Recent studies indicate that microsomal triglyceride transfer protein (MTP) and apolipoprotein B (apoB) interact physically via two specific binding sites located within the amino-terminal globular region of apoB100. The first site is thought to be within the first 5.8% of the amino-terminal sequence, and the second site is between 9 and 16% of the amino-terminal sequence. It is not clear from prior studies whether these sites have unique or overlapping functions. Furthermore, there are no data differentiating between lipid transfer and potential chaperone functions of MTP. In the present study we have attempted to further characterize the physiologic interaction between apoB and MTP and to determine the relationship between the binding and lipid transfer aspects of the interaction. HepG2 cells were transiently transfected with apoB cDNAs, and MTP binding to apoB polypeptides was determined by two-step immunoprecipitation. MTP bound equally well to apoB polypeptides with (apoB13, 16, 16p, apoB34, and apoB42) or without (apoB16, apoB13, and 16 or apoB13, 13, and 16) \( \beta \) sheet domains. When proteasomal degradation of newly synthesized apoB polypeptides was blocked, MTP binding to all of the apoB polypeptides was only modestly affected by lipid availability and was independent of MTP-associated lipid transfer. Furthermore, MTP did not bind directly to a portion of the first \( \beta \) sheet domain. We created two apoB constructs (apoB16\text{del} and apoB34\text{del}) by deleting the first 210 amino acids of apoB16 and apoB34. These apoB polypeptides, therefore, lacked the putative first MTP binding site. MTP binding to apoB16\text{del} and apoB34\text{del} was decreased significantly. However, the secretion of apoB16\text{del} was not different from apoB16, whereas the secretion of apoB34\text{del} was impaired significantly. Our results indicate that the interaction between MTP and apoB involves independent binding and lipid transfer activities but that both activities are required for the secretion of apolipoprotein B from liver cells.

Microsomal triglyceride transfer protein (MTP)\(^1\) is a heterodimer consisting of protein disulfide isomerase and the 97-kDa-large subunit and is found mainly in the endoplasmic reticulum (ER) lumen of liver and intestinal cells (1–3). The essential role of MTP in the translocation of apolipoprotein B (apoB) across the ER membrane and the assembly of apoB with lipids has been demonstrated clearly by co-expressing apoB and MTP in cells that do not normally express either protein and by the characterization of mice lacking MTP. The expression of apoB alone in cells without MTP resulted in the intracellular degradation of nascent apoB and no secretion; co-expression of apoB with MTP resulted in the secretion of apoB on lipoprotein particles (4–6). Furthermore, the inhibition of MTP activity in cultured liver cells blocked the assembly and secretion of apoB-containing lipoproteins (7–11). We demonstrated that inhibition of MTP activity was associated with increased ubiquitination of apoB, particularly when proteasomal degradation was inhibited (12). Those results suggested that, at least in HepG2 cells, MTP facilitated the translocation of apoB across the ER membrane. Lack of expression of the \( Mtp \) gene in mice is associated with an absence of lipid droplets in the ER lumen (13) and defective secretion of apoB (13–14). On the other hand, increases in MTP protein and activity are associated with increased assembly and secretion of apoB lipoproteins in mice (15) and hamsters (16).

apoB100 (4536 amino acids) is predicted to be comprised of an amino-terminal globular domain followed by three amphipathic \( \alpha \)-helical domains alternating with two hydrophobic \( \beta \) sheet domains (17). Triglyceride-rich lipoprotein assembly is not a simple size-related lipidation of apoB but seems to be determined by the presence of hydrophobic sequences present within apoB (18–20). In most cell lines, carboxyl-terminally truncated forms of apoB smaller than apoB23, which lack significant \( \beta \) sheet domains, are secreted with little or no associated lipid and do not require MTP to attain a secretion-competent status, whereas truncations longer than apoB23 require active MTP and are secreted as lipoproteins (4, 5, 11). We recently demonstrated that the presence of a \( \beta \) sheet domain seems sufficient to make MTP function essential for secretion (21).

We and others (22–25) have demonstrated that MTP and apoB physically interact during the assembly of triglyceride-rich lipoproteins. Indeed, recent studies with both in vitro and yeast two-hybrid systems have identified two probable binding sites for MTP within the amino-terminal globular region of apoB (25–27). The first site is located within the initial 5.8% of apoB, and the second site is between 9–16% of the apoB polypeptide. The second site is close to the beginning of the first lipid-binding domain of apoB (17) and could be the site of initial lipid transfer via MTP. However, several questions related to the interaction of apoB and MTP in hepatocytes remain unanswered. For example, does MTP binding to apoB depend on the...
presence of an adjacent \( \beta \) sheet domain? Does MTP binding to apoB depend on active lipid synthesis and/or transfer? Does MTP bind directly to \( \beta \) sheet domain sequences? Are the two MTP binding sites on apoB functionally independent of each other?

We sought to answer these questions by using cultured liver cells expressing the following human apoB constructs (Fig. 1): carboxy-terminal truncations of full-length apoB including apoB16, apoB34, and apoB42; apoB13,16 and apoB13,13,16, which contain repeats of the amino-terminal globular domain but no \( \beta \) sheet domains; apoB13,16,\( \beta \), in which the \( \beta \) sheet domain between apoB28 and B34 in full-length apoB100 has been inserted into apoB13,16 at either 9.5 or 22.5% of the construct; insertion of the \( \beta \) sheet domain at either site gave the same results in preliminary cell experiments, and only the construct with the \( \beta \) sheet domain at 9.5% of apoB13,16 was used in the studies presented in this report. The length of the apoB13,16,\( \beta \) construct was equal to 35% of full-length apoB100. ApoB16\( ^{del} \) and apoB34\( ^{del} \) were prepared by digestion of the apoB16 and apoB34 expression vectors with \( Ehe \)I and \( BcI \)I to liberate the first 210 residues of apoB followed by subsequent ligation. ApoB \( \beta \) was created by directly linking the amphipathic \( \beta \) strand between apoB28 and B34 to a signal peptide of apoB.

**EXPERIMENTAL PROCEDURES**

**Reagents**—N-Acetyl-leucinyl-leucinyl-norleucinal (ALLN), oleic acid (OA), Triton X-100 and protein A-Sepharose CL 4B were purchased from Sigma. ApoB34 and apoB42; apoB13,16 and apoB13,13,16, which contain repeats of the amino-terminal globular domain but no \( \beta \) sheet domains; apoB13,16,\( \beta \), in which the \( \beta \) sheet domain between apoB28 and B34 in full-length apoB100 has been inserted into apoB13,16; apoB\( \beta \), in which the \( \beta \) sheet domain between apoB28 and B34 has been linked directly to a signal peptide of apoB; and apoB16\( ^{del} \) and apoB34\( ^{del} \), in which the putative first MTP binding site (the first 210 residues of apoB) (25–27) was deleted from apoB16 and apoB34. The latter two constructs lacked the first 5.5% of the apoB sequence.
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RESULTS AND DISCUSSION

Does the Binding of MTP to the Amino Terminus of apoB Depend on the Presence of β Sheet Domains and the Availability of Core Lipids?—Our previous studies demonstrated that the intracellular levels of newly synthesized apoB peptides with anti-apoB antibody after adjusting the SDS concentration to 0.1%. The samples were finally analyzed on either 3–15% or 7% SDS-polyacrylamide gels. Samples were loaded onto the SDS-polyacrylamide gels after correcting for total trichloroacetic acid precipitable radioactivity from each cell lysate.

ing for three times, the precipitated immune complex was denatured by boiling 4 min in 2% SDS and subjected to second immunoprecipitation with anti-apoB antibody after adjusting the SDS concentration to 0.1%. The samples were finally analyzed on either 3–15% or 7% SDS-polyacrylamide gels. Samples were loaded onto the SDS-polyacrylamide gels after correcting for total trichloroacetic acid precipitable radioactivity from each cell lysate.

FIG. 2. The interaction of apoB and apoB polypeptides with MTP was independent of the presence of β sheet domains. The OA-induced increase in MTP binding to β sheet domain-containing apoB polypeptides was minimal when proteasomal degradation was inhibited. A, HepG2 cells were transiently transfected with apoB cDNAs (lanes 1 and 2, apoB34; lanes 3 and 4, apoB42; lanes 5 and 6, apoB13,13,16; lanes 7 and 8, apoB13,16; lanes 9 and 10, apoB16). Thirty-six h after transfection, the cells were preincubated with or without OA (0.4 mM) for 2 h and labeled with [3H]leucine for 0.5 h in the absence or presence of OA. After labeling, the association of apoB polypeptides with MTP was determined by two-step immunoprecipitation as described under “Experimental Procedures.” B, HepG2 cells were pretreated with ALLN (40 μg/ml) for 2 h in the presence or absence of OA (0.4 mM). Other experimental conditions were the same as described above. Lanes 1 and 2, ApoB34; lanes 3 and 4, apoB13,16; lanes 5 and 6, apoB16. The data shown are from one of three separate experiments.

3, 5, 7, and 9, relative densitometric units (RDU) 4.21 ± 0.89; n = 3 separate experiments, p < 0.05). Additionally, all of the apoB peptides with (apoB34, lanes 1 and 2; apoB42, lanes 3 and 4) or without (apoB16, lanes 9 and 10; apoB13,16, lanes 7 and 8; apoB13,13,16, lanes 5 and 6) β sheet domains bound to MTP. Of interest, MTP binding to apoB peptides containing a β sheet domain (apoB34, apoB42) was increased markedly by OA treatment (lane 2 versus 1, RDU 3.35 ± 0.65, p < 0.05; lane 4 versus 3, RDU 3.64 ± 0.11, p < 0.05), whereas MTP binding to apoB peptides without any β sheet domains (apoB13,13,16, apoB13,16, or apoB16) was not affected by OA (lane 6 versus 5, RDU 1.10 ± 0.25; lane 8 versus 7, RDU 0.97 ± 0.21; lane 10 versus 9, RDU 0.95 ± 0.19). These results suggested that the binding of MTP to apoB was independent of the presence of lipid-binding β sheet domains. However, the effect of OA suggested that the association of MTP with the amino terminus of apoB constructs that did contain β sheet domains might be regulated by the availability of newly synthesized core lipids. To understand how OA treatment could increase the association of MTP with apoB, we carried out an additional experiment.

Cells were pretreated for 2 h with the proteasome inhibitors ALLN (40 μg/ml) or lactacystin (10 μM) to prevent degradation of inefficiently translocated apoB (33, 34) in the presence or absence of OA and labeled for 30 min with [3H]leucine. As indicated in Fig. 2B, ALLN treatment minimized the OA-induced increase in the association of MTP with apoB100 and apoB34. As noted above, in the absence of ALLN, OA increased the association of apoB100 and MTP by 320% (Fig. 2A). When...
ALLN was present (Fig. 2B), the association of apoB100 with MTP was only increased by 35% (lanes 2, 4, and 6 versus lanes 1, 3, and 5, RDU 1.35 ± 0.29; n = 3). Similarly, whereas OA increased the association of MTP with apoB34 by 235% without ALLN (Fig. 2A), the increase was only about 74% (lane 2 versus lane 1, RDU 1.74 ± 0.78; n = 3) when ALLN was present (Fig. 2B). The association of either apoB13,16 or apoB16 with MTP, which was not affected by OA alone (Fig. 2A), was also not affected by OA treatment in ALLN-pretreated HepG2 cells (lane 4 versus lane 3, RDU 1.23 ± 0.42; lane 6 versus lane 5, RDU 1.12 ± 0.35; both not significant, n = 3). These data indicate that the increased association of MTP with apoB100 and the apoB constructs containing β sheet domains in the presence of OA (demonstrated in Fig. 2A) was mediated mainly by the ability of OA to protect apoB from proteasomal degradation. Thus, the majority of the effect of OA to increase binding of MTP to apoB seemed to result from decreased proteasomal degradation of nascent apoB rather than by any direct effect of OA or lipid availability on the binding process. Overall, therefore, the studies shown in Fig. 2 indicated that the interaction between MTP and apoB was mainly independent of both the presence of lipid-binding β sheet domains and the availability of core lipids.

**Does the Binding of MTP to the Amino Terminus of apoB Depend on Lipid Transfer Activity?—**Because MTP activity seems to be required for efficient apoB translocation when a β sheet domain is present (21), our finding that increased lipid availability did not directly affect the interaction between MTP and apoB was surprising. Therefore, our next experiment examined the effect of inhibiting MTP lipid transfer activity on the association of MTP with apoB peptides. Thirty-six h after transfection with apoB cDNAs, the cells were preincubated for 1 h with and without an MTP inhibitor (CP-10447, 50 μM) (28) and labeled for 30 min with [3H]leucine. Cell lysates were analyzed by two-step immunoprecipitation. Consistent with our first experiment, apoB peptides with (apoB13,16,β, lanes 3 and 4; apoB42, lanes 5 and 6; apoB100, lanes 1–10) or without (apoB16, lanes 1 and 2; apoB13,16, lanes 9 and 10; apoB13,16, lanes 7 and 8) β sheet domains all bound to MTP (Fig. 3A). When the MTP activity was inhibited by CP-10447, MTP binding to apoB peptides with β sheet domains decreased dramatically. Specifically, apoB100 binding to MTP was reduced by 72% (lanes 2, 4, 6, 8, and 10 versus lanes 1, 3, 5, 7, and 9, RDU 0.28 ± 0.08; p < 0.05, n = 3), apoB13,16,β binding to MTP was reduced by 71% (lane 4 versus 3, RDU 0.29 ± 0.12, p < 0.05, n = 3), and apoB42 binding to MTP was reduced by 65% (lane 6 versus 5, RDU 0.35 ± 0.11; p < 0.05, n = 3). In contrast, MTP binding to apoB peptides without β sheet domains was not affected by the inhibition of MTP (apoB16, lane 2 versus lane 1, RDU 0.88 ± 0.24; apoB13,16, lanes 8 versus 7, RDU 0.94 ± 0.29; apoB13,16, lane 10 versus 9, RDU 0.97 ± 0.33; all not significant, n = 3) (Fig. 3A). Thus, although simple insertion of a small portion of the first β sheet domain of apoB100 into apoB13,16 did not affect MTP binding to apoB in control medium, it appeared to make the interaction between MTP and apoB13,16,β responsive to the inhibition of MTP activity.

The results presented in Fig. 2B indicated, however, that the reduced interaction between MTP and apoB constructs containing β sheet domains observed during inhibition of MTP activity (in Fig. 3A) could have resulted from either 1) increased apoB degradation of nascent apoB by the proteasome and fewer apoB molecules available for binding to MTP or 2) decreased binding affinity of MTP for apoB. We therefore de-
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FIG. 4. MTP does not bind directly to the β sheet domain. HepG2 cells were transiently transfectected with apoB β (lanes 1 and 2), apoB16 (lanes 3 and 4), and apoB34 (lanes 5 and 6) cDNAs. Thirty-six h after transfection, the cells were pretreated for 1 h and labeled for 0.5 h with [3H]leucine in the presence or absence of MTP inhibitor. The cells were lysed in non-denaturing buffer, and two-step immunoprecipitation was carried out as described under “Experimental Procedures.” A, anti-apoB antiseraum was used for immunoprecipitation in both steps 1 and 2 to demonstrate adequate expression of apoB β. B, anti-MTP large subunit antiseraum was used for immunoprecipitation in step 1, and anti-apoB antiseraum was used in step 2. This two-step immunoprecipitation demonstrated that MTP did not bind to apoB β.

determined the effects of inhibiting MTP lipid transfer activity on the binding of MTP to apoB in the absence or presence of lactacystin (Fig. 3B). Thirty-six h after HepG2 cells were transiently transfectected with apoB cDNAs, the cells were preincubated for 2 h with or without MTP inhibitor (10 μM) in the presence or absence of lactacystin and labeled for 30 min with [3H]leucine. In the presence of lactacystin, MTP binding to the apoB species containing β sheet domains, apoB13,16,β and apoB100, was unaffected by the MTP inhibitor. Thus Fig. 3B, lane 2 versus 1 and lane 5 versus 4, confirms the effects of the MTP inhibitor on the apoB100 binding to MTP shown in Fig. 3A, but this effect is reversed by lactacystin (lane 3 versus 1 and lane 6 versus 4, RDU 0.91 ± 0.33; not significant, n = 3). Similarly, Fig. 3B, lane 2 versus lane 1, confirms the effect of the MTP inhibitor on binding of apoB13,16,β to MTP, but this effect is reversed by lactacystin (lane 3 versus lane 1, RDU 0.89 ± 0.29; not significant, n = 3). On the other hand, the binding of apoB13,16 to MTP, which was not affected by the MTP inhibitor (Fig. 3A), was also not altered by the MTP inhibitor plus lactacystin (Fig. 3B, lane 6 versus lane 4, RDU 0.92 ± 0.28). These results indicate clearly that if proteasomal degradation is inhibited, the ability of MTP to interact physically with apoB is independent of MTP lipid-transfer activity.

Does MTP Bind Directly to the β Sheet Domain of apoB?—The results described above indicated that the binding of MTP to the amino-terminal globular domain of apoB is independent of the presence of β sheet domains. On the other hand, β sheet domains play a critical role in determining the response of apoB to OA-treatment and MTP-inhibition. Furthermore, it appears that although binding of MTP to apoB is mainly independent of both lipid availability and lipid transfer activity, the latter are both required for the translocation of apoB peptides containing β sheet domains into the ER lumen where they are assembled into lipoproteins. These findings raised the question whether MTP, in addition to binding to the amino-terminal globular domain of apoB, binds directly to the β sheet domains, where lipid transfer may occur. In an attempt to address this question, we created apoBβ in which the β sheet domain between apoB28 and apoB34 of full-length apoB is attached directly to the apoB signal peptide without any intervening amino-terminal globular region. ApoBβ was expressed in HepG2 cells (Fig. 4A, lanes 1 and 2). Consistent with our previous data, binding of MTP to apoB34 and apoB100 (in the absence of lactacystin) was decreased markedly by MTP inhibition (Fig. 4B, lanes 2, 4, and 6 versus lanes 1, 3, and 5; for apoB34: Fig. 4B, lane 6 versus lane 5), whereas MTP binding to apoB16 was not affected (Fig. 4B, lane 4 versus lane 3). We could not, however, detect any binding of MTP to apoBβ either in the presence or absence of the MTP inhibitor (Fig. 4B, lanes 1 and 2). This was not caused by a lack of translocation of apoBβ, because it was associated only with microsomes (data not shown). These data suggest that MTP does not bind directly to the first β sheet domain in apoB100. Although apoBβ may not be fully representative of the β sheet domains that are present within native apoB, we have shown previously that insertion of this portion of the first β sheet domain into the globular amino-terminal domain of apoB100 is sufficient to make the amino-terminal polypeptide sensitive to lipid availability and MTP activity. Additionally, the observation that MTP does not bind to apoBβ is consistent with the recent reports using in vitro (24, 25) and yeast two-hybrid systems (26, 27) that identified two putative MTP binding sites within the first 16% of apoB before the start of the first β sheet domain.

Are the Two MTP Binding Sites on apoB Functionally Independent of Each Other?—The preceding experiments suggested that the physical interaction between MTP and the amino-terminal domain of apoB was independent of the presence of β sheet domains, lipid availability, and lipid transfer activity. However, it is clear from numerous studies that all three are critical in determining the need for MTP in the assembly and secretion of apoB from liver cells. One explanation for this seeming paradox would be that MTP and apoB interacted in a sequential manner, with binding preceding lipid transfer. More importantly, our results indicated that apoB might contain at least two independent functional domains involved in MTP binding and MTP lipid transfer, respectively. Because the putative second MTP binding site on apoB (between 9 and 16% of apoB) (24, 27) is close to the beginning of the lipid-binding domain of apoB, that site could be the site of initial lipid transfer via MTP. Indeed, when a salt bridge within the putative second MTP binding site in apoB was disrupted by mutation of the arginine 531 residue, the MTP-apoB interaction in the yeast two-hybrid system and the secretion of apoB from COS-1 cells were both reduced markedly (27). The other putative MTP binding site is located within the first 5.8% of apoB, further away from the start of the first β sheet domain (26). This site might play an important role in facilitating the initial translocation of apoB across the ER membrane, an event that could be the necessary prerequisite for lipid transfer to occur at
apoB16del and apoB34del from HepG2 cells was also studied. The deletion of the first 5.8% of apoB markedly inhibited MTP binding. HepG2 cells were transiently transfected with apoB34 (lane 1), apoB34del (lane 2), apoB16 (lane 3), and apoB16del (lane 4) cDNAs. Thirty-six h after transfection, the cells were labeled with [3H]leucine for 0.5 h. After labeling, the association of apoB peptides with MTP was determined by two-step immunoprecipitation as described under “Experimental Procedures.” A, anti-MTP large subunit antiserum was used for immunoprecipitation in step 1, and anti-apoB antiserum was used in step 2. B, anti-apoB antiserum was used for immunoprecipitation in both steps 1 and 2 to demonstrate that apoB16del and apoB34del were expressed adequately. Lane 5 was an untransfected control.

The intracellular assembly of endogenous apoB100 and apoB48 with lipids in hepatocytes and enterocytes, respectively, absolutely requires MTP activity (22, 28, 35, 36). The role of the amino terminus of apoB in the interaction with MTP has been demonstrated in various experimental systems (6, 22–27). In Sf-21 insect cells, Gretch et al. (6) demonstrated that the secretion of apoB48 was increased significantly by the co-expression of MTP, whereas secretion of apoB17 was minimally affected. When the amino-terminal 17% of longer apoB polypeptides was deleted, however, the secretion of these apoB polypeptides was reduced markedly, and the response to MTP stimulation was lost. In a heterologous expression system, Patel and Grundy (23) found that when MTP was expressed at very high levels, it was physically associated with all forms of apoB intracellularly, but only the secretion of apoB larger than B29 was affected by MTP and OA treatment.

Several recent reports (24–27) have focused on the identification of MTP binding sites within the amino-terminal globular domain of apoB. The first binding site was localized by Mann et al. (26), using a two-hybrid system, to residues 1–264 of apoB (apoB5.8), which interacts with the predicted β barrel of MTP. Bradbury et al. (27) used the same method to localize a second binding site in the region of residues 512–721 of apoB, which interacts with helices 13–17 (residues 517–603) of MTP. This second site is in the same region as the site of MTP binding to apoB16del and apoB34del, in which the first MTP binding site (the first 210 residues of apoB) was deleted. Thirty-six h after transfection of HepG2 cells with apoB16del and apoB34del cDNAs, the cells were labeled for 30 min with [3H]leucine. The cell lysates were first immunoprecipitated with the anti-MTP large subunit antibody followed by secondary immunoprecipitation with a polyclonal anti-apoB antibody. In HepG2 cells, MTP binding to either apoB34del or apoB16del was decreased significantly compared with its binding to apoB34 and apoB16 (Fig. 5A, lane 2 versus 1 and lane 4 versus 3). The marked decrease in ability to immunoprecipitate apoB that had interacted with MTP was not caused by decreased immunoreactivity of either apoB16del or apoB34del, thus, when the anti-apoB antibody was used in both steps of the two-step immunoprecipitation, apoB34 and apoB16 with deletions were precipitated as well as their counterparts without the deletions (Fig. 5B, lane 2 versus 1 and lane 4 versus 3. Lane 5 was an untransfected control). Secretion of apoB16del and apoB34del from HepG2 cells was also studied. Thirty-six h after transfection, HepG2 cells were labeled with [3H]leucine for 2 h. After labeling, the cell medium and lysates were collected and analyzed by a single-step immunoprecipitation with anti-apoB antibodies (Fig. 6). Secretion of apoB34 was significantly inhibited when the first 5.5% of the amino terminus of that polypeptide was deleted (Fig. 6B, lane 4). The data in Fig. 5A indicate that the first MTP binding site is required for the interaction between apoB and MTP. Furthermore, the results presented in Fig. 6 (medium) demonstrate that this first binding site plays a critical role in apoB translocation and secretion only when a β sheet domain is present.

**SUMMARY**

The secretion of apoB significantly inhibited secretion of apoB34del but not apoB16del. HepG2 cells were transiently transfected with apoB34 (lanes 1 and 5), apoB34del (lanes 2 and 6), apoB16 (lanes 3 and 7), and apoB16del (lanes 4 and 8) cDNAs. Thirty-six h after transfection, HepG2 cells were labeled with [3H]leucine for 2 h. After labeling, the cell medium and lysates were collected and analyzed by immunoprecipitation.
apoB reported by Hussain et al. (24), who demonstrated an in vitro interaction of amino acids 430–570 in apoB with MTP.

Our present experiments extend these previous results to a human liver cell line and provide the basis for a physiologic model of the interaction between apoB and MTP. Thus, we have been able to demonstrate that although MTP binding and the concomitant transfer of newly synthesized lipids are required for efficient translocation across the ER membrane of apoB100 and apoB constructs that contain β sheet domains, the actual binding of MTP to the amino-terminal globular domain of apoB is independent of both the presence of β sheet domains and lipid transfer activity. Our results also indicate that MTP binding is mainly independent of lipid availability. Of particular note, our results demonstrate that the first MTP domain and lipid transfer activity. Bakillah et al. (37) concluded that MTP both chaperone and lipid transfer activities. In summary, our present studies indicate that the interaction between MTP and apoB involves independent binding and lipid transfer activities but that both activities are required for the secretion of apoB lipoproteins from liver cells.

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