The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways

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Highlights

- Pattern recognition receptors (PRRs) of the mammalian innate immune system mediate the first line of defense against pathogens and danger signals
- PRRs signal via oligomeric signaling complexes that assemble via co-operative assembly mechanisms
- Signaling by co-operative assembly formation (SCAF) allows PRRs to respond rapidly and amplify the response to a low level of stimulus
- The molecular mechanisms of SCAF by NLR, PYHIN family, TLR and RIG-I receptors are reviewed
- Conservation of SCAF in plants and fungi is discussed

Keywords

higher-order assembly signaling, inflammasome, nucleotide binding and oligomerization domain, leucine-rich repeat-containing/nucleotide and oligomerization domain-like receptor (NLR), RIG-1-like receptor (RLR), signaling by co-operative assembly formation (SCAF), Toll-like receptor (TLR)

Abbreviations

CARD, caspase recruitment domain; CC, coiled-coil; DAMP, danger-associated molecular pattern; DD, death domain; DED, death effector domain; NBD, nucleotide-binding domain; NLR, nucleotide binding and oligomerization domain (NOD), leucine-rich repeat (LRR)-containing/nucleotide and oligomerization domain-like receptor; OB, oligonucleotide/oligosaccharide binding; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptors; PYD, pyrin domain; RLR, RIG-1-

like receptor; SCAF, signaling by co-operative assembly formation; TIR domain, Toll/interleukin-1 receptor domain; TLR, Toll-like receptor

ABSTRACT

The innate immune system is the first line of defense against infection and responses are initiated by pattern recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs). PRRs also detect endogenous danger-associated molecular patterns (DAMPs) that are released by damaged or dying cells. The major PRRs include the Toll-like receptor (TLR) family members, the nucleotide binding and oligomerization domain, leucine-rich repeat containing (NLR) family, the PYHIN (ALR) family, the RIG-1-like receptors (RLRs), C-type lectin receptors (CLRs) and the oligoadenylate synthase (OAS)-like receptors and the related protein cyclic GMP-AMP synthase (cGAS). The different PRRs activate specific signaling pathways to collectively elicit responses including the induction of cytokine expression, processing of pro-inflammatory cytokines and cell-death responses. These responses control a pathogenic infection, initiate tissue repair and stimulate the adaptive immune system. A central theme of many innate immune signaling pathways is the clustering of activated PRRs followed by sequential recruitment and oligomerization of adaptors and downstream effector enzymes, to form higher-order arrangements that amplify the response and provide a scaffold for proximity-induced activation of the effector enzymes. Underlying the formation of these complexes are co-operative assembly mechanisms, whereby association of preceding components increases the affinity for downstream components. This ensures a rapid immune response to a low-level stimulus. Structural and biochemical studies have given key insights into the assembly of these complexes. Here we review the current understanding of assembly of immune signaling complexes, including inflammasomes initiated by NLR and PYHIN receptors, the myddosomes initiated by TLRs, and the MAVS CARD filament initiated by RIG-1. We highlight the co-operative assembly mechanisms during assembly of each of these complexes.

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1. Introduction

The innate immune system has evolved a number of pattern recognition receptors (PRRs) that sense the presence of pathogens via recognition of conserved pathogen-associated molecular patterns (PAMPs). PRRs also respond to endogenous molecules released from damaged or dying cells, which are referred to as danger-associated molecular patterns (DAMPs). Major families of PRRs include the nucleotide binding and oligomerization domain, leucine-rich repeat (LRR) containing (NLR) family (also referred to as the nucleotide binding and oligomerization domain (NOD)-like receptor family), the Toll-like receptor (TLR) family of transmembrane receptors, the PYHIN (pyrin and HIN200 domain-containing) family, the RIG-I-like receptor (RLR) family, the C-type lectin receptors (CLRs) and oligoadenylate synthase (OAS) proteins and the related protein cyclic GMP-AMP (cGAMP) synthase (cGAS). Upon activation, these PRRs initiate signaling pathways to induce immediate host defense mechanisms and also promote the adaptive immune response (Pandey et al., 2015).

Signaling via assembly of multi-protein complexes that enable proximity-induced activation of effector enzymes has emerged as a mode of signaling by many PRRs (Hauenstein et al., 2015; Lu and Wu, 2015; Wu, 2013; Yin et al., 2015). Activated PRRs cluster to recruit and nucleate the oligomerization of adaptor proteins, which in turn recruit and nucleate oligomerization of effector enzymes. Recent structural and biochemical studies of PRRs and components of their signaling complexes have revealed co-operative assembly mechanisms during their oligomerization (Lin et al., 2010; Lu et al., 2016; Lu et al., 2014b; Xu et al., 2014). In most cases, oligomerization of preceding components presents multiple interaction sites to downstream proteins to mediate co-operative assembly. Co-operative assembly can also be mediated by induction of a conformational change in a protein that facilitates interaction with downstream components. We refer to this mode of signaling as **“signaling via co-operative assembly formation”** (SCAF). SCAF provides a scaffold for proximity-induced activation of downstream effector enzymes and also enables PRRs to respond rapidly to low levels of DAMPs and PAMPs. Here we highlight the mechanism of SCAF as revealed from structures of components of the inflammasome initiated by NLR and PYHIN proteins, the MAVS CARD filament initiated by RIG-I, and the myddosome initiated by TLR4.

2. Co-operative assembly formation in the inflammasome signaling pathway

2.1 The inflammasome is a filamentous network

Inflammasomes are cytosolic signaling complexes that were originally shown to recruit and activate procaspase-1, which processes the proinflammatory cytokines, interleukin-1 β (IL-1 β) and IL-18, and also cleaves gasdermin D to mediate pyroptotic cell death (Martinon et al., 2002; Rathinam and Fitzgerald, 2016; Shi et al., 2015). However, more recent studies have shown that inflammasomes also recruit and activate procaspase-8 to mediate apoptotic cell death and pro-IL-1 β processing (Antonopoulos et al., 2015; Chung et al., 2016; Martin et al., 2016; Pierini et al., 2012; Sagulenko et al., 2013). Deregulated inflammasome activation contributes to the pathology of many common diseases (Guo et al., 2015) and thus all aspects of inflammasome biology, including its activation and mechanism of assembly, are of interest. Canonical inflammasomes generally consist of a PRR, the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment

domain) and the downstream effectors enzymes caspases-1 and -8. Notably, most components of inflammasomes have death-fold domains, which generally interact homotypically to form helical filaments using conserved interaction modes (Hauenstein et al., 2015; Kersse et al., 2011; Lu and Wu, 2015). Inflammasome assembly (Fig. 1) involves ligand-induced activation and oligomerization of the PRR, which subsequently nucleates filaments of the adaptor ASC, a major component of inflammasomes (Fernandes-Alnemri et al., 2007; Lu et al., 2014b). ASC filaments in turn nucleate filaments of procaspases-1 and -8 (Lu et al., 2016; Lu et al., 2014b; Vajjhala et al., 2015). The filamentous nature of the inflammasome is visible by electron microscopy of isolated inflammasomes (Franklin et al., 2014). Assembly into filaments amplifies the response (Dick et al., 2016) and provides a scaffold for the proximity-induced activation of the caspases. The entire cascade of events involves multiple steps of co-operative assembly. We review the mechanisms of activation of the AIM2 and NAIP2/NLRC4 inflammasomes, which are currently the best-characterized structurally, and highlight the mechanisms of co-operative assembly.

2.2 Inflammasome-initiating PRRs oligomerize via co-operative assembly mechanisms

Inflammasomes are initiated upon activation of structurally diverse PRRs including certain NLR family members, the PYHIN family protein AIM2, and the TRIM family protein pyrin (Rathinam and Fitzgerald, 2016). Although the structures of these protein families are quite diverse, they all have a domain that mediates the oligomerization of the PRR. In the NLR family proteins, the NOD mediates oligomerization (Hu et al., 2015; Zhang et al., 2015), while the PYHIN protein AIM2 has a HIN domain that binds to and oligomerizes along double-stranded (ds) DNA (Lu et al., 2015; Morrone et al., 2015). The pyrin inflammasome is less well-characterized, however, a coiled-coil (CC) domain is proposed to mediate its oligomerization (Yu et al., 2006). Another common feature of most inflammasome-initiating PRR complexes is the presence of a death-fold domain, either a pyrin domain (PYD) or a caspase recruitment domain (CARD) (Sharma and Kanneganti, 2016).

2.2.1 The PYHIN family member AIM2

The PYHIN family proteins all have an N-terminal PYD-type death-fold domain and one or two copies of a HIN domain, each of which consists of two oligonucleotide/oligosaccharide binding (OB) folds (Fig. 2A). Members of the human family include AIM2 (absent in melanoma), IFI16, IFIX and MNDA (Cridland et al., 2012). AIM2 is the only member with a well-characterized role in inflammasome activation. AIM2 has been proposed to be in an auto-inhibited state due to interaction between the PYD and HIN domains until it senses and binds to dsDNA via the HIN domain (Jin et al., 2013). However, more recent work disputes this auto-inhibitory mechanism (Morrone et al., 2015).

2.2.1.1 Oligomerization of the AIM2 HIN domain on dsDNA. Initial structural studies of the AIM2 HIN domain/dsDNA complex revealed the structure of the HIN domain and the molecular details of its interaction with DNA (Jin et al., 2012). The structure revealed that each HIN domain consists of two β -barrel sub-domains, each of which is characteristic of the OB fold (Arcus, 2002). The two OB folds are stacked one above the other along the DNA and both OB folds and the linker between them are involved in binding to both strands of the dsDNA (Fig. 2A). Basic residues in the concave surface formed by the HIN domain bind to the negatively charged DNA backbone. More recent EM studies

showed that the AIM2 HIN domains oligomerize along the length of dsDNA (Lu et al., 2015; Morrone et al., 2015). Modeling of the dsDNA/HIN polymer (Lu et al., 2015) using the crystal structure of the HIN domain/dsDNA complex as a template yielded a helical model whereby each HIN domain occupies 4 base-pairs (bp) of DNA and is surrounded by six adjacent HIN domains (Lu et al., 2015).

2.2.1.2 Clustering of the AIM2 PYD upon AIM2 oligomerization on DNA. As the N-terminus of the HIN domain is oriented away from the DNA in the crystal structure, the PYDs are expected to extend from the side of the HIN domain/dsDNA filament. The AIM2 PYD, similar to other death-fold domains, consists of six α -helices arranged in an anti-parallel arrangement (Fig. 2B) (Jin et al., 2013; Kersse et al., 2011). It has been shown to form helical filaments *in vitro* and the structure of the filament was proposed based on negative-stain EM and homology modeling, using the structure of the ASC PYD filament (Lu et al., 2015) as a template (Morrone et al., 2015). This suggested that like many other death-fold domains, the AIM2 PYD filament assembly uses six surfaces, to mediate three modes of interaction referred to as type I, II and III (Kersse et al., 2011; Park et al., 2007).

In vivo, it is likely that when full-length AIM2 binds to DNA, the PYDs extending from the side of the dsDNA/HIN domain cluster and assemble into a short helical segment (Lu et al., 2015; Morrone et al., 2015) (Fig. 2C). The length of the linker between the HIN and PYD is ~50 amino acids, and this restricts the number of PYDs able to contribute to this short filament, of currently undefined length.

2.2.1.3 Oligomerization of AIM2 on dsDNA involves multiple steps of co-operative assembly. Because each HIN domain contacts six adjacent HIN domains (Lu et al., 2015), its assembly into a filament on dsDNA is likely to be co-operative, as once it is initiated, subsequent AIM2 molecules can bind to the DNA as well as to multiple sites on the elongating HIN domain filament. This ensures a rapid response once the DNA is sensed. Furthermore, co-operative assembly allows compact packing of the HIN domains on DNA that may present an excess of possible binding sites, and consequently maximizes AIM2 PYD clustering, which is required for the recruitment of the adaptor protein ASC. In addition, the AIM2 PYD promotes binding of AIM2 to DNA, because full-length AIM2 binds dsDNA more efficiently than the HIN domain alone (Morrone et al., 2015). Although this appears to be in some contradiction to previous studies suggesting that AIM2 PYD-HIN interaction mutually inhibits binding of AIM2 HIN domain to DNA and clustering of the PYDs (Jin et al., 2013), it is possible that the PYD-HIN interaction keeps AIM2 in an auto-inhibited state, but that once the HIN domain binds to DNA, the PYDs cluster and stabilize the complex.

2.2.2 The NLR family

The central NOD of the NLRs comprises a nucleotide-binding domain (NBD), an adjacent helical domain (HD1) and a winged helical domain (WHD) (Fig. 3A). The NBD and HD1 are common to AAA+ family ATPases while the WHD is unique to the signal transduction ATPases with numerous domains (STAND) subfamily to which the NLRs belong (Danot et al., 2009; Leipe et al., 2004). A second helical domain (HD2), which is also referred to as an arm domain, links the NOD to the C-terminal LRR domain (Fig. 3A). The NOD of NLRs is also referred to as a NACHT domain named after NAIP, CIITA, HET-E and TP-1 proteins (Koonin and Aravind, 2000). Three types of NLRs are

associated with inflammasomes. NLRs with an N-terminal PYD are referred to as NLRP, NLRs with an N-terminal CARD are referred to as NLRC, and NLRs with an N-terminal BIR domain are referred to as NLRB or more commonly as NAIP (NLR family, apoptosis inhibitory protein) (Ting et al., 2008).

NLRP family members that are known to form inflammasomes include NLRP1 and 3. NLRP3 in particular has been shown to be activated by a wide range of stimuli, including ATP, potassium ionophores, and particulate material (Rathinam and Fitzgerald, 2016), and has been shown to be associated with the pathology of many common diseases. However, the precise mechanism of NLRP3 activation is not known. Although NLRP6, 7 and 12 are proposed to form inflammasomes, the evidence for these inflammasomes is less substantial. Negative-stain EM of NLRP1 has indicated the formation of an oligomeric ring (Faustin et al., 2007); however, there are no detailed structural studies of NLRP-type NLRs. NAIP and NLRC4 NLRs assemble together to initiate inflammasomes (Kofoed and Vance, 2011). The NAIPs are activated by ligand binding and then recruit NLRC4 to initiate inflammasome assembly. There is a single NAIP protein in humans and it is activated by flagellin (Kortmann et al., 2015) and by needle proteins of the type III secretion system (T3SS) (Yang et al., 2013), which transfers bacterial proteins into eukaryotic host cells. There are seven NAIP proteins in mouse. NAIP1 is suggested to function as the mouse homologue of human NAIP and is also activated by needle proteins of T3SS (Yang et al., 2013). Mouse NAIP2 recognizes the T3SS rod protein PrgJ while NAIP5 is activated by bacterial flagellin (Kofoed and Vance, 2011; Zhao et al., 2011). Recent structural studies have given key insights into the assembly of the NAIP2/NLRC4 and NAIP5/NLRC4 inflammasome initiator complexes (Diebold et al., 2015; Hu et al., 2015; Zhang et al., 2015).

2.2.2.1 Activation of NAIP2 and oligomerization with NLRC4 via the NOD. The only high-resolution structure of an NLR monomer is that of NLRC4 without the CARD (Hu et al., 2013) and it revealed that the C-terminal LRR domain is bent over to contact the NBD and maintain it in an auto-inhibited state (Fig. 3B). In addition, WHD and HD2 also interact with the NBD to auto-inhibit it. Interestingly, the NBD-associated domains of NAIP2 and NAIP5, which include HD1, WHD and HD2, are the critical determinants for binding to PrgJ and flagellin (Tenthorey et al., 2014). The activated NAIPs then recruit NLRC4 molecules to form an inflammasome initiation platform (Kofoed and Vance, 2011; Zhao et al., 2011). As there is no evidence that NLRC4 senses the PAMP, it may be regarded as an adaptor (Hauenstein et al., 2015), while NAIPs are the PRRs. However, NAIPs cannot initiate inflammasome assembly in the absence of NLRC4. Thus the two together form an inflammasome initiation platform.

Cryo-EM analysis of complexes with PrgJ/NAIP2 and NLRC4 lacking the CARD (NLRC4^{CARD}), indicated the formation of oligomeric rings with 10, 11 and 12-fold symmetry (Hu et al., 2015; Zhang et al., 2015). The NAIP and NLRC4 molecules were indistinguishable in these experiments due to their similar NOD and LRR domains. However, immunogold labeling indicated that each complex contained a single NAIP2 molecule (Hu et al., 2015). Furthermore, intermediates of the assembling ring could be detected by EM, consistent with sequential recruitment of NLRC4 molecules. Complexes with full-length NLRC4 formed double-ring structures, which are likely due to interactions mediated

between the CARDs, and these mainly had 11 and 12-fold symmetry. In both reports (Hu et al., 2015; Zhang et al., 2015), a complex of NLRC4^{ΔCARD} with 11-fold symmetry was selected for detailed analysis and this was stated to be very similar to the complex with 11-fold symmetry formed by full-length NLRC4 (Hu et al., 2015) (Fig. 3C).

The interactions between the monomers in the ring are mediated by both the NOD and the LRR domain, which form an inner and outer ring, respectively. A basic patch formed by the NOD of one monomer interacts with an acidic patch formed by the NOD of an adjacent monomer and provides the major interaction that mediates oligomerization. The LRR domains also interact via complementary charged surfaces. Notably, the conformation of the NLRC4 monomer in the oligomeric ring differs from that of the inactive monomer by a rotation of 87.5° in the HD1/WH1 region that shifts the HD2/LRR away from the NBD (Hu et al., 2015; Zhang et al., 2015) (Fig. 3B).

Cryo-electron tomography of flagellin-induced NAIP5/full-length NLRC4 complexes (Diebolder et al., 2015) also revealed the formation of a double ring structure similar to that of the PrgJ-induced NAIP2/full-length NLRC4 complex. However, this study also identified a few helical polymers of NAIP5/NLRC4. While this is intriguing, it is unclear whether the helical polymers will form in cells expressing endogenous levels of protein. This study also noted a rotation of the LRR-HD2 domains away from the NBD of NLRC4 in the NAIP5/NLRC4 oligomer (Diebolder et al., 2015), compared to the NLRC4 monomer (Hu et al., 2013).

2.2.2.2 Oligomerization of the NAIP2/NLRC4 complex is a co-operative assembly process. In an inactive NLRC4 monomer, the acidic surface of the NOD is available for interaction, while the basic surface is sterically hindered by the LRR domain (Hu et al., 2015; Zhang et al., 2015). Although the structure of NAIP2 has not been solved, a homology model suggests it is likely to be similarly auto-inhibited (Tenthorey et al., 2014). When NAIP2 interacts with PrgJ, it is proposed to undergo a conformational change that frees the basic surface formed by the NBD and WH1 and allows interaction with the acidic surface formed by NBD, HD1 and WH1 of an auto-inhibited NLRC4. Upon recruitment, the inactive NLRC4 is proposed to undergo a similar conformational change to the active conformation that allows it to use its basic surface on the NOD to recruit another NLRC4 molecule (Fig. 3D). This nucleated assembly is a co-operative mechanism, because the initial interaction of a NAIP/PrgJ complex with an NLRC4 molecule facilitates subsequent recruitment of NLRC4 molecules to form a ring. This mechanism allows a single PrgJ PAMP molecule to initiate an inflammasome and activate multiple effector caspases.

2.2.2.3 Clustering of the NLRC4 CARDs in the NAIP2/NLRC4 oligomer. Because the N-terminus of the NBD faces the core of the ring, this brings the N-terminal CARDs into close proximity (Fig 3E) (Zhang et al., 2015). The NLRC4 CARD fused to GFP forms a filament *in vitro* (Zhang et al., 2015), although the structure of this filament has not been determined. Based on this and by analogy to other death-fold domain filaments, the NLRC4 CARDs are predicted to form a short filament above the core of the oligomeric ring (Zhang et al., 2015). However, filament assembly will depend on the constraints imposed by the linker between the CARD and NOD.

2.3 The inflammasome adaptor ASC amplifies the signal and bridges the interaction of PRRs with effector caspases

The inflammasome adaptor ASC consists of an N-terminal PYD and a C-terminal CARD, with which it can be recruited to inflammasome initiators with PYDs or CARDS, respectively. The presence of a flexible linker between the two domains allows each domain to interact independently (de Alba, 2009) (Fig. 4A) and each domain has the ability to form filaments *in vitro* (Lu et al., 2014b; Sborgi et al., 2015; Schmidt et al., 2016) and upon overexpression *in vivo* (Masumoto et al., 2001).

2.3.1 Nucleation of ASC PYD filaments by PYD-containing PRRs

The structure of the ASC PYD filament was solved by cryo-EM (Lu et al., 2014b) and also by solid state NMR (Sborgi et al., 2015), revealing a triple helical filament (Fig. 4B), which exemplifies the use of the conserved death-fold interactions by six faces of each monomer. The type I interaction mediates the dominant interactions forming a single helical strand; a type Ia surface interacts with the type Ib surface of an adjacent domain in an individual helical strand. The type II and III interactions mediate interactions between the three strands of the triple helix. This process of filament formation is a co-operative assembly mechanism, because the molecules recruited downstream have multiple surfaces to bind to (Fig. 4C). Once initiated, the filaments can continue extending in a prion-like manner (Cai et al., 2014) and all the ASC in a cell can become polymerized within minutes (Cheng et al., 2010; Fernandes-Alnemri et al., 2007). ASC CARDS on the side of the ASC PYD filament are predicted to cluster using similar death-fold interactions (Lu et al., 2014b) (Fig. 4C). Because extension of the ASC PYD filament increases the capacity for caspase-1 recruitment and subsequent activation, continued ASC PYD polymerization would amplify the response.

AIM2 PYD can initiate formation of ASC PYD filaments *in vitro* and consistent with a “seeding” role, AIM2 PYDs are found at one end of the filament (Lu et al., 2014b). Thus *in vivo*, the AIM2 PYDs that extend from the side of the AIM2 HIN filament are predicted to nucleate radiating ASC PYD filaments (Fig. 5A). Although the mechanism of NLRP3 activation is unknown, a truncated NLRP3 construct with the NOD and PYD can also induce ASC PYD filaments (Lu et al., 2014b). Thus, NLRP-type PRRs are likely to oligomerize similarly to NLRC4 and allow clustering of the associated PYD and subsequently induce ASC PYD filaments. In agreement, AIM2 and NLRP3 activation can initiate ASC PYD filaments *in vivo* (Dick et al., 2016). Mutations of the type I, II and III surfaces of AIM2 and NLRP3 PYDs diminish their ability to induce ASC PYD filaments (Lu et al., 2014a). Furthermore, mutations of ASC PYD that diminish its self-association also diminish the interaction with AIM2 and NLRP3 PYDs (Vajjhala et al., 2015; Vajjhala et al., 2012). These data are consistent with the self-association surfaces being used for the interaction between the PYDs of the PRRs and ASC. Furthermore, the structural similarity between the AIM2 PYD and ASC PYD filaments (Morrone et al., 2015) is consistent with the short helical segment generated by AIM2 PYD forming a template that recruits and nucleates an ASC PYD filament using the same interaction surfaces used by each PYD to self-associate.

2.3.2 Nucleation of ASC PYD filaments by the NAIP/NLRC4 oligomer

ASC filaments can be detected upon activation of a NAIP/NLRC4 inflammasome (Schmidt et al., 2016). It is proposed that the clustered CARDs of a NAIP2/NLRC4 oligomer first recruit ASC molecules via CARD interactions and then the ASC PYDs cluster and nucleate an ASC PYD filament in a manner similar to nucleation by PYD-containing PRRs (Fig. 5B).

2.3.3 Condensation of ASC filaments by ASC CARD interactions to form a compact speck.

ASC CARDs are exposed on the ASC PYD filament (Fig. 4C) and have been shown to mediate the condensation of ASC filaments into a speck *in vivo* (Dick et al., 2016; Proell et al., 2013; Schmidt et al., 2016). Although ASC CARDs can form a filament *in vitro* and upon high-level expression *in vivo* (Morrone et al., 2015; Schmidt et al., 2016), the CARDs linked to the ASC PYD filament are likely to be restrained to form only a short helical segment *in vivo*. The ASC speck is visible by fluorescence microscopy (Broz et al., 2010; Sagulenko et al., 2013; Schmidt et al., 2016) and has become synonymous with inflammasome activation.

2.3.4 Nucleation of procaspase-1 CARD filaments by ASC CARDs.

ASC CARDs also recruit procaspase-1, which consists of an N-terminal CARD and a catalytic domain. Knowing that GFP-ASC CARDs can nucleate caspase-1 CARD filaments *in vitro* (Lu et al., 2014b), the clustered ASC CARDs are expected to nucleate procaspase-1 CARD filaments *in vivo* and allow proximity-induced dimerization and activation of procaspase-1 (Fig. 6). However, the specific orientation of caspase-1 required for its activation, and whether this is achieved within a single filament as depicted in Figure 6, has not been established. Activated caspase-1 can then process the pro-inflammatory cytokines proIL-1 β and proIL-18, which initiate inflammation (Rathinam and Fitzgerald, 2016), and gasdermin D, which induces pyroptosis (Kayagaki et al., 2015; Shi et al., 2015).

2.3.5 Nucleation of procaspase-1 CARD filaments by NLRC4 CARDs of the NAIP/NLRC4 oligomer.

The NAIP/NLRC4 oligomer as well as GFP-NLRC4 CARDs, which form a filament, can induce procaspase-1 CARD filaments *in vitro* (Zhang et al., 2015). This suggests direct recruitment and activation of procaspase-1 by the NAIP2/NLRC4 oligomer is possible *in vivo*. In some agreement, activation of NAIP/NLRC4 in the absence of ASC polymerization induces pyroptosis *in vivo* (Dick et al., 2016; Schmidt et al., 2016). However, proIL-1 β processing was greatly diminished. Furthermore, processing of procaspase-1 was not detected upon activation of NLRC4-dependent inflammasomes in the absence of ASC (Mariathasan et al., 2004). Thus, ASC is required for optimal activation and processing of procaspase-1 to mediate both proIL-1 β cleavage and pyroptosis and in its absence, procaspase-1 is not processed but is activated to a form that is only capable of mediating pyroptosis (Dick et al., 2016; Lage et al., 2014; Mariathasan et al., 2004).

2.3.6 Nucleation of procaspase-8 DED filaments by ASC PYD.

Caspase-8 recruitment and activation by death receptor signaling complexes is well established (Salvesen and Walsh, 2014) and the inflammasome is a newly recognized platform for caspase-8 activation (Man et al., 2013; Pierini et al., 2012; Sagulenko et al., 2013). Procaspase-8 consists of a C-terminal catalytic domain and two N-terminal death effector domains (DEDs), which also belong to the

death-fold family. Procaspase-8 DEDs form filaments with a similar helical symmetry to that of the ASC PYD filament (Fu et al., 2016). ASC PYD interacts with the procaspase-8 DEDs via an unusual non-homotypic interaction to nucleate procaspase-8 DED filaments that extend from the ASC speck (Sagulenko et al., 2013; Vajjhala et al., 2015) (Fig. 6). *In vitro*, ASC promotes polymerization of procaspase-8 DED filaments and is detected at one end of the procaspase-8 filament (Vajjhala et al., 2015). Thus, recruitment of procaspase-8 DEDs may terminate the ASC PYD filament. However, ASC CARDs can recruit additional ASC molecules to nucleate an ASC PYD filament via a mechanism similar to that proposed for the nucleation of ASC PYD filaments by the NAIP/NLRC4 oligomer (Fig. 5B) (Schmidt et al., 2016). Interestingly, when co-expressed with ASC and AIM2, full-length procaspase-8 does not form radiating filaments *in vivo* like the DEDs, but instead is condensed to form a speck. This suggests that the catalytic domains from separate procaspase-8 filaments interact to cross-link the filaments (Vajjhala et al., 2015). Inter-filament interactions may be important for proximity-induced dimerization and activation of procaspase-8 to induce apoptotic cell death.

3.0 Co-operative assembly formation in the RLR and TLR signaling pathways

Oligomerization of members of the death-fold superfamily has also been shown to be central to the signaling pathways downstream of RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs).

3.1 The RLR family

The RLR family members recognize viral RNA in the cytosol and activate antiviral signaling pathways, leading to the production of type-I-interferons (IFNs) and other pro-inflammatory cytokines (Andrejeva et al., 2004; Yoneyama et al., 2004). The family consists of three members: retinoic acid-inducible gene 1 (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology-2 (LGP2). RIG-I and MDA5 share the same domain architecture, consisting of N-terminal tandem CARDs (2-CARD), a central DExD/H motif helicase domain and a C-terminal domain (CTD). The latter two domains together function as an RNA-recognition unit, forming a ring-like structure around dsRNA (Jiang et al., 2011; Kowalinski et al., 2011; Luo et al., 2011; Wu et al., 2013), while the N-terminal CARDs interact with the mitochondrial anti-viral signaling (MAVS) adaptor (also known as CARD adapter inducing IFN β (Cardif), virus-induced signaling adapter (VISA), or IFN β promoter stimulator 1 (IPS-1)), leading to activation of antiviral signaling pathways (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). Although RIG-I and MDA-5 share the same domain architecture and the same signaling adaptor, they recognize distinct features of viral RNAs (Baum et al., 2010; Feng et al., 2012; Goubau et al., 2014; Hornung et al., 2006; Kato et al., 2008; Pichlmair et al., 2006; Triantafilou et al., 2012) and detect different groups of viruses (Gitlin et al., 2006; Kato et al., 2006; Loo et al., 2008), and thus play non-redundant roles in the antiviral innate immune response. LGP2 contains a helicase domain and a CTD, but lacks CARDs, which are usually critical for downstream signaling. The specific role of LGP2 in RLR signaling has therefore been more difficult to elucidate, but growing evidence suggest a role for LGP2 as a positive regulator of antiviral responses (Bruns et al., 2014; Satoh et al., 2010; Venkataraman et al., 2007).

3.1.1 Receptor oligomerization

Upon binding to viral dsRNA, both RIG-I and MDA5 can cooperatively assemble into a filament (Berke and Modis, 2012; Berke et al., 2012; Patel et al., 2013; Peisley et al., 2011; Peisley et al., 2013; Wu et al., 2013), resulting in clustering of the N-terminal CARDs, which forms a scaffold for binding and oligomerization of the MAVS CARDs. In the inactive state, the tandem RIG-I CARDs have been shown to be protected from self-association by interaction with the helicase domain. Upon viral RNA binding, conformational changes release the CARDs from the helicase domain, enabling them to self-associate and interact with MAVS (Jiang et al., 2011; Kowalinski et al., 2011; Luo et al., 2011). RIG-I and MDA5 tandem CARD oligomerization is further promoted by both non-covalent and covalent K63-linked polyubiquitination (Gack et al., 2007; Jiang et al., 2012; Peisley et al., 2014; Zeng et al., 2010).

3.1.2 MAVS filament formation

MAVS activates RIG-I and MDA5-dependent antiviral signaling by recruiting the E3 ubiquitin ligases TRAF2, 5 and 6, which in turn activate cytosolic kinases, TBK1 and IKK, and subsequently the transcription factors IRF3/7 and NF- κ B (Vazquez and Horner, 2015). MAVS contains an N-terminal CARD involved in oligomerization and RIG-I/MDA5 interaction, a central non-structured region of approximately 400 amino acids with TRAF2- and TRAF5-binding sites, and a C-terminal transmembrane (TM) domain required for anchoring to the mitochondrial membrane. Recent studies have shown that, similar to the inflammasomes, MAVS-dependent RIG-I/MDA-5 signaling involves a SCAF mechanism resulting in filament formation of MAVS on the mitochondria (Hou et al., 2011) (Fig. 7). The filament is formed by the MAVS CARDs, and is necessary for recruitment of TRAF2 and TRAF5 and activation of the downstream signaling pathway.

The structure of the MAVS^{CARD} filament was solved by cryo-EM at 3.6 Å resolution and revealed a left-handed single-stranded helix of MAVS^{CARD} subunits (Wu et al., 2014). Similar to inflammasome PYD/CARD filaments, each MAVS^{CARD} subunit interacts with six nearest neighbours, using six distinct surface areas forming three types of intermolecular interactions. Crystal structures of the RIG-I tandem CARDs alone (Peisley et al., 2014) and in complex with MAVS^{CARD} (Wu et al., 2014) revealed that tandem RIG-I CARDs act as a template for assembly formation of MAVS^{CARD}, by forming a helical tetrameric structure and recruiting MAVS^{CARD} along its helical trajectory, in what has been termed the lock-washer mechanism. Although crystal structures are not available for MDA5 oligomers alone or in complex with MAVS^{CARD}, it is likely that MDA5 induces formation of MAVS filaments via a similar mechanism.

3.2 The TLR family and TIR domain-containing adaptor proteins

TLRs are single-pass transmembrane receptors that contain an extracellular LRR domain involved in PAMP/DAMP recognition, and a cytosolic Toll/interleukin-1 receptor (TIR) domain required for signal transduction (Akira et al., 2006). TLRs localize to the cell surface or to the intracellular membrane-bound compartments such as the endosomes. The TLR family members recognize a wide variety of PAMPs, including lipopolysaccharides, lipopeptides and flagellin (surface TLRs) and viral and bacterial nucleic acids (endosomal TLRs). PAMP recognition by TLRs leads to activation of

transcription factors NF- κ B, AP-1 and IRFs to induce anti-pathogen responses and inflammation (Gay et al., 2014).

PAMP recognition by TLRs leads to recruitment of a single, or specific combination of, intracellular TIR domain-containing adaptor proteins via homotypic TIR-domain interactions (Ve et al., 2012). The adaptors involved in TLR signaling comprise MyD88 (myeloid differentiation primary response gene 88), MAL (MyD88 adaptor-like protein), TRIF (TIR domain-containing adaptor protein inducing IFN), TRAM (TRIF-related adaptor molecule) and SARM (sterile- α and armadillo motif).

MyD88 is required for signaling by all TLRs except for TLR3, which uses TRIF alone. The C-terminal TIR domain of MyD88 interacts directly with the TIR domains of activated receptors, or indirectly via the adaptor protein MAL (Medzhitov et al., 1998; Yamamoto et al., 2002). MyD88 also has a N-terminal death domain (DD), which recruits IRAK4 (interleukin-1 receptor-associated kinase 4) through DD interactions (Wesche et al., 1997). This results in activation of the MAP kinase and NF- κ B pathways, leading to production of inflammatory cytokines. MAL, also called TIRAP (Toll/interleukin-1 domain-containing adaptor protein) is a bridging adaptor between TLRs and MyD88 (Fitzgerald et al., 2001). PIP2 (phosphatidylinositol-4,5-bisphosphate) binding localizes MAL to the plasma membrane, where it recruits MyD88 to TLR2 and TLR4 via homotypic TIR-domain interactions (Kagan and Medzhitov, 2006). MAL has recently also been shown to be required for endosomal MyD88-dependent TLR9 signaling in response to viral infection (Bonham et al., 2014).

TRIF, also called TICAM-1 (TIR-containing adaptor molecule-1) is the sole signaling adaptor for TLR3, and also associates with TLR4 *via* TRAM (Hoebe et al., 2003; Oshiumi et al., 2003a; Yamamoto et al., 2003). TRIF activates both NF- κ B and IRF3, resulting in production of both pro-inflammatory cytokines and type-1-IFN. TRAM, also called TICAM-2, is responsible for recruitment of TRIF to the activated and endocytosed TLR4 via homotypic TIR-domain interactions and operates both at the plasma membrane and the endosomes (Kagan et al., 2008; Oshiumi et al., 2003b; Rowe et al., 2006). The fifth adaptor, SARM, is the most conserved TLR adaptor protein with homologs identified in *Drosophila* and *Caenorhabditis elegans*. Human SARM can act as a specific inhibitor of TLR3 and TLR4 signaling mediated by TRIF (Carty et al., 2006), but recently SARM has also been shown to have functional roles in apoptosis and antiviral innate immune responses (reviewed by (Panneerselvam and Ding, 2015)), and to be an executioner of axon degeneration, a self-destruction program that promotes the localized clearance of damaged axon segments (Gerdts et al., 2013; Osterloh et al., 2012).

3.2.1 Receptor dimerization triggers MyD88 and TRIF oligomerization

Ligand binding to a TLR causes dimerization of the receptors or conformational changes of existing dimers. To date, the structures of TLR3-5 and TLR7-8 extracellular domain homodimers and the TLR1/2 and TLR1/6 extracellular domain heterodimers in complex with their physiological or synthetic ligands have been reported (Liu et al., 2008; Song and Lee, 2012; Tanji et al., 2013; Yoon et al., 2012; Zhang et al., 2016). The dimeric arrangements of the LRR domains are similar in all of the TLR-ligand complex structures, with the two C termini converging in the middle, which presumably drives the intracellular TIR domains to self-associate and subsequently recruit TIR domain-containing

adaptor proteins via homotypic TIR-domain interactions to initiate signaling (Gay et al., 2014) (Fig. 8). Recruitment of MyD88 to the activated membrane-bound TLR induces formation of an oligomeric MyD88-IRAK complex called the myddosome. Biophysical studies have shown that the DD domain of MyD88 exists in a rapid monomer-oligomer equilibrium in solution, but in the presence of the IRAK-4 DD, stable assemblies with stoichiometries of 7:4 and 8:4 are formed (Lin et al., 2010; Motshwene et al., 2009). The crystal structure of a MyD88, IRAK4 and IRAK2 DD complex revealed a helical assembly consisting of six MyD88, four IRAK4, and four IRAK2 DDs (Lin et al., 2010) (Fig. 8). As observed in the inflammasome and MAVS filaments, the DDs in the myddosome interact with each other through three types of asymmetric interaction modes. The structure suggests a sequential assembly process in which a receptor-induced homo-oligomer of MyD88 forms first and provides a platform for recruitment of four IRAK4 and then four IRAK2 molecules. Similar to the lock-washer mechanism described for the RIG-I/MDA5-MAVS interaction, the DDs of MyD88 form a helical template that is extended first by IRAK4 and then IRAK2. myddosome formation brings the kinase domains of IRAKs into proximity for phosphorylation and activation.

In comparison to MyD88 signaling, much less is known about TRIF signaling (Ullah et al., 2016), but live-cell imaging and confocal immunofluorescence analyses have shown that TRIF alters its distribution profile from a diffuse cytoplasmic form to a speckle-like structure in response to TLR3 interaction with dsRNA, which suggests that oligomeric TRIF complexes are also formed in response to ligand activation (Funami et al., 2007). Yeast two-hybrid and co-immunoprecipitation studies using TRIF deletion mutants revealed that both the TIR domain and the C-terminal region, but not the N-terminal domain are required for TRIF oligomerization (Funami et al., 2008). TRIF does not contain a DD, but the C-terminal region harbors a RIP homotypic interaction motif (RHIM), which in RIP1 and RIP3 kinases have been shown to facilitate the assembly of the functional filamentous signaling complex that mediates necroptosis (Li et al., 2012).

3.2.2 The TIR domain signalosome

The adaptor MAL has been shown to be required for TLR4-induced myddosome assembly and is present within it (Bonham et al., 2014). Forced dimerization of the MyD88 TIR domain constitutively activates signaling (Fekonja et al., 2012), suggesting that MAL and MyD88 form an oligomeric platform through TIR-domain interactions upon TLR activation, which in turn promotes the assembly of the myddosome via DD interactions. The molecular mechanisms underpinning adaptor recruitment to TLRs via homotypic TIR-domain interactions are currently not well understood due to the difficulty of reconstituting stable TIR domain oligomers. This suggests that weak interactions are general feature of the TIR-domain function, and that a high local concentration is required for stabilizing the interaction, which in normal signaling can be achieved either by membrane attachment or in the context of a larger assembly.

Crystal structures are available for the TIR domains of human TLR1-2, TLR6, TLR10, IL-1RAPL, MyD88 and MAL (Jang and Park, 2014; Khan et al., 2004; Nyman et al., 2008; Snyder et al., 2013; Valkov et al., 2011; Xu et al., 2000), and the Toll-related receptor TRR-2 from the lower metazoan

Hydra magnipapillata (PDB ID 4W8G, 4W8H). In addition, NMR structures have been determined for the TIR domains of MyD88, TRAM and TRIF (Enokizono et al., 2013; Ohnishi et al., 2009). Crystal contact analysis, and site directed mutagenesis-restrained docking of crystal and NMR structures or homology models have been used extensively to gain insight into the TIR domain assembly process (Bovijn et al., 2013; Bovijn et al., 2012; Enokizono et al., 2013; Guven-Maiorov et al., 2015; Khan et al., 2004; Nunez Miguel et al., 2007; Ohnishi et al., 2009; Tao et al., 2002; Toshchakov et al., 2011; Vyncke et al., 2016). Although some common broad trends in homotypic TIR-domain association modes have been identified, there is overall little consensus among the different assembly models (Nimma et al., 2017; Ve et al., 2015). Many of the earlier models assume a 1:1 or 2:1 receptor:adaptor stoichiometry, which fails to explain how receptor dimerization can induce myddosome formation (Bovijn et al., 2013; Bovijn et al., 2012; Nunez Miguel et al., 2007). However, two more recent distinct models try to rationalize how receptor dimerization can lead to myddosome formation (Guyen-Maiorov et al., 2015; Vyncke et al., 2016). In one of these models, which is based on docking restrained by extensive mutagenesis data, MyD88 TIR domains assemble into a left-handed helix by combining a symmetric and an asymmetric mode of homotypic TIR-domain interactions (Vyncke et al., 2016). Extension of the helix would allow assembly of multiple myddosomes, which is consistent with the ability of TLRs to activate a large transcriptional response in response to extremely low concentrations of PAMPs (Cheng et al., 2015; Gioannini and Weiss, 2007; Stylianou et al., 1992), and is similar to the SCAF models for inflammasome activation and MAVS-dependent RIG-I/MDA5 signaling (Lu et al., 2014b; Wu et al., 2014). Although this model is consistent with observed homotypic TIR-domain interaction modes, the significant variations observed in TIR:TIR interactions in different mammalian proteins could give rise to TIR-domain oligomers with different architectures. To fully elucidate the molecular mechanisms of TIR-domain assembly formation in TLR signaling and the exact nature of the interfaces, structural information on stable oligomeric assemblies will be required.

4. Conservation of SCAF in plants and fungi

4.1 NLRs in other kingdoms of life

NOD-like receptors can be found in kingdoms of life outside of animals; both fungi and plants have NLR proteins analogous to those found in animals. No NLRs or PRR-like proteins have been identified to date in bacteria; however, proteins containing singular domains associated with PRRs, such as TIR domains, have been identified in some bacterial species.

4.2 Plant NLRs

NLR proteins have important roles in the immune systems of plants. These proteins are intracellular receptors employed to detect virulence proteins, such as those secreted into the host by fungal, bacterial and oomycete pathogens, and consequently signal to activate cell death. Unlike animal NLRs, plant NLRs are highly ligand-specific, recognizing only strain-specific virulence proteins (Dangl and Jones, 2001). Regardless of this, plant NLRs have a highly similar domain arrangement to animal NLRs, consisting of an N-terminal signaling domain, central NOD domain, and a C-terminal LRR domain. The major difference from animal NLRs is in the type of domains used to make up the

receptor. Plant NLRs can be seen as constructed from a medley of components from animal PRRs and apoptotic proteins (Bentham et al., 2016). They often contain an N-terminal TIR domain, also present in animal TLRs, but many instead have a CC domain with a four-helix bundle fold that bears some similarities to a death-fold domain structure (Jones and Dangl, 2006). The central NOD is most similar to that of APAF-1 or CED4, which is referred to as NB-ARC (nucleotide-binding adaptor shared by APAF-1 [apoptotic protease-activating factor 1], R proteins and CED-4) domain (van der Biezen and Jones, 1998). The NB-ARC domain is closely related to but distinct from the NACHT domain of animal NLRs. Proteins with the NACHT or NB-ARC domains make up the two major subfamilies of the STAND family of ATPases (Leipe et al., 2004). Homotypic interactions between N-terminal TIR and CC effector domains have been established as an essential event for the NLR to effectively activate cell death (Bernoux et al., 2011; Schreiber et al., 2016; Williams et al., 2014). Interestingly, these N-terminal interactions have been demonstrated to be weak and transient, and therefore it has been proposed that the signaling domain self-association is mediated by NOD oligomerization (Bernoux et al., 2011; Schreiber et al., 2016; Williams et al., 2014). Currently, there is no structural evidence for NOD oligomerization in plant NLRs; however, recent biochemical analysis of the Arabidopsis NLR "Resistance to *Peronospora Parasitica* 1" (RPP1) demonstrated that it is capable of ligand-induced, NB-ARC domain-dependent self-association, and this process is vital for RPP1-mediated cell death (Schreiber et al., 2016). Similarities between plant and animal NLR structure and function have led to the hypothesis that plant NLRs signal via cooperative assembly to produce a "resistosome" signaling complex, similar to that of the inflammasome (Bentham et al., 2016).

4.3 Fungal NLRs

Analyses of fungal genomes have identified extensive repertoires of NLR and STAND-like proteins. Fungal NLRs have been implicated in a process called heterokaryon incompatibility that involves non-self recognition and activation of cell death. These proteins share a similar tripartite domain architecture as their plant and animal counterparts, with an N-terminal signaling domain, central NOD-like domain, and C-terminal repeat super-structure (Koonin and Aravind, 2000; Saupe et al., 1995). Unlike animals and plants however, there is high variability in the potential domain arrangement of fungal NLRs, with the exception of the NOD domain, which is primarily dominated by the NACHT domain, although NB-ARC-containing fungal NLRs have also been identified (Dyrka et al., 2014). There are >12 different N-terminal domains associated with fungal NLRs, and three major types of C-terminal repeat domains (WD, ANK and TPR) (Dyrka et al., 2014). The N-terminal domains of fungal NLRs can be separated into two classes based on proposed functions. Class 1 N-terminal domains have proposed enzymatic functions and have potential involvement in cell-death activation. Class 2 N-terminal domains are more analogous to plant and animal N-terminal domains, acting as adaptors that may signal through homotypic interactions to recruit downstream signaling partners (Dyrka et al., 2014). Two domains in class 2 NLRs are noteworthy: the prion HeLo domain of Het-s, which can substitute the PYD of NLRP3 to create a chimeric protein capable of activating pro-caspase-1 (Cai et al., 2014); and the HET domain of HET-E, which shares remote structural similarities to the TIR domains of plant and animal PRRs (Dyrka et al., 2014). Biochemical and structural information of fungal NLRs is scarce and so it is unclear if these proteins assemble into higher-order oligomers;

however, considering the available data and their shared similarities to animal NLRs, this is a likely possibility.

5. Conclusions

The architecture of the signaling complexes induced by PRRs allows rapid co-operative assembly, signal amplification and provides a scaffold for proximity-induced activation. There are two types of co-operative assembly mechanisms observed in the different complexes. In the case of the death-fold filaments, including the PYD, CARD and DD filaments and the AIM2 HIN domain/dsDNA oligomer, co-operative assembly occurs via the presentation of multiple interaction sites by the preceding components that facilitate downstream recruitment. However, a second type of mechanism observed in the NAIP2/NLRC4 complex is induction of conformational changes in NLRC4 upon recruitment to the complex, which enables recruitment of a subsequent NLRC4 monomer.

There are many advantages of SCAF. First, the low affinity of the receptors and downstream components prevents interaction of the receptors in the absence of activation-induced clustering and ensures a PAMP-triggered signaling event. Second, it allows a rapid amplification process, whereby low-level PAMP concentration and activation of just a few receptors can recruit multiple downstream adaptors and effectors and amplify the signal immensely (Hauenstein et al., 2015). Third, in the case of the inflammasome for example, it initiates a self-perpetuating system that continues to recruit downstream proteins. Furthermore, because the complex is self-perpetuating, it can continue signaling upon transfer from one cell to another (Franklin et al., 2014). Thus, the SCAF mechanism is perfectly suited to enhance the effect of PRRs as a first line of defense in innate immunity.

Conflict of interest

The authors have no financial or personal interests to disclose.

Submission declaration

This article has not been published previously elsewhere although some background information included in the paper is widely available elsewhere. The authors included in this paper have contributed significantly to the overall knowledge and information included in this article.

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Figure legends

Fig. 1. Overview of inflammasome assembly. Activation and oligomerization of pattern recognition receptors (PRRs) enables them to recruit and nucleate filaments of the adaptor protein ASC. The ASC filament in turn nucleates filaments of procaspases -1 and -8.

Fig. 2. The AIM2/dsDNA inflammasome initiation complex. *A*, Structure of the AIM2 HIN domain bound to dsDNA (PDB ID 3RN2) (Jin et al., 2012). Two HIN domains (colored pink and brown) are shown and the individual oligosaccharide binding domains (OB1 and OB2) are indicated on each HIN domain. *B*, Structure of the AIM2 PYD (PDB ID 3VD8) (Jin et al., 2013), which is a typical death-fold domain containing six α -helices in an anti-parallel arrangement. *C*, Model to illustrate the polymerization of the AIM2 HIN domains (OB1 and OB2 shown as pink, brown rectangles) along dsDNA and the clustering of the AIM2 PYDs (yellow hexagons).

Fig. 3. The NAIP2/NLRC4 inflammasome initiation complex. *A*, Schematic representation of NLRC4 and NAIP2, indicating the position of the nucleotide binding and oligomerization domains (NODs), which comprise the nucleotide binding domain (NBD), helical domain 1 (HD1), and the winged helical domain (WHD); helical domain 2 (HD2); the leucine-rich repeat (LRR) domain; and the N-terminal CARD or BIR domain. *B*, Structure of the NLRC4 monomer in the inactive conformation (PDB ID 4KXF) (Hu et al., 2013) and a subunit of the NAIP2/NLRC4 oligomer (subunit from PDB ID 3JBL) (Zhang et al., 2015) in the active conformation. *C*, structure of the NAIP2/NLRC4 oligomer (PDB ID 3JBL). Domains are colored as in *A* and *B*. *D*, Model for co-operative assembly of the NAIP2/NLRC4 oligomer via an induced conformational change. An activated monomer of NLRC4 (subunit from PDB ID 3JBL) is proposed to use a basic surface of the NOD to interact with an acidic surface of the NOD from an inactive monomer of NLRC4 (PDB ID 4KXF). The interaction induces conformational changes in the inactive monomer to the active conformation that exposes the basic surface, which can then interact with another monomer. Some of the charged residues in the basic and acidic surfaces are indicated in blue and red, respectively. *E*, the NAIP2/NLRC4 oligomer (PDB ID 3JBL), with one subunit colored red to indicate a NAIP2 monomer, showing the predicted position of the NLRC4 CARDS.

Fig. 4. The ASC filament. *A*, Structure of ASC (PDB ID 2KN6) showing ASC PYD and ASC CARD separated by a flexible linker. *B*, Structure of the ASC PYD triple helical filament (PDB ID 3J63) (Lu et al., 2014b). One monomer is boxed to show the six interaction surfaces (type Ia, Ib, IIa, IIb, IIIa, IIIb), which are typical of death-fold domains. *C*, Schematic diagram of the ASC PYD filament showing how a downstream recruited ASC PYD is presented with multiple interaction sites to assemble co-operatively. The CARDS on the side of the ASC PYD filament that cluster to form a short filament are also shown.

Fig. 5. Model for initiation of ASC filaments by inflammasome initiation complexes. *A*, AIM2 HIN domain polymerizes on dsDNA and the AIM2 PYDs cluster and recruit ASC via its PYD to nucleate

an ASC PYD filament. *B*, The clustered CARDs of the NAIP2/NLRC4 oligomer recruit ASC via its CARD. Clustering of ASC CARDs drives clustering of ASC PYDs, which can then recruit further ASC PYDs to initiate an ASC PYD filament.

Fig. 6. Model for recruitment and activation of procaspases-1 and -8. The AIM2/dsDNA inflammasome initiation complex nucleates an ASC PYD filament. Clustered ASC CARDs on the side of the ASC filament recruit procaspase-1 and nucleate procaspase-1 CARD filaments. Catalytic domains on the sides of the procaspase-1 CARD filament can dimerize and become activated. Procaspase-8 DEDs are recruited to the ASC PYD filament and a DED filament is nucleated. The procaspase-8 catalytic domains on the side of the filament can also dimerize and become activated.

Fig. 7. Model of activated RIG-I/MDA-5:MAVS complexes. Viral RNA binding induces conformational changes in the RIG-I/MDA-5 receptors resulting in release of the tandem CARDs from the helicase domain. The receptor CARDs can then self-associate and form a template for MAVS recruitment and filament formation on the mitochondria.

Fig. 8. Working model for activation of MyD88 dependent TLR signaling. PAMP binding to TLRs results in self-association of the cytosolic TIR domains and recruitment of adaptor proteins such as MAL and MyD88 via TIR:TIR domain interactions. MAL is membrane associated through interaction with PIP2?? *Need some comment on that feature of the diagram.* Recruitment of multiple MyD88 molecules subsequently leads to recruitment of IRAKs via DD:DD interactions and formation of the myddosome. The structural basis for myddosome formation and PAMP recognition by many TLRs has been established, but how receptor and adaptor TIR domains assemble to promote formation of the myddosome is poorly understood.

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A **B** **C**

TLR4:MD-2-LPS complex

Myddosome