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Rosetta stone of NLR innate immunity

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Abstract

The formation of NLR inflammasomes is a central step in the initiation of the innate immune response. Two recent publications describe the structure of the NAIP2-NLRC4 inflammasome and derive an elegant model of NLR inflammasome formation, whereby binding of the pathogen-molecule-bound NLR NAIP2 to NLRC4 leads to the activation of NLRC4 and initiation of self-propagating NLRC4 inflammasome formation.

Keywords

inflammasome; innate immunity; inflammation; cryo-EM

NLR inflammasomes are disc-shaped multi-protein assemblies that are crucial to the initiation of the innate immune response [1, 2]. They specifically form upon recognition of danger- or pathogen-associated molecular patterns (DAMPs/PAMPs) in the host cell. NAIP and NLRC4 are two members of the NLR [nucleotide-binding domain (NBD) and leucine-rich repeat (LRR) containing] subfamily of proteins that together compose NAIP-NLRC4 inflammasomes [1, 2]. NLRs typically comprise an N-terminal protein-protein interaction domain belonging to either the CARD (caspase activation and recruitment domain) or PYD (pyrin domain) family [3], a central nucleotide-binding and oligomerization domain (NOD) that belongs to the family of AAA+ ATPases, as well as the C-terminal LRR region that exerts an autoinhibitory function [4]. The NOD itself contains the NBD (nucleotide binding domain), followed by a helical domain (HD1), a winged helix domain (WHD) and a second helical domain (HD2). Sensing of pathogens by NAIPs [5-7] results in NOD-mediated oligomerization of NLRC4, and subsequent activation of caspase-1, ultimately leading to processing of pro-IL-1 β and release of the proinflammatory IL-1 β [1, 2]. Structural insight on inflammasomes or their components until recently was limited to the crystal structure of autoinhibited NLRC4. This structure revealed that autoinhibited NLRC4 is maintained in a compact closed conformation in an ADP-dependent manner [4].

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Two recent cryo-electron microscopy (cryo-EM) studies by the Chai and Wu laboratories shed the first light on the formation of the activated pathogen molecule-associated PrgJ-NAIP2-NLRC4 inflammasome [8, 9]. These studies reveal that the mouse NAIP2-NLRC4 inflammasome mainly consists of 11 NLR protomers arranged in an 11-fold symmetric ring, which the authors conclude contains a single molecule of NAIP2 and 10 copies of NLRC4. Both groups propose a mechanism in which binding of the *Salmonella Typhimurium* PAMP PrgJ to NAIP2 induces the NAIP2-mediated initiation of nucleation of the particle. Although the resolution of the cryo-EM structures is not high enough to individually resolve the PrgJ-NAIP2 complex within the inflammasome ring, the structures together with other studies [5, 6] suggest that the binding of PrgJ to the NOD of NAIP2 results in a conformational change in NAIP2 leading to the presentation of a basic nucleating (or ‘catalytic’) surface (Figure 1A). Autoinhibited NLRC4, by contrast, features a complementary acidic receptor surface (Figure 1A). Binding of the basic surface of activated NAIP2 to the NLRC4 acidic surface leads to a conformational change in NLRC4 and exposure of a basic patch on NLRC4 itself (Figure 1B). This basic patch can then act very much like the initial nucleating surface on NAIP2 and bind to the acidic patch of another autoinhibited NLRC4, in turn creating a new basic patch and thus leading to self-propagating oligomerization of NLRC4 molecules until inflammasome formation is complete (Figure 1C). The resulting NLRC4 arrangement naturally orients the NLRC4 CARD protein-protein interaction domains so that they can oligomerize the caspase-1 CARD and catalytic domains and ultimately trigger the innate immune response. This model describes an elegant mechanism in which a single pathogen-derived molecule is able to induce the formation of the full NAIP-NLRC4 inflammasome disc by activating one NAIP molecule and initiating its oligomerization with ~10 NLRC4 molecules (Figure 1C).

Previous research suggests that in mice different NAIP molecules have evolved as sensors for different pathogen-derived molecules [5-7]. Interestingly, sequence analysis shows that key residues of the NLRC4-binding (nucleating) surface in different members of the NAIP subfamily are conserved [8, 9], suggesting that the observed mechanism of NAIP2-NLRC4 interaction and activation is universal amongst different NAIP PAMP sensors. This notion is supported by additional biochemical and biophysical experiments in both manuscripts, showing that NAIP5, the sensor of bacterial flagellin in mice, affects NLRC4 inflammasome formation similarly to PrgJ NAIP2, and forms structures with NLRC4 that resemble the PrgJ-NAIP2-NLRC4 complex in negative staining EM [8, 9]. Thus, different NAIP molecules similarly act as important receptors and mediators between a variety of pathogen molecules and the NLRC4 molecules in the inflammasome. Importantly, in humans only a single NAIP orthologue is known and it is believed to be activated only by proteins of the bacterial type III secretion system, but not flagellin [7]. It will be interesting to see whether human NAIP activates NLRC4 in a similar fashion as the NAIP orthologues in mice.

As mentioned, ADP is necessary to maintain the autoinhibited form of NLRC4. It has been demonstrated that mutation of residues in the ADP binding pocket can result in constitutively active NLRC4 [4] and disease in humans [10], highlighting the importance of ADP in NLRC4 regulation and the necessity of keeping inflammasome activation in check. However, in light of the new cryo-EM structures and derived models, it remains to be seen

whether ADP release or ADP/ATP exchange have additional more active roles in NLRC4 activation and how these potential roles are executed on a molecular level. One of these additional functions, for example, could be to assist in the conformational change induced by the binding of the autoinhibited NLRC4 to the nucleating surface.

The cryo-EM structures of the PrgJ-NAIP2-NLRC4 inflammasome studied by the Chai and Wu groups suggest an elegant model for the activation and formation of the mixed NLR [2, 5] NAIP- and NLRC4-containing inflammasomes. For other inflammasomes, however, most prominently the NLRP3 inflammasome (considered one of the best-studied inflammasomes [1]), it is believed that they only contain one species of NLR proteins [2]. This raises the question of whether the proposed activation mechanism of the NAIP2-NLRC4 inflammasome is universal amongst the different types of NLR inflammasomes. If a similar mechanism activates the NLRP3 inflammasome, then it needs to be seen whether there exists an as-yet unknown receptor molecule taking over the part of NAIP or whether a single NLR species acts both as receptor and as self-propagating entity.

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Figure 1. Schematic model of PrgJ-NAIP2-NLRC4 inflammasome formation

(A) Binding of the *Salmonella Typhimurium* protein PrgJ (orange) to NAIP2 [5] (grey; model based on NLRC4 monomer from PDB ID 3JBL [9]) leads to formation of an initial nucleating (or 'catalytic') basic surface (blue) on NAIP2. This basic surface can bind to an acidic 'receptor' surface (red) on the autoinhibited NLRC4 (cyan; PDB ID 4KXF [4]). (B) Binding of PrgJ-NAIP2 to NLRC4 induces a conformational change in NLRC4 and opening of the leucine-rich repeat (LRR) domain, which in turn exposes a newly formed nucleating (basic) surface (blue) on the NLRC4 NOD (nucleotide-binding and oligomerization domain). This basic surface can now further interact with the acidic surface of other autoinhibited NLRC4 molecules, leading to self-propagating oligomerization of NLRC4 (inflammasome formation). (C) Fully formed PrgJ-NAIP2-NLRC4 inflammasome (based on PDB ID 3JBL [9]).