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VACCINIA VIRUS MOTILITY

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Key Words microtubules, actin, intracellular transport, virus dissemination

■ **Abstract** Vaccinia virus (VV), the virus smallpox vaccine, replicates in the cytoplasm of infected cells. The intracellular movement of this large virus would be inefficient without specific transport mechanisms; therefore, VV uses microtubules for movement during both entry and egress. In addition, the dissemination of virus from infected cells to adjacent cells is promoted by the polymerization of actin beneath cell surface virions to drive virus particles away from the cell. Last, the roles of different VV particles in virus movement within and between hosts are discussed.

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INTRODUCTION

Vaccinia virus (VV) is the best-studied member of the orthopoxvirus genus of the poxvirus family. Other members of this genus include variola virus, the cause of smallpox, monkeypox virus, cowpox virus, and ectromelia virus. All these viruses are morphologically indistinguishable and antigenically cross-reactive, such that prior infection with any one of them confers some protection against other members of the genus (26).

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VV has played seminal roles in the history of medicine and biomedical research. It is the only vaccine to have eradicated a disease (25) and yet its natural host and origin are unknown (4). Shortly after the eradication of smallpox recombinant VVs were constructed that expressed foreign genes (64, 79) and had potential as genetically engineered live vaccines (100). More recently, the study of VV virulence revealed a wide range of immunomodulatory proteins and has increased our knowledge of virus pathogenesis and mammalian physiology (101). Last, VV interactions with the host cell have illustrated how viruses can modify and exploit host cell biology to achieve the efficient transport of virus particles by use of cytoskeletal components. The latter topic and how VV spreads within and between hosts are considered in this review.

THE VACCINIA VIRUS LIFE CYCLE

Like other viruses, VV must bind to a susceptible cell, penetrate into the cell interior, replicate and then be transmitted to other susceptible cells or hosts. VV replication within the cytoplasm has been studied extensively [for review see (75)], but less is known about virus binding, penetration, and egress. VV morphogenesis is complex and produces several types of virion that have different roles in the virus life cycle [for review see (102)]. After entry and the coordinated expression of virus genes intracellular mature virus (IMV) particles are assembled in cytoplasmic factories. IMV move on microtubules to sites near the microtubule-organizing center (MTOC) where they become wrapped by intracellular membranes to form intracellular enveloped virions (IEV). IEV move to the cell periphery on microtubules and fuse with the plasma membrane to expose cell-associated enveloped virions (CEV) on the cell surface. CEV induce the polymerization of actin, which propels the virion away from the cell and into surrounding cells. Extracellular enveloped virus (EEV) is released from the cell and mediates the long-range dissemination of virus in cell culture and probably in vivo.

INITIATION OF INFECTION

Any discussion of VV binding and entry must define which form of VV is being considered because IMV and EEV are antigenically distinct and are surrounded by different numbers of lipid membranes. Most early studies used IMV particles because they are abundant and physically robust. However, EEV is the more important form for dissemination within the host and more recently was also used for binding and entry studies.

Virus Binding

IMV and EEV bind to different receptors (118) consistent with the different proteins on their surfaces, but the nature of these receptors remains largely unknown.

Reports that VV binds to the epidermal growth factor receptor (24) and that myxoma virus uses chemokine receptors (59) have been refuted (46, 68). More progress has been made identifying receptors used by IMV than EEV. Among the several proteins on the IMV surface, three have been shown to bind to glycosoaminoglycans (GAGs). The A27L and H3L gene products bind to heparan sulfate (44, 62, 119) and the D8L protein binds to chondroitin sulfate (45). Virus binding to GAGs is only the initial interaction of the virus with the cell, and additional host and virus molecules are likely to be involved in virus penetration.

For EEV, four virus-encoded proteins (A33R, A34R, A56R, B5R) are present on the virion surface [for review see (102)], but there is only indirect evidence for interactions of these proteins with cell receptors. First, the virus hemagglutinin (HA, gene A56R) causes agglutination of erythrocytes from some species (7). Second, the loss of A34R reduced the specific infectivity of EEV fivefold (70), suggesting a role for this protein in either binding or entry. Third, EEV can be neutralized by antibody (Ab) directed against protein B5R (30, 61). Last, mutations in the A33R (55), A34R (11, 70), and B5R (38, 55, 69) proteins influence whether virions remain attached as CEV or are released as EEV. Evidence of physical interactions between specific EEV proteins and cell surface antigens is needed.

The binding of IMV and EEV particles affect the cell differently. Binding of IMV induces signaling cascades and the production of actin-containing cell surface projections, whereas the binding of EEV does not (58). The IMV-induced projections may promote the entry of virions because cores can sometimes be seen within these processes.

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Virus Penetration

After virus binding the core has to enter the cytoplasm to initiate replication and this requires the removal of membranes. A fundamental issue is the number of membranes surrounding the IMV particle. Originally, a single membrane was reported (22), but subsequently two closely apposed lipid bilayers were described (105). Other studies supporting either one (37, 42, 74), two (35, 36, 89), or several (35, 36, 89) membranes have been published. Irrespective of the number of membranes around an IMV particle, EEV is surrounded by one more membrane than IMV; therefore IMV and EEV face a different topological problem during entry. Consequently, the entry mechanisms are likely to be different and evidence for this has been presented (116). Several mechanisms have been proposed for IMV and EEV entry (Figure 1).

If IMV has a single membrane, a single fusion event would release the core into the cytosol, but if IMV has two membranes, fusion of the outer membrane would release an enveloped virion into the cytosol (Figure 1). Evidence for virus-plasma membrane fusion was provided by electron microscopy (2, 18) and by the dispersal of fluorescent dye in prelabeled IMV particles after binding and entry (23). Others proposed that IMV is internalized by endocytosis (21, 85) or is uncoated outside the

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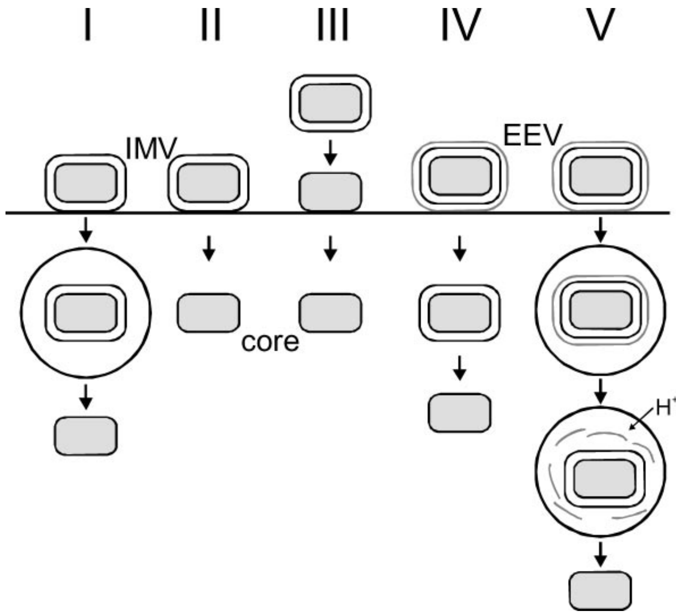

Figure 1 Possible IMV and EEV entry mechanisms. (I) Bound IMV enters by endocytosis after which the IMV surface membrane fuses with the vesicular membrane to release a core into the cytosol. (II) Bound IMV fuses with the plasma membrane releasing a core into the cytosol. (III) IMV uncoats outside the cell and the core somehow crosses the plasma membrane. (IV) Bound EEV fuses with the plasma membrane. The IMV within the cytosol then loses its membrane to release the core. (V) EEV is endocytosed and the outer EEV membrane is destroyed within an acidified vesicle releasing an IMV. The IMV then fuses with the vesicle membrane releasing the core into the cytosol.

cell followed by transfer of the core across the plasma membrane (106). IMV entry is slower than EEV despite it having to shed fewer membranes (23, 58, 85, 116).

Fusion of EEV with the plasma membrane would release an IMV particle still surrounded by at least one lipid membrane into the cytoplasm (Figure 1). To explain EEV entry, Ichihashi (47) proposed that the EEV particle is endocytosed, the outer envelope is destroyed within acidified intracellular vesicles, and the IMV particle released into the vesicle then fuses with the endosomal membrane to introduce a core into the cytoplasm. In support of this model, the integrity of the EEV outer envelope is sensitive to low pH, and the appearance in the cytosol of cores derived from EEV but not IMV is inhibited by drugs that raise the pH of intracellular vesicles (47, 116). Others reported that low pH is not required for EEV entry (23, 58, 85). When interpreting data from these different studies, the method used

to obtain EEV should be considered because centrifugation, sonication, osmotic shock, or freeze/thawing can rupture the outer envelope such that the virion may function as an IMV rather than as an EEV.

MOVEMENT OF VIRUS CORES

After cores have entered the cytosol, they move inward to sites where virus factories develop. The appearance of virus cores has been investigated by electron microscopy (2, 18, 21, 42, 58) and confocal microscopy using Ab specific for core antigens (58, 66, 116). The mechanism of core movement was addressed recently using VV cores labeled with enhanced green fluorescent protein (EGFP) fused to the A5L protein (90). After binding to cells, EGFP-cores from these virions moved inward from the cell periphery. The process was inhibited reversibly by nocodazole but not cytochlasin D and the mean velocity of core movement was 52 μm/min, consistent with movement on microtubules. In addition, confocal microscopy showed that cores and microtubules colocalized (66, 90). The nature of the core proteins that interact with microtubules remains to be defined, but in vitro studies suggested that A10L and L4R might be involved (86).

IMV FORMATION AND MOVEMENT

Within 20 min after VV entry early mRNAs are transcribed within the core (75), extruded (54), transported on microtubules, and organized into discrete granular structures in association with host translation factors and polyribosomes (66). Consistent with a role for microtubules, nocodazole reduced the synthesis and stability of viral but not host mRNA in HeLa cells (66). The VV L4R protein has been implicated in VV mRNA movement because it interacts with both ssRNA and microtubules (5, 66, 86). Host cell mRNAs frequently associate with the cytoskeleton rather than being randomly distributed in the cytoplasm. In most somatic cell types including fibroblasts, myoblasts, and neuroblasts, microfilaments (actin) mediate mRNA-cytoskeleton interactions, although in neurons, oligodendrocytes, and oocytes intact microtubules are important for mRNA localization (49).

Early virus proteins mediate further core uncoating to release the DNA genome (43, 53). Viral DNA replication and virion assembly occur in virus factories from which cellular organelles are largely excluded (16, 22). Viral DNA synthesis starts 1 to 2 h postinfection (76) near the uncoated core and this site becomes surrounded by endoplasmic reticulum (ER), resembling nuclear envelope assembly in late anaphase/telophase of the cell cycle (65, 111). Subsequently, the ER membranes dissociate from the virus factories during virus assembly (~5–6 h postinfection) (111). The events after core entry are illustrated in Figure 2.

The first visible structures within virus factories are crescents (cupulae in three dimensions) that are composed of lipids and viral proteins [for review see (75)].

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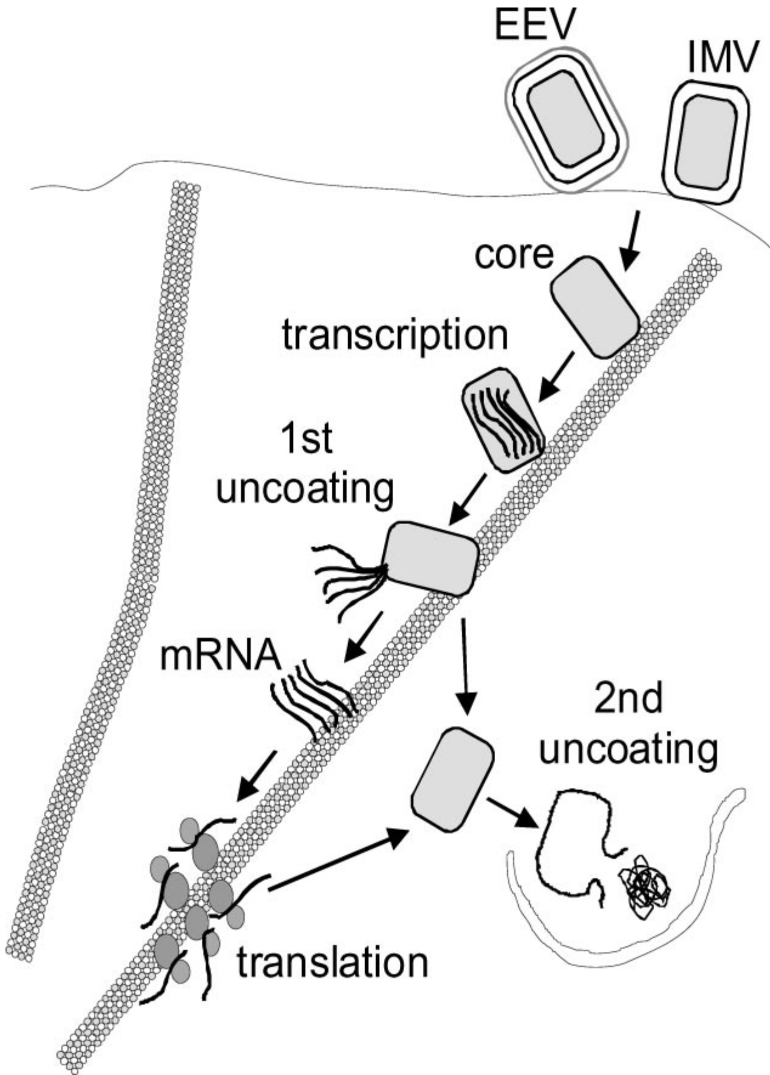

Figure 2 Movement of virus core and mRNAs in association with microtubules. After entry the core moves on microtubules. Meanwhile, the early virus genes are transcribed using enzymes packaged during virus assembly. Uncoating of the core releases early mRNAs that are transported on microtubules. These mRNAs localize to discrete granules in the cytosol and are translated using host translation machinery. The early proteins induce further uncoating of the core to release viral DNA for subsequent replication. ER membranes surround the viral DNA.

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The number of membranes in virus crescents and hence around IMV is disputed, but these crescents grow to form ovals into which virus DNA and proteins are packaged to form immature virus (IV). Maturation of IV into IMV requires the proteolytic cleavage of several capsid proteins and core condensation (75).

Most IMV particles remain intracellular and are released only upon cell lysis. However, some IMV particles move to sites near the MTOC where they become wrapped by membranes of the *trans*-Golgi network (TGN) or early endosomes to form IEV (102). Movement of IMV from virus factories requires microtubules and the VV protein A27L because treatment of VV-infected cells with nocodazole or colchicine, or repression of VV gene A27L, prevented the dispersal of IMV from virus factories (96). Similarly, accumulation of IMV near the MTOC was inhibited by overexpressing p50/dynamitin that disrupts the function of dynein-dynactin (86).

The A27L protein is multifunctional (Figure 3*A*) and is attached to the IMV surface via interactions with proteins A17L and A14L (92, 122). It is involved in IMV attachment (19), virus-cell fusion (23), cell-cell fusion (34, 44, 94), and wrapping of IMV to form IEV. It is also a target for IMV neutralization by Ab (93). A27L forms a coiled-coil trimer, similar to the fusion proteins of HIV and influenza virus (94, 120). Three regions of A27L were predicted to fold into helical structures (120). The first and second regions (residues 29–37 and 44–72) form a random coil and a rigid helix, respectively (63). The third helix (residues 77–98) contains a leucine-zipper motif that is important for interacting with A17L (120). The N-terminal 20 amino acids are cleaved in the mature protein (110) to expose a lysine- and arginine-rich region (residues 21–32) that binds to heparan sulfate (44, 119). The membrane fusion domain lies within residues 29–43 and the second helix is important for formation of the coiled-coil trimer (119, 120). However, the region responsible for IMV transport is not known.

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IEV FORMATION AND MOVEMENT

IEV particles are formed by wrapping IMV particles with membranes derived from early endosomes (112, 114) or the TGN (99), and markers of both compartments can be detected in EEV (56). Early during infection, most IMV particles become wrapped to form IEV, but the IMV particle is mostly unwrapped later during infection (113). IEV serves as an intermediate structure to transport virus particles from the site of wrapping to the cell periphery (in the opposite direction to IMV), and to protect released particles from Ab and complement, to which IMV is sensitive.

Seven VV proteins (F12L, F13L, A33R, A34R, A36R, A56R, and B5R) are absent from IMV but are located on IEV, and most of these (A33R, A34R, A56R, B5R, and F13L) are also present on CEV and EEV [for review see (102)]. Two of these proteins, B5R and F13L, and the IMV A27L protein are needed for wrapping. A virus mutant with an A27L Ala25 to Asp substitution permitted IMV transport but not wrapping (96), and N-terminally truncated A27L does not make EEV (119). Similarly, mutant viruses lacking B5R or F13L form 5- or

10-fold less EEV owing to a defect in wrapping, and wrapping is also inhibited by the drug N_1-isonicotinoyl-N_2-3-methyl-4-chlorobenzoylhydrazine (IMCBH) [for review see (102)].

The VV particle (250 × 350 nm) is estimated to take 5.7 h to diffuse across 10 μm of cytoplasm (104). Thus, to hasten virion movement, the virus exploits the host cellular mechanisms to transport virions to the cell surface. Late during VV infection actin-containing microvilli are formed with a virion at their tip (9, 39, 40, 57, 109), and it was proposed that the polymerization of actin propels IEV through the cell, as observed in the intracellular bacteria *Shigella* and *Listeria* (20, 28, 29). However, evidence indicated that actin filament formation was not used for IEV transport. Addition of cytochalasin D, an inhibitor of actin filament formation, did not prevent CEV formation (83). Furthermore, several mutants are unable to form actin tails but can nonetheless form CEV and EEV (102). CEV are formed in the presence of the Src-kinase inhibitor PP1, which inhibits tyrosine phosphorylation of A36R and actin tail formation (41). Indeed, mutant VVs expressing A36R in which the sites of tyrosine phosphorylation required for actin tail formation were mutated could still move to the cell periphery (123). Finally, actin tails are formed in a polarized fashion on one side of the virus particle (20). Whereas *Listeria* and *Shigella* have a protein at one end of the bacterium to drive actin polymerization, no protein has been found with a polarized distribution on IEV (115). Taken together these data indicate that actin tail formation is not the means by which IEV is transported to the cell surface.

More recently, it was shown that IEV move to the cell surface on microtubules and actin tails are formed beneath CEV particles at the plasma membrane of infected cells, where the A36R protein is concentrated (31, 41, 88, 124). By fusing GFP to the EEV proteins B5R (91, 124) or F13L (31, 88), it was seen that virus particles move in a saltatory fashion. This movement could be inhibited reversibly by nocodazole (31, 41, 124) but not by the myosin inhibitor 2,3-butanedione monoxime (31). In addition, an F13L-GFP-tagged virus was visualized along microtubules in cells expressing tubulin-GFP (88). The speed of virion transport ranged from 40 to 180 μm/min, with an average speed of 60 μm/min, which is similar to that of microtubule transport of cellular vesicles but is 20 times faster than the speeds of movement via actin tails, which measured at 2.8 μm/min (20).

Two VV proteins (F12L and A36R) have been implicated in IEV movement and are termed transport proteins (102). The VV F12L protein (Figure 3*B*) is conserved in chordopoxviruses and its deletion caused a small plaque phenotype, sevenfold less EEV and a highly attenuated virus (129). Microscopic examination of ΔF12L virus-infected cells showed that IEV are formed but not transported to the cell surface (114). Colocalization of epitope-tagged F12L and microtubules has been observed (114). The second protein (A36R) is a type 1b membrane protein with the majority of the protein in the cytosol (95, 115) (Figure 3*C*). A mutant virus that lacks A36R forms small plaques, produces fivefold less EEV, and is attenuated in vivo (80). The A36R protein is required for actin polymerization to drive CEV away from the cell surface.

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The F12L and A36R proteins were shown by immunoelectron microscopy to be absent from both CEV and EEV but present on the outer IEV membrane (56, 114, 115). How this distribution occurs is unknown, but it remains possible that the proteins are also present in CEV and EEV particles in a conformation inaccessible to these monoclonal antibodies (mAbs). The apparent exclusion of A36R and F12L from CEV and EEV warrants further investigation. During wrapping of IMV, these proteins might be excluded from the space between the IMV surface by steric hindrance because the majority of their polypeptide chains are located in the cytosol. In contrast, only small parts of the EEV proteins B5R, A33R, A34R, and A56R are located in this space [for discussion see (102)]. Alternatively, it is possible that their location is dependent on differences in lipid concentration that exist on the different sides of intracellular organelles or possibly due to lipid microdomains that exist in the Golgi apparatus, for example (32, 107).

The movement of IEV from the site of wrapping to the cell surface follows an anterograde pattern, suggesting movement via kinesin (33, 121). Consistent with this, Rietdorf et al. (88) reported that kinesin transports IEV to the cell periphery, and residues 71 to 100 of the A36R protein were suggested to have a role in this movement. However, a virus lacking A36R forms CEV (41, 114, 115, 126), indicating movement to the plasma membrane. Moreover, viruses lacking A36R but with secondary mutations in A33R or B5R released more EEV than wild-type virus, showing that transport does not require the A36R protein (55). This discrepancy might be explained by differences in time after infection at which cells were examined. While Rietdorf et al. (88) examined cells 6 to 8 h postinfection, others looked later during infection. Perhaps the loss of A36R reduces the efficiency of IEV transport but does not prevent it, so at later times of infection CEV are seen.

During infection with wild-type VV the heavy and light chains of kinesin colocalize with IEV particles (88), and the tetratricopeptide region (TPR) of the kinesin light chain (KLC) was implicated in this interaction. This region binds cargo to kinesin and overexpression of KLC TPRs inhibits movement of kinesin cargo (121) and egress of IEV to the cell surface (88). Although the A36R protein was suggested to be involved in movement, no physical interaction between kinesin and A36R was found (88). Another possibility is that the F12L protein, which is needed for IEV movement, binds microtubules (114). Consistent with this possibility, both kinesin and F12L, but not A36R, are excluded from the tips of actin tails.

FORMATION OF ACTIN TAILS

The importance of actin tail formation in VV spread is highlighted by the fact that all mutant viruses unable to form actin tails have a reduced plaque size (60, 91, 102). The A36R protein has a vital role in actin tail formation and is situated just underneath the CEV with the majority on the cytosolic side of the plasma membrane (115). A36R is phosphorylated on serine, threonine, and tyrosine (127).

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Although the sites of tyrosine phosphorylation have been identified as residues 112 and 132 (29, 98, 123), which serine and threonine residues are phosphorylated is unknown (127).

Phosphorylation of A36R by the Src family of kinases is essential in the signaling cascade that leads to actin tail formation and can be inhibited by the drug PP1 and kinase-deficient Src mutants (29). Following recruitment of Nck, the Wiskott-Aldrich syndrome protein (WASP) family member N-WASP is also recruited to the site of actin assembly (29) (Figure 4). Further investigation revealed that the WASP-interacting protein (WIP) recruits N-WASP to the site of actin nucleation, though it does not activate N-WASP to trigger the Arp2/3 complex (67, 73). The WIP binding site of N-WASP was mapped to the wasp homology 1 domain (73) and the sequence ESRFYFHPISD of WIP binds to N-WASP, with

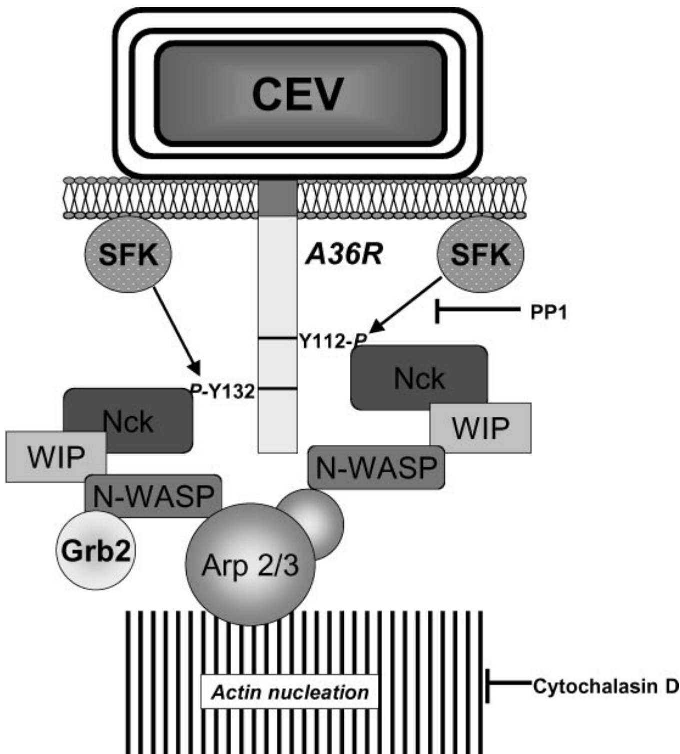

Figure 4 The formation of actin filaments beneath CEV particles. VV causes actin nucleation to drive CEV away from the cell to infect neighboring cells. The cascade begins with phosphorylation of the cytoplasmic face of A36R by Src-family kinases (SFK), which can be inhibited by PP1. Through subsequent recruitment of Nck, WIP, and N-WASP followed by the Arp 2/3 complex, actin nucleation occurs, which is inhibited by cytochalasin D.

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the phenylalanine residues being particularly important (128). Recruitment of N-WASP and Grb2 to the tip of actin tails is dependent on phosphorylation of A36R tyrosine 132. This pathway is unable to initiate actin nucleation alone, but it is important for efficient actin tail formation (98). Thereafter, the actin-nucleating protein Arp 3 is recruited (28), which is part of the Arp 2/3 complex that nucleates actin monomers to form actin filaments (17). These pathways were deduced using dominant-negative mutants and ectopic expression of constructs. However, the involvement of N-WASP, Nck, and WIP has also been demonstrated in cells from N-WASP-deficient embryos (103).

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VIRUS DISSEMINATION

Once VV particles reach the cell surface they may disseminate to other susceptible cells. Dissemination within a host and between hosts is achieved by different forms of virion and is summarized in Figure 5.

Spread Within a Host

For dissemination within a host the virus must replicate and spread to the neighboring or distant cells without being eliminated by the host immune response. The CEV and EEV forms are responsible for this type of spread, and evasion of the host immune response is aided by the expression of a wide array of viral immunomodulators [for review see (101)]. The possible mechanisms of spread in vitro or in vivo are considered below.

DIRECT CELL-TO-CELL SPREAD CEV are important for efficient cell-to-cell spread by VV and this might explain why VV retains significant amounts of virus particles on the cell surface (10). In contrast, some other viruses have mechanisms to enhance their release and prevent retention. For instance, influenza virus expresses neuraminidase that removes the sialic acid receptors from infected cells and virions. CEV are required for actin tail formation that mediates efficient cell-to-cell VV spread in an Ab-resistant manner [for review see (102)].

There is also an Ab-resistant mechanism of virus cell-to-cell spread that is independent of actin tails. In the presence of VV-neutralizing Ab, all VV mutants that are unable to make actin tails still form plaques, except a virus with gene A33R deleted (60). The A33R protein therefore mediates this resistance directly or indirectly, possibly by a mechanism similar to HIV and alpha herpesviruses that disseminate preferentially across cell contacts/junctions to escape the surveillance of antibodies (52).

LONG-RANGE SPREAD Systemic spread of orthopoxviruses may occur within a host either by infected leukocytes or as free virus, and this has been studied best with ectromelia virus in mice and rabbitpox virus (a VV strain) in rabbits. After

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replication at the primary sites of infection the virus spreads to regional lymph nodes and replicates further. Subsequently, the virus enters the blood (primary viremia) and spreads to other major organs [for review see (15)]. With ectromelia virus and rabbitpox virus, infectivity is mostly cell-associated (leukocytes) during viremia (6, 8, 125). The exact cell types in which the virus is carried and replicated is unknown, although primary monocytes and macrophages have been suggested (50, 71). However, activated rabbit macrophages (3, 14, 71), primary mouse and human macrophages (12, 77), and dendritic cells (13, 51, 78) do not support virus replication. On the other hand, rabbit leukocytes were once used for the cultivation of VV in vitro (27) and phytohemagglutinin-treated human leukocytes also support VV growth (72). Cultured human B cells support VV replication, whereas T cell lines produce little virus (1, 108). However, in vivo mouse lymphocytes were uninfected by VV (78). More work is needed to determine whether poxviruses can infect leukocytes productively or remain latent in these cells, and recombinant viruses expressing EGFP might be useful for this purpose.

In addition to the possible systemic spread of VV by infected leukocytes, VV may disseminate as free virus. Payne & Kristensson (84) demonstrated that EEV was released from epithelial cells of mice infected intranasally. Also, VV spread to distant organs correlated with the levels of EEV made by different VV strains (with the exception of the Western Reserve strain that is neurotropic), and EEV was detected in blood of rabbits that were immunosuppressed using cyclophosphamide (82). In other studies antibodies raised against inactivated IMV were ineffective in protecting animals against virus challenge, whereas antibodies against EEV offered good protection [for review see (102)]. These studies suggest that EEV is important in the long-range virus spread.

EEV is better suited than IMV for long-range dissemination. It is released early during infection and dispersed by convection currents (60). Antiserum resulting from a live VV infection contains higher neutralizing antibodies against IMV than EEV (61). IMV is very immunogenic and there are many mAbs that neutralize IMV but none that neutralize EEV. The extra layer of EEV membrane shields these immunodominant IMV surface antigens. In addition, host proteins, such as complement regulatory proteins, are incorporated into this extra membrane and protect EEV from inactivation by complement (117).

SPREAD BY VIRUS-INDUCED MOTILE CELLS VV-infected cells develop extensive cytopathic effect (CPE) long before cell death. Sanderson et al. (97) noted that VV-induced CPE included morphological changes such as the formation of long, branched projections and also that cells became more motile than noninfected cells. These virus-induced cell changes require the expression of VV early genes but not late genes or virus assembly. Why VV induces cell movement and formation of long protrusions is not known, but it is possible that these induced motile cells may assist virus spread. Cell migration and morphology changes require the activation of various signal transduction cascades. How VV manipulates these and which virus and host proteins are involved remain unknown.

Host-to-Host Spread

For dissemination between hosts, different orthopoxviruses species use different routes. Variola virus and VV are transmitted via the respiratory route as aerosols or dust generated from dry scabs that contain the virus, whereas ectromelia virus spreads mechanically among mice by skin abrasions (25). IMV is well suited for host-to-host spread because it is robust, stable at ambient temperature, and remains infectious even under desiccation. In contrast, the EEV/CEV membrane is fragile and easily disrupted by physical factors, but once broken, it will release an infectious IMV particle. In some orthopoxviruses, such as cowpox virus and ectromelia virus, a large proportion of IMV becomes occluded in A-type inclusion bodies (ATIs). The ATIs are proteinaceous structures that appear late in infection and are composed predominantly of a single polypeptide (48, 81) that enhances IMV stability after cell death and aids virus transmission between hosts. However, the majority of orthopoxviruses, including VV, do not make ATIs because the gene encoding the 160-kDa protein is disrupted by mutation.

CONCLUSIONS

This article illustrates how VV exploits host cell biology for entry into and egress from cells. Movement of virus cores and IMV and IEV particles involves microtubules and molecular motors. Thereafter, a virus-induced signaling cascade that leads to actin polymerization promotes cell-to-cell spread of CEV. Different forms of VV play different roles in the virus replication cycle. IMV are used for transmission between hosts, whereas CEV and EEV are important for virus spreading within a host.

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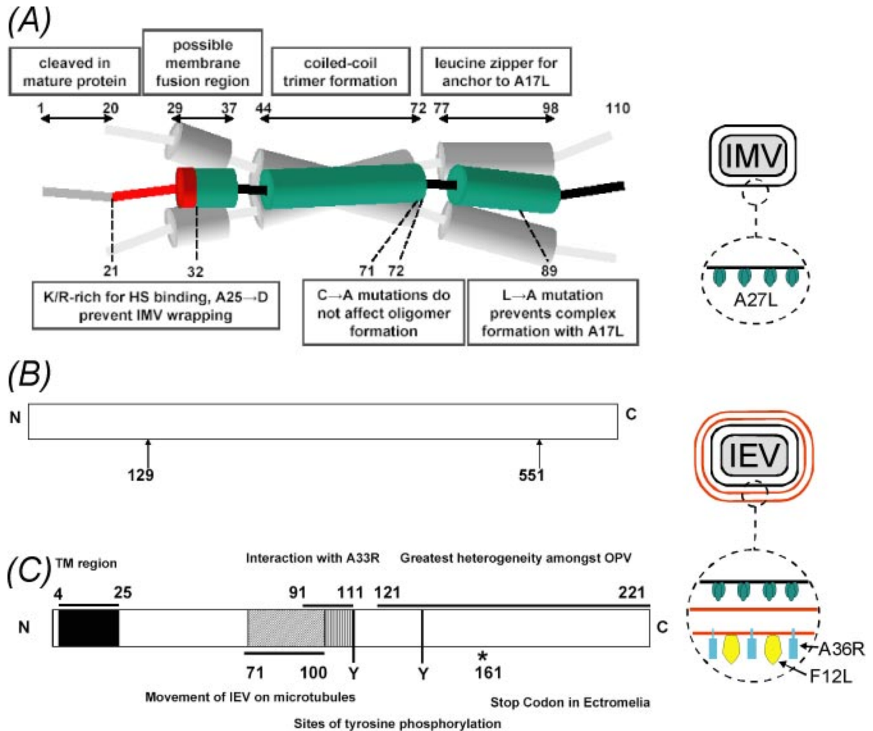

Figure 3 Schematic representations of VV proteins A27L, A36R, and F12L. (*A*) A27L is a 110-amino-acid protein that forms a coiled-coil trimer. The three helical regions are shown in green cylinders: the first helix forms a random coil and may contain the membrane fusion domain; the second region is a rigid helix for the formation of the coiled-coil trimer; and the last helix contains a leucine-zipper motif for interacting with A17L. Other features are marked. (*B*) F12L is a 635-amino-acid protein. Two putative myristoylation sites are indicated. (*C*) A36R is a 221-amino-acid type 1b transmembrane protein with the majority of the protein in the cytosol. Regions of interest are indicated and include a transmembrane region (TM), a stop codon (*) found in ectromelia virus (87), and sites of tyrosine phosphorylation (residues 112 and 132). The location of these proteins on virions is illustrated on the right.

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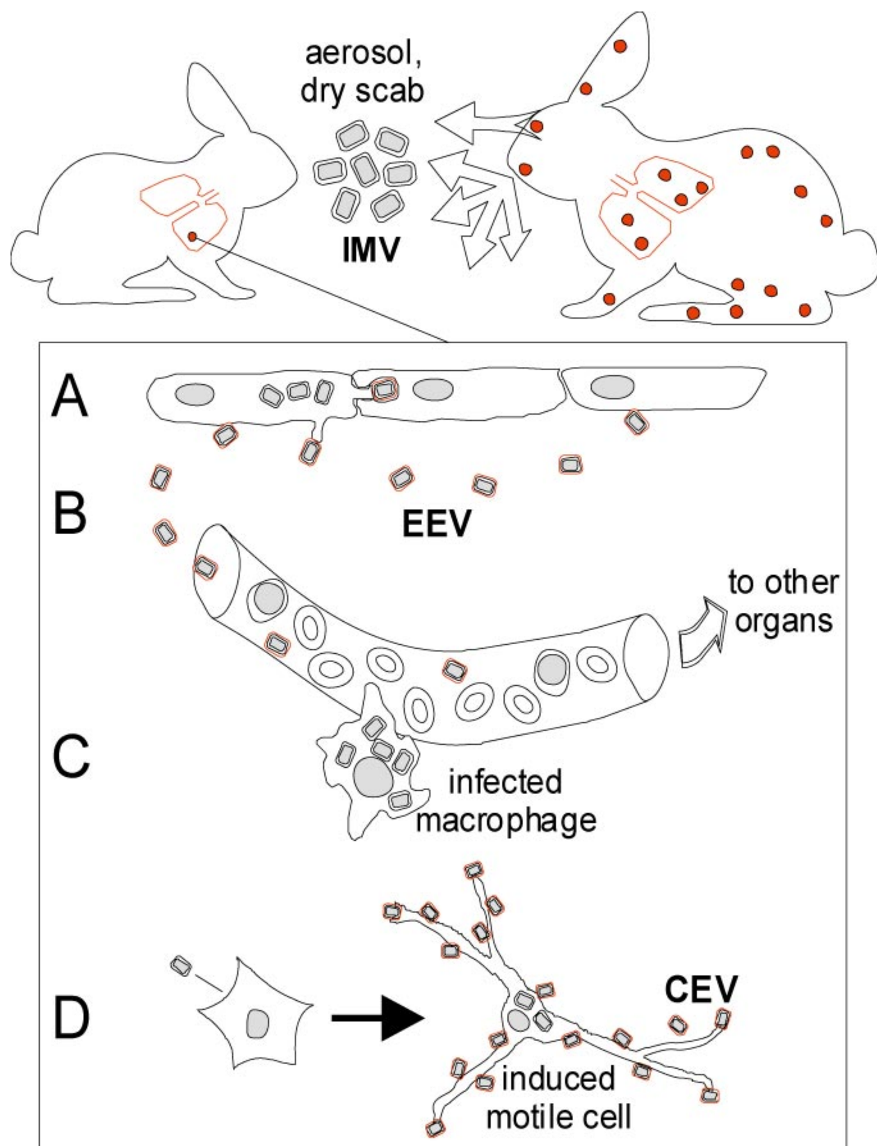

Figure 5 VV dissemination. VV may disseminate within the host by (*A*) direct cell-to-cell spread using actin tails, (*B*) as free virus, (*C*) infected leukocytes, and/or (*D*) virus-induced cell motility. A naïve host is infected via the respiratory route with aerosols or dust generated from dry scabs that contain virus produced from an infected animal.

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