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Regulation of microsomal triglyceride transfer protein

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Abstract

Microsomal triglyceride transfer protein (MTP) facilitates the transport of dietary and endogenous fat by the intestine and liver by assisting in the assembly and secretion of triglyceride-rich apolipoprotein B-containing lipoproteins. Higher concentrations of apolipoprotein B lipoproteins predispose individuals to various cardiovascular and metabolic diseases such as atherosclerosis, diabetes, obesity and the metabolic syndrome. These can potentially be avoided by reducing MTP activity. In this article, we discuss regulation of MTP during development, cellular differentiation and diurnal variation. Furthermore, we focus on the regulation of MTP that occurs at transcriptional, post-transcriptional and post-translational levels. Transcriptional regulation of MTP depends on a few highly conserved *cis*-elements in the promoter. Several transcription factors that bind to these elements and either increase or decrease MTP expression have been identified. Additionally, MTP is regulated by macronutrients, hormones and other factors. This article will address the many ways in which MTP is regulated and advance the idea that reducing MTP levels, rather than its inhibition, might be an option to lower plasma lipids.

Keywords

apolipoprotein B; lipid; lipoprotein; microsomal triglyceride transfer protein; regulation; transcription

Cardiovascular diseases are a major cause of mortality and morbidity in Western countries. A major underlying basis for the high incidence of these diseases is excess plasma lipids that have been associated with a shift in the nutritional balance towards an over-consumption of carbohydrates and lipids. Plasma lipids are carried in apolipoprotein (apo)B-containing lipoproteins. Accumulation of remnant or modified apo B-lipoproteins is considered atherogenic as their deposition in the arterial wall is a major event in the initiation of the disease. The apoB-containing lipoproteins chylomicron and VLDL are assembled and secreted by the intestine and liver, respectively. Besides lipids, biosynthesis of these lipoproteins requires apoB and microsomal triglyceride transfer protein (MTP). ApoB is a structural protein always found associated with these lipoproteins. MTP binds and chaperones lipids to the nascent apoB to prevent aberrant folding and degradation by proteasomes and assists in the intracellular assembly of apoB-lipoproteins [1,2].

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Microsomal triglyceride transfer protein is an endoplasmic reticulum resident heterodimeric complex of a unique functional MTP subunit and ubiquitously expressed protein disulfide isomerase subunit that transfers various lipids, interacts with apoB and associates with lipid vesicles [2]. Abetalipoproteinemia subjects that carry mutations in their MTP gene have no plasma apoB-lipoproteins [3]. Because of its role in lipoprotein assembly, MTP inhibition is considered an important modality to treat hyperlipidemia and reduce risk for atherosclerosis [4]. In this regard, several MTP inhibitors have been identified that lower plasma lipids [4,5]; however, they have not reached clinical use due to associated adverse events mainly related to accumulation of lipids in the liver. Therefore, attempts are underway to explore intestine-specific inhibition of MTP to lower plasma lipids. The main purpose of summarizing regulation of MTP is to draw attention to the possibility that MTP inhibition, may be achieved indirectly or in a tissue-specific fashion. It is possible that new knowledge about the regulation of MTP will pave the way for novel approaches to inhibit its activity while avoiding associated side effects.

The liver and intestine are the major organs that express MTP and secrete apoB-containing lipoproteins. Other tissues also express MTP but at much lower levels. For example, placenta, heart, ovary, testis, kidney, pancreas, adipose tissue and retina express measurable MTP mRNA [6–10]. Mechanisms controlling different levels of MTP expression in various tissues are unknown. In general, there is a good agreement between MTP mRNA, protein levels and activity indicating that transcriptional control is the main mechanism of MTP regulation. In this article, we will summarize current knowledge about the MTP promoter, transcriptional control mechanisms and post-transcriptional regulatory events that alter MTP activity and apoB-lipoprotein production.

cis-Elements & transcription factors important for MTP expression

The human MTP gene, *MTTP*, is approximately 55 kb in length comprised of 18 exons and 17 introns [11]. It has been mapped to q22–24 on chromosome 4 by PCR analysis of rodent/human somatic cell hybrids followed by FISH [11,12], while the mouse MTP gene, *Mttp*, was localized to a distal region of chromosome 3 by Southern blotting [13]. Studies indicate that mice express two forms of MTP; MTP A and MTP B [14,15]. They arise due to alternate splicing of exon 1. The minor MTP B utilizes an alternate promoter and has two extra N-terminal amino acids. Low levels of MTP expression in antigen-presenting cells might arise from the low activity of the alternate promoter [14,16,17]. A high fat diet in mice with cardiac deficiency of the MTP A isoform results in increased expression of the MTP B form suggesting that both these promoters respond to high fat diet [18].

In contrast to mice, humans express one MTP isoform. *In vitro* promoter-reporter expression studies indicate that the expression of this isoform in the liver and intestine-derived cells depends on approximately 150 bp upstream of the transcription start site [19]. Comparative alignment of these upstream promoter sequences from different species identified several highly conserved motifs [20]. Deletion analysis followed by expression in hepatic cells revealed that the promoter sequence contains critical positive (hepatic nuclear factor [HNF]-1, HNF-4, direct repeat [DR]1 and FOX) and negative regulatory sterol and insulin response element (SRE/IRE) (Figure 1).

Hepatic nuclear factor-1 element binds to HNF-1α and HNF-1β [Dai K & Hussain M, Unpublished Data], whereas HNF-4 element interacts with the HNF-4α transcription factor [19,21,22]. HNF-4α knockout mice do not express MTP, indicating that it is absolutely required for expression [23]. HNF-1α/β synergistically activate MTP expression along with HNF-4α [22,24]. Hence, HNF-1 and HNF-4 elements and their interactions with HNF-1α/β and HNF-4α transcription factors are critical for basal and synergistic MTP expression.

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Several transcription factors bind to the DR1 element. In cells that do not express MTP, this site is occupied by either NR2F1 (also known as COUP-TF1) or NR2F2 (or COUP-TFII). NR2F2 reduces MTP in hepatoma cells [25], whereas NR2F1 suppresses MTP expression in intestinal cells [22]. However, in MTP-expressing cells, RXR-α binds to this site [25]. DR1 sequences are also known to constitute peroxisome proliferator-activated receptor (PPAR) response elements. In fact, MTP expression is increased after treatment with PPARα agonist but not by PPARγ agonist in the liver [26]. Thus, binding of different transcription factors to DR1 might modulate tissue- and cell-specific expression ofMTP.

Overexpression studies [27,28] have identified binding sites for FoxO1 and FoxA2 in the upstream region of the MTP promoter (Figure 1). These transcription factors enhance MTP activity. They respond to insulin signaling and are excluded from the nucleus. Therefore, they might be involved in insulin regulation of MTP (see later).

Microsomal triglyceride transfer protein promoter also harbors a SRE/IRE that negatively regulates MTP expression. Deletion of this element increases MTP promoter activity. Insulin-mediated MTP suppression in HepG2 cells requires this element [19,29]. Sterol regulatory element-binding proteins (SREBPs) bind to this element and reduce MTP expression [29]. However, overexpression of SREBPs in the mouse liver is associated with increased MTP expression [30] most likely due to a secondary effect, as MTP was not identified to be a direct target of SREBPs by microarray analysis [31]. Therefore, it is unclear whether sterol regulation of MTP involves SRE/IRE elements *in vivo*.

Besides transcription factors that bind to *cis*-elements in the MTP promoter, several co-activators and co-repressors that bind to these transcription factors and modulate their activity have been identified. Forced expression of PGC-1α and PGC-1β co-activators increases MTP expression [32]. PGC-1β acts as a co-activator with FoxA2 to increase MTP activity [27]. On the other hand, small heterodimeric partner (SHP) represses HNF-4α, LRH-1 and HNF-1α activities by interacting with these factors [21,33,34]. Overexpression and knockdown of SHP reduces and increases MTP activity in mouse liver [33,34]. NCOR1 has been shown to interact with NR2F1 and suppress MTP activity in intestinal cells [22]. Thus, MTP expression is modulated by various co-activators and co-repressors.

In summary, a short MTP promoter with few *cis*-elements appears to be sufficient for its regulation in cultured cells. HNF-1/HNF-4 and Fox elements are involved in positive regulation of MTP, whereas the SRE/IRE element might be involved in negative regulation. The DR1 element can be engaged to either increase or decrease MTP expression. It remains to be determined whether this small promoter sequence is sufficient for *in vivo* MTP expression.

Regulation of MTP by macronutrients

MTP gene expression is altered by changes in dietary components. High sucrose diet increases MTP mRNA in the liver but not in the intestine [35] whereas a fructose enriched diet increases MTP mRNA and activity in both the liver and intestine [36]. High saturated fat (hydrogenated coconut oil) [35,37] and cholesterol [38] increase MTP expression in the intestine. In hamsters, long-term high fat diet increases MTP mRNA in the liver and intestine [35]. Increases in MTP activity by saturated fat and cholesterol could potentially involve decreased binding of SREBPs to the SRE/IRE element in the MTP promoter, but this has not been demonstrated *in vivo*. Recently, Iqbal *et al.* have proposed that intestinal inositol requiring enzyme 1β (IRE1β) might play a role in this process [38]. In the absence of IRE1β, mice express more MTP in the intestine and develop more hyperlipidemia than control mice when fed a Western diet.

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Cell culture experiments suggest regulation of MTP by sterols. Sterol depletion and pravastatin decrease MTP mRNA and protein levels in HepG2 cells [29]. The regulation of MTP by sterols in these cells involves direct interaction of SREBP-2 with the SRE/IRE in the promoter [29]. Oleic acid has been shown to up-regulate MTP in HepG2 cells. This regulation was suggested to not involve SRE/IRE [36]. More studies are needed to determine how MTP is regulated *in vitro* and *in vivo* by macronutrients.

Regulation by insulin

Insulin acutely reduces VLDL secretion. Since MTP is essential for VLDL secretion, it has been speculated that insulin-induced MTP reduction could contribute to decreased VLDL production. Insulin reduces *MTP* gene transcription in HepG2 cells [19] in a dose- and time-dependent manner [39]. Using different $MEK^{1/2}$ inhibitors, the dominant negative Raf/MEK1 as well as constitutively active Raf/MEK1 enzymes, Au *et al.* showed that MTP regulation by insulin involves $MAPK^{erk}$ cascade (Figure 2) [40]. This was confirmed by Allister *et al.* who also showed that naringenin, a citrus flavonoid, uses the same pathway to suppress MTP expression [41]. Both these studies excluded the PI3-kinase signaling pathway that involves phosphorylation of Akt. It is known that insulin also stimulates $MAPK^{p38}$, which inhibits the $MAPK^{erk}$ cascade. In fact, inhibition of $MAPK^{p38}$ increases insulin-mediated MTP suppression involving the $MAPK^{erk}$ cascade [40,41]. The $MAPK^{erk}$ cascade involves phosphorylation and translocation of $ERK^{1/2}$ (p44/p42) to the nucleus. In the nucleus, these enzymes phosphorylate several transcription factors that ultimately bind to various *cis*-elements in different genes [42,43]. Subsequent targets of $ERK^{1/2}$ that ultimately interact with the MTP promoter have not been identified.

A different type of insulin signaling has been proposed (Figure 2) to involve forkhead transcription factors FoxA2 and FoxO1 [27,28], which probably bind the same binding site within the MTP promoter (Figure 1). These factors are phosphorylated in response to insulin signaling and are excluded from the nucleus. Overexpression of FoxA2 increases MTP expression in *ob/ob* mice [27]. Its activity is synergistically increased in the presence of PGC1β. Kamagate *et al.* showed that overexpression of FoxO1 also increases MTP expression and this increased expression is inhibited by insulin or constitutively active Akt [27,28]. Moreover, FoxO1 RNAi decreased hepatic MTP and VLDL production in normal and *db/db* mice. These data suggest that FoxO1 and FoxA2 play a role in basal hepatic MTP expression.

The above studies indicate that insulin negatively regulates MTP expression. Therefore, it is anticipated that hepatic MTP should be significantly reduced in hyperinsulinemic animals. However, this is not the case. High sucrose fed hamsters show increased MTP expression in the liver [35], whereas fructose-fed hamsters have high hepatic [44] as well as high intestinal [45] MTP. Treatment of fructose-fed hamsters with rosiglitazone improves insulin sensitivity and normalizes MTP expression [46]. Young (6 weeks old) Otsuka Long-Evans Tokushima Fatty (OLETF) rats do not show hyperglycemia and hyperinsulinemia but still have more hepatic MTP [47]. High hepatic MTP levels persist in these rats after the development of hyperglycemia or hyperinsulinemia. Similarly, hyperglycemic and hyperinsulinemic *ob/ob* mice have high hepatic MTP levels [48]. Also, Zucker obese (fa/fa) rats that show hyperinsulinemia have higher levels of MTP in the liver and intestine [49]. In contrast to these animals, streptozotocin-treated rats [50] and mice [48] or alloxan-treated rabbits [51] show increased intestinal MTP with no change in hepatic MTP expression. A general consensus from these studies is that hyperglycemia and hyperinsulinemia are generally associated with increased, not decreased, MTP expression. This may be the consequence of insulin resistance rather than insulin action. Further studies are necessary to explain the role of insulin and insulin resistance in MTP regulation.

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To test the direct effect of insulin on MTP expression, Sparks *et al.* injected insulin into fasted *Apobec1*$^{-/-}$ mice that synthesize only apoB100 [52]. Analysis after 2 h revealed reductions in plasma glucose and hepatic FoxO1 and PGC-1α mRNA. There was no significant change in MTP mRNA and activity in these mice suggesting that acute administration of insulin does not decrease MTP expression in these mice, as it does in HepG2 cells. Therefore, it remains to be determined whether insulin is a negative regulator of MTP expression *in vivo*.

Briefly, MTP expression is regulated by insulin in cultured hepatoma cell lines. Insulin-mediated suppression of MTP in these cells requires the SRE/IRE element. Additionally, loss of the binding of Fox transcription factors to their binding sites also contributes to reduced MTP expression. However, regulation of hepatic MTP by insulin has not been demonstrated *in vivo*.

Regulation by bile acids

Hirokane *et al.* have demonstrated that chenodeoxycholate decreases MTP expression in HepG2 cells [21]. Moreover, they showed that the suppressive effect of chenodeoxycholate was mediated by increased SHP expression. Further studies demonstrated that SHP suppresses HNF-4α activity and reduces MTP expression [21]. Thus, bile acids act as negative regulators of MTP expression.

Regulation by leptin

Using various mouse models, Iqbal *et al.* demonstrated that global leptin receptor deficiency is associated with decreased intestinal MTP expression [53]. Furthermore, they showed that hepatic expression was resilient to leptin receptor deficiency. Increased intestinal MTP expression did not require central leptin signaling in the hypothalamus. Instead, they showed that intestinal cells respond to leptin and regulate MTP levels because they express leptin receptors and its downstream target genes. It remains to be determined how leptin differentially regulates MTP expression in intestinal and hepatic cells and what signaling mechanisms are involved.

Developmental regulation

Expression of MTP is first detected at day 7.5 after gestation [54]. In early development expression mainly occurs in the liver. As the embryo matures the relative expression in the intestine increases compared with the liver and reaches levels higher than liver as seen in the postnatal stage [55]. During embryonic development MTP plays a pivotal role as homozygous knockout mice are not viable and at least suffer from a non-closing anterior neuropore [56]. This phenotype was also observed in apoB knockout mice indicating that MTP is perhaps important for apoB protein stabilization and lipoprotein assembly in embryonic development [57]. Although it cannot be ruled out that homozygous knockout of MTP has a direct intracellular effect on embryonic development it is more likely that visceral endodermal cells that line the inner layer of the yolk sac are involved, since they are embryo derived and produce apoB-containing lipoproteins [58]. These lipoproteins may be used to nurture the embryo and therefore are important for embryonic development. On the other hand, MTP has also been shown to be required for yolk lipid utilization and absorption of dietary lipids in zebrafish larvae [59,60]. However, MTP is not essential for human embryo development as evidenced by the presence of abetalipoproteinemia subjects. Thus, requirement of MTP during embryo development differs in vertebrates.

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Although liver is the first organ to express MTP during development, liver-specific MTP knockout mice obtained after crossing with Alb-Cre mice are viable [Khatun I *et al.*, Unpublished Data]. This might be related to late expression of albumin during development. In contrast, deletion of intestine-specific MTP using Villin-Cre transgenic mice does not result in viable progeny. Davidson and associates have obtained intestine-specific knockout mice by postnatal activation of the villin promoter [61]. A clear explanation for a defect in embryonic development in intestine-specific MTP knockout mice is missing, since intestinal function in dietary lipid transport is considered to be important after birth.

Differentiation-dependent induction of MTP in enterocytes

The small intestine is divided anatomically into basal crypts and apical villi. Stem/progenitor cells are present as a layer between crypt and villus cells. Stem/progenitor cells divide, migrate and differentiate into four types of cells; enterocytes, Goblet cells and enteroendocrine cells, all present in the villi, and Paneth cells, at the base of the crypt. Several molecular mechanisms involved in the differentiation of stem cells into villus cells have been identified [62]. An important adaptation during enterocyte maturation is acquisition of the absorptive phenotype necessary for uptake and transport of dietary fat.

To understand differentiation-dependent induction of lipid transport mechanisms, Dai *et al.* used Caco-2, human colon adenocarcinoma cells [22] that are used extensively to study differentiation and various intestinal functions [63–65] because they possess an inherent genetic ability to become enterocyte-like cells. Quantitative PCR and microarray analyses show strong correlations between gene expression profiles during Caco-2 cell differentiation and that of intestinal epithelia across the crypt–villus axis [66,67]. Differentiation of these cells results in reorganization of cytoskeletal structures, formation of tight junctions, development of cellular polarity and enterocyte-associated functions [65,66,68–70]. Undifferentiated Caco-2 cells do not synthesize or secrete apoB-lipoproteins [71–73]. However, differentiated cells produce chylomicron-size apoB-lipoproteins when media are supplemented with oleic acid [64,71–77]. Dai *et al.* demonstrated that differentiation-dependent induction of apoB-lipoprotein secretion in Caco-2 cells correlates with the expression of MTP, and not apoB [22]. Mechanistic studies revealed that the MTP promoter engages HNF-4α/HNF-1α in undifferentiated cells and yet is inactive because the DR1 element is occupied by the NR2F1 repressor. During differentiation, NR2F1 expression declines, possibly leading to its decreased binding to the DR1 element and increased expression of MTP. In addition to the transcriptional suppression of MTP by NR2F1, Dai *et al.* reported that post-transcriptional mechanisms involving IRE1β are also operative in undifferentiated cells [22]. IRE1β expression is high in undifferentiated cells and its levels drop during differentiation. Hence, transcriptional and post-transcriptional mechanisms ensure low expression of MTP in undifferentiated intestinal cells.

To determine whether NR2F1 and IRE1β play a role in MTP expression in mouse intestine, Dai *et al.* studied the expression of these proteins by immunohistochemistry. There was an inverse relationship in the expression of MTP with NR2F1 and IRE1β along the jejunum to colon axis. Similarly, a reciprocal expression pattern was also seen along the villus to crypt axis in the jejunum. Furthermore, isolated enterocytes expressed higher amounts of MTP and lower amounts of NR2F1 compared with villus cells. They further demonstrated that NR2F1 associates with the MTP promoter in cells that do not express MTP. Indeed, binding of NR2F1 to the MTP promoter was high in crypt but low in villus cells. Therefore, low association of NR2F1 with MTP promoter in villi might contribute to enhanced MTP expression.

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In short, these studies have shown that both transcriptional and post-transcriptional mechanisms are involved in differentiation-dependent induction of MTP in enterocytes. In undifferentiated cells, MTP expression is suppressed by the binding of NR2F1 to the DR1 element. Furthermore, high expression of IRE1β might ensure low MTP mRNA levels. During differentiation, expression of these proteins is reduced. Decreased expression of these regulatory proteins increases MTP expression and induces apoB-lipoprotein assembly in differentiated Caco-2 cells.

Circadian regulation

Several biological, physiological and behavioral activities such as feeding, thermogenesis and sleep–wake cycle exhibit circadian rhythms that recur with 24 h intervals and are attuned to sunrise and sunset. Plasma triglyceride and cholesterol concentrations are maintained within a narrow range by balancing lipoprotein production and catabolism and exhibit circadian rhythmicity in humans and rodents [78–82]. Pan and Hussain showed that plasma lipids and MTP expression exhibit synchronized circadian changes in rats and mice and have suggested that changes in intestinal and hepatic MTP might contribute to daily variations in plasma lipids [83]. Changes in MTP and plasma lipids were abrogated when mice were placed in total light or dark for 5 days indicating involvement of light-entrained regulation [84]. Furthermore, surges in both MTP and plasma lipids were altered when mice were subjected to food entrainment [34,83,84]. These diurnal and food-entrained variations were absent in *Clock* mutant mice that have difficulty in maintaining circadian rhythms. Thus, both light and food entrainment mechanisms regulate plasma lipids and these regulatory mechanisms require normal Clock activity. Pan *et al.* further showed that Clock regulates diurnal MTP expression at the transcriptional level [34]. MTP gene transcription was high at night and low in the day. Cell culture studies showed that Clock reduces MTP expression at the transcription level [34]. Further studies revealed that Clock increases the expression of SHP, a repressor of MTP [34]. It was further demonstrated that the binding of Clock to the SHP promoter enhances its expression at the onset of light. This leads to increased binding of SHP to the MTP promoter and suppression of its expression during the day. Therefore, SHP is a Clock-controlled gene upregulated by Clock. Higher amounts of SHP suppress MTP expression.

In brief, daily variations in plasma triglyceride have been correlated with changes in intestinal and hepatic MTP. Mechanistic studies revealed that Clock plays an important role in the daily regulation of MTP that is entrained by light as well as food. Clock binds to the SHP promoter and enhances its expression. High levels of SHP negatively regulate MTP expression by interacting and suppressing the activity of different activators.

Post-transcriptional regulation by IRE1β

IRE1β, a homolog of ubiquitously expressed IRE1α, which plays a critical role in unfolded protein response, is primarily expressed in the intestine [85]. Within the intestine, IRE1β protein is detectable in the epithelial cells of stomach, small intestine and colon [22,85]. Its expression increases along the jejunum–colon axis as well as from villus to crypt [22]. Iqbal *et al.* observed that high cholesterol and Western diets reduce jejunal expression of IRE1β and enhance MTP expression [38]. *Ire1b*$^{-/-}$ mice express more MTP, absorb more lipids and develop more pronounced hyperlipidemia than controls when challenged with high cholesterol or high fat diets. Mechanistic studies revealed that IRE1β post-transcriptionally cleaves *Mttp* mRNA initiating its degradation. Hence, IRE1β might act to dampen fat-induced upregulation of intestinal MTP and lipid absorption and act as an anti-lipidemic gene.

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Qiu *et al.* demonstrated that liver-specific ablation of phosphatase and tensin homolog (PTEN) in mice and overexpression of a dominant negative form of PTEN in HepG2 cells lower MTP activity and protein levels by 36–37% [86]. However, under these conditions, reductions in MTP mRNA were modest (−8%). These studies indicate that PTEN might affect MTP protein involving post-transcriptional mechanisms. Further studies are needed to explain how PTEN and AKT lower MTP protein.

Translational & post-translational control

Very little is known about the translational control of MTP. It is known that MTP is a hetero-dimer of 97 and 55 kDa subunits. It is also known that dissociation of the complex leads to irreversible loss of activity. This led to the suggestion that association of these two subunits occurs during or soon after the translation of these peptides. Since, the 55-kDa subunit PDI exists as an independent protein, the translation of this subunit does not require the 97-kDa M subunit. In contrast, the biosynthesis, proper folding and maturation of the 97-kDa subunit requires the 55-kDa subunit.

Pan *et al.* have demonstrated that CCl_4 decreases plasma apoB-lipoproteins and increases hepatic and intestinal lipids in mice in a time-dependent manner [87]. Mechanistic studies revealed that CCl_4 decreased apoB-lipoprotein secretion by reducing MTP activity and protein levels without affecting its lipid transfer activity and mRNA levels indicating that CCl_4 is not an antagonist and that reductions in MTP activity do not involve transcriptional mechanisms. Furthermore, CCl_4 had no effect on MTP biosynthesis but it induced post-translational degradation involving ubiquitinylation and endoplasmic reticulum-associated proteasomal degradation. They also showed that prevention of MTP degradation by proteasomal inhibitors protects against CCl_4 toxicity. Further studies showed that free radicals arising from CCl_4 by the action of cytochrome P450 oxygenases covalently attach with MTP, inducing its degradation by proteasomes. Thus, MTP degradation after short-term exposure to CCl_4 is the major mechanism leading to cellular accumulation of lipids and that covalent modification of MTP leading to its destruction is a key early event in the onset of CCl_4-induced steatosis.

The preferential and rapid degradation of MTP after CCl_4 exposure suggest that MTP might be involved in the transfer of reactive species and needs further evaluation. It is possible that MTP attempts to transfer damaged lipids and in turn gets damaged. More studies are needed to understand susceptibility of MTP to CCl_4 attack. It is unknown whether MTP has residues that are highly susceptible to CCl_4 modification.

In short, MTP undergoes post-translational degradation in hepatocytes exposed to CCl_4. An unknown metabolite of CCl_4 attaches covalently to MTP. This leads to ubiquitinylation and proteasomal degradation of MTP.

Conclusion

In this article, we have discussed regulation of MTP at the transcriptional, post-transcriptional and post-translational levels. The data reviewed indicate that MTP regulation is complex. The major mode of regulation occurs at the transcriptional level. Although several transcription factors, co-activators and co-repressors of MTP have been identified, there is a need for additional information about the mechanisms that control MTP levels in different tissues. This information could be useful in strategically targeting MTP expression and function.

Increased MTP expression during enterocyte differentiation has been shown to involve two proteins, NR2F1 and IRE1β, which negatively regulate MTP expression. The expression of

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these proteins is high when MTP expression is low. Increases in MTP expression correlate with reduced levels of these proteins.

Bile acid and circadian regulation studies also underscore the importance of repressors in the regulation of MTP. Bile acids increase SHP and reduce MTP expression. Circadian mechanisms also utilize SHP to regulate MTP expression. SHP levels are high in the daytime, which is associated with low MTP expression in rodents. Hence, a major mechanism of MTP regulation involves changes in proteins that suppress MTP expression. It remains to be determined whether these repressors can be augmented to reduce MTP expression.

We have also summarized evidence for the regulation of MTP by lipids, macronutrients and hormones. The exact mechanisms by which these and various other factors exert their effects on MTP levels are unknown. Specifically, molecular mechanisms in the regulation of MTP by insulin, leptin and diurnal variations need to be elucidated.

Future perspective

Direct inhibition of MTP has been associated with undesirable outcomes. There is a need to re-evaluate and come up with new approaches for MTP therapy. The purpose of summarizing MTP regulation was to draw attention to the possibility that MTP reduction, perhaps, can be achieved indirectly or in a tissue-specific fashion involving strategies that do not involve inhibition of lipid transfer activity. If such a mechanism is identified then it might be possible to reduce MTP levels partially in all or a specific tissue to lower plasma lipids and avoid risk of cardiovascular and metabolic diseases. An option to achieve such a goal is to target upstream regulators of MTP. In this regard, transcription factors that regulate MTP expression can be targeted. Perhaps interfering with the synergistic activation of MTP by HNF1/HNF-4a can be used to lower MTP expression. Another possibility is to upregulate proteins that negatively regulate MTP. If MTP levels can be reduced with acceptable side effects, MTP suppressors, besides lowering lipids, might also be useful in weight reduction. Additionally, because of its role in CD1 biosynthesis, MTP suppressors/ reducers may be useful in the treatment of natural killer T-cell mediated disorders.

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▪ of interest

▪▪ of considerable interest

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Executive summary

- Microsomal triglyceride transfer protein (MTP) regulation involves a simple promoter that harbors few conserved *cis*-elements.
- Activators, suppressors, co-activators and co-suppressors that regulate MTP have been identified.
- MTP is regulated by lipids and hormones; however, the molecular mechanisms involved have not been explained.
- MTP is critical for the development of the embryo in mice but not in humans.
- MTP gene expression during enterocyte differentiation depends on decreased expression of NR2F1 suppressor and IRE1β endoribonuclease.
- CLOCK and small heterodimeric partner play an important role in the diurnal regulation of MTP expression.
- CCl_4-induced steatosis involves post-translational degradation of MTP.

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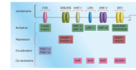

Figure 1. A basic microsomal triglyceride transfer protein promoter depicting various *cis*-elements and transcription factors that bind to these elements

Various regulatory *cis*-elements are located in the 600 bp upstream of the transcription start site. These elements bind to various transcription factors (activators) resulting in increased microsomal triglyceride transfer protein promoter activity. They also bind to repressors reducing expression. Additionally, various co-activators and co-repressors modulate microsomal triglyceride transfer protein expression.

DR: Direct repeat; HNF: Hepatic nuclear factor; PGC: Peroxisome proliferator-activated receptor γ coactivator; SHP: Small heterodimeric partner; SRE/IRE: Sterol and insulin response element.

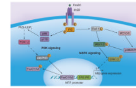

Figure 2. Microsomal triglyceride transfer protein regulation by insulin

Two different pathways regulating microsomal triglyceride transfer protein (MTP) expression have been proposed. In the MAPK signaling pathway, insulin increases phosphorylation of Raf-1, $MEK^{1/2}$ and $ERK^{1/2}$, which culminates in reduced expression of MTP in cells. This signaling arm is negatively regulated by $MAPK^{p38}$. In the PI3K pathway, insulin activates PI3-kinase and Akt/PKB. This leads to increased phosphorylation of forkhead transcription factors, their export from the nucleus and reduced *MTP* gene transcription.

INSR: Insulin receptor; SRE/IRE: Sterol and insulin response element.

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