Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9161–9165, October 1992
Applied Biological Sciences

Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line

RICHARD N. THRIFT*, JODI DRISKO, SVEIN DUELAND, JOHN D. TRAWICK*, AND ROGER A. DAVIS*†

Atherosclerosis Research Center, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262

Communicated by Richard J. Havel, June 29, 1992 (received for review February 4, 1992)

ABSTRACT **To explore the process of lipoprotein assembly, plasmids encoding truncated forms of apolipoprotein B (apoB) were transfected into Chinese hamster ovary (CHO) fibroblasts. (One, encoding apoB53, the N-terminal 53% of apoB100, can direct the assembly and secretion of lipoproteins when expressed in hepatoma cells, while the other, encoding the shorter apoB15, does not direct lipoprotein assembly.) Expression of apoB15 in CHO cells resulted in the accumulation of apoB15 protein in both medium and cells. In contrast, apoB was not detectable in medium or within CHO cells transfected with the plasmid encoding apoB53, despite the expression of apoB53 mRNA. ApoB53 did accumulate within transfected cells incubated with the thiol protease inhibitor *N*-acetylleucylleucylnorleucinal (ALLN), suggesting that it is synthesized but completely degraded in the absence of the inhibitor. ApoB53 was not secreted despite its presence within ALLN-treated cells. Essentially all the apoB53 that accumulated in microsomes from ALLN-treated cells was associated with the membrane and was susceptible to degradation by exogenous trypsin, indicating exposure on the cytoplasmic face of the membrane. Thus, translocation of apoB53 across the endoplasmic reticulum membrane is blocked. However, the apoB53 bound to concanavalin A, suggesting that it is glycosylated and therefore partly exposed to the lumen as well. ApoB requires a unique process, not expressed in CHO fibroblasts, for its complete translocation and entrance into the secretory pathway. This process might account for the inability of abetalipoproteinemic patients to secrete apoB.**

Apolipoprotein B (apoB) is a large amphipathic protein responsible for the assembly of very low density lipoprotein and chylomicrons by the liver and intestine (1). Abetalipoproteinemia is a recessive disease characterized by the absence of plasma apoB (2), although the disease is not linked to the gene encoding apoB (3). Livers of patients with abetalipoproteinemia have apoB mRNA of normal size and elevated abundance (4). ApoB is synthesized (5, 6); however, almost no apoB is secreted (2). Triglyceride-rich lipoproteins are not demonstrable in any organelle of the secretory pathway (2, 6), suggesting that the block in secretion occurs prior to translocation or assembly into lipoproteins. The inability to secrete apoB is not caused by a general impairment of protein secretion, since the plasma concentrations of other liver-derived proteins are not altered in abetalipoproteinemics (2). Thus, apoB requires a unique process, absent in homozygous abetalipoproteinemia, for its secretion.

When expressed in hepatoma cells, apoB53 (the N-terminal 53% of apoB100, encompassing the sequence of the naturally occurring apoB48) assembles lipoproteins and is secreted as a lipoprotein complex (7). In marked contrast, when apoB53 is expressed in nonhepatic COS cells, it is not secreted (7). While the inability of COS cells to secrete apoB53 could be an artifact of overexpression, it might instead reflect tissue specificity of a process (such as the one lacking in abetalipoproteinemia) required for the secretion of apoB. We investigated whether a stably transformed cell line would exhibit a block in secretion of apoB, and, to characterize the block further, two truncated forms of apoB (apoB15 and apoB53) were expressed in nonhepatic Chinese hamster ovary (CHO) fibroblasts at levels comparable to those seen in hepatoma cells. ApoB15 was chosen for comparison to apoB53 because it is less than the minimum length required to assemble lipoproteins (7, 8) and thus might not require the same unique processes for its secretion.

We show here that transfected CHO cells secrete apoB15 but do not secrete apoB53. The inability to secrete apoB53 is due to a block in the translocation of the complete apoB53 peptide chain across the endoplasmic reticulum (ER). As a result, apoB53 is degraded by a process that can be blocked by a thiol protease inhibitor. Since apoB15 is able to be translocated and secreted, the inability of CHO cells to translocate and secrete apoB53 is likely due to the property that allows the larger form to assemble lipoproteins. The process missing from CHO cells, but present in liver and intestinal cells, may normally function to regulate the assembly of apoB-containing lipoproteins.

MATERIALS AND METHODS

Cell Culture and Transfection. All tissue culture supplies, chemicals, and radioactive chemicals were obtained from suppliers described (9–13) or as indicated. CHO-K1 cells were obtained from the Eleanor Roosevelt Cancer Research Institute (Denver). Cells were maintained in minimum essential medium with 1.5% fetal bovine serum and 3.5% newborn calf serum (Gemini Bioproducts, Calabasas, CA) (MEM plus serum) at 37°C in an atmosphere of 5% CO_2/95% air. Plasmids encoding human apoB15 and apoB53 were generously supplied by Brian McCarthy and Zemin Yao (Gladstone Research Institute, San Francisco) and were constructed by using the expression vector pCMV5 (14). Cells were plated at 20% confluence and transfected the next day with one of the apoB expression plasmids and a neomycin-resistance plasmid (pRSV2-neo) in an 18:1 molar ratio (7). Cells were cultured in the presence of G418 (400 μg/ml) for 2 weeks. (Nontransfected CHO cells were killed when the same concentration of G418 and the same time course were used.) Surviving cells were single cell plated into 96-well plates, grown out, and characterized as described below.

RNA Isolation and Analysis. Cellular RNA was extracted using the guanidinium isothiocyanate/phenol/chloroform method (15) and quantitated by slot blot analysis (12). The relative abundance of human apoB mRNA was determined by using a plasmid containing a cDNA encoding the N-ter-

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Abbreviations: apoB, apolipoprotein B; ALLN, *N*-acetylleucylleucylnorleucinal; ER, endoplasmic reticulum; HMGCoA, 3-hydroxy-3-methylglutaryl CoA.

*Present address: Department of Biology, San Diego State University, San Diego, CA 92182.
†To whom reprint requests should be addressed.

minal portion, generously supplied by Lusis *et al.* (16). The cDNA insert was gel purified using low melting point agarose and ^{32}P-labeled with a random priming kit (Boehringer Mannheim).

Membrane and Protein Analysis. CHO cells were grown in MEM plus serum to 80% confluence. On the night before the experiment, the culture medium was changed to MEM plus serum with or without 100 μg of *N*-acetylleucylleucylnorleucinal (ALLN) (calpain inhibitor I; Boehringer Mannheim) per ml, as indicated. After 18 h, cells were scraped into phosphate-buffered saline (PBS) containing 2 mM phenylmethylsulfonyl fluoride, 5 μM ALLN, 100 μM leupeptin, 5 mM EDTA, and 5 mM EGTA (17) and then resuspended in 250 mM sucrose/10 mM Tris·HCl, pH 7.4 containing the same inhibitors. Microsomes were prepared by subjecting cells to nitrogen cavitation and subsequent ultracentrifugation (10). For carbonate extraction, 100 μg of microsomes was diluted into 1 ml of 100 mM Na_2CO_3 and centrifuged 20 min at 100,000 rpm in a Beckman TLA 100.2 rotor at 4°C. The membrane pellet was resuspended in SDS, while the supernatant was precipitated with 10% trichloroacetic acid. For proteolysis, microsomes were washed twice with sucrose/Tris to remove protease inhibitors, resuspended at 400 μg/ml, Dounce homogenized, and incubated 30 min at room temperature with or without trypsin (120 μg/ml). Inhibitors were added (2 mM phenylmethylsulfonyl fluoride and leupeptin and aprotinin, each at 20 μg/ml), and then the sample was precipitated with trichloroacetic acid. Samples were solubilized in SDS, separated on a 2–20% polyacrylamide gradient, and then Western blotted as described in detail (9). Rabbit antiserum against human apoB was generously supplied by John Elovson and Verne Schumaker (University of California, Los Angeles). The antibody against protein disulfide isomerase was obtained from Steven D. Fuller (European Molecular Biology Laboratory, Heidelberg). Monoclonal antibodies against 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase were obtained from S. R. Panini (ERICR, Denver). Detection of blots was by the chemiluminescent method as described by the manufacturer (Amersham). For triglyceride analysis, cells were grown to 80% confluence in MEM plus serum, and then the medium was changed to serum-free MEM plus 1% bovine serum albumin or 1% albumin/1 mM oleic acid. After 24 h, cells were harvested, lipids were extracted, and triglyceride content was determined by enzymatic assay using a triglyceride assay kit (UV-340; Sigma) as described (13), except the GK start procedure was used.

Concanavalin A Binding. Microsome fractions were solubilized in 2% SDS/100 mM dithiothreitol/100 mM Tris·HCl, pH 7.4 and boiled to denature and dissociate proteins. The samples were then diluted with 20 vol of concanavalin A buffer (1.5 M NaCl/1% Triton X-100/1 mM $CaCl_2$/1 mM $MnCl_2$/100 mM Tris·HCl, pH 7.4), with protease inhibitors added as described above. To show binding was specific, some samples included 500 mM methyl α-D-mannopyranoside. Samples were precleared with Sepharose Cl-4B and then rocked overnight at 4°C with concanavalin A-Sepharose beads. Beads were pelleted in a microcentrifuge, washed three times with concanavalin A buffer, washed once with PBS, and then Western blotted.

RESULTS

Cells Expressing ApoB15 mRNA Contain ApoB15, Whereas Cells Expressing ApoB53 mRNA Contain No ApoB53. Three independent isolates of transfected CHO cells expressing apoB53 mRNA and one isolate expressing apoB15 were characterized in detail. The apoB mRNA levels were similar for all transfected cells (Fig. 1) and ≈70% (data not shown) of that of HepG2 cells, which secrete apoB (19). As expected,

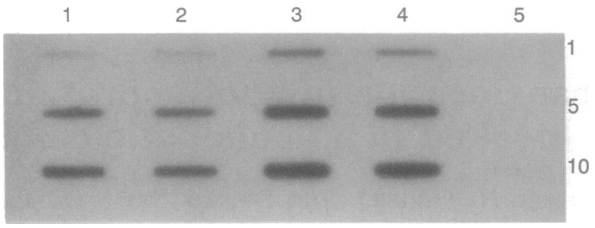

FIG. 1. Slot-blot analysis for the presence of apoB mRNA. Extracted RNA was applied to nylon membranes in the amounts indicated in μg on the far right and hybridized with a ^{32}P-labeled cDNA probe encoding the N terminus of human apoB (16). Lanes: 1–3, three independent clones of CHO cells transfected with the plasmid encoding apoB53; 4, a clone of CHO cells transfected with the plasmid encoding apoB15; 5, CHO-K1 cells (not transfected). Not shown: the blot was stripped and rehybridized using a β-actin cDNA probe. All transfected cells displayed the same level of β-actin mRNA, indicating that the loading of RNA was similar.

the nontransfected CHO cells did not express detectable levels of apoB mRNA. While there were no significant differences in the relative abundance of apoB mRNA in CHO cells transfected with the plasmids encoding either apoB15 or apoB53, only cells transfected with apoB15 contained the expected translation product (Fig. 2).

Based on previous results showing that the nontranslocated pool of apoB in rat liver is degraded (9), we hypothesized that the lack of detectable translation product in the CHO cells transfected with apoB53 might be the result of complete intracellular degradation of the newly synthesized protein. Incubation of CHO cells with the membrane-permeable thiol protease inhibitor ALLN inhibits the degradation of HMGCoA reductase (17, 20). We examined whether the degradation of apoB53 could be blocked by this inhibitor. When incubated for 24 h with ALLN, the CHO cells expressing apoB53 mRNA accumulated proteins that were recognized by the human apoB-specific antiserum (Fig. 3). In all three CHO cell clones expressing apoB53 mRNA, the major protein recognized by the antiserum had an estimated size of ≈290 kDa, the size expected for apoB53. These data show that CHO cells expressing apoB53 mRNA syn-

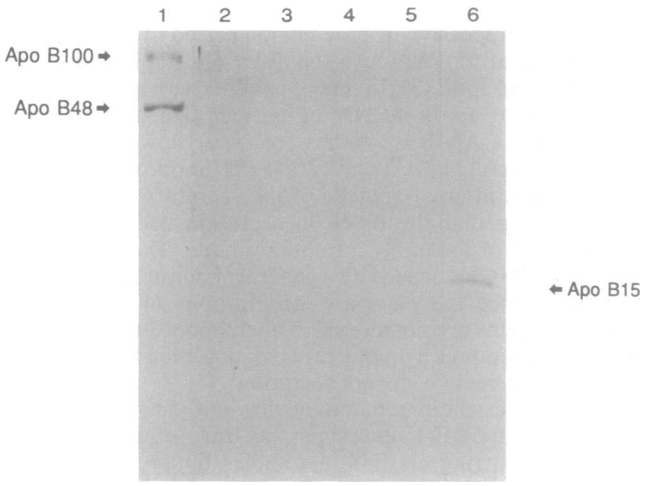

FIG. 2. Analysis of CHO cells for the presence of apoB. Western blots of lysates of cells grown in the absence of ALLN. The blot was probed with an antiserum to human apoB100. Lanes: 1, apoB molecular weight standards obtained from plasma lipoproteins; 2, CHO-K1 (nontransfected cells); 3–5, three independent clones of CHO cells expressing apoB53 mRNA; 6, a clone expressing apoB15 mRNA.

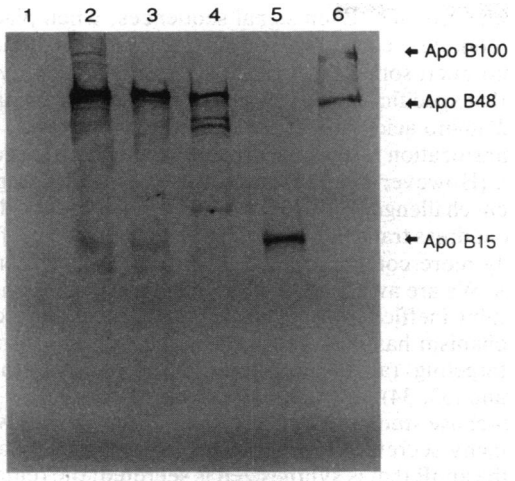

FIG. 3. Accumulation of apoB in cells in the presence of ALLN. Western blots of lysates of cells incubated in the presence of the protease inhibitor ALLN. The blot was probed with an antiserum to human apoB100. Lanes: 1, CHO-K1 (nontransfected cells); 2–4, three independent clones of CHO cells expressing apoB53 mRNA; 5, a clone expressing apoB15 mRNA; 6, apoB molecular weight standards obtained from plasma lipoproteins.

thesize the protein but that in the absence of ALLN it is completely degraded.

The Secretion of ApoB53, but Not ApoB15, Is Blocked in CHO Cells. Although incubation with ALLN caused apoB53 to accumulate in cells, none was secreted into serum-free culture medium (Fig. 4). In marked contrast, apoB15 was easily detected in the culture medium (Fig. 4). Thus, CHO cells can secrete apoB15, but not apoB53. The amount of apoB53 contained in ALLN-treated CHO cells is less than the amount of apoB100 contained in HepG2 cells. (When equal amounts of microsomal protein are applied to Western blots, the bands from HepG2 microsomes are much darker; data not shown.) Thus, overexpression of apoB does not account for the inability of ALLN-treated transfected CHO cells to secrete apoB53. ALLN itself does not inhibit secretion, since apoB15 is secreted in its presence.

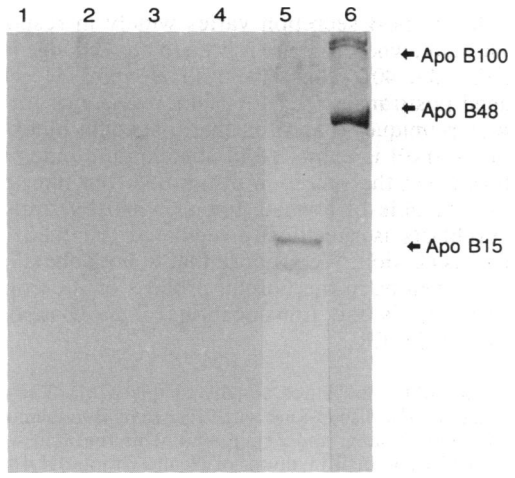

FIG. 4. Accumulation of apoB in culture medium of cells in the presence of ALLN. The culture medium was obtained from confluent 60-mm dishes of cells after a 24-h incubation in serum-free medium with ALLN. Medium was precipitated with trichloroacetic acid, Western blotted, and probed with an antiserum to human apoB100. Lanes: 1, CHO-K1 (nontransfected) cells; 2–4, three independent clones of CHO cells expressing apoB53 mRNA; 5, a clone expressing apoB15 mRNA; 6, apoB molecular weight standards obtained from plasma lipoproteins.

We examined whether apoB53 might require additional lipid for its secretion by CHO cells. Cells were incubated overnight with oleic acid (1 mM complexed with bovine serum albumin). Oleic acid caused an accumulation of Sudan black-stainable cytoplasmic lipid droplets (data not shown) and increased the cellular content of triglyceride 17-fold (5.9 ± 0.2 vs. 99 ± 2 μg of triglyceride per mg of cell protein for control and oleic acid-supplemented cells, respectively; n = 3 plates assayed for each condition). Despite the dramatically increased triglyceride content, CHO cells remained unable to secrete apoB53 (data not shown). Furthermore, adding oleic acid together with ALLN also failed to promote the secretion of apoB53 (data not shown). Thus, insufficient triglyceride cannot account for the inability of CHO cells to secrete apoB53.

ApoB53 Remains Exposed on the Cytoplasmic Surface of the ER: Evidence for Blocked Translocation. CHO cells expressing apoB53 (see Fig. 3) were incubated with ALLN for 24 h, after which the cells were harvested and microsomes were isolated. Reisolation of microsomes in the sucrose buffer resulted in a quantitative recovery of apoB53 as well as the lumenal marker protein disulfide isomerase in the pellet, indicating that the microsomes remained intact in the absence of carbonate disruption (Fig. 5, lanes 1 and 2). After extraction with sodium carbonate, which opens microsomal vesicles and releases the soluble contents (21), nearly all the apoB53 (as well as HMGCoA reductase; data not shown) remained in the membrane pellet (lane 4). In contrast, nearly all protein disulfide isomerase was released by carbonate, indicating that the microsomes were in fact opened by the treatment (lane 3). Thus, the apoB53 in transfected CHO cells is associated with the microsomal membrane.

To examine the topography of apoB53, microsomes were subjected to trypsin, an impermeable protease. Trypsin degraded essentially all the apoB53 present in microsomes obtained from ALLN-treated cells (Fig. 5, lane 6). We could not clearly demonstrate that any fragments of apoB were resistant to proteolysis (that is, protected by being within the microsomal lumen). (However, we cannot rule out the possibility that segments not recognized by our antiserum may have been protected.) In contrast, the majority of apoB15 was resistant to trypsin (unpublished results). Protein disulfide isomerase was not degraded by trypsin, indicating that the microsomes remained intact. Adding SDS to disrupt the microsomes before incubating with trypsin resulted in a complete disappearance of both apoB and protein disulfide isomerase bands (data not shown).

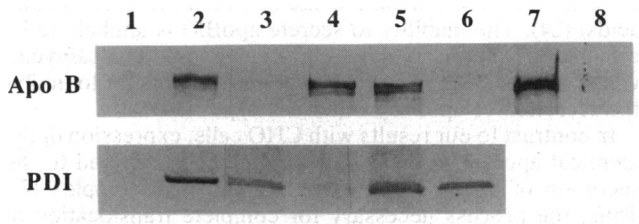

FIG. 5. Analysis of microsomes obtained from ALLN-treated CHO cells expressing apoB53. CHO cells expressing apoB53 were grown in the presence of ALLN. Microsomes were isolated, fractionated, or proteolyzed; Western blotted; and probed for apoB (*Upper*). Then the nitrocellulose was reprobed for protein disulfide isomerase (PDI; *Lower*). Lanes: 1 and 2, supernatant and pellet, respectively, from microsomes extracted with 250 mM sucrose/10 mM Tris, pH 7.4; 3 and 4, supernatant and pellet, respectively, from microsomes extracted with sodium carbonate (pH 11.5); 5, untreated microsomes; 6, microsomes incubated with trypsin (120 μg/ml) at room temperature for 30 min; 7, pellet from microsomes solubilized and then bound to concanavalin A-Sepharose; 8, pellet from microsomes bound to concanavalin A-Sepharose in the presence of 500 mM methyl α-D-mannopyranoside.

ApoB53 Is Glycosylated and Partially Translocated into the Lumen of the ER. The combined data show that in CHO cells the translocation of apoB53 is blocked, resulting in its accumulation on the cytoplasmic surface of the ER. Since apoB15 and apoB53 share the identical N terminus, one might expect that apoB53 would be partially translocated, at least to the point corresponding to the length of apoB15. Both apoB15 and apoB53 contain consensus sites for N-linked glycosylation. Glycosylation, which occurs in the lumen of the ER, would require translocation of at least a portion of the apoB molecule. We tested whether apoB53 was glycosylated and therefore intralumenal. Solubilized microsomes from ALLN-treated cells were incubated with concanavalin A-Sepharose and the bound material was subjected to Western blotting. ApoB53 bound to the concanavalin A beads (Fig. 5, lane 7). Nearly all of the microsomal apoB53 was recovered in this fraction (data not shown). Moreover, this protein was blocked from binding to concanavalin A by methyl α-D-mannopyranoside, a specific competitor (lane 8). These data show that the apoB53 in microsomes from ALLN-treated cells is glycosylated. Thus, apoB53 is partially translocated across the ER membrane of transfected CHO cells.

DISCUSSION

This study shows (*i*) that nonhepatic CHO cells cannot translocate apoB53 completely into the lumen of the ER—the product is not soluble as expected for secretory proteins but rather is an integral membrane protein (by the criterion of resistance to carbonate extraction) and is exposed on the cytoplasmic face of the ER; (*ii*) that as a result of its incomplete translocation apoB53 is degraded; (*iii*) that the intracellular degradation of apoB53 can be blocked by a thiol protease inhibitor; and (*iv*) that molecules of apoB15, a form of apoB that is too short to assemble lipoproteins, can be translocated completely. From these combined data we propose that the translocation of apoB53 occurs as a two-step process. The first step (active in both hepatic and nonhepatic cells) can translocate apoB15 but cannot completely translocate the apoB53 molecule. The second step, which is absent in CHO and perhaps other nonhepatic cells, is required to translocate the portion of apoB53 that is not shared with apoB15.

CHO-K1 cells have been used in a number of studies examining the secretion of mammalian proteins. When transfected with the appropriate expression plasmids, these cells are able to synthesize and secrete apoA-1 (22) and apoE (23). CHO cells are capable of efficiently translocating proteins as large as the von Willebrand factor precursor (2813 amino acids) (24). The inability to secrete apoB53 is unlikely to be caused by altered N-linked glycosylation. Tunicamycin, which blocks glycosylation, has been reported not to inhibit the secretion of apoB by other cell types (14, 25).

In contrast to our results with CHO cells, expression of the identical apoB53 plasmid in hepatoma cells did lead to the secretion of apoB53 as part of a lipoprotein complex (7). Thus, the process necessary for complete translocation of apoB53 is cell-type specific. It is possible that this process is specifically required for apoB53 because of the properties that allow apoB to assemble lipoproteins. It is not required for translocation of apoB15, which contains the identical signal sequence and N-terminal domain but which cannot assemble lipoproteins, nor is it required for translocation of the portion of apoB53 that is glycosylated by CHO cells.

It is not known how lipids become associated with apoB to form a lipoprotein. ApoB contains multiple hydrophobic domains (18, 26–28), many of which must interact with lipoprotein lipids. It is conceivable that during biosynthesis the hydrophobic domains of apoB interact with the ER membrane or with the ER proteins involved in integration of membrane proteins. Even signal sequences, when placed in certain contexts, can cause the integration of chimeric proteins into microsomal membranes (29). It has recently been shown that specific short sequences from apoB15, containing charged amino acids, are able to transiently "pause" the *in vitro* translocation of apoB15 as well as of chimeric proteins (30, 31). [However, the presence of pause signals in apoB17 has been challenged (32).] While our experiments do not directly address transient phenomena, the biogenesis of apoB is clearly more complex than that of many other secretory proteins. We are aware of only two proteins other than apoB that exhibit inefficient translocation *in vivo*. In both cases, the mechanism has been attributed to inefficient cotranslational targeting rather than to interactions with the ER membrane (33, 34).

Pulse–chase studies in cultured rat hepatocytes show that, unlike many secretory proteins (e.g., albumin), only a fraction of the apoB that is synthesized is secreted; the remainder is degraded intracellularly (10). Similar observations have been made in HepG2 cells (35), in rat hepatoma cells (7), and in rat liver (11). Since in the present study blocking degradation of apoB with ALLN did not result in translocation, the latter step was the major factor regulating apoB53 secretion by CHO cells. Furthermore, the finding in liver of a nontranslocated pool of apoB that becomes degraded (9) is consistent with the proposal that, in liver as in CHO cells, translocation is the active process regulating secretion, and that molecules that fail to become translocated are degraded by default.

Degradation of apoB in the ER may be related to the processes responsible for the degradation of the α subunit of the T-cell antigen receptor (36) and of HMGCoA reductase (17, 20, 37). The signal in the α subunit of the T-cell receptor responsible for its degradation in the ER has been identified as a charged amino acid within a hydrophobic domain (36). ApoB may contain sequences that function analogously. However, in contrast to the degradation of the α subunit of the T-cell receptor (38), incubation of CHO microsomes at 37°C does not cause degradation of apoB (unpublished results). The protease responsible for the degradation of apoB has not yet been identified. Since it is inhibited by ALLN, it may be similar to the protease responsible for the degradation of HMGCoA reductase.

The rate of apoB secretion varies widely in response to metabolic state, whereas there is little (if any) change in apoB mRNA (12, 39, 40). Thus, the rate of apoB secretion is determined posttranscriptionally. Other evidence for a regulatable step unique to apoB is that a specific block in the secretion of apoB is exhibited in abetalipoproteinemia. We have shown that the secretion of apoB53 (but not apoB15, which is incapable of forming lipoproteins) by transfected CHO fibroblasts is specifically regulated (blocked) at the level of translocation. We propose that in both abetalipoproteinemia as well as in the normal process of secretion, the regulatory step is the translocation of apoB across the membrane of the ER.

R.N.T. and J.D. contributed equally to this work. The authors acknowledge helpful discussions with Drs. Juan Bonifacino, Brian McCarthy, Steve Young, and Zemin Yao. This research was supported in part by National Institutes of Health Grants HL41624 and DK34914 and by a grant in aid from the American Heart Association.

1. Kane, J. P. (1983) *Annu. Rev. Phys.* **45**, 637–651.
2. Herbert, P. N., Assman, G., Gotto, A. M. & Fredrickson, D. S. (1983) in *Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S. (McGraw-Hill, New York), 5th Ed., pp. 589–691.
3. Talmud, P. J., Lloyd, J. K., Muller, D. R., Collins, D. R., Scott, J. & Humphries, S. (1988) *J. Clin. Invest.* **82**, 1803–1806.

4. Lackner, K. J., Monge, J. C., Gregg, R. E., Hoeg, J. M., Triche, T. J., Law, S. W. & Brewer, H. B., Jr. (1986) *J. Clin. Invest.* **78,** 1707–1712.
5. Dullaart, R. P. F., Speelberg, B., Schuurman, H. J., Milne, R. W., Havekes, L., Marcel, Y. L., Genze, H. J., Hulshox, M. M. & Erkelens, D. W. (1986) *J. Clin. Invest.* **78,** 1397–1404.
6. Bouma, M. E., Beucler, I., Pessah, M., Heinzmann, C., Lusis, A. J., Naim, H. Y., Ducastelle, T., Leluyer, B., Schmitz, J., Infante, R. & Aggerbeck, L. P. (1990) *J. Lipid Res.* **31,** 1–15.
7. Yao, Z., Blackhart, B. D., Linton, M. F., Taylor, S. M., Young, S. G. & McCarthy, B. J. (1991) *J. Biol. Chem.* **266,** 3300–3308.
8. Graham, D. L., Knott, T. J., Jones, T. C., Pease, R. J., Pullinger, C. R. & Scott, J. (1991) *Biochemistry* **30,** 5616–5621.
9. Davis, R. A., Thrift, R. N., Wu, C. C. & Howell, K. E. (1990) *J. Biol. Chem.* **265,** 10005–10011.
10. Borchardt, R. A. & Davis, R. A. (1987) *J. Biol. Chem.* **262,** 16394–16402.
11. Davis, R. A., Prewett, A. B., Chan, D. C. F., Thompson, J. J., Borchardt, R. A. & Gallaher, W. R. (1989) *J. Lipid Res.* **30,** 1185–1196.
12. Leighton, J. K., Joyner, J., Zamarripa, J., Deines, M. & Davis, R. A. (1990) *J. Lipid Res.* **31,** 1663–1668.
13. Lang, C. A. & Davis, R. A. (1990) *J. Lipid Res.* **31,** 2079–2086.
14. Suita-Mangano, P., Janero, D. R. & Lane, M. D. (1982) *J. Biol. Chem.* **257,** 11463–11467.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
16. Lusis, A. J., West, R., Mehrabian, M. & Reuben, M. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4597–4601.
17. Inoue, S., Bar-Nun, S., Roitelman, J. & Simoni, R. D. (1991) *J. Biol. Chem.* **266,** 13311–13317.
18. 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., & Chan, L. (1989) *Arteriosclerosis* **9,** 96–108.
19. Thrift, R. N., Forte, T. M., Cahoon, B. E. & Shore, V. G. (1986) *J. Lipid Res.* **27,** 236–250.
20. Roitelman, J., Bar-Nun, S., Inoue, S. & Simoni, R. D. (1991) *J. Biol. Chem.* **256,** 16065–16090.
21. Fujiki, Y., Hubbard, A. L., Fowler, S. & Lazarow, P. B. (1982) *J. Cell Biol.* **93,** 97–102.
22. Forte, T. M., McCall, M. R., Amacher, S., Nordhausen, R. W., Vigne, J. L. & Mallory, J. B. (1990) *Biochim. Biophys. Acta* **1047,** 11–18.
23. Wernette-Hammond, M. E., Lauer, S. J., Corsini, A., Walker, D., Taylor, J. M. & Rall, S. C., Jr. (1989) *J. Biol. Chem.* **264,** 9094–9101.
24. Dorner, A. J., Wasley, L. C. & Kaufman, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7429–7432.
25. Bell-Quint, J., Forte, T. & Graham, P. (1981) *Biochem. J.* **200,** 409–414.
26. Knott, T. J., Pease, R. J., Powell, L. M., Wallis, S. C., Rall, S. C., Milne, R., Johnson, D., Fuller, M., Lusis, A. J., McCarthy, B. J., Mahley, R. W., Levy-Wilson, B. & Scott, J. (1986) *Nature (London)* **323,** 734–740.
27. Chen, G. C., Handman, D. A., Hamilton, R. L., Mendel, C. M., Schilling, J. W., Zhu, S., Lau, K., Wong, J. S. & Kane, J. P. (1989) *Biochemistry* **9,** 2477–2484.
28. Cladaras, C., Hadzopoulou-Cladaras, M., Nolte, R. T., Atkinson, D. & Zannis, V. I. (1986) *EMBO J.* **5,** 3495–3507.
29. Andrews, D. W., Young, J. C., Mirels, L. F. & Czarnota, G. J. (1992) *J. Biol. Chem.* **267,** 7761–7769.
30. Chuck, S. L., Yao, Z., Blackhart, B. D., McCarthy, B. J. & Lingappa, V. R. (1992) *Nature (London)* **346,** 382–385.
31. Chuck, S. L. & Lingappa, V. R. (1992) *Cell* **68,** 9–21.
32. Pease, R. J., Harrison, G. B. & Scott, J. (1991) *Nature (London)* **353,** 448–450.
33. von Heijne, G., Liljestrom, P., Mikus, P., Andersson, H. & Ny, T. (1991) *J. Biol. Chem.* **266,** 15240–15243.
34. Ulmer, J. B. & Palade, G. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6992–6996.
35. Sato, R., Imanaka, T., Takatsuki, A. & Takano, T. (1990) *J. Biol. Chem.* **265,** 11880–11884.
36. Bonifacino, J. S., Cosson, P., Shah, N. & Klausner, R. D. (1991) *EMBO J.* **10,** 2783–2793.
37. Gil, G., Faust, J. R., Chin, D. J., Goldstein, J. L. & Brown, M. S. (1985) *Cell* **41,** 249–255.
38. Stafford, F. J. & Bonifacino, J. S. (1991) *J. Cell Biol.* **115,** 1225–1236.
39. Davidson, N. O., Powell, L. M., Wallis, S. C. & Scott, J. (1988) *J. Biol. Chem.* **203,** 13482–13485.
40. Pullinger, C. R., North, J. D., Teng, B.-B., Rificini, V. A., Ronhild de Brito, A. E. & Scott, J. (1989) *J. Lipid Res.* **30,** 1065–1077.