Rev. Med. Virol. 2016; **26**: 146–160.
Published online 28 January 2016 in Wiley Online Library
(wileyonlinelibrary.com)
DOI: 10.1002/rmv.1872

REVIEW

Membrane dynamics associated with viral infection

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SUMMARY

Viral replication and spreading are fundamental events in the viral life cycle, accounting for the assembly and egression of nascent virions, events that are directly associated with viral pathogenesis in target hosts. These processes occur in cellular compartments that are modified by specialized viral proteins, causing a rearrangement of different cell membranes in infected cells and affecting the ER, mitochondria, Golgi apparatus, vesicles and endosomes, as well as processes such as autophagic membrane flux. In fact, the activation or inhibition of membrane trafficking and other related activities are fundamental to ensure the adequate replication and spreading of certain viruses. In this review, data will be presented that support the key role of membrane dynamics in the viral cycle, especially in terms of the assembly, egression and infection processes. By defining how viruses orchestrate these events it will be possible to understand how they successfully complete their route of infection, establishing viral pathogenesis and provoking disease. © 2015 The Authors *Reviews in Medical Virology* Published by John Wiley & Sons, Ltd.

Received: 30 September 2015; Revised: 14 December 2015; Accepted: 16 December 2015

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Abbreviations used

ADP, adenosine diphosphate; ALIX, ALG-2 (apoptosis-linked gene 2)-interacting protein X; APOBEC3, apolipoprotein B mRNA-editing enzyme-catalytic, polypeptide-like 3; Arf, ADP-ribosylation factor; Arf-GEF, Arf-GTP exchange protein; ASFV, African swine fever virus; Atg, autophagy-related protein; BFA, Brefeldin A; CCR, C-C chemokine receptor; CD, cluster of differentiation; COPI I and II, clathrin, coatomer protein complex I and II; CPV, cytopathic vacuole; CVB3, Coxsackievirus B3; CXCR, C-X-C chemokine receptor; C3-PI3K, lipid class III phosphatidylinositol 3-kinase complex; DC, dendritic cell; DENV, dengue virus; DMVs, double-membrane vesicles; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; Env, envelope; ERGIC, ER–Golgi intermediate compartment; ESCRT, endosomal-sorting complex required for transport; GALT, gut-associated lymphoid tissue; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GTPase, guanosine triphosphatase; HDAC6, histone deacetylase 6; ISG-15, interferon-stimulated gene 15 protein; LC3-I, microtubule-associated protein 1A/1B-light chain 3; LC3-II, the phosphatidylethanolamine-conjugated from LC3-I; LHBs, large HBV. surface protein; MA, matrix viral protein; MLV, murine leukaemia virus; MVB, multivesicular body; MT, microtubule; MTOC, microtubule organizing centre; NCLDVs, nucleocytoplasmic large DNA viruses; Nef, negative factor; NS5A, non-structural 5A protein; NS5B, non-structural 5B protein; PI4P5-K Iα, phosphatidylinositol-4-phosphate 5-kinase Iα; PIP_2, phosphatidylinositol-4,5-bisphosphate; prM, precursor membrane; RC, replication complex; RUBV, rubella virus; SAMHD1, sterile alpha motif (SAM) and histidine-aspartate (HD) domain-containing protein 1; SFV, Semliki forest virus; SQSTM1, sequestosome-1 (or p62); ssRNA, single-stranded RNA; SVP, spherical or filamentous envelope particles; TGN, trans-Golgi network; Tsg101, tumour susceptibility gene 101; UPR, unfolded protein response; VAMP, vesicle-associated membrane protein; VAP, VAMP-associated protein; Vif, viral infectivity factor; Vps, vacuolar protein sorting-associated protein; VF, viral factory; VS, Virological synapse; 5ptaseIV, polyphosphoinositide 5-phosphatase IV.

INTRODUCTION

Viruses are small structures that lack the metabolic pathways and structures necessary to ensure their own survival, relying on their host's machinery to replicate their genome and spread their progeny. Accordingly, viruses have developed strategies to enter cells and exploit their structures to replicate. These strategies also serve to evade immune responses, such as those involving toll-like receptors and autophagic-mediated antigen presentation [1–4]. Similarly, viruses use the target cell's main trafficking pathways to ensure their propagation, exploiting the endosome or vesicular compartments by recruiting the clathrin, coatomer protein complex (COPI) I and II (Figure 1), the endosomal-sorting complex required for transport (ESCRT) and their accessory proteins (reviewed by [1,2,5]: Figure 2), as well as small guanosine triphosphatases (GTPases) [2]. This is evident during neutrophil-mediated

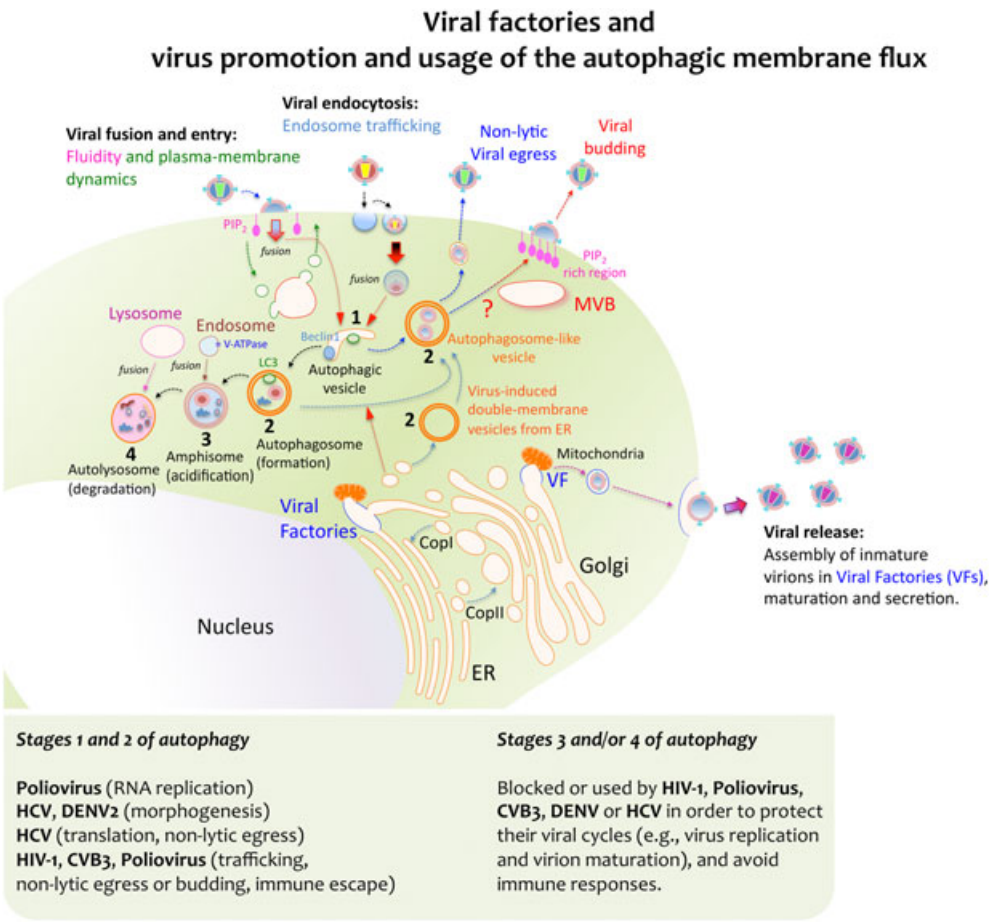

Figure 1. Viral factories and virus-triggered autophagic membrane flux for replication and egression. Some viruses achieve replication by exploiting the cell's membrane transport pathways, thereby generating membrane organelles named Viral Factories (VFs). These VFs are organised by different viral proteins, and they represent specialized compartments for viral-gene replication, morphogenesis, export, maturation and release. Moreover, these compartments also serve to override or evade the immune responses directed against viral genomes. Viral proteins can enter secretory pathways by co-translational translocation into the ER in order for them to be further transported to the Golgi complex, either in vesicles or in a coatomer protein complex (COP) II-dependent manner. Viral complexes formed inside the VFs communicate with vesicles, mitochondria, Golgi cisternae and ER membranes. This interaction allows viral complexes to be transported through the Golgi network to the plasma membrane and it promotes their final release as viral particles. Alternatively, some viruses take advantage of the host's autophagic machinery for their own replication and pathogenesis. Viruses first initiate the formation of vesicles that bear key autophagic proteins, such as Beclin-1 and LC3, capturing portions of membranes from the ER and other cytoplasmic elements. This assembly evolves toward an immature double-membrane vesicle (DMV) that serves as an aggresome compartment to recruit viruses or newly formed viral replication complexes. Several RNA viruses induce the formation of these autophagosome-like vesicles (also referred to as DMVs) to enhance viral replication and non-lytic egression, such as poliovirus and CVB3, HIV-1 and HCV. How these viruses trigger the accumulation of autophagosome-like vesicles and DMVs remains unclear. Some theories involve blocking the fusion of nascent autophagosomes with late endosomes and lysosomes, as in the case of HIV-1 Nef, which appears to cause autophagosome accumulation by inhibiting their progression towards more mature stages. Indeed, autophagosome-like vesicles may represent a trafficking pathway for these viruses, connecting to multivesicular bodies (MVBs), and assuring virus assembly and budding at the cell surface while protecting them from intrinsic antiviral factors and immune responses. The morphogenesis and release of mature and infectious HBV particles also require Tsg101 and depend on the ESCRT-MVB system. Under standard conditions the lumen of autophagosomes acidifies after fusion with endosomes that carry vacuolar (H^+)-ATPase (V-ATPase) to form amphisomes. The autophagic membrane flux progresses by fusing with lysosomes in order to form the autolysosome that contains the former's proteinases. Poliovirus inhibition of autophagosome formation attenuates viral replication while inhibiting autolysosome formation, and thus, catalytic activity does not affect the virus. However, degradation of cellular triglycerides by autophagy benefits DENV replication and autolysosome degradation dampens IFN activation following HCV infection

phagocytosis, where microorganisms can be cleared by granule and vesicle secretion [6]. Therefore, determining how viruses use and rearrange intracellular organelles during their biological cycle is an important goal that will aid the development of new antiviral strategies, and our understanding

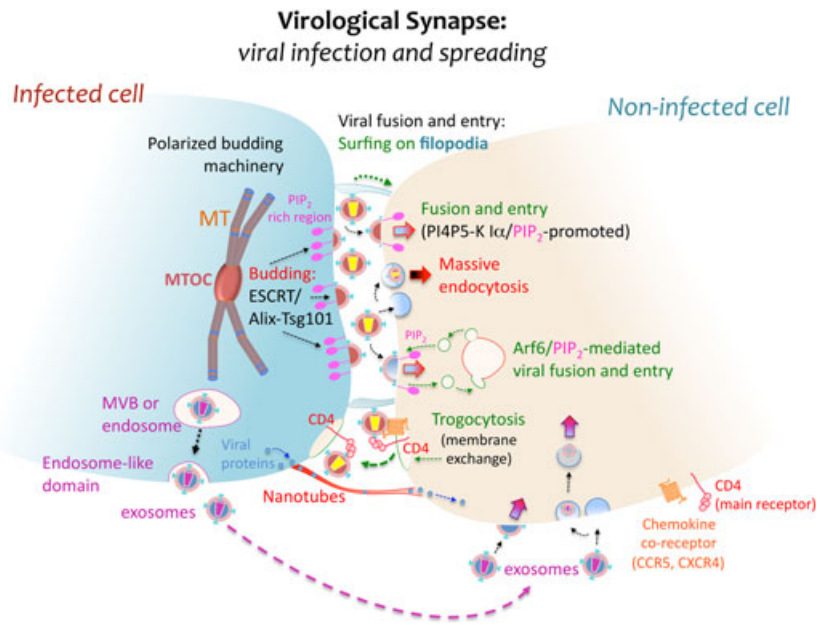

Figure 2. Virological synapse and spreading. At the virological synapse (VS), some viruses attach structural polyproteins to PIP_2-rich membrane regions of the infected cell for further budding and release into the intercellular space. PIP_2 confers fluidity to the cell membrane and favours virus–cell fusion. These virions then bind to specific receptors in order to infect the neighbouring target cell at the VS, fusing with its plasma membrane directly or after surfing on actin-structured filopodia, or being internalized by endocytosis as is believed to occur with HIV-1. The VS represents an efficient environment for viral budding. It typically arises in PIP_2-enriched plasma membrane domains, where the membrane of the infected cell is polarized towards the synaptic junction through the movement of vesicles governed by the ESCRT/Alix-Tsg101 machinery or by MVBs coordinating the translocation of the MTOC. This scaffolding facilitates subsequent viral infection and spread from the infected to the nearby uninfected cell. In addition, long membrane nanotubes may also form between neighbouring cells, promoting viral protein trafficking. Other dynamic membrane events involved in viral infection and spreading are trogocytosis, Arf6/PIP_2-mediated membrane dynamics and exosomal transport. Trogocytosis involves the exchange of cell surface membrane patches that may contain receptor clusters associated to viral particles, while exosomes are vesicles formed from MVBs that could participate in viral infection and spreading between cells

of these pathologies. Indeed, there is growing evidence that cell's modify their membranes to defend themselves against pathogens and infection, altering their spatial reorganization and vesicle trafficking. In this review, we focus on the importance of the membrane flux triggered by viruses to achieve replication and egression, and to ensure their propagation.

Membrane dynamics during viral replication

Several of the cell's organelles and membrane structures are involved in viral replication and in fact, many viruses use specific cellular compartments to replicate, referred to as viral factories (VFs: Figure 1). These VFs provide a physical scaffold that brings together elements required for genome replication and morphogenesis [1,7]. VFs are usually formed by rearranging the host's cell membranes, reorganizing the cytoskeleton and recruiting specific organelles, like mitochondria (reviewed in [8]). These viral driven events involve the association of replication complexes (RCs) with ER derived membranes to form a VF. Hence, intracellular membrane dynamics appear to be crucial for viral replication and survival.

A well-known example of a VF is that used by vaccinia virus, an enveloped pathogen of the Poxvirus family that replicates in the cytoplasm by assembling small rough ER-derived cisternae into a microenvironment that resembles a cytoplasmic mini-nucleus for viral replication [9]. Similarly, the RCs of Togaviruses associate with endocytic membranes, while Nodavirus RCs associate with mitochondrial membranes (reviewed in [1]). Thus, specific membrane compartments can be used as VFs by RNA viruses to concentrate viral replicases and key cofactors, and ensure efficient viral genome replication [10]. In this context, both rubella virus (RUBV), a relevant human teratogenic *Togaviridae* virus [11], and Semliki forest virus (SFV), a member

of the Alphavirus group of this family [12], couple their RNA synthesis to endosome and lysosome membranes modified by the association of virus specific components. The subsequent fusion of these late endosomes and lysosomes generates cytopathic vacuoles (CPVs) [13,14] that are lined with small vesicular invaginations or spherules (viral RNA replication sites) [13,14]. CPVs establish complex and reversible contact with endocytotic vesicles through internal membranes interconnected with transport endosomes [8]. For example RUBV forms VFs around CPVs via the recruitment of membrane structures from the ER cisternae, Golgi stacks and mitochondria [8] (Figure 1). The Golgi apparatus is a highly dynamic organelle with a sustained, functional flux of membrane proteins [15], and it can serve as a morphogenic mould for Rubiviruses, Coronaviruses, Arteriviruses and Bunyaviruses [1,8,16,17]. These RUBV factories connect viral replication with the assembly and maturation of nascent virions at Golgi membranes, contributing to the virus escaping from the host cell's defences [16].

Some viruses induce the formation of double-membrane vesicles (DMVs) and/or autophagosomes for replication [1–4,18,19], such as the positive RNA viruses of the *Flaviviridae* family and *Nidovirales* order [20,21] (Figure 1). The RNA polymerase of the human poliovirus, a *Picornaviridae* family member responsible for poliomyelitis, can also assemble DMVs [22]. Infection by this virus triggers the modification of different intracellular membrane structures and organelles (but not mitochondria), converting them into virus replication vesicles. In fact, poliovirus-associated DMVs resemble autophagosomes, as also described for another *Picornaviridae* family member, Coxsackievirus B3 (CVB3 [23,24]: Figure 1). Autophagosomes are DMVs generated by membrane trafficking and they are related to the catabolic process of autophagy, which involves the degradation of cytoplasmic components within lysosomes [25,26]. Autophagy maintains the organism's homeostasis by sequestering undesired intracellular elements for lysosomal degradation and recycling [25,26]. Viruses often use autophagy to complete their lifecycle and evade immune responses, even though it is based on catalytic pathways [3,23,24]. Poliovirus, like other positive RNA viruses, has evolved the capacity to convert autophagy into a key cellular motor for replication [3,10,23]. During autophagy, a cytosolic form of the microtubule-associated protein 1A/1B light chain 3 (LC3-I) conjugates with phosphatidylethanolamine to form LC3-II and associate with autophagosomal membranes, ultimately producing the degradation of LC3-II during the late steps of autophagy [27]. Conversely, the p62 protein (or sequestosome-1, SQSTM1) interacts with ubiquitinated proteins, LC3 and other proteins to ensure the correct degradation of undesired material. LC3-II augments during active autophagy when p62 is degraded [28,29]. In this context, Poliovirus or CVB3 infection triggers the generation of autophagosomes with a higher LC3-II/LC3-I ratio and with LC3 foci, structures that support the RNA RC without promoting lysosome degradation (evident through p62 stabilization [23,24]: Figure 1). However, it is unclear whether these viruses block autophagosome maturation into amphisomes, avoiding autophagosome fusion with endosomes [30]. Such events override the appearance of degradative autolysosomes [31] or they may provoke the formation of autophagosome-like structures disconnected from catalytic pathways. It is also thought that these autophagosomes may ultimately serve as a membrane scaffold to permit the egression of nascent virions from infected cells, preventing cell lysis [30] (Figure 1).

All HCV viral genotypes (1a, 1b and 2a), positive RNA flaviviruses that are a major cause of chronic liver disease [32], induce autophagosome accumulation [33,34]. This involves regulation of the unfolded protein response (UPR), which relieves ER stress and prevents the formation of catalytic autolysosomes by suppressing their fusion with lysosomes [33,34] (Figure 1). Apparently, the success of viral replication relies on the recruitment of membrane-trafficking proteins to ER-derived membrane scaffolds [35–41]. Hence, domain 1 of the non-structural 5A (NS5A) protein and the helicase domain of NS3 are sufficient to achieve efficient DMV formation, which also depends on tightly regulated *cis* cleavage of the HCV-polyprotein precursor [35] and requires cyclophilin A isomerase activity [36]. NS5A associates with NS5B, a RNA-dependent RNA polymerase, a complex that interacts with VAMP (vesicle-associated membrane protein)-associated proteins (VAPs) [37,38] and recruits Ras-like small GTPases (e.g. Rab1, Rab5 and Rab7), enlarging the viral replication compartment by docking membrane vesicles [39–41]. This process also regulates autophagy [42], given that HCV-

induced autophagosomes support viral replication and the delivery of incoming viral RNA to the translation apparatus, and/or the recruitment of cellular factors for translation. However, some controversy still surrounds this issue, autophagosomes can mature into acidic amphisomes in HCV-infected cells [43,44], and subsequently fuse with late endosomes or lysosomes [44] (Figure 1). Autophagic membrane flux appears to be necessary to translate the HCV genome, yet it appears to be dispensable once viral infection has begun [45]. Moreover, no changes in either p62 or the degradation of long-lived proteins are observed [33], despite the enhanced autolysosome formation in cells expressing HCV replicons [46]. While specific silencing of autophagy genes does not affect viral translation and RNA replication, it does apparently alter HCV morphogenesis [47]. However, the silencing of factors critical for autophagosomes formation, like LC3 or Atg7, appears to suppress HCV RNA replication [48], while HCV replication is apparently potentiated when the UPR promotes autophagy [49]. Conversely, HCV infection seems to promote autophagy without concomitant stimulation of the UPR and autophagy does not appear to be required as a platform for HCV RNA replication [50]. Thus, doubts remain about the role of autophagy and the UPR in HCV replication, although the distinct interactions between autophagy and HCV replication suggest that such membrane flux promotes viral replication.

The dengue virus (DENV) is a mosquito-borne single positive-stranded RNA virus of the *Flaviviridae* family that causes dengue fever [51]. There are five antigenically related but distinct DENV virus genotypes (DENV-1 to DENV-5) [51,52]. Like HCV, there is evidence that autophagy may be implicated in DENV replication. Following cell entry and nucleocapsid uncoating, DENV RNA is translated into a single polyprotein that passes into the ER lumen where the different viral proteins are processed. In fact, DENV-2 proteins involved in translation and replication are found in or in close proximity to autophagosomes during viral infection [53,54]. Accordingly, inhibition of autophagosome formation dampens the production of infectious DENV-2 particles [53], while stabilizing autophagosomes and/or amphisomes by impeding their fusion with lysosomes enhances viral egression [55]. Indeed, DENV-3 seems to promote autophagy during early infection [54], while inhibition of autophagosome formation also dampens the production of infectious DENV-3 [54]. Hence, DENV-2 and -3 appear to interact with the autophagy machinery in a different manner, and while it is conceivable that amphisomes or autophagosomes represent the site of DENV-2 translation/replication [54,55], autophagolysosomes could be the crucial site for DENV-3 viral replication [54]. The distribution of NS1 or DENV-2 and DENV-3 double-stranded RNA (dsRNA) in the different autophagy-associated membrane structures confirms these observations (Figure 1). Moreover, nascent viral particles are formed and mature in these structures, then travelling through the trans-Golgi network (TGN) to egress [56,57]. Remarkably, the precursor membrane (prM) protein of the DENV-1–4 genotypes behaves similarly and it is cleaved by the TGN-protease furin in the secretory pathway [58], assuring viral assembly and the infectivity of nascent viral particles [59].

HIV is a single-stranded RNA virus (*Lentivirus* genus of the *Retroviridae* family) that causes AIDS. HIV type 1 (HIV-1) alters the autophagic membrane flux of the host cell's organelles, thereby modulating the intracellular milieu in favour of viral replication and propagation [3] (Figure 1). When CD4+ T cells, monocytes and dendritic cells (DCs) are infected with HIV-1, autophagic vacuole formation is blocked and the expression of autophagy proteins down-regulated (e.g. LC3 and Beclin1 [19,60]: Figure 1). Remarkably, the HIV-1 protein Nef (negative factor) blocks the autophagic flux of membranes, especially during the autolysosome stage of autophagy, resulting in an accumulation of autophagosomes and LC3 in macrophages (Figure 1). Thus, Nef prevents autophagic degradation of HIV-1 biosynthetic intermediates of virions by targeting the lipid class III phosphatidylinositol 3-kinase (C3-PI3K) complex and by associating with Beclin1 (Atg6—autophagy-related protein 6—in yeast). Significantly, Beclin1 is actually part of the C3-PI3K complex, together with the vacuolar protein sorting-associated proteins 34 (Vps34) and 15 (p150). Nef therefore alters the sub-cellular distribution of Vps34, potentially ensuring the survival of the viral progeny [3,61]. Indeed, Nef is thought to promote the appropriate HIV-1 Gag membrane localization and processing, thereby facilitating viral cell-to-cell transfer [62]. Although the catalytic activity of autophagy appears to be impeded by HIV-1, autophagosome formation or accumulation is still promoted. Hence, the HIV-1 Gag protein promotes early stages of

autophagosome formation by directly interacting with LC3 in macrophages, enhancing HIV-1 yields and Gag processing, a critical step in virion assembly and release [61] (Figure 1). Notably, newly identified components of the ubiquitin-like conjugation system all seem to be involved in HIV-1 replication (e.g. Atg7, Atg8—LC3 is its best characterized mammalian homologue—Atg12 and Atg16L2—responsible for vesicle elongation) [63]. However, it remains unclear how these factors actually affect HIV-1 replication, which occurs in the nucleus of infected cells. Moreover, while autophagic vacuoles would appear to be fundamental for HIV-1 morphogenesis and egression, how HIV-1 overrides or uses autophagy to persist remains poorly understood. Hence, the infectious capacity of nascent HIV-1 virions depends on the uptake of the viral infectivity factor (Vif) during viral budding, a process influenced by histone deacetylase 6 (HDAC6), which promotes autophagic clearance of Vif [64]. Other positive RNA viruses exploit the formation of ER-derived membrane scaffolds and membrane autophagic flux to replicate (e.g. the Norwalk virus), because the membrane-bound nsp48 protein also binds to VAP-A [65].

RNA replication may occur in endosomes, lysosomes (Togaviruses), peroxisomes and chloroplasts (Tombusvirus), or mitochondria (Nodaviruses), shielded from immune responses. All positive RNA viruses transform cytoplasmic membranes into specialized viral replication sites [10]. The antiviral effect of Brefeldin A (BFA), an inhibitor of anterograde ER–Golgi network membrane dynamics, suggests that membrane trafficking must be active for enterovirus replication, as reported for Picornaviruses, poliovirus and Coxsackievirus [66,67]. BFA prevents the membrane flux required to form replication compartments, blocking virus secretion from infected cells [68] by inhibiting ADP (adenosine diphosphate)-ribosylation factor (Arf)-GTP exchange proteins (Arf-GEFs). This blockade negatively affects COPI coat generation at the Golgi by diminishing and sequestering Arf1-GTP [69]. For several Picornaviruses, COPII-coated vesicles may provide membranes suitable for replication [70], although autophagosomes may also contribute at this point [23] (Figure 1). Reovirus and SFV also promote coated-pit formation [71]. Moreover, the small GTPase Rab7 is soon recruited for SFV internalization when associated to intermediate endosomes [72], which in turn induces the formation of CPVs that is an important event for viral RNA synthesis in target cells [13].

An important biological process common to the recently proposed *Megavirales* order is viral replication within cytoplasmic VFs [73]. Giant viruses (also called nucleocytoplasmic large DNA viruses—CLDVs) belonging to this order are double-stranded DNA (dsDNA) viruses with a genome and particle size comparable to those of small bacteria [74]. African swine fever virus (ASFV; from the *Asfarviridae* family), poxviruses and iridoviruses are the three families of NCLDVs that terminate or undergo their entire replication cycle in the cytoplasm [75–77]. This feature is not observed in herpes viruses or baculoviruses, other large DNA viruses of eukaryotes that undergo nuclear DNA replication and transcription [78]. Giant viruses provoke VF formation in the cytoplasm of infected cells to permit genome replication and morphogenesis [73,79]. ASFV factories are similar to the aggresomes formed at the MTOC (microtubule organizing centre) [80], and they provoke a rearrangement of the intermediate vimentin cytoskeleton at the MTOC into a star shaped structure that resembles the microtubule aster formed during mitosis, a structure required for late gene expression [81]. Together with an ASFV chaperone, the hsp70 cell chaperone is recruited to ASFV factories, along with mitochondria, facilitating the folding of viral structural proteins like the major capsid protein p72 [82,83]. Nascent ASFV virions are formed from VF membranes through the assembly and recruitment of viral proteins in VFs. Thus, the viral membranes in VFs may be connected to cellular organelles, particularly given that resident ER markers are detected with the viral p17, p54 and pB318L proteins in new viral particles [84–87]. ASFVs are thought to reorganize cell membranes through viral proteins that contain a KDE motif, inducing the redistribution of ER-associated proteins [88] and the viral p54 protein. The latter is required for the correct VF localization of the membranes and the collapse of the ER-derived cisternae [89]. ASFV infection is achieved by redistributing membranes from the secretory pathway and TGN [90]. Therefore, these common biological features of giant virus replication and virion architecture could reflect a common origin, and the sharing of a large set of ancestral genes [74,91].

Membrane dynamics during viral assembly and budding

Budding is an important event in the life cycle of enveloped viruses, influencing their morphology

and infectiousness. During budding, successful infection is achieved by adjustment and distortion of the target cell's plasma membrane [4]. The structural Gag polyprotein is common to several retroviruses, like HIV-1 and the murine leukaemia virus (MLV), representing the minimal plasma membrane component required for viral assembly [92]. HIV-1 Gag localizes to phosphatidylinositol-4,5-bisphosphate (PIP_2) rich plasma membrane regions, where PIP_2 plays a critical role in HIV-1 virion assembly [93] (Figures 1 and 2). In fact, the matrix viral protein (MA) within the unprocessed HIV-1 Gag polypeptide drives Gag towards these PIP_2 membrane domains [92,94] in a myristoylation-dependent manner [95], raft domains where HIV-1 buds [95–97]. Phosphate hydrolysis by polyphosphoinositide 5-phosphatase IV (5ptaseIV) diminishes the plasma membrane PIP_2 [98], causing the Gag polypeptide to translocate from HIV-1 budding sites at the membrane to CD63 rich compartments, thereby inhibiting viral release [93]. Similarly, Arf6/Q67L expression, a GTP-bound mutant of Arf6, alters the trafficking of Arf6/PIP_2-associated vesicles, provoking their accumulation in the cytoplasm to where Gag is redirected. These complexes lie far from the budding sites at the membrane, thereby dampening virus release [93]. Although the assembly of HIV-1 at the cell surface is only partially understood, several key steps in the membrane trafficking of viral proteins have been defined, shedding light on both the viral assembly and budding processes [92,99].

Enveloped viruses like HIV-1, Vesicular stomatitis virus (VSV), Ebola virus (EBOV) and Hepatitis B virus (HBV), and other RNA and DNA viruses, mainly emerge from cells by co-opting the host's ESCRT machinery [100,101], which plays a vital role in cellular abscission and in multivesicular body (MVB) biogenesis (a process by which ubiquitinated misfolded or damaged proteins enter endosomes to be destroyed). In addition, MVBs are important intermediates in endolysosomal transport [102] (Figures 1 and 2). Gag activity drives ESCRT-III complex formation at the budding site of HIV-1, which binds to and recruits the ESCRT-I complex and the ALG-2 (apoptosis-linked gene 2)-interacting protein X (ALIX). This ESCRT-III complex promotes the excision of nascent virions at the cell surface, an event potentially equivalent to the cleavage of intraluminal vesicles from MVBs [103,104] (Figures 1 and 2). Moreover, the tumour susceptibility gene 101 (Tsg101) is a subunit of the ESCRT-I complex that drives viral RNA transport and envelope fusion to late endosomes, processes required for infection and RNA release [105] (Figure 2). However, the interaction of viral Gag protein with the ESCRT machinery appears not to be absolutely required for HIV-1 viral budding [106–108]. Nevertheless, interferon-stimulated gene 15 protein (ISG-15) inhibits HIV-1 egression by interfering with ESCRT-III protein membrane flux during budding [109,110].

Remarkably, morphogenesis and the release of HBV particles also require Tsg101 [111], although this DNA virus lacks a viral protein bearing the late (L) domain necessary to interact with the ESCRT-machinery [101]. However, α-taxilin interacts directly with Tsg101 and with the large HBV surface protein (LHBs), thereby recruiting the viral capsids to ESCRT complexes, thus permitting correct viral formation and egression [111]. Therefore, HBV maturation and egression depends on the ESCRT-MVB system. Notably, HBV infected cells also produce large amounts of non-infectious spherical or filamentous envelope particles (SVPs). These SVPs are a mixture of lipids and viral surface proteins that accumulate in an ER–Golgi intermediate compartment (ERGIC), budding into the lumen and provoking release through the general secretory pathway [112].

Many other enveloped RNA viruses bud in an ESCRT-dependent manner [5,100,113], as do most negative-strand non-segmented single-stranded RNA (ssRNA) viruses, such as Rhabdoviruses, Filoviruses and most Paramyxoviruses, all of which recruit ESCRTs for viral egression [114,115]. Even the budding of negative-strand segmented-ssRNA Arena viruses involves an ESCRT-dependent pathway [116,117]. However, no evidence for the participation of ESCRTs has yet been reported in Nipah, Measles, HRSV or *Bornaviridae* budding ([5]). Indeed, the enveloped influenza virus buds in an ESCRT-independent manner as its matrix protein is devoid of an ESCRT-binding domain [118,119]. Other viruses are also released from the host's plasma membrane through their MAs, such as the Newcastle disease virus or VSV. In these cases, bud formation and excision from the membrane are matrix-dependent processes [120,121], as for influenza virus. However, much work is still required to determine how membrane dynamics affect the trafficking and assembly of these viruses, particularly in terms of the cellular factors that control the

trafficking of the structural proteins of these viruses to the plasma membrane [92,122].

Given all of these findings, membrane dynamics has a crucial influence on the assembly and budding of numerous viruses, and it may represent an important and complex target to limit the viral life cycle.

Membrane dynamics and viral spreading

Viruses use various cell communication pathways to achieve effective cell-to-cell dissemination [123]. First described for type 1 HTLV (HTLV-1) [124], the virological synapse (VS) is a complex structure found at the interface of infected:uninfected cells. Viral receptors and the egression machinery accumulate at the VS [125], making the infection and spread of HTLV-1 through T lymphocytes cell–cell dependent. Direct cell-to-cell transmission facilitated by the formation of stable cellular junctions has several advantages, including faster replication rates [126], successful transmigration of infected cells across mucosal barriers [123] and viral protection from host responses. However, such transmission is still to be confirmed for HIV [127,128].

Cell-to-cell spreading of HIV-1 (Figure 2) is considered to involve microtubule-mediated polarization and substantial budding, followed by the entry of free viral particles into target cells [129]. Thus, it involves pathways that regulate cell-free virus entry by modifying membrane dynamics. In this regard, most HIV-1-infected GALT (gut-associated lymphoid tissue) cells in intestinal crypts are infected by concentrated pools of free HIV-1 viral particles in HIV-1-infected humanized mice. Fewer infected cells are found in the mucosal regions and the lamina propria, where VS presumably occur [130], explaining why infection of permissive cells by free viral particles is crucial for HIV-1 replication and pathogenesis *in vivo*. This is consistent with the recent identification of the key cell signals required for efficient early HIV-1 infection and the establishment of latency in CD4+ T cells [131–136]. Interference with retroviral cell-to-cell transmission is not only produced by blocking cytoskeletal motility [137] and depleting membrane-cholesterol [138] but also, by interfering with Arf6-governed plasma membrane dynamics. Moreover, restricting plasma membrane fluidity caused by altering early HIV-1-triggered phosphatidylinositol-4-phosphate 5-kinase (PI4P5-K) Iα activation and the ensuing detrimental effects PIP_2 production on HIV-1 transmission [4,133,135]. In fact, Arf6-coordinated membrane trafficking is required for efficient HIV-1 fusion, entry and infection of CD4+ T lymphocytes [135] (Figures 1 and 2). The flux and turnover of PIP_2-enriched vesicles from the plasma membrane, driven and coordinated by the Arf6-GTP/GDP cycle, ensures cell surface membrane regeneration and it allows membrane exchange between the viral and target cell surfaces. This type of membrane trafficking, coupled with enhanced fluidity, is in strong synergy with the key HIV-1/receptor (CD4 and C–X–C or C–C chemokine receptor type 4 or 5—CXCR4 or CCR5), interactions that promote fusion pore formation in target cells. These interactions take place between the non-regenerative HIV-1 viral membrane and the dynamic cell surface membrane, favouring efficient virus–cell fusion, entry and infection, both for the free virus and in the context of the VS [4,133,135] (Figures 1 and 2).

Despite these similarities, some contact-specific events that affect membrane flux should also be considered. During cell–cell HIV transmission, intense viral endocytosis drives entry into neighbouring cells even if they are in contact [139] (Figure 2). Remarkably, biofilm-like structures at the surface of infected cells concentrate HTLV-1 viruses for their efficient transmission to target cells [140] and cellular projection is used to transmit pseudo-rabies virus. Retroviruses also travel along membrane protrusions that contact adjacent cells and MLV surfs on the filopodia of fibroblasts before entering cells [141] (Figure 2). HIV-1 also takes advantages of filopodia for cell-to-cell transmission [141], similarly surfing on the narrower membranous nanotubes that connect cells separated no more than 100 μm [142] and facilitating the transfer of viral proteins to the inner side of the membrane. These actin structures extend from HIV-infected cells to target cells irrespective of receptor-envelope interactions [142] (Figure 2).

The take up of larger membrane invaginations at the VS of connected cells [129] is known as trogocytosis, an event that may also control the extent and stability of the synapse, regulating its duration [143]. HIV particles, like CD4 molecules and other membrane components, are transferred by trogocytosis from uninfected to infected cells in a manner triggered by the HIV-1 envelope (Env)/CD4 [128] (Figure 2). This mechanism could be very significant and render cells permissive to HIV infection, as recently proposed [144].

The cell–cell contacts and signalling induced by the HIV-1 Env complex that occur at the VS can

activate autophagic membrane flux, leading to apoptotic cell death of uninfected CD4+ T cells [60,145]. This lethal autophagy may provoke or enhance immunodeficiency, as observed *in vivo* where the majority of CD4+ T cells undergoing apoptosis, as well as the peripheral blood and lymph nodes of HIV patients, remain uninfected [146]. Simultaneously, autophagy can be suppressed in infected CD4+ T cells [60], thereby antagonizing Env-mediated apoptosis and allowing viral replication to occur in infected CD4+ T cells. In this context, HIV-1 evades immune responses in an HIV-1 Env-CD4-dependent manner by efficiently impairing autophagy in DCs when early contacts are established [19]. Taking into account the role autophagy plays in viral replication, HIV-1 can enhance or suppress autophagy at different stages of its viral cell cycle, favouring persistence and the evasion of immune responses, and therefore, its pathogenesis [19] (Figure 1). Finally, like other viruses (e.g. CMV), HIV-1 stably associates with professional APCs during infection (such as DCs) to further infect lymphocytes during T-cell scanning or antigen presentation [147]. In fact, HIV-1 enters DCs by exploiting exosomal trafficking during antigen presentation [148] (Figure 2).

CONCLUDING REMARKS

This review examines the intracellular trafficking of viruses that occurs in association with cell-membrane structures, some of which may be newly assembled by viruses to ensure their replication and budding. Membranes derived from the ER, mitochondria, lysosomes and endosomes are sculpted by viruses to generate VFs, acquiring their own functional morphology. These structures help ensure RNA replication is accomplished without alerting the host's defence mechanisms.

Although the importance of membrane dynamics during viral infection has been established, several questions remain unanswered. It remains unclear how proteins from distinct viruses and host cells use the same intracellular membrane compartments or events (e.g. autophagy) to achieve viral replication, without affecting important cellular processes. Conversely, it is not clear why viruses replicate in different subcellular membrane compartments, how they move across membranes and which host factors are involved in these events. Similarly, we still do not know how these changes in membrane dynamics enable viruses to avoid immune responses. Indeed, it remains unclear whether rearranging intracellular organelles enables viruses to escape the anti-replicative activity of natural restriction factors, such as apolipoprotein B mRNA-editing enzyme-catalytic, polypeptide-like 3 (APOBEC3) proteins, Tetherin (BST-2/CD317/HM1.24) or SAMHD1 (sterile alpha motif (SAM) and histidine-aspartate (HD) domain-containing protein 1) for HIV-1 [149]. Resolving these issues will help decipher how viruses rearrange membranes during their infection cycle, thereby aiding the design of new antiviral strategies that target these dynamic viral-cell interactions and combat viral infection. These findings may also produce innovations in non-viral gene delivery systems to tackle tumours and immune diseases.

New technical developments, such as more powerful microscopy systems [4,8,135,150], will allow dynamic viral trafficking and egression to be studied in cells with better spatial and temporal resolution. Such information will further our understanding of the viral infection process and of how viruses succeed in deceiving the host's immune responses.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

ACKNOWLEDGEMENTS

This work and A.V.-F. are supported by the European Regional Development Fund (ERDF), SAF2008-01729 and SAF2011-24671 (MICINN and MINECO, respectively, Spain), UNLL10-3E-783 (ERDF) and Fundación CajaCanarias, and by the Project RD12/0017/0034 integrated in the "Plan Nacional I+D+i" and co-funded by ISCIII-"Subdirección General de Evaluación" and ERDF (RIS-RETIC) grants. M-S.V and L.A-R are supported by RD12/0017/0034-(RIS-RETIC) and SAF2011-24671-FPI-associated grants and fellowships, respectively. We thank Dr Mark Sefton (Biomedred SL) for his linguistic revision of the manuscript. We apologize for all research studies and reviews that we have not discussed or cited in this *Review*. We have tried to avoid any and all such omissions but space limitations have surely made this an impossible endeavour.

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