Translocation Efficiency, Susceptibility to Proteasomal Degradation, and Lipid Responsiveness of Apolipoprotein B Are Determined by the Presence of β Sheet Domains

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Apolipoprotein (apo) B100 is an atypical secretory protein in that its translocation across the endoplasmic reticulum membrane is inefficient, resulting in the partial translocation and exposure of apoB100 on the cytoplasmic surface of the endoplasmic reticulum. Cytosolic exposure leads to the association of nascent apoB with heat shock protein 70 and to its predisposition to ubiquitination and proteasomal degradation. The basis for the inefficient translocation of apoB100 remains unclear and controversial. To test the hypothesis that β sheet domains present in apoB100 contribute to its inefficient translocation, we created human apoB chimeric constructs apoB13,16 and apoB13,13,16, which contain amino-terminal α globular domains but no β sheet domains, and apoB13,16,β, which has an amphipathic β sheet domain of apoB100 inserted into apoB13,16. These constructs, along with carboxyl-terminal truncations of apoB100, apoB34 and apoB42, were used to transfect HepG2 and Chinese hamster ovary cells. In contrast to the lack of effect of proteinase K on apoB13,16 and apoB13,13,16, the levels of apoB34, apoB42, and apoB13,16,β were decreased by 70–85% after proteinase K-induced proteolysis in both HepG2 and Chinese hamster ovary cells. Either oleic acid or proteasomal inhibitors (N-acetyl-leucinyl-leucinyl-norleucinal and lactacystin) significantly increased the cell levels of apoB13,16,β, apoB34, apoB42, and full-length apoB100 but had no effect on the cell levels of apoB13,16 and apoB13,13,16. When HepG2 cells were incubated with a microsomal triglyceride transfer protein inhibitor, the cellular levels of apoB13,16,β, apoB34, and apoB42 were decreased by 70–80%, whereas the levels of apoB13,16 and apoB13,13,16 were unaffected. The effects of microsomal triglyceride transfer protein inhibition were reversed by lactacystin. Our results clearly demonstrate that the translocation efficiency, susceptibility to proteasomal degradation, and lipid responsiveness of apoB were determined by the presence of a lipid binding β sheet domain. It is possible that β sheet domains may at least transiently facilitate the interaction of apoB with the lipid bilayer surrounding the translocation channel.

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Apolipoprotein (apo) B100 is the major protein of atherogenic very low density and low density lipoproteins (1). There is a wide range of apoB100 secretion from the liver in humans, and overproduction of very low density lipoprotein and low density lipoprotein is a common feature of human dyslipidemia (2). ApoB100 is a constitutively synthesized protein (3), and the secretion of apoB is regulated at the posttranslational level, where the availability of newly synthesized core lipids seems to facilitate the translocation of the nascent polypeptide and targets it for secretion rather than degradation via the proteasomal pathway (3–8). The basis for the posttranslational regulation of apoB100 seems to derive from the fact that it is an atypical secretory protein whose translocation across the endoplasmic reticulum (ER) membrane is inefficient, resulting in only a partial translocation of a significant portion of nascent apoB100 and hence the exposure of apoB100 on the cytoplasmic surface of the ER (9–14). Cytosolic exposure of apoB100 leads to its association with heat shock protein 70 (7, 15) and a predisposition to ubiquitination and proteasomal degradation (7, 16). Indeed, we have recently shown that ubiquitination of apoB can occur cotranslationally (17).

The basis for the inefficient translocation of apoB100 remains unclear and controversial (18–21). ApoB100 contains 4536 amino acids and is predicted to be comprised of three amphipathic α helix domains and two amphipathic β sheet domains (22). One of the β sheet domains is located between amino acids at about 18 and 45% of full-length apoB100 and may participate in apoB100 translocation arrest by a hydrophobic interaction with the ER membrane (23–25). The β sheet domain also seems to be responsible for lipid binding and very low density lipoprotein assembly (26). To directly test the hypothesis that β sheet domains determine apoB100 translocation efficiency, we created human apoB chimeric constructs containing repeats of the first 13–16% of full-length apoB100. These constructs, apoB13,16 and apoB13,13,16, contain the amino-terminal α globular domains of apoB100, but no β sheet domains. These constructs were equal in length to the first 29 and 42% of apoB100, respectively. We also constructed apoB13,16,β in which the amphipathic β sheet domain situated between 28 and 34% of full-length apoB100 was inserted into apoB13,16 at either 9.5% of the construct or at 22.5% of the construct. The length of the apoB13,16,β constructs was equal to 35% of full-length apoB100. The efficiency of translocation, susceptibility to proteasomal degradation, responsiveness to oleic acid (OA) treatment, and sensitivity to the inhibition of...
microsomal triglyceride transfer protein (MTP) activity of these chimeric proteins were compared with the same properties for full-length apoB100 and for the carboxyl-terminal truncations apoB34 and apoB42 in HepG2 and Chinese hamster ovary (CHO) cells. Our results demonstrate that the presence of a β sheet domain near the amino-terminal of apoB100 is necessary and sufficient to cause the inefficient translocation of at least the first 42% of this atypical secretory protein.

**EXPERIMENTAL PROCEDURES**

**Reagents—**N-Acetyl-leucyl-norleucinal (ALLN), OA, Triton X-100 and protein A-Sepharose CL 4B were purchased from Sigma Chemical Co. ALLN was used at a concentration of 100 μM. OA was used at a concentration of 0.4 mM. Triton X-100 was used at 0.5%, and protein A-Sepharose CL 4B was used at 0.2%. Synthetic lactacystin and rabbit anti-human apoB polyclonal antibody were purchased from Calbiochem-Novabiochem International. Lactacystin was used at a concentration of 10 μM. Sheep anti-human apoB polyclonal antibody was purchased from Boehringer Mannheim. Transfectam was purchased from Promega; LipofectAMINE was purchased from Life Technologies, Inc. MTP inhibitor CP-10447 was provided by Pfizer Inc. 

**Growth of Cells—**HepG2 cells and CHO cells obtained from the American Type Culture Collection were grown as described previously (5). Briefly, cells were maintained at 37 °C, 5% CO₂ in 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum. The medium was changed every 3 days, and experiments were started after the cells reached 70–90% confluence. During the experiments, cells were maintained at 37 °C, 5% CO₂ in serum-free minimum Eagle’s medium containing 1.5% bovine serum albumin with the indicated additions or treatments.

In transfection experiments, HepG2 or CHO cells were treated with Transfectam or LipofectAMINE with or without apoB cDNAs. After 36 h, cells were labeled with [1-4]5-H)leucine for 2 h in the presence or absence of the indicated additions or treatments. Cell lysates and conditioned medium were analyzed by immunoprecipitation with either rabbit anti-human apoB polyclonal antibodies or sheep anti-human apoB polyclonal antibodies. Both antibodies gave similar immunoprecipitation results.

**Construction of ApoB34 and ApoB13 Constructs—**ApoB34 and apoB42 are carboxyl-terminal truncations of apoB100 that have no β sheet domains. The two chimeric constructs are equal in length to the first 29% and the first 42% of apoB100, respectively. ApoB13,16,β is a construct in which the amphipathic β sheet domain situated between 28 and 34% of full-length apoB100 was inserted into apoB13,16 at either 9.5% of the construct or 22.5% of the construct. The length of the apoB13,16,β construct was equal to 35% of full-length apoB100. apoB34 and apoB42, carboxyl-terminal truncations of apoB100, contain the amino-terminal α globular and β sheet domain of apoB100.

**Immunoprecipitation**—Immunoprecipitation of apoB in medium and

**Fig. 1. Comparison of apoB chimeric constructs and apoB carboxyl-terminal truncations with apoB100.** The figure depicts the structure of apoB100 as described by Segrest et al. (22). Human apoB chimeric constructs apoB13,16, apoB13,13,16, and apoB13,16,β, and a carboxyl-terminal truncation of apoB100, apoB34, were constructed as described in Experimental Procedures. ApoB13,16 and apoB13,13,16 contain repeated amino-terminal α globular domains of the amino-terminal region of apoB100 but no β sheet domains. The two chimeric constructs are equal in length to the first 29% and the first 42% of apoB100, respectively. ApoB13,16,β is a construct in which the amphipathic β sheet domain situated between 28 and 34% of full-length apoB100 was inserted into apoB13,16 at either 9.5% of the construct or 22.5% of the construct. The length of the apoB13,16,β construct was equal to 35% of full-length apoB100. apoB34 and apoB42, carboxyl-terminal truncations of apoB100, contain the amino-terminal α globular and β sheet domain of apoB100.
apoB13,16,β was significantly reduced by the PK digestion of microsomes isolated from HepG2 cells, but apoB13,16,β was not affected. HepG2 cells were transiently transfected with apoB13,16 cDNAs (left three lanes) or apoB13,16,β cDNA (right three lanes) as indicated at the bottom of the figure. Thirty-six h after transfection, the cells were labeled for 2 h with [3H]leucine. Microsomes were isolated as described under “Experimental Procedures.” Microsomes were incubated with or without PK (50 μg/ml) or Triton X-100 (0.5%) for 30 min on ice. After digestion, phenylmethylsulfonyl fluoride (3 mM) was added, and the samples were incubated for another 5 min on ice. The samples were then recentrifuged, and the microsome pellets were dissolved in lysis buffer and analyzed by immunoprecipitation as described under “Experimental Procedures.”

RESULTS

Translocation Efficiency of ApoB Across the ER Membrane Is Determined by the Presence of β Sheet Domains—In our initial experiments, we wished to determine whether the translocation of nascent apoB was affected by the presence or absence of β sheet domains. We isolated microsomes and used the sensitivity of nascent apoB to exogenous protease digestion as a measure of translocation (9–10, 12, 14). Under the conditions of this assay, apoB100 and apoB constructs that are fully protected from exogenously added PK should be those that have been completely translocated, whereas any apoB species that are sensitive to proteolysis should be in a partially translocated, bitopic orientation. Based on our previous studies (12), and those of others (9, 10, 13, 14), we expected that the majority of nascent apoB100 molecules would be partially translocated and susceptible to PK; endogenous apoB100 could therefore serve as a positive control against which to compare the sensitivity to proteolysis of other transfected apoB species in each experiment.

Thirty-six h after HepG2 cells were transiently transfected with apoB cDNAs, cells were labeled for 2 h with [3H]leucine. Microsomes were then isolated and subjected to PK digestion in vitro to provide an estimate of translocation efficiency. After PK digestion, immunoreactive bands of full-length apoB100 were quantified, and those of others (9, 10, 13, 14), we expected that the majority of apoB100 was significantly reduced by PK in all transfected constructs was further examined in CHO cells, which lack MTP activity. When CHO cells were co-transfected with apoB13,16 and apoB13,16,β or with apoB13,16 and apoB34, the β sheet domain-containing constructs, apoB13,16,β and apoB34, were reduced by 85% (0.15 ± 0.04 relative densitometric units; n = 3) and 79% (0.21 ± 0.03 relative densitometric units; n = 3), respectively, after the PK digestion of isolated microsomes, whereas apoB13,16 was unaffected (0.92 ± 0.23 relative densitometric units; n = 3) (Fig. 4). The same results were obtained with the apoB13,16,β construct in which the β sheet had been inserted in the second-third of apoB13,16. HepG2 cells were transfected with either apoB13,16 or apoB13,16,β, and 36 h later, they were labeled for 2 h with [3H]leucine. Microsomes were then isolated and subjected to PK digestion in vitro. In contrast to the lack of effect of PK on apoB13,16 (0.94 ± 0.25 relative densitometric units compared with undigested control; n = 4), apoB13,16,β was decreased by 75% (0.25 ± 0.09 relative densitometric units; n = 4) after PK digestion (Fig. 3). Again, apoB100 was significantly reduced by PK in all transfected cells, and all bands disappeared when Triton X-100 was present together with PK in the media.

Inefficient translocation of β sheet domain-containing constructs was further examined in CHO cells, which lack MTP activity. When CHO cells were co-transfected with apoB13,16 and apoB13,16,β or with apoB13,16 and apoB34, the β sheet domain-containing constructs, apoB13,16,β and apoB34, were reduced by 85% (0.15 ± 0.04 relative densitometric units; n = 3) and 79% (0.21 ± 0.03 relative densitometric units; n = 3), respectively, after the PK digestion of isolated microsomes, whereas apoB13,16 was unaffected (0.92 ± 0.23 relative densitometric units; n = 3) (Fig. 4). The same results were obtained with the apoB13,16,β construct in which the β sheet had been inserted in the second-third of apoB13,16 (data not shown; see “Experimental Procedures”).

Susceptibility to Proteasomal Degradation and Responsiveness to OA Treatment Are Determined by the Presence of β Sheet Domains in ApoB—The results described above indicated that the addition of a β sheet domain to an apoB construct otherwise containing only a globular domains (apoB13,16,β versus apoB13,16) was sufficient to cause translocation to become inefficient. If this is true, then the simple addition of a β sheet domain should convert apoB constructs with only a globular domains to ones that are dependent on the availability of core lipids and are sensitive to proteasomal degradation.

To directly assess the involvement of the β sheet domain in determining the responsiveness to lipid availability and sensitivity to proteasomal degradation, HepG2 cells were transiently transfected with apoB cDNAs. Thirty-six h after transfection, the cells were preincubated for 2 h with and without OA (0.4 mM) to stimulate lipid synthesis or with and without ALLN (100 μM) to inhibit proteasomal degradation. The cells were then labeled with [3H]leucine for 2 h. OA significantly increased both the cell and medium levels of full-length apoB100 and apoB42 but not apoB13,13,16 (Fig. 5). OA and ALLN both increased the cell levels of full-length apoB100 and apoB13,16,β but had no effect on the cell levels of apoB13,16.
FIG. 4. ApoB13,16,β and apoB34 were significantly reduced by the PK digestion of microsomes isolated from CHO cells, but apoB13,16 was not affected. CHO cells were transiently co-transfected with apoB13,16 and apoB13,16 cDNAs (left three lanes) or apoB13,16 and apoB34 cDNA (right three lanes) as indicated at the bottom of the figure. Thirty-six h after transfection, the cells were labeled for 2 h with [3H]leucine. Microsomes were isolated as described under “Experimental Procedures.” The digestion conditions for isolated microsomes were the same as those described in the Fig. 2 legend. Digested samples were analyzed by immunoprecipitation and densitometry as described under “Experimental Procedures.”

FIG. 5. ApoB42 was significantly increased by OA treatment in HepG2 cells, but apoB13,13,16 was not affected. HepG2 cells were transiently transfected with apoB42 or apoB13,13,16 cDNAs as indicated at the bottom of the figure. Thirty-six h after transfection, the cells were preincubated with or without OA (0.4 mM) for 2 h and labeled with [3H]leucine for 2 h in the absence or presence of OA. After labeling, cell lysates and medium were analyzed by immunoprecipitation as described under “Experimental Procedures.” The lowest band in the medium lanes is a nonspecifically immunoprecipitated protein. (Fig. 6). apoB100 and apoB13,16,β were increased approximately 3× by OA (apoB100, 2.82 ± 0.23 relative densitometric units compared with control; apoB13,16,β, 3.01 ± 0.65 relative densitometric units, n = 4) and 2× by ALLN (apoB100, 2.02 ± 0.40 relative densitometric units; apoB13,16,β, 2.13 ± 0.32 relative densitometric units, n = 4) (Fig. 6).

To further test the sensitivity of β sheet domain-containing species to proteasomal degradation, similar experiments were carried out in CHO cells using another proteasomal inhibitor, lactacystin. Thirty-six h after CHO cells were transiently transfected with apoB cDNAs, the cells were preincubated for 2 h and labeled with [3H]leucine for 2 h in the presence or absence of lactacystin (10 μM) to inhibit proteasomal degradation. Cell levels of apoB42 and apoB13,16,β were increased approximately 2.5× by lactacystin treatment (apoB42, 2.32 ± 0.32 relative densitometric units; apoB13,16,β, 2.53 ± 0.33 relative densitometric units, n = 3), whereas apoB13,16 and apoB13,13,16 levels were unchanged (Fig. 7).

These data suggest that the amphipathic β sheet domain plays a critical role in the response of apoB to OA and to either ALLN or lactacystin treatment. Furthermore, these results provide compelling evidence to support the hypothesis that β sheet domains determine translocation efficiency, lipid binding, and sensitivity to proteasomal degradation of apoB100.

The Sensitivity of ApoB to MTP Inhibition Is Determined by the Presence of β Sheet Domains—The role of MTP in the translocation of apoB100 and its assembly with lipids has been demonstrated in both hepatic cells and in nonhepatic cells expressing truncated apoB100 fragments (6, 30, 31). MTP and apoB100 form complexes that can be co-immunoprecipitated (29, 32), and this interaction requires only the amino-terminal 13% of apoB100 (32). Recent studies by Bakillah et al. (33) and Hussain et al. (34) have identified specific binding sites within the first 16% of apoB100. Despite the fact that the interaction of MTP with apoB100 seems to occur within the α globular region of apoB100, the presence of β sheet domains seems to make MTP essential for secretion. We therefore sought to determine whether the simple addition of a β sheet domain would make apoB constructs dependent on MTP.

HepG2 cells were transiently transfected with apoB cDNAs and labeled with [3H]leucine for 2 h in the presence or absence of MTP inhibitor. When HepG2 cells were incubated with 100 μM of the MTP inhibitor CP-10447, the cell levels of apoB42, apoB13,16,β, and full-length apoB100 decreased more than 80% (apoB100, 0.19 ± 0.07 relative densitometric units compared with control; apoB42, 0.18 ± 0.08 relative densitometric units, n = 4; apoB13,16,β, 0.17 ± 0.07 relative densitometric units, n = 5). Increased apoB degradation associated with MTP inhibition was significantly blocked by the co-incubation of cells with lactacystin (Fig. 8). By contrast, the levels of apoB13,16 and apoB13,13,16 were unaffected by the inhibition of MTP. Secretion of apoB species containing β sheet domains (i.e. full-length apoB100, apoB42, and apoB13,16,β) was also significantly decreased by the inhibition of MTP (data not shown). These results indicate that the presence of β sheet domains makes MTP activity essential for the efficient translocation of apoB across the ER membrane, and that the increased degradation of β sheet domain-containing apoB species that occurs when MTP is inhibited is mediated by the proteasomal degradation pathway.

DISCUSSION

Efficient co-translational insertion into the translocon and translocation across the ER membrane have been a well-accepted model for the initial processing of secretory proteins (35, 36). Evidence accumulated during the past 10 years, however, has indicated that the initial intracellular transport of
apoB100 does not fit this paradigm (5, 37). In particular, it appears that after normal insertion of the amino-terminal of nascent apoB into the translocon, further translocation is either relatively slow or actually stops, resulting in a breakdown of the typical segregation of secretory proteins from the cytosol. In the present studies, we have attempted to gain insight into the molecular basis for the inefficient translocation across the ER membrane of the secretory protein apoB100. The importance of this “aberrant” processing of apoB100 is that inefficient translocation is likely to be a major regulatory step that determines how many apoB100-containing lipoproteins are assembled and secreted by the liver (5, 37). Our present results strongly indicate that hydrophobic β sheet domains present in apoB100 play a crucial role in determining the translocation efficiency of this important lipid carrier molecule.

The results from PK digestion experiments in isolated microsomes indicated that the difference in sensitivity to PK-induced degradation between apoB42, apoB34, and apoB13,16,13,16, which contain β sheet domains, and apoB13,16 or apoB13,13,16, which have no β sheet domains, derives directly from the effects of the β sheet on translocation efficiency (Figs. 2–4). Thus, apoB13,16 and apoB13,13,16 were intraluminal and were not accessible to PK, whereas apoB34, apoB42, apoB13,16,β, and full-length apoB100 were incompletely translocated and were accessible to exogenously added protease in isolated microsome preparations. Our demonstration that the simple addition of a β sheet domain to apoB13,16 significantly reduced translocation efficiency clearly indicates that the β sheet domain between 28 and 34% of full-length apoB is directly involved in the translocation process.

Incomplete translocation of apoB100 across the ER membrane results in a bitopic orientation of apoB100 in which some portion of the amino-terminal of nascent apoB is present in the lumen of the ER (9–14,38). Although the availability of lipoprotein core lipids is regarded as the most crucial physiological factor governing the proportion of nascent apoB polypeptides that are secreted or degraded (3–7), the regulatory roles of molecular chaperones, such as heat shock protein 70 in the cytosol and MTP in the lumen of the ER have also been demonstrated (6,7,15). In a recent report by Chen et al. (39), calnexin was also shown to be involved in the translocation of apoB100. In the absence of adequate core lipid availability, apoB associates with heat shock protein 70 and undergoes rapid intracellular degradation by the ubiquitin-mediated proteasomal degradation pathway (7,15,16). Using the human apoB chimeric constructs (apoB13,16, apoB13,13,16, and apoB13,16,β) and the carboxyl-terminal truncations (apoB34 and apoB42), we have demonstrated that the amphipathic β sheet domain plays a critical role in the response of apoB to OA and either ALLN or lactacystin treatment (Figs. 5–7). We did not test whether the β sheet domains are important for the interaction of apoB100 with calnexin.

MTP is required for efficient apoB translocation (6, 29, 30), and MTP and apoB100 physically interact (29, 32). We previously showed that both posttranslational and co-translational ubiquitination and proteasomal degradation of apoB100 are significantly increased when apoB translocation is arrested by an inhibition of MTP (7,17). Our present studies addressed the question of whether MTP inhibition affects apoB β sheet domain-containing constructs and apoB constructs lacking β-sheet domains differently. Treatment of HepG2 cells with a MTP inhibitor significantly decreased the intracellular levels of newly synthesized apoB42, apoB34, and apoB13,16,β, whereas levels of apoB13,16 and apoB13,13,16 were unaffected (Fig. 8), indicating that the presence of β sheet domains makes MTP activity essential for the efficient translocation of apoB100 across the ER membrane. Our demonstration that lactacystin prevents the degradation of β sheet domain-containing constructs during MTP inhibition is consistent with prior data indicating that the degradation of inefficiently translocated apoB100 is mediated by the proteasomal degradation pathway. Although MTP is required for the translocation of β sheet domain-containing apoB species, recent unpublished data from our laboratory suggest that the binding of MTP to apoB100 is not dependent on the presence of β sheet domains, and that β sheet domains do not bind to MTP in the absence of the amino terminus of apoB.2 Our unpublished work is consistent with recent studies by Bakillah et al. (33) and Hussain et al. (34) demonstrating binding sites for MTP in the first 16% of apoB100.

Although the possibility that the putative topogenic “pause transfer sequences” (18,19) may lead to a transmembrane topology is not excluded by these studies, our results have demonstrated clearly that the translocation efficiency of apoB100 can be determined by the presence of hydrophobic β sheet domains. Indeed, because apoB13,16 and apoB13,13,16 contain numerous pause transfer sequences (40,41), it seems unlikely that those sequences play independent roles in the translocation of apoB100 in cultured cells. Our direct comparison of apoB13,16 and apoB13,16,β, which contain similar numbers of disulfide bonds and glycosylation sites, also indicates that the inefficient translocation of apoB100 is not linked exclusively to either amino-terminal disulfide bond formation or glycosylation. Support for the importance of β sheet domains for the assembly of triglyceride-enriched lipoproteins comes from the recent studies by Mcleod et al. (26), who found that the addition of a β sheet domain to apoA-I was associated with the secretion of the chimeric protein on a triglyceride-enriched lipoprotein. Of note, those authors found that the addition of the same β sheet domain to the carboxyl-terminal domain of apoA-I did not cause significant sensitivity of the chimeric protein to exogenous protease digestion of isolated microsomes. They concluded, therefore, that the initial translocation of apoB100 was not determined by β sheet domains. It is likely that because they placed the β sheet domain at the very carboxyl-terminal end of their chimeric construct, the translocation of their apoA-I/apoB chimera was nearly complete before the β sheet domain could interact with the translocation channel. In our constructs, the β sheet domain was positioned either in the first or second third of the chimeric protein; therefore, translocation arrest would have resulted in the exposure of a significant portion of apoB to the cytosol.

1. J. Liang, H. Jiang, L.-S. Huang, S. L. Sturley, and H. Ginsberg, unpublished data.
In conclusion, our results from experiments with both carboxyl-terminal truncations of full-length apoB100 and chimeric apoB constructs with or without a β sheet domain demonstrate clearly that the translocation efficiency, susceptibility to proteasomal degradation, responsiveness to OA treatment, and sensitivity to MTP inhibition of apoB are mediated by the presence of hydrophobic, lipid binding β sheet domains. We and others have recently demonstrated that apoB100 is associated with the translocon proteins, Sec61β (39) and Sec61α (39). It is possible that the β sheet domain interacts not only with Sec61 proteins, but also with TRAM (42). We speculate that such an interaction could result in the significant slowing or cessation of translocation and the exposure of more carboxyl-terminal regions of apoB100 to the cytosol. Studies to test this hypothesis are the focus of our current experiments.

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