Pulse-Chase Studies of the Synthesis and Intracellular Transport of Apolipoprotein B-100 in Hep G2 Cells*

(Received for publication, May 20, 1986)

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The synthesis and secretion of apolipoprotein B-100 (apoB-100) have been studied in a human hepatoma cell line, the Hep G2 cells.

The time needed for the synthesis of apoB-100 was estimated to be 14 min, which corresponds to a translation rate of approximately 6 amino acids/s.

ApoB-100 was compared with albumin and α2-macroglobulin as to the distribution between the membrane and the luminal content in the endoplasmic reticulum (ER) and the Golgi apparatus. The results suggested that apoB-100 approximately followed the distribution of these secretory proteins in the Golgi, while the ratios between the percent membrane-bound apoB-100 and percent membrane-bound albumin or α2-macroglobulin were 3–4:1 in the ER. This may suggest that apoB-100 occurs in a membrane-associated form in ER prior to the integration in the lipoproteins.

Pulse-chase studies combined with subcellular fractionation was used to investigate the kinetics for the intracellular transfer of apoB-100. A 3-min pulse of [35S]methionine was followed by an increase in apoB-100 radioactivity in the ER during the first 10–15 min of chase. The following 10–15 min of chase were characterized by linear decrease in apoB-100 radioactivity with a decay rate of approximately 6%/min. The residence kinetics for apoB-100 in the ER differed from that of transferrin and probably also from that of albumin.

By comparing the time for the pulse maximum in ER with that in the denser Golgi fractions the time needed for the transfer between ER and Golgi could be estimated to be 10 min.

The time needed for the secretion of newly synthesized apoB-100 was estimated to be 30 min. This indicates that the transfer of the protein through the Golgi apparatus to the extracellular space requires 20 min.

The major protein component of the low density lipoproteins (LDL)3 of human plasma is referred to as apoB-100 (1).

This protein has a molecular mass of more than 300 kDa (1–3) and is synthesized in the liver. It is coded for by a 20-kilobase mRNA (4–10) but in contrast to some other proteins coded for by large messengers, apoB-100 is secreted without undergoing any major proteolytic processing (3) (compare, for example, the insulin receptor (11, 12), the C3 (13), and C4 (14, 15) complement components). The newly completed apoB-100 molecule is secreted after a delay of 30 min (3).

Secretory proteins in general are synthesized on polysomes bound to the rough endoplasmic reticulum (ER) and cotranslationally transferred to the lumen of this compartment. The proteins are then transported from ER through the Golgi apparatus and secreted (for review, see Refs. 16 and 17).

This kinetic model has recently been questioned by Yeo et al. (20), who have presented results that indicate that the residence time for a glycoprotein in the rough ER explains less than 50% of the residence time in the cell.

It is unclear whether the intracellular transport of apoB-100 follows that of other secretory proteins, as apoB-100 is integrated into a complex particle, the lipoprotein. The processes involved in the assembly and modification (21–24) of the lipoprotein are still not completely elucidated. In view of these considerations we have undertaken this study to characterize the intracellular transport of apoB-100.

ApoB-100 is a hydrophobic protein (8) with physical-chemical properties that have been suggested to resemble those of membrane proteins more than those of the other apolipoproteins (25). In addition structural analyses based on sequences of cloned cDNA have revealed the presence of hydrophobic β sheets (5, 8) and it has been suggested that the protein is woven in and out of the hydrophobic portion of the lipoprotein similar to an integral membrane protein (5). To investigate whether apoB-100 follows the classical route of a secretory protein (see above) or if it may at some stage of the secretion appear as the membrane proteins, we have compared the distribution of apoB-100 between the membrane and content of the ER and Golgi with that to two secretory proteins, albumin and α2-macroglobulin.

MATERIALS AND METHODS

Eagle’s minimum essential medium and Eagle’s minimum essential medium without leucine were from Flow Laboratories (Irvine, United Kingdom). FBS was from Gibco (Paisley, United Kingdom). UDP-[3H]galactose, L-[4,5-3H]leucine, [14C]methylated protein standards, and Amplify were purchased from Amersham International (Amersham, United Kingdom). [35S]Methionine, L-[14C(U)]leucine, Aquasol 2, Econofluor, and Protosol were from New England Nuclear. NADPH and cytochrome c were from Boehringer Mannheim. UDP galactose, ATP,
ovalbumin (V), and Triton X-100 were from Sigma. Antisera to albumin, transferrin, and α1-macroglobulin were from DAKOPATTS (Glostrup, Denmark). Immunoprecipitin was purchased from Bethesda Research Laboratories. All chemicals used for polyacrylamide gel electrophoresis were from Bio Rad. All chemicals were of analytic grade.

Cell Culture—Hep G2 cells (26, 27) were made available by the courtesy of Drs. B. B. Knowles and D. P. Aden (The Wistar Institute, Philadelphia, PA). The cells were cultured as described earlier (3).

Pulse-Chase Studies—The first set of experiments was undertaken to investigate the intracellular transport of apoB-100. Confluent cells in 28-cm² culture dishes were incubated with methionine-free Eagle's minimum essential medium for 2 h. The cells were then pulsed for 5 min with 500 μCi of [35S]methionine and chased for 0, 2.5, 5, 7.5, 10, 20, 30, 40, and 90 min or in a second set of experiment for 0, 10, 15, 20, 25, 30, 35, and 45 min (19). After each chase period the cells were lysed and the cell lysate was fractionated on a linear sucrose gradient (see below). ApoB-100 was recovered from each fraction as well as from the culture medium by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (3). The band corresponding to apoB-190 was identified by autoradiography, cut out, and the radioactivity measured as described earlier (3).

The second set of experiments was carried out to explore the residence kinetics for apoB-100 in the ER with that of transferrin and albumin. The cells were cultured and preincubated as described above and pulsed with 300 μCi of [35S]methionine for 10 min. The pulse was followed by a chase for 0, 10, 20, 30, 40, and 90 min in 1000 μCi of 35S-labeled methionine and the cells were recovered and lysed (3) after each chase period. A fraction enriched in membranes derived from the ER was isolated from the cell lysate by centrifugation on a discontinuous sucrose gradient (see below). ApoB-100, transferrin, and albumin were recovered from the ER fraction by consecutive immunoprecipitation (anti-apoB-100 followed by anti-transferrin and finally anti-albumin) and polyacrylamide gel electrophoresis. The radioactivity in the bands corresponding to apoB-100, albumin, and transferrin were determined as described above.

Subcellular Fractionation on a Linear Sucrose Gradient—The cells were harvested and lysed as described by Fries et al. (19) with the exception that all buffers used contained 1 mM phenylmethylsulfonyl fluoride and 0.1 mM leupeptin.

The homogenization (20 strokes with a tight fitting pestle in a Dounce homogenizer at 0 °C) resulted in less than 5% intact cells as judged by dye exclusion test or counting in a cell counter (A. J. Cellcounter 134, Analys Instrument, Lidingo, Sweden). The sucrose concentration of the cell lysate (500 μl) was adjusted to 250 mM. The lysate was then centrifuged at 19,000 g for 10 min at +4 °C, and the supernatant (sup I) was recovered. The pellet was gently suspended by vortexing in 250 μl of 250 mM sucrose, and a supernatant (sup II) was recovered by centrifugation as described above. The pellet was again suspended in 250 μl of 250 mM sucrose and centrifuged as described above. The recovered supernatant was combined with supernatants I and II and layered on top of a 250 μl cushion of 350 mM sucrose. A 2.5-ml cushion of 32-34% (w/w) sucrose was then layered on top of a 0.6-ml cushion of 49% (w/w) sucrose. This system was adopted from Beaufay and co-workers (28) but was scaled down to fit the purpose of subfractionation of lysed Hep G2 cells from one 28-cm² culture dish. Further, the gradient was adjusted to obtain a maximal separation of the gradient fractions (32-34). These results indicate that fractions 16-19 are enriched in membranes derived from the Golgi apparatus and will be referred to as Golgi in the following presentation.

The membrane and the luminal content were isolated from the ER and Golgi vesicles after disruption of the vesicles in 100 mM sodium carbonate at pH 11.5. The method of Fujiki and co-workers (35) was used with the following modifications: (i) the isolated subcellular fractions were not pelleted before sodium carbonate treatment; (ii) bovine serum albumin was added (final concentration 0.5%) to the sample after the 30 min incubation on ice; (iii) the pellet was washed with 50 mM sodium phosphate, pH 7.3, with 150 mM sodium chloride and 0.5% bovine serum albumin. In our hands these modifications (i-iii) increased the recovery of apoB-100 from approximately 10% to more than 90%.

The radioactivity in apoB-100, albumin, and α1-macroglobulin was determined after isolation of the proteins by immunoprecipitation and polyacrylamide gel electrophoresis (3).

Sudhar Fractionation on a Discontinuous Sucrose Gradient—We have used the system described by Fries and co-workers (19) with the following exceptions: (i) all buffers contained 1 mM phenylmethylsulfonyl fluoride and 0.1 mM leupeptin and (ii) one fraction corresponding to the bottom one-third of the tube was collected. This fraction contained 69 ± 10% (X ± S.D., n = 10) of the NADPH-cytochrome c reductase activity recovered after the centrifugation, corresponding to 38 ± 7% (X ± S.D., n = 15) of the activity present in the cell lysate loaded on the gradient. Only small amounts (7 ± 4, X ± S.D., n = 10) of the galactosyltransferase activity recovered after centrifugation was recovered in this fraction. Based on these findings we regard this fraction I as being enriched in membranes derived from the endoplasmic reticulum.

Estimation of Half-transit Time—Half-transit time for apoB-100 was determined by the double isotope method of Palmier (36) under conditions to minimize the influence of RNase (37). Cells at confluence in 28-cm² culture dishes were incubated with 50 μCi of [14C]leucine for 2 h. This was followed by pulses with 75 μCi of [3H]leucine for 18, 19, and 20 min. After each pulse period the cells were harvested with a rubber policeman and lysed (37). A postmitochondrial supernatant was recovered (38) and the proteins in an aliquot of the postmitochondrial supernatant were precipitated with 20% trichloroacetic acid. The protein pellet was solubilized in 1 M NaOH, and the 1H and 14C activity determined (see below). To isolate the completed proteins the polymers were removed from the postmitochondrial supernatant by centrifugation (3) and the supernatant recovered. ApoB-100 was
isolated from the supernatant by immunoprecipitation and electrophoresis in 3–15% polyacrylamide gradient gels with SDS. The gels were cut in 1-mm pieces and apoB-100 was eluted from the gel slices by shaking in 10 ml of 3% Protosol in Econofluor at 37 °C for 24 h. The gels were then removed and the 3H- and 14C-activity measured (see below).

Radioactivity was determined in a liquid scintillation counter (LKB Rack-Beta) in a system of 3% Protosol in Econofluor (for extracts from polyacrylamide gels) or 10% Protosol in Aquasol 2 (for solubilized trichloroacetic acid precipitate). Channel settings in the liquid scintillation counter had been adjusted to optimize separation between the 14C and the 3H channels under the quenching conditions induced by the extraction of the polyacrylamide gels or the solubilization of the trichloroacetic acid precipitates. Quenching levels were checked for each sample with the external standard channels ratio method and was found to be compatible with the 14C/3H separation intended. Under the conditions used the 14C efficiency was 82% for the extract from the polyacrylamide gels and 73% for the solubilized trichloroacetic acid precipitates. The 3H efficiency was 37% for the extract from the polyacrylamide gels and 22% for the solubilized trichloroacetic acid precipitates.

Electrophoresis—This procedure was carried out in vertical polyacrylamide gradient (3–15%) slab gel containing SDS (39). Autoradiography was carried out by fluorography (40) using Amplify as fluorographic reagent.

Electron Microscopy—The subcellular fractions were fixed in glutaraldehyde, post-fixed in osmium tetroxide, and stained en bloc with 1% uranyl acetate in 70% ethanol (33). After dehydration in a graded series of ethanol solutions, the samples were embedded in agar resin 100 and cut into ultrathin sections on an LKB Ultrotome V. The samples were counterstained with an alkaline bismuth solution (41), and examined in a Philips 400 transmission electron microscope.

RESULTS

Pulse-Chase Studies Combined with Subcellular Fractionation on a Linear Sucrose Gradient—Most of the apoB-100 radioactivity was recovered in fractions 6–19 from the linear sucrose gradient, while only little radioactivity was present in fractions 1–5 and 20–24.

The residence kinetics for apoB-100 in the ER fractions (6–11) was characterized by an increase in radioactivity during the first 10 min followed by a rapid decrease during the next 10 min (Fig. 3).

The residence kinetics in ER was further characterized in three different experiments (Fig. 4) where chase periods of 0, 10, 15, 20, 25, 30, 35, and 40 min were used. The choice of the chase period was based on the observations in Fig. 3 indicating that the pulse maximum in ER appeared after 10 or more than 10 min of chase. The results (Fig. 4) showed a pulse maximum occurring between 10 and 15 min (mean: 11 min) of chase followed by a almost linear decay until 25 min of chase (Y = 166.5X; r = −0.93, n = 11). The curves leveled off after 30 min of chase (compare also Fig. 3). In the denser Golgi fractions (16 and 17) the apoB-100 radioactivity reached a maximum after 20 min of chase. A comparison with the radioactivity maximum in the ER fraction, suggested that the time needed for the transfer of apoB-100 between these two compartments is approximately 10 min. This was confirmed in three additional experiments using chase periods of 0, 10, 15, 20, 25, 30, 35, and 40 min.

In agreement with our earlier findings (3), apoB-100 could first be detected in the culture medium after 30 min chase (five experiments) (Fig. 3). This indicates that the transfer time of apoB-100 through the cell is approximately 30 min (23). ApoB-100 radioactivity in the medium reached its maximum after 45 min of chase (not shown) and leveled off, suggesting a transfer time through the cell of 35 min. Thus, all these data indicate that the time needed for the intracellular transport of apoB-100 is approximately 30 min. Since we have estimated that 10 min is required for the transfer between ER and the Golgi apparatus, this means that 20 min is required for the transfer through the Golgi apparatus to the extracellular space.

Comparison between the Residence Kinetics for ApoB-100 in ER with That for Transferrin and Albumin—The apoB-100 radioactivity in the ER fraction, isolated by the discontinuous gradient, increased during the first 10 min of chase. This was followed by a relatively rapid decrease during the next 20 min (Fig. 5). This residence kinetics did not differ substantially from that found in the ER fractions isolated by the linear sucrose gradient. The residence kinetics for apoB-
FIG. 3. ApoB-100 associated radioactivity in fractions 6–11 (ER) and 16–19 (Golgi) from the linear sucrose gradient (cf. Fig. 1) and in the medium. Hep G2 cells were pulsed with 500 µCi of [35S]methionine for 3 min and chased for 0, 2.5, 5, 7.5, 10, 20, 30, 40, and 90 min. The cells were recovered after each period of chase and fractionated into 24 fractions on a linear sucrose gradient. ApoB-100 was recovered by immunoprecipitation and SDS-polyacrylamide gel electrophoresis from each fraction as well as from the medium. Results are given as counts/min (y axis) as a function of chase period (minutes on x axis).

100 in the ER fraction differed from that of transferrin and probably also from that of albumin (Fig. 5). In the latter case, however, it should be noticed that the initial increase in apoB-100 associated radioactivity may impair the comparison between the two curves.

Estimation of the Translation Time for apoB-100—A linear increase in 3H:14C with time was found between 18 and 20 min for apoB-100 recovered from the pool of completed proteins (Fig. 6). The linear extrapolation (36) based on pulse lengths between 18 and 20 min gave an estimated translation time for apoB-100 of 14 min as the mean of four experiments (the range was 11–17 min).

Distribution of ApoB-100 Albumin and α2-Macroglobulin between the Membrane and Content of the ER and Golgi Vesicles—In the experiment shown in Table I, the Hep G2 cells were incubated with [35S]methionine for 2 h, the ER and Golgi vesicles were recovered with ultracentrifugation in a linear sucrose gradient. The vesicles were disrupted and apoB-100, albumin, and α2-macroglobulin were recovered from the membrane as well as from the luminal content. Only a minor portion of the albumin and α2-macroglobulin (Table I) were recovered from the ER membrane, while on the other hand a substantial amount of apoB-100 was associated with the membrane. The ratio between percent membrane bound apoB-100 and percent membrane bound albumin or α2-macroglobulin were 3.9 and 2.7, respectively. A somewhat larger portion of albumin and α2-macroglobulin was associated with the membrane in the Golgi fractions than it was in the ER fractions (Table I). The results obtained for albumin are in agreement with those reported by Howell and Palade (34). A significantly lower proportion of apoB-100 was associated with the membrane in the Golgi fractions than in the ER fractions (Table I). However, it was still larger than the corresponding values for albumin (the ratio was 1.7) and α2-macroglobulin (ratio 1.3). The significance of this observation is difficult to evaluate. It probably represents a slightly
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FIG. 4. The residence kinetics of apoB-100 in ER. Hep G2 cells were pulsed for 3 min with 500 μCi of [35S]methionine/28-cm² culture dish and chased for 0, 10, 15, 20, 25, 30, 35, and 40 min. The ER fractions were recovered by centrifugation on the linear sucrose gradient and apoB-100 were isolated by immunoprecipitation and polyacrylamide gel electrophoresis. The results are given as percent of the maximal radioactivity in the compartment in three different experiments (□, O, and ●) and each point represents the mean of 2 ER fractions. The solid line represents the regression line (Y = 166 - 5.9X, r = -0.93) for the values between 10 and 25 min.

FIG. 5. Residence kinetics of apoB-100 (●), albumin (○), and transferrin (□) in ER. Hep G2 cells were pulsed for 10 min with 300 μCi of [35S]methionine and chased for 0, 10, 20, 30, 40, and 90 min. A fraction enriched in membranes derived from ER was recovered by centrifugation on a discontinuous sucrose gradient after each period of chase. ApoB-100, albumin, and transferrin were isolated from the ER fraction by consecutive immunoprecipitations (a-apoB-100 followed by a-albumin and finally a-transferrin) and SDS-polyacrylamide gel electrophoresis. The results are given as percent of the initial radioactivity in the compartment (y axis), as a function of time (minutes on x axis).

The change of methodology from continuous labeling (Table I) to pulse-chase labeling (Table II) had no major influence on the proportion of membrane-bound albumin or α₂-macroglobulin. Nor did the resuspension of the membrane pellet in 50 mM Tris-HCl (pH 7.4) with 500 mM KC1, 5 mM MgCl₂, 250 mM sucrose, and 0.5% bovine serum albumin (34) affect these results. On the contrary the proportion of membrane-bound apoB-100 increased considerably (compare Tables I and II). The ratios between the proportion of membrane-bound apoB-100 versus membrane-bound albumin and α₂-macroglobulin was 4.8 and 3.4, respectively.

DISCUSSION

This paper deals with the synthesis and intracellular transport of apoB-100 (1) in an established human liver cell line (Hep G2) (26, 27).
The amount of radioactivity recovered in the ER membrane (percent of total amount in the fraction $X \pm S.D., n = 5$) after a 10-min pulse with 300 $\mu$Ci of $[^{35}]$Smethionine/28-cm$^2$ culture dish followed by chase for 15 min

| ApoB-100 Albumin 2-Macro-globulin |
|----------------------------------|
| LS 67 ± 7 14 ± 2 20 ± 3 |
| HS 63 ± 7 15 ± 3 21 ± 3 |

The time needed for the synthesis of apoB-100 was estimated (36) to be 14 min. Information for the calculation of the translation rate could be obtained from recent studies on the structure of apoB-100 mRNA. ApoB-100 cDNA has recently been cloned and to a large extent sequenced (4-10). Northern blot analysis with such cDNA clones have suggested that apoB-100 mRNA has a size of 15-20 kilo bases (4-10). This messenger appear to contain less than 3% non-coding regions (8, 10), suggesting that it has the capacity to code for a protein of at least 5000 amino acids. Our results would therefore suggest a translation rate of approximately 6 amino acids/s. This translation rate is similar to that reported for other proteins (36, 38, 43). The intracellular transport of apoB-100 in Hep G2 cells was studied by combining pulse-chase methodology with subcellular fractionation on a linear sucrose gradient. The apoB-100 radioactivity peaked in the ER after 10-15 min chase. Using this decay, we estimated the efflux of apoB-100 from ER to be 6%/min. It has to be kept in mind that apoB-100 unspecifically interacts with the ER membrane (percent of total amount in the fraction $X \pm S.D., n = 5$) after a 10-min pulse with 300 $\mu$Ci of $[^{35}]$Smethionine/28-cm$^2$ culture dish followed by chase for 15 min. This is in agreement with an estimated translation time of 14 min. An approximately linear decay of apoB-100 radioactivity was found between 11 and 25 min chase. Based on the finding of a linear decay of apoB-100 radioactivity was found between 11 and 25 min chase. Based on the finding of this linear decay, we estimated the efflux of apoB-100 from ER to be 6%/min. It has to be kept in mind that these results may be influenced by the contamination of the ER fractions with Golgi vesicles. To estimate the influence of such a contamination, we made the assumption that the majority of the apoB-100 radioactivity present in the ER fractions after chase periods of 30 min or more (when the curves had leveled off) was due to contamination of Golgi vesicles. Making this assumption, we could estimate that an ER fraction was at the most contaminated with 3% of the apoB-100 radioactivity present in the Golgi vesicles. The maximal theoretical effect of such a contamination was an increase of the slope from 6 to 6.3%/min.

The peak of apoB-100 radioactivity was recovered in the denser Golgi fractions after 20 min chase. This suggests that newly synthesized apoB-100 molecules are transported from ER to Golgi within 10 min. Since the time needed for the intracellular transport of apoB-100 was estimated to be approximately 30 min, our results indicate that approximately one-third of the time needed for intracellular transport of apoB-100 is due to the transfer from ER to Golgi. These results are in agreement with the findings for other secretory proteins by Yeo et al. (20). Stein and Stein (45) have presented results from electron microscopy studies indicating that $[\text{H}]$palmitate or $[\text{H}]$glycerol that had been injected into rats could be found in the ER fraction of the liver 2 min after the injection, and in Golgi, in lipoprotein form, 10 min after the injection. These in vivo data on the lipid moiety of the lipoprotein are in good agreement with our results on apoB-100.

The residence kinetics for apoB-100 in ER differed from that of transferrin and probably also from that of albumin. This result is consistent with the report by other authors (18, 19, 46), indicating that different secretory proteins leave ER with different kinetics. It should be noticed that the results obtained for albumin and transferrin are in agreement with those presented by Fries and co-workers (19) using the same system for subcellular fractionation.

The results obtained indicate that the transfer of apoB-100 through the latter part of the secretory pathway accounts for approximately two-thirds of the time needed for the intracellular transport of apoB-100. This relatively long transfer time through the Golgi apparatus to the cell surface may be the explanation for the finding of a pulse maximum after 30 min chase in the less dense Golgi fractions (cf. Fig. 3). It should, however, be noticed that the Hep G2 cells express the LDL receptor (47-49). Thus it is possible that the results obtained in the Golgi fractions after more than 30 min chase, may be influenced by apoB-100 in endocytosis vesicles. Such vesicles have been shown to be contaminants in the density fractions containing Golgi vesicles (50). In order to estimate the influence of this contamination we carried out the following experiment: Hep G2 cells were labeled with 200 $\mu$Ci of $[^{35}]$Smethionine/28-cm$^2$ culture dish for 1 h. The medium were changed and the metabolically labeled apoB-100 were chased into the medium for 1 h. The chase medium was transferred to another 28-cm$^2$ culture dish with confluent Hep G2 cells and incubated for 2 h. Cells and medium were collected and apoB-100 were recovered by immunoprecipitation and polyacrylamide gel electrophoresis and the radioactivity determined. Only 1% of the apoB-100 radioactivity was associated with the cells. Together with the finding of Hornick et al. (50) that 1.6% of the endocytosed LDL was found in the Golgi fractions, our finding suggests a very minor influence of endocytosed apoB-100 radioactivity on the apoB-100 radioactivity recovered from the Golgi fractions. Furthermore, most of the results presented in this paper are based on chase periods shorter than 30 min, i.e. before the apoB-100 radioactivity has appeared in the medium. Even under the conditions where the cells had been labeled continuously for 2 h, less than 1% of the radioactivity recovered in apoB-100 from the Golgi could be derived from endocytosed apoB-100.

The relatively long residence time for apoB-100 in the later part of the secretory pathway may explain the observations from ultrastructural studies (21, 45, 51) that VLDL particles are concentrated within the Golgi. The distribution of apoB-100 between the membrane and the content of the Golgi vesicles followed approximately that of the two secretory proteins; albumin and $\alpha_2$-macroglobulin. This was, however, not the case in the ER vesicles where apoB-100 appeared to be associated with the ER membrane to a greater extent. The possibility that the association is a function of the long translation time, i.e. is due to nascent polypeptide chains, is less likely since (i) only the band corresponding to the completed protein have been excised and counted, (ii) the polysomes are stripped from the membranes during the disruption, and (iii) the proportion of membrane-bound apoB-100 increased rather than decreased when the cells were pulse labeled and chased for 15 min, although the nascent polypeptides disappear after this chase period. The main problem in interpreting the results is, however, the possibility that apoB-100 unspecifically interacts with the ER.
membrane during the preparation. It is possible that the finding that a somewhat higher proportion of apoB-100 than of albumin or α2-macroglobulin is associated with the Golgi membrane is due to a higher affinity of apoB-100 for the membranes of the vesicles. The ratios between the proportion of membrane-bound apoB-100 versus albumin or α2-macroglobulin are, however, 2–3 times higher in the ER than in the Golgi. It is therefore less likely that artificial binding explains the entire association between apoB-100 and the ER membrane. The observation of a higher proportion of a membrane-associated apoB-100 in ER after 15 min chase than after 2 h continuous labeling of the cells, could be consistent with a model in which apoB-100 is co-translationally integrated into a membrane associated “pool” and from this pool transferred to the lumen. This model, as well as its relation to the lipoprotein assembly process, is now under investigation in our laboratory.

Acknowledgments—We thank Anita Magnusson, Margareta Ewaldsson, Ulla-Britt Rignell, and Ann-Sofi Anderson for skilled technical assistance and Eva Landegren and Ulla Wallström for typing the manuscript. We would also like to thank Dr. Stanley Fowler and Dr. Erik Fries for valuable discussions concerning the subcellular fractionation.

REFERENCES

1. Kane, J. P., Hardman, D. A., and Paulus, H. E. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2465–2469
2. Elvovson, J., Jacobs, J. C., Schumaker, V. N., and Puppione, D. L. (1985) Biochemistry 24, 1569–1578
3. Wessetsten, N., Boström, K., Bondjers, G., Jarfeldt, M., Norfeldt, P.-J., Carrelli, M., Wiklund, O., Borén, J., and Olofsson, S.-O. (1985) Eur. J. Biochem. 149, 461–466
4. Deeb, S. S., Motulsky, A. G., and Alberts, J. J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4983–4986
5. Knott, T. J., Rall, S. C., Jr., Innerarity, T. L., Jacobson, S. F., Urdea, M. S., Levy-Wilson, B., Powell, L. M., Pease, R. J., Eddy, R., Nakai, H., Byers, M., Priestley, L. M., Robertson, E., Golub, J., and Olofsson, S.-O. (1985) Cell 41, 1317–1327
6. Lusis, A. J., West, R., Mehrabian, M., Reuben, M. A., LeBoeuf, R. C., Kaptein, J. S., Johnson, D. F., Schumaker, V. N., Yuhassa, M. P., Schotz, M. C., and Elvovson, J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4597–4601
7. Carlsson, P., Olofsson, S.-O., Bondjers, G., Darnfors, C., Wiklund, O., and Bjursell, G. (1985) Nuclear Acid Res. 13, 8813–8826
8. Wei, C.-F., Chen, S.-H., Yang, C.-Y., Marcel, Y. L., Milne, R. W., Li, W.-H., Sparrow, J. T., Gott, A. M., Jr., and Chan, L. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7265–7269
9. Law, S. W., Lackner, K. J., Hospettanakar, A. V., Anchors, J. M., Sakaguchi, A. Y., Naylor, S. L., Brewer, H. B. Jr., (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8340–8344
10. Procter, A. A., Hardman, D. A., Schilling, J. W., Miller, J., Appelby, V., Chen, G. C., Kirsher, S. W., McEnroe, G., and Kane, J. P. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1467–1471
11. Ullrich, A., Bell, J. R., Chen, E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Gray, A., Cousens, L., Liao, Y.-C., Tsubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M., and Ramachandran, J. (1985) Nature 313, 756–761
12. Salzman, A., Wan, C. F., and Rubin, C. S. (1984) Biochemistry 23, 6555–6565
13. Ooi, Y. M., and Colton, H. R. (1979) J. Immunol. 123, 2494–2498
14. Rosa, P. A., and Shreffler, D. C. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2332–2336
15. Karp, D. R. (1983) J. Biol. Chem. 258, 14490–14495
16. Walter, P., Gilmore, R., and Blobel, G. (1984) Cell 38, 5–8
17. Sabatini, D. D., Kreibich, G., Morimoto, T., and Adesnik, M. (1982) J. Cell Biol. 92, 1–22
18. Lodish, H. F., Kong, N., Snider, M., and Strous, G. J. A. M. (1985) Nature 314, 80–83
19. Vale, R. W., Guastafosin, L., and Peterson, P. A. (1984) Eur. Mol. Biol. Org. J. 3, 147–157
20. Yeo, K.-T., Parent, J. B., Yeo, T.-K., and Olden K. (1985) J. Biol. Chem. 260, 7896–7902
21. Howell, K. E., and Palade, G. E. (1982) J. Cell Biol. 92, 833–845
22. Janero, D. R., and Lane, M. D. (1983) J. Biol. Chem. 258, 14496–14504
23. Janero, D. R., Siuta-Mangano, P., Miller, K. W., and Lane, M. D. (1984) J. Cell. Biochem. 24, 131–152
24. Higgins, J. A., and Hutson, J. L. (1984) J. Lipid Res. 25, 1295–1305
25. Walsh, M. T., and Atkinson, D. (1983) Biochemistry 22, 3170–3178
26. Aden, D. P., and Kervina, M. (1982) Methods Enzymol. 31, 105–107
27. Wibo, M., Amar-Costesec, A., Berthet, J., and Beaufay, H. (1971) J. Cell Biol. 51, 52–71
28. Fleischer, S., and Kervina, M. (1974) Methods Enzymol. 31, 6–41
29. Howell, K. E., and Palade, G. E. (1982) J. Cell Biol. 92, 822–832
30. Folk, J., Hubbard, A. L., Fowler, S., and Lazarow, R. B. (1982) J. Cell Biol. 93, 97–102
31. Palmiter, R. D. (1972) J. Biol. Chem. 247, 6770–6780
32. Palacios, R., Palmiter, R. D., and Schimke, R. T. (1972) J. Biol. Chem. 247, 2316–2321
33. Gehlke, L., Bast, R. E., and Ilan, J. (1981) J. Biol. Chem. 256, 2514–2521
34. Laemmli, U. K. (1970) Nature 227, 680–685
35. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88
36. Altschuler, S. K., and Karnovsky, M. J. (1972) J. Histochem. Cytochem. 20, 225–229
37. Siuta-Mangano, P., Howard, S. C., Lennarz, W. J., and Lane, M. D. (1982) J. Biol. Chem. 257, 4292–4300
38. Roper, M. D., and Wicks, W. D. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 140–144
39. Hille, M. B., and Albers, A. A. (1979) Nature 280, 469–471
40. Stein, O., and Stein, Y. (1967) J. Cell Biol. 33, 319–339
41. Fitting, T., and Kabat, D. (1982) J. Biol. Chem. 257, 14011–14017
42. Havelkes, L., Van Hinsbergh, V., Kempen, H. J., and Emeis, J. (1983) Biochem. J. 214, 951–958
43. Leichtner, A. M., Krieger, M., and Schwartz, A. L. (1984) Hepatology 4, 897–901
44. Wu, G. Y., Wu, C. H., Rifichi, V. A., and Stockert, R. D. (1984) Hepatology 4, 1190–1194
45. Hornick, C. A., Hamilton, R. L., Spazian, E., Enders, G. H., and Havel, R. J. (1985) J. Cell Biol. 100, 1558–1569
46. Alexander, C. A., Hamilton, R. L., and Havel, R. J. (1976) J. Cell Biol. 69, 241–268