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Lipids at the interface of virus-host interactions

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Abstract

Viruses physically and metabolically remodel the host cell to establish an optimal environment for their replication. Many of these processes involve the manipulation of lipid signaling, synthesis and metabolism. An emerging theme is that these lipid-modifying pathways are also linked to innate anti-viral responses and can be modulated to inhibit viral replication.

Introduction

In recent years, we have gained tremendous appreciation that viruses interact with and modulate host cell lipids at several stages of their life cycle. This facilitates efficient entry, replication and egress of the virus. This review will specifically highlight some of the virus-mediated physical and metabolic remodeling of lipids that are required for efficient replication and cellular countermeasures. The viruses discussed in this review along with their family and abbreviations are listed in Table 1 for reference.

Physical remodeling of membranes

Most viruses that replicate in cytoplasm tend to do so in specific membranous compartments that are induced by the virus (reviewed in [1]). Even though the origin and morphology of these replication compartments differ between viruses, they all are proposed to aid replication by: concentrating viral and cellular proteins involved in replication, providing a physical scaffold on which to form the replication complex, as well as providing a physical barrier separating replicating RNA from innate immune sensors.

Modulation of lipid synthesis

Early studies defined a requirement for lipid synthesis and modifying enzymes in the replication of (+) strand RNA viruses. Some picornaviruses and a number of other (+) strand RNA viruses require phospholipid and/or sterol biosyntheses for efficient replication [2–7]. Brome mosaic virus (BMV) replication requires OLE1, a fatty acid desaturation enzyme that promotes membrane fluidity [8]. BMV has also been recently shown to utilize ACB1-encoded acyl coA binding protein (ACBP), which promotes lipid synthesis, for efficient replication [9*]. The morphology of the BMV-induced replication structures, termed spherules, is perturbed in cells deficient in ACBP. In addition to requiring lipid synthetic enzymes to alter membrane composition (and possibly curvature), there is likely a requirement for viral or host proteins that induce membrane curvature. In the case of BMV

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replication complex formation, the interaction of the viral 1a protein with cellular reticulon homology proteins promotes spherule formation [10**].

In addition to simply requiring lipid biosynthetic pathways, some viruses, such as flaviviruses, actively manipulate lipid biosynthesis to establish sites of replication (Fig. 1A). Kunjin subtype of West Nile Virus (WNV) manipulates cholesterol biosynthesis pathway to efficiently replicate and evade anti-viral response. WNV redistributes cholesterol-synthesizing enzymes to replication sites and also reduces cholesterol at the plasma membrane leading to defective anti-viral signaling [11]. Similarly, Dengue virus (DENV) replication requires cholesterol biosynthesis and transport [12,13]. Additionally, DENV manipulates cellular fatty acid synthesis. DENV NS3 binds to fatty acid synthase (FASN), relocalizes it to sites of viral replication, and stimulates its activity [14**]. The consequences of FASN manipulation by NS3 appear to include an altered lipid composition for replication complex formation. Membrane fractions of DENV-infected mosquito cells have a FASN-dependent enrichment of unsaturated phospholipids, ceramide and lysophospholipids and signaling molecules like sphingomyelin [15*]. WNV and yellow fever virus (YFV) also require fatty acid biosynthesis for replication [14**]. Similar to DENV, WNV infection has also been reported to result in the relocalization of FASN to sites of replication [16]. Thus, it is now increasingly clear that many viruses induce changes to lipid synthesis. This modulation likely influences the composition, fluidity, and curvatures of membrane compartments; and plays an important role for efficient replication of RNA viruses.

Additional roles for fatty acid biosynthesis in viral infection include post-translational modifications of viral or host cofactors [17,18] and virion envelopment. Human cytomegalovirus (HCMV) stimulates fatty acid synthesis to enhance the assembly of infectious HCMV virions [19]. Since flavivirus replication complex structures are physically linked to sites of assembly [20,21], the lipid alterations noted for viral replication may also impact the efficiency of virion assembly and the lipid content of virion envelopes.

Viral modulation of lipid signaling

In addition to lipid synthesis, some (+) strand RNA viruses modify cellular lipid signaling to establish sites of replication (Fig. 1B). Hepatitis C Virus (HCV) and enteroviruses such as Poliovirus and Coxsackievirus stimulate phosphatidylinositol (PI)-4 kinases to generate PI4-phosphate (PI4P) at sites of replication. Synthesis of PI4P is essential for replication of these viruses. While HCV engages the ER-associated PI-4-kinase-III α (PI4KA) [22*–25], enteroviruses use the Golgi isoform PI4-kinase III β (PI4KB) [26**]. In the case of HCV, viral protein NS5A specifically interacts and recruits PI4KA to stimulate PI4P synthesis [22*–24]. In the case of Picornaviridae, although no direct interaction has been described, enterovirus 3A protein is required for recruiting PI4KB to replication compartments [26**]. Recently, viral proteins of Aichivirus, another member of Picornaviridae have been shown to interact with acyl-coenzyme A binding domain containing 3 (ACBD3), which in turn can interact with and recruit PI4KB to replication compartments [27].

The role of PI4P in replication complex remains unclear. Numerous cellular proteins contain canonical PI4P binding domains, such as PH domains [28]. For example, lipid transfer proteins, like oxysterol binding protein, which is required for HCV replication and release and ceramide transfer protein have PI4P binding domains and may facilitate cholesterol and sphingomyelin recruitment to these compartments [29,30]. Alternatively, PI4P or further modification of PI4P such as PI(4,5)P₂ may be bound by viral proteins to establish a platform for replicase formation. In the case of poliovirus, the viral polymerase is thought to directly interact with PI4P and this interaction may stabilize viral replication complexes [26**]. At least in the case of HCV, silencing PI4KA in the context of viral protein

expression results in the aggregation of viral replicase proteins and large clusters of homogenous double membrane vesicles [22*–24,31]. This suggests that PI4KA contributes to the fidelity of membranous web organization. It is interesting that not just viruses, but intracellular bacteria including Chlamydia and Legionella replicate in cytosolic vacuoles enriched in PI4P [32,33]. In addition to promoting protein interaction and stabilization, lipids participate in a variety of signaling events as second messengers for cellular processes like apoptosis, endocytosis, and actin rearrangements [34]. Many viruses utilize such lipids or lipid domains to efficiently enter, replicate and egress the cell. For a detailed review, see [35].

Lipid Metabolic Remodeling

Several viruses manipulate or alter the metabolic state of the host for their survival. Induction of the Warburg effect by Kaposi's Sarcoma Herpes Virus (KSHV), in which energy production via glycolysis is enhanced, has been implicated in maintaining the latently infected cells [36*]. Similarly HCMV also alters the host metabolome, including lipid metabolism [37–39]. A number of microbes co-opt lipid droplets for their replication (reviewed in [40]). Lipid droplets store excess cholesterol esters and fatty acids in the form of triacylglycerides, which can subsequently be used as an energy source during starvation [41]. HCV assembly is closely linked with lipids and the lipid droplet (reviewed in [42]). HCV core localizes to lipid droplets and stimulates lipid droplet accumulation, possibly via an interaction with diacylglycerol acyltransferase 1 [43*]. The purpose of HCV assembly on or near lipid droplets is not entirely clear; however, HCV virions are lipo-viral particles, with a distinctive low-density lipid envelope composition and are associated with apolipoproteins E to enhance infectivity [44]. Large lipid droplets were also observed in chronically infected individuals with steatosis [45].

The processing of lipid droplet triacylglycerides by lipases release free fatty acids, which can undergo β -oxidation in mitochondria, providing ATP and other intermediates for the cell. In addition to cytosolic lipases, a selective autophagy called lipophagy has also been implicated in the degradation of lipids in the lipid droplet [46]. DENV infection induces lipophagy to deplete lipid droplets and stimulate β -oxidation [47**]. Thus, DENV may signal to the infected cell that it is in starvation condition to deplete its energy stores. The role of β -oxidation of fatty acids in HCV replication is still unclear. While microarray analysis revealed a decrease in genes involved in fatty acid oxidation [48], proteomic and lipidomic analysis revealed an increase in β -oxidation [49]. A fatty acid oxidation enzyme dodecenoyl coenzyme A delta isomerase is essential for HCV replication [50]. It was also recently shown that ATP is enriched in Hepatitis C virus (HCV) replication compartments while the cytosolic ATP is dramatically reduced in these infected cells [51*]. This suggests that there is an active need for ATP during HCV replication.

HCMV on the other hand seems to reduce ATP production by recruiting the interferon stimulated gene viperin to mitochondria to inhibit β -oxidation [52**]. Low ATP levels are then thought to disrupt the cytoskeleton and promote viral replication. Similarly, Adenovirus type 36, but not type 2, decreases fatty acid oxidation and increases lipid droplet accumulation [53]. Thus different viruses and even subtypes of the same virus have differential requirements for β -oxidation in infection.

The strategy of co-opting the lipid droplet is not limited to viruses: a number of intracellular bacteria, including Chlamydia and Mycobacterium employ similar mechanisms [54–57]. In addition to mobilizing lipids from lipid droplets, several viruses also manipulate peroxisomes for their replication either for β -oxidation of fatty acids or for other purposes. For recent detailed review, see [58].

Inhibition of lipid synthesis: an innate antiviral response

An emerging theme is that pathways regulating lipid biosynthesis may perform innate antiviral functions. Adenosine 5' monophosphate-activated protein kinase (AMPK) is a master regulator of multiple cellular metabolic pathways, including lipid metabolism. During cellular stress, including nutrient deprivation or viral infection, AMPK is activated and shuts down anabolic pathways, such as lipid biosynthesis, while enhancing catabolic pathways such as autophagy and β -oxidation for ATP production (Fig. 2). AMPK is activated upon Rift Valley fever virus (RVFV) entry and restricts its replication by inhibiting fatty acid synthesis, independent of interferon stimulation [59**]. Additionally, Sindbis, WNV and vesicular stomatitis virus (VSV) are also restricted by AMPK. Thus, AMPK appears to have intrinsic innate immune functions, at least against RNA viruses.

Some viruses have evolved mechanisms to manipulate AMPK activation, analogous to viral innate immune antagonists (reviewed in [60]). HCV infection activates protein kinase B to phosphorylate AMPK, thus inhibiting AMPK activation. This is required for HCV-induced lipid accumulation, as pharmacological activation of AMPK prevents viral stimulation of lipid synthesis and viral replication [61*]. Human Immunodeficiency Virus (HIV) also inhibits AMPK activation [62]. Alternatively, Simian Vacuolating virus 40 (SV40) and Avian reovirus activate AMPK, which may enhance cell survival even in nutrient-deprived conditions [63,64]. Interestingly, Vaccinia virus entry requires AMPK to control actin dynamics, suggesting possible metabolic-independent functions for AMPK [65]. HCMV induces calcium/calmodulin-dependent protein kinase kinase (CaMKK) activation of AMPK to stimulate ATP generating catabolic processes, while at the same time inducing acetyl co-A carboxylase (ACC) expression and lipid biosynthesis for virion assembly [19,66,67*]. This suggests that HCMV can uncouple the catabolic and anabolic regulation by AMPK. Similarly, DENV stimulates both catabolic (lipophagy) and anabolic process (lipid biosynthesis), albeit with distinct kinetics [14**,47**]. The exact mechanism by which DENV induces and/or regulates these pathways is unknown.

While AMPK is an example of possible intrinsic immunity, it was recently shown that the interferon actively represses sterol biosynthesis as an antiviral strategy [68**]. Upon infection of macrophages with multiple viruses, or interferon treatment, the transcription factor sterol regulatory element binding protein 2 (SREBP2) involved in coordinating sterol biosynthesis is reduced. This in turn reduces SREBP2 activated sterol biosynthetic gene expression in an interferon α/β receptor-dependent manner. This can inhibit viral replication by limiting the synthesis of mevalonate/isoprenoid, in the case of murine CMV, or cholesterol. Similarly, the interferon stimulated gene viperin has been reported to have anti-viral activity by disrupting lipid raft domains [69]. As discussed above, HCMV co-opts viperin to inhibit β -oxidation [52**].

Conclusions and future directions

Our appreciation of the requirements and manipulation of distinct lipid species in viral replication is expanding as our toolbox, particularly RNA interference technology and mass spectrometry based lipidomics, improves. Lipids play several essential roles in every stage of the viral life cycle [70]. Due to space limitations, we focused on examples of lipids in replication in this review. Of particular interest many lipid metabolic enzymes are potential drug targets, with FDA-approved drugs in existence, in some cases. Additionally, the general requirements for lipid metabolism in viral infections suggest the possibility for broad-spectrum antivirals targeting these enzymes. For example, drugs that target fatty acid synthase activity can inhibit DENV, WNV, YFV, HCV, Epstein-Barr Virus (EBV), HCMV and Influenza A replication in human cells [14**–16,39,71,72]. Similarly, anti-PI4K drugs

can be used to inhibit enterovirus and HCV replication [73*,74*]. Thus, understanding how viruses manipulate lipid metabolism and signaling will have huge implication in developing new therapeutics.

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Highlights

- Viruses physically remodel the cell to create replication compartments by manipulating cellular lipid signaling and/or synthesis.
- Viruses manipulate cellular lipid metabolism to facilitate replication and virion assembly.
- Lipid synthesis is emerging as a target of intrinsic and innate anti-viral immunity.

A. LIPID SYNTHESIS

B. LIPID SIGNALING

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Figure 1. Roles for lipids in viral replication compartment formation

A. Lipid synthesis. Flaviviruses recruit lipid synthesis machinery to expand surface area of membranes to accommodate replication machinery. Specific example of lipids enriched in DENV replication compartments is shown [15*]. In addition to general lipid synthesis, membrane fluidity is either reduced by enrichment of cholesterol and sphingomyelin in certain domains, while unsaturated phospholipids are enhanced to increase fluidity in other areas of replication compartments. Membrane curving lipids such as ceramide that induces negative curvature and lysophosphatidylcholine (Lyso PC) that induces positive curvature are also enhanced. B. Lipid signaling. The enteroviruses and HCV stimulate phosphatidylinositol signaling. HCV and enteroviruses specifically recruit PI-4-kinases to phosphorylate PI to PI4P [22*–26**]. This can then be bound by viral or cellular PI4P-binding proteins to facilitate replication complex formation.

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Figure 2. AMPK signaling and lipid metabolism

AMPK is a central regulator of cellular metabolism. Specific activators and inhibitors of AMPK and the downstream effectors that specifically modulate lipid metabolism are shown. In general, AMPK senses low energy conditions and once activated it inhibits lipid anabolic pathways to conserve energy, while stimulating lipid catabolism to generate. This is differentially manipulated in a number of viral infections. During HCV infection, AMPK activation is inhibited via activation of Akt/PKB [61*]. HCMV is also thought to activate Akt, however, recently it has been shown that AMPK is activated by HCMV via cAMKK [66,67]. HIV Tat may inhibit AMPK through Sirtuin 1 (SIRT1) inhibition [60,62]. SV40 inhibits protein phosphatase 2 A (PP2A) leading to AMPK activation [63]. Vaccinia and Avian reovirus also activate AMPK through unknown mechanisms [64–65].

Table 1

List of viruses discussed in this review

Family	Virus	Abbrev.
Adenoviridae	Adenovirus	-
Bromoviridae	Brome Mosaic Virus	BMV
Bunyaviridae	Rift Valley Fever Virus	RVFV
Flaviviridae	Genus Flavivirus:	
	West Nile Virus	WNV
	Dengue Virus	DENV
	Yellow Fever Virus	YFV
	Genus Hepacivirus:	
	Hepatitis C Virus	HCV
Herpesviridae	Human cytomegalovirus	HCMV
	Kaposi's Sarcoma-associated Herpesvirus	KSHV
	Epstein-Barr Virus	EBV
Orthomyxoviridae	Influenza A Virus	-
Picornaviridae	Genus Enterovirus:	
	Poliovirus	-
	Coxsackievirus	-
	Genus Kobuvirus:	
	Aichivirus	-
Polyomaviridae	Simian Vacuolating virus 40	SV40
Poxviridae	Vaccinia Virus	-
Reoviridae	Avian Reovirus	-
Retroviridae	Human Immunodeficiency Virus	HIV
Rhabdoviridae	Vesicular Stomatitis Virus	VSV
Togoviridae	Sindbis Virus	-