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Viral rewiring of cellular lipid metabolism to create membranous replication compartments

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Positive-strand RNA (+RNA) viruses (*e.g.* poliovirus, hepatitis C virus, dengue virus, SARS-coronavirus) remodel cellular membranes to form so-called viral replication compartments (VRCs), which are the sites where viral RNA genome replication takes place. To induce VRC formation, these viruses extensively rewire lipid metabolism. Disparate viruses have many commonalities as well as disparities in their interactions with the host lipidome and accumulate specific sets of lipids (sterols, glycerophospholipids, sphingolipids) at their VRCs. Recent years have seen an upsurge in studies investigating the role of lipids in +RNA virus replication, in particular of sterols, and uncovered that membrane contact sites and lipid transfer proteins are hijacked by viruses and play pivotal roles in VRC formation.

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Introduction

Viruses are obligate intracellular pathogens that depend on host cell metabolism for their replication. Viruses interact with host membranes and lipids at various stages of their life cycle. All positive-strand RNA (+RNA) viruses, which comprise many medically and economically important pathogens of humans, animals, plants and unicellular eukaryotes (Table 1), remodel cellular membranes into unique 'viral replication compartments' (VRCs) to support replication of the viral genome (for review, see *e.g.* Ref. [1]). VRCs are generally assumed to support replication by serving as a platform on which proteins involved in genome replication are concentrated and assembled into active replication complexes. Additionally, VRCs are thought to shield the viral RNA from cellular defence systems that patrol the cytoplasm to detect and eliminate intruders (*e.g.* pattern recognition receptors, RNases). The structure of VRCs and the mechanisms underlying their formation vary greatly between +RNA viruses, but universally depend on cellular lipid and membrane homeostasis. Here we will review how +RNA viruses rewire host lipid metabolism and describe contributions of different lipid categories to VRC formation and functioning.

The structure of VRCs

Recent advances in 3D electron microscopy have revolutionised our understanding of the structure of VRCs. VRCs can be subdivided into two morphological classes (Figure 1) (reviewed in Ref. [2]). Invagination-type VRCs are formed by bending of the donor membrane away from the cytoplasm (*i.e.* by induction of negative membrane curvature), resulting in a secluded environment that contains all factors required for genome replication. These VRCs are usually connected to the cytoplasm by a narrow pore that allows passing of small molecules such as nucleotides but restricts access of the antiviral defence machinery. Viruses with invagination-type VRCs include flaviviruses, tombusviruses and nodaviruses (Table 1).

Protrusion-type VRCs are generated by bending of the donor membrane into the cytoplasm (*i.e.* through induction of positive membrane curvature), resulting in single-membrane and double-membrane structures. Viruses that generate protrusion-type VRCs include hepatitis C virus (HCV), coronaviruses and enteroviruses (*e.g.* poliovirus, coxsackievirus, and rhinovirus) (Table 1). Protrusion-type VRCs often form a network of tightly packed membranes, which has been proposed to protect and hide the viral RNA [3–8]. Biochemical support for this idea comes from work with poliovirus. In isolated poliovirus VRC networks, the viral RNA is protected from RNase digestion, but it becomes RNase-sensitive upon reversible disruption of the network (without the use of detergents, thus retaining membrane integrity) [4], indicating that the RNA does not reside inside the VRCs. In line with this, pores allowing access to the VRC lumen appear to be absent from single/double-membrane protrusion-type VRCs [5–7]. Collectively, these data indicate that RNA replication takes place on the cytoplasmic face of protrusion-type VRCs. Importantly, for enteroviruses and HCV it was shown that VRCs are dynamic structures that gradually transform from single-membrane structures into double-membrane and multilamellar structures,

Table 1

Examples of well-known pathogenic +RNA viruses

Family/Genus	Examples	Hosts	Symptoms/disease	VRCs[a]
Bromoviridae				
Bromovirus	Brome mosaic virus (BMV)	Plants, yeast[b]		Invag
Coronaviridae				
Betacoronavirus	MERS coronavirus	Humans[c]	Severe respiratory disease	Protr
	SARS coronavirus	Humans[c]	Severe respiratory disease	Protr
Flaviviridae				
Hepacivirus	Hepatitis C virus (HCV)	Humans	Hepatitis; liver cancer	Protr
Flavivirus	Dengue virus (DENV)	Humans[d]	Haemorrhagic fever; death	Invag
	West Nile virus (WNV)	Birds, humans[d]	Encephalitis; meningitis	Invag
	Yellow fever virus	Primates, humans[d]	Jaundice; liver and kidney damage; bleeding	Invag
	Zika virus	Primates, humans[d]	Microcephaly; Guillain-Barré syndrome	Invag
Nodaviridae				
Alphanodavirus	Flock House virus	Insects	Aberrant development	Invag
Picornaviridae				
Enterovirus	Coxsackievirus B3 (CVB3)	Humans	Encephalitis; myocarditis	Protr
	Enterovirus-A71	Humans	Hand, foot and mouth disease; paralysis	Protr
	Enterovirus-D68	Humans	Severe respiratory disease; paralysis	Protr
	Poliovirus	Humans	Poliomyelitis (paralysis)	Protr
	Rhinovirus	Humans	Common cold; exacerbations of chronic pulmonary diseases like asthma	Protr
Cardiovirus	Encephalomyocarditis virus (EMCV)	Rodents, pigs, elephants[e]	Encephalitis; premature abortions; death	Protr
	Saffold virus	Humans	Respiratory symptoms; gastrointestinal disease	
Aphthovirus	Foot-and-mouth disease virus	Cloven-hoofed ruminants	Foot-and-mouth disease	Protr
Hepatovirus	Hepatitis A virus	Humans	Hepatitis; jaundice; acute liver failure	Protr
Kobuvirus	Aichivirus	Humans	Diarrhoea; vomiting	
Parechovirus	Human parechovirus 1	Humans	Meningitis; sepsis	Protr
Tombusviridae				
Tombusvirus	Tomato bushy stunt virus (TBSV)	Tomato, yeast[b]	Stunting of growth; deformed or absent fruit	Invag
	Carnation Italian ringspot virus	Dianthus, yeast[b]	Stunting of growth; spots on leaves	Invag

Viruses are grouped by ***family*** and *genus* to indicate evolutionary relationships. Some of the relevant hosts, and a selection of symptoms or diseases associated with infection by the virus are listed.

[a] Type of VRC generated by the virus (Protr = protrusion-type; Invag = invagination-type).

[b] The natural hosts for bromoviruses and tombusviruses are plants, but many of those viruses can also infect yeast as a surrogate host.

[c] Viruses having a zoonotic origin, likely originating from bats and being transmitted to humans through camels (MERS) or civet cats (SARS).

[d] Viruses are transmitted via mosquito bites.

[e] The natural hosts for encephalomyocarditis virus are rodents, but the virus can cause zoonotic infections in many other animal species with devastating results.

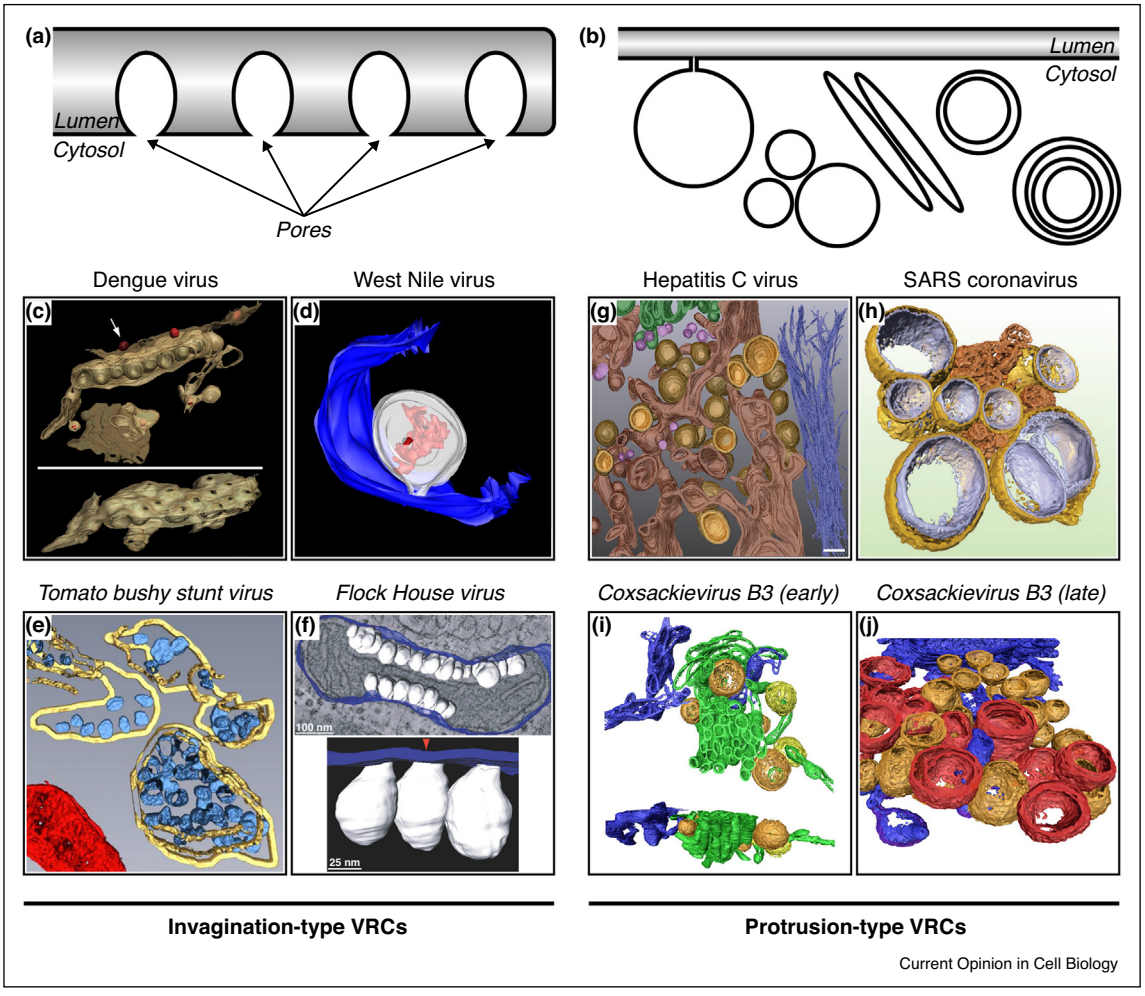
reminiscent of autophagic structures [5–7]. The single/double-membrane structures likely primarily support genome replication. The role of the multilamellar structures is less clear. In enterovirus-infected cells, they have been implicated in the *en bloc* release of virions in extracellular vesicles [9•]. In HCV-infected cells, they may be the result of a cellular stress response [7].

Sterols

Intracellular sterol distribution

Sterols are important components of eukaryotic membranes, which affect membrane properties like thickness and lipid packing, and are enriched particularly in late Golgi, endocytic compartments and the plasma membrane [10]. While mammalian cells incorporate cholesterol in membranes, plants and fungi use other sterols like ergosterol. Sterols are synthesised in the endoplasmic reticulum (ER) and from there redistributed to other organelles, keeping ER cholesterol levels low. Sterol biosynthesis is tightly regulated depending on extracellular supplies, which are taken up and redistributed intracellularly through the endosomal system. A significant portion of sterols is redistributed through non-vesicular transport by lipid transfer proteins, which often operate at membrane contact sites (MCSs), where membranes of two distinct organelles approximate closely (typically <30 nm). Sterol

Figure 1

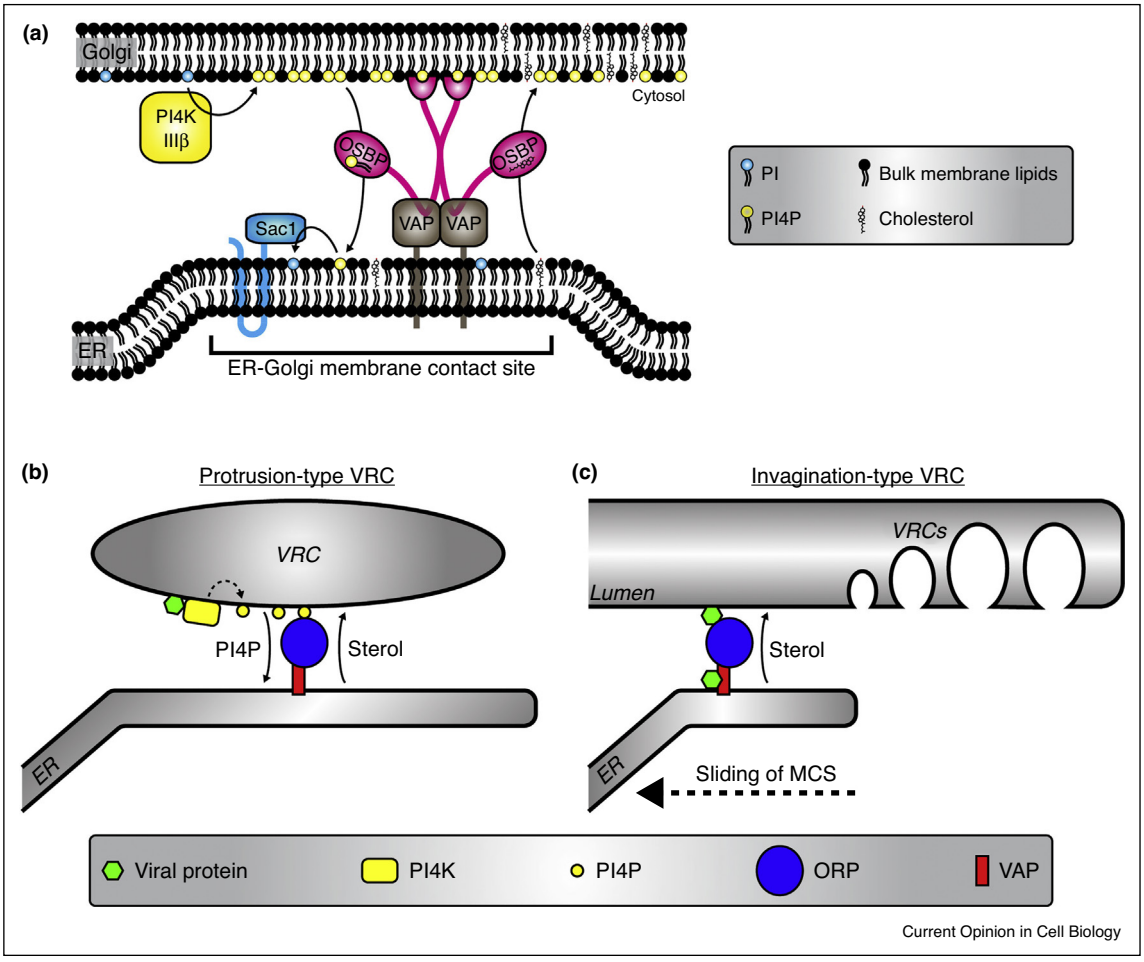


Overview of different VRC morphologies.
(a–b) Schematic overview of **(a)** invagination-type VRCs and **(b)** protrusion-type VRCs ([networks of] single- and double-membrane vesicles, multilamellar vesicles, tubules). (c–f) Three-dimensional reconstructions of invagination-type VRCs from various viruses. **(c)** VRCs of dengue virus in ER membranes. The tilted bottom panel is rotated by 90° to highlight the pores in the ER membrane that connect the VRC interior to the cytoplasm. **(d)** VRC (white) of West Nile virus in the lumen of the ER (blue). Viral RNA in the VRC lumen is displayed in red. **(e)** VRCs (blue) of tomato bushy stunt virus in the lumen of the peroxisome (yellow). A mitochondrion is shown in red. **(f)** VRCs (white) of Flock House virus in the intermembrane space of a mitochondrion connected to the outer mitochondrial membrane (blue). (g–j) Three-dimensional reconstructions of protrusion-type VRCs from various viruses. **(g)** Early stage of hepatitis C virus VRCs showing single-membrane (pink) and double-membrane (yellow inner membrane, light brown outer membrane) structures interspersed with ER membranes (dark brown). Golgi apparatus is shown in green, intermediate filaments are coloured dark blue. **(h)** Double-membrane vesicle VRCs of SARS-coronavirus (outer membrane in gold, inner membrane in silver) connected to so-called convoluted membranes (bronze). **(i)** Early stage tubular (green) and vesicular (orange, yellow) VRCs of the enterovirus coxsackievirus B3. ER is depicted in blue. **(j)** Late-stage VRCs of coxsackievirus B3 showing double-membrane vesicles (orange) and multilamellar vesicles (red). C has been reprinted from Ref. [79] with permission from Elsevier, D has been reproduced from Ref. [80] with permission from American Society for Microbiology, and E has been reproduced with permission from Journal of Cell Science from Ref. [81]. f–j are reproduced from open access publications [82] (f), [7] (g), [83] (h) and [5] (i, j).

transporters include oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) [11].

OSBP is a mammalian protein that operates at ER-Golgi MCSs. OSBP docks to *trans*-Golgi membranes through the small GTPase Arf1 and phosphatidylinositol 4-phosphate (PI4P) lipids, primarily produced by phosphatidylinositol 4-kinase type IIIβ (PI4KIIIβ). Simultaneously, OSBP binds to the ER via the transmembrane proteins VAP-A/B. OSBP shuttles cholesterol from the ER to the Golgi against the concentration gradient, which is powered by a counterflux of PI4P along the concentration gradient from the Golgi to the ER, where Sac1 removes the phosphate [12••] (Figure 2a).

Figure 2



Schematic depiction of cholesterol shuttling at MCSs.
(a) Schematic depiction of OSBP-mediated cholesterol shuttling at ER-Golgi MCSs (based upon the model presented in [12••]). PI4KIIIβ produces PI4P lipids at the Golgi. PI4P serves as a docking site for OSBP dimers. VAP-A/B transmembrane proteins link OSBP to the ER. OSBP transports cholesterol against the concentration gradient from ER to Golgi. A counterflux of PI4P along the concentration gradient provides the driving force for cholesterol transport. In the ER, Sac1 hydrolyses PI4P into PI to keep the PI4P gradient intact. **(b)** Model of cholesterol shuttling at ER-VRC MCSs as proposed for enteroviruses, cardioviruses and HCV (recently reviewed in [84]). Viral proteins (i.e. enterovirus 3A, cardiovirus 3A or HCV NS5A) recruit a PI4K (i.e. PI4KIIIβ for enteroviruses, PI4KIIIα for HCV and cardioviruses) to enrich the VRC membranes in PI4P lipids (dotted arrow). Reminiscent of the physiological situation at the Golgi, the PI4P lipids anchor ORPs (in this case OSBP) to the VRCs and drive and OSBP-mediated cholesterol accumulation. **(c)** Model of cholesterol transport to invagination-type VRCs as proposed for TBSV [38••]. The viral protein p33 recruits ORPs (in this case Osh3, Osh5, Osh6 and Osh7) to the MCS between ER and the peroxisomes, while p33 also binds VAP at the ER. The ORPs mediate cholesterol accumulation at the peroxisome. Of note, Osh6 and Osh7 were shown to exchange PS instead of cholesterol for PI4P [85•], suggesting that also PS may be shuttled to peroxisomes through the ER-peroxisome MCS. It has been hypothesised that the MCS slides along the surface of the peroxisome and that cholesterol accumulation primes the membrane for VRC formation in the wake of the sliding MCS [39].

Roles of sterols in VRC formation and function

Several evolutionary unrelated viruses, both with protrusion-type and invagination-type VRCs accumulate sterols at their VRCs. Several picornaviruses and HCV all employ a similar mechanism depending on PI4Ks, PI4P and OSBP (Figure 2b). Enteroviruses, through their 3A protein, recruit PI4KIIIβ to VRCs to enrich them in PI4P [13••]. The PI4P lipids serve as an anchor for OSBP and drive OSBP-mediated cholesterol accumulation at VRCs [14•,15•,16•]. Another picornavirus, Aichi virus, also recruits PI4KIIIβ and enriches VRCs in PI4P, whereas encephalomyocarditis virus (EMCV) hijacks PI4KIIIα instead of PI4KIIIβ to enrich PI4P at VRCs to mediate OSBP docking and cholesterol shuttling to VRCs [17,18,19•]. HCV has evolved a similar mechanism as EMCV to acquire cholesterol, involving PI4KIIIα recruitment by viral protein NS5A (although some genotypes also use PI4KIIIβ) to enrich PI4P at VRCs (reviewed in Ref. [20]) and drive OSBP-dependent cholesterol accumulation [21••].

In line with the essential role of the PI4K-PI4P-OSBP axis in recruiting cholesterol, enteroviruses, EMCV, human Saffold virus (which like EMCV belongs to the cardiovirus genus), and HCV are all sensitive to pharmacological inhibitors of the respective PI4K and OSBP [13••,15•,16•,19•,21••,22–28]. Importantly, not all viruses with protrusion-type VRCs depend on the PI4K-PI4P-OSBP axis. Picornaviruses from several other genera (*e.g.* foot-and-mouth disease virus, hepatitis A virus and human parechovirus) are insensitive to inhibitors of PI4KIIIα/β and/or OSBP [16•,19•,26,29–31], and also mouse hepatitis virus, a coronavirus, is insensitive to OSBP inhibition [29].

The involvement of OSBP in cholesterol accumulation at VRCs suggests that cholesterol, at least in part, originates from the ER. Since cholesterol is synthesised in the ER, it would be logical to assume that VRC cholesterol is newly synthesised. Alternatively, cholesterol from other organelles may be distributed through the ER to the VRCs. Enteroviruses and cardioviruses likely do not rely on newly synthesised cholesterol, but instead depend on redistribution of pre-existing cholesterol pools [32]. Enteroviruses may mobilise cholesterol stores from lipid droplets [15•]. Furthermore, they enhance endocytosis to increase uptake of cholesterol, which is delivered to VRCs via recycling endosomes [33••,34]. This latter pathway may also involve a role of OSBP, since OSBP was recently shown to operate at ER-endosome MCSs as well [35•].

For HCV, inhibition of PI4K-PI4P-OSBP-mediated cholesterol shuttling disrupts the lipid environment of the viral proteins and alters VRC ultrastructure [21••]. The PI4K-PI4P-OSBP-cholesterol system is likely also involved in picornavirus VRC formation, since inhibition of PI4KIIIα or OSBP alters global EMCV VRC organisation [19•]. Viral rewiring of sterols may support multiple aspects of virus replication, as inhibition of the PI4K-PI4P-OSBP axis and treatments that reduce VRC cholesterol availability also alter proteolytic processing of the enteroviral polyprotein – which is a membrane-dependent process [36] – by viral proteinases [33••,37].

A number of unrelated viruses that generate invagination-type VRCs also rely on sterols, while there is little if any evidence that they use PI4Ks or PI4P. This process is best understood for the tombusviruses tomato bushy stunt virus (TBSV) (Figure 2c), a plant virus that in experimental settings can also replicate in yeast. TBSV forms VRCs in peroxisomes and recruits several ORPs to ER-peroxisome MCSs through its viral protein p33 [38••]. Moreover, TBSV interacts with the ER-localised VAP homologue Scs2. Observations pointing to direct recruitment of ORPs were also made for the tombusvirus carnation Italian ringspot virus, which forms its VRCs in mitochondria [38••]. Thus, tombusviruses shuttle sterols to peroxisomes apparently independent of PI4P. How they fuel sterol accumulation is unknown. Unlike protrusion-type VRCs, invagination-type VRCs are formed in a pre-existing organelle. Therefore, viruses that build invagination-type VRCs may rely on – and perhaps reinforce – MCSs and lipid transport mechanisms that already exist in uninfected cells.

Whereas protrusion-type VRCs can directly form MCSs with the ER, this is topologically impossible for invagination-type VRCs. Instead, in TBSV-infected cells MCSs form between ER and peroxisomes close to the VRCs. It has been proposed that these MCSs slide along the peroxisome surface, possibly mediated by actin, to locally enrich sterols and prime the membrane for VRC formation [39,40]. Likely, TBSV requires sterols for the assembly, stability and functioning of the replication machinery [38••,41], although it is not known whether this is related to VRC formation.

The closely related flaviviruses West Nile virus (WNV) and dengue virus (DENV) also replicate independently of PI4Ks and PI4P, but are still sensitive to disruptions of cholesterol homeostasis [42–44,45••,46,47]. Unfortunately, the role of cholesterol in flavivirus replication has not been comprehensively studied. WNV accumulates cholesterol at its VRCs [42], but similar studies are missing for DENV. DENV replication is insensitive to the OSBP inhibitor OSW-1 [21••], but there are no data for WNV. Assuming similar replication mechanisms, these findings suggest that flaviviruses accumulate cholesterol at VRCs independent of the PI4K-PI4P-OSBP axis. It remains to be studied whether flaviviruses build ER-VRC MCSs at all and usurp other cholesterol transfer proteins to accumulate cholesterol at VRCs, or whether they depend on disparate mechanisms like vesicular cholesterol delivery.

Glycerophospholipids

Glycerophospholipids (*e.g.* phosphatidylcholine [PC], phosphatidylethanolamine [PE], phosphatidylserine [PS], phosphatidylinositol [PI]) are major constituents of most membranes. While the different head groups are prime determinants of lipid properties like charge, shape and interactions with peripheral membrane proteins, FAs varying in length and saturation affect membrane properties like lipid packing and fluidity [48].

PC

Both protrusion- and invagination-type VRC-generating viruses (*e.g.* poliovirus, EMCV, Flock House virus, HCV, DENV and brome mosaic virus [BMV]) increase cellular levels of PC, the most prevalent glycerophospholipid [49–53,54••]. In fact, poliovirus, EMCV, HCV and BMV accumulate PC at VRCs, while BMV has even been shown to recruit PC biosynthetic machinery to VRCs for local PC production [54••]. Other viruses likely also

modulate local lipid synthesis at or near VRCs. For example, DENV and WNV recruit fatty acid synthase (FASN), the key enzyme in FA biogenesis, to VRCs [44,55,56]. In line with a requirement for *de novo* FA biosynthesis, several viruses, including enteroviruses, DENV, WNV and HCV, are sensitive to pharmacological inhibition of FASN and in some cases upregulate FASN [44,53,55–60].

As an alternative strategy to support increased membrane lipid biosynthesis, several enteroviruses and EMCV increase uptake of FAs [61••]. The underlying mechanism has been investigated in detail in poliovirus-infected cells, which revealed specific increases in PC species with FAs with 16 or 18 carbons, likely matching VRC membrane properties. To increase uptake and activation of those FAs in particular, poliovirus harnesses the long chain acyl-CoA synthase Acsl3. Furthermore, while in non-infected cells most of the absorbed FAs are stored in lipid droplets, poliovirus reroutes them to membrane lipids at VRCs [61••].

PC is synthesised via two routes catalysed by ER-localised enzymes, either by addition of the choline head group to a diacylglycerol backbone (Kennedy pathway), or from PE by three subsequent methylation events of the head group (reviewed in Ref. [62]). In yeast, PE methylation is the major route for PC production, which may (partly) occur at MCSs, at least at ER-plasma membrane MCSs [63]. In contrast, the Kennedy pathway is the major pathway for PC biosynthesis in most mammalian cell types. Nevertheless, it is tempting to speculate that viruses alter host lipid metabolism to locally produce PC via *in trans* PE methylation at ER-VRC MCSs, which would allow *in situ* PC production at VRCs without the need for relocalisation of the enzymes from ER to VRCs. Which biosynthetic pathway mediates PC biosynthesis at VRCs and whether this involves virus-induced MCSs, remains to be established.

PE

+RNA viruses use a variety of other glycerophospholipids during replication, although their role in the viral life cycle and the involvement of MCSs in their homeostasis is often poorly understood. For example, TBSV accumulates PE at VRCs, possibly as a docking station for the viral replication machinery [64••]. Correspondingly, replication of TBSV is sensitive to disruptions of PE biosynthesis. The requirement for PE is likely more widespread among tombusviruses, including some that form VRCs in other organelles. It has been speculated that, apart from serving a role in the recruitment of viral replication proteins, PE through its small head group and resulting conical shape may contribute to the induction of the negative membrane curvature that is important for the invagination-type VRCs [65], but proof for this is lacking.

PS

Enteroviruses accumulate PS at VRC subdomains [9•]. Later in infection, PS is found at autophagosome-like double-membrane structures, which have been proposed to mediate non-lytic virus release. The resulting virus-containing extracellular vesicles are enriched in PS, which facilitates PS-receptor dependent uptake and infection in other cells [9•]. Numerous other viruses employ PS-dependent uptake in a process termed 'apoptotic mimicry' [66]. How PS accumulates at VRCs is unknown. Recently, the MCS proteins ORP5 and ORP8 were shown to facilitate a PS/PI4P exchange at ER-plasma membrane MCSs [67•]. Possibly, ORP5/8 are hijacked to facilitate PS accumulation at VRCs. It remains to be determined whether PS also has a role in enterovirus VRC formation and/or function.

PI(4,5)P_2

Besides having functions of its own, PI4P is also a direct precursor for the PI-bisphosphates PI(3,4)P_2 and PI(4,5)P_2 [68•]. PI(4,5)P_2 plays a role in cellular lipid transport by anchoring MCS proteins at multiple MCSs (*e.g.* ER-plasma membrane, lysosome-peroxisome) [69,70]. HCV accumulates PI(4,5)P_2 at its VRCs and for this it requires PIP5KIα [71••], suggesting that PI(4,5)P_2 is locally produced from PI4P. PI(4,5)P_2 interacts with a novel amphipathic helical motif in viral NS5A and enhances the interaction between NS5A and the cellular ER-localised Rab1 GTPase activating protein TBC1D20, which is an important host factor for HCV replication [71••,72,73]. Interestingly, the enteroviral protein 2C harbours a similar motif. Whether the presence of this motif is indicative of the accumulation of PI(4,5)P_2 at enterovirus VRCs, and what the role of PI(4,5)P_2 in enterovirus replication would be, remains to be investigated. Whether other PI-phosphates are in any way involved in +RNA virus replication is yet also unknown.

Sphingolipids

Sphingolipids represent a third major category of membrane lipids. This category of lipids is enriched in the plasma membrane, late Golgi and endolysosomal compartments [48]. MCSs and PI4P play crucial roles in the biosynthesis of sphingolipids (reviewed in Ref. [74]). The ceramide transporter (CERT) docks to the Golgi through PI4P and delivers ceramide, a substrate for sphingomyelin (SM) synthesis, from ER to Golgi at MCSs. Emerging evidence points to a role of sphingolipids in +RNA virus replication. This is best studied for HCV. Replication of HCV is sensitive to inhibitors of SM biosynthesis. SM binds the viral polymerase NS5B *in vitro*, supposedly to recruit it to SM-enriched detergent-resistant membranes, and activates the polymerase, although this may differ between genotypes [75]. However, there is no evidence yet that SM accumulates at VRCs and recruits and activates NS5B in HCV-infected cells.

The four-phosphate adaptor protein 2 (FAPP2) docks to the Golgi through PI4P and shuttles the glycosphingolipid glucosylceramide (GlcCer), a substrate for the synthesis of lactosylceramide (LacCer) and a subset of complex glycosphingolipids. FAPP2 localises to HCV VRCs and mediates LacCer accumulation [76]. FAPP2 depletion impairs HCV replication and disrupts VRC formation [76]. The adverse effect of replication of FAPP2 depletion can be efficiently rescued by addition of different (complex) glycosphingolipids, but not all, implying that HCV has specific requirements for sphingolipids at its VRC.

Also some viruses with invagination-type VRCs may use sphingolipids. SM localises to WNV VRCs and WNV replication is sensitive to inhibition of SM biosynthesis, albeit moderately [77]. Correspondingly, ceramide is redistributed to VRCs and inhibition of ceramide biosynthesis impairs WNV replication [78]. For DENV, two studies report contrasting results on the accumulation and importance of sphingolipids [53,78], which may represent differences in biology or experimental approach. This leaves the matter whether these closely related flaviviruses have similar or different interactions with sphingolipids currently unresolved.

Outlook

Although lipids are essential components of membranes and thus indispensible for the replication +RNA viruses, their diverse roles are quite poorly studied. Recent years have seen an upsurge of the field, spurred by emerging insights in basic lipid and membrane biology. In the near future, novel technologies (*e.g.* lipidomics, fluorescent probes to detect lipids) will spur further studies into how +RNA viruses rewire host lipid metabolism to optimally support genome replication. Such fundamental insights into virus replication may provide a basis for the development of novel antiviral drugs.

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