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Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability

(lipoprotein assembly/lipoprotein secretion/lipid transfer)

DAVID A. GORDON*†, HARIS JAMIL*, DARU SHARP*, DAVID MULLANEY*, ZEMIN YAO‡, RICHARD E. GREGG*, AND JOHN WETTERAU*

*Department of Metabolic Diseases, Bristol-Myers Squibb, Princeton, NJ 08543-4000; and ‡Lipid and Lipoprotein Research Group, Heritage Medical Research Center, University of Alberta, Edmonton, AB, Canada, T6G2S2

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ABSTRACT **To elucidate the role of the microsomal triglyceride transfer protein (MTP) in lipoprotein assembly, MTP and apolipoprotein B-53 (apoB 53; the N-terminal 53% of apoB) were expressed in HeLa cells. The results showed that apoB-53 could be expressed in HeLa cells with or without expression of MTP. In contrast, efficient secretion of apoB-53 required expression of MTP. Ultracentrifugal density flotation analysis showed that apoB-53 was secreted predominantly as a particle with the density of high density lipoprotein. An essentially identical apoB-53 particle density distribution was obtained after transient expression of apoB-53 in McArdle RH-7777 rat hepatoma cells. The mass of apoB-53 secreted was greater, and the flotation density was lower, from cells fed lipid, suggesting that apoB secretion in HeLa cells was regulated by lipid availability, similar to what has been described for lipoprotein-producing cell lines. These results indicate that MTP is necessary and sufficient to direct the regulated secretion of apoB-53 in HeLa cells.**

Very low density lipoproteins (VLDLs) are macromolecular complexes composed of a neutral lipid core [≈90% triglyceride (TG)] surrounded by a layer of phospholipid, free cholesterol, and the large (512 kDa) hydrophobic protein, apolipoprotein B (apoB). After secretion from the liver, VLDLs are converted in the circulation to low density lipoproteins (LDLs). While LDL receptor-mediated removal of LDL from the circulation is well understood (1), the details of VLDL assembly and secretion remain obscure. Identification of the specific molecular factors involved in VLDL assembly and secretion will provide a more fundamental understanding of this process.

While it is clear that assembly and secretion of VLDL particles require synthesis of apoB, phosphatidylcholine, and neutral lipids (2), recent evidence indicates that at least one other factor must also be involved. This evidence comes from expression of apoB in cultured cell lines. ApoB-53 (the N-terminal 53% of apoB-100) expressed in hepatocyte-derived cell lines was secreted as a lipoprotein particle (3), while apoB-53 expressed in non-lipoprotein-producing cells was not secreted but rather degraded intracellularly (3, 4).

The nature of the missing factor(s) in non-lipoprotein-producing cells remains unknown. A candidate for this missing factor is the microsomal TG transfer protein (MTP). MTP is a soluble microsomal protein that catalyzes the transfer of TG, cholesterol ester, and phophatidylcholine between membranes (5). Structurally, MTP is a heterodimer composed of the multifunctional enzyme protein disulfide isomerase (PDI; 58 kDa) and a unique 97-kDa large subunit (6). While PDI is a multifunctional ubiquitous protein (7), the large subunit of MTP has been found only in the liver and intestine (8). Studies have shown that the large subunit confers lipid transfer activity to the MTP complex (9).

Recent studies showing that a defect in the MTP is the proximal cause of abetalipoproteinemia indicate that this protein is required for assembly and secretion of apoB-containing lipoproteins (10, 11). Abetalipoproteinemic patients have only trace amounts of plasma apoB-containing lipoproteins, resulting in extremely low TG and cholesterol levels (12). The cause of this phenotype is a defect in the pathway responsible for assembly and secretion of apoB-containing lipoproteins since the apoB gene (13, 14) and lipid synthesis (12) have been shown to be normal. Thus, it is clear that MTP is required for the efficient assembly and secretion of apoB-containing lipoprotein particles.

Although studies of abetalipoproteinemic patients indicate that MTP is required for the production of plasma lipoproteins containing apoB, the role of MTP in this process remains unclear. Also, it is not known whether MTP is the only tissue-specific factor needed by hepatocytes and enterocytes to synthesize and secrete these particles. To address these issues, apoB-53 was expressed in either a non-lipoprotein-producing cell line (HeLa) or a derivative cell line stably expressing the large subunit of MTP and MTP activity. These cells were evaluated for their ability to secrete apoB-containing lipoproteins into the tissue culture medium.

MATERIALS AND METHODS

Cell Cultures, Plasmids, and Transfections. All tissue culture media, serum, and reagents for tissue culture were obtained from GIBCO. All other chemicals and reagents were from Sigma unless otherwise indicated. HeLa-229, McArdle RH-7777 rat hepatoma, and HepG2 human hepatoblastoma cells were obtained from the American Type Culture Collection and were maintained as recommended by the supplier under standard cell culture conditions (37°C with a 5% CO_2/95% air atmosphere). A 3.2-kb fragment containing the entire coding sequence of the large subunit of MTP, extending from nucleotide -64 to 3138 (11), was subcloned into plasmid pRC/neo (Invitrogen) to yield plasmid pRC/hMTP. Expression of the large subunit of MTP in this plasmid is under the control of the cytomegalovirus promoter. The apoB-53 expression plasmid, pB53, was constructed by inserting the cDNA coding for the N-terminal 53% of human apoB-100 between the *Eco*RI and *Hin*dIII sites on plasmid

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Abbreviations: apoB, apolipoprotein B; MTP, microsomal triglyceride transfer protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; AUC, area under the curve; PDI, protein disulfide isomerase; TG, triglyceride.

†To whom reprint requests should be addressed.

pCMV-5 (15). A cDNA encoding a secretable form of human placental alkaline phosphatase (16) was obtained from Kevin Felsenstein (Bristol-Myers Squibb). This was subcloned downstream of the cytomegalovirus promoter in plasmid pCMV-βGal (Clontech) after removal of the β-galactosidase coding sequences to yield plasmid pHPLAP-543. Alkaline phosphatase activity (1 unit of activity equals a change in A_{405} of 1.0 per min caused by the hydrolysis of *p*-nitrophenyl phosphate) was measured in cell culture medium heat treated for 20 min at 65°C to inactivate endogenous alkaline phosphatase activity as described (16). All transfections were carried out with the Lipofectin reagent (Bethesda Research Laboratories).

To stably express the large subunit of MTP, HeLa-229 cells were plated at 15% confluency in 100-mm dishes, and transfected 48 hr later with 50 μg of pRC/hMTP in the presence of 120 μl of Lipofectin reagent. After 20 hr the transfection mixture was removed and growth medium [Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum/nonessential amino acids] was added for an additional 24 hr before selection. Stable transfectants were selected for resistance to the cytotoxic neomycin analog Geneticin (1.5 mg/ml) (Bethesda Research Laboratories) for 2 wk, after which time the concentration was reduced to 1 mg/ml. A Geneticin-resistant cell line (HL-neo) without MTP was produced in the same manner after transfection with plasmid pRC/neo. Resistant colonies were isolated, grown for analysis, and maintained on medium containing 1.0 mg of Geneticin per ml.

Transient expression of apoB-53 was carried out by transfecting a total of 150 μg of DNA per 100-mm plate in the presence of 360 μl of lipofectin as described above. Twenty hours later, the transfection mixture was removed and lipid-rich medium (DMEM/3% fatty acid-free bovine serum albumin/0.8 mM sodium oleate/1 mM glycerol/0.05 mg of cholesterol per ml) or control medium (DMEM/3% fatty acid-free bovine serum albumin) was added. After an additional 24 hr, medium and cells were harvested for analysis. In cases in which <150 μg of the apoB-53 expression plasmid was tranfected, the difference was made up with plasmid pCMV-5. Mock-transfected cultures were treated with plasmid pCMV-5 only.

Protein Analyses. Geneticin-resistant cell lines were screened for the presence of the large subunit of MTP as follows. Confluent cultures in six-well plates were rinsed twice with phosphate-buffered saline. After addition of 0.25 ml of homogenization buffer (0.05 M Tris·HCl, pH 7.4/0.15 M NaCl/0.005 M EDTA/0.0625 M sucrose/0.5% Triton X-100/0.5% sodium deoxycholate/0.001 M phenylmethylsulfonyl fluoride/0.001 M benzamidine/50 mg of aprotinin per ml/0.1 mg of pepstatin per ml/0.1 mg of leupeptin per ml), cells were scraped from the dishes, homogenized for 20 sec at 20,000 rpm with a Polytron model PT-3000 (Brinkmann), and centrifuged at 10,000 × *g* to remove debris. Supernatants were mixed 1:1 with 2× SDS/PAGE sample buffer [0.125 M Tris·HCl, pH 6.8/4% SDS/2% 2-mercaptoethanol/20% (vol/vol) glycerol/0.01 mg of bromphenol blue per ml] and assayed for the presence of the large subunit of MTP by Western immunoblot analysis as described (10). Protein was quantitated on an LKB Ultroscan XL densitometer. Data are expressed as the integrated area under the curve (AUC) from the densitometer trace.

To measure MTP activity, cells were homogenized and treated as described (10). Lipid transfer activity was measured as the rate of [^{14}C]TG transfer from donor small unilamellar vesicles to acceptor small unilamellar vesicles. HLM-40 is the HeLa cell clone carrying the large subunit of MTP that was selected for further study.

Extracts from cells transiently transfected with the apoB-53 expression plasmid were prepared for analysis of intracellular apoB-53 expression as described above for MTP expression in Geneticin-resistant HeLa cell lines. In this case, the protein concentrations of 10,000 × *g* supernatants were measured by the Bradford assay (17) and 300 μg of total protein was analyzed on a SDS/polyacrylamide gel 5% by Western immunoblot as described (3) with the following changes: (*i*) a goat polyclonal antiserum to human apoB (Biodesign International, Kennebunkport, ME) was used at 1:500 dilution as the primary antiserum; and (*ii*) bands were visualized with a secondary antibody coupled to horseradish peroxidase and a colorimetric reaction. To analyze for apoB-53 secreted from HLM-40 cells, medium from 100-mm culture dishes was treated with Cab-O-Sil (Kodak) as described (18) except the eluates were dialyzed against a buffer containing 0.125 M Tris·HCl (pH 6.8) and 2% SDS prior to electrophoresis. Immunoblot analysis was performed as described above.

Lipoprotein Buoyant Density Analysis. Secreted apoB lipoprotein particles were isolated from 6 ml of medium from McArdle RH-7777 and HLM-40 cells in 100-mm culture dishes by a variation of the method of Arbeeny *et al.* (19). After harvesting, media were adjusted to 1 mM phenylmethylsulfonyl fluoride and centrifuged for 5 min at 1500 × *g* to remove loose cells and debris. Two milliliter aliquots of media were placed in polycarbonate ultracentrifuge tubes (8 × 30 mm), overlayered with 1 ml of 0.85% NaCl and spun in a TLA 100.3 rotor at 100,000 rpm in a Beckman TLA centrifuge at 16°C for 2 hr. The top 1 ml, the VLDL density fraction ($d < 1.006$ g/ml), was isolated with a tube slicer. The infranatant was adjusted to $d = 1.063$ g/ml by addition of solid KBr, brought to 3 ml with a $d = 1.063$ g/ml solution of KBr, centrifuged again under identical conditions, and the top 1 ml, the LDL density fraction ($d = 1.006$–1.063 g/ml), was isolated. To isolate the high density lipoprotein (HDL) ($d = 1.063$–1.21 g/ml) and $d > 1.21$ g/ml fractions, the infranatant was adjusted to a density of 1.21 g/ml and a vol of 3 ml by addition of solid KBr and a 1.21 g/ml solution of KBr, respectively. Samples were centrifuged as described above except the time was extended to 4 hr. The top 1 ml (HDL density fraction) and bottom 2 ml ($d > 1.21$ g/ml) were isolated. Fractions containing apoB were concentrated by the method of Vance *et al.* (18). ApoB-53 was analyzed by immunoblot as described above.

RESULTS

Characterization of a HeLa Cell Line Stably Expressing the Large Subunit of MTP. HeLa cells were chosen as the cell line for this study because the steady-state levels of PDI and lipid synthesis capabilities were found to be similar to those of the lipoprotein-producing HepG2 cell line (data not shown). In addition, preliminary transient transfection experiments showed that expression of the large subunit of MTP in HeLa cells led to a commensurate level of MTP activity. To obtain a stable HeLa cell line expressing MTP, cells were transfected with plasmid pRC/hMTP and selected for resistance to the neomycin analog Geneticin. Primary screening for the presence of the large subunit of MTP by Western immunoblot analysis identified several positive clonal cell lines. Of these, the cell line designated HLM-40 had the highest level of expression. To more precisely define the steady-state levels of MTP large subunit and activity, HLM-40 cells were further characterized. Fig. 1*A* shows that triglyceride transfer activity in HLM-40 cells was ≈1/4 the level found in HepG2 cells. These results are consistent with Western blot analyses of the same cell extracts that showed that HLM-40 cells contained ≈1/4 the amount of the large subunit of MTP measured in HepG2 cells (Fig. 1*B*). These results suggest that the large subunit of MTP is efficiently incorporated into an active complex with PDI in HeLa cells. Similar to wild-type

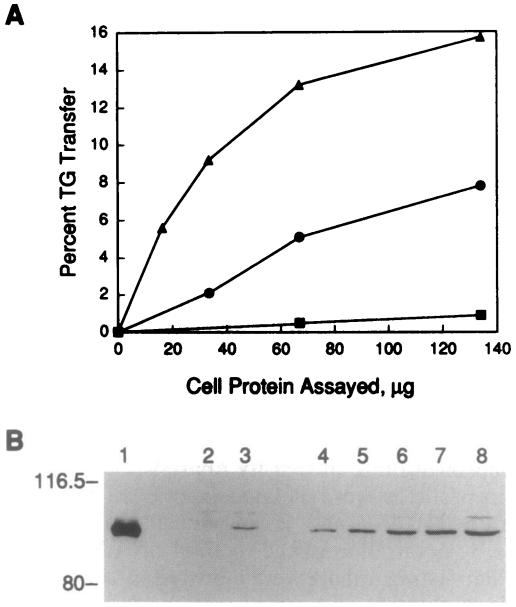

FIG. 1. Stable expression of MTP activity and protein in HeLa Cells. (*A*) MTP activity. Soluble protein extracts of HepG2 (▲), HLM-40 (●), and HeLa (■) cells were assayed for MTP-mediated transfer of [^{14}C]TG from donor to acceptor small unilamellar vesicles. Data are expressed as percentage radiolabeled TG transferred as a function of protein. (*B*) MTP protein. Aliquots of the same extracts assayed for MTP activity were analyzed by a Western immunoblot with a polyclonal antiserum against the large subunit of MTP. Lanes: 1, 1 μg of purified bovine MTP; 2 and 3, 100 μg of protein from HeLa and HLM-40 cell extracts, respectively; 4–8, 25, 33, 50, 75, and 100 μg of protein from HepG2 cell extracts.

HeLa cells, no MTP protein or activity was detected in HL-neo cells (data not shown).

ApoB-53 Is Produced Intracellularly in HeLa Cell Lines but Secreted Efficiently Only from HLM-40 Cells. A plasmid containing a cDNA encoding apoB-53 under the control of the cytomegalovirus promoter was transiently expressed at two different concentrations (15 and 150 μg per plate) in HL-neo cells and HLM-40 cells. Twenty hours after initiation of transfection, the cultures were fed medium containing oleic acid, cholesterol, glycerol, and bovine serum albumin to increase intracellular lipid synthesis. Cells and media were analyzed for the presence of apoB-53 by Western immunoblot analysis with a polyclonal antibody raised to human apoB-100. Fig. 2*A* shows that apoB-53 was detectable in cell extracts from both the HL-neo and HLM-40 cell lines. Densitometric estimation of the amount of apoB-53 visualized on the immunoblot showed that the HL-neo cells contained 1.7-fold (AUC = 0.092 vs. 0.054) and 3.3-fold (AUC = 0.561 vs. 0.172) more protein than the HLM-40 cells when transfected with 15 and 150 μg of pB53, respectively. This difference is most likely due to a higher efficiency of transfection in the former, since these cells produce 3- to 4-fold more alkaline phosphatase activity when transiently transfected with pHPLAP-543 under identical conditions (data not shown).

In contrast to the cell extracts, Fig. 2*B* shows that apoB-53 was not detected in the medium from HL-neo cells transfected with 15 μg of pB53 and only a trace was detected from cells transfected with 150 μg. However, substantial quantities of apoB-53 were clearly observed in the medium from HLM-40 cells transfected with both 15 and 150 μg of plasmid. In fact, the amount secreted by the HLM-40 cells transfected with 15 μg of plasmid was 3-fold greater than the amount secreted from the HL-neo cells that were transfected with 10-fold more plasmid (AUC = 0.06 vs. 0.019) even though the

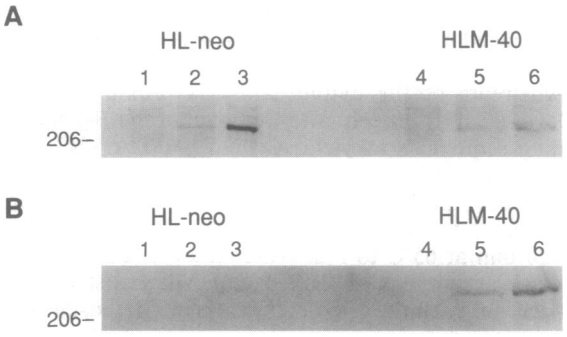

FIG. 2. Transient expression of apoB-53 in HL-neo and HLM-40 cells. (*A*) Cell extracts. Aliquots (300 μg of protein) of cell extracts from HL-neo or HLM-40 cells transfected with pCMV-5 (lanes 1 and 4), 15 μg of pB53 (lanes 2 and 5), or 150 μg of pB53 (lanes 3 and 6) were analyzed for apoB-53 by Western immunoblot that was probed with a goat anti-human apoB-100 polyclonal antiserum. The electrophoretic mobility of a 206-kDa size marker is shown on the left. (*B*) Culture medium. ApoB-53 was concentrated from cell culture medium and an aliquot corresponding to the entire volume of medium from each culture dish was analyzed by Western immunoblot. Lane assignments and size markers are as in *A*.

latter cells expressed >10-fold (AUC = 0.054 vs. 0.561) more protein intracellularly. Thus, the MTP-expressing cell line secreted apoB-53 at least 30 times more efficiently than the control.

HLM-40 Cells Secrete ApoB-53 as a HDL-Like Particle. The size of apoB-containing lipoprotein particles secreted in cell culture has been reported to vary depending on the length of the apoB polypeptide, the availability of lipid, and the type of cell or cell line used (3, 20, 21). Thus, it was of interest to determine the nature of the apoB-53 particles secreted from HLM-40 cells. For comparison, apoB-53 was also expressed in McArdle RH-7777 cells under identical conditions. Sequential buoyant density flotation analysis was carried out on cell culture media from HLM-40 and McArdle RH-7777 cells transfected with 150 μg of plasmid pB53. The media were fractionated into four density classes: VLDL (d < 1.006 g/ml), LDL (d = 1.006–1.063 g/ml), HDL (d = 1.063–1.210 g/ml), and the bottom fraction (d > 1.210 g/ml). Lipoproteins in each fraction were concentrated on fumed silica (Cab-O-Sil) and the protein components were extracted. Proteins were analyzed for apoB by Western immunoblot. The majority (90%) of the apoB-53 secreted from HLM-40 cells was observed in the HDL fraction (Fig. 3, lane 7). The remainder was present in the LDL fraction (lane 6). The pattern of distribution of native rat apoB-48 from the McArdle RH-7777 cells was similar to that previously reported (22); 70% was observed in the HDL fraction (lane 3), 22% was observed in

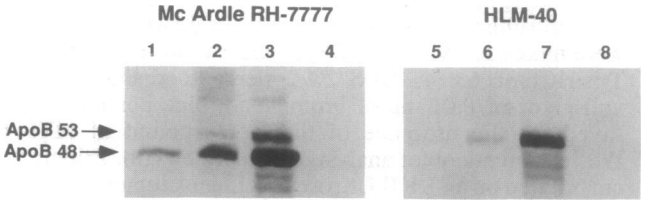

FIG. 3. Buoyant density analyses of lipoproteins secreted from McArdle RH-7777 and HLM-40 cells transfected with apoB-53. Conditioned medium from McArdle RH-7777 cells and HLM-40 cells, both transfected with apoB-53 under identical conditions, was separated into four density fractions by a sequential buoyant density ultracentrifugation technique. ApoB-containing lipoproteins were concentrated and analyzed by Western immunoblot that was probed with a goat anti-human apoB-100 polyclonal antiserum. Lanes: 1 and 5, VLDL fractions; 2 and 6, LDL fractions; 3 and 7, HDL fractions; 4 and 8, bottom fractions.

the LDL fraction (lane 2), and a small amount was observed in the VLDL fraction (lane 1). Transiently expressed human apoB-53 distributed similar to that secreted from HLM-40 cells; 90% was observed in the HDL fraction (lane 3), 10% was observed in the LDL fraction (lane 2), and only a trace was observed in the VLDL fraction (lane 1).

ApoB-53 Secretion from HLM-40 Cells Is Regulated by Lipid. A characteristic of apoB-containing lipoprotein production in cell culture is that apoB secretion increases when lipid is added to serum-free medium (2). The regulation of apoB secretion by lipid availability was investigated in HLM-40 cells. Cultures of HLM-40 cells were cotransfected with 150 μg of apoB-53 expression plasmid and 5 μg of human placental alkaline phosphatase plasmid for 20 hr in serum-free medium. Experimental cultures were then fed a lipid-rich medium containing 3% bovine serum albumin complexed with oleic acid (final concentration, 0.8 mM), cholesterol (50 μg/ml), and 1 mM glycerol for 24 hr, while control cultures were fed medium containing 3% fatty acid-free bovine serum albumin. The media were harvested and aliquots were immediately assayed for alkaline phosphatase activity. The remaining media were separated into VLDL + LDL, HDL, and bottom fractions by sequential buoyant density centrifugation, and apoB-53 was quantitated via Western immunoblot at two dilutions.

Two effects of lipid-supplemented media on apoB secretion by HLM-40 cells expressing apoB-53 were observed (Fig. 4). First, the total amount of apoB-53 secreted increased 2-fold. Second, proportionately increased amounts of apoB-53 were detected in the LDL fraction where only a trace was detected in the absence of lipid. The amount of alkaline phosphatase activity secreted into the medium did not change (without lipid, 9.3 milliunits/mg ± 1.5 SD; with lipid, 8.9 milliunits/mg ± 3.6 SD). Also, incubation of apoB-53-containing lipoprotein particles secreted from unstimulated HLM-40 cells with lipid-rich medium did not change the buoyant density profile (data not shown). This eliminates the possibility that the shift of apoB-53 toward the lower density upon feeding with lipid-rich medium was not due to direct transfer of lipid to high density particles after secretion into the medium.

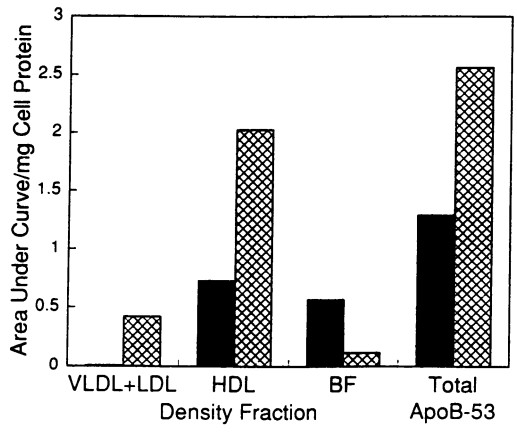

FIG. 4. Regulation of apoB-53 secretion by lipid availability in HLM-40 cells. HLM-40 cells were transfected with the apoB-53 expression plasmid and incubated in lipid-poor (solid bars) or lipid-rich (cross hatched lane) medium for 24 hr. Media were fractionated into VLDL + LDL, HDL, and bottom fractions (BF) and were analyzed for the content of apoB-53 by Western immunoblot analysis as in Fig. 3. ApoB-53 was quantitated by densitometry. Data shown are from a single experiment and are representative of two independent trials. Each sample was measured for apoB-53 at two different dilutions to ensure linearity. Data are expressed as integrated AUC from the densitometer trace normalized to total cell protein.

DISCUSSION

In this report, we describe the results of experiments designed to determine whether MTP is sufficient to reconstitute the assembly and secretion of apoB-containing lipoproteins in a nonhepatic, nonintestinal cell line. This study clearly shows that MTP activity expressed in HeLa cells is sufficient to reconstitute the efficient assembly of apoB-53 and lipid into a macromolecular lipoprotein particle that is secreted from the cell. Except for trace amounts of apoB-53 that were observed in medium from control cells in which apoB was highly overexpressed, lipoprotein secretion from HeLa cells required the expression of MTP. The ability of small amounts of apoB to escape the cells in this experiment may be similar in nature to the exceedingly low levels (<1.5% of normal) of apoB observed in the plasma of homozygous abetalipoproteinemic patients (23).

Similar to McArdle RH-7777 cells, apoB-53 lipoprotein particles produced by HLM-40 cells were predominantly of the HDL density class, although their buoyant densities varied somewhat depending on the availability of lipid. This reconstituted system also displayed upregulation of apoB-53 secretion after addition of lipid to the cell culture medium. Such upregulation of apoB secretion is characteristic of various lipoprotein-producing cell lines (20, 24, 25).

An important observation, with implications concerning the role of MTP in lipoprotein assembly, is that apoB-53 was secreted primarily as a HDL particle from both McArdle RH-7777 and HLM-40 cells. This result is consistent with the observations of Spring *et al.* (21) showing that transient expression of apoB-48 in HepG2 cells yields a particle with the density of HDL. Thus, transient expression of these truncated apoB polypeptides in HeLa-derived cells yielded the same lipoprotein particle density distribution generated by two hepatoma cell lines. This demonstrated that MTP is not only sufficient to direct secretion of apoB-containing lipoproteins in a non-lipoprotein-producing cell but is also sufficient to confer a differentiated hepatoma cell phenotype on the density distribution of the secreted lipoprotein particles.

While MTP does confer a hepatoma-like phenotype on non-lipoprotein-producing cells, it does not recreate a normal *in vivo* phenotype. Both the small intestine (26) and the liver (27) are clearly capable of secreting apoB-48 on very large, lipid-rich particles, while hepatoma cells are not. In the case of the HepG2 cell, this deficiency is even more pronounced since even full-length apoB-100 is secreted as a LDL density particle (28), while McArdle RH-7777 cells can secrete apoB-100 on VLDL density particles (22).

Several explanations for these observations have been considered. First, it is unlikely that this relates to the overall capacity of transformed cells to synthesize and store lipid. In the case of the HepG2 cell, Wang *et al.* (29) have shown that HepG2 cells grown in serum-containing medium actually have a higher content of TG and phospholipid than normal human liver. The cholesterol content was similar. Also, we have made the observation that lipid droplets accumulate in the cytoplasm of all three cell lines upon feeding precursors to lipid synthesis. What may be deficient is the ability of transformed cells to provide sufficient neutral lipid for export from the cell. This may be due to the increased requirements of growing cells for lipids to produce new membranes or for use as an energy source. In addition, these cells may have limiting amounts of other necessary metabolites for lipoprotein assembly. Indeed, increasing the concentration of glucose in the medium of HepG2 cells in the presence of oleate induces formation of a VLDL-like particle containing 3-fold more TG per molecule of apoB than with basal medium. In contrast addition of oleate alone simply increases the number of small apoB-containing particles secreted (29).

An alternative explanation could be that there is a structural deficiency that precludes delivery of sufficient neutral lipid to the site of lipoprotein maturation from cytoplasmic storage sites. For instance, Alexander *et al.* (30) have suggested that the majority of neutral lipid is added to nascent lipoproteins via coalescence of large lipid droplets present in the smooth endoplasmic reticulum (SER) with nascent apoB-containing phospholipid particles. HepG2 cells are clearly deficient in SER (28). While it is known that HeLa cells have SER (31), it is not known whether it has the necessary components to produce the lumenal neutral lipid droplets required to lipidate nascent small dense apoB-containing particles.

Finally, it is possible that transformed cells are deficient in a factor responsible for synthesis or delivery of additional neutral lipid to the site of lipoprotein maturation. Genetic support for this is provided by chylomicron retention disease (12) in which affected individuals are not capable of secreting TG-rich chylomicrons. While an additional "factor" involved in lipoprotein assembly may remain to be identified, our results make it likely that this factor is involved in particle maturation and not in the critical initial addition of lipid that directs apoB into the lipoprotein assembly and secretion pathway.

The posttranslational assembly and secretion of apolipoprotein B-containing lipoproteins is a process distinct from other secretory proteins in general. Studies have shown that a robust level of triglyceride, cholesterol, cholesterol ester, and/or phospholipid synthesis (2) is essential to this process in order to form a stable nascent apoB-containing lipoprotein particle in the endoplasmic reticulum, which otherwise would be degraded. These previous studies have clearly established the importance of lipid synthesis in lipoprotein assembly and defined many of the kinetic parameters of this pathway. The results presented in this report indicate that MTP mediates the addition of sufficient lipid to apoB to facilitate the assembly of this large polypeptide into a lipoprotein particle destined for secretion. That the particles produced in HLM-40 cells were predominantly of the HDL density class suggests that MTP participates at a very early, critical step in lipoprotein assembly. In addition, MTP may be involved in the bulk addition of neutral lipids to apoB-containing particles, but this was not apparent in this system. Additional experimentation will be required to determine whether this is the case.

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