

REVIEW

The NLRP3–inflammasome as a sensor of organelle dysfunction

Paula I. Seoane^{1,4*}, Bali Lee^{1,4*}, Christopher Hoyle^{1,4*}, Shi Yu^{1,4}, Gloria Lopez-Castejon^{2,4}, Martin Lowe³, and David Brough^{1,4}

Diverse pathogen- and damage-associated stresses drive inflammation via activation of the multimolecular NLRP3–inflammasome complex. How the effects of diverse stimuli are integrated by the cell to regulate NLRP3 has been the subject of intense research, and yet an accepted unifying hypothesis for the control of NLRP3 remains elusive. Here, we review the literature on the effects of NLRP3-activating stimuli on subcellular organelles and conclude that a shared feature of NLRP3-activating stresses is an organelle dysfunction. In particular, we propose that the endosome may be more important than previously recognized as a signal-integrating hub for NLRP3 activation in response to many stimuli and may also link to the dysfunction of other organelles. In addition, NLRP3–inflammasome-activating stimuli trigger diverse posttranslational modifications of NLRP3 that are important in controlling its activation. Future research should focus on how organelles respond to specific NLRP3-activating stimuli, and how this relates to posttranslational modifications, to delineate the organellar control of NLRP3.

Inflammation and the NLRP3–inflammasome

Inflammation is an organism’s primary response to infection or injury and is generally a protective response. In mammals, inflammation is characterized by vasodilation and permeation of the vascular endothelium and the recruitment and activation of immune cells to fight infection and clear debris. An inflammatory response ensures removal of detrimental stimuli, as well as restoration of tissue homeostasis through tissue and wound repair. Insufficient inflammation results in persistent unresolved infection, while excessive inflammation contributes to chronic inflammatory disease and can also lead to sepsis and subsequent multiorgan failure. For these reasons, the regulation of inflammation is tightly controlled by the host (e.g., [Netea et al., 2017](#); [Weavers and Martin, 2020](#)).

The initiation of an inflammatory response relies on the recognition of a diverse range of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs; [Takeuchi and Akira, 2010](#)). Different classes of PRRs include the Toll-like receptors (TLRs), C-type lectins, the nucleotide-binding oligomerization domain-like receptors (NLRs), DNA sensors including absent in melanoma 2 (AIM2), and the RNA-sensing retinoic acid-inducible gene I-like helicases ([Schroder and](#)

[Tschopp, 2010](#)). PRRs are found on both immune and nonimmune cells, can be both membrane associated or soluble, and respond to PAMPs and DAMPs by initiating downstream signaling cascades, including the production and secretion of signaling mediators such as cytokines. Cytokines can be either pro- or anti-inflammatory and modulate the immune response, ensuring pathogen elimination and removal of cellular debris while also limiting excessive tissue damage.

Inflammasomes are an important component of an inflammatory response to pathogens or damage ([Liston and Masters, 2017](#)). Inflammasomes are multimolecular protein complexes comprising a soluble PRR, commonly an adaptor protein called ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), and the protease caspase-1 that form when the scaffolding PRR senses or binds to its activating stimuli. Following activation on inflammasomes, caspase-1 cleaves the inactive cytokine precursors pro-interleukin (IL)-1 β and pro-IL-18, which subsequently leads to release of their active forms. Caspase-1 also cleaves gasdermin D, which forms pores in the plasma membrane and in many settings can induce a rapid form of cell death called pyroptosis ([He et al., 2015](#); [Shi et al., 2015](#)).

Although several inflammasomes have been described, the best-characterized inflammasome is formed by NLR family

¹Division of Neuroscience and Experimental Psychology, School of Biological Sciences, Faculty of Biology, Medicine, and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; ²Division of Infection, Immunity, and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine, and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; ³Division of Molecular and Cellular Function, School of Biological Sciences, Faculty of Biology, Medicine, and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; ⁴The Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, UK.

*P.I. Seoane, B. Lee, and C. Hoyle contributed equally to this paper; Correspondence to David Brough: david.brough@manchester.ac.uk.

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pyrin domain containing 3 (NLRP3). The NLRP3 structure consists of three domains: an amino-terminal PYRIN (PYD) domain, a nucleotide-binding NACHT domain containing ATPase activity, and a carboxy-terminal leucine-rich repeat (LRR) domain (Mangan et al., 2018; Fig. 1). The importance of NLRP3 to human disease was first revealed when gain-of-function mutations in NLRP3, resulting in its constitutive activation, were ascribed a causative role in cryopyrin-associated periodic syndromes, neonatal-onset multisystem inflammatory disease, Muckle-Wells syndrome, and familial cold auto-inflammatory syndrome (Aksentijevich et al., 2002; Hoffman et al., 2001). NLRP3 senses a diverse range of PAMP and DAMP stimuli, which seemingly converge on NLRP3 by disrupting cellular homeostasis (Liston and Masters, 2017). Experimental evidence supports a role for NLRP3 in generating the damaging inflammation that is associated with numerous diseases, including Alzheimer's disease (Heneka et al., 2013), gout (Martinon et al., 2006), and atherosclerosis (Düweil et al., 2010). Furthermore, the Canakinumab Anti-inflammatory Thrombosis Outcomes Study, which targeted IL-1 β with the monoclonal antibody canakinumab in patients recovering from myocardial infarction, found that treatment significantly reduced rates of recurrent cardiovascular events (Ridker et al., 2017). Thus, NLRP3 represents a promising therapeutic target.

The pathways activating the NLRP3-inflammasome have been classified to some extent on the nature of the stimulus and the cell type and are variously described as the canonical, non-canonical, and alternative pathways (Mangan et al., 2018). The canonical pathway describes NLRP3 activation in macrophages where two stimuli are required (Fig. 1). In this model, the first stimulus is regarded as the priming step and serves to up-regulate the expression of NLRP3 and pro-IL-1 β . In vitro, priming is usually achieved via the activation of TLR4 by lipopolysaccharide (LPS), an endotoxin found on the outer membrane of Gram-negative bacteria (Hornung and Latz, 2010). However, priming can occur after the activation of various TLRs and cytokine receptors, such as IL-1R and TNF receptor, since they are all upstream of the pro-inflammatory transcription factor NF- κ B (Bauernfeind et al., 2009). As well as initiating gene transcription, priming induces the rapid licensing (a pre-activation step) of NLRP3 in preparation for its activation (Juliana et al., 2012). Activation, the second step in the canonical pathway, is triggered by a broad spectrum of stimuli. The identification of the cellular event responsible for NLRP3 activation via the canonical pathway is complicated by the fact that the diverse NLRP3-activating stimuli initiate multiple cellular stress pathways that are not mutually exclusive. The non-canonical NLRP3 activation pathway results from the sensing of intracellular LPS (Kayagaki et al., 2011) through a mechanism that depends on TLR4-TRIF (TIR domain-containing adaptor-inducing interferon- β) and type I interferons and guanylate-binding proteins, leading to activation of caspase-11 (Santos et al., 2018), which ultimately leads to NLRP3 activation (Kayagaki et al., 2011, 2015; Shi et al., 2014; Rühl and Broz, 2015; Viganò et al., 2015; Yang et al., 2015b). The "alternative" pathway of NLRP3-inflammasome activation is only described in primary human and porcine monocytes and bypasses the

requirement for two-step activation, instead occurring in response to extracellular LPS alone (Gaidt et al., 2016; Netea et al., 2009). In human cells, alternative splicing of the human NLRP3 gene is also reported to regulate NLRP3 activity (Hoss et al., 2019).

There are several aspects of NLRP3-inflammasome activation that remain unanswered. Of particular relevance to this review, the literature regarding the subcellular localization of NLRP3 and the contribution of different cellular organelles to its activation has become complex. Here, we address the relationship between subcellular organelles, the stresses inflicted by NLRP3-activating stimuli, and how these ultimately contribute to NLRP3-inflammasome activation.

Posttranslational modifications (PTMs) of NLRP3

Before describing the literature related to organelles and NLRP3, it is pertinent to discuss the specific signaling events that regulate NLRP3 activation. NLRP3 is tightly controlled through a complex array of PTMs (Fig. 1). The first PTM to consider is phosphorylation. Soluble agonists of G α_s protein-coupled receptors, such as dopamine, forskolin, prostaglandins, and bile acids, raise cellular cAMP and inhibit NLRP3 activation (Guo et al., 2016; Lee et al., 2012; Sokolowska et al., 2015; Yan et al., 2015). cAMP activates protein kinase A (PKA), which directly phosphorylates NLRP3 at S295 (S291 in mouse) and switches off NLRP3 ATPase activity (Mortimer et al., 2016). PKA-phosphorylated NLRP3 is also proposed to be tagged by the E3 ubiquitin ligase MARCH7 and eventually degraded by autophagy (Yan et al., 2015). PKD also appears to phosphorylate NLRP3 at S295, but instead of inhibiting NLRP3, PKD-dependent phosphorylation promotes inflammasome activation (Zhang et al., 2017). It is unclear how phosphorylation at the same site differentially regulates inflammasome activation. c-Jun N-terminal kinase 1 (JNK1) and spleen tyrosine kinase phosphorylate ASC and promote NLRP3-inflammasome assembly. Dual inhibition of JNK and spleen tyrosine kinase by kinase inhibitors R406 and SP600125 inhibits NLRP3-inflammasome activation (Gross et al., 2009; Hara et al., 2013). JNK1 directly phosphorylates NLRP3 at S198 (S194 in mouse) between the NLRP3 PYD and NACHT domain, strongly promoting NLRP3 oligomerization (Song et al., 2017). Kinases that indirectly modulate NLRP3 are also reported to regulate inflammasome activation. For example, selective inhibitors of Bruton's tyrosine kinase inhibit NLRP3-dependent IL-1 β release (Ito et al., 2015). Inhibition of TAK1 blocks inflammasome activation in various cell types (Compan et al., 2012; Gong et al., 2010; Manna et al., 2018; Ninomiya-Tsuji et al., 2003), although prolonged inhibition of TAK1 causes necroptotic cell death and activates NLRP3 (Malireddi et al., 2018; Muendlein et al., 2020; Orning et al., 2018). Dephosphorylation of NLRP3 also promotes its activation. Protein phosphatase 2A (PP2A) inhibitors block NLRP3-inflammasome activation (Luheshi et al., 2012; Perregaux et al., 1996). PP2A alleviates the phosphorylation of NLRP3 at S5 to enable NLRP3 to self-oligomerize and to interact with ASC through PYD-PYD interactions (Stutz et al., 2017). Furthermore, dephosphorylation of NLRP3 at Y861 by protein tyrosine phosphatase nonreceptor type 22 (PTPN22) promotes NLRP3-inflammasome activation

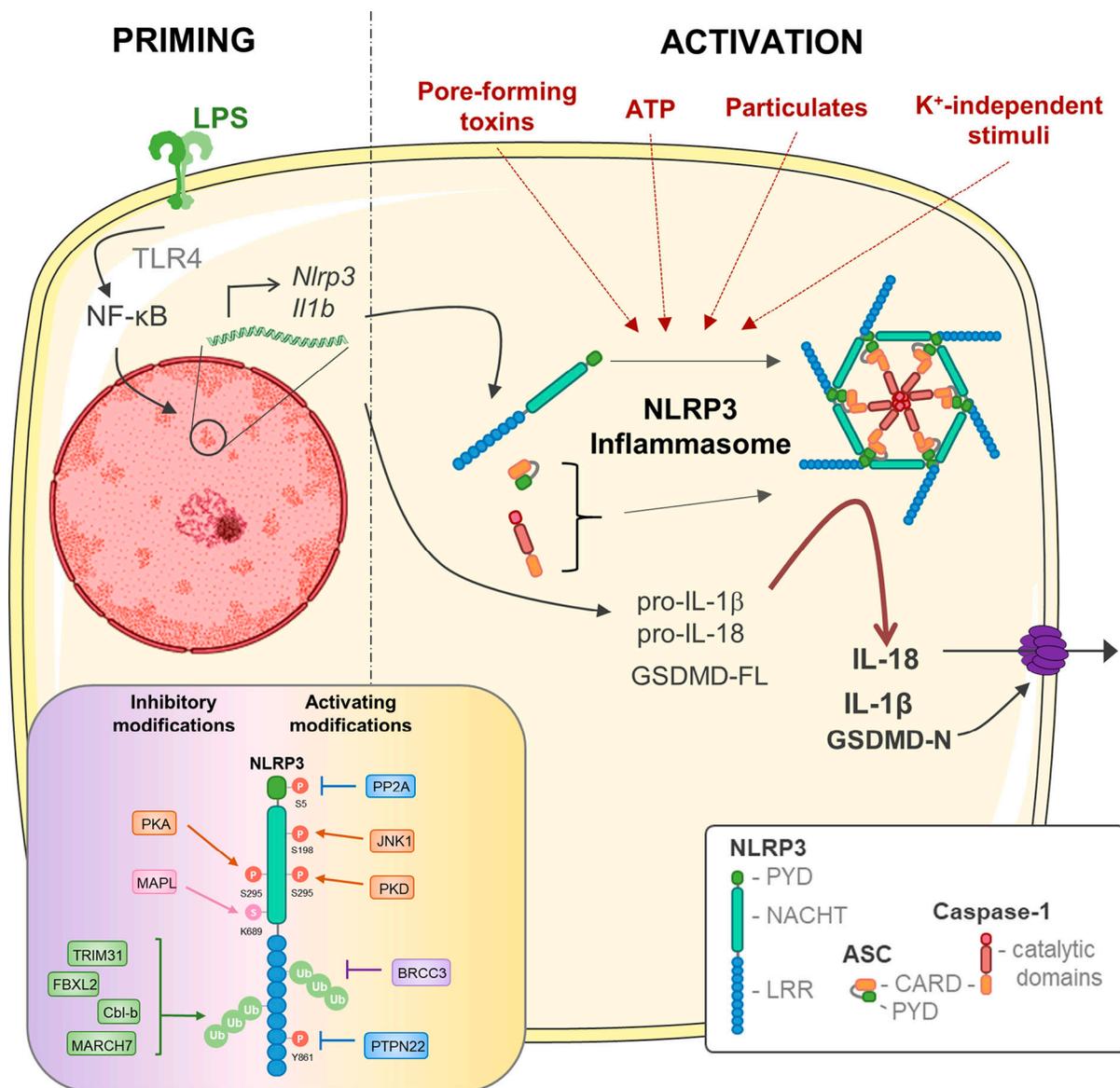


Figure 1. **The canonical pathway of NLRP3-inflammasome activation.** Priming stimuli such as LPS drive NF-κB-dependent expression of NLRP3 and pro-IL-1β, as well as NLRP3 licensing. NLRP3 is composed of an LRR, NACHT, and PYD domain. Numerous PTMs have been described that promote either activation or inhibition of NLRP3, including phosphorylation (P), ubiquitination (Ub), and sumoylation (S). Following the priming step, a broad spectrum of PAMP or DAMP stimuli triggers NLRP3 activation. Active NLRP3 oligomerizes and forms an inflammasome complex by nucleating the adaptor protein ASC to form a speck, leading to the recruitment and activation of the inflammasome effector protein caspase-1. Caspase-1 processes pro-IL-1β and pro-IL-18 into their active forms. Caspase-1 also cleaves gasdermin D (GSDMD), which forms pores in the membrane that may serve as the conduit for IL-1β release, as well as leading to pyroptotic cell death. The noncanonical and alternative pathways of NLRP3 activation are not shown. GSDMD-FL, GSDMD full length. GSDMD-N, GSDMD N-terminus. CARD, caspase activation and recruitment domain.

potentially through switching off inflammasome regulation by autophagy (Spalinger et al., 2016; Spalinger et al., 2017). Phosphatase and tensin homologue dephosphorylates NLRP3 in response to chemotherapy treatment, promoting NLRP3-inflammasome activation and enhancing the anti-tumor response (Huang et al., 2020).

In addition to phosphorylation, ubiquitination plays an important role in the regulation of the NLRP3-inflammasome. The importance of ubiquitination was originally demonstrated using broad spectrum deubiquitinase (DUB) inhibitors that block NLRP3-inflammasome activation (Juliana et al., 2012; Lopez-Castejon et al., 2013). This was followed by the identification

of the DUBs BRCA1/BRCA2-containing complex subunit 3 (BRCC3) that mediates K63 deubiquitination of NLRP3, and the ubiquitin-specific peptidases 7 and 47 as positive regulators of NLRP3-inflammasome activation (Palazón-Riquelme et al., 2018; Py et al., 2013). However, NLRP3 can be positively regulated by K63 ubiquitination. For example, the E3 ubiquitin ligase Pellino 2 acts as a positive regulator of NLRP3 by promoting its K63 ubiquitination in the priming step (Humphries et al., 2018). In contrast, other DUBs such as CYLD, A20, and POH1 act as negative regulators of NLRP3. CYLD deubiquitinates PLK4 and indirectly prevents interaction of NIMA-related kinase 7 (NEK7)

and NLRP3 at the centrosome, reducing inflammasome assembly (Yang et al., 2020). POH1 and A20 deubiquitinate pro-IL-1 β , resulting in a decreased ability of caspase-1 to cleave pro-IL-1 β and hence reduced amounts of the active mature form (Duong et al., 2015; Zhang et al., 2018). A20-deficient mice have enhanced NLRP3-inflammasome and caspase-1 activation, suggesting that A20 regulates NLRP3 (Vande Walle et al., 2014). Several E3 ubiquitin ligases, such as MARCH7, tripartite motif-containing protein 31 (TRIM31), F-box/LRR repeat protein 2 (FBXL2), and Cbl-b (Han et al., 2015; Yan et al., 2015; Song et al., 2016; Tang et al., 2020), add K48-linked ubiquitin chains to NLRP3 to signal its degradation at the proteasome or via autophagy. Thus, the regulation of NLRP3 by ubiquitination and deubiquitination is complex, with both processes able to positively and negatively regulate NLRP3. Sumoylation is reported to negatively regulate NLRP3 via E3 ligase mitochondrial-anchored protein ligase (Barry et al., 2018). More recently, acetylation of NLRP3 has been shown to regulate inflammasome activation during inflammaging (He et al., 2020). Thus, a wide range of PTMs regulating NLRP3 activity have been described, suggesting tight cellular control of NLRP3-inflammasome activation. How these signaling events are coordinated in space and time, however, is largely unknown.

Organelle involvement in NLRP3-inflammasome activation

In addition to transcriptional and posttranslational control of NLRP3-inflammasome activation, evidence suggests that NLRP3 is regulated by its subcellular localization (Hamilton and Anand, 2019). Early observations of NLRP3-inflammasome activation allude to an importance of spatial regulation, with ASC specks reported to form in a perinuclear cell compartment (Bryan et al., 2009; Fernandes-Alnemri et al., 2007; McConnell and Vertino, 2000), and numerous studies have since described interactions between NLRP3 and cellular organelles (Fig. 2). However, the precise subcellular site of NLRP3-inflammasome activation and the contributions of different organelles to this activation remain unclear. Interactions with, and an involvement of, nearly every cellular organelle and NLRP3 has been described. How can all of these observations be consolidated or explained by a model of NLRP3-inflammasome activation? In this section, we look at the evidence for organelle involvement and propose the hypothesis that NLRP3 acts as a sensor of organelle stress.

Lysosomes and NLRP3

Lysosomes were the first organelle reported to be important in NLRP3-inflammasome activation. Phagocytosis of particulates induces NLRP3-inflammasome activation through lysosomal membrane damage, which can cause K⁺ efflux from the cell, a previously acknowledged trigger of NLRP3 activation by the canonical pathway (Muñoz-Planillo et al., 2013). Lysosomal acidification appears important for triggering K⁺ efflux, as an increase in lysosomal pH caused by the vacuolar H⁺-ATPase inhibitor bafilomycin blocks NLRP3-inflammasome activation (Hornung et al., 2008; Muñoz-Planillo et al., 2013). Release of lysosomal cathepsin proteins into the cytosol following lysosomal damage is suggested to contribute to NLRP3-inflammasome activation, although there is much conflicting

literature and the precise role of cathepsins or their substrates remains to be determined (Campden and Zhang, 2019; Dostert et al., 2009; Hornung et al., 2008; Orłowski et al., 2015). The extent of lysosomal membrane permeabilization appears to regulate NLRP3, as limited permeabilization facilitates NLRP3-inflammasome activation, whereas complete loss of lysosomal integrity inhibits activation (Katsnelson et al., 2016). Sphingosine induces NLRP3-dependent IL-1 β release through a mechanism dependent on lysosomal acidification, protein phosphatase activity, and K⁺ efflux, yet is independent of lysosomal membrane destabilization per se (Luheshi et al., 2012). Activation of cyclic GMP-AMP synthase stimulator of interferon genes (STING) by cytosolic DNA in human monocytes induces STING-mediated lysosomal permeabilization and subsequent K⁺ efflux, leading to NLRP3-inflammasome activation (Gaidt et al., 2017). There is little to suggest that NLRP3 localization or recruitment to the lysosome is required for activation. It is, however, clear that perturbations of the lysosomes, or lysosomal stress, can lead to NLRP3-inflammasome activation.

The ER and NLRP3

Following the reported association between NLRP3-inflammasome activation and lysosome membrane destabilization, further studies began to suggest that localization of NLRP3 within the cell plays a more important role in orchestrating its activation than previously anticipated. Under resting conditions, NLRP3 is found in the cytosol and on the ER in the THP-1 human monocytic cell line (Zhou et al., 2011). During inflammasome activation with particulate stimuli or with the K⁺ ionophore nigericin, both NLRP3 and ASC relocate to the perinuclear region, where they colocalize with mitochondria-associated ER membranes (MAMs; Saitoh et al., 2008; Zhou et al., 2011). MAMs consist of tightly associated mitochondrial outer membranes and ER and provide a platform for several cellular functions that require the rapid exchange of metabolites between these organelles to maintain homeostasis (Missiroli et al., 2018).

Transport of inflammasome components between mitochondria and the ER has also been reported. Acetylation of α -tubulin promotes microtubule-dependent mitochondrial transport to the perinuclear region, where it enables the spatial approximation of ASC on mitochondria and NLRP3 on the ER to promote inflammasome activation (Misawa et al., 2013). The deacetylase sirtuin 2 (SIRT2) was identified as a potential regulator of NLRP3-inflammasome activation, acting on α -tubulin to reduce microtubule-dependent transport of mitochondria to the perinuclear region (Misawa et al., 2013). Decreased SIRT2 activity was recently implicated in enabling acetylation of NLRP3 and inflammasome activation during inflammaging, suggesting multiple roles for SIRT2 in the regulation of NLRP3 (He et al., 2020).

NLRP3 interacts with STING and localizes at the ER. In THP-1 cells and HeLa cells transfected to express NLRP3-inflammasome components, STING promotes ER localization of NLRP3, and this localization is enhanced during infection with herpes simplex virus type 1 (HSV-1). During HSV-1 infection or transfection with DNA, STING facilitates activation of NLRP3 through NLRP3 deubiquitination and enhanced ASC speck formation (Wang et al., 2020).

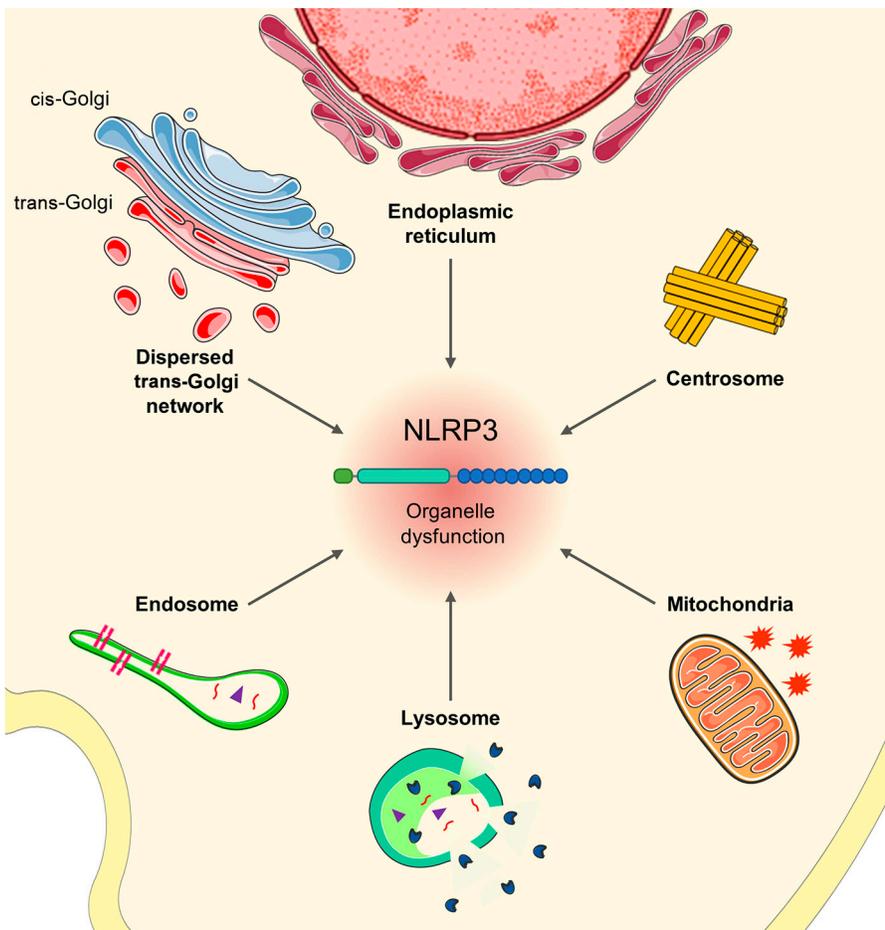


Figure 2. Organelle localization and activation of NLRP3. NLRP3–inflammasome activation occurs in response to multiple stimuli, with the common feature being organelle stress and dysfunction. Indeed, NLRP3-activating stimuli induce some or all of the following: ER stress, MAM formation, mitochondrial ROS production and mtDNA release, dispersal of the TGN, lysosomal rupture, and disruption to endosomal pH. NLRP3-activating stimuli are also reported to trigger NLRP3 recruitment to MAMs, mitochondria, the TGN, and endosomes, suggesting that organelles may serve as both sensor and platform for NLRP3 activation.

The ER carries out several functions within the cell, including protein folding and modification, lipid biosynthesis, and Ca^{2+} homeostasis, and disturbance of ER function leads to “ER stress.” Some have speculated that localization of NLRP3 to the ER or ER-associated membrane could allow NLRP3 to sense ER stress, perhaps even as an indirect measure of cellular distress. For example, accumulation of unfolded or misfolded proteins within the ER lumen will cause ER stress and trigger the unfolded protein response, aimed at restoring ER homeostasis, and this may lead to NLRP3 activation (Lerner et al., 2012). However, conflicting evidence suggests that ER stress induces NLRP3 activation independently of the unfolded protein response (Menu et al., 2012). ER stress-induced NLRP3 activation was observed during infection with *Brucella abortus*, and canonical NLRP3–inflammasome activation using LPS and ATP did not elicit ER stress (Bronner et al., 2015).

The centrosome and NLRP3

The centrosome is a membrane-less cellular organelle composed of two centrioles surrounded by an amorphous protein matrix termed pericentriolar material. The pericentriolar material contains hundreds of proteins, including cell cycle and signaling regulators, as well as proteins responsible for organizing and anchoring microtubules. The centrosome is the main microtubule-organizing center of the cell (Conduit et al., 2015).

An important link between the centrosome and NLRP3 activation is the centrosome-associated protein kinase NEK7, which is involved in spindle formation during mitosis (O’Regan and Fry, 2009) and centriole duplication in interphase (Kim et al., 2011). With regard to NLRP3, NEK7 acts downstream of K^+ efflux (Schmid-Burgk et al., 2016; Shi et al., 2016). The requirement of NEK7 for NLRP3–inflammasome activation was identified in a genome-wide CRISPR/Cas9 screen (Schmid-Burgk et al., 2016). CRISPR/Cas9 targeting of NEK7 dampens nigericin-induced NLRP3–inflammasome activation (Schmid-Burgk et al., 2016). NEK7 is also reported to directly interact with NLRP3 (Sharif et al., 2019; Shi et al., 2016) and promote its oligomerization and ASC speck formation (Shi et al., 2016). Given the role of NEK7 in spindle formation and mitosis (O’Regan and Fry, 2009), the interplay between NLRP3–inflammasome activation and cell cycle dynamics was investigated. NLRP3–inflammasome activation is severely blunted in mitotic cells compared with cells in interphase, and overexpression of NEK7 partially restores production of $\text{IL-1}\beta$ in mitotic cells, suggesting that both mitosis and NLRP3–inflammasome activation do not occur simultaneously due to the limited availability of free NEK7 within the cell (Shi et al., 2016). Recently, the centrosomal adaptor protein Spata2 was identified as a negative regulator of NLRP3–inflammasome activation. Spata2 is shown to recruit the DUB CYLD to the centrosome, where it acts on the centrosomal protein kinase

PLK4. Deubiquitinated PLK4 then phosphorylates NEK7 at S204, which in turn attenuates its interaction with NLRP3, leading to a reduction in NLRP3–inflammasome activation (Yang et al., 2020). Further supporting the involvement of the centrosome in NLRP3–inflammasome activation, the microtubule affinity-regulating kinase 4 (MARK4), a kinase involved in microtubule dynamics, is shown to directly interact with NLRP3 and transport it to the centrosome, where a single inflammasome speck per cell is formed (Li et al., 2017). An absence of MARK4 reduced, rather than abolished, NLRP3–inflammasome activation and IL-1 β secretion, suggesting that MARK4 may be responsible for trafficking only a fraction of the available NLRP3 (Li et al., 2017). Recently, histone deacetylase 6 (HDAC6) was found to be indispensable for NLRP3–inflammasome activation at the centrosome (Magupalli et al., 2020).

Mitochondria and NLRP3

The reported importance of ER–mitochondrial contact sites in inflammasome activation, and the observation that reactive oxygen species (ROS) can activate the inflammasome (Zhou et al., 2011), prompted further interest in the contribution of mitochondria to NLRP3–inflammasome regulation, with mitochondria also being suggested as a subcellular site of NLRP3–inflammasome activation. NLRP3-activating stimuli are reported to trigger NLRP3 recruitment to the outer mitochondrial membrane through interaction of the NLRP3 LRR domain with the mitochondrial phospholipid cardiolipin (Iyer et al., 2013). Disruption of cardiolipin synthesis through either palmitate treatment or knockdown of cardiolipin synthase reduces NLRP3 recruitment to mitochondria and subsequent activation (Iyer et al., 2013). Mitochondrial recruitment of NLRP3, as well as pro-caspase-1, is reported to occur during the priming step, which induces translocation of cardiolipin from the inner to the outer mitochondrial membrane due to mitochondrial stress (Dudek, 2017), and this is at least partly dependent on ROS production (Elliott et al., 2018). Once NLRP3 is localized to mitochondria, nigericin stimulation is required for subsequent ASC recruitment to the mitochondrial fraction and caspase-1 activation (Elliott et al., 2018). A small heterodimer partner, an orphan member of the nuclear receptor superfamily, has been proposed to promote translocation of NLRP3 from the cytosol to the mitochondria, upon which greater ASC binding occurs followed by inflammasome activation. In accordance with this view, small heterodimer partner deficiency decreases the mitochondrial recruitment of NLRP3 (Yang et al., 2015a).

NLRP3 is proposed to be recruited to mitochondria by mitochondrial antiviral signaling protein (MAVS). Initially, stimulation with nigericin was suggested to trigger NLRP3 mitochondrial recruitment and activation that is partly dependent on MAVS. ATP and poly(I:C) also induce NLRP3 activation that is partially MAVS dependent, whereas NLRP3 activation by crystals of alum or mono sodium urate is less dependent on MAVS (Subramanian et al., 2013). However, subsequent studies suggest that MAVS-dependent NLRP3–inflammasome activation specifically requires RNA viral infection (Franchi et al., 2014; Park et al., 2013). Treatment of THP-1 cells with Sendai virus induces NLRP3–inflammasome activation, and this is reduced by MAVS knockdown (Park et al., 2013). Experiments in murine

bone marrow–derived macrophages (BMDMs) further suggest that the interaction between NLRP3 and MAVS in response to Sendai virus infection may in fact only prime NLRP3 responses, perhaps through recruitment to the mitochondria, before subsequent activation with a second stimulus such as nigericin (Park et al., 2013), in a similar vein to cardiolipin-mediated NLRP3 recruitment (Elliott et al., 2018). Cytosolic poly(I:C) is suggested to trigger NLRP3–inflammasome activation through MAVS-dependent membrane permeabilization and K⁺ efflux (Franchi et al., 2014). RNA viral infection is also reported to trigger NLRP3 interaction with mitofusin 2, a mitochondrial outer membrane protein that regulates mitochondrial fusion and ER–mitochondrial tethering (Ichinohe et al., 2013).

The importance of mitochondria in NLRP3–inflammasome activation is further implied by the association between mitochondrial dysfunction and NLRP3 activity, reviewed recently by Holley and Schroder (2020). Several studies suggest a role for ROS production in NLRP3–inflammasome activation, perhaps facilitated by the close proximity of NLRP3 to mitochondria (Martinon, 2010; Tschopp and Schroder, 2010). Similarly, NLRP3 activators imiquimod (also known as R837) and CLO97 are reported to drive ROS production through N-ribosylidihydronicotinamide:quinone reductase 2 (NQO2) and mitochondrial complex I inhibition, leading to NLRP3–inflammasome activation (Groß et al., 2016). ROS are suggested to promote the interaction of NEK7 with NLRP3 (Shi et al., 2016). ROS production may also be involved in NLRP3 priming (Bauernfeind et al., 2011). Mitochondrial dysfunction may also result in the release of oxidized mitochondrial DNA (mtDNA) into the cytosol, where it activates NLRP3 (Shimada et al., 2012), and which is preceded by a TLR-driven increase in mtDNA synthesis during the NLRP3–inflammasome priming step (Zhong et al., 2018). ROS production in response to NLRP3–inflammasome-activating stimuli may oxidize newly synthesized mtDNA, driving subsequent NLRP3–inflammasome activation (Zhong et al., 2018), although the mechanism of mitochondrial permeabilization that facilitates mtDNA release is currently unclear (Holley and Schroder, 2020). As with ROS, the specific contribution of mtDNA to NLRP3–inflammasome activation is complicated. For example, NLRP3–inflammasome activation drives mitochondrial damage and depolarization (Yu et al., 2014), and NLRP3–inflammasome activation has been suggested to drive the release of mtDNA into the cytosol, which is then able to potentiate NLRP3–inflammasome activation (Nakahira et al., 2011). Furthermore, autophagic removal of dysfunctional mitochondria may negatively regulate NLRP3–inflammasome activation and mtDNA release (Nakahira et al., 2011), possibly via NF- κ B-induced p62 accumulation (Zhong et al., 2016). Thus, mitochondrial damage and subsequent mtDNA release may be a downstream consequence of NLRP3–inflammasome activation, rather than an upstream trigger. Hence, several lines of evidence suggest a role for mitochondria and mitochondrial dysfunction or stress in the regulation of NLRP3–inflammasome activation.

The Golgi apparatus and NLRP3

Traditionally considered to be a hub for vesicle trafficking, the Golgi apparatus is now regarded as an important platform in the

coordination of multiple innate immune responses (Tao et al., 2020). Indeed, this is not surprising, as the Golgi is functionally connected with numerous other organelles including the ER and endosomes, and it plays an important role in autophagosome biogenesis (Glick and Nakano, 2009; Huang and Wang, 2017). Levels of DAG at the Golgi apparatus can influence NLRP3-inflammasome activation, and NLRP3 is proposed to be present on MAMs in close proximity to the Golgi (Zhang et al., 2017). Upon NLRP3 stimulation, diacylglycerol production at the Golgi membrane results in activation of its agonist PKD, which in turn phosphorylates NLRP3 and promotes its release from MAMs, thereby allowing assembly of NLRP3 into a functional inflammasome (Zhang et al., 2017).

Treatment of cells with Brefeldin A (BFA), which disrupts ER to Golgi trafficking and causes Golgi disassembly, attenuates NLRP3-inflammasome activation in mouse BMDMs (Hong et al., 2019; Zhang et al., 2017). BFA inhibits LPS-induced priming of the NLRP3-inflammasome, and since BFA does not affect LPS-induced TLR4 internalization, it was hypothesized that BFA must be exerting its effects on priming via disruption of ER to Golgi trafficking (Hong et al., 2019). shRNA knockdown of the Golgi-associated proteins BFA-inhibited guanine nucleotide exchange protein 1 (BIG1) and Golgi BFA-resistant guanine nucleotide exchange factor 1, both molecular targets of BFA, was also found to inhibit NLRP3-inflammasome activation (Hong et al., 2019). Because BIG1 depletion did not appear to affect Golgi structure, it was proposed that BFA is acting via disruption of Golgi trafficking to attenuate NLRP3 activation, as opposed to an effect on Golgi organization (Hong et al., 2019). However, other studies have reported that in addition to disrupting trafficking at the TGN (Ishizaki et al., 2008; Manolea et al., 2008), BIG1 depletion disrupts Golgi organization (Boal and Stephens, 2010; Shen et al., 2007). Similarly, depletion of GBF-1, which acts at earlier Golgi compartments, also appears to disrupt Golgi structure (Manolea et al., 2008; Szul et al., 2007). Thus, although ER to Golgi trafficking may play a role in NLRP3 priming, the structure of the Golgi apparatus, and in particular the TGN, may also be important.

NLRP3 forms a ternary complex, via its NACHT domain, with the cholesterol regulator SCAP-SREBP2, a sterol sensor that translocates from the ER to the Golgi upon sterol depletion (Goldstein et al., 2006; Guo et al., 2018). SREBP2 then undergoes proteolytic processing by site-1 and site-2 proteases (S1P and S2P), which results in SREBP2 activation and SREBP cleavage-activating protein (SCAP) recycling back to the ER (Guo et al., 2018). Inhibition of SCAP-SREBP2 translocation impairs NLRP3-inflammasome activation, suggesting a potential importance of Golgi trafficking (Guo et al., 2018). Using subcellular fractionation and Western blotting, NLRP3 was detected in the Golgi fraction of LPS-primed THP1-V5-SCAP macrophages and was enriched after 10 min of nigericin stimulation before gradually diminishing (Guo et al., 2018). Similar levels of NLRP3 were detected in the mitochondrial fraction, although unlike in the Golgi fraction, NLRP3 was only present on mitochondria following nigericin stimulation. Interestingly, super-resolution and 3D structured illumination microscopy revealed that a portion of NLRP3 is closely localized to the COPII protein

Sec23, suggesting that COPII-coated vesicles are used by SCAP to transport NLRP3 to the Golgi during inflammasome activation (Guo et al., 2018). In this study, it was found that BFA treatment of LPS-primed BMDMs was sufficient to induce IL-1 β release in the absence of a second activating stimulus (Guo et al., 2018). It was hypothesized that this was due to BFA-induced S1P activation, as S1P inhibition abolished this response, suggesting that S1P-driven SREBP2 activation and SCAP release is sufficient to drive NLRP3-inflammasome activation (Guo et al., 2018).

Further evidence for an involvement of the Golgi apparatus in NLRP3-inflammasome activation was provided with the demonstration that multiple NLRP3-inflammasome-activating stimuli can disrupt Golgi structure, resulting in a dispersed TGN (dTGN; Chen and Chen, 2018). The dTGN appears to act as a platform for NLRP3 recruitment, which occurs through binding of an internal polybasic region to the lipid phosphatidylinositol 4-phosphate (PtdIns4P), which is enriched at the TGN. Once recruited to dTGN, NLRP3 can oligomerize ASC to form an inflammasome (Chen and Chen, 2018).

Endosomes and NLRP3

Endosomes are heterogeneous membrane-bound organelles that facilitate the sorting and transport of proteins and lipids internalized at the plasma membrane. In addition to controlling the number of competent receptors at the plasma membrane, endosomes are also important sites of intracellular cell signaling in their own right (Villaseñor et al., 2016). Recently, endosomes have been implicated in NLRP3 signaling, as complement membrane attack complex internalization by endothelial cells was found to initiate NLRP3 recruitment to early endosomes, which is dependent on the formation of a Rab5*Akt*Nik* signalosome (Xie et al., 2019). Prior studies have shown that recruitment of the adaptor protein phosphotyrosine interacting with pleckstrin homology domain and leucine zipper 1 (APPL1) to the early endosome is essential for NF- κ B-inducing kinase stabilization and Akt activation (Hupalowska et al., 2012). Since Akt and Nik signaling at the early endosome is essential for NLRP3 recruitment, it is possible that APPL1 may also be involved in NLRP3 recruitment to early endosomes. Interestingly, PtdIns4P is present on endosomal membranes in addition to the TGN (Hammond et al., 2014), raising the possibility that NLRP3 may also undergo endosomal recruitment through binding to this lipid. Although dTGN was proposed as a site for NLRP3 recruitment (Chen and Chen, 2018), it should be considered that the marker used for this study, TGN38/46, undergoes endosome-TGN transport (Progida and Bakke, 2016). This transport is sensitive to alteration of endosomal pH (Chapman and Munro, 1994; Reaves and Banting, 1994), which by coincidence also occurs upon treatment of cells with a number of known NLRP3-inflammasome-activating stimuli, including K⁺ ionophores such as nigericin, hypoosmotic stress, imiquimod, and CLO97 (Chapman and Munro, 1994; Reaves and Banting, 1994; Schreiber et al., 1996), raising the possibility that the cell compartment described as dTGN in fact corresponds to endosomes that contain accumulated TGN38/46. It is therefore possible that NLRP3 oligomerization may also occur on endosomes, which may be a consequence of endosomal dysfunction (Fig. 3).

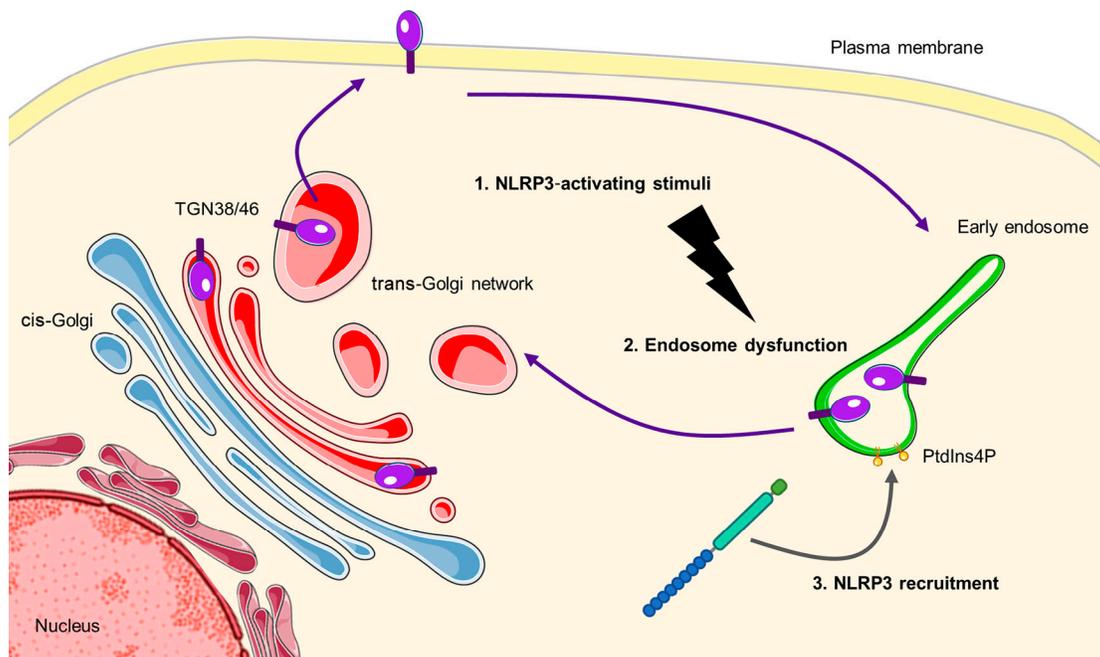


Figure 3. Endosome dysfunction may promote activation of NLRP3. Stimuli that activate the NLRP3–inflammasome (1) cause endosomal stress and dysfunction (2). Such endosomal dysfunction results in impaired recycling of proteins such as TGN38/46 to the TGN. Under conditions of endosomal stress, we propose that NLRP3 is recruited to the endosomal membrane, which may be driven in part by association with the inositol lipid PtdIns4P (3). Thus, the endosome membrane may serve as a platform for NLRP3 recruitment and subsequent inflammasome activation.

Furthermore, caspase-1 activation is also reported to cleave the endosomal tethering protein EEA1, which is reported to be important for IL-1 β secretion (Baroja-Mazo et al., 2019).

Conclusions

As is evident from the literature discussed, the diverse NLRP3-activating stimuli reported exert specific stresses on organelles. We propose that these stresses manifest themselves in organelle dysfunction, which is directly communicated to NLRP3 via an array of specific PTMs, coordinating NLRP3–inflammasome activation and a subsequent period of NLRP3-driven inflammation. It is possible that many NLRP3-activating stimuli exert stress on multiple organelles and NLRP3 activation results as a combination of multiple organelle dysfunction. Alternatively, specific stress may result in the activation of NLRP3 at one organelle. How an NLRP3-activating stimulus affects organelles may be influenced by additional factors such as cell type and the nature of the stimulus as well as its concentration and duration of exposure, adding potential variation to results observed and their interpretation. Another caveat that must be considered is whether NLRP3 localizes with organelles in a monomeric or multimeric form, which has important implications for how the localization could affect activity.

It is also possible that stress in one organelle results in dysfunction of another, leading to NLRP3 activation. That is to say, a specific organelle acts as a sentinel for sensing stress in other organelle systems. In this respect, the endosome is a particularly interesting candidate. Endosome cycling of cargoes such as TGN38/46, which is shuttled from the Golgi to the plasma membrane and back by endosomes, is disrupted by K⁺

ionophores, leading to the cargoes becoming trapped in the endosome (Chapman and Munro, 1994; Reaves and Banting, 1994). As discussed, NLRP3 colocalizes with TGN38/46 following cell stimulation with NLRP3-activating DAMPs (Chen and Chen, 2018), suggesting this may indeed be the case. It is possible therefore that stimuli driving K⁺ efflux to activate NLRP3 may rely on endosome dysfunction. Thus, particulate stimuli, which cause lysosome membrane destabilization and NLRP3 activation via K⁺ efflux (Muñoz-Planillo et al., 2013), may impact endosome trafficking and thus recruit endosomes to facilitate NLRP3–inflammasome activation. It is also worth noting that stimuli that activate NLRP3 independent of K⁺ efflux, and through reported effects on mitochondria, such as imiquimod and CLO97, also perturb endosome pH (Groß et al., 2016; Schreiber et al., 1996). However, a role for endosomes as proposed here (Fig. 3) remains to be proven experimentally. In conclusion, it is clear that organelle stress and dysfunction is central to the coordination of NLRP3-driven inflammation. The challenge is now to unravel the cell biology to fully understand organellar control of NLRP3–inflammasome activation.

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