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Entangled in a Membranous Web: ER and Lipid Droplet Reorganization during Hepatitis C Virus Infection

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Abstract

Hepatitis C virus (HCV) is a major cause of liver disease worldwide. To establish and maintain chronic infection, HCV extensively rearranges cellular organelles to generate distinct compartments for viral RNA replication and virion assembly. Here, we review our current knowledge of how HCV proliferates and remodels ER-derived membranes while preserving and expanding associated lipid droplets during viral infection. Unraveling the molecular mechanisms responsible for HCV-induced membrane reorganization will enhance our understanding of the HCV life-cycle, the associated liver pathology, and the biology of the ER:lipid droplet interface in general.

Introduction

Hepatitis C virus (HCV) is a significant health burden: 170–185 million individuals are infected worldwide [1,2]. Most infections result in a life-long condition that increases risk of liver cirrhosis and hepatocellular carcinoma [1]. New anti-HCV drugs that eradicate the virus do not reverse end-stage liver disease, are very expensive, and unavailable in resource-poor countries where most infected people live [3].

HCV is an enveloped, positive-strand RNA virus of the family of *Flaviviridae* [4]. Unlike other *Flaviviridae*, such as yellow fever virus and dengue virus, HCV is not mosquito-borne and infects mainly hepatocytes after contact with infected blood. The virus replicates continuously to high titers within the cytoplasm of infected cells, a process that revolves around the endoplasmic reticulum (ER) and associated lipid droplets (LDs). HCV engages these organelles in multiple ways (Figure 1): 1) After viral entry, the ~9.6-kb single-stranded HCV RNA genome is translated at the rough ER into a single large polyprotein that is proteolytically processed into 10 functional HCV proteins, which are all, except the NS3 protease, firmly integrated in or associated with the ER membrane (Box 1). 2) The C-terminal region of the polyprotein gives rise to nonstructural viral proteins, which form a replicase complex that propagates the viral RNA genome within a newly formed ER-derived web-like membranous compartment that consumes considerable space and membrane resources in the cytoplasm of infected cells. 3) The N-terminal part of the polyprotein

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produces structural viral proteins that reside on the surface of LDs or are firmly anchored within the ER membrane to assemble immature progeny virions that eventually bud into the ER. 4) Within the ER lumen, progeny virion production intersects with intraluminal LDs to produce mature “lipoviroparticles” that are released from producer cells via the lipoprotein pathway [3].

Here, we focus on points 2 and 3 and on recent insights into how HCV infection induces proliferation of ER membranes and manipulates LDs within the cytoplasm of infected hepatocytes.

Molecular architecture of the membranous web

Positive-strand RNA viruses induce extensive cytoplasmic membrane proliferation and remodeling [5]. Induced membrane structures provide favorable microenvironments for compartmentalization of viral RNA replicase complexes. Here, the local concentration of host and viral factors required for efficient RNA replication is increased, and replication is protected from nuclease-mediated degradation and host cell antiviral responses [3,6–9]. HCV is unique as it induces a matrix of cytoplasmic double-membrane vesicles (DMVs) (Figure 1) [10,11]. This is different from other flaviviruses, which mainly form invaginated vesicles within the ER [5,12]. DMVs have diameters of 150–200 nm [13,14] and contain active replicase complexes, supporting the model that they form to support viral RNA replication [8,13]. The outer membranes of ~50% of DMVs connect to the ER membrane via a neck-like structure [13,15]. Biochemical analyses of purified membranes reveal ER-resident calnexin and calreticulin proteins, confirming the ER as a major membrane source for DMVs [15–18].

For some time, viral NS4B protein, an integral membrane protein in the ER, was believed to induce DMVs [12]. NS4B expression induces membrane remodeling via a mechanism dependent on NS4B oligomerization, mediated by its N- and C-terminal α-helices [10,19,20]. However, more sophisticated EM techniques showed that NS4B induces formation of single-membrane vesicles (SMVs) [13]. Only combined expression of all HCV replicase proteins induces DMVs morphologically similar to HCV-infected cells [13]. The contribution of SMVs to HCV replication is unknown, but one model postulates that local, HCV-induced exvaginations form SMVs and, while the vesicles remain attached to the ER, a secondary invagination produces DMVs [13,14].

Critical players in DMV biogenesis are NS5A and the prolyl-peptidyl *cis-trans* isomerase cyclophilin A that binds to the D2 domain of NS5A [21–24], an intrinsically unstructured domain required for efficient HCV replication [25]. Cellular expression of NS5A alone induces small DMV formation albeit with low efficiency [9,13]. Pharmacological inhibition of cyclophilin A or treatment with antiviral compounds targeting NS5A abrogates *de novo* DMV formation in cells expressing the viral replicase complex, underscoring the critical role of both factors in membranous web formation [9,13,24,26,27]. The compounds likely prevent a conformational change in NS5A required for membrane rearrangements otherwise induced by active cyclophilin A. In support of this model, catalytically active cyclophilin A is required to induce DMVs [24,27], and the NS5A N-terminal amphipathic helix exhibits

membranolytic properties, suggesting a direct role in membrane remodeling [28–30]. NS4B and NS5A interact with the proline-serine-threonine phosphatase-interacting protein 2 (PSTPIP2), a host protein that induces positive membrane curvature and is required for DMV formation [31].

Lipid signaling and viral RNA replication

Local lipid concentrations recently emerged as a second regulatory layer of membranous web formation. During HCV infection, phosphatidylinositol-4-phosphate-3 kinase (PI4KIIIα), a critical enzyme within the phosphoinositide synthesis pathway, is trafficked from the Golgi apparatus and the plasma membrane to the ER via interaction with NS5A [32–35]. Intracellular phosphatidylinositol 4-phosphate (PI4P), the product of PI4KIIIα activity and the ligand of pleckstrin-homology domains present in many cellular coat and accessory proteins, increases in concentration concomitantly with this redistribution, particularly at local sites of HCV RNA replication [32,36]. Transient depletion or inhibition of PI4KIIIα suppresses viral RNA replication and causes aggregation of atypically small DMVs [32–34]. This phenotype is mirrored by NS5A mutations that impair interaction with PI4KIIIα, supporting the concept that NS5A recruits PI4KIIIα to the ER, where PI4P accumulates to enhance proper DMV formation and HCV replication [35].

One consequence is delivery of sphingholipids, cholesterol, and fatty acids to budding DMVs and formation of detergent-resistant lipid rafts [33,37]. Lipid rafts are membranous microdomains involved in compartmentalization of HCV replicase complexes within DMVs and signal transduction to modulate host-cellular processes [37]. Glycosphingolipids access PI4P-enriched membranes by interacting with four-phosphate adaptor protein 2 (FAPP2), which contains functional binding domains for PI4P and glycosphingolipids [38]. FAPP2 knockdown impairs HCV replication and prevents formation of DMVs, while addition of exogenous glycosphingolipids restores viral RNA replication [38].

In one model, viral proteins interact with specific host factors (i.e, PI4KIIIα) to induce lipid changes that recruit host factors (i.e., FAPP2 and associated lipids) to promote membrane curvature and DMV biogenesis (Figure 1). Cholesterol is delivered to DMVs by interaction of oxysterol-binding protein (OSBP) with PI4P and vesicle-associated membrane protein-associated protein A (VAPA), a host protein interacting with NS5A and NS4A [39–41]. Inhibitiing OSBP or depleting cholesterol results in aggregated, atypically small DMVs in HCV-infected cells, underscoring the critical contribution of this process to web formation [8,39]. Fatty acid synthase co-localizes with the viral NS5B polymerase within lipid rafts, and its transient knockdown or inhibition also impairs HCV replication [42].

However, fatty acids play a two-faced role in HCV infection. In hepatoma cells and primary hepatocytes, adding polyunsaturated fatty acids (PUFA), targets of reactive oxygen species in lipid peroxidation reactions, inhibits RNA replication of HCV strains that are not adapted to replication in cell culture [43,44]. Conversely, adding antioxidants, such as vitamin E, enhances replication of the same strains [43,44]. Expressing the vitamin E transporter SEC14L2, naturally lacking in hepatoma cells, or inhibiting sphingosine kinase 2 (SPHK2) phenocopies this effect, pointing to lipid peroxidation as a critical barrier to viral RNA replication in *in vitro* cultures [44,45]. The mechanism by which SPHK2 regulates HCV

replication remains unknown but it is likely that SEC14L2 expression serves to locally deliver vitamin E to sites of HCV replication. In some strains, resistance to PUFA treatment maps to adaptive mutations located in membrane-proximal residues of viral NS3/4A or NS5B proteins, suggesting that peroxidation could impair viral RNA replication by preventing viral protein oligomerization or generally by altering membrane fluidity [44].

Lipid storage and virion assembly

Packaging of the HCV genome into nucleocapsids, a process called assembly, also depends on lipids [46]. In particular, HCV assembly is dependent on lipid droplets (LDs), which are cytosolic lipid storage organelles involved in many cellular processes [47,48]. LDs are composed of a neutral lipid core consisting of triglycerides and cholesterol esters, surrounded by a phospholipid monolayer likely derived from the ER outer leaflet of the ER. Juxtaposed to the ER within the membranous web, LDs are regarded as platforms for HCV virion assembly (Figure 1) [12].

Two HCV-encoded proteins, the nucleocapsid core and NS5A, associate with LDs during virion assembly; NS5A may traffic the RNA genome out of DMVs to the LD surface where encapsidation by core is initiated (Figure 1) [46]. Notably, in HCV-infected or core-expressing cells, LDs cluster around the nucleus, which may further condense the sites of viral RNA replication and virion assembly [49,50]. Why LDs are required for assembly is unclear, as virion formation does not involve LDs directly, but instead occurs at adjacent ER membranes where envelope proteins E1 and E2 reside [51]. Mutations that prevent core from localizing to LDs inhibit HCV assembly [47,48,52], but viral p7 and NS2 are thought to eventually recruit LD-bound core to the ER to enable infectious virion formation [53].

Core undergoes two proteolytic cleavages at positions 191 and 179 that generate mature core protein (179 amino acids), which is loosely anchored within the cytosolic leaflet of the ER via a C-terminal signal peptide [54]. It then traffics onto the surface of LDs [55–58] and other closely associated organelles, such as the mitochondria [59,60]. Localization of core to the ER, mitochondria, or LDs is dependent on its D2 domain, which harbors an amphipathic helix-turn-helix motif found in other LD-binding proteins [60,61].

NS5A attaches to intracellular membranes through its N-terminal amphipathic helix [62]. This helix interacts with the LD-resident protein tail-interacting protein 47 (TIP47), which connects ER and LD membranes and supports HCV RNA replication [63–66]. This and the phosphorylation status of NS5A might serve as an important rheostat for NS5A's dual function in viral RNA replication and virion assembly (Figure 1) [67,68]. Interestingly, transfer of core and NS5A to LDs is linked via common host factors (e.g., diacylglycerol acyltransferase-1 (DGAT1), Ras-related protein 18 (Rab18) and apolipoprotein J).

DGAT1 is one of two known enzymes that catalyze the final step in triglyceride synthesis and fuel LD generation. Core and NS5A bind DGAT1 within the ER and associate with DGAT1-generated LDs, a process that enables proper colocalization of both factors to support assembly [69,70]. Similarly, Rab18 binds NS5A and promotes its association with LDs [63], while the role for Rab18 in the trafficking of core to LDs remains unclear [63,71].

Apolipoprotein J, a very low-density lipid (VLDL)-associated molecular chaperone, stabilizes the core-NS5A complex and supports infectious virion production [72].

LDs also harbor antiviral host factors such as viperin, an interferon-induced host factor involved in the antiviral immune response [73]. Viperin interacts with core and NS5A and is thought to disrupt the interaction of NS5A with the host VAPA protein at replication sites, thus suppressing viral RNA replication [74]. Heterologous nuclear ribonucleoprotein K (HNRNPK) is a poly(C)-binding protein exerting anti-HCV effects through localization near the ER and LDs [75]. Silencing of HNRNPK reduced HCV entry/replication steps, but enhanced virus assembly/release steps, suggesting complex involvement of the factor in HCV infection [75–78]. One model is that HNRNPK is redistributed to sites of viral particle production where it sequesters HCV RNA, blocking HCV assembly [75].

Ups and downs of lipid droplets

Remarkably, cellular LDs are preserved and enriched in HCV infection [79]. This is in contrast to the consumption of LDs that is observed during infection with the related dengue virus [80]. HCV infection is often characterized by liver steatosis, a complex feature involving aberrant accumulation of LDs recapitulated in HCV-infected or core-expressing cells [46]. Steatosis is more frequently associated with HCV genotype 3, and genotype 3a core protein induces formation of large LDs by downregulating phosphatase and tensin homolog (PTEN) [81] or increasing LD cholesterol ester content with sphingolipid biosynthesis [82]. Core expression in mouse liver induces expression of sterol regulatory element binding protein 1c (SREBP-1c), a critical transcription factor in fatty acid, triglyceride, and phospholipid biosynthesis [83]. Moreover, SREBP transcription is induced upon HCV infection via interaction of the 3′-UTR of the viral genome with the host protein DEAD box polypeptide 3, X-linked and the IκB kinase-α, an innate immune response that couples lipogenesis and inflammation [84,85]. NS5A and core associate with apolipoproteins A1 and A2, respectively, which regulate triglyceride content in hepatocytes [58,86], and core interacts with nuclear receptor retinoid X receptor alpha to promote lipogenesis [87]. Also, core modulates expression of two transcription factors regulating liver lipid metabolism, peroxisome proliferator-activated receptors (PPAR) alpha and gamma [88,89], and inhibits the microsomal triglyceride transfer protein, preventing lipid secretion through the VLDL pathway in hepatocytes [90].

Cellular LD homeostasis is maintained through a balance between lipogenesis and lipase-dependent processing of LDs (lipolysis). Lipolysis produces free fatty acids, which are transported into the mitochondria to undergo β-oxidation. Reports on β-oxidation during HCV infection are conflicting: HCV infection and core expression downregulate PPARα [88,91–93] and the cellular energy sensor AMP-activated protein kinase [94,95], two important activators of β-oxidation. In addition, levels of two enzymes involved in β-oxidation, medium-chain acyl coenzyme A dehydrogenase (MCAD) and short-chain acyl coenzyme A dehydrogenase (SCAD), decrease following HCV infection or core expression, supporting a model where β-oxidation is downregulated during HCV infection and LD content is increased [96]. However, Diamond et al. reported a paradoxical increase in both fatty acid oxidation and lipid biosynthesis in HCV-infected cells, which points to a more

complex regulation of both processes during HCV infection [97]. Furthermore, HCV particle production depends on the activity of dodecenoyl coenzyme A delta isomerase, an inner mitochondrial enzyme that catalyzes the breakdown of long-chain fatty acids during β-oxidation [98]. Similarly, inhibiting the mitochondrial import of fatty acids blocks HCV replication, further linking intact β-oxidation to efficient HCV infection [80,98].

Core directly interferes with lipolysis in cultured cells and murine livers [99,100]. Lipases linked to HCV infection are the hormone-sensitive lipase (HSL) [96], the putative lipase arylacetamide deactylase (AADAC) [101], and the adipose triglyceride lipase (ATGL) [102]. ATGL directly hydrolyzes triglycerides in LDs, but this activity is inhibited by core when both proteins are located on the same LD surface *in vitro* [102]. Core expression strengthens the interaction between ATGL and its activator CGI-58 and increases recruitment of the complex to core-coated LDs, a paradoxical finding pointing to the dynamics of lipase recruitment to LDs as a critical regulator of its activity [102]. Interestingly, a variant (I148M) of the closely related patatin-like phospholipase family 3 protein (PNPLA3) is linked to nonalcoholic fatty liver disease in genome-wide association studies [103] and increases steatosis risk in HCV-infected individuals in some, but not all, studies [104–108]. Mice carrying a knockin of the *pnpla3I148M* variant develop liver steatosis accompanied by a marked accumulation of the enzyme on LDs similar to what is observed with ATGL in core-expressing cells [109]. The possibility exists that lipases abnormally residing at the LD surface sequester an essential lipolytic factor, leading to an overall decrease in LD turnover [109].

Conclusions and Future Perspectives

HCV biology is closely tied to the lipid biology of infected cells, resulting in rearrangement of membranes and LDs. The past few years have seen a prolific increase in our understanding of the molecular mechanisms governing these changes with implications far beyond HCV infection. This progress was possible after break-through discoveries in *in vitro* viral replication systems in 1999 (viral RNA replication system) and 2005 (full-length infectious clone). While these systems propelled research in the area of viral RNA replication and viral assembly, they remain limited to certain viral genotypes and a handful of cancer cell lines. The next step will be to examine all HCV genotypes in primary hepatocytes. The finding that core of genotype 3 in a new replication system is not found at LDs is an indication of system complexity [110]. Similarly, findings pointing to lipid peroxidation as a major limiting factor for viral RNA replication in primary or hepatoma cells might open the door for future studies.

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Bibliography

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis c virus infection: New estimates of age-specific antibody to hcv seroprevalence. Hepatology. 2013; 57(4): 1333–1342. [PubMed: 23172780]
2. Thomas DL. Global control of hepatitis c: Where challenge meets opportunity. Nat Med. 2013; 19(7):850–858. [PubMed: 23836235]
3. Paul D, Madan V, Bartenschlager R. Hepatitis c virus rna replication and assembly: Living on the fat of the land. Cell Host Microbe. 2014; 16(5):569–579. *This review provides an extensive summary of the function and structure of the HCV viral proteins; the construction, composition, and purpose of the membranous web; and two major models for virion assembly. Published in 2014, this review highlights many earlier studies probing these aspects of HCV infection that were not discussed in the current manuscript. [PubMed: 25525790]
4. Moradpour D, Penin F, Rice CM. Replication of hepatitis c virus. Nat Rev Microbiol. 2007; 5(6): 453–463. [PubMed: 17487147]
5. Romero-Brey I, Bartenschlager R. Membranous replication factories induced by plus-strand rna viruses. Viruses. 2014; 6(7):2826–2857. [PubMed: 25054883]
6. Schwartz M, Chen J, Janda M, Sullivan M, den Boon J, Ahlquist P. A positive-strand rna virus replication complex parallels form and function of retrovirus capsids. Mol Cell. 2002; 9(3):505–514. [PubMed: 11931759]
7. Lyle JM, Clewell A, Richmond K, Richards OC, Hope DA, Schultz SC, Kirkegaard K. Similar structural basis for membrane localization and protein priming by an rna-dependent rna polymerase. J Biol Chem. 2002; 277(18):16324–16331. [PubMed: 11877407]
8. Paul D, Hoppe S, Saher G, Krijnse-Locker J, Bartenschlager R. Morphological and biochemical characterization of the membranous hepatitis c virus replication compartment. J Virol. 2013; 87(19): 10612–10627. [PubMed: 23885072]
9. Romero-Brey I, Berger C, Kallis S, Kolovou A, Paul D, Lohmann V, Bartenschlager R. Ns5a domain 1 and polyprotein cleavage kinetics are critical for induction of double-membrane vesicles associated with hepatitis c virus replication. MBio. 2015; 6(4):e00759. [PubMed: 26152585]
10. Egger D, Wolk B, Gosert R, Bianchi L, Blum HE, Moradpour D, Bienz K. Expression of hepatitis c virus proteins induces distinct membrane alterations including a candidate viral replication complex. J Virol. 2002; 76(12):5974–5984. [PubMed: 12021330]
11. Gosert R, Egger D, Lohmann V, Bartenschlager R, Blum HE, Bienz K, Moradpour D. Identification of the hepatitis c virus rna replication complex in huh-7 cells harboring subgenomic replicons. J Virol. 2003; 77(9):5487–5492. [PubMed: 12692249]
12. Chatel-Chaix L, Bartenschlager R. Dengue virus- and hepatitis c virus-induced replication and assembly compartments: The enemy inside–caught in the web. J Virol. 2014; 88(11):5907–5911. [PubMed: 24623440]
13. Romero-Brey I, Merz A, Chiramel A, Lee JY, Chlanda P, Haselman U, Santarella-Mellwig R, Habermann A, Hoppe S, Kallis S, Walther P, et al. Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis c virus replication. PLoS Pathog. 2012; 8(12):e1003056. *Utilizing confocal microscopy, electron microscopy, and electron tomography, this study differentiated the effects of individual HCV proteins on membranous web induction and structure. DMVs were shown to be derived from and often connected to the ER and were induced with a low efficiency by NS5A, but required expression of the entire HCV replicon for high levels of induction. [PubMed: 23236278]
14. Ferraris P, Beaumont E, Uzbekov R, Brand D, Gaillard J, Blanchard E, Roingeard P. Sequential biogenesis of host cell membrane rearrangements induced by hepatitis c virus infection. Cell Mol Life Sci. 2013; 70(7):1297–1306. *One of the first studies to probe the structure of the membranous web in hepatoma cells infected with full-length HCV using a variety of EM techniques. This study meticulously differentiated between the different types of vesicles that are produced. Small SMVs (here called contiguous vesicles) accumulate early in infection with increased RNA replication, while DMVs accumulate over time, suggesting that SMVs precede and may be used to generate DMVs. [PubMed: 23184194]

15. Ferraris P, Blanchard E, Roingeard P. Ultrastructural and biochemical analyses of hepatitis c virus-associated host cell membranes. J Gen Virol. 2010; 91(Pt 9):2230–2237. [PubMed: 20484561]
16. Miyanari Y, Hijikata M, Yamaji M, Hosaka M, Takahashi H, Shimotohno K. Hepatitis c virus non-structural proteins in the probable membranous compartment function in viral genome replication. J Biol Chem. 2003; 278(50):50301–50308. [PubMed: 12963739]
17. Huang H, Sun F, Owen DM, Li W, Chen Y, Gale M Jr, Ye J. Hepatitis c virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proc Natl Acad Sci U S A. 2007; 104(14):5848–5853. [PubMed: 17376867]
18. El-Hage N, Luo G. Replication of hepatitis c virus rna occurs in a membrane-bound replication complex containing nonstructural viral proteins and rna. J Gen Virol. 2003; 84(Pt 10):2761–2769. [PubMed: 13679611]
19. Paul D, Romero-Brey I, Gouttenoire J, Stoitsova S, Krijnse-Locker J, Moradpour D, Bartenschlager R. Ns4b self-interaction through conserved c-terminal elements is required for the establishment of functional hepatitis c virus replication complexes. J Virol. 2011; 85(14):6963–6976. [PubMed: 21543474]
20. Gouttenoire J, Roingeard P, Penin F, Moradpour D. Amphipathic alpha-helix ah2 is a major determinant for the oligomerization of hepatitis c virus nonstructural protein 4b. J Virol. 2010; 84(24):12529–12537. [PubMed: 20926561]
21. Chatterji U, Lim P, Bobardt MD, Wieland S, Cordek DG, Vuagniaux G, Chisari F, Cameron CE, Targett-Adams P, Parkinson T, Gallay PA. Hcv resistance to cyclosporin a does not correlate with a resistance of the ns5a-cyclophilin a interaction to cyclophilin inhibitors. J Hepatol. 2010; 53(1): 50–56. [PubMed: 20451281]
22. Foster TL, Gallay P, Stonehouse NJ, Harris M. Cyclophilin a interacts with domain ii of hepatitis c virus ns5a and stimulates rna binding in an isomerase-dependent manner. J Virol. 2011; 85(14): 7460–7464. [PubMed: 21593166]
23. Rosnoblet C, Fritzinger B, Legrand D, Launay H, Wieruszeski JM, Lippens G, Hanoulle X. Hepatitis c virus ns5b and host cyclophilin a share a common binding site on ns5a. J Biol Chem. 2012; 287(53):44249–44260. [PubMed: 23152499]
24. Madan V, Paul D, Lohmann V, Bartenschlager R. Inhibition of hcv replication by cyclophilin antagonists is linked to replication fitness and occurs by inhibition of membranous web formation. Gastroenterology. 2014; 146(5):1361–1372. e1361–1369. [PubMed: 24486951]
25. Tellinghuisen TL, Foss KL, Treadaway JC, Rice CM. Identification of residues required for rna replication in domains ii and iii of the hepatitis c virus ns5a protein. J Virol. 2008; 82(3):1073–1083. [PubMed: 18032500]
26. Berger C, Romero-Brey I, Radujkovic D, Terreux R, Zayas M, Paul D, Harak C, Hoppe S, Gao M, Penin F, Lohmann V, et al. Daclatasvir-like inhibitors of ns5a block early biogenesis of hepatitis c virus-induced membranous replication factories, independent of rna replication. Gastroenterology. 2014; 147(5):1094–1105. e1025. [PubMed: 25046163]
27. Chatterji U, Bobardt M, Tai A, Wood M, Gallay PA. Cyclophilin and ns5a inhibitors, but not other anti-hepatitis c virus (hcv) agents, preclude hcv-mediated formation of double-membrane-vesicle viral factories. Antimicrob Agents Chemother. 2015; 59(5):2496–2507. [PubMed: 25666154]
28. de Witte L, Bobardt MD, Chatterji U, van Loenen FB, Verjans GM, Geijtenbeek TB, Gallay PA. Hsv neutralization by the microbicidal candidate c5a. PloS one. 2011; 6(5):e18917. [PubMed: 21573158]
29. Bobardt MD, Cheng G, de Witte L, Selvarajah S, Chatterji U, Sanders-Beer BE, Geijtenbeek TB, Chisari FV, Gallay PA. Hepatitis c virus ns5a anchor peptide disrupts human immunodeficiency virus. Proc Natl Acad Sci U S A. 2008; 105(14):5525–5530. [PubMed: 18378908]
30. Cheng G, Montero A, Gastaminza P, Whitten-Bauer C, Wieland SF, Isogawa M, Fredericksen B, Selvarajah S, Gallay PA, Ghadiri MR, Chisari FV. A virocidal amphipathic {alpha}-helical peptide that inhibits hepatitis c virus infection in vitro. Proc Natl Acad Sci U S A. 2008; 105(8):3088–3093. [PubMed: 18287023]
31. Chao TC, Su WC, Huang JY, Chen YC, Jeng KS, Wang HD, Lai MM. Proline-serine-threonine phosphatase-interacting protein 2 (pstpip2), a host membrane-deforming protein, is critical for

membranous web formation in hepatitis c virus replication. J Virol. 2012; 86(3):1739–1749. [PubMed: 22130530]

32. Reiss S, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, et al. Recruitment and activation of a lipid kinase by hepatitis c virus ns5a is essential for integrity of the membranous replication compartment. Cell Host Microbe. 2011; 9(1):32–45. [PubMed: 21238945]
33. Hsu NY, Ilnytska O, Belov G, Santiana M, Chen YH, Takvorian PM, Pau C, van der Schaar H, Kaushik-Basu N, Balla T, Cameron CE, et al. Viral reorganization of the secretory pathway generates distinct organelles for rna replication. Cell. 2010; 141(5):799–811. [PubMed: 20510927]
34. Berger KL, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase iii alpha in hepatitis c virus replication. Proc Natl Acad Sci U S A. 2009; 106(18):7577–7582. [PubMed: 19376974]
35. Reiss S, Harak C, Romero-Brey I, Radujkovic D, Klein R, Ruggieri A, Rebhan I, Bartenschlager R, Lohmann V. The lipid kinase phosphatidylinositol-4 kinase iii alpha regulates the phosphorylation status of hepatitis c virus ns5a. PLoS Pathog. 2013; 9(5):e1003359. [PubMed: 23675303]
36. Bianco A, Reghellin V, Donnici L, Fenu S, Alvarez R, Baruffa C, Peri F, Pagani M, Abrignani S, Neddermann P, De Francesco R. Metabolism of phosphatidylinositol 4-kinase iiialpha-dependent pi4p is subverted by hcv and is targeted by a 4-anilino quinazoline with antiviral activity. PLoS Pathog. 2012; 8(3):e1002576. [PubMed: 22412376]
37. Shi ST, Lee KJ, Aizaki H, Hwang SB, Lai MM. Hepatitis c virus rna replication occurs on a detergent-resistant membrane that cofractionates with caveolin-2. J Virol. 2003; 77(7):4160–4168. [PubMed: 12634374]
38. Khan I, Katikaneni DS, Han Q, Sanchez-Felipe L, Hanada K, Ambrose RL, Mackenzie JM, Konan KV. Modulation of hepatitis c virus genome replication by glycosphingolipids and four-phosphate adaptor protein 2. J Virol. 2014; 88(21):12276–12295. [PubMed: 25122779]
39. Wang H, Perry JW, Lauring AS, Neddermann P, De Francesco R, Tai AW. Oxysterol-binding protein is a phosphatidylinositol 4-kinase effector required for hcv replication membrane integrity and cholesterol trafficking. Gastroenterology. 2014; 146(5):1373–1385. e1371–1311. [PubMed: 24512803]
40. Mesmin B, Bigay J, Moser von Filseck J, Lacas-Gervais S, Drin G, Antonny B. A four-step cycle driven by pi(4)p hydrolysis directs sterol/pi(4)p exchange by the ergolgi tether osbp. Cell. 2013; 155(4):830–843. [PubMed: 24209621]
41. Ramage HR, Kumar GR, Verschueren E, Johnson JR, Von Dollen J, Johnson T, Newton B, Shah P, Horner J, Krogan NJ, Ott M. A combined proteomics/genomics approach links hepatitis c virus infection with nonsense-mediated mrna decay. Mol Cell. 2015; 57(2):329–340. [PubMed: 25616068]
42. Huang JT, Tseng CP, Liao MH, Lu SC, Yeh WZ, Sakamoto N, Chen CM, Cheng JC. Hepatitis c virus replication is modulated by the interaction of nonstructural protein ns5b and fatty acid synthase. J Virol. 2013; 87(9):4994–5004. [PubMed: 23427160]
43. Huang H, Chen Y, Ye J. Inhibition of hepatitis c virus replication by peroxidation of arachidonate and restoration by vitamin e. Proc Natl Acad Sci U S A. 2007; 104(47):18666–18670. [PubMed: 18003907]
44. Yamane D, McGivern DR, Wauthier E, Yi M, Madden VJ, Welsch C, Antes I, Wen Y, Chugh PE, McGee CE, Widman DG, et al. Regulation of the hepatitis c virus rna replicase by endogenous lipid peroxidation. Nat Med. 2014; 20(8):927–935. **This study robustly demonstrates that endogenous lipid peroxidation represses replication of some HCV isolates, but not others. Isolate resistance was mapped to mutations in membrane-proximal residues of three HCV proteins, indicating adaptive evolution. These results provide novel, compelling evidence that HCV autoregulates its replication to reduce ROS production and tissue injury under high oxidative stress. [PubMed: 25064127]
45. Saeed M, Andreo U, Chung HY, Espiritu C, Branch AD, Silva JM, Rice CM. Sec14l2 enables pan-genotype hcv replication in cell culture. Nature. 2015; 524(7566):471–475. **Transfection of hepatoma cells with lentivirus-based human cDNA library and HCV subgenomic replicons revealed SEC14L2 as a host factor present in primary human hepatocytes but absent in hepatoma

cells that promotes replication of non-adapted HCV genotypes. Few other differences in host protein profiles between immortal, carcinogenic versus primary hepatocytes have been directly linked to regulating HCV replication and SEC14L2's antioxidant properties strengthen the association of lipid peroxidation with impairment of HCV replication. [PubMed: 26266980]

46. Filipe A, McLauchlan J. Hepatitis c virus and lipid droplets: Finding a niche. Trends Mol Med. 2015; 21(1):34–42. *This review provides an extensive summary of the various roles of lipid droplets in HCV infection; the host proteins and structural motifs within viral proteins that target HCV proteins to lipid droplets; and the mechanisms by which HCV alters lipid metabolism to promote steatosis. Published in early 2015, this review highlights many earlier studies probing lipid droplet-centric aspects of HCV infection that were not discussed in the current manuscript. [PubMed: 25496657]
47. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis c virus production. Nat Cell Biol. 2007; 9(9):1089–1097. [PubMed: 17721513]
48. Shavinskaya A, Boulant S, Penin F, McLauchlan J, Bartenschlager R. The lipid droplet binding domain of hepatitis c virus core protein is a major determinant for efficient virus assembly. J Biol Chem. 2007; 282(51):37158–37169. [PubMed: 17942391]
49. Boulant S, Douglas MW, Moody L, Budkowska A, Targett-Adams P, McLauchlan J. Hepatitis c virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner. Traffic. 2008; 9(8):1268–1282. [PubMed: 18489704]
50. Depla M, Uzbekov R, Hourioux C, Blanchard E, Le Gouge A, Gillet L, Roingeard P. Ultrastructural and quantitative analysis of the lipid droplet clustering induced by hepatitis c virus core protein. Cell Mol Life Sci. 2010; 67(18):3151–3161. [PubMed: 20422251]
51. Lindenbach BD, Rice CM. The ins and outs of hepatitis c virus entry and assembly. Nat Rev Microbiol. 2013; 11(10):688–700. [PubMed: 24018384]
52. Boulant S, Targett-Adams P, McLauchlan J. Disrupting the association of hepatitis c virus core protein with lipid droplets correlates with a loss in production of infectious virus. J Gen Virol. 2007; 88(Pt 8):2204–2213. [PubMed: 17622624]
53. Boson B, Granio O, Bartenschlager R, Cosset FL. A concerted action of hepatitis c virus p7 and nonstructural protein 2 regulates core localization at the endoplasmic reticulum and virus assembly. PLoS Pathog. 2011; 7(7):e1002144. [PubMed: 21814513]
54. Roingeard P, Hourioux C. Hepatitis c virus core protein, lipid droplets and steatosis. J Viral Hepat. 2008; 15(3):157–164. [PubMed: 18086178]
55. Targett-Adams P, Hope G, Boulant S, McLauchlan J. Maturation of hepatitis c virus core protein by signal peptide peptidase is required for virus production. J Biol Chem. 2008; 283(24):16850–16859. [PubMed: 18424431]
56. Moradpour D, Englert C, Wakita T, Wands JR. Characterization of cell lines allowing tightly regulated expression of hepatitis c virus core protein. Virology. 1996; 222(1):51–63. [PubMed: 8806487]
57. Hope RG, McLauchlan J. Sequence motifs required for lipid droplet association and protein stability are unique to the hepatitis c virus core protein. J Gen Virol. 2000; 81(Pt 8):1913–1925. [PubMed: 10900028]
58. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Brechot C. Hepatitis c virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A. 1997; 94(4):1200–1205. [PubMed: 9037030]
59. Schwer B, Ren S, Pietschmann T, Kartenbeck J, Kaehlcke K, Bartenschlager R, Yen TS, Ott M. Targeting of hepatitis c virus core protein to mitochondria through a novel c-terminal localization motif. J Virol. 2004; 78(15):7958–7968. [PubMed: 15254168]
60. Suzuki R, Sakamoto S, Tsutsumi T, Rikimaru A, Tanaka K, Shimoike T, Moriishi K, Iwasaki T, Mizumoto K, Matsuura Y, Miyamura T, et al. Molecular determinants for subcellular localization of hepatitis c virus core protein. J Virol. 2005; 79(2):1271–1281. [PubMed: 15613354]

61. Boulant S, Montserret R, Hope RG, Ratinier M, Targett-Adams P, Lavergne JP, Penin F, McLauchlan J. Structural determinants that target the hepatitis c virus core protein to lipid droplets. J Biol Chem. 2006; 281(31):22236–22247. [PubMed: 16704979]

62. Hinson ER, Cresswell P. The antiviral protein, viperin, localizes to lipid droplets via its n-terminal amphipathic alpha-helix. Proc Natl Acad Sci U S A. 2009; 106(48):20452–20457. [PubMed: 19920176]

63. Salloum S, Wang H, Ferguson C, Parton RG, Tai AW. Rab18 binds to hepatitis c virus ns5a and promotes interaction between sites of viral replication and lipid droplets. PLoS Pathog. 2013; 9(8):e1003513. *Using an unbiased proteomic approach, this study identified Rab18 as a host binding partner of HCV NS5A. During HCV infection, NS5A was shown to localize to Rab18-postiive LDs where Rab18 appears to promote the association of NS5A and other replicase components with LDs. These results support a model where Rab18 helps to enhance virion assembly by mediating an interaction between HCV membranous webs and LDs, thus bringing together the sites of HCV replication and assembly. [PubMed: 23935497]

64. Vogt DA, Camus G, Herker E, Webster BR, Tsou CL, Greene WC, Yen TS, Ott M. Lipid droplet-binding protein tip47 regulates hepatitis c virus rna replication through interaction with the viral ns5a protein. PLoS Pathog. 2013; 9(4):e1003302. [PubMed: 23593007]

65. Ploen D, Hafirassou ML, Himmelsbach K, Sauter D, Biniossek ML, Weiss TS, Baumert TF, Schuster C, Hildt E. Tip47 plays a crucial role in the life cycle of hepatitis c virus. J Hepatol. 2013; 58(6):1081–1088. [PubMed: 23354285]

66. Ploen D, Hafirassou ML, Himmelsbach K, Schille SA, Biniossek ML, Baumert TF, Schuster C, Hildt E. Tip47 is associated with the hepatitis c virus and its interaction with rab9 is required for release of viral particles. European journal of cell biology. 2013; 92(12):374–382. [PubMed: 24480419]

67. Tellinghuisen TL, Foss KL, Treadaway J. Regulation of hepatitis c virion production via phosphorylation of the ns5a protein. PLoS Pathog. 2008; 4(3):e1000032. [PubMed: 18369478]

68. Masaki T, Matsunaga S, Takahashi H, Nakashima K, Kimura Y, Ito M, Matsuda M, Murayama A, Kato T, Hirano H, Endo Y, et al. Involvement of hepatitis c virus ns5a hyperphosphorylation mediated by casein kinase i-alpha in infectious virus production. J Virol. 2014; 88(13):7541–7555. [PubMed: 24760886]

69. Camus G, Herker E, Modi AA, Haas JT, Ramage HR, Farese RV Jr, Ott M. Diacylglycerol acyltransferase-1 localizes hepatitis c virus ns5a protein to lipid droplets and enhances ns5a interaction with the viral capsid core. J Biol Chem. 2013; 288(14):9915–9923. [PubMed: 23420847]

70. Herker E, Harris C, Hernandez C, Carpentier A, Kaehlcke K, Rosenberg AR, Farese RV Jr, Ott M. Efficient hepatitis c virus particle formation requires diacylglycerol acyltransferase-1. Nat Med. 2010; 16(11):1295–1298. [PubMed: 20935628]

71. Dansako H, Hiramoto H, Ikeda M, Wakita T, Kato N. Rab18 is required for viral assembly of hepatitis c virus through trafficking of the core protein to lipid droplets. Virology. 2014:462–463. 166–174. [PubMed: 25248160]

72. Lin CC, Tsai P, Sun HY, Hsu MC, Lee JC, Wu IC, Tsao CW, Chang TT, Young KC. Apolipoprotein j, a glucose-upregulated molecular chaperone, stabilizes core and ns5a to promote infectious hepatitis c virus virion production. J Hepatol. 2014; 61(5):984–993. [PubMed: 24996046]

73. Mattijssen S, Pruijn GJ. Viperin, a key player in the antiviral response. Microbes and infection/Institut Pasteur. 2012; 14(5):419–426.

74. Helbig KJ, Eyre NS, Yip E, Narayana S, Li K, Fiches G, McCartney EM, Jangra RK, Lemon SM, Beard MR. The antiviral protein viperin inhibits hepatitis c virus replication via interaction with nonstructural protein 5a. Hepatology. 2011; 54(5):1506–1517. [PubMed: 22045669]

75. Poenisch M, Metz P, Blankenburg H, Ruggieri A, Lee JY, Rupp D, Rebhan I, Diederich K, Kaderali L, Domingues FS, Albrecht M, et al. Identification of hnrnpk as regulator of hepatitis c virus particle production. PLoS Pathog. 2015; 11(1):e1004573. [PubMed: 25569684]

76. Hsieh TY, Matsumoto M, Chou HC, Schneider R, Hwang SB, Lee AS, Lai MM. Hepatitis c virus core protein interacts with heterogeneous nuclear ribonucleoprotein k. J Biol Chem. 1998; 273(28):17651–17659. [PubMed: 9651361]

77. de Chassey B, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugue S, Meiffren G, Pradezynski F, Faria BF, Chantier T, Le Breton M, et al. Hepatitis c virus infection protein network. Molecular systems biology. 2008; 4(230)

78. Upadhyay A, Dixit U, Manvar D, Chaturvedi N, Pandey VN. Affinity capture and identification of host cell factors associated with hepatitis c virus (+) strand subgenomic rna. Molecular & cellular proteomics: MCP. 2013; 12(6):1539–1552. [PubMed: 23429521]

79. McLauchlan J. Lipid droplets and hepatitis c virus infection. Biochim Biophys Acta. 2009; 1791(6):552–559. [PubMed: 19167518]

80. Heaton NS, Randall G. Dengue virus-induced autophagy regulates lipid metabolism. Cell Host Microbe. 2010; 8(5):422–432. [PubMed: 21075353]

81. Clement S, Peyrou M, Sanchez-Pareja A, Bourgoin L, Ramadori P, Suter D, Vinciguerra M, Guilloux K, Pascarella S, Rubbia-Brandt L, Negro F, et al. Down-regulation of phosphatase and tensin homolog by hepatitis c virus core 3a in hepatocytes triggers the formation of large lipid droplets. Hepatology. 2011; 54(1):38–49. [PubMed: 21465511]

82. Loizides-Mangold U, Clement S, Alfonso-Garcia A, Branche E, Conzelmann S, Parisot C, Potma EO, Riezman H, Negro F. Hcv 3a core protein increases lipid droplet cholesteryl ester content via a mechanism dependent on sphingolipid biosynthesis. PloS one. 2014; 9(12):e115309. [PubMed: 25522003]

83. Moriishi K, Mochizuki R, Moriya K, Miyamoto H, Mori Y, Abe T, Murata S, Tanaka K, Miyamura T, Suzuki T, Koike K, et al. Critical role of pa28gamma in hepatitis c virus-associated steatogenesis and hepatocarcinogenesis. Proc Natl Acad Sci U S A. 2007; 104(5):1661–1666. [PubMed: 17234812]

84. Li Q, Pene V, Krishnamurthy S, Cha H, Liang TJ. Hepatitis c virus infection activates an innate pathway involving ikk-alpha in lipogenesis and viral assembly. Nat Med. 2013; 19(6):722–729. **This study uniquely demonstrates that HCV subverts the IκB kinase-α (IKK-α) to suppress the innate antiviral response. This subversion further promotes HCV infection, as the association of HCV with IKK-α induces a transcriptional program involving SREBPs that induces lipogenic genes and enhances lipid drop formation essential for HCV assembly. These important results elucidate a pathway by which HCV avoids host recognition and simultaneously induces lipogenesis essential for the viral life cycle. [PubMed: 23708292]

85. Pene V, Li Q, Sodroski C, Hsu CS, Liang TJ. Dynamic interaction of stress granules, ddx3x, and ikk-alpha mediates multiple functions in hepatitis c virus infection. J Virol. 2015; 89(10):5462–5477. [PubMed: 25740981]

86. Shi ST, Polyak SJ, Tu H, Taylor DR, Gretch DR, Lai MM. Hepatitis c virus ns5a colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. Virology. 2002; 292(2):198–210. [PubMed: 11878923]

87. Tsutsumi T, Suzuki T, Shimoike T, Suzuki R, Moriya K, Shintani Y, Fujie H, Matsuura Y, Koike K, Miyamura T. Interaction of hepatitis c virus core protein with retinoid × receptor alpha modulates its transcriptional activity. Hepatology. 2002; 35(4):937–946. [PubMed: 11915042]

88. Dharancy S, Malapel M, Perlemuter G, Roskams T, Cheng Y, Dubuquoy L, Podevin P, Conti F, Canva V, Philippe D, Gambiez L, et al. Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis c virus infection. Gastroenterology. 2005; 128(2):334–342. [PubMed: 15685545]

89. Kim KH, Hong SP, Kim K, Park MJ, Kim KJ, Cheong J. Hcv core protein induces hepatic lipid accumulation by activating srebp1 and ppargamma. Biochemical and biophysical research communications. 2007; 355(4):883–888. [PubMed: 17331464]

90. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, Koike K, Pessayre D, Chapman J, Barba G, Brechot C. Hepatitis c virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: A model of viral-related steatosis. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2002; 16(2):185–194. [PubMed: 11818366]

91. Blackham S, Baillie A, Al-Hababi F, Remlinger K, You S, Hamatake R, McGarvey MJ. Gene expression profiling indicates the roles of host oxidative stress, apoptosis, lipid metabolism, and intracellular transport genes in the replication of hepatitis c virus. J Virol. 2010; 84(10):5404–5414. [PubMed: 20200238]

92. Cheng Y, Dharancy S, Malapel M, Desreumaux P. Hepatitis c virus infection down-regulates the expression of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl acyl-coa transferase 1a. World journal of gastroenterology: WJG. 2005; 11(48):7591–7596. [PubMed: 16437683]

93. de Gottardi A, Pazienza V, Pugnale P, Bruttin F, Rubbia-Brandt L, Juge-Aubry CE, Meier CA, Hadengue A, Negro F. Peroxisome proliferator-activated receptor-alpha and -gamma mrna levels are reduced in chronic hepatitis c with steatosis and genotype 3 infection. Alimentary pharmacology & therapeutics. 2006; 23(1):107–114. [PubMed: 16393287]

94. Mankouri J, Tedbury PR, Gretton S, Hughes ME, Griffin SD, Dallas ML, Green KA, Hardie DG, Peers C, Harris M. Enhanced hepatitis c virus genome replication and lipid accumulation mediated by inhibition of amp-activated protein kinase. Proc Natl Acad Sci U S A. 2010; 107(25):11549–11554. [PubMed: 20534540]

95. Yu JW, Sun LJ, Liu W, Zhao YH, Kang P, Yan BZ. Hepatitis c virus core protein induces hepatic metabolism disorders through down-regulation of the sirt1-ampk signaling pathway. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases. 2013; 17(7):e539–545. [PubMed: 23510538]

96. Bose SK, Kim H, Meyer K, Wolins N, Davidson NO, Ray R. Forkhead box transcription factor regulation and lipid accumulation by hepatitis c virus. J Virol. 2014; 88(8):4195–4203. *This study interrogated the role of forkhead box transcription factors in the regulation of lipid metabolism during HCV infection. The authors describe an HCV-induced transcriptional program that results in the disruption of normal lipid metabolism, ultimately promoting lipid droplet accumulation and efficient viral replication. [PubMed: 24478438]

97. Diamond DL, Syder AJ, Jacobs JM, Sorensen CM, Walters KA, Proll SC, McDermott JE, Gritsenko MA, Zhang Q, Zhao R, Metz TO, et al. Temporal proteome and lipidome profiles reveal hepatitis c virus-associated reprogramming of hepatocellular metabolism and bioenergetics. PLoS Pathog. 2010; 6(1):e1000719. [PubMed: 20062526]

98. Rasmussen AL, Diamond DL, McDermott JE, Gao X, Metz TO, Matzke MM, Carter VS, Belisle SE, Korth MJ, Waters KM, Smith RD, et al. Systems virology identifies a mitochondrial fatty acid oxidation enzyme, dodecenoyl coenzyme a delta isomerase, required for hepatitis c virus replication and likely pathogenesis. J Virol. 2011; 85(22):11646–11654. [PubMed: 21917952]

99. Harris C, Herker E, Farese RV Jr, Ott M. Hepatitis c virus core protein decreases lipid droplet turnover: A mechanism for core-induced steatosis. J Biol Chem. 2011; 286(49):42615–42625. [PubMed: 21984835]

100. Piodi A, Chouteau P, Lerat H, Hezode C, Pawlotsky JM. Morphological changes in intracellular lipid droplets induced by different hepatitis c virus genotype core sequences and relationship with steatosis. Hepatology. 2008; 48(1):16–27. [PubMed: 18570290]

101. Nourbakhsh M, Douglas DN, Pu CH, Lewis JT, Kawahara T, Lisboa LF, Wei E, Asthana S, Quiroga AD, Law LM, Chen C, et al. Arylacetamide deacetylase: A novel host factor with important roles in the lipolysis of cellular triacylglycerol stores, vldl assembly and hcv production. J Hepatol. 2013; 59(2):336–343. [PubMed: 23542347]

102. Camus G, Schweiger M, Herker E, Harris C, Kondratowicz AS, Tsou CL, Farese RV Jr, Herath K, Previs SF, Roddy TP, Pinto S, et al. The hepatitis c virus core protein inhibits adipose triglyceride lipase (atgl)-mediated lipid mobilization and enhances the atgl interaction with comparative gene identification 58 (cgi-58) and lipid droplets. J Biol Chem. 2014; 289(52):35770–35780. [PubMed: 25381252]

103. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in pnpla3 confers susceptibility to nonalcoholic fatty liver disease. Nature genetics. 2008; 40(12):1461–1465. [PubMed: 18820647]

104. Ampuero J, Del Campo JA, Rojas L, Garcia-Lozano JR, Sola R, Andrade R, Pons JA, Navarro JM, Calleja JL, Buti M, Gonzalez-Escribano MF, et al. Pnpla3 rs738409 causes steatosis according to viral & il28b genotypes in hepatitis c. Annals of hepatology. 2014; 13(4):356–363. [PubMed: 24927606]

105. Zampino R, Coppola N, Cirillo G, Boemio A, Pisaturo M, Marrone A, Macera M, Sagnelli E, Perrone L, Adinolfi LE, Miraglia del Giudice E. Abdominal fat interacts with pnpla3 i148m, but

not with the apoc3 variant in the pathogenesis of liver steatosis in chronic hepatitis c. J Viral Hepat. 2013; 20(8):517–523. [PubMed: 23808989]

106. Cai T, Dufour JF, Muellhaupt B, Gerlach T, Heim M, Moradpour D, Cerny A, Malinverni R, Kaddai V, Bochud M, Negro F, et al. Viral genotype-specific role of pnpla3, pparg, mttp, and il28b in hepatitis c virus-associated steatosis. J Hepatol. 2011; 55(3):529–535. [PubMed: 21236304]
107. Huang CF, Chen JJ, Yeh ML, Huang CI, Hsieh MY, Yang HL, Dai CY, Huang JF, Lin ZY, Chen SC, Chuang WL, et al. Pnpla3 genetic variants determine hepatic steatosis in non-obese chronic hepatitis c patients. Scientific reports. 2015; 5:11901. [PubMed: 26139292]
108. Nakamura M, Kanda T, Nakamoto S, Miyamura T, Jiang X, Wu S, Yokosuka O. No correlation between pnpla3 rs738409 genotype and fatty liver and hepatic cirrhosis in japanese patients with hcv. PloS one. 2013; 8(12):e81312. [PubMed: 24349054]
109. Smagris E, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, Cohen JC, Hobbs HH. Pnpla3i148m knockin mice accumulate pnpla3 on lipid droplets and develop hepatic steatosis. Hepatology. 2015; 61(1):108–118. *This study provided the first direct evidence that physiological expression of the I148M variant of PNPLA3, which exhibits impaired enzymatic activity, causes nonalcoholic fatty liver disease (NAFLD) in mice. Furthermore, the authors found that the accumulation of catalytically inactive PNPLA3 on the surfaces of LDs is associated with increased hepatic triglyceride (TG) levels. These data suggest that PNPLA3 I148M may prevent TG hydrolysis by restricting access to LDs or by sequestering a critical lipolytic factor. [PubMed: 24917523]
110. Kim S, Date T, Yokokawa H, Kono T, Aizaki H, Maurel P, Gondeau C, Wakita T. Development of hepatitis c virus genotype 3a cell culture system. Hepatology. 2014; 60(6):1838–1850. **Takaji Wakita is responsible for the development of the first full-length HCV vector, an adapted genotype 2a strain, that replicated efficiently in hepatoma cells. Here, his group designs full-length, adapted, genotype 3a strains that replicate efficiently in this same system. This allows for the in vitro study of the HCV genotype (3a) that is of highest clinical importance as it is closely associated with increased liver steatosis. [PubMed: 24797787]

Box 1

HCV Proteins and Functions

Protein	Function
Structural Proteins	
Core	A multifunctional protein, core forms a viral capsid that protects HCV RNA. Its N-terminal domain (D1) contains three basic subdomains that bind RNA and other proteins, while its C-terminal domain (D2) is highly helical and mediates lipid binding. Mature core resides at the cytosolic side of the ER membrane and traffics to cLDs to facilitate viral assembly.
E1 E2	Viral surface glycoproteins, E1 and E2 form non-covalent heterodimers within infected cells, but assemble as large covalent complexes stabilized by disulfide bonds on viral particles. E2 mediates binding to receptors at hepatocyte surfaces to promote viral entry. E1 and E2 may mediate fusion between the viral envelope and endosomal host cell membranes.
p7	A short, 63-residue ion channel protein, p7 protects nascent HCV particles while in transit through acidic intracellular compartments to facilitate maturation and particle release. p7 also modulates capsid assembly and the envelopment of viral particles.
Non-Structural (NS) Proteins	
NS2	A Cys-protease that mediates cleavage at the NS2/NS3 junction of the viral polyprotein, NS2 is not required for RNA replication. Rather, NS2 co-localizes with core and NS5A at punctate sites near cLDs and recruits E2 to promote viral assembly.
NS3	A bifunctional molecule comprised of N-terminal serine-protease and C-terminal helicase domains. The NS3 protease domain catalyzes polyprotein cleavage between non-structural proteins, while the role of the helicase domain is unknown.
NS4A	A 54-residue transmembrane protein that is a cofactor for NS3. NS4A stimulates NS3 protease and helicase activities, and regulates NS3 localization to ER and mitochondrial membranes.
NS4B	A poorly-characterized protein with four transmembrane helices and N- and C-terminal amphipathic α-helices. NS4B is critical for efficient RNA replication and HCV particle assembly. The protein, as homo-oligomers, facilitates membranous web formation.
NS5A	A multifunctional protein that associates with membranes via an N-terminal amphipathic α-helix. NS5A localizes to both DMVs and cLDs and serves critical roles in both HCV RNA replication and virion assembly. A phosphoprotein, basal NS5A promotes HCV RNA replication, whereas hyperphosphorylated NS5A promotes HCV assembly.
NS5B	NS5B is the RNA-dependent RNA polymerase required for RNA replication. Its N-terminal catalytic domain connects to a transmembrane domain anchored to intracellular membranes.

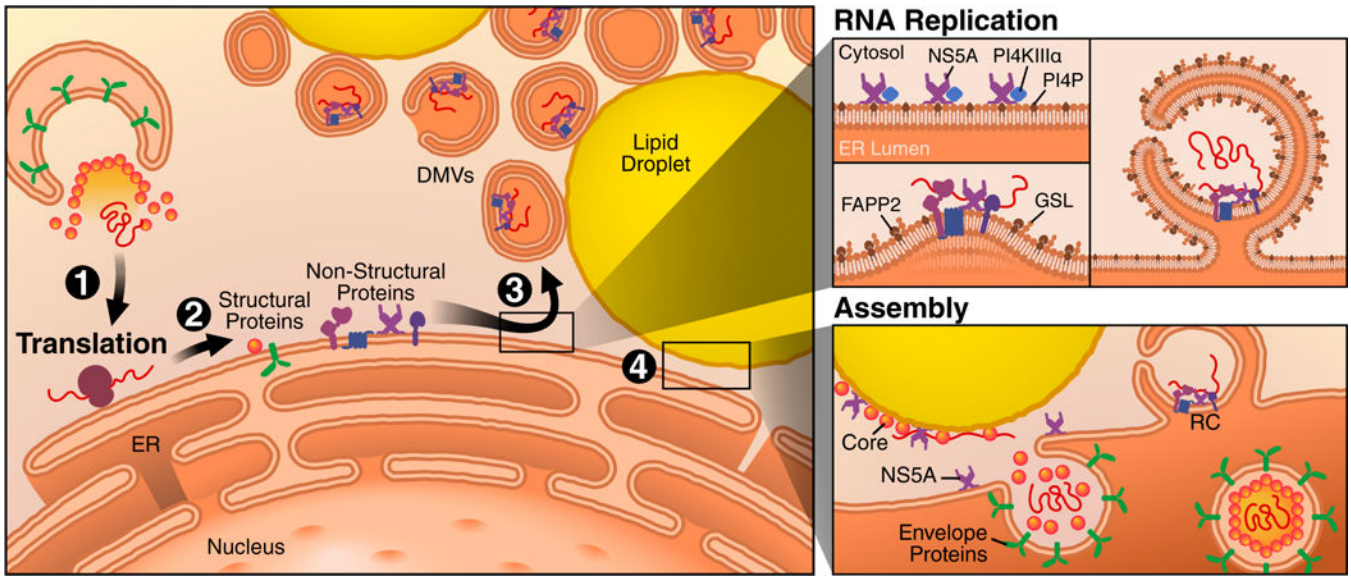

FIGURE 1. HCV replication and assembly are coordinated via intracellular organelles

Left, HCV infection stimulates production of DMVs and LDs. 1) After viral entry and uncoating, HCV RNA is released into the cytoplasm. 2) The HCV RNA genome is translated at the rough ER into a single large polyprotein, which is cleaved into structural and nonstructural proteins. 3) Viral proteins NS4B and NS5A, along with host factors, induce changes in the ER membrane to produce DMVs. DMVs remain attached to the ER or bud off into the cytosol and form a membranous web hosting viral RNA replicase complexes. 4) LD production increases during infection to serve as a scaffold for assembly. NS5A and core proteins are loaded onto LDs and promote HCV RNA translocation from DMVs.

Upper right, DMV formation from ER membranes. Following translation and processing at ER membranes, NS5A activates PI4KIIIα, locally enriching the membrane in PI4P. FAPP2 is recruited to these sites via interaction with PI4P recruiting associated glycosphingolipids. Membrane curvature is induced by these lipid changes, along with the coordinated actions of NS5A and NS4B.

Lower right, HCV assembly at LDs. The core-NS5A complex recruits replication complexes to ER membranes close to LDs. Eventually, LDs connect with ER regions containing the viral glycoproteins. Assembly begins when core and viral RNA, mediated by NS2 and NS3/4A, are transferred back to the cytosolic membrane of the ER.

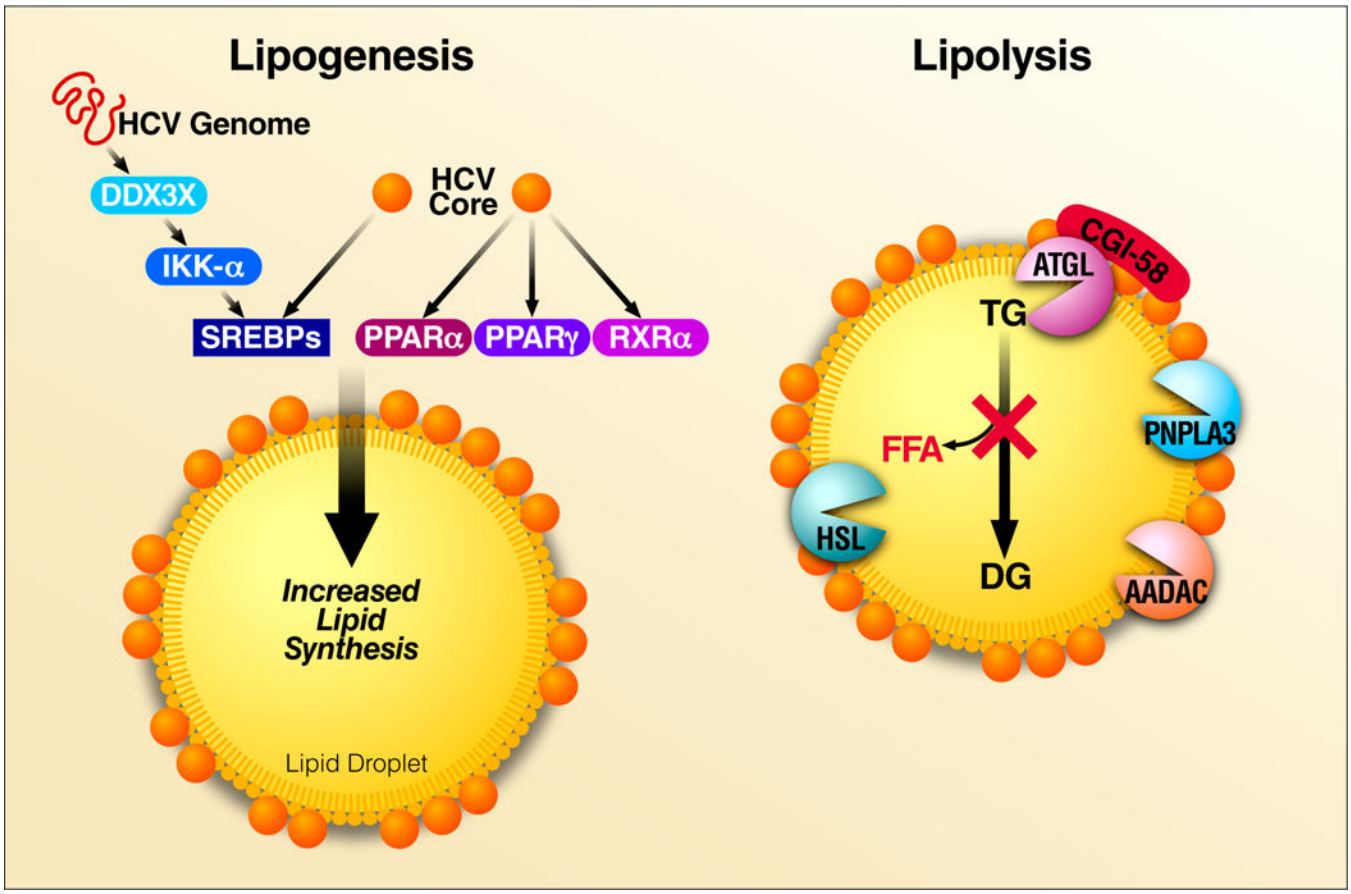

FIGURE 2. The modulation of lipogenesis and lipolysis by HCV

Left, Lipogenesis is induced during HCV infection. Interaction of the HCV genome 3′-UTR with DEAD box polypeptide 3, X-linked activates IκB kinase-α, which results in SREBP-mediated expression of lipogenic genes, such as fatty acid synthase (FASN).

Right, Lipolysis is inhibited during HCV infection. HCV infection has been linked to several lipases, including ATGL, HSL, AADAC and PNPL3. Core strengthens the interaction between ATGL and its activator CGI-58, and increases the recruitment of the complex to LDs.

Importantly, this interaction results in suppression of ATGL activity. Core expression and HCV infection modulate HSL phosphorylation to potentially impair its activity. HCV infection also regulates the temporal expression of the putative lipase AADAC. The I148M variant of PNPLA3 causes liver steatosis in mice, which is accompanied by accumulation of the enzyme on LDs, similar to what is observed with ATGL.