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Caging NLRP3 tames inflammasome activity

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Abstract/summary:
How the danger sensor NLRP3 is activated is intensively debated. Using cryo-electron microscopy (EM) approaches, Andreeva and colleagues made the remarkable discovery that inactive NLRP3 forms a double ring of 12-16 monomers that shield its pyrin domains from the cytosol. We discuss this surprising new mechanism of inflammasome regulation.

Main text:
NACHT, LRR and PYD domain-containing protein 3 (NLRP3) is a cytosolic sensor of disrupted cellular homeostasis such as ionic flux. NLRP3 consists of an N-terminal pyrin (PYD) domain, a central NACHT domain that mediates ATP hydrolysis, and a C-terminal leucine rich repeat (LRR) domain (Figure 1A). Prior to activation, NLRP3 interacts with the protein NEK7, which facilitates NLRP3 signaling. Upon activation, NLRP3 assembles an inflammasome complex via PYD-PYD interactions with the adapter Apoptosis-associated speck-like protein containing a CARD (ASC), forming a platform for caspase-1 dimerization and activation. Active caspase-1 cleaves interleukin(IL)-1β, IL-18, and Gasdermin D (GSDMD), resulting in cytokine release, cell death and a highly inflammatory milieu. NLRP3-driven inflammation accompanies the pathogenesis of many human diseases and NLRP3 is an attractive drug target (Swanson et al., 2019). Defining the structure and activation mechanisms of NLRP3 are thus of great interest. In this issue of Cell, Andreeva and colleagues (Andreeva et al., 2021) describe the first structure of full-length NLRP3 in its inactive state, providing new insights into how NLRP3 is prevented from inadvertent activation and providing a basis for rationale design of drugs to lock NLRP3 in its inactive state.

Cryo-EM imaging showed that amazingly, murine NLRP3 spontaneously forms a large oligomer consisting of a circular assembly of six to eight NLRP3 dimers. The six-dimer ring was resolved to 4.2 Å. Here, the NACHTs are barely in contact, while the LRRs of each NLRP3 molecule interlock with another monomer forming a pair; each pair forms a back-to-back interface with other pairs to form a double ring (Figure 1B). This 'cage' structure is consistent with a pre-print cryo-EM structure of inactive human NLRP3 that forms a decamer (Hochheiser et al., 2021). Why different NLRP3 stoichiometries are reported is unclear but could reflect species differences or technical aspects.

Although the PYD domains could not be resolved by Andreeva and co-workers, their presumed density in the cryo-EM model suggests that the PYDs are contained inside the ring of NACHT-LRRs. PYD shielding within the NACHT-LRR cage may prevent aberrant interactions with ASC when NLRP3 is in its inactive, closed conformation (Figure 1B), and indeed, the double ring NLRP3 cage could not nucleate ASC. The NLRP3 NACHT-LRR domains bind to NEK7 (Sharif et al., 2019), but importantly, cannot do so within the NLRP3 cage because the NLRP3 double ring structure forms via LRR interactions along similar interfaces to those required for NEK7. A major structural rearrangement must occur for

NLRP3 to transit from the inactive cage structure to the active NLRP3 structure, which is likely a single ring formed by NACHT-NACHT interactions, akin to the NAIP-NLRC4 inflammasome (Duncan and Canna, 2018). It is possible that the cage may provide a mechanism for rapid NLRP3 activation, as NLRP3 monomers are assembled and on standby to sense a signal and convert to their active conformation.

Given that NLRP3 recruitment to the *trans*-Golgi network (TGN) may be necessary for NLRP3 signaling (Chen and Chen, 2018), the current study investigated the cellular location of the NLRP3 cage. Membrane extracts from inflammasome-resting cells indeed contained large NLRP3 oligomers consistent with the purified double ring, while cytosolic NLRP3 was monomeric or dimeric. The authors confirmed previous findings (Chen and Chen, 2018) that the NLRP3 polybasic motif (Figure 1A) mediates TGN membrane association, and discovered that this motif is also required for NLRP3 to form the double ring oligomer. Using a tag to force NLRP3 relocation to the Golgi membrane induced the double ring structure to form, suggesting that TGN membrane association is sufficient for NLRP3 cage assembly.

NLRP3-activating stimuli cause TGN dispersal concomitant with NLRP3 activation (Chen and Chen, 2018). Andreeva et al., report that NLRP3, and the NLRP3 cage in particular, are necessary for TGN dispersal, as an NLRP3 activator fails to induce TGN dispersal in *NLRP3*-deficient cells, or cells expressing an NLRP3 mutant that is unable to form the cage. Future work could address whether NLRP3 double ring cages are associated with other membrane types, and how this contributes to NLRP3 signaling. Endosomes are of particular interest as pre-prints report that NLRP3 activators disrupt endosomal trafficking (Lee et al., 2021; Zhang et al., 2021).

The cage structure has interesting implications for NLRP3 activation by ionic changes. The NLRP3 PYD-NACHT linker includes a fish-specific NACHT associated domain (FISNA; Figure 1A) that senses ionic flux and allows NLRP3 to adopt an active configuration (Tapia-Abellán et al., 2021) and an overlapping polybasic motif that enables membrane interaction (Chen and Chen, 2018). In the NLRP3 double ring structure, the arrangement of the PYD-NACHT linker will dictate how the PYD is positioned inside the cage structure. It is tempting to speculate that the FISNA must be exposed in the NLRP3 cage, so that it can sense ionic flux and respond by triggering the opening of NLRP3 monomers, and ATP hydrolysis, to enable NLRP3 to adopt its active quaternary conformation.

It is interesting to consider whether the NLRP3 cage structure is influenced by post-translation modifications or natural variants. The authors modelled the LRR phosphorylation sites S803 and Y858 and propose that these may disrupt LRR interactions in the double ring structure. Point mutations in NLRP3 cause hyperactive signaling leading to autoinflammatory syndromes. It is possible that such LRR mutations, while rare, may alter the stability of the double ring structure to influence the NLRP3 activation threshold. The HSP90-SGT1 chaperone complex binds to NLRP3 LRRs and is required for maintaining NLRP3 in an inactive state that is poised for signaling (Mayor et al., 2007). Whether HSP90-SGT-1 associates with the NLRP3 cage or regulates its trafficking is unclear. Intriguingly, the NLRP3 LRR domain is reportedly dispensable for NLRP3 signaling (Hafner-Bratkovič et al., 2018). It must therefore be possible to activate NLRP3 without the prior formation of the double ring cage.

This study significantly advances our knowledge of NLRP3 biology by demonstrating how NLRP3 remains inactive, adding a novel regulatory step to a complex activation mechanism. Alongside two recent reports of NLRP3 structures containing the inhibitor MCC950 (Dekker et al., 2021; Hochheiser et al., 2021), this study will advance the rational design and development of new chemical entities that keep NLRP3 safely tamed inside its cage.

Acknowledgements
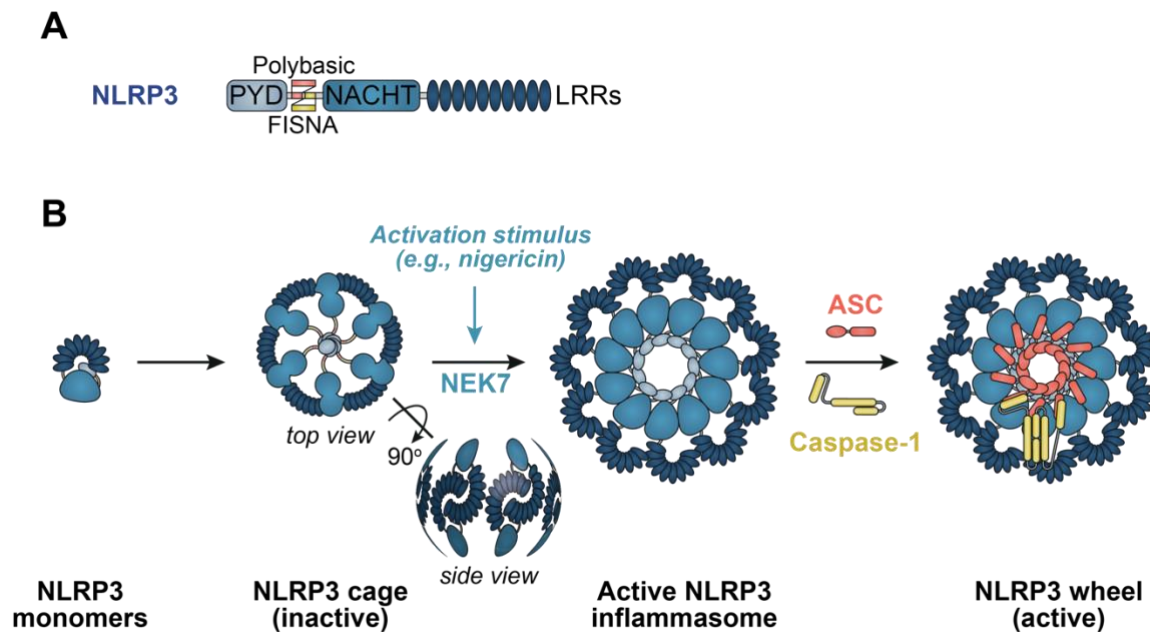
Declaration of interests

Figure 1. Proposed model for NLRP3 activation leading to inflammasome signaling. A. NLRP3 domain structure showing the pyrin domain (PYD), NACHT domain (NACHT), leucine rich repeats (LRRs), polybasic motif (Polybasic) and fish-specific NACHT associated domain (FISNA) **B.** An NLRP3 cage shields the PYDs from signaling in resting cells. When caged NLRP3 monomers sense an activation stimulus (e.g., nigericin), NLRP3 conformation rapidly switches to its active, single ring oligomeric structure that can recruit ASC and nucleate ASC polymerization. ASC fibers then recruit caspase-1 monomers, leading to caspase-1 clustering, proximity-induced dimerization, and activation, resulting in the generation of the proteolytically active inflammasome complex.

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