Chinese Hamster Ovary Cells Require the Coexpression of Microsomal Triglyceride Transfer Protein and Cholesterol 7α-Hydroxylase for the Assembly and Secretion of Apolipoprotein B-containing Lipoproteins*

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Due to the absence of microsomal triglyceride transfer protein (MTP), Chinese hamster ovary (CHO) cells lack the ability to translocate apoB into the lumen of the endoplasmic reticulum, causing apoB to be rapidly degraded by an N-acetyl-leucyl-leucyl-norleucinal-inhibitable process. The goal of this study was to examine if expression of MTP, whose genetic deletion is responsible for the human recessive disorder abetalipoproteinemia, would recapitulate the lipoprotein assembly pathway in CHO cells. Unexpectedly, expression of MTP mRNA and protein in CHO cells did not allow apoB-containing lipoproteins to be assembled and secreted by CHO cells expressing apoB53. Although expression of MTP in cells allowed apoB to completely enter the endoplasmic reticulum, it was degraded by a proteolytic process that was inhibited by dithiothreitol (1 mM) and chloroquine (100 μM), but resistant to N-acetyl-leucyl-leucyl-norleucinal. In marked contrast, coexpression of the liver-specific gene product cholesterol 7α-hydroxylase with MTP resulted in levels of MTP lipid transfer activity that were similar to those in mouse liver and allowed intact apoB53 to be secreted as a lipoprotein particle. These data suggest that, although MTP-facilitated lipid transport is not required for apoB translocation, it is required for the secretion of apoB-containing lipoproteins. We propose that, in CHO cells, MTP plays two roles in the assembly and secretion of apoB-containing lipoproteins: 1) it acts as a chaperone that facilitates apoB53 translocation, and 2) its lipid transfer activity allows apoB-containing lipoproteins to be assembled and secreted. Our results suggest that the phenotype of the cell (e.g. expression of cholesterol 7α-hydroxylase by the liver) may profoundly influence the metabolic relationships determining how apoB is processed into lipoproteins and/or degraded.

The assembly and secretion of lipoproteins containing apoB

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The assembly and secretion of lipoproteins containing apoB occur in several tissues (liver, intestine, yolk sac, and heart) and are essential for the transport and delivery of fat during development and in the adult (reviewed in Refs. 1–4). There are at least three distinct human familial disorders associated with developmental and nutritional abnormalities caused by the inability of the liver and/or intestine to assemble and secrete apoB-containing lipoproteins (5). Hypobetalipoproteinemia is caused by mutations of the apoB gene leading to forms that are incapable of forming lipoprotein particles and/or to impaired synthesis of apoB by the liver and intestine (1, 6). The expression of human hypobetalipoproteinemic apoB genes in mice recapitulates many of the phenotypic abnormalities observed in human (7, 8). Abetalipoproteinemia is caused by mutations in the MTP1 gene (9–12). The absence of MTP lipid transfer activity in the lumen of the endoplasmic reticulum (ER) causes apoB to be degraded rather than secreted as a lipoprotein particle by the liver and intestine (12, 13). The recent demonstration that inactivation of a single allele of the MTP gene in mice causes significant impairment in the secretion of apoB-containing lipoproteins by the liver suggests that, in the mouse, MTP plays an essential role in one or more of the rate-limiting steps in the lipoprotein assembly/secretion pathway (14). In contrast to mice, heterozygous abetalipoproteinemic humans appear phenotypically normal (12, 13). These findings suggest that the role of MTP in regulating lipoprotein assembly/secretion may depend upon complex metabolic relationships that are defined by the genetic background of species and individuals. The third disorder (chylomicron retention disease) is unique in that it disables intestinal, but not hepatic, lipoprotein secretion (5). Although the gene defect responsible for chylomicron retention disease remains unknown, its recessive mode of inheritance (5) suggests that its phenotype is caused by a loss-of-function mutation.

Based on what is known about the chylomicron retention disease phenotype, we have considered the possibility that hepatic and intestinal lipoprotein assembly/secretion pathways may have distinct requirements. This interpretation is contrary to studies indicating that apoB and MTP are the only tissue-specific gene products required for reconstituting the lipoprotein assembly/secretion pathway in somatic cells. (In COS-1 cells (15, 16) and HeLa cells (17), which normally do not express MTP, plasmid-driven expression of apoB and MTP is sufficient to allow the secretion of apoB-containing lipopro-

1 The abbreviations used are: MTP, microsomal triglyceride transfer protein; ER, endoplasmic reticulum; CHO, Chinese hamster ovary; ALLN, N-acetyl-leucyl-leucyl-norleucinal; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol.
teins, albeit inefficiently.) We developed a somatic Chinese hamster ovary (CHO) cell model that reflects many of the characteristics that are thought to occur in the livers of abetalipoproteinemic patients. Like the livers of abetalipoproteinemics, CHO cells do not express MTP and display blocked translocation of apoB53 into the lumen of the endoplasmic reticulum (18). The incompletely translocated apoB53 is rapidly degraded by an ALLN-inhibitable process (18). Recent studies showed that the expression of liver-specific 7α-hydroxylase (cholesterol:NADPH:oxygen oxidoreductase (7α-hydroxylation), EC 1.14.13.17) profoundly influenced the processing of apoB53 by CHO cells (i.e. the ALLN-inhibitable ubiquitin-dependent proteasome degradation of apoB was blocked in these cells (designated as JF7 cells) (19)). Additional studies showed that the translocation-arrested apoB was N-linked and glycosylated (18) and assumed an orientation in which 69 kDa of the N terminus of apoB was in the lumen, whereas the C-terminal portion(s) was exposed to the cytoplasm (20). In the absence of ALLN, proteolysis caused the N terminus to be released from the ER membrane, to resume translocation, and to become secreted as an 85-kDa N-terminal peptide (20). Identiﬁed truncated N-terminal apoB peptides are enriched ~2000-fold in the plasma of abetalipoproteinemics, leading to a phenotype similar to that of the livers of abetalipoproteinemics (21).

In this study, we examined how complementation of CHO cells with MTP with or without liver-specific gene 7α-hydroxylase affected the cellular processing of apoB. The results provide new insights into how phenotypic gene expression can influence MTP function and the apoB-containing lipoprotein assembly/secretion pathway.

MATERIALS AND METHODS

Cell Culture—B53 cells were cultured in Dulbecco's modiﬁed Eagle's medium (Life Technologies, Inc.) containing 1.5% fetal bovine serum, 3.5% enriched calf serum, and antibiotics as described (18).

Transfections—B53 cells (18) were co-transfected with an expression plasmid encoding rat 7α-hydroxylase driven by the constitutive cyto-megalovirus promoter (22) and a plasmid conferring puromycin resistance to hygromycin (23) in a 12:1 ratio. Single cell clones of cells selected for resistance to 500 μg/ml hygromycin (i.e. JF7 cells) were found to express 7α-hydroxylase mRNA, protein, and enzyme activity that were comparable to those of rat liver (19). B53 cells were co-transfected with a pcDNA3 plasmid (Invitrogen) that was constructed to contain a cDNA encoding hamster MTP (a gift from David Gordon) and a plasmid conferring puromycin resistance in a 12:1 ratio. Cells were selected for resistance to puromycin (500 μg/ml). Three independent clones were grown out and shown to express MTP mRNA and protein and to process apoB as described by the cells designated as B53+ MTP cells. JF7 cells were co-transfected with the pcDNA3 plasmid encoding hamster MTP and a plasmid conferring puromycin resistance in a 12:1 ratio as described above. Cells were selected for resistance to puromycin (500 μg/ml). Three independent clones were grown out and shown to express MTP mRNA and protein and to process apoB as described by the cells designated as JF7+ MTP cells.

Western Blotting—Cells were grown to 85% conﬂuence and homogenized by subjecting them to 500 p.s.i. for 20 min at 4 °C and then rapidly releasing the pressure at atmosphere. The disrupted cell homogenate was centrifuged for 5 min at 1000 rpm to remove unlysed cells. The supernatant was then centrifuged for 20 min at 10,000 rpm in a Beckman J2–20 centrifuge using a JA-20 rotor. The supernatant from this spin was aliquoted into fresh tubes and centrifuged for 2 h at 45,000 g in a Beckman TL–45 rotor equipped with a Mircotubular supernatant removed, and the microsomal pellet was used for immunoprecipitation.

Immunoprecipitation—Cells were washed with cold phosphate-buffered saline. Cellular proteins were then solubilized in 1 ml of TET buffer (25 mM Tris, pH 7.5, 5 mM EDTA, 1% Triton X-100, and 250 mM NaCl) containing the following protease inhibitors: aprotinin (100 μg/ml), leupeptin (100 μg/ml), and phenylmethylsulfonyl fluoride (100 μM). Protein A-Sepharose beads were preincubated with unlabeled cell extract overnight at 4 °C. A rabbit antiseraum specific for human apoB (5 μl) was then incubated with 20 μl of protein A-Sepharose beads (dry volume) in 1 ml of TET buffer overnight at 4 °C. The antibody-bound protein A-Sepharose conjugates were incubated at 4 °C with the labeled cellular protein samples. Beads were recovered by centrifugation and washed three times in ice-cold TET buffer. The immunoprecipitates were then rinsed with phosphate-buffered saline, dissolved in 100 μl of sample buffer containing SDS and β-mercaptoethanol, and separated on a 1–20% gradient SDS-polyacrylamide gel.

Isolation of [35S]Methionine-labeled Lipoproteins—B53, JF7, B53+ MTP, and JF7+ MTP cells were cultured to 80% confluency and then cultured with oleic acid conjugated to albumin ([35S]Methionine (100 μCi/ml) for 24 h. The medium was harvested in the presence of the following proteolytic inhibitors: aprotinin (100 μg/ml), leupeptin (100 μg/ml), phenylmethylsulfonyl fluoride (100 μM), and EDTA (0.1%). The medium was then centrifuged for 5 min at 1000 rpm to remove any cells. The medium samples were adjusted to a density of 1.065 g/ml with KBr and subjected to ultracentrifugation in a Beckman L5–50 rotor using an SW 40 rotor at 39,000 rpm for 48 h at 5 °C. The lipoprotein fractions were then obtained by tube slicing and desalted using Sepharose CL-4B beads. Fractions containing radioactivity were separated via SDS-PAGE and analyzed by autoradiography.

MTP-facilitated Lipid Transfer Activity—The MTP lipid transfer assay was similar to the procedure established by Wetterau and Zilversmit (26) with minor modifications (14). Cells were grown to confluence in a 75-cm2 flask in a buffer containing 50 mM KCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4, and protease inhibitor mixture (Boehringer Mannheim). Samples were diluted to 1.75 mg/ml total cell protein, and deoxycholate was added to a ﬁnal concentration of 0.05%. After a 30-min incubation on ice, samples were centrifuged at 100,000 g for 1 h at 4 °C. The supernatant, containing the luminal contents (i.e. MTP), was pipetted off and dialyzed against sample buffer (50 mM Tris, pH 7.4, 40 mM NaCl, and 1 mM EDTA). Donor vesicles (40 nM egg phosphatidylcholine, 0.08 nM [14C]cholesterol, 0.08 nM [3H]triolein (NEN Life Science Products), and 3.0 mM cardiolipin) and acceptor vesicles (240 nM egg phosphatidylcholine and 0.48 nM triolein) were prepared in sample buffer. Aliquots of the cell extracts were added to the vesicle preparation and assayed for triglyceride transfer activity at 37 °C in sample buffer as described (14, 26). The reaction was terminated by adding DEAE-cellulose beads. After pelleting the DEAE-bound donor vesicles, triglyceride transfer was quantitated by measuring the radioactivity in the supernatant. Transfer activity was calculated as percentage triglyceride transferred per h and 100 μg of total cell protein assuming first-order kinetics (14, 26).

[14C]Acetate Labeling—Cells were incubated in serum-containing culture medium with [2-14C]acetate (5 μCi, specific activity of 47 mCi/ mol) for 1 h. Cells were harvested by scraping them off the dish with a rubber policeman and centrifuged at 400 x g for 10 min, and the pellet was used for extraction and protein assay. The culture medium was centrifuged at 400 x g for 10 min to remove cell debris. Cells and medium were extracted with chloroform/methanol and separated on silica gel TLC plates, and the silica gel containing the individual lipid classes was scraped into scintillation vials for radioactivity quantitation using β-scintillation counting (27).

Statistical Analysis—Results are given as means ± S.D. Statistical analysis was determined by Student’s t test. Values of p ≤ 0.05 were considered to be signiﬁcant.

RESULTS

Stable Expression of Cholesterol 7α-Hydroxylase and MTP in CHO Cells Affects the Intracellular Fate of ApoB53 in CHO Cells—Similar to previous results (18, 20), in the absence of the proteolytic inhibitor ALLN, CHO cells expressing apoB53 mRNA (B53 cells) contained little apoB53 (Fig. 1, lane 1). Adding ALLN caused intact apoB53 to accumulate in B53 cells
In JF7 cells (JF7 cells with the proteolytic inhibitor ALLN did not cause intact apoB53 to be degraded. Furthermore, treating these cells showed no detectable level of intact apoB53 (Fig. 1, lane 5), indicating that it was degraded. In contrast, B53 cells stably expressing MTP (B53 MTP cells) did not affect the intracellular content of apoB53 (Fig. 1, lane 2).

To investigate the effect of MTP on apoB processing in CHO cells, hamster MTP was expressed in B53 and JF7 cells. Unexpectedly, B53 cells stably expressing MTP (B53+MTP cells) showed no detectable level of intact apoB53 (Fig. 1, lane 5), suggesting that it was degraded. Furthermore, treating these cells with the proteolytic inhibitor ALLN did not cause intact apoB to accumulate (Fig. 1, lane 6). Stable expression of MTP in JF7 cells (JF7+MTP cells) did not affect the intracellular content of intact apoB53 either in the absence (Fig. 1, lane 7) or presence (lane 8) of ALLN. These results were found in three individual single clones of each type of stably expressing CHO cells (data not shown) and thus are not a unique characteristic of a single clone.

In CHO Cells, MTP Requires the Coexpression of 
7α-Hydroxylase to Secrete Intact ApoB53 as a Lipoprotein Particle—Similar to previous results (18, 20), either in the absence (Fig. 2, lane 1) or presence (lane 2) of ALLN, no intact apoB53 was secreted into the culture medium by B53 cells. Although there was a marked accumulation of intact apoB53 in JF7 cells (Fig. 1, lanes 3 and 4), no detectable apoB53 was secreted into the culture medium by JF7 cells treated with and without ALLN (Fig. 2, lanes 3 and 4).

B53 cells expressing MTP (B53+MTP cells) also did not secrete intact apoB53 either in the absence (Fig. 2, lane 5) or presence (lane 6) of ALLN. However, JF7 cells expressing MTP (JF7+MTP cells) did secrete intact apoB53 (Fig. 2, lane 7). ALLN did not affect the amount of intact apoB53 secreted by these cells (Fig. 2, lane 8).

Further analysis of the apoB53 secreted by JF7+MTP cells and incubated with oleic acid (1 mM) showed that it had a density <1.065 g/ml (Fig. 3). These data suggest that coexpression of 7α-hydroxylase and MTP allows CHO cells to assemble and secrete apoB-containing lipoproteins.

In CHO Cells, MTP-Facilitated Lipid Transfer Activity Requires 7α-Hydroxylase—In both groups of cells transfected with the MTP expression plasmid, the levels of both MTP mRNA (Fig. 4A) and protein (Fig. 4B) were similar. Thus, differences in the expression of MTP protein cannot account for the differences between B53+MTP and JF7+MTP cells in secreting apoB-containing lipoproteins. In CHO cells not transfected with the MTP expression plasmid, there were no detectable levels of MTP mRNA (Fig. 4A) or protein (Fig. 4B).

MTP-facilitated lipid transfer activity was essentially absent in extracts from B53, JF7, or B53+MTP cells (Fig. 5). In marked contrast, fairly high levels of MTP-facilitated lipid transfer activity were present in extracts of JF7+MTP cells (Fig. 5). The level of MTP lipid transfer activity expressed by JF7+MTP cells was similar to the activity observed in primary cultured mouse hepatocytes (Fig. 5). These findings were obtained with four separate preparations of cell extracts from
four individual plates of cells. Thus, there was a concordance in the ability of MTP to allow the secretion of apoB-containing lipoproteins and the enzymatic lipid transfer activity of cell extracts (compare results in Fig. 3 with the results in Fig. 5).

To examine the possibility that, in B53-MTP cells, the lack of MTP-facilitated lipid transfer activity was due to the presence of an inhibitor, we performed mixing experiments (Fig. 5). Adding extracts of B53-MTP cells to extracts from JF7-MTP cells caused the same “dilution” of activity as adding buffer alone (Fig. 5). These data indicate that the inability of B53-MTP cells to display MTP-facilitated lipid transfer activity is not likely to be caused by the presence of an inhibitor that can readily associate with or dissociate from MTP in the assay buffer.

In B53 Cells, Expression of MTP Allows ApoB to be Translocated into the ER and Subsequently Targeted to Degradation by a DTT- and Chloroquine-inhibitable Process—It is now established that ALLN inhibits the degradation of translocation-arrested apoB by blocking ubiquitin-dependent proteasome degradation (19, 28–31). ALLN also inhibits the degradation of apoB in the lumen of the ER by blocking ER-60 protease (32). The inability of ALLN to block the degradation of apoB53 in B53 cells expressing MTP (Fig. 1, lane 6) suggests that it is degraded in an intraluminal compartment in which apoB degradation is not dependent upon ALLN-inhibitable proteases. There are at least two additional proteolytic processes that have been reported to degrade apoB and that are resistant to inhibition by ALLN: 1) a DTT-inhibitable process that degrades apoB, which is completely translocated into the ER lumen (33); and 2) a chloroquine-inhibitable protease that resides in the lysosomal/endosomal compartment (34). To examine if the apoB53 expressed by B53-MTP cells was degraded by either of these proteolytic processes, these cells were labeled with [35S]methionine to detect intact apoB53 in the absence and presence of chloroquine (100 μM) and DTT (1 mM) for 4 h. Immunoprecipitation and autoradiography showed that there was an ~8-fold increase in the amount of intact apoB53 in the whole cell extract of cells treated with DTT and an ~20-fold increase in the amount of intact apoB53 in the whole cell extract of cells treated with chloroquine compared with untreated cells (Fig. 6A). Ultra-centrifugation of the whole cell extract followed by immunoprecipitation showed that most (>85%) of the intact apoB53 that accumulated in cells treated with chloroquine was in the 10,000 × g pellet (Fig. 6B). The amount of intact apoB53 that accumulated in the microsomal fraction (100,000 × g pellet) was ~5% of the amount in the 10,000 × g pellet. No detectable intact apoB53 was found in the cytoplasm (data not shown). These data suggest that, in B53-MTP cells, a significant portion of apoB53 enters the ER lumen of the secretory pathway and is transported to an acidic compartment (i.e. lysosomes and endosomes), where it is degraded.

Coexpression of 7α-Hydroxylase with MTP Increases the Rate of Synthesis of Lipoprotein Lipids—In McArdle rat hepatoma cells, plasmid-driven expression of 7α-hydroxylase increased the synthesis and secretion of all very low density lipoprotein lipids (22). We examined if 7α-hydroxylase may have affected lipid synthesis in CHO cells by measuring the incorporation of [14C]acetate into cellular and secreted phospholipids, triglycerides, cholesterol, and cholesterol esters (Fig. 7). In B53-MTP cells, the synthesis of phospholipids, triglycerides, cholesterol, and cholesterol esters was significantly less than that in JF7-MTP cells (Fig. 7A). The decrease in lipid biosynthesis displayed by B53-MTP cells was further demonstrated by a decrease in the secretion of these lipids (Fig. 7B).

**FIG. 5.** Analysis of microsomal triglyceride transfer protein-facilitated lipid transfer activity. A, B53, JF7, B53+MTP, and JF7+MTP cells and primary cultured mouse hepatocytes were harvested and analyzed for MTP lipid transfer activity using the procedure established by Wetterau and Zilversmit (26) with minor modifications (14). Values represent the means ± S.D. of three separate cell extracts in each group of cells. BSA represents the lipid transfer activity without the cell extracts (background). This experiment was repeated three additional times (using different plates of cells); the results were similar to those shown. Wild-type CHO-K1 cells displayed the same level of MTP lipid transfer activity as B53 cells. B, extracts from JF7+MTP cells were assayed for MTP lipid transfer activity in undiluted samples, in samples that were diluted 50% with buffer, and in samples that were diluted 50% with extracts from B53+MTP cells. Values represent the means ± S.D. of three separate cell extracts in each group of cells. This experiment was repeated three additional times (using different plates of cells); the results were similar to those shown. TC, triglyceride.

**FIG. 6.** Effect of DTT and chloroquine on the accumulation of apoB53 in B53 cells expressing MTP (A) and the accumulation of apoB53 in subcellular membrane fractions prepared from cells treated with chloroquine (B). A, B53-MTP cells were grown to 85% confluence. Cells were incubated with culture medium containing [35S]methionine (400 μCi/ml) with or without DTT (1 mM) or chloroquine (100 μM) for 4 h. Aliquots were immunoprecipitated, separated by SDS-PAGE, and developed using a PhosphorImager. The analysis of duplicate plates of cells is shown for each culturing condition. B, B53+MTP cells were grown to 85% confluence. Cells were incubated with culture medium containing [35S]methionine (400 μCi/ml) with or without chloroquine (100 μM) for 4 h. Cells were scraped off the culture dishes, disrupted by nitrogen cavitation, and separated into membrane fractions by ultracentrifugation (49). Equal aliquots of each fraction were immunoprecipitated, separated by SDS-PAGE, and developed using a PhosphorImager.

**DISCUSSION**

Our results show for the first time that, in CHO cells, MTP plays two distinct roles in the cellular processing of apoB: 1) facilitating translocation across the ER and 2) providing lipid transfer for the assembly and secretion of apoB-containing lipoprotein particles. Although both functions are required to assemble and secrete apoB-containing lipoprotein particles, they do not occur in a concerted manner in CHO cells expressing MTP protein (i.e., without 7α-hydroxylase, translocation occurs, whereas lipid transfer does not). Based on these unique findings, we propose that MTP functions as an intraluminal “chaperone” to facilitate apoB translocation and as a lipid transfer protein in the assembly of apoB-containing lipoproteins.
the cellular content of intact apoB53 (Fig. 1). Since, in the absence of MTP expression in B53 cells, ALLN blocks the degradation of translocation-arrested apoB53 (20), these findings suggest that MTP removed apoB53 from this first step in the secretory pathway. Second, in B53 cells expressing MTP without 7α-hydroxylase, the lysosomal inhibitor chloroquine blocks the degradation of apoB53 (Fig. 6). As a result, apoB53 accumulates in the 10,000 × g fraction, suggesting that at least a portion of the apoB53 that has entered the ER lumen can be transported to a compartment containing acidic (lysosomal) proteases. In marked contrast, essentially all of the intact apoB53 that accumulates in ALLN-treated B53 cells not expressing MTP is in the microsomal membrane fraction (i.e. 100,000 × g fraction) in a form that is both glycosylated and susceptible to trypsin digestion, suggesting that it has a transmembrane, translocation-arrested orientation in the ER and cannot move further through the secretory pathway (18, 20). The simplest interpretation of these combined data is that expressing MTP in B53 cells allows intact apoB to be completely translocated across the ER membrane and to enter the secretory pathway. However, in the absence of 7α-hydroxylase expression, B53 cells expressing MTP do not display detectable MTP-facilitated transfer activity and lipoprotein assembly, and secretion remains blocked. In marked contrast, JF7 cells expressing MTP both display MTP-facilitated lipid transfer activity and secrete apoB-containing lipoproteins. These combined data indicate that, in CHO cells, MTP-facilitated translocation and MTP-facilitated lipid transfer are separable functions and that both are required for the efficient assembly and secretion of apoB-containing lipoproteins.

Our conclusions that MTP functions to facilitate both apoB translocation and the addition of lipid to the nascent lipoprotein particle are consistent with results obtained from studies using chemical inhibitors of MTP lipid transfer. Chemical inhibition of MTP lipid transfer activity has been reported to impair the assembly and secretion of apoB-containing lipoproteins by blocking an early event in the secretory pathway (30, 35–38). Additional pulse-chase studies in HepG2 cells suggested that chemical inhibition of MTP lipid transfer blocks the translocation of apoB100, causing it to be cotranslationally degraded by a process attributed to the proteasome (30). These conclusions were further supported by studies showing that inhibition of MTP results in translocation arrest and rapid cotranslational degradation of apoB100 by a ubiquitin-dependent proteasome process (39). In addition to blocking apoB translocation, inhibition of MTP lipid transfer also impairs a later process in the secretory pathway (i.e. the addition of lipid to form a more fully lipilated lipoprotein particle). In separate studies using McArdle rat hepatoma cells, chemical inhibition of MTP lipid transfer activity was shown to block the oleic acid stimulation of apoB48 secretion (40) and the formation and secretion of very low density lipoprotein containing apoB100 (41).

The combined data suggest that MTP may act as a multifunctional chaperone via both lipid transfer-independent and -dependent processes. MTP lipid transfer-independent processes, which occur in B53 + MTP cells, may facilitate the folding and cotranslational disulfide formation of apoB (16, 42, 43). MTP lipid transfer-dependent processes may also facilitate apoB folding in a manner that may be coupled to lipoprotein particle formation (40, 41, 44).

Previous studies showed that transfecting HeLa and COS cells with MTP expression plasmids, similar to one we used in our studies, results in MTP lipid transfer activity and the assembly and secretion of apoB-containing lipoproteins (15, 17). In other studies using mammary-derived mouse cells (C127), which do not express MTP, apoB-containing lipoproteins were assembled and secreted (45). In contrast, our findings indicate that CHO cells require both MTP and 7α-hydroxylase to display MTP lipid transfer activity and the assembly and secretion of apoB53-containing lipoprotein particles. These combined results suggest that factors present in each cell type and experimental system profoundly influence the requirements for the translocation and processing of apoB and lipid components into lipoprotein particles.

There are sufficient data to indicate that 7α-hydroxylase significantly influences several processes that, depending upon the cell phenotype, may be rate-limiting in the lipoprotein assembly/secretion pathway. In McArdle rat hepatoma cells, expression of 7α-hydroxylase increases the cellular content of mature SREBP1 and the expression of MTP mRNA and protein (22). As a result, McArdle rat hepatoma cells expressing 7α-hydroxylase become resistant to oleic acid stimulation of apoB100 secretion (22). In CHO cells, the coexpression of 7α-hydroxylase with MTP is required for MTP-facilitated lipid transfer activity (Fig. 5). Expression of 7α-hydroxylase in CHO cells also changes many other phenotypic characteristics: it increases the synthesis and secretion of all very low density lipoprotein lipids by CHO cells (Fig. 7), and it blocks ubiquitin conjugation and subsequent proteasome degradation of translocation-arrested apoB53 (19). Phenotypic metabolic relationships that determine the process(es) that may be rate-limiting for lipoprotein assembly/secretion are likely to include the rate of apoB synthesis, the rate of lipogenesis, the expression of MTP protein and lipid transfer activity, and the activity of apoB-degradative processes. With the proviso that our results obtained studying the cellular processing of apoB53 in CHO cells may reflect similar processes that occur in the liver expressing apoB100 and the intestine expressing apoB48, our findings may provide a new insight into explaining why intestinal and hepatic processing of apoB may have different constraints. One of the most provocative differences is chylomicron retention disease, which blocks the secretion of apoB-containing lipoproteins by the intestine, but not the liver (5). Affected patients produce immunoreactive apoB48 in enterocytes, process apoB mRNA to the intestinal (edited) form, and express MTP (46–48). These data suggest that the inability of the intestines of chylomicron retention disease patients to secrete apoB-containing lipoproteins is due to the functional loss of a gene product other than apoB or MTP. Our findings showing that the liver-specific enzyme 7α-hydroxylase is required for CHO cells expressing apoB and MTP to secrete apoB-containing lipoproteins support the possibility that expression of 7α-
hydroxylase in the livers of chylomicron retention disease patients may complement a defective process that is common to both intestinal and hepatic lipoprotein assembly/secrecion pathways.

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