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RESEARCH HIGHLIGHT

The lock-washer: a reconciliation of the RIG-I activation models

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RIG-I belongs to a type of intracellular pattern recognition receptors involved in the recognition of viral RNA by the innate immune system. A report by Peisley *et al.* published in *Nature* provides the crystal structure of human RIG-I revealing a tetrameric architecture of the RIG-I 2-CARD domain bound by three K63-linked ubiquitin chains, uncovering its activation mechanism for downstream signaling.

The recognition of microbial-derived nucleic acids and the correct and specific activation of the molecular machinery governing the mammalian immune response are paramount to host survival during viral infection. Viral RNA represents a key trigger for the activation and mobilization of a series of pattern recognition receptors (PRRs) such as the Toll-like receptor (TLR) and retinoic acid-inducible gene 1 (RIG-I)-like receptor (RLR) families. While the TLRs are restricted to the cell surface or inside endosomal compartments, the RLRs are present in the cytosol and act as the key sentinels of actively invading and replicating viruses.

The RLR family of receptors, RIG-I and Melanoma Differentiation-Associated protein 5 (MDA-5), are characterised by 3 distinct signaling domains critical for viral RNA recognition and response. The C-terminal repressor domain and the internal ATPase-containing DExD/H-box helicase domain of RIG-I function together to facilitate binding of viral dsRNA which contain either a 5′-ppp motif or 5′ blunt-end base-paired RNA with a triphosphate motif, moieties absent on self-nucleic acids [1]. Upon viral RNA ligation, two N-terminal caspase activation and recruitment domains (CARD), known as 2-CARD, on RIG-I propagate signal transduction via interactions with mitochondrial antiviral signaling protein (MAVS) [2]. Recent molecular and structural studies have elucidated the mechanisms by which RLR-activated MAVS mediates the antiviral response. During RIG-I signaling, MAVS forms large multimeric prion-like filaments on the mitochondrial membrane which are essential for RIG-I-mediated type I interferon (IFN) production [3]. Such functional aggregates are capable of recruiting key downstream signaling components such as members of TNF receptor associated factors (TRAF) family, resulting in the activation of the MAPKs, the NF-κB pathway and interferon regulatory factor 3/7 (IRF3/7) and consequently culminating in the upregulation of protective IFNs and pro-inflammatory cytokines. Viral infection is sufficient to convert nearly all endogenous detectable MAVS to functionally active aggregates, and interestingly this phenomenon can be recapitulated *in vitro* using only mitochondria, RIG-I and K63-linked ubiquitin chains, underscoring the functional importance of polyubiquitination events during RIG-I signaling [4].

In contrast to the well-documented and -accepted paradigm of MAVS activation, the model of RIG-I-mediated activation has remained incompletely understood. The classical model holds that RIG-I remains in an auto-repressed state in the absence of ligand. Upon viral recognition, the E3 ubiquitin ligase tripartite motif 25 (TRIM25) binds to the 2-CARD domain of RIG-I, resulting in the covalent conjugation of K63-linked polyubiquitin chains to induce a conformation change in the receptor and facilitate a "release" of the 2-CARD domain for MAVS interaction and activation [5]. However, this simple release model of the 2-CARD domain does not reconcile with recent compelling reports that RIG-I can act as a receptor for unanchored, non-covalently attached ubiquitin chains and that polyubiquitination of RIG-I induces the oligomerization of a heterotetrameric complex consisting of 4 RIG-I and 4 K63-ubiqutin chain molecules [6, 7]. In addition, although K63-ubiquitination is essential for the signaling potential of isolated 2-CARD molecules, full-length RIG-I can form filaments around the ends of dsRNA molecules, allowing 2-CARD regions of RIG-I molecules to come into close proximity to each other and facilitate MAVS aggregation in an ubiquitin-independent manner [8].

Although such conflicting reports seem to propose vastly different models of RIG-I activation, an elegent study published in *Nature* by Peisley *et al*. [9] uses biochemical and structural studies to reconcile the different models and they finally offer a unified understanding of RIG-I receptor activation. They resolved the crystal structure of human RIG-I 2-CARD in complex with K63-ubiquitin at 3.7 Å. The structure revealed the tetrameric architecture of RIG-I 2-CARD bound by three K63 ubiquitin chains (Figure 1). Crystallization and structure determination reveal that four 2-CARD subunits form a tetrameric helical assembly, termed the "lock washer", with the two ends displaced by half the thickness of 2-CARD.

Two key questions arise from the RIG-

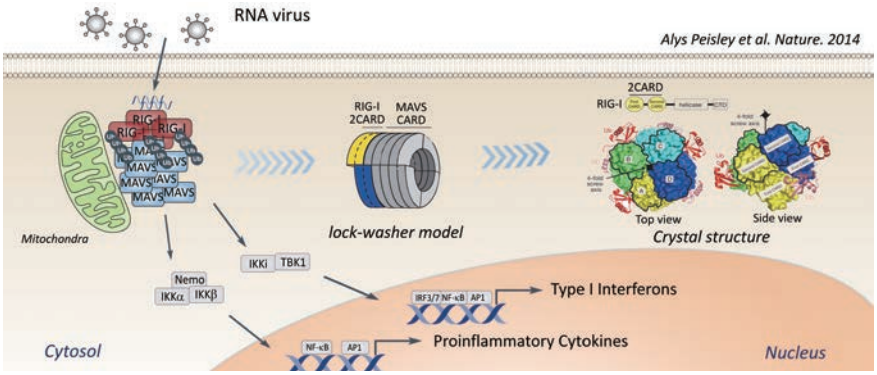

Figure 1 A model of RIG-I-mediated antiviral response.

I 2-CARD structure. First, how does the tetrameric architecture of RIG-I serve as a platform to activate downstream signaling? The CARD domain belongs to the death domain (DD) superfamily, members of which have a similar three-dimensional fold. The structures of other DD oligomers such as Myddosome, PIDDosome, or FAS-FADD complex have recently been resolved. The assembly of DD oligomers is usually mediated at six surface areas, with the helical oligomeric structure of upstream signaling molecules serving as a scaffold to assemble the downstream DD oligomers through helical extension. In the current study, the authors show that the assembly and stability of the tetramer and its IFN-β signaling potential are dependent on several intermolecular and intramolecular CARD interactions by generating mutants on different interaction surfaces and analyzing their tetramer formation and IFN-β induction abilities. MAVS filament formation assays indicate that the helical tetrameric structure of RIG-I 2-CARD serves as the platform for MAVS-CARD filament assembly, with the top surface of the second CARD as the primary site for MAVS recruitment [9].

The second pertinent question addressed is how the interaction between ubiquitin and 2-CARD contributes to downstream signaling? Unlike other DD oligomers, tetramer formation of isolated RIG-I 2-CARD requires K63-linked ubiquitin chains. The structure predicts that longer ubiquitin chains might wrap around the 2-CARD tetramer at 1:4 or 2:4 molar ratios to stabilize the 2-CARD tetramerization. Another key problem addressed in this study is the relationship between the covalent conjugation and non-covalent binding of K63-ubiquitin in stabilizing 2-CARD tetramers during RIG-I signaling. The authors challenge previous publications on the significance of 6 lysine (K) residues in both covalent conjugation and non-covalent K63-ubiquitin binding.

The authors show that only K6 is covalently conjugated with K63-ubiquitin chains and that non-covalent binding of K63-ubiquitin to 2-CARD can induce a further stabilization of the tetramer complex. RIG-I filament formation on dsRNA with appropriate length can also compensate for the requirement of both covalent and non-covalent K63-ubiquitin binding. Thus they arrive at the conclusion that these three mechanisms might act synergistically for signal activation. This compensatory mechanism could guarantee the detection of foreign pathogen RNA in case of the absence of one or two of the mechanisms or may allow an amplification of the signal potential. One could speculate that such functional redundancy in the initiation stage of signal activation may be a common theme in other innate immunity pathways.

The significance of this study lies in the resolution of the structural basis of the activated RIG-I 2-CARD tetramer and its initiation of MAVS aggregation and filament formation — the first elements of the dsRNA sensing signaling cascade that lead to production of type I IFNs and pro-inflammatory cytokines. It provides another detailed example of DD oligomers and adds to the growing realization of a common role of oligomeric molecular scaffolds in mediating innate immune signaling. Such exciting findings will no doubt instigate further study into the exact molecular interactions and mechanisms controlling dsRNA sensing. For example, the authors use a crystallized K115A/R117A 2-CARD double mutant for structural analysis; although it retains the ability to tetramerize with K63-ubiquitin and activate type I IFNs, the structure might still not be consistent with the wild-type 2-CARD and this may warrant further investigation. Furthermore, whether the RIG-I signaling activation mechanism that derived from this structure could be generalized and applied to other CARD domain receptors such as MDA-5, NOD1, NOD2, IPAF and NLRP1 will require further investigation. By utilizing advanced structural determination techniques coupled with sophisticated *in vitro* assays such as those described in this study, these questions will no doubt be addressed in the near future.

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