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The hierarchical structural architecture of inflammasomes, supramolecular inflammatory machines

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Inflammasomes are caspase-1 activating, molecular inflammatory machines that proteolytically mature pro-inflammatory cytokines and induce pyroptotic cell death during innate immune responses. Recent structural studies of proteins that constitute inflammasomes have yielded fresh insights into their assembly mechanisms. In particular, these include a crystal structure of the CARD-containing NOD-like receptor NLRC4, the crystallographic and electron microscopy (EM) studies of the dsDNA sensors AIM2 and IFI16, and of the regulatory protein p202, and the cryo-EM filament structure of the PYD domain of the inflammasome adapter ASC. These data suggest inflammasome assembly that starts with ligand recognition and release of autoinhibition followed by step-wise rounds of nucleated polymerization from the sensors to the adapters, then to caspase-1. In this elegant manner, inflammasomes form by an 'all-or-none' cooperative mechanism, thereby amplifying the activation of caspase-1. The dense network of filamentous structures predicted by this model has been observed in cells as micron-sized puncta.

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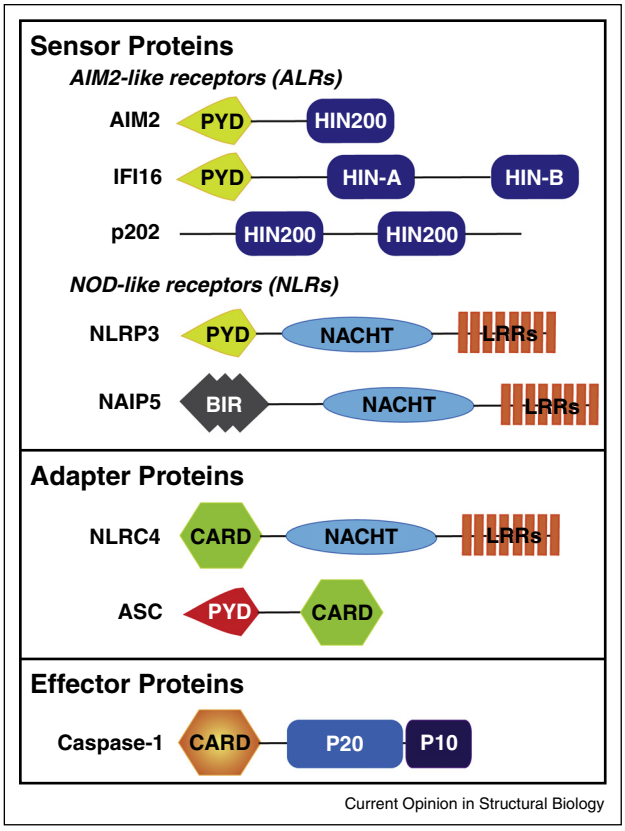
Introduction

The immune system protects organisms from infections and other types of insults; It consists of an innate immune component and an adaptive immune component. Innate immunity offers the first line of defense and is mediated by germ line encoded pattern recognition receptors (PRRs) that recognize pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) [1–3]. PRRs include cell surface and endosomal Toll-like receptors (TLRs), as well as cytosolic PRRs such as RIG-I-like receptors (RLRs), AIM2-like receptors (ALRs), NOD-like receptors (NLRs) and cyclic GMP-AMP synthase (cGAS) [4•]. PRR signal transduction induces a plethora of cellular reactions to counter immediate dangers, including cytokine secretion, cell death and interferon response. It also helps to initiate adaptive immunity for antigen-specific defense mechanisms.

Over the last decade, the classical model of signal transduction as a series of binding events that trigger the allosteric activation of enzymes and other downstream effector molecules has been expanded to include the formation of large supramolecular assemblies that explain the threshold kinetics and signal amplification observed in many innate, as well as adaptive, immune signaling pathways [5••,6••,7,8]. These assemblies, which we recently named supramolecular organizing centers (SMOCs), often manifest as large, heterogeneous micron-sized puncta in cells [6••]. Recent advances in cryo-electron microscopy (cryo-EM) and single-molecule fluorescence microscopy have begun to bring the structural characterization of these puncta from the micrometer-scale to the sub-nanometer-scale [9,10]. In this review, we will focus on one class of SMOCs, known as inflammasomes, that are caspase-1 activating machines [11••,12]. The hierarchical assembly mechanism illustrated here, involving successive steps of nucleated polymerization, may be general to other innate immune signaling pathways.

Canonical inflammasomes are formed by the assembly of three classes of molecules: sensors in the ALR and NLR family, adapters such as apoptosis-associated, speck-like protein containing a CARD (ASC) [13], and effectors such as caspase-1 (Figure 1). Caspase-1 activation leads to processing of the pro-forms of interleukin-1β (IL-1β) and IL-18 into their mature forms for secretion, and induces an inflammatory cell death known as pyroptosis [11••,12]. ALRs include absent in melanoma 2 (AIM2) and interferon-inducible protein 16 (IFI16), and are composed of an N-terminal pyrin domain (PYD) and a C-terminal, ~200 amino acid hematopoietic, interferon-inducible, nuclear localization (HIN) domain [14–17]. Although the HIN domain detects phagocytosed or actively replicating viral dsDNA in the cytosol, the PYD recruits the adapter ASC through homotypic PYD/PYD interactions. Negative regulators of ALR

Figure 1



Domain structures of inflammasome proteins. Domain abbreviations are as follows: PYD: Pyrin domain (yellow and red symbols); HIN: hematopoietic, interferon-inducible, nuclear localization domain (dark blue rounded rectangles); NACHT: nucleotide-binding and oligomerization domain (cyan elongated ovals); LRRs: leucine-rich repeats (repeating orange rectangles); BIR: baculovirus IAP repeat (dark gray overlapping diamonds); and CARD: caspase recruitment domain (light green and orange hexagons). Caspase domain: p20 and p10 as the large and small subunits, respectively (blue and purple rounded rectangles).

inflammasome formation, such as p202, have only two HIN domains and lack the PYD [14]. ASC also contains a CARD domain, which is responsible for caspase-1 recruitment and activation [13]. NLRs have a more complex domain architecture with variable N-terminal domains, a central nucleotide binding and oligomerization (NACHT) domain that shares homology with the AAA+ superfamily of ATPases, and a C-terminal leucine rich repeat (LRR) domain [11••,12]. The biggest subfamily of NLRs is the NLRP family, with the 'P' representing the N-terminal PYD domain. The NLRP inflammasomes require the adapter ASC to mediate caspase-1 activation. The N-terminal BIR domain-containing NLRs, such as NAIPs, detect bacterial flagellin and type III secretion proteins: NAIP5 detects flagellin, and NAIP2 specifically detects the rod protein of the type III secretion system [18–20]. Although named as an NLR, the N-terminal CARD-containing protein NLRC4 is now known to function as an adapter to the NAIP sensors [18–22]. Upon ligand binding, NAIPs can interact with NLRC4 to form a NAIP/NLRC4/caspase-1 inflammasome in the absence of ASC [18–22]. However, recent studies suggest that NLRC4 also interacts with ASC, and that the NAIP inflammasome co-localizes with NLRP3 in a single speck in THP-1 macrophages upon infection with *Salmonella typhimurium* derived flagellin [23].

Inflammasome assembly and caspase activation proceed in several steps. First, in the resting state, sensor molecules, either ALRs or NLRs, appear to exist in autoinhibited conformations. Second, upon encountering PAMPs and DAMPs such as flagellin, lipoproteins, viral dsDNA, uric acid crystals and extracellular ATP, ALR or NLR sensors undergo conformational changes that overcome the autoinhibition. Third, for sensor proteins with a PYD such as AIM2 and NLRP3, clustering of PYDs ensues, which recruits ASC and promotes multivalent PYD/PYD interactions. Alternatively, for the NAIP inflammasomes, recruitment of NLRC4 follows, which leads to clustering of NLRC4 CARDs. Fourth, clustered ASC and NLRC4 CARDs recruit pro-caspase-1, whose expression is upregulated by prior NF-κB driven transcription. Activated caspase-1 cleaves pro-IL-1β and pro-IL-18 to generate the mature forms of these proinflammatory cytokines. It may also cleave a collection of additional substrates to induce the pyroptotic cell death that is associated with swelling and rupture of cellular membranes. The following sections will use existing structural information to illustrate each of these steps in inflammasome assembly and activation.

Auto-inhibition in ALR and NLR sensor and adapters through intramolecular interactions

Both inflammasome sensors and adapters have been proposed to exist in an autoinhibited conformation before activation. A structure of the mouse NLRC4 adapter lacking the N-terminal CARD (ΔCARD mNLRC4) provides insights about the mechanism of autoinhibition in this NLR. NLRC4 functions as an adapter protein in NAIP/NLRC4/caspase-1 inflammasome formation [24••]. The NACHT domain of NLRC4 is composed of a nucleotide-binding domain (NBD), a helical domain 1 (HD1), a winged helix domain (WHD), and a helical domain 2 (HD2) (Figure 2a). The autoinhibited mNLRC4 assumes a closed conformation with intricate intramolecular interactions that sequester mNLRC4 in a monomeric state (Figure 2a). Without oligomerization, the CARD of NLRC4 is unable to recruit caspase-1 through CARD–CARD interactions, thereby affording a safety mechanism against auto-activation in the absence of proper stimulation. In the autoinhibited ΔCARD mNLRC4 structure, an ADP molecule is bound at the interface between the NBD and WHD (Figure 2a). The