Previously, based on distinct requirement of microsomal triglyceride transfer protein (MTP) and kinetics of triglyceride (TG) utilization, we concluded that assembly of very low density lipoproteins (VLDL) containing B48 or B100 was achieved through different pathways (Wang, Y., McLeod, R. S., and Yao, Z. (1997) J. Biol. Chem. 272, 12272–12278). To test if the apparent dual mechanisms were accounted for by apolipoprotein B (apoB) length, we studied VLDL assembly using transfected cells expressing various apoB forms (e.g. B64, B72, B80, and B100). For each apoB, enlargement of lipoprotein to form VLDL via bulk TG incorporation was induced by exogenous oleate, which could be blocked by MTP inhibitor BMS-197636 treatment. While particle enlargement was readily demonstrable by density ultracentrifugation for B64- and B72-VLDL, it was not obvious for B80- and B100-VLDL unless the VLDL was further resolved by cumulative rate flotation into VLDL1, (Sf > 100) and VLDL2 (Sf 20–100). BMS-197636 diminished B100 secretion in a dose-dependent manner (0.05–0.5 μM) and also blocked the particle enlargement from small to large B100-lipoproteins. These results yield a unified model that can accommodate VLDL assembly with all apoB forms, which invalidates our previous conclusion. To gain a better understanding of the MTP action, we examined the effect of BMS-197636 on lipid and apoB synthesis during VLDL assembly. While BMS-197636 (0.2 μM) entirely abolished B100-VLDL assembly/secretion, it did not affect B100 translation or translocation across the microsomal membrane, nor did it affect TG synthesis and cell TG mass. However, BMS-197636 drastically decreased accumulation of [14C]glycerol-labeled TG and TG mass within microsomal lumen. The decreased TG accumulation was not a result of impaired B100-VLDL assembly, because in cells treated with brefeldin A (0.2 μg/ml), the assembly of B100-VLDL was blocked yet luminal TG accumulation was normal. Thus, MTP plays a role in reducing accumulation of TG within microsomes, a prerequisite for the post-translational assembly of TG-enriched VLDL.

The major function of hepatic very low density lipoproteins (VLDL)1 is to deliver triacylglycerol (TG) from the liver to peripheral tissues. Each VLDL particle contains a single copy of apolipoprotein B (apoB) and variable amounts of TG (1). There are two forms of apoB proteins present in the plasma, the full-length B100 and a truncated B48 collinear with the N-terminal 48% of B100 (2). In humans, B100 and B48 are produced in the liver and intestine, respectively. In rats, however, the liver produces both B100 and B48, and both forms have the ability to assemble VLDL (1). Accumulating evidence suggests that formation of B100-VLDL (3) and B48-VLDL (4–7) is accomplished through two steps. Known as the “two-step” model, it postulates that the initial product is a primordial small, dense particle, formed during or immediately after apoB translation in the endoplasmic reticulum (ER). Subsequently, bulk lipid is incorporated into the primordial particle to form a mature VLDL (7). Under certain conditions, such as insufficient lipid supply or treatment of a low dose of brefeldin A (BfA), the assembly/secretion of mature VLDL are impaired whereas those of small, dense particles remain normal (7, 8). Apparently, multiple factors are involved for the maturation of VLDL.

One factor that plays an obligatory role in VLDL assembly is microsomal triglyceride transfer protein (MTP) (9). Defective MTP is the cause of abetalipoproteinemia, a recessive genetic disorder manifested by extremely low concentrations of plasma apoB (10, 11). MTP is a heterodimer that consists of protein disulfide isomerase and the 97-kDa catalytic subunit (9). The 97-kDa subunit is expressed mainly in intestine and liver, and has the ability to catalyze the transfer of various lipids between lipid surfaces in vitro (12). Physical interaction between MTP and apoB has been observed (13–19), but it is not clear if MTP also catalyzes lipid transfer onto apoB in vivo. Inactivation of MTP in hepatic or intestinal cells invariably impairs apoB assembly and secretion (7, 20, 21). On the other hand, co-expression of the MTP 97-kDa subunit with recombinant apoB facilitates assembly of small, dense lipoproteins containing apoB in heterologous cells (22–25). Although these studies have provided strong evidence that MTP is essential for apoB secretion as lipoproteins, our knowledge on the mechanism by which MTP facilitates VLDL assembly is incomplete and controversial.

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§ Scientist of Medical Research Council of Canada. To whom correspondence should be addressed. Tel.: 613-798-5555 (ext. 8711); Fax: 613-761-5281; E-mail: zyao@ottawaheart.ca.

The abbreviations used are: VLDL, very low density lipoprotein; TG, triacylglycerol; apoB, apolipoprotein B; BfA, brefeldin A; ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein; ALLN, N-acetyl-leucyl-leucyl-norleucinal; DMEM, Dulbecco's modified Eagle's medium; PAGE, polyacrylamide gel electrophoresis; TLC; thin layer chromatography; PC, phosphatidylcholine; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein.
Initial transfection studies with non-hepatic cell lines (22–25) suggested that the major function of MTP was to assist translocation of apoB across the membrane of ER. Since it was believed that the formation of primordial dense particles took place during apoB translocation, some researchers postulated that MTP activity was essential only in the early stage of apoB lipiddation (26). In the recent work showing post-translational B100-VLDL assembly, the notion that MTP was required for the “early phase” of assembly but not for bulk TG incorporation at the “late phase” was reinforced (3). Although plausible that MTP acts as a facilitator for apoB translocation, other studies cast some doubts on this conclusion. Experiments with cells (27) or microsomal membranes (28) that lack MTP expression have shown that apoB translocation and assembly into a primordial particle do not need MTP activity. Furthermore, emerging evidence suggests that the demand for MTP is much greater for bulk TG incorporation during VLDL assembly than that for primordial particle formation (7, 21). These data raise the possibility that MTP may play a role other than assisting apoB translocation.

Significant new insight into the requirement of MTP activity in VLDL assembly has been gained using stable McA-RH7777 cells. McA-RH7777 cells are the only hepatoma available that produces authentic VLDL but the level of endogenous apoB expression is low. Fortunately, these cells are suitable for stable transfection with recombinant apoBs. Studies conducted so far have consistently demonstrated that the ability of recombinant human B100 and B48 to assemble lipoprotein is indistinguishable from that of endogenous apoBs (29, 30). Working with B48-transfected cells, we previously observed marked differences in the kinetics of TG utilization and in the sensitivity toward MTP inhibition between B48- and B100-VLDL assembly/secretion (7). On the basis of these observations, we concluded that dual mechanisms operated in McA-RH7777 cells; while B48-VLDL assembly was achieved post-translationally, assembly of B100-VLDL was co-translational (7). However, in these studies, no attention was given to the heterogeneity of B100-VLDL; hence, they were dealt with as a uniform fraction (d < 1.02 g/ml) without consideration of size differences (7). Because of this technical caveat, potential size enlargement accompanied with bulk TG incorporation into B100-VLDL was overlooked.

In the current work, we studied VLDL assembly using transfected cells expressing various apoB forms (e.g. B64, B72, B80, and B100) to inquire if there were indeed dual mechanisms for the assembly of VLDL containing small or large apoB. Moreover, we resolved VLDL subclasses by size using cumulative rate flotation to determine if MTP activity was required for the formation of large, TG-rich VLDL. The present study has yielded a unified model that accommodates VLDL assembly with all apoB forms and nullifies the conclusion of dual assembly mechanisms. Also, we have found that MTP activity is crucial in the maintenance of a metabolically dynamic microsomal TG pool, which is essential for bulk TG incorporation during the final stage of B100-VLDL assembly.

**EXPERIMENTAL PROCEDURES**

**Materials**—ProMix™ ([35S]methionine/cysteine, 1000 Ci/mmol); [2-3H]glycerol (1 Ci/mmol), [3H]oleic acid (9 Ci/mmol), glycerol tri[1-14C]oleate (80 mCi/mmol), and CNBr-activated Sepharose 4B beads were obtained from Amersham Pharmacia Biotech. Oleic acid, fatty-acid free bovine serum albumin and standard lipids were obtained from Sigma. N-Acetyl-leucyl-leucyl-norleucinal (ALLN) and BfA were from Biomol and Epicenter Technologies, respectively. The ECL immunodetection system, trypsin, and soybean trypsin inhibitor were obtained from Roche Molecular Biochemicals. Monoclonal antibody specific for human apoB (1D1) was a gift of R. Milne and Y. Marcel (University of Ottawa Heart Institute).

**Cell Culture and Stock Solution**—Stable McA-RH7777 cells that express human B72, B80, or B100 (30) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) plus 20% serum. Cell line that expressed B64 was prepared as follows. A DNA fragment extending from the MluI to MspI sites at nucleotides 7011 and 8848, respectively, was excised from pB100-L (30). After the MspI site was ligated with a KpnI linker, the fragment was inserted into pB53-L (31) that had been digested with MluI and KpnI to create pB48-L, which was then used to generate stable transfectants in McA-RH7777 cells as described previously (30). Protocols for metabolic labeling with [35S]methionine/cysteine, [3H]glycerol, or [3H]oleate are described in the figure legends. Stock solution of BFA (5 mg/ml) and MTP inhibitor BMS-197636 (20 mM) (designated compound 7) (32) were prepared in absolute ethanol and dimethyl sulfoxide, respectively.

**MTP Activity Assay**—The TG transfer activity of MTP was measured using [14C]triolein and the deoxycholate extract of total cell homogenate according to the published protocol (10). Since the extensive dialysis required for the deoxycholate extract precluded the assessment of BMS-197636 (a reversible inhibitor) effect on MTP, an alternative protocol (referred to as sonication extraction) was developed in which the cell homogenate was sonicated for 2 min at 4°C. After centrifugation (400,000 × g, 16 min) of the sample, the supernatant that contained the MTP activity was used for TG transfer assay. The MTP activity in McA-RH7777 cells measured by the two protocols was comparable (data not shown).
Fig. 2. Effect of oleate on secretion of B100-VLDL1, A, after labeling cells for 3 h with [35S]methionine/cysteine in DMEM (20% serum) ± 0.4 mM oleate, the conditioned media were fractionated by cumulative rate flotation. The [35S]B100 in each fraction was analyzed as in Fig. 1B, B, analysis of apoB-lipoprotein size by non-denaturing gradient gel electrophoresis. B100-VLDL1, B100-VLDL2, B48-VLDL (d < 1.02 g/ml) and B48-HDL (d > 1.02 g/ml), isolated from conditioned media, were separated on a 2–8% gradient polyacrylamide gel and analyzed by Immunoblotting with antibody 1D1. Positions of markers are indicated on the left.

TABLE I

Composition of VLDL1 and VLDL2 secreted from human B100-transfected McA-RH7777 cells

| Lipoproteins | [35S]B100 | [3H]TG | [3H]PC |
|--------------|-----------|--------|--------|
| B100-VLDL1   | 187.1 ± 9.8 | 227.3 (1.2) | 16.5 (0.9) |
| B100-VLDL2   | 546.7 ± 41.5 | 81.9 (0.15) | 9.0 (0.02) |
| VLDL1/VLDL2  | 0.34       | 2.8    | 1.8    |

**RESULTS**

A Unified VLDL Assembly Model for All ApoB Forms—Our previous study suggested that assembly of B100-VLDL and B48-VLDL took different paths (7). Here we systematically analyzed assembly/ssecretion of VLDL with B64, B72, B80, or B100 in an attempt to determine if the apparent dual mechanisms were accounted for by apoB length. Under basal culture conditions (i.e. DMEM ± 20% serum), the buoyant density of secreted apoB-lipoproteins, as expected, was inversely related to apoB length. Thus, B64, B72, and B80/B100 were found predominantly in fractions with densities resembling those of...
high density lipoproteins (HDL), low density lipoproteins (LDL), and VLDL, respectively (Fig. 1A, panels labeled –OA). Upon supplementation with oleate, however, all apoB forms were secreted as VLDL (Fig. 1A, panels labeled +OA). The enlargement of lipoprotein size was readily demonstrable for B64 and B72 by ultracentrifugation in a sucrose density gradient, but it was less evident for B80 and B100 because these proteins were already observed in VLDL (d < 1.02 g/ml) fraction even in the absence of oleate. Likewise, while the effect of MTP inhibitor BMS-197636 (0.2 μM) on oleate-stimulated [3H]oleate-labeled TG as B100-VLDL 1 by 5-fold and evidence of the BMS-197636 effect on [35S]B100-VLDL secretion was not as clear although still discernible (Fig. 1B, b). (The inhibitor BMS-197636, unlike the photoactivated inhibitor BMS-192951 used previously (7), did not require ultraviolet treatment yet inhibited B100 secretion within 10 min of administration (data not shown).) These results led us to suspect that the supposed co-translational B100-VLDL assembly was a misinterpretation of data resulting from inadequate resolution of B100-VLDL (7), and that there was nothing but an imaginary dual mechanism for VLDL assembly in McA-RH7777 cells.

In the following experiments, we used cumulative rate flotation technique to resolve B100-VLDL1 (Sₜ > 100) and -VLDL2 (Sₜ 20–100) from intermediate density lipoproteins (IDL)/LDL. Fig. 2A shows that, without oleate, B100 was secreted as VLDL1 and IDL/LDL, and that secretion of VLDL1, particularly VLDL1, was stimulated by exogenous oleate. (Secretion of [35S]B72-VLDL secretion was certainly observable (Fig. 1A, panels labeled –OA)). Oleate also increased secretion of [3H]PC as B100-VLDL 1 by 8- and 5-fold, respectively, greater than those in VLDL2. Thus, the oleate-induced size enlargement of B100-VLDL1 (equivalent to a 2-fold increase in diameter) was accompanied with bulk TG incorporation, which was observable only after VLDL1 and VLDL2 were resolved.

Previous pulse-chase experiments suggested that assembly of B48-VLDL utilized pre-existing TG but B100-VLDL1 did not (7). Here we re-examined the utilization of pre-existing TG for B100-VLDL1 assembly with the same pulse-chase protocol (7) but using the cumulative flotation to separate VLDL1 and VLDL2. Fig. 2C shows that exogenous oleate increased secretion of [3H]oleate-labeled TG as B100-VLDL1 by 5-fold and concomitantly decreased its secretion as B100-VLDL2 by 30%. Oleate also increased secretion of [3H]PC as B100-VLDL1 by 8-fold, yet had no effect on [3H]PC associated with B100-VLDL2 (Fig. 2C). Thus, through the improved resolution of B100-VLDL1 particles, oleate-induced bulk TG incorporation, the hallmark of VLDL assembly irrespective of apoB length, became manifest.

To inquire whether B100-VLDL, like B48-VLDL, is also assembled post-translationally, we performed metabolic labeling (Fig. 3A) and pulse-chase experiments (Fig. 3B) under conditions that were optimal for VLDL assembly (i.e. 0.4 mM oleate was supplemented throughout the experiment). Fig. 3A shows that at the end of 10 or 20 min of labeling, [35S]B100 was found mainly in IDL/LDL fraction with small amount in VLDL2 fraction within the microsomal lumen. It also shows that VLDL1-

Fig. 3. Analysis of assembly of B100-VLDL, within microsomal lumen. Cells were pretreated with 0.4 mM oleate in DMEM (20% serum) for 30 min, and either continuously labeled with [35S]methionine/cysteine for 10, 20, and 40 min (A), or else pulse-labeled for 20 min, and then incubated in chase medium ± BMS-197636 for 15, 45 or 60 min (B). The microsomal fraction was fractionated into VLDL1, VLDL2, IDL/LDL, and HDL, respectively (Fig. 1A, panels labeled –OA)). The B100-VLDL1 contained ~25% of total secreted [35S]B100 and contained 74% and 65% of respective total [3H]TG and [3H]PC associated with B100 (Table I). As a result, the ratios of [3H]TG/[35S]B100 and [3H]PC/[35S]B100 in VLDL1 are 8- and 5-fold, respectively, greater than those in VLDL2. Thus, the oleate-induced size enlargement of B100-VLDL1 (equivalent to a 2-fold increase in diameter) was accompanied with bulk TG incorporation, which was observable only after VLDL1 and VLDL2 were resolved.

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B100 became detectable at 40 min of labeling. Since translation of B100 completes within 20 min (36), the delayed B100-VLDL1 assembly suggests a post-translational mechanism. Next, we inquired whether or not B100-VLDL1 assembly required MTP activity by pulse-chase analysis. After 20 min of pulse, the association of [35S]B100 with VLDL1 gradually became apparent within microsomal lumen at 15, 45, and 60 min of chase (Fig. 3B). Inclusion of BMS-197636 in the chase medium completely abolished the formation of B100-VLDL1 (Fig. 3B, bottom). These combined results, reminiscent of the post-translational TG incorporation and MTP requirement observed for B48-VLDL assembly (7), provide evidence that B100-VLDL1 assembly is achieved via a path similar to that for B48-VLDL.

The requirement of MTP for B100-VLDL1 assembly was also manifest by examining the effect of BMS-197636 on lipoprotein secretion. With doses increasing from 0.05 to 0.5 μM, BMS-197636 not only diminished secretion of [35S]B100 to <1% of control, but also decreased the size of B100-lipoproteins from VLDL to HDL (Fig. 4A and B). The BMS-197636 doses that abolished secretion of B100-VLDL1 and -VLDL2 were 0.05 μM and 0.2 μM, respectively, corresponding to 62% and 50% of normal MTP activity (Fig. 4C). (Although treatment with 0.2 μM BMS-197636 resulted in only partial inhibition of cell-associated MTP activity, adding the same concentration of MTP inhibitor BMS-197636 to the assay mixture entirely abolished MTP activity (see Fig. 4C, closed square.).) In contrast to that of B100, secretion of B48 (mainly as HDL) was relatively unaffected by BMS-197636 (Fig. 4B). These data showed clearly that the demand for MTP activity is positively correlated with the extent of apoB
lipidation. The inhibited B100-VLDL secretion by BMS-197636, as expected, was accompanied with decreased VLDL-TG secretion (Fig. 4D). However, under no circumstances did BMS-197636 affect synthesis or accumulation of cell TG and PC (Table II).

**MTP Activity Is Required for Mobilizing TG into Microsomes**—Knowing that BMS-197636 did not affect TG synthesis, we hypothesized that it might impair TG mobilization into microsomes. To test this hypothesis, we determined the effect of BMS-197636 on TG accumulation and on [3H]TG secretion was also apparent at the end of 1 h of labeling. Thus, BMS-197636 specifically impairs the accumulation and attainment of newly synthesized TG within the microsomal lumen. When [3H]TG accumulation in the microsomal lumen was plotted against the dose of BMS-197636 (Fig. 6B), an inverse relationship, similar to the residual MTP activity within the cells as a function of MTP inhibitor (Fig. 4C), was observed. The abolished B100-VLDL1 assembly/secretion (Fig. 4A) coincided with 55% decrease in lumenal [3H]TG accumulation at 0.05 mM BMS-197636. Lipid associated with B100-VLDL1 or -VLDL2 was extracted, and radioactivity of [3H]TG were quantified. (The high proportion of 3H-labeled PC (Fig. 5B, top) or PC mass (Fig. 5B, bottom) among different subcellular compartments.

| Condition | Lipid synthetic rate | Lipid mass | 
|------------|----------------------|------------|
|            | [3H]TG | [3H]PC | TG | PC |
| 1. No oleate | ND | ND | 5.3 ± 0.6 | 43.3 ± 2.5 |
| 2. + 0.4 mM oleate | 5.0 ± 0.5 | 2.8 ± 0.7 | 27.9 ± 4.1 | 41.3 ± 2.6 |
| 3. Same as 2, + 0.2 μM BMS-197636 | 5.4 ± 0.4 | 3.0 ± 0.6 | 31.4 ± 1.1 | 44.8 ± 4.8 |
| 4. Same as 2, + 0.2 μg/ml brefeldin A | ND | ND | 31.9 ± 2.0 | 39.8 ± 1.9 |

**FIG. 4. Effect of BMS-197636 on secretion of apoB and TG.** A, cells were labeled for 3 h with [35S]methionine/cysteine in DMEM (20% serum) in the presence of 0.4 mM oleate + indicated dose of BMS-197636. The secreted [35S]B100 and [35S]B48 were analyzed as in Fig. 2A. B, radioactivity associated with secreted [35S]B100 and [35S]B48 was quantified. Data are presented as % of control (i.e. no BMS-197636). C, MTP activity assay. The microsomal content was isolated from cells treated for 30 min with various doses of BMS-197636 and 0.4 mM oleate, and was used to measure the TG transfer activity (cell). Data are presented as % of control (n = 4). Addition of 0.2 μM BMS-197636 directly to the assay mixture abolished the TG transfer activity (in vitro). D, cells were labeled with [3H]glycerol for 2 and 4 h in DMEM (20% serum) ± 0.4 mM oleate or oleate + 0.2 μM BMS-197636. Lipid associated with B100-VLDL1 or -VLDL2 was extracted, and radioactivity of [3H]TG were quantified.
gests strongly that the demand of MTP activity for B100-VLDL assembly correlates closely with the influx of TG into microsomal lumen.

The alternate possibility of diminished lumenal [3H]TG being a consequence of impaired VLDL assembly was tested using cells treated with a low dose of BFA (0.2 μg/ml). As shown previously (7, 8), BFA effectively blocked bulk TG incorporation into VLDL, which was confirmed here by its effect on B100-VLDL assembly within the microsomal lumen (Fig. 6C). Under this condition, however, influx of [3H]TG into microsomes and [3H]TG secretion decreased marginally as compared with control (i.e. no BFA) (Fig. 6A, a, b, and d). The un-impaired accumulation of lumenal TG by BFA treatment was confirmed by prolonged lipid labeling (Fig. 6D, a) and mass measurement (data not shown). In these cells, however, the [3H]TG associated with microsomal lumen (Fig. 6D, a) and secreted into the medium (Fig. 6D, b) were also sensitive to BMS-197636 treatment, providing another evidence that MTP activity is essential for TG accumulation within microsomes. Thus, the influx of TG into microsomal lumen may not be tightly coupled with VLDL assembly. Together, these data suggest that the diminished lumenal [3H]TG upon BMS-197636 treatment is unlikely attributable to an inhibited VLDL assembly.

Finally, we tested (by pulse-chase experiments) the possibility, although unlikely, that the decreased lumenal TG accumulation by BMS-197636 was the result of impaired B100 translocation across the ER membrane (Fig. 7). The experiment was done under conditions where B100-VLDL assembly was maximized (with exogenous oleate) yet degradation of B100 was minimized (with ALLN). Between control and BMS-197636 (0.2 μM)-treated cells, equal amounts of full-length [35S]B100 were found during 0, 15, and 30 min of chase (Fig. 7, compare lanes 1, 4, and 7 between A and B). Thus, MTP inhibition per se does not affect apoB translation. We then determined translocation of pulse-labeled (15 min) [35S]B100 during chase by trypsin digestion of the isolated microsomes. In control cells 40–50% of [35S]B100 was sensitive to trypsin at 0, 15, and 30 min of chase (Fig. 7A, lanes 2, 5, and 8). Under these conditions, inhibition of MTP did not have an effect on the attainment of trypsin resistance in [35S]B100 (Fig. 7B, lanes 2, 5, and 8). (The integrity of microsomal vesicles was verified by nearly 100% trypsin resistance of the ER-resident protein disulfide isomerase (data not shown).) Fragments of apoB that were resistant to exogenous trypsin (indicated by a bracket to the right of lanes 2 of Fig. 7, A and B) were observed whose intensity decreased with time (compare 0, 15, and 30 min of chase). These fragments might be derived from partially translocated B100 as reported previously (39). Thus, decreasing MTP activity by half (at 0.2 μM BMS-197636) did not impede B100 translation/translocation.

**DISCUSSION**

Previously, based on the apparent differences in the kinetics of TG utilization into B100-VLDL and B48-VLDL, we concluded that the two VLDL species were assembled through different paths (7). Although the TG kinetics lends some plausibility to this conclusion, it does not stand up well to scrutiny. Attempts to delineate these distinct mechanisms using various C-terminal truncated apoB variants in this study failed to detect any multiplicity in the assembly pathway. Rather, a unified model that can accommodate VLDL assembly with all apoB forms (i.e. B48, B64, B72, B80, and B100) has emerged which invalidates our previous conclusion. By revealing the enlargement of VLDL particle size using cumulative rate flotation together with monitoring TG content (Fig. 2 and Table I), the oleate-inducible, MTP-dependent, post-translational TG incorporation becomes clearly demonstrable for B100-VLDL formation. Two critical points of this model are noteworthy.

![Fig. 5. Effect of oleate and BMS-197636 on subcellular distribution of TG and PC.](image-url)
is a function of MTP activity. Experimental evidence supporting this conclusion includes (a) measurement of metabolically labeled TG (Figs. 5A and 6A) and of TG mass (Fig. 5A), and (b) demonstration of a direct correlation between the attainment of luminal TG and the MTP activity (Fig. 6B). In principle, the steady state level of luminal TG is determined by the rate of influx of TG through mobilization and the efflux via secretion. Since MTP inactivation inhibits TG secretion, yet it does not inhibit TG synthesis, the decreased influx TG is the most likely explanation for the diminished luminal TG content. Although the origin of TG being mobilized into microsomal lumen is not determined in the current study, our result does suggest that the maintenance of a microsomal TG pool, regardless which being derived from de novo synthesis or from hydrolysis-reesterification of a storage pool, requires normal MTP activity. Another important, although less conclusive, result derived from this study is the demonstration that accumulation of luminal TG can be independent of VLDL assembly in BfA-treated cells (Fig. 6, A and C). Observation of “TG particles” within the secretory compartments in cells where VLDL assembly is abolished (by genetically inactivating apoB expression) has been reported recently (40). Our data are in accord with this observation and provide indirect evidence that the MTP-mediated TG mobilization may lead to the formation of apob-free “TG droplets” (41). A point of note is that in mice in which hepatic MTP was inactivated by gene targeting, lipid droplets were completely absent within the secretory compartment (42). These in vitro and in vivo findings together argue strongly that accumulation of bulk TG within microsomes is associated with normal MTP activity. A major challenge ahead to this theory, however, is to characterize these “TG particles” biochemically and to demonstrate that they are indeed precursors for VLDL assembly. The core difficulty with all VLDL assembly models harks back to the old question concerning the path that is taken by bulk TG eventually being incorporated into apoB. The current studies indicate that MTP may play a role in facilitating the accumulation and attainment of TG in the microsomes. However, they did not address the question of whether MTP activity is required for incorporation of the microsomal TG into VLDL. It is clear that the demand for MTP is much greater for the assembly of TG-rich VLDL than for the assembly of dense particle. For instance, VLDL_{11} assembly was abolished at 0.05
from the present study that assembly of small B100-lipoprotein assembly was unaware of that the demand for MTP is determined does not occur if the activity of MTP is inhibited. Second, the requirement of MTP activity for B100-VLDL assembly is achieved through a process insensitive to MTP inhibition. However, the three caveats associated with these experiments are noteworthy and could potentially jeopardize the conclusion. First, the post-translational B100-VLDL assembly is achieved through a process insensitive to MTP inhibition. Therefore, caution must be exercised in concluding that MTP activity is not required for bulk lipid incorporation during B100-VLDL assembly.

In summary, we have demonstrated that MTP plays an important role in the accumulation and attainment of bulk TG from the microsomal lumen, an event that can be separated from TG incorporation into mature VLDL but represents an indispensable requisite for the olate-induced, post-translational assembly of VLDL.

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