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Review

The role of signalling and the cytoskeleton during Vaccinia Virus egress

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ABSTRACT

Viruses are obligate intracellular parasites that are critically dependent on their hosts to replicate and generate new progeny. To achieve this goal, viruses have evolved numerous elegant strategies to subvert and utilise the different cellular machineries and processes of their unwilling hosts. Moreover, they often accomplish this feat with a surprisingly limited number of proteins. Among the different systems of the cell, the cytoskeleton is often one of the first to be hijacked as it provides a convenient transport system for viruses to reach their site of replication with relative ease. At the latter stages of their replication cycle, the cytoskeleton also provides an efficient means for newly assembled viral progeny to reach the plasma membrane and leave the infected cell. In this review we discuss how Vaccinia virus takes advantage of the microtubule and actin cytoskeletons of its host to promote the spread of infection into neighboring cells. In particular, we highlight how analysis of actin-based motility of Vaccinia has provided unprecedented insights into how a phosphotyrosine-based signalling network is assembled and functions to stimulate Arp2/3 complex-dependent actin polymerization. We also suggest that the formin FHOD1 promotes actin-based motility of the virus by capping the fast growing ends of actin filaments rather than directly promoting filament assembly. We have come a long way since 1976, when electron micrographs of vaccinia-infected cells implicated the actin cytoskeleton in promoting viral spread. Nevertheless, there are still many unanswered questions concerning the role of signalling and the host cytoskeleton in promoting viral spread and pathogenesis.

Contents

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1. Introduction

The hijacking of the cytoskeleton is a common strategy employed by viruses infecting virtually all organisms including bacteria, plants and animals (Dodding and Way, 2011; Erb and Pogliano, 2013; Niehl et al., 2013; Taylor et al., 2011). However, one of the most striking examples of viral subversion of the host actin and microtubule cytoskeletons occurs during Vaccinia virus infection (Dodding and Way, 2011; Welch and Way, 2013). Vaccinia is a large double stranded DNA virus, which replicates exclusively in the cytoplasm of infected cells and is the most studied member of the Orthopoxviridae (Moss, 2007). Vaccinia is perhaps best known for its use as the vaccine to protect against smallpox, a deadly human disease caused by its close relative Variola virus (Jacobs et al., 2009; Walsh and Dolin, 2011). Smallpox was eradicated more than 30 years ago. Nevertheless, Vaccinia is increasingly being used as a vaccine vector for a wide range of different diseases as well as for oncolytic therapies (Jacobs et al., 2009; Kirn and Thorne, 2009; Thorne, 2011; Volz and Sutter, 2013; Walsh and Dolin, 2011). The vaccinia virus genome consists of ~200 kbp encoding for some 260 proteins, only about 80 of which, end up in infectious intracellular mature virus (IMV) particles (Chung et al., 2006; Resch et al., 2007; Yoder et al., 2006). This large coding capacity, which allows Vaccinia to infect and replicate in many different cell types, is in part due to its complex replication cycle, which involves the assembly of two morphologically distinct types of cytoplasmic virus particles (Fig. 1). The large genome also reflects the prodigious number of viral proteins Vaccinia uses to inhibit or suppress the antiviral activity of its host at all stages of its replication cycle (Haller et al., 2014). This includes inhibiting apoptosis of infected cells before new viral progeny are assembled and minimizing detection by the host immune system (Bahar et al., 2011; Mohamed and McFadden, 2009; Postigo and Ferrer, 2009).

After binding to the cell membrane, virus entry occurs either by direct fusion with the plasma membrane (Carter et al., 2005; Law et al., 2006) or by low-pH endosomal entry pathway (Huang et al., 2008; Townsley et al., 2006). Moreover, Vaccinia actually promotes its uptake by stimulating actin-dependent macropinocytosis (Mercer and Helenius, 2008; Mercer et al., 2010; Schmidt et al., 2011). Having gained access to the cell cytoplasm, expression of early proteins is initiated allowing viral cores to uncoat and release their DNA (Kilcher et al., 2014; Mercer et al., 2012; Schmidt et al., 2013). This early protein expression is required for viral DNA replication, which occurs in viral factories located in a perinuclear region near the microtubule-organizing centre of the infected cell (Ploubidou et al., 2000; Roberts and Smith, 2008). Only after DNA replication does intermediate and late gene expression start, resulting in the assembly of intracellular mature virus (IMV) particles. IMV which represent the majority of viral progeny are infectious but are only released when the infected cell undergoes lysis (Roberts and Smith, 2008). Alternatively, some IMV can become intracellular enveloped virus (IEV) by being 'wrapped' by membrane cisternae derived from trans-Golgi or endosomal compartments containing a subset of viral proteins (Roberts and Smith, 2008; Smith et al., 2002). The molecular basis for this envelopment remains to be established, but it involves multiple integral viral membrane proteins as well as the Vaccinia E2 and F12 proteins (Dodding et al., 2009; Domi et al., 2008; Roper et al., 1998; Röttger et al., 1999; Sanderson et al., 1998; Smith et al., 2002; Wolffe et al., 1997; Wolffe et al., 1998). Once formed, IEV are transported to the cell periphery on microtubules by kinesin-1 before fusing with the plasma membrane (Fig. 1). Fusion of IEV with the plasma membrane results in two outcomes that have a different impact on the subsequent spread of infection. The extracellular enveloped virus (EEV), which are infectious and released from the cell, promote the long-range spread of vaccinia. Alternatively, after their fusion with the plasma membrane, some virions remain attached to the outside of the cell and are known as the cell-associated enveloped virus (CEV). It is the CEV that are responsible for the local actin-dependent cell-to-cell spread of vaccinia (Fig. 1). In this review, we will discuss our current understanding of how vaccinia uses and manipulates the cytoskeleton of the cell to enhance its spread.

2. Microtubule-based transport of Vaccinia

2.1. IMV and IEV move on microtubules

Microtubule-based transport is the primary way in which cargoes are moved over micron distances in a directed fashion throughout the cell (Franker and Hoogenraad, 2013; Fu and Holzbaur, 2014; Stephens, 2012). It is perhaps not surprising then that viruses have developed numerous strategies to take advantage of this cellular transport system at all stages of their infection cycles (Dodding and Way, 2011; Greber and Way, 2006; Radtke et al., 2006). Moreover, the ability of large viruses, such as Vaccinia, to hijack this rapid and efficient transport system is essential, as their size precludes their movement by diffusion (Greber and Way, 2006; Sodeik, 2000). For the virologist, examining how viruses use the microtubule cytoskeleton and its associated motors is necessary to understand how the infection cycle is established, as well as the mechanisms underlying viral replication, assembly and spread. For the cell biologist, the same analysis promises to uncover fundamental insights into the molecular basis of microtubule motor recruitment and regulation.

Immunofluorescence analysis of vaccinia-infected cells reveals that intracellular mature virions (IMV) are capable of dispersing from their peri-nuclear site of assembly throughout the cell at the latter stages of the viral replication cycle (Fig. 2). This movement to the cell periphery is more apparent in viral backgrounds that result in an absence of IEV formation (Fig. 2). The extent of viral dispersal in fixed samples provided the first suggestion that IMV are capable of moving on microtubules, as it has been calculated that they would only diffuse 10 μm in ~5 h (Sodeik, 2000). Subsequent live-cell imaging of infected cells demonstrated that YFP-tagged IMV are capable of undergoing rapid, linear movements at speeds approaching 3 μm/s (Ward, 2005). IMV movement is saltatory in nature and was abolished when cells were treated with the microtubule depolymerizing agent nocodazole. Direct imaging of IMV moving on microtubules still remains to be performed and the identity of the motor responsible for transporting IMV has yet to be established. In contrast, GFP-tagged IEV have been imaged rapidly moving in a microtubule dependent fashion as well as along microtubules towards the cell periphery prior to their fusion with the plasma membrane (Dodding et al., 2009; Geada et al., 2001; Herrero-Martinez et al., 2005; Hollinshead et al., 2001; Rietdorf et al., 2001; Ward and Moss, 2001a; Ward and Moss, 2001b). IEV movement is dependent on kinesin-1 (also known as Kif5B or conventional kinesin), which uses the power of ATP hydrolysis to transport cargoes towards the plus end of microtubules usually located at the cell periphery (Dodding and Way, 2011; Rietdorf et al., 2001).

2.2. The basis of kinesin-1 recruitment to IEV

Kinesin-1 is recruited to IEV through an interaction between the tetratricopeptide repeats (TPR) of its light chain (KLC) and A36, an integral IEV membrane protein that has a cytoplasmic domain of ~195 residues exposed on the surface of the virus (Röttger et al., 1999; van Eijl et al., 2000; Ward and Moss, 2004). Fluorescence resonance energy transfer (FRET) experiments have confirmed that

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