Hepatocyte Nuclear Factor 4 Inhibits the Activity of Site A from the Rat Apolipoprotein AI Gene†

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ABSTRACT: The pivotal role of apolipoprotein AI (Apo AI) in mediating reverse cholesterol transport has lead us to the study of transcription factors that influence the expression of this gene. Previous studies show that rat HNF-4 enhances the activity of a cis-acting site C in the rat Apo AI promoter. Since sites C and A share 80% homology, we have examined whether HNF-4 binds to and modulates the transcriptional activity of the A-motif. Results show that HNF-4 binds to site A. The transcriptional activity of site A in a human hepatoma cell line, HuH-7, increases 2−2.5-fold in the presence of antisense HNF-4, but the sense construct has no effect on the activity of the reporter template. The lack of an effect of HNF-4 on site A activity may be due to high endogenous levels of the factor in HuH-7 cells. However, in BHK cells HNF-4 clearly inhibits the transcriptional activity of site A. Together these findings suggest that in contrast to the enhancing effects of HNF-4 on site C, the same factor inhibits site A activity. Since hepatocytes normally contain the T3 receptor and this nuclear factor increases site A action, cotransfection of T3 receptor along with antisense HNF-4 further augments the activity of p5′A.CAT. In summary, rat HNF-4 binds to site A from rat Apo AI DNA, and this factor suppresses site A activity. HNF-4 interferes with the enhancer role of the T3 receptor and thus contributes negatively to the net expression of the Apo AI gene.

Apolipoprotein AI (Apo AI)[1] is the major protein component of the serum high density lipoprotein particles (HDL) (Glomset, 1968). These particles mediate the efflux of cholesterol from extrahepatic tissues in a process called "reverse cholesterol transport (RCT)" (Miller et al., 1985; Franceschini et al., 1991). Enhanced RCT lowers total body cholesterol. Since hypercholesterolemia is a modifiable risk factor associated with coronary arterial disease (CAD), there is widespread interest in effective ways to lower serum cholesterol (Castelli et al., 1986; Kane et al., 1990; Arntzenius et al., 1985). A better understanding of Apo AI gene regulation should help us develop new ways to increase abundance of the protein. To this end, we have been studying the nuclear factors that regulate the expression of Apo AI gene expression.

Previous studies from our laboratory and those of others have shown that several members of the nuclear thyroid/steroid hormone receptor superfamily interact with sites A (−208 to −193, AACCCTTGATCCCAGC) and C (−136 to −119, GAGCTGATCCTTGAACTC) in the rat Apo AI promoter (Romney et al., 1992; Chan et al., 1993; Taylor et al., 1996; Ladias & Karathanasis, 1991; Rottman et al., 1991; Widom et al., 1991; Mangelsdorf et al., 1995; Mietus-Synder et al., 1992). Since both sites interact with members belonging to the same superfamily, it is not surprising that they share 80% sequence homology (Chan et al., 1993). One member of the superfamily, HNF-4, modulates the activity of both cis-acting elements (Chan et al., 1993; Sladek et al., 1991; Fuernkranz et al., 1994). HNF-4 is an orphan receptor that regulates the expression of genes which play important roles in the control of cellular metabolism. For example, HNF-4 influences both glucose and lipid metabolism by enhancing the expression of PEPCK and Apo CIII or AI, respectively (Chan et al., 1993; Hall et al., 1995; Shih et al., 1995). We have previously shown that rat HNF-4 binds to site C in rat Apo AI DNA and increases the activity of this cis-acting element (Chan et al., 1993). In addition, recent results of others show that rat HNF-4 may also bind to site A in human Apo AI DNA (Fuernkranz et al., 1994). Although the ability of HNF-4 to bind site A is clear, its role in modulating the activity of this site is not. The conflicting information arises from the results of two studies (Fuernkranz et al., 1994; Ginsburg et al., 1995). The first report claimed that HNF-4 expressed in yeast enhanced the activity of site A from human Apo AI DNA (Fuernkranz et al., 1994). However, a more recent set of experiments showed that HNF-4 had no effect on site A activity in the hepatoma cell line Hep G2 (Ginsburg et al., 1995). In light of these discordant findings, we have re-examined the role

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[1] Abbreviations: Apo, apolipoprotein; HDL, high-density lipoprotein; T3, the thyroid hormone L-triiodothyronine; TR, T3 receptor; CAT, chloramphenicol acetyltransferase; RXRα, 9-cis retinoic acid receptor α; EMSA, electrophoretic mobility shift assay; ARP-1, apolipoprotein repressor protein-1; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; HNF-4, hepatocyte nuclear factor 4.

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of rat HNF-4 on the activity of site A derived from rat Apo AI DNA.

MATERIALS AND METHODS

Plasmid DNA Used in Studies. To produce rat HNF-4 protein, a construct containing the cDNA (Sladek et al., 1991; gift from J. E. Darnell, Jr., New York, NY) was inserted into pT7-7 for expression in *E. coli*, BL21(DE3). In addition, the protein was also produced using *in vitro* transcription/translation of HNF-4 cDNA inserted into pBluescript-KS+ (pBS-KS+, Stratagene). Transient transfection studies to determine the activity of site A used a previously described reporter construct, p5′A.CAT, which contained duplicate site A's fused in tandem to the SV40 promoter (Romney et al., 1992). The activity of p5′A.CAT was determined in the presence or absence of cotransfection with eukaryotic expression vectors containing either the sense (pLEN4S) or the antisense HNF-4 (pLEN4A) (Sladek et al., 1991; gifts from J. E. Darnell, Jr.). The reporter construct, pAPF-HIV.CAT (Sladek et al., 1991; gift from J. E. Darnell, Jr.), contains a HNF-4 binding site that activates the promoter. In cotransfection studies with thyroid hormone receptor, the human TRα1 cDNA was contained in the eukaryotic expression vector PECE (Graupner et al., 1989; gift from M. Pfahl, La Jolla, CA).

Bacterial Synthesis of HNF-4. *E. coli* BL21(DE3) cells were transformed with pT7-7 carrying the cDNA encoding the rat HNF-4 protein (Sladek et al., 1991). Expressed proteins were partially purified using heparin−agarose chromatography as previously described (Forman & Samuels, 1990) prior to use in electrophoretic mobility shift assays. To radiolabel HNF-4 with [^{35}S]methionine, *E. coli* containing the cDNA were grown in LB overnight and used to inoculate a 5 mL aliquot of LB broth. This culture was grown until the OD_{590} reached 4 before treating with IPTG (final concentration 0.4 mM) for 30 min to induce HNF-4 expression. The cells were then exposed to rifampicin (Sigma, 200 μg/mL) for 2 h to amplify the abundance of the induced protein. The cells were pelleted by centrifugation (2000 rpm in a Beckmann J6A at 4 °C), washed with M9 media (Sambrooke et al., 1989), and then resuspended in 500 μL of M9 media. These cells were permitted to recover by shaking at 37 °C for 20 min, followed by the addition of 10 μCi of [^{35}S]methionine (NEN, 400 Ci/mmol) and further incubation for 10 min. The relative mass of radiolabeled HNF-4 in the cells was assessed by using SDS−polyacrylamide gel electrophoresis (SDS−PAGE).

In Vitro Transcription and Translation of HNF-4. The pBS-KS+ vector carrying HNF-4 cDNA was transcribed *in vitro* with T7 RNA polymerase (Gibco-BRL) according to the manufacturer's instructions. The RNA product was translated with a rabbit reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine. Radiolabeled product(s) from the reaction was (were) analyzed by SDS−PAGE followed by autoradiography of the dried gel using Kodak XAR-5 film and in the presence of intensifying screens.

Electrophoretic Mobility Shift Assay (EMSA). For these studies, a synthetic oligonucleotide (Gibco-BRL) spanning site A (−232 to −187) from the rat Apo A1 gene was radiolabeled by incubating with [α-^{32}P]ATP (NEN) and the Klenow fragment of DNA polymerase I as described previously (Taylor et al., 1996). Electrophoretic mobility shift assays were performed according to procedures described previously (Wong et al., 1989), and the radiolabeled material was detected using autoradiography.

Transfection of Cultured Cells and Assay for CAT Activity. The culturing conditions for the human hepatoma cell line HuH-7 and the transfection of these cells with DNA have been described previously (Chan et al., 1993). The assay for CAT activity and normalization of these data against β-galactosidase activity by including 2 μg of RSV-β-galactosidase in transfected cells were performed according to procedures described previously (Herbomel et al., 1984; Nakabayashi et al., 1991). The differences between the activities of the various treatment groups were analyzed by ANOVA to determine whether they were significant.

RESULTS

HNF-4 Expressed in Bacterial Cells or by in Vitro Transcription/Translation Bind to Site A. To determine whether rat HNF-4 binds to the A-motif from rat Apo A1 DNA, the protein was expressed in *E. coli* or using an *in vitro* transcription/translation system. Incubation of bacteria carrying HNF-4 cDNA with [^{35}S]methionine yielded a radiolabeled protein product that migrated as a single band with an estimated mass of 56 kDa (Figure 1A). Radiolabeled protein of the same mass was not detected in lysate from bacteria carrying empty vector, pT7-7 (data not shown). In separate studies, *in vitro* transcribed HNF-4 mRNA was translated in the presence of [^{35}S]methionine and yielded a major radiolabeled product with a mass of 56 kDa. This value matched the known molecular mass of rat HNF-4 (Sladek et al., 1991). For comparison, radiolabeled rat TRα1 (48 kDa; Thompson et al., 1987) and a 65 kDa control protein are shown (Figure 1A). These findings demonstrate that proteins expressed in bacteria or by transcription/translation had a molecular mass the same as that predicted from the rat HNF-4 cDNA.

Next, we used the EMSA to examine whether the partially purified HNF-4 could bind radiolabeled site A from rat apo AI DNA. Results (Figure 1B, lane 1) showed that heparin−agarose-purified extract from bacteria carrying the empty vector, pT7-7, did not bind to the DNA, but that containing HNF-4 formed a single complex with radiolabeled site A (Figure 1B, lane 2). HNF-4 expressed using transcription/translation also bound site A (Figure 1B, lane 4) and formed a single complex with a mobility similar to that arising from the use of HNF-4 expressed in bacteria. Competition studies revealed that formation of the retarded complex was inhibited when excess (100-fold) unlabeled site A (Figure 1C, lane 2) but not nonspecific DNA homologous to rat Apo AI site B (Figure 1C, lane 4) was added to the reaction. In agreement with previous findings (Chan et al., 1993), the addition of rat Apo AI site C oligomer also inhibited binding of HNF-4 to site A (Figure 1C, lane 3). Together these studies show that HNF-4 expressed using either method binds specifically to site A from the rat Apo AI promoter. These results are consistent with those of rat HNF-4 binding to site A from the human Apo AI gene (Fuernkranz et al., 1994; Ginsburg et al., 1995).

HNF-4 Represses the Activity of Site A in HuH-7 Cells. Although HNF-4 binds to site A, the effect of this factor on the activity of the motif is controversial. There is evidence to support both an enhancer and no effect of HNF-4 on this

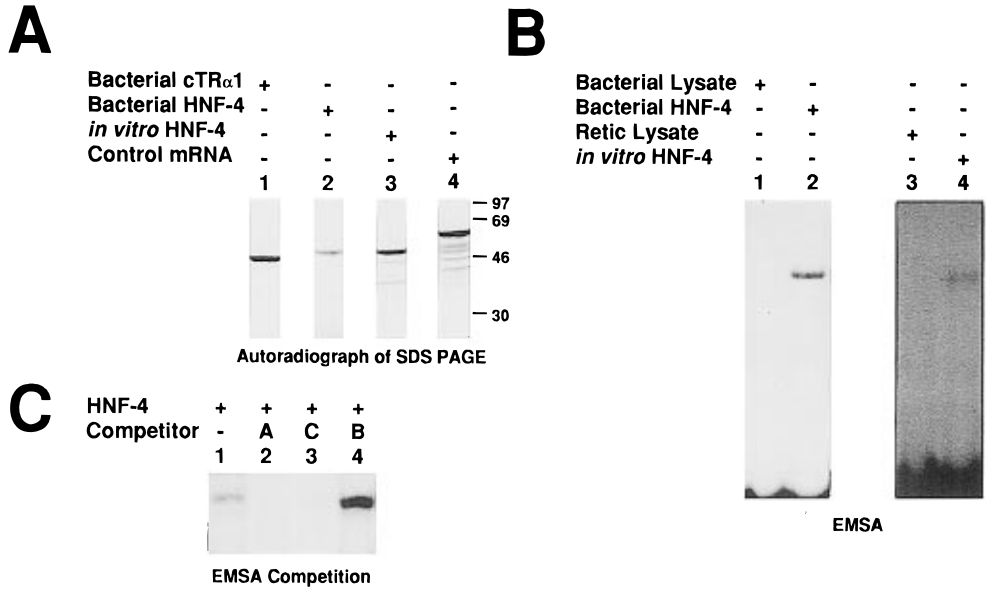

FIGURE 1: Rat HNF-4 expressed in bacteria or by *in vitro* transcription/translation binds to radiolabeled site A from rat Apo AI DNA. Panel A: [^{35}S]Methionine-labeled protein products from bacteria transformed with pT7-7 carrying cDNAs encoding rat TRα1 (lane 1, 48 kDa) or rat HNF-4 (lane 2). Labeled protein product from *in vitro* transcription/translation reactions directed by mRNA encoding rat HNF-4 (lane 3) or control mRNA (Promega) that encodes a 65 kDa marker (lane 4). Panel B shows autoradiographs of EMSA reactions containing protein lysate from bacteria carrying empty vector (lane 1) or vector with HNF-4 cDNA (lane 2). Reactions containing reticulocyte lysate without (lane 3) and with (lane 4) HNF-4 mRNA. Panel C shows autoradiographs of EMSA studies with HNF-4 expressed in bacteria bound to radiolabeled site A in the absence of competitor (lane 1) or in the presence of competitor DNA; site A (lane 2), site C (lane 3), and site B (lane 4) from the rat Apo AI gene. Increased intensity of the complex in lane 4 is likely due to uneven sample loading.

cis-acting site (Fuernkranz et al., 1994; Ginsburg et al., 1995). Therefore, we attempted to define the effect of HNF-4 on the activity of site A. The HuH-7 cells were cotransfected with the reporter construct p5′A.CAT and a vector carrying the HNF-4 cDNA in a sense or antisense orientation. Results (Figure 2A,B) showed that expression of sense HNF-4 had no significant effect on the activity of p5′A.CAT compared to control, i.e., p5′A.CAT cotransfected with empty vector. In contrast, cotransfection of antisense HNF-4 yielded a 2−2.5-fold increase in the activity of the p5′A.CAT.

To determine whether antisense HNF-4 augmented CAT activity in a dose-dependent fashion, we cotransfected the HuH-7 cells with a fixed amount of p5′A.CAT and progressively increasing quantities of antisense HNF-4. The ratio of reporter template to antisense HNF-4 was 4:1, 2:1, or 1:1. Results (Figure 2C) showed a rise in CAT activity to 155%, 193%, and 227%, respectively, and these increases varied with the quantity of antisense HNF-4 in a dose-dependent manner. This observation suggests that the presence of antisense HNF-4 mRNA enhances the activity of p5′A.CAT.

In order to determine whether sense and antisense HNF-4 expression plasmids were indeed active in HuH-7, we cotransfected these cells with the expression vectors and a reporter template, pARP-HIV.CAT. The activity of this template is stimulated by the actions of HNF-4 (Sladek et al., 1991). Cells that overexpressed sense HNF-4 had 2−2.5-fold higher CAT activity compared to control (Figure 2D,E). In contrast, CAT activity in cells cotransfected with antisense HNF-4 decreased to one-fourth of that in the control cells, i.e., HuH-7 transfected with pARP-HIV.CAT alone. These data show that sense and antisense HNF-4 stimulated and repressed, respectively, the activity of pARP-HIV.CAT.

HNF-4 Represses the Activity of Site A in BHK Cells. The preceding studies imply that depletion of endogenous HNF-4 using antisense mRNA increases the activity of site A. However, we have not demonstrated clearly that HNF-4 directly inhibits the activity of this element. In order to address this question, we measured site A activity in a cell line, BHK derived from kidney. In contrast to liver, kidney expresses low levels of both HNF-4 and Apo AI (Sladek et al., 1991; Elshourbagy et al., 1985). Low levels of HNF-4 in BHK cells are consistent with our observation that the activity of pARP-HIV.CAT in HuH-7 cells is much higher than that in BHK cells (data not shown). The reporter template, p5A.CAT, was active in the BHK cells. Cotransfection of p5A.CAT and sense HNF-4 at a ratio of 4:1 or 2:1 lead to decreases in CAT activity of 56% and 28%, respectively (Figure 3A,B). This observation clearly shows that rat HNF-4 inhibits the activity of site A.

Opposing Effects of HNF-4 and Thyroid Hormone Receptor on Site A Activity. To try and understand the contribution of HNF-4 to site A activity, we wondered whether the removal of this inhibitory factor would enhance the actions of TRα1 in HuH-7 cells (Romney et al., 1992; Taylor et al., 1996). Therefore, we cotransfected a fixed amount of the reporter p5′A.CAT and TRα1 with increasing amounts of antisense HNF-4 into HuH-7 cells treated with T3. Results of studies (Figure 4) where the p5′A.CAT:antisense HNF-4 ratio was 2:1 and 1:1 revealed a dose-dependent increase in CAT activity of 139% and 186%, respectively, compared to control cells transfected with reporter and TRα1 only. These findings show that expression of antisense HNF-4 mRNA adds to the ability of TRα1 to enhance the activity of site A.

DISCUSSION

In this report, we have examined the effect of rat HNF-4 on a cis-acting element contained in rat Apo AI DNA because of conflicting reports claiming HNF-4 activates or has no effect on site A activity (Fuernkranz et al., 1994;

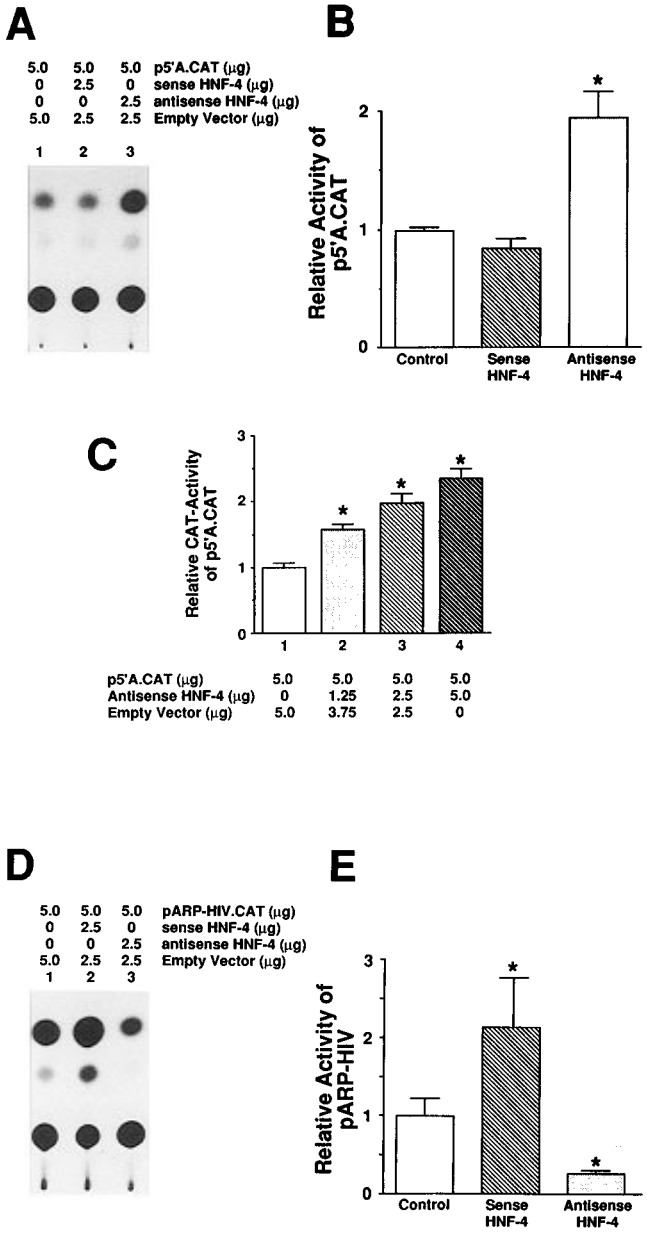

FIGURE 2: Sense and antisense HNF-4 inhibits and enhances, respectively, site A activity. Panel A shows an autoradiograph of CAT assay results from HuH-7 cells cotransfected with p5′A.CAT and empty vector (lane 1), pLEN4S (a template that expresses sense HNF-4) (lane 2), or pLEN4A (a template that expresses antisense HNF-4) (lane 3). Panel B shows a graph of the mean ($n = 4$ for each data point) and SD of CAT activity corrected for transfection efficiency relative to β-gal levels. The asterisk indicates a signficant difference ($p < 0.05$) from the control. Panel C shows a graph of the mean ($n = 4$ for each data point) and SD of CAT activity corrected for transfection efficiency relative to β-gal levels in HuH-7 cells cotransfected with p5′A.CAT and an empty vector or progressively higher amounts of pLEN4A, a vector that expresses antisense HNF-4. Amount of pLEN4A represented in column: 1 is 0 μg, 2 is 1.25 μg, 3 is 2.5 μg, and 4 is 5.0 μg. Panel D shows an autoradiograph of CAT assay results from HuH-7 cells cotransfected with pARP-HIV.CAT and an empty vector (lane 1), pLEN4S (a template that expresses sense HNF-4) (lane 2), or pLEN4A (a template that expresses antisense HNF-4) (lane 3). Panel E shows a graph of the mean ($n = 4$ for each data point) and SD of CAT activity corrected for transfection efficiency relative to β-gal levels.

Ginsburg et al., 1995). The data presented here indicate that rat HNF-4 binds to and inhibits the activity of site A from the rat Apo AI gene. EMSA studies showed that HNF-4 bound to site A (Figure 1) and formed a single complex.

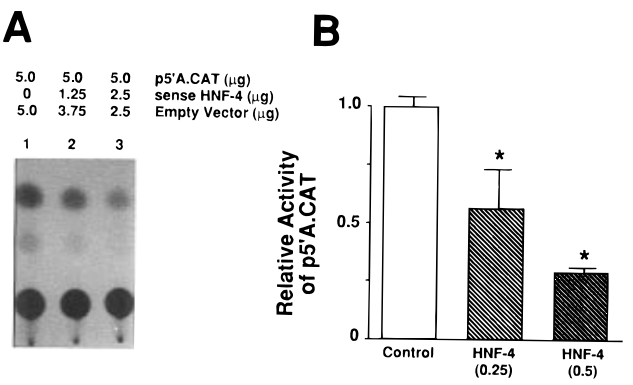

FIGURE 3: Sense HNF-4 decreases p5′A.CAT activity in BHK cells. Panel A shows an autoradiograph of CAT assay results from BHK cells cotransfected with p5′A.CAT and an empty vector or progressively higher amounts of pLEN4S, a template that expresses sense HNF-4. Amount of pLEN4S represented in lane: 1 is 0 μg, 2 is 1.25 μg, and 3 is 2.5 μg. Panel B shows a graph of the mean ($n = 4$ for each data point) and SD of CAT activity corrected for transfection efficiency relative to β-gal levels. The asterisk indicates a significant difference ($p < 0.05$) from the control.

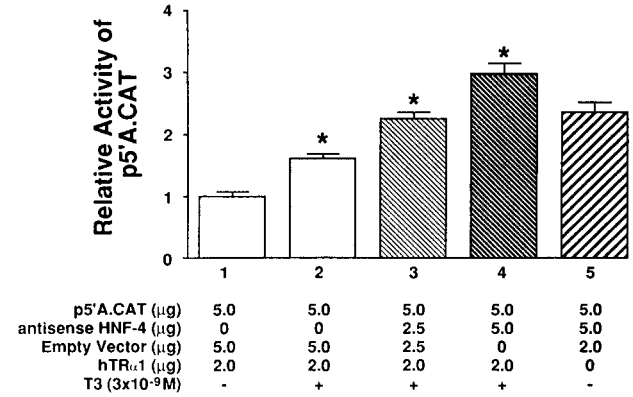

FIGURE 4: Antisense HNF-4 augments the activity of TRα1 on site A activity. Graph of results from HuH-7 cells cotransfected with fixed amounts of p5′A.CAT (5.0 μg) plus human TRα1 (2.0 μg) and an empty vector or progressively higher amounts of pLEN4A, a vector that expresses antisense HNF-4. Amount of pLEN4A represented in column: 1 and 2 is 0 μg, 3 is 2.5 μg, and 4 and 5 are 5.0 μg. Column 5 is CAT activity in cells containing TRα1 and p5′A.CAT treated with T3.

This observation is consistent with previous results of others showing that rat HNF-4 binds to site A from human Apo AI DNA to yield a single complex (Fuernkranz et al., 1994). Since HNF-4 is known to form homodimers in solution or when bound to DNA (Jiang et al., 1995), the single protein−DNA complex likely represents HNF-4 homodimer bound to site A.

The initial observation (Figure 2A, lane 2) in HuH-7 cells transfected with p5′A.CAT and sense HNF-4 showed no significant effect on the activity of site A. However, in cells containing p5′A.CAT and antisense HNF-4, there was a 2−2.5-fold increase in activity of p5′A.CAT. Several explanations for these findings are plausible. First, the observation that sense HNF-4 does not affect site A activity may be due to high endogenous levels of the factor in HuH-7 cells. This idea is consistent with previous results from our studies which suggest HuH-7 cells have high levels of the factor (Chan et al., 1993). Therefore, the added expression of sense HNF-4 in the HuH-7 cells is unlikely to further alter the activity of site A. This scenario may account for the lack of an effect of sense HNF-4 on the activity of rat

site A in HuH-7 cells and possibly explain the inability of HNF-4 to alter human site A activity in Hep G2 cells observed by Ginsburg and others (Ginsburg et al., 1995). Second, the unexpected enhancement of p5′A.CAT activity in HuH-7 cells containing antisense HNF-4 suggests that increased CAT activity arises from lower endogenous levels of HNF-4. This finding is consistent with the idea that rat HNF-4 represses the activity of rat site A.

To be certain that the findings in the preceding studies correlated with HNF-4 expression, we cotransfected HuH-7 cells with sense or antisense HNF-4 and pARP-HIV.CAT, a reporter template whose activity was increased by HNF-4 (Figure 2D,E). As expected, overexpression of sense HNF-4 enhanced but antisense HNF-4 inhibited the activity of pARP-HIV.CAT (Sladek et al., 1991). Since HNF-4 increases pARP-HIV.CAT activity, it seems to argue against the idea that levels of HNF-4 are so high in HuH-7 cells such that added expression leads to no significant effects on site A activity. If HNF-4 acts alone, then this hypothesis is incorrect. However, it is possible that the inhibitory effects of HNF-4 require an auxilary factor(s) the abundance of which may limit its actions on site A. The possibility that the actions of HNF-4 may require an auxilary factor(s) is supported by the studies of the colonic cancer cell line, CaCO2. In these cells, HNF-4 increases the activity of site A (Ginsburg et al., 1995). Given the differential function of HNF-4 in hepatoma vs colonic cells, perhaps the presence of auxiliary factor(s) in these cells enables HNF-4 to have divergent activities.

Although we have provided a reasonable explanation for why sense HNF-4 has no effect on site A activity in hepatoma cells, the ability of HNF-4 to enhance site A activity in yeast remains a puzzle (Fuernkranz et al., 1994). Perhaps yeast cells lack the necessary features, such as the ligand for HNF-4 required by this protein to exert a negative effect. A similar finding has been observed for another member of the nuclear receptor superfamily, TRβ1, which normally represses promoter activity in the absence of T3 (Ohashi et al., 1991). When TRβ1 is expressed in yeast, it has exactly the opposite effect on T3-responsive elements when compared to that in liver cells (Murray et al., 1988). Thus, a mechanism similar to that which reverses the actions of TRβ1 in yeast, may operate on HNF-4 and thus reverse its role in these cells.

The likelihood that HNF-4 is an inhibitor of site A activity was further investigated by the insertion of p5′A.CAT into BHK cells. This cell line comes from kidney, a tissue that normally expresses small amounts of Apo A1, and the levels of HNF-4 are lower than that in liver (Sladek et al., 1991; Elshourbagy et al., 1985). The results of these studies (Figure 3A,B) showed that the activity of site A was repressed in the presence of high levels of HNF-4. This finding provides added support for the inhibitory role of HNF-4 on the activity of site A.

Since TR is normally present in the liver and this receptor increases the activity of site A in the presence of the hormone (Romney et al., 1992; Chan et al., 1993; Taylor et al., 1996; Murray et al., 1988), we wondered whether the repressing effects of HNF-4 contribute to the overall activity of the promoter. To answer this question, we coexpressed both TRα1 and antisense HNF-4 in the same cells (Figure 4). In transfected cells, the activity of p5′A.CAT more than doubled compared to their respective controls. This finding suggests that removal of endogenous HNF-4 by expressing antisense HNF-4 mRNA increases the activity of site A. An extrapolation of this observation to the *in vivo* situation implies that HNF-4 exerts a negative effect on site A derived from rat Apo AI DNA. The finding that HNF-4 represses site A activity is novel because we are unaware of any other examples of a cis-acting element whose activity is down-regulated by HNF-4.

In addition to the results of the current studies showing that HNF-4 binds to and decreases the activity of site A, we and others have noted several proteins belonging to the same superfamily of hormone receptors which also modulate the activity of this motif, including TRα1, 9-*cis*-retinoic acid receptor (RXRα), and Apo AI repressor protein (ARP-1) (Romney et al., 1992; Chan et al., 1993; Taylor et al., 1996; Ladias & Karathanasis, 1991; Rottman et al., 1991). The repressive activities of HNF-4 on site A are not unique, because ARP-1 also decreases the activity of this site (Ladias & Karathanasis, 1991). Given the host of nuclear proteins that have the potential to interact with the A motif, perhaps this element serves as a focal point to coordinate the actions of multiple transcription factors. Other cis-acting elements that have features similar to those of site A, i.e., interact with HNF-4 and other members of the same superfamily, have been reported. For example, the C-motif in rat liver Apo AI and the C3P element of Apo CIII promoters interact with HNF-4 and ARP-1 to activate and repress, respectively, the actions of these sites (Chan et al., 1993; Mietus-Synder et al., 1992). In addition, the erythropoietin gene 5′ promoter and 3′ enhancer contain multiple sites with hexanucleotide motifs that bind HNF-4 or EAR3/COUP-TF to enhance or repress, respectively, the activity of this promoter (Galson et al., 1995). Another element, RAREMCAD, from the medium chain acyl-CoA dehydrogenase gene promoter is similarly up-regulated by HNF-4 and retinoic acid receptors (Carter et al., 1993). The existence of cis-acting elements with features similar to those of site A raises the possibility that these so-called "switches" are sites where different members of the same superfamily of nuclear receptors act in opposite fashions to modulate the activity of the gene (Mietus-Synder et al., 1992; Ktistaki et al., 1994; Ginsburg et al., 1995).

In conclusion, the novel findings in this report include (a) HNF-4 binds to site A of the rat apo A1 promoter and (b) HNF-4 appears to function as a repressor of site A activity. When this information is viewed in light of our previous results showing that HNF-4 also binds to site C of the rat apo AI promoter, it is apparent that this factor interacts with two different cis-acting sites on the same promoter. However, HNF-4 inhibits site A activity but it enhances the activity of site C.

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REFERENCES

Arntzenius, A. C., Krombout, D., Barth, J. D., Reiber, J. H., Bruschke, A. V., Buis, B., van Gent, C. M., Kempen,-Voogd, N., Strikwerda, S., & van der Velde, E. A. (1985) *N. Engl. J. Med. 312,* 805−811.

Carter, M. E., Gulick, T., Raisher, B. D., Caira, T., Ladias, J. A., Moore, D. D., & Kelly, D. P. (1993) *J. Biol. Chem. 268*, 13805−13810.
Castelli, W. P., Garrison, R. J., Wilson, P. W., Abbott, R. D., Kalousdian, S., & Kannel, W. B. (1986) *JAMA 256*, 2835−2838.
Chan, J., Nakabayashi, H., & Wong, N. C. W. (1993) *Nucleic Acids Research 21*, 1205−1211.
Elshourbagy, N. A., Boguski, M. S., Liao, W. S., Jefferson, L. S., Gordon, J. I., & Taylor, J. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8242−8246.
Forman, B. M., & Samuels, H. H. (1990) *Genes 105*, 9−15.
Franceschini, G., Maderna, P., & Sirtori, C. R. (1991) *Atherosclerosis 88*, 99−107.
Fuernkranz, H. A., Wang, Y., Karathanasis, S. K., & Mak, P. (1994) *Nucleic Acids Res. 25*, 5665−5671.
Galson, D. L., Tsuchiya, T., Tendler, D. S., Huang, L. E., Ren, Y., Ogura, T., & Bunn, H. F. (1995) *Mol. Cell. Biol. 15*, 2135−2144.
Gaudet, F., & Ginsburg, G. S. (1995) *J. Biol. Chem. 270*, 29916−29922.
Ginsburg, G. S., Ozer, J., & Karathanasis, S. K. (1995) *J. Clin. Invest. 96*, 528−538.
Glomset, J. A. (1968) *J. Lipid Res. 9*, 155−167.
Graupner, G., Willis, K. N., Tzukerman, M., Zhang, X. K., & Pfahl, M. (1989) *Nature 340*, 653−656.
Hall, R. K., Sladek, F. M., & Granner, D. K. (1995) *Proc. Nat. Acad. Sci. U.S.A. 92*, 412−416.
Herbomel, P., Bourachot, B., & Yaniv, M. (1984) *Cell 39*, 653−662.
Jiang, G., Nepomuceno, L., Hopkins, K., & Sladek, F. M. (1995) *Mol. Cell. Biol. 15*, 5131−5143.
Kane, J. P., Malloy, M. J., Ports, T. A., Phillips, N. R., Diehl, J. C., & Havel, R. J. (1990) *JAMA 264*, 3007−3012.
Ktistaki, E., Lacorte, J. M., Katrakili, N., Zannis, V. I., & Talianidis, I. (1994) *Nucleic Acids Res. 22*, 4689−4696.
Ladias, J. A., & Karathanasis, S. K. (1991) *Science 251*, 561−565.
Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumber, B., Kastner, P., Mark, M., Chambon, P., & Evans, R. M. (1995) *Cell 83*, 835−839.
Mietus-Synder, M., Sladek, F. M., Ginsburg, G. S., Kuo, C. F., Ladias, J. A., Darnell, J. E., Jr., & Karathanasis, S. K. (1992) *Mol. Cell Biol. 12*, 1708−1718.
Miller, N. E., Laville, A., & Crook, D. (1985) *Nature 314*, 109−111.
Murray, M. B., Zilz, N. D., McCreary, N. L., MacDonald, M. J., & Towle, H. C. (1988) *J. Biol. Chem. 263*, 12770−12777.
Nakabayashi, H., Hashimoto, T., Miyao, Y., Tjong, K. K., Chan, J., & Tamaoki, T. (1991) *Mol. Cell. Biol. 11*, 5885−5893.
Ohashi, H., Yang, Y. F., & Walfish, P. G. (1991) *Biochem. Biophys. Res. Commun. 178*, 1176−1175.
Romney, J. S., Chan, J., Carr, F. E., Mooradian, A. D., & Wong, N. C. W. (1992) *Mol. Endocrinol. 6*, 943−950.
Rottman, J. N., Widom, R. L., Nadal-Ginard, B., & Mahdavi, V. (1991) *Mol. Cell. Biol. 11*, 3814−3820.
Sambrooke, J., Fritsch, E. F., & Maniatis, T. (1989*) Molecular cloning: A Laboratory Manual*, 2nd ed., pp 7.37−7.52, Cold Spring Harbor Laboratory Press, Plainview, NY.
Shih, H. M., Liu, Z., & Towle, H. C. (1995) *J. Biol. Chem. 270*, 21991−21997.
Sladek, F. M., Zhong, W., Lai, E., & Darnell, J. E. (1991) *Genes Dev. 4*, 2353−2365.
Taylor, A. H., Wishart, P., Lawless, D. E. Raymond, J., & Wong, N. C. W. (1996) *Biochemistry* (in press).
Thompson, C. C., Weinberger, C., Lebo, R., & Evans, R. M. (1987) *Science 237*, 1610−1614.
Widom, R. L., Ladias, J. A., Kouidou, S., & Karathanasis, S. K. (1991) *Mol. Cell Biol. 11*, 677−687.
Wong, N. C. W., Perez-Castillo, A., Sanders, M. M., Schwartz, H. L., & Oppenheimer, J. H. (1989) *J. Biol. Chem. 264*, 4466−4470.

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