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To cite this article: Diego E Alvarez & Hervé Agaisse (2014) A role for the small GTPase Rac1 in vaccinia actin-based motility, *Small GTPases*, 5:2, e29038, DOI: [10.4161/sgtp.29038](https://doi.org/10.4161/sgtp.29038)

To link to this article: <http://dx.doi.org/10.4161/sgtp.29038>



Published online: 31 Oct 2014.



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

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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

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

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Submitted: 03/19/2014

Revised: 04/24/2014

Accepted: 04/28/2014

Published Online: 05/09/2014

<http://dx.doi.org/10.4161/sgtp.29038>

Commentary to: Alvarez DE, Agaisse H. The formin FHOD1 and the small GTPase Rac1 promote vaccinia virus actin-based motility. *J Cell Biol* 2013; 202:1075-90; PMID:24062339; <http://dx.doi.org/10.1083/jcb.201303055>

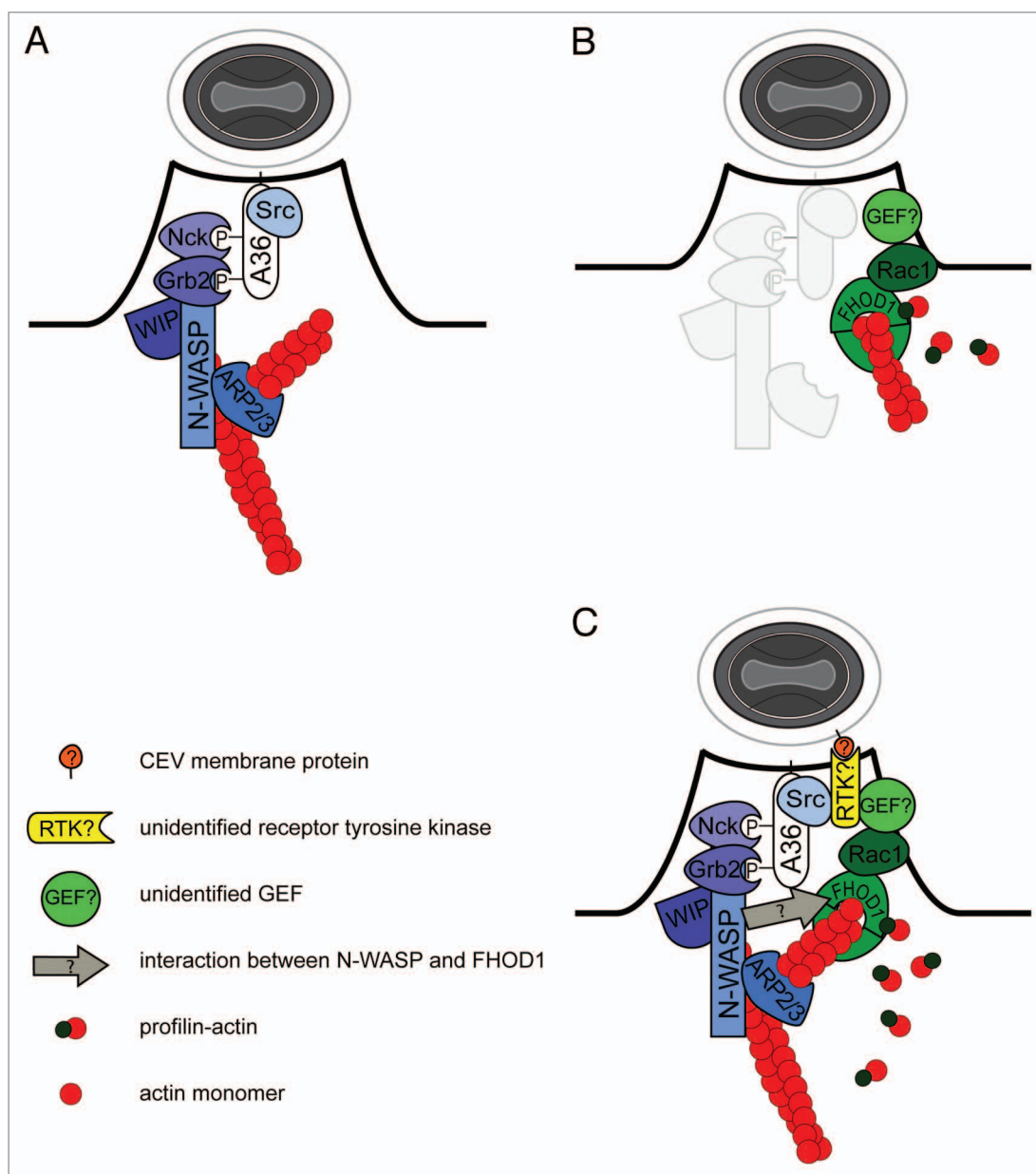


Figure 1. (A) Canonical model for vaccinia actin tail formation by the activation of the N-WASP/ARP2/3 pathway. Phosphorylation of the cytoplasmic domain of A36 underneath CEVs (gray and white oval) by Src and Abl families of non-receptor tyrosine kinases mediates the recruitment of Nck1 and Grb2 adaptor 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).

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.¹⁴ Formins exist in an auto-inhibited conformation due to interactions between the N-terminal FH3 domain and the C-terminal DAD

domain.¹⁵⁻¹⁸ 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.¹⁹ In the activated form, the FH2 domain of formins is able

to nucleate, elongate and cap the plus end of actin filaments.²⁰⁻²⁶ The elongation activity of formins is stimulated through binding of profilin-actin to the FH1 domain.^{27,28} 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.¹⁴ 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.²⁹⁻³¹ 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 FHOD1.¹⁴ 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 promoting GTP hydrolysis. Dominant-negative versions of small GTPases act by stabilizing the interaction with and sequestering the upstream GEF involved in GTPase activation.³² Thus, the effect of dominant-negative Rac1 overexpression on vaccinia actin tail formation suggested the involvement of a Rac1 GEF in the activation of Rac1 (Fig. 1B). The identity of this putative Rac1 GEF remains unknown.

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

In addition to the A36-mediated N-WASP/ARP2/3 pathway (Fig. 1A),

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.^{33,34} 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 FHOD1,¹⁴ which may be mediated through the interaction of N-WASP with adaptor proteins, such as WISH^{35,36} (Fig. 1C). Importantly, the recruitment and activation of Rac1 to CEVs did not rely on the viral protein A36.¹⁴ This critical result uncovered that, although required for FHOD1 recruitment, the N-WASP/ARP2/3 pathway is likely dispensable for Rac1 recruitment and activation underneath CEVs. It is noteworthy that the signaling events leading to Src activation, which presumably involves the activation of a member of the receptor tyrosine kinase (RTK) family, are unknown. We note that RTKs are well suited to integrate the activities of the N-WASP/ARP2/3 and Rac1/FHOD1 pathways. RTKs not only activate Src,^{37,38} but also modulate the activity of small GTPases of the Rho/Rac/Cdc42 family through recruitment and activation GTP exchange factors (GEFs) to the plasma membrane.³⁹ In fact, recent data indicate that CEVs recruit the GEF intersectin-1 prior to vaccinia actin tail formation.⁴⁰ Intersectin-1 regulates the N-WASP/ARP2/3 pathway through activation of Cdc42.^{10,40} Whether intersectin-1 may function as a GEF for Rac1 remains to be tested. In conclusion, we suggest the existence of RTK(s) that would integrate the activity of the N-WASP/ARP2/3 and Rac1/FHOD1 pathways through the activities of Src and a yet unidentified GEF for Rac1 (Fig. 1C). The identification of the putative GEF(s) and RTK(s), as well as putative viral components engaging outside-in RTK signaling, will unveil the missing link in vaccinia actin-based motility (Fig. 1C).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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