Possible role of the Golgi apparatus in the assembly of very low density lipoprotein

(triacylglycerol/apolipoprotein B/secretion/hepatocyte)

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Contributed by M. Daniel Lane, January 4, 1990

ABSTRACT Transit of newly synthesized triacyl[³H]-glycerol through organelles of the secretory system leading to assembly into nascent very low density lipoproteins (VLDLs) or to cytoplasmic storage was investigated in chick hepatocytes. Cells in monolayer culture were pulse-labeled with [2-³H]glycerol, and after different periods of chase with unlabeled glycerol, the movement of triacyl[³H]glycerol through the endoplasmic reticulum (ER) and Golgi and the incorporation into nascent VLDL and cytoplasmic triacylglycerol-rich vesicles (TGRVs) were determined. Initially, newly synthesized triacyl[³H]glycerol is tightly associated with the ER. Movement from the ER of triacyl[³H]glycerol destined for cytoplasmic storage (as TGRVs) is extremely rapid and virtually complete within 8 min of chase. After 8 min of chase, triacyl[³H]glycerol lost from organelles of the secretory system was accounted for entirely as triacyl[³H]glycerol secreted as VLDL. Comparison of the rates of movement of triacyl[³H]glycerol, apolipoprotein B, apolipoprotein II, and apolipoprotein A-I through the ER and Golgi and of their secretion in nascent VLDL suggests that assembly of triacylglycerol with apolipoproteins occurs in the Golgi. Experiments with permeabilized hepatocytes supplemented with cytosol show that newly synthesized triacyl[³H]glycerol and [³H]phospholipid moves from the ER through the full-density range of Golgi elements and is dependent upon supplementary ATP.

Very low density lipoprotein (VLDL), secreted by the hepatocyte, is a major carrier of triacylglycerol in the bloodstream of higher animals. The VLDL particle consists of a neutral hydrophobic core of triacylglycerol and cholesterol ester surrounded by an amphipathic shell of phospholipid, cholesterol, and specific apolipoproteins. Each of these components is synthesized by a distinct membrane-bound enzyme system associated with the endoplasmic reticulum (ER) (1, 2). The apolipoproteins, like other secretory proteins, are synthesized and cotranslationally translocated through the membrane of the ER into its lumen (3, 4). Since the lipid components of VLDL are synthesized by enzymes situated on the cytoplasmic face of the ER (2), the question is raised as to how these lipids cross the ER membrane to gain access to the luminal apolipoproteins with which they must assemble? Also unknown is the site within the secretory system at which this assembly process occurs.

Recently we determined that three apolipoproteins (apoB, apoII, and apoA-I) of nascent VLDL move through the ER of the chick hepatocyte at markedly different rates (5). In each case transit through the Golgi was found to be rate-limiting for the overall process of apolipoprotein secretion. Thus, it can be concluded that these apolipoproteins do not associate with each other or with the same particle during movement through the ER; apoB and apoII are hydrophobic

and difficult to dissociate from the triacylglycerol core of VLDL once this association has occurred (6, 7). It would be expected, therefore, that upon binding to the nascent triacylglycerol particle, these apolipoproteins would remain tightly bound to this lipid during the remainder of their transit through the secretory system. Taken together, these observations and the fact that the apolipoproteins traverse the Golgi at comparably slow rates (5) suggest that the assembly process may occur late in the secretory pathway, perhaps in the Golgi. Lacking, however, is information on the residence times of triacylglycerol in the ER and Golgi as it moves through the secretory pathway.

The present investigation was undertaken to measure the rate of transit of pulse-labeled triacyl[³H]glycerol (relative to the apolipoproteins) through the ER and Golgi of the chick hepatocyte. Comparison of the rates of movement of triacyl[³H]glycerol, apoB, apoII, and apoA-I through these organelles and their secretion as nascent VLDL supports the view that assembly of these components occurs in the Golgi. A cell-free system was used to demonstrate the ATP-dependent movement of triacyl[³H]glycerol (and [³H]phospholipid) from the ER to the Golgi.

EXPERIMENTAL PROCEDURES

Labeling of Primary Chick Hepatocytes with [2-³H]Glycerol, Cell Fractionation, and [³H]Glycerolipid Analysis. Hepatocytes were isolated from estrogen-induced chicks and were plated and treated as described (8). Cellular triacylglycerol was labeled by incubating cell monolayers for 2 min at 37°C with serum-free medium containing 100 μ Ci (1.85 mg/ml; 1 Ci = 37 GBq) of [2-³H]glycerol (9) per ml. To end the pulse, cell monolayers were washed and then chased with medium containing 9.25 mg of unlabeled glycerol per ml for 0, 8, 23, 43, or 83 min. To terminate the chase, the medium was collected, and the cell monolayers were washed once with ice-cold 250 mM sucrose/10 mM Hepes, pH 7.4/2 mM EDTA (SH buffer) and then twice with SH buffer minus EDTA.

Cells were scraped into SH buffer containing protease inhibitors (5) and then were homogenized by 10 passages through the ball-bearing homogenizer (5) described by Balch and Rothman (10). One-half milliliter of 0.22 M sucrose was layered over the cell homogenate, and the sample was centrifuged for 10 min at 10,000 $\times g$. Nuclei and debris from the cell homogenate were in the pellet, whereas cytoplasmic triacylglycerol-rich vesicles (TGRVs; see ref. 11) were in the

Abbreviations: VLDL, very low density lipoprotein; ER, endoplasmic reticulum; TGRVs, triacylglycerol-rich vesicles; apoB, apoII, and apoA-I, apolipoproteins B, II, and A-I.

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layer of 0.22 M sucrose. This layer, containing membrane vesicles derived from the ER and Golgi, was placed on a six-step sucrose gradient containing protease inhibitors at 1 μ l/ml (5). After centrifugation (5), TGRVs, which floated to the top of the gradient, were removed and combined with those from the initial centrifugation. Each of 10 fractions collected from the bottom of the tube were diluted with SH buffer containing protease inhibitors, and the membrane vesicles in each fraction were pelleted by centrifugation (5). Membrane pellets were resuspended in SH buffer, and the [3 H]glycerolipids extracted from the membrane pellets (and the cytoplasmic TGRVs) were separated and quantitated as described below. Organelle marker analyses conducted on membrane vesicles from the sucrose density gradient (5) showed that fraction 2 contained primarily rough ER, fractions 2–4 contained the entire ER, and fractions 5–8 contained the Golgi. [3 H]Leucine-labeled apoB nascent chains, NADPH-cytochrome *c* reductase activity, and galactosyltransferase activity served as markers for the rough ER, ER, and Golgi, respectively (5).

Triacyl[3 H]glycerol and [3 H]phosphoglycerides were extracted from cell fractions as described by Bligh and Dyer (12). Labeled [3 H]phospholipid and triacyl[3 H]glycerol were separated by thin-layer chromatography on silica gel IB2 plates (Baker) using a petroleum ether/diethyl ether/glacial acetic acid (75:25:1, vol/vol) solvent system, located with I_2 vapor, and quantitated by scintillation counting.

Translocation of [3 H]Glycerolipids from ER to Golgi in Permeabilized Chick Hepatocytes. Estrogen-induced chick hepatocytes in monolayer culture were prepared as described above except that the 10-cm culture dishes were treated for 1 hr with a solution containing 50 μ g of fibronectin before addition of the hepatocytes. Cell monolayers were labeled at 37°C with serum-free medium containing 100 μ Ci (1.85 mg/ml) of [3 H]glycerol per ml. After 2 min, the monolayers were washed twice with ice-cold medium containing unlabeled glycerol (9.25 mg/ml). The monolayers were washed twice at 5°C with SH buffer (pH 7.2) without EDTA. The cells were then permeabilized with nitrocellulose filters essentially as described by Balch and co-workers (13). Nitrocellulose filters were wetted with SH buffer without EDTA and then blotted. The filters were then layered onto the monolayers and after 10 min were lifted. Permeabilized cells were scraped from the culture dishes and filters into SH buffer and then were pelleted by centrifugation at 1000 $\times$ *g* and washed once with SH buffer. Permeabilized hepatocytes were gently resuspended in SH buffer and stored on ice until use (5–15 min). Greater than 90% of resuspended cells were permeable to trypan blue.

To monitor movement of tritiated lipids from ER to Golgi, permeabilized hepatocytes (1.5 mg of protein) were incubated at 37°C in 700 μ l of 250 mM sucrose/25 mM Hepes, pH 7.2/50 mM KCl/250 μ M magnesium acetate/200 μ M EDTA/9.25 mg of glycerol per ml/1 mM glycerophosphate. Some assays included cytosol (6 mg of protein) and an ATP-regenerating system consisting of 100 μ M ATP (Na form), 100 μ M magnesium acetate, 2 mM creatine phosphate, and 7 units of creatine phosphokinase per ml. To prepare cytosol, livers from 9- to 12-day-old chicks treated with estrogen as above were homogenized in SH buffer (10% wt/vol), and the homogenate was centrifuged at 10,000 $\times$ *g* for 10 min. The supernate was centrifuged at 100,000 $\times$ *g* for 60 min, and the clear supernatant was concentrated by using Centricon-10 filters (Amicon). When cytosol was added without ATP, 2 mM glucose and 1 unit of hexokinase were included to deplete the cytosol of endogenous ATP. Assays were terminated by chilling the reaction mixture to 2°C. After disruption of permeabilized cells with the ball-bearing homogenizer (5, 10), the homogenate was layered over a six-step sucrose gradient and fractionated as described above. Before frac-

tions were collected from the bottom of the tube, the cytoplasmic TGRVs were removed from the top of the gradient and combined with fraction 10, which itself contained little labeled triglyceride (see Fig. 1).

RESULTS AND DISCUSSION

Transit of Triacyl[3 H]glycerol Through the Secretory Pathway of the Intact Hepatocyte. Previously we compared the transit rates of three apolipoproteins of nascent VLDL through the secretory system of the chick hepatocyte (5). In the present investigation, pulse-chase experiments with [3 H]glycerol were conducted to determine the rate of movement of newly synthesized triacyl[3 H]glycerol through organelles of the secretory system under conditions that permit comparison with the rates of movement of the apolipoproteins. An earlier study (14) showed that virtually all labeled triacylglycerol secreted by chick hepatocytes is recovered in the culture medium in VLDL particles immunoprecipitable (in the absence of detergent) with antibodies directed against apoB and apoII. An important consideration not pertinent to studies with the apolipoproteins is that triacyl[3 H]glycerol, which is synthesized exclusively by the ER (2), can enter two major pathways: (i) the secretory pathway in which assembly into VLDL occurs and (ii) a cytoplasmic storage pathway outside of the secretory system. Cytoplasmic storage of triacyl[3 H]glycerol occurs within cytoplasmic TGRVs, which turn over quite slowly[‡] (11, 15). Importantly, tritium label from TGRV triacyl[3 H]glycerol cannot be incorporated *en bloc* into VLDL triacylglycerol since mobilization of TGRVs involves hydrolysis of triacyl[3 H]glycerol to [3 H]glycerol (which is diluted in the present experiments by the addition of unlabeled glycerol to chase medium) and fatty acids (11, 15). In the present study [3 H]TGRVs, which have an extremely low density, were resolved (by flotation during subcellular fractionation) from organelles of the secretory system, which have higher densities.

Fig. 1 illustrates a representative [3 H]glycerol pulse-chase experiment with hepatocytes subjected to subcellular fractionation after various periods of chase. By the end of the 2-min pulse with [3 H]glycerol, 60–65% of cellular triacyl[3 H]glycerol moved from its site of synthesis in the ER into cytoplasmic TGRVs. The remaining triacyl[3 H]glycerol is associated with membrane vesicles of the secretory system, primarily ($\geq$ 70%) ER membrane vesicles (fractions 2–4 of the sucrose density gradient). By 8 min of chase (Fig. 1), the level of triacyl[3 H]glycerol in the ER (fractions 2, 3, and 4) decreased by 50% with virtually all of this lipid having been directed into TGRVs. After 8 min of chase, however, no further transport of triacyl[3 H]glycerol from the ER into TGRVs can be detected. Thus, the movement of newly synthesized triacylglycerol destined for cytoplasmic storage in TGRVs is extremely rapid and comprises 80–85% of the triacyl[3 H]glycerol synthesized during the pulse.[‡] These results are consistent with previous observations (9, 11). The other 15–20% of triacyl[3 H]glycerol synthesized during the 2-min pulse is retained within the secretory system—i.e., gradient fractions 1–10 (Fig. 1)—and ultimately is secreted as VLDL triacylglycerol. From 8 min of chase to the end of the experiment, the loss of triacyl[3 H]glycerol from these gradient fractions accounts for virtually all of the triacyl[3 H]glycerol secreted into the medium as VLDL.

It is evident, however (Fig. 1), that triacyl[3 H]glycerol does not accumulate in Golgi-containing fractions 5, 6, and 7 prior to its secretion into the medium as nascent VLDL. We have determined (5) that fractions 5, 6, and 7 contain the highest

[‡]TGRVs appear to store triacylglycerol temporarily in the hepatocyte when the rate of triacylglycerol synthesis exceeds the capacity of the secretory system to assemble this lipid into VLDL (11).

FIG. 1. Movement of pulse-labeled [^3H]triglyceride through the secretory pathway of the chick hepatocyte. Estrogen-induced chick hepatocytes in primary monolayer culture were pulse-labeled with medium containing [$2\text{-}^3\text{H}$]glycerol for 2 min and then were washed and incubated with medium containing unlabeled glycerol for various periods of time. Cells were homogenized, the homogenate was subjected to subcellular fractionation, and the distribution of triacyl[^3H]glycerol in sucrose gradient fractions, medium, and cytoplasmic TGRVs was analyzed. Chase times in min: T_0 , 0; T_8 , 8; T_{23} , 23; T_{43} , 43; T_{83} , 83 min. (Inset) Movement of radiolabeled apoB for fractions 5 through 10 with the same fractionation protocol and chase times as given above for [^3H]triglyceride. The apoB data are from ref. 5.

concentration of galactosyltransferase, a trans-Golgi marker enzyme. However, the level of triacyl[^3H]glycerol does increase slightly in fraction 8 between 8 and 23 min of chase, the time interval during which secretion of triacyl[^3H]glycerol begins (Fig. 1). The lack of significant accumulation of triacyl[^3H]glycerol in the Golgi of chick hepatocytes is in contrast to the pattern observed for [^3H]apoB and [^3H]apoII, which accumulate in these fractions during this time interval in pulse-chase experiments with [^3H]leucine (5). For comparison, the pattern of accumulation of apoB in the sucrose density gradient, taken from our earlier paper (5) in which the same fractionation protocol and chase times were used, is shown in Fig. 1 Inset. It is evident that [^3H]apoB accumulates in Golgi-containing fractions 5, 6, and 7 between 8 and 23 min and moves in a sequential manner from the more-dense to the less-dense fractions during the chase. An analysis of the kinetics of apoB movement through the ER and Golgi of the chick hepatocyte showed that transit through the Golgi is the rate-limiting step in apoB secretion (5). The fact that the residence time of apoB and apoII in the Golgi is much longer than that of triacylglycerol strongly suggests that assembly of triacylglycerol with these apolipoproteins does not occur before these components reach the Golgi. The rates of movement of these components should be identical beyond the site of assembly. The relatively short residence time of triacylglycerol in the Golgi suggests that the apolipoproteins, held up in the Golgi, are released upon the arrival of and assembly with triacylglycerol. Another possibility, although not as likely, is that triacylglycerol somehow bypasses the early—i.e., cis and perhaps medial—elements of the Golgi. It is conceivable that triacyl[^3H]glycerol moves into the cytoplasm from the surface of the ER (where triacylglycerol synthesis occurs) and then enters medial or trans-Golgi elements, where assembly may occur. Further insight into this process was gained by use of a cell-free system derived from chick hepatocytes.

Translocation of Triacyl[^3H]glycerol from ER to Golgi in a Cell-Free System Derived from Hepatocytes. To address the questions raised above concerning the route traversed by triacylglycerol in its movement from ER to Golgi and to characterize the processes by which the VLDL particle is assembled, we set out to develop a suitable cell-free system. After numerous unsuccessful attempts to obtain cell-free translocation of triacyl[^3H]glycerol from ER to Golgi with

hepatocytes disrupted by gentle homogenization procedures or by digitonin, we successfully adapted the cell-permeabilization procedure developed recently in Balch's laboratory (13). This technique involves stripping the plasma membrane from hepatocyte monolayers by use of nitrocellulose filters (ref. 13; see *Experimental Procedures*), thereby releasing cytosolic components. This approach permits selective supplementation of the permeabilized cells with the released cytosolic components to assess their role(s) in the translocation of triacylglycerol (and other constituents of nascent VLDL) through organelles of the secretory system.

Initially, intact hepatocyte monolayers were pulse-labeled for 2 min at 37°C with medium containing [$2\text{-}^3\text{H}$]glycerol to label triacylglycerol and phospholipid within the ER (see T_0 zero time of chase in Fig. 1). Cell monolayers were then cooled to 2°C and washed, and the cells were disrupted with nitrocellulose filters. The labeled permeabilized cells, freed of cytosol, were then incubated (in the presence of unlabeled glycerol and glycerol 3-phosphate to prevent further incorporation or reincorporation of [^3H]glycerol) at 4°C or 37°C in the absence or presence of ATP and cytosol. After incubation, the permeabilized cells were homogenized, organelles were fractionated, and the tritiated lipid content of each fraction was analyzed.

When labeled permeabilized cells were incubated at 4°C for 50 min, either in the presence (Figs. 2A and 3A) or absence (results not shown) of ATP and cytosol, little movement of triacyl[^3H]glycerol into Golgi-derived vesicles (fractions 5, 6, and 7) occurred. This distribution of triacyl[^3H]glycerol within the sucrose density gradient was similar to that of intact cells immediately after the 2-min pulse (compare the dashed line in Fig. 3A with T_0 in Fig. 1). A similar pattern is also obtained with pulse-labeled intact cells that have been chilled to 4°C and then chased with unlabeled glycerol at 4°C for 60 min (results not shown). As was the case with intact cells that have been pulse-labeled for 2 min (Fig. 1), a substantial fraction of the cellular triacyl[^3H]glycerol is found in cytoplasmic TGRVs (fraction 10 in Fig. 2A); $\approx 35\%$ of the total triacyl[^3H]glycerol is found in ER vesicles (fractions 1–4) and 55% in TGRVs (fraction 10). Even when the incubation of the labeled permeabilized cells is conducted at 37°C in the absence of ATP and cytosol (Figs. 2B and 3A), movement of triacyl[^3H]glycerol to Golgi-derived vesicles does not occur. When both ATP and cytosol are added,

FIG. 2. Translocation of triacyl[^3H]glycerol from ER to Golgi in permeabilized chick hepatocytes. Intact estrogen-induced chick hepatocytes in primary monolayer culture were pulse-labeled with medium containing [^3H]glycerol for 2 min at 37°C and cooled to 4°C, and their plasma membranes were permeabilized by treatment with nitrocellulose filters. Permeabilized cells were incubated under various conditions for 50 min and subjected to subcellular fractionation, and the triacyl[^3H]glycerol content of the fractions was analyzed. (A) Regions of autoradiograms corresponding to triacylglycerol from sucrose density gradient fractions from labeled permeabilized cells incubated at 4°C in the presence of ATP and cytosol. The 10 sucrose gradient fractions are shown, with fraction 10 representing the cytoplasmic TGRVs. (B) Labeled permeabilized cells incubated at 37°C in the absence of both ATP and cytosol. (C) Labeled permeabilized cells incubated at 37°C in the presence of ATP and cytosol.

however, and the incubation is carried out at 37°C (Figs. 2C and 3C), the level of triacyl[^3H]glycerol in the ER-containing fractions decreases markedly, particularly in fraction 3 which contains primarily smooth ER vesicles (as compared with fraction 2, which contains primarily rough ER vesicles; see ref. 5). This loss of labeled triacylglycerol from the ER during a 5-min incubation is accompanied by movement of triacyl[^3H]glycerol into less-dense Golgi-derived vesicles (fractions 5, 6, and 7 in Fig. 3C) and into TGRVs (fraction 10 in Fig. 3C). When the incubation is extended to 50 min, triacyl[^3H]glycerol moves to an even less-dense region of the sucrose density gradient, peaking in fraction 7 (Figs. 2C and 3C). This pattern is similar to the movement of apoB and triacylglycerol in the intact cell (see Fig. 1).

The rapid movement of triacyl[^3H]glycerol from the ER to Golgi appears to be ATP-dependent, since deleting this nucleotide from the incubation medium results in a marked decrease in labeled triacylglycerol in the low-density region (fractions 5, 6, and 7) of the gradient after 5 or 50 min of incubation (compare with Fig. 3B and C). Despite the omission of ATP, movement of triacyl[^3H]glycerol from the ER into cytoplasmic TGRVs continues (Fig. 3B and C, fraction 10).

The movement of [^3H]phospholipid from ER to Golgi was also measured in labeled permeabilized cells. Most of the [^3H]phospholipid synthesized during the 2-min pulse with [^3H]glycerol is found in the ER (fractions 2, 3, and 4; Fig. 4A). Previous experiments with intact chick hepatocytes (9, 14) showed that only a small fraction of this labeled phospholipid is ultimately secreted in VLDL, most of the labeled phospholipid being incorporated into cellular membranes (9, 14). Nevertheless, it is of interest that movement of the pulse-labeled phospholipid from ER membranes to Golgi membranes (and into cytoplasmic TGRVs, albeit to a lesser extent) in permeabilized cells exhibits the same requirements

FIG. 3. Factors affecting the translocation of triacyl[^3H]glycerol from ER to Golgi in permeabilized chick hepatocytes. Hepatocytes were pulse-labeled with [^3H]glycerol, permeabilized, and fractionated as described in Fig. 2. Triacyl[^3H]glycerol in the sucrose gradient fractions was analyzed by TLC (see the legend to Fig. 2). The dashed line (fractions 1-9) and hatched bar (fraction 10) represent the distribution of labeled triacyl[^3H]glycerol in cells held at 4°C in the presence of ATP and cytosol for 50 min to serve as a control and are reproduced in each panel of Fig. 3 to aid comparison. (A) Distribution of triacyl[^3H]glycerol in permeabilized cells incubated at 37°C in the absence of ATP and cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min. (B) Distribution of triacyl[^3H]glycerol in permeabilized cells incubated at 37°C in the presence of cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min. (C) Distribution of triacyl[^3H]glycerol in permeabilized cells incubated at 37°C in the presence of both ATP and cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min.

as the transit of triacyl[^3H]glycerol from ER to Golgi (and into cytoplasmic TGRVs). Both processes are temperature-dependent, occurring at 37°C but not at 4°C (Fig. 4). Also, movement from ER to Golgi is ATP-dependent (compare Fig. 4B and C). Finally, movement from ER into cytoplasmic TGRVs requires a cytosolic factor(s) (compare fraction 10 in Fig. 4A and B).

Experiments with permeabilized cells showed, more clearly than experiments with intact cells, that newly synthesized triacyl[^3H]glycerol moves from the ER through the full density range of Golgi elements (Fig. 3C). In intact cell experiments (Fig. 1), it had not been possible to detect accumulation of triacyl[^3H]glycerol in the early Golgi elements (fractions 5 and 6), whereas accumulation was readily detected in the permeabilized cell experiments (Fig. 3C). Possibly the rate of transit of triacyl[^3H]glycerol through the Golgi (fractions 5, 6, and 7) is slower in permeabilized cells than in intact cells. Nonetheless, it is evident that triacyl[^3H]glycerol traverses the Golgi. This process occurs at the

FIG. 4. Factors affecting the translocation of [^3H]phospholipid from ER to Golgi in chick permeabilized hepatocytes. Hepatocytes were pulse-labeled with [^3H]glycerol, permeabilized, and fractionated as described in Fig. 2. [^3H]Phospholipid in the sucrose gradient was analyzed by TLC and scintillation counting (see *Experimental Procedures*). The dashed line (fractions 1–9) and hatched bar (fraction 10) represent the distribution of labeled phospholipid in cells held at 4°C in the presence of ATP and cytosol for 50 min to serve as a control and are reproduced in each panel of Fig. 4 to aid in comparison. (A) Distribution of [^3H]phospholipid in permeabilized cells incubated at 37°C in the absence of ATP and cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min. (B) Distribution of [^3H]phospholipid in permeabilized cells incubated at 37°C in the presence of cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min. (C) Distribution of [^3H]phospholipid in permeabilized cells incubated at 37°C in the presence of both ATP and cytosol for either 5 (● and solid bar) or 50 (△ and open bar) min.

same rate and exhibits the same temperature- and ATP-dependence as movement of [^3H]phospholipid-containing membranes through the Golgi elements (Fig. 4C). The similarity of these properties suggest that the same ER-derived vesicles carry both triacylglycerol and membrane phospholipid to the Golgi.

Evidence presented in this and an earlier paper (5) suggests that the assembly of triacylglycerol with apolipoproteins to form nascent VLDL particles does not occur in the ER immediately after synthesis but rather occurs in the Golgi or in another compartment that fractionates with the Golgi.

Since the rates of transit of newly synthesized apoB, apoII, and apoA-I through the ER differ widely (5), it is evident that these apolipoproteins neither associate with each other nor with a common triacylglycerol particle during movement through the ER. The fact that transit through the Golgi is the rate-limiting step in the movement of apoB through the secretory pathway (refs. 5 and 16; Fig 1) suggests that this apolipoprotein may be retained in the Golgi while some event necessary for maturation of the VLDL particle occurs. Perhaps apoB is modified or its orientation is altered, allowing association with triacylglycerol. If this is the case, the assembly process must be rapid, since triacylglycerol does not accumulate in the Golgi. The mechanisms by which the apolipoproteins are held up in the Golgi for assembly and then released for secretion are unknown. Two recent findings may provide insight into this process. It has been observed that several apolipoproteins (5, 16), including apoB and apoII, are membrane-bound while resident in the early dense Golgi elements and that this membrane association decreases as the apolipoproteins move into the late, less-dense Golgi elements (5). It has also been determined (17) that several apolipoproteins, including apoB and apoA-I, become fatty acylated in the secretory pathway. Fatty acylation of the apolipoproteins could provide the hydrophobic anchor for their membrane association and retention in the Golgi during assembly with triacylglycerol. Further investigations will be necessary to test this hypothesis.

This work was supported by Research Grant DK-14575 from the National Institute of Diabetes and Digestive and Kidney Diseases. M.J.B. was supported by National Institutes of Health Postdoctoral Fellowship HL-07038.

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