

VIRAL INFECTION

Rhinoviruses usurp STING for replication

Stimulator of interferon genes (STING) is a proviral host factor for all rhinoviruses.

Rutger D. Luteijn and Frank J. M. van Kuppeveld

The cGAS–STING (cyclic GMP–AMP synthase–stimulator of interferon genes) pathway is a cytosolic DNA-sensing pathway that is an integral part of the innate immune response in animals¹. cGAS–STING is activated directly by viral DNA (DNA virus infection), but for RNA viruses, which are not directly recognized by the cGAS–STING pathway, cGAS–STING can be activated indirectly by mislocalized host cell DNA that is released when RNA virus infection damages mitochondria and host nuclei². Rhinovirus species A and C, but not species B, have previously been shown to rely on STING for replication. Reporting in *Nature Communications*, Triantafilou et al.³ show that STING is a proviral factor for all rhinoviruses (RVs) and how RVs redirect STING to support replication in bronchial and airway epithelial cells.

In uninfected cells, the STING protein is tethered to the endoplasmic reticulum (ER) by the calcium ion (Ca²⁺)-sensor protein stromal interaction molecule 1 (STIM1). Following activation of innate immune responses — for example, due to infection with a pathogenic microorganism (including viruses) — STING is released and trafficked to the ER–Golgi intermediate compartment (ERGIC) and trans-Golgi network, where it interacts with TANK-binding kinase 1 (TBK1), interferon regulatory factor 3 (IRF3) and nuclear factor kappa B (NF-κB). Activation of the transcription factors IRF3 and NF-κB results in a proinflammatory response, the function of which is to clear viral infections. STING relocalization to the ERGIC also results in a non-canonical autophagy pathway that clears viruses from the cytosol¹. Many viruses have developed strategies to target components of the cGAS–STING pathway to dampen host cell antiviral responses. Findings published recently reveal that RVs not only subvert cGAS–STING antiviral responses, but also co-opt STING for virus replication (Fig. 1).

Rhinoviruses belong to the genus *Enterovirus* in the Picornaviridae family, which also contains important human pathogens such as poliovirus, coxsackievirus, enterovirus A71 and

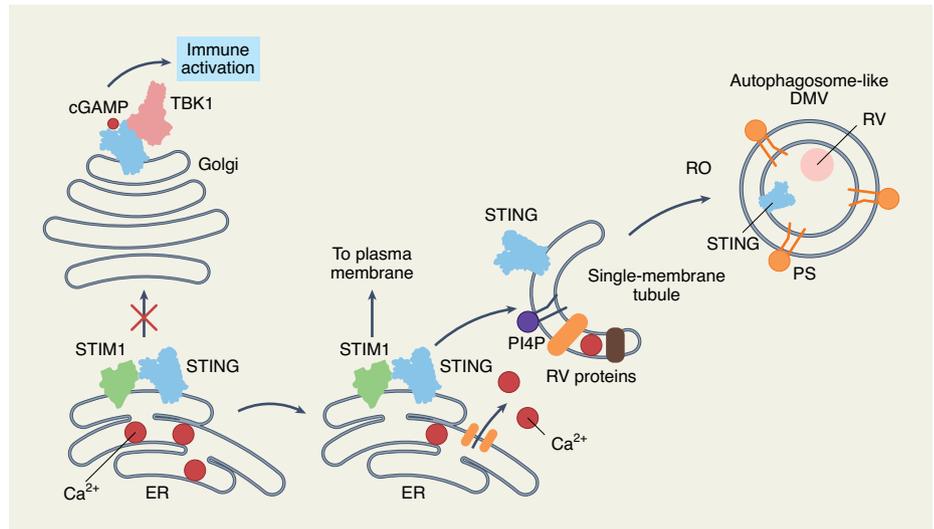


Fig. 1 | STING relocation in RV infection. In uninfected cells, STING is complexed with the Ca²⁺ sensor STIM1 in the ER. Following RV infection, Ca²⁺ release, which is catalysed by viral 2B protein, results in the dissociation of STIM1 from STING. After STING is released from STIM1, it localizes to PI4P-rich ROs, where virus replication and assembly take place. Later in infection, STING localizes to PS-rich autophagosomes, where RVs further mature. By relocating STING, TBK1-mediated immune activation of STING in the Golgi is substantially reduced in RV-infected cells.

enterovirus D68 (ref. 4). Rhinoviruses can be classified into three species — RV-A, RV-B and RV-C — and are the main cause of the common cold in humans. Infections due to these viruses have a huge economic impact, costing billions of euros in losses every year owing to worker sickness and reduced productivity. Rhinovirus infections have the potential to cause severe lower respiratory tract infections, wheezing in infants and exacerbation of asthma and chronic obstructive pulmonary disease⁴.

Enterovirus RNA replication takes place on the outer surface of virus-induced, rearranged host cell membrane structures that accumulate in the cytosol. These rearranged membranes initially consist of single-membrane tubules, which transform into double-membrane vesicles (DMVs) later in infection. These membranes contain the viral replication complexes and are named replication organelles (ROs). Viral membrane proteins 2B, 2C and 3A have essential roles in the

formation and function of these ROs through interaction with membranes or recruitment of lipid-modifying cellular proteins. By interacting with ACBD3, protein 3A recruits lipid kinase PI4KB to ROs, resulting in phosphatidylinositol 4-phosphate (PI4P)-enriched membranes that serve as a platform for the binding of oxysterol-binding protein (OSBP), which delivers cholesterol to ROs^{5,6}. The ROs have been proposed to serve as scaffolds for efficient replication, protecting RV ROs from cytosolic sensors of non-self viral double-stranded RNA. Replication organelles probably also serve as a platform for virus assembly and may supply the membranes for pre-lytic release of virus progeny, which occurs via phosphatidylserine (PS)-enriched extracellular vesicles⁷.

In 2020, McKnight, Lemon and co-workers⁸ reported that STING could function as a proviral host factor for RV-A and RV-C, but not for RV-B. They showed

that STING was rerouted to PI4P-enriched viral ROs, and that STING expression stimulated viral genome replication. Human STING, but not mouse STING, was required for productive virus infection. Adaptation to use of mouse STING occurred through two mutations in the viral 2C protein, suggesting that 2C interacts with STING, but direct binding between 2C and STING was not shown. A second study from Gagliardi, Scull and co-workers⁹ strengthened the evidence base for STING as a proviral factor by showing that STING is required for replication of RV-A and RV-C, but not RV-B, in human airway epithelial cells.

Now, the study from Triantafilou et al.³ provides additional evidence for the role of STING in the RV lifecycle. The work supports a proviral role for STING in bronchial and airway epithelial cells. Remarkably, they find that in these cells, STING has a proviral role in the replication of RV-A, RV-C and RV-B viruses. The reason for the difference in STING dependency for RV-B with the previous studies is unclear. Whether this is due to differences in cell lines or viral preparations used, or due to other unanticipated variations, remains to be established.

Using non-invasive microscopy techniques, including stimulated Raman scattering (SRS) and fluorescence resonance energy transfer (FRET) microscopy, the authors show that STING relocates from the ER to PI4P-enriched ROs in RV-infected cells. The authors provide evidence that STING relocation is triggered by the RV 2B protein, a viroporin that was recognized to deplete Ca²⁺ in the ER¹⁰ —

but the relevance of this activity for the viral lifecycle remained poorly understood. Indeed, 2B expression released the STING-retention protein STIM1 from the ER, although the effect on STING release was not studied.

The authors also report that STING can directly bind to PI4P via charged residues in the ligand binding domain of STING. Release of STING from STIM1 may expose these residues for subsequent PI4P binding at ROs. How STING promotes virus replication at the RO is still unclear. Importantly, a proviral role for STING may not be restricted to PI4P-enriched ROs, because later in infection STING localizes to PS-enriched autophagosomes, where virus particles mature for pre-lytic release via extracellular vesicles. Whether and how STING contributes to the formation of these PS-enriched autophagosomes remains to be established.

Finally, the authors show that immune activation by cytosolic DNA or other bona fide STING agonists is prevented during RV infection. Several RNA viruses, including picornavirus EMCV, can activate STING by triggering mitochondrial DNA leakage into the cytosol². By rewiring the STING response, RVs may not only promote viral replication but, at the same time, also limit undesirable STING-induced immune responses.

The three studies on the proviral role of STING in RV infection raise several points that warrant further investigation. First, the differential requirement of STING for RV-B replication observed in the different studies. Second, in contrast to the study from Triantafilou et al.³, McKnight et al.⁸ showed that inhibition of autophagy, or

treatment with the STING antagonist H-151, had no effect on RV replication. Elucidating the underlying reasons for these differences may provide important insights into the intriguing role(s) of STING in RV replication. Moreover, it would be instructive to investigate the role of STING in the replication of other enteroviruses, including the emerging respiratory pathogen enterovirus D68 as well as other RNA viruses that build PI4P-enriched ROs, such as other picornaviruses (for example, cardioviruses and kobuviruses), and hepatitis C virus. □

Rutger D. Luteijn and
Frank J. M. van Kuppeveld  

Virology Section, Division of Infectious Diseases & Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

✉e-mail: f.j.m.vankuppeveld@uu.nl

Published online: 4 May 2022
<https://doi.org/10.1038/s41564-022-01117-9>

References

1. Ablasser, A. & Hur, S. *Nat. Immunol.* **21**, 17–29 (2020).
2. Fan, Y. M. et al. *Rev. Med. Virol.* **2022**, e2343 (2022).
3. Triantafilou, M. et al. *Nat. Commun.* **13**, 1406 (2022).
4. Baggen, J. et al. *Nat. Rev. Microbiol.* **16**, 368–381 (2018).
5. Roulin, P. S. et al. *Cell Host Microbe* **16**, 677–690 (2014).
6. Hsu, N.-Y. et al. *Cell* **141**, 799–811 (2010).
7. Chen, Y.-H. et al. *Cell* **160**, 619–630 (2015).
8. McKnight, K. L. et al. *Proc. Natl Acad. Sci. USA* **117**, 27598–27607 (2020).
9. Gagliardi, T. B. et al. *PLoS Pathog.* **18**, e1010159 (2022).
10. van Kuppeveld, F. J. M. et al. *Trends Microbiol.* **13**, 41–44 (2005).

Competing interests

The authors declare no competing interests.