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Lipids and RNA virus replication

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Abstract

Most viruses rely heavily on their host machinery to successfully replicate their genome and produce new virus particles. Recently, the interaction of positive-strand RNA viruses with the lipid biosynthetic and transport machinery has been the subject of intense investigation. In this review, we will discuss the contribution of various host lipids and related proteins in RNA virus replication and maturation.

Introduction

Viruses have evolved to utilize cellular membranes for entry, replication of their genome and production of nascent particles. Notably, positive-strand RNA viruses have developed this unique ability to co-opt cytosolic membranes to build a novel RNA replication factory. The factory contains the replicase complex, which includes viral RNA, proteins and host factors [1,2]. Additionally, this platform is thought to protect the virus from host immune defenses. Previous reports indicate that the endoplasmic reticulum (ER), Golgi complex, mitochondria and endosome membranes are reorganized by various RNA viruses to build their replication complex [1-9]. For hepatitis C virus, the replication factory consists of double-membrane vesicles (DMVs) organized in a membranous web (MW) structure [10-15]. Efforts to understand the organization and function of RNA virus replication platforms have initially focused on virus and host factors. However, there is overwhelming evidence that host lipids play a crucial role in the functioning of these replication factories. This review will underscore the recent findings on the roles that lipids play in virus RNA replication and particles production. Lipids are thought to help organize a functional virus replication factory by facilitating membrane curvature or binding to and stimulating the activity of the virus polymerase in the replication complex (Figure 1). Alternatively, lipids recruit core proteins to facilitate virus particles formation (Figure 2).

Lipids are essential for the integrity of cellular membranes and major alterations in lipid composition can negatively impact cellular homeostasis. However, subtle changes in

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membrane lipids pose a special challenge for RNA viruses that rely heavily on membranes for efficient genome replication and virus particles production. While these viruses need cytosolic membranes to replicate their genome, they all display specific lipid requirements. Hence, positive-strand RNA viruses employ multifaceted strategies to hijack host machinery involved in lipid biosynthesis and transport. This review will underscore the recent findings on the crucial roles of lipids in virus RNA replication and particles production. Due to space limitation, the review will feature the wealth of knowledge gained from studying the *Flaviviridae* and *Picornaviridae* families of viruses

Fatty acids and virus genome replication

Fatty acids are constituents of triglycerides and phospholipids. They contribute to energy production and storage, generation of lipid droplets and structural integrity of membranes. Inhibitors of fatty acids synthesis (e.g. cerulenin, C75) severely impede the replication of enteroviruses (e.g. poliovirus), flaviviruses (e.g. West Nile virus or WNV) or hepatitis C virus (HCV) [16-19]. Fatty acid synthase (FASN) is a key lipogenic enzyme involved in fatty acids synthesis (Table 1; Figure 1A). Indeed, FASN protein levels are increased in HCV-infected patients [20,21] or HCV-infected cells [22,23]. Notably, Nasheri et al. found that HCV NS4B was involved in FASN induction and concomitant increase in fatty acids production [23]. However, NS4B does not appear to bind to FASN protein or the regulatory sequences for FASN expression. Instead, NS4B activates the sterol regulatory element-binding protein (SREBP-1c), a major transcription factor for FASN expression [24,25]. Under this scenario, FASN-derived fatty acids, but not FASN itself, are recruited to build HCV replication factory [23], which consists of double-membrane vesicles (DMVs) organized into a membranous web [5,11,12,14,15,26]. The generation of DMVs likely requires additional phospholipids production to ensure minimal alteration in the lipid content of ER membrane. Indeed, HCV infection leads to increased levels of phosphatidylcholine and phosphatidylethanolamine [22]. Thus, while fatty acids may be directly recruited to the HCV replication factory, they can be converted into phospholipids to generate the DMVs. Alternatively, a recent report shows that FASN binds to, and stimulates HCV NS5B polymerase (RdRp) activity in the replication factory [27]. While this report needs to be confirmed, it implies that FASN plays at least two different roles during HCV replication: providing the building block for phospholipids and stimulating virus polymerase.

FASN function is also required for WNV or dengue virus (DENV) RNA replication [18,28]. Nevertheless, these viruses do not induce an increase in FASN protein level. Instead, they cause a redistribution of FASN into their replication platform [18,28]. The DENV NS3 protein which has protease and RNA helicase activity, interacts with, and activates FASN to generate fatty acids, which are utilized to either build or stimulate the activity of DENV replicase complex [28]. There is no report yet that FASN affects poliovirus replication. However, poliovirus 2A protein is reported to stimulate the activity of another lipogenic enzyme, long chain acyl-CoA synthetase 3, thus providing the fatty acids required for poliovirus replication [29].

Phosphatidylinositol 4-phosphate and virus genome replication

Phosphatidylinositol 4-phosphate (PI4P) regulates membrane trafficking via interaction with effector proteins [30,31]. Its role in RNA virus replication (Figure 1B) was revealed in several studies in which phosphatidylinositol 4-kinase (PI4K) knockdown led to a significant decrease in enterovirus or HCV RNA synthesis [32-39]. There are four PI4K isoforms, with PI4KIIIα and PI4KIIIβ functioning in the ER and Golgi complex, respectively [30] (Table 1). HCV replication complex is ER-derived [5,6,10,14,40,41] and favors PI4KIIIα function for replication [34,35,39]. By contrast, enteroviruses build their replication platform on Golgi-derived membranes [3,7,42], hence require PI4KIIIβ for replication [33,43].

Following infection, enteroviruses and HCV recruit PI4KIIIα/β to their replication complex, leading to increased PI4P production [33,34,36,44]. For HCV, several studies have shown that NS5A protein interacts with, and activates PI4KIIIα [34,36,44]. A more recent report by Reiss et al. [45] shows that a conserved sequence in the C-terminal end of NS5A domain 1 is crucial for NS5A interaction with PI4KIIIα. NS5A is a phosphoprotein with two phosphorylation states based on its mobility on SDS-PAGE. The basal NS5A phosphorylation, p56, facilitates HCV genome replication [46] whereas the hyperphosphorylation state, p58, impedes HCV replication but appears to enhance virus production [46-48]. Notably, Reiss et al. [45] reported that PI4KIIIα plays a crucial role in NS5A phosphorylation. Indeed, mutations within the C-terminal end of NS5A domain 1 resulted in both a decrease in HCV genome replication and increased NS5A hyperphosphorylation. Hence, the NS5A-PI4KIIIα interaction is a crucial checkpoint for regulating the transition from HCV genome replication to virus particles production.

The role of enterovirus proteins in the recruitment of PI4KIIIβ remains controversial. Two studies found that enteroviruses 3A protein interacts with PI4KIIIβ [33,49] while coxsackievirus 3A mutation rendered the virus resistant to the inhibitors of PI4KIIIβ, enviroxime and GW5074 [43]. However, Teterina et al. [50] were unable to confirm the interaction between 3A and PI4KIIIβ.

Membranous web (MW) vesicles are formed during HCV infection [10-15,51] and appear as dots or foci in confocal microscopy [6,10,13,41,52]. PI4KIIIα knockdown causes a drastic change in the MW or the disruption of HCV replicase foci [35,36,39,44,45], implying a role for PI4KIIIα in the organization of the HCV replication factory. Alternatively, the PI4P, generated by PI4K, may help to organize or stimulate virus replicase complex (RC) because dephosphorylation of PI4P with SacI impedes HCV or poliovirus RNA synthesis [33,53]. In poliovirus, PI4P binds to the 3D polymerase either to anchor it in the virus RC or stimulate its enzymatic activity [33]. There is also evidence implicating PI4P effector proteins (e.g. oxysterol-binding protein or OSBP; Ceramide transport protein or CERT; four phosphate adaptor protein 2 or FAPP2) function in the transport of cholesterol or sphingolipids required for virus replication (Table 1). These effectors will be discussed below.

Cholesterol and sphingolipids in virus genome replication

Cholesterol and sphingolipids are major constituents of non-ionic detergent resistant lipid rafts [54,55]. The replicase complexes from HCV, enteroviruses or flaviviruses co-fractionate with lipid rafts while disruption of the lipid rafts interferes with RNA synthesis [56-60]. Consistent with these findings, pharmacological inhibition of the enzymes involved in cholesterol biosynthesis impedes virus RNA synthesis [60-63]. An enzyme involved in cholesterol synthesis, HMGCR (3-hydroxy-methyglutaryl-CoA reductase), relocalizes to WNV replication complex, whereas DHCR24 (24-dehydrocholesterol reductase) level is upregulated during HCV infection [64,65] (Table 1).

How cholesterol is recruited to the viral replicase complex is just beginning to be elucidated. Studies with HCV and enteroviruses show that OSBP (oxysterol-binding protein) is responsible for cholesterol transport [66,67]. Notably, knockdown or pharmacological inhibition of OSBP interferes with RNA replication and disrupts virus replication factory [66-68]. In poliovirus, cholesterol also plays a role in processing precursor 3CD into mature 3D polymerase [60]. Notably, PI4P binds to, and recruits OSBP to the virus replication complex [30]. Additionally, HCV NS5A also binds to OSBP, implying a concerted effort by host and virus factors to bring cholesterol into the virus replication platform [69].

High levels of sphingolipids have been reported in DENV or HCV-infected cells [19,70-74]. Specifically, sphingomyelin levels are elevated in the HCV replicase membrane fraction [70]. Yet, the role of sphingolipids during HCV replication is highlighted by the findings that inhibition of serine palmitoyltransferase (SPT), the first enzyme in the sphingolipid biosynthetic pathway, or knockdown of sphingomyelin synthase, leads to lower HCV replication efficiency [70-73]. Accordingly, sphingomyelin binds to, and activates HCV NS5B polymerase [70,75], implying that sphingomyelin stimulates the function of virus replicase complex. Recently, we have found that lactosylceramide, a glycosphingolipid, colocalizes with HCV replicase complex. Lactosylceramide production requires FAPP2, a PI4P effector protein. Our study suggests that FAPP2, or its transported glycosphingolipid, is crucial for generating functional HCV replication factory [52]. Hence, sphingolipids may contribute to the organization and function of virus replicase complex.

Role of lipids in virus production

The importance of VLDL (Figure 2) in virus particles formation has been reported previously [76-79]. This section will therefore underscore recent developments in the role of other lipids and proteins in regulating the transition from replication to virus assembly. Lipid droplets (LDs) are ER-derived organelles enriched in cholesterol esters and triglycerides [80,81]. LDs are recognized as sites for HCV (e.g. JFH1 strain) and DENV assembly [82,83]. Pharmacological inhibition of triglycerides and cholesterol esters synthesis impedes HCV assembly by decreasing the cellular LDs content or increasing LDs size [84]. Virus core proteins get recruited to LDs via interaction with diacylglycerol acyltransferase-1 (DGAT1) (Table 1) or tail-interacting protein of 47 kD (TIP47) [85,86], two proteins required for LDs biogenesis [87,88]. Knockdown or inhibition of DGAT1 activity hinders HCV assembly [86]. Additionally, the interaction of HCV NS5A with core protein is

believed to transfer HCV RNA from replication to assembly sites [82,89] and two LDs-associated proteins, DGAT1 and Rab18, were reported recently to play a crucial role in NS5A-core interaction [90-92]. Nevertheless, the assembly of the highly infectious HCV Jc1 virus takes place on ER membranes and requires p7 function [93-96]. Hence, HCV may utilize LDs to modulate virus infectivity.

As an enveloped virus, host lipids also play a crucial role in the composition of the infectious HCV particles. Recent studies show elevated levels of sphingolipids in cell culture-derived HCV particles [77,97] (Figure 2). Furthermore, inhibition of cholesterol and sphingolipids biosynthesis, or knockdown of proteins involved in the transport of these lipids (OSBP and CERT) hampers HCV production [56,69,98]. Note that OSBP is also required for HCV RNA synthesis as discussed above [67].

Conclusions

Despite the replication factories of RNAs vary widely in structure, positive-strand RNA viruses co-opt similar host lipids and related proteins to amplify their genomes. Since RNA synthesis is a crucial step in viral lifecycle, drugs targeting host lipid metabolic pathways are likely to increase the efficacy of currently available direct acting antivirals. However, several questions remain unanswered. For example, are the discussed lipids and related proteins playing distinct roles during virus replication? Are the lipids involved in membrane curvature required for generating membrane vesicles in the replication factories? How does palmitoylation of HCV proteins contribute to their roles in virus replication and assembly [99,100]? Finally, do most viruses modulate host lipid metabolism to alter the innate or acquired immune response to infection [64]?

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Highlights

- Fatty acids provide additional phospholipids in the replicase factory
- PI4P stimulates virus proteins and recruits effectors crucial for RNA replication
- Cholesterol and sphingolipids help to organize a functional replicase factory
- Virus assembly on LDs requires interaction between virus and LDs-bound proteins

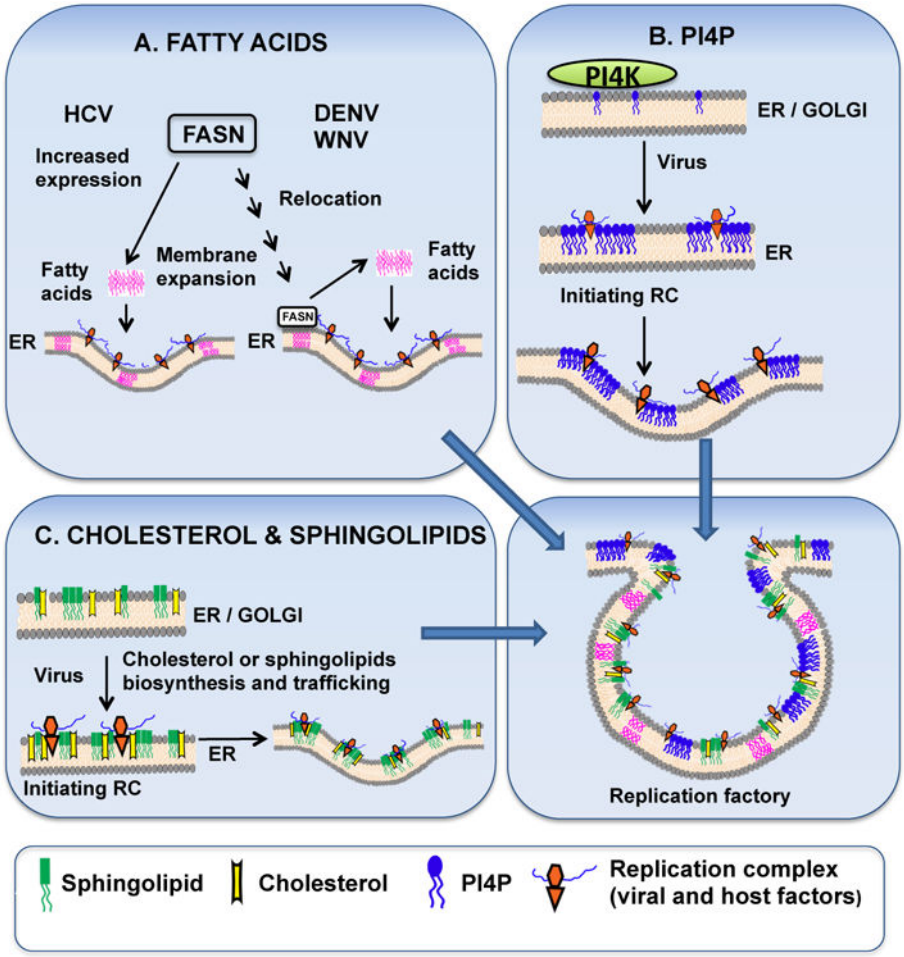

Figure 1. Lipids in virus RNA synthesis

A. Fatty acids. The host protein, FASN, is a key enzyme in the biosynthesis of fatty acids, which are incorporated at sites of viral RNA replication and cause membrane expansion necessary to form the replication factory. FASN expression increases after HCV infection [23] while FASN is recruited to the sites of viral RNA replication after WNV or DENV infection [18,19,28]. **B.** PI4P. Following virus infection, PI4P is produced on ER membranes by PI4KIIIα or Golgi membranes by PI4KIIIβ. PI4P may bind to virus replicase proteins and contribute to membrane curvature. Alternatively, PI4P can recruit effector proteins via their PH domains, triggering a cascade of events which can lead to the organization of a functional replicase complex [101]. **C.** Cholesterol and sphingolipids. Biosynthesis and trafficking of cholesterol or sphingolipids are essential for optimal functioning of virus replicase complex. By changing the membrane composition and fluidity, these lipids can contribute to membrane bending and stimulation of the replicase complex.

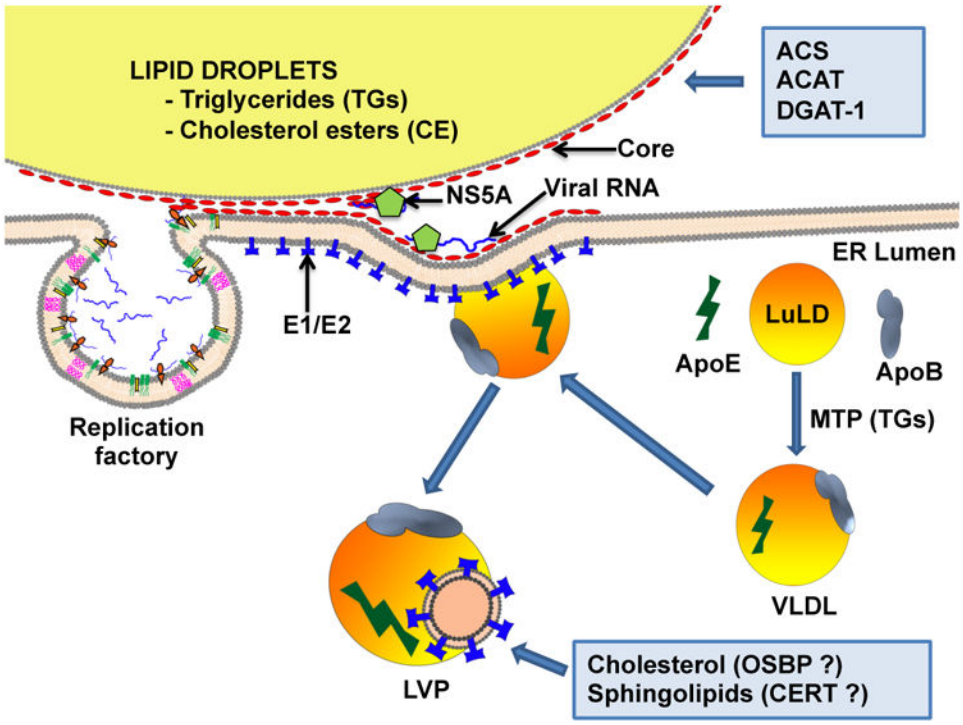

Figure 2. Lipids in HCV maturation

The first step in HCV assembly often takes place on LDs. HCV core localizes to LDs that are in proximity to ER-associated virus replication factory. Following replication, HCV NS5A brings the nascent RNA to LDs via interaction with DGAT1 and core proteins, thus initiating virus assembly [90]. The two enzymes, ACS and ACAT, are crucial for LDs biogenesis and facilitate HCV assembly [84]. Virus particles maturation, in the form of LVP, requires cholesterol and sphingolipids, which may be provided by OSBP and CERT, two PI4P effector proteins [98]. E1/E2: HCV glycoproteins; LuLD: luminal LDs; VLDL: very low density lipoprotein; LVP: lipo-viro-particle.

Table 1

Host factors and related lipids in RNA virus replication.

PROTEIN	LOCATION	FUNCTION	REFERENCES
FASN	Cytosol	Fatty acid synthesis for membrane expansion in virus replication factory.	[23,78]
PI4KIIIα	ER	PI4P synthesis; required for membranous web vesicles formation	[39,45]
PI4KIIIβ	Golgi	PI4P synthesis and stimulation of viral $3D^{Pol}$ in enteroviruses.	[101,102]
OSBP	ER and Golgi	Transports cholesterol to the HCV replication complex.	[67]
CERT	ER and Golgi	Transports ceramide from ER to Golgi for sphingomyelin biosynthesis; required for virus production.	[103]
DHCR24	ER	Contributes to cholesterol synthesis; required for HCV genome replication.	[65]
DGAT1	ER	Recruits HCV core and NS5A to lipid droplets: required for virus assembly.	[79,90,104]
ACS	ER	Fatty acyl-CoA synthesis for incorporation into triglycerides and cholesterol esters in lipid droplets; required for virus production.	[84]
ACAT	ER	Conversion of cholesterol to cholesterol esters in lipid droplets; required for virus production.	[84]