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Membrane fusion during poxvirus entry

Bernard Moss

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

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ABSTRACT

Poxviruses comprise a large family of enveloped DNA viruses that infect vertebrates and invertebrates. Poxviruses, unlike most DNA viruses, replicate in the cytoplasm and encode enzymes and other proteins that enable entry, gene expression, genome replication, virion assembly and resistance to host defenses. Entry of vaccinia virus, the prototype member of the family, can occur at the plasma membrane or following endocytosis. Whereas many viruses encode one or two proteins for attachment and membrane fusion, vaccinia virus encodes four proteins for attachment and eleven more for membrane fusion and core entry. The entry-fusion proteins are conserved in all poxviruses and form a complex, known as the Entry Fusion Complex (EFC), which is embedded in the membrane of the mature virion. An additional membrane that encloses the mature virion and is discarded prior to entry is present on an extracellular form of the virus. The EFC is held together by multiple interactions that depend on nine of the eleven proteins. The entry process can be divided into attachment, hemifusion and core entry. All eleven EFC proteins are required for core entry and at least eight for hemifusion. To mediate fusion the virus particle is activated by low pH, which removes one or more fusion repressors that interact with EFC components. Additional EFC-interacting fusion repressors insert into cell membranes and prevent secondary infection. The absence of detailed structural information, except for two attachment proteins and one EFC protein, is delaying efforts to determine the fusion mechanism.

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E-mail address: bmoss@nih.gov

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1. Introduction

Many viruses contain an outer lipoprotein envelope that fuses with a cellular membrane allowing entry of the nucleoprotein core into the cytoplasm [1]. The viral fusion pathway usually involves membrane apposition, hemifusion and pore formation, which is similar to that occurring during fusion of cellular membranes although the molecular mechanisms vary [2]. The study of viral fusion, in addition to being vital for elucidating the biology of viruses, may provide important clues that can increase understanding of the fusion of cellular vesicles and organelles. One or two proteins are required for entry of most viruses although three or four are needed for herpesviruses [1]. Viral fusion mechanisms have been divided into three types. Class I fusion proteins, as exemplified by the influenza virus hemagglutinin, form trimers of hairpins that contain a central α-helical coiled-coil domain and a N-terminal hydrophobic fusion peptide that inserts into the cell membrane. Class II fusion proteins, encoded by flaviviruses, have β-structures with an internally located hydrophobic fusion loop. Class III fusion proteins, including both the vesicular stomatitis G protein and the herpes simplex gB protein, have an internal bipartite fusion loop and some characteristics of both class I and class II fusion proteins. For all three classes, a conformational change in the fusion protein brings the viral and cellular membranes together [1].

This review provides current information related to the fusion of poxviruses with cell membranes during entry, which is distinct from that of other viruses and requires at least 11 viral proteins that form an entry fusion complex (EFC) in addition to four attachment proteins [3]. Other aspects of poxvirus cell entry will be covered only briefly as they have been subjects of other recent reviews [3–6].

2. Poxvirus biology

2.1. Poxvirus family

Poxviruses comprise a large family of enveloped double-stranded DNA viruses that replicate entirely within the cytoplasm of vertebrate and invertebrate cells [7]. Variola virus, the most notorious member of the family, was responsible for smallpox until eliminated from nature by vaccination with the closely related vaccinia virus (VACV). Members of the chordopoxvirus subfamily have genomes of 150,000 to 300,000 base pairs encoding approximately 200 proteins. About half of the proteins are highly conserved and dedicated to essential functions including cell entry, genome replication, transcription and virion assembly [8]; the remainder are more variable and many engage host defense mechanisms [9]. This review focuses on VACV, the prototype poxvirus.

2.2. Virion structure

The VACV mature virion (MV) has a barrel shape with dimensions of $\sim 360 \times 270 \times 250$ nm and consists of an internal nucleoprotein core flanked by protein structures called lateral bodies and an outer membrane envelope composed of a single lipoprotein bilayer [10–12]. Purified MVs contain at least 80 viral proteins of which 20 or more are associated with the membrane [13–15]. The MV membrane appears to be derived by modification of the endoplasmic reticulum with viral proteins [6]. During late steps in morphogenesis some MVs becomes surrounded by an additional double-membrane derived from trans-Golgi or endosomal membranes [16–18] and are propelled through the cytoplasm on microtubules [19,20]. Upon reaching the periphery, the outermost membrane fuses with the plasma membrane resulting in exocytosis. The MV has a single lipoprotein membrane whereas the extracellular virion (EV) has one additional membrane containing at least six viral proteins distinct from those of the MV. MVs, which are released by cell lysis, are stable and thought to be important for animal-to-animal spread of infection, whereas EVs provide efficient cell-to-cell spread and resistance to antibody neutralization.

Lipids, mostly phospholipids and cholesterol, constitute approximately 5% of the MV mass [21]. Phosphatidylcholine and lesser amounts of phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and semilysobisphosphatidic acid (also known as acylbis(monoacylglycero)phosphate or acylphosphatidylglycerol) are present [22–25]. Extraction of virion-associated lipids with a non-ionic detergent renders MVs non-infectious, but incubation with either uninfected cell membrane preparations or phosphatidylserine-containing liposomes partially restores infectivity [26,27]. More recently, these findings were extended and an apoptotic mimicry model for poxvirus entry, in which virion-associated phosphatidylserine serves to flag virions as apoptotic debris for cell uptake by macropinocytosis, was proposed [28]. However, a specific phosphatidylserine receptor for VACV has not been identified and reconstitution of detergent-extracted MVs with some other anionic phospholipid species also facilitate virus entry [29]. Nonetheless, these studies indicated the importance of the MV membrane lipid composition, specifically the presence of anionic phospholipid species, in cell entry of VACV MVs.

3. Entry

3.1. Entry pathways

Entry of poxviruses is defined as the step in which the nucleoprotein core of the virion passes into the cytoplasm. MVs can enter cells either at the plasma membrane at neutral pH or through a low pH-dependent endocytic route [30,31] depending in part on the virus strain and cell type [32,33]. Modification of entry pathways could have occurred during extensive cell culture passaging of VACV strains. The endocytic route appears to be the primary one used by orthopoxviruses that have been recently isolated from nature [32] and may provide an advantage in facilitating transit through the dense cell cortex. Engulfment of MVs occurs by macropinocytosis or fluid phase endocytosis and is dependent on actin dynamics and cell signaling [28,34,35].

EVs have been described as entering through the plasma membrane by fusion of a MV-like particle released upon shedding the outer membrane at the cell surface [36] and by macropinocytosis [37]. Since most entry studies have been carried out with purified MVs and the fusion proteins are located in the MV membrane, there will be little further discussion of EVs in this review.

3.2. Cell binding and internalization

The binding of MVs to the cell surface is mediated by at least four viral proteins: D8 binds chondroitin [38], A27 and H3 bind heparan [39–42] and A26 binds laminin [43]. The crystal structure of a trimeric form of A27 [44] and a dimeric form of H3 [45] have been solved. Inactivation of any one of the attachment proteins does not prevent entry, though infectivity is severely reduced by deletion of the genes encoding A27 or H3. The four proteins have additional roles unrelated to attachment. Another protein, L1, has been reported to bind an unidentified cell surface protein for entry [46] and its crystal structure has been solved [47]. As discussed below, L1 is a component of the EFC.

The binding of MVs to cells also involves association with integrin β1 and CD98 receptor molecules and activation of several serine/threonine kinases that induce the formation of actin-enriched membrane protrusions that engulf virus particles during