Phospholipid Transfer Activity of Microsomal Triacylglycerol Transfer Protein Is Sufficient for the Assembly and Secretion of Apolipoprotein B Lipoproteins*

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Human microsomal triacylglycerol transfer protein (hMTP) is essential for apolipoprotein B (apoB)-lipoprotein assembly and secretion and is known to transfer triacylglycerols, cholesterol esters, and phospholipids. To understand the relative importance of each lipid transfer activity, we compared the ability of hMTP and its Drosophila ortholog (dMTP) to assemble apoB lipoproteins and to transfer various lipids. apoB48 secretion was induced when co-expressed with either hMTP or dMTP in COS cells, and oleic acid supplementation further augmented secretion without altering particle density. C-terminal epitope-tagged dMTP (dMTP-FLAG) facilitated the secretion of apoB polypeptides in the range of apoB48 to apoB72 but was ~50% as efficient as hMTP-FLAG. Comparison of lipid transfer activities revealed that although phospholipid transfer was similar in both orthologs, dMTP was unable to transfer neutral lipids. We conclude that the phospholipid transfer activity of MTP is sufficient for the assembly and secretion of primordial apoB lipoproteins and may represent its earliest function evolved for the mobilization of lipid in invertebrates. Identification of MTP inhibitors, which selectively affect transfer of a specific lipid class, may have therapeutic potential.

Lipoproteins are lipid–protein complexes that transport lipids, fatsoluble vitamins, and other hydrophobic molecules in the plasma. Apo- lipoprotein B (apoB) is a structural protein embedded in the phospholipid monolayer on the surface of triglyceride-rich lipoproteins. It has been hypothesized that apoB contains amphipathic α-helical and β-sheet domains (1). Lipidation of the β-sheets is necessary for the assembly of larger apoB polypeptides into lipoprotein emulsion particles. Lipoprotein assembly begins co-translationally and microsomal triglyceride transfer protein (MTP) plays a pivotal role in this process (for reviews, see Refs. 2–6). MTP is absent in abetalipoproteinemia, a disease characterized by the deficit of plasma apoB lipoproteins and low plasma cholesterol levels (7,8). Reconstitution of MTP in heterologous systems rescues apoB secretion, whereas tissue-specific liver knock-out mice (9,10). The signature activity of MTP is its ability to transfer lipids between membranes. It has been demonstrated that MTP lipid transfer activity is necessary for apoB lipoprotein assembly and secretion (for reviews, see Refs. 2–6). More recent evidence suggests that MTP lipid transfer activity is also responsible for lipid accretion within the secretory pathway and that MTP potentially stabilizes lipid vesicles in the endoplasmic reticulum (reviewed in Refs. 2 and 3).

MTP transfers several lipids including triacylglycerols, cholesterol esters, and phospholipids between vesicles in vitro (11–14). It has been suggested that MTP transiently interacts with a membrane, extracts lipids, and then delivers them to another lipid acceptor or to nascent apoB lipoproteins (15). Kinetic studies indicate the presence of two lipid-binding sites: one site binds triacylglycerols, cholesterol esters, and phospholipids with a preference for neutral lipids, whereas a second site binds only phospholipids (16). It is not clear whether all MTP lipid transfer activities are required for lipoprotein assembly.

Recently, we identified a human MTP (hMTP) ortholog from the genome of the fruit fly, Drosophila melanogaster, that supported the secretion of human apoB34 and apoB41 (17). However, the mechanisms involved in the secretion of apoB lipoproteins by the Drosophila MTP (dMTP) have not been elucidated. In this paper, we show that although dMTP is defective in triacylglycerol transfer activity, the phospholipid transfer activity is equivalent to that of hMTP. Thus, phospholipid, but not the triacylglycerol transfer activity, may be necessary for the assembly of primordial lipoproteins.

**MATERIALS AND METHODS**

**Construction of MTP-FLAG Chimeras**—hMTP and dMTP expression vectors have been previously described (17). C-terminal FLAG tag forms of Drosophila and hMTP were produced by PCR, utilizing 3 antisense primers encoding the FLAG sequence (DYKDDDDK) followed by an in-frame termination codon.

**Cell Culture and apoB Secretion**—COS-7 cells were grown in Dulbecco’s modified Eagle’s medium (CellGrow) containing 10% fetal bovine serum (BSA) supplemented with l-glutamine and antibiotics. The cells were initially dislodged from the plate with trypsin and seeded in six-well plates (400,000 cells/well). Transfections were performed using FuGENE 6 transfection reagent (Roche Applied Sciences) according to the manufacturer’s instructions. For sequential transfections, apoB expression vectors were initially introduced into COS cells using FuGENE 6 in T175 flask (7.2 × 106 cells). After 8 h, the cells were detached by trypsin treatment, seeded in six-well plates, and transfected with MTP expression plasmids. At 48 h post-transfection, the media were aspirated and either 1 ml of Dulbecco’s modified Eagle’s medium or 1 ml of lipid-containing medium (Dulbecco’s modified Eagle’s medium including 0.4 mM oleic acid complexed with 1.5% BSA and 1 mM glycerol) were added. Following additional 18 h of incubation, the media were collected, protease inhibitors (Sigma) were added, the samples were centrifuged (2,500 rpm, 4 °C, 10 min) to pellet cell debris, and the apoB contents were measured in the supernatants by ELISA (18, 19).

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‡ The abbreviations used are: apoB, apolipoprotein B; BSA, bovine serum albumin; MTP, microsomal triglyceride transfer protein; dMTP, Drosophila MTP; hMTP, human MTP; OA, oleic acid; PDI, protein disulfide isomerase; SREBP, sterol regulatory element-binding protein; ELISA, enzyme-linked immunosorbent assay.
The media were also subjected to ultracentrifugation to separate lipoproteins (20). Conditioned media (4 ml) were adjusted to 1.30 g/ml with KBr and overlaid with 2 ml each of 1.24, 1.15, and 1.063 g/ml KBr solutions and 1 ml each of 1.019 and 1.006 g/ml. After ultracentrifugation (SW41 rotor, 40,000 rpm, 17 h, 15 °C), 1-ml fractions were collected, and their apoB content was determined by ELISA. The density in each fraction was measured using a refractometer (Fisher).

Immunofluorescence—COS-7 cells grown on coverslips in 24-well tissue culture plates were transfected with apoB48 and either hMTP- or dMTP-FLAG expression plasmids. Forty-eight hours post-transfection, the cells were fixed and permeabilized in methanol for 15 min at -20 °C. The fixed cells were blocked with phosphate-buffered saline containing 1 mM MgCl2, 0.5 mM CaCl2, 3% BSA, and 1% goat serum and incubated with antibodies diluted 1:100 (unless stated otherwise) in the same buffer at room temperature for 1 h. The cells were incubated with mouse anti-FLAG M2 (Sigma) and either rabbit anti-calnexin (Stressgen) or anti-α-mannosidase II (USBiological; 1:25) followed by treatment with Alexa Fluor 488 (green fluorescence)-conjugated goat anti-mouse IgG and Alexa Fluor 594 (red fluorescence)-conjugated goat anti-rabbit IgG (Molecular Probes). The coverslips were mounted in phosphate-buffered saline containing 10% glycerol and 12% triethyldiamine (Sigma) to prevent fluorescent bleaching and visualized using a Bio-Rad Radiance 2000 confocal microscope.

Affinity Purification of MTP-FLAG—COS-7 cells in 150-mm dishes were transiently transfected with either hMTP- or dMTP-FLAG expression plasmids, washed with phosphate-buffered saline, and lysed by incubating in hypotonic buffer (1 mM Tris, pH 7.4, 1 mM MgCl2, 1 mM EGTA, and protease inhibitor mixture) as previously described (13, 14). The lysates were then centrifuged (SW55 Ti rotor, 50,000 rpm, 1 h, 4 °C), and the supernatants were adjusted to 10 mM Tris, pH 7.4, 150 mM NaCl and mixed by rotation with M2-agarose beads (Sigma) for 3 h at 4 °C. The beads were pelleted by centrifugation (10,000 rpm, 4 °C, 10 s) and washed three times with 10 mM Tris, pH 7.4, 150 mM NaCl and protease inhibitor. To elute the bound proteins, the beads were rotated with 300 ng/μl of FLAG peptide (DYKDDDDK) in the same buffer for 2 h at 4 °C. The samples were centrifuged (10,000 rpm, 4 °C, 10 s), and the supernatants containing MTP-FLAG were collected.

Lipid Transfer Assays—Radiolabeled lipid transfer assays were performed according to the method described by Wetterau and Zilversmit (11, 12). To prepare unilamellar donor vesicles 1800 nmol of phosphatidylcholine (Avanti Polar Lipids), 3.6 nmol of either [14C]triaclylglycerol, [14C]phosphatidylcholine (New England Nuclear), or [3H]cholesterol.
ester (Amersham Biosciences), and 135 nmol of cardiolipin (Sigma) were dried under nitrogen, resuspended in 4.5 ml of buffer (15 mM Tris, 40 mM NaCl, 1 mM EDTA, and 0.02% sodium azide, pH 7.4), and sonicated. Acceptor vesicles containing 10,800 nmol of phosphatidylcholine were prepared similarly. The vesicles were then centrifuged (50,000 rpm, 15 °C, 1 h, SW55 Ti rotor), and the top 4-ml fraction was collected for activity determinations. The reaction mixture (final volume, 0.5 ml) contained 100 μl of donor and acceptor vesicles in 10 mM Tris-HCl, pH 7.4, 0.1% BSA, and 150 mM NaCl. M2 affinity-purified MTP (100 μl) was added to the reaction mixture and incubated for 2 h at 37 °C. The reaction was stopped by the addition of 0.5 ml of DE52 anion exchange resin (1:1, v/v, suspension in 15 mM Tris, 1 mM EDTA, 0.02% sodium azide, pH 7.4), and sonicated. Radiolabeled lipids in supernatants (500 μl) were measured by scintillation counting. Blank assays were performed in the absence of purified proteins. Total radioactivity present in the assays was measured by omitting DE52 from the stop buffer. Lipid transfer (% transfer/2 h) was determined.

Transfer of fluorescently labeled lipids was assayed as described previously (13, 14). Briefly, donor vesicles containing nitrobenzoaxadiazole-labeled triacylglycerols (Chylos, Inc.) and phosphatidylethanolamine (14) were incubated with acceptor vesicles in the presence of equal amounts of total cell protein lysates or purified hMTP- and dMTP-FLAG proteins for the indicated times. Increases in fluorescence during transfer were recorded, and the percentage of transfer was calculated.

**MTP Gene Deletion Studies—**MTP<sup>−/−</sup>Drosophila mexicano Cre mice (21) were obtained from Jackson Laboratories and bred at SUNY Downstate Medical Center. The mice were injected once with either phosphate-buffered saline or plpc (250 μg). After 48 h, the livers were collected and used to measure lipid transfer activities of MTP as described before (13, 14).

**RESULTS**

We previously showed that dMTP promotes human apoB34 and apoB41 secretion (17). Here we explored how dMTP aids in the secretion of apoB. First, we asked whether apoB secretion supported by dMTP is modulated by oleic acid (OA) supplementation. Transfection of COS cells with apoB48 expressing plasmids resulted in barely detectable apoB levels in the media with or without OA supplementation (Fig. 1, A and B). However, co-transfection with hMTP significantly increased (~20-fold) the secretion of apoB48, and OA supplementation further enhanced its secretion by 25.6 ± 6.9% (p = 0.003) (Fig. 1A). Similar to hMTP, co-expression of dMTP and apoB48 resulted in increased secretion of apoB48 (~7-fold) that was further augmented (80.8 ± 21.6%, p = 0.004) by OA supplementation (Fig. 1B). Next, we explored the effect of OA supplementation on the density of secreted lipoproteins. In the absence of OA, apoB secreted from COS cells expressing hMTP or dMTP was present in fractions 3–6 corresponding to a density of 1.1–1.15 g/ml (Fig. 1, C–E). Although OA increased the total apoB secreted, it did not change the density of the secreted lipoproteins (Fig. 1, compare C and D). These studies indicate that apoB secretion driven by dMTP expression is responsive to OA supplementation and produces lipoproteins with properties similar to those secreted in the presence of hMTP.

We observed that dMTP can render apoB48 secretion-competent, but cells expressing dMTP consistently secreted less apoB than those expressing hMTP (Fig. 1). To understand why dMTP was less efficient in promoting apoB secretion, we considered two possibilities. First, the reduction might be due to the lower expression of dMTP compared with hMTP in COS cells. Second, different levels of apoB expression might be due to variations in co-transfection efficiency. To obtain equal expression, the cells were transfected with expression plasmids containing sequences for apoB18 through apoB72 and subsequently transfected with expression vectors containing either hMTP-FLAG or dMTP-FLAG. The relative expression of the different MTPs was determined by Western blotting. apoB18-expressing cells synthesized similar amounts of hMTP- and dMTP-FLAG (Fig. 2A, inset), and apoB18 secretion was not affected by the co-expression of either MTP (Fig. 2A) consistent, with previous studies (22, 23). Next, COS cells were transfected with apoB48 and subsequently transfected with...
FIGURE 3. Subcellular localization of human and Drosophila MTP-FLAG. COS cells transfected with apoB48 and either hMTP-FLAG (d–f) or dMTP-FLAG (g–i) were grown on coverslips in 24-well tissue culture dishes. After 48 h, the cells were fixed and treated as described under "Materials and Methods." A, cells were stained with anti-FLAG M2 to label MTP (panels a, d, and g; green fluorescence) and anti-calnexin (panels b, e, and h; red fluorescence) as a marker for endoplasmic reticulum. Co-localization of MTP with the endoplasmic reticulum was demonstrated in the merged images (panels c, f, and i; yellow fluorescence). B, cells were incubated with anti-FLAG (panels a and d; green) and anti-α-mannosidase II (panels b and e; red) to label the Golgi. The merged images (panels c and f; yellow) illustrated co-localization of MTP with the Golgi marker.
either hMTP- or dMTP-FLAG. The amount of apoB48 secreted by dMTP-transfected cells was \( \frac{1}{3} \) of the level observed in hMTP-expressing cells (Fig. 2B). However, after correcting for MTP protein levels (Fig. 2B, inset), we calculated that dMTP was \( \frac{1}{2} \) as effective as hMTP in promoting apoB48 secretion. Utilizing similar correction methods it was calculated that dMTP-FLAG was \( \frac{1}{2} \) and \( \frac{2}{3} \) as efficient as the hMTP-FLAG in assisting apoB53 (Fig. 2C) and apoB72 (Fig. 2D) secretion, respectively. These studies suggest that dMTP can support the secretion of longer apoB polypeptides; however, it is less efficient compared with hMTP.

Consideration was given to the possibility that dMTP is less proficient in supporting apoB secretion because of incorrect subcellular distribution. MTP is present in the endoplasmic reticulum due in part to association with protein disulfide isomerase (PDI) (24, 25) and has also been localized to the Golgi apparatus (26, 27). The subcellular distri-
Lipid transfer (Fig. 5) of orthologs were equipotent in time-dependent initial and maximum fluorescently labeled phosphatidyl-ethanolamine showed that both reached saturation with time. Similar studies with donor vesicles containing fluorescence in the presence of dMTP were minimal and did not appear to saturate with time. In addition to endoplasmic reticulum, hMTP- and dMTP-FLAG (Fig. 3B, panels a and d) co-localized with the Golgi marker protein α-mannosidase II (Fig. 3B, panels b and e). This was confirmed in the merged images (Fig. 3B, c and f, yellow fluorescence). These studies indicate similar distribution of dMTP- and hMTP-FLAG chimeras in the endoplasmic reticulum and Golgi apparatus.

Mammalian MTP is a heterodimeric complex of a 97-kDa “M” subunit and a 55-kDa PDI subunit (28, 29). We therefore determined whether dMTP also associates with endogenous PDI. hMTP-FLAG and dMTP-FLAG were affinity-purifed using M2 (anti-FLAG monoclonal antibody)-agarose from transiently transfected COS cell lysates (Fig. 4). Polyacrylamide gel electrophoresis followed by silver staining demonstrated two predominant protein bands of ~95 and 55 kDa in dMTP- and hMTP-transfected cells, but not in control cells (Fig. 4A, compare control lane 2 with lanes 3 and 4). These were shown to be the M subunit and PDI by Western blotting using specific antisera (Fig. 4B), indicating that dMTP interacts with endogenous PDI similarly to hMTP.

We then sought to understand which property of dMTP is critical for its ability to render apoB secretion competent by comparing the specificity of lipid transfer between hMTP and dMTP. Although the lysates from cells that transiently expressed hMTP demonstrated a measurable ability to transfer triacylglycerols (0.71 ± 0.04% triacylglycerol transfer/µg of protein/h), no significant increase was observed using lysates obtained from cells that expressed dMTP (0 ± 0.04% triacylglycerol transfer/µg of protein/h) (Fig. 5A). The lack of triacylglycerol transfer activity in dMTP is similar to what we previously reported (17). To demonstrate that dMTP was expressed, we performed similar experiments using lysates from hMTP- or dMTP-FLAG-expressing cells (Fig. 5B). Once more, the lysates from cells expressing hMTP-FLAG, but not dMTP-FLAG transfected triacylglycerols. Furthermore, equal amounts of MTP were present in the lysates as assessed by Western blot (Fig. 5B, inset). Thus, dMTP appeared to lack the ability to transfer triacylglycerols.

Next, we studied the phospholipid transfer activities of human and Drosophila MTP. We were unable to measure significant phospholipid transfer in cell lysates obtained from COS cells expressing either of the MTP orthologs (data not shown). We therefore assayed (13, 14) for these activities using purified hMTP and dMTP (Fig. 5, C and D). The transfer of triacylglycerols by hMTP-FLAG increased with time and reached saturation (Fig. 5C). However, increases in triacylglycerol fluorescence in the presence of dMTP were minimal and did not appear to saturate with time. Similar studies with donor vesicles containing fluorescently labeled phosphatidyl-ethanolamine showed that both orthologs were equipotent in time-dependent initial and maximum lipid transfer (Fig. 5D). These data indicate that hMTP and dMTP can transfer phospholipids, but dMTP is deficient in triacylglycerol transfer activity.

The lipid transfer activities of purified proteins were also measured employing a classical radiolabeled vesicle transfer assay (11, 12). hMTP-FLAG showed a robust triacylglycerol transfer activity (19.2 ± 1.1%), whereas only minimal transfer was detected using purified dMTP-FLAG (0.6 ± 0.2%) (Fig. 6A), confirming data from Fig. 5. Likewise, hMTP could transfer cholesterol esters (3.7 ± 0.2%); however, this activity was undetectable in dMTP-FLAG (Fig. 6B). In contrast to the transfer of triacylglycerols and cholesterol esters, dMTP and hMTP were equally active in transferring phospholipids (2.6 ± 0.4 and 3.1 ± 0.1%, respectively; Fig. 6C). These studies showed that dMTP was deficient in the transfer of neutral lipids, compared with hMTP, but was as efficient as hMTP in phospholipid transfer.

It has been reported that MTP antagonist, BMS 200150, does not affect phospholipid transfer but does inhibit triacylglycerol transfer activity at low concentrations in HepG2 cell lysates (30). We evaluated the effect of BMS 200150 on both phospholipid and triacylglycerol transfer activities using HepG2 cell lysates and purified MTP. Low concentrations of BMS 200150 inhibited triacylglycerol transfer without inhibiting phospholipid transfer activity (Fig. 7A) and reduced apoB secretion (Fig. 7B) in HepG2 cells. However, low concentrations of the compound inhibited both phospholipid and triacylglycerol transfer activities of the purified MTP (Fig. 7C). In addition, we studied the effect of MTP gene deletion on cellular lipid transfer activities. Conditional deletion of MTP gene resulted in a 72% decrease in triacylglycerol activity but had no effect on phospholipid transfer activity in liver homoge-
Using the fluorescence-based assay. In assays are represented as the means apoB secretion was measured by ELISA. Triplicate cells were treated with inhibitor overnight, and lysates (respectively). MTP lipid transfer activities of cell activities were measured using 20 acylglycerol and phospholipid transfer specific additions (Fig. 7). We conclude that incomplete inhibition of phospholipid transfer activity by BMS 200150 in HepG2 cell lysates and no inhibition in Mttp gene deleted liver homogenates is due to the presence of other phospholipid transfer activities.

**DISCUSSION**

The data presented here show that dMTP promotes the secretion of apoB polypeptides but is less efficient than hMTP. Furthermore, dMTP responds to lipid availability, as does its human ortholog. However, although dMTP and hMTP can both transfer phospholipids, dMTP displays virtually no neutral lipid transfer activity. Based on these observations, we propose that the phospholipid transfer activity of MTP may be necessary and sufficient for the assembly and secretion of primordial apoB lipoproteins. The presence of neutral lipid transfer activity may increase the efficiency by which apoB lipoproteins are assembled, or alternatively, might be involved primarily in lipid accretion and second step particle core expansion.

Recently, we showed that dMTP was able to support the secretion of apoB34 and apoB41 (17). These apoB polypeptides do not contain the complete hydrophobic β-sheet domains critical for the assembly of larger lipoproteins. Thus, we had hypothesized that dMTP would not support the secretion of longer apoB polypeptides, which contain more extensive lipid-binding β-sheet domains. Instead, we found that dMTP rescued the secretion of apoB48, which contains the complete β1 domain, as well as apoB72, which contains both the β-1 and a major portion of the β2 domain. These studies suggest that dMTP is capable of lipidating apoB polypeptides with long hydrophobic β-sheets, rendering them secretion-competent.

The nascent apoB polypeptide is believed to interact with the inner leaflet of the endoplasmic reticulum membrane. Its release from the membrane and conversion to a lipoprotein particle is critically dependent on MTP (reviewed in Refs. 2, 6, and 31–34). In the absence of MTP, nascent apoB folds incorrectly and undergoes proteasomal degradation. Although mammalian MTP has been shown to transfer triacylglycerols, cholesteryl esters, and phospholipids between membrane vesicles in vitro (11–14), its predominant activity is toward triacylglycerols (12, 14). Several antagonists of the lipid transfer activity of MTP are potent inhibitors of the triacylglycerol transfer activity (14, 30). Similarly, a missense mutation, N780Y, in MTP abolishes triacylglycerol transfer activity and also does not support apoB secretion (35, 36). Hence, it has been assumed that the triacylglycerol transfer activity of MTP is critical for the assembly of nascent apoB into lipoproteins. Here, we have presented evidence that dMTP lacks the ability to transfer triacylglycerols and yet is able to assist in the assembly and secretion of apoB lipoproteins. Thus, phospholipidation of the nascent apoB-polypeptide by MTP may be sufficient to inhibit proteasomal degradation and promote lipoprotein assembly.

It has long been suggested that apoB lipoprotein assembly occurs in two steps. These steps involve the synthesis of primordial lipoprotein particles that contain only small amounts of neutral lipid, followed by the bulk addition of neutral lipid via fusion with triacylglycerol droplets. Based on the present data, we propose that the first step may involve phospholipidation of nascent apoB by MTP followed by the budding of primordial particles from the endoplasmic reticulum membrane. In our previous studies, we observed that the apoB particles secreted in the presence of dMTP and hMTP contained a similar content of triacylglycerols (17). How did these particles acquire triacylglycerols in the absence of a neutral lipid transfer activity? It is known that neutral lipids have low solubility within phospholipid bilayers and tend to diffuse rapidly within the plane of the membrane (37). It is possible that after MTP-dependent priming with phospholipids, the initiating domain of apoB (38) inserts into the membrane and functions to trap neutral lipids
in the form of a small oil droplet between the leaflets of the bilayer. At a critical stage during its translation, apoB is released from the endoplasmic reticulum membrane in the form of a nascent triglyceride core-containing emulsion particle. Subsequently, the particle can be further enriched with neutral lipids by MTP, particularly during second step core expansion.

Comparisons of the lipid transfer activities in hMTP and dMTP revealed that both orthologs were equipotent in transferring phospholipids. Using a classical radiolabel assay, we were unable to measure any neutral lipid transfer activity associated with dMTP. However, employing a more sensitive fluorescent assay, we did detect the transfer of small amounts of triacylglycerols (Fig. 6D). This transfer activity, however, did not appear to saturate with time and was ~10-fold lower than that observed in hMTP. Thus, we conclude that the activity detected in the fluorescent assay most likely represents a background, nonspecific transfer and that dMTP lacks the robust neutral lipid transfer activity, which is a hallmark of hMTP.

At first glance, the data obtained with BMS 200150 appear to contradict the conclusion that phospholipid is sufficient for apoB lipoprotein assembly because BMS 200150 inhibits triacylglycerol transfer activity but not the phospholipid transfer activity in HepG2 cell lysates (Fig. 7A). However, inhibition of purified MTP by BMS 200150 and MTP gene deletion studies clearly show that the liver expresses other proteins that can transfer phospholipids. In fact, phosphatidyl-choline and phosphatidylinositol transfer proteins have been purified, and their tissue distributions and biochemical characterizations have been reported (for review, see Refs. 39). Thus, with respect to phospholipid transfer, it is difficult to interpret studies using MTP antagonists and whole cell lysates. In contrast, the data presented here show clearly that purified hMTP and dMTP have comparable phospholipid transfer activities. Because dMTP cannot transfer triacylglycerol (Figs. 5 and 6), we suggest that phospholipid transfer activity may be sufficient for the assembly and secretion of apoB lipoproteins.

It was surprising to discover that dMTP lacks neutral lipid transfer activity because insects require exogenous sterols for their growth and viability (40). The major function of mammalian MTP is to assist in the transfer of dietary fat and sterols via chylomicron assembly and secretion. In insects, dietary lipids are transported by lipid transfer particles called lipophorins. Insect fat body, which performs the functions equivalent to vertebrate liver and adipose tissue, synthesizes and secretes high density lipophorins into the hemolymph. These particles acquire lipids from the midgut and fat body via the action of an extracellular lipid transfer particle and become low density lipophorins. These particles then interact with cell surface receptors to deliver lipids to their target tissues (40). Based on the data presented here and previous studies (17, 41), we propose that dMTP may play a crucial role in the intracellular assembly of high density lipophorins by the fat body and that this process may involve the phospholipidation of nascent apolipoporphin by MTP.

The phospholipid transfer activity of MTP may also be involved in the secretion of vitellogenin. Sellers et al. (41) have recently shown that a mutant hMTP, deficient in triacylglycerol transfer activity, can promote the secretion of Xenopus vitellogenin A1. It remains to be determined whether phospholipidation of vitellogenin is required for its secretion. We have recently shown that hMTP transfers phospholipids, but not triacylglycerols, to CD1d, a lipid antigen presenting molecule (42). Therefore, the phospholipid transfer activity of hMTP may also be important for the biogenesis and cell surface expression of CD1d and perhaps other CD1 family members (43).

MTP is a member of the large lipid transfer protein gene family that includes apoB, apolipoporphin and vitellogenin (44). It has been suggested that these genes are derived from a common ancestral MTP gene (41, 45). Based on the data presented here, we hypothesize that phospholipid transfer activity might have been evolutionarily the earliest lipid transfer activity acquired by MTP. During evolution, MTP might have acquired neutral lipid transfer activity as an adaptation to accommodate the expanding requirement for triacylglycerol transport in vertebrates.

There are interesting parallels between the evolution of lipid transfer activities and the regulation of lipid biosynthesis. The major pathway that regulates cholesterol and unsaturated fatty acid biosynthesis in mammalian cells is the SREBP pathway, which involves proteolysis of membrane bound transcription factors (46). Seegmiller et al. (47) showed that the Drosophila genome encodes all the components of the SREBP pathway. However, in contrast to mammals, the Drosophila SREBP pathway regulates enzymes involved in the biosynthesis of saturated fatty acids and not sterols. In turn, SREBP function is regulated by phosphatidylethanolamine in Drosophila and not sterols (48). It has been suggested that the SREBP pathway evolved to maintain the phospholipid composition of membranes and subsequently acquired sterol regulatory function following gene duplication (47). Thus, both lipid transfer and regulatory mechanisms might have been first developed for phospholipids and later evolved to include other lipids.

Potent MTP antagonists that inhibit both triglyceride and phospholipid transfer activities of MTP and decrease apoB secretion have been identified (49). Most of these compounds have been associated with specific adverse effects, primarily related to hepatic lipid accumulation. Based on the studies presented here, it might be useful to identify compounds that selectively inhibit either of the two lipid transfer activities. Compounds that inhibit only phospholipid transfer and, hence, only the first step in apoB assembly may reduce the number of apoB precursor particles competent to acquire lipids without perturbing MTP-dependent flux of triacylglycerols into the secretory pathway. Such classes of inhibitors may reduce the number of apoB-containing lipoproteins produced by the liver with a minimal disruption in the net export of triacylglycerols. Indeed, antisense oligonucleotide-mediated reduction in apoB expression in mouse liver was recently shown to reduce plasma apoB and cholesterol, without causing liver steatosis (50). Compounds that only inhibit triacylglycerol transfer activity might allow synthesis and secretion of primordial particles with small amounts of neutral lipids and help avoid overt toxicities found associated with MTP antagonists that inhibit both the lipid transfer activities.

In summary, although hMTP can transfer triacylglycerols and cholesterol esters, this activity is absent in dMTP. However, the ability to transfer phospholipids is retained at comparable levels in both the dMTP and hMTP orthologs. Because dMTP can support human apoB secretion, we conclude that phospholipid transfer activity is sufficient to support primordial apoB assembly and to promote lipoprotein assembly in response to lipid availability. The robust neutral lipid transfer activity present in mammalian MTPs might have evolved to increase the efficiency of lipoprotein assembly and to aid in the bulk transport of neutral lipids.

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