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The many intersecting pathways underlying apolipoprotein B secretion and degradation

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Abstract

Because the levels of secreted apolipoprotein B (apoB) directly correlate with circulating serum cholesterol levels, there is a pressing need to define how the biosynthesis of this protein is regulated. Most commonly, the concentration of a secreted, circulating protein corresponds to transcriptionally and/or translationally regulated events. By contrast, circulating apoB levels are controlled by degradative pathways in the cell that select the protein for disposal. This article summarizes recent findings on two apoB disposal pathways, endoplasmic reticulum (ER)-associated degradation and autophagy, and describes a role for post-ER degradation in the increased circulating lipid levels in insulin-resistant diabetics.

Introduction

All atherogenic lipoproteins that transport cholesterol and other lipids to peripheral tissues contain apolipoprotein B (apoB). Cholesterol is required for mammalian cell survival because it is a vital plasma membrane component and is also the precursor of bile acids and steroid hormones. However, excess cholesterol can accumulate both intracellularly and extracellularly, most notably in coronary arteries, which contributes to the growth and pathophysiology of atherosclerotic plaques.

ApoB-containing lipoproteins fall into select classes based on their densities and lipid and protein compositions. These classes – in order of increasing density – include chylomicrons, very low density lipoproteins (VLDLs), and low density lipoproteins (LDLs). Intestinal cells produce chylomicrons, which transport dietary-derived lipids. The liver produces VLDLs, which transport endogenously synthesized lipids. After the secretion of either chylomicrons or VLDLs, the delivered triacylglycerols are hydrolyzed by endothelium-bound lipoprotein lipase, and freed fatty acids enter cells to provide energy (e.g. in muscle) or to be re-esterified and stored (e.g. in adipose tissue). The triacylglycerol-depleted particles are termed remnants and are cholesterol-enriched. Remnants can be taken up by cells (including those in the atherosclerotic plaque) and VLDL remnants can be converted to LDLs [1]. LDLs are particularly atherogenic because they are highly enriched in cholesterol, and their relatively small size facilitates plaque entry. After entry into plaques, a fraction of the particles are retained and modified and subsequently taken up by endothelial cells, macrophages, and smooth muscle cells. This activates or provides substrates to several pathways that trigger plaque initiation and growth [2].

ApoB is synthesized in two isoforms. In the mammalian intestine, apoB48 is the major isoform. In human liver, apoB100 predominates, but in rats and mice both isoforms are synthesized in the liver. ApoB100 is an ~550 kD single chain polypeptide, and apoB48 represents an apoB100 fragment encompassing the N-terminal 48% of the polypeptide that results from the selective editing of the apoB-encoding mRNA [3,4]. In the rat or mouse liver, both apoB48 and apoB100 can assemble into VLDLs, although a greater percentage of apoB100 is found in this lipoprotein. By contrast, most apoB48 is found in denser, less lipidated particles [5].

Given its vital role in forming and trafficking lipoproteins, apoB levels are highly regulated. However, unlike most secreted proteins, apoB levels are primarily regulated via degradation, a phenomenon first observed in both human HepG2 cells and in rat primary hepatocytes [6,7]. Multiple degradative pathways for apoB have been discovered, and each pathway appears to be regulated by distinct metabolic factors [8]. Moreover, dysregulation of some of these pathways may contribute to the overproduction of VLDLs in insulin-resistant states, such as metabolic syndrome and type 2 diabetes [9]. The resulting VLDL overproduction may contribute to the increased risk of coronary artery disease in these populations.

Early events during apoB biogenesis: a lipoprotein runs the gauntlet

Protein translocation into the endoplasmic reticulum

Like all secreted proteins or protein particles, apoB-containing VLDLs and chylomicrons are synthesized at the surface of the endoplasmic reticulum (ER). Proteins are targeted to the ER membrane via the signal sequence, which spans amino acids 1–27 in apoB. Many polymorphisms in this region of apoB lead to alterations in plasma lipids, a phenomenon that most likely arises from compromised import or ‘translocation’ into the ER [10].

The translocation of apoB, like other secreted proteins, occurs through a proteinaceous channel in the ER membrane, known as the translocon. The translocon is formed primarily by the Sec61 protein, and binds with high affinity to ribosomes [11,12] (Figure 1). The role of the translocon in catalyzing apoB import into the ER has been established by cross-linking studies [13,14].

In theory, proteins can be translocated into the ER cotranslationally (during synthesis, as shown in Figure 1a–b), or posttranslationally (after synthesis and release into the cytosol). It is also possible that the high-affinity seal between the ribosome and Sec61 may be temporarily loosened such that a gap forms. The existence of a gap might permit the deposition of newly synthesized polypeptide loops into the cytosol. In addition, the ribosome may not continuously synthesize proteins once it is engaged at the ER but may ‘sputter’ during polypeptide synthesis and translocation, which is known as pause-transfer. In fact, both events occur during apoB synthesis. The deposition of cytosolic loops [15] is essential for the regulated degradation of the protein (Figure 1b), and pause-transfer sequences within apoB [16] may permit time for ER luminal segments of apoB to acquire lipids. However, recent data suggest that mutations in the pause-transfer sequences might introduce secondary structural changes [17].

The N-terminal assembly of lipids onto apoB during translocation requires the microsomal triglyceride transfer protein (MTP). MTP resides within the ER lumen and is a membrane-associated dimer between protein disulfide isomerase (PDI) and a 97 kd polypeptide (the ‘M’ subunit) that catalyzes the transfer of phospholipids, cholesterol esters, and triglycerides to apoB (reviewed in [18,19]). Individuals lacking MTP activity have abetalipoproteinemia [20], which is characterized by an absence of some circulating lipoproteins, but more modest pharmacological inhibition of MTP results in lower circulating LDL levels in individuals

with familial hypercholesterolemia [21]. At first, the transfer of macromolecules onto apoB was thought to facilitate translocation, but more recent evidence suggests that MTP does not directly catalyze apoB import [22].

In addition to MTP, apoB interacts with an ensemble of molecular chaperones during and after translocation. These chaperones include PDI, BiP (an ER luminal Hsp70 chaperone), Grp94 (an Hsp90 chaperone), calnexin and calreticulin (chaperone-like lectins), and cyclophilin (an enzyme with peptidyl prolyl *cis-trans* isomerase activity) [14,23–27]. Although molecular chaperones are thought to maintain polypeptide solubility and augment folding, most chaperones exhibit diverse activities and can target substrates for posttranslational modification or degradation, or can help insert polypeptides into multi-protein complexes. To date, it has been difficult to discern the roles played by the many chaperones with which apoB associates. In addition, because ER chaperones exist in multiprotein complexes [28], it is possible that some of these factors associate indirectly with apoB.

ER-associated degradation

Protein synthesis and folding are inherently error-prone. Because of the acquisition of mutations, because of cellular stress conditions, or because some proteins fold with low efficiency, the ER must respond to the presence of misfolded secretory proteins. If a misfolded protein is ignored, it may aggregate and cause irreparable damage. To prevent the formation of cytotoxic aggregates, misfolded proteins can be targeted for ER-associated degradation (ERAD) [29]. During ERAD, chaperones select aberrant polypeptide substrates and facilitate the acquisition of a poly-ubiquitin ‘tag’ that permits interaction with the proteasome [30], a large (~2.5 MD) multicatalytic machine that destroys ERAD substrates and select cytoplasmic proteins. If the concentration of misfolded proteins rises in the ER such that the ERAD pathway cannot keep up, the unfolded protein response (UPR) is initiated [31,32]. The UPR is induced by transmembrane UPR sensors in the ER that trigger the increased synthesis of ER chaperones, augment ERAD efficiency, and enhance other degradative pathways, including autophagy (see below). A strong, nonspecific induction of the UPR reduces apoB secretion in various cell models, most likely because of the increased concentration of apoB-interacting chaperones and heightened level of ERAD [27,33]. By contrast, more modest UPR induction via oleic acid supplementation increases apoB secretion. Only after prolonged time points and higher levels of oleic acid treatment (and UPR induction) is an inhibitory effect on apoB secretion evident [34].

ERAD and apoB

The ERAD pathway is not only an ER quality control process but is also used to regulate metabolic processes. For example, in the lipid-ligand deficient state, which occurs if MTP activity is low [35], or when lipid availability or synthesis is reduced [36,37], apoB is cotranslationally targeted for ubiquitinylation and degradation by the proteasome (Figure 1b). As observed for some ERAD substrates [29], the cytosolic Hsp70 and Hsp90 molecular chaperones are critical for this process; by contrast, the Hsp110 chaperone protects apoB from degradation [37–40]. The ER luminal chaperone, BiP, also facilitates apoB degradation [27], as does a BiP-interacting co-chaperone, known as p58 [41,42]. Although apoB has hydrophobic interactions with the ER membrane, its removal to the cytosol is most likely via retrotranslocation based on its persistent association with Sec61 [13,41].

After chaperone-dependent selection, apoB is ubiquitinated, and the E3 ubiquitin ligase that accomplishes this is gp78 [35,37,38,43–45]. Nevertheless, it is likely that additional, as-yet undiscovered factors catalyze apoB degradation, especially given the size and

hydrophobic content of the lipoprotein and given the fact that most ERAD substrates are degraded posttranslationally and not cotranslationally.

Post-ER control of apoB secretion: are we there yet?

Lipid loading and ER exit

When sufficient triacylglycerols, phospholipids, and cholesterol are loaded onto apoB in the ER, the primordial lipoprotein particle becomes competent for ER exit and transport to the Golgi apparatus (reviewed in [46]). Secreted proteins are shuttled between organelles in vesicles, and the biogenesis of these vesicles requires a specialized machine that recognizes and integrates secretory proteins into the coatomer. Coatomers are necessary and sufficient for membrane deformation and vesicle budding, and the coatomer that mediates ER exit is known as COPII [47]. Because the size of COPII vesicles is ~50 nm, and the apoB-containing particles that leave the ER may be larger [48], the coatomer must expand [49]. To date, it remains unclear how this occurs and whether apoB requires a unique factor that directs coat expansion.

One protein that triggers the formation and liberation of COPII vesicles from the ER is a GTPase known as Sar1. Mutations in the gene encoding one Sar1 isoform cause chylomicron retention disease, which leads to chylomicron deposition in enterocytes [50]. Consistent with a role for Sar1 in chylomicron biogenesis, mutant forms of Sar1 block the ER exit of apoB in rat hepatoma cells [51]. A role for Sar1 in mediating the fusion of chylomicron-containing vesicles into the Golgi apparatus in intestinal cells has also been suggested [52].

Interestingly, we found that much of the ER-derived apoB lipoproteins are unexpectedly dense in liver cells [51]. Therefore, it was hypothesized by us [53] and by others [25,54–58] that additional lipids are added after ER exit and that this event is essential to achieve VLDL density. In this model, the additional lipids would either have to be transported with a nascent particle from the ER or from the cytosol, or synthesized in the Golgi and then transferred directly to the pre-VLDL particle (for more discussion of these possibilities and data to support them, see [55,58,59]). Nevertheless, there are other data to support an alternate model in which the ER is the major site for final VLDL lipidation [60–62]. The most likely scenario is that there are specific processes in both the ER and Golgi that are required for maximal formation of fully lipidated VLDL.

ApoB can be targeted for autophagy

ERAD is not the only mechanism used to regulate apoB levels (Figure 1a). For example, dietary polyunsaturated fatty acids (PUFAs), particularly the n-3 fatty acids enriched in fish oils, lower VLDL levels. The major PUFAs in these oils (eicosapentaenoic acid and docosahexaenoic acid) stimulate a nonproteasomal, post-ER form of apoB degradation dubbed PERPP (post-ER, presecretory proteolysis) [63]. In contrast to ERAD, which destroys apoB when lipids are limiting, PERPP occurs when triglyceride formation is normal. Of note, the PUFA-stimulated PERPP of apoB appears to be selective for apoB that is incorporated into VLDL particles. PERPP also requires lipid peroxidation and increased oxidant tone, and was observed both *in vitro* and in mice receiving DHA infusions [64]. Incubation of hepatic cells with PUFAs leads to oxidative modification and aggregation of apoB. Because VLDL can acquire additional lipids and expands in the Golgi (see above), it is likely that the VLDL particles formed there in cells exposed to PUFAs contain lipid peroxides, and the resulting contact between damaged lipids and apoB leads to apoB aggregation.

Because the ERAD machinery cannot access protein aggregates in the Golgi, it was not clear how these apoB-containing particles were destroyed. However, it was discovered that the turnover of the apoB aggregates was altered when the autophagic pathway was manipulated [65]. Originally, autophagy was thought to operate only during nutrient starvation to reclaim essential amino acids from cytosolic proteins. It is now recognized that autophagy mediates the degradation of damaged organelle fragments, cytosolic proteins, and protein aggregates, each of which can be engulfed by double-membrane vesicles (autophagosomes) that deliver these cargo for degradation in the lysosome [66,67]. Although many genes are known to be required for autophagosome formation, the molecular determinants that select apoB and other substrates for autophagy are poorly understood [68].

Defects in proteasome function or in ERAD can lead to the aggregation of misfolded proteins. Consistent with this view, proteasome inhibition increases autophagosome formation and apoB levels in the lysosome in a human liver cell line [69]. Further evidence supporting a link between ERAD and autophagy derives from the finding that UPR induction increases the efficiency of both pathways [70–72]. 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. Recent evidence also suggests that the formation of aggregates in the liver is regulated by p62, a protein whose levels are regulated by autophagy [73]. In the future, it will be important to examine whether p62 controls the levels of apoB-containing aggregates in PUFA-treated cells and whether plasma levels of apoB-containing lipoproteins are altered in mice with defects in autophagy-requiring genes [74,75].

Insulin resistance and apoB degradation

As described in the preceding section, PUFAs stimulate the PERPP of apoB, but these molecules may not be the only factors that enhance the post-ER destruction of apoB. Other evidence indicates that insulin stimulates a closely related process: 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 [76,77]. A link between these phenomena is also provided by the fact that PUFA-rich diets improve insulin sensitivity, perhaps by decreasing the activity of lipogenic enzymes and desaturases [78,79].

The effects of insulin on apoB secretion require PI3-kinase activity [80], MAP kinase, and TNF α [81,82]. Because apoB degradation is decreased in the liver or in the primary hepatocytes of insulin-resistant animal models (e.g. rats [83] and hamsters [84]; also reviewed in [85]), the overproduction of VLDL in insulin-resistant patients may be a consequence of decreased activity of insulin-stimulated apoB degradation. Although it is not clear whether insulin decreases apoB levels via PERPP-related autophagy, a recent study suggested that insulin shunts a post-ER, apoB-containing lipoprotein population to autophagosomes in hepatocytes [86].

Concluding remarks

The number of VLDL particles assembled is determined by apoB availability. This, in turn, is regulated not by apoB synthesis but by its degradation. Depending on the lipidation state, apoB degradation is accomplished either by ERAD (when lipids are rare, i.e. lipid insufficiency) or by PERPP (when lipids are abundant, i.e. lipid sufficiency). The former explains how a portion of apoB is constitutively turned over and is relevant in patients who are placed on MTP inhibitor therapy [20]. The latter also contributes to constitutive apoB turnover [64,65] and to the control of VLDL production by dietary fatty acids and perhaps by insulin. A continued definition of the molecular mechanisms of both pathways will lead

to novel insights into protein quality control and to the identification of new therapeutic targets to lower the production of atherogenic lipoproteins.

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Figure 1.

Schematic summary of apoB secretory regulation. ApoB is initially synthesized on ER membrane-bound ribosomes. **(a)** With adequate lipid ligands, apoB is translocated into the ER where it is folded and stabilized in the proper conformation. Primordial VLDL particles are formed (pre-VLDL) and transported to the Golgi apparatus in COPII vesicles. Insulin promotes a post-ER transfer of apoB-lipoproteins to a nonproteasomal degradation process, possibly autophagy [86], either before or after entry into the Golgi. When polyunsaturated fatty acids (PUFA) comprise a critical complement of lipid species associated with apoB lipoproteins, lipid peroxides are produced that damage and cross-link apoB, most likely in the Golgi as part of the VLDL maturation process. Eventually, aggregates are formed that are degraded by autophagy. At subcritical PUFA levels, VLDL matures normally and is secreted. With insufficient lipid ligands **(b)**, nascent apoB translocation into the ER lumen is inefficient and domains of apoB are exposed to the cytosol where the particle is selected by chaperones for ERAD. The ubiquitin E3-ligase gp78 attaches poly-ubiquitin, and the protein is degraded by the proteasome.