Lipoprotein assembly capacity of the mammary tumor-derived cell line C127 is due to the expression of functional microsomal triglyceride transfer protein

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Abstract C127, a murine mammary tumor-derived cell line, is capable of lipidating and secreting apolipoprotein B-41 (apoB-41) in the apparent absence of microsomal triglyceride transfer protein (MTP). Using a semiquantitative reverse transcriptase-coupled polymerase chain reaction, mouse MTP mRNA was detected in C127 cells at ~10–20% of the relative abundance of human MTP in HepG2 cells. Radiolabeling of C127 cells with [^{35}S]methionine and [^{35}S]-cysteine followed by immunoprecipitation with anti-MTP antibodies identified a band with an electrophoretic mobility identical to that of authentic mouse MTP. Cotransfection of apoB-41 and the MTP 97-kDa subunit in C127 cells enhanced apoB secretion by ~5-fold relative to apoB-41 transfection alone, suggesting that MTP is limiting in these cells. To establish that MTP expression is responsible for apoB-containing lipoprotein assembly in C127 cells, the effects of the MTP inhibitor BMS-200150 were examined. Secretion of apoB-41 by C127 cells was inhibited to the same extent observed in COS-1 cells cotransfected with apoB-41 and MTP. These results suggest that low MTP expression, and not the expression or overexpression of another known or novel factor(s), is responsible for apoB assembly and secretion in C127 cells and further supports the essential nature of MTP in the biogenesis of apoB-containing lipoproteins.—Sellers, J. A., and G. S. Shelness. **Lipoprotein assembly capacity of the mammary tumor-derived cell line C127 is due to the expression of functional microsomal triglyceride transfer protein.** ***J. Lipid Res.*** **2001.** 42: **1897–1904.**

Supplementary key words endoplasmic reticulum • MTP inhibitors • protein disulfide isomerase • protein secretion • very low density lipoprotein

The assembly of apolipoprotein B (apoB) with lipids to form hepatic triglyceride-rich VLDL requires the dedicated cofactor microsomal triglyceride transfer protein (MTP) (1). MTP is composed of a 97-kDa unique subunit complexed with the ubiquitous endoplasmic reticulum (ER)-localized folding enzyme protein disulfide isomerase (2). Although the complete dimensions of its functional roles in VLDL formation are not fully understood, MTP may transfer lipids to apoB during its translation and translocation into the ER and may participate in the post-translational enlargement of nascent VLDL particles (3–7). As with apoB, the MTP 97-kDa subunit is thought to be expressed primarily in lipoprotein-producing tissues, namely, liver, intestine, and heart (8–10). The essential nature of MTP in VLDL formation is observed in the human autosomal recessive disorder abetalipoproteinemia, in which MTP gene mutations virtually abolish apoB-containing lipoprotein assembly and secretion (8, 10).

In addition to apoB and MTP, evidence also exists for the presence of other tissue-specific factor(s) that play a role in the intracellular processing of apoB-containing lipoproteins. In the human genetic disorder Anderson's or chylomicron retention disease, intestinal apoB-48-containing lipoproteins are retained within the enterocytes in the face of an apparently intact hepatic VLDL assembly and secretion pathway (11). This disorder is unlinked to the genes for apoB, MTP, and several other candidate genes involved in lipid and lipoprotein metabolism (12, 13). Further evidence of the existence of an additional factor required for apoB assembly has come from studies performed in the murine mammary tumor-derived cell line C127. These cells were reported to have no detectable MTP protein or neutral lipid transfer activity and yet were capable of assembling apoB-41 into lipoprotein particles containing a triglyceride-rich core (14, 15). In other such evidence, liver-specific disruption of the gene for MTP in mice led to an almost complete loss of hepatic apoB-100 secretion; however, apoB-48 secretion was only partially inhibited (4, 16). Finally, studies utilizing selective inhibitors of MTP also support the notion that MTP may not be essential for the assembly of apoB-48 and smaller recombinant forms of apoB (17–19).

To assess the basis for the assembly and secretion of

Abbreviations: apoB, apolipoprotein B; ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein.

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apoB-containing lipoproteins in a cell line that apparently lacks MTP, with the ultimate goal of identifying the factor(s) responsible, we characterized apoB-41 processing in C127 cells. Using methodologies more sensitive than those employed previously, we demonstrated that C127 cells express sufficient MTP to support apoB-41 assembly and secretion. These results suggest that, while smaller forms of apoB may display a lower requirement for MTP, MTP is an essential cofactor required for all forms of intracellular apoB-containing lipoprotein precursor formation.

EXPERIMENTAL PROCEDURES

Cell culture

Except where indicated, COS-1, C127, and HeLa cell lines were grown in DMEM containing high glucose (4.5 g/l), 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml). HepG2 cells were cultured in MEM containing 10% FBS and antibiotics. Cells were maintained at 37°C in an atmosphere containing 5% CO_2.

Plasmid construction

A mouse MTP cDNA clone in pBluescript (Stratagene, La Jolla, CA) was obtained from L. Chan (Baylor College of Medicine, Houston, TX). The cDNA insert was excised from the pBluescript vector with *Not*I (5′ flanking site) and *Apa*I (internal site and 3′ flanking site). The two MTP fragments were gel purified and ligated to *Not*I- and *Apa*I-digested pcDNA3 (Invitrogen, San Diego, CA). ApoB-6.6F, which contains the native apoB signal peptide and the first 300 amino acids of the mature protein, followed by a C-terminal FLAG epitope (DYKDDDDK) (20), was produced by PCR with apoB-28F as a template. The 5′ sense oligonucleotide primer was positioned in the vector upstream of the insert. The 3′ antisense primer contained sequences that hybridized to DNA corresponding to amino acids 291–300 of apoB followed by the 8-amino acid FLAG epitope, a termination codon, and an *Xba*I restriction site. The PCR product was cloned into the *Eco*RI (5′) and *Xba*I (3′) sites of expression plasmid pCMV5 (21). Correct sequence and orientation of the MTP and apoB-6.6F plasmids was verified by automated DNA sequencing. ApoB-41F was constructed by digesting apoB-50F (22) with *Cla*I (internal site corresponding to amino acid 1881 of mature apoB) and *Kpn*I (3′ flanking site). A double-stranded oligonucleotide containing the eight-amino acid FLAG epitope and a termination codon was phosphorylated and ligated into *Kpn*I- and *Cla*I-digested apoB-50F to form apoB-41F. Sequence analysis revealed an intact apoB-41-coding region and C-terminal FLAG epitope. However, the engineered termination codon was lost, adding an additional six amino acids (YRHAYR) downstream of the FLAG sequence.

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted with Trizol (Life Technologies, Rockville, MD) according to the manufacturer protocol. Where applicable, RNA samples were diluted with yeast tRNA to maintain identical total concentrations of RNA in all samples. RT-PCR was performed with the Access RT-PCR system (Promega, Madison, WI) according to the manufacturer protocol. Assays were performed in 50-μl reactions containing 1 mM $MgSO_4$, a 0.2 mM concentration of each dNTP, 1 μg of each oligonucleotide primer, and 1 μg of input RNA. The sequence of the sense strand primer positioned in exon 9 is 5′-GGACTTTTTGGA TTTCAAAAGTGAC-3′. The sequence of the antisense primer positioned in exon 13 is 5′-GGAGAAACGGTCATAATTGTG-3′. The reverse transcription reaction was carried out at 48°C for 45 min followed by 94°C for 2 min to heat inactivate the enzyme. The cDNA was then amplified, using 35 cycles of 94°C for 40 sec, 53°C for 1 min, and 68°C for 2 min. After the last cycle the samples were incubated at 68°C for an 8-min final extension and then held at 4°C. Ten microliters of each reaction was electrophoresed on a 0.7% agarose gel containing ethidium bromide (0.5 μg/ml). Amplification of cyclophilin mRNA was performed as described above with the cyclophilin-specific primers CYCF and CYCR (23). For sequencing of the RT-PCRs, two 50-μl PCRs were combined, gel purified, and subjected to automated DNA sequence analysis. The African green monkey MTP sequence was obtained by PCR amplification of African green monkey kidney total RNA under the conditions described above.

Cell transfection, metabolic radiolabeling, and immunoprecipitation

COS-1 and C127 cells were transfected at 50–60% confluence by the FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN) method, using a 2:1 (volume:mass) ratio of FuGENE 6 to DNA. For coexpression, cells were transfected with apoB-41F or apoB-28F (24) and an equal mass of human MTP 97-kDa subunit (+MTP) or truncated human placental alkaline phosphatase (−MTP). Cells were incubated with transfection mixture for 24 h and then radiolabeled with $[^{35}S]$Met/Cys (100 μCi/ml; NEN Life Sciences Products, Boston, MA) in Met/Cys-deficient DMEM. After labeling, cells were washed with PBS and scraped. Cell lysis and immunoprecipitation of cell and medium samples with anti-FLAG M2 monoclonal antibody (Sigma-Aldrich, St. Louis, MO) or anti-human apoB polyclonal antibodies (Roche Molecular Biochemicals) was performed as described (22, 25).

For detection of MTP by immunoprecipitation, C127 or HepG2 cells were metabolically radiolabeled with $[^{35}S]$Met/Cys (100 μCi/ml) for 48 h, in a 9:1 mixture of serum-free Met/Cys-deficient medium and complete medium containing 10% FBS. Twenty-four hours posttransfection, COS-1 cells were radiolabeled for 24 h under the same conditions. Immunoprecipitation of MTP with anti-human or bovine MTP serum was performed as described above, with the exception that cell lysates were precleared by addition of a 10-μl bed volume of protein G-Sepharose. After incubation with inversion for 4 h, the protein G-Sepharose beads were removed by centrifugation and supernatants were transferred to fresh tubes for immunoprecipitation.

Effect of MTP inhibitor on apoB secretion

The MTP inhibitor BMS-200150 (Bristol-Myers Squibb, Princeton, NJ) was dissolved in dimethyl sulfoxide at a concentration of 10 mM. Inhibitor was added to cells at the indicated concentrations such that the final dimethyl sulfoxide concentration in all dishes was 0.5%. For inhibition experiments, COS-1 cells in six-well dishes were transfected with the 97-kDa subunit of human MTP and apoB-41F or apoB-6.6F. C127 cells, also in six-well dishes, were transfected with apoB-41F or apoB-6.6F. Twenty-four hours after transfection, cells were washed with PBS and fresh DMEM containing 0–20 μM BMS-200150 was added. After 24 h, medium samples were removed, centrifuged briefly to remove cells and debris, transferred to fresh tubes, and adjusted to 1 mM PMSF. The relative apoB-6.6F and apoB-41F content of media was measured by ELISA as described (26). Total cell protein was measured by the bicinchoninic acid method (Pierce, Rockford, IL).

ApoB-41F density gradient ultracentrifugation analysis

C127 cells in 150-mm dishes were cotransfected with 25 μg of apoB-41F and 25 μg of MTP or alkaline phosphatase. Twenty-four hours posttransfection cells were labeled for 48 h with

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[^{35}S]Met/Cys at 100 μCi/ml in a 9:1 mixture of serum-free Met/Cys-deficient DMEM and serum-free complete DMEM. After 48 h, medium was collected, adjusted to 1 mM PMSF, and centrifuged to removed cells and debris. Medium was concentrated to approximately 500 μl, using a 10,000 MW cutoff Ultrafree-15 protein concentrator (Millipore, Bedford, MA). The sample was then adjusted to 1.20 g of KBr per ml and a final volume of 1 ml and analyzed by density gradient ultracentrifugation as described previously (24). After centrifugation, twelve 1-ml samples were collected and concentrated to ~200 μl, using a Centricon-10 centrifugal concentrator (Millipore). Samples were diluted with 1 ml of lysis buffer (1% Triton X-100, 300 mM NaCl, 25 mM Tris-HCl, 1 mM PMSF) and subjected to immunoprecipitation with sheep anti-human apoB antibodies. Samples were analyzed by 6% SDS-PAGE. After drying, gels were exposed at −70°C to BioMax MS film (Eastman Kodak, Rochester, NY), using a BioMax TranScreen-LE intensifying screen (Eastman Kodak).

RESULTS

Comparison of truncated apoB secretion in COS-1 and C127 cells

COS-1 and C127 cells were transfected with apoB-28F (N-terminal 28.8% of apoB with C-terminal FLAG tag) with or without cotransfection with the 97-kDa subunit of human MTP. Forty-eight hours posttransfection cells were labeled with [^{35}S]Met/Cys for 5 h and cell lysates and medium samples were subjected to immunoprecipitation with anti-FLAG antibody. Without MTP coexpression COS-1 cells secreted a small amount of apoB-28F into the medium (**Fig. 1A**, lane 2). This agrees with previous studies showing that smaller forms of apoB can be secreted independently of MTP, presumably in the lipid-poor state (27, 28). However, an ~3-fold increase in apoB-28F secretion was observed in COS-1 cells cotransfected with MTP (lane 4). C127 cells transfected with apoB-28F displayed a level of secretion similar to that observed in MTP-transfected COS-1 cells (Fig. 1A, compare lanes 4 and 6). In apoB-41F-transfected COS-1 cells, no secretion was observed without MTP cotransfection (Fig. 1B, compare lanes 2 and 4). In contrast to COS-1 cells, transfected C127 cells were capable of secreting apoB-41F, although not to the same extent as observed in MTP-transfected COS-1 cells (Fig. 1B, lane 6). Conditioned media from parallel dishes of transfected COS-1 and C127 cells were examined for apoB-41F content by ELISA (Fig. 1C). This analysis further confirmed that apoB-41F secretion from transiently transfected C127 cells was well above the background observed in COS-1 cells transfected with apoB-41 alone (compare solid columns in Fig. 1C).

Detection of MTP mRNA by RT-PCR

To characterize the basis for the secretion of apoB-41F in C127 cells, RT-PCR analysis was performed with primers to regions of the MTP gene conserved in mice and humans and separated by several introns. For the RT-PCR assay, total RNA was extracted from the various cell lines and subjected to 10-fold serial dilution as indicated. As seen in **Fig. 2A**, the expected 699-bp RT-PCR product was detected in HepG2 cell RNA at up to a 100-fold dilution.

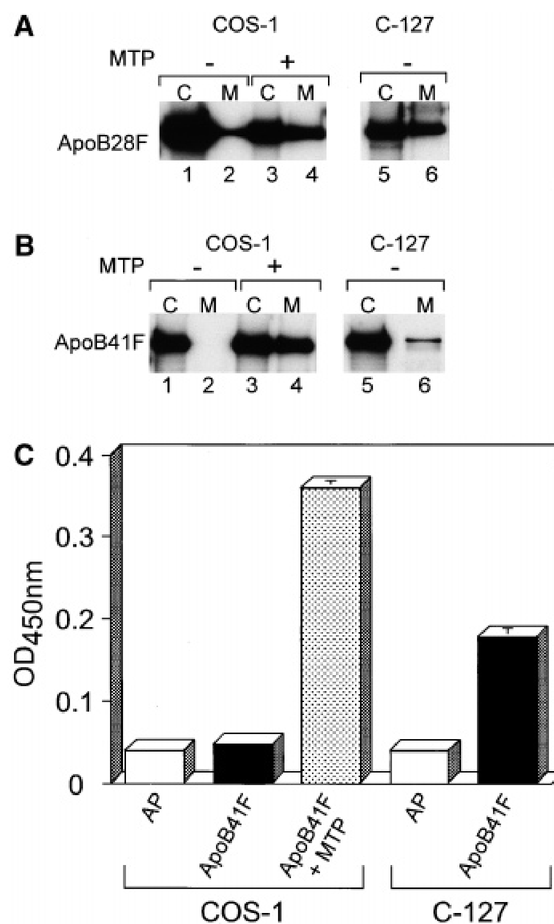

Fig. 1. Secretion of apoB from COS-1 and C127 cells. COS-1 cells in 100-mm dishes were transfected with 5 μg of apoB28F (A) or apoB-41F (B) and 5 μg of MTP (+) or human placental alkaline phosphatase (−). C127 cells were transfected with 20 μg of apoB28F or apoB-41F. Twenty-four hours posttransfection, cells were radiolabeled with [^{35}S]Met/Cys for 5 h. Cell lysates (C) and medium (M) samples were subjected to immunoprecipitation with anti-FLAG M2 monoclonal antibody and analyzed by SDS-PAGE and fluorography. C: Media from dishes transfected in parallel to those in (B) were harvested 48 h posttransfection. Relative apoB-41F content was measured by ELISA. Each bar represents the mean of three independent measurements of medium from a single transfected dish of cells ±SD.

Surprisingly, MTP mRNA was also detected in C127 cells; however, the titration experiment suggested that the amount of MTP message in these cells is ~10–20% of that observed in HepG2 cells. In addition, the MTP RT-PCR product was also detected in undiluted COS-1 cell RNA. In contrast to the other lines tested, HeLa cells did not display detectable MTP mRNA. The cyclophilin PCR product was detected at similar levels in each of the samples, confirming the integrity of the RNA preparations.

To further verify the validity of the RT-PCR results, products were subjected to automated DNA sequencing. C127 and HepG2 sequences were identical to the published mouse and human MTP sequences, respectively. The COS-1 sequence was identical to the sequence of MTP obtained by sequencing an RT-PCR product, using

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Fig. 2. MTP mRNA is present in C127 cells. A: Total RNA extracted from the indicated cell lines was diluted 0- to 1,000-fold, as indicated, with yeast tRNA and analyzed by RT-PCR with gene-specific primers. The cyclophilin reaction was used to verify the integrity of the RNA samples. B: Sequence relationships among the cell line-derived PCR products and the known MTP sequence from the corresponding species. Thick vertical lines indicate differences between species.

African green monkey kidney RNA as template (Fig. 2B). These data demonstrate that the MTP mRNA detected in each cell line was not due to cross-contamination and, specifically, that C127 cells express endogenous murine MTP mRNA.

Detection of MTP 97-kDa subunit by immunoprecipitation

Consistent with previous reports (15), we were unable to detect MTP 97-kDa subunit in C127 cells by Western blot analysis (data not shown). To detect MTP protein more sensitively, immunoprecipitation of metabolically radiolabeled cell extracts was performed. In **Fig. 3A** the indicated untransfected or transfected cells were labeled with [^{35}S]Met/Cys for 24 or 48 h and cell lysates were subjected to immunoprecipitation with either anti-human or anti-bovine MTP rabbit serum. As a positive control, mouse (Fig. 3A, lanes 2 and 3) or human (Fig. 3A, lanes 5 and 6) MTP was transfected into COS-1 cells. Both MTP orthologs were immunoprecipitated with anti-bovine (Fig. 3A, lanes 2 and 5) or human (Fig. 3A, lanes 3 and 6) MTP sera and comigrated with the endogenous human MTP protein expressed by HepG2 cells. Similarly, when C127 cell lysate was subjected to immunoprecipitation with bovine MTP antibody a band that comigrated with authentic mouse MTP expressed in transfected COS-1 cells was observed (Fig. 3A, lane 4). Despite the fact that a small amount of MTP mRNA was detected in COS-1 cells (Fig. 2), no immunoprecipitable protein was observed (Fig. 3A, lane 1), consistent with the inability of COS-1 cells to secrete transfected apoB-41F (Fig. 1B, lane 2). On the basis of a comparison of relative band intensities of MTP, and the fact that two dishes of C127 cells and one dish of HepG2 cells were used, it appears that C127 cells possess ~25% of the MTP protein content seen in HepG2 cells.

To further confirm the validity of MTP protein expression in C127 cells, we took advantage of the slight mobility difference between human and mouse MTP that becomes apparent when protein loads were better controlled and an 8% polyacrylamide gel was used. Endogenous C127 MTP comigrated with authentic mouse MTP expressed in transfected COS-1 cells (Fig. 3B, lanes 2 and 3). By comparison, both endogenous HepG2 cell and transfected human MTP (Fig. 3B, lanes 4 and 5) display a slightly more rapid mobility than mouse MTP (Fig. 3B, lane 2). These results further support the conclusion that MTP detected in C127 cells is derived from transcription and translation of the endogenous mouse MTP gene.

MTP expressed by C127 cells promotes apoB-41F secretion

It was previously reported that MTP lipid transfer activity was undetectable in C127 cells (15). We therefore tested whether MTP activity could be detected in C127 cells using an apoB secretion assay coupled with MTP inhibition. C127 or COS-1 cells were transiently transfected with apoB-41F or apoB-6.6F and incubated with various concentrations of the MTP inhibitor BMS-200150 (29, 30). As seen previously in Fig. 1B, apoB-41F secretion from COS-1 cells is completely dependent on exogenous MTP expression. Therefore, cells expressing apoB-41F and MTP should display reduced apoB secretion on addition of an MTP inhibitor. ApoB-6.6F, which lacks sequences required for lipoprotein formation and does not require MTP for efficient secretion, was used as a control protein. Media from COS-1 cells transfected with apoB and MTP were analyzed for apoB content by ELISA. ELISA values were normalized to total cell protein and expressed as a percentage of secretion observed in untreated cells. As expected, the COS-1 cells transfected with apoB-6.6F showed relatively unchanged secretion over the range of inhibitor concentrations tested (**Fig. 4A**). The COS-1 cells transfected with apoB-41F, however, showed a dose-dependent decrease in apoB secretion with an IC_{50} of ~5 μM. Figure 4B shows the results of MTP inhibition in apoB-6.6F- or apoB-41F-transfected C127 cells. The C127 cells show a dose-dependent decrease in apoB-41F secretion nearly identical to the pattern seen in the MTP-transfected COS-1 cells. The IC_{50} for both the COS-1 and C127 cells was ~5 μM, close to the IC_{50} of 3 μM reported for apoB-100 secretion in HepG2 cells (29). To further verify the effects of BMS-200150, a separate experiment was performed in which the IC_{50} dose of 5 μM was compared in MTP- and apoB4F-cotransfected COS-1 cells and

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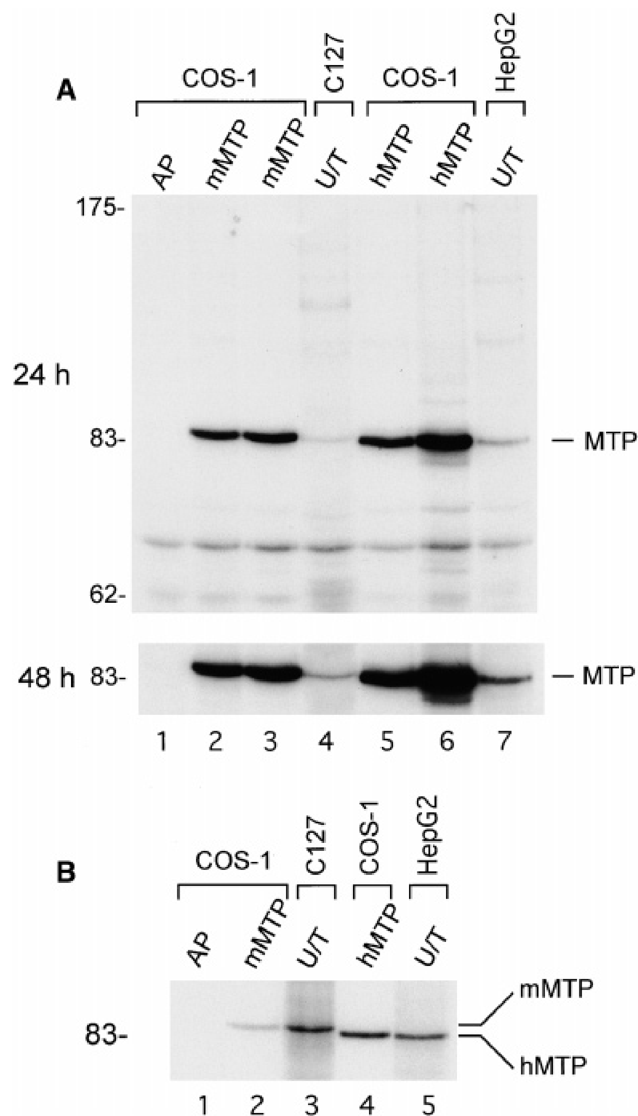

Fig. 3. Detection of MTP 97-kDa subunit in C127 cells. A: Untransfected (U/T) C127 and HepG2 cells, and COS-1 cells transfected with mouse MTP (mMTP), human MTP (hMTP), or human alkaline phosphatase (AP), were metabolically radiolabeled with [^{35}S]Met/Cys for 48 h (C127 and HeG2 cells) or 24 h (transfected COS-1 cells). MTP was immunoprecipitated from cell extracts with anti-bovine (lanes 1, 2, 4, 5, and 7) or anti-human (lanes 3 and 7) MTP polyclonal antibodies and analyzed by 6% SDS-PAGE and fluorography. Both 24- and 48-h exposures of the relevant gel area are shown. The positions of the MTP bands are indicated. Each gel lane represents immunoprecipitation of one 100-mm dish except for the C127 cell lane, which shows immunoprecipitation from two 100-mm dishes. Although the calculated molecular mass of MTP is 97 kDa (34), it migrates during SDS-PAGE as an ~87-kDa protein (2). B: MTP from the indicated cell extracts was immunoprecipitated with anti-bovine MTP antibody and subjected to 8% SDS-PAGE and fluorography. For COS-1 cells, one 100-mm dish was labeled for 8 h. For HepG2 cells one 100-mm dish was labeled for 48 h. For C127 cells, one 150-mm dish was radiolabeled for 48 h.

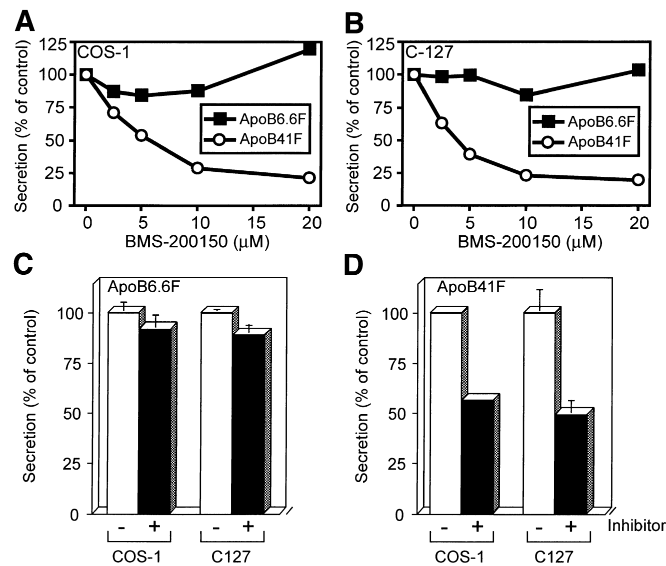

Fig. 4. The MTP inhibitor BMS-200150 inhibits apoB-41F secretion from C127 cells. In (A) and (B), duplicate wells of six-well plates of cells were treated with increasing concentrations of the MTP inhibitor BMS-200150 and medium samples were analyzed for relative apoB content by ELISA. A: COS-1 cells transfected with MTP and apoB6.6F or apoB-41F. B: C127 cells transfected with apoB-41F or apoB6.6F. Averages of duplicate experiments are plotted. In (C) and (D), cells were treated in triplicate with 5 μM BMS-200150 and medium samples were analyzed for relative apoB content by ELISA. C: Comparison of COS-1 cells cotransfected with MTP and apoB6.6F with C127 cells transfected with apoB6.6F. D: Comparison of COS-1 cells cotransfected with MTP and apoB-41F and C127 cells transfected with apoB-41F. ELISA values from each dish of cells were determined in triplicate. Mean values were normalized to cell protein and expressed as a percentage of secretion observed in control cells ±SD.

apoB-41F-transfected C127 cells. Figure 4C and D demonstrates a selective ~50% inhibition of apoB-41F secretion in both cell types with little or no effect on apoB-6.6F secretion. These inhibition studies indicate that the underlying basis for the ability of C127 cells to secrete apoB is related to their expression of functional MTP.

Density gradient analysis of apoB-41F secreted from C127 cells

To further examine the hypothesis that C127 secretion of apoB-41F was due to a low level of MTP expression, apoB-41F-containing particles secreted from these cells in the presence and absence of exogenous MTP expression were examined. As was seen in Fig. 1, apoB-41F secretion from C127 cells is quantitatively less than from MTP-transfected COS-1 cells. When MTP was cotransfected into C127 cells, however, a stimulation of secretion was observed (**Fig. 5A**, lanes 2 and 4). This quantitative difference in secretion of apoB-41F from C127 cells in the presence or absence of cotransfected MTP suggests that, although MTP may be present in these cells, it is limiting (31).

Next, the density of apoB-41F-containing lipoprotein particles secreted from C127 cells was analyzed. As is shown in Fig. 5B, apoB-41F-containing particles secreted from C127 cells in the presence or absence of cotransfected MTP have the same overall density gradient profile, with each displaying a peak density of d ≈ 1.15–1.17 g/ml, a value similar to that reported previously in apoB-41-

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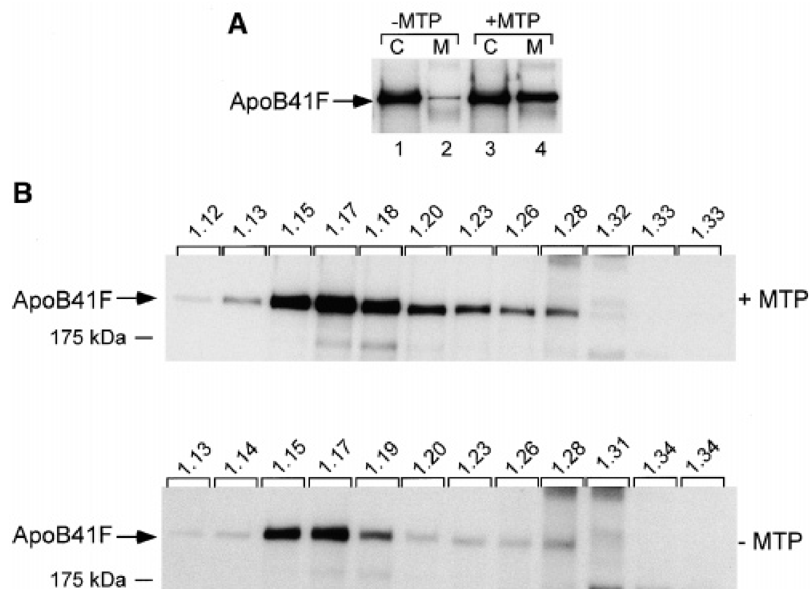

Fig. 5. ApoB-41F particle characteristics. A: C127 cells in 100-mm dishes were cotransfected with apoB-41F and MTP (+MTP) or alkaline phosphatase (−MTP). Cells were radiolabeled with [^{35}S]Met/Cys for 4 h and cell (C) and medium (M) samples were subjected to immunoprecipitation with anti-FLAG monoclonal antibody followed by SDS-PAGE and fluorography. B: Density gradient analysis of apoB-41F-containing particles secreted from C127 cells cotransfected with (+) or without (−) MTP. Each gradient was generated with media from one 150-mm dish of transfected cells. The density of each fraction (g/ml) was determined gravitometrically and is displayed above each gel lane. The film exposures of the +MTP and −MTP panels were adjusted to give similar band intensities.

transfected C127 cells (15). These results demonstrate that the characteristics of apoB-41F-containing particles produced by C127 cells are similar with or without additional MTP expression. This similarity in particle densities further supports the conclusion that apoB-41-containing particles formed by C127 arise by an MTP-dependent mechanism.

DISCUSSION

Early studies of apoB-containing lipoprotein assembly revealed that nonhepatic cells were incapable of secreting all but the smallest N-terminal forms of transfected apoB (28, 32, 33). The underlying basis for this phenomenon was solved when the gene for the 97-kDa subunit of MTP was cloned and shown to be defective in patients with the apoB assembly and secretion disorder abetalipoproteinemia (10, 34). When nonhepatic cells such as COS-1 or HeLa were transfected with the 97-kDa subunit of MTP, their capacity to assemble and secrete transfected forms of apoB as VLDL precursor particles was reconstituted (35). Hence, MTP was identified as an essential tissue-specific cofactor required for apoB assembly and secretion.

Although it is clear the MTP is necessary for efficient assembly and secretion of apoB, the details of MTP function have not been fully elucidated (36). In particular, the relationship between the size of apoB translated and the requirement for MTP has been controversial, with many examples of a limited role for MTP in the assembly of apoB-48 or smaller C-terminal-truncated forms. For example, studies of oleate- or palmitate-treated CaCo-2 cells revealed a dose-dependent effect of the MTP inhibitor BMS-200150 on apoB-100 secretion with virtually no change in apoB-48 secretion (19). Consistent with this finding, it was observed that apoB-100 secretion from mouse hepatocytes with a disrupted MTP gene demonstrated a complete loss of apoB-100 secretion but only a small reduction in apoB-48 (4). Nicodeme et al. (18) performed a systematic analysis of the relationship between C-terminal truncation of apoB and susceptibility to MTP inhibition in HepG2 cells and noted that as the length of apoB increased to apo53, lipoprotein assembly and secretion became disproportionately more sensitive to MTP inhibition. Conversely, the assembly and secretion of small forms of apoB, for example, apoB-29, were essentially unaffected by MTP inhibitor even at the highest concentrations used. Finally, C127 cells, which are considered to be devoid of MTP, were found capable of secreting apoB-41 as a nascent VLDL particle containing a triglyceride core (14, 15).

In the present study the underlying basis for the ability of C127 cells to assemble apoB into triglyceride-containing lipoprotein particles in the apparent absence of MTP was examined. It was demonstrated by a number of criteria that C127 cells do indeed express MTP and that MTP expression represents the sole underlying basis for apoB-41 secretion. Most convincingly, it was observed that apoB-41 secretion in C127 cells was inhibited by BMS-200150 to the same extent as in MTP-transfected COS-1 cells. As the secretion of apoB-41 in COS-1 cells is completely dependent on exogenous MTP expression, the similar inhibition profiles suggest that apoB-41 secretion by C127 cells is also MTP dependent.

The level of MTP mRNA and protein expressed by C127 cells was estimated to be ~10–25% of that observed in HepG2 cells, an amount perhaps too low to be reproducibly detected by MTP assays and immunoblots but enough to affect the intracellular processing of apoB-41. For example, when varying amounts of MTP expression plasmid were cotransfected into COS-1 cells along with a constant amount of apoB-41, apoB-41 secretion was observed above the minus MTP background when using as little as 3 ng of MTP plasmid (data not shown). Hence, the promotion of apoB secretion is easily the most sensitive means of assaying functional MTP expression.

The demonstration of MTP in C127 cells confirms that MTP is essential for the intracellular processing of even

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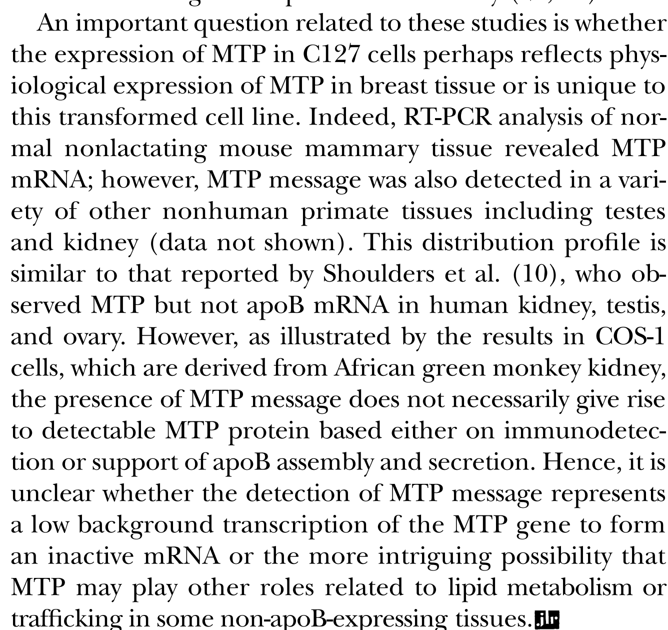
small forms of apoB and that it is unlikely that another factor or factors can substitute for this requirement (37). However, there is still the important question of why inhibition of MTP has such dramatically different effects on the capacity of small versus larger forms of apoB. This perhaps reflects the possibility that MTP may play different roles at different stages of the cotranslational assembly process. It has been suggested, for example, that MTP may transfer only a few molecules of lipid to apoB early in its translation, which in turns allows apoB to achieve lipid recruitment by a mechanism independent of MTP lipid transfer activity (1). Alternatively, it has been predicted that MTP plays a structural role in the completion of a lipid-binding pocket in apoB (38). In these scenarios, the lipid transfer activity of MTP is less important in the early stages of apoB assembly, perhaps explaining why inhibitors that primarily function to block lipid transfer have negligible effects on the assembly and secretion of small forms of apoB. Along these lines, it is of interest that the MTP inhibitor AGI-517 was shown to inhibit apoB-100 secretion by inhibiting intracellular apoB-MTP interactions, without affecting MTP lipid transfer activity (26, 39).



An important question related to these studies is whether the expression of MTP in C127 cells perhaps reflects physiological expression of MTP in breast tissue or is unique to this transformed cell line. Indeed, RT-PCR analysis of normal nonlactating mouse mammary tissue revealed MTP mRNA; however, MTP message was also detected in a variety of other nonhuman primate tissues including testes and kidney (data not shown). This distribution profile is similar to that reported by Shoulders et al. (10), who observed MTP but not apoB mRNA in human kidney, testis, and ovary. However, as illustrated by the results in COS-1 cells, which are derived from African green monkey kidney, the presence of MTP message does not necessarily give rise to detectable MTP protein based either on immunodetection or support of apoB assembly and secretion. Hence, it is unclear whether the detection of MTP message represents a low background transcription of the MTP gene to form an inactive mRNA or the more intriguing possibility that MTP may play other roles related to lipid metabolism or trafficking in some non-apoB-expressing tissues.

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