Intrahepatic Role of Exchangeable Apolipoproteins in Lipoprotein Assembly and Secretion

Meenakshi Sundaram, Zemin Yao

Abstract—Exchangeable apolipoproteins, composed mainly of amphipathic α-helices, are associated with various plasma lipoproteins and play an important role in the metabolism of those lipoproteins to which they bind. Accumulating experimental evidence suggests that exchangeable apolipoproteins, such as apoE, apoA-IV, and apoC-III, also play a role intracellularly in facilitating lipid recruitment at different stages of very low-density lipoprotein assembly and trafficking through the endoplasmic reticulum-Golgi secretory compartments. This review summarizes findings related to the modulation of intracellular assembly of very low-density lipoprotein and high-density lipoprotein by exchangeable apolipoproteins. (Arterioscler Thromb Vasc Biol. 2012;32:1073-1078.)

Key Words: apolipoproteins ■ atherosclerosis ■ lipids ■ lipoproteins ■ molecular biology

The last decade has witnessed a renewed interest in the study of the exchangeable apolipoprotein family, especially in the intracellular roles they play in the process of assembly and secretion of triglyceride (TG)-rich very low-density lipoprotein (VLDL) and cholesterol-rich high-density lipoprotein (HDL). Exchangeable apolipoproteins, as the name suggests, are proteins that are able to dissociate from 1 lipoprotein and reassociate with another lipoprotein in the circulation. This property is in sharp contrast to nonexchangeable apoB that remains associated with TG-rich lipoproteins from the beginning of lipoprotein assembly/secretion to the end of remnant particle clearance. The exchangeability of exchangeable apolipoproteins is attributable to their high content of α-helical structure, in which 11- and 22-mer amphipathic helices are arranged in tandem, and frequently punctuated by proline residues. These helices display varied affinity toward phospholipids on the surface of lipoproteins. Recent experimental data have suggested that exchangeable apolipoproteins not only play a role extracellularly (in circulation) in modulating lipoprotein catabolism and clearance but also exert an impact intracellularly on the rate and efficiency of lipoprotein assembly/secretion. Although underlying mechanisms responsible for the intracellular role of exchangeable apolipoproteins remain to be further defined, accumulating evidence suggests that they play a role in lipid mobilization and recruitment during lipoprotein assembly and trafficking through the endoplasmic reticulum (ER)-Golgi secretory compartments. This review summarizes the current understanding of the intracellular role of 5 exchangeable apolipoproteins, namely apoE, apoA-IV, apoC-III, apoA-V, and apoA-I, in the process of hepatic lipoprotein assembly/secretion.

Apolipoprotein E

Human apoE is an O-linked glycoprotein (299 amino acids) and is expressed in many cell types including hepatocytes and neuronal cells. Three isoforms of apoE exist (E2, E3, and E4), which are determined by single amino acid substitutions at residues 112 and 158, and they have a profound influence on the susceptibility to atherosclerosis, Alzheimer disease, cerebral amyloid angiopathy, Parkinson disease, and multiple sclerosis. The plasma concentration of apoE is in the range of 3 to 8 mg/dL; most of plasma apoE is found in association with VLDL, chylomicrons, chylomicron remnants, and subclasses of HDL. A major role of apoE in circulation is to facilitate the clearance of lipoproteins by acting as a ligand for members of the low-density lipoprotein receptor family. Intracellularly, expression of apoE is intimately involved in hepatic VLDL assembly/secretion. Experiments with cultured human hepatocellular carcinoma (HepG2) or rat hepatoma (McA-RH7777) cells expressing human apoE showed that upon stimulation of lipogenesis and VLDL production, apoE became associated with apoB-VLDL intracellularly with no effect on HDL production. Although cell culture studies were unable to detect the differential effect of apoE isoforms (ie, E2, E3, or E4) on VLDL secretion, studies on mice expressing normal apoE3 or a mutant form apoE3-Leiden showed that the mutant apoE expression was associated with impaired VLDL-TG secretion and hepatosteatosis, suggesting
that the stimulatory effect of apoE on VLDL production may be isofrom specific. Comparative analysis between Apoe-null and control mice, either in whole animals or using perfused livers and isolated primary hepatocytes, has shown that the rate of in vivo VLDL-TG production was markedly reduced (by >60%) in Apoe-null mice. Production of VLDL was stimulated many fold in Apoe-null mice by restoring apoE3 expression through adenovirus-mediated gene transfer and in transgenic rabbits expressing human apoE3. Moreover, Apoe-null mice are prone to hepatosteatosis, probably as a consequence of impaired mobilization of lipids for VLDL assembly along the ER-Golgi secretory compartments. Accumulation of lipiddic bodies in the ER and ER-derived vesicles was observed in Apoe-null liver cells, suggesting that apoE may facilitate utilization of lipids during VLDL assembly. The hepatic VLDL assembly process is initiated during or immediately after apoB translation and translocation across the ER membrane. Once a pre-VLDL particle is assembled within the ER, it traverses through post-ER compartments (eg, the Golgi apparatus) for further lipidation to form mature VLDL2 (S 20-100) or VLDL1 (S>100) (Figure 1). Maturation of VLDL during trafficking through the Golgi apparatus no longer requires apoE. Current experimental evidence thus suggests that apoE may facilitate the early stage of VLDL assembly process in the ER (Figure 1).

It is known that hepatitis C viral production opportunistically uses the VLDL assembly/secretion pathway. Virions of hepatitis C virus were assembled as apoE-enriched lipoprotein particles, and production and infectivity of hepatitis C virus were increased by apoE expression. Monoclonal antibodies specific to human apoE can efficiently neutralize hepatitis C virus infectivity, making apoE a potential target for hepatitis C virus treatment.

**Apolipoprotein A-IV**

Human apoA-IV, the largest member of the exchangeable apolipoprotein family, is a 376-amino acid glycoprotein present in plasma at concentrations of ~15 mg/dL. The primary structure of apoA-IV contains 13 tandem amphipathic α-helical domains, which are punctuated by proline residues. In humans, apoA-IV is mainly synthesized in intestinal enterocytes and secreted as a constituent of chylomicrons. Synthesis of apoA-IV increased 5-fold under conditions where chylomicron assembly was stimulated upon absorption of long-chain fatty acids, suggesting an involvement of apoA-IV in chylomicron assembly. Expression of apoA-IV in porcine intestinal epithelial cells promoted TG secretion when cell culture media contained exogenous oleate, which was associated with upregulation of microsomal triglyceride transfer protein. It has been shown that apoA-IV was associated with cytosolic lipid droplets in intestinal enterocytes. The mechanism by which apoA-IV can exist as a cytosolic protein has not been defined, nor is it clear whether the cytosolic lipid droplet-bound apoA-IV exerts an impact on chylomicron assembly.

In rodents, apoA-IV is also expressed in the liver, and is found in the plasma in association with VLDL. Evidence suggesting a role of apoA-IV in hepatic VLDL assembly has been obtained from transfection experiments using McA-RH7777 cells expressing rat or human apoA-IV. Under lipid-poor conditions (ie, serum-free media plus 0.1 mM oleate), expression of apoA-IV had no effect on VLDL secretion, and the majority of apoA-IV was secreted as HDL. When cells were

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**Figure 1.** Proposed mechanism for hepatic assembly of triglyceride (TG)-rich very low-density lipoprotein (VLDL). Initial lipid recruitment occurs during and immediately after apoB translation and translocation across the endoplasmic reticulum (ER) membrane. Lipids used for apoB lipidation were made available through the activity of microsomal triglyceride transfer protein (MTP). ApoE appears to facilitate lipid utilization at the early stage of apoB lipidation. Maturation of VLDL is achieved during transit of the nascent pre-VLDL particle from ER through the Golgi apparatus. The rate of transit and particle expansion (through additional lipidation) is modulated by apoA-IV. Final lipid recruitment to form TG-rich VLDL, is enhanced by apoC-III, which promotes TG partitioning into ER-Golgi lumen and promotes incorporation of bulk TG into VLDL.
cultured in lipid-rich media, expression of apoA-IV resulted in increased secretion of TG-rich VLDL, albeit the number of apoB-containing lipoproteins secreted was reduced.33 The apoA-IV-promoted TG-rich VLDL assembly/secretion was not attributable to increased microsomal triglyceride transfer protein activity.34 Rather, kinetic studies suggested that expression of human apoA-IV prolonged the residence time of apoB within the ER-Golgi secretory compartments, allowing sufficient time to undergo core expansion before secretion.34 If this view is correct, then apoA-IV may act as a molecular chaperon escorting nascent pre-VLDL particle traversing through the ER-Golgi secretory compartment (Figure 1). Whether or not apoA-IV achieves this role by directly or indirectly binding to apoB-100 remains to be determined experimentally.

### Apolipoprotein C-III

Expressed mainly in the liver and intestine, apoC-III is a small (79 amino acids) O-linked glycoprotein composed of 6 amphipathic α-helices.35,36 Normal plasma apoC-III concentration is about 10 mg/dL.37 ApoC-III is a major protein component of the VLDL (40% of protein mass) and HDL. In circulation, apoC-III attenuates the activity of lipoprotein lipase38 and interferes with clearance of TG-rich lipoproteins through receptor-dependent or receptor-independent mechanisms.39 Thus, elevated plasma apoC-III concentrations are closely correlated with hypertriglyceridemia in humans and mice.40 An ApoC3-null mutation identified in Lancaster Amish population was associated with hypotriglyceridemia.41

An intrahepatic role of human apoC-III in promoting VLDL assembly/secretion was discovered in transfection studies with human apoC-III-expressing McA-RH7777 cells cultured under lipid-rich conditions (eg, media supplemented with 20% serum plus 0.4 mM oleate) and in ApoC3-null mice in which human apoC-III expression was restored by adenovirus-mediated gene transfer under high-fat diet feeding.52-44 The stimulatory effect of human apoC-III expression was most pronounced for TG-rich VLDL1, assembly/secretion, whereas production of VLDL2 and other TG-poor apoB-containing lipoproteins (eg, intermediate-density lipoprotein and low-density lipoprotein) was unaffected.42-44 The requirement of apoC-III for VLDL1 production suggests that this exchangeable apolipoprotein plays a role in the recruitment of TG during the late state (ie, post-ER) VLDL1 assembly process. There was no evidence that apoC-III directly interacted with apoB-100 during this process. Metabolic labeling experiments showed that expression of human apoC-III in McA-RH7777 cells was associated with enhanced partitioning of newly synthesized TG (as well as diacylglycerol and phosphatidylcholine) into ER-Golgi microsomes.42-44 A mutant form of apoC-III, Lys58Glu, originally identified in 2 Mayan Indians with hypotriglyceridemia,46 also failed to promote TG-rich VLDL1 assembly/secretion under lipid-rich conditions.46 Unlike Lys58Glu, the Ala23Thr mutant exhibited no impairment in promoting partitioning of TG into microsome. However, the microsome-associated TG in Ala23Thr mutant cells was unable to be recruited into VLDL1. It appears that the Ala23Thr mutation at the N-terminal region of apoC-III abolished its function in promoting incorporation of TG substrates during the late stage of VLDL1 assembly. These results raise the possibility that enhanced expression of apoC-III, often under conditions with excessive influx of fatty acids57 or carbohydrates,48 may represent a hepatic protective mechanism to prevent steatosis under stress conditions through purging TG in the form of VLDL1. The intricacy of pathophysiological consequences associated with an intrahepatic role apoC-III—on one hand increasing production of atherogenic TG-rich VLDL1, contributing to hypertriglyceridemia, and on the other hand promoting TG secretion to protect liver from steatosis—is the topic that merits further investigation.

### Apolipoprotein A-V

The apoA-V (368 amino acids) is expressed mainly in the liver and exists in plasma at low concentrations (0.1–0.4 μg/mL).59,60 The low plasma concentration of apoA-V is likely attributable to its inefficient secretion.51 Plasma apoA-V is mainly associated with VLDL and HDL, and its main function appears to counter that of apoC-III and accelerate the rate of plasma TG catabolism.51 A truncated form of apoA-V (Q139X), caused by a nonsense mutation, ablates binding of apoA-V to VLDL and HDL, and is associated with severe hypertriglyceridemia.60,62 Studies on genetically modified mice, such as Apoc3-null, Apoa5-null, Apoc3/Apoa5-null, and mice expressing human apoA-V, provided strong evidence that apoC-III and apoA-V were functionally antagonistic with regard to plasma TG concentrations.53-55 Thus, there was an inverse relationship between apoA-V expression and plasma TG concentration. However, such a relationship did not hold true in patients with hypertriglyceridemia.55,56

The mechanism by which apoA-V could attenuate the production or secretion of TG-rich VLDL is unclear. Experiments with Hep3B cells suggested no direct interaction between apoA-V and apoB-100, thus implying that the association occurred extracellularly after secretion.57 Similar to what was observed for apoA-IV, apoA-V was also present on cytosolic lipid droplets in Hep3B and McA-RH7777 cells.57,58 suggesting that apoA-V may play a role in sequestering TG for storage.51 The functional significance of apoA-V binding to cytosolic lipid droplets and its relationship to TG-rich lipoprotein secretion are yet to be determined.

### Apolipoprotein A-I

Human apoA-1 is a 243-amino acid protein which is mainly synthesized in the liver and small intestine and is a major
protein constituent of HDL. Plasma concentration of apoA-I is \( \approx 100 \) mg/dL in humans.\(^5\) Multiple functions have been ascribed to plasma apoA-I, such as acting as a cofactor of lecithin-cholesterol acyltransferase,\(^6\) inhibiting low-density lipoprotein oxidation, stabilizing paraoxonase/arylesterase (PON1) in HDL,\(^7\) and antioxidant and anti-inflammatory properties in mice.\(^8\) In addition, apoA-I may exert an anti-obesity effect in mice by increasing overall energy expenditure and uncoupling protein expression in brown adipose tissue.\(^9\)

Studies of ATP-binding cassette transporter A1 (ABCA1) have revealed that the liver is the most important site of apoA-I/HDL production, and that the formation of apoA-I/HDL required the activity of ABCA1.\(^10\) Acquisition of lipids (eg, phospholipids) by apoA-I was probably through interaction between apoA-I and ABCA1 at cell surface.\(^11\) However, lipidation of apoA-I also occurred intracellularly in liver hepatocellular carcinoma (HepG2) cells\(^12\) and in primary mouse hepatocytes.\(^13,14\) Intracellularly, acquisition of phospholipids and cholesterol by apoA-I in mouse hepatocytes could be achieved through ABCA1-dependent and ABCA1-independent mechanisms.\(^15\) Intracellular lipidation of apoA-I in mouse hepatocytes appeared to involve both lumen and membrane of the ER and medial Golgi.\(^16\) Studies with some cell types also suggested that lipidation of apoA-I could occur through a process termed retroendocytosis, in which the ABCA1-dependent process was accompanied by endocytosis and resecretion of exogenous apoA-I.\(^17\) (Figure 2). Whether or not the retroendocytosis process occurs in hepatocytes is currently unknown.

Hepatic HDL production may also exert an effect on VLDL secretion. Enhanced cholesterol efflux, as a consequence of overexpression of ABCA1, resulted in diminished secretion of apoB-containing lipoproteins, likely attributable to diversion of lipids (eg, cholesterol) to the HDL pathway.\(^18\) Cell culture studies suggested that this inverse relationship between HDL and VLDL secretion from hepatocytes was related to phosphatidylinositol-3 kinase activation.\(^19\) It appears that hepatic HDL particles, assembled by ABCA1, could elicit a phosphatidylinositol-3 kinase–mediated autocrine signal that attenuated VLDL secretion.\(^20\) These results raise an intriguing possibility that hepatic lipid homeostasis is coordinately regulated intracellularly by secretion of VLDL and HDL as 1 of the major elements.

**Concluding Remarks and Perspectives**

It is increasingly recognized that the secretory, exchangeable apolipoproteins, such as apoE, apoA-IV, apoC-III, and apoA-I, contribute to the overall regulation of hepatic lipid homeostasis during their transit from the ER through the Golgi apparatus, which coincides with the lengthy journey of assembly and secretion of VLDL and HDL. In the case of VLDL, apoE appears to facilitate lipid recruitment at the early and intermediate stages of apoB lipidation, whereas apoA-IV (probably through interacting with apoB) regulates the rate of apoB trafficking (and lipoprotein expansion) through the ER-Golgi compartments. The final stage of apoB lipidation (ie, formation of TG-rich VLDL, \( \text{VLDL}_{\text{II}} \)) is enhanced by the expression of apoC-III under lipid-rich conditions, presumably through effective mobilization and incorporation of bulk TG into VLDL. In the case of HDL, acquisition of lipid by apoA-I occurs as early as in the ER and continues throughout the ER-Golgi-plasma membrane secretory pathway. Because secretion and efflux of lipids represent an important component of overall hepatic lipid homeostasis, and because secretion and efflux of lipids are invariably assisted by proteins, particularly the exchangeable apolipoproteins, unraveling the temporal and spatial events during the protein-lipid interactions will lead to better understanding of how these processes are regulated. What remains to be determined is a comprehensive view of protein and lipid factors involved in the complex lipoprotein assembly/secretion process.\(^21\) It is anticipated that the next decade will witness a surge in the studies of protein-protein, protein-lipid, and lipid-lipid interactions during intracellular lipoprotein assembly using systems biology approaches. It is also anticipated that a deeper understanding of structural determinants within the exchangeable apolipoproteins will be gained through the use of new physicochemical and imaging tools.\(^22\)

**Figure 2. Proposed mechanism for hepatic production and secretion of apoA-I/high-density lipoprotein (HDL).** Lipidation of apoA-I occurs in the endoplasmic reticulum (ER) and Golgi apparatus via ATP-binding cassette transporter A1 (ABCA1)-dependent and ABCA1-dependent mechanisms in hepatocytes (steps 1–3). Acquisition of lipids by apoA-I also occurs at the cell surface in an ABCA1-dependent fashion (step 4). In some cell types, apoA-I lipidation has been achieved through a process termed retroendocytosis that also requires active ABCA1 (step 5).
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References
3. Elshourbagy NA, Liao WS, Mahley RW, Taylor JM. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. Proc Natl Acad Sci USA. 1985;82:203–207.


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