Towards a crystal-clear view of the viral RNA sensing and response by RIG-I-like receptors

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Abstract

The RIG-I-like receptors (RLRs) – RIG-I, MDA5, and LGP2 – detect intracellular pathogenic RNA and elicit an antiviral immune response during viral infection. The protein architecture of the RLR family consists of multiple functional domains, including N-terminal Caspase Activation and Recruitment Domains (CARDs) for signaling initiation, a central RNA helicase core and a C-terminal domain for RNA sensing. With these specialized sensing-and-responding modules, RLRs are able to selectively bind non-self RNA species and trigger downstream signaling events leading to interferon production. This article summarizes the recent progress towards defining the precise mechanisms of RNA recognition and subsequent signal induction by RLRs.

RIG-I like receptors activate the antiviral immune response.

Upon the breakage of the physical and chemical barriers - skin, mucous membranes, body fluids, the immediate defense against pathogenic infections in our body is the innate immune response. In our immune system, pattern recognition receptors (PRRs) are primarily responsible for the detection of pathogens by recognizing pathogen associated molecular patterns (PAMPs), pathogen-derived molecules that are different from those normally found in the host [1]. PAMPs are diverse in their origin, chemical and structural nature, and subcellular localization, and therefore demand a broad spectrum of PRRs for their detection. Based on gene structure, there are four major classes of PRRs: Toll-like-receptors (TLRs), nucleotide-binding oligomerization domain receptors (NLRs), RIG-I-like-receptors (RLRs), and C-type lectin receptors (CLRs) [1].

RLRs are specialized intracellular PRRs that detect pathogenic RNA species generated during infection by viruses and other foreign organisms. There are three members of the RLR family: retinoic acid-inducible gene 1 (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [2]. RIG-I and MDA5 are similar to each other both structurally and functionally. Both contain two tandem caspase activation and recruitment domains (CARDs; CARD1 and CARD2) at the N-terminus, which initiate a downstream signaling relay; a central double-stranded RNA stimulated ATPase (DRA) motor domain, and a C-terminal domain (CTD) that facilitates viral RNA recognition **(Fig 1)** [3-7]. However, they have different preferences towards the RNA species they recognize; RIG-I recognizes short, duplex RNA with a 5'end triphosphate, whereas longer and non-triphosphorylated substrates are also able to efficiently activate RIG-I. In contrast, MDA5 cooperatively binds long duplex RNAs with no preference in 5'end character[8]. Therefore it is not surprising that RIG-I and MDA5 recognize different but overlapping groups of RNA viruses [2]. LGP2 does not contain the N-terminal CARDs and is thought to serve as a feedback regulator. However, the exact function and preferred RNA ligands of LGP2 are not clearly defined [9, 10]. RLRs are less abundant and remain inactive in resting cells. Detection and binding of viral RNA stimulates the ATPase activity of RIG-I and MDA5 and initiates a signalling cascade leading to type I interferon (IFN) production [8, 11]. The adaptor protein for signal transduction is MAVS, also known as IPS-1, VISA or CARDIF [12-15], which is found on the outer mitochondrial membrane. Activated RIG-I or MDA5 trigger MAVS oligomerization through their exposed CARDs. This activation process is assisted by post-translational modifications like phosphorylation, ubiquitination and non-covalent poly-ubiquitin binding [16, 17]. Oligomeric MAVS then activates several transcription factors – including IRF3, IRF7 and NF-κB – that induce the production of

type I IFN and inflammatory cytokines [3, 18, 19], which in turn help to engage the adaptive immune system to eliminate the infection and build immunological memory [20]. The molecular mechanism of MAVS mediated IFN/Cytokine expression has been subjected to extensive study[21]. The latest findings suggest that the activated MAVS is able to recruit and activate E3 ligases TRAF2, TRAF5, and TRAF6 to ubiquitinate TRAF2 and other proteins, which then through the adaptor NEMO recruit kinases -IKK and TBK1[22]. These kinases phosphorylate IκBα and IRF3 and therefore activate the transcription factors – IRF3 and NF-κB – to switch on the gene expression of IFN and cytokines[11, 21, 22].

As a subgroup of the Superfamily 2 (SF2) nucleic acid dependent ATPases, RLRs contain a helicase core in the middle of the gene [7]. This core domain harbors the conserved structural and functional elements of Superfamily 1 and 2 (SF1 and SF2) nucleic acid dependent ATPases: two RecA-like domains containing a set of conserved sequence features: motifs Q, I, II, and VI forms the ATP binding and hydrolysis pocket; motifs Ia, Ib, Ic, IV, IVa, V and Vb mediate RNA binding; motifs III and Va coordinate nucleic acid binding with ATP hydrolysis (**Fig 1F**)[23, 24]. Despite these similarities, RLRs do not fall into the classical helicase category. They thus belong to a subgroup of superfamily 2 RNA helicases, namely Duplex RNA-activated ATPases (DRAs), which also include Dicer and Dicer-related helicases from worms [7]. As the name implies, both the ATPase activity and subsequent cellular functions of these proteins are stimulated by interaction with dsRNA (**Fig 2A**). Furthermore, DRAs do not appear to display RNA unwinding activity. They lack the β-hairpin motif on the second helicase domain which has been shown to be the duplex opener in the hel308 DNA helicase (**Fig 2C**) [25]. Similar hairpin structures are also found in viral SF2 helicases and they also seem to function as nucleic acid duplex opener (**Fig 2DE**)[26, 27]. Rather, DRAs contain accessory domains specialized for duplex

RNA recognition, including an insertion domain (HEL2i), a CTD, and additional motifs (IIc and Vc) found in the helicase core (**Fig 1, 2A**)[7]. As a distinct group of macromolecular machines, the mechanical functions of DRAs upon binding to RNA and ATP are related to other processes, such as the release of signaling domains (CARDs of RIG-I) and activation of partner proteins (MAVS).

RIG-I: two-component molecular machine for sensing and responding to viral infections

The RNA sensing process is the key step in the initiation of an antiviral innate immune response. It is logical to think that selecting the correct RNA PAMPs rapidly and with high fidelity would be required for a highly efficient host defense system. RIG-I has evolved to comprise specific RNA recognition modules to handle this task (**Fig 1**). The CTD provides the specificity for both the 5'triphosphate of RNA targets through electrostatic interactions, and for the blunt ends of the RNA duplex through hydrophobic stacking to the first base pair (**Fig 1**)[28, 29]. The 5'triphosphate of the RNA establishes strong electrostatic interactions with the conserved positively charged residues of RIG-I. This provides the structural basis for the high affinity and specificity of RIG-I for the duplex RNAs bearing 5' triphosphates that are generated during viral replication, as opposed to host RNAs generated during normal cellular metabolism (**Fig 1AB**). The helicase domain displays specificity for the RNA duplex stem, albeit to a much lower extent than the CTD-duplex end interaction (**Fig 1F**). Both the conserved helicase core and the HEL2i domain, a novel insertion domain in HEL2 with five α-helices, contain specialized motifs that allow RIG-I to bind the top or 5' strand of the RNA duplex (the conventional RNA-binding SF2 motifs interact with only the bottom or 3' tracking strand) (**Fig 1**). Interestingly, there are two opposing functional surfaces on HEL2i: one RNA binding surface with a conserved residue

(Q511 in human RIG-I) that dynamically samples the RNA ligand (**Figs 1,3**)[30] and one inhibitory surface that interacts with the CARD2 domain to maintain the auto-inhibited state of RIG-I [5]. Thus, at the molecular level, HEL2i serves as a molecular transmitter, bridging the sensing and signaling processes of RLRs (**Fig 3**).

The CTD and the helicase domain act together in 5' triphosphorylated duplex RNA recognition.

These two RNA sensing modules are connected and coordinated by the pincer domain [4-6]. Unique to RLRs, this pincer domain is made of two long α helices, which emanate from the HEL2 domain of the helicase core, stack back across the HEL1 domain and lead into the CTD with a non-structured proline-rich linker [4, 6]. For 5'triphosphorylated substrates, RNA binding is likely nucleated by duplex end capture mediated by an interaction between the triphosphate and the CTD. Presumably, complete binding of the CTD:dsRNA terminus then brings the helicase domain and duplex RNA stem into close proximity, effectively increasing their local concentration and promoting their interaction. Alternatively, these two steps may be non-sequential: in the *apo* enzyme, the RNA binding surfaces from both the CTD and the helicase domains are well exposed and capable of simultaneous recognition of RNA PAMPs[5]. The length and flexibility of the second pincer arm helps sandwich the duplex RNA between the CTD and the HEL1 domain of the helicase core to form a hetero-molecular rigid body (**Figs 1,3**)[6]. The HEL2 and HEL2i domains form a second rigid body, which displays significant degrees of freedom across the RNA-free state, the RNA sensing state and the activated state of RIG-I (**Fig 3**). This design allows RIG-I to examine the correct chemistry and structure of the RNA ligand [30-33].

RIG-I prefers capping the ends of RNA duplex rather than binding internally.

Like other RNA helicases, ATP binding closes the helicase core and subsequent hydrolysis and product release allows cycling back to an open conformation (**Fig 1a, 3**). An interpretation of this repetitive intra-molecular motion is that RIG-I may be able to translocate along duplex RNA [34], allowing multiple copies of RIG-I to assemble and reside on a single duplex RNA to provide stronger stimulatory signals for IFN production [34-36]. However, several conflicting reports related to this proposed mechanism of RIG-I signaling still need to be reconciled. First, in order for RIG-I to translocate along RNA duplex, the CTD must no longer cap the RNA end and the high-affinity interaction between the CTD and the triphosphates must be disrupted. Further, RIG-I also displays no intermolecular cooperativity for RNA binding, prefers short RNA, including ten base pair hairpins and siRNA, for its enzymatic activity, and can be effectively stimulated by short and long RNA with respect to IFN production[30]. Finally, in all known RIG-I:dsRNA complexes, RIG-I was crystallized at the ends of all RNA duplexes of different length and sequence composition, even in the absence of the CTD domain (**Fig 3**), further suggesting that RIG-I preferentially binds at the duplex RNA terminus. Therefore, it seems plausible that the large conformational changes resulting from the intra-molecular structural flexibility between the two rigid bodies of RIG-I (HEL1-dsRNA-CTD versus HEL2-HEL2i) (**Fig 3**) coupled to cycles of ATP hydrolysis may provide an alternative explanation to the results from the single molecule experiments by Myong et al., 2009 that were initially interpreted to be RIG-I translocation.

Internal binding of the RNA duplex stem by RIG-I is not required for strong monomeric binding at the 5’ end[30]. Indeed, our recent work allowed us to define the consensus, minimal

functional RNA PAMP for RIG-I. It is clear that RIG-I requires only the 5' terminus of duplex RNA, along with an adjacent ten to twelve base pairs for binding (**Fig 1a**), ATP hydrolysis, and cell-based IFN production [30]. Interestingly, new evidence from EM studies in the Hur lab suggests that, despite the lack of cooperativity, internal binding could be induced by a nucleation effect initiated by RIG-I capping at the end of the RNA duplex which could lead to a more robust interferon response [35, 36]. Conceivably, at lower concentrations of RIG-I in the resting state, RIG-I surveys and binds only the ends of 5'trisphorphylated RNA, but upon IFN induction and the concomitant increase in cellular levels of RIG-I protein, RIG-I molecules may start to bind internally near the RIG-I-capped RNA[35, 37]. A distinct conformation of RIG-I bound internally to the duplex RNA is therefore highly desirable, which may provide a second – similar but not identical – means to activate RIG-I. Carefully designed experiments are needed to further clarify the functional implications of this RNA end capping preference versus the duplex internal binding activity of RIG-I *in vivo*.

The N-terminal CARDs of RIG-I turn on IFN production.

The N-terminal tandem CARDs of RIG-I comprise the signaling domain (**Fig 4**), which alone triggers robust IFN production when ectopically expressed in cells [38]. The RIG-I CARDs, specifically the first CARD domain – CARD1, turn on the intracellular signaling cascade by interacting with the mono CARD domain of the mitochondrial adaptor protein MAVS and inducing its oligomerization [39]. In the resting cells of ducks, *apo* RIG-I adopts an auto-repressed conformation in which CARD2 interacts with the HEL2i domain of the helicase [5]. This intra-molecular inhibitory interaction locks the CARDs in an inaccessible conformation for MAVS activation; a strategy that is likely employed in mammalian RIG-I as well [5, 38]. Both K63-linked

covalent ubiquitination and non-covalent polyubiquitin binding of the RIG-I CARDs have been shown to mediate the MAVS activation process [16]. Although the exact molecular mechanism is unclear, ubiquitination and polyubiquitin binding might further rearrange the conformation of the CARDs of RIG-I to promote CARD-CARD interactions between RIG-I and MAVS or might facilitate RIG-I oligomerization (**Fig 4**)[36]. It is thought that once MAVS oligomerizes and recruits the downstream signaling kinases, the signaling cascade becomes essentially irreversible [36, 39, 40].

Activation of RIG-I signaling is a carefully regulated process.

Through the aforementioned molecular modules, RIG-I senses viral infection by binding to the viral RNA PAMP and subsequently rearranges its conformation to initiate the intracellular signaling cascade leading to IFN expression. IFNs then reset the body's metabolism and promote both innate and adaptive immunity to defend against pathogens [41]. This process is very costly and often dangerous to the host; therefore it must be tightly regulated. RIG-I is such a well-designed nano-mechanical device that it allows regulation at multiple levels. The auto-repressed state sets the threshold for activation (**Fig 4**). The structural dynamics upon RNA binding and sensing provides a means to examine the strength of the danger signal (**Fig 1a, 3**). The ATP dependent conformational changes of the helicase domain switch off the auto-inhibition of the CARDs [7, 42, 43]. Ubiquitin or phosphorylation mediated post-translational modifications further potentiate RIG-I activation [16, 40, 44]. Lastly, multiple copies of activated RIG-I:RNA complex are probably needed to activate the adaptor protein MAVS [40].

MDA5 (and LGP2) complements RIG-I in targeting a broad spectrum of viral infections.

Despite the fact that MDA5 overlaps functionally with RIG-I, the two RLRs are not entirely redundant when facing viral infections [2, 8]. Early RLR studies proposed RNA and length preferences for MDA5 and RIG-I which helped to explain the ability of each RLR to detect different but in some cases overlapping families of viruses [2, 8, 45]. MDA5 shares the same protein architecture and approximately 33% sequence identity with RIG-I. The number of structural biology studies of MDA5 is catching up with those for RIG-I, providing us with insightful comparisons between these two proteins, and allowing us to better understand the molecular bases of their similarities and differences.

Unlike RIG-I, MDA5 binds cooperatively to long RNA duplex.

The corporative binding of MDA5 to long RNA duplex molecules has been demonstrated by biochemical, biophysical and structural methods [46-50]. This cooperativity is independent of the CARDs and the interface has been mapped to HEL1 of one molecule of MDA5 and the CTD of the neighboring molecule [47, 50]. This cooperativity is necessary for activation of MDA5, as MDA5 displays poor cellular activity on short RNA species [40, 45, 46]. The structure of MDA5 lacking the CARDs in complex with a 12mer RNA duplex exhibits several differences from those of similar RIG-I:RNA complexes: first, the MDA5 CTD in the structure binds only to the backbone of the RNA duplex but not to the end (**Fig 1**)[50]; second, unlike the RIG-I CTD, mutations that remove the RNA capping loop from the CTD of MDA5 do not weaken RNA binding of MDA5 [50]. Therefore the respective CTDs of RIG-I and MDA5 play a major role in distinguishing these proteins. Interestingly, encephalomyocarditis virus (EMCV), a *picornavirus*, is recognized by MDA5 but not RIG-I, presumably because of the peptidyl modification at the 5' end of the viral genome that would block RIG-I binding [2, 8]. In addition to differences in the

CTD, a HEL2 loop of MDA5 inserts in and widens the major groove of the RNA, whereas the corresponding HEL2 loop of RIG-I actually resides near the dsRNA end.

Using EM reconstitution, Berke et al. revealed a helical envelop of MDA5 on an RNA duplex [47]. Interestingly, although the data were obtained for both full length and CARD-less MDA5, the authors could not locate the CARDs [47]. It is likely that in the activated MDA5:RNA complex, the CARDs do not form a stable interaction with the HEL-CTD domains, but are physically tethered to the HEL-CTD:RNA complex through the 100 amino acid long non-structured linker between CARD2 and the HEL-CTD domains. The Hur group suggested that at least 6-8 copies of the activated MDA5 (on a long duplex RNA of more than a hundred base pairs) form the minimal activation unit – comprising a multimeric head-to-tail filament with the free CARDs able to cluster together (**Fig 4**). This CARDs complex is then able to recruit MAVS and trigger its oligomerization [50]. This theory could explain the length preference of MDA5; a longer RNA duplex is simply able to recruit and activate more copies of MDA5. However, this putative CARDs complex as the initiator of the MDA5-mediated IFN activation requires further study as the interface for self-association and the interface for MAVS interaction are not well-defined at a structural level. In addition, like RIG-I, MDA5 also appears to require ubiquitination or polyubiquitin binding for its function, most likely after MDA5 binds and oligmerizes on RNA [40, 51, 52].

The structure and function of LGP2 is still poorly defined.

As LGP2 lacks the N-terminal CARDs found in other RLRs, it likely retains no capacity for signaling. Being able to bind dsRNA with its HEL-CTD structure might suggest a mechanism of inhibition of RIG-I/MDA5 signaling [10, 53, 54]. However, LGP2 knockout mice were

defective in responding to virus infection, particularly for picornaviruses like EMCV. Curiously, synthetic RNAs (including polyIC) and *in vitro* transcribed RNAs elicited comparable IFN production in both WT and LGP2$^{-/-}$ mice [9, 55]. Despite these contradictory results, LGP2 appears to positively regulate MDA5 signaling through an as yet unclear mechanism while perhaps also indirectly modulating RIG-I signaling[9, 55, 46, 10, 53, 54]. Unfortunately, there is only one structure of the LGP2 CTD in complex with a short RNA duplex (**Fig 1e**) [53], making it difficult to infer the molecular basis of LGP2 function.

A recent structural study nicely demonstrated how paramyxovirus V protein selectively inhibits MDA5 and LGP2 [56]. The viral protein actively disrupts the fold of the HEL2 domain of MDA5, and consequently likely disrupts LGP2 based on protein sequence conservation analysis, thereby impairing the RNA binding and ATP hydrolytic functions of both proteins [56]. This evasion strategy, while apparently specific for MDA5 and LGP2, may also indirectly act against RIG-I through LGP2 via an undefined mechanism[56, 57].

Very little is known about how similar or different LGP2 is from RIG-I or MDA5 in recognizing various RNA ligands. However, recently Bruns et al. demonstrated that robust basal ATPase activity allows LGP2 to diversify its RNA recognition capacity [58]. This result is interesting in light of the fact that both RIG-I and MDA5 completely lack basal ATPase activity. Moreover, the duck apo RIG-I structure suggests that ATP binding and hydrolysis can only occur in the presence of RNA. Therefore, a structure of the apo and RNA bound LGP2 helicase domain would help shed some light on this issue. Future *in vitro* and cellular studies will hopefully answer how LGP2 recognizes RNA and whether its regulatory roles are performed by directly acting on RIG-/MDA5. This will provide a clearer picture of LGP2 function in RLR signaling.

Conclusions and prospects

Over the past few years, tremendous efforts have been invested in better understanding the molecular biology of RLRs and in attempting to answer several important questions: How do RLRs sense viral infection? What are the PAMPs of RLRs? What is the activation mechanism? How is the downstream signaling cascade activated for the production of IFN? What are the similarities and differences between RLRs, across cell types, tissues, and species? How do viruses counter RLR recognition and response? What are the potential therapeutic applications in targeting the RLR systems for antivirals, vaccines, and anticancer drugs? Moving forward, there are still plenty of interesting and urgent questions that need to be addressed in the studies of RLRs and more generally in host-and-pathogen interactions. Here are some unanswered questions about the RLR activation pathway (**Fig 4**): (1) What is/are the conformation (s) of RIG-I binding internally to dsRNA? And what is the relationship between the internally bound RIG-I versus end-capped RIG-I? How are they different in their activation mechanism? (2) What is the *apo* state of MDA5? Is RNA binding alone able to activate MDA5? What is the function of ATP in MDA5 activation? Can RIG-I and MDA5 work together on the same RNA in the same subcellular environment?(3) What are the minimum copies of activated RIG-I for initiating MAVS oligomerization? (4) How are the CARDs of RIG-I and MDA5 different from each other? What are the 3D molecular rearrangements of CARDs for turning on the MAVS oligomerization. (5) What is the molecular basis of the ubiquitination/polyubiquintin binding in RIG-I and/or MDA5 activation process? (6) What are the 3D molecular rearrangements of the MAVS fiber on the mitochondria membrane? How does this fiber turn on the downstream signaling effectors? (7) What is the molecular role of LGP2 in RLR mediated antiviral immune response ?

293

294

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Conflict of Interest

The author declares no competing financial interests.

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Figure Captions:

Figure 1. Molecular features of RNA recognition by RLRs. (A) Schematic view of the RLR genes (upper panel) and the RIG-I:dsRNA interface (lower panel). The HEL2 and HEL2i domains are connected as one rigid body and move along the RNA, which is presumably regulated by the ATP binding and hydrolysis state of the protein. The HEL1-dsRNA-CTD forms another sandwich like rigid body, capping the blunt end of the duplex RNA. **(B)** The CTD of RIG-I is responsible for the recognition of the blunt end of dsRNA. The 5' triphosphate group of the RNA specifically interacts with the conserved positively charged residues on the CTD. Color codes: PDB-4AY2 in red, 3LRR in green, 3LRN in magenta, and 3NCU in blue. **(C)** Electrostatic surface view of the RIG-I CTD, highlighting the mode of RNA-protein interaction: the positively charged pocket for RNA 5' triphosphate group recognition and RNA end capping. **(D)** Electrostatic surface view of the MDA5 CTD, highlighting the mode of RNA-protein interaction: the positive patch for RNA backbone recognition and the absence of RNA end capping. **(E)** Electrostatic surface view of the LGP2 CTD. Although the dsRNA end is still capped, there is no positively charged surface at the 5'end of the dsRNA. **(F)** Electrostatic surface view of the duck RIG-I helicase domain in complex with a synthetic 19 mer RNA duplex and an ATP analog (PDB:4A36). The helicase domain is in the most closed conformation and RIG-I binds at the end of RNA duplex.

Figure 2. Structural features of the Superfamily 2 helicases. (A) RIG-I:dsRNA interaction. Duplex RNA remains base paired. The HEL2i domain, instead of separating the duplex, interacts with the dsRNA backbone [4-6]. **(B)** DEAD-box RNA helicase eIF4A3 binds to the single stranded RNA [59, 60]. **(C)** Archaeal Hel308 DNA helicase separates the DNA duplex with the beta-hairpin instertion in red on the helicase domain 2 [25]. (**D**) HCV NS3 helicase binds to the single stranded DNA and the beta-hairpin instertion on the helicase domain 2 prevents the complementary strand from baseparing with the single stranded nucleic acid [26]. (**E**) Dengue NS3 helicase binds to the single stranded RNA. Similarly, the beta-hairpin instertion on the helicase domain 2 is thought to function as the RNA duplex opener[27]. The helicase core domains 1 and 2 are colored in wheet yellow, the HEL2i domain or the beta-hairpin insertion in red, nucleic acids in magenta, and the accessory domains are in gray.

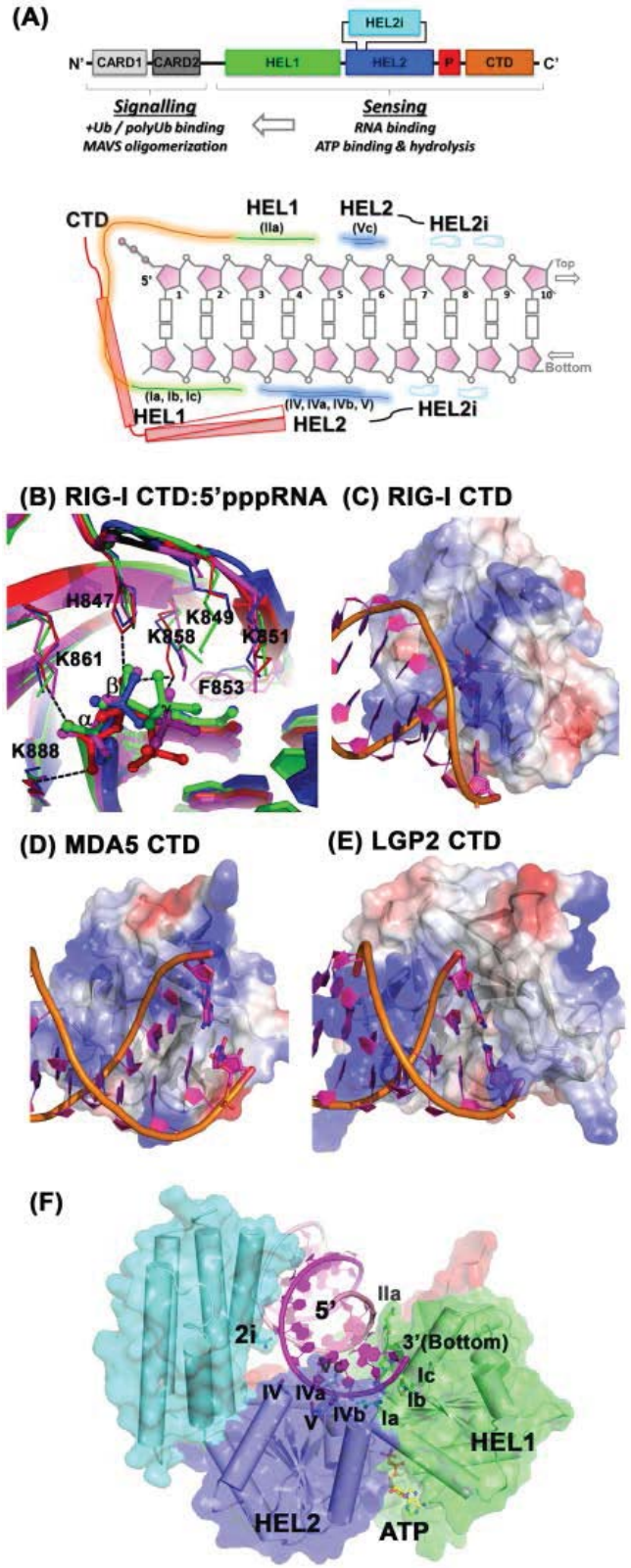
Figure 3. Superposition of the RIG-I structures. (A) Alignment of the RIG-I structures based on the HEL1 domain. The structures highlight that HEL2-HEL2i as a rigid domain moving relative to the HEL1-dsRNA-CTD tri-party fold. **(B)** Top view of the aligned structures. Left: relative locations of the HEL2i domain and dsRNA. Right: relative locations of the HEL2i domain and CTD. **(C)** A model of the relative HEL2i locations (solid circles) in RIG-I structure as an indicator for the gating mechanism of RIG-I activation. The figure is a schematic representation of the HEL2i motions (the yellow trajectory that goes through the center of the circles) shown in (B) with the same color codes. In the resting cells, RIG-I remains in the sensing state where the HEL2i domain does not interact with the RNA, as indicated in the *apo* RIG-I

structures 3TBK (black), 4A2W (dark gray), and 4A2Q (light gray). At the checkpoint state (dashline), RIG-I encounters foreign RNA species and the HEL2i domain participates in the RNA recognition process as indicated in the RIG-I RNA complexes 3ZD6 (cyan), 2YKG (blue) and 3ZD7 (light blue). Effective activation of RIG-I requires additional trigger by ATP to disrupt the HEL2i-CARD2 interaction as indicated in the ternary complexes of RIG-I, RNA and ATP analogs, 3TMI (pink) and 4A36 (red).

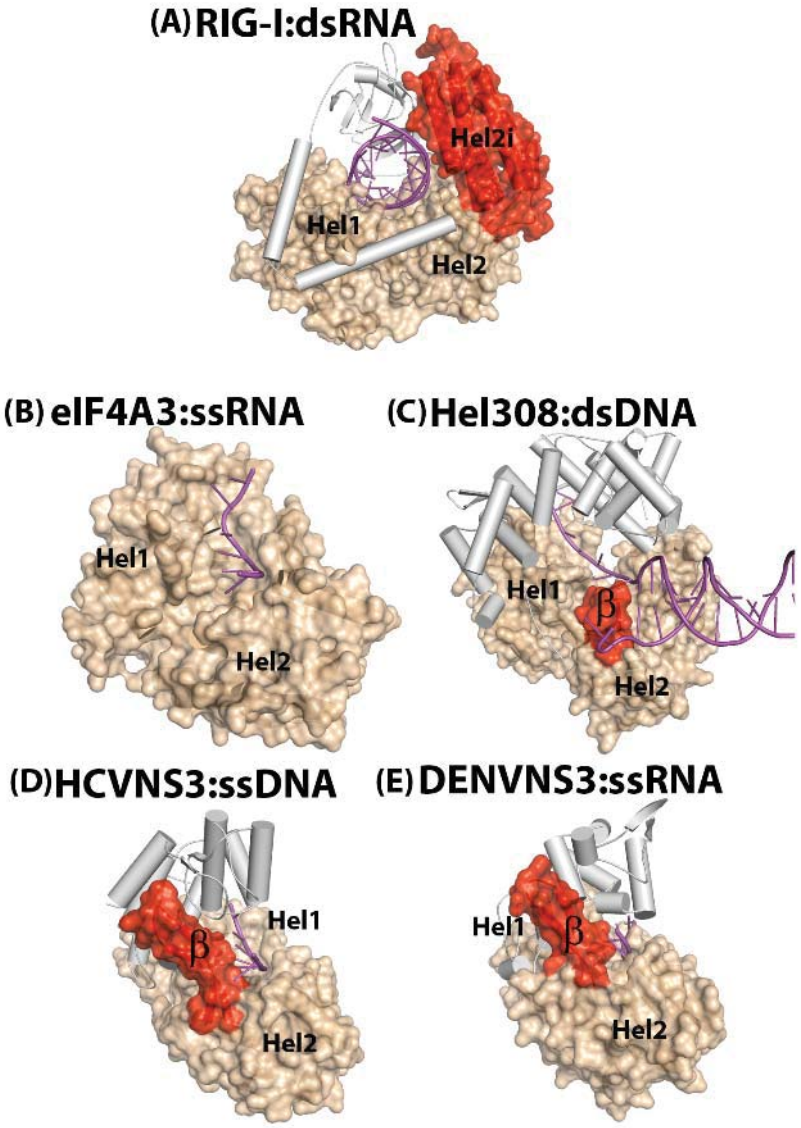
Figure 4. Current opinions on the molecular basis of the RLR signaling pathway. (A) RNA sensing and activation of RIG-I **(B)** RNA sensing and activation of MDA5. All the crystal structures of RIG-I reside at the end of the duplex RNA, at least suggesting RIG-I prefers RNA ends, while MDA5 has been shown to bind RNA duplex internally and oligomerizes cooperatively on long duplex RNA. Ubiquitination and polyubiquitin chain non-covalent binding to the RIG-I CARDs are essential for RIG-I activation, which presumably occurs downstream of the molecular events of RNA binding and ATP binding to RIG-I. MAVS resides on the outer membrane of mitochondria and is activated by the RIG-I or MDA5 CARDs through the formation of fiber-like high order oligomers. This leads to the activation of IFN production through the NFkB and IRF3 signaling pathway.

Figures

Figure 1.



487 **Figure 2.**



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492 **Figure 3.**

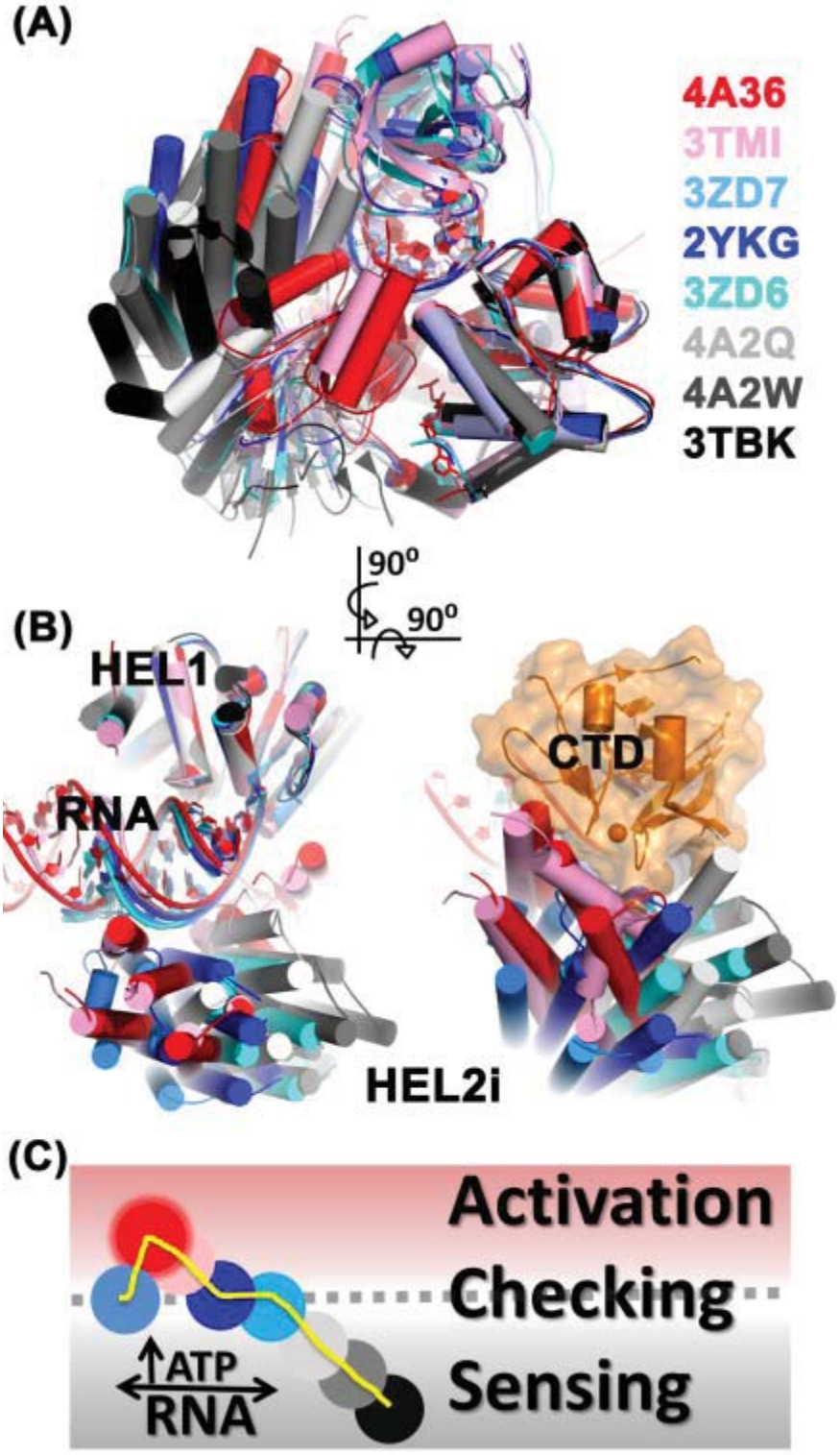

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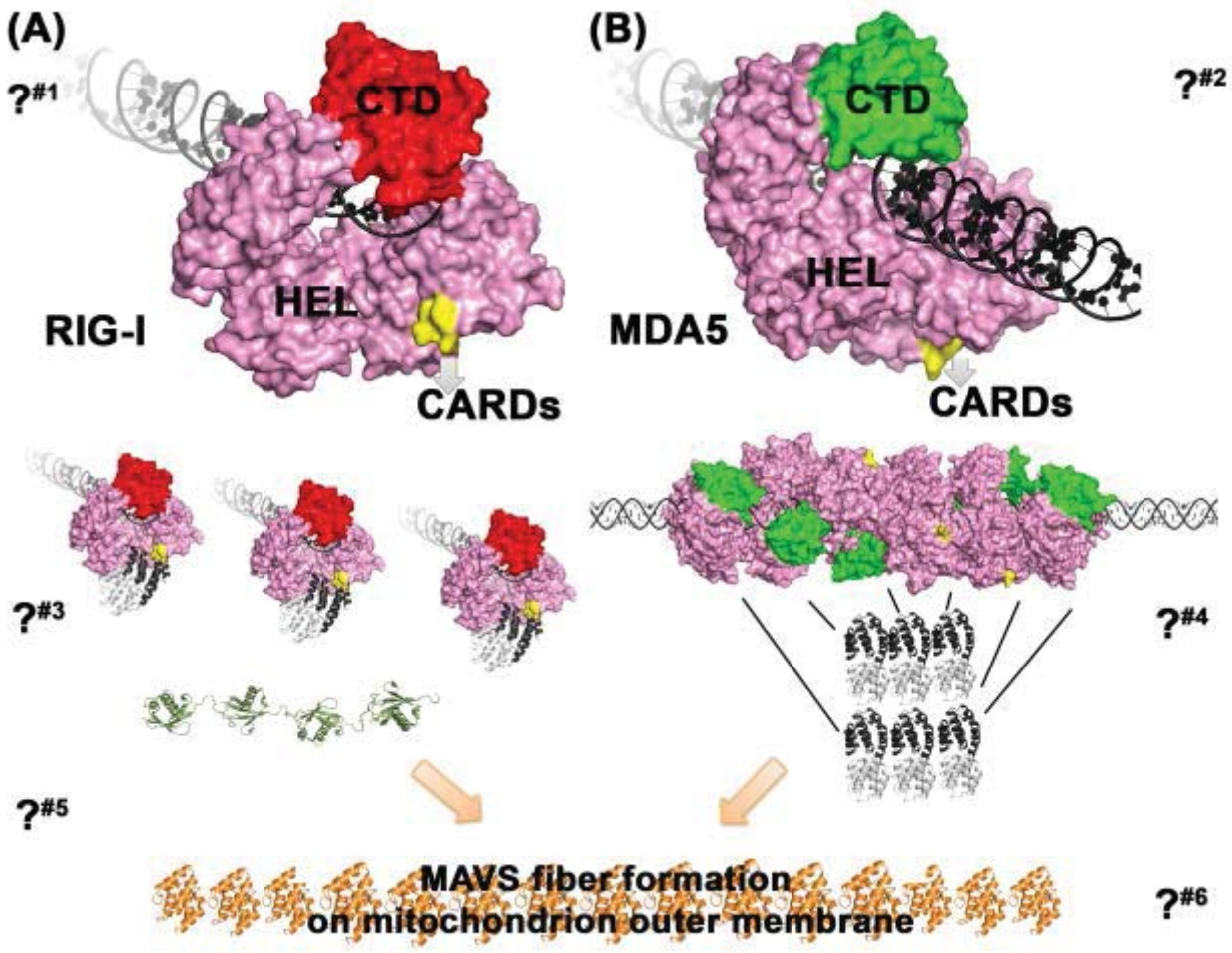
Figure 4.


(A)
?#1
CTD
HEL
RIG-I
CARDs
(B)
?#2
CTD
HEL
MDA5
CARDs
?#3
?#4
?#5
MAVS fiber formation
on mitochondrion outer membrane
?#6