review

Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly

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Abstract **Apolipoprotein B (apoB) and microsomal triglyceride transfer protein (MTP) are necessary for lipoprotein assembly. ApoB consists of five structural domains, $\beta\alpha_1$-β_1-α_2-β_2-α_3. We propose that MTP contains three structural motifs (N-terminal β-barrel, central α-helix, and C-terminal lipid cavity) and three functional domains (lipid transfer, membrane associating, and apoB binding). MTP's lipid transfer activity is required for the assembly of lipoproteins. This activity renders nascent apoB secretion-competent and may be involved in the import of triglycerides into the lumen of endoplasmic reticulum. In addition, MTP binds to apoB with high affinity involving ionic interactions. MTP interacts at multiple sites in the N-terminal $\beta\alpha_1$ structural domain of apoB. A novel antagonist that inhibits apoB-MTP binding decreases apoB secretion. Furthermore, site-directed mutagenesis and deletion analyses that inhibit apoB-MTP binding decrease apoB secretion. Lipids modulate protein-protein interactions between apoB and MTP. Lipids associated with MTP increase apoB-MTP binding whereas lipids associated with apoB decrease this binding. Thus, specific antagonist, site-directed mutagenesis, deletion analyses, and modulation studies support the notion that apoB-MTP binding plays a role in lipoprotein biogenesis. However, specific steps in lipoprotein assembly that require apoB-MTP binding have not been identified. ApoB-MTP binding may be important for the prevention of degradation and lipidation of nascent apoB.**—Hussain, M. M., J. Shi, and P. Dreizen. **Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly.** ***J. Lipid Res.*** **2003.** 44: **22–32.**

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Plasma lipoproteins are absent in abetalipoproteinemia due to mutations in the microsomal triglyceride transfer protein (MTP) gene, and plasma lipoprotein levels are low in hypobetalipoproteinemia due to mutations in the apolipoprotein B (apoB) gene (1, 2). These genetic disorders clearly underscore the importance of these two proteins in lipoprotein biogenesis, and recent findings indicate that MTP and apoB physically interact during this process. The aim of this review is to discuss specific molecular interactions between these proteins and their role in the biosynthesis of triglyceride-rich lipoproteins. A brief review of apoB and MTP is provided to aid in the understanding of protein-protein interactions between these proteins. In-depth discussion of apoB, MTP, and lipoprotein assembly can be found in several recent reviews and references therein (3–14).

STRUCTURAL AND FUNCTIONAL DOMAINS IN APOB

Apolipoprotein B (apoB) is a non-exchangeable apolipoprotein found associated exclusively with plasma lipoproteins. In the human genome there is one *apob* gene of ~45 kb. In the liver, it is transcribed into a single mRNA of 15 kb and is translated into a single polypeptide of 4536 amino acids called apoB-100. In the intestine, the apoB mRNA is post-transcriptionally edited, resulting in the conversion of a glutamine codon into a stop codon. The edited mRNA is translated into a single polypeptide of 2,152 amino acids called apoB48. By comparing mean hydrophobic moments per amino acid residue and the average hydrophobicity of the hydrophobic face of the helices, Segrest et al. have proposed a pentapartite secondary structure for apoB-100 (3, 15–17). According to this model, apoB100 is comprised of three amphiphatic α-helical domains alternat-

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Abbreviations: ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein; M subunit, 97-kDa subunit of the MTP complex; P subunit, the 55-kDa PDI subunit of the MTP complex; PDI, protein disulfide isomerase.

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ing with two amphiphatic β-sheet domains in an NH_2-α_1-β_1-α_2-β_2-α_3-COOH configuration.

The α_1 domain (B:58–795) is an independent globular domain. It associates with lipids but is incapable of forming a lipoprotein (18, 19). Nonetheless, it is required for lipoprotein assembly because its absence ablates lipoprotein assembly (20). It contains 12 cysteine residues that form six disulfide linkages (21). Proper disulfide bond formation between some cysteine residues is essential for the assembly of apoB-containing lipoproteins (22, 23). Based on sequence homology with lipovitellin, this region has been predicted to consist of a β-barrel (B:1–263) and an α-helical (B:294–592) domains (24) and has subsequently be called $\beta\alpha_1$ domain (3). As discussed below, this domain contains MTP binding site.

The other four domains (β_1-α_2-β_2-α_3) are also comprised of several short amphiphatic β-strands and α-helices. The β-sheet domains (B:827–2001 and B:2571–4032) are essential for lipoprotein assembly and bind lipids nonreversibly. The assembly of these β-sheets into lipoproteins requires α_1 domain (20). The LDL receptor binding and heparin binding sites are in the β_2 domain. ApoB-48 contains β_1 domain only, whereas apoB-100 contains both the β_1 and β_2 domains. These two proteins are used for the assembly of two different lipoproteins, chylomicrons and VLDLs. The α_2 (B:2045–2587) and α_3 (B:4017–4515) domains consist of several amphiphatic helices that can reversibly associate with lipids, a characteristic property of exchangeable apolipoproteins. Their role in lipoprotein assembly is unknown.

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN

Evidence for the presence of a protein in the endoplasmic reticulum (ER) responsible for the transfer of neutral lipids between vesicles was first provided by Wetterau and Zilversmit (25). Subsequently, they purified MTP to homogeneity and showed that it consists of two non-covalently bound polypeptides of 97 (M subunit) and 55 (P subunit) kDa (26–28). The small 55-kDa "P" subunit was the ubiquitous ER resident enzyme protein disulfide isomerase (PDI). The P subunit is inactive with respect to its isomerase activity in the MTP complex (27, 29). Moreover, the isomerase activity is not essential for its association with the larger M subunit and for MTP activity, as PDI mutants lacking enzyme activity are fully functional in lipid transfer activity in association with a normal M subunit (30). The large 97-kDa M subunit was unique and was essential for the lipid transfer activity. The kinetics of lipid transfer from membranes to lipoproteins has not yet been studied in detail.

MTP enhances the rate of lipid transfer between vesicles (31, 32). Kinetic studies with model membranes suggest that MTP transfers lipids by a shuttle mechanism (33). In this mechanism, each MTP molecule is proposed to interact transiently with a membrane, extract lipid molecules, dissociate from the membrane, bind transiently with another membrane, deliver lipids rapidly to the second membrane, and become available for another cycle of lipid transfer. The lipid transfer activity was shown to be optimum with neutrally charged membranes and decreased in the presence of negatively charged lipids in vesicles (33). Kinetic studies suggest that MTP has two, one fast and one slow, lipid binding sites (34, 35). The fast site is implicated in lipid transfer (34).

FUNCTIONAL DOMAINS IN THE HETERODIMERIC COMPLEX

The M subunit is a single polypeptide of 894 amino acids (36). Based on sequence homology with lipovitellin, it is proposed (24, 37, 38) that the M subunit contains three domains: N-terminal β-barrel, central α-helical domain, and C-terminal lipid-binding cavity (**Fig. 1**). We propose that MTP may contain at least three (lipid transfer, membrane associating, and apoB binding) functionally independent domains (Fig. 1). Kinetic studies indicate for the presence of two, one low and one high affinity, lipid-binding domains in MTP (34). The high affinity domain binds few molecules of neutral lipids and phospholipids and may represent the lipid transfer domain. The lipid transfer activity antagonists probably bind at this site and inhibit lipid transfer activity. Precise information about the lipid transfer domain in MTP is not available but based on the homology with lipovitellin (39), Read et al. (38) have suggested that MTP contains a C-terminal lipid binding cavity. The walls of the lipid binding cavity in MTP are formed by the A and C β-sheets present in the M subunit (Fig. 1). The α-helical domain holds these sheets together. The back of the cavity is probably covered by the P subunit (not shown in the figure). The lipid transfer domain may be involved in the loading and unloading of lipid molecules, a step necessary for their transfer. A nonsense mutation in the A sheet, Asn780Tyr, does not affect its binding to the P subunit but abolishes MTP's lipid transfer activity (40). Thus, the C-terminal 1/3rd of the M subunit and the P subunit may form a lipid transfer domain in MTP.

Studying the binding of ^{125}I-MTP to lipid vesicles and separating MTP from vesicles by density gradient ultracentrifugation, we provided evidence for the stable association of MTP with membranes (41). Similarly, Read et al. (38) have shown association of MTP with lipid vesicles. The low affinity lipid-binding domain identified in the kinetic experiments may be involved in membrane association. At present, the structural properties of the membrane-associating domain are not known. We propose that the region between the N and the A sheets may form a membrane-association domain. It should be pointed out that the lipovitellin (LV) structure contains a lipid moiety at this site (39).

We showed that apoB-binding domain in MTP is different from the lipid transfer domain because the lipid transfer activity inhibitors do not inhibit apoB-MTP binding, and inhibitors that inhibit apoB-MTP binding have no effect on lipid transfer activity of MTP (42). Furthermore, im-

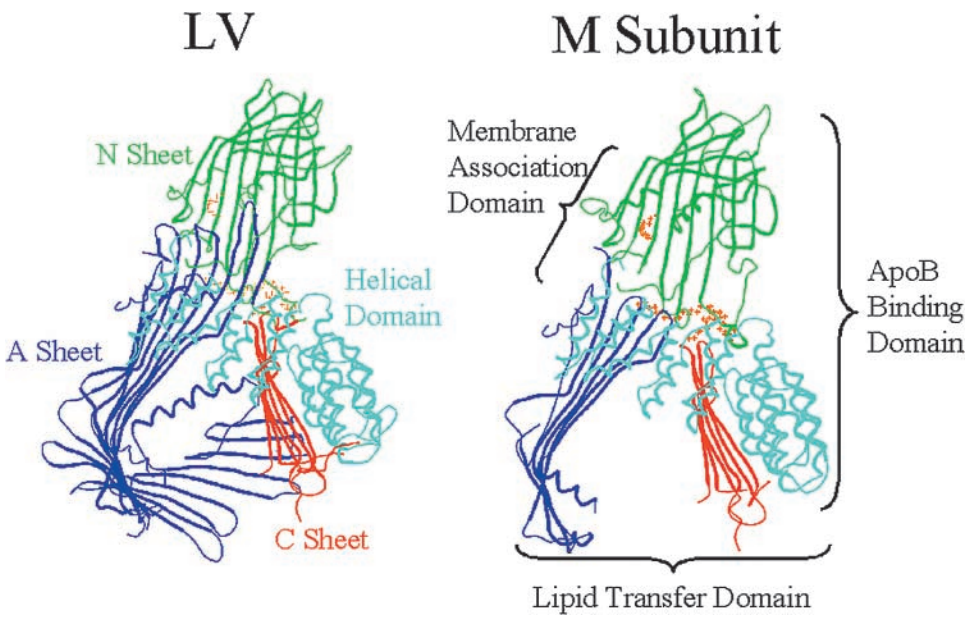

Fig. 1. Structures of lipovitellin (LV) and the 97-kDa subunit of the MTP complex (M subunit) of microsomal triglyceride transfer protein (MTP):LV. X-ray structure of lamprey LV contains N sheet (green), C sheet (red), A sheet (blue), helical domain (cyan), and associated lipids (orange) (39). M subunit: Molecular model of the M subunit of MTP based upon sequence homology with LV:15-521 using CLUSTAL W 1.8 and modified alignment for LV:522–907 as described (24, 38). We have retained LV β-sheets and α-helices, defined by ϕ/ψ angles, to deduce the structure of the M subunit. Various sheets and helices have been either truncated or deleted to accommodate the absence of homologous sequences in the M subunit. Side chains are replaced using dictionary definitions, keeping the main chain intact. Sequence gaps and insertions assigned to loops and β-turns are rebuilt with the fewest residues needed based on loop searches of known PDB structures. Using the AMBER force field (72), the structure was energy minimized using the SIMPLEX method to keep maximum force on any atom below 1,000 kcal/mol D^2, followed by the conjugate gradient method to convergence at 0.5 kcal/mol D. Atomic coordinates are deposited in the Protein Data Bank. Calculations and graphics were done using SYBYL software (Tripos Inc.). The structure of the M subunit has largely conserved N sheet, C sheet, and helical domain and a truncated A sheet. Three functional domains have been identified. The membrane-association domain is hypothesized to be the region between the N and the A sheets. The apoB-binding domain has been shown to involve both the N-sheet and the helical domain. The lipid transfer domain is lined by the A and C sheets. The major structural difference between LV and MTP is in the lipid transfer domain due to the absence of several β-strands of the A sheet. In the M subunit, the lipid transfer domain is larger and more open and may be responsible for the transfer of greater number of lipid molecules compared to LV.

mobilization of MTP results in partial loss of MTP's lipid transfer activity but has no effect on apoB-MTP binding (43, 44). Similarly, apoB-binding and membrane associating domains in MTP appear to be dissimilar. Evidence for the independent membrane associating and apoB binding domains also comes from the modulation of apoB-MTP interactions by lipids (41). If apoB and membrane binding domains were the same, then apoB-MTP binding would have decreased in the presence of lipids. Contrary to this expectation, association of MTP with lipids resulted in increased binding to apoB. Thus, apoB-binding domain in MTP appears to be different from both the lipid transfer and membrane associating domains in MTP.

DOMAINS INVOLVED IN SUBUNIT INTERACTIONS

The M subunit requires the P subunit for its solubility, retention in the ER, and for lipid-transfer activity (27, 29). These two subunits are held together by non-covalent interactions. Early evidence for the P subunit-binding site in the M subunit came from the identification of genetic mutations in abetalipoproteinemia. Ricci et al. (45) sequenced the *mttp* gene, which codes for the M subunit, from an abetalipoproteinemia patient and showed that the C-terminal 30 amino acids are required for its interaction with the P subunit. Using yeast two-hybrid system, Bradbury et al. (37) showed that P:1–274 bind to the central α-helical region of the M (M:297–603) subunit. Within this region, M:520–598 showed maximum binding to the P subunit. The binding between the N-terminus of the P subunit and the middle region of the M subunit may constitute a nucleation site for the heterodimerization of the two subunits. Subsequent binding of other regions in the P subunit with the C-terminal region in the M subunit are probably important for the formation of a soluble and biochemically active heterodimeric MTP complex.

PROTEIN-PROTEIN INTERACTIONS BETWEEN APOB AND MTP

Three independent approaches (coimmunoprecipitation, solid-liquid inter-phase binding assays, and yeast two-hybrid system) have been used to demonstrate protein-protein interactions between apoB and MTP. Wu et al. (46) presented the first evidence for interactions between these proteins in 1996 using co-immunoprecipitation technique. They immunoprecipitated MTP from [^{3}H]leucine-labeled HepG2 cells and found that about 5–10% of the nascent apoB was associated with MTP. Furthermore, co-immunoprecipitation of apoB was also demonstrated by Western blot analysis of proteins immunoprecipitated with anti-MTP antibodies. In the same year Patel and Grundy transfected COS cells with various C-terminally truncated apoB polypeptides with MTP, immunoprecipitated apoB with anti-apoB antibodies, and observed that MTP was precipitated with various apoB peptides (47).

We published a solid-liquid inter-phase binding assay in 1997 to study apoB-MTP binding. First, we immobilized different lipoproteins to microtiter plates and incubated them with ^{125}I-labeled heterodimeric MTP. The amounts of MTP bound to LDL and VLDL were significantly higher than those bound to HDL (43). Next, the binding of MTP to different lipoproteins was compared with the binding of PDI. Lipoproteins bound to MTP but not to PDI. These studies indicated that the M subunit plays an important role in lipoprotein binding. Subsequently, we showed that immobilized heterodimeric MTP also interacted with lipoproteins present in solution. Kinetic studies demonstrated that protein-protein interactions between these proteins were of high affinity (K_d 10–30 nM).

In 1999, Shoulders and associates used baculoviral expression system and yeast two-hybrid system to study protein-protein interactions (24, 37). They expressed apoB-17 with M subunit, P subunit, or both M and P subunits in Sf9 cells, immunoprecipitated apoB, and looked for the co-precipitation of the M and P subunits (24). PDI was not precipitated with apoB. However, the M subunit was immunoprecipitated with apoB-17 when expressed with the P subunit. These studies reinforced the notion that apoB interacts with the M subunit and the P subunit is probably not required for apoB binding.

NATURE OF INTERACTIONS

Patel and Grundy made the first attempt to understand the nature of interactions between apoB and MTP (47). They washed coimmunoprecipitated apoB-MTP complexes with high salt concentrations, observed that the protein complexes were not disrupted by these washes, and concluded that the protein-protein interactions were hydrophobic in nature. We also studied the effect of salt on apoB-MTP binding (43). Presence of salt during binding inhibited protein-protein interactions, indicating that the binding was ionic in nature. Furthermore, in agreement with the studies of Patel and Grundy, pre-formed apoB-MTP complexes could not be disrupted by high salt washes (43). Thus, it appears that protein-protein interactions between apoB and MTP initially involve ionic interactions. Subsequently, these interactions become resistant to salt washes, most likely due to additional hydrophobic interactions.

The importance of ionic interactions was substantiated by chemical modification of functional groups in apoB using group specific reagents (48). Modification of 38–44% of the aspartic and glutamic acid residues in LDL by glycine methyl ester had no effect on apoB-MTP binding. Alteration of all histidine residues by diethyl pyrocarbonate in LDL decreased, but did not abolish, apoB-MTP binding. Treatment with 1,2-cyclohexanedione resulted in the modification of 54% of the arginine residues in LDL and completely abolished its binding to MTP. Similarly, modification of 74% of lysine ε-amino groups by acetoacetylation abolished LDL-MTP binding. More importantly, hydroxylamine treatment of the modified LDL regenerated all modified arginine and lysine residues and completely restored MTP binding. Modification of the ε-amino groups by reductive methylation and acetylation further substantiated the involvement of positive charges. Reductive methylation does not alter the positive charges, whereas acetylation neutralizes the positive charges. Reductive methylation of 88% of lysine residues did not affect apoB-MTP binding. In contrast, acetylation of LDL completely abolished apoB-MTP binding. These studies showed that positive charges on the functional side groups of arginine and lysine in apoB are critical for apoB-MTP binding. Most likely, they interact with negatively charged residues in MTP.

Positively charged amino acid residues in apoB are known to interact with heparin and LDL receptors (49, 50). To determine whether the MTP binding site overlaps with the heparin binding site, Bakillah et al. studies the inhibition of LDL-MTP binding by heparin, chondroitin sulfate, and suramin (48). Heparin and chondroitin sulfate had no significant inhibitory effect on LDL-MTP binding. However, suramin, a highly charged polysulfated polycyclic hydrocarbon, inhibited apoB-MTP binding. Inhibition by suramin indicates that apoB-MTP binding involves ionic interactions. Lack of inhibition of apoB-MTP binding by heparin indicates that heparin binding and MTP binding sites are independent and different from each other. Thus, lysine and arginine residues crucial for MTP binding are different from those involved in heparin and LDL receptor binding.

DOMAINS IN APOB THAT INTERACT WITH MTP

Patel and Grundy (47) showed that MTP co-immunoprecipitates with apoB polypeptides as small as apoB13. This was the first study to demonstrate that MTP binding site may be present in the N-terminus of apoB. To identify MTP binding site in apoB, we compared the binding of equimolar concentrations of several C-terminally truncated apoB polypeptides to MTP (43). ApoB-100 and apoB-42 bound to similar extent. However, decreasing the length

TABLE 1. MTP binding site in apoB

ApoB Sequences	Binding
	% of B:270–570
Amino acids	
C-terminal truncations[a]	
270–570	100
270–509	58
270–430	9
270–394	0
N-terminal truncations[b]	
270–570	100
291–570	80
341–570	67
430–570	0

[a] The data is from (44).
[b] Unpublished observations.

of apoB from apoB-42 to apoB-28 resulted in 2.6-fold increased binding. Further decrease in length from apoB-28 to apoB-17 resulted in another 2-fold increased binding to MTP. These studies indicated that the binding of apoB to MTP decreases with increases in the length of apoB polypeptide. Recently, Liang and Ginsberg (51) showed that MTP binds to the N-terminal apoB-16 but not to β_1 domain of apoB (51). Thus, it can be concluded that MTP binds to the N-terminal $\beta\alpha_1$-globular domain of apoB.

To further delineate the MTP binding site in the $\beta\alpha_1$-domain, we expressed different apoB sequences as FLAG-apoB chimeras in COS cells (44). Two chimeras, B:1–300 and B:270–570 were secreted to similar extent, but their binding to MTP was significantly different. B:270–570 bound robustly to MTP but the binding of B:1–300 was considerably low (4–25% of B:270–570 in different experiments, unpublished observations). We concluded that B:270–570 contains a high affinity binding site for MTP. Attempts were then made to identify the minimum sequence that could bind to MTP. For this purpose, we subjected B:270–570 to N- and C-terminal truncations at proline residues (**Table 1**). C-terminal deletion to amino acid 430 resulted in significant loss of MTP binding. These studies indicated that amino acids 430–570 are critical for MTP binding. Progressive N-terminal truncations of amino acids from 270 to 341 resulted in progressive decrease in MTP binding, and truncations to amino acid 430 resulted in significant loss of MTP binding. More importantly, these studies showed that B:430–570 do not bind to MTP. Thus, it appears that amino acids 270–570 are required for optimum binding.

Mann et al. (24) showed that B:1–152 interacted with MTP significantly more than the binding of apoA-I to MTP. They also used yeast two-hybrid system to study apoB-MTP interactions (37). By co-expressing various combinations of apoB and M subunit sequences in yeast, they showed that B:349–583 and B:512–721 also bind to MTP. Based on the data discussed above from different groups, it can be concluded that MTP interacts at multiple sites in the $\beta\alpha_1$-domain of apoB. Critical amino acids in this region that interact with MTP are not known.

DOMAINS IN MTP THAT INTERACT WITH APOB

Mann et al. (24) and Bradbury et al. (37) identified two regions in MTP that interact with two different binding sites in apoB; B:1–264 interact with amino-terminal β-barrel of MTP (M:22–303) and B:512–721 interact with M:517–603 in MTP. It needs to be determined whether the binding characteristics of these two binding sites are similar or dissimilar. Moreover, contribution of these interactions in lipoprotein assembly needs to be determined.

FACTORS MODULATING APOB-MTP BINDING

Inhibition of apoB-MTP binding by sequences within apoB

As discussed above MTP binds to the N-terminal 17% of apoB. We observed that increasing the length of apoB from apoB-17 to apoB-28, and from apoB-28 to apoB-42 resulted in substantial decrease in MTP binding (43). The reasons for decrease in MTP binding with increase in apoB length are not known and need further investigation. We suggested two reasons for incremental decreases with increases in the length of apoB (43). First, decrease in MTP binding by increasing the length from apoB-17 to apoB-28 may be due to the presence of a sequence that might inhibit apoB-MTP binding. Second, region between apoB-28 and apoB-42 may bind lipids and decrease apoB-MTP binding.

Effect of PDI on apoB-MTP binding

In yeast two-hybrid system, Bradbury et al. observed that M:520–598 interact with the P subunit and apoB-17 (37). They expressed M subunit and apoB-17 in the presence and absence of P subunit in insect cells and observed that the P subunit decreased the binding of apoB to the M subunit. These studies indicate that the P subunit may affect apoB binding to M subunit of MTP most likely prior to the formation of heterodimeric MTP complex. It remains to be determined whether the P subunit can modulate apoB binding to the heterodimeric MTP. Most likely, it may not for the following reasons. There is excess of PDI in the ER lumen and yet apoB-MTP complexes have been isolated by immunoprecipitations. The M subunit probably does not exist in the ER unassociated with the P subunit and thus has a limited ability to interact with apoB in the absence of the P subunit.

MODULATION OF APOB-MTP BINDING BY LIPIDS

Lipids associated with MTP increase apoB-MTP binding

Wu et al. (46) showed that, at basal levels, ~10% of nascent apoB is associated with MTP in HepG2 cells. Inhibition of intracellular apoB degradation by proteosomal inhibitors prolonged the duration of apoB-MTP binding without increasing the amounts of apoB bound to MTP. In contrast, increases in triglyceride synthesis by the supplementation of oleic acid increased the amounts of apoB as-

sociated with MTP by 3–5-folds. Thus, these studies provided initial evidence that lipids may modulate apoB-MTP binding.

We observed that lipids have a profound effect on apoB-MTP binding (41). Zwitterionic phospholipids, phosphotidylcholine, and sphingomyelin increased apoB-MTP binding and negatively charged phospholipids, phosphatidylinositol, and phosphatidylserine and decreased apoB-MTP binding (41). The negatively charged lipids, most likely, competed with ionic interactions between these proteins. However, the effect of zwitterionic lipids was intriguing. Kinetic analysis revealed that phosphatidylcholine vesicles increased affinity between proteins without affecting the number of binding sites. To understand the mechanisms for increased affinity between proteins, we pre-incubated apoB and MTP with lipids. Pre-incubation of apoB with lipids had no effect on MTP binding. In contrast, pre-incubation of MTP with phosphatidylcholine vesicles increased apoB binding by 4-fold (41). During pre-incubation, MTP stably associated with phospholipid vesicles and the MTP-lipid vesicles bound better to apoB. These studies clearly indicate that the association of MTP with phospholipid vesicles results in increased affinity for apoB. It is likely that apoB first interacts with MTP via ionic interactions. Next, lipids bound to MTP may interact either with lipid-binding domains in apoB or with lipids present in apoB-containing lipoproteins. Thus, protein-protein interactions may bring lipids into close proximity leading to additional hydrophobic interactions between lipids. Combination of ionic and hydrophobic interactions may result in increased affinity.

Lipids associated with apoB decrease apoB-MTP binding

Pre-incubation of apoB with phospholipids had no effect on apoB-MTP binding (41). However, apoB-associated lipids decrease MTP binding. Partial de-lipidation of VLDL with Tween-20 increased the binding of VLDL-apoB to MTP (43). Similarly, partial de-lipidation of LDL with taurocholate increased its binding to MTP. These studies suggest that the amounts of lipids associated with apoB have a negative effect on apoB-MTP binding. Hence, increased apoB lipidation may decrease apoB-MTP binding.

MTP AND APOB-LIPOPROTEIN ASSEMBLY

Role of MTP's lipid transfer activity in lipoprotein assembly

Three independent approaches led to the conclusion that the lipid transfer activity of the heterodimeric MTP complex is essential for the assembly and secretion of apoB-containing lipoproteins. First, mutations in the M subunit were correlated with defective in vitro lipid transfer activity and an absence of apoB-containing lipoproteins in the plasma of abetalipoproteinemia patients (6, 7, 9, 10, 32). Second, direct correlation between MTP activity and lipoprotein assembly was obtained by coexpressing apoB and MTP in non-hepatic, non-intestinal cells that neither express apoB nor MTP (20, 52, 53). Expression of apoB alone in most studies resulted in the intracellular synthesis and degradation of apoB polypeptides, but no secretion. In contrast, co-transfection of apoB with MTP resulted in the synthesis and secretion of apoB (20, 52, 53). The secretion efficiency of apoB was low and apoB was secreted as HDL-size particles, indicating that other factors are needed for optimal lipoprotein assembly and secretion. In Chinese hamster ovary cells, in addition to MTP, cholesterol 7α-hydroxylase has been shown to be necessary for apoB secretion (54). Third, the importance of the lipid transfer activity in the lipidation of apoB polypeptides was reinforced by using specific inhibitors (55–60). MTP inhibitors that inhibited lipid transfer activity in vitro decreased apoB secretion in vivo. Thus, MTP's lipid transfer activity is essential for the assembly and secretion of apoB-containing lipoproteins. However, it is still not clear how this lipid transfer activity of MTP results in a net transfer of lipids to nascent apoB. So far, no in vitro assay has been reported that demonstrates net transfer of lipids to apoB-lipoproteins resulting in the assembly of larger lipoproteins.

Substantial evidence exists to suggest that MTP's lipid transfer activity is essential for two processes in lipoprotein assembly. First, it is required for rendering apoB-secretion competent. Rusinol et al. have shown that nascent apoB can be assembled into lipoproteins in a cell-free system in the absence of MTP (61). The secretion competency is achieved by the addition of lipids to nascent apoB and inhibition of intracellular degradation. The lipidation process inhibits the degradation of nascent apoB and promotes lipoprotein assembly. The dependency of apoB on MTP with respect to its lipidation and secretion appears to depend on the length of apoB. Specific inhibitor and gene ablation studies have shown that the larger apoB peptides are more dependent on MTP compared to smaller apoB peptides (62–64). In fact, there are reports indicating that apoB-48 may be secreted in the absence of MTP (64). Nicodeme et al. (62) have suggested that a region between apoB-51 and apoB-53 has a high requirement for lipids and for the lipid transfer activity of MTP.

Evidence is accumulating to suggest that MTP's lipid transfer activity may be required for the accumulation of triglycerides in the lumen of the ER. It has been shown that the absence of MTP or inhibition of MTP leads to decreased triglycerides in the lumen of the ER (64, 65). It has been suggested that MTP may in involved in the formation and stabilization of lumenal lipid droplets (41). The accumulation of the triglycerides in the ER is probably required for the "core expansion" of the nascent primordial particle (see below).

Importance of apoB-MTP binding in lipoprotein assembly

We observed that MTP's lipid transfer activity antagonists had no effect on apoB-MTP binding (42). These studies encouraged us to look for compounds that might inhibit protein-protein interactions without affecting lipid transfer activity of MTP. Screening of several compounds from the

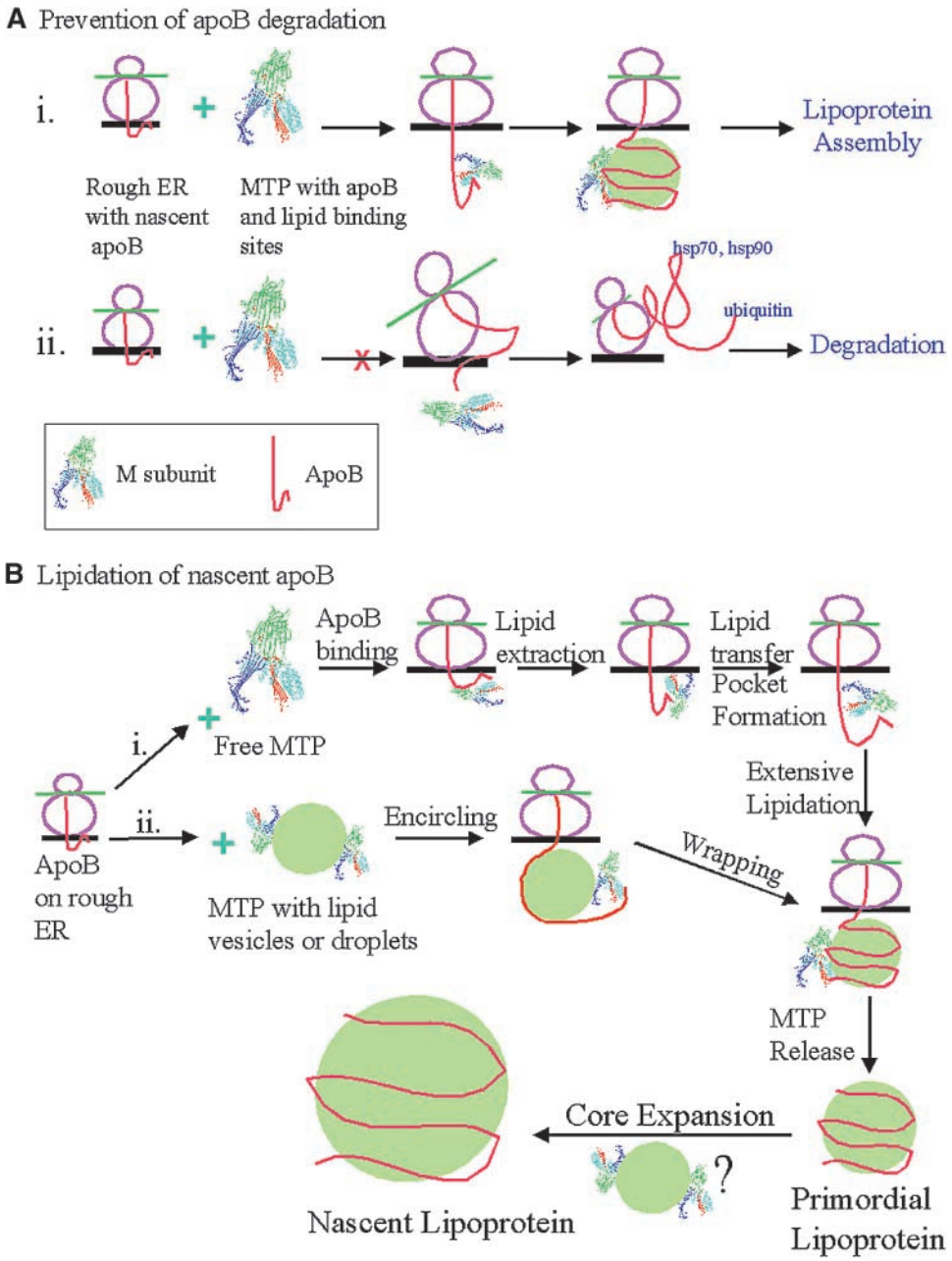

Fig. 2. Possible roles of apoB-MTP binding in lipoprotein assembly. A: Prevention of apoB degradation: *i*) nascent apoB is depicted interacting with the inner leaflet of the endoplasmic reticulum (ER). MTP consists of "M" and "P" subunits. The M subunit contains independent apoB and lipid binding sites. MTP is shown to interact with nascent apoB. This binding may facilitate lipidation of nascent apoB. *ii*) If the apoB-MTP binding is inhibited by inhibitors or mutations in the binding sites, then apoB is predicted to undergo degradation involving hsp70 and hsp90 binding, ubiquitination, and proteosomal degradation. B: Lipidation of nascent apoB: lipidation of apoB can occur by two different pathways: *i*) Free MTP that is not associated with lipid droplets or vesicles may bind nascent apoB. Subsequently, the apoB bound MTP can extract lipid molecules from the membrane and transfer them to nascent apoB polypeptide. Note that this process may involve formation of a pocket. Several rounds of this process will result in extensive lipidation of apoB. MTP molecules that are not physically associated with apoB can further assist this step and the pocket may serve as a nucleation site for lipid deposition. *ii*) MTP associated with lipid vesicles or droplets may bind to apoB and provide a lipid core for the nascent apoB to encircle and wrap around it. This would result in an efficient and faster assembly of apoB-containing lipoproteins and may predominate during excess availability of fat. Release of MTP would result in the formation of secretion-competent primordial lipoproteins. Primordial lipoproteins can be supplied with a large dose of fat resulting in their core expansion and formation of nascent lipoproteins. Droplets containing MTP molecules can efficiently accomplish the process of core expansion by fusing with primordial lipoproteins.

Atherogenics Inc. resulted in the identification of a compound, AGI-S17, that inhibited apoB-MTP binding by ~70% at 40 μM and had no effect on MTP's lipid transfer activity (42). Next, we studied the effect of AGI-S17 in HepG2 cells. Incubation of HepG2 cells with AGI-S17 had no effect on the lipid transfer activity of MTP but decreased its binding to apoB. These studies indicated that AGI-S17 inhibited intracellular apoB-MTP binding with-

out affecting lipid-transfer activity. In addition, AGI-S17 inhibited the secretion of total and nascent apoB by ~70%. Most likely, inhibition of apoB secretion by AGI-S17 is a consequence of the inhibition of intracellular apoB-MTP binding. These studies provide strong evidence that protein-protein interactions between apoB and MTP may be important for lipoprotein assembly and secretion.

Evidence for the importance of apoB-MTP binding in lipoprotein assembly also comes from the studies of Bradbury et al. (37). They showed that mutations in B:521–721 decrease MTP binding and apoB secretion. Moreover, Liang and Ginsberg (51) showed that deletion of B:1–210 that contains the first MTP binding site decreased the secretion of apoB-34 but had no effect on apoB-16 secretion. As discussed before, modulation of apoB-MTP binding by lipids also indicates that it may be a physiologically significant event. It should be pointed out that apoB has been shown to bind several other proteins (66), but modulation for the binding of these proteins to apoB has not yet been demonstrated. Thus, specific antagonists, site-directed mutagenesis, deletion analysis, and modulation studies lend strong support to the notion that apoB-MTP binding plays an important role in lipoprotein biogenesis.

We propose that apoB-MTP binding may play different roles during lipoprotein biosynthesis (**Fig. 2**). For clarity, apoB-MTP binding is shown to play an important role in two steps, *A*) prevention of apoB degradation and *B*) lipidation of nascent apoB. We have suggested that the emerging nascent apoB polypeptide interacts with MTP prior to its lipidation due to the localization of its binding site in the N-terminal 17% of the molecule (42, 43). It is known that apoB undergoes dislocation from the ER and degradation by proteosomes if not assembled into lipoproteins (67, 68). It is proposed that MTP binding may facilitate the import of nascent apoB into the ER lumen, prevent dislocation from the ER, and inhibit proteosomal degradation. These processes can be facilitated further by the concomitant lipidation of the emerging apoB peptide by MTP (Fig. 2A, i). If inhibitors or mutations inhibit apoB-MTP binding, apoB is predicted to undergo degradation involving hsp70 (69) and hsp90 binding (70), ubiquitination, and proteosomal degradation (Fig. 2A, ii).

Formation of primordial lipoproteins requires MTP. MTP may participate in this process by two ways. First, free MTP (unassociated with lipid droplets or vesicles) may bind nascent apoB, extract lipids from membrane, and transfer to apoB (Fig. 2B, i). This may result in the formation of "lipid pocket" as has been suggested by Segrest et al. (3, 17) similar to that observed in LV, which accommodates several lipid molecules. As shown in Fig. 2, the lipid pocket could be an important intermediate acceptor. This pocket could be a nucleation site to accommodate lipid influx. The apoB-MTP binding may sequester MTP to apoB, leading to a preferential unidirectional transfer of lipids to apoB. Thus, apoB-MTP binding may help in net transfer of lipids from membrane to apoB. It should be noted that MTP molecules that are not bound to apoB could facilitate transfer of lipids to the pocket. Second, it is also possible that this process occurs in one step where MTP delivers lipids in bulk (Fig. 2B, ii). MTP associated with lipid vesicles or droplets may bind to apoB and provide a lipid core for the nascent apoB to encircle and wrap around it. This would result in efficient and faster assembly of apoB-containing lipoproteins and may predominate during excess lipid availability. Release of MTP would result in the formation of secretion-competent "primordial lipoproteins."

Very little is known about the lipid droplet formation. This process may require efficient lipid synthesis. In addition, it may require MTP because mice deficient in MTP expression do not accumulate lipid droplets in the ER lumen (64). Wang et al. suggested that MTP activity might be necessary for the accumulation of triglycerides into the ER lumen (65). We have shown that MTP exists associated with lipids in the ER lumen (41). Thus, MTP may play a role in the formation and stabilization of lipid droplets. Obviously, this property of the MTP does not require apoB binding.

The role of MTP in the fusion of lipids droplets with primordial lipoproteins to form nascent lipoproteins in a process called "core expansion" is not known. The apoB-MTP binding may bring lipids associated with apoB and MTP into close proximity and facilitate their fusion. It is also possible that MTP may initiate the fusion of lipids. MTP has been proposed to contain sequences that may have fusogenic properties (38). Thus, apoB-MTP binding may also play an important role during core expansion of primordial lipoproteins.

CONCLUDING REMARKS

Major advances have been made in the understanding of the molecular interactions between apoB and MTP, identification of binding sites, and regulation of these interactions. In addition, data is available to support the idea that apoB-MTP binding plays a role in lipoprotein biosynthesis. However, very little progress has been made in the identification of different steps in lipoprotein assembly that require apoB-MTP binding. We have discussed various steps that might need apoB-MTP binding (Fig. 2) and hope that this will spur new investigations to delineate them. Knowledge of these steps will establish that MTP acts as a chaperone during lipoprotein assembly.

It remains to be determined whether inhibition of apoB-MTP will increase intracellular triglyceride accumulation. This is important because MTP's lipid transfer activity inhibitors have not yet proven to be useful in controlling plasma lipid levels because they significantly decrease lipoprotein secretion and cause increased accumulation of lipids in the liver. The structural information may be useful in obtaining compounds that partially inhibit lipoprotein assembly and secretion. Sub-optimal inhibition of lipoprotein assembly may provide a more desirable phenotype of lowering plasma lipid levels coupled with low lipid accumulation in the liver.

ApoB has been shown to interact with several other chaperones in the ER (66). At present, no information is

available about the modulation of the binding of different chaperones to apoB. Particularly, it is not known how the binding of these chaperones to apoB affects MTP binding and vice versa. Modulation of the binding of various chaperones to apoB may play an important role in the maturation of apoB into secretion-competent lipoprotein, and their understanding will provide new insights into lipoprotein biosynthesis. A proteomic approach has resulted in the identification of several apoB-binding proteins (71). It remains to be determined whether these proteins play a role in lipoprotein assembly.

The role of MTP in the import of triglycerides into the lumen of ER also needs further investigation. The acceptors involved in the import need to be identified. It remains to be determined how these acceptors compete with apoB and how MTP discriminates between different acceptors and avoids futile cycle of triglyceride transfer between membranes.

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REFERENCES

1. Havel, R. J., and J. P. Kane. 1995. Introduction: Structure and metabolism of plasma lipoproteins. *In* The Metabolic and Molecular Bases of Inherited Disorders. C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, editors. McGraw-Hill, Inc., New York. 1841–1851.
2. Kane, J. P., and R. J. Havel. 1995. Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins. *In* The Metabolic and Molecular Bases of Inherited Disorders. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, Inc., New York. 1853–1885.
3. Segrest, J. P., M. K. Jones, H. De Loof, and N. Dashti. 2001. Structure of apolipoprotein B-100 in low density lipoproteins. *J. Lipid Res.* **42:** 1346–1367.
4. Fisher, E. A., and H. N. Ginsberg. 2002. Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. *J. Biol. Chem.* **277:** 17377–17380.
5. Shelness, G. S., and J. A. Sellers. 2001. Very-low-density lipoprotein assembly and secretion. *Curr. Opin. Lipidol.* **12:** 151–157.
6. Davidson, N. O., and G. S. Shelness. 2000. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu. Rev. Nutr.* **20:** 169–193.
7. Berriot-Varoqueaux, N., L. P. Aggerbeck, M. Samson-Bouma, and J. R. Wetterau. 2000. The role of the microsomal triglygeride transfer protein in abetalipoproteinemia. *Annu. Rev. Nutr.* **20:** 663–697.
8. Olofsson, S. O., L. Asp, and J. Borén. 1999. The assembly and secretion of apolipoprotein B-containing lipoproteins. *Curr. Opin. Lipidol.* **10:** 341–346.
9. Davis, R. A. 1999. Cell and molecular biology of the assembly and secretion of apolipoprotein B-containing lipoproteins by the liver. *Biochim. Biophys. Acta.* **1440:** 1–31.
10. Gordon, D. A., and H. Jamil. 2000. Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. *Biochim. Biophys. Acta.* **1486:** 72–83.
11. Ginsberg, H. N. 1997. Role of lipid synthesis, chaperone proteins and proteasomes in the assembly and secretion of apoprotein B-containing lipoproteins from cultured liver cells. *Clin. Exp. Pharmacol. Physiol.* **24:** A29–A32.
12. Yao, Z., K. Tran, and R. S. McLeod. 1997. Intracellular degradation of newly synthesized apolipoprotein B. *J. Lipid Res.* **38:** 1937–1953.
13. Hussain, M. M., M. H. Kedees, K. Singh, H. Athar, and N. Z. Jamali. 2001. Signposts in the assembly of chylomicrons. *Front. Biosci.* **6:** D320–D331.
14. Hussain, M. M. 2000. A proposed model for the assembly of chylomicrons. *Atherosclerosis.* **148:** 1–15.
15. Segrest, J. P., M. K. Jones, V. K. Mishra, G. M. Anantharamaiah, and D. W. Garber. 1994. ApoB-100 has a pentapartite structure composed of three amphipathic alpha-helical domains alternating with two amphipathic beta-strand domains - Detection by the computer program LOCATE. *Arterioscler. Thromb.* **14:** 1674–1685.
16. Segrest, J. P., M. K. Jones, V. K. Mishra, V. Pierotti, S. H. Young, J. Borén, T. L. Innerarity, and N. Dashti. 1998. Apolipoprotein B-100: conservation of lipid-associating amphipathic secondary structural motifs in nine species of vertebrates. *J. Lipid Res.* **39:** 85–102.
17. Segrest, J. P., M. K. Jones, and N. Dashti. 1999. N-terminal domain of apolipoprotein B has structural homology to lipovitellin and microsomal triglyceride transfer protein: a "lipid pocket" model for self-assembly of apoB-containing lipoprotein particles. *J. Lipid Res.* **40:** 1401–1416.
18. Herscovitz, H., A. Derksen, M. T. Walsh, C. J. McKnight, D. L. Gantz, M. Hadzopoulou-cladaras, V. Zannis, C. Curry, and D. M. Small. 2001. The N-terminal 17% of apoB binds tightly and irreversibly to emulsions modeling nascent very low density lipoproteins. *J. Lipid Res.* **42:** 51–59.
19. DeLozier, J. A., J. S. Parks, and G. S. Shelness. 2001. Vesicle-binding properties of wild-type and cysteine mutant forms of alpha(1) domain of apolipoprotein B. *J. Lipid Res.* **42:** 399–406.
20. Gretch, D. G., S. L. Sturley, L. Wang, B. A. Lipton, A. Dunning, K. A. A. Grunwald, J. R. Wetterau, Z. Yao, P. Talmud, and A. D. Attie. 1996. The amino terminus of apolipoprotein B is necessary but not sufficient for microsomal triglyceride transfer protein responsiveness. *J. Biol. Chem.* **271:** 8682–8691.
21. Yang, C. Y., T. W. Kim, S. A. Weng, B. R. Lee, M. L. Yang, and A. M. Gotto, Jr. 1990. Isolation and characterization of sulfhydryl and disulfide peptides of human apolipoprotein B-100. *Proc. Natl. Acad. Sci. USA.* **87:** 5523–5527.
22. Huang, X. F., and G. S. Shelness. 1997. Identification of cysteine pairs within the amino-terminal 5% of apolipoprotein B essential for hepatic lipoprotein assembly and secretion. *J. Biol. Chem.* **272:** 31872–31876.
23. Tran, K., J. Borén, J. Macri, Y. W. Wang, R. McLeod, R. K. Avramoglu, K. Adeli, and Z. Yao. 1998. Functional analysis of disulfide linkages clustered within the amino terminus of human apolipoprotein B. *J. Biol. Chem.* **273:** 7244–7251.
24. Mann, C. J., T. A. Anderson, J. Read, S. A. Chester, G. B. Harrison, S. Köchl, P. J. Ritchie, P. Bradbury, F. S. Hussain, J. Amey, B. Vanloo, M. Rosseneu, R. Infante, J. M. Hancock, D. G. Levitt, L. J. Banaszak, J. Scott, and C. C. Shoulders. 1999. The structure of vitellogenin provides a molecular model for the assembly and secretion of atherogenic lipoproteins. *J. Mol. Biol.* **285:** 391–408.
25. Wetterau, J. R., and D. B. Zilversmit. 1984. A triglyceride and cholesteryl ester transfer protein associated with liver microsomes. *J. Biol. Chem.* **259:** 10863–10866.
26. Wetterau, J. R., and D. B. Zilversmit. 1985. Purification and characterization of microsomal triglyceride and cholesteryl ester transfer protein from bovine liver microsomes. *Chem. Phys. Lipids.* **38:** 205–222.
27. Wetterau, J. R., K. A. Combs, S. N. Spinner, and B. J. Joiner. 1990. Protein disulfide isomerase is a component of the microsomal triglyceride transfer protein complex. *J. Biol. Chem.* **265:** 9800–9807.
28. Wetterau, J. R., L. P. Aggerbeck, P. M. Laplaud, and L. R. McLean. 1991. Structural properties of the microsomal triglyceride-transfer protein complex. *Biochemistry.* **30:** 4406–4412.
29. Wetterau, J. R., K. A. Combs, L. R. McLean, S. N. Spinner, and L. P. Aggerbeck. 1991. Protein disulfide isomerase appears necessary to maintain the catalytically active structure of the microsomal triglyceride transfer protein. *Biochemistry.* **30:** 9728–9735.
30. Lamberg, A., M. Jauhiainen, J. Metso, C. Ehnholm, C. Shoulders, J. Scott, T. Pihlajaniemi, and K. I. Kivirikko. 1996. The role of protein disulphide isomerase in the microsomal triacylglycerol transfer protein does not reside in its isomerase activity. *Biochem. J.* **315:** 533–536.
31. Gregg, R. E., and J. R. Wetterau. 1994. The molecular basis of abetalipoproteinemia. *Curr. Opin. Lipidol.* **5:** 81–86.
32. Wetterau, J. R., M. C. M. Lin, and H. Jamil. 1997. Microsomal triglyceride transfer protein. *Biochim. Biophys. Acta.* **1345:** 136–150.

33. Atzel, A., and J. R. Wetterau. 1993. Mechanism of microsomal triglyceride transfer protein catalyzed lipid transport. *Biochemistry.* **32:** 10444–10450.
34. Atzel, A., and J. R. Wetterau. 1994. Identification of two classes of lipid molecule binding sites on the microsomal triglyceride transfer protein. *Biochemistry.* **33:** 15382–15388.
35. Jamil, H., J. K. Dickson, Jr., C-H. Chu, M. W. Lago, J. K. Rinehart, S. A. Biller, R. E. Gregg, and J. R. Wetterau. 1995. Microsomal triglyceride transfer protein. Specificity of lipid binding and transport. *J. Biol. Chem.* **270:** 6549–6554.
36. Sharp, D., L. Blinderman, K. A. Combs, B. Kienzle, B. Ricci, K. Wager-Smith, C. M. Gil, C. W. Turck, M-E. Bouma, D. J. Rader, L. P. Aggerbeck, R. E. Gregg, D. A. Gordon, and J. R. Wetterau. 1993. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinemia. *Nature.* **365:** 65–69.
37. Bradbury, P., C. J. Mann, S. Köchl, T. A. Anderson, S. A. Chester, J. M. Hancock, P. J. Ritchie, J. Amey, G. B. Harrison, D. G. Levitt, L. J. Banaszak, J. Scott, and C. C. Shoulders. 1999. A common binding site on the microsomal triglyceride transfer protein for apolipoprotein B and protein disulfide isomerase. *J. Biol. Chem.* **274:** 3159–3164.
38. Read, J., T. A. Anderson, P. J. Ritchie, B. Vanloo, J. Amey, D. Levitt, M. Rosseneu, J. Scott, and C. C. Shoulders. 2000. A mechanism of membrane neutral lipid acquisition by the microsomal triglyceride transfer protein. *J. Biol. Chem.* **275:** 30372–30377.
39. Anderson, T. A., D. G. Levitt, and L. J. Banaszak. 1998. The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein. *Structure.* **6:** 895–909.
40. Ohashi, K., S. Ishibashi, J. Osuga, R. Tozawa, K. Harada, N. Yahagi, F. Shionoiri, Y. Iizuka, Y. Tamura, R. Nagai, D. R. Illingworth, T. Gotoda, and N. Yamada. 2000. Novel mutations in the microsomal triglyceride transfer protein gene causing abetalipoproteinemia. *J. Lipid Res.* **41:** 1199–1204.
41. Bakillah, A., and M. M. Hussain. 2001. Binding of microsomal triglyceride transfer proten to lipids results in increased affinity for apolipoprotein B: Evidence for stable microsomal MTP-lipid complexes. *J. Biol. Chem.* **276:** 31466–31473.
42. Bakillah, A., N. Nayak, U. Saxena, R. M. Medford, and M. M. Hussain. 2000. Decreased secretion of apoB follows inhibition of apoB-MTP binding by a novel antagonist. *Biochemistry.* **39:** 4892–4899.
43. Hussain, M. M., A. Bakillah, and H. Jamil. 1997. Apolipoprotein B binding to microsomal triglyceride transfer protein decreases with increases in length and lipidation: implications in lipoprotein biosynthesis. *Biochemistry.* **36:** 13060–13067.
44. Hussain, M. M., A. Bakillah, N. Nayak, and G. S. Shelness. 1998. Amino acids 430-570 in apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *J. Biol. Chem.* **273:** 25612–25615.
45. Ricci, B., D. Sharp, E. Orourke, B. Kienzle, L. Blinderman, D. Gordon, C. Smithmonroy, G. Robinson, R. E. Gregg, D. J. Rader, and J. R. Wetterau. 1995. A 30-amino acid truncation of the microsomal triglyceride transfer protein large subunit disrupts its interaction with protein disulfide-isomerase and causes abetalipoproteinemia. *J. Biol. Chem.* **270:** 14281–14285.
46. Wu, X. J., M. Y. Zhou, L. S. Huang, J. Wetterau, and H. N. Ginsberg. 1996. Demonstration of a physical interaction between microsomal triglyceride transfer protein and apolipoprotein B during the assembly of ApoB-containing lipoproteins. *J. Biol. Chem.* **271:** 10277–10281.
47. Patel, S. B., and S. M. Grundy. 1996. Interactions between microsomal triglyceride transfer protein and apolipoprotein B within the endoplasmic reticulum in a heterologous system. *J. Biol. Chem.* **271:** 18686–18694.
48. Bakillah, A., H. Jamil, and M. M. Hussain. 1998. Lysine and arginine residues in the N-terminal 18% of apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *Biochemistry.* **37:** 3727–3734.
49. Mahley, R. W., T. L. Innerarity, R. E. Pitas, K. H. Weisgraber, J. H. Brown, and E. Gross. 1977. Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B apoproteins. *J. Biol. Chem.* **252:** 7279–7287.
50. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053–9062.
51. Liang, J., and H. N. Ginsberg. 2001. Microsomal triglyceride transfer protein binding and lipid transfer activities are independent of each other, but both are required for secretion of apolipoprotein B lipoproteins from liver cells. *J. Biol. Chem.* **276:** 28606–28612.
52. Gordon, D. A., H. Jamil, D. Sharp, D. Mullaney, Z. Yao, R. E. Gregg, and J. Wetterau. 1994. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. *Proc. Natl. Acad. Sci. USA.* **91:** 7628–7632.
53. Leiper, J. M., J. D. Bayliss, R. J. Pease, D. J. Brett, J. Scott, and C. C. Shoulders. 1994. Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. *J. Biol. Chem.* **269:** 21951–21954.
54. Fleming, J. F., G. M. Spitsen, T. Y. Hui, L. Olivier, E. Z. Du, M. Raabe, and R. A. Davis. 1999. Chinese hamster ovary cells require the coexpression of microsomal triglyceride transfer protein and cholesterol 7 α-hydroxylase for the assembly and secretion of apolipoprotein B containing lipoproteins. *J. Biol. Chem.* **274:** 9509–9514.
55. Haghpassand, M., D. Wilder, and J. B. Moberly. 1996. Inhibition of apolipoprotein B and triglyceride secretion in human hepatoma cells (HepG2). *J. Lipid Res.* **37:** 1468–1480.
56. Jamil,H., D. A. Gordon, D. C. Eustice, C. M. Brooks, J. K. Dickson, Jr., Y. Chen, B. Ricci, C-H. Chu, T. W. Harrity, C. P. Ciosek, Jr., S. A. Biller, R. E. Gregg, and J. R. Wetterau. 1996. An inhibitor of the microsomal triglyceride transfer protein inhibits apoB secretion from HepG2 cells. *Proc. Natl. Acad. Sci. USA.* **93:** 11991–11995.
57. Gordon, D. A., H. Jamil, R. E. Gregg, S. O. Olofsson, and J. Borén. 1996. Inhibition of the microsomal triglyceride transfer protein blocks the first step of apolipoprotein B lipoprotein assembly but not the addition of bulk core lipids in the second step. *J. Biol. Chem.* **271:** 33047–33053.
58. Benoist, F., E. Nicodeme, and T. Grand-Perret. 1996. Microsomal triacylglycerol transfer protein prevents presecretory degradation of apolipoprotein B-100 - A dithiothreitol-sensitive protease is involved. *Eur. J. Biochem.* **240:** 713–720.
59. Benoist, F., and T. Grand-Perret. 1997. Co-translational degradation of apolipoprotein B100 by the proteasome is prevented by microsomal triglyceride transfer protein - Synchronized translation studies on HepG2 cells treated with an inhibitor of microsomal triglyceride transfer protein. *J. Biol. Chem.* **272:** 20435–20442.
60. Wang, Y., R. S. McLeod, and Z. Yao. 1997. Normal activity of microsomal triglyceride transfer protein is required for the oleate-induced secretion of very low density lipoproteins containing apolipoprotein B from McA- RH7777 cells. *J. Biol. Chem.* **272:** 12272–12278.
61. Rusiñol, A. E., H. Jamil, and J. E. Vance. 1997. *In vitro* reconstitution of assembly of apolipoprotein B48-containing lipoproteins. *J. Biol. Chem.* **272:** 8019–8025.
62. Nicodeme, E., F. Benoist, R. McLeod, Z. M. Yao, J. Scott, C. C. Shoulders, and T. Grand-Perret. 1999. Identification of domains in apolipoprotein B100 that confer a high requirement for the microsomal triglyceride transfer protein. *J. Biol. Chem.* **274:** 1986–1993.
63. Van Greevenbroek, M. M. J., M. G. Robertus-Teunissen, D. W. Erkelens, and T. W. A. De Bruin. 1998. Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with saturated and unsaturated dietary fatty acids. *J. Lipid Res.* **39:** 173–185.
64. Raabe, M., M. M. Véniant, M. A. Sullivan, C. H. Zlot, J. Björkegren, L. B. Nielsen, J. S. Wong, R. L. Hamilton, and S. G. Young. 1999. Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. *J. Clin. Invest.* **103:** 1287–1298.
65. Wang, Y., K. Tran, and Z. Yao. 1999. The activity of microsomal triglyceride transfer protein is essential for accumulation of triglyceride within microsomes in McA-RH7777 cells. A unified model for the assembly of very low density lipoproteins. *J. Biol. Chem.* **274:** 27793–27800.
66. Linnik, K. M., and H. Herscovitz. 1998. Multiple molecular chaperones interact with apolipoprotein B during its maturation - The network of endoplasmic reticulum-resident chaperones (ERp72, GRP94, calreticulin, and BiP) interacts with apolipoprotein B regardless of its lipidation state. *J. Biol. Chem.* **273:** 21368–21373.
67. Liang, J., X. Wu, E. A. Fisher, and H. N. Ginsberg. 2000. The

amino-terminal domain of apolipoprotein B does not undergo retrograde translocation from the endoplasmic reticulum to the cytosol. Proteosomal degradation of nascent apolipoprotein B begins at the carboxyl terminus of the protein, while apolipoprotein B is still in its original translocon. *J. Biol. Chem.* **275:** 32003–32010.

68. Pariyarath, R., H. Wang, J. D. Aitchison, H. N. Ginsberg, W. J. Welch, A. E. Johnson, and E. A. Fisher. 2001. Co-translational interactions of apoprotein B with the ribosome and translocon during lipoprotein assembly or targeting to the proteasome. *J. Biol. Chem.* **276:** 541–550.
69. Fisher, E. A., M. Y. Zhou, D. M. Mitchell, X. J. Wu, S. Omura, H. Wang, A. L. Goldberg, and H. N. Ginsberg. 1997. The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. *J. Biol. Chem.* **272:** 20427–20434.
70. Gusarova, V., A. J. Caplan, J. L. Brodsky, and E. A. Fisher. 2001. Apoprotein B degradation is promoted by the molecular chaperones hsp90 and hsp70. *J. Biol. Chem.* **276:** 24891–24900.
71. Rashid, K. A., S. Hevi, Y. Chen, F. Le Caherec, and S. L. Chuck. 2002. A proteomic approach identifies proteins in hepatocytes that bind nascent apolipoprotein B. *J. Biol. Chem.* **277:** 22010–22017.
72. Cornel, W. D., P. Cieplak, C. I. Bayley, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, and P. A. Kollman. 1995. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117:** 5179–5197.