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Murine mammary-derived cells secrete the N-terminal 41% of human apolipoprotein B on high density lipoprotein-sized lipoproteins containing a triacylglycerol-rich core

(oleate/electron microscopy/brefeldin A)

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ABSTRACT **The cDNA encoding the N-terminal 41% of human apolipoprotein B (apoB), apoB-41, was transfected into nonhepatic, nonintestinal, mammary-derived mouse cells (C127) to generate stably transfected cells expressing human apoB-41 (C127B-41). As determined by centrifugation, apoB-41 is secreted exclusively on lipoproteins (LPs) having a peak density of 1.13 g/ml. Electron microscopy of apoB-41-containing LPs purified by immunoaffinity chromatography showed round particles about 12 nm in diameter. No discoidal particles were observed. Characterization of apoB-41-associated lipids after labeling C127B-41 cells with [^{3}H]oleate and immunoprecipitating the secreted LPs with antibodies to apoB showed that ^{3}H-labeled triacylglycerols were a major lipid class and accounted for about 54% of the total labeled lipids. Cholesterol esters and phospholipids accounted for about 6% and 22%, respectively. Incubation of cells with 0.4 mM oleate resulted in an increased incorporation of the added oleate into lipids associated with secreted apoB-41, along with a 2- to 3-fold increased secretion of apoB-41. The newly formed LPs appear to be transported through the Golgi complex, as brefeldin A (1 μg/ml) and monensin (1 μM) greatly reduced (>90%) the secretion of labeled apoB-41 and the amount of triacylglycerol and phospholipid associated with it. Microsomal triacylglycerol transfer protein (MTP) was not detected in these cells. Taken together, the data presented demonstrate that apoB-41 can direct the assembly and secretion of LPs that contain a triacylglycerol-rich core in nonhepatic cells that apparently lack MTP. These cells, therefore, represent an important model for studying LP assembly and may offer some advantages over cultured hepatic or intestinal cells that express their endogenous apoB gene.**

Apolipoprotein B (apoB) is a large hydrophobic glycoprotein (1–4) synthesized in the liver and the intestine that is essential for the assembly and secretion of triacylglycerol (TAG)-rich lipoproteins (LPs) (5, 6). Two forms of apoB are found in plasma, apoB-100 and apoB-48. ApoB-100 is a 4536 amino acid protein synthesized in the liver and secreted on very low density lipoproteins (VLDL) (1–4). It is the major protein constituent of low density lipoprotein (LDL) and is the ligand for the receptor-mediated clearance of LDL (7). ApoB-48 is a truncated form corresponding to the N-terminal 48% of apoB-100, generated by a posttranscriptional modification of apoB-100 mRNA that results in a stop codon at Gln-2153 (8, 9). ApoB-48 is synthesized mainly in the intestine and is secreted on chylomicrons (10).

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ApoB-48 contains the structural elements required to direct the formation of chylomicrons and VLDL (8). Peptides as short as the N-terminal 37% of apoB also appear sufficient to direct VLDL formation (11). Information derived from cultured hepatoma cells expressing truncated forms of apoB suggests that the length of apoB is directly correlated with the size and inversely correlated with the density of the secreted LPs (12–17).

Early steps in the synthesis of apoB, as with the synthesis of most secretory proteins (18), involve the cotranslational translocation of the nascent peptide across the endoplasmic reticulum (ER) membrane into the ER lumen. However, the appearance of apoB in the ER lumen occurs more slowly than is typical for other secreted proteins, as it is preceded by additional steps. These include cotranslational binding of apoB to the ER membrane (19–21), where segments of apoB are exposed on the cytosolic surface of the ER (22–25), and cotranslational association of apoB with lipids (15) to form small LPs of high density lipoprotein (HDL) and LDL densities (19). The complete translocation of apoB into the ER lumen is followed by a rapid transport to the Golgi complex (21). Some have suggested that VLDL-sized particles are formed in the ER (19, 26, 27) and, more specifically, at the junction between the rough and smooth ER (26), while others have suggested that lipids are added to apoB-containing LPs in the Golgi complex (20, 28).

The regulation of apoB secretion appears to be post- or cotranslational (29), as factors affecting the secretion levels of apoB do not significantly affect steady state mRNA levels or the synthesis of apoB (14, 30, 31). Degradation of newly synthesized apoB is a major regulatory mechanism, as only a fraction of the newly synthesized apoB (5–41%) gets translocated through the ER membrane, becomes associated with lipids, and is secreted (22, 32–34). The nonsecreted portion remains membrane-bound and is targeted for degradation (23) by proteolytic enzymes residing in a pre-Golgi compartment (22, 34). Lipid availability partly determines the efficiency of secretion, as oleate appears to increase apoB secretion by reducing its degradation (14, 32).

The assembly of apoB with lipids appears to be mediated by microsomal triacylglycerol transfer protein (MTP). Wetterau and colleagues showed that the absence of the large subunit of MTP that is associated with mutations in its gene is linked to the inability to secrete apoB-containing LPs in patients with abetalipoproteinemia (35, 36). Additional factor(s) may be involved in LP assembly, since patients with chylomicron retention disease express normal apoB and MTP (35) but are

Abbreviations: apoB, apolipoprotein B; LP, lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TAG, triacylglycerol; CE, cholesterol ester; BFA, brefeldin A; MTP, microsomal triacylglycerol transfer protein; ER, endoplasmic reticulum.

unable to secrete chylomicrons (6). Thus, while we have a general knowledge about LP assembly and secretion, the factors and the details of the path from translocation of apoB to secretion of TAG-rich LPs are incompletely defined.

Cultured liver cells have been widely used in secretion studies; however, these cells normally synthesize and secrete a variety of apolipoproteins, including apoB. To determine if LP assembly is mediated by factors that are unique to liver or intestinal cells, we have used a mouse mammary tumor-derived cell line (C127) that does not synthesize apoB or secrete LPs to generate cell lines that express C-terminally truncated forms of human apoB (37, 38). The line expressing the N-terminal 41% of apoB (apoB-41) assembles apoB-41 into LP particles that contain a TAG-rich core.

MATERIALS AND METHODS

Cell Cultures. Permanently transfected C127 cells expressing a human apoB-41 cDNA (Fig. 1) were grown as described (37, 39). In all experiments, cells were incubated in serum-free (SF) medium (37, 39). When oleate was to be added, the cells were preincubated for 24 h with oleate/bovine albumin (4:1, mol/mol) or fatty acid-free albumin in serum-containing medium. The cells were then washed and incubated for 24 h in SF medium with oleate/albumin or albumin. Lipids and proteins were labeled with [^{3}H]oleate and [^{35}S]methionine, respectively. Medium was collected and adjusted to 5 mM EDTA/0.02% NaN_3 containing leupeptin and aprotinin, each at a final concentration of 10 μg/ml. Medium was used directly for immunoprecipitation or was first subjected to either density-gradient (40) or sequential ultracentrifugation (41). Cells were washed in cold phosphate-buffered saline (PBS; 10 mM sodium phosphate/120 mM NaCl/2.7 mM KCl), pH 7.4 and then lysed in 1% Triton X-100 in PBS containing protease inhibitors as above. Cellular protein was determined according to Markwell *et al.* (42) or Lowry *et al.* (43) by using a kit from Bio-Rad.

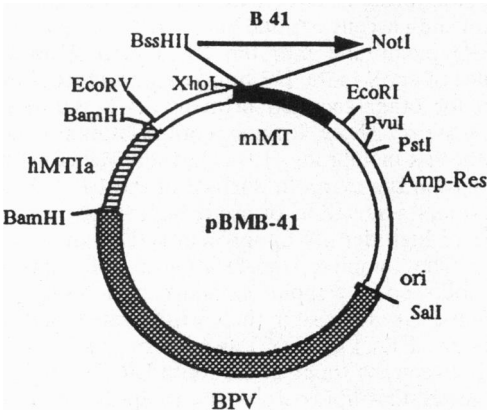

FIG. 1. Schematic representation of the 22.2-kb expression plasmid pBMB-41. To construct this plasmid, overlapping apoB cDNA sequences covering the entire length of apoB-100 cDNA were initially assembled into a pUC18 derivative designated pUB-100. This plasmid was digested with *Not* I and *Cla* I. The resulting 8.7-kb fragment containing the origin of replication and the ampicillin-resistance region of the pUC18 plasmid was ligated to the following adaptor sequence.

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Cla I          Not I
CGATagcttcaagtcaGC
  TAtcgaagttcagtCGCCGG
```

(Upper case letters at the 5′ and 3′ ends indicate the *Cla* I and *Not* I restriction sites, respectively.) This adaptor sequence complements the *Cla* I and *Not* I sites of the 8.7-kb apoB-containing fragment. Digestion with *Not* I and religation generated an apoB-41-containing plasmid designated pUB-41. pUB-41 was digested with *Bss*HII and *Not* I to release a 5.5-kb fragment containing the apoB-41 sequence, which was inserted into the corresponding restriction sites of the pBMT3XM vector.

The pBMT3XM vector was constructed by inserting the synthetic oligonucleotide

```
TCGATCTCGAG GCGCGC GCGGCCGC TGA T TGA T TGA
     Xho I  BssHII   Not I
```

into the *Xho* I site of the parent pBMT3X vector. This generated unique cloning sites (*Xho* I, *Bss*HII, and *Not* I) and TGA termination signals in all reading frames. The characteristics of the bovine papilloma virus (BPV)-derived pBMT3X vector and the generation of permanent cells lines by using this vector were as described (37).

Immunoprecipitation and Immunoblotting. Cell lysates or culture medium samples were precleared with protein G-Sepharose, incubated overnight at 4°C with goat antiserum to human apoB (BioDesign), and then incubated for 45 min with protein G-Sepharose. The protein G-Sepharose beads that were incubated with culture medium were washed with PBS. The beads that were incubated with cell lysates were first washed with PBS/1% Triton X-100, then with PBS/0.5% SDS/0.5% Triton X-100 and then with PBS alone. Lipids were isolated from culture medium by two extractions of the samples with 1 ml of chloroform/methanol (2:1, vol/vol). The protein was extracted by boiling the Sepharose beads in sample buffer (44) for 3–5 min and then resolved by SDS/7% PAGE (44) and autoradiography. Immunoblotting was performed as described (37).

Analysis of Lipids Associated with ApoB-41. Culture medium conditioned by C127B-41 cells in the presence of [^{3}H]oleate for 24 h was collected, adjusted to ρ = 1.25 g/ml with solid KBr, and ultracentrifuged in an SW55Ti rotor (Beckman) at 50,000 rpm for 40 h at 8°C. The $\rho < 1.25$ g/ml fractions were immunoprecipitated, and the lipids were separated by TLC and quantified by liquid scintillation spectrometry (37, 39). To identify the mass of lipids associated with apoB-41, culture medium was subjected to immunoaffinity chromatography as described below, followed by ultracentrifugation at ρ = 1.25 g/ml. The lipids in the $\rho < 1.25$ g/ml fractions were separated by TLC and identified by charring with 50% H_2SO_4.

Immunoaffinity Chromatography. Conditioned medium was incubated successively with gelatin-Sepharose (to remove fibronectin), protein G-Sepharose, and with an immunoadsorbent constructed by cross-linking apoB antibodies to protein G-Sepharose modified as by Schneider *et al.* (45). After extensive washing, apoB-containing LPs were eluted from the beads with 0.1 M glycine, pH 2.5. Fractions were neutralized, adjusted to 1 mM EDTA/0.02% NaN_3, and centrifuged at ρ = 1.25 g/ml as described above. The top ($\rho < 1.25$ g/ml) and bottom ($\rho > 1.25$ g/ml) fractions were collected and analyzed.

Negative Stain EM. Aliquots from the $\rho < 1.25$ g/ml fraction obtained after immunoaffinity chromatography were negatively stained (37) and analyzed with a Phillips CM-12 transmission electron microscope.

RESULTS

C-127 clones that express human apoB-41 cDNA were generated by transfection and selection. Autoradiographic analysis of intracellular and secreted apoB-41 demonstrated that several clones can synthesize and secrete apoB-41. The clone expressing the highest amount of apoB-41 was used for the studies described below.

Medium conditioned by cells labeled with [^{35}S]methionine was subjected to density-gradient ultracentrifugation and SDS/PAGE without prior immunoprecipitation. ApoB-41 was recovered in fractions of densities in the HDL range with the highest amount recovered in the ρ = 1.13 g/ml fraction (Fig. 2*A*). The $\rho > 1.21$ g/ml fractions contain all the other proteins secreted by these cells. ApoB-41 is not present in this fraction, as determined in a similar experiment using C127 cells transiently expressing apoB-41, where density fractions were subjected to immunoprecipitation prior to SDS/PAGE (data not shown). This experiment showed that there was no apoB-41 in

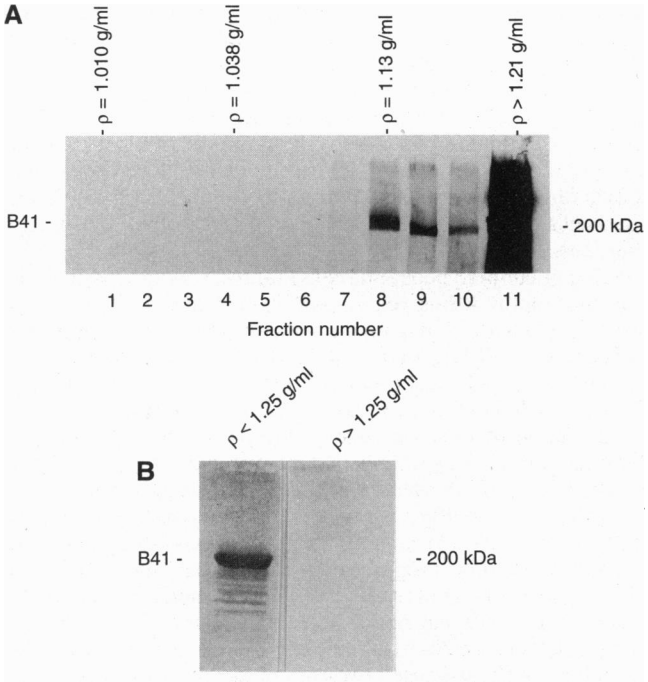

FIG. 2. Centrifugal analysis of apoB-41 secreted by C127B-41 cells. (*A*) Cells were labeled with [^{35}S]methionine for 6 h. Conditioned medium was subjected to density gradient ultracentrifugation, and density fractions were analyzed by SDS/PAGE and autoradiography. The bulk of apoB-41 floats at 1.13 g/ml. (*B*) Medium conditioned by cells for 24 h was subjected to immunoaffinity chromatography and then centrifugation at $\rho = 1.25$ g/ml. Fractions ($\rho < 1.25$ g/ml and $\rho > 1.25$ g/ml) were subjected to SDS/PAGE and immunoblotting. Note: no apoB-41 is present in the $\rho > 1.25$ g/ml bottom fraction. B41 indicates the position of apoB-41.

the $\rho > 1.21$ g/ml fraction (data not shown). Similarly, apoB-41 obtained by immunoaffinity chromatography and ultracentrifugation at $\rho = 1.25$ g/ml was exclusively recovered in LP fractions ($\rho < 1.25$ g/ml). There was no detectable apoB-41 in LP-deficient fractions ($\rho > 1.25$ g/ml) (Fig. 2*B*).

Analysis of apoB-41-associated ^{3}H-labeled lipids showed that labeled TAG, cholesterol ester (CE), and phospholipid accounted for about 54, 6, and 22%, respectively, of total labeled lipids (Table 1). Inclusion of 0.4 mM oleate resulted in about a 20-fold increase in the relative intracellular composition of oleate-labeled TAG and CE (data not shown), and increased incorporation of added [^{3}H]oleate into lipid associated with apoB-41 (Table 1). Despite increased incorporation of added oleate, there was no distribution of lipids into the LDL density range (data not shown). The presence or absence of oleate did not affect the relative composition of ^{3}H-labeled lipid associated with apoB-41 (Table 1). Taken together, the data show that apoB-41 must be assembled with lipids, especially TAG and phospholipid, to be secreted.

Composition analysis of the lipids associated with apoB-41 (obtained by immunoaffinity chromatography followed by centrifugation at $\rho = 1.25$ g/ml) by TLC and charring with H_2SO_4 confirmed the presence of TAG as a major lipid (data not shown).

Oleate (0.4 mM) increased the secretion of apoB-41 2- to 3-fold as measured by laser densitometry of blots of the $\rho <$ 1.25 g/ml fractions of conditioned medium. Similar results were obtained by measuring the secretion of ^{35}S-labeled apoB-41 after incubation with oleate (data not shown). Thus, the amount of apoB-41 that could be assembled with lipids prior to its secretion by C127 cells is closely correlated with lipid availability.

Negative stain EM showed that apoB-41-containing LPs were roughly round particles, usually ranging from 8 to 25 nm in diameter. In some fields rare particles with a diameter of 35–50 nm were seen (Fig. 3). The size distribution showed that particles isolated from cells incubated with oleate were slightly larger (12.6 ± 2.8 nm) than those isolated from cells incubated without added oleate (10.9 ± 3.1 nm) (mean ± SD, 250 particles measured). This size difference is not statistically significant. There were no discoidal particles observed in any field. Therefore, consistent with the high content of core lipids, we conclude that apoB-41 is secreted on emulsion-like LPs.

Brefeldin A (BFA) and monensin are drugs that perturb the transport of newly synthesized proteins from the ER to the Golgi complex (46, 47) and lead to diminished secretion. BFA almost abolished the secretion of ^{35}S-labeled apoB-41 (> 90%) without

Table 1. Composition of oleate-labeled lipids associated with secreted apoB-41

	pmol of labeled oleate per mg of cell protein (% of total)			
			Oleate	
Lipid class	None	Albumin	0.4 mM	0.8 mM
PL	0.52 (23)	0.25 (19)	6.40 (22)	9.40 (23)
MAG	0.06 (3)	0.03 (2)	0.61 (2)	0.95 (2)
DAG	0.12 (5)	0.04 (3)	0.90 (3)	2.00 (5)
FA	0.04 (2)	0.05 (4)	1.80 (6)	4.50 (11)
TAG	1.15 (50)	0.81 (62)	17.60 (60)	22.47 (54)
X*	0.25 (11)	0.08 (6)	1.10 (4)	1.31 (3)
CE	0.14 (6)	0.05 (4)	0.90 (3)	0.96 (2)

Cells cultured in unsupplemented medium (None) or in medium supplemented with albumin (Albumin) or oleate and albumin (Oleate) were labeled with [^{3}H]oleate for 24 h. Medium was centrifuged at $\rho =$ 1.25 g/ml. Lipids in the $\rho < 1.25$ g/ml fractions were analyzed after immunoprecipitation. The values are given as the mean of three separate experiments. The SEM for the major lipids (PL, TAG, X, and CE) is <15%. MAG, monoacyglycerols; DAG, diacylglycerols; FA, fatty acids; PL, phospholipid.

*This lipid migrates on TLC between TAG and CE. A preliminary characterization of this lipid spot was carried out by eluting the lipid from the silica gel with chloroform. The extracted lipid was then saponified in 5% KOH in ethanol, extracted with hexane, and separated by TLC. After hydrolysis the lipid comigrated with *O*-alkyl MAG on TLC. Therefore, the original lipid is probably monoalkyl DAG.

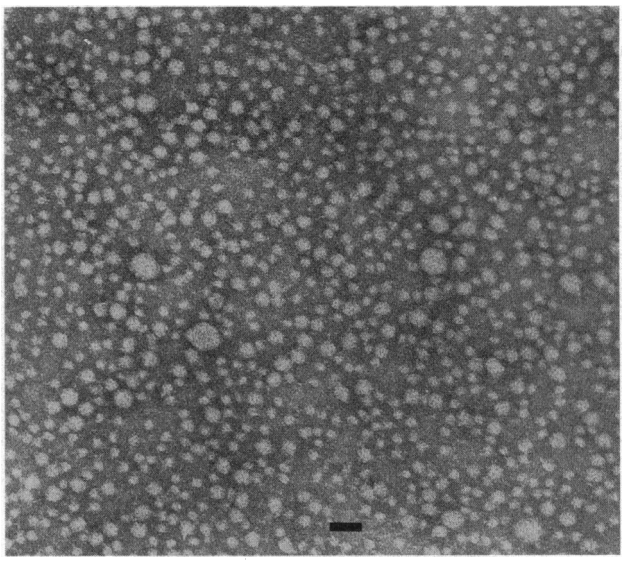

FIG. 3. Electron micrograph of apoB-41-containing LPs. Culture medium conditioned by cells in the presence of 0.4 mM oleate was subjected to immunoaffinity chromatography and centrifugation at $\rho =$ 1.25 g/ml. Aliquots from the $\rho < 1.25$ g/ml fraction were analyzed by EM after negative staining. The mean particle diameter was 12.6 nm ± 2.8 nm (250 particles measured). (Bar = 35 nm.)

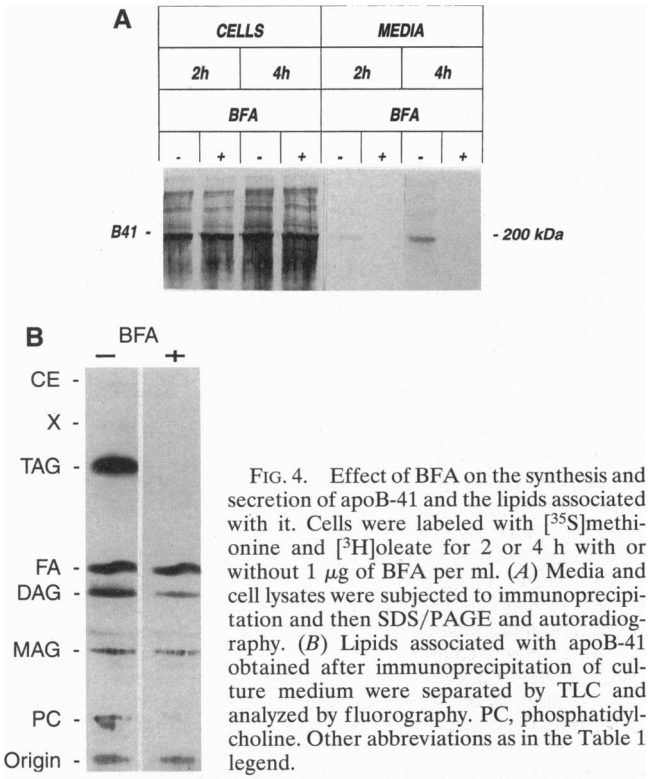

FIG. 4. Effect of BFA on the synthesis and secretion of apoB-41 and the lipids associated with it. Cells were labeled with [^{35}S]methionine and [^{3}H]oleate for 2 or 4 h with or without 1 μg of BFA per ml. (*A*) Media and cell lysates were subjected to immunoprecipitation and then SDS/PAGE and autoradiography. (*B*) Lipids associated with apoB-41 obtained after immunoprecipitation of culture medium were separated by TLC and analyzed by fluorography. PC, phosphatidylcholine. Other abbreviations as in the Table 1 legend.

reducing its synthesis (Fig. 4*A*). Similarly, secretion of ^{3}H-labeled TAG was almost abolished (>90%) and secretion of phosphatidylcholine was greatly reduced in the presence of BFA (Fig. 4*B*), without their synthesis being affected (data not shown). Despite the lack of TAG and phosphatidylcholine in medium obtained from cells treated with BFA, there were some other lipids (mainly fatty acids) immunoprecipitated from the medium (Fig. 4*B*). These lipids could have bound nonspecifically to protein G-Sepharose or to some components in the antiserum. These lipids were not found when medium was subjected to ultracentrifugation prior to immunoprecipitation.

The effect of BFA and monensin on the secretion of apoB-41 was studied in a pulse–chase experiment. Fig. 5 shows that BFA and monensin reduced secretion of apoB-41 by 86 and 70%, respectively. The results of both experiments suggest that apoB-41 is transported from the ER to the Golgi complex prior to its secretion. It is important to note that after a 2-h chase, only about 20% of the radioactivity present at the beginning of the chase was recovered. Thus, the major fraction, about 80% of the newly synthesized apoB-41, is degraded.

DISCUSSION

The present report documents that cells that are neither hepatic nor intestinal in origin are able not only to express a relatively large segment of human apoB but also to carry out the complex process of assembling apoB-41 with lipids to form LPs containing a TAG-rich core.

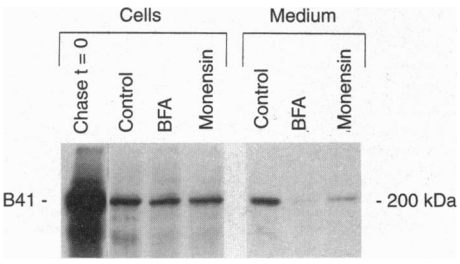

FIG. 5. Effect of BFA and monensin on the secretion of apoB-41. Cells were pulsed with [^{35}S]methionine for 2 h (chase t = 0) and chased for 2 h in the absence of BFA and monensin (control) or in the presence of BFA or monensin. Cell lysates and culture medium were immunoprecipitated and processed for autoradiography. B41, apoB-41.

In contrast to apoB-17 (37) or apoB-29 (38), apoB-41 expressed by C127 cells was secreted exclusively in LPs (Fig. 2*B*). ApoB-41-containing LPs secreted by C127 cells are very similar to truncated apoB-containing LPs secreted by cultured hepatoma cells in terms of density, size, and composition (12–15, 48). Thus, C127 cells secreted apoB-41-containing LPs in the HDL range (Fig. 2*A*), like apoB-41-, apoB-42-, or apoB-48-containing LPs secreted by cultured hepatoma cells (13, 14). In contrast, similarly truncated apoB forms (e.g., apoB-40 and apoB-46) found in plasma obtained from patients with hypobetalipoproteinemia associate with VLDL, LDL, and HDL (11). These data suggest that fatty acid availability in cultured cell lines is probably limiting. Oleate supplementation of rat hepatoma cells resulted in the redistribution of LPs containing apoB-37 or apoB-48 to lower densities (14). However, rat hepatoma cells did not secrete either apoB-37 or apoB-48 on LPs in the VLDL density range (14). Thus, the effect of fatty acids in these experiments is limited. LPs secreted by C127B-41 cells incubated in oleate-supplemented medium were only insignificantly larger than those secreted without oleate (12.6 nm vs. 10.9 nm mean diameter, respectively). Perhaps C127 cells may not be able to form larger LPs, although rare particles in the 35- to 40-nm diameter range were seen (Fig. 3). In light of the proposed two-step model for VLDL assembly (12, 49, 50), it is possible that C127B-41 cells carry out only the first step which takes place in the rough ER, where apoB is assembled with a small core of neutral lipids, TAG, and CE, to form "primordial" LP particles of HDL and LDL densities (12, 49, 50). The formation of these particles is both required and sufficient to release the apoB bound to the ER membrane into the lumen (21) and permit its secretion. Unlike apoB-100, truncated forms apoB-37 to apoB-48 appear to be stable on small, dense HDL-like particles. These particles, therefore, either could be targeted for secretion via the Golgi complex or they could acquire additional lipids later (26), forming large, mature VLDL-like particles (12, 26, 49, 50). Perhaps this later step is nonfunctional in C127 cells due to reduced amounts of smooth ER (as is the case for HepG2 cells) (51). Thus, newly formed apoB-41-containing LPs of HDL density, once released into the lumen, are rapidly targeted for secretion through the Golgi complex. The involvement of the Golgi complex is supported by the experiments with BFA and monensin, which block the secretion of apoB-41 and its lipids (Figs. 4 and 5). Oleate supplementation of culture media results in an increased secretion of apoB-41-associated lipids and a 2- to 3-fold increase in apoB-41 secretion. Increased lipid availability and complexing with apoB-41 allow for increased apoB-41 to be secreted, thus escaping degradation by proteolytic enzymes possibly residing in a pre-Golgi compartment (22, 34). Under these conditions it appears that the number of apoB-41-containing particles increases, but the size and composition of the particles do not change significantly.

The mean diameter of apoB-41-containing LPs is in the range of 11–13 nm (Fig. 3), consistent with the expected size of LPs containing apoB-42 secreted by hepatic cells (12). Thus, it appears that C127 cells express all the factors necessary to mediate the assembly of apoB-41 with lipids to form and to secrete LPs. In contrast, CHO cells fail to secrete apoB-53, despite normal expression of the protein (52), probably due to the inability of these cells to mediate lipoprotein assembly.

The mechanism for the assembly of apoB-41 with lipids to form LPs is not known at the present time. It is clear that MTP plays an important role in the assembly of apoB with lipids to form TAG-rich LPs (35). Since the present study clearly shows that C127B-41 cells can form LPs, it was important to determine whether MTP can be detected in these cells. Western blot analysis of increasing amounts of cell protein by using an antibody to the large subunit of MTP (shown to detect MTP in mouse liver) showed strong reactivity in HepG2 cells, but

none in C127 or C127B-41 cells (J. Wetterau and D. Gordon, personal communication). Further, analysis of TAG transfer activity (35) showed only background activity, which did not increase with increasing protein concentration in C127 and C127B-41 cells. In contrast, high and increasing TAG transfer activity was present in HepG2 cells (J. Wetterau and D. Gordon, personal communication). Two possible explanations may account for the failure to detect MTP: (*i*) C127 cells express very low levels of MTP that are below the detection limits of the assays used. It is possible, however, that low levels of MTP may be sufficient for the assembly of small amounts of apoB-41 with lipids in C127B-41 cells. (*ii*) MTP does not play a role in C127B-41 cells in the assembly of lipids with apoB-41 to form LPs, either because MTP is not required for the formation of small dense LPs or because an alternative mechanism plays a role in LP assembly in C127 cells.

In conclusion, apoB-41 can direct the assembly of LPs that contain a TAG-rich core in C127 cells. Thus, these cells represent an important model in which to study the domains of apoB that allow it to assemble intracellularly with lipids to form LPs as well as the cellular activities that may be required for LP assembly and secretion.

Note Added in Proof. After this manuscript was accepted for publication, two groups showed that simultaneous expression of MTP and a truncated form of apoB (apoB-53 in HeLa cells and apoB-41 in COS-1 cells) are sufficient to allow secretion of the expressed, truncated apoB in the HDL density range (53, 54).

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