Investigating the RIG-1 Receptor: Mechanisms, Behavior, and Parallel Pathways

Authors: Nathan Ko, Makeda Tamrat, Samuel Chen, Vidhur Thirupathi

George Mason University Center for Infectious Disease Research

Abstract:

The immune system serves a diverse array of functions, pertaining to protecting its host from foreign organisms, viruses, and other threats, but many do not know of the multiple lines of defense the immune system has set in place to ensure viability. First are the physical and chemical barriers that work to prevent foreign organisms from entering. The second, innate immunity, uses non-specific cellular and molecular responses to fight off invaders. Finally, the third line, adaptive immunity, involves specific lymphocytes and antibodies that target and eliminate pathogens. In this study, however, we are going to delve into the role that RIG-1 receptors have in activating the innate immune system.

Introduction:

According to Garland Science, the innate immune system blocks many pathogens before they even reach the body, preventing infections and serious pathogens from infecting you.
An example of such a molecule that stimulates the activation of the innate immune system is the Retinoic acid-inducible gene 1 (RIG-I) receptor, which, when induced, can recognize specific sequences related to viral RNA while also triggering downstream signaling pathways that cascade into further, more intensive immune responses (Ramos & Gale, 2011). These viral

RNAs often attach themselves to host cells and become parasites, hijacking the cells' membranes and machinery to create more viral RNA instead of their original duty. Without the help of RIG-I and other innate immune system receptors, humans would not be able to fight off many infections and would succumb more easily to disease.

The RIG-I receptor is one of many molecules classified as a pattern-recognition receptor (PRR). PRRs, which include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs), specialize in the recognition of molecular structures on cells, such as pathogens and damaged cells, through the binding of ligands, and they can trigger appropriate immune responses as a result of these interactions. For example, all groups of PRRs can activate inflammatory responses, CLRs can phagocytize pathogens, and RLRs can trigger the production of antiviral interferons, a group of cytokines that impair viral replication and alert nearby cells to the infection.

RIG-I serves as the frontline sensor in the innate immune system, becoming activated when it detects specific molecule patterns on viral RNA. Since RIG-I is autoinhibited (blocks its activity in unneeded scenarios) in resting cells, RIG-I will undergo a conformational change that exposes its CARD domains when needed, specifically when detecting viral RNA with a 5' triphosphate or diphosphate dsRNA (Yoneyama & Fujita, 2009). Once these CARD domains have been exposed, the domains will interact with specific MAVS on mitochondria, which activate transcription factors such as IRF3/7 and NF-κB to induce type I interferons (Liu et al., 2015). These interferons can induce antiviral states in neighboring cells, effectively containing the virus before adaptive immunity kicks in. Additionally, they can enhance immune cell activity, enhancing antigen presentation and preparing infected cells to be recognized by cytotoxic T cells for apoptosis (McNab et al., 2015). These are just a few examples of the potential triggers for RIG-I

The specific RIG-I recognition of viral RNA is largely determined by molecular features, particularly 5’ triphosphate ends and short double-stranded structures. RIG-I recognizes short double-stranded RNA (dsRNA) with a base pair range of 10-20, which is a relatively short sequence. (Schlee et al., 2009; Luo et al., 2023). While they recognize these motifs, host RNA usually contains 5’ caps to distinguish host cells from viral cells. Host cells can also do this by removing key features that RIG-I recognizes, such as in 5’ppp groups and short dsRNA groups, which are typically only found in viral RNA, and not our own. This allows for RIG-I to complete its tasks without accidentally harming the host (Hornung et al., 2006). An additional example of sequence motifs is regions rich in polyuridine or uridine-cytidine-rich. Hepatitis C virus (HCV) is a genome that contains such poly (U/UC) sequences, which, when paired with a 5’-ppp, act as a signature for RIG-I to begin its processes (Saito et al., 2008). These motifs and sequences are what truly allow for RIG-I to help defend against foreign attacks without inducing adaptive immunity.

Upon recognition of viral RNA, RIG-I undergoes a series of structural changes that enable signal transduction through MAVS. Firstly, RIG-I proteins primarily begin in their C-terminal domain (CTD), which has a high affinity for specific RNA with 5’-ppp. This, as stated before, is a signature common in viral RNA, but not in host cells, allowing for the RNA to be recognized without harming the original host. When RIG-I binds to this RNA, it undergoes a conformational change, exposing the helicase domain and CARDs (caspase activation and recruitment domains), which are normally hidden in inactive cells (Luo et al., 2023). Once bound, the helicase domain binds and hydrolyzes ATP, which allows for the RIG-I molecule to translocate along the RNA strand, allowing for oligomerization, which is critical for signal amplification, as well as preparing RIG-I to activate downstream signaling molecules (Tang & Hur, 2020). To finally activate downstream immune signaling, the CARDs located at the N-terminus are stabilized through k63-linked ubiquitination, creating an "open" conformation of the terminus. This happens through molecular tagging through E3 ligases such as TRIM25 and Riplet, which help change the shape of the ubiquitin to expose the CARDS and activate the antiviral response. Once ubiquitinated, CARDs may bind to MAVS on the mitochondrial membrane, triggering a signaling reaction that leads to interferon production and eventually, an antiviral response (Cadena et al., 2019; Hayman et al., 2019). However, in uninfected cells, RIG-I remains inactive due to the phosphorylation of its CARDs, which prevents premature signaling. Additionally, acetylation of the CTD, particularly at lysine 909 (K909), reduces RNA-binding affinity, keeping RIG-I inactive until viral RNA is present (Tang & Hur, 2020).

While the RIG-I pathway is important for activating antiviral measures, it does not work alone; instead, it cooperates with other mechanisms to coordinate their responses. When RIG-I detects viral RNA, it has to interact with the adaptor proteins called MAVS, which in turn use the kinases TBK1 and IKKε, both of which are transcription factors that increase type 1 interferon production, which is essential for launching the immune response (Seth et al., 2005; Liu et al., 2015). Additionally, there are cGAS-STING pathways, which, dissimilarly to RIG-I, detect cytosolic DNA and not RNA, but intersect with RIG-I because they both activate the kinase TBK1, as well as lead to IRF3 phosphorylation. While they sense different types of pathogens, they both converge downstream and contribute to a coordinated immune response (Ishikawa & Barber, 2008; Decout et al., 2021). Similar to cGAS-STING pathways are endosomal toll-like receptors (TLRs), such as TLR3 and TLR7/8, recognize both single and double-stranded RNA. These receptors are located in endosomes and also activate IRF3/7 and NF-κB, which can be used with RIG-I to work together to create anti-viral responses, or compensate when one system fails, adding a level of redundancy to the defense (Kawai & Akira, 2006). Lastly, there are host proteins that help to enhance or suppress RIG-I signalling when the occasion calls for it. LGP2 is a similar receptor that can modulate RIG-I and MDA5 activity, enhancing or dampening the signals. RNF125 and RNF122 are E3 ligases that add K48-linked ubiquitin to RIG-I, which marks it for proteasomal degradation (breakdown of proteins by way of a proteasome), in turn halting the signal. ZFP36 can help to enhance RIG-IU signalling by supporting any riplet-mediated K63-linked ubiquitination, keeping RIG-I in its active form without allowing for it to deactivate (Zheng et al., 2024). While many types of helpful pathways intersect with RIG-I, there are also some hindering ones, such as viral RNA with evasion techniques. For example, the Influenza A virus can bind to and inhibit RIG-I and TRIM25, preventing activation and thereby weakening the innate antiviral system. An additional example is SARS-CoV-2, which can target MAVS to suppress downstream signaling and reduce interferon production (Thorne et al., 2021; Tang & Hur, 2020).

In this paper, we will investigate the full triggering process of the RIG-I receptor by viral RNA, including an in-depth examination of its motifs/sequences, mechanisms, and pathways. Not only will this explain the full potential of RIG-I in the immune response process, but it will also provide future insight into how RIG-I can be utilized for immunotherapy developments.

Methods:

To conduct this study, we compiled articles published between 2005 and 2024 that we found from databases including PubMed, Google Scholar, and ScienceDirect. The articles were originally published in journals such as *Nature*, *Science*, *Cell*, *Frontiers in Immunology,* and *Virology Journal*. Additional relevant articles were found in journals published by Portland Press. Some keywords used were “RIG-I”, “PRRs”, “MAVS”, “viral RNA”, “innate immunity”, and “CARD”.

Results:

I. Signatures for viral recognition in the RIG-1 response pathway:

One of the most important features of the RIG-1 pathway process is the PAMPs (pathogen-associated molecular patterns) that the RIG-1 receptor detects to create an immune response.

A key example of such a PAMP is the uncapped 5’ triphosphate (5’-ppp) end of viral RNAs (Hornung et al., 2006). This uncapped end is extremely crucial in helping RIG-1 distinguish between self and non-self RNA, as most host RNAs have an m7G cap at the end of their 5’ ends, which is mainly used for stability during RNA translation. Host cells can also differentiate themselves from accidental RIG-1 binding by removing key features that RIG-I recognizes, such as in 5’ppp groups and short dsRNA groups, which are typically only found in viral RNA, and not our own. This allows for RIG-I to complete its tasks without accidentally harming the host (Hornung et al., 2006). The RIG-1 receptor is also highly specific, since the RIG-1 receptor has a CDT (C-terminal domain) that is specific for binding to the 5’ppp ends of viral RNAs (Hornung et al., 2006; Kowalinski et al., 2011). While 5’-ppp ends of viral RNA appear to be the primary trait detected by cellular receptors, an additional example of a key motif is regions rich in polyuridines or uridine-cytidines. For example, Hepatitis C virus (HCV) contains a genome with a large amount of such poly (U/UC) sequences, which, when paired with a 5'-ppp, act as a clear and direct signature for RIG-I's activation (Saito et al., 2008).

It must also be noted that the RIG-1 pathway specializes in detecting short, blunt dsRNAs (double-stranded RNAs) with a length of around 10-20 base pairs. (Schlee et al., 2009; Luo et al., 2023). This allows researchers to distinguish the RIG-1 pathway from the MDA5 pathway, a pathway that also results in the production of a downstream immune response, but relies on the detection of long dsRNAs rather than short dsRNAs (Yoneyama and Fujita, 2009).

II. Mechanisms of RNA binding in the RIG-1 response pathway:

Between the detection of viral RNA and the triggering of RIG-I-associated pathways involved in the immune response, the RNA must bind to a RIG-I receptor. While 5'-triphosphate (5'-ppp) ends of viral RNA appear to be the primary trait detected by cellular receptors (Hornung et al., 2006), further investigation in this area has led to the conclusion that other factors, namely the replication intermediate (RI) poly-U/UC region, are also required to ultimately trigger RIG-I activation (Saito et al., 2010), as discussed previously.

When RIG-I is activated by the presence of viral RNA, it undergoes substantial modifications in structure to facilitate optimal binding. RIG-I's CTD binds with the 5'-ppp, and the helicase domains (particularly Hel2i) connect along the ribose backbone (Kowalinski et al., 2011; Juang et al., 2011; Luo et al., 2011). While the CTD stabilizes the conformation, the helicases then facilitate ATP binding to RIG-I, which is critical for activating further conformational changes (Kowalinski et al., 2011; Juang et al., 2011; Luo et al., 2011). Following these changes, the CARDs are revealed and activated (Zeng et al., 2010; Luo et al., 2023), initiating the RIG-1 CARD tetramerization.

III. RIG-1 response pathway: ubiquitination and oligomerization:

After viral RNA has been detected and bound, RIG-I undergoes ubiquitination along the length of the ribose backbone. As RIG-1's N-terminus CARDs are now exposed and activated outside of their normal autorepressed state, several E3 ubiquitin ligases such as Riplet (also called RNF135) and TRIM25 attach and bind to facilitate ubiquitination (Cadena et al., 2019). Riplet can recognize and bind to RIG-1's helicase-CTD region, which allows Riplet to produce K63-linked polyubiquitin chains at lysine 788. These polyubiquitin chains can stabilize RIG-1 CARD interactions and promote oligomerization and tetramerization of the RIG-1 CARDs. Riplet can also act as a scaffolding protein, offering physical stabilization. TRIM25 is also an important ligase for ubiquitination, though Riplet is considered more essential (Cadena et al., 2019). Though TRIM25 can still contribute to ubiquitination by using Ubc13 and Uev1A to add K63-linked polyubiquitin chains to lysine 172, further promoting RIG-1 CARDs for oligomerization.

Oligomerization, which is facilitated by ubiquitination, refers to the formation and assembly of monomers into a larger complex called an oligomer, which creates a linear sequence. In the RIG-1 pathway, oligomerization is the stabilization and tetramerization of the RIG-1 CARD via the K63-linked polyubiquitin chains (Cadena et al., 2019). Oligomerization has been shown to increase sensitivity to viral dsRNA of much more substantial lengths, and is a vital step for RIG-1 to undergo to continue downstream immune signalling (mainly interactions with MAVS).

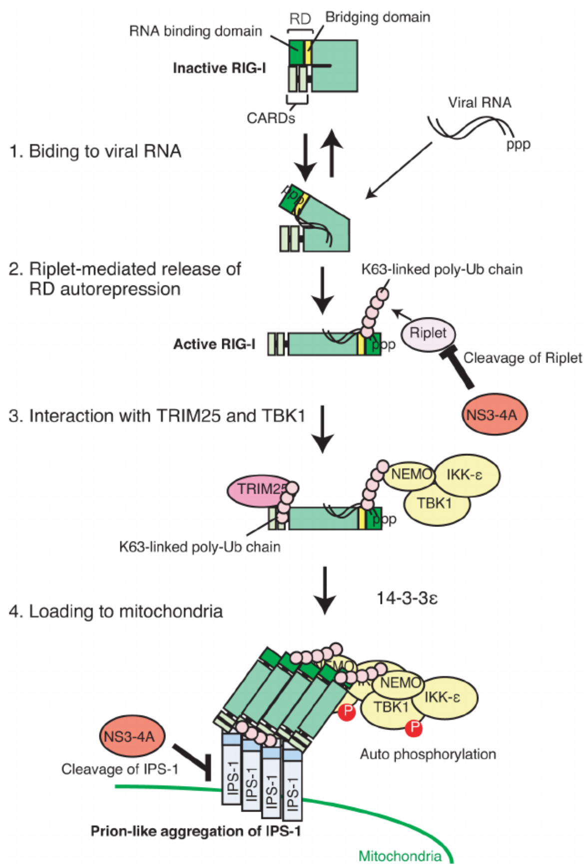


Figure 1 (Cadena et al. 2019): The RIG-1 pathway, indicating ubiquitination/oligomerization

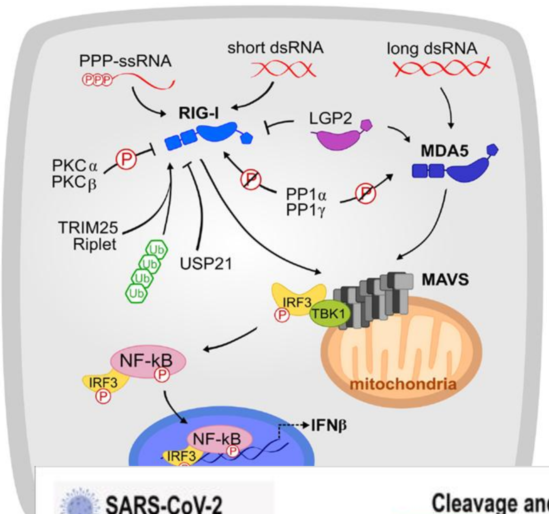
<u>IV. Role and significance of MAVS in the RIG-1 response pathway:</u>

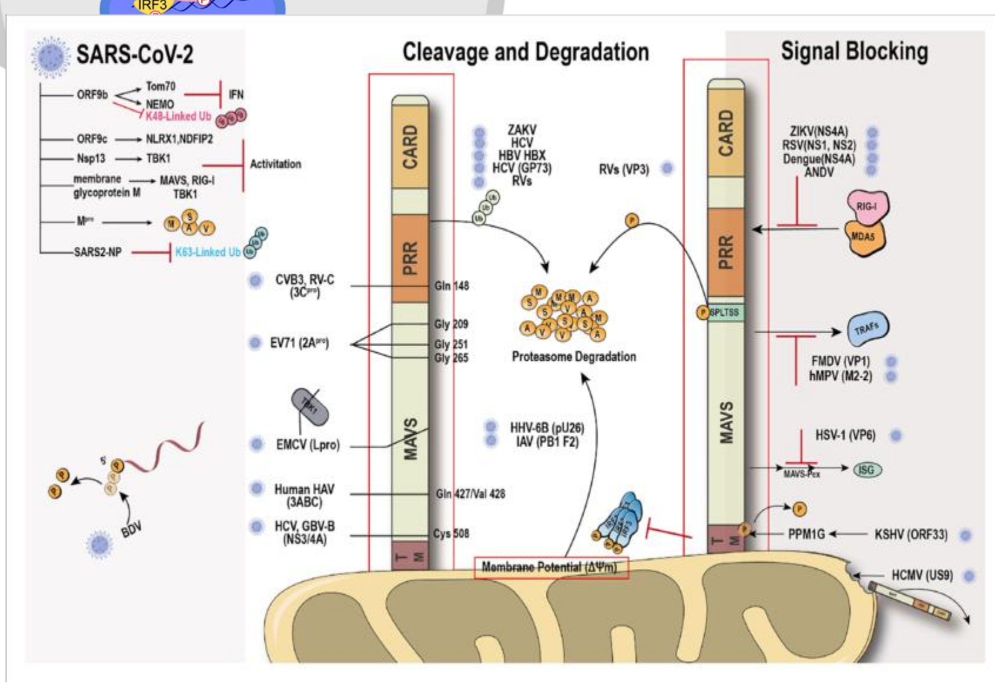
MAVS (mitochondrial antiviral signalling), a type of mitochondrial membrane protein, can be deduced as a key factor involved in the downstream signalling of the RIG-1 pathway.

RIG-1 interacts with MAVS throughout the pathway to activate transcription factor kinases such as TBK1 and IKKε, which ultimately results in type-1 interferon and inflammatory cytokine production (Seth et al., 2005; Liu et al., 2015). First, after the RIG-1 receptor undergoes ubiquitination through conformational changes when detecting viral RNA, the now activated and tetramerized receptor binds with MAVS through CARD-CARD domain interactions. The interaction between the RIG-1 CARD domains and the proline-rich N-terminus CARD of the MAVS is highly specific. (Seth et al., 2005). When CARDs interact and bind, MAVS then form large functional aggregates on the outer mitochondrial membrane, which in turn allows the MAVS to convert to an active state. Through the activation of MAVS, the aggregates recruit kinases such as TBK1 and IKKε, which then activate transcription factors IRF3/7 and NF-kB, thus initiating an increase in the production of type-1 interferons and cytokines. This is why researchers consider MAVS to be an efficient and potent antiviral protein (Seth et al., 2005).

Figure 2 (Cadena et al., 2019): The RIG-1 pathway, indicating MAVS

However, despite its strong antiviral qualities, MAVS still has some slight but important features that could counteract its antiviral abilities. For example, it is noted that the CARD domains of MAVS are essential to their role in the RIG-1 pathway, and are rendered functionally useless without their CARD domains (Seth et al., 2005). A lack of CARD domains means a lack of CARD-CARD domain interactions with RIG-1, resulting in the lack of an activated MAVS activation. Without the activation of MAVS, there will be no production of type-1 interferons and other kinases, ultimately resulting in the lack of an immune response against invading viral RNAs. Speaking of viral RNAs, certain viruses have evolved capabilities and counter-measures against the initiation of MAVS signalling in the RIG-1 pathway, such as proteolytic cleavages,

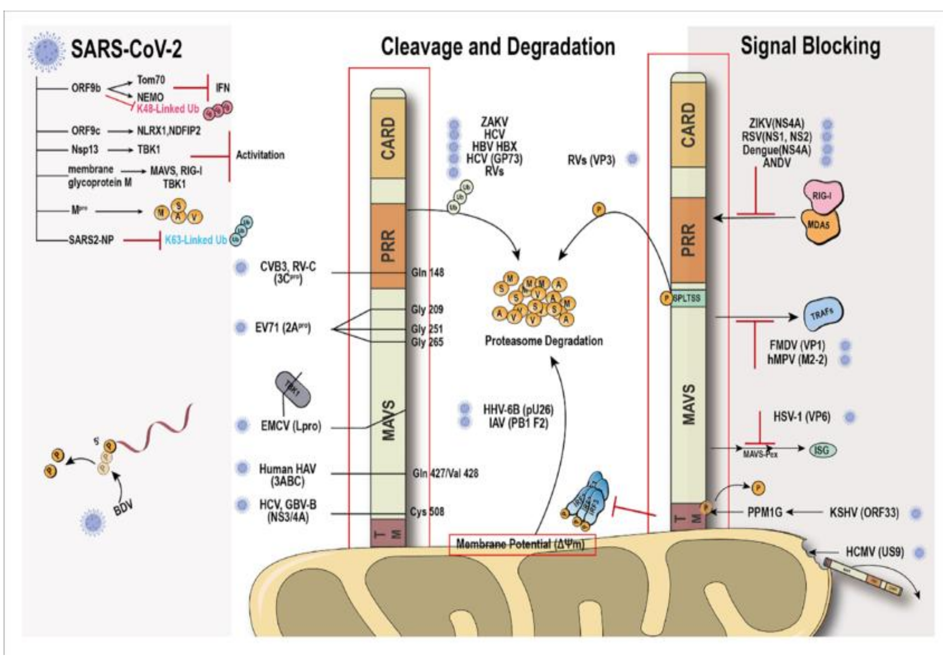




proteasomal/autophagy degradation, inhibition of ubiquitination, and various other strategies. For example, certain viruses have been known to use influenza A virus (IAV) NS1, which is able to suppress TRIM25-mediated RIG-1 CARDs from ubiquitination by binding to TRIM25 amino acids and affecting its coil-coiled domain (Liu et al., 2015). Another example discusses the hepatitis A virus (HAV), which uses proteolytic cleavages via protease 3Cpro/2Apro to remove MAVS from mitochondria, preventing downstream signalling (Liu et al., 2015). An additional example is SARS-CoV-2, which can target MAVS to suppress downstream signaling and reduce interferon production through using ORF9b, an accessory protein, to target MAVS-TOM70 interactions, as well as ORF3b, which blocks MAVS aggregation. (Thorne et al., 2021; Ahmadi et al., 2023).

Figure 3 (ResearchGate): Sars-Cov2 Viral Antagonism of the RIG-1 Pathway

Ultimately, MAVS is regarded as a key component of the RIG-1 pathway and an important factor when discussing the downstream signalling of an immune response. Viruses seem to understand the importance of MAVS as well, considering the various methods they use to antagonize MAVS signalling. The continued investigation of MAVS in the RIG-1 pathway and viral antagonism against MAVS can provide researchers with new approaches to immunotherapies to enhance the effectiveness of the RIG-1 pathway and MAVS signalling.



V. Parallel pathways: cGAS-STING & endosomal TLRs:

Other pathways interact similarly to the RIG-1 pathway, such as the cGAS-STING pathway and the numerous types of TLR pathways.

The cGAS-STING pathway somewhat resembles the RIG-1 pathway, but focuses on the detection of cytoplasmic/cytosolic viral DNAs rather than viral RNAs. The cGAS-STING pathway relies on cGAS as its receptor and uses a downstream adaptor STING as opposed to the MAVS used in the RIG-1 pathway. When cGAS detects viral DNA, rather than undergoing ubiquitination, cGAS's conformational changes allow it to produce a secondary messenger called cGAMP using ATP (Sun et al., 2013). cGAMP then activates STING, receptors located on the endoplasmic reticulum (ER). As STING activation occurs, STING translocates from the ER to the Golgi body, where palmitoylation occurs, allowing STING to recruit and activate TBK1. TBK1 then activates transcription factors such as IRF3, increasing the production of type-1 interferons (Tanaka & Chen, 2012). Though the cGAS-STING pathway and the RIG-1 pathway rely on different initial receptors, adaptors, and triggers, both of these downstream reactions can be considered parallel reactions, as they both rely on TBK1 to activate transcription factors, and they both result in the increased production of type-1 interferons and inflammatory cytokines, indicating a coordinated immune response (Ishikawa & Barber, 2008; Decout et al., 2021).

Toll-like receptor (TLR) pathways also act similarly to both the RIG-1 pathway and the cGAS-STING pathway. There are numerous types of TLRs, such as TLR3, TLR7, TLR8, and TLR9, each acting as PRRs just like RIG-1 receptors. Though the TLR types are all found in endosomes, each has slightly different pathways from one another. Some, like TLR3, are activated through the detection of dsRNAs, while others, like TLR7/8, are activated through the detection of ssRNAs (Kawai & Akira, 2006). Each of the TLR pathways also relies on numerous types of adaptors, such as MyD88 and TRIF (TICAM-1), to facilitate the downstream signalling process. Each of the TLR pathways also has differential cytokine activation profiles, as some rely on NF-kB and IRF7, while others use IRF3 to produce type-1 interferons (Kawai & Akira, 2006). Overall, the TLR pathways are also an important factor when producing a coordinated immune response, just like cGAS-STING pathways and RIG-1 pathways, all within the context of the in-depth examination of the innate immune response system.

VI. Host protein regulation in the RIG-1 response pathway:

Besides TRIM25 and Riplet (RNF135)'s involvement in RIG-1 ubiquitination, the RIG-1 pathway also uses and interacts with numerous other host proteins to regulate or suppress the expression of immune responses.

One example of such a protein is ZFP36, a zinc finger RNA-binding protein, which plays a novel role in enhancing antiviral responses. Despite not relating to its RNA-binding function, through testing co-immunoprecipitation assays, ZFP36 is shown to be able to boost RIG-1 signalling by directly interacting with Riplet (RNF135), one of the ligases key to the K63-linked ubiquitination of RIG-1. ZFP36 enhances the enzymatic function of Riplet, increasing the rate of RIG-1 ubiquitination and, in turn, increasing the production of MAVS and other downstream processes. Research also suggests that ZFP36 can act as a scaffolding protein or cofactor, stabilizing the interaction between RIG-1 and Riplet (Zheng et al., 2024).

Another example is LGP2, which is a modulator and regulatory receptor similar to RIG-1. Though LGP2 lacks CARD domains and cannot facilitate signaling by itself, it can compete for RNA ligands with RIG-1, interfering with RNA binding and affecting the oligomerization process of RIG-1. Thus, LGP2 can often be considered as a negative regulator of RIG-1 with suppressive qualities, though in certain situations it could act as a positive modulator by stabilizing MDA5-RNA complexes for dsRNA detection (Yoneyama & Fujita, 2009; Liu et al., 2015).

Other examples include RNF122 and RNF125, both of which are E3 ubiquitin ligases that act as negative regulators of the RIG-1 pathway. Both of these proteins mark RIG-1 for proteasomal degradation, mediating K48-linked ubiquitination. These act as homeostatic control mechanisms to prevent an overreaction and an overproduction of type-1 interferons in an immune response. Furthermore, RNF122 is also known to be able to destabilize MAVS, suppressing excess downstream signalling (Yoneyama & Fujita, 2009; Liu et al., 2015).

Lastly, many viruses also have methods to suppress the RIG pathway. Besides the methods that viruses have to target MAVS as discussed previously, viruses are able to target many other key areas of the pathway as well, whether through directly interfering with ubiquitization or evading RIG-1 activation altogether. For example, viruses such as SARS-CoV PLpro and Foot-and-mouth disease virus (FMDV PLpro) use viral deubiquitinase (DUBs) to remove ubiquitin from RIG-1, while other viruses use proteins such as Vaccinia virus E3L or the Hantavirus nucleocapsid proteins to directly bind dsRNAs away and shield viral RNA ligands from detection by RIG-1 (Liu et al., 2015; Tang & Hur, 2020).

Conclusion:

RIG-I is a critical cytosolic sensor of viral RNA that most notably serves in detecting patterns in these nucleic acids, such as blunt-ended double-stranded structure or 5'-triphosphate ends. Following this recognition, RIG-I binds to the RNA, and ATP hydrolysis drives conformational changes that expose its CARD domains, in turn triggering further signaling via MAVS that mounts an antiviral response. In addition, the significance of the ubiquitination of

RIG-I by E3 ligases, including TRIM25 and RIPLET, has been thoroughly characterized; these factors are crucial for higher-specificity containment of viral RNA and stronger signaling cascades. Furthermore, the RIG-I pathway functions alongside other PRR pathways, including those of cGAS-STING and endosomal TLRs, to form a layered antiviral defense system, intersecting in their reliance on common molecules such as the transcription factor TBK1. Future research should delve deeper into the mechanisms through which RIG-I differentiates between self and viral RNA, post-translational modifications beyond ubiquitination that modulate RIG-I's activity, and ways in which certain viruses, such as HIV, may bypass the detection of RIG-I or other PRRs.

Works Cited:

Cadena, C., Ahmad, S., Xavier, A., Willemsen, J., Park, S., Park, J., ... & Hur, S. (2019). Ubiquitin-dependent and -independent roles of E3 ligase Riplet in RIG-I activation. *Immunity, 51*(3), 573–586. https://pmc.ncbi.nlm.nih.gov/articles/PMC6525047/

Decout, A., Katz, J. D., Venkatraman, S., Ablasser, A. (2021). The cGAS–STING pathway as a therapeutic target in inflammatory diseases. *Nature Reviews Immunology, 21*(9), 548–569. https://pmc.ncbi.nlm.nih.gov/articles/PMC8029610/

Hornung, V., Ellegast, J., Kim, S., Brzózka, K., Jung, A., Kato, H., ... & Hartmann, G. (2006). 5′-Triphosphate RNA is the ligand for RIG-I. *Science, 314*(5801), 994–997. https://doi.org/10.1126/science.1132505

Ishikawa, H., & Barber, G. N. (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature, 455*(7213), 674–678. https://doi.org/10.1038/nature07317

Kawai, T., & Akira, S. (2006). Innate immune recognition of viral infection. *Nature Immunology, 7*(2), 131–137. https://doi.org/10.1038/ni1303

Liu, Y., Olagnier, D., Lin, R. (2015). Host and viral modulation of RIG-I-mediated antiviral immunity. *Frontiers in Immunology, 6*, 529. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2016.00662/full

Luo, D., Peisley, A., Liu, M., & Hur, S. (2023). Proofreading mechanisms of the innate immune RNA sensor RIG-I. *Biochemical Society Transactions, 52*(3), 1131–1142. https://doi.org/10.1042/BST20230724

Solotchi, M., & Patel, S. S. (2024, June 26). *Proofreading mechanisms of the innate immune receptor rig-I: Distinguishing self and viral RNA*. Biochemical Society transactions. https://pmc.ncbi.nlm.nih.gov/articles/PMC11346460/

Saito, T., Owen, D. M., Jiang, F., Marcotrigiano, J., & Gale, M. (2008). Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature, 454*(7203), 523–527. https://doi.org/10.1038/nature07106

Schlee, M., Roth, A., Hornung, V., Hagmann, C. A., Wimmenauer, V., Barchet, W., ... & Hartmann, G. (2009). Recognition of 5′-triphosphate by RIG-I helicase requires short blunt double-stranded RNA as an auxiliary ligand. *Immunity, 31*(1), 25–34. https://doi.org/10.1016/j.immuni.2009.05.008

Seth, R. B., Sun, L., Ea, C. K., & Chen, Z. J. (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF-3. *Cell, 122*(5), 669–682. https://doi.org/10.1016/j.cell.2005.08.012

Hongliang Dong, Jie Shen. MAVS Ubiquitylation: Function, Mechanism, and Beyond. *Front. Biosci. (Landmark Ed)* **2024**, 29(2), 72. https://doi.org/10.31083/j.fbl2902072

Tang, E. D., & Hur, S. (2020). Mechanisms of RIG-I-like receptor activation and manipulation by viral pathogens. *Current Opinion in Immunology, 66*, 42–52. https://doi.org/10.1016/j.coi.2020.06.002

Li, M., Ramage, H., & Cherry, S. (2020). Deciphering flavivirus-host interactions using quantitative proteomics. Current opinion in immunology, 66, 90–97. https://doi.org/10.1016/j.coi.2020.06.002

Yoneyama, M., & Fujita, T. (2009). RNA recognition and signal transduction by RIG-I-like receptors. *Immunity, 29*(2), 178–190. https://doi.org/10.1016/j.immuni.2008.08.010

Zheng, Z., Liu, M., Wang, T., Liu, Y., Yuan, W., & Zhang, L. (2024). ZFP36 enhances Riplet-dependent K63 ubiquitination of RIG-I and antiviral signaling. *Virology Journal, 21*(1), 34. https://doi.org/10.1186/s12985-024-02503-x

McNab, F., Mayer-Barber, K., Sher, A., Wack, A., & O'Garra, A. (2015). *Type I interferons in infectious disease*. Nature Reviews Immunology, 15(2), 87–103. https://doi.org/10.1038/nri3787

Ahmadi, S., Bazargan, M., Elahi, R., & Esmaeilzadeh, A. (2023, April). *Immune evasion of severe acute respiratory syndrome coronavirus-2 (SARS-COV-2); molecular approaches*. Molecular immunology. https://pmc.ncbi.nlm.nih.gov/articles/PMC9684099/

Sun, L., Wu, J., Du, F., Chen, X., & Chen, Z. J. (2013). *Cyclic GMP–AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway.* Science, 339(6121), 786–791. https://doi.org/10.1126/science.1232458

Tanaka, Y., & Chen, Z. J. (2012). *STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway.* Science Signaling, 5(214), ra20. https://doi.org/10.1126/scisignal.2002521

Zeng, W., Sun, L., Jiang, X., Chen, X., Hou, F., Adhikari, A., Xu, M., & Chen, Z. J. (2010). Reconstitution of the RIG-I pathway reveals a signaling role of unanchored polyubiquitin chains in innate immunity. Cell, 141(2), 315–330. https://doi.org/10.1016/j.cell.2010.03.029

Delves, P. J., Martin, S. J., Burton, D. R., & Roitt, I. M. (2017). *Overview of the immune system*. In P. J. Delves, S. J. Martin, D. R. Burton, & I. M. Roitt (Eds.), *Roitt's Essential Immunology* (13th ed.). Wiley-Blackwell. https://www.ncbi.nlm.nih.gov/books/NBK459455/

Kowalinski, E., Lunardi, T., McCarthy, A. A., Louber, J., Brunel, J., Grigorov, B., Gerlier, D., & Cusack, S. (2011). Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. Cell, 147(2), 423–435. https://doi.org/10.1016/j.cell.2011.09.039

Jiang, F., Ramanathan, A., Miller, M. T., Tang, G. Q., Gale, M., Jr, Patel, S. S., & Marcotrigiano, J. (2011). Structural basis of RNA recognition and activation by innate immune receptor RIG-I. Nature, 479(7373), 423–427. https://doi.org/10.1038/nature10537

Luo, D., Ding, S. C., Vela, A., Kohlway, A., Lindenbach, B. D., & Pyle, A. M. (2011). Structural insights into RNA recognition by RIG-I. Cell, 147(2), 409–422. https://doi.org/10.1016/j.cell.2011.09.023