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Poxvirus entry and membrane fusion

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Abstract

The study of poxvirus entry and membrane fusion has been invigorated by new biochemical and microscopic findings that lead to the following conclusions: (1) the surface of the mature virion (MV), whether isolated from an infected cell or by disruption of the membrane wrapper of an extracellular virion, is comprised of a single lipid membrane embedded with non-glycosylated viral proteins; (2) the MV membrane fuses with the cell membrane, allowing the core to enter the cytoplasm and initiate gene expression; (3) fusion is mediated by a newly recognized group of viral protein components of the MV membrane, which are conserved in all members of the poxvirus family; (4) the latter MV entry/fusion proteins are required for cell to cell spread necessitating the disruption of the membrane wrapper of extracellular virions prior to fusion; and furthermore (5) the same group of MV entry/fusion proteins are required for virus-induced cell–cell fusion. Future research priorities include delineation of the roles of individual entry/fusion proteins and identification of cell receptors.
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Contents

Introduction

Poxviruses constitute a large family of enveloped DNA viruses that replicate entirely in the cytoplasm of infected vertebrate or invertebrate cells (Moss, 2001). In a recent tabulation of viral membrane fusion proteins (Earp et al., 2005), poxviruses and the distantly related iridoviruses had the dubious distinction of being the only virus families with the entry "Not Determined." Poxviruses also did not make a list of viruses with known cell receptors (Sieczkarski and Whittaker, 2005). Happily, progress in understanding poxvirus entry has accelerated, and recent biochemical and microscopic discoveries are bringing order and direction to the field. Readers of this review may wish to consult an article on the formation and function of extracellular virions (Smith et al., 2002).

Mature virions

Investigations of the mechanism(s) used by vaccinia virus (VACV), the prototype poxvirus, to enter cells have been complicated by the existence of multiple infectious forms that

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differ from one another by their outer membrane (Fig. 1). Nevertheless, each form contains the same DNA–protein core, with numerous enzymes and factors for early gene expression, that is surrounded by a lipid membrane with more than a dozen viral proteins, none of which are glycosylated (Moss, 2001). The "simplest" and most abundant form has no additional membranes and is referred to here as a mature virion (MV), although it has previously been called the intracellular mature virion. Until recently, it was accepted that the MV membrane is comprised of a single lipid bilayer. This view was based on electron micrographs published by Dales and Mosbach (1968) and other investigators over many years. Griffiths et al. (2001a, b), however, proposed an alternative model in which the surface of the MV consists of two closely apposed membrane bilayers. The latter model originated with the plausible idea that the immature viral membrane forms from a flattened cisterna derived from a cellular organelle (Sodeik et al., 1993). Nevertheless, only a single membrane bilayer has been resolved even with modern transmission electron microscopic methods (Hollinshead et al., 1999) and the latest cryo-electron tomography techniques (Cyrklaff et al., 2005). Moreover, freeze fracture studies provide no evidence for more than a single bilayer (Heuser, 2005). Therefore, although the two-membrane model is appealing with regard to the genesis of the viral membrane, current data support the existence of a single membrane at the surface of the MV. Moreover, the presence of a single membrane is consistent with a fusion mode of entry for which there is considerable evidence.

MVs purified from infected cells by sucrose gradient sedimentation are most often used for entry experiments. However, it should be recognized that some MVs may be damaged or not completely mature, perhaps accounting in part for the relatively high (~50) ratio of particles to plaque forming units. Ichihashi and colleagues (Ichihashi and Oie, 1980, 1982; Oie et al., 1990, Ichihashi, 1996) reported that the majority of MVs isolated at late times after infection are in a "protected" state and must be activated by proteolysis in order to penetrate cells efficiently.

Extracellular virions

The MV may undergo additional membrane wrapping by a trans-Golgi (Hiller and Weber, 1985; Schmelz et al., 1994) or endosomal (Tooze et al., 1993) cisterna that has been modified by the insertion of at least seven viral proteins (Smith et al., 2002). The usual name of this form, which has two membranes surrounding the MV, is "intracellular enveloped virion." However, the descriptive name wrapped virion (WV) will be used here to eliminate possible confusion as the MV already has a membrane envelope (Fig. 1). The WV moves along microtubules to the periphery of the cell (Moss and Ward, 2001; Smith et al., 2002). Upon reaching the plasma membrane, the outer WV membrane fuses with the plasma membrane resulting in exocytosis. Extracellular virions (EVs) may be divided into cell-associated (Fig. 1) and unattached forms of which the former is usually predominant (see next paragraph) and chiefly responsible for cell-to-cell spread with the aid of actin tails (Blasco and Moss, 1992; Roper et al., 1998; Sanderson et al., 1998; Wolffe et al., 1997; Wolffe et al., 1998). The EV can be thought of as consisting of an MV surrounded by one additional membrane wrapper, though there are differences between an MV released by lysis of cells and disruption of the EV wrapper (Ichihashi and Oie, 1982; Ulaeto et al., 1996) which may in part account for the enhanced ability of EV to penetrate and fuse with cells (Doms et al., 1990; Ichihashi, 1996; Payne and Norrby, 1978). The EV wrapper contains at least four glycoproteins and one non-glycosylated protein, none of which are present in the enclosed MV membrane. The fragility of the EV wrapper can cause problems in the study of EV entry as particles with broken membranes are present even in fresh medium and their numbers increase after freeze and thaw or purification (Ichihashi, 1996).

In the case of the commonly used Western Reserve (WR) strain of VACV, the predominant form of EV remains cell-associated even at late times after infection (Blasco and Moss, 1992). The unattached form of EV (i.e. the form released into the culture medium) constitutes only about 0.5% of the total infectious WR strain of VACV (Payne, 1979). In contrast, the

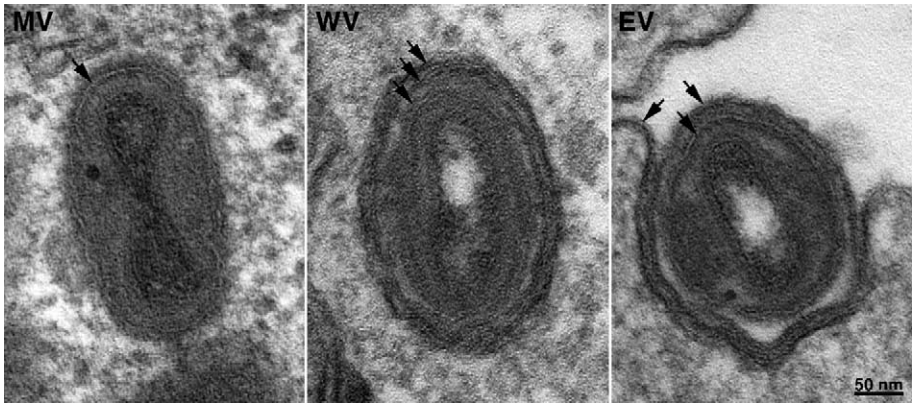

Fig. 1. Transmission electron micrographs of the MV (left panel), WV (middle panel), and EV (right panel) forms of VACV. Left panel: arrow points to the single membrane of MV. Middle panel: one arrow points to the MV membrane and two to the outer wrapping membranes. Right panel: one arrow points to the outer wrapping membrane that has fused with the plasma membrane and the others to the MV membrane and remaining EV wrapper. Electron microscopic images were kindly provided by Andrea S. Weisberg.

unattached EV constitutes from 8 to 27% of the total IHD-J strain, depending on the cell type (Payne, 1979). Marker rescue studies showed that the dissimilarity in release of EV by IHD-J and WR strains is largely due to a single amino acid difference in the A34 glycoprotein component of the EV wrapper (Blasco et al., 1993). Because the A34 proteins of other orthopoxviruses resemble WR rather than IHD-J, it seems likely that the mutation arose during tissue culture passage of the latter virus. Nevertheless, nearly all investigations on EV entry have been carried out with EVs isolated from the medium of cells infected with the IHD-J strain of VACV. Mutations of other EV membrane proteins can also enhance release of EV into the medium (Katz et al., 2003).

Entry of the MV

Enveloped viruses typically enter cells by fusion of their surface membrane with either the plasma membrane or the membrane of an endocytic vesicle (Earp et al., 2005; Sieczkarski and Whittaker, 2005). Following VACV infection, MVs have been seen in vesicles (Dales, 1963) and fusing with the plasma membrane (Armstrong et al., 1973; Carter et al., 2005; Chang and Metz, 1976). The inhibition of MV entry by fluoride and cytochalasin B supports an internalization mechanism (Dales and Kajioka, 1964; Payne and Norrby, 1978) perhaps involving macropinocytosis (Sieczkarski and Whittaker, 2002). However, endosomal entry usually involves a low pH step that is inhibited by lysosomotropic drugs, but such inhibition was not observed for infection by MVs (Chillakuru et al., 1991; Janeczko et al., 1987; Vanderplasschen et al., 1998). Furthermore, low pH did not enhance the fusion of MVs with HeLa cell membranes as determined by lipid mixing (Doms et al., 1990). Studies of the effect of low pH on acceleration of MV entry, however, produced mixed results (Ichihashi, 1996; Vanderplasschen et al., 1998). It is possible that both plasma membrane and endosomal entry paths are used depending on the virus strain and cell type. Precedence for a dual entry pathway exists with herpesviruses (Spear and Longnecker, 2003).

Fusion mechanisms of MV entry are inconsistent with the MV double membrane hypothesis because the remaining lipid bilayer would still surround the core. Images of MVs with disrupted membranes near the cell surface have led to the suggestion that fusion is not needed for free cores to enter the cytoplasm though putative pores in the plasma membrane (Locker et al., 2000; Sodeik and Krijnse-Locker, 2002). In view of both indirect and direct data supporting a fusion model, additional evidence would be needed to seriously consider such an alternative entry mechanism.

MV attachment and entry proteins

Entry of enveloped viruses typically involves at least three steps: virus attachment, fusion protein activation, and membrane fusion. There is evidence that certain MV membrane proteins bind to cell surface glycosaminoglycans and thereby facilitate virion attachment. D8 has been reported to bind to chondroitin sulfate (Hsiao et al., 1999) and the A27 and H3 proteins to heparan sulfate (Chung et al., 1998; Hsiao et al., 1998; Lin et al., 2000; Vazquez and Esteban, 1999). But, at least individually, these proteins are not essential, and the inhibitory effect of heparin on virus entry appears to be incomplete and cell-type-dependent (Carter et al., 2005).

Esteban and co-workers called the A27 (P14) protein the fusion protein because of the ability of specific antibodies and soluble A27 to block MV entry and cell–cell fusion (Gong et al., 1990; Hsiao et al., 1998; Rodriguez et al., 1985, 1987; Vazquez and Esteban, 1999). The A27 protein forms a triple coiled-coil structure and interacts with the A17 virus membrane protein through a C-terminal alpha helix. The crucial test regarding the role of A27, however, requires analysis of a null mutant. When this experiment was done, the A27 deletion mutant was found to enter cells and induce syncytia (Senkevich and Moss, 2005), demonstrating that it is not required for fusion. In fact, the major defect of A27 null mutants is a failure of MV wrapping and consequent effects on virus egress (Rodriguez and Smith, 1990; Ward, 2005). Therefore, the virus neutralizing activity of antibodies to A27 might be due to steric effects produced by their binding to MVs. A role for the L1 membrane protein in penetration was suggested by the ability of a monoclonal antibody to inhibit MV entry after cell binding (Ichihashi et al., 1994; Wolffe et al., 1995). However, because conditional lethal L1 null mutants exhibit an assembly block (Wolffe et al., 1995), it has not been possible to produce MVs lacking the L1 protein to determine if it has a direct role in entry as well as assembly.

Recently, a group of previously uncharacterized VACV proteins (A21, A28, H2, and L5) was shown to have roles in MV entry (Senkevich et al., 2004a, 2004b; Townsley et al., 2005a, 2005b). Common features of these proteins, which are conserved in all sequenced poxviruses, include an N-terminal or near N-terminal hydrophobic domain and invariant cysteines. The proteins are expressed late in infection, contain intramolecular disulfide bonds that are formed by the poxvirus-encoded cytoplasmic redox system (Senkevich et al., 2002), and are surface components of the MV membrane. Studies with inducible mutants indicated that each of the above proteins is essential and that their null phenotypes are indistinguishable. In the absence of any one of the four proteins, normal-looking but non-infectious MVs are produced. In each case, addition of the mutant MV to cells results in their attachment, but the cores fail to penetrate into the cytoplasm, indicating a membrane-fusion block. The requirement for each protein for VACV entry as well as their conservation in all poxviruses indicates that they have non-redundant essential functions. The proteins are associated with each other and additional MV membrane proteins (Senkevich and Moss, 2005; Senkevich, unpublished data), suggesting that they function as an entry/fusion complex.

Entry of EV

The key question regarding EV entry concerns the fate of the wrapper. Fusion of the EV wrapper with the cell membrane seems most unlikely on general principles because it would

result in the entry of an MV with an intact membrane. Moreover, the finding that the MV entry proteins are required for spread of VACV infection by EV necessitates the removal of the wrapper before fusion can occur (Senkevich et al., 2004a, 2004b; Townsley et al., 2005a, 2005b). The EV membrane is fragile, and progeny particles with disrupted wrappers are commonly seen on the surface of infected cells (M. Husain and B. Moss, unpublished). Furthermore, electron microscopic images of MVs fusing with the plasma membrane beneath a disrupted EV wrapper have been obtained (M. Hollinshead and G.L. Smith, personal communication; J. Heuser, personal communication). Disruption of the EV wrapper within endosomes has also been suggested (Ichihashi, 1996; Vanderplasschen et al., 1998). It should be recognized that investigations of EV entry have used particles isolated from the medium of cells infected with the IHD-J strain of VACV and that there are no studies of how cell-associated EV enter adjacent cells.

Cell surface receptors

Virus entry is generally facilitated by interaction of viral proteins with specific cell surface proteins or polysaccharides, which can determine tropism (Sieczkarski and Whittaker, 2005). Receptors can enhance attachment, induce signaling, and activate metastable viral fusion proteins. Although no specific receptors have been identified for any poxvirus, the following information has been obtained. Depletion of plasma membrane cholesterol inhibits entry of MVs, suggesting a role of lipid rafts (Chung et al., 2005). VACV MVs and EVs attach to cells differently (Vanderplasschen and Smith, 1997). In addition, MVs but not EVs trigger cell signaling events, and entry of the former requires actin dynamics and tyrosine and protein kinase C phosphorylation (Locker et al., 2000). A monoclonal antibody that prevents attachment of VACV MVs was isolated (Chang et al., 1995), but the target has not yet been shown to be a specific receptor. The receptors for many other viruses have been identified by complementation of non-permissive cells. However, VACV can infect a wide variety of cultured cells, and replication blocks usually occur at a post-entry stage. An exception may be resting primary human T lymphocytes, where there appears to be a block in virus binding that is reversed by activation (Chahroudi et al., 2005).

VACV-induced syncytia

Cell–cell fusion is unnoticeable during a normal VACV infection of cultured cells but occurs spontaneously with certain mutants and can be triggered by briefly lowering the pH of the medium below 6. Though the phenomenon is artificial, it can provide important insights for virus entry. Syncytia form at neutral pH when infected with mutants containing a disruption of either the A56R (Ichihashi and Dales, 1971) or K2L (Law and Smith, 1992; Turner and Moyer, 1992; Zhou et al., 1992) open reading frame encoding the hemagglutinin (HA) and serine protease inhibitor 3 (SPI-3), respectively. The HA is a transmembrane protein that is present on the plasma membrane of infected cells and the EV wrapper but was not detected in the MV membrane (Payne, 1979). The SPI-3 gene co-localizes with HA, even though it does not have a transmembrane segment of its own (Brum et al., 2003). As SPI-3 has a signal sequence, it probably associates with HA as it transits the secretory pathway. Fusion occurs late after infection with HA or SPI-3 mutants and is mediated by progeny virions on the cell surface. The requirement for progeny virions is due to the mechanism of poxvirus morphogenesis. The MV membrane forms within the cytoplasm, and the constituent proteins do not traffic to the plasma membrane through the secretory pathway as with many other viruses. Antibodies to MV membrane proteins block fusion (Ichihashi et al., 1994; Turner and Moyer, 1992), suggesting that the EV wrapper is disrupted prior to syncytia formation. Moreover, HA-induced fusion is dependent on expression of the A28 MV entry protein (T. Wagenaar, personal communication) and presumably the other members of the entry protein complex. Thus, HA and SPI-3 negatively regulate the normal entry process, perhaps to prevent re-infection of cells with progeny virions.

Taken together, the above data suggest that cell–cell fusion occurs as outlined in Fig. 2: (1) progeny EV collect on the plasma membrane; (2) the EV wrapper is disrupted to expose the MV membrane; (3) the MV membrane fuses with the

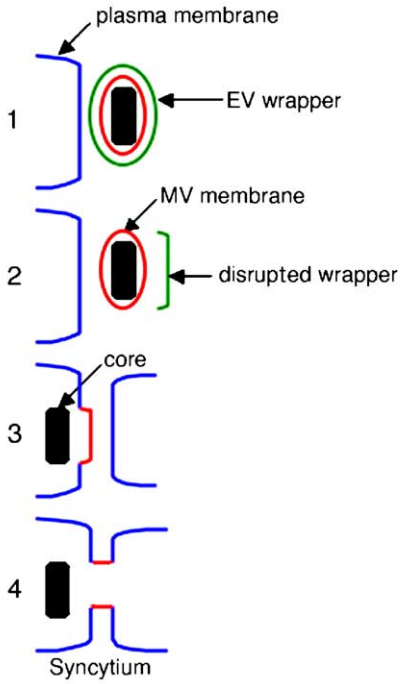

Fig. 2. Steps in formation of syncytia. (1) Progeny EV are displayed on the surface of the plasma membrane. (2) The EV membrane is disrupted exposing the MV membrane. (3) The MV membrane fuses with the plasma membrane. (4) The plasma membrane, now containing embedded viral entry/fusion proteins, fuses with the plasma membrane of an adjacent cell. In the case of HA or SPI-3 mutants, syncytia form spontaneously. In fusion from within, low pH triggers disruption of the CEV membrane and synchronizes fusion of MV with the plasma membrane. In fusion from without, purified MVs induce low pH-triggered fusion so that the process starts after step 2. For visual simplicity, cell–cell fusion is depicted as being induced by a single virion, but actually many virions are required to form syncytia.

plasma membrane resulting in the insertion of the viral entry/fusion complex; (4) the plasma membrane containing the viral entry/fusion complex fuses with the plasma membrane of an adjacent cell to form a syncytium. Although, for visual simplicity, a single virion is shown to induce cell–cell fusion in Fig. 2, it is likely that this only occurs after many MV fuse with the cell membrane and large amounts of viral proteins are inserted into the membrane. Parenthetically, it would be difficult to provide a mechanism for cell–cell fusion if the MV membrane was discarded outside of the cell during entry as has been proposed (Locker et al., 2000; Sodeik and Krijnse-Locker, 2002). Moreover, if poxviruses do not employ fusion for entry, the fusion apparatus would have evolved solely to form syncytia—a most unlikely situation.

The mechanism of HA/SPI-3 inhibition of fusion remains to be elucidated, but it does not depend on serine protease inhibitor activity of SPI-3 (Turner and Moyer, 1995). Theoretically, the inhibition could occur at several different stages including wrapper disruption or MV fusion. Ichihashi and colleagues (Ichihashi and Oie, 1980; Ichihashi and Oie, 1982; Oie et al., 1990) suggested that HA inhibits MV infectivity and that protease treatment activates MVs by digesting traces of bound HA. One interesting hypothesis is that HA or SPI-3 inhibits fusion by interacting with MV entry/fusion proteins.

Brief low pH treatment of cells at late times after infection with wild type VACV (expressing both HA and SPI-3) also results in the formation of syncytia (Doms et al., 1990; Gong et al., 1990). Although referred to as fusion from within, syncytia formation is dependent on the presence of progeny virions on the cell surface (Blasco and Moss, 1991) just as with HA and SPI-3 mutants. Fusion can also be induced by adding large numbers of MV to cells and briefly lowering the pH, a phenomenon called fusion from without (Gong et al., 1990). Both types of low pH-triggered cell–cell fusion are dependent on the entry/fusion proteins A21, A28, H2, and L5 (Senkevich et al., 2004b; Townsley et al., 2005a, 2005b). Therefore, low pH triggered syncytia formation may follow the same scheme outlined in Fig. 2, except for low pH treatment and omission of the EV wrapper disruption step for fusion from without. Low pH has at least two effects: it disrupts the EV wrapper (Vanderplasschen et al., 1998) and synchronizes the fusion of MVs with the plasma membrane (J. Heuser, personal communication), consistent with accelerated MV entry (Ichihashi, 1996). Although MV entry occurs at neutral pH, presumably, the amount of viral protein present on the plasma membrane at any one time is insufficient for cell–cell fusion to occur. Although the triggering of fusion at the plasma membrane by lowering the pH of the medium is clearly artificial, it suggests that endosomes are a natural route of VACV infection at least in some cells since the pH occurring in these organelles is similar to that needed to trigger syncytia formation.

Summary and future directions

Taken together, most of the published data are consistent with the following: (1) the surface of the MV is comprised of a single lipid membrane embedded with non-glycosylated viral proteins; (2) the MV membrane fuses with the cell membrane during virus entry, allowing the core to enter the cytoplasm and initiate gene expression; (3) fusion is mediated by a newly recognized group of viral protein components of the MV membrane, which are conserved in all members of the poxvirus family; (4) the latter MV entry/fusion proteins are required for cell to cell spread, necessitating the disruption of the membrane wrapper of extracellular virions prior to fusion; and (5) the same group of MV entry/fusion proteins is required for virus-induced cell–cell fusion, which in important ways mimics virus entry. Additional work is needed to identify other protein components of the viral entry/fusion complex and especially to delineate their roles. Given the large numbers of proteins involved, it seems likely that poxvirus entry/fusion will involve unique features that distinguish it from the well-characterized types 1 and 2 fusion mechanisms (Earp et al., 2005). The discovery of bona fide cell receptors will also be of crucial importance to the field.

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References

Armstrong, J.A., Metz, D.H., Young, M.R., 1973. The mode of entry of vaccinia virus into L cells. J. Gen. Virol. 21, 533–537.

Blasco, R., Moss, B., 1991. Extracellular vaccinia virus formation and cell-to-cell virus transmission are prevented by deletion of the gene encoding the 37,000 Dalton outer envelope protein. J. Virol. 65, 5910–5920.

Blasco, R., Moss, B., 1992. Role of cell-associated enveloped vaccinia virus in cell-to-cell spread. J. Virol. 66, 4170–4179.

Blasco, R., Sisler, J.R., Moss, B., 1993. Dissociation of progeny vaccinia virus from the cell membrane is regulated by a viral envelope glycoprotein: effect of a point mutation in the lectin homology domain of the A34R gene. J. Virol. 67, 3319–3325.

Brum, L.M., Turner, P.C., Devick, H., Baquero, M.T., Moyer, R.W., 2003. Plasma membrane localization and fusion inhibitory activity of the cowpox virus serpin SPI-3 require a functional signal sequence and the virus encoded hemagglutinin. Virology 306, 289–302.

Carter, G.C., Law, M., Hollinshead, M., Smith, G.L., 2005. Entry of the vaccinia virus intracellular mature virion and its interactions with glycosaminoglycans. J. Gen. Virol. 86, 1279–1290.

Chahroudi, A., Chavan, R., Koyzr, N., Waller, E.K., Silvestri, G., Feinberg, M.B., 2005. Vaccinia virus tropism for primary hematolymphoid cells is determined by restricted expression of a unique virus receptor. J. Virol. 79, 10397–10407.

Chang, A., Metz, D.H., 1976. Further investigations on the mode of entry of vaccinia virus into cells. J. Gen. Virol. 32, 275–282.

Chang, W., Hsiao, J.-C., Chung, C.-S., Bair, C.-H., 1995. Isolation of a monoclonal antibody which blocks vaccinia virus infection. J. Virol. 69, 517–522.

Chillakuru, R.A., Ryu, D.D., Yilma, T., 1991. Propagation of recombinant vaccinia virus in HeLa cells: adsorption kinetics and replication in batch cultures. Biotechnol. Prog. 7, 85–92.

Chung, C.-S., Hsiao, J.-C., Chang, Y.-S., Chang, W., 1998. A27L protein

mediates vaccinia virus interaction with cell surface heparin sulfate. J. Virol. 72, 1577–1585.
Chung, C.S., Huang, C.Y., Chang, W., 2005. Vaccinia virus penetration requires cholesterol and results in specific viral envelope proteins associated with lipid rafts. J. Virol. 79, 1623–1634.
Cyrklaff, M., Risco, C., Fernandez, J.J., Jimenez, M.V., Esteban, M., Baumeister, W., Carrascosa, J.L., 2005. Cryo-electron tomography of vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. 102, 2772–2777.
Dales, S., 1963. The uptake and development of vaccinia virus in strain L cells followed with labeled viral deoxyribonucleic acid. J. Cell Biol. 18, 51–72.
Dales, S., Kajioka, R., 1964. The cycle of multiplication of vaccinia virus in Earle's strain L cells. I. Uptake and penetration. Virology 24, 278–294.
Dales, S., Mosbach, E.H., 1968. Vaccinia as a model for membrane biogenesis. Virology 35, 564–583.
Doms, R.W., Blumenthal, R., Moss, B., 1990. Fusion of intra- and extracellular forms of vaccinia virus with the cell membrane. J. Virol. 64, 4884–4892.
Earp, L.J., Delos, S.E., Park, H.E., White, J.M., 2005. The many mechanisms of viral membrane fusion proteins. Curr. Top. Microbiol. Immunol. 285, 25–66.
Gong, S.C., Lai, C.F., Esteban, M., 1990. Vaccinia virus induces cell fusion at acid pH and this activity is mediated by the N-terminus of the 14-kDa virus envelope protein. Virology 178, 81–91.
Griffiths, G., Roos, N., Schleich, S., Locker, J.K., 2001a. Structure and assembly of intracellular mature vaccinia virus: thin-section analyses. J. Virol. 75, 11056–11070.
Griffiths, G., Wepf, R., Wendt, T., Locker, J.K., Cyrklaff, M., Roos, N., 2001b. Structure and assembly of intracellular mature vaccinia virus: isolated-particle analysis. J. Virol. 75, 11034–11055.
Heuser, J., 2005. Deep-etch EM reveals that the early poxvirus envelope is a single membrane bilayer stabilized by a geodetic "honeycomb" surface coat. J. Cell Biol. 169, 269–283.
Hiller, G., Weber, K., 1985. Golgi-derived membranes that contain an acylated viral polypeptide are used for vaccinia virus envelopment. J. Virol. 55, 651–659.
Hollinshead, M., Vanderplasschen, A., Smith, G.L., Vaux, D.J., 1999. Vaccinia virus intracellular mature virions contain only one lipid membrane. J. Virol. 73, 1503–1517.
Hsiao, J.C., Chung, C.S., Chang, W., 1998. Cell surface proteoglycans are necessary for A27L protein-mediated cell fusion: identification of the N-terminal region of A27L protein as the glycosaminoglycan-binding domain. J. Virol. 72, 8374–8379.
Hsiao, J.C., Chung, C.S., Chang, W., 1999. Vaccinia virus envelope D8L protein binds to cell surface chondroitin sulfate and mediates the adsorption of intracellular mature virions to cells. J. Virol. 73, 8750–8761.
Ichihashi, Y., 1996. Extracellular enveloped vaccinia virus escapes neutralization. Virology 217, 478–485.
Ichihashi, Y., Dales, S., 1971. Biogenesis of poxviruses: interrelationship between hemagglutinin production and polykaryocytosis. Virology 46, 533–543.
Ichihashi, Y., Oie, M., 1980. Adsorption and penetration of the trypsinized vaccinia virion. Virology 101, 50–60.
Ichihashi, Y., Oie, M., 1982. Proteolytic activation of vaccinia virus for penetration phase of infection. Virology 116, 297–305.
Ichihashi, Y., Takahashi, T., Oie, M., 1994. Identification of a vaccinia virus penetration protein. Virology 202, 834–843.
Janeczko, R.A., Rodriguez, J.F., Esteban, M., 1987. Studies on the mechanism of entry of vaccinia virus into animal cells. Arch. Virol. 92, 135–150.
Katz, E., Ward, B.M., Weisberg, A.S., Moss, B., 2003. Mutations in the vaccinia virus A33R and B5R envelope proteins that enhance release of extracellular virions and eliminate formation of actin-containing microvilli without preventing tyrosine phosphorylation of the A36R protein. J. Virol. 77, 12266–12275.
Law, K.M., Smith, G.L., 1992. A vaccinia serine protease inhibitor which prevents virus-induced cell fusion. J. Gen. Virol. 73, 549–557.
Lin, C.L., Chung, C.S., Heine, H.G., Chang, W., 2000. Vaccinia virus envelope H3L protein binds to cell surface heparan sulfate and is important for intracellular mature virion morphogenesis and virus infection in vitro and in vivo. J. Virol. 74, 3353–3365.
Locker, J.K., Kuehn, A., Schleich, S., Rutter, G., Hohenberg, H., Wepf, R., Griffiths, G., 2000. Entry of the two infectious forms of vaccinia virus at the plasma membrane is signaling-dependent for the IMV but not the EEV. Mol. Biol. Cell 11, 2497–2511.
Moss, B., 2001. Poxviridae: the viruses and their replication. In: Knie, D.M., Howley, P.M. (Eds.), Fields Virology, 4th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 2849–2883.
Moss, B., Ward, B.M., 2001. High-speed mass transit for poxviruses on microtubules. Nat. Cell Biol. 3, E245–E246.
Oie, M., Shida, H., Ichihashi, Y., 1990. The function of the vaccinia hemagglutinin in the proteolytic activation of infectivity. Virology 176, 494–504.
Payne, L.G., 1979. Identification of the vaccinia hemagglutinin polypeptide from a cell system yielding large amounts of extracellular enveloped virus. J. Virol. 31, 147–155.
Payne, L.G., Norrby, E., 1978. Adsorption and penetration of enveloped and naked vaccinia virus particles. J. Virol. 27, 19–27.
Rodriguez, J.F., Smith, G.L., 1990. IPTG-dependent vaccinia virus: identification of a virus protein enabling virion envelopment by Golgi membrane and egress. Nucleic Acids Res. 18, 5347–5351.
Rodriguez, J.F., Janeczko, R., Esteban, M., 1985. Isolation and characterization of neutralizing monoclonal antibodies to vaccinia virus. J. Virol. 56, 482–488.
Rodriguez, J.F., Paez, E., Esteban, M., 1987. A 14,000-Mr envelope protein of vaccinia virus is involved in cell fusion and forms covalently linked trimers. J. Virol. 61, 395–404.
Roper, R., Wolffe, E.J., Weisberg, A., Moss, B., 1998. The envelope protein encoded by the A33R gene is required for formation of actin-containing microvilli and efficient cell-to-cell spread of vaccinia virus. J. Virol. 72, 4192–4204.
Sanderson, C.M., Frischknecht, F., Way, M., Hollinshead, M., Smith, G.L., 1998. Roles of vaccinia virus EEV-specific proteins in intracellular actin tail formation and low pH-induced cell–cell fusion. J. Gen. Virol. 79, 1415–1425.
Schmelz, M., Sodeik, B., Ericsson, M., Wolffe, E.J., Shida, H., Hiller, G., Griffiths, G., 1994. Assembly of vaccinia virus: the second wrapping cisterna is derived from the trans Golgi network. J. Virol. 68, 130–147.
Senkevich, T.G., Moss, B., 2005. Vaccinia virus H2 protein is an essential component of a complex involved in virus entry and cell–cell fusion. J. Virol. 79, 4744–4754.
Senkevich, T.G., White, C.L., Koonin, E.V., Moss, B., 2002. Complete pathway for protein disulfide bond formation encoded by poxviruses. Proc. Natl. Acad. Sci. U.S.A. 99, 6667–6672.
Senkevich, T.G., Ward, B.M., Moss, B., 2004a. Vaccinia virus A28L gene encodes an essential protein component of the virion membrane with intramolecular disulfide bonds formed by the viral cytoplasmic redox pathway. J. Virol. 78, 2348–2356.
Senkevich, T.G., Ward, B.M., Moss, B., 2004b. Vaccinia virus entry into cells is dependent on a virion surface protein encoded by the A28L gene. J. Virol. 78, 2357–2366.
Sieczkarski, S.B., Whittaker, G.R., 2002. Dissecting virus entry via endocytosis. J. Gen. Virol. 83, 1535–1545.
Sieczkarski, S.B., Whittaker, G.R., 2005. Viral entry. Curr. Top. Microbiol. Immunol. 285, 1–23.
Smith, G.L., Vanderplasschen, A., Law, M., 2002. The formation and function of extracellular enveloped vaccinia virus. J. Gen. Virol. 83, 2915–2931.
Sodeik, B., Krijnse-Locker, J., 2002. Assembly of vaccinia virus revisited: de novo membrane synthesis or acquisition from the host? Trends Microbiol. 10, 15–24.
Sodeik, B., Doms, R.W., Ericsson, M., Hiller, G., Machamer, C.E., van't Hof, W., van Meer, G., Moss, B., Griffiths, G., 1993. Assembly of vaccinia virus: role of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks. J. Cell Biol. 121, 521–541.
Spear, P.G., Longnecker, R., 2003. Herpesvirus entry: an update. J. Virol. 77, 10179–10185.
Tooze, J., Hollinshead, M., Reis, B., Radsak, K., Kern, H., 1993. Progeny vaccinia and human cytomegalovirus particles utilize early endosomal cisternae for their envelopes. Eur. J. Cell Biol. 60, 163–178.

Townsley, A., Senkevich, T.G., Moss, B., 2005a. The product of the vaccinia virus L5R gene is a fourth membrane protein encoded by all poxviruses that is required for cell entry and cell–cell fusion. J. Virol. 79.

Townsley, A., Senkevich, T.G., Moss, B., 2005b. Vaccinia virus A21 virion membrane protein is required for cell entry and fusion. J. Virol. 79, 9458–9469.

Turner, P.C., Moyer, R.W., 1992. An orthopoxvirus serpin-like gene controls the ability of infected cells to fuse. J. Virol. 66, 2076–2085.

Turner, P.C., Moyer, R.W., 1995. Orthpoxvirus fusion inhibitor glycoprotein SPI-3 (open reading frame K2L) contains motifs characteristic of serine protease inhibitors that are not required for control of cell fusion. J. Virol. 69, 5978–5987.

Ulaeto, D., Grosenbach, D., Hruby, D.E., 1996. The vaccinia virus 4c and A-type inclusion proteins are specific markers for the intracellular mature virus particle. J. Virol. 70, 3372–3375.

Vanderplasschen, A., Smith, G.L., 1997. A novel virus binding assay using confocal microscopy: demonstration that intracellular and extracellular vaccinia virions bind to different cellular receptors. J. Virol. 71, 4032–4041.

Vanderplasschen, A., Hollinshead, M., Smith, G.L., 1998. Intracellular and extracellular vaccinia virions enter cells by different mechanisms. J. Gen. Virol. 79, 877–887.

Vazquez, M.I., Esteban, M., 1999. Identification of functional domains in the 14-kilodalton envelope protein (A27L) of vaccinia virus. J. Virol. 73, 9098–9109.

Ward, B.M., 2005. Visualization and characterization of the intracellular movement of vaccinia virus intracellular mature virions. J. Virol. 79, 4755–4763.

Wolffe, E.J., Vijaya, S., Moss, B., 1995. A myristylated membrane protein encoded by the vaccinia virus L1R open reading frame is the target of potent neutralizing monoclonal antibodies. Virology 211, 53–63.

Wolffe, E.J., Katz, E., Weisberg, A., Moss, B., 1997. The A34R glycoprotein gene is required for induction of specialized actin-containing microvilli and efficient cell-to-cell transmission of vaccinia virus. J. Virol. 71, 3904–3915.

Wolffe, E.J., Weisberg, A.S., Moss, B., 1998. Role for the vaccinia virus A36R outer envelope protein in the formation of virus-tipped actin-containing microvilli and cell-to-cell virus spread. Virology 244, 20–26.

Zhou, J., Sun, X.Y., Fernando, G.J.P., Frazer, I.H., 1992. The vaccinia virus K2L gene encodes a serine protease inhibitor which inhibits cell–cell fusion. Virology 189, 678–686.