



## REVIEW ARTICLE OPEN

# Regulation of RIG-I-like receptor-mediated signaling: interaction between host and viral factors

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Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are RNA sensor molecules that play essential roles in innate antiviral immunity. Among the three RLRs encoded by the human genome, RIG-I and melanoma differentiation-associated gene 5, which contain N-terminal caspase recruitment domains, are activated upon the detection of viral RNAs in the cytoplasm of virus-infected cells. Activated RLRs induce downstream signaling via their interactions with mitochondrial antiviral signaling proteins and activate the production of type I and III interferons and inflammatory cytokines. Recent studies have shown that RLR-mediated signaling is regulated by interactions with endogenous RNAs and host proteins, such as those involved in stress responses and posttranslational modifications. Since RLR-mediated cytokine production is also involved in the regulation of acquired immunity, the deregulation of RLR-mediated signaling is associated with autoimmune and autoinflammatory disorders. Moreover, RLR-mediated signaling might be involved in the aberrant cytokine production observed in coronavirus disease 2019. Since the discovery of RLRs in 2004, significant progress has been made in understanding the mechanisms underlying the activation and regulation of RLR-mediated signaling pathways. Here, we review the recent advances in the understanding of regulated RNA recognition and signal activation by RLRs, focusing on the interactions between various host and viral factors.

**Keywords:** RIG-I-like receptors; Viral infection; Innate immunity; Stress response

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## INTRODUCTION

Interferons (IFNs), the family of cytokines first discovered, were identified as host factors secreted by virus-infected cells to “interfere” with viral replication.<sup>1,2</sup> Based on the subsequent identification of their corresponding genes, they were further classified into three types in humans: type I IFNs [multiple IFN- $\alpha$  forms (1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, and 21), IFN- $\beta$ , IFN- $\epsilon$ , IFN- $\kappa$ , and IFN- $\omega$ ], type II IFN (a single form, IFN- $\gamma$ ), and type III IFNs (IFN $\lambda$ 1-4).<sup>3</sup> Among these types, type I and type III IFNs play essential roles in innate antiviral immunity. Infections caused by microbes, including viruses and bacteria, transiently induce the secretion of type I IFNs, which associate with the type I IFN receptor complex (IFNAR1 and IFNAR2) expressed by the infected cells themselves and surrounding noninfected cells, thereby activating cytoplasmic Janus kinases (JAK1 and TYK2). Signal transducer and activator of transcription (STAT1) and STAT2, activated by JAK-mediated tyrosine phosphorylation, together with the DNA-binding component IFN regulatory factor (IRF)-9, form a tripartite complex, referred to as IFN-stimulated gene factor 3 (ISGF3). ISGF3 then is translocated to the nucleus, where it binds to the IFN-stimulated response element (ISRE) on the promoters of hundreds of IFN-inducible genes (ISGs) and activates their transcription. Proteins encoded by ISGs induce strong antiviral innate immunity to eliminate invading viruses.<sup>4</sup> Since the major histocompatibility complex (MHC) and costimulatory molecules are also ISGs, IFNs play critical roles in regulating acquired immunity (Fig. 1).

Type III IFNs, similar to type I IFNs, are also activated in response to pathogenic infections; however, these cytokines are recognized by a distinct receptor complex comprising IFNLR1 and interleukin (IL)-10R2.<sup>5</sup> The signaling pathway downstream of the type III IFN receptor complex is nearly identical to that of type I IFNs, resulting in similar antiviral activity via JAK-STAT-induced ISG production.<sup>3</sup> The functional differences between type I and type III IFNs might be caused by the different cell types producing IFNs and their cognate receptors.<sup>5</sup> Notably, type III IFN receptors are predominantly expressed on epithelial cells and certain myeloid cells, suggesting their critical roles in these cell types.<sup>5</sup>

IFNs have been used for the treatment of several viral infections and various immunological disorders and cancers due to their antiviral effects and cell growth inhibitory properties.<sup>6</sup> Although type I IFNs have previously comprised the sole treatment strategy for hepatitis C virus (HCV), they have more recently been replaced by nonstructural protein (NS)3/4A and NS5A inhibitors, which have proven effective for the complete elimination of HCV. IFN therapy has been proposed as a potential strategy for the treatment of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>7</sup> Indeed, mouse models of SARS-CoV, another pathogenic coronavirus, have exhibited delayed induction of type I IFNs during the early onset of infection, leading to potential excessive cytokine secretion, called a “cytokine storm”, which is associated with exacerbated disease.<sup>8</sup> This outcome is also observed in COVID-19,

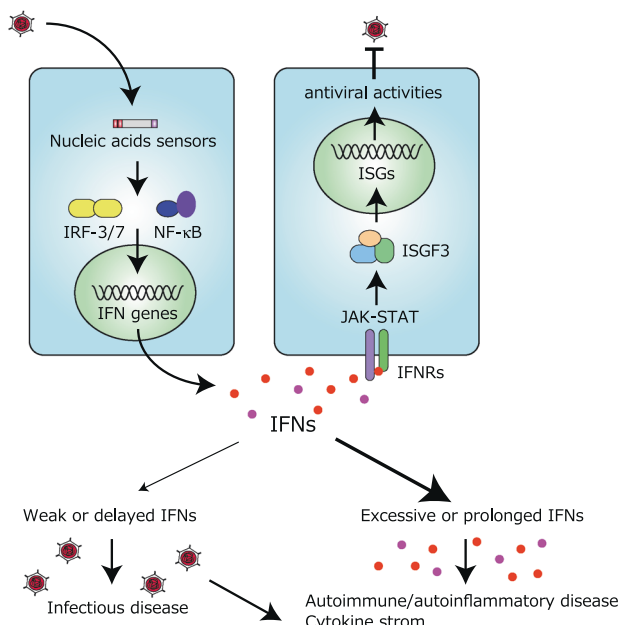
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**Fig. 1** Interferon-mediated antiviral responses and diseases. In an antiviral innate immune system, nucleic acid sensors detect viral nucleic acids intracellularly and extracellularly, which then activate intracellular signaling pathways, leading to transcriptional activation of type I and type III interferon (IFN) genes upon the nuclear translocation of the transcription activators IFN regulatory factor (IRF)-3/7 and nuclear factor-κB (NF-κB). Secreted type I and type III IFNs activate a secondary signaling pathway by binding to their cognate IFN receptor (IFNR) complexes in an autocrine and paracrine manner. The downstream JAK-STAT pathway activates the transcription complex IFN-stimulated gene factor 3 (ISGF3), which leads to the expression of hundreds of IFN-stimulated genes (ISGs), resulting in strong antiviral responses. Since IFN-induced antiviral activity is critical for eliminating infected viruses, its weak or delayed response leads to uncontrolled infectious disease. In contrast, IFNs also play essential roles in regulating the subsequently induced adaptive immunity, and thus, their excessive or chronic activation can lead to the development of autoimmune or autoinflammatory diseases as, well as the initiation of cytokine storm, which exacerbates inflammation

suggesting a potential therapeutic strategy to enhance the initial production of IFNs during the early stages of SARS-CoV-2 infection.<sup>9</sup> However, excessive IFN production in the late stages of infection is associated with the development of cytokine storm, suggesting that the therapeutic use of IFNs requires careful consideration in terms of timing and dosage.<sup>10,11</sup> The use of type III IFNs in COVID-19 treatment has also been suggested due to their limited action on cell types such as epithelial cells;<sup>7</sup> however, recent reports have shown that they may be detrimental to COVID-19 patients,<sup>12,13</sup> and thus, further investigations into their suitability as therapeutic agents are warranted.

In higher vertebrates, pathogenic infections are detected by pattern recognition receptors (PRRs), which are part of the innate immune system. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are extracellular PRRs that detect pathogens on the plasma membrane or endosomal compartment. In addition, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), Nod-like receptors (NLRs), and cytoplasmic DNA sensors serve as intracellular PRRs. The production of type I and type III IFNs is explicitly mediated by PRRs, such as endosomal TLRs and RLRs that detect nucleic acids. Among these PRRs, TLR-3 recognizes double-stranded RNA (dsRNA), TLR-7/8 recognizes single-stranded RNA (ssRNA), and TLR-9 recognizes unmethylated CpG DNA in the endosomal compartment. RLRs recognize cytosolic RNAs, and

cyclic GMP-AMP synthase (cGAS) acts as a cytosolic DNA sensor.<sup>14</sup> The transcription factors IRF-3/7 and nuclear factor-κB (NF-κB), downstream of IFN-inducing PRRs, are commonly activated and transiently induce IFN-encoding genes. Genes encoding proinflammatory cytokines, such as IL-6 and tumor necrosis factor (TNF)-α, are also induced by activated NF-κB. In this review, we focus on RLRs that detect cytoplasmic viral RNAs and the molecular mechanisms underlying RNA-mediated activation of RLRs and signaling regulation performed by host and viral factors.

## ACTIVATION OF RLR-MEDIATED SIGNALING

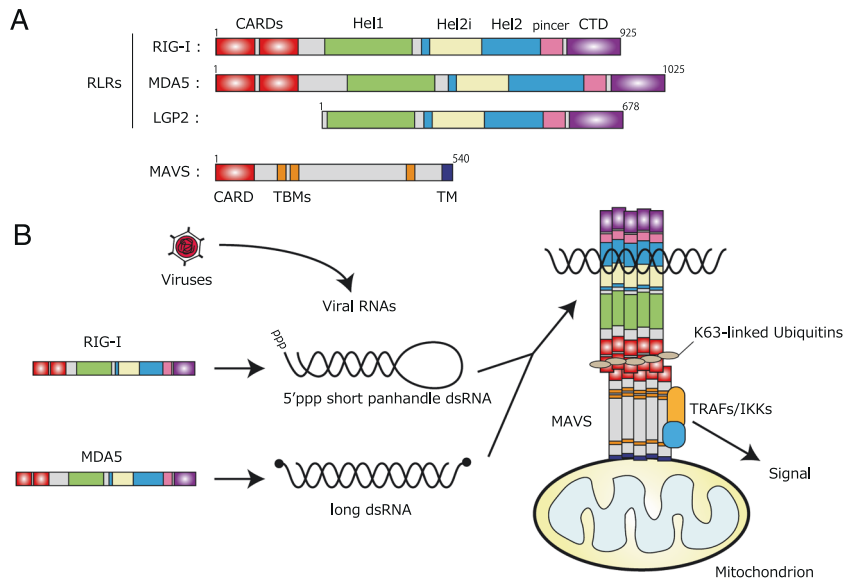
### Association between RLRs and MAVS

RIG-I, which enhances IFN production in response to RNA virus infection, was isolated via expression cloning.<sup>15</sup> The human genome encodes three RLRs, RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), which are DEXD/H box-containing RNA helicases and ubiquitously expressed in the cytoplasm.<sup>16</sup> These RLRs commonly have a central helicase domain and a C-terminal domain (CTD) critical for RNA recognition. RIG-I and MDA5 also contain a tandem caspase recruitment domain (CARD) in their N-terminal region, which is required for their association with the adapter molecule mitochondrial antiviral-signaling protein (MAVS; also known as IPS-1, VISA, and Cardif), which also contains a single CARD at its N-terminus (Fig. 2a). The interaction between the CARDS of RIG-I/MDA5 and MAVS leads to the recruitment of downstream signaling molecules, including TNF receptor-associated factor (TRAF)3/6 and inhibitor of NF-κB kinase (IKK) family members (IKKε, TBK1, and IKKα/β), to activate IRF-3/7 and NF-κB, leading to the transcriptional activation of IFN and proinflammatory cytokine genes (Fig. 2b).<sup>17,18</sup> More importantly, since RLR genes are ISGs, a positive feedback loop leads to further enhancement of IFN production.<sup>15</sup>

RIG-I detects substrate RNAs that have a panhandle double-stranded structure with a 5'-triphosphate or 5'-diphosphate moiety and activates downstream signaling in an ATP-dependent manner (Fig. 2b).<sup>19</sup> A gene knockout (KO) study in mice revealed that RIG-I can be used for detecting multiple viral species in many cell types.<sup>20</sup> The study showed that RIG-I KO in mice results in embryonic lethality resulting from liver degeneration; however, another study showed that KO mice with a different genomic region targeted were viable but developed spontaneous colitis.<sup>21</sup> Although the mechanism underlying colitis development in these mice has not been fully elucidated, recent studies have demonstrated that RIG-I might participate in the maintenance of homeostasis in digestive organs by interacting with commensal microorganisms.<sup>22,23</sup>

MDA5 recognizes relatively long dsRNAs.<sup>24</sup> Specifically, studies with MDA5-KO mice have demonstrated that MDA5 is primarily involved in detecting viruses belonging to the *Picornaviridae* family;<sup>25</sup> however, it can also recognize several other viral species. In addition to their virus-sensing roles, RIG-I/MDA5 are both capable of controlling viral replication by exhibiting an "effector-like" function that competitively block the interaction between viral proteins and RNA.<sup>26–28</sup>

Another RLR, LGP2, does not harbor N-terminal CARD and therefore does not function as a signaling molecule. Although in vitro studies, including our own, suggested that LGP2 acts as a negative regulator of RIG-I/MDA5-mediated signaling,<sup>16,29,30</sup> and subsequent studies with KO mice revealed a positive regulatory function for LGP2.<sup>31,32</sup> These reports revealed that LGP2 deficiency impairs IFN production in response to picornaviruses, including encephalomyocarditis virus (EMCV), suggesting that LGP2 is involved in the MDA5-mediated signaling activation (the molecular mechanisms are described as follows). In contrast, the physiological significance of LGP2 in RIG-I-mediated signaling remains unclear because LGP2 deficiency has different effects



**Fig. 2** RIG-I-like receptors and the associated activation machinery. **a** Primary structure of retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and mitochondrial antiviral-signaling protein (MAVS). All RLR family members contain a DExD/H box-containing RNA helicase domain composed of Hel1, Hel2i, Hel2, and pincer domains and the C-terminal domain (CTD). RIG-I and melanoma differentiation-associated gene 5 (MDA5), but not laboratory of genetics and physiology 2 (LGP2), contain repeated caspase recruitment domains (CARDs) in their N-terminal regions. MAVS has an N-terminal single CARD, three TNF receptor-associated factor (TRAF)-binding motifs (TBMs), and a transmembrane (TM) domain in the C-terminus. **b** RIG-I and MDA5 recognize 5'-triphosphate (ppp)-containing panhandle double-stranded (ds)RNA and long dsRNA, respectively. Recognition of substrate RNAs induces ATP-dependent conformational changes of RLRs, which induce filamentous oligomer formation on substrate RNAs. The N-terminal CARDs adopt a "lock-washer"-like tetramer with K63-linked ubiquitin chains and form signal-competent oligomers with the CARD of MAVS, which is expressed on the outer membrane of mitochondria. MAVS oligomers recruit downstream signaling molecules, such as TRAFs and inhibitors of NF- $\kappa$ B kinases (IKKs), which leads to the activation of IRF-3/7 and NF- $\kappa$ B

depending on the viral infection.<sup>31,32</sup> LGP2 has also been reported to regulate RNA silencing machinery. Specifically, it can associate with Dicer, an endoribonuclease for microRNA (miRNA) production, suggesting that LGP2 might indirectly regulate antiviral signaling by regulating gene expression.<sup>33</sup> Moreover, LGP2 has been reported to interact with transactivation response RNA-binding protein (TRBP), a positive regulator of Dicer-mediated miRNA maturation, to inhibit the production of several miRNAs and induce the apoptotic pathway by enhancing the expression of specific genes.<sup>34,35</sup> In the case of West Nile virus (WNV) infection, LGP2 plays an essential role in the survival and adaptation of CD8<sup>+</sup> T cells but not in MAVS-mediated IFN production.<sup>36</sup> Taken together, these observations suggest that LGP2 might be involved in a broad range of antiviral effects, and hence, further analysis is required.

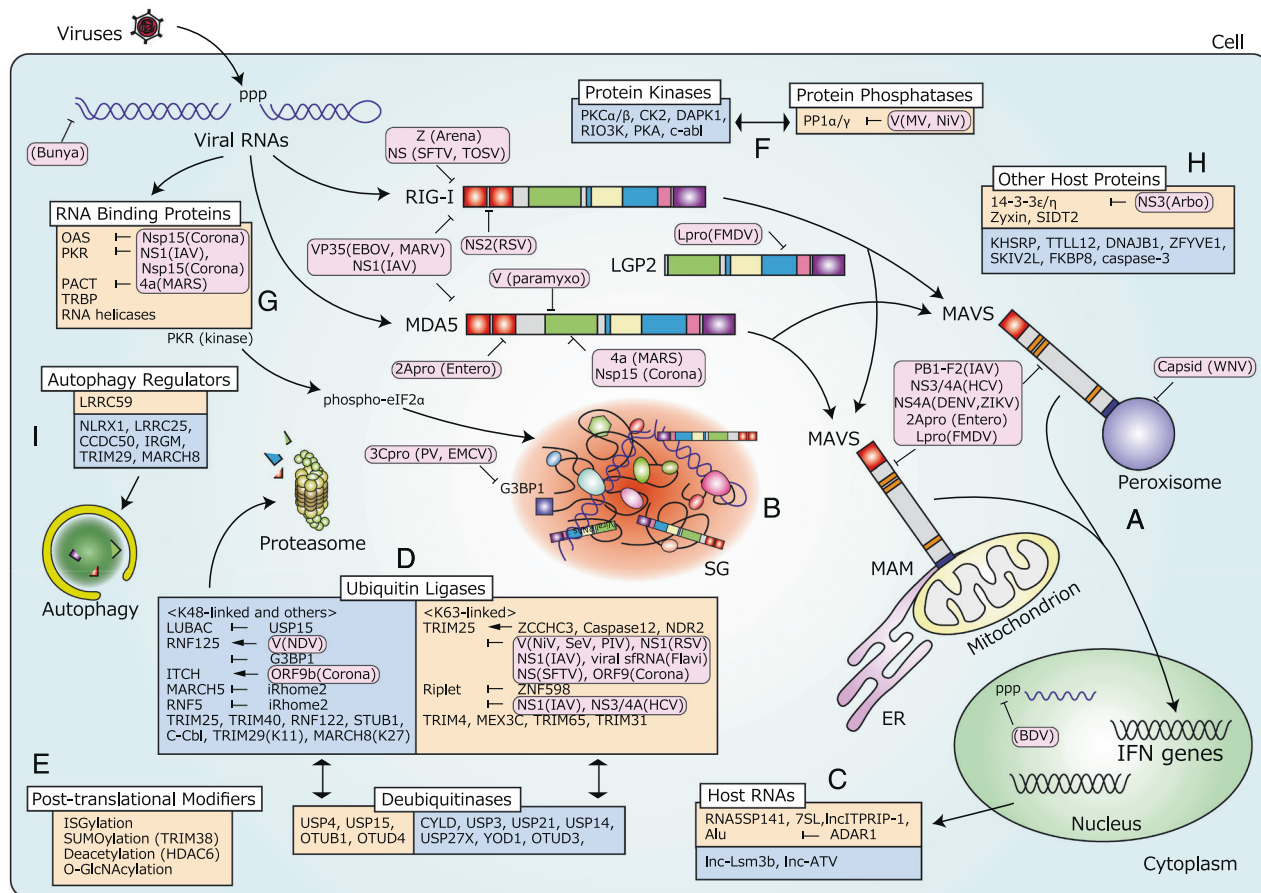
MAVS is an adapter molecule for RLR-mediated signaling that contains an N-terminal CARD and C-terminal transmembrane (TM) domain required for its localization to intracellular membranes, including those of mitochondria, and three TRAF-binding motifs (TBMs) required for signal transduction. A MAVS-KO mouse model was used to clearly identify the essential role of MAVS in RIG-I/MDA5-mediated IFN production.<sup>37</sup>

Several studies have suggested that RLRs/MAVS are involved in inflammasome-mediated proinflammatory cytokine production, such as IL-1 $\beta$  and IL-18.<sup>38</sup> RIG-I has been reported to be directly associated with apoptosis-associated speck-like protein containing CARD (ASC) and caspase-1 of the inflammasome to form virus-specific inflammasomes in response to vesicular stomatitis virus (VSV) and influenza A virus (IAV) infection.<sup>39,40</sup> MAVS is also reportedly involved in the formation of the NLRP3 inflammasome in mitochondria.<sup>41,42</sup> Furthermore, MAVS-mediated signaling is required to increase cell membrane permeability and the subsequent K<sup>+</sup> ion efflux that leads to the formation of the NLRP3-containing inflammasome.<sup>43</sup> Thus, further analysis is warranted to better understand virus-induced inflammasomes.

#### Activation of RLR-mediated signaling

In the steady state, RIG-I is likely to adopt an autorepressed conformation via direct interactions between CARDs and the helical insertion domain (Hel2i) located in the helicase domains.<sup>44</sup> An initial structural analysis of RIG-I CARD showed that the CTD with substrate dsRNA forms a rigid proteolysis-resistant structure, indicating a critical role of the CTD in substrate recognition.<sup>45,46</sup> The positively charged surface of the RIG-I CTD was also shown to be critical for recognizing the 5'-triphosphate signature of substrate dsRNA. Subsequent structural analysis of the helicase domain and CTD of RIG-I with substrate RNA revealed that both wrap around dsRNA with CTD sheathing the end.<sup>44,47–49</sup> The interaction between RIG-I and its dsRNA substrate induces an ATP-dependent intramolecular conformational change and the release of N-terminal CARDs, allowing the RIG-I CARD to associate with the MAVS CARD on the mitochondria. At the same time, the translocase activity of RIG-I allows it to migrate along the RNA substrate; thus, the accumulation of multiple RIG-I molecules on RNA results in the formation of filamentous oligomers.<sup>50–52</sup> Furthermore, the CARDs of the activated RIG-I proteins on the RNA form a helical tetrameric "lock washer" structure, allowing the MAVS CARD to form signal-competent aggregates (Fig. 2b).<sup>53</sup>

In contrast, the constitutive activity of MDA5 is repressed by phosphorylation.<sup>54</sup> In contrast to RIG-I, the MDA5 CTD forms a relatively open structure, indicating that the MDA5 CTD cannot function independently to recognize RNA.<sup>55</sup> A structural analysis of MDA5 with substrate dsRNA demonstrated that the helicase domain and CTD of MDA5 recognize internal duplexes rather than the end of the dsRNA substrate structures.<sup>49</sup> Moreover, MDA5 molecules accumulate on long dsRNA in a head-to-tail arrangement, forming a filamentous structure and transmitting a signal by the N-terminal CARDs protruding from the filaments.<sup>49,56</sup> Furthermore, the formation of the MDA5 filament is essential for distinguishing between self- and non-self-dsRNA via assembly/disassembly dynamics that depend on the length of the substrate dsRNA.<sup>57,58</sup>



**Fig. 3** Regulation of RLR-mediated signal transduction. A summary of the regulatory mechanisms of RLR/MAVS-mediated signaling by host and viral factors. **a** Activated RLRs interact with MAVS, localize to mitochondria, mitochondria-associated endoplasmic reticulum membranes (MAMs), and peroxisomes, where they activate downstream signaling. **b** RLRs accumulate in virus-induced stress granules (SGs) with viral RNAs, resulting in enhanced signaling. **c** Several host RNAs can be recognized by RLRs and are involved in the regulation of autoimmunity and cancer. Moreover, RLR/MAVS-mediated signaling is regulated by various host molecules: ubiquitin ligases/deubiquitinases (**d**), posttranslational modifiers (**e**), protein kinases/phosphatases (**f**), RNA-binding proteins (**g**), other host proteins (**h**), and autophagy regulators (**i**). These molecules are classified in square boxes (orange boxes: positive regulators; blue boxes: negative regulators). The molecules that inhibit or promote these regulatory molecules are shown on the right side of each column. Regulatory molecules of viral origin are shown in pink rounded rectangles

However, the precise mechanisms by which LGP2 regulates RIG-I/MDA5-mediated signaling remain unclear. The CTD of LGP2 adopts a structure similar to that of RIG-I and recognizes the blunt end of dsRNA in a 5'-triphosphate-independent manner, reflecting the differential function of LGP2 in RIG-I- and MDA5-mediated signaling.<sup>55,59,60</sup> A structural analysis of full-length LGP2 suggested that it forms a complex with the MDA5-like helicase domain and a RIG-I-like CTD.<sup>61</sup> LGP2 can augment MDA5-mediated filament formation.<sup>62</sup> Further analysis is required to elucidate the molecular machinery involved in the cooperative recognition performed by these RLRs.

Ectopic overexpression of MAVS *in vitro* causes constitutive IFN production, suggesting that the activity of MAVS as a signaling adapter is tightly regulated. Although several molecules have been identified as negative regulators of MAVS, their repression does not induce constitutive activation of MAVS, thus suggesting alternative inhibitory mechanisms. Qi et al. reported that the expression of multiple N-terminal truncated MAVS isoforms produced by alternative translation initiation suppresses the constitutive activation of full-length MAVS.<sup>63</sup> The constitutive signaling capacity of full-length MAVS is repressed upon association with inactive truncated MAVS isoforms through the TM domain. However, the intramolecular interaction of MAVS suppresses constitutive activation.<sup>64</sup> In response to the activation

of RIG-I or MDA5, MAVS CARDs form protease-resistant prion-like aggregates on intracellular membranes such as mitochondria. A structural analysis revealed that tetrameric RIG-I/MDA5 CARDs act as templates for MAVS CARD filaments to which downstream signaling molecules are recruited.<sup>65–67</sup>

#### Intracellular localization of RLRs and MAVS

In the inactivated state, RLRs are uniformly expressed in the cytoplasm. Since MAVS is expressed on mitochondrial outer membranes, the activated RIG-I/MDA5 in filamentous aggregates is located close to mitochondria. MAVS is also localized in mitochondrial-associated endoplasmic reticulum membranes (MAMs) and peroxisomes and regulates RLR-mediated signaling (Fig. 3a).<sup>68,69</sup> Peroxisomal MAVS is primarily involved in type III IFN induction.<sup>70</sup> In addition, a recent study that performed high-resolution assays reported that microsomal fractions, such as endoplasmic reticulum membranes, but not mitochondria, play central roles in RLR-mediated signaling.<sup>71</sup> Nuclear-localized RIG-I is required for detecting IAV, which replicates in the nucleus;<sup>72</sup> however, the mechanism by which it activates MAVS-mediated signaling is unclear. Further analysis is required to understand the intracellular localization of RLRs.

Stress granules (SGs) are membrane-less cytoplasmic aggregates to which RLRs are localized, and they can enhance MAVS-



mediated signaling by acting as platforms for the recognition of foreign viral RNAs by RLRs (Fig. 3b).<sup>73</sup> Under various stress conditions, including viral infection, cells produce SGs to inhibit cellular protein synthesis and store the protein synthesis machinery (translationally stalled mRNAs and RNA-binding proteins) to reinitiate translation after recovery from the stress condition.<sup>74</sup> During viral infection, protein kinase R (PKR), a well-known dsRNA-dependent kinase that is also an antiviral ISG, phosphorylates the eukaryotic translation initiation factor eIF-2 $\alpha$  and induces the production of SGs.<sup>75–78</sup> The inhibition of virus-induced and PKR-dependent SG formation significantly impairs IFN production when exposed to NS1-defective IAV but not wild-type IAV, indicating that IAV-induced SGs are critical for IFN response activation and that NS1 can function as a viral inhibitor for SG-mediated signaling.<sup>73</sup> The accumulation of MAVS-enriched mitochondria around RIG-I-containing aggregates supports the hypothesis that SGs serve as platforms for viral detection.<sup>79,80</sup> In the case of Newcastle disease virus (NDV) infection, RIG-I is localized to the viral replication complex (vRC) to initiate primary IFN induction during the early postinfection phase, and its subsequent accumulation in SGs is required for the robust upregulation of IFN genes, suggesting the importance of intracellular dynamics of RIG-I proteins for efficient activation of the signaling pathway.<sup>80</sup> Furthermore, growing evidence suggests that multiple RNA-binding proteins are localized in virus-induced SGs and are involved in IFN production, which strongly suggests an association between SG formation and RLR-mediated signal enhancement.<sup>75,81–87</sup> Moreover, Ras-GTPase-activating protein SH3-domain-binding protein 1 (G3BP1), which is a critical component of SG formation, is implicated in RLR-mediated signaling, suggesting a functional interaction between RLRs and SG components in innate antiviral responses.<sup>88–90</sup> However, several reports have revealed that RLR localization in SGs is not necessary for IFN production; thus, the requirement for SGs in RLR-mediated signaling remains unclear.<sup>91–94</sup> Although many viruses have evolved strategies to inhibit SG formation, some exploit SG-containing proteins for their proliferation.<sup>76,95</sup> Thus, it remains unclear whether PKR-induced SG formation is directly involved in the detection of viral RNAs by RLRs.<sup>96</sup> To clarify the function of SGs in antiviral responses, further analysis of the relationship between the life cycle of each virus and the requirement of SG formation is needed. In addition, because SGs are membrane-less aggregates, the lack of an appropriate assay system to investigate their function has been a major problem. However, recent progress based on an in vitro reconstitution system using liquid-liquid phase separation has enabled an understanding of the molecular mechanisms of SG formation.<sup>97,98</sup> Future analyses including those using this in vitro system are expected to clarify the physiological significance of SGs in antiviral responses.

RLRs and their involvement in autoimmune and autoinflammatory diseases

Since RLRs play essential roles in the initial production of IFNs and in regulating acquired immunity, their aberrant activation leads to various disorders, including autoimmune and autoinflammatory diseases.<sup>99–101</sup> A genome-wide analysis revealed that single-nucleotide polymorphisms (SNPs) in *IFIH1*, which encodes MDA5, have been associated with these diseases. Smyth et al. demonstrated that an Ala to Thr mutation at 946 (A946T) of MDA5 (rs1990760) is significantly correlated with the risk of type 1 diabetes.<sup>102</sup> Subsequent studies demonstrated that the A946T mutation is also associated with Graves' disease,<sup>103</sup> rheumatoid arthritis,<sup>104</sup> multiple sclerosis,<sup>105</sup> systemic lupus erythematosus (SLE),<sup>106–108</sup> selective IgA deficiency,<sup>109</sup> and vitiligo.<sup>110,111</sup> In addition, a functional analysis of A946T-containing MDA5 revealed that this mutant exhibits gain-of-function properties and constitutively activates IFN production.<sup>112</sup> Furthermore, Funabiki et al. developed a mouse strain bearing a Glu821Ser mutation that

exhibited a gain-of-function phenotype similar to that in the A946T model. The mutant mice spontaneously developed autoinflammatory symptoms, including lupus-like nephritis.<sup>112</sup> A more recent study developed a knock-in mouse model with MDA5 carrying the A946T mutation, and the results showed that it exacerbated the mouse autoimmune disease through the upregulation of basal IFN and hypersensitivity to self-RNA.<sup>113</sup> Multiple MDA5 SNPs, in addition to A946T, have also been reported to be associated with autoimmune and autoinflammatory diseases.<sup>114,115</sup> Several gain-of-function mutations in *IFIH1* are also associated with Aicardi-Goutieres syndrome (AGS)<sup>116,117</sup> and Singleton-Merten syndrome (SMS).<sup>118</sup> In addition, a loss-of-function mutation in *IFIH1*, Lys365Glu, has been shown to cause severe recurrent respiratory viral infections.<sup>119</sup>

SNPs in *DDX58*, which encodes RIG-I, are also associated with SMS.<sup>120,121</sup> Specifically, Lässig et al. reported that the Glu373Ala and Cys268Phe RIG-I mutants found in SMS, which are devoid of ATPase activity, interact with endogenous ribosomal RNA and activate unintentional ATP-independent IFN production, resulting in SMS development.<sup>122,123</sup> However, little is known about the relationship between MAVS and autoimmune diseases, and although a loss-of-function mutation (Cys79Phe) found in MAVS has been associated with SLE, the molecular mechanism remains unclear.<sup>124</sup>

## REGULATION OF RLR-MEDIATED SIGNALING BY RNA RECOGNITION

RNA recognition by RIG-I

An analysis of RIG-I-KO mice revealed that RIG-I is required for the detection of RNA viruses, including IAV, NDV, and Sendai virus (SeV).<sup>20</sup> Subsequent analyses revealed that multiple types of RNA viruses, including HCV,<sup>125</sup> WNV,<sup>126</sup> dengue virus (DEV), Zika virus (ZIKV),<sup>127</sup> hepatitis D virus (HDV),<sup>128</sup> hepatitis E virus (HEV),<sup>129</sup> and Hantaan virus (HTNV), can be recognized by RIG-I.<sup>130</sup> Since most RNA viruses replicate their own genome using viral-encoded RNA-dependent RNA polymerase (RdRP) within the infected cells, RIG-I recognizes viral genomic RNAs based on their 5'-triphosphate signature.<sup>131,132</sup> The double-stranded structures that cannot be unwound by RIG-I helicase activity, and the short panhandle blunt-ended structures with 5'-triphosphate ends, are preferential structures for RIG-I recognition.<sup>45,133,134</sup> A pregenomic RNA, which has been reverse-transcribed from the genomic DNA of hepatitis B virus (*Hepadnaviridae*) genomic DNA, is also recognized by RIG-I.<sup>27</sup> Moreover, incoming full-length viral IAV RNAs can be recognized by RIG-I.<sup>135–137</sup> Since the IAV RNA genome associates with the virus-derived nucleocapsid protein (NP) and tripartite RdRP complex at the 5'-terminal panhandle structure,<sup>138</sup> the mechanism by which RIG-I recognizes the protected viral RNA-protein complex (RNP) remains unclear. A recent study showed that several host cosensor(s) or regulatory molecule(s) are involved in RIG-I-mediated signaling; however, the molecular machinery critical for the coordinated detection of viral RNP by RIG-I and its cofactors remains unclear. Interestingly, recent reports have demonstrated that small RNAs, such as miniviral RNA (mvRNA), produced by the aberrant replication of viral RNA via RdRP form aberrant viral RNPs, are preferentially recognized by RIG-I.<sup>139,140</sup> In the case of SeV infection, RNA with an abnormal panhandle structure derived from defective interfering (DI) particles can be detected by RIG-I.<sup>141</sup> Additionally, the uridine-rich region of the HCV RNA genome can be recognized by RIG-I.<sup>125</sup> The short viral RNA suppressor of virus RNA (svRNA), generated by host ribonuclease L (RNase L)-mediated cleavage of the HCV RNA genome, is also efficiently detected by RIG-I.<sup>142</sup>

In addition to the 5'-triphosphate structure, the 5'-diphosphate moiety characteristic of the reovirus (RV) RNA genome can also be recognized by RIG-I.<sup>143</sup> This substrate specificity for RNA recognition by RIG-I clearly shows that the 5'-cap and 5'-monophosphate

structures of endogenous RNAs, including that of mRNA, tRNA, and rRNA, cannot be recognized by RIG-I as non-self-RNA.<sup>144</sup> Schuberth-Wagner et al. further demonstrated that, in addition to the 5'-cap structure of the mRNA, 2'-O-methylation at the +1 base neighbor of the 5'-cap, the N1 position, is critical for evading recognition by RIG-I.<sup>145</sup> They also showed that the conserved His830 residue of the RIG-I RNA-binding pocket is critical for distinguishing the 2'-O-methylated RNA at the N1 site, whereas substituting His830 with Ala enhances IFN production via the recognition of 2'-O-methylated endogenous mRNA by RIG-I. Yellow fever virus (YFV), belonging to the *Flaviviridae* family, encodes a 2'-O-methyltransferase that allows it to escape detection by RIG-I.<sup>145</sup> Many viruses have developed this type of evasion strategy to avoid detection by RIG-I. For example, viruses belonging to the *Picornaviridae* family cannot be recognized by RIG-I due to the covalent bonding of the viral protein Vpg (viral protein genome-linked) at the 5'-end of the viral genome.<sup>146</sup> Crimean-Congo hemorrhagic fever virus (CCHFV) and HTNV, belonging to the *Bunyvirales* order; and Borna disease virus (BDV), belonging to the *Bornaviridae* family, have a 5'-monophosphate end that is not recognized by RIG-I.<sup>147</sup> A recent study demonstrated that the RNA of human metapneumovirus (HMPV), belonging to the *Pneumoviridae* family, undergoes N6-methyladenosine modification to evade recognition by RIG-I.<sup>148</sup>

Furthermore, RIG-I can also recognize RNA secreted from bacteria. Specifically, RNA of intracellular pathogenic bacteria, such as *Listeria monocytogenes*, which is released into the cytoplasm, can be detected by RIG-I.<sup>149,150</sup> IFN production via RIG-I signaling has been observed in response to other intracellular bacteria, including *Salmonella enterica* serovar Typhimurium<sup>151</sup> and *Mycobacterium tuberculosis*.<sup>152</sup> A recent study showed that secreted *L. monocytogenes* RNA complexed with the bacterial RNA-binding protein *Imo2686/zea* can also be recognized by RIG-I.<sup>153</sup> Furthermore, *M. tuberculosis* RNA is delivered into the extracellular vesicles of macrophages, leading to the activation of RIG-I-mediated signaling in uninfected cells.<sup>154</sup> These observations strongly suggest that RIG-I-mediated signaling has a broad function in the self-defense machinery against pathogenic infection. Notably, further analysis of the maintenance of homeostasis in the intestinal and respiratory tracts via commensal microbes and RIG-I-mediated signaling is necessary.<sup>22,23,155</sup>

#### Interactions between RIG-I and endogenous RNAs

Since the 5'-triphosphate signature in host RNAs is apparent during transcription by RNA polymerases, RIG-I can recognize host endogenous RNAs (Fig. 3c). An analysis of IFN production in response to dsDNA showed that 5'-triphosphate RNA transcribed by RNA polymerase III from intracellular poly(dA-dT) DNA can induce IFNs via RIG-I.<sup>156,157</sup> This pol III-mediated activation of RIG-I has been analyzed in host responses against DNA virus infections. Next-generation sequencing of RNA samples interacting with RIG-I during herpes simplex virus (HSV; *Herpesviridae*) infection led to the identification of endogenous RNA transcribed from 5S ribosomal RNA pseudogene 141 (*RNA5SP141*). *RNA5SP141*, transcribed by RNA pol III in the nucleus, is translocated to the cytoplasm and activates RIG-I-mediated signaling.<sup>158</sup> In cells infected with Kaposi's sarcoma-associated herpesvirus (KSHV; belonging to the *Herpesviridae* family), an endogenous RNA designated "vault RNA" transcribed by RNA pol III accumulates as a 5'-triphosphate-containing RNA due to the reduced expression of DUSP11 (dual-specificity phosphatase 11) and induces RIG-I-mediated signaling to inhibit lytic reactivation of KSHV.<sup>159</sup> However, RIG-I can recognize KSHV RNA in a pol III-independent manner.<sup>160</sup> These findings indicate that activation of the RIG-I pathway via pol III-induced endogenous RNA is a critical host strategy to fight against DNA virus infections. In addition, endogenous 7SL RNA transcribed by RNA pol III can activate RIG-I-mediated signaling in cancer cells. 7SL RNA, a signal

recognition particle (SRP) component, is upregulated in stromal cells by Notch-Myc signaling from breast cancer cells, and an excess of 7SL RNA is secreted in exosomes, which in turn activates RIG-I in breast cancer cells, leading to increased cancer growth, metastasis, and treatment resistance.<sup>161</sup> However, *Alu*-derived RNA, an endogenous retrotransposon sequence originating from 7SL RNA, exhibits anticancer effects via RIG-I activation. The inhibition of heterogeneous nuclear ribonucleoprotein C (HNRNPC), which is highly expressed in cancer cells, suppresses HNRNPC-mediated cellular splicing, resulting in the accumulation of *Alu* sequences within the pre-mRNA intron and *Alu*-mediated activation of RIG-I to inhibit cancer growth.<sup>162</sup> Several long noncoding (lnc)RNAs repress the RIG-I pathway. Mouse-specific lnc-Lsm3b and human-specific lncATV, which are upregulated by viral infection, compete with viral RNAs for detection by RIG-I, thereby inhibiting RIG-I-mediated IFN production.<sup>163,164</sup> However, microRNAs involved in posttranscriptional regulation are believed to be unrecognized by RIG-I, as they do not contain 5'-triphosphate and have a 3'-overhang. However, several miRNAs enriched in uridines are potential RIG-I agonists.<sup>165,166</sup> For example, miR136, the expression of which is induced by IAV infection, can inhibit IAV growth by activating RIG-I-mediated signals. Moreover, the introduction of exogenous circular RNAs (circRNAs) into cells can activate RIG-I in a 5'-triphosphate- or double-stranded structure-independent manner.<sup>167</sup> Chen et al. further showed that endogenous circRNAs, generated by back-splicing to covalently link the 3' and 5' ends of the RNA exon, avoid RIG-I recognition by exhibiting N6-methyladenosine modification.<sup>168</sup> However, several reports have also demonstrated that contaminating short ssRNAs generated during the production of circRNAs are critical for circRNA-mediated RIG-I activation.<sup>169</sup> Further analysis is required to elucidate the physiological significance of circRNA-mediated RIG-I activation.

Finally, based on accumulating evidence highlighting the physiological significance and molecular machinery of RIG-I-mediated signaling, research on the agonist/antagonist targeting of RIG-I has been initiated. For example, 5'-triphosphate dsRNA has been shown to exhibit antiviral activity against lethal IAV infection.<sup>170</sup> Furthermore, a unique RIG-I agonist has been reported to exhibit significant antitumor activity.<sup>171</sup> RIG-I antagonists have also been identified with the aim of controlling excessive IFN production.<sup>172</sup>

#### RNA recognition by MDA5

An analysis of MDA5-KO mice revealed that MDA5 is involved in detecting viruses belonging to the *Picornaviridae* family, including EMCV and Theiler's encephalomyelitis virus (TMEV).<sup>25</sup> A subsequent study revealed that MDA5 can recognize relatively long dsRNAs.<sup>24</sup> In addition to picornaviruses, MDA5 also recognizes DEV (*Flaviviridae*), RV (*Reoviridae*), a segmented dsRNA virus,<sup>173</sup> murine norovirus (MNV; *Caliciviridae*), with Vpg at the 5'-end of the viral RNA,<sup>174</sup> murine hepatitis virus (MHV) belonging to the *Coronaviridae* family with a 5' cap,<sup>175</sup> as well as other viruses<sup>101</sup>. The non-self-RNA signature of these viruses is a double-stranded structure generated as a replication intermediate.<sup>176</sup> However, the exact RNA structure recognized by MDA5 is unclear, and further analysis is required to determine the specific properties of MDA5 ligands.<sup>177-179</sup> In the case of coronavirus infection, the 2'-O-methylation of viral RNA by 2'-O-methyltransferase encoded by viral nonstructural protein 16 (nsp16) suppresses MDA5-mediated IFN production;<sup>180</sup> however, it is unclear whether 2'-O-methylation directly impacts the binding of viral RNA to MDA5.<sup>145</sup> A recent report on MHV showed that the panhandle dsRNA structure containing 5'-polyuridines of negative-sense RNA (PUN RNA) is an agonist of MDA5.<sup>181</sup> Since SARS-CoV-2 belongs to the *Coronaviridae* family as MHV, it might be recognized by MDA5 via a similar mechanism. Degradation of PUN RNA by MHV nsp15, coding for a polyU-specific endoribonuclease (EndoU), limits MDA5-mediated

IFN production, suggesting a viral strategy to escape antiviral activity.<sup>181</sup> Considering that it is conserved in the SARS-CoV-2 genome, the EndoU gene might serve as an effective potential therapeutic target for COVID-19 treatment. In addition, MDA5 can detect RNA from malaria parasites. *Plasmodium* infection induces type I IFN in an MDA5- and MAVS-dependent manner and limits the propagation of parasitemia.<sup>182,183</sup> These observations suggest that MDA5-mediated IFN production modulates innate immune responses to a wide range of pathogen infections.

#### Interaction between MDA5 and endogenous RNA

It is becoming clear that long dsRNAs, which are substrates of MDA5, can be generated from endogenous transposable repetitive sequences, such as *Alu* and retroviral elements (ERVs).<sup>101,184</sup> *Alu* repeats are present at more than one million sites in the human genome. *Alu* RNAs transcribed from inverted repeats in the genome can be recognized by MDA5 when they form a dsRNA structure.<sup>185</sup> However, in the steady state, A-to-I modification by adenosine deaminase acting on RNA (ADAR)1 prevents the formation of immunostimulatory dsRNAs. ADAR1-deficient mice are embryonic lethal due to global IFN induction;<sup>186</sup> however, this phenotype is partially rescued by the deletion of MAVS or MDA5 but not RIG-I,<sup>187,188</sup> suggesting that ADAR1 is a specific suppressor of MDA5/MAVS signaling. A recent report showed that dysfunction caused by a mutation in the *ADAR1* gene leads to the abnormal accumulation of *Alu*-derived dsRNA, resulting in the overproduction of IFNs, which leads to autoimmune diseases, including AGS.<sup>117,185,188–190</sup> In addition, dsRNAs generated by the activation of intrinsic ERVs following treatment with DNA demethylating drugs or via the bidirectional transcription of specific ERV regions can also be recognized by MDA5, leading to enhanced antitumor activity through IFN production.<sup>191–193</sup> In adult human cells, tripartite motif (TRIM) protein 28 (TRIM28, also known as KAP1) represses ERV transcription via epigenetic modification. However, the depletion of TRIM28 induces the RLR-mediated pathway, presumably as a result of dsRNA generation from ERV-derived RNAs.<sup>194</sup>

MDA5 can also recognize mitochondrial dsRNAs. The translocation of unstable mitochondrial dsRNAs from the mitochondria to the cytoplasm is suppressed by the mitochondrial RNA helicase SUV3 and the polynucleotide phosphorylase PNPase; however, loss of function of either protein induces cytoplasmic accumulation of dsRNAs, leading to the activation of MDA5-mediated IFN production.<sup>195</sup> The noncoding RNA ITPRIP-1 also plays an auxiliary role in supporting MDA5 oligomer formation by binding to the C-terminus of MDA5.<sup>196</sup>

## REGULATION BY HOST PROTEINS

Regulation by ubiquitin ligases and deubiquitinases  
Posttranslational modification plays an essential role in regulating RLR/MAVS-mediated signaling. In particular, regulation by ubiquitination has been extensively analyzed (Fig. 3d). In 2007, Gack et al. reported that Lys63 (K63)-linked ubiquitination at the Lys172 residue of RIG-I CARD by TRIM25, a RING-finger-containing E3 ubiquitin ligase, was required for CARD-mediated signaling activation.<sup>197</sup> Subsequent analysis using an in vitro reconstitution assay system demonstrated that the unanchored K63-linked ubiquitin chain added by TRIM25 is also involved in RIG-I activation.<sup>198</sup> The crystal structure of the RIG-I CARD with K63-linked ubiquitin revealed that both anchored and unanchored ubiquitin chains might participate in the stabilization of the “lock washer”-like tetramer structure formed by RIG-I CARD.<sup>53</sup> Moreover, TRIM25 regulates RIG-I-mediated signaling in coordination with multiple host factors. Specifically, the mitochondrial-targeting chaperone 14-3-3 $\epsilon$  forms a complex with RIG-I and TRIM25 to regulate the transition from the cytoplasm to MAM, which is implicated in the RIG-I/MAVS interaction.<sup>199</sup> ZCCHC3, a CCHC-type

zinc finger protein, recruits TRIM25 to RIG-I/MDA5 and enhances the ubiquitin-mediated activation of RIG-I/MDA5.<sup>200</sup> Caspase-12, an inflammatory caspase, contributes to the innate response to WNV infection by enhancing the K63-linked ubiquitination of RIG-I by TRIM25.<sup>201</sup> Furthermore, by associating with RIG-I and TRIM25, nuclear Ddf2-related 2 (NDR2), an NDR family serine/threonine kinase, also positively regulates the addition of ubiquitin chains by TRIM25.<sup>202</sup> However, the linear ubiquitin assembly complex (LUBAC), consisting of the E3 ligase heme-oxidized IRP2 ligase-1L (HOIL-1L), and HOIL-1L-interacting protein (HOIP), negatively regulates RIG-I signaling by adding a K48-linked ubiquitin chain onto TRIM25, leading to its proteasomal degradation.<sup>203</sup> This K48-linked ubiquitin chain on TRIM25 is eliminated by a deubiquitinating enzyme ubiquitin-specific peptidase (USP)15.<sup>204</sup> A recent study showed that NLRP12, a member of the NLR family, binds to TRIM25 and inhibits the TRIM25-mediated ubiquitination of RIG-I while simultaneously enhancing the degradation of RIG-I via K48-linked ubiquitination of RIG-I by another E3 ligase, ring finger protein (RNF)125, demonstrating the functional interaction between the two PRR families.<sup>205</sup> Notably, recent studies have reported that TRIM25 is capable of RNA binding, which is required for regulating signals by a mechanism that does not involve ubiquitin ligase activity.<sup>206–209</sup>

Riplet (also known as RNF135 and REUL) is another critical K63-linked E3 ligase for RIG-I.<sup>210–212</sup> Riplet conjugates ubiquitin on multiple Lys residues in the CARD and CTD of RIG-I. Although a previous study suggested that Riplet functions upstream of TRIM25 through the ubiquitination of Lys788 in the RIG-I CTD,<sup>82</sup> more recent studies have shown that Riplet, but not TRIM25, may be an essential regulator of RIG-I activation.<sup>213–215</sup> Mechanistically, Cadena et al. proposed a model in which Riplet is involved in upregulating RIG-I-mediated signaling via the initial ubiquitination of oligomerized RIG-I on substrate dsRNAs and the subsequent cross-bridging of RIG-I filaments on longer dsRNAs, leading to signal amplification.<sup>214</sup> Furthermore, Ube2D3, an E2 ligase, has been identified as a coregulator of Riplet-mediated RIG-I activation.<sup>213</sup> However, another E3 ligase, zinc finger protein (ZNF)598, has been identified as a negative regulator of K63-linked ubiquitination by Riplet. ZNF598 conjugates the ubiquitin-like protein FAT10 (also known as ubiquitin D) on RIG-I to inhibit Riplet-mediated ubiquitination of RIG-I.<sup>216</sup>

In addition to TRIM25 and Riplet, TRIM4 and MEX3C have also been identified by expression screening methods as positive regulatory E3 ligases of RIG-I.<sup>83,217</sup> However, recent studies suggest that these ligases are not involved in RIG-I-mediated MAVS aggregation in vitro.<sup>213</sup> The physiological function of the four E3 ligases, specifically TRIM25, Riplet, TRIM4 and MEX3C, in the activation of RIG-I remains unclear. This issue has been discussed in detail in recent excellent review articles.<sup>218,219</sup> Briefly, although all of these molecules may be involved in the positive regulation of RIG-I activation, differing analytical conditions of recent reports has led to conflicting interpretations. Future analysis that takes into account differences in viral, animal, and cell types is expected to reveal the exact regulatory mechanisms based on specific usage and/or redundancy of the molecules in RIG-I-mediated signal activation.

Several E3 ligases that negatively regulate RIG-I have been identified. For example, TRIM40 promotes proteasomal degradation by conjugating K27- and K48-linked ubiquitin chains on both RIG-I and MDA5,<sup>220</sup> whereas RNF125 and RNF122 negatively regulate RIG-I activation via K48-linked ubiquitination of RIG-I CARD.<sup>221,222</sup> The RNF125-mediated degradation of RIG-I is antagonized by the SG component G3BP1 via enhanced self-ubiquitination of RNF125.<sup>89</sup> After viral infection, the STUB1 E3 ligase associates with the CARD of RIG-I via the trithorax group protein mixed lineage leukemia (MLL5), promoting K48-linked ubiquitin-mediated degradation of RIG-I.<sup>223</sup> Another E3 ligase, c-Cbl, is recruited, together with RIG-I, to Siglec-G, which is



expressed on the plasma membrane and promotes the proteasomal degradation of RIG-I via K48-linked ubiquitin conjugation on the Lys813 residue of RIG-I.<sup>224</sup> Moreover, Siglec-G belongs to the CLR family, suggesting an interaction between different PRRs.

Regulation by several deubiquitinating enzymes has been demonstrated. USP4 and ovarian tumor-domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) stabilize RIG-I proteins by eliminating the K48-linked ubiquitin chains conjugated to RIG-I.<sup>225,226</sup> Conversely, *CYLD*, a tumor suppressor gene expressed in cylindromatosis, negatively regulates RIG-I activation by deubiquitinating the K63-linked ubiquitin chains on RIG-I.<sup>227</sup> Moreover, syndecan-4 (SDC4), which is a TM protein, complexes with both RIG-I and CYLD to promote the CYLD-mediated deubiquitination of RIG-I, leading to subsequent signaling inhibition.<sup>228</sup> Furthermore, USP3,<sup>229</sup> USP21,<sup>230</sup> USP14,<sup>231</sup> and USP27X<sup>232</sup> also deubiquitinate the K63-linked ubiquitin of RIG-I to inhibit RIG-I function.

An in vitro reconstitution analysis revealed that MDA5 CARD also requires K63-linked ubiquitin chains to activate the signaling pathway.<sup>233</sup> However, the precise mechanism associated with ubiquitin-mediated regulation of MDA5 has remained unclear. TRIM65 promotes K63-linked ubiquitination at Lys743 of MDA5 to enhance the oligomerization of MDA5 on dsRNAs.<sup>234</sup> Moreover, arrestin domain-containing 4 (ARRDC4) interacts with MDA5 to recruit TRIM65 in response to enterovirus 71 (EV71) infection, leading to the activation of MDA5-mediated signaling via Lys63-linked ubiquitination of MDA5.<sup>235</sup>

The regulation of MAVS by ubiquitin has also been extensively analyzed. K63-linked ubiquitination at Lys19, Lys311, and Lys461 of MAVS by TRIM31 is critical for the formation of prion-like aggregates of MAVS.<sup>236</sup> The scaffold protein FAS-associated factor 1 (FAF1) negatively regulates TRIM31-mediated ubiquitination of MAVS by disrupting the interactions between MAVS and TRIM31.<sup>237</sup> The phosphorylation of FAF1 by IKK $\epsilon$ , which is activated by RLR-mediated signaling, induces FAF1 degradation and promotes the activation of MAVS-mediated signaling. OTUD4, a deubiquitinase, enhances MAVS-mediated signaling by stabilizing MAVS and by eliminating K48-linked ubiquitins.<sup>238</sup>

Several studies have demonstrated that the ubiquitination of MAVS negatively regulates MAVS-mediated signaling. In contrast to RIG-I, TRIM25 induces the degradation of MAVS via K48-linked ubiquitination at Lys7 and Lys10 of MAVS.<sup>239</sup> The HECT domain-containing E3 ligase ITCH (also known as AIP4) binds to MAVS via poly(C)-binding protein (PCBP) 1/2 and TAX1 binding protein (TAX1BP1), which triggers K48-linked ubiquitination of MAVS and its subsequent degradation.<sup>240–242</sup> Membrane-associated ring-CH-type finger 5 (MARCH5), a mitochondrial ubiquitin ligase, and RNF5 promote MAVS degradation via K48-linked ubiquitination at Lys7 and 500, as well as at Lys362 and 461, respectively. Interestingly, ER-associated inactive rhomboid protein (irhom) 2 inhibits the MARCH5- and RNF5-mediated degradation of MAVS and positively regulates MAVS-mediated signaling.<sup>243</sup> The TRIM29 protein conjugates with the K11-linked ubiquitin chain on MAVS for its degradation.<sup>244</sup> However, MARCH8 is recruited by Tetherin (also known as BST2) to bind to MAVS and promote K27-linked ubiquitination at Lys7 of MAVS.<sup>245</sup> Moreover, RNF34 conjugates with K27- and K29-linked ubiquitin chains at multiple Lys/Arg positions of MAVS. This K27/K29-linked ubiquitination induces NDP52/CALCO2-dependent autophagic degradation of MAVS.<sup>246</sup> The activity of MAVS is also regulated by deubiquitinases, including YOD1,<sup>247</sup> and OTUD3,<sup>248</sup> which repress MAVS-mediated signaling by removing the K63-linked ubiquitin chains. Although a considerable number of studies have reported the regulation of RLR/MAVS-mediated signaling via ubiquitination/deubiquitination, the mechanisms underlying these regulatory processes remain unclear, and further studies are warranted.

#### Roles of other ubiquitin-like modifiers

Regulation via ubiquitin-like modifications has also been demonstrated (Fig. 3e). An early study showed that RIG-I protein levels are negatively regulated by conjugation with the IFN-inducible ubiquitin-like protein ISG15 (ISGylation).<sup>249</sup> Very recently, it has been reported that ISGylation of MDA5 is essential for triggering IFN production in response to several RNA viruses, including coronaviruses.<sup>250</sup> Furthermore, TRIM38 positively regulates both RIG-I and MDA5 via SUMOylation at K43/K865 and K96/K888, respectively.<sup>251</sup> The SUMOylation of RLRs inhibits degradation by K48-linked ubiquitination. However, in the late stage of infection, de-SUMOylation by SUMO-specific protease 2 (SEN2) enhances degradation, which leads to disrupted signaling. As mentioned earlier, Riplet-mediated RIG-I activation is attenuated by the conjugation of FAT10 with RIG-I.<sup>216</sup> Moreover, the noncovalent interaction between FAT10 and RIG-I suppresses RIG-I activation.<sup>252</sup>

#### Regulation by kinases and phosphatases

RIG-I is negatively regulated by phosphorylation at Thr170 and Ser8 (Fig. 3f).<sup>253,254</sup> Protein kinase C (PKC)- $\alpha$  and PKC- $\beta$  are involved in phosphorylation, which suppresses the TRIM25 interaction and K63-linked ubiquitination, resulting in the inhibition of RIG-I-mediated signaling.<sup>255</sup> However, as mentioned earlier, the requirement of TRIM25 in RIG-I activation remains unclear and requires further analysis.<sup>213–215</sup> Casein kinase 2 (CK2) phosphorylates RIG-I at Thr770, Ser854, and Ser855,<sup>256</sup> which might be necessary for maintaining RIG-I in the autorepressed state. Phosphorylation of RIG-I by DAPK1 at Thr667, located within the helicase domain, also suppresses RIG-I-mediated signaling. Since DAPK1 activity is induced by RIG-I-mediated signaling, negative feedback regulation by DAPK1 is suggested.<sup>257</sup> However, MDA5 is phosphorylated by RIO kinase 3 (RIOK3) at Ser828, which inhibits MDA5 oligomerization.<sup>258</sup> Moreover, phosphorylation at Ser88 in the MDA5 CARD also inhibits MDA5 activation.<sup>54</sup> Importantly, dephosphorylation of RIG-I and MDA5 by PP1 $\alpha$  and PP1 $\gamma$  induces the transition of RLRs to an activated state, indicating that dephosphorylation is one of the fundamental regulatory mechanisms for RLR activation.<sup>54</sup> The regulation of MAVS by phosphorylation has also been reported. Protein kinase A (PKA) promotes the degradation of MAVS by MARCH5 by phosphorylating Thr54 of MAVS and suppressing IFN induction.<sup>259</sup> In contrast, phosphorylation of MAVS Tyr by c-Abl positively regulates MAVS-mediated IFN production.<sup>260,261</sup>

#### Regulation by other modifications

RIG-I is regulated by acetylation (Fig. 3e). For example, the deacetylation of the Lys858 and Lys909 residues of RIG-I via the deacetylating enzyme HDAC6 is implicated in viral RNA detection by RIG-I;<sup>262,263</sup> however, the molecular mechanisms underlying RIG-I acetylation remain unclear. Moreover, MAVS is positively regulated by O-GlcNAcylation.<sup>264,265</sup> In fact, Li et al. demonstrated that O-GlcNAcylation at Ser366 of MAVS in response to VSV infection promotes K63-linked ubiquitination of MAVS by TRIM31, leading to increased IFN production.

#### Regulation by RNA-binding proteins

Since RLRs are RNA-binding proteins, activation of RLR-mediated signaling is regulated by the coordination of various host RNA-binding proteins (Fig. 3g). Members of the 2'-5' oligoadenylate (2-5 A) synthetase (OAS) family, which are classical antiviral ISGs, are activated by viral dsRNA and eliminate viral RNA via the activation of the host ribonuclease RNase L.<sup>266</sup> Interestingly, human OAS-like protein (OASL) is a member of the OAS family that contains a unique ubiquitin-like domain but lacks oligoadenylation activity. The ubiquitin-like domain of OASL mimics the K63-linked polyubiquitin moiety and induces the oligomerization



of RIG-I on RNA substrates, thereby enhancing RIG-I-mediated signaling.<sup>267</sup>

PKR, another classic antiviral ISG, has been extensively analyzed.<sup>268</sup> PKR inhibits viral growth by potently repressing host translation through the phosphorylation of eIF-2 $\alpha$ . In terms of the regulatory function of RLRs, as mentioned earlier, SGs formed via PKR mediation might play critical roles as platforms for viral RNA detection by RLRs, suggesting an indirect regulatory function of PKR in RLR-mediated signaling.<sup>269,270</sup> Moreover, the RNA helicase DHX36 facilitates PKR-dependent SG formation, resulting in increased RLR-mediated IFN production.<sup>75</sup>

Protein activators of PKR (PACT) and TRBP are dsRNA-binding proteins that exert positive and negative regulatory functions of PKR, respectively.<sup>271</sup> Initially, PACT was reported to participate in IFN production<sup>272</sup> but was subsequently shown to also physically associate with both RIG-I and MDA5, effectively augmenting their signaling by enhancing ATPase function and oligomerization, respectively.<sup>273,274</sup> Moreover, recent studies have indicated that the PACT-LGP2 interaction is required for upregulated MDA5-mediated signaling.<sup>275,276</sup> However, the regulation of RLR-mediated signaling by PACT is antagonized by viral factors, including VP35 of Ebola virus (EBOV), NS1 of IAV, and NP of arenavirus.<sup>277–279</sup> Importantly, the 4a protein of Middle East respiratory syndrome coronavirus (MERS-CoV) and the nucleocapsid (N) protein of MHV and SARS-CoV directly interact with PACT to inhibit RLR-mediated signaling,<sup>280,281</sup> suggesting that it is a possible therapeutic target for COVID-19. In addition to the inhibitory function of PKR, TRBP enhances MDA5-mediated innate responses by physically interacting with LGP2 in response to infection by cardiovirus, which belongs to the *Picornaviridae* family.<sup>282</sup> However, TRBP plays an essential role in miRNA processing from precursor RNAs in conjunction with the ribonuclease Dicer.<sup>283</sup> Recent studies have shown that the enhanced expression of LGP2 in response to viral infection suppresses Dicer-mediated miRNA maturation via the LGP2-induced sequestration of TRBP from the Dicer-TRBP complex.<sup>34</sup> Genome-wide analysis revealed that the suppression of RNA silencing by TRBP-dependent miRNA leads to enhanced specific gene expression, including cell death-related gene expression, suggesting a potential novel antiviral defense mechanism induced by the LGP2-TRBP interaction.<sup>35</sup>

The involvement of multiple RNA helicases in RLR-mediated signaling has been demonstrated. For example, DDX60 can enhance RIG-I-mediated signaling by associating with RIG-I, indicating its role as a sentinel RNA sensor.<sup>284,285</sup> However, another report revealed that DDX60 deficiency does not affect IFN production in response to several RNA viruses or dsRNAs, suggesting that it might function in antiviral responses against very specific viral species.<sup>286</sup> In addition, DDX6, DHX29, and DHX15 can also function as cosensors of RIG-I and MDA5 by directly interacting with RLRs.<sup>287–290</sup> However, DDX3, which was initially identified as a MAVS-interacting helicase via two-hybrid screening,<sup>291</sup> participates in the detection of aberrant human immunodeficiency virus type 1 (HIV-1) RNA in dendritic cells (DCs), which induces MAVS-mediated signaling independent of RLRs.<sup>292</sup> Furthermore, DHX9 is also involved in the RLR-independent activation of MAVS-mediated signaling in response to dsRNA in DCs.<sup>293</sup> Since the molecular mechanisms of these observations remain unclear, further analysis is necessary.

#### Regulation by other host proteins

14-3-3 proteins, including 14-3-3 $\epsilon$  and 14-3-3 $\eta$ , can function as mitochondrial transporting chaperones for RIG-I and MDA5, respectively (Fig. 3h).<sup>199,294</sup> NS3 proteins of DENV, ZIKV, and presumably WNV antagonize RLR-mediated signaling by sequestering 14-3-3 proteins.<sup>295</sup> Zyxin, which is involved in actin polymerization, localizes with MAVS on the mitochondria and functions as a scaffold protein for the interaction between RLRs

and MAVS.<sup>296</sup> SIDT2, the mammalian ortholog of the *Caenorhabditis elegans* dsRNA transporter systemic RNA interference defective protein 1 (SID-1), has been shown to enhance RLR signaling by enhancing the transport of internalized dsRNA from endosomal compartments to the cytoplasm for viral detection by RLRs.<sup>297</sup>

RLR/MAVS-mediated signaling is inhibited by multiple host factors. Soonthornvacharin et al. identified K-homology splicing regulatory protein (KHSRP) as a negative regulatory molecule for RIG-I-mediated signaling by genome-wide RNAi screening.<sup>298</sup> KHSRP suppresses the early activation of RIG-I by directly associating with RIG-I CTD. The predicted methyltransferase TTL12, which was identified as a host regulator that inhibits SeV-induced RIG-I-mediated signaling, binds to MAVS to suppress signaling.<sup>299</sup> DNAJ homolog subfamily B member 1 (DNAJB1), which is a heat shock protein (HSP) 40 family member protein, was identified as an MDA5-interacting molecule.<sup>87</sup> In response to viral infection, DNAJB1 is translocated with HSP70 to SGs, where it inhibits the formation of signal-competent MDA5/MAVS filaments. The zinc finger FYVE domain-containing protein ZFYVE1 specifically associates with MDA5 and inhibits MDA5-mediated signaling.<sup>300</sup> FK506-binding protein 8 (FKBP8) inhibits the RLR-MAVS pathway by directly binding to MAVS.<sup>301</sup> Eckard et al. demonstrated that SKIV2L, which is a cytosolic 3'-5' RNA exosome, prevents the constitutive activation of RLRs via the degradation of endogenous RNAs, which are produced by inositol requiring enzyme-1 (IRE-1)-mediated cytoplasmic splicing in response to the unfolded protein response.<sup>302</sup> They also observed that patients with human trichohepatoenteric syndrome (THES), caused by a SKIV2L loss-of-function mutation, exhibit an excessive IFN signature, indicating that the metabolic machinery of endogenous RLR substrates consists of exosomal RNAs.<sup>303</sup> NLRX1, a mitochondrial-localized NLR family member, suppresses RIG-I-mediated signaling by interrupting RIG-I and MAVS.<sup>304,305</sup> A subsequent study demonstrated that NLRX1 not only suppresses RIG-I-mediated signaling but also promotes autophagosome formation in association with mitochondrial Tu translation elongation factor (TUFM), leading to enhanced VSV replication.<sup>306</sup> The involvement of autophagy and related proteins, such as Atg5-Atg12, in RLR-mediated signaling has been demonstrated (Fig. 3i).<sup>307,308</sup> Recently, leucine-rich repeat-containing protein 25 (LRRC25) has been reported to interact with ISGylated RIG-I and to promote the degradation of RIG-I in autophagosomes to suppress IFN production.<sup>309</sup> However, LRRC59 inhibits the degradation of ISGylated RIG-I by competing with LRRC25.<sup>310</sup> Coiled-coil domain-containing 50 (CCDC50), a novel autophagy receptor, also induces the degradation of RIG-I/MDA5 by recruiting it as an autophagic cargo in a K63-linked ubiquitin-dependent manner to inhibit the IFN pathway.<sup>311</sup> Immunity-related GTPase M (IRGM), in which loss-of-function mutations are known to be genetically associated with various immune diseases,<sup>312</sup> binds to RIG-I/MAVS to promote autophagic degradation.<sup>313</sup> Finally, a recent study revealed that caspase-3 negatively regulates this signaling pathway via the degradation of MAVS and IRF-3.<sup>314</sup>

#### REGULATION BY VIRAL PROTEINS

Most viruses have evolved strategies that favor their proliferation by suppressing the host innate immune system.<sup>315</sup> In particular, the target molecules range from RLR/MAVS to downstream signaling molecules such as TRAFs, IKKs, IRFs, NF- $\kappa$ Bs, IFNRs, JAKs, and STATs. In this section, we summarize recent findings regarding direct interactions between RLR/MAVS and pathogenic viruses (Fig. 3).

#### Negative-sense ssRNA viruses

RNA viruses with single-stranded, negative-sense genomes inhibit RLR/MAVS in various ways. For example, the V protein of viruses

belonging to the *Paramyxoviridae* family suppresses IFN production by interacting with MDA5 and LGP2 but not with RIG-I.<sup>316–318</sup> Moreover, the V proteins of measles virus (MV) and Nipah virus (NiV) inhibit dephosphorylation-mediated activation of MDA5 by directly associating with PP1 $\alpha/\gamma$ .<sup>319</sup> However, since paramyxoviruses have a 5'-triphosphate moiety, which is a non-self-RNA signature specific for RIG-I, the biological significance of the V-mediated repression of MDA5 is unclear. Notably, MDA5 has been suggested to be involved in SeV-induced IFN production in the late stages of infection; thus, the mechanism of action of V proteins might differ depending on the time points of the antiviral response.<sup>320</sup> The V proteins of NiV, SeV, and parainfluenzavirus (PIV) can suppress RIG-I-mediated signaling by preventing TRIM25-mediated ubiquitin conjugation on RIG-I via complex formation with both RIG-I and TRIM25.<sup>321</sup> However, the V proteins of several paramyxoviruses, including NDV, suppress IFN signaling by enhancing MAVS degradation by the RNF5 E3 ligase.<sup>322</sup>

Moreover, the NS1 protein of human respiratory syncytial virus (RSV), a member of the *Pneumoviridae* family, also interacts with TRIM25 and inhibits ubiquitin-conjugation of RIG-I.<sup>323</sup> In addition, the NS2 protein of RSV directly binds to RIG-I CARD, which inhibits the association between activated RIG-I and MAVS.<sup>324</sup> The VP35 protein of EBOV and Marburgvirus (MARV), which belongs to the *Filoviridae* family, can bind dsRNA and antagonize RLR-mediated signaling.<sup>325,326</sup> In addition, VP35 can suppress RIG-I-mediated signaling by suppressing RIG-I-PACT interactions.<sup>277</sup> BDV, a member of the *Bornaviridae* family, has a strategy to trim several nucleotides at the 5' end of the viral genome to evade recognition by RIG-I, resulting in latent infection.<sup>147,327</sup>

Viruses belonging to the *Orthomyxoviridae* family, including IAV, also have negative-sense ssRNAs. The IAV NS1 protein, derived from the smallest viral genome of the eight segments that make up the IAV genome, is a well-known multifunctional virulence factor. NS1 contains an N-terminal dsRNA-binding domain and C-terminal effector domain that suppress antiviral responses through distinct actions.<sup>328</sup> While the N-terminal dsRNA-binding domain antagonistically represses RIG-I-mediated IFN production,<sup>132,329</sup> NS1 also inhibits RIG-I via direct interaction in an RNA-independent manner.<sup>330</sup> NS1 also associates with TRIM25 and Riplet and represses the K63-linked ubiquitination of RIG-I.<sup>331,332</sup> The C-terminal effector domain of NS1 interacts with several host factors, including PKR.<sup>333</sup> IAVs that lack NS1 can efficiently activate PKR and enhance RIG-I-mediated IFN production via SG formation.<sup>73</sup> The PB1-F2 protein is a mitochondrial-localized peptide (87 amino acids) translated from the PB1-encoding RNA segment and contributes to virulence.<sup>334</sup> Although PB1-F2 inhibits MAVS-mediated signaling via direct interaction,<sup>335</sup> PB1-F2 of H7N9 highly pathogenic IAVs inhibits K63-linked ubiquitination of MAVS by suppressing the TRIM31-MAVS interaction.<sup>336</sup> PB1-F2 can also repress MAVS-mediated signaling by degrading MAVS via mitophagy.<sup>337</sup> However, M2, a membrane protein generated from the M-encoding RNA segment, enhances MAVS-mediated signaling by antagonizing reactive oxygen species (ROS)-induced autophagy activation and increasing the number of MAVS aggregates on the mitochondria.<sup>338</sup>

#### Positive-sense ssRNA viruses

HCV, a member of the *Flaviviridae* family, encodes protease NS3/4A, which is essential for the cleavage of the viral polyprotein. NS3/4A inhibits IFN production via the degradation of MAVS.<sup>339,340</sup> NS3/4A also disrupts RIG-I-mediated signaling by degrading Riplet.<sup>82</sup> Since many flaviviruses are transmitted by arthropods (known as arboviruses), they share several standard features. The phosphomimetic amino acid sequences in the NS3 proteins of ZIKV, DENV, and WNV repress RLR-mediated signaling by associating with the scaffold proteins 14-3-3 $\epsilon$  and  $\eta$ .<sup>295,341</sup> The capsid proteins of DENV and WNV suppress IFN- $\lambda$  gene expression via the degradation of peroxisome-localized MAVS in virus-

infected cells.<sup>342</sup> NS4A of DENV and ZIKV inhibits IFN signaling by directly interacting with MAVS.<sup>343,344</sup> Flaviviruses produce unique subgenomic flavivirus RNA (sfRNA), a noncoding RNA derived from the 3' UTR, which is associated with viral pathogenicity.<sup>345</sup> A comparative analysis of the DENV viral clade revealed that an increase in sfRNA inhibits the deubiquitination of the K48-linked ubiquitin chains of TRIM25, which suppresses the RIG-I-mediated signaling induced by TRIM25.<sup>206</sup>

Viruses belonging to the *Picornaviridae* family terminate innate immune signaling using their protease activity, which is essential for processing viral polyproteins. Enteroviruses have two proteases, 2A and 3C. EV71, coxsackievirus 3 (CBV3), and poliovirus (PV) degrade MDA5 via their respective 2A protease.<sup>346</sup> Moreover, MAVS can also be degraded by 2A and 3C proteases.<sup>346–349</sup> In the case of PV infection, degradation of MDA5 by the host proteasome and caspase has been suggested.<sup>350</sup> Furthermore, RIG-I can also be processed by enteroviral proteases; however, its biological significance is unclear.<sup>351</sup> In addition, 3C of PV and EMCV inhibits SG formation by degrading G3BP1 (an essential regulator of SGs)<sup>352</sup> and represses SG-mediated enhancement of RLR-mediated signaling.<sup>353</sup> Foot-and-mouth disease virus (FMDV), an *Aphthovirus* genus member, encodes leader protease (Lpro), a unique protein that degrades LGP2 and indirectly suppresses MDA5-mediated signaling.<sup>354</sup> A recent study indicated that Lpro inhibits signaling by degrading MAVS and TBK1.<sup>355</sup> However, Lpro has been shown to deubiquitinate RIG-I, thereby terminating RIG-I-mediated signaling.<sup>356</sup> Furthermore, the 2B protein of FMDV specifically inhibits RIG-I;<sup>357</sup> however, it remains unclear why these picornavirus proteins target RIG-I.

Viruses belonging to the *Bunyavirales* order replicate their genomic RNA using a “prime-and-realign” machinery.<sup>358</sup> The genomic RNA of CCHFV (*Nairoviridae*) and HTNV (*Hantaviridae*) avoids RIG-I recognition upon the removal of the triphosphate moiety at the 5' end of the single G overhang and generating a 5'-monophosphate by “prime-and-realign” replication.<sup>147</sup> However, viruses belonging to the *Arenaviridae* family have been reported to escape RIG-I detection precisely because of its 5' overhang structure, even though the 5'-triphosphate G is not removed.<sup>359</sup> Moreover, the Z protein of *Arenaviridae* viruses such as Junin virus (JUNV) suppresses the IFN response via direct interaction with RIG-I.<sup>360</sup> This suppression mechanism is common among pathogenic arenaviruses, suggesting that it is an essential factor for their pathogenicity. However, the NS protein of severe fever with thrombocytopenia syndrome virus (SFTSV), a family member of the *Phenuiviridae* family, associates with RIG-I and TRIM25 and suppresses RIG-I-mediated signaling by repressing the K63-linked ubiquitination of RIG-I.<sup>361,362</sup> Although inhibition of RIG-I by its direct interaction with the NS protein of Toscana virus (TOSV) has been suggested,<sup>363</sup> a recent report revealed that NS can function as an E3 ubiquitin ligase and degrade the RIG-I protein to terminate signaling.<sup>364</sup>

Finally, we describe recent findings on the interaction between *Coronaviridae* family members and RLR/MAVS. Pathogenic human coronaviruses, including SARS-CoV, SARS-CoV-2, and MERS-CoV, are classified as betacoronaviruses. Since the production of IFNs is significantly attenuated in cells infected with these viruses,<sup>365–367</sup> suppression of the RLR/MAVS pathway by viral proteins might be a critical therapeutic target for coronaviral infectious diseases, including COVID-19. ORF9 of SARS-CoV, which encodes the N protein, interacts with TRIM25 and inhibits RIG-I via the ubiquitination of RIG-I.<sup>368</sup> SARS-CoV ORF-9b, a short peptide with 98 amino acids translated based on a discordant ORF in the N gene, localizes to the mitochondria, altering their morphology by inhibiting mitochondrial fission and thereby promoting the PCBP2-mediated degradation of MAVS by the E3 ubiquitin ligase ITCH/AIP4.<sup>369</sup> In addition, MERS-CoV is closely related to SARS-CoVs; however, the genomic structures differ slightly. Through its dsRNA-binding activity, the unique 4a protein of MERS-CoV

represses MDA5 signaling.<sup>370</sup> However, 4a also associates with PACT to suppress IFN production independent of RIG-I/MDA5.<sup>280</sup> Since 4a can act antagonistically on PKR,<sup>371</sup> it might be involved in PKR/PACT-mediated SG formation to regulate IFN production. Currently, reports demonstrating the suppression of RLR-mediated signaling by SARS-CoV-2-encoded proteins are accumulating. Recent studies revealed that ORF-9b of SARS-CoV-2 represses IFN induction via an interaction with the mitochondrial protein TOM70, which is a critical regulator of MAVS-mediated antiviral signaling.<sup>372</sup> ORF-3b, a 22-amino acid short peptide encoded by SARS-CoV-2, was identified as a potent antagonist of IFN production; however, the molecular machinery associated with its antagonistic function remains unclear.<sup>373</sup> Very recently, it was demonstrated that membrane protein (M), encoded by ORF5 of SARS-CoV-2, functions as an IFN antagonist via direct interactions with RLRs and MAVS to prevent RLR/MAVS/TRAF3/TBK1-containing signal complex formation.<sup>374,375</sup> Furthermore, multiple viral factors have been reported to inhibit RLR/MAVS and its downstream signaling pathway.<sup>376–378</sup> Further analysis of these interactions might reveal effective therapeutic targets for COVID-19 treatment.

### CONCLUDING REMARKS

The world is currently battling a significant health crisis in the form of the COVID-19 pandemic caused by SARS-CoV-2. In addition, infectious diseases caused by pathogenic RNA viruses such as EBOV, avian IAV, and other unidentified viruses are expected to occur in the future. The RLR/MAVS-mediated signaling pathway plays an essential role in the antiviral innate immune response against these viruses. However, RLR/MAVS pathway activation can also cause autoimmune diseases and trigger cytokine storms, as observed in COVID-19 patients, indicating that this pathway functions as a double-edged sword in the human immune system. Moreover, as the regulation of this pathway is significantly affected by the interaction between individual viruses and host factors, elucidating the detailed regulatory mechanisms underlying each viral infection from a spatiotemporal perspective and understanding them in an integrated manner are crucial. An improved understanding of the regulatory mechanisms associated with this pathway can inform the development of novel therapeutic strategies against infectious diseases, cancer, and immunological disorders in the future.

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### ADDITIONAL INFORMATION

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