Review

Vaccinia virus morphogenesis and dissemination

Kim L. Roberts and Geoffrey L. Smith

Department of Virology, Faculty of Medicine, Imperial College London, St. Mary's Campus, Norfolk Place, London, W2 1PG, UK

Vaccinia virus is the smallpox vaccine. It is the most intensively studied poxvirus, and its study has provided important insights about virus replication in general and the interactions of viruses with the host cell and immune system. Here, the entry, morphogenesis and dissemination of vaccinia virus are considered. These processes are complicated by the existence of two infectious vaccinia virus particles, called intracellular mature virus (IMV) and extracellular enveloped virus (EEV). The IMV particle is surrounded by one membrane, and the EEV particle comprises an IMV particle enclosed within a second lipid membrane containing several viral antigens. Consequently, these virions have different biological properties and play different roles in the virus life cycle.

Introduction

Vaccinia virus (VACV) (see Box 1 for taxonomy and a brief historical background) has a complex morphogenic pathway that culminates in the formation of two distinct infectious virions that are surrounded by different numbers of membranes. The first virion produced, which is called intracellular mature virus (IMV), is surrounded by a single membrane and remains within the cell until cell lysis. The other virion is surrounded by a second membrane and is exported from the cell before cell death. This virion is called cell-associated enveloped virus (CEV) if it is retained on the cell surface and extracellular enveloped virus (EEV) if it is released from the cell surface. The mechanism of the formation of both the IMV and EEV membranes is still debated (see Box 2 and, for a review, see Ref. [1]). What is clear, however, is that different viral proteins are found within either membrane, and this makes IMV and EEV structurally, antigenically and functionally different. The CEV and EEV forms are important for virus dissemination. CEVs induce the formation of actin tails from the cell surface that drive virions towards uninfected cells, and EEVs mediate longer-range dissemination *in vitro* and probably within the host also [2]. In addition to their roles in virus dissemination, CEVs and EEVs help VACV to evade host antibody and complement, by being wrapped in a host-derived membrane. Although several IMV proteins, especially A27 and H3, are targets for neutralizing Abs that prevent infection [3], the only EEV protein that induces neutralizing Ab is B5 [3]. The differences between IMVs and EEVs affect virus attachment and entry into cells, egress from cells and virus dissemination. This short review addresses the morphogenesis and dissemination of VACV.

Corresponding author: Smith, G.L. (glsmith@imperial.ac.uk).

VACV replication cycle

An outline of the VACV replication cycle in a single cell is illustrated in Figure 1 and has been reviewed [4]. The replication of VACV begins with the binding of virions to, and entry into, a susceptible cell. However, because there are two structurally distinct forms of virus, IMV and EEV, virus entry is considered last, after the formation and structure of these virions have been described. Recently, the IMV and EEV forms of VACV were named mature virus (MV) and extracellular virus (EV), respectively [5].

Virus gene expression

After the removal of the IMV membrane or both EEV membranes, the remaining part of the infecting virion, the core, enters the cell and is transported on microtubules deeper into the cytoplasm [6]. The virus protein(s) on the surface of the core that is (or are) responsible for interacting with microtubules and the microtubule motor that is needed for this transport are unknown. The core contains viral structural proteins, the tightly compacted viral DNA genome and transcriptional enzymes that are necessary to initiate replication (for a review, see Ref. [1]).

Cores accumulate in the perinuclear region of the cell and are partially uncoated to enable the virus genome to be transcribed into early mRNAs by the virus-associated DNA-dependent RNA polymerase. Approximately half of the 200 genes encoded by VACV are transcribed early during infection, and a recent study has indicated that these genes are subdivided into immediate and delayed early classes [7]. Proteins translated from these immediate and delayed early mRNAs serve to replicate the virus DNA, modify the host cell to the advantage of the virus and aid virus escape from the host innate immune response. After DNA replication has begun, the transcription of intermediate genes commences. Intermediate genes are fewer in number and encode mostly regulatory proteins that induce the transcription of late genes. The late genes encode most of the virus proteins that make up new virus particles and also enzymes that are packaged into virions to initiate transcription in the next infected cell. For a review of virus transcription, see Ref. [8].

Morphogenesis

The formation of progeny virions begins in areas of the cytoplasm called virus factories, from which cellular organelles are largely excluded. The first visible structure is crescent-shaped, contains lipid and protein, and grows to form an oval or spherical structure that encloses the virus core components and is called an immature virion (IV).

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Box 1. Poxviridae

VACV is a member of the *Orthopoxvirus* (OPV) genus of the *Poxviridae*. Poxviruses have large, ovoid or brick-shaped virions and large double-stranded DNA genomes, and replicate in the cytoplasm. They encode their own enzymes for replication and transcription. VACV has often been confused with cowpox virus (CPXV), the vaccine that Edward Jenner used to immunize against smallpox in 1796 [82]. However, it was recognized in 1939 that the smallpox vaccines being used in the 20th century were not CPXV but a distinct OPV species that became known as VACV [83]. Although Jenner predicted the eradication of smallpox in 1801 [84], this prophecy was not fulfilled until 1977 and was certified by the WHO in 1980 [85]. So VACV is an enigma of virology because it is the only vaccine to have been used to eradicate a disease, and yet its origin and natural host are unknown [86].

The double-stranded DNA genome is packaged into the IV, and the proteolytic cleavage of core proteins transforms the virion into the characteristic brick-shaped IMV, the first form of infectious progeny. For the majority of virions, morphogenesis ends at this point and IMV are released upon cell lysis.

Box 2. IMV membrane debate

During VACV morphogenesis, the first structure seen by electron microscopy is a crescent, but the origin and structure of this have been debated since the 1960s. Early studies reported that the crescent contained a single lipid bilayer with a layer of protein on the convex surface and that they were synthesized *de novo* within the cytoplasm [87]. This view was challenged by a report that claimed that the crescent was a double lipid bilayer that was derived from and continuous with the intermediate compartment between the endoplasmic reticulum and Golgi stack [88]. But a further electron microscopy study reported only a single membrane with a thickness of 5 nm (the same as other single membranes within the infected cell) [89]. The serial sectioning of samples and tilt series analysis found no continuity with cellular membranes. Furthermore, freeze fracturing of immature virus and IMV and deep-etch electron microscopy also demonstrated only a single membrane [90]. Lastly, studies of virus entry showed unequivocal images of fusion between the plasma membrane and the IMV envelope, and no other virus membrane was visible [75]. Collectively, these data show that IMV is surrounded by a single lipid membrane.

The questions for VACV IMV morphogenesis, then, are, 'How does a structure with a single lipid membrane form in the cytoplasm?', 'How are the ends of such structures stabilized in an aqueous environment?' and 'How can the disulphide bonds that are found in several IMV membrane proteins form in the reducing environment of the cytoplasm?' The latter question was solved by the demonstration that VACV encodes its own cytoplasmic disulphide-bond-formation system [91]. The three proteins O2, G4 and A2.5 are essential for the formation of disulphide bonds on the IMV surface and for virus morphogenesis. The first and second questions pose a greater challenge. Cell biology dogma proposes that membranes grow from existing membranes and do not form *de novo*. So how can this happen? Recently, a combination of genetics and electron microscopy showed that the lipid crescent was associated with a lattice of protein made up of trimers of the D13 protein [90,92]. Mutations in this protein were identified that did not prevent lattice formation but inhibited interaction of the lattice with a lipid bilayer. Instead, a double protein lattice formed [92]. Notably, the D13 protein does not remain on the surface of mature virions, so that it seems to be a scaffold against which a lipid bilayer can form and be stabilized. Although it seems that the D13 protein helps the formation of a lipid bilayer, it is also evident that proteins synthesized in the endoplasmic reticulum can be transported into the growing lipid bilayer of the immature virus [93,94]. These observations are not inconsistent because once a lipid bilayer is formed, lipid vesicles can fuse with it and transport proteins to that bilayer.

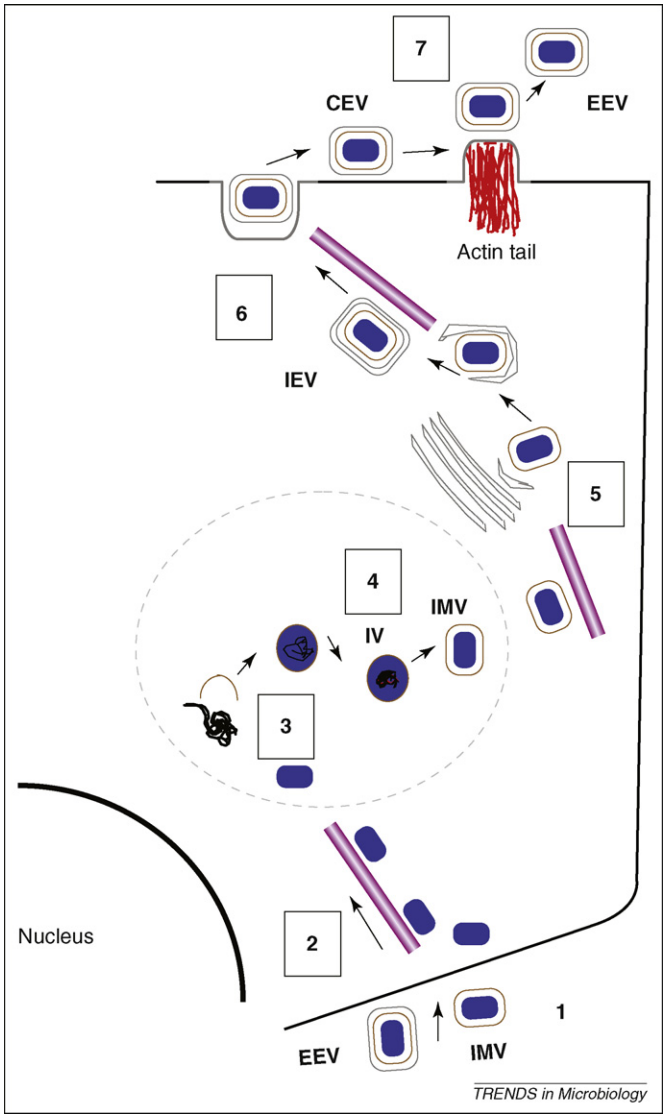

Figure 1. Overview of the VACV life cycle. This cartoon diagram illustrates the VACV single-cell life cycle. (1) The virus binds and enters the cell (see Figure 2), losing the membrane(s) and releasing the naked viral core into the cytoplasm. (2) The core is transported on microtubules deeper into the cell. (3) Transcription of the early mRNAs leads to core uncoating and subsequent DNA replication. (4) Within the viral factory, immature virions (IV) assemble and are processed to form IMV. The majority of IMVs are released from the cell by cell lysis. (5) Some IMVs are transported to sites of wrapping by early endosomes and the trans-Golgi network. (6) IMV particles are wrapped by a double membrane to form IEVs that are then transported to the cell surface on microtubules. (7) The outer IEV membrane fuses with the plasma membrane to expose a CEV at the cell surface. Polymerization of an actin tail beneath the CEV can occur to drive the virus into a neighbouring cell, or the virus is released as an EEV.

The remaining virions are transported away from the viral factory to sites where they become enveloped by a double cellular membrane. Although the distance between the viral factory and wrapping membranes is on average 1.9 μm [9], simple diffusion to cover this distance would be inefficient and has been estimated to take ~1 h (extrapolated from Ref. [10]). IMV transport requires microtubules [9,11]. At present, neither the viral protein(s) nor molecular motor(s) that are required for IMV transport have been identified. However, live digital video microscopy was used to visualize IMVs moving bidirectionally, with speeds approaching 2.8 μm/s, and this was inhibited reversibly by the microtubule-depolymerizing drug nocodazole [9].

The wrapping membranes are derived from endosomal [12] or trans-Golgi cisterna [13,14], and an IMV that is enveloped by these membranes is called intracellular enveloped virus (IEV), or wrapped virus (WV) using the alternative nomenclature [5]. The mechanism for IEV formation is not understood fully, but the viral proteins required include A27, B5 and F13; for reviews, see Refs [1,15]. A27 is an IMV-associated protein, and when the gene is suppressed [16] or deleted [9], IEV formation is inhibited. Similarly, the deletion of B5 [17,18] or F13 [19] inhibits IEV formation. B5 and F13 are two of at least nine viral proteins that are incorporated into or associated with the wrapping membranes. The others are A33 [20], A34 [21], A36 [22], A56 [23], F12 [24], K2 [25,26] and E2 [27]. The deletion of any one of these genes (except *K2R* and *A56R*) causes a small plaque phenotype that indicates reduced cell-to-cell spread. Deletion of *B5R* reduces IEV and, consequently, EEV formation [17,18]. F13 is located on the cytoplasmic side of the wrapping membranes and is anchored to the membrane through palmitoylation of cysteines 185 and 186 [13,28]. F13 has similarity with phospholipases [29], has phospholipase activity [30] and induces the formation of post-Golgi vesicles that is dependent on a HKD motif that is characteristic of phospholipases [31]. The mutation of this motif results in a small plaque phenotype [32,33], and treatment with a phospholipase inhibitor, butanol-1, reduced EEV production [31]. After their formation, IEVs are transported to the cell periphery on microtubules.

IEV transport

The transport of IEVs to the cell periphery has been visualized using green fluorescent protein (GFP) fused to either B5 or F13 [34–38]. IEVs move at speeds that are consistent with microtubule-based transport, which is inhibited reversibly by nocodazole [34–36,38,39]. Two VACV proteins, F12 and A36, have been implicated in IEV movement. Confocal and electron microscopy has shown that the deletion of *F12L* arrests virus morphogenesis after the formation of IEV particles so that CEV formation at the cell surface is reduced dramatically [24,35,40]. However, an interaction between F12 and the microtubule transport system has not been reported. In the absence of A36, IEV were reported to exhibit little [36] or no [39] movement. Moreover, using yeast two-hybrid and glutathione S-transferase (GST) pull-down assays, an interaction was found between the cytoplasmic domain of A36 and the N-terminal tetratricopeptide repeat region of the light chain of the microtubule motor kinesin-1 [41]. This indicates that a function of A36 is the recruitment and binding of kinesin-1 to IEV. However, in the absence of A36, CEVs are visible at the cell surface by confocal and electron microscopy [35,42,43], and EEVs are also produced [22]. Four possible reasons for this discrepancy have been postulated: IMV might be enveloped close to the plasma membrane [41]; IMV might bud out of the plasma membrane, as has been described for the IHD-W strain of VACV [44] and fowlpox virus [45]; IEV proteins other than A36 might interact with kinesin; and lastly, another form of intracellular transport might be used [41]. Recently, the roles of F12 and A36 in IEV transport were compared directly, and it was found that the deletion of *F12L* had a profound effect on CEV formation, whereas the deletion of *A36R* did not [38]. Additionally, visualizing the intracellular movement of a virus lacking *A36R* but with B5 fused to enhanced GFP showed that in the absence of A36, IEVs are transported at speeds consistent with microtubular-based movement, and the movement is inhibited reversibly by nocodazole [38]. Therefore, although A36 interacts with kinesin-1, A36 is not essential for IEV transport to the cell surface, whereas F12 is.

Actin tail formation

At the cell periphery, the IEV particle must cross the layer of cortical actin to reach the plasma membrane. It has been reported that to achieve this, VACV modulates both peripheral microtubule dynamics and cortical actin by the F11-mediated inhibition of RhoA signalling [46,47]. However, the VACV strain MVA lacks the F11 protein and yet can produce CEV and EEV efficiently [48]. Once the IEV reaches the plasma membrane, the outer IEV membrane fuses with the plasma membrane to expose an enveloped virion on the cell surface. During this process, protein A36 becomes localized underneath the CEV, with the majority of the polypeptide chain on the cytosolic side of the plasma membrane [43]. Here, it can be phosphorylated [49] by src kinases to induce virion dissociation from kinesin-1 [50] and to initiate a signalling cascade that leads to actin polymerization beneath the CEV [51]. This nucleation of actin forms projections, called actin tails, which drive CEV away from the cell to infect neighbouring cells. The importance of actin tail formation for the cell-to-cell spread of virus is shown by virus mutants that are unable to induce actin tails and that form small plaques. For instance, the *A36R* deletion mutant makes CEV and EEV but is unable to initiate actin tail formation and forms a small plaque [22,42]. A drug that inhibits Abl-family tyrosine kinases blocked CEV release, reduced viral dissemination and protected mice against a lethal dose of VACV [52].

It is reported that an outside-in signal that is dependent on B5 activates Src [50]; however, actin tails can still form in cells infected with virus that lack B5, albeit at a highly reduced frequency compared to wild-type infection [42]. The dramatic reduction in actin tail formation in the absence of B5 is largely attributable to the failure to make IEV and, therefore, CEV, a prerequisite for actin tail formation. Other VACV proteins are, at least indirectly, required for actin tail formation. The A34 protein regulates the incorporation of proteins, such as B5 and A33, into EEV [53,54]. Similarly, A33 chaperones A36 into the wrapping membranes [49]; therefore, when A34 or A33 are absent from IEV membranes so is A36, and actin tails do not form. Actin tails do not form or are rare in the absence of A34 or F13 and, although this has been attributed to reduced IEV formation, it was found that phosphorylation of A36 was reduced in the absence of either protein [42,49]. Therefore, both A34 and F13 might contribute directly to actin tail formation.

Recently, it was shown that a small fraction of enveloped virions exposed on the cell surface have a ruptured outer membrane and that the A34 protein is involved in this rupture because in its absence the membrane remains

intact [55]. Theoretically, the IMV particle released from a ruptured CEV and EEV might fuse with and reinfect the infected cells, but VACV has a method to prevent this. It was discovered long ago that the deletion of the *A56R* or *K2L* gene induced syncitium formation, so these gene products prevented cell-to-cell fusion and both were required (reviewed in Ref. [15]). Later, these two proteins were found to form a complex on the cell surface and on the EEV surface [25]. Recently, it was shown that these proteins prevent fusion by binding to the fusion machine on the IMV surface [26]. Therefore, the presence of the A56/K2 complex on the cell surface inhibits entry of new IMVs.

EEV release

Enveloped virus on the cell surface is either released (EEV) or retained (CEV). The balance between EEV and CEV is influenced by the host cell [56] and strain of virus. For example, a point mutation within the A34 protein of the IHD-J strain of VACV is responsible for a 50-fold increase in EEV production compared to the Western Reserve (WR) strain [57,58]. Mutations in B5 and A33 that increase EEV release have also been described [59–62]. It might seem peculiar that most virions are retained on the cell surface. By contrast, some viruses encode a receptor-destroying enzyme to enable virus release and dissemination, such as the neuraminidase of influenza viruses. One plausible reason for the retention, rather than the immediate release, of CEV is to enable actin tail formation to occur to drive the virus from cell to cell. However, actin tail formation is not always induced by CEV, and some virus mutants that are unable to make actin tails form enhanced EEV levels. By contrast, the deletion or mutation of several of the proteins in the IEV or EEV membranes reduces EEV release, although often this is due to defects occurring earlier in the morphogenic pathway; for a review, see Ref. [15].

Exocytosis of an IEV particle is not the only way that EEV might be formed, and the budding of IMV through the plasma membrane has also been documented with the VACV IHD-W strain from FL cells [44] and the WR, IHD-J and MVA VACV strains from HeLa cells [63]. This seems to be a major route of release with fowlpox virus [45] but is rare with VACV and most easily observed late during infection. The budding of these VACV strains occurred at sites that contained B5 [63], but it is unclear whether these virions contain the same protein composition within the EEV membrane as those formed by intracellular wrapping and exocytosis.

VACV dissemination

Cell-to-cell spread of VACV can occur in several ways in tissue culture. Under liquid overlay, released EEV can either infect adjacent cells or spread in a convection-mediated, unidirectional manner to distal cells [64], and this forms characteristic comet-shaped plaques, with comet tails that are formed by secondary plaques. Comet plaque formation is inhibited by Abs directed against EEV [65], and strains of virus that produce greater yields of EEV have more pronounced comet plaques [66]. Interestingly, although polyclonal anti-VACV Ab (containing Abs to both IMV and EEV) inhibits comet plaque formation, the primary plaque is only slightly reduced in size [64], which indicates that the virus can spread by an Ab-resistant mechanism. This type of spread can be independent of actin tail formation because viruses that lack either A56 or A36 and, therefore, do or do not make actin tails, respectively, can spread by this Ab-resistant mechanism [64]. In addition, VACV can also spread by an Ab-sensitive mechanism because in the absence of A33, plaque formation was inhibited by Ab directed against EEV [64]. VACV spread can also occur after lysis of the infected cell and the release of IMV. Lastly, VACV increases cell motility, and it was proposed that this might aid the dissemination of virus infection [67]. More recently, the cell motility was attributed to protein F11-mediated inhibition of RhoA signalling [48].

Dissemination within host

The dissemination of VACV *in vivo* is thought to be mediated predominantly by EEV. This is supported by several observations. First, antibodies that neutralize EEV are more effective at providing protection against orthopoxvirus (OPV) challenge than antibodies that neutralize IMV, although both play a part and together they are more effective than either alone [65]. Second, electron microscopy showed that EEVs are formed *in vivo* after infection with cowpox virus [2]. Third, EEV seems well adapted to spread infection outside the cell because it is relatively resistant to neutralization by antibody [68] and is also resistant to complement [69]. By contrast, IMV is easily neutralized by antibody and can be destroyed by complement even without specific antibody. Fourth, virus strains that only produce IMV are avirulent and unable to spread *in vivo* (reviewed in Ref. [15]). Lastly, a correlation has been reported between the virulence of different VACV strains in a mouse model and their ability to produce EEV, although this correlation is not absolute and the WR strain is a notable exception in being virulent in mice and yet

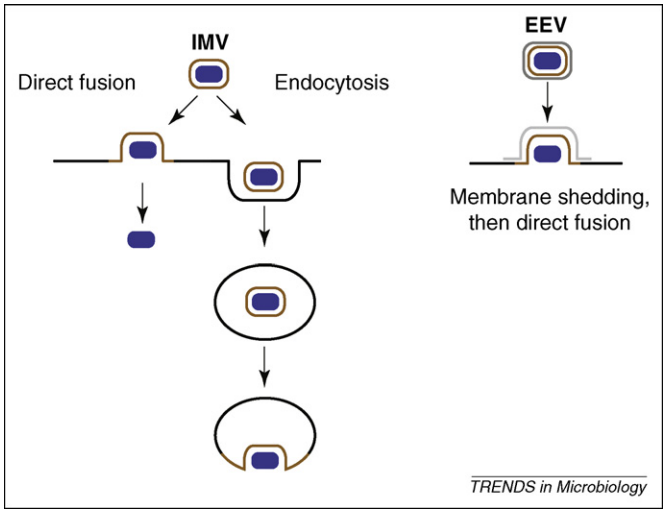

Figure 2. IMV and EEV entry. This cartoon diagram illustrates the proposed models for IMV and EEV entry. There are two nonexclusive models for IMV entry. The naked viral core could be deposited into the cytoplasm by either direct fusion of the IMV membrane with the plasma membrane, or endocytosis and/or macropinocytosis into a vesicle and then fusion of the IMV membrane with the vesicle membrane. EEVs enter cells by shedding the external membrane outside of the cell and then fusion of the IMV membrane with the plasma membrane.

producing low levels of EEV [66]. The spread of infection within the body might be cell associated or as free virions. This issue has been reviewed [70].

VACV entry

The entry of VACV has been reviewed recently [5] and is complicated by the existence of two forms of virus that bind to different (unknown) receptors and have to shed differing numbers of membranes during entry. IMV has been reported to bind to the cell surface via the interaction of H3, A27 or D8 with glucosoaminoglycans (GAGs) [71–73] or via p4c interaction with laminins [74]. However, GAGs are non-essential for infection [75]. After binding, two entry pathways have been reported (Figure 2). The simpler is that the IMV membrane fuses with the plasma membrane in a pH-independent manner to enable the core to enter the cytoplasm, and electron micrographs showing this have been published [75] (Figure 3a–c). The second is that IMV is endocytosed and, thereafter, the virus and vesicular membrane fuse [76] (Figure 2). It seems that both pathways can be used and that the entry mechanism is influenced by the virus strain and cell type [76]. Furthermore, evidence for the requirement for low pH at two stages during IMV entry has been presented [77]. Recently,

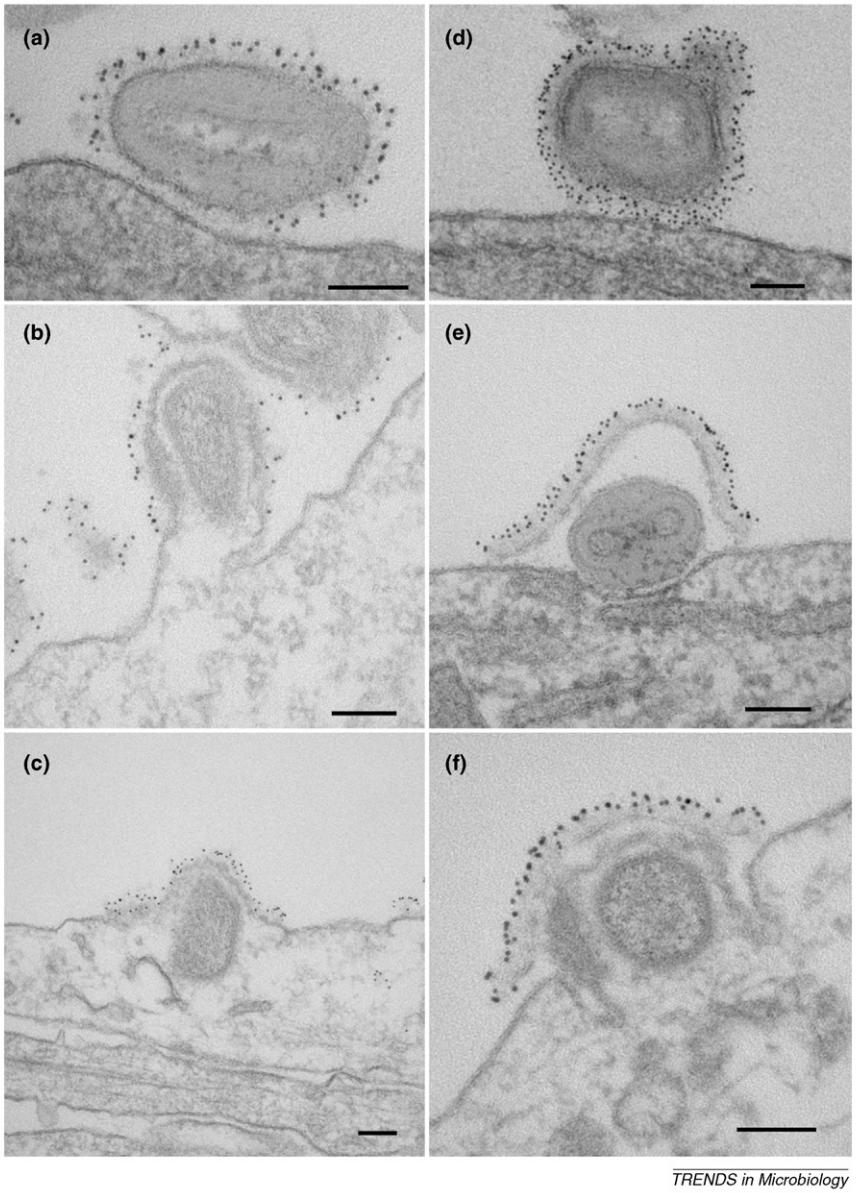

Figure 3. Electron micrographs of VACV entry. (a–c) Electron micrographs showing the entry of VACV IMV by fusion at the cell surface. Purified VACV strain Western Reserve (WR) IMV (325 plaque-forming units per cell) was spinoculated onto PtK2 cells to study the binding and entry of IMV, and samples were either retained on ice or incubated at 37 °C for 10 min. The IMV surface antigen D8 was labelled with D8-specific mAb for immuno-EM. Images show **(a)** IMV particles bound to a cell, **(b)** the IMV membrane fusing with the plasma membrane and **(c)** the core entering into the cytosol. Reproduced, with permission, from the Society for General Microbiology. Published originally in Ref. [75]. (d–f) Electron micrographs showing the entry of EEV into cells by ligand-induced non-fusogenic dissolution of the EEV outer envelope followed by fusion of the IMV with the plasma membrane, as in panels (a–c). Fresh EEV of VACV strain WR was spinoculated onto PtK2 cells at 4 °C and then incubated at 37 °C. The EEV surface was labelled by rat anti-B5 mAb 19C2 followed by rabbit anti-rat IgG and then 6-nm protein A-gold conjugate, then the samples were processed for electron microscopy. **(d)** EEV bound to cell surface with membranes intact. **(e)** The EEV outer membrane has disrupted at the point of contact, enabling the IMV particle to contact the cell. **(f)** The IMV membrane has now fused with the plasma membrane, enabling the core to enter the cell. Note that the EEV membrane remains outside the cell. Scale bars for all panels =100 nm. Reproduced, with permission, from Ref. [81].

it was shown that IMV induces cell-surface blebbing characteristic of apoptosis to aid the macropinocytosis of virions into the cell [78].

The fusion of the IMV membrane with either the plasma membrane or endosomal membrane is mediated by a complex of at least nine proteins (A16, A21, A28, F9, G3, G9, H2, J5 and L5) [4,5,79] which are all essential for fusion but not for binding. This situation is distinct from the entry of other enveloped viruses in which fusion is generally mediated by a single protein [80]. Several proteins of the VACV fusion machine were generated by ancient gene duplication, yet each protein is now essential for fusion, indicating that they have non-redundant functions [79]. Each protein is also conserved in poxviruses, which indicates a common entry mechanism for these viruses and that this entry mechanism must have evolved before vertebrate and invertebrate poxvirus species diverged.

The study of EEV entry is hampered by the low amount of EEV that is produced by most VACV strains and by the fragility of the EEV membrane. EEV entry was a topological puzzle: for EEV particles to infect cells, the viral core has to be released from within two viral membranes and deposited into the cytosol. In a recent electron microscopy study, it was shown that after binding to the cell, the EEV membrane is disrupted outside of the cell [81] (Figures 2,3). This disruption exposes the IMV membrane to the plasma membrane, leading to their fusion and the entry of the viral core into the cytoplasm (Figure 3d–f). It was found that EEVs become sensitive to IMV-neutralizing antibody when treated with certain poly-anionic compounds, such as heparin, and that larger, more negative compounds were more effective at disrupting the EEV membrane [81]. The disruption of the EEV membrane also required the B5 and A34 proteins.

Concluding remarks and future directions

VACV has a complicated replication cycle that produces two different types of virion, IMV and EEV, which are structurally, antigenically and functionally distinct. IMV particles are robust and are released in large numbers by cell lysis but are more susceptible than EEV to neutralizing antibodies and complement. The wrapping of a subset of the progeny virions in an additional cell-derived membrane enables EEV to better evade neutralizing antibodies and spread through the host. The extra envelope of EEV also complicates virus entry. The cellular receptor(s) for IMV and EEV are not known. IMV entry requires a large complex of viral proteins for membrane fusion, and these proteins are lacking from the EEV membrane. EEVs enter cells by shedding their external membrane outside of the cell, exposing the IMV membrane and fusion complex to the plasma membrane.

There are several aspects of the VACV entry, morphogenesis, transport and exit that remain poorly understood. First, the nature of the receptors for IMV and EEV remain to be identified. Second, during at least three stages during virus replication, a virus structure is transported on microtubules: the virus core after entry, the IMV particle from factories to sites of wrapping, and the IEV particle to the cell surface. These structures all have different surfaces and so must engage the microtubules and their motor proteins in different ways, but the molecular details of these interactions remain largely uncharacterized. Third, the details of how a single membrane structure is formed within the cytoplasm remains of great interest to virologists and cell biologists. Lastly, the mechanism by which the IMV particle becomes wrapped by cellular membranes to form IEV needs further study. Overall, VACV represents an excellent model to study the interactions of a virus and the cell.

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