In Vitro Reconstitution of Assembly of Apolipoprotein B48-containing Lipoproteins*

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Human apolipoprotein B48 (apoB48) and apoB15 (the NH2-terminal 48 and 15% of apoB100, respectively) were translated in vitro from their respective mRNAs using a rabbit reticulocyte lysate and microsomes derived from rat liver or dog pancreas. Synthesis of phosphatidylcholine and triacylglycerols was reconstituted in freshly isolated microsomes by the addition of precursors of these glycerolipids (acylcarnitine, glycerol 3-phosphate, and CDP-choline) before, during, or after translation. Assembly of apoB15 and apoB48 with newly synthesized phospholipids and triacylglycerols was favored by active, co-translational lipid synthesis. Moreover, translocation of apoB48 but not apoB15 into the microsomal lumen was increased in the presence of co-translational lipid synthesis. When apoB48 was translated in vitro, approximately 50% of apoB48 was buoyant at a density of <1.10 g/ml in the lumen of liver microsomes only when lipid synthesis was reconstituted during translation. Microsomal triacylglycerol transfer protein has been proposed to be essential for lipidation and/or translocation of apoB48. However, apoB48 was translocated into the lumen of dog pancreas microsomes in which the activity of the microsomal triacylglycerol transfer protein was not detectable. These data indicate that (i) apoB15 and apoB48 bind newly synthesized phosphatidylcholine during translocation; (ii) apoB48 but not apoB15 associates co-translationally with triacylglycerols; (iii) translocation of apoB48 but not apoB15 is stimulated by lipid synthesis; (iv) assembly of buoyant apoB48-containing lipoproteins can be reconstituted in vitro in the presence of active lipid synthesis; and (v) even in microsomes lacking microsomal triacylglycerol transfer protein activity, apoB48 is translocated into the lumen.

Transport of apoB out of the ER appears to be the rate-limiting step in apoB secretion (23), and it has been suggested that translocation of apoB across the ER membrane is a key regulatory step in VLDL secretion (1, 6, 7, 24). One unique feature of apoB secretion is that, in contrast to the widely accepted model for secretion of typical secretory proteins, newly synthesized apoB appears to exist in two intracellular populations: a lipoprotein-associated form wholly within the ER lumen, and a membrane-associated form in which portions of apoB are exposed to the cytosol and are susceptible to degradation (7, 23, 25, 26). One hypothesis is that lipid supply determines how much apoB is translocated into the ER lumen, and apoB that is not translocated is subsequently degraded (27, 28). Evidence in support of exposure of some apoB to the cytosol is: (i) in intact microsomes some apoB is degraded by exogenously added proteases (1, 25, 29, 30), and (ii) in HepG2 cells apoB can be co-immunoprecipitated with the cytosolic heat-shock protein, hsp70 (31). However, the existence of cytosolic domains of apoB has been questioned (32, 33).

Recent studies by Lingappa and colleagues (34–37) on in vitro translation/translocation of carboxyl-truncated apoB variants have suggested that apoB undergoes an unusual translocation process in which the protein pauses transiently at distinct sites along the nascent chain during translocation. During translocation pausing, translation continues, and domains of apoB become exposed to the cytosol. ApoB chains that pause but fail to restart during translocation are proposed to remain }

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spanning the microsomal membrane, generating segments of apoB which are exposed to the cytosol and are degraded upon proteolysis of intact microsomes. A new class of topogenic sequences, pause transfer sequences, which are rich in charged amino acids, has been shown to mediate translational pausing (35, 37). The reason why apoB but not other secretory proteins contains pause transfer sequences is not known. However, we and others (34) speculate that translational pausing might provide time for apoB to associate co-translationally with lipids and/or to become folded into a secretion-competent form.

In the present study we have reconstituted in a cell-free system the assembly of apoB with triacylglycerols and phosphatidylcholine and have generated bovuitant lipoprotein particles in the microsomal lumen. Translocation of apoB48 but not the truncated apoB variant apoB15 is stimulated by active lipid synthesis. In addition, the ability of apoB48 to translocate in vitro into the lumen of dog pancreas microsomes, which lack MTP activity, suggests that MTP is not absolutely required for translocation of apoB48 into the ER lumen.

**EXPERIMENTAL PROCEDURES**

**Materials—**[35S]Methionine, [14C]CDP-choline, [14C]triolein, and Amplify were purchased from Amersham Canada. and Tran35S-label from ICN. [3H]Oleoyl-CoA was chemically synthesized (38). Polyvinylidene difluoride membranes were obtained from Millipore. The cDNA encoding β-lactamase and reagents used for transcription of cDNAs were from Promega as was the rabbit reticulocyte lysate used for *in vitro* translation. Triton X-100, tosylphenylalanyl chloromethyl ketone-treated trypsin, soybean trypsin inhibitor, proteinase K, proteinase A-Sepharose, and micrococcal nucleases were purchased from Sigma. Sepharose 4B was from Pharmacia. The sheep anti-human apoB100 polyclonal antibody was purchased from Boehringer Mannheim. The polyclonal antibody directed against β-lactamase was as described previously (35). All reagents used for polyacrylamide gel electrophoresis were from Bio-Rad. The BCA protein assay kit was from Pierce. All reagents used for polyacrylamide gel electrophoresis were from the respective companies as previously described (35, 37). The reason why apoB but not other secretory proteins was released from the Sepharose by boiling the beads for 3 min in buffer containing 8 mM urea and 2% SDS.

**Reconstitution of Lipid Synthesis in Rat Liver Microsomes—**Freshly isolated microsomes were included in the translation mixture (39) with precursors of glycerolipid biosynthesis: oleoyl-CoA (50 μM), glycerol 3-phosphate (100 μM), and CDP-choline (1.5 μM). Lipid precursors were added either for 1 h at 30 °C or for 1 h at 30 °C after initiation of translation or were added for 1 h at 30 °C before the addition of mRNA. In some instances, lipid precursors were added post-translationally for 1 h at 30 °C after translation had been terminated by the addition of emetine (0.1 μM) for 15 min at 25 °C (35). In some translation experiments, microsomes were omitted. To determine the extent of co-translational association of triacylglycerols or phosphatidylcholine with newly translated proteins, 1 μCi of [3H]Oleoyl-CoA or 1 μCi of [14C]CDP-choline, respectively, was included in the translation reaction. After translation, microsomes were reisolated and luminal contents released with sodium carbonate as above. Newly synthesized apoB and β-lactamase were isolated from luminal contents by immunoprecipitation under nondenaturing conditions using Sepharose 4B linked covalently to polyclonal antibodies directed against either human apoB100 or β-lactamase (1). Lipids were extracted from the beads (42), and phosphatidylcholine and triacylglycerols were isolated by thin layer chromatography in a two-solvent system consisting of chloroform:methanol:acetic acid:water, 70:30:12:4:2 (v/v) followed by hexane:isopropyl ether:acetic acid:water, 80:20:12:4:2 (v/v). Protein degradation was measured by measurement of radioactivity in the supernatant and in the precipitate (1). Radiolabeling of proteins with [35S]S and of lipids (phosphatidylcholine with [14C]triacylglycerol with [3H]associated with newly translated proteins, was determined.

**Electrophoresis and Autoradiography—**Proteins were separated by electrophoresis on 7% polyacrylamide minigels containing 0.1% SDS and then transferred to polyvinylidene difluoride membranes for 60 min at 100 volts, and used for autoradiography (1). In some experiments, minigels were impregnated with Amplify and dried. Membranes or dried gels were autoradiographed for 1–16 h at −70 °C.

**Assay of MTP Activity—**Lipid transfer from donor membranes to acceptor membranes was measured in an assay similar to that described previously by Wetterau et al. (19). Donor and acceptor small unilamellar vesicles were prepared by bath sonication in 15 mM Tris- HCl (pH 7.5) containing 1 mM EDTA, 40 mM NaCl, and 0.02% sodium azide. The lipid transfer assay mixture contained donor membranes (40 nmol of egg phosphatidylcholine, 7.5 mol % cardiolipin, and 0.25 mol % glycerol tri-[1-14C]oleate), acceptor membranes (240 nmol of egg phosphatidylcholine), and 5.0 mg of bovine serum albumin in a total volume of 0.7 μl. The reaction was started by the addition of a source of MTP. After 60 min the reaction was terminated by the addition of 0.5 ml of DE32 buffer (15 mM Tris-HCl (pH 7.5) containing 1 mM EDTA, 40 mM NaCl, and 0.02% sodium azide). The mixture was agitated for 5 min and then centrifuged at maximum speed in a Biofuge B centrifuge (Baxter Scientific, McGraw Park, IL) for 3 min to pellet the DE32 containing bound donor vesicles. Radioactivity was measured in 0.5 ml of supernatant. Background transfer in the absence of MTP was subtracted to calculate MTP-mediated transfer of triacylglycerol from donor vesicles.
RESULTS
ApoB15 and ApoB48 Co-translationally Bind Newly Synthesized Phosphatidylcholine—As a prerequisite for secretion, apoB100 and apoB48 are assembled into lipoprotein particles consisting of a hydrophobic core of neutral lipids (primarily triacylglycerols with some cholesteryl esters) covered by a surface monolayer of phospholipids, unesterified cholesterol, and apoproteins. Unlike large native apoB, apoB15 (the recombinant NH2-terminal 15% of human apoB100) has a density of >1.2 g/ml when secreted, implying that it is associated with little neutral core lipid. However, several hydrophobic domains, representative of those occurring throughout the apoB100 molecule, are present within apoB15. These regions are assumed to bind lipids in the assembled lipoprotein particle (43–45). The possibility exists, therefore, that some lipids, particularly phospholipids, are associated with secreted apoB15. Indeed, apoB17 is secreted in association with some triacylglycerols and cholesteryl esters (46), and the co-translational assembly of phospholipids with apoB has been suggested previously (1, 47).

The co-translational association of phospholipid with apoB15 was investigated in in vitro translation experiments in which apoB15 mRNA was translated in the presence of [35S]methionine and in the absence or presence of rat liver microsomes. Precursors of phosphatidylcholine biosynthesis (oleoyl-CoA, glycerol 3-phosphate, and [14C]CDP-choline) were included either before or during translation. Alternatively, lipid precursors were added post-translationally, after the addition of emetine, which terminates translation (35). After the reaction was complete, microsomes were reisolated, and luminal contents and loosely bound membrane proteins were released by sodium carbonate treatment. ApoB15 was isolated from the sodium carbonate extract by immunoaffinity chromatography under non-denaturing conditions using anti-human apoB100 antibody linked to Sepharose 4B (1). ApoB15-bound lipids were extracted from the beads, and incorporation of 35S into phosphatidylcholine was measured. Subsequently, 35S-proteins were extracted from the Sepharose. When microsomes were omitted from the translation mixture, very little [14C]phosphatidylcholine was bound to apoB15 (Table I). However, when microsomes were present and precursors of phosphatidylcholine biosynthesis were added either before or during translation, luminal apoB15 readily associated with [14C]phosphatidylcholine. In contrast, when lipid precursors were added post-translationally, the amount of [14C]phosphatidylcholine bound to apoB15 was less than 14% of that when the protein was translated in the presence of lipid precursors. The 14C/35S ratio in immunoprecipitated apoB15 (Table I) is indicative of how much newly synthesized phosphatidylcholine is co-translationally associated with newly translated apoB15. This ratio was 9–11-fold higher when precursors of glycerolipid biosynthesis were added during (ratio = 1.14 ± 0.12) or before (ratio = 1.42 ± 0.03) translation than when added post-translationally (ratio = 0.13 ± 0.06) (Table I). These data imply that once apoB15 is translocated into the lumen relatively little phosphatidylcholine subsequently binds to apoB15. The amount of luminal [35S]apoB15 was similar whether lipid precursors were present or absent during translation.

In parallel experiments, lipid precursors were added during translation of the typical secretory protein β-lactamase in the presence of microsomes. Negligible amounts of [14C]phosphatidylcholine were associated with immunoprecipitated β-lactamase (Table I). These observations demonstrate that apoB15 but not β-lactamase co-translationally associates with phosphatidylcholine.

ApoB48 was also successfully translated in vitro in both the presence and absence of microsomes (Table II). As was the case for apoB15, more [14C]phosphatidylcholine was associated with apoB48 when lipid precursors were added pre- or co-translationally (14C/35S ratio = 1.77 ± 0.61 and 1.75 ± 0.49, respectively), than post-translationally (14C/35S ratio = 1.19 ± 0.72) (Table II). These experiments also reveal that relatively more phosphatidylcholine associates post-translationally with apoB48 (14C/35S ratio = 1.19 ± 0.72, Table II) than with apoB15 (14C/35S ratio = 0.13 ± 0.06, Table I). Although we cannot fully explain this phenomenon, the greater post-translational association of phospholipid with apoB48 than with apoB15 might be related to a less efficient translation/translocation of apoB48 (because of its large size of ~225,000 kDa) compared with apoB15 (~70 kDa). Alternatively, the greater association of lipids post-translationally with B48 than with B15 might reflect the presence of more lipid binding domains in apoB48 than in apoB15 (43, 35), allowing more post-translational assembly of phosphatidylcholine with apoB48. Nevertheless, these experiments demonstrate that both apoB15 and apoB48 bind phosphatidylcholine during translation/translocation.

The data in Table II also show that when active lipid synthesis was reconstituted during translation, approximately...
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Twice as much \(^{35}S\)apoB48 was present in the sodium carbonate extract (either translocated into the microsomal lumen or loosely membrane bound) as when lipid synthesis was reconstituted after translation. A similar conclusion can be drawn from the experiments depicted in Table III and Fig. 1 (see below).

**ApoB48 Co-translationally Binds Triacylglycerols More Efficiently Than Does ApoB15**—Since in vivo apoB48 but not apoB15 is secreted in association with a neutral lipid core, we next investigated whether or not these apoproteins bound newly synthesized triacylglycerols during *in vitro* translation/translocation. Precursors of triacylglycerol biosynthesis (\(^{[3}H\)oleoyl-CoA and glycerol 3-phosphate) were added to the translation mixture before, during, or after translation. ApoB48 co-translationally associated with newly synthesized triacylglycerols (Table III). When lipid precursors were added after, rather than before or during translation, markedly less \(^{[3}H\)triacylglycerol bound to apoB48. When lipid precursors were added co-translationally or pre-translationally, the \(^{3}H/\(^{35}S\) ratio in immunoprecipitated apoB48 was 0.82 ± 0.30 and 0.79 ± 0.26, respectively, whereas this ratio was −45% less when lipid precursors were added post-translationally (\(^{3}H/\(^{35}S\) ratio = 0.43 ± 0.15). A negligible amount of \(^{[3}H\)triacylglycerol co-translationally associated with \(\beta\)-lactamase (data not shown). Moreover, as is the situation with apoB15 secreted from intact cells, far less newly synthesized triacylglycerol co-translationally associated with apoB15 (\(^{3}H/\(^{35}S\) ratio = 0.09 ± 0.01) than with apoB48 (ratio = 0.82 ± 0.30) (Table III).

These experiments indicate that apoB48 binds newly synthesized phosphatidylcholine and triacylglycerols during translation/translocation, whereas apoB15 associates co-translationally with newly synthesized phosphatidylcholine but not triacylglycerols.

**Active Lipid Synthesis Stimulates ApoB48 Translocation**—Tables II and III indicate that translocation of newly synthesized apoB48 into the ER lumen was stimulated −2-fold when the translation mixture was supplemented with precursors of glycerolipid synthesis pre-translationally or co-translationally, rather than post-translationally. In contrast, translocation of apoB15 was not enhanced when lipid biosynthetic precursors were present during translation (Table I).

The impact of active lipid synthesis on apoB48 translocation was also assessed by analysis of the mass of apoB48 present in the microsomal lumen. Lipid precursors were either absent or present during translation. After translation was complete, microsomes were reisolated, luminal contents and loosely bound membrane proteins were separated from membranes by sodium carbonate treatment, and proteins were separated by polyacrylamide gel electrophoresis. The distribution of apoB48 between microsomal membranes and luminal contents was determined by autoradiography and densitometric scanning of the films. As shown in Fig. 1, when lipid precursors were added post-translationally, the majority (85.7%) of newly translated apoB48 was tightly associated with microsomal membranes, with only a small amount (14.3%) being extractable by sodium carbonate and therefore being either in the lumen or loosely membrane-associated. In contrast, when lipid synthesis was active during translation, the majority of apoB48 (74.5%) was translocated into the microsomal lumen, with a smaller amount (25.5%) being membrane-associated. These experiments demonstrate that active glycerolipid synthesis promotes apoB48 translocation into the microsomal lumen.

**MTP Is Not Required for Translocation of ApoB48 into the Lumen of Dog Pancreas Microsomes**—One factor that might be expected to control the extent of apoB lipiddation and the rate of apoB translocation is the ER luminal protein, MTP. This protein is expressed in liver and intestine but not in pancreas (12). Therefore, the dependence of translocation of apoB48 on the presence of MTP was determined by *in vitro* translation of apoB48 in the presence of lipid precursors and either dog pancreas microsomes or rat liver microsomes. After translation, the extent of translocation of apoB48 into the microsomal lumen was assessed by a protease protection assay in which proteins of intact microsomes were assumed to be luminal if they were resistant to proteolysis by exogenously added protease (48). Fig. 2 shows that translation of apoB48 occurred approximately equally efficiently in the presence or absence of rat liver microsomes or dog pancreas microsomes. In the *in vitro* translation system, endogenous mRNAs are stripped from membranes before translation is initiated; therefore only species of mRNA that are added to the translation mixture (in this case for apoB48 and SLSTgG) are translated. Fig. 2 shows that the incubation without microsomes, or in incubations with rat liver microsomes, by a translation product corresponding to apoB48 was detected on the gel. However, with dog pancreas microsomes a doublet was evident in the region of apoB48. The upper band of the doublet clearly corresponds to full-length apoB48 as synthesized in incubations without microsomes or with liver microsomes. The lower band of the doublet is probably an alternative translation product derived from apoB48.

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**Table III**

| Construct | Lipid synthesis reconstitution | \(^{3}H\)TG | \(^{3}S\)-Protein | \(^{3}H/\(^{35}S\) (× 10⁻¹) |
|-----------|-------------------------------|-------------|----------------|------------------|
| ApoB48    | No microsomes                 | 0.70 ± 0.01 | 12.01 ± 1.98   | 0.09 ± 0.02      |
| ApoB48    | Co-trans                      | 8.23 ± 3.33 | 10.01 ± 2.03   | 0.82 ± 0.30      |
| ApoB48    | Post-trans                    | 2.02 ± 0.90 | 4.58 ± 1.31    | 0.43 ± 0.15      |
| ApoB48    | Pre-trans                     | 6.61 ± 2.30 | 8.41 ± 2.02    | 0.79 ± 0.06      |
| ApoB15    | Co-trans                      | 1.05 ± 0.02 | 11.86 ± 2.01   | 0.09 ± 0.01      |

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mRNA, since apoB48 mRNA and SLSTgG mRNA were the only mRNAs present. One possible explanation is that the smaller apoB48 mRNA translation product was generated by translation from an alternative start site. Another possible origin of apoB48 mRNA translation product was generated by translation of a truncated species of apoB48 is from translation of a partial degradation product of full-length apoB48 mRNA.

When rat liver microsomes were treated with proteinase K after translation, nearly all apoB48 was resistant to digestion and was therefore luminal. The lack of digestion of apoB48 by proteinase K was not due either to apoB48 being inherently resistant to proteolysis by proteinase K or to the protease being inactive, because in the presence of the detergent Triton X-100 all apoB48 was degraded by proteinase K. In dog pancreas microsomes, the upper band of the apoB48 doublet (corresponding to full-length apoB48) was almost completely resistant to digestion by exogenously added proteinase K. In contrast, the protein corresponding to the lower band of the doublet was not protected from exogenous protease and was therefore exposed on the outside of the microsomes. These data indicate that the truncated apoB48 species might not contain the NH₂-terminal region of the full-length apoB48 and might therefore lack the signal sequence. Such a protein would be translated but not translocated across the membrane.

As a control to confirm the protease susceptibility of cytosolic portions of a transmembrane protein in our assay, the chimeric protein SLSTgG was co-translated with apoB48 in the same incubation mixtures. SLSTgG contains a stop transfer sequence and therefore has a transmembrane orientation (35). Almost all of the SLSTgG was cleaved when microsomes from dog pancreas and rat liver were treated with proteinase K, and a ~14-kDa fragment of the protein was generated which was resistant to proteolysis and was therefore luminal (Fig. 2). In the presence of detergent, the luminal fragment of SLSTgG was completely degraded by proteinase K. These data confirm the transmembrane disposition of this chimera and demonstrate that proteinase K had access to cytosolic domains of newly synthesized polypeptide chains. These experiments indicate that apoB48 is translocated efficiently into the microsomal lumen, even in pancreatic microsomes that have been reported to lack MTP (12). In vitro translated apoB15 was also translocated into the microsomal lumen equally efficiently in the presence of microsomes derived from either dog pancreas or rat liver (data not shown).

The mRNA for MTP is not detectable in human pancreas (12). As confirmation that dog pancreas microsomes also lack MTP activity, we compared the ability of microsomes from rat liver and dog pancreas to transfer triacylglycerols between membranes in vitro in a standard assay for MTP activity (19). Table IV shows that dog pancreas microsomes, both those purchased from Promega and those prepared in our laboratory, possessed only ~0.1% of the triacylglycerol transfer activity of rat liver microsomes.

These experiments suggest that in the in vitro translation system apoB48 translocates across microsomal membranes equally efficiently in the presence or absence of MTP activity.

**TABLE IV**

| Source of microsomes | MTP activity (% transfer/100 μg protein) |
|----------------------|----------------------------------------|
| Rat liver            | 33.77 ± 5.19                          |
| Dog pancreas (Vance) | 0.02 ± 0.01                            |
| Dog pancreas (Promega)| 0.04 ± 0.05                            |

**DISCUSSION**

**Role of Glycerolipid Synthesis in Translocation of ApoB15 and ApoB48**—We have examined the involvement of synthesis of triacylglycerols and phosphatidylcholine in translocation of apoB across microsomal membranes using an *in vitro* translation system. Human apoB48 (a component of triacylglycerol-rich lipoprotein particles) and apoB15 (which is secreted from cells in association with very little lipid at a density of >1.2 g/ml) were translated *in vitro* in the presence of rat liver microsomes. Translocation of newly synthesized proteins across the microsomal membrane was assessed by a protease protection assay and by isolation of microsomal luminal contents. Both apoB15 and apoB48 were translated and translocated across the membranes. During translation/translocation, glycerolipid synthesis was reconstituted, and newly synthesized phosphatidylcholine, which is the major phospholipid of all plasma lipoproteins, became co-translationally associated with both apoB15 and apoB48. In contrast, newly synthesized triacylglycerols bound to apoB48 but not to apoB15. Our finding that triacylglycerols assemble with apoB48 co-translationally *in vitro* agrees with the suggestion of Boström et al. (49) that
contain significant amounts of triacylglycerols (50, 51). How-
g/ml, whereas apoB molecules smaller than apoB28 are se-
in association with a neutral lipid core with density of
8024
buoyant lipoproteins. ApoB48 mRNA was translated for 1 h at 30 °C
approximately the NH2-terminal 28% of apoB100 are secreted
consistent with the observation that apoB variants longer than
begins before translation is complete. Our data are also con-
association of apoB with newly synthesized triacylglycerols
Triacsin D does not inhibit phosphatidylcholine synthesis,
apoB secretion (5). However, even under conditions for which
association of apoB48 but not apoB15 was stimulated when glycero-
phatidylcholine whereas triacylglycerols associate with apoB48
apoB15 and apoB48 co-translationally associate with phos-
phospholipid synthesis, for the following reasons. First, both
lipid synthesis were added co-translationally, with a corresponding reduction in the phosphati-
data indicate, therefore, that decreased synthesis of phosphati-
dylcholine content of microsomal membranes, does not restrict
apoB translocation.
The source of triacylglycerols used for assembly of apoB-
containing lipoproteins such as VLDL and the mechanism by
which these lipids are delivered to apoB are not yet clear.
Gibbons and co-workers (55–57) and Yang et al. (58, 59) have
proposed that the bulk of triacylglycerols associated with apoB
are derived from intracellular stores rather than from de novo
synthesis. The proposal is that cytosolic triacylglycerols are
hydrolyzed by a triacylglycerol lipase, the lipolysis products are
recycled in the ER, and the resulting triacylglycerols are
assembled with apoB. Some triacylglycerols associated with
apoB, however, are believed to be produced by de novo synthe-
sis from glycerol 3-phosphate in the ER membrane. In support
of this hypothesis, HepG2 cells do not assemble typical VLDL
particles but instead secrete apoB-containing particles having
densities corresponding to low and high density lipoproteins
(60) which are triacylglycerol-poor compared with VLDL. Triacy-
lipid synthesis were added co-translationally, ~50% of luminal apoB48
was in the form of lipoprotein particles with a density of <1.17
g/ml. In contrast, translocation of apoB15 into the microsomal
lumen was not stimulated by active glycerolipid synthesis. These
data support the hypothesis that active synthesis of triacylglycerols
and/or phosphatidylcholine stimulates the translocation of apoB species that form lipoprotein particles
containing a neutral lipid core. In contrast, translocation of
smaller truncated variants of apoB, which do not assemble into
triacylglycerol-rich lipoprotein particles, is not stimulated by
ongoing glycerolipid synthesis.
Although both triacylglycerol and phospholipid
synthesis were reconstituted in the in vitro translation
reactions, it is more likely that enhanced translocation of apoB48
was due to stimulation of triacylglycerol synthesis, rather than
phospholipid synthesis, for the following reasons. First, both
apoB15 and apoB48 co-translationally associate with phos-
atidylcholine whereas triacylglycerols associate with apoB48
but not significantly with apoB15. Correspondingly, transloca-
tion of apoB48 but not apoB15 was stimulated when glycerol-
lipid synthesis was reconstituted. Second, treatment of HepG2
cells with Triacsin D inhibits triacylglycerol synthesis and
apoB secretion (5). However, even under conditions for which
Triacsin D does not inhibit phosphatidylcholine synthesis,
though MTP protein and activity are apparently absent (63). Although the exact function of MTP in lipoprotein assembly has not been defined, MTP is thought to transfer triacylglycerols to newly synthesized apoB in the ER lumen. Indeed, MTP catalyzes the transfer of neutral lipids (triacylglycerols and cholesteryl esters) (16), and to a lesser extent phospholipids (18), between membranes in vitro. MTP also associates with apoB in HepG2 cells (61). Even though MTP transfers phosphatidylcholine between membranes with only ~10% of the efficiency of triacylglycerols (18), the number of molecules of phosphatidylcholine with which MTP would come into contact on the microsomal membrane is ~10 times higher than the number of triacylglycerol molecules at this location (64).

In our experiments, apoB48 was translocated approximately equally efficiently into the lumen of microsomes derived from rat liver (which contains robust MTP activity) and dog pancreas (which contains robust MTP activity) and dog pancreatic microsomes was in buoyant lipoprotein particles of density 1.10 g/ml in dog pancreas microsomes (data not shown). These observations suggest that although MTP might not be required for translocation of apoB48 across the membrane, MTP might be required for assembly of apoB48 with sufficient lipid for formation of lipoprotein particles that are stable in the aqueous environment of the ER lumen. In previous experiments using cultured cells transfected with truncated forms of apoB100 we have also observed that apoB translocation can be dissociated from assembly of apoB with a neutral lipid core (27). Our experiments do not, however, eliminate the possibility that our in vitro reconstituted system of lipoprotein assembly might be lacking some normal requirements for lipoprotein assembly which are present in an intact animal or that MTP might augment apoB translocation in vivo.

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