Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in abetalipoproteinemia

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Abstract Abetalipoproteinemia (ABL) is an autosomal recessive disease characterized by the inability of the liver and intestine to secrete apolipoprotein B (apoB). Mutations in the microsomal triglyceride transfer protein (MTP) gene, but not the apoB gene, are responsible for the ABL phenotype. It is not clear how loss of MTP in ABL patients leads to a complete, but specific, block in the secretion of apoB. It is to this question that our work is directed. In cultured cells lacking MTP, translocation of apoB is completely arrested, leading to the hypothesis that apoB requires MTP in order to completely enter the lumen of the endoplasmic reticulum, the site of lipoprotein assembly. We examined this hypothesis by determining the presence in plasma of distinct N-terminal apoB peptides, produced exclusively from translocation arrested apoB, in the plasma of six ABL patients and six normal subjects. The data show that N-terminal apoB peptides are present in the plasma of six ABL patients, whereas intact apoB-100 was barely detectable. Moreover, the plasma of all six ABL patients displayed a 2000-fold increase in the amount of an 85 kDa N-terminal apoB peptide relative to apoB-100. These data provide the first in vivo data supporting the essential role that MTP plays in apoB translocation. In normal humans, varied expression of MTP may be responsible for the post-transcriptional regulation of apoB secretion.—**Du, E. Z., S-L. Wang, H. J. Kayden, R. Sokol, L. K. Curtiss, and R. A. Davis.** Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in abetalipoproteinemia. *J. Lipid Res.* 1996. **37:** 1309–1315.

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The secretion of apoB by the liver is a major determinant of plasma low density lipoprotein (LDL) levels, one of the most important risk factors for atherosclerotic cardiovascular disease. Hepatic apoB secretion is regulated post-transcriptionally by a mechanism that determines whether de novo synthesized apoB is secreted or is degraded intracellularly (1–4). There are several sites along the secretory pathway where apoB is degraded (2–5). The first step in the secretory pathway, translocation of apoB across the endoplasmic reticulum, plays a critical role in determining the fate of de novo synthesized apoB. In livers of rats (6) and rabbits (7), primary rat hepatocytes (6), avian hepatocytes (8) and several lines of hepatoma cells (9–11), a significant portion of de novo synthesized apoB is inefficiently translocated across the endoplasmic reticulum. Incompletely translocated apoB is rapidly degraded in the endoplasmic reticulum (10–13). In human hepatoma HepG2 cells, translocation determines how much apoB enters the rapid degradation pathway (11). Variation in the translocation of apoB in response to metabolic state can explain how apoB secretion displays rapid changes while hepatic levels of mRNA remain nearly constant (14–16).

One candidate gene that may control the rate of apoB translocation and lipoprotein secretion is MTP, a protein found in the endoplasmic reticulum of the liver and intestine (17–19). The ability of MTP to transfer lipid to the nascent apoB lipoprotein particle changes in parallel with the rate of apoB secretion by both liver and intestine (17–19). Genetic loss of functioning MTP is the basis of the human recessive disorder abetalipoproteinemia (ABL) (17–19). This disease results from an inability of

Abbreviations: ABL, abetalipoproteinemia; apoB, apolipoprotein B; SDS/PAGE, sodium dodecyl sulfate, polyacrylamide gel electrophoresis; LDL, low density lipoproteins; CHO, Chinese hamster ovary; MTP, microsomal triglyceride transfer protein.

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both the liver and intestine to secrete apoB-containing lipoproteins (20). As the plasma levels of most other liver-derived proteins are normal in ABL patients, MTP is required for the secretion of apoB, but not other proteins. A similar phenotype in regard to the specific block in the secretion of apoB exhibited by the livers and intestines of ABL patients has been recapitulated in cultured cells that express apoB, but not MTP. Cells that do not express MTP cannot translocate apoB or secrete apoB-containing lipoproteins (12, 13, 21, 22). However, expression of MTP in these cells allows apoB to be secreted as a lipoprotein particle (21, 22).

Topographical analysis of translocation-arrested apoB that accumulates in cells lacking MTP shows that the extreme N-terminus is lumenal, whereas the C-terminus remains on the cytoplasmic surface of the endoplasmic reticulum (13). Similar topographical orientation of translocation-arrested apoB occurs in cells (i.e., HepG2) that express MTP, but display inefficient translocation of apoB (13). These data indicate that structural determinants present in apoB act as a barrier to translocation and that MTP abrogates this obstacle. Proteolytic cleavage of translocation-arrested apoB releases lumenal N-terminal forms of apoB that are secreted as three distinct apoB peptides (13). These N-terminal apoB peptides, identified by their complement of specific immunoreactive epitopes, are found in plasma of normal humans and serve as an indicator of translocation arrest. If, as proposed, MTP is essential for apoB translocation, these N-terminal apoB peptides, but little or no intact apoB, should be present in the plasma of ABL patients. In the present study we examined plasma from six ABL patients and six normal subjects for the presence of intact apoB and N-terminal apoB peptides.

MATERIALS AND METHODS

ABL diagnosis

All six hypolipidemic patients (**Table 1**) were diagnosed as being ABL based on the finding that their parents were normolipidemic. Moreover, as shown below (Results section) small amounts of apoB-100 could be detected in all of the plasma samples, indicating the validity of the ABL diagnosis as the only reasonable explanation for their severe hypolipidemia.

Plasma sample analysis

Plasma samples were obtained with informed consent from six ABL patients and six normolipidemic controls (graduate student volunteers) after an overnight fast. Plasma samples were immediately frozen at -20°C until use. The analyses for triglycerides and cholesterol were determined in Lipid Research Centers at the University of Colorado Health Sciences Center (ABL patients 1–3), New York University Medical Center (ABL patients 4 and 6), University of Texas Southwestern Medical Center (ABL patient 5) and at the University of California, San Diego Medical Center (all normal controls) using standard protocols.

Western blotting

Western blotting was performed as described in detail (13). Plasma proteins were denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 1–20% gradient SDS/PAGE and electroblotted onto nitrocellulose membranes. Nonspecific binding sites of the membranes were blocked using defatted milk proteins followed by addition of primary antibody. The relative amount of primary antibody bound was detected with species-specific horseradish peroxidase-conjugated second antibody. Blots were developed using an ECL kit (Amersham).

Ratios of the 85 kDa apoB peptide to apoB-100 were determined by Western blotting with monoclonal antibody 1D1 (described below) and densitometric analysis using a PhosphorImager (Molecular Dynamics). Preliminary experiments showed that within the conditions used, there was a linear relationship between the density of the apoB band and the relative amount of plasma sample used. Using the software supplied by the manufacturer, the densitometry for each analysis was on the linear portion of the standard curve under all conditions used.

Immunoprecipitation

Ten mg of ABL plasma protein or 100 μg of normal plasma protein was solubilized in 1 ml phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and 100 μg/ml aprotinin, 100 μg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride. Antibodies specific for human apoB (3 μg of monoclonal antibodies or 5 μl of antiserum) were preincubated with 20 μl of protein A-Sepharose beads (dry volume) in 1 ml PBS buffer at room temperature for 2 h. The beads were then washed with PBS buffer three times to remove free antibodies. The antibody-bound protein A-Sepharose conjugates were incubated overnight at 4°C with the plasma protein samples in an amount determined empirically to completely bind the apoB present in each sample. Beads were recovered by centrifugation in a microfuge and were washed three times with the PBS buffer. The immunoprecipitates were dissolved in 100 μl sample buffer containing SDS and β-mercaptoethanol and separated on a 1–20% gradient SDS-PAGE. The gels were then subjected to Western blotting, as described above.

TABLE 1. Plasma lipid and apoB analyses for ABL patients (1–6) and normals (7–12)

Patient	Plasma Triglyceride	Plasma Cholesterol	Ratio 85 kDa/ApoB-100
	mg/dl		
1	10	23	43
2	10	17	153
3	10	20	127
4	20	30	586
5	0	38	330
6	6	40	154
Mean ± SD	9 ± 7	28 ± 10	232 ± 151
7	83	218	0.086
8	33	156	0.142
9	54	131	0.127
10	55	150	0.143
11	24	181	0.090
12	20	160	0.091
Mean ± SD	45 ± 24	154 ± 17	0.1 ± 0.03

There were significant differences among all parameters measured in the ABL plasma compared to those of normolipidemic plasma: $P < 0.01$.

Statistical analysis

All values are reported as the mean ± standard deviation. Differences between groups are calculated using unpaired Student's *t* test; double-tailed *P* values are reported.

RESULTS

ABL patients were diagnosed by the following criteria: consistent near absence of LDL in plasma of patients having normal lipidemic parents (recessive hypobetalipoproteinemia) (20). All six ABL plasma samples showed reduced concentrations of cholesterol and triglyceride (Table 1).

In order to identify any apoB present in the ABL plasma, we used two methods to concentrate apoB. One method involved immunoprecipitating plasma with a rabbit polyclonal apoB-specific antiserum, separating the immunoprecipitates on SDS-PAGE, and Western blotting with monoclonal antibody 1D1, which recognizes an epitope residing between amino acids 474–539 (23) of human apoB. The other method involved passing the plasma through a rabbit polyclonal apoB-specific antibody affinity column. The eluted proteins were then Western blotted using monoclonal antibody 1D1. Both methods gave us the same results (the results of the first method are shown in **Fig. 1**). In the three plasma samples obtained from ABL patients (ABL patients 1–3) there were very low levels of apoB-100. Small amounts of apoB-100 in ABL plasma have been described by others (24). In the remaining three ABL plasma apoB-100 was barely detectable (100 times more protein was used for the ABL plasma than was used for normal subject samples, Fig. 1). All ABL plasma samples displayed three truncated forms of apoB having sizes similar to the N-terminal apoB peptides secreted by cells lacking MTP (Fig. 1: 126 kDa, 104 kDa, and 85 kDa). All three peptides were derived from the N-terminus of apoB-100 as demonstrated by their recognition by monoclonal antibody 1D1 (Fig. 1). When the same blot was probed with C-terminal epitope specific antibodies MB3 (residues 1022–1031) and MB43 (residues 4027–4081), there was no recognition (data not shown), indicating that these peptides do not contain these C-terminal epitopes.

In marked contrast to their affected children, apoB-100 was the major apoB form in both non-affected parents of ABL patients 1–3 (plasma from the father is shown: lane 1, Fig. 1). N-terminal apoB peptides were detected in the plasma from the unaffected father and mother with longer development of the Western blot (data not shown).

Based on previous studies showing that an 85 kDa N-terminal apoB peptide is produced from translocation-arrested apoB and is secreted by both hepatic and non-hepatic cells, we determined its ratio to intact apoB-100 in plasma from ABL patients and normal subjects (Table 1). When monoclonal antibodies that recognized epitopes on the extreme N-terminus of human apoB were used, the 85kDa peptide appeared to be the major form of apoB peptide present in ABL plasma (**Fig. 2**). In contrast, monoclonal antibody 1D1 (epitope 474–539) immunoprecipitated all three N-terminal peptides: 126, 104, and 85 kDa (lane 4, Fig. 2). Epitopes residing on the C-terminal side of 126 kDa of the N-terminal portion of apoB-100 were not present in any of

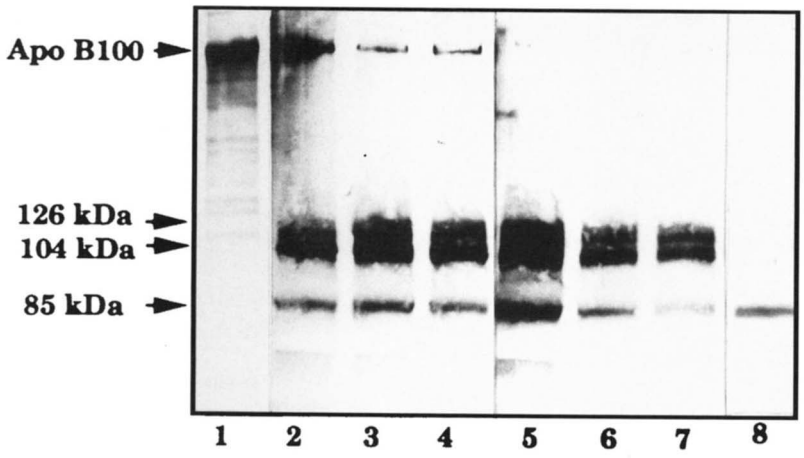

Fig. 1. Small apoB peptides predominate in ABL plasma. Plasma from the non-affected father of ABL patients 1–3 (100 μg protein, lane 1) and six ABL patients (10 mg protein each, lanes 2–7) were immunoprecipitated with a rabbit anti-human apoB antiserum as described in Methods. The immunoprecipitate was separated by SDS-PAGE and Western blotted with monoclonal antibody 1D1, which recognizes an epitope between residues 474 and 539 in human apoB-100 (23). Lane 2, ABL patient 1; lane 3, ABL patient 2; lane 4, ABL patient 3; lane 5, ABL patient 4; lane 6, ABL patient 5; lane 7, ABL patient 6; lane 8, purified sample (2 μg) of the N-terminal 85 kDa apoB peptide secreted by CHO cells expressing apoB-53 (13).

the apoB peptides smaller than apoB-100 and included residues 1022–1031 (Fig. 2, lane 5) and 4027–4081 (Fig. 2, lane 6). However, apoB-100 present in normal plasma samples did react with these antibodies (Fig. 2, lane 7–8). Additional control experiments showed that when ABL plasma was subjected to the immunoprecipitation procedure without antibody or with pre-immune rabbit serum, there was no detection of any immunoreactive apoB peptides (Fig. 2, lane 9–10). Identical results were obtained from all six ABL patients.

The combined data indicate that the 85 kDa apoB peptide present in plasma from ABL patient 1 contained apoB epitopes residing throughout the boundaries expected, if it was derived from the extreme N-terminal portion of apoB-100 (Fig. 2, lanes 2–4). These data indicate that: *1)* the 85 kDa apoB peptide present in ABL plasma was the same as that secreted by translocation-arrested apoB in CHO cells and HepG2 cells and is present in normal plasma (13); and *2)* there were no apoB peptides detected that were derived from the C-terminal portion of apoB-100 beyond 126 kDa of the N-terminus. In addition, the data suggest that the 104 and 126 kDa apoB peptides are also derived from the N-terminus of apoB-100; they do not contain C-terminal epitopes. As these peptides are not recognized by antibodies having epitopes at the extreme N-terminus of apoB (1–71, lane 2; and 12–27, lane 3, Fig. 2), but do contain the N-terminal 1D1 antibody epitope (lane 4, Fig. 2) they probably lack the extreme N-terminus. N-terminal apoB peptides lacking the extreme N-terminus have been observed to be secreted by cells treated with a specific inhibitor of MTP (25).

Based on the data that the 85 kDa apoB peptide was identical to the N-terminal apoB peptide produced from translocation-arrested apoB, we determined its presence in plasma relative to that of intact apoB-100 (Table 1). In six normal subjects, this ratio was relatively similar: 0.1 ± 0.03. Moreover, in plasma from all ABL patients the ratio was 2000-fold greater (232 ± 151; Table 1). These data strongly support the concept that translocation of apoB is blocked in ABL patients.

DISCUSSION

Our results show the presence of N-terminal, but not C-terminal, apoB peptides in ABL plasma. One apoB peptide having a molecular weight of 85 kDa is identical in terms of size and complement of specific antibody epitopes to the 85 kDa apoB peptide derived exclusively from translocation-arrested apoB (13). Two apoB peptides (104 and 126 kDa) were also present in ABL plasma. Both peptides were similar to those secreted by cells that lack MTP expression (13) or function (using an MTP inhibitor) (25). As other than intact apoB-100, no apoB peptides present in ABL plasma contained epitopes residing on the C-terminal side of 126 kDa of apoB-100, the N-terminal apoB peptides were not derived from proteolysis of intact apoB. Control experiments in which plasma was incubated with thrombin, trypsin, and proteinase C showed the presence of truncated apoB peptides bearing C-terminal epitopes (data not shown). Thus, had these N-terminal apoB peptides been produced by proteolysis of intact apoB already present in plasma, our assays would have detected C-terminal epitopes. ApoB variants having a size smaller than apoB-37 have been shown not to be capable of assembling VLDL (26). Thus, the secretion of these truncated N-terminal apoB peptides as a result of proteolytic cleavage of translocation-arrested apoB can explain the inability of ABL patients to secrete triglyceride-rich lipoproteins by the liver and intestine.

Based on previous findings showing that the 85 kDa N-terminal apoB peptide is produced by translocation-arrested apoB (13), its 2000-fold enrichment relative to apoB-100 (Table 1) in ABL plasma compared to plasma

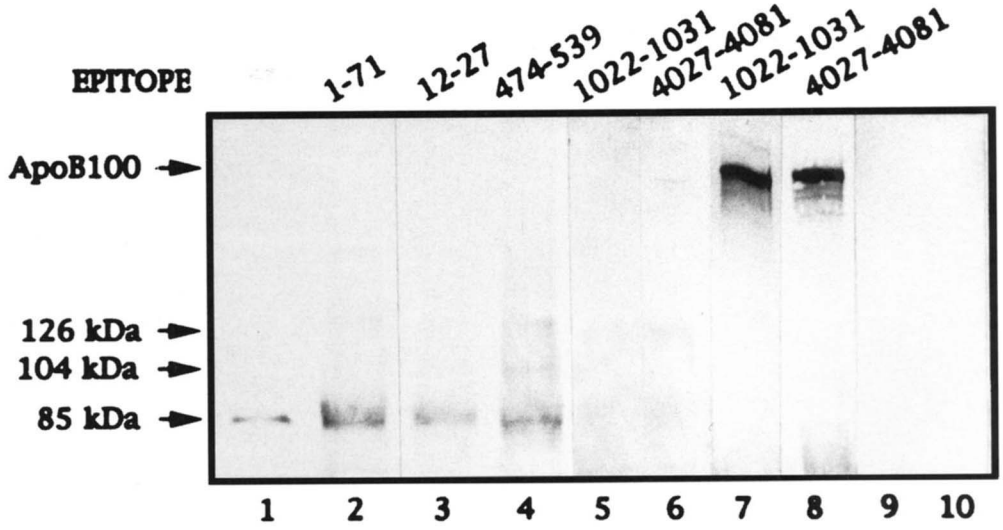

Fig. 2. Epitope mapping of 85kDa apoB peptide in ABL plasma. Plasma from ABL patient 1 (lanes 2–6) was immunoprecipitated with the following apoB-specific antibodies: monoclonal antibody MB19 recognizing an epitope mapped to around residue 71 (36), a rabbit antiserum prepared against a synthetic peptide containing residues 12–27 (37), monoclonal antibody 1D1 recognizing an epitope mapped to residues 474–539 (23), monoclonal antibody MB3 recognizing an epitope mapped to residues 1022–1031 (38), and monoclonal antibody MB43 recognizing an epitope mapped to residues 4027–4081 (39). The immunoprecipitates were separated by SDS-PAGE and Western blotted with 1D1 (lanes 2–4) or an apoB-100 specific affinity-purified rabbit antiserum (13) (lanes 5–6). As a control, plasma from non-affected father of ABL patients 1–3 was immunoprecipitated with monoclonal antibody MB3 and MB43 to show they did recognize apoB-100 (lanes 7–8). In addition, ABL plasma was immunoprecipitated without antibody (lane 9) or with rabbit pre-immune serum (lane 10). Lane 1, purified N-terminal 85 kDa apoB peptide secreted by CHO cells expressing apoB-53 (13) (2 μg).

from normal subjects suggests that ABL patients lack the ability to translocate apoB across the endoplasmic reticulum. Estimation of the mass of the 85 kDa apoB peptide compared to apoB-100 indicates that the molar amount of the 85 kDa N-terminal apoB peptide in ABL plasma is not equivalent to the amount of apoB-100 present in plasma from control subjects. This finding is expected as previous studies showed that the majority of the 85 kDa N-terminal apoB peptide is degraded intracellularly and is not efficiently secreted.

Our findings and interpretations are consistent with earlier studies describing the inability of the liver and intestine of ABL patients to secrete intact apoB. Liver and intestine biopsies obtained from ABL patients showed the production of intact and truncated forms of apoB; however, little intact apoB was secreted (27–31). Additionally, one study noted that apoB produced by an intestinal biopsy obtained from an ABL patient was degraded compared to that produced by biopsies from non-affected controls (30). Thus, in ABL apoB is synthesized by both the liver and intestine, but it is not secreted and is subsequently proteolytically degraded into truncated forms.

How can a fully lumenal protein like MTP block the movement of a protein across the ER? There are examples where the loss of intralumenal proteins blocks translocation (e.g., Bip (32) and Sec61p (33)), suggesting that the translocation machinery senses both cytoplasmic and intralumenal events. With Bip and Sec61p deletions, there are general impairments of translocation, whereas loss of MTP is selective for apoB. The unusual amphipathic qualities of apoB, thought to be necessary for lipoprotein assembly (2, 34) and/or the presence of pause/transfer sequences (35), may necessitate additional requirements for its translocation. Our finding that translocation of apoB is blocked in ABL patients suggests that MTP-facilitated lipidation of this amphipathic protein may be a unique requirement for translocation across the endoplasmic reticulum. Based on studies showing that MTP activity varies in parallel with apoB secretion (17–19), it is reasonable to propose that varied functional expression of MTP may be responsible for the post-translational regulation of apoB secretion.

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REFERENCES

1. Borchardt, R. A., and R. A. Davis. 1987. Intrahepatic assembly of very low density lipoproteins. Rate of transport out of the endoplasmic reticulum determines rate of secretion. *J. Biol. Chem.* **262:** 16394–16402.
2. Davis, R. A. 1991. Lipoprotein structure and secretion. *In* Biochemistry of Lipids and Membranes. D. E. Vance and J. E. Vance, editors. Elsevier, Amsterdam, London, New York, Tokyo. 403–424.
3. 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.
4. Bjornsson, O. G., J. D. Sparks, C. E. Sparks, and G. F. Gibbons. 1994. Regulation of VLDL secretion in primary culture of rat hepatocytes. *Eur. J. Clin. Invest.* **24:** 137–148.
5. Adeli, K. 1994. Regulated degradation of apolipoprotein B in semipermeable HepG2 cells. *J. Biol. Chem.* **269:** 9166–9175.
6. 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.
7. Wilkinson, J., J. A. Higgins, P. Groot, E. Gherardi, and D. E. Bowyer. 1992. Determination of the intracellular distribution and pool sizes of apolipoprotein B in rabbit liver. *Biochem. J.* **288:** 413–419.
8. Dixon, J. L., R. Chattapadhyay, T. Huima, C. M. Redman, and D. Banerjee. 1992. Biosynthesis of lipoprotein: location of nascent apoA-I and apoB in the rough endoplasmic reticulum of chicken hepatocytes. *J. Cell Biol.* **117:** 1161–1169.
9. Rusinol, A. E., E. Y. W. Chan, and J. E. Vance. 1993. Movement of apolipoprotein B into the lumen of microsomes from hepatocytes is disrupted in membranes enriched in phosphatidylmonomethylethanolamine. *J. Biol. Chem.* **268:** 25168–25175.
10. 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.
11. Bonnardel, J. A., and R. A. Davis. 1995. In HepG2 Cells, translocation, not degradation, determines the fate of the de novo synthesized apolipoprotein B. *J. Biol. Chem.* **270:** 28892–28896.
12. 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 non-hepatic cell line. *Proc. Natl. Acad. Sci. USA.* **89:** 9161–9165.
13. Du, E. Z., J. Kurth, S-L. Wang, P. Humiston, and R. A. Davis. 1994. Proteolysis-coupled secretion of the N-terminus of apolipoprotein B: characterization of a transient, translocation-arrested intermediate. *J. Biol. Chem.* **269:** 24169–24176.
14. Pullinger, C. R., J. D. North, L. M. Powell, S. C. Wallis, and J. Scott. 1989. The apolipoprotein B gene is constitutively expressed in HepG2 cells: regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J. Lipid Res.* **30:** 1065–1077.
15. Leighton, J. K., J. Joyner, J. Zamarripa, M. Deines, and R. A. Davis. 1990. Fasting decreases apolipoprotein B mRNA editing and the secretion of small molecular weight apoB by rat hepatocytes: evidence that the total amount of apoB secreted is regulated post-transcriptionally. *J. Lipid Res.* **31:** 1663–1668.
16. Davidson, N. O., R. C. Carlos, M. J. Drewek, and T. G. Parmer. 1988. Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* **29:** 1511–1522.
17. Gregg, R. E., and J. R. Wetterau. 1994. The molecular basis of abetalipoproteinemia. *Curr. Opin. Lipidol.* **5:** 81–86.
18. Shoulders, C. C., D. J. Brett, J. D. Bayliss, T. M. E. Narcisi, A. Jaruz, T. T. Grantham, P. R. D. Leoni, S. Bhattacharya, R. J. Pease, P. M. Cullen, S. Levi, P. G. H. Byfield, P. Purkiss, and J. Scott. 1993. Abetalipoproteinemia is caused by defects of the gene encoding the 97 kDa subunit of a microsomal triglyceride transfer protein. *Hum. Molec. Genet.* **2:** 2109–2116.
19. Sharp, D., L. Blinderman, K. A. Combs, B. Kienzle, B. Ricci, S. K. Wager, C. M. Gil, C. W. Turck, M. E. Bouma, D. J. Rader, L. P. Aggerbeck, R. E. Gregg, D. A. Gordon, and J. R. Wetterau. 1993. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinemia. *Nature.* **365:** 65–69.
20. Kane, J. P., and R. J. Havel. 1989. Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1139–1164.
21. 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.
22. Leiper, J. M., J. D. Bayless, 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.
23. Marcel, Y. L., T. L. Innerarity, C. Spilman, R. W. Mahley, A. A. Protter, and R. W. Milne. 1987. Mapping of human apolipoprotein B antigenic determinants. *Arteriosclerosis.* **7:** 166–175.
24. Menzel, H. J., H. Dieplinger, C. Lackner, F. Hoppichler, J. K. Lloyd, D. R. Muller, C. Labeur, P. J. Talmud, and G. Utermann. 1990. Abetalipoproteinemia with an ApoB-100-lipoprotein[a] glycoprotein complex in plasma. Indication for an assembly defect. *J. Biol. Chem.* **265:** 981–986.
25. Gordon, D. A., Z. Yao, H. Jamil, M. Yan, S. Wang, R. McLeod, and J. Wetterau. 1995. The microsomal triglyceride transfer protein mediates both translocation of apolipoprotein B into the endoplasmic reticulum and the addition of core lipid in the formation of apolipoprotein B-100 very low density lipoprotein. *Circulation.* **92 (Suppl. 1):** 1–362.
26. Yao, Z., 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.
27. Dullaart, R. P. F., B. Speelberg, H. J. Schuurman, R. W. Milne, L. M. Havekes, Y. Marcel, H. J. Geuze, M. M. Hulshof, and D. W. Erkelens. 1986. Epitopes of apolipoprotein B-100 and B-48 in both liver and intestine. Expres-

sion and evidence for local synthesis in recessive abetalipoproteinemia. *J. Clin. Invest.* **78:** 1397–1404.
28. Lackner, K. J., J. C. Monge, R. E. Gregg, J. M. Hoeg, T. J. Triche, S. W. Law, and H. B. Brewer, Jr. 1986. Analysis of the apolipoprotein B gene and messenger ribonucleic acid in abetalipoproteinemia. *J. Clin. Invest.* **78:** 1707–1712.
29. Bouma, M. E., I. Beucler, M. Pessah, C. Heinzmann, A. J. Lusis, H. Y. Naim, T. Ducastelle, B. Leluyer, J. Schmitz, R. Infante, and L. P. Aggerbeck. 1990. Description of two different patients with abetalipoproteinemia: synthesis of a normal-sized apolipoprotein B-48 in intestinal organ culture. *J. Lipid Res.* **31:** 1–15.
30. Glickman, R. M., J. N. Glickman, A. Magun, and M. Brin. 1991. Apolipoprotein synthesis in normal and abetalipoproteinemic intestinal mucosa. *Gastroenterology.* **101:** 749–755.
31. Black, D. D., R. V. Hay, N. P. Rohwer, H. Ellinas, J. K. Stephens, H. Sherman, B. B. Teng, P. F. Whitington, and N. O. Davidson. 1991. Intestinal and hepatic apolipoprotein B gene expression in abetalipoproteinemia. *Gastroenterology.* **101:** 520–528.
32. Nguyen, T. H., D. T. S. Law, and D. B. Williams. 1991. Binding protein BiP is required for translocation of secretory proteins into the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA.* **88:** 1565–1569.
33. Sanders, S. L., K. M. Whitfield, J. P. Vogel, M. D. Rose, and R. W. Schekman. 1992. Sec61p and BiP directly facilitate polypeptide translocation into the ER. *Cell.* **69:** 353–365.
34. Segrest, J. P., M. K. Jones, V. K. Mishra, G. M. Anantharamaiah, and G. W. Garber. 1994. ApoB-100 has a pentapartite structure composed of three amphipathic alpha-helical domains alternating with two amphipathic beta-strand domains. Detection by the computer program LOCATE. *Arterioscler. Thromb.* **14:** 1674–1685.
35. 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.
36. Young, S. G., S. J. Bertics, L. K. Curtiss, D. C. Casal, and J. L. Witztum. 1986. Monoclonal antibody MB19 detects genetic polymorphism in human apolipoprotein B. *Proc. Natl. Acad. Sci. USA.* **83:** 1101–1105.
37. Innerarity, T. L., S. G. Young, K. S. Poksay, R. W. Mahley, R. S., R. W. Milne, Y. L. Marcel, and K. H. Weisgraber. 1987. Structural relationship of human apolipoprotein B-48 to apolipoprotein B-100. *J. Clin. Invest.* **80:** 1794–1798.
38. Curtiss, L. K., and T. S. Edgington. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. I. Apolipoprotein B binding of mouse hybridoma antibodies. *J. Biol. Chem.* **257:** 15213–15221.
39. Young, S. G., J. L. Witztum, D. C. Casal, L. K. Curtiss, and S. Bernstein. 1986. Conservation of the low density lipoprotein receptor-binding domain of apoprotein B. Demonstration by a new monoclonal antibody, MB47. *Arteriosclerosis.* **6:** 178–88.