Transcriptional Regulation of Human and Hamster Microsomal Triglyceride Transfer Protein Genes

CELL TYPE-SPECIFIC EXPRESSION AND RESPONSE TO METABOLIC REGULATORS*

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In order to characterize the molecular mechanisms that dictate microsomal triglyceride transfer protein (MTP) gene transcription in human and hamster, two species with similar plasma lipoprotein profiles, the MTP gene promoters were cloned, sequenced, and functionally characterized by transient transfection analysis. The results presented in this report indicate that the 5' ends of human and hamster MTP genes share similar structural features. The promoter sequences are well conserved and consist of similar functional elements. Transient transfection analysis of MTP promoter-driven luciferase gene expression showed that the promoter is active in liver and intestinal cells but not in epithelial cells, consistent with endogenous MTP gene expression. The -123 to -85 bp region of the human promoter is critical for the expression and contains the consensus recognition sequences for liver cell-specific factors HNF-1 and HNF-4 and activator protein AP-1. The promoter contains a modified sterol response element and a negative insulin response element. The human promoter activity is positively regulated by cholesterol and negatively regulated by insulin. From the functional analysis of MTP promoters, it is concluded that the elements that regulate the cell type-specific expression in human and hamster are well conserved and that insulin and cholesterol can regulate the activity of the MTP promoter in opposite directions.

Microsomal triglyceride transfer protein (MTP) catalyzes the transfer of triglycerides, cholesteryl esters, and phospholipids between the phospholipid surfaces, and it is found in the endoplasmic reticulum of hepatocytes and enterocytes where it is involved in the assembly of apolipoprotein B (apoB) containing particles (1 and see references therein). MTP is a heterodimer consisting of an 88-kDa catalytic domain non-covalently associated with the 58-kDa multifunctional protein disulfide isomerase (PDI). The human MTP large subunit cDNA and gene have been cloned and the gene, about 55 kb in length, is localized to the short arm of chromosome 4 at position 4q24 (2). This single copy gene, made up of 18 exons, is transcribed primarily in liver and intestine and the expression in hamster is modulated by various dietary conditions and metabolic regulators (3–5). It has been recently demonstrated that MTP is absent in human patients with the rare autosomal recessive disease, abetalipoproteinemia (3, 4, 6). These patients show a defect in the assembly and secretion of plasma lipoproteins containing apoB and a deficiency in fat-soluble vitamin absorption, transport, and utilization (7 and references therein). The studied patients have nonsense mutations/deletions in the MTP gene coding sequences leading to the absence of functional protein.

The mammalian tissue-specific gene expression and response to metabolic regulators are controlled at the level of transcription by the complex interaction of tissue-specific trans-acting factors bound to upstream cis-acting elements, with the transcription machinery formed over the basal promoters and initiator elements (8 and references therein). In order to characterize the molecular mechanisms that dictate the MTP tissue-specific transcription and regulation by metabolic regulators in humans and hamsters, two species with similar plasma lipoprotein profiles, their MTP 5' regulatory elements were cloned, sequenced, and functionally characterized by transient transfection analysis.

MATERIALS AND METHODS

Cloning and Mapping of the Promoter Region—a dash II recombinant phage containing human MTP gene fragment and hamster genomic DNA library in a λ fix vector (Stratagene, La Jolla, CA), respectively, were screened by hybridization at 65 °C in 6× SSC buffer containing 0.25% SDS with a randomly primed, 32P-labeled 0.5-kb human and hamster EcoRI and NheI cDNA fragment containing exons 1–4 (9). The filters were washed in 1.0× SSC buffer containing 0.1% SDS at 65 °C. The DNA from positive clones was isolated, digested with EcoRI, and exon 1-bearing fragments were identified by Southern blot hybridization with a 68-nucleotide, exon 1 probe. The 1.5-kb human and 4.3-kb hamster EcoRI fragments that hybridized to the exon 1 probes were gel purified and subcloned into an EcoRI-digested Bluescript BSII vector (Stratagene, La Jolla, CA) and sequenced. For hamster, the genomic DNA fragment that hybridized to exon 2 and 3 was also isolated and mapped by restriction digestion, exon-specific hybridization, and sequencing.

Promoter-Luciferase Reporter Gene Constructs—MTP promoter fragments containing varying lengths of 5' flanking sequences and the nontranslated region of exon 1 were generated by polymerase chain reaction. The first exon in the human MTP gene is 146 bp long and in the hamster gene is 148 bp long, and they contain 84 and 85 bp, respectively, of noncoding sequences (2, 5). The 3' end of the human promoter fragment was terminated at +85 bp and the hamster promoter at +78 bp relative to transcription start site, both just short of the MTP transcription initiation codon. The polymerase chain reaction fragments, tailored to contain a KpnI site at the 5' end and a BstII site at the 3' end, after ethanol precipitation were digested with these enzymes, gel purified.
through Gene Clean (Bio101, La Jolla, CA), and cloned 5' to the promoterless luciferase reporter gene into the KpnI and BglII sites in the pGNeo 5' vector (Promega, Madison, WI). In addition, human promoter regions corresponding to -129 bp to -76 bp (normal and mutants) were synthesized with KpnI restriction enzyme recognition ends (Genosys, Woodlands, TX). This oligonucleotide was cloned into the KpnI site present 5' to the MTP-69 bp basal promoter, the -50 bp TATA box minus promoter, and the SV40 early minimal promoter in a pGL2Pro vector (Promega, Madison, WI). All promoter deletion constructs and site-specific mutant constructs were confirmed by sequencing in an automated sequencer (ABI, Foster City, CA). In the pRL30-CAT reporter gene vector used for normalizing the transient transfection, the promoterless CAT gene is driven by the strong housekeeping promoter of the mouse ribosomal protein gene, pRL30 (10). The LDL receptor promoter-luciferase vector was generated by introducing the -146 to +36 bp human LDL receptor promoter DNA fragment 5' to the promoterless luciferase gene in the pGNeo 5' vector (11).

**Cell Culture**—The human cell line HepG2 (liver) was grown in RPMI 1640 and the human CaCo2 cell line (intestinal) and HeLa cell line (epithelial) in Dulbecco's modified Eagle's medium, both media containing 10% fetal bovine serum (FBS), at 37 °C, 5% CO2. Cells were split every 2 to 3 days at 0.05% trypsin-EDTA solution. All cell culture reagents were purchased from Life Technologies, Inc.

**Endogenous MTP Gene Expression**—For the quantitation of relative MTP mRNA levels, total cytoplasmic RNA was prepared (12) and Northern blot hybridized with a random primed 32P-labeled full-length human MTP cDNA probe. Typically, 20 μg of total RNA was separated on a formaldehyde, 1% agarose gel, blotted onto a nitrocellulose filter, and hybridized with the human MTP cDNA and mouse rpL30 cDNA probes. After washing the cells twice with serum-free medium, fresh growth medium was added, the cells were over-layered with formaldehyde, 1% agarose gel, blotted onto a nitrocellulose filter, and further hybridized bands was quantitated by direct counting in a Betascope counter (Betagen, Waltham, MA). The triglyceride transfer activity in the cells was determined using a small unilamellar membrane vesicle assay which determines the transfer of [14C]-labeled triglycerides between donor and recipient vesicles (14)

**Transient Transfection Analysis**—The relative transcriptional activities of MTP promoter fragments were determined by transient transfection analysis in monolayer cultures of HepG2, CaCo2, and HeLa cells. Typically, cells were grown overnight in a 35-mm plate and washed twice with serum-free medium. DNA-lipofectin complex, containing 10 μg/ml MTP promoter-luciferase vector, 5 μg/ml pRL30-CAT vector, and 75 μg/ml lipofectin (Life Technologies, Inc.), was allowed to form for 15 min at room temperature. The complexes were then added to the DNA-lipofectin complex and incubated for 4-6 h at 37 °C. After washing the cells twice with serum-free medium, fresh growth medium containing 10% FBS was added. The transfected cells were harvested after 48 h of incubation at 37 °C, washed twice with ice-cold phosphate-buffered saline, treated in lysis buffer (Promega, Madison, WI), and aliquots of the lysates were assayed for luciferase and CAT enzyme activities. The luciferase enzyme activities were determined according to the manufacturer's (Promega, Madison, WI) instructions, and the luciferase activity was quantitated in a EG-Berthold luminometer. The CAT activity was determined according to standard procedures using [14C]chlo伦amphenicol and ascending thin layer chromatography (13). The radioactivity in the acetylated and non-acetylated chloramphenicol spots was quantitated by direct counting in a Betascope counter (Betagen, Waltham, MA).

**Insulin Effect**—For determining the effect of insulin, the HepG2 cells were transiently cotransfected with MTP promoter-luciferase and pRL30-CAT gene constructs and grown in medium containing 2.5% fetal bovine serum and bovine insulin. An insulin stock solution (1.0 mM) was prepared by resuspending bovine insulin (Life Technologies, Inc.) in 0.25 M Tris-HCl, pH 7.5, buffer. Transfected cells were harvested after 48 h and assayed for enzyme activities. The experiment was done at reduced amount of serum in order to minimize the effect of insulin present in the serum.

**Cholesterol Effect**—For determining the effect of sterols, the HepG2 cells were transiently cotransfected with MTP-luciferase or LDL receptor-luciferase and pRL30-CAT reporter constructs and grown in medium containing 2.5% de-derivatized FBS, 1.0 μg/ml 25-OH cholesterol, and 10 μM N-acetylcysteine as described previously (11). An equal volume of car-rier ethanol was added to control cells. Transfected cells were harvested after 48 h and assayed for enzyme activities.

**RESULTS**

**Organization of the 5' End of Human and Hamster MTP Genes**—It has been recently reported that the human MTP gene is approximately 55 kb long, contains 18 exons, and is on chromosome 4 at position 4q24 (2). The organization of the first three exons and introns in human and hamster are comparable (Fig. 1a). The first exon is made up of both noncoding and coding sequences. Transcription of the human MTP is initiated from a purine present in a string of pyrimidine nucleotides, and transcription of the hamster MTP is initiated from a purine, present six nucleotides further upstream (2, 5). The analysis of human and hamster MTP 5'-flanking sequence revealed over 70% sequence homology in the first 200 bp, and beyond this the conservation was found only in small pockets 10-12 bp long (Fig. 1b). The cDNA sequences are conserved to the extent of 84% (5). The high sequence conservation in the first 200 bp from the transcription start site suggests that regulatory elements required for the tissue-specific expression of the MTP gene may be present in this proximal promoter region.

The human MTP promoter contains an AT-rich sequence at -33 to -30 bp, a region typically occupied by a TATA box in polymerase II-transcribed genes. The hamster promoter contains a TATA box at -33 to -30 bp. In addition, the hamster promoter also contains a CCAT box at -81 bp. The human -97 to -85 bp region is homologous to the liver-specific transcription factor HNF-4-binding site present in the human apoB promoter (14), while the -104 to -99 bp region is homologous to another liver-specific transcription factor, HNF-1-binding site (15). In hamster promoter the -91 to -79 bp region and -98 to -93 bp region are homologous to the HNF-4 and HNF-1-binding sites, respectively. The -110 to -105 bp region in human and -104 to -99 bp region in hamster is homologous to the activator protein AP-1-binding site (16). The -123 to -112 bp region and -117 to -106 bp region in human and hamster, respectively, is homologous to the putative negative insulin response element (IRE) present in the promoters of human protein disulfide isomerase (PDI) and phosphoenol pyruvate carboxylase kinase (PEPCK) genes (17 and references therein).

Further upstream, the MTP promoters contain a sequence element at -175 to -164 bp in human and -174 to -163 bp in hamster that shows sequence homology to the human LDL receptor promoter sterol response element (SRE) (11).

**Cell Type-specific Expression of MTP Gene**—The human and hamster MTP genes are primarily expressed in the liver and intestine (3-5). MTP mRNA is also detectable in the testes, ovaries, and kidneys. To determine the minimal promoter region necessary for the liver and intestinal-specific expression of the MTP gene, nested 5' deletions of the MTP promoter DNA were made and cloned 5' to a promoterless luciferase gene. The activities of these promoter fragments were tested in HepG2, CaCo2, and HeLa cells by transient transfection analysis. The initial human promoter construct tested contained 612 bp and the hamster construct contained 580 bp, respectively, of 5'-flanking sequences. The quantitation of the luciferase activity in the transfected cells showed that MTP promoters are active in a human liver and intestinal cell line but not in a human epithelial cell line (Fig. 2). The promoterless luciferase construct was inactive in all three cell types tested. Interestingly, while the human promoter was only about 40-50% as active in CaCo2 cells as compared with HepG2 cells, the hamster promoter was about 2-fold more active in CaCo2 cells as compared with HepG2 cells. In order to detect additional 5' upstream regulatory elements, a hamster promoter construct with 1249 bp of 5'-flanking sequence was made and tested for expression. The results suggested that the sequence between -1249 and -580 bp contained no additional regulatory elements because
FIG. 1. MTP gene locus, 5' elements. a, the human and hamster MTP gene 5' regions are diagrammatically represented. The shaded boxes are coding sequences while the open boxes are noncoding transcribed sequences. e, exon; i, intron; RI, EcoRI restriction site. b, comparison of the human and hamster promoter sequences. The top line is human, and the bottom line is hamster. Gaps were introduced to obtain the best sequence fit between human and hamster. The transcription start site is base 1. The functional elements and translation initiation codon are indicated in bold letters above the underlined sequences. The intron sequences are written in lowercase letters.

the activity of the hamster -1249 bp construct was not significantly different from the -580 bp promoter construct (data not shown).

To determine if the relative level of cell type-specific activation of the luciferase gene by MTP promoters is comparable to the endogenous MTP promoter strengths in HepG2 and CaCo2 cells, the following experiments were done. First, the total cytoplasmic RNA was prepared from HepG2, CaCo2, and HeLa cells and Northern blot hybridized with a human MTP cDNA probe. The probe detected a 4.4-kb RNA from both HepG2 and CaCo2 cells, and the level in CaCo2 cells was about 20-25% of that in HepG2 cells (Table I). The HeLa cells contained no detectable MTP mRNA. Second, the relative MTP activity in HepG2 and CaCo2 cells was determined. Consistent with the promoter activity and mRNA data, the CaCo2 cells contained reduced levels of MTP activity (Table I). These results are consistent with the MTP promoter strength, as determined by transient transfection analysis (Table I). The results demonstrated that the deletion of sequences 5' to -239 bp for human and -173 bp for hamster had no effect on promoter activities in HepG2 cells. Further deletion of human promoter sequences from -239 to -142 bp and -121 bp, however, increased the promoter activity to approximately 250 and 350%, respectively, in HepG2 cells. It is interesting to note that the putative negative IRE is interrupted in the -121 bp mutant. The deletion of sequences from -173 bp to -123 bp for hamster, similarly, increased the activity of the promoter to 360%. These deletions, however, had very little effect on promoter activity in CaCo2 cells. For example, the human -142 bp construct showed activity similar to the construct with -379 bp of 5'-flanking sequence and the hamster -123 bp construct showed activity similar to the construct with -173 bp of 5'-flanking sequence.

The MTP Promoter Contains Both Positive and Negative Response Elements—In order to delineate the regulatory elements in the MTP promoter, the deletion mutants with 379, 239, 142, 121, 88, 69, and 30 bp of 5'-flanking sequences for human, and with 216, 173, 123, 81, 58, and 25 bp of 5'-flanking sequences for hamster were generated and cloned 5' to the promoterless luciferase gene. The effect of these deletions on promoter function was investigated in HepG2 and CaCo2 cells by transient transfection analysis (Table II). The results demonstrated that the deletion of sequences 5' to -239 bp for human and -173 bp for hamster had no effect on promoter activities in HepG2 cells. Further deletion of human promoter sequences from -239 to -142 bp and -121 bp, however, increased the promoter activity to approximately 250 and 350%, respectively, in HepG2 cells. It is interesting to note that the putative negative IRE is interrupted in the -121 bp mutant. The deletion of sequences from -173 bp to -123 bp for hamster, similarly, increased the activity of the promoter to 360%. These deletions, however, had very little effect on promoter activity in CaCo2 cells. For example, the human -142 bp construct showed activity similar to the construct with -379 bp of 5'-flanking sequence and the hamster -123 bp construct showed activity similar to the construct with -173 bp of 5'-flanking sequence.
levels. The triglyceride transfer activity was determined by SW assay.

MTP expression in HepG2 cells was defined as 100%.

human and hamster promoters, respectively (Table I). The promoter deletion mutants analysis, therefore, suggests that a conserved liver cell-specific repressor element is present in the human MTP promoter -239 to -142 bp region and the hamster promoter -173 to -123 bp region. It is interesting to note that a putative sequence element homologous to the human LDL receptor promoter SRE is present at -175 and -174 bp in the human and the hamster promoters, respectively.

Further deletions from -121 to -88 or -69 bp for the human promoter reduced the promoter activity in HepG2 cells by over 95% as compared to the -612 bp construct (Table II). The activities of the comparable hamster deletion mutants, -123 to -81 or -58 bp were also decreased in HepG2 cells, however, the decrease was less dramatic. The difference between human and hamster promoter deletion mutants in the liver cells is unlikely to the presence of a CCAT box at the -81 bp region in hamster because further deletion from -81 to -58 bp had no detrimental effect on hamster promoter activity. The -69 bp human and -58-bp hamster promoters were both about half as active in CaCo2 cells as compared to the -142 and -123 bp human and hamster promoters, respectively (Table III). The region of the MTP promoter at -123 to 85 bp in human and -117 to -79 bp in hamster, as mentioned above, is complex and shows sequence homology to consensus binding sites for liver cell-specific factors HNF-1 and HNF-4, the activator factor AP-1, and the putative negative IRE of PDI and PEPCK genes.

**Analysis of 5′ deletions of the MTP promoter in HepG2 cells**

Unidirectional 5′ deletions for human and hamster MTP promoters were generated by polymerase chain reaction and inserted 5′ to the promoterless luciferase reporter gene. The 3′ ends of human and hamster promoters were terminated at +85 and +78 bp, respectively. HepG2 cells were transiently cotransfected with the MTP-luciferase construct and a pRL30-CAT control construct. Cells were harvested after 48 h, and cell lysates were assayed for luciferase and CAT activities. Luciferase values were normalized to CAT activity. Promoter activities of the -612 bp construct for human and the -58-bp construct for hamster were defined as 100%. ND, not detected. The values given are the average of data from four or more transfections.

**TABLE I**

<table>
<thead>
<tr>
<th>Human constructs Activity</th>
<th>% of control</th>
<th>Hamster constructs Activity</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2 (control) 100</td>
<td>100</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>CaCo2 40-50</td>
<td>50-60</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>HeLa 0</td>
<td>0</td>
<td>pGL2 ND</td>
<td>ND</td>
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(Fig. 2b). The human -30 bp and hamster -25 bp mutants in which the TATA box was removed showed no promoter activity. All these deletion mutants were also tested in HeLa cells and found to be inactive (data not shown).

**TABLE II**

<table>
<thead>
<tr>
<th>Human constructs Activity</th>
<th>% of control</th>
<th>Hamster constructs Activity</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-612 (control) 100</td>
<td>100</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>-779 107</td>
<td>100</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>-739 100</td>
<td>100</td>
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<td>ND</td>
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<tr>
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</tr>
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<td>41</td>
<td>pGL2 ND</td>
<td>ND</td>
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<tr>
<td>-30 ND</td>
<td>25</td>
<td>pGL2 ND</td>
<td>ND</td>
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</table>

**TABLE III**

<table>
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<tr>
<th>Human constructs Activity</th>
<th>% of control</th>
<th>Hamster constructs Activity</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-379 (control) 100</td>
<td>100</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>-142 95</td>
<td>123</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>-69 48</td>
<td>58</td>
<td>pGL2 ND</td>
<td>ND</td>
</tr>
<tr>
<td>-30 ND</td>
<td>25</td>
<td>pGL2 ND</td>
<td>ND</td>
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The -129 to -76 bp Region Is Promoter-Specific—The human MTP promoter region from -129 to -85 bp is complex, and deletion map analysis suggested that this region is essential for the activity of the human promoter in liver cells. To evaluate the possibility that it can function as a hepatocyte-specific enhancer, the DNA region corresponding to the human -129 to -76 bp region was synthesized and cloned 5′ to the heterologous SV40 early minimal promoter and the -69 and -30 bp human MTP minimal promoters. In the -30 bp promoter, the TATA box was deleted. Transient transfection analysis of these constructs in HepG2 cells, as presented in Fig. 3, showed that this region can stimulate the expression from the MTP -69 bp minimal promoter but not from the -30 bp promoter. This MTP activation region showed no effect on transcription from the heterologous SV40 minimal promoter, thereby suggesting that it is not an enhancer but an integral part of the MTP promoter.

The -129 to -76 bp Region Consists of Both Positive and Negative Elements—To determine the effect of mutations within the individual putative regulatory elements in the -123 to -85 bp MTP activation region on transactivation from the minimal promoter, random nucleotide substitutions were introduced into the putative elements, generally following the A to C and G to T rule. The mutant oligonucleotides with changes in the putative negative IRE (M1), AP-1 (M2), HNF-1 (M3), or
The MTP activator region is promoter-specific. HepG2 cells were transiently cotransfected with a luciferase vector, in which the -129 to -76 bp human MTP activator region was linked 5′ to the SV40 early minimal promoter (pGL2Pro) or to the human MTP minimal promoter (MTP-69 bp and MTP-30 bp), and with a rpl30-CAT control construct. 1, vector; 2, vector with activator region. Luciferase values were normalized to the CAT activity. The promoter activities given are levels relative to the SV40 early minimal promoter or human MTP -69 and -30 bp promoter, respectively. The values given are the average of data from three transfections.

HNF-4 (M4) sites were cloned 5′ to the -69 bp human minimal promoter and the effect of the mutations on transactivation was analyzed by transient transfection in HepG2 cells. The results, given in terms of fold increase in transcriptional activity relative to the -69 bp minimal promoter construct, are shown in Fig. 4. The promoter activity of the IRE mutant M1 was higher than the wild type (42-versus 29-fold relative to the -69 bp construct). This is consistent with the higher activity shown by -121 bp promoter construct as compared to the -142 bp construct, thereby suggesting that a negative element is indeed present in this region. The AP-1 mutant M2 and HNF-1 mutant M3 were transcriptionally almost completely inactive. The activity of the HNF-4 mutant M4, was reduced to half as compared with the wild type. These data, therefore, suggest that the human MTP activation region is indeed complex and consists of multiple positive elements and a negative element.

MTP Promoter Contains a Modified SRE—The functional deletion of the mutant humans of human and hamster promoters suggested that a liver cell-specific repressor element is present in the -239 to -142 bp region and -173 to -123 bp region of the human and hamster MTP promoter, respectively. As shown in Fig. 5a, the MTP promoter contains a putative SRE at -175 and -174 bp in human and hamster, respectively. This SRE, however, differs from human LDL receptor SRE at positions 5 (A to T transversion in human and hamster) and 11 (C to A transversion in hamster) critical for the interaction of SREBP1 with SRE within the context of the LDL receptor promoter. The mutation at these positions decreased the LDL receptor promoter activity to 22 and 7%, respectively, of control and abolished sterol regulation (11, 18). As described above, the deletion of the putative SRE-containing region in both human and hamster increased the promoter activity. To determine the effect of sterols on the human MTP promoter, the HepG2 cells were transiently cotransfected with the human MTP -229 bp promoter or the human SRE-containing LDL receptor promoter driving luciferase constructs and rpl30 CAT constructs, and grown in the presence of 25-OH cholesterol and cholesterol. As expected, the LDL receptor promoter activity was suppressed (66% as compared with the control) in the presence of sterols (Fig. 5b). The MTP promoter activity, surprisingly, was moderately increased (172% as compared with the control) rather than decreased in presence of sterols.

The MTP Gene Is Negatively Regulated by Insulin—Many genes respond either positively or negatively to the insulin receptor-mediated signal transduction cascade (17, 19, and references therein). Both PDI and PEPCK have been reported to be negatively regulated by insulin and contain a negative IRE in their 5′-flanking sequences. Human and hamster MTP promoters contain a sequence element at -123 to -112 bp and -121 bp promoter. The mutated elements are underlined. b, HepG2 cells were transiently cotransfected with the -69 bp minimal promoter construct or wild type or mutant constructs and a rpl30-CAT control construct. Luciferase values were normalized to CAT activity. The promoter activities given are levels relative to the human MTP -69 bp promoter. The values given are the average of data from three transfections, and the error bars represent the maximum and minimum transactivation of luciferase gene expression, as compared to the control constructs, obtained in independent experiments.

As shown in Fig. 5a, the human -129 to -76 bp region is shown with the functional elements in bold letters. WT, the -129 to -76 bp wild type sequence linked 5′ to the -69 bp minimal promoter; M1, the IRE mutated; M2, the AP-1 site mutated; M3, the HNF-1 site mutated; M4, the HNF-4 site mutated. The mutated elements are underlined. HepG2 cells were transiently cotransfected with the -69 bp minimal promoter construct or wild type or mutant constructs and a rpl30-CAT control construct. Luciferase values were normalized to the CAT activity. The promoter activities given are levels relative to the human MTP -69 bp promoter. The values given are the average of data from three transfections, and the error bars represent the maximum and minimum transactivation of luciferase gene expression, as compared to the control constructs, obtained in independent experiments.
**Transcriptional Regulation of MTP Genes**

**Fig. 5. MTP gene expression: effect of cholesterol in HepG2 cells.** a, the human LDL receptor SRE, the putative MTP SRE, and human cholesterol 7α-hydroxylase- (CYP7) modified SRE sequences are aligned to show their homology. b, HepG2 cells were transiently cotransfected with LDL receptor-luciferase construct (LDL-R) or -239 bp human MTP promoter-luciferase construct and the rpL30 promoter-CAT construct. Cells were grown in 2.5% delipidated serum containing 1 μg/ml 25-OH cholesterol and 10 μg/ml cholesterol. The control cells were grown in medium supplemented with carrier ethanol. Cells were harvested after 48 h, and cell lysates were assayed for luciferase and CAT activities. Luciferase values were normalized to CAT activity. Promoter activities of the respective constructs in the absence of added sterol was considered as 100%. The error bars represent the maximum and minimum effects obtained in the presence of added cholesterol in independent experiments. The values shown are the average of data from three transfections for the LDL receptor promoter and five transfections for the MTP promoter.

**Fig. 6. MTP gene expression: effect of insulin in HepG2 cells.** a, the putative MTP negative IRE and the PDI and PEPCCK-negative IRE sequences are aligned and the consensus sequences is given below (R, purine; Y, pyrimidine; N, any nucleotide; W, G or C). The PDI and PEPCCK proximal promoter negative IRE sequences are given in the reverse orientation indicated by an asterisk above the sequence. b, HepG2 cells were transiently cotransfected with human MTP promoter-luciferase constructs -142 bp, -121 bp, or IRE mutant M1, and a rpL30-CAT control construct. Cells were then grown in 2.5% FBS containing medium supplemented with or without 1.0 μg/ml bovine insulin. Cells were harvested after 48 h, and cell lysates were assayed for luciferase and CAT activities. Luciferase values were normalized to CAT activity. Promoter activity of the respective promoter constructs in the absence of added insulin was taken as 100%. The error bars represent the maximum and minimum effects obtained in the presence of added insulin in independent experiments. The values given are the average of data from five and 10 transfections, respectively, for -142 and -121 bp constructs and two transfections for IRE mutant M1.

**DISCUSSION**

The analysis of the human and hamster MTP genes show that the genes are evolutionarily conserved as evidenced by gene organization, promoter structure, and protein coding sequence. The human MTP transcription is initiated at a purine nucleotide present in a string of pyrimidines 30 bp downstream of the TATA box. Such initiator sequences, found in many polymerase II-transcribed genes, bind initiator-specific factors that determine the site of transcription initiation (20). The hamster MTP transcription is initiated at a purine nucleotide present 30 bp downstream of the TATA box. However, unlike the human promoter this initiation site is in a string of purines. Therefore, it is likely that the TATA box plays a significant role in determining the site of MTP transcription initiation. The high sequence conservation in the first 200 bp of sequence 5′ to the transcription start site suggests that important regulatory elements are present in this proximal promoter region. Indeed, the functional analysis by transient transfections in liver and large intestine-derived cell lines show that cell type-restricted expression of the MTP gene is regulated by a compact promoter present within this 200 bp sequence. The relatively higher activity of the hamster promoter in the intestinally derived cell line is consistent with the recent observation that the hamster intestine contains more MTP mRNA and MTP activity as compared with the liver (5). The relative levels of MTP gene expression in human liver and intestine is not known. In general, the various deletion mutants of human and hamster MTP promoters showed comparable levels of activity in liver- and intestinal-derived cell lines. The exception was the deletion that removed the -123 to -85 bp human and -117 to -79 bp hamster activator region in that the effect of deletion on human pro-
The human and hamster MTP promoters contain consensus recognition sequences for hepatocyte-specific tranfactors, HNF-1 and HNF-4, an AP-1 recognition sequence, and a negative response element for the metabolic regulator insulin in the −123 to −86 bp and −117 to −79 bp region, respectively (Figs. 1 and 8). Deletion of the promoter sequence containing the putative HNF-1, HNF-4, and AP-1 recognition sites almost completely abolishes the human promoter activity and markedly reduces the hamster promoter activity in human liver cells. The isolated human −129 to −76 bp region was able to stimulate transcription from the −69 bp MTP basal promoter but not from the heterologous SV40 minimal promoter in liver cells. It is, therefore, unlikely that the MTP promoter region is an enhancer in the classical sense, but is an integral part of the MTP promoter that is expressed primarily in the liver and intestine.

The HNF-1 and HNF-4 transcription factors are primarily expressed in liver, with some expression also observed in intestine and kidney, and they regulate the expression of many liver-specific genes (21–23). HNF-4 is a major activator of genes of lipoprotein metabolism including apolipoprotein A-I (apoA-I), apolipoprotein C-III (apoC-III), and apoB, HNF-1 and HNF-4 are also involved in an autoregulation loop in which HNF-1 down-regulates the HNF-4-activated transcription from both intact apoA-I and apoC-III genes as well as chimeric promoter-reporter genes in liver cells (23). Therefore, the presence of such close/overlapping HNF-1 and HNF-4 sites may offer regulatory opportunities under different developmental and metabolic conditions and availabilities of trans factors. The presence of an AP-1 site partially masked by the liver specific factor binding sites is interesting and may provide another level of regulatory response analogous to the activation of the apoB gene by C/EBP α and HNF-4 binding in its proximal promoter (14). It is also worth noting that HNF-4 is a member of the steroid hormone receptor super family of transcription factors and that certain members of this family have been found to respond to fatty acids (24). Indeed, both apoB and MTP expression is positively affected by dietary fat (5, 25), and it is possible that this is through transactivation mediated by HNF-4. It would be very interesting to determine the identity and the sequential nature of binding of the individual transacting factors that bind to the MTP activator region and their potential to coordinate regulate MTP and apoB gene transcription and lipoprotein synthesis and secretion.

Human and hamster promoter deletion mutant analysis suggests that the −180 to −160 bp region negatively influences the promoter function in liver cells. The modified SRE present in the human and hamster promoters at −175 and −174 bp, respectively, differs from the human LDL receptor SRE at positions previously been demonstrated to be critical for the activity of the promoter, the interaction with SREBP-1 and feedback transcriptional regulation of the LDL receptor by sterols (11, 18). Surprisingly, when tested for sterol response the activity of the MTP promoter was moderately enhanced rather than repressed in the presence of 25-OH cholesterol and cholesterol. The effect of cholesterol on the endogenous MTP gene expression in HepG2 cells, as determined by RNA analysis, is however, minimal.

This is probably due to the inability of the assay condition to detect the moderate up-regulation of MTP gene by cholesterol. It is interesting to note that the gene coding for the enzyme 7-α-hydroxylase, CYP7, that catalyzes the first reaction of the catalytic pathway of cholesterol to bile acids, contains a modified SRE as shown in Fig. 5a (26–28). The expression of this gene is also moderately induced by cholesterol and suppressed by bile acids. Therefore, it is possible that both the 7-α-hydroxylase and MTP genes modified SREs bind a novel member of the SRE-binding protein family and up-regulate transcription in response to cholesterol. It will be important to determine whether the pathways of cholesterol catabolism and export/secretaion share a common transcriptional regulatory mechanism. In addition, the sequence element that causes the liver cell-specific repression of the MTP promoter needs further investigation.

Insulin can regulate gene expression positively or negatively at the level of transcription, mRNA processing, degradation, and translation efficiency (17, 19 and references therein). The insulin-sensitive transacting factors that stimulate or inhibit gene expression have not been fully characterized to date. The MTP promoter analysis in liver cells shows that MTP gene transcription is under negative insulin regulation. The differential response of human deletion mutants −142 and −121 bp

![Fig. 7. MTP gene expression: dose-response for the insulin effect in HepG2 cells. HepG2 cells were transiently transfected with luciferase vector in which the −129 to −76 bp human MTP activator region was linked 5’ to the human MTP −69 bp minimal promoter, and a rpL30-CAT construct. Cells were grown in 2.5% FBS containing media supplemented with the indicated amounts of bovine insulin. Cells harvested after 48 h were lysed and assayed for luciferase and CAT activities. Promoter activity in the absence of added insulin was considered as 100%.

![Fig. 8. MTP gene promoter region: conserved sequence elements in the human and hamster promoters. The putative functional elements in the MTP promoters are diagrammatically represented. Base 1 is the transcriptional start site. Open boxes are the noncoding transcribed sequences while the shaded boxes are coding sequences.](image-url)
and IRE mutant M1 to insulin confirmed the location of the negative insulin-response element, and additionally showed that the sequence integrity of the element at −123 to −112 bp is essential for an insulin response. The partial insulin effect on −121 bp promoter construct is probably due to the ability of the truncated negative IRE to interact with the insulin sensitive trans-acting factor with lower affinity. It is interesting to note that both the −121 bp and IRE mutant M1 promoter constructs showed higher transcriptional activity as compared with the −142 bp and wild type constructs, respectively, in the absence of added insulin. This was probably due to the removal of the negative regulation by insulin present in the serum. The MTP-negative IRE, that shares no sequence homology with the previously characterized upstream IRE in the PEPCK gene promoter, may be recognized by a novel insulin-regulated transfactor. It has been recently demonstrated that expression of the endogenous MTP gene is also down regulated by insulin in HepG2 cells.

It has been previously demonstrated in diabetic rats, in which the β cells of the pancreas were destroyed by streptozotocin treatment, that the PDI gene is activated at the transcriptional level (29). Insulin decreases this transcriptional activation within 30 min of its administration to these animals, demonstrating that the PDI gene can be negatively regulated by insulin. Functional MTP is a heterodimer consisting of a type I and type I1 diabetic animal models and in human patients should shed important insights into the regulation of these genes by insulin and into their common molecular mechanisms of regulation. It is also interesting to note that insulin therapy in human type I diabetic patients results in a decrease in plasma very low density lipoprotein levels, and that type II diabetic patients with insulin resistance have increased plasma very low density lipoprotein levels (30 and references therein).

In conclusion, sequence and deletion analysis show that the MTP promoters of human and hamster are organized similarly. The promoter activity is cell type specific, it has a modified sterol regulatory element and a negative insulin response element, and it has potential binding sites for HNF-1, HNF-4, and AP-1. The human promoter activity is positively regulated by cholesterol and negatively regulated by insulin.

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