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Virus assembly factories in a lipid world

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Many viruses build specialized structures known as viral factories, a protected environment in which viral genome replication and morphogenesis take place. Recent findings show that viruses manipulate lipid flows to assemble these replication platforms. Viruses are thus able to create new membranes by interfering with lipid metabolism, targeting and transport; they make use of specific lipid transfer proteins (LTP) at membrane contact sites, and frequently recruit endoplasmic reticulum (ER), ER export sites, and mitochondria. Some factories, such as those built by plant and certain animal viruses, are motile membranous structures involved in intracellular or intercellular transport of the replicated viral genome. The identification of lipids and LTP subverted by viruses might lead to better understand and fight viral infections.

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Introduction

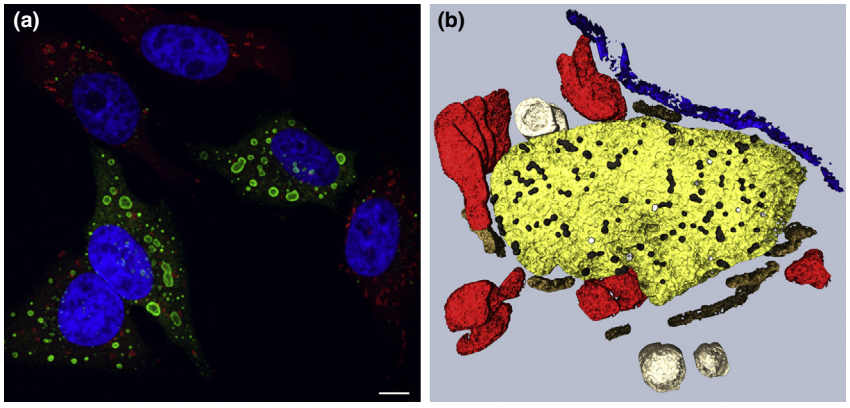
Viruses modify cell organization to build their replication factories. These structures are complex and very dynamic [1] (Figure 1). Live cell imaging has revealed that some factories are motile and fuse with a behavior characteristic of a liquid state [2,3]; others, like those of plants, are able to travel long distances to reach uninfected cells [4•]. Viral and cellular proteins are both key players in the biogenesis of these structures. Viral polymerases can produce direct membrane rearrangements [5] that often involve a number of cell membrane-remodeling proteins such as ESCRT (endosomal sorting complexes required for transport) and reticulons. These proteins induce the membrane curvature necessary for assembly of viral replication complexes (VRC) [6–8]. In some cases, the viral genome can also determine the size of the vesicles or spherules that harbor active VRC [9], to which chaperones, helicases and translational effectors are recruited. Recent reviews describe these effectors in detail [1,10,11]; here we focus on lipids and membranes.

Although many cell structures have no membranes, as for example stress granules, the centrosome, the nucleolus or the cytoskeleton, a virus generally uses membranes to assemble the replication complex. During virus infections, notable changes take place in the up- and downregulation of host genes involved in lipid synthesis, metabolism and transport, as identified by genetic screenings, biochemistry and microscopy [12,13]. In virus-infected cells, membranes are modified in a variety of ways. Viruses can induce the synthesis of new membranes, mix pre-existing membranous compartments, or even induce membrane rupture [14,15]. Alterations in lipid distribution are so pronounced that phosphatidylserine, a phospholipid usually found on the inner leaflet of the plasma membrane, becomes exposed in virus-infected cells. This characteristic that could be associated with virus-induced cell activation or apoptosis, has been used in the design of antivirals [16•]. In this review, we focus on three main topics that summarize important recent findings on how viruses harness lipids and manipulate cell membranes.

Viruses manipulate lipid synthesis and flow

Viruses can control membrane fluidity and plasticity by desaturating lipid tails or through cholesterol accumulation. Correct membrane fluidity is essential for viral RNA replication, as has been seen in cells infected by brome mosaic virus (BMV), whose replication requires unsaturated fatty acids [17••]. As a matter of fact, most nonstructural viral proteins are hydrophobic and integrate into cell membranes, where they induce changes in membrane composition and fluidity [18]. Viruses not only recruit specific lipids, but can also stimulate *de novo* lipid synthesis. Some pathogenic DNA and RNA viruses of the families *Hepadnaviridae*, *Herpesviridae*, *Retroviridae*, *Coronaviridae*, *Flaviviridae* and *Togaviridae* induce synthesis of highly ordered, three-dimensional nano-periodic structure, termed cubic membranes. Virus-induced cubic membranes are thought to be linked to cell cholesterol metabolism by activating HMG-CoA-reductase, the rate-limiting enzyme for cholesterol synthesis [18]. Alterations in cholesterol metabolism are common during viral infections. For example, West Nile virus (WNV) redistributes cholesterol-synthesizing enzymes as well as cellular cholesterol to replication sites. This alters the cholesterol biosynthetic pathway and modifies cholesterol levels at the plasma membrane, which in addition interferes with antiviral signaling [19•].

Figure 1



Confocal microscopy and 3D transmission electron microscopy (TEM) of factories assembled by the human reovirus in HeLa cells. **(a)** Cells were labeled with antibodies to viral proteins (green), P32 mitochondrial protein with specific antibody (red), and nuclei were stained with DAPI (blue). **(b)** Factory as visualized by TEM of serial sections, three-dimensional reconstruction and image processing. Mitochondria (red), rough ER (brown) and lipid droplets (white) surround a network of membranes (yellow) that contain mature virions (black) and empty viral cores (white). Nuclear envelope is blue.

Another example is dengue virus (DENV) whose manipulation of cholesterol biosynthesis and transport has been characterized in detail [20,21]. The DENV non-structural protein NS3 binds to fatty acid synthase (FASN) and this interaction relocalizes and activates the cellular enzyme in viral replication sites [22••]. Analysis of membranes from DENV-infected cells showed FASN-dependent enrichment in unsaturated phospholipids such as ceramide and lysophospholipids, and in signaling molecules such as sphingomyelin [23].

In addition to lipid synthesis, some (+) strand RNA viruses modify cellular lipid signaling to create new replication sites. Local increase in phosphatidylinositol 4-phosphate (PI4P) thus has important effects on viral replication, through formation of membrane structures that allow VRC assembly, while protecting the viral genome from degradation. To this purpose, hepatitis C virus (HCV), as well as some enteroviruses and picornaviruses stimulate PI4 kinases to generate PI4P at replication sites. PI4P synthesis is essential for replication of these viruses [24,25••], although its specific function in VRC remains unknown. In the case of HCV, a link between PI4P and glycosphingolipids, that are important in HCV RNA synthesis, has been recently proposed [26]. For accumulating specific lipids in replication organelles viruses recruit lipid transfer proteins (LTP) to PI4P-rich regions where they play a major role in VRC assembly. For example, oxysterol-binding protein (OSBP) that is necessary for HCV and poliovirus replication and ceramide transfer protein, have PI4P-binding domains and can facilitate cholesterol and sphingomyelin recruitment to these compartments [27,28•,29]. Inhibition of OSBP and OSBP-related protein 4 (ORP4) with the anti-fungal agent itraconazole perturbs the virus-induced membrane structures necessary for enterovirus replication [30]. Another example is the human rhinovirus, whose replication depends on OSBP1, the LTP which transports cholesterol and PI4P between the endoplasmic reticulum and the Golgi complex at membrane contact sites (MCS) [31]. LTP probably facilitate the recruitment of specific lipid species and the formation of MCS during the assembly of viral replication organelles and factories of many other viruses. Similar membrane expansion processes are likely to underlie the generation of membranes for making viral envelopes.

Viruses use lipid droplets

Lipid droplets (LD) are multifunctional dynamic organelles that regulate the storage and hydrolysis of neutral lipids, participate in lipid energy balance and signaling, cell immunity, protein storage and trafficking [32]. They interact with other organelles such as endoplasmic reticulum (ER) and mitochondria, interactions that facilitate lipid and protein transport. LD are especially relevant for RNA viruses [33], and we describe several examples below.

Rotavirus factories or viroplasms colocalize with LD-associated lipids and proteins, such as peripilin and ADRP (adipose differentiation-related protein). These interactions appear to be required for the assembly of functional viroplasms because dispersing LD with chemical compounds or blocking LD biogenesis significantly impair viroplasm size, viral RNA replication and the production of infectious viruses [34]. Lipidome analysis