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Activation and assembly of the inflammasomes through conserved protein domain families

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Abstract

Inflammasomes are oligomeric protein complexes assembled through interactions among the death domain superfamily members, in particular the CARD and PYD domains. Recent progress has shed lights on how the ASC PYD can polymerize to form filaments using multiple domain:domain interfaces, and how the caspase4 CARD can recognize LPS to activate the non-classical inflammasome pathway. Comprehensive understanding of the molecular mechanisms of inflammasome activation and assembly require more extensive structural and biophysical dissection of the inflammasome components and complexes, in particular additional CARD or PYD filaments. Because of the variations in death domain structures and complexes observed so far, future work will undoubtedly shed lights on the mechanisms of inflammasome assembly as well as more surprises on the versatile structure and function of the death domain superfamily.

Keywords

Inflammasome; Death domain superfamily; CARD; PYD; Filament formation

Introduction

The terminology of inflammasome was first proposed by the Tschopp group in 2002 to describe a protein complex involved in pro-IL-1 β processing [1]. To date, many different inflammasome complexes have been reported, and they are denoted by the presumed sensory molecules within such complexes, such as the NOD-like receptor (NLR) family members NLRP1, NLRP3, NLRC4, and the PYHIN family member AIM2 [2]. In addition to these proteins, most of these inflammasomes include the adaptor molecule ASC and effector enzymes such as caspase-1 or caspase-8. The consequence of inflammasome

activation is not limited to the processing of pro-IL-1 β , but also pro-IL-18 and pro-IL-33 [3]. Recently, a type of non-canonical inflammasome has been discovered, including the intracellular LPS sensing caspase-11, which can process pro-IL-1 α [4–6].

Many different stimuli can activate the inflammasome pathways, including complement component C3a [7], soluble polymerized C3b and C3b bound to Sepharose [8], pathogen virulence factors [9], LPS [4–6], mezerein [10], calcium flux [11], as well as ATP, reactive oxygen species (ROS), crystals, and DNA [2]. Activated inflammasomes promote the maturation and secretion of proinflammatory cytokines, leading to cascades of inflammation and a form of cell death termed pyroptosis. As an important component of the innate immune system, inflammasomes are targeted by microbes as a mechanism of immune evasion. For example, *Leishmania donovani* can suppress the macrophage IL-1 production in response to LPS [12]; the virulence factor YopM from *Yersinia pestis* can directly bind caspase-1 and inhibit the activation inflammasome [13]. Conversely, inappropriate activation of the inflammasomes contributes to a number of immunopathological conditions: elevated extracellular IL-1 β is related to the pathogenesis of systemic autoinflammatory diseases [14]; blockade of IL-1 has been shown to be effective and safe treatment for many of the above conditions [15].

Despite the rapid progress of inflammasome research in the past decade, the molecular mechanisms of inflammasome activation remain poorly understood. In this review, we discuss recent reports on the activation and assembly of inflammasomes through conserved death domain superfamily members.

Conserved protein domain families in inflammasome assembly

The death domain superfamily is characterized by a globular fold of anti-parallel six-helix bundles, the surface of which often display polarized charge distribution. There are four families including the death domains (DD), death effector domains (DEDs), caspase recruiting domains (CARDs) and pyrin domains (PYDs). Two of these subfamilies, namely the CARDs and PYDs, play critical roles in the assembly of the inflammasomes through homotypic protein–protein interactions (Fig. 1).

The CARD domain

There are 32 genes in the human genome that encode 35 predicted CARD domains, including caspase-1, ICEBERG/ CARD18, caspase-2, caspase-4, caspase-5, caspase-9, caspase-12, APAF1, Nucleolar protein 3, DLG5, RIG-I (2CARDs), MDA5 (2CARDs), MAVS/IPS1, BinCARD, Bcl10, ASC, NLRP1, NLRC4/IPAF, NOD1, NOD2 (2CARDs), RIPK2, RAIDD, CARD6, CARD8, CARD9, CARMA1-3, cIAP1/BIRC2, cIAP2/BIRC3, CARD16/ COP1 and CARD17/INCA (www.deathdomain.org). These can be grouped into several subsets such as the caspases, NLRs, RLRs, CARMAs, and their adaptors or regulators. Many of them have known to play roles in inflammatory pathways.

Caspase-1, caspase-4/caspase-11 and caspase-8 are the three known caspases involved in inflammasome function. Caspase-1 plays critical roles in the canonical inflammasome pathways. It is thought to be recruited to the inflammasome assembly through homotypic

CARD:CARD interactions with the adapter molecule ASC [16]. But the mechanism of caspase-1 self-inhibition and subsequent activation is not well understood. In a recent report of the non-canonical caspase-8 inflammasome, the processing of pro-IL1 β is not dependent on the activation of caspase-1, but the MALT1-caspase-8 and ASC complex presumably mediated by the association of the death domain superfamily members within these three proteins [17]. In the non-canonical inflammasome pathway, caspase-4 or murine caspase-11 can directly recognize LPS in the cytosol, and LPS can induce the oligomerization of their N-terminal CARD domains, thus leading to the activation of the C-terminal caspase domain that promotes the maturation and secretion of pro-IL- α [4–6]. The mechanism of the LPS binding-induced CARD oligomerization is still unknown due to the lack of structure information.

Structures for eleven of the human CARD domains have been determined, including those from APAF-1 (1CY5), ICEBERG/CARD18 (1DGN), Bcl10 (2MB9), caspase-9 (3YGS), RAIDD (2O71), cIAP2 (2I9 M), CARMA1 (4JUP), NLRP1 (4IFP), ASC (2KN6), NOD1 (2NZ7), CARD8 (4IKM). Two of these, ASC [18] and NLRP1 [19], are implicated in inflammasome assembly (Fig. 2a). Importantly, the homotypic CARD:CARD interactions in inflammasomes have not been recapitulated using recombinantly expressed and purified proteins, even though such interactions have been demonstrated through cellular assays. This may be partially due to the instability of the inflammasome complexes in solution. Nonetheless, structural and biophysical characterization of the CARD domain complexes is essential for detailed understanding of their interactions.

The PYD domain

The PYD domain was first described in the pyrin protein implicated in the familial mediterranean fever [20, 21], and its potential roles in inflammation have been increasingly recognized. To date, the PYD domain has been identified in many protein families such as the NLRs (NLRP1-14), PYHIN proteins (AIM2, IFI16, PYHIN1 and MNDA), PYD only proteins (POP1-3) and the adaptor ASC.

Based on analogy to the CARD:CARD interactions in apoptosis, the PYD domains were predicted to function through homotypic PYD:PYD interactions [22]. As an example, the PYD domain of the adapter ASC associates with those from pyrin, NLRP1, NLRP3, NLRP10, NLRP12 and AIM2 to assemble the respective inflammasomes [2]. It is worth noting that recent reports suggested that the ASC PYD domain did not interact with the PYD domains from NLRP4 [23], NLRP7 [24], NLRP14 [25], or other PYHIN family members [26], suggesting highly specific PYD:PYD interactions.

Structures of the PYD domains

Because of the important roles of the PYD domain in inflammasome assembly, here we focus on recently reports of the PYD domain structures and their complexes. Currently, there are 10 published PYD domain structures determined through either nuclear magnetic resonance spectroscopy (NMR) or X-ray crystallography (Fig. 2b). These include the PYD domains from ASC [18], ASC2 or POP1 [27], AIM2 [28, 29], NLRP3 [30], NLRP4 [23], NLRP7 [24], NLRP10 [31], NLRP12 [32], NLRP14 [25] and pyrin [33].

An important progress in the field is a recent study of the ASC inflammasome through cryo-electron microscopy, which revealed that both the N-terminal PYD domain and the C-terminal CARD domain could form filament structures [34]. In particular, the structure of the ASC PYD cluster could be refined to 3.8 Å resolution (Fig. 2c), allowing for fairly detailed analysis of the PYD domain complex. The helical ASC clusters were proposed to be nucleated by the inflammasome-forming receptors AIM2 and NLPR3, which further recruit the caspase-1 filaments through the CARD:CARD domain interactions. Similar filament formation was also observed in the activation of the RIG-I like receptors [35, 36]. It seems that polymerization through filament formation underlies the fundamental signaling mechanisms during inflammasome activation and antiviral immune defense.

Host and microbial PYD domains regulate inflammasome activation

The human genome encodes several proteins that harbor only the PYD domains, such as POP1-3. These were proposed to regulate inflammasome formation through their interaction with other PYD containing proteins. For instance, POP1 can inhibit inflammasome activation through its direct interaction with ASC [37]. Mapping of the POP1:ASC interface through NMR demonstrates that complementary electrostatic charge surface plays an important role in mediating these PYD:PYD interactions [27, 38]. In comparison, POP2 appears to regulate the activation of both inflammasome and the NF-κB pathway [39]. Recently, Khare et al. found that POP3 can compete with ASC through direct interaction with the PYD domains of AIM2 and IFI16, thus suppressing DNA virus-induced inflammasome activation [40].

In addition to host-derived regulatory mechanisms, pathogens have evolved inhibitors for host inflammasomes. A poxvirus encoded protein vPYD was shown to directly interact with the host adapter ASC to suppress inflammasome activation and the processing of IL-1β and IL-18 [41]. Notably, the predication of such microbial PYD domains are based on limited sequence similarity with host PYD domains. Future structural studies of the microbial PYD domains will shed light on their structural and functional similarities with the host PYD domains.

Variation of the death domain structures and complexes

With about a third of structures solved to date for the death domain superfamily, variations to the canonical structure of six-helix bundle have emerged. For example, the CARD domain of NOD1 forms a homodimer through a swapping of their α6 helix [42, 43]. Similarly, the FAS DD was shown to adopt dramatic structural changes through reorganization of its last two helices upon complex formation with the FADD death domain [44]. Such variations may arise from dynamic structures for certain death domain superfamily members, as was also demonstrated for the PYD domain of IFI16 [45]. The above observations also emphasize the importance of determining the structures for other death domain superfamily members and their complexes through experimental methods, given increasing recognition of their important biological function.

The death domain superfamily is characterized by homotypic interactions within the same subfamily to assemble large signaling complexes in apoptosis and inflammation. Increasing

evidence suggests that DD may interact with non-death domain protein modules. Because most of the DD are components of multi-domain proteins, they are often sequestered by other protein domains through intramolecular domain:domain interactions. Recent full-length structure of duck RIG-I demonstrated that its CARD2 domain interacts extensively with its C-terminal Hel2i domain, which functions to retain the receptor in a self-inhibited state [46]. A similar autoinhibition mechanism was reported for the cytosolic DNA sensor AIM2 through intramolecular interactions between its N-terminal PYD domain and C-terminal HIN domain [28]. It is clear that the death domain superfamily members are versatile assemblers of protein or domain complexes, and can engage different binding partners from within and outside the superfamily.

Summary

Deep understanding of the molecular mechanisms of inflammasome activation and assembly require more extensive structural and biophysical dissection of the inflammasome components and complexes. Recent progress on the structure of the ASC PYD complexes has provided novel insights regarding the multiple interfaces required for the PYD filament formation. Future studies of additional CARD and PYD filaments will undoubtedly shed lights on how other inflammasomes are assembled, and perhaps reveal more surprises on the structural variations of death domain complexes.

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Fig. 1.

Cartoon presentation of inflammasome assembly. **a** Individual domains involved in inflammasome. Different domains were defined by *distinct colors and shapes*. **b** Resting states of the inflammasome components. **c** Domain organization for two of the best-studied inflammasomes in their activated states. Activated caspase domains are denoted with *stars*

Fig. 2.

Structures of death domain superfamily members involved in inflammasome assembly. **a** Structure superposition of the CARDS from NLRP1 (*cyan*) and ASC (*green*). **b** Superposition of PYD structures onto the PYD of ASC. The ASC PYD structure is colored in *grey* and presented in the same orientation for all the panels. **c** Cryo-EM structure of the ASC PYD clusters in two different views