Received: 25 February 2022 | Revised: 18 May 2022 | Accepted: 21 May 2022

DOI: 10.1111/mmi.14946

MICRO REVIEW

WILEY

The unconventional way out—Egress of HCMV through multiviral bodies

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Funding information
Leibniz Science Campus InterACt, Grant/Award Number: W6/2018; Federal Ministry of Health; Studienstiftung des deutschen Volkes; Deutsche Forschungsgemeinschaft, Grant/Award Number: FOR 5200 DEEP DV / BO 4158/5-1 and RESIST EXC2155 / 390874280; Wellcome Trust, Grant/Award Number: 209250/Z/17/Z; Free and Hanseatic City of Hamburg

Abstract

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus and the leading cause of congenital disabilities as well as a significant cause of disease in immunocompromised patients. The envelopment and egress of HCMV particles is an essential step of the viral life cycle as it determines viral spread and potentially tropism. Here we review the current literature on HCMV envelopment and egress with a particular focus on the role of virus-containing multivesicular body-like vesicles for virus egress and spread. We discuss the difficulties of determining the cellular provenance of these structures in light of viral redistribution of cellular marker proteins and provide potential paths to illuminate their genesis. Finally, we discuss how divergent egress pathways could result in virions of different tropisms.

KEYWORDS
egress, envelopment, HCMV, MVB, MViB

1 | INTRODUCTION

Human cytomegalovirus (HCMV) is an important human pathogen. Primary HCMV infection is typically asymptomatic in immunocompetent individuals, yet the virus establishes lifelong latency and subclinical reactivation (Forte et al., 2020). In immunosuppressed individuals, such as transplant recipients, AIDS, and chemotherapy patients, viral primary infection or reactivation can cause severe diseases and morbidity (Azevedo* et al., 2015; Gianella et al., 2015; Griffiths, 2006). Moreover, HCMV can be vertically transmitted from the mothers to the fetus, leading to congenital disabilities (Manicklal et al., 2013). There is no licensed vaccine available, and antivirals suffer from nephrotoxicity and resistance development (Griffiths, 2020; Lurain & Chou, 2010).

HCMV can infect a variety of different cell types, including but not limited to fibroblasts, epithelial cells, endothelial cells, and hematopoietic progenitor cells (Jarvis & Nelson, 2002; Maciejewski et al., 1992; Maciejewski & St Jeor, 1999; Schrier et al., 1985; Sinzger et al., 1995; Söderberg et al., 1993). The wide range of cell types susceptible to HCMV infection is also reflected by the breadth of clinical HCMV manifestations such as pneumonia, hepatitis, colitis, atherosclerosis, and multiorgan disease (reviewed in Azevedo* et al., 2015; Gianella et al., 2015).

Linda Wedemann and Felix J. Flomm contributed equally to this study.

HCMV extensively restructures the cell's secretory system by forming a donut-shaped membranous structure around the microtubule-organizing center called the assembly compartment (AC) (Sanchez, Greis, et al., 2000). Newly formed capsids leave the host nucleus through an envelopment-de-envelopment step called primary envelopment. Electron microscopy data suggest that unenveloped capsids traffic from the nucleus to the AC for final envelopment (Figure 1; Schauflinger et al., 2013). Here, the cumulating morphogenesis step, called secondary envelopment, is mediated. All components, capsid, tegument, amorphous protein layer, and the host-derived viral membrane studded with glycoproteins come together. This envelopment step results in forming a mature virion inside an exocytic vesicle that needs to fuse with the plasma membrane to release the infectious virion (Severi et al., 1988).

2 | HCMV USES MViBs FOR INTERMITTENT BULK RELEASE OF VIRUS PARTICLES

Although current data (Read et al., 2019; Schauflinger et al., 2013; Shaga Devan et al., 2021; Taisne et al., 2019) have mainly described that HCMV capsids individually bud into small vesicles in the AC (Figure 1a,b), several studies have described large vesicles filled with virus particles (Bughio et al., 2013; Fraile-Ramos et al., 2010; Momtaz et al., 2021; Schauflinger et al., 2011; Severi et al., 1988). However, the relevance of these structures has remained unclear. Notably, large virus-filled multivesicular structures are also found in cells infected with the related mouse cytomegalovirus (MCMV) (Maninger et al., 2011) as well as another beta-herpesvirus such as Human Herpesvirus 6A (HHV6-A) (Mori et al., 2008). These vesicles are often named multivesicular bodies (MVBs), even though it remains unclear if they stem from bona fide cellular MVBs or are a novel entity created in the infection process. We will, therefore, dub them multiviral bodies (MViBs) in the following.

We now provide evidence that HCMV capsids can envelop the surface of large vesicles, resulting in MViBs filled with hundreds of virus particles Flomm et al., 2022) (Figure 1a,c). Using an integrated imaging approach combining live-cell imaging and 3D correlative light and electron microscopy (CLEM), we show that MViBs are subsequently released by fusion at the plasma membrane, where they form extracellular viral accumulations (EVAs) intermittently (Figure 1d). We find EVAs on more than 85% of all infected cells such that we conclude that intermittent bulk release of virus

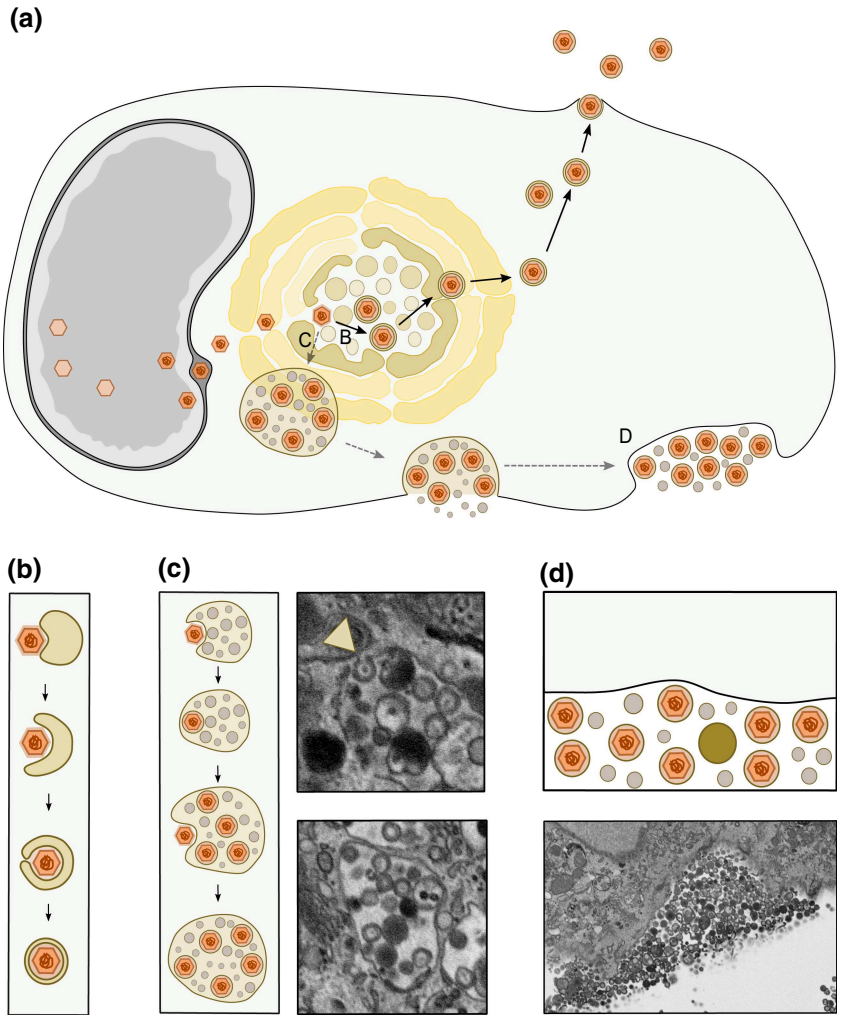

FIGURE 1 Human cytomegalovirus (HCMV) envelopment and egress pathways in fibroblasts. (a) Schematic overview of individual vesicle and MViB-mediated HCMV egress. (b) Details of the virus envelopment process at individual vesicles. (c) Details of the virus envelopment process at MViBs with EM slices depicting capsid budding into MViBs (upper image, arrow) and an MViB containing several enveloped virus particles as well as other viral and cellular material (lower image). (d) Schematic drawing and corresponding EM image of an extracellular viral assembly

particles into EVAs is a so far overlooked HCMV egress pathway. While investigating the provenance of these MViBs, we also found that they carry CD63, which is also present at the release events. The presence of CD63 potentially links MViBs to the endosomal/exosomal pathway, which would be in line with earlier studies, which reported multivesicular structures filled with HCMV virions (Bughio et al., 2013; Fraile-Ramos et al., 2007, 2010; Momtaz et al., 2021; Schauflinger et al., 2011; Severi et al., 1988). However, in our hands, the MVB inhibiting drug U18666A does not inhibit EVA generation, which indicates that the properties of MViBs are different from classical cellular MVBs.

3 | THE PROVENANCE OF MViBs: WHERE DO THEY COME FROM, WHAT DO THEY CARRY? OR HOW TO FIT A SQUARE INTO A CIRCLE

To delineate the provenance of membranes involved in secondary envelopment and egress, studies have typically relied on cellular marker proteins that are enriched in cellular compartments in non-infected cells. Very early studies found that endosomal and recycling processes might be involved in the generation of the HCMV envelope (Tooze et al., 1993; Tugizov et al., 1999). After the discovery of the HCMV AC, mostly trans-Golgi network (TGN), Golgi, ER-Golgi-intermediate compartments (ERGIC), and other secretory markers were associated with HCMV maturation processes (Homman-Loudiyi et al., 2003; Sanchez, Greis, et al., 2000; Sanchez, Sztul, et al., 2000).

Recently, Momtaz et al. analyzed cellular markers at MViBs in fibroblasts and endothelial cells. They found that MViBs vary depending on the cell type regarding protein markers and molecular cargo. Based on the presence of the cellular markers LBPA, RAB5, CD63, ALIX, and clathrin adaptor proteins, the authors concluded that MViBs in fibroblasts originate from the classical endocytic pathway with the involvement of UL71. In contrast, MViBs in endothelial cells originate from a nonclassical MVB biosynthesis pathway with the involvement of UL135 (Momtaz et al., 2021).

However, it has become clear that HCMV uses membranes containing a complex mixture of cellular markers originating from several cellular compartments for envelopment. In addition to the secretory pathways, markers of endosomal, lysosomal, and recycling processes are found throughout the assembly process (Cepeda et al., 2010; Cepeda & Fraile-Ramos, 2011; Das et al., 2007; Das & Pellett, 2011; Fraile-Ramos et al., 2007, 2010). This mixing of organelle-specific markers and the association of viral factors essential for envelopment with different trafficking processes led to the conclusion that HCMV generates a novel compartment tailored to HCMV virion production (Cepeda et al., 2010; Henaff et al., 2012; Moorman et al., 2010). An elegant spatial proteomics study from the Cristea lab further supported this conclusion. The authors found significant global relocalization of cellular marker proteins, indicating the large-scale reorganization of organelles. Some of the novel organelle clusters, which emerged during this process, were associated with viral assembly proteins and suggested that they are involved in HCMV envelopment and egress (Jean Beltran et al., 2016). Deep proteomics methods have been developed to analyze the roles of viral and host proteins involved in viral morphogenesis (reviewed in Jean Beltran & Cristea, 2014), and their complexes (Hashimoto et al., 2020). Tools to analyze the depth of information obtained will be essential as illustrated recently (Federspiel et al., 2020). In the following years, autophagy and exosomal pathways were added to the list of pathways involved in HCMV assembly and further complicated the picture (Taisne et al., 2019; Turner et al., 2020). Since cellular organelles are so dramatically reorganized during HCMV infection, compartment identity is unlikely to be conserved after the extensive remodeling of the secretory system during HCMV infection.

4 | PROTEIN COMPLEXES INVOLVED IN THE GENESIS OF MViBs

To guide future research into the function of cellular and viral proteins in the viral MViB compartment, we will briefly highlight some cellular complexes that likely play a role in MViBs.

It has been shown that in recent years, many enveloped viruses hijack the ESCRT machinery to perform the budding process in their envelopment stage reviewed (Alonso Y Adell et al., 2016; Martin-Serrano & Neil, 2011). Nevertheless, due to complex and conflicting results in studies of HCMV's dependency on ESCRT components, its role in HCMV morphogenesis remains controversial (Fraile-Ramos et al., 2007; Streck et al., 2018; Tandon et al., 2009). However, instead of hijacking complete cellular pathways, viruses can also shortcut them by introducing viral proteins. A recent study described that the HSV-1 homolog of HCMV UL71, UL51 resembles the ESCRT-III component CHMP4B, which mediates the scission after outwards budding (Butt et al., 2020). Furthermore, an earlier study hypothesized that UL71 mimics an ESCRT-III component (Streck et al., 2018), and perturbations of pUL71 result in viral particles stalled at the scission step in fibroblasts. It is, therefore, possible that pUL71 performs ESCRT-like functions in HCMV-infected cells.

Since some structural proteins of HCMV appear to be shuttled through the PM, trafficking of those toward assembly sites is a crucial process in HCMV assembly (Moorman et al., 2010; Tugizov et al., 1999). The tetraspanin CD63 seems to be a key player in this process (van Niel et al., 2011). Due to its essential role in both ESCRT-dependent and -independent formation of MVBs, the presence of CD63 on MViBs could give mechanistic hints about their biogenesis. The tetraspanin was identified on the surface of virions and MViBs in fibroblasts by immuno-EM (Flomm et al., 2022; Fraile-Ramos et al., 2007) as well as light microscopy (Momtaz et al., 2021). Interestingly, no correlation between viral capsid markers and CD63 could be found in endothelial cells, suggesting the absence of CD63 on endothelial MViBs (Momtaz et al., 2021). Moreover, analyzing the functional role of CD63 in HCMV virion production has led to conflicting results (Hashimoto et al., 2020; Streck et al., 2020). Other major regulators of intercompartmental

vesicle transport are Rab GTPases. Due to their essential function, Rab GTPases are expected to play a critical role during HCMV envelopment and egress. HCMV virions contain Rab27a, Rab4B, and Rab11 on their surface, and a knockdown of Rab4B and Rab27a has led to a reduction of HCMV titers (Fraile-Ramos et al., 2010; Krzyzaniak et al., 2009; McCormick et al., 2018; Turner et al., 2020). However, a mechanistic model on how Rab-mediated trafficking guides HCMV assembly and egress is still lacking.

Several steps during HCMV morphogenesis likely involve the attachment and fusion of membranes. A significant group of mediators of such processes are SNARE proteins and are highly interesting in HCMV infection. Early studies found SNAP-23 in HCMV virions (Liu et al., 2011) and a role for syntaxin 3 in virion maturation (Cepeda et al., 2010). Later, also syntaxin 5 was described to play a critical role during HCMV infection (Cruz et al., 2016). Together with the recent identification of the v-SNARE VAMP3 as a factor in HCMV replication, the involvement of SNARE factors in HCMV virion generation and release of progeny is likely (Turner et al., 2020).

5 | POTENTIAL RELEVANCE OF DIVERGING HCMV EGRESS PATHWAYS FOR GENERATING VIRIONS OF DIFFERENT DIVERSITY

HCMV can infect a wide range of organs and cell types. To this end, the virus codes for an extensive range of genes that are essential to mediate divergent cell tropism. Many are rapidly lost if the virus is cultured in fibroblasts (Dargan et al., 2010). At least two glycoprotein complexes dubbed trimer and pentamer mediate divergent entry modes into different cell types. The trimeric complex consists of the glycoproteins gH/gL and gO and binds to the cellular receptor PDGFR alpha. It is sufficient to mediate entry into fibroblasts (Kabanova et al., 2016; Wu et al., 2017). The pentameric complex consists of gH/gL and the genes UL128, UL130, and UL131A (Ciferri et al., 2015; Wang & Shenk, 2005). NRP2 acts as one of the cellular receptors for pentamer and this complex is needed to infect endothelial, epithelial, and myeloid lineage cells (Martinez-Martin et al., 2018). Although the pentameric complex can mediate cell-associated spread to endothelial and epithelial cells, both pentamer and trimer are required on the viral surface to infect the same cell types in a cell-free manner (Laib Sampaio et al., 2016; Wille et al., 2010; Zhou et al., 2015). The trimer and pentamer ratio seems to influence virion tropism, and the viral gene UL148 has been implicated in regulating the trimer/pentamer ratio (Li et al., 2015). Significantly, HCMV virions from the same host cell can be separated into distinct populations based on their trimer/pentamer content (Adler, 2015; Li et al., 1995; Scrivano et al., 2011), implying that infected cells release divergent virus populations with different tropism. However, this remains a topic of controversy to date (Schultz et al., 2021; Vlasak et al., 2016). It is currently not understood how this diversity is produced. Diverging envelopment and egress pathways in individual cell types and between cell types could be involved in producing virions with different glycoprotein contents. For example, an endothelial cell-produced virus is largely cell-associated and mediates cell-to-cell spread, whereas a fibroblast-produced virus is released into the supernatant (Scrivano et al., 2011). Moreover, a study reported HCMV spread in the absence of UL99, an essential component of the secondary envelopment complex is formed by

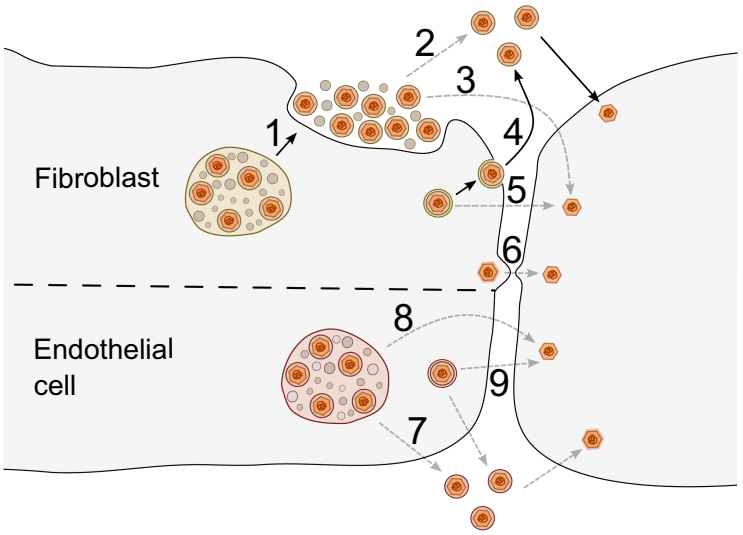

FIGURE 2 Potential links between viral egress and spread pathways. (1) Only MViB fusion with the plasma membrane in fibroblasts has been shown directly, resulting in extracellular viral accumulations (EVAs). It is unclear if EVAs contribute to the (2) cell-free virus population or lead to (3) cell-to-cell spread. (4) Cumulative data strongly suggest that individually wrapped particles result in the cell-free virus. How virus mediating cell-to-cell spread is generated is unclear. Likely is (5) by exocytosis of wrapped virions and subsequent fusion. Alternatively, (6) direct budding at the plasma membrane and subsequent fusion is hypothetically possible. No data exist on MViB-mediated egress in endothelial cells. It could contribute to (7) the cell-free population or (8) direct cell-to-cell spread. Moreover, (9) exocytosis of wrapped virions and subsequent fusion is likely

UL99 and UL94 (Maninger et al., 2011; Phillips et al., 2012; Phillips & Bresnahan, 2012; Seo & Britt, 2008; Silva et al., 2003, 2005). These data might indicate that HCMV cell-to-cell spread is independent of intracellular secondary envelopment. Instead, budding at the plasma membrane is an avenue that should be explored. It is currently unclear which role divergent egress pathways play in viral spread modes or in determining tropism (Figure 2). Further research is needed to link envelopment and egress pathways to spread modes and tropism.

Recent developments in live-cell imaging techniques like lattice-light-sheet microscopy that permit long-term volumetric imaging with minimal phototoxicity are now allowing to illuminate how virus morphogenesis determines viral spread. In addition, 3D correlative light- and electron microscopy closes the gap between ultrastructure and specific labeling. Proteomics of purified virion populations will be valuable tools to assess their genesis by inferring their cellular protein content. Finally, first organoid models are used to study HCMV infection in more complex tissue models (Sun et al., 2020). Imaging technologies capable of using these models will be vital to understand how HCMV uses its swiss army knife-like genome to infect its human host.

ACKNOWLEDGMENTS

We thank the Bosse lab for the critical reading of the manuscript. This work was funded by the Wellcome Trust through a Collaborative Award (209250/Z/17/Z) to JBB. JBB is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2155—project number 390874280 as well as by FOR 5200 DEEP DV (BO 4158/5-1). FJF is holding a graduate student fellowship by the Studienstiftung des deutschen Volkes. The Leibniz Institute for Experimental Virology is supported by the Free and Hanseatic City of Hamburg and the Federal Ministry of Health. This work is further funded by the Leibniz ScienceCampus InterACt (Grant Agreement No. W6/2018). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Wedemann, L., Flomm, F. J. & Bosse, J. B. (2022). The unconventional way out—Egress of HCMV through multiviral bodies. *Molecular Microbiology*, 117, 1317–1323. https://doi.org/10.1111/mmi.14946