A role for the small GTPase Rac1 in vaccinia actin-based motility

Diego E Alvarez¹ and Hervé Agaisse²,*

¹Instituto de Investigaciones Biotecnológicas Dr. Rodolfo A. Ugalde; Universidad Nacional de San Martín-CONICET; San Martín, Buenos Aires, Argentina;
²Department of Microbial Pathogenesis; Boyer Center for Molecular Medicine; Yale School of Medicine; New Haven, CT USA

Vaccinia virus dissemination relies on the recruitment of the nucleation promoting factor N-WASP underneath cell-associated extracellular virus (CEVs) and subsequent recruitment and activation of the ARP2/3 complex, a major actin nucleator of the host cell. We have recently discovered that, in addition to the N-WASP/ARP2/3 pathway, vaccinia actin-based motility also relies on the small GTPase Rac1 and its downstream effector the formin-type actin nucleator FHOD1. Here we discuss the potential signaling mechanisms supporting the integration of the N-WASP/ARP2/3 and Rac1/FHOD1 pathways. We suggest the existence of a receptor tyrosine kinase family member that would integrate the Src-dependent activation of the N-WASP/ARP2/3 pathway and the GTP exchange factor-dependent activation of the Rac1/FHOD1 pathway.

Vaccinia Virus

Vaccinia virus is a large enveloped virus with a double-stranded DNA genome that belongs to the genus Orthopoxvirus in the Poxviridae family of viruses. Orthopoxviruses include animal pathogens such as variola virus, monkeypox virus, and cowpox virus. Variola virus infection causes smallpox and was declared eradicated in 1980 after a worldwide immunization campaign using vaccinia virus as a vaccine strain.¹ Vaccinia virus replication in the cytoplasm of an infected cell yields two infectious forms, intracellular mature virus (IMV) and extracellular virus (EV). Viral dissemination is supported by the propagation of IMVs upon cell lysis and by direct cell-to-cell spread of EVs through actin-based motility.² Here, we discuss the role of the small GTPase Rac1 in vaccinia virus actin-based motility.

Vaccinia Actin-Based Motility: The N-WASP/ARP2/3 Pathway

Similar to intracellular pathogens such as Listeria monocytogenes and Shigella flexneri, vaccinia virus achieves motility through manipulation of the ARP2/3 complex, a major actin nucleator of the host cell.³ Upon egress of intracellular enveloped virus through fusion with the plasma membrane, the viral protein A36 is positioned in the plasma membrane underneath the cell-associated extracellular virus (CEV).⁴ Non-receptor tyrosine kinases of the Src/Abl families phosphorylate A36,⁵-⁸ thereby generating docking sites for the adaptor proteins Nck1 and Grb2. Nck1 and Grb2 mediate the recruitment of a complex of the WASP-interacting protein WIP and the nucleation-promoting factor N-WASP, that in turn recruits and activates the ARP2/3 complex⁹-¹¹(Fig. 1A). ARP2/3-dependent actin nucleation leads to the generation of a branched actin network that supports the formation of virus-tipped membrane protrusions. This results in the release of EVs into the extracellular environment or the propagation to the neighboring cells.²,¹²,¹³

Vaccinia Actin-Based Motility: The Rac1/FHOD1 Pathway

In order to gain insight into the mechanisms controlling vaccinia virus dissemination, we have previously shown that vaccinia virus dissemination relies on the recruitment of the nucleation promoting factor N-WASP underneath cell-associated extracellular virus (CEVs) and subsequent recruitment and activation of the ARP2/3 complex, a major actin nucleator of the host cell. We have recently discovered that, in addition to the N-WASP/ARP2/3 pathway, vaccinia actin-based motility also relies on the small GTPase Rac1 and its downstream effector the formin-type actin nucleator FHOD1. Here we discuss the potential signaling mechanisms supporting the integration of the N-WASP/ARP2/3 and Rac1/FHOD1 pathways. We suggest the existence of a receptor tyrosine kinase family member that would integrate the Src-dependent activation of the N-WASP/ARP2/3 pathway and the GTP exchange factor-dependent activation of the Rac1/FHOD1 pathway.

Keywords: Dissemination; spread from cell to cell; actin-based motility; vaccinia virus; Rac1; FHOD1; N-WASP; ARP2/3 complex

¹Correspondence to: Hervé Agaisse;
Email: herve.agaisse@yale.edu
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actin-based motility we developed an RNAi-based screen for host factors required for vaccinia spread from cell to cell. Screening of a siRNA library covering regulators of the actin cytoskeleton led to the identification of the formin FHOD1 as a cellular factor required for vaccinia dissemination. We observed that silencing FHOD1 resulted in fewer CEVs with actin tails and a slower rate of elongation of the formed actin tails. Formins exist in an auto-inhibited conformation due to interactions between the N-terminal FH3 domain and the C-terminal DAD domain. Binding of activated small GTPases of the Rho/Rac/Cdc42 family to the GTPase binding domain (GBD) domain of formins relaxes the auto-inhibited conformation, which contributes to formin activation. In the activated form, the FH2 domain of formins is able

Figure 1. (A) Canonical model for vaccinia actin tail formation by the activation of the N-WASP/ARP2/3 pathway. Phosphorylation of the cytoplasmatic domain of A36 underneath CEVs (gray and white oval) by Src and Abl families of non-receptor tyrosine kinases mediates the recruitment of Nck1 and Grb2 adaptor proteins that in turn recruit a complex of WIP and N-WASP to activate the ARP2/3 complex promoting actin tail formation. (B) Vaccinia actin tail formation involves the activation of the Rac1/FHOD1 pathway. The small GTPase Rac1 is activated underneath CEVs by a yet unidentified guanine-nucleotide exchange factor (GEF). Downstream of Rac1, recruitment and activation of FHOD1 promotes vaccinia actin tail formation. Profilin stimulates formin activities of FHOD1. (C) A model for the integration of N-WASP/ARP2/3 and Rac1/FHOD1 pathways. A vaccinia protein exposed on the outer membrane of CEVs engages a yet unidentified receptor tyrosine kinase (RTKs) leading to the activation of Src and subsequent phosphorylation of A36, as well as the recruitment of a GEF for Rac1, thereby integrating the activities of the N-WASP/ARP2/3 and Rac1/FHOD1 pathways. N-WASP interacts with FHOD1 through an unknown mechanism (arrow).
to nucleate, elongate and cap the plus end of actin filaments. The elongation activity of formins is stimulated through binding of profilin-actin to the FH1 domain. We observed that the functional GBD and FH2 domains of FHOD1 were required for both actin tail formation and localization of the formin to vaccinia actin tails. In addition, we found that actin tail formation requires both the FH1 domain of FHOD1 and profilin. In previous studies, the GBD domain of FHOD1 was shown to physically interact with the activated form of Rac1 and expression of active Rac1 led to the recruitment of FHOD1 to the plasma membrane. In vaccinia-infected cells, we observed that GFP-tagged Rac1 was enriched and activated at the plasma membrane surrounding actin tails. In agreement with previous studies, we confirmed a role for Rac1 in the recruitment and activation of FHOD1. First, silencing Rac1 or overexpression of dominant-negative Rac1 mimicked the phenotype of FHOD1 silencing. Second, silencing Rac1 impaired the recruitment of FHOD1 to vaccinia actin tails. Third, the effect of dominant-negative Rac1 on vaccinia actin tail formation could be rescued by overexpression of full-length or constitutively active versions of FHOD1. Like other GTP binding proteins, small GTPases of the Rho/Rac/Cdc42 family cycle between a GTP-bound active form and a GDP-bound inactive form. Guanine-nucleotide exchange factors (GEFs) convert the inactive GDP-bound GTPase to an active GTP-bound form. GTPase activating proteins (GAPs) inactivate GTPases by converting the active GTP-bound active form. GTPase activating factors (GEFs) convert the GDP-bound inactive form to the active GTP-bound form. Like other GTP binding proteins, small GTPases such as Rac1 are notorious for their role in recruitment of N-WASP/WAVE family members to the plasma membrane. However, we found that in the context of vaccinia actin tail formation, silencing Rac1 had no effect on the recruitment of N-WASP. By contrast, silencing N-WASP affected the recruitment of FHOD1, which may be mediated through the interaction of N-WASP with adaptors, proteins, such as WISH. Importantly, the recruitment and activation of Rac1 to CEVs did not rely on the viral protein A36. This critical result uncovered that, although required for FHOD1 recruitment, the effect of dominant-negative Rac1 on vaccinia actin tail formation, silencing Rac1 impaired the recruitment of FHOD1 to CEVs. Second, silencing N-WASP impaired the recruitment of FHOD1, even though Rac1 was enriched and activated in the context of vaccinia actin tail formation. Third, overexpression of full-length or constitutively active Rac1 rescued the effect of dominant-negative Rac1 on vaccinia actin tail formation, silencing Rac1 had no effect on the recruitment of N-WASP. By contrast, silencing N-WASP impaired the recruitment of FHOD1.

Integration of the N-WASP/ARP2/3 and Rac1/FHOD1 Pathways

In addition to the A36-mediated N-WASP/ARP2/3 pathway, our recent studies thus revealed that robust actin-based motility relies on the activation of the Rac1/FHOD1 pathway (Fig. 1B). What mechanisms potentially integrate the activation of these two signaling pathways? Small-GTPases such as Rac1 are notorious for their role in the recruitment of N-WASP/WAVE family members to the plasma membrane. However, we found that in the context of vaccinia actin tail formation, silencing Rac1 had no effect on the recruitment of N-WASP. By contrast, silencing N-WASP affected the recruitment of FHOD1, which may be mediated through the interaction of N-WASP with adaptors, proteins, such as WISH. Importantly, the recruitment and activation of Rac1 to CEVs did not rely on the viral protein A36. This critical result uncovered that, although required for FHOD1 recruitment, the effect of dominant-negative Rac1 on vaccinia actin tail formation, silencing Rac1 impaired the recruitment of FHOD1 to CEVs. Second, silencing N-WASP impaired the recruitment of FHOD1, even though Rac1 was enriched and activated in the context of vaccinia actin tail formation. Third, overexpression of full-length or constitutively active Rac1 rescued the effect of dominant-negative Rac1 on vaccinia actin tail formation, silencing Rac1 had no effect on the recruitment of N-WASP. By contrast, silencing N-WASP impaired the recruitment of FHOD1.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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