

The ever-expanding role of degradation in the regulation of apolipoprotein B metabolism

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Abstract Apolipoprotein B (apoB) is the essential protein required for the assembly and secretion of chylomicrons from the small intestine and VLDLs from the liver. These lipoproteins, as well as their remnants and LDL, play key roles in the transport of dietary and endogenously synthesized lipids throughout the body. However, they can be involved in the initiation of atherosclerotic lesions in the vessel wall. Therefore, it is not surprising that the assembly of apoB-containing lipoproteins in the small intestine and liver is a highly regulated process. In particular, cotranslational and posttranslational targeting of apoB for degradation, regulated largely by the availability of the core lipids carried in the lipoprotein, by the types of dietary fatty acids consumed, and by the hormonal milieu, determines the number of chylomicrons or VLDL that are secreted. In this review, we summarize both older and more recent findings on the pathways of apoB degradation, focusing on events in the liver.—Ginsberg, H. N., and E. A. Fisher. The ever-expanding role of degradation in the regulation of apolipoprotein B metabolism. *J. Lipid Res.* 2009. S162–S166.

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Hydrophobic neutral lipids, such as cholesterol esters and triglycerides (TGs), must be transported through the plasma as core components of macromolecular spherical complexes called lipoproteins, which have amphipathic phospholipids and hydrophilic free cholesterol on their surfaces. Lipoproteins can be characterized by their densities, by their core lipid composition, and, importantly, by the major apolipoproteins present on their surfaces. Apolipoprotein B (apoB100), a complex protein of 4,536 amino acids and a molecular mass of ~550 kDa that is synthesized in the liver, is the essential apolipoprotein present in VLDLs, intermediate density lipoproteins, and LDLs. In humans, the intestine synthesizes a truncated form of apo-

lipoprotein B (apoB48), which is the essential apolipoprotein on chylomicrons, the lipoproteins that transport dietary lipids. In rodents, apoB48 is made both in the liver and the intestine.

The role of both hepatic-derived VLDL and intestinal-derived chylomicrons is to deliver energy, in the form of TG, to peripheral tissues, particularly adipose tissue and skeletal muscle. This redistribution of energy also protects the liver from excessive accumulation of TG and/or precursors of TG that may be involved in lipotoxicity. Delivery of energy, in the form of free FAs, results from interaction of those lipoproteins with lipoprotein lipase. After uptake of FA by adipose and muscle cells, intermediate density lipoproteins (also called VLDL remnants) and chylomicron remnants return to the liver where they are taken up in part (VLDL remnants) or completely (chylomicron remnants). Remnant uptake delivers the remaining TG and probably most of the cholesterol originally contained in those lipoproteins to the liver; the delivery of cholesterol to the liver by these pathways plays a key role in regulating hepatic cholesterol metabolism. A variable but significant proportion of VLDL remnants undergoes further loss of TG in the hepatic circulation and is converted to LDL. HL plays an important role in the latter process. The removal of VLDL remnants and LDL from the circulation occurs mainly via their interaction with the LDL receptor, which recognizes apoB100.

In addition to their critical roles in lipid transport and metabolic regulation, all of the apoB-containing lipoproteins, with the exception of nascent chylomicrons and possibly very large, nascent VLDLs, can permeate through the endothelial layer of arteries and initiate atherogenesis. LDLs are particularly atherogenic because they are com-

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Abbreviations: apoB, apolipoprotein B; ER, endoplasmic reticulum; ERAD, endoplasmic-reticulum-associated degradation; MTP, microsomal triglyceride transfer protein; OA, oleic acid; PERPP, post-ER, presecretory proteolysis; TG, triglyceride; UPR, unfolded protein response.

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posed mainly of cholesterol, and their relatively small size facilitates plaque entry. On the other hand, chylomicron and VLDL remnants, which are much larger than LDLs, actually carry more cholesterol esters per particle even though that lipid makes up a smaller proportion of the core. After entry into plaques, a fraction of the particles are retained, modified, and subsequently taken up by endothelial cells, macrophages, and smooth muscle cells. This activates or provides substrates to a number of pathways that trigger plaque initiation and growth (1).

Given the critical role that apoB plays in lipoprotein transport, it is not surprising that regulation of the secretion of apoB-containing lipoproteins is complex, occurring at several stages in the assembly of VLDL and at several intracellular sites. Availability of the lipid components of VLDL and hormonal factors play critical roles in determining the fate of apoB in hepatocytes and intestinal cells. Indeed, early studies in both human HepG2 cells and rat primary hepatocytes indicated that a significant proportion of newly synthesized apoB was not secreted, but degraded intracellularly (2, 3). Since then, multiple degradative pathways for apoB have been discovered, and each pathway appears to be regulated by distinct metabolic factors and pathways (4). Moreover, dysregulation of some of these pathways may contribute to the overproduction of VLDL that is characteristic of insulin-resistant states, such as metabolic syndrome and type-2 diabetes mellitus (5). The resulting VLDL overproduction contributes to the increased risk of coronary artery disease in these populations. In this article, we will review the published studies that have described the various pathways for apoB degradation, focusing on the metabolic circumstances that contribute, at each critical pathway, to the targeting of apoB for either secretion or degradation.

ENDOPLASMIC-RETICULUM-ASSOCIATED DEGRADATION OF apoB

Cotranslational endoplasmic-reticulum-associated degradation of ApoB

Like all secreted proteins, apoB is synthesized at the surface of the endoplasmic reticulum (ER), after targeting of the N terminus of the protein and its associated ribosome to that organelle via the signal sequence, which spans amino acids 1 to 27 in apoB. Once the signal sequence has contacted the ER membrane, translocation of apoB occurs through the translocon, a proteinaceous channel in the ER membrane that is formed primarily by the Sec61 protein, which binds with high affinity to ribosomes (6). Polymorphisms in the signal sequence of apoB lead to alterations in plasma lipids, a phenomenon that most likely arises from compromised import or “translocation” into the ER (7).

Entry of the N terminus of most secretory proteins into the translocon causes reinitiation of translation, which is coordinated with translocation until the full-length protein enters the ER lumen for further processing and transport. During cotranslational translocation, it is thought that a

high affinity “seal” is formed between the ribosome and the cytosol, prohibiting exposure of the secretory protein to the cytosol. A consequence of this typical translocation process is that when (for whatever reason) a secretory protein is degraded, this degradation occurs after translation and translocation are completed. However, studies by several laboratories support a very different itinerary for apoB in hepatocytes, most likely because of the complex structure of this protein. As will be discussed below in more detail, this atypical itinerary derives from 1) a stronger than usual interaction of apoB with translocon proteins, 2) the requirement for microsomal TG transfer protein (MTP)-mediated cotranslational lipidation of the N terminus of apoB, and 3) the interaction between sequences of nascent apoB that are exposed to the cytosol and chaperone proteins. These three unusual aspects of the itinerary of apoB predispose it to cotranslational targeting for degradation.

First, the unusually strong interaction between apoB and proteins of the translocon, which is the result of the presence of very hydrophobic β -sheet sequences in several regions of apoB, can lead to slowing of translocation while translation continues. The latter may result in the “backing up” of apoB in the translocon that leads to the “looping out” of sequences of apoB into the cytosol (8) through gaps that exist between the ribosome and the ER membrane (9). ApoB also has several “pause-transfer” sequences (10) that may contribute to an atypical itinerary of the nascent protein in the translocon, although recent evidence indicates the predominant role of the β -sheet sequences in this process (11).

Second, the requirement for lipidation of the N-terminal sequences of apoB by an ER chaperone, MTP, can lead to further slowing of apoB translocation. MTP is a membrane-associated dimer comprised of protein disulfide isomerase and a 97 kDa polypeptide (the “M” subunit) that catalyzes the transfer of phospholipids, cholesterol esters, and TGs from the ER membrane to apoB [reviewed in (12)]. The transfer of lipids onto apoB by MTP facilitates translocation of the protein (13, 14), although recent evidence suggests that MTP is required for the import of the N-terminal 20% of apoB into the ER lumen (15). In any event, inhibition of MTP activity is associated with exposure of apoB to the cytosol, despite some concomitant slowing of translation. The requirement of MTP for the efficient translocation of apoB is perhaps most dramatically demonstrated by the human disease, abetalipoproteinemia, in which the absence of MTP activity results in the absence of plasma apoB lipoproteins (16).

The third atypical aspect of the translocation of apoB across the ER is related to the interaction of apoB with the cytosolic chaperones, especially when core lipid availability is low. For example, in the lipid-ligand-deficient state, which occurs if MTP activity is low (17), or when lipid availability or synthesis is reduced (18), apoB is cotranslationally targeted for ER-associated degradation (ERAD). As observed for other substrates of ERAD (19), the cytoplasmic Hsp70 and Hsp90 molecular chaperones are critical for this process, and both interact with apoB when its translocation is interrupted or inefficient and sequences of

apoB become exposed to the cytosol. By contrast, the Hsp110 chaperone protects apoB from degradation (20). The ER luminal chaperone BiP may also facilitate apoB degradation (21), as does a BiP-interacting co-chaperone, known as p58 (22).

After chaperone-dependent targeting, apoB is ubiquitinated, and the E3 ubiquitin ligase that accomplishes this is gp78 (23). Ubiquitination of apoB can occur after ~50% of the protein has been translated (20). The ubiquitinated apoB becomes a target for the proteasome, a large (~2.5 MDa) multicatalytic machine that destroys ERAD substrates and select cytoplasmic proteins (24). The targeting includes the retrograde translocation of apoB out of the same translocon within which it was inserted during signal sequence targeting. Proteasomes located at the cytosolic surface of the ER can then “ingest” ubiquitinated apoB as it emerges, in a retrograde manner, from the translocon. Of note, the N-terminal 70 kDa sequence of apoB appears, at least in part, to undergo proteolytic processing and escape degradation by the proteasome (25). Indeed, this N-terminal peptide of apoB has been isolated from plasma of individuals with abetalipoproteinemia (26).

Posttranslational ERAD of apoB

Although apoB can be targeted for degradation cotranslationally via retrotranslocation, probably because of its persistent association with sec61 (14), most secretory or ER-associated proteins are targeted for proteasomal degradation after completion of translation and translocation. The molecular mechanisms involved in the retrograde translocation of a fully translated protein that is in the lumen of the ER have been studied extensively (27) but are not fully characterized. There is also evidence that ERAD can occur within the ER itself. For apoB, depending on the experimental model, 50% or more of apoB degradation occurs after translocation, and this is mainly nonproteasomal degradation. ER60 (28), or other ER proteases (4), may be involved at this stage. Some studies suggest that full-length apoB may be degraded by the proteasome after retrograde translocation (29, 30). The role of ER chaperones in apoB-Lp formation has been well documented (28), but recent work suggests that high levels of Grp78 (BiP) can also target apoB for degradation (21).

ER-stress-associated ERAD

The accumulation of mutant or misfolded proteins in the ER, or simply cellular stress, can induce ERAD. In such cases, the ER must respond to the presence of misfolded secretory proteins before they aggregate and cause irreparable damage. The response is to degrade them by increasing ERAD (19). If the concentration of misfolded proteins rises in the ER, such that the ERAD pathway cannot keep up, the unfolded protein response (UPR) is triggered (31) leading to increased synthesis of ER chaperones, augmented ERAD efficiency, and induction of other degradation pathways, including autophagy. A strong UPR has been shown to target apoB for degradation in various cell

models, most likely because of the increased concentration of apoB-interacting chaperones (21).

FA-induced ER stress and ERAD of apoB

Recently, we demonstrated another way in which ER stress leading to apoB-ERAD could be induced; for example, both in vitro and in vivo, delivery of FA to hepatocytes induced a modest UPR that targeted apoB for degradation and decreased its secretion (32). This finding was unexpected since oleic acid (OA), as well as other FAs, typically stimulates the secretion of TG, and often apoB, from cultured liver cells when provided at relatively low concentrations, such as 0.4 mM, for short periods of time (2–4 h) (33). We and others have also demonstrated that increased delivery of FAs to the liver can stimulate the secretion of apoB in vivo (34). However, when higher doses of OA are provided to cultured liver cells for long periods of time, there is a dose- and duration-related induction of ER stress, which is accompanied by not only loss of stimulation, but actual inhibition of the secretion of apoB (32). This inhibition of apoB secretion results from increased degradation of nascent protein; our data support both cotranslational degradation by the proteasome and posttranslational non-proteasomal degradation in this process. Additionally, as in the cultured cells, the duration of increased FA supply is an important determinant of apoB secretion in vivo: whereas 6 h infusions of OA stimulate the secretion of apoB, 9 h infusions, which induce greater ER stress, are associated with loss of the stimulation seen at 6 h (32). Finally, the inhibitory effects of OA, both in cultured cells and in mice, are reversed by phenylbutyric acid, which concomitantly inhibits ER stress. Ongoing studies are focused at better characterization of the pathways that are active in the degradation of apoB during ER stress. Additionally, we are conducting studies with palmitic acid and docosahexanoic acid; the former is well known as a cause of ER stress, while we have shown that the latter inhibits apoB secretion via generation of lipid peroxidation products and induces a post-ER degradation pathway for apoB (see below) (35).

POST-ER, PRESECRETORY PROTEOLYSIS OF apoB

In addition to ERAD, there are other mechanisms whereby secretion of apoB is regulated by degradation. An example is a nonproteasomal, post-ER form of apoB degradation dubbed post-ER, presecretory proteolysis (PERPP) (36). Of note, PERPP occurs when TG availability and incorporation into VLDL is normal; this is in contrast with ERAD, which plays a major role in the degradation of apoB that occurs when lipids are limiting. Factors that stimulate PERPP include PUFA, particularly n-3 PUFA, and insulin.

N-3 FA-induced PERPP of apoB

We have identified dietary n-3 PUFA, eicosapentaenoic acid, and docosahexaenoic acid as inducers of PERPP. Thus, incubation of hepatic cells with n-3 PUFAs leads to oxidative modification and aggregation of apoB after VLDL is formed (35). Infusion of n-3 PUFA into mice also

inhibits apoB secretion (35). N-3 PUFA induction of PERPP most likely occurs in the Golgi, where the VLDL apoB undergoes aggregation after exposure to n-3 lipid peroxides. ERAD cannot process protein aggregates in the Golgi, so it was not clear how these apoB-containing particles were destroyed. We recently determined that the apoB aggregates were targeted for autophagy (37). Although autophagy was originally thought to operate only during nutrient starvation, we now know that autophagy mediates the degradation of damaged organelle fragments, cytosolic proteins, and protein aggregates. Autophagosomes, double membrane vesicles, deliver these cargo for degradation in the lysosome (38). The molecular determinants that select apoB and other substrates are poorly understood. Of interest are recent data indicating a link between ERAD and autophagy; UPR induction increases the efficiency of both pathways (39). Thus, ERAD may prevent the catastrophic accumulation of aggregates in or beyond the Golgi, but if this pathway is overcome, then protein aggregates can be handled by autophagy.

Insulin-induced PERPP of apoB

Acute increases in insulin *in vivo* or in cell culture (to simulate the postprandial setting) decrease hepatic VLDL and apoB secretion via a post-ER, nonproteasomal process (40). The effects of insulin on apoB secretion require PI3-kinase activity (41); mitogen-activated protein kinase and tumor necrosis factor- α may also be involved (42). However, it is unclear if those findings are relevant to humans where systemic hyperinsulinemia is typically associated with increased production of VLDL. A likely explanation for the discrepancy between experiments in cultured liver cells demonstrating that insulin targets apoB for degradation and the human, *in vivo*, data showing that systemic hyperinsulinemia is associated with increased VLDL production is that hepatic insulin resistance is present in the pathway linking insulin signaling to apoB degradation in the human situation. This hypothesis is supported by studies in insulin-resistant animal models [e.g., rats (43) and hamsters (44); also reviewed in (45)] in which overproduction of VLDL is associated with decreased apoB degradation in livers or primary hepatocytes. Our recent demonstration of increased apoB secretion in mice lacking any hepatic insulin receptors (liver insulin receptor knock-out mice) (46), a model of pure and complete hepatic insulin resistance, is powerful evidence in support of both a critical role of insulin in the targeting of apoB for degradation and the role of hepatic insulin resistance in limiting this pathway. Although a role for autophagy in insulin-mediated PERPP has not been clearly determined, recent data suggest that insulin shunts apoB-containing lipoproteins from a post-ER compartment to autophagosomes (47).

CONCLUDING REMARKS

The assembly and secretion of VLDL lay the foundation for the levels of circulating atherogenic, apoB-containing lipoproteins. The metabolic role of VLDL is to carry excess

energy, in the form of FA esterified as TG, from the liver to the periphery. Therefore, it is not surprising that a complex system of regulation has evolved to control the assembly and secretion of apoB-containing lipoproteins from both the intestine and the liver. Regulation appears to mainly derive from the availability of the major core lipid, TG, by the types of dietary fatty acids entering the system and by hormones, such as insulin, that signal the status of whole-body energy balance. Of note, the major regulatory pathways involve degradation of apoB or VLDL; apoB itself is constitutively synthesized. In this review, we have described several pathways for degradation of apoB that have been characterized to varying extents; it is clear that the path for apoB from translation to secretion is characterized by several forks in the road, quality checkpoints, and many “dead ends.” We are certain that further studies of these pathways will reveal not only more detail about each but uncover additional regulatory steps in VLDL secretion. A continued definition of the molecular mechanisms of the pathways of degradation for apoB should provide insights that lead to the identification of novel therapeutic targets to lower the production of atherogenic lipoproteins. **■**

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