Structures and functions of the inflammasome engine

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Inflammasomes are molecular machines that carry out inflammatory responses on challenges by pathogens and endogenous dangers. Dysregulation of inflammasome assembly and regulation is associated with numerous human diseases from autoimmunity to cancer. In recent years, significant advances have been made in understanding the mechanism of inflammasome signaling using structural approaches. Here, we review inflammasomes formed by the NLRP1, NLRP3, and NLRC4 sensors, which are well characterized structurally, and discuss the structural and functional diversity among them. (J Allergy Clin Immunol 2021;147:2021-9.)

Key words: *Inflammasome, NLRP3, NLRP1, NLRC4, pyroptosis, IL-1, caspase-1*

The innate immune system is at the forefront of the host defense against pathogens and endogenous danger.[1] Through surveillance by pattern recognition receptors, phagocytes, dendritic cells, and epithelial cells recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), and initiate appropriate innate immune responses.[1,2] One type of innate immune response lifts the transcription level of interferons, proinflammatory cytokines, and other essential players required for host defense pathway activation. Inflammasomes belong to another type of innate immune response, which often requires priming, and are activated when the danger signals have breached the cytosol.[2] Inflammasome activation can lead to processing and release of proinflammatory cytokines, as well as the inflammatory programmed cell death called pyroptosis.[2] These innate immune processes bring the host inflammatory machineries to the site of infection or injury, and are responsible for the recruitment of additional immune cells such as lymphocytes to bridge innate and adaptive immunity.[2,3]

Canonical inflammasomes are higher-order signaling complexes that consist of sensors, adaptors, and effectors, which are thought to assemble in a hierarchical fashion.[4] There are 2 major classes of inflammasome sensor proteins, nucleotide-binding domain (NBD) and leucine-rich repeat (LRR)-containing proteins (NLRs) and AIM2-like receptors.[2] Oligomerization of NLR and AIM2-like receptor sensors facilitates the oligomerization of adapters such as apoptosis-associated, speck-like protein containing a caspase recruitment domain (ASC).[5] Assembled adaptors then trigger the recruitment of effectors such as pro–caspase-1.[2] Oligomerized pro–caspase-1 molecules are activated and cleaved into their mature forms, and are responsible for the cleavage of proinflammatory cytokines such as pro–IL-1β, pro–IL-18 as well as the pore-forming protein gasdermin D.[2,3,6,7] The resulting inflammasome machineries are megadalton supramolecular organizing centers often with specific cellular localizations.[4,5]

Recent advances in cryo-electron microscopy (cryo-EM) enabled elucidation of the molecular architectures of inflammasomes. In this review, we discuss structures of important inflammasome signaling complexes solved in recent years, and the mechanistic insights they provide. In particular, we contrast the diverse mechanisms of oligomerization used by NLR sensors and adaptors, which are tailored to sense different PAMPs or DAMPs, with the universal involvement of death domain fold for interactions between an activated sensor and an adaptor or between an adaptor and caspase-1.

THE NLR FAMILY OF APOPTOSIS INHIBITORY PROTEIN-NLRC4 INFLAMMASOMES

The NLR family of apoptosis inhibitory proteins (NAIPs) differs from other NLRs by the presence of 3 baculovirus inhibitor-of-apoptosis repeats at their N-terminal regions.[8,9] In mice, NAIPs are encoded within a small gene cluster and vary significantly in number and sequence among different mouse strains, whereas in humans, there is only 1 NAIP.[8,9] Different mouse NAIPs directly recognize bacterial proteins, with NAIP5 and NAIP6 for bacterial flagellin, the major protein in the bacterial flagellum, and NAIP1 and NAIP2 for needle and inner rod proteins, respectively, of the bacterial type III secretion system.[8-13] Human NAIP has been shown to respond to needle protein of type III secretion system as well as flagellin, perhaps depending on splicing isoforms.[11,14]

On ligand recognition, an activated NAIP coassembles with NLRC4 to form the NAIP-NLRC4 inflammasome.[9,10] NLRC4 is

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Abbreviations used
ADP: Adenosine diphosphate
ASC: Apoptosis-associated, speck-like protein containing a caspase recruitment domain
CARD: Caspase recruitment domain
CARD8: CARD-containing protein 8
cryo-EM: Cryo-electron microscopy
CT: C-terminal
DAMP: Damage-associated molecular pattern
DPP: Dipeptidyl peptidase
FIIND: Function-to-find domain
GSDMD: Gasdermin D
HD: Helical domain
LRR: Leucine-rich repeat
NACHT: NAIP, CIITA, HET-E, and TP1
NAIP: NLR family of apoptosis inhibitory protein
NBD: Nucleotide-binding domain
NLR: Nucleotide-binding domain and leucine-rich repeat-containing protein
PAMP: Pathogen-associated molecular pattern
PYD: Pyrin domain
UPA: Conserved in UNC5, PIDD and ankyrins
VbP: Val-boro-pro
ZU5: Initially found in Zonula occludens-1 and UNC5

an adaptor and a member of the NLR family with an N-terminal caspase recruitment domain (CARD), which can directly interact with and activate caspase-1 independent of ASC, although NLRC4 activation is modulated by ASC.[15,16] (Fig 1). Cryo-EM structures of the PrgJ-NAIP2-NLRC4 inflammasome, in which PrgJ is the inner rod protein from *Salmonella typhimurium* and NLRC4 is CARD-deleted, revealed a disk-like oligomeric state with 11 to 12 copies of NAIP2 or NLRC4[17,18] (Fig 2, *A* and *B*). A rotation occurs between NBD-helical domain 1 (HD1) and the winged helix domain (WHD)-helical domain 2 (HD2) within the NAIP, CIITA, HET-E, and TP1 (NACHT) domain, which moves the LRR domain to an open conformation, contrasted by the autoinhibited closed conformation observed in the crystal structure[19] (Fig 2, *C*). Because it is challenging to distinguish NAIP2 and NLRC4 structurally, the authors adopted biochemical methods to reveal that there is only 1 copy of the PrgJ-NAIP2 complex per disk.[17,18] These pieces of evidence suggest an oligomerization mechanism in which the ligand-bound NAIP undergoes conformational changes to expose a nucleation interface, onto which NLRC4 can be recruited to undergo conformational changes and expose a new interface for the recruitment of another NLRC4 molecule. The nucleation process results in the oligomerization of NLRC4 (Fig 1, and Fig 2, *B*), which then dimerizes and activates caspase-1 through CARD-CARD interactions.

This mechanism is further supported by 2 cryo-EM structures of flagellin-NAIP5-NLRC4 complexes in an open conformation.[20-23] The structure by Tenthorey et al[20] contains NAIP5 in complex with full-length *Legionella* flagellin FlaA together with 2 subunits of NLRC4 at 5.2 Å resolution, and the structure by Yang et al[22] comprises NAIP5, a truncated *Salmonella* flagellin FliC with D0N, D0C, and D0N' fragments, and an oligomerization-deficient mutant of NLRC4 at 4.3 Å resolution (Fig 2, *D*). These structures convincingly demonstrate that the C-terminal (CT) helix (D0C) of flagellin binds to a deep pocket formed by multiple domains of NAIP5 (Fig 2, *D*), which pries NAIP5 into an active conformation, and that simultaneous recognition of multiple ligand surfaces may limit pathogen evolution to prevent immune escape. However, details of the flagellin-NAIP5 interaction differ between the 2 studies, and higher resolution structures may be required to resolve the discrepancies.

THE NLRP3 INFLAMMASOME

NLRP3 is one of the most extensively studied inflammasome. However, it is also one of the most mysterious for its mechanism of activation. The NLRP3 inflammasome is activated by various seemingly unrelated stimuli, including pore-forming toxins, crystals, aggregates, and ATP[2,24] (Fig 1). A common mechanism by which these PAMPs and DAMPs activate the NLRP3 inflammasome, likely indirectly, is through inducing potassium (K^+) efflux.[25] A number of very interesting reports investigated NLRP3 inflammasome activation and discovered that a common downstream event of K^+ efflux is the association of the serine/threonine NIMA-related kinase 7 (NEK7) with NLRP3[25-29] (Fig 3, *A*). They further found that the catalytic domain of NEK7 interacted with NLRP3; however, the catalytic activity of NEK7 was not required for activation of the NLRP3 inflammasome.[26,27] Intriguingly, recent studies showed that NLRP3 activation does not necessarily lead to pyroptosis; instead, under certain conditions, cells can survive and become hyperactivated to continuously secrete mature IL-1β, likely due to the ability of cells to repair their membranes on gasdermin D pore formation.[30-33]

NLR proteins are notoriously difficult to purify and stabilize under *in vitro* conditions. They often aggregate because of their inherent tendency to form higher-order oligomers and challenged atomic resolution structure determination. The NLRC4 crystal structure was solved in complex with adenosine diphosphate (ADP), which keeps the NLRC4 NACHT domain in an autoinhibited conformation.[19] For NLRP3, ADP binding, complex formation with NEK7,[26,27,29] and use of the small-molecule NLRP3 inhibitor MCC950[34] all helped to stabilize NLRP3 to facilitate structural studies. The cryo-EM structure of the NLRP3-NEK7-ADP-MCC950 complex was solved at 3.8 Å resolution.[35] The structure revealed that the NEK7 CT lobe interacts directly with the LRR and NACHT domains of NLRP3 (Fig 3, *B*). The NEK7 N-lobe was disordered, and rigid fitting of the N-lobe to the structure did not show an interaction with NLRP3, rationalizing earlier observations that both active and catalytically dead NEK7 activate NLRP3.[26,27] Furthermore, a bound ADP molecule was found at the NACHT domain of NLRP3,[35] which exhibits a similar closed conformation as observed in the NLRC4 autoinhibited structure.[19] MCC950, which greatly stabilized the protein complex, was not visible in the cryo-EM map, likely due to the modest resolution.[35] These data suggest that the NLRP3 structure is in an inactive conformation despite its complexation with NEK7.

To understand the NLRP3 activation mechanism, a model based on the activated NLRC4 structure was proposed.[35] Drastic conformational rearrangements of the NLRP3 NBD-HD1 module place NBD, HD1, and winged helix domain of NACHT in a direct interaction between neighboring NLRP3 molecules in an oligomeric assembly similar to NLRC4[35] (Fig 3, *B*). This

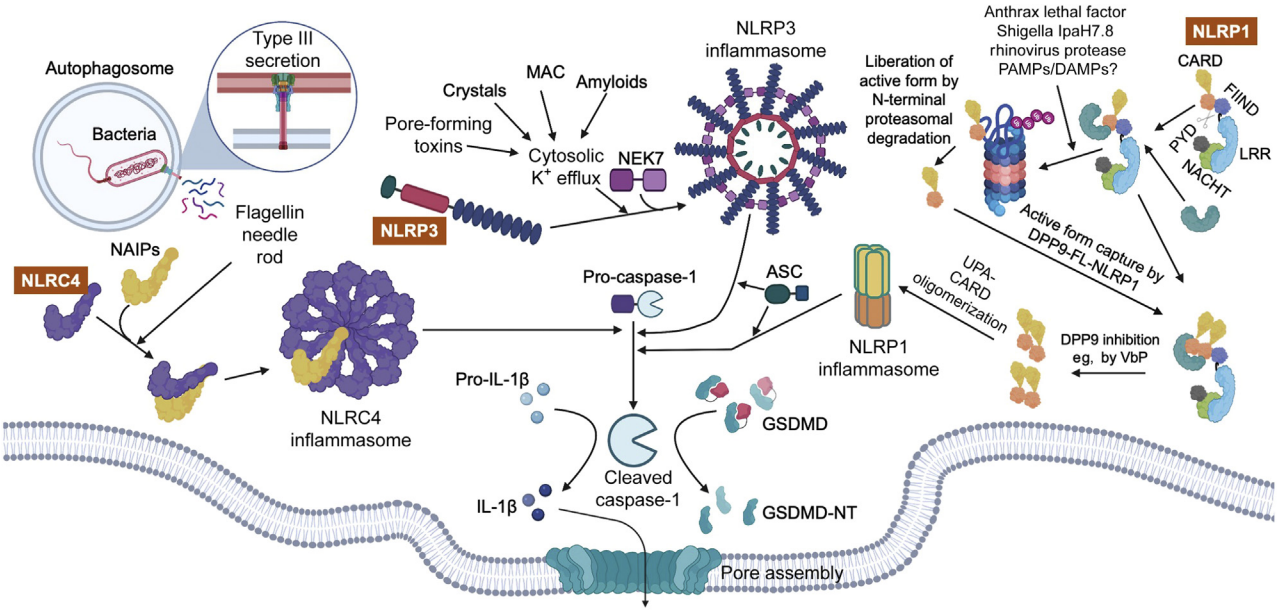

FIG 1. The NAIP-NLRC4, NLRP3, and NLRP1 inflammasome pathways. Gram-negative bacteria can use the type III secretion system to release various products into the cytosol, which are detected by NAIPs to seed the NLRC4 inflammasome. Broader insults to the cell that convergently result in K^+ efflux activate NLRP3, which binds NEK7 to assemble the NLRP3 inflammasome. NLRP1 undergoes autoproteolysis within the FIIND, leaving noncovalently associated NT domain and the CT UPA-CARD. Bacterial and viral products directly proteolyze or ubiquitinate the NT domain to promote functional proteasomal degradation to liberate the active UPA-CARD. NLRP1 is further regulated by DPP9 and its small-molecule inhibitor VbP. All inflammasomes incorporate pro–caspase-1, either directly or through the adaptor protein ASC, which results in proximity-driven activation of caspase-1. Active caspase-1 cleaves pro–IL-1β into mature IL-1β and FL-GSDMD into NT and CT fragments. GSDMD-NT binds to acidic lipids, oligomerizes, and inserts into the membrane to form the GSDMD pore through which IL-1β is secreted. The GSDMD pore can also increase permeability of the membrane to various ions and water, which can ultimately result in osmotic swelling and pyroptosis. *NT*, N-terminal; *MAC*, membrane attack complex.

activation model does not affect the NEK7 binding surface; instead, in a surprising discovery, it puts NEK7 at the oligomerization interface. The trans-surface of NEK7 (opposite to NLRP3 interaction site) plays an essential role in mediating interactions with adjacent NLRP3 protomers in a growing disk-like assembly of the NLRP3 inflammasome (Fig 3, *B*). This novel hotspot of interaction was further validated by mutational analysis in cell-based inflammasome assays and *in vitro* pull-downs of purified proteins.[35] Thus, unlike the NLRC4 disks with both NACHT-NACHT and LRR-LRR interactions between adjacent molecules (Fig 2, *B*), it appears that the shorter LRR of NLRP3 uses its bound NEK7 to bridge the LRR of the next molecule (Fig 3, *B*).

NEK7 belongs to a family of kinases that also include NEK9.[36-41] During cell division, these kinases function in regulation of cell cycle signaling.[36-43] NEK7 interacts with NEK9 during mitosis and serves as a molecular switch that dictates the mutual exclusivity between NLRP3 inflammasome activation and cytokinesis.[27] The molecular basis of this exclusivity was further discerned by analyzing the crystal structure of NEK7 bound to NEK9,[44] which showed that the NEK9-binding surface of NEK7 partially overlaps with its NLRP3-binding surface.[35] Superposition of NLRP3-NEK7 with NEK7-NEK9 structures revealed a mutually exclusive interaction of NEK7 with NLRP3 or NEK9, but not both (Fig 3, *C*). Because of its limited expression in macrophages, NEK7 cannot be engaged in both these NEK7-mediated processes. Although mitotic cells do not support NLRP3 inflammasome activation, danger signal–induced NLRP3 inflammasome activation also would likely prevent mitosis.[27,35]

Collectively, the cryo-EM structure of the NLRP3-NEK7 complex suggests a bimodal activation mechanism of NLRP3 requiring NEK7 binding and an inducer of conformational change.[35] The latter could be an exchange of ADP to ATP or some unknown trigger, leading to nucleated oligomerization of NLRP3 similar to that in the Flagellin-NAIP5-NLRC4 inflammasome.[8,9,18,20] Consequently, the role of NEK7 is redefined because it is required but not sufficient for NLRP3 activation, leading to its "licensing" role in inflammasome activation rather than as the sole activator.[35]

THE NLRP1 INFLAMMASOME

NLRP1 was the first NLR to be characterized as an inflammasome-forming pattern recognition receptor,[45] but its distinct mechanism of activation and its physiological functions have only recently been revealed. Human NLRP1 has an N-terminal pyrin domain (PYD) followed by NACHT and LRR domains[45] (Fig 4, *A*). This domain architecture is similar to NLRP3, but unlike NLRP3 or any other NLRs, NLRP1 has an additional CT function-to-find domain (FIIND) followed by a CARD[45-48] (Fig 4, *A*). Compared with human NLRP1, rodent genome encodes 3 paralogs of NLRP1, NLRP1a, NLRP1b, NLRP1c, all of which lack N-terminal PYD.[48] The FIIND is a

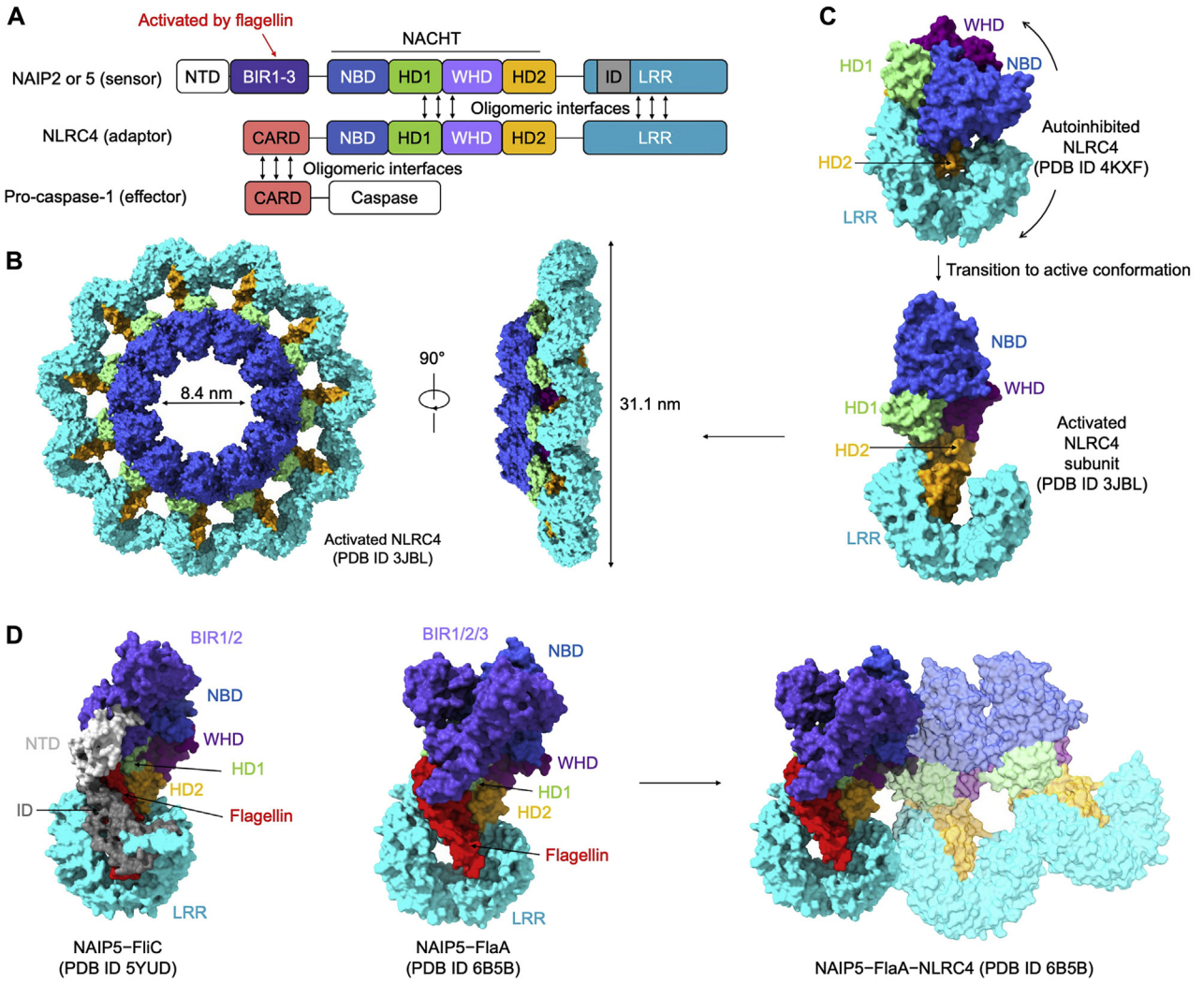

FIG 2. Structural insights on NAIP-NLRC4 inflammasomes. **A**, Domain architectures and protein-protein interactions. **B**, Activated NLRC4 forms disk-like oligomers, which are assembled by NACHT(NLRC4)-NACHT(NLRC4) and LRR(NLRC4)-LRR(NLRC4) interactions. **C**, *Top*, NLRC4 in an autoinhibited state. *Bottom*, rotation of the NBD and HD1 relative to the rest of the protein required for activation of NLRC4. **D**, *Left*, Structure of NAIP5 bound to *Salmonella* flagellin FliC. Right, *Legionella* flagellin FlaA binds to NAIP5 and pries NAIP5 into an active conformation, exposing surfaces to propagate the oligomerization of NLRC4. The CT helix of flagellin binds to a deep pocket in NAIP5. *BIR*, Baculovirus inhibitor of apoptosis repeat; *ID*, insertion domain.

particularly unusual domain because it is encoded by only 3 proteins in the human proteome—NLRP1, the CARD-containing protein 8 (CARD8),[49-52] and p53-induced protein with a death domain.[53] NLRP1 FIIND undergoes autoproteolysis between its ZU5 (initially found in Zonula occludens-1 and UNC5) and UPA (conserved in UNC5, p53-induced protein with a death domain, and ankyrin) subdomains.[49-51] As a result, 2 polypeptides—N-terminal PYD-NACHT-LRR-ZU5 and CT UPA-CARD—that remain associated by noncovalent interactions are generated.[49-51]

NLRP1 activation relies on N-terminal "functional degradation," in which NLRP1 is targeted to the proteasome for N-terminal degradation on sensing PAMPs and DAMPs.[46,54] NLRP1 N-terminus is susceptible to protease cleavage and posttranslational modifications, such as those by microbial proteases and microbial ubiquitin ligases.[46,54-56] Mouse NLRP1b can be proteolyzed by *Anthrax* lethal factor or ubiquitinated by *Shigella* ligase IpaH7.8, whereas human NLRP1 can be proteolyzed by 3C proteases from diverse picornaviruses including the human rhinovirus. Autoproteolysis is thus a critical step for NLRP1 activation because the CT is liberated when the proteasome reaches the autocleavage site. The liberated CT fragment containing the UPA subdomain and CARD then interacts with ASC and pro–caspase-1 to form a supramolecular inflammasome complex.[45,46,54]

Interestingly, unlike specific targeting by pathogenic proteases, all NLRP1 paralogs in rodents and humans share an activation mechanism that requires inhibition of the serine/threonine dipeptidyl peptidase 8 (DPP8) and dipeptidyl peptidase 9 (DPP9) by small-molecule inhibitors such as Val-boro-pro

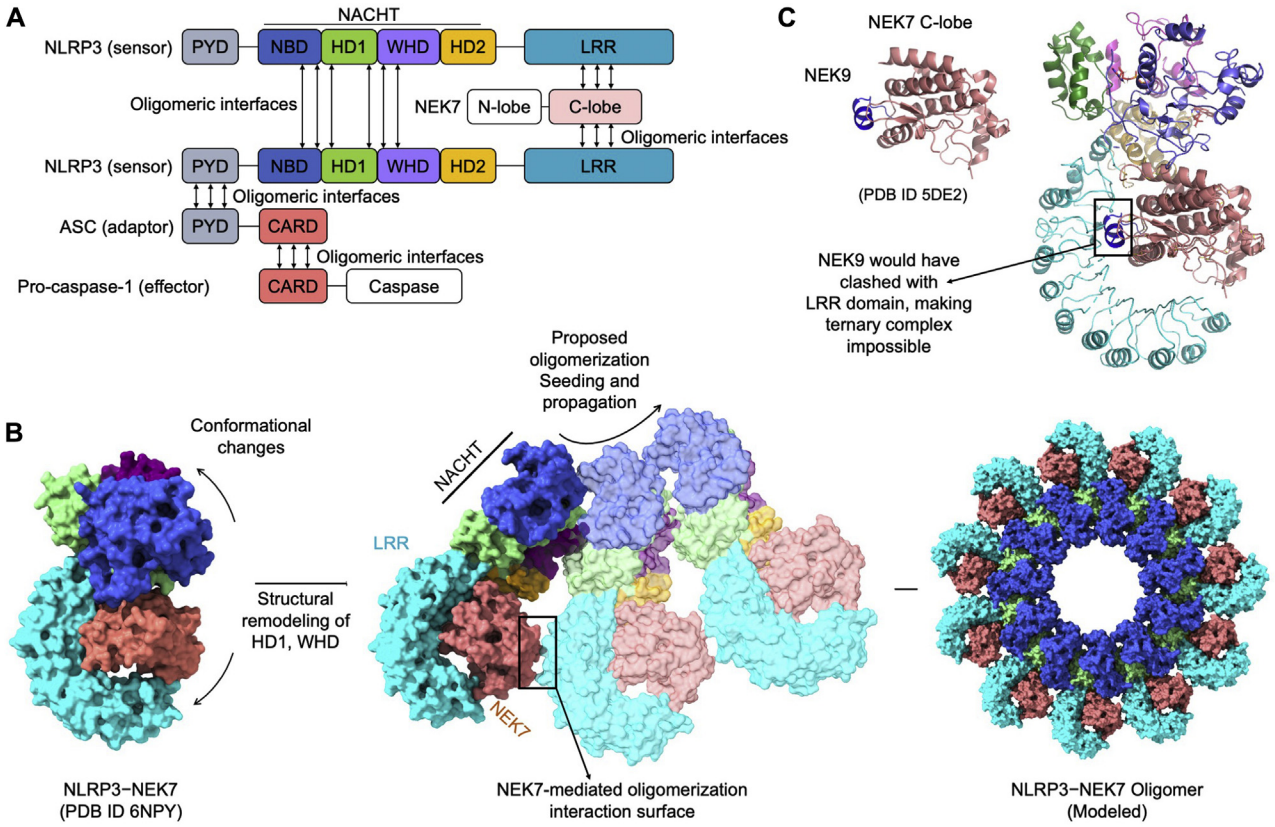

FIG 3. Structural insights on the NLRP3 inflammasome. **A**, Domain architectures and protein-protein interactions among them for the inflammasome assembly. **B**, *Left,* NLRP3 bound with NEK7. Domains are color coded as in Fig 3, *A*. *Middle,* Modeled active conformation of NLRP3 based on NLRC4 may be required for proposed oligomerization of NLRP3. *Right,* Once activated, oligomerization of the NLRP3-NEK7 complex is propagated by NACHT(NLRP3)-NACHT(NLRP3) and NEK7-LRR(NLRP3) interactions to form full disk-like oligomers. **C**, *Left,* Crystal structure of NEK7 (C-lobe only) bound to NEK9. *Right,* NEK9 interacts with NEK7 at the surface where NLRP3 interacts, making NEK7-NLRP3 and NEK7-NEK9 interactions mutually exclusive.

(VbP).[57-59] In the absence of inhibition, DPP8 or DPP9 acts as an intracellular suppressor of NLRP1. Any perturbation in cells that depletes the available pool of free DPP9 or DPP9 active-site inhibition unleashes the NLRP1 inflammasome by an unknown mechanism. This distinct mechanism of action is exclusive to NLRP1s among all NLRs, and was unclear until the recent structural studies.

Two back-to-back articles reported NLRP1 inflammasome activation and regulation by DPP9 and shed fascinating mechanistic insights on the system.[60,61] Cryo-EM structures of human and rat NLRP1, respectively, both bound to DPP9, revealed a tripartite complex: 1 molecule of DPP9 bound to 1 full-length NLRP1 ($NLRP1^{FL}$) and 1 already truncated, autoprocessed CT fragment ($NLRP1^{CT}$)[60,61] (Fig 4, *B*). This review will focus on the human NLRP1-DPP9 complex, but the rat NLRP1-DPP9 complex presented similar mechanisms. Human NLRP1 ($NLRP1^{FL}$) was coexpressed with DPP9 in HEK293 cells. The purified sample contained both $NLRP1^{FL}$ and autoprocessed protein in almost equal amounts, in agreement with previously described observations that about half of overexpressed NLRP1 is autoprocessed and half remains intact.[48,50,60] One important observation from the structural studies is that functional N-terminal degradation already exists under homeostatic protein turnover to produce $NLRP1^{CT}$, which is however captured by $NLRP1^{FL}$ and DPP9 to suppress the inflammasome activation.[60,61]

In the human NLRP1-DPP9 tripartite complex, only the FIIND (ZU5-UPA) and UPA are visible in the DPP9-bound $NLRP1^{FL}$ and $NLRP1^{CT}$, respectively, suggesting that the other domains are flexibly linked. Surprisingly, the unstructured N-terminal segment (S1212-N1224) of the UPA domain of $NLRP1^{CT}$ threads into the active-site tunnel of DPP9 (Fig 4, *B*), and differs from its structure in $NLRP1^{FL}$ where it folds into a β-strand.[60] DPP9 targets and cleaves flexible N-termini of proteins with signature sequences of NH_2-X-A or NH_2-X-P.[62,63] $NLRP1^{CT}$ has an NH_2-S-P dipeptide sequence, which is a *bona fide* DPP9 substrate sequence. However, $NLRP1^{CT}$ does not get cleaved by DPP9, although it induces conformational changes at the R-helix, which becomes ordered on substrate interaction.[64] This is because of the difference in its binding pose with how substrates binds.[60] Thus, the structure shows that DPP9 sequesters $NLRP1^{CT}$ rather than acts as a peptidase.[60]

Another notable aspect of the study is that it explored NLRP1 activation by active-site–directed DPP9 inhibitor VbP (Fig 4, *C*).[60] Because the apo structure showed that $NLRP1^{CT}$ binds directly in the active-site tunnel of DPP9, presence of VbP might interfere with this interaction and destabilize the NLRP1-DPP9 complex. Indeed, the NLRP1-DPP9 structure

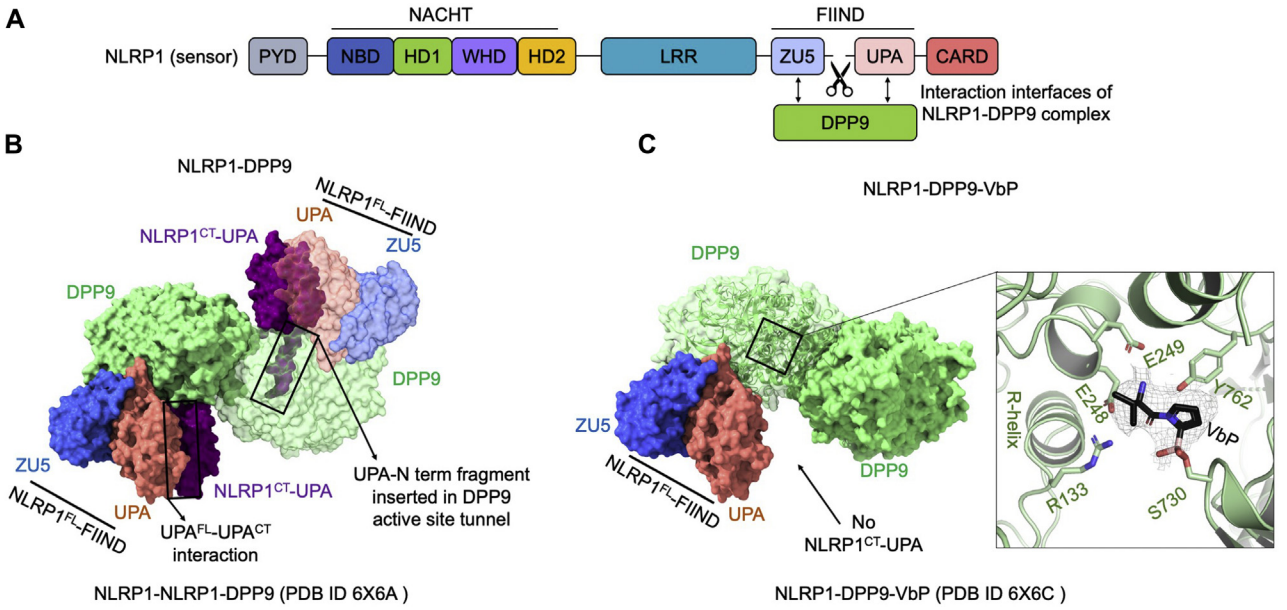

FIG 4. Structural insights on the NLRP1 inflammasome. **A**, Domain architecture of NLRP1. Interactions of NLRP1 with DPP9 are shown with lines. **B**, Cryo-EM structure of the NLRP1-DPP9 ternary complex, composed of NLRP1^{FL}-FIIND, NLRP1^{CT}-UPA, and DPP9. NLRP1^{CT}-UPA N-terminal peptide interacts with DPP9 by inserting itself in the DPP9 active-site tunnel. The UPA^{FL}-UPA^{CT} interaction interface is marked. Domains are color coded as in Fig 4, *A*. **C**, Cryo-EM structure of NLRP1-DPP9 bound with VbP. VbP interacts with the active-site tunnel residues of DPP9 and displaces NLRP1^{CT}-UPA.

solved in the presence of VbP revealed that VbP sits in the active-site pocket, forms a covalent bond with the catalytic residue S730, and interacts with key DPP9 residues including R133 in the R-helix and E248 and E249 necessary for substrate binding. VbP interaction changes the NLRP1-DPP9 complex stoichiometry by greatly reducing bound NLRP1^{CT} (Fig 4, *C*): only very weak density of NLRP1^{CT} was present in the cryo-EM map of the NLRP1-DPP9-VbP complex, further supporting that VbP displaces NLRP1^{CT} from the NLRP1-DPP9 complex[60] and rationalizing earlier reports that VbP destabilizes the complex.[65,66]

In addition to the distinct interfaces between NLRP1 and DPP9, there is a UPA-UPA interaction between NLRP1^{FL} and NLRP1^{CT} in the tripartite NLRP1-DPP9 complex. This interaction is largely mediated by a translational relationship in which one surface of UPA in one NLRP1 molecule interacts with the opposing surface of UPA in another NLRP1 molecule. This interaction is strikingly important for both NLRP1 repression and NLRP1 activation. NLRP1 mutations at this surface abolished gasdermin D activation and ASC speck formation and abrogated NLRP1 CT-mediated pyroptosis.[60]

Physiologically, because of its high expression in keratinocytes, germline mutations of human NLRP1 lead to skin-related inflammatory diseases: multiple self-healing palmoplantar carcinoma, familial keratosis lichenoides chronica,[67] vitiligo,[68,69] and autoinflammation with arthritis and dyskeratosis.[66,70] Among these deleterious mutations, A54T, A66V, M77T,[67] L155H,[68,69] R726W,[70] ΔF787-R843,[68] and M1184V[68] lie in the autoinhibitory N-terminal, whereas P1214R[66,70] localizes at the DPP9 substrate motif of the inflammatory CT, causing constitutive NLRP1 activation and downstream pyroptosis.[66,67]

DEATH DOMAIN FOLD OLIGOMERIZATION IN INFLAMMASOME SIGNALING

CARD and PYD belong to the death domain superfamily, and are present in most inflammasome proteins, including sensors, adaptors, and caspase-1 (Fig 2, *A*, Fig 3, *A*, and Fig 4, *A*). On stimulation, the sensor proteins mediate the oligomerization of adaptors, resulting in the oligomerization and activation of associated effectors such as caspase-1. Taking the NLRP3 inflammasome as an example, ligand-induced oligomerization of the sensor protein NLRP3 provides a PYD-PYD interaction platform to nucleate the helical packing of its specific adaptor ASC, which in turn serves as the template for caspase-1 helical filaments assembly through CARD-CARD interactions. The structures of ASC PYD filament, ASC CARD filament, and caspase-1 CARD filament provided mechanisms of assembly and polymerization among these inflammasome proteins[5,71-74] (Fig 5, *A* and *B*).

Recent reports on NLRP1 and CARD8 inflammasomes revealed additional insights into the rules of CARD-mediated polymerization.[75,76] The NLRP1 CT and the analogous CARD8 CT released on functional N-terminal degradation contain the UPA subdomain of the FIIND and a CARD (UPA-CARD) (Fig 5, *C*). Interestingly, the UPA-UPA interaction between NLRP1^{FL} and NLRP1^{CT} observed in the tripartite NLRP1-DPP9 complex structure is also important for NLRP1 and CARD8 UPA-CARD filament formation. UPA subdomain alone in fact can oligomerize likely by propagating the near-translational relationship (Fig 5, *D*), which facilitates the oligomerization of the CARD to form filaments.[75,76] Without UPA, much higher protein concentrations of the CARDs of NLRP1 and CARD8 are needed for polymerization to occur.

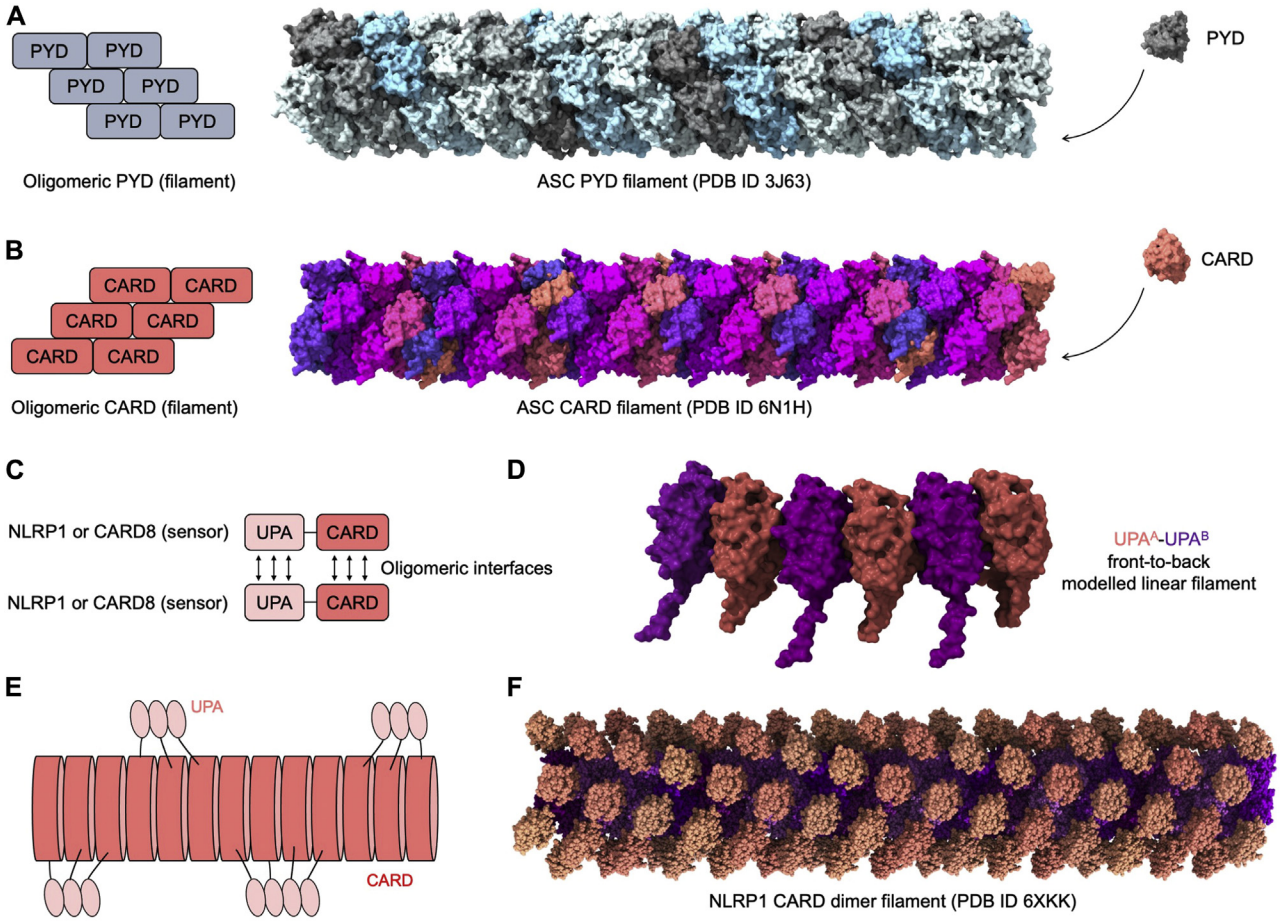

FIG 5. Structural insights on filamentous assemblies in inflammasomes. **A,** Polymerization of ASC PYDs forms a helical filament. **B**, Polymerization of ASC CARDs forms a helical filament. **C**, Domain architecture of cleaved CT fragment of CARD8 and NLRP1. Oligomerization of CARD is facilitated by UPA-UPA interactions. **D**, UPA-UPA filament modeled by UPA-UPA dimer observed in the NLRP1-DPP9 ternary complex (PDB ID 6X6A). **E**, Schematic diagram of how UPA oligomerization facilitates CARD filament formation. **F**, A variant of CARD filament formed by NLRP1 CARD dimers that is about twice the thickness of a conventional CARD filament.

Structurally, UPA is not ordered in the UPA-CARD filament structures, but most likely surrounds the CARD filament core and promotes CARD oligomerization (Fig 5, *E*). Another twist is that NLRP1 CARD filament comprises CARD dimers, whereas the CARD8 CARD filament is like other CARD filaments with monomer CARD units.[75,76] In NLRP1, the core filament is formed by one CARD and the dimeric partner of the core CARD protrudes outside the core filament (Fig 5, *F*). This helical assembly of CARD dimer is a novel mode of filament formation, which adds a variation to the existing scheme.[75,76]

The discovery of CARD and PYD filaments has deep biological implications. Unlike traditional signaling pathways in which protein molecules form interactions as monomers, dimers, or oligomers, the innate immune system forms higher-order protein complexes for signal transduction. These higher-order complexes are often assembled through homotypic domain-domain interactions into large puncta or specks as signaling hubs. Because of the high cooperativity, these higher-order assemblies have a characteristic threshold or all-or-none signaling behavior in innate immune responses.[77] Because there is a large number of death domain superfamily proteins, it is not surprising that higher-order assembly presents a common signaling scheme within and beyond innate immunity.

SUMMARY AND OUTLOOKS

In this review, we provide a brief overview of the progress in structure determinations and structure-driven mechanistic understandings on inflammasomes. There is growing evidence that inflammasome activation mechanisms are diverse, contrary to what was thought previously. Increasing structural insights in addition to biochemical and cellular studies have demonstrated the importance of understanding molecular details of inflammasome activation and their diverse mechanisms. Recent structures of NLRC4,[17-20,78,79] NLRP3,[35] and NLRP1[60,61,75,76] directly reveal and support diverse activation mechanisms of inflammasomes. There are, however, still many open questions on how these inflammasomes are regulated.

The biggest remaining question for the NLRP3 inflammasome is how it gets activated in cells. This question is especially intriguing because NLRP3 can be activated by many PAMPs or DAMPs, raising the suggestion that none of these signals are direct NLRP3 activators. Together with NEK7, which plays a

critical role[26-28,35] as discussed above, a true mediator that would release NLRP3 from an inhibited form to an active form still remains to be discovered. The importance of NLRP3 trafficking from Golgi to the microtubule organizing center for its activation[80-82] is consistent with the microtubule organizing center localization of NEK7, but how the trafficking is mediated and regulated remains enigmatic. For the NLRP1 inflammasome, a recent study showed that it also acts as a direct sensor of double-stranded RNA,[83] in addition to its sensing of proteases and ubiquitin ligases that induce functional NLRP1 degradation. Future structural work is required to elucidate these and other questions in inflammasome activation and regulation.

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