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The NLRP1 inflammasome: new mechanistic insights and unresolved mysteries

Patrick S. Mitchell[a,†], **Andrew Sandstrom**[a,b,†], **Russell E. Vance**[a,b,*]

[a]Division of Immunology & Pathogenesis, Department of Molecular & Cell Biology, and Cancer Research Laboratory, University of California, Berkeley, California, USA.

[b]Howard Hughes Medical Institute, University of California, Berkeley, California, USA.

Abstract

Nucleotide-binding domain, leucine-rich repeat (NLR) proteins constitute a diverse class of innate immune sensors that detect pathogens or stress-associated stimuli in plants and animals. Some NLRs are activated upon direct binding to pathogen-derived ligands. In contrast, we focus here on a vertebrate NLR called NLRP1 that responds to the enzymatic activities of pathogen effectors. We discuss a newly proposed 'functional degradation' mechanism that explains activation and assembly of NLRP1 into an oligomeric complex called an inflammasome. We also discuss how NLRP1 is activated by non-pathogen-associated triggers such as the anti- cancer drug Val-boroPro, or by human disease-associated mutations. Finally, we discuss how research on NLRP1 has led to additional biological insights, including the unexpected discovery of a new CARD8 inflammasome.

Introduction

A key function of the innate immune system is to detect and respond to pathogens. Central to this process is the ability of germ-line encoded receptors to distinguish accurately between self and infectious non-self [1]. Many innate immune receptors directly recognize microbial ligands called pathogen-associated molecular patterns (PAMPs), such as bacterial flagellin or double-stranded RNA. However, PAMPs are also produced by harmless microbes, and therefore, detection of PAMPs does not by itself provide a means to initiate responses specifically to pathogens. Studies performed initially in plants [2,3], and later in animals, have led to the idea that the immune system may also sense pathogens via detection of their unique *activities* [4]. These activities are associated with the toxins or effector enzymes that are secreted by pathogens to manipulate their hosts. In this review, we focus on an immune sensor in vertebrates called NLRP1. We highlight recent work demonstrating how this sensor recognizes and responds to pathogen-associated activities.

*Correspondence to: rvance@berkeley.edu.
†These authors contributed equally to this work.

NLRP1 and the discovery of inflammasomes

In 2002, Martinon, Burns and Tschopp described an intracellular molecular platform that could recruit, dimerize and activate the pro-inflammatory protease Caspase-1 (CASP1)—the first inflammasome [5]. This protein complex consisted of NLRP1 and an adaptor protein called ASC (apoptosis-associated speck-like protein). Since this landmark finding, multiple additional inflammasomes have been discovered that together are now appreciated to constitute a major arm of the innate immune system [6,7]. Active CASP1 triggers downstream inflammatory responses by cleavage of inflammatory cytokines interleukin-1β (IL-1β) and IL-18 to their active forms, and by the cleavage and activation of the pore-forming protein Gasdermin-D, which induces a pyroptotic cell death. Some inflammasomes are activated upon direct detection of pathogen-derived ligands. For example, the NAIP/NLRC4 inflammasomes are activated by recognition of components of the bacterial type III secretion system or flagellin [8–13]. However, other NLR sensors, including NLRP1, detect and respond to pathogen-associated activities.

NLRP1 belongs to the Nucleotide Binding Domain (NBD), Leucine-Rich Repeat (LRR) containing (NLR) protein superfamily (Figure 1) [3,14]. Typically, the NLRs that recruit CASP1 do so either directly via a Caspase Activation and Recruitment Domain (CARD), or indirectly via a Pyrin Domain (PYD) that recruits CASP1 via ASC. Human NLRP1 contains both a CARD and a PYD, though only the CARD appears to be required for CASP1 recruitment [15]. In contrast to most NLRs, one unusual feature of the NLRP1 CARD is its location at the NLRP1 C-terminus (Figure 1). The other unusual feature of NLRP1 is a FIIND (Function-to-find domain), which undergoes constitutive self-cleavage (henceforth referred to as auto-processing) resulting in two distinct polypeptides that remain non-covalently associated with each other after auto-processing [16]. For reasons that have been unclear until recently (see below), FIIND auto-processing is required for NLRP1 function.

Unlike humans, which are believed to encode a single NLRP1 gene, mice typically encode multiple NLRP1 paralogs. A major breakthrough in our understanding of NLRP1 came when the mouse NLRP1B paralog was discovered to activate CASP1 in response to the *Bacillus anthracis* Lethal Toxin (LeTx) [17]. LeTx is a two-component toxin consisting of the lethal factor (LF) protease and the channel-forming protective antigen (PA) protein that delivers LF into cells. Macrophages from certain mouse strains, e.g., 129S1 or BALB/c, carry a LeTx ‘susceptible’ *Nlrp1b* allele and undergo *Nlrp1b*-dependent pyroptosis in response to LeTx, whereas macrophages from mice with a LeTx ‘resistant’ *Nlrp1b* allele (e.g., C57BL/6J) do not. Note that the ‘resistant’ and ‘susceptible’ designations refer to the phenotype of macrophages treated with LeTx. In fact, mice carrying a ‘susceptible’ *Nlrp1b* allele are actually *resistant* to anthrax spore challenge. This seeming paradox is explained by clear evidence that inflammasome activation and pyroptotic cell death leads to the release of IL-1β, a cytokine that orchestrates a potent anti- anthrax neutrophil response [18–20].

The protease activity of LF is required for LeTx to activate NLRP1B [21,22]. Evidence in mice and rats demonstrated that direct cleavage of the NLRP1B N-terminus is necessary for LF to activate NLRP1B [23–25]. Moreover, insertion of a tobacco etch virus (TEV) protease site into the N-terminus of mouse NLRP1B [23] or human NLRP1 [26] renders both

proteins sensitive to activation by the TEV protease. Together, these results imply that NLRP1 is a direct sensor of a pathogen-encoded *activity* (i.e., proteolysis), as opposed to functioning as a receptor of pathogen-associated ligands. However, the mechanism connecting N-terminal cleavage to NLRP1 inflammasome activation remained mysterious. Strangely, inhibitors of aminopeptidases [22], the N-end rule [27] and the proteasome [21,27–30] were found to block LeTx activation of NLRP1B, whereas these inhibitors have no effect on the activation of other inflammasomes. However, it was not apparent how these observations relate to the mechanism of NLRP1 activation.

The molecular mechanism of NLRP1 inflammasome activation

Prior studies proposed that the NLRP1B N-terminus mediated its auto-inhibited state, and that proteolytic removal of the N-terminus relieved this inhibition, resulting in inflammasome activation [31,32]. In opposition to this model, the N-terminus of NLRP1B was found to be dispensable to maintain an inactive state. For example, auto-inhibition was maintained in an NLRP1 mutant with its N-terminus replaced by GFP [26]. Instead of removing an auto-inhibitory domain, N-terminal cleavage was found to markedly destabilize NLRP1, resulting in its ubiquitylation and proteasome-mediated degradation [33,34]. Counterintuitively, degradation of NLRP1 positively correlates with its activation, as both degradation and activation are blocked by proteasome inhibitors [33,34]. The specific factors linking NLRP1B N-terminal cleavage to NLRP1B degradation were uncovered by a genome-wide CRISPR/Cas9 screen for host genes required for LeTx-induced pyroptosis [33]. The screen identified multiple genes involved in a protein quality control pathway called the N-end rule. The N-end rule pathway is complex, but, generally speaking, relates protein stability to the identity of its N-terminal amino acid [35]. Destabilizing N-termini, such as those generated following protease cleavage, are recognized by N-recognins [36,37], which target their substrates for ubiquitylation and proteasomal degradation. In particular, UBR2, an N-end rule E3 ubiquitin ligase, was found to be critical for LeTx-mediated NLRP1B activation—*Ubr2*$^{-/-}$ cells are highly desensitized to LF-induced pyroptosis despite normal cleavage of NLRP1B [33].

How then does proteasome degradation of NLRP1B result in its activation? The answer lies in the FIIND and C-terminally located CARD, two defining features of NLRP1. The FIIND is comprised of two subdomains: ZU5 (found in ZO-1 and UNC5 domain) and UPA (conserved in UNC5, PIDD, and Ankyrin domain) [15,38,39]. FIIND auto-processing results in two non- covalently associated polypeptides: the NBD-LRR-ZU5 and UPA-CARD. It was proposed [33,34] that N-terminal cleavage of NLRP1 (e.g., by LeTx) leads to proteasomal degradation of the N-terminal domains. Since the proteasome is a processive protease, the break in the polypeptide chain within the FIIND prevents the proteasome from degrading the C-terminal NLRP1 domains and instead results in release of the bioactive C-terminal UPA-CARD fragment (Figure 2). The UPA-CARD then self-assembles and forms a platform for CASP1 recruitment and activation. We refer to this model of proteasome-dependent inflammasome activation as ‘functional degradation.’ [34]

The ‘functional degradation’ model has several appealing features. First, it explains the unique susceptibility of the NLRP1 inflammasome to proteasome inhibitors. Second, it

accounts for the requirement for FIIND auto-processing—an unprocessed FIIND would result in proteasome degradation of the entire protein. And third, it provides a functional rationale for the C-terminally located CARD, as a more typically located N-terminal CARD would be cleaved from NLRP1 or degraded. Consistent with the 'functional degradation' model, the UPA-CARD was found to form high molecular weight oligomers [34] and is sufficient to induce CASP1 activation [31,33,34,38,40]. Moreover, the UPA-CARD, but not the NBD-LRR-ZU5, was found to specifically associate with the ASC speck following NLRP1B inflammasome activation [34]. Interestingly, a protein consisting solely of the ZU5-UPA-CARD domains is still auto-inhibited, suggesting that the NLRP1 NBD-LRR domains are not required for auto-inhibition and that the ZU5 subdomain is sufficient to prevent the spurious release of the UPA-CARD [34]. This does not rule out a role for the NBD-LRR domains in pathogen detection or in sensing other NLRP1 agonists.

Taken together, the 'functional degradation' model suggests that NLRP1 senses its own protein stability to detect and respond to pathogen-associated activities. Indeed, using an auxin- interacting degron (AID)-tagged NLRP1B, which is specifically and inducibly degraded via the proteasome upon addition of the plant hormone indole-3-acetic acid (IAA, or auxin), NLRP1B degradation was found to be not only necessary but also *sufficient* to induce inflammasome activation [34]. In principle, this observation suggests that NLRP1 could recognize pathogens not only via proteolysis of its N-terminus, but through any process that directly or indirectly leads to its degradation.

Additional pathogens (and effectors) sensed by NLRP1

The 'functional degradation' model derived from efforts to understand the mechanism of LeTx-induced activation of the NLRP1B inflammasome. However, other pathogens that have been reported to activate NLRP1 may do so via a similar mechanism. For example, *Shigella flexneri* was recently reported to activate NLRP1 [41]. The *S. flexneri* factor responsible for this activation appears to be IpaH7.8 [34], an E3 ubiquitin ligase effector secreted by *S. flexneri* [42,43]. Interestingly, IpaH7.8 was previously proposed to activate the NLRC4 and NLRP3 inflammasomes[44,45]. The relevance of these observations for IpaH7.8-mediated activation of NLRP1 remains uncertain. Using a reconstituted ubiquitylation assay, IpaH7.8 was shown to directly and specifically ubiquitylate NLRP1B (Figure 2) [34]. Moreover, *Shigella* infection was found to induce IpaH7.8-dependent pyroptosis in WT but not *Nlrp1b*$^{-/-}$ macrophages. Of note, pyroptosis induced by IpaH7.8 also occurs in *Ubr2*$^{-/-}$ cells, likely because IpaH7.8 itself can directly ubiquitylate NLRP1B and therefore bypasses the requirement for N-end rule E3 ligases. *Toxoplasma gondii* [46–48] and *Listeria monocytogenes* [41] have also been reported to activate NLRP1, though whether cellular perturbations (e.g., ATP depletion [41,49]) associated with NLRP1 activation by these pathogens also activates NLRP1 via 'functional degradation' is unclear. The identification of pathogen-encoded effectors that activate human NLRP1 also remains an important, unresolved question.

Rare inflammatory skin disorders highlight a tissue-specific role for NLRP1

Recently, the causative germline-activating mutations underlying three distinct monogenic skin disorders were mapped to *NLRP1*: multiple self-healing palmoplantar carcinoma (MSPC), familial keratosis lichenoides chronica (FKLC) and autoinflammation with arthritis and dyskeratosis (AIADK) [31,50,51]. In one pedigree, the disease-associated variant was a deletion in the NLRP1 LRR, reminiscent of the auto-activity of a NLRP1 LRR truncation mutation [5,40]. However, a new class of heterozygous dominant gain-of-function mutations were also identified in the NLRP1 PYD [31]. Reconstitution of inflammasome components in 293T cells or immortalized keratinocytes demonstrated that the disease-associated NLRP1 PYD variants A54T, A66V and M77T were auto-active, spontaneously forming high molecular weight NLRP1 oligomers and inducing CASP1-dependent processing of IL-1β.

Consistent with robust expression of NLRP1 in human keratinocytes, MSPC, FKLC and AIADK present primarily as epidermal disorders. However, NLRP1 is also expressed in PBMCs [31,52], raising the question of why NLRP1 auto-activating mutations fail to induce a more systemic inflammatory disease, such as seen in patients with activating mutations in other inflammasomes [53]. Nevertheless, the function of NLRP1 in the skin is intriguing, and may be analogous to the barrier role of the NAIP/NLRC4 inflammasome in the intestinal epithelium [54].

The disease-associated NLRP1 mutants also provide insight into the mechanism of the PYD. The PYD of other NLRs (e.g., NLRP3) functions to recruit ASC and CASP1. Although the NLRP1 PYD was originally proposed to also have this function [5], more recent results indicate that this is not the case [26,31]. Instead, ASC and CASP1 are recruited to NLRP1 via the C-terminal UPA-CARD. Interestingly, disease-associated mutations that activate NLRP1 were found to destabilize the PYD fold, as measured with 2D-NMR and circular dichroism [31]. Although the authors proposed that destabilization of the PYD eliminates a PYD-mediated auto-inhibitory interaction, an alternative explanation is that misfolding of the PYD results in NLRP1 activation via proteasome-mediated ‘functional degradation’ of NLRP1 by the protein quality control machinery. Future studies are required to differentiate these models of human NLRP1 activation.

Interestingly, NLRP1, and the PYD in particular, has been subject to intense positive selection, an adaptive genomic signature consistent with pathogen-driven evolution [26,55]. Positive selection can highlight protein surfaces that are directly engaged as the targets of pathogen activities in host-pathogen ‘arms races’ [56]. Therefore, an intriguing possibility is that the NLRP1 PYD, and perhaps the LRRs and NBD, acts as a molecular tripwire for pathogen detection. For example, the PYD may have evolved as a ‘decoy’ target that mimics the true substrates of pathogen-secreted effectors. An analogous mechanism exists in plants. For example, the *Arabidopsis* NLR RRS1 contains an ‘integrated decoy’ WRKY transcription factor domain that is modified by the activity of pathogen effectors, leading to RRS1 activation. The true (‘intended’) target of the effectors, however, are the WRKY transcription factors themselves, which are critical for initiation of host immune responses (Figure 3) [57,58]. For NLRP1, a putative effector might trigger NLRP1 activation upon cleaving, modifying or otherwise inducing the unfolding of the PYD (or perhaps any of its

N-terminal domains). The capacity for the PYD to sense pathogen-encoded activities would explain both the dispensable nature of the PYD for NLRP1 function and its continued presence in diverse NLRP1 orthologs over evolutionary time. Interestingly, the NLRP1 PYD has been lost in multiple species, including mouse-like rodents, suggesting that the adaptive benefits of the NLRP1 PYD may also confer a fitness cost (e.g., a lower threshold to undergoing spontaneous activation), which perhaps becomes unbalanced in the prolonged absence of PYD-sensed pathogens.

Val-BoroPro provides unexpected insights into NLRP1 function and regulation

Val-boroPro (VbP, or PT-100, Talabostat) is a small molecule inhibitor of post-proline cleaving proteases, including the fibroblast activation protein (FAP) and the dipeptidyl peptidases DPP4, DPP7, DPP8 and DPP9. In mice, VbP administration induces cytokine production and reduces tumor burden [59–61] and also causes cell death of cultured macrophages [62,63]. These effects of VbP were recently determined to occur via activation of a CASP1-dependent, NLRP1B-dependent inflammasome response [64]. Similarly, genetic deletion of *Dpp8/9* results in NLRP1B- and CASP1-dependent pyroptosis. Consistent with the highly conserved nature of DPP8/9, VbP triggers NLRP1 activation across multiple, divergent NLRP1 alleles, and thus appears to be the first universal activator of the NLRP1 inflammasome.

Interestingly, VbP-mediated inflammasome activation is not dependent on UBR2 or other N-end rule factors [33], suggesting that DPP8/9-mediated inhibition of NLRP1 is distinct from that of N-terminal proteolysis of LeTx. Moreover, unlike other direct activators (e.g., LeTx), VbP- induced pyroptosis can take over 48 hours in some cell lines [60]. What, then, is the mechanism by which DPP8/9 inhibits NLRP1? Using a mass-spectrometry approach, DPP9 was found to differentially interact with NLRP1 in untreated versus VbP-treated cells [50]. Accordingly, immunoprecipitation assays clearly demonstrate binding between DPP9 and NLRP1, which was reported to be abrogated by VbP treatment. These findings suggest that direct DPP9 binding may be an important aspect of NLRP1 inhibition [50].

However, whether or not the mechanism of VbP-mediated cell death is due simply to the disruption of a DPP8/9-NLRP1 interaction is unclear. Indeed, VbP inhibits the enzymatic activity of DPP8/9, and enzymatically dead DPP9 point mutants fail to inhibit NLRP1, implying that DPP8/9 inhibits NLRP1 via cleavage of an as yet undefined substrate [50,62,64]. Importantly, the S759A DPP9 mutant, which abolishes the enzymatic activity of DPP9, maintains binding to NLRP1, suggesting that binding and enzymatic activity—at least in an over-expression system—are separable [50]. In addition, spontaneously active NLRP1 variants (e.g., PYD auto-active mutants) maintain an association with DPP8/9. One possibility is that DPP8/9 binding to NLRP1 increases its local concentration, which is required for processing of its as yet identified substrate(s), which either directly or indirectly induces NLRP1 inflammasome activation. The identification of the DPP8/9 substrate(s) that regulate NLRP1 therefore constitutes a major outstanding question in the field.

A tempting hypothesis is that NLRP1 itself is the substrate of DPP8/9. Interestingly, the NLRP1 P1214R mutation, like auto-activating mutations in the NLRP1 PYD, results in spontaneous NLRP1 activation [50]. P1214R occurs at the P2' position after the FIIND auto- proteolysis site i.e., the second amino acid in the C-terminal UPA-CARD peptide. Given that P2 prolines constitute a recognition motif for DPP8/9, a reasonable prediction was that mutation of this proline eliminates DPP8/9 targeting and inhibition of NLRP1. However, a recent study using Chemical Enrichment of Protease Substrates (CHOPS) to profile DPP8/9 substrates found no evidence that NLRP1B is a direct DPP8/9 substrate [65]. Instead, the DPP9-NLRP1 interaction, which occurs through contacts between DPP9 and both FIIND subdomains (i.e., ZU5 and UPA), is abrogated by the P1214R mutation [50], suggesting that P1214R may disrupt the DPP9- NLRP1(UPA) interface.

DPP8/9-mediated inhibition of NLRP1 may have evolved in response to its unique mechanism of activation. Since degradation of NLRP1 leads to its activation, constitutive turnover of NLRP1 might result in release of sufficient amounts of bioactive UPA-CARD to induce CASP1 activation, even in the absence of pathogen infection. We therefore speculate that the role of DPP8/9 may be to prevent the constitutive and deleterious accumulation of bioactive UPA-CARD. Indeed, the delayed kinetics of VbP-induced inflammasome activation [33,62,64] is consistent with a model in which activation requires the accumulation of the UPA- CARD upon its infrequent and spontaneous release from the N-terminal domains of NLRP1. If correct, 'active' pathogen-induced NLRP1 inflammasome activation must somehow bypass the DPP8/9 blockade, either by mass action or concomitant degradation/release of DPP8/9.

CARD8: a new inflammasome.

Surprisingly, NLRP1 is dispensable for VbP-induced pyroptosis in some human AML-derived monocytic cell lines. Instead, another FIIND-CARD protein, CARD8 (also known as TUCAN or CARDINAL), was found to be required for VbP-induced cell death in THP-1, OCI- AML2 and MV4;11 cells [60] (Figure 4). CARD8 was shown to activate CASP1 to induce GSDMD-mediated cell death both in AML monocytic cell lines and in inflammasome reconstituted 293T cells [50]. Interestingly, CARD8 activation requires the proteasome and FIIND auto-processing [16,60], and the C-terminal UPA-CARD fragment constitutes the bioactive component of CARD8. Accordingly, the 'functional degradation' model likely explains CARD8 inflammasome activation [60]. Indeed, CARD8 could be considered a 'minimized' NLRP1 ortholog that contains the key FIIND-CARD domains but lacks the NBD-LRR [16], consistent with the idea that the N-terminal NBD-LRR domains of NLRP1 are dispensable for inflammasome assembly and activation. In another parallel to NLRP1, the CARD8 FIIND also associates with DPP9, and VbP treatment abolishes this interaction [50]. Thus, the unresolved mechanism of DPP8/9 inhibition is also shared between NLRP1 and CARD8. A key outstanding question is whether CARD8 plays a role in innate immune sensing, and if so, what pathogens (and pathogen-encoded enzymes) activate CARD8.

The NLRP1 inflammasome: What's next?

Overall, despite some unresolved mysteries, NLRP1 provides a fascinating example of an immune sensor that is able to detect diverse pathogen and non-pathogen-encoded activities. The 'functional degradation' model for NLRP1 inflammasome activation may also offer insights into proteins with similar domain architectures. Certainly, clear parallels exist between NLRP1 and the newly minted CARD8 inflammasome [60]. Interestingly, in addition to NLRP1 and CARD8, the FIIND is found in a handful of other proteins with C-terminal death domains [66], including PIDD [67], Ankyrins [68,69] and multiple Netrin-1 receptors [70] (Figure 4). In addition, a FIIND-like domain, sometimes called a GAIN domain, is found in proteins without death domains [66]. We anticipate that 'functional degradation' may also regulate signaling in these diverse FIIND-containing proteins.

Like PAMPs, the enzymatic activity of pathogen effectors is an essential and therefore highly immutable aspect of pathogen replication. Additionally, secretion of effectors is a uniquely pathogen-specific event. As such, effector activity is not only an appealing target for innate recognition, but for the discrimination of pathogens from otherwise harmless microbes. It will be of interest to determine how widespread this mode of pathogen recognition is in vertebrate immunity.

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Highlights

- NLRP1 is a sensor of diverse pathogen-encoded activities
- ‘Functional degradation’ provides a mechanism for NLRP1 activation
- New insights into NLRP1 have been revealed by non-pathogen triggers, including Val- boroPro and auto-active disease-associated human mutations
- CARD8 is a novel NLRP1-like inflammasome

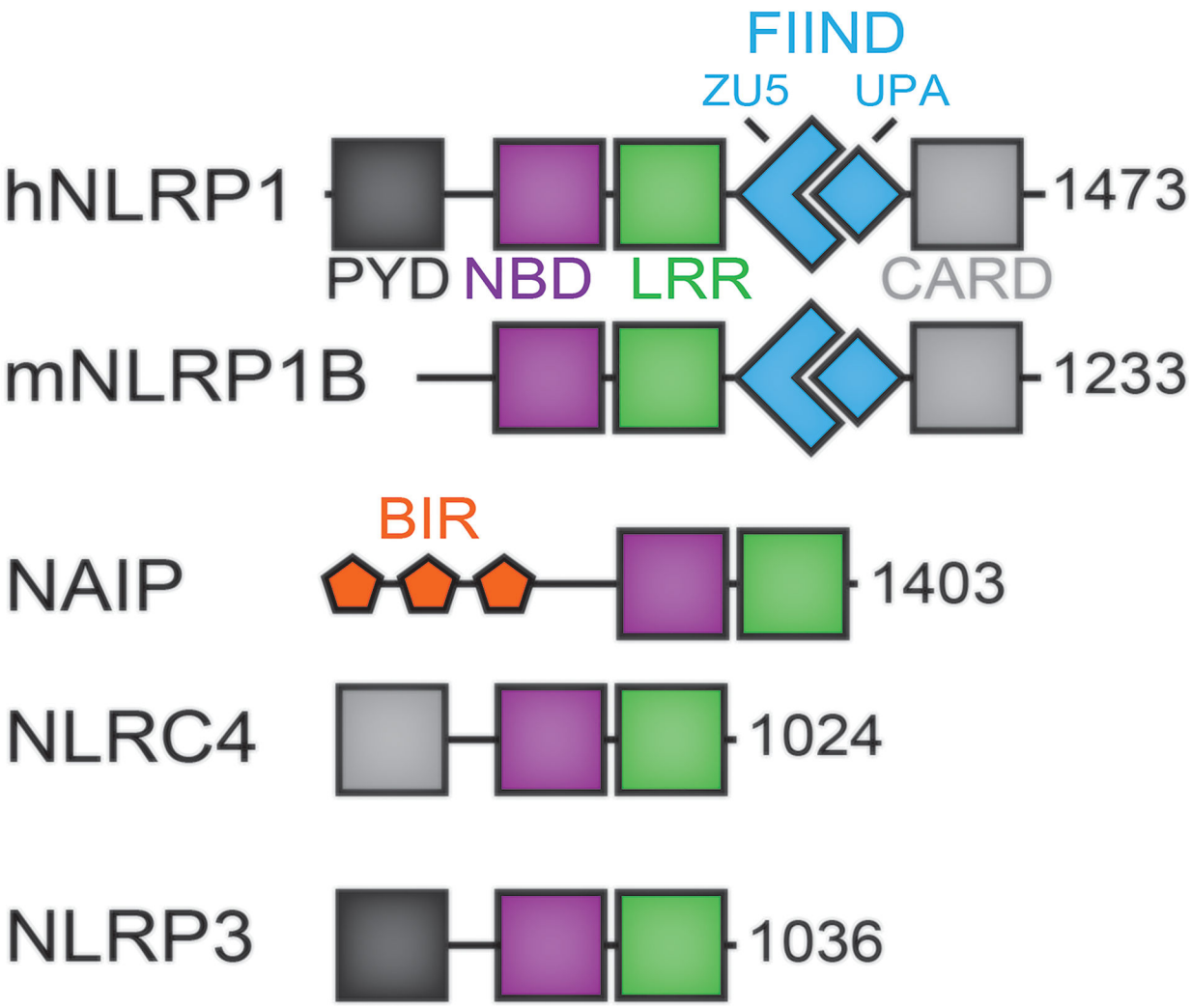

Figure 1. Domain architecture of representative NLRs.

NLRs that form an inflammasome contain Nucleotide Binding Domain (NBD, purple) and Leucine-Rich Repeat (LRR, green) domains. Upon pathogen detection, NLRs assemble and recruit CASP1 via N-terminally located death domains: either directly through a CARD (grey) or indirectly through a PYD (black) via the adaptor ASC. Distinct from all other NLRs, NLRP1 has a C-terminal CARD and an N-terminal PYD, which has been lost in some species, including mouse. NLRP1 also contains a Function-to-find domain (FIIND), comprised of ZU5 and UPA subdomains, which undergoes constitutive self-cleavage (i.e., auto-processing). mNLRP1B, mouse NLRP1B; hNLRP1, human NLRP1; BIR, baculovirus inhibitor of apoptosis protein repeat.

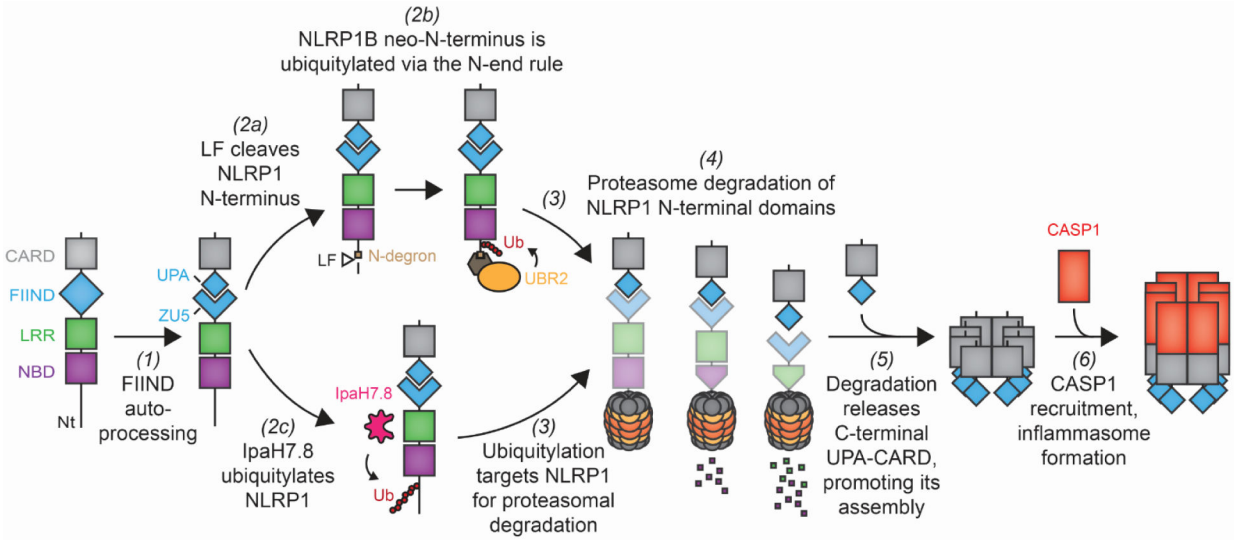

Figure 2. NLRP1 recognizes and responds to diverse pathogen-encoded effectors via 'functional degradation.'

In this model, the FIIND domain, consisting of ZU5 and UPA subdomains, undergoes constitutive auto-processing, resulting in two non-covalently associated peptides (1). N-terminal proteolysis by Lethal Factor (LF) protease exposes a destabilizing N- degron (2a), which is recognized by a UBR2-containing complex resulting in NLRP1B ubiquitylation (2b). Subsequent proteasomal degradation of the N-terminal domains (NBD-LRR- ZU5) (3, 4) liberates the C-terminal UPA-CARD (5), which allows for its assembly and recruitment of CASP1 (6). The *Shigella* E3 ubiquitin ligase IpaH7.8 can directly ubiquitylate NLRP1B (2c), which also promotes NLRP1B degradation and inflammasome activation. Ub, ubiquitin; Nt, N-terminus.

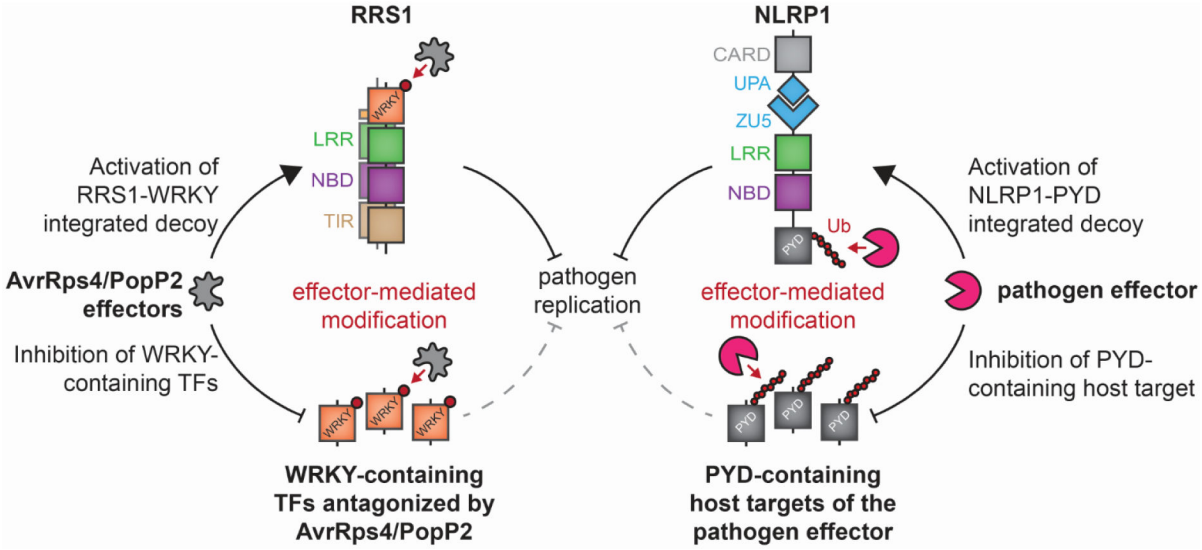

Figure 3. Pathogen recognition via an integrated decoy.

In the ‘integrated decoy’ strategy of pathogen detection, innate sensors harbor a domain that is similar to the ‘intended’ target of pathogen effectors. Host sensor activation occurs when the integrated decoy is triggered by the same enzymatic activity used by the pathogen effector to antagonize other host proteins. In plants (left), immune signaling requires numerous WRKY-containing transcription factors (TFs). The pathogen effectors AvrRps4 and PopP2 inhibit WRKY-containing TFs through post- translational modification. This same enzymatic activity is sensed by the host sensor RRS1 through its WRKY-integrated decoy, resulting in its activation and a protective immune response. We hypothesize that the NLRP1 PYD may also function as an integrated decoy for pathogen detection (right). Note that any NLRP1 N-terminal domain could theoretically serve as an integrated decoy. TIR, Toll–interleukin-1 receptor domain; Ub, ubiquitin.

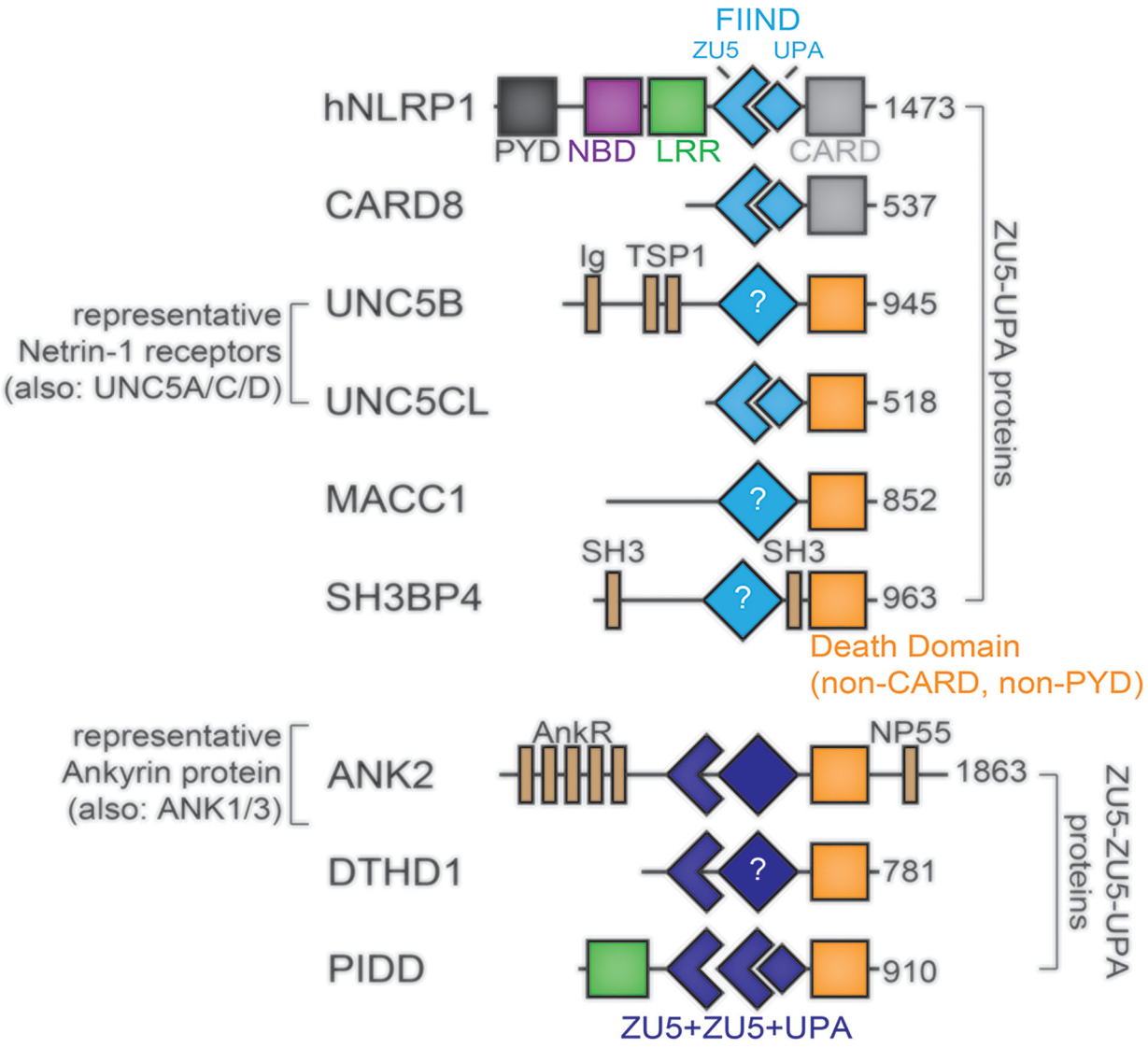

Figure 4. FIIND (ZU5-UPA) containing proteins.

The Function-to-find domain (FIIND) is comprised of two subdomains, ZU5 and UPA. In NLRP1 and CARD8, the FIIND is followed by a C-terminal CARD, a death-fold domain. This domain architecture is found in several other human proteins. The NLRP1 and CARD8 FIIND is structurally similar to that of UNC5B (pdb 3G5B) and other Netrin receptors, MACC1 and SH3BP4. In contrast, ANK2 (pdb 4D8O), DTHD1 and PIDD all have a second ZU5 preceding the FIIND, which may itself be sufficient for a second auto-processing event. FIIND auto-processing is required for the function of NLRP1, CARD8 and PIDD, but is apparently not essential for the function of UNC5CL. Likewise, some FIIND-containing proteins, like ANK2, do not appear to undergo auto-processing (closed diamond). FIIND auto-processing has not been experimentally tested in many cases (indicated by a “?”). In addition, FIIND-containing proteins are decorated with a variety of accessory domains, suggesting that ‘functional degradation’ may be utilized across diverse biological processes.

Ig, immunoglobulin domain; TSP1, Thrombospondin type 1 repeats; AnkR, ankyrin repeat; NP55, Neuroendocrine-specific golgi protein P55; SH3, Src Homology 3 domain.