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CELL SCIENCE AT A GLANCE

Inflammasome activation and assembly at a glance

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ABSTRACT

Inflammasomes are multimeric protein complexes that typically comprise a sensor, an adaptor and the zymogen procaspase-1. An inflammasome assembles in response to a diverse range of pathogen-associated or danger-associated molecular patterns (PAMPs or DAMPs). The inflammasome platform leads to activation of caspase-1 through proximity-induced self-cleavage, which further induces maturation of interleukins 1β and 18 (IL-1β and IL-18) through proteolytic cleavage of pro-IL-1β and pro-IL-18. Activated caspase-1 also cleaves gasdermin D, which leads to a particular form of cell death called pyroptosis. Mutations in genes that encode inflammasome components are associated with many inflammatory disorders, and studies in the past decade have highlighted the importance of appropriate activation of the inflammasome in homeostasis and disease pathogenesis. Therefore, much attention is being paid to uncover the modulators and regulators of inflammasome assembly and pyroptosis. This Cell Science at a Glance article and accompanying poster outlines the concepts in the activation of inflammasome sensors and assembly of the inflammasome platform. We also discuss recent insights into the mechanisms of regulation of inflammasome activity and the induction of cell death by pyroptosis.

KEY WORDS: Inflammasome, Pyroptosis, Cell death, IL-1 cytokines

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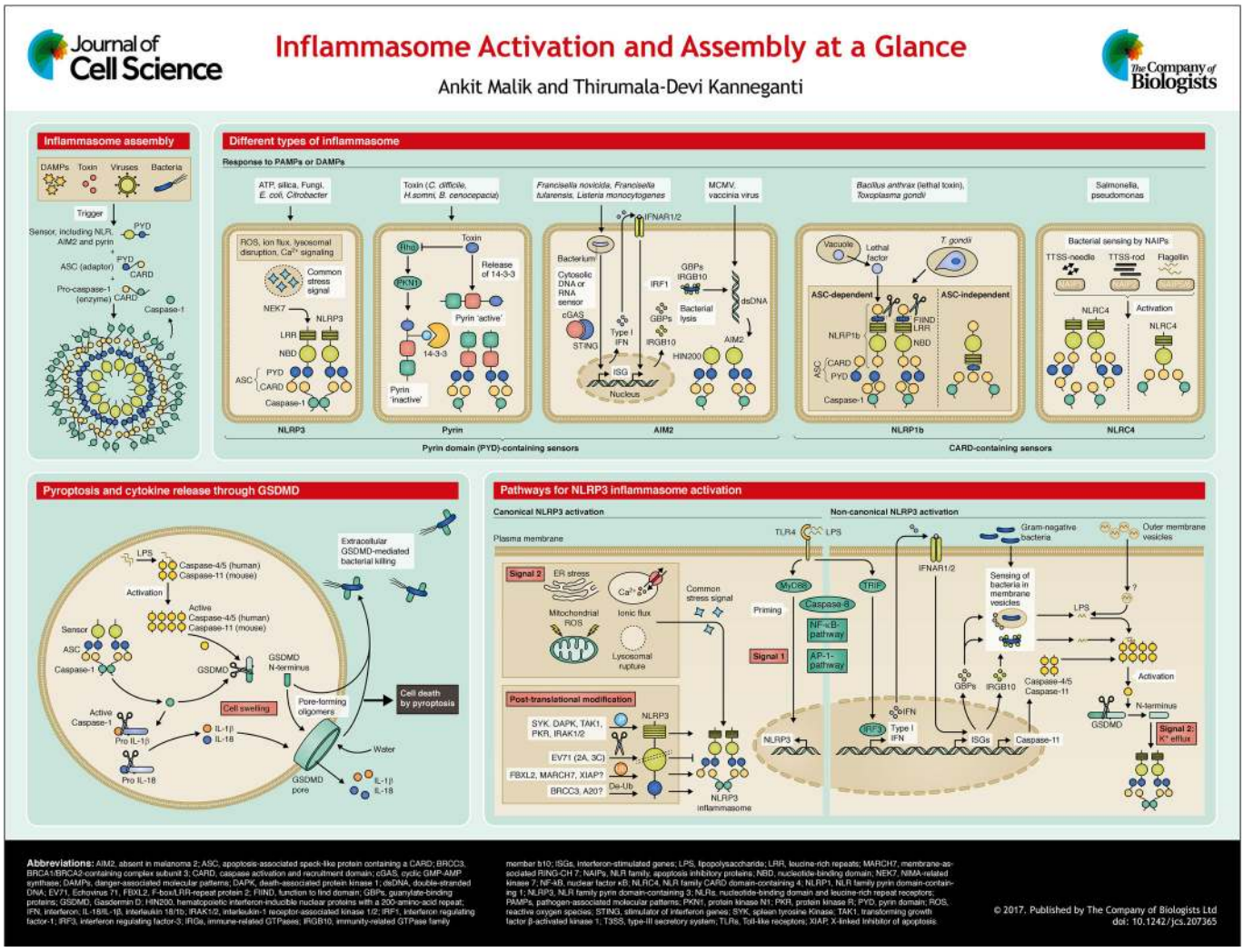

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Introduction

Sensors of PAMPs and DAMPs in the cytoplasm include nucleotide-binding oligomerization domain (NOD)- and leucine-rich repeat (LRR)-containing receptors (NLRs), the absent in melanoma-2 (AIM2)-like receptors (ALRs) and proteins that contain a tripartite motif (TRIM), including pyrin. Among these sensors, members of the evolutionary conserved NLRs, together with AIM2 and pyrin, can assemble into a multimeric protein complex that is called the inflammasome (see poster). Inflammasomes are typically composed of a sensor, the adaptor molecule ASC [apoptosis-associated speck-like protein containing a caspase-activation and recruitment domain (CARD); also known as PYCARD] and the cysteine protease procaspase-1. Upon detection of specific stimuli, the sensor, which includes NLRs, AIM2 or pyrin, recruits ASC to form a multimeric complex that is referred to as 'speck'. Oligomerized or 'nucleated' ASC recruits procaspase-1 into the complex, which then is converted into bioactive caspase-1 by proximity-induced self-cleavage. Following this, the active caspase-1 subunits p20 and p10 cleave pro-IL-1β and pro-IL-18 to yield bioactive cytokines and activate pore-forming gasdermin D (GSDMD) to induce a form of cell death called 'pyroptosis' (see poster and Box 1). Inflammasome activation serves critical functions in pathogen defense; it helps to remove damaged and transformed host cells and stimulates an adaptive immune response. Conversely, aberrant inflammasome activation is linked to many inflammatory disorders, infectious diseases and cancer. Therefore, inflammasome activation is a tightly regulated event that encompasses many molecular and cellular signals. Here, we summarize the mechanisms that regulate the activation of the inflammasome sensors and the assembly of the inflammasome platform. We also discuss recent insights on the induction of pyroptosis and open questions in inflammasome biology.

Inflammasome assembly

All members of the NLR family of proteins contain a central nucleotide-binding domain (NBD), and most also have a variable N-terminal domain and a C-terminal LRR domain. Based on the presence of an N-terminal pyrin domain (PYD) or CARD, this family is further divided into NLRP or NLRC receptors. The human and mouse genomes encode 22 and 34 NLRs, respectively (Harton et al., 2002). Among them, NLRP1, NLRP3 and NLRC4 are the NLRs capable of inducing the formation of the inflammasome as a platform to activate caspase-1. NLRP12, NLRP6 and NLRP9b have also been suggested to form inflammasomes, but their role as inflammasome sensors is not well defined (Anand et al., 2012; Elinav et al., 2011; Mamantopoulos et al., 2017; Vladimer et al., 2012; Zhu et al., 2017).

Inflammasome assembly requires homotypic CARD–CARD or PYD–PYD interactions between its components, and both the PYD and CARD domains can induce oligomerization, which is the basis of inflammasome assembly (Cai et al., 2014; Lu et al., 2014; Sborgi et al., 2015). When the ligand is detected, the sensor is relieved from an inhibitory state and oligomerizes or nucleates ASCs by inducing homotypic interactions between their PYDs. Next, ASC recruits pro-caspase-1 through interactions between its CARD domain with that of the caspase. The resultant multimeric inflammasome complex contains the sensor, the adaptor and the enzyme at increasing stoichiometric ratios (Lu et al., 2014) (see poster). The assembly of NLRP3, AIM2 and pyrin inflammasomes is strictly dependent on the adaptor ASC. In contrast, NLRP1 and NLRC4 possess a CARD domain and can recruit caspase-1 directly (Jin et al., 2013a; Nour et al., 2009; Ponomareva et al., 2013). Therefore, NLRP1 and NLRC4 can induce inflammasome assembly and pyroptosis independently of ASC. However, ASC recruitment into the complex still promotes the efficient processing of IL-1β and IL-18 (Broz et al., 2010; Guey et al., 2014; Mariathasan et al., 2004; Van Opdenbosch et al., 2014). As inflammasome assembly requires homotypic CARD–CARD and PYD–PYD interactions, certain PYD-only proteins (POPs) and CARD-only proteins (COPs) can act as dominant-negative regulators of inflammasome assembly (Matusiak et al., 2015).

Box 1. Pyroptosis

Pyroptosis is an inflammatory form of cell death that is induced by inflammasome activation; it is characterized by swelling of the cell that is followed by lysis and release of intracellular contents. Loss of osmotic potential had been previously implicated as a critical event in cell death that is induced by pyroptosis (Fink et al., 2008; Fink and Cookson, 2006), but how inflammasome activation induced loss of osmotic potential was not known. Gasdermin D (GSDMD) was recently identified as the missing link between cell death and caspase-1 and/or caspase-11 activation (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015b). The N-terminal domain of GSDMD exhibits robust and specific binding to membrane lipids, phosphoinositides and cardiolipin. It is held in an inhibited form by its C-terminal domain (Aglietti et al., 2016; Ding et al., 2016; Liu et al., 2016; Sborgi et al., 2016). Pyroptotic caspases 1 and 11 (caspase-4 and caspase-5 in humans) cleave GSDMD at D276 in the linker region, thereby relieving the intramolecular inhibitory effect of the C-terminal domain (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015b). Following the binding to membrane lipids, this N-terminal region of GSDMD (GSDMD-N) oligomerizes to form pores with an inner diameter of 10 to 14 nm (see poster) (Ding et al., 2016). The formation of these pores disrupts the osmotic potential of the cell, thereby causing its swelling and lysis. Therefore, whereas IL-1β is processed normally in GSDMD-deficient cells, its release is abrogated. Expression of the N-terminal fragment is also sufficient for killing bacterial cells (He et al., 2015; Shi et al., 2015b).

GSDMD belongs to the gasdermin family that shares 45% sequence homology amongst its six members. Except for autosomal recessive deafness type 59 protein (DFNB59), all other GSDM proteins have a GSDMD-like two-domain structure. In addition, similar to GSDMD, their N-terminus can induce pyroptosis (Ding et al., 2016; Shi et al., 2015b). However, unlike GSDMD, other members lack the pyroptotic caspase cleavage site (Shi et al., 2015b), which underscores its importance in pyroptosis. Although GSDMD can be cleaved by caspase-3 (Wang et al., 2017), if and how other GSDM molecules are also cleaved to release the pore-forming N-terminus is not yet known. It is also not clear why pyroptosis follows inflammasome activation in macrophages (Fink et al., 2008), dendritic cells (Edgeworth et al., 2002) and epithelial cells (Sellin et al., 2014), but not in neutrophils or monocytes (Chen et al., 2014; Gaidt et al., 2016; Miao et al., 2010a). Whether these cell type-specific differences are due to differences in GSDMD expression and regulation is another unanswered question.

Inflammasome sensors

NLRP1

The first sensor that was identified as being capable of forming the inflammasome was NLRP1 (Martinon et al., 2002). Human NLRP1 contains a PYD, NBD and LRR domain, a 'function-to find' domain (FIIND) and a C-terminal CARD. However, the mouse genome encodes for three NLRP1 paralogs (a–c), all of which lack the PYD. NLRP1b is activated by cleavage at its N-terminal and FIIND domains by lethal factor (LF), a component of the anthrax lethal toxin (LeTx) produced by *Bacillus anthracis* (Boyden and Dietrich, 2006; Chavarría-Smith and Vance, 2013; Finger et al., 2012; Frew et al., 2012). This NLRP1b activation by LeTx has been shown to protect mice from *Bacillus anthracis* infection (Moayeri et al., 2010; Terra et al., 2010). Single-nucleotide polymorphisms (SNPs) in *NLRP1*

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are associated with congenital toxoplasmosis, and, furthermore, NLRP1 activation is required to limit *Toxoplasma* infectivity (Witola et al., 2011). Although the NLRP1b inflammasome is activated by *T. gondii*, no cleavage product for NLRP1b is detectable in response to the parasitic stimuli (Cavailles et al., 2014; Ewald et al., 2014; Gorfu et al., 2014), which suggests that NLRP1b proteolysis, or cleavage, may not be a global mechanism for its inflammasome assembly. As mentioned above, NLRP1 can also induce ASC-independent inflammasome assembly (see poster).

Whereas the specific trigger(s) for NLRP1a-mediated inflammasomes are unknown, mice homozygous for a dominant-negative Q593P mutation in NLRP1a develop an autoinflammatory disease that is dependent on caspase-1 and IL-1β. These mice (*Nlrp1a*$^{Q593P/Q593P}$) have aberrant myelopoiesis and display an increased susceptibility to chemoablation and infection with lymphocytic choriomenigitis virus (LCMV) (Masters et al., 2012); in contrast, *Nlrp1a*$^{-/-}$ mice demonstrate enhanced hematopoietic recovery in response to the same stresses. However, how NLRP1 affects these processes is largely unknown.

NLRP3

NLRP3 is composed of an N-terminal PYD, a central NBD and a C-terminal LRR domain. Mutations in *NLRP3* have been observed in autoinflammatory disorders such as cryopyrin-associated periodic syndromes (CAPS) that are characterized by episodic skin rashes and fever (Feldmann et al., 2002; Hoffman et al., 2001; Neven et al., 2004). NLRP3 was eventually discovered to function as a NLR that forms inflammasomes and senses a vast array of infectious and endogenous DAMPs. These include microbial cell wall components, nucleic acids, pore-forming toxins, environmental crystalline agents such as silica and endogenous molecules, including ATP and uric acid crystals (Cassel et al., 2008; Cruz et al., 2007; Dostert et al., 2008; Hornung et al., 2008; Kanneganti et al., 2006a,b). As the NLRP3 inflammasome assembles in response to a vast range of DAMPs, it is likely that it senses a common cellular distress signal that is induced by these molecules, instead of undergoing a direct interaction with all of these triggers. Changes in cell volume, rupture of lysosomes, production of reactive oxygen species (ROS), K^+ efflux and Ca^{2+} signaling have all been proposed as the distress signals that are sensed by NLRP3 (Compan et al., 2012; Halle et al., 2008; Kanneganti et al., 2007; Munoz-Planillo et al., 2013; Schorn et al., 2011; Zhou et al., 2011).

Two distinct steps – priming (denoted signal 1 on poster) and inflammasome assembly (signal 2 on poster) – are required to activate the NLRP3 inflammasome (see the poster section on pathways for NLRP3 inflammasome activation). Priming involves activation of myeloid differentiation primary response protein (MyD88) or of other nuclear factor (NF)-κB- or activator protein 1 (AP-1)-activating pathways, which upregulate the expression of *Nlrp3* and other inflammasome components. Apoptotic caspase-8 has also been implicated in inflammasome priming (Gurung et al., 2014; Lemmers et al., 2007), its assembly (Gringhuis et al., 2012; Gurung et al., 2014) and IL-1β processing (Maelfait et al., 2008). In human monocytes, an axis comprising toll-like receptor 4 (TLR4), the adaptor TIR-domain-containing adapter-inducing interferon-β (TRIF; also known as TICAM1), receptor-interacting serine/threonine-protein kinase 1 (RIPK1), FAS-associated death domain protein (FADD) and caspase-8 has been described for NLRP3 inflammasome activation that is independent of K^+ efflux and ASC (Gaidt et al., 2016). In addition to promoting the activity of the NLRP3 inflammasome, caspase-8 can act redundantly with caspase-1 to process IL-1β in a model of osteomyelitis (Gurung et al., 2016; Lukens et al., 2014). Whereas this pathway suggests an integration of inflammasome activation with extracellular ligand recognition signaling, further studies are required to define the molecular basis of the assembly of the NLRP3 inflammasome.

Caspase-11 and the NLRP3 inflammasome

Caspase-11 (which has two orthologs in humans, caspase-4 and caspase-5, and only the ortholog caspase-4 in mice) is an inflammatory caspase that can bind to caspase-1 (Wang et al., 1998). The genes encoding caspase-1 (*Casp1*) and caspase-11 display linkage disequilibrium, and the caspase-11 gene (*Casp4*) was lost when a primary knockout for caspase-1 was generated (in a 129 mouse strain background) (Kayagaki et al., 2011). Therefore, the functional significance of caspase-11 was initially assigned to caspase-1. Caspase-11 was eventually identified to have a non-redundant role in the NLRP3 inflammasome activation and pyroptosis after infection of mice with Gram-negative bacteria *E. coli* and *Citrobacter rodentium* (Kayagaki et al., 2011; Rathinam et al., 2012). Further studies described caspase-11 as a sensor of endotoxin [i.e. lipopolysaccharide (LPS)] in the cytoplasm and a critical regulator of susceptibility to endotoxemia (Kayagaki et al., 2011). Caspase-11 directly binds to the penta- and hexa-acylated lipid A-component of LPS (Hagar et al., 2013) through its CARD domain (Shi et al., 2014). This interaction of LPS and caspase-11 in the cytoplasm is independent of the extracellular detection of LPS, which is mediated by TLR4, MD2 (also known as LY96) and CD14 (Hagar et al., 2013; Kayagaki et al., 2013). In contrast to the upregulation of NLRP3 and ASC by MyD88 and NF-κB-activating signals, expression of caspase-11 is upregulated by TRIF and interferon (IFN)-mediated signaling (Gurung et al., 2012; Rathinam et al., 2012).

The mechanism by which caspase-11 promotes the activation of NLRP3 is, however, unclear. Whereas caspase-11 can bind to caspase-1 (Wang et al., 1998), it is dispensable for inflammasome assembly following the activation of NLRP3 with canonical triggers, such as silica and uric acid crystals. One hypothesis is that caspase-11 instigates an effector molecule that induces NLRP3 inflammasome assembly. For example, caspase-11 can cleave pannexin-1, leading to a drop in intracellular K^+ levels, which then induces activation of the NLRP3 inflammasome (Rühl and Broz, 2015; Schmid-Burgk et al., 2015; Yang et al., 2015). However, K^+ efflux is also associated with NLRP1b activation (Pétrilli et al., 2007). Therefore, a specific link between caspase-11 and the NLRP3 inflammasome is currently unknown. The human genome encodes two orthologs of caspase-11: caspase-4 and caspase-5, either of which can functionally provide the caspase-11 function in murine cells (Shi et al., 2014). However, caspase-4 and caspase-5 may not be redundant, as both are required for cell death and IL-1β release following *Salmonella* infection. In contrast, only caspase-4 is required for inflammasome activation by cytosolic LPS (Baker et al., 2015).

The mechanism by which LPS is delivered into the cytoplasm for recognition by caspase-11 is one of the major questions in the field. A recent study demonstrated that LPS that is contained within the bacterial outer membrane vesicles (OMVs) is detected by caspase-11 (Vanaja et al., 2016) (see poster). However, how OMV-associated LPS is transported into the cytoplasm is unclear. Another study identified a role for interferon-induced molecules in the liberation of LPS from bacteria. The signal transducer and activator of transcription 1 (STAT1)–IRF9 signaling axis downstream of IFNs upregulates the transcription factor IRF1, which in turn promotes the expression of guanylate-binding proteins (GBPs) and immunity-related GTPase family member B10 (IRGB10; UniProt U5NFV2) (Man et al., 2016). These proteins target intracellular bacteria for lysis and enhance the release of LPS into the cytosol, which increases its detection by

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caspase-11 and induces subsequent activation of the NLRP3 inflammasome (Man et al., 2016; Pilla et al., 2014) (see poster). Therefore, IFN signaling promotes both caspase-11 upregulation and liberation of LPS into the cytoplasm. Consistent with its role as a cytosolic LPS sensor and an inducer of the NLRP3 inflammasome, caspase-11 is particularly important in the defense against intracellular bacterial infections (Aachoui et al., 2013; Gurung et al., 2012).

In addition to the above mechanisms of activation, the NLRP3 inflammasome is also regulated by many different types of post-translational modifications and several other cellular mechanisms (Box 2).

NLRC4

NLRC4 was characterized as an NLR capable of binding to and activating caspase-1 through its CARD-domain (Poyet et al., 2001). Subsequently, NLRC4 was identified as inducing inflammasome formation following *Salmonella* infection (Mariathasan et al., 2004). NLRC4 can be activated by the bacterial flagellin (Franchi et al., 2006; Miao et al., 2006; Molofsky et al., 2006; Ren et al., 2006) and components of the flagellin-associated secretion systems (Miao et al., 2010b; Yang et al., 2013; Zhao et al., 2011). However, NLRC4 is not a direct sensor of these ligands. Instead, NLR family apoptosis inhibitory proteins (NAIPs) are the sensors of NLRC4 ligands and are therefore critical for NLRC4 inflammasome activation. Whereas only a single NAIP has been identified in humans (Endrizzi et al., 2000), the mouse genome encodes seven NAIP proteins. Mouse NAIP1 and NAIP2 bind to the bacterial needle and inner rod proteins of the type 3 secretion system (T3SS), whereas NAIP5 and NAIP6 recognize flagellin (Kofoed and Vance, 2011; Rayamajhi et al., 2013; Yang et al., 2013; Zhao et al., 2011) (see poster). The sole human NAIP can sense both flagellin and the components of bacterial T3SS (Kortmann et al., 2015; Yang et al., 2013). The function of other mouse NAIPs is currently unknown.

Inflammasome assembly is initiated by a single activated NAIP molecule that provides a platform for NLRC4 self-oligomerization (Hu et al., 2015; Zhang et al., 2015). In the absence of a ligand, NLRC4 is maintained in an inactive state by its NBD and the winged helix domain (WHD) that stabilize its closed conformation, while the LRR domain provides steric hindrance to its oligomerization (Diebolder et al., 2015; Tenthorey et al., 2014). The importance of these auto-inhibitory mechanisms is underscored by the description of auto-inflammation and spontaneous colitis in humans and mouse models with gain-of-function mutations in *NLRC4* (Canna et al., 2014; Kitamura et al., 2014; Romberg et al., 2014).

Unsurprisingly, NAIPs and NLRC4 are critical components of the host defense against flagellated bacteria. In addition to maturation of IL-1β and IL-18, NLRC4-mediated pyroptosis promotes shedding of infected epithelial cells, which help to control the pathogen load during *Salmonella* infection (Sellin et al., 2014). Apart from pyroptosis and maturation of IL-1β and IL-18, NLRC4 activation also affects other aspects of cell biology such as the release of eicosanoids, including prostaglandins and leukotrienes (von Moltke et al., 2012). Whether the function of NLRC4 in eicosanoid production can be extended to other inflammasomes and cell death pathways is currently unclear.

AIM2

AIM2 is a highly conserved member of the ALR family that contains an N-terminal PYD and a C-terminal hematopoietic interferon-inducible nuclear protein with a 200-amino-acid repeat (HIN200) domain (Cridland et al., 2012). The PYD and HIN200 domain, when they occur together, are referred to as the PYHIN domain. The ALR family has 14 members in mice and four in humans. Unlike other members of the ALR family, AIM2 lacks a nuclear localization domain and interacts with ASC through its PYD (Hornung et al., 2009).

Although AIM2 was initially seen as an IFN-inducible tumor suppressor, subsequent studies identified it as a cytosolic sensor of double-stranded (ds)DNA that can assemble into inflammasomes (Bürckstümmer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009). The AIM2 inflammasome assembles during viral and bacterial infections of, for example, vaccinia virus, mouse cytomegalovirus (MCMV) (Rathinam et al., 2010), *Francisella tularensis* (Fernandes-Alnemri et al., 2010; Jones et al., 2010) and *Listeria monocytogenes* (Kim et al., 2010; Rathinam et al., 2010) (see poster). In the absence of dsDNA, AIM2 exists in the cytoplasm in an auto-inhibitory state with the HIN200 domain bound to the PYD. Binding to dsDNA by the HIN200 domain relieves this auto-inhibition, which allows the PYD to undergo homotypic interaction with ASC (Jin et al., 2013b).

Box 2. Regulation of the NLRP3 inflammasome

Post-translational modifications are integral to the assembly of the NLRP3 inflammasome, including deubiquitylation of NLRP3 by BRCA1/BRCA2-containing complex subunit 3 (BRCC3) (Py et al., 2013), dephosphorylation of PYD of NLRP3 by phosphatase 2A (Stutz et al., 2017), linear ubiquitin chain assembly complex (LUBAC)-induced ubiquitylation of ASC (Rodgers et al., 2014) and the phosphorylation of ASC by Syk kinase (Hara et al., 2013; Lin et al., 2015). Interleukin-1 receptor-associated kinase 1 (IRAK1) was also identified to have a role in the transcription-independent, early activation of the NLRP3 inflammasome (Fernandes-Alnemri et al., 2013; Lin et al., 2014). Other kinases such as death-associated protein kinase (DAPK) (Chuang et al., 2011) and double stranded RNA-dependent protein kinase (PKR) (Lu et al., 2012) promote NLRP3 assembly through unknown mechanisms. On the other hand, ubiquitylation of NLRP3 by the E3 ligases FBXL2 (Han et al., 2015), MARCH7 (Yan et al., 2015) and XIAP (Yabal et al., 2014) restricts the level and activation of the NLRP3 inflammasome. NLRP3 is also targeted and inhibited by various host factors including Enterovirus 71 (EV71) proteases 2A and 3C that cleave NLRP3 and dampen the innate immune response (see poster).

Inflammasome activation is tightly regulated by multiple cellular mechanisms. Under homeostatic conditions, ASC localizes in the cytosol and the nucleus, whereas NLRP3 is localized on the membrane of the endoplasmic reticulum (ER) (Zhou et al., 2011). Therefore, changes in subcellular localization of NLRP3 and ASC are essential for inflammasome nucleation. Dynein-mediated transport of the mitochondria towards the ER has been proposed as a mechanism for bringing ASC and NLRP3 into close proximity (Misawa et al., 2013). Recent studies have also identified the serine-threonine kinase NEK7, a protein involved in mitotic spindle formation and cytokinesis, as a critical regulator of the assembly of NLRP3 inflammasome (He et al., 2016; Schmid-Burgk et al., 2016; Shi et al., 2015a). NEK7 interacts with the LRR domain of NLRP3 independently of its kinase activity and promotes nucleation (He et al., 2016; Shi et al., 2015a). During cell division, NEK7 is localized on mitotic spindles, which prevents NLRP3 inflammasome assembly (Shi et al., 2015a). It can therefore be hypothesized that NEK7 functions to safeguard dividing cells from the activation of the NLRP3 inflammasome because during cell division, the cytoplasm is enriched with endogenous ligands that could be misrepresented as stress signals.

Autoinflammatory disorders associated with NLRP3 often involve mutations in or around the NBD (Dodé et al., 2002; Feldmann et al., 2002; Hoffman et al., 2001; Neven et al., 2004; Touitou et al., 2004) and display NLRP3 auto-activation (Brydges et al., 2013, 2009; Meng et al., 2009; Mortimer et al., 2016), which suggests an auto-inhibitory function of the NBD in NLRP3 activation. NLRP3 inflammasome, therefore, has multiple levels of regulation including post-translational modifications and structural restriction. Understanding and employing strategies to modulate NLRP3 activation would likely be beneficial in multiple inflammatory disorders.

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The HIN200 domain of AIM2 binds to dsDNA independently of its sequence identity; however, it does require the DNA to be of a certain length (about 80 bp) (Jin et al., 2012; Li et al., 2014). Therefore, AIM2 inflammasome activation is induced by cytoplasmic dsDNA from both pathogenic and host sources.

Interferon signaling is required to induce the activation of AIM2 after infection with the bacterium *Francisella*, but not the DNA virus MCMV. Whereas MCMV actively releases its genomic DNA into the cytoplasm, cGAS-STING-mediated IFN signaling IFN signaling is required to induce the expression of GBPs and IRGB10, which mediate bacterial lysis and release of genomic DNA into the cytoplasm (Man et al., 2015a, 2016; Meunier et al., 2015) (see poster). Intriguingly, AIM2 deficiency actually results in increased type-I IFN production (Corrales et al., 2016; Fernandes-Alnemri et al., 2010; Hornung et al., 2009). Whether this is because of a direct inhibition of interferon-producing pathways by AIM2 is not clear. As AIM2 binds to dsDNA in a sequence-independent manner, it has also been implicated in recognition of host DNA during inflammatory diseases (Choubey, 2012; Dihlmann et al., 2014a; Dombrowski et al., 2011) and cancer (Dihlmann et al., 2014b; Ponomareva et al., 2013). However, the function of AIM2 as a tumor suppressor was found to be independent of its inflammasome activity, but instead dependent on its ability to regulate Akt kinase signaling and cellular proliferation (Man et al., 2015b; Wilson et al., 2015).

Other members of the ALR family can inhibit AIM2: p202 (also known as IFI202) lacks the PYD but has two HIN domains (HIN1 and HIN2). HIN1 can bind to and sequester dsDNA from AIM2, whereas HIN2 can bind to the HIN domain of AIM2 and inhibit inflammasome assembly (Roberts et al., 2009; Yin et al., 2013). In contrast, POP3 lacks a HIN domain, but does have a PYD through which it can bind to AIM2 and so block ASC binding and oligomerization, which blunts the immune response to DNA viruses (Khare et al., 2014). Furthermore, alternatively spliced isoforms of AIM2 that lack the PYD or HIN domain have been predicted (Choubey et al., 2010). These putative, truncated proteins may similarly inhibit AIM2 activation and associated immune responses.

Pyrin

Pyrin was first described as the protein associated with familial Mediterranean fevers (FMF), an autoinflammatory disorder characterized by episodic fever and joint inflammation (Bernot et al., 1998). Recently, pyrin was characterized as an inflammasome sensor (Gavrilin et al., 2012; Xu et al., 2014). In humans, pyrin is composed of an N-terminal PYD, central B-box and coiled-coil domain, and a C-terminal B30.2/SPRY domain. In mice, the C-terminal B30.2/SPRY domain is absent. A mouse model in which the human B30.2 domain was spliced into mouse pyrin to generate a mouse–human chimeric protein develops a severe autoinflammatory disorder (Chae et al., 2011). As evidence for pyrin forming an inflammasome, the autoinflammatory disorder in these mice could be rescued by deletion of caspase-1, ASC and IL-1β, but not NLRP3, caspase-8 or IL-1α (Chae et al., 2011; Sharma et al., 2017).

Recent studies have demonstrated that the pyrin inflammasome assembles upon the modification of cytoskeletal proteins. Toxins produced by various bacterial species, such as *Clostridium difficile* (TcdB), *Vibrio parahemolyticus* (VopS), *Clostridium botulinum* (C3), *Burkholderia cenocepacia* and *Bordetella pertussis* (PT), induce covalent modifications within the switch I region of Rho family members. These modifications include glycosylation, adenylylation and ADP-ribosylation and lead to assembly of the pyrin inflammasome (Xu et al., 2014). The modification of Rho was found to be essential for pyrin inflammasome activation, even though a direct interaction between Rho and pyrin was not detected (Dumas et al., 2014; Xu et al., 2014). Mice with an inactive mutant of the actin-associated WD repeat-containing protein 1 (Wdr1) also develop a pyrin- and IL-18-mediated autoimmune disorder (Kim et al., 2015). Furthermore, defects in the mevalonate kinase pathway (MVK), which is required for synthesis of geranyl pyrophosphate, a substrate involved in geranylgeranylation and function of small cellular GTPases, promotes pyrin inflammasome activation (Akula et al., 2016; Park et al., 2016). The pyrin inflammasome is inhibited by microtubule disruption in response to pyrin-activating stimuli (Gao et al., 2016; Park et al., 2016), although peripheral blood mononuclear cells (PBMCs) from human patients with FMF are recalcitrant to colchicine-mediated inhibition of IL-1β release (Van Gorp et al., 2016). Whereas it has been posited that microtubule disruption reverses the inactivation of Rho, the exact mechanism of colchicine-mediated pyrin inhibition is unknown. These findings suggest that pyrin senses a common stress signal induced by cytoskeletal modifications instead of interacting with the inducers directly, which is similar to the proposed way of action for NLRP3.

Similar to other inflammasomes, cellular machinery exists to prevent pyrin activation under resting conditions. In homeostasis, pyrin is phosphorylated at S242 and bound by 14-3-3 proteins (Masters et al., 2016). TcdB induces dephosphorylation at S242, which relieves the binding of 14-3-3 and allows inflammasome assembly (Akula et al., 2016; Gao et al., 2016; Masters et al., 2016; Park et al., 2016) (see poster). However, the molecular events that control the phosphorylation, dephosphorylation and subsequent nucleation events are unknown. Furthermore, whether auto-activating mutations that are associated with pyrin bypass this regulatory inhibition has also not been studied.

Conclusion and future directions

Inflammasomes play a critical role in health and disease. They are required for maturation and release of IL-1β, IL-18 and other cellular contents, removal of malignant or damaged cells and host defense from infectious agents. Inflammasome activation intersects with many critical cellular processes that include inflammatory signaling, metabolism, cell division and cell death pathways. Given their high inflammatory potential, many cellular processes are also devoted to the regulation of inflammasome activation. In spite of recent progress in elucidating these mechanisms, many fundamental questions still remain. For example, the exact ligand and mechanisms that activate NLRP3- and pyrin-mediated inflammasomes are not entirely known. Furthermore, the mechanisms that regulate sensing of DAMPs and/or PAMPs present within membrane vesicles by sensors that are localized in the cytoplasm are still unclear. Investigating these and other fundamental questions highlighted in this article will help to significantly improve our understanding of the inflammasome biology, and likely yield novel therapeutic options for the associated diseases and conditions.

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Conflict of interest

The authors declare no competing or financial interests.

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Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.207365.supplemental.

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