Transcriptional regulation of human microsomal triglyceride transfer protein by hepatocyte nuclear factor-4α

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Abstract Microsomal triglyceride transfer protein (MTP) catalyzes the assembly of triglyceride (TG)-rich apolipoprotein B-containing liver (e.g., VLDL) and intestinal (e.g., chylomicron) lipoproteins. The human MTP gene promoter is reported here to associate in vivo with endogenous hepatocyte nuclear factor-4α (HNF-4α) and to be transactivated or transsuppressed by overexpressed or by dominant negative HNF-4α, respectively. Human MTP (hMTP) transactivation by HNF-4α is accounted for by the concerted activity of distal (−83/−70) and proximal (−50/−38) direct repeat 1 elements of the hMTP promoter that bind HNF-4α. Transactivation by HNF-4α is specifically antagonized by chicken ovalbumin upstream promoter. Transcriptional activation of hMTP by HNF-4α is mediated by HNF-4α domains engaged in ligand binding and ligand-driven transactivation and is further complemented by HNF-4α/HNF-1α synergism that involves the HNF-4α activation function 1 (AF-1) domain. hMTP transactivation by HNF-4α is specifically inhibited by β,β-tetramethyl-hexadecanedioic acid acting as an HNF-4α antagonist ligand. hMTP transactivation by HNF-4α may account for the activation or inhibition of MTP expression and the production of TG-rich lipoproteins by agonist (e.g., saturated fatty acids) or antagonist [e.g., (n-3) PUFA, hypolipidemic fibrates, or Methyl-substituted dicarboxylic acid (Medica) compounds] HNF-4α ligands.—Sheena, V., R. Hertz, J. Nousbeck, I. Berman, J. Magenheim, and J. Bar-Tana. **Transcriptional regulation of human microsomal triglyceride transfer protein by hepatocyte nuclear factor-4α.** ***J. Lipid Res.*** **2005.** 46: **328–341.**

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Microsomal triglyceride transfer protein (MTP) is expressed in liver and intestine, where it plays a central role in the assembly and secretion of triglyceride (TG)-rich, apolipoprotein B (apoB)-containing lipoproteins (e.g., liver VLDL and intestinal chylomicrons). MTP catalytic subunit heterodimerizes with protein disulfide isomerase to catalyze the lipidation of the newly synthesized apoB with TG, cholesteryl esters, and phospholipids in the endoplasmic reticulum lumen (reviewed in 1, 2). In the absence of MTP, lipoprotein assembly is blocked, resulting in apoB ubiquitination and its subsequent proteolysis (3, 4). Blocking lipoprotein assembly may lead to hypo/abetalipoproteinemia, hypotriglyceridemia, and hypocholesterolemia at the expense of hepatic steatosis (5–8). On the other hand, overexpression of MTP may result in increased apoB lipidation, its incorporation into plasma apoB-containing lipoproteins, and hyperlipidemia (9). In addition to apoB lipidation and lipoprotein assembly, MTP has recently been implicated in regulating the ability of CD1d-bearing cell types (e.g., hepatocytes, intestinal epithelial cells) to effect CD1d-restricted antigen presentation (10). Thus, abrogation of MTP function may lead to protection from CD1d-mediated hepatitis and/or colitis.

The 5′-flanking 200 bp of the MTP gene promoter are highly conserved in human and hamster (11). The human MTP (hMTP) promoter consists of consensus sequences for sterol regulatory element binding protein (SREBP; −124/−116), insulin (−122/−111), activator protein 1 (AP-1; −109/−104), and hepatocyte nuclear factor-1α (HNF-1α; −103/−98). Transfection analysis has indicated that the hMTP promoter activity was reduced by more than 95% by deleting the promoter to −87 bp or by mutating its HNF-1α or AP-1 elements (11). The hMTP promoter is further controlled by more distal promoter elements. Of particular interest is the G493T mutation, which results in higher promoter activity with a concomitant increase in the production of postprandial chylomicrons and VLDL (12, 13).

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Abbreviations: AF-1, activation function 1; AP-1, activator protein 1; apoB, apolipoprotein B; CHIP, chromatin immunoprecipitation; CI-DICA, α,α′-tetrachloro-tetradecanedioic acid; COUP-TF, chicken ovalbumin upstream promoter transcription factor; D-DR1, distal direct repeat 1; D-mut, mutated distal direct repeat 1; DR1, direct repeat 1; GFP, green fluorescent protein; hMTP, human microsomal triglyceride transfer protein; HNF-1α, hepatocyte nuclear factor-1α; HNF-4α, hepatocyte nuclear factor-4α; Medica 16, β,β-tetramethyl-hexadecanedioic acid; MTP, microsomal triglyceride transfer protein; P-DR1, proximal direct repeat 1; P-mut, mutated proximal direct repeat 1; PPARα, peroxisome proliferator-activated receptor α; PUFA, polyunsaturated fatty acid; RXR, retinoid X receptor; SREBP, sterol regulatory element binding protein; TG, triglyceride.

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The expression of MTP is regulated by dietary cholesterol and fatty acids. Thus, sterol depletion results in a decrease (14, 15), whereas saturated fat (e.g., hydrogenated coconut oil, tripalmitin, or trimyristin) leads to an increase (16, 17), in MTP expression and its protein content. MTP suppression by cholesterol depletion is mediated by binding of SREBP to the sterol response element in the MTP promoter (15). Modes of activation of MTP expression by saturated fatty acids remain to be investigated.

HNF-4α is a member of the superfamily of nuclear receptors. Mammalian HNF-4α is expressed in liver, kidney, intestine, and pancreas, where it controls the expression of nuclear receptors (e.g., HNF-1α), enzymes, and proteins involved in lipoprotein and lipid metabolism, carbohydrate metabolism, blood coagulation, and others (reviewed in 18). Transcriptional activation by HNF-4α is mediated by its binding as a homodimer to direct repeat 1 (DR1) promoter sequences of target genes. HNF-4α binding to DR1 sequences may be competed out by peroxisome proliferator-activated receptor/retinoid X receptor (PPAR/RXR), retinoic acid receptor/RXR, and chicken ovalbumin upstream promoter transcription factor I or II (COUP-TFI or COUP-TFII) (19–22). Transcriptional modulation by HNF-4α may further be mediated by its physical interaction with other transcription factors (e.g., HNF-1α, COUP) (23, 24).

Until recently, HNF-4α was considered to be an orphan nuclear receptor. However, truncated HNF-4α ligand binding domain (LBD) recombinants have recently been reported to consist of bound long-chain fatty acids (25). Furthermore, various long-chain saturated or unsaturated fatty acyl-CoA thioesters longer than C12 have now been reported by us to specifically bind to the full-length HNF-4α or its LBD recombinants with K_d values in the 2.0–4.0 nM range (26, 27). Also, the transcriptional activity of HNF-4α in transfected cells is affected by added fatty acids as a function of their chain length, unsaturation, or extent of substitution (26, 28). Thus, saturated fatty acids of C14–C16 activate HNF-4α transcriptional activity, whereas its activity is robustly suppressed by (n-3)PUFAs or hypolipidemic xenobiotic amphipathic carboxylates [e.g., fibrates, Methyl-substituted dicarboxylic acid (Medica) analogs] (26, 29).

Selective activation of MTP expression by saturated fatty acids (16, 17) and suppression of TG-rich lipoprotein production by hypolipidemic xenobiotic amphipathic carboxylates (30, 31), together with the capacity of these effectors to activate or inhibit HNF-4α transcriptional activity, respectively (26–29), prompted us to analyze the putative role played by HNF-4α in directly modulating the transcription of the human MTP gene.

EXPERIMENTAL PROCEDURES

hMTP promoter constructs

The (−611/+87)hMTP(WT) wild-type promoter fragment was generated by PCR from genomic DNA. Forward and reverse primers were 5′-TCCCCCGGGTGTGCTAATGACAGACAATGC-3′ and 5′-TCCCCCGGGTATTGACCAGCAATCCTCAAC-3′, respectively. After digestion with *Sma*I, the resulting fragment was cloned into a promoterless CAT reporter vector (32). The (−74/+87) hMTP(WT) wild-type promoter fragment was generated by restriction of the (−611/+87)hMTP *Ava*II site, followed by filling the overhang using the Klenow fragment of DNA polymerase I. The resulting 161 bp fragment was cloned into the *Sma*I site of the promoterless CAT reporter vector. (−611/+87)hMTP(P-mut)-CAT and (−74/+87)hMTP(P-mut)-CAT were generated by in vitro mutagenesis (QuikChange Site-Directed Mutagenesis kit; Stratagene) of (−611/+87)hMTP(WT)-CAT and (−74/+87) hMTP(WT)-CAT, respectively. The mutated P-DR1 (P-mut) primers consisted of 5′-GGAGTTTGGAGTCTGTGCTTTCCCCAAAGATAAAC-3′ (forward) and 5′-GTTTATCTTTGGGGAAAGCACAGACTCCAAACTCC-3′ (reverse) sequences. (−611/+87)hMTP-(D-mut)-CAT was generated by in vitro mutagenesis of (−611/+87)hMTP-(WT)-CAT. The mutated D-DR1 (D-mut) primers consisted of 5′-GTGAGCCCTTCAGTGTTGTTACCTCCTGATTTTGGAG-3′ (forward) and 5′-CTCCAAAATCAGGAGGTAACAACACTGAAGGGCTCAC-3′ (reverse) sequences. Oligonucleotide primers for PCR were prepared by IDT (Coralville, IA). All promoter constructs were confirmed by sequencing.

Expression plasmids

pSG5-HNF-4α wild-type and pSG5-HNF-4α(C179W) were constructed as previously described (28, 33). pSG5-HNF-4α(Y6D, Y14D,F19D) was constructed in three steps using the Quick Change Site-Directed Mutagenesis kit. The following primers were used: Y6D sense (5′-CATGGACATGGCTGACGACAGTGCTGCCTTGG-3′) and antisense; Y14D sense (5′-GCCTTGGACCCAGCCGACACCACCCTGGAGTTTG-3′) and antisense; and F19D sense (5′-CACCACCCTGGAGGATGAAAATGTGCAGGTG-3′) and antisense. Mutant constructs were verified by sequencing. pcDNA3-DN-HNF-4α was constructed by cloning the HNF-4α gene fragment encoding amino acids 112–455, prepared by PCR, into the pcDNA3 *Kpn*I/*Eco*RV sites. The pSG5-mPPARα expression plasmid was from S. Green (34). pSG5-COUP-TFI and pSG5-COUP-TFII were from Y. Bergman (35).

Cell cultures and transfection assays

HepG2 cells grown in MEM-EAGLE medium containing 10% fetal calf serum were transfected for 6 h by calcium phosphate precipitation with 5.0 μg of hMTP promoter-CAT reporter plasmids, washed, and further incubated for 24–40 h. HeLa cells grown in DMEM containing 10% fetal calf serum were transfected overnight by calcium phosphate precipitation with 5.0 μg of hMTP promoter-CAT reporter plasmids, followed by glycerol shock and further incubation for 24 h. When cotransfecting expression vectors, the total amount of DNA was kept constant by supplementing with the pSG5 or pcDNA3 empty vector. CAT values were normalized to β-galactosidase activity.

Chromatin immunoprecipitation analysis

HepG2 cells plated in 100 mm dishes were fixed with 1% formaldehyde, neutralized with glycine for 5 min to a final concentration of 125 mM, rinsed with PBS, and harvested. The cell pellet was rinsed once in TBS (150 mM NaCl, 20 mM Tris-HCl, pH 7.6), frozen in liquid nitrogen, and kept at −70°C. The cell pellet was sonicated to yield DNA fragments of ∼700 bp on average, immunoprecipitated with 3 μg of anti-HNF-4α antibody (Santa Cruz Sc-8987) or with control IgG, and the precipitated DNA was prepared according to Chakravarty et al. (36). The purified DNA isolated by immunoprecipitation was analyzed by PCR using the forward (5′-CTGGTTTGGTTTAGCTCTC-3′) and reverse (5′-GACCCTCTTCAGAACCTG-3′) primers for the (−211/−1) hMTP gene promoter, the forward (5′-CAGCCTGTTTGGGAA-

CTCAG-3′) and reverse (5′-CATACCACCACCAATCACTTC-3′) primers for the (−689/−482)hMTP gene promoter, and the forward (5′-GCCAACGCCAAAACTCTCCCTCC-3′) and reverse (5′-CGAGCCATAAAAGGCAACTTTCG-3′) primers for the β-actin gene promoter. The amplified ^{32}P-labeled DNA fragments were separated by electrophoresis using 8% acrylamide gel and analyzed with a PhosphorImager.

Cell infection

The parent plasmids pAdEasy-1 and pAdTrack-cytomegalic virus (CMV) were provided by H. Giladi (Gene Therapy Department, Hadassah Hospital, Jerusalem, Israel). Recombinant adenoviruses were prepared as described by He et al. (37). Ad-HNF-4α was constructed by cloning a *Bsm*I/*Sph*I fragment of pSG5-HNF-4α encoding amino acids 1–455 into pAdTrack-CMV followed by recombination with pAdEasy-1 to yield an adenovirus harboring the HNF-4α and green fluorescent protein (GFP) cDNAs in tandem, downstream of separate CMV promoters. Viruses were propagated in 293 cells. Hep3B cells were infected with Ad-virus (vector backbone containing the GFP cDNA but lacking HNF-4α cDNA) or Ad-HNF-4α as indicated and cultured for 48 h. Infection efficacy amounted to 70–100%.

hMTP and human apoA-I mRNA levels

Total RNA was prepared using the EZ-RNA kit (Biological Industries, Beit Haemek, Israel). hMTP mRNA levels were determined by semiquantitative RT-PCR. First-strand cDNA used as template was synthesized by reverse transcription using oligo(dT) as primer and Moloney murine leukemia virus RT (Invitrogen). PCR was performed in a total volume of 25 μl containing 1 μl of the RT reaction as template, 10 mM Tris-HCl (pH 9 at 25°C), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 1 μCi of [^{32}P]dCTP, 0.5 μM forward and reverse hMTP primers, 0.05 μM forward and reverse β-actin primers, and 1.25 units of *Taq* DNA polymerase (Promega). PCR conditions consisted of denaturation (94°C, 1 min), annealing (56°C, 1 min), and extension (72°C, 1 min). Fifteen to 18 cycles of this program were within the linear range of amplification for both MTP and β-actin. The following primers were used: MTP forward (5′-TGTGTCAGAATGAAGGCTGC-3′) and reverse (5′-AAGGTCTTCTTCACCTCATC-3′); β-actin forward (5′-TCACCAACTGGGACGACTAG-3′) and reverse (5′-GTACAGGGATAGCACAGCCT-3′). Product sizes were 256 and 200 bp for MTP and β-actin, respectively. The products were separated by electrophoresis on a 10% polyacrylamide gel and quantified with a PhosphorImager. Results are expressed as a ratio of MTP to β-actin.

Human apoA-I was determined by Northern blot analysis. ApoA-I mRNA was probed using the human apoA-I cDNA.

Gel electrophoretic mobility shift assay

Double-stranded radiolabeled oligonucleotides were prepared by the Klenow fragment of DNA polymerase I and [^{32}P]dCTP using the following double-stranded oligonucleotides,

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(P-DR1)hMTP:
5′-AGTTTGGAGTCTGACCTTTCCCCAAAGATA
        CCTCAGACTGGAAAGGGGTTTCTATTTGTA;

(D-DR1)hMTP:
5′-TCAGTGAACTTAGGTCCTGATTTTGGAGTTTGGA
   AGTCACTTGAATCCAGGACTAAAACCTCAAACCTCAGAC;

(−96/−84)hMTP:
5′-ACGTTTAATCATTAATAGTGAGCCCTTCAGTGAA
        ATTAGTAATTATCACTCGGGAAGTCACTT.
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Nuclear extracts were prepared from COS7 cells with or without HNF-4α overexpression and from HepG2 cells. Harvested cells were suspended in hypotonic buffer (10 mM Hepes, pH 7.9, 10% glycerol, 1.5 mM $MgCl_2$, 10 mM KCl, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM DTT, 10 μg/ml aprotinin, and 2 μg/ml leupeptin), incubated for 10 min on ice, homogenized, and centrifuged at 4,000 rpm for 15 min. The pellet was resuspended in low-salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 20 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM DTT, 10 μg/ml aprotinin, and 2 μg/ml leupeptin) followed by dropwise addition of high-salt buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 1.2 M KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM DTT, 10 μg/ml aprotinin, and 2 μg/ml leupeptin) (0.5× packed nuclear volume), incubated for 30 min on ice with continuous mixing, and centrifuged at 25,000 *g*. The supernatant was stored at −70°C.

Nuclear extracts were incubated with or without added unlabeled competitor oligonucleotides or anti-HNF-4α antiserum as indicated in a total volume of 20 μl containing 10 mM Hepes, pH 7.8, 50 mM KCl, 2.5 mM $MgCl_2$, 10% glycerol, 1 mM DTT, and 3.0 μg of poly(dI-dC). After 30 min of incubation on ice, the radiolabeled oligonucleotide was added, and the reaction mixture was further incubated for 15 min at room temperature. Protein-DNA complexes were separated with a 5% polyacrylamide gel and analyzed with a PhosphorImager.

RESULTS

Transactivation of the hMTP gene promoter by HNF-4α

Modulation of hMTP gene expression by HNF-4α was evaluated in Hep3B cells infected with Ad-HNF-4α (38) compared with Ad-virus. Expression of the HNF-4α-responsive gene apoA-I served as positive control. Ad-HNF-4α infection resulted in 60% and 70% increases in human apoA-I and MTP mRNA levels, respectively (**Fig. 1**), indicating that expression of the endogenous hMTP gene was modulated by added HNF-4α.

Transactivation of the hMTP gene promoter by HNF-4α was evaluated in HepG2 cells transfected with a CAT reporter plasmid promoted by the 5′-flanking −611 bp sequence of the hMTP gene promoter [(−611/+87)hMTP-CAT]. Cotransfection of the reporter plasmid together with an expression vector for HNF-4α resulted in a 5-fold increase in hMTP promoter activity (**Fig. 2**), indicating that the 5′-flanking −611 bp sequence of the hMTP gene promoter may account for the transcriptional regulation of the hMTP gene by transfected HNF-4α. Transactivation of the hMTP gene promoter by the endogenous HNF-4α was evaluated by transfecting HepG2 cells with dominant negative HNF-4α (DN-HNF-4α) that lacks the DNA binding domain, thus forming nonfunctional homodimers with the endogenous HNF-4α (38). Cotransfection of the (−611/+87)hMTP-CAT reporter plasmid together with an expression vector for DN-HNF-4α resulted in a 60% decrease in hMTP promoter activity (Fig. 2), indicating that endogenous HNF-4α may limit hMTP transcription in transfection assays.

HNF-4α interaction in vivo with the hMTP gene promoter was evaluated by chromatin immunoprecipitation (CHIP) analysis of HepG2 DNA using anti-HNF-4α antiserum. Endogenous HNF-4α was found to associate with the

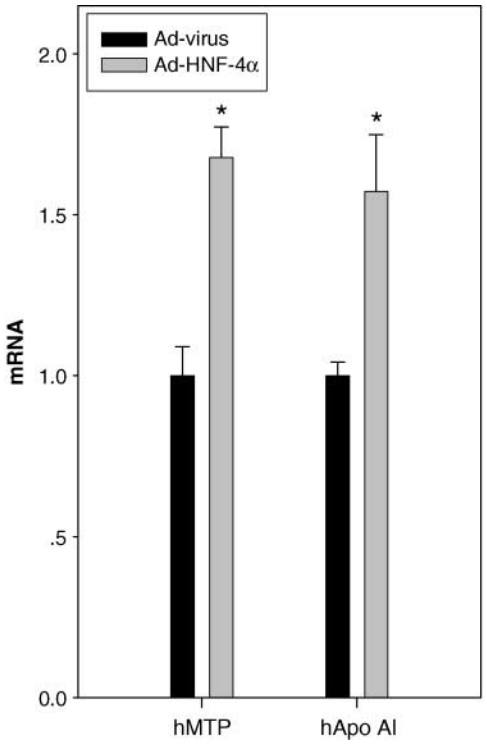

Fig. 1. Effect of hepatocyte nuclear factor-4α (HNF-4α) on microsomal triglyceride transfer protein (MTP) mRNA levels. Hep3B cells were infected with 20–30 multiplicity of infection of Ad-virus or Ad-HNF-4α as described in Experimental Procedures. Human MTP (hMTP) mRNA levels were determined by semiquantitative RT-PCR as described in Experimental Procedures. MTP mRNA (arbitrary units) was normalized to β-actin mRNA levels. Human apolipoprotein A-I (hApoAI) mRNA (arbitrary units) was determined by Northern blot hybridization and normalized to GAP mRNA. Values shown are means ± SEM for three independent experiments. * Differs significantly from MTP or apoA-I mRNA in Ad-virus-infected cells ($P < 0.05$).

(−211/−1)hMTP 5′-flanking promoter sequence but not with the (−689/−482)hMTP promoter sequence or the β-actin gene promoter (**Fig. 3**), indicating that the endogenous HNF-4α specifically interacted in vivo with the hMTP proximal promoter. These results corroborate and extend the recently reported association of HNF-4α with the (−700/+200)hMTP promoter (39).

DR1 elements of the proximal hMTP gene promoter

The (−96/−84)hMTP promoter sequence GTGAGCCCTTCAG has previously been reported by Hagan et al. (11) to serve as a response element for HNF-4α, based on its apparent homology with the HNF-4α response element in the apoB gene promoter (40). Two other putative DR1 sequences that may serve as response elements for HNF-4α in the (−611/+87)hMTP promoter context were indicated by MatInspector analysis: a distal (−83/−70)hMTP DR1 element (D-DR1) consisting of TGAACTTAGGTCCT and a proximal (−50/−38)hMTP DR1 element (P-DR1) consisting of TGACCTTTCCCCA.

HNF-4α binding to DR1 elements of hMTP was evaluated by gel mobility shift assay using the wild-type P-DR1

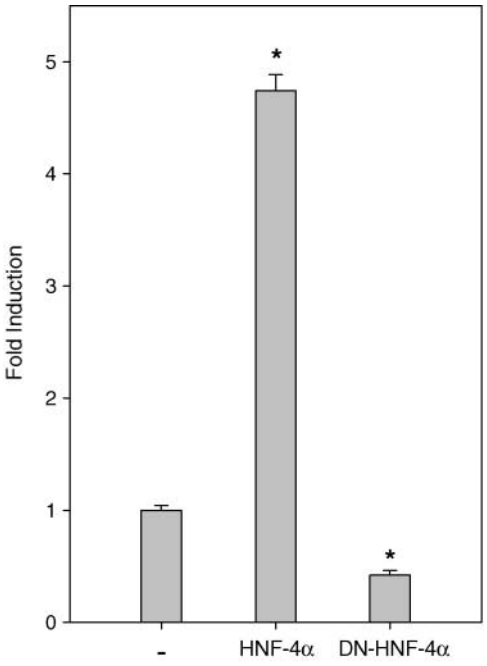

Fig. 2. Transcriptional regulation of hMTP by HNF-4α. HepG2 cells were cotransfected with (−611/+87)hMTP-CAT reporter plasmid and with expression vectors for HNF-4α (0.05 μg), dominant negative (DN)-HNF-4α (0.05 μg), or the pSG5 or pcDNA3 empty vectors as described in Experimental Procedures. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activity in cells transfected with the empty vectors taken as 1.0. Values shown are means ± SEM for four independent experiments. * Differs significantly from CAT activity of pSG5- or pcDNA3-transfected cells ($P < 0.05$).

and D-DR1 oligonucleotides as well as their respective P-mut and D-mut mutants (**Fig. 4A**). Nuclear extract of COS7 cells overexpressing HNF-4α bound to labeled P-DR1, and binding was effectively competed out by nonlabeled P-DR1

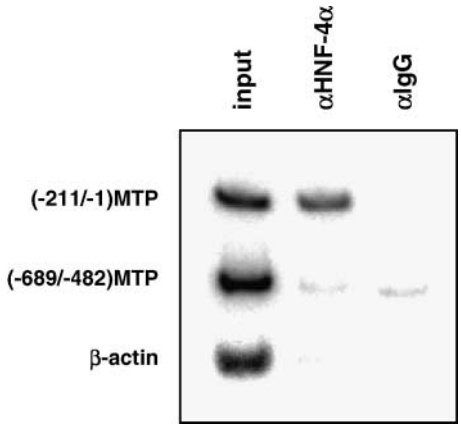

Fig. 3. Chromatin immunoprecipitation analysis of liver hMTP. Soluble chromatin prepared from HepG2 cells was immunoprecipitated with anti-HNF-4α antibody or with control IgG as described in Experimental Procedures. The immunoprecipitated DNA was amplified using primers flanking the (−211/−1)hMTP gene promoter, the (−689/−482)hMTP gene promoter, and the β-actin gene promoter as a negative control as described in Experimental Procedures. Results shown are from one representative experiment out of four independent experiments.

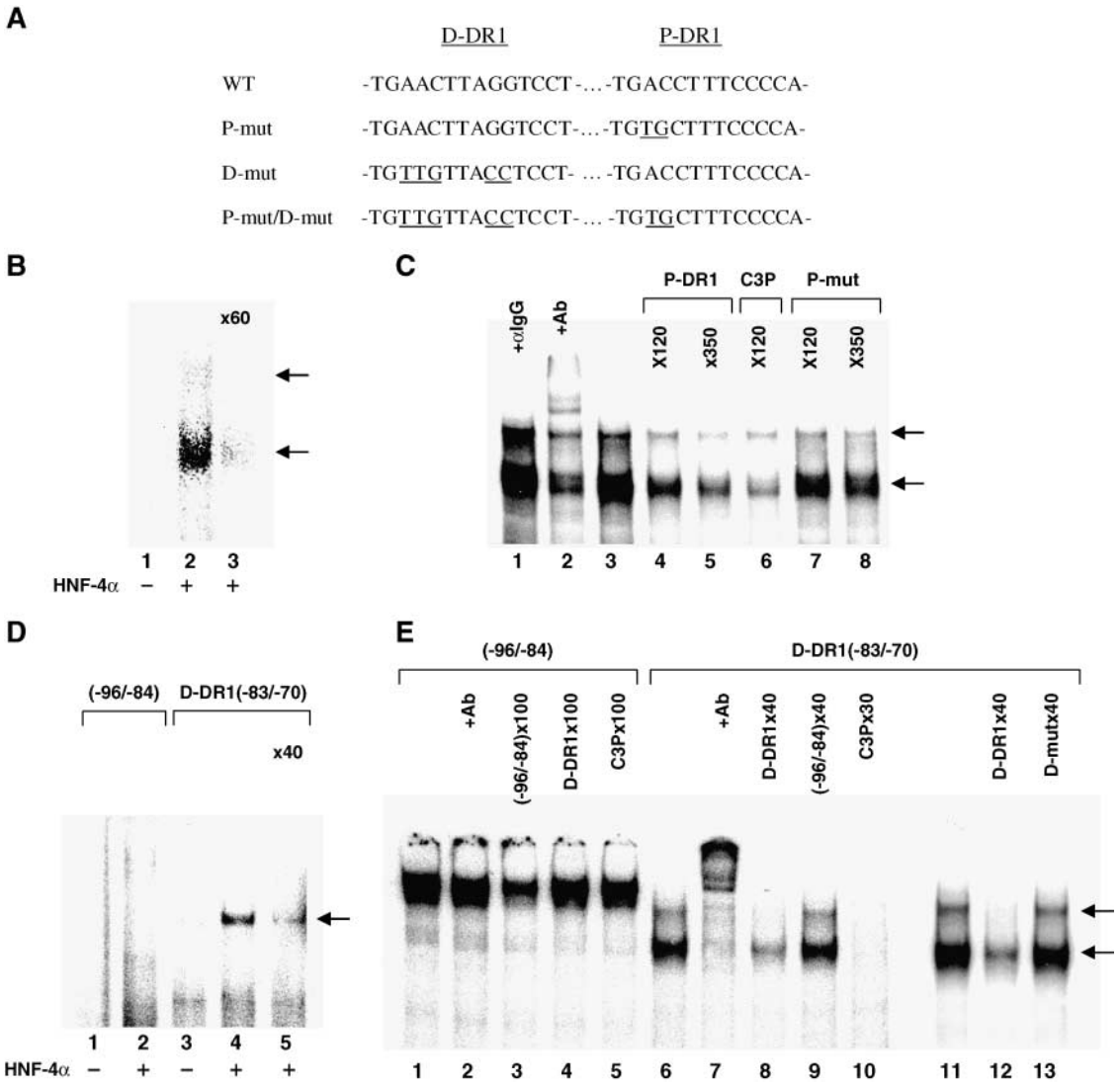

Fig. 4. Binding of HNF-4α to direct repeat 1 (DR1) elements of the hMTP proximal promoter. Gel electrophoretic mobility shift assays were carried out as described in Experimental Procedures. HNF-4α-DNA complexes are indicated by arrows. A: DR1 elements of the hMTP proximal promoter. D-mut, distal DR1 mutant; D-mut/P-mut, double P-DR1 and D-DR1 mutant; P-mut, proximal DR1 mutant; WT, wild-type. B: Nuclear extracts of COS7 cells lacking (lane 1) and overexpressing HNF-4α (lanes 2 and 3) were incubated with double-stranded ^{32}P-end-labeled P-DR1 oligonucleotide. Excess unlabeled self competitor oligonucleotide was added as indicated to confirm specific binding (lane 3). C: Nuclear extracts of HepG2 cells were incubated with double-stranded ^{32}P-end-labeled P-DR1 oligonucleotide in the absence (lanes 1–3) or presence (lanes 4–8) of excess unlabeled P-DR1 competitor, C3P, or P-mut oligonucleotides as indicated. HNF-4α was supershifted by anti-HNF-4α antiserum (Ab; lane 2). D: Nuclear extracts of COS7 cells lacking (lanes 1 and 3) or overexpressing HNF-4α (lanes 2, 4, and 5) were incubated with double-stranded ^{32}P-end-labeled D-DR1 oligonucleotide (lanes 3–5) or the (−96/−84)hMTP oligonucleotide (lanes 1 and 2). Excess unlabeled self-competitor oligonucleotide was added as indicated to confirm specific binding (lane 5). E: Nuclear extracts of HepG2 cells were incubated with double-stranded ^{32}P-end-labeled (−96/−84)hMTP oligonucleotide (lanes 1–5) or D-DR1 oligonucleotide (lanes 6–13) in the absence (lanes 1, 2, 6, 7, and 11) or presence (lanes 3–5, 8–10, 12–13) of excess unlabeled D-DR1, D-mut, C3P or (−96/−84)hMTP oligonucleotide competitors as indicated. HNF-4α supershift was analyzed by adding anti-HNF-4α antiserum (lanes 2 and 7).

(Fig. 4B) but not by P-mut (data not shown). Similarly, HNF-4α of HepG2 nuclear extract bound to P-DR1, and the respective band was supershifted by anti-HNF-4α antiserum (Fig. 4C). HNF-4α binding to P-DR1 was specific, being effectively competed out by nonlabeled P-DR1 or by the nonlabeled HNF-4α/C3P element of the apoC-III gene promoter (20, 33), but less by nonlabeled P-mut (Fig. 4C). Nuclear extract of COS7 cells overexpressing HNF-4α bound as well to labeled D-DR1, and binding was effectively competed out by nonlabeled D-DR1 (Fig. 4D). In contrast to P-DR1 and D-DR1, nuclear extract of COS7 cells overexpressing HNF-4α did not bind to the (−96/−84)hMTP promoter sequence previously reported as a putative response element for HNF-4α (11) (Fig. 4D). Similarly, HepG2 nuclear extract did bind to D-DR1, and the respective band was supershifted by anti-HNF-4α antiserum (Fig. 4E). HNF-4α binding to D-DR1 was specific, being competed out by nonlabeled D-DR1 or by nonlabeled C3P but not by the nonlabeled (−96/−84) hMTP promoter sequence or by D-mut (Fig. 4E). Also, in

agreement with the inefficacy of the (−96/−84)hMTP promoter sequence to compete out HNF-4α binding to D-DR1, HNF-4α did not bind to the labeled (−96/−84) hMTP element, as verified by the lack of supershift by anti-HNF-4α antiserum and by the lack of competition by nonlabeled D-DR1 or C3P (Fig. 4E). Hence, the (−611/+87)hMTP promoter consists of two binding sites for HNF-4α, D-DR1 and P-DR1, whereas the previously reported (−96/−84) hMTP sequence does not serve as a response element for HNF-4α.

P-DR1 and D-DR1 as response elements for HNF-4α

The functional role of P-DR1 and D-DR1 in the context of the hMTP gene promoter was evaluated in HepG2 cells transfected with a CAT reporter plasmid promoted by the (−611/+87)hMTP(WT) wild-type promoter, the (−611/+87) hMTP(P-mut) promoter consisting of the P-mut sequence, the (−611/+87)hMTP(D-mut) promoter consisting of the D-mut sequence, or the (−611/+87)hMTP(P-mut/D-mut) promoter consisting of the P-mut and D-mut sequences replacing the wild-type P-DR1 and D-DR1 se-

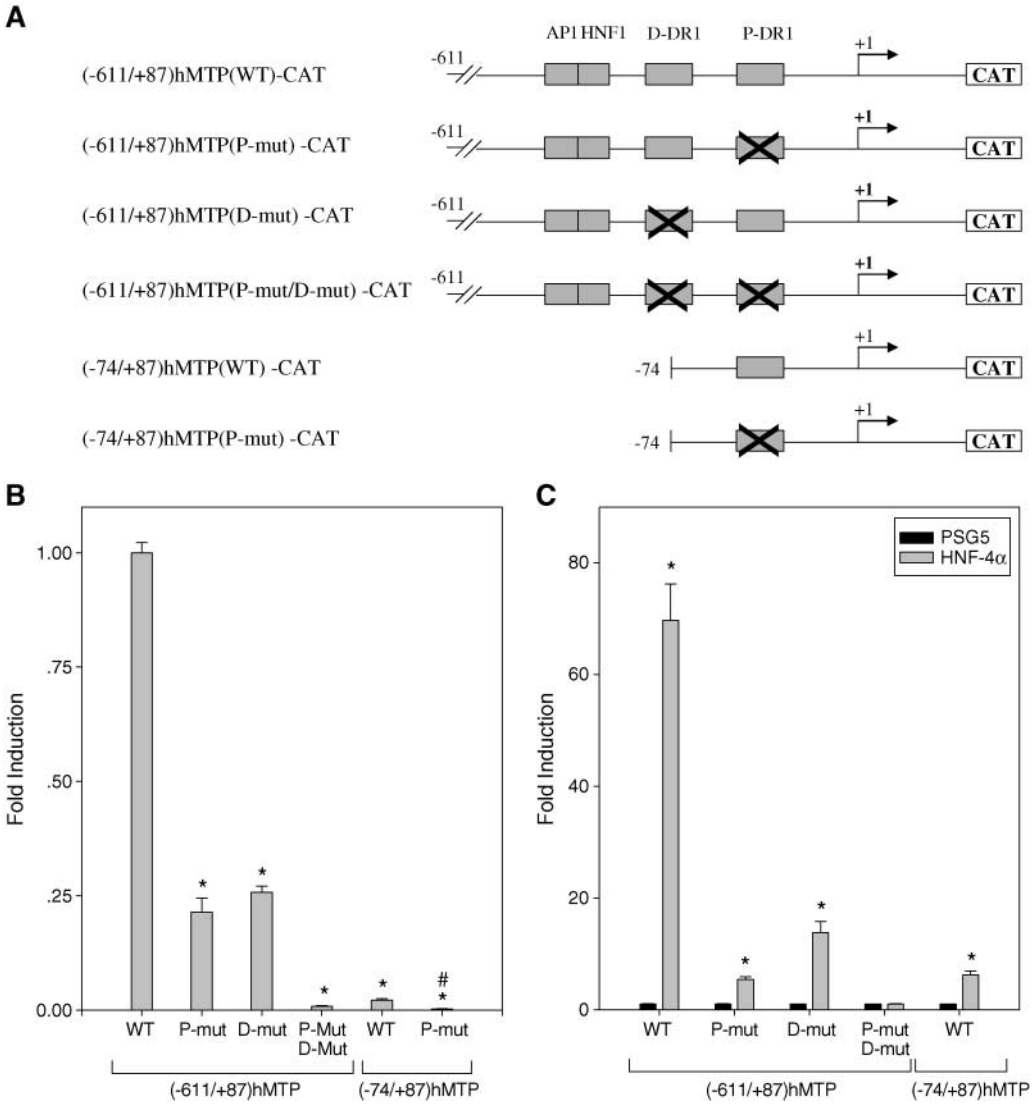

Fig. 5. Transactivation of wild-type and mutated hMTP promoter by HNF-4α. A: hMTP reporter plasmids. B: HepG2 cells were cotransfected with wild-type (WT), P-mut, D-mut, or P-mut/D-mut (−611/+87)hMTP-CAT or with wild-type or P-mut (−74/+87)hMTP-CAT reporter plasmids as indicated. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activity of cells transfected with wild-type (−611/+87)hMTP-CAT taken as 1.0. Values shown are means ± SEM for four independent experiments. * Differs significantly from CAT activity in cells transfected with wild-type (−611/+87)hMTP-CAT ($P < 0.05$); # differs significantly from CAT activity in cells transfected with wild-type (−74/+87)hMTP-CAT ($P < 0.05$). C: HeLa cells were cotransfected with wild-type, P-mut, D-mut, or P-mut/D-mut (−611/+87)hMTP CAT or with wild-type (−74/+87)hMTP-CAT reporter plasmids as indicated and expression vectors for HNF-4α (0.05 μg) or the empty pSG5 vector as described in Experimental Procedures. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activities of cells transfected with pSG5 taken as 1.0. Values shown are means ± SEM for three independent experiments. * Differs significantly from the pSG5 value ($P < 0.05$).

quences, respectively. These studies were complemented by evaluating the promoter activities of the truncated (−74/+87)hMTP-CAT reporter plasmid and of its (−74/+87)hMTP(P-mut)-CAT mutant, consisting of the P-DR1 response element or its respective mutant in the context of (−74/+87)hMTP promoter, devoid of D-DR1 (**Fig. 5A**).

The promoter activity of the (−611/+87)hMTP-CAT reporter plasmid mutated either in its P-DR1 or D-DR1 element was reduced by 75–80% compared with wild-type (−611/+87)hMTP(WT)-CAT (Fig. 5B), thus indicating that each of the two elements was required for the wild-type promoter activity in HepG2 cells. Also, the (−611/+87)hMTP promoter activity was essentially eliminated by mutating both DR1 elements (Fig. 5B). The function of the P-DR1 element was further realized in the context of the truncated (−74/+87)hMTP promoter. In agreement with Hagan et al. (11), the promoter activity of (−74/+87)hMTP was decreased by 95% compared with (−611/+87)hMTP as a result of the deletion of the AP-1 (−109/−104) and HNF-1α (−103/−98) elements. The residual activity of the truncated (−74/+87)hMTP promoter, however, was essentially eliminated in the (−74/+87)hMTP (P-mut) promoter context (Fig. 5B). Hence, the hMTP promoter activity was robustly affected in HepG2 cells by both its P-DR1 and D-DR1 elements.

The contribution made by P-DR1 and D-DR1 to the full promoter activity of (−611/+87)hMTP in response to transfected HNF-4α was evaluated in HeLa cells devoid of endogenous HNF-4α that do not express MTP (11). Cotransfection of (−611/+87)hMTP with an expression plasmid for HNF-4α resulted in a 70-fold increase in the promoter activity. The HNF-4α-dependent promoter activity of (−611/+87)hMTP mutated either in P-DR1 or D-DR1 was decreased by 90% and 80%, respectively, compared with the wild-type promoter, whereas the doubly mutated promoter was essentially not activated by HNF-4α (Fig. 5C). Also, the HNF-4α-dependent promoter activity of the truncated (−74/+87)hMTP-CAT reporter was 10-fold decreased compared with (−611/+87)hMTP-CAT (Fig. 5C). Hence, each of the two elements was required for full transactivation of the (−611/+87)hMTP promoter by HNF-4α.

HNF-4α/HNF-1α synergism

In addition to the HNF-4α response elements P-DR1 and D-DR1, the (−611/+87)hMTP gene promoter consists of a critical (−103/−98)HNF-1α response element necessary for the promoter activity, as previously verified by the 95% decrease in the activity of the hMTP promoter mutated in its HNF-1α element (11). HNF-4α and HNF-1α have previously been reported to physically interact (23) and to transcriptionally antagonize (23) or synergize (41) each other, thus prompting our interest in studying their putative interaction in the context of the hMTP gene promoter.

Putative HNF-4α/HNF-1α interactions in the context of the hMTP gene promoter and the contribution made by P-DR1 and D-DR1 to that interaction were verified in HeLa cells cotransfected with hMTP-promoted CAT reporter plasmids together with expression vectors for HNF-4α and/or HNF-1α (**Fig. 6**). The (−611/+87)hMTP(WT) promoter was 64-fold transactivated by HNF-4α in the absence of transfected HNF-1α but was marginally transactivated by HNF-1α in the absence of transfected HNF-4α (Fig. 6A). However, the wild-type promoter was robustly activated (1,250-fold) by cotransfecting HNF-4α and HNF-1α (Fig. 6A), indicating that the wild-type hMTP promoter activity was limited by each and could be synergistically activated by HNF-4α and HNF-1α. The synergistic interaction between HNF-4α and HNF-1α was independently maintained by each of the two HNF-4α response elements, P-DR1 and D-DR1 (Fig. 6B, C), but was essentially eliminated in the context of the doubly mutated (−611/+87)hMTP(P-mut/D-mut) (Fig. 6D). Hence, HNF-4α docking to at least one of the two HNF-4α response elements of the hMTP promoter was required and sufficient for the HNF-4α/HNF-1α synergistic interaction. Furthermore, the HNF-4α/HNF-1α synergistic interaction maintained by HNF-4α docking to P-DR1 required additional promoter elements, as verified by the lack of activation of the truncated (−74/+87)hMTP promoter by HNF-1α in the absence or presence of added HNF-4α (data not shown). Docking of HNF-1α to its response element in the context of HNF-4α/HNF-1α synergism remains to be verified.

Transcriptional regulation of hMTP by HNF-4α mutants

The requirement for functional ligand-dependent domains of HNF-4α for hMTP transactivation by HNF-4α and for its synergism with HNF-1α was evaluated in HeLa cells cotransfected with wild-type HNF-4α or with the HNF-4α(C179W) mutant, previously shown to be defective in acyl-CoA binding and in recruiting HNF-4α transcriptional coactivators and to lack ligand-dependent transcriptional activity (28). In the absence of transfected HNF-1α, transactivation of the wild-type (−611/+87)hMTP promoter and its P-DR1 or D-DR1 mutants by HNF-4α (C179W) was reduced by 2- to 5-fold compared with wild-type HNF-4α (**Fig. 7**), indicating that ligand-dependent domains of HNF-4α were involved in transactivating the (−611/+87)hMTP promoter. Furthermore, HNF-4(C179W) synergism with HNF-1α was reduced by 40–45% in the contexts of the wild-type, P-mut, and D-mut (−611/+87) hMTP promoters compared with HNF-4α(WT)/HNF1α (Fig. 7), indicating that ligand-dependent domains of HNF-4α could be involved as well in synergizing hMTP transcription by HNF-4α/HNF-1α.

The putative role played by the ligand-independent activation function 1 (AF-1) domain of HNF-4α (42–44) in hMTP transactivation by HNF-4α and in synergizing the activity of HNF-1α was evaluated in HeLa cells transfected with wild-type HNF-4α or the HNF-4α(Y6D,Y14D,F19D) mutant consisting of mutations previously reported to abort AF-1-mediated transcriptional activity of HNF-4α (42, 44). In the absence of transfected HNF-1α, transactivation of the wild-type (−611/+87)hMTP gene promoter or its P-DR1 and D-DR1 mutants by HNF-4α(Y6D,Y14D, F19D) was similar to that of wild-type HNF-4α (Fig. 7), indicating that the ligand-independent AF-1 domain of

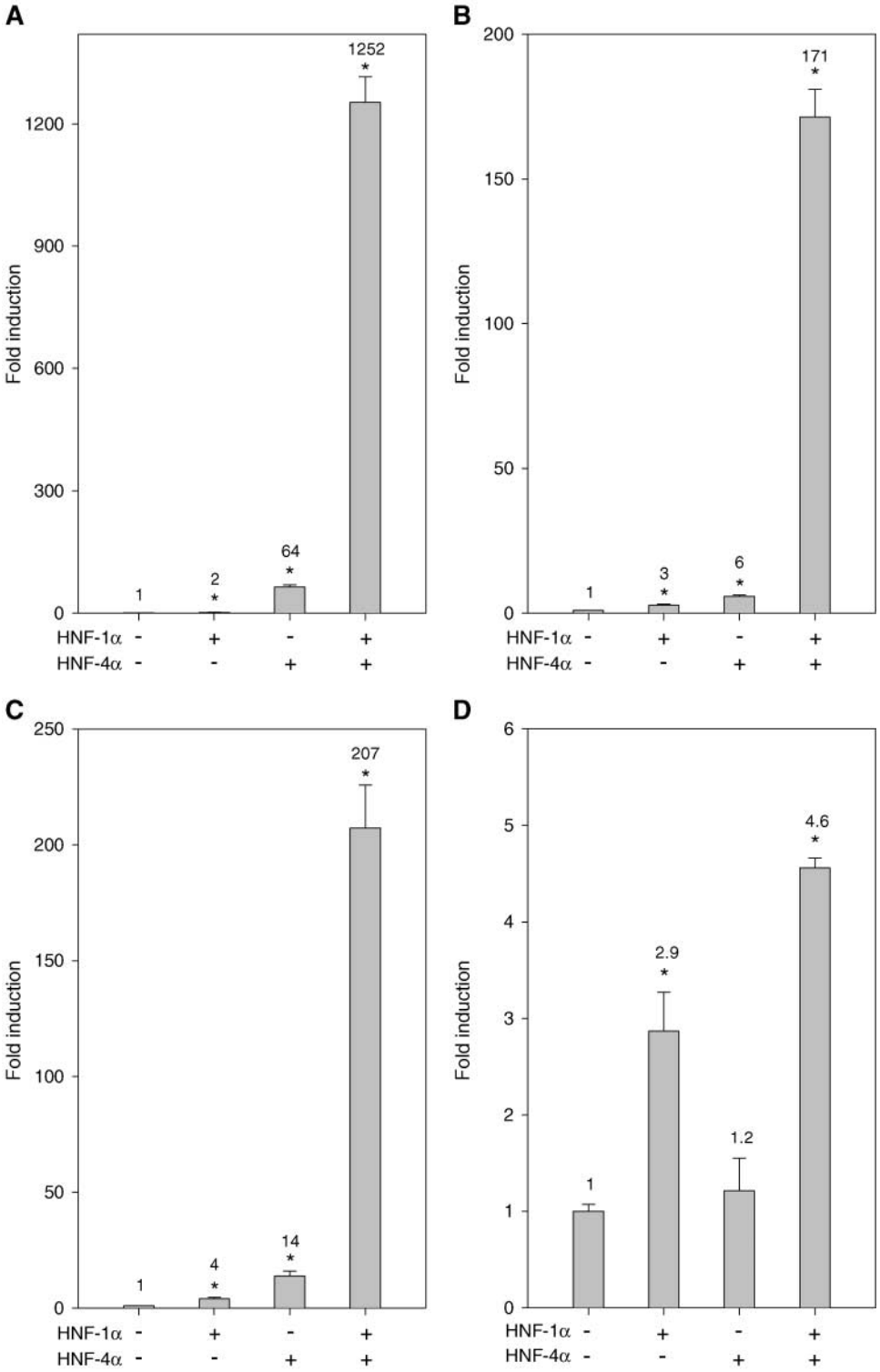

Fig. 6. HNF-4α/HNF-1α synergism. HeLa cells were cotransfected with wild-type (−611/+87)hMTP-CAT (A), (−611/+87)hMTP(P-mut)-CAT (B), (−611/+87)hMTP(D-mut)-CAT (C), or (−611/+87)hMTP(P-mut/D-mut)-CAT (D) reporter plasmids and the indicated expression vectors for HNF-4α (0.05 μg), HNF-1α (0.07 μg), or the empty pSG5 vector as described in Experimental Procedures. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activities of cells transfected with pSG5 taken as 1.0. Fold changes are indicated above the bars. Values shown are means ± SEM for three independent experiments. * Differs significantly from pSG5 values ($P < 0.05$).

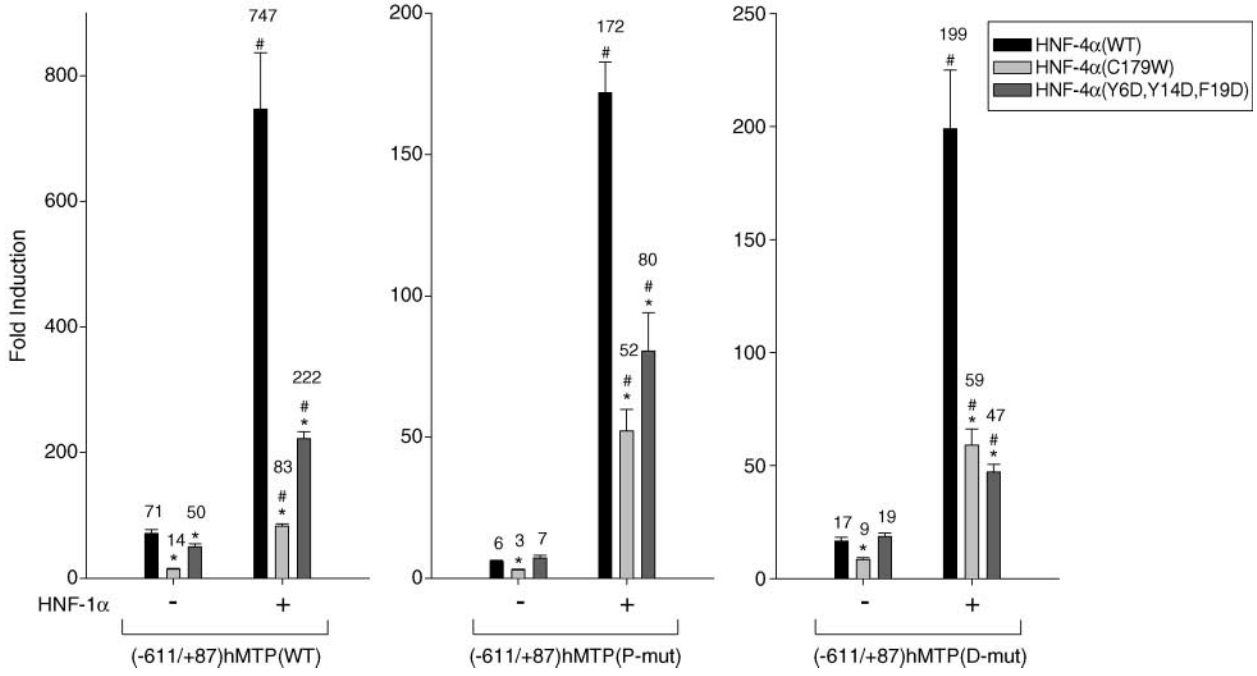

Fig. 7. hMTP transactivation by HNF-4α mutants. HeLa cells were cotransfected with wild-type, P-mut, or D-mut (−611/+87)hMTP-CAT reporter plasmids as indicated and expression vectors for wild-type HNF-4α (0.05 μg), HNF-4α(C179W) (0.05 μg), HNF-4α(Y6D,Y14D,F19D) (0.05 μg), and HNF-1α (0.07 μg), or the empty pSG5 vector as described in Experimental Procedures. HNF-4α mutants were expressed to a similar extent as wild-type HNF-4α, as verified by Western blot analysis of extracts of transfected cells. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activities of cells transfected with pSG5 taken as 1.0. Fold changes are indicated above the bars. Values shown are means ± SEM for three independent experiments. * Differs significantly from HNF-4α wild-type values ($P < 0.05$); # differs significantly from CAT activities in cells transfected with no added HNF-1α ($P < 0.05$).

HNF-4α was not involved in transactivating the (−611/+87)hMTP promoter by HNF-4α. However, HNF-4α(Y6D,Y14D,F19D) synergism with HNF-1α was reduced by 2.5- to 4.5-fold in the context of the wild-type, P-mut, and D-mut (−611/+87)hMTP promoters compared with wild-type HNF-4α synergism with HNF-1α (Fig. 7), indicating that the ligand-independent AF-1 domain of HNF-4α was specifically involved in synergizing hMTP transcription by HNF-4α/HNF-1α.

Transcriptional specificity of P-DR1 and D-DR1

DR1 is shared by PPAR, HNF-4, RXR, and COUP isoforms, prompting our interest in evaluating the transcriptional specificity of P-DR1 and D-DR1 in the context of the (−611/+87)hMTP and the truncated (−74/+87)hMTP promoters in HepG2 cells. HNF-4α appeared to be the only DR1 transactivating factor of the wild-type (−611/+87)hMTP promoter, whereas PPARα was marginally active and COUP-TFI and COUP-TFII were strongly inhibitory (**Fig. 8A**). In contrast to wild-type (−611/+87)hMTP, (−611/+87)hMTP(P-mut) and (−611/+87)hMTP(D-mut) were transactivated by HNF-4α but not inhibited by COUP-TFI or COUP-TFII (Fig. 8B, C). Furthermore, P-DR1 was robustly activated by COUP-TFI or COUP-TFII in the context of the truncated (−74/+87)hMTP promoter (Fig. 8D). Hence, the promoter activities of P-DR1 and D-DR1 acting in concert appear to be positively and negatively dominated by HNF-4α and COUP, respectively. Transactivation by HNF-4α is evident in the context of P-DR1 and D-DR1 acting in concert as well as when acting individually in the context of the P-mut or D-mut mutant of (−611/+87)hMTP or the truncated (−74/+87)hMTP promoters. In contrast, transsuppression by COUP requires P-DR1 and D-DR1 concerted activity, whereas COUP acts as a transactivator (45) rather than a suppressor in the context of the DR1 enhancers acting individually .

Suppression by COUP as a result of specifically antagonizing HNF-4α-mediated transactivation was further verified in HeLa cells overexpressing HNF-4α. As shown in **Fig. 9**, COUP-TFII suppressed the transcriptional activity of HNF-4α, both in the absence of added HNF-1α and in terms of HNF-4α/HNF-1α synergism.

Suppression of hMTP expression and promoter activity by HNF-4α ligand antagonists

HNF-4α ligand agonists (e.g., fatty acids of C14–C16) have been repeatedly reported to induce VLDL production and hyperlipidemia ascribed to stabilizing nascent apoB and promoting its lipidation with hepatic TGs. In contrast, HNF-4α ligand antagonists [e.g., (n-3)PUFAs, fibrates, Medica analogs] induce hypolipidemia ascribed to the suppression of VLDL production (30, 31) together with activation of its plasma clearance (30, 33). The putative role played by direct inhibition of hMTP transcription in the hypolipidemic activity induced by HNF-4α ligand antagonists was evaluated here by analyzing the effect of Medica analogs in suppressing hMTP expression and its

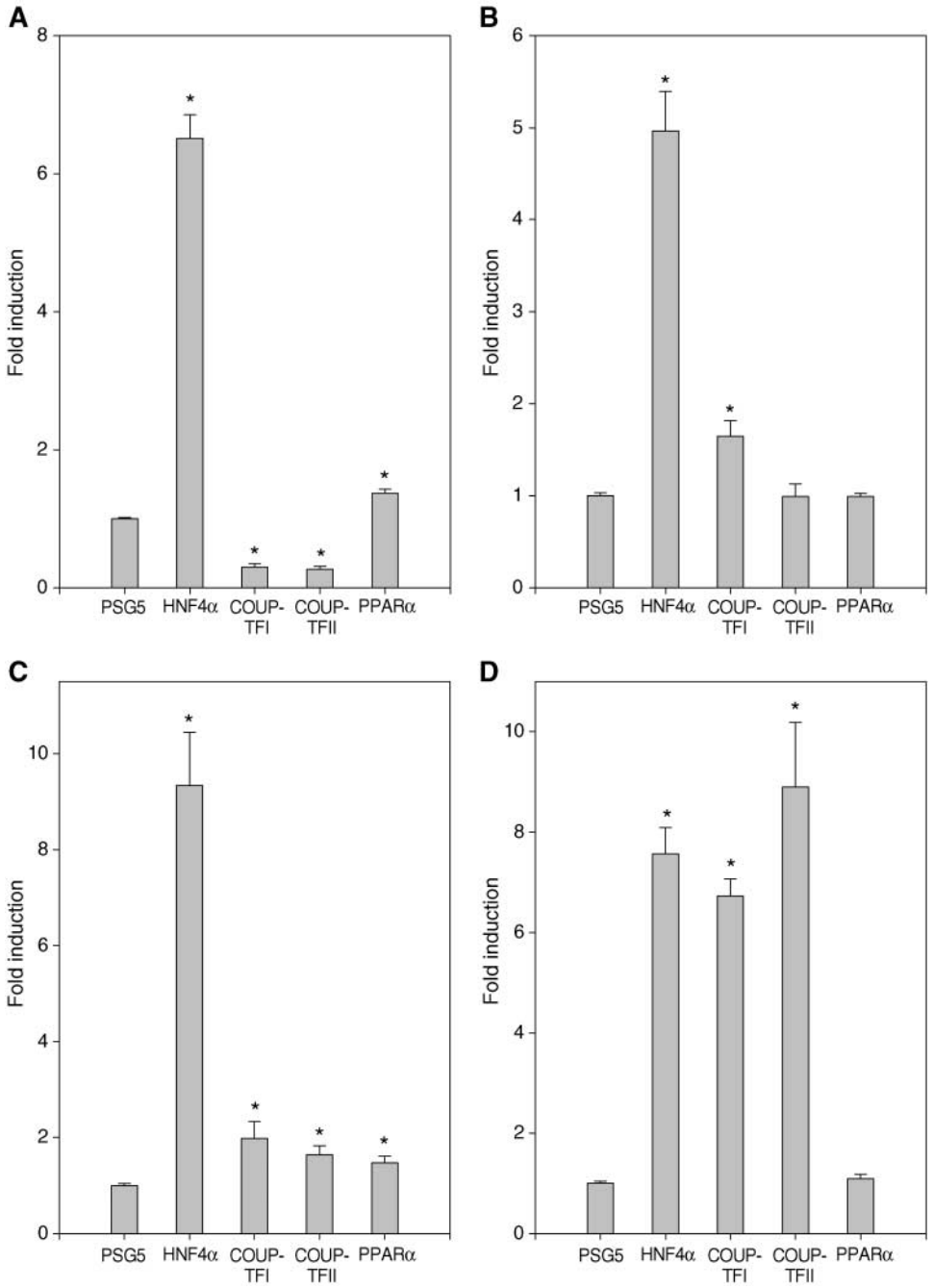

Fig. 8. hMTP transactivation/transsuppression by DR1 nuclear receptors. HepG2 cells were cotransfected with wild-type (−611/+87)hMTP-CAT (A), (−611/+87)hMTP(P-mut)-CAT (B), (−611/+87)hMTP(D-mut)-CAT (C), or the truncated (−74/+87)hMTP-CAT (D) reporter plasmids and the indicated expression vectors (0.1 μg) for HNF-4α, chicken ovalbumin upstream promoter transcription factor I or II (COUP-TFI or COUP-TFII), peroxisome proliferator-activated receptor α (PPARα), or the empty pSG5 vector as described in Experimental Procedures. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activity of cells transfected with pSG5 taken as 1.0. Values shown are means ± SEM for three independent experiments. * Differs significantly from pSG5 values ($P < 0.05$).

promoter activity. hMTP expression in HepG2 cells was robustly inhibited by β,β-tetramethyl-hexadecanedioic acid (Medica 16) or β,β-tetramethyl-octadecanedioic acid (**Fig. 10A**) but not by the structurally related α,α′-tetrachlorotetradecanedioic acid (Cl-DICA) (29), which fails to suppress HNF-4α activity as a result of its nonavailability for ATP-dependent CoA-thioesterification (29). Suppression of hMTP expression by Medica 16 but not by Cl-DICA, was similar to that of apoA-I and apoC-III, serving as a positive control for HNF-4α-responsive genes engaged in lipoprotein metabolism (Fig. 10A). hMTP suppression by Medica 16 was further verified in the context of the (−611/+87)

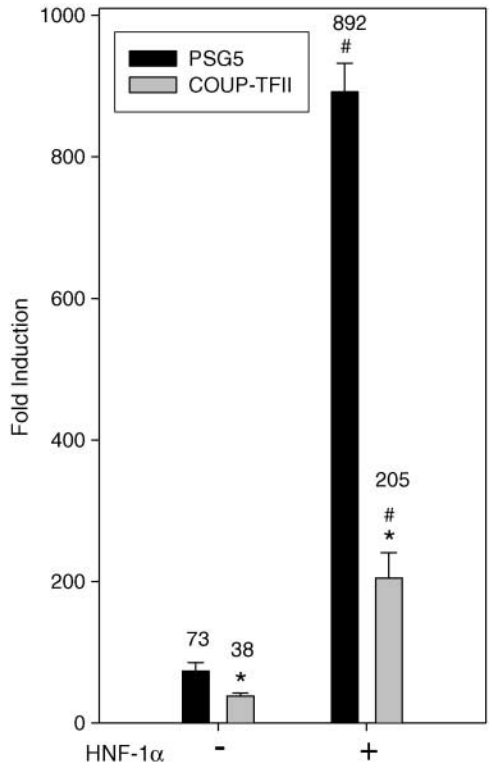

Fig. 9. hMTP transsuppression by COUP-TFII. HeLa cells were co-transfected with (−611/+87)hMTP-CAT reporter plasmid and expression vector for HNF-4α (0.05 μg) in the absence or presence of the indicated expression vectors for HNF-1α (0.07 μg) or COUP-TFII (0.05 μg) as described in Experimental Procedures. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activities of cells transfected with pSG5 taken as 1.0. Fold changes are indicated above the bars. Values shown are means ± SEM for three independent experiments. * Differs significantly from CAT activities in the absence of transfected COUP-TFII ($P < 0.05$); # differs significantly from CAT activities in the absence of transfected HNF-1α ($P < 0.05$).

hMTP promoter activity in HepG2 cells (Fig. 10B) as well as in HeLa cells overexpressing HNF-4α or HNF-4α/HNF-1α (Fig. 10C). Medica 16 suppressed hMTP transactivation by HNF-4α, both in the absence of HNF-1α and in the presence of HNF-4α/HNF-1α. Hence, HNF-4α ligand antagonists may specifically and directly inhibit hMTP promoter activity, which suppresses hMTP expression. Direct inhibition of hMTP promoter activity by HNF-4α ligand antagonists may offer a molecular basis for their hypolipidemic activity.

DISCUSSION

Endogenous HNF-4α was shown here by CHIP analysis to associate in vivo with the 5′-flanking proximal promoter of the liver hMTP gene. Furthermore, the hMTP gene promoter was shown in HepG2 cells to be transactivated or transsuppressed by overexpressed HNF-4α or dominant negative HNF-4α, respectively, indicating that liver hMTP transcription was limited but not saturated by endogenous HNF-4α. Also, the hMTP proximal promoter consists of distal (−83/−70)hMTP and proximal (−50/−38)hMTP DR1 elements that bind HNF-4α. Each of the two DR1 elements was required for HNF-4α-dependent transactivation of the hMTP gene promoter, whereas the doubly DR1-mutated promoter was essentially inactive. Hence, the two DR1 elements of the hMTP promoter were complementary in transactivating hMTP by HNF-4α. These findings corroborate the previously reported robust suppression of MTP in liver HNF-4α knockouts (46) and may further indicate that HNF-4α is directly required for hMTP promoter activity.

Transactivation of the hMTP gene promoter by HNF-4α is mediated by both HNF-4α domains engaged in ligand-dependent transactivation and by HNF-4α/HNF-1α synergism. The role played by HNF-4α domains engaged in ligand-dependent transactivation and recruitment of transcriptional coactivators was demonstrated by the hampered transcriptional activity of the HNF-4α(C179W) mutant (28) compared with wild-type HNF-4α. HNF-4α synergism with HNF-1α was demonstrated by the robust transactivation of hMTP by the HNF-4α/HNF-1α couple under conditions of marginal transcriptional activity of HNF-1α per se. The characteristics of hMTP transactivation by HNF-4α/HNF-1α are worth noting. First, the HNF-1α response element of the hMTP promoter was previously reported to be required for transactivation by HNF-1α, as verified by the 95% decrease in the activity of the hMTP promoter mutated in its HNF-1α element (11). The lack of HNF-4α/HNF-1α synergism in the context of the truncated (−74/+87)hMTP promoter, devoid of an HNF-1α response element, may indicate that docking of HNF-1α to its response element may be required as well for HNF-4α/HNF-1α synergism. HNF-4α/HNF-1α synergism mediated by docking of both to the hMTP promoter is in agreement with the HNF-4α/HNF-1α synergism previously reported in the context of the α_1-antitrypsin gene promoter (41). It is worth noting that the previously reported inhibition of HNF-4α transcriptional activity by its interaction with HNF-1α concerns a promoter devoid of an HNF-1α response element (23) and therefore is not applicable to the hMTP promoter. Second, the synergistic HNF-4α/HNF-1α interaction was mediated by HNF-4α binding to either one of the two DR1 elements of the hMTP proximal promoter. Third, transactivation by HNF-4α in the absence of HNF-1α remained unaffected by mutating the HNF-4α AF-1 domain, whereas the AF-1 domain was specifically involved in HNF-4α/HNF-1α synergism, as verified by the decreased activity of HNF-4α(Y6D,Y14D,F19D)/HNF-1α. Hence, transactivation by HNF-4α/HNF-1α is mediated by distinct HNF-4α domains and distinct DR1 elements of the hMTP promoter.

Similarly to the COUP antagonism of HNF-4α transactivation of the apoC-III, apoB, and apoA-II gene promoters (19, 20), liver hMTP transcription was shown here to be robustly inhibited by overexpressed COUP. Because COUP may compete with HNF-4α for binding to DR1 elements, liver hMTP suppression by COUP may be ascribed to COUP displacement of the endogenous (Fig. 8) or trans-

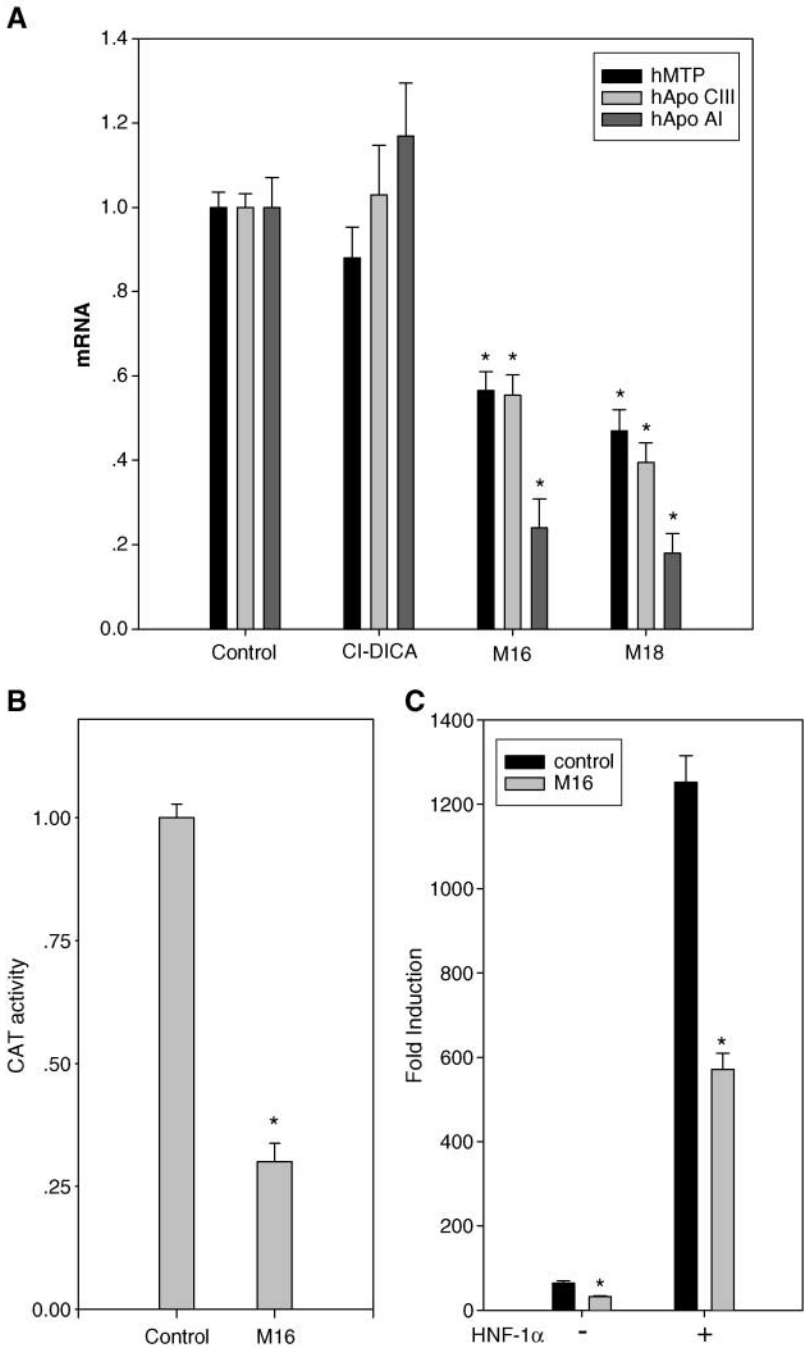

Fig. 10. Suppression of hMTP expression and promoter activity by Methyl-substituted dicarboxylic acid (Medica) analogs. A: HepG2 cells were incubated for 48 h in the absence or presence of 250 μM α,α′-tetrachloro-tetradecanedioic acid (Cl-DICA), β,β-tetramethyl-hexadecanedioic acid (Medica 16; M16), or β,β-tetramethyl-octadecanedioic acid (M18). hMTP mRNA levels were determined by semiquantitative RT-PCR as described in Experimental Procedures and normalized to β-actin mRNA levels. Human apoA-I and apoC-III (hApo AI and hApo CIII) mRNA levels were determined by Northern blot hybridization and normalized to GAP mRNA levels. Values shown are means ± SEM for three to five independent experiments for each ligand. * Differs significantly from the nontreated value ($P < 0.05$). B: HepG2 cells were transfected with (−611/+87)hMTP-CAT reporter plasmid as described in Experimental Procedures and further cultured for 40 h in MEM-EAGLE medium containing 10% fetal calf serum supplemented with 200 μM M16 or empty vehicle. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activity in the absence of M16 taken as 1.0. Values shown are means ± SEM for five independent experiments. * Differs significantly from the nontreated value ($P < 0.05$). C: HeLa cells were cotransfected with (−611/+87)hMTP-CAT reporter plasmid and with expression vector for HNF-4α (0.05 μg) as described in Experimental Procedures in the absence or presence of transfected HNF-1α (0.07 μg) as indicated. Cells were then cultured for 40 h in DMEM containing 10% fetal calf serum supplemented with 200 μM M16 or empty vehicle. CAT activities normalized to β-galactosidase are presented as fold induction relative to CAT activity of cells transfected with pSG5 taken as 1.0. Values shown are means ± SEM for five independent experiments. * Differs significantly from the nontreated value ($P < 0.05$).

fected (Fig. 9) HNF-4α bound to DR1 elements of the hMTP proximal promoter. Suppression of rat MTP by COUP-TFII has indeed been previously reported by Kang et al. (47) and ascribed to COUP binding to the (−45/−33) DR1 element of the rat MTP promoter. It is worth noting, however, that suppression of the hMTP promoter activity by COUP required both the P-DR1 and D-DR1 elements. Thus, mutating only one of the two elements or truncation of the distal element resulted in transactivation rather than transsuppression by COUP. Hence, HNF-4α-mediated transactivation as well as COUP-mediated transsuppression of the hMTP gene promoter may require cross-talk between respective transcription factors bound to the proximal and distal DR1 elements of the hMTP promoter.

Suppression of VLDL synthesis by PPARα agonists [e.g., fibrate drugs, (n-3)PUFAs] has been traditionally ascribed to PPARα-induced α-, β-, and ω-oxidation of fatty acids, resulting in curtailing their availability for hepatic TG synthesis and VLDL production. The role played by HNF-4α in transactivating hMTP in the human liver context combined with inhibition of HNF-4α transcriptional activity by HNF-4α ligand antagonists implies a direct suppression of VLDL synthesis by hypolipidemic drugs independently of PPARα.

In conclusion, the hMTP promoter consists of two DR1 elements that may specifically bind HNF-4α. Binding of HNF-4α to the two sites is required for full transactivation of hMTP by HNF-4α. Transactivation by HNF-4α is mediated by coactivator recruitment as well as by interaction with HNF-1α. Transactivation of MTP by HNF-4α may account for the activation of MTP expression by long-chain saturated fatty acids (16, 17) acting as agonist ligands of HNF-4α (26, 28). Suppression of HNF-4α transcriptional activity by its antagonistic fatty acyl ligands [e.g., (n-3)PUFAs] or by xenobiotic amphipathic carboxylates (e.g., fibrate or Medica drugs) (26, 29) may account for MTP suppression and the hypolipidemic activity exerted by these nutrients or drugs. Furthermore, suppression of HNF-4α by its antagonistic ligands may mediate their prospective use in protecting from CD1d-mediated hepatitis or colitis (10).

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