Intracellular Assembly of Very Low Density Lipoproteins Containing Apolipoprotein B100 in Rat Hepatoma McA-RH7777 Cells*

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Previous studies with McA-RH7777 cells showed a 15–20-min temporal delay in the oleate treatment-induced assembly of very low density lipoproteins (VLDL) after apolipoprotein (apo) B100 translation, suggesting a post-translational process. Here, we determined whether the post-translational assembly of apoB100-VLDL occurred within the endoplasmic reticulum (ER) or in post-ER compartments using biochemical and microscopic techniques. At steady state, apoB100 distributed throughout ER and Golgi, which were fractionated by Nycodenz gradient centrifugation. Pulse-chase experiments showed that it took about 20 min for newly synthesized apoB100 to exit the ER and to accumulate in the cis/medial Golgi. At the end of a subsequent 20-min chase, a small fraction of apoB100 accumulated in the distal Golgi, and a large amount of apoB100 was secreted into the medium as VLDL. VLDL was not detected either in the lumen of ER or in that of cis/medial Golgi where apoB100 was membrane-associated and sensitive to endoglycosidase H treatment. In contrast, VLDL particles were found in the lumen of the distal Golgi where apoB100 was resistant to endoglycosidase H. Formation of luminal VLDL almost coincided with the appearance of VLDL in the medium, suggesting that the site of VLDL assembly is proximal to the site of secretion. When microsomal triglyceride transfer protein activity was inactivated after apoB had exited the ER, VLDL formation in the distal Golgi and its subsequent secretion was unaffected. Lipid analysis by tandem mass spectrometry showed that oleate treatment increased the masses of membrane phosphatidylcholine (by 26%) and phosphatidylethanolamine (by 27%) and altered the membrane phospholipid profiles of ER and Golgi. Taken together, these results suggest that VLDL assembly in McA-RH7777 cells takes place in compartments at the distal end of the secretory pathway.

The very low density lipoprotein (VLDL) synthesized in the liver carries various amounts of triacylglycerol (TG) in the neutral lipid core surrounded by phospholipids, cholesterol, and apolipoproteins. Each VLDL particle contains a single copy of apolipoprotein (apo) B100, an extremely hydrophobic and glycosylated polypeptide of ~550 kDa (1). Rat liver secretes VLDL that contains either apoB100 or apoB48 (N-terminal 48% of apoB100). We previously showed that in rat hepatoma McA-RH7777 cells, assembly of apoB48- or apoB100-VLDL could be induced by exogenous oleate and was achieved after apoB translation (2). The TG-rich VLDL (e.g. VLDL1, S4 > 100) that contained 35S-labeled apoB100 was undetectable within the microsomal membrane until 20–40 min after continuous labeling (2). Results from pulse-chase experiments also demonstrated that it took about 35 min (20-min pulse and 15-min chase) for VLDL1 to appear in the lumen of microsomes after apoB synthesis (2). The time spent for VLDL1 assembly is equivalent to that required for newly synthesized apoB100 to traverse through the secretory pathway (3, 4). These results demonstrate that bulk TG is not incorporated into VLDL immediately after apoB100 translation and suggest the existence of post-translational events.

A model that describes the post-translational event and is supported by a good number of experimental evidence is the two-step VLDL assembly model (5–9). According to this model, the newly synthesized apoB100 polypeptides start to recruit, with the assistance of microsomal triglyceride transfer protein (MTP) (2, 10, 11), surface lipids and a small quantity of neutral lipids during and immediately after translation. At this stage, apoB100 polypeptide remains associated with the endoplasmic reticulum (ER) membranes (11, 12), and the resulting lipoprotein particle has buoyant density resembling that of high density lipoproteins (HDL) (13, 14). Normally, these HDL-like particles are not secreted as such. Rather, they combine with

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1 The abbreviations used are: VLDL, very low density lipoprotein; apo, apolipoprotein; Bip, immunoglobulin-binding protein; β-COP, β subunit of coatamer-protein I; COPII, coater-protein-II; DMEM, Dulbecco’s modified Eagle’s medium; EE1, early endosomal antigen 1; Endo H, endoglycosidase H; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum Golgi intermediate compartment; FBS, fetal bovine serum; Grp, glucose-regulated protein; HDL, high density lipoprotein; Hsp, heat shock protein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; ManII, α-mannosidase II; MTP, microsomal triglyceride transfer protein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PNGase F, peptide-N-glycosidase F; TEM, transmission electron microscopy; TG, triacylglycerol; TGN, trans-Golgi network.

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bulk neutral lipids (the second step) to form buoyant low density lipoproteins (LDL), intermediate density lipoproteins (IDL), or VLDL that are detectable in the lumen of microsomes (2, 12). Acquisition of bulk neutral lipids in the second step appears to be independent of the MTP activity (10, 11, 15).

What remains unclear is the subcellular compartments where bulk neutral lipids are incorporated into VLDL containing apoB100. To date, two models have been proposed, and both are supported by experimental evidence. The first model suggests that VLDL assembly takes place in the ER. The “ER assembly” model postulates that the newly synthesized apoB is retained within the rough ER until VLDL, whose size, buoyancy, and lipid composition are indistinguishable from that of secreted VLDL, is fully assembled (16). The resulting ER-derived VLDL is then traversed through the secretory pathway and secreted. The second model theorizes that VLDL assembly occurs in post-ER compartments. This “post-ER assembly” model was suggested by experimental data where the rates of intracellular trafficking between apoB and lipids were compared (4, 17) and the lipid contents of apoB-containing lipoproteins between different subcellular compartments were determined (7, 9). Kinetic analysis provided evidence that the rate of

FIG. 1. Distribution of marker proteins and apoB100 among Nycodenz fractions. Fractionation of subcellular microsomes by Nycodenz gradient centrifugation was achieved as described under “Experimental Procedures.” Proteins of the fractionated samples were resolved by SDS-PAGE (3–15% gel), transferred to nitrocellulose membranes, and immunoblotted with various antibodies. A, ManII, calnexin (Cnx), and TGN38. B, COPII, protein disulfide isomerase, and β-COP. C, p58 (a rat analog of human ERGIC53), MTP, and EEA1. D, apoB100. The bands on immunoblots were semi-quantified by scanning densitometry, and the intensity was plotted as the percentage of the maximum value in which 100% corresponds to the highest value.

FIG. 2. Merging confocal images of apoB and markers. The cells were permeabilized and stained either with anti-human apoB antibody alone or else with anti-apoB antibody plus antibodies against calnexin (Cnx), COP-II, ManII, β-COP, or EEA1. The secondary antibodies for apoB were conjugated with Alexa Fluor™ 488 (green), and that for marker proteins was conjugated with Alexa Fluor™ 594 (red). Scale bar, 10 μm for all panels.
TG transit from ER to Golgi was distinct from that of apoB, which ruled in the possibility of a post-ER event (4, 17). Biochemical studies showed the highest amount of lipids associated with apoB in trans-Golgi as compared with cis-Golgi and rough and smooth ER (7, 9), suggesting a stepwise acquisition of lipids along the secretory pathway (18). Recently, results suggesting post-ER assembly of VLDL containing apoB48 in McA-RH7777 cells have been reported (12). The present study aims to determine the assembly site for VLDL containing apoB100.

**EXPERIMENTAL PROCEDURES**

**Materials**—Glycerol [14C]trioleate (57 mCi/mmol), [35S]methionine/cysteine (1000 Ci/mmol), [3H]palmitic acid (52 Ci/mmol), protein A-Sepharose™ CL-4B beads, and horseradish peroxidase-linked anti-mouse or anti-rabbit IgG antibodies were purchased from Amersham Biosciences. Labeled goat anti-mouse (Alexa Fluor™ 488) or anti-rabbit (Alexa Fluor™ 594) IgG antibodies were purchased from Molecular Probes. Endoglycosidase H (Endo H) and peptide:N-glycosidase F (PNGase F) were obtained from New England BioLabs. Fibronectin, oleic acid, triacylglycerol, and phospholipid standards were obtained from Sigma and Avanti Polar Lipids. Monoclonal antibody against TGN38 and polyclonal anti-H9252-COP, -COPII, or -early endosomal antigen 1 (EEA1) were obtained from Affinity Bioreagents. Monoclonal antibody recognizing proteins containing the KDEL motif (Bip, Grp94, and Hsp47) and polyclonal anti-calnexin antiserum were obtained from StressGen. Monoclonal antibody against human apoB antibody 1D1 was a gift of R. Milne and Y. Marcel (University of Ottawa Heart Institute). Polyclonal anti-H9251-mannosidase II (ManII) and anti-MTP antiserum were gifts from M. G. Farquhar (University of San Diego) and C. C. Shoulders (Hammersmith Hospital, London, UK), respectively. Polyclonal antiserum against human LDL was produced in our laboratory. The MTP inhibitor BMS-197636 was a gift of D. Gordon (Bristol-Myers Squibb). Protease inhibitor mixture and chemiluminescent blotting substrate were purchased from Roche Diagnostics. Culture plate inserts (0.4 MILLICELL™-CM, 30-mm diameter) were purchased from Millipore.

**Cell Culture**—Transfected McA-RH7777 cells stably expressing human apoB100 (19) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), 10% horse serum, and 200 μg/ml G418. During experiments, the cells were kept in DMEM containing 20% FBS plus other reagents as indicated in the figure legends.

**Subcellular Fractionation**—Two to four 100-mm dishes of cells were harvested in 2 ml of ice-cold homogenizing buffer (10 mM Tris-HCl, pH 7.4, 250 mM sucrose, 5 mM EDTA, and serine/cysteine protease inhibitor mixture) and homogenized by passing 10 times through a ball-bearing homogenizer. Post-nuclear supernatant was obtained by centrifugation (9,500 rpm, 10 min, 4 °C, Sorvall SS-34 rotor) and subjected to fractionation by centrifugation in a Nycodenz gradient as described previously (20, 21). First, a step gradient was created in Beckman SW41 centrifuge tubes by loading (top to bottom) 2.5 ml of 10, 14.66, 19.33, and 24% of Nycodenz solution in saline buffer. The solutions were prepared from 27.6% Nycodenz stock solution and 0.75% NaCl (both in 10 mM Tris-HCl, pH 7.4, 3 mM KCl, 1 mM EDTA, 0.02% NaN3). The tube was sealed with Parafilm and placed horizontally for 45 min at room temperature followed by centrifugation (37,000 rpm, 4 h, 15 °C, SW41 rotor). One a nonlinear gradient was formed after centrifugation, 2 ml of the post-nuclear supernatant was layered on top of the gradient and fractionated by centrifugation (37,000 rpm, 1.5 h, 15 °C). After centrifugation, 15 fractions (0.8 ml each) were collected from top of the tube (see Fig. 4A, left three columns). An aliquot of each fraction (50 μl) was mixed with an equal volume of two-time concentrated protein sample buffer and resolved by SDS-PAGE (5–15% gel). After electrophoresis, the proteins were transferred onto nitrocellulose membranes and probed with anti-FIG. 3. Trafficking of radiolabeled apoB100 along the secretory pathway. The cells were pulse-labeled with [35S]methionine/cysteine for 10 min and chased in the presence of cycloheximide for up to 120 min. At each chase time, medium was collected, and the cells were homogenized followed by Nycodenz fractionation. See “Experimental Procedures” for details. A, representative fluorograms of 35S-apoB100 that was secreted into medium (lanes M) or associated with the 15 Nycodenz fractions. B, quantification of radioactivity associated with 35S-apoB100 by scintillation counting.

**FIG. 3.** Trafficking of radiolabeled apoB100 along the secretory pathway.
bodies for marker proteins of various subcellular compartments.

**Immunocytochemistry**—The cells were plated on coverslips for 24 h, fixed with 3% paraformaldehyde in phosphate-buffered saline for 20 min, and permeabilized with 0.1% Triton X-100 (in phosphate-buffered saline) for 3 min. The cells were incubated with 10% FBS (in phosphate-buffered saline) for 20 min prior to probing with antibodies. Monoclonal antibody 1D1 (1:1000 dilution) was used to probe the recombinant human apoB (1 h) followed by incubation with goat anti-mouse IgG antibody (1 h). Subcellular compartments were probed with antibodies against calnexin (1:500 dilution) for ER, COPII (1:100 dilution) for Golgi anterograde vesicles, ManII (1:100 dilution) for cis/medial Golgi, β-COP (1:100 dilution) for Golgi anterograde/retrograde vesicles, and EEA1 (1:100 dilution) for endosomes. The secondary antibody was Alexa Fluor 594 conjugated with anti-rabbit IgG (1:200 dilution) as a secondary antibody. The coverslips were mounted onto a glass slide using SlowFade Antifade kits (Molecular Probes). The images were captured by a MRC-1024 laser scanning confocal imaging system.

**Pulse-Chase Experiments**—In pulse-chase experiments where luminal apoB100 particles of different subcellular fractions were determined, the cells in two 100-mm dishes were labeled with [35S]methionine/cysteine (200 μCi/ml in 3 ml of methionine- and cysteine-free DMEM containing 20% FBS and 0.4 mM oleate) for 20 min. The cells were then incubated with chase medium (DMEM containing 20% FBS and 0.4 mM oleate) for 15, 30, and 45 min. At the end of each chase time, the medium was collected and subjected to cumulative rate flotation centrifugation (2) to recover apoB100-VLDL1 (S > 100) and apoB100-VLDL2 (S ~ 20–100) from other lipoproteins (i.e. IDL, LDL, and HDL). The [35S]-apoB100 in each fraction was recovered by immunoprecipitation using polyclonal antisera raised against human LDL as described previously (22). Also, at the end of each chase time, the radiolabeled cells were harvested in 2 ml of ice-cold homogenization buffer, mixed with two 100-mm dishes of unlabeled cells, and subjected to subcellular fractionation and carbonate treatment as described below.

In experiments where transit of newly synthesized apoB100 along the secretory pathway was determined, the cells were pulse-labeled for 10 min, washed, and incubated with chase medium containing 10 μM cycloheximide for 10, 20, 40, 80, and 120 min. The medium and cell samples were processed at the end of chase time as described above, except that the one dish of labeled cells was mixed with one dish of unlabeled cells prior to subcellular fractionation. In experiments where intracellular distribution of membrane- and lumen-associated apoB100 was determined, the cells were pulse-labeled for 20 min and incubated with chase medium for 0, 15, 30, and 45 min. apoB100 associated with membrane and lumenal content was isolated and analyzed as described below.

In experiments where MTP was inactivated by BMS-197636, two protocols were used. In the first protocol, MTP activity was inhibited prior to apoB synthesis. To do this, cells were incubated with 0.2 μM BMS-197636 for 30 min, pulse-labeled with 200 μCi/ml [35S]methionine/cysteine for 20 min, and chased first for 15 min and second with fresh medium for additional 30 min. Oleate (0.4 mM) and BMS-197636 (0.2 μM) were present throughout pulse and chase. In the second protocol, MTP activity was inhibited after apoB had exited the ER. To achieve this, the MTP inhibitor was added to the medium during the second chase (30 min).

**Analysis of apoB100 Associated with Membranes and Lumenal Contents of Microsomes**—Each Nycodenz fraction was added with an equal

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**Fig. 4.** Isolation and separation of VLDL from the content of subcellular compartments. A, a protocol used for analyzing buoyancy of lipoproteins containing apoB100 in the fractionated microsomal lumen. B, distribution of [3H]palmitate-labeled sphingomyelin among the 15 Nycodenz fractions. The cells were labeled with [3H]palmitic acid (3 μCi) for 4 h prior to subcellular fractionation. The data are presented as the ratio of [3H]sphingomyelin/[3H]PC. C, protein profile of the 15 Nycodenz fractions. The gel was stained with Coomassie Blue. D, pooled Nycodenz fractions (1–3, 4–8, and 9–15) were mixed with or without equal volume of 0.2 nM Na2CO3, pH 12.4, for 30 min and subjected to ultracentrifugation to separate membranes (as pellet, P) from lumenal content (as supernatant, S). The proteins were resolved by SDS-PAGE and blotted using anti-calnexin (Cnx) antibody or anti-KDEL antibody to visualize Grp94, Bip, and Hsp47.
Subcellular Distribution of apoB100—Subcellular compartments were fractionated using a Nycodenz gradient, and each fraction was probed with antibodies specific to marker proteins by immunoblot analysis (Fig. 1, A−C). Three distinct subcellular compartments, namely ER, cis/medial Golgi, and distal Golgi, were separated. Fractions 9−15 were designated ER by their possessing of calnexin, MTP, protein disulfide isomerase, Grp78 (Bip), Grp94, and Hsp47 (see Fig. 4D). Fractions 4−8 were designated cis/medial Golgi because they contained ManII and COPII (marker for ER-to-Golgi anterograde vesicles). The intermediate compartment between ER and Golgi...
marker p58 (a rat analog of human ERGIC53) had a bimodal distribution with two peaks at ER (fraction 15) and cis/medial Golgi (fraction 6), respectively. Fractions 1–3 represent a mix of trans-Golgi network, early endosome, and Golgi-derived retrograde/anterograde vesicles by appearance of TGN38, EEA1, and Hsc70/COP. At steady state, apoB100 distributed throughout the entire secretory pathway (Fig. 1D). Merging confocal images of immunocytochemistry confirmed co-localization of apoB (green color) with ER (calnexin), cis/medial Golgi (COPII and ManII), and distal Golgi (Hsc70/COP) markers (red color) (Fig. 2). However, apoB100 did not co-localize with the endosomal marker EEA1.

Intracellular Trafficking of apoB100—Intracellular trafficking of apoB100 was monitored by pulse-chase experiments (cycloheximide was included in chase medium to prevent protein elongation) in conjunction with subcellular fractionation. At the end of the 10-min pulse, the majority of 35S-apoB100 was located in the ER, whereas a small portion appeared in cis/medial Golgi (Fig. 3A, 0 min chase). The presence of apoB100 in cis/medial Golgi after 10 min of labeling was not unexpected because translation was unsynchronized in these cells. Accumulation of 35S-apoB100 in cis/medial Golgi became obvious at 10 min and peaked at 20 min during chase. At the end of a 40-min chase, 35S-apoB100 appeared in distal Golgi (fraction 3), and secretion of 35S-apoB100 into the medium was detectable. Prolonged chase (i.e. 80 and 120 min) resulted in further accumulation of 35S-apoB100 in the medium but did not result in accumulation of 35S-apoB100 in distal Golgi. These results suggest that newly synthesized apoB100 traverses at a relatively slow rate through cis/medial Golgi but transits rather rapidly through distal Golgi.

VLDL in Distal Golgi Lumen—To determine which Nycodenz fraction(s) contained VLDL, we analyzed the buoyancy of lipoproteins containing apoB100 within the lumenal of pooled ER (fractions 9–15), cis/medial Golgi (fractions 4–8), and distal Golgi (fractions 1–3) microsomes (Fig. 4A). In addition to marker distributions shown in Fig. 1, pooling of these microsomal membranes was justified by the distribution of [3H]palmitate-labeled sphingomyelin (a Golgi-synthesized lipid) (Fig. 4B) and by overall protein patterns of the Nycodenz fractions (Fig. 4C). Separation of lumen from membrane after sodium carbonate treatment was complete, as evidenced by the appearance of Grp94, Bip, and Hsp47 in the supernatant and that of calnexin in the pellet of the ER microsomes (Fig. 4D, panels marked + Na2CO3). Using the protocol depicted in Fig. 4A, we analyzed the kinetics of apoB100-VLDL assembly and secretion at various chase times. At the beginning of chase, the majority of 35S-
apoB100 was associated with the membrane of ER (Fig. 5A). The amount of 35S-apoB100 radioactivity in the ER membranes decreased during chase (between 15 and 45 min), and the lost radioactivity could be quantitatively recovered in cis/medial Golgi membranes (Fig. 5A) and in distal Golgi lumen (Fig. 5B). Thus, degradation of newly synthesized 35S-apoB100 was insignificant within this time frame. Trace amount of 35S-apoB100 in the form of VLDL could be detected in the medium at 15-min chase, although apoB100-VLDL was not detectable in the lumen of distal Golgi at this time (Fig. 6A, left panel). By the time of 30- and 45-min chase, the amount of 35S-apoB100 associated with VLDL increased in the distal Golgi as well as in the medium (Figs. 5B and 6, B and C, left panels). Notably, the amount of 35S-apoB100 associated with VLDL in the lumen was 10–20-fold lower than that secreted in the medium at all chase times, indicating rapid release of VLDL once they are assembled. During the entire chase, only trace amounts of 35S-apoB100 were detectable in the lumen of ER or cis/medial Golgi (Fig. 5B), even though a considerable amount of apoB100 was present in these compartments (Figs. 1D, 3, and 5A). The low abundance of 35S-apoB100 in the ER lumen was unlikely due to incomplete treatment by sodium carbonate, because the ER residence proteins Grp94, Bip, and Hsp47 were effectively released into the lumen under the same conditions (Fig. 4D).

The near absence of 35S-apoB100 in the ER or cis/medial Golgi lumen suggested that apoB100 in the early secretory compartments was mainly membrane-bound and could not readily be removed by carbonate treatment. We attempted to remove the membrane-associated apoB100 from fractionated microsomes with sodium carbonate plus sodium deoxycholate and potassium chloride (11, 22). Under these conditions, the amount of 35S-apoB100 particles associated with lipoproteins of high buoyant density was increased in the lumen of ER, cis/medial Golgi, and distal Golgi (Fig. 6, A–C, compare right panels and left panels). However, no increase in 35S-apoB100 was found in fractions containing VLDL. These data suggest that the membrane-associated apoB100 is poorly lipidated within the early secretory compartments.

To ascertain that the membrane-bound apoB100 was indeed associated with microsomes of early secretory pathway, we determined the glycosylation status of apoB100 by Endo H digestion. In cells where lipogenesis was maximized by exogenous oleate, membrane-bound apoB100 in ER and cis/medial Golgi was Endo H-sensitive (Fig. 7A). However, once apoB100 reached distal Golgi, it became associated with lipoproteins of...
Lipoprotein diameter changes with transit through consecutive Golgi saccules, the TGN, and secretory granules

TEM processing of cells and measurements of lipoprotein diameter were performed as described Experimental Procedures. The diameters of a total of 1025 lipoprotein particles were measured.

| Golgi saccules | Average diameter (nm) | Range (nm) | Median (nm) |
|----------------|-----------------------|------------|-------------|
| 1              | 33 ± 11 (n = 98)       | 24–40      | 37          |
| 2              | 36 ± 12 (n = 112)      | 12–196     | 31          |
| 3              | 44 ± 15 (n = 112)      | 12–123     | 37          |
| 4              | 44 ± 19 (n = 138)      | 12–148     | 39          |
| 5              | 42 ± 16 (n = 233)      | 19–148     | 43          |
| 6              | 46 ± 19 (n = 95)       | 19–148     | 43          |

Distribution of Lipoprotein Particles—To ascertain that VLDL particles were not formed within the ER, we analyzed the distribution of lipoproteins within the secretory pathway of McA-RH7777 cells by single and serial section transmission electron microscopy (TEM). The apparent absence of lipoprotein particles in Golgi-associated ER was noted. However, electron-dense particles were found in dilations of Golgi saccules (from cis-trans), in the TGN, and in secretory granules (Fig. 8). These electron-dense particles are mainly apolipoprotein-containing particles as demonstrated previously (26) but may include lipid droplets devoid of apolipoproteins. The Golgi stacks sampled contained on average 11 particles/sectioned stack, and about 40% of the stacks contained less than 5 particles/stack. It was noted that lipoproteins in the cis-Golgi frequently were membrane-associated, whereas those in the trans-Golgi, TGN, and secretory granules were not (Fig. 8).

The average diameter of pooled lipoproteins from all Golgi saccules (saccules 1–6 in Table I) was 40 ± 17 nm (n = 656). An incremental increase in the average diameter of particles occurred in each sacule (except for sacule 5); between the cis-most (sacule 1) and the trans-most (sacule 6) elements, the increase was 1.4-fold (Table I). In post-Golgi compartments, the average lipoprotein diameter decreased. Thus, the average diameter of particles in the TGN was 9% smaller than that in sacule 6, and a further 5% decrease in particle size occurred between the TGN and secretory granules. The increase in lipoprotein size from cis to trans sacculles was also evident when data were presented in the form of histograms (Fig. 9). Five species of particles with increasing size denoted as *1 to *5 were identified for sacculles 1–3 (Fig. 9A) and for sacculles 4–6 plus TGN (Fig. 9B). The incidence of species *1, *2, *3, and *4–5 (relative to total lipoprotein particles between 10–75 nm) was 6.2, 66.5, 18.8, and 8.5%, respectively, in the cis elements (sacculles 1–3) and 52, 29.6, and 14%, respectively, in the trans elements (sacculles 4–6 plus TGN). Thus, between cis and trans sacculles, a shift occurred from two smaller diameter species (*1 and *4) toward three larger diameter species (*3, *4, and *5).

Assembly of VLDL after apoB Exits ER Requires No MTP Activity—Knowing that VLDL assembly possibly occurred in post-ER compartments, we then inquired whether the MTP activity is required at this stage. Because MTP was found predominantly in the ER (Fig. 1C), we hypothesized that the post-ER VLDL assembly required no MTP activity. To test this hypothesis, we designed two pulse-chase protocols by which the MTP was inactivated either before or after metabolic labeling of apoB100 under conditions where lipogenesis was maximized by exogenous oleate. When the MTP inhibitor BMS-197636 was added to the medium 30 min before the pulse labeling, secretion of 35S-apoB as VLDL during chase was virtually abolished as compared with the control (i.e. no MTP inhibition) (Fig. 10A, compare top and middle panels). Inactivation of MTP before metabolic labeling also blocked formation of VLDL containing
After chase commenced, secretion of 35S-apoB100 as VLDL and HDL (Table VI). Although to a lesser extent, other PC species are stimulated when exogenous oleate is added to the medium (Fig. 6). At the dose of BMS-197636 used in these experiments, the translation of apoB100 was not affected (Fig. 6). The results of these experiments are evidence that once apoB100 has exited ER, the exogenous oleate-induced VLDL assembly requires no MTP activity and therefore is insensitive to MTP inhibition.

**Molecular Species of Phospholipids in Subcellular Compartments**—In McA-RH7777 cells, molecular species of phospholipids are regulated by deacylation and reacylation processes that are stimulated when exogenous oleate is added to the medium to induce VLDL assembly and secretion (22). We hypothesized that oleate treatment might produce a unique membrane micro-environment that is composed of phospholipids with molecular species especially suitable for VLDL assembly. As a first attempt to test this hypothesis, we determined the effect of oleate treatment on the PC and PE molecular species associated with secreted lipoproteins. Oleate treatment resulted in increased PC (by 56%) and PE (by 108%) mass in the lumen of all subcellular compartments with the highest increase in PC and PE mass by oleate treatment was detected here. In fact, lumenal PC and PE mass was increased in all subcellular compartments. For instance, lumen PC (mainly species with 18:1) was increased by 52, 42, and 91% in ER, cis/medial Golgi, and distal Golgi membranes, respectively (Fig. 11C and Table IV). Similarily, elevation of most PE species occurred in the lumen of all subcellular compartments with the highest increase in the distal Golgi lumen (Fig. 11D and Table V). The increased lumenal PC and PE mass cannot be an artifact resulting from membrane rupture by homogenization or carbon-accine treatment, because the molecular species of PC and PE in the lumen were obviously distinct from those associated with the membranes (Tables II–V). It is possible that the ER lumenal PC and PE are part of the previously reported lipid entities devoid of apoB (27). We also determined molecular species of PC that are associated with secreted lipoproteins. Oleate treatment markedly increased PC species with 18:1 in VLDL and VLDL₃, but had no effect in IDL/LDL and resulted in decrease in HDL (Table VI). Although to a lesser extent, other PC species were also increased in VLDL and decreased in HDL by oleate

![Comparison of the size distribution of lipoprotein particles between cis- and trans-Golgi](image-url)

**Fig. 9. Comparison of the size distribution of lipoprotein particles between cis- and trans-Golgi.** Histograms of pooled lipoprotein diameter data for sacules 1–3 (A) and sacules 4–6 plus TGN (B) revealed five species of particles of increasing size denoted as *1* to *5*. Each species was represented by multiple columns, where the highest column corresponds to the diameter of that species at its equator, whereas the stepwise decreasing columns to the left likely represent cross-sections of that species away from its equator.
treatment. The secreted PE species were not determined because of low abundance. Together, data of lipid analysis revealed that the oleate-induced VLDL assembly and secretion was associated with drastically altered phospholipid content and composition in the membranes of the secretory pathway.

**DISCUSSION**

The rat hepatoma McA-RH7777 cells retain the ability to synthesize and secrete TG-rich VLDL (i.e. VLDL₁, S₄ > 100) when cultured in the presence of exogenous oleate. By transfecting human apoB100 into these cells, we have been able to investigate the biochemical events during assembly of VLDL containing human apoB100 (19). The current study was intended to determine the subcellular compartments where the oleate-induced assembly of apoB100-VLDL (i.e. incorporation of bulk TG) was achieved. Using comprehensive biochemical approaches, we have determined the path through which the membrane-bound nascent apoB100 polypeptides are converted into buoyant VLDL. The transition from membrane-bound apoB100 to VLDL occurs clearly as the nascent apoB100 polypeptides move from ER to the distal Golgi (Figs. 3, 5, and 6). In this study, the identities of ER and Golgi microsomes have been authenticated not merely by exhaustive immunolocalization of the marker proteins among the Nycodenz fractions (e.g. calnexin, MTP, protein disulfide isomerase, Grp94, Bip, Hsp47, ERGIC53, β-COP, ManII, COPII, and TGN38) (Figs. 1, A–C, and 4D). In addition, they are validated by the distribution of sphingomyelin with respect to PC (Fig. 4B) and by the glycosylation status of human apoB100 at various subcellular compartments (Fig. 7). The demonstration that the appearance of VLDL in the lumen coincided with apoB100 gaining Endo H resistance suggests strongly that assembly of VLDL must be achieved in post-ER compartments in these cells. Thus, the current work, as a sequel of our previous studies showing the temporal features associated with post-translational VLDL formation (2), has provided new insights into the spatial perspectives of apoB100-VLDL assembly in McA-RH7777 cells.

Three observations from the current study are noteworthy. First, the appearance of apoB100-VLDL observed within the distal Golgi lumen occurs almost concurrently with the secretion of apoB100-VLDL into the medium (Fig. 6). This observation provides solid evidence that the compartment where apoB100-VLDL is assembled must be in close proximity to the site for its secretion. Moreover, this observation also indicates that mature apoB100-VLDL particles, once assembled with bulk neutral lipids, are immediately secreted and not retained within the Golgi. The identical glycosylation status of VLDL-associated apoB100 between distal Golgi lumen and medium (Fig. 7, B and C) indicates the former being direct precursors of the latter. The rapid release of apoB100-VLDL after full assembly is also supported by the pulse-chase data that the amount of 35S-apoB100 in the lumen is 10–20-fold lower than that in the medium during chase. The low abundance of apoB100-VLDL within the microsomal lumen has also been observed in cultured rat hepatocytes (28). Thus, the current results, in agreement with conclusions drawn previously by Bamberger and Lane (4, 17) that VLDL assembly occurs in the Golgi, indicate that apoB100-VLDL has a transient nature within the distal Golgi.

**FIG. 10.** MTP activity is not required for the VLDL assembly and secretion after apoB exited ER. The cells were pretreated with or without 0.2 μM of MTP inhibitor (BMS-197636) for 30 min, pulse-labeled with [35S]methionine/cysteine for 20 min, and chased for 45 min. In one set of experiments, the inhibitor was present throughout pulse and chase (BMS (pulse & chase)). In the other set, the inhibitor was added during the last 30 min of chase (BMS (chase)). Medium (A) and lumenal content of distal Golgi (fractions 1–3) (B) were collected and subjected to cumulative rate flotation centrifugation. 35S-apoB100 was immunoprecipitated with anti-human apoB antiserum and resolved in SDS-PAGE/fluorography. C, cells were incubated with various concentrations of MTP inhibitor (0–0.5 μM) for 30 min in the presence of 0.4 mM oleate. The cell lysates were subjected to MTP assay. Note that about 80% of MTP was inactivated during 30 min of incubation with the inhibitor. D, the cells were pretreated with 0.2 μM of BMS-197636 for 30 min and then labeled with [35S]methionine/cysteine for 10, 20, and 30 min in the presence of MTP inhibitor. Oleate (0.4 mM) was present throughout the experiment. The cells were solubilized, and total 35S-apoB100 was immunoprecipitated and detected by SDS-PAGE/fluorography.
Second, transit of lipoprotein particles through the Golgi results in a 1.4-fold increase in the average diameter (and a 2.7-fold increase in volume, assuming spherical particles) and a shift from two smaller toward three larger diameter species. These observations are compatible with lipid recruitment across the stacked Golgi. The average diameter (40 nm) of

![Distribution of PC and PE in membranes and lumenal contents of subcellular compartments.](image)

**FIG. 11.** Distribution of PC and PE in membranes and lumenal contents of subcellular compartments. The cells were incubated with (hatched bars) or without (black bars) 0.4 mM oleate for 18 h. The subcellular compartments were fractionated by the Nycodenz gradient centrifugation, and the membranes and lumenal contents of distal Golgi (fractions 1–3), cis/medial Golgi (fractions 4–8), and ER (fractions 9–15) were isolated by sodium carbonate treatment followed by ultracentrifugation. Lipids of the membranes (A and B) and lumenal contents (C and D) of subcellular compartments were extracted and subjected to electrospray tandem mass spectrometry for the analysis of PC (A and C) and PE (B and D) as described under “Experimental Procedures.”

**TABLE II**

Analysis of membrane PC species

Single underlines indicate species that showed mass increase, and double underlines indicate species that showed mass decrease by treatment with oleate (OA).

| Species                        | Distal Golgi Control | Distal Golgi OA | cis/medial Golgi Control | cis/medial Golgi OA | ER Control | ER OA |
|-------------------------------|----------------------|----------------|--------------------------|---------------------|------------|-------|
| 14:0–14:0*                    | 0.14                 | 0.14           | 0.14                     | 0.14                | 0.14       | 0.14  |
| 14:0–16:0                     | 0.24                 | 0.45           | 1.56                     | 0.45                | 0.86       | 1.00  |
| 16:0–16:1                     | 0.80                 | 1.04           | 5.34                     | 1.87                | 6.69       | 5.80  |
| 16:0–16:0                     | 0.81                 | 0.94           | 2.37                     | 1.54                | 2.12       | 3.23  |
| 16:1–18:2                     | 0.82                 | 1.13           | 4.48                     | 3.11                | 6.59       | 10.33 |
| 16:0–18:1                     | 2.51                 | 5.33           | 13.21                    | 9.60                | 16.44      | 33.37 |
| 16:0–20:5, 18:2–18:3          | 0.08                 | 0.01           | 0.38                     | 0                   | 0.60       | 0     |
| 16:0–20:4, 18:2–18:2          | 0.13                 | 0.01           | 0.63                     | 0                   | 1.04       | 0     |
| 18:1–18:2, 16:0–20:3          | 0.30                 | 0.47           | 1.24                     | 0.91                | 1.90       | 3.49  |
| 18:0–18:1, 18:0–18:2          | 0.94                 | 4.72           | 5.32                     | 11.74               | 7.04       | 43.25 |
| 18:0–18:1                     | 0.83                 | 2.11           | 3.47                     | 3.74                | 3.32       | 12.17 |
| 18:0–18:0                     | 0.13                 | 0.01           | 0.48                     | 0                   | 0          | 0     |
| 18:2–20:5                     | 0.01                 | 0.01           | 0.00                     | 0                   | 0          | 0     |
| 18:0–22:6, 18:2–20:4          | 0.10                 | 0.16           | 0.35                     | 0.30                | 0.85       | 1.05  |
| 18:1–20:4, 16:0–22:5, 18:0–20:5 | 0.09             | 0.19           | 0.47                     | 0.59                | 0.86       | 1.50  |
| 18:0–20:4, 18:1–20:3          | 0.08                 | 0.17           | 0.40                     | 0.43                | 0.72       | 1.42  |
| 18:0–20:3                     | 0.15                 | 0.24           | 0.51                     | 0.47                | 0.68       | 1.58  |
| 18:1–20:1                     | 0.47                 | 1.27           | 1.75                     | 1.59                | 1.42       | 4.50  |
| 18:2–22:6                     | 0.03                 | 0.05           | 0.04                     | 0.05                | 0.04       | 0.11  |
| 18:1–22:6, 18:2–22:5          | 0.03                 | 0.15           | 0.32                     | 0.34                | 0.43       | 0.82  |
| 18:0–22:6, 18:1–22:5          | 0.14                 | 0.10           | 0.42                     | 0.10                | 0.46       | 0.70  |

* Internal standard.
Single underlines indicate species that showed mass increase, and double underlines indicate species that showed mass decrease by treatment with oleate (OA).

| Table III |
| Analysis of membrane PE species |

| Species | Distal Golgi | cis/medial Golgi | ER |
|---------|-------------|-----------------|----|
| 14.0–14.0 | 0.14 | 0.14 | 0.14 |
| 14.0–16.0 | 0 | 0.14 | 0.14 |
| 16.0–16.1 | 0.02 | 0.07 | 0.07 |
| 16.0–16.0* | 0.15 | 0.15 | 0.15 |
| 16.1–18.2 | 0.20 | 0.20 | 0.20 |
| 16.0–18.1 | 0.26 | 0.26 | 0.26 |
| 16.0–20.5, 18.2–18.3 | 0.29 | 0.29 | 0.29 |
| 16.0–20.4, 18.2–18.2 | 0.31 | 0.31 | 0.31 |
| 18.1–18.2, 16.0–20.3 | 0.37 | 0.37 | 0.37 |
| 18.1–18.1, 18.0–18.2 | 0.50 | 0.50 | 0.50 |
| 18.0–18.0 | 0.68 | 0.68 | 0.68 |
| 18.0–20.5 | 0 | 0 | 0 |
| 18.0–22.6, 18.2–20.4 | 0.30 | 0.30 | 0.30 |
| 18.1–20.4, 16.0–22.5, 18.0–20.5 | 0.30 | 0.30 | 0.30 |
| 18.0–20.4, 18.1–20.3 | 0.27 | 0.27 | 0.27 |
| 18.0–20.3 | 0.54 | 0.54 | 0.54 |
| 18.0–18.0 | 0.19 | 0.19 | 0.19 |
| 18.0–20.5 | 0 | 0 | 0 |
| 18.0–22.6, 18.2–20.4 | 0.30 | 0.30 | 0.30 |
| 18.1–20.4, 16.0–22.5, 18.0–20.5 | 0.30 | 0.30 | 0.30 |
| 18.0–20.4, 18.1–20.3 | 0.16 | 0.16 | 0.16 |
| 18.0–18.0 | 0.53 | 0.53 | 0.53 |
| 18.0–18.1 | 0.39 | 0.39 | 0.39 |
| 18.0–18.2 | 0 | 0 | 0 |
| 18.0–20.5 | 0 | 0 | 0 |
| 18.0–22.6, 18.2–20.4 | 0.05 | 0.05 | 0.05 |
| 18.1–20.4, 16.0–22.5, 18.0–20.5 | 0.05 | 0.05 | 0.05 |
| 18.0–20.4, 18.1–20.3 | 0.06 | 0.06 | 0.06 |
| 18.0–20.3 | 0.07 | 0.07 | 0.07 |
| 18.1–20.1 | 0.16 | 0.16 | 0.16 |
| 18.2–22.6 | 0 | 0 | 0 |
| 18.1–22.6, 18.2–22.5 | 0.05 | 0.05 | 0.05 |
| 18.0–20.4, 18.1–22.5 | 0.05 | 0.05 | 0.05 |

*Internal standard.

| Table IV |
| Analysis of lumen PC species |

| Species | Distal Golgi | cis/medial Golgi | ER |
|---------|-------------|-----------------|----|
| 14.0–14.0 | 0.04 | 0.04 | 0.04 |
| 14.0–16.0 | 0.14 | 0.14 | 0.14 |
| 16.0–16.1 | 0.23 | 0.23 | 0.23 |
| 16.0–16.0 | 0.31 | 0.31 | 0.31 |
| 16.1–18.2 | 0.27 | 0.27 | 0.27 |
| 16.0–18.1 | 0.54 | 0.54 | 0.54 |
| 16.0–20.5, 18.2–18.3 | 0.29 | 0.29 | 0.29 |
| 16.0–20.4, 18.2–18.2 | 0.28 | 0.28 | 0.28 |
| 18.0–20.3 | 0.28 | 0.28 | 0.28 |
| 18.0–18.0 | 0.42 | 0.42 | 0.42 |
| 18.0–20.5 | 0 | 0 | 0 |
| 18.0–22.6, 18.2–20.4 | 0.05 | 0.05 | 0.05 |
| 18.1–20.4, 16.0–22.5, 18.0–20.5 | 0.05 | 0.05 | 0.05 |
| 18.0–20.4, 18.1–20.3 | 0.06 | 0.06 | 0.06 |
| 18.0–20.3 | 0.07 | 0.07 | 0.07 |
| 18.1–20.1 | 0.20 | 0.20 | 0.20 |
| 18.2–22.6 | 0 | 0 | 0 |
| 18.1–22.6, 18.2–22.5 | 0.05 | 0.05 | 0.05 |
| 18.0–20.4, 18.1–22.5 | 0.05 | 0.05 | 0.05 |

* Internal standard.

Lipoprotein particles in the Golgi of McA-RH7777 cells expressing human apoB100 resembles that of negatively stained lipoproteins, isolated from the lumen of rat liver Golgi fractions (39 nm) (16) or viewed within mouse liver Golgi fractions (35 nm) (18). The five lipoprotein species (~20-, 40-, 50-, 60-, and 75-nm diameter) identified within the Golgi (Fig. 9) cannot be placed in a maturation continuum until the lipid/apolipoprotein content of each species is known. However, isolated HDL-type particles have a maximum diameter of 25 nm, whereas isolated VLDL-type particles range between 30 and 80 nm (29). Thus, the shift from two smaller species in cis elements toward the three larger species in trans elements is compatible with the biochemical data of assembly of VLDL occurring in the Golgi. The decreases in average lipoprotein particle diameter between saccule 6 and the TGN and between the TGN and the secretory granules are compatible with lipid recruitment ceasing to occur past the stacked Golgi and may reflect remodeling of assembled VLDL.

Third, the newly synthesized apoB100 polypeptides enter the cis/medial Golgi compartments as membrane-associated forms that remain Endo H-sensitive. Unlike what was originally thought, that the membrane-associated apoB polypeptides were secretion-incompetent and destined for degradation (30), recent experimental evidence has indicated that the membrane-associated apoB are the direct precursors of secreted VLDL both in McA-RH7777 cells and in cultured primary rat...
hepatocytes (11, 12, 28). Thus, the near absence of apoB100 in the lumen of ER and cis/medial Golgi (Figs. 5B and 6), the frequent detection by electron microscopy of membrane-associated lipoproteins in the cis-, but not trans-Golgi (Fig. 8), together with the narrow time window between VLDL assembly and VLDL secretion (Fig. 6) suggested strongly that the secretion-competent VLDL particles utilize membrane-associated apoB100 during assembly. The observed difference in Endo H sensitivity between medium (Endo H-resistant) and membrane-associated apoB100 (Endo H-sensitive) is reminiscent of a previous report that membrane-bound apoB in rat hepatocytes had oligosaccharide moieties distinct from that of apoB in the plasma (31).

Although evidence abounds, the significance of apoB association with membranes during VLDL assembly is unknown, nor is the physical nature of apoB-membrane interactions clear. We have recently postulated that membrane phospholipid remodeling plays an important role in apoB100-VLDL assembly in oleate-treated McA-RH7777 cells (22). In these cells, remodeling of phospholipids is mediated primarily by Ca$^{2+}$-independent phospholipase A$_2$ (22). As an attempt to unravel the mechanisms underlying the oleate-induced apoB100-VLDL assembly and secretion, we have quantified PC and PE molecular species in the membranes and lumen of the secretory compartments. Of note was the observation that oleate treatment resulted in increased PC and PE mass in the distal Golgi and ER membranes, respectively, with a concomitant decrease in the cis/medial Golgi membranes (Fig. 11, A and B). In the case of PC, the mass increase was accompanied with noticeable changes in membrane phospholipid composition may regulate membrane association and proper folding of apoB100 in

### TABLE V

| Species | Distal Golgi | cis/medial Golgi | ER |
|---------|-------------|-----------------|----|
|         | Control     | OA              | OA | Control | OA | OA | Control | OA |
| 14:0–16:0 | 0            | 0               | 0  | 0       | 0  | 0  | 0       | 0  |
| 16:0–16:1 | 0.06         | 0.20            | 0.10 | 0.14   | 0.26 | 0.32 |
| 16:0–16:0$^a$ | 0.31         | 0.31            | 0.31 | 0.31   | 0.31 | 0.31 |
| 16:1–18:2 | 0.02         | 0.06            | 0.15 | 0.12   | 0.05 | 0.07 |
| 16:0–18:2 | 0.12         | 0.33            | 0.62 | 0.67   | 0.61 | 0.67 |
| 16:0–18:1 | 0.20         | 0.48            | 0.62 | 0.87   | 0.52 | 0.79 |
| 16:0–20:5 | 0.08         | 0.30            | 0.18 | 0.34   | 0.34 | 0.18 |
| 16:0–20:4 | 0.09         | 0.31            | 0.18 | 0.40   | 0.35 | 0.38 |
| 18:1–18:2 | 0.11         | 0.56            | 0.57 | 1.00   | 0.49 | 0.75 |
| 18:1–18:1 | 0.24         | 1.55            | 0.91 | 2.97   | 0.98 | 3.03 |
| 18:0–18:1 | 0.11         | 0.85            | 0.29 | 1.00   | 0.62 | 0.90 |
| 18:0–18:0 | 0.06         | 0.14            | 0.10 | 0.10   | 0.15 | 0.18 |
| 18:2–20:5 | 0            | 0               | 0   | 0      | 0   | 0   |
| 18:0–22:6 | 0.11         | 0.38            | 0.60 | 0.64   | 0.39 | 0.61 |
| 18:1–20:4 | 0.17         | 0.93            | 0.68 | 1.31   | 0.55 | 1.48 |
| 18:0–20:4 | 0.24         | 0.83            | 0.60 | 1.24   | 0.53 | 1.78 |
| 18:0–20:3 | 0.11         | 0.43            | 0.38 | 0.78   | 0.44 | 0.77 |
| 18:2–20:1 | 0.02         | 0.05            | 0.07 | 0.06   | 0.09 | 0.07 |
| 18:1–22:6 | 0.04         | 0.32            | 0.14 | 0.40   | 0.19 | 0.55 |
| 18:0–22:6 | 0.15         | 0.42            | 0.40 | 0.64   | 0.56 | 0.77 |

$^a$Internal standard.

### TABLE VI

| Species | VLDL1 | VLDL2 | IDL | LDL | HDL |
|---------|-------|-------|-----|-----|-----|
|         | Control | OA | Control | OA | Control | OA | Control | OA |
| 14:0–16:0 | 2 | 3 | 2 | 3 | 3 | 3 | 14 | 13 | 30 | 11 |
| 16:0–16:1 | 4 | 5 | 7 | 7 | 6 | 6 | 19 | 16 | 64 | 20 |
| 16:0–18:2 | 3 | 12 | 10 | 12 | 10 | 15 | 48 | 44 | 338 | 170 |
| 16:0–18:1 | 4 | 21 | 10 | 21 | 25 | 36 | 73 | 79 | 292 | 122 |
| 16:0–20:4 | 1 | 3 | 1 | 4 | 3 | 6 | 3 | 5 | 84 | 31 |
| 18:1–18:2 | 2 | 4 | 3 | 6 | 5 | 8 | 14 | 15 | 142 | 53 |
| 18:1–18:1 | 3 | 28 | 9 | 35 | 25 | 34 | 91 | 90 | 696 | 260 |
| 18:0–18:1 | 4 | 11 | 7 | 12 | 26 | 32 | 71 | 73 | 253 | 101 |
| 18:0–18:0 | 2 | 2 | 1 | 2 | 3 | 3 | 14 | 13 | 50 | 19 |
| 18:0–20:4 | 3 | 3 | 4 | 4 | 6 | 10 | 14 | 13 | 96 | 38 |
| 18:0–20:3 | 2 | 2 | 2 | 4 | 6 | 12 | 18 | 20 | 70 | 26 |
| 18:1–20:1 | 2 | 6 | 4 | 8 | 12 | 14 | 29 | 28 | 50 | 18 |
| 18:0–20:6 | 1 | 2 | 1 | 2 | 2 | 4 | 8 | 5 | 40 | 15 |
the ER and cis/medial Golgi and facilitate acquisition of bulk neutral lipids in distal Golgi. Correct folding of integral membrane proteins by membrane PE (acting as a molecular chaperon) has been reported (33, 34). Alternatively, changes in membrane phospholipid composition may also modulate apoB100 interaction with various molecular chaperons (12, 35) during trafficking and post-ER assembly. Clearly, the purpose of membrane phospholipid remodeling in oleate-induced apoB100-VLDL assembly and secretion merits further study.

Following its movement, the initial apoB100 associated with ER membrane was gradually transferred to the lumen of distal Golgi (Figs. 5 and 6). Although the Endo H-resistant apoB100 associated with various lipoproteins observed within the distal Golgi lumen is suggestive of apoB100-VLDL being assembled here, it by no means rules out the possibility that assembly commences at cis/medial Golgi compartments. Although apoB100 was present in ER fractions (Figs. 1, 2, and 7), visible lipoprotein particles were not detectable in ER of McA-RH7777 cells expressing human apoB100, using the same TEM protocol that detected particles as small as 20 nm in diameter in ER of rat primary hepatocytes (data not shown). This may imply that expressed human apoB100 only starts to recruit lipid post-ER. Alternatively, poorly lipidated apoB100 particles are formed in cis/medial Golgi. How- ever, the fact that only highly buoyant dense apoB100 particles, instead of VLDL, were releasable from cis/medial Golgi membrane by treatment with mild detergent and high salt concentrations, together with the frequent detection of lipoprotein particles associated with the membranes of cis-Golgi by TEM, suggests a rapid transit of mature VLDL into the distal Golgi. It should be noted that the Nycodenz fractions we considered as distal Golgi (fractions 1–3) are contaminated by endosomes as observed previously by others (36). Immunocolo-

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