STAT proteins, ROR-γt, GATA-3, T-bet, Bcl-6 and Foxp3, are regulated by ubiquitination[12]. The fact that E3 ligases and deubiquitinating enzymes such as Itch, Cbl-b, A20 or CYLD function as critical negative regulators of inflammatory responses by modulating different cell types and pathways makes them potentially attractive drug targets in conditions such as chronic infections or for the immunotherapy of cancer. New strategies for targeting these previously 'undruggable' molecules[13] will help to fully realize this potential. Going forward it will be critical to further elucidate the complex co-regulation of individual substrates and pathways by E3 ligases and deubiquitinating enzymes and to discern scaffolding function from enzymatic activity. By doing so, researchers might eventually be able to 'fine-tune' the ubiquitination state and, hence, pathway activity for therapeutic intervention.

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A dRAStic RHOAdblock of Pyrin inflammasome activation

Andrea Dorfleutner & Christian Stehlik

The Pyrin inflammasome guard is disabled if the activity of small cellular GTPase is compromised in response to defects in the mevalonate pathway and is disabled directly by mutations in the gene encoding Pyrin, which results in the interleukin 1β (IL-1β)-driven autoinflammatory diseases MKD and FMF.

The recognition of microbial ligands by pattern-recognition receptors (PRRs) triggers the formation of a cytosolic protein platform referred to as the 'inflammasome'. During inflammasome assembly, the adaptor ASC and the protease caspase-1 are recruited to PRRs, followed by the activation of caspase-1, then processing and release of the pro-inflammatory cytokines IL-1β and IL-18 and the induction of pyroptotic cell death. While inflammasome formation is instrumental for host defense, it is detrimental if dysregulated, as is the case in human autoinflammatory diseases, including cryopyrinopathy and familial Mediterranean fever (FMF)[1]. Several PRRs have been described as assembling an inflammasome, including NLRs, ALRs and Pyrin. Although the mechanism and function of NLR and ALR inflammasomes are quite well understood, the precise role of Pyrin in inflammasome signaling has been controversial. Initially, contradicting studies suggested that Pyrin either promoted or inhibited inflammasome activation, and potential ligands for Pyrin remained elusive until a distinct mode of operation for the Pyrin inflammasome was identified. It is now known that Pyrin senses modification and inactivation of GTPases of the Rho family by bacterial toxins, which promotes assembly and activation of the Pyrin inflammasome[2] (**Fig. 1a**). Two papers in this issue of *Nature Immunology*, by Park *et al.*[3] and Akula *et al.*[4], now provide exciting insights into the mechanism by which the Pyrin inflammasome is regulated by small cellular GTPases through geranylgeranylation, a post-translational lipid modification. Thus, defects in the fundamental metabolic mevalonate kinase (MVK) pathway that synthesizes geranylgeranyl pyrophosphate, the substrate of geranylgeranylation, lead to activation of the Pyrin inflammasome. Therefore, the two seemingly unrelated autoinflammatory diseases FMF (caused by a mutation in the gene encoding Pyrin (*MEFV*)) and MVK deficiency (MKD (also known as hyperimmunoglobulinemia D syndrome (HIDS)), caused by a loss-of-function mutation in the gene encoding MVK) both share a common mechanism: activation of the Pyrin inflammasome.

MEFV was the first inflammasome-encoding gene linked to a human disease (FMF). Its amino-terminal protein-binding domain, which has a key role in inflammasome assembly, is now referred to as the 'PYRIN domain' and is present in many inflammasome components[5]. Due to its recessive mode of inheritance, FMF was originally considered a disease caused by loss-of-function mutations, a view that has since been challenged[1]. In addition to its role as an inflammasome sensor, Pyrin also interacts with caspase-1 through its B30.2 (SPRY) domain to modulate the release of IL-1β and is itself cleaved by caspase-1, which results in truncated Pyrin that activates the transcription factor NF-κB; this might further amplify autoinflammatory responses, since proteins encoded by FMF-linked mutations are cleaved more efficiently. However, such mouse studies have been complicated by the fact that mouse Pyrin lacks the B30.2 (SPRY) domain, which is encoded by sequence that contains the majority of FMF-linked mutations[1]. In contrast, MKD is an autosomal recessive autoinflammatory disease that also has a wide clinical spectrum, depending on the level of MVK activity. MVK-mediated geranylgeranylation promotes the release of IL-1β[6], but the underlying mechanism has remained elusive.

Akula *et al.* investigate the pathology of MKD. The fundamental metabolic mevalonate pathway is responsible for the biosynthesis of cholesterol and other metabolites, whereby MVK-mediated phosphorylation of mevalonate is a key step and defects in MVK activity cause HIDS[7]. Geranylgeranylation of small GTPases, which is facilitated by the geranylgeranyl transferase GGTase I, functions at least in part as

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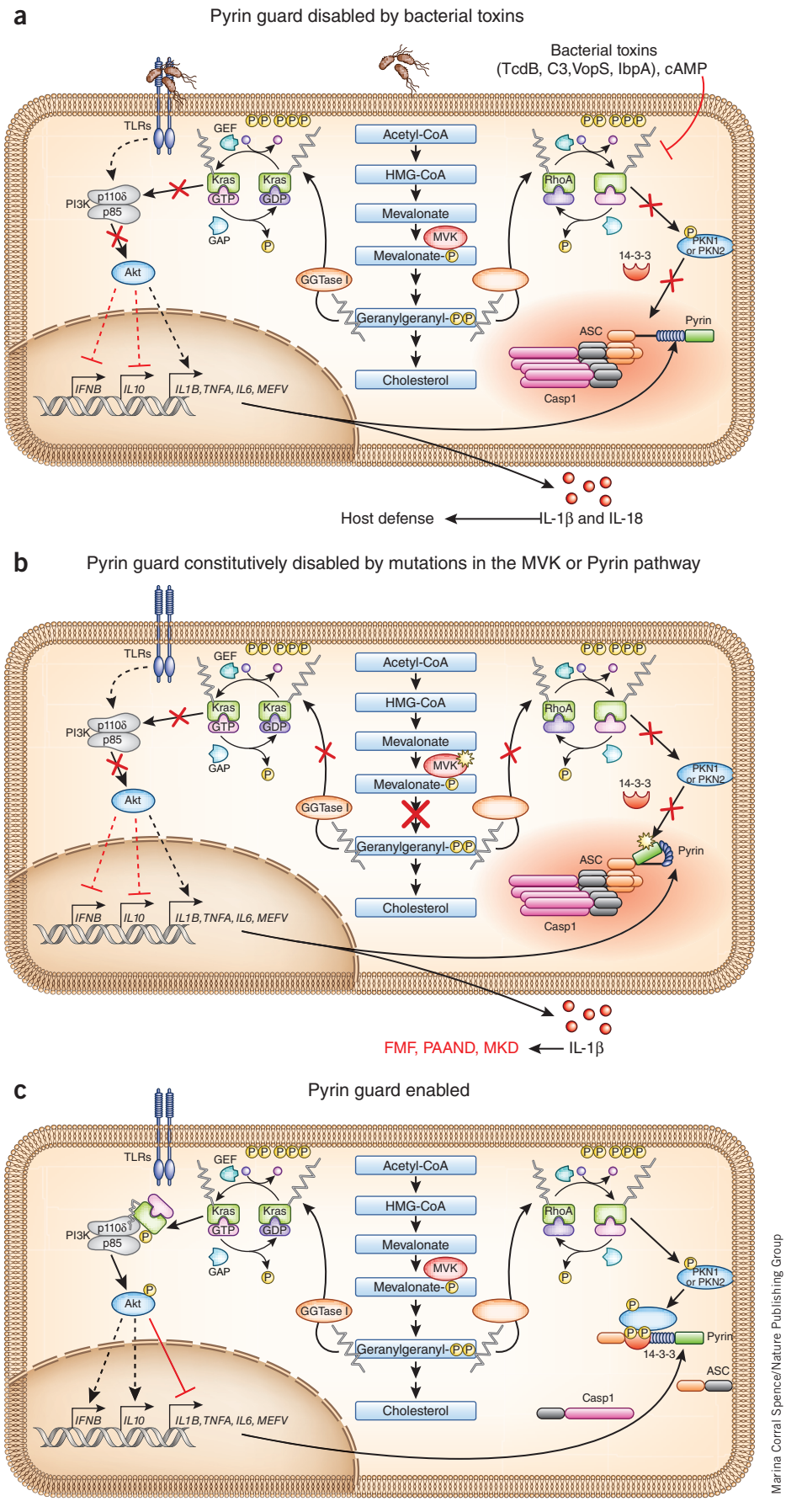

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Figure 1 The guard-type Pyrin inflammasome. (**a**) Bacterial toxins or intracellular cAMP cause inactivation of RhoA and disrupt PKN-mediated phosphorylation of Pyrin, which releases the 14-3-3 guards from Pyrin to promote assembly of the Pyrin inflammasome and release IL-1β and IL-18 to support host defense against bacterial pathogens. GEF, guanine-nucleotide–exchange factor; p85, PI3K subunit; GAP, GTPase-activating protein. (**b**) Deficiency in MVK (as in patients with MKD) impairs geranylgeranylation of Kras and subsequently causes enhanced release of pro-inflammatory cytokines and transcription of *MEFV*. In parallel, geranylgeranylation-dependent activation of RhoA is impaired, which releases the 14-3-3 guard from Pyrin and causes constitutive assembly of the Pyrin inflammasome and release of IL-1β. Hereditary mutations in the gene encoding Pyrin (as observed in patients with FMF) prevent the interaction of Pyrin with PKNs and 14-3-3 proteins or directly destroy the Ser242-phosphorylation site (as observed in patients with PAAND), which results in activation of the Pyrin inflammasome. Hence, temporary disruption of any part of this guard pathway causes Pyrin-dependent host defense responses. (**c**) During homeostasis, geranylgeranyl pyrophosphate is generated via the metabolic mevalonate pathway through the activities of MVK and GGTase I to appropriately geranylgeranylate small GTPases, including Kras and RhoA, and tether them to the plasma membrane. Active, geranylgeranylated Kras regulates TLR-mediated activation of PI3K-Akt1 to balance the transcription of genes encoding pro-inflammatory and anti-inflammatory mediators and downregulates *MEFV* expression. At the same time, activation of PKN1 and PKN2 results in phosphorylation of Pyrin at Ser208 and Ser242 and enables interaction with members of the 14-3-3 family, which guard against activation of Pyrin.

a membrane anchor. The authors show that geranylgeranylation enables activation of the lipid kinase PI3K induced by Toll-like receptors (TLRs). GGTase I–deficient (*Pggt1b*$^{-/-}$) macrophages have a greater abundance of pro-inflammatory cytokines than do wild-type mice, and *Pggt1b*$^{-/-}$ mice are highly susceptible to lipopolysaccharide-induced septic shock. The cytokine profile of *Pggt1b*$^{-/-}$ mice is similar to that of mice deficient in PI3K or the kinase Akt1, and *Pggt1b*$^{-/-}$ macrophages display impaired Akt1 activation and are a phenocopy of macrophages deficient in the PI3K catalytic subunit p110δ (*Pik3cd*$^{-/-}$). Ras GTPases directly bind to and activate PI3K catalytic subunits, but small-interfering-RNA screen by Akula *et al.* specifically identifies the small GTPase Kras as being necessary for the activation of Akt1, and they find that geranylgeranylation of Kras is essential for its binding to and activation of p110δ. *Pggt1b*$^{-/-}$ and *Pik3cd*$^{-/-}$ macrophages exhibit constitutive

release of IL-1β dependent on Pyrin, ASC and caspase-1 but independent of the PRRs NLRP3, AIM2 or NLRC4, which demonstrates that the Pyrin inflammasome is specifically activated. Both *Pggt1b*$^{-/-}$ macrophages and *Pik3cd*$^{-/-}$ macrophages display higher basal and inducible expression of *Mefv* than that of wild-type macrophages. Congruent with that, patients with HIDS have higher expression of pro-inflammatory cytokines than that of healthy subjects, reminiscent of that of healthy peripheral blood mononuclear cells treated with blockade of the mevalonate pathway. Hence, the authors conclude that in the absence of protein geranylgeranylation, PI3K activity is compromised and therefore promotes unchecked TLR-induced inflammatory responses, which causes constitutive activation of the Pyrin inflammasome and IL-1β release (**Fig. 1b**). Hence, GGTase I is able to control activation of the Pyrin inflammasome through a PI3K-p110δ–dependent pro-inflammatory transcriptional response.

In the related paper, Park *et al* [3]. follow up on a published report [2] and investigate the mechanism by which bacterial toxins that inactivate Rho GTPases induce the release of IL-1β by the Pyrin inflammasome. Clostridial toxins induce glucosylation, adenylation, ADP-ribosylation or deamidation of the switch I region of RhoA, which prevents the binding of guanine nucleotides and GTPase activity. Therefore, these authors hypothesize that signaling downstream of RhoA regulates the Pyrin inflammasome. They find that silencing of the RhoA effector kinases kinases PKN1 and PKN2 or chemical inhibition of PKN induces the release of IL-1β, and activation of PKN prevents this. It is known that Pyrin is phosphorylated and interacts with guard proteins of the 14-3-3 family, but the kinase responsible and the functional relevance of this has remained elusive[8]. Park *et al.* now show that PKN1 and PKN2 phosphorylate Pyrin at Ser208 and Ser242. Notably, the mutation that results in the S242R substitution also causes Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND)[9]. PKN1- and PKN2-dependent phosphorylation of human Pyrin containing a B30.2 (SPRY) domain is much less than that of truncated Pyrin lacking that domain and is diminished even further for Pyrin encoded by a gene with FMF-associated mutations, which suggests that this domain negatively regulates the binding of PKN to and phosphorylation of Pyrin. Furthermore, binding of 14-3-3 proteins is diminished in the presence of FMF-associated mutations. Hence, 14-3-3 proteins maintain Pyrin in an inactive conformation, and loss of phosphorylation and the resulting failure of binding of 14-3-3 proteins promotes FMF (**Fig. 1b**). A similar scenario has been identified for PAAND-associated mutations, in which 14-3-3 proteins bind only to phosphorylated Pyrin but not to mutant Pyrin with the PAAND-associated S242R substitution[9]. Geranylgeranylation of RhoA is necessary for its activation, and Park *et al.* therefore investigate the link of Pyrin to MVK and observe that statins cause dose-dependent release of IL-1β that is dependent on Pyrin but independent of NLRP3, NLRC4 and AIM2 (ref. 3).

These two groundbreaking reports[3,4], although they originate from opposite 'directions', arrive at a similar conclusion by highlighting the importance of prenylation and small GTPases in activation of the Pyrin inflammasome and by connecting disruptions of this pathway to PAAND, FMF and HIDS. However, some distinctions exist. While Akula *et al.* propose that MVK-dependent prenylation of Kras is needed to prevent activation of the Pyrin inflammasome and release of IL-1β[4], Park *et al.* conclude that MVK-dependent geranylgeranylation and other forms of RhoA activation are needed to prevent activation of the Pyrin inflammasome via maintenance of the phosphorylation of Pyrin and interaction with inhibitory 14-3-3 protein guards[3] (**Fig. 1c**). Hence, Pyrin functions as a sensor of small GTPase activity, which is frequently targeted by bacterial toxins to impair fundamental cellular processes. Depending on which GTPase activity is disrupted, the signals elicited modulate the transcription of *Mefv* and pro-inflammatory mediators or directly regulate the phosphorylation and subsequent activity of Pyrin. Hence, synergistic mechanisms that induce expression of the Pyrin inflammasome and disrupt the 14-3-3 guards on Pyrin to culminate in activation of the Pyrin inflammasome and release of IL-1β would support successful bacterial clearance. Due to the high carrier frequency of FMF-associated *MEFV* mutations in Mediterranean and Middle Eastern populations, there has long been speculation about the possible advantage of such mutations. Fine-tuning of Pyrin inflammasome activity might be beneficial under certain circumstances for the promotion of host defense. FMF-linked mutations in the sequence encoding B30.2 (SPRY) diminish or prevent the phosphorylation of Pyrin and activate Pyrin, probably through altered intramolecular interactions to block interaction with PKN1 and PKN2 and, subsequently, 14-3-3 proteins, contrary to the intramolecular interactions between leucine-rich repeats and the nucleotide-binding and oligomerization domain NACHT in NLRs or the interaction of the DNA-binding domain HIN200 and PYRIN domain in ALRs, which keep these sensors inactive. However, how mice that completely lack the B30.2 (SPRY) domain would then undergo promotion of Pyrin activation is currently unknown, but Park *et al.* speculate that phosphatases might be necessary [3], and their identification would be the next logical step in further elucidation of the regulation of the Pyrin inflammasome. Such an innate immunological guard-type mechanism is well established in plant disease-resistance R proteins during the hypersensitive response, which involves the phosphorylation of negative regulators, and plant 14-3-3 proteins have a prominent role in this response[10,11]. Such effector-triggered immunity for the sensing bacterial toxin activity by Pyrin represents a new paradigm in mammalian innate immunity in which host-defense responses are indirectly triggered by a guard-type mechanism to enable the detection of broad GTPase-inactivating toxin activities of live pathogenic bacteria. However, defects in any part of this guard system cause uncontrolled production of IL-1β and systemic inflammatory disease. Consequently, these new insights might have important clinical implications, as traditionally FMF and HIDS have not been linked, but placing them into the category of 'inflammasomopathies' might enable the application of the well-established cadre of IL-1β-targeting drugs. These two studies therefore provide an important piece in the puzzle of understanding mammalian pathogen sensing and how defects in this system cause autoinflammatory disease.

COMPETING FINANCIAL INTERESTS
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