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Atherosclerosis Is a Liver Disease of the Heart

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Abstract**—**The production of apolipoprotein B (apoB)–containing lipoproteins by the liver is regulated by a complex series of processes involving apoB being cotranslationally translocated across the endoplasmic reticulum and assembled into a lipoprotein particle. The translocation of apoB across the endoplasmic reticulum is facilitated by the intraluminal chaperone, microsomal triglyceride transfer protein (MTP). MTP facilitates the translocation and folding of apoB, as well as the addition of lipid to lipid-binding domains (which consist of amphipathic β sheets and α helices). In the absence of MTP or sufficient lipid, apoB exhibits translocation arrest. Thus, apoB translation, translocation, and assembly with lipids to form a core-containing lipoprotein particle occur as concerted processes. Abrogation of $\geq$1 of these processes diverts apoB into a degradation pathway that is dependent on conjugation with ubiquitin and proteolysis by the proteasome. The nascent core-containing lipoprotein particle that forms within the lumen of the endoplasmic reticulum can be "enlarged" to form a mature very low density lipoprotein particle. Additional studies show that the assembly and secretion of apoB-containing lipoproteins are linked to the cholesterol/bile acid synthetic pathway controlled by cholesterol 7α-hydroxylase. Studies in cultured cells and transgenic mice indicate that the expression of cholesterol 7α-hydroxylase indirectly regulates the expression of lipogenic enzymes through changes in the cellular content of mature sterol response element binding proteins. Oxysterols and bile acids may also act via the ligand-activated nuclear receptors LXR and FXR to link the metabolic pathways controlling energy balance and lipid metabolism to nutritional state. **(*Arterioscler Thromb Vasc Biol*. 2001;21:887-898.)**

Key Words: apolipoprotein B ■ lipoprotein assembly/secretion ■ cholesterol-7α-hydroxylase ■ microsomal triglyceride transfer protein ■ ubiquitin-dependent proteasome degradation

Atherosclerotic cardiovascular disease is the major cause of death in technically advanced societies.[1–3] The hallmark of atherosclerosis is the accumulation of cells containing excessive lipids (ie, foam cells) within the arterial wall.[4] Plasma lipoproteins are a major source of the lipid that accumulates in atherosclerotic lesions.[5] Within the arterial wall, many processes act in a seemingly concerted manner to initiate the formation of lesions that ultimately result in the occlusion of blood flow, ischemia, and tissue injury.[6–8] These processes include injury to the endothelium, retention of lipoproteins within the arterial wall, oxidation of lipids, and inflammation and proliferation of smooth muscle cells.[9]

The liver is the major organ responsible for the production[10] and degradation[11,12] of apoB-100–containing lipoproteins. In response to genotype and nutrition, the balance in these 2 pathways determines the plasma levels of LDL, an important determinant of susceptibility to atherosclerosis.[1,13] For example, patients with familial combined hyperlipidemia exhibit increased rates of production of apoB-containing lipoproteins by the liver and increased susceptibility to atherosclerosis.[14,15] Similarly, patients with familial hypercholesterolemia with functional loss of hepatic LDL receptors display marked hypercholesterolemia and increased susceptibility to atherosclerosis.[16] In addition, the type of dietary fatty acid consumed influences the hepatic levels of cholesterol esters, the amount of cholesterol esters that are secreted (which affects plasma levels of LDL),[17] and the susceptibility to atherosclerosis.[18]

The Liver Is a Therapeutic Target for Atherosclerosis

Based on the central role of the liver in determining plasma lipoprotein levels, several therapeutic strategies that act on hepatic lipid metabolism have been developed to ameliorate several forms of hyperlipidemia and reduce the susceptibility to atherosclerosis. Bile acid–binding resins, such as cholestyramine, induce the hepatic expression of cholesterol 7α-hydroxylase (CYP7A1),[19] increase hepatic LDL receptor expression and LDL uptake,[20] and cause a slight, but significant, reduction of plasma LDL.[21] Fibrates activate hepatic peroxisome proliferator–activated receptor-α, resulting in increased β-oxidation of fatty acids, decreased plasma triglycerides, and increased plasma HDL levels.[22] β-Hydroxy-β-methylglutaryl coenzyme A reductase inhibitors (ie,

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statins) block cholesterol biosynthesis, increase the hepatic expression of LDL receptors and hepatic LDL uptake, and decrease plasma LDL.[23,24] A large long-term clinical trial has established for the first time that simvastatin, a β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitor, significantly decreased mortality and morbidity from cardiovascular disease.[25] On the basis of these combined findings demonstrating the therapeutic importance of the liver in ameliorating hyperlipidemia and cardiovascular disease, we propose that "atherosclerosis is a liver disease of the heart."

For the past several years, our research has concentrated on 2 seemingly unrelated aspects of hepatic lipid metabolism: (1) how the production of apoB-containing lipoproteins by the liver is regulated and (2) the regulation of the expression of cholesterol-7α-hydroxylase and how this gene/enzyme plays a central role in regulating lipid and lipoprotein metabolism. The present review will summarize how these 2 independent lines of inquiry converged, leading to new insights integrating hepatic lipoprotein metabolism with biliary function.

In Mammals, Assembly and Secretion of ApoB-Containing Lipoproteins Are Coordinately Regulated in Response to Nutritional State via the SREBP Family of Transcription Factors

Lipoprotein transport systems are essential for the survival and reproduction of all metazoan species. In submammalian species, sex hormones and developmental signals coordinately induce all the processes necessary for delivering essential lipid nutrients for egg and sperm production (see review[26]). As a result of estrogen-induced hyperlipidemia during spawning, several species of salmon indigenous to the Pacific West Coast of North America die from arteriosclerosis soon after their single reproductive act.[27,28] These observations emphasize that evolutionary development favors maximizing the transport of lipids from the liver to reproductive tissues rather than protection from hyperlipidemia-induced artery disease.

In mammals, coordinate induction of the lipoprotein assembly/secretion pathway occurs in response to metabolic signals in a manner that is reminiscent of the sex-linked hormone induction displayed by submammalian species.[29] Compared with hepatocytes from chow-fed rats, those from sucrose-fed rats displayed induced synthesis of all VLDL lipids (ie, cholesterol, cholesterol esters, triglycerides, and phospholipids) as well as increased assembly and secretion of apoB-containing lipoproteins.[29] Conversely, hepatocytes from fasted rats displayed reduced synthesis of all VLDL lipids, together with a decrease in assembly and secretion of apoB-containing lipoproteins.[30,31] These findings suggest that the nutritional state causes a coordinated response in the lipoprotein assembly/secretion pathway by the liver. These coordinate changes include the rates of synthesis of all VLDL lipids and the capacities of processes necessary to package these lipids into VLDL particles. Additional studies have shown that the expression of apoB mRNA remains nearly constant, whereas the amount of de novo synthesized apoB secreted varies in parallel with the rate of lipogenesis. These data suggest that posttranslational processing of apoB plays a critical role in the coordinate control of hepatic VLDL assembly/secretion.[31–33]

Attempts to recapitulate the coordinate induction of lipogenesis and VLDL assembly/secretion caused by carbohydrate-feeding animals by adding fructose or glucose to the medium of cultured rat hepatocytes were unsuccessful.[29,30] Although glucose, fructose, and fatty acids increased the amount of glycerolipids secreted as VLDL, the amount of apoB secreted remained unchanged.[29,30] In addition, adding glucose directly to the medium of HepG2 cells[34] or fructose directly to the medium of primary cultured hamster hepatocytes[35] did not increase the secretion of apoB along with increased lipid secretion. The combined data suggest that increased availability of carbon unit substrates derived from hexose is not sufficient for the coordinate induction of lipoprotein assembly/secretion. We have proposed that metabolic signals produced in response to the nutritional state act to coordinately regulate the genetic expression of genes controlling the processes necessary for VLDL assembly and secretion.[26]

The discovery of the sterol response element binding protein (SREBP) family of transcription factors provided new insights into how the expressions of genes involved in regulating the synthesis of most lipids and many other processes controlling lipoprotein production and metabolism are coordinately regulated.[36,37] The ability of SREBP to activate gene transcription is regulated by oxysterols and fatty acids,[38–42] providing mammalian lipoprotein metabolism a metabolic control independent of reproductive status. There are several examples showing that increased SREBP-mediated gene expression is associated with a coordinate induction of hepatic lipogenesis, the expression of hepatic lipogenic enzymes, and the assembly and secretion of apoB-containing lipoproteins; such examples are SREBP1a transgenic mice,[43,44] hepatoma cells that express a CYP7A1 transgene,[45] carbohydrate-fed mice,[46] and mice that express a CYP7A1 transgene (R.A. Davis, unpublished data, 2001). These combined findings support the proposal that metabolic signals coordinately regulate the apoB-containing lipoprotein assembly/secretion pathway by acting through changes in SREBP.

Efficiency of ApoB Translocation Across the Endoplasmic Reticulum Determines Whether ApoB Enters VLDL Assembly and Secretion or Is Cotranslationally Degraded

To gain insight into which processes may be rate limiting for VLDL assembly, we determined the relative rate constants describing the movement of apoB through the secretory pathway of cultured rat hepatocytes.[32] Our results (summarized in Figure 1) indicate that (1) the rate-limiting step is movement out of the rough endoplasmic reticulum and (2) only a fraction of de novo synthesized apoB is secreted, with the remainder being degraded within the hepatocyte.[32] Subsequent studies have shown that the majority of apoB detected by epitope-specific monoclonal and polyclonal antibodies resides within the endoplasmic reticulum.[33] Additional findings indicating that small peptides ($\approx$70 kDa) were present in the endoplasmic reticulum but absent in the Golgi led to the proposal that apoB was degraded within the endoplasmic reticulum.[33] Surprisingly, in rat livers, a major portion of the apoB that accumulated in the rough endoplas-

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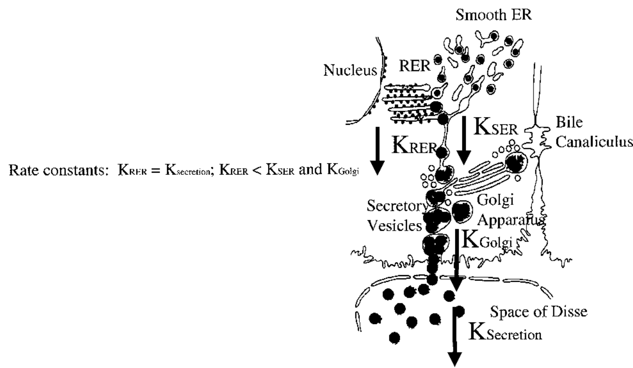

Figure 1. K_{RER}, $K_{Secretion}$, K_{SER}, and K_{Golgi} are rate constants (K), where RER indicates rough endoplasmic reticulum (ER), SER indicates smooth ER, K_{RER}=$K_{Secretion}$, and K_{RER}<K_{SER} and K_{Golgi}. The movement of apoB out of the RER is the rate-limiting step determining the ultimate rate of apoB secretion because K_{SER} and K_{Golgi} exceed K_{RER}. Only a portion of de novo synthesized apoB is secreted, whereas the remainder is degraded within the hepatocyte.

mic reticulum was membrane-associated and susceptible to degradation by exogenous proteases.[47] In addition, specific apoB epitopes were present on the cytoplasmic surface of rat liver microsomes, as demonstrated by binding to specific monoclonal antibodies.[47] Inasmuch as the microsomal membrane vesicles used for these studies were shown to be intact and impermeable to proteases and small molecular weight molecules (ie, mannose phosphate), it was concluded that apoB was exposed on the cytoplasmic surface of the endoplasmic reticulum.[47] These combined data led to the proposal that unlike most other "secretory proteins," apoB had the capacity to become a "transmembrane" protein in the endoplasmic reticulum (ie, display translocation arrest).[47] Additional data supporting the proposal that apoB can exist as a stable transmembrane protein has been obtained by studies using different models of hepatocytes, including rat liver,[48] HepG2 cells,[49–52] chicken hepatocytes,[53] and rabbit livers.[54]

The absence in apoB of predictable amphipathic α helices that are of sufficient length to span a membrane bilayer (ie, transmembrane domain)[55–57] argued against a typical "stop-transfer" sequence being responsible for translocation arrest.[58,59] One explanation for a transient arrest of apoB translocation was the presence of "pause-transfer" sequences.[60–62] In addition, the unusual characteristic of apoB being a nonexchangeable protein associated with VLDL and LDL may provide the basis for its ability to reside in the endoplasmic reticulum as a stable transmembrane protein. With this consideration, we proposed that the amphipathic β sheets in apoB, which exhibited structural features similar to those that allow porins to integrate into membranes, allow apoB to integrate into lipoproteins and act to block translocation.[47]

Secretion of ApoB-Containing Lipoproteins by Cells Lacking Microsomal Triglyceride Transfer Protein Is Blocked Because of an Inability of ApoB-53 to be Completely Translocated Across the Endoplasmic Reticulum

Analysis of the sequence and the structure of apoB showed that it contains many lipid-binding domains located throughout its unusually long (>500 000-kDa) peptide length.[55–57,63] Structure/function analysis of various truncated forms of apoB expressed in hepatoma cells established that a minimum size of apoB was necessary to form a core-containing lipoprotein particle.[64] For example, although apoB-15 was abundantly expressed in rat hepatoma cells, it did not assemble lipoprotein particles because it was too short.[64] In contrast, apoB-53 was abundantly expressed in rat hepatoma cells and, as a result, was assembled and secreted in small VLDL-sized lipoprotein particles.[64] Subsequent studies further established the importance of size and lipid-binding domains as essential characteristics of apoB necessary for the assembly and secretion of core-containing lipoproteins.[65–68] Extending similar studies to mice provided a compelling mechanism for the hypobetalipoproteinemic phenotype (ie, mutations in the apoB gene that result in apoB forms that are too short to assemble core-containing lipoproteins).[69,70]

Further studies expressing various forms of apoB have provided compelling evidence for a cell-type–specific process that is necessary for the assembly of apoB-containing lipoproteins. When expressed in rat hepatoma cells, human apoB-53 assembles core-containing lipoprotein particles that are secreted.[64] In marked contrast, although apoB-53 can be produced in abundance in nonhepatic cells (ie, Chinese hamster ovary [CHO] cells), it is degraded instead of being secreted as a lipoprotein particle.[71] To identify the cell-type–specific process and its role in lipoprotein assembly/secretion, 2 distinct forms of apoB, having different abilities to assemble lipoproteins, were expressed in CHO cells.[71] Although the expression of apoB-15 in CHO cells resulted in the secretion of apoB-15 in a lipid-deficient form, no detectable lipoproteins containing apoB-53 were secreted into the culture medium.[71] These data show that apoB that is too short to form a lipoprotein particle behaves as a generic secretory protein and is secreted by CHO cells. In contrast, the structural features that allow apoB-53 to form a lipoprotein particle prevent its assembly into lipoproteins and secretion by CHO cells. Moreover, because the same apoB-53 construct facilitated the production and secretion of apoB-53 lipoprotein particles from rat hepatoma cells,[64] we concluded that nonhepatic CHO cells lacked a process necessary for the assembly of apoB-containing lipoprotein particles.[71]

N-acetyl-Leu-Leu-norleucinal (ALLN) blocked the degradation of translocation-arrested apoB-53, causing it to accumulate in the endoplasmic reticulum of CHO cells. This discovery indicated that the cell-type–specific process missing in nonhepatic cells functions to translocate apoB across the endoplasmic reticulum and subsequently assemble it into a lipoprotein particle.[71–73] Proteolytic mapping using epitope-specific antibodies revealed that ≈70 kDa of the N-terminus of translocation-arrested apoB-53 was in the lumen of the endoplasmic reticulum, whereas the remaining C-terminus resided in the cytoplasm.[73] In the absence of ALLN, ≈85 kDa of the N-terminal portion of apoB was cleaved and secreted.[73] These studies have shown that CHO cells lack a process that is essential for the translocation of apoB.

In hepatoma cells, translocation of apoB and lipoprotein assembly vary inversely with cotranslational degradation. Pulse-chase experiments using HepG2 cells showed that although ALLN blocked the intracellular degradation of apoB-100 and caused it to accumulate in microsomes, secretion was not increased.[51,74] These data suggest that apoB degradation does not determine how much was secreted but

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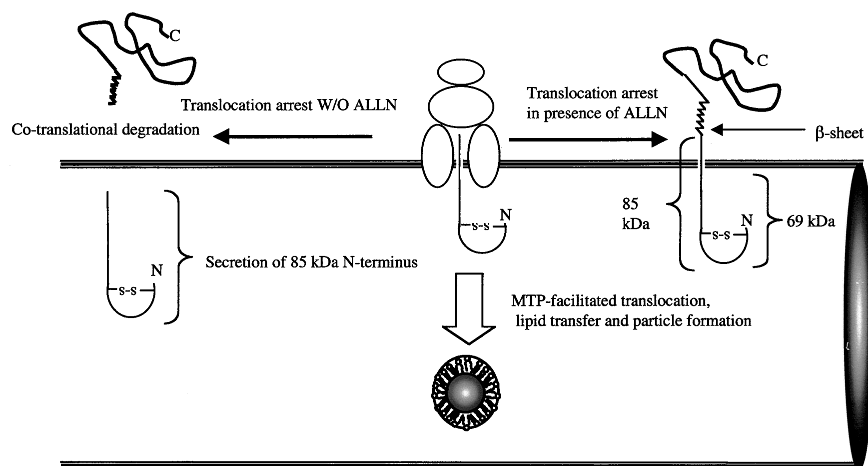

Figure 2. Translocation across the endoplasmic reticulum determines the fate of apoB. C and N indicate the C- and N-termini, respectively; S-S, disulfate bond. In the presence of sufficient lipid, the lipid-binding domains of apoB fold in a manner allowing apoB to be assembled and translocated into the lumen of the endoplasmic reticulum.

that translocation determined how much apoB was either degraded (translocation arrest) or assembled into a lipoprotein (completely translocated).

Because the formation of N-terminal apoB-100 peptides, produced from translocation-arrested apoB-100, occurred before completing translation, the data also indicated that degradation of translocation-arrested apoB-100 occurred cotranslationally.[51] In other experiments, it was observed that adding oleic acid to the medium of cultured hepatocytes increased the efficiency of apoB translocation across the endoplasmic reticulum and the amount that was assembled and secreted into lipoproteins.[74] These data suggest that oleic acid–stimulated glycerolipid biosynthesis facilitates the translocation of apoB across the endoplasmic reticulum and the assembly of lipoprotein particles.

The topographical orientation of translocation-arrested apoB-100 in ALLN-treated HepG2 cells was found to be similar to that of apoB-53 in CHO cells (ie, ≈69 kDa of the N-terminus was in the lumen, whereas the remaining C-terminal portion was exposed to the cytoplasm).[51,73] In other epitope mapping studies, it was concluded that apoB may assume an orientation having multiple transmembrane domains that weave in and out of the endoplasmic reticulum.[75] These data suggest that similar structural motifs in apoB are responsible for its ability to assemble a core-containing lipoprotein particle and to assume a transmembrane orientation in the endoplasmic reticulum. Recent studies suggest that sequences in apoB-41 responsible for "binding" phospholipids are different from the amphipathic β sheets that "bind" triglycerides.[76]

Cotranslation, Translocation, or Degradation Determines the Initial Fate of De Novo Synthesized ApoB

On the basis of the combined data, we proposed a model that integrates apoB translocation with lipidation (Figure 2). This model predicts that the structures in apoB that allow it to assemble stable lipid/protein emulsions containing amphipathic and hydrophobic lipids provide the signals that determine its metabolic fate within the endoplasmic reticulum, which is lipoprotein particle assembly or degradation. Thus, in the presence of sufficient lipid, the lipid-binding domains of apoB fold in a manner that allows particle assembly and translocation into the lumen of the endoplasmic reticulum (Figure 2). There are several dynamic features of this model that provide adaptation to the genetic, metabolic, and nutritional environment in which the lipoprotein assembly/secretion pathway is expressed. In situations in which the assembly and secretion of apoB-containing lipoproteins is most efficient (eg, dietary carbohydrate induction[29]), translation, translocation, protein modification, protein folding, and lipid addition occur in a concerted manner. As a result, no intermediates accumulate in the endoplasmic reticulum, and the integrity of its functions is maintained. In contrast, metabolic situations in which ≥1 of these individual steps is impaired (eg, fasting[30,31] or abetalipoproteinemia[77]), apoB is rapidly and cotranslationally degraded by a process that is inhibited by ALLN (Figure 2).

This model accurately predicts that the hepatic VLDL assembly/secretion pathway is intimately linked to the lipogenic state. Thus, nutritional and metabolic conditions leading to the induction of hepatic lipogenesis would drive the predicted lipid-facilitated translocation of apoB and the compensatory decrease in its degradation. Metabolic coordinate regulation of mammalian VLDL assembly/secretion is reminiscent of the estrogen induction of hepatic VLDL secretion in avian species.[78–80] However, unlike estrogen induction, which is linked to reproduction, this metabolic regulation involves diverse signals that provide greater specificity.

There has been remarkable progress in elaborating the details of the VLDL assembly pathway. Of particular importance has been the identification and characterization of the processes responsible for apoB translocation and degradation of translocation-arrested apoB.

Intraluminal Protein MTP Facilitates Translocation of ApoB and Its Assembly of Lipoprotein Particles

Microsomal triglyceride transfer protein (MTP) is a lipid transfer protein that is present in the lumen of the endoplasmic reticulum of liver.[81,82] Its predicted role in the assembly of lipoproteins was shown when the genetic basis for abetalipoproteinemia was found to be caused by mutations in the MTP gene.[83] MTP has the ability to facilitate the transfer of neutral and amphipathic lipids between membranes and vesicles.[84] It is likely that MTP plays a role in the folding of apoB in addition to transferring lipid to the nascent lipoprotein particle.[85–95]

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The finding that plasma from patients with abetalipoproteinemia is enriched with the same N-terminal peptide produced from proteolytic clipping of translocation-arrested apoB-53 from transfected cultured cells led to the conclusion that MTP lipid transfer facilitates apoB translocation.[77] This interpretation was subsequently supported by the finding that cells treated with chemical inhibitors of MTP lipid transfer activity displayed an inability to translocate apoB across the endoplasmic reticulum, which led to its rapid degradation.[86,96,97] Additional support for the essential role of MTP in apoB translocation was provided by transfection studies using cell culture systems. The inability of CHO cells,[71,73,93] COS cells,[98] and HeLa cells[99] to translocate apoB across the endoplasmic reticulum and to assemble apoB-containing lipoprotein particles can be corrected by plasmid-driven expression of MTP. However, it has also been reported that transfected apoB-41 could be secreted by the mouse mammary cell line C127, which displays no detectable MTP expression.[100] In addition, in vitro translation/translocation assays showed that pancreatic microsomes could translocate apoB-48 in the absence of detectable MTP.[101] Although these studies suggest that detectable amounts of apoB-48 can be translocated across the endoplasmic reticulum without MTP, it is possible that the efficiency is low. The phenotype of the single gene (MTP) disorder, familial abetalipoproteinemia, in which there is an almost, but not quite, complete inability to secrete apoB-100 and apoB-48 lipoprotein particles, supports this interpretation.[77,102]

The developments of chemical and genetic methods to inhibit MTP function have provided new insights into its essential role in the assembly and secretion of apoB-containing lipoprotein particles. Chemical inhibition of MTP lipid transfer activity was shown to block the early step in the VLDL assembly/secretion pathway.[96,103–105] Irreversible inhibition of MTP transfer activity in HepG2 cells showed that the level of MTP lipid transfer activity was correlated with apoB-100 secretion.[105] These results support the proposal that MTP controls the rate-limiting step in VLDL assembly/secretion. The finding showing that plasma levels of apoB-100 were reduced by 28% in heterozygous MTP gene–deleted mice further supports this proposal.[106] Subsequent studies showed that the concentration of MTP within the endoplasmic reticulum, not the MTP-to-apoB ratio, is the key determinant of the amount of apoB-100 secreted by the liver.[107] The additional finding that overexpression of MTP via an adenovirus transgene increased the secretion of apoB provides further evidence supporting the rate-limiting role of MTP in VLDL assembly/secretion.[108] It has been recently reported that an MTP inhibitor (AGI-S17) blocked MTP-apoB binding and the secretion of apoB without interfering with MTP lipid transfer activity.[109] These data are consistent with the proposal that MTP facilitates the translocation of apoB across the endoplasmic reticulum by acting as a chaperone.[93]

MTP Lipid Addition to Lipid-Binding Domains in ApoB Facilitates Translocation and Lipoprotein Particle Assembly

Functional mutagenesis experiments indicate that β-sheet lipid-binding domains in apoB are intimately linked to the MTP requirement for translocation across the endoplasmic reticulum.[110] Additional studies suggest that a particular sequence, which resides between apoB-51 and apoB-53 and contains a predicted amphipathic α helix surrounded by amphipathic β sheets, displays an usually high requirement for MTP.[95] These findings suggest that the addition of lipid to these structures occurs in concert with protein folding and translocation. This interpretation is consistent with additional studies showing that the translocation of apoB-100[97] and apoB-53[71,73] requires functional MTP, whereas shorter forms of apoB (apoB-41[100] and apoB-48[101]) can be translocated, albeit inefficiently, independently of MTP. Additional studies in which hepatic MTP gene expression was knocked out with the use of cre-recombinase in mice showed that apoB-100 virtually disappeared from plasma, whereas detectable levels of apoB-48 remained.[111,112] In 1 study, liver-specific knockout of the MTP gene in mice mainly blocked the secretion of apoB-100, with almost no effect on the secretion of apoB-48.[111] In another study, liver-specific knockout of the MTP gene in mice blocked the secretion of apoB-100 and apoB-48.[112] With the proviso that the apoB-48 was not of intestinal origin, these findings provide further support for the proposal that the translocation of apoB-48 is not completely dependent on MTP.

Cytoplasmic C-Terminal Portion of Translocation-Arrested ApoB Is Degraded by Ubiquitin-Dependent Proteasome

If the amount of energy consumed by the degradation of de novo synthesized apoB (protein synthesis followed by degradation[72]) is commensurate with the importance of this process, one would predict that the cotranslational degradation of apoB is likely to be essential for maintaining vital cellular function(s). One obvious benefit of degrading translocation-arrested apoB is preventing “constipation” of the secretory pathway by sequestering common factors used for processing secretory proteins. Several processes involving the proteolytic degradation of several proteins in addition to apoB have been proposed as the means to maintain “quality control” of the endoplasmic reticulum.[113–117]

In a series of elegant experiments from several different laboratories, the proteolytic process responsible for degrading translocation-arrested apoB in the endoplasmic reticulum was identified and characterized. This information provides compelling evidence supporting the hypothesis that metabolic fate (translocation and lipoprotein particle assembly or degradation) occurs cotranslationally (Figure 3). Lactacystin, which specifically inhibits proteolysis by the proteasome,[118] blocked the degradation of apoB-100 in HepG2 cells in a manner similar to ALLN.[119] These findings led to the conclusion that ubiquitin conjugation and proteasome degradation is responsible for the rapid degradation of apoB.[119] Subsequent studies have shown that abrogation of translocation across the endoplasmic reticulum diverts apoB to ubiquitin-dependent proteasome degradation.[97,120–124] The recent finding that ubiquitin-dependent proteasome degrades translocation-arrested apoB in primary hamster hepatocytes suggests that this pathway is relevant to in vivo physiology.[125] (There are many additional proteolytic degradation pathways for degrading apoB [see reviews[126–128]]. It is likely that the phenotype and metabolic state of the cell play an important role in determining the fate of apoB.)

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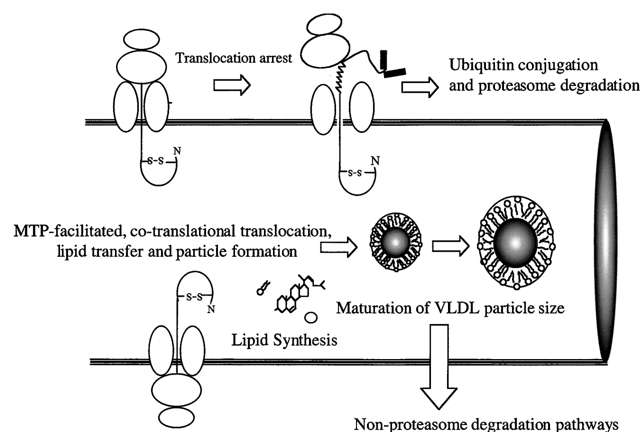

Figure 3. The production of apoB-containing lipoproteins by the liver is regulated by a complex process involving apoB being cotranslationally translocated across the endoplasmic reticulum and assembled into a lipoprotein particle. The translocation of apoB across the endoplasmic reticulum is facilitated by the intraluminal chaperone, MTP. MTP facilitates the translocation, folding of apoB, and addition of lipid to lipid-binding domains (which consist of amphipathic β sheets and α helices). In the absence of MTP or sufficient lipid, apoB exhibits translocation arrest. Thus, apoB translation, translocation, and assembly with lipids to form a core-containing lipoprotein particle occur as concerted processes. Abrogation of $\geq$1 process necessary to form a fully translocated lipoprotein particle diverts apoB into a degradation pathway that is dependent on conjugation with ubiquitin and proteolysis by the proteasome. The nascent core-containing lipoprotein particle that forms within the lumen of the endoplasmic reticulum can be "enlarged" to form a mature VLDL particle.

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The discovery that the ubiquitin-dependent proteasome was responsible for degradation of apoB in the endoplasmic reticulum provides strong support for the proposal that this process occurred in the cytoplasm, as proposed in Figure 3. The inability to detect nonglycosylated forms of the apoB reporter that were degraded by the proteasome led to the proposal that apoB is transferred back into the cytoplasm via retrograde translocation processes after entering the lumen of the endoplasmic reticulum.[129] However, compelling evidence indicating that apoB does not undergo a retrograde translocation process but is cotranslationally degraded by the ubiquitin-dependent proteasome was obtained by using an apoB chimera that had antigen reporters on the N-terminus and C-terminus.[130] By use of a variety of techniques, it has been shown that the N-terminus remains within the lumen of the endoplasmic reticulum, whereas the C-terminus residing in the cytoplasm is degraded by the proteasome.[130] Additional studies have shown that apoB associates with sec61β of the translocon complex while it is being translated and attached to the ribosome.[131] This complex is subsequently released by a process dependent on MTP lipid transfer activity.[132] These data support the proposal that apoB translation, translocation, MTP lipid transfer, and lipoprotein particle assembly occur as a concerted reaction. Abrogation of any 1 of these processes diverts apoB into a pathway that leads to ubiquitin conjugation and cotranslational degradation by the proteasome.

Some of the first studies using electron microscopy are generally considered seminal in recognizing how adaptations of "the generic secretory pathway" provide specialized functions, such as hepatic VLDL assembly/secretion.[133–135] With the use of antibodies that recognize human LDL, electron microscopy immunolocalization of epitopes presumably representing apoB has provided important information on the intracellular itinerary of apoB in the hepatic VLDL assembly/secretion pathway. The results suggested that (1) apoB was synthesized in the rough endoplasmic reticulum, (2) VLDL-sized lipid particles, without immunodetectable apoB, appeared in the lumen of the smooth endoplasmic reticulum, and (3) VLDL-sized lipid particles containing immunodetectable apoB appeared in the lumen of the junctions between the rough and smooth endoplasmic reticulum.[135] These findings led the authors to conclude that VLDL was assembled in the endoplasmic reticulum via a process in which apoB was joined together with a nascent lipoprotein particle produced in the lumen.[135] The recent discovery of lipoprotein particles visibly present in the endoplasmic reticulum of intestines in apoB knockout mice provided additional support for the proposal that apoB is not essential for the formation of core-containing lipoprotein particles within the endoplasmic reticulum.[136]

Based on the ability to discriminate at least 2 separate steps, the 2-step model of the VLDL assembly/secretion pathway was proposed.[137] Experimental evidence supporting a 2-step model of the VLDL assembly/secretion pathway in which an HDL-sized particle is transformed into a VLDL-sized particle has been recently published.[138,139] The second step (in which an HDL-sized particle is transformed into a VLDL-sized particle by oleic acid–stimulated lipogenesis) has been shown to require ADP ribosylation factor-1 and its activation of phospholipase D.[127,140] The ADP ribosylation factor-1 requirement for the second step explains its inhibition by brefeldin A.[141] These findings and those showing that in hepatoma cells the oleic acid stimulation of the second step requires a phospholipase A_2 rearrangement of membrane phospholipids[142] suggest that the second step may involve the formation of a specialized vesicle. These combined findings indicate that the first step (ie, apoB translocation and initial particle assembly[131,132]) and the second step require oleic acid.

The oleic acid requirement may be more complicated than merely supplying substrate for glycerolipid biosynthesis. Mice lacking functional stearoyl-coenzyme A desaturase-1 show a nearly complete inability to secrete apoB-containing lipoproteins.[143] Moreover, treatment of primary hepatocytes from these mice with oleic acid does not overcome the defect in the secretion of apoB-containing lipoproteins.[143] Because the expression of stearoyl-coenzyme A desaturase-1 is SREBP1c dependent,[144,145] it may play an indirect role in VLDL secretion through a regulatory loop with SREBP.

CYP7A1 Regulates Catabolism of Cholesterol to Bile Acids, Which Subsequently Determines Cholesterol Homeostasis and Intestinal Lipid Absorption and Lipoprotein Production

The liver-specific gene product CYP7A1 is the rate-limiting enzyme controlling the synthesis of bile acids from cholesterol.[146] This pathway controls cholesterol homeostasis and indirectly influences the production of intestinal and hepatic lipoproteins. In the rat, the CYP7A1-dependent cholesterol catabolic pathway accounts for $\approx$85% of the cholesterol that

is removed from the body.[147] The role of CYP7A1 in intestinal lipoprotein production is emphasized by findings showing that its deletion in CYP7A1$^{-/-}$ knockout mice results in postnatal lethality that is reversed by dietary bile acids and fat-soluble vitamins.[148,149] Furthermore, the size and content of the endogenous bile acid pool is an important determinant of intestinal lipid digestion, absorption, and assembly into lipoprotein particles.[150–152] Finally, because some fat-soluble vitamins inhibit the oxidation of apoB-containing lipoproteins, a process that contributes to the formation of atherosclerosis,[153–155] bile acid–facilitated intestinal absorption of antioxidants may have a significant effect on the metabolism of lipoproteins.

Cholesterol/Bile Acid Biosynthetic Pathway Indirectly Regulates Production and Metabolism of Hepatic Lipoproteins

Bile acids are essential for the digestion and absorption of essential lipid nutrients.[146] By providing dietary fatty acids and cholesterol to the liver as substrates for the production of hepatic lipoproteins, the production of bile acids indirectly influences VLDL assembly/secretion. Furthermore, the CYP7A1 bile acid synthetic pathway indirectly induces the expression of hepatic LDL receptors, the major pathway responsible for removing apoB-containing lipoproteins from plasma. As a result, the LDL receptor expression level varies in parallel with CYP7A1 expression.[20,23,156]

Hepatic Lipogenesis and VLDL Production Is Linked to Cholesterol/Bile Acid Biosynthetic Pathway via Oxysterols

Stable expression of CYP7A1 in CHO cells led to an induction in the expression of LDL receptor mRNA.[157] The increased expression of LDL receptors in CHO cells expressing CYP7A1 was accompanied by a significant increase in the cellular content of free and esterified cholesterol.[157] In additional studies, stable expression of a CYP7A1 transgene in CHO cells resulted in increasing the cellular content of SREBP1, the expression of mRNAs encoding lipogenic enzymes, and the synthesis of cholesterol, cholesterol esters, triglycerides, and phospholipids.[41] Because the rate of metabolism of radiolabeled 25-hydroxycholesterol by CHO cells was increased by CYP7A1 expression, it has been proposed that CYP7A1 indirectly induces SREBP-dependent gene expression by metabolizing and inactivating oxysterol repressors.[41,157] Early studies failed to show that CYP7A1 could metabolize oxysterols.[158] However, subsequent studies have provided compelling evidence that CYP7A1 is capable of 7α-hydroxylating many oxysterols, some of which were better substrates than cholesterol.[159,160]

The role of CYP7A1 in regulating hepatic VLDL assembly/secretion was demonstrated by expressing CYP7A1 in rat hepatoma cells. Transfected cells showed a marked induction in the expression of mRNAs encoding lipogenic enzymes and MTP. Consequently, the assembly and secretion of apoB-100–containing lipoproteins were also increased.[45] The induction of lipogenesis and of VLDL secretion was linked to the increased cellular content of mature SREBP1, which is directly proportional to the level of CYP7A1 mRNA expression.[45] This is further supported by the results obtained from constitutive expression of CYP7A1 in the livers of transgenic mice. In addition to an induction of mRNAs encoding lipogenic enzymes and MTP, CYP7A1 transgenic mice exhibit an increase in the assembly and secretion of apoB-100–containing lipoproteins (R.A. Davis, unpublished data, 2001). Together, these data provide convincing evidence linking the anabolic VLDL assembly/secretion pathway to the CYP7A1 cholesterol catabolic pathway in the liver. It is interesting to note that CYP7A1 transgenic mice display no hyperlipidemia in spite of having increased hepatic VLDL production. It should also be noted that CYP7A1 transgenic mice display increased hepatic expression of the LDL receptor, a gene whose transcription is SREBP dependent.[161] Apparently, the increased expression of the LDL receptor and CYP7A1 in transgenic mice was sufficient to compensate for the increased hepatic VLDL production. These combined findings emphasize that the balance between the hepatic anabolic and catabolic pathways is a critical determinant of plasma levels of lipoproteins. Our findings support the proposal that SREBP-mediated gene expression links the anabolic VLDL production pathway to the cholesterol/bile acid catabolic pathway.[45]

The metabolic relationship between the bile acid synthetic pathway and VLDL production may help to explain some forms of hyperlipidemia. In several types of hypertriglyceridemic patients, the production of hepatic triglyceride-rich lipoproteins varies in parallel with rates of bile acid synthesis.[162–165] Reduced absorption of bile acids displayed by type IV hypertriglyceridemic patients may be responsible for increased bile acid synthesis.[166] Moreover, the findings showing that treating type IV hyperlipidemic patients with agents that either increase (cholestyramine[167]) or decrease (chenodeoxycholic acid[168]) CYP7A1 expression results in parallel changes in VLDL triglyceride production provide strong evidence indicating the importance of this relationship to human physiology.

A Look Toward the Future

The complex processes controlling hepatic assembly and secretion of lipoproteins begin at the endoplasmic reticulum, where the metabolic fate of de novo synthesized apoB is determined. ApoB can undergo a concerted cotranslational translocation step that is coupled to lipoprotein particle assembly and entrance into the secretory pathway. Conversely, the cotranslational translocation of apoB can become arrested, leading to ubiquitin conjugation and cotranslational degradation by the proteasome. Which of these 2 paths are taken by apoB is a “choice” that is determined by many different parameters, including MTP activity, the appropriate folding and modifications of apoB, and the association of apoB with lipids, which is dependent on their availability. Thus, the metabolic fate of apoB is coordinately linked to the expression of genes controlling hepatic lipid metabolism and the availability of lipids for lipoprotein assembly.

The transcription of many of the genes that encode enzymes regulating energy and lipid metabolism are controlled in part by transcription factors whose activity is dependent on substrates and products of the bile acid biosynthetic pathway. Because CYP7A1 controls hepatic levels of cholesterol, it indirectly affects the content of mature SREBP, an important determinant of the transcription of several regulatory lipo-

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genic enzymes. CYP7A1 also affects hepatic levels of oxysterols and bile acids, which are the ligands that activate the nuclear receptors LXR[169-171] and FXR,[172–175] respectively. The additional finding showing that LXR induces the expression of CYP7A1[176] and SREBP1c[42,177] further indicates the possibility of an additional mechanism linking the bile acid biosynthetic pathway and the VLDL production pathway. Thus, the number and types of metabolic pathways that may be linked to the cholesterol/bile acid synthetic pathway must be expanded from the already diverse group that is regulated by genes whose transcription is controlled by the SREBP family.[36] Gaining an understanding of how these diverse metabolic pathways are linked should provide important new insights linking energy balance and lipid metabolism to nutritional state.

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