Hepatitis C virus: viral proteins on the move

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Abstract

There is now increasing evidence that LDs (lipid droplets) play a central role in the production of infectious HCV (hepatitis C virus) and participate in virus assembly. Two viral proteins, namely core, which forms the capsid, and NS5A (non-structural 5A protein), a component of complexes engaged in viral RNA synthesis, are detected at LD surfaces in infected cells. Interactions between the two proteins may be critical for anchoring RNA replication sites to droplets for initiating virus assembly. The requirements for targeting of core in particular has received considerable attention since the nature of its interaction with LDs could play a key role in determining the efficiency of virion production. As well as attaching to droplets, core is able to alter their intracellular distribution and direct them towards the microtubule organizing centre. Inhibitors that disrupt microtubules block this redistribution by core and there is a concomitant decrease in virus production. Therefore altered dynamics of LDs may contribute to HCV assembly and release. The purpose of targeting LDs by HCV may be linked to their contribution to the formation of VLDLs (very-low-density lipoproteins) in hepatocytes since virus circulating in infected patients is associated with lipoprotein. Thus HCV may utilize the role played by LDs in the formation of lipoprotein particles as part of its life cycle and access this pathway by direct interaction of viral components with these intracellular storage organelles.

Introduction

Present estimates predict that 2.2% of the world's population has been infected with HCV (hepatitis C virus), which equates to approx. 130 million people [1,2]. Therefore HCV is a global health care problem and is likely to remain so for the foreseeable future. The virus typically establishes a chronic infection in the liver that can promote serious hepatic disorders over a period of decades [3,4]. Consequently, HCV infection is now a lead indicator for liver transplantation. Currently, the only approved therapy is a combination of PEGylated IFNα (interferon α) and ribavirin that successfully eradicates the virus in approx. 54% of infected individuals [5].

HCV has a positive-sense, single-stranded RNA genome of approx. 9.6 kb, which encodes a polyprotein of approx. 3010 amino acids [6,7]. The open reading frame encoding the polyprotein is flanked by 5'-UTR (5'-untranslated region) and 3'-UTR elements that are important for translation and replication of the viral RNA (Figure 1A). The N-terminal component of the polyprotein encodes three structural proteins: core, which forms the capsid, and two envelope glycoproteins called E1 and E2. The C-terminal region of the polyprotein contains the NS (non-structural) proteins that form replication complexes for synthesis of viral RNA [8,9]; many of the NS proteins also have a role in virion assembly [10–16]. The structural components are released from the polyprotein by cellular proteases, whereas the mature NS proteins are produced by virus-encoded proteases [6,7] (Figure 1A).

Targeting of the HCV proteins to lipid droplets

Before the availability of a tissue culture system for producing infectious HCV, two viral proteins, core and NS5A, were reported to associate with LDs (lipid droplets) [17–20]. Detecting NS5A at the surface of LDs is less obvious compared with core as much of the protein is located at the ER (endoplasmic reticulum) membrane [21]. NS5A can be separated into three domains (D1–D3) with D1 and D2 playing key roles in replication of viral RNA, whereas D3 is necessary for assembly of infectious virus (Figure 1B) [16,22–26]. From tissue culture studies with the system that produces infectious virions, mutations in D1 impair LD localization but D3 can be largely removed without affecting NS5A detection at the surface of droplets [27]. However, additional studies are required to define more precisely the sequence requirements and nature of the interaction of NS5A with droplets.

By contrast, there is much more information available about the targeting of core to LDs. First, association of core with LDs relies on the release of the protein from the HCV polyprotein [28]. Maturation of core requires processing by two cellular proteases: signal peptidase and signal peptide peptidase [28–30]. Signal peptidase cleaves at the C-terminus of a signal peptide that lies between core and the E1 glycoprotein, thereby producing an immature form of core and the N-terminus of E1 (Figure 1A). This immature form of core is unable to attach to droplets. The second cleavage event is executed by signal peptide peptidase, which cuts within

Key words: fluorescence recovery after photobleaching (FRAP), hepatitis C virus (HCV), lipid droplet, viral protein, virus assembly.

Abbreviations used: ADAR, adenosine differentiation-related protein; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ER, endoplasmic reticulum; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; HCV, hepatitis C virus; LD, lipid droplet; MTP, microsomal transfer protein; NS, non-structural protein; UTR, untranslated region; VLDL, very-low-density lipoprotein.

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the signal peptide at the C-terminal end of immature core to give the mature form of the protein (Figure 1A). This mature form of core is then directed from the ER membrane, where these cleavage events occur, to the surface of LDs [28]. After signal peptide peptidase cleavage, attachment of core to LDs is mediated by the C-terminal domain of the protein, which is termed D2 [31]. D2 is approx. 55 amino acids in length and possesses two amphipathic α-helices (H1 and H11) separated by a hydrophobic segment termed the hydrophobic loop ('HL' in Figure 1B) [32]. From modelling predictions, it is anticipated that D2 interacts in-plane with the surface of droplets [32]. In support of this model, mutation of hydrophobic amino acids to hydrophilic residues in H1, H11 and HL can prevent association of core with LDs [32]. By contrast, mutation of hydrophilic residues in H1 and H11 does not impair LD attachment. The N-terminal segment of core upstream from D2, called D1, is dispensable for targeting to droplets but has important functions in RNA binding and protein–protein interactions [31].

Attachment of core and NS5A to lipid droplets is necessary for production of infectious virus

Studies with an infectious HCV system have demonstrated that core initially attaches to a punctate site on droplets after processing by signal peptide peptidase and then progressively coats the entire organelle [33,34]. This observation has led to speculation for a defined loading site for core, possibly at points of contact between LDs and the ER membrane [33]. The ability of core to coat the entire surface of droplets correlates with virus production since mutations in the D2 domain that impair this process do not yield infectious virions [33]. Similarly, blocking LD localization of core by disrupting maturation of the protein by signal peptide peptidase lowers the titre of infectious virus released from cells to barely detectable levels [35]. For NS5A, the mutations in its D1 domain that block LD attachment prevent production of infectious progeny [27]. Interestingly, removal of most of the D3 domain of NS5A does not impair its targeting to LDs but the protein is found on droplets that do not contain core [10]. Mutations at serine residues towards the C-terminal end of D3 also disrupt interaction with core [36]. Thus it would appear that it is not only the targeting of core and NS5A to LDs that is critical for virus production, but also the ability of the two proteins to interact on the organelle.

The nature of the interaction between core and lipid droplets affects virus production

As discussed above, there is more information available concerning the attachment of core to LDs as compared
with NS5A. Since the D1 domain of core is dispensable for targeting, the interaction of D2 with droplets can be examined by linking it to a heterologous protein, such as GFP (green fluorescent protein) [32]. This approach has proved highly beneficial for identifying amino acids that are critical for LD targeting. An added advantage to using GFP to examine targeting is that it also enables analysis of the dynamics of proteins attached to organelles by biophysical methods such as FRAP (fluorescence recovery after photobleaching) (Figure 2A) [13,37,38]. Application of this method has revealed that even single amino acid substitutions in D2 alter the trafficking of GFP–D2 chimaeric proteins between LDs [39]. Changes in the rate of exchange between LDs are presumed to reflect the strength of binding of D2 to the organelle (Figure 2B). Thus GFP–D2 molecules with slower exchange are bound more tightly to droplets. Changes to strength of binding characterized by this approach also affect virus production; in one example, mutation of alanine to a valine residue (amino acid position 147 in HCV core) in the hydrophobic loop lowered D2 mobility, suggesting increased adherence to LDs, and this substitution also enhanced virus production [39]. Whether increasing the strength of binding to droplets by mutation at other residues in D2 has a similar effect on virus production remains to be determined. Nonetheless, these studies illustrate that even subtle changes to the D2 sequence, which do not impair targeting to droplets but do modify the nature of the interaction with the organelle, can be a contributory factor in virus assembly and release.

Core protein alters the intracellular distribution of lipid droplets

LDs are typically located throughout the cytoplasm of cells and are probably attached to the ER membrane. Several reports have also highlighted their capacity for rapid movement by transient attachment to microtubules [40–44]. Interestingly, attachment of core changes the intracellular distribution of droplets such that they have a tendency to aggregate towards the periphery of the nucleus [45]. This aggregation occurs principally around the microtubule-organizing centre but can be blocked by either disrupting the microtubule network or impairing the function of the dynein motor protein [45]. Hence, it is thought that core protein modifies the microtubule–dependent mobility of LDs. Core is also able to displace ADRP (adipocyte differentiation-related protein), the major surface protein on droplets [45]. Therefore ADRP or other droplet-associated proteins that are removed by core could regulate the intracellular distribution of LDs and their interaction with microtubules.

The ability of core to modify the distribution of droplets appears to be important for virus production since disrupting the microtubule network reduces virus production [45]. One possibility is that core changes the intracellular location of droplets to aid their close apposition with sites of viral RNA synthesis that contain newly synthesized genomes and the NS proteins. HCV RNA synthesis occurs at modified locations on the ER membrane but, in cells producing virus progeny, viral RNA is detected also in fractions containing LDs [27]. Moreover, close apposition of HCV RNA and LDs in infected cells is dependent on the association of core with droplets [35,46]. Such sites may represent locations for transfer of viral RNA to core protein to initiate genome packaging and nucleocapsid assembly, the first stages in the production of virions. Since core and NS5A form protein-protein interactions [36], these two viral proteins may form the bridge between LDs and the sites of viral RNA replication.

Possible role of lipid droplets in the assembly and release of infectious HCV

The purpose of directing viral proteins to LDs potentially to initiate virus assembly is a subject of some debate. Infectious virus particles released from tissue culture cells have low density and are approx. 70 nm in diameter [47]. These characteristics are similar to those of HCV virions isolated from patients, which are known to be complexed with lipoprotein [48,49]. Virion assembly and release from tissue culture cells is sensitive to inhibition of MTP (microsomal transfer protein) and reductions in the abundance of apolipoproteins ApoB (apolipoprotein B) and ApoE (apolipoprotein E) [50–52]. Hence, current models predict that assembly and release of HCV virions occur in concert with the production of VLDL.
Figure 3 | Proposed model for assembly of VLDL and HCV virions in hepatocytes

(A) VLDL assembly occurs through initial lipidation of translocated ApoB by MTP to create a pre-VLDL particle. Bulk triacylglycerol from cytosolic LDs is added to pre-VLDL particles through a process that is thought to produce luminal LDs. Additional lipoprotein components are added (e.g. ApoE; results not shown) to generate mature VLDL that is released from the cell. (B) Co-assembly of HCV virion-lipoprotein particles to produce lipoviroparticles [48]. HCV core associates with cytosolic LDs and its interaction with NS5A at sites of HCV RNA replication enables engagement of viral genomes with core to initiate virus assembly, possibly creating nucleocapsids. Assembly proceeds through unknown processes in which the viral envelope glycoproteins are added to nucleocapsids and combine also with the VLDL assembly pathway to yield lipoviroparticles that are released from the cell.

References
1 Alter, M.J. (2007) Epidemiology of hepatitis C virus infection. World J. Gastroenterol. 13, 2436-2441

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