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Review

Viruses that ride on the coat-tails of actin nucleation

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ABSTRACT

Actin nucleation drives a diversity of critical cellular processes and the motility of a select group of viral pathogens. Vaccinia virus and baculovirus, *Autographa californica* multiple nucleopolyhedrovirus, recruit and activate the cellular actin nucleator, the Arp2/3 complex, at the surface of virus particles thereby instigating highly localized actin nucleation. The extension of these filaments provides a mechanical force that bestows the ability to navigate the intracellular environment and promote their infectious cycles. This review outlines the viral and cellular proteins that initiate and regulate the signalling networks leading to viral modification of the actin cytoskeleton and summarizes recent insights into the role of actin-based virus transport.

Contents

1. Introduction 156
2. Actin-based motility of vaccinia virus 156
   2.1. Background and post-replicative transport of virus to the cell surface 156
   2.2. Initiation of actin nucleation at the virus-to-cell interface 156
   2.3. Cellular components in the core cascade leading to actin nucleation 157
   2.4. Regulating the core cascade 158
   2.5. Functional outcomes of vaccinia virus-induced actin nucleation 159
   2.6. Actin nucleation by other poxviruses 160
3. Actin-based motility of baculovirus 160
   3.1. Background and the mechanism of baculovirus-induced actin nucleation 160
   3.2. Functional outcomes of baculovirus-induced actin nucleation 160
4. Conclusions 161
   Acknowledgements 161
   References 161

Abbreviations: AcMNPV, *Autographa californica* multiple nucleopolyhedrovirus; CK2, Casein kinase 2; CRIB, Cdc42- and Rac-interactive binding; hpi, hours post infection; ITSN1, Intersectin-1; KLC, Kinesin light chain; MV, Mature virus; N-WASP, Neural Wiskott-Aldritch Syndrome Protein; NPF, Nucleation Promotion Factors; SCR, Short ensus Repeat; SH2, Src Homology 2; SH3, Src Homology 3; SIM, Structured Illumination Microscopy; VACV, Vaccinia virus; VCA, Verprolin-like Central Acidic; WH1, WASP homology region 1; WIP, WASP Interacting Protein; WV, Wrapped virus.

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1. Introduction

Actin – what is it good for? Absolutely everything, at least from a cellular perspective. Actin is responsible for giving a cell its shape, anchoring the cell and organelles within it, facilitating the transport of contents within the cell, mediating interactions with the extracellular milieu, and driving cell movement. During the course of an infection, viruses are able to interface with actin at many levels to promote their replication cycle. As a result, many of them have developed diverse mechanisms to manipulate actin in its varied forms. Actin is a small (42 kDa) protein that is present in globular (G-actin) and filamentous (F-actin) forms. Monomeric actin may self-assemble into actin filaments *in vitro*, although nucleation factors and associated proteins are thought to be critical for actin nucleation and polymerization *in vivo*. Examples of cellular actin nucleators include the Arp2/3 complex and the formins, which are primarily regulated by Rho GTPases and nucleation promotion factors (NPFs) [1].

The subversion of the host cytoskeleton by viral pathogens can take many forms. Cellular microtubule-based motor complexes are the primary transport systems that mediate subcellular movement of virus particles following entry and during egress [2–4]. Interactions with the actin cytoskeleton have also been well documented and include the elicitation of changes to cell shape and behaviour and the association with induced or existing actin structures [5,6]. For example, during entry HIV triggers virus-to-cell membrane fusion and a host of cytoskeletal rearrangements, in part mediated by Rac effectors such as WAVE2, resulting in activity of the Arp2/3 complex [7]. HIV also associates with formin (Diaphanous 2)-induced filopodia in infected dendritic cells, enabling budding particles to explore the local extracellular environment [8]. Individual virus particles of African swine fever virus induce thin cytoplasmic extensions rich in F-actin during exit, but currently an actin nucleator has yet to be identified in these structures [9]. In addition, several viruses also manipulate nuclear actin and lamins as part of their replication cycles [10]. Here we will focus on the induction of actin nucleation that is localized to the surface of virus particles.

The propulsion of viral pathogens by the localized stimulation of actin nucleation at the viral/host interface has been a powerful research model, leading to significant insights into the regulation of actin dynamics, as well as deepening our understanding of novel pathogenesis mechanisms. During normal cellular functioning, actin nucleation is a highly dynamic and seemingly capricious process. In contrast, the assembly of actin filaments by vaccinia virus (VACV) and the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is robust and highly localized, while also being amenable to genetic manipulation. Recent studies have begun to shed light on the role of actin-based motility as a virulence mechanism in the replication cycle of these viruses.

2. Actin-based motility of vaccinia virus

2.1. Background and post-replicative transport of virus to the cell surface

Vaccinia virus was the live vaccine used in the eradication of smallpox, caused by the related orthopoxvirus, variola virus [11]. It is the virus with the best-characterized molecular mechanism for how cellular actin nucleation pathways are repurposed for the promotion of virus transport. Infection by VACV generates oblong virus particles of 250–350 nm that house a 200 kb genome encoding over 200 proteins [12,13]. Considering the recent expansion of the nucleocytoplasmic large DNA virus group of viruses [14], VACV can be considered a historically large virus with significant genomic complexity.

Virus-associated comets of F-actin are readily visible in infected cells 7–9 h post infection (hpi) (Fig. 1) and bear remarkable similarity to disruptions of the actin cytoskeleton associated with *Listeria monocytogenes*-induced pathogen motility [15,16] (THERIOT, this issue). In fact, induction of actin comets by VACV is more akin to actin pedestals formed by enteropathogenic *E. coli* (EPEC), which, as is the case for VACV, are induced by extracellular pathogens adhered to the surface of cells [17,18]. A productive replication cycle of the prototypal VACV strain Western Reserve takes a minimum of 6–8 hpi to produce two morphologically distinct but mature infectious forms of the virus (for a detailed overview of the VACV replication cycle, see [19,20]). Mature virus (MV) particles are generated from an established perinuclear replication centre, referred to as the virus factory. These particles have a single membrane, derived from the endoplasmic reticulum, and comprise over 100 viral proteins with a range of post-translational modifications [19,21–23]. A subset of MV acquires two additional membrane layers from the early endosome/trans-Golgi network compartment. These are referred to as wrapped virus (WV). Being the only viral form able to promote actin nucleation, WV are the focus of this review.

In acquiring additional membranes of a different origin to MV, WV possess an additional complement of viral proteins that are integral to, or associated with, these membranes; these are referred to as WV-specific proteins. Three WV-specific proteins A36, F12 and E2 recruit and stabilize the microtubule motor complex kinesin-1 at the cytoplasmic virus surface. This interaction acts to haul virus cargo from the site of WV wrapping, typically located between the host nucleus and virus factory, to the cell periphery [24–31]. A36 is a type Ib integral membrane protein of 221 amino acids that lies at the heart of WV transport events, mediating interactions with both microtubule and actin cytoskeletons [24,32,33]. From the N-terminus, a short transmembrane domain anchors the protein to the WV outer envelope, with the remainder of the protein protruding into the cytoplasm. Although lacking recognized domains, two WD/WE motifs associate with the tetratricopeptide repeats (TPR) of kinesin light chain (KLC), a component of kinesin-1 [30]. Efficient anterograde virus transport also requires a second pathway, involving a complex of F12 and E2 that also binds KLC (specifically the KLC-2 isoform) [31]. How the cytoplasmic proteins F12 and E2 are tethered to the virus is not yet fully understood, but part of the answer may be an interaction between F12 and A36 [34].

2.2. Initiation of actin nucleation at the virus-to-cell interface

Anterograde transport mediated by kinesin-1 translocates WV to the vicinity of the cell surface. Access to the plasma membrane is granted by the cytoplasmic viral protein F11 that globally downregulates RhoA GTPase signalling, thereby clearing a path for the virus through the dense cortical F-actin [35].

Upon reaching the cell periphery, the outer WV membrane fuses with the plasma membrane leaving a cell-associated extracellular virus. Following this fusion event, there is an abrupt rearrangement to the complement of virus-associated proteins. Clathrin and the clathrin adaptor AP-2 accumulate on the cytoplasmic surface of extracellular virus [36,37] (Fig. 1). Clathrin is a cellular scaffold protein known for pinching off membrane into baskets of clathrin lattice, often involved in the uptake of extracellular molecular complexes, or viruses, such as influenza virus [38–40]. Here, it is associated with the reverse phenomenon – the exocytic release of virus particles. In parallel to clathrin accumulation there is an abrupt disassociation of F12, E2 and kinesin-1 from WV [28,36,41].