Hepatitis C virus (HCV) infects more than 170 million people worldwide. According to the Centers for Disease Control and Prevention, 4.1 million Americans are estimated to be infected by HCV, 3.2 million of whom become chronically infected. These individuals account for most cases of liver failure in the United States. The most effective therapy for HCV infection involves inhibiting a HCV-encoded enzyme. However, the HCV genome rapidly acquires mutations that render the virus drug resistant because of the low fidelity of the viral replication machinery. Thus, these inhibitors must be combined with interferon to significantly improve treatment outcome of HCV infection. Because of the expense and severe side effects that accompany interferon treatment, the search for new strategies to treat HCV infection is merited.

HCV is a single-stranded positive-sense RNA virus of the Flaviviridae family. The 9.6-kb HCV genome encodes a single polyprotein that is posttranslationally processed into at least 10 structural and nonstructural (NS) proteins (Figure 1). The amino-terminal one-third of the polyprotein encodes 3 virion structural proteins: core, E1, and E2. The remainder of the genome encodes NS proteins that are not found in viral particles but instead are required for replication and assembly of the virus. The NS3, NS4A, NS4B, NS5A, and NS5B proteins, which are necessary and sufficient for replication of viral RNA, form a viral replication complex on endoplasmic reticulum (ER) membranes.

Association of HCV With Very Low-Density Lipoprotein
An intriguing feature of HCV is that the viral particles are found in complex with very low-density lipoprotein (VLDL), which plays an important role in transporting cholesterol and triglyceride from the liver to peripheral tissues. VLDL contains a hydrophobic core of neutral lipids consisting of triglycerides and cholesteryl esters surrounded by a surface coat containing phospholipids, free cholesterol, and lipoproteins, including apolipoprotein B (apoB) and apoE. HCV particles isolated from the serum of virus-infected patients exhibited a density similar to that of VLDL. Moreover, these particles were rich in triglyceride and contained apoB and apoE. Recently, Merz et al developed a strain of HCV in which the E2 protein was tagged with a FLAG epitope and purified the HCV virion produced from the human hepatoma Huh7 cells through affinity purification with anti-FLAG. The purified HCV virion appeared to contain more apoE than viral proteins at the surface of the particles. Lipidomic analysis revealed that cholesteryl esters made up almost half of the total lipid content in the affinity-purified viral particles. In sharp contrast, the viral envelope derived from host cell membranes is predominantly composed of phospholipids. The triglyceride content in the purified viral particles was not determined because of the technical difficulty.

Electron microscopy (EM) analysis of HCV either isolated from patient serum or affinity purified from the culture medium of virus-infected Huh7 cells revealed structures that contained lipid-rich cores resembling lipoprotein particles rather than the classic viral capsid structure. These observations suggest that the viral genome and capsid may be hidden within the hydrophobic core of VLDL. This structure may allow HCV to evade B cell/antibody–mediated immune surveillance during circulation, thereby providing a natural advantage.
plausible explanation as to why the viral infection cannot be effectively prevented by vaccination. In contrast to these studies, EM analysis revealed structures resembling enveloped viral particles in a fraction of culture medium enriched in HCV infectivity. Thus, HCV may exist as multiple forms, and more sophisticated EM analyses, such as cryo-EM, which is capable of visualizing structures within the hydrophobic cores of VLDL, may be necessary to identify capsid structure of HCV.

**Assembly of HCV-VLDL Complex**

The hepatic synthesis of VLDL requires generation of lipid droplets enriched in neutral lipids, such as triglycerides and cholesteryl esters, in the ER lumen. These lipid droplets are produced by reactions catalyzed by microsomal triglyceride transfer protein (MTP). Although it has not been formally demonstrated, apoE might also play an important role in generating these lipid droplets. On fusion with apoB, these lipid droplets can be secreted out of cells as nascent VLDL through exocytosis. In addition to generating lipid droplets in the ER lumen, MTP also stabilizes apoB during translation by transferring lipids to the nascent polypeptide chain of apoB. In the absence of this lipid transfer, the secretion of apoB is blocked, and the protein is rapidly degraded in cells. VLDL secretion also requires hepatic synthesis of phosphatidylcholine, the major phospholipid on the surface of the lipoprotein particles. In human hepatoma Huh7 cells, long chain acyl-coenzyme A synthetase 3 (ACSL3)–mediated phosphatidylcholine synthesis is required for secretion of apoB.

Proteomic analysis of ER membrane vesicles containing HCV RNA and viral replication complex composed of viral proteins NS3 to NS5B revealed that these vesicles were enriched in apoB, apoE, MTP, and ACSL3. The reason for colocalization of the HCV replication and VLDL assembly appears to lie in a requirement for coassembly and secretion of VLDL and HCV particles. Thus, secretion of HCV virion from virus-infected Huh7 cells was inhibited when cells were treated with pharmacological inhibitors of MTP. Secretion of HCV was also inhibited in cells transfected with a small interfering RNA targeting apoE or ACSL3. The results regarding apoB are not consistent: knockdown of apoB was shown to block HCV secretion in 2 studies, but had no effect on release of HCV virion in another study. This discrepancy is most likely caused by the different HCV infection system used in the studies. In the reports showing that apoB was required for secretion of HCV, care was taken to ensure that HCV infection did not result in cellular apoptosis so that viral particles were only released through exocytosis. In contrast, the study showing the opposite result used a system known to cause apoptosis of virus-infected cells. Consequently, intracellular infectious HCV particles containing NS5A were released into culture medium from dying cells. Because apoB is not required to produce intracellular HCV particles, it is not surprising to observe apoB-independent production of infectious HCV particles in culture medium using this system.

A puzzling question regarding assembly of the HCV-VLDL complex is how viral genome synthesized at cytosolic face of the ER is transported across the membrane bilayers to reach the ER lumen, where it is packaged into VLDL. A clue to the question may come from a unique property of the HCV-encoded capsid core protein. The HCV core protein contains 2 domains: an NH2-terminal hydrophilic domain (D1) that binds viral RNA, and a COOH-terminal hydrophobic domain (D2) that interacts with neutral lipid. In HCV-infected Huh7 cells, the majority of core proteins are localized at the surface of cytosolic lipid droplets that are in contact with ER membranes containing the HCV replication complex, which is also the site for VLDL assembly. Thus, HCV may replicate at an ER domain enriched in neutral lipids that can bud toward cytosol or lumen to form lipid droplets in both locations. A hypothetic model is proposed in Figure 2 to explain translocation of HCV capsid based on this localization. Core is targeted to cytosolic lipid droplets through its D2 domain after it is cleaved from the viral polyprotein. The hydrophilic D1 domain is exposed to the cytosol, ready to accept viral RNA synthesized by the viral replication complex (Figure 2A). Once associated with viral RNA, core protein undergoes a conformational change so that hydrophilic residues that bind viral RNA are folded inside, whereas hydrophobic residues are exposed at the surface. This conformational change allows the core-RNA complex to become completely embedded in the hydrophobic core of lipid droplets (Figure 2B). The viral capsid-RNA complex then travels through the neutral lipid-rich ER membrane to reach lipid droplets in the ER lumen. The HCV-containing luminal lipid droplets then fuse with apoB, acquire 2 other lipoprotein-like viral structural proteins, E1 and E2, and are secreted out of the cells through exocytosis (Figure 2C).

The model shown in Figure 2 predicts that HCV capsids are able to enter the hydrophobic core of lipid droplets. Although there have not been many studies characterizing localization of cellular proteins in hydrophobic cores of lipid droplets, such localization was reported through EM analysis. Additional studies are required to determine whether these host proteins facilitate translocation of HCV capsids. This model also predicts that HCV capsids travel across the membrane through neutral lipid-rich domain of the ER. ApoB has been reported to translocate from the ER lumen into the cytosol though a mechanism that involves lipid droplets. This observation implies that lipid droplets across ER membranes are continuous and that proteins may be able to transport across ER membranes through lipid droplet intermediates.
is not required for entry of HCV pseudoparticles, which are assembled by displaying HCV structural proteins E1 and E2 onto retroviral core particles that are not in complex with lipoproteins.55

Mutations disrupting the function of the LDLR have been identified in humans. These mutations produce autosomal dominant familial hypercholesterolemia, which affects 0.2% of the world’s population.56 Affected individuals have elevated plasma levels of low-density lipoprotein cholesterol, which causes premature coronary atherosclerosis. However, during evolution when dietary cholesterol was scant, these mutations may not have produced such a severe phenotype but may actually have protected these individuals from infection with HCV or an HCV-related virus. An analysis comparing the frequency of HCV infection in people expressing normal LDLR versus those affected by familial hypercholesterolemia will be needed to test this hypothesis. This hypothesis may also be tested with mice containing human liver grafts, an animal model successfully used to study HCV infection.55-57 If this hypothesis is correct, mice grafted with human liver derived from patients affected by familial hypercholesterolemia are expected to resist infection by HCV.

Treating HCV Infection With Drugs Targeting VLDL Metabolism

The dependence on VLDL in the life cycle of HCV offers opportunities to treat the viral infection with drugs targeting VLDL metabolism. Several MTP inhibitors have already been tested in clinical trials because of their ability to block VLDL secretion, thereby lowering the plasma levels of VLDL triglycerides and low-density lipoprotein cholesterol.58,59 Long-term treatment with MTP inhibitors leads to the accumulation of fat in livers, thus hampering the approval of these drugs for treatment of hypercholesterolemia, which may require lifelong administration in the case of familial hypercholesterolemia.58,59 However, short-term treatment (up to several weeks) reduced the plasma level of VLDL with only minor adverse effects, which disappeared after drug removal.58 Because the standard treatment for HCV infection with drugs targeting the viral enzymes lasts only for about 12 weeks, MTP inhibitors may be combined with these drugs to treat HCV infection. MTP inhibitors also have an advantage in that they target a host protein rather than viral proteins, so they are less likely to face the drug-resistance problem caused by mutations in the viral genome.

Another drug that inhibits VLDL assembly is an antisense RNA drug targeting apoB.50 Unlike MTP inhibitors, apoB antisense RNA lowered VLDL secretion in the absence of accumulation of fat in livers.58 However, in cultured cells, knockdown of apoB by small interfering RNA was less potent than MTP inhibitors to inhibit HCV production.12,28 Thus, more studies are required to determine the efficacy of apoB antisense RNA on treatment of HCV infection in vivo.

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References


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