Microsomal triglyceride transfer protein is essential for hepatic secretion of apoB-100 and apoB-48 but not triglyceride

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Abstract **Despite a complete lack of microsomal triglyceride transfer protein (MTP), L35 rat hepatoma cells secrete triglyceride-containing lipoproteins, albeit at a rate 25% of that of parental FAO hepatoma cells, which express high levels of MTP. The inability to express MTP was associated with a complete block in the secretion of both apolipoprotein (apo)B-100 and apoB-48. Stable expression of a MTP transgene restored the secretion of both apoB-100 and apoB-48 in L35 cells, indicating that MTP is essential for the secretion of both forms of apoB. Treatment with the MTP inhibitor BMS-200150 reduced the secretion of triglyceride by 70% in FAO cells, whereas the inhibitor did not affect the secretion of triglycerides by L35 cells. Thus, in the presence of the MTP inhibitor, both cell types secreted triglycerides at similar rates. Essentially, all of the triglycerides secreted by L35 cells were associated with HDL containing apoA-IV and apoE but devoid of apoB-100 or apoB-48. These results suggest that these triglyceride-containing lipoproteins are assembled and secreted via a pathway that is independent of both apoB and MTP. Our findings support the concept that apoB and MTP co-evolved and provided a means to augment the secretion of triglyceride through the formation of lipoproteins containing large hydrophobic cores enriched with triglycerides.**—Hui, T. Y., L. M. Olivier, S. Kang, and R. A. Davis. **Microsomal triglyceride transfer protein is essential for hepatic secretion of apoB-100 and apoB-48 but not triglyceride.** ***J. Lipid Res.*** **2002.** 43: **785–793.**

Supplementary key words high density lipoproteins • liver lipoproteins • MTB inhibitors

Triglycerides are the most efficient form of substrate for transport and storage of metabolic energy (1). Because of their inability to form hydrogen bonds, triglycerides are packaged into the hydrophobic core of lipoproteins for secretion and subsequent transport through plasma (1, 2). The co-evolution of apolipoprotein (apo)B and microsomal triglyceride transfer protein (MTP) provided a means for packaging triglycerides into VLDL, the major vehicle through which triglycerides are transported from their site of synthesis in the liver to peripheral tissues for energy utilization and anabolic purposes (1, 2). ApoB is a large (4563 amino acids) amphipathic protein which serves as the major structural protein in VLDL (3–6). Two major forms of apoB are produced through a unique mRNA editing step (7, 8). In humans, apoB-100 is made exclusively by the liver, whereas apoB-48 is made in the intestine (9–12). MTP, a resident protein in the lumen of the endoplasmic reticulum, facilitates the transfer of triglycerides from their site of synthesis in the endoplasmic reticulum into the lumen during the assembly of VLDL (13). Genetic loss of either apoB (hypobetalipoproteinemia) or MTP (abetalipoproteinemia) results in the inability of both the liver and intestine to secrete VLDL (14). The small amount of triglycerides found in the plasma of abetalipoproteinemics is in the HDL fraction (15). The defect in VLDL production by abetalipoproteinemics is associated with mild hepatic steatosis and reduced plasma lipoprotein levels, and sometimes deficiencies in fat-soluble vitamins (14, 15).

The ability to alter the genome of mice has provided new insights about the mechanisms responsible for several human genetic disorders (16). Unlike humans (hypobetalipoproteinemics and abetalipoproteinemics), homozygous knockout of either the apoB gene or the MTP gene in mice is embryonic lethal (17, 18). This may be due to a difference in embryonic development between the two species. In mice, the assembly/secretion pathway of apoB-containing lipoproteins plays an essential role in transporting fat-soluble nutrients to the yolk sac (19). To overcome the lethality associated with functional deletion of MTP, two individual research groups have created liver-specific knockout mice using cre/lox recombinase systtem (20, 21). While both studies clearly showed that liver-specific deletion of MTP nearly abolished the secretion of apoB-100, one showed that a similar dramatic decrease in apoB-48 (20), whereas only ~20% decrease in apoB-48

Abbreviations: apo, apolipoprotein; CMV, cytomegalovirus; FPPS, farnesyl pyrophosphate synthase; MTP, microsomal triglyceride transfer protein.

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was observed in the other study (22). Since MTP deficiency in humans is associated with an almost complete absence of both apoB-100 and apoB-48 in the plasma (14), the findings showing that liver-specific MTP knockout caused only a slight reduction in plasma apoB-48 raised the possibility that MTP may not be essential for the secretion of apoB-48 in mice (22). This interpretation has important implications in evaluating many studies of assembly and secretion of apoB-containing lipoproteins performed in mice and rats.

In the present study, we examine lipoprotein assembly and secretion by rat hepatoma L35 cells that express no detectable MTP mRNA, protein, or lipid transfer activity. L35 cells are a stable cell line derived from parental FAO cells following DNA-mediated gene transfer and screening for resistance to 25-hydroxycholesterol (23). The ability of L35 cells to express several liver-specific genes (e.g., CYP7A1, CYP3E1, PIG receptor) not expressed by FAO cells is thought to be due to the alteration of a *trans*-acting factor that affects gene transcription (23, 24). As shown below, the inability of L35 cells to express MTP was associated with a complete block in the secretion of apoB-100 and apoB-48. Our data indicate that in rats MTP is required for the secretion of both apoB-100 and apoB-48.

MATERIALS AND METHODS

Cell culture and transfection

L35 cells were cultured as previously described (25). FAO cells were maintained in DMEM containing 4% ECS and 4% FBS, in 5% CO_2 at 37°C. HepG2 cells were cultured in DMEM containing 10% FBS. McArdle RH-7777 cells were maintained in DMEM with 15% FBS. Two MTP expressing plasmids were used for stable expression in L35 cells. The hamster MTP cDNA and human MTP expression vector containing the human promoter (−612 to +84) driving the expression of human MTP cDNA sequence were obtained from Dr. Narayanan Hariharan, (Bristol-Myers/Squibb). To generate the cytomegalovirus (CMV)-driven expression plasmid, the hamster MTP cDNA was sub-cloned into pcDNA3 (Invitrogen).

L35 cells were transfected with a CMV promoter-driven expression plasmid containing the coding region of hamster MTP previously shown to allow HeLa cells to express MTP and secrete apoB-53 (26). Following transfection, cells were screened for resistance to G418 (400 μg/ml). Antibiotic resistant cells were single-cell cloned. For each stable transfectant, at least three individual single cell clones showed dexamethasone-inducible expression of cholesterol-7α-hydroxylase (CYP7A1), a unique phenotype for L35 cells (23), as well as the lack of MTP expression, as reported below.

Immunoprecipitation and SDS-PAGE

Media from the stable transfectants were incubated overnight with 50 μl of a 50% (v/v) solution Sepharose CL-6B beads (Pharmacia). The samples were centrifuged for 5 min at 12,000 rpm and the supernatant was incubated with shaking for 2 h with anti-apoB antibodies. Protein A-Sepharose beads (Sigma) were added to each sample and incubated for an additional hour. The samples were centrifuged for 5 min at 12,000 rpm and the antigen/antibody protein A-sepharose pellet was washed four times with immunoprecipitation buffer (25 mM Tris/HCl, 5 mM EDTA, 250 mM NaCl, 1% Triton X-100, pH 7.5), and once with PBS. The pellets were solubilized in sample buffer (0.13 M Tris, 4% SDS, 20% glycerol, 0.4 mM EDTA, 5% β-mercaptoethanol), boiled for 5 min, and separated on a linear 1–15% SDS-polyacrylamide gel as described. Proteins were analyzed by Western blotting using specific antibodies to apoB.

Lipoprotein isolation and characterization

FAO and L35 cells were cultured to ~80% confluence. Labeling with [^{35}S]methionine/cysteine protein labeling mixture (NEN) was performed for 24 h at the concentration of 100 μCi/ml. The total lipoprotein fraction was obtained by ultracentrifugation of the culture medium adjusted to the density >1.21 g/ml with KBr. Proteins in dialyzed samples were separated by SDS-PAGE and detected by autoradiography. Labeling with [^{3}H]glycerol was performed for 24 h at the concentration of 20 μCi/ml. The total lipoprotein fraction was obtained by ultracentrifugation of the culture medium adjusted to the density >1.21 g/ml with KBr. Dialyzed samples were fractionated by fast protein liquid chromatography with a Superose 6 column.

Northern blot analysis

Poly(A)$^+$ RNA from FAO and L35 cells were isolated by a modified guanidinium isothiocynate method according to Chomczynski (27). Seven and a half micrograms of the resulting mRNA were resolved by 0.8% formaldehyde/agarose gel electrophoresis, followed by transfer to a nylon membrane and hybridized with ^{32}P-labeled cDNA probes.

Transient transfection assay

On the day before transfection, 1.2×10^5 cells were plated in each well of a 12-well dish. Transfection by lipofection was performed under optimized conditions. Briefly, 650 ng DNA was mixed with 4 μl lipofectamine in 0.4 ml DMEM for 30 min before addition to cells. After 4 h, 0.4 ml DMEM containing 8% FBS and 8% ECS were added. Cells were switched to regular growth media the next day. Transfected cells were maintained for 48 h before lysis for reporter assays using the Dual Luciferase Kit (Promega). Transfection efficiencies were normalized by co-transfecting with pRL-CMV (Promega), a control vector containing a sea pansy (*Renilla reniformis*) luciferase gene driven by a CMV promoter in the ratio of 100:1. Results from triplicate transfection were presented as mean ± SD.

Lipid extraction and analysis

Cultured medium or total lipoprotein fraction was extracted with 5 v of chloroform-methanol (2:1, v/v) and 5 v of pure chloroform. The combined organic phase was washed with water to remove radioactive precursors and then dried under reduced pressure. Extracted lipids were dissolved in chloroform and resolved by TLC using silica gel plates and hexane-diethyl ether-acetic acid (85:15:1, v/v/v). The bands of interest were scraped into counting vials and the amount of radioactivity was determined by liquid scintillation counter.

Triglyceride secretion and MTP inhibitor studies

MTP inhibitor BMS-200150 is a generous gift from Dr. John Wetterau (Bristol-Myers/Squibb). L35 cells were grown to ~80% confluence in 60-mm dishes and labeled with 20 μCi [^{3}H]glycerol in the presence of 0.25 mM oleate for 2 h unless otherwise indicated. In the case of MTP inhibitor studies, cells were pretreated with 10 μM BMS-200150 (or DMSO in the control group) prior to switching to the labeling medium containing the MTP inhibitor (or DMSO in the control group). At the end of the incubation, media were collected for lipid extraction and analysis.

Statistics analysis

Results are given as mean ± SD. Statistical analysis was determined using Student's *t*-test. Values of $P \leq 0.05$ were considered to be significant.

RESULTS

The lack of apoB secretion is due to MTP gene inactivation

Unlike parental FAO cells, which have been shown to assemble and secrete apoB-containing lipoprotein particles (28), L35 cells secrete neither apoB-100 nor apoB-48 (**Fig. 1A**). This inability of L35 cells to secrete apoB-containing lipoproteins persisted even in the presence of 1 mM oleic acid (Fig. 1A). In contrast, oleic acid caused a 4-fold increase in the secretion of apoB-100 by FAO cells (Fig. 1A). The selective stimulation of apoB-100 secretion by oleic acid has previously been reported (29, 30). Adding oleic acid (1 mM) to the medium of L35 cells resulted in a 38.7-fold increase in the synthesis of [^{3}H]triglycerides (Fig. 1B). This oleic acid stimulation of [^{3}H]triglyceride synthesis displayed by L35 cells was similar to the 32.4-fold increase displayed by FAO cells (Fig. 1B). Also, L35 cells and FAO cells contain similar amounts of triglyceride (Fig. 1C). These combined data indicate that the inability of L35 cells to secrete apoB is not caused by a lack of triglyceride synthesis or availability.

While the relative abundance of apoB mRNA was similar in L35 cells and FAO cells, MTP mRNA was virtually absent in L35 cells, but highly expressed by FAO cells (**Fig. 2A**). These findings suggested that the lack of MTP might be responsible for the inability of L35 cells to secrete apoB. Based on findings indicating that alteration of a transacting factor that affects gene transcription is responsible for the phenotype of L35 cells (23, 24), we examined if the transcription of the MTP gene is reduced in L35 cells. For these studies we obtained a 612-bp promoter sequence from the human MTP promoter (31). This promoter element contains sequences that are conserved (i.e., present in the hamster MTP promoter) and has been shown to provide transcriptional regulation in regards to tissue specificity and hormone response (31).

Transient transfection studies showed that the transcriptional activity of MTP promoter in L35 cells was 10-

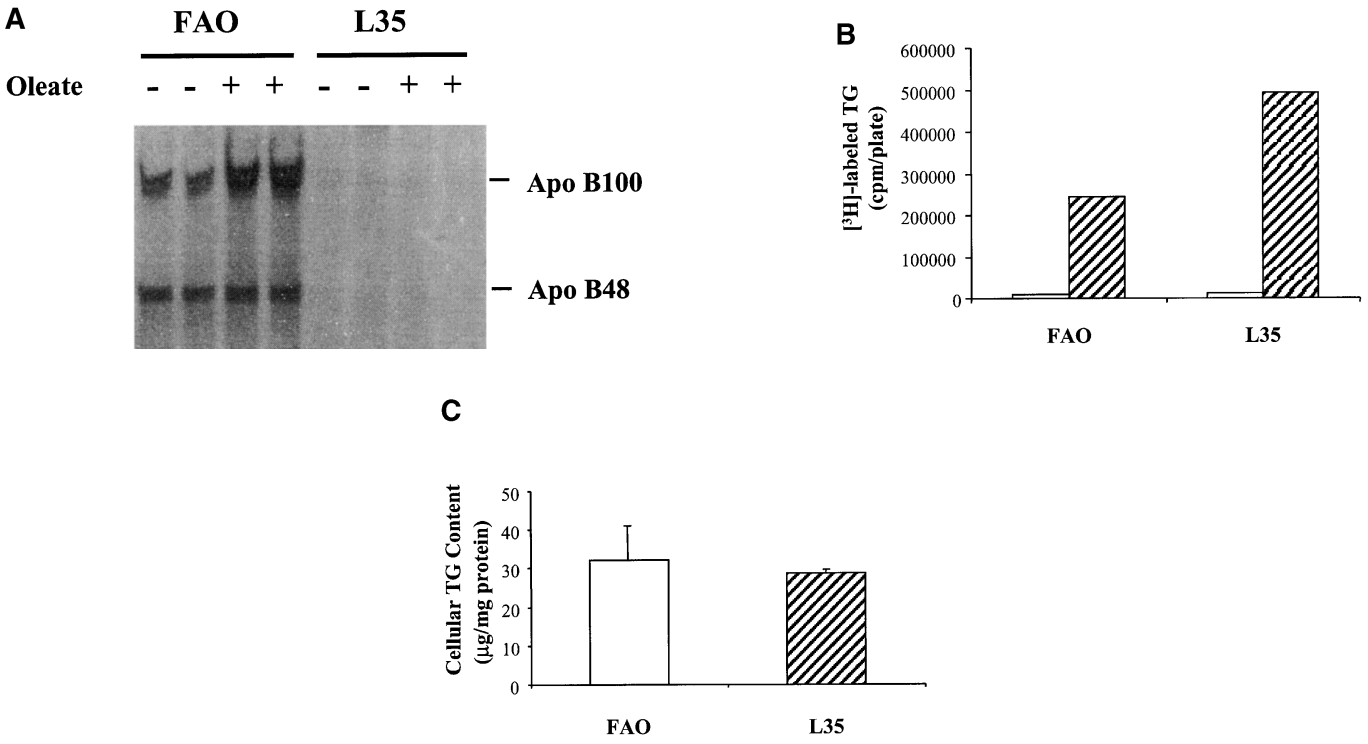

Fig. 1. A: Secretion of apolipoprotein (apo)B by L35 and FAO cells. L35 and FAO cells were cultured to ~80% confluence and labeled with [^{35}S]methionine (100 μCi/ml) overnight in the presence or absence of 1 mM oleate. The cultured medium was collected the following day and immunoprecipitated with polyclonal anti-apoB antibodies, followed by protein A-Sepharose. The immunoprecipitated complex was then resolved by SDS-PAGE and visualized by autoradiography. B: Induction of triglyceride synthesis by oleate in L35 and FAO cells. FAO and L35 cells were labeled with [^{3}H]glycerol (10 μCi/ml) for 2 h in the absence (open bars) or presence (hatched bars) of 1 mM oleate. Cells were washed three times with PBS before their lipids were extracted and resolved by TLC. Bands corresponding to triglyceride were scraped and counted by liquid scintillation counter. Results represent the mean from duplicate dishes of cells. C: Cellular triglyceride content in L35 and FAO cells. L35 and FAO cells were cultured to ~80% confluence in DMEM containing 4% ECS and 4% FBS. Cells were washed three times with PBS, resuspended, and sonicated in 1 ml PBS in the presence of protease inhibitors. Amount of triglyceride in the cell lysate was determined by enzymatic assay kit (Sigma, St. Louis, MO). Results are presented as mean ± SD (n = 4).

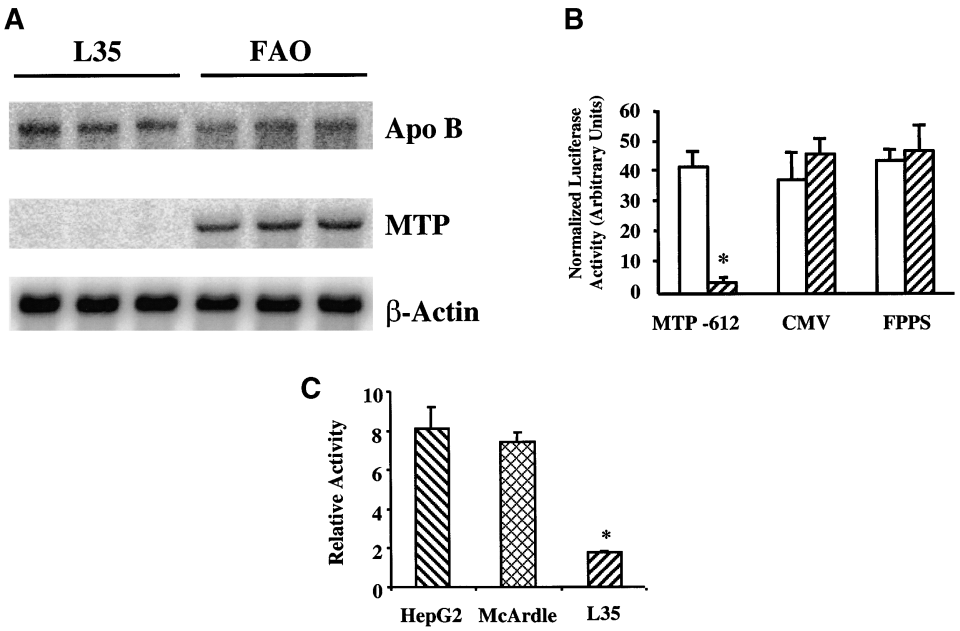

Fig. 2. A: Relative expression of apoB and microsomal triglyceride transfer protein (MTP) in L35 and FAO cells. Total RNA was isolated from L35 and FAO cells. 7.5 μg poly(A)$^+$ RNA was loaded on each lane and hybridized with the ^{32}P-labeled probe as indicated. B: Transcriptional activity of the 612-bp MTP promoter in L35 and FAO. Firefly luciferase constructs driven by the promoter indicated were co-transfected with pRL-CMV into FAO (open bars) and L35 (hatched bars) cells respectively. Cells were lysed and assayed for luciferase activity 48 h after transfection. Normalized luciferase are shown as mean ± SD (n = 3). *Denotes significant statistical difference, $P < 0.001$. C: Relative ability of hepatoma cells to transcribe a 612-bp MTP promoter luciferase reporter. Transient transfection assays were performed under optimized conditions in HepG2, McArdle RH-7777, and L35 cells respectively. Normalized promoter activity of the human 612-bp promoter was compared with that of the promoterless vector (pGL3-Basic). The strength of the human MTP promoter in each cell line is expressed as fold of increase above the background (i.e., pGL3-Basic). Results are presented as mean ± SD (n = 3).

fold less than in FAO cells (Fig. 2B). Since the expression of two other promoter luciferase reporters (CMV promoter and farnesyl pyrophosphate synthase promoter) displayed similar activities in both cell types, the diminished MTP promoter activity displayed by L35 cells is specific to the MTP promoter sequence and is not due to a general impairment in gene transcription. In addition, the relative activity of the 612 bp-MTP luciferase reporter in L35 cells was also reduced by ~80% when compared with its activity in two other hepatoma cells (HepG2 cells and McArdle RH-7777 cells) that express MTP and secrete apoB-containing lipoproteins (Fig. 2C). Thus, the relative ability of these four different hepatoma cell lines to transcribe the 612 bp-MTP luciferase reporter correlates with their expression of MTP. The combined data suggest that the lack of MTP expression by L35 cells is due to their relative inability to transcribe the MTP gene.

Complementation of L35 cells with a MTP transgene allows secretion of both apoB-100 and apoB-48

If the inability of L35 cells to secrete apoB-48 or apoB-100 is solely caused by a lack of MTP expression, expression of a MTP transgene in L35 cells should correct this defect and restore apoB secretion. To test this hypothesis, two stable L35 cell lines expressing MTP cDNA (one driven by CMV promoter, the other by the 612-bp human MTP promoter) were generated and their abilities to secrete apoB were determined. L35 cells transfected with the MTP plasmid driven by the CMV promoter expressed high levels of MTP protein (**Fig. 3A**) and the secretion of both apoB-100 and apoB-48 was restored (Fig. 3B). As predicted from the data of transient transfection assays (Figs. 2B and 2C), L35 cells transfected with the MTP plasmid driven by the 612 bp-human MTP promoter failed to express MTP protein (Fig. 3A) and hence did not secrete apoB-48 nor apoB-100 (Fig. 3B).

L35 cells secrete triglycerides in the absence of apoB and MTP

In spite of the absence of detectable MTP and the ability to secrete apoB-48 or apoB-100, L35 cells secreted triglycerides, albeit at a rate 25% of FAO cells (**Fig. 4A**). To substantiate the apparent MTP/apoB-independent secretion of triglycerides by L35 cells, we determined the effect of the MTP inhibitor (BMS-200150) (32) on the secretion of [^{3}H]triglycerides. Treatment of FAO cells with 10 μM BMS-200150 reduced the secretion of [^{3}H]triglyceride by 70% (Fig. 4B). In contrast, the MTP inhibitor had no effect on the secretion of [^{3}H]triglyceride by L35 cells (Fig. 4B). Thus, in the presence of the MTP inhibitor, both cell types secrete [^{3}H]triglyceride at a similar rate.

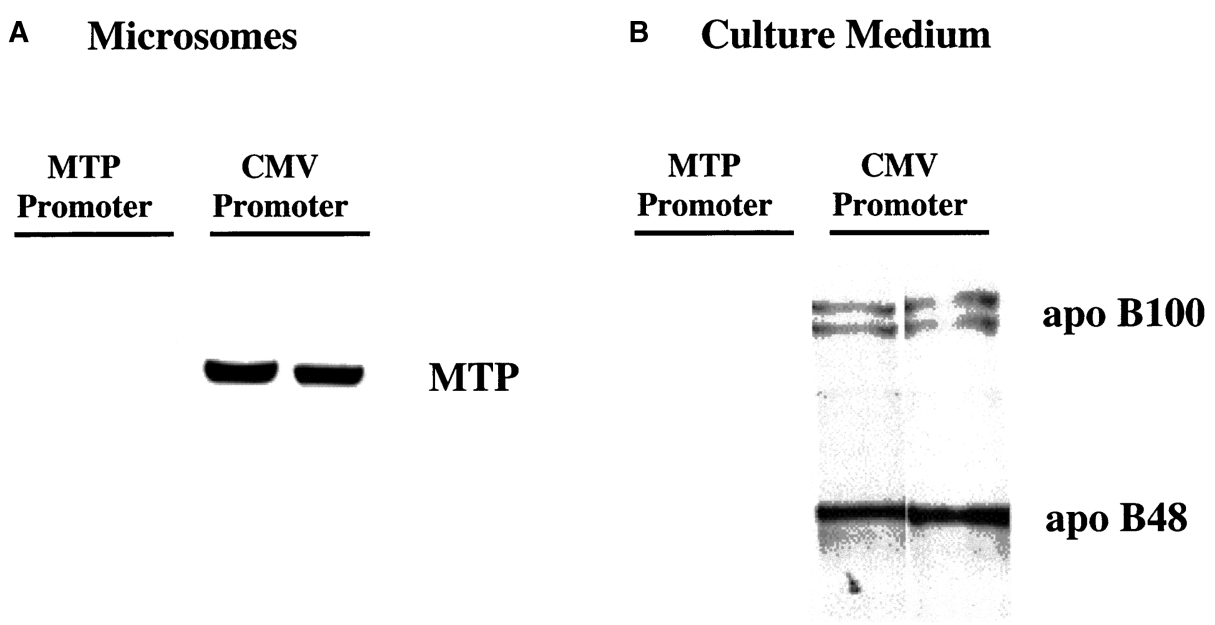

Fig. 3. Expression of MTP in L35 cells restores apoB secretion. L35 cells were transfected with a neomycin-resistance plasmid and a MTP expression plasmid driven either by a 612-bp human MTP promoter or by a CMV promoter. G-418 resistant colonies were picked and characterized for their ability to secrete apoB-100 and apoB-48. A: Microsomes were isolated from each cell type and were subjected to Western blotting using anti-MTP antibodies. B: Culture medium was obtained from each cell type and subjected to Western blotting using anti-apoB antibodies.

Triglyceride is secreted as HDL-sized particles

To further characterize the MTP/apoB independent secretion of triglycerides displayed by L35 cells, a total lipoprotein ($d < 1.21$ g/ml) fraction was isolated from culture media obtained from L35 cells incubated with [^{3}H]glycerol. Essentially, all the de novo synthesized ^{3}H-labeled phospholipids and triglycerides were found to be associated with lipoprotein particles having a size similar to mouse HDL (**Fig. 5**). These HDL particles lacked detectable apoB-48 and apoB-100, but contained apoA-IV and apoE (**Fig. 6**).

DISCUSSION

The data from this study indicate that there are at least two distinct pathways to assemble and secrete lipoproteins

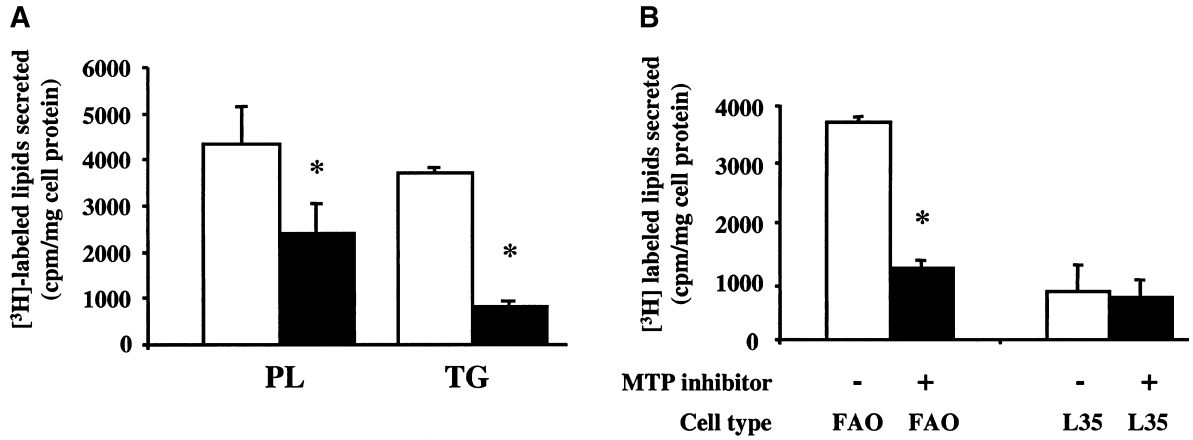

Fig. 4. Analysis of ^{3}H-labeled lipid secretion by L35 and FAO cells. A: Secretion of de novo ^{3}H-labeled glycerolipids by L35 and FAO cells. FAO (open bars) and L35 (filled bars) cells were labeled with [^{3}H]glycerol (10 μCi/ml) for 2 h in the presence of 0.25 mM oleate. Lipids in the media were extracted and resolved by TLC. Bands corresponding to triglyceride and phospholipids were scraped and counted by liquid scintillation counter. Results represent the mean ± SD of three separate dishes of cells. B: Effects of MTP inhibitor on the secretion of de novo ^{3}H-labeled glycerolipids. Cells were pre-treated with 10 μM BMS-200150 for 30 min prior to labeling. Control groups received equivalent amount of DMSO. Labeling with [^{3}H]glycerol (10 μCi/ml) was performed in the presence of the same amount of inhibitor or DMSO for 2 h in the presence of 0.25 mM oleate. Lipids in the media were extracted and resolved by TLC. Bands corresponding to triglyceride and phospholipids were scraped and counted by liquid scintillation counter. Results represent the mean ± SD of three separate dishes of cells. PL, Phospholipid; TG, triglyceride. *Denotes significant statistical difference, $P < 0.05$.

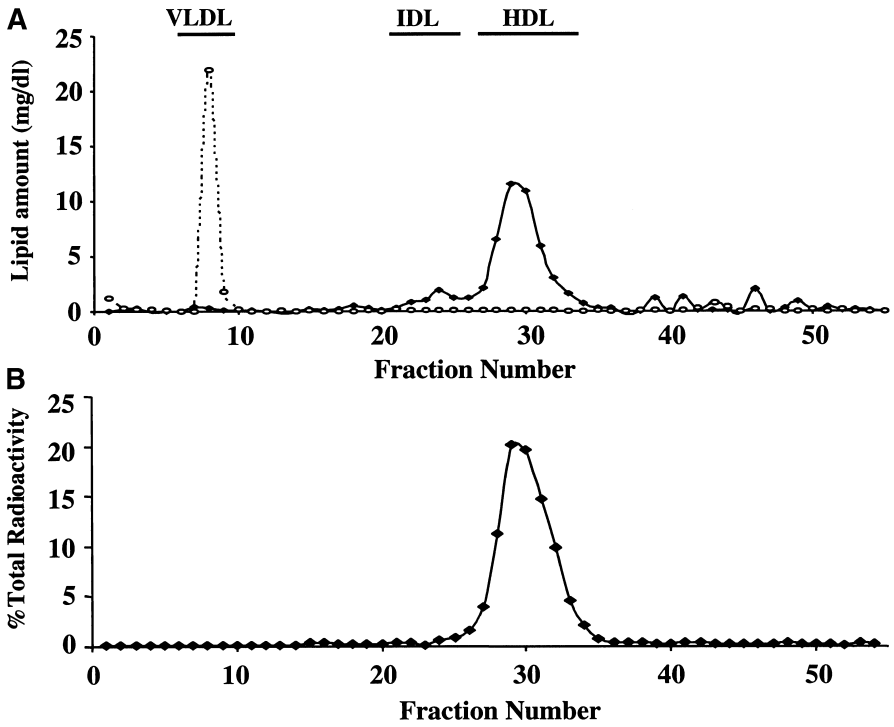

Fig. 5. Fast protein liquid chromatography (FPLC) separation of the de novo ^{3}H-labeled glycerolipids in the lipoproteins secreted by L35 cells. Aliquots of pooled plasma from fasted C57/BL6 mice were fractionated by FPLC (A). The amount of triglyceride (open circles) and cholesterol (filled diamonds) in each fraction was determined by enzyme assay kit (Sigma, St. Louis). The horizontal lines indicate the elution of the indicated lipoprotein fraction obtained from mouse plasma. Cultured medium from L35 cells was collected after labeling overnight with [^{3}H]glycerol (10 μCi/ml). Total lipoproteins ($d < 1.21$ g/ml) were prepared by ultracentrifugation, dialyzed, and fractionated by FPLC (B). Amount of [^{3}H]glycerolipids secreted by L35 cells was determined in each FPLC fraction and is expressed as the percentage of total tritium counts recovered.

containing triglycerides: *1*) an apoB/MTP-independent HDL pathway, and *2*) a MTP/apoB dependent VLDL pathway. The findings showing that L35 cells secreted triglyceride at 25% the levels displayed by FAO cells (Fig. 4A) suggest that the MTP/apoB-independent pathway has a reduced capacity to secrete triglycerides compared with the MTP/apoB-dependent pathway. We propose that MTP and apoB may have co-evolved to augment the capacity of the primordial MTP/apoB independent pathway by providing an efficient method to package triglycerides via enlarging the hydrophobic core of lipoproteins.

The requirement of MTP for the secretion of individual forms of apoB has been a subject of much study; however, due to differing results and conclusions reported by various research groups, this subject remains controversial and unresolved. There are several studies suggesting that the secretion of apoB-48 is less dependent on MTP than apoB-100 is (1, 2). The recent findings indicating that liver-specific MTP knockout mice still secrete apoB-48 suggest that the secretion of rodent (mouse) apoB-48 may not require MTP (22). Our findings show for the first time that rat hepatoma cells display an absolute requirement for MTP in order to secrete apoB-48, as well as apoB-100.

Metazoans produce lipoproteins as means to transport lipids among different cell types as well as to maintain lipid/cholesterol homeostasis. Many mammalian lipoprotein transport systems do not require apoB nor MTP. For example, nascent HDL particles are produced by most mammalian cells via an ABCA1/apoA-1-facilitated cholesterol efflux pathway (33–36). Chinese hamster ovary cells without any detectable MTP lipid transfer activity are also capable of secreting lipoprotein particles containing triglycerides and cholesteryl esters (37). Also, de novo synthesis of triglyceride-containing HDL by perfused rat livers has been described in detail (38), suggesting these lipoproteins may be the source of a subclass of triglyceride-containing HDL isolated from human plasma (39). The central nervous system also produces distinct types of lipoproteins via processes not requiring apoB or MTP; these lipoproteins play an important role in lipid and cholesterol homeostasis (40–42). Thus, there are a large number of variations in lipoprotein assembly/secretion pathways that utilize similar structural components of apolipoproteins (e.g., amphipathic α-helical apoproteins).

It is generally accepted that the assembly/secretion pathway of apoB-containing lipoproteins evolved from primordial pathways (43–50). Primitive lipoproteins used by insects, fish, birds, and other non-mammalian species (e.g., lipophorin) (45–47) do not require apoB or MTP. These lipoproteins have a hydrophobic core that is much smaller than that of VLDL. Since neither apoB nor MTP can function independently of each other, it is likely that

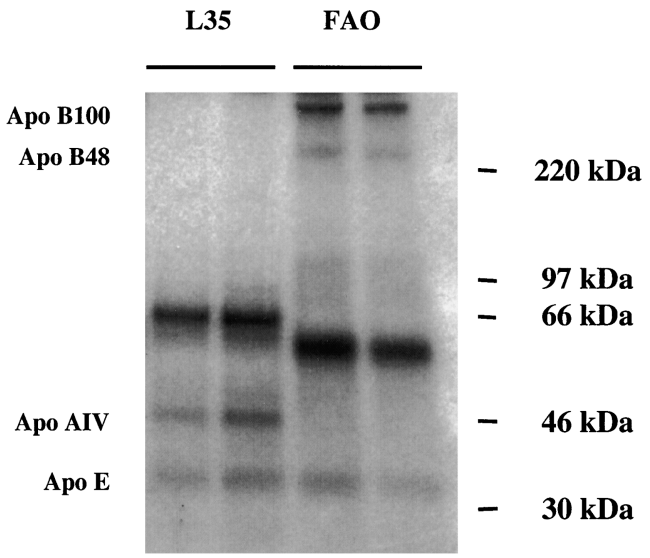

Fig. 6. Characterization of proteins in HDL secreted by L35 cells. L35 cells were labeled with [^{35}S]methionine overnight. The medium was harvested and total lipoproteins fraction (d < 1.21 g/ml) was isolated by ultracentrifugation. Dialyzed samples were then resolved by SDS-PAGE. Labeled protein bands were visualized by autoradiography. Designations of protein molecular weight standards are indicated on the left. Identities of radiolabeled apolipoproteins were confirmed by Western blotting analysis.

they co-evolved from gene products utilized in the assembly of primitive lipoproteins (e.g., vitellogenin) (43, 48). The evolution of the apoB/MTP lipoprotein assembly/secretion pathway markedly enhanced the capacity to export triglyceride. This is demonstrated by a dramatic increase in the triglyceride content of lipoproteins produced by the apoB/MTP pathway (i.e., VLDL) compared with lipoproteins produced by primitive pathway (i.e., vitellogenin) (49). Perhaps one of the most striking examples supporting the increased ability of the apoB/MTP pathway to package triglycerides into lipoproteins is an 8-fold increase in the secretion of triglycerides by insect cells caused by the expression of apoB and MTP (50).

In a similar complementary manner, the expression of apoB and MTP may enhance the secretion of triglyceride-containing lipoproteins that are initially formed replete of apoB. Early electron microscopy studies of liver suggested that immunoreactive apoB was present throughout the rough endoplasmic reticulum membrane unassociated with lipid (51). Surprisingly, apoB was not detected to be associated with the lipid particles present in the lumen of the smooth endoplasmic reticulum, whereas it was observed to be associated with the lipoprotein particles in the terminal junctions between the rough and smooth endoplasmic reticulum (51). These data were interpreted to indicate that: *1*) apoB is synthesized on the rough endoplasmic reticulum; *2*) the lipid core is produced independent of apoB; and *3*) apoB becomes associated with lipoprotein particles in the terminal junctions of the smooth endoplasmic reticulum (51). Subsequent studies have provided further evidence supporting the proposal that VLDL is assembled in at least two separate steps. The first step, inhibited by inactivation of MTP lipid transfer activity, involves the MTP facilitated formation of a HDL-sized particle in the endoplasmic reticulum (52–54). The second step requires ADP-ribosylation factor 1 and facilitates the apparent fusion of the nascent HDL sized particle with the "apoB-free core" (55). In the absence of the second step, apoB is secreted as a HDL particle. To our knowledge there have been no reports indicating that a core-containing lipoprotein lacking apoB, observed by immunodetection (51), is secreted in the absence of the second step. It is possible that the apoB/MTP-independent secretion of triglyceride secreted by L35 cells may represent this particle.

The authors thank Drs. Narayanan Hariharan and John Wetterau (Bristol-Myers-Squibb) for their generous gifts of MTP gene constructs and the MTP inhibitor. We also thank Monica Gaya and Bill Strauss for their technical assistance. This work was supported by National Institutes of Health grant HL-51648.

Manuscript received 31 October 2001 and in revised form 7 February 2002.

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