Minireview

Complexity in the Secretory Pathway: The Assembly and Secretion of Apolipoprotein B-containing Lipoproteins*

Published, JBC Papers in Press, March 21, 2002, DOI 10.1074/jbc.R100068200

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Apolipoprotein B100 (apoB100) is expressed primarily in mammalian liver. It has 4536 amino acids, 25 cysteines (16 of which are in intramolecular disulfide bonds), and 20 N-linked glycosylation sites. A smaller form, apoB48, is expressed in mammalian intestine and in the livers of some non-human mammals. ApoB48 results from a post-transcriptional modification of the apoB mRNA at codon 2153 that converts a glutamine codon to a stop codon at ~48% of the full-length coding sequence. Both forms of apoB have a complex structure that includes a globular amphipathic NH2 domain spanning the first 15–20% (using B100 as a standard length) of the polypeptide followed by an extended hydrophobic β-sheet domain from about 20 to 48%. In apoB100, the rest of the polypeptide is comprised of an α-helical amphipathic region, another long β-sheet domain, and another α-helical domain.

In humans, apoB100 is an essential component of liver-derived very low density lipoproteins (VLDL) and low density lipoproteins (LDL), whereas apoB48 is essential for the formation of chylomicrons in the small intestine. VLDL, LDL, and chylomicrons transport the majority of cholesterol and triglyceride (TG) in the circulation of humans, and because increased plasma levels of these lipoproteins are associated with increased risk for the development of atherosclerosis, regulation of their assembly and secretion is of considerable interest.

In this review, we summarize how this large, complex protein assembles together with lipid molecules to form lipoprotein particles that are then secreted. We emphasize the post-transcriptional regulation of hepatic apoB lipoprotein formation and in particular, the regulated intracellular degradation of apoB (for a recent review of the transcriptional regulation of the apoB gene, see Ref. 1).

Regulated Translation of ApoB

Two studies have shown regulation at the level of elongation. The first focused on rats made diabetic by injection of streptozotocin. Their primary hepatocytes exhibited a profound decrease in apoB synthesis with no change in apoB mRNA abundance (2). Using ribosomal transit time analysis, it was shown that the decreased synthesis could be explained by a prolonged elongation rate for apoB.

The second example is the report that the elongation of apoB mRNA in HepG2 cells slows when translocation of nascent apoB across the endoplasmic reticulum (ER) is inhibited by preventing microsomal triglyceride transfer protein (MTP)-associated transfer of lipids to the nascent polypeptide (3). The molecular mechanisms responsible for the slowing of apoB translation in either study are not known but may be related to unusual physical properties of hepatic polysomes containing apoB mRNA (4).

Regulation of ApoB Targeting to ER by the Signal Peptide

ApoB is unusual in that there is common sequence variation in the human population of the signal peptide (SP) (5). The “wild type” SP is 27 amino acids long (SP27); a commonly occurring variant is 24 amino acids long (SP24) and is the result of deletion of Leu-Ala-Leu residues. This occurs at a carrier frequency of about 30% in Caucasians, 21% in Blacks, and 19% in Chinese. Loss of Leu-Ala-Leu may reduce the efficiency of insertion of the apoB SP into the ER (6), consistent with the secretion in yeast of a fusion protein consisting of apoB SP and invertase differing between the SP24 and SP27 versions (7). Population studies, however, have not shown consistent associations between the two polymorphisms and plasma lipid levels.

The Bitopic Orientation of ApoB across the ER Membrane

Most secretory proteins undergo efficient co-translational translocation into the lumen of the ER (8, 9). Starting in the early 1990s, evidence accumulated that the translocation of apoB may not conform to the classic paradigm (10). For example, in isolated microsomes, domains of apoB were digested by exogenous proteases, suggesting that apoB was exposed to the cytosol at some point(s) during synthesis or intracellular transport. Although apoB does not have a classical transmembrane domain, studies confirmed a “bitopic” orientation in which there is simultaneous exposure of apoB domains to the ER lumen and the cytosol (11–13). One basis for the bitopic topology of apoB was suggested by studies in a cell-free system in which putative “pause-transfer” sequences interrupted apoB translocation but not translation (14). An alternative view of translocational pauses proposing transient difficulties posed by the secondary structure of apoB mRNA (15). Regardless of the exact molecular bases, inefficient translocation, together with continued translation, could result in the escape of the nascent polypeptide from the translocon into the cytosol (16). Of interest, then, are recent studies that have identified lateral openings between the ribosome and the translocon that could provide an entrance into the cytosol for nascent polypeptide chains (17, 18).

Yet another basis for inefficient translocation is the demonstration that the presence of a portion of the first β-sheet domain is sufficient to cause exposure of nascent apoB to the cytosol (19). The β-sheet domain also made apoB secretion responsive to the presence of oleic acid in the medium and dependent on MTP activity. Of note, sequences containing numerous pause-transfer sequences, but lacking a β-sheet domain, were efficiently translocated and not exposed to the cytosol.

ApoB has a prolonged interaction with translocon-associated proteins (20), and completion of translation may be delayed until a lipid-associated signal for secretion occurs (21). The exact nature of the interaction between β-sheet domains of apoB and the translocon is undefined, but it does provide a potential mechanism for regulation of translocation by lipid availability.

As noted, MTP activity is required for apoB lipoprotein assembly and secretion. MTP is a heterodimer consisting of protein-disulfide isomerase and a 97-kDa large subunit that transfers neutral lipids between membranes. Mutations in the gene encoding the large subunit are the basis for the human syndrome, abetalipoproteinemia, in which neither intestinal nor hepatic apoB lipoproteins are found in the plasma (22). In addition to its involvement in the translocation of β-sheet-containing apoB molecules (see above), MTP has also been shown to bind to two sites at the N-terminal end of apoB (24–28). One binding site is within the first 5–6% of the N-terminal end whereas the second is within the first 10% of the

*This minireview will be reprinted in the 2002 Minireview Compendium, which will be available in December, 2002. The work mentioned in this minireview was supported by National Institutes of Health Grants HL 58541 (to E. A. F.) and HL 55638 (to H. N. G.).

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1 The abbreviations used are: VLDL, very low density lipoprotein(s); LDL, low density lipoproteins; TG, triglyceride; ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein; SP, signal peptide; Ub, ubiquitin; ERAD, ER-associated degradation; HIV, human immunodeficiency virus.
Regulated Co- and Post-translational Degradation of ApoB

Early pulse-chase studies in HepG2 cells (40) and in rat primary hepatocytes (41) showed that a significant amount of newly synthesized apoB is degraded. A number of laboratories over the past 10 years has shown that this degradation is accomplished by both proteasomal and non-proteasomal pathways and that each type of degradation is regulated by one or more metabolic factors.

Proteasomal Degradation of ApoB—Studies of apoB degradation in HepG2 cells indicated that the turnover of the protein was rapidly and negatively regulated by triglyceride synthesis. By applying specific inhibitors of the proteasome (42–44), it was found that when conditions are not favorable for apoB assembly with lipids, apoB is ubiquitinylated and degraded by the proteasome (35, 45, 46). This degradation involves the chymotrypsin-like activity of the proteasome in a process facilitated by cytosolic chaperones (hsp70, hsp90) that begins co-translationally (34, 35, 39, 46). In contrast to ER-associated degradation (ERAD) of other proteins (47) targeting of apoB was not regulated by a mutation in its primary amino acid sequence. The lack of co-translational lipida
tion leads to the targeting of the nascent, apoB polypeptide to quality control mechanisms designed to prevent the ER exit of misfolded proteins (48).

The route by which apoB enters the Ub-proteasome pathway has been investigated. A commonly accepted model for ERAD substrates was full translocation into the ER, followed by retrotranslocation ("dislocation") back to the cytosol (49–51). Indeed, additional work suggests that unique sequences within the major translocon protein, sec61, are required for dislocation of certain abnormal proteins (52, 53).

Although there are some data consistent with a translocation-retrotranslocation model (54), the bulk of evidence favors an alternative model (Fig. 1A) in which apoB is targeted co-translationally for degradation while it remains bound to the ribosome and becomes engaged by cytosolic factors that lead to its extraction from the translocon into the Ub-proteasome pathway (20, 21, 34, 55, 56).

The level of degradation of apoB by the proteasome is regulated by a number of metabolic factors. As noted above, a lack of triglyceride synthesis or proteasome activity increases apoB ERAD. Although the supply of exogenous fatty acids influences triglyceride synthesis in cultured cells, SREBP-1c-regulated lipogenesis also seems to be important in protecting apoB from proteasomal degradation (57). Besides hsp70, hsp90 can be a factor in the ERAD process (e.g. Refs. 58 and 59). Recently, it was found to promote apoB degradation by the Ub-proteasome pathway (39) at a step distal to the effects of hsp70. It has been proposed that one role of hsp90 in proteasome function is to facilitate unfolding of the “doomed” substrate into the narrow mouth of the 26 S cap (60).

Non-proteasomal Degradation of ApoB—The induction of apoB degradation in rat primary hepatocytes by n-3 fatty acids (found...
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mainly in certain fish oils) and insulin are examples of a non-proteasomal pathway. These metabolic perturbations stimulate a process that not only degrades apoB but preferentially decreases the secretion of the most buoyant apoB lipoproteins (61–63). Proteasomal inhibitors do not affect apoB degradation stimulated by either fish oils or insulin (61). A variety of experimental data indicate that these stimuli induce the degradation of apoB after translocation, assembly with lipids, and exit from the ER (61, 64). Interestingly, inhibition of phosphatidylinositol 3-kinase reduces the degradation stimulated by either fish oils or insulin (61, 65), making it tempting to speculate that overproduction of hepatic apoB lipoproteins in the insulin-resistant state reflects decreased activity of this degradation pathway. Other examples of apoB degradation that appear to occur post-ER include regulation by choline deficiency (66), dexamethasone (67), and the interaction of newly assembled apoB lipoproteins with LDL receptors in the secretory pathway or at the cell surface (61, 68, 69). In the latter case, cell surface heparin sulfate proteoglycans may also serve an LDL receptor-like function.

There are also data suggesting intra-ER degradation of apoB (30, 70–73). Of note, in three of these studies, dithiothreitol inhibited the degradation. In addition to a dithiothreitol-sensitive protease, another candidate is ER-60, which has been shown to have serine protease activity (74) and to interact with apoB in HepG2 cells (75). However, there are no reports that inhibitors that decrease the proteolytic activity of ER-60 increase apoB recovery. An alternative role for ER-60 is that it may act as an ER chaperone for apoB, as it does for other proteins (e.g., see Ref. 76).

Overall, the data support the occurrence of non-proteasomal apoB degradation, but much remains to be determined, including the number of distinct processes, the factors targeting apoB to this pathway, and the proteolytic activities involved.

Regulated Lipoprotein Assembly in the ER

Little is known about how the mature, TG-enriched lipoprotein is assembled, particularly the process of bulk lipid addition, the site where that addition occurs, and the role of MTP in the final stages of maturation. There are data (21) consistent with either the complete (or nearly complete) formation of a TG-rich apoB lipoprotein while the complex is still associated with the translocon or the formation of a TG-poor particle at the translocon and rapid conversion to an apoB VLDL. Thus, the possibility would be consistent with an early study (37), which suggested that a lipid-poor form of apoB present in the rough ER could “fuse” with lipid droplets in the smooth ER, as well as with more recent studies (37, 78–83).

The further lipidation of the TG-poor, dense, apoB lipoprotein may require its lateral diffusion to a specialized compartment of the ER or transport to a pre-Golgi compartment (37, 80). MTP activity must be present preceding and during the early stages of apoB lipoprotein formation to have bulk lipid addition occur at a later stage (36, 84, 85). Thus, MTP may provide bulk lipid to the site where it is finally added (86, 87). Alternatively, the addition of bulk lipid during the later stages of assembly may be independent of MTP activity and ongoing TG synthesis (20, 79, 83, 84, 88), suggesting that oleic acid, besides stimulating core lipid synthesis, acts as a signaling molecule that targets lipoproteins to the site of bulk lipid addition.

Small GTP-binding proteins and phospholipase D may function in the later stages of apoB lipoprotein assembly (89). Palmitoylation of apoB may target the protein to where bulk lipid addition can occur (90). The possibility that oleic acid works together with phospholipase A2 to generate oleyl-enriched ER membrane phospholipids that stimulate movement of a lipid droplet into the ER lumen was also raised recently (91). Whether n-3 fatty acids and insulin decrease or apoE increases VLDL secretion (64, 65, 93–96) by inhibition or stimulation, respectively, of the later steps of apoB lipoprotein formation remains to be determined.

Role of the Golgi in Lipoprotein Assembly

There are studies that suggest that the final maturation of the lipid-enriched apoB lipoprotein occurs in the Golgi (97–100). For example, Golgi lipoproteins were of the size of VLDL, whereas those in the ER were smaller (101). In other studies, the size ranges of Golgi- and ER-derived rat hepatic apoB lipoproteins were similar (102), but the larger size of secreted particles implied lipids had been added in the Golgi (103). Potential restraints in the ER export processes (104) may be avoided if full lipid loading occurred after leaving the ER. We have incorporated some of the possible places of the assembly of apoB with lipids as shown in Fig. 1B.

Physiological Relevance of the Regulation of ApoB Lipoprotein Assembly and Secretion

Examples in humans likely to involve post-transcriptional regulation of apoB include: 1) those with combined hyperlipidemia (105) or the insulin resistance syndrome (106) who have increased hepatic apoB lipoprotein secretion; 2) settings in which increased fatty acid flux to the liver increases apoB secretion in VLDL (e.g., Ref. 107); and 3) the reports that hyperinsulinemia can acutely inhibit VLDL apoB secretion in normal subjects (but not in obese insulin-resistant subjects) (108).

Of particular note is the recent report that HIV protease inhibitors, agents that cause a dyslipidemia characterized by increased VLDL levels (109), are inhibitors of the proteasome (110). In a variety of human and rodent hepatic cell types treated with HIV protease inhibitors, there was increased protection of nascent apoB from degradation and, in the presence of oleic acid, increased secretion of apoB lipoproteins (111). These data suggest a significant role for proteasomal degradation in the in vivo regulation of apoB secretion in mammals.

There are also animal models of post-transcriptional regulation of apoB secretion. For example, hamsters fed a high fructose diet had increased assembly and secretion of VLDL apoB with no change in apoB mRNA levels but with decreased non-proteasomal degradation of apoB (112). There is also post-transcriptional regulation of apoB secretion in human apoB transgenic mice lacking brown adipose tissue (92). Insulin resistance was present in both of these animal models, underscoring the relevance to the data obtained in humans.

Summary

ApoB is essential for the production of hepatic atherogenic lipoproteins. The major role of post-transcriptional degradation of apoB is surprising. The same lipophilic sequences enabling apoB to partake in lipoprotein formation also appear to participate in its targeting to proteasomal degradation when lipid export by the liver is not favored. Even after the translocation of nascent apoB is completed, several quality controls appear to be in place to further regulate lipoprotein secretion. Indeed, the cellular “bureaucracy” developed to regulate apoB lipoprotein assembly and secretion matches the complexity of the lipoprotein itself.

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J. Biol. Chem. 2002, 277:17377-17380.
doi: 10.1074/jbc.R100068200 originally published online March 21, 2002

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