Proteasome-mediated Degradation of Apolipoprotein B Targets Both Nascent Peptides Cotranslationally Before Translocation and Full-length Apolipoprotein B After Translocation into the Endoplasmic Reticulum*

(Received for publication, July 17, 1998, and in revised form, August 19, 1998)

Wei Liao‡§, Sai-Ching Jim Yeung‡, and Lawrence Chan¶

From the Departments of Medicine and Cell Biology, Division of Endocrinology, Baylor College of Medicine, Houston, Texas 77030

A major portion of newly synthesized apolipoprotein B (apoB) is degraded intracellularly. This degradation has been demonstrated to be mediated largely by the ubiquitin-proteasome pathway. We examined whether nascent apoB polypeptides or full-length apoB is selectively retrotranslocated from the endoplasmic reticulum into the cytosol for degradation. Herein, we found that full-length apoB as well as partial-length apoB peptides are ubiquitinated in HepG2 cells, and ubiquitination is an exclusively cytosolic process. Calnexin, which binds specifically to glycoproteins, has been postulated to promote apoB folding and complete translocation; we found that ubiquitinated apoB is bound to calnexin, suggesting that ubiquitinated apoB is glycosylated. In addition to calnexin binding, we have other pieces of evidence that the full-length intracellular ubiquitinated apoB is glycosylated, because (i) it binds to concanavalin A, and (ii) glycan can be demonstrated in the full-length ubiquitinated apoB by a chemical detection method involving oxidation of adjacent hydroxyl groups in the glycan moiety. Because glycosylation occurs inside the endoplasmic reticulum, the full-length glycosylated apoB must have been retrotranslocated into the cytosol for ubiquitination and proteasome-mediated degradation. Next we synchronized translation in HepG2 cells by puromycin treatment. A pulse-chase experiment using [35S]methionine labeling of intracellular apoB in these synchronized cells demonstrated that nascent partial-length apoB peptides are also ubiquitinated cotranslationally. We conclude that the ubiquitin-proteasome-mediated degradation of apoB targets both nascent peptides cotranslationally before translocation as well as full-length apoB after its translocation into the endoplasmic reticulum.

As the sole protein component in low density lipoproteins, apoB-100 is an important determinant of atherosclerosis susceptibility (1, 2). The plasma concentration of apoB-100 balances between its production rate in the liver and its removal from the circulation by receptor- and nonreceptor-mediated pathways (1). It has been known for over a decade that apoB-100 production in the liver is regulated almost exclusively at the posttranscriptional level (3). ApoB mRNA levels do not change substantially in response to various stimuli. However, there appears to be significant variation in the proportion of the newly translated apoB-100 that is degraded intracellularly before secretion (3–6). The intracellular degradation of apoB is thus an important factor determining apoB production in the liver. Recently, the ubiquitin-proteasome pathway has been identified as a major mechanism for the intracellular degradation of apoB-100 (7–9). In the last few years, it has become evident that the proteasome is responsible for the degradation of a number of intracellular proteins, usually resulting in the complete degradation of the target proteins (10–12). The site of proteasome-mediated degradation appears to be cytosolic, requiring the retrograde translocation of the proteins from the endoplasmic reticulum (ER) into the cytosol for degradation (10, 12). ApoB is one of the largest secretory proteins known, and it has a complex structure (2, 13, 14). The proteolytic removal of apoB by proteasomes appears to be a mechanism by which the cell removes apoB molecules that are misfolded, especially when lipid supply is limiting. However, it is presently known whether only incomplete nascent apoB peptides or full-length apoB molecules that are completely translocated into the ER are targeted for proteasome-mediated degradation. In this study, we have examined this mechanistic issue in the biogenesis of apoB-100 in a human hepatoma cell line, HepG2. We found that both incomplete nascent apoB peptides and the full-length apoB that has undergone glycosylation in the ER can be retrotranslocated to the cytosol for ubiquitination and proteasome degradation.

MATERIALS AND METHODS

Reagents—Trypsin, soybean trypsin inhibitor, N-acetyl-L-leucinyl-L-leucinyl-L-norleucinal (ALLN), N-ethylmaleimide (NEM), and rabbit anti-bovine ubiquitin polyclonal antibody were from Sigma. Gamma-bind G (protein G-Sepharose), concanavalin A-Sepharose, and cyanogen bromide-activated Sepharose were from Amersham Pharmacia Biotech. Lactacystin was obtained from Dr. E. J. Corey (Harvard Medical School). Mouse anti-bovine ubiquitin monoclonal antibody and goat anti-human albumin polyclonal antibody were from Research Diagnostic, Inc. Rabbit anti-human albumin polyclonal antibody was from Bio-Rad Laboratories. Grifols, Inc. Rabbit anti-human apoB polyclonal antibody was from Chemicon International, Inc. Rabbit anti-human albumin polyclonal antibody was from Chemicon International, Inc. Rabbit anti-human albumin polyclonal antibody was from Chemicon International, Inc. Rabbit anti-human albumin polyclonal antibody was from Chemicon International, Inc.
(Ottawa Heart Institute). The peroxidase labeling kit was from Pierce. The anti-goat IgG peroxidase, anti-rabbit IgG peroxidase, and oxidative glycan detection kit were from Boehringer Mannheim. The polyvinylidene difluoride membrane was from Bio-Rad. Prestained molecular weight standards and ECL chemiluminescence development solution were from Amersham Pharmacia Biotech. The Rabbit anti-calnexin polyclonal antibody was kindly provided by Dr. Ari Helenius (Yale University Medical School).

Cell Culture and Microsome Preparation—HepG2 cells from American Type Culture Collection were maintained at 37 °C in an atmosphere with 5% CO2 in RPMI 1640 medium containing 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Microsomes were prepared as described by Cosgrove et al. (15). In brief, the cells were scraped in phosphate-buffered saline after washing once with ice-cold phosphate-buffered saline. They were pelleted by centrifugation at 15,000 × g for 5 min. The supernatant was discarded and the pellet washed once with ice-cold phosphate-buffered saline containing 0.1 mM ALLN, 5 mM NEM, 1 mM phenylmethylsulfonyl fluoride, and complete protease inhibitors, and finally suspended in 1% SDS in HEPES-buffered saline. They were then homogenized with a glass Dounce homogenizer in 0.25% sucrose and 10 mM HEPES (pH 7.4) on ice. The cell homogenate was first centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatant was centrifuged at 100,000 × g for 60 min at 4 °C to pellet the microsomes.

Trypsin Digestion—Trypsin digestion of microsomes was done according to Bonnardel and Davia (16). Microsomes were suspended in 0.25% sucrose and 10 mM HEPES, pH 7.4. Trypsin was then added to a final concentration of 200 μg/ml. The mixture was incubated at room temperature for 20 min. After incubation, the microsome tryptic digest (final concentration, 5 mg/ml) was added to stop digestion. The microsomes were then lysed 2.5 h at 4 °C by adding an equal volume of 2% sodium cholate in HEPES-buffered saline (50 mM HEPES, 200 mM NaCl, pH 7.4) containing 2 mM phenylmethylsulfonyl fluoride, 0.2 mM ALLN, and 10 mM NEM, and a protease inhibitor mixture (1 tablet for 50 ml, complete protease inhibitor tablet, Boehringer Mannheim). The lystate was then centrifuged at 10,000 × g for 5 min. The supernatant was used for immunoprecipitation.

Immunoprecipitation and Immunoblot Analysis—Single immunoprecipitation and sequential immunoprecipitation were done as described previously (7). Cells were lysed for 2.5 h at 4 °C in 2% sodium cholate in HEPES-buffered saline (50 mM HEPES, 200 mM NaCl, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 0.1 mM ALLN, and 10 mM NEM, and complete protease inhibitors (Boehringer Mannheim). The cell lystate was then centrifuged at 10,000 × g for 10 min. The supernatant was incubated with anti-apoB antibody at 4 °C on a rotary platform for 1 h. Gamma-Bind G beads were then added and the mixture was incubated for 5 h. After 3 washes with 2% cholate, for single immunoprecipitation, the immunoprecipitate was heated to 100 °C for 5 min in 2× SDS-polyacrylamide gel electrophoresis (PAGE) buffer containing 5% 2-mercaptoethanol and analyzed by SDS-PAGE. For sequential immunoprecipitation, the first antibody immunoprecipitation was done as described above. The Immunobead-antigen complex was washed 3 times and then incubated in 1% SDS in HEPES-buffered saline (0.1 ml) at 95 °C for 3 min to dissociate the antigen from the immunoprecipitate. The non-concanavalin A-bound apoB was recovered from the supernatant by immunoprecipitation using a goat anti-apoB polyclonal antibody. To dissociate the concanavalin A-bound apoB, the concanavalin A-Sepharose was washed 3 times and incubated in 1% SDS in HEPES-buffered saline (0.1 ml) at 95 °C for 3 min. After centrifugation, the supernatant was diluted to 1 ml with 1% Triton X-100 in HEPES-buffered saline containing 0.1 mM ALLN, 5 mM NEM, 1 mM phenylmethylsulfonyl fluoride, and complete protease inhibitors, and then incubated with 50 μl of anti-apoB-Sepharose beads. The column was prepared by conjugating rabbit polyclonal anti-apoB antibody with cyanogen bromide-activated Sepharose according to the manufacturer's procedure (Amersham Pharmacia Biotech). The bound apoB was eluted by 2 bed volumes of 50 mM sodium acetate, pH 4.0. The eluate was then added to 2× SDS-PAGE buffer as described above for the immunoblot assay.

Oxidative Glycan Detection—Three 162-cm2 flasks of HepG2 cells were solubilized in 1% SDS-PAGE PAGE buffer and homogenized in a glass Dounce homogenizer. The lysate was then centrifuged at 10,000 × g for 10 min to pellet any cellular debris. The lysate was then passed through a column (4 ml) of anti-apoB-Sepharose beads. The column was prepared by conjugating rabbit polyclonal anti-apoB antibody with cyanogen bromide-activated Sepharose. The control Sepharose was prepared by quenching the reactive sites on cyanogen bromide-activated Sepharose with cyanogen bromide. The control apoB was eluted the same way. The eluate was then concentrated down to 500 μl by using Centricon (molecular mass cutoff, 20 KDa). 30 μl of each sample in SDS sample buffer was loaded onto the gel. After electrophoresis, the proteins were blotted onto nitrocellulose membranes, and oxidative glycan detection was performed using a kit (Boehringer Mannheim). Briefly, the membrane was incubated with an oxidizing agent, which oxidized the adjacent hydroxyl groups in carbohydrates. The oxidized group was then conjugated to digoxigenin. Detection was then performed with anti-digoxigenin-alkaline phosphatase with color development using 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT/x-phosphate solution). Carboxypeptidase Y, a glycoprotein, and albumin, a nonglycoprotein, were used as positive and negative controls, respectively.

ApoB Translation Following Proteasome Synchronization—Pyruvomycin synchronization was performed as described by Benoist and Grand-Perret (17). HepG2 cells were preincubated in the methionine-free medium for 20 min, and puromycin was added (10 μM) for another 10 min. The cells were then washed 3 times with ice-cold medium. A 5-min pulse label was initiated by the addition of [35S]methionine (100 μCi/ml) in methionine-free medium followed by a 10-min chase by the addition of cold methionine (15 μg/ml). The cells were lysed and ubiquitinated apoB was immunoprecipitated by goat polyclonal anti-apoB then by anti-ubiquitin antibody as described above. The immunoprecipitates were run on 6% SDS-PAGE, the gels were dried, and the autoradiographic image was captured using a storage phosphor system (Cyclone, Packard). In some parallel pyruvomycin synchronization experiments, lactacystin (10 μM) was included in the culture medium.

RESULTS

Polyubiquitination of ApoB in the Absence and Presence of Lactacycin—In the first experiments, we studied the effect of a specific proteasome inhibitor, lactacycin, on the fate of intracellular apoB in cultured HepG2 cells (Fig. 1). In agreement with experiments using other less specific proteasome inhibitors (ALLN and carbobenzoxyl-leucyl-leucyl-norvalinal-H guarana (MG115)) in our laboratory (7), lactacycin (10 μM) treatment led to an accumulation of total intracellular apoB (compare lanes 3 and 4), as well as ubiquitinated apoB (compare lanes 1 and 2). Both in the absence and presence of lactacycin, the ubiquitin-immunoreactive material co-precipitated by the apoB antisera is quite heterogeneous in size. The largest ubiquitinated apoB molecules have an apparent molecular mass significantly larger than 550 kDa, the size of native full-length unubiquitinated apoB, reflecting the presence of multiple 8.5-kDa ubiquitin conjugates covalently linked to the apoB molecule. Importantly, there are substantial amounts of ubiquitinated apoB immunoreactive material that are significantly smaller than full-length apoB-100. These short mole-
Ubiquitinated ApoB in HepG2 Cells Is Bound to Calnexin—Calnexin is a ubiquitous resident ER protein that binds to newly synthesized N-linked glycoproteins (20–22). Chen et al. (9) demonstrated that inhibition of calnexin binding of glycosylated apoB by castanospermine was associated with the proteasome-mediated degradation of apoB. They speculated that calnexin safeguards glycosylated apoB from degradation by promoting correct folding and complete translocation. We investigated whether ubiquitinated apoB, which is tagged for proteasome degradation, would still be bound to calnexin in HepG2 cells. Ubiquitinated apoB was isolated from HepG2 cells by immunoprecipitation as described under “Materials and Methods.” Lactacystin (10 μM) treatment results in the accumulation of ubiquitinated apoB. In addition, the relative molecular mass of the ubiquitinated apoB detected varies from >550 kDa, the size of full-length unubiquitinated apoB, to ~100 kDa in the absence of lactacystin, and <46 kDa in the presence of lactacystin. Blotting antibodies are: α-Ub, anti-ubiquitin antibody; α-ApoB, polyclonal anti-human apoB antibody.

Polyubiquitination Is a Cytosolic Process—To further characterize the ubiquitination process, we isolated microsomes from HepG2 cells and treated them with trypsin. As shown in Fig. 2, trypsin treatment results in a small decrease in the amount of full-length apoB-100, indicating that some of the apoB is on the cytosolic side of the ER. However, the persistence of a substantial amount of apoB following trypsin treatment indicates that only a portion of the full-length apoB under steady-state conditions is accessible to digestion by exogenously added trypsin, and the rest is within the lumen of the ER and not susceptible to the enzyme digestion. In agreement with the observations of Du et al. (18), upon trypsin treatment of HepG2 microsomes, we observed the accumulation of an 85-kDa N-terminal fragment, which is not ubiquitinated. No C-terminal fragment was detected when we used a C-terminal specific monoclonal antibody (Bsal 22) (19) for detection. Furthermore, trypsinization completely removed the ubiquitinated apoB, whereas under the identical experimental conditions, albumin was completely resistant to the proteolytic action of trypsin. Therefore, trypsin is excluded from the ER lumen and cannot access proteins within the lumen. These results indicate that polyubiquitination is an exclusively cytosolic process.

Ubiquitinated ApoB in HepG2 Cells Is Bound to Calnexin—Calnexin binding per se does not provide absolute protection of apoB against ubiquitination. Furthermore, because calnexin only binds to glycoproteins (22), this experiment indicates that ubiquitinated apoB is glycosylated. As a control, inhibition of apoB glycosylation by tunicamycin was found to completely abolish calnexin binding to the unglycosylated apoB in HepG2 cells.

Ubiquitinated ApoB Is Glycosylated—Calnexin binding to ubiquitinated apoB constitutes only indirect evidence for the presence of carbohydrate conjugates in the ubiquitinated protein. To obtain more direct evidence of the presence of carbohydrate, we used two other independent methods to analyze for the presence of carbohydrate conjugates in intracellular ubiquitinated apoB. In the first series of experiments, we used concanavalin A-Sepharose to affinity purify intracellular apoB from cultured HepG2 cells. Concanavalin A binds to apoB molecules that display covalently linked α-D-glucopyranosyl and/or α-D-mannopyranosyl on the surface that is available for interaction with the lectin; therefore, all concanavalin A-bound apoB molecules would be glycosylated. The apoB recovered in the unbound fraction would contain unglycosylated apoB as well as glycosylated apoB whose D-glucose or D-mannose moieties are inaccessible for concanavalin A binding. By immuno-
Proteasome Degradation of apoB

**Fig. 4. Experiments on the glycosylation state of ubiquitinated apoB.** A, concanavalin A binding of ubiquitinated apoB. apoB was isolated from the culture medium and cellular lysate of HepG2 cells previously incubated in 0.1 mM ALLN for 2 h. Glycosylated apoB fractions were isolated by concanavalin A-Sepharose binding as described under "Materials and Methods." The bound apoB from the secreted (Sec) and intracellular (Intracell) compartments and the unbound intracellular apoB were analyzed by 4–15% gradient SDS-PAGE and detected by Western blot analysis. Note the presence of ubiquitin immunoreactivity in the concanavalin A-bound and unbound apoB. α-Ub, anti-ubiquitin antibody; α-ApoB, anti-human apoB monoclonal antibody 1D1. B, chemical detection of glycan on intracellular ubiquitinated apoB. Ubiquitinated apoB was isolated from cellular lysates of cultured HepG2 cells by sequential immunoprecipitation using anti-ubiquitin and anti-apoB. The ubiquitinated apoB was analyzed by 4–15% gradient SDS-PAGE. The presence of glycan in the apoB protein (lane 3) was detected by an oxidative glycan detection method described under "Materials and Methods." Carboxypeptidase Y, a glycoprotein, was used as a positive control (lane 1). Albumin, a nonglycoprotein (lane 2), and control beads (i.e. nonimmune beads instead of anti-ubiquitin beads in the second immunoprecipitation) (lane 4) were used as negative controls. Glycan staining was readily detected in ubiquitinated apoB in the full-length apoB-100 region and in a lower molecular mass region, which probably represents a proteolytic fragment of ubiquitinated apoB.

**Fig. 5. Presence of ubiquitinated nascent apoB polypeptides in synchronized HepG2 cells.** Control HepG2 cells were pulse-labeled for 5 min with [35S]methionine and chased for 10 min with cold methionine. Total and ubiquitinated apoB were isolated (lanes 1 and 2) and analyzed by SDS-PAGE and fluorography. The [35S]-labeled ubiquitinated apoB consists of both full-length and partial-length apoB in these unsynchronized cells. Synchronization was performed in the presence of puromycin as described under "Materials and Methods." In these synchronized HepG2 cells, total and ubiquitinated apoB were isolated in the absence (lanes 3 and 4) and presence (lanes 6 and 7) of lactacystin (10 μM). Ubiquitinated apoB was detected in puromycin-synchronized HepG2 cells in both cases but was more intense in the presence of lactacystin. Therefore, newly initiated incomplete apoB polypeptides are substrates for polyubiquitination during translation. Control, use of control beads instead of anti-ubiquitin antibody in the second immunoprecipitation. α-ApoB, anti-apoB; α-Ub, anti-ubiquitin antibody.

In a second set of experiments to complement the concanavalin A binding studies, we used a chemical approach to document that ubiquitinated apoB contains glycan. Ubiquitinated apoB isolated from HepG2 cells was fractionated on SDS-PAGE, and the presence of glycan on the ubiquitinated apoB was determined by an oxidative glycan detection method as described under "Materials and Methods." An unglycosylated protein, albumin, and a glycosylated protein, carboxypeptidase Y, were used as negative and positive controls, respectively. By using this method, there is unequivocal evidence that intracellular ubiquitinated apoB is glycosylated, because clear glycan staining is seen in the ubiquitinated apoB lane (Fig. 4B, lane 3). The glycan detection method is specific for glycoproteins; another glycoprotein, carboxypeptidase Y, also shows positive staining, but an unglycosylated protein, albumin, remains unstained. We note that the carbohydrate conjugates are found almost exclusively on the full-length ubiquitinated apoB protein, indicating that the full-length glycosylated apoB-100 is a substrate for retrotranslocation and polyubiquitination. A smaller ubiquitinated apoB fragment that is also glycosylated probably represents a proteolytic fragment of apoB.

**Nascent Partial-length ApoB Peptides Are Also Ubiquitinated**—The evidence presented thus far strongly indicates that full-length apoB that has undergone glycosylation, a luminal event in the ER, is the substrate for ubiquitination and proteasome degradation. We also addressed the question whether some of the nascent apoB chains are also targeted for degradation by ubiquitination during translation and prior to completing synthesis of the molecule. apoB-100 translation in HepG2 cells was synchronized by treatment with puromycin prior to pulse labeling with [35S]methionine. By this technique, only newly initiated apoB polypeptide chains were labeled. As shown in Fig. 5, nascent apoB chains of sizes ~220–400 kDa were labeled under these pulse-chase conditions. After purifying the ubiquitinated nascent chains with an anti-ubiquitin antibody and fractionating them on SDS-PAGE, we observed that a proportion of these [35S]-labeled nascent polypep-
tides is ubiquitinated. Therefore, significant ubiquitination occurs on nascent apoB chains during translation. Because we showed above that apoB ubiquitination occurs exclusively in the cytosolic compartment, ubiquitination of these apoB polypeptides must happen cotranslationally before the emerging nascent chain is translocated into the ER.

**DISCUSSION**

We first reported that the intracellular degradation of apoB-100 in HepG2 cells occurs largely via the ubiquitin-proteasome pathway, because it has the following characteristics: (i) it is ATP-dependent, (ii) the intracellular apoB destined for intracellular degradation is ubiquitinated, and (iii) proteasome inhibitors inhibit the degradation leading to the accumulation of ubiquitinated apoB (7). These observations were subsequently confirmed and extended by other laboratories (8, 9, 17). Fisher et al. (8) showed that induced overexpression of hsp70 in HepG2 cells promotes apoB degradation, suggesting a possible role of this chaperone in apoB degradation. Benoist and Grand-Perret (17) demonstrated that inhibition of lipid transfer by a microsomal triglyceride transfer protein inhibitor promotes the degradation of nascent apoB peptides by proteasomes. Chen et al. (9) found that calnexin protects against proteasome-mediated degradation. They also presented experiments that suggest that apoB that has entered the ER far enough to be recognized by the glycosylation machinery may still undergo proteasomal degradation.

In this study, we have demonstrated conclusively that the ubiquitin-proteasome-mediated degradation of apoB affects both incomplete nascent apoB polypeptides cotranslationally as well as the full-length glycosylated apoB protein, which could only have happened to molecules that have completely translocated into the ER. In puromycin-synchronized HepG2 cells, we showed that some nascent partial-length apoB polypeptides are targeted for degradation by ubiquitination during translation before they are translocated into the ER. The evidence for the involvement of proteasomes in the degradation of the incomplete nascent chains corroborates that presented by Benoist and Grand-Perret (17) on microsomal triglyceride transfer protein inhibitor-treated cells. Our experiments demonstrate that proteasome-mediated degradation of newly synthesized partial-length apoB peptides during translation occurs also in the absence of microsomal triglyceride transfer protein inhibitors. Our observations on the fate of the full-length glycosylated apoB are supported by the study of Chen et al. (9) using [3H]mannose labeling, although they did not show that the mannose-labeled apoB was actually targeted for degradation by ubiquitination. Herein we showed directly that full-length ubiquitinated apoB is glycosylated. We have provided three pieces of evidence for the presence of carbohydrate conjugates on ubiquitinated apoB: (i) ubiquitinated apoB retains its binding affinity to calnexin, a glycoprotein-specific chaperone (20–22), an interaction that can be abolished by tunicamycin treatment; (ii) ubiquitinated apoB displays binding to concanavalin A; and (iii) the presence of glycan on ubiquitinated apoB is demonstrated by glycan-specific oxidative staining. The fact that the full-length ubiquitinated apoB has covalently linked carbohydrate indicates that the protein has completely translocated into the ER before it is retrotranslocated back to the cytosol for degradation. Finally, we note that calnexin is a membrane protein of the ER that associates with newly synthesized N-linked glycoproteins (21, 22). The luminal domain of calnexin binds to glycoproteins only if they are at least monoglucosylated (22), and this lectin-like chaperone is thought to assist the folding and oligomeric assembly of glycoproteins and possibly to prevent premature degradation. Here we showed that if calnexin offers protection of apoB from proteasome-mediated degradation, the protection is incomplete, and ubiquitinated apoB actually has affinity for the chaperone.

It is difficult from the available evidence to quantify how much of the apoB tagged for degradation by ubiquitination consists of incomplete nascent chains and how much of it consists of full-length glycosylated apoB protein. As discussed earlier, although concanavalin A only binds to and thus selectively purifies glycosylated apoB that possesses accessible d-glucose and/or d-mannose moieties on the surface, the unbound fraction may be a mixture of unglycosylated and glycosylated apoB polypeptides. Furthermore, glycoproteins in the ER are partially deglycosylated by the action of peptide:N-glycanase, prior to proteolytic degradation in the cytosol (23–25), which would further impair concanavalin A binding and oxidative glycan detection. Therefore, the substantially lower amount of glycosylated versus unglycosylated apoB among the ubiquitinated apoB pool observed in these experiments represents an underestimate of the actual amount of the full-length, once fully glycosylated apoB protein that is targeted for proteasome degradation.

The partially translated polypeptides and the fully translocated apoB could be transported to the cytosolic compartment for proteasome-mediated degradation via one or more of three possible pathways (Fig. 6): (i) the partially translated nascent polypeptides are mostly cytosolic in location when they are ubiquitinated and are presumably fed into the proteasomes by retrotranslocation through the same translocon in which the nascent chain is partially partitioned; (ii) the fully translocated and fully glycosylated apoB-100 proteins could be retrograde-translocated, i.e. via the same translocon through which they have entered the ER; or (iii) these molecules could have been completely discharged into the lumen of the ER and be translocated back into the cytosol in a retrograde fashion via a different retrotranslocon. Our experiments do not allow us to...
make a distinction among these possibilities, which will be the subject for future investigations.

Acknowledgments—We thank Dr. Ross Milne (Ottawa Heart Institute, Ottawa, Canada) for supplying anti-apoB monoclonal antibodies, Dr. Ari Helenius (Yale University Medical School) for supplying anti-calnexin antibody, Benjamin Tseng and Alexander Bamias for excellent technical assistance, and Sylvia Ledesma for expert secretarial assistance.

REFERENCES
1. Kane, J. P., and Havel, R. J. (1995) in The Metabolic and Molecular Bases of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1853–1885, McGraw-Hill, Inc., New York
2. Chan, L. (1992) J. Biol. Chem. 267, 25621–25624
3. Borchardt, R. A., and Davis, R. A. (1987) J. Biol. Chem. 262, 16394–16402
4. Dixon, J. L., and Ginsberg, H. N. (1993) J. Lipid Res. 34, 167–179
5. Ginsberg, H. N. (1995) Curr. Opin. Lipidol. 6, 275–280
6. Yao, Z., Tran, K., and McLeod, R. S. (1997) J. Lipid Res. 38, 1937–1953
7. Yeung, S. J., Chen, S.-H., and Chan, L (1996) Biochemistry 35, 13843–13848
8. Fisher, E. A., Zhou, M., Mitchell, D. M., Wu, X., Omura, S., Wang, H., Goldberg, A. L., and Ginsberg, H. N. (1997) J. Biol. Chem. 272, 20427–20434
9. Chen, Y., Le Cahérec, F., and Chuck, S. L. (1998) J. Biol. Chem. 273, 11887–11894
10. Ciechanover, A., and Schwartz, A. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2727–2730
11. Baumeister, W., and Lupas, A. (1997) Curr. Opin. Struct. Biol. 7, 273–278
12. Suzuki, T., Yan, Q., and Lennarz, W. J. (1998) J. Biol. Chem. 273, 10683–10686
13. Yang, C.-Y., Gu, Z.-W., Weng, S.-A., Kim, T. W., Chen, S.-H., Pownall, H. J., Sharp, P. M., Liu, S.-W., Li, W.-H., Gotto, A. M., Jr., and Chan, L (1989) Arteriosclerosis 9, 96–108
14. Segrest, J. P., Jones, M. K., Mishra, V. K., Anantharamaiah, G. M., and Garber, D. W. (1994) Arterioscler. Thromb. 14, 1674–1685
15. Cosgrove, P. G., Gaynor, B. J., and Harwood, J., Jr. (1998) J. Lipid Res. 39, 1983–2003
16. Bonnardel, J. A., and Davis, R. A. (1995) J. Biol. Chem. 270, 28892–28896
17. Benoist, F., and Grand-Perret, T. (1997) J. Biol. Chem. 272, 20435–20442
18. Du, E. Z., Kurth, J., Wang, S.-L., Humiston, P., and Davis, R. A. (1994) J. Biol. Chem. 269, 24169–24176
19. Chen, P.-F., Marcel, Y. L., Yang, C.-Y., Gotto, A. M., Jr., Milne, R., Sparrow, J. T., and Chan, L (1988) Eur. J. Biochem. 175, 111–118
20. Bergeron, J. J. M., Brenner, M. B., Thomas, D. Y., and Williams, D. B. (1994) Trends Biochem. Sci. 19, 124–128
21. Hammond, C., and Helenius, A. (1995) Curr. Opin. Cell Biol. 7, 523–529
22. Zapun, A., Petrescu, S. M., Rudd, P. M., Dwek, R. A., Thomas, D. Y., and Bergeron, J. J. (1997) Cell 88, 29–38
23. Halaban, R., Cheng, E., Zhang, Y., Moellmann, G., Hanlon, D., Michhalak, M., Setaluri, V., and Hebert, D. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6210–6215
24. Hughes, E. A., Hammond, C., and Cresswell, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1896–1901
25. Wiertz, E. J. H. J., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J., and Ploegh, H. L. (1996) Cell 84, 769–779