NR2F1 disrupts synergistic activation of the MTTP gene transcription by HNF-4α and HNF-1α

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Abstract  Regulation of microsomal triglyceride transfer protein (MTP) expression mainly occurs at the transcriptional level. We have previously shown that MTTP gene expression was repressed in nondifferentiated intestinal cells by nuclear receptor 2 family 1 (NR2F1). However, mechanisms involved in the repression of MTP by NR2F1 were not elucidated. Here, we show that MTP expression requires hepatic nuclear factor (HNF)-4α transcription factor. Different HNF-1 proteins synergistically enhance MTP promoter activity along with HNF-4α by binding to different cis elements. NR2F1 does not alter individual effects of HNF-4α and HNF-1 proteins on the MTTP gene promoter. However, NR2F1 suppresses synergistic activation of the MTP promoter by HNF-4α/HNF-1α by binding to a direct repeat 1 (DR1) element. This suppression is further enhanced in the presence of nuclear receptor corepressor 1. In short, these studies identified a novel mechanism of MTP repression that involves binding of NR2F1 to the DR1 element and recruitment of corepressors. In this mechanism, NR2F1 does not affect activities of individual transcription factors; instead, it abrogates synergistic activation by HNF-4α and HNF-1 proteins. —Dai, K. and M. M. Hussain. NR2F1 disrupts synergistic activation of the MTTP gene transcription by HNF-4α and HNF-1α. J. Lipid Res. 2012. 53: 901–908.

Supplemental key words  apolipoprotein • microsomal triglyceride transfer protein • repression • Caco-2 • intestine

Microsomal triglyceride transfer protein (MTP) is an essential chaperone for the assembly and secretion of triglyceride-rich apoB-containing lipoproteins (1–5). As in most of the other protein-encoding genes, transcription of the MTTP gene relies on the RNA polymerase II (Pol II) machinery (6). The Pol II-mediated gene transcription involves recruitment of the basal initiation complex, which leads to transcriptional activation (7). The recruitment process is complex but well studied and is influenced by various promoter- and/or enhancer-specific transcription factors. Activity of these transcription factors is further modulated by activators/co-activators and repressors/corepressors, resulting in activation or repression of the target genes, respectively.

The MTTP gene expression is largely controlled at the transcriptional level and is dependent on the presence of an evolutionarily conserved minimal 204 bp MTTP promoter that contains several cis elements (4, 6, 8, 9). Three critical elements involved in MTP regulation are hepatic nuclear factor 1 (HNF-1), HNF-4, and direct repeat 1 (DR1) (4, 6). As the name implies, the HNF-1 and HNF-4 elements interact with HNF-1 and HNF-4 family members. The binding of HNF-4α to HNF-4 element is essential for MTP expression, inasmuch as HNF-4α deficiency abrogates MTP expression in mice (10). The role of the HNF-1 site in MTP expression has not been fully elucidated. There are two major isoforms of HNF-1, HNF-1α and HNF-1β, which bind to the putative HNF-1 sequences as a homo- or hetero-dimer (11). HNF-1α has been shown to synergistically activate MTP expression in hepatic cells with HNF-4α and postulated to be essential for its tissue-specific expression (12). But HNF-1α gene deficiency in mice (13) has no apparent effect on MTP expression. This could be due to the possibility that HNF-1β might interact with the MTTP promoter in the absence of HNF-1α. A further complicating aspect of this hypothesis is that HNF-1β exists in two isoforms, HNF-1β(a) and HNF-1β(b), which arise due to differential splicing (14). Therefore, we tested the hypothesis that either one or both isoforms of HNF-1β interact with the MTP promoter and synergistically activate its activity similar to HNF-1α. The third element, DR1, could bind to HNF-4α and/or many other transcription factors, mostly retinoid X receptor (RXR) heterodimers, in hepatic cells (12, 15). It has been hypothesized that this element can be critical for both activation and suppression of MTP expression (4, 9).

Abbreviations: ChIP, chromatin immunoprecipitation; DR1, direct repeat 1; HDAC3, histone deacetylase 3; HNF, hepatic nuclear factor; MTP, microsomal triglyceride transfer protein; NCOR1, nuclear receptor corepressor 1; NR2F1, nuclear receptor 2 family 1; Pol II, polymerase II; RXR, retinoid X receptor; WT, wild type.

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We (6) and others (16) have shown that MTP expression is induced during differentiation of a human adenocarcinoma cell line, Caco-2 cells. During cellular differentiation, the binding of HNF-4α and HNF-1α to the MTP promoter did not change significantly, but there was a significant increase in the binding of Pol II and acetylated histone 3 to the MTTP promoter (6). Therefore, induction of the MTTP gene expression was not related to increases in the binding of different transcription factors to the promoter. Instead, the induction was dependent on the reduced expression and binding of nuclear receptor 2 family 1 (NR2F1), a putative transcriptional repressor, to the MTTP promoter. However, the molecular mechanisms of how NR2F1 represses MTTP transcription were not illustrated. In this study, we studied the binding of NR2F1 to the cis elements in the minimal MTTP promoter, evaluated the activation potential of the two HNF-1β isoforms, and elucidated the mechanism by which NR2F1 represses MTTP gene expression.

METHODS

Chemicals
Most chemicals, including DNA oligonucleotides, were purchased from Sigma (St. Louis, MO), whereas most primary antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), unless indicated otherwise.

The cDNA plasmids of HNF-1α (GenBank: BC104910), HNF-1β (GenBank: BC017714), and nuclear receptor corepressor 1 (NCOR1; GenBank: BC167451) were obtained from Open Biosystems, Inc. (Huntsville, AL), and the inserts were subcloned into pcDNA3.1 vector. The HNF-1β(b) expression plasmid was generated with the following primers: GAGGATCCCTCGAGACACGGTCAAGCACAGG and GCTCGTTGATGACGTCGCAGAGATCTC, using the QuikChange kit (Stratagene; Santa Clara, CA), according to the manufacturer’s instructions. The resultant HNF-1β(b) vector lacked 78 bp (e.g., 26 amino acids), from position 740 to 817 of the HNF-1β(a) mRNA sequence. All of these vectors were sequenced by Genewiz, Inc. (South Plainfield, NJ) and were found to match with those in the GenBank.

Cell culture and transfection
HEK293 and Caco-2 cells were maintained in Dulbecco’s modified medium supplemented with 10% FBS, streptomycin, ampicillin, and glutamine in a humid 37°C cell culture incubator aired with 5% CO2. For transient transfection, cells were seeded at 40% confluence in 60 mm dishes. Fresh growth media containing 8 µg/ml polybrene (Sigma) and ~1 × 10^6 viral particles were added the following morning. After overnight incubation, the virus-containing media was replaced with fresh growth media and the transduced cells were allowed to grow for 2 more days before collection for RNA or protein extractions.

Western blot
Cell lysates from HEK293 or Caco-2 cells were resolved on 5–15% gradient SDS PAGE and then transferred onto a nitrocellulose membrane. The primary antibodies anti-β-actin (Sigma), anti-HNF-1α (sc-10791), anti-HNF-1β (sc-22840), anti-HNF-4α (sc-8978), anti-NR2F1 (sc-6575), and NCOR1 (Bethyl Laboratories, Inc.) were used to detect their target proteins with an Amersham ECL kit (GE Life Sciences; Pittsburg, PA). Next, the ECL-developed membranes were exposed to X-ray films. The imaging data were scanned and then processed using Photoshop (Adobe; San Jose, CA).

Chromatin immunoprecipitation
Chromatin immunoprecipitation (ChIP) was used to study the binding of transcription factors to the MTTP promoter (6). In brief, Caco-2 cell cultures 4 and 14 days old were fixed with 1% formaldehyde. After 15 min at room temperature, cells were harvested, and nuclear fractions were isolated and sonicated. Approximately 25 µg of cross-linked genomic DNA in 1 ml 1% BSA/PBS was incubated overnight at 4°C with 10 µl of antibodies against NR2F1, HNF-4α, HNF-1α, or a control rabbit IgG. The following day, 50 µl of protein G beads (GE Life Sciences) were used to precipitate DNA. Then DNA was extracted by phenol-chloroform after reversal of cross-linkage by incubating at 65°C for 4 h. PCR (95°C, 3 min; 25 cycles of 95°C, 30 s; 58°C 30 s; 72°C 30 s; 72°C 7 min) was carried out using primers described previously (6) to determine binding of these factors to the MTTP promoter.

RNA isolation and quantitative RT-PCR
Extraction of total RNA, synthesis of the first-strand cDNA, and quantitative PCR, as well as primers for MTP, ARPP0, NR2F1, and NR2F2, were described previously (6). Primers used for NCOR1 are 5′-CTGACAGGCTCAAGAAGG-3′ and 5′-AACCTGTCGAGGCTTGTC-3′.

Statistics
Experiments were performed in triplicate and repeated at least twice. Values are presented as means ± SEM. Statistical significance (P<0.05) was determined using Student’s t test (GraphPad Prism; La Jolla, CA).

RESULTS

Three HNF-1 family members synergistically enhance HNF-4α-mediated activation of the MTTP promoter

The minimal 204 bp MTTP promoter contains cis elements (HNF-1, HNF-4, and DR1) that are critical for differentiation-dependent activation of MTP expression in Caco-2 cells (4, 6, 9). Putative binding of HNF-4α and different HNF-1 family members to various cis elements is

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that the two HNF-1 isoforms also synergistically activated the MTP promoter in the presence of HNF-4, although their efficiency was slightly less than HNF-1α. These findings suggest that MTP promoter activity is

illustrated in Fig. 1A. We compared MTP promoter sequences from seven species using CLUSTAL W and found that these elements are conserved across vertebrates (Fig. 1B), as has been reported before (4, 6, 8, 9). To identify transcription factors that bind to the three conserved cis elements, we cloned HNF-4, HNF-1α, HNF-1β(a), and HNF-1β(b) and studied their expression in HEK293 cells that do not endogenously express these factors (data not shown). Transfection of HNF-1α expression plasmid resulted in the appearance of a protein of ~80 kDa, whereas transfection of HNF-1β(a) and HNF-1β(b) plasmids revealed two protein bands with slightly different molecular mass around 60 kDa (Fig. 2A), consistent with a previous report (14) that HNF-1β(b) is 26 amino acids shorter than HNF-1β(a). As expected, transfection of HNF-4 yielded proteins of ~55 kDa (Fig. 2B). These studies indicate successful cloning and expression of various HNF proteins.

Next, we studied the effect of these transcription factors on the MTP promoter activity using a dual luciferase reporter assay. As shown in Fig. 2C, the minimal MTP promoter activity was weak in HEK293 cells, probably due to the absence of critical transcription factors in these cells, but was robust in undifferentiated Caco-2 cells (see DISCUSSION). Expression of HNF-1β had no effect on the MTP promoter; however, expression of HNF-1α or HNF-1β(a) slightly (~2-fold) increased this activity. By contrast, HNF-4α enhanced the promoter activity by ~15-fold, consistent with mouse genetics and cell culture studies (8, 10); this activity was comparable to that present in undifferentiated Caco-2 cells. Moreover, we coexpressed different HNF-1 isoforms with HNF-4α. HNF-1α synergistically (~30-fold) activated MTP promoter activity when expressed with HNF-4α, consistent with the studies reported in hepatoma cells by Sheena et al. (12). For the first time, we observed that the two HNF-1β isoforms also synergistically activated the MTP promoter in the presence of HNF-4α, although their efficiency was slightly less than HNF-1α. These findings suggest that MTP promoter activity is

![Fig. 1. Evolutionarily conserved cis elements in the MTP promoter. A: Schematic illustration of HNF-1, HNF-4, and DR1 elements in the 204-bp MTP promoter. Possible activators and repressors that could bind to these elements were identified using online software MatInspector (Genomatix). MatInspector predicts that HNF-1α binds to the MTP site and HNF-4α binds to DR1 and MTP sites. Binding of HNF-1β(a) and HNF-1β(b) to the HNF-1 site is based on the data presented in this study. B: The MTP promoter sequences from seven species, including human (1), mouse (2), dog (3), rat (4), hamster (5), chicken (6) and zebrafish (7), were compared to identify conserved homologous sequences using CLUSTAL W (UCSD Biology Workbench). Consensus sequences for different cis elements is shown in bold underneath the original sequences, whereas mutated sequences are shown in italics above the original sequence.](https://example.com/fig1)

![Fig. 2. Synergistic activation potential of HNF-1 family proteins with HNF-4α. A: Expression of three different HNF-1 proteins, HNF-1α, HNF-1β(a), and HNF-1β(b), in HEK293 cells was determined by Western blotting using specific antibodies. B: Western blotting showed transient overexpression of HNF-4α in HEK293 cells. C: Activation of the 204-bp MTP promoter by various HNF-1 proteins. Minimal MTP promoter firefly luciferase (pMTP-204-luciferase) constructs were expressed together with a control plasmid pCMV-RL expressing Renilla luciferase in undifferentiated Caco-2 (open bar) and 293 cells (solid bars). Additionally, these constructs were introduced along with plasmids expressing different combinations of transcription factors as indicated. The control plasmid was used to normalize the transfection efficiency among experiments.](https://example.com/fig2)
synergistically activated by HNF-4α in combination with any one of the three HNF-1 family proteins.

HNF-1, HNF-4, and DR1 elements are important for synergistic activation of MTP expression

To identify cis elements necessary for the minimal and synergistic activation of MTP, we mutated these conserved elements individually or in combination and expressed them along with plasmids expressing HNF-4α alone or together with HNF-1α. Again, the MTP promoter showed negligible activity when expressed alone in HEK293 cells (Fig. 3A, control). Coexpression with HNF-4α enhanced the MTP promoter activity by 15-fold. HNF-4α and HNF-1α synergistically activated the promoter activity by approximately 40-fold. Mutation of the HNF-1 element had no effect on the activation of the MTP promoter by HNF-4α; however, it significantly curtailed the synergistic activation with HNF-1α (Fig. 3B, HNF1m). Mutagenesis of the HNF-4 element significantly reduced but did not abolish the HNF-4α-mediated enhancement of MTP promoter activity (Fig. 3B, HNF4m). Nevertheless, the residual activity was synergistically activated by HNF-1α (Fig. 3B, HNF4m). Similar results were obtained with the mutant DR1 element (Fig. 3B, DR1m). DR1m had significantly low MTP promoter activity in the presence of HNF-4α, and the residual activity was synergistically activated by HNF-1α. Double mutagenesis of the HNF-4 and DR1 elements completely abolished the expression of MTP promoter activity by HNF-4α and its synergistic activation by HNF-1α (Fig. 3B, HNF-4m+DR1m). We interpret these studies to suggest that HNF-4α is able to bind to both the HNF-4 and DR1 elements. The binding of HNF-1α to the HNF-1 element synergistically activates both of these activities.

NR2F1 represses gene transcription by disrupting synergistic activation of the MTP promoter by HNF-4α/HNF-1α

We have previously shown that overexpression of NR2F1 in Caco-2 cells repressed the MTP promoter (6). Hence, attempts were made to find out the molecular basis of MTP gene repression by NR2F1, which has a molecular mass of ~46 kDa when expressed in HEK293 cells (Fig. 4A). We hypothesized that NR2F1 might counteract the activation of the MTP promoter by either HNF-4α or HNF-1α. Therefore, we first studied the effect of NR2F1 on HNF-1α activity (Fig. 4B). Similar to that shown in Fig. 2C, HNF-1α increased MTP promoter activity by about 2-fold. NR2F1 had no effect on the HNF-1α activity. Next, we studied the effect of NR2F1 on HNF-4α activity (Fig. 4C). HNF-4α significantly enhanced the MTP promoter activity, and again NR2F1 had no effect on the HNF-4α activity. These studies eliminated the possibility that NR2F1 might interact separately with these activators and repress their activities.

We then hypothesized that NR2F1 might suppress the synergistic activation of MTP transcription by HNF-4α and HNF-1α. Consistent with data presented earlier (Figs. 2, 3), coexpression of HNF-4α and HNF-1α synergistically activated the MTP promoter activity (Fig. 4D). NR2F1 repressed this synergistic activation by 40% (Fig. 4D). Because we obtained only partial inhibition, we studied the effect of higher concentrations of NR2F1 (Fig. 4E). Transfections with higher amounts of plasmid DNA increased NR2F1 protein levels with no change in β-actin. NR2F1 showed a dose-dependent reduction of synergistic activation of the MTP promoter by HNF-1α/HNF-4α at lower concentrations (Fig. 4E). But, again, at several higher concentrations, it only reduced the synergistic activation by ~40%. These studies indicated that NR2F1 partially suppresses the synergistic activation by HNF-4α/HNF-1α.

Because NR2F1 did not completely repress, we hypothesized that corepressors might be required to abolish synergistic activation by HNF-4α/HNF-1α. Several lines of evidence have indicated that NCOR1 enhances the transrepressive potential of NR2F1 (17). Hence, we asked whether a corepressor is necessary for the repression of the MTP promoter activity. Expression of NCOR1 resulted in the synthesis of a protein corresponding to ~450 kDa (Fig. 4F, arrow). When NCOR1 was expressed along with NR2F1, the MTP promoter was further suppressed (Fig. 4D). However, expression of NCOR1 in the absence of NR2F1 had no effect on synergistic activation of MTP promoter activity by HNF-1α/HNF-4α. These data suggest that NR2F1 represses MTP promoter activity by disrupting the synergy between HNF-4α and HNF-1α and additional recruitment of NCOR1 augments this repression.
NR2F1 represses synergistic activation by binding to the DR1 element

Next, attempts were made to identify the cis element(s) crucial for NR2F1-mediated MTPP repression. MTP promoter luciferase construct and HNF-4α/HNF-1α expression plasmids were coexpressed with or without NR2F1-expressing plasmids. Synergistic activation of the MTP promoter by the HNF-4α/HNF-1α was normalized to 1. As before, NR2F1 partially reduced the synergistic activation of the MTP promoter [Fig. 5A, wild type (WT)]. Similarly, NR2F1 repressed the synergistic activation when the HNF-4 site was mutated (Fig. 5A, HNF-4m). In contrast, DR1m was resistant to NR2F1 repression (Fig. 5A, DR1m). These data indicate that the DR1 element is required for MTPP gene repression by NR2F1.

Then we asked whether NR2F1 binds directly to the DR1 element on the MTPP promoter in Caco-2 cells. ChIP analysis showed that the binding of HNF-4α and HNF-4β to the MTPP promoter was similar in both undifferentiated (Fig. 5B, D4) and differentiated (Fig. 5B, D14) Caco-2 cells. In contrast, NR2F1 and NCOR1 binding was observed only in undifferentiated Caco-2 cells. Experiments were then performed to determine whether this binding suppresses MTPP expression in undifferentiated cells. To test this, we expressed shRNA against NR2F1 and/or NCOR1 in undifferentiated Caco-2 cells. As shown in Fig. 5C, shRNA against NR2F1 (NR2F1-KD) reduced NR2F1 but had no effect on NR2F2 and NCOR1 mRNA levels. Similarly, shRNA against NCOR1 (NCOR1-KD) knocked down only NCOR1 expression, with no effect on NR2F1 and NR2F2. Importantly, MTP mRNA levels were increased ~1.5- to 2-fold after knockdown of either NR2F1 or NCOR1; their double knockdown synergistically enhanced MTPP expression by ~6-fold compared with the control cells. Consistent with its mRNA, MTP protein was also induced in the undifferentiated cells (Fig. 5D). These studies indicate that loss of NR2F1 and NCOR1 derepresses MTPP gene expression in undifferentiated Caco-2 cells.

On the basis of these observations, we propose that the MTPP promoter contains one binding site for HNF-4α, HNF-4β, and NR2F1 (Fig. 5E, top). NR2F1 binds to the DR1 element and represses the synergistic activation of MTPP transcription by HNF-4α/HNF-1α with the help of NCOR1 in undifferentiated Caco-2 cells (Fig. 5E, top). If NR2F1 levels are low, then MTPP transcription is permitted due to derepression (Fig. 5E, bottom).

DISCUSSION

We have previously shown that NR2F1 suppresses MTP expression in undifferentiated Caco-2 cells. Furthermore, there was an inverse relationship between the expression of NR2F1 and MTP at the jejunum-colon and villus-crypt axes in mouse intestines (6). Loss of NR2F1 after differentiation of Caco-2 cells coincided with increases in MTP expression. These studies were interpreted to suggest that high expression of NR2F1 in undifferentiated cells suppresses MTP expression (6). To understand how NR2F1 could inhibit MTP expression, we used HEK293 cells that are deficient in several transcription factors important for MTP expression. These cells were transfected with various MTPP promoter reporter constructs along with different transcription factors. Furthermore, different cis elements in the MTPP promoter were mutagenized to evaluate their

Fig. 4. Mechanisms involved in the repression of the MTPP promoter by NR2F1. A: Western blot analysis showing overexpression of NR2F1 in HEK293 cells; B: NR2F1 does not affect the activation potential of HNF-1α. Cells were transfected with different combinations of plasmids as indicated. C: NR2F1 has no effect on HNF-4α activity. D: NR2F1 represses the synergistic activation of HNF-4α and HNF-1α. WT pMTP-204 and pCMV-RL were transfected together in HEK293 cells. Various combinations of plasmids expressing different transcription factors were cotransfected as indicated, and the promoter activity was scored. E: Effect of different concentrations of NR2F1 on the synergistic activation of the MTPP promoter by HNF-4α/HNF-1α. Various amounts of NR2F1 expression plasmid were introduced into cells, along with MTP promoter and HNF-1α and HNF-4α expression plasmids. Overexpression of NR2F1 in HEK293 cells was determined by Western blot analysis. F: Expression of NCOR1 in HEK293 cells. Lane 1: Cells were transfected with plasmids expressing NCOR1, and cell homogenates (40 µg protein) were separated on 10% SDS-PAGE. Lane 2: Cell homogenates (1 mg total protein) were immunoprecipitated using NCOR1 antibodies, separated on 4–12% gradient gels, and subjected to Western blot analysis. Lane 3: Mock-transfected cells. The anti-NCOR1 antibodies were used for Western blot analysis.
role in NR2F1-mediated suppression of MTP expression. Additionally, direct binding of NR2F1 to the MTP promoter was studied by ChIP assays. Using these approaches, we show that NR2F1 directly binds to the DR1 element in the MTP promoter. Binding of NR2F1 to this element does not affect the individual activation potentials of HNF-4α/HNF-1α. However, it significantly diminishes the synergistic activation of MTP promoter by HNF-4α/HNF-1α. Moreover, NR2F1 in the presence of the corepressor NCOR1 further suppresses the synergistic activity of HNF-4α/HNF-1α. We then extended these studies to Caco-2 cells and showed that the binding of NR2F1 and NCOR1 is high in undifferentiated Caco-2 cells. After differentiation, NR2F1 and NCOR1 did not interact with MTP, suggesting that MTP suppression in undifferentiated Caco-2 cells involves binding of NR2F1 to the DR1 element and recruitment of corepressors to the MTP promoter. To further substantiate that NR2F1/NCOR1 suppress MTP expression in undifferentiated Caco-2 cells, we reduced their expression using shRNA. Knockdown of NR2F1 and NCOR1 significantly increased MTP expression in differentiated Caco-2 cells. These studies show that MTP expression is suppressed in undifferentiated cells due to the binding of NR2F1 to the DR1 element and recruitment of NCOR1 to this site.

The NR2F family consists of two homologous members, NR2F1 (also known as COUP-TF1 or EAR3) and NR2F2 (COUP-TF2 or ARP-1). Although they were identified as activators, it is now generally believed that they act as repressors. These transcription factors homodimerize or heterodimerize with other nuclear receptors and regulate gene expression involving four different mechanisms (18, 19). First, they bind to a variety of direct repeats and directly compete for the binding of different transcription factors to these repeats. Second, they interact with RXR, thereby interfering with its binding to other receptors. Third, they can actively repress gene expression by interacting with corepressors. Fourth, NR2Fs can transrepress gene expression by interacting with the ligand binding domains of various nuclear hormone receptors. Our studies indicate that the repression of MTP expression by NR2F1 involves a novel mechanism that includes a combination of the first and third mechanisms. In this mechanism, NR2F1 directly interacts with the DR1 element and recruits corepressors to cause gene silencing.

NR2F1/NCOR1 repressed MTTP promoter activity to levels seen in the presence of HNF-4α alone in transfected cells (Fig. 4E) but did not completely silence it. It is known that NCOR1 interacts with histone deacetylase 3 (HDAC3) and this activity is essential for its repressor activity (20). Additionally, the NCOR1 complex contains chromatin remodeling enzymes and CpG methylation machinery (20, 21). We do not know whether these enzymes are present in HEK cells used in this study. Therefore, one possibility is that absence of these enzymes might have avoided complete silencing of the MTP promoter activity. Alternatively, these enzymes may not be recruited to and may not silence a promoter activity that is present extrachromosomally, as

Fig. 5. NR2F1 binds to the DR1 element and represses synergistic activation of MTTP promoter by HNF-4α/HNF-1α. A: WT and mutant pMTP-204 plasmids as illustrated were cotransfected with HNF-4α/HNF-1α expression vectors along with or without NR2F1-expressing plasmids. Expression of NR2F1 reduces the activity of the WT and HNF-4m promoters but not that of the DR1m promoter. ** P < 0.01; NS, not significant. B: ChIP assay showing the binding of NR2F1 and NCOR1 to the MTP promoter in nondifferentiated Caco-2 (D4) cells. These transcription factors did not bind to MTP in differentiated cells (D14). C: Knockdown of NCOR1 and NR2F1 in undifferentiated Caco-2 cells increases MTP mRNA level. Caco-2 cells were transduced with lentivirus or viruses expressing shRNA against NR2F1 (NR2F1-KD) and NCOR1 (NCOR1-KD) as indicated. Cells were collected 2 days after infection and used to measure MTP, NR2F1, NR2F2, and NCOR1 by quantitative RT-PCR. ** P < 0.01. (D) Western blot analysis of MTP expression after NR2F1 and NCOR1 knockdown in undifferentiated Caco-2 cells. E: A proposed model explaining the mode of action of NR2F1 in regulating the MTTP promoter in undifferentiated and differentiated Caco-2 cells. Top: Three major cis elements in the MTP promoter are shown. These are occupied by different transcription factors. Despite the binding of HNF-4α and HNF-1α to their putative cis elements in the MTP promoter, binding of NR2F1 and NCOR1 to the DR1 element suppresses MTP gene expression. Bottom: In the absence of NR2F1/NCOR1, MTP gene transcription occurs in differentiated Caco-2 cells.
in promoter-reporter plasmids. This is supported by the observation that the expression of promoter-reported constructs in undifferentiated Caco-2 cells results in significant expression of the promoter activity despite lack of endogenous gene expression. These studies suggest that endogenous gene silencing might involve recruitment of deacetylases and chromatin remodeling enzymes by NCOR1. In contrast, extra-chromosomal plasmids are not subjected to chromatin remodeling to achieve maximum suppression of the endogenous gene silencing might involve recruitment of NCOR1/HDAC3 to deacetylate and remodel chromatin structure to silence the gene.

NCOR1 is now well recognized as transcriptional repressor of multiple genes. Recently, Doyon et al. (22) showed that NCOR1 protein levels are high in intestinal crypt cells and that its protein levels are low in the uppermost villus cells. We have previously (6) shown that MTP expression is low in crypt cells and high in villus cells. Thus, there is a reciprocal relationship between MTP expression and NCOR1 along the villus-crypt axis. This relationship is consistent with the idea that NCOR1 represses MTP expression. Doyon et al. (22) also showed that NCOR1 expression is high in proliferating Caco-2 cells and that its protein levels decrease during differentiation. Our observation that NCOR1 binds less to the MTTP promoter in differentiated cells might be related to decreases in its protein levels. Thus, reduced levels of NCOR1 might play a role in the expression of MTP during differentiation of Caco-2 cells and possibly during the differentiation of crypt cells into enterocytes.

It has been shown that HNF-1α synergistically activates HNF-4α in cell culture studies (12); however, MTP expression is not altered in HNF-1α knockout mice (13). This could either be due to the fact that HNF-1α is not involved in MTP expression or another transcription factor could substitute for its activity. To delineate between these two possibilities, we tested the hypothesis that HNF-1β might compensate for the loss of HNF-1α by cloning two different isoforms of HNF-1β. Individually, both the isoforms had very little activation potential for MTP promoter, similar to HNF-1α. Analogous to HNF-1α, both HNF-1β isoforms were able to synergistically activate MTP expression. These studies indicate that in the absence of HNF-1α, either of the two HNF-1β isoforms can bind to the HNF-1 cis element in the MTTP promoter and synergistically enhance HNF-4α activity and provide an explanation for the lack of effect of HNF-1α deficiency on MTP expression in HNF-1α knockout mice.

Functional interactions between HNF-1α and HNF-4α have been described for several genes involving different mechanisms [(23) and references therein]. In general, when HNF-1α and HNF-4α proteins interact with two different cis elements, they synergistically enhance promoter activity. The possibility that HNF-1β could substitute for HNF-1α for the synergistic activation of promoter activity with HNF-4α has been examined for the α1-antitrypsin gene. In this case, HNF-1β does not substitute for HNF-1α in synergistically activating α1-antitrypsin promoter with HNF-4α (24).

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