ERα and the apoAI enhancer.

MATERIALS AND METHODS

Plasmid Constructions—The −2500AI.LUC.CIIIAIV construct was created by transferring a 3.0-kilobase HindIII 5′ apoAI DNA fragment and a 7-kilobase BamHI 3′ apoAI fragment from the previously reported construct −2500ALUC(CIIIAIV) (21) into their respective sites in pGL2-Basic (Promega). The −2500AI.LUC construct was created by transferring only the 3.0-kilobase HindIII fragment into pGL2-Basic. The −256AI.LUC, −220/−110AI.LUC, and −41/−397LUC constructs were created by transferring their respective HindIII fragments from −256AI.CAT, −222Δ−110/−41AI.CAT, and −41AI.CAT, respectively (18), into pGL2-Basic. Construct −220/−110ABC.LUC was generated by transferring an 110-base pair BamHI fragment from −222Δ−110/−41AI.CAT (18) into −41LUC (19). The apoAI-enhancer-type mutants were created by transferring the approximately 110-base pair BamHI fragments from the corresponding chloramphenicol acetyltransferase (CAT) constructs (18) into −41LUC (19). Construct TK.LUC was generated by cloning a −105/+10 NheI/HindIII TK promoter fragment into pGL2-Basic. Construct −220/−110ABCTK.LUC was generated by transferring the 110-base pair BamHI DNA fragment from −222Δ−110/−41ALCAT (18) into TKLUC. The A.LUC, B.LUC, and ERR.LUC construct were described previously (19, 48).

The pMT2-ERα construct was created by transferring an EcoRI fragment containing the coding region of ERα from the HEO plasmid (49) into the pMT2 expression vector. The ERα mutant expression vectors AP1-DBD-X, X-DBD-AF2, and X-DBD-X were generated in pCDNA3 (Invitrogen) as described previously (50). The ERα expression vector was created by introducing three nucleotide substitutions within the ERα DNA binding domain (DBD) (51), and the pCDNA3-ERα expression vector was described previously (48).

Stable Cell Line Creation—HepG2 cells stably expressing ERα (Hep89) were created by transfecting the pCDNA3-ERα expression vector into HepG2 cells by electroporation using the BTX Electro Cell Manipulator 600 according to the manufacturer’s recommended settings. Stably expressing cells were selected by resistance to G418 (400 μg/ml). Distinct, well isolated colonies were picked using Bellco cloning cylinders (6 × 8 mm) and assessed for the presence of ERα.

Cell Transfections—Plasmid DNAs were purified on Qiagen columns and transfected into HepG2 cells by the calcium phosphate coprecipitation method as described previously (26). The cells were seeded in deficient growth media (phenol red-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with heat-inactivated 10% fetal bovine serum, 1% Glutamax, 1% minimum essential medium nonessential amino acids, 100 units/ml penicillin and 100 μg/ml streptomycin) at 2.5 × 10⁶ cells/well in a 12-well dish (Falcon) before transfection. Different amounts of the expression vectors pMT2-ERα, pMT2-HNF4-A2, pMT2-HNF4-Δ2, pCMV.HNF-3β (53), pEFPRII (53), or ECV-CBP-HA (38) were cotransfected as indicated. Luciferase and β-galactosidase activity was determined as described previously (26). The data shown represent the mean ± S.E. from at least three independent experiments, each in duplicate. Statistical analysis of the data was carried out using the Dunnett’s method (54) to compare treated versus control samples.

Northern Analysis—HepG2 and Hep89 cells were seeded in deficient growth media and treated over 72 h in the presence or absence of 1 μM E2. Total RNA was isolated (Biotex Labs), subjected to electrophoresis, and hybridized with 32P-labeled apoAI Pet1 DNA fragment (55) or 32P-labeled human glyceraldehyde-3-phosphate dehydrogenase cDNA (Stratagene). The relative intensities of the hybridized signals were quantitated by phosphoimaging (Molecular Dynamics).

Electrophoretic Mobility Shift Assays—Protein-DNA complexes were analyzed by incubation of bacterially expressed HNF-4, HepG2 nuclear extracts (26), or baculovirus-expressed human ERα (Panvera) with 32P-labeled DNA probes corresponding to either the 110-base pair apoAI enhancer or the vitellogenin ERE followed by electrophoresis in low ionic strength polyacrylamide gels as described previously (26).

Western Blot Experiments—HepG2 cells stably expressing ERα were prepared as described previously (26). Protein concentrations were determined by the BCA method. Proteins were transferred from a 4% SDS-polyacrylamide gel to nitrocellulose and blotted using affinity-purified human monoclonal ER antibody (Stress Gen), rabbit anti-HNF-3β antibody (gift of R. Costa), or rabbit anti-HNF4 serum (from F. Sladek) as the primary antibodies followed by peroxidase-conjugated goat anti-rabbit IgG antibody (Zymed Laboratories Inc.). Detection was performed using the Enhanced Chemiluminescence Western blotting Detection System (Amersham Pharmacia Biotech).

RESULTS

Estrogen Represses apoAI mRNA Levels in HepG2 Cells Stably Expressing ERα—Human hepatoma HepG2 cells retain many liver-specific functions; however, they no longer express EROs. Therefore, HepG2 cells stably expressing ERα were created to monitor the regulation of the apoAI gene by estrogen. The resulting cell line, Hep89, expressed ERα by Western blot (Fig. 1A). Further, the activity of a synthetic vitellogenin estrogen response element luciferase reporter (ERE.LUC) was simulated 25-fold by 100 nM 17β-estradiol (E2) in Hep89 cells, whereas no E2-dependent promoter activity was observed in the parental HepG2 cells (Fig. 1B). Northern blotting demonstrated a 2–3-fold decrease in apoAI mRNA steady state levels in Hep89 cells after a 72-h treatment with 1 μM E2, whereas apoAI mRNA levels remained unchanged in HepG2 cells (Fig. 1, C and D). Therefore, apoAI mRNA steady state levels are regulated by E2 in a receptor-dependent manner.

Estrogen Represses apoAI Promoter Activity in HepG2 Cells—The ERα effects on apoAI mRNA levels could be due to changes in mRNA stability or apoAI transcription. To determine the potential of ERα to regulate apoAI gene transcription, a luciferase reporter under the control of both 5′- and 3′-flanking regulatory sequences of the human apoAI gene (reporter−2500 ALUC.CIIIAIV, Ref. 21) was cotransfected with an ERE expression vector into HepG2 cells. After transcription, the cells were treated with estrogen agonists and antagonists, and the reporter activity in cell extracts was determined. Dose-response experiments with E2 resulted in a 75% maximal repression of apoAI promoter activity with an EC₅₀ value of approximately 12 nM (Fig. 2A). The repression was both ligand- and receptor-dependent, since it did not occur with either ERα or E2 alone (Fig. 2B). The estrogen receptor antagonist ICI 182,780 (1 μM) also repressed promoter activity but to a lesser extent (30% repression). A 10-fold molar excess of ICI (1 μM) over E2 (100 nM) effectively competed the E2-mediated repression to the level seen with ICI alone. ERE.LUC reporter activity was also regulated by ERα in a ligand-dependent fashion, except that in contrast to the apoAI promoter, ICI acted as a pure antagonist for ERE activation. The mechanism of ERα repression on the apoAI promoter may be distinct from those involved in ERα activation of an ERE.

Mapping of the apoAI Promoter Estrogen Response Element—A collection of apoAI promoter reporters (18) was used to delineate elements involved in ERO-induced repression of the apoAI promoter. Deletion of the entire 5′-flanking region of the apoAI gene (reporter−2500 ALUC) or deletion of both the 5′- and 3′-flanking regions and approximately 2.25 kilobases of the 5′ region (reporter−2506ALUC) did not affect ERα and E2-induced repression (Fig. 3A). A reporter containing only the apoAI hepatocyte-specific enhancer driving the expression of the apoAI basal promoter (reporter−220/−110ALUC) was also repressed approximately 60% by ERα plus E2. The activity of the apoAI enhancer in a heterologous reporter containing the
thymidine kinase promoter was also repressed by ERα plus E2, whereas the thymidine kinase basal promoter reporter activity remained unaffected (Fig. 3B), demonstrating that ERα regulation occurs directly on the apoAI enhancer.

ApoAI enhancer activity in hepatocytes depends on synergistic interactions between transcription factors bound to three distinct sites designated A (−214 to −192), B (−169 to −146), and C (−134 to −119) (18). To determine the contribution of each of these sites to the ERα and E2-induced repression, nucleotide mutations (denoted by X, Fig. 3B) were introduced into sites A, B, or C, rendering them incapable of binding their cognate transcription factors (18). Although the basal activity of these reporters was reduced compared with the intact enhancer, the range of repression was 48–69% in response to ERα and E2. These results suggest that repression occurs at a level distinct from inhibition of individual transcription factors bound to the enhancer.

The ERα DNA Binding Domain and Transcription Activation Functions 1 or 2 Are Necessary for apoAI Enhancer Repression—To further probe the mechanism by which ERα and E2 repressed the apoAI enhancer, vectors expressing ERα with a deletion in the N-terminal transcription activation function (AF1) or point mutations in the C-terminal transcription activation function (AF2) or in the DBD were cotransfected with either the −220/−110 ABC.LUC or ERE.LUC reporter into HepG2 cells (Fig. 4). Deletion of AF1 (mutant X-DBD-AF2) had no effect on apoAI repression but inhibited ERE activation by 80%. In contrast, inactivation of AF2 (mutant AF1-DBD-X) diminished both apoAI repression and ERE activation. Inactivation of both AF1 and AF2 completely abolished both apoAI repression and ERE activation. Finally, point mutations within the DNA binding domain that converts its binding selectivity from an ERE to a glucocorticoid response element (mutant AF1-X-AF2) eliminated repression of the apoAI enhancer. As shown previously (51), this ERα DNA binding domain mutant activated a reporter driven by a glucocorticoid
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Under these conditions, ERα did not bind to the apoAI enhancer but bound efficiently to the vitellogenin ERE as expected (Fig. 5, A and B). Control experiments showed that HNF-4 bound strongly to the apoAI enhancer as expected (20, 25) (Fig. 5A) and weakly to the vitellogenin ERE (Fig. 5B). Since HNF-4 does not activate the ERE.LUC reporter in HepG2 cell transfections (data not shown), this binding interaction is unlikely to occur in the intact cell. The presence of additional cellular proteins did not overcome the inability of ERα to bind to the apoAI enhancer, as demonstrated in experiments comparing extracts from HepG2 cells and Hep89 cells (Fig. 5C) or when HepG2 extracts were supplemented with ERα (Fig. 5D). In both instances, no ERα binding to the enhancer could be detected, although binding to the ERE was observed. Thus even under conditions where nonspecific binding of HNF-4 to a non-cognate sequence could be detected, no direct binding of ERα to the apoAI enhancer was observed.

ERα and E2 Do Not Influence HNF-4 or HNF-3β Abundance or Function—HNF-4, which binds to sites A and C in the apoAI enhancer (20, 22, 25), and HNF-3β, which binds to site B (19), are two fundamental transcription factors involved in apoAI enhancer function. Ligand-activated ERα could repress apoAI enhancer activity by reducing the abundance or by inhibiting the activation properties of endogenous HNF-4, HNF-3β, or both (see for example Refs. 42 and 56). To examine the first possibility, the effects of E2 on the relative abundance of HNF-4 and HNF-3β in Hep89 cells was determined by Western blotting analysis. As shown in Fig. 6, A and B, neither HNF-4 nor HNF-3β protein levels were altered after treatment with E2.

To determine whether ERα affects transcription factor activity, HepG2 cells were cotransfected with a reporter gene driven by the apoAI basal promoter and site A (reporter A.LUC) along with HNF-4 and ERα expression vectors. Cotransfection of the HNF-4 expression plasmid increased A.LUC activity 4-fold. This activation was not influenced by the presence of ERα and E2 (Fig. 6C). Similarly, a reporter driven by the apoAI basal promoter and site B (reporter B.LUC) was stimulated 8-fold by cotransfection of an HNF-3β expression vector, and ERα and E2 did not affect this activity (Fig. 6D). Thus, the repressive effects of ERα is not due to reduction in abundance or activity of HNF-4 or HNF-3β. These findings raise the possibility that ERα may affect a non-DNA-bound factor required for optimal apoAI enhancer activity.

RIP140 Is a Coactivator for the apoAI Enhancer—Although optimal transcriptional activity of the apoAI enhancer depends on both DNA-bound transcription factors and non-DNA-bound coactivators (18, 26), there is no information regarding the identity of these coactivators. We tested two well characterized ERα coactivators, CBP (38) and RIP140 (33) for their possible involvement in apoAI enhancer activity. Specifically, the apoAI enhancer reporter (−220/−110 ABC.LUC) was cotransfected with increasing amounts of either RIP140 or CBP expression vectors. CBP did not alter apoAI enhancer activity. In contrast, RIP140 repressed activity in a dose-dependent fashion (Fig. 7), reminiscent of its squelching affects on ERα activation of ERE reporters (33). RIP140 did not affect the function of an SV40 enhancer driven reporter (data not shown), demonstrating that the RIP140 effect was specific to the apoAI enhancer.

The Role of Coactivator Partitioning in ERα-mediated Repression of the apoAI Enhancer—Transcriptional interference among nuclear receptors is due, at least in part, to partitioning of limited amounts of shared transcriptional coactivators (36, 39). It is therefore possible that ERα and the apoAI enhancer share common cofactors and that partitioning of these cofactors to ERα results in repression of the enhancer. To test this

response element (data not shown).

These data suggest that the DNA binding domain of ERα cooperates with either AF-1 or AF-2 to repress the activity of the apoAI enhancer. Moreover, the observation that the X-DBD-AF2 mutant is as efficacious as wild type ERα in apoAI repression but not ERE reporter activation suggests that different mechanisms underlie ERα activation of an ERE reporter and repression of the apoAI reporter.

ERα Does Not Bind to the apoAI Enhancer—The requirement of an intact DNA binding domain for ERα repression of the apoAI enhancer suggested that ERα could bind directly to the enhancer and interfere with the synergistic interactions between bound transcription factors. We therefore determined if partially purified ERα binds to the −220/−110 apoAI enhancer fragment by electrophoretic mobility shift assays. Un-
possibility, HepG2 cells were cotransfected with the pMT2-ERα expression vector into HepG2 cells. The structure of the constructs is indicated. Bent lines were used to indicate plasmid integrity. After transfection, the cells were cultured in the absence (open bars) or presence of 100 nM E2 (filled bars) for 24 h. Luciferase activities were determined as in Fig. 2B. A, a series of apoAI enhancer reporter plasmids were analyzed as above. A, B, and C denote transcription factor binding sites in the enhancer, with X indicating nucleotide substitutions that disrupt nuclear factor binding to these sites. The luciferase values are reported as relative luciferase activities with the activity of the reporter −41.LUC defined as 1. TK, thymidine kinase.

FIG. 3. ApoAI enhancer repression by ERα and 17β-estradiol. A, a series of apoAI promoter deletion reporter plasmids were cotransfected with the pMT2-ERα expression vector into HepG2 cells. The structure of the constructs is indicated. Bent lines were used to indicate plasmid integrity. After transfection, the cells were cultured in the absence (open bars) or presence of 100 nM E2 (filled bars) for 24 h. Luciferase activities were determined as in Fig. 2B. B, a series of apoAI enhancer reporter plasmids were analyzed as above. A, B, and C denote transcription factor binding sites in the enhancer, with X indicating nucleotide substitutions that disrupt nuclear factor binding to these sites. The luciferase values are reported as relative luciferase activities with the activity of the reporter −41.LUC defined as 1. TK, thymidine kinase.

FIG. 4. ER α DNA binding and activation domains contribute to apoAI repression. HepG2 cells were cotransfected with 0.5 μg of −220/−110ABC.LUC or ERE.LUC and 0.2 μg of expression vectors encoding ERα with the indicated truncations or point mutations (denoted by X). After transfection, the cells were cultured in the absence (open bars) or presence (filled bars) of 100 nM E2. The activities obtained from cells transfected with −220/−110ABC.LUC and wild type ERα in the absence of E2 (left panel) or from cells transfected with the ERE.LUC reporter and wild type ERα cultured in the presence of E2 (right panel) were defined as 100%.

stimulated 3-fold by ERα in an E2-dependent fashion (Fig. 8B). RIP140 does not appear to repress ERα production as determined by transient transfections in which different promoter enhancers (i.e. CMV enhancer or adenovirus major late promoter) used to drive expression of ERα gave similar results (data not shown). Together, these data indicate that ERα can affect apoAI gene expression via transcription coactivators and that RIP140 or an endogenous RIP140-like protein may be involved in apoAI enhancer regulation by ERα and E2.
DISCUSSION

The apoAI liver-specific enhancer plays a central role in integration of diverse physiological and environmental signals affecting apoAI gene expression. Our working hypothesis is that signal-induced transient multiprotein complexes containing both DNA binding factors and factors not directly bound to DNA assemble onto the apoAI enhancer and regulate one or more steps in transcription initiation.

In this study, estrogenic signals influenced apoAI expression at the mRNA level by repressing apoAI gene transcription. More specifically, ERα and E2 potently repressed apoAI liver-specific enhancer activity in a ligand- and receptor-dependent manner. Although gel shift experiments did not provide evidence for ERα binding to the enhancer, the DBD was essential for apoAI repression. In addition, the ERα transcription activation functions AF1 and AF2, although not sufficient by them-
selves, were individually essential for repression when associated with the DBD. Experiments with ERα mutants showed that the combination of the DBD and AF2 domain is as effective in enhancer repression as the wild type ERα, whereas the combination of the DBD and AF1 domain is only 50% as effective as full-length ERα. All ERα mutants tested were capable of ligand binding (51, 57, 58), and apoAI enhancer repression by them was strictly ligand-dependent. Thus, it appears that a ligand-induced change in the receptor cooperates with the DBD and either AF1 or AF2 to impart an apoAI enhancer repressing activity.

How does ERα and E2 repress apoAI enhancer function? Previous studies showed that maximal activity of the apoAI enhancer depends on synergistic interactions between transcription factors bound to enhancer sites A, B, and C (18). Because ERα does not bind to the enhancer under our gel-shifting conditions, repression mechanisms involving transcription interference by quenching (59) seem unlikely. ERα may repress the apoAI enhancer by inhibiting the activity of transcription factors required for enhancer function (see for example Refs. 42 and 56). For example, we have recently observed that adenovirus E1A inhibits apoAI enhancer activity by selective inactivation of HNF-3, the factor that binds to site B. To test this possibility, two liver-specific transcription factors, HNF-4 and HNF-3β, involved in apoAI enhancer function were assayed. The results showed that neither HNF-3β nor HNF-4 activities or protein levels were influenced by ERα plus E2, suggesting that ERα represses the apoAI enhancer at some other level. Although the possibility that ERα activates a repressing transcription factor that binds to the enhancer and inhibits its function cannot be unequivocally excluded, the observation that ERα could mediate apoAI enhancer repression independent of individual cis-elements mutations within the enhancer suggests that repression occurs at a level secondary to DNA binding.

An alternative explanation for the ERα- and E2-induced repression of the apoAI enhancer is that a coactivator common to the enhancer and ERα is partitioned to ERα, leading to transcriptional interference with the enhancer. HNF-4, which binds to site A, is a member of the nuclear receptor superfamily and could share coactivators with ERα similar to other nuclear receptors.

2 E. Kilbourne and S. K. Karathanasis, unpublished data.
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RIP140 expression vector levels reversed the ERα or presence (apoAI enhancer activity, reminiscent of the RIP140 squelching experiments with RIP140 showed that this cofactor repressed does not affect apoAI enhancer activity. However, similar experiments with RIP140 showed that this cofactor repressed apoAI enhancer activity. Therefore, RIP140-like factors may be one class of non-DNA binding coactivators involved in apoAI enhancer function that are partitioned to ERα.

The observation that two different activation functions in ERα (i.e. AF1 and AF2) can in conjunction with the DBD repress apoAI enhancer activity independently of each other raises the possibility that ERα partitions an additional cofactor(s) involved in apoAI enhancer function. Consistent with this, RIP140 interaction with ERα requires a functional AF2 domain (60) that is inactive in the ERα mutant AF1-DBD-X that represses enhancer activity. Therefore a model whereby, in addition to RIP140, ERα shares other cofactors with the apoAI enhancer and RIP140 binding to ERα alters its conformation, so that some, but not all, of these factors are released and used by the enhancer is possible.

How could the disparities regarding ERα regulation of apoAI and HDL plasma levels between different cell and animal systems (see the Introduction) be reconciled? It is clear from our previous work that the multiprotein complexes assembled onto the apoAI enhancer are transient, and their protein composition is influenced by the prevailing developmental, physiological, and environmental factors affecting apoAI gene expression. For example, although apoAI enhancer activity does not depend on retinoids, prior repression of enhancer activity by the nuclear receptor apolipoprotein AI regulatory protein-1 converts the enhancer into a retinoid-responsive element (61). A similar phenomenon may be occurring with E2 in which the state of the apoAI enhancer due to intracellular coactivator levels determines the mode of ERα regulation. When cofactors shared by the apoAI enhancer and ERα are present in limiting amounts compared with ERα, their partitioning by ERα will result in enhancer inhibition. This latter case appears to be operating in the Hep89 cell system. In contrast, when these cofactors are in excess compared with ERα and apoAI enhancer activity is partially repressed due to endogenous squelching, estrogen-activated ERα will alleviate repression by partitioning cofactors in excess and result in enhancer stimulation. Consistent with this, the low level enhancer activity obtained at high RIP140 expression vector levels was stimulated 3-fold by ERα in a ligand-dependent fashion. In addition, differences in ERα affinity for these coactivators, induced by different ligands, may explain the opposite effects of different estrogen agonists and antagonists on apoAI plasma levels (62, 63). Therefore, we propose that the effects of estrogen on apoAI gene transcription will depend upon the balance between coactivators shared by the apoAI enhancer and ERα and may explain the disparities observed for apoAI gene regulation by E2 obtained using different cell and animal systems.

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REFERENCES

1. Karathanasis, S. K. (1992) *Monogr. Hum. Genet.* 14, 140–171
2. Sorci-Thomas, M., Prack, M. M., Dashti, N., Johnson, F., Rudel, L. L., and Williams, D. L. (1989) J. Lipid Res. 30, 1397–1403
3. Vandenbroucke, Y., Lambert, G., Janvier, B., Girlich, D., Bereziat, G., and Mangeney-Andreani, M. (1995) *FEBS Lett.* 376, 99–102
4. Jin, F.-Y., Kamanna, V. S., Chuang, M.-Y., Morgan, K., and Kashyap, M. L. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 1052–1062
5. Azrolan, N., Odaka, H., Breslow, J., and Fisher, E. A. (1995) J. Biol. Chem. 270, 19833–19838
6. Tang, J., Sriwastava, R. A. K., Kral, E. S., Baumann, D., Pfleger, B. A., Kitchens, R. T., and Schonfeld, G. (1991) J. Lipid Res. 32, 1571–1581
7. Ettinger, W. H., Varma, V. K., Sorci-Thomas, M., Parks, J. S., Sigmon, R. C., Smith, T. K., and Verdery, R. B. (1994) Arterioscler. Thromb. 14, 8–13
8. Kaptein, A., deWit, E. C. M., and Prince, H. M. G. (1993) *Arterioscler. Thromb.* 13, 1505–1514
9. The Writing Group for the PEPI Trial (1995) *J. Am. Med. Assoc.* 273, 199–208
10. Barrett-Connor, E. (1996) *Maturitas* 23, 227–234
11. Rich-Edwards, J. W., and Hennekens, C. H. (1996) *Carr. Opin. Cardiol.* 11, 440–446
12. Britton, E. A. (1996) *Arterioscler. Thromb. Vasc. Biol.* 16, 431–440
Estrogen Regulation of the apoAI Enhancer

13. Walsh, B., Li, H., and Sacks, F. (1994) J. Lipid Res. 35, 2083–2093
14. Applebaum-Bowden, D., McLean, P., Steinmetz, A., Fontana, D., Matthys, C., Warrick, G. R., Chang, E. P., Helgason, J. A., and Hazzard, W. R. (1998) J. Lipid Res. 35, 1895–1906
15. Williams, J. K., Anthony, M. S., Houore, E. K., Herrington, D. M., Morgan, T. M., Register, T. C., and Clarkson, T. B. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1145–1154
16. Clarkson, T. B., Shibuya, C. A., Morgan, T. M., Kortnik, D. R., Adams, M. R., and Kaplan, J. R. (1990) Obstet. Gynecol. 75, 217–222
17. Staels, B., Auwerx, J., Chan, L., van Tol, A., Rossenew, M., and Verhoogen, G. (1989) J. Lipid Res. 30, 1137–1145
18. Widom, R. L., Ladias, J. A., Krous, S., and Karathanasis, S. K. (1991) Mol. Cell. Biol. 11, 677–687
19. Harnish, D., Malik, S., and Karathanasis, S. K. (1994) J. Biol. Chem. 269, 28220–28226
20. Ge, R., Rhee, M., Malik, S., and Karathanasis, S. K. (1994) J. Biol. Chem. 269, 13185–13192
21. Ginsburg, G. S., Ozer, J., and Karathanasis, S. K. (1995) J. Clin. Invest. 96, 528–538
22. Chan, J., Nakabayashi, H., and Wong, N. C. W. (1991) Nucleic Acids Res. 19, 1205–1211
23. Rottman, J., Widom, B., Nadal-Ginard, B., Mahdavi, V., and Karathanasis, S. (1991) Mol. Cell. Biol. 11, 3814–3820
24. Ladias, J. A., and Karathanasis, S. K. (1991) Science 251, 561–565
25. Malik, S., and Karathanasis, S. K. (1995) Nucleic Acids Res. 23, 1536–1543
26. Harnish, D., Malik, S., Kilbourne, E., Costa, R., and Karathanasis, S. K. (1996) J. Biol. Chem. 271, 13621–13628
27. Green, S., Walter, P., Kumar, V., Krust, A., Bornert, J.-M., Argos, P., and Chambon, P. (1986) Nature 320, 134–139
28. Kroezer, G. R., Cho, E. P., Ho, H. Y., Nilsson, S., and Gustafsson, J.-A. (1996) Biochemistry 35, 5925–5930
29. Hassel, S., Walter, P., Kumar, V., Krouse, S., Bornert, J.-M., Argos, P., and Chambon, P. (1986) Nature 320, 134–139
30. Lee, J. W., Ryan, P., Swaffield, J. C., Johnston, S. A., and Moore, D. D. (1995) Nature 374, 827–834
31. vom Baur, E., Zechel, C., Garnier, J. M., Darnell, J., and Karathanasis, S. K. (1996) Mol. Cell. Biol. 16, 7134–7143
32. Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C., and Auricchio, F. (1994) Cancer Res. 54, 4516–4521
33. Bodine, P. V., Green, J., Harris, H. A., Bhat, R. A., Stein, G. S., Lian, J. B., and Korn, B. S. (1997) J. Cell. Biochem. 65, 368–387
34. Kumar, V., Green, S., Stack, G., Berry, M., Jin, R. J., and Chambon, P. (1987) Nature 334, 271–274
35. Kroezer, G. R., Sladek, F., Ginsburg, G., Koon, C. F., Ladias, J., Darnell, J., and Karathanasis, S. K. (1992) Mol. Cell. Biol. 12, 1708–1718
36. Pani, L., Overdier, D. G., Porello, A., Qian, X., Lai, E., and Costa, R. H. (1992) Mol. Cell. Biol. 12, 3723–3732
37. Breslow, J. L., Ross, D., McPherson, J., Williams, H. W., Kurnit, D., Nassbaum, A. L.,Karathanasis, S. K., and Zannis, V. I. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 6861–6865
38. Stein, B., and Yang, M. X. (1995) Mol. Cell. Biol. 15, 4971–4979
39. Lees, J. A., Gawel, S. C., and Parker, M. G. (1989) Nucleic Acids Res. 17, 4577–4588
40. Danielian, P. S., White, R., Lees, J. A., and Parker, M. G. (1992) EMBO J. 11, 1025–1033
41. Levine, M., and Manley, J. L. (1989) Cell 58, 405–408
42. Danielian, P. S., Cavailles, V., Balaguer, P., and Nicolas, J. C. (1997) Mol. Endocrinol. 11, 193–202
43. Widom, R. L., Rhee, M., and Karathanasis, S. K. (1992) Mol. Cell. Biol. 12, 3980–3989
44. Saarinen, T., Blomqvist, C., Ehnholm, C., Taskinen, M. R., and Elomaa, I. (1996) J. Clin. Oncol. 14, 429–433
45. Smith, C. L., Oates, S. A., Tasi, M., and O'Malley, B. W. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 8884–8888
46. Tzukerman, M. T., Esty, A., Santiso-Mere, D., Danielian, P., Parker, M. G., Stein, R. B., Pike, J. W., and McDonnell, D. P. (1994) Mol. Endocrinol. 8, 21–30
47. Mader, S., Kumar, V., L'Hermet, H., and Chambon, P. (1989) Nature 338, 597–601
48. Saarto, T., Blomqvist, C., Ehnholm, C., Taskinen, M. R., and Elomaa, I. (1996) J. Cell. Biochem. 65, 110–124
49. Danielian, P. S., White, R., Lees, J. A., and Parker, M. G. (1992) EMBO J. 11, 1025–1033
50. Levine, M., and Manley, J. L. (1989) Cell 58, 405–408
51. Danielian, P. S., Cavailles, V., Balaguer, P., and Nicolas, J. C. (1997) Mol. Endocrinol. 11, 193–202
52. Widom, R. L., Rhee, M., and Karathanasis, S. K. (1992) Mol. Cell. Biol. 12, 3980–3989
53. Saarinen, T., Blomqvist, C., Ehnholm, C., Taskinen, M. R., and Elomaa, I. (1996) J. Clin. Oncol. 14, 429–433
54. Anthony, M. S., Clarkson, T. B., Hughes, J. C., L. Morgan, T. M., and Burke, G. L. (1996) J. Nutr. 126, 43–50