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Review

Microbes and Metabolism

Multifaceted roles for lipids in viral infection

Nicholas S. Heaton and Glenn Randall

Department of Microbiology, The University of Chicago, Chicago, IL 60637, USA

Viruses have evolved complex and dynamic interactions with their host cell. In recent years we have gained insight into the expanding roles for host lipids in the virus life cycle. In particular, viruses target lipid signaling, synthesis, and metabolism to remodel their host cells into an optimal environment for their replication. This review highlights examples from different viruses that illustrate the importance of these diverse virus–lipid interactions.

Roles for lipids in the virus life cycle

The importance of cellular lipids in viral infection has long been appreciated, particularly in regards to membrane fusion during the entry process and virion envelopment during particle maturation. More recently our view of the roles of lipids in viral infection has greatly expanded. We now recognize that lipids organize the subcellular localization of key events in the virus life cycle. Viruses encode proteins that coopt lipid signaling and synthesis machinery to remodel the host cell. This serves to establish protected sites of replication and generate lipids for envelopment. Furthermore, there are numerous mechanisms for how viruses modulate host-cell lipid metabolism to promote efficient virus replication.

The focus of this review will be on recent publications that describe viral modulation of lipid metabolism, lipid composition, or lipid signaling to facilitate increased virus entry, replication or assembly/secretion. Owing to space limitations it is impossible to have an exhaustive discussion of this topic, and thus we will concentrate on specific examples to demonstrate concepts. An emerging theme is that uncovering new virus–lipid interactions not only enhances our understanding of the virus–host interaction, it expands antiviral therapeutic approaches to include drugs that target lipid metabolism.

Lipids in endocytosis

Cellular lipid membranes are the first barrier to viral infection of the host cell and an important initial point of interaction for viruses. In some cases, viruses can exploit lipids to use them as viral receptors (Figure 1a). For non-enveloped viruses, the virus capsid can interact directly with specific components of host-cell membranes, usually glycosphingolipids, as receptors to initiate infection (reviewed in [1]). Polyomaviruses, for example, bind glycosphingolipids upon association with a host cell and the specific bound ganglioside will determine the internalization pathway used [2,3]. Alternatively, lipid trafficking receptors can function as indirect viral receptors (Figure 1b). Members of the *Flaviviridae,* including hepatitis C virus (HCV) and bovine viral diarrheal virus (BVDV), have been shown to require functional low-density lipoprotein receptor (LDL-R) for virus entry [4,5]. Although additional high-affinity receptors are known to be required for HCV entry, the requirement for an early, low-affinity LDL-R interaction is probably a reflection of virion association with apolipoproteins which serves to bring the virus in proximity to other entry cofactors (reviewed in [6]) (Figure 1b). Similarly, the scavenger receptor class B type I (SR-BI) is an HCV receptor that is known to interact with the high-density lipoprotein HDL [7]. Although the virus glycoproteins directly interact with SR-BI, there is evidence that HDL/HCV/SR-B1 interactions can also influence the infectivity of HCV (reviewed in [8]).

Many viral processes require specific lipid compositions in distinct compartments for appropriate membrane composition and dynamics. Lipid microenvironments influence the entry of some viruses, frequently by influencing the clustering of receptors in cholesterol-rich subdomains (Figure 1c). Human immunodeficiency virus-1 (HIV-1) fails to enter host cells that have been depleted for cholesterol via treatment with β-cyclodextran [9], implicating a lipid as a required cofactor for HIV-1 entry. Further mechanistic work showed that cholesterol is essential to bring the viral receptors into proximity and initiate entry complex formation [10]. Host-cell lipid microdomains are similarly required for murine coronavirus entry [11] and pseudorabies virus entry [12]. In addition, some viruses require cholesterol in the virus envelope for efficient virus entry. This has been reported for influenza A virus [13] and human herpes virus 6 [11], however, the exact role for virus envelope cholesterol remains unclear.

Endocytic events frequently require lipid signaling. Many viruses rely on phosphatidylinositol (PI)-3 kinase signaling for their appropriate internalization (reviewed in [14]). Following internalization, virions can be sorted into appropriate endosomal compartments from which they eventually fuse out. Specific lipids can influence the compartment of virion uncoating (Figure 1d). Recent work on dengue virus (DENV) has shown that virus fusion with late endosomes is specifically induced by the presence of a late endosome-specific lipid species bis(monoacylglycero)phosphate (BMP) [15]. Interestingly, low pH alone is ineffective in stimulating virus fusion in the absence of BMP. Similarly, alphaviruses require cholesterol and sphingolipids for

Corresponding author: Randall, G. (grandall@bsd.uchicago.edu).

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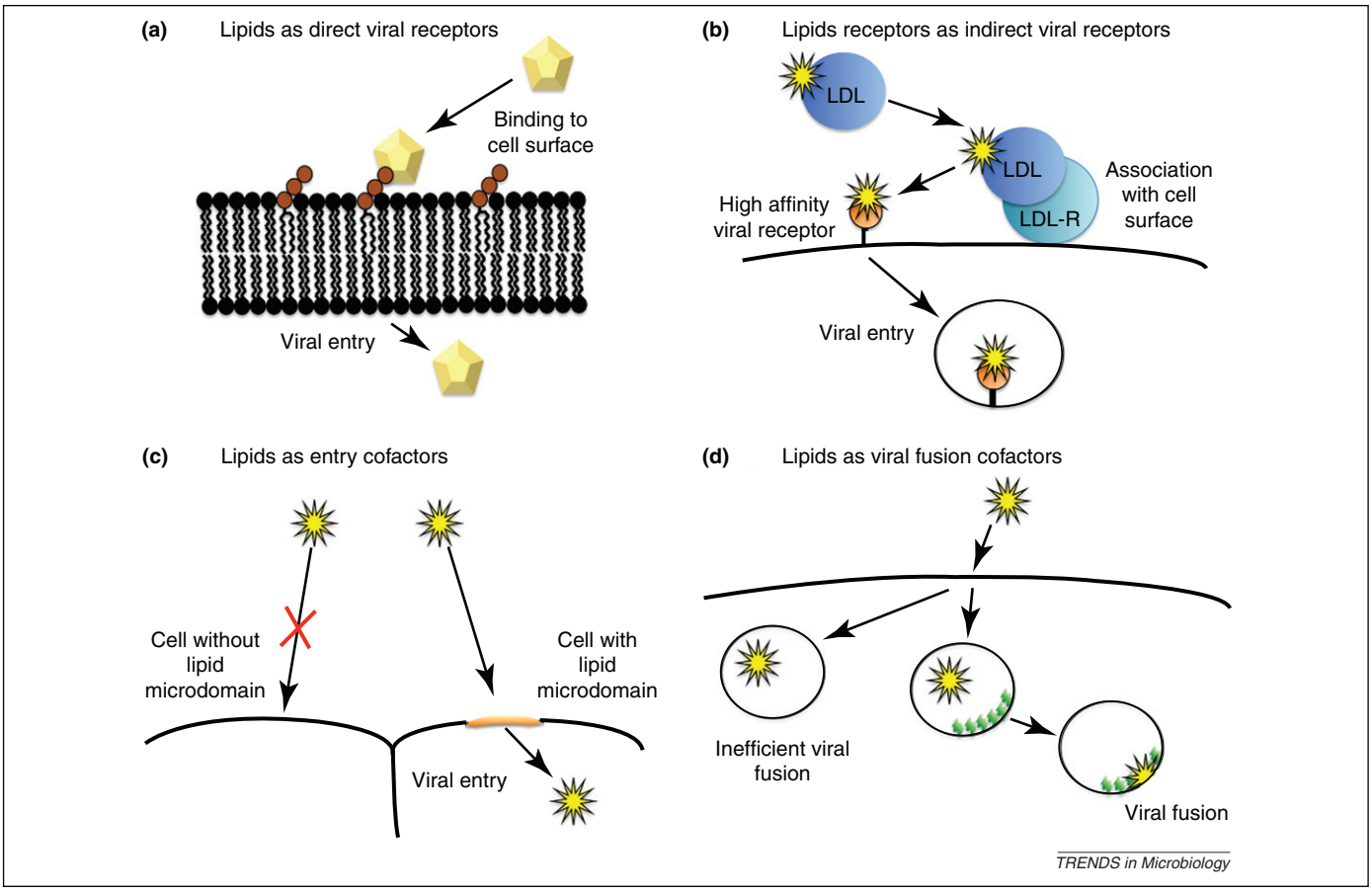

Figure 1. Roles for lipids in virus entry. **(a)** Lipids as direct viral receptors. The virus protein coat of non-enveloped viruses, such as polyomaviruses, can associate with lipids on the cell surface [1]. In many cases these lipids display a carbohydrate moiety (brown) that directly interacts with the virus protein coat (yellow). This interaction leads to internalization of the virus and the initiation of infection. **(b)** Lipid receptors as indirect viral receptors. Viruses (yellow) such as HCV and BVDV, which associate with LDL in the blood, can be brought into proximity of a target cells via the association between LDL and its receptor (LDL-R) [4,5]. Subsequently, interaction with high-affinity cell-surface viral receptors (orange) facilitates infection. **(c)** Lipids as entry cofactors. Lipids can modulate the ability of a virus to enter the cell after initial receptor interactions. Many viruses (yellow) such as influenza, HIV, and Ebola virus enter cells at lipid microdomains (orange) or require lipid microdomains for some step of virus entry (reviewed in [86]). **(d)** Lipids as virus fusion cofactors. Some viruses (yellow), such as DENV, utilize specific lipids (green) in order to stimulate virus envelope fusion with target membranes [15].

optimal virus endosomal fusion, (reviewed in [16]). Thus, specific lipid species appear to influence the ability of viruses to initiate infection at multiple stages of the virus entry process.

Membrane remodeling for viral replication complex formation

Viruses that replicate in the cytosol have a shared requirement for reorganizing cellular membranes to establish sites of replication. Although the morphology of these structures are distinct, they are thought to serve three common purposes: (i) provide a physical scaffold on which the virus replicase can assemble, (ii) increase the local concentration of the viral and cellular cofactors required for replication, and (iii) provide a protected environment that limits recognition of virus protein and nucleic acid by the innate immune system. Positive-stranded RNA viruses can form replication complexes from different cytosolic membrane sources including the endoplasmic reticulum (ER), Golgi, endosomes and/or lysosomes, and mitochondria; and their mechanism of formation probably differs (reviewed in [17,18]). The importance of the source of membrane is unclear. At least in the case of Flock House virus (FHV), replication is not perturbed by targeting replication complex formation to an abnormal subcellular location [19]. Advances in electron tomography have allowed the first appreciation of the 3D structure of virus replication factories [20–25]. For these viruses, the replication membranes are contiguous with either the ER or mitochondria. They are severely remodeled, producing enclosed pockets or invaginations for the viruses to replicate inside, with a neck to release the RNA, frequently into an associated virion-assembly compartment. Other viruses, such as HCV and the picornaviruses, appear to have a different structure involving the potential clustering of heterogeneous vesicles (reviewed in [17,18]).

Research into the mechanism of membrane remodeling has advanced at a slower pace. Initially the question of how membranes are reorganized was addressed by identifying virus requirements for the formation of these membrane structures. Individual virus proteins were overexpressed in cells, which were then examined by electron microscopy to identify membrane structures that resemble those found in infected cells. This approach has identified HCV NS4B [26,27], flavivirus NS4A [28,29] and enterovirus nonstructural protein 2BC, possibly in combination with the 3A protein [30–33], as being capable of inducing membrane alterations. The exact mechanism by which these virus proteins alter membrane organization however, is generally unclear. Further complexities have been revealed by

work on brome mosaic virus (BMV) which has shown that not only the virus proteins but their interactions and relative expression levels can lead to different membrane morphologies [34].

Although virus proteins clearly contribute to the formation of these membrane structures, there is an increasing appreciation for the roles of host-cell cofactors. Specifically, much work has been carried out on host-cell cofactors that have functions in host lipid-membrane metabolism. Both enteroviruses and HCV appear to coopt lipid signaling to influence the formation of these structures (Figure 2a). It has been shown by several groups that host genes involved in phosphatidylinositol (PI) signaling influence HCV and enterovirus replication [35–40]. These reports have led to a model wherein the modulation of these lipid signaling molecules directs cellular membrane trafficking to coalesce with virus proteins to generate replication complex (RC) formation [41]. HCV infection or NS5A expression stimulates cellular PI4P production by the PI4-kinase III-alpha [42]. This activity is required for the formation of appropriate membrane RCs. Enteroviruses require the related PI4-kinase III-beta. Further, the enterovirus RNA polymerase $3D^{pol}$ physically binds PI4P, which could influence both its association with cellular membranes and its ability to synthesize RNA (Figure 2a) [40].

Host cofactors can facilitate the formation of virus RCs either by structurally supporting membrane curvature or by generating components of the RCs. BMV engages reticulon homology domain proteins which function to physically induce membrane curvature, and are required for RC formation (Figure 2b) [43]. In addition to reticulon interactions, BMV requires membrane lipid processing events to facilitate the formation of fully functional RCs [44,45]. The host gene *OLE1*, which introduces a double bond into an unsaturated fatty acid tail, is required for virus replication by altering the fluidity and plasticity of membranes for viral replication complexes (Figure 2d).

Viruses not only require certain lipid compositions for RC formation, but they can also stimulate *de novo* lipid synthesis (Figure 2c,d). In West Nile virus (WNV) infection, the cholesterol-synthesizing enzyme 3-hydroxymethyglutaryl-CoA reductase as well as cellular cholesterol are redistributed to virus RCs during replication [46]. The related DENV also depends on cholesterol biosynthesis [47]. In addition to the importance of cholesterol, DENV NS3 recruits the host enzyme fatty acid synthase to sites of

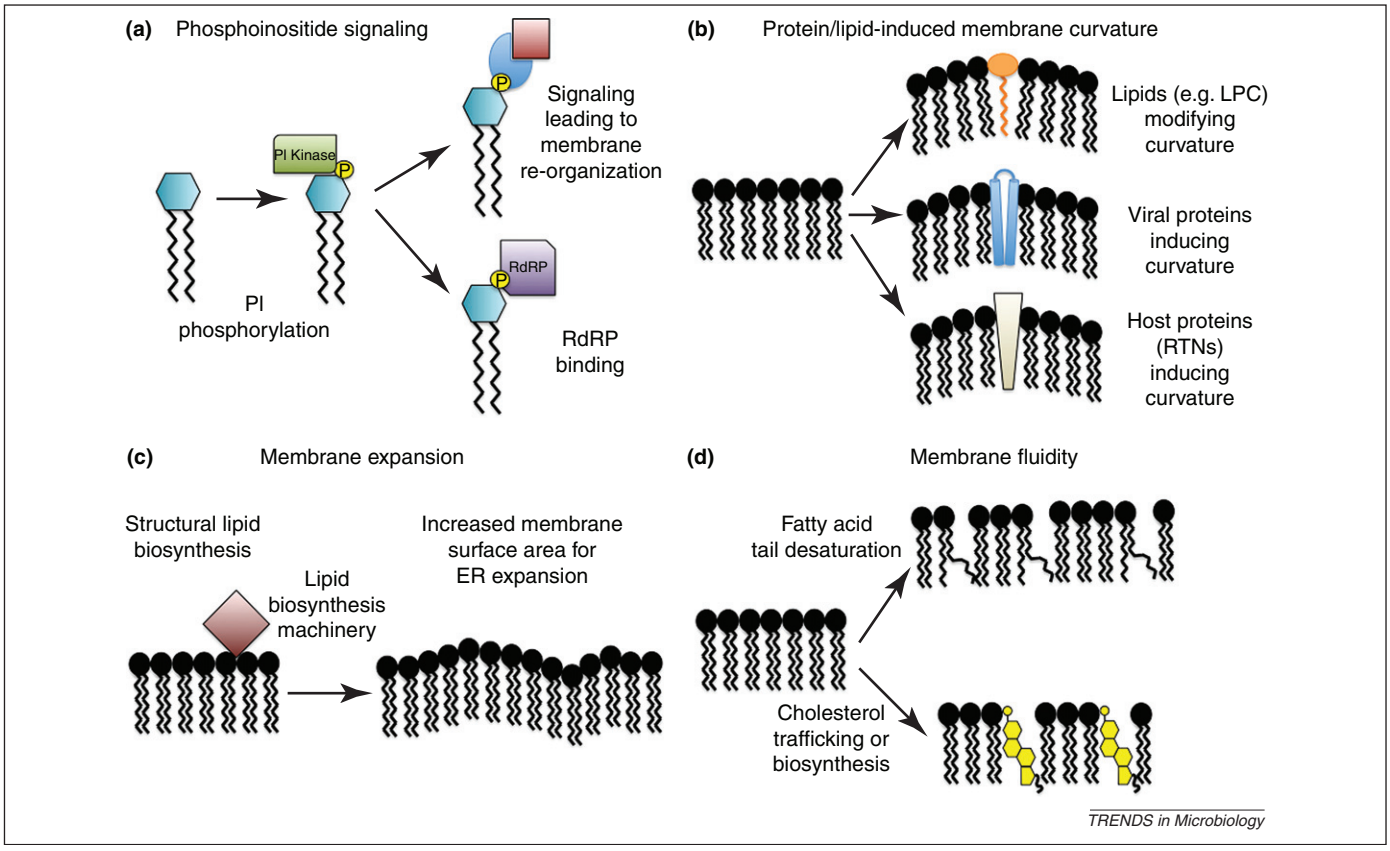

Figure 2. Roles for lipids and lipid signaling in replication complex formation and function. **(a)** Phosphoinositide signaling. Phosphoinositides (PIs) are negatively charged lipids with a specific head-group that can be phosphorylated at different positions to generate PI-phosphates (PIPs). PIPs can direct cell signaling in a pro-viral manner or directly bind to virus proteins during HCV and enterovirus infection [40,42]. **(b)** Membrane curvature. There are several ways in which viruses can induce membrane curvature. First, the virus can cause the accumulation of cone-shaped lipids (such as lysophosphatidylcholine, LPC) in one leaflet of the membrane bilayer, and this can lead to membrane bending (top). Second, the virus can encode a membrane-associated protein that induces membrane curvature (e.g. HCV NS4B and flavivirus NS4A, middle) [26,28]. Third, the virus can utilize host membrane proteins that induce membrane curvature, such as reticulons (RTNs, bottom) [43], which have been shown to be required for BMV membrane rearrangements. It is important to note that these are not mutually exclusive and a virus could use several strategies to accomplish membrane bending. **(c)** Membrane expansion. WNV and DENV actively recruit lipid-biosynthesis machinery to generate lipids at sites of replication [46,48]. This helps to form the replication complexes. Further, membrane expansion might be important in generating lipids for the virus envelope. **(d)** Membrane dynamics. Viruses such as DENV or WNV, which recruit lipid-biosynthesis machinery to RCs, can modulate membrane fluidity and plasticity of replication complex membranes by desaturating lipid tails or leading to the accumulation of cholesterol, as has been seen during BMV infection [44,45]. These modulations probably lead to a membrane environment that is conducive to viral replication complex formation or function.

virus replication and stimulates its activity to synthesize fatty acids [48]. A major theme of these studies is that simply inducing membrane curvature is probably insufficient to create the functional replication complexes seen in virus replication. There are additional requirements for altering both the total membrane content and specific lipid composition that impact upon membrane fluidity. A second emerging theme is that the formation of these structures might not simply be the function of one virus protein. Although HCV NS4B expression drives membrane remodeling, NS5A modulation of PI4-kinase activity clearly influences the process [42]. Similarly, DENV NS3 modulation of fatty acid synthesis probably influences the ability of NS4A to drive the formation of RC structures.

Although it is intuitive that lipids can play physical roles in virus-induced membrane rearrangements, their utility is not limited to that arena. Lipid post-translational modifications of virus proteins and host-cell cofactors are important for productive infection. The roles of post-translational lipid modifications with some viral examples is reviewed in [49]. Using HCV as an example, it has been shown that the virus protein NS4B is palmitoylated in an overexpression system [50] and this palmitoylation is required for virus replicase formation. In addition, palmitoylation of the HCV core protein is required for virus particle formation [51]. Geranylgeranylation of the host cofactor FBL2 is also required for HCV replication [52]. This gave hope that drugs targeting cholesterol synthesis such as statins could be useful for treating HCV infection. Although successful in inhibiting HCV replication *in vitro*, this approach only modestly improves the sustained antiviral response in patients [53].

Viruses provoke global changes in cellular metabolism

An emerging topic in virology is the reprogramming of cell metabolism to facilitate virus replication. Metabolomic analysis of cytomegalovirus (CMV) infection showed that metabolites involved in glycolysis, the citric acid cycle, and pyrimidine nucleotide biosynthesis as well as transcripts for genes involved in these processes were markedly increased [54,55]. There were also specific lipid metabolism changes; in particular, there was an increase in the flux of metabolites channeled into fatty acid biosynthesis pathway [56]. The authors further showed that inhibiting fatty acid biosynthesis inhibited CMV infection and thus identified a possible antiviral target. James Alwine and colleagues discuss the metabolic changes in CMV infection in much greater detail in another review in this themed issue [57]. Another herpesvirus, Kaposi's sarcoma-associated herpes virus, provokes similar changes in cell metabolism that resemble the Warburg effect – an increase in aerobic glycolysis and a decrease in oxidative phosphorylation – as part of its latent infection [58].

Microarray analyses of HCV-infected cells showed that there are significant changes in the expression of genes involved in biosynthesis, degradation, and transport of intracellular lipids [59]. Interestingly, proteomic analysis of infected cells revealed that many of the key regulators of lipid metabolism reprogramming were found to be post-transcriptionally regulated [60]. For example, the proteins involved in fatty acid oxidation are more abundant in HCV-infected cells but show no differential regulation in microarray studies. Complementary lipidomic analysis of HCV-infected cells also revealed that there were changes in specific lipid species (e.g. phospholipids and sphingomyelins), and these are predicted to play roles in virus replication as well as assembly and secretion events [60]. The authors of this study propose a model in which the infected cell is reprogrammed to maintain homeostasis and cell viability by modulating cellular metabolism to deal with the elevated energy demands during virus replication (Figure 3).

The mechanism by which HCV modulates lipid metabolism is partially understood. It has been shown that HCV infection or NS4B protein expression leads to the activation of transcription factors that stimulate the transcription of genes involved in sterol biosynthesis [61,62]. These studies taken together indicate that alterations in lipid metabolism can take place via transcriptional and post-transcriptional mechanisms and probably reflect complex regulation of lipid metabolism during HCV infection.

Lipids presumably can be mobilized for oxidation via canonical lipase pathways. In addition, it was recently shown that DENV infection induces a form of autophagy that specifically targets lipid droplet stores. This results in the depletion of cellular triglycerides and the release of fatty acids, which are then transported to the mitochondria where they undergo β-oxidation to generate ATP [63] (Figure 3). In essence, DENV signals the infected cell to react to perceived starvation conditions, triggering depletion of energy stores to stimulate robust virus replication. Thus, it appears that diverse viruses have evolved distinct mechanisms to modulate the metabolic state of the cell to facilitate virus replication.

Lipids in virion assembly and budding

In addition to being a source of energy, lipid droplets can function as sites of virus assembly. Lipid droplets are well characterized as the sites of HCV assembly [64] and have also been proposed to be involved in DENV assembly [65]. HCV core localizes to lipid droplets that are in proximity to ER-associated virus structural proteins. A current model is that HCV replication occurs at the membranous web discussed earlier and then nascent RNA is transported to lipid droplets by the virus replicase proteins NS3 and NS5A. Assembly of virus RNA into capsids is thought to take place at lipid droplets and requires the virus NS2 and p7 proteins [66]. It is thought that assembly at lipid droplets might influence lipid-associated secretion of HCV (discussed later). Interestingly, HCV core protein expression enhances the accumulation of lipid droplets in the perinuclear region. This phenomenon appears to be genotype-specific and linked to assembly efficiency [67]. One possible mechanism for core and lipid-droplet association is an interaction of HCV core with DGAT-1, which promotes lipid droplet formation [68].

For viruses that bud at the plasma membrane, lipids play a central role in organizing this event. HIV budding occurs at the plasma membrane at specific lipid microdomains rich in cholesterol and sphingolipids termed lipid rafts [69]. These lipid rafts are actively modulated by HIV, which increases cholesterol synthesis in infected cells and

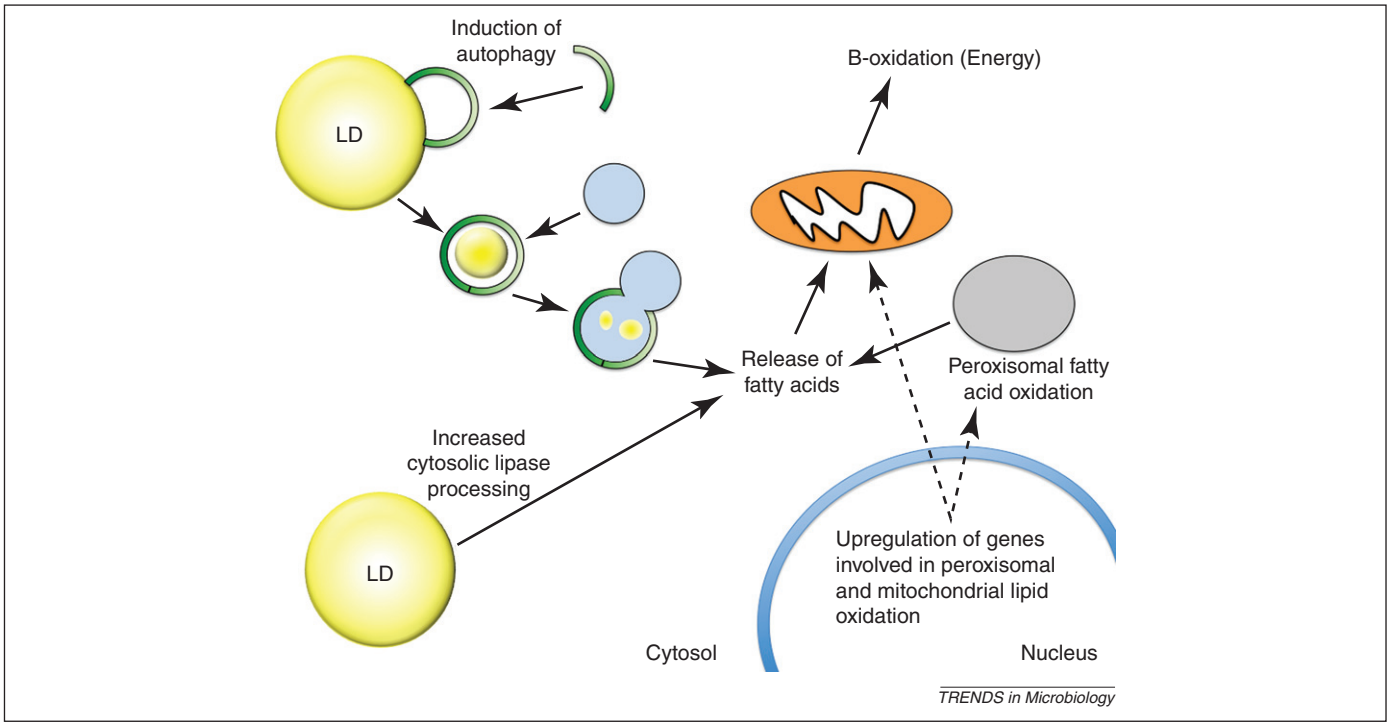

Figure 3. Modulating lipid metabolism can lead to the generation of energy for virus replication. The virus replication cycle is an energy-intensive process. Several reports have shown that the metabolic state of the infected cell can be shifted towards energy generation. Lipids and lipid stores represent vast pools of energy that are exploited during virus replication. Some viruses, such as HCV, appear to induce lipid metabolism both transcriptionally [59] and post-transcriptionally [60] to facilitate lipid oxidation and ATP generation. In addition, some viruses, such as DENV, can induce cytosolic processes, such as autophagy, which can lead to the degradation of lipid droplets (LDs) [63]. The lipids released from LDs are oxidized at mitochondria, leading to oxidative phosphorylation of ADP to generate ATP.

traffics it to lipid rafts [70] (Figure 4a). Lipid signaling also helps to organize HIV budding sites by recruiting the major HIV structural protein, Gag. The localization of Gag to lipid rafts at the plasma membrane is dependent on the interactions of the Gag protein with the lipid signaling molecule PI-(4,5)-bisphosphate, which is enriched at the plasma membrane [71–73]. The lipid interaction with Gag is thought to ensure the appropriate subcellular localization of virus structural proteins for virion assembly and budding. Similarly, the trafficking of influenza virus glycoproteins to the plasma membrane and subsequent budding depends on the interaction of virus components with lipid microdomains [74].

In addition to forming a scaffold or organizing center for virus assembly, lipids can be actively synthesized to provide membranes for virus envelopment. The enhanced fatty acid biosynthesis that occurs during CMV infection discussed earlier is thought to contribute to virion budding by increasing available membranes [56]. It is also important to note that any kind of membrane modulations that influence virus replication (such as those described earlier for DENV) could also play additional roles in virion envelopment because these compartments are frequently spatially linked.

A final step in the budding of enveloped viruses is membrane scission to release progeny virions. There are two major characterized pathways, those that are ESCRT (endosomal sorting complex required for transport)-dependent and those that are ESCRT-independent, whereby virus proteins can induce the membrane curvature required for release of the virus (Figure 4a). HIV secretion is a well-characterized example of an ESCRT-dependent pathway. HIV encodes proteins that contain 'L' or 'late' domains (reviewed in [75]) which interact with ESCRT machinery leading to release of the virus (reviewed in [76]). The ESCRT pathway normally functions to generate endosomal vesiculation, which leads to the formation of multivesicular bodies. It appears that this cellular machinery performs a similar function to facilitate the budding of HIV. This has been characterized as the canonical budding pathway for late-domain-containing viruses.

By contrast, influenza A virus does not appear to require host machinery for membrane scission; instead, a virus protein performs the same function. It was recently shown that the influenza protein M2 localizes to the 'neck' of budding virions and is necessary and sufficient for budding in the absence of ESCRT machinery (Figure 4b) [77]. This requires an amphipathic helix of M2 that induces membrane curvature, and this is thought to result in membrane scission. Several other viruses have been shown to use late domains for virus budding (reviewed in [78]). It will be interesting to see whether those viruses without obvious late domains use a mechanism similar to that of influenza A.

Roles for lipids in virion secretion and enhanced infectivity

Lipids and lipid trafficking can also play roles in virus secretion and influence the infectivity of released virions. The best-characterized model for lipid-associated egress is HCV, which appears to use the VLDL secretion machinery to facilitate its exit from cells (reviewed in [79]). HCV virions display a broad density range, with the low-density lipid-associated fractions being the most highly infectious [80]. Recently, the lipidomic profile of HCV virions was

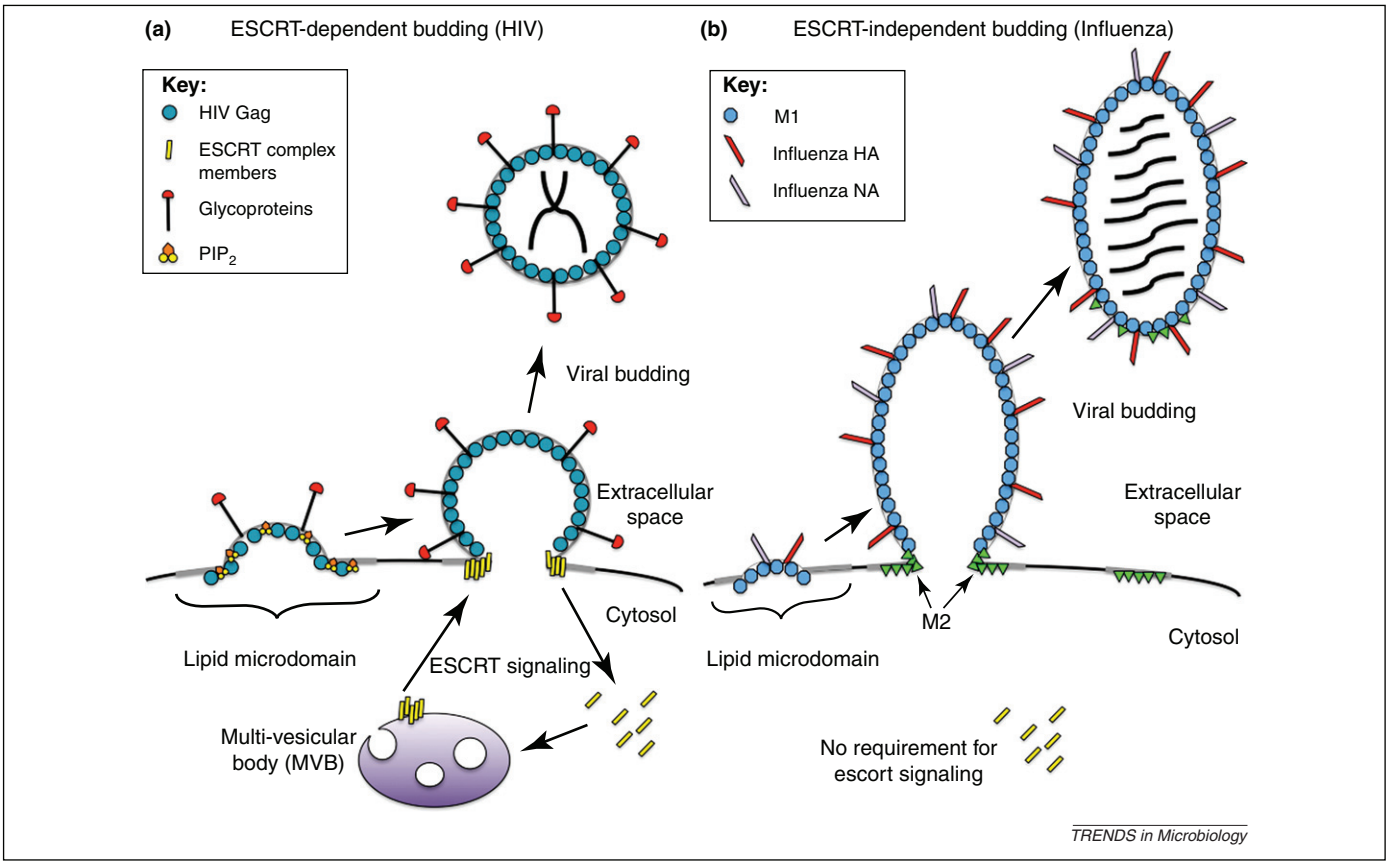

Figure 4. Lipids, lipid signaling and membrane scission in virus assembly and release. **(a)** Lipids in HIV assembly and release. HIV assembly and release requires specific lipids at multiple stages. Initially, the structural protein Gag is recruited to pre-budding sites by its association with the lipid phosphatidylinositol-(4,5)-bisphosphate (PIP_2) [72]. In addition, the membrane composition of these sites is specific, containing high amounts of sphingolipids and cholesterol [69]. As the virus begins to bud, there is significant membrane curvature that needs to be induced and then resolved, a process known as membrane scission. In the case of HIV, membrane scission is accomplished by coopting cellular machinery known as the ESCRT machinery. The ESCRT complex facilitates membrane scission and the release of the virus [76]. **(b)** Lipids in influenza virus assembly and release. Influenza A similarly associates with specific lipid microdomains during the budding process, however, the mechanism of membrane scission is significantly different. Instead of utilizing the ESCRT complex, the virus genome encodes the M2 protein, which serves an analogous function to the ESCRT complex and facilitates virus budding without a requirement for host machinery [77].

determined, which closely resembles that of low- and very-low-density lipoproteins [81]. HCV isolated from infected patients contained components of the VLDL machinery, including apolipoproteins (Apo) B and E [82]. Further, knockdown of ApoB and ApoE decreased the amount of intracellular as well as secreted infectious virus, indicating that apolipoproteins play a role in both secretion and infectivity [83,84]. Thus, the lipids required for HCV secretion also influence the characteristics of the mature virion that allow for enhanced entry of the virus.

Conclusions and future directions

It was recently written that the 1990s were the decade of DNA, the 2000s the decade of RNA, and the 2010s could be that of the lipid [85]. We feel that this could not be truer for the field of virology. Research interests are frequently shaped by the availability of tools, and we have an increasingly potent arsenal for studying the manipulation of lipids by viral infection. As always, the challenge will be to apply this technology to unmask the underlying mechanisms of virus–host interaction. An appealing outgrowth of this research is the concept of broad-spectrum antiviral therapeutics that target lipid metabolism. As an example, many viruses require active fatty acid biosynthesis. Existing FDA-approved drugs target this pathway and could be adapted to serve as broad-spectrum antiviral therapeutics. Because these drugs target host proteins, they could prove less susceptible to the development of virus drug resistance.

Much work remains to be done to better appreciate virus–lipid interactions. Many of the studies have relied heavily on drug or RNA interference inhibition of a lipid metabolic process to determine whether or not it is required during viral infection. However, only rarely have the essential lipid or lipid species been identified. Additionally, even in cases where we can identify distinct lipid species that are modified during viral infection, the proposed mechanism for how they contribute to virus replication is often left to speculation. Questions remain as to why specific lipid species are required, in addition to their physical orientation and biochemical function within these complexes. Finally, most of the studies described here have focused on the virus–cell interaction. Much remains to be determined how alterations in cell metabolism influence the host response to viral infection on an organismal level, from hormone production to immune responses. With increased research in this area, our understanding of how and why lipids are manipulated in the host cell continues to expand. This not only increases our basic understanding of virology and cell biology, but could also have practical implications for novel antiviral agents.

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