Regulation of antiviral innate immune signaling and viral evasion following viral genome sensing

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A harmonized balance between positive and negative regulation of pattern recognition receptor (PRR)-initiated immune responses is required to achieve the most favorable outcome for the host. This balance is crucial because it must not only ensure activation of the first line of defense against viral infection but also prevent inappropriate immune activation, which results in autoimmune diseases. Recent studies have shown how signal transduction pathways initiated by PRRs are positively and negatively regulated by diverse modulators to maintain host immune homeostasis. However, viruses have developed strategies to subvert the host antiviral response and establish infection. Viruses have evolved numerous genes encoding immunomodulatory proteins that antagonize the host immune system. This review focuses on the current state of knowledge regarding key host factors that regulate innate immune signaling molecules upon viral infection and discusses evidence showing how specific viral proteins counteract antiviral responses via immunomodulatory strategies.

Experimental & Molecular Medicine (2021) 53:1647–1668; https://doi.org/10.1038/s12276-021-00691-y

INTRODUCTION

Viruses need to hijack the host cell machinery to replicate effectively; however, they must first overcome the host’s defenses. The efficacy of a viral infection depends on the comparative potency of the effector molecules used by the virus and the host. A critical determinant of whether a host succumbs to or can subvert a viral infection is the speed at which the host’s defenses are activated. Almost all innate immune responses require an extended sequence of actions: pathogen sensing, signal transduction, transcription, translation, protein folding, and transport to the site of action. To initiate signaling upon viral infection, host cells detect viral DNA or RNA using a set of PRRs; these include retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (RNA sensors), cyclic GMP-AMP (cGAMP) synthase (cGAS), interferon gamma-inducible protein 16 (IFI16), absent in melanoma 2 (AIM2), and double-stranded RNA-stimulated antiviral signaling protein (MAVS) or stimulator of interferon genes (STING), which induce expression of interferon (IFN)-stimulated genes via autocrine or paracrine mechanisms; the products of genes (proinflammatory cytokines, chemokines, and IFNs) inhibit viral replication and spread and induce activation of adaptive immune responses. These antiviral signaling pathways play a crucial role in achieving an optimal outcome for the host; therefore, much attention has been devoted to identifying and understanding the signaling pathways and regulatory factors involved in antiviral innate immunity.

Conventional posttranslational modifications such as polyubiquitination and phosphorylation, unconventional posttranslational modifications such as acetylation and methylation, and other regulatory mechanisms such as physical interactions and translocations affect the production of IFN-β and inflammatory cytokines by targeting innate immune sensors and downstream signaling molecules (e.g., receptors, adaptors, enzymes, and transcription factors). These aforementioned modifications play a critical role in regulating the production of IFNs and inflammatory cytokines, which can, if production is unchecked, have deleterious effects on the host by promoting the development of autoimmune disorders, allergies, and other immunopathologies, as well as by activating and regulating the cellular status to exacerbate the severity of viral disease.

It is not surprising that viruses exploit numerous strategies to enhance their replication. To establish efficient, lifelong infection and to initiate viral pathogenesis, a large portion of the viral genome encode numerous immunomodulatory proteins; the function of these proteins is to evade/disrupt the host immune system and ensure viral persistence. From the perspective of the virus, these actions are critically important because viruses depend on living cells for replication. This review focuses on current knowledge regarding two factors. First, we summarize the posttranslational modifications (PTMs) and other regulatory mechanisms of signaling molecules downstream of the RNA/DNA sensing cascade that regulate efficient IFN responses and/or maintenance of host immune homeostasis. Second, we summarize how RNA/DNA viruses evade transduced host innate immune signals, which are initiated by PRRs, to establish a permissive state in host cells.

ROLE OF PTMS IN REGULATING SIGNAL TRANSDUCTION

PTMs play an important role in regulating the stability, activity, subcellular localization, and folding of proteins. Advances in...
Experimental techniques used to map and quantify PTMs have led to marked progress in these areas. Such techniques have identified a number of PTMs that alter the innate immune response by regulating protein function, abundance, catalysis, interactions, or subcellular localization without necessarily requiring induction of a new transcriptional program. Additionally, some of these PTMs are highly dynamic and fully reversible, allowing both initiation and resolution of responses.

Phosphorylation, a process by which a phosphoryl group is attached to a serine, threonine, tyrosine, histidine, or aspartate residue, is a well-studied PTM regulated by the opposing actions of protein kinases and phosphatases; this PTM plays an important role in innate immunity. The introduction of a phosphoryl group imparts a negative (–2) charge at physiological pH, resulting in a major biophysical perturbation of protein structure. This is manifested by conformational changes that alter enzymatic activity and/or protein–protein interactions. Ubiquitination is another important PTM. During ubiquitination, proteins are modified via covalent attachment of a small 76-amino acid protein called ubiquitin, which (as the name implies) is expressed ubiquitously and is highly conserved in all eukaryotes. Ubiquitination is inversely regulated by ubiquitin activating (E1), ubiquitin-conjugating (E2), and ubiquitin protein ligase (E3) enzymes and by deubiquitinating enzymes (DUBs); thus, it plays a critical role in regulating innate immune signal transduction. In contrast to phosphorylation, a single target site can be modified by a single ubiquitin molecule (monoubiquitination) or by chains of linked ubiquitin molecules (polyubiquitination). Ubiquitin chains can be classified topologically into one of four types according to architecture: homogeneous chains, multiple chains (in which one substrate is separately modified by distinct chains), mixed chains (in which a tandem chain contains two linkage types), and branched chains. Lysine 48 (K48)-linked polyubiquitination induces proteasomal degradation of the target protein, whereas K63-linked polyubiquitination mediates signal transduction. Monoubiquitination, linear polyubiquitination, and K6-, K11-, K27-, K29-, and K33-linked ubiquitination are being investigated intensely to determine their divergent roles in innate immunity. Similar to conventional PTMs, unconventional PTMs also play a role in innate immune signal transduction. The transfer of acetyl groups from acetyl coenzyme A (acetyl-CoA) to the ε-amino acid groups of lysine residues (a process termed acetylation) results in charge neutralization, which alters the biological properties of proteins; in addition, lysine and arginine residues are inversely regulated by methyltransferases (a process termed methylation) and demethylases, and both acetylation and methylation play important roles in innate immune signaling.

Below we summarize the PTMs and other regulatory mechanisms of signaling molecules downstream of the RNA/DNA sensing cascade (also see Tables 1, 2, and 3).

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**Fig. 1** Regulatory host factors and interacting viral proteins of the RLR-mediated antiviral signaling pathway. Schematic representation of positive and negative regulatory host factors of Mitochondrial antiviral signaling protein (MAVS), TNF receptor-associated factor (TRAF3), TANK-binding kinase 1 (TBK1), NF-kappa-B essential modulator (NEMO), and IκB kinase (IKK) through posttranslational modifications (PTMs) or other regulatory mechanisms and viral proteins interacting with MAVS, TRAF3, TBK1, NEMO, or IKKε for viral evasion of the host immune response. The RLR-MAVS pathway consists of RIG-I and MDA5 as the main viral RNA sensors and the downstream signaling molecules MAVS and TRAF3, which activate IRF3/IRF7 via the kinases IKK and TBK1/IKKε. (Note: Host factors and viral proteins involved in TBK1 regulation upon infection with both RNA and DNA viruses are indicated as being common regulators in the figure.)
INNATE IMMUNE EVASION STRATEGIES USED BY RNA AND DNA VIRUSES

Viruses that have evolved with their host develop strategies to evade the innate immune system and ensure their replication and survival. Individual viruses or virus families use different strategies. This review explores the different mechanisms used by RNA and DNA viruses to subvert the functions of individual signaling molecules of the type 1 interferon (IFN) pathway. Many viruses use proteases to cleave target proteins, while some viral proteins promote the degradation of target innate immune signaling molecules. Furthermore, viral deubiquitinase enzymes remove K63-linked polyubiquitin chains from signaling molecules to prevent their activation, and viral E3 ubiquitin ligases transfer K48-linked polyubiquitin moieties to target molecules to trigger their proteasomal degradation. Some viral proteins recruit host E3 ubiquitin ligases to polyubiquitinate signaling molecules and increase their proteasomal degradation. The formation of signaling molecule complexes is crucial for downstream transduction of innate immune signals. Direct interactions with viral proteins inhibit the formation of signaling complexes such as the TRAF3, TANK, and TBK1 complexes. Another important mechanism of immune evasion is physical interaction between viral proteins and host signaling molecules, which prevents activation, dimerization, phosphorylation, or nuclear translocation. Below, we summarize the mechanisms underlying innate immune evasion mediated by viral proteins (also see Tables 4 and 5).

RNA-INDUCED SIGNAL TRANSDUCTION AND MECHANISMS UNDERLYING VIRAL EVASION OF HOST IMMUNITY

RLR (RIG-I-like receptor) family receptors are the main PRRs that detect intracellular viral RNA. The RLR family comprises RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). RIG-I and MDA5 are typical PRRs, whereas LGP2 is a regulator of RIG-I and MDA5-mediated signal transduction. RIG-I and MDA5 contain two N-terminal caspase-recruitment domains, a central DExD/H-box helicase domain, and a C-terminal domain. RIG-I and MDA5 bind to viral RNA in the cytoplasm via an RNA binding motif, after which the signaling domain interacts with the downstream adapter molecule MAVS via a CARD-CARD-mediated interaction. This interaction causes aggregation of MAVS to form a prion-like protein complex, which relays the signal to kinases such as TBK1, IKK-ε, and IRF-7. Activation of this cascade results in phosphorylation of the transcription factors IFN-regulating factor 3 (IRF-3) and IRF-7.

Fig. 2 Regulatory host factors and interacting viral proteins of the cGAS-mediated antiviral signaling pathway. Schematic representation of positive and negative regulatory host factors of 2',3'-cyclic GMP-AMP (2',3'-cGAMP), stimulator of interferon gene (STING), Interferon regulatory factor 3 (IRF-3), and IRF-7 through posttranslational modifications (PTMs) or other modifications and viral proteins interacting with cGAMP or STING for viral evasion of the host immune response. The STING-mediated signaling pathway includes cGAS as the key sensor molecule that is mainly involved in the recognition of viral DNA. This recognition triggers cGAMP production and binding of cGAMP with STING, which leads to activation of IRF-3/IRF-7 and induction of type 1 IFNs. TBK1, IRF-3, and IRF-7 are involved in the IFN signaling cascade initiated upon sensing of RNA and DNA viruses. (Note: Host factors and viral proteins involved in IRF-3/IRF-7 are indicated as being common regulators in the figure.).
Table 1. Host regulators of RLR-initiated antiviral signaling.

| Signaling molecule | Classification | Regulator | Function | Ref. |
|--------------------|----------------|-----------|----------|------|
| MAVS | PTMs | Positive | MAVS | Aggregation | 45 |
| | | | TRIM31 | Aggregation | 47 |
| | | | TRIM21 | K27-linked ubiquitination | 49 |
| | | | OGT | K63-linked ubiquitination | 48 |
| | | | OTUD4 | Deubiquitination | 51 |
| | | | TBK1 and IKKβ | Recruitment of IRF3 for its phosphorylation by TBK1 | 12 |
| | | Negative | RNF125 | Ubiquitination | 68 |
| | | | MARCH8 | K27-linked ubiquitination | 69 |
| | | | PCBP2 | K48-linked ubiquitination | 65 |
| | | | RNF5 | K48-linked ubiquitination | 52 |
| | | | TRIM25 | K48-linked ubiquitination | 59 |
| | | | Smurf1 | K48-linked ubiquitination | 60 |
| | | | Smurf2 | K48-linked ubiquitination | 61 |
| | | | RNF115 | K48-linked ubiquitination | 58 |
| | | | PCBP1 | K48-linked ubiquitination | 50 |
| | | | pVHL | K48-linked ubiquitination | 62 |
| | | | MARCH5 | K48-linked ubiquitination | 63 |
| | | | OTUD1 | K48-linked ubiquitination | 67 |
| | | | ITCH | K48-linked ubiquitination | 66 |
| | | | TAX1BP1 | K48-linked ubiquitination | 66 |
| | | | YOD1 | Deubiquitination | 64 |
| | | | NLK | Phosphorylation and degradation | 70 |
| | | | PPM1A | Dephosphorylation | 71 |
| | Other regulatory mechanisms | Positive | MFN1 | Abrogation of virus-induced redistribution of MAVS | 55 |
| | | | IFIT3 | Induction of bridging between MAVS and TBK1 | 168 |
| | | | NAC1 | Induction of bridging between MAVS and TBK1 | 56 |
| | | | FAK | Activation | 57 |
| | | | TRAF3 | Activation | 54 |
| | | Negative | PLK1 | Disruption of the MAVS-TRAF3 interaction | 77 |
| | | | UBXN1 | Interference with MAVS oligomerization and disruption of the MAVS/TRAFL3/TRAFL6 signalosome | 74 |
| | | | GPATCH3 | Disruption of virus-induced MAVS signalosome formation | 76 |
| | | | gC1qR | Physical interaction | 79 |
| | | | Mitofusin 2 | Physical interaction | 80 |
| | | | TTLL12 | Direct interaction with MAVS, TBK1 and IKKβ; inhibition of the interactions of MAVS with other signaling molecules | 73 |
| | | | Lactate | Direct interaction with MAVS to prevent MAVS aggregation | 72 |
| | | | ASC | Physical interaction | 81 |
| | | | PSMA7 | Physical interaction | 82 |
| | | | Rac1 | Inhibition of MAVS ubiquitination, aggregation, and activation | 78 |
| | | | LGP2 | Inhibition of IKKβ binding | 75 |
| TRAF3 | PTMs | Positive | RNF166 | Ubiquitination | 115 |
| | | | OPN | Deubiquitination | 117 |
| | | | cIAP1 | K63-linked ubiquitination | 111 |
| | | | cIAP2 | K63-linked ubiquitination | 111 |
| | | | TRIM24 | K63-linked ubiquitination | 113 |
| | | | LGALS3BP | K63-linked ubiquitination | 112 |
| | | | DDX3 | K63-linked ubiquitination | 110 |
| | | | TRIM35 | K63-linked ubiquitination | 114 |
| | | | CK1ε | Phosphorylation and promotes K63-linked ubiquitination | 116 |
| | | Negative | ERα | K48-linked ubiquitination | 128 |
the major innate signaling molecules, along with the immunomodulatory mechanisms by which viruses evade them.

REGULATION OF MAVS BY HOST FACTORS

MAVS, also called IPS1, VISA, and CARDIF, is a key adaptor protein for RIG-I-like receptor-initiated signal transduction. Upon viral infection, RIG-I and MDA5 bind to MAVS, thereby activating downstream signal transduction. The MAVS protein, which contains 540 amino acids encoded by the nuclear genome, is localized predominantly on the mitochondrial outer membrane. However, experimental evidence shows that it also localizes to mitochondrial-associated endoplasmic reticulum membranes and peroxisomes. MAVS contains three domains: a CARD, a middle proline-rich region, and a C-terminal transmembrane domain. The CARD interacts with CARDs in RIG-I and MDA5, activating MAVS, whereas the proline-rich region interacts with the tumor necrosis factor-related factor (TRAF) family members TRAF2, TRAF3, TRAF5, and TRAF6 to activate downstream signaling. The TM domain plays a crucial role by ensuring the localization of MAVS to the mitochondrial outer membrane. Upon binding to the CARDs of RIG-I and MDA5, MAVS rapidly forms prion-like aggregates, which convert other MAVS proteins present on the mitochondrial outer membrane into prion-like aggregates. Activation of MAVS through aggregation recruits TRAF2, TRAF3, TRAF5, and TRAF6 via the PRR to promote the formation of the TBK1 complex (comprising TBK1, IkKε, IKKε, and NEMO). It is not surprising that the expression of MAVS is regulated to ensure that RLR-mediated signaling cascades are not activated rapidly upon stimulation; indeed, its function at this stage of viral infection is to prevent rapid viral replication.

Self-association and prion-like aggregate formation are markers of MAVS activation. The E3 ubiquitin ligase Tripartite motif-containing protein (TRIM) 31 interacts with MAVS and catalyzes K63-linked polyubiquitination of aa residues K10, K311, and K461 in MAVS to promote the formation of aggregates. Interestingly, this phenomenon occurs upon viral infection in the presence of RIG-I; thus, recruitment of RIG-I may be required for TRIM31-mediated MAVS aggregation upon viral infection. Moreover, K63-linked polyubiquitination is enhanced by O-linked N-acetyl glucosamine (O-GlcNAc) transferase (OGT)-mediated glycosylation of MAVS. Another recent study suggested that K27-linked polyubiquitination of K325 in MAVS by the E3 ubiquitin ligase TRIM21 promotes downstream signaling activation. The PRY-SPRY domain of TRIM21 interacts with MAVS, while the RING (Really Interesting New Gene) domain transfers the E3 ubiquitin protein complex to MAVS. K48-linked ubiquitination of MAVS leads to its proteasomal degradation; thus, proteins that inhibit MAVS K48-linked ubiquitination are positive regulators of MAVS-mediated signaling. Ovarian tumor family deubiquitinase 4 (OTUD4) removes K48-linked ubiquitin chains from MAVS to inhibit its degradation. Moreover, the expression of cyclophilin A is upregulated upon viral infection; cyclophilin A competes with TRIM25 for binding to MAVS. Inhibiting TRIM25 promotes MAVS ubiquitination and degradation.

Phosphorylation is an important PTM that regulates MAVS signaling. Activated MAVS recruits TBK1 and IKKε to the complex. These kinases mediate the phosphorylation of MAVS, enabling the recruitment of IRF3. Recruited IRF3 is phosphorylated by TBK1, which increases its homodimerization and nuclear translocation. Similar to PTMs, non-PTMs play a crucial role in regulating MAVS signaling. Importantly, TRAF3 interacts with MAVS (aa 450–468),

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Table 1 continued

| Signaling molecule | Classification | Regulator | Function | Ref. |
|--------------------|----------------|-----------|----------|-----|
| Parkin             | K48-linked ubiquitination | 130       |
| Triad3A            | K48-linked ubiquitination | 131       |
| WDR82              | K48-linked ubiquitination | 129       |
| DUBA               | Deubiquitination       | 121       |
| MYSM1              | Deubiquitination       | 120       |
| USP19              | Deubiquitination       | 122       |
| FOSL1              | Deubiquitination       | 125       |
| OTUB1              | Deubiquitination       | 123       |
| OTUB2              | Deubiquitination       | 123       |
| UCHL1              | Deubiquitination       | 124       |
| SRA                | Deubiquitination       | 127       |
| HSCARG             | Deubiquitination       | 126       |

Other regulatory mechanisms

Positive

| DOK3              | TRAF3/TBK1 complex formation | 118       |
| RAβ18             | Facilitation of the interaction with MAVS | 119       |
| NEMO              | Disruption of the MAVS-TRAF3 complex | 132       |

Negative

| MARCH2            | K48-linked ubiquitination | 140       |
| TRIM29            | K48-linked ubiquitination | 141       |
| Rubicon           | Inhibition of ubiquitination | 142       |
| PGRN/A20          | Deubiquitination       | 143       |

IKKε

PTMs

Negative

| DDX19             | Degradation       | 210       |

Other regulatory mechanisms

Positive

| SPL                | Physical interaction | 208       |
| DDX3              | Activation         | 209       |

Negative

| Fascin1           | Physical interaction | 211       |

Self-association and prion-like aggregate formation are markers of MAVS activation. The E3 ubiquitin ligase Tripartite motif-containing protein (TRIM) 31 interacts with MAVS and catalyzes K63-linked polyubiquitination of aa residues K10, K311, and K461 in MAVS to promote the formation of aggregates. Interestingly, this phenomenon occurs upon viral infection in the presence of RIG-I; thus, recruitment of RIG-I may be required for TRIM31-mediated MAVS aggregation upon viral infection. Moreover, K63-linked polyubiquitination is enhanced by O-linked N-acetyl glucosamine (O-GlcNAc) transferase (OGT)-mediated glycosylation of MAVS. Another recent study suggested that K27-linked polyubiquitination of K325 in MAVS by the E3 ubiquitin ligase TRIM21 promotes downstream signaling activation. The PRY-SPRY domain of TRIM21 interacts with MAVS, while the RING (Really Interesting New Gene) domain transfers the E3 ubiquitin protein complex to MAVS, resulting in recruitment of TBK1 to MAVS. K48-linked ubiquitination of MAVS leads to its proteasomal degradation; thus, proteins that inhibit MAVS K48-linked ubiquitination are positive regulators of MAVS-mediated signaling. Ovarian tumor family deubiquitinase 4 (OTUD4) removes K48-linked ubiquitin chains from MAVS to inhibit its degradation. Moreover, the expression of cyclophilin A is upregulated upon viral infection; cyclophilin A competes with TRIM25 for binding to MAVS. Inhibiting TRIM25 promotes MAVS ubiquitination and degradation.

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resulting in activation of MAVS signaling. Mitofusin 1 (MFN1) binds to MAVS to increase MAVS redistribution; MFN1 positively regulates the RLR-mediated innate antiviral response. Furthermore, nucleus accumbens-associated 1 (NAC1), a member of the BTB/POZ family, acts as a bridge between MAVS and TBK1, thereby activating downstream signaling. In addition, focal adhesion kinase (FAK) interacts with MAVS at the mitochondrial membrane in a viral infection-dependent manner to potentiate MAVS-mediated signaling via a kinase-independent mechanism.

Negative regulation of MAVS is mediated mainly by K48-linked ubiquitination of MAVS, signaling blockade, autophagy, and apoptosis. K48-linked polyubiquitination of MAVS triggers its proteasomal degradation and abrogates RLR-mediated signal transduction. Experimental evidence has shown that several E3 ubiquitin ligases are involved in K48-linked ubiquitination of MAVS and its proteasomal degradation; these ligases include RING finger protein 5 (RNF5), RNF115, TRIM25, Smurfl, Smurf2, von Hippel-Lindau protein (pVHL), and membrane-associated RING finger protein 5 (MARCH5). Importantly, the ubiquitin thioesterase OTU1 (YOD1) cleaves the K63-linked ubiquitin moiety and abrogates the formation of prion-like aggregates by MAVS, thereby attenuating IRF3-mediated production of IFN-β. Moreover, interactions between several proteins mediate MAVS ubiquitination and degradation via recruitment of E3 ubiquitin ligases. For example, poly(RC) binding protein 1/2 (PCBP1/PCBP2) and tax1-binding protein 1 (TAX1BP1)-mediated K48-linked ubiquitination of MAVS via AIP4/ITCH triggers proteasomal degradation of MAVS. Similarly, Smurfl-mediated K48-linked ubiquitination is upregulated by OTUD1. The E3 ubiquitin ligase RNF125 conjugates ubiquitin to MAVS, thereby suppressing its function, and K27-linked ubiquitination of MAVS mediated by the E3 ubiquitin ligase MARCH8 recruits the autophagy protein NDP52, resulting in lysosomal degradation of MAVS. Additional mechanisms that negatively regulate MAVS-mediated RLR signaling are phosphorylation and degradation of MAVS via Nemo-like kinase (NLK) and 2-BP. Protein phosphatase magnesium-dependent 1A (PPM1A; also called PP2Cα) is an inherent component of the TBK1/IKKe complex, which targets both MAVS and TBK1/IKKe for dephosphorylation, thereby disrupting MAVS-driven formation of the signaling complex.

Direct protein–protein interactions and signal blockade are other mechanisms that downregulate MAVS-mediated RLR signaling:

### Table 2. Host regulators of cGAS-initiated antiviral signaling.

| Signaling molecule | Classification | Regulator | Function | Ref. |
|--------------------|---------------|----------|----------|------|
| 2',3'-cGAMP        | Positive      | LRRC8    | Transportation | 290 |
|                    | Negative      | ENPP1    | Hydrolysis | 291 |
|                    |               |          | Physical interaction & hydrolysis | 292 |
| STING              | PTMs          | AMFR/INSIG1 | K27-linked ubiquitination | 304 |
|                    |               | MUL1     | K63-linked ubiquitination | 301 |
|                    |               | TRAF6    | K63-linked ubiquitination | 302 |
|                    |               | UBXN3B   | K63-linked ubiquitination | 303 |
|                    |               | RNF115   | K63-linked ubiquitination | 59  |
|                    |               | CYLD     | Deubiquitination | 308 |
|                    |               | OTUD5    | Deubiquitination | 309 |
|                    |               | USP44    | Deubiquitination | 307 |
|                    |               | USP20/USP18 | Deubiquitination | 305 |
|                    |               | USP20    | Deubiquitination | 306 |
|                    |               | iRhom2   | Deubiquitination | 310 |
|                    |               | CSK      | Phosphorylation | 315 |
|                    |               | TBK1     | Phosphorylation | 313 |
|                    |               | STING    | Palmitoylation | 312 |
|                    | Negative      | USP13    | K33-linked ubiquitination | 324 |
|                    |               | TRIM30α  | K48-linked ubiquitination | 321 |
|                    |               | TRIM29   | K48-linked ubiquitination | 320 |
|                    |               | RNF90    | K48-linked ubiquitination | 319 |
|                    |               | RNF5     | K48-linked ubiquitination | 318 |
|                    |               | USP49    | Deubiquitination | 323 |
|                    |               | USP21    | Deubiquitination | 322 |
|                    |               | PTPN1/2  | Dephosphorylation & degradation | 325 |
|                    |               | PPM1A    | Dephosphorylation | 314 |
|                    |               | 2-BP     | Inhibition of palmitoylation | 311,312 |
|                    | Other regulatory mechanisms | ZDHHC1 | Physical interaction | 315 |
|                    |               | TMED2    | Physical interaction | 316 |
|                    |               | SNX8     | Translocation | 317 |
|                    | Negative      | Atg9a    | Colocalization | 329 |
|                    |               | MRP      | Physical interaction | 326 |
|                    |               | NLRX1    | Physical interaction | 327 |
|                    |               | RIG-1/IL-6 | Degradation | 328 |
Table 3. Host regulators commonly involved in RLR/cGAS-initiated antiviral signaling.

| Signaling molecule | Classification | Regulator | Function | Ref. |
|--------------------|----------------|----------|----------|------|
| TBK1               | PTMs           | Positive | MIB      | K63-linked ubiquitination | 161 |
|                    |                |          | TBK1     | K63-linked ubiquitination | 154 |
|                    |                |          | Nrdp1    | K63-linked ubiquitination | 163 |
|                    |                |          | RNF128   | K63-linked ubiquitination | 162 |
|                    |                |          | USP1–UA1 complex | Deubiquitination | 164 |
|                    |                |          | UBQLN2   | Phosphorylation | 159 |
|                    |                |          | Src      | Autophosphorylation | 160 |
|                    |                |          | TBK1     | Autophosphorylation | 155 |
|                    |                |          | BK1P     | Autophosphorylation | 158 |
|                    |                |          | GSK3β    | Self-association and autophosphorylation | 157 |
|                    |                |          | Dnmt3a   | Recruitment of HDAC9 for deacetylation | 165 |
|                    |                |          | HDAC3    | Deacetylation | 166 |
|                    |                |          | TRIM9    | Recruitment of GSK3β for activation | 157 |
|                    |                | Negative | ASB8     | K48-linked ubiquitination | 172 |
|                    |                |          | USP38    | K48-linked ubiquitination | 176 |
|                    |                |          | DYRK2    | K48-linked ubiquitination | 174 |
|                    |                |          | THOC7    | K48-linked ubiquitination | 175 |
|                    |                |          | TRIP     | K48-linked ubiquitination | 173 |
|                    |                |          | Siglec1  | Recruitment of TRIM27 for K48-linked ubiquitination | 177 |
|                    |                |          | NLRP4    | Recruitment of DTX4 for K48-linked ubiquitination of TBK1 | 178 |
|                    |                |          | A20 and TAX1BP1 | Inhibition of K63-linked ubiquitination | 179 |
|                    |                |          | UBE2S    | Recruitment of USP15 for deubiquitination | 181 |
|                    |                |          | USP2b    | Deubiquitination | 180 |
|                    |                |          | CYLD     | Deubiquitination | 37 |
|                    |                |          | TIPARP   | ADP-ribosylation & TBK1 deactivation | 183 |
|                    |                |          | Lck/Hck/Fgr | Disruption of dimerization and activation | 182 |
|                    |                |          | PPM1B    | Dephosphorylation | 185 |
|                    |                |          | PP4      | Dephosphorylation and Deactivation | 186 |
|                    |                |          | Cdc25A   | Dephosphorylation | 184 |
|                    | Other regulatory mechanisms | Positive | MSX1     | Induction of the assembly of TBK1-associated complexes | 118 |
|                    |                |          | DOK3     | Facilitation of TRAF3/TBK1 complex formation | 170 |
|                    |                |          | IFIT3    | Bridging of TBK1 to MAVS on mitochondria | 168 |
|                    |                |          | BTN3A1   | Transport of the TBK1/IRF3 complex to the perinuclear region | 167 |
|                    |                |          | PLA1A    | Phosphorylation and modulation of mitochondrial morphology | 171 |
|                    |                | Negative | TRIM26   | Induction of TBK1/NEMO interaction | 169 |
|                    |                |          | NLRP2    | Disruption of IRF3 binding | 187 |
|                    |                |          | MIP-T3   | Inhibition of TRAF3/TBK1 complex formation | 189 |
|                    |                |          | ISG56    | Disruption of the interaction between MITA and MAVS or TBK1 | 190 |
|                    |                |          | ERRα     | Inhibition of the TBK1-IRF3 interaction | 188 |
|                    |                |          | INKIT    | Physical interaction | 191 |
| IRF3               | PTMs           | Positive | NSD3     | Methylation | 223 |
|                    |                |          | HSPD1    | Phosphorylation and dimerization | 61 |
|                    |                |          | lnClrrc55-AS | Phosphorylation | 221 |
|                    |                | Negative | RBCK1    | Ubiquitination | 226 |
|                    |                |          | RAUL     | K48-linked ubiquitination | 229 |
|                    |                |          | TRIM26   | K48-linked ubiquitination | 230 |
signaling. Recent studies have shown that lactate, the end product of anaerobic glycolysis, acts as a negative regulator of RLR signal transduction by interacting with the TM domain of MAVS and preventing its mitochondrial localization and aggregation. Tubulin tyrosine ligase-like protein 1 (TTLL12) interacts with MAVS, TBK1, and IKKε to prevent interactions between MAVS and other molecules. However, upon viral infection, TTLL12 expression decreases, thereby activating downstream MAVS signaling via the release of MAVS blockade. During the late stage of viral infection, MAVS function is negatively regulated by UBX-domain-containing protein 1 (UBXN1). The expression of UBXN1 increases at the late stage of infection, and it then competes with TRAF3/TRAF6 for binding to MAVS. Additionally, the gpatch domain-containing protein 3 (GPATCH3) binds to MAVS to prevent MAVS/TRAF6/TBK1 complex formation, whereas binding of polo-like kinase 1 (PLK-1) to MAVS disrupts its interaction with TRAF3. The Rho family small guanosine triphosphatase Ras-related C3 botulinum toxin substrate 1 (Rac1) limits the interaction between MAVS and the E3 ubiquitin ligase-like activity of rotavirus NSP1 means that its interaction with the MAVS CARD or TM domain leads to ubiquitin-dependent proteasomal degradation of MAVS. Addition-ally, the structural protein VP3 of RV upregulates the phosphorylation of MAVS, leading to its K48-linked ubiquitination and degradation. Hepatitis B virus (HBV) protein X (HBX) binds to MAVS and promotes its ubiquitination and proteasomal degradation via an unknown E3 ubiquitin ligase. Additionally, HBV-induced Parkin recruits the linear ubiquitin assembly complex to mitochondria and abrogates IFN-β synthesis. Severe acute respiratory syndrome coronavirus (SARS-CoV-2) open reading frame 9b (ORF-9b) catalyzes K48-linked ubiquitination of MAVS via the PCBP2-AIP4 axis. Moreover, HCV infection induces the expression of Golgi protein 73 (GP73), which mediates the proteasomal degradation of MAVS. HCV infection upregulates NLRX1 and recruits PCBP2 to MAVS, thereby triggering K48-linked ubiquitination and degradation of MAVS with the help of AIP4. In addition, the interaction between the HCV NS5A protein and MAVS prevents the binding of the latter to TRAF3 and TRAF6. The Nipah virus (NiV) V protein...

### Table 3 continued

| Signaling molecule | Classification | Regulator | Function | Ref. |
|-------------------|----------------|-----------|----------|-----|
| Ro52              | Ubiquitination | 227       |          |     |
| Pin1              | Ubiquitination | 225       |          |     |
| OTUD1             | Deubiquitination | 231     |          |     |
| Mst1              | Phosphorylation | 235       |          |     |
| PP2A              | Deyphosphorylation | 234     |          |     |
| MKP5              | Deyphosphorylation | 233     |          |     |
| DD5X5             | Deyphosphorylation | 237     |          |     |
| FBXO17            | Deyphosphorylation | 236     |          |     |
| HDAC4             | Inhibition of phosphorylation | 238     |          |     |
| IFITM3            | Autophagic degradation | 232     |          |     |
| SENP2             | DeSUMOylation   | 239       |          |     |

Other regulatory mechanisms:
- **Positive**
  - USP22: Nuclear translocation
  - IRF1: Activation
- **Negative**
  - A20: Deubiquitination
  - TRIM28: SUMOylation
  - TRIM21/RO52: Degradiation

IRF7

- **PTMs**
  - **Positive**
    - TRAF6: K63-linked ubiquitination
  - **Negative**
    - Nmi: K48-linked ubiquitination
    - A20: Deubiquitination
    - TRIM28: SUMOylation
    - TRIM21/RO52: Degradiation

Other regulatory mechanisms:
- **Negative**
  - IFI204: Physical interaction
  - ATF4: Physical interaction
  - HSP70: Physical interaction

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**REGULATION OF MAVS BY VIRAL PROTEINS**

From the perspective of the virus, it is important to avoid the host innate immune response during the early stage of infection. Since MAVS plays a critical role as a central adaptor molecule in the RLR-mediated signaling cascade, the genomes of many viruses encode proteins that interfere with MAVS. For example, enterovirus 71 (EV71) cysteine protease 2Apro cleaves MAVS at Gly209, Gly251, and Gly265. This was the first viral protein found to cleave MAVS at multiple aa residues. The small RNA viruses human rhinovirus C, coxsackievirus B3 (CVB3), and Seneca Valley virus (SVV) encode a cysteine protease, 3Cpro, which cleaves MAVS at Gln148 to prevent signal transduction. In addition, CVB3 encode another MAVS-cleaving protease named 2Apro; however, its cleavage site is unclear. Porcine reproductive and respiratory syndrome virus (PRRSV) produces a 3C-like serine protease (3CLSP) that cleaves MAVS at Glu268. Additionally, NS3-4A of hepatitis C virus (HCV38,89 and the 3ABC precursor of 3C90 of hepatitis A virus91 cleave MAVS to disrupt activation of its downstream signaling. The E3 ubiquitin ligase-like activity of rotavirus NSP1 means that its interaction with the MAVS CARD or TM domain leads to ubiquitin-dependent proteasomal degradation of MAVS. Additionally, the structural protein VP3 of RV upregulates the phosphorylation of MAVS, leading to its K48-linked ubiquitination and degradation. Hepatitis B virus (HBV) protein X (HBX) binds to MAVS and promotes its ubiquitination and proteasomal degradation via an unknown E3 ubiquitin ligase. Additionally, HBV-induced Parkin recruits the linear ubiquitin assembly complex to mitochondria and abrogates IFN-β synthesis. Severe acute respiratory syndrome coronavirus (SARS-CoV-2) open reading frame 9b (ORF-9b) catalyzes K48-linked ubiquitination of MAVS via the PCBP2-AIP4 axis. Moreover, HCV infection induces the expression of Golgi protein 73 (GP73), which mediates the proteasomal degradation of MAVS. HCV infection upregulates NLRX1 and recruits PCBP2 to MAVS, thereby triggering K48-linked ubiquitination and degradation of MAVS with the help of AIP4. In addition, the interaction between the HCV NS5A protein and MAVS prevents the binding of the latter to TRAF3 and TRAF6. The Nipah virus (NiV) V protein...
| Signaling molecules | Virus | Virulence factor | Function | Ref. |
|---------------------|-------|------------------|----------|-----|
| MAVS                | HCV   | NS3-4A           | Cleavage | 38,89 |
|                     | HAV   | 3ABC             | Cleavage | 91  |
|                     | CVB3  | 3C<sub>pro</sub> | Cleavage | 85  |
|                     | EV71  | 2A<sub>pro</sub> | Cleavage | 83  |
|                     | CVB3  | 2A<sub>pro</sub> | Cleavage | 87  |
|                     | PRRSV | 3CLSP            | Cleavage | 88  |
|                     | SVV   | 3C<sub>pro</sub> | Cleavage | 86  |
|                     | HBV   | HBX              | Ubiquitination | 95  |
|                     | RV    | NSP1             | Degradation | 93  |
|                     | SARS-CoV | ORF9b        | Degradation | 97  |
|                     | SARS-CoV-2 | M         | Inhibition of RIG-I, MAVS, TRAF3 and TBK-1 complex formation | 26  |
|                     | SARS-CoV-2 | M         | Inhibition of MAVS aggregation | 105 |
|                     | RV    | VP3             | Proteosomal degradation | 94  |
|                     | RSV   | NS1             | Inhibition of the MAVS-RIG-I interaction | 102 |
|                     | hMPV  | M2-2            | Inhibition of TRAF3-, TRAF5- and TRAF6-mediated recruitment of MAVS | 104 |
|                     | HBV   | Recruitment of LUBAC & disruption of MAVS signalosome formation | 96  |
|                     | HCV   | Recruitment of PCBP2 to MAVS and induction of K48-linked ubiquitination | 25  |
|                     | HCV   | Regulate the interaction between GP73 and MAVS for proteasomal degradation | 98  |
|                     | NiV   | V               | Stabilization of UBXN1 and enhancement of its interaction with MAVS | 100 |
|                     | HCV   | N55A            | Inhibition of the MAVS-TRAF3 interaction | 99  |
|                     | FMDV  | VP1             | Inhibition of the TRAF3-MAVS interaction | 101 |
|                      | TRAF3 | SARS-CoV | M | Inhibition of TRAF3, TANK, and TBK1/IKKe complex formation | 27  |
|                     | FMDV  | L<sub>pro</sub> | Deubiquitination | 133 |
|                     | HSV   | UL36            | Deubiquitination | 134 |
|                     | EV-D68 | 2A<sub>pro</sub> | Cleavage | 135 |
|                     | NEMO  | PDCoV           | nsp5     | Cleavage | 150 |
|                     | FMDV  | 3C<sub>pro</sub> | Cleavage | 144 |
|                     | PRRSV | NSP4            | Cleavage | 146,147 |
|                     | HAV   | 3C<sub>pro</sub> | Cleavage | 145 |
|                     | PEDV  | NSP5            | Cleavage | 149 |
|                     | EAV   | NSP4            | Cleavage | 147 |
|                     | FIP   | NSP5            | Cleavage | 148 |
|                     | Influenza virus | – | Enhancement of the PGRN level to inhibit K63-linked ubiquitination | 143 |
|                     | SARS-CoV-2 | ORF9b | Deubiquitination of NEMO | 151 |
|                     | TBK1  | GCRV            | –        | K48-linked ubiquitination | 24  |
|                     | MHV   | PLP2            | Deubiquitination | 195 |
|                     | FMDV  | L<sub>pro</sub> | Inhibition of TBK1 ubiquitination and activation | 133 |
|                     | SFTSV | NS              | Sequestration of the TBK1/IKKe complex into inclusion bodies | 197,198 |
|                     | SARS-CoV | PLpro | Disruption of the STING-TRAF3-TBK1 interaction | 204 |
|                     | DENV  | NS              | Inhibition of phosphorylation | 194 |
|                     | HRTV  | NS              | Inhibition of TBK1 and IRF3 interaction | 201 |
|                     | PEDV  | N               | Inhibition of the association between TBK1 and IRF3 by sequestration | 200 |
|                     | MCV   | MC159/MC160     | Impairment of activation | 205 |
|                     | ZIKV  | NSS             | Impairment of activation | 202 |
|                     | SARS-CoV-2 | NSP13 | Inhibition of phosphorylation | 192 |
|                     | SARS-CoV-2 | NSP13 | Disruption of the TBK1-MAVS interaction | 203 |
|                     | HRTV  | NS              | Inhibition of phosphorylation | 193 |
|                     | IKKe  | MERS-CoV | ORF8b | Inhibition of HSP70-dependent activation | 213 |
|                     | DENV  | NS2B            | Binding and inhibition of kinase activity | 212 |
|                     | HCV   | NS2             | Inhibition of IRF3 phosphorylation via interaction with IKKe | 216 |
interacts directly with UBXN1 to enhance the interaction between MAVS and UBXN1 via protein stabilization. A recent study showed that the wild-type VP1 (83E) but not the mutant VP1 (83K) protein of foot and mouth disease (FMDV) subverts MAVS signaling by disrupting the interaction between MAVS and TRAF3. Moreover, the NS1 and N proteins of respiratory syncytial virus attenuate the production of type I IFNs during infection by inhibiting the MAVS/RIG-I interaction and by localizing MAVS in inclusion bodies, respectively. The human metapneumovirus (hMPV) M2-2 protein prevents recruitment of the MAVS downstream adaptors TRAF3, TRAF5, and TRAF6. Interestingly, a recent study showed that the M protein of SARS-CoV-2 impairs MAVS aggregation and the recruitment of downstream TRAF3, TBK1, and IRF3, while another study reported that SARS-CoV-2 M2 inhibits RIG-I/MAVS/TRAF3 and TBK-1 complex formation and subsequent nuclear translocation of IRF3. Viral proteins known to interact with or affect MAVS are listed in Table 4.

**REGULATION OF TRAF3 BY HOST FACTORS AND VIRAL PROTEINS**

TRAF3 (also called Amn, CAP-1, CD40bp, CRAF1, LAP1, or T-BAM) is one of the most enigmatic, ubiquitously expressed members of the TRAF family. The protein contains 568 amino acids (64.295 kDa) and a typical C3HC4 RING finger domain upstream of five zinc fingers, an isoleucine zipper, and a TRAF3 domain in the C-terminal region. The TRAF domain is critical for binding to the cytoplasmic domain of tumor necrosis factor receptor (TNFR) family members and intracellular signaling mediators and for the formation of homo- or heterodimers. TRAF3 forms a stable complex with MAVS, which recruits kinases and IRF3 to itself, ultimately leading to IRF3 activation and nuclear translocation. The E3 ubiquitin ligases DEAD-box helicase 3 (DDX3), cIAP1, cIAP2, galectin 3 binding protein (LGALS3BP), TRIM24, and TRIM35 trigger K63-linked polyubiquitination of TRAF3. This modification of TRAF3 enables its association with MAVS and TBK1, which activates downstream antiviral signaling. Moreover, the E3 ubiquitin ligase RING finger protein 166 transfers ubiquitin to TRAF3 upon RNA virus infection, thereby activating IFN-β production. The serine-threonine kinase CK1ɛ interacts with TRAF3 and phosphorylates it on Ser349, which promotes Lys63 (K63)-linked ubiquitination of TRAF3 and subsequent recruitment of the kinase TBK1 to TRAF3. Osteopontin (OPN) interacts with TRAF3 to inhibit Triad3A-mediated K48-linked polyubiquitination and degradation of TRAF3. Downstream of kinase 3 (DOK3) interacts with TRAF3 through its tyrosine-rich CTD to induce TRAF3/TBK1 complex formation, whereas the interaction...
between TRAF3 and the GTPase-trafficking protein RAB1B facilitates the formation of the TRAF3/MAVS complex. As mentioned above, K63-linked polyubiquitination plays a critical role in activating TRAF3. Therefore, the deubiquitinases MYSM11120, DUBA1121, USP191122, OTUB1, OTUB21123, UCHL11124, and FOSL11125 remove ubiquitin chains from TRAF3 to negatively regulate its function. In addition, scavenger receptor A (SRA) and HSCARG1126 negatively regulate the stability of the TRAF3 protein by promoting recruitment of OTUB1 to TRAF31127. K48-linked polyubiquitination and degradation of TRAF3 mediated by estrogen receptor-alpha (ERα)1128, WD repeat domain (WDR) 82139, Parkin130, and Triad3A131 is another mechanism that downregulates IFN production via targeting of TRAF3. Linear-ubiquitinated NEMO associates with TRAF3 and disrupts the MAVS-TRAF3 complex, thereby inhibiting IFN activation132.

Since K63-linked polyubiquitination plays an important role in TRAF3-mediated signaling, it comes as no surprise to see that viruses encode proteins that inhibit TRAF3 ubiquitination to overcome host innate responses. The leader proteinase (Lpro) of FMDV133 and the ubiquitin-specific protease (UL36) of herpes simplex virus 1 (HSV-1)134 act as viral deubiquitinases that mediate TRAF3 deubiquitination, leading to downregulation of TRAF3 signaling. The nonstructural protein 2A protease (2Apro) of human enterovirus D68 (EV-D68) cleaves TRAF3 at G462135. The M protein of SARS-CoV forms a complex with TRAF3, TANK, and the TBK1/IKKε complex to inhibit TBK1/IKKε-dependent activation of the IRF3/IRF7 transcription factors27.

### REGULATION OF NEMO BY HOST FACTORS AND VIRAL PROTEINS

NF-κB essential modulator (NEMO or IKKγ), which contains 419 aa, is the integral regulatory scaffolding protein of the canonical IKK complex located at the center of both the NF-κB and type I IFN signaling cascades136. The IKK complex comprises two kinases, IKKa and IKKB, and a regulatory subunit, NEMO137. For appropriate assembly of the IKK complex, NEMO associates with TRAF3 and disrupts the MAVS-TRAF3 complex, thereby inhibiting IFN activation132.

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domain from the protein and impairing the ability of NEMO to noncanonical kinase IKK. Furthermore, the structure of TBK1 is similar to that of the acts as a regulatory domain by binding to the functional domains mieties from NEMO, thereby inhibiting its activation and subsequent signal transduction upon viral infection. Additionally, progranulin (PGRN) is expressed during influenza virus infection; PGRN interacts directly with NEMO and removes conjugated ubiquitin chains from K264 of NEMO, resulting in impaired activation of downstream signaling.

Viruses can escape antiviral immune responses by promoting cleavage or degradation of NEMO. Many viruses encode proteases that cleave NEMO independent of proteasomal degradation or apoptosis to inhibit RLR signaling. For example, 3C90 of FMDV specifically targets NEMO at Gln383, cleaving the C-terminal ZF domain from the protein and impairing the ability of NEMO to activate downstream IFN production. Additionally, the HAV 3C protease (3Cpro) cleaves NEMO at Q304, thereby abolishing its signaling adaptor function and abrogating the induction of IFN-β synthesis. NSP4, a viral 3C-like serine protease of PRRSV, cleaves NEMO at E166, E171, and E349, while NSP4 of equine arteritis virus, which is similar to NSP4 of PRRSV, cleaves NEMO at E166, E171, Q205, and E349 to inhibit downstream signaling and maintain viral infection. NSP5 of feline infectious peritonitis virus and NSP5 encoded by porcine epidemic diarrhea virus (PEDV) cleave NEMO at Q132, Q205, Q231, and Q231, resulting in downregulation of immune signaling. Similarly, NSP5 of porcine deltacoronavirus (PDCoV) cleaves NEMO at Q231 to impair the ability of NEMO to activate the IFN response and downstream signaling. Furthermore, ORF9b of SARS-CoV-2 disrupts K63-linked polyubiquitination of NEMO, thereby downregulating IFN production during SARS-CoV-2 infection.

REGULATION OF TBK1 BY HOST FACTORS

TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1, also called NAK or T2K) is one of two noncanonical IKKs implicated in regulating the activation of IRF3/IRF7 and the NF-κB signaling pathway. TBK1 is a 729 aa protein (84 kDa) containing an N-terminal kinase domain (KD), a ubiquitin-like domain (ULD), and two C-terminal coiled-coil domains. The ULD acts as a regulatory domain by binding to the functional domains of TBK1 as well as to substrates such as IRF3/IRF7, thereby enabling the KD to phosphorylate target substrate proteins. Furthermore, the structure of TBK1 is similar to that of the noncanonical kinase IKKε; indeed, both kinases always work together. Cellular expression of TBK1 is ubiquitous; thus, it plays an indispensable role in antiviral innate immunity. Upon infection with RNA viruses, TBK1 is activated by the upstream protein MAVS, and activated TBK1 recruits IRF3 and IRF7; these proteins undergo TBK1-mediated C-terminal phosphorylation to trigger their dimerization and nuclear translocation, an event followed by induction of IFN secretion.

As a vital kinase that regulates the activation of IRF3/IRF7 and the subsequent expression of IFN, the function of TBK1 must be regulated to maintain immune homeostasis and suppress viral replication. Therefore, several regulatory factors target TBK1 to control its function, while viruses have evolved mechanisms to disable it. Moreover, TRAF family E3 ubiquitin ligase-mediated K63-linked polyubiquitination of intact dimerized TBK1 at Lys30 and Lys401 results in transautophosphorylation on Ser172, which marks TBK1 for phosphorylation-mediated activation. Glycogen synthase kinase 3β (GSK3β) facilitates the aforementioned autophosphorylation of TBK1 at Ser172. TRIM9 short isofrom (TRIM9s) facilitates the recruitment of GSK3β to TBK1 upon viral infection, and Raf kinase inhibitory protein serves as a positive regulator; both of these proteins promote autophosphorylation of TBK1. Moreover, ubiquitin 2 (UBQLN2) promotes the stability and facilitates the phosphorylation of TBK1. Ubiquitination also plays a critical role in the activation of TBK1. Mindbomb E3 ubiquitin-protein ligase 1 (MIB1) and MIB2, ring finger protein 128 (RNF128), and neuregulin receptor degradation protein 1 (Nrdp1/RNF41) activate TBK1 by promoting its K63-linked ubiquitination. The deubiquitinase complex comprising ubiquitin-specific peptidase 1 (USP1) and USP1-associated factor 1 (UAF1), binds to TBK1 to remove K48-linked polyubiquitination and reverse the degradation process. The DNA methyltransferase Dnmt3a maintains high expression of the histone deacetylase HDAC9, which maintains deacetylation of TBK1 and increases its kinase activity, whereas HDAC3 positively regulates TBK1 in the same manner as HDAC9. Additionally, butyrophilin 3A1 (BTN3A1) interacts with TBK1 to facilitate its dynein-dependent transport to the perinuclear region to promote its association with IRF3 after viral infection. INI3-associated protein with tetratricopeptide repeats 3 (IFIT3) mediates the bridging of TBK1 to MAVS on mitochondria. Additionally, the E3 ubiquitin ligase TRIM26 bridges the interaction between NEMO and TBK1, which facilitates immune activation upon viral infection. Moreover, the homeobox protein MSX1 and docking protein 3 (DOK3) positively regulate TBK1 function to facilitate complex formation, and PLA1A upregulates TBK1 recruitment to mitochondria via modulation of mitochondrial morphology. In contrast, several TBK1-regulating proteins negatively impact TBK1. K48-linked polyubiquitination of TBK1 induced by E3 ubiquitin ligases such as SOCS box-containing 8 (ASB8), TRAF-interacting protein, dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2), and THO complex subunit 7 homolog (THOC7) triggers proteasomal degradation of TBK1 and ultimately terminates immune activation. Interestingly, USP38 permits K48-linked ubiquitination and subsequent degradation of TBK1 by specifically removing K33-linked ubiquitin chains from the same lysine site on TBK1. Additionally, Siglecs recruit TRIM27 and NLRP4 recruits DTX4 to trigger K48-linked polyubiquitination of TBK1. As noted above, K63-linked polyubiquitination plays a crucial role in activating TBK1. Therefore, any protein that disrupts the ubiquitin chain can be considered a negative regulator. For example, the deubiquitinating enzyme cylindromatosis (CYLD) removes K63-linked polyubiquitin moieties from TBK1, and the A20 regulatory complex (comprising the ubiquitin-editing enzyme A20, Taxi-binding protein 1 (TAX1BP1, also called T6BP or TXBP151), and ubiquitin-specific protease (USP) 2b (USP2b) antagonize K63-linked polyubiquitination of TBK1. Moreover, UBE2S recruits USP15 to TBK1, thereby removing K63-linked polyubiquitin chains. The Src family kinases Lck, Hck, and Fgr phosphorylate TBK1 directly at Tyr354/394 to prevent its dimerization and activation. The ADP-ribosylase TIPARP interacts with TBK1 to suppress its activity via ADP-ribosylation. The phosphatase Cdc25A dephosphorylates TBK1 at its activation site (S172) upon viral infection. Moreover, upon infection with RNA viruses, protein phosphatase 1B (PPM1B), Cdc25A, and protein phosphatase 4 (PP4) dephosphorylate Ser172 of TBK1 to prevent continuous activation of TBK1. Preventing protein–protein interactions is another method of inhibiting TBK1-driven immune activation. NOD-like receptors (e.g., NLRP2) and estrogen-related receptor α (ERRα) inhibit the interaction between TBK1 and IRF3, while MIP-T3 prevents the formation of the TRAF3/TBK1 complex. Additionally, ISG56
prior characteristic of the innate immune system, by activating the pattern recognition receptors (PRRs) on the cell surface, and by producing type I interferons (IFNs) to induce antiviral responses.

**REGULATION OF TBK1 BY RNA VIRAL PROTEINS**

TBK1 is a member of the IκB kinase (IKK) family that has been studied extensively due to its ability to activate type I IFN responses. It is a 716 aa protein comprising a KD, a ULD, and a scaffold dimerization domain. The KD of IKK is a 47.2 kDa protein that is expressed ubiquitously in tissues. IRF3 (also called IIAE7) is a master transcription factor responsible for the induction of innate antiviral immunity. It is a 427 aa (47.219 kDa) protein that is expressed ubiquitously in tissues. IRF3 contains an N-terminal DNA binding domain (DBD) and a C-terminal transactivation domain. After IRF3 activation, it is not surprising that IRF3 function is both positively and negatively regulated by host proteins or that viruses have evolved mechanisms to abolish protein expression. The long noncoding RNA (lncRNA) lncLrrc55-AS recruits methylesterase 1 (PME-1) to promote the interaction between PME-1 and the phosphatase PP2A, an inhibitor of IRF3 phosphorylation. Similarly, IRF1 interacts with IRF3 to augment the activation of IRF3 by blocking the interaction between IRF3 and PP2A. Heat shock protein family D (Hsp60) member 1 facilitates the phosphorylation and dimerization of IRF3 and increases IFN-β induction induced by SeV infection. The lysine methyltransferase nuclear receptor-binding SET domain 3 (NSD3) binds directly to the IRF3 C-terminal region through its PWWP1 domain and methylates IRF3 at K366. Monomethyltransferase maintains IRF3 phosphorylation by promoting the dissociation of IRF3 from the protein phosphatase PP1cc, thereby promoting the production of type I IFN. The deubiquitinating enzyme USP22 deubiquiti nates and stabilizes KPNA2 after viral infection, thereby facilitating efficient nuclear translocation of IRF3.

**REGULATION OF IKKe BY HOST FACTORS AND VIRAL PROTEINS**

IKKe (originally called IKKi) is a noncanonical member of the IkB kinase family that has been studied extensively due to its ability to promote type I IFN responses. It is a 716 aa protein comprising a KD, a ULD, and a scaffold dimerization domain. The KD of IKKe shares 49% identity and 65% similarity with that of TBK1. Activation of TBK1 and IKKe promotes phosphorylation and nuclear translocation of IRF3 and 7, leading to transcriptional upregulation of type I IFNs during the induction of the innate immune response. During the innate immune response, TBK1 and IKKe exhibit functional redundancy, although TBK1 appears to be more important than IKKe. The IKK subunit NEMO promotes activation of TBK1 and IKKe downstream of cytoplasmic DNA signaling, whereby ubiquitinated NEMO recruits IKKB to facilitate activation of TBK1 or IKKe.

Biochemical analysis has revealed that the interaction between sphingosine 1-phosphate (S1P) lyase and IKKe leads to IKKe-driven activation of IFN signaling. Viral infection triggers an interaction between DDX3 and IKKe. Expression of DDX3 amplifies TBK1/IKKe-mediated induction of the IFN-β promoter. DEX/D/H-box RNA helicase 19 (DDX19) recruits Lamtor2 to form the TBK1/IKKe-Lamtor2/DDX19/IRF3 complex, which suppresses IFN production by promoting degradation of TBK1 and IKKe. Fasclin1, an actin-bundling protein, interacts with IKKe to suppress the RIG-I-mediated signaling cascade in colon cancer cells.

To date, few studies have been conducted on viral proteins that interfere with the signaling mechanisms of IKKe. NS2B/3 of DENV interacts directly with IKKe; computational analysis revealed that via this interaction, NS2B/3 masks the KD of IKKε and potentially affects its functionality, thereby impairing the phosphorylation and nuclear translocation of IRF3. Interestingly, NS2 of HCV interacts physically with the IKKε/TBK1 kinase complex, thereby inhibiting IRF3 phosphorylation. Moreover, the VP35 protein of Ebola virus (EBOV) interacts with IKKε and TBK1 during the early phase of viral infection; this physical interaction with IKKε further prevents the interaction of IKKε with IRF3, IRF7, and MAVS.

**REGULATION OF IRF3 BY HOST FACTORS**

IRF3 (also called IIAE7) is a master transcription factor responsible for the induction of innate antiviral immunity. It is a 427 aa (47.219 kDa) protein that is expressed ubiquitously in tissues. IRF3 contains an N-terminal DNA binding domain (DBD) and a C-terminal transactivation domain. After IRF3 activation, it is not surprising that IRF3 function is both positively and negatively regulated by host proteins or that viruses have evolved mechanisms to abolish protein expression. The long noncoding RNA (lncRNA) lncLrrc55-AS recruits methylesterase 1 (PME-1) to promote the interaction between PME-1 and the phosphatase PP2A, an inhibitor of IRF3 phosphorylation. Similarly, IRF1 interacts with IRF3 to augment the activation of IRF3 by blocking the interaction between IRF3 and PP2A. Heat shock protein family D (Hsp60) member 1 facilitates the phosphorylation and dimerization of IRF3 and increases IFN-β induction induced by SeV infection. The lysine methyltransferase nuclear receptor-binding SET domain 3 (NSD3) binds directly to the IRF3 C-terminal region through its PWWP1 domain and methylates IRF3 at K366. Monomethyltransferase maintains IRF3 phosphorylation by promoting the dissociation of IRF3 from the protein phosphatase PP1cc, thereby promoting the production of type I IFN. The deubiquitinating enzyme USP22 deubiquiti nates and stabilizes KPNA2 after viral infection, thereby facilitating efficient nuclear translocation of IRF3.

Regarding the negative regulation of IRF3-mediated signaling, the E3 ubiquitin ligase interacting protein peptidyl-prolyl cis/trans isomerase, NIMA-interacting 122, and RBCC protein interacting with PKC1 (RBCK1), Ros2/TrIM21, the HECT domain ubiquitin-ε ligase RAUL, and TRIM26 catalyze the K48-linked polyubiquitination and subsequent proteasomal degradation of IRF3. Moreover, OTUD1 removes viral infection-induced K6-linked ubiquitin moieties from IRF3, resulting in dissociation of IRF3 from the promoter region of its target genes without affecting its protein stability, dimerization, or nuclear translocation. IFN-induced transmembrane protein 3 (IFITM3) associates with IRF3 and regulates the homeostasis of IRF3 by mediating its autophagic degradation. Phosphorylation of IRF3 is the key modification that leads to its activation. Therefore, dephosphorylation of IRF3 via phosphatases such as MAPK phosphatase 5 (MKP5) and the serine/threonine phosphatase PP2A inactivates IRF3. However, Mst1 associates with IRF3 and phosphorylates IRF3 directly at Thr75 and Thr253, which prevents IRF3 homodimerization.
reduces its ability to occupy chromatin, and dampens IRF3-mediated transcriptional responses.\textsuperscript{235} Interestingly, the F-box protein FBXO17 decreases IRF3 dimerization and nuclear translocation by recruiting protein phosphatase 2A (PP2A), resulting in dephosphorylation of IRF3.\textsuperscript{236} A recent study showed that open reading frame 6 (ORF6) of SARS-CoV-2 binds to the importin karyopherin (KPNA3 and KPNA4) and one of their cargo molecules, IRF3.\textsuperscript{237} JEV downregulates IRF3 phosphorylation and nuclear translocation, an effect that became more pronounced when the molar ratio of SFRNA to genomic RNA ultimately leading to the induction of type 1 IFN genes and other antiviral genes.\textsuperscript{238} Although other proteins, such as IFI16, DDX41, Ro52/TRIM21, also mediate DNA-induced IFN-β expression, these mechanisms, along with the immunomodulatory mechanisms by which viruses evade them.

REGULATION OF IRF7 BY HOST FACTORS AND RNA VIRAL PROTEINS

IRF7 is a 503 aa (55 kDa) protein containing an N-terminal DBD, an AID, a nuclear export sequence, an autoinhibitory domain, and a signal response domain composed of key serine residues.\textsuperscript{237,238} Unlike IRF3, IRF7 is not expressed ubiquitously in cells; instead, its expression is induced upon pathogen infection or stimulation. However, it is a master regulator of type I IFN gene expression and IFN-dependent innate immune responses.\textsuperscript{239} IKKe and TBK1 are the major kinases responsible for IRF7 phosphorylation and activation.\textsuperscript{240} Nuclear translocation and accumulation of IRF7 trigger the induction of IFN-β and IFN-α expression.\textsuperscript{241} K63-linked polyubiquitination of IRF7 on lysines 444, 446, and 452, a process that is important for its activation prior to its phosphorylation and nuclear translocation, is triggered by TRAF6.\textsuperscript{242} Research has shown that the regulation of IRF7 activity by several negative regulators maintains immune homeostasis. N-MyC and STAT inhibitor (Nme) promote K48-linked ubiquitination of IRF7 and its subsequent proteasome-dependent degradation, whereas Ro52/TRIM21 mediates its ubiquitination-promoted degradation upon upstream signaling activation.\textsuperscript{243} TRIM28 interacts with the SUMO E2 enzymes to increase the SUMOylation of IRF7. TRIM28-mediated SUMOylation of IRF7 increases during viral infection, resulting in transcriptional repression.\textsuperscript{244} The N-terminal deubiquitinase domain of the enzyme A20 interacts physically with IRF7 to reduce its K63-linked ubiquitination and negatively regulates transcriptional function.\textsuperscript{245} Moreover, physical interactions between IRF7 and the IFN-inducible p200 family protein IFI204,\textsuperscript{246} activating transcription factor 4 (ATF4), and HSP70\textsuperscript{247,248} downregulate IRF7 activity, leading to downregulation of innate immune activation. Different RNA viral proteins inhibit IRF7. VP35 of EBOV increases PIAS1-mediated SUMOylation of IRF7, thereby repressing IFN transcription.\textsuperscript{249} In addition, HCV infection impairs the nuclear translocation of IRF7.\textsuperscript{250} The Zn-binding domain of the CSFV Npro protein interacts directly with IRF7 to subvert its function.\textsuperscript{251} In particular, \(3_{\text{cpr}}\) of SVV was found to reduce IRF7 protein expression and phosphorylation in PK-15 cells.\textsuperscript{252} DNA VIRUS-INDUCED SIGNAL TRANSDUCTION AND IMMUNE EVASION MECHANISMS

Upon infection with DNA viruses, viral DNA is released into the host cell cytoplasm prior to viral protein synthesis. Cytosolic viral DNA is recognized mainly by cyclic GMP-AMP (cGAMP) synthase (cGAS), which contains a nucleotidyltransferase (NTase) domain. After DNA binding, cGAS synthesizes a second messenger molecule, cyclic GMP-AMP (cGAMP). This cGAMP isomer, called \(2',3'-\text{cGAMP}\), functions as a second messenger that binds to the ER membrane adaptor STING,\textsuperscript{253,254} to induce a conformational change that presumably results in activation of STING. STING then traffics from the ER to the ER-Golgi intermediate compartment and then to the Golgi apparatus.\textsuperscript{255,256} During this process, the carboxyl terminus of STING recruits and activates the kinase TBK1, which in turn phosphorylates the transcription factor IRF3. Phosphorylated IRF3 dimerizes and then enters the nucleus, ultimately leading to the induction of type 1 IFN genes and other antiviral genes.\textsuperscript{257} Although other proteins, such as IFI16, DDX41, and MRE11, also mediate DNA-induced IFN-β production in a STING-dependent manner, only cGAS, which enzymatically generates cGAMP as a second messenger that activates STING, provides a clear molecular mechanism for DNA-stimulated IFN-β production.\textsuperscript{258} However, DNA viruses exploit strategies to evade innate immune responses. Below, we describe the activation and regulation of these mechanisms, along with the immunomodulatory mechanisms by which viruses evade them.

REGULATION OF 2',3'-CGAMP BY HOST FACTORS AND VIRAL PROTEINS

Upon DNA recognition, cGAS generates the second messenger \(2',3'-\text{cGAMP}\) by using ATP and GTP.\textsuperscript{259,260} Unlike the secondary messengers in classical bacterial signaling (c-di-GMP and c-di-AMP), \(2',3'-\text{cGAMP}\) contains mixed phosphodiester bonds (\(G(2',5')pA\) and \(A(3',5')pG\)). The intermediate product, called \(5'-\text{pppG}(2',5')pA\), is generated by cGAS prior to synthesis of cyclic \(2',3'-\text{cGAMP}\).\textsuperscript{42} Next, \(2',3'-\text{cGAMP}\) interacts with STING to activate downstream signaling, resulting in strong induction of IFNs, which confer antiviral efficacy.\textsuperscript{261} To date, few studies have examined host factors and viral proteins that

REGULATION OF IRF3 BY RNA VIRAL PROTEINS

Due to genomic constraints, the immunomodulatory efforts of most viruses focus on host targets that are key players in the antiviral response. It is not surprising, therefore, that IRF3 is one of these targets. The NS1 proteins of influenza A virus (IAV)\textsuperscript{243} and porcine hemagglutinating encephalomyelitis virus (PHEV)\textsuperscript{244} are phosphorylated (P) of rabies virus (RABV)\textsuperscript{245}, the PLpro protein (with ubiquitination activity) of SARS-CoV-2, the NS1 β protein of PMPV\textsuperscript{246} in the N protein of Pestivirus.\textsuperscript{247} The NSP15 protein of PEDV\textsuperscript{248} and the NSP1 protein of PEDV\textsuperscript{249} inhibit activation of IRF3 to downregulate nuclear translocation. A recent study reported that open reading frame 6 (ORF6) of SARS-CoV-2 binds to the importin karyopherin α2 (KPNA2), thereby inhibiting the nuclear translocation of IRF3.\textsuperscript{250} In addition, the ORF6, NSP12, and NSP5 proteins inhibit the nuclear translocation of IRF3 to prevent IFN production,\textsuperscript{249,250,251} while the NSP3/papain-like protease cleaves IRF3 to subvert IFN production.\textsuperscript{252} Moreover, NS5 of Japanese encephalitis virus (JEV) interacts with the nuclear transport proteins KPNA2, KPNA3, and KPNA4, which competitively block the interactions between KPNA3 and KPNA4 and one of their cargo molecules, IRF3.\textsuperscript{253} JEV downregulates IRF3 phosphorylation and nuclear translocation, an effect that became more pronounced when the molar ratio of sRNA to genomic RNA was increased.\textsuperscript{254} The V protein of Sendai virus (SeV) inhibits IRF3 translocation to the nucleus\textsuperscript{255} and the 3Cpro protein of SVV degrades IRF3 via its protease activity.\textsuperscript{256} The Npro protein of classical swine fever virus (CSFV)\textsuperscript{257} and the NS1 protein of RV\textsuperscript{258} trigger proteasomal degradation of IRF3. FMDV 3A interacts with DDX56 to inhibit type I IFN production by reducing the phosphorylation of IRF3.\textsuperscript{259} Hantavirus\textsuperscript{260} oncoprotein Tax of human T-cell leukemia virus type 1 (HTLV-1)\textsuperscript{261} and the NS protein of DENV\textsuperscript{262} and the M protein of MERS-CoV\textsuperscript{263} downregulate IRF3 phosphorylation. Moreover, two reports revealed that the ML protein of Thogoto virus (TOV) and the NSP1 protein of RV block the dimerization and subsequent nuclear translocation of IRF3.\textsuperscript{264,265}
regulate 2',3'-cGAMP function during innate immune activation. A recent study of HSV-1 infection showed that Leucine-rich repeat-containing protein (LRRC) LRRCA8/LRRC8ε-containing-ubiquitin-regulated anion channels transport cGAMP across the plasma membrane to initiate effective antiviral innate immunity.

In contrast, 2',3'-cGAMP is hydrolyzed predominantly by ectonucleotide pyrophosphatase/phosphodiesterase (ENPP1), thereby preventing STING activation. In general, viruses have evolved mechanisms to antagonize host innate immune activation. However, the antiviral second messenger 2',3'-cGAMP can be packaged into viral particles, including those of poxviruses, herpesviruses, and retroviruses, thereby enabling its transfer to newly infected cells, where it activates the immune response. Once 2',3'-cGAMP-carrying virions infect neighboring cells, they activate a STING-dependent antiviral program.

Moreover, the poxvirus immune nuclease (poxin) family, a member of the 2',3'-cGAMP-degrading enzymes, has been identified. Vaccinia virus poxin degrades 2',3'-cGAMP through metal-independent cleavage of the 3'-5' bond, thereby converting 2',3'-cGAMP into linear Gp [2'-5']Ap[3']. Furthermore, the same study revealed that deletion of the poxin gene (B2R) attenuates vaccinia virus replication in vivo, thereby restricting STING-dependent signaling.

REGULATION OF STING BY HOST FACTORS

STING, also called MITA, ERIS, TMEM173, or MPYS, is an ER membrane protein of 379 aa; it harbors a predicted TM portion (aa residues 1–173) at the N-terminus, which regulates its cellular localization and homodimerization, since the TM domains cross the ER membrane. It also harbors an intracellular soluble portion (aa residues 174–379) in the CTD, which functions to dock downstream molecules such as TBK1/IKKe and IRF3/IRF7296,297. To initiate signaling, the native ligand cGAMP binds to the V-shaped hydrophilic pocket in the STING dimer. The resulting conformational change exposes the hidden CTT of STING to TBK1 and IRF3.

Due to this conformational change, STING is transported from the ER to the ER-Golgi intermediate compartment and then to the Golgi apparatus and perinuclear region.

Since STING is essential for innate immune responses to cytosolic nucleic acids, its activity is tightly regulated to maintain immune homeostasis while enabling timely activation of down-stream signaling to fight against viral infections. Several PTMs are involved in regulating STING function. Among them, K63-linked polyubiquitination plays a critical activating role. Mitochondrial E3 involved in regulating STING function. Among them, K63-linked stream signaling to cytosolic nucleic acids, its activity is tightly regulated to maintain immune homeostasis.

RNF11559 also conjugate K63-linked polyubiquitin chains to STING, to dock downstream molecules such as TBK1/IKKε domains cross the ER membrane. It also harbors an intracellular homedimerization, since the TM N-terminus of STING332, while UL46 of HSV-1, one of the most common viral polymerase of HSV is translocated to the cytoplasm during stimulation328. Autophagy-related gene 9a (Atg9a) colocalizes with STING to suppress its activation337. The viral polymerase of HBV encodes a product referred to as M152, which interacts with STING to inhibit IRF3 activation. The HSV-1 γ34 protein downregulates STING trafficking from the ER to Golgi by interacting with the N-terminus of STING332, while UL46 of HSV-1, one of the most abundant HSV tegument proteins, interacts with STING to suppress its activation333. The HSV-1 VP1-2 protein deubiquitinates STING and inhibits its downstream signaling344. The human T lymphotropic virus type 1 (HTLV-1) Tax protein also deubiquitinates STING to inhibit its downstream signaling, while NS4B of HCV cleaves STING directly345, and virR1 of KSHV impairs the STING/TBK1 interaction336. Murine CMV (MCMV) encodes a product referred to as M152, which interacts with STING to suppress its activation. The viral polymerase of HBV interferes with K63-linked polyubiquitination of STING via its reverse transcriptase domains346. The HCMV tegument protein UL82 negatively regulates STING signaling by interacting directly with STING. It then inhibits STING trafficking from the ER to perinuclear punctate structures. The IE86 protein of HCMV facilitates proteasome-dependent degradation of STING to suppress the secretion of IFN-β and CXCL10340,341, and UL42 of HCMV impairs the translocation of STING from the ER to perinuclear punctate structures, which is required for STING activation342. Duck Embus virus (DTMUV) NS2B3 cleaves STING by interacting with aa residues 221–225; this method of STING cleavage is not strictly species-specific343.
REGULATION OF TBK1 BY DNA VIRAL PROTEINS
To complete their life cycles in the host, DNA viruses use numerous strategies to evade host immune signaling initiated by RLRs; they do this by targeting TBK1. The Us11 protein of HSV-1 and the endogenous Hsp90 to disrupt the Hsp90/TBK1 complex, which blocks TBK1 activation. Furthermore, Us11 induces destablization of TBK1 through a proteasome-dependent pathway that ultimately blocks phosphorylation of IRF3134. In addition, the UL46 protein of HSV-1 interacts with the C-terminal region of TBK1 to inhibit the interaction of TBK1 and STING343, whereas the gamma(1)34.5 protein forms a complex with TBK1 and disrupts the TBK1/IRF3 interaction, thereby preventing downstream signaling344. ORF11 of murine gammaherpesvirus 68 (MHV-68) gamma(1)34.5 protein forms a complex with TBK1 and disrupts IRF7348. Varicella-zoster virus (VZV) is an important alpha herpesvirus that infects only humans. Several VZV viral proteins interfere with IRF3 activity. VZV viral immediate-early protein 62 (IE62) inhibits IRF3 phosphorylation at key serine residues but does not interfere with the IRF3/TBK1 interaction349. ORF47 interacts directly with IRF3, thereby inhibiting subsequent signal transduction, while ORF61 interacts directly with IRF3 and induces its ubiquitination and proteasomal degradation350,351. The nuclear early protein N2 of vaccinia virus inhibits the phosphorylation and nuclear translocation of IRF3351.

REGULATION OF IRF3 BY DNA VIRAL PROTEINS
A number of DNA viral proteins inhibit IRF3 to suppress innate immune signaling. The VP24 protein of HSV-1 and the LANA2 (also called vIRF3) protein of Kaposi's sarcoma-associated herpesvirus (KHSV) limit the induction of IFN-β by interacting with IRF3 to inhibit its dimerization and phosphorylation29,347. The ICP0 protein (bICP0) encoded by bovine herpesvirus 1 (BoHV-1) induces proteasomal degradation of IRF3 but not IRF7346. Varicella-zoster virus (VZV) is an important alpha herpesvirus that infects only humans. Several VZV viral proteins interfere with IRF3 activity. VZV viral immediate-early protein 62 (IE62) inhibits IRF3 phosphorylation at key serine residues but does not interfere with the IRF3/TBK1 interaction349. ORF47 interacts directly with IRF3, thereby inhibiting subsequent signal transduction, while ORF61 interacts directly with IRF3 and induces its ubiquitination and proteasomal degradation350,351. The nuclear early protein N2 of vaccinia virus inhibits the phosphorylation and nuclear translocation of IRF3351.

REGULATION OF IRF7 BY DNA VIRAL PROTEINS
Different viral proteins inhibit and activate IRF7. The interaction of the Epstein-Barr virus oncoprotein LMP1 with IRF7 catalyzes RIP-dependent K63-linked polyubiquitination and subsequent activation of IRF7352. The VP23 protein of Marek's disease virus interacts with IRF7 and blocks its binding to TBK1, thereby inhibiting IRF7 phosphorylation and nuclear translocation, resulting in reduced IFN-β production353. The immediate-early nuclear transcription factor RTA encoded by KSHV and HHV8 acts as an ubiquitin E3 ligase to catalyze the polyubiquitination and proteasomal degradation of IRF7354. KSHV vIRF3 interacts specifically with either the DBD or the central IAD of IRF7, which inhibits the DNA binding activity of IRF7355. KSHV vIRF4 interacts specifically with IRF7, thereby inhibiting IRF7 dimerization and ultimately suppressing IRF7-mediated activation of type I IFNs356. LANA2 (also called vIRF3) of KSHV limits the induction of IFN-β by interacting with IRF7 and inhibiting its phosphorylation29.

CONCLUSIONS
Over the past few decades, tremendous research progress has been made in identifying and characterizing two antiviral innate immunity pathways: the RLR-MAVS pathway for cytoplasmic RNA sensing and the cGAS-cGAMP-STING pathway for cytosolic DNA recognition. In this review, we summarize the current knowledge of the mechanisms that positively and negatively regulate PRR-mediated immune responses. We also discuss the molecules involved in the two abovementioned signaling pathways, which maintain immune homeostasis to achieve the most favorable outcome for the host. Finally, we explain how viral proteins adapt to escape host antiviral mechanisms to maintain active infection. Due to advanced biomedical techniques such as fluorescence imaging, mass spectrometry, and nuclear magnetic resonance imaging, we now know much more about the molecular mechanisms and the host and viral factors that regulate signaling. Moreover, each new regulatory and molecular mechanism identified brings the inspiring possibility that we may identify and develop novel immunostimulatory agents, anti-inflammatory agents, vaccines, and antiviral agents that tilt the host-pathogen interaction in favor of the host. Despite tremendous advances in our knowledge regarding the functions and mechanisms of positive and negative regulatory molecules and of escape mechanisms used by viruses to evade innate immune signaling, several intriguing and important aspects regarding the regulation of RNA- and (especially) DNA-initiated signaling pathways and viral escape mechanisms remain elusive. These will be interesting topics for future investigations.

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K. Chathuranga et al.
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