The microsomal triglyceride transfer protein (MTP) is a heterodimeric protein consisting of a 97-kDa catalytic subunit noncovalently linked to protein-disulfide isomerase (10). The ability of MTP to transfer TG between lipid membranes has been demonstrated in vitro (11), and deficiency in the MTP activity is associated with human abetalipoproteinemia (12, 13). In HepG2 cells, a physical association between the hydrophobic sequences of apoB and MTP has been detected (14). It is unclear, however, whether the direct interaction between MTP and apoB is essential for the recruitment of lipid. The functional role of MTP in the secretion of lipoproteins containing apoB has been demonstrated by co-expression of MTP and apoB in heterologous cells that normally produce neither pro-

Two forms of apolipoprotein B (apoB) are synthesized by the rat liver, the full-length apoB100 and apoB48, which represents the N-terminal 48% of apoB100 (1). Although the physiological significance of having two forms of apoB in rat liver is not clear (2), both forms of apoB have the ability to assemble very low density lipoproteins (VLDL) (3). The mechanism by which hepatic VLDL is synthesized has not been completely defined. However, significant progress has been made over the past several years concerning the formation and secretion of VLDL containing apoB48 (B48-VLDL) (4). Biochemical evidence has been obtained through studies with primary rat hepatocytes (5, 6) and the rat hepatoma cell line McA-RH7777 (7) that B48-VLDL is assembled via two discontinuous lipidation stages in the endoplasmic reticulum (ER). In the first stage, apoB48 is associated with a small amount of lipid to form a primordial particle with high buoyant density. These high density lipoprotein (HDL) particles (designated B48-HDL) may be secreted from the cells if further lipid recruitment does not occur. Alternatively, the B48-HDL particle can undergo a second lipidation stage, expanding its lipid content, primarily triacylglycerol (TG), to form VLDL. This “two-step” assembly model is consistent with the early immunohistochemical studies of hepatic VLDL assembly in rats (8). In rat hepatoma cells, the conversion of B48-HDL into B48-VLDL is associated with increased synthesis of cellular lipid, and the process can be inhibited by brefeldin A (9) or cycloheximide (7). These results suggest that in addition to active TG synthesis, other factors involved in vesicular trafficking or lipid mobilization may participate in the second stage of B48-VLDL formation.

The requirement of the activity of microsomal triglyceride transfer protein (MTP) for very low density lipoprotein (VLDL) secretion was determined using McA-RH7777 cells stably transfected with human apoB48 (hB48). Secretion of VLDL containing hB48 (hB48-VLDL) by the transfected cells was induced by exogenous oleate (0.4 mM), and oleate-dependent VLDL secretion was selectively inhibited by brefeldin A (0.2 μg/ml). Two protocols were used to determine the effect of MTP inhibition on VLDL secretion. In the first protocol, cell protein and lipid were labeled with radioactive amino acids and oleate prior to MTP inhibition (using 5 μM of the photoaffinity inhibitor BMS-192951 to reduce MTP activity by 65–70%), and secretion of prelabeled apoB and triacylglycerol (TG) associated with lipoproteins was monitored during oleate-supplemented chase. In control cells, a 6-fold increase in incorporation of prelabeled TG into hB48-VLDL was observed after oleate supplement, while incorporation of prelabeled TG into VLDL containing endogenous rat apoB100 (rB100-VLDL) was unaffected. Inhibition of MTP activity abolished the oleate-induced utilization of prelabeled TG (by 80%) and hB48 (by 70%) for hB48-VLDL secretion but decreased utilization of pre-existing TG (by <25%) and B100 (by 45%) for rB100-VLDL secretion to a lesser extent. Inhibition of MTP did not affect incorporation of prelabeled TG or hB48 into high density lipoproteins containing hB48 (hB48-HDL). In the second protocol, MTP was inactivated prior to metabolic labeling of protein and lipid, and secretion of newly labeled apoB and TG as lipoproteins was monitored after oleate supplement. Under this condition, MTP inhibition decreased incorporation of newly labeled TG (by 80%) and hB48 (80%) into hB48-VLDL but did not affect their incorporation into hB48-HDL. Additionally, MTP inhibition decreased incorporation of newly labeled TG (by 50%) and rB100 (by 90%) into rB100-VLDL. Thus, normal activity of MTP is required for the oleate-induced secretion of hB48-VLDL from McA-RH7777 cells.

The abbreviations used are: apo B, apolipoprotein B; VLDL, very low density lipoprotein(s); B48-VLDL, VLDL containing apoB48; ER, endoplasmic reticulum; HDL, high density lipoprotein(s); B48-HDL, HDL containing apoB48; DMEM, Dulbecco’s modified Eagle’s medium; rB100-VLDL, VLDL containing rB100; hB48-VLDL, VLDL containing hB48; hB48-HDL, HDL containing hB48; rB48, rat apoB48; PC, phosphatidylcholine.
tein (15–18). Data from these reconstitution experiments clearly indicate that MTP indeed plays an important role in the assembly and secretion of lipoproteins containing apoB. However, since cell lines used for the reconstitution experiments lacked the ability to synthesize and secrete VLDL, the requirement for MTP activity in VLDL assembly, particularly in the second step assembly, could not be determined. Thus, an alternative approach to assess the involvement of MTP in VLDL synthesis is to use inhibitors that can specifically inactivate MTP in situ.

Inactivation of MTP using specific MTP inhibitors has recently been reported by several laboratories to inhibit apoB secretion from cells of hepatic or intestinal origin (19–22). Invariably, the inhibition of MTP activity markedly decreased secretion of the full-length apoB100. However, the effect of MTP inhibition on apoB48 secretion was less consistent. In Caco-2 cells, while MTP inhibition resulted in significantly decreased secretion of apoB100, secretion of apoB48 was unaffected (22). In McA-RH7777 cells, inactivation of MTP seemed to only affect formation of B48-HDL (i.e. the product of the first step assembly) but had no effect on the conversion of B48-HDL into B48-VLDL (19). The differential effect of MTP inhibition on apoB48 secretion in different cells has not been explained.

Several laboratories including ours (23–26) have presented experimental evidence that the size of lipoproteins is positively correlated with the length of the associated apoB polypeptide during the first step assembly. However, the length of apoB does not seem to play a major role in the conversion of apoB-HDL into apoB-VLDL during the second step assembly. In McA-RH7777 cells stably expressing recombinant human apoB variants or apoAIB chimeric proteins, HDL that carried either truncated apoB variants (e.g. as short as apoB34) or apoAIB chimeras containing a segment of apoB (e.g. as short as ~5% of apoB100) were readily converted into VLDL in the presence of exogenous oleate (27). Thus, conversion of apoB-HDL into apoB-VLDL in the second step may be determined primarily by synthesis of lipid and by protein factors that mobilize the lipid during assembly rather than specific apoB sequences. Since MTP plays an important role in lipid transfer, we hypothesize that the MTP activity is required for the mobilization of lipid that is utilized for the oleate-induced B48-VLDL assembly. This hypothesis was tested in the current work.

EXPERIMENTAL PROCEDURES

Materials—Culture media and sera were obtained from Life Technologies, Inc. Reagents for polycarboxamidyl gel electrophoresis were obtained from Bio-Rad. Sheep anti-human apoB antisera was obtained from Boehringer Mannheim. CNBr-activated Sepharose 4B beads and protein A-Sepharose CL-4B beads were obtained from Pharmacia Biotech Inc. [9,10-3H]Oleate (9.2 Ci/mmol) was obtained from DuPont, [2-14C]Glycerol (0.6 Ci/mmol) was obtained from IC Pharmaceuticals Canada Ltd. ProMix294 (a mixture of [35S]methionine and [32P]cysteine, 1000 Ci/mmol) and [1-14C]oleic acid (57 Ci/mmol) were obtained from Amersham Corp. Oleic acid, fatty acid-free bovine serum albumin, and standard lipids were obtained from Sigma. Silica gel 60 thin layer chromatography (TLC) plates and organic solvents used for TLC were obtained from BDH chemicals. Brefeldin A was obtained from Epicenter Technologies. The photoactive MTP inhibitor BMS-192951 was a gift of D. Gordon (Bristol-Myers Squibb). Antibody (1D1) specific for human apoB was obtained from R. Milne and Y. Marcel (University of Ottawa Heart Institute).

Cell Culture and MTP Inhibition—McA-RH7777 cells stably transfected with the human apoB48 (hB48) cDNA were generated and cultured in Dulbecco’s modified Eagle’s medium (DMEM) plus 20% serum and 1% sodium bicarbonate. MTP inhibition was introduced prior to the initiation of chase to deplete any residual intracellular [3H]oleate pool, and inactivation of MTP with BMS-192951 was performed during the 1-h delay period. VLDL and HDL were separated by ultracentrifugation, and lipoproteins containing rat B100 (rB100-VLDL, d < 1.02 g/ml) or hB48 (hB48-VLDL, d < 1.02 g/ml or hB48-HDL, d = 1.08–1.13 g/ml) were separated by immunoaffinity chromatography. Lipids associated with these particles were extracted with chloroform/methanol. Separation of phospholipids and neutral lipids was performed on silica gel 60 plates as described (29) using egg yolk lipids as a carrier. Cell lipids were also extracted and separated by TLC. The radioactivity associated with individual lipid species was quantified by liquid scintillation counting (Wallac 1409 counter).

Immunoaffinity Chromatography and Lipid Analysis—Monoclonal antibody 1D1 (4 mg) was coupled to CNBr-activated Sepharose 4B beads (1 g) according to the manufacturer’s instructions. Twelve fractions (i.e. combined fractions of density 1.02 g/ml or hB48-HDL, rB100-VLDL, and hB48-HDL) were subjected to lipid analysis. The recovery of hB48-VLDL from the conditioned medium by immunoaffinity purification was greater than 90%, and the purified hB48-VLDL contained less than 15% endogenous rB100-VLDL as determined by Western blot analysis and by quantification of 35S-labeled apolipoproteins (data not shown).

RESULTS

Oleate-induced B48-VLDL Secretion by Human B48-transfected McA-RH7777 Cells—In McA-RH7777 cells transfected with recombinant hB48, the secretion of hB48-VLDL is dependent upon oleate supplementation (27). This event is similar to the oleate-induced secretion of VLDL containing rat B48 (rB48) in nontransfected McA-RH7777 cells (7). As reported previously, overexpression of hB48 suppressed endogenous rB48 secretion (28). To demonstrate further that hB48-VLDL secretion was comparable with endogenous rB48-VLDL secretion, we tested the response of the transfected cell line to brefeldin A. It has been reported that the oleate-induced rB48-VLDL assembly (the second step) was sensitive to low doses of brefeldin A (9). We found that in hB48-transfected McA-RH7777 cells, secretion of hB48-VLDL was also sensitive to brefeldin A. Fig. 1A shows fluorograms of 35S-labeled apoBs that were secreted as lipoproteins from the cells treated with (bottom) or without (top) 0.2 μg/ml brefeldin A, and Fig. 1B shows the quantitative assessment of apolipoprotein secretion. In the absence of brefeldin A, hB48 was secreted as both VLDL and HDL (Fig. 1A, top). In the presence of brefeldin A, secretion of hB48-VLDL was decreased by 60% compared with control, whereas secretion of hB48-HDL was not decreased (Fig. 1A, bottom). As expected, endogenous rB100-VLDL secretion was also decreased by 60% by the brefeldin A treatment (Fig. 1A, bottom). In these experiments, the amount of radioactivity associated with intracellular 35S-labeled B100 was 40–50% lower in the brefeldin A-treated cells than control (during a 2-h labeling period), but the radioactivity associated with intracellular 35S-

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labeled hB48 was unchanged (data not shown). Synthesis of TG and phosphatidylcholine (PC) was not affected by brefeldin A treatment, as measured by the incorporation of [3H]glycerol during a 4-h labeling period (data not shown). These results together indicate that mechanisms responsible for the second step of hB48-VLDL assembly and secretion are preserved in the transfected McA-RH7777 cells. Thus, in the subsequent experiments, the hB48-transfected cells were used to examine the requirement of MTP activity for the oleate-induced B48-VLDL secretion.

Utilization of Pre-existing Triacylglycerol for B48-VLDL Secretion—To monitor lipid incorporation into secreted B48-VLDL, we first labeled the intracellular lipid pool with [14C]oleate under basal conditions (i.e., DMEM plus 20% serum). During this labeling period, additional oleate mass was not present; therefore, the cells did not produce B48-VLDL. After [14C]oleate labeling, the cells were washed and immediately incubated with DMEM (20% serum) containing 0.4 mM oleate to induce the second step. Oleate was not present in the medium of control cells. In addition, [3H]glycerol was included in both chase media to label newly synthesized lipid. Fig. 2A shows that incorporation of [3H]glycerol-labeled TG (newly synthesized) into secreted rB100-VLDL, hB48-VLDL, or hB48-HDL was increased by 4-, 16-, and 2-fold, respectively, by exogenous oleate. Similarly, incorporation of [3H]glycerol-labeled PC into secreted rB100-VLDL and hB48-VLDL (but not hB48-HDL) was increased by the oleate treatment (Fig. 2A). These results suggest that oleate supplementation, newly synthesized TG is utilized for both hB48-VLDL and rB100-VLDL secretion.

However, a striking difference was observed between incorporation of prelabeled [14C]TG into secreted hB48-VLDL or rB100-VLDL. While oleate treatment had no apparent stimulatory effect on incorporation of [14C]TG into rB100-VLDL, it increased incorporation of [14C]TG into hB48-VLDL more than 6-fold (Fig. 2B). Measurement of [14C]TG associated with hB48-VLDL could be an underestimate of secretion of prelabeled TG, since supplementation of the medium with oleate stimulated TG synthesis and inevitably decreased the specific activity of the [14C]TG pool. There was a concomitant 2-fold decrease in the incorporation of [14C]TG into hB48-HDL at the end of a 4-h chase (Fig. 2B). The effect of oleate on the incorporation of prelabeled [14C]PC into the secreted lipoproteins, however, was similar to that for newly synthesized [3H]PC. These results demonstrate that the unique feature associated with the oleate-stimulated hB48-VLDL secretion is the utilization of pre-existing TG. In the following experiments, we used incorporation of pre-existing TG into hB48-VLDL as a marker to assess the requirement of MTP activity in the second step assembly.

Activity of MTP Is Required for Oleate-induced hB48-VLDL Secretion—A photoactivated MTP inhibitor, designated BMS-192951 (19), was used to inactivate MTP in hB48-transfected McA-RH7777 cells. After incubation with cells for 1 h and subsequent photoactivation (under ultraviolet light for 15 min), BMS-192951 at 5 or 10 μM reduced the MTP activity by 65–70%. The inhibitory effect persisted for at least 8 h (data not shown).

The effect of MTP inhibition on the recruitment of prelabeled TG during oleate-induced second step was determined by pulse labeling of the cells with [3H]oleate (4 h), inactivating MTP with BMS-192951 (1½ h), and monitoring lipoprotein secretion during oleate-supplemented chase. In preliminary experiments, we found that introducing a 1- or 2-h delay period between pulse and chase (Fig. 3A) did not affect the oleate-induced secretion of pre-existing TG as hB48-VLDL. After a 1- or 2-h delay, incorporation of prelabeled TG into secreted hB48-VLDL was again increased by 5–7-fold upon oleate supplementation (Fig. 3B), while incorporation of prelabeled TG into rB100-VLDL was unchanged (Fig. 3C). Increased TG synthesis during oleate-supplemented chase did not significantly alter the intracellular pool of the prelabeled TG or PC, although a small increase in labeled TG and a slight decrease in labeled PC were consistently observed (Fig. 3, D and E). These results indicate that the oleate-stimulated recruitment of pre-existing TG for hB48-VLDL secretion is not diminished after a 1- or 2-h delay.

However, recruitment of pre-existing TG for hB48-VLDL secretion was abolished by MTP inhibition (Fig. 4). At 5 μM BMS-192951, incorporation of [3H]TG into secreted hB48-VLDL or rB100-VLDL decreased by 45 and 70%, respectively, as compared with cells treated with no inhibitor (Fig. 4, A and B). MTP inhibition had little effect on secretion of [35S]labeled hB48 with HDL. Inactivation of MTP also decreased the incorporation of lipid into VLDL. Secretion of prelabeled [3H]TG and [3H]PC associated with hB48-VLDL was decreased by 80 and 85%, respectively, at the end of a 4-h chase (Fig. 4C). Inhibition of MTP did not affect secretion of [3H]TG associated with hB48-HDL or [3H]PC with rB100-VLDL, and it only slightly (<25%) decreased the secretion of [3H]TG with rB100-VLDL or [3H]PC with hB48-HDL (Fig. 4C). These results provide evidence that utilization of pre-existing TG for the oleate-induced hB48-VLDL secretion is sensitive to MTP inhibition. The relatively small effect on secretion of pre-existing TG as rB100-VLDL suggests that a considerable amount of rB100-VLDL particles are probably formed before the oleate-induced second step.
The turnover of prelabeled [3H]TG and [3H]PC in MTP-inactivated cells during oleate-supplemented chase was identical to that in untreated cells (see Fig. 3, D and E), indicating that MTP inactivation did not alter the pools of prelabeled lipid. Nor did MTP inhibition affect secretion of endogenous rat apoAI as HDL (data not shown).

We then examined the effect of MTP inhibition on VLDL secretion by inactivating MTP prior to metabolic labeling of apoB and lipid. Inactivation of MTP diminished the secretion of [35S]-labeled apoB proteins associated with hB48-VLDL (by 80%) or rB100-VLDL (by 90%) as compared with cells treated without inhibitor (Fig. 5, A and B). Similarly, MTP inhibition decreased secretion of [3H]TG associated with hB48-VLDL (by 6-fold) or rB100-VLDL (by less than 2-fold) (Fig. 5C). The greater decrease in radiolabeled rB100 than in radiolabeled TG in the rB100-VLDL fraction indicates that the trace amount of secreted rB100-VLDL is enriched with newly labeled TG. However, similar to our observations in pulse-chase experiments (Fig. 4), secretion of [35S]-labeled B48 or [3H]TG associated with hB48-HDL was unaffected by MTP inhibition (Fig. 5, B and C). These results are reminiscent of the inhibitory effect of brefeldin A on hB48-VLDL secretion (Fig. 1) and demonstrate further that MTP activity is required for the oleate-induced secretion of hB48-VLDL. Under these experimental conditions, incorporation of [3H]glycerol into cellular TG was decreased by 30% (69.8 ± 9.8% of control, n = 8) during a 4-h labeling period in the inhibitor-treated cells compared with untreated cells, whereas incorporation of [3H]glycerol into PC was not affected (103.0 ± 14.5% of control, n = 8).

**DISCUSSION**

Normal MTP Activity Is Required for the Second Step of B48-VLDL Assembly—In the current work we have inquired whether or not MTP activity is required for B48-VLDL secretion using two experimental protocols: MTP was inactivated either before or after metabolic labeling to assess secretion of pre-existing or newly synthesized apoB and lipid as lipoproteins. We took advantage of the fact that hB48-VLDL could be readily purified from the culture media of hB48-transfected McA-RH7777 cells, which retained the ability to secrete hB48-VLDL upon oleate supplementation. We also took advantage of the fact that pre-existing TG was preferentially utilized for hB48-VLDL secretion, which could be used to monitor the oleate-induced second step. In the present study, we found that under conditions where MTP activity was reduced by 65–70%, secretion of either newly synthesized (Fig. 5) or prelabeled hB48 and TG (Fig. 4) as hB48-VLDL was abolished, whereas their secretion as hB48-HDL was unaffected. Most strikingly, secretion of pre-existing TG as hB48-VLDL that was specifically enhanced during the oleate-induced second step (Figs. 2
and 3) was extremely sensitive to MTP inhibition (Fig. 4). These results suggest strongly that expanding the neutral lipid core during hB48-VLDL assembly could be achieved only with normal MTP activity.

Recently, we found that secretion of VLDL containing other truncated apoB variants (e.g. B37) or apoAI/B chimeric proteins (e.g. AI/B29–34) was also induced by oleate supplementation. Moreover, secretion of VLDL containing B37 or AI/B29–34 by the transfected McA-RH7777 cells upon oleate supplementation was also abolished by MTP inhibition.2 These results provide additional evidence indicating that the oleate-induced VLDL secretion is not solely determined by apoB length and that MTP is an important component of the second step VLDL assembly and secretion.

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2 R. S. McLeod and Z. Yao, unpublished results.
Different Assembly Pathways for hB48-VLDL and rB100-VLDL—There are two important differences between hB48-VLDL and rB100-VLDL in the lipid recruitment and its sensitivity to MTP inhibition. The first difference was in the kinetics of incorporation of pre-existing TG upon oleate supplementation. Although both newly synthesized and pre-existing TG could be utilized for rB100-VLDL and hB48-VLDL secretion, pre-existing TG seemed to be preferentially incorporated into hB48-VLDL upon oleate-induced VLDL secretion. Thus, while there was a 6-fold increase in the incorporation of pre-existing TG into hB48-VLDL, there was no difference in secretion of pre-existing TG as rB100-VLDL upon oleate supplementation (Figs. 2 and 3). These results indicate clearly that assembly and secretion of B48-VLDL and B100-VLDL must be achieved through different pathways. The second difference was observed in the response of pre-existing TG recruitment to MTP inhibition. Although inactivation of MTP (by ~70%) decreased secretion of both newly synthesized and pre-existing TG as B48-VLDL and B100-VLDL, the most remarkable effect of MTP inhibition was to abolish the incorporation of pre-existing TG into B48-VLDL during oleate-induced second step. Thus, while secretion of pre-existing TG as hB48-VLDL was decreased by 80%, incorporation of pre-existing TG into rB100-VLDL was only slightly affected (<25%) by MTP inhibition (Fig. 4). The oleate-stimulated incorporation of pre-existing TG into B48-VLDL and its extreme sensitivity to MTP inhibition provide evidence to support the assembly model, suggesting that bulk lipid is added to a primordial B48-HDL particle in the oleate-induced second step (4). In contrast to B48, the inability of B100 to mobilize additional pre-existing TG in response to oleate and its relative insensitivity to MTP inhibition would support the hypothesis that B100-VLDL is assembled primarily through a “one-step” process even before oleate supplementation (7, 30), and that post-translational lipid recruitment may not play a major role in B100-VLDL assembly.

The MTP-mediated TG Mobilization Is Probably Associated with a Pathway Sensitive to Brefeldin A—The present observation that reduced MTP activity results in decreased hB48-VLDL secretion is reminiscent of the similar inhibitory effect of brefeldin A on VLDL secretion (Fig. 1). It has been reported that B48-VLDL secretion induced by exogenous oleate in McA-RH7777 cells can be specifically inhibited by a low dose of brefeldin A (9). Since brefeldin A interferes with the formation of coatamer essential for vesicular transport (31), this result suggests that vesicularization of ER or other intracellular trafficking events may also be components of the second step. Phenotypically, the inhibitory effect of a low dose of MTP inhibitor on lipoprotein secretion was similar to that of brefeldin A: suppressed secretion of hB48-VLDL (and rB100-VLDL) without affecting secretion of hB48-HDL (Figs. 1 and 5). Thus, although speculative, MTP may play a role in facilitating formation of ER-associated TG droplets at the site of the second step by mobilization of the cellular stored TG, processes that could also be sensitive to brefeldin A. Since the current study was not designed to reveal the precursor-product relationship between hB48-HDL and hB48-VLDL, the effect of MTP inactivation on this conversion was not directly examined. The relationship between brefeldin A-sensitive vesicular movement and MTP-facilitated TG mobilization during the second step VLDL assembly needs further evaluation.

Although their overall effects on hB48-VLDL secretion were similar, brefeldin A and MTP inhibitor exerted different effects on the apparent synthesis of intracellular TG. While brefeldin A (0.2 μg/ml) had no effect on cell TG synthesis, inactivation of MTP by the inhibitor BMS-192951 (5 μM) consistently decreased (by 30%) the incorporation of radiolabeled tracer into cell TG (when metabolic labeling was initiated after MTP inhibition). The apparent decrease in TG synthesis could not be explained by increased turnover of the labeled TG, since the pulse-chase experiment showed that MTP inhibition had no effect on the level of the prelabeled cell TG pool, nor was the decreased TG synthesis the result of impairment of lipid synthesis in general, since incorporation of radiolabeled tracer into cell PC was not affected. Currently, there is no satisfactory explanation for the impaired TG synthesis by MTP inhibition.

In the present experiments, the use of a low dose of MTP inhibitor (5 μM) preserved 30–35% of the initial cellular MTP activity. Apparently, the residual MTP activity and the retained active TG synthesis were sufficient to allow normal secretion of the products of first step assembly, such as hB48-HDL. Whether or not the decreased TG synthesis (by 30%) that was associated with MTP inactivation also contributed to the impaired second step hB48-VLDL assembly needs to be determined. Since heterozygotes for human abetalipoproteinemia are asymptomatic, the activity of the MTP expressed from the functional allele is presumably sufficient for normal lipoprotein production. Further experiments using MTP inhibitors in whole animals such as transgenic mice expressing human apoB (2) or using mice bearing nonfunctional MTP mutations will provide additional insights into the requirement of MTP in VLDL production in vivo.

Possible Roles of MTP in the First Step Assembly—The current model for the mode of MTP action in hepatic B48-VLDL synthesis includes but extends beyond the proposed role that MTP plays in the early stage of lipoprotein assembly (19). In cultured cell lines such as COS and HeLa, cells that do not normally synthesize apoB or MTP, coexpression of recombinant MTP and truncated apoB variants resulted in enhanced secretion of most apoB variants examined (15–18). In these cells, the requirement of MTP expression and oleate-induced lipogenesis appeared to be a function of apoB length, suggesting that there is an important interplay between lipid availability, MTP activity, and the hydrophobic lipid-binding regions of apoB. In transfected cells that lacked MTP activity, the expressed apoBs were unable to translocate across the microsomal membrane or were degraded immediately after translocation (32). Thus, inactivation of MTP in McA-RH7777 cells would be expected to somehow diminish apoB synthesis, either by premature termination of chain elongation or by rapid degradation of newly synthesized polypeptides (18). The extent of MTP inhibition on apoB synthesis and on the secretion of the first step products (i.e., HDL- and LDL-like particles) also appeared to be a function of apoB length (33). In Caco-2 cells treated with an MTP inhibitor, secretion of B48 was not affected, whereas secretion of B100 was abolished (22). Furthermore, in a murine mammary-derived cell line that lacks MTP activity, assembly and secretion of the transfected N-terminal 41% of human apoB on HDL-sized lipoproteins has been observed (34). These data together reinforce the notion that the requirement of MTP activity for apoB secretion is correlated positively with the length of apoB and with the extent of lipid recruitment (18).

The current study, therefore, presents evidence that the activity of MTP, together with the brefeldin A-sensitive ER vesicularization and other protein factors, may constitute the complex second step of B48-VLDL assembly and subsequent B48-VLDL secretion. These events, induced by exogenous oleate in McA-RH7777 cells, may represent an enhanced mobilization of pre-existing TG for the expansion of the neutral lipid core of VLDL. In addition, the enhanced lipid mobilization may also facilitate the first step lipid assembly by apoB, a step that has been documented to be assisted by MTP. Thus, MTP activity is required for the entire VLDL assembly process,
including both co-translational and post-translational addition of bulk neutral lipid.

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