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Filament assemblies in foreign nucleic acid sensors

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Summary

Helical filamentous assembly is ubiquitous in biology, but was only recently realized to be broadly employed in the innate immune system of vertebrates. Accumulating evidence suggests that the filamentous assemblies and helical oligomerization play important roles in detection of foreign nucleic acids and activation of the signaling pathways to produce antiviral and inflammatory mediators. In this review, we focus on the helical assemblies observed in the signaling pathways of RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs). We describe ligand-dependent oligomerization of receptor, receptor-dependent oligomerization of signaling adaptor molecules, and their functional implications and regulations.

Introduction

Pattern Recognition Receptors (PRRs) in the innate immune system serves as the front line of defense against pathogen infection. PRRs detect a broad range of pathogens by recognizing conserved molecular patterns (also known as Pathogen Associated Molecular Patterns, PAMPs), and activate the inflammatory innate immune response to restrict infection. These PAMPs are bacterial cell membrane or cell wall components, or common features of viral or bacterial nucleic acids absent in the host nucleic acids. These include, but are not limited to, the endosomal localization of RNA/DNA, cytoplasmic location of DNA, duplex structure of RNA, and often times a combination of these features. While their activities are often transient and restricted to the infected state, an increasing number of studies have shown that dysregulated function of PRRs can also lead to a variety of auto-inflammatory or chronic inflammatory diseases^{1–3}, and have led to the on-going efforts to therapeutically inhibit PRRs for the treatment of these immune disorders. Over the last decade or so, there has been a rapid progress in our understanding of the structures of PRRs in the ligand-free and ligand-bound states, and their interactions with downstream signaling adaptors, activators and regulators^{4–9}. In doing so, one common observation made by many laboratories, including ourselves, is that PRRs and many molecules in the signaling pathway often aggregate *in vitro*, which has posed technical challenges in biochemical and structural characterization. The accumulating evidence, however, suggest that underlying the apparent

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aggregation phenomena are ordered structural assemblies, in particular helical assemblies, that are key to their functions.

In this review, we will focus on the helical assemblies observed in the signaling pathways of RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs). We will describe ligand-dependent oligomerization of receptor, receptor-dependent oligomerization of signaling adaptor molecules, and their functional implications and regulations. We will also describe our speculation and perspectives about the common occurrence of the helical oligomers or filamentous assemblies in innate immune signaling. Due to the space limitation, we will not discuss Toll-like receptors and other helical assemblies in cell death as outstanding reviews are available elsewhere^{7, 10}.

RIG-I-like receptors (RLRs): RIG-I and MDA5

RIG-I and MDA5 are non-membrane bound, soluble receptors that recognize viral double-stranded RNAs (dsRNAs) and activate antiviral immune response by stimulating transcriptional up-regulation of type I interferons¹¹. RIG-I and MDA5 share a high sequence similarity and the same domain architecture, consisting of the N-terminal tandem caspase activation and recruitment domain (2CARD), central DExD/H box motif helicase domain and zinc-binding C-terminal domain (CTD). As with many proteins with a similar helicase domain, RIG-I and MDA5 appear to lack the duplex unwinding activity¹²(unpublished data for MDA5) although there is a conflicting report on RIG-I¹³. 2CARD mediates signal activation by interacting with the downstream signaling adaptor molecule, MAVS, while the helicase domain and CTD are responsible for RNA recognition (Fig. 1A). Despite the shared domain organization and the downstream signaling pathway, RIG-I and MDA5 play non-redundant functions by recognizing largely distinct types of viral RNAs^{14, 15}. MDA5 recognizes long (>~1 kb) dsRNA that are produced in the form of replication intermediates of picornaviruses and other positive strand viruses^{16–18}. In contrast, RIG-I recognizes relatively short duplex RNA or hairpin structure with the 5' tri- or di-phosphate, as a sign of the lack of 5'-processing that normally occurs in cellular RNAs^{19–24}. These RNAs are often formed from defective interfering particles or genomic single-stranded RNA of both positive and negative strand viruses^{19–24}.

dsRNA-triggered receptor oligomerization of MDA5

Recognition of viral dsRNAs by MDA5 involves helical filament assembly of the RNA-binding domain (helicase-CTD), which in turn triggers the second step of oligomerization by 2CARD²⁵. Upon dsRNA binding, the helicase-CTD cooperatively assembles into a filament that extends along the length of dsRNA^{26, 27} (Fig. 1B). This filament formation is required for high affinity interaction with dsRNA as binding of a monomeric MDA5 is inefficient²⁸. The MDA5 filament nucleates on a dsRNA interior with no obvious sequence preference, and propagates to the RNA termini²⁸ (Fig. 1C). A combination of crystallography, electron microscopy and protein-protein crosslinking studies provided a detailed model of the MDA5 filament: the helicase domain and CTD form a ring-like structure around dsRNA, and this monomeric ring stacks head-to-tail along the length of dsRNA every ~14 bp to form a filament^{25, 29}. Mutations in the filament interface decreased MDA5's ability to induce IFN,

suggesting its importance in its signaling function²⁵. Interestingly, the filament displays dynamic instability in that filament formation triggers ATP hydrolysis, which in turn triggers disassembly from the filament termini²⁸. As a result of the ATP-driven end-disassembly, the stability of the MDA5 filament increases with the dsRNA length, in a manner that recapitulates the dsRNA length-dependent signaling activity of MDA5 in cells¹⁶.

How does the filament assembly of MDA5 contribute to its signaling activity? Several lines of evidence support that the filament formation of the RNA-binding domain brings 2CARD of nearby MDA5 molecules (MDA5^{2CARD}) into close proximity and promotes their oligomerization²⁵ (Fig. 1C). The MDA5^{2CARD} oligomer can then activate MAVS by inducing its own filament formation (to be discussed more in the next section). However, our understanding of MDA5 signaling remains largely incomplete. Studies showed that additional factors, such as K63-linked polyubiquitin (K63-Ub_n)³⁰ and dephosphorylation of MDA5^{2CARD} by PP1α/γ³¹, play a role in MDA5 activation, and it is unclear how these factors interplay with proximity-induced oligomerization of MDA5^{2CARD}. In addition, the current structural models of the MDA5^{2CARD} oligomer and its activation of MAVS are derived from that of RIG-I (to be discussed below), and they remain to be validated.

dsRNA-triggered receptor oligomerization of RIG-I

As with MDA5, RIG-I also requires oligomerization (*i.e.* tetramerization) of 2CARD, in order to activate MAVS. RIG-I appears to achieve it through at least two non-mutually exclusive mechanisms: *via* filament formation of the helicase-CTD on relatively long dsRNA and *via* binding to K63-linked polyubiquitin chains (K63-Ub_n) (Fig. 1D).

Filament formation of RIG-I involves a mechanism distinct from MDA5^{32, 33}. Unlike MDA5, RIG-I binds the dsRNA end with 5'ppp (or 5'pp) in a manner independent of ATP. During ATP hydrolysis, RIG-I translocates from the dsRNA end to the interior¹², and accumulates near the dsRNA end forming helical oligomers that closely resemble short segments of the MDA5 filament³² (Figs. 1B & 1D). It is yet unclear how the translocation leads to oligomerization and what prevents the filament from propagating further. Regardless of the detailed mechanism, filament formation of the RNA-binding domain of RIG-I (as with MDA5) promotes proximity-induced tetramerization of RIG-I^{2CARD} that is competent to activate MAVS³². As each RIG-I occupies ~10 bp, ~40 bp is the minimum length for tetramerization of RIG-I^{2CARD} on dsRNA in the absence of K63-Ub_n³².

Unlike MDA5, filament formation on dsRNA is not the absolute requirement for RIG-I signaling. When stimulated with shorter (*e.g.* <~40 bp) dsRNA or with isolated RIG-I^{2CARD}, RIG-I utilizes K63-Ub_n to oligomerize RIG-I^{2CARD} relies on the cofactor, K63-Ub_n^{30, 32, 34}. RIG-I^{2CARD} is known to be covalently conjugated with K63-Ub_n³⁵ and at the same time can bind unanchored K63-Ub_n³⁴. The combination of the structural and biochemical studies showed that K63-Ub_n bridges between adjacent RIG-I^{2CARD} domains through non-covalent interaction, and promote its tetramerization by wrapping around the periphery of the RIG-I^{2CARD} tetramer³⁶ (Fig. 1D). While the covalent conjugation of K63-Ub_n is not absolutely required, it markedly enhances the tetramerization efficiency and MAVS stimulatory activity of RIG-I^{2CARD}, as expected from the conversion of the intermolecular interaction to an intramolecular interaction³⁶.

It is important to note that the two mechanisms described above (*i.e.* filament-induced and K63-Ub_n-mediated mechanisms) are not mutually exclusive, and that they instead cooperate for stable tetramerization of RIG-I^{2CARD}^{32, 36} (Fig. 1D). When stimulated by longer dsRNA (> 40 bp), the requirement for Ub conjugation or Ub binding decreases, consistent with the notion that filament formation on dsRNA compensates for the loss of K63-Ub_n. In further support of the importance of the synergism, ~10 bp dsRNA that can accommodate only a single RIG-I molecule is generally a poor ligand for RIG-I^{20, 37}, although conflicting reports exist³⁸. We suspect that this discrepancy largely stems from the different expression level of RIG-I used in different experimental settings, where a high concentration of RIG-I could potentially compensate for the lack of RNA-mediated RIG-I oligomerization. The contribution of RNA-mediated oligomerization is clearly seen from the observation that RIG-I is better stimulated by longer dsRNA (up to ~500 bp) when compared at the equivalent molar concentration, *i.e.* with same number of 5'ppp end^{33, 39}. The plateau around ~500 bp, which is significantly earlier than the plateau observed with MDA5, likely reflects the inefficient propagation of the RIG-I filament along dsRNA³². Why then is RIG-I thought to be poorly stimulated by long dsRNA? One should keep in mind that this notion is true only when the comparison is made using the equivalent mass concentration of RNA¹⁶, in which case longer dsRNA samples would have fewer 5'ppp and thus would be limited in recruiting RIG-I to dsRNA³². All in all, given the requirement for the oligomerization of RIG-I^{2CARD} for its signaling activity, it is not surprising to see the positive impact (but non-obligatory role) of RIG-I filament formation on its signaling.

Receptor-triggered oligomerization of MAVS

MAVS contains a single N-terminal CARD, the central linker of ~400 amino acid and the C-terminal transmembrane (TM) domain (Fig. 1A). TM anchors MAVS to the outer membrane of mitochondria, peroxisomes and mitochondria-associated membranes^{40–45}. Studies showed that RIG-I and MDA5 activate MAVS by inducing its filament formation on mitochondrial surface⁴⁶ (Fig. 2). The MAVS CARD (MAVS^{CARD}) is both necessary and sufficient to interact with 2CARD of RIG-I and MDA5 and to form filament in response to these interactions⁴⁶. The MAVS^{CARD} filament can self-propagate *in vitro*, which led to the notion of prion-like filament. However, the MAVS^{CARD} filament differs from amyloid filaments in that it lacks cross-beta structures^{46, 47}, and does not involve conformational change of the protomer^{47, 48}.

How do the RIG-I/MDA5^{2CARD} oligomers (tetramer for RIG-I) induce MAVS^{CARD} filament formation? CARD belongs to the Death Domain superfamily, members of which often form homotypic oligomeric structures. While their oligomerization often involves common molecular interfaces (that are conserved in location but not in sequence), their oligomeric structures vary and provide key clues to their functions⁷. This was also the case for RIG-I and MAVS. That is, the three oligomeric structures of RIG-I^{2CARD} and MAVS^{CARD} showed how RIG-I^{2CARD} tetramer induces MAVS^{CARD} filament formation^{36, 47}. First, the structure of the RIG-I^{2CARD} tetramer (in complex with K63-Ub₂) showed that the tetramer forms a helical assembly³⁶. There is no conformational change in the protomer compared to the auto-repressed, monomeric state⁴⁹. However, the protomer repeats itself in a manner that the four 1st CARDS of RIG-I forms the first helical turn,

which is extended by the four 2nd CARD domains³⁶ (Fig. 2B). This helical architecture of RIG-I^{2CARD} differs from those of other members of Death Domain superfamily⁷. Interestingly the structure of the MAVS^{CARD} filament displayed a matching helical symmetry⁴⁷, suggesting that the RIG-I^{2CARD} tetramer may serve as the template to nucleate the MAVS^{CARD} filament (Fig. 2B). In further support of this model, the structure of the RIG-I^{2CARD} tetramer in complex with the first helical turn of MAVS^{CARD} showed that MAVS^{CARD} is recruited to the top of the RIG-I^{2CARD} tetramer (on the 2nd CARD side) in a manner that extends the helical trajectory pre-defined by the RIG-I^{2CARD} tetramer⁴⁷ (Fig. 2B). While the crystallization of the RIG-I^{2CARD}: MAVS^{CARD} complex inevitably required protein engineering to prevent the propagation of the MAVS^{CARD} filament, functional analyses supports the validity of the structure⁴⁷. The protein-engineering scheme used for this structure also provides a generalizable strategy to crystallize nucleator-filament complex.

The filamentous MAVS activates the downstream signaling pathways by recruiting the E3 ubiquitin ligases TRAF2, 5 & 6, which in turn activate cytosolic kinases, TBK1 and IKK, and subsequently the transcription factors, IRF3/7 and NF- κ B^{46, 50}. The TRAF-binding sites reside within the linker that tethers MAVS^{CARD} to the outer membrane of mitochondria (Figs. 1A & 2A). The mechanism by which TRAFs bind to filamentous, but not monomeric MAVS, is yet unclear. Previous studies showed that the trimeric TRAF prefers pre-oligomerized substrate peptides (through the avidity effect) as binding of individual peptide is inefficient⁵¹. This observation can explain why filamentous MAVS, not monomeric MAVS, can recruit TRAFs. Recently, MAVS was shown to directly facilitate phosphorylation of IRF3 by TBK1 by recruiting both molecules to MAVS and by placing them in close proximity⁵². Accordingly, filamentous architecture of MAVS could play an additional role by further amplifying the clustering effect of TBK1 and IRF3. Furthermore, a recent study proposed a model where MAVS filament formation releases an auto-inhibition exerted by the *cis* inhibitory elements⁵³. Although the precise nature of the auto-inhibition and the mechanism by which MAVS filament assembly releases the inhibition remain to be investigated, filament formation of MAVS appears to act at multiple steps for the signal activation.

Production of type I interferons by RIG-I/MDA5 is transient and this transiency is important to avoid chronic inflammation. As such, the MAVS filament must be resolved shortly after the activation of the downstream signaling pathways. In keeping with this, the level of MAVS was shown to decrease shortly after its activation through several distinct mechanisms. This includes Atg5-mediated autophagy of mitochondria (mitophagy)⁵⁴, ubiquitin-mediated proteosomal degradation of MAVS (through the actions of E3 ligases, Smurf2⁵⁵, March5⁵⁶ and pVHL⁵⁷). Other more complex regulatory mechanisms involving miniMAVS⁵⁸, Trim25⁵⁹ and IRTKS⁶⁰ were also proposed. The relative importance of each of these regulatory mechanisms and their relationships remain to be further investigated. It would be also important to understand how the actions of these regulatory elements are restricted to post-activation (*i.e.* filamentous MAVS) as opposed to pre-activation (*i.e.* monomeric MAVS).

AIM2-like Receptors (ALRs): AIM2 and IFI16

AIM2 and IFI16 recognize dsDNA from invading viruses and bacteria (e.g. HIV, HSV-1, and *F. tularensis*) and activate a series of inflammatory responses^{61–72}. AIM2 detects dsDNA in the cytoplasm and assembles into a filamentous oligomer, which then triggers the polymerization of the signaling adaptor, ASC^{69, 73–75}. The ASC filament in turn recruits and activates caspase-1 (converting the latent pro-caspase-1 to the active caspase-1), which processes the latent form of pro-inflammatory cytokine interleukin-1 β (IL-1 β) and induces pyroptosis (Fig. 3A). This ternary complex (receptor•ASC•pro-caspase-1) is also dubbed the inflammasome (Fig. 3A)^{69, 73–75}. Unlike AIM2, IFI16 operates in both the nucleus and cytoplasm^{62–66, 76–79}, and was initially reported to stimulate IFN production independent of ASC and pro-caspase-1^{67, 69, 74}. More recent studies, however, reported that IFI16 can also interact with ASC and pro-caspase-1, forming an IFI16 inflammasome and processing pro-IL-1 β similarly to the AIM2 inflammasome^{61, 64, 66, 80}. The IFI16 and AIM2 inflammasomes appear to be mutually exclusive and selectively assembled in response to different pathogenic infections. For instance, the AIM2 inflammasome assembles upon both cytosolic bacterial and viral infections (e.g. vaccinia virus and *F. tularensis*)^{69, 73–75}, whereas the IFI16 inflammasome is only found in response to nuclear viruses (e.g. HSV and KSHV) and certain retroviruses such as HIV^{61, 64, 66}.

Both AIM2 and IFI16 contain an N-terminal pyrin domain (PYD), which is important for activation of ASC (Fig. 3A, box). AIM2 contains one dsDNA binding HIN domains (HIN: hematopoietic interferon inducible nuclear antigen), whereas IFI16 has two tandem HIN domains (Fig. 3A, box); the HIN domains are composed of two oligo-binding folds and nonspecifically bind dsDNA. As with CARD of RLRs, PYD belongs to the Death Domain superfamily and functions as the oligomerization and signaling domain. Both AIM2 and IFI16 bind dsDNA in a sequence-independent manner, and require at least ~70-bp dsDNA for robust signaling^{67, 81}. It is noteworthy that the host cytoplasm is normally free of dsDNA, which in turn marks any invading pathogen dsDNA to be targeted as “nonself” by both AIM2 and IFI16; however, nuclear IFI16 must distinguish between host and viral dsDNA through a mechanism to be described below.

dsDNA-triggered receptor oligomerization of IFI16

Upon invasion of viral dsDNA in the nucleus (e.g. HSV and KSHV), individual IFI16 molecules rapidly locate one another on large viral genomes and assemble into filaments^{61, 64, 66}, while leaving the host dsDNA intact. It has been hypothesized that the chromatinization plays a key role in preventing the host DNA from being targeted, while invading viral DNA is relatively “naked” and thus susceptible to recognition by IFI16^{62, 64, 66, 77, 82}. Indeed, Knipe and colleague have observed that transfected plasmid is more readily recognized by IFI16 than intact virions that contain additional dsDNA binding proteins^{62, 82}. Studies showed that a “naked” stretch of dsDNA needs to be ~60–70 bp to allow stable formation of the IFI16 filament^{83, 84}. However, longer DNA increases the assembly rate^{83, 84}, which is likely important for facilitating foreign DNA recognition while suppressing the inadvertent assembly of the IFI16 filament on the host DNA.

How does IFI16 assemble the filament so that its assembly rate increases with the DNA length? Unlike RLRs where the dsRNA-binding domains (helicase-CTD) largely drive receptor oligomerization^{26, 27}, the HIN domains of IFI16 one dimensionally tracks dsDNA without forming a stably bound complex^{83, 84}. However, the tracking ability of the HIN domains allows multiple IFI16 to dynamically associate on dsDNA and cluster into filaments via PYD-PYD interactions^{83, 84}. Consequently, not only does IFI16 bind longer dsDNA more stably⁸⁴, but it also assembles filaments faster on the longer duplexes^{83, 84}. Importantly, we found that nucleosomes directly block these one-dimensional movements, thereby preventing IFI16 from clustering⁸³. This tracking-assisted oligomerization mechanism would then allow IFI16 to distinguish friend from foe and assemble into signaling platforms efficiently and selectively on foreign DNA.

dsDNA-mediated oligomerization of AIM2

As with IFI16, AIM2 assembles into filaments on dsDNA^{69, 73–75} (Figs. 3A step 1 and 3B-b), and its filament formation and signal activation are dependent on the oligomerization of the PYD (AIM2^{PYD})⁸⁵. Despite the apparent similarity in the domain composition and overall filament architecture, AIM2 and IFI16 display different oligomerization properties, which may be linked to their distinct functions. While AIM2 forms the filament more efficiently with DNA, it can also self-oligomerize at high protein concentrations in the absence of DNA (Fig. 3B-c)⁸⁵. This differs from IFI16, for which DNA-independent filament formation has been rarely observed even at high protein concentrations⁸⁴. More detailed studies showed that unlike IFI16, the HIN of AIM2 can oligomerize in the absence of DNA, and that full-length AIM2 can self-oligomerize better than either of isolated PYD or HIN domain without DNA⁸⁵. These observations suggest that AIM2 does not utilize auto-repression as previously proposed^{81, 86}, and its cellular regulation is mediated by other mechanisms. The concentration-dependent and DNA-independent oligomerization property of AIM2 also raises the possibility that the auto-inflammatory disorders associated with high level of AIM2⁸⁷ could be caused by its spontaneous filament assembly rather than by detection of certain cytosolic DNA. Interestingly, mouse encodes p202⁸⁸, which consists of two HIN domains and inhibits the oligomerization of AIM2 by HIN•HIN interactions. Currently, there is no known ortholog of p202 in human⁸⁹. Considering that all HIN domains share high structural homology (RMSD < 2 Å)⁸⁹, the difference in oligomerization activity of the HINs of IFI16 and AIM2 suggests that the dsDNA-binding and oligomerization activities of nuclear and cytosolic ALRs have been tuned differently by amino acid variations in key areas (the sequence similarity between the HIN domains of AIM2 and IFI16 is less than 50%). Future investigation of whether the DNA-tracking property is unique to IFI16 HINs or shared with AIM2 would further provide additional insights into the functional versatility of HINs.

For both AIM2 and IFI16, the precise architecture of the filaments on dsDNA remains to be further investigated. For example, it is unclear whether the HIN domains are arranged in a helical manner on dsDNA, and whether the PYD filaments extend parallel to or co-axially with DNA. Regardless, it is noteworthy that this PYD-driven oligomerization is distinct from RLRs, of which receptor oligomerization is mainly driven by the RNA binding domain (helicase-CTD), and can also occur without 2CARD.

Receptor-induced oligomerization of ASC

How does the oligomerized ALR PYDs activate ASC, and how does the activated ASC in turn promote cleavage of pro-caspase-1 to form a mature caspase-1? Studies suggested that a series of step-wise filament assembly underlie the signal propagation from ALR to ASC (Fig. 3A step 2), and to caspase-1 (Fig. 3A step 3)^{90,91}. ASC consists of an N-terminal PYD and a C-terminal CARD, whereas pro-caspase-1 consists of the N-terminal CARD and C-terminal caspase domain (Fig. 3A). The AIM2^{PYD} filament activates ASC by nucleating the filament formation of PYD of ASC (ASC^{PYD}) (Fig. 3A step 2). In this filamentous architecture of ASC, CARD of ASC (ASC^{CARD}) in turn recruits pro-caspase-1 through the ASC^{CARD}:pro-caspase-1^{CARD} interaction and nucleates the filament assembly of pro-caspase-1 (Fig. 3A step 3). Filament assembly of pro-caspase-1 then brings together the caspase domains in close proximity, thereby promoting its dimerization and activation, likely resulting in the release of active caspase-1 from the inflammasome.

Structure of the filament assembled by the AIM2^{PYD} and ASC^{PYD} showed that both filaments display the same helical symmetry (~9 nm wide, six start helix with C3 symmetry)⁸⁵. This is in line with the model that AIM2^{PYD} oligomer serves as the helical template that nucleates the ASC^{PYD} filament (step 1), and is analogous to the mechanism by which the RIG-I^{2CARD} tetramer nucleates the MAVS^{CARD} filament. By contrast, the mechanism by which ASC^{CARD} initiates the pro-caspase-1 filament formation (in step 2) is less clear. While it is tempting to suggest that AIM2^{CARD} forms a helical template to nucleate pro-caspase-1^{CARD}, oligomeric or filamentous structure of ASC^{CARD} or pro-caspase-1^{CARD} is currently unavailable to demonstrate the helical template mechanism. It is also unclear whether the signal propagation mechanism shown with AIM2 is applicable to IFI16 and other receptors that regulate the polymerization of ASC, which remains as a topic of future investigation.

The regulatory mechanisms of the ALR/ASC-signaling pathway also require further studies. In particular, how spontaneous filament assembly of ASC is suppressed in the absence of infection, and how the filament is resolved after its activation to prevent chronic inflammation. It was initially thought that ASC is autosuppressed by having an intramolecular interaction between PYD and CARD, thereby avoiding spontaneous filament formation and activation of caspase-1. However, the NMR studies of full-length ASC suggested that the PYD and CARD of ASC do not interact with each other under the experimental condition⁹². It is possible that the maintenance of ASC at low concentration and having multiple steps of oligomerization to assemble the fully activated inflammasome may together provide a sufficient blockade for aberrant activation of the inflammatory response. Nevertheless, it is also likely that there could be a yet uncharacterized *trans* regulatory elements or post-translational modification that suppress ASC's activity in the absence of infection. Indeed, recent studies suggest that IL-1 β and IFN inducible PYD-only proteins (POPs) can suppress the oligomerization of ALRs and ASC^{93,94}, providing a negative feedback mechanism to prevent persistent inflammatory responses (e.g. POP3 inhibits the oligomerization of both AIM2 and IFI16, and POP1 inhibits ASC). Nonetheless, the detailed mode of actions for POPs and the mechanism by which the assembled inflammasome is resolved to prevent chronic inflammation needs future investigation.

Concluding remarks

Helical assemblies are ubiquitous in biology, ranging from structural elements (viral capsids and cytoskeletal actins) to DNA repair and recombination machineries (RecA and Rad51). It was only recently realized that helical architectures are employed in a number of innate immune signaling pathways, either during foreign nucleic acid sensing or during signal propagation. While cellular imaging of these molecules often reveals punctated or aggregated structures, rather than filaments that resemble cytoskeletons, the shape of the aggregate at the macroscale does not necessarily inform about the structure of the molecular assemblies at the microscale. Our observations so far suggest that filamentous molecules can often tangle and aggregate, and that the filament propagation can be also limited in cells, both of which can lead to the appearance of non-linearity in cellular imaging. If so, how can one be confident about the *in vivo* relevance of filaments observed *in vitro*? High resolution structures of the filaments have enabled specific mutational studies, which in turn have revealed strong correlations among filament formation *in vitro*, puncta (or aggregate) formation in cells and the cellular signaling activity of many of these molecules^{47, 63, 90, 91, 95}. More importantly, *in vitro* reconstitution of some of these systems allowed direct examination of the importance of the filament assemblies in their signaling activities^{25, 34, 46}. We expect that recent advances in cellular imaging techniques (*e.g.* cryo-tomography⁹⁶ and super-resolution fluorescence microscopy⁹⁷) would further help defining detailed assembly architecture of these large “aggregate” in cells.

Why are these helical assemblies so common in innate immune signaling pathways? Could there be a rationale for their frequent occurrence? Helical assembly formed by the nucleic acid sensors is understandably common as it partly reflects the helical symmetry of the bound nucleic acid (*i.e.* dsRNA and dsDNA), and allows these sensors to measure the length of the naked nucleic acids, a common criterion for self vs non-self discrimination. Helical assembly by the signaling domains, such as CARDs and PYDs of RLRs, ALRs and their signaling adaptors, are also common despite the fact that they do not directly contact the nucleic acids. These assemblies contribute to rapid signal amplification by allowing a small number of upstream molecules to induce oligomerization of a large number of downstream molecules. Although there are foreign nucleic acid sensors, such as OAS1⁹⁸ and cGAS⁹⁹, that are currently not known form helical oligomers, our observations with RLRs and ALRs represent a growing number of receptors where helical assembly is utilized for foreign nucleic acid detection and signal propagation.

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Highlights

- RIG-I and MDA5 assemble into filamentous oligomers along viral dsRNA.
- They then form the helical oligomers of 2CARD to nucleate the MAVS filament.
- IFI16 and AIM2 assemble into filamentous oligomers on bacterial and viral dsDNA.
- The AIM2 filament provides a structural template for assembling the ASC filament.

Fig. 1. dsRNA-dependent oligomerization of RIG-I and MDA5

(A) Domain organization of RIG-I, MDA5 and MAVS. CTD refers to the C-terminal domain. TBS and TM refer to the TRAF-binding site and transmembrane domain, respectively.

(B) The electron micrographs of MDA5 filament and RIG-I filament formed on 112 bp dsRNA. The images were adapted from ²⁶ and ³².

(C) A schematic of the filament assembly process of MDA5. The MDA5 filament nucleates on the dsRNA interior and unidirectionally propagates to the dsRNA end. Filament formation brings 2CARDS of nearby MDA5 molecules into close proximity and promotes 2CARD oligomerization, which is a pre-requisite for activation of MAVS.

(D) Schematics of the filament- and ubiquitin-dependent oligomerization of RIG-I 2CARD. (I) On > 40 bp dsRNA, RIG-I forms a signaling-competent filament that assembles from the dsRNA end and propagates to the dsRNA interior. Within this filament, 2CARD oligomerization can occur independent of K63-Ub_n through the proximity-induced mechanism as with MDA5. (II) On short dsRNA (<20 bp), only a single RIG-I molecule can bind per RNA molecule, and thus 2CARD oligomerization exclusively depends on K63-

Ub_n. (III) In general, the two mechanisms synergize to obtain stable tetramerization of 2CARD and robust signal activation.

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Fig. 2. Oligomerization of receptors and adaptor during RIG-I & MDA5 signal activation

(A) Three steps of oligomerization involved during RNA recognition and signaling by RIG-I and MDA5. First, the RNA-binding domains (helicase-CTD) of RIG-I and MDA5 oligomerize on dsRNA (as shown in Fig. 1). Second, the signaling domain (2CARD) of RIG-I/MDA5 oligomerizes through proximity-induced and/or K63-Ub_n-mediated mechanisms. Finally, the oligomerized 2CARD nucleates the MAVS filament through the interaction between RIG-I^{2CARD} and MAVS^{CARD}. The filamentous MAVS then activates the downstream signaling pathway by recruiting TRAF molecules to TRAF-binding sites (TBSs) that are clustered on the surface of mitochondria. The image was modified from Ref. ⁴⁷.

(B) A composite structure obtained by overlaying the structures of the helical tetramer of the RIG-I^{2CARD}:MAVS^{CARD} complex and the MAVS^{CARD} filament. These structures were reported in Ref. ⁴⁷ and the image was modified from this paper.

Fig. 3. Activation of the ALR inflammasome pathway

(A). The stepwise assembly of ALR inflammasomes. Step 1: ALRs assemble into receptor filaments on foreign dsDNA. Step 2: the PYD oligomers of ALRs induce the polymerization of ASC^{PYD} by providing a structural template, which in turn induces the polymerization of ASC^{CARD} . Finally, step 3, ASC^{CARD} promotes the polymerization of procaspase-1, consequently activating the protease. The box shows the domain organization of ALRs. The figure is modified from Ref. ⁸⁵.

(B) Electron micrographs of ALR filaments. The images were adapted from Refs. ⁸⁴ and ⁸⁵.

(C) The average power spectra generated from negatively stained electron micrographs of the $AIM2^{PYD}$ and ASC^{PYD} filaments. The power spectrum was generated by Fourier transformation of the filament images, and it contains the full information of the helical symmetry of the filament. That is, each layer line represents periodicity of a family of helical lattice lines, and the n value refer to the Bessel order of the layer line. The figure is adapted from Ref. ⁸⁵.