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Poxvirus A51R: A microtubule maestro and virulence virtuoso

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Seo et al.[1] shed light on virus-host interactions as they reveal how poxvirus A51R stabilizes microtubules in infected cells, which impacts vaccinia virus virulence in mice by potentially inhibiting reactive-oxygen-species-dependent antiviral responses in macrophages.

Viruses exhibit remarkable proficiency in manipulating cellular machinery, particularly exploiting microtubules for essential viral processes such as replication and immune evasion.[2] Microtubules, dynamic polymers intricately woven throughout the cell, act as essential architectural pillars for maintaining cellular organization.[3] Viruses have evolved strategies to hijack host-cell microtubule motors and microtubule-associated proteins (MAPs) to facilitate their intracellular transport and dissemination.[4] Additionally, viruses deploy their own arsenal of viral MAPs, enhancing their ability to manipulate these cellular highways. Recent findings published by Seo et al.[1] shed light on the pivotal role of the poxvirus A51R protein in modulating microtubule stability and evading antiviral responses within macrophages.

Poxviruses, encompassing pathogens like variola virus and monkeypox, fascinate with their sophisticated survival strategies. Among them, vaccinia virus (VV) stands out due to its historical use as a smallpox vaccine. VV's replication initiates in the cytoplasm, where specialized viral factories produce immature virions that mature into brick-shaped intracellular mature virions.[5] Some mature virions cloak themselves with host-cell-derived membrane "crescents" and are trafficked to the plasma membrane. Upon reaching the plasma membrane, virions are released into the extracellular milieu by membrane fusion, facilitating long-range dissemination. A subset of enveloped mature virions remains tethered to the extracellular surface of host cells, initiating actin-tail assembly to propagate viral spread from cell to cell.

VVs harness host-cell microtubules for their replication and transport, with key proteins like A36, F12, and E2 facilitating intracellular VV transport along microtubules to the plasma membrane via interactions with kinesin-1.[4] Moreover, F11L stimulates microtubule dynamics to enhance virus release from infected cells, while VV infection stabilizes perinuclear microtubules, safeguarding them from depolymerization.[6] Despite previous identifications of viral MAPs like A10L and L4R, the broader significance of VV-induced microtubule stability remains unclear.

Gammon et al. highlighted the critical role of A51R in mediating VV-induced microtubule stabilization.[7] A51R acts as a linchpin, bundling and stabilizing microtubules. Seo et al. extend this work by validating A51R as a viral MAP, demonstrating its direct interaction with microtubules to promote growth and prevent depolymerization (Figure 1A).[1] Conserved positively charged residues in A51R mediate these interactions, suggesting a mechanism where A51R's self-association drives microtubule bundling.

The physiological consequences of A51R-microtubule interactions are profound. Mutations in A51R's microtubule-binding/bundling residues diminish VV virulence in mice (Figure 1B), highlighting its role in evading host defenses. Mice infected with the A51R-microtubule interaction mutant VV (A51R^{Triple}) exhibit perturbed immune responses, suggesting impaired replication within B cells and macrophages/monocytes. Although the A51R^{Triple} VV was able to replicate normally in both human U2OS osteosarcoma cells and murine FL83B hepatocytes, it failed to do so in alveolar macrophages—the lung's primary defense against respiratory infection. A similar phenomenon was observed in RAW 264.7 murine macrophages. Electron microscopy reveals pronounced defects in virion morphogenesis in macrophages infected with A51R^{Triple}, emphasizing the importance of these interactions in viral replication. Thus, A51R-microtubule interactions are crucial for VV to counteract a cell-intrinsic antiviral reactive oxygen species (ROS) response in macrophages, which typically inhibits VV morphogenesis and replication.

It remains unknown how pathogens manipulate microtubules to inhibit ROS. However, an intriguing hypothesis could relate to the effect of viral infection on the intracellular positioning of mitochondria, which are major sources of ROS production. Mitochondria contribute to redox signaling by producing ROS, such as hydrogen peroxide, which can modify the activity of target proteins.[8] Given that hypoxia alters the microtubule-dependent transport of mitochondria, leading to increased ROS, it is pertinent to investigate whether the A51R-dependent increase in perinuclear stable microtubules alters mitochondria-dependent ROS generation. Interestingly, VV can induce a hypoxic response in infected cells dependent upon the viral protein C16.[9] It is curious that microtubule detyrosination, a post-translational modification that removes the C-terminal glutamic acid residue from α-tubulin in stable microtubules, regulates the ability of striated muscle to produce ROS as a function of stretch-mediated activation of nicotinamide adenine dinucleotide phosphate oxidase (NOX2).[10] Consequently, it will be

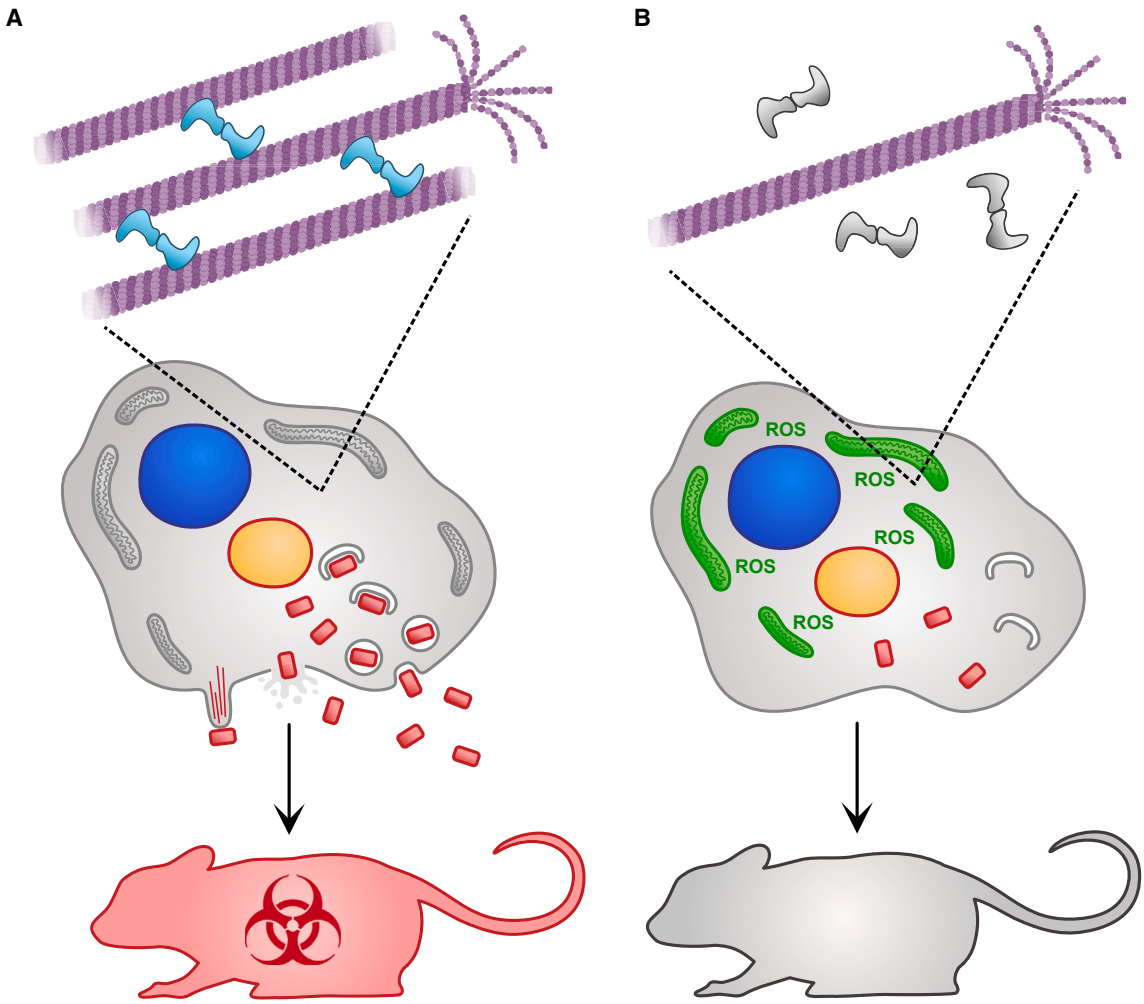

Figure 1. A51R-microtubule interactions are required for VV morphogenesis, replication in macrophages, and virulence in mice

(A) VV replication in macrophages with wild-type A51R. A51R binds to microtubules as dimers; this promotes microtubule stability and bundling. In a macrophage that is infected with VV, a viral factory (orange circle) forms near the cell nucleus (blue circle). VV virions (red rectangles) leaving the viral factory take a variety of routes out of the cell. Some virions are cloaked in membrane crescents derived from the host cell and exit the cell via membrane fusion. Other virions exit the cell through lysis. Some virions remain tethered to the host cell and are propelled by actin tails. Wild-type VV infection is lethal in mice.

(B) Mutating three conserved residues in A51R results in the loss of A51R's ability to bind microtubules. This prevents microtubule bundling and results in microtubule destabilization. Macrophages infected with an engineered VV that contains these mutations in A51R are still able to make viral factories, but the VV maturation process is severely disrupted. Mitochondria (green oblong shapes) release ROS, which inhibit virion morphogenesis. Mice survive infection with this mutant VV.

important to test the prospective roles for microtubule detyrosination and NOX2 in VV infection in macrophages.

Several aspects of A51R-microtubule interactions remain ambiguous and warrant additional investigation. Questions persist regarding the significance of A51R self-association for microtubule bundling and stabilization, as well as its impact on other MAPs (cellular or viral) or motors. The effect of the potential phosphorylation sites predicted near crucial microtubule-binding residues raises questions about the regulation of A51R-microtubule interactions. Lastly, identifying the antiviral strategy impacted by A51R-microtubule interactions in B cells would be very exciting. Likewise, the role of A51R in sculpting VV virion morphogenesis and its interplay with macrophage ROS antiviral activity present intriguing research prospects. Understanding how pathogens manipulate microtubules to inhibit ROS production and investigating microtubule post-translational modifications like detyrosination in VV infection offer promising directions for future studies.

In summary, A51R is a microtubule maestro and a virulence virtuoso. Its decisive role in viral pathogenesis underscores the significance of microtubule dynamics in virus-host interactions. The evolving narrative of A51R and microtubules holds promise for unraveling secrets that could reshape antiviral therapeutic strategies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Seo, D., Brito Oliveira, S., Rex, E.A., Ye, X., Rice, L.M., da Fonseca, F.G., and Gammon, D.B. (2024). Poxvirus A51R proteins regulate microtubule stability and antagonize a cell-intrinsic antiviral response. Cell Rep. *43*, 113882. https://doi.org/10.1016/j.celrep.2024.113882.
2. da Silva, E.S., and Naghavi, M.H. (2023). Microtubules and viral infection. Adv. Virus Res. *115*, 87–134. https://doi.org/10.1016/bs.aivir.2023.02.003.
3. Borisy, G., Heald, R., Howard, J., Janke, C., Musacchio, A., and Nogales, E. (2016). Microtubules: 50 years on from the discovery of tubulin. Nat. Rev. Mol. Cell Biol. *17*, 322–328. https://doi.org/10.1038/nrm.2016.45.
4. Seo, D., and Gammon, D.B. (2022). Manipulation of Host Microtubule Networks by Viral Microtubule-Associated Proteins. Viruses *14*, 979. https://doi.org/10.3390/v14050979.
5. Liu, L., Cooper, T., Howley, P.M., and Hayball, J.D. (2014). From crescent to mature virion: vaccinia virus assembly and maturation. Viruses *6*, 3787–3808. https://doi.org/10.3390/v6103787.
6. Ploubidou, A., Moreau, V., Ashman, K., Reckmann, I., González, C., and Way, M. (2000). Vaccinia virus infection disrupts microtubule organization and centrosome function. EMBO J. *19*, 3932–3944. https://doi.org/10.1093/emboj/19.15.3932.
7. Gammon, D.B., Duraffour, S., Rozelle, D.K., Hehnly, H., Sharma, R., Sparks, M.E., West, C.C., Chen, Y., Moresco, J.J., Andrei, G., et al. (2014). A single vertebrate DNA virus protein disarms invertebrate immunity to RNA virus infection. Elife *3*, e02910. https://doi.org/10.7554/eLife.02910.
8. Xu, Q., Huff, L.P., Fujii, M., and Griendling, K.K. (2017). Redox regulation of the actin cytoskeleton and its role in the vascular system. Free Radic. Biol. Med. *109*, 84–107. https://doi.org/10.1016/j.freeradbiomed.2017.03.004.
9. Mazzon, M., Peters, N.E., Loenarz, C., Krysztofinska, E.M., Ember, S.W.J., Ferguson, B.J., and Smith, G.L. (2013). A mechanism for induction of a hypoxic response by vaccinia virus. Proc. Natl. Acad. Sci. USA *110*, 12444–12449. https://doi.org/10.1073/pnas.1302140110.
10. Kerr, J.P., Robison, P., Shi, G., Bogush, A.I., Kempema, A.M., Hexum, J.K., Becerra, N., Harki, D.A., Martin, S.S., Raiteri, R., et al. (2015). Detyrosinated microtubules modulate mechanotransduction in heart and skeletal muscle. Nat. Commun. *6*, 8526. https://doi.org/10.1038/ncomms9526.