Heterologous expression of apolipoprotein B carboxyl-terminal truncates: a model for the study of lipoprotein biogenesis[1]

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Abstract Expression of proteins in cells that lack specific chaperones, or subunits required for correct assembly results in the degradation of these proteins early on after synthesis, probably in the endoplasmic reticulum (ER) or an ER-related compartment. We have explored heterologous expression as a model to study the complex process involved in the biogenesis of apolipoprotein B (apoB)-containing lipoproteins. As an initial step, a series of carboxyl-terminal truncated apoB in COS cells were expressed to characterize this system. ApoB proteins ranging from apoB-13 to apoB-41 were expressed. Truncates larger than apoB-29 were completely retained and degraded within the cells; hence these cells have a 'secretory defect' for the larger truncates. Degradation of these apoB proteins is likely to be in the endoplasmic reticulum or ER-related compartment, as shown by sensitivity to endo β-N-acetylglucosaminidase, by lack of an effect on the degradation kinetics by brefeldin A treatment, and by ImmunoGold EM. Degradation in COS cells was not affected by a variety of protease inhibitors, including N-acetyl-leucyl-leucyl-norleucinal (ALLN). Addition of oleate to the culture medium did not alter their metabolic fate. In comparison to the larger truncates, apoB proteins less than apoB-29 were partially secreted; the majority of these, too, were also retained and degraded intracellularly in a similar compartment. Secreted apoB-17 protein was shown to have been processed correctly in the Golgi and was secreted as a minimally lipidated protein. This model, based on the expression of apoB truncates larger than B-29 in COS cells, may therefore allow for the identification and study of the requirements of specific factors that may be required for both apoB secretion and lipoprotein biogenesis by in vivo complementation of this 'secretory' defect.—**Patel, S. B., and S. M. Grundy.** Heterologous expression of apolipoprotein B carboxyl-terminal truncates: a model for the study of lipoprotein biogenesis. *J. Lipid Res.* 1995. **36:** 2090–2103.

Supplementary key words apolipoprotein degradation • endoplasmic reticulum • apoB-29

The biogenesis of apolipoprotein (apo) B-containing lipoprotein is a multi-step process (1–3). Two species of apoB, apoB-48 and apoB-100, have evolved as the major lipid transporting forms of the apolipoprotein. Both species are utilized for lipoprotein secretion by the livers of mice and rats, but only apoB-100 is secreted by primate liver. The liver cell synthesizes considerably more full length apoB than is secreted; the rest is degraded early on after synthesis. Much of this process takes place in the ER or in an ER-related compartment (4–7). Lipid is added to the apolipoprotein both in the ER and in the Golgi (8–12). The Golgi, in addition to modifying the terminal mannose residues of the N-linked sugars, may also be the site where other associated proteins of the nascent lipoprotein are added (13, 14). The synthesis and secretion of apoB require both the constitutive machinery for translocation and maturation, as do all secretory proteins, and in addition, specific proteins for the formation of a complete lipoprotein particle. The early steps involved in the synthesis and maturation of the apoB into a lipoprotein particle have not been fully characterized (4, 15–21), and it is uncertain as to how many specific factors are needed for the secretion of a VLDL-sized lipoprotein. Hepatocytes and hepatoma cell lines provide a valuable tool for the study of many of these processes (9, 11, 22–25). From these studies, it is known that a large proportion of newly synthesized apoB is degraded in the ER or in an ER-related compartment. Furthermore, the post-translational control of apoB secretion from such cells is at

Abbreviations: apoB, apolipoprotein B; MTP, microsomal triglyceride transfer protein; Endo H, endoβ-N-acetyl glucosaminidase; PNG'ase F, peptidyl-N-glycosidase F; ER, endoplasmic reticulum; CHO, Chinese hamster ovary; BFA, brefeldin A; ALLN, N-acetyl-leucyl-leucyl-norleucinal; FCS, fetal calf serum; VLDL, very low density lipoprotein.

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this stage, at least for the effects of oleate, at the expense of degradation. Genetic disorders of apoB secretion suggest that at least two different loci, other than the apoB locus, are specifically involved in the secretion of lipoproteins containing apoB (26). One of these factors has recently been identified as microsomal triglyceride transfer protein (MTP). Defects in the MTP gene have now been shown to be responsible for abetalipoproteinemia, a condition where apoB is synthesized but not secreted from the liver or the intestine (27, 28).

However, as hepatocytes and intestinal cells already express most, if not all, of the specific factors required for VLDL synthesis, it is difficult to identify and study any one of these factors independent of the others. As an example of this difficulty, oleate supplementation leads to increased secretion of apoB and decreased degradation. However, it is not clear how this is mediated; perhaps oleate has a direct effect on the folding of apoB, or its effects are mediated through MTP, or by increased synthesis of triglyceride.

For this reason, we have sought to develop and characterize another system for the investigation of lipoprotein biogenesis. This system uses the expression of carboxyl-terminal truncated apoBs in a heterologous cell, COS-1. Expression of the full-length apoB-100 cDNA is possible, but has proven relatively more difficult than the carboxyl-terminal truncated forms that have been previously expressed in lipoprotein-secreting cells (29, 30) and have been shown to form lipoprotein particles. Indeed, truncated apoBs have been previously expressed in heterologous cell lines, such as CHO and COS cells (20, 29). For the latter, an apparent secretory defect was detected for apoBs larger than B-26, but this was not further characterized (29). In the case of stable expression in CHO cells, apoB-53 protein could not be detected unless the cells were pre-treated with a protease inhibitor, and no secretion of the apoB-53 was detected under any of these conditions (20). There would therefore appear to be a 'secretory' block for heterologously expressed apoB.

Expression of apoB truncates in COS cells has a number of advantages. There is a high level of expression of apoB cDNAs and the proteins can be readily detected in the absence of protease inhibitor treatment of the cells. COS cells are unlikely to express hepatocyte-specific functions, especially those that are involved in lipoprotein biogenesis. Co-expression of such candidate factors with such apoB truncates in a heterologous cell should allow us both to identify specific factors required for lipoprotein secretion and to study the interaction between them in a defined cell system. To this end, we have characterized apoB expression in the COS cell to investigate the following: *a)* what is the range of truncated apoB that can be secreted by these cells; *b)* where are the larger truncates retained within the cell; *c)* is this process of retention and degradation affected by oleate supplementation; *d)* of the apoB that is secreted, is it secreted as a lipoprotein, and is this affected by oleate supplementation; and *e)* if not, are the secreted apoproteins capable of forming lipoproteins?

EXPERIMENTAL PROCEDURES

Reagents

Tissue culture media were obtained from Gibco-BRL (UK/USA); Protein A-Sepharose, Triton X-100, phenylmethylsufonylfluoride (PMSF), and oleate-cyclodextrin complexes were obtained from Sigma Chemical Co., (St. Louis, MO); brefeldin A was obtained from Epicenter Technologies (Madison,WI); iodoacetamide from Aldrich Chemicals (Fairlawn, NJ); [^{35}S]methionine/cysteine mix for cell labeling was obtained from Amersham (UK) or NEN-DuPont (Boston, MA); antisera to apolipoprotein B was obtained from Boehringer Mannheim Corp. (Germany, sheep antiserum) or Dako Corp. (Denmark, rabbit antiserum); Protein A-Gold from Biocell (UK); all other protease inhibitors and the deglycosylases were obtained from Boehringer Mannheim Corp. Rabbit anti-serum to H2a was a gift from Drs. Bishoff and Shia (Whitehead Institute, Cambridge, MA). All other chemicals were of reagent grade or better.

DNA constructs

The pSV7D-based expression plasmids containing carboxyl-truncated apoB fragments [numbering using the centile system, full length 550 kD apoB is B-100 (31)], apoB-41, apoB-29, apoB-23, apoB-17, and apoB-13 were as previously described (30). ApoB-29.75 was constructed by recloning the Eco RI–Xba I fragment from apoB-41 into pSV7D. The in-frame stop translation signals in all of these constructs was provided by allowing the translation to run into the polylinker sequences containing an in-frame stop translation in all of the three frames. This resulted, in some cases, in the addition of a short novel peptide tail at the carboxyl end (see Fig. 1). The asialoglycoprotein receptor large subunit H2a (32), in the expression vector pcDNAI (Invitrogen, CA), was obtained from Dr. Harvey Lodish (Whitehead Institute, Cambridge, MA).

Cell culture and transfection

COS-1 cells, maintained in 90% D-MEM and 10% FCS, were harvested at 80% confluence for transfection by electroporation using 10×10^6 cells/ml and 20 μg of supercoiled DNA as described (33). The cells were grown for 36–48 h prior to harvesting. Transfection efficiency of 30–50% was routinely obtained, monitored

by parallel transfection with a beta-galactosidase expression plasmid, pCH110 (Pharmacia). The 2–18 cells were grown as previously described (32).

Brefeldin A or oleate treatments

Oleate-cyclodextrin complexes were dissolved in medium at a final concentration of 0.8 mM with respect to oleate. Oleate was added at the pre-incubation step and was present throughout the chase period. For brefeldin A treatment, cells were incubated in brefeldin A for 1 h prior to harvesting and brefeldin A was present throughout the experiment at 5 μg/ml. For incorporation of oleate into cellular lipids, [9,10-^{3}H]oleate (sp act 60 Ci/mM) was added to cells at a concentration of 10 μCi/ml and incubated for 24 h. The cells were collected and total lipids were extracted by the method of Folch, Lees, and Sloane Stanley (34) and separated by thin-layer chromatography, and the amount of label incorporation into triglycerides, phospholipid, and cholesteryl ester was measured by scintillation counting.

Metabolic labeling and immunoprecipitation

All media were pre-warmed to 37°C prior to use. Cells were harvested by PBS/EDTA, washed once in methionine-free D-MEM, pre-incubated in methionine-free medium for 30 min, pulse-labeled in methionine-free D-MEM containing 200 μCi/ml of [^{35}S]methionine (sp act > 1000 Ci/mmol) for 10 min, washed, aliquoted, and chased in complete medium for the times indicated in the Results. In all experiments where a metabolic treatment such as brefeldin A or oleate was performed, to prevent differences in transfection efficiencies from affecting the results, the transfected cell population was split into two equal portions, one of which was used as a control and the other was exposed to the various treatments. At various times into the chase, cells were harvested by centrifugation, the media were collected where appropriate, and the cell pellets were stored on dry ice until lysis. Cell lysis was accomplished by rapid thawing and vortexing in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM PMSF, 20 mM iodoacetamide, and 1% Triton X-100) and incubation on ice for 45 min. The samples were pre-cleared by centrifugation at 14,000 *g* for 15 min at 4°C. Aliquots were taken for total protein assays. The lysates were incubated with 1:100 dilution of apoB sheep or rabbit antiserum on ice for 1 h; Protein-A Sepharose was added and the samples were gently rocked at 4°C overnight. The immunoprecipitates were collected by centrifugation, washed three times with the lysis buffer, resuspended in sample buffer, and analyzed by SDS-PAGE on 6% or 8% gels (35). The gels were fixed, dried, and analyzed using a phosphorimaging screen and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). All the data were normalized by using the total protein estimated from the cell lysates.

Endoglycosidase H and peptide N-glycosidase treatment

After immunoprecipitation, the washed Protein A-immunoprecipitates were further washed once in 150 mM NaCl, resuspended in Endo H buffer (10 mM sodium phosphate buffer, pH 6, 1% mercaptoethanol, 0.04% SDS, 0.2% Triton X-100, and 10 mM EDTA), heated at 100°C for 5 min, adjusted to 1 mM PMSF on cooling, and incubated with either 0.1 mU/ml endoglycosidase H (Endo H) or 0.17 U/ml peptide N-glycosidase F (PNG'ase F) for 8–18 h at 37°C. The incubation was stopped by the addition of sample buffer, heated, and analyzed by SDS-PAGE.

Cesium chloride density gradient centrifugation

After pulse-labeling, cells were chased in the presence or absence of fetal calf serum, and supernatants were collected and cleared of any cellular debris by centrifugation at 2000 *g* for 5 min. The supernatant was split into two fractions and adjusted with solid CsCl to 1.1 g/ml and 1.3 g/ml, respectively. The lighter fraction was overlaid on the denser one and spun in a 70Ti rotor at 55 k for 40 h at 20°C in a Beckman ultracentrifuge, essentially as described (36). For mixing experiments, conditioned media were incubated with 10% FCS (final concentration) or 10% BSA/0.8 mM oleate (final concentration) for 3–4 h at 37°C and then subjected to CsCl gradient centrifugation.

Fractions were collected from the bottom and aliquots were accurately weighed for density estimations. Aliquots were also analyzed for radioactivity by trichloroacetic acid extraction and scintillation counting. The remaining fractions were de-salted by centrifugation in centricon 30 tubes (Amicon, Lexington, MA), adjusted to 1% Triton, immunoprecipitated for apoB, and analyzed by SDS-PAGE.

Electron microscopy

Cells, released from tissue culture by incubation with PBS/EDTA, were fixed in suspension in freshly prepared 8% paraformaldehyde, 100 mM phosphate buffer, pH 7.4, on ice for a minimum of 4 h. The fixative was removed and quenched by washing in 50 mM ammonium chloride for 30 min at room temperature. Dehydration in Lowicryl K4M (Agar Scientific, UK) was carried out as previously described (37) except methanol was substituted for ethanol. Cell were embedded in fresh resin by polymerization at -20°C with UV light for 24–36 h. Ultrathin sections were collected on formvar/carbon-coated copper grids and immunolabeled on 20-μl droplets on parafilm. The sections were

pre-incubated in PBS-3% BSA for 1 h, incubated with 1:50 diluted protein G affinity-column purified sheep antiserum for 1 h, washed 3 × 5 min in PBS/BSA, incubated with Protein A-gold 20 nm (Biocell) diluted 1:100 in PBS/BSA for 1 h, washed as above, rinsed in a distilled water stream, stained with uranyl acetate 15 min, lead citrate for 1.5 min and examined in a JEOL 1200EX electron microscope. Each grid was methodically searched; at least 20 grid squares were examined in detail.

RESULTS

Secretion of truncated forms of apoB

To study the expression of apoB in a heterologous system, a series of carboxyl-truncated apoB expression plasmids (**Fig. 1**) were transfected into COS-1 cells, and the cell lysates and media were analyzed for apoB by quantitative immunoprecipitation (**Fig. 2**). Although apoB-13, apoB-17, and apoB-23 were secreted by the cells, very little apoB-29 or apoB-41 was secreted (Fig. 2). Occasionally some apoB-41 (< 1%) in the media was detectable but this might be attributable to an occasional decline of about 1–5% in cell viability and spontaneous lysis over the experimental period. All the truncates were readily detectable within the cells. Some of the apoB bands appeared as doublets on SDS-PAGE under reducing conditions; these are thought to be due to incomplete cleavage of the signal peptide resulting in both the pro-apoB and apoB proteins (see ref. 38). Pulse chase experiments were carried out to characterize the pattern of expression of apoB on COS cells (**Fig. 3**).

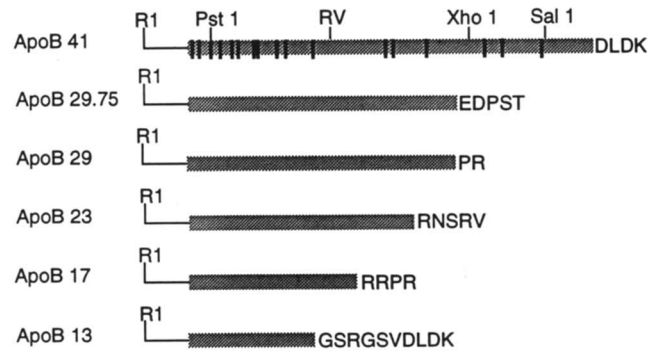


Fig. 1. Constructs of truncated apolipoprotein B cDNA. ApoB cDNA fragments were cloned into the expression vector pSV7D as described in Methods and ref. 30. All the constructs share the same amino terminal end but vary at the carboxyl end. Due to the cloning procedure, a short non-apoB peptide sequence is translated before a stop translation codon is encountered. The solid vertical bars on the apoB-41 construct indicate the position of the cysteine residues.

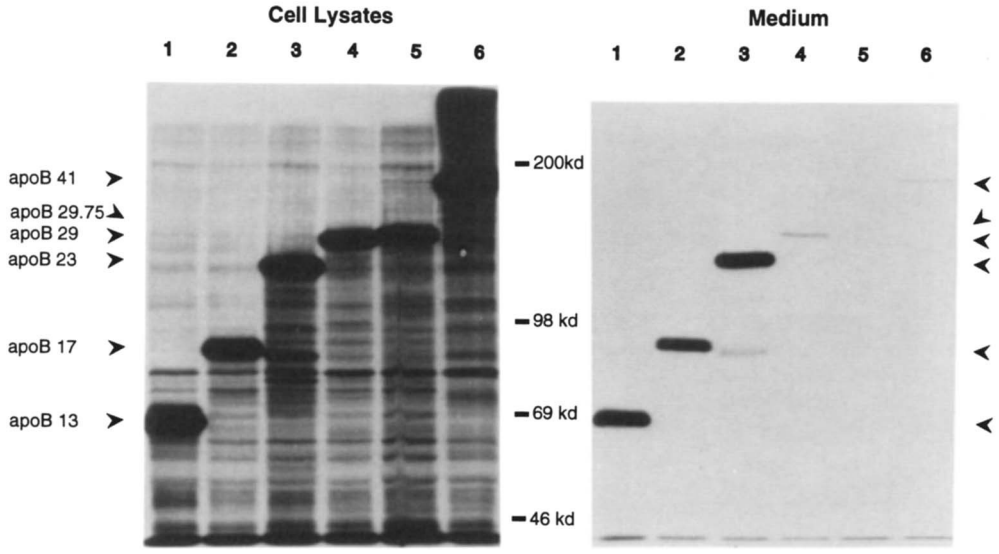


Fig. 2. Expression of apoB truncates in COS cells. COS cells were transfected with the apoB constructs and pulse-labeled for 30 min and chased for 2 h. Cells were harvested immediately after the pulse (Cell lysates), the media (Medium) after a 2-h chase period, and apoB was immunoprecipitated and analyzed by SDS-PAGE as described in Methods. Lanes 1–6 are cells transfected with apoB-13, -17, -23, -29, -29.75, and -41, respectively; the arrowheads indicate the position of migration of the respective proteins. No secretion of apoB-41 is normally detectable, although, on this occasion, cell viability at the end of the experiment had dropped by 5%, which accounts for the faint band seen in lane 6, Medium.

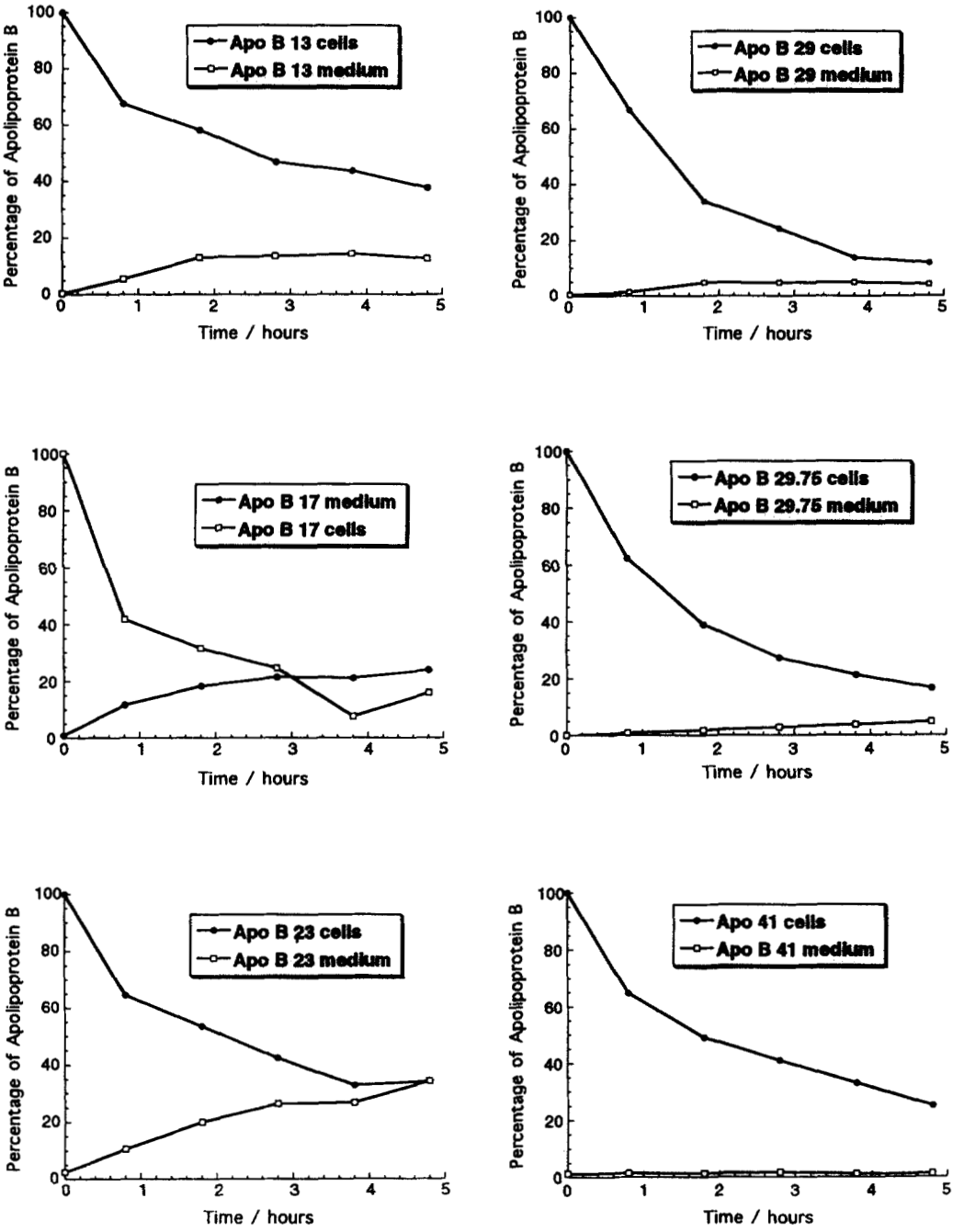


Fig. 3. Pulse-chase analysis of apoBs -13, -17, -23, -29.75, -29, and apoB-41. COS cells were transfected with apoB-13, -17, -23, -29.75, or apoB-41, pulsed for 10 min, and chased for the various times as indicated. The apoB was quantitatively immunoprecipitated and analyzed by SDS-PAGE. A representative experiment for each is shown. The data are expressed as a percentage of the initial labeled pool. About 20–30% of the apoB-13, -17, and -23 was secreted by 5 h, but very little (< 5%) of apoB-29 was secreted by this time. Note that although the apoB-29s have different carboxyl terminal peptide sequences, they appear to exhibit similar kinetics (see text for discussion).

Although full-length apoB-41 was readily synthesized, no secretion was detectable in the medium. In contrast, truncating the apoB further resulted in a progressive ability of the cells to secrete these proteins. Hence apoB-13, apoB-17, and apoB-23, and to a much lesser extent two variants of apoB-29 were secreted into the

medium. However, most of the newly synthesized apoBs, regardless of the size, were degraded intracellularly. At best only about 30% of apoB-13–23 were secreted, but only 5% of the apoB-29 variants were secreted (Fig. 3). Negligible uptake of secreted protein was present, as indicated by incubations of untransfected cells with labeled secreted apoB-17 (data not shown), excluding the possibility of rapid re-uptake to account for these results.

There are only two naturally occurring apoB proteins, apoB-100 and apoB-48, and hence only two natural carboxyl-terminal ends. All of the apoB truncates result in the formation of 'new' C-terminal ends as a result of the cloning procedure (Fig. 1) and these may alter the intracellular processing of these proteins. Short peptide sequences at the carboxyl ends of proteins are known to affect the intracellular metabolism, such as ER retention, targeting to peroxisomes, lysosomes, or the nucleus, etc. (39–42). However, none of the apoB truncate C-terminal sequences show any similarity to such peptide signals, and despite the divergent ends, no apparent effect of these peptide sequences was seen for the apoB truncates relative to each other in their degradation/secretion rates.

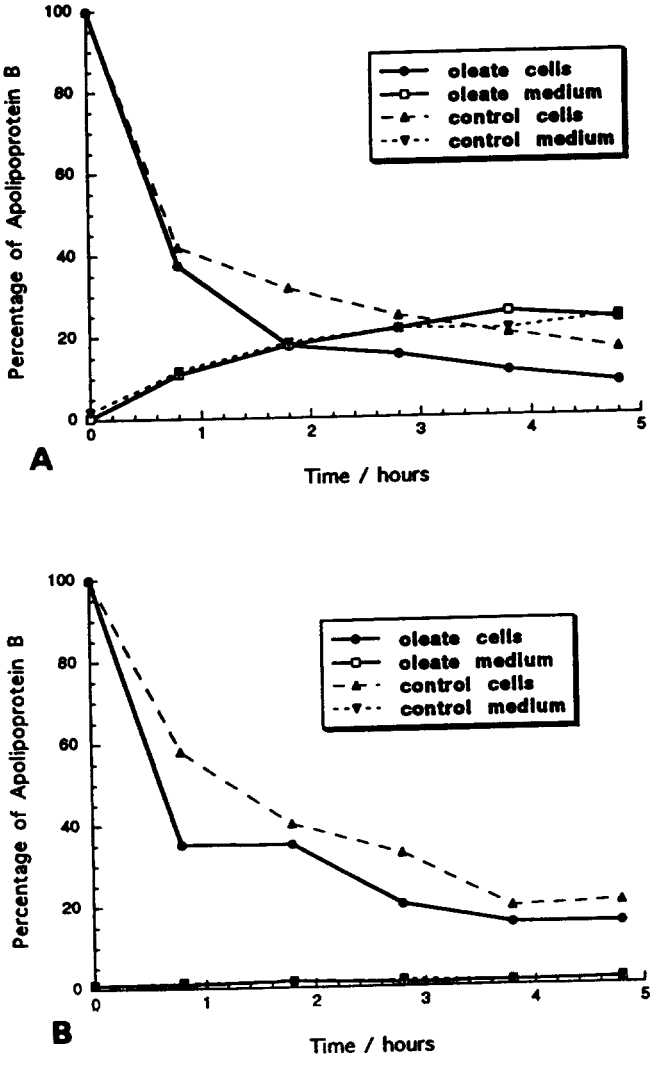


Fig. 4. Effect of oleate treatment on the secretion of apoB-17 and -41. COS cells were transfected with apoB-17 or apoB-41, labeled, and chased in the presence or absence of oleate as described in Methods. The top panel (A) shows a representative experiment for apoB-17 and the bottom panel (B) that for apoB-41. No significant changes were observed in the amounts of apoB-17 secreted or in the rates at which apoB-41 was degraded intracellularly after oleate treatment.

Addition of oleate to the cells did not result in secretion of apoB-41, nor were the kinetics of degradation significantly altered (**Fig. 4**). Addition of oleate to cells transfected with apoB-17 did not increase secretion of apoB-17. The ability of COS cells to metabolize oleate was not impaired as judged by the incorporation of labeled oleate into cellular triglycerides (38 pmol/24 h per 10^6 cells), cholesteryl ester (1 pmol/24 h per 10^6 cells) and phospholipids (188 pmol/24 h per 10^6 cells). To examine whether apoB-17 was secreted as a lipoprotein particle or could function as a lipid acceptor, transfected cells were labeled and chased in the presence or absence of 10% fetal calf serum. The serum was used as a ready supply of both lipid and protein factors for lipid exchange. The medium was collected, subjected to CsCl density ultracentrifugation, and fractionated, and apoB was immunoprecipitated and analyzed by SDS-PAGE (**Fig. 5**). In the absence of serum, the secreted apoB-17 floats at a slighter lower density than total secreted protein. An unidentified protein (protein X, Fig. 5) serves as a marker for non-lipid associated proteins. This protein cross-reacted with the anti-serum used. When apoB-17 was pulse-chased in the presence of 10% fetal calf serum, a marked shift in the density of apoB-17 was noted (Fig. 5, panel A). In mixing experiments, when apoB-17, secreted in the absence of FCS, was collected, incubated with 10% FCS, and subjected to ultracentrifugation, it attained a density of 1.10 g/ml (panel C, Fig. 5). However, when apoB-17 was mixed with 10% BSA/0.8 mM oleate, the peak density of apoB-17 remained at around 1.20 g/ml (panel D, Fig. 5). These results would suggest that the apoB-17 was secreted as a minimally lipidated protein, but subsequently either obtained further lipid from the serum by transfer, perhaps mediated by CETP, or became physically associated with the lipoproteins in the serum and hence attained greater buoyancy. As apoB-17 is about 94 kD in size, it is likely that it, like apoB-100 and apoB-48, is non-exchangeable among other lipoproteins, favoring the latter possibility.

Intracellular sites of apoB degradation

To investigate the site of degradation of the apoB within the cell, the glycosylation pattern was studied. In keeping with many N-glycosylated secreted proteins,

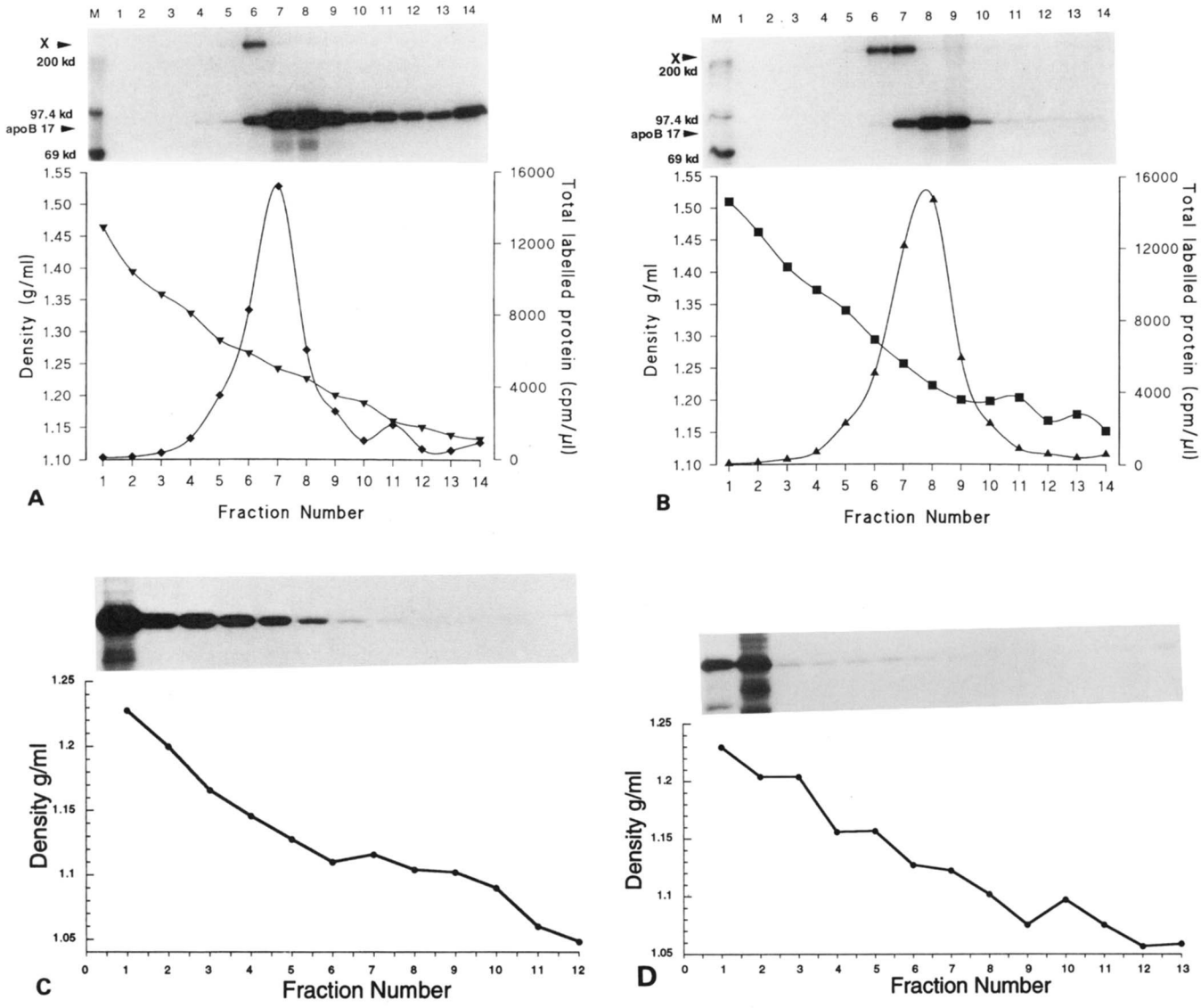


Fig. 5. Density gradient centrifugation of secreted apoB-17. ApoB-17 was transfected into COS cells, pulse-labeled for 30 min, and chased for 2 h in the presence (panel A) or absence (panels B, C, and D) of 10% fetal calf serum. The media were subjected to cesium chloride density centrifugation, fractionated, and the apoB-17 was immunoprecipitated and analyzed as described in Methods. The density profiles as well as the total secreted protein radioactivity profiles are shown. The insets above the profiles show the immunoprecipitated apoB-17 profiles. In the absence of FCS in the incubation medium (panel B), apoB-17 has a peak density of 1.20 g/ml. In the presence of FCS in the chase medium, it attains a spectrum of densities, the lowest is about 1.10 g/ml (panel A). Panels C and D show the result of mixing apoB-17, secreted in the absence of FCS, with 10% FCS (C), or 10% BSA/0.8 mM oleate (D) and then subjected to density gradient centrifugation. ApoB-17 attained a buoyant density as low as 1.10 g/ml in the presence of FCS, but remained at around 1.20 g/ml in the presence of BSA/oleate. The apoB antiserum immunoprecipitated an unidentified protein, X, which serves as a marker for the buoyancy of non-lipidated secretory proteins (panels A and B).

apoB undergoes terminal modification of its mannose residues in the Golgi under normal circumstances. This Golgi modification renders the sugars resistant to endoglycosidase H (Endo H) digestion, but these sugars can be cleaved by peptidyl-N-glycosidase F (PNG'ase F) (43). Pulse-chased apoB-41 was subjected to endoglycosidase H (Endo H) or peptidyl N-glycosidase F (PNG'ase F) digestion and analyzed by SDS-PAGE (**Fig. 6A**). All of apoB-41 remained fully sensitive to Endo H, even after a chase time of 2 h, suggesting that this protein does not reach the Golgi, indicating an ER or ER-related compartment for the site of degradation. In contrast, apoB-17, which is partially secreted, showed that the secreted form was fully resistant to Endo H digestion, as would be expected if the N-glycosylated sugars were modified in the Golgi (Fig. 6B). The majority of the intracellular apoB-17 remains Endo H-sensitive, suggesting that its degradation may also be in an ER or ER-related compartment. All of the intracellular apoB-29 was also Endo H-sensitive (data not shown), suggesting this truncate is

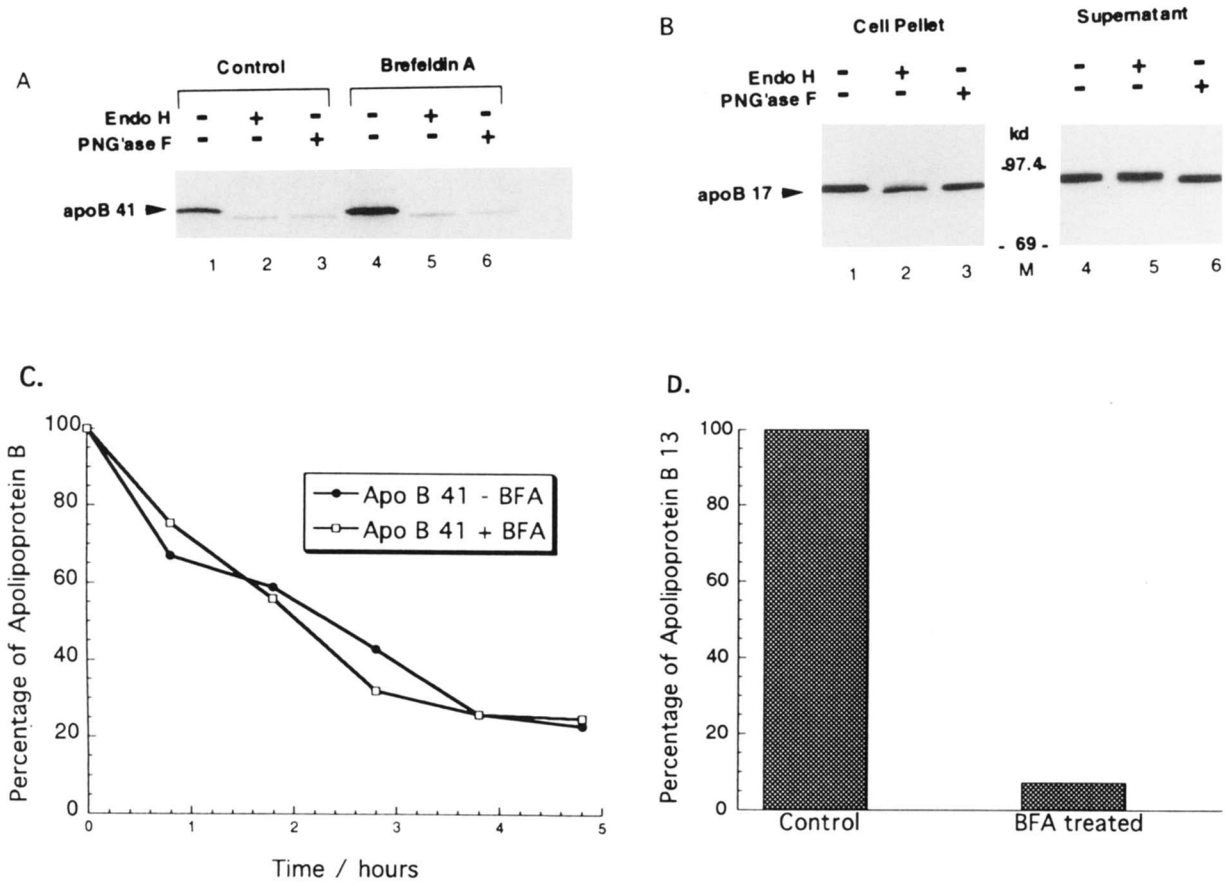
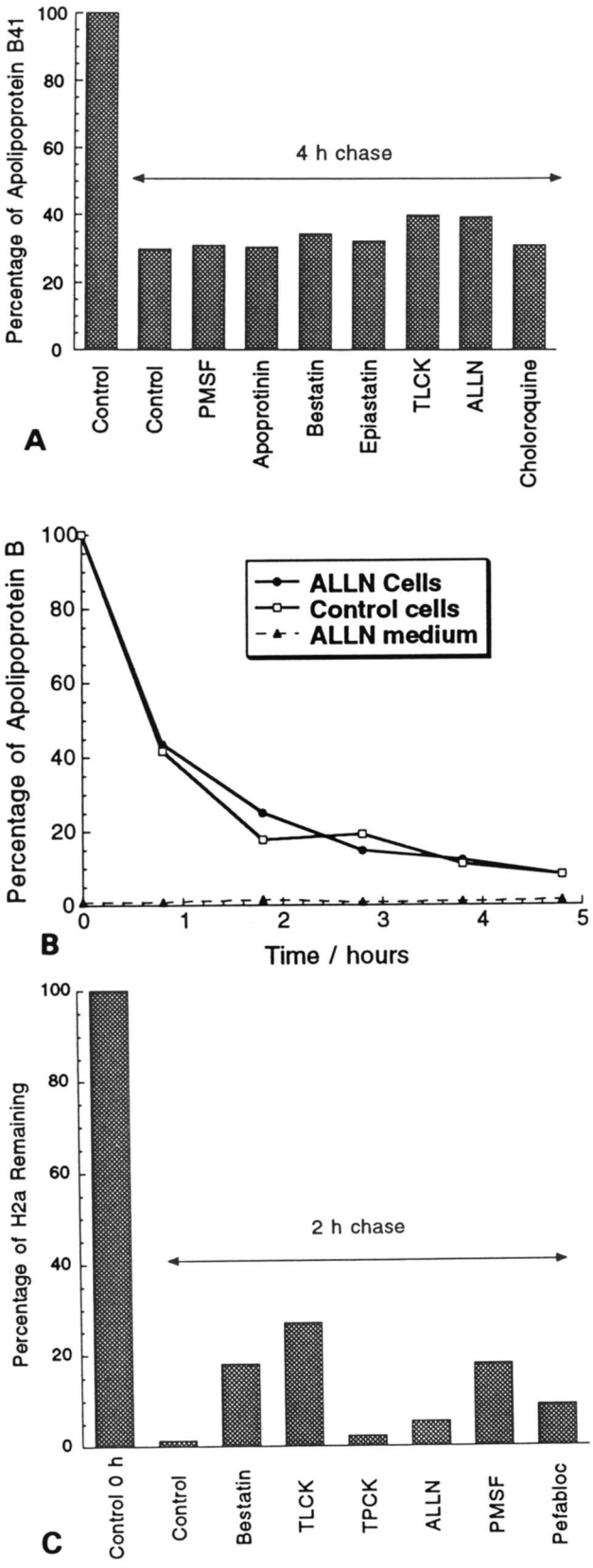


Fig. 6. Endoglycosidase sensitivity of intracellular apoB-41 and apoB-17. After transfection with apoB-41, COS cells were either treated with brefeldin A at 5 μg/ml (panel A, lanes 4–6) or incubated under standard conditions (panel A, lanes 1–3), labeled for 10 min, and chased for 1 h, and apoB-41 was isolated by immunoprecipitation and digested with Endo H (lanes 2 and 5) or peptide N-glycosidase F (PNG'ase F), lanes 3 and 6. Controls are lanes 1 and 4. The protein appears to be fully Endo H sensitive, with no effects by brefeldin A treatment. Panel B shows the glycosidase treatment of intracellular (lanes 1–3) or the secreted (lanes 4–6) forms of apoB-17. A large proportion of intracellular apoB-17 remains Endo H sensitive but the secreted apoB-17 is resistant. Panel C shows a pulse chase analysis of apoB-41 in the presence and absence of treatment by brefeldin A. No significant differences in the intracellular kinetics were observed. Panel D shows the effects of brefeldin A on the secretion of apoB-13. Compared to control cells (normalized as 100%), brefeldin A inhibited secretion by more than 90%.

also confined to a similar compartment.

To investigate whether the newly synthesized proteins were shuttling between compartments, such as the *cis*-Golgi and the ER, the cells were treated with brefeldin A (BFA) (44). Brefeldin A treatment blocks the anterograde transport between the ER and the Golgi, hence would affect the metabolism of apoB if it followed this path. BFA treatment did not alter the sensitivity of apoB-41 to digestion by Endo H (Fig. 6A, lanes 4–6) or its rate of degradation (Fig. 6C). In contrast, brefeldin A blocked the secretion of apoB-13 by 93% (Fig. 6D).

Protease inhibitors, and in particular N-acetyl-leucyl-leucyl-norleucinal (ALLN, also known as Calpain I), have been used to modulate ER degradation of apoB in HepG2 cells (45) as well as apoB and other proteins in CHO cells (4, 15–21, 46–48). In contrast, a variety of protease inhibitors appear to have no significant effect on the degradation of apoB-41 in COS cells (**Fig. 7A**). In particular, ALLN showed no significant inhibition, and a detailed pulse-chase analysis using ALLN also failed to alter the degradation kinetics (Fig. 7B). Chloroquine did not affect degradation, suggesting that lysosomal degradation is unlikely to play a role in apoB degradation. The asialoglycoprotein receptor subunit H2a has been shown to undergo ER degradation in the fibroblast line 2–18, and this process can be inhibited by TLCK, but not by many other protease inhibitors used (49). Degradation of the H2a sub-unit in the 2–18 cell line was observed by TLCK and to lesser extents by ALLN and PMSF (Fig. 7C). We expressed H2a in COS cells and found that it too was retained and degraded in the ER, as judged by Endo H sensitivity (**Fig. 8**). However, no sensitivity to TLCK or TPCK nor to a variety of other protease inhibitors was found in COS cells. This would suggest that at least for this feature of ER degradation, COS cells may differ significantly from hepatocytes, or that of the active protease inhibitors, none of these penetrated into the cells. Indeed, there is evidence



that the penetration of such inhibitors is dependent on the cell's resistance to a variety of drugs (46).

To localize the site of apoB degradation further, transfected COS cells were also examined by immunogold-EM. ApoB-41 was found only in the ER, and although apoB-17 was also found predominantly in the ER, some was detected in the Golgi and in vesicles, presumably en route to secretion from the cells (**Fig. 9, A and B**). ApoB-17 staining was also different in that the gold grains appeared to be in 'clumps' (see Fig. 9, middle panels) compared to the somewhat uniform labeling seen for apoB-41. We have no explanation for this, although we speculate that perhaps most of the apoB-41 may be membrane bound, whereas the apoB-17 may be lumenal and may therefore aggregate. No immunogold staining was detected when non-immune serum was used or when untransfected cells were stained with immune serum (Fig. 9, lower panels).

DISCUSSION

The purpose of this study was to characterize a system for the investigation of lipoprotein biogenesis that uses truncated forms of apoB expressed in a heterologous cell line, COS-1. ApoB truncates, ranging from 77 kD (apoB-13) to 220 kD (apoB-41) were expressed in these cells. We have hypothesized that since apolipoprotein B (apoB) undergoes a complex series of processes to form a lipoprotein requiring specific proteins, heterologous expression would lead to its retention and ER degradation. A variety of proteins, when expressed heterologously, are degraded in the ER because they require co-expression of specific proteins for maturation and/or secretion (50–52). Complementation of this 'secretory defect' by introducing factors necessary for some of the vital steps in the biogenesis of the lipoprotein particle should therefore lead to the identification and further study of these interactions.

Full length apoB truncates were readily synthesized

Fig. 7. Sensitivity of the degradation of apoB-41 in COS cells to various protease inhibitors. COS cells were transfected with apoB-41, pulse-labeled for 30 min, and chased for 4 h in the presence of the various protease inhibitors as indicated. The data are expressed as a percentage of the initial pool of apoB-41 synthesized. No significant effect was observed for any of the protease inhibitors indicated in panel A. Panel B shows a pulse chase analysis of cells transfected with apoB-41 treated with ALLN. No changes in the kinetics were observed, and no increase in apoB-41 was detected in the medium. Panel C shows that the protease inhibitors used can be effective, as judged by inhibition of H2a sub-unit stably expressed in the cell line 2-18 (see text for discussion).

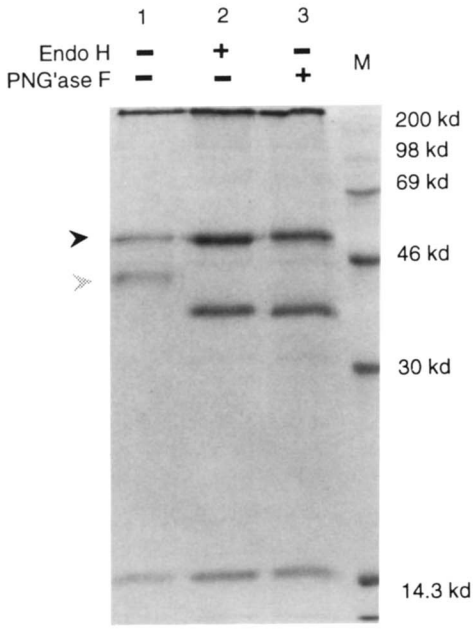


Fig. 8. Endoglycosidase H sensitivity of H2a subunit. The H2a subunit of the asialoglycoprotein receptor was expressed in COS cells, pulse labeled, chased for an hour, and the protein was isolated by immunoprecipitation and subjected to Endo H digestion. The H2a subunit remains fully sensitive to Endo H (mottled arrowhead, lane 2). The antiserum crossreacts with an unidentified COS cell protein (solid arrowhead).

by COS cells. ApoB truncates equal to or larger than apoB-29 were almost completely retained within the cells and degraded. The smaller constructs, apoB-13, -17, and -23, on the other hand were partially secreted. However, more than 60% of these too were degraded intracellularly. The site of degradation appears to be the ER or an ER-related compartment, as judged by sensitivity of the proteins to digestion by Endo H, by a lack of an effect on degradation kinetics by pre-treatment with brefeldin A, and by immunogold EM localization.

Stop translation signals for all of the cDNAs were generated by allowing the translation to run into the vector sequence containing a stop codon in all three frames. This results in a variety of short peptide sequences added to the carboxyl end not normally present in apoB. Peptide sequences at such ends can act as 'signals' that could account for the misdirection of the apoB proteins (40, 53, 54). However, none of the extra peptide sequences show similarities to known peptide signals. Furthermore, all of the apoB fragments have different carboxyl peptide sequences, but still display a similar biochemical fate. In particular, no differences were observed between apoB-29 and apoB-29.75, which are almost equal in size but have differing ends. This would suggest that the mechanism of ER retention and degradation is not due to the novel carboxyl-terminal ends generated by our cloning procedures. Novel short peptide sequences at the carboxyl end are frequently predicted to occur naturally in patients with hypobetalipoproteinemias (55), where a truncated apoB species results from a variety of genetic mechanisms. Expression in COS cells might be a useful initial step in investigating whether these novel sequences are the cause of abnormal intracellular processing, as has been proposed to explain very low levels in the peripheral blood (55).

One question that can be asked is whether the smaller apoB truncates are secreted as lipoproteins or as lipid-free proteins. Our results suggest that they are capable of forming rudimentary lipoproteins but are not secreted from COS cells bearing sufficient lipid to cause a change in buoyant density. Secreted apoB-17, in the absence of lipid, was found to have a buoyant density only a fraction lower than the bulk secreted proteins (1.20 g/ml vs. 1.25 g/ml). However, in the presence of fetal calf serum, the apoB-17 had a density into the HDL range (1.15 g/ml). We believe that this change in density takes place after the protein is secreted and acquires the lipid from the medium, suggesting it is capable of functioning as a lipid acceptor. A similar observation has been made for apoB-17 when heterologously expressed in murine C127 cells (56). Secreted apoB-17 was resistant to Endo H digestion but fully sensitive to PNG'ase F, indicating that its N-linked sugars have been terminally modified in the Golgi.

The results of these studies suggest that the retention and degradation of the larger apoB truncates may be a result of their failure to fold correctly. The folding of these apoBs may require the addition of sufficient lipid to form a 'core' particle. The shorter apoB truncates may not require lipid for folding correctly, and a sufficient proportion of this may be able to fold spontaneously to escape the ER's quality control (57). An explanation for this behavior may lie in the elegant studies of Yao et al. (29) and Spring et al. (58) who have shown that there is a linear relationship between the size of the truncated protein and the lipoprotein radius or the core circumference, as well as an inverse linear relationship between the size of the truncate and the buoyant density of the apoB truncates secreted from hepatocytes. However, this linearity seems to deviate once the size of the apoB truncate is smaller than apoB-26. These smaller proteins (apoB < 29) may not require as much lipid for stability of the tertiary and higher structures and hence are secreted as minimally lipidated proteins. These smaller apoBs appear to have the capacity to obtain lipids from their milieu, as shown by our results and by Herscovitz et al. (56).

We propose that in the heterologous system used

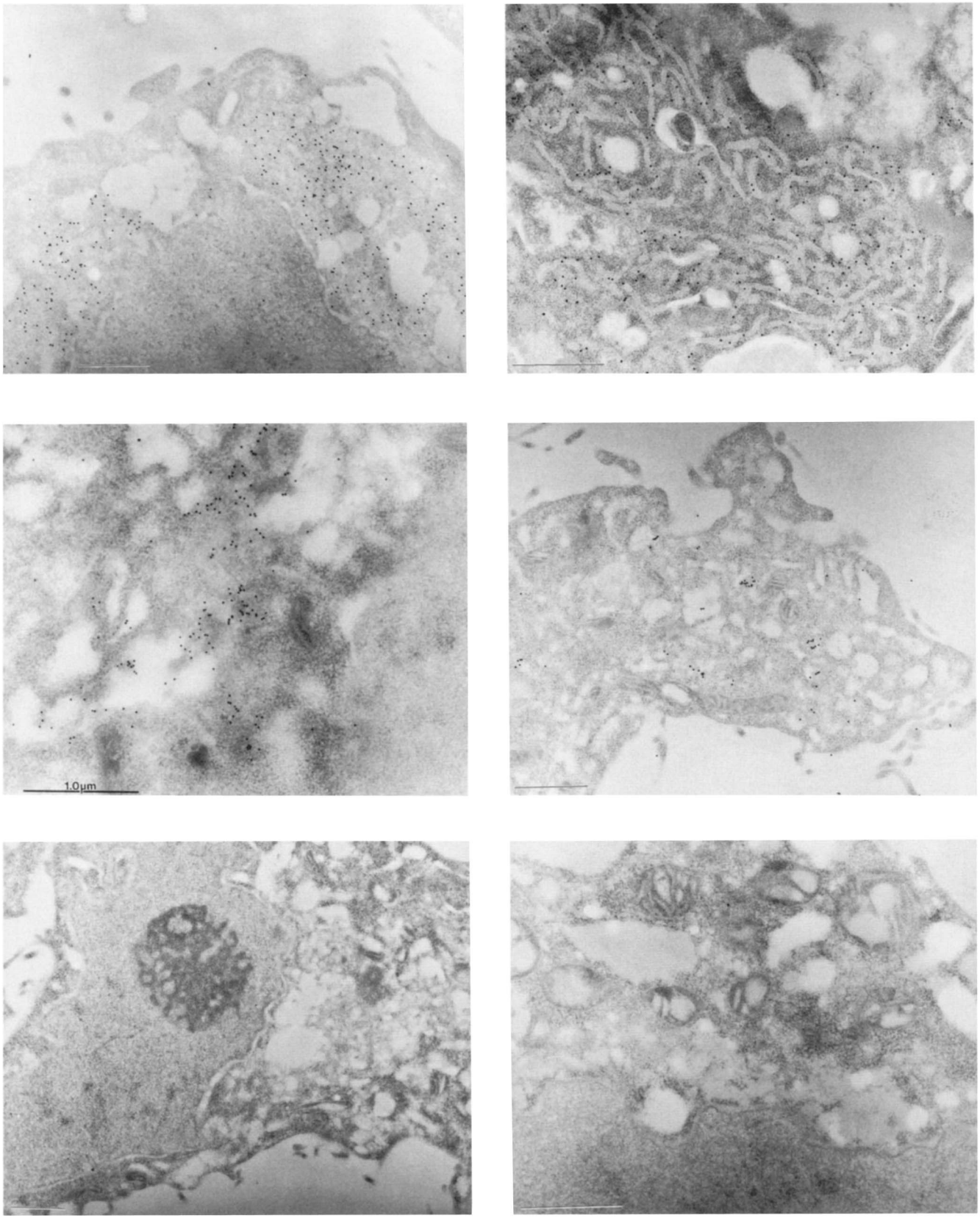


Fig. 9. Immunogold electron microscopy localization of intracellular apoB. COS cells were transfected with apoB-41 (top panels, right and left) or apoB-17 (middle panels, right and left) or controls (bottom panels) and processed for immunogold staining and examined by electron microscopy as described in Methods. When the cells were transfected with apoB-41, no gold particles were found in any other organelle except the ER. The majority of the gold particles are found in the ER in cells transfected with apoB-17, although a few were also found in vesicular bodies and the Golgi. In many of the fields for cells transfected with apoB-17, the gold particles appeared to be in 'clumps,' in contrast to the pattern seen for apoB-41 (compare the right middle panel with the top panels). Control experiments showed no significant staining with non-immune serum on apoB transfected cells (lower panel, right), or untransfected cells with immune serum (lower panel, left). The bars indicate 1 μm.

here, the specific factors required for lipoprotein assembly are deficient, hence those proteins that require a significant amount of lipid for structural stability, i.e., apoB > 29, cannot fold sufficiently correctly. These proteins will therefore be degraded. The smaller truncates can escape this as their requirements are reduced. One prediction of our system would therefore be that the complementation of this system by any factor(s) involved in the simultaneous addition of lipid to facilitate the folding of nascent apoB into a core lipoprotein particle should lead to the secretion of the larger truncates.

Indeed, apoB-53 can be secreted from HeLa cells stably expressing MTP (59), and co-expression of apoB-41 and MTP in COS cells, resulting in secretion, has also been reported (60). Gordon et al. (59) have shown that HeLa cells that do not express MTP do not efficiently secrete apoB-53 when transfected and analyzed after 24 h. In the presence of stably expressed MTP, apoB-53 was secreted as a lipoprotein with a density in the HDL range (59). The intracellular sites of apoB degradation are not reported. Leiper et al. (60) co-expressed apoB-41 and MTP in COS cells and found that in the absence of MTP, no apoB-41 was secreted, but in its presence apoB-41-carrying lipoprotein in the HDL range was secreted. These authors also found no effect of ALLN on apoB-41 degradation, but found that ALLN, when apoB-41 was co-expressed with MTP, resulted in 2.5-fold more secretion.

There are some similarities and some significant differences between the behavior of newly synthesized apoB, both full length or truncates, in the hepatoma cell lines such as HepG2, or McArdle 7777 cells and COS cells. In both cases, the full-length protein is synthesized, N-glycosylated, and appears to be degraded in the ER or ER-related compartment. No effect of brefeldin A is seen on the degradation kinetics in either case. However, degradation has been shown to be sensitive to protease inhibitors, especially ALLN, in intact or permeabilized HepG2 cells (45, 61), but we could not demonstrate a similar effect in COS cells. Heterologously expressed apoB in the hamster-derived cell line CHO has also been reported to be sensitive to ALLN (20). In our efforts to explore the lack of an effect of ALLN in COS cells, we examined the effects of protease inhibitors on the degradation of the asialoglycoprotein receptor (ASGP) H2a subunit expressed in COS cells. ASGP H2a is also retained and degraded in COS cells. However, we could not alter the degradation kinetics of this process by any of the protease inhibitors used. The degradation of H2a, expressed in a mouse fibroblast line, has been shown to be inhibited by TPCK and TLCK (49). We have been able to inhibit the degradation of the H2a in a 3T3 cell line, 2–18, stably expressing the H2a protein, as has been shown previously (32, 49). Our lack of such an effect in the monkey kidney-derived fibroblastic COS cell leads us to conclude that there may either be differences in the accessibility of these inhibitors when added to different living cells (see ref. 46) or that the ER degradation pathway is different in the various cell lines. We favor the former explanation because the protease inhibitors used have been used in other cell lines to inhibit ER degradation in vivo and there is evidence that the accessibility of peptides and drugs into cells is dependent on the activity of the P-glycoprotein, responsible for multiple drug resistance (46).

We therefore propose a model for the study of lipoprotein biogenesis using heterologous expression of apoB truncates in COS cells. The high level of expression, together with the ability to introduce specific cDNAs into this system, allows for the rapid evaluation of the roles some of these factors play in lipoprotein biogenesis.

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REFERENCES

1. Vance, J. E., and D. E. Vance. 1990. Lipoprotein assembly and secretion by hepatocytes. *Annu. Rev. Nutr.* **10:** 337–356.
2. Gibbons, G. F. 1990. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* **268:** 1–13.
3. Schumaker, V. N., M. L. Phillips, and J. E. Chatterton. 1994. Apolipoprotein B and low-density lipoprotein structure: implications for biosynthesis of triglyceride-rich lipoproteins. *Adv. Protein Chem.* **45:** 205–248.
4. Davis, R. A., R. N. Thrift, C. C. Wu, and K. E. Howell. 1990. Apolipoprotein B is both integrated into and

translocated across the endoplasmic reticulum membrane. Evidence for two functionally distinct pools. *J. Biol. Chem.* **265:** 10005–10011.
5. Boren, J., M. Wettesten, A. Sjoberg, T. Thorlin, G. Bondjers, O. Wiklund, and S. O. Olofsson. 1990. The assembly and secretion of apoB-100-containing lipoproteins in HepG2 cells. Evidence for different sites for protein synthesis and lipoprotein assembly. *J. Biol. Chem.* **265:** 10556–10564.
6. Dixon, J. L., S. Furukawa, and H. N. Ginsberg. 1991. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from HepG2 cells by inhibiting early intracellular degradation of apolipoprotein B. *J. Biol. Chem.* **266:** 5080–5086.
7. Sato, R., T. Imanaka, A. Takatsuki, and T. Takano. 1990. Degradation of newly synthesized apolipoprotein B-100 in a pre-Golgi compartment. *J. Biol. Chem.* **265:** 11880–11884.
8. Bamberger, M. J., and M. D. Lane. 1990. Possible role of the Golgi apparatus in the assembly of very low density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **87:** 2390–2394.
9. Alexander, C. A., R. L. Hamilton, and R. J. Havel. 1976. Subcellular localization of B apoprotein of plasma lipoproteins in rat liver. *J. Cell Biol.* **69:** 241–263.
10. Stein, O., and Y. Stein. 1967. Lipid synthesis, intracellular transport, storage, and secretion. I. Electron microscopic radioautographic study of liver after injection of tritiated palmitate or glycerol in fasted and ethanol-treated rats. *J. Cell Biol.* **33:** 319–339.
11. Bostrom, K., M. Wettesten, J. Boren, G. Bondjers, O. Wiklund, and S. O. Olofsson. 1986. Pulse-chase studies of the synthesis and intracellular transport of apolipoprotein B-100 in HepG2 cells. *J. Biol. Chem.* **261:** 13800–13806.
12. Boren, J., S. Rustaeus, M. Wettesten, M. Andersson, A. Wiklund, and S. O. Olofsson. 1993. Influence of triacylglycerol biosynthesis rate on the assembly of apoB-100-containing lipoproteins in HepG2 cells. *Arterioscler. Thromb.* **13:** 1743–1754.
13. Hamilton, R. L., A. Moorehouse, and R. J. Havel. 1991. Isolation and properties of nascent lipoproteins from highly purified rat hepatocytic Golgi fractions. *J. Lipid Res.* **32:** 529–543.
14. Ye, S. Q., C. A. Reardon, and G. S. Getz. 1993. Inhibition of apolipoprotein E degradation in a post-Golgi compartment by a cysteine protease inhibitor. *J. Biol. Chem.* **268:** 8497–8502.
15. Chuck, S. L., Z. Yao, B. D. Blackhart, B. J. McCarthy, and V. R. Lingappa. 1990. New variation on the translocation of proteins during early biogenesis of apolipoprotein B. *Nature.* **346:** 382–385.
16. Chuck, S. L., and V. R. Lingappa. 1992. Pause transfer: a topogenic sequence in apolipoprotein B mediates stopping and restarting of translocation. *Cell.* **68:** 9–21.
17. Chuck, S. L., and V. R. Lingappa. 1992. Apolipoprotein B intermediates [letter]. *Nature.* **356:** 115–116.
18. Chuck, S. L., and V. R. Lingappa. 1993. Analysis of a pause transfer sequence from apolipoprotein B. *J. Biol. Chem.* **268:** 22794–22801.
19. Pease, R. J., G. B. Harrison, and J. Scott. 1991. Cotranslocational insertion of apolipoprotein B into the inner leaflet of the endoplasmic reticulum. *Nature.* **353:** 448–450.
20. Thrift, R. N., J. Drisko, S. Dueland, J. D. Trawick, and R. A. Davis. 1992. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line. *Proc. Natl. Acad. Sci. USA.* **89:** 9161–9165.
21. Davis, R. A., A. B. Prewett, D. C. Chan, J. J. Thompson, R. A. Borchardt, and W. R. Gallaher. 1989. Intrahepatic assembly of very low density lipoproteins: immunologic characterization of apolipoprotein B in lipoproteins and hepatic membrane fractions and its intracellular distribution. *J. Lipid Res.* **30:** 1185–1196.
22. Adeli, K., and C. Sinkevitch. 1990. Secretion of apolipoprotein B in serum-free cultures of human hepatoma cell line, HepG2. *FEBS Lett.* **263:** 345–348.
23. Davis, R. A., and M. Malone-McNeal. 1985. Dietary cholesterol does not affect the synthesis of apolipoproteins B and E by rat hepatocytes. *Biochem. J.* **227:** 29–35.
24. Dixon, J. L., and H. N. Ginsberg. 1993. Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: information obtained from cultured liver cells. *J. Lipid Res.* **34:** 167–179.
25. Hamilton, R. L., L. S. Guo, T. E. Felker, Y. S. Chao, and R. J. Havel. 1986. Nascent high density lipoproteins from liver perfusates of orotic acid-fed rats. *J. Lipid Res.* **27:** 967–978.
26. Kane, J. P., and R. J. Havel. 1989. Disorders of the biogenesis and secretion of lipoproteins containing the B lipoproteins. *In* The Metabolic Basis of Inherited Disease. Vol. 1. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Inc., New York. 1129–1164.
27. Shoulders, C. C., D. J. Brett, J. D. Bayliss, T. M. Narcisi, A. Jarmuz, T. T. Grantham, P. R. Leoni, S. Bhattacharya, R. J. Pease, P. M. Cullen, et al. 1993. Abetalipoproteinemia is caused by defects of the gene encoding the 97 kDa subunit of a microsomal triglyceride transfer protein. *Hum. Mol. Genet.* **2:** 2109–2116.
28. Sharp, D., L. Blinderman, K. A. Combs, B. Kienzle, B. Ricci, K. Wager-Smith, C. M. Gil, C. W. Turck, M. E. Bouma, D. J. Rader, et al. 1993. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. *Nature.* **365:** 65–69.
29. Yao, Z. M., B. D. Blackhart, M. F. Linton, S. M. Taylor, S. G. Young, and B. J. McCarthy. 1991. Expression of carboxyl-terminally truncated forms of human apolipoprotein B in rat hepatoma cells. Evidence that the length of apolipoprotein B has a major effect on the buoyant density of the secreted lipoproteins. *J. Biol. Chem.* **266:** 3300–3308.
30. Graham, D. L., T. J. Knott, T. C. Jones, R. J. Pease, C. R. Pullinger, and J. Scott. 1991. Carboxyl-terminal truncation of apolipoprotein B results in gradual loss of the ability to form buoyant lipoproteins in cultured human and rat liver cell lines. *Biochemistry.* **30:** 5616–5621.
31. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
32. Shia,M. A., and H. F. Lodish. 1989. The two subunits of the human asialoglycoprotein receptor have different fates when expressed alone in fibroblasts. *Proc. Natl. Acad. Sci. USA.* **86:** 158–162.
33. Chu, G., H. Hayakawa, and P. Berg. 1987. Electroporation for the efficient transfection of mammalian cells with DNA. *Nucleic Acids Res.* **15:** 1311–1326.
34. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
35. Sambrook, J., E. F. Fritsch, and T. Maniatis, ed. 1989. Molecular Cloning: a Laboratory Manual. Second ed.

Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY.
36. Christofori, G., and W. Keller. 1988. 3′ Cleavage and polyadenylation of mRNA precursors in vitro requires a poly(A) polymerase, a cleavage factor, and a snRNP. *Cell.* **54:** 875–889.
37. Durer, U., H. Franke, R. Dargel, and J. Ude. 1986. A post-embedding immunoelectron-microscopic demonstration of apolipoprotein-B-containing lipoprotein particles in hepatocytes of fetal rats. *Histochemistry.* **84:** 263–270.
38. Dixon, J. L., R. Battini, S. Ferrari, C. M. Redman, and D. Banerjee. 1989. Expression and secretion of chicken apolipoprotein A-I in transfected COS cells. *Biochim. Biophys. Acta.* **1009:** 47–53.
39. Pelham, H. R. 1990. The retention signal for soluble proteins of the endoplasmic reticulum. *Trends Biochem. Sci.* **15:** 483–486.
40. Nilsson, T., and G. Warren. 1994. Retention and retrieval in the endoplasmic reticulum and the Golgi apparatus. *Curr. Opin. Cell Biol.* **6:** 517–521.
41. Pelham, H. R., and S. Munro. 1993. Sorting of membrane proteins in the secretory pathway. *Cell.* **75:** 603–605.
42. Boulikas, T. 1993. Nuclear localization signals (NLS). *Crit. Rev. Eukaryotic Gene Expr.* **3:** 193–227.
43. Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54:** 631–664.
44. Fujiwara, T., K. Oda, S. Yokota, A. Takatsuki, and Y. Ikehara. 1988. Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. *J. Biol. Chem.* **263:** 18545–18552.
45. Sakata, N., X. Wu, J. L. Dixon, and H. N. Ginsberg. 1993. Proteolysis and lipid-facilitated translocation are distinct but competitive processes that regulate secretion of apolipoprotein B in HepG2 cells. *J. Biol. Chem.* **268:** 22967–22970.
46. Sharma, R. C., S. Inoue, J. Roitelman, R. T. Schimke, and R. D. Simoni. 1992. Peptide transport by the multidrug resistance pump. *J. Biol. Chem.* **267:** 5731–5734.
47. Inoue, S., R. C. Sharma, R. T. Schimke, and R. D. Simoni. 1993. Cellular detoxification of tripeptidyl aldehydes by an aldo-keto reductase. *J. Biol. Chem.* **268:** 5894–5898.
48. Sherwood, S. W., A. L. Kung, J. Roitelman, R. D. Simoni, and R. T. Schimke. 1993. In vivo inhibition of cyclin B degradation and induction of cell-cycle arrest in mammalian cells by the neutral cysteine protease inhibitor N-acetylleucylleucylnorleucinal. *Proc. Natl. Acad. Sci. USA.* **90:** 3353–3357.
49. Wikstrom, L., and H. F. Lodish. 1991. Nonlysosomal, pre-Golgi degradation of unassembled asialoglycoprotein receptor subunits: a TLCK- and TPCK-sensitive cleavage within the ER. *J. Cell Biol.* **113:** 997–1007.
50. Amara, J. F., G. Lederkremer, and H. F. Lodish. 1989. Intracellular degradation of unassembled asialoglycoprotein receptor subunits: a pre-Golgi, nonlysosomal endoproteolytic cleavage. *J. Cell Biol.* **109:** 3315–3324.
51. Lippincott-Schwartz, J., J. S. Bonifacino, L. C. Yuan, and R. D. Klausner. 1988. Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. *Cell.* **54:** 209–220.
52. Bonifacino, J. S., C. K. Suzuki, J. Lippincott-Schwartz, A. M. Weissman, and R. D. Klausner. 1989. Pre-Golgi degradation of newly synthesized T-cell antigen receptor chains: intrinsic sensitivity and the role of subunit assembly. *J. Cell Biol.* **109:** 73–83.
53. Pelham, H. R. 1992. The Florey Lecture. The secretion of proteins by cells. *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **250:** 1–10.
54. Saraste, J., and E. Kuismanen. 1992. Pathways of protein sorting and membrane traffic between the rough endoplasmic reticulum and the Golgi complex. *Semin. Cell Biol.* **3:** 343–355.
55. Linton, M. F., R. Farese, Jr., and S. G. Young. 1993. Familial hypobetalipoproteinemia. *J. Lipid Res.* **34:** 521–541.
56. Herscovitz, H., M. Hadzopoulou-Cladaras, M. T. Walsh, C. Cladaras, V. I. Zannis, and D. M. Small. 1991. Expression, secretion, and lipid-binding characterization of the N-terminal 17% of apolipoprotein B [published erratum appears in *Proc. Natl. Acad. Sci. USA.* 1991. **88:** 9375]. *Proc. Natl. Acad. Sci. USA.* **88:** 7313–7317.
57. Hammond, C., and A. Helenius. 1994. Quality control in the secretory pathway: retention of a misfolded viral membrane glycoprotein involves cycling between the ER, intermediate compartment, and Golgi apparatus. *J. Cell Biol.* **126:** 41–52.
58. Spring, D. J., L. W. Chen-Liu, J. E. Chatterton, J. Elovson, and V. N. Schumaker. 1992. Lipoprotein assembly. Apolipoprotein B size determines lipoprotein core circumference. *J. Biol. Chem.* **267:** 14839–14845.
59. Gordon, D. A., H. Jamil, D. Sharp, D. Mullaney, Z. Yao, R. E. Gregg, and J. Wetterau. 1994. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. *Proc. Natl. Acad. Sci. USA.* **91:** 7628–7632.
60. Leiper, J. M., J. D. Bayliss, R. J. Pease, D. J. Brett, J. Scott, and C. C. Shoulders. 1994. Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. *J. Biol. Chem.* **269:** 21951–21954.
61. Adeli, K. 1994. Regulated intracellular degradation of apolipoprotein B in semipermeable HepG2 cells. *J. Biol. Chem.* **269:** 9166–9175.