Identification of a Novel Isoform of Microsomal Triglyceride Transfer Protein*

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Microsomal triglyceride transfer protein (MTP) has been studied extensively, primarily because of its role in the assembly of very low density lipoproteins by the liver and chylomicrons by the intestine. Recent studies have suggested that MTP may also play key roles in other cellular processes. In this paper we report the identification of a novel splice variant of MTP in mice. This isoform, MTP-B, has a unique first exon located ~2.7 kilobases upstream of canonical MTP (MTP-A) exon 1. The alternative exon encodes 35 amino acids compared with 20 amino acids encoded by exon 1 of MTP-A. MTP-B represents ~90% of total MTP mRNA in mouse adipocytes and 3T3-L1 cells and <5% in mouse liver and intestine. Expression of the alternate isoform in mouse liver was confirmed by mass spectrometry. Co-transfection of COS cells with truncated forms of apoB and either MTP-A or MTP-B demonstrated that both isoforms are effective in the assembly and secretion of nascent apoB-containing lipoproteins. Confocal microscopy of 3T3-L1 cells transfected with enhanced green fluorescent protein or DsRed fusions of the two proteins revealed that MTP-A is localized to the endoplasmic reticulum, whereas MTP-B localizes primarily to the Golgi complex in these cells. We conclude that MTP-B functions similarly to MTP-A in lipoprotein assembly. However, in nonlipoprotein-secreting cells, such as the adipocyte, MTP-B may have different localization properties, perhaps reflecting a distinct role in lipid storage and mobilization.

Microsomal triglyceride transfer protein (MTP) exists as a heterodimeric protein complex consisting of a unique 97-kDa subunit that possesses lipid transfer activity and the multifunctional 58-kDa protein disulfide isomerase (PDI) (1). The complex facilitates the transfer of triglycerides, cholesteryl esters, and phospholipids between membrane vesicles in vitro (2) and is essential for the assembly of very low density lipoproteins by the liver and chylomicrons by the small intestine (3, 4). Mutations in the human MTP gene cause abetalipoproteinemia, an autosomal recessive disorder characterized by a near absence of apolipoprotein (apo)B-containing lipoproteins in the plasma of homozygous carriers (5). Pharmacologic inhibition of MTP activity leads to a dose-dependent decrease in the secretion of apoB-containing lipoproteins by HepG2 cells (6). In addition, administration of MTP inhibitors to Watanabe heritable hyperlipidemic rabbits leads to a reduction of plasma triglycerides and cholesteryl secondary to decreases in very low density lipoproteins and low density lipoproteins (7). An MTP inhibitor also reduced apoB production and plasma apoB-containing lipoproteins in patients with homozygous familial hypercholesterolemia (8).

MTP is expressed primarily in tissues that synthesize apoB-containing lipoproteins. It is abundantly expressed in liver and small intestine (9, 10) as well as in the myocardium (11–13) and yolk sac (14, 15). In the mouse deletion of Mttp is incompatible with embryonic development, as MTP is essential for the transport of nutrients from the yolk sac to the developing embryo (14). MTP expression in ovary, testis, kidney, and retina has also been reported (9, 16). Because some of these tissues do not express apoB, their specialized needs may require MTP for other aspects of lipid trafficking and/or storage. In addition to its role in bulk lipid transport, MTP may also be important in the lipidation of the CD1 family of lipid-antigen-presenting proteins and may, in fact, regulate their function (17).

Recently, we reported the presence of MTP in adipocytes of mouse brown and white fat as well as in 3T3-L1 cells and demonstrated triglyceride transfer activity in adipose tissue microsomes (18). Our confocal microscopy studies demonstrated prominent MTP fluorescence in juxtanuclear areas of undifferentiated 3T3-L1 cells that appeared to parallel individual Golgi cisternae. In contrast, MTP is localized primarily within the endoplasmic reticulum (ER) in hepatocytes and enterocytes (19, 20). Based on this unusual distribution of MTP within the adipocyte, a cell that does not produce apoB or assemble lipoproteins, we hypothesized that adipocyte MTP may be different from MTP found in liver and intestine.

In this paper we report the identification of a novel MTP isoform, MTP-B, and demonstrate that this new isoform functions similarly to canonical MTP (MTP-A) in the assembly of
nascent apoB-containing lipoproteins. However, our finding that the alternate isoform is the major form of MTP in adipocytes coupled with earlier findings that MTP may be involved in the biogenesis of lipid droplets (18) suggests that MTP-B may be important in triglyceride metabolism within the fat cell.

**EXPERIMENTAL PROCEDURES**

**Animals—**C57BL/6 mice (4–8 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). The animals were maintained in the Animal Care facility on a 12-h light/12-h dark cycle and were fed a normal mouse chow diet (RF5015, PMI Feeds Inc., St. Louis, MO). All animal procedures were carried out in accordance with institutional guidelines with approval from the Animal Care Committee at Vanderbilt University.

**Cell Culture—**3T3-L1 cells were obtained from American Type Culture Collection (Manassas, VA) and were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 4 mM glutamine, 4500 mg/liter glucose, 1 mM sodium pyruvate, and 1500 mg/liter sodium bicarbonate. To maintain cells in a predifferentiated state, they were not allowed to reach confluence. Differentiation of 3T3-L1 cells into adipocytes was initiated by the addition of 10 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 μg/ml insulin to confluent cells for 2 days. The cells were then cultured in medium containing 10 μg/ml insulin for 2 days after which they were maintained in complete media. The medium was changed every 2 days until differentiation was complete (~day 8).

**RNA Preparation—**Total tissue or 3T3-L1 cell RNA was prepared using Trizol (Invitrogen) at 1 ml per 100 mg of tissue/cells according to the manufacturer’s instructions. RNA pellets were dried and dissolved in water.

**Reverse Transcription—PCR—**cDNA was synthesized using oligo(dT) priming and the Superscript II reverse transcriptase kit (Invitrogen) according to the protocol provided by the manufacturer. PCR (Advantage II protocol, BD Biosciences, Clontech) was performed using 40–200 ng of cDNA as template and desalted sense and antisense oligonucleotides (Operon Biotechnologies, Inc., Huntsville, AL). Oligonucleotides for full-length cDNA amplification were ATGATCCTTTGGCACTGCTT (MTP-A, sense), ATGAGACTGTGATGGGAATGT (MTP-B, sense), and CCAGCTCCAGTCAAAAACCATCCACCCGAGTTATC (MTP-A and -B, antisense). Oligonucleotides for analytical PCR, used to screen relative tissue expression of the isoforms were, ATGATCCTTTGGCACTGCTT (MTP-A, sense), ATGAGACTGTGATGGGAATGT (MTP-B, sense), and CCAGCTCCAGTCAAAAACCATCCACCCGAGTTATC (MTP-A and -B, antisense). Oligonucleotides for analytical PCR, used to screen relative tissue expression of the isoforms were, ATGATCCTTTGGCACTGCTT (MTP-A, sense), ATGAGACTGTGATGGGAATGT (MTP-B, sense), and CCAGCTCCAGTCAAAAACCATCCACCCGAGTTATC (MTP-A and -B, antisense). Oligonucleotides for analytical PCR, used to screen relative tissue expression of the isoforms were, ATGATCCTTTGGCACTGCTT (MTP-A, sense), ATGAGACTGTGATGGGAATGT (MTP-B, sense), and CCAGCTCCAGTCAAAAACCATCCACCCGAGTTATC (MTP-A and -B, antisense).

**Quantitative Real-time—PCR—**Total RNA was extracted from tissues (liver, intestine, white fat, brown fat) and from 3T3-L1 cells using TRizol as described above. cDNA was synthesized using a gene-specific primer and Superscript II reverse transcriptase (Invitrogen). Real-time PCR was performed using the iQ SYBR Green Supermix (Bio-Rad) in a Bio-Rad iCycler iQ real-time PCR detection system. Cycling conditions were 95 °C for 5 min followed by 40 cycles of 30 s at 95 °C and 1 min at 60 °C. PCR amplifications were analyzed by agarose gel electrophoresis to confirm product size. Four serial dilutions of cDNA from each tissue were used to determine linearity and efficiencies of amplification. Standard curves were generated for each isoform using dilutions of a pcDNA vector into which full-length cDNA for either MTP-A or MTP-B had been cloned. The efficiencies of amplification were greater than 95%. The threshold cycles for equivalent concentrations of vectors containing either cDNA differed by an average of 0.18 ± 0.25 cycles (A < B). The relative amount of the mRNA for the two isoforms in each sample was calculated using the 2−ΔΔCt method corrected for the difference in efficiency of the primers. Data were obtained from tissue samples from three to five different mice and are expressed as the mean ± S.D.

**Identification of Alternate MTP Isoform by Mass Spectrometry—**MTP was isolated from mouse liver microsomes by immunoprecipitation and SDS-PAGE. The band, visualized using colloidal Coomassie stain (ProtoBlue Safe, National Diagnostics, Atlanta, GA) was excised and digested with trypsin (21). Liquid chromatography-MS-MS analyses were performed using a Thermo Finnigan LTQ ion trap mass spectrometer equipped with a Thermo Finnigan Surveyor HPLC pump and autosampler, nanospray source, and Xcalibur 2.0 instrument control and a Thermo LTQ-Orbitrap mass spectrometer equipped with an Eksigent NanoLC 1D plus HPLC and autosampler as previously described (22). The full MS data obtained with the LTQ-Orbitrap mass spectrometer were collected at 60,000 resolution, and MS/MS data were collected on the LTQ portion of the instrument similarly to the settings for the LTQ instrument. Some expected masses were targeted to trigger MS/MS spectra throughout the run to verify the initial results. Proteins were identified using the TurboSEQUEST Version 27 (revision 12) algorithm (Thermo Electron, San Jose, CA (23)) using the mouse subset of the Uniref 100 data base. Data were also searched for unanticipated modifications using the P-Mod algorithm (24).

**Transfection of COS-1 Cells and Analysis of ApoB Secretion—**C-terminal His6-tagged form of human apoB34 and native MTP were cloned into the pcCMV5 expression vector (25). Mouse MTP-A and MTP-B were cloned into the pcDNA3.1 expression vector. COS cells were cultured as described (26) in 100-mm dishes and transiently co-transfected with 3 μg of human apoB34 and 3 μg of human MTP, mouse MTP-A, mouse MTP-B, or human placental alkaline phosphatase (negative control) using the FuGENE 6 transfection reagent (Roche Applied Science) (27). Twenty-four hours post-transfection, cells were metabolically radiolabeled with 100 μCi/ml [35S]Met/Cys (EasyTag Express protein labeling mix, PerkinElmer Life Sciences) in Met/Cys-deficient DMEM for 3 h. Cell lysates and media samples were immunoprecipitated with anti-apoB antibodies and analyzed by SDS-PAGE and fluorography, as described (27).

**Flotation and Density Gradient Analysis of Lipoproteins—**COS cells in 100-mm dishes were co-transfected with apoB34 (3 μg) and an equivalent mass of human MTP, MTP-A, or MTP-B. Twenty-four hours post-transfection, cells were metabolically radiolabeled with 100 μCi/ml [35S]Met/Cys for 4 h. After the addition of protease inhibitors, the medium (1 ml) was
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Identification of MTP Splice Variant—The published mRNA sequence for mouse Mtp (NM008642) was compared with sequences in the Expressed Sequence Tag (EST) database (NCBI; blastn), and multiple “hits” were observed across the Mtp mRNA sequence (see Fig. 1, A and B). We identified 28 ESTs that matched the Mtp mRNA sequence; however, only 24 of these were identical to the Mtp 5′ end. The remaining four matches (Fig. 1C) contained a unique 5′ sequence of 85 nucleotides. Comparison of the sequence of these four clones with the intron-exon organization of mouse Mtp revealed that the break in alignment with the published Mtp mRNA sequence was located at the junction between exons 1 and 2. Inspection of mouse genomic sequence revealed that the novel 5′ sequence matched a region of mouse chromosome 3, 2702 bp upstream of Mtp exon 1. These data suggest that the novel 5′ sequence contained within the four similar ESTs may have arisen from transcription initiation at an alternate upstream exon 1.

Amplification of Full-length MTP-B cDNA from Mouse Cells and Tissues—Sense strand PCR primers specific for the 5′ ends of either canonical MTP (MTP-A) or the novel MTP (MTP-B) were generated. These were used in combination with a 3′ antisense strand primer common to both forms of MTP mRNA. Full-length MTP-A (∼2.7 kilobases) was amplified from mouse liver, white fat, brown fat, and intestine cDNA (Fig. 2, lanes 2, 8, 10, 12, and 14). MTP-A PCR product was also amplified from undifferentiated or differentiated 3T3-L1 cells (Fig. 2, lanes 4 and 6). Sequence analysis of the MTP-A-specific PCR reactions subjected to KBr density gradient ultracentrifugation as described (25). Twelve fractions (1 ml each) were collected, subjected to immunoprecipitation with apoB antibodies, and analyzed by SDS-PAGE and fluorography. The mean densities of two blank gradients were determined gravimetrically.

Transfection of 3T3-L1 Cells—Full-length MTP-A and MTP-B cDNAs were cloned into pEGFP-N2 and pDsRed-Monomer N1 (BD Biosciences Clontech) using standard molecular techniques. The integrity of the resulting clones was confirmed by DNA sequencing. 3T3-L1 cells were cultured at 20% confluence on 35 mm Mattek plates. Twelve hours post-transfection, cells were washed, and serum-containing (10%) DMEM was added. Twenty-four hours later cells were fixed and processed for imaging as described below.

Confocal Microscopy—Images were collected and analyzed on a Zeiss LSM 510 Meta confocal laser microscope (40-power oil 1.3 NA (Zeiss) or 63-power oil 1.4 NA (Zeiss), pinhole equals 1.0 Airy Disc) using Carl Zeiss Imaging software. Both channels were collected on PMT3 using calibrations to confirm that fluorescence was collected in the linear range. For display, images were converted into TIFF format and processed using Adobe Photoshop software (Version 7.0).

Transfection of CHO Cells—CHO cells were plated at ~20% confluence. Twenty-four hours later they were transfected with the pcDNA3.1 vector expressing full-length MTP-A or -B as described above. Twelve hours post-transfection cells were washed, and serum-containing (10%) DMEM was added. Forty-eight hours later the cells were collected and lysed in 100 mM Tris-HCl, pH 7.9, containing 0.5% Nonidet P-40. Protein was determined using the bicinchoninic acid (BCA) assay, and aliquots were taken for co-immunoprecipitation studies.

Co-immunoprecipitation Studies—Co-immunoprecipitations were carried out in deoxycholate buffer (62.5 mM sucrose, 0.5% sodium deoxycholate, 5 mM EDTA, 50 mM Tris-HCl, 150 mM NaCl, and 1% Triton X-100, pH 7.4). Antibody to MTP or PDI (mouse monoclonal IgG2a, Affinity BioReagents, Golden, CO) was allowed to bind with protein A-agarose (Calbiochem), and the pellets were washed 5 times with ice-cold deoxycholate buffer. Cell lysates (~200 μg of protein) were added to the antibody/protein A-agarose suspension and incubated for 2 h at 4 °C. The complexes were recovered by centrifugation, and the pellets were washed 5 times with ice-cold deoxycholate buffer. The immunoprecipitates were solubilized in NuPAGE LDS sample buffer, separated by SDS-PAGE using NuPAGE Bis-tris gels (4–12% gradients) (Invitrogen), and transferred to nitrocellulose membranes. Bands were visualized by enhanced chemiluminescence (Amersham Biosciences) after incubations with appropriate antibodies.

RESULTS

Identification of MTP Splice Variant—The published mRNA sequence for mouse Mtp (NM008642) was compared with sequences in the Expressed Sequence Tag (EST) database (NCBI; blastn), and multiple “hits” were observed across the
revealed 2685 nucleotide products that corresponded to the published mRNA sequence for mouse MTP (NM008642) (data not shown).

Using the primer set designed to amplify the unique MTP-B sequence, an abundant PCR product slightly larger than the MTP-A product was generated in all tissues examined (Fig. 2, lanes 1, 3, 5, 7, 9, 11, and 13). Evaluation of the unique 5′ sequence revealed several ATG codons; however, only the upstream ATG was found within the context of a well defined translation initiation consensus sequence (28). This MTP-B initiator codon is positioned 106 nucleotides upstream from the start of exon 2, compared with 61 nucleotides for canonical MTP-A (Fig. 3). These data together further support the conclusion that the unique 5′ sequence present in MTP-B arises from transcription initiation at an alternative upstream exon (exon 1B), which is spliced directly to exon 2. MTP mRNA sequences derived from exons 2–18 are identical for both the MTP-A and -B isoforms (Fig. 3).

As a result of the alternate first exons, the size of the MTP-A and MTP-B mRNAs and proteins are different. Canonical MTP-A encodes an 894-amino acid protein, with the first 20 amino acids encoded by canonical exon 1A (exon 1A), which contains the initiator codon for MTP-A. B, mRNA sequence of exons 1A (61 bp) and 1B (106 bp) for Mtp, N-terminal sequences of MTP-A and MTP-B as deduced from mRNAs. The underlined sequences represent predicted signal peptides. Arrows denote possible cleavage sites for MTP-B signal peptide with probabilities of 0.65, 0.5, and 0.2 (1–3), respectively.

**FIGURE 3. Comparison of MTP-A and MTP-B.** A, exon organization of mouse Mtp. Novel exon 1B is located near 9 kilobases 5′ of exon 2 and ~2.7 kilobases (kb) 5′ of exon 1A, which contains the initiator codon for MTP-A. B, mRNA sequence of exons 1A (61 bp) and 1B (106 bp) for Mtp. C, N-terminal sequences of MTP-A and MTP-B as deduced from mRNAs. The underlined sequences represent predicted signal peptides. Arrows denote possible cleavage sites for MTP-B signal peptide with probabilities of 0.65, 0.5, and 0.2 (1–3), respectively.

**FIGURE 4. Expression of MTP-A and MTP-B in tissues and 3T3-L1 cells.** Total RNA was extracted from liver, ileum, jejunum, white fat, brown fat, undifferentiated (D−), and differentiated (D+) 3T3-L1 cells as described under “Experimental Procedures.” A, reverse transcription-PCR using primers specific for MTP-A and -B mRNA was performed. Mouse β-actin primers were included for normalization. B, quantitative real-time PCR was performed using SYBR Green in a Bio-Rad iCycler iQ system as described under “Experimental Procedures.” Relative amounts of mRNA for the two isoforms were calculated using the 2−ΔΔCt method corrected for the efficiencies of the primers. Data represent the mean ± S.D. obtained from the analyses of three to five different samples. Black bars, MTP-A; gray bars, MTP-B.

were designed with similar GC content, melting temperatures, and ΔG (6.0 kcal/mol). Mouse β-actin primers, generating a ~500-bp product, were included as a reference control. PCR products for both isoforms were generated in all tissues examined as well as in 3T3-L1 cells (Fig. 4A). Real-time PCR was used to quantitate the relative expression levels of the two isoforms. In liver, jejunum, and ileum, MTP-A PCR product represented >97% of the total (Fig. 4B). In contrast, in white and brown fat as well as in 3T3-L1 cells, MTP-B predominated, composing at least 90% of the total PCR product.

**Mass Spectrometric Identification of MTP-B—**Liquid chromatography-MS analysis of the digest of the MTP band confirmed the presence of MTP. Approximately 46% of the entire sequence was identified after filtering the Sequest data for confidently identified peptides. Additional analysis of the data with the P-Mod algorithm (24) revealed a match to a doubly charged peptide, QNSGHTTGLSLNNER, with a mass shift of approximately 17 Da (Fig. 5A). Peptides containing an N-terminal glutamine can cyclize and lose NH₃ to form a pyroglutamate moiety (31), consistent with this MS/MS spectrum. Additional confirmatory evidence for the identity of this peptide was obtained using the LTQ-Orbitrap mass spectrometer which showed that the peptide was doubly charged and within 2.2 ppm of the predicted mass (Fig. 5B). This peptide could only arise from the B isoform of MTP (Fig. 3). In addition, it would not likely be formed by trypsin digestion (which cleaves at arginines and lysines), supporting its identification as the N-terminal peptide of mature MTP-B.

**Functional Properties of MTP-A and MTP-B—**Studies in our laboratory have demonstrated abundant triglyceride transfer activity in lysates from 3T3-L1 cells, which predominantly express MTP-B (Fig. 4). To explore further the functional prop-
Properties of the MTP-B isoform, COS cells were co-transfected with human apoB34 and either human MTP, mouse MTP-A, or mouse MTP-B followed by metabolic radiolabeling with [35S]Met/Cys and immunoprecipitation (25). As observed in Fig. 6A, cotransfection of apoB34 with the control plasmid, alkaline phosphatase (AP), resulted in abundant apoB34 synthesis (lane 1) but undetectable secretion (lane 2). Cotransfection with human MTP and the mouse MTP isoforms revealed that each was capable of inducing apoB34 secretion to a similar extent (compare lanes 4, 6, and 8). However, immunoblot analysis of the same COS cell lysates analyzed for apoB content in Fig. 6A revealed that expression of MTP-B was greater than that of either human MTP or MTP-A (Fig. 6B, upper panel). A second independent transfection experiment confirmed this differential pattern of expression (Fig. 6B, lower panel) with equivalent induction of apoB34 secretion (data not shown). Hence, whereas each MTP isoform appears equivalent in terms of its ability to promote apoB34 secretion, the efficiencies of the different isoforms may not be identical.

To confirm directly that MTP-B promoted the assembly of apoB34 into lipoprotein particles, density gradient ultracentrifugation was performed. These analyses confirmed that apoB34 secreted by cotransfected COS cells was assembled into buoyant lipoprotein particles with a peak density of ~1.169 g/ml (25) and that the density profiles produced by human MTP and mouse MTP-A and -B were virtually identical (Fig. 7).

Subcellular Localization of MTP-A and MTP-B—MTP-A and MTP-B were differentially localized when transfected into 3T3-L1 cells (Fig. 8). Fluorescence from EGFP-MTP-A was observed throughout the cells in a reticulated pattern consistent with a location in the ER. In contrast, cells transfected with cDNA encoding DsRed-MTP-B demonstrated fluorescence in juxtanuclear regions of the cell, consistent with Golgi localization (Fig. 8). There was little overlap of fluorescence from MTP-A and MTP-B, suggesting unique subcellular locations for the two MTP isoforms.

Interactions of MTP-A and MTP-B with PDI—Transfection of CHO cells with MTP-A or MTP-B led to production of the respective proteins (Fig. 9A). Immunoprecipitation of either MTP-A or MTP-B resulted in co-immunoprecipitation of PDI (Fig. 9B). In addition, immunoprecipitation of PDI resulted in co-immunoprecipitation of both MTP isoforms (Fig. 9C). MTP bands were not observed in non-transfected control cells.

DISCUSSION

MTP was first isolated from bovine liver microsomes by Wetterau and Zilversmit (1). Subsequent studies led to the conclusion that MTP was localized primarily within the ER of the cell (19) and was essential for the assembly of triglyceride-rich lipoproteins by the liver and the small intestine (3, 5, 19).
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FIGURE 6. Induction of apoB34 secretion by human MTP and mouse MTP-A and -B. A, COS cells (100-mm dishes) were co-transfected with 3 μg of human apoB34 and 3 μg of the indicated plasmids (AP, alkaline phosphatase; hMTP, human MTP; MTP-A, mouse MTP form A; mMTP-B, mouse MTP form B). Twenty-four hours post-transfection cells were labeled with [35S]Met/Cys for 3 h. Cell lysates (C) and media (M) samples were immunoprecipitated with anti-apoB antibodies and analyzed by SDS-PAGE and fluorography. B, immunoblot of COS cell lysates demonstrating the expression of human MTP (Hu), MTP-A (A), or MTP-B (B). The upper panel corresponds to aliquots of lysates analyzed for apoB expression in panel A. The lower panel shows results of a second independent transfection.

FIGURE 7. Density gradient ultracentrifugation of apoB34-containing lipoproteins. COS cells were co-transfected with apoB34 and the indicated MTP isoform. Media was subjected to KBr density gradient ultracentrifugation as described (25). Twelve 1-ml fractions were collected, subjected to immunoprecipitation with apoB antibodies, and analyzed by SDS-PAGE and fluorography. Mean densities of two blank gradients (g/ml) are displayed.

FIGURE 8. MTP-A and MTP-B display unique subcellular distributions in 3T3-L1 cells. Full-length MTP-A (A) and MTP-B (B) cDNAs were transfected into 3T3-L1 cells as described under “Experimental Procedures.” Twenty-four hours after transfection, cells were fixed and processed for imaging. EGFP-MTP-A and DsRed-MTP-B fluorescent signals do not overlap (right panel) suggesting unique subcellular locations for the two proteins.

Recently, we reported that MTP is present in adipocytes from mouse brown and white fat as well as in 3T3-L1 cells. Furthermore, in undifferentiated 3T3-L1 cells MTP was almost exclusively localized within the Golgi complex (18). The latter observation led us to hypothesize that adipocyte MTP may be different from the major form of MTP found in hepatocytes and enterocytes.

A search of the mouse EST data base led to the identification of four clones identical to mouse Mttp (NM008642) beginning at exon 2 (nucleotide 86) but with a unique 5’ sequence. The sources of these clones were liver, brain, “upper head,” and whole eye. The unique 5’ nucleotide sequence of these clones was found on mouse chromosome 3, ~2.7 kilobases upstream of exon 1A of mouse Mttp. Our studies provide strong evidence that this novel isoform of MTP arises from transcription initiation at an alternative upstream exon 1 (exon 1B), which is directly spliced to exon 2 (thereby skipping exon 1A) (Fig. 3). The tissue distribution of the two isoforms is distinct. In mouse liver and intestine (jejunum and ileum) MTP-A is the predominant isoform composing ~97% of the total Mtp mRNA (Fig. 4). In contrast, in adipocytes from white and brown fat of mice and in undifferentiated and differentiated 3T3-L1 cells MTP-B mRNA is the primary isoform composing ~90% of total MTP mRNA (Fig. 4). Although we have not analyzed mouse brain or eye for either MTP isoform, the fact that the alternate form of MTP was identified via EST clones suggests that it might also be abundant in these tissues.

While this manuscript was under review, Dougan et al. (32) reported the identification of a novel splice variant (MTPv1) of mouse MTP, which is identical to MTP-B that we have identified in our studies. They reported that MTPv1 is ubiquitously expressed, including the small intestine and hepatocytes, whereas canonical MTP (MTP-A) was restricted to intestine, liver, and NKT cell hybridoma. However, our studies clearly demonstrate that MTP-A is expressed, albeit in low relative abundance, in white and brown fat as well as in 3T3-L1 cells.

MTP-B mRNA is predicted to encode a 909-amino acid protein, whereas the message for MTP-A encodes an 894-amino acid protein. Mass spectrometric analysis not only confirmed expression of the alternate isoform in mouse liver (Fig. 5) but also suggested that the N terminus of the mature protein is QNS (Fig. 3). This sequence would not arise from trypsin digestion; however, cleavage between the methionine and glutamine represents one of the three predicted signal peptide cleavage sites (Fig. 3). Thus, the N terminus for MTP-B is distinctly dif-
ferent from the predicted N-terminal sequence of VK for MTP-A (Fig. 3). In contrast, Dougan et al. (32) reported that the signal peptidase cleavage site on MTPv1 is between amino acids 33 and 34, giving an N terminus of NS, whereas the signal peptidase cleavage site on MTP (MTP-A) is between amino acids 22 and 23, giving an N terminus of HTT. It is possible that alternate signal peptide cleavage sites exist for both isoforms, and the N termini are different depending on the cell type. Regardless of the precise cleavage sites, the differences in the primary sequences of MTP-A and MTP-B do not grossly affect MTP function as both forms of MTP promote the secretion of apoB34 in cotransfected COS cells (Fig. 6). Furthermore, density gradient analyses confirmed that lipoprotein particles produced by each MTP displayed virtually identical buoyant density profiles (Fig. 7). Whereas the two isoforms appear to function to promote apoB secretion, they may not function with equal efficiencies, as the expression levels of MTP-B were greater than MTP-A (Fig. 6). One possible explanation for the apparent lower efficiency of apoB34 assembly by MTP-B may relate to the fact that a large amount of MTP-B is localized to the Golgi rather than the ER (Fig. 8 and below). Additional evidence that MTP-B is functional comes from unpublished studies in our laboratory demonstrating abundant triglyceride transfer activity in microsomes from 3T3-L1 cells, which express predominantly MTP-B. Together, these results indicate that the novel MTP-B isoform is capable of engaging in triglyceride transfer and apoB-containing lipoprotein assembly.

An interesting difference between MTP-A and -B is the differential subcellular distributions of the two proteins in 3T3-L1 cells. Studies using EGFP and DsRed analogs of the two proteins revealed that MTP-A localized primarily to the ER as evidenced by the reticulated fluorescent signals visible throughout the cell (Fig. 8). In contrast, cells transfected with cDNA encoding MTP-B demonstrated fluorescence in the juxtanuclear regions of the cell, consistent with a location in the Golgi apparatus. Furthermore, when cells were simultaneously transfected with cDNAs encoding both proteins, there was little evidence of overlap of the two signals. The results are consistent with previous studies from our laboratory (18) which demonstrated that endogenous MTP in 3T3-L1 cells was localized primarily in a juxtanuclear region, consistent with the Golgi complex. Given the fact that MTP-B is the primary isoform in these cells (Fig. 4), our results showing differential localization of the two fluorescent analogs is not unexpected.

The localization of MTP-B within the Golgi compartment led us to question if this isoform interacts with PDI, since it is the KDEL retention sequence on PDI (33) that is thought to play a key role in targeting canonical MTP (MTP-A) to the ER (19). Co-immunoprecipitation studies, however, clearly demonstrated that MTP-B can associate with PDI (Figs. 9, B and C), but the results do not preclude the possibility that the alternate isoform associates with other protein(s) that target it to the Golgi complex. These results demonstrating Golgi localization of MTP are not without precedent. Previous studies from our laboratory suggested that MTP was localized to both the ER and the Golgi apparatus of mouse liver (34). Confocal microscopic studies subsequently confirmed that MTP was present within both the cis and trans aspects of the Golgi apparatus in McArdle RH7777 cells (20). Levy et al. (35) also demonstrated the presence of MTP in the Golgi of rat enterocytes, and Slight et al. (36) found evidence for MTP in the brush border membranes of rat enterocytes. Clearly, MTP is not completely localized within the ER of the cell, but the determinants of the different subcellular localizations have not been defined. In addition, it is not clear if the MTP in compartments other than the ER is solely the alternate isoform, as would be suggested by our studies.

In contrast to our results demonstrating different subcellular locations for the two isoforms, Dougan et al. (32) reported that both MTP and MTPv1 were localized to the ER as demonstrated by the co-localization of FLAG-tagged proteins with the ER marker calnexin. However, these studies were carried out in Ld cells, a fibroblast cell line, transduced with MTPv1-FLAG or MTP-FLAG. Hence, it is possible that the isoforms localize differently in different cell lines. In this regard it is important to note that we have also shown the differential localization of the two isoforms in CHO cells transfected with pcDNA vectors expressing either native MTP-A or MTP-B (data not shown). Additional studies will be required to understand the differences in localization of the isoforms and the possibility that these differences are cell specific and functionally significant.

Deletion of the Mttp gene in mice is embryonically lethal (14); consequently, Cre-lox technology has been utilized to generate tissue-specific MTP knock-out mice (37, 38). Raabe et al. (37) generated liver specific MTP knock-out mice by targeted deletion of Mttp exon 1. Theoretically, these mice should still express MTP-B. It is interesting to note that Raabe et al. (37) reported that neither the Cre adeno virus approach nor the MX1-Cre transgene approach completely inactivated Mttp gene expression. They speculated that some hepatocytes may not express Cre or simply escape the period of high level Cre expression without undergoing complete excision of both alleles. Based on our results reported here, we would speculate that residual Mttp mRNA represents the alternate MTP isoform. In contrast, Chang et al. (38) developed liver-specific MTP knock-out mice with loxp sites flanking exons 5 and 6. In these mice Cre recombinase administration would lead to deletion of exons 5 and 6 and total disappearance of liver MTP-A and MTP-B mRNA and protein. Interestingly, Raabe et al. (37) found marked reductions in plasma apoB100 levels but no significant reductions in apoB48 levels, whereas Chang et al. (38) reported that apoB100 was undetectable in the plasma of their mice and that apoB48 was barely detectable. One explanation for this difference is that the residual MTP-B isoform in the livers of the mice from the study of Raabe et al. (37) is sufficient to support the assembly and secretion of apoB48-containing lipoproteins, whereas in the complete absence of either MTP isoform, the assembly and secretion of both apoB100- and apoB48-containing lipoproteins are nearly eliminated.

In summary, our studies have identified a novel form of MTP that comprises a small fraction (~5%) of the total MTP in mouse liver and intestine but appears to represent the predominant form of MTP in mouse adipocytes. The alternate form is expressed in mouse liver and is functional in the assembly of apoB-containing lipoproteins in transfected cells. We speculate that both isoforms also function in the assembly of apoB-con-
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taining lipoproteins in liver and intestine. The predominance of the alternate form in adipose tissue suggests a role in adipocyte biology that may be distinct from its role in apoB-containing lipoprotein assembly. Defining the role of this triglyceride transfer protein within the adipocyte may prove important for understanding the biogenesis of lipid droplets and the basic mechanisms underlying fat storage.

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