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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 (≈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-

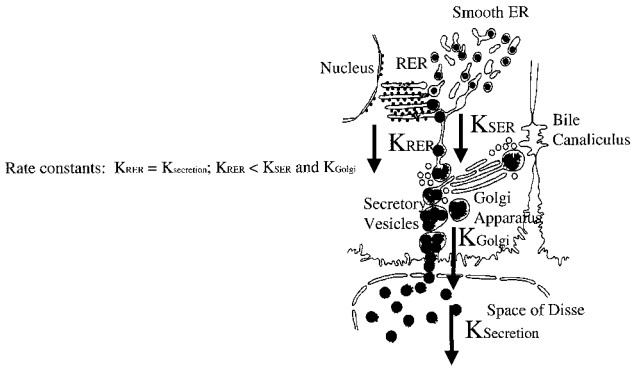

Figure 1. K_{RER}, $K_{Secretion}$, K_{SER}, and K_{Golgi} are rate constants (K), where RER indicates rough endoplasmic reticulum (ER), SER indicates smooth ER, $K_{RER}=K_{Secretion}$, and $K_{RER}<K_{SER}$ and K_{Golgi}. The movement of apoB out of the RER is the rate-limiting step determining the 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

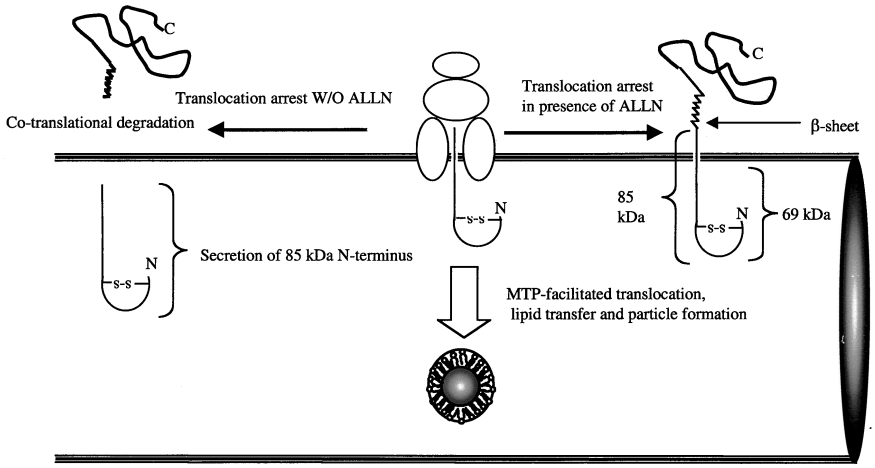

Figure 2. Translocation across the endoplasmic reticulum determines the fate of apoB. C and N indicate the C- and N-termini, respectively; S-S, disulfate bond. In the presence of sufficient lipid, the lipid-binding domains of apoB fold in a manner allowing apoB to be assembled and translocated into the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

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.)

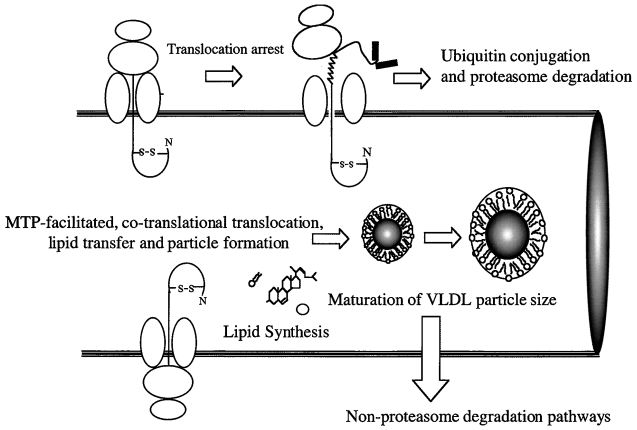

Figure 3. The production of apoB-containing lipoproteins by the liver is regulated by a complex process involving apoB being cotranslationally translocated across the endoplasmic reticulum and assembled into a lipoprotein particle. The translocation of apoB across the endoplasmic reticulum is facilitated 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.

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 ≈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-

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.

References

1. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality: 30 years of follow-up from the Framingham study. *JAMA*. 1987;257: 2176–2180.
2. Schaefer EJ, Lamon FS, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP, Wilson P. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *J Lipid Res*. 1994;35: 779–792.
3. Kreger BE, Odell PM, D'Agostino RD, Wilson P. Long-term intraindividual cholesterol variability: natural course and adverse impact on morbidity and mortality: the Framingham Study. *Am Heart J*. 1994;127:1607–1614.
4. Smith EB. The relationship between plasma and tissue lipids in human atherosclerosis. *Adv Lipid Res*. 1974;12:1–49.
5. Small DM. George Lyman Duff memorial lecture: progression and regression of atherosclerotic lesions: insights from lipid physical biochemistry. *Arteriosclerosis*. 1988;8:103–129.
6. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest*. 1991;88:1785–1792.
7. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
8. Lusis AJ. Atherosclerosis. *Nature*. 2000;407:233–241.
9. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation*. 1995;91:2488–2496.
10. Kane JP. Apolipoprotein B: structural and metabolic heterogeneity. *Annu Rev Physiol*. 1983;45:637–650.
11. Pittman RC, Carew TE, Attie AD, Witztum JL, Watanabe Y, Steinberg D. Receptor-dependent and receptor-independent degradation of low density lipoprotein in normal rabbits and in receptor-deficient mutant rabbits. *J Biol Chem*. 1982;257:7994–8000.
12. Carew TE, Pittman RC, Steinberg D. Tissue sites of degradation of native and reductively methylated [14C]sucrose-labeled low density lipoprotein in rats: contribution of receptor-dependent and receptor-independent pathways. *J Biol Chem*. 1982;257:8001–8008.
13. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease: new perspectives based on the Framingham study. *Ann Intern Med*. 1979;90:85–92.
14. Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler Thromb*. 1993;13:1110–1118.
15. Castellani LW, Weinreb A, Bodnar J, Goto AM, Doolittle M, Mehrabian M, Demant P, Lusis AJ. Mapping a gene for combined hyperlipidaemia in a mutant mouse strain. *Nat Genet*. 1998;18: 374–377.
16. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science*. 1986;232:34–47.
17. Woollett LA, Spady DK, Dietschy JM. Mechanisms by which saturated triacylglycerols elevate the plasma low density lipoprotein-cholesterol concentration in hamsters: differential effects of fatty acid chain length. *J Clin Invest*. 1989;84:119–128.
18. Rudel LL, Kelley K, Sawyer JK, Shah R, Wilson MD. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor–null, human apoB100-overexpressing transgenic mice. *Arterioscler Thromb Vasc Biol*. 1998;18:1818–1827.
19. Straka MS, Junker LH, Zaccaro L, Zogg DI, Dueland S, Everson GT, Davis RA. Substrate stimulation of 7alpha-hydroxylase, an enzyme located in the cholesterol-poor endoplasmic reticulum. *J Biol Chem*. 1990;265:7145–7149.
20. Chao YS, Yamin TT, Alberts AW. Catabolism of low density lipoproteins by perfused rabbit livers: cholestyramine promotes receptor-dependent hepatic catabolism of low density lipoproteins. *Proc Natl Acad Sci U S A*. 1982;79:3983–3986.
21. Spady DK, Bilheimer DW, Dietschy JM. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc Natl Acad Sci U S A*. 1983;80:3499–3503.
22. Bisgaier CL, Essenburg AD, Barnett BC, Auerbach BJ, Haubenwallner S, Leff T, White AD, Creger P, Pape ME, Rea TJ, et al. A novel compound that elevates high density lipoprotein and activates the peroxisome proliferator activated receptor. *J Lipid Res*. 1998;39:17–30.
23. Bilheimer DW, Grundy SM, Brown MS, Goldstein JL. Mevinolin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Proc Natl Acad Sci U S A*. 1983;80:4124–4128.
24. Chao YS, Kroon PA, Yamin TT, Thompson GM, Alberts AW. Regulation of hepatic receptor-dependent degradation of LDL by mevinolin in rabbits with hypercholesterolemia induced by a wheat starch-casein diet. *Biochim Biophys Acta*. 1983;754:134–141.
25. Pedersen TR. Coronary artery disease: the Scandinavian Simvastatin Survival Study experience. *Am J Cardiol*. 1998;82:53T–56T.
26. Davis RA. Evolution of processes and regulators of lipoprotein synthesis: from birds to mammals. *J Nutr*. 1997;127:795S–800S.
27. House EW, Dornauer RJ, Van Lenten BJ. Production of coronary arteriosclerosis with sex hormones and human chorionic gonadotropin (HCG) in juvenile steelhead and rainbow trout, *Salmon gairdneri*. *Atherosclerosis*. 1979;34:197–206.
28. Farrell AP, Johansen JA. Reevaluation of regression of coronary arteriosclerotic lesions in repeat-spawning steelhead trout. *Arterioscler Thromb*. 1992;12:1171–1175.
29. Boogaerts JR, Malone MM, Archambault SJ, Davis RA. Dietary carbohydrate induces lipogenesis and very-low-density lipoprotein synthesis. *Am J Physiol*. 1984;246:E77–E83.
30. Davis RA, Boogaerts JR, Borchardt RA, Malone-McNeal M, Archambault-Schexnayder J. Intrahepatic assembly of very low density lipoproteins varied synthetic response of individual apolipoproteins to fasting. *J Biol Chem*. 1985;260:14137–14144.
31. Leighton JK, Joyner J, Zamarripa J, Deines M, Davis RA. Fasting decreases apolipoprotein B mRNA editing and the secretion of small molecular weight apoB by rat hepatocytes: evidence that the total amount of apoB secreted is regulated post-transcriptionally. *J Lipid Res*. 1990;31:1663–1668.
32. Borchardt RA, Davis RA. Intrahepatic assembly of very low density lipoproteins: rate of transport out of the endoplasmic reticulum determines rate of secretion. *J Biol Chem*. 1987;262:16394–16402.
33. Davis RA, Prewett AB, Chan DC, Thompson JJ, Borchardt RA, Gallaher WR. Intrahepatic assembly of very low density lipoproteins: immunologic characterization of apolipoprotein B in lipoproteins and hepatic membrane fractions and its intracellular distribution. *J Lipid Res*. 1989;30:1185–1196.
34. Jiang H, Ginsberg HN, Wu X. Glucose does not stimulate apoprotein B secretion from HepG2 cells because of insufficient stimulation of triglyceride synthesis. *J Lipid Res*. 1998;39:2277–2285.
35. Taghibiglou C, Carpentier A, Van Iderstine SC, Chen B, Rudy D, Aiton A, Lewis GF, Adeli K. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance: evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *J Biol Chem*. 2000;275:8416–8425.
36. Brown MS, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci U S A*. 1999;96:11041–1108.
37. Nohturfft A, Yabe D, Goldstein JL, Brown MS, Espenshade PJ. Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. *Cell*. 2000;102:315–323.
38. Thewke DP, Panini SR, Sinensky M. Oleate potentiates oxysterol inhibition of transcription from sterol regulatory element-1-regulated promoters and maturation of sterol regulatory element-binding proteins. *J Biol Chem*. 1998;273:21402–21407.
39. Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ. Polyunsaturated fatty acids decrease expression of promoters with sterol

regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J Biol Chem*. 1998;273:25537–25540.
40. Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids: a mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem*. 1999;274: 23577–23583.
41. Spitsen G, Dueland S, Krisans SK, Slattery C, Miyake JH, Davis RA. In non-hepatic cells, cholesterol-7α-hydroxylase induces the expression of genes regulating cholesterol biosynthesis, efflux and homeostasis. *J Lipid Res*. 2000;41:1347–1355.
42. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev*. 2000;14:2819–2830.
43. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest*. 1996;98:1575–1584.
44. Horton JD, Shimano H, Hamilton RL, Brown MS, Goldstein JL. Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J Clin Invest*. 1999;103:1067–1076.
45. Wang S-L, Du E, Martin TD, Davis RA. Coordinate regulation of lipogenesis and the assembly and secretion of apolipoprotein B-containing lipoproteins by sterol response element binding protein 1. *J Biol Chem*. 1997;272:19351–19364.
46. Horton JD, Bashmakov Y, Shimomra I, Shiman H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci U S A*. 1998;95:5987–5992.
47. Davis RA, Thrift RN, Wu CC, Howell KE. Apolipoprotein B is both integrated into and translocated across the endoplasmic reticulum membrane: evidence for two functionally distinct pools. *J Biol Chem*. 1990;265:10005–10011.
48. Verkade HJ, Fast DG, Rusinol AE, Scraba DG, Vance DE. Impaired biosynthesis of phosphatidylcholine causes a decrease in the number of very low density lipoprotein particles in the Golgi but not the endoplasmic reticulum of rat liver. *J Biol Chem*. 1993;268: 24990–24996.
49. Furukawa S, Sakata N, Ginsberg HN, Dixon JL. Studies of the sites of intracellular degradation of apolipoprotein B in Hep G2 cells. *J Biol Chem*. 1992;267:22630–22638.
50. Boren J, Rustaeus S, Wettesten M, Andersson M, Wiklund A, Olofsson S-O. Influence of triacylglycerol biosynthesis rate on the assembly of apoB-100–containing lipoproteins in HepG2 cells. *Arterioscler Thromb*. 1993;13:1743–1754.
51. Bonnardel JA, Davis RA. In HepG2 cells, translocation, not degradation, determines the fate of de novo synthesized apolipoprotein B. *J Biol Chem*. 1995;270:28892–28896.
52. Macri J, Adeli K. Studies on intracellular translocation of apolipoprotein B in a permeabilized HepG2 system. *J Biol Chem*. 1997; 272:7328–7337.
53. Dixon JL, Chattapadhyay R, Huima T, Redman CM, Banerjee D. Biosynthesis of lipoprotein: location of nascent apoAI and apoB in the rough endoplasmic reticulum of chicken hepatocytes. *J Cell Biol*. 1992;117:1161–1169.
54. Wilkinson J, Higgins JA, Groot P, Gherardi E, Bowyer DE. Determination of the intracellular distribution and pool sizes of apolipoprotein B in rabbit liver. *Biochem J*. 1992;288:413–419.
55. Knott TJ, Pease RJ, Powell LM, Wallis SC, Rall SC, Innerarity TL, Blackhart B, Taylor WH, Marcel Y, Milne R, et al. Complete protein sequence and identification of structural domains of human apolipoprotein B. *Nature*. 1986;323:734–738.
56. Yang C-Y, Chen S-H, Gianturco SH, Bradley WA, Sparrow JT, Tanimura M, Li W-H, Sparrow DA, DeLoof H, Rosseneu M, et al. Sequence, structure, receptor-binding domains and internal repeats of human apolipoprotein B-100. *Nature*. 1986;323:738–742.
57. Cladaras C, Hadzopoulou-Cladaras M, Nolte RT, Atkinson D, Zannis VI. The complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48 forms. *EMBO J*. 1986;13:3495–3507.
58. Lingappa VR, Katz FN, Lodish HF, Blobel G. A signal sequence for the insertion of a transmembrane glycoprotein: similarities to the signals of secretory proteins in primary structure and function. *J Biol Chem*. 1978;253:8667–8670.
59. Mize NK, Andrews DW, Lingappa VR. A stop-transfer sequence recognizes receptors for nascent chain translocation across the endoplasmic reticulum membrane. *Cell*. 1986;47:711–719.
60. Chuck SL, Yao Z, Blackhart BD, McCarthy BJ, Lingappa VR. New variation on the translocation of proteins during early biogenesis of apolipoprotein B. *Nature*. 1990;346:382–385.
61. Chuck SL, Lingappa VR. Pause transfer: a topogenic sequence in apolipoprotein B mediates stopping and restarting of translocation. *Cell*. 1992;68:9–21.
62. Chuck SL, Lingappa VR. Analysis of a pause transfer sequence from apolipoprotein B. *J Biol Chem*. 1993;268:22794–22801.
63. Segrest JP, Jones MK, Mishra VK, Anantharamaiah GM, Garber DW. ApoB-100 has a pentapartite structure composed of three amphipathic alpha-helical domains alternating with two amphipathic beta-strand domains: detection by the computer program LOCATE. *Arterioscler Thromb*. 1994;14:1674–1685.
64. Yao Z, Blackhart BD, Linton MF, Taylor SM, Young SG, McCarthy BJ. Expression of carboxyl-terminally truncated forms of human apolipoprotein B in rat hepatoma cells: evidence that the length of apolipoprotein B has a major effect on the buoyant density of the secreted lipoproteins. *J Biol Chem*. 1991;266:3300–3308.
65. Graham DL, Knott TJ, Jones TC, Pease RJ, Pullinger CR, Scott J. Carboxyl-terminal truncation of apolipoprotein B results in gradual loss of the ability to form buoyant lipoproteins in cultured human and rat liver cell lines. *Biochemistry*. 1991;30:5616–5621.
66. Spring DJ, Chen-Liu LW, Chatterton JE, Elovson J, Schumaker VN. Lipoprotein Assembly: Apolipoprotein B determines lipoprotein core circumference. *J Biol Chem*. 1992;267:14839–14845.
67. Wu MJ, Chen LL, Xiao Q, Phillips ML, Elovson J, Linton MF, Young SG, Schumaker VN. Secretion from cell culture of HDL and VLDL bearing apoB-33 with a large internal deletion. *J Lipid Res*. 1997;38:2473–2482.
68. Xiao Q, Elovson J, Schumaker VN. Rat McA-RH7777 cells efficiently assemble rat apolipoprotein B-48 or larger fragments into VLDL but not human apolipoprotein B of any size. *J Lipid Res*. 2000;41:116–125.
69. Young SG. Recent progress in understanding apolipoprotein B. *Circulation*. 1990;82:1574–1594.
70. Schonfeld G. The hypobetalipoproteinemias. *Annu Rev Nutr*. 1995; 15:23–34.
71. Thrift RN, Drisko J, Dueland S, Trawick JD, Davis RA. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line. *Proc Natl Acad Sci U S A*. 1992; 89:9161–9165.
72. Davis RA. The endoplasmic reticulum is the site of lipoprotein assembly and regulation of secretion. In: *Subcellular Biochemistry*. New York, NY: Plenum Press; 1993:169–183.
73. Du E, Kurth J, Wang SL, Humiston P, Davis RA. Proteolysis-coupled secretion of the N-terminus of apolipoprotein B: characterization of a transient, translocation arrested intermediate. *J Biol Chem*. 1994;269:24169–24176.
74. Sakata N, Wu X, Dixon JL, Ginsberg HN. Proteolysis and lipid-facilitated translocation are distinct but competitive processes that regulate secretion of apolipoprotein B in Hep G2 cells. *J Biol Chem*. 1993;268:22967–22970.
75. Du X, Stoops JD, Mertz JR, Stanley CM, Dixon JL. Identification of two regions in apolipoprotein B100 that are exposed on the cytosolic side of the endoplasmic reticulum membrane. *J Cell Biol*. 1998;141: 585–599.
76. Carraway M, Herscovitz H, Zannis V, Small DM. Specificity of lipid incorporation is determined by sequences in the N-terminal 37 of apoB. *Biochemistry*. 2000;39:9737–9745.
77. Du EZ, Wang S-L, Kayden HJ, Sokol R, Curtiss LK, Davis RA. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in abetalipoproteinemia. *J Lipid Res*. 1996;37:1309–1315.
78. Dolphin PJ, Ansari AQ, Lazier CB, Munday KA, Akhtar M. Studies on the induction and biosynthesis of vitellogenin, an oestrogen-induced glycolipophosphoprotein. *Biochem J*. 1971;124:751–758.
79. Luskey KL, Brown MS, Goldstein JL. Stimulation of the synthesis of very low density lipoproteins in rooster liver by estradiol. *J Biol Chem*. 1974;249:5939–5947.
80. Chan L, Jackson RL, Means AR. Female steroid hormones and lipoprotein synthesis in the cockerel: effects of progesterone and nafoxidine on the estrogenic stimulation of very low density lipoproteins (VLDL) synthesis. *Endocrinology*. 1977;100:1636–1643.

81. Wetterau JR, Zilversmit DB. A triglyceride and cholesteryl ester transfer protein associated with liver microsomes. *J Biol Chem*. 1984;259:10863–10866.
82. Wetterau JR, Zilversmit DB. Purification and characterization of microsomal triglyceride and cholesteryl ester transfer protein from bovine liver microsomes. *Chem Phys Lipids*. 1985;38:205–222.
83. Sharp D, Blinderman L, Combs KA, Kienzle B, Ricci B, Wager SK, Gil CM, Turck CW, Bouma ME, Rader DJ, et al. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. *Nature*. 1993;365:65–69.
84. Wetterau JR, Lin MC, Jamil H. Microsomal triglyceride transfer protein. *Biochim Biophys Acta*. 1997;1345:136–150.
85. Gretch DG, Sturley SL, Wang L, Lipton BA, Dunning A, Grunwald KA, Wetterau JR, Yao Z, Talmud P, Attie AD. The amino terminus of apolipoprotein B is necessary but not sufficient for microsomal triglyceride transfer protein responsiveness. *J Biol Chem*. 1996;271:8682–8691.
86. Wang S, McLeod RS, Gordon DA, Yao Z. The microsomal triglyceride transfer protein facilitates assembly and secretion of apolipoprotein B-containing lipoproteins and decreases cotranslational degradation of apolipoprotein B in transfected COS-7 cells. *J Biol Chem*. 1996;271:12124–12133.
87. Wu X, Zhou M, Huang LS, Wetterau J, Ginsberg HN. Demonstration of a physical interaction between microsomal triglyceride transfer protein and apolipoprotein B during the assembly of apoB-containing lipoproteins. *J Biol Chem*. 1996;271:10277–10281.
88. Benoist F, Nicodeme E, Grand-Perret T. Microsomal triacylglycerol transfer protein prevents presecretory degradation of apolipoprotein B-100: a dithiothreitol-sensitive protease is involved. *Eur J Biochem*. 1996;240:713–720.
89. Hussain MM, Bakillah A, Jamil H. Apolipoprotein B binding to microsomal triglyceride transfer protein decreases with increases in length and lipidation: implications in lipoprotein biosynthesis. *Biochemistry*. 1997;36:13060–13067.
90. Wang L, Fast DG, Attie AD. The enzymatic and non-enzymatic roles of protein-disulfide isomerase in apolipoprotein B secretion. *J Biol Chem*. 1997;272:27644–27651.
91. Ingram MF, Shelness GS. Folding of the amino-terminal domain of apolipoprotein B initiates microsomal triglyceride transfer protein-dependent lipid transfer to nascent very low density lipoprotein. *J Biol Chem*. 1997;272:10279–10286.
92. Hussain MM, Bakillah A, Nayak N, Shelness GS. Amino acids 430–570 in apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *J Biol Chem*. 1998;273:25612–25615.
93. Fleming JF, Spitsen GM, Hui TY, Olivier L, Du EZ, Raabe M, Davis RA. Chinese hamster ovary cells require the co-expression of microsomal triglyceride transfer protein and cholesterol-7α-hydroxylase for the assembly and secretion of apolipoprotein B-containing lipoproteins. *J Biol Chem*. 1999;274:9509–9514.
94. Bradbury P, Mann CJ, Kochl S, Anderson TA, Chester SA, Hancock JM, Ritchie PJ, Amey J, Harrison GB, Levitt DG, et al. A common binding site on the microsomal triglyceride transfer protein for apolipoprotein B and protein disulfide isomerase. *J Biol Chem*. 1999;274:3159–3164.
95. Nicodeme E, Benoist F, McLeod R, Yao Z, Scott J, Shoulders CC, Grand PT. Identification of domains in apolipoprotein B100 that confer a high requirement for the microsomal triglyceride transfer protein. *J Biol Chem*. 1999;274:1986–1993.
96. Gordon DA, Jamil H, Gregg RE, Olofsson SO, Boren J. Inhibition of the microsomal triglyceride transfer protein blocks the first step of apolipoprotein B lipoprotein assembly but not the addition of bulk core lipids in the second step. *J Biol Chem*. 1996;271:33047–33053.
97. Benoist F, Grand PT. Co-translational degradation of apolipoprotein B100 by the proteasome is prevented by microsomal triglyceride transfer protein: synchronized translation studies on HepG2 cells treated with an inhibitor of microsomal triglyceride transfer protein. *J Biol Chem*. 1997;272:20435–20442.
98. Leiper JM, Bayless JD, Pease RJ, Brett DJ, Scott J, Shoulders CC. Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. *J Biol Chem*. 1994;269:21951–21954.
99. Gordon DA, Jamil H, Sharp D, Mullaney D, Yao Z, Gregg RE, Wetterau JR. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. *Proc Natl Acad Sci U S A*. 1994;91:7628–7632.
100. Herscovitz H, Kritis A, Talianidis I, Zanni E, Zannis V, Small DM. Murine mammary-derived cells secrete the N-terminal 41% of human apolipoprotein B on high density lipoprotein-sized lipoproteins containing a triacylglycerol-rich core. *Proc Natl Acad Sci, U S A*. 1995;92:659–663.
101. Rusinol AE, Jamil H, Vance JE. In vitro reconstitution of assembly of apolipoprotein B48-containing lipoproteins. *J Biol Chem*. 1997;272:8019–8025.
102. Menzel HJ, Dieplinger H, Lackner C, Hoppichler F, Lloyd JK, Muller DR, Labeur C, Talmud PJ, Utermann G. Abetalipoproteinemia with an ApoB-100-lipoprotein(a) glycoprotein complex in plasma: indication for an assembly defect. *J Biol Chem*. 1990;265:981–986.
103. Jamil H, Gordon DA, Eustice DC, Brooks CM, Dickson JJ, Chen Y, Ricci B, Chu CH, Harrity TW, Ciosek CJ, et al. An inhibitor of the microsomal triglyceride transfer protein inhibits apoB secretion from HepG2 cells. *Proc Natl Acad Sci U S A*. 1996;93:11991–11995.
104. Wang Y, McLeod RS, Yao Z. Normal activity of microsomal triglyceride transfer protein is required for the oleate-induced secretion of very low density lipoproteins containing apolipoprotein B from McA-RH7777 cells. *J Biol Chem*. 1997;272:12272–12278.
105. Jamil H, Chu CH, Dickson JK Jr, Chen Y, Yan M, Biller SA, Gregg RE, Wetterau JR, Gordon DA. Evidence that microsomal triglyceride transfer protein is limiting in the production of apolipoprotein B-containing lipoproteins in hepatic cells. *J Lipid Res*. 1998;39:1448–1454.
106. Raabe M, Flynn LM, Zlot CH, Wong JS, Veniant MM, Hamilton RL, Young SG. Knockout of the abetalipoproteinemia gene in mice: reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc Natl Acad Sci U S A*. 1998;95:8686–8691.
107. Leung GK, Veniant MM, Kim SK, Zlot CH, Raabe M, Bjorkegren J, Neese RA, Hellerstein MK, Young SG. A deficiency of microsomal triglyceride transfer protein reduces apolipoprotein B secretion. *J Biol Chem*. 2000;275:7515–7520.
108. Tietge UJ, Bakillah A, Maugeais C, Tsukamoto K, Hussain M, Rader DJ. Hepatic overexpression of microsomal triglyceride transfer protein (MTP) results in increased in vivo secretion of VLDL triglycerides and apolipoprotein B. *J Lipid Res*. 1999;40:2134–2139.
109. Bakillah A, Nayak N, Saxena U, Medford RM, Hussain MM. Decreased secretion of ApoB follows inhibition of ApoB-MTP binding by a novel antagonist. *Biochemistry*. 2000;39:4892–4899.
110. Liang JS, Wu X, Jiang H, Zhou M, Yang H, Angkeow P, Huang LS, Sturley SL, Ginsberg H. Translocation efficiency, susceptibility to proteasomal degradation, and lipid responsiveness of apolipoprotein B are determined by the presence of beta sheet domains. *J Biol Chem*. 1998;273:35216–35221.
111. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkegren J, Nielsen LB, Wong JS, Hamilton RL, Young SG. Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. *J Clin Invest*. 1999;103:1287–1298.
112. Chang BH, Liao W, Li L, Nakamuta M, Mack D, Chan L. Liver-specific inactivation of the abetalipoproteinemia gene completely abrogates very low density lipoprotein/low density lipoprotein production in a viable conditional knockout mouse. *J Biol Chem*. 1999;274:6051–6055.
113. Klausner RD, Sitia R. Protein degradation in the endoplasmic reticulum. *Cell*. 1990;62:611–614.
114. Hammond C, Helenius A. Quality control in the secretory pathway. *Curr Opin Cell Biol*. 1995;7:523–529.
115. Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell*. 1996;87:391–404.
116. Kopito RR. ER quality control: the cytoplasmic connection. *Cell*. 1997;88:427–430.
117. Brown MS, Ye J, Rawson RB, Goldstein JL. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell*. 2000;100:391–398.
118. Fenteany G, Standaert RF, Lane WS, Choi S, Corey EJ, Schreiber SL. Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science*. 1995;268:726–731.
119. Yeung SJ, Chen SH, Chan L. Ubiquitin-proteasome pathway mediates intracellular degradation of apolipoprotein B. *Biochemistry*. 1996;35:13843–13848.

120. Fisher EA, Zhou M, Mitchell DM. Wu X, Omura S, Wang H, Goldberg AL, Ginsberg HN. The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. *J Biol Chem*. 1997;272:20427–20434.
121. Liao W, Yeung S, Chan L. Proteasome-mediated degradation of apolipoprotein B targets both nascent peptides cotranslationally before translocation and full-length apolipoprotein B after translocation into the endoplasmic reticulum. *J Biol Chem*. 1998;273: 27225–27230.
122. Chen Y, Le CF, Chuck SL. Calnexin and other factors that alter translocation affect the rapid binding of ubiquitin to apoB in the Sec61 complex. *J Biol Chem*. 1998;273:11887–11894.
123. Du EZ, Fleming JF, Wang S-L, Spitzen GM, Davis RA. Translocation-arrested apolipoprotein B evades proteasome degradation via a sterol-sensitive block in ubiquitin conjugation. *J Biol Chem*. 1999;274:1856–1862.
124. Cavallo D, Rudy D, Mohammadi A, Macri J, Adeli K. Studies on degradative mechanisms mediating post-translational fragmentation of apolipoprotein B and the generation of the 70-kDa fragment. *J Biol Chem*. 1999;274:23135–23143.
125. Taghibiglou C, Rudy D, Van Iderstine SC, Aiton A, Cavallo D, Cheung R, Adeli K. Intracellular mechanisms regulating apoB-containing lipoprotein assembly and secretion in primary hamster hepatocytes. *J Lipid Res*. 2000;41:499–513.
126. Yao Z, Tran K, McLeod RS. Intracellular degradation of newly synthesized apolipoprotein B. *J Lipid Res*. 1997;38:1937–1953.
127. Olofsson SO, Asp L, Boren J. The assembly and secretion of apolipoprotein B-containing lipoproteins. *Curr Opin Lipidol*. 1999;10: 341–346.
128. Davidson NO, Shelness GS. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu Rev Nutr*. 2000;20:169–193.
129. Huang XF, Shelness GS. Efficient glycosylation site utilization by intracellular apolipoprotein B: implications for proteasomal degradation. *J Lipid Res*. 1999;40:2212–2222.
130. Liang J, Wu X, Fisher EA, Ginsberg HN. The amino-terminal domain of apolipoprotein B does not undergo retrograde translocation from the endoplasmic reticulum to the cytosol: proteasomal degradation of nascent apolipoprotein B begins at the carboxyl terminus of the protein, while apolipoprotein is still in its original translocon. *J Biol Chem*. 2000;275:32003–32010.
131. Pariyarath R, Wang H, Aitchison JD, Ginsberg HN, Welch WJ, Johnson AE, Fisher EA. Co-translational interactions of apoprotein B with the ribosome and translocon during lipoprotein assembly or targeting to the proteasome. *J Biol Chem*. 2001;276:541–550.
132. Mitchell DM, Zhou M, Pariyarath R, Wang H, Aitchison JD, Ginsberg HN, Fisher EA. Apoprotein B100 has a prolonged interaction with the translocon during which its lipidation and translocation change from dependence on the microsomal triglyceride transfer protein to independence. *Proc Natl Acad Sci U S A*. 1998; 95:14733–14738.
133. Claude A. Growth and differentiation of cytoplasmic membranes in the course of lipoprotein granule synthesis in the hepatic cell, I: elaboration of elements of the Golgi complex. *J Cell Biol*. 1970;47: 745–766.
134. Ehrenreich JH, Bergeron JJ, Siekevitz P, Palade GE. Golgi fractions prepared from rat liver homogenates, I: isolation procedure and morphological characterization. *J Cell Biol*. 1973;59:45–72.
135. Alexander CA, Hamilton RL, Havel RJ. Subcellular localization of B apoprotein of plasma lipoproteins in rat liver. *J Cell Biol*. 1976;69: 241–263.
136. Hamilton RL, Wong JS, Cham CM, Nielsen LB, Young SG. Chylomicron-sized lipid particles are formed in the setting of apolipoprotein B deficiency. *J Lipid Res*. 1998;39:1543–1557.
137. Hamilton RL, Erickson SK, Havel RJ. Nascent VLDL assembly occurs in two steps in the endoplasmic reticulum (ER) of hepatocytes. In: *Atherosclerosis X*. New York, NY: Elsevier Science BV; 1995:414–418.
138. Rustaeus S, Lindberg K, Stillemark P, Claesson C, Asp L, Larsson T, Boren J, Olofsson SO. Assembly of very low density lipoprotein: a two-step process of apolipoprotein B core lipidation. *J Nutr*. 1999; 129:463S–466S.
139. Stillemark P, Boren J, Andersson M, Larsson T, Rustaeus S, Karlsson KA, Olofsson SO. The assembly and secretion of apolipoprotein B-48-containing very low density lipoproteins in McA-RH7777 cells. *J Biol Chem*. 2000;275:10506–10513.
140. Asp L, Claesson C, Boren J, Olofsson SO. ADP-ribosylation factor 1 and its activation of phospholipase D are important for the assembly of very low density lipoproteins. *J Biol Chem*. 2000;275: 26285–26292.
141. Rustaeus S, Lindberg K, Boren J, Olofsson SO. Brefeldin A reversibly inhibits the assembly of apoB containing lipoproteins in McA-RH7777 cells. *J Biol Chem*. 1995;270:28879–28886.
142. Tran K, Wang Y, DeLong CJ, Cui Z, Yao Z. The assembly of very low density lipoproteins in rat hepatoma McA-RH7777 cells is inhibited by phospholipase A2 antagonists. *J Biol Chem*. 2000;275: 25023–25030.
143. Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, Ntambi JM. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J Biol Chem*. 2000;275:30132–30138.
144. Tabor DE, Kim JB, Spiegelman BM, Edwards PA. Transcriptional activation of the stearoyl-CoA desaturase 2 gene by sterol regulatory element-binding protein/adipocyte determination and differentiation factor 1. *J Biol Chem*. 1998;273:22052–22058.
145. Tabor DE, Kim JB, Spiegelman BM, Edwards PA. Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. *J Biol Chem*. 1999;274:20603–20610.
146. Edwards PA, Davis RA. Isoprenoids, sterols and bile acids. In: *New Comprehensive Biochemistry*. Amsterdam, Netherlands: Elsevier; 1996:341–362.
147. Siperstein MD, Jayko ME, Charkoff IL, Dauben WE. Nature of the metabolic products of 14C-cholesterol excreted in bile and feces. *Proc Soc Exp Biol Med*. 1952;81:720–724.
148. Ishibashi S, Schwarz M, Frykman PK, Herz J, Russell DW. Disruption of cholesterol 7α-hydroxylase gene in mice, I: postnatal lethality reversed by bile acid and vitamin supplementation. *J Biol Chem*. 1996;271:18017–18023.
149. Schwarz M, Lund EG, Setchell KDR, Kayden HJ, Zerwekh JE, Björkhem I, Herz J, Russell DW. Disruption of cholesterol 7 α-hydroxylase gene in mice, II: bile acid deficiency is overcome by induction of oxysterol 7α-hydroxylase. *J Biol Chem*. 1996;271: 18024–18031.
150. Davidson NO, Kollmer ME, Glickman RM. Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J Lipid Res*. 1986;27:30–39.
151. Oelkers P, Kirby LC, Heubi JE, Dawson PA. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). *J Clin Invest*. 1997;99: 1880–1887.
152. Small DM. Point mutations in the ileal bile salt transporter cause leaks in the enterohepatic circulation leading to severe chronic diarrhea and malabsorption. *J Clin Invest*. 1997;99:1807–1808.
153. Witztum JL. The role of oxidized LDL in the atherogenic process. *J Atheroscler Thromb*. 1994;1:71–75.
154. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, et al. The Yin and Yang of oxidation in the development of the fatty streak: a review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol*. 1996;16:831–842.
155. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem*. 1997;272:20963–20966.
156. Angelin B, Raviola CA, Innerarity TL, Mahley RW. Regulation of hepatic lipoprotein receptors in the dog: rapid regulation of apolipoprotein B, E receptors, but not of apolipoprotein E receptors, by intestinal lipoproteins and bile acids. *J Clin Invest*. 1983;71: 816–831.
157. Dueland S, Trawick JD, Nenseter MS, MacPhee AA, Davis RA. Expression of 7-alpha-hydroxylase in non-hepatic cells results in liver phenotypic resistance of the low density lipoprotein receptor to cholesterol repression. *J Biol Chem*. 1992;267:22695–22698.
158. Toll A, Wikvall K, Sudjana-Sugiaman E, Kondo KH, Bjorkhem I. 7-Alpha hydroxylation of 25-hydroxycholesterol in liver microsomes: evidence that the enzyme involved is different from cholesterol 7-alpha-hydroxylase. *Eur J Biochem*. 1994;224:309–316.
159. Norlin M, Andersson U, Bjorkhem I, Wikvall K. Oxysterol 7alpha-hydroxylase activity by cholesterol 7alpha-hydroxylase (CYP7A). *J Biol Chem*. 2000;275:34046–34053.
160. Norlin M, Toll A, Bjorkhem I, Wikvall K. 24-Hydroxycholesterol is a substrate for hepatic cholesterol 7alpha-hydroxylase (CYP7A). *J Lipid Res*. 2000;41:1629–1639.

161. Briggs MR, Yokoyama C, Wang X, Brown MS, Goldstein JL. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter, I: identification of the protein and delineation of its target nucleotide sequence. *J Biol Chem*. 1993;268: 14490–14496.
162. Angelin B, Einarsson K, Hellstrom K, Leijd B. Bile acid kinetics in relation to endogenous triglyceride metabolism in various types of hyperlipoproteinemia. *J Lipid Res*. 1978;19:1004–1010.
163. Angelin B, Hershon KS, Brunzell JD. Bile acid metabolism in hereditary forms of hypertriglyceridemia: evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia. *Proc Natl Acad Sci U S A*. 1987;84:5434–5438.
164. Duane WC. Measurement of bile acid synthesis by three different methods in hypertriglyceridemic and control subjects. *J Lipid Res*. 1997;38:183–188.
165. Duane WC, Hartich LA, Bartman AE, Ho SB. Diminished gene expression of ileal apical sodium bile acid transporter explains impaired absorption of bile acid in patients with hypertriglyceridemia. *J Lipid Res*. 2000;41:1384–1389.
166. Duane WC. Abnormal bile acid absorption in familial hypertriglyceridemia. *J Lipid Res*. 1995;36:96–107.
167. Beil U, Crouse JR, Einarsson K, Grundy SM. Effects of interruption of the enterohepatic circulation of bile acids on the transport of very low density-lipoprotein triglycerides. *Metabolism*. 1982;31: 438–444.
168. Camarri E, Marcolongo R, Zaccherotti L, Marini G. The hypotriglyceridemic effect of chenodeoxycholic acid in type IV hyperlipemia. *Biomedicine*. 1978;29:193–198.
169. Lehmann JM, Kliewer SA, Moore LB, Olivier BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, Willson TM. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem*. 1997;272:3137–3140.
170. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc Natl Acad Sci U S A*. 1999;96:266–271.
171. Russell DW. Nuclear orphan receptors control cholesterol catabolism. *Cell*. 1999;97:539–542.
172. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, et al. Bile acids: natural ligands for an orphan nuclear receptor. *Science*. 1999;284:1365–1368.
173. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. *Science*. 1999;284:1362–1365.
174. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell*. 2000;102:731–744.
175. Chawla A, Saez E, Evans RM. "Don't know much bile-ology." *Cell*. 2000;103:1–4.
176. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro J-M, Hammer RE, Mangelsdorf DJ. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR. *Cell*. 1998;93:693–704.
177. DeBose-Boyd RA, Ou J, Goldstein JL, Brown MS. Expression of sterol regulatory element-binding protein 1c (SREBP-1c) mRNA in rat hepatoma cells requires endogenous LXR ligands. *Proc Natl Acad Sci U S A*. 2001;98:1477–1482.

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