Microreview

Lipid interactions during virus entry and infection

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Summary

For entry and infection viruses have developed numerous strategies to subjugate indispensable cellular factors and functions. Host cell lipids and cellular lipid synthesis machinery are no exception. Not only do viruses exploit existing lipid signalling and modifications for virus entry and trafficking, they also reprogram lipid synthesis, metabolism, and compartmentalization for assembly and egress. Here we review these various concepts and highlight recent progress in understanding viral interactions with host cell lipids during entry and assembly.

Introduction

Lipids are a highly diverse group of naturally occurring hydrophobic biomolecules indispensable for cellular life. The biological functions of lipids range from membrane formation to energy storage and signalling (Voelker, 1991; van Meer et al., 2008; Vanhaesebroeck et al., 2012). As the main building block of biological membranes in eukaryotic cells, lipids serve for compartmentalization of organelles and their diverse functions. The plasma membrane, for instance, serves to selectively separate the intra- and extracellular environments and acts as the first barrier against invading pathogens (van Meer et al., 2008).

Investigation of the interaction between viruses and their host cells provides invaluable insights into the molecular mechanisms of viral pathogenesis and on host cell biology. How viruses enter host cells and systematically reprogram the cellular environment is one of the most compelling subjects in host–pathogen interaction. Recent work on multiple aspects of the role played by cellular lipids during the infection process has revealed their importance throughout the entire lifecycle of viruses (Heaton and Randall, 2011; Lorizate and Krausslich, 2011). These interactions range from virus binding to the host cell plasma membrane, to the release of new infectious progeny into the extracellular space. The emerging picture suggests that viruses take advantage of cell lipids by two means: subjugation and reprogramming. During early stages of infection, viruses subvert pre-existing cellular lipids and lipid signalling mechanisms for entry and trafficking. Once infection is initiated and viral genes expressed, extensive reprogramming of lipid synthesis and remodelling of lipid distribution serves to promote viral replication, assembly, and egress. This review focuses on how viruses exploit cellular lipids to promote entry and reorganize cell lipid composition, localization, and metabolism for the generation of progeny virions capable of propagating infection (illustrated in Fig. 1).

Cellular lipids and virus entry

The first encounter between a virus and a target cell occurs at the plasma membrane. It is here that viruses first engage binding and entry receptors to initiate the infection process. In addition to acting as the main barrier between the cells and their external environment, the plasma membrane controls and co-ordinates the internalization of particles and fluids through different endocytic uptake mechanisms (reviewed in Doherty and McMahon, 2009); not surprisingly, viruses have learned to subjugate most of these for their own entry (Mercer et al., 2010b).

In animal cells, phospholipids account for more than half of the lipids in cellular membranes. The plasma membrane also contains glycolipids and cholesterol, the latter important for regulating membrane fluidity. While lipids mostly diffuse freely in the plasma membrane, cholesterol, glycosphingolipids (glycolipids and sphingomyelin), glycosylphosphatidylinositol (GPI)-anchored proteins, and transmembrane proteins can cluster into discrete domains, called lipid rafts (Simons and Ikonen, 1997; Levental et al., 2010; Simons and Sampaio, 2011). Lipid rafts play an important role in signalling and membrane organization, but also serve as an important platform used by both enveloped and non-enveloped viruses to enter cells (Marsh and Helenius, 2006; Mercer et al., 2010b; Ewers and Helenius, 2011).

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Adhesion and attachment

For most viruses, enveloped and non-enveloped, entry is preceded and enhanced by weak ionic interactions between the virion and cell surface glycosaminoglycans (e.g. heparan and chondroitin sulfate) or glycosphingolipids (Lorizate and Krausslich, 2011). These interactions promote adhesion of the virion to the cell membrane, allowing the virus to surf along the cell surface until recognition of specific binding or internalization receptors occurs. Trafficking on the cell surface or through the endosome network serves to bring the virus to its preferred site of uncoating, while providing the cues needed to activate the virus fusion machinery (Marsh and Helenius, 2006). While normally non-specific in nature, different glycosphingolipids on the viral surface have been reported to confer cell specificity: the sialyllactose moiety on human immunodeficiency virus (HIV) membrane gangliosides, for instance, has been suggested to promote uptake of HIV into mature dendritic cells (mDCs) (Izquierdo-Useros et al., 2012). Gangliosides are acidic glycosphingolipids carrying one or more terminal sialic acid; by using liposomes mimicking the composition and size of HIV, the authors demonstrated that gangliosides GM1, GM2 and GM3 are the key molecules that mediate liposome uptake in mDCs, with the sialyllactose moiety on gangliosides acting as a molecular recognition pattern. Recognition and uptake of sialyllactose moieties on gangliosides is likely to be a common mechanism of particle internalization by DCs, leading to antigen processing and presentation; however, in the case of HIV, the antigen-processing function of mDCs is subverted, as the virus rather uses these for virus transmission (Izquierdo-Useros et al., 2012).

Lipid-mediated signalling for entry

While most viruses use surface proteins or sugars to promote entry, lipids can also function as virus entry
receptors. Low-density lipoprotein receptors, negatively charged phospholipids and gangliosides have all been shown to assist entry of different viruses (Agnello et al., 1999; Tsai et al., 2003; Campanero-Rhodes et al., 2007; Roth and Whittaker, 2011; Izquierdo-Useros et al., 2012; Meisen et al., 2012).

Gangliosides, in particular, have been shown to be required for entry of the non-enveloped polyomavirus simian virus 40 (SV40). Upon specific binding to ganglioside GM1, SV40 reduces GM1’s diffusion rate. This stabilized SV40-GM1 complex then recruits cholesterol to generate a lipid raft. The interaction induces actin-dependent immobilization of the virus-ganglioside complex, followed by virus-induced invagination of the plasma membrane. An elegant study by Ewers et al. has demonstrated that association of SV40, or the isolated pentameric receptor VP1, with GM1 is sufficient to induce membrane curvatures that lead to the formation of deep invaginations in the plasma membrane of the cell (Ewers et al., 2010). Other polyomaviruses, as well as Shiga and cholera toxin, can also induce plasma membrane curvature and promote their endocytic uptake (Romer et al., 2007; Wolf et al., 2008).

Low-density lipoprotein receptors, also known as cholesterol receptors, are used by several members of the Flaviviridae family, including hepatitis C virus (HCV) (Agnello et al., 1999). The interaction is likely to occur through lipoproteins associated with the virion, such as cholesteryl esters and apolipoproteins (ApoB and ApoE) acquired during the assembly and budding of virus (Targett-Adams et al., 2010; Alvisi et al., 2011), as discussed below.

Recent reports have also indicated that the transmembrane, cholesterol-sensing receptors Niemann-Pick C1 (NPC1) and Niemann-Pick C1-like 1 (NPC1L1) are important entry factors for filovirus (Carette et al., 2011) and HCV (Sainz et al., 2012) respectively. For filoviruses, silencing of NPC1 prevents fusion from lysosomal compartments. In contrast to NPC1L1-mediated HCV entry (Sainz et al., 2012), this was independent of the cholesterol binding activity of NPC1 (Carette et al., 2011). Whether the cholesterol transport function of NPC1 receptors is required for infectivity is unclear, but these results suggest that the NPC1 receptors can facilitate virus entry in a cholesterol-dependent and -independent fashion.

Finally, negatively charged phospholipids have been implicated as virus receptors for vesicular stomatitis virus (VSV). Entry of VSV has been suggested to require the interaction between the viral glycoprotein G and the negatively charged phospholipid phosphatidylserine (PS) on the cell surface (Carneiro et al., 2006). However, whether these molecules are the actual receptor for VSV entry has been challenged and is not clear (Coil and Miller, 2004).

### Lipid signalling during virus endosomal trafficking

Attachment to cellular receptors commonly activates signalling pathways that induce endocytosis of the virus. Regardless of the mechanism used for uptake, trafficking within the endosomal network is a highly co-ordinated process. After formation, endosomes go through maturation, a process that involves defined changes in cellular location, pH, protein and lipid composition (Fig. 1 for details).

The importance of lipids in co-ordinating these various events has come to light in the past decade. Phosphatidylinositol (PI) is the least abundant phospholipid in the cell membrane, but it is also one of the most versatile signalling molecules in cells and plays a central role in endosome trafficking and maturation. Differential phosphorylation of PI, regulated by specific PI kinases and phosphatases, results in the formation of different PI phosphate (PIP) species (reviewed in Vicinanza et al., 2008; Vanhaesebroeck et al., 2012). The constrained localization of these enzymes leads to differential concentrations of the PIPs within various cellular compartments; in turn, this compartmentalization influences PIPs signalling and protein recruitment activities.

Many viruses have evolved to exploit PI-mediated signalling at different stages of infection, and in particular, to co-ordinate virus entry and trigger downstream reprogramming of the host cell. The Class I PI3-kinase (PI3K) signalling pathway is one of the most important PI-mediated signalling cascades activated during virus entry. Activation of PI3K and subsequent generation of PIP3 serves as docking platform for proteins carrying lipid-binding domains, including Akt, the main effector of PI3K signalling (Das et al., 2010). While PI3K is involved in all forms of endocytosis (Doherty and McMahon, 2009; Mercer et al., 2010b; Antonescu et al., 2011), it is best characterized for its role in macrophagocytosis, where PI3K serves to co-ordinate signalling and cytoskeletal modulation during protrusion, extension, and closure phases of macrophagosome formation (Lindmo and Stenmark, 2006; Bohdanowicz and Grinstein, 2013). Activation of PI3K upon virus binding has been observed for a number of viruses using this entry mechanism, including influenza (Fujikawa et al., 2011; Marjuki et al., 2011), Herpes simplex virus type 1 (HSV-1) (Zheng et al., 2014), HCV (Berger et al., 2009), Zaire Ebola Virus (ZEBOV) (Saud et al., 2008), and vaccinia virus (VACV) (Mercer and Helenius, 2008; Mercer et al., 2010b; Izmailyan et al., 2012).

In addition to its crucial role in virus entry and trafficking, many viruses activate PI3K signalling for modulation of post-internalization events such as virus replication and assembly (highlighted in Diehl and Schaal, 2013). VACV, for example, requires activation of PI3K early for...
macropinocytic internalization (Mercer and Helenius, 2008; Mercer et al., 2010a; Izmailyan et al., 2012), and PI3K-Akt late to attenuate apoptosis of infected cells (Soares et al., 2009). In line with this, VACV is acutely sensitive to pharmacological inhibition of PI3K or Akt activity, which reduces viral yield up to 90% (Mercer and Helenius, 2008; Soares et al., 2009; Mercer et al., 2010a). The collective evidence indicates that activation of the PI3K signalling pathway is a widespread strategy used by multiple viruses to different ends (Diehl and Schaal, 2013).

Lipids, membrane curvature and virus fusion

The molecular shape of membrane lipids within endosomes can strongly influence virus fusion activities. For fusion from endosomes, after internalization and activation of viral fusion machinery, the viral fusion peptide inserts into the target endosome membrane. Fusion of viral and cell membranes then proceeds through a transient hemifusion intermediate where the outer leaflet of the viral membrane mixes with the inner leaflet of the endosome membrane. Subsequently, the inner viral and outer endosomal membrane leaflets merge and the hemifusion stalk opens, forming the fusion pore and thus completing the fusion process (Lorizate and Krausslich, 2011). Interestingly, lipid composition influences from which endosomal compartment a virus will fuse. For instance, dengue virus (DENV) exploits the late endosome specific anionic lipid bis(monoacylglycerol) phosphate (BMP) to promote fusion from late endosomes. The lipid-dependence of DENV fusion machinery assures that DENV does not fuse prematurely (Zaitseva et al., 2010). Endosomal lipid content has also been shown to be important for West Nile and alphavirus fusion, requiring either cholesterol or both cholesterol and sphingolipids respectively (Kielland et al., 2010; Moesker et al., 2010). Given the importance of lipids during this stage of virus entry, it comes to no surprise that host cells have developed strategies to counteract the interaction between viral and cellular membranes. Recent description of interferon-inducible transmembrane (IFITM) proteins and 25-hydroxycholesterol as viral restrictions factors serve as good examples (see Appendix 1).

Cellular lipids during viral infection

Subjugation of cellular lipids by viruses is not only used to promote entry and intracellular trafficking. In fact viruses also use lipids to modify their own proteins as well as cellular factors to promote viral replication complex formation, production of new viral particles, viral egress and spread of infection.

Lipids and viral replication complex formation

Viruses that replicate in the cytosol tend to reorganize cellular membranes to create sites of active replication. These sites, called viral factories or replication complexes (RC) provide a scaffold for the viral replication machinery, serve to concentrate the viral and cellular factors needed for assembly of new virions, and provide a protective environment for avoidance of cellular innate immune responses. Examples include the large dsDNA poxviruses which transiently recruit endoplasmic reticulum (ER)-derived cisternae around viral RCs (Condit et al., 2006; Krijnse Locker et al., 2013), and positive stranded RNA viruses which reorganize ER, Golgi, endosomal, lysosomal or mitochondrial membranes to form specialized membrane-bound RCs (Miyananri et al., 2007; Miller and Krijnse-Locker, 2008; Welsch et al., 2009; den Boon et al., 2010; Heaton et al., 2010).

A number of studies are now shedding light on virus-mediated modification of lipid profiles in order to shape the cellular membrane environment to promote infection. Membrane remodelling induced by DENV, for instance, is directly linked to a shift in the lipid repertoire during infection (Perera et al., 2012). High-resolution mass-spectrometry studies in mosquito cells indicates that together with lipids involved in controlling membrane fusion, fission, trafficking and cytoskeletal function, those able to change membrane curvature or permeability are enriched in DENV infected cells (Heaton et al., 2010; Perera et al., 2012).

Recent work has revealed that several of these viruses co-opt cellular cofactors to facilitate virus-mediated membrane remodelling. The NS5A protein of Hepatitis C virus (HCV), for instance, recruits PI4K-IIIα to virus replication sites to increase local levels of PI(4)P (Alvisi et al., 2011), while the picornavirus protein 3A recruits PI4K-IIIβ to the same end (Greninger et al., 2012). It has been proposed that high levels of PI(4)P directly contribute to RC formation by influencing membrane bending or by regulation of intracellular processes including vesicle fusion, budding and sorting (Berger et al., 2009; Alvisi et al., 2011).

In addition to subverting lipids for its replication, HCV subjugates existing lipid droplets (LDs) for assembly of infectious viral particles (Miyananri et al., 2007). Under normal conditions, lipid droplets serve as storage organelles for neutral lipids. During HCV infection viral core protein associates and accumulates on LDs. These virus-modified structures become surrounded by HCV RC-containing membranes and associated viral RNA and non-structural proteins. Although not fully understood, co-ordinated events occur between HCV RCs containing replicated genomes, and the viral core containing LDs for complete genome packaging and virus assembly (Miyananri et al., 2007).
Virus subversion of lipid metabolism

That viruses impact cell metabolism has been known for many years. However, advances in the field of metabolomics have only recently allowed us to fully appreciate the extent to which viruses reprogram host cell metabolism. Recent studies on several different viruses indicate that metabolic pathways and enzymes tend to be manipulated in a virus specific fashion. This is likely dependent upon the metabolic precursors the individual viruses find most advantageous for their replication. Not too surprisingly, all of these studies have identified important alterations in lipid synthesis upon viral infection. By altering the activity of the tricarboxylic acid (TCA) cycle or by manipulating the carbon source used by the cells to generate energy and macromolecules, several viruses take control of central energy metabolism to promote synthesis of cholesterol and fatty acids. This phenomenon has been described for two large enveloped viruses: HCMV (Vastag et al., 2011; Yu et al., 2011) and VACV (Greseth and Traktman, 2014). This is likely to be required to assure sufficient viral lipid membrane for building new viral progeny, and for remodelling of viral and cellular membranes to enhance viral replication, egress and entry into neighbouring cells.

Large enveloped viruses are not the only viruses that manipulate cellular lipid metabolism. Microarray analysis of HCV infected cells show significant changes in the expression of genes involved in lipid metabolism (Woodhouse et al., 2010). Recent transcriptomic and proteomic analyses indicate that the expression of host genes involved in lipid biosynthesis, degradation, and transport is profoundly altered by HCV; in particular cholesterol biosynthesis genes were found to be upregulated (Woodhouse et al., 2010). Parallel lipidomics analysis also showed changes in selected lipid species, particularly phospholipids and sphingomyelin (Diamond et al., 2010). This suggests that HCV reprogramming of host lipid metabolism attempts to maintain host homeostasis in spite of the elevated demand of metabolic precursors by the virus.

Modulation of cell lipids for virus assembly and infectivity

To complete the cycle of infection, newly assembled virions must have the ability to infect naive cells. Thus the importance of lipids during this last stage of the viral life cycle is twofold: not only are they required for assembly and release of new viral particles, in some cases the lipid content of the viral particles dictates virus infectivity.

Most enveloped viruses acquire their envelope by budding. This can occur either at the plasma membrane or internal membranes. The majority of retroviruses, paramyxoviruses, and orthomyxoviruses bud from the plasma membrane; flaviviruses bud from the ER membrane, and HSV gains its envelope from trans Golgi network (TGN) or endosomes (Lorizate and Krausslich, 2011). As lipids play a central role in this process, it is not surprising that viruses promote lipid synthesis and reorganization of cellular membrane lipid composition for virus assembly and exit. HIV, which buds from plasma membrane lipid rafts provides an interesting example. HIV actively modulates lipid rafts by increasing the synthesis and trafficking of cholesterol to these sites (Aloia et al., 1993). Analysis of the viral membrane has revealed that it is enriched in sphingolipids and cholesterol at the expense of phosphatidyCho lines, confirming its raft origin (Aloia et al., 1993). Also, the major HIV structural protein responsible for assembly of the budding structure, Gag, localizes in lipid rafts through interactions with the lipid signalling molecule PIP2 to ensure the correct localization of Gag for viral assembly and budding (Aloia et al., 1993; Barrero-Villar et al., 2008; Lorizate et al., 2013).

In addition to modifying the lipid content of existing membranes to co-ordinate virus budding, viruses can promote the active synthesis of lipids to provide membranes for the viral envelope. The increased biosynthesis of fatty acids, observed during HCMV and VACV infections is thought to increase the available membranes for viral membrane wrapping and assembly respectively. Interestingly in these cases the viral lipid content is altered with respect to host cell membranes. HCMV has been shown to contain more phosphatidylethanolamines and less phosphatidylserine than the host cell membrane. This lipid content, which resembles that of synaptic vesicles, is thought to facilitate HCMV budding and release (Liu et al., 2011).

In contrast, the VACV membrane is enriched for phosphatidylserine (PS) (Ichihashi and Oie, 1983) facilitating the next round infection by PS-dependent macropinocytic entry (Mercer and Helenius, 2008). With the exposure of PS, VACV mature viruses (MV)s resemble apoptotic bodies. As apoptotic clearance can occur by macropinocytosis (Hoffmann et al., 2001) we postulated that VACV employs apoptotic mimicry (Mercer and Helenius, 2010). Despite our demonstration that the virus can directly bind to the PS receptor Axl (Frei et al., 2012), the PS receptors (TAMs, TIMs, stablin-2, MFGE-8) and bridging molecules (serum protein S and Gas6) (Scott et al., 2001; Hanayama et al., 2002; Hafizi and Dahlback, 2006; Miyashita et al., 2007; Park et al., 2008) used by VACV remain elusive. It will be of interest to determine if VACV PS receptor(s) bind phosphatidylserine or the D-stereoisomer of PS, two lipids absent from the MV membrane that can functionally substitute for the naturally occurring PS (Laliberte and Moss, 2009).
Since its inception, viral apoptotic mimicry has proven to be a widespread lipid-mediated entry mechanism used by several enveloped viruses including: pichinde, cytomegalo, lassa fever, lenti, dengue, ebola and Marburg viruses (Callahan et al., 2003; Shimojima et al., 2006; Soares et al., 2008; Hunt et al., 2011; Kondratowicz et al., 2011; Mercer, 2011; Meertens et al., 2012; Jemielity et al., 2013; Moller-Tank et al., 2013; Morizono and Chen, 2014). For several of these viruses the PS-receptors and bridging molecules required for entry have now been defined (Shimojima et al., 2006; Hunt et al., 2011; Kondratowicz et al., 2011; Mercer, 2011; Meertens et al., 2012; Jemielity et al., 2013; Moller-Tank et al., 2013). That antibodies directed against PS can neutralize lethal pichinde and cytomegalo virus infections (Soares et al., 2008), and virus engagement of PS receptors can dampen innate immune responses to infection (Bhattacharya et al., 2013) suggests that the therapeutic potential of PS targeting deserves further investigation.

Future perspectives

The fundamental role of lipids in virus biology and infection is becoming increasingly clear. New technologies, such as metabolomics, and advances in mass spectrometry-based lipidomics are allowing for systematic characterization of the alterations in host lipid metabolism, as well as cellular and viral lipid profiles induced by viral infection.

As highlighted in this review, lipids serve to orchestrate different stages of viral replication, ranging from entry to spread. Viruses take control of lipid-mediated signalling to co-ordinate viral entry and intracellular trafficking. Later during infection, they actively modify intracellular membrane for replication, re-direct lipid metabolism to produce sufficient membrane for the assembly of new particles, and modify cell membrane lipid content to ensure infectivity of those virions.

Systematic characterization of how viruses take control of and alter lipid metabolism is now needed to unravel the common strategies used by these different viruses. Such studies may serve for the identification of infection biomarkers; and together with the development of therapeutics targeting subsets of lipid synthesis enzymes it may be possible to identify novel broad-spectrum therapeutic agents that target virus modified lipid metabolism. Overall, a deeper understanding of how viruses manipulate the host cells lipid program will serve to further our understanding of the cellular mechanisms that govern lipid modification, compartmentalization and metabolism.

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Appendix 1

**IFITMs and 25-HC as viral restriction factors**

About 20 years after their identification as interferon stimulated genes (ISGs) (Lewin et al., 1991), interferon-inducible transmembrane (IFITM) proteins were characterized as viral restriction factors (Brass et al., 2009). Interestingly, while most known restriction factors act after viral entry, IFITM proteins were shown to restrict viral fusion. Since the initial study describing the role of IFITM proteins as influenza A virus (IAV) and flavivirus restriction factors (Brass et al., 2009), several other studies have followed showing the importance of these proteins in inhibiting entry of many other viruses including filoviruses (Huang et al., 2011), rabdoviruses (Weidner et al., 2010; Smith et al., 2013), bunyaviruses (Mudhasani et al., 2013), and the non-enveloped reoviruses (Anafu et al., 2013).

Each of the three IFITM proteins has different cellular localizations which determine the range of viruses they restrict. IFITM1 is located primarily at the plasma membrane; IFITM2 and 3 localize in intracellular compartments, with IFITM3 colocalizing with endosomal markers (reviewed in Smith et al., 2014). IFITM3 has been shown to block fusion of viral and cell membranes – plasma or endosomal – via reduction of membrane fluidity (Li et al., 2013). While the exact mechanism of action is not clear, one study suggests that IFITM3 reduces membrane fluidity and increase positive curvature in the outer leaflet of the host membrane: by interacting with vesicles membrane protein associated protein A (VAPA), IFITM3 would disrupt the association between VAPA and an oxysterol binding protein that regulates the content of cholesterol in endosomal membrane, therefore restricting viral fusion (Amini-Bavil-Olyaee et al., 2013). More recent reports have however contradicted this hypothesis, demonstrating that an increase in the concentrations of cholesterol in cellular membranes is not itself sufficient to inhibit fusion, and suggests instead that, directly or indirectly, IFITM3 might interfere with pore formation in the cytoplasmic leaflet of the hemifusion intermedi- ate or, alternatively, that they might re-direct viral fusion to a non-productive pathway (Desai et al., 2014). Inhibition of non-enveloped viruses as reovirus, and lack of inhibition of enveloped viruses like arenaviruses (Brass et al., 2009) and some major DNA viruses like papilloma virus, human cytomegalovirus and adenovirus (Warren et al., 2014) also suggest that much more still needs to be discovered, and also introduces the possibility that some viruses might have evolved to specifically evade this antiviral mechanism.

Recent findings also indicate that repression of the sterol biosynthetic pathway is an important component of the interferon-inducible antiviral response (Blanc et al., 2011). An overexpression screen aimed at identifying IFN-induced antiviral effectors has clarified a role for 25-hydroxycholesterol (25HC) in inhibiting infection of several enveloped viruses (Liu et al., 2012). 25HC was reported to inhibit virus-cell fusion through direct alteration of cellular membrane composition (Liu et al., 2013), although it is also possible that viral infection is modulated at multiple post-entry events (Schoggin and Randall, 2013).

References

Agnello, V., Abel, G., Elfahal, M., Knight, G.B., and Zhang, Q.X. (1999) Hepatitis C virus and other flaviviridae viruses
Heaton, N.S., and Randall, G. (2011) Multifaceted roles for lipids in viral infection. Trends Microbiol 19: 368–375.

Heaton, N.S., Perera, R., Berger, K.L., Khadka, S., Lacount, D.J., Kuhn, R.J., and Randall, G. (2010) Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. Proc Natl Acad Sci USA 107: 17345–17350.

Hoffmann, P.R., Decathelineau, A.M., Ogden, C.A., Leverrier, Y., Bratton, D.L., Daleke, D.L., et al. (2001) Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. J Cell Biol 155: 649–659.

Huang, I.C., Bailey, C.C., Weyer, J.L., Radoshitzky, S.R., Becker, M.M., Chiang, J.J., et al. (2011) Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog 7: e1001258.

Hunt, C.L., Kolokoltsov, A.A., Davey, R.A., and Maury, W. (2011) The Tyro3 receptor kinase Axl enhances cellular fatty acid synthase signalling. Nature 472: 341–349.

Izmailyan, R., Hsao, J.C., Chung, C.S., Chen, C.H., Hsu, Ichihashi, Y., and Oie, M. (1983) The activation of vaccinia virus entry by endocytosis through phosphatidylserine from the plasma membrane. Virology 130: 306–317.

Izquierdo-Useros, N., Lorizate, M., Contreras, F.X., Kondratowicz, A.S., Lennemann, N.J., Sinn, P.L., Davey, R.A., Hunt, C.L., Moller-Tank, S., et al. (2011) TIM-family proteins promote infection of multiple enveloped viruses through virion-associated phosphatidylserine. PLoS Pathog 9: e1003232.

Jemielity, S., Wang, J.J., Chan, Y.K., Ahmed, A.A., Li, W., Monahan, S., et al. (2013) TIM-family proteins promote infection of multiple enveloped viruses through virion-associated phosphatidylserine. PLoS Pathog 9: e1003232.

Kielian, M., Chanell-Vos, C., and Liao, M. (2010) Alphavirus Entry and Membrane Fusion. Viruses 2: 796–825.

Kondratowicz, A.S., Lennemann, N.J., Sinn, P.L., Davey, R.A., Hunt, C.L., Moller-Tank, S., et al. (2011) T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. Proc Natl Acad Sci USA 108: 8426–8431.

Krijnse Locker, J., Chlanda, P., Sachsenhaemier, T., and Brugger, B. (2013) Poxvirus membrane biogenesis: rupture not disruption. Cell Microbiol 15: 190–199.

Laliberte, J.P., and Moss, B. (2009) Appraising the apoptotic mimicry model and the role of phospholipids for poxvirus entry. Proc Natl Acad Sci USA 106: 17517–17521.

Levental, I., Grzybek, M., and Simons, K. (2010) Greasing their way: lipid modifications determine protein association with membrane rafts. Biochemistry 49: 6305–6316.

Lewin, A.R., Reid, L.E., McMahon, M., Stark, G.R., and Kerr, I.M. (1991) Molecular analysis of a human interferon-inducible gene family. Eur J Biochem 199: 417–423.

Liu, K., Markosyan, R.M., Zheng, Y.M., Golffeto, O., Bungart, B., Li, M., et al. (2013) IFITM proteins restrict viral membrane hemifusion. PLoS Pathog 9: e1003124.
Miyanari, Y., Atsuzawa, K., Usuda, N., Watashi, K., Hishiki, T., Zayas, M., et al. (2007) The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 9: 1089–1097.

Miyanami, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., and Nagata, S. (2007) Identification of Tim4 as a phosphatidylserine receptor. *Nature* 450: 435–439.

Moesker, B., Rodenhuis-Zybert, I.A., Meijerhof, T., Wilschut, J., and Smit, J.M. (2010) Characterization of the functional requirements of West Nile virus membrane fusion. *J Gen Virol* 91: 389–393.

Moller-Tank, S., Kondratowicz, A.S., Davey, R.A., Rennert, P.D., and Maury, W. (2013) Role of the phosphatidylserine receptor TIM-1 in enveloped-virus entry. *J Virol* 87: 8327–8341.

Morizono, K., and Chen, I.S. (2014) Role of phosphatidylserine receptors in enveloped virus infection. *J Virol* 88: 4275–4290.

Mudhasani, R., Tran, J.P., Retterer, C., Radoshitzky, S.R., Kota, K.P., Altamura, L.A., et al. (2013) IFITM-2 and IFITM-3 but not IFITM-1 restrict Rift Valley fever virus. *J Virol* 87: 8451–8464.

Park, S.Y., Jung, M.Y., Kim, H.J., Lee, S.J., Kim, S.Y., Lee, B.H., et al. (2008) Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. *Cell Death Differ* 15: 192–201.

Perera, R., Riley, C., Isaacs, G., Hopf-Jannasch, A.S., Moore, R.J., Weitz, K.W., et al. (2012) Dengue virus infection perturbs lipid homeostasis in infected mosquito cells. *PLoS Pathog* 8: e1002584.

Romer, W., Berland, L., Chambon, V., Gaus, K., Windschiegl, B., Tenza, D., et al. (2007) Shiga toxin induces tubular membrane invaginations for its uptake into cells. *Nature* 450: 670–675.

Roth, S.L., and Whittaker, G.R. (2011) Promotion of vesicular stomatitis virus fusion by the endosome-specific phospholipid bia( monoacylglycero)phosphate (BMP). *FEBS Lett* 585: 865–869.

Saeed, M.F., Kolokoltsov, A.A., Freiberg, A.N., Holbrook, M.R., and Davey, R.A. (2008) Phosphoinositide-3-kinase-Akt pathway controls cellular entry of Ebola virus. *PLoS Pathog* 4: e1000141.

Sainz, B., Jr, Barreto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., et al. (2012) Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. *Nat Med* 18: 281–285.

Schoggins, J.W., and Randall, G. (2013) Lipids in innate antiviral defense. *Cell Host Microbe* 14: 379–385.

Scott, R.S., McMahon, E.J., Pop, S.M., Reap, E.A., Caricchio, R., Cohen, P.L., et al. (2001) Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 411: 207–211.

Shimoyama, M., Takada, A., Ebihara, H., Neumann, G., Fujikawa, K., Irimura, T., et al. (2006) Tyro3 family-mediated cell entry of Ebola and Marburg viruses. *J Virol* 80: 10109–10116.

Simons, K., and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* 387: 569–572.

Simons, K., and Sampaio, J.L. (2011) Membrane organization and lipid rafts. *Cold Spring Harb Perspect Biol* 3: a004697.

Smith, S., Weston, S., Kelam, P., and Marsh, M. (2014) IFITM proteins—cellular inhibitors of viral entry. *Curr Opin Virol* 4: 71–77.

Smith, S.E., Gibson, M.S., Wash, R.S., Ferrara, F., Wright, E., Temperton, N., et al. (2013) Chicken interferon-inducible transmembrane protein 3 restricts influenza viruses and lyssaviruses in vitro. *J Virol* 87: 12957–12966.

Soares, J.A., Leite, F.G., Andrade, L.G., Torres, A.A., De Sousa, L.P., Barcelos, L.S., et al. (2009) Activation of the PI3K/Akt pathway early during vaccinia and cowpox virus infections is required for both host survival and viral replication. *J Virol* 83: 6883–6899.

Soares, M.M., King, S.W., and Thorpe, P.E. (2008) Targeting inside-out phosphatidylserine as a therapeutic strategy for viral diseases. *Nat Med* 14: 1357–1362.

Targett-Adams, P., Boulant, S., Douglas, M.W., and McLauchlin, J. (2010) Lipid metabolism and HCV infection. *Viruses* 2: 1195–1217.

Tsai, B., Gilbert, J.M., Heine, T., Lencer, W., Benjamin, T.L., and Rapoport, T.A. (2003) Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J* 22: 4346–4355.

Vanhaesebroeck, B., Stephens, L., and Hawkins, P. (2012) PI3K signalling: the path to discovery and understanding. *Nat Rev Mol Cell Biol* 13: 195–203.

Vastag, L., Koyuncu, E., Grady, S.L., Shenk, T.E., and Rabinowitz, J.D. (2011) Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism. *PLoS Pathog* 7: e1002124.

Vicinanza, M., D’angelo, G., Di Capelli, A., and De Matteis, M.A. (2008) Function and dysfunction of the PI system in membrane trafficking. *EMBO J* 27: 2457–2470.

Voelker, D.R. (1991) Organelle biogenesis and intracellular lipid transport in eukaryotes. *Microbiol Rev* 55: 543–560.

Warren, C.J., Griffin, L.M., Little, A.S., Huang, I.C., Farzan, M., and Pyeon, D. (2014) The antiviral restriction factors IFITM1, 2 and 3 do not inhibit infection of human papillomavirus, cytomegalovirus and adenovirus. *PLoS ONE* 9: e96579.

Weidner, J.M., Jiang, D., Pan, X.B., Chang, J., Block, T.M., and Guo, J.T. (2010) Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. *J Virol* 84: 12646–12657.

Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C.K., Walther, P., et al. (2009) Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe* 5: 365–375.

Wolf, A.A., Jobling, M.G., Saslowsky, D.E., Kern, E., Drake, K.R., Kenworthy, A.K., et al. (2008) Attenuated endocytosis and toxicity of a mutant cholera toxin with decreased ability to cluster ganglioside GM1 molecules. *Infect Immun* 76: 1476–1484.

Woodhouse, S.D., Narayan, R., Latham, S., Lee, S., Antarbus, R., Gangadharan, B., et al. (2010) Transcriptome sequencing, microarray, and proteomic analyses reveal cellular and metabolic impact of hepatitis C virus infection in vitro. *Hepatology* 52: 443–453.
Yu, Y., Clippinger, A.J., and Alwine, J.C. (2011) Viral effects on metabolism: changes in glucose and glutamine utilization during human cytomegalovirus infection. *Trends Microbiol* **19**: 360–367.

Zaitseva, E., Yang, S.T., Melikov, K., Pourmal, S., and Chernomordik, L.V. (2010) Dengue virus ensures its fusion in late endosomes using compartment-specific lipids. *PLoS Pathog* **6**: e1001131.

Zheng, K., Xiang, Y., Wang, X., Wang, Q., Zhong, M., Wang, S., *et al*. (2014) Epidermal growth factor receptor-PI3K signaling controls cofillin activity to facilitate herpes simplex virus 1 entry into neuronal cells. *mBio* **5**: e00958–13.