Molecular Immunology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

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

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ARTICLE INFO

*Article history:*
Received 12 January 2017
Received in revised form 16 February 2017
Accepted 19 February 2017
Available online xxx

*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)

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.



## 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).

*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.

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http://dx.doi.org/10.1016/j.molimm.2017.02.012
0161-5890/

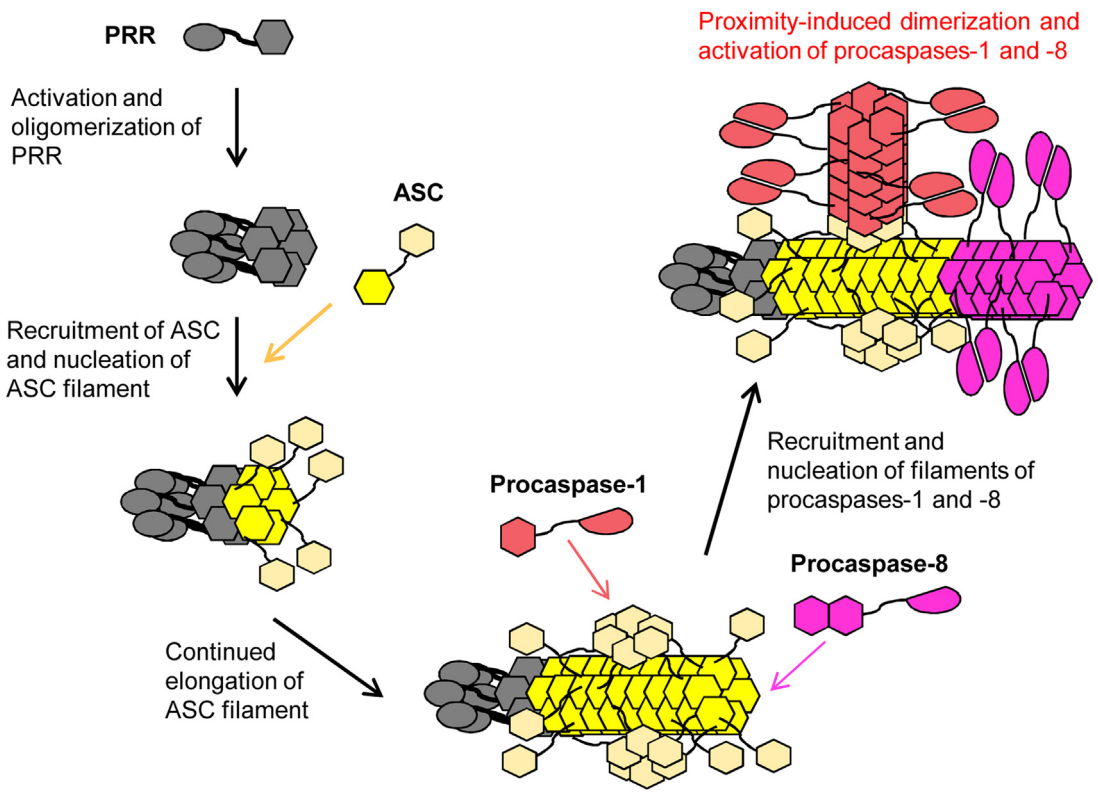


**Fig. 1.** Overview of inflammasome assembly. Activation and oligomerization of pattern recognition receptors (PRRs) enables them to recruit and nucleate filaments of the adaptor protein ASC. The ASC filament in turn nucleates filaments of procaspases −1 and −8.

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., 2014b; Lu et al., 2016; 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-1β (IL-1β) 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-1β 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., 2014b; Lu et al., 2016; 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

Please cite this article in press as: Vajjhala, P.R., et al., The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways. Mol. Immunol. (2017), http://dx.doi.org/10.1016/j.molimm.2017.02.012