Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Viral RNA detection by RIG-I-like receptors

Mitsutoshi Yoneyama[1], Koji Onomoto[1], Michihiko Jogi[1], Teppei Akaboshi[1] and Takashi Fujita[2,3]

In higher vertebrates, recognition of the non-self signature of invading viruses by genome-encoded pattern recognition receptors initiates antiviral innate immunity. Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) detect viral RNA as a non-self pattern in the cytoplasm and activate downstream signaling. Detection of viral RNA also activates stress responses resulting in stress granule-like aggregates, which facilitate RLR-mediated antiviral immunity. Among the three RLR family members RIG-I and melanoma differentiation-associated gene 5 (MDA5) recognize distinct viral RNA species with differential molecular machinery and activate signaling through mitochondrial antiviral signaling (MAVS, also known as IPS-1/VISA/Cardif), which leads to the expression of cytokines including type I and III interferons (IFNs) to restrict viral propagation. In this review, we summarize recent knowledge regarding RNA recognition and signal transduction by RLRs and MAVS/IPS-1.

Addresses
[1] Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan
[2] Laboratory of Molecular Genetics, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan
[3] Laboratory of Molecular Cell Biology, Graduate School of Biostudies, Kyoto University, Kyoto 606-8507, Japan

Corresponding authors: Yoneyama, Mitsutoshi (myoneyam@faculty.chiba-u.jp) and Fujita, Takashi (tfujita@virus.kyoto-u.ac.jp)

Current Opinion in Immunology 2015, **32**:48–53

This review comes from a themed issue on **Innate immunity**

Edited by **Zhijian J Chen** and **Sebastian Amigorena**

http://dx.doi.org/10.1016/j.coi.2014.12.012

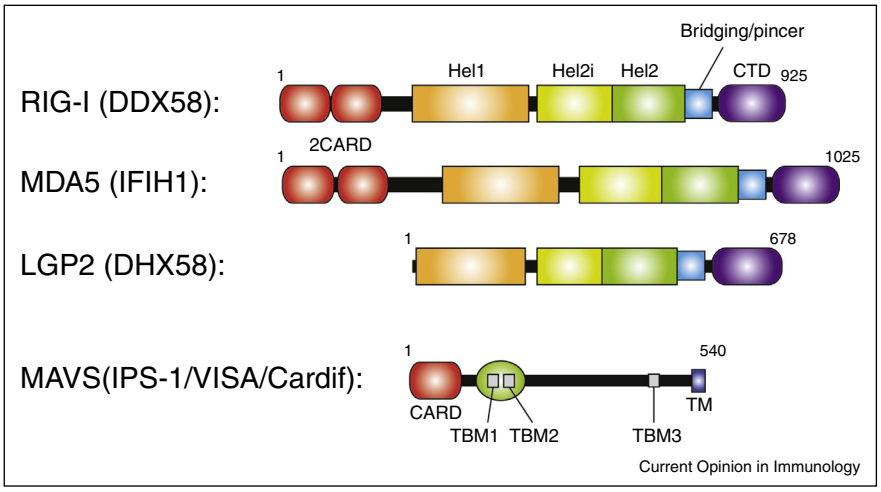
Introduction

In 2004, RLRs were identified as RNA sensors to trigger innate immune responses against viral infection [1]. Mammalian RLRs are composed of three family members; RIG-I (DDX58), MDA5 (IFIH1) and laboratory of genetics and physiology 2 (LGP2; DHX58), and all are expressed in the cytoplasm of ubiquitous types of cells [2]. These RLRs all share a DExD/H-box RNA helicase domain and a C-terminal domain (CTD), while RIG-I and MDA5, but not LGP2, have a N-terminal caspase recruitment domain (CARD), which is responsible for interacting with a downstream adaptor molecule, MAVS/IPS-1 (Figure 1). The C-terminal RNA helicase and CTD are implicated in the detection of viral RNA, and ATP-dependent conformational change allows CARDs to interact with MAVS/IPS-1. For RIG-I activation, conjugation of Lys63-linked ubiquitin chain (Ubs) by tripartite motif protein 25 (TRIM25) and/or association of unanchored Ubs with CARD are required [3]. A recent *in vitro* study revealed that MDA5 also interacts with unanchored Ubs [4]. The CARDs accumulated on the mitochondrial surface recruits signaling adaptors and kinases, including IκB kinase (IKK) family kinases, IKKα/β/γ, TBK1 and IKKε IKKα/β/γ activates NF-κB and TBK1 and IKKε activates IFN regulatory factor (IRF)-3 and 7. The activated NF-κB and IRF-3/7 can translocate into the nucleus, and interact with the promoter regions of target genes, including IFNs and inflammatory cytokines. Secreted IFNs transmit a signal *via* cognate receptors and induce the expression of hundreds of IFN-stimulated genes (ISGs), including, double-stranded RNA dependent protein kinase (PKR), 2′-5′-oligoadenylate synthetase (OAS) and RLRs, leading to the establishment of an antiviral state [5]. Here we review recent advances in our knowledge of the molecular machinery for RLR activation and RLR-mediated signal transduction.

RNA recognition and signal activation by RLRs

RIG-I is activated by infection by a variety of RNA viruses, such as influenza A virus (IAV), Newcastle disease virus, Sendai virus, vesicular stomatitis virus (VSV), measles virus (MV), and hepatitis C virus [2,6]. The non-self signature of these viruses is a 5′-triphosphate (5′ppp)-containing short double-stranded (ds) structure with a complementary end and/or a poly-U/UC rich ds-stretch. Recently, it has been demonstrated that incoming 5′ppp-containing viral RNA with nucleocapsid proteins can be recognized by RIG-I [7]. A more recent study reports that the 5′-diphosphate (5′pp) dsRNA genome of reovirus or synthesized 5′pp-dsRNA can also be recognized by RIG-I [8•]. Indeed, structural analysis of CTD has demonstrated that the basic groove of RIG-I CTD interacts with the negatively charged α and β, but not γ, phosphate group of 5′ppp [9]. The crystal structure of RIG-I with substrate RNA indicates that the RIG-I protein surrounds A-form dsRNA and the 5′ppp-containing end is

Figure 1



Schematic representation of the domain structures of RLRs and MAVS/IPS-1. Three RLRs are DExD/H box-containing RNA helicases, and comprised of Helicase domains 1 (Hel1) and 2 (Hel2), Helicase insertion domain (Hel2i), bridging/pincer domain and CTD. RIG-I and MDA5, but not LGP2, has a N-terminal tandem CARDs. MAVS/IPS-1 is composed of CARD, PRR, three TBMs and TM domains.

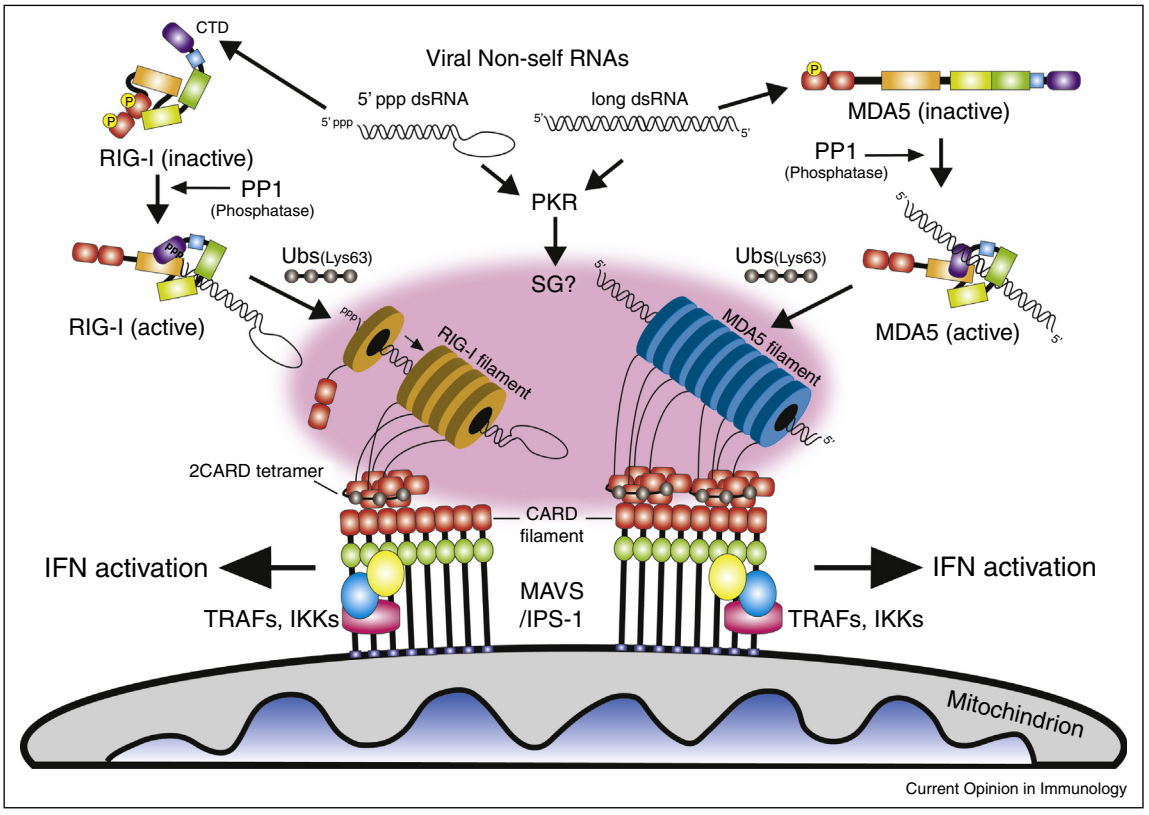
capped by CTD [9–12]. The current model of RIG-I activation obtained from high-resolution crystal structural analysis is that; i) an intramolecular interaction between the second CARD and Hel-2i domain inactivates the RIG-I protein without ligand RNA, ii) once viral 5′ppp-dsRNA is recognized by CTD, ATP-dependent conformational change induces a packed complex formation of the helicase domain/CTD with dsRNA, iii) the tandem CARDs (2CARD) are released from auto-repression and are available for interaction with MAVS/IPS-1 (Figure 2) [12].

MDA5 detects infection by picornaviruses, such as polio and encephalomyocarditis viruses (EMCV). The structural signature recognized by MDA5 is known as long dsRNA (>1 kbp), and is known to be generated by picornaviruses. Reo, West Nile and Dengue viruses are recognized by both RIG-I and MDA5. A recent study using next-generation sequencing revealed that MDA5 can also recognize AU-rich viral RNA species generated in MV-infected cells [13]. The molecular machinery of MDA5 activation is not yet well understood. A previous report indicated that the C-terminal region of MDA5 does not play a role as an auto-repressor [14], suggesting a differential repression mechanism(s) different to RIG-I in the absence of viral dsRNA (see below). The crystal structure of MDA5 lacking the 2CARD with substrate dsRNA reveals that the helicase domain and CTD surround dsRNA in the same manner as RIG-I, but since MDA5 CTD does not have a 5′ end-capping loop, which is essential for the recognition of 5′ppp for RIG-I CTD [15], MDA5 CTD interacts with the dsRNA stem, allowing both 5′ ends of dsRNA to be free from interaction (Figure 2) [16••]. This structural difference between RIG-I and MDA5 could explain the diverged substrate specificity of these two RLRs.

Importantly, recent reports revealed a link between MDA5 and autoimmune diseases. Funabiki *et al.* demonstrated that a gain-of-function mutation of MDA5 (Gly^{821} to Ser^{821}) induces lupus-like nephritis in mice [17•]. In a more recent study, Rice *et al.* found gain-of-function mutations in Aicardi-Goutières syndrome patients [18], suggesting an important role for MDA5-mediated IFN upregulation in the onset of inflammatory disorders.

LGP2 has no N-terminal CARD but interacts with dsRNA with high affinity, thus it was initially reported as a negative regulator for RIG-I and MDA5 [19]. However, studies using LGP-deficient mice demonstrated that LGP2 facilitates IFN production in response to infection by several RNA viruses, such as EMCV and VSV [20,21]. Indeed, a recent study revealed that LGP2 associates with certain RNA species, which can stimulate MDA5 in EMCV-infected cells, suggesting cooperative recognition of picornavirus RNA by these two RLRs [22]. Furthermore, a recent biochemical study demonstrated that LGP2 assists MDA5-RNA interaction and MDA5 filament formation (see below) [23•]. A more recent study suggested that RNA-binding protein, pumilio, plays a role as a chaperon for RNA recognition by LGP2 [24]. However, since LGP2 KO studies have presented confusing observations in response to several RNA viruses [20,21,25], further analysis will be necessary to understand LGP2-mediated regulation of viral RNA recognition and signal activation.

Figure 2



Model of RNA recognition by RIG-I and MDA5 and MAVS/IPS-1 mediated signal activation. Details are described in the text.

MAVS/IPS-1 was identified as a downstream adaptor molecule for RLRs. MAVS/IPS-1 is composed of a N-terminal single CARD, a central proline-rich region (PRR), which contains two Tumor necrosis factor Receptor-Associated Factor (TRAF) binding motifs (TBFs: TBF1 and TBF2), and a third TBM at the C-terminal region (TBM3) and a transmembrane domain (TM), which specifically restricts mitochondrial localization of MAVS/IPS-1. CARD interacts with the 2CARD of RIG-I or MDA5, and thereby induces the oligomer formation of MAVS/IPS-1 on the mitochondrial surface. Artificial oligomerization of TBF3 is sufficient to trigger IFN production, suggesting that the primary role of CARD and TM is its efficient oligomerization on mitochondrion [26]. Expression of MAVS/IPS-1 is also observed on the peroxisome and mitochondrial-associated membranes [27,28]. A recent study demonstrated that human miniMAVS, which is produced by bicistronic translational initiation and devoid of a CARD, interferes with full-length MAVS/IPS-1-mediated IFN production [29].

Signal activation *via* aggregate formation of RLRs

Previously, it was suggested that dimerization or oligomerization of RLRs is required for signal activation [14]. Recent *in vitro* studies proposed a model in which RIG-I, MDA5 and MAVS/IPS-1 signal through multimolecular aggregates (Figure 2). RIG-I forms a complex with dsRNA in a 5′ppp and ATP-dependent, but also 2CARD-independent manner [30,31]. Since sliding RIG-I on dsRNA was reported [32], ATP hydrolysis-driven translocation may allow RIG-I to form a beads-on-a-string complex on viral dsRNA. Following the complex formation, 2CARD adopts a helical tetrameric 'lock washer' structure, and interacts with MAVS/IPS-1 CARD [33,34,35••]. The MAVS/IPS-1 aggregate, which exhibits similarities to prion aggregates, is required for the recruitment of downstream signaling molecules, such as TRAF family members [26,33]. MDA5 forms a helical filamentous structure on dsRNA *in vitro* [36–39]. Since MDA5 has no end preference for RNA recognition, MDA5 randomly initiates the binding, however cooperativity allows the elongation of the MDA5 oligomer on dsRNA (Figure 2). Unlike RIG-I, ATP hydrolysis by MDA5 induces dissociation of MDA5 from the filament. Peisley *et al.* propose a model in which the formation of a long filamentous oligomer of MDA5 is critical for long dsRNA recognition by MDA5 [37]. The oligomer formations mentioned above are observed *in vitro* by using purified components,

however their presence in virus-infected cells has not been demonstrated.

Stress response and RLR signaling

Recent studies demonstrate that infection by various viruses induces the formation of stress granule (SG)-like aggregates, termed antiviral SG (avSG), in the cytoplasm [40]. In many cases, PKR is responsible for sensing viral infection and initiating avSG formation (Figure 2). An avSG contains RLRs and several antiviral molecules, including PKR and OAS as well as viral ribonucleoprotein complex (RNP) [41•]. Inhibition of the avSG formation impairs the virus-induced activation of IFN genes, suggesting a critical role of SGs in the antiviral innate response and their function as a platform for viral RNP recognition by RLRs. On the other hand, many viruses have acquired means to inhibit the formation of SGs [41•]. Unlike RLR, avSG formation is not an absolute determinant for triggering innate immunity [42], so avSGs may have a limited function in facilitating efficient viral RNA recognition by RLRs.

Regulation of RLRs-mediated signaling by ubiquitin chains

In vitro experiments showed that Lys-63-linked Ub chains are critical for the oligomerization of RIG-I and MDA5. However, the involvement of Lys63-Ubs has been controversial. Although the initial report indicated that ubiquitination of 2CARD at Lys172 by TRIM25 is required for RIG-I activation [3], subsequent studies have shown that RIG-I with Arg172 is fully active [43] and that the interaction between 2CARD and unanchored Ubs is important for the signaling-competent tetramer formation of 2CARD [4,44]. It was shown that RIG-I forms a complex with dsRNA in an Ubs-independent manner [30]. Further structural and biochemical analyses revealed that Lys63-linked Ubs interacts with the 2CARD tetramer at the outer rim, thereby stabilizing the tetrameric conformation, and covalent Ub-conjugation could give an enhanced filament formation of MAVS/IPS-1 CARD (Figure 2) [45••]. The data suggests that the requirement of Ubs-binding is compensated for by the filament formation of RIG-I, suggesting a complex regulatory mechanism(s) by Ubs. On the other hand, the requirement of unanchored Ubs in MDA5 activation has yet to be fully understood.

In addition to TRIM25, several lines of evidence indicate that RLR-mediated signaling is regulated by both the conjugation and dissociation of Ub-chains. An E3 ligase, Riplet (also known as RNF135/REUL), which can directly conjugate Lys63-Ubs at Lys-788 of RIG-I CTD, helps to release RIG-I from its auto-repressed state after viral detection, and to enhance TRIM25 function [46]. A recent study identifies an E3 ligase, MEX3C, which can also conjugate Lys63-Ubs at Lys99 and Lys169 of RIG-I 2CARD independently of TRIM25, and enhance RIG-I-mediated signaling. Interestingly, the presented data indicates that MEX3C interacts with viral RNA and accumulates in SGs together with RIG-I and viral RNA [47]. Systematic analysis focusing on TRIM family E3 ligases revealed that a large number of TRIM proteins positively regulate RLR-mediated signaling at differential steps of the signal [48]. A more recent study indicates that TRIM13 plays a negative role in MDA5-mediated signaling, but not in RIG-I [49]. TRAF family E3 ligase family members, TRAF2, 5, 6, are also responsible for the regulation of RLR-mediated signaling in multiple ways [50].

On the other hand, recent studies revealed the involvement of ubiquitin-specific protease (USP) family members in RLR-mediated signaling. Both USP3 and USP21 negatively regulate RIG-I activation *via* the cleavage of Lys63-linked Ubs conjugated on the 2CARD [51,52]. It is known that TRIM25 is regulated by proteasomal degradation *via* Lys48-linked ubiquitination catalyzed by linear ubiquitin assembly complex (LUBAC), USP15 positively regulates RIG-I signaling by deubiquitination of the Lys48-Ubs from TRIM25 [53]. USP4 can also function as a positive regulator for RIG-I by the dissociation of Lys48-Ubs from RIG-I to stabilize the protein [54].

Regulation by phosphorylation of RLRs

It was suggested that RIG-I signaling is attenuated by phosphorylation at the 2CARD and CTD of RIG-I by protein kinase C (PKC) α/β and casein kinase II (CKII) respectively [55]. On the other hand, protein phosphatase 1 (PP1), PP1α and PP1γ, directly interact with and dephosphorylate RIG-I and MDA5 and virus-induced signaling [56••]. In the case of MDA5, phosphorylation at the Ser88 residue in 2CARD attenuates MDA5 signaling and it is dephosphorylated by PP1, suggesting a role for the phosphorylation in suppressing the basal activity of MDA5. However, neither kinase nor phosphatase is regulated by viral infection and dephosphorylation alone is not sufficient to trigger RLR signaling.

Concluding remarks

Recent advances in biochemical and structural analysis elegantly elucidates the molecular machinery underlying non-self RNA recognition and signal activation by RLRs and MAVS/IPS-1. Although RIG-I and MDA5 differentially recognize distinct RNA species, both induce filamentous aggregates on dsRNA and the relieved 2CARDs form an oligomer to interact with the prion-like aggregates of MAVS/IPS-1 CARDs. Furthermore, virus-induced SG-like aggregates might also be involved in some parts of these processes. These intriguing observations bring up several questions; why do RLRs and MAVS/IPS-1 have to form these aggregates for sufficient signal activation? And how are these rigid structures dissolved after the completion of viral eradication? On

the other hand, knowledge about viral antagonistic functions targeting RLR/MAVS-mediated signaling and immunological disorders caused by dysregulated RLR/MAVS signaling has also been accumulating [17•,18,57•]. Thus, current advances in the molecular machinery of RLR activation will help us to develop novel therapeutic strategies against infectious diseases and immune disorders.

Acknowledgements

This work was supported by grants from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, including Innovative Area "Infection competency" (No. 24115004 and 25115503), Scientific Research "A" (No. 23249023) and "B" (No. 26293101), The Ministry of Health, Labor and Welfare (MHLW) of Japan, the Uehara Memorial Foundation, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the Takeda Science Foundation and the Naito Foundation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T: **The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses**. *Nat Immunol* 2004, **5**:730-737.

2. Yoneyama M, Fujita T: **Recognition of viral nucleic acids in innate immunity**. *Rev Med Virol* 2010, **20**:4-22.

3. Gack MU, Shin YC, Joo C-H, Urano T, Liang C, Sun L, Takeuchi O, Akira S, Chen Z, Inoue S *et al.*: **TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity**. *Nature* 2007, **446**:916-920.

4. Jiang X, Jiang X, Kinch LN, Kinch LN, Brautigam CA, Brautigam CA, Chen X, Du F, Grishin NV, Chen ZJ: **Ubiquitin-induced oligomerization of the RNA sensors RIG-I and MDA5 activates antiviral innate immune response**. *Immunity* 2012, **36**:959-973.

5. Sadler AJ, Williams BRG: **Interferon-inducible antiviral effectors**. *Nat Rev Immunol* 2008, **8**:559-568.

6. Takeuchi O, Akira S: **Pattern recognition receptors and inflammation**. *Cell* 2010, **140**:805-820.

7. Weber M, Gawanbacht A, Habjan M, Rang A, Borner C, Schmidt AM, Veitinger S, Jacob R, Devignot S, Kochs G *et al.*: **Incoming RNA virus nucleocapsids containing a 5'-triphosphorylated genome activate RIG-I and antiviral signaling**. *Cell Host Microbe* 2013, **13**:336-346.

8. • Goubau D, Schlee M, Deddouche S, Pruijssers AJ, Zillinger T, Goldeck M, Schuberth C, Van der Veen AG, Fujimura T, Rehwinkel J *et al.*: **Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates**. *Nature* 2014, **514**:372-375.

This study demonstrates that RIG-I can recognize viral RNA bearing 5'pp moiety as well as 5'ppp-RNA

9. Wang Y, Ludwig J, Schuberth C, Goldeck M, Schlee M, Li H, Juranek S, Sheng G, Micura R, Tuschl T *et al.*: **Structural and functional insights into 5′-ppp RNA pattern recognition by the innate immune receptor RIG-I**. *Nat Struc Mol Biol* 2010, **17**:781-787.

10. Jiang F, Ramanathan A, Miller MT, Tang G-Q, Gale M, Patel SS, Marcotrigiano J: **Structural basis of RNA recognition and activation by innate immune receptor RIG-I**. *Nature* 2011, **479**:423-427.

11. Civril F, Bennett M, Moldt M, Deimling T, Witte G, Schiesser S, Carell T, Hopfner K-P: **The RIG-I ATPase domain structure reveals insights into ATP-dependent antiviral signalling**. *EMBO Rep* 2011, **12**:1127-1134.

12. Kowalinski E, Lunardi T, McCarthy AA, Louber J, Brunel J, Grigorov B, Gerlier D, Cusack S: **Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA**. *Cell* 2011, **147**:423-435.

13. Runge S, Sparrer KMJ, Lässig C, Hembach K, Baum A, García-Sastre A, Söding J, Conzelmann K-K, Hopfner K-P: **In vivo ligands of MDA5 and RIG-I in measles virus-infected cells**. *PLoS Pathog* 2014, **10**:e1004081.

14. Saito T, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, Akira S, Fujita T, Gale M: **Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2**. *Proc Natl Acad Sci* 2007, **104**:582-587.

15. Takahasi K, Kumeta H, Tsuduki N, Narita R, Shigemoto T, Hirai R, Yoneyama M, Horiuchi M, Ogura K, Fujita T *et al.*: **Solution structures of cytosolic RNA sensor MDA5 and LGP2 C-terminal domains: identification of the RNA recognition loop in RIG-I-like receptors**. *J Biol Chem* 2009, **284**:17465-17474.

16. •• Wu B, Peisley A, Richards C, Yao H, Zeng X, Lin C, Chu F, Walz T, Hur S: **Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5**. *Cell* 2013, **152**:276-289.

This paper reports on the crystal structure of MDA5 bound to dsRNA, and reveals the differential RNA recognition mechanisms between MDA5 and RIG-I.

17. • Funabiki M, Kato H, Miyachi Y, Toki H, Motegi H, Inoue M, Minowa O, Yoshida A, Deguchi K, Sato H *et al.*: **Autoimmune disorders associated with gain of function of the intracellular sensor MDA5**. *Immunity* 2014, **40**:199-212.

This study demonstrates that dysregulation of MDA5 is linked to auto-inflammatory disorder in mice.

18. Rice GI, del Toro Duany Y, Jenkinson EM, Forte GMA, Anderson BH, Ariaudo G, Bader-Meunier B, Baildam EM, Battini R, Beresford MW *et al.*: **Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling**. *Nat Genet* 2014, **46**:503-509.

19. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo Y-M, Gale M, Akira S *et al.*: **Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity**. *J Immunol* 2005, **175**:2851-2858.

20. Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, Tsujimura T, Fujita T, Akira S, Takeuchi O: **From the Cover: LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses**. *Proc Natl Acad Sci* 2010, **107**:1512-1517.

21. Venkataraman T, Valdes M, Elsby R, Kakuta S, Caceres G, Saijo S, Iwakura Y, Barber GN: **Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses**. *J Immunol* 2007, **178**:6444-6455.

22. Deddouche S, Goubau D, Rehwinkel J, Chakravarty P, Begum S, Maillard PV, Borg A, Matthews N, Feng Q, van Kuppeveld FJM *et al.*: **Identification of an LGP2-associated MDA5 agonist in picornavirus-infected cells**. *eLife* 2014, **3**:e01535.

23. • Bruns AM, Bruns AM, Leser GP, Leser GP, Lamb RA, Lamb RA, Horvath CM, Horvath CM: **The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly**. *Mol Cell* 2014, **55**:771-781.

This study describes the molecular basis of cooperation between LGP2 and MDA5, revealing the LGP-mediated modulation of MDA5-dsRNA interaction and the filament assembly of MDA5.

24. Narita R, Takahasi K, Murakami E, Hirano E, Yamamoto SP, Yoneyama M, Kato H, Fujita T: **A novel function of human pumilio proteins in cytoplasmic sensing of viral infection**. *PLoS Pathog* 2014, **10**:e1004417.

25. Suthar MS, Ramos HJ, Brassil MM, Netland J, Chappell CP, Blahnik G, McMillan A, Diamond MS, Clark EA, Bevan MJ *et al.*: **The RIG-I-like receptor LGP2 controls CD8(+) T cell survival and fitness**. *Immunity* 2012, **37**:235-248.

26. Takamatsu S, Onoguchi K, Onomoto K, Narita R, Takahasi K, Ishidate F, Fujiwara TK, Yoneyama M, Kato H, Fujita T: **Functional**

characterization of domains of IPS-1 using an inducible oligomerization system. *PLoS ONE* 2013, **8**:e53578.

27. Dixit E, Boulant S, Zhang Y, Lee ASY, Odendall C, Shum B, Hacohen N, Chen ZJ, Whelan SP, Fransen M *et al.*: **Peroxisomes are signaling platforms for antiviral innate immunity**. *Cell* 2010, **141**:668-681.

28. Horner SM, Liu HM, Park HS, Briley J, Gale M: **Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus**. *Proc Natl Acad Sci* 2011, **108**:14590-14595.

29. Brubaker SW, Gauthier AE, Mills EW, Ingolia NT, Kagan JC: **A bicistronic MAVS transcript highlights a class of truncated variants in antiviral immunity**. *Cell* 2014, **156**:800-811.

30. Peisley A, Bin Wu Yao H, Walz T, Hur S: **RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner**. *Mol Cell* 2013, **51**:573-583.

31. Patel JR, Jain A, Chou Y-Y, Baum A, Ha T, García-Sastre A: **ATPase-driven oligomerization of RIG-I on RNA allows optimal activation of type-I interferon**. *EMBO Rep* 2013, **14**:780-787.

32. Myong S, Cui S, Cornish PV, Kirchhofer A, Gack MU, Jung JU, Hopfner K-P, Ha T: **Cytosolic viral sensor RIG-I is a 5′-triphosphate-dependent translocase on double-stranded RNA**. *Science* 2009, **323**:1070-1074.

33. Hou F, Sun L, Zheng H, Skaug B, Jiang Q-X, Chen ZJ: **MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response**. *Cell* 2011, **146**:448-461.

34. Xu H, He X, Zheng H, Huang LJ, Hou F, Yu Z, la Cruz de MJ, Borkowski B, Zhang X, Chen ZJ *et al.*: **Structural basis for the prion-like MAVS filaments in antiviral innate immunity**. *eLife* 2014, **3**:e01489.

35. •• Wu B, Peisley A, Tetrault D, Li Z, Egelman EH, Magor KE, Walz T, Penczek PA, Hur S: **Molecular imprinting as a signal-activation mechanism of the viral RNA sensor RIG-I**. *Mol Cell* 2014, **55**:511-523.

This study describes the crystal structure of the RIG-I 2CARD and the MAVS CARD complex, representing that the tetramer of RIG-I 2CARD activates MAVS CARD filament formation.

36. Peisley A, Lin C, Wu B, Orme-Johnson M, Liu M, Walz T, Hur S: **Cooperative assembly and dynamic disassembly of MDA5 filaments for viral dsRNA recognition**. *Proc Natl Acad Sci* 2011, **108**:21010-21015.

37. Peisley A, Jo MH, Lin C, Wu B, Orme-Johnson M, Walz T, Hohng S, Hur S: **Kinetic mechanism for viral dsRNA length discrimination by MDA5 filaments**. *Proc Natl Acad Sci* 2012, **109**:E3340-E3349.

38. Berke IC, Yu X, Modis Y, Egelman EH: **MDA5 assembles into a polar helical filament on dsRNA**. *Proc Natl Acad Sci* 2012, **109**:18437-18441.

39. Berke IC, Modis Y: **MDA5 cooperatively forms dimers and ATP-sensitive filaments upon binding double-stranded RNA**. *EMBO J* 2012, **31**:1714-1726.

40. Onomoto K, Jogi M, Yoo J-S, Narita R, Morimoto S, Takemura A, Sambhara S, Kawaguchi A, Osari S, Nagata K *et al.*: **Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity**. *PLoS ONE* 2012, **7**:e43031.

41. • Onomoto K, Yoneyama M, Fung G, Kato H, Fujita T: **Antiviral innate immunity and stress granule responses**. *Trends Immunol* 2014, **35**:420-428.

A Review of the interactions between virus-induced stress responses and innate immune responses.

42. Langereis MA, Feng Q, van Kuppeveld FJ: **MDA5 localizes to stress granules, but this localization is not required for the induction of type I interferon**. *J Virol* 2013, **87**:6314-6325.

43. Shigemoto T, Kageyama M, Hirai R, Zheng J, Yoneyama M, Fujita T: **Identification of loss of function mutations in human genes encoding RIG-I and MDA5: implications for resistance to type I diabetes**. *J Biol Chem* 2009, **284**:13348-13354.

44. Zeng W, Sun L, Jiang X, Chen X, Hou F, Adhikari A, Xu M, Chen ZJ: **Reconstitution of the RIG-I pathway reveals a signaling role of unanchored polyubiquitin chains in innate immunity**. *Cell* 2010, **141**:315-330.

45. •• Peisley A, Wu B, Xu H, Chen ZJ, Hur S: **Structural basis for ubiquitin-mediated antiviral signal activation by RIG-I**. *Nature* 2014, **509**:110-114.

This study reports on the crystal structure of tetrameric RIG-I 2CARD with K63-Ubs, and reveals the molecular basis of ubiquitin-mediated RIG-I activation.

46. Oshiumi H, Miyashita M, Matsumoto M, Seya T: **A distinct role of Riplet-mediated K63-Linked polyubiquitination of the RIG-I repressor domain in human antiviral innate immune responses**. *PLoS Pathog* 2013, **9**:e1003533.

47. Kuniyoshi K, Takeuchi O, Pandey S, Satoh T, Iwasaki H, Akira S, Kawai T: **Pivotal role of RNA-binding E3 ubiquitin ligase MEX3C in RIG-I-mediated antiviral innate immunity**. *Proc Natl Acad Sci* 2014, **111**:5646-5651.

48. Versteeg GA, Rajsbaum R, Sánchez-Aparicio MT, Maestre AM, Valdiviezo J, Shi M, Inn K-S, Fernandez-Sesma A, Jung J, García-Sastre A: **The E3-ligase TRIM family of proteins regulates signaling pathways triggered by innate immune pattern-recognition receptors**. *Immunity* 2013, **38**:384-398.

49. Narayan K, Waggoner L, Pham ST, Hendricks GL, Waggoner SN, Conlon J, Wang JP, Fitzgerald KA, Kang J: **TRIM13 is a negative regulator of MDA5-mediated type I interferon production**. *J Virol* 2014, **88**:10748-10757.

50. Liu S, Chen J, Cai X, Wu J, Chen X, Wu Y-T, Sun L, Chen ZJ: **MAVS recruits multiple ubiquitin E3 ligases to activate antiviral signaling cascades**. *eLife* 2013, **2**:e00785.

51. Cui J, Song Y, Li Y, Zhu Q, Tan P, Qin Y, Wang HY, Wang RF: **USP3 inhibits type I interferon signaling by deubiquitinating RIG-I-like receptors**. *Cell Res.* 2014, **24**:400-416.

52. Fan Y, Mao R, Yu Y, Liu S, Shi Z, Cheng J, Zhang H, An L, Zhao Y, Xu X *et al.*: **USP21 negatively regulates antiviral response by acting as a RIG-I deubiquitinase**. *J Exp Med* 2014, **211**:313-328.

53. Pauli E-K, Chan YK, Davis ME, Gableske S, Wang MK, Feister KF, Gack MU: **The ubiquitin-specific protease USP15 promotes RIG-I-mediated antiviral signaling by deubiquitylating TRIM25**. *Sci Signal* 2014, **7**:ra3-ra3.

54. Wang L, Zhao W, Zhang M, Wang P, Zhao K, Zhao X, Yang S, Gao C: **USP4 positively regulates RIG-I-mediated antiviral response through deubiquitination and stabilization of RIG-I**. *J Virol* 2013, **87**:4507-4515.

55. Sun Z, Ren H, Liu Y, Teeling JL, Gu J: **Phosphorylation of RIG-I by casein kinase II inhibits its antiviral response**. *J Virol* 2011, **85**:1036-1047.

56. •• Wies E, Wang MK, Maharaj NP, Chen K, Zhou S, Finberg RW, Gack MU: **Dephosphorylation of the RNA sensors RIG-I and MDA5 by the phosphatase PP1 is essential for innate immune signaling**. *Immunity* 2013, **38**:437-449.

This work demonstrates that dephosphorylation of the CARD of both RIG-I and MDA5 by PP1α/γ is critical for antiviral signaling.

57. • Goubau D, Deddouche S, Reis e Sousa C: **Cytosolic sensing of viruses**. *Immunity* 2013, **38**:855-869.

A review of cytosolic virus sensors and host–virus-interactions.